Root Exudates in the Grassland Ecosystem

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Table of Content

Summary

The rhizosphere is the immediate vicinity of the plant root. Therein, plant roots exude inter alia metabolites of the primary and secondary metabolism into the rhizosphere to affect the nutrient availability as well as to interact with other plants, microbes and herbivores. These exogenous environmental and endogenous factors such as species-specific and plant specific traits influence the chemical composition of exuded metabolites. In most cases, these findings were obtained mostly from interaction studies, using an one-factorial design with single compartments of the rhizosphere and intact plants or with artificial exudate profiles. While this approach allows elucidating the role of specific exudates, it neglects the intertwined parts of the rhizosphere, and thus, is highly artificial. Moreover, as the whole plant metabolome is largely unknown, the focus of metabolome studies has been on specific metabolite classes or specific metabolites, so far. This targeted metabolite-profiling approach, however, cannot reflect the complexity of the root exudate profile.

By investigating the exudation of plants under natural rhizosphere conditions, this study aims to describe exudate patterns in natural environments. Ten perennial grassland plant species were planted as phytometers into existing grasslands of the German Biodiversity Exploratories and examined for their exudate profiles. In 2014 and 2015, the exudates were collected directly form the roots, using a method that had been tested under laboratory conditions, and analysed by two mass spectrometric approaches. The combination of gas and liquid chromatography coupled to mass spectrometry and subsequent untargeted metabolite profiling allowed the observation of metabolite profiles consisting of classes of both, the primary and secondary metabolites. Furthermore, a high number of substances could be assigned to chemical substance classes by a novel identification method, as well as identified by other methods. Exudate patterns were related to different drivers, including information on plant functional traits, plant species composition of the neighbourhood, abiotic soil and climate conditions and land use intensity.

The results show that endogenous as well as exogenous factors affect the exudation of plant roots. The relative impact of the different factors was found to be highly metabolite-specific. Both primary and secondary metabolites show a response to abiotic factors such as soil conditions. For the exudation of secondary metabolites, the species identity of the target plant played the most important role. While here forbs displayed a higher interspecific variability, grasses were very similar to each other. In contrast, climate and land use intensity had only a minor impact on shaping the exudate composition of the

phytometer plants. Similarly, the local plant neighbourhood did affect the metabolite composition only marginally.

The results of this thesis show that an investigation of exudates under realistic non-controlled field conditions is not only feasible but also highly informative.

Zusammenfassung

Die Rhizosphäre ist der die Pflanzenwurzel unmittelbar umgebene Lebensraum. In ihm können Pflanzen durch die Exsudation von u.a. Metaboliten des primären und sekundären Stoffwechsels über die Wurzeln die Nährstoff Verfügbarkeit beeinflussen und mit den sie umgebenden Pflanzen, Mikroorganismen und Herbivoren interagieren. Diese exogenen Umweltfaktoren sowie auch endogene Faktoren wie artspezifische und Individuen-spezifische Merkmale beeinflussen das chemische Muster der exsudierten Metabolite. Diese Erkenntnisse beruhen vor allem auf Interaktionsstudien, die zumeist mit einfaktoriellem Design zwischen einzelnen Kompartimenten der Rhizosphäre und intakten Pflanzen oder mit artifiziellen Exsudat Profilen durchgeführt wurden. Diese Vorgehensweise erlaubt es zwar, die Rolle spezifischer Exsudate aufzuklären, durch das Vernachlässigen des Ineinandergreifens der verschiedenen Bestandteile der Rhizosphäre ist sie jedoch sehr artifiziell. Da das gesamte pflanzliche Metabolom weitestgehend unbekannt ist, wurde zudem meist das Augenmerk auf einzelne Metabolit-Klassen oder einzelne Metabolite gelegt. Dieses *targeted metabolite profiling* spiegelt jedoch nicht die Komplexität des Exsudat-Profils wider.

Durch die Untersuchung der Exsudation unter natürlichen Rhizosphären-Bedingungen, hat diese Arbeit das Ziel, die Exsudat-Muster unter realistischen Bedingungen zu beschreiben. Dazu wurden zehn mehrjährige Pflanzenarten als Phytometer in existierende Grünländer der „deutschen Biodiversitäts-Exploratorien“ gepflanzt und auf ihre Exsudat-Profile untersucht. In den Jahren 2014 und 2015 wurden die Exsudate der Pflanzen direkt von den intakten Wurzeln mittels einer unter kontrollierten Bedingungen getesteten Methode gesammelt und mit zwei massenspektrometrischen Methoden analysiert. Die gewählte Kombination aus Gas- und Flüssigkeits-Chromatographie gekoppelter Massenspektrometrie und anschließendem *untargeted metabolite profiling* erlaubte dabei die Generierung von Metabolit-Profilen der verschiedenen Klassen sowohl des primären als auch des sekundären Pflanzenstoffwechsels. Zudem konnte mithilfe einer neuen Klassifizierungsmethode eine große Anzahl an Metaboliten Substanzklassen zugeordnet und, sowie durch weitere Methoden identifiziert werden. Die Exsudat-Profile wurden in Beziehung zu endogenen und exogenen Faktoren gesetzt, wofür funktionelle Pflanzen-Merkmalen, die Pflanzenarten-Zusammensetzung der Nachbarschaft, die abiotische Boden- und Klima-Faktoren sowie die Landnutzungsintensität herangezogen wurden

Die Ergebnisse zeigen, dass unter diesen natürlichen Bedingungen sowohl endogene als

auch exogene Faktoren die Exsudation der Pflanzenwurzeln beeinflussen. Die Bedeutung dieser verschiedenen Einflussfaktoren ist dabei vom jeweiligen Metabolit abhängig. So konnten sowohl primäre und sekundäre Metabolite abhängig von abiotische Faktoren wie z.B. des Bodens bestimmt werden. Bei den sekundären Metaboliten spielt dagegen die Art-Identität die bedeutendste Rolle. Während Kräuter hier eine höhere interspezifische Variabilität ihrer Exsudationsmuster aufwiesen, waren sich Gräser in ihrer Exsudation sehr ähnlich. Dagegen hatten Klima und Landnutzungsintensität nur einen geringen Einfluss auf die Exsudat-Zusammensetzung der jeweiligen Phytometer-Pflanze. Auch die lokale Pflanzen-Nachbarschaft änderte die Metabolit Zusammensetzung nur unwesentlich.

Die Ergebnisse dieser Arbeit zeigen, dass eine Analyse von Exsudaten unter realistischen unkontrollierten Freiland-Bedingungen nicht nur möglich, sondern auch höchst aufschlussreich ist.

I. Introduction

1.1. The grassland ecosystem

Ecosystems are defined as 'a community made up of living (biotic) and non-living (abiotic) components' (Chapin et al., 2002), as well as the interactions between those parts. Each ecosystem includes a specific habitat e.g., the grasslands. Grasslands are an area with climatic, anthropogenic and other environmental conditions that inhibit the growth of trees (White, 1983). This ecosystem cover 26 % of global land area (Herz, 2017) and is dominated up to 90 % by grasses (monocotyledons) (Boval and Dixon 2012). However, other growth forms like forbs (dicotyledons) also occur within it (Box, 1996). Accordingly, grasslands have a high biodiversity at a small spatial scale (Herz, 2017) and offer multi functionality at different ecosystem levels (Hector and Bagchi, 2007, Isbell et al., 2011). However, only a small percentage of grasslands are still in their natural state. Anthropogenic land use, which includes mowing, grazing by cattle and sheep, and/or fertilization, has transformed 47 % of all existing natural grasslands to pastoral areas, while 26 % occurring in marginal areas (for instance, global dry zones) are reduced to a semi-natural condition (Kruska et al., 2003). Thus, biodiversity, ecosystem functioning and functional stability are strongly affected (Fischer et al., 2010, Laliberté and Tylianakis, 2011). A significant body of evidence, gathered through the use of ecological model experiments, has discussed the impact of this type of human intervention on grassland ecosystem (Cardinale et al., 2012, Roscher et al., 2004, Tilman et al., 2006). Agricultural activity changes the soil chemistry, and alters or disturbs the productivity of grasslands (Laliberté and Tylianakis, 2011). For instance, Foley et al. (2005) and Allan et al. (2014) observed a reduced diversity of many taxa that provide ecosystem services to grasslands, another indication of an indirect effect on ecosystem functioning. The shift in functional vegetation composition is driven by the agricultural preference and dissemination of fast-growing species at the expense of slow growing species (de Vries et al., 2012, Lavorel et al., 2011). This supports the postulation that this development decreases not only biodiversity, but ecosystem functionality as well (Fischer et al., 2010, Laliberté and Tylianakis, 2011). However, none of these studies has taken into consideration one important level of the grassland ecosystem: the subterranean metabolomics of plants.

1.2. Metabolomics and Ecology

Metabolites are substances synthesised by the cell to response to physiological and environmental events (Fiehn, 2002, Oliver et al., 1998). The 'the quantitative complement of all of these molecules present in cells' (Oliver et al., 1998) is known as metabolome, while the investigation of the aggregate metabolome is called metabolomics (Kopka et al.,

2004). It is estimated that the global metabolome of all plant species is upwards of 1,000,000 metabolites, of which 51,179 (as listed in the KNApSAcK database 2018/08/07) have so far been found in higher plants (Saito and Matsuda, 2010). These organic compounds are roughly classified as either primary or secondary metabolites or, based on their chemical characteristics, as polar, semi-polar or a-polar metabolites. Because metabolites are involved in a vast number of biological and ecological functions, their investigation is of great importance. In a relatively new and evolving field, eco-metabolomics, their ecophysiological adjustments are investigated and quantified using metabolomics techniques (Peters et al., 2018, Webster et al., 2008). This allows the revelation of the biochemical mechanisms that are involved in the interactions of species with their environment and surrounding organisms (Sardans et al., 2011, Viant, 2009). In contrast to typical metabolomics approaches where investigations are conducted on model plants such as *Arabidopsis thaliana* (Monchgesang et al., 2016b, Strehmel et al., 2016), or crop plants such as maize (*Zea mays*), *Solanacea* species (Dobritzsch et al., 2016, Yoshihara et al., 1978) or rice (*Oryza sativa*) (Aulakh et al., 2001), eco-metabolomics focusses its investigations on non-model organisms (Peters et al., 2018). The advantage of this approach is the unearthing of a high number of novel compounds and the exploration of their potential purpose and interactions within the natural ecosystem (Viant et al., 2017). One of these ecosystem interactions is the release of metabolites into the rhizosphere via roots: the exudation.

1.3. Rhizosphere, roots and exudates

The term rhizosphere was developed by Hiltner (1904), and describes the “soil compartment influenced by plant roots”. It consists of the plant root and the soil as well as the organisms in proximity to the root. It is thereby defined as the belowground region where processes take place that are important for plant growth and health (Badri et al., 2009b, Lynch, 1987).

The plant root itself is a non-leaf, non-nodes bearing organ of the plant (Eshel and Beeckman, 2013). The roots’ main functions were traditionally determined to be anchorage of the plant, as well as the uptake of nutrients and water supply for the metabolic and developmental processes of the plant (Badri and Vivanco 2009). In fulfilling these functions, plant roots change the chemical and biochemical properties of the surrounding soil, and alter the availability of e.g., nitrogen, phosphorus, iron and water (Badri and Vivanco, 2009, Ziegler et al., 2016). This interference with the abiotic part of the rhizosphere impacts the biotic community as well by the competition for nutrients, space

and interaction partners (Badri et al., 2009b, Bardgett et al., 2014, Lambers et al., 2008). Plants developed different methods to successfully improve their performance and fitness in this competing environment. One strategy is to alter their plant traits. Those comprises 'physiological, morphological and phenological' plant characteristics (Pérez-Harguindeguy et al., 2013, Reich and Cornelissen, 2014) and were referred as plant functional traits in this thesis. For example, plants evolve two different root system architectures (Straßburger, 2002). Roots consisting of a primary (tap) root system with lateral roots were typically found in dicotyledonous species, whereas a less branched primary root system is typical for monocotyledonous species (Badri and Vivanco, 2009, Straßburger, 2002). Both root systems fulfil specific functions in the resource economic spectrum (Reich and Cornelissen, 2014). The root system of monocotyledons adapts more quickly to the surrounding soil condition, and allows for a faster acquisition of nutrients (Bardgett et al., 2014). This enhances the phenotypical plasticity of these plants in comparison to dicotyledons (Herz, 2017, Siebenkas et al., 2015). On the other hand, dicotyledons can use their roots to store nutrients (Straßburger, 2002), and with this they are able to better cope with changing abiotic conditions (Herz et al., 2017b).

Both types of root systems secrete various inorganic and organic molecules. This covers a spectrum from low to high molecular weight compounds (Bais et al., 2006b). This thesis focus on the root released low molecular weighted organic molecules, hereafter called metabolites, compounds, root exudates, or simply exudates. Therefore, the higher molecular weighted and inorganic compounds are not further considered.

The release of exudates occurs either actively by transporters and ion channels through the cellular membrane in the rhizosphere, or passively by diffusion or detaching of root cells (Badri et al., 2009a). Although the primary exuding region of the root is considered to be the root tip and the zone directly abutting the root tip (Badri and Vivanco, 2009, Baetz and Martinoia, 2014), an exudation from other parts of the root cannot be excluded (McDougall and Rovira, 1970). The exuded organic compounds alter the local surrounding environment of the plant root (Chaparro et al., 2013), and are secreted as a reaction to both positive and negative influences (Badri and Vivanco, 2009). The following sections will provide a closer look at the chemical characteristics and the function of exudates in the rhizosphere.

1.4. Chemical characteristics of plant metabolites

The chemical cocktail of exudates comprises a high variety of different volatile and non-volatile metabolites. Non-volatile metabolites, both primary (mostly polar) and secondary

(often semi-polar), are the most common and will be the subject of analysis in this thesis.

Primary metabolites are required for the growth and maintenance of cellular function, for instance, the provision of energy, development of structural elements and primary growth processes (Buchanan et al., 2015). These metabolites are derived from fundamental metabolic pathways or cycles such as glycolysis, the Krebs Cycle or the Calvin Cycle. A broad range of metabolites, such as amino acids, biogenic amines (amines in this thesis), lipids, nucleotides and nucleic bases, organic acids, and sugars (also called carbohydrates), are categorized as primary metabolites.
Amino acids are the building blocks of proteins and are the precursors for a number of secondary metabolites e.g., the phenylpropanoids (Buchanan et al., 2015). The enzymatic decarboxylation of amino acids results in biogenic amines, which can function as synthetic precursor of alkaloids and hormones (Guggenheim, 1951). Lipids are a chemically heterogeneous class of metabolites (Gurr, Harwood et al. 2016). Members of this class include among others: fatty acids, fats, oils, steroids (sterols), glycerophospholipids (phospholipids), glycerol glycolipids, terpenes, and tocopherols (Gurr et al., 2016). Their function ranges from participating in a structural element of membranes, signalling, the transport of substances through the membrane, to the storage of metabolic energy and electrons (Gurr, Harwood et al. 2016). Nucleic bases are components of the nucleosides, which are in turn components of the nucleotides. Nucleotides are themselves precursors of nucleic acids, which are the components of ribonucleic acids (RNA) and of deoxyribonucleic acid (DNA) (Buchanan et al., 2015). Organic acids are a class of metabolites described as intermediates in carbon metabolism, e.g., the Krebs Cycle. They are also a key component in mechanisms of plants to cope with biotic and abiotic stresses (Jones et al., 2004). Carbohydrates, such as monosaccharides and disaccharides, are a class of highly water soluble metabolites (Aulakh et al., 2001) and are synthesised during photosynthesis. They are storage units of energy for plant developmental processes and play a role in plant interaction with the biotic community (Jones et al., 2004).

Secondary metabolites are primarily involved in plant processes that are highly dependent on specific situations. These processes include defence or attraction, as well as processes of inhibition and interaction (Badri et al., 2009b, Strehmel et al., 2014, van Dam and Bouwmeester, 2016). Based on their chemical characteristics, the most prominent classes are the nitrogen containing alkaloids, the phenolic phenylpropanoids and the terpenes (Rhodes, 1994). Phenylpropanoids and terpenes will be further described due to their relevance in this thesis.
Phenylpropanoids are widely distributed in the plant kingdom (Graham, 1991). Most members of this chemical class originate from the mevalonate / shikimate pathway (Vogt,

2010), through which phenylalanine and tyrosine are transformed into a variety of metabolites. Coumarins, flavonoids, polyphenolic acids and polymer are structured form the sub-classification of these metabolites (Vogt, 2010). Coumarins are found ubiquitously in higher plants (Baxter and Harborne, 1999). Although their primary function is to provide antimicrobial protection (Bourgaud et al., 2006), Schmid et al (2014) identified the coumarin esculetin as an important component in the recovery of iron from the soil. The hydroxyl group of esculetin serves as catechol-type siderophore residue, building complexes with Fe^{3+} that assist in the nutrient acquisition of plants. Flavonoids are another group that occurs widely in plants (Treutter, 2006). They are sub-grouped into anthocyanins, flavonols, flavones, flavanols, flavanones, chalcones, dihydrochalcones and dihydroflavonols (Treutter, 2006). Flavonoids play a role in UV protection (Harborne, 1999), frost hardiness and drought resistance in aboveground plant parts (Treutter, 2006). Belowground, they provide defensive functions (see next section) (Gershenzon and Dudareva, 2007, Hassan and Mathesius, 2012, Langenheim, 1994, Treutter, 2006). Polyphenolic acids, e.g. hydroxycinnamic acids, comprise a variety of different metabolites inter alia: cinnamic acid, p-coumaric acid, ferulic acid, caffeic acid and chlorgenic acid (Vogt, 2010). By combining derivatives of these metabolites to polymers (e.g., p-cumaryl alcohol, coniferyl alcohol and sinapyl alcohol to lignin), they contribute substantially to the capability of gymnosperms and angiosperms to deal with mechanical or environmental damage (Vogt, 2010). Moreover, hydroxycinnamic acid derivatives also fulfil a variety of defence and adjustment functions related to biotic and abiotic stimuli outside of the plant cell (Dobritzsch et al., 2016).

The terpenes form the largest class of plant defence chemicals and are also called terpenoids or isoprenoids (Degenhardt et al., 2009, Gershenzon and Dudareva, 2007, Langenheim, 1994). They are subdivided into lower terpenes, those with less than 20 carbon (C) atoms, and higher terpenes, those with 20 or more C atoms (Langenheim, 1994). Terpenes with 20 or more C atoms are non-volatile and water soluble (Langenheim, 1994). They are also included in this thesis. Terpenes serve as toxic, repelling or attracting agents and with this fulfil various ecological functions in plant-environment interaction (Gershenzon and Dudareva, 2007). For instance, the iridoid glycosides inhibit the growth of leave feeding insect and grazing animals (Yamane et al., 2010). Other higher terpenes are secreted into the rhizosphere as defensive metabolites (Dixon, 2001, Rasmann et al., 2005).

Most secondary metabolites carry the potential to be toxic for both the enemy organism as well as for the plant of origin. To prevent the plant of origin from damage, these metabolites are often inactivated by chemical modifications, e.g., a linkage to carbohydrates by an o-

glyosidic bond (Zhu and Schmidt, 2009). The derivatives of this chemical modification are summarised as glycosides or glycosylated compounds. By hydrolysing the glyosidic bond, the metabolite (aglycone) becomes active and can fulfil its function, e.g., the inhibition of the growth of neighbouring plants (Bais et al., 2006a).

Besides primary and secondary metabolites, the phytohormons also play a crucial role in plants' adaption to their environment (Fahad et al., 2014). Jasmonate, for instance, is part of a plant's defence system (Schweiger et al., 2014, Zhang et al., 2017). In case of an attack of herbivores and necrotrophic pathogens, plants produce jasmonate and methyl-jasmonate and initiate the jasmonate response cascade (Schweiger et al., 2014). The derivatives are also involved in tolerance to abiotic stresses and developmental processes, e.g., root growth (Huang et al., 2017).

1.5. The functional role of exudates in the rhizosphere

There are a number of endogenous and exogenous factors that are related to the exudation of metabolites by plants, and that impact the variety of functions exudates perform.

An often cited endogenous factor that impacts root exudation is the plant species (Aulakh et al., 2001, Chaparro et al., 2013, Hoekenga et al., 2003, Monchgesang et al., 2016a). Mönchgesang et al (2016a) and Hoekenga et al. (2003) observed qualitative and quantitative differences in the levels of metabolites present in the root exudates of *Arabidopsis thaliana* ecotypes. This underlines that there can be intraspecific variation in exudate profiles of the same species. Many studies have also shown the individual exudate profiles of single species, e.g. *Arabidopsis thaliana* (Strehmel et al., 2014) or *Zea maize* (Petriacq et al., 2017). Those studies observed specificity in exuding certain chemical compound classes (e.g., glycosinolates for *Brassicaceae*). However, experimental studies investigating the interspecific variation of the overall root exudate profile of a plant are rare.

Another factor impacting exudation is the developmental stage of plants. Whereas seedlings of plants, e.g., rice (*Oryza sativa*), produce the lowest quantity of root exudates, the qualitative and quantitative amount of released metabolites increases until flowering and decreases again during maturity (Aulakh et al., 2001, Chaparro et al., 2013).
Moreover, certain plant-specific traits, to some extend related to developmental stage, have an effect on plant root exudation. Garcia et al. (2001) showed that root exudation is positively correlated with root growth. Aulakh et al. (2001) states that a higher root weight

correlates with quantitatively higher and the certain release of specific organic acids. Thus, an actively growing root system secretes more exudates. Other plant traits, such as nutrient content and aboveground traits, however, have not yet been linked to root exudation.

Two different types of exogenous factors that impact exudation can be distinguished: abiotic and biotic influences. Abiotic factors are not living aspects of the environmental system. For instance, soil moisture has an impact on plants since a high moisture limits the availability of oxygen and causes a hypoxia condition in the plant (Rivoal and Hanson, 1994, Xia and Robert, 1994). By releasing metabolic products of the resulting anaerobic metabolism, e.g., organic acids and alcohols, plants can protect their cells from the consequences of this impact. The presence (or absence) of certain minerals and toxic metals in the soil can also alter the composition of root exudates. It has been shown that plant roots secrete organic acids to detoxify aluminium that was present in the soil (Ma 2000; Liao et al. 2006; Wang et al. 2006). Furthermore, physiochemical soil properties can strongly influence root morphology and exudation of roots (Neumann et al., 2014). When plants are suffering from a deficiency of phosphorous due to e.g. absorption by the soil, organic acids or phenolic compounds are released by the plant root to enhance the acquisition of insoluble inorganic phosphate (Badri and Vivanco, 2009, Tawaraya et al., 2014, Ziegler et al., 2016). Nitrogen (N)-limiting conditions can lead to the secretion of more flavones and flavonols by legume plants to attract and initiate legume–rhizobia symbiosis (Coronado et al., 1995, Zhang et al., 2009). Another abiotic factor is temperature, which impacts exudation indirectly. High temperatures and/or drought conditions lead to difficulties in plant metabolism. Drought tolerance can be raised indirectly when plants trigger arbuscular mycorrhiza (AM) fungi to enhance the accumulation of osmotic metabolites, thus lowering the water potential of the host plant (Latef et al., 2016, Rapparini and Peñuelas, 2014).

A further important abiotic factor, especially for grasslands, is agriculture. Studies have shown that depending on its intensity, agricultural land use alters plant root volume and root nutrient concentration (Blüthgen et al., 2012, Herz et al., 2017b) Thus, these findings suggest that agricultural land use also alters exudate profile composition. However, studies verifying this impact do not yet exist.

In contrast to abiotic factors, biotic factors comprise all living parts of an ecosystem, e.g., microorganisms (bacteria and fungi), herbivores (nematodes), insect and animals, and competing plants (Badri et al., 2009b, van Dam and Bouwmeester, 2016). When biotic factors affect the plant, these interactions can trigger the root to exude metabolites that result in either positive or negative interactions. Exudates, such as benzoxazinoids (Neal

et al., 2012) and carbohydrates (Huang et al., 2014), promote the attraction and colonialization of the plant root by growth promoting bacteria. Another example is when roots attract fungi by the secretion of flavonoids (Treutter, 2006). Such beneficial interactions (symbioses) play an important role in a number of vital ecosystems functions, e.g., carbon sequestration and nitrogen nutrient cycling (Jones et al., 2004, Kiers et al., 2011, Singh et al., 2004).These interactions could also increase the plants´ immunity to abiotic and biotic stresses (Badri et al., 2013, Huang et al., 2014, Strehmel et al., 2016), e.g., by producing a biofilm or antibiotics (Bais et al., 2004).

In addition to the advantageous interactions with the soil microbial community, plants also defend against or regulate the population of the organisms that neighbour the plant by phytoalexins (Dixon, 2001, Jandova et al., 2015, Mommer et al., 2016). For instance, phenolic acids or isoflavonoids, improve the resistance of watermelon against *Fusarium oxysporum f.sp.niveum* (Ling et al., 2013) or protect pea (*Pisum sativum*) plants against infection with *Nectria haematococca* (Wu and VanEtten, 2004), respectively. Moreover, when plants are wounded by herbivores or necrotrophic microorganisms, they activate the jasmonic acid defence pathway leading to an increased release of exudates (Carvalhais et al., 2013, Carvalhais et al., 2017, Zhang et al., 2017). Besides fungi, bacteria and herbivores, plants must also assert themselves against other plants in the rhizosphere. Rice plants exude momilactones to inhibit the growth of lettuce and grass (Xu et al., 2012), whereas the giant hogweed (*Heracleum mantegazzianum*) releases a blend of different chemicals of which 15 were identified as being responsible for the inhibition of the germination and root length of *Plantago lanceolata* (Jandova et al., 2015). Even the self/non-self-recognition of plants is affected by exudates (Biedrzycki et al., 2010). In the case of *Ambrosia dumosa*, roots stop growing when encountering roots of other plants from the same population, but not when encountering roots from the same physiological individual (Biedrzycki et al., 2010).

1.6. Metabolite profiling

In order to understand the role of exudates in the rhizosphere, they must be identified and characterized as a whole. The investigation of such a metabolome requires analytical methods that are highly sensitive and accurate. The results of these investigations must also have the possibility of high throughput in routine applications (Arens et al., 2015), in order to differentiate between various chemically related substances. Therefore, methods such as nuclear magnetic resonance (NMR) and mass spectrometry (MS) are often used.

Because of its high sensitivity (Viant and Sommer, 2013) mass spectrometry was applied in this thesis and will be introduced in the following.

In mass spectrometry, a sample of a more or less complex mixture of substances (the analyte) is investigated for their single molecules. This can be performed in a targeted way, in which a selected metabolites or chemical class is examined for, e.g., its quantative occurrence in the sample or due to its relation to a specific process (Dobritzsch et al., 2016, Döll et al., 2018). The opposite strategy is to measure as many as possible metabolites without apriori selection and, thus, get an overview of the metabolome of e.g. less considered biological systems (Peters et al., 2018, van Dam and Bouwmeester, 2016). This strategy is called untargeted metabolite profiling and was the method of choice for this thesis. In both strategies, untargeted and targeted profiling, the analyte can be directly injected into the mass spectrometer, which allows the investigation of inter alia gaseous metabolites in a rapid way (Biasioli et al., 2011), or it can be pre-separated by chromatographic methods like gas chromatography (GC) or liquid chromatography (LC). In both chromatographic methods, the compounds of the analyte (mobile phase) are separated due to different physical- and/ or chemical properties and their respective interactions with a column (stationary phase). Each compound is thereby recorded in a chromatogram as a peak by the time it needs to pass the distance from the injection to the detection (retention time, RT) and their number of impingement at the detector (intensity or relative abundance). In contrast to a direct injection, a pre-separation allows the differentiation of isobars and isomers (Kopka et al., 2004). The combination of chromatographic techniques (like in this thesis) with mass spectrometry improves the characterisation of the analyte by a further discrimination of co-eluting substances by their molecular size (Kopka et al., 2004). In general, eluting compounds are directly transferred to the mass spectrometer where they are firstly transformed into ions by an ion source (Viant and Sommer, 2013). Afterwards, the ions were accelerated to be transferred into the mass analyser that sorts them by their mass-to-charge ratios (m/z), which is proportional to the molecular size. Finally, ions are recorded by a detector for their abundance (ion count / intensity) (Kopka et al., 2004, Tautenhahn et al., 2008). The result is a mass spectrum (MS_1) for each compound with intensity against the m/z. The nearly coincide recording of chromatographic and mass spectrometric data allows the annotation of a compound with its RT and m/z information (feature) and the designation to its intensity in the sample. However, the MS_1 allows not the distinct identification. Therefore, further methods are needed. The most relevant (for this thesis) are presented in following **sections 2.1.1.** and **2.1.2.** .

The investigation of complex biological samples e.g. exudates, requires more than one technology to allow a comprehensive, selective, and sensitive investigation of as many as

possible signals (Kopka et al., 2004, Weckwerth, 2003). Thus, GC as well as LC techniques coupled to mass spectrometry with different ion sources and mass analysers were used in this thesis and will be presented in the subsequent **sections 2.1.1.** and **2.1.2.,** too.

1.6.1. Gas chromatography coupled to mass spectrometry

Gas chromatography coupled to mass spectrometry is one of the most commonly used techniques in metabolomics (Dunn and Ellis, 2005, Viant and Sommer, 2013). The separation of the metabolites takes place in a gaseous phase. The technique takes advantage of the differences in the vapour pressure and polarity of molecules and their interaction with the stationary phase of the column. This allows the investigation of metabolites that are stable in the gaseous phase, e.g. volatiles or lipids. By chemical modification via derivatization (see Chapter 2.2. and 3.4.) also other, mostly polar metabolites, such as alcohols, amino acids, carbohydrates and organic acids, can be investigated with this approach (Badri et al., 2013, Chaparro et al., 2013). The preferred ionisation technique in GC-MS is electron impact (EI) ionisation in which the analyte is strongly fragmented by colliding with electrons at an acceleration voltage of 70 eV (Viant and Sommer, 2013). In this thesis, the emerging fragment ions were sorted by a linear quadrupole (Q) mass analyser. Its four rods are arranged in a circle and create an electric field that filters ions with the frequency of the induced electric field. Thus, the trajectory of ions is used for the assignment of the ions to an m/z value, resulting in a characteristic ion spectrum for each metabolite (Tautenhahn et al., 2008). This information can be compared with information of databases such as those of the National Institute of Standards and Technology (NIST,(National Insitute of Standards and Technologies), MassBank of North America (MoNA) (Fiehn Lab) or the Golm Metabolome Database (GMD) (Kopka et al., 2004). The assignment of retention index values (R_i) by alkanes spiked to the sample can improve the identification of metabolites and makes the designation much easier than in case of the LC-MS investigation. Thus, GC-MS is frequently used for the analyses in ecotoxicology, trees and plant-animal interaction (Kopka et al., 2004, Viant and Sommer, 2013), but also for investigations of exuded metabolites (Neumann et al., 2014).

1.6.2. Liquid chromatography coupled to mass spectrometry

Another technique used to analyse metabolites is the liquid chromatography coupled to mass spectrometry (LC-MS). In this method, analytes can be investigated without prior chemical modification. This provides a more comprehensive profile than the profile provided by GC-MS (Arens et al., 2015, Viant and Sommer, 2013).
The separation of metabolites in this thesis is achieved by a C18 (reversed phase, RP) stationary phase, which retents hydrophobic substances. The metabolites are eluted with increasing hydrophobicity in solvent gradients starting with water towards increasing proportions of acetonitrile. By using an ultra-high performance liquid chromatography instrument (UPLC), a rapid separation of the analyte can be obtained, which is sufficient for further investigation using an MS metabolomics approach (Viant and Sommer, 2013). The UPLC technique provides a higher resolution than other chromatographic methods with sharper peaks and better compound separation (Viant and Sommer, 2013).
The connected mass spectrometer of this thesis was an electron spray (ESI) quadrupole (Q) time of flight (ToF) mass spectrometer, which is a well-established instrumental setup in metabolomics (Döll et al., 2018). In ESI, metabolites are ionized in a capillary held at a high voltage, whereby positive ([M+H]+) and negative ions ([M-H]-) occur, followed by the evaporation of the liquid around the ions. This smooth ionisation prevents molecules from fragmentation in most cases. In dependency on the MS acquisition mode, positive or negative ions are transferred to the MS. The ToF mass analyser takes advantage of the different velocities of ions in the field-free flight tube that is proportional to their molecular size (Tautenhahn et al., 2008). Out of this, the m/z is calculated and combined with the recorded intensity by the detector. This instrumental setup allows the investigation of metabolites with high scan rates, higher mass accuracies (up to 5 ppm in this thesis) and higher resolution than by the GC-MS techniques of this thesis. It also allows a deeper investigation of single metabolites than in GC-MS due to the integrity of the ions and therefore a more comprehensive identification (Döll et al., 2018, Kopka et al., 2004). The molecules of interest were thereby selected for fragmentation within the mass spectrometer by e.g. collision-induced dissociation (CID). CID is performed in the collision cell by the collision of the feature of interest with differentially accelerated and neutral charged gaseous molecules (Döll et al., 2018). This leads to fragmentation of the ion with a subsequent fragment ion spectra (MS/MS) (Döll et al., 2018, Kopka et al., 2004). CID can be either performed by a first scan for the existing ions and a following selective fragmentation of ions of choice within milliseconds (data dependent acquisition) or of a previous selected list of targeted features (data independent acquisition) (Arens et al., 2015, Döll et al., 2018). The MS/MS can then be used for structure elucidation (Ruttkies

et al., 2016, Strehmel et al., 2014), chemical classification (Treutler et al., 2016, Vaniya and Fiehn, 2015) or the comparison with data bases such as MoNA or analytical standards (Petriacq et al., 2017, Strehmel et al., 2014). In comparison with those of GC-MS, the metabolite databases for LC-MS measured metabolites are quite small (Fiehn, 2017).

1.7. The Experimental System

The majority of the thesis´ experiments were performed in the DFG project "German Biodiversity Exploratories" under the pseudonym "BELOW" and in cooperation with different scientists e.g. Katharina Herz (Geobotany research group of the Martin-Luther-University Halle-Wittenberg). The "German Biodiversity Exploratories" project is a large-scale experiment with the focus on effects of a varying gradient of land use on biodiversity and ecosystem functioning in forest and grassland ecosystems (Fischer et al., 2010). Different levels of ecosystem functions are analysed by focussing on either local (Plots) or regional scale (Sites). The "Biodiversity Exploratories" project comprises three sites (Schorfheide-Chorin, Hainich-Dün, Swaibian Alb, Figure 1) representing most of the variation in land use typical for grasslands in Germany (Fischer et al., 2010). This is accompanied by the dfferences in climate and soil characteristics (Blüthgen et al., 2012, Fischer et al., 2010).The UNESCO Biosphere Reserve Schorfheide-Chorin (SCH) is situated in the lowlands of north-eastern Germany. This site represents a young glacial landscape with many wetlands (Fischer et al., 2010, Herold, 2013). This characteristic determines the types of soil of this grassland site. Glacio-fluvial sand and glacial till soil, as well as drained histosols and gleysols are common in this environment (Fischer et al., 2010, Herold, 2013). The second site, the National Park Hainich (HAI) and its surrounding areas, is situated in the hilly lands of central Germany. Due to its geological substrate,

Figure 1: The German Biodiversity Exploratories. The map presents the locations of the three sites in Germany.[1]

[1] Figure cited from website of the university of Ulm (University Ulm, 2017).

loess over triassic limestone, the grassland soils frequently have a loamy or clayey texture (Fischer et al., 2010, Herold, 2013). The third site is situated in the low mountain ranges of southwestern Germany and is called UNESCO Biosphere Reserve Swaibian Alb (ALB). This site is characterized by swaibian jura and is therefore rich in clay and preferentially used for sheep pasture (Fischer et al., 2010, Herold, 2013). Since land use is a crucial factor in this large-scale experiment, Blüthgen et al. (2012) invented an index ("land use index", LUI) taking all components of German grassland management into consideration. With this, LUI includes the various aspects and gradations of mowing, grazing and fertilization (Blüthgen et al., 2012).

20 perennial grassland species were cultivated under greenhouse conditions from seeds of the Biodiversity Exploratories or commercial suppliers. The phytometers were planted (by Katherina Herz) into 18 experimental grasslands in each site (in sum 54 plots) of the Biodiversity Exploratories. They were chosen based on their different land use types: six times meadow, six times pasture and six times mown-pasture. In each grassland, an equal-sized area (Plot) was chosen for planting the phytometer in the existing local plant neighbouring community ("Local Plant Neighbourhood", LNH). One replicate of each species was planted in random order and at a distinct distance of 25cm from each other (Figure 2). This setup was repeated two times per plot in separated subplots (blocks) for two sampling campaigns, 2014 and 2015. The full experimental setup is presented in Herz et al. (Herz et al., 2017b). The sampling methods can be found in the **Chapter 2.1 to 2.3**. The experimental investigations of this thesis were done on ten of the twenty species (see Table 1).

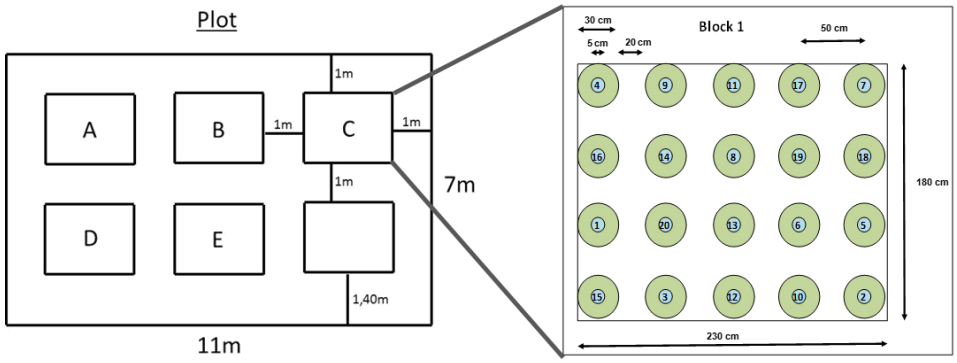

Figure 2: Scheme of the implemented setup in each plot of the grasslands of the German Biodiversity Exploratories (Herz et al., 2017b). Figure is not true to actual scale.

1.8. Plant species

Table 1: Ten target species with their affiliation to one of the two growth forms and their systematic family.

Growth Form	Species	Family
Forb	*Achillea millefolium* L.	*Asteraceae*
Forb	*Galium mollugo* L.	*Rubiaceae*
Forb	*Galium verum* L.	*Rubiaceae*
Forb	*Plantago lanceolata* L.	*Plantaginaceae*
Forb	*Ranunculus acris* L.	*Ranunculaceae*
Grass	*Alopecurus pratensis* L.	*Poaceae*
Grass	*Arrhenatherum elatius* (L.) P.Beauv. ex J.Presl & C.Presl.	*Poaceae*
Grass	*Dactylis glomerata*L.	*Poaceae*
Grass	*Lolium perenne* L.	*Poaceae*
Grass	*Poa pratensis* L.	*Poaceae*

The ten plant species (Table 1) can be subdivided into grasses and forbs. These growth forms differ in various plant functional traits (see also section 1.3.). The combined analysis of aboveground and belowground traits is to some extend pivotal for the grouping (Herz, 2017). Grasses exhibit a higher interspecific variation in aboveground plant traits e.g. leaf dry matter content (LDMC) and low leaf area ratio (LAR) and lower interspecific variation in belowground traits than forbs (Siebenkas et al., 2015). Belowground, however, they show a higher variation in root carbon concentration (RCC) and root carbon to nitrogen ratio (RCNR).This permits faster adaption to environmental influences and a higher phenotypical plasticity than forbs (Herz et al., 2017b, Siebenkas et al., 2015). Forbs, instead variate more in plant functional traits, such as specific leaf area (SLA), root to shoot ratio (RSR), root volume (Rvol), root nutrient concentration of nitrogen (RNC), phosphate (RPC), potassium (RKC), magnesium (RMgC) and calcium (RCaC) content of the root (Herz et al., 2017b), as well as the performance traits leaf dry matter content (LDMC) and root dry matter content (RDMC) (Herz et al., 2017a). This reflects the function of this root system as storage roots (Herz, 2017). Furthermore, the ten chosen species differ phylogenetically (Table 1, Figure 3), as well as in their environmental preferences. All chosen grasses belong to the *Poaceae*, the sweet grasses, and prefer nutrient enriched moderately wet meadow.(Klapp and Opitz von Boberfeld, 2006) Moreover, species such

as *Dactylis glomerata* and *Lolium perenne* nutrient rich soil in moisture meadows, *Galium mollugo* and *Galium verum* prefer calcareous soil in moderately moisture meadow with low nutrient content.

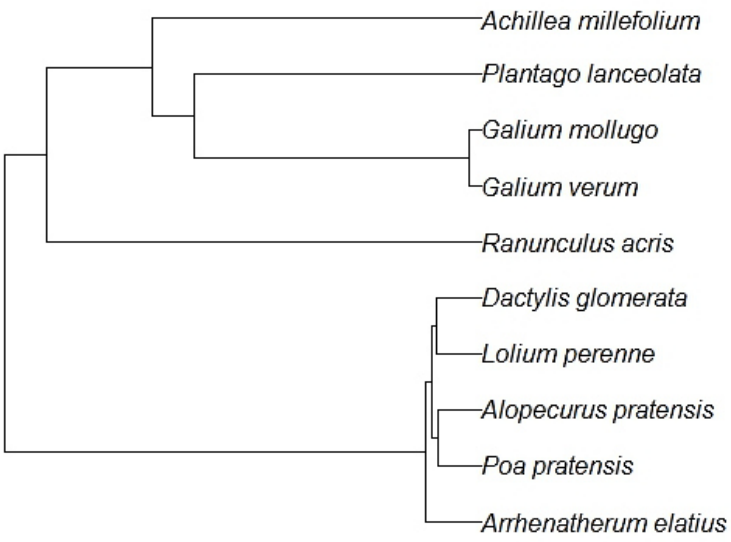

Figure 3: Phylogenetic tree[1] of ten target species according to their genetic distance to each other.

Many investigations have been conducted with regard to the genetic variation of these plants and their distribution (Cole, 2003, Guo et al., 2005, Mason et al., 2011), as well as the impact of environmental factors (such as management and nutrients) on grassland ecosystems (Dostalek and Frantik, 2012, Gubsch et al., 2011, Le Roux et al., 2003, Roscher et al., 2007, Trinder et al., 2012). Most of these studies, however, focus on ecological issues with little attention being given to metabolomics. On the other hand, metabolomics-orientated investigations of some of these species largely ignore ecology aspects and/or the exudation in the rhizosphere network (Agnihotri et al., 2005, Sabais et al., 2012, Sutter and Muller, 2011, Viljoen et al., 2012, Zhang et al., 2012).

1.9. Objectives

Various experiments have broadened our knowledge of ecological processes linked to grasslands and land use influences (Allan et al., 2014, Cardinale et al., 2012, Herz, 2017, Herz et al., 2017a, Kruska et al., 2003, Lavorel et al., 2011, Roscher et al., 2004, Tilman et al., 2006). There have also been a number of investigations designed to explain the function of exudates under different beneficial and impairing interactions (Aulakh et al., 2001, Carvalhais et al., 2015, Chen et al., 2017, Eisenhauer et al., 2017, Jandova et al., 2015, Jones et al., 2004, Neumann et al., 2014, Singh et al., 2004, Wang et al., 2016). However, little is known about the role of exudates in a complex rhizosphere environment of grasslands. Attempts to understand this part of an ecosystem have been conducted under controlled conditions with one or rarely two interaction partners (Aulakh et al., 2001, Neumann et al., 2014, Schmid et al., 2014, Strehmel et al., 2016, Ziegler et al., 2016), or in systems mimicking the environmental conditions (Eisenhauer et al., 2017, Liese et al., 2018) but not fully reflecting them. However, neither strategies can provide a realistic impression of the rhizosphere since they neglect various aspects that are critical in the interaction network.

This thesis present an investigation of plant exudation under field conditions in the complex ecosystem grasslands. With its observational character, in which the focus lies more on pattern than on identification of metabolites, it will try to provide answers to three important questions (see also Figure 4).

1. Is it possible to detect and analyse plant root exuded metabolites under field conditions?

A field based investigation of exuded metabolites requires a soil based cultivation and thereon adapted a exudate collection method (Oburger and Jones, 2018). Therefore, an exudate sampling method presented by Aulakh et al. (Aulakh et al., 2001) was adapted and expanded by using an untargeted metabolite profiling approach of exudates and root metabolites using LC-MS and GC-MS methods, followed by identification and classification techniques. The methodology was first tested on plants grown under controlled condition in a phyto chamber (**Chapter 2.1.**), and later on plants grown under field condition (**Chapter 2.2., 3.3., 3.4.**). The results should verify the suitability of this soil based exudate collection method, as well as their applicability under field conditions.

2. What has more impact on exudation of plant roots: the growth form or the species?

The comparison of previous investigations of scientists leads to the suggestion that the exudation of plants is inter alia impacted by the species identity (Badri et al., 2009b, Bais et al., 2006b, van Dam and Bouwmeester, 2016). This assumption was mostly obtained from comparisons of a limited number of metabolites, or explorative comparisons of different studies. However, a study specifically demonstrating this linkage has not yet been conducted. This thesis tests the evidence of this assertion on less investigated plants and their exudate profiles of polar (primary) (**Chapter 2.2., 3.4.**) and semi-polar (secondary) (**Chapter 2.3., 3.4.**) metabolites by using different statistical methods. Moreover, the results will give an impression of the root exudate compositions of these perennial grassland species.

3. Which further factors can explain the root exudate pattern in the field?

While a variety of endogenous factors impact root exudation (Aulakh et al., 2001), the surrounding environment of a plant also alters the profile root exudates (Carvalhais et al., 2015, Eisenhauer et al., 2017, Jandova et al., 2015, Jones, 1998, Jones et al., 2004, Neumann et al., 2014, Neumann and Römerheld, 2007). Experimental data of the plant functional and performance traits (**Chapter 2.2., 3.3.**) were correlated to the exudate composition of the target plants to reveals their impact on plant exudation in grasslands. Moreover, exogenous abiotic and biotic factors were correlated to the obtained exudate data (**Chapter 2.2.-2.4.**) to test their relation to plant exudation. In the case of abiotic factors, precipitation, soil nutrient content of carbon (C) nitrogen (N), soil types and soil texture according to the Worlds Reference Base (WRB) as well as aboveground temperature were taken into consideration.

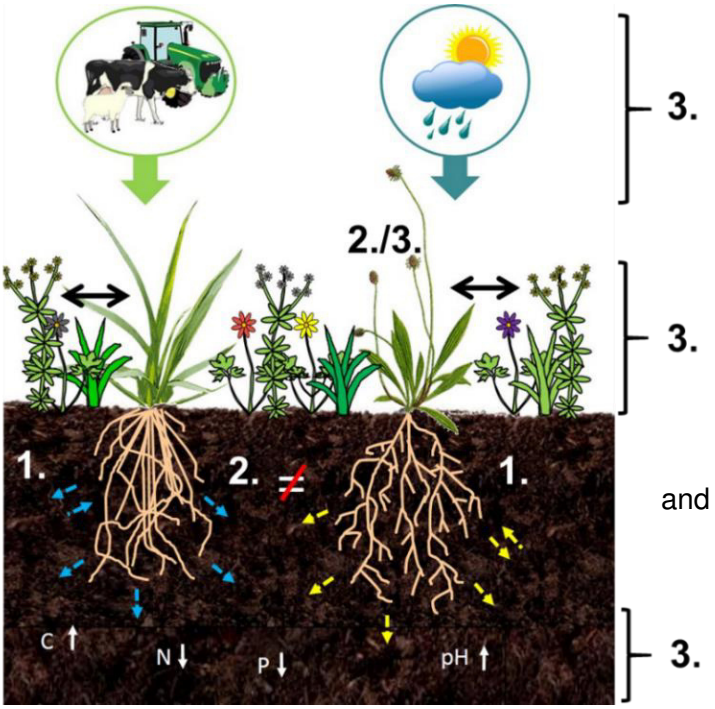

Figure 4: Scheme of the grassland interaction network. Numbers reperesent the questions investigated in this thesis.

Moreover, the land use intensity index (LUI) (with its three corresponding variables mowing, land use intensity index (LUI) (with its three corresponding variables mowing, grazing and fertilization), as well as the biotic factors of the transplants´ local neighbouring plant community (“Local Plant Neighbourhood”, LNH) with the covered area (Cover), the number of plants (Richness) of a certain species and the neighbouring species diversity (Shannon), were also taken into account.

II. Results

Chapter 2.1.: Extraction of root exudates in a soil based system: a field applicable method

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Author Contributions

SD and DS designed the phyto-cabinet experiment. The conduction of the plant cultivation, the exudate and root metabolite collection as well as the extraction and analyses where performed by SD with contribution of NH. Annotation where performed by SD. Statistical analysis was performed by SD with input from DS. The manuscript was written by SD with input from all co-authors.

The manuscript is written in publication style but is not submitted so far.

Abstract

Exudates are part of the plant metabolome and were released for the interaction with their surrounding habitat by for instance roots. The investigation of exudates under natural rhizosphere condition is challenging due to the lack of a methods to collect them under soil based conditions. The current study presents an exudate collection method on plant roots grown under soil conditions. The analysis of inner root and root exudates were measured by mass spectrometry. The results verify that the approach do not alter the exudate profile substantially by other metabolites than those exuded by the root.

Keywords: Exudates; soil condition; exudate collection method; liquid chromatography; mass spectrometry

Introduction

The terrestrial plant root is an important organ. Besides its function as anchor into the ground, it releases metabolites into the belowground habitat, the rhizosphere (Lynch, 1987). These low and high molecular weight compounds, called root exudates, fulfil diverse functions (Badri and Vivanco, 2009, Badri et al., 2009b, van Dam, 2009, van Dam and Bouwmeester, 2016). They alter the soil properties e.g. changing the water content, reduce the amount of phytotoxic metals and improve the plant´s nutrient acquisition (Badri and Vivanco, 2009). They also act as mediator for plant-microbial and plant-plant interactions (Bais et al., 2004, Bais et al., 2006b, Oburger et al., 2014, Oburger and Jones, 2018, Oburger et al., 2009, Oburger and Schmidt, 2016). Their elucidation, however, is challenging since exudation is a continuous process and the metabolite composition is affected by the mentioned factors. Many different approaches were taken into consideration trying to investigate exudates without altering the original profile. On the one hand, soil free nutrient solution (hydroponic) cultures were used to collect root exudates (Neumann et al., 2009, Neumann and Römerheld, 2007, Strehmel et al., 2014). These hydroponic methods benefit from the lack of soil adsorption and microbial degradation of metabolites in a natural soil environment (Oburger and Jones, 2018). However, they lack of plant interaction partners and cause artificial root development in comparison to natural growth conditions (Oburger and Jones, 2018). This makes the results questionable for their reflection of the natural rhizosphere situation.

On the other hand, soil based approaches were used to collect exudates. Plants were cultivated in soil under (semi) natural conditions and the exudates collected either by rinsing the root with water : methanol solution (Petriacq et al., 2017) or by suction of water from the close vicinity of the root (Eisenhauer et al., 2017, Oburger et al., 2013, Oburger

et al., 2014). The latter one was performed by either a vacuum system (Oburger et al., 2013, Oburger et al., 2014) or micro - suction cups (Eisenhauer et al., 2017). These approaches may reflect the exudation of plants better than hydroponic approaches since they minimize disturbances of the rhizosphere. They, however, also include probably alteration of the exudate profile due to microbial and soil interaction and components as well as exudate concentration differences due to soil water (Oburger and Jones, 2018). Moreover, the listed methods are either not suitable for field experiments due to their complicated setup and used solvents or not applicable for high resolution investigations of the belowground metabolomics due to their low analyte concentration or impurity with interfering substances. Besides the use of less selective metabolome analysis methods e.g. HPLC (Eisenhauer et al., 2017), many root exudate studies focused on single compounds or compound classes (Eisenhauer et al., 2017, Jaitz et al., 2011, Oburger et al., 2014, Schmid et al., 2014). This targeted metabolomics approaches, however, cannot reveal the different facets of the complex chemical cocktail released by a plant root.

This study presents a soil based untargeted metabolite collection and profiling approach based on mass spectrometry, which is suitable for root exudate investigations in field experiments.

To validate the methodology, six perennial grassland plant species were cultivated under controlled conditions and investigated for (i) the possibility to collect root exudates from them, and (ii) the integrity of their roots after exudate collection. By comparing the results to those from field experiments (Dietz et al., 2019) also (iii) the applicability under field conditions was tested.

Methods

Plants and cultivation condition

In total, the six following species were raised in three independent experiments: three forbs (*Achillea millefolium* L. [Asteraceae], *Galium mollugo* L., *Plantago lanceolata* L. [Plantaginaceae],) and three grasses (*Arrhenatherum elatius* [L.] P.Beauv. ex J.Presl & C.Presl., *Dactylis glomerata*L, *Poa pratensis* L. [all Poaceae]). Seeds were either received from the German Biodiversity Exploratories or bought from commercial suppliers. In each experiment, seeds of each species were sawed out on pots filled with steam sterilized sand : silt mixture (50:50) and cultivated for 28 days under long day conditions (light phase: 16 hours, 150 µMol/m^2s, 22 °C, 60 % humidity; dark phase: 8 hours, 0 µMol/m^2s, 18 °C, 60 % humidity) in a phyto chamber. Plants were sprinkled with tap water once a week to minimize the destruction of seedlings. Afterwards plants were separated into pots each filled with sand : silt mixture (50:50) and arranged in shells in random order. One plant of each species was placed in one shell. Plants were further cultivated under the same

condition such as seedlings for 56 days. During this period, plants were watered with nutrient medium (0.1 M KH_2PO_4, 0.1 M $MgSO_4$* 7 H_2O, 0.025 M K_2SO_4, 0.1 M $CaCl_2$ * 2 H_2O, 1 M NH_4NO_3, 0.1 M Na-Fe-EDTA, 0.05 M KCl, 0.03 M H_3BO, 0.005 M $MnSO_4$* H_2O, 0.001 M $ZnSO_4$ ** H_2O, 0.001 M $CuSO_4$* 5 H_2O, $NaMoO_4$* 2 H_2O in deionized water, pH = 5.84) from once a week after separation to every two days till sampling.

Sampling of plant roots and exudates

Plants together with their bulk soil were removed from the pots. Bulk and rhizosphere soil were carefully removed followed by a gentle wash step of the roots with tap water. A second wash step of the roots was performed with deionised water to reduce the content of ions from the tap water. Afterwards, the whole root systems of the intact plants were placed in 250 ml brown plastic vessels (Nalgene, Nalge Nunc International, Rochester, New York, USA) containing 200 ml of deionised water of HPLC quality for 2 hours exudation. Valentinuzzi et al. (Valentinuzzi et al., 2015) found water to be the most effective solvent for short-term collection of exudates. It is also the analyte with the lowest interference in mass spectrometric analysis. Water samples of 200 ml deionised water of HPLC quality in brown plastic vessels (Nalgene, Nalge Nunc International, Rochester, New York, USA) without exudation were used for process control (“water blanks”) and treated exactly like the exudate samples. All samples were frozen and stored at -20 °C until further processing. To collect root material, roots were photographed, separated from aboveground plant part, filled into 30 ml plastic vials (Zinsser, Eschborn, Germany) and immediately frozen in liquid nitrogen. The root samples were stored at -80 °C until further processing. The number of harvested plants for collection of exudate and roots is provided in the Supplementary Table 2.

Extraction of exuded metabolites

After thawing the exudate solution at room temperature, it was filtered (Sartorius, Göttingen, Germany; 185mm, 80g/cm^3). The water was successively evaporated under reduced pressure (30 mbar) at 40 °C to dryness using a 100 ml round-bottom flask and a vacuum rotary evaporator. The metabolites were dissolved two times in 3 ml 100 % methanol (Sigma-Aldrich, Taufkirchen, Germany), sonicated for 10 min at 20 °C and transferred into 5 ml glass tubes (Agilent Technologies, Santa Clara, USA). The extracts were evaporated to dryness at 40 °C using a vacuum centrifuge and reconstituted in 1.1 ml 80 % MeOH containing 20 µg/mL 2,4-Dichlorophenoxy acetic acid (2,4-D) and 100 µM Ribitol as internal standards. Aliquots of 100 µL were transferred into glass vials (Waters, Eschborn, Germany) and subjected to liquid chromatography coupled to mass spectrometry (LC-MS).

Extraction of root metabolites

The root samples were filled into grinding vials containing 2 steel balls of 7 mm size and ground in a Cryo Grinder robot (Labman Automatics, Stokesley, North Yorkshire, United Kingdom). Each root sample was ground for 30 s at – 80°C with a 30 s break to avoid warming up of the sample. This cycle was repeated 10 times in case of forbs and 15 times in case of grasses to obtain fine powders. Empty tubes with two steel balls were used as process control and treated like the grass root samples. An aliquot of 100 mg of each of the root samples was transferred into cold reaction tubes and mixed with 200 µL of extraction solution (3 µM kinetin, 3 µM phlorizin, 3 µM IAA valine, 3 µM biochanin, 100 µM Ribitol as internal standards in 80: 20 methanol : water (v/v)). The extracts were homogenized (Vortex mixer, Staufen, Germany), chilled on ice for 5 min and homogenized a second time followed by centrifugation for 5 min at RT and 15000 rpm. The supernatant was transferred into brown glass vials (Waters, Eschborn, Germany). Grinding and extraction controls were treated like the root samples. Aliquots of 20 µL of samples from each species as well as from the controls were transferred into glass vials (Waters, Eschborn, Germany) and subjected to LC-MS see below) to test for detection limit. According to these results, samples were diluted 1:10 with extraction solution and 20 µL of each sample were analysed by LC-MS.

LC-MS analysis and data processing

Exudates, root metabolites and controls were analysed by non-targeted metabolite profiling with ultra performance liquid chromatography coupled to electrospray ionisation quadrupole time of flight mass spectrometry (UPLC/ESI-Q-ToF-MS). Appliance performance was supervised by measurements of standard mix of eight substances (MM8: 10 µM α-phenylglycin, 10 µM kinetin, 10 µM rutin, 10 µM o-anisic acid, 10 µM phlorizin, 10 µM IAA-valine, 10 µM indolacetonitril, 10 µM biochanin) every ten samples.

Samples were applied to a UPLC platform (ACQUITY UPLC; Waters, Eschborn, Germany) equipped with a C18 column (ACQUITY UPLC HSS T3 Column, 100Å, 1.8 µm, 3 mm X 100 mm, 1/pkg; Waters, Eschborn, Germany) with 2 µL full loop injection at 40°C. The following gradient was used with a flow rate of 150 µL/min: 0–1 min, isocratic 95 % A (water/formic acid, 99.9/0.1 (v/v)), 5 % B (acetonitrile/formic acid, 99.9/0.1 (v/v)); 1–14 min, linear from 5 to 95 % B; 14–18 min, isocratic 95 % B; 18–20 min, isocratic 5 % B. The eluting ions were detected from m/z 90 to 1000 using a MicrOTOF–Q II hybrid quadrupole time-of-flight mass spectrometer equipped with an Apollo II electrospray ion source (Bruker Daltonics, Billerica, Massachusetts, USA) in negative ion mode. Following instrument settings were used: nebulizer gas, nitrogen, 1.6 bar; dry gas, nitrogen, 6 L/min, 190 °C; capillary, +4000 V; end plate offset, -500 V; funnel 1 RF, 200 Vpp; funnel 2 RF,

200 Vpp; in- source CID energy, 0 eV; hexapole RF, 100 Vpp; quadrupole ion energy, -5 eV; collision gas, nitrogen; collision energy, -7 eV; collision 150 Vpp; transfer time, 70 µs; pre pulse storage, 5 µs; spectra rate, 3 Hz.

Data pre-processing

Data files of the measurements of exudate samples were pre-processed separately from the data files of the root sample measurements due to their size. However, all samples were pre-processed in the same way described afterwards to allow their combined statistical analysis. The individual data files were subjected to MetaboScape 3.0 (Bruker Daltonics, Billerica, Massachusetts, USA) for feature extraction and grouping. The individual raw data files where recalibrated according there m/z scale on lithium formiate cluster ions obtained by automatic infusion of 20 µL 10 mM lithium hydroxide in isopropanol/water/formic acid, 49.9/ 49.9/ 0.2 (v/v/v) at a gradient time of 18.03 min to 18.3 min and by using a diverter valve. Peak picking was performed by T-ReX 3D algorithm with an intensity threshold of 700 counts, minimum peak length of 7 spectra and an exclusion of features occurring only one time in all samples. Feature grouping was performed within the retention time (RT) range of 0.01 to 18.00 min and mass range of 90 to 1000 mass to charge ratio (m/z). Adducts of M-H, M-H2O-H, M+NA-H2, M+K-H2 and M+HCHOOH-H were annotated as fragment spectra. No background subtraction was performed. According to these settings precursor masses were recalculated and previously found features of the same type in the selected range were deleted. The annotation with m/z and RT was done automatically by MetaboScape 3.0 (Bruker Daltonics, Billerica, Massachusetts, USA).

Data purification was performed for statistical analysis. Features occurring in 50 % of the controls (water, grinding, extraction) were regarded as artefacts and excluded from the feature list. The remaining features were referred as compounds in the further description and were subjected to statistical analysis.

Statistical Analysis

Statistical analyses were performed either with R (version 3.2.3, R Core Team, 2015) or excel 2010. All analyses were carried out on relative compound intensities, normalized to root weight in case of exudates and transformed by log2 to reach normal distribution in case of all measurements.

The experimental setup consists of different levels (Figure 1): experimental replication (experiment), different species (species) and biological replicates (plant).

At first, overall variation between experimental replication (experiment, Figure 1) was tested. Therefore, variance homogeneity was proven by levene test (levene, package car,

Fox et al., 2018). If variance homogeneity was given, ANOVA (aov, R Core Team, 2015) followed by post hoc sheffée test (sheffe.test, package agricolae, de Mendiburu, 2017) was performed to test for significant differences between experiments. If variance homogeneity was not given, welsh anova (aov.test.test, package onewaytests, Dag et al., 2018) followed by welsh test (welsh.test, package onewaytests, Dag et al., 2018) was performed.

Moreover, each of these levels could affect the observed compound profiles of roots and exudates individually leading to different degree of variation within the dataset. To test for the proportion of variation at these levels, a hierarchical variance partitioning analysis was performed. Therefore, linear mixed effect models (lme, package lmerTest, Bates et al., 2015) of exudate or root compounds, respectively, effected by plant nested in species nested in experiment, were calculated. The individual variances were extracted (varComp, package vegan, Oksanen et al., 2016) scaled and plotted as stack plot (barplot, package ggplot2, Wickham, 2009). The same procedure was repeated for calculation of the explained variance of species and plant in the different experiments of exudates and roots, respectively.

The overlapping number and identity of compounds detected in exudates and roots were calculated by using excel 2010. M/z values of root compounds and exuded compounds were rounded to the third decimal number by the “round up”. The theoretical recovery of root compounds in exudate measurements was calculated by the function “find duplicates”. The verification of the actual recovery of root compounds in the exudate dataset was analysed manually by comparison of measured m/z value and RT value of each duplicate pair in species and experiment dependent manner. The number of recovered root compounds was divided by the number of measured exudates per species and experiment to calculate the percentage of overlap per species and experiment. Furthermore, the relative intensities of overlapping compounds in root and exudate measurements was compared to examine if their appearance in the exudates has a critical influence on the reliability of the exudate collection method.

The same procedure was used to check for applicability of the harvest and exudate collection method in the field. For that purpose, species-specific compounds of the six chosen species obtained by the analysis of the field dataset of 2015 were analysed for recovery in the phyto chamber dataset. The mass spectra of compounds detected in both datasets were analysed for their fragment similarity.

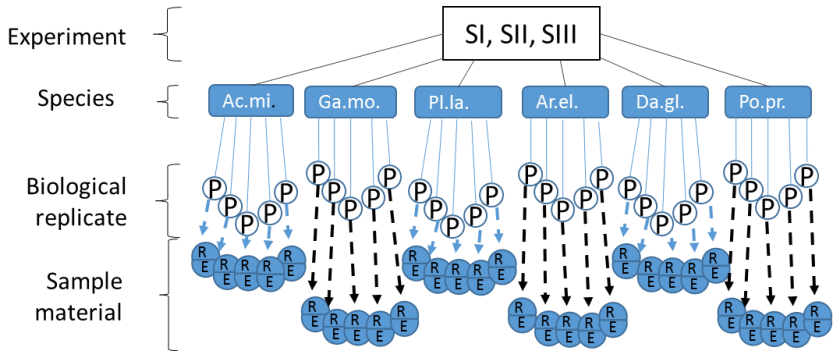

Figure 1: Experimental setup. SI – SIII = number of experimental replication, Ac.mi. = *Achilea millefolium*, Ga.mo. = *Galium mollugo*, Pl.la. = *Plantago lanceolata*, Ra.ac. = *Ranunculus acris*, Ar.el. = *Arrhenatherum elatius*, Da.gl. = *Dactylis glomerata*, Po.pr. = *Poa pratensis*, P = plant or biological replicate of the species, R = root, E = exudate

Results

Hierarchical variation in exudate and root samples

The number of features observed in the root samples, further referred to as root compounds, (1,865) was lower than the number of features observed in the exudate samples, henceforth called exuded compounds (4,536). The variation between the experimental replications (experiment), was also lower in root samples (3.78 x10^{-4} %) than in exudate samples (31.61 %) (Figure 2, Table 1). Therefore, root compounds showed a higher variation between the six different species (species) (92.58 %) than between those of the exuded compounds (25.49 %). In case of variation between the different plants of one species (biological replicates), the samples possessed a higher variation between the exuded compound patterns (7.09 %) than between the root compound patterns (36.02 %). These results are reflected by the analysis of variance (ANOVA and Welsh-ANOVA, Figure S1 A-F) applied to the datasets.

Especially the variation between the experiments led to the decision to perform a separate hierarchical analysis for each of the three experiments (Figure 3 A, B). Also here, the overall variation of the composition of root compounds stayed similar between the three experiments (Figure 3A, Table 2). Species explains most variance, which is driven by the significant difference between *Galium mollugo* and the other species as well as between *Achilea millefolium* and *Plantago lanceolata* in all three experiments (Figure 4 A, C, E). The grass species *Arrhenatherum elatius, Dactylis glomerata* and *Poa pratensis* showed no significant differences in the three experiments. The variation within the species is heterogenic depending on the experiment.

The results differ in case of exudates (Figure 3 B, Table 2). Depending on the experiment, either the compound patterns between species (Exp I) or the biological replicates (Exp II

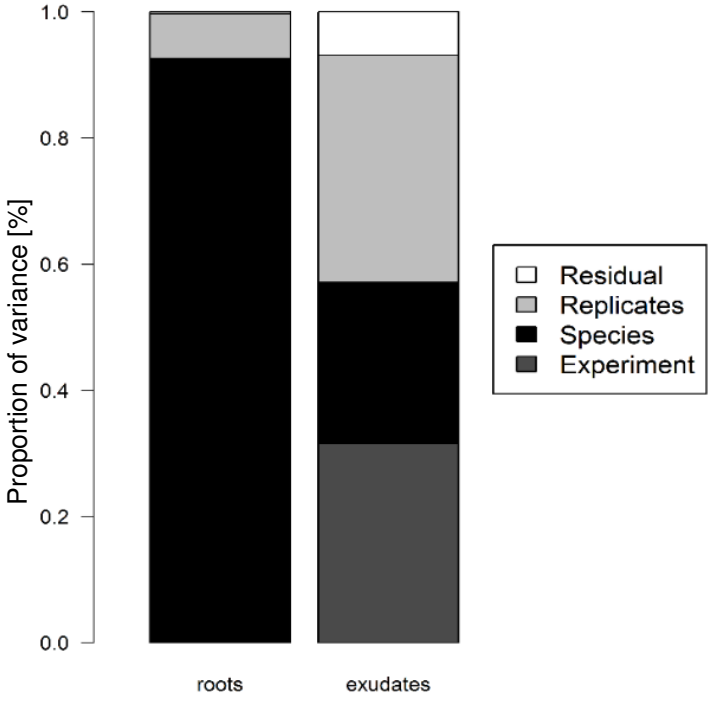

Figure 2: Hierarchical variance analysis of all exudates and roots samples. Stack plot shows the proportion of variation in root exuded compound composition explained by the experimental levels, experiment = variance between experimental replication, species = variance between different species, and replicates = variance between different biological replicates of each species. The variation explained by here not considered factors is represented by the residuals.

Table 1: Hierarchical variance analysis of all exudates and root samples. Values show percentage of total variance explained by each level, experimental replication (Experiment), different species (Species), biological replication of each species (Replicates), and remaining unexplained variance (Residuals).

Level/Data matrix	Roots [%]	Exudates [%]
Experiment	3.78×10^{-6}	31.61
Species	92.58	25.49
Replicates	7.09	36.02
Residuals	3.31×10^{-3}	6.88

and III) vary the most. In experiment I, this is mainly caused by *G. mollugo* being significantly different from all species with exception of *A. millefolium* and *P. pratensis* being significantly different from *G. mollugo* (Figure 4 B). This effect was not observed in experiment II and III (Figure 4 D, E).

Comparison of root and exudate composition

The overall number of compounds annotated for each sample set, root and exuded compounds, revealed a similar result (Table 4). A higher number of compounds was measured in exudates than in roots and also in experiment three compared to the other two experiments. Despite of this, the ranking of species with the highest number of compounds varied between the experiments. In experiment I, *G. mollugo* followed by *P. pratensis* possessed the highest number of compounds in exudates, whereas *A. elaitus* and *D. glomerata* had the lowest

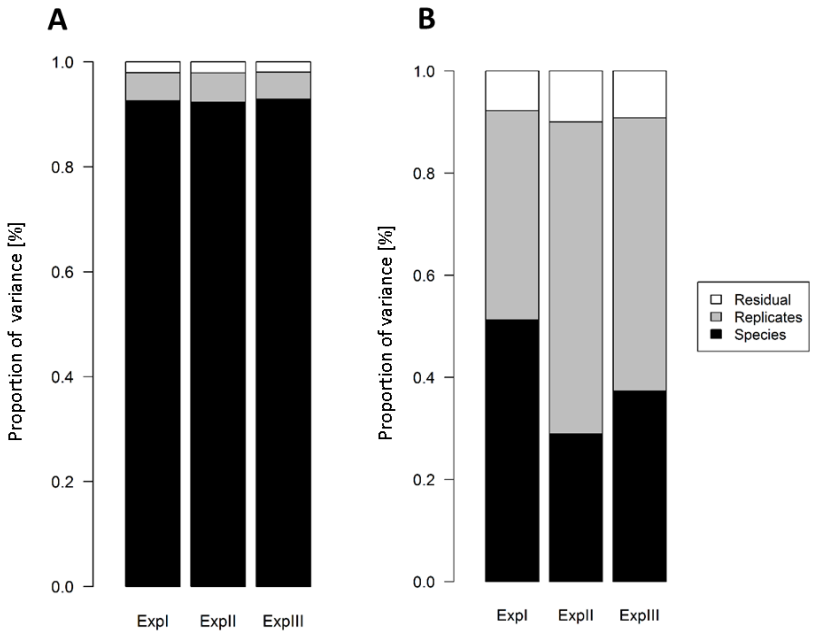

Figure 3: Hierarchical variance analysis of exudate and root samples of each experiment. Stack plot shows the proportion of variation in **A** root and **B** exuded compound composition explained by the experimental levels, species = variance between different species, and replicates = variance between different biological replicates of each species. The variation explained by here not considered factors is represented by the residuals.

Table 2: Hierarchical variance analysis of all exudate and root samples for each experiment. Values show percentage of total variance explained by each level, different species (Species), biological replication of each species (Replicates), and remaining unexplained variance (Residuals) for each of the three experiments.

Data matrix	Level	Experiment I	Experiment II	Experiment III
Roots	Species [%]	92.58	92.28	92.89
	Replicates [%]	5.38	5.59	5.13
	Unexplained [%]	2.04	2.12	1.97
Exudates	Species [%]	51.20	28.92	37.28
	Replicates [%]	41.06	61.12	53.53
	Unexplained [%]	7.78	9.96	9.18

number. In experiment II*, D. glomerata* and *P. pratensis* showed the highest number, whereas *A. millefolium* and *G. mollugo* had the highest number of exuded compounds in experiment III. The comparison of both sample sets, root compounds and exuded compounds, of the same species and experiment revealed 1 to 24 % of annotated compounds overlap, while in average 13 % overlap was found between the two data sets (Table 3, Figure 5 A-F). It depends on

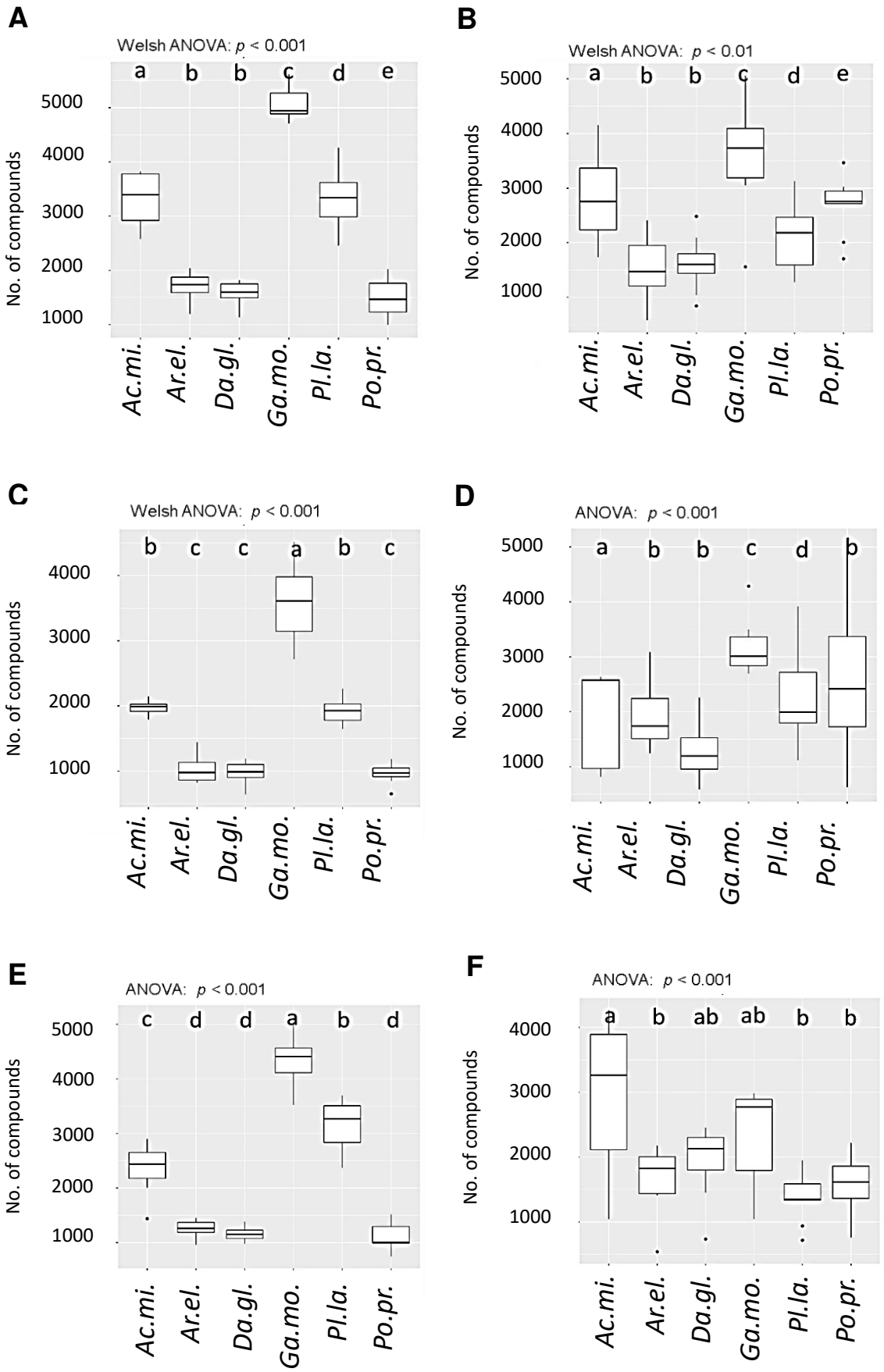

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Figure 4: Analysis of variance within the level species of each experiment. The Boxplots presents the number of measured compounds per species for the root compounds of **A** experiment I, **C** experiment II, and **E** experiment III as well as of the exuded compounds of **B** experiment I, **D** experiment II and **F** experiment III of the six species, 1 = *Achilea millefolium,* 2 = *Galium mollugo*, 3 = *Plantago lanceolata*, 4 = *Arrhenatherum elatius*, 5 = *Dactylis glomerata,* 6 = *Poa pratensis*. The significance of the influence of the level species on the composition of root and exuded compounds by analysis of variance (ANOVA or Welsh ANOVA) is indicated below each plot. Sheffée Posthoc test or welsh t-test, respectively, revealed significant differences between the species, which is indicated by letters. Boxes with the same letters are not significantly different from each other.

Table 3: Compounds in roots and exudates. Table presents number (No.) of compounds observed in root and exudate dataset as well as the No. of shared compounds of each of the six species (for abbreviation see Figure 1) and three experiments (Exp).

Species	Exp	No. of compounds		Shared compounds		
		R	E	No.	%	%
Ac.mi.	1	433	906	56	6	4.66
	2	341	643	45	7	
	3	414	1903	26	1	
Ga.mo.	1	627	1300	52	4	4
	2	516	1031	81	7	
	3	601	1752	107	1	
Pl.la.	1	554	837	83	9	16.33
	2	340	966	86	24	
	3	547	1034	81	16	
Ar.el.	1	276	1012	33	3	2.33
	2	177	1039	16	2	
	3	203	1350	26	2	
Da.gl.	1	285	858	30	12	13.33
	2	216	1446	29	14	
	3	244	1691	33	14	
Po.pr.	1	258	1024	38	16	16.66
	2	150	1084	24	16	
	3	196	1124	34	18	

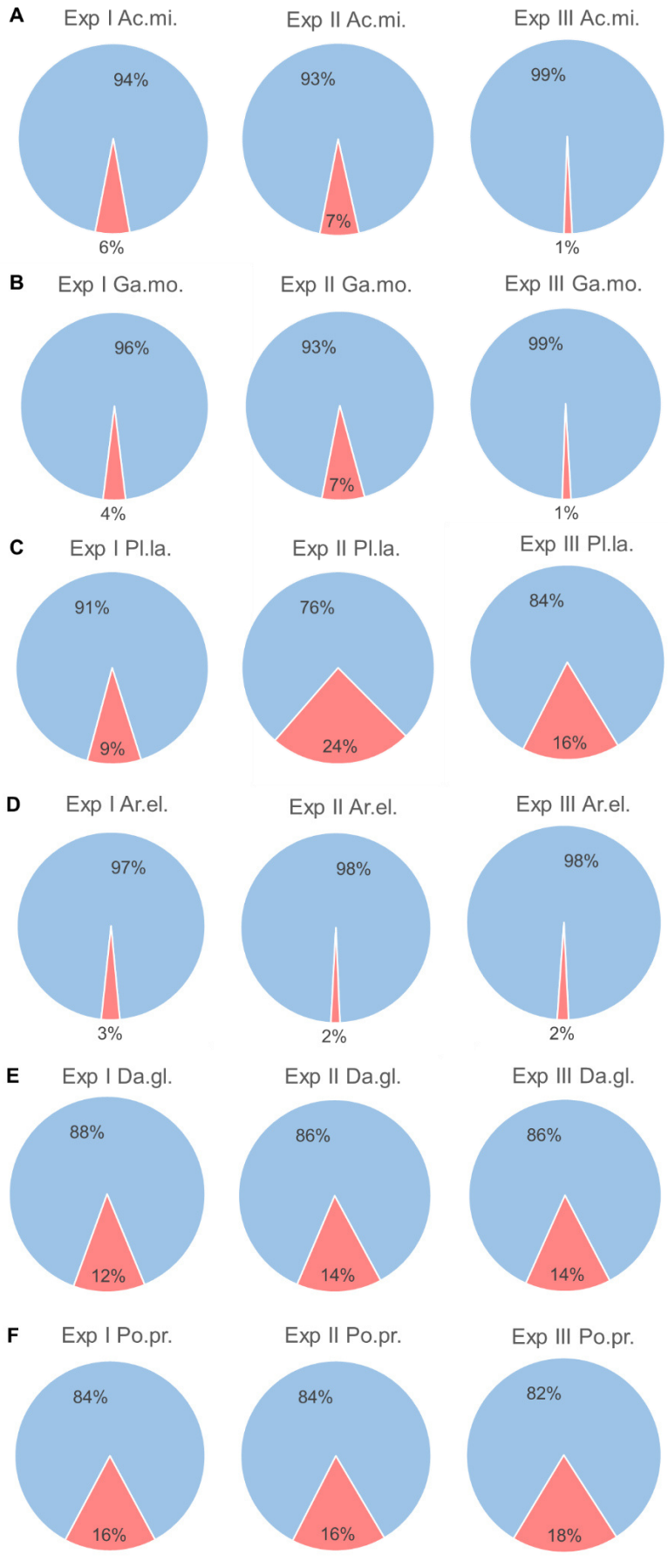

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Figure 5: Overlap of root and exudate datasets of the three experiment and six species. Pie charts present the comparison of root and exuded compound datasets for each of the three experiments (exp I,II,III) of the species, **A** *Achilea millefolium* (Ac.mi.), **B** *Galium mollugo* (Ga.mo.), **C** *Plantago lanceolata* (Pl.la.), **D** *Arrhenatherum elatius* (Ar.el.), **E** *Dactylis glomerata* (Da.gl.), **F** *Poa pratensis* (Po.pr.). The whole circle represents the total number of compounds detected in the exudate samples. The red section represents the percentage of root compounds being identical with the exudate compounds. The values are presented in table 4.

thereby what kind of species is taken into consideration. *A. millefolium* (Exp I: 6 %, Exp II: 7 %, Exp III: 1 %), *G. mollugo* (Exp I: 4 %, Exp II: 7 %, Exp III: 1 %) and *A. elatius* (Exp I: 3 %, Exp II: 2 %, Exp III: 2 %) showed low percentages of overlap. In case of *P. lanceolata* (Exp I: 9 %, Exp II: 24 %, Exp III: 16 %), *D. glomerata* (Exp I: 12 %, Exp II: 14 %, Exp III: 14 %) and *P. pratensis* (Exp I: 16 %, Exp II: 16 %, Exp III: 18 %), the number of shared compounds of root and exudate samples was higher than in the other species. A closer look, however, on the relative intensities of all shared compounds revealed that only five compounds (Table 5) occurred with a higher relative intensity in exudates than in roots. In some cases they also occur only in roots or in exudates and also in not all samples of each species (Table 4, Figure S2).

Comparison with field results

To test the applicability of the untargeted metabolite collection and analysis method in the field, the species-specific compounds observed in the six species (Dietz et al., 2019 (accepted)) were compared with exudates found in the phyto chamber experiment. 61 out of 237 possible compounds (Table S2) occurred in both data sets. This accounts for a conformity of 25.74 %. The highest number of shared compounds was found in the species *G. mollugo* (27) and *P. lanceolata* (22), whereas *A. elatius* (6), *A. millefolium* (4), *and P. pratensis* (1) shared less compounds with their field grown relatives. A comparison of mass spectra of the matching compounds between phyto chamber and field grown plants resulted in 14 % (33 compounds) spectral similarity. These compounds were considered as being identical. Further 18 compound pairs have certain fragments in common but also a lot of fragments being unique for one of the two compared plant groups.

Table 4: Critical root compounds. Table present the compounds occurring with a higher intensity in exudates than in roots. The corresponding samples are shown in column “biological samples”.

Identifier root compound	Identifier exudate compound	Biological samples
327.21717_6.43	327.21747_6.45	SI_Po.pr09, SII_Po.pr07, SII_Po.pr10, SIII_Ar.el07, SIII_Ar.el08, SIII_Ar.el09, SIII_Da.gl04, SIII_Da.gl09, SIII_Pl.la09, SIII_Po.pr01, SIII_Po.pr05, SIII_Po.pr06, SIII_Po.pr07, SIII_Po.pr09, SI_Po.pr03, SI_Po.pr04, SI_Po.pr05, SI_Po.pr10, SII_Po.pr02, SII_Po.pr03, SII_Po.pr05, SII_Po.pr06, SII_Po.pr08, SII_Po.pr09, SIII_Ac.mi01, SIII_Ac.mi03, SIII_Ac.mi05, SIII_Ac.mi07, SIII_Ac.mi08, SIII_Ac.mi10, SIII_Ar.el01, SIII_Ar.el02, SIII_Ar.el04, SIII_Ar.el06, SIII_Ar.el10, SIII_Da.gl01, SIII_Da.gl02, SIII_Da.gl05, SIII_Da.gl08, SIII_Da.gl10, SIII_Pl.la03, SIII_Pl.la04, SIII_Pl.la06, SIII_Pl.la10, SIII_Po.pr02, SIII_Po.pr03,
309.20564_8.23	309.20585_8.25	SIII_Ga.mo02, SIII_Ga.mo05, SIII_Ga.mo07,
311.2215_8.79	311.22265_8.76	SI_Ga.mo02, SI_Ga.mo06, SI_Ga.mo09,
551.17758_3.88	551.17835_3.88	SIII_Ac.mi01, SIII_Ac.mi02, SIII_Ac.mi03, SIII_Ac.mi05, SIII_Ac.mi06, SIII_Ac.mi07, SIII_Ac.mi08, SIII_Ac.mi10,
328.22301_6.43	328.21909_6.44	SIII_Ga.mo01, SIII_Ga.mo02, SIII_Ga.mo03, SIII_Ga.mo05, SIII_Ga.mo06, SIII_Ga.mo07, SIII_Ga.mo08, SIII_Ga.mo09

Discussion

The investigation of root exudates is of great importance to understand rhizosphere interaction networks of plants and their surrounding organisms (Oburger and Jones, 2018, van Dam and Bouwmeester, 2016). However, the main challenge is to find methods for the unbiased investigation of these root derived compounds under natural growth conditions. Previously applied methods used either special apparatuses to collect exudates from plants remaining in the soil e.g. the rhizoboxes (Oburger et al., 2013) or mesocosms in tubes (Eisenhauer et al., 2017, Petriacq et al., 2017).

The here presented results show a further suitable method for the investigation of root released compounds under soil growth condition (i). In contrast to previously mentioned methods, roots were here removed from the soil with little impact on root integrity (Herz et al., 2018)and contamination of exudates with inner root compounds. This contradicts in

part the statements of Oburger and Jones (2018) and the general opinion of other scientists (Eisenhauer et al., 2017, Petriacq et al., 2017). They remarked the inevitability of damage of the roots and with this root cell leakage when growing them in soil, remove them out of it and letting them exude in deionized water. The here presented method caused slight wounding of root hairs, as shown in Herz et al. (2018). However, this wounding did not lead to a considerable leakage since the results of the present study showed only an overlap of exuded and root contained compounds of 13 % in average. Only five compounds had a higher relative intensity in the exudate than in root compound dataset. The percentage of inner root compounds observed in exudate profiles of *P. lanceolata* and *P. pratensis* are higher than the average value. A possible explanation could be the differences in the root architecture of different plant species (Bardgett and van der Putten, 2014) and with this there species-specific susceptibility to root injury. Tests proofing this hypothesis would be of great interest.

Nevertheless, the results point to an insignificant alteration of the exudate profiles by this collection method and demonstrate the reliability of the method (ii). Also the applied trapping solution is of no disadvantage, as emphasized by Petriacq et al. (2017) and Aulakh et al. (2001). They observed remarkable cell damage and with this leakage of inner root metabolites when roots exuded in deionized water. According to Valentinuzzi et al. (2015), exudation in deionized water for two hours did not result in an osmotic shock, which matches the present results. It could be that the three wash step of the presented approach adjusted the root stepwise to the collection solution and prevented thereby bursting of cells. This is in accordance with the opinion of Oburger and Jones (2018).

The main disadvantage of most of the already implemented exudate collection methods is the impossibility to apply them to a field approach. They are either too complicated in their setup (Oburger et al., 2013) or disturb the integrity of the rhizosphere network (Eisenhauer et al., 2017, Petriacq et al., 2017). The here presented method is simple enough to apply it to field experiments without altering the ecosystem before the harvest, as successfully shown for the investigation of different kind of exuded compounds (Dietz et al., 2019 (accepted), Dietz et al., 2019, Herz et al., 2018). The comparability of exudate patterns obtained under phyto-cabinet and field conditions was proven by a recovery rate of 14 % and the same statistical results for semi-polar compounds: forbs differ from grasses and the species within a growth form also from each other (Dietz et al., 2019 (accepted), Dietz et al., 2019). The valued recovery rate might be rated as low. Under field conditions, however, a lot of different factors influence the exudation process (Bais et al. 2004, Bais et al. 2006, Badri and Vivanco 2009, Badri et al. 2009, Oburger et al. 2009, Oburger et al. 2014, Oburger and Schmidt 2016, van Dam 2009, van Dam and Bouwmeester 2016). This

could lead to a different exudation profile compared to plants grown under consistent conditions in a phyto chamber, especially in case of semi-polar metabolites. A comparison of polar metabolites, which are less variable in their overall composition of both growth conditions might help to underline the conclusions of this study.
Besides this, the phyto chamber experiments did also revealed a further interesting point: the variation within the species is impacted by the identity of the biological replicate. This was also shown by Mönchgesang et al. (2016b) for *Arabidopsis thaliana*. There, the resulting metabolite profile was highly influenced dependent by the experimental setup. Additionally, also the experimental replication itself has an influence on the outcome of the investigation. This is reflected by the differences in the number of annotated compounds, the different values of variance and the altered percentage of overlapping compounds, especially in forbs e.g. *P. lanceolata*. All of this emphasizes the special attention on the statistical analysis of the results to avoid misleading interpretations.

Overall, the collection of exudates is not easy and holds a lot of pitfalls. In agreement with Oburger and Jones (2018), a complete unaffected investigation of exudates is hardly possible. The method presented here, however, represents an opportunity for the investigation of exudates in a natural rhizosphere interaction scenario.

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Chapter 2.2.: Linking root exudates to functional plant traits

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Author contributions

KH, SD, UJ and SH conducted the field experiment and collected the phytometers. Exudate extraction and analyses where performed by SD whereas annotation and identification where performed by SD and KG. Trait analysis was performed by KH. SD performed the staining, microscopic analysis of plant roots and editing of the images. The manuscript was written by KH and SD with input from all co-authors. SH, UJ, DS and HB designed and prepared the field experiment. HB carried out the statistical analyses.

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Abstract

Primary and secondary metabolites exuded by plant roots have mainly been studied under laboratory conditions, while knowledge of root exudate patterns of plants growing in natural communities is very limited. Focusing on ten common European grassland plant species, we asked to which degree exuded metabolite compositions are specific to species or growth forms (forbs and grasses), depend on environments and local neighbourhoods, and reflect traditional plant functional traits. Root exudates were collected under field conditions and analysed using a non-targeted gas chromatography coupled mass spectrometry (GC-MS) approach. In total, we annotated 153 compounds of which 36 were identified by structure and name as metabolites mainly derived from the primary metabolism. Here we show by using variance partitioning, that the composition of exuded polar metabolites was mostly explained by plot identity, followed by plant species identity while plant species composition of the local neighbourhood played no role. Total and root dry biomass explained the largest proportion of variance in exudate composition, with additional variance explained by traditional plant traits. Although the exudate composition was quite similar between the two growth forms, we found some metabolites that occurred only in one of the two growth forms. Our study demonstrated the feasibility of measuring polar exudates under non-sterile field conditions by mass spectrometry, which opens new avenues of research for functional plant ecology.

Keywords: Biodiversity Exploratories; gas chromatography; mass spectrometry; grassland; growth form; plant functional traits; root exudates; root traits

Introduction

Plant roots constantly exude compounds of the primary and secondary metabolism into the rhizosphere. Such exudates serve for nutrient acquisition and interaction of the plant with the root surrounding environment (De-la-Pena et al., 2010, De-la-Pena et al., 2008). Their release is controlled and adjusted to the needs of a plant as well as to abiotic and biotic factors (Aulakh et al., 2001, Chaparro et al., 2013, van Dam and Bouwmeester, 2016). Until now, analyses of the composition of such metabolites were mostly done on model plants, such as Arabidopsis thaliana, for which up to 130 primary (Badri et al., 2013, Chaparro et al., 2013) and 103 secondary metabolites (Strehmel et al., 2014) have been described. These are sugars, sugar alcohols, phenolic compounds, organic acids, fatty acids as well as aliphatic and aromatic amino acids (Badri et al., 2013, Chaparro et al., 2013). The functions described for those metabolites comprise (i) mobilization of soil nutrients with low availability, such as phosphorus; (ii) stimulation of external detoxification

of metals; and (iii) mediation of positive and mutualistic interactions with beneficial, plant-growth promoting microorganisms, such as endophytic and rhizobial bacteria or mycorrhizal fungi (Badri and Vivanco, 2009, Bouwmeester et al., 2007, Faure et al., 2008, Jones et al., 2004).

In addition, the metabolic composition of exudates can be expected to be also affected by different neighbour plant species (Badri and Vivanco, 2009) via direct or indirect interaction with other plant roots (van Dam and Bouwmeester, 2016). Another potential factor influencing root exudation patterns might be the functional traits of the exuding plant individual. Plant functional traits have been found to be closely linked to individual plant performance (Freschet et al., 2015, Gubsch et al., 2011, Wilson et al., 1999). For example, a high specific leaf area is linked to high relative growth rate (Pérez-Harguindeguy et al., 2013), and thus might result in larger amounts of exuded carbon, as carbon exudation has been found to positively correlate with plant biomass (Aulakh et al., 2001). Under nutrient deficiency more biomass is allocated to roots (Bloom et al., 1985, Lambers et al., 2008) which might be reflected by more exuded substances for nutrient mobilization (Badri and Vivanco, 2009, Jones, 1998). Establishing links between exudate composition and plant traits would be very useful in ecological research since the impact of exudates in natural grasslands was underestimated so far. Combining morphological or anatomical traits with exudate pattern would allow for mechanistic explanations in ecosystem functioning which have been described so far for few selected substances only, but not for the overall exudate composition (Aulakh et al., 2001). Furthermore, such analysis of causal relationships in the belowground ecosystem compartment is still heavily understudied (van Dam and Bouwmeester, 2016) due to another challenge in this kind of investigation: the extraction of root exudates from field-grown plants under non-sterile conditions (van Dam and Bouwmeester, 2016). Here, we present a successful procedure to obtain root exudates from plants planted into natural grassland communities. Grassland communities consist of two main growth forms: grass and forbs. As the ecological functions of grass and forb species are distinct and they differ in several functional traits (Herz et al., 2017b, Siebenkas et al., 2015), we hypothesized 1) that these two growth forms differ in exudation patterns. Furthermore, we tested the hypotheses that root exudate composition of a target plant is correlated 2) with the plant's functional traits and 3) depends on local neighbour plant species as well as conditions varying at the plot level.

Methods

Experimental setup

The experiment took place during spring and summer 2014 in the three regions of the German Biodiversity Exploratories (Fischer et al., 2010): Schorfheide-Chorin, Hainich-Dün and Schwäbische Alb. Eighteen out of the 50 experimental grassland plots in each of the three Exploratories were selected varying in land use intensity, resulting in a total of 54 experimental plots, see also Herz et al. (Herz et al., 2017b). Each plot comprised an area of 7 x 11 m and included five blocks. Each block contained one individual (=one phytometer) of each of the total 20 study species. Of these, the present study used ten species: *Alopecurus pratensis* L., *Arrhenatherum elatius* (L.) P.Beauv. ex J.Presl & C.Presl., *Dactylis glomerata*L., *Lolium perenne* L., *Poa pratensis* L. (all Poaceae) and Achillea millefolium L. (Asteraceae), *Galium mollugo* L., *Galium verum* L. (Rubiaceae), *Plantago lanceolata* L. (Plantaginaceae), *Ranunculus acris* L. (Ranunculaceae). These perennial species are among the most frequent and abundant species in all plots of the Exploratories' grasslands. Within blocks, the individuals of each species were planted using random planting positions. An overview of the ten species and their sample sizes is given in S1. For detailed descriptions of the raising conditions, planting process and experimental setup see Herz et al. (2017b).

As access to some plots was restricted at the time of harvest and in some plots mortality was too high, we could not harvest the full set of planted phytometers. The phytometer species (five grasses and five forbs) were harvested in 46 of the 54 established plots (in total 304 individual plants) as follows. We dug a hole of ca. 20 cm depth and 15 cm length and width to extract one individual, carefully removed the rhizosphere soil and roots of other plants and gently washed the roots with tap water. As the plants were only three months in the field by the time of harvest, the roots did not grow into other patches and could be easily extracted from the soil and surrounding plants. Therefore, it was possible to extract the phytometer plants completely without damaging their roots. We measured several above- and belowground traits (S2) as well as polar exuded metabolites (see below) on these phytometers.

Additionally, the composition of neighbour plant species at the location of each phytometer was obtained by recording the number of plant species and cover per species growing in a 15 cm radius (707 cm^2) around each phytometer plant. These vegetation records were used to calculate species richness and Shannon diversity of neighbour plant species, in addition to species composition as obtained from the first four axes of a detrended correspondence analysis (DCA).

Extraction of exudates

We successfully adapted a procedure developed by Aulakh et al. (2001) to collect exudates directly form the roots in field. To reduce the content of ions from the tap water, we performed a second wash step of the roots with deionised water. Afterwards, the complete roots of the intact plant were placed in 250 ml brown plastic vessels (Nalgene) containing 200 ml of deionised water of HPLC quality for 2 hours exudation. Water was found to be the most effective extracting solution for collecting exudates also by Vallenrinuzzi et al.(Valentinuzzi et al., 2015), who compared different trap solutions. Water samples of 200 ml deionised water of HPLC quality in brown plastic vessels (Nalgene) without exudation were used for process control ("water blanks") and treated exactly like the exudate samples. All samples were frozen and stored at -20 °C until further processing. After thawing the exudate solution, it was filtered (Sartorius; 185mm, 80g/cm3) and the water was successively evaporated under reduced pressure (30 mbar) at 40 °C to dryness using a 100 ml round-bottom flask and a vacuum rotary evaporator. The extraction of metabolites was obtained by dissolving them two times in 3 ml 100 % methanol (Sigma-Aldrich), sonicating them for 10 min at 20 °C and then transferring the solution into 5 ml glass tubes (Agilent Technologies). The residuum was evaporated to dryness at 40 °C using a vacuum centrifuge and reconstituted in 1.1 ml 80 % MeOH containing 20 µg/mL 2- (2,4-dichlorophenoxy) acetic acid and 10 µM Ribitol as internal standards. An aliquot of 200 µl of each sample was centrifuged to precipitate remaining particles followed by the transfer of the supernatant to a new tube and drying in a vacuum concentrator. The metabolites in these samples were derivatized by methoxylamination with 50 µl methoxylamin-hydrochloride (20 mg/ml in pyridine, Sigma Aldrich) for 90 min at 37 °C and subsequently silylated with 50 µl BSTFA (Macherey–Nagel) with added alkane retention time indices (C12, C15, C19, C22, C28 (each 0.1 mg/ml final concentration; Sigma Aldrich) and C32 (0.4 mg/ml final concentration); Sigma Aldrich)) for 30 min at 37 °C (Gorzolka et al., 2012).

GC-MS analysis and data processing

Derivatized exudates and water controls were analysed by non-targeted plant metabolite profiling with gas chromatography coupled to mass spectrometry.

The measurements were performed using a gas chromatograph (6890N GC; Agilent Technologies) equipped with a ZB-5 Zebron GuardianTM Capillary GC column (30 m + 10 m ZebronTM, iD 0.25 mm, df 0.25 µm; Phenomenex) and coupled to mass spectrometer (5975 MSD; Agilent Technologies) with settings and method adapted to Gorzolka et al. (Gorzolka et al., 2012). Samples (2 µl) were injected automatically by multipurpose sampler (MPS 2XL; Gerstel) at 230 °C injector temperature and separated

chromatographically with 1ml/min flow and the following oven program: 1 min 70 °C, ramp with 7 °C per minute up to 300 °C, 5 min 300 °C. The transfer line temperature was set at 300 °C and ion source at 230 °C. Mass spectra were recorded with 20 Hz with MS calibration obtained by daily automated tuning of the MS on PSTFA. Single samples were derivatized and measured separately with intervals of at least one day. A “chemical blank” (derivatization agents without biological sample) was interspersed every five to six samples to check for a potential carryover of metabolites during measurement. Constant chromatographic performance and sensitivity was checked by tune evaluation with PSTFA. The raw data were converted to cdf-files by the Data Analysis software (Agilent Technologies) and uploaded to the MeltDB software (Neuweger et al., 2008). In MeltDB, peak detection with SN = 5 and FWHM = 6 using the warped-algorithm and metabolite profiling with threshold = 0.75 for compound conformation was done (Neuweger et al., 2008). Identification of metabolites by mass spectra similarity was performed in MeltDB based on customized spectral and index libraries. Gaps in metabolite annotation were manually filled with the help of MeltDB, spectral and index libraries as well as Data Analysis (Agilent Technologies). Unidentified compounds were manually annotated by their mass to charge ratio (m/z) and retention time (RT). Metabolites and compounds occurring in 50 % of water controls as well as in 50 % of chemical blanks were regarded as artefacts and excluded from the metabolite list. Classification of metabolites was done according to their affiliation to natural substance classes.

Ex-situ experiment on potential root damage by the exudate sampling procedure

To reveal the potential damage caused by our harvest and exudation procedure in the field we assembled a phyto-cabinet experiment which fully mimicked the field harvest procedure. The forb species *Plantago lanceolata* and the grass species *Arrhenatherum elatius* were grown from seeds under long daytime growth conditions (light phase: 16 hours, 150 µMol/m2s, 22 °C, 60 % humidity; dark phase: 8 hours, 0 µMol/m2s, 18 °C, 60% humidity) in pots filled with a mixture of steam sterilized sand and silt (50:50) in phyto-cabinets for 56 days. After root cleaning and exudate collection analogous to the field experiment, dead and damaged cells were stained with lactophenol-trypanblue according to Koch et al. (Koch and Slusarenko, 1990). Afterwards one half of the vertically separated roots was immediately transferred into Trypan blue colour solution (0.1% Trypan blue dye (Sigma, Darmstadt, Germany) in one part of lactophenol (25 % (v/v) Glycerine, 25 % (v/v) Lactic acid, 25 % (v/v) Phenol (all Roth, Karlsruhe, Germany), 25 % (v/v) tape water) and 2 parts of Ethanol (Roth, Karlsruhe, Germany)). To stain dead and damaged cells, roots were incubated for 3 hours under shaking at room temperature. Background staining of the root was removed by three to four washing steps with Chloral hydrate (2.5 g/mL,

Sigma-Aldrich, Darmstadt, Germany). Whereas the first washing step was used to remove remaining colour solution, following washing steps where performed over night by shaking at room temperature. Afterwards, roots were washed two times with deionized distilled water and stored in glycerine-water solution (1:1 v/v) at 4°C until microscopy. Samples from each root were selected randomly, cut from the root system with a razor blade, transferred onto object slides (Menzel) with glycerine-water solution (1:1 v/v), covered with a coverslip (IDL) and subjected immediately to microscopic analysis.

Microscopic analysis and image editing of the ex-situ experiment

Three independent bright-field microscopic images were recorded of three different regions (root tip and two different upper regions) of the selected stained root with an AZ 100 Multi-Purpose Zoom Microscope and the NIS Elements Imaging Software (both Nikon Instruments Inc., Melville, NY, USA). Overview images were conducted with an AZ-Plan Apo 1x (NA: 0.1/WD: 35 mm) objective with 5 x magnification. Furthermore, detailed images of root hairs were made using an AZ-Plan Apo 4x (NA: 0.4/WD: 20 mm) objective with 8 x magnification. Images were processed equally in contrast and brightness with the Gimp software (v. 2.8.14). The same program was used to adapt scale bar in color, font and position to scale bar automatically generated by the NIS Elements Imaging Software.

Trait analyses

Root nutrient concentrations were obtained using at least 50 mg root powder for a digestion with nitric acid and analysing the samples with photometric phosphate assay (for P) and atomic absorption spectrometry (AAS vario 6; Analytik Jena, Germany) to obtain K, Ca and Mg. Samples with less than 50 mg root powder could not be used for the digestion and values of these samples were predicted by using near infrared spectroscopy (NIRS; OPUS version 7.0, Bruker Optics) similar to the methods used by Mir-Marqués et al. (Mir-Marques et al., 2016). As all samples were subjected to NIRS, the spectra of samples which were also digested were used for calibration, allowing the predictions for samples with insufficient sample amount for digestion.

Root N and C content as well as C to N ratio were obtained from root powder using a C/N-analyser (vario EL cube; Elementar, Hanau, Germany). In total, the following above- and below-ground plant traits were measured on a total of the same 304 phytometers planted into 46 grassland communities (plots): specific leaf area (SLA), leaf area ratio (LAR), leaf and root dry matter content (LDMC, RDMC), root to shoot ratio (RSR), root volume (RVol), root mass per volume (RMV) as well as the dry mass of roots (DM roots), leaves (DM leaves), all aboveground organs (DM above) and the whole plant (DM total), root nutrient contents (C, N, P, K, Ca and Mg) and root C to N ratio (RCNR) (S2). By using redundancy

analysis (RDA), variance partitioning, procrustes analysis and principal component analysis (PCA), we identified the factors that accounted for variation in plant exudate composition of polar metabolites. A more detailed description of the trait analysis is given in Herz et al. (Herz et al., 2017b).

Statistical analysis

Statistical analyses were performed with R (version 3.2.3, R Core Team, 2015)). We carried out all analyses on exudates based on peak intensities and on presence/absence of metabolites. As the amount of single exudates varied strongly among samples, we standardized peak areas by an internal standard (Ribitol m/z= 217), log- or square-root-transformed them and scaled them by column (exudate compound) and/or row (sample). This still resulted in highly heterogeneous data that were driven by single exudates in single samples, when we were using the metabolite peak area as input value. Thus, we confined all analyses to the presence/absence of polar metabolites. In total, 35 samples of all traits were identified as outliers and excluded from the analyses. At first we excluded values of root P, K, Ca and Mg smaller than zero (wrong NIRS predictions) and values of RCC smaller than 1 as the latter were caused by an error in peak area estimation of the C/N-analyser. Then LDMC, SLA, LAR, RDMC, RSR, RVol, RMV, RNC, RKC, RCaC, DM roots, DM leaves, DM above and DM total were transformed by natural logarithm, RPC and RMgC by square root. To test for growth form-specificity of exudation patterns we conducted a redundancy analysis where the polar metabolites were tested against a presence/absence matrix of growth form (grass or forb). Furthermore, we used variance partitioning (varpart, package vegan, Oksanen et al., 2016) to detect how much variance was explained by either target species identity, the plot identity, composition of neighbour plant species of each phytometer or the trait composition of the phytometer plant. To test for differences in variation between grasses and forbs we made the described variance partitioning analysis separately for the two growth forms. Finally, all exudates and traits (S2) were subjected to a principal component analysis (PCA). The two PCAs of exudates and traits were then compared with a procrustes analysis (function protest, package vegan, Oksanen et al., 2016) to assess the correlation between exudate and trait patterns.

Results and Discussion

We measured the root exudate composition of polar metabolites mainly derived from the primary metabolism and related it to 18 plant traits. To evaluate root damage caused by the exudate collection and the consequent potential contamination of exudates by root metabolites we performed trypan blue staining and microscopic analysis of representative species (grown in the phyto-cabinet) that underwent the same sampling procedure as in

the field (S3). The roots were intact and damage to root hairs was insignificant or very minor, which indicated that the measured metabolites were not released from damaged cells but were exuded.

Out of the 153 compounds encountered by mass spectrometry, 36 could be identified and were classified into alcohols (4), amino acids (10), lipids (4), nucleic bases (1), nucleotides (1), organic acids (11) and sugars (5; S4). To detect differences in exudate composition between grasses and forbs, a presence/absence matrix of these substances was subjected to a RDA with a presence/absence matrix of growth form as constraint.
Grasses and forbs showed a largely common exudate composition which states the considerable low variation explained by the first RDA-axis (1.67%, Fig. 1a, b). Nevertheless, grasses and forbs also showed differences in exudate patterns (Fig. 1b) caused by many metabolites released predominantly by one of the two growth forms (S4, S5), and thus confirming our first hypothesis. While for example adenosine and homoserine were released in more grass than forb samples, the opposite was true for e.g. scyllo-inositol and some compound compounds (S5, S6).

To investigate which factors drive the variation in exudate composition in both growth forms, we used variance partitioning. In separate analyses for grasses and forbs, we used species identity, plot identity (which comprised all differences in ecological conditions between plots) and either species composition of the local plant neighbourhood or traits of the target plant as predictors for exudate composition. Similar to the results of Herz et al. (Herz et al., 2017b) more variation was explained in forb species (28.8 % to 32.9 %, Fig. 2B, D) than in grass species (26.8 % to 28.9 %, Fig. 2A, C).

Furthermore, interspecific variation of forbs was higher than that of grasses (Fig. 2). This highlights the differences between these two growth forms and confirms the results of Herz et al. (2017b) for another important plant characteristic: exudate composition. In addition, the variance partitioning showed that the exudation behaviour of both, grasses and forbs, is mainly driven by the plot into which they were planted (20.9 % and 21.1 %, respectively). Species identity of the phytometer (2 % in grasses and 6.7 % in forbs) or species composition of the local neighbourhood in a 15 cm radius around the exuding plant (0.3 % in grasses and 0 % in forbs) contributed little to the total explained variation. The shared variation between plot and neighbouring plants of 4.7 % in grasses and 2.5 % in forbs points to a simultaneous account of effects of neighbour species and plot environment. This means that conditions varying at the plot level might be of greater importance for influencing root exudation of polar metabolites than the local plant neighbours or the species identity of the target plant. However, we cannot exclude that such species-specific

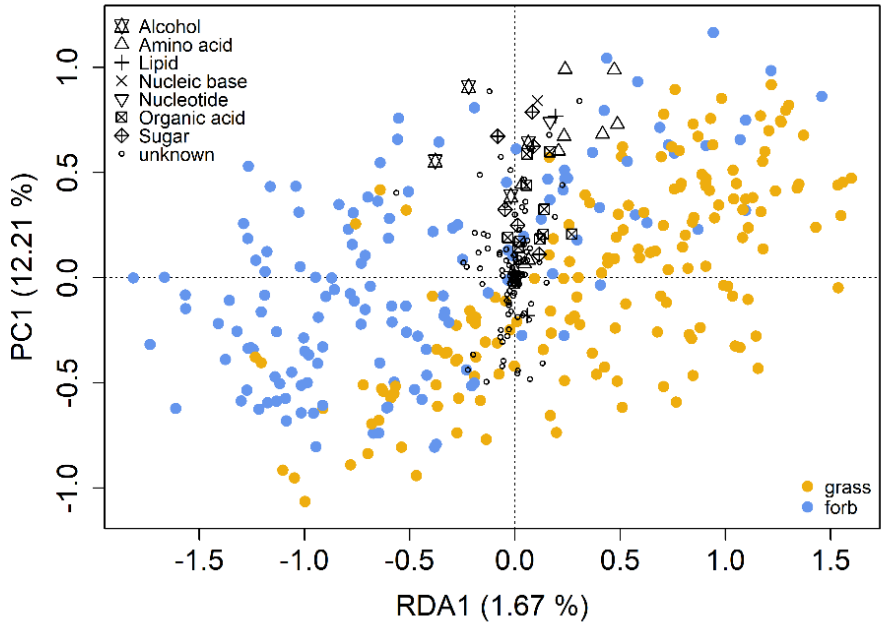

Fig. 1a. Redundancy analysis of polar metabolites and a presence/absence matrix of growth form. Symbols show compounds that could be attributed to the seven substance classes (see S4) and compound compounds.

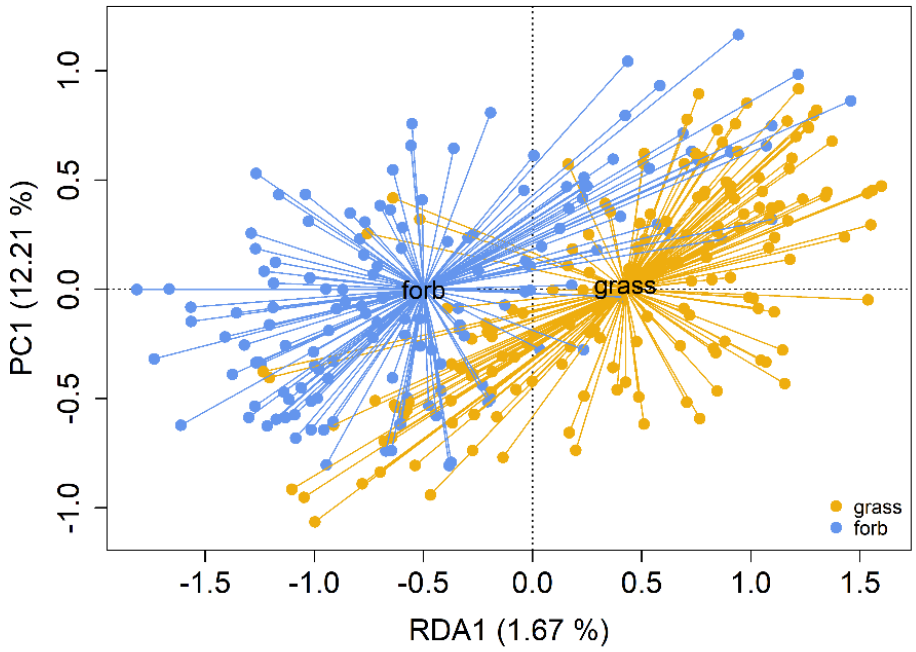

Fig. 1b. Redundancy analysis of polar metabolites and a presence/absence matrix of growth form. Points are grouped by growth form.

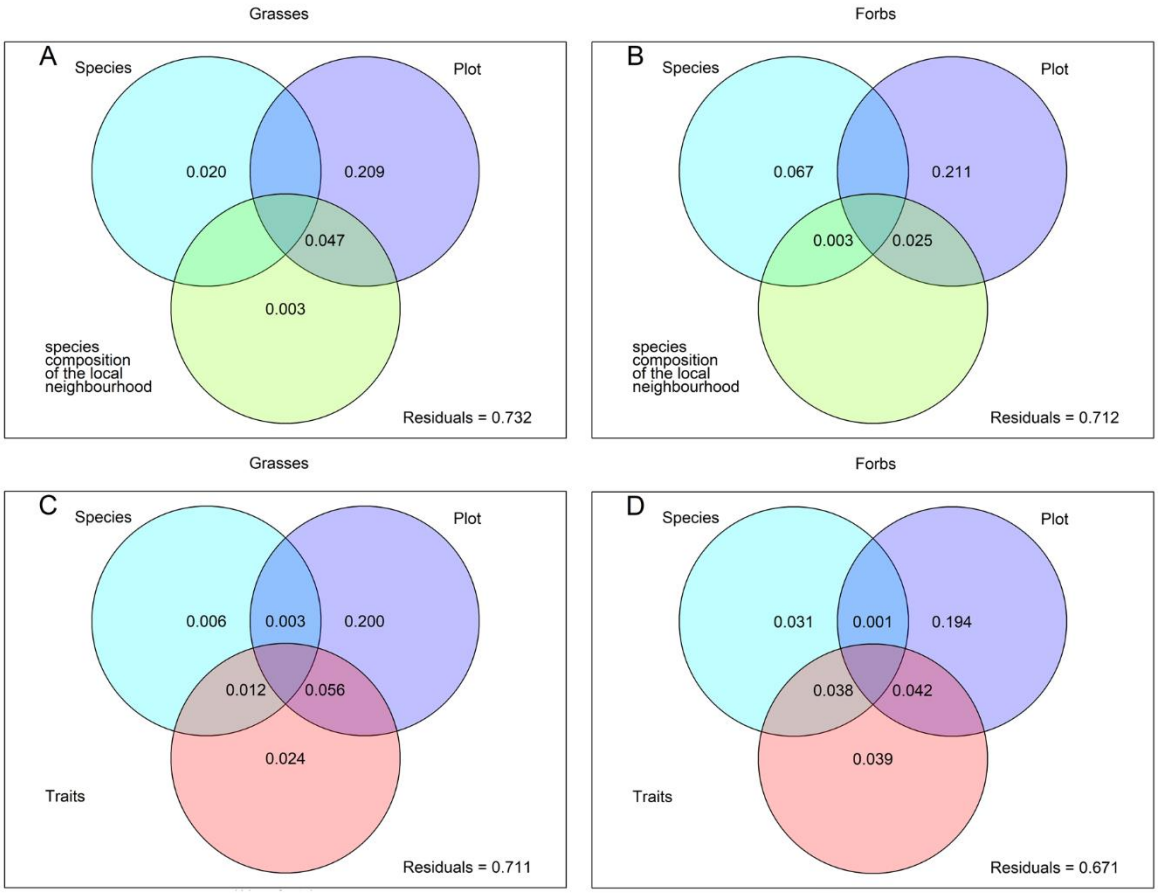

Fig. 2. Variance partitioning with proportion of explained variance of all polar metabolites of Fig. 1 separately for grasses as forbs. Species = species identity of the phytometers, Plot = plot, where the phytometers were located between April and September 2014, species composition of the local neighbourhood = variables varying at the subplot level of 15 cm radius around the phytometer, including species richness, Shannon diversity, total cover and species composition of the local neighbourhood (obtained from the first four axes of a detrended correspondence analysis). The traits used as predictors in C and D are the same as in S7. Values below 0 are not shown.

adjustments to neighbour plants will not occur after a longer residence time of a plant like for example in the Jena experiment, where species richness effects on belowground organs were only visible four years after planting (Ravenek et al., 2014).

When we included phytometer traits instead of local plant neighbourhood composition the total amount of explained variation increased with traits explaining 2.4 % in grasses and 3.9 % in forbs (Fig. 2). The identity of the plot still explained best the variation in plant exudation (20 % in grasses and 19.4 % in forbs), whereas species identity explained less than 4 %.

We further compared the PCA of functional traits (S7) with the PCA of exudates (S8) in a procrustes analysis (Fig. 3) to relate functional traits and exudates to each other. It

revealed a high congruence between trait and exudate composition (R^2=0.2995, p=0.001) thus supporting our second hypothesis. By comparing the effects of each single trait on exudate composition using variance partitioning we revealed total and root dry mass as the most important traits (S9, S10) which pointed to shifts in exudate composition with plant biomass. This finding conforms to the reports that exudate composition of a plant changes with developmental stage and biomass (Aulakh et al., 2001). For rice plants Aulakh et al. (2001) reported an increase of exudation rates from the seedling stage till panicle initiation and a subsequent decrease at maturity. Therefore, young plants with low biomass can acquire more nutrients while adult plants with higher biomass invest more into storage. Furthermore, it was shown by Aulakh et al. that seedlings exuded a lower absolute amount of metabolites than adult plants (Aulakh et al., 2001). However, the explained variation did not exceed 2.48 % for any single trait included in our analysis and thus shows that exudate composition is not driven by one specific trait alone.

Overall we could show that exudates of plants growing in a natural community were much more determined by the environmental conditions of the plot rather than by species identity, by species-specific traits or neighbouring plants in this study. In contrast to our third hypothesis, root exudation patterns were almost unaffected by neighbour plant species composition. Thus the match between traits and exudates as shown in the procrustes analysis (Fig. 3) was probably mainly driven by the environmental conditions of a plot that affected both traits and exudates. Nevertheless, to unravel the role of such environmental conditions as climate, soil properties or inclination on root exudation (Kuijken et al., 2015, Moles et al., 2014, Ordoñez et al., 2009, Siebenkas et al., 2015) is still a challenge. This is particularly true for our approach of analysing root exudate pattern on This is particularly true for our approach of analysing root exudate pattern on individual plants in natural communities as compared to root exudates at the level of artificially assembled communities in microcosms (see Eisenhauer et al., 2017). The challenge of analysing the drivers of root exudation also increases with the number of analysed compounds. While Eisenhauer et al. (2017) measured a total of 15 exudates with HPLC, we annotated 153 compounds with mass spectrometry and included them in the analysis. With our results we could already account for up to 32.9 % of the total variation in exudate composition by the factors included in our study, although our phytometers have been exposed to their environment only for one vegetation season. The remaining proportion indicates that further variation in exudate composition might be explained by other factors such as seasonal variation, but also stochastic events within plots such as grazers'

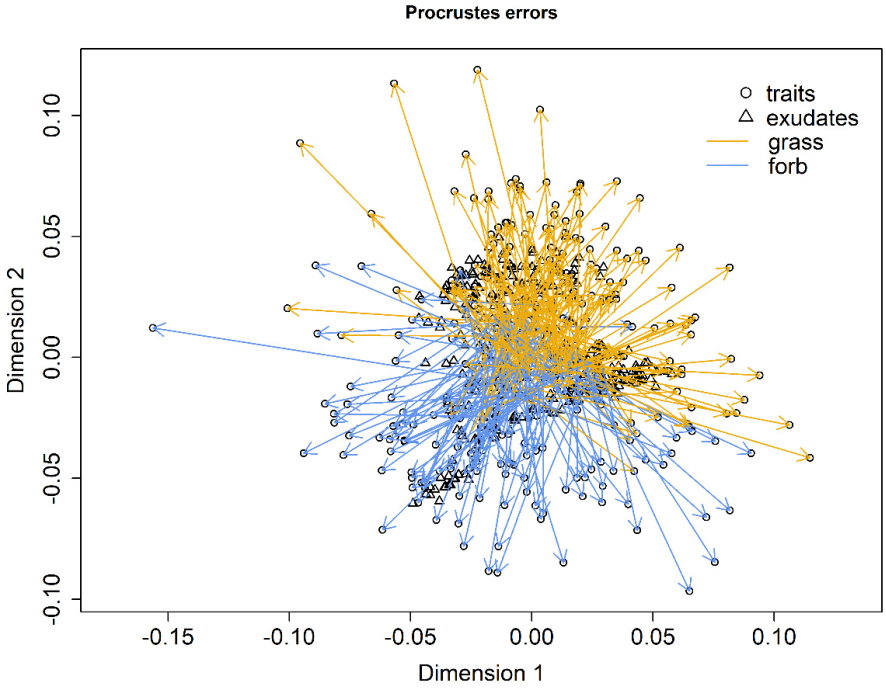

Fig. 3. Procrustes correlation of S7 and S8. Arrows indicate in which direction the ordination is stretched to fit the ordination of traits to the ordination of the exudates. The two axes correspond to the principal components of the principal component analysis (PCA) of polar metabolites (S8). Correlation of the symmetric procrustes rotation = 0.2995, p = 0.001, number of permutations = 999.

trampling or defecating on single target plants or plot- and species-specific effects of the microbial community composition, herbivory, pathogens or endophytes. It would be of great interest to examine their influence on plant exudation behaviour, also for short and long time exposure in a natural environment.

Finally, plant exudate composition comprises not only the presented polar metabolites but also semi-polar metabolites characteristic of the secondary metabolism (Aulakh et al., 2001). The technique of planting phytometers, retrieving them and collecting their root exudates could also allow for analysing such secondary metabolites, which is work in progress. Secondary metabolites have been found to be of considerable importance for plant interactions as they for example inhibit the growth of competing plant species (Biedrzycki et al., 2010) but also stimulate the germination of parasite seeds (Auger et al., 2012). As root exudation depends on as yet unpredictable interactions in the rhizosphere, further integration of such metabolic data on root exudates with information on rhizosphere microbial community composition may fundamentally change our understanding of belowground interactions.

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Chapter 2.3.: Semi-polar root exudates in natural grassland communities

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Authors contribution:
HB, UJ, SH and DS designed the field experiment. KH, SD, UJ and SH conducted the field experiment and collected the phytometers. Trait analysis was performed by KH. Exudate extraction and analyses where performed by SD. Annotation and identification where performed by SD with contribution by StD. Statistical analysis was performed by SD with input from HB, KH and DS. The manuscript was written by SD with input from all co-authors.

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Abstract

In the rhizosphere, plants are exposed to a multitude of different biotic and abiotic factors, to which they respond by exuding a wide range of secondary root metabolites. So far, it has been unknown to which degree root exudate composition is species-specific and is affected by land use, the local impact and local neighborhood under field conditions. In this study, root exudates of 10 common grassland species were analyzed, each five of forbs and grasses, in the German Biodiversity Exploratories using a combined phytometer and untargeted liquid chromatography-mass spectrometry (LC-MS) approach. Redundancy analysis and hierarchical clustering revealed a large set of semi-polar metabolites common to all species in addition to species-specific metabolites. Chemical richness and exudate composition revealed that forbs, such as *Plantago lanceolata* and Galium species, exuded more species-specific metabolites than grasses. Grasses instead were primarily affected by environmental conditions. In both forbs and grasses, plant functional traits had only a minor impact on plant root exudation patterns. Overall, our results demonstrate the feasibility of obtaining and untargeted profiling of semi-polar metabolites under field condition and allow a deeper view in the exudation of plants in a natural grassland community.

Keywords: Exudates, grassland community, liquid chromatography coupled to mass spectrometry, plant functional traits, semi-polar metabolites, untargeted metabolite profiling

Introduction

The roots of terrestrial plants are embedded in a belowground “black box” surrounded by soil and a multitude of living organisms. This small habitat, the rhizosphere, is characterized by complex chemical, biological and ecological processes (Bais et al., 2006a). The chemical interactions are mediated by among others low-molecular-weight metabolites (Dakora and Phillips, 2002, Faure et al., 2008) released by the plant root and thus called plant root exudates. Those consist of polar, semi-polar and a-polar metabolites. The semi-polar metabolites, which in most cases are products of the plant secondary metabolism, are highly diverse and involved in different kinds of interactions with the rhizosphere habitat. They are a part of the plant stress responses to abiotic factors, such as temperature, light and soil conditions (Badri and Vivanco, 2009, Lambers et al., 2015, Lambers et al., 2008) but also fertilizer treatments (Vogt, 2010). In case of land use, however, the involvement is not fully understood. Herz et al. (2017) observed an increased root volume and lower root carbon-to-nitrogen-ratio in case of higher land use. So, they

postulated that this is linked to high resource acquisition of fast growing grassland species. However, the link between exudation of a plant root and land use intensity has not been found so far (Herz et al., 2018).

With regard to biotic relations, the release of semi-polar metabolites mediates a multitude of interactions with the microbial soil communities (Bardgett and van der Putten, 2014, Lambers et al., 2009), and also with neighbouring plants (Bais et al., 2004, Bais et al., 2006a, Broeckling et al., 2008, van Dam and Bouwmeester, 2016, Weir et al., 2004). Exuded phytotoxins reduce the establishment, growth, or survival of susceptible neighbours by altering respiration, membrane transport, germination or shoot and root growth (Bais et al., 2006a). *Arabidopsis thaliana* seedlings for instance have a reduced root length of primary root but an increased number of lateral roots when exposed to exudates of other plant species (Biedrzycki et al., 2010). Also hogweed (*Heracleum mantegazzianum*) root exudates inhibit the germination and growth of other plant species (Jandova et al., 2015). Most of these abiotic and biotic factors, however, have been related to plants in “one plant – one factor” experiments under controlled conditions (Strehmel et al., 2014, van Dam and Bouwmeester, 2016) or in ecological field experiments without the investigation of belowground exudation (Herz et al., 2017b, Ravenek et al., 2014, Ravenek et al., 2016).

Similarly, the impact of plant´s characteristics such as species’ identity, developmental stage (Aulakh et al., 2001, Chaparro et al., 2013) or plant functional traits (Aulakh et al., 2001, Herz et al., 2018, Herz et al., 2017b) on the exudation pattern or the plants relation to its habitat, respectively, were performed under controlled conditions. Plant functional traits are thereby ‘characteristics, or trait values, at tissue-to-organismal scales that reflect their evolutionary history and mold their performance’ (Reich and Cornelissen, 2014). In case of belowground traits, this includes root length, root volume, root respiration, nutrient uptake kinetics, root tissue nutrient content and also the release of exudates (Bardgett et al., 2014). Making use of such traits is a very attractive approach as it would allow to generalize patterns observed across different species, and thus, bringing them together in a comprehensive framework. In a preceding study (Herz et al., 2018), the relation between polar root exuded metabolites and plant traits was investigated under natural conditions. The authors demonstrated that the exudate pattern was correlated with the root weight of the phytometers (Herz et al., 2018). However, these results only refer to a limited number of exuded metabolites. So far, it is unknown whether such correlations also hold true for the much more diverse semi-polar secondary compounds released by a plant root (Dixon, 2001, Monchgesang et al., 2016a).

The listed gaps in the knowledge of root exuded semi-polar metabolites and their relationships to the environment are mainly due to the challenge in collecting root exudates

in the field. Techniques as the collection of exudates in hydroponic or rhizobox systems are not applicable in field experiments. They are either collected under artificial growth conditions, and thus, have only limited relevance for soil conditions, or the technique is too complicated for a field approach (Oburger et al., 2013). Another often limiting point is the frequent use of targeted metabolic profiling. Most studies of exudates use targeted analyses of a predefined set of selected compounds, such as the phytoalexin camalexin (Millet et al., 2010), or compound classes, e.g. coumarins (Fourcroy et al., 2014) and strigolactones (Kohlen et al., 2011). Although this approach allows studying the role of a particular metabolite in the rhizosphere network, it cannot reflect the complexity of the functional linkage of metabolites such as semi-polar compounds in a complete exudate profile. An untargeted metabolomics approach, instead, allows the detection and, to some extent, identification of metabolites which otherwise would be neglected (Peters et al., 2018, van Dam and Bouwmeester, 2016).

Whereas the study of Herz et al. (2018) demonstrated that that polar metabolites can be analysed under field condition, the present study tested whether the collection, sample preparation and untargeted metabolite profiling method is applicable for semi-polar metabolites under field conditions. Ten species of two different growth forms (forb and grass) were planted as phytometers in 54 existing grassland communities in the regions of the Biodiversity Exploratories in Germany (Fischer et al., 2010) to distinguish between species-specific and environmentally induced exudate patterns. Their root exudate profiles were investigated for their composition, specific compounds and correlation with biotic and abiotic influences. Considering the characteristics of semi-polar metabolites we hypothesised that (i) there are differences in metabolite composition between the growth forms. We further hypothesised (ii) that these differences are mainly driven by species specificity in the exudate profiles. The significant species-specific compounds were further analysed for a putative chemical metabolite classification. We further postulate, that (iii) semi-polar metabolite composition is influenced by biotic factors, here the local neighbourhood of the plant community, and by abiotic factors, such as the locational impact (Plot), and land use intensity (LUI). Finally, (iv) we tested whether the correlations observed between 18 different above- and belowground plant functional traits and polar root exuded metabolites (Herz et al., 2018) also hold for semi-polar metabolites compositions of the phytometer exudates.

Material and Methods

Experimental Setup

The experiment was performed during March and September 2014 in the three regions of the German Biodiversity Exploratories (Fischer et al., 2010): Schorfheide-Chorin, Hainich-

Dün and Swabian Alb as described in Herz et al. (2017). In total, 18 experimental grassland plots in each exploratory were selected varying in land use intensity, leading to a total number of 54 plots. In total, 10 species were investigated in this experiment: five forbs (*Achillea millefolium* L. [Asteraceae], *Galium mollugo* L., *Galium verum* L. [Rubiaceae], *Plantago lanceolata* L. [Plantaginaceae], *Ranunculus acris* L. [Ranunculaceae]) and five grasses (*Alopecurus pratensis* L., *Arrhenatherum elatius* [L.] P.Beauv. ex J.Presl & C.Presl., *Dactylis glomerata,* L., *Lolium perenne* L., *Poa pratensis* L. [all Poaceae]). These perennial species are among the most frequent and abundant species in all Exploratory grassland plots. Seeds of all species were collected in the Exploratories and raised under greenhouse conditions. Each species was planted into five subplots established in each of the 54 plots. Within a subplot, the plants were planted at a distance of 50 cm to each other at random locations. A full description of the field design and the planting procedure is given in Herz et al. (2017).Due to restricted access to some plots at the time of sampling and mortality in some plots, it could not be sampled the full set of planted phytometers. In total, 389 plants (*A. millefolium*: 38, *G. mollugo:* 41, *G. verum:* 37, *P. lanceolata:* 39, *R. acris:* 28, *A. pratensis:* 40, *A. elatius:* 40, *D. glomerata:* 48, *L. perenne:* 37, *P. pratensis:* 41) of 46 of the 54 experimental plots were sampled using the same approach described in Herz et al. (2018).

Collection of Exudates

The procedure developed by Aulakh et al. (2001) was adapted for a field targeted analysis of semi-polar metabolites. The phytometer plants were excavated after being exposed to field conditions for three months and the roots carefully washed with tap water to remove bulk and rhizosphere soil. After the reduction of possible ions from the tap water by a second wash step with deionised water, the complete roots of the intact plant were placed in 250 ml brown plastic vessels containing 200 ml of deionised water of HPLC quality for 2 hours exudation. To distinguish exudates from procedure artefacts 200 ml water samples without exudation (“blank”) were treated exactly like the exudate samples. All samples were frozen and stored at -20 °C until further processing. The samples were filtered after a slowly thawing process and reduced to soluble substances by evaporating the water under reduced pressure at 40 °C in a rotary evaporator. The metabolites were resolved by dissolving them two times with 100 % methanol (Sigma-Aldrich, Taufkirchen, Germany), sonicating them for 10 min at 20 °C and then transferring the solution into new tubes. Followed by a second evaporation step (under pressure, 40°C) in a vacuum centrifuge, the metabolites were reconstituted in 80 % methanol containing 20 µg/mL 2,4-dichlorophenoxyacetic acid and 10 µM Ribitol as internal standards. An aliquot of 100 µl of exudate was transferred into a glass vial (Waters, Eschborn, Germany) and subjected

to mass spectrometry. Further details and additional tests on the appropriateness of the approach are given in Herz et al. (2018).

LC-MS analysis and data processing

Exudates and controls were measured by non-targeted metabolite profiling with ultra performance liquid chromatography coupled to electron spray ionisation quadrupole time of flight mass spectrometry (UPLC/ESI-Q-ToF-MS). Performance was supervised by measurements of a standard mix of eight substances (MM8: 10µM α-Phenylglycin, 10µM Kinetin, 10µM Rutin, 10µM o-Anisic acid, 10µM Phlorizin, 10µM IAA Valine, 10µM Indolacetonitril, 10µM Biochanin) every ten samples.

The separation of metabolites was performed by ultra performance liquid chromatography (ACQUITY UPLC; Waters) equipped with aC18 column (ACQUITY UPLC HSS T3 Column, 100Å, 1.8 µm, 1 mm x 100 mm; Waters) with 2 µL full loop injection at 40°C. The following gradient was utilized: flow rate of 150 µL/min 0–1 min, isocratic 95 % A (water/formic acid, 99.9/0.1 (v/v)), 5 % B (acetonitrile/formic acid, 99.9/0.1 (v/v)); 1–14 min, linear from 5 to 95 % B; 14–18 min, isocratic 95 % B; 18–20 min, isocratic 5 % B. The eluting compounds were detected from m/z 90 to 1000 using a MicrOTOF–Q II hybrid quadrupole time-of-flight mass spectrometer equipped with an Apollo II electrospray ion source (Bruker Daltonics, Billerica, MA, USA) in negative ion mode. The following instrument settings were used: nebulizer gas, nitrogen, 1.6 bar; dry gas, nitrogen, 6 L/min, 190 °C; capillary, -4000 V; end plate offset, -500 V; funnel 1 RF, - 200 Vpp; funnel 2 RF, - 200 Vpp; in- source CID energy, 0 eV; hexapole RF, - 100 Vpp; quadrupole ion energy, - 5 eV; collision gas, nitrogen; collision energy, -7 eV; collision - 150 Vpp; transfer time, 70 µs; pre pulse storage, 5 µs; spectra rate, 3 Hz.

The individual raw data files were recalibrated on lithium formate cluster ions obtained by automatic infusion of 20 µL 10mM lithium hydroxide in isopropanol/water/formic acid, 49.9/ 49.9/0.2 (v/v/v) at a gradient time of 18 min and by using a diverter valve. Peak picking was performed with Compass DataAnalysis 4.4.2 software (Bruker Daltonics) and a signal-to-noise threshold of 2, correlation coefficient threshold 0.7, minimum feature length 7 spectra, smoothing width of 3. Automated detection of adducts was enabled for M-H, M-H2O-H, M+Na-H2, M+K-H2 and M+HCHOOH-H. No background subtraction was performed. Retention time alignment and feature extraction was performed by Compass ProfileAnalysis 2.3 (Bruker Daltonics) within the retention time (RT) range of 0.01 to 18.00 min and mass range of 90 to 1000 mass to charge ratio (m/z). Peak grouping was performed with an allowed deviation of 0.1 min for the retention time and 200 mDa for the mass. Features occurring only once in all samples were excluded from further analysis. An automated annotation of the feature list by m/z and RT was done with MetaboScape

2.0 (Bruker Daltonics). Features occurring in 50 % of water controls were excluded from the metabolite list. The feature list was subjected to statistical analysis (see below).

LC-MS/MS analysis and data processing

For the acquisition of collision induced dissociation (CID) mass spectra, exudate samples and water controls were pooled according to their affiliation to the ten species and three Exploratory sites. The measurements were performed by UPLC/ESI-Q-ToF-MS with an ultra performance chromatographic system (ACQUITY UPLC; Waters) equipped with a C18column (ACQUITY UPLC HSS T3 Column, 100Å, 1.8 µm, 1 mm x 100 mm; Waters) and a MicrOTOF–Q I hybrid quadrupole time-of-flight mass spectrometer equipped with an Apollo II electrospray ion source (Bruker Daltonics). The separation and MS measurement was performed as described above. CID mass spectra were acquired at first by automated data dependent MS/MS and, if necessary, using a scheduled precursor ion list with an isolation width of ± 3-15 m/z and fragmentation inside the collision cell with an applied collision energy in the range of 15–70 eV. Argon was used as collision gas. Product ions were detected using the same parameter settings as described above. MS as well as tandem MS measurements of pooled samples were processed with MetaboScape 3.0.1 software (Bruker Daltonics). The T-Rex 3D algorithm (Bruker Daltonics) was applied for peak picking, alignment and automated assignment of MS2 spectra with following settings: Intensity threshold: 1500, minimum peak length: 7 spectra, minimum peak length recursive: 7 spectra, minimum of compounds for extraction: 2, no log mass calibration, primary ion: M-H-, expected ions: M+Cl-, M+Na-H-, M+K-H-, ions for pseudo spectra: M-H-H2O, M+ HCOOH-H-, EIC correlation; 0.8, mass range: 90 – 1001, RT range: 0.01-18.

Identification approach

In a first step, significant species specific compounds were annotated according their m/z and RT manually. Secondly, the most probable elemental composition was calculated with MetaboScape 3.1's Smart Formula algorithm (Bruker Daltonics). In a third step, MS/MS spectra of compounds (limited to the species specific metabolites) were exported and The spectral library of species-specific compounds was processed with the MetFamily metabolite classification online software (Treutler et al., 2016). There, fragment spectra were deconvoluted, reduced to fragments with an intensity above 1000. Afterwards fragment intensities were normalized within each MS/MS spectrum to a maximum of 1 (base peak) (Treutler et al., 2016). Fragments with a normalized intensity higher 0.1 and neutral losses were annotated according to their m/z similarity to those of an in-house database of possible characteristic fragments and neutral losses measured on LC-MS systems (Appendix Table A1). Using this data base and further chemical knowledge, a

putative classification of compounds was performed. The results were summarized in Appendix Table A2.

Plant functional trait analysis and ecological data

After sampling exudates, the plant material was used for analysing plant functional traits (Appendix Table A3) as described in detail in Herz et al. (2017b). Fresh and dry mass of roots were assessed, shoots and leaves separately. Roots and leaves were scanned fresh on a HP Scanjet Flatbed Scanner at 600 dpi and analysed with the programs WinRHIZO (v Pro 2008a; Regent Instruments, Quebec, Canada) and WinFOLIA (v Pro 2004a Regent Instruments). From these measurements, root volume, root mass per volume and specific leaf are (SLA, leaf area per total dry weight) were obtained. We are aware that root mass per volume differs from root tissue density measurements made by diameter class (Rose, 2017). Given the large amount of samples that had to process, it was not possible to analyse all roots systems by diameter class. However, comparisons between plants are not affected, as all samples were treated in the same way. Additionally, the root and leaf dry matter content (root and leaf dry mass per fresh mass, respectively) as well as root to shoot ratio (RSR) was calculated. The dried roots were ground to assess C, N (C/N-analyzer vario EL cube; Elementar, Hanau, Germany) and after a digestion with nitric acid also P (photometric phosphate assay), K, Mg and Ca (atom absorption spectrometry with AAS vario 6; Analytik Jena, Jena, Germany) content as well as C to N ratio.

The composition of the local neighbourhood of the phytometers was obtained by recording the number of plant species and cover per species growing in a 15cm radius [707 cm^2] around each phytometer plant. These records were used to calculate species richness and Shannon diversity of the local neighbourhood, in addition to species composition as obtained from the first four axes of a detrended correspondence analysis [DCA].

Statistical Analysis

All statistical analyses were performed with R (version 3.4.4; R Core Team, 2015). All analyses were carried out on exudates based on a presence/absence matrix of these compounds since compound number instead of intensity were in focus of this study, similar to the analysis of Herz et al. (Herz et al., 2018). To calculate the chemical richness of each species, the mean of number of measured metabolites per group were calculated. This was used as base for calculation of the overall significance of the difference between species by an ANOVA (function aov, R Core Team, 2015). A Scheffé posthoc test (function scheffe.test, package agricolae, de Mendiburu, 2017) was performed to test for the exact significant differences between groups and presented in a violin plot (function ggplot and geom_violin, package ggplot2, Wickham, 2009). To investigate the exudate composition

of the ten species a hierarchical clustering (function dist and hclust, R Core Team, 2015) was performed. Moreover, a redundancy analysis (RDA) (function rda, package vegan, Oksanen et al., 2016) was conducted to relate the matrix of semi-polar metabolites to the presence/absence matrix of the species from which the exudates were obtained.

To calculate the number of shared compounds between all species, all compounds that occurred at least twice in all analysed samples were taken into consideration and summed up per species. The species-specificity of compounds was analysed by calculating the frequency of all compounds per species and using performing an exact binomial test (function binom.test, R Core Team, 2015). We tested the probability of every compound to be more frequent in a particular species than in the other nine species together ($p > 0.95$). These species-specific compounds were further used for the tandem MS approach and the putative identification approach. To test for the relationship between above- and belowground traits and exudation patterns of semi-polar exudates matrix of 302 of the 389 samples of each traits (Appendix Table A3) and exudates was subjected to a Principal Component Analysis (PCA) (function rda, package vegan, Oksanen et al., 2016) and then compared both matrices with a Procrustes analyses (function protest, package vegan; Oksanen et al., 2016). The trait matrix was thereby rotated to reach maximum similarity with the exudate matrix. The Procrustes rotation minimizes the sum of squared differences between both matrices. Then, a permutation test is used to obtain the correlation between both matrices Furthermore, the data were subjected to variance partitioning (function varpart, package vegan, Oksanen et al., 2016) to detect how much variance was explained by either the combination of target species identity (Species), the environment and geographic location (Plot), composition of the local neighbourhood of each phytometer (LNH) or the combination of Species, Plot and plant functional traits of the phytometer (Trait). Variance partitioning was performed separately for the two growth forms to reveal the differences in variation between grasses and forbs. Furthermore, the explained variance for each single variable of the predictors Trait, LNH and LUI (Blüthgen et al., 2012) was calculated, together with Species and Plot using variance partioning (function varpart, package vegan; Oksanen et al., 2016). Here, LUI is the mean of land use intensity index of the years 2006 to 2014. Each LUI consists of the sums of measurements of each plot (i) of fertilization (F, in kg nitrogen ha^{-1} $year^{-1}$), the frequency of mowing ($year^{-1}$) and grazing intensity which were standardized relative to their means (r) within the sites (Blüthgen et al., 2012).

$$LUI = \frac{Fi}{Fr} + \frac{Mi}{Mr} + \frac{Gi}{Gr}$$

Results

Profiles of semi-polar metabolites differ more due to the species identity than due to growth form

Using the untargeted metabolite profiling approach, 5,414 features were annotated as putative compounds among the 389 phytometer exudate samples. An analysis of variance (ANOVA) of the chemical richness of each species revealed that the total exuded number of compounds of each species differs significantly between species ($p < 0.001$, Figure 1), but not between the two growth forms ($p = 0.0684$). Thus, *P. lanceolata* displays a significantly higher chemical richness than *A. millefolium, D. glomerata, P. pratensis* and *R. acris*, whereas *G. mollugo* has a significantly higher chemical richness than *P. pratensis* and *R. acris* but not higher than *P. lanceolata*. Moreover, *R. acris* showed a significant lower chemical richness than *A. elatius*. All other species did not differ significantly in the number of exuded metabolites. This trend was also observed in a redundancy analysis (RDA) (Appendix Figure A1) where separation of grass exudation profiles was less pronounced than that of forbs. There, the samples of the species *G. mollugo* and *Galium*

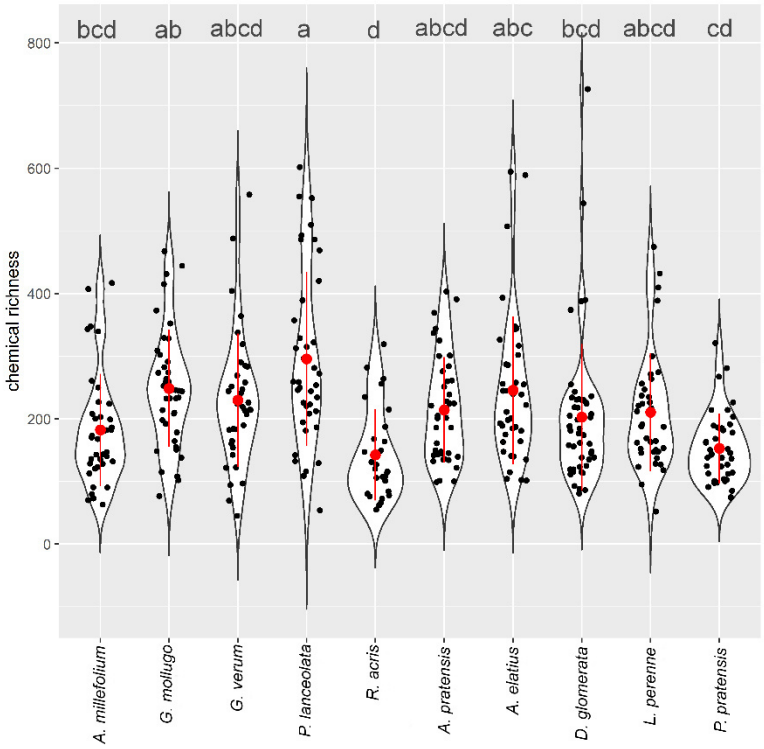

Figure 1: Chemical richness of semi-polar metabolites in root exudates of ten grassland species. Violin plot presents the number of measured compounds per species (chemical richness) of the 389 exudate samples. The shape of the violins represents the distribution of the number of metabolites. The black points show the value of the specific samples. Red points represent the median of the chemical richness, whereas the lines represent the quantiles. ANOVA with the median of chemical richness as response and species as predictor revealed a significant influence of species with a p-value of 2.42e-10 ***. The Scheffé Posthoc test uncovered significant differences between the species, presented by letters. Violins with the same letters are not significantly different from each other.

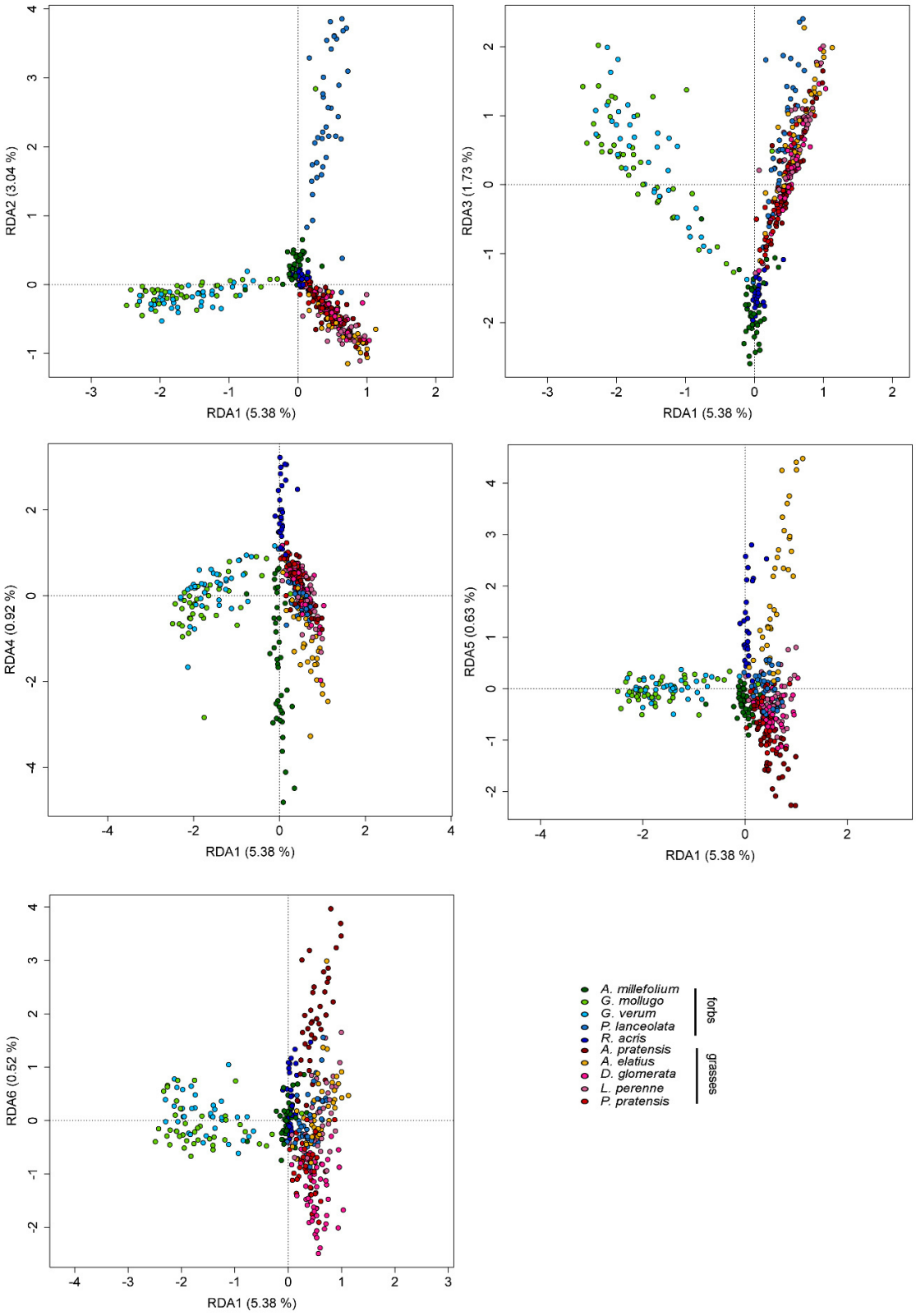

Figure 2: Redundancy analysis of semi-polar metabolites in root exudates. RDA was performed with 389 samples plotted against a presence/absence matrix of species. Axis one to six are displayed. The ten species are represented by colour (see legend). RDAs coloured by growth form are presented in Appendix Figure A1.

verum cluster together on the first axis (5.38 %), whereas the separation of *P. lanceolata* occurred on the second axis (3.04 %) apart from the Galium species and the other species (Figure 2).

A further separation of *A. millefolium* and *R. acris* was observed on axis three (1.72 %), axis four (0.92 %) and less prominent on axis five (0.63 %) (Figure 2). The exudate profiles of two of the grass species were separated at first on the axis five (*Arrhenatherum elatius*), and more or less on the axis six (0.52 %, *A. pratensis* as well as a cluster of *D. glomerata*, *Lolium perenne* and *P. pratensis*). Distance-based hierarchical clustering of exudate patterns resulted in a similar picture (Figure 3) indicating that the exudation profiles are species-dependent. It also points to more similar exudation patterns among grasses than among forbs.

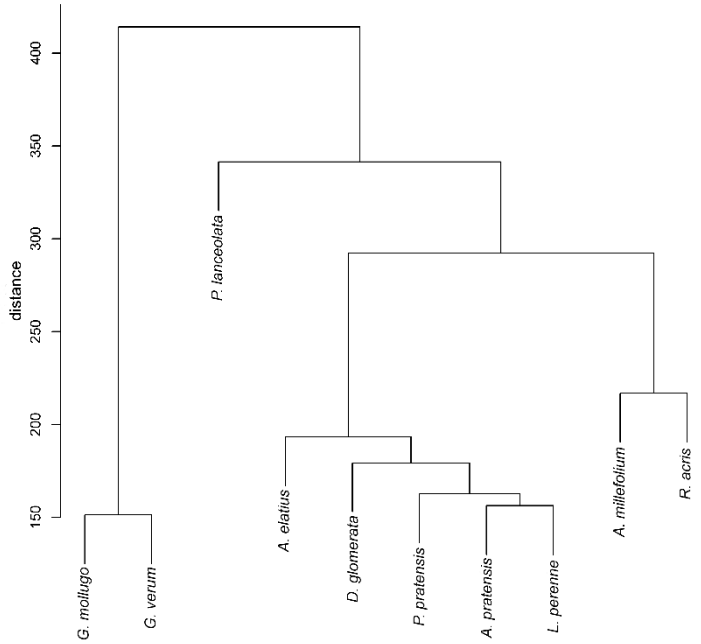

Figure 3: Hierarchical clustering of phytometer sample according to their semi-polar exudate composition. The 389 samples were clustered according to their differences in the semi-polar metabolite composition by a distance-based analysis of all annotated compounds.

Species-specific semi-polar metabolites

The analysis of samples concerning shared and unique semi-polar metabolites revealed that 270 compounds occurred in both growth forms, whereas 625 compounds were significantly species-specific. Specific metabolites were more pronounced in forbs (534) than in grasses (91). In addition, 150 of exuded compounds were specific for the two species of the *Galium* genus (*Galium* spp.). Specific metabolites were more pronounced in forbs (534) than in grasses (91). In addition, 150 of exuded compounds were specific for the two species of the *Galium* genus (*Galium* spp.). Following our identification approach, 200 of these compounds were chosen for further identity elucidation (Appendix Table A2). A total of 102 of these compounds could be assigned to the following metabolite

families (Table 1, Appendix Table A2): glycosides (26; one sulfated glycoside, one sulfated and phosphorylated glycoside, one diglycoside), phenylpropanoids (64) with one glycosylated coumarin (1), polyphenols, such as flavonoids (23; 12 of them glycosylated), other polyphenols (10; 8 of them are hydroxycinnamic acids and one of them is a glycosylated hydroxycinnamic acid), as well as other not further sub-classified phenylpropanoids (29; 11 of them are glycosylated). Furthermore, compounds of the classes polyketides (3), terpenes (6; two were glycosylated) were found, as well as three iridoid glycosides. Furthermore, a total of 104 unclassified compounds with different functional groups was also detected. Many of the compound classes occurred in different species at the same time. Glycosides were mainly detected in *A. millefolium* and *P. lanceolata*, but occurred also in *G. mollugo* as well as in both *Galium* spp. together and one time in *R. acris* and *A. elatius*. Flavonoids occurred mainly in *Galium* spp. and specific ones in *G. mollugo* and to a lower extent in *G. verum*, but also in *P. lanceolata, A. elatius* und *P. pratensis*. Glycosylated flavonoids were predominantly observed in exudate samples of *Galium* spp. and *P. lanceolata*. Also hydroxycinnamic acid and glycosylated phenylpropanoids were mainly detected in *Galium* spp and *P. lanceolata*, whereas unglycosylated phenylpropanoids mainly occurred in *P. lanceolata*. Glycosylated terpenes could only be annotated in *P. lanceolata* and *A. elatius* samples.

Beside shared classes, there were also some of them exclusively exuded by one out of ten species (Table 1, Appendix Table A2). The three putatively classified iridoid glycosides (931.2832 m/z, 4.03 min; 667.15112 m/z, 4.04 min; 585.16223 m/z, 4.84 min) and two putative jasmonate related metabolites (661.29873 m/z, 5.19 min; 499.23636 m/z, 5.88 min; 485.2021 m/z, 5.79 min), as well as the sulfated (701.23201 m/z, 4.84 min) and sulfated and phosphorylated glycosides (591.22857 m/z, 4.42 min) exclusively occurred in *P. lanceolata* samples. Also a glycosylated hydroxycinnamic acid (431.1292 m/z, 3.45 min) was detected only in *P. lanceolata* samples. The polyketides only occurred in the *Galium* spp. samples. *A. elatius* was the only species in which one putative terpene (563.2187 m/z, 5.92 min) and a diglycoside (975.5086 m/z, 6.76 min) were detected.

Biotic and abiotic impact on exudate pattern based on growth form

Many biotic and abiotic factors have an impact on the exudation profiles of plants (Eisenhauer et al., 2017; van Dam and Bouwmeester, 2016). However, it is unknown what kind of factors affect semi-polar metabolite exudate pattern of plants under field conditions. Therefore, correlational analyses of semi-polar root exudates as well as ecological conditions and plant functional traits were measured on 302 of the same 389 phytometers (Appendix Figure A2). A Procrustes correlation (Figure 4) of a Principal Component Analysis (PCA) of exuded semi-polar metabolites (Appendix Figure A3, A5, A6) and a PCA

Table 1: Putative classification of species-specific compounds. The table contains the total number of compounds (in brackets) putatively classified as one of the respective metabolite classes as well as the occurrences in the samples of the ten different species. * = fragment spectrum of compound contains characteristic ion which could also account for Agmatine classification.

			A. millefolium	*G. mollugo*	*G. verum*	*Galium* species	*P. lanceolata*	*R. acris*	*A. pratensis*	*A. elaitus*	*D. glomerata*	*L. perenne*	*P. pratensis*
Glycoside (26)		Glycoside (23)	8	2		3	8	1		1			
		Glycoside, sulfated (1)					1						
		Glycoside, sulfated, phosphorylated (1)					1						
		Diglycoside (1)								1			
Phenylpropanoid (64)		Coumarin , glycosilated (1)				1							
	F. (23)	Flavonoid (11)		2	1	5	1			1			1
		Flavonoid, glycosilated (12)	1	1		3	6						1
	Other Polyphenols (10)	Other Polyphenol (1)				1							
		Hydroxy-cinnamic acid (8)	1			2	3		1	1			
		Hydroxy-cinnamic acid, glycosylated (1)					1						
	Other P. (29)	Phenylpropanoid (18)*	4			2 (1)*	9	1				2	
		Phenylpropanoid, glycosylated (11)	1			4	4	1		1			
Polyketide, aromatic acetate (3)				1	1	1							
Jasmonate conjugate (2)							2						

		A. millefolium	*G. mollugo*	*G. verum*	*G. species*	*P. lanceolata*	*R. acris*	*A. pratensis*	*A. elatius*	*D. glomerata*	*L. perenne*	*P. pratensis*
Terpene (6)	Terpene (1)								1			
	Terpene, glycosylated (2)					1			1			
	Iridio glycoside (3)					3						
Unclassified (104)	Unclassified (73)	12	6		25	18	11			1		
	Unclassified, aromatic acid fragment (5)					4			1			
	Unclassified, Imine (2)				2							
	Unclassified, phosphorylated (3)				1	1	1					
	Unclassified, phosphorylated, glycosylated (1)								1			
	Unclassified, sulfated (11)	1			1	6	2		1			
	Unclassified, glycosylated (5)					2	2		1			
	Unclassified, sulfated, phosphorylated (1)					1						
	Unclassified, sulfated, glycosylated (3)					2	1					

of plant functional traits (Appendix Figure A4, A7, A8) indicated a connection between plant functional traits and exudation patterns of semi-polar metabolites (R2 = 0.79, p = 0.001). The variance partitioning of exudate composition with the predictors' species identity (Species), the locational impact (Plot) and above- and belowground plant functional traits (Traits) (Figure 5, Appendix Table A3) could only partly explain these results. Traits explained less of the variance in exudate pattern of both growth forms (forbs: 3.3 % and grasses: 1.6 %) than the other predictors. In forbs (Figure 5 a), Plot explained

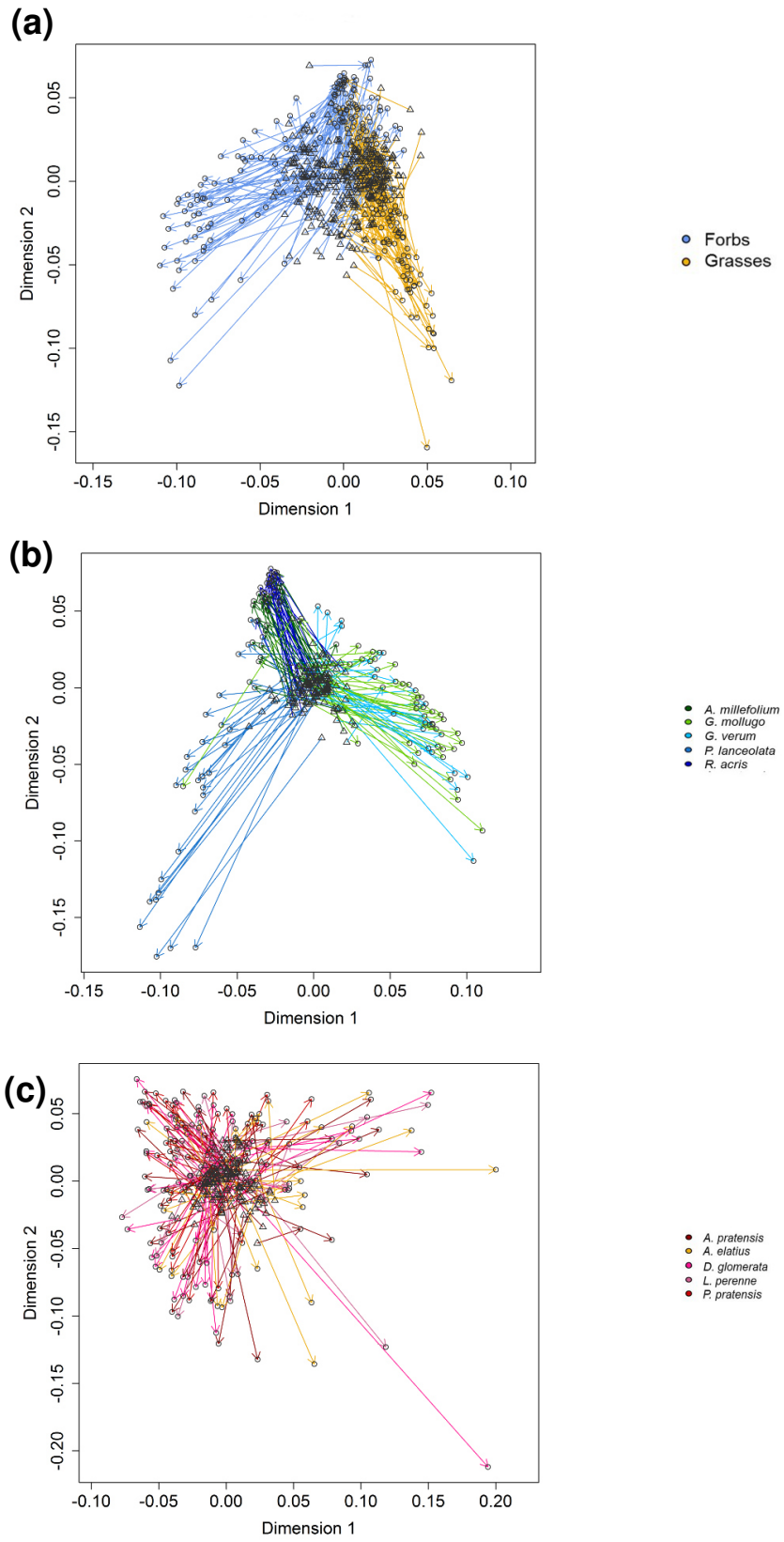

Figure 4: Procrustes analysis of PCA of plant functional traits and PCA of semi-polar metabolites in root exudates. PCAs of supplementary Figures S4 and S5 were correlated to each other. Direction of stretch of the ordination of plant functional trait composition (triangles) to the ordination of the exuded semi-polar metabolite composition (circles) is shown by arrows. **(a)** Procrustes analysis coloured by growth form (see legend), Correlation of the symmetric Procrustes rotation = 0.4582, p = 0.001, Number of permutations = 999. **(b)** Procrustes plot of the forb samples coloured by species (see legend) Correlation of the symmetric Procrustes rotation = 0.1919, p = 0.001, Number of permutations = 999 **(c)** Procrustes plot of the grass samples coloured by species (see legend). Correlation of the symmetric Procrustes rotation = 0.2592, p = 0.001, Number of permutations = 999.

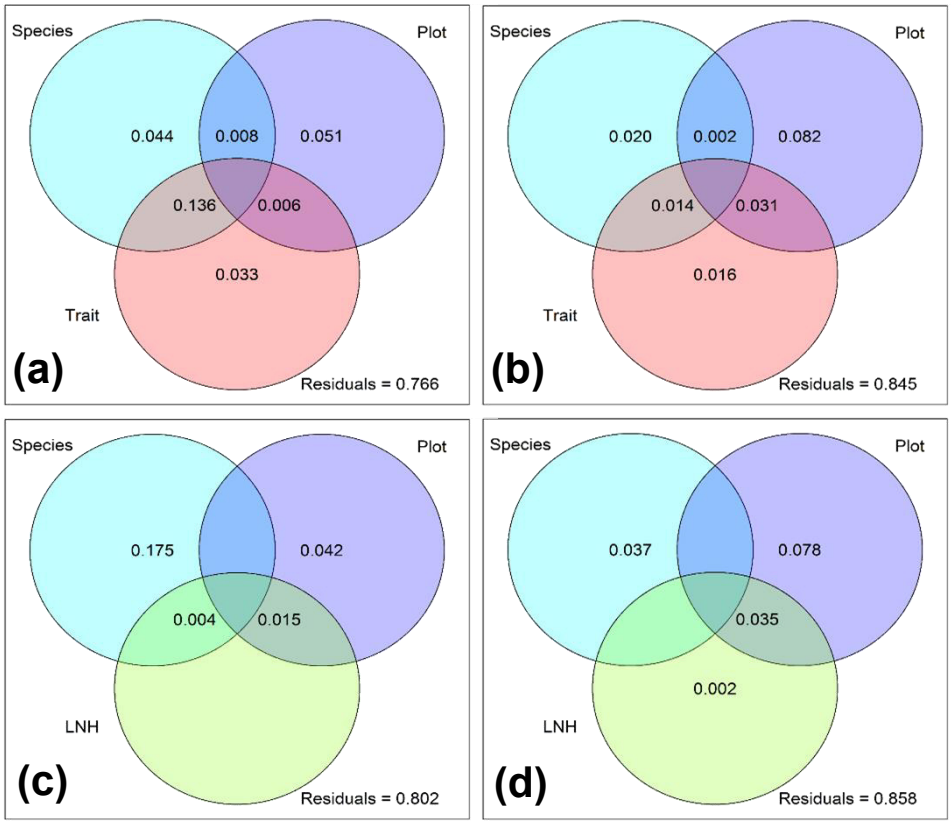

Figure 5: Variance partitioning for the composition of semi-polar metabolites in root exudates. Graphs represent the proportion of explained variance in the semi-polar metabolites composition of forbs (**(a)** and **(c))** and forbs (**(b)** and **(d))**. Predictor variables: Species = species identity of the phytometer; Plot = impact of environment and geographical location of the plot; LNH = species composition of the local neighbourhood (containing variables determined in 15 cm radius around the phytometer, including species richness, Shannon diversity, total cover and species composition of the local neighbourhood, Traits = plant functional traits presented in Table S3. Values below 0 are not shown.

5.1 % of variance and Species 4.4 %, whereas a large proportion of variance in exudate composition was simultaneously related to Species and Traits (13.6 %). In Grasses (Figure 5 b), Plot explained the exudate composition variance best (8.2 %), followed by Species (2.0 %), whereas Plot and Traits together explained only a minor part of variance (3.1 %). This overall observation did not change when plant local neighbourhood community (LNH) was included. Instead, LNH accounted for none of the variance in exudate composition in forbs (0 %, Figure 5 c) and for only a small proportion of variance in grasses (0.2 %, Figure 5 d). The ranking of predictors did not change in grasses (Plot: 7.8 %, Species: 3.7 %, Plot and LNH: 3.5 %, Figure 5 d), whereas in forbs (Figure 5 c) the predictor Species had the highest explanatory power (17.5 %) followed by Plot (4.2 %) and simultaneously explained variance by Plot and LNH (1.5 %). A marginal shared explained variance occurred due to

Species and LNH" (0.4 %). A more detailed analysis of the individual explained variances of the contributing variables to the predictors Traits and LNH (Table 2) revealed that in case of forbs dry mass of roots and leaves (1.5 % each), carbon (C) concentration of roots (1.5 %) and specific leaf area (1.0 %) contributed each to a minor extent to the total explained variance of the Traits. In case of grasses C concentration of roots explained 1.4 % of variance whereas the proportion of explained variance given by dry mass of leaves and roots (2.0 % each) was higher. The contributing variables for predictor LNH and LUI, as an indirect contributing variable to predictor Plot, did not contribute to the explained variance at all (Table 2).

In conclusion, the variance partitioning of both predictor combinations revealed that forb exudates were more related to species identity and displayed a higher inter-specific variation than those of grasses. Grass exudation patterns instead were more responsive to geographic and environmental impacts than forbs.

The variance partitioning also revealed that a higher total variation in root exudation was explained in case of forbs (27.8 % to 23.6 %, Figure 5 a, c) than in case of grasses (16.5 % to 15.2 %, Figure 5 b, d) although the proportion of unexplained variance was high in both growth forms (76.6 % to 80.2 % in case of forbs, 84.5 % to 85.8 % in case of grasses). This points to further influencing factors in addition to those analysed in this study.

Discussion

The analysis of plants´ belowground biochemistry is challenging especially under field conditions (van Dam and Bouwmeester, 2016). For instance, the proximity of a target root to those of other plants makes it difficult to collect the roots of interest without damaging them. Therefore, the collection of exudates of plants is usually performed under hydroponic (Monchgesang et al., 2016) or field mimicking conditions (Eisenhauer et al., 2017; Petriacq et al., 2017). In contrast, the approach presented here and in Herz et al. (2018) allowed the sampling of root exudates from plants grown in soil in their natural habitat. It could be shown that the collection method caused only insignificant micro-injuries of plant root tissues (see Herz et al., 2018). This was made possible by the application of an early stage phytometer approach, where the roots did not grow into other root networks (Clements and Goldsmith, 1924; Dietrich et al., 2013). This prevented root tissue injury due to intertwined roots during the sampling and allows an actual insight into the rhizosphere network. Thus, to our knowledge, this is one of the first investigations of the exudate composition of semi-polar metabolites in a complex environmental context.

The applied statistical analysis of exudate composition of the ten different species showed that the exuded semi-polar metabolite patterns of forbs are different from those of grasses. However, the here presented results point to a higher inter-specific variation in exudation

Table 2: Proportion of explained variance of single variables implemented in the predictors of the variance partitioning of semi-polar metabolite composition of root exudates in both growth forms. The Explained variance (in %) is given for single variables for each predictor and both growth forms, a) forbs and b) grasses, separately. LUI = Land use intensity index, Richness = species richness of local neighbourhood, Shannon = index for local neighbourhood diversity, Cover = cover of all vascular plant species in a 15-cm radius around each phytometer, DCA1-4 = first four axes of a detrended correspondence analysis of all plants surrounding each phytometer, plant functional traits = see Table S3. Negative amounts of explained variance are caused by unbalanced sample sizes and can be considered to be 0.

a) Forbs

Predictor	single Variable (SV)	Species	Plot	SV	Species +Plot	Plot + SV	Species + SV	Species +Plot+ SV	Residu-als
Plant functional traits	SLA	14.56	5.47	1.03	-1.89	0.21	3.4	-1.71	78.93
	LDMC	16.17	5.7	0.04	-2.43	-0.02	1.78	-1.17	79.93
	LAR	17.96	5.59	-0.07	-3.61	0.09	-0.01	0.01	80.03
	RSR	18.07	5.79	0.25	-3.58	-0.1	-0.11	-0.02	79.71
	RDMC	17.69	5.73	0.11	-3.6	-0.05	0.26	-0.01	79.85
	RMV	17.79	5.71	0.19	-3.49	-0.03	0.16	-0.12	79.78
	RVol	13.97	6.12	0.43	-1.08	-0.43	3.98	-2.52	79.53
	RCC	16.79	6.33	1.54	-3.07	-0.65	1.16	-0.53	78.42
	RNC	17.56	5.68	0.24	-3.22	0	0.39	-0.38	79.72
	RCNR	17.3	5.49	-0.07	-3.21	0.2	0.65	-0.39	80.03
	RPC	10.59	5.61	0.07	-1.39	0.07	7.37	-2.21	79.89
	RKC	17.99	5.54	-0.11	-3.61	0.15	-0.03	0	80.08
	RMgC	17.54	4.58	-0.14	-3.16	1.11	0.42	-0.44	80.1
	RCaC	17.66	5.07	-0.08	-3.4	0.62	0.29	-0.2	80.04
	DM_leaves	15.27	5.63	1.49	-2.06	0.05	2.69	-1.54	78.47
	DM_roots	15.37	5.6	1.52	-2.25	0.09	2.58	-1.35	78.44
	DM_total	14.96	5.45	0.5	-2.11	0.24	2.99	-1.49	79.47
	DM_above	15.66	5.45	0.48	-2.49	0.24	2.29	-1.11	79.48
	LUI	17.95	5.49	0	-3.47	0.19	0	-0.14	79.96
Plant community local neighborhood	Cover	17.99	5.54	-0.11	-3.61	0.15	-0.03	0	80.08
	DCA1	17.54	4.58	-0.14	-3.16	1.11	0.42	-0.44	80.1
	DCA2	17.66	5.07	-0.08	-3.4	0.62	0.29	-0.2	80.04
	DCA3	17.91	5.55	-0.09	-3.55	0.14	0.04	-0.05	80.05

	DCA4	17.58	5.66	0.08	-3.23	0.02	0.38	-0.38	79.89
	Richness	18.13	5.61	-0.05	-3.68	0.08	-0.18	0.07	80.01
	Shannon	18.2	5.6	0.01	-3.69	0.09	-0.25	0.09	79.95

b) Grasses

Predictor	single Variable (SV)	Species	Plot	SV	Species +Plot	Plot + SV	Trait + SV	Species +Plot+ SV	Residu-ales
Plant functional traits	SLA	3.33	10.78	1.93	-0.77	0.58	0.14	-0.14	84.15
	LDMC	3.28	11.2	-0.06	-0.69	0.16	0.2	-0.22	86.14
	LAR	3.46	11.12	-0.05	-0.75	0.24	0.01	-0.15	86.13
	RSR	3.41	11.4	0.08	-0.89	-0.05	0.06	-0.02	86
	RDMC	3.29	11.3	0.11	-0.8	0.06	0.18	-0.11	85.97
	RMV	3.43	11.33	-0.01	-0.87	0.02	0.04	-0.03	86.09
	RVol	3.32	11.39	0.04	-0.83	-0.04	0.15	-0.07	86.04
	RCC	3.38	11.46	1.39	-0.77	-0.1	0.09	-0.13	84.69
	RNC	3.19	11.56	0.35	-0.79	-0.2	0.28	-0.12	85.73
	RCNR	3.3	10.17	0.08	-0.62	1.19	0.17	-0.28	85.99
	RPC	3.24	10.72	0.17	-0.67	0.63	0.23	-0.24	85.91
	RKC	3.48	11.3	0.07	-0.9	0.06	-0.01	0	86.01
	RMgC	3.52	9.55	-0.07	-0.91	1.8	-0.05	0	86.15
	RCaC	3.52	10.42	-0.01	-0.92	0.93	-0.05	0.01	86.08
	DM_leaves	3.16	10.72	1.98	-0.69	0.64	0.31	-0.21	84.1
	DM_roots	3.15	10.76	2.01	-0.68	0.59	0.32	-0.22	84.07
	DM_total	3.34	10.72	0.83	-0.77	0.64	0.13	-0.13	85.25
	DM_above	3.34	10.65	0.62	-0.77	0.71	0.13	-0.14	85.46
	LUI	3.47	10.92	0	-0.92	0.43	0	0.02	86.08
Plant local neighborhood community (LNH)	Cover	3.48	11.3	0.07	-0.9	0.06	-0.01	0	86.01
	DCA1	3.52	9.55	-0.07	-0.91	1.8	-0.05	0	86.15
	DCA2	3.52	10.42	-0.01	-0.92	0.93	-0.05	0.01	86.08
	DCA3	3.52	10.86	0.05	-0.92	0.5	-0.05	0.01	86.03
	DCA4	3.48	11.27	0.09	-0.88	0.09	-0.01	-0.03	85.99
	Richness	3.52	10.5	-0.01	-0.82	0.86	-0.05	-0.09	86.09
	Shannon	3.48	10.44	0.02	-0.77	0.92	0	-0.13	86.06

patterns in forb than in grass species, since *P. lanceolata* and *Galium* species differ also from A. millefolium and *R. acris*. This partly contradicts the first hypothesis. At the same time, it supports the second hypothesis, since the differences between growth forms originate from specificity of particular species. Species specificity of root exudates has already been described in earlier studies (Badri and Vivanco, 2009; Monchgesang et al., 2016; van Dam and Bouwmeester, 2016).

The grass species, however, did not differ from each other to a large extent. On the one hand, that might be traced back to the closer phylogenetic relation between the chosen grasses (all are members of the Poaceae family) than those between the investigated forbs. On the other hand, it could also be that the responses of grasses are more similar to each other as they are the dominant life form, and thus, best reflect the ecological selection pressure present in grasslands. The higher similarity among grasses is in accordance with the result of the variance partitioning and published ecological trait studies (Aerts and Chapin, 1999; Craine et al., 2001; Freschet et al., 2010; Herz et al., 2017; Pérez-Harguindeguy et al., 2013; Roumet et al., 2006; Siebenkas et al., 2015; Tjoelker et al., 2005). For instance, Herz et al. (2017) and Siebenkäs et al. (2015) investigated variation in belowground traits between forbs and grasses and found that grasses have a higher plasticity in their traits due to their better adjustment in this habitat. In accordance with this, forbs have to integrate much more into this habitat which fits the explained variance of Plot. The explained variance of grass exudate composition, however, was low for all predictors. This also points to further factors affecting exudate composition than the edaphic and climatic plot conditions included in our study.

The data presented here provide furthermore evidences that plant functional traits are linked to exudation of these plants, which confirms hypothesis iv. Biomass related traits, such as dry mass of roots and leaves or carbon concentration, contribute to explained variance of exudate profiles of both growth forms, grasses and forbs. This is in accordance with the result of Aulakh et al. (2001) who observed that plant biomass alter the exudate pattern of rice plants. In future, investigation of the effects of the single functional traits on the composition of exudates and the occurrence of single exuded compounds could help to better understand their role in the process of exudation.

Interestingly, there was no evidence that the exudate composition of the phytometers are affected by the plant local neighbourhood community, which contradicts our hypothesis iii and the literature (Biedrzycki et al., 2010; Cheng et al., 2007; Jandova et al., 2015; Vogt, 2010). One reason for this lack of neighbourhood effect might be the short exposure time of the phytometers to the new habitat. The findings of Ravenek et al. (2014) point to such a possibility, since they observed changes in morphological belowground traits only after

four years in their analysis of long-term influences of biodiversity effects on belowground biomass. However, this findings were determined for root functional traits and not for exudates. Thus, the correlation between exudates and exposure time to a certain environment has to be investigated in follow up experiments with a longer field growing period.

It is also of great interest, that there was no observed impact of LUI to the exudate pattern of the phytometers. This also contradicts the hypothesis iii, and stands in contrast to other studies presenting an interaction between LUI (Blüthgen et al., 2012) and e.g. soil biota (Blüthgen et al., 2012) or plant traits (Herz et al., 2017) in the Biodiversity Exploratory (Fischer et al., 2010). The LUI represents a broad combination of fertilization, mowing, and livestock grazing at each site in one parameter. Unfortunately, it accounts only for an annual average of each parameter, whereas measurements for parameters at the exact sampling time, such events as trampling and number of fertilization per plant are missing. This limitation could account for the lack of measurable influence.

Overall, the total amount of explained variation was unexpectedly low. This points to further factors influencing the exudation of plant roots. For instance, it could be possible that the semi-polar exudates detected in this study are more responsible for communication and defence (Badri et al., 2009; Strehmel et al., 2014). This is supported by the classification results and published functions of semi-polar exuded metabolites. Phenylpropanoids such as coumarins are involved in the regulation of oxidative stress and hormonal regulation (Treutter, 2006). Flavonoids protect plants against phytopathogens (Bourgaud et al., 2006; Treutter, 2006), and activate nodulation genes in various rhizobia species (Long, 1989). They have also been described as chemoattractants for Rhizobium leguminosarum biovar meliloti (Caetano-Anolles et al., 1988). Hydroxycinnamic acids act as phytoalexins in soil and inhibit the growth of other plants, as described for the solanaceous crops, eggplant (*Solanum melongena*) and tomato (*Lycopersicum esculentum*) (Yoshihara et al., 1978). Terpenes and terpenoids are involved in the plant defence against herbivores. Rasmann et al. (2005) identified (E)-*β*-caryophyllene exuded by maize roots into the soil as a 'herbivore-induced underground signal that strongly attracts entomopathogenic nematodes'. Also iridoid glycosides and the two jasmonate related metabolites, which both were detected exclusively in *P. lanceolata* exudates in this study, function as signals in plant herbivore interactions (Rosenthal and Berenbaum, 2012, Schweiger et al., 2014). Furthermore, terpenoids act as phytoalexins against fungi and bacteria but are also used as energy source by bacteria (Langenheim, 1994). So far, it is unclear which role these metabolites fulfil in the field belowground network of the chosen phytometer. So, it would be of great interest to further investigate the identity of these

metabolites by structure elucidating methods and their function by bioassays. An implementation of information of other abiotic, e.g. soil and climate characteristics, and biotic factors, for instance microbial community, could further help to define their role in the belowground network in future experiments. However, the classification method of semi-polar exuded compounds presented here is a substantial progress in untargeted profiling of metabolites and a good base for the investigation of the unknown compounds of such exudates.

The results on semi-polar exudates contradict in some part those of Herz et al. (2018) on polar metabolites. Whereas LNH and LUI were of minor importance in both cases, the observed higher dependence of root exudation of polar metabolites to environmental impacts irrespective of the growth form (Herz et al., 2018) could not be confirmed in case of semi-polar metabolites. This suggests that the different functions of these two types of metabolites depend differently on the environment. Although both kinds of metabolites are involved in the acquisition of nutrients and the attraction of beneficial interaction partners, semi-polar metabolites are also released for the defence against harmful microorganisms, herbivores and competing plants (Badri et al., 2009; Biedrzycki et al., 2010; Jandova et al., 2015; van Dam, 2009). Since this mechanisms evolved over time and in a species–specific manner, the semi-polar metabolite patterns are more diverse than those of polar metabolites (Dixon, 2001).

In conclusion, the results presented here provide information on the root exudate composition of plants exposed to a complex environment. Thereby, an unknown diversity and specificity of semi-polar metabolites was demonstrated across these so far not inspected species. A novel method for classification of unknown compounds was employed, which otherwise would not have been classified. With this, the study provides a deeper insight in the exudation of forbs and grasses in a natural grassland community and demonstrates the feasibility of investigating semi-polar metabolites in field studies.

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Chapter 2.4.: Root exudate composition of grass and forb species in natural grasslands

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Author Contributions

HB, UJ and DS designed the field experiment. KH, SD and UJ conducted the field experiment and collected the target plants. Trait and soil parameter analysis were performed by KH. Exudate extraction and analyses where performed by SD. Annotation and identification where performed by SD with input from KG. Statistical analysis were performed by SD with input from HB and KG. The manuscript was written by SD with input from all co-authors.

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Abstract

Plants exude a diverse cocktail of metabolites into the soil as response to exogenous and endogenous impacts. So far, root exudates have only been studied under artificial conditions due to methodological difficulties. In this study, each five perennial grass and forb species were investigated for polar and semi-polar metabolites in exudates under field conditions. Metabolite collection and untargeted profiling approaches combined with a novel classification method allowed the designation of 182 metabolites. The composition of exuded polar metabolites depended mainly on the local environment, especially soil conditions, whereas the pattern of semi-polar metabolites was primarily affected by the species identity. The profiles of both polar and semi-polar metabolite differed between growth forms, with grass species being generally more similar to each other and more responsive to the abiotic environment than forb species. This study demonstrated the feasibility of investigating exudates under field condition and their compositional changes by impacting factors.

Introduction

Plants adjust their phenotype in response to environmental conditions and interactions with other organisms (Badri et al., 2009b, Chaparro et al., 2013, Jandova et al., 2015, van Dam and Bouwmeester, 2016). So far, studies on phenotypic adjustment have mainly focused on morphological and physiological characteristics. One result was that the species' phenotypic plasticity differs between forbs or grasses, which are the dominating growth forms of grasslands. These two growth forms vary with respect to plant functional traits (Herz et al., 2017a, Herz et al., 2017b, Ravenek et al., 2014, Ravenek et al., 2016, Siebenkas et al., 2015). Grasses are thereby the dominant organisms in grasslands, due to their tolerance to mowing and grazing, fast spread and responses to environmental changes (Herz et al., 2017b, Siebenkas et al., 2015). Forbs instead, are less affected by changes in nutrient conditions than grasses due to their ability to store nutrients in their roots (Herz et al., 2017b). However, it is unknown so far whether these two growth forms also differ with respect to another fundamental aspect of the plant's phenotype: the metabolites exuded by plant roots.

These exudates include organic compounds of low molecular weight (Faure et al., 2008) which belong either to the primary metabolism, which contain polar compounds, e.g. alcohols, aldehydes, amino acids, lipids, nucleic bases and nucleotides, organic acids and carbohydrates, or the secondary metabolism, which contain semi-polar compounds, such

as alkaloids, phenylpropanoids and terpenes (Badri and Vivanco, 2009, Bais et al., 2004, Bais et al., 2006b, Strehmel et al., 2014, van Dam and Bouwmeester, 2016).

Various experiments revealed that the composition of the exuded metabolites into the rhizosphere (Badri et al., 2009b, Jones et al., 2004, Long, 1989, Rosenthal and Berenbaum, 2012, van Dam, 2009) depends on different endogenous and exogenous factors. Endogenous factors such as growth form (Herz et al., 2018), species (Aulakh et al., 2001, Chaparro et al., 2013, van Dam and Bouwmeester, 2016), and the plant functional traits (Aulakh et al., 2001, Dietz et al., 2019, Herz et al., 2018) turned out to alter the exudate composition in the rhizosphere. In addition, exogenous biotic factors e.g., microbial rhizosphere community (Jones et al., 2004), herbivores (van Dam, 2009) and neighbouring plants (Biedrzycki et al., 2010, Jandova et al., 2015, Xu et al., 2012) also define the metabolite profile. For instance, rice plants inhibit the growth of neighbouring lettuce and grass species by releasing lactones (Xu et al., 2012). The noxious weed (*Centaurea maculosa*) secretes the allelochemical (±)-catechin into the surrounding rhizosphere to delay germination of seeds and thus prevents intraspecific sibling competition (Biedrzycki et al., 2010). However, studies investigating the effect of the neighbouring plants on the exudates pattern of target plants in a natural environment are rare so far (Dietz et al., 2019, Eisenhauer et al., 2017, Herz et al., 2018).

Besides responding to biotic factors, root metabolites are also released as a reaction to abiotic environment such as light and temperature, as well as soil pH, moisture, nutrient supply and organic matter (Inderjit, 1996, Inderjit and Malik, 1997, Neumann et al., 2014, Rivoal and Hanson, 1994, Tawaraya et al., 2014, Xia and Robert, 1994, Ziegler et al., 2016). For example, nutrient deficiency often results from a complex production by metal ions or absorption by the soil (Schmid et al., 2014, Ziegler et al., 2016) which can be overcome by the release of organic acids (Haase et al., 2007) or phenolic compounds (Neumann et al., 2014, Ziegler et al., 2016). These chelating substances enhance the acquisition of insoluble nutrients, and thus, interfere with the nutrient cycles (Inderjit, 1996). Furthermore, anthropogenic land use was assumed to impact the exudate profiles of plants (Herz et al., 2018). High amounts of nutrients, such as nitrogen and phosphor, are introduced into the soil by fertilization and grazing. This modifies the soil nutrient status (Alt et al., 2011, Blüthgen et al., 2012, Laliberté and Tylianakis, 2011) and, thus, probably the root exudation. Specific evidences for this are, however, missing.

Most of these findings were obtained under controlled laboratory conditions, some of those mimic the natural ecosystem conditions (Eisenhauer et al., 2017, Liese et al., 2018), with an one- or two-factorial designs (Biedrzycki et al., 2010, Jandova et al., 2015, Jones et al., 2004, Oburger et al., 2014, Petriacq et al., 2017, Xu et al., 2012). Furthermore, due to the tremendous variety of metabolites in the plant kingdom (Peters et al., 2018, Saito and

Matsuda, 2010, van Dam and Bouwmeester, 2016), these studies have mainly focused on specific metabolites or metabolite classes (Eisenhauer et al., 2017, Jandova et al., 2015, Strehmel et al., 2014). As result, a great part of the exuded plant metabolome remains unconsidered. On the other side, most studies under field conditions neglect the role of exudates. Thus, both types of strategies deliberately disregarded important components of the complex natural ecosystems. To fully understand those networks, a comprehensive understanding of the metabolite profile of a plant and the combination of metabolomics and ecological techniques under natural conditions are of great importance (Peters et al., 2018). So far, there are two studies about root exudation of either polar (Herz et al., 2018) or semi-polar root metabolites (Dietz et al., 2019) applying the untargeted metabolite profiling approach to field grown plants. These two studies focus mainly on the impacts of different endogenous factors in a natural ecosystem.

In this study, the effects of different exogenous factors such as climate, soil, neighbouring plants and anthropogenic land use, as well as the endogenous factors species and growth form were investigated for their impact on the composition of both, polar and semi-polar, root exuded metabolites. Therefore, a large field experiment was carried out in which each five typical grass and forb species were transplanted in more than 50 different grassland communities in the three sites (Schorfheide-Chorin, Hainich and Schwäbische Alb) of the German Biodiversity Exploratories. Those differ in various environmental factors e.g. soil, climate and land use (Blüthgen et al., 2012, Fischer et al., 2010, Herold et al., 2014). After being exposed for more than one year to the surrounding environment, the transplants` root exudates were analysed by untargeted metabolite profiling mass spectrometric approaches. Moreover, as the identification of semi-polar metabolites is challenging due to their high chemical diversity in plants (Strehmel et al., 2014), a novel approach of classifying metabolites to chemical classes was tested (Treutler et al., 2016).

The main issues of this paper are:

(1) Which of the factors, growth from (grass or forb), species identity and site, affect the root exudate richness under field condition significantly?

(2) What are the impacts of biotic growth conditions, species identity and neighbouring transplants' on root exudate composition?

Results

Chemical richness and composition of polar (primary) metabolites

The untargeted metabolite profiling of the investigated samples revealed an annotation of 285 compounds (Supplementary Table 1), of which 66 were identified and classified as alcohols (6), aldehydes (1), alkaloids (1), amines (2), amino acids (19), carbohydrates (10),

lipids (4), nucleic bases or nucleotides (3) and organic acids (19). Five compounds were classified as unidentified carbohydrates (4) and unidentified lipid (1), respectively, due to their mass spectra similarity to other compounds of these classes (Table 1, Supplementary Table 1).

Linear mixed-effects models showed that the number of exuded metabolites, mostly of the primary metabolism (chemical richness, Fig. 1), significantly depended on species ($p < 0.05$), site ($p < 0.001$; Schorfheide, Hainich or Swabian Alb) and their interaction ($p < 0.01$; Supplementary Table 2 a). In contrast, the two growth forms grass and forb as a whole did not differ in chemical richness, whereas those of forbs and grasses of the sites differ significantly from each other ($p < 0.001$; Fig. 1A, Supplementary Table 2 a). Chemical richness was thereby highest in the Schorfheide (SCH), in particular for grasses ($p < 0.001$) and the forb *Ranunculus acris* ($p < 0.01$; Fig. 1A, Supplementary Table 2 b). The pattern in chemical richness was also reflected in the multivariate analysis of the metabolites composition, as revealed by a Redundancy Analysis (RDA, Fig. 2A) with species and site as constraining variables. Here, the samples of SCH were separated from those of HAI and ALB on first axis (12.59 % of total variance explained, Fig. 2A), while ALB and HAI differed in their scores on the second axis (2.57 %; Fig. 2A). In contrast to site, differences among species or growth forms played a subordinate role, and were only apparent on the lower axes (Supplementary Fig.1).

The loadings of the exuded compounds (Fig. 2 B) indicated a common set of metabolites in the exudate profiles of the transplants. Those comprised metabolites of the classes: alcohols, amino acids, carbohydrates, lipids, nucleic bases, organic acids, and also unidentified compounds. Some of the class members, however, showed a higher probability to occur in specific sample groups. Grasses exuded the highest number of group discriminating metabolites compared to forbs (Supplementary Table 3 a)). The highest number of those metabolites was thereby observed in plots of the ALB like N-Acetylglucosamine, favourably exuded by *Poa pratensis,* and Succinate, specially exuded by *Lolium perenne,* and a number of unidentified compounds (Supplementary Table 3).

Discriminating exudates of grass plants grown in SCH or HAI plots showed no species-specificity, but the number of those metabolites was higher in SCH than in HAI. Instead, forb exudate profiles revealed the highest number of discriminating metabolites in plots of the HAI. Most of those could be related to a specific species, e.g. 3-Caffeoyl-trans quinic acid preferentially exuded by *Galium mollugo*, and a lot of unidentified compounds (Supplementary Table 3). Forbs grown on SCH and ALB plots exuded nearly the same number of discriminating metabolites. In all sample groups, a lot of metabolites were preferentially exuded by a specific growth form in a specific site, but not by a specific

Table 1: Overview of the polar metabolites. The table shows the number of detections of a compounds of each class per site and species as well as site and growth form. The numbers in brackets represent the number of metabolite identities in each chemical class. u_ = unidentified

		alkaloid (1)	alcohol (6)	aldehyde (1)	amine (2)	amino acid (19)	carbohydrates (10)	lipid (4)	nuclic base/ nucleotide (3)	organic acid (18)	phenylpropanoid (1)	u_carbohydrates (4)	u_lipid (1)	u_compounds (215)
forb	ALB	0	139	0	1	266	160	7	7	219	0	129	19	1260
	HAI	0	181	2	0	369	219	13	15	314	1	118	21	2184
	SCH	0	80	0	0	189	82	23	15	140	0	61	16	1242
grass	ALB	1	140	2	1	371	153	17	22	232	0	123	28	1105
	HAI	0	114	0	1	343	127	9	24	206	0	84	20	1161
	SCH	0	104	0	1	385	116	42	37	235	0	81	26	2004
A. millefolium	ALB	0	32	0	0	61	34	2	3	42	0	22	4	227
	HAI	0	35	0	0	81	40	4	5	58	0	26	5	419
	SCH	0	10	0	0	22	11	3	2	16	0	9	2	155
G. mollugo	ALB	0	32	0	0	67	38	1	4	53	0	24	3	298
	HAI	0	38	1	0	99	50	2	3	75	1	26	3	504
	SCH	0	25	0	0	64	27	8	6	43	0	17	6	402
G. verum	ALB	0	29	0	0	57	37	2	0	47	0	32	7	246
	HAI	0	36	0	0	85	53	1	1	70	0	23	4	436
	SCH	0	18	0	0	44	21	5	6	34	0	9	4	257
P. lanceolata	ALB	0	25	0	1	44	31	1	0	48	0	35	2	324
	HAI	0	31	1	0	29	33	4	0	48	0	24	5	421
	SCH	0	17	0	0	42	17	6	1	31	0	20	3	282
R. acris	ALB	0	21	0	0	37	20	1	0	29	0	16	3	165
	HAI	0	41	0	0	75	43	2	6	64	0	19	4	404
	SCH	0	10	0	0	17	6	1	0	16	0	6	1	146
A. pratensis	ALB	0	11	1	0	31	12	0	3	18	0	11	1	93
	HAI	0	19	0	0	73	28	3	4	41	0	18	5	238
	SCH	0	13	0	0	46	14	4	4	30	0	11	3	249

		alkaloid (1)	alcohol (6)	aldehyde (1)	amine (2)	amino acid (19)	carbohydrates (10)	lipid (4)	nuclic base/ nucleotide (3)	organic acid (18)	phenylpropanoid (1)	u_carbohydrates (4)	u_lipid (1)	u_compounds (215)
A. elatius	ALB	1	48	0	0	123	48	8	10	81	0	32	9	420
	HAI	0	16	0	0	49	19	1	2	25	0	12	2	148
	SCH	0	18	0	0	75	25	10	8	45	0	13	4	391
D. glomerata	ALB	0	32	1	0	92	38	5	4	57	0	37	10	265
	HAI	0	33	0	0	98	32	1	9	57	0	22	6	304
	SCH	0	28	0	1	117	33	13	13	70	0	23	9	601
L. perenne	ALB	0	27	0	0	60	29	2	2	41	0	23	5	158
	HAI	0	23	0	1	67	24	3	6	44	0	14	4	250
	SCH	0	26	0	0	88	26	8	7	54	0	19	5	464
P pratensis	ALB	0	22	0	1	65	26	2	3	35	0	20	3	169
	HAI	0	23	0	0	56	24	1	3	39	0	18	3	221
	SCH	0	19	0	0	59	18	7	5	36	0	15	5	299

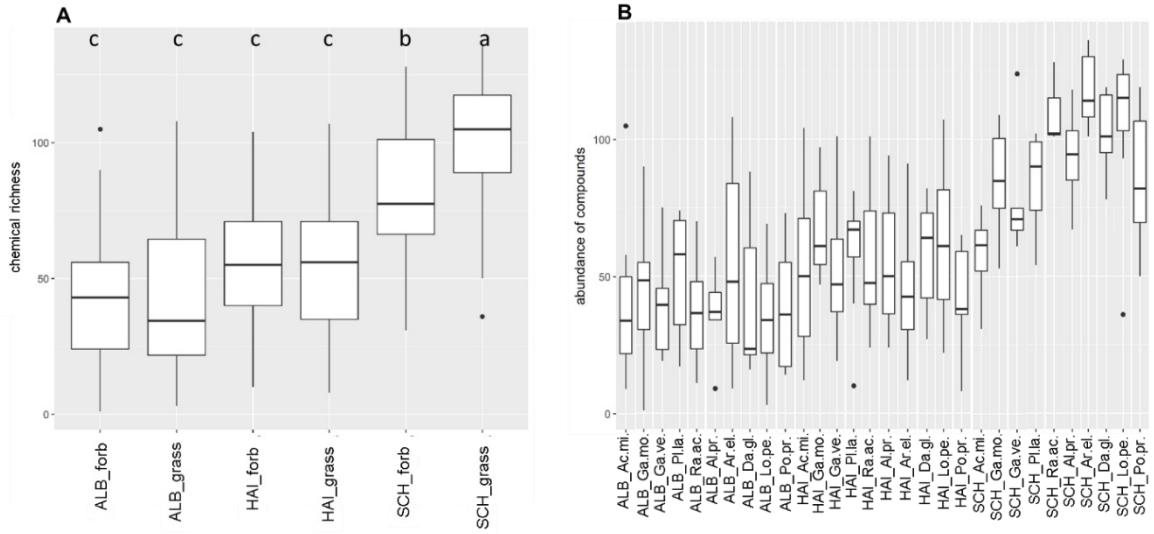

Fig. 1: Chemical richness of polar metabolites. Boxplots consists of the median chemical richness (line in the box), the distribution (box) and the upper and lower quantile (lines) of the chemical richness of polar metabolites of each samples within the sample group **A** site-growth form and **B** site-species. Points above and below the box represent samples having a much higher or lower chemical richness than all other samples. A scheffé post hoc test was performed to reveal the significant difference between the sample groups. Sample groups with the same letter are not significantly different.

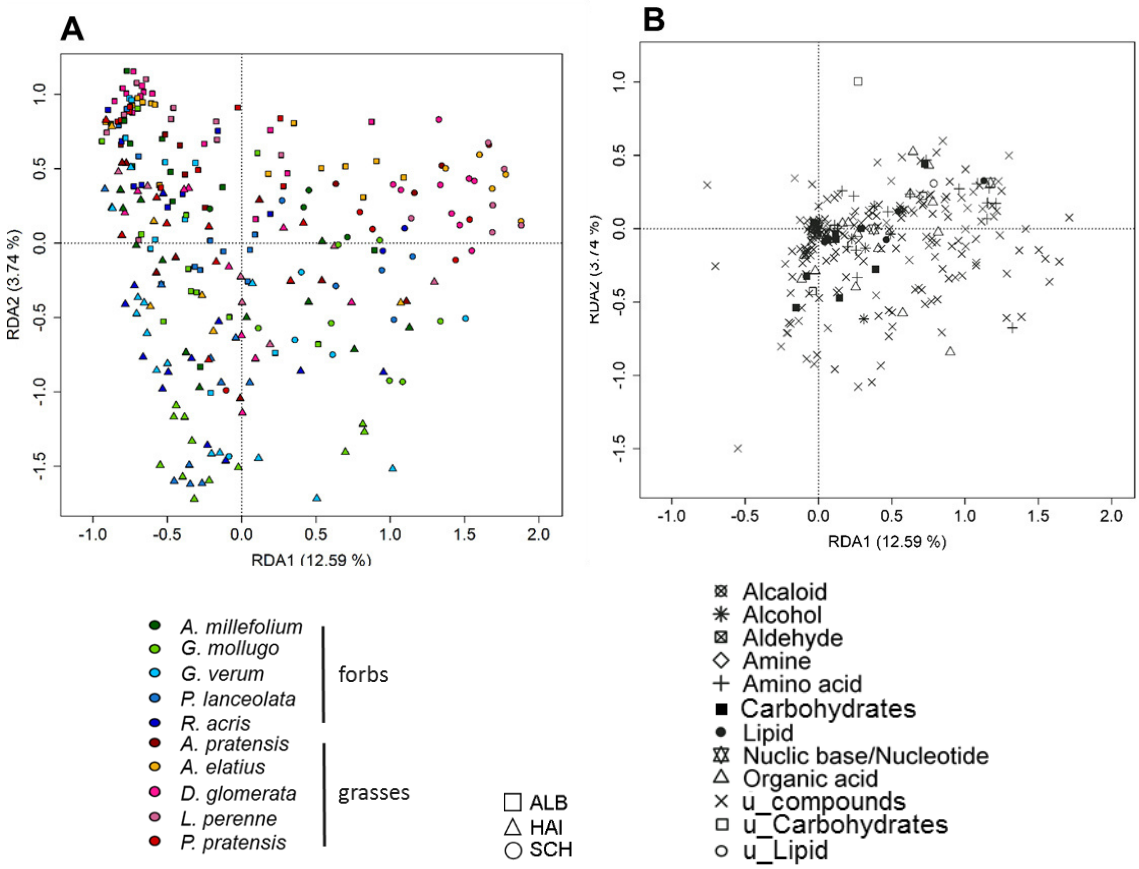

Fig. 2: Redundancy analysis of polar metabolites. RDA was performed with 257 samples plotted against a presence/absence matrix of species per site. Plot **A** presents axis 1 against axis 2. The ten species are represented by colour, whereas the points are grouped by site (see legend). **B** represents the loadings of the exuded compounds of the corresponding RDA levels. The points represent the different chemical classes (see legend).

species. Simultaneously, compounds were preferentially exuded by plants of a specific growth form or species without an influence of the site factor. For instance, Octadecatrienoic acid is exuded by *Plantago lanceolata* of all plots, or 2-Aminoadipate, which is preferentially exuded by *A. elatius* plants in all plots (Supplementary Table 3).

Polar metabolite composition and exogenous factors

The importance of the place plants resident (e.g. the site) compared to species in explaining exuded primary metabolite composition is also reflected in the variance partitioning analysis both for forbs and grasses (Fig. 3). Here, plot explained most of the variance (forbs: 23.8 %, grasses: 24.4 %; Fig. 3A, B). While in forbs (Fig. 3A) the second most important factor was species (8.8 %), in grasses it was the interaction with the local neighbouring plants (LNH) and the plot (7.0 %) together. The effect of LNH on polar metabolite composition was mainly brought about by covered area of the neighbouring

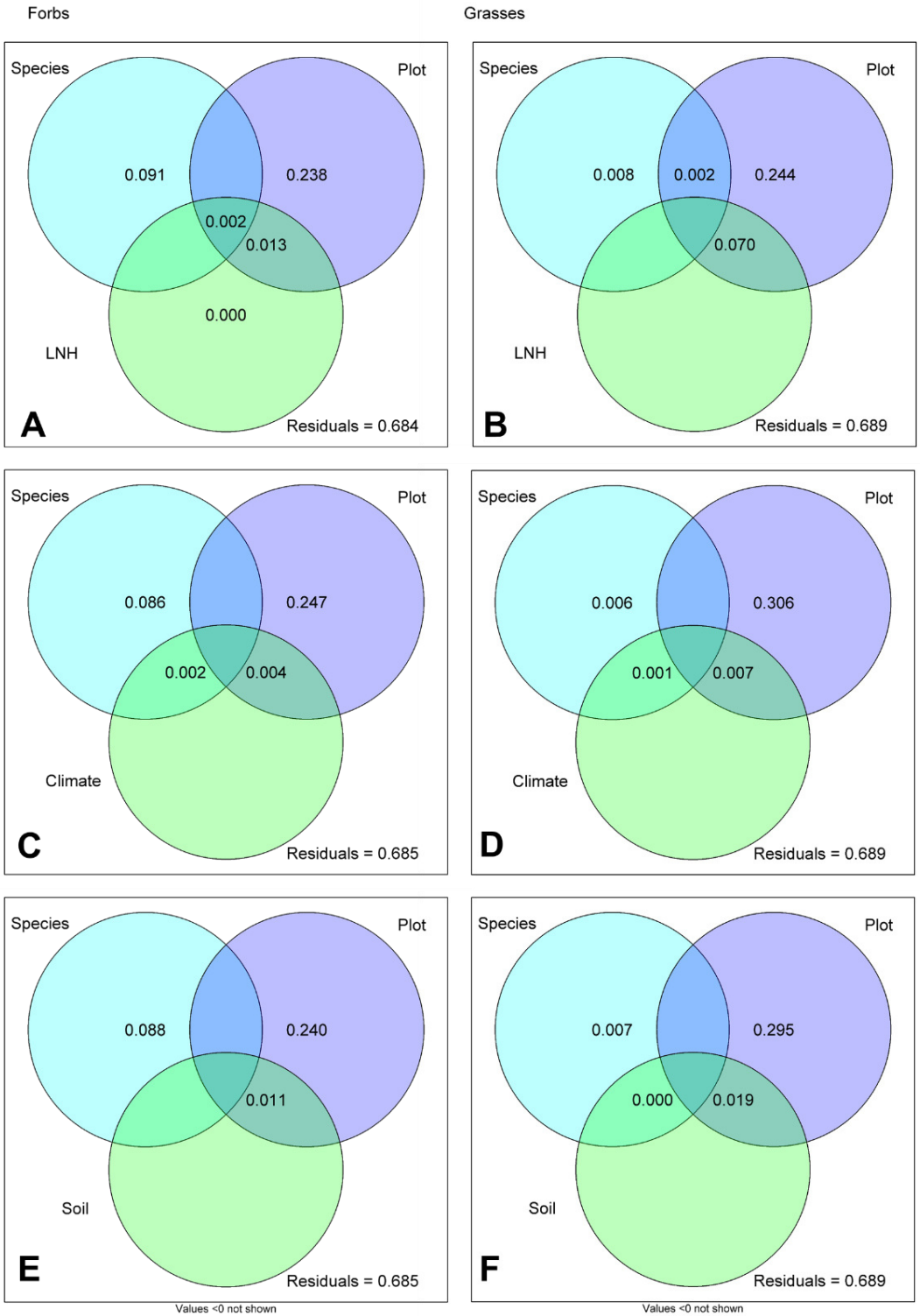

Fig. 3: Variance partitioning of polar metabolite composition. Venn diagrams present the proportion of variance in metabolite pattern of forbs (left) and grasses (right) explained by different predictors: Species = species identity of the target plant, Plot = local impact, LNH (**A, B**) = plant local neighbourhood in a radius of 25 cm around the target plan, Climate (**C, D**) = cumulated variables for temperature and precipitation, Soil (**E, F**) = cumulated variables describing the soil of the location where the target plant was planted.

plants (Cover, Supplementary Table 4). When LNH was replaced by environmental factors, either by climate and soil factors (Env, Supplementary Fig. 2, Supplementary Table 4), or by climate (Climate) and soil (Soil) separately (Fig. 3 C-F), the previously described pattern remained essentially the same. Also here, plot explained most of the variation in the exudate metabolite pattern (24.0 % to 24.7 % in case of forbs, 29.5 % to 30.6 % in case of grasses), while neither climate nor soil turned out to be important at all. With minor exception, the same holds true for impacts of the factor land use and the single variables cumulated in the environmental factors (Supplementary Table 4). The highest amount of shared variation between plot and single variables was brought about by the combination of plot and total carbon content of the soil (TC; 2.85 % and 7.38 % in case of forbs and grasses, respectively; Supplementary Table 4).

The correlation of the single metabolites to the different environmental drivers, however, revealed that 65.14 % and 69.72 % of metabolites in forb and grass exudate samples, respectively, responded significantly to the environment (Supplementary Table 5 a), c)). Soil variables showed the highest number of impacted metabolites followed by LNH and climate, whereas the lowest number of metabolites is linked to LUI effects in forbs as well as in grasses (Supplementary Fig. 3, Supplementary Table 5 a), c)). For instance, 101 of the polar metabolites exuded each by forbs and grasses, were significantly affected by soil moisture (moisture), whereas the number (Richness) and the diversity (Shannon) of species neighbouring the exuded plant and the annual temperature in 2 m height above the plant (T(200)) affected the exudation of round about 80 metabolites (Supplementary Fig. 3, Supplementary Table 5 a). In accordance with the results of the variance partitioning and in case of all LUI variables, grazing impacted most strongly the primary metabolites.

Semi-polar (secondary) metabolites occur in a species-dependent manner in exudates

Untargeted metabolite profiling by LC-MS revealed 2,947 features as putative compounds of the secondary metabolism. The chemical richness of these compounds was independent of the growth form ($p = 0.630$), but significantly depended on site and species ($p < 0.001$, Supplementary Table 2). This was mainly driven by the grass species displaying a higher chemical richness in SCH plots than in all other exploratory plots (Figure 4B, Supplementary Table 2b)). Moreover exudates from grasses and forbs grown on SCH plots showed a significantly higher chemical richness than in the other Exploratories (Fig. 4A, Supplementary Table 2b). In case of forbs, this was mainly driven

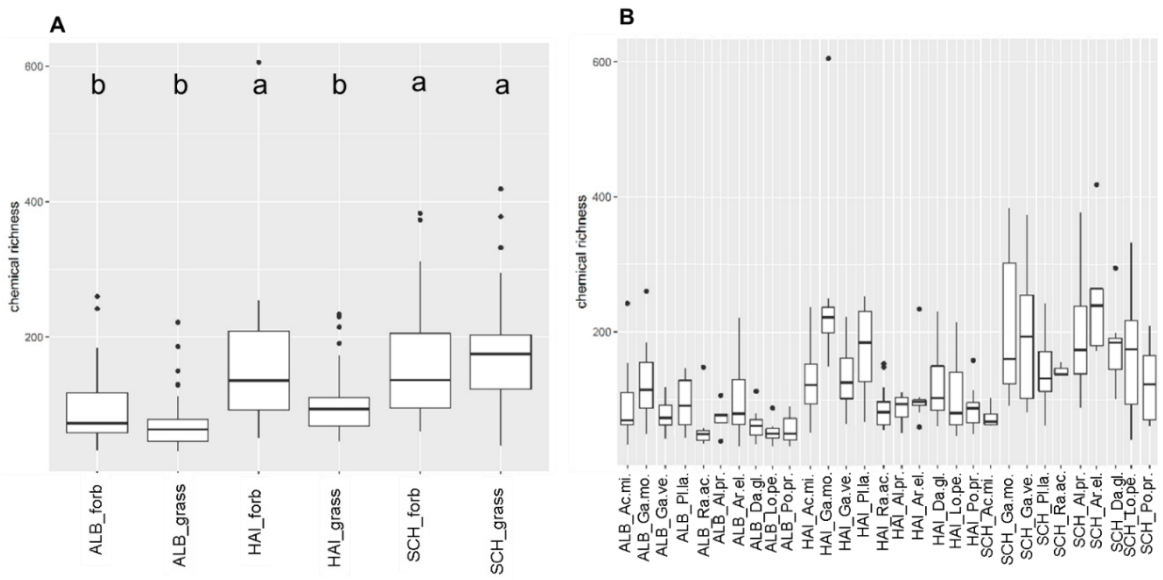

Fig. 4: Chemical richness of semi-polar metabolites. Boxplots consists of the median chemical richness (line in the box), the distribution (box) and the upper and lower quantile (lines) of the chemical richness of semi-polar metabolites of each samples within the sample group **A** site-growth form and **B** site-species. Points above and below the box represent samples having a much higher or lower chemical richness than all other samples. A scheffé post hoc test was performed to reveal the significant difference between the sample groups. Sample groups with the same letter are not significant different.

by the *Galium* species. The composition of semi-polar compounds in the RDA partly reflected these results (Fig. 5). Although a discrimination of the plant samples by site was not observed, a species-specific pattern occurred (Fig. 5A). While the two *Galium* species were separated from the other species on axis one (8.72 %), *P. lanceolata* was separated from the other species on axis three (2.42 %, Supplementary Fig. 4). Furthermore, *A. millefolium* samples were discriminated from the other species on axis four (1.71 %, Supplementary Fig. 4) whereas the separation of *R. acris* samples occurred on axis six (1.05 %, Supplementary Fig. 4). Axis five (1.44 %, Supplementary Fig. 4) was the only dimension in which *A. elatius*, was separated from all other species, while all other grass species always clustered together. Moreover, the loadings of the exuded semi-polar metabolites (Fig. 5B) indicated a common set of exuded metabolites of all species, but also a higher degree of metabolite diversity in exudate patterns of forbs than of grasses. This is reflected by the calculation of the significant specific compounds per species or the Genus *Galium* (*Galium* spp.) together, respectively. 229 of these significant species-specific compounds were thereby observed in metabolite profiles of forbs (*A. millefolium*: 40, *G. mollugo*: 69, *G. verum*: 2, *P. lanceolata*: 89, *R. acris*: 29), whereas 47 were observed in grass profiles. Further 76 significant specific compounds were observed

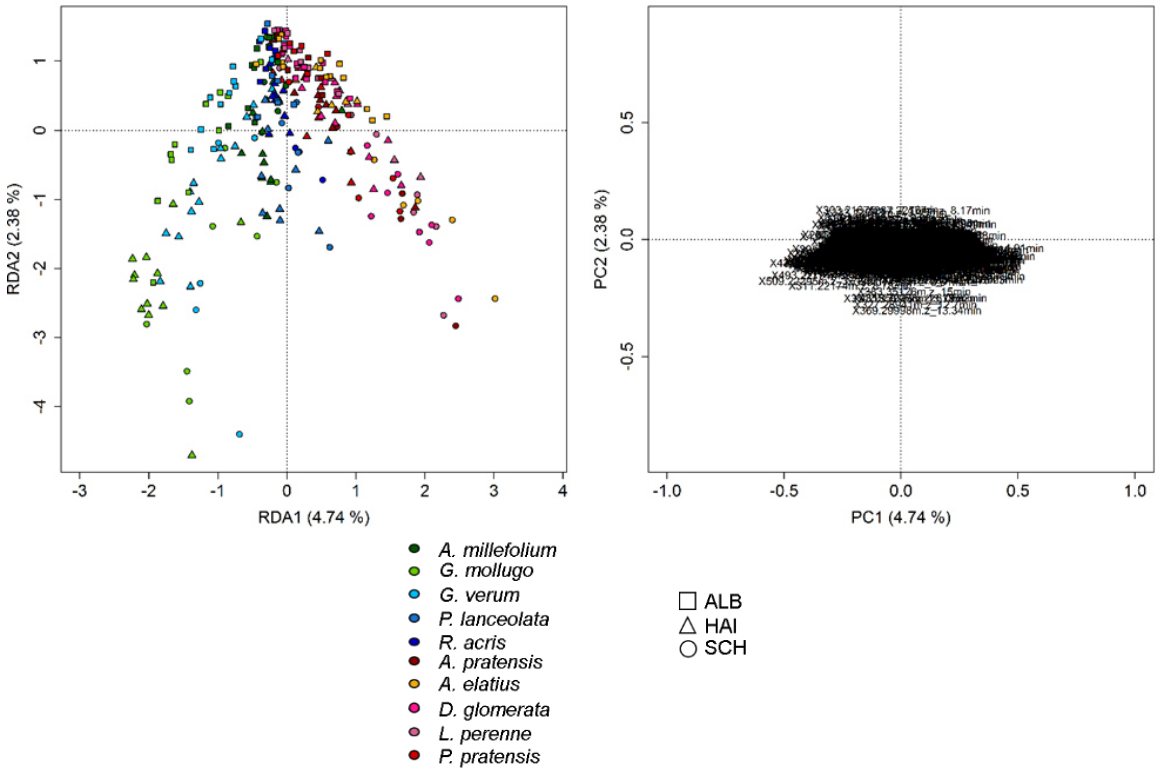

Fig. 5: Redundancy analysis of semi-polar metabolites. RDA was performed with 257 samples plotted against a presence/absence matrix of species per site. **A** The ten species (p = 0.001) are represented by colour, whereas the points are grouped by site (see legend). The plot **B** represents the loadings of semi-polar compounds.

in *Galium* spp. samples.

The identity of species-specific exuded semi-polar metabolites

The tandem mass spectrometry allowed the gain of fragment mass spectra of 217 of the 352 significant species-specific compounds (Supplementary Table 6). The chemical classification of those assigned 116 compounds which were grouped into seven chemical classes with different subclasses (Table 2): glycosides (20) with different residues (acid (2), sulfate (2), hydroxycarbonic acid (1)), jasmonate derivatives (2), phenylpropanoids such a coumarin derivative (1), flavonoids (15) (glycosylated (3), kaempferol derivatives (3)), hydroxycinnamic acids (39) (glycosylated (15), not glycosylated (21) amide residues (3)), polyketides (5), terpenes (12) and compounds which could not be assigned to one of these semi-polar metabolite families but carried different chemical residues (aliphatic (7), imine residues (2), methoxy-groups (3), sulfates or phosphate groups (10)).

Hierarchical clustering of the compounds according to their mass spectral fragment similarities resulted in a dendrogram with nine main branches (Fig. 6). Seven of those

corresponded to the substance classes listed above, whereas two branches contained members of all chemical families and unclassified compounds (Fig. 6, Supplementary Fig. 5-12). Furthermore, the spectra of species-specific compounds showed a clustering due to species identity. Sulfated or phosphorylated compounds clustered in branch one together. They were predominantly exuded by *P. lanceolata* (Fig. 6, Supplementary Fig. 5). The majority of glycosylated compounds and glycosides clustered in main branch two and were exuded by *A. millefolium*, *G. mollugo*, *G. verum*, *P. lanceolata*, *A. elatius*, *A. pratensis*, and *R. acris* plants, (Fig. 6, Supplementary Fig. 6). The annotated polyketides and some potential flavonoids released by *G. mollugo*, *G. verum*, *Galium* spp and *A. millefolium* roots clustered in branch four (Fig. 6, Supplementary Fig. 8). There were two branches containing structures that resembled terpenes (Fig. 6, Supplementary Fig. 9 and 11), mainly exuded by *Galium* spp (branch 5) or *A. elatius* (branch 8). The latter also contained one of the two annotated jasmonate derivatives. Compounds of phenylpropanoid-, flavonoid- and hydroxycinnamic acid-like structures were predominantly clustered in branches six and seven, whereas branch six contained fragment spectra of *Galium* spp., *P. lanceolata* and *A. pratensis* specific compounds and branch seven fragment spectra of *R. acris* and *P. lanceolata* specific compounds (Fig. 6, Supplementary Fig.10). Branches three and eight instead contained compounds of either different classes of chemically unrelated or unclassified compounds (Fig. 6, Supplementary Fig. 7 and 12). These branches are heterogeneous in the species origin.

Two compounds (931.2829m/z at 3.67 min, 501.1253m/z at 4.07min) were exclusively exuded by *P. lanceolata* roots and might represent irido glycosides (Table 2).

Semi-polar metabolite exudation of forbs and grasses is differentially environmental affected

The results of variance partitioning of the semi-polar metabolites strongly differed between the two growth forms. In sum, the predictors explained less of the variation in semi polar exudate profiles of grasses than those of forbs (up to 15.9 % and up to 24.9 % for grasses and forbs, respectively, Fig. 7). Moreover, while for grasses the largest proportion of variance was explained by plot, in forbs most of the variation was accounted by species identity (Fig. 7 A, C, E, Supplementary Fig. 2 C). The predictors LNH, Climate, Soil and Env did not have any explanatory power at all, whereas single environmental variables could explain the variability in semi-polar metabolite profiles to a minor extent (Supplementary Table 8). The inclusion of LUI as predictor resulted in a minor amount of explained variance (0.34 and 0.96 % for forbs and grasses, respectively). This is caused by the effect of fertilization and grazing on the exudation of grasses and forbs (Supple-

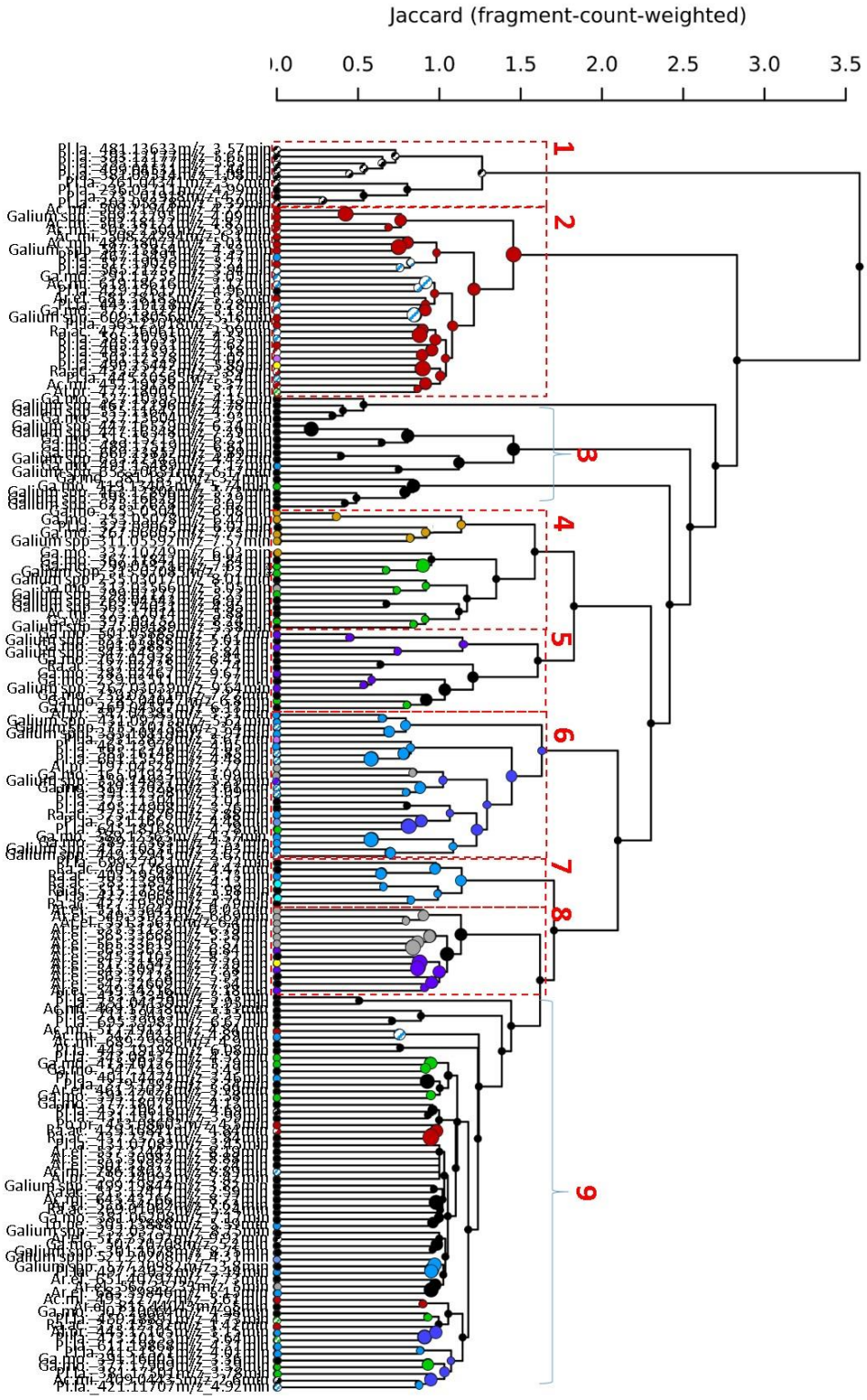

Fig. 6: Hierarchical clustering of species-specific semi-polar compounds. Hierarchical clustering was performed on the tandem-mass spectra (CID and DDA) of the significant species-specific compounds. Spectra cluster are based on fragment similarity rested on Jaccard dissimilarity and fragment-count-weighted value rating. The numbers represent the clusters being shown in supplement Supplementary Fig. 5-12 for more details. The classification of metabolites is given in the legend.

Table 2: Putative classification of species-specific semi-polar compounds. The table contains the total number of compounds (in brackets) of each class as well as the occurrences in the samples of the ten different species. Numbers in brackets behind the species represent the total amount of specific compounds per species.

		Classes	*A. millefolium* (22)	*G. mollugo* (40)	*G. verum* (5)	*Galium spp.* (37)	*P. lanceolata* (59)	*R. acris* (19)	*A. pratensis* (6)	*A. elatius* (25)	*D. glomerata* (0)	*L. perenne* (1)	*P. pratensis* (1)
Glycosides (19)		Glycoside, acidified (2)						2					
		Glycoside, sulfated/phosphorylated (2)	1				1						
		o. Glycoside[1] (14)	5			3	3	1	1				1
Phenylpropanoid (46)		Phenylpropanoid, Coumarin derivative[1] (1)	1										
	Flavonoids (15)	Flavonoid, glycosylated (3)		1			2						
		Flavonoid, Kaempferol derivative[2] (3)		2		1							
		o. Flavonoid[1] (9)		4	1	1	3						
	Hydroxy-cinnamic acid (40)	Hydroxycinnamic acid amid[1] (3)					2	1					
		Hydroxycinnamic acid, glycosylated (16)	2	2		2	9	1					
		Hydroxycinnamic acid[1] (21)	1	4		4	6	2	2	1		1	
Jasmonate (2)		Jasmonate derivative, glycosylated (1)					1						
		Jasmonate derivative[1] (1)								1			
Polyketide (5)		Polyketide[1] (5)		4		1							

1 Chemical classes contain compounds being classified on the base of one identifier fragment.

2 The annotation of compounds as a kaempferol derivative bases on identifier fragments of kaempferol and spectral similarity. This has to be confirmed by analytical standards.

	Classes	*A. millefolium* (22)	*G. mollugo* (40)	*G. verum* (5)	*Galium spp.* (37)	*P. lanceolata* (59)	*R. acris* (19)	*A. pratensis* (6)	*A. elatius* (25)	*D. glomerata* (0)	*L. perenne* (1)	*P. pratensis* (1)
unclassified (122)	Unclassified, aliphatic acid[1] (7)								7			
	Unclassified, imin (1)		1									
	Unclassified, imin, aliphatic acid (1)				1							
	Unclassified, methoxylated (2)		1					1				
	Unclassified, sulfate/phosphate residue[1] (9)	1				8						
	Unclassified, sulfate/phosphate residue, aliphatic acid (1)								1			
	o. Unclassified compounds (101)	11	19		23	22	12	2	12			

[1] Chemical classes contain compounds being classified on the base of one identifier fragment.

[2] The annotation of compounds as a kaempferol derivative bases on identifier fragments of kaempferol and spectral similarity. This has to be confirmed by analytical standards.

mentary Table 8).

The correlation of semi-polar metabolites with single environmental variables revealed a strong environmental impact on the exudation of many secondary metabolites (Supplementary Table 5 b), c)). 21.90 % and 17.49 % of compounds detected in forb and grass exudate samples, respectively, could be linked to one of the environmental variables (Supplementary Fig. 13). Soil variables such as moisture and the soil texture but also the climate variable precipitation and T(200) were most frequently significantly correlated to semi-polar compounds (Supplementary Fig. 13, Supplementary Table 5 b), c)). In general, LUI and LNH variables had a similar effect on the metabolite exudation. In particular, mowing is the variable of LUI with the highest number of affected compounds with 89 (forbs) and 80 (grasses) significantly correlated compounds, whereas Cover was involved in the exudation of 106 compounds and Shannon in 101 compounds in forbs and grasses, respectively (Supplementary Fig. 13, Supplementary Table 5 b), c)). Interestingly, there are species-specific compounds among these correlated compounds (Supplementary Table 5b)). For instance, LUI traits could be linked to compounds of the phenylpropanoid metabolism and glycosides of various species. Furthermore, compounds of hydroxycinnamic acid like character exuded by *A. millefolium* (619.1862m/z_3.12min), *G.*

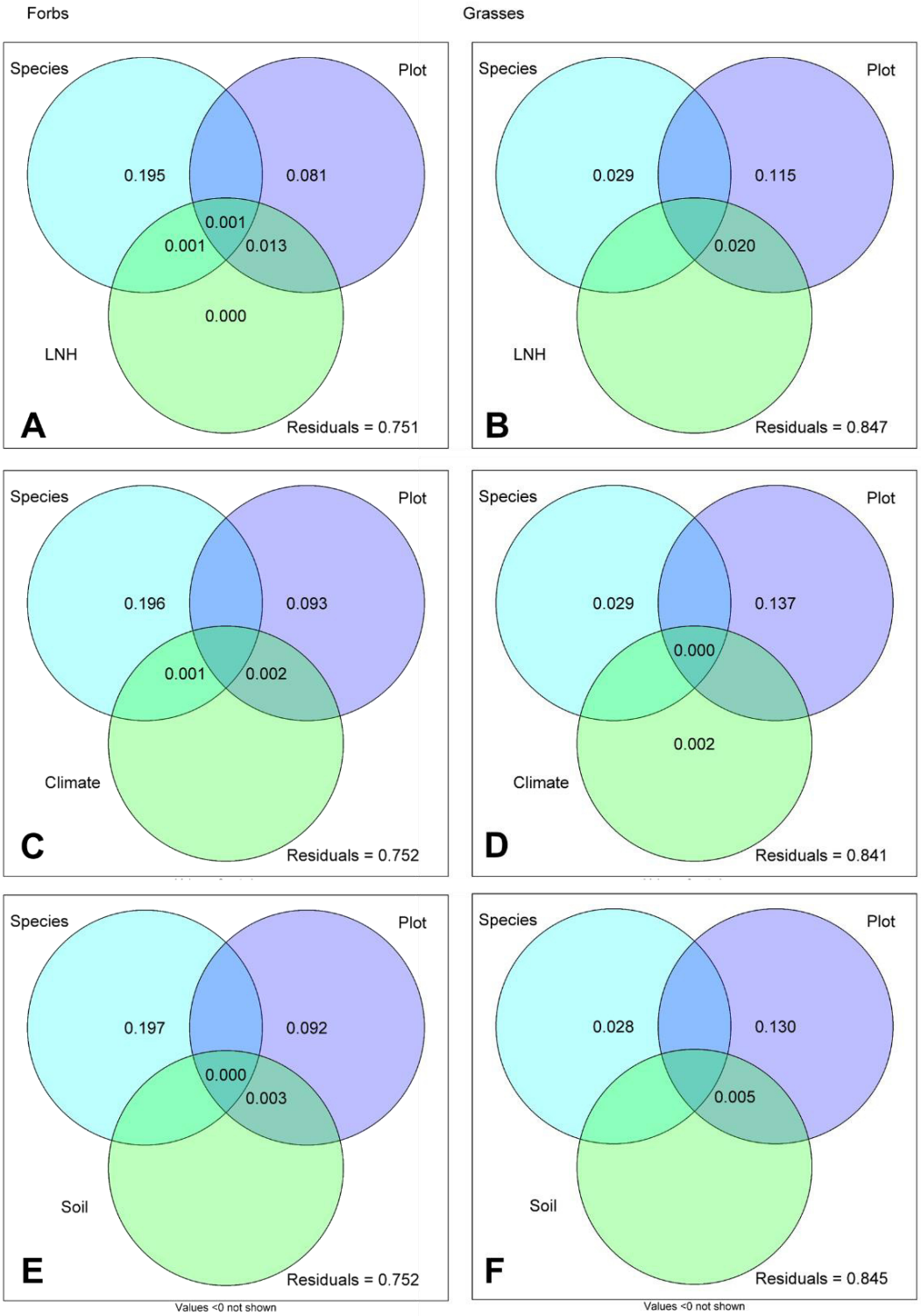

Fig. 7: Variance partitioning of semi-polar metabolite composition. Venn diagrams present the proportion of variance in metabolite pattern of forbs (left) and grasses (right) explained by different predictors: Species = species identity of the target plant, Plot = local impact, LNH **(A, B)** = plant local neighbourhood community in a radius of 25 cm around the target plant, climate **(C, D)** = combined characteristics describing the temperature and precipitation, soil **(E, F)** = combined characteristics describing the soil of the location where the target plant was growing.

mollugo (381.0621m/z_7.17min) *and P. lanceolata* (445.2057m/z_3.4min) were significantly correlated to climate variables, whereas flavonoids exuded by *G. mollugo* (507.2066m/z_4.98min)*, G. verum* (389.1117m/z_3.09min) and *P. lanceolata* (645.1817m/z_4.78min) or potential terpenes released by the *Galium spp.* (267.0304m/z_9.64min) and *A. elatius* (549.3422m/z_7.18min) were significantly correlated with soil variables.

Discussion

Root exudation is a complex process in which a diverse chemical cocktail of substances is released into the rhizosphere. Most studies focus on the investigation of either single substances or specific chemical families (Aulakh et al., 2001, Neumann et al., 2014, Oburger et al., 2014, Petriacq et al., 2017, Strehmel et al., 2014, Ziegler et al., 2016) and with this they neglect the complexity of exudate profiles. The untargeted metabolite profiling approach presented here allowed not only the detection of 3,185 compounds but also the classification of 182 substances into various chemical families and represents a highly comprehensive metabolite profile of plants being of minor interest, so far. The semi-polar metabolites that have been designated by chemical classification were grouped according to spectral and fragment similarities (Treutler et al., 2016). A traditional identification and chemical categorisation of such a metabolite set is time consuming and challenging due to the lack of appropriate analytical standards and the large gap in the knowledge of the majority of metabolites (Peters et al., 2018, Strehmel et al., 2014, van Dam and Bouwmeester, 2016). Traditionally, this is the bottle neck in almost all untargeted metabolomics investigations (Peters et al., 2018, Petriacq et al., 2017, Strehmel et al., 2014, Treutler et al., 2016). A clustering by fragment similarity and classification by shared fragments could help to overcome this obstacle. Thus, this method provides a basis for the further elucidation of such metabolites and their characterization.

The overall composition of the metabolite profiles of the investigated transplants showed a quite common set of compounds in all ten species, but also differences due to various impacting factors. Moreover, it played a role if the metabolites were referred to the polar (primary) or semi-polar (secondary) metabolite profile. The chemical composition of polar metabolites was qualitatively similar between the species and less impacted by growth form (issue 1). Semi polar metabolite compositions, however, showed major differences between forbs and grasses (issue 1). Here, forbs had a higher diversity between the different profiles. This can be linked to the high impact of species identity and the tendency of forbs to exude more species-specific metabolites than grasses. The high importance of this factor on forbs´ exudate composition could be explained by the phylogenetic distance

between the species of both growth forms. It was shown that the genus of a species can impact the diversity in the metabolite profiles (Monchgesang et al., 2016a). Thus, the larger phylogenetic distance between the forbs compared to the *Poaceae* grasses could impact the result. This assumption is consistent with the results of Herz et al.(2018) and Dietz et al. (2019). Both studies investigated the impact of endogenous factors on plant root exudation of the same ten target species. In contrast to Herz et al. (2018), the present study revealed the factor growth form of minor importance for polar metabolites (issue 1). This might be due to the different exposure times in the field. The transplants of Herz et al. (2018) and Dietz et al. (2019) stayed three month in the field, whereas the plants analysed here were exposed to field conditions for more than one year.

Another explanation for the differences in the role of growth form in polar metabolite exudation might be the inclusion of further aspects of the experiment: the site (issue 1). The German Biodiversity Exploratories were set up along environmental gradients (Fischer et al., 2010), in which Schorfheide occupied a special position. This was particularly obvious at the level of soil, nutrient cycles and organismic interactions (Alt et al., 2011, Herold et al., 2014). The Schorfheide-Chorin exhibits a higher soil moisture and lower pH as well as higher nitrogen and carbon content than the Swabian Alb and Hainich-Dün (Alt et al., 2011, Herold et al., 2014). Low pH and high soil moisture trigger the exudation of alcohols, amino acids and organic acids (Rivoal and Hanson, 1994, Xia and Robert, 1994) to overcome the acidification of the plant cells (Herold et al., 2014, Rüdy, 2014) which are caused by anaerobic soil conditions. Previous studies also showed that the release of metabolites containing nitrogen, such as amino acids, contribute to the nitrogen content of the soil (Moe, 2013, Phillips et al., 2004), which in turn trigger the increased release of carbohydrates, organic acids (Haase et al., 2007) and phenylpropanoids e.g. coumarin (Ziegler et al., 2016). Those mediate the uptake of nutrients by enhancing their absorption or interact with decomposing organisms (Hättenschwiler and Vitousek, 2000). This causality might explain the occurrence of some of the amino acids, carbohydrates, organic acids and phenylpropanoids in the exudate profiles of the plants investigated here. The inclusion of site might also be the reason for the divergence in the impact of growth form between this study and Herz et al.(2018), where the site factor was neglected.

The present study revealed furthermore that grasses showed a higher chemical richness in exuded polar metabolites in SCH compared to forbs. This implies that grasses exhibit a higher environmental adjustment than forbs (Herz et al., 2017b, Siebenkas et al., 2015), which is supported by further results of this study. As in case of the findings of Herz et al. (2018) and Dietz et al. (2019), the variance partitioning shows that plot characteristics are

the main drivers for semi-polar metabolite exudation of grasses, but also polar metabolite released of both growth forms (issue 2). In addition, the present study further resolved the impact of the individual environmental traits to shed more light on their influence (issue 2). Single variables of the predictors' soil and climate altered the exudate composition. Soil variables, such as soil moisture and the soil texture had thereby a high relevance for the exudation of polar and semi-polar metabolites in both growth forms. The relation of soil moisture and pH to not only polar but also semi-polar compounds as e.g. hydroxycinnamic acids or terpenes is remarkable and not described so far. Also the relation of climatic drivers like aboveground temperature to polar and semi-polar compounds needs further investigations.

In previous exudate studies (Dietz et al., 2019, Herz et al., 2018) of the ten target species in grasslands, minor impact was found by neighbouring plants and no impact by land use. The results of the present study revealed instead a contribution of single variables of these predictors to the variance in plant polar and semi-polar metabolite exudation. The impact given by LNH underlines the suggestion that longer residential time could play a role in the exudation of plants in a plant community in the field (Dietz et al., 2019, Herz et al., 2018). Therefore, a better adaptation and a stronger interaction with their locally neighbouring plants, as described for other species (Biedrzycki et al., 2010, Cheng et al., 2007, Jandova et al., 2015, Vogt, 2010) is highly likely. An impact of the plant neighbourhood on a plant after a long residential time was already investigated for belowground root development and plant fitness by Ravenek et al. (2014). The impact given by LUI also fits the statements of different scientists to the impact of land use on ecosystems (Blüthgen et al., 2012, Herz et al., 2017b). The results would match the functions of semi-polar metabolites as mediators of interaction with and defence against neighbouring plants (Biedrzycki et al., 2010, Jandova et al., 2015, Xu et al., 2012), but also polar and semi-polar metabolites as adaptive agents to abiotic factors (Haase et al., 2007, Moe, 2013, Phillips et al., 2004, Rivoal and Hanson, 1994, Xia and Robert, 1994) such as LUI. So far, the nature of these interactions of exudates and LUI or LNH factors, respectively, is not clear. Thus, the further investigation of the correlated exudates with variables of the predictors LUI and LNH might expand the knowledge of plant-plant interaction and land use impact, respectively.

It has to be noted that the presented relations between environmental factors and exudates are to the greatest extent not the result of the typical relation of cumulated predictors to the dataset of interest (here the exudates) (Blüthgen et al., 2012, Dietz et al., 2019, Herz et al., 2018) (issue 3). The findings discussed here are the result of the impact of single variables on the compound composition and single compounds, respectively. Therefore, quenching effects might be the reason. Those effects might result from either

less explanatory power of single variables or the number of compounds being not correlated with the endogenous and exogenous factors. This might reduce the overall explanation power of the predictors. On the other hand, the higher impact of logistic models compared to variance partitioning is also reasonable. In logistic models, the non-linear character of the analysis can result in a much earlier significant correlation of two variables, here an exudate and an environmental factor, than by the variance partitioning. However, variance partioning is affected by the presence of a metabolite in comparison to the overall metabolite profile, whereas logistic models compare each compound individually with the specific environmental variable. Further statistical methods could help to clarify this point. A further interesting point are those compounds that showed a linkage to different factors, whose identity, however, is unknown so far. It would be of great interest to reveal their chemical identity by the identification approaches provided by different analytical techniques (Kopka et al., 2004, Ruttkies et al., 2016, Ziegler et al., 2016).

Last but not least, although plot together with different individual variables of the neighbouring plants, land use, climate and soil contributed to explain the polar and semi-polar exudate profiles of the ten species (up to 31.6 % and 24.9 %, respectively), an unexplained variance of 68.4 % to 68.9 % in polar metabolites and of 75.1 % to 84.7 % in semi-polar metabolites remained. This points to the fact that there have to be further unrevealed variables influencing the exudation. For instance, single aboveground events (trampling and erosion) or further root surrounding organisms like bacteria, fungi and herbivores might explain the appearance of certain exuded metabolites and the exudate composition. This is especially of huge interest for some polar metabolites, as amino acids, organic acids and carbohydrates, known to be released as response to the microbial community surrounding the root (Aulakh et al., 2001). But also several semi-polar metabolites are described as interaction mediators between plants and as defence agents against bacteria or fungi (Bourgaud et al., 2006, Langenheim, 1994, Long, 1989, Treutter, 2006). They also act as inhibitors of the growth of plants (Yoshihara et al., 1978) and as toxins for herbivores (Rasmann et al., 2005, Rosenthal and Berenbaum, 2012, Schweiger et al., 2014).

In conclusion, this study provides a hint to the diversity of exudate profiles of polar and semi-polar metabolites of different forb and grass species in the field. Only the combined investigation of a broad set of metabolites and different ecosystem components can help to find the most probable explanation why plants release a part of their metabolome into the soil.

Methods

Experimental Setup

The experiment was performed during Mai 2014 and August 2015 in the three regions of the German Biodiversity Exploratories (Fischer et al., 2010): Schorfheide-Chorin, Hainich-Dün and Swabian Alb. The sites differ in their location, climatic and soil properties (Alt, 2013, Birkhofer et al., 2012, Fischer et al., 2010, Herold et al., 2014, Rüdy, 2014).

18 experimental grassland plots in each Exploratory (54 in total) varying in land use intensity were selected for the analyses. In total, 10 species were raised for the experiment: five forbs (*Achillea millefolium* L. [Asteraceae], *Galium mollugo* L., *Galium verum* L. [Rubiaceae], *Plantago lanceolata* L. [Plantaginaceae], *Ranunculus acris* L. [Ranunculaceae]) and five grasses (*Alopecurus pratensis* L., *Arrhenatherum elatius* [L.] P.Beauv. ex J.Presl & C.Presl., *Dactylis glomerata* L., *Lolium perenne* L., *Poa pratensis* L. [all Poaceae]). The cultivation and planting was performed in 2014 as described in Herz et al. (Herz et al., 2017b). Supplementary Table 9 presents the number of samples per site and species.

Environmental Factors and data collection

Climate data of the precipitation (in %) and temperature in 10 cm and 2 m height (T(10) and T(200), in °C) as well as soil moisture (moisture, in %) were provided by Biodiversity Local Management teams (Fischer et al., 2010). Soil pH (pH), the total carbon (TC, in %) and total nitrogen content (TN, in %) of the soil of each site were measured on soil samples of bulk soil of each target plant. The soil was collected, sieved (2 mm mesh size), dried at 105 °C and ground. pH was determined by mixing 10 g soil powder and 25 ml demineralized water. 1.86 g KCl was added and pH was measured by a glass electrode. TC and TN were determined by weighing 10 mg soil powder into tin capsules and analysed using a C/N-analyser (vario EL cube; Elementar).

Agricultural management was investigated by calculating the Land use intensity index of 2015 (LUI) according the following formula of Blüthgen et al (Blüthgen et al., 2012):

$$\mathrm{LUI} = \frac{\mathrm{Fi}}{\mathrm{Fr}} + \frac{\mathrm{Mi}}{\mathrm{Mr}} + \frac{\mathrm{Gi}}{\mathrm{Gr}}$$

i = factor per plot

r = mean factor within the site

The factors account for the amount of N fertilizer in kg per ha for the growth time of the target plants (fertilization, F), the annual mowing rate in (mowing, M) and annual grazing frequency in livestock units days of grazing per ha per year (grazing, G). All abbreviations are summarized in Supplementary Table 10.

Exudate sample collection

The sample collection took place from June to August 2015. A field exudate collection method was adapted to those of Herz et al. (Herz et al., 2018) and Dietz et al. (Dietz et al., 2019) to collect the polar and semi-polar metabolites. A wash step of the roots in 0.5 % sodium chloride solution (NaCl) for 10 min was inserted between wash step one and two to remove rhizosphere microorganisms form the root surface. The exudate collection was performed in deionised water of HPLC quality from the complete root. Water samples without root exudation were used as process control ("water blanks").

An internal standard stock solution containing 20 µg/mL 2,4-dichlorophenoxyacetic acid and 10 µM Ribitol were added immediately after exudate collection in the field.

The exudate solution was purified by using the approach described in Herz et al. (2018) and Dietz et al. (2019) and measured with two different non-targeted plant metabolite profiling approaches. Aliquots of 100 µL of each sample were analysed by LC-MS according to Dietz et al. (2019). Aliquots of 200 µL of each sample were derivatized as described in Herz et al. (2018) and subjected to GC-MS analysis.

GC-MS analysis and data processing

Derivatized exudates and water controls were analysed by non-targeted plant metabolite profiling with a gas chromatograph (6890N GC; Agilent Technologies, Santa Clara, USA) equipped with a ZB-5 Zebron Guardian™ Capillary GC column (30 m + 10 m Zebron™, iD 0.25 mm, df 0.25 µm; Phenomenex, Torrance, USA) and coupled to mass spectrometer (5975 MSD; Agilent Technologies). Settings and method of measurement as well as data processing were applied as described in Herz et al. (2018).

LC-MS and MS/MS analysis and data processing

Exudate samples as well as water controls were analysed by ultra performance liquid chromatography coupled to electron spray ionisation quadrupole time of flight mass spectrometry (UPLC/ESI-Q-ToF-MS). An ultra performance liquid chromatography (ACQUITY UPLC; Waters, Eschborn, Germany) equipped with an Acquity UPLC® HSS T3 column (ACQUITY UPLC HSS T3 Column, 100Å, 1.8 µm, 1 mm x 100 mm; Waters) coupled to MicrOTOF–Q II hybrid quadrupole time-of-flight mass spectrometer equipped with an Apollo II electrospray ion source (Bruker Daltonics) was used for MS mode. To obtain CID mass spectra (MS/MS) of exuded compounds UPLC/ESI-Q-ToF-MS with an ultra performance Acquity UPLC platform (ACQUITY UPLC; Waters) equipped with an Aquity UPLC® H5S T3 column (Acquity UPLC® HSS T3 column (ACQUITY UPLC HSS T3 Column, 100Å, 1.8 µm, 1 mm x 100 mm; Waters) and a MicrOTOF–Q I hybrid

quadrupole time-of-flight mass spectrometer equipped with an Apollo II electrospray ion source (Bruker Daltonics). Detailed description were provided in the publication of Dietz et al. (2019).

Compound classification and identification

Data were processed as described in Herz et al. (2018) and Dietz et al.(2019). The identification of GC-MS measured compounds based on the National Institute of Standards and Technology (NIST) data base, the Golm metabolome database (GMD) and reference standards measured on the same instrument like the samples. The classification of LC-MS measured compounds based on comparison of qualifier ions of each MS/MS spectra of each compound with a fragment library of measured reference standards (see also Dietz et al. (2019)). The specific identifier ions are given in Supplementary Table 7. The hierarchical clustering of mass spectra was done according to their fragment spectra similarity by the MetFamily tool (Treutler et al., 2016). The colouration of the endpoints were done manually due to family classes and fragments.

Statistical Analysis

The statistical analysis were adjusted to Dietz et al. (2019) and were performed either with excel 2010 or with R (version 3.4.4, R Core Team, 2015). Firstly, metabolites and compounds occurring in 50 % of water controls as well as in 50 % of chemical blanks (GC-MS analysis) were regarded as artefacts and excluded from the metabolite dataset. For investigation of the overall metabolite profile, data were transformed to a presence/absence matrix. This allows the observation of metabolite composition without the heterogeneity already described in former investigations (Dietz et al., 2019, Herz et al., 2018). The chemical richness as well as the significance of differences were calculated by the mean of number of measured metabolites per group, here site and species or site and plant, using ANOVA (function aov, R Core Team, 2015) and scheffe posthoc test (function sheffe, package agricolae, de Mendiburu, 2017). The dependency on traits as species, site and growth form were calculated by linear mixed effect models (function lmer, package lmerTest, Kuznetsova et al., 2018)) including site with plot nested in species or species and plot nested in growth form as random factors. Results were visualized using boxplots (function qplot, package ggplot2, Wickham, 2009).

The exudate composition was analysed by redundancy analysis (function rda, package vegan, Oksanen et al., 2016) of the presence/absence matrix of metabolite composition against a presence/absence matrix of species and site together. GC-MS measured exudates were also analysed for their quantitative occurrence in growth form, site, and growth form and site as well as species, and species and site. First, the counts of each

substance over all samples of the group members were summed up and divided by the number of samples per group. This percentage of occurrence of one group member e.g. ALB, was than divided by the sum of percentage of occurrence of all other group members, e.g. SCH and HAI. Metabolites with a ratio of at least 2 were accepted as linked to this group member. LC-MS measured exudates were analysed for the significant species-specific compounds by calculating the mean of the compound composition of each species and subjecting it to a t-test (function binom.test, R Core Team, 2015) with alternative hypothesis that a compound not occurs in one out of ten species. In a second test, *Galium mollugo* and *Galium verum* data were combined as *Galium* species due to their phylogenetic and compound composition similarity and investigated with the alternative hypothesis that a compound occurs at least in two species.

Polar and semi-polar metabolite matrices were subjected to variance partitioning (function varpart, package vegan, Oksanen et al., 2016) for the calculation of explained variance by target species identity (Species), the plot as local impact (Plot) and either parameters of the neighbour plants around each target plant (LNH) and the environmental conditions (either as cumulated predictors of soil and climate parameters (Env), or soil conditions (Soil), and climate conditions (Climate) separately). The single variables contained in LNH (Cover, Richness and Shannon), LUI (mowing, grazing, fertilization), Soil (pH, soil moisture, soil core, soil type, TC, TN) and Climate (precipitation, T(10) and T(200)) were investigated for their explanatory power of exudate composition in the same way as the cumulated predictors. Furthermore, logistic regression models (glmer, lme4 package, Bates et al., 2015) were performed with the particular metabolites as dependent variable and a single environmental variable as predictor, using plot and species as random factors. Correlations with an alpha error below 0.05 in anova and correlation analysis were considered as significant. The correlated compounds were summed up for each variable in a bar plot (excel 2010) and divided by the total number of compounds found in the dataset of forb or grass exudates, respectively, for calculation of the percentage of affected compounds per cumulated predictor.

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III. Discussion

The central topic of this thesis is the investigation of plant root exudation in the rhizosphere of grassland species. Therefore, an exudate collection method (Aulakh et al., 2001) was modified and combined with an untargeted-metabolite profiling approach by gas and liquid chromatography coupled to mass spectrometry. The reliability of this combined approach was tested under controlled **(Chapter 2.1.)** as well as field conditions **(Chapter 2.2. – 2.4.)**. In the field based studies, an experimental setup was used that was designed and established by Katharina Herz (2017) in different grassland plots of the "German Biodiversity Exploratories". Ten perennial species planted in these plots were analysed for their polar and semi-polar metabolite profiles and how they were related to endogenous and exogenous influences **(Chapter 2.2. – 2.4.)**. Six of these species were also cultivated under controlled condition and used for the verification of the collection method **(Chapter 2.1.)**.

All in all, the following results were obtained:

1. The established methods for the investigation of root exudates under soil condition were proven to be valid for the analysis of root exudates from soil grown plants under controlled as well as field conditions.
2. The composition of root exudates is impacted by endogenous factors such as species identity, growth form and plant functional traits. Growth form was always of lower importance than the other considered factors.
3. The exudation pattern is also affected by exogenous factors, whereby the range of impact differed between the metabolite profiles investigated by LC-MS and GC-MS.

The following section discusses these statements in a more detailed way.

3.1. Exudate collection in a soil based system

The collection of plant root exudates is a challenging issue. To date, two collection procedures have been used: the collection in sterile hydroponic systems (Strehmel et al. 2014, Monchgesang et al. 2016, Ziegler et al. 2016), and the collection from plants grown in soil based systems by using suction caps or solvent elution (Oburger et al. 2013, Eisenhauer et al. 2017, Petriacq et al. 2017).
The collection method presented in this thesis **(Chapter 2.1. – 2.4.)** uses techniques from both systems. It combines the growth of the plants under soil conditions with an exudate collection method used in hydroponic systems (Oburger and Jones 2018). The postulated destruction of root cell with subsequent leakage of root metabolites into the exudate profiles by such an approach (Eisenhauer et al. 2017, Petriacq et al. 2017, Oburger and

Jones 2018) was thereby of no considerable relevance for thes thesis´ results. Staining of root that were treated with the collection method **(Chapter 2.2, Figures in the Appendix)** showed that the method cause insignificant injuries on the root and root hairs of the plants. This resulted in a low percentage of overlap (in average 13 %) between compounds of the roots and exudates **(Chapter 2.1.)**.

On the contrary, the method has several advantages compared to previous techniques. The collection of exudates from plants grown in their natural habitat, the soil, allows the investigation of root exudates under natural conditions (Oburger and Jones 2018), e.g., the rhizosphere of a existing grassland. Previous methods such as self-contained systems (Eisenhauer et al., 2017, Oburger et al., 2014, Petriacq et al., 2017) or sterile growth of plants (Strehmel et al., 2014, Ziegler et al., 2016) were not feasible under field conditions. The simplicity of the thesis´ collection set up, however, allows such an implementation, which has been proven by the results of **Chapter 2.2.** to **2.4.**. Thus, this collection method provides a basis for further field investigations of root exudates, which are rare and need to be extended (Peters et al., 2018).

Furthermore, the use of water as a trapping solution allows the application of high resolution techniques such as mass spectrometry. In previous studies the choice of exudate solution (Aulakh et al. 2001) limited the method of detection. Nutrient or $CaSO_4$ solution (Aulakh et al., 2001) as well as the suction of root surrounding soil water (Eisenhauer et al., 2017) might prevent root cells from burst, but add further substances, e.g. salt and humic acids, which could interfere with the metabolites of interest by either increasing the back ground of the measurement (Valentinuzzi et al., 2015) or causing ion suppression (Annesley, 2003). The investigation by mass spectrometry, however, is susceptible to such impacts (Annesley, 2003, Kopka et al., 2004). Thus, the detection spectrum was limited to methods such as high performance liquid chromatography (HPLC) or UV-VIS spectrophotometer (Aulakh et al., 2001, Eisenhauer et al., 2017) and metabolite classes e.g. carbohydrates, organic acids and phenylpropanoids (Aulakh et al., 2001, Eisenhauer et al., 2017, Oburger et al., 2009). An exudate profile comprises more than these classes (Strehmel et al. 2014, Petriacq et al. 2017), which means that methods that cover a broader spectrum of those (e.g., mass spectrometry) and trapping solutions (e.g., distilled water), which interfere as little as possible with the method of metabolite measurement, are preferable (Kopka et al., 2004, Valentinuzzi et al., 2015).

3.2. Plant root exudate composition in the grassland

The chemical identity and/or composition of exuded compounds of various species was an often touched upon topic (Aulakh et al., 2001, Eisenhauer et al., 2017, Oburger et al., 2014, Oburger et al., 2009, Petriacq et al., 2017, Schmid et al., 2014, Strehmel et al., 2014, Ziegler et al., 2016). In most cases, scientists focused their investigation on specific classes or metabolite groups (primary or secondary metabolites) using specific techniques. Ziegler et al. (2016), Schmid et al. (2014), and Strehmel et al. (2014) analysed secondary metabolites by LC-MS, whereas Oburger et al. (2009) and Aulakh et al. (2001) analysed the primary metabolites by scintillation counter or HPLC, respectively. This type of experimentation, however, reduces the information to only a small part of the exudate profile of a plant (Peters et al., 2018, van Dam and Bouwmeester, 2016). The results of this thesis **(Chapter 2.2. - 2.4.)** allowed not only the observation of more than 8,700 compounds, but also the designation of 297 of those to various metabolite classes and metabolite identities at the same time**.** Among those are compounds of the primary and the secondary metabolism, namely; alcohols, aldehydes, alkaloids, amines, amino acids, carbohydrates, glycosides, jasmonate derivatives, lipids, nucleic base or nucleotides, organic acids, phenylpropanoids, polyketides and terpenes. The gain of this results can be assigned to the application of GC-MS and LC-MS with the subsequent untargeted metabolite profiling of the same sample. Both, the two mass spectrometry techniques as well as their combination with the profiling method, cover a broader spectrum of metabolite classes (Kopka et al., 2004, van Dam and Bouwmeester, 2016) than in most of the previous mentioned studies. This is particularly of great importance for metabolomics studies where less investigated species (like in this thesis) rather than model plants (Badri et al., 2013, Strehmel et al., 2014, Ziegler et al., 2016) or crop plants (Aulakh et al., 2001, Petriacq et al., 2017) are of great interest (Peters et al., 2018). These unrevealed plants are of great relevance for the detection of new metabolites and the assesment of their purpose and interactions within the natural ecosystem (Viant et al., 2017).

The observed metabolite classes **(Chapter 2.2. – 2.4.)** match to a great extent the exuded substances of plant species already described in existing investigations (Aulakh et al., 2001, Petriacq et al., 2017, Strehmel et al., 2014). This underlines the feasibility of such an analysis. It is, however, important to mention that the investigations did not discovered all possible metabolite classes which were observed so far (Petriacq et al., 2017, Strehmel et al., 2014, Ziegler et al., 2016). For instance, no dipeptides, indolic derivatives and salicylic acid derivatives, as in the case of *A. thaliana* (Strehmel et al., 2014), were observed with the selected methods. These substances might not be exuded by the species of this thesis. An absence of e.g., antimicrobial and antifungal substances as

salicylic acid (van Dam and Bouwmeester, 2016), however, is hardly conceivable, especially under field conditions. It has to be noted, that in the case of semi-polar metabolites **(Chapter 2.3., 2.4.)** only the species-specific compounds were taken into consideration for the classification. So, it might be that such compounds were among the omitted substances.

The comparison of the exudate compositions of both sampling campaigns, 2014 and 2015, revealed slight differences. Certain aldehydes and one alkaloid were only detected in plant exudates sampled in 2015 **(Chapter 2.4.)**, whereas a specific diglycoside was only detected in profiles of the 2014 campaign **(Chapter 2.3.)**. Furthermore, an apparently broader range of exudates were released by the plant roots in 2014 than in those of 2015 **(Chapter 2.2. - 2.4.)**. In contrast, a higher number of species-specific semi-polar compounds was observed in 2015 (352 substances; **Chapter 2.4.**) than in 2014 (270 substances; **Chapter 2.3.**). These details must be considered with caution, since a method prior the exudate collection was altered in the 2015 campaign. The roots were equilibrated in an osmotic NaCl solution after the first washing step in the 2015 sampling campaign **(Method section Chapter 2.4.)**, which improved the collection of the microbial community of the root rhizosphere (Vieira et al., unpublisched). Ion suppression supposed to be no impact due to the second wash step with distilled water. This could, however, have also affected the exudation of the plant root and caused some metabolites to be exuded before the exudate collection step. The collection method presented here **(Chapter 2.1.)** is a time-dependent method where the abundance (and with this the occurrence of exuded metabolites above the detection limit of the instruments) depends on the time range of exudation in a certain media. Simultaneously, there was a time difference between the measurements of the sample sets of 2014 and 2015, which impact the performance and the detection of single metabolites. This was caused by the sampling time points and the number of samples of each campaign, which resulted (depended on the MS method) in long time ranges for the measurements. Thus, a detailed comparison of both sampling campaigns is not feasible. An overall comparison of the classified and identified metabolites of both years **(Chapter 2.1. - 2.4.)**, however, revealed an overlap of 31 of the identified polar metabolites and 62 of the species-specific semi-polar compounds (**Appendix Discussion Table 2, 3**). Moreover, the species-specific metabolites were detected in the same species in both years. With this, these results indicate that a set of polar and semi-polar metabolites is fixed in the exudate metabolome of the target species.

3.3. Endogenous and exogenous factors affecting the root exudation

Plant root exudation is the response to certain endogenous (Bais et al. 2006, Badri and Vivanco 2009, van Dam and Bouwmeester 2016) and exogenous abiotic (Haase et al. 2007, Neumann et al. 2014, Tawaraya et al. 2014, Ziegler et al. 2016) and biotic factors (Xu et al. 2012, Jandova et al. 2015). Existing studies investigated these interactions separately and under controlled conditions.

The experiments conducted for this thesis take a broader look at exudation under grassland conditions, where many of the above mentioned factors can interact with the plant simultaneously (Badri and Vivanco, 2009, van Dam and Bouwmeester, 2016). In general, the results drawn from a variety of existing studies (Aulakh et al., 2001, Badri and Vivanco, 2009, van Dam and Bouwmeester, 2016) support the findings presented in this thesis **(Chapter 2.2. - 2.4.)**. This demonstrates the transferability of knowledge gained under controlled condition to the field rhizosphere. However, the results **(Chapter 2.2.-2.4.)** also show that the impacts are lower than expected. This might be related to the simultaneous exposure of the plant root to all of the investigated factors, which might reduce the impact of a single effect such as shown in **Chapter 2.4.**. Furthermore, the results of the here presented investigation reveal that the impact of the factors varies between the GC- and LC-MS detected metabolites (polar/primary or semi-polar/secondary), as well as species and growth form **(Figure 5)**. The next section will take a closer look at these findings.

The Exploratories sites

The different sites of the Exploratories are known for their edaphic and climatic differences and their varying effect on ecosystem functioning (Alt, 2013, Blüthgen et al., 2012, Fischer et al., 2010, Herold et al., 2014). For instance, the soil of the Schorfheide has a higher nutrient content and moisture than soils in the HAI and ALB (Herold et al., 2014). The abiotic condition of the SCH exploratory are of adverse impact for microorganisms whereas enzymatic reactions that transform nutrients in the different cycles are promoted (Alt et al., 2011, Herold, 2013). With this, this Exploratory should have a special position among the Exploratories site with regard to exudation, too. This could only partly captured by the studies presented in this thesis. Polar metabolites, in combination with species and growth form, showed a linkage to site specific characteristics **(Chapter 2.4., Figure 1)**. This might coincide with the role of exudates improving unfavourable environmental conditions to the best for the plant (Aulakh et al., 2001, Ma et al., 2016, Tawaraya et al., 2014). Also semi-polar metabolites displayed a higher chemical richness in SCH and with this a dependence on the Exploratory site **(Chapter 2.4., Figure 4)**. In contrast to other

considered factors of this study, this is, however, of lower importance for the composition as shown by the analyses of **Chapter 2.4.**. The following paragraphs will have a closer look on the relations of polar and semi-polar metabolites to the other endogenous and exogenous factors considered in this thesis.

The growth form and species identity

Species identity is often listed as an important characteristic that influences the exudate composition (Monchgesang et al., 2016a, Strehmel et al., 2014, van Dam and Bouwmeester, 2016, Ziegler et al., 2016). However, it has never been investigated for many different species in one study. This thesis contributes to close this gap by exploring the exudate profiles of ten different species, which refer to two growth forms: forbs and grasses. Previous ecological studies showed trait differences between these two plant groups (Craine et al., 2001, Herz, 2017, Siebenkas et al., 2015). While Crain et al. (Craine et al., 2001) observed differences in root and leaf morphologies, Siebenkäs et al. (Siebenkas et al., 2015) displayed differences in the trait response of both growth forms to light and nutrient supply. Also Herz (Herz, 2017) recorded those variations, but remarked that a clear differentiation is not always possible. This assumption holds also true for the impact of growth form onto plant root exudation. Although there were differences in the chemical composition of polar and semi-polar exudate profiles of forb and grasses **(Chapter 2.2. - 2.4.)**, the impact of growth form was not significant **(Chapter 2.3., 3.4.)**. The investigation of polar metabolite composition showed that specific metabolites occurred more often in one or the other of the two growth forms **(Chapter 2.2.)**. However, these observed differences were mostly attributed to location impact and in some cases also to species identity **(Figure 5 A; Chapter 2.4.)**. Thus, the conclusion drawn by Katharina Herz (2017) that polar metabolites differ due to their growth form must be extended to include the differentiation caused by species specificity and site characteristics **(Chapter 2.4.)**.

In case of semi-polar metabolites, the profiles of forbs and grasses showed differentiation in their overall substance composition **(Chapter 2.3., 2.4.)**. This is supported by results of the phyto-cabinet experiment **(Chapter 2.1.)**. Further investigations attributed this to a higher interspecific variation in exudation of forbs **(Figure 5)** and linked to this, a higher number of species-specific compounds **(Chapter 2.3., 2.4.)**. Thus, the variability on the level of species overruled the impact of the level of growth form. A reasonable explanation for this is the different phylogenetic heterogeneities between forb and grass species **(Introduction, Figure 3 and Table 1)**.

The type of impact caused by different endogenous and exogenous factors also varies between forbs and grasses **(Chapter 2.2. – 2.4.)**. The differing impacts of the chosen endogenous and exogenous factors that were analysed in this thesis hold more explanatory power for grasses than forbs, accounting for the hypothesis of higher plasticity and faster adaption of grasses to environmental situations (Bardgett et al., 2014, Herz et al., 2017b, Siebenkas et al., 2015). The higher chemical richness of grasses under challenging conditions **(Chapter 2.4.)**, as in the SCH site, support this assumption. However, since exudate profiles of forb species also differ due to various other factors, species identity alone does not explain the differences in all released exudates.

Polar metabolites

Plot specific characteristics were the most effective determinant in explaining the variety in polar metabolite profiles for up to 30.6 % of the grasses and 24.64 % of the forbs **(Figure 5A)**. The role of soil variables was thereby higher than the variables of climate **(Chapter 2.4.)**. Particularly, soil moisture, the soil cores and the different nutrient contents of the soil accounted for the variation in polar metabolite exudation **(Figure 5C)**. Such correlations fit the function of different polar metabolites in nutrient recovery and soil characteristic adjustment in the rhizosphere of the plant (Haase et al., 2007, Tawaraya et al., 2014, Xia and Robert, 1994). Especially organic acids, amino acids and alcohols are known to promote the uptake of nutirents e.g., nitrogen (Haase et al., 2007) or allow plants to overcome acidification due to hypoxia effect (Xia and Robert, 1994). Those metabolites were found in exudate profiles of the target plants. The grasses showed thereby a higher dependence on location impacts than forbs in various analyses **(Chapter 2.2., 2.4.)**. The higher explained variation concerning plot and single soil and climatic variables fits to the observation that grasses adapt faster and are more plastic to their surrounding (Herz, 2017, Herz et al., 2017b, Pérez-Harguindeguy et al., 2013, Siebenkas et al., 2015). This could contribute to the explanation why grasses dominate the ecosystems grassland
The impact of the plant performance and functional traits, whose investigation was the subject of the PhD thesis of Katharina Herz (2017), support to some extend the relations to soil conditions. The determined contribution of those traits to the explanation of variation of 3.9 % in forbs and 2.4 % in grass exudate profiles **(Figure 5A,B)** was mainly driven by the biomass of the roots. This was attributed to the development-related differences in the root system (Herz, 2017). Indeed, younger plants have smaller roots and receive more nutrients for the development of the plant, whereas roots of older plants possess a bigger root system with more storage function (Aulakh et al., 2001). In this study (**Chapter 2.2.;**

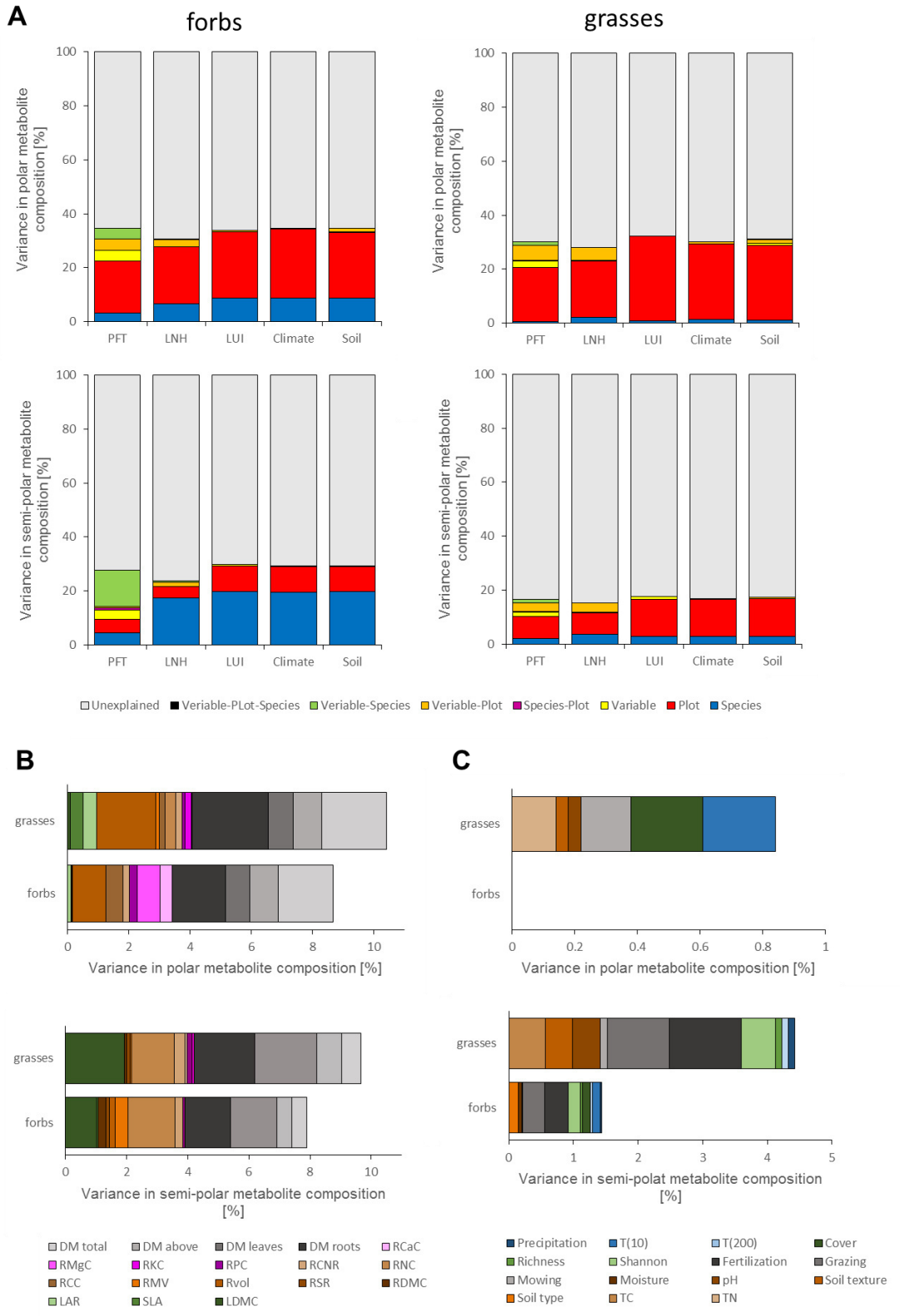

Figure 5: Overview of the proportions of explained variation within the polar and semi-polar metabolite profiles of exuded metabolites. Stack plot presents the explained variances of the polar and semi-polar metabolite composition of grasses and forbs by **A** the cumulated predictors, **B** single variables nested in plant functional traits (PFT), and **C** single variables nested either in climate, LNH, LUI or soil. For abbreviations see the section **VI. Abbreviation**.

also part of Katherina Herz thesis (2017)), however, investigations were obtained on plants of nearly the same developmental stage. Thus, this statement has to be more restricted to the morphological differences. It is hypothesised that bigger roots have a larger surface (Bardgett et al., 2014) and with this a higher probability to release metabolites. So it would be more reasonable to hypothesize that the root surface has an impact on the alteration of the investigated exudate profile. Because an investigation of the root surface or root architecture was not implemented in the study of Katharina Herz (2017), a verification of this assumption is not possible. Further studies focusing on this issue would help clarify this hypothesis.

The results of the present thesis showed also that other traits, such as the content of different nutrients in the roots, have a greater impact on the exudation of polar metabolites in grasses than in forbs **(Chapter 2.2., Figure 2 and Table S10 in the Appendix)**. When higher root volume and the impact of soil conditions are considered together, the observations account for the fast acquisition and low storage capacity theory of grass species (Herz et al., 2017b, Siebenkas et al., 2015, Straßburger, 2002).

The minor impact found for LNH **(Chapter 2.2., 2.4)** and its variables was surprising due to the postulated competition pressure by resource exploration in the belowground (Ravenek et al., 2016) and the function of polar metabolites for resource acquisition (Jones et al., 2004). Thus, a higher relation than 0.3 % and 0 % in case of grasses and forbs **(Figure 5)** would be expected. However, since some of the variation in metabolite profiles are captured by LNH and plot characteristics together, a more indirect effect of e.g., nutrient competition between neighbouring plants (Ravenek et al., 2016), could be possible. Moreover, the investigation of each factor of LNH and the single metabolites in **Chapter 2.4.**, respectively, suggests that the occurrence of specific metabolites is influenced by the neighbouring plant community. Further investigations in this direction could verify this assumption.

The results presented in this thesis reveal also that agricultural management has a minor impact on plant exudation of polar metabolites. **Figure 5A** and the results of **Chapter 2.4.** suggest that especially grazing frequency alters the overall composition of exudates in the grassland. This is surprising because the described changes of soil characteristics, as well as organismic diversity and developmental changes, have been linked to agricultural management in grasslands (Allan et al., 2015, de Vries et al., 2012, Lavorel et al., 2011, Soliveres et al., 2016). The different spatial and temporal scales of LUI and exudate information might explain this. LUI levels were elevated throughout the Exploratory site and during the time frame of a year (Blüthgen et al., 2012), whereas exudate collection occurred on one plant and over a time range of two hours. However, the linkage found

between polar metabolites and soil variables would point to variations in plot level factors driving the relationship between LUI and exudation. Further investigations targeting this issue and on the same scale could help to evaluate this impact.

Semi-polar metabolites

Besides the previously mentioned high impact of species, other predictors shape the exudate profile of semi-polar metabolites, too. For instance, 3.3 % of the exudation in forbs and 1.6 % of the variability in the exudate profiles of grass species **(Chapter 2.3.; Figure 5A,B)** were explained by plant functional traits. For forbs, plant functional traits are connected to species **(Chapter 2.3., Figure 5)** emphasizing the mentioned impact of the phylogenetic heterogeneity (Herz, 2017). In contrast, the combined effect of location impact and plant traits and the higher overall explanatory power of single plant traits underline the higher plasticity of grasses to environmental influences (Herz et al., 2017b, Pérez-Harguindeguy et al., 2013, Siebenkas et al., 2015). Also, the aboveground plant traits and root biomass of grasses have a stronger effect on semi-polar metabolite exudation than in case of forbs **(Chapter 2.3., Table 2)**. This further accounts for a faster adaption of grasses to their surrounding environment (Herz, 2017, Pérez-Harguindeguy et al., 2013).

The observations and analyses found in this thesis reveal furthermore the high impact and explanatory power of specific plot characteristics **(Chapter 2.3., 2.4.)**. Soil variables altered thereby the exudate profile more than any other plot specific predictor. Many of the semi-polar metabolites are described as being linked to abiotic environmental influences (Badri et al., 2013, Oburger et al., 2014, Ziegler et al., 2016). Phenylpropanoids (like coumarins) are involved in phosphorus and iron acquisition (Schmid et al., 2014, Ziegler et al., 2016), whereas phenolic acids (e.g., p-coumaric, ferulic, p-hydroxybenzoic, and protocatechuic acids) promote the availability of organic and inorganic ions (Inderjit and Malik, 1997). The observed relation of flavonoids and terpenes with soil and climate variables were instead quite surprising. Although flavonoids play a role in UV protection (Baxter and Harborne, 1999), frost hardness and drought resistance in aboveground plant parts (Treutter, 2006), they are mainly known for their functionality in positive and negative interactions with the biotic community belowground (Gershenzon and Dudareva, 2007, Hassan and Mathesius, 2012, Langenheim, 1994, Treutter, 2006). Thus, their function in belowground abiotic relations would be of great interest.

The Biotic interactions with neighbouring plants might be linked to the exudate composition with differing strengths of influence. Whereas the results discussed in **Chapter 2.3.** point

to a minor dependence of the exudation of semi-polar metabolites to the local neighbouring plants, **Chapter 2.4.** showed that the exudation of some of those metabolites can be linked to the single variables of this biotic factor **(Figure 5A)**. This meets the experimental observations of Eisenhauer et al. (2017) and other scientists (Biedrzycki et al., 2010, Jandova et al., 2015) who have shown that exudation is influenced by the species diversity of the plant community. Neighbouring plants alter thereby the occurrence of certain forb-exuded metabolites, whereas the Shannon diversity of those plants was linked more particularly to the presence or absence of grass exudates. The effect of the neighbouring plants on the exudation was, however, lower than expected since secondary metabolites are described as defensive and communication compounds to neighbouring organisms (Bais et al., 2006a, Inderjit and Weiner, 2001, Jandova et al., 2015, Xu et al., 2012). Thus, additional impacts have to be considered. These assumption is supported by the fact that the combined impact of both LNH and plot characteristics explain a significant degree of the variation of exudate profiles **(Chapter 2.4., Figure 7)**. This suggests that a plant community surrounding the target plant alters the exudate profile via the edaphic conditions by e.g., reducing the amount of nutrients (Ravenek et al., 2016). However, since no study exists that investigates such complex relationships under natural conditions, a final answer for this phenomenon cannot be given. A deeper exploration of these relationships would shed more light on this issue.

The low impact of land use on the variation of the exuded secondary metabolite composition of grasses and forbs somehow met the expectations. Because soil conditions alter the exudate profile, the impact caused by fertilization is reasonable (**Figure 5)** since this alters the nutrient content of the soil (Blüthgen et al., 2012).Thus, this linkage could explain the impact of land use on the exudation. This thesis also revealed that when exclusively land use factors (especially grazing, but also mowing) were considered, the exudation of forbs was impacted the most **(Chapter 2.4., Figure 9 and Supplementary Table 5)**. This relation could possibly be linked to altered manifestation of aboveground plant traits due to LUI (Breitschwerdt et al., 2018, Herz et al., 2017b). For instance, LDMC decreases under land use (Breitschwerdt et al., 2018). A reduction of the leaf mass by mowing or grazing can be linked to a reduction of the photosynthetic capacity in the broadest sense (Herz, 2017). This leads to an alteration in nutrient demand. Since aboveground plant traits were also correlated to the release of semi-polar metabolites **(Figure 5A,B)**, it might be that the impact of mowing and grazing manifests in an altered exudate composition to react to the changed nutrient requirement. Thus, since forbs are thought to invest more of their resources into the leaf biomass than grasses (Siebenkas et al., 2015), an alteration of that trait might have a higher effect on forbs than on grasses.

However, this assumption cannot be proven within the frame of this study due to missing experiments addressing this issue.

It has to be mentioned that although more factors demonstrated an impact on the grasses' exudation, the percentage of correlated compounds to one of the factors was almost always higher in forbs than in grasses **(Chapter 2.4.)**. This could, however, also be attributed to the higher phylogenetic variability within the forb species which was mentioned earlier in this thesis.

The impact of the experimental level

The outcome of an investigation is highly impacted by the experimental level that is considered for analysis. This observation has already been demonstrated in the analysis of inner root metabolites (Monchgesang et al., 2016b). In this thesis, this impact on exudation was also shown under controlled conditions (**Chapter 2.1.**), but also by the relation of different levels of the endogenous and exogenous factors that were subjected, both simultaneously and individually, to plant exudation (**Chapter 2.2. – 2.4.**). When factors that describe the same abiotic or biotic influences were combined into one descriptive predictor, the explained variation of the exudate composition was lower, than subjecting the factor individually to the overall exudate profiles. The metabolites also showed different relations to specific factors when each metabolite individually instead of the overall profile was investigated **(Chapter 2.4.)**. This could be explained in two ways. On the one hand, the range of explained variation in exudate composition might be altered by the composition of each predictors. For instance, the cumulated predictor LUI displayed no impact on the exudate profile **(Chapter 2.3.),** whereas the single factors of LUI, e.g. total mowing or grazing frequency could be related to the release of different flavonoids and glycosides **(Chapter 2.4.)**, respectively. Thus, any investigation of these complex relationships should consider the hierarchy of already known impacts in order to draw the appropriate conclusions. On the other hand, the choice of statistical method might affect the impact of predictors. The analyses of **Chapter 2.4.** revealed that the choice of the statistical methods, variance partitioning or logistic models **(Chapter 2.4.)** might alter the height of influence. However, the results of both statistical methods can only give a first clue, which in the following can be further investigated.

3.4. Overall synthesis

The investigation of exudates has occupied scientists for decades. In the course of these investigations, a number of characteristics and functions of these plant metabolites have been determined. Many studies examined both the root-released metabolites of different sometimes genetically modified species (Monchgesang et al., 2016a, Petriacq et al., 2017, Schmid et al., 2014, Ziegler et al., 2016), as well as the impact of different abiotic and biotic factors on plant root exudation (Aulakh et al., 2001, Eisenhauer et al., 2017, Liese et al., 2018). All of these studies were conducted under controlled laboratory conditions, or in the field imitating experiments. However, the investigation of exudates has not yet been combined with ecological analysis that reveal their role in a grassland community. This was mainly addressed to the missing possibility to measure root exudates under soil conditions (Oburger et al., 2013, Oburger and Jones, 2018, Strehmel et al., 2014).

The present thesis offers an approach to fill this gap. The **first issue** addressed by this study is to **determine if an investigation of root exudates is possible under field conditions**. Indeed, by successfully applying a combined method of collection and untargeted metabolite profiling, this study was able to reveal a broad spectrum of root exuded metabolites referring to the polar and semi-polar substances types. This thesis also deals simultaneously with many different factors impacting root exudation than described before. By doing so, this analysis addresses **two other issues** related to the exploration of exudates, namely: **does growth form or species have a greater impact on exudation,** and **what other observable environmental factors might impact root exudation in the field.**

In this thesis, ten different species were explored for their polar and semi-polar root metabolite exudation. The findings show that the results are highly dependent on the type of metabolites, the kind of growth form and the various stages of the experimental levels. Although semi-polar (secondary) metabolites have a shared metabolite pattern, they differ more on the level of species, and are to some extent also variable between the growth form. When polar (primary) metabolites were examined, the answer is not as simple as that. In one of the analyses, the differences in the occurrence of polar metabolites was more dependent on location rather than growth form or species. In a second study, growth form played a more critical role in the differentiation of this type of metabolite, and was less impacted by species identity.

It is also important to note that the impact of the different investigated effectors is highly dependent on which growth form and which metabolite group (polar or semi-polar) were taken into consideration. Although the species identity of grasses effect the metabolite

release of both polar and semi-polar compounds, they exuded their metabolites based primarily on the impact of a specific location and its environmental conditions. The release of forbs polar metabolites is mainly impacted by the plot characteristics, whereas semi-polar metabolite release is primarily affected by the species identity, and secondarily by other factors. In all cases, the impact of the local neighbouring plants, the land use and the climatic and edaphic characteristics of the locations were lower than expected.

However, it is important to note that all of the investigated factors of the rhizosphere were related to each other. Plant functional traits of plants do impact root exudation, but are highly dependent on the species and the provided environmental conditions of the location. While the environmental conditions impact the exudation, these factors are also influenced by agricultural management and neighbouring plants, and by the climatic and soil characteristics of the different sites. Based on these findings, it is not useful to divide the rhizosphere into compartmentalized sections for the investigation of the overall relation of exudates to this habitat.

Moreover, a single investigation of a specific group or class of exuded metabolites is not recommended. Many current studies concentrate on the influence of secondary metabolites released by the plant root to reveal how these substances interact with the surrounding plant root community (Eisenhauer et al., 2017, Lange et al., 2015, van Dam, 2009, van Dam and Bouwmeester, 2016). This thesis, however, shows that primary metabolites released by plant roots also have an interesting and important impact on the function of exudates in the rhizosphere community. Previous studies have already shown that organic acids not only serve as chelating substances for the acquisition of nutrients (Bais et al., 2006b, Jones et al., 2004, Neumann et al., 2014). Together with carbohydrates, they also serve as nutrient bases for microbial and fungal species (Badri et al., 2013, Eisenhauer et al., 2017, Kiers et al., 2011). Since the findings of these studies were obtained under controlled conditions, investigations of these relationships under field conditions were also needed to reveal the full functional diversity of exudates. This study addresses this need by providing a basis for the investigation of root exudates under natural ecosystem conditions.

IV. Outlook and future perspectives

The investigations and results found in this thesis provide a good basis for further research. For instance, the direction of influence onto plant exudate composition that is triggered by different environmental factors was not sufficiently address by this thesis. A deeper analysis of the relationship between exudates and the overall impact of the different factors present in a specific ecosystem is warranted. An investigation using linear effects model of intensity based instead of present/absent based data might provide us with results that determine the intensity of the direction of influence given by a certain impacting factor. The results and observations offered in this thesis could be used for a more targeted investigation of specific interactions under controlled conditions. This is essential for the full identification of the function of certain metabolites.

Another interesting topic is the identity of the metabolites observed during the course of the investigations presented in this thesis. The majority of the observed metabolites are annotated by their mass-to-charge ratio or a spectral similarity to other known metabolites, as well as classified by specific fragment ions typically observed in known metabolite classes. Their exact identity, however, is mostly unknown. Metabolomics provides a variety of possible methods to examine the exact chemical identity of these substances. Elucidation of structure by insilico fragmentation (Ruttkies et al., 2016), mass spectra chemical analysis of fragmented metabolites, and/or a comparison to analytical standards are only a few of the available methods of analysis. Moreover, investigations using analytical methods such as Atmospheric Pressure Chemical Ionisation Mass Spectrometry (APCI-MS) could help to elucidate the unknown carbohydrates and fatty acids of the polar metabolites in a less destructive way than the harsh electron impact ionization of the GC-MS method. Moreover, this method could extend the metabolite profiles by the investigation of (further) terpenes, carotenoids and aliphatic substances (Kopka et al., 2004) Another way to enhance the results presented in this thesis is to use triple quadrupole or ion-trap mass spectrometry, rather than the single quadrupole time of flight instrument method used in this thesis. This might gain higher levels of mass fragmentation (Döll et al., 2018, Kopka et al., 2004). Moreover, nuclear magnetic resonance spectroscopy could increase the number of identified metabolites (Döll et al., 2018).

Yet another area requiring deeper analyses is the impact of some of the investigated predictors. For instance, the LUI supplied by the Biodiversity Exploratories might not be the preferred method for root exudates and warrants repetition using other recording methods. The differences in temporal and spatial recording between LUI and exudates mentioned in this thesis and the thesis of Katharina Herz (Herz, 2017) could distort the investigation of their correlation. A more detailed report based on daily mowing, grazing and fertilization events (as well as exact amounts of fertilization) would give a more exact picture of their influence on

plant root exudation. Another critical point is the information provided for the local neighbouring plant community. The predictor contains a lot of information about below and aboveground traits, as well as the diversity and specific species richness of ambient plants, but lacks information about the exudation of these plants or the exact composition of neighbouring plants around the target plant. It would be of great interest to include this factor in the investigation of root exudate with regard to plant-plant interaction, too.

Last but not least, all analyses presented a high degree of unexplained variation (**Figure 4, Chapter 2.2. – 2.4.**). On the one hand, this could be attributed to the sum of variations in cumulated predictors. On the other hand, however, the interaction of microbial as well as fungal and herbivore communities (which were not included in this thesis) in relation to exudate composition and the other included endogenous and exogenous traits could be addressed. An investigation of these relationships would probably account for some if not the greatest amount of semi-polar metabolite variation. Their known relationship in interaction and defence (Badri and Vivanco, 2009, Carvalhais et al., 2015, van Dam and Bouwmeester, 2016) could help to provide a more comprehensive picture of the rhizosphere ecosystem. They also could contribute to a certain amount of the unexplained variation in polar metabolites due to their already mentioned function as nutrient supply for these community members (Huang et al., 2014).

Another aspect of unexplained variation might also be attributed to intraspecific variability. As shown by Katharina Herz (Herz, 2017, Herz et al., 2018), intraspecific variation overruled interspecific variation between growth forms in polar metabolites. This could be attributed to biological variations between plants and their exudation, such as shown in the phyto chamber experiment **(Chapter 2.1).** It, however, could also be attributed to other factors such as genetic variability of root inner and exuded metabolite (Monchgesang et al., 2016a, Monchgesang et al., 2016b), respectively.

V. Abbreviation

Abbriviation	Description
(±)	In the range of
[M+H]+	Positive charged mother ion, due to a additional proton
[M-H]-	Negative charged mother ion, due to the loss of a proton
2,4-D	2,4- Dichlorophenoxyacetic acid
A. elatius, Ar.el.	*Arrhenatherum elatius* (L.) P.Beauv. ex J.Presl & C.Presl.
A. millefolium, Ac.mi.	*Achillea millefolium* L.
A. pratensis, Al.pr.	*Alopecurus pratensis* L.
AAS	atomic absorption spectrometry
AM	arbuscular mycorrhiza
ANOVA	analysis of variance
BSTFA	N,O-Bis(trimethylsilyl)trifluoroacetamide
C	Carbon
C/N	Carbon-nitrogen-ratio
C12	Chain of 12 to 32 carbon atoms, respectively
C15	
C18	
C19	
C22	
C28	
C32	
Ca	Calcium
$CaCl_2$	Calcium chloride
CID	Collision-induced dissociation
-COOH	Carboxy group
$CuSO_4$	Copper sulphate
D. glomerata, Da.gl.	*Dactylis glomerata* L.
DCA	Detrended Correspondence Analysis
DDA	Data dependent Acquisition
DIMS	Differential ion mobility spectrometry
DNA	Deoxyribonucleic acid

Abbriviation	Description
EI	Electron Impact
EIC	Extracted Ion Chromatogram
Env	Environment
ESI	Electron Spray Ionisation
et al.	et alia
Fe3+	Ferredoxin 3 ion
funnel RF	Funnel radial force
GC	Gas Chromatography
GC-Q-MS	Gas chromatography coupled to quadrupole Mass spectrometry
G. mollugo, Ga.mo.	*Galium mollugo* L.
G. verum, Ga.ve.	*Galium verum* L.
Galium *spp.*	*Galium* species
GMD	Golm Metabolome Database
H	Hydrogen
H_2O	Water
H_3BO	Boric acid
HAI	Hainich-Dün Exploratory
HPLC	High-Performance Liquid Chromatography
K	Potassium
K_2SO_4	Potassium sulphate
KCl	Potassium chloride
KH_2PO_4	Potassium phosphate monobasic
L. perenne, Lo.pe.	*Lolium perenne* L.
LC– MS	Liquid Chromatography coupled to Mass spectrometry
LDMC	Leaf Dry Matter Content
LNH	Local Plant Neighbourhood
log2	Binary logarithm
LUI	Land Use Index
m/z	Mass to charge ratio
M+HCHOOH-H	Formic acid adduct
M+K-H2	Potassium adduct
M+Na-H2	Sodium adduct
mg	Milligram

Abbriviation	Description
$MgSO_4$	Magnesium sulphate
M-H	Loss of a proton
M-H2O-H	Loff of water
$MnSO_4$	Manganese sulphate
MoNA	MassBank of North America
MS	Mass spectrometry
N	Nitrogen
Na-Fe-EDTA	Ferric Sodium Ethylenediaminetetraacetate; enhance ferric complex
$NaMoO_4$	Sodium molybdate dihydrate
-NH2	Amino group
NH_4NO_3	Ammonium Nitrate
NIRS	Near Infrared spectroscopy
NIST	National Institute of Standards and Technology
NMR	Nuclear Magnetic Resonance
o-glyosidic	oxygen links the glycoside to the aglycone
P. lanceolata, Pl.la.	*Plantago lanceolata* L.
P. prantensis, Po.pr.	*Poa pratensis* L.
PCA	Principal Component Analysis
p-coumaric	4-Hydroxy cinnamic acid
Plot	Edaphic and climatic conditions of the location the plant is growing
Q	Quadrupole
R group	side chain of an amino acid
R. acris, Ra.ac.	*Ranunculus acris* L.
RCaC	Root nutrient concentration of calcium
RCC	Root nutrient concentration of carbon
rcf	relative centrifugal force
RCNR	Root carbon to nitrogen ratio
RDA	Redundancy Analysis
RDMC	Root Dry Matter Content
RKC	Root nutrient concentration of potassium
RMgC	Root nutrient concentration of magnesium
RNA	Ribonucleic Acids
RNC	Root nutrient concentration of nitrogen

Abbriviation	Description
RP	Reversed-phase
RPC	Root nutrient concentration of phosphate
RSR	Root Shoot Ratio
Rvol	Root Volume
SCH	Schorfheide-Chorin Exploratory
SLA	Specific Leaf Area
Ta_10	Temperature in 10 cm height above the ground
Ta_200	Temperature in 200 cm height above the ground
TC	Total Carbon content of the soil
TN	Total Nitrogen content of the soil
TOF	Time of Flight
UNESCO	United Nations Educational, Scientific and Cultural Organization
UPLC	Ultra Performance Liquid Chromatography
UPLC/ESI-Q-ToF-MS	Ultra Performance Liquid Chromatography coupled to Electron spray ionization Quadrupole Time of Flight Mass spectrometry
$ZnSO_4$	Zinc sulphate

VI. Literature references

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VII. List of Figures

All page numbers marked with an A refer to the corresponding pages in the Appendix.

Introduction:

Results:

Chapter 2.1.:

Chapter 2.2.:

Chapter 2.3.:

Chapter 2.4.:

Discussion:

VIII. List of Tables

All page numbers marked with an A refer to the corresponding pages in the Appendix.

Introduction:

Chapter 2.1.:

Chapter 2.2.:

Chapter 2.3.:

Discussion:

IX. Appendix

Supplementary figures and tables can be found on the attached cd.

Curriculum Vitae

Education

06/2014 – Present day

PhD in Mass spectrometry, Biology

Focus: plant biochemistry, metabolomics, root exudates, mass spectrometry (LC-MS und GC-MS Hybrid-Techniques), rhizosphere environmental science, statistics

Thesis: "Root-derived Exudates and their Relationship to the Belowground Ecosystem in Grassland communities" (writing)

In the research group of Prof. Dr. D. Scheel ("Metabolomics Research Group", Department of Stress- and Developmental Biology*) Leibniz Institute of Plant Biochemistry, Halle (Saale)*

In cooperation with the research group of Prof. Dr. H. Bruelheide ("Geobotany and Botanical Garden", Institute for Biology), MLU Halle-Wittenberg, Halle (Saale)

03/2014 – 05.2014

Scientific assistant

Focus: biochemistry and molecular biology, protein purification and characterisation, Mass spectrometry (MALDI-TOF MS), HPLC, UPLC

In the research group of Dr. H.-P. Mock („Applied Biochemistry", Department of Physiology and Cell Biology), Leibniz-Institute for Plant Genetics and Crop Plant Research (IPK), Gatersleben

09/2011 – 11/2013

Master of Science in Biochemistry, Martin-Luther-University Halle-Wittenberg (MLU Halle-Wittenberg)

Focus: plant biochemistry, plant physiology, protein biochemistry

Thesis: "Untersuchungen zur Wirkung von Effektorproteinen des Phytopathogenen Oomyzeten Phytophthora infestans auf das pflanzliche Phospholipidsystem"

In the research group of Prof. Dr. I. Heilmann ("Cellular Biochemistry", Institute for Biochemistry and Biotechnology*) MLU Halle-Wittenberg, Halle (Saale*

In cooperation with the research group of Prof. Dr. S. Rosahl and Prof. Dr. D. Scheel ("Induced Pathogen Defense", Department of Stress- and Developmental Biology*), Leibniz-Institute of Plant Biochemistry (IPB), Halle (Saale)*

09/2007 - 07/2011	Bachelor of Science in Biochemistry, MLU Halle - Wittenberg *Thesis: „Affinity based Protein Profiling - Charakterisierung von Sonden und Anwendung in pflanzlichen Systemen“* *In the research group of Dr. T. Vogt (formerly: “Plant biochemistry & Metabolite profiling“,* Department of Cell and Metabolic Biology*), IPB, Halle (Saale), 0345 5582 1530*
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Publications

Dietz, S., Herz, K., Gorzolka, K., Jandt, U, Bruelheide, H., Scheel, D. (2019): „Root exudate composition of grass and forb species in natural grasslands." Nature communications (submitted)

Dietz, S., Herz, K., Döll, S., Haider, S., Jandt, U, Bruelheide, H., Scheel, D. (2019): Semi-polar root exudates in natural grassland communities. Ecology and Evolution (accepted)

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08.2018 **Dietz S.**, Gorzolka K., Döll S., Scheel D. (2018): “Eco-Metabolomics of Root Exudates in German Grassland Communities”. IMSC Florence, 22th Internationale Mass spectrometry Conference. (Florence, Italy)

12.2017 **Dietz S.**, Gorzolka K., Herz K., Bruelheide H., Jandt U., Scheel D.: “Effects of biodiversity on exuded root metabolites in grassland communities Root-derived Exudates and their Relationship to Plant functional Traits in Grassland communities.” 47th Annual Meeting of the der Ecological Society of Germany, Austria and Switzerland, British Ecological Society, European Ecological Federation, Dutch-Flemish Ecological Society (Gent, Belgium)

06.2017 **Dietz S.**, Gorzolka K., Herz K., Bruelheide H., Jandt U., Scheel D.: “Effects of biodiversity on exuded root metabolites in grassland communities. “13th Plant Student Sience Conference, Leibniz Institute of Plant Biochemistry, (Halle -Saale, Germany)

02.2016 **Dietz S.,** Gorzolka K., Herz K., Bruelheide H., Jandt U., Scheel D.: “Effects of biodiversity on exuded root metabolites in grassland communities.“ 12th Annual Metabolomics Conference of the Metabolomics Society, (Dublin, Republik Irland)

02.2016 **Dietz S.**, Herz K., Bruelheide H., Jandt U., Scheel D.: “BE LOW - Analysis of root traits and root exudates in grassland communities.” 13th Annual Meeting of the German Biodiversity Exploratories, (Wernigerode, Germany)

12.2015 Herz K., **Dietz S.**, Bruelheide H., Jandt U., Scheel D. (2015): ”Effects of biodiversity and land use on root traits and root exudates in grassland communities.” 1st Annual Conference of the German Centre for Integrative Biodiversity Research (iDiv); Leipzig, Germany. *Talk contribution*

02.2015 **Dietz S.**, Herz K., Bruelheide H., Jandt U., Scheel D. (2015): “BE LOW - Analysis of root traits to test for environmental filtering and niche complementarity in grassland communities.” 12th Annual Meeting of the German Biodiversity Exploratories, (Wernigerode, Germany

Declaration / Eigenständigkeitserklärung

Hiermit erkläre ich an Eides statt, dass die Arbeit mit dem Titel „Root Exudates in the Grassland Ecosystem“ bisher weder bei der Naturwissenschaftlichen Fakultät I Biowissenschaften der Martin-Luther-Universität Halle-Wittenberg noch einer anderen wissenschaftlichen Einrichtung zum Zweck der Promotion vorgelegt wurde.

Darüber hinaus erkläre ich, dass ich die vorliegende Arbeit eigenständig und ohne fremde Hilfe verfasst sowie keine anderen als die im Text angegebenen Quellen und Hilfsmittel verwendet habe. Textstellen, welche aus verwendeten Werken wörtlich oder inhaltlich übernommen wurden, wurden von mir als solche kenntlich gemacht.

Ich erkläre weiterhin, dass ich mich bisher noch nie um einen Doktorgrad beworben habe.

Halle (Saale),

Sophie Dietz

VI. Appendix

Chapter 2.1.

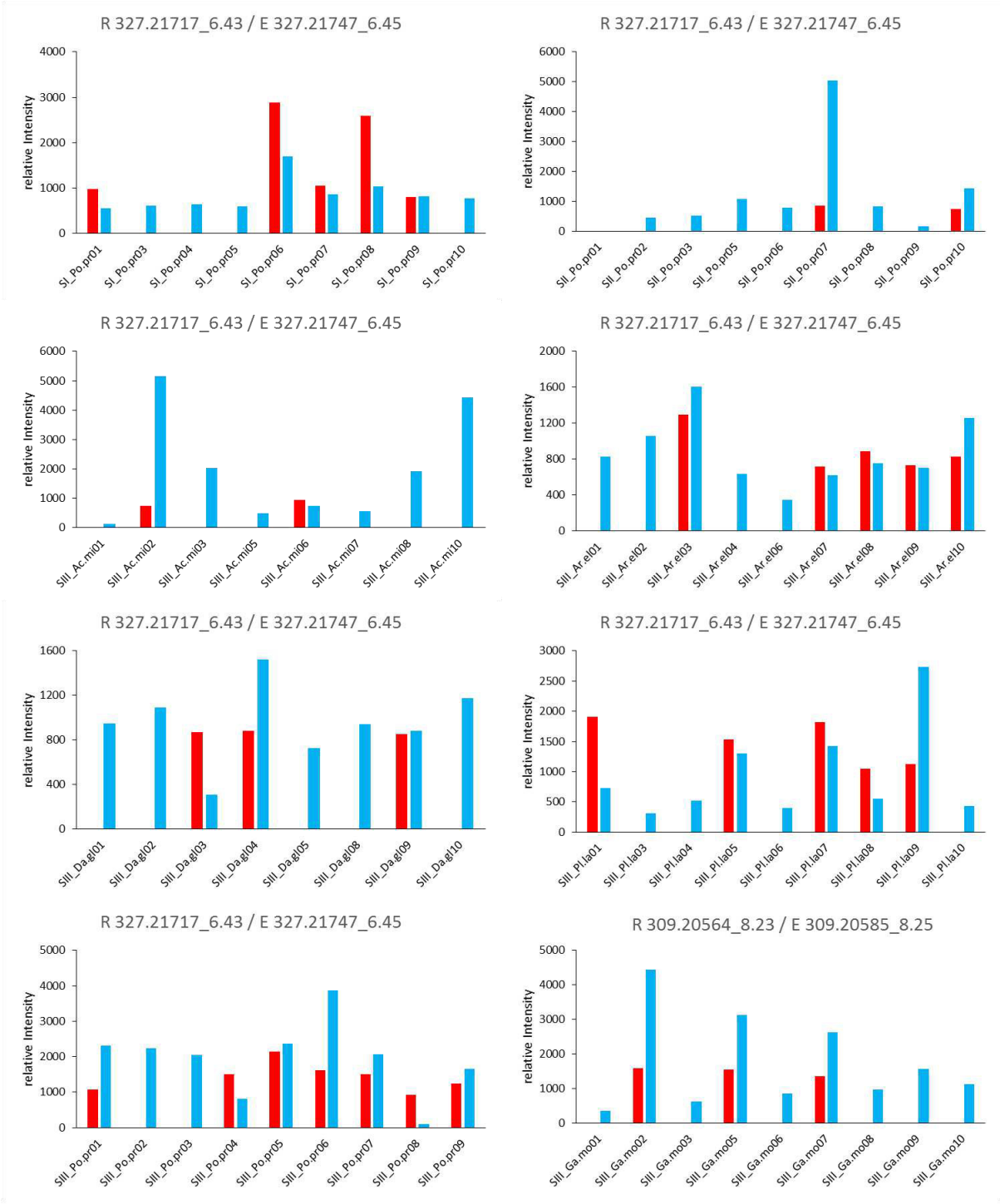

Figure will be continued on the next page

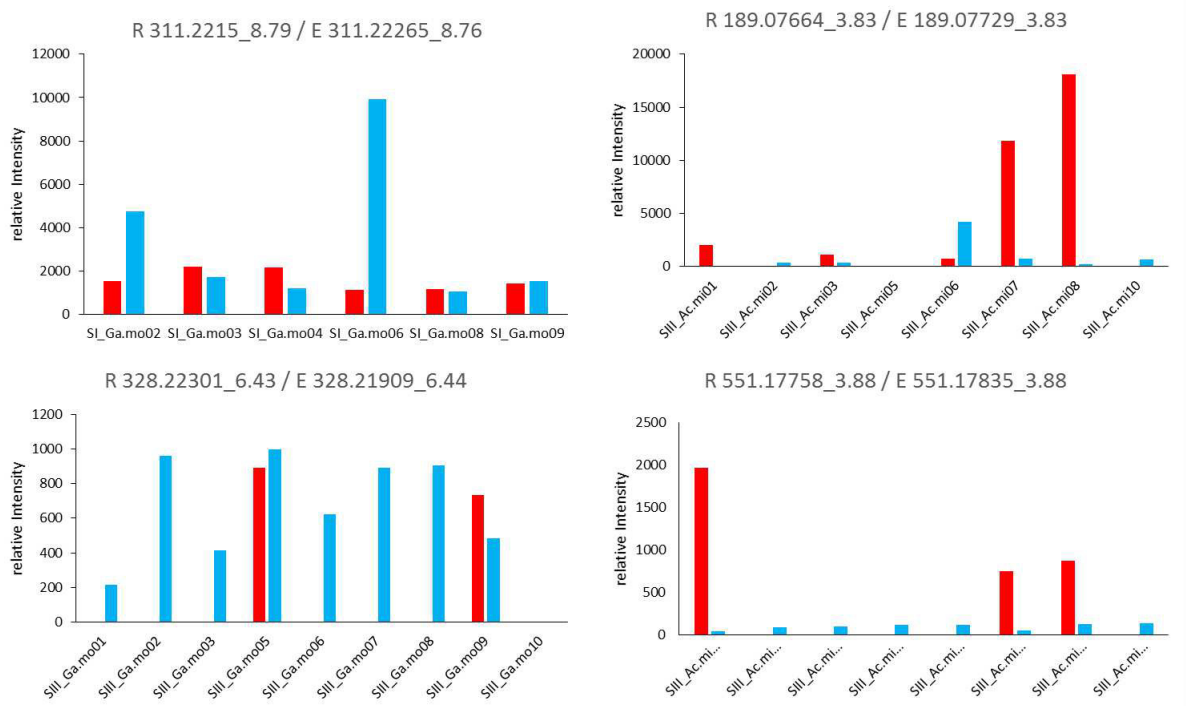

Figure S1: Critical root compounds. Bar charts present the metabolites presenting a higher relative intensity in exudate than root metabolite data sets. Blue = exuded compound, red = inner root compound. Headline comprises the compound annotation of the root (R) and exudate (E) consisting of the mass to charge ratio and retention time (in minutes).

Table S1: Distribution of samples per species and experiment. Exp = Experiment

Species	Exp I	Exp II	Exp III
A.millefolium (Ac.mi.)	4	5	8
G.mollugo (Ga.mo.)	6	8	9
P.lanceolata (Pl.la.)	8	9	9
A.elatius (Ar.el.)	8	8	9
D.glomerata (Da.gl.)	10	8	8
P.pratensis (Po.pr.)	9	9	9

Table S2: Matching compounds between field and phyto-cabinet experiments. The table presents species-specific compounds observed in phyto-cabinet experiment from the same plants. Species = Species identity, sand/Feld m/z_RT = compound identifier with m/z and RT [min] value of the phyto-cabinet and the field experiment, sample = samples, in which compound was detected, compliance = presence if compounds have a similar mass spectrum (yes) or not (empty field), question marks mark mass spectra where a compliance is not clear. SI- SIII represent the three independent experimental replications, AEG (ALB), HEG (HAI) and SEG (SCH) represent the three locations investigated in the field approaches (Dietz et al. 2018a, Dietz et al. 2018b, Herz et al. 2018).

Species	Sand/Feld mz_RT[min]	Samples in which compound deteced	Compliance
A. millefolium	177.05518_5.91 / 177.05545_5.91	SEG_Ac.mi., HEG_Ac.mi., HEG_Ac.mi., HEG_Ac.mi., HEG_Ac.mi., HEG_Ac.mi., AEG_Ac.mi.,	x
		SI_Ac.mi, SIII_Ac.mi, SIII_Ac.mi,	x
	273.17014_5.88 / 273.17061_5.86	SEG_Ac.mi., SEG_Ac.mi., HEG_Ac.mi., HEG_Ac.mi., HEG_Ac.mi., HEG_Ac.mi., HEG_Ac.mi., AEG_Ac.mi., AEG_Ac.mi., AEG_Ac.mi., SEG_Ga.mo., HEG_Ga.mo., AEG_Ga.mo., AEG_Ga.mo.,	?
		SI_Ac.mi, SI_Ac.mi, SI_Ac.mi, SI_Ac.mi, SI_Ga.mo, SI_Ga.mo, SI_Ga.mo, SI_Ga.mo, SI_Ga.mo, SI_Ga.mo, SI_Pl.la, SI_Ar.el, SI_Ar.el, SI_Ar.el, SI_Ar.el, SI_Ar.el, SI_Ar.el, SI_Da.gl, SI_Da.gl, SI_Da.gl, SI_Da.gl, SI_Da.gl, SI_Da.gl, SI_Da.gl, SI_Da.gl, SI_Da.gl, SI_Da.gl, SI_Po.pr, SI_Po.pr, SI_Po.pr, SI_Po.pr, SI_Po.pr, SI_Po.pr, SI_Po.pr, SII_Ac.mi, SII_Ac.mi, SII_Ac.mi, SII_Ac.mi, SII_Ac.mi, SII_Ga.mo, SII_Ga.mo, SII_Ga.mo, SII_Ga.mo, SII_Ga.mo, SII_Ga.mo, SII_Ga.mo, SII_Ga.mo, SII_Pl.la, SII_Pl.la, SII_Pl.la, SII_Pl.la, SII_Ar.el, SII_Ar.el, SII_Ar.el, SII_Ar.el, SII_Ar.el, SII_Ar.el, SII_Ar.el, SII_Ar.el, SII_Ar.el, SII_Da.gl, SII_Da.gl, SII_Da.gl, SII_Da.gl, SII_Da.gl, SII_Da.gl, SII_Po.pr, SII_Po.pr, SII_Po.pr, SII_Po.pr, SII_Po.pr,	?

		SII_Po.pr, SII_Po.pr, SII_Po.pr, SIII_Ac.mi, SIII_Ac.mi, SIII_Ac.mi, SIII_Ac.mi, SIII_Ac.mi, SIII_Ac.mi, SIII_Ac.mi, SIII_Ac.mi, SIII_Ga.mo, SIII_Ga.mo, SIII_Ga.mo, SIII_Ga.mo, SIII_Ga.mo, SIII_Ga.mo, SIII_Ga.mo, SIII_Ga.mo, SIII_Ga.mo, SIII_Ga.mo, SIII_Ar.el, SIII_Ar.el, SIII_Ar.el, SIII_Ar.el, SIII_Ar.el, SIII_Ar.el, SIII_Ar.el, SIII_Ar.el, SIII_Da.gl, SIII_Da.gl, SIII_Da.gl, SIII_Da.gl, SIII_Da.gl, SIII_Po.pr, SIII_Po.pr, SIII_Po.pr, SIII_Po.pr, SIII_Po.pr, SIII_Po.pr,	
	409.04435_2.6 / 409.04517_2.63	SEG_Ac.mi., SEG_Ac.mi., HEG_Ac.mi., HEG_Ac.mi., HEG_Ac.mi., HEG_Ac.mi., HEG_Ac.mi., HEG_Ac.mi., HEG_Ac.mi., AEG_Ac.mi., AEG_Ac.mi., AEG_Ac.mi., AEG_Ac.mi., HEG_Da.gl.,	x
	577.2099_3.72 / 577.21022_3.78	SI_Ac.mi, SI_Ac.mi, SIII_Ac.mi, SIII_Ac.mi, SIII_Ac.mi, SIII_Ac.mi, SIII_Ac.mi, SEG_Ac.mi., HEG_Ac.mi., HEG_Ac.mi., HEG_Ac.mi., HEG_Ac.mi., HEG_Ac.mi., HEG_Ac.mi., HEG_Ac.mi., HEG_Ac.mi., HEG_Ac.mi., AEG_Ac.mi., AEG_Ac.mi., AEG_Ac.mi., AEG_Ac.mi., SEG_Ar.el., HEG_Da.gl., SEG_Ga.mo., SEG_Ga.mo., SEG_Ga.mo., HEG_Ga.mo., HEG_Ga.mo., AEG_Ga.mo.,	x ?
G. mollugo	239.03511_7.27 / 239.03514_7.27	SI_Ga.mo, SI_Ga.mo, SIII_Ga.mo, SIII_Ga.mo, SIII_Ga.mo, SIII_Ga.mo, SEG_Ga.mo., SEG_Ga.mo., SEG_Ga.mo., HEG_Ga.mo., HEG_Ga.mo., HEG_Ga.mo., HEG_Ga.mo., HEG_Ga.mo., HEG_Ga.mo., HEG_Ga.mo., HEG_Ga.mo., HEG_Ga.mo., HEG_Ga.mo., AEG_Ga.mo., AEG_Ga.mo., AEG_Ga.mo., AEG_Ga.mo., AEG_Ga.mo., SI_Ga.mo, SIII_Ga.mo, SIII_Ga.mo, SIII_Ga.mo,	? ? ?

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	SI_Ga.mo, SIII_Ga.mo,	x
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	SIII_Ga.mo, SIII_Ga.mo,	x
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	SI_Ga.mo, SI_Ga.mo, SI_Ga.mo, SI_Ga.mo, SI_Ga.mo, SI_Ga.mo, SII_Ga.mo, SII_Ga.mo, SII_Ga.mo, SII_Ga.mo, SIII_Ga.mo, SIII_Ga.mo, SIII_Ga.mo, SIII_Ga.mo, SIII_Ga.mo, SIII_Ga.mo,	x
501.05885_7.27 / 501.05948_7.28	SEG_Ga.mo., SEG_Ga.mo., HEG_Ga.mo., HEG_Ga.mo., HEG_Ga.mo., HEG_Ga.mo., HEG_Ga.mo., HEG_Ga.mo., HEG_Ga.mo., HEG_Ga.mo., SI_Ar.el, SI_Ar.el, SI_Ar.el, SII_Ar.el, SII_Ar.el, SII_Ar.el,	
515.15213_6.25 / 515.15235_6.23	SEG_Ga.mo., SEG_Ga.mo., SEG_Ga.mo., SEG_Ga.mo., HEG_Ga.mo., HEG_Ga.mo., HEG_Ga.mo., HEG_Ga.mo., HEG_Ga.mo., HEG_Ga.mo., HEG_Ga.mo., HEG_Ga.mo., HEG_Ga.mo., HEG_Ga.mo., AEG_Ga.mo., AEG_Ga.mo., AEG_Ga.mo., AEG_Ga.mo., AEG_Ga.mo.,	x
	SI_Ga.mo, SI_Ga.mo, SI_Ga.mo, SI_Ga.mo, SI_Ga.mo, SII_Ga.mo, SII_Ga.mo, SII_Ga.mo, SII_Ga.mo, SIII_Ga.mo, SIII_Ga.mo, SIII_Ga.mo, SIII_Ga.mo, SIII_Ga.mo, SIII_Ga.mo,	x
519.17023_3.01 / 519.17003_3.01	HEG_Ga.mo., HEG_Ga.mo., HEG_Ga.mo., HEG_Ga.mo., HEG_Ga.mo., HEG_Ga.mo., HEG_Ga.mo., HEG_Ga.mo., HEG_Ga.mo., HEG_Ga.mo., AEG_Ga.mo., AEG_Ga.mo., AEG_Ga.mo., AEG_Ga.mo., AEG_Ga.mo.,	x
	SI_Ga.mo, SI_Ga.mo, SI_Ga.mo, SI_Ga.mo, SIII_Ga.mo, SIII_Ga.mo,	x
557.04896_9.66 / 557.04889_9.65	SEG_Ga.mo., SEG_Ga.mo., HEG_Ga.mo., HEG_Ga.mo., HEG_Ga.mo., HEG_Ga.mo., HEG_Ga.mo., HEG_Ga.mo., HEG_Ga.mo., AEG_Ga.mo.,	x
	SI_Ga.mo, SI_Ga.mo, SI_Ga.mo, SI_Ga.mo, SII_Ga.mo, SIII_Ga.mo, SIII_Ga.mo, SIII_Ga.mo, SIII_Ga.mo, SIII_Ga.mo, SIII_Ga.mo, SIII_Ga.mo,	x
581.1875_3.4 / 581.18661_3.39	SEG_Ga.mo., SEG_Ga.mo., SEG_Ga.mo., HEG_Ga.mo., HEG_Ga.mo., HEG_Ga.mo.,	x

		HEG_Ga.mo., HEG_Ga.mo., AEG_Ga.mo., AEG_Ga.mo.,	
		Sl_Ac.mi, Sl_Ac.mi, Sl_Ga.mo, Sl_Ga.mo, Sl_Ga.mo, Sl_Ga.mo, Sl_Ga.mo, Sl_Ga.mo, Sll_Ga.mo, Sll_Ga.mo, Sll_Ga.mo, Sll_Ga.mo, Sll_Ga.mo, Sll_Ga.mo, Sll_Ga.mo, Sll_Ga.mo, Slll_Ac.mi, Slll_Ac.mi, Slll_Ac.mi, Slll_Ac.mi, Slll_Ga.mo, Slll_Ga.mo, Slll_Ga.mo, Slll_Ga.mo, Slll_Ga.mo, Slll_Ga.mo, Slll_Ga.mo, Slll_Ga.mo,	x
	547.1437_5.49 / 547.14487_5.48	SEG_Ga.mo., SEG_Ga.mo., SEG_Ga.mo., SEG_Ga.mo., HEG_Ga.mo., HEG_Ga.mo., HEG_Ga.mo., HEG_Ga.mo., HEG_Ga.mo., HEG_Ga.mo., HEG_Ga.mo., HEG_Ga.mo., HEG_Ga.mo., HEG_Ga.mo., AEG_Ga.mo., AEG_Ga.mo., AEG_Ga.mo., AEG_Ga.mo., AEG_Ga.mo., AEG_Ga.mo., AEG_Ga.mo., AEG_Ga.mo.,	x
		Sl_Ga.mo, Sl_Ga.mo, Sl_Ga.mo, Sll_Ga.mo, Slll_Ga.mo, Slll_Ga.mo, Slll_Ga.mo, Slll_Ga.mo, Slll_Ga.mo, Slll_Ga.mo,	x
	669.23852_5.89 / 669.23933_5.88	SEG_Ga.mo., SEG_Ga.mo., HEG_Ga.mo., HEG_Ga.mo., HEG_Ga.mo., HEG_Ga.mo., HEG_Ga.mo., HEG_Ga.mo., HEG_Ga.mo., HEG_Ga.mo., AEG_Ga.mo., AEG_Ga.mo., AEG_Ga.mo., AEG_Ga.mo., AEG_Ga.mo.,	x
		Sl_Ga.mo, Sl_Ga.mo, Sl_Ga.mo, Sll_Ga.mo, Sll_Ga.mo, Sll_Ga.mo, Sll_Ga.mo, Slll_Ga.mo, Slll_Ga.mo, Slll_Ga.mo, Slll_Ga.mo, Slll_Ga.mo, Slll_Ga.mo, Slll_Ga.mo,	x
P. lanceolata	236.03711_4.99 / 236.03678_5.02	SEG_Pl.la., SEG_Pl.la., SEG_Pl.la., HEG_Pl.la., HEG_Pl.la., HEG_Pl.la., HEG_Pl.la., AEG_Pl.la., AEG_Pl.la., AEG_Pl.la., AEG_Pl.la.,	
		Sl_Pl.la, Sl_Pl.la, Sl_Pl.la, Sll_Pl.la, Sll_Pl.la, Sll_Pl.la, Slll_Pl.la, Slll_Pl.la, Slll_Pl.la, Slll_Pl.la,	

343.08532_4.52 / 343.08529_4.52	HEG_Pl.la., HEG_Pl.la., AEG_Pl.la., AEG_Pl.la., SIII_Ga.mo	
373.11304_2.01 / 373.11395_2.01	SEG_Da.gl., HEG_Ga.mo., HEG_Ga.mo., HEG_Ga.mo., HEG_Ga.mo., AEG_Ga.mo., AEG_Ga.mo., SEG_Pl.la., SEG_Pl.la., SEG_Pl.la., HEG_Pl.la., HEG_Pl.la., HEG_Pl.la., HEG_Pl.la., HEG_Pl.la., HEG_Pl.la., HEG_Pl.la., HEG_Pl.la., HEG_Pl.la., AEG_Pl.la., AEG_Pl.la., AEG_Pl.la., AEG_Pl.la., AEG_Pl.la.,	x
	SI_Ga.mo, SI_Ga.mo, SI_Ga.mo, SI_Ga.mo, SI_Ga.mo, SI_Ga.mo, SI_Pl.la, SI_Pl.la, SI_Pl.la, SI_Pl.la, SI_Pl.la, SI_Pl.la, SI_Pl.la, SII_Ga.mo, SII_Ga.mo, SII_Ga.mo, SII_Ga.mo, SII_Ga.mo, SII_Ga.mo, SII_Ga.mo, SII_Pl.la, SII_Pl.la, SII_Pl.la, SII_Pl.la, SII_Pl.la, SII_Pl.la, SII_Pl.la, SII_Pl.la, SII_Pl.la, SIII_Ga.mo, SIII_Ga.mo, SIII_Ga.mo, SIII_Ga.mo, SIII_Ga.mo, SIII_Ga.mo, SIII_Ga.mo, SIII_Ga.mo, SIII_Pl.la, SIII_Pl.la, SIII_Pl.la, SIII_Pl.la, SIII_Pl.la, SIII_Pl.la, SIII_Pl.la, SIII_Pl.la,	x
379.1593_3.34 / 379.16001_3.33	SEG_Da.gl., SEG_Pl.la., HEG_Pl.la., HEG_Pl.la., AEG_Pl.la., AEG_Pl.la.,	?
	SI_Pl.la, SI_Pl.la, SI_Pl.la, SIII_Ac.mi, SIII_Pl.la, SIII_Pl.la, SIII_Pl.la, SIII_Pl.la, SIII_Pl.la,	?
381.17501_3.78 / 381.17503_3.77	HEG_Pl.la., HEG_Pl.la., HEG_Pl.la., AEG_Pl.la.,	x
	SI_Ga.mo, SI_Ga.mo, SI_Ga.mo, SI_Pl.la, SI_Pl.la, SI_Pl.la, SI_Pl.la, SI_Pl.la, SI_Pl.la, SIII_Ac.mi, SIII_Pl.la, SIII_Pl.la, SIII_Pl.la, SIII_Pl.la, SIII_Pl.la, SIII_Pl.la, SIII_Pl.la,	x
391.12398_1.09 / 391.12395_1.09	AEG_Ac.mi., SEG_Ar.el., SEG_Da.gl., HEG_Da.gl., AEG_Da.gl., SEG_Pl.la., SEG_Pl.la., SEG_Pl.la., HEG_Pl.la., HEG_Pl.la., HEG_Pl.la., HEG_Pl.la., HEG_Pl.la., HEG_Pl.la.,	?

	HEG_Pl.la., HEG_Pl.la., AEG_Pl.la., AEG_Pl.la., AEG_Pl.la., AEG_Pl.la., AEG_Pl.la., AEG_Pl.la., AEG_Pl.la., SEG_Po.pr.,	
	SI_Pl.la, SI_Pl.la, SI_Pl.la, SI_Pl.la, SI_Pl.la, SI_Pl.la, SI_Pl.la, SI_Pl.la, SII_Pl.la, SII_Pl.la, SII_Pl.la, SII_Pl.la, SII_Pl.la, SII_Pl.la, SII_Pl.la, SII_Pl.la, SII_Pl.la, SII_Pl.la, SIII_Ga.mo, SIII_Ga.mo, SIII_Pl.la, SIII_Pl.la, SIII_Pl.la, SIII_Pl.la, SIII_Pl.la, SIII_Pl.la, SIII_Pl.la, SIII_Pl.la,	?
393.12177_5.65 / 393.12228_5.64	SEG_Pl.la., SEG_Pl.la., HEG_Pl.la., HEG_Pl.la., HEG_Pl.la., SI_Ar.el, SI_Ar.el, SI_Ar.el, SII_Ar.el, SII_Ar.el, SII_Ar.el,	
401.14474_3.46 / 401.145_3.43	SEG_Da.gl., SEG_Pl.la., SEG_Pl.la., SEG_Pl.la., SEG_Pl.la., HEG_Pl.la., HEG_Pl.la., HEG_Pl.la., HEG_Pl.la., HEG_Pl.la., HEG_Pl.la., HEG_Pl.la., AEG_Pl.la., AEG_Pl.la., AEG_Pl.la., AEG_Pl.la., AEG_Pl.la., AEG_Pl.la.,	?
	SI_Ac.mi, SI_Ga.mo, SI_Ga.mo, SI_Pl.la, SI_Pl.la, SI_Pl.la, SI_Pl.la, SI_Pl.la, SI_Pl.la, SI_Pl.la, SII_Ac.mi, SII_Pl.la, SII_Pl.la, SII_Pl.la, SII_Pl.la, SII_Pl.la, SII_Pl.la, SII_Pl.la, SII_Pl.la, SIII_Ac.mi, SIII_Ac.mi, SIII_Ac.mi, SIII_Ga.mo, SIII_Ga.mo, SIII_Pl.la, SIII_Pl.la, SIII_Pl.la, SIII_Pl.la, SIII_Pl.la, SIII_Pl.la, SIII_Pl.la, SIII_Pl.la, SIII_Pl.la, SIII_Pl.la,	?
403.15957_4.36 / 403.16098_4.35	SEG_Ac.mi., HEG_Ac.mi., HEG_Ac.mi., AEG_Ac.mi., AEG_Ar.el., SEG_Da.gl., SEG_Da.gl., SEG_Da.gl., HEG_Da.gl., SEG_Pl.la., SEG_Pl.la., SEG_Pl.la., SEG_Pl.la., HEG_Pl.la., HEG_Pl.la., HEG_Pl.la., HEG_Pl.la., HEG_Pl.la., HEG_Pl.la., HEG_Pl.la., HEG_Pl.la., AEG_Pl.la., AEG_Pl.la., AEG_Pl.la., SEG_Po.pr., HEG_Po.pr.,	?

	SI_Ac.mi, SI_Pl.la, SI_Pl.la, SI_Pl.la, SI_Pl.la, SI_Pl.la, SI_Pl.la, SIII_Ac.mi, SIII_Ac.mi, SIII_Ac.mi, SIII_Ac.mi, SIII_Ac.mi,	?
415.15965_3.9 / 415.16009_3.88	SEG_Da.gl., SEG_Pl.la., SEG_Pl.la., HEG_Pl.la., HEG_Pl.la., HEG_Pl.la., AEG_Pl.la., AEG_Pl.la., AEG_Pl.la., AEG_Pl.la.,	?
	SI_Pl.la, SI_Pl.la, SI_Pl.la, SI_Pl.la, SI_Pl.la, SI_Pl.la, SI_Pl.la, SII_Ac.mi, SII_Pl.la, SII_Pl.la, SII_Pl.la, SII_Pl.la, SIII_Ac.mi, SIII_Pl.la, SIII_Pl.la, SIII_Pl.la, SIII_Pl.la, SIII_Pl.la,	?
417.21199_4.85 / 417.21213_4.84	HEG_Ac.mi., SEG_Ar.el., SEG_Ar.el., SEG_Ar.el., SEG_Ar.el., AEG_Ar.el., AEG_Ar.el., SEG_Da.gl., SEG_Da.gl., SEG_Da.gl., SEG_Da.gl., SEG_Da.gl., HEG_Da.gl., HEG_Da.gl., HEG_Da.gl., HEG_Da.gl., AEG_Da.gl., SEG_Pl.la., SEG_Pl.la., SEG_Pl.la., SEG_Pl.la., HEG_Pl.la., HEG_Pl.la., HEG_Pl.la., HEG_Pl.la., HEG_Pl.la., HEG_Pl.la., HEG_Pl.la., HEG_Pl.la., AEG_Pl.la., AEG_Pl.la., AEG_Pl.la., AEG_Pl.la., SEG_Po.pr., SEG_Po.pr., SEG_Po.pr., HEG_Po.pr., HEG_Po.pr., SI_Ac.mi, SI_Ga.mo, SI_Pl.la, SI_Pl.la, SI_Pl.la, SI_Pl.la, SI_Ar.el, SI_Ar.el, SI_Ar.el, SI_Ar.el, SI_Ar.el, SI_Ar.el, SI_Da.gl, SI_Da.gl, SI_Da.gl, SI_Da.gl, SI_Da.gl, SI_Da.gl, SI_Da.gl, SI_Da.gl, SI_Da.gl, SI_Po.pr, SI_Po.pr, SI_Po.pr, SI_Po.pr, SI_Po.pr, SI_Po.pr, SII_Pl.la, SII_Pl.la, SII_Ar.el, SII_Ar.el, SII_Da.gl, SII_Da.gl, SII_Da.gl, SII_Po.pr, SII_Po.pr, SII_Po.pr, SII_Po.pr, SII_Po.pr, SIII_Ga.mo, SIII_Pl.la, SIII_Pl.la, SIII_Pl.la, SIII_Pl.la, SIII_Pl.la, SIII_Ar.el, SIII_Ar.el, SIII_Po.pr, SIII_Po.pr, SIII_Po.pr,	

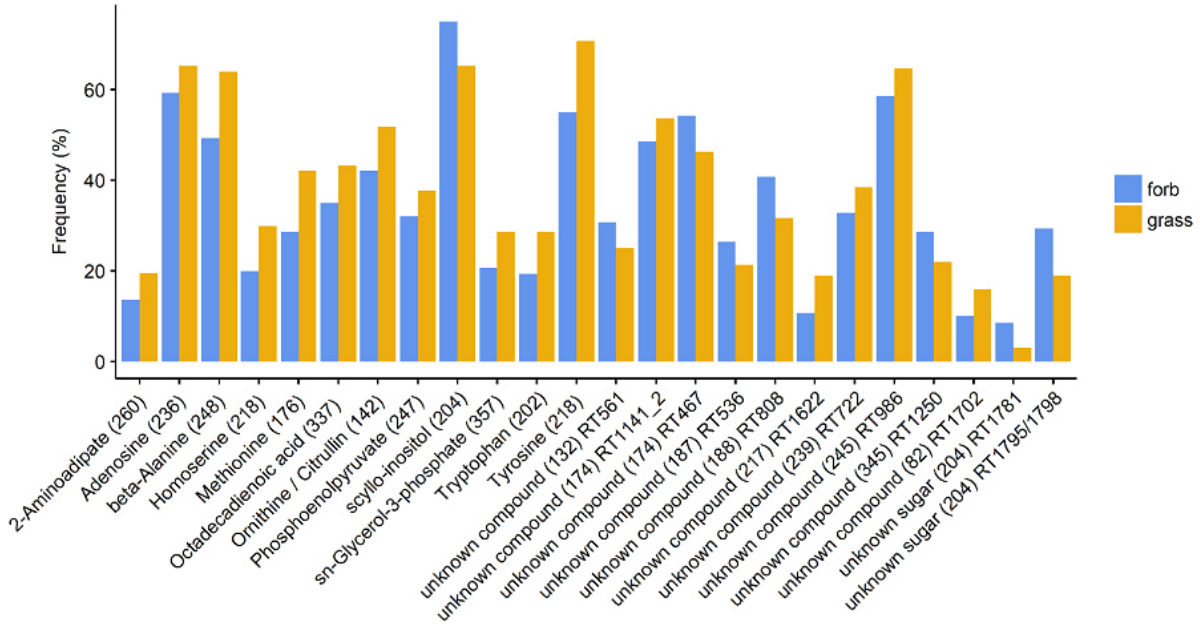
427.19668_5.51 /	SEG_Ar.el., SEG_Da.gl., SEG_Da.gl.,	x
427.19726_5.51	SEG_Pl.la., SEG_Pl.la., SEG_Pl.la.,	
	SEG_Pl.la., HEG_Pl.la., HEG_Pl.la.,	
	HEG_Pl.la., HEG_Pl.la., HEG_Pl.la.,	
	HEG_Pl.la., HEG_Pl.la., HEG_Pl.la.,	
	HEG_Pl.la., AEG_Pl.la., AEG_Pl.la.,	
	AEG_Pl.la., AEG_Pl.la., AEG_Pl.la.,	
	SEG_Po.pr., SEG_Po.pr.,	
	SII_Ar.el, SII_Ar.el,	x
433.13348_3.13 /	SEG_Ar.el., HEG_Da.gl., SEG_Pl.la.,	?
433.13332_3.13	SEG_Pl.la., SEG_Pl.la., SEG_Pl.la.,	
	HEG_Pl.la., HEG_Pl.la., HEG_Pl.la.,	
	HEG_Pl.la., HEG_Pl.la., HEG_Pl.la.,	
	HEG_Pl.la., AEG_Pl.la., AEG_Pl.la.,	
	AEG_Pl.la., AEG_Pl.la., AEG_Pl.la.,	
	AEG_Pl.la.,	
	SI_Pl.la, SI_Pl.la, SIII_Ac.mi, SIII_Pl.la,	?
	SIII_Pl.la, SIII_Pl.la, SIII_Pl.la,	
439.05638_1.79 /	HEG_Ac.mi., HEG_Ac.mi., HEG_Ac.mi.,	
439.05542_1.79	HEG_Ac.mi., HEG_Ac.mi., HEG_Ac.mi.,	
	HEG_Ac.mi., AEG_Ac.mi., SEG_Da.gl.,	
	SEG_Da.gl., SEG_Da.gl., SEG_Da.gl.,	
	HEG_Da.gl., SEG_Ga.mo., SEG_Pl.la.,	
	SEG_Pl.la., SEG_Pl.la., HEG_Pl.la.,	
	HEG_Pl.la., HEG_Pl.la., HEG_Pl.la.,	
	HEG_Pl.la., HEG_Pl.la., HEG_Pl.la.,	
	AEG_Pl.la., AEG_Pl.la., AEG_Pl.la.,	
	AEG_Pl.la., AEG_Pl.la., AEG_Pl.la.,	
	SEG_Po.pr.,	
	SI_Ac.mi, SI_Ac.mi, SI_Pl.la, SI_Pl.la,	
	SI_Po.pr, SI_Po.pr, SI_Po.pr, SI_Po.pr,	
	SI_Po.pr, SI_Po.pr, SIII_Ac.mi, SIII_Ac.mi,	
	SIII_Ac.mi, SIII_Ac.mi, SIII_Pl.la, SIII_Po.pr,	
	SIII_Po.pr,	
443.19128_5.28 /	SEG_Da.gl., SEG_Pl.la., SEG_Pl.la.,	?
443.19097_5.27	HEG_Pl.la., HEG_Pl.la., HEG_Pl.la.,	
	HEG_Pl.la., HEG_Pl.la., HEG_Pl.la.,	
	HEG_Pl.la., AEG_Pl.la., AEG_Pl.la.,	
	AEG_Pl.la.,	
	SI_Pl.la, SI_Pl.la, SI_Pl.la, SII_Pl.la,	?
	SII_Pl.la, SII_Pl.la, SIII_Pl.la, SIII_Pl.la,	

465.13996_3.67 / 465.14007_3.66	HEG_Da.gl., AEG_Da.gl., SEG_Pl.la., SEG_Pl.la., SEG_Pl.la., HEG_Pl.la., HEG_Pl.la., HEG_Pl.la., HEG_Pl.la., HEG_Pl.la., HEG_Pl.la., HEG_Pl.la., HEG_Pl.la., AEG_Pl.la., AEG_Pl.la., AEG_Pl.la., AEG_Pl.la., AEG_Pl.la., AEG_Pl.la., AEG_Pl.la., AEG_Pl.la.,	x
	SI_Pl.la, SI_Pl.la, SI_Pl.la, SI_Pl.la, SI_Pl.la, SI_Pl.la, SI_Pl.la, SI_Pl.la, SII_Pl.la, SII_Pl.la, SII_Pl.la, SII_Pl.la, SII_Pl.la, SII_Pl.la, SII_Pl.la, SII_Pl.la, SIII_Pl.la, SIII_Pl.la, SIII_Pl.la, SIII_Pl.la, SIII_Pl.la, SIII_Pl.la, SIII_Pl.la, SIII_Pl.la, SIII_Pl.la, SIII_Ar.el,	x
465.13976_4.05 / 465.14051_4.04	HEG_Da.gl., SEG_Pl.la., HEG_Pl.la., HEG_Pl.la., HEG_Pl.la., HEG_Pl.la., AEG_Pl.la., AEG_Pl.la., AEG_Pl.la.,	?
	SI_Pl.la, SI_Pl.la, SI_Pl.la, SI_Pl.la, SI_Pl.la, SI_Pl.la, SI_Pl.la, SII_Pl.la, SII_Pl.la, SII_Pl.la, SII_Pl.la, SII_Pl.la, SII_Pl.la, SII_Pl.la, SIII_Pl.la, SIII_Pl.la, SIII_Pl.la, SIII_Pl.la, SIII_Pl.la, SIII_Pl.la,	?
487.18118_4.84 / 487.18083_4.83	SEG_Ar.el., SEG_Pl.la., SEG_Pl.la., SEG_Pl.la., SEG_Pl.la., HEG_Pl.la., HEG_Pl.la., HEG_Pl.la., HEG_Pl.la., HEG_Pl.la., HEG_Pl.la., HEG_Pl.la., AEG_Pl.la., AEG_Pl.la., AEG_Pl.la., AEG_Pl.la., AEG_Pl.la., AEG_Pl.la., AEG_Pl.la.,	x
	SI_Pl.la, SI_Pl.la, SI_Pl.la, SI_Pl.la, SII_Pl.la, SII_Pl.la, SIII_Pl.la, SIII_Pl.la, SIII_Pl.la, SIII_Pl.la, SIII_Pl.la, SIII_Pl.la,	x
491.17597_3.37 / 491.17596_3.35	HEG_Pl.la., HEG_Pl.la., HEG_Pl.la., HEG_Pl.la., HEG_Pl.la., AEG_Pl.la.,	x
	SI_Pl.la, SI_Pl.la, SI_Pl.la, SI_Pl.la, SI_Ar.el, SI_Ar.el, SII_Ar.el, SIII_Pl.la, SIII_Pl.la, SIII_Pl.la, SIII_Pl.la, SIII_Da.gl, SIII_Da.gl,	x
511.14537_3.64 / 511.14452_3.63	HEG_Da.gl., HEG_Pl.la., HEG_Pl.la., AEG_Pl.la., AEG_Pl.la.,	x

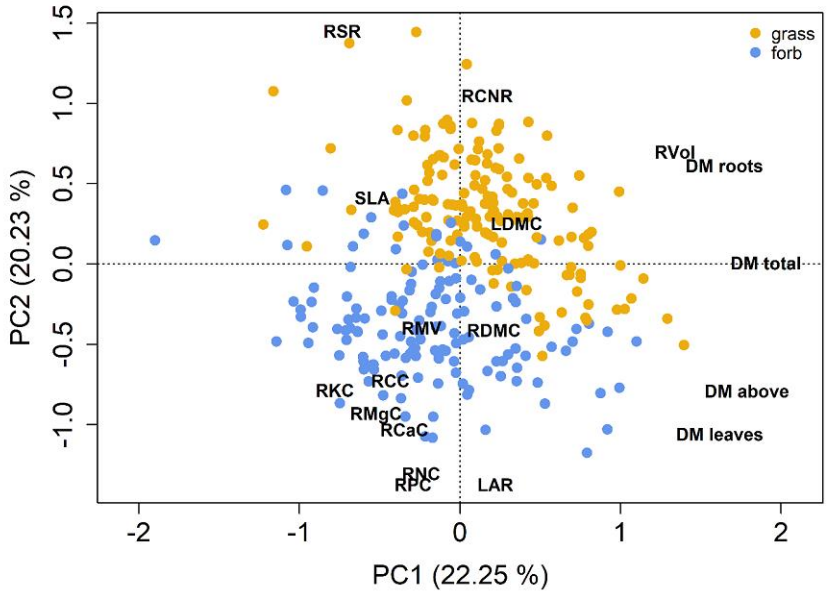
		SI_Pl.la, SI_Pl.la, SI_Pl.la, SI_Pl.la, SII_Pl.la, SIII_Pl.la, SIII_Pl.la, SIII_Pl.la, SIII_Pl.la,	x
	549.12301_3.55 / 549.12265_3.54	SEG_Pl.la., SEG_Pl.la., SEG_Pl.la., HEG_Pl.la., HEG_Pl.la., HEG_Pl.la., HEG_Pl.la., HEG_Pl.la., HEG_Pl.la., AEG_Pl.la., AEG_Pl.la., AEG_Pl.la., AEG_Pl.la., AEG_Pl.la.,	x
		SI_Pl.la, SI_Pl.la, SI_Pl.la, SI_Pl.la, SII_Pl.la, SII_Pl.la, SII_Pl.la, SII_Pl.la, SII_Pl.la, SII_Pl.la, SIII_Pl.la, SIII_Pl.la, SIII_Pl.la, SIII_Pl.la, SIII_Pl.la,	x
	585.16148_4.85 / 585.16067_4.85	HEG_Ac.mi., SEG_Pl.la., HEG_Pl.la., HEG_Pl.la., HEG_Pl.la., AEG_Pl.la., AEG_Pl.la.,	x
		SI_Pl.la, SI_Pl.la, SII_Pl.la, SII_Pl.la, SII_Pl.la, SIII_Pl.la, SIII_Pl.la, SIII_Pl.la,	x
A. elatius	341.10725_5.74 / 341.10669_5.74	SEG_Ar.el., SEG_Ar.el., SEG_Ar.el., AEG_Ar.el., AEG_Ar.el., AEG_Ar.el., HEG_Ga.mo., AEG_Ga.mo.,	?
		SI_Ga.mo, SI_Ga.mo, SI_Ga.mo, SI_Ga.mo, SI_Ar.el, SI_Ar.el, SI_Ar.el, SI_Ar.el, SI_Ar.el, SI_Da.gl, SI_Da.gl, SII_Ga.mo, SII_Ar.el, SII_Ar.el, SII_Ar.el, SII_Ar.el, SII_Ar.el, SII_Ar.el, SIII_Ga.mo, SIII_Ga.mo, SIII_Ga.mo, SIII_Ga.mo, SIII_Ga.mo, SIII_Ga.mo, SIII_Ar.el, SIII_Ar.el, SIII_Ar.el, SIII_Ar.el, SIII_Ar.el, SIII_Da.gl, SIII_Da.gl, SIII_Da.gl, SIII_Po.pr,	?
	501.31977_7.74 / 501.32007_7.72	SEG_Ar.el., SEG_Ar.el., SEG_Ar.el., SEG_Ar.el., AEG_Ar.el., AEG_Ar.el., AEG_Ar.el., SIII_Ga.mo	
	529.3155_9.18 / 529.31656_9.17	SEG_Ar.el., SEG_Ar.el., SEG_Ar.el., HEG_Ar.el., AEG_Ar.el., AEG_Ar.el., AEG_Ar.el., AEG_Ar.el.,	x

P. pratensis		SI_Ar.el, SI_Ar.el, SI_Ar.el, SII_Ar.el, SII_Ar.el, SII_Ar.el, SII_Ar.el, SIII_Da.gl, SIII_Da.gl, SIII_Da.gl,	x
	535.30982_8.88 / 535.30997_8.86	SEG_Ar.el., SEG_Ar.el., SEG_Ar.el., HEG_Ar.el., AEG_Ar.el., SI_Pl.la, SI_Pl.la,	
	537.18177_3.46 / 537.18217_3.45	SEG_Ar.el., SEG_Ar.el., HEG_Ar.el., HEG_Ar.el., HEG_Ar.el., AEG_Ar.el., AEG_Ar.el., AEG_Ar.el., AEG_Ar.el., AEG_Ar.el., AEG_Ar.el., AEG_Ar.el., SEG_Po.pr., HEG_Po.pr.,	?
		SI_Ar.el, SI_Ar.el, SI_Ar.el, SI_Ar.el, SI_Ar.el, SI_Ar.el, SII_Ar.el, SII_Ar.el, SII_Ar.el, SIII_Ac.mi, SIII_Ac.mi, SIII_Ac.mi, SIII_Ac.mi, SIII_Da.gl, SIII_Da.gl, SIII_Da.gl, SIII_Da.gl, SIII_Da.gl,	?
	545.30973_7.28 / 545.30975_7.32	SEG_Ar.el., SEG_Ar.el., SEG_Ar.el., SEG_Ar.el., AEG_Ar.el., AEG_Ar.el., AEG_Ar.el., AEG_Ar.el., SI_Ar.el, SI_Ar.el, SII_Ar.el, SII_Ar.el,	
	545.31105_8.32 / 545.31107_8.32	SEG_Ar.el., SEG_Ar.el., AEG_Ar.el., AEG_Ar.el., AEG_Ar.el., SEG_Da.gl.,	?
		SI_Pl.la, SI_Pl.la, SI_Pl.la, SII_Pl.la, SIII_Pl.la, SIII_Pl.la, SIII_Pl.la, SIII_Pl.la, ,	?
	555.03733_3.72 / 555.03687_3.74	AEG_Ar.el., SEG_Da.gl., SEG_Da.gl., SEG_Po.pr., SEG_Po.pr., HEG_Po.pr., HEG_Po.pr., HEG_Po.pr., AEG_Po.pr.,	x
		SI_Ar.el, SI_Po.pr, SI_Po.pr, SI_Po.pr, SI_Po.pr, SI_Po.pr, SII_Ar.el, SII_Po.pr, SIII_Da.gl, SIII_Da.gl, SIII_Po.pr, SIII_Po.pr, SIII_Po.pr,	x

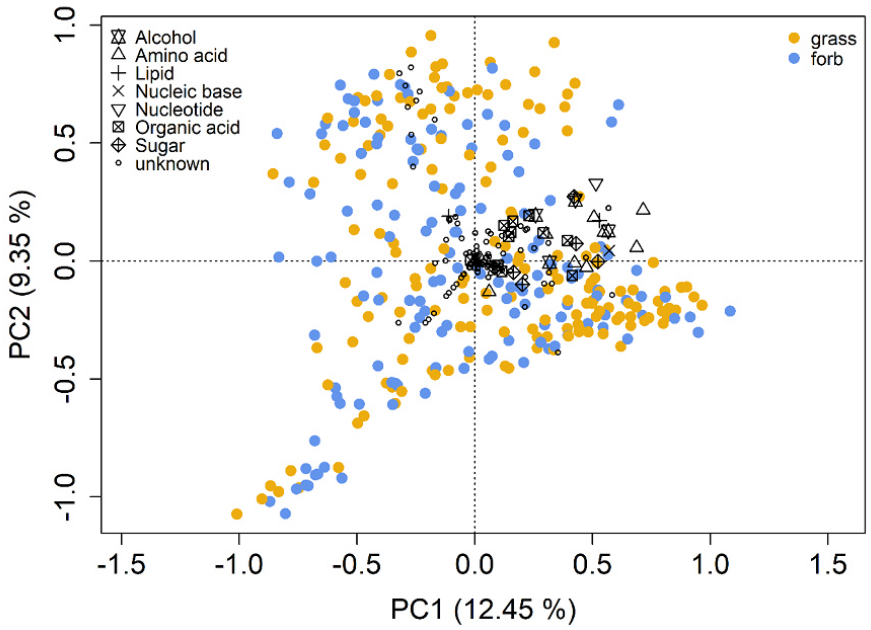
Chapter 2.2.



S1 Fig. Frequency of selected metabolites occurring in the two growth forms. The values present the number of samples of forbs or grasses, respectively, in which a specific metabolite was detected, divided by the total number of analysed samples per growth form. Thereby, the graph presents all metabolites with a difference of more than 5 % in total occurrence between the two growth forms.

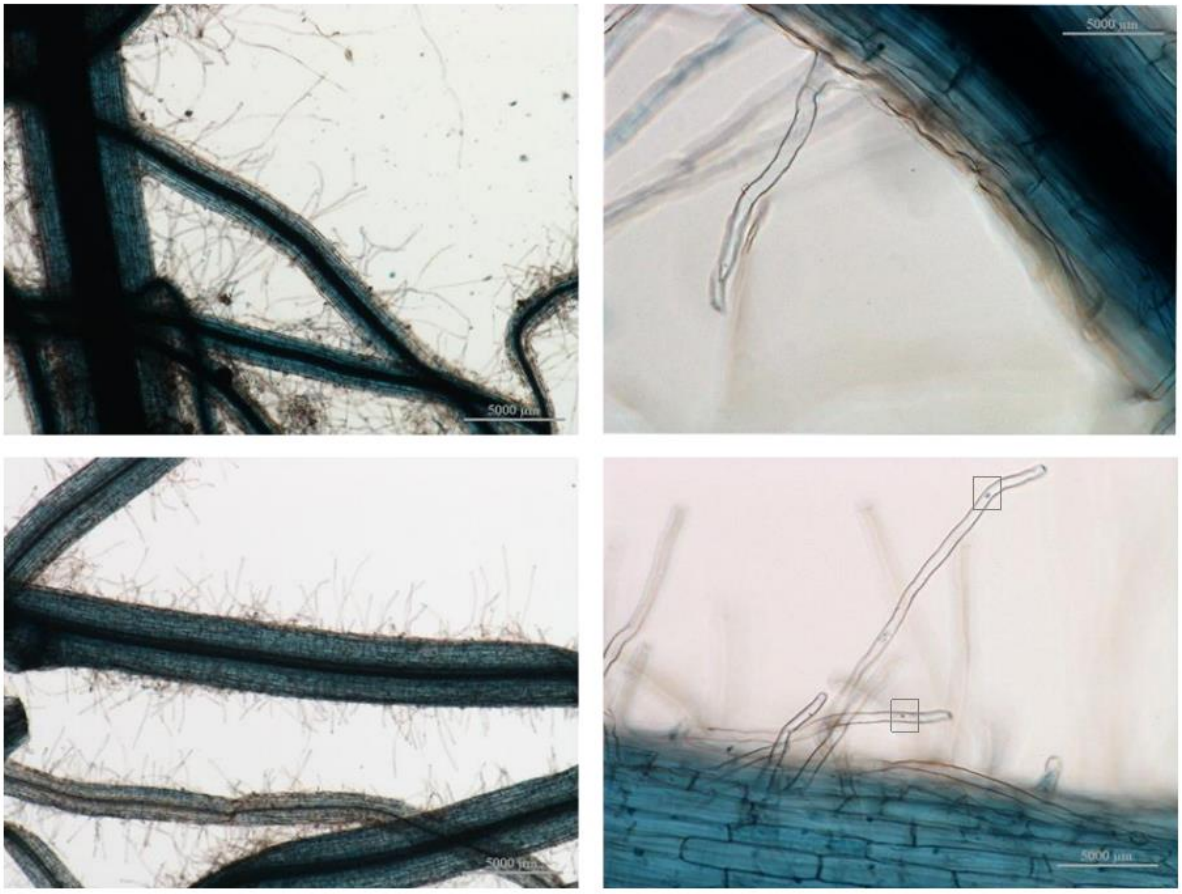


S2 Fig. Principal component analysis (PCA) of plant traits. For abbreviations see S2 Table. Colours represent the two growth forms.

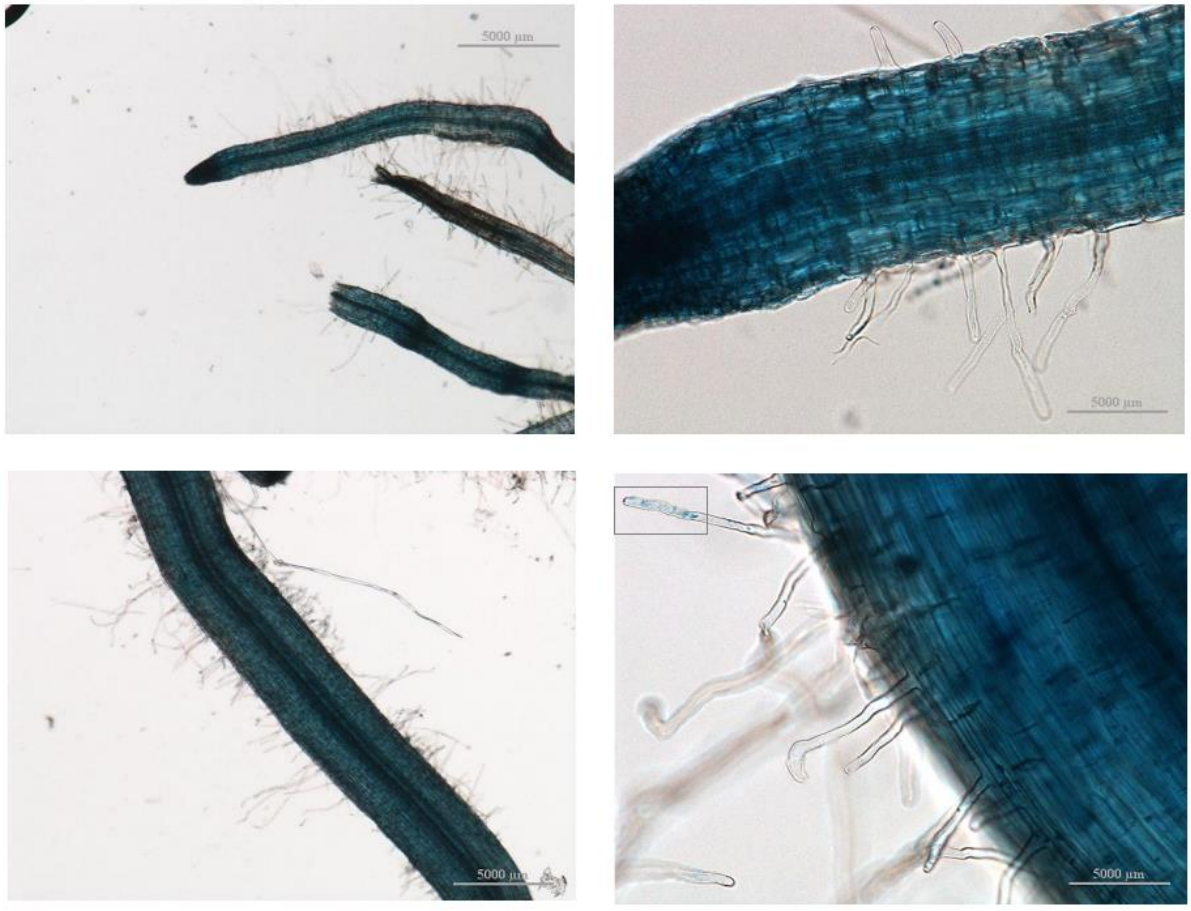


S3 Fig. Principal component analysis (PCA) of polar metabolites. Exudates were standardized by an internal standard (Ribitol (217)) and transformed into a presence/absence matrix. Colours represent the two growth forms. Symbols show compounds that could be attributed to the 7 substance classes (see S3 Table) and unknown compounds. This ordination was used to run a procrustes analysis (Fig. 3). To check for spatial autocorrelation we calculated Moran's I by using the scores of the first and second axis of the PCA as response variable and across all speciesfound a marginal significant spatial autocorrelation on the first (observed difference in scores - 0.008028366, expected -0.00330033, p = 0.091), but not on the second axis (observed difference in scores - 0.0007582542, expected - 0.00330033, p = 0.431). To exclude the plot effect, we calculated Moran's I for the first axis by species, which gave a marginal significant positive spatial autocorrelation for 1 out of the 10 species, *Ranunculus acris* (p=0.0501).

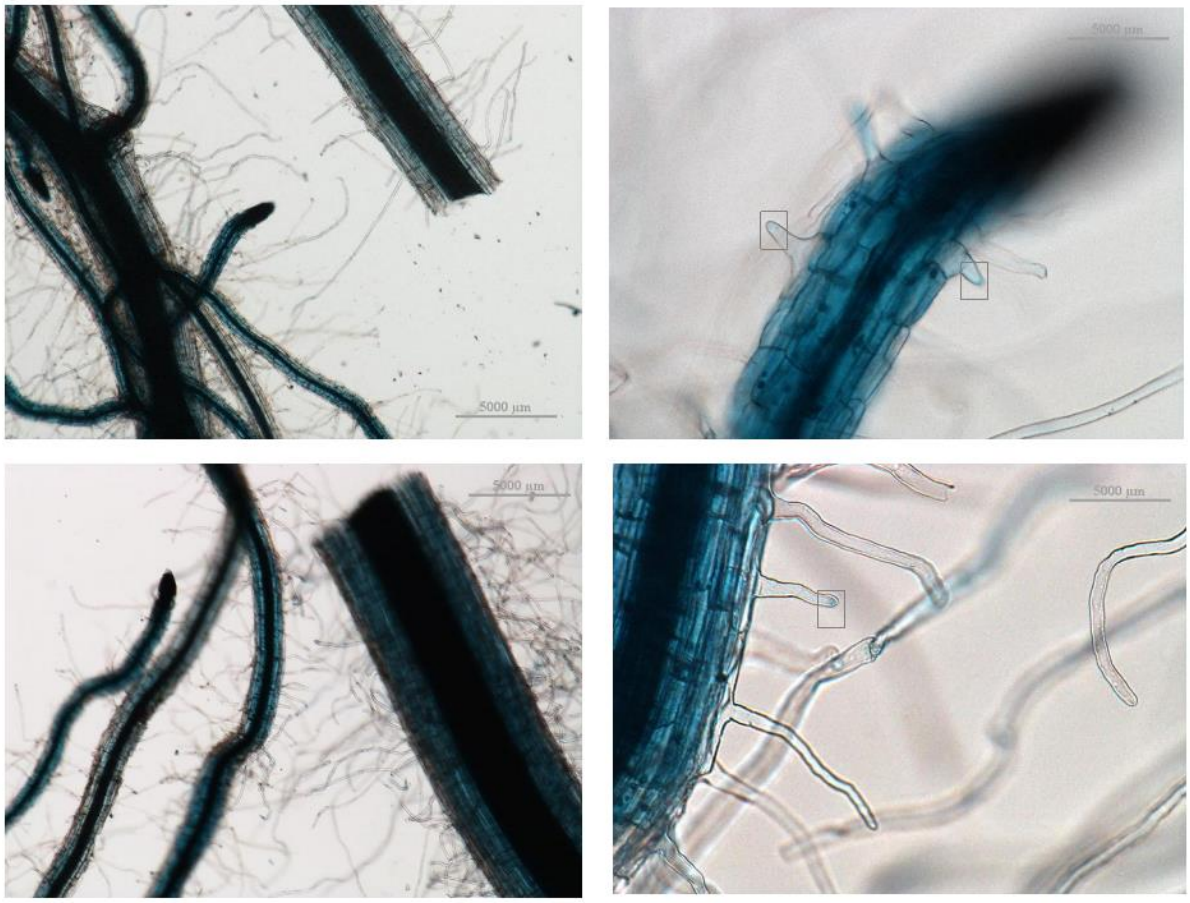
S1 File. Representative microscopic images of exuded plant roots stained by Trypan blue. Dead and damaged root hair cells are coloured blue and marked by squares, whereas undamaged cells are colourless. Blue colour of the main root is provoked by the sum of staining of the cell layers.



a. Top left: Overview image of *Plantago lanceolata* main root. Top right: Zoomed image of *P. lanceolata* root hairs. Bottom left: Overview image of *Arrhenatherum elatius* main root. Bottom right: Zoomed image of *A. elatius* root hairs.



b. *P. lanceolata*: Top left: Overview image of *P. lanceolata* main root and root tip. Top right: Zoomed image of *P. lanceolata* root hairs at the root tip; bottom left: Overview image of *P. lanceolata* main root. Bottom right: Zoomed image of *Plantago lanceolata* root hairs.



c. *A. elatius*: Top left: Overview image of *A. elatius* main root and root tip. Top right: Zoomed image of *A. elatius* root hairs at the root tip; bottom left: Overview image of *A. elatius* main root. Bottom right: Zoomed image of *A. elatius* root hairs.

S1 Table. Number of samples from each of the ten study species. No. individuals show the number of individuals which could be analysed in this study. In total 304 phytometer individuals were analysed, 164 grasses and 140 forbs.

Growth form	Family	Species	No. individuals
Grass	*Poaceacea*	*Alopecurus pratensis* L.	37
Grass	*Poaceacea*	*Arrhenatherum elatius* (L.) P.Beauv. ex J.Presl & C.Presl.	29
Grass	*Poaceacea*	*Dactylis glomerata* L.	39
Grass	*Poaceacea*	*Lolium perenne* L.	30
Grass	*Poaceacea*	*Poa pratensis* L.	29
Forb	*Asteraceae*	*Achillea millefolium* L.	29
Forb	*Rubiaceae*	*Galium mollugo* L.	33
Forb	*Rubiaceae*	*Galium verum* L.	27
Forb	*Plantaginaceae*	*Plantago lanceolata* L.	29
Forb	*Ranunculaceae*	*Ranunculus acris* L.	22

S2 Table. List of used plant traits including abbreviations, category, unit and description.

Trait	Abbreviation	Unit	Description
Leaf dry matter content	LDMC	mg/g	Leaf dry mass per leaf fresh mass
Specific leaf area	SLA	m^2/kg	Leaf area per leaf dry mass
Leaf area ratio	LAR	cm^2/g	Leaf area per total dry mass
Root dry matter content	RDMC	mg/g	Root dry mass per root fresh mass
Root to shoot ratio	RSR		Root dry mass per aboveground dry mass
Root volume	RVol	cm^3	Root volume
Root mass per volume	RMV	g/cm^3	Root dry mass per scanned root volume
Root carbon content	RCC	%	Root carbon content
Root nitrogen content	RNC	%	Root nitrogen content
Root carbon to nitrogen ratio	RCNR		Root carbon to nitrogen ratio
Root phosphorus content	RPC	µmol/g	Root phosphorus content
Root potassium content	RKC	µmol/g	Root potassium content
Root magnesium content	RMgC	µmol/g	Root magnesium content
Root calcium content	RCaC	µmol/g	Root calcium content
Root dry mass	DM roots	g	Root dry mass
Leaf dry mass	DM leaves	g	Leaf dry mass
Aboveground dry mass	DM above	g	including shoots, leaves and flowers
Total dry mass	DM total	g	Dry mass of whole plant

S3 Table. **List of identified polar metabolites of the gas chromatography coupled mass spectrometry approach.** Columns show the substance, the affiliation to natural substance classes, the mass of quantification, the corresponding retention index and the number of plant samples per growth form in which the metabolites occur, out of a total of 164 and 140 for grass and forb species, respectively.

Metabolite	Metabolite class	Quantification mass [m/z]	Retention index [RI]	No. of grass phytometers	No. of forb phytometers
Pinitol (260)	Alcohol	260	1868	120	97
scyllo-inositol (204)	Alcohol	204	2072	107	124
scyllo-inositol (305)	Alcohol	305	1995	73	64
Xylitol (307)	Alcohol	307	1735	82	89
Arginine (256)	Amino acid	256	1843	7	2
Asparagine (245)	Amino acid	245	1697	16	8
beta-Alanine (248)	Amino acid	248	1473	105	49
Glutamine (155)	Amino acid	155	1485	1	1
Homoserine (218)	Amino acid	218	1463	49	24
Lysine (156)	Amino acid	156	1939	28	21
Methionine (176)	Amino acid	176	1532	69	23
Ornithine / Citrullin (142)	Amino acid	142	1840	85	52
Tryptophan (202)	Amino acid	202	2250	47	20
Tyrosine (218)	Amino acid	218	1960	116	57
Digalactosylglycerol (204)	Lipid	204	3218	143	117
Octadecadienoic acid (337)	Lipid	337	2218	71	44
Octadecatrienoic acid (335)	Lipid	335	2230	1	2
sn-Glycerol-3-phosphate (357)	Lipid	357	1797	47	33
Adenine (264)	Nuclic base	264	1883	101	77
Adenosine (236)	Nucliotide	236	2679	107	77
2-Aminoadipate (260)	Organic acid	260	1739	32	13
2-Isopropylmalate (275)	Organic acid	275	1600	7	4
3-Caffeoyl-trans-quinic acid (345)	Organic acid	345	3166	2	2

Metabolite	Metabolite class	Quantification mass [m/z]	Retention index [RI]	No. of grass phytometers	No. of forb phytometers
4-Aminobutanoate [GABA] (174)	Organic acid	174	1543	157	122
Aminomalonic acid (218)	Organic acid	218	1483	26	12
Gluconate (333)	Organic acid	333	2049	157	137
Phosphoenol-pyruvate (247)	Organic acid	247	1623	62	48
Ribonic_acid-gamma-lactone_like (204) RT1888	Organic acid	204	2784	43	32
Salicylic acid (267)	Organic acid	267	1533	11	8
Shikimate (204)	Organic acid	204	1841	153	119
Syringic acid (342)	Organic acid	342	1912	66	33
Glucose 6-phosphate (387)	Sugar	387	2393	14	2
Lactose (361)	Sugar	361	2734	48	48
Melibiose (361)	Sugar	361	2946	140	112
Myo-Inositol-1-phosphate (318)	Sugar	318	2487	13	10
Rhamnose (117)	Sugar	117	1756	130	115

S4 Table. List of all metabolites occurring in the two growth forms. Values (in percent) for grasses and forbs show in how many samples of these two growth forms all detected metabolites occur. Metabolites with a difference of more than 5 % are marked with an asterisk and presented in the bar plot in S1 Fig.

Metabolite	Growth form		Difference	
	grass	forb		
2-Aminoadipate (260)	19.512	13.571	5.941	*
2-Isopropylmalate (275)	4.268	3.571	0.697	
3-Caffeoyl-trans-Quinic acid (345)	1.220	2.857	1.638	
4-Aminobutanoate [GABA] (174)	95.732	92.143	3.589	
Adenine (264)	61.585	57.143	4.443	
Adenosine (236)	65.244	59.286	5.958	*
Aminomalonic acid (218)	15.854	12.857	2.997	
Arginine (256)	4.268	3.571	0.697	
Asparagine (245)	9.756	7.143	2.613	
beta-Alanine (248)	64.024	49.286	14.739	*
Digalactosylglycerol (204)	87.195	87.857	0.662	
Gal_spec unknown (204)	26.220	23.571	2.648	
RT1888_Ribonic_acilactone				
Gluconate (333)	95.732	95.000	0.732	
Glucose-6-phosphate (387)	8.537	5.000	3.537	
Glutamine (155)	0.610	1.429	0.819	
sn-Glycerol-3-phosphate (357)	28.659	20.714	7.944	*
Homoserine (218)	29.878	20.000	9.878	*
Lactose (361)	29.268	30.714	1.446	
Melibiose (361)	85.366	80.714	4.652	
Lysine (156)	17.073	14.286	2.787	
Methionine (176)	42.073	28.571	13.502	*
Myo-Inositol-1-phosphate (318)	7.927	7.143	0.784	
Octadecadienoic acid (337)	43.293	35.000	8.293	*
Octadecatrienoic acid (335)	0.610	0.714	0.105	
Ornithine / Citrullin (142)	51.829	42.143	9.686	*
Phosphoenolpyruvate (247)	37.805	32.143	5.662	*
Pinitol (260)	73.171	73.571	0.401	
Rhamnose (117)	79.268	79.286	0.017	

Metabolite	Growth form		Difference	
	grass	forb		
scyllo-inositol (305)	44.512	47.143	2.631	
Shikimate (204)	93.293	92.857	0.436	
Syringic acid (342)	40.244	40.000	0.244	
Tryptophan (202)	28.659	19.286	9.373	*
Tyrosine (218)	70.732	55.000	15.732	*
Xylitol (307)	50.000	54.286	4.286	
unknown compound (184) RT704	0.610	0.714	0.105	
unknown compound (261) RT1755	7.317	9.286	1.969	
unknown compound (306) RT832	4.268	7.143	2.875	
unknown compound (349) RT1833	6.098	7.857	1.760	
unknown compound (217) RT1622	18.902	10.714	8.188	*
unknown sugar (319) RT1278	0.000	2.143	2.143	
unknown sugar (319) RT1481	2.439	2.143	0.296	
unknown sugar (319) RT1485	68.293	69.286	0.993	
unknown sugar (204) RT1613	91.463	92.857	1.394	
unknown sugar (204) RT1781	3.049	8.571	5.523	*
unknown sugar (261) RT1789	3.659	7.143	3.484	
unknown sugar (204) RT1795/1798	18.902	29.286	10.383	*
unknown sugar (361) RT1972, Melibiose	0.000	4.286	4.286	
unknown compound (214) RT436_2	19.512	17.143	2.369	
unknown compound (214) RT437	17.073	15.000	2.073	
unknown compound (175) RT438	1.220	4.286	3.066	
unknown compound (153) RT445	21.341	23.571	2.230	
unknown compound (153) RT445_2	0.610	0.000	0.610	

Metabolite	Growth form		Difference	
	grass	forb		
unknown compound (174) RT467	46.341	54.286	7.944	*
unknown compound (75) RT468	1.220	2.143	0.923	
unknown compound (71) RT483	31.098	31.429	0.331	
unknown compound (130) RT503	1.220	0.000	1.220	
unknown compound (138) RT511	2.439	2.857	0.418	
unknown compound (133) RT514	1.829	2.857	1.028	
unknown compound (355) RT528	9.146	8.571	0.575	
unknown compound (187) RT536	21.341	26.429	5.087	*
unknown compound (177) RT541	2.439	2.857	0.418	
unknown compound (188) RT544	32.317	34.286	1.969	
unknown compound (281) RT550	0.610	1.429	0.819	
unknown compound (369) RT550	2.439	2.857	0.418	
unknown compound (132) RT561	25.000	30.714	5.714	*
unknown compound (169) RT599	32.317	35.000	2.683	
unknown compound (266) RT602	77.439	76.429	1.010	
unknown compound (281) RT602	0.610	0.714	0.105	
unknown compound (369) RT609	18.293	18.571	0.279	
unknown compound (211) RT629_2	9.756	7.857	1.899	
unknown compound (341) RT696	2.439	2.143	0.296	
unknown compound (239) RT722	38.415	32.857	5.557	*
unknown compound (239) RT723	0.610	1.429	0.819	

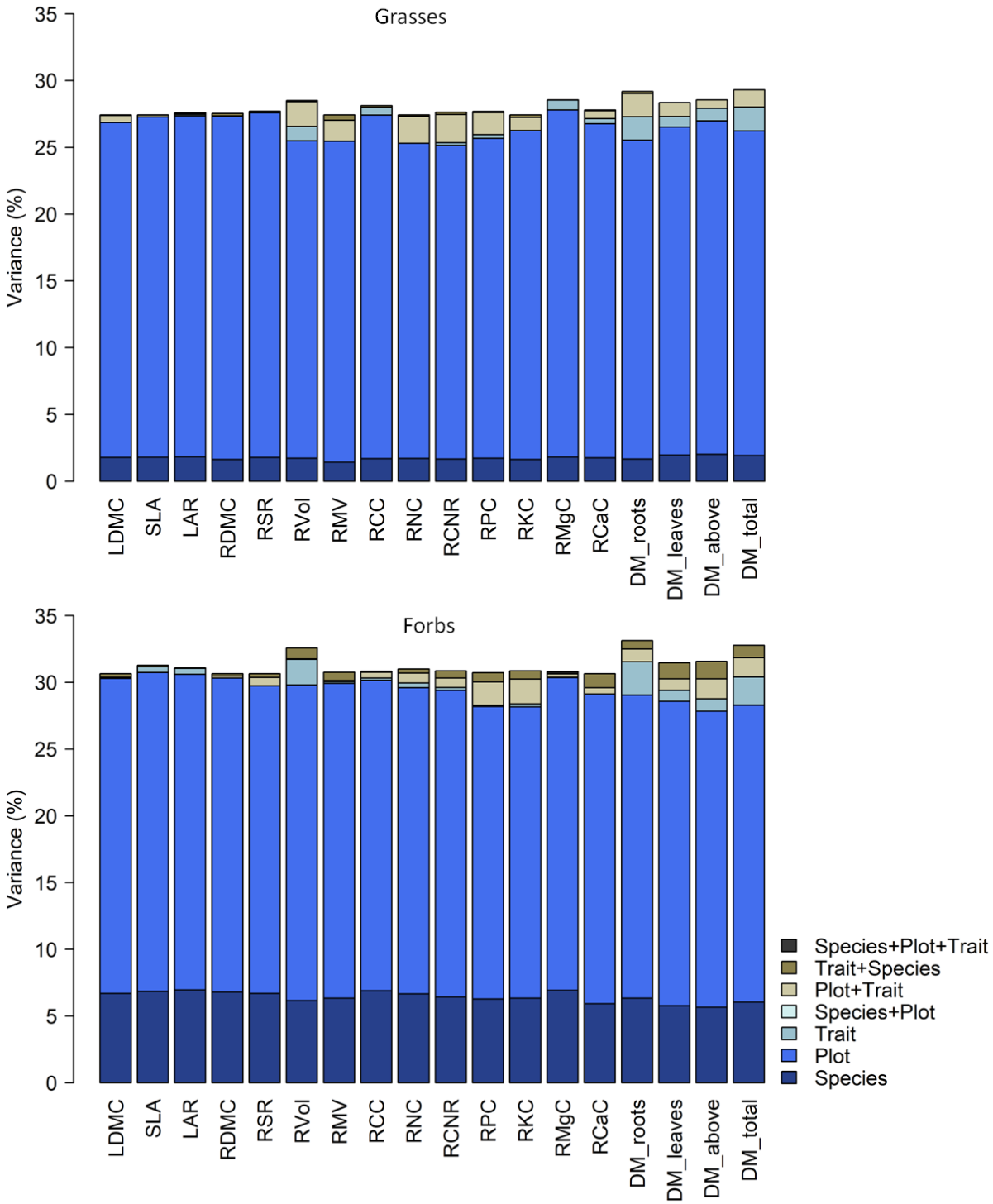
Metabolite	Growth form		Difference	
	grass	forb		
unknown compound (234) RT730	31.707	30.714	0.993	
unknown compound (156) RT781	44.512	42.143	2.369	
unknown compound (116) RT783	0.610	0.714	0.105	
unknown compound (261) RT784	4.878	2.857	2.021	
unknown compound (213) RT790	3.659	2.143	1.516	
unknown compound (234) RT801	12.805	8.571	4.233	
unknown compound (188) RT808	31.707	40.714	9.007	*
unknown compound (160) RT809	4.878	5.000	0.122	
unknown compound (188) RT837	0.000	0.000	0.000	
unknown compound (143) RT854	1.220	0.714	0.505	
unknown compound (188) RT854	0.610	0.714	0.105	
unknown compound (255) RT855	0.000	0.714	0.714	
unknown compound (247) RT865	0.610	0.714	0.105	
unknown compound (231) RT866	1.829	0.714	1.115	
unknown compound (247) RT866_2	11.585	8.571	3.014	
unknown compound (174) RT886	0.000	0.000	0.000	
unknown compound (174) RT886_2	4.268	7.857	3.589	
unknown compound (174) RT887	2.439	2.143	0.296	
unknown compound (239) RT936	16.463	15.000	1.463	
unknown compound (227) RT943	24.390	23.571	0.819	
unknown compound (129) RT968	1.220	0.000	1.220	

Metabolite	Growth form		Difference	
	grass	forb		
unknown compound (245) RT986	64.634	58.571	6.063	*
unknown compound (223) RT987	4.268	5.714	1.446	
unknown compound (245) RT987	23.780	23.571	0.209	
unknown compound (262) RT1020	0.610	5.000	4.390	
unknown compound (158) RT1048	3.049	5.714	2.666	
unknown compound (245) RT1048	1.829	2.857	1.028	
unknown compound (260) RT1063	3.049	5.714	2.666	
unknown compound (274) RT1114	9.146	6.429	2.718	
unknown compound (221) RT1115	0.610	0.714	0.105	
unknown compound (103) RT1120	0.000	0.000	0.000	
unknown compound (271) RT1139	9.146	7.143	2.003	
unknown compound (174) RT1141_2	53.659	48.571	5.087	*
unknown compound (174) RT1142	7.927	5.000	2.927	
unknown compound (217) RT1161	0.610	0.000	0.610	
unknown compound (193) RT1196	3.659	2.143	1.516	
unknown compound (174) RT1210_2	3.049	3.571	0.523	
unknown compound (174) RT1211	3.049	0.714	2.334	
unknown compound (286) RT1213	3.049	5.714	2.666	
unknown compound (374) RT1240	1.220	1.429	0.209	
unknown compound (330) RT1241	7.927	5.714	2.213	
unknown compound (345) RT1250	21.951	28.571	6.620	*

Metabolite	Growth form		Difference	
	grass	forb		
unknown compound (299) RT1308_2	0.000	0.714	0.714	
unknown compound (299) RT1308	7.317	9.286	1.969	
unknown compound (204) RT1389	2.439	2.857	0.418	
unknown compound (82) RT1481	2.439	3.571	1.132	
unknown compound (82) RT1486	0.000	0.000	0.000	
unknown compound (167) RT1517	4.268	5.714	1.446	
unknown compound (148) RT1541	2.439	2.857	0.418	
unknown compound (148) RT1541_2	1.220	2.143	0.923	
unknown compound (129) RT1551	0.610	1.429	0.819	
unknown compound (82) RT1558	3.659	2.857	0.801	
unknown compound (357) RT1570	0.610	1.429	0.819	
unknown compound (285) RT1632	2.439	2.143	0.296	
unknown compound (287) RT1632	1.220	0.714	0.505	
unknown compound (167) RT1639	3.659	5.714	2.056	
unknown compound (239) RT1642	4.878	2.857	2.021	
unknown compound (217) RT1655	92.073	92.143	0.070	
unknown compound (297) RT1661	1.829	1.429	0.401	
unknown compound (82) RT1702	15.854	10.000	5.854	*
unknown compound (97) RT1714	14.024	13.571	0.453	
unknown compound (56) RT1715	0.610	1.429	0.819	
unknown compound (199) RT1720	0.000	0.714	0.714	

Metabolite	Growth form		Difference
	grass	forb	
unknown compound (55) RT1748	0.610	0.714	0.105
unknown compound (149) RT1786	4.268	5.714	1.446
unknown compound (85) RT1847	2.439	2.857	0.418
unknown compound (85) RT1847_2	1.829	2.857	1.028
unknown compound (361) RT1927	0.000	0.714	0.714
unknown compound (204) RT1934	1.829	1.429	0.401

S2 Files. Explained variance of exudate composition using single traits. We applied variance partitioning to a model containing target species identity, plot and one of the traits on the x-axis as predictors separately for grasses and forbs. Residual variance is not shown. For abbreviations see S1. For specific values see S7.



S3 Files. Explained variance of exudate data in grasses and forbs using single traits.

a. Explained variance of exudate data in grasses using single traits. The columns show exclusively explained variance by target species identity, plot and one of the listed traits, as well as the variance jointly explained by the combinations of these factors.

	Species	Plot	Trait	Species+Plot	Plot+Trait	Trait+Species	Species+Plot+Trait	Residuals
LDMC	1.77	25.08	0.00	0.00	0.53	0.04	0.00	73.77
SLA	1.80	25.47	0.00	0.00	0.14	0.02	0.00	73.83
LAR	1.82	25.53	0.11	0.00	0.08	0.00	0.04	73.41
RDMC	1.63	25.70	0.03	0.00	0.00	0.18	0.00	73.49
RSR	1.78	25.81	0.03	0.00	0.00	0.04	0.05	73.48
Rvol	1.72	23.76	1.08	0.00	1.85	0.09	0.00	72.44
RMV	1.42	24.04	0.00	0.00	1.57	0.39	0.00	73.65
RCC	1.69	25.73	0.57	0.00	0.00	0.13	0.00	72.94
RNC	1.71	23.60	0.00	0.00	2.01	0.11	0.00	73.64
RCNR	1.65	23.49	0.20	0.00	2.12	0.16	0.00	73.32
RPC	1.72	23.96	0.27	0.00	1.65	0.09	0.00	73.25
RKC	1.63	24.63	0.00	0.00	0.98	0.19	0.00	73.51
RMgC	1.81	25.99	0.74	0.00	0.00	0.00	0.00	72.77
RCaC	1.75	25.03	0.38	0.00	0.58	0.07	0.00	73.14
DM_roots	1.66	23.87	1.76	0.00	1.74	0.16	0.00	71.75
DM_leaves	1.94	24.57	0.79	0.00	1.04	0.00	0.00	72.72
DM_above	2.01	24.97	0.93	0.00	0.64	0.00	0.01	72.58
DM_total	1.91	24.32	1.79	0.00	1.30	0.00	0.00	71.73

b. Explained variance of exudate data in forbs using single traits. The columns show exclusively explained variance by target species identity, plot and one of the listed traits, as well as the variance jointly explained by the combinations of these factors.

	Species	Plot	Trait	Species+Plot	Plot+Trait	Trait+Species	Species+Plot+Trait	Residuals
LDMC	6.69	23.60	0.01	0.00	0.08	0.27	0.00	71.01
SLA	6.85	23.89	0.42	0.00	0.00	0.12	0.00	70.59
LAR	6.95	23.65	0.44	0.00	0.02	0.02	0.00	70.58
RDMC	6.81	23.51	0.00	0.00	0.17	0.16	0.00	71.26
RSR	6.69	23.05	0.00	0.00	0.63	0.28	0.00	71.04
Rvol	6.16	23.65	1.92	0.00	0.03	0.81	0.00	69.10
RMV	6.34	23.58	0.12	0.00	0.10	0.63	0.00	70.90
RCC	6.90	23.25	0.18	0.00	0.43	0.07	0.00	70.84
RNC	6.66	22.94	0.36	0.00	0.73	0.31	0.00	70.66
RCNR	6.43	22.97	0.21	0.00	0.71	0.54	0.00	70.81
RPC	6.27	21.92	0.08	0.00	1.76	0.70	0.00	70.94
RKC	6.34	21.83	0.22	0.00	1.85	0.63	0.00	70.80
RMgC	6.92	23.43	0.03	0.00	0.25	0.05	0.12	70.98
RCaC	5.92	23.21	0.00	0.00	0.47	1.05	0.00	71.15
DM_roots	6.34	22.71	2.48	0.00	0.97	0.62	0.00	68.54
DM_leaves	5.76	22.82	0.82	0.00	0.85	1.21	0.00	70.20
DM_above	5.67	22.18	0.92	0.00	1.50	1.30	0.00	70.10
DM_total	6.05	22.24	2.12	0.00	1.43	0.92	0.00	68.90

Chapter 2.3.

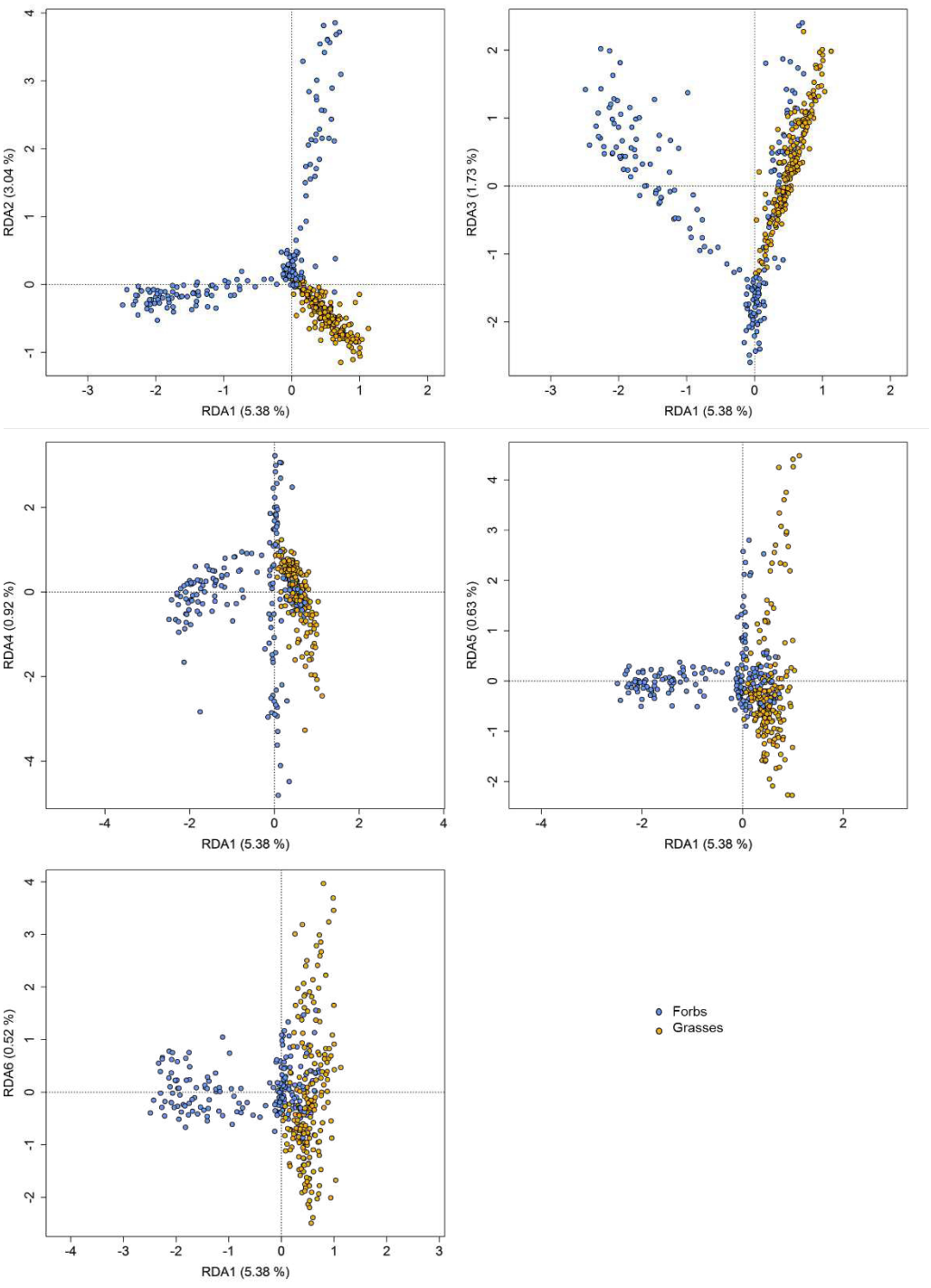

RDA2 (3.04 %)
RDA1 (5.38 %)
RDA3 (1.73 %)
RDA1 (5.38 %)
RDA4 (0.92 %)
RDA1 (5.38 %)
RDA5 (0.63 %)
RDA1 (5.38 %)
RDA6 (0.52 %)
RDA1 (5.38 %)
Forbs
Grasses

Figure A1: Redundancy analysis of semi-polar metabolites in root exudates. RDA was performed with 389 samples plotted against a presence/absence matrix of species. Axis one to six are displayed. The two growth forms are represented by colour (see legend).

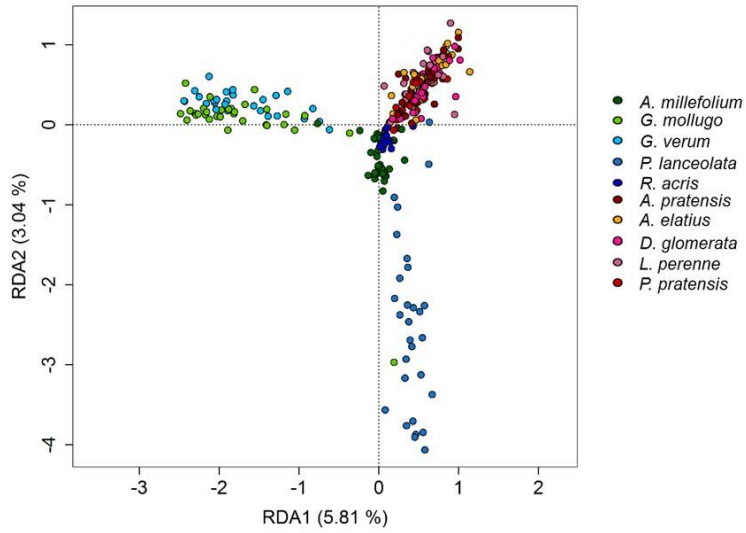


Figure A2: Redundancy analysis with semi-polar metabolites of root exudates. RDA was performed with the 302 samples for which also trait data were available. Metabolite compositions of the samples were plotted against a presence/absence matrix of species per site. The ten species are represented by colour (see legend).

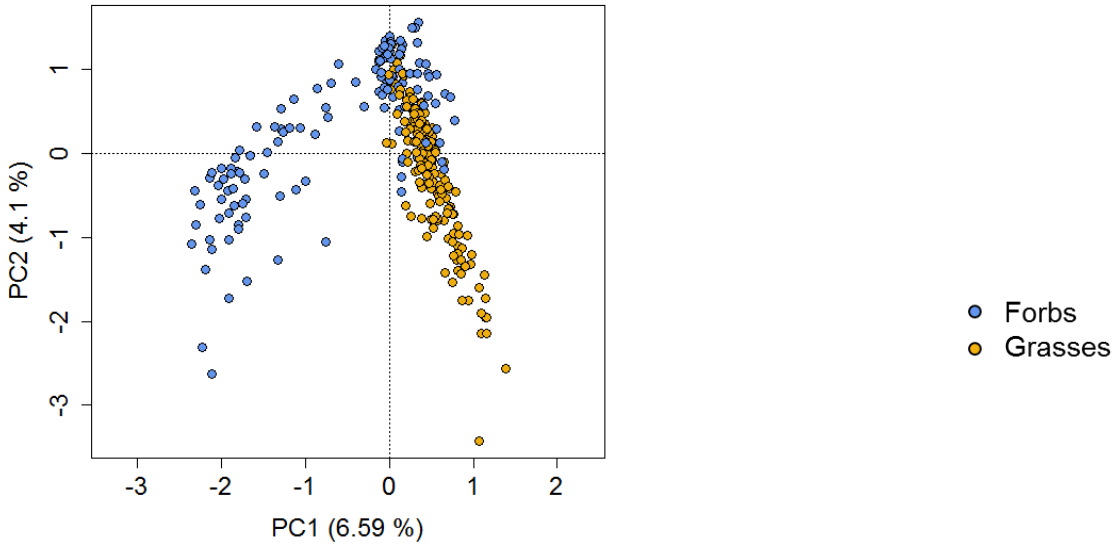


Figure A3: Principal component analysis of semi-polar metabolites in root exudates. PCA was performed with the 302 samples for which semi-polar metabolite data were also available. Colours represent growth form (see legend).

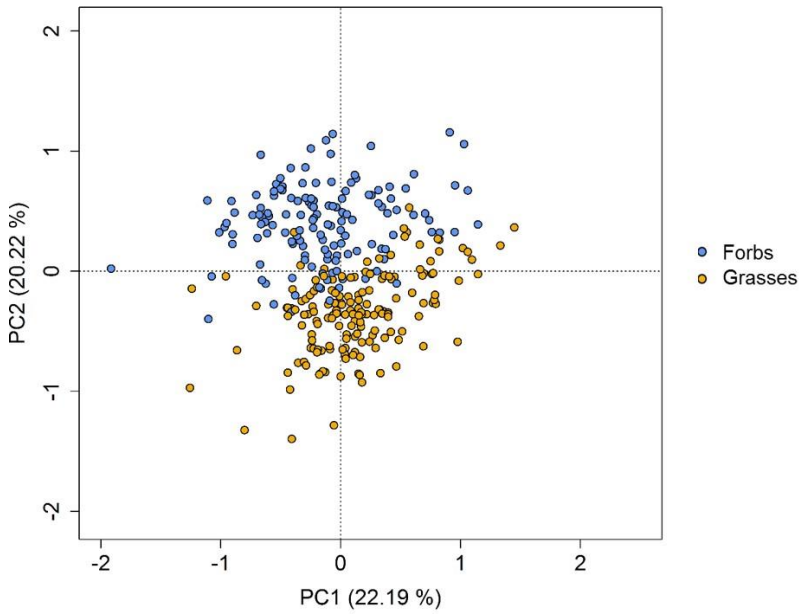


Figure A4: Principal component analysis of plant functional traits. PCA was performed with the 302 samples for which trait data were also available. Colours represent growth forms (see legend).

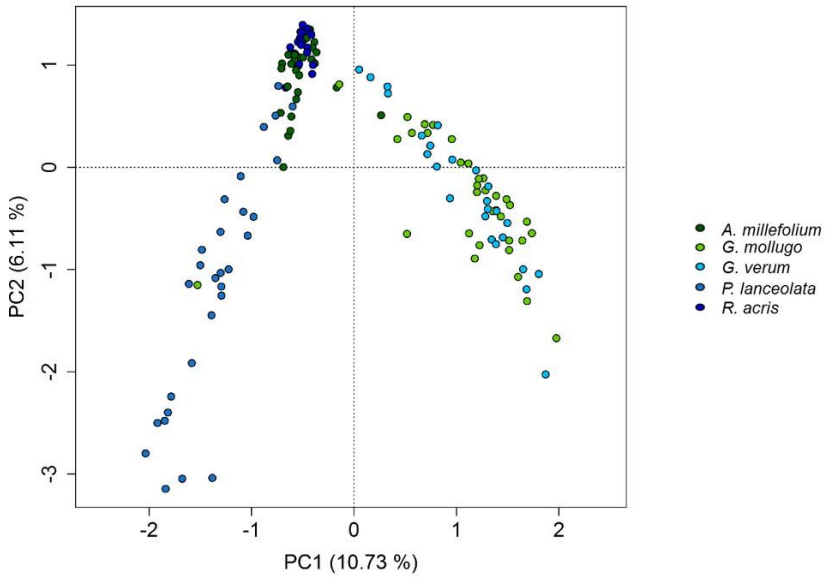


Figure A5: Principal component analysis of semi-polar metabolites in root exudates of forbs. PCA was performed with the 138 forb samples for which semi-polar metabolite data were also available. Colours represent species (see legend).

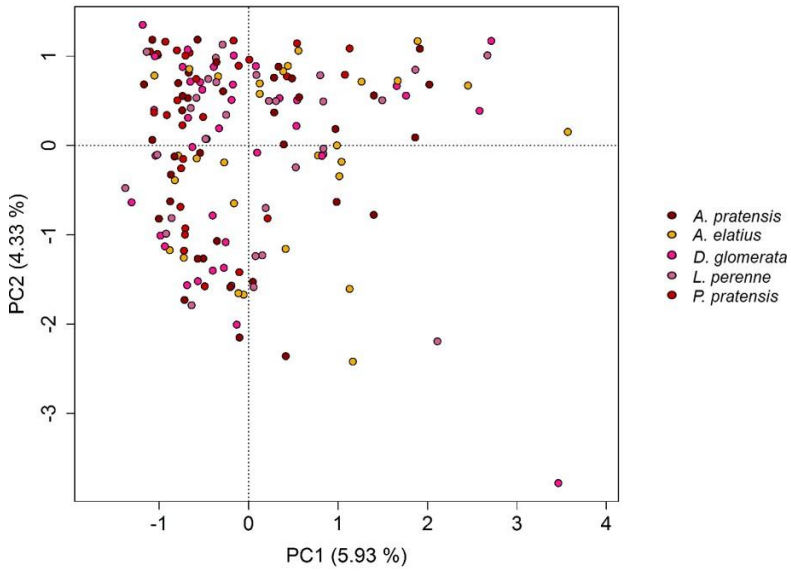


Figure A6: Principal component analysis of semi-polar metabolites in root exudates of grasses. PCA was performed with the 164 grass samples for which semi-polar metabolite data were also available. Colours represent species (see legend).

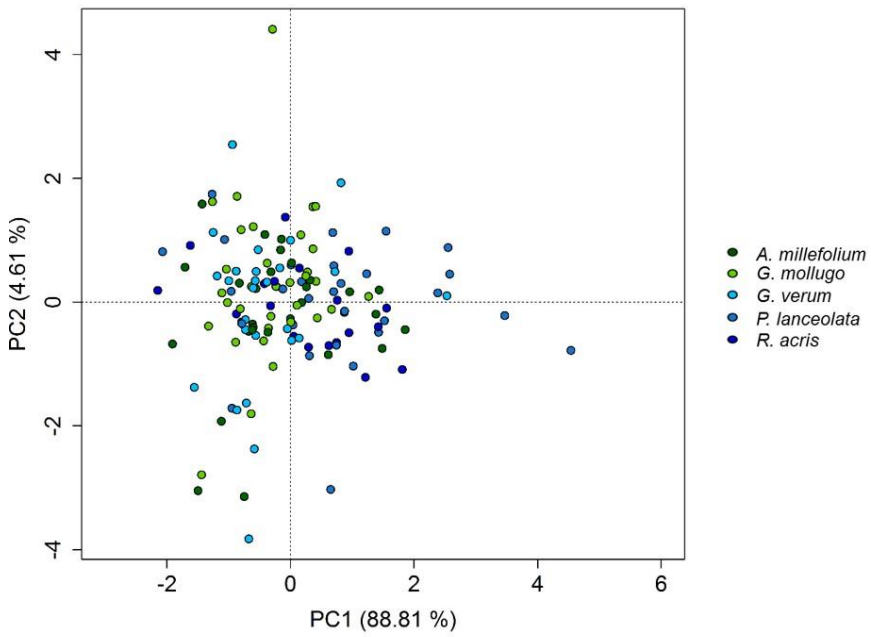


Figure A7: Principal component analysis of plant functional traits of forbs. PCA was performed with the 138 forb samples for which trait data were also available. Colours represent species (see legend).

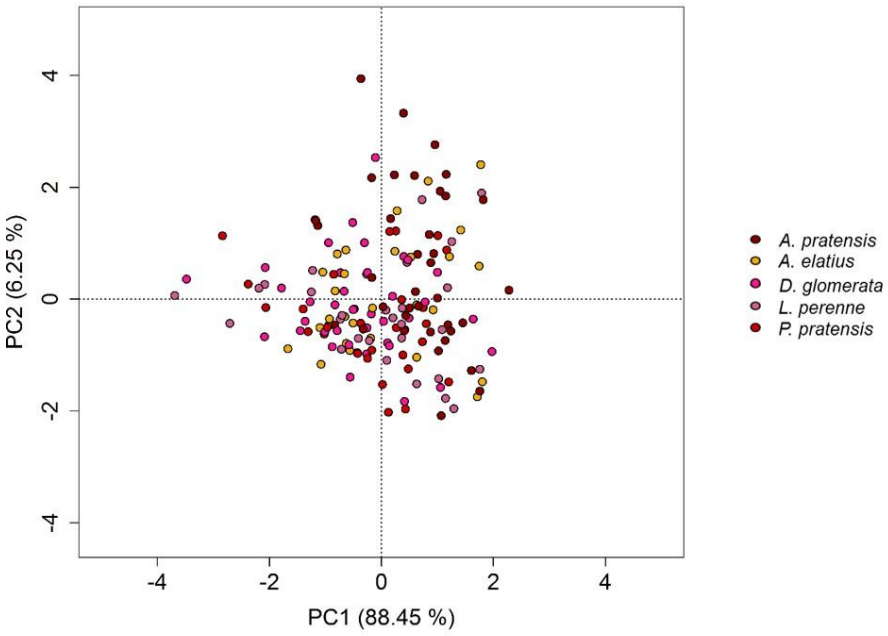


Figure A8: Principal component analysis of plant functional traits of grasses. PCA was performed with the 164 grass samples for which trait data were also available. Colours represent species (see legend).

Table A1: Identifier fragments and characteristic masses. The table contains all neutral losses and fragment ions which were used for classification of compounds in table A2.

identifier fragments	masses (m/z)
neutral losses	
Methoxylated aromatic compounds 30.0454	30.0454
Polyketides 42.0117	42.0117
Imin /Cholin ester 59.0133	59.0133
O-sulfated compounds 79.9615	79.9615
Hexose (-H_2O)162.0484	162.0484
Hexose 180.0581	180.0581
Fragment ions	
Carboxylic acid 89.0284	89.0284
Phosporous group ($H_2PO_4^-$) 96.9582	96.9582
Sulfate fragment (HSO_4^-) 96.9590	96.9590
Sugar fragment 101.023	101.023
Sugar fragment 113.0227	113.0227
Coumaroyl fragment 119.0499	119.0499
Caffeoyl fragment 135.0432	135.0432
Salicylate fragment 137.0231	137.0231
Caffeoyl fragment 145.0283	145.0283
Sinapoyl fragment 149.0226	149.0226
Dihydroxybenzoic acid, Gentisate 153.0087	153.0087
Phosphorylated fragment 158.9942	158.9942
Desoxyglucosilated fragment 161.045	161.045
Coumaroyl fragment 163.0359	163.0359
Caffeoyl fragment 163.0491	163.0491
Rhamnoside fragment 163.0715	163.0715
Sinapoyl fragment 164.0452	164.0452
Feruloyl fragment or Methoxy-coumarin fragment 175.038	175.038
Ferulic acid fragment 178.0264	178.0264
Caffeoyl fragment 179.0338	179.0338
Hexose fragment 179.0561	179.0561
Sinapic acid fragment 179.0713	179.0713
Sinapoyl-related fragment 190.0182	190.0182
Quinic acid fragment 191.057	191.057

Ferulic acid fragment 193.0514	193.0514
Sinapoyl-related fragment 205.0538	205.0538
Fraxetin fragment 207.0333	207.0333
Glycosides_1-O-methyl-β-D-glucuronate fragment 207.0657	207.0657
Sinapic acid fragment 223.0602	223.0602
Aglycon of sesquiterpene glycosides 239.0293	239.0293
Phosphatidylcholine headgroup 255.0144	255.0144
Kaempferol backbone fragment 255.029	255.029
Hexose phosphate 259.0262	259.0262
Naringenine fragment 271.0634	271.0634
Kaempferol backbone 283.0553	283.0553
Kaempferol, Luteolin, Cyanidin fragment 284.0352	284.0352
Kaempferol fragment, Anthocyanidine-backbone 285.0397	285.0397
Catechin/Epicathechin 289.0995	289.0995
Jasmonate fragment 291.1991	291.1991
Quercetin fragment 299.0585	299.0585
Quercetin fragment 300.0661	300.0661
Quercetin fragment 301.0291	301.0291
2xMe-Kaempferol fragment 313.0761	313.0761
Me-Quercetin fragment 315.0592	315.0592
Disaccharide fragment 323.0947	323.0947
2xMe-Kaempferol fragment 327.0813	327.0813
2xMe-Quercitin fragment 329.0709	329.0709
Caffeoyl-Glucose fragment 341.0821	341.0821
4xMe-Kaempferol fragment 341.1084	341.1084
Succrose fragment 341.1454	341.1454
2xMe-Myricitin fragment 345.0545	345.0545
Esculetin(4-O-8)G fragment 355.0729	355.0729
3xMe-Myricetin fragment 359.0822	359.0822
4xMe-Myricitin fragment 373.0995	373.0995
Esculetin(4-O-8)S fragment 385.0927	385.0927
Sesquiterpene diglycoside malonylated fragment 443.2687	443.2687
Agnuside fragment 465.1391	465.1391

Table A2: Putative classification of the significantly species-specific semi-polar metabolites

The table contains no. of metabolites, species, retention time (RT), m/z value, p value for significance of occurrence in single species, type of adduct, putative elemental composition, putative classification, observed neutral losses and fragment ions upon CID, whereas identifier ions (see table S2) were marked in bold. * = precursor ion in addition to fragment ions and neutral losses used for annotation of compound. All measurements were obtained in negative ionisation mode.

No.	**Species**	**RT [min]**	**m/z**	**p-value**	**Type of adduct**	**Putative Elemental composition**	**Putative Class**	**Fragment ions and neutral losses detected in CID (identifier masses in bold)**	
								Neutral losses	**Fragment ion**
1	*A. millefolium*	2.62	409.04451	$4{,}55 \times 10^{-12}$	[M-H]-	C12H16N3O11S	Unclassified, sulfated	198.0589, 212.0011, 312.0866, 227.0244	210.9914, 197.0441, **96.9582,** 182.0228
2	*A. millefolium*	3.11	619.18815	$6{,}03 \times 10^{-03}$	[M-H]-	C26H36O17	Flavonoid, glucosilated	474.1619, 502.1567, 278.0821, 506.1664, 456.1454	145.0283, 117.0312, **341.1084**, **113.0227**, **163.0491, 119.0499, 179.0561**

3	*A. millefolium*	4	421.1582	$8{,}52x10^{-04}$	[M-H]-	C25H27O4P	Glycoside	184.105, **180.0581**, 308.1367, 200.0463, **162.0484**	237.0533, 241.1095, **113.0227**, 221.1108
4	*A. millefolium*	4.43	413.14458	$2{,}14x10^{-03}$	[M-H]-	C14H26N2O12	Unclassified	221.0659, 220.0538	192.0732, 193.0845
5	*A. millefolium*	4.44	559.27218	$7{,}13x10^{-04}$	[M-H]-	C27H44O12	Phenylpropa-noid, glucosilated	208.0522, 360.2372, 207.0535, 307.0735, 349.1792, 208.1591, 358.2673, 362.1566, 259.0752, 334.2148, 283.0589, 360.1399, 476.3299, 378.1646, 353.2364, 344.1622	351.2203, 199.0348, 252.1994, 210.0863, 351.1057, 201.0071, 197.1206, 300.1976, 225.0548, 276.2086, 199.1277, 82.9457, 206.0411, 181.1174, 215.1089, **113.0227, 179.0713, 175.038**
6	*A. millefolium*	4.64	491.21285	$3{,}90x10^{-04}$	[M-H]-	C22H36O12	Glycoside	192.0655, 378.1885,	299.146, **113.0227**, **161.045**, 247.0785

								330.1615, 244.1306	
7	*A. millefolium*	4.77	509.22072	$7{,}91 \times 10^{-14}$	[M-H]-	C22H38O13	Glycoside	178.0490, 276.1558, 348.1760, 307.1988, 396.1989, 384.1977, 177.0437, 366.1900, 350.1904, 306.1959, 46.0083	331.1782, 233.0644, **161.0450**, 202.0248, **113.0227**, 125.0235, 143.0330, 159.0343, 203.0295, 463.2102, **101.0230**
8	*A. millefolium*	4.82	503.17797	$3{,}15 \times 10^{-16}$	[M-H]-	C22H32O13	Glycoside	355.1230, 340.1032, 378.1529, 390.1506, 354.1208, 270.1108	148.0485, **163.0715**, 125.0235, **113.0227**, 149.0596, 233.0644
9	*A. millefolium*	4.86	517.18406	$1{,}24 \times 10^{-07}$	[M+HCOOH-H]-, [M-H]-	C23H28N4O7	Unclassified	369.1411, 354.1208, 270.1108, 374.1600	148.0485, 163.0715, 247.0785, 143.0330, 125.0235, 113.0227

10	*A. millefolium*	4.86	537.16869	$4{,}03x10^{+00}$	[M-H]-	C32H26O8	Polyphenole, Hydroxy-cinnamicacid	390.1186, 199.0862, 214.1195, 344.1622, 410.1210, 423.1057, 279.0868, 243.0697, 350.0774, 428.1306, 342.0776, 344.1192, 241.5948, 349.0797, 399.1010	147.0460, 338.0599, 323.0545, 192.9962, 127.0393, 258.0749, 114.0531, 294.0901, 187.0943, 109.0263, 195.0830, 193.0407, 295.5674, 188.0813, **163.0359**,
11	*A. millefolium*	4.89	547.20258	$1{,}29x10^{-27}$	[M+HCOOH-H]-, [M-H]-, [M+Cl]-	C23H34O12	Phenylpropa-noid	354.1208, 369.1411, 422.1756, 384.1622, 353.1117, 300.1251	193.0845, 178.0677, 125.0235, **163.0359**, 194.0906, 247.0785, **179.0713**
12	*A. millefolium*	4.89	647.29147	$6{,}91x10^{-03}$	[M-H]-	C25H50N3O14S	Unclassified	502.2059	145.0942

13	*A. millefolium*	4.9	689.29637	$7{,}57x10^{-13}$	[M-H]-	C30H48N3O15	Unclassified	466.0796, 481.1047, 465.0809, 448.1388	223.0602, 208.0390. 224.0616, 241.0031
14	*A. millefolium*	4.91	307.10868	$2{,}40x10^{-05}$	[M-H]-	C10H18N3O8	Unclassified	15.8486, 103.0292, 88.1494, 37.6603, 46.6628, 163.5876, 225.2302, , 66.1459, 258.6425, 203.4779, 244.6099, 125.9688, 134.1051, 162.8712, 46.7496	307.0735, 291.2591, 204.0788, 218.9630, 143.5200, 260.4449, 269.4474, 81.8818, 48.4640, 240.9707, 62.4977, 103.6298, 172.9970, 181.1363, 144.2365
15	*A. millefolium*	5.02	487.18126	$3{,}00x10^{-21}$	[M-H]-, [M+Cl]-	C22H32O12	Glycoside	326.1378, 362.1566	**113.0227**, 115.0035, **161.045**
16	*A. millefolium*	5.13	401.13835	$1{,}96x10^{-09}$	[M-H]-	C18H26O10	Phenylpropa-noid	208.0811, 223.0694, 224.1493, 307.1988, 162.1258, 154.0709, 207.0731,	193.0656, 178.0677, 176.9997, 93.9404, 247.0785, 239.0293, 194.0562, 199.0748,

								202.0608, 128.9881, 61.9278, 289.3524, 238.0954, 160.0478, 214.1071, 327.7048	272.1528, 339.1974, 111.7927, **163.0491**, […]-, **373.0995**, 267.0266,
17	*A. millefolium*	5.13	469.1706	$4{,}15x10^{-14}$	[M-H]-, [M+HCOOH-H]-	C22H30O11	Unclassified	252.044, 208.0598, 251.044	217.1226.261.1088, 218.1309
18	*A. millefolium*	5.19	471.18573	$5{,}74x10^{-07}$	[M-H]-	C23H36O6S2	Unclassified	254.0618, 208.0598, 252.0539	217.1226.263, 1309, 219.1300, 66.2180, 1309, 261.1088
19	*A. millefolium*	5.36	452.19105	$6{,}58x10^{-05}$	[M-H]-	C23H27N5O5	Unclassified	254.0618, 208.0598, 252.0539, 210.0718	217.1226, 263.1309, 219.1366, 218.1309, 261.1088
20	*A. millefolium*	5.51	499.17737	$3{,}91x10^{-09}$	[M-H]-	C23H32O12	Phenylpropa-noid	321.1186	178.0677, 193.0845, **163.0359, 179.0713**, 194.0906

21	*A. millefolium*	5.51	567.18974	$3{,}90x10^{-04}$	[M-H]-	C33H28O9	Glycoside	**324.1046**	243.0664, 199.0748
22	*A. millefolium*	5.54	477.22204	$2{,}19x10^{+00}$	[M-H]-	C21H34O12	Phenylpropa-noid	240.1343, 253.1528, 236.1168, 224.1493, 209.1373, 105.1423, 284.1416, 278.1279, 135.5813, 212.0921, 176.0448, 308.1529, 221.1056, 208.0347, 223.2450	237.0799, 224.0455, 267.0680, 241.0880, 253.0495, 268.0693, 372.0577, 193.0656, 199.0748, 341.6239, 265.1088, 301.1637, 169.0459, […]-, **137.0331, 153.0087**
23	*A. millefolium*	5.95	445.23445	$1{,}10x10^{-13}$	[M-H]-	C29H34O4	Glycoside	160.0283, 253.1925, 238.1699, 295.2107, 244.1189, 332.2063, 207.0535, 343.1352,	285.1942, 192.0411, **207.0657**, 150.0288, 201.1082, **113.0227**, 238.1843,

								204.0904, 334.1868	102.1033, 241.1420, 111.0498
24	*A. millefolium*	6.1	508.24348	$2{,}37 \times 10^{-18}$	[M-H-H]-2	C50H74N4O18	Unclassified	267.1878, 208.1432, 266.1808	241.0499, 300.0969, 242.0526
25	*A. millefolium*	6.15	451.15528	$4{,}87 \times 10^{+00}$	[M-H]-	C22H28O10	Glycoside	326.1378, 338.1439, 328.1141, **324.1046**, 350.1334	125.0235, **113.0227**, 123.0437, 127.0599, **101.0230**
26	*A. millefolium*	6.5	289.12929	$6{,}91 \times 10^{-03}$	[M-H]-	C13H22O7	Unclassified		
27	*A. millefolium*	7.22	303.1444	$7{,}23 \times 10^{-02}$	[M-H]-	C15H20N4O3	Unclassified	174.0497, 190.0396, 146.0531	129.0969, 113.1028, 157.0894
28	*A. millefolium*	8.94	286.18069	$9{,}36 \times 10^{-19}$	[M-H]-, [M+HCOOH-H]-	C18H25NO2	Unclassified	120.0623	286.1750, 166.1286
29	*A. pratensis*	3.3	347.04423	$1{,}05 \times 10^{-03}$	[M-H]-	C27H8O	Polyphenole, Hydroxy-cinnamic acid	153.9980, 213.0175, 169.0206,	**193.0514**, 134.0357, **178.0264**, 194.0562, 149.0596

								152.9913, 197.9887	
30	*A. elatius*	3.69	417.1763	$3{,}61x10^{-04}$	[M-H]-	C26H26O5	Glycoside	206.0316, **180.0581**, 224.0503, 218.1546, 254.0618, 289.1342, 287.0805, 223.0400, 302.1690, 232.0961, 206.1083, 188.9991, 250.1350, 204.0904, 150.0739	211.1394, 237.1120, 193.1251, 199.0149, 163.1143, 128.0329, 130.0882, 194.1225, 115.0035, 185.0768, 211.0557, 228.1714, 167.0372, 213.0870, 267.0962
31	*A. elatius*	4.92	571.22054	$3{,}10x10^{+00}$	[M-H]-	C27H41O11P	Unclassified, phosphorylated, glucosilated	336.1860, 458.2057, 201.1309, 306.0875, 326.0443, 347.1402, 378.1885, 332.1020,	235.0498, **113.0227**, 370.0970, 265.1502, 245.1879, 224.0941, 193.0407, 239.1345,

								323.1052, 346.1186, 384.1333, 322.0820, 338.1194, 432.2342, 446.1397	248.1310, 225.1095, 187.0943, 249.1596, 233.1143, 139.0060, 125.0969, 311.1003, **179.0561**,
32	*A. elatius*	5.37	545.25984	$1{,}04x10^{-01}$	[M-H-H]-2	C59H80O19	Phenylpropa-noid, glucosilated	288.1951, 435.2376, 351.2195, 333.0900, 331.1918, 397.2122, 444.1984, 299.1887, 400.3703, 271.0627, 282.1632, 346.2394, 420.1722, 434.1783, 454.3455, 267.7391, 404.1959,	257.0639, 194.0360, 110.0155, 212.1623, 214.0614, 101.0548, 148.0485, 246.0590, 263.0813, 274.1905, 199.0149, 144.8829, […]-, **190.0182**, 311.0494, 344.0910, 218.0779, 250.0742,

								251.1830 234.2122, 355.2432, 201.1721	216.9399, […]-, **373.0995**, 298.1836, […]-, **163.0715**
33	*A. elatius*	5.62	577.25563	8,53x10^{-02}	[M-H-H]-2	C59H82NO22	Terpene	41.9252, 214.0391, 336.2472, 375.1691, 356.0985, 332.0615, 372.1222, 331.1691, 344.1192, 298.1251, 426.1724, 310.1815, 338.0836	535.3287, 363.2249, 241.0031, 202.0803, 221.1555, 245.1879, 205.1253, 246.0945, 233.1330, 279.1302, 151.0798, 267.0680, **239.0293**
34	*A. elatius*	5.62	600.26278	1,04x10^{-02}	[M-H]-	C24H59NOS7	Unclassified, glucosilated, sulfated	379.1935, 62.9272, 499.2358, 184.5433, 184.0594, 439.2198, 34.9453, 317.1984, 351.2195, 441.2322, 246.1422,	221.0671, 537.3438, **101.0230**, 415.7166, 416.1974, 161.0450, 565.3227, 283.0553, 249.0378,

								326.1542, 305.1261, 371.1090,	159.0343, [….]-, **113.0227**, [….]-, 554.3342, **96.9701**,
35	*A. elatius*	5.69	567.26537	$1{,}04x10^{-02}$	[M-H]-	C25H44O14	Unclassified, glucosilated	176.0448, 338.0836, 328.1309, 268.0672, 238.0502, 270.0939, 45.9238, 314.0875, 250.0623, 216.0324, 454.2374, 352.1821, 232.1181, 194.0543, 237.061	391.2188, 229.1727, 239.1345, 299.2021, 329.2184, 297.1781, 521.3447, 253.1796, 317.2055, 351.2203, **113.0227**, 215.0864, 373.2153, 335.1512, 330.2065
36	*A. elatius*	5.92	563.31879	$1{,}03x10^{+00}$	[M-H]-	C37H44N2O3	Terpene, glucosilated	176.0448, 338.0836, 328.1309, 268.0672,	391.2188, 229.1727, 239.1345, 299.2021,

								238.0502, 270.0939, 45.9238, 314.0875, 250.0623, 216.0324, 454.2374, 352.1821, 194.0543, 232.1181, 237.0610, 452.2092, 249.9808, 346.1655,	329.2184, 297.1781, 521.3447, 253.1796, 317.2055, 351.2203, **113.0227**, 215.0864, 373.2153, 335.1512, 330.2065, 115.0550, 221.1108, **443.2687**
37	*A. elatius*	6.76	975.50862	1,06x10^{-06}	[M+HCOOH-H]-, [M-H]-	C47H78O18	Diglycoside	754.4519, 208.0598, 712.4419, 207.0535, 502.1567, 370.1182, 814.4722, 796.4613, 652.4214, 832.4821, 862.4923, 753.4483,	221.0671, 767.4565, 263.0813, 473.3597, 605.4013, 161.0450, 179.0561, **323.0947**, 143.0330, **113.0227**, 222.0683,

38	*A. elatius*	7.57	547.32609	1,06x10^{-05}	[M-H]-	C31H48O8	Unclassified, Aromatic acid	43.9839, 42.9853, 234.1019, 260.1177, 338.2024, 296.1606,	503.3342, 313.2108, 287.1985, 209.1166, 251.1720,
39	*A. elatius*	8.09	299.07602	7,56x10^{-01}	[M-H]-	C16H12O6	Flavonoid	89.9010, 92.0235, 108.0220, 59.0133, 107.0533, 15.0215, 27.9912, 123.0423,	209.1502, **207.0333**, 191.0361, 240.0408, 192.0070, **284.0352**, **271.0634**, 176.0118,
40	*D. glomerata*	3.93	245.13851	3,13x10^{-04}	[M-H]-	C10H20N3O4	Unclassified	122.0567, 104.0515, 130.9931, 134.0537, 22.2797, 62.0349, 90.0298, 178.4211, 74.0352, 115.0552, 95.1331, 110.1478, 59.1660, 86.4543, 86.8307	123.0811, 141.0882, 114.1437, 111.0800, 222.8570, 155.1107, 183.1058, 66.7238, 130.0882, 171.1040, 150.0037, 134.9882,

									158.6784, 185.9703, 158.3060
41	*G. mollugo*	3.92	611.24752	$3{,}18 \times 10^{-06}$	[M-H]-	C27H40N4O12	Unclassified	360.1238, 404.1125, 359.1208, 403.1087,	251.1261, 207.1380, 252.1254, 208.1377
42	*G. mollugo*	4.08	509.21506	$2{,}26 \times 10^{-08}$	[M-H]-	C22H38O13	Glycoside	178.0490, 348.1760, 177.0437, 396.1989, 276.1558, 46.0083	331.1782, **161.0450**, **113.0227**, 233.0644, 463.2102
43	*G. mollugo*	4.13	377.17599	$1{,}71 \times 10^{-07}$	[M-H]-	C17H30O9	Flavonoid, glucosilated	174.1894, 189.212, 243.1335, 163.0362, 78.1171, **162.0484**, 191.1474, 79.0928, 170.0986, 160.1037, 204.0904, 75.1080, 33.4871, 132.1216, 168.2815, 204.9187,	202.9945, 187.9662, 134.0470, 214.1438, **299.0585**, 215.1294, 186.0360, 298.0767, […]-, 188.0625, 217.1526, 222.0426, 242.0731,

									253.1076, 313.0420, **113.0227**
44	*G. mollugo*	4.78	467.11897	$4{,}09 \times 10^{-08}$	[M-H]-	C21H24O12	Unclassified	265.0892, 264.0863	202.0248, 203.0295
45	*G. mollugo*	5.73	419.13406	$5{,}27 \times 10^{-15}$	[M-H]-	C21H24O9	Flavonoid	207.0535, 163.0602, 206.0509, 192.1030, 162.0590	212.0847, 256.0754, 213.0870, 227.0351, 257.0800, **255.0290**
46	*G. mollugo*	5.75	563.1378	$8{,}96 \times 10^{-01}$	[M-H]-	C26H28O14	Unclassified	295.1032, 294.0928, 310.0864, 312.1032, 340.0923, 323.0883	268.0361, 269.0481, 253.0495, 251.0338, 223.0434, 240.0408
47	*G. mollugo*	6.09	297.04047	$9{,}71 \times 10^{-21}$	[M-H]-	C14H8N3O5	Unclassified	43.9916, 87.0083, 42.9853	253.0495, 210.0334, 254.0539
48	*G. mollugo*	6.1	253.05029	$2{,}27 \times 10^{-14}$	[M+K-H]-, [M+Na-H]-	C10H15O5	Polyketide, Aromatic acetate	43.0179, **42.0117**	210.0334, 211.0377

49	*G. mollugo*	6.14	283.05943 *	8,68x10^{-07}	[M-H]-	C16H12O5	Flavonoid	30.0051, 44.0247, 73.0253, 72.0167, 29.0036, 43.0179, 15.0215	253.0495, 239.0293, 210.0334, 211.0377, 254.0539, 240.0408, 268.0361, **283.0553**
50	*G. mollugo*	6.4	691.23246	2,15x10^{-01}	[M-H]-	C33H40O16	Glycoside	**324.1046**, 323.1052, 339.1370, 382.1837	367.1275, 352.0933, 309.0403
51	*G. mollugo*	6.83	489.17542	3,98x10^{-13}	[M-H]-, [M+Cl]-	C26H26N4O6	Unclassified	205.0779, 264.0863, 260.1284, 282.0966, 248.1243, 204.0625, 220.0992	284.1042, 225.0840, 229.0428, 207.0831, 241.0499, 285.1061, 269.0796
52	*G. mollugo*	7.74	269.0503	3,10x10^{-06}	[M-H]-	C16H6N4O	Unclassified		
53	*G. verum*	6.24	299.02009	4,41x10^{-01}	[M-H]-	C15H8O7	Flavonoid	43.9839, 87.9814, 44.9635, 88.9932, 71.9868, 18.0110, 42.9853	**255.0290**, 211.0377, 254.0539, 210.0334,

									227.0351, 280.9980, 256.0316
54	*G. verum*	7.62	311.00608	$2{,}11x10^{-01}$	[M-H]-	C17H12O6	Polyketide, aromatic acetate	43.0179, **42.0117**	268.0361, 269.0481
55	*Galium spp.*	2.68	241.07151	$9{,}28x10^{-07}$		C14H12NO3	Unclassified, Imin fragment	62.0006, **59.0133**	
56	*Galium spp.*	2.68	449.12732	$6{,}71x10^{-38}$	[M+HCOOH-H]-, [M-H]-, [M+Cl]-	C17H24O11	Phenylpropa-noid, glucosilated	310.0864, 208.0522, 240.0812, 206.0395, 348.1023, 258.0909, 322.0820, 254.0618, **324.1046**, 226.0637, 238.0668, 328.0940, 288.1027, 338.1194	139.0371, 241.0726, 209.0445, 243.0891, **101.0230**, 191.0361, 127.0393, 195.0663, 125.0235, **223.0602**, 211.0694, 121.0294, **205.0538, 119.0499**

57	*Galium spp.*	2.96	355.09956 *	5,88x10^{-27}	[M-H]-	C16H20O9	Polyphenole, Hydroxycinnamic acid	**162.0484**, 221.0560 , 206.0316, 177.0710, 161.0488	193.0514, 134.0357, 149.0596, 178.0264, 194.0562, **113.0227, 255.0290, 179.0561**
58	*Galium spp.*	3.05	391.15882	3,56x10^{-08}	[M-H-H2O]-, [M+Na-H]-	C17H30O11	Flavonoid, glucosilated	198.1012, 190.1682, 168.1210, 272.1215, 280.0749, 76.1030, 131.0847, 160.0283, 62.0765, 75.0302, 343.5816, 189.0825, 278.1279, 174.0331, 144.1086	**193.0514**, 200.9925, 223.0434, 119.0333, 111.0800, 315.0592, 260.0752, 231.1375, **329.0709**, 316.1216, 47.5766, 202.0803, 217.1226, **178.0264, 355.0729**
59	*Galium spp.*	3.08	389.11408	7,53x10^{-11}	[M-H]-	C16H22O11	Polyphenole, Hydroxycinnamicacid	188.1065, 268.0785, 268.0449, 224.0395, 266.0629,	200.9925, 121.0294, 121.0645, 165.0557, 123.0437,

								250.1043, 174.0813, 247.1258, 130.9602, 246.0678, 196.0587, 174.0071, 234.0257, 181.0460, 127.9720,	139.0060, 386.1923, 215.0292, 141.9803, 258.1450, 143.0451, **193.0514**, 215.1089, 155.0812, 208.0636, 261.1441
60	*Galium spp.*	3.11	583.17685	$1{,}47 \times 10^{-14}$	[M-H]-	C19H36O20	Flavonoid, glucosilated	410.1052, 451.1437, 248.0447, 291.0576, 371.0893, 338.0907, 468.1725, 456.1361, 247.0408, 453.1068, 286.0631, 392.1121, 300.1251, 382.0773,	173.0767, 132.0300, 335.1225, 292.1146, 212.0847, 245.0744, 115.0035, 127.0393, 336.1404, […]-, **283.0553**, […]-, **113.0227**, […]-, **345.0545,** **161.0450**, **299.0585,**

								409.0971, 352.1153,	**313.0761, 163.0359,**
61	*Galium spp.*	3.26	671.20884	$5{,}26x10^{-11}$	[M-H]-	C25H40N2O19	Unclassified	371.1181, 370.1106, 430.1709,	300.0969, 301.1032, 241.0499,
62	*Galium spp.*	3.48	539.23314	$3{,}56x10^{-08}$	[M+HCOOH-H]-, [M-H]-	C22H38O12	Coumarin, glucosilated	208.0522, 378.1885, 426.2025, 438.2069, 294.1636, 332.2063, 428.2173, 276.1458, 396.1989, 400.2250,	331.1782, **161.0450**, **113.0227**, **101.0230**, 245.0744, **207.0333**, **111.0096**, 263.0813, 143.0330, 139.0060, 175.0228
63	*Galium spp.*	3.49	433.13439	$5{,}80x10^{-07}$	[M-H]-	C18H26O12	Glycoside	226.0637, 310.0864, 332.1020, 208.0522, 225.0871,	**207.0657**, 123.0437, **101.0230,** 225.0840, 208.0390,
64	*Galium spp.*	3.69	377.1741	$9{,}37x10^{-17}$	[M-H]-	C20H29NO4P	Flavonoid, glucosilated	162.0831, 205.1117, 171.1213, 157.0813,	215.0864, 172.0551, 206.0663, 220.0881,

								151.1725, 238.5506, 48.1162, 194.0887, 237.2491, 303.0122, 317.1016, 78.1523, 118.1453, 134.1254	138.6255, 226.0036, **329.0709**, **285.0397**, 183.0870, 139.9273, 74.1584, **259.0262**, 60.0759, […]-, **271.0634**
65	*Galium spp.*	3.7	345.15503	$2{,}42x10^{-05}$	[M-H]-	C16H26O8/C17H22N4O4	Phenylpropa-noid/ Agmatine	215.0950, 193.0944, 180.0441, 117.0119, 276.5944, 212.7553, 65.9882, 88.0636, 148.1353, 149.1106, 118.1453, 168.0674, 229.0701, 196.0587, 88.9490	130.0592, 152.0418, 165.0905, 228.1451, 68.5488, 132.3910, 196.0337, 257.0800, 197.0184, 279.1562, 177.0788, 226.9998, 116.0722, **193.0514, 241.0031**
66	*Galium spp.*	3.77	553.24547	$1{,}57x10^{-20}$	[M-H]-	C31H38O9	Flavonoid	406.1987, 222.0578, 404.2148, 358.1354,	147.046, 331.1782, **149.0226**, 195.1101, 206.0663,

								371.1988, 347.1727, 406.9088, 339.1885, 296.2380, 431.2060, 354.1740, 334.1688,	60.3026,	182.0439, 146.3428, 257.0001, 214.0614, 122.0410, […]-, […]-, **313.0761,**
67	*Galium spp.*	3.78	499.19377	$2{,}17x10^{-27}$	[M-H]-	C34H28O4	Flavonoid	296.1893, 214.1071, 392.1121, 286.1062, 259.1703, 276.0535, 277.0726, 314.0668, 272.0884, 248.1432, 218.1926, 418.0122, 293.1743		202.9945, 285.0761, 107.0821, 213.0870, 223.1343, 240.0148, 185.1181, 222.1187, 227.0937, 251.0494, 280.9980, 224.0616, […]-, **359.0822**
68	*Galium spp.*	4.13	493.17993	$1{,}44x10^{-13}$	[M-H]-	C22H30N4O9	Unclassified	226.1253, 291.1656,		267.068, 202.0248,

69	*Galium spp.*	4.18	509.22311	$3{,}64 \times 10^{-11}$	[M-H]-	C29H34O8	Glycoside	178.0490, 348.1760, 177.0437, 396.1989, 276.1558, 46.0083,	331.1782, **161.0450**, **113.0227**, 233.0644, 463.2102, **101.0230**
70	*Galium spp.*	4.25	899.3127	$3{,}56 \times 10^{-08}$	[M-H]-	C36H56N2O24	Unclassified	402.19, 697.2910, 686.2632, 401.1878, 696.2861, 672.2834, 498.1376,	497.1286, 202.0248, 213.0493, 203.0295, 227.0351, 401.1807,
71	*Galium spp.*	4.36	389.12107	$1{,}36 \times 10^{-01}$	[M-H]-	C24H24N4O5	Unclassified	238.0502, 178.0234, 240.0202, 222.0145, 196.0382, 209.1373,	209.1166, 269.1404, 207.1380, 225.155, 251.1261, 238.0251,
72	*Galium spp.*	4.4	447.16464	$3{,}96 \times 10^{-13}$	[M+HCOOH-H]-, [M-H]-, [M+Cl]-	C32H36NO11	Unclassified	371.1181, 370.1182, 277.1296,	284.1042, 285.1061, 378.0986,

73	*Galium spp.*	4.67	549.25373	$4{,}88 \times 10^{-14}$	[M-H]-	C25H42O13	Phenylpropa-noid, glucosilated	340.0923, 178.0364, 388.2034, 436.2201, 177.0437, 448.2231, 316.1849, 406.2136, 339.0904,	209.1502, 371.2017, **161.045**, **113.0227**, 372.2127, **101.023**, 233.0644, 143.033, **191.0570**
74	*Galium spp.*	4.78	437.10837	$1{,}37 \times 10^{-09}$	[M-H]-	C20H22O11	Phenylpropa-noid	258.0723, 242.0462, 164.9614, 206.0316, 185.0637, 220.1309, 198.0589, 235.0903, 168.1210, 228.0864, 255.9914	**179.0338**, 195.0663, 272.1528, 231.0859, 216.9834, 252.0411, 239.0477, 202.0248, 209.0290, 124.0170, 181.1174, 268.9853
75	*Galium spp.*	4.94	563.13922	$2{,}38 \times 10^{-26}$	[M-H]-	C27H24N4O10	Unclassified	312.1032, 294.0928, 311.0984, 326.0844,	251.0338, 269.0481, 252.0411, 237.0533,

								298.0878, 293.0882,	265.0429, 270.0456,
76	*Galium spp.*	5.07	625.17515	$1{,}60 \times 10^{-16}$	[M-H]-	C26H32N3O15	Unclassified	369.1042, 413.0866, 412.0838, 368.0952,	256.0754, 212.0847, 213.0870, 257.08,
77	*Galium spp.*	5.15	609.1758	$1{,}37 \times 10^{-09}$	[M-H]-	C28H34O15	Phenylpropa-noid, glucosilated	488.1460, 340.1277, 478.1328, 460.1191, 342.1113, 358.1453, 496.1614, 418.1268, 487.1496, 495.1459, 402.1900	121.0294, 269.0481, 131.0515, 149.0596, 267.0680, 251.0338, **113.0227**, **191.0570**, 122.0301, […]-, **175.0380,** **161.0450**
78	*Galium spp.*	5.28	595.16638	$5{,}26 \times 10^{-11}$	[M-H]-	C25H30N3O14	Unclassified		
79	*Galium spp.*	5.38	275.0922	$2{,}54 \times 10^{-45}$	[M-H]-	C15H16O5	Unclassified	88.0496, 87.0449, 116.0442,	187.0375, 188.0456, 159.0343,

80	*Galium spp.*	5.47	595.16624	$1{,}49 \times 10^{-39}$	[M-H]-	C27H32O15	Phenylpropa-noid, glucosilated	339.0904, 383.0827, 338.0907, 368.1377,	256.0754, 212.0847, 257.0800, 227.0351, **191.0570**
81	*Galium spp.*	5.48	547.14532	$1{,}57 \times 10^{-20}$	[M-H]-	C24H26N3O12	Unclassified	295.1032, 294.0928, 310.0864, 356.0899, 416.1069, 434.1299, 293.0978, 398.0991,	252.0411, 253.0495, 237.0533, 191.0570, 131.0346, 113.0227, 254.0539, 149.0439,
82	*Galium spp.*	5.73	463.12566	$8{,}50 \times 10^{-34}$	[M-H]-	C23H20N4O7	Unclassified	207.0535, 251.0440, 250.0345, 206.0509, 236.0889,	256.0754, 212.0847, 213.0870, 257.0800, 227.0351,
83	*Galium spp.*	5.84	547.14493	$6{,}52 \times 10^{-23}$	[M-H]-	C26H28O13	Unclassified	294.0928, 293.0882,	253.0495, 254.0539,
84	*Galium spp.*	5.85	431.13376	$8{,}61 \times 10^{-19}$	[M-H]-	C20H22N3O8	Unclassified	207.0464, 163.0602, 218.1151,	224.0941, 268.0693, 213.0201,

								222.0671, 206.0509,	209.0729, 225.0840,
85	*Galium spp.*	6.08	463.15973	$1{,}93x10^{-41}$	[M-H]-	C21H26N3O9	Unclassified	222.1078, 163.0602, 221.1056,	241.0499, 300.0969, 242.0526,
86	*Galium spp.*	6.18	507.23321	$7{,}51x10^{-36}$	[M-H-H]-2	C35H71N17O14PS	Glycoside	192.0877, 266.1808, 394.2140, 207.1353, 296.1606, 191.0838, 382.2117, 392.2306, 265.1807,	315.1512, 241.0499, **113.0227**, 300.0969, 211.0694, 125.0235, 115.0035, 242.0526,
87	*Galium spp.*	6.25	239.0351	$1{,}87x10^{-24}$	[M-H]-	C15H4N4	Unclassified	27.9912, 43.9916,	239.0293, 211.0377, 195.0456,
88	*Galium spp.*	6.28	283.02542	$2{,}29x10^{-05}$	[M-H]-, [M-H-H2O]-	C16H4N4O2	Unclassified	43.9916, 71.9868, 42.9853, 87.9814,	239.0293, 211.0377, 240.0408, 195.0456,

89	*Galium spp.*	6.62	267.02998	$1,87x10^{-24}$	[M-H]-	C16H4N4O	Unclassified	43.9916, 71.9868, 42.9853,	223.0434, 195.0456, 224.0455,
90	*Galium spp.*	6.88	807.41276	$1,37x10^{-09}$	[M-H]-	C42H64O15	Flavonoid, glucosilated	478.1938, 482.2877, 694.3974, 678.3998, 602.3362, 650.4003, 522.3185, 409.3394, 489.3883, 503.1910, 652.3350, 591.3814, 520.3743, 477.1850, 552.3432, 479.3235, 448.7728, 624.3880, 668.4130, 393.2433	329.2184, 325.1251, **113.0227**, 129.0161, 205.0864, […]-, 183.0286, 139.0060, **315.0592**, 537.3438, 414.1778, […]-, **175.0228**, 261.0195, 175.0808, 60.1830, 475.3423, […]-, 251.1033, 123.4200, 116.0062, 77.3653, **289.0396**

91	*Galium spp.*	6.94	269.04756	$1{,}34x10^{-23}$	[M-H]-, [M-H-H2O]-	C15H10O5	Unclassified	18.011, 17.0095,	251.0338, 252.0411,
92	*Galium spp.*	7.03	253.05071	$8{,}61x10^{-19}$	[M-H]-	C13H8N3O3	Polyketide	15.0215, 43.0179, 14.0187, **42.0117**	238.0251, 210.0334, 239.0293, 211.0377,
93	*Galium spp.*	7.19	491.1554	$2{,}38x10^{-22}$	[M+HCOOH-H]-, [M-H]-, [M+Cl]-	C18H26N2O11	Unclassified	208.0598, 267.0734, 268.0785, 209.0635, 240.0812, 266.0629, 282.0966, 284.0717, 207.0535,	283.1019, 224.0941, 223.0748, 282.0856, 251.0756, 225.0840, 209.0729, 207.0831, 284.1042,
94	*Galium spp.*	7.22	313.0712	$8{,}36x10^{-02}$	[M-H]-	C17H14O6	Flavonoid	**30.0454**, 15.0215, 58.0398, 29.0422,	283.0176, 298.0496, 255.0290, **284.0352**, 299.0585
95	*Galium spp.*	7.66	**299.05504** *	$1{,}64x10^{-02}$	[M-H]-	C16H12O6	Flavonoid	87.9814, **30.0454**, 15.0215, 43.9916, 58.0398, 86.9764,	211.0694, 269.0070, **284.0352**, 255.0564,

									241.0031, 212.0847, **285.0397, 299.0585**
96	*Galium spp.*	7.87	269.04591	$1{,}14x10^{-16}$	[M-H]-	C13H8N3O4	Unclassified	43.9916, 15.0215, 27.9912, 43.0179, 46.0083, 44.9985, 15.999, 61.9693, 18.011, 59.0133,	225.0548, 254.0189, 241.0499, 226.0282, 223.0434, 224.0455, 253.0495, 207.0831, 268.0693, 251.0338, 210.0334,
97	*Galium spp.*	8.05	255.03005	$1{,}03x10^{-11}$	[M-H]-	C14H8O5	Flavonoid	**27.9912**	**255.0290**, 227.0351,
98	*Galium spp.*	8.67	328.05884	$3{,}56x10^{-08}$	[M-H]-	C20H11NO4	Unclassified, Imin fragment	102.0324, **59.0133**, 88.0155, 118.0246, 101.0244, 117.0119, 62.0349, 132.9201, 87.0083, 44.8614, 106.0308, 90.0298, 73.0361, 126.9422,	226.0282, 269.0481, 240.0408, 239.0293, 210.0334, 327.2024, 227.0351, 211.0377,

								134.0225, 46.8822, 58.0105,	266.0199, 195.1414, 241.0499, 283.1922, 222.0222, 238.0251, **255.0290**
99	*Galium spp.*	8.79	301.10803	$1{,}90x10^{-40}$	[M-H]-	C15H16N3O4	Unclassified	101.0987, 113.0983, 69.0713, 100.0955, 141.0937,	200.0177, 188.0203, 232.0334, 201.0071, 160.0198,
100	*Galium spp.*	8.8	232.03736	$2{,}49x10^{-22}$	[M-H]-	C12H9O5	Unclassified	29.0036, 124.0171, 89.1289, 88.0496, 90.242, 111.4848, 134.0815, 131.6694, 153.5161, 69.4696, 174.1599, 149.4254, 40.7212, 79.4318, 174.6441,	203.0295, 108.0200, 142.9091, 231.1463, 231.0269, 143.9877, 141.7874, 120.5614, 97.9631, 100.3686, 78.5295, 162.5684, 57.8822, 82.6151, 191.3154, 152.6063, 57.3881,

101	*Galium spp.*	9.87	367.11863	$4{,}74x10^{-06}$	[M-H]-	C21H20O6	Unclassified	58.0815, 70.0786, 57.0765, 15.0215, 69.0713,	309.0403, 297.0396, 310.0412, 352.0933, 298.0496,
102	*Galium spp.*	9.93	388.11854	$1{,}64x10^{-02}$	[M-H]-	C23H19NO5	Unclassified	180.0822, 179.072,	208.039, 209.0445,
103	*Galium spp.*	10.46	354.13392	$1{,}64x10^{-02}$	[M-H]-	C20H21NO5	Unclassified	146.0927, 145.0897,	208.039, 209.0445,
104	*L. perenne*	5.6	305.13879	$5{,}58x10^{-03}$	[M-H]-	C17H22O5	Phenylpropa-noid	96.0258, 198.1012, 144.0835, 100.0955, 102.0324, 62.0121, 103.0685, 88.0636, 103.0846, 197.0835, 102.0746, 80.0064, 76.0539	209.1166, 107.0503, 305.1371, 161.0598, **205.0538**, 203.1052, 243.1404, 202.0803, 217.0795, 202.0568, 108.0518, **163.0359**, **149.0226**
105	*L. perenne*	8.28	289.14387	$4{,}61x10^{-02}$	[M-H]-	C17H22O4	Phenylpropa-noid	62.0006, 43.9916, 60.9952, 170.0986, 60.0239, 182.0952,	227.144, 245.1449, 228.1451, **119.0499,** 229.1136,

								43.0504, 150.0315, 18.9471	107.0503, 246.0945, 139.1144
106	*P. lanceolata*	1.09	381.09511	$1{,}21 \times 10^{-14}$	[M-H]-	C25H18O2S	Unclassified, sulfated	140.0932, 284.1295, 138.0811, 120.0623, 251.0021,	241.0031, **96.9582**, 243.0006, 261.0195, 130.0882
107	*P. lanceolata*	1.45	409.04766	$3{,}02 \times 10^{-06}$	[M-H]-	C14H18O12S	Unclassified, sulfated	168.049, 312.0866,	241.0031, **96.9582**
108	*P. lanceolata*	1.83	439.05513	$4{,}63 \times 10^{-11}$	[M-H]-	C15H20O13S	Unclassified, sulfated	198.0589, 342.0951,	241.0031, **96.9582**
109	*P. lanceolata*	2.03	373.11345	$6{,}91 \times 10^{-08}$	[M-H]-	C17H18N4O6	Unclassified	206.072, 250.0623, 221.1056, 265.0892,	167.0372, 123.0437, 152.0125, 108.02,
110	*P. lanceolata*	2.8	373.11301	$8{,}33 \times 10^{-04}$	[M-H]-	C16H22O10	Glycoside	176.0663, 164.0684, **180.0662**, **162.0484**, 220.0538	197.0441, 209.0445, 193.0407, 211.0557, 153.0559
111	*P. lanceolata*	3.13	433.14145	$4{,}03 \times 10^{-09}$	[M-H]-	C18H26O12	Unclassified, Aromatic acid	236.0889, 251.1112, 295.1032,	197.0441, 182.0228, **138.0290**,

112	*P. lanceolata*	3.19	563.2283	$6{,}34x10^{-01}$	[M-H]-	C29H40O9S	Unclassified, sulfated, glucosilated	450.2041, 356.0899, 358.1094, 374.0974, 388.2034, 440.1885, 449.1997, 366.1900, 400.1185, 390.1721, 462.2080,	**113.0227**, 207.1380, 205.1253, 189.1234, 175.0228, 123.0437, 197.0441, 163.1143, 173.0545, 101.0230, 198.0488, 208.1377, 561.2070, […]-, **96.95820**,
113	*P. lanceolata*	3.37	319.14009	$2{,}61x10^{+00}$	[M-H-H2O]-, [M+K-H]-	C24H20NO	Unclassified	163.0602, 107.0802, 102.0949, 127.0366, 206.0884, 42.9853, 93.9886, 151.9486, 226.1042, 85.9998, 205.0779, 233.0633, 96.0730, 160.1037	212.0509, 156.0701, 217.038, 192.106, 113.0543, 276.1546, 225.1366, 167.1936, 93.0325, 233.133, 114.0531, 86.071, 223.0602, 159.0343, 56.7092,

114	*P. lanceolata*	3.42	401.14426	$2{,}20 \times 10^{-20}$	[M-H]-	C18H26O10	Polyphenole, Hydroxycinnamic acid	208.0963, 267.1050, 252.0821, 223.1152,	**193.0514**, 134.0357, 149.0596, **178.0264**,
115	*P. lanceolata*	3.45	431.12924	$5{,}90 \times 10^{-05}$	[M-H]-	C18H24O12	Polyphenol, Hydroxy-cinamic acid, glucosilated	306.0961, 210.0398, 206.0395, 238.0668, 228.0484,	125.0235, 221.0823, 225.0840, **193.0514**, 203.0696, **113.0227**
116	*P. lanceolata*	3.49	613.21169	$9{,}77 \times 10^{-07}$	[M-H]-	C23H38N2O17	Terpene, glucosilated	286.0763, 432.1611, 285.0889, 420.1549, 283.1095, 414.2023, 474.1705, 284.0949, 402.1762, 486.1702, 397.2122, 415.1350, 348.3331	327.1156, 181.0567, 193.0514, 330.0960, 199.0149, 139.0371, 329.1183, 211.0377, 127.0393, 215.9960, […]-, **101.0230**, **456.1391**
117	*P. lanceolata*	3.53	963.27139	$1{,}49 \times 10^{-21}$	[M-H]-	C44H52O24	Unclassified, Aromatic acid	482.1475, 682.2155,	481.1281, 281.0653,

								481.1454, 681.2131, 724.2269,	282.0650, 239.0554, **137.0231**
118	*P. lanceolata*	3.54	544.13066	$7{,}60 \times 10^{-03}$	[M-H]-	C19H29O18	Phenylpropa-noid	313.9847, 227.2967, 267.0734, 340.1032, 388.4210, 92.0767, 398.6884, 469.6728, 476.3490, 123.0123, 224.9926, 359.0406, 430.8272, 448.3846, 182.9811, 193.0199, 336.9861, 332.9850, 335.092,	230.1503, 316.8281, 277.0525, 204.0206, 452.0478, 155.7037, 145.4338, 319.1341, 421.1125, 185.0938, 74.4519, 67.7800, 95.7401, 207.1380, 361.1472, 351.1057, 113.3052, 209.0290, **193.0541**
119	*P. lanceolata*	3.65	459.15046	$1{,}07 \times 10^{-08}$	[M-H]-	C25H22N3O6	Polyphenole, Hydroxy-cinnamic acid	186.0320, 166.0478, 214.0391, 250.1043, 198.0716, 63.1931,	208.0390, 273.1158, 293.1044, 245.1178, 209.0445,

								266.1291, 280.0749, 292.0852, […]-, 350.4543, 331.6202, 295.1032, 231.0468	261.0683, 395.9545, 193.0197, **179.0713**, 167.0718, 115.0827, […]-, 127.5274, **164.0452**,
120	*P. lanceolata*	3.66	533.12805	$2{,}49 \times 10^{-25}$	[M-H]-	C32H22O8	Phenylpropa-noid	252.0539, 294.0667, 396.1114, 354.0959, 362.1182,	281.0653, 239.0554, **137.0231**, **179.0338**, 171.0102
121	*P. lanceolata*	3.67	669.10185	$6{,}53 \times 10^{-16}$	[M-H]-	C28H46O18	Flavonoid, glucosilated	386.1610, 446.1635, **180.0581**, 384.1752, 354.1929, 404.1564, 532.2356, 448.1810, 460.2142, 462.2401,	283.1019, 223.0978, 489.2019, 285.0761, **315.0592**, 265.1088, 137.0231, 221.0823, 209.0445, **175.038**0

122	*P. lanceolata*	3.68	261.0437	$8{,}50x10^{-03}$	[M-H]-	C10H14O6S	Unclassified, sulfated	164.0885	**96.9582**, 261.0397,
123	*P. lanceolata*	3.77	525.16087	$9{,}27x10^{-06}$	[M-H]-	C24H30O13	Phenylpropa-noid	244.0894, 328.1141, 388.1335, 286.1062, 243.0908,	281.0653, 197.0441, **137.0231**, 239.0554, 282.0650, **341.0821, 179.0338**
124	*P. lanceolata*	3.88	399.16576	$6{,}34x10^{-01}$	[M-H]-	C21H24N2O6	Unclassified	194.0329, 303.8632, 190.1183, 121.0062, 186.0979, 205.0395, 179.0262, 107.0355, 180.0822, 64.0058, 188.0424, 327.3932, 59.7085, 172.0128	205.1253, 95.2950, 209.0445, 213.0493, 278.1513, 194.1225, 220.1339, 292.1146, 219.0665, 335.1512, 211.1110, 71.7649, 227.1440, 339.4497, 334.9702, 261.4741
125	*P. lanceolata*	3.92	565.22467	$1{,}92x10^{-07}$	[M-H]-	C27H36NO12	Unclassified	342.1249, 300.1165,	223.0978, 265.1088,

								386.1154, 344.1074,	179.1067, 221.1235,
126	*P. lanceolata*	4	415.13734	$2{,}04x10^{-02}$	[M-H]-	C22H26NO7	Unclassified		
127	*P. lanceolata*	4.03	931.28229	$1{,}39x10^{-17}$	[M-H]-	C44H52O22	Iridoglycoside	466.1498, 646.2148, 465.1496, 628.2046, 645.2119,	**465.1391**, 285.0761, 303.0864, **137.0231**
128	*P. lanceolata*	4.04	667.15112	$7{,}06x10^{-06}$	[M-H]-	C36H28O13	Iridoglycoside	382.0677, 466.1498, 202.0042, 464.1505, 530.1196, 381.0654, 364.0579,	285.0761, 200.9925, **465.1391**, 202.9945, **137.0231**, 303.0864,
129	*P. lanceolata*	4.05	501.11652	$1{,}36x10^{-16}$	[M-H]-	C17H42O2S7	Unclassified, sulfated, glucosilated	216.0324, 364.0757, 365.0964, 260.0257, 352.0039, 380.0752, 318.0482, 293.0724, 305.0745,	285.0761, 137.0231, 136.0156, 241.0726, 149.0956, 121.0294, 183.0544, 208.0269, 196.0337,

								263.0319, 215.0254,	238.0780, **113.0227, 956.9582**
130	*P. lanceolata*	4.11	611.22769	$9{,}56x10^{-04}$	[M-H]-	C26H44O16	Phenylpropa-noid	360.1238, 404.1125, 359.1208,	251.1261, 207.1380, 252.1254, **223.0602**
131	*P. lanceolata*	4.12	431.19138	$2{,}00x10^{-05}$	[M-H]-	C18H30N3O9	Unclassified	226.0637, 225.0653,	205.1253
132	*P. lanceolata*	4.16	495.13374	$1{,}64x10^{-16}$	[M-H]-	C13H36O13S3	Unclassified, sulfated, glucosilated	328.094, 374.0974, 180.0441, 358.1094, 329.1000, 344.1192, 373.0954, 360.0966, 256.0780, 290.0670, 382.1066, 343.1195, 372.0847, 346.0628, 342.1113, 286.0763, 199.0729, 283.0925,	167.0372, 121.0294, 315.0853, 137.0231, 166.0260, 151.0105, 122.0301, 135.0267, 239.0477, 205.0538, **113.0227**, 152.0125, […]-, **96.9582**

133	*P. lanceolata*	4.16	495.13374	$1{,}64 \times 10^{-16}$	[M-H]-	C25H24N2O9	Unclassified	328.1047, 374.1104, 180.0441, 344.1303, 372.0958, 238.0668, 329.1173, 254.1281, 316.1153, 358.1094	167.0372, 121.0294, 315.0853, 151.0105, 123.0437, 257.0800, 166.0260, 241.0031, 179.0338, 122.0410
134	*P. lanceolata*	4.16	755.19918	$8{,}50 \times 10^{-03}$	[M-H]-	C36H52O15S	Unclassified, sulfated	466.2613, 412.1719, 568.1836, 411.1702, 550.1708, 658.3337, 574.2259,	289.0396, 343.1210, 187.1123, 344.1169, 205.1253, **96.95820**, 181.0567,
135	*P. lanceolata*	4.22	725.28149	$1{,}16 \times 10^{-10}$	[M-H]-	C32H46N4O15	Unclassified, glucosilated	412.1719, 520.1635, 476.1718, 538.1718, 436.2461, 574.2259, **180.0662**, 478.1574,	313.1029, 205.1253, 249.1120, 187.1123, 289.0396, 151.0569, 545.2193, 247.1304,

								496.1614, 411.1702,	229.1228, 314.1236, **179.0561**
136	*P. lanceolata*	4.25	332.05639	$6{,}34x10^{-01}$	[M-H-H]-2	C32H26O16	Unclassified	58.0105, 130.8984, 174.9605, 76.0664, 117.9909, 183.0018, 190.7880, 59.0133, 85.8280, 167.9738, 234.5058, 158.3924, 188.7560, 51.4247	274.0412, 201.1614, 157.0894, 255.9908, 149.0596, 214.0614, 141.2637, 273.0481, 246.2293, 164.0803, 97.5515
137	*P. lanceolata*	4.31	987.47169	$7{,}68x10^{-08}$	[M-H]-	C52H76O18	Glycoside	766.4291, 808.4418, 826.4512, 765.4254,	221.0671, **179.0561**, **161.0450,** 222.0683
138	*P. lanceolata*	4.34	403.1541	$3{,}44x10^{-11}$	[M-H]-	C18H28O10	Glycoside	180.0260, 179.0262, **162.0484**, 206.0395,	223.1343, 224.1387, 241.1095, 197.1206
139	*P. lanceolata*	4.3	611.19774	$2{,}35x10^{-04}$	[M-H]-	C28H36O15	Phenylpropa-noid	388.1335, 416.1286, 403.1583,	**223.0602,** 195.0663, 208.0390,

								386.1154, 401.1443, 387.1343,	225.0840, 210.0557, 224.0616,
140	*P. lanceolata*	4.42	591.22857	$7{,}53 \times 10^{-09}$	[M-H]-	C27H45O8PS2	Glycoside, sulfated, phosphorylated	386.1029, 342.1249, 404.1125, 362.0969, 385.0982, 218.1025, 372.0847, 430.0969, 300.1067, 384.1622, 390.0991, 388.0924, 332.1020	205.1253, 249.1120, 187.1123, 229.1228, 373.1294, 219.1366, **161.1346,** 291.1183, 207.0657, 201.1311, **196.0337**, **113.0227**, **96.9582**
141	*P. lanceolata*	4.42	641.20718	$8{,}50 \times 10^{-03}$	[M-H]-	C40H34O6S	Unclassified	418.1446, 432.1298, 433.1690, 434.1413, 417.1445, 424.0966,	223.0602, 209.0729, 208.0390, 207.0657, 224.0616, 217.1076,

142	*P. lanceolata*	4.5	395.16622	$5{,}17x10^{-04}$	[M-H]-	C17H33O8P	Unclassified, phosphorylated	236.2041, 203.1180, 339.388, 134.9786, 208.0811, 160.0734, 176.8273, 287.1648, 180.1185, 177.1379, 106.0145, 132.0464, 283.6564, 238.1911, 268.1105, 272.1598, 27.7607, 60.0774,	**158.9942**, 192.0732, 55.8039, 260.2117, 187.1123, 108.0200, 235.1195, 218.3653, 289.1785, 263.1309, 218.0588, 215.0682, 111.5362, 123.0437, 157.0084, 127.0783, 367.4328,
143	*P. lanceolata*	4.5	689.13778	$8{,}85x10^{-01}$	[M-H]-	C24H34O23	Phenylpropanoid	466.0796, 481.1047, 465.0809, 448.1388,	**223.0602**, 208.0390, 224.0616, 241.0031,
144	*P. lanceolata*	4.55	595.20091	$2{,}02x10^{-20}$	[M-H]-	C28H36O14	Phenylpropanoid, glucosilated	386.1154, 372.1388, 388.1335, 401.1443, 195.0756,	209.0729, **223.0602**, 207.0657, 194.0562, 400.1157,

								180.0581, 387.1602, 371.1294, 403.1583, 210.1032, 385.1166,	415.1369, 208.0390, 224.0616, 192.0411, **385.0927**, **205.0538, 179.0338**
145	*P. lanceolata*	4.57	447.12876	$7{,}53 \times 10^{-09}$	[M-H]-	C22H24O10	Flavonoid, glucosilated	**162.0484**, 268.0944, 161.0488, 161.9374, 296.0894, 206.0395, 265.0892, 163.0839, 258.0909, 192.0655, **180.0581**	285.0761, **179.0338**, 285.1942, 151.0379, 241.0880, 182.0439, **284.0352**, 189.0402, 255.0564,
146	*P. lanceolata*	4.57	609.18212	$1{,}36 \times 10^{-16}$	[M-H]-	C28H34O15	Phenylpropa-noid	386.1258, 401.1443, 385.1262,	**223.0602**, 208.0390, 224.0616, **205.0538**
147	*P. lanceolata*	4.59	543.13373	$4{,}12 \times 10^{-01}$	[M-H]-	C30H24O10	Phenylpropa-noid	258.0523, 406.0995, 240.0381,	285.0761, **137.0231**, 303.0864, 136.0156, **119.0499**

								407.1069, 257.0479,	
148	*P. lanceolata*	4.59	575.13916	$3{,}84x10^{-06}$	[M-H]-	C22H28N2O16	Unclassified, Aromatic acid	290.0670, 438.1202,	285.0761, **137.0231**,
149	*P. lanceolata*	4.6	507.15065	$1{,}23x10^{-12}$	[M-H]-	C24H28O12	Unclassified, Aromatic acid	222.0671, 370.1182, 371.1294, 266.0538, 386.1154, 221.0659,	285.0761, **137.0231**, 136.0156, 241.0880, 121.0294,
150	*P. lanceolata*	4.63	341.12297	$7{,}53x10^{-09}$	[M-H]-	C16H22O8	Phenylpropa-noid, glucosilated	**162.0484**, 206.0316,	**179.0713**, 135.0797,
151	*P. lanceolata*	4.69	537.1457	$3{,}23x10^{-05}$	[M-H]-	C17H30O19	Flavonoid, glucosilated	400.1051, 414.0853, 392.0994, 416.1069, 370.0907, 390.0823, 344.0671, 340.0726, 332.0010, 238.0668, 318.0482,	137.0231, 123.0437, **145.0283**, 121.0294, 167.0372, 147.0460, 193.0656, 197.0607, 205.1253, **299.0585**, […]-, 80.9676, 138.0290,

								469.9658, 294.9488, 322.1009, 332.0615,	**78.9915, 163.0491,** […]-, **341.1454, 175.038**
152	*P. lanceolata*	4.7	451.16717	$1{,}36x10^{-13}$	[M-H]-	C22H28O10	Unclassified		
153	*P. lanceolata*	4.72	235.02909	$1{,}51x10^{-22}$	[M-H]-	C8H12O6S	Unclassified, sulfated	138.0811	9**6.9582**
154	*P. lanceolata*	4.72	459.19136	$8{,}18x10^{-04}$	[M-H]-	C21H32O11	Flavonoid, glucosilated	240.0509, 228.0484, 258.0523, 252.0821, 294.1010, 233.1670, 132.0464, 174.1434, 239.0519, 245.0620, 217.1044, 253.0089, 294.1892,	219.1366, 231.1375, 201.1311, 207.1031, 165.0905, 226.0282, 327.1514, **285.0397**, 220.1339, 214.1185, 242.0731, […]-, **113.0227**, […]-, **301.0291**
155	*P. lanceolata*	4.74	715.24055	$1{,}45x10^{-14}$	[M-H]-	C32H44O18	Flavonoid	548.2101, 492.1495,	167.0372, 223.0978,

								563.2347, 468.1505, 450.1416, 486.1621, 512.1427, 547.2085,	152.0125, 247.0965, 265.1088, 229.0857, 203.1052, 168.0371, **289.0995**
156	*P. lanceolata*	4.77	645.18102	9,77x10^{-07}	[M-H]-	C31H34O15	Phenylpropa-noid	448.1388, 406.1239, 360.1102, 434.1118, 346.1055, 508.1584, 338.1568, 304.0911, 447.1317,	197.0441, 239.0554, 285.0761, 211.0694, 299.0744, 137.0231, 307.0299, **341.0821**, 198.0488,
157	*P. lanceolata*	4.77	825.4243	8,00x10^{-06}	[M-H]-	C42H66O16	Glycoside	368.0952, 712.4032, 664.3807, 121.6460, 724.4035, 367.0945, 682.3956, 706.39326, 666.39488,	457.3456, **113.0227**, **161.0450**, 179.0561, **101.0230**, 458.3335, 143.0330, 119.0333,

								694.39740, 604.3544	159.0343, 341.0821, **89.0284**
158	*P. lanceolata*	4.83	487.18138	$1{,}96x10^{-24}$	[M-H]-	C22H32O12	Glycoside	374.1533, 372.1754,	**113.0227**, 115.0035, **161.0450**
159	*P. lanceolata*	4.84	585.16223	$1{,}47x10^{-16}$	[M-H]-	C29H30O13	Iridoglycoside	300.0815, 448.1310, 346.0976, 304.0911, 406.1239, 299.0726, **120.0207**, 344.0671, 449.1422, 303.0869,	285.0761, 137.0231, 239.0554, 281.0653, **179.0338**, **465.1391**, 241.0880, 136.0156, 282.0650,
160	*P. lanceolata*	4.84	701.23201	$8{,}74x10^{-05}$	[M-H]-	C28H46O18S	Glycoside, sulfated	480.1687, 592.2047, 180.0822, 548.2163, 224.0596, 386.1610, 432.0326, 540.1900,	221.0671, 109.0263, 108.0200, 521.1496, 153.0242, 477.1843, 315.0592, 152.0125,

								479.1146, 492.1834, 498.1636, 564.2050, 588.2029, 522.1348, 502.1369, 404.1458,	269.1975, **161.0450**, 222.1187, 209.0445, **137.0331**, 203.0696, **113.0338**, […]-, **101.023, 96.9582**
161	*P. lanceolata*	4.96	429.17621	$5{,}32 \times 10^{-10}$	[M-H]-	C23H28NO7	Unclassified	208.0963, 226.1042, 224.0503, 207.0946,	221.0823, 203.0696, 205.1253,
162	*P. lanceolata*	5.01	411.20153	$8{,}27 \times 10^{-08}$	[M-H]-	C28H28O3	Glycoside	**180.0581**, 174.0813, 218.0609, 288.1159, 204.2085, 290.1331, 211.2030, 265.3599, 179.0720, 145.1187, 89.0485, 203.1585, 188.5481, 144.1323,	231.1375, 237.1120, 193.1251, 123.0811, 206.9934, 121.0645, 200.0007, 145.8358, 232.1373, 266.0738, 322.1630, 208.0390, 222.6476,

								214.1195, 86.0736, 154.0411, 64.0247,	267.0680, 197.0799, 325.1251, 257.1503, 347.1655, **113.1028**
163	*P. lanceolata*	5.01	495.06676	$6{,}66 \times 10^{-06}$	[M-H]-	C15H30NO17	Unclassified	272.0884, 386.0780, 402.1088, 179.0542, 274.0569, 272.1354, 326.0743, 291.9968, 242.0114, 262.0051, 288.0575, 374.1104, 377.7153, 447.1951, 246.0678, 220.0538	223.0602, 109.0651, 93.0325, 316.0904, 221.0823, 222.9975, 169.0636, 203.1439, 207.0831, 253.1273, 233.1330, 121.0294, 117.4256, 47.9458, 249.0706, 241.0031
164	*P. lanceolata*	5.05	387.12623	$5{,}90 \times 10^{-05}$	[M-H]-	C12H14N13O3	Phenylpropa-noid, glucosilated	178.085, 163.0602, 177.071, **162.0484**, 15.0215, 221.0659,	209.0445, 224.0616, 210.0557, 225.0840,

									372.1154, 166.0574, **223.0602**
165	*P. lanceolata*	5.08	489.19669	$1{,}64x10^{-16}$	[M-H]-	C27H30N4O3S	Phenylpropa-noid, glucosilated	376.1727, 254.1491, 316.1577, 374.1924, 338.0836,	**113.0227**, 235.0498, 173.0545, 115.0035, 151.1110, **191.0570, 101.0230, 161.0450**
166	*P. lanceolata*	5.19	661.29873	$8{,}74x10^{-05}$	[M-H]-	C31H50O15	Jasmonate conjugate	208.0598, 370.1106, 452.2622, 207.0535, 376.2285, 350.2251,	**291.1991**, 209.0445, 285.0761, 311.0794, 436.2371, 239.0554, 269.0481, 447.1288, **113.0227**, 309.2160, 273.1882, 205.0864, **179.0561**
167	*P. lanceolata*	5.24	433.14949	$3{,}02x10^{-06}$	[M-H]-	C22H26O9	Glycoside	226.0869, 225.1122, 224.0707,	**207.0657**, 208.0390, 209.0729,

								210.0920, 239.0958, 242.1122, 241.1100,	223.0602, 194.0562, 191.0361, 192.0411,
168	*P. lanceolata*	5.24	515.24891	$7{,}37x10^{-10}$	[M-H]-	C25H40O11	Flavonoid, glucosilated	238.0668, 164.0256, 237.0610, 182.0381, 256.0780, 402.2242, 378.1885, 226.0321,	277.1788, 351.2203, 333.2146, 259.1747, 513.2601, **113.0227**, 137.0588, 289.2157, 150.0691, 126.0156, […]-, **301.0291, 161.0450**
169	*P. lanceolata*	5.31	497.27506	$8{,}50x10^{-03}$	[M-H]-	C26H42O9	Polyphenole, Hydroxycinnamicacid	345.2560, **180.0662**, 360.4007, 377.2505, 308.2621, 336.3652, 235.1936, 281.2246, 362.2288, 272.2292,	152.0125, 317.2055, 136.8697, 120.0167, 189.0120, 160.9046, **135.0432**, […]-, **175.0371**, 389.1669,

								314.4139, 89.1710, 241.0656	330.0960, 304.0708, 234.0175
170	*P. lanceolata*	5.37	417.16027	$8{,}68x10^{-02}$	[M-H]-	C19H30O10	unclassified, glucosilated	**180.0662**, 256.078, 212.0921, 179.0542, 304.1529	237.1120, 161.0966, 205.0864, 238.1105, **113.0227**,
171	*P. lanceolata*	5.57	357.15623	$7{,}60x10^{-03}$	[M-H]-	C12H26N2O10	Unclassified	61.9693, 174.1599, 156.1637, 125.0251, 120.0623, 185.0488, 75.1796, 156.0563, 104.032, 31.2981, 31.0489, 132.1735, 303.8424, 61.1324	295.1835, 182.9941, 200.9925, 232.1373, 237.0799, 172.1104, 281.9806, 201.1082, 253.1273, 325.8621, 326.1111, 224.984, 53.3178, 296.0315
172	*P. lanceolata*	5.59	469.24233	$8{,}68x10^{-02}$	[M-H]-	C24H38O9	Unclassified	222.1078, 266.0916,	247.1304, 203.1439,
173	*P. lanceolata*	5.63	519.20732	$8{,}68x10^{-02}$	[M-H]-, [M+HC	C20H32N3O10	Unclassified	226.0637, 270.0602, 338.1194,	247.1304, 203.1439, 135.0797,

					OOH-H]-			272.0738, 269.0565, 225.0653,	201.1311, 248.1310,
174	*P. lanceolata*	5.79	485.2021	$8{,}85x10^{-01}$	[M-H]-	C23H34O11	Unclassified	244.2044, 255.0548, 382.4200, 230.1984, 298.1505, 198.3316, 362.1566, 213.2209, 377.6380, 205.0561, 283.1821, 223.1980, 267.1246, 297.1251, 317.2331, 364.1725	241.0031, 230.1503, 255.0144, 187.0679, 102.7854, 484.1997, 286.8736, 123.0437, 271.9867, 280.1451, 107.5641, 202.0248, 121.0294, 262.0039, 218.0779, 188.0813, 167.9754, 284.9143, 254.0189, 119.0499, 148.0485, 218.1987,

175	*P. lanceolata*	5.88	401.16258	$1{,}76x10^{-02}$	[M-H]-	C13H28N3O11	Unclassified	248.0848, 285.0889, 117.9715, 135.9825, 192.0511, 258.0909, 232.0752, 134.9786, 288.1027, 250.1043, 133.1276, 192.1804, 292.1586,	153.0751, 116.0722, 283.1922, 265.1845, 209.1166, 143.0712, 169.0861, 266.1760, 113.0543, 151.0569, 268.0361, 208.9816, 109.0098,
176	*P. lanceolata*	5.88	499.23635	$6{,}66x10^{-06}$	[M+HCOOH-H]-, [M-H]-	C24H38O8	Jasmonate conjugate	208.0522, 226.0637, 278.0821, 207.0535, 45.9943, 270.0801, 299.3244, 320.2154, 44.2115, 362.1717, 172.1443, 390.1881, 312.1465, 205.5912,	**291.1991**, 273.1882, 221.1555, 453.2537, 229.1727, 199.9259, **179.0338**, 455.0388, 137.0846, 327.1156, 187.1123, 109.0651,

								235.6828, 292.2795, 404.8488,	293.6591, 263.5676, 206.9708, 214.0227, 249.1865
177	*P. lanceolata*	6.07	389.21706	$8{,}33 \times 10^{-04}$	[M-H]-	C17H32N3O7	Unclassified	45.1791, 223.1476, 188.1586, 110.1478, 202.1343, 239.1771, 90.0886, 149.1389, 147.0897, 178.2899, 304.9272, 158.1076	344.0284, 166.0574, 201.0552, 187.0679, 279.0583, 150.0288, 299.1152, 240.0632, 242.1178, 210.9177, 84.2804, 231.1065
178	*P. lanceolata*	6.08	443.19255	$8{,}16 \times 10^{-05}$	[M-H]-	C21H32O10	Glycoside	256.078, 224.0596, **180.0662**, 255.0792, 258.1009	187.1123, 219.1366, 263.1309, 188.1093, 185.0938, **113.0227, 101.0230**
179	*P. pratensis*	4.11	621.10908	$1{,}06 \times 10^{-04}$	[M-H]-	C27H26O17	Flavonoid, glucosilated	352.0665, 508.0875, 386.0557,	269.0481, **113.0227**, 235.0498,

								351.0645, 424.0709, 494.0733, 298.0519, 336.0793, 398.0523, 270.0602, 412.0165, 399.0113, 443.6389, 446.0849, 384.0645, 353.9442, 421.0113, 433.0829,	270.0456, 197.0441, 127.0393, **285.0397**, 323.0545, 223.0602, 351.0488, 209.0966, 222.0879, **175.0228**, 177.4714, 267.1647, 237.0533, 188.0203, **163.0359**	[…]-,
180	*P. pratensis*	4.23	651.12143	1,31x10^{-04}	[M-H]-	C28H28O18	Flavonoid	352.0665, 300.0667, 454.0776, 458.0846, 367.0945, 538.1011, 366.0783, 249.9423, 351.0645, 537.0940, 449.097,	**299.0585**, 351.0488, 197.0441, 193.0407, **284.0352**, **113.0227**, **285.0397**, 401.1807, **300.0661**, 202.0248,	

								536.1202, 362.0736, 318.0797,	115.0035, 289.0396, 333.0454,
181	*R. acris*	3	477.16081	$1{,}79x10^{-07}$	[M-H]-, [M+Cl]-, [M+HCOOH-H]-	C22H26NO8	Unclassified	182.0952, 157.0641, 302.1690, 35.0362, 223.1152, 260.0689, 306.1315, 168.0490, 285.4405, 191.5267, 202.0911, 294.0928, 238.0668, 83.2776, 153.0899, 190.1519, 240.0670, 106.6210	249.0551, 274.0830, 128.9830, 396.1143, 208.0390, 125.0235, 171.0768, 263.1035, 239.6238, 145.7075, 229.0568, 137.0588, 347.8809, 193.0845, 241.0031, 191.0772
182	*R. acris*	3.27	577.21217	$3{,}00x10^{+00}$	[M-H]-	C28H36NO12	Unclassified	370.1106, 371.1181, 369.1042,	207.1031, 206.1004, 208.1073,

183	*R. acris*	3.37	559.17941	$1{,}60x10^{-02}$	[M-H]-	C25H36O14	Unclassified	369.1042, 368.0952, 326.0844,	190.0953, 191.1063, 233.1143,
184	*R. acris*	3.63	405.20243	$6{,}10x10^{-02}$	[M-H]-	C19H34O9	Unclassified, glucosilated	**162.0484**, **180.0581**, 161.0488,	243.1604, 225.1550, 244.1767, **113.0227,** 179.0542
185	*R. acris*	3.83	437.23277	$3{,}97x10^{-07}$	[M-H]-	C20H38O10	Unclassified	196.0805, 264.1700, 233.1051, 316.1577, 219.1051, 211.0677, 169.1731, 244.1499, 176.1273,	241.1420, 173.0545, 204.1264, 121.0645, 218.1309, 226.1580, 268.0693, 193.0845, 261.1088,
186	*R. acris*	3.84	420.09682	$3{,}46x10^{+00}$	[M-H]-	C19H19NO10	Unclassified	250.0716, 224.0503,	170.0267, 196.0491,
187	*R. acris*	3.85	567.25966	$1{,}70x10^{-07}$	[M-H]-	C25H44O14	Phenylpropa-noid, glucosilated	340.0923, 334.1868, 344.1436, 178.0364, 346.1957, 406.2136,	227.1640, 233.0644, 223.0978, 389.2190, 221.0671, **161.0450**,

								342.1388, 302.1480, 454.2374, 344.1303, 177.0338, 388.1428, 376.1961, 442.2289, 420.2961, 466.2313	225.1095, 265.1088, 565.2573, **113.0227**, 223.1343, 390.2307, 179.1067, **191.0570**, 125.0235, 146.9501, **101.0230**
188	*R. acris*	4.19	471.18079	$5{,}12x10^{-19}$	[M-H]-	C22H33O9P	Unclassified, phosphorylated	67.9759, 208.0522, 248.0447, 210.0718, 166.0318, 230.0456, 232.1181, 66.9768, 164.0493, 224.0830, 247.0408, 205.0779, 216.1620, 270.1247, 252.0235	403.1997, 263.1309, 223.1343, 261.1088, 305.1371, 241.1420, 239.0554, 307.1193, 224.1387, 247.0965, 266.1090, **255.0144**, 201.0552, 219.1527, 263.1941

189	*R. acris*	4.42	381.12368	$3{,}06x10^{-05}$	[M-H]-	C19H26O9	Glycoside	206.0395, 262.1084, 205.0395, **162.0484**	191.1063, 135.0432, 192.1060
190	*R. acris*	4.47	405.17841	$2{,}18x10^{+00}$	[M-H]-	C18H30O10	unclassified, glucosilated	**180.0581**, 224.0503, 182.0381, 179.0542,	225.1095, 181.1174, 223.1343, 226.1165,
191	*R. acris*	4.68	541.19507	$2{,}45x10^{+01}$	[M-H]-	C22H38O13S	Unclassified, sulfated	444.2385, 77.9504, **79.9615**, 239.9993, 242.0196, 76.9472,	**96.9582**, 463.2533, 461.2334, 301.2047, 299.1736, 464.2307,
192	*R. acris*	4.78	427.19573	$6{,}23x10^{-05}$	[M-H]-	C20H30NO9	Unclassified	206.1083, 224.1234, 224.0503, 266.0538,	221.0823, 203.0696, 203.1439, 161.1346,
193	*R. acris*	4.85	429.15844	$5{,}30x10^{+01}$	[M-H]-	C18H28N3O9	Unclassified	208.0963, 226.1042, 224.0503, 207.0946,	221.0823, 203.0696, 205.1253,
194	*R. acris*	4.96	427.14879	$2{,}18x10^{+00}$	[M-H]-	C20H30NO9	Unclassified	224.0395, 266.0538, 223.04,	203.1439, 161.1346,

								206.1083, 222.0578,	221.0823, 205.1253,
195	*R. acris*	5.07	499.23821	1,21x10^{-01}	[M-H]-	C25H40O8S	Unclassified, sulfated, glucosilated	386.2082, 290.2499, 443.3236, 174.3693, 338.1807, 269.2135, 442.0312, 208.0811, 156.0563, 140.0479, 315.1747, 40.0953, 261.1846, 295.1862, 180.1450, 353.1346,	**113.0227**, 208.9816, 55.9070, 324.8613, 161.0450, 230.0083, 57.1994, 291.1478, 359.1826, […]-, 209.0966, 112.9376, 353.1090, **96.9582**, **101.023**0
196	*R. acris*	5.23	381.14863	8,46x10^{-01}	[M-H]-	C16H30O8S	Unclassified, sulfated	284.2012, **79.9615**,	**96.9582**, 301.2047,
197	*R. acris*	5.24	269.00934	1,21x10^{-16}	[M-H]-	C17H4NO3	Unclassified		269.0070
198	*R. acris*	5.62	415.17489	6,40x10^{-02}	[M-H]-	C23H28O7	Unclassified	211.1051, 62.0006, 318.2258,	415.1766, 204.0788, 353.1760, 96.9582,

199	*R. acris*	6.16	219.05794	$3{,}06x10^{-05}$	[M-H]-	C15H10NO	Unclassified	62.0006, 46.0083, 43.9916, 59.0133, 60.0239, 44.9985, 71.9868, 87.9814, 60.9952, 111.0559, 74.0352, 42.9853, 85.0308, 18.011,	157.0667, 173.0545, 175.0808, 160.0535, 159.0343, 174.0717, 219.0665, 147.0827, 172.0551, 131.0854, 158.0715, 108.0200,
200	*R. acris*	6.76	355.11841	$1{,}72x10^{+00}$	[M-H]-	C20H20O6	Phenylpropa-noid	236.0595, 210.0920, 192.0877,	**119.0499**, **145.0283**, **163.0359**

Table A3: List of plant traits including abbreviations, unit and description.

Trait	Abbreviation	Unit	Description
Leaf dry matter content	LDMC	mg/g	Leaf dry mass per leaf fresh mass
Specific leaf area	SLA	m^2/kg	Leaf area per leaf dry mass
Leaf area ratio	LAR	cm^2/g	Leaf area per total dry mass
Root dry matter content	RDMC	mg/g	Root dry mass per root fresh mass
Root to shoot ratio	RSR	g/g	Root dry mass per aboveground dry mass
Root volume	RVol	cm^3	Root volume
Root mass per volume	RMV	g/cm^3	Root dry mass per scanned root volume
Root carbon content	RCC	%	Root carbon content
Root nitrogen content	RNC	%	Root nitrogen content
Root carbon to nitrogen ratio	RCNR	g/g	Root carbon to nitrogen ratio
Root phosphorus content	RPC	µmol/g	Root phosphorus content
Root potassium content	RKC	µmol/g	Root potassium content
Root magnesium content	RMgC	µmol/g	Root magnesium content
Root calcium content	RCaC	µmol/g	Root calcium content
Root dry mass	DM roots	g	Root dry mass
Leaf dry mass	DM leaves	g	Leaf dry mass
Aboveground dry mass	DM above	g	including shoots, leaves and flowers
Total dry mass	DM total	g	Dry mass of whole plant

Chapter 2.4.

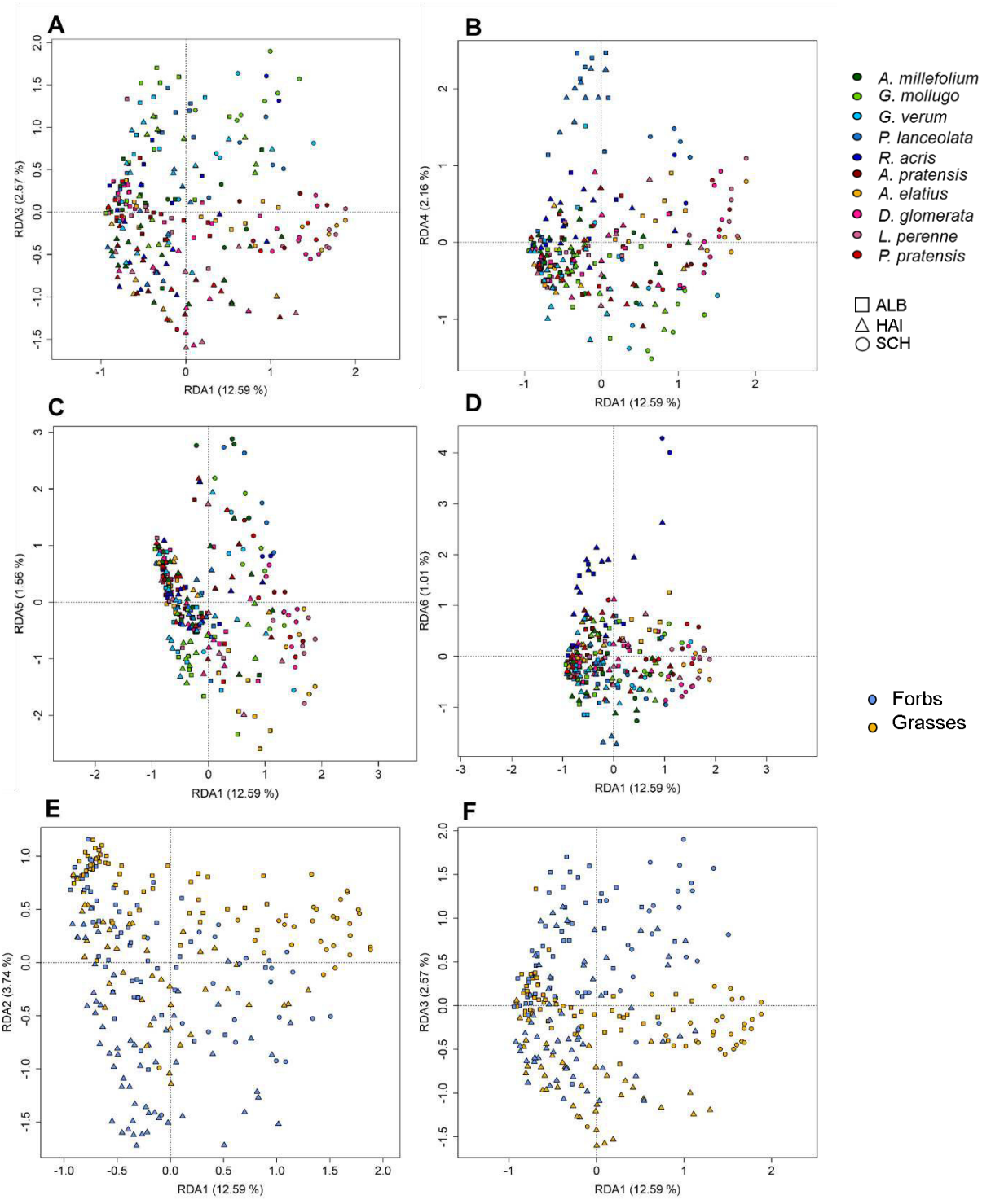
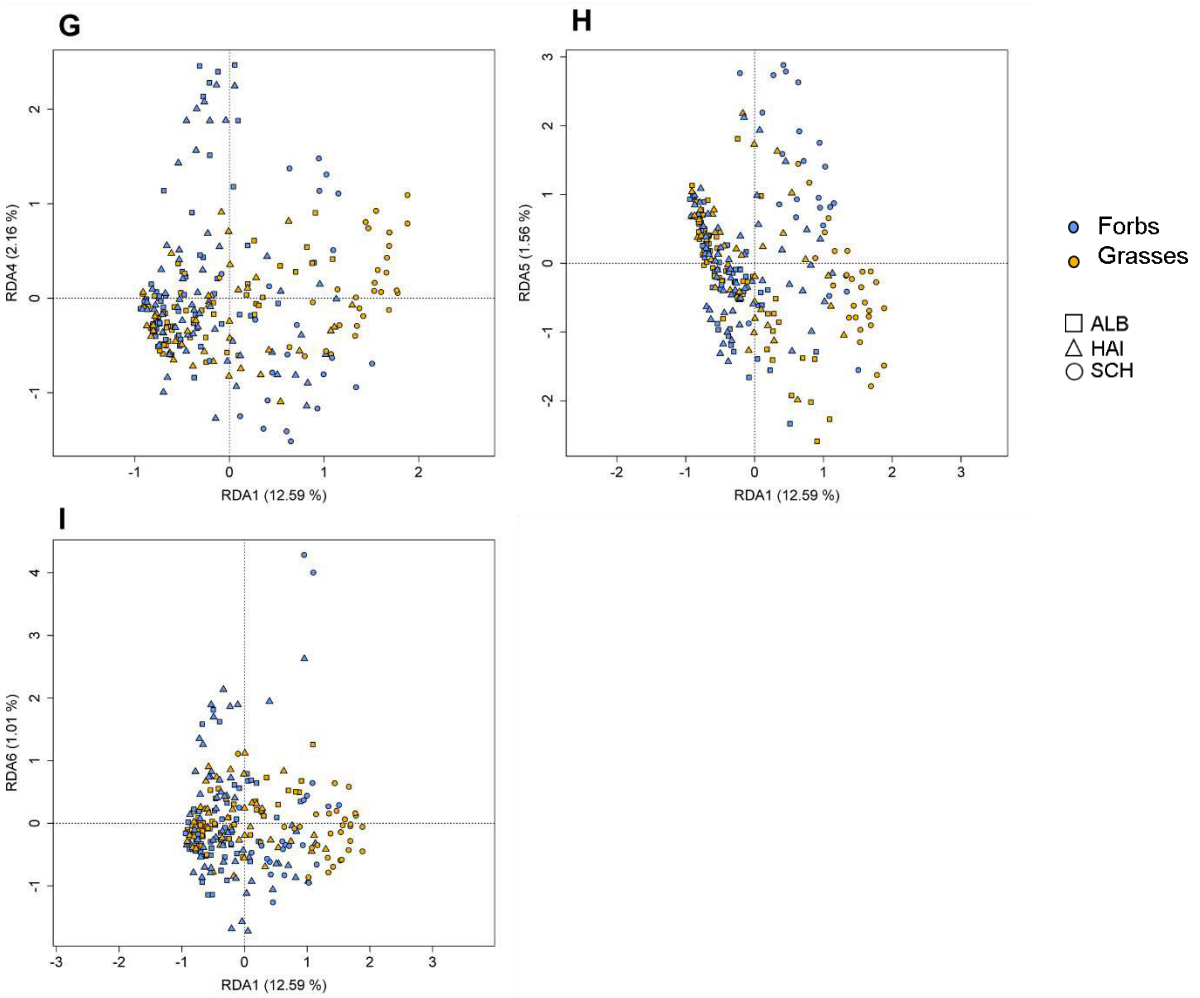
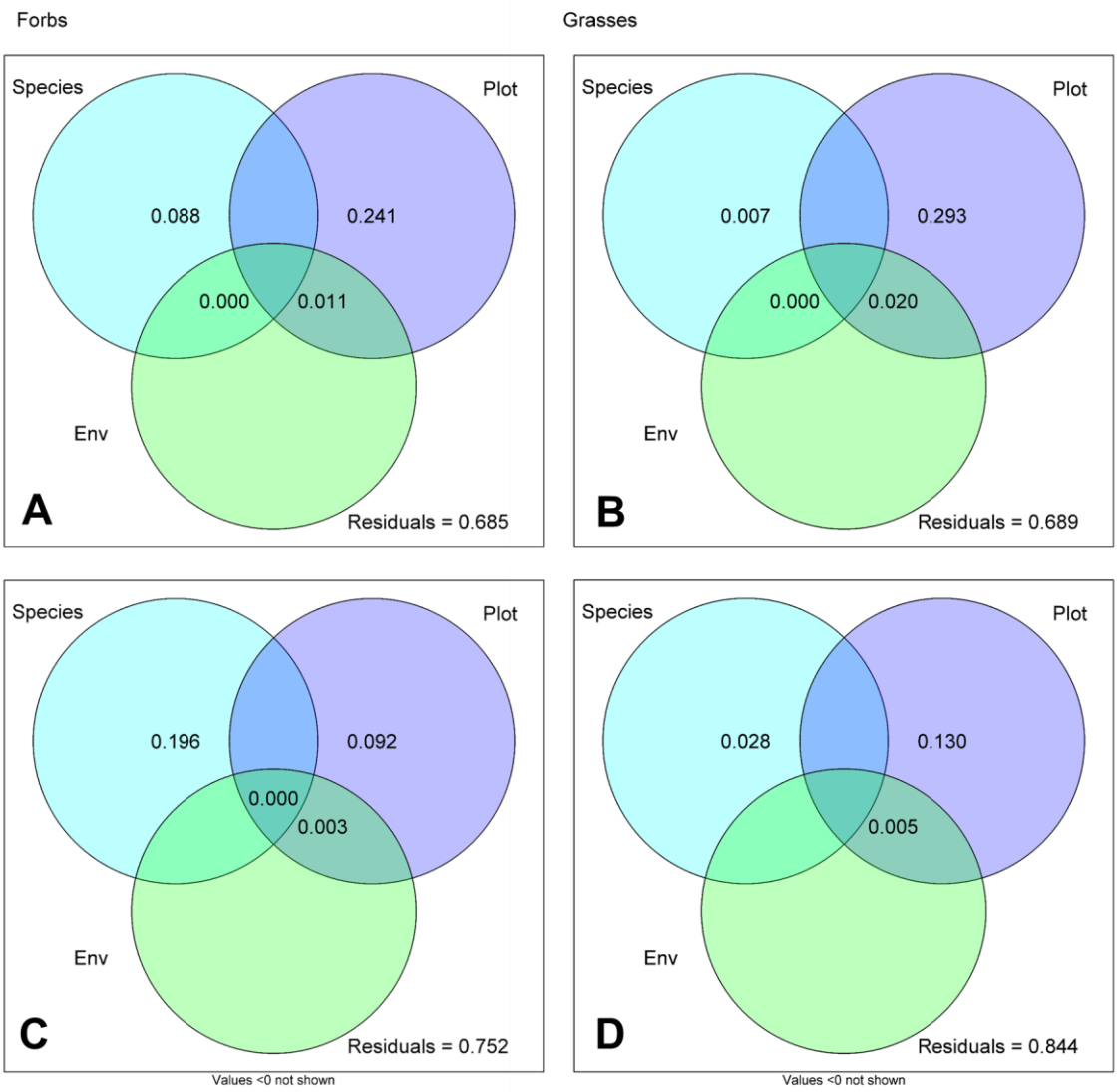


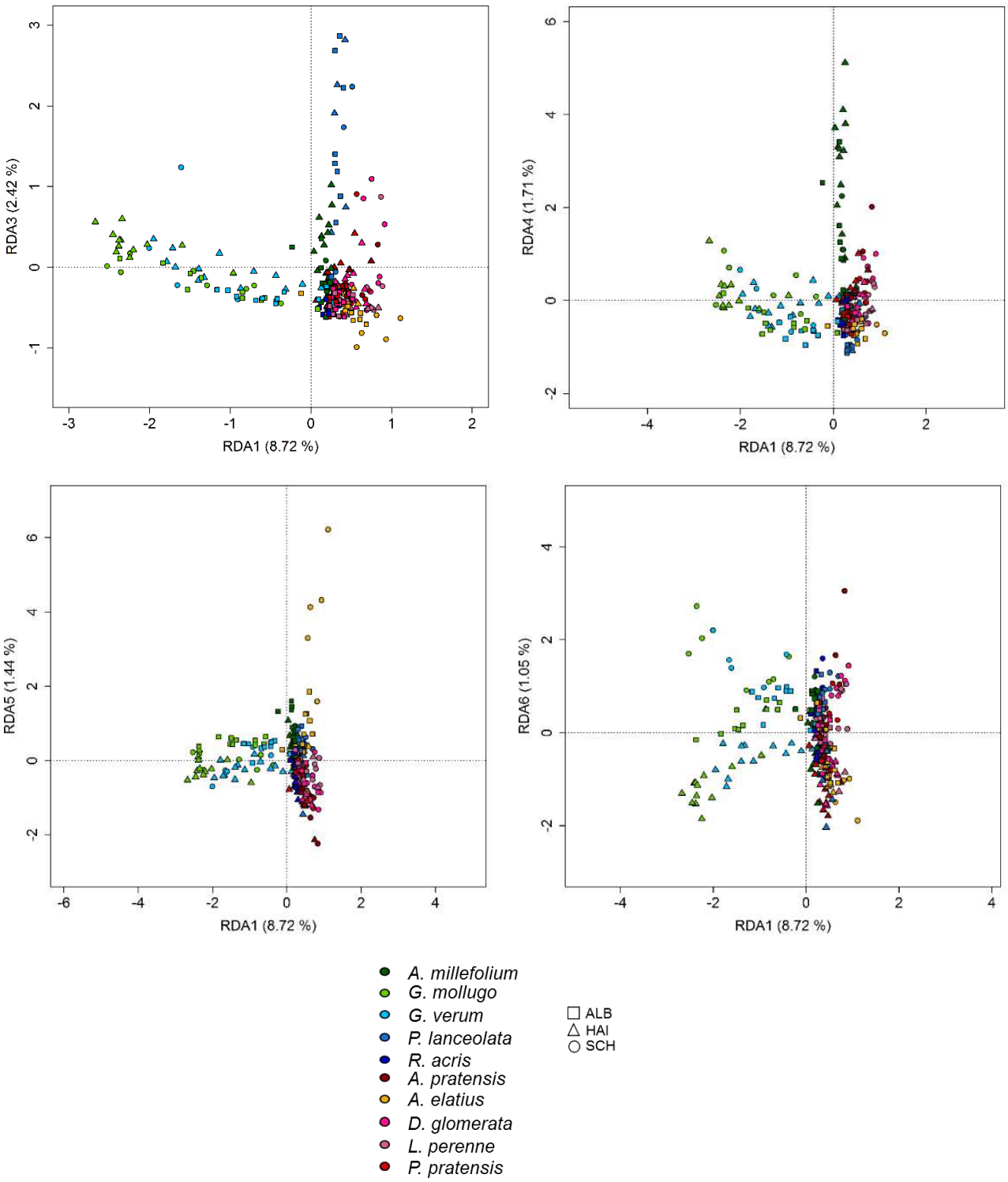
Fig. is continued on the following page



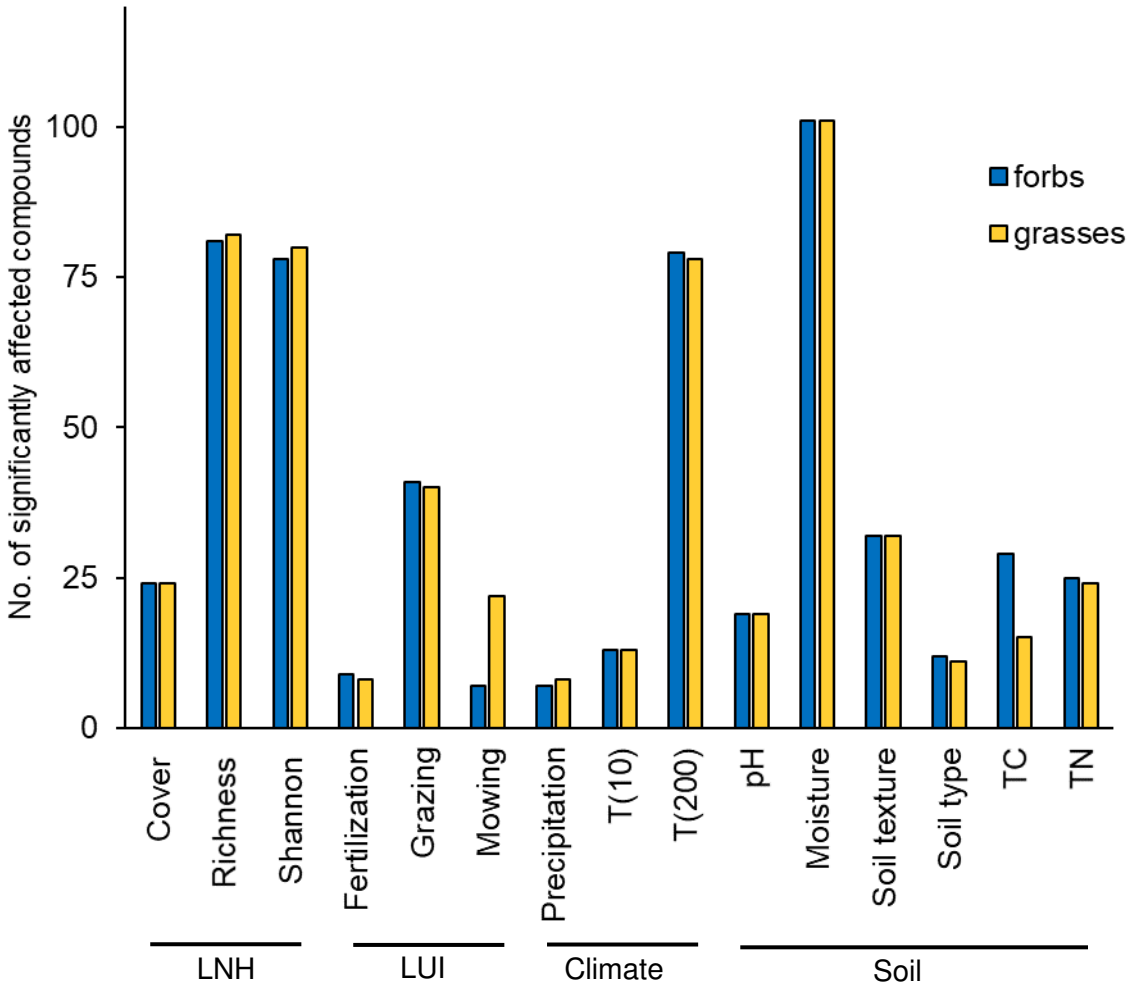
Supplementary Fig. 1: Redundancy analysis of polar metabolites. RDA was performed with 257 samples matching with impacting environmental factor information. Metabolite compositions of the samples were plotted against a presence/absence matrix of species per site. The plots A-D represents the RDA axis three to six plotted against rda axis one and were coloured by species (see legend), whereas plots E-I represents the RDA axis one against two to six and were coloured by growth form (see legend). Symbols represent the three Exploratories (see legend).



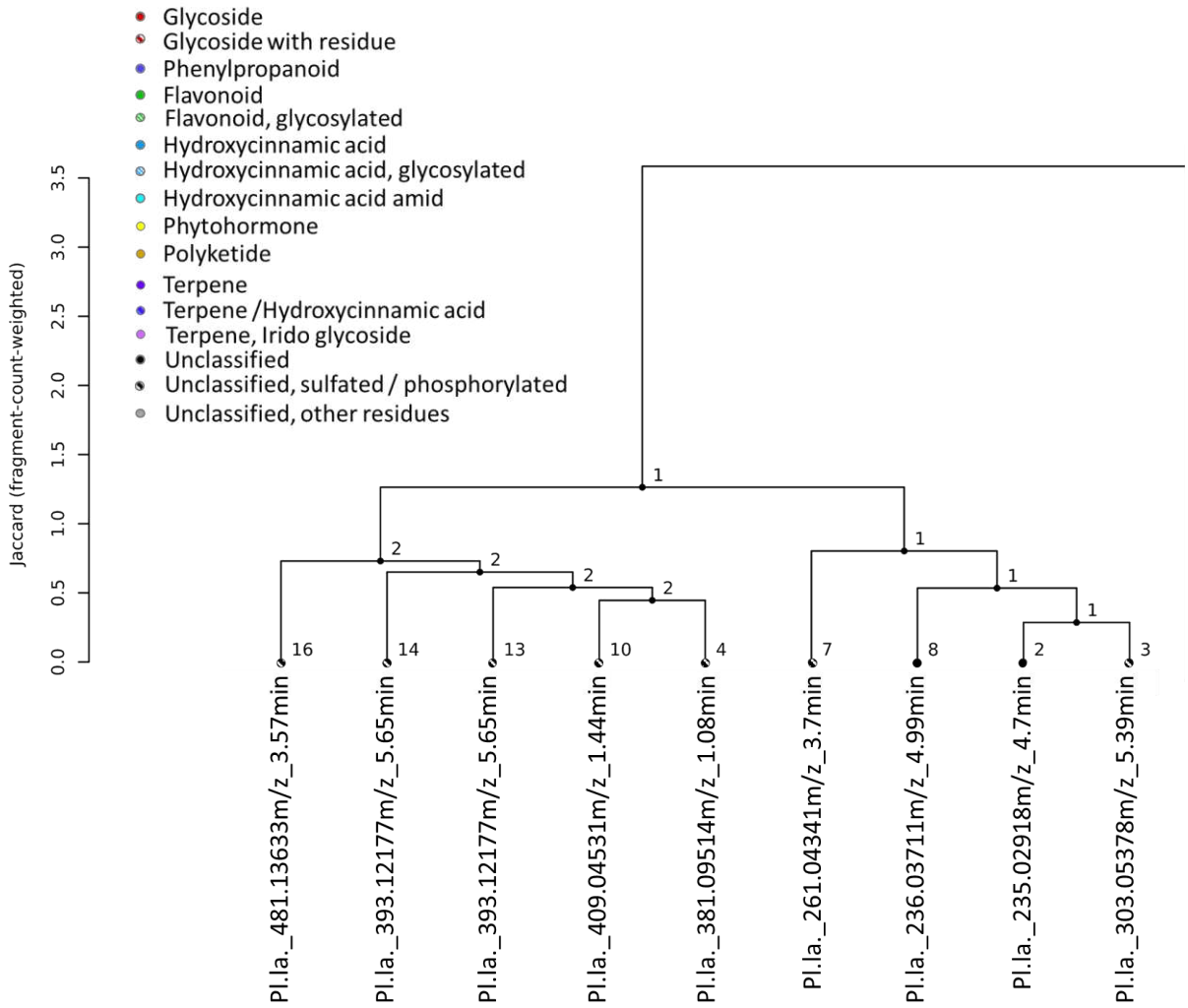
Supplementary Fig. 2: Variance partitioning of polar and semi-polar metabolites. Venn diagrams present the proportion of variance in A, B polar or C, D semi-polar metabolite pattern, respectively, of forbs (left) and grasses (right) explained by different predictors: Species = species identity of the target plant, Plot = local impact, Env= Environmental properties summarizing climate and soil variables.



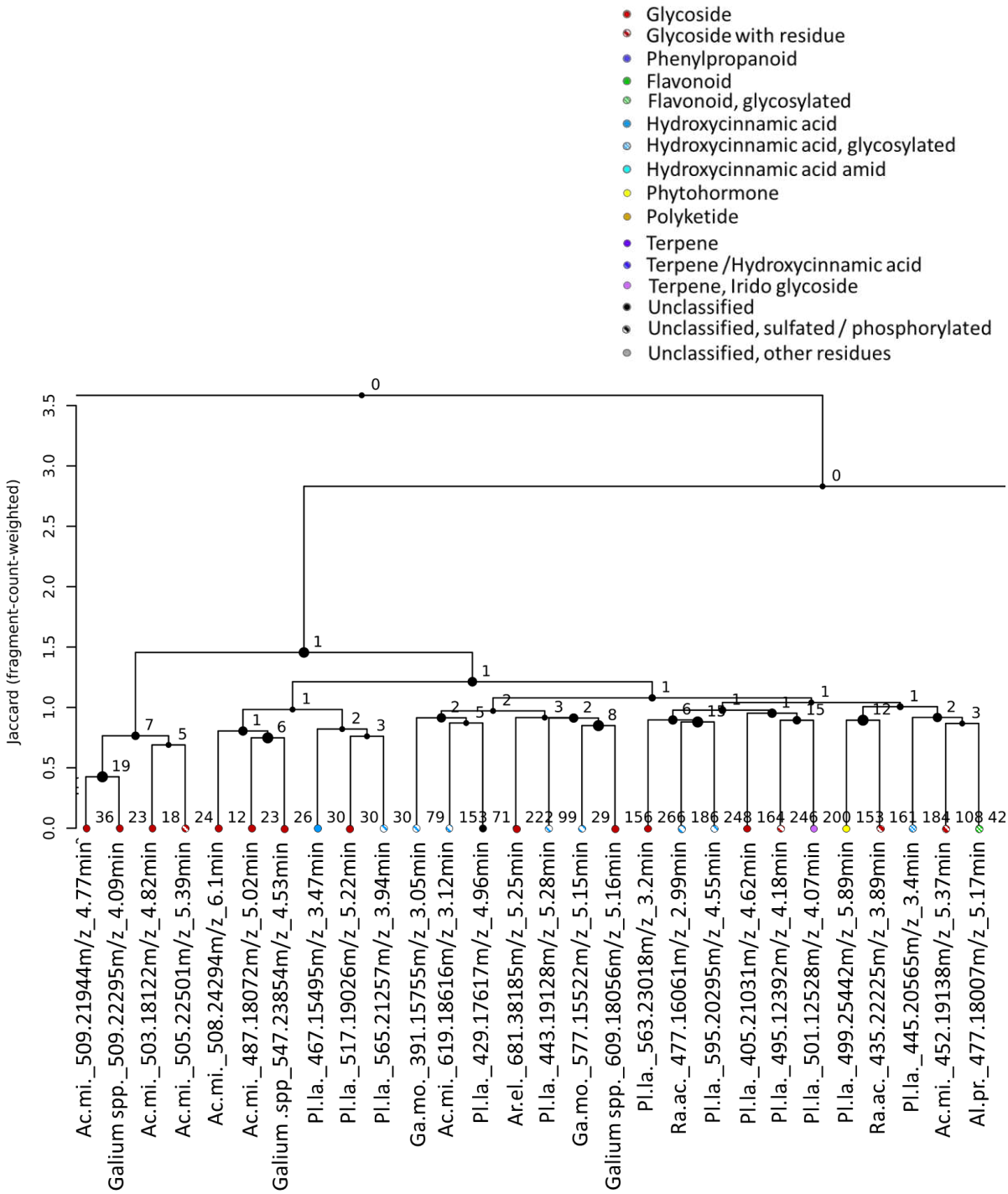
Supplementary Fig. 3: Redundancy analysis of semi-polar metabolites. RDA was performed with 257 samples matching with impacting environmental factor information. Metabolite compositions of the samples were plotted against a presence/absence matrix of species per site. The ten species are represented by colour, whereas the symbols indicate the sites (see legend). Axis three to six were plotted against axis one.



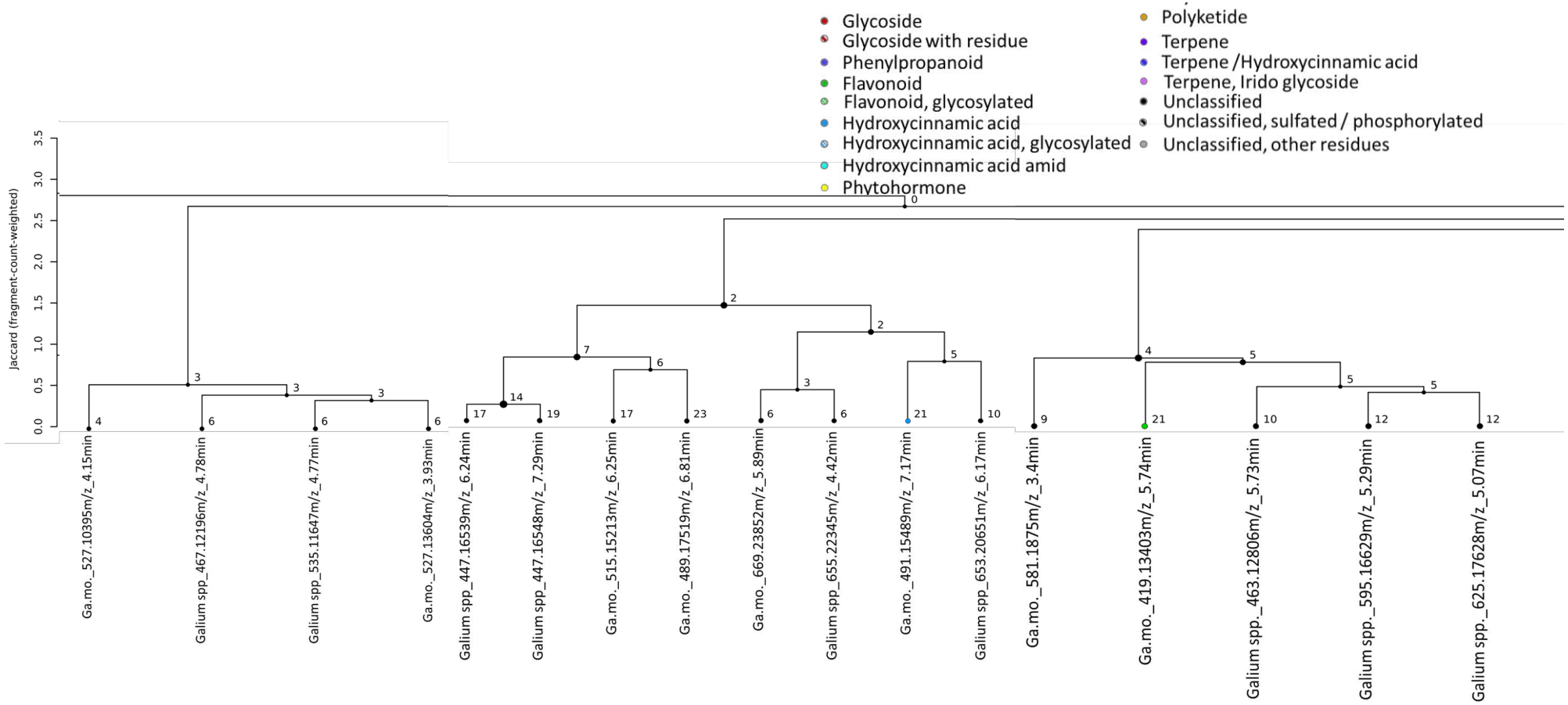
Supplementary Fig. 4: Environmental characteristics affecting polar compounds. Bar plot presents the number of significantly affected polar compounds correlated to the different single variables of the environmental factors LNH, LUI, Climate and Soil. The numbers were calculated for each growth form (see legend). A detailed description of the abbreviation is given in Supplementary Table 10.



Supplementary Fig. 5: Detailed view of branch 1 of hierarchical clustering of species specific semi-polar compounds. Hierarchical clustering was performed on the tandem-mass spectra (CID and DDA) of the significant species specific compounds. Spectra cluster are based on fragment similarity by jaccard algorithm and fragment-count-weighted value rating. The branch contains sulphated or phosphorylated compounds.



Supplementary Fig. 6: Detailed view of branch 2 of hierarchical clustering of species specific semi-polar compounds. Hierarchical clustering was performed on the tandem-mass spectra (CID and DDA) of the significant species specific compounds. Spectra cluster are based on fragment similarity by jaccard algorithm and fragment-count-weighted value rating.



Supplementary Fig. 7: Detailed view of branch 3 of hierarchical clustering of species specific semi-polar compounds. Hierarchical clustering was performed on the tandem-mass spectra (CID and DDA) of the significant species specific compounds. Spectra cluster are based on fragment similarity by jaccard algorithm and fragment-count-weighted value rating.

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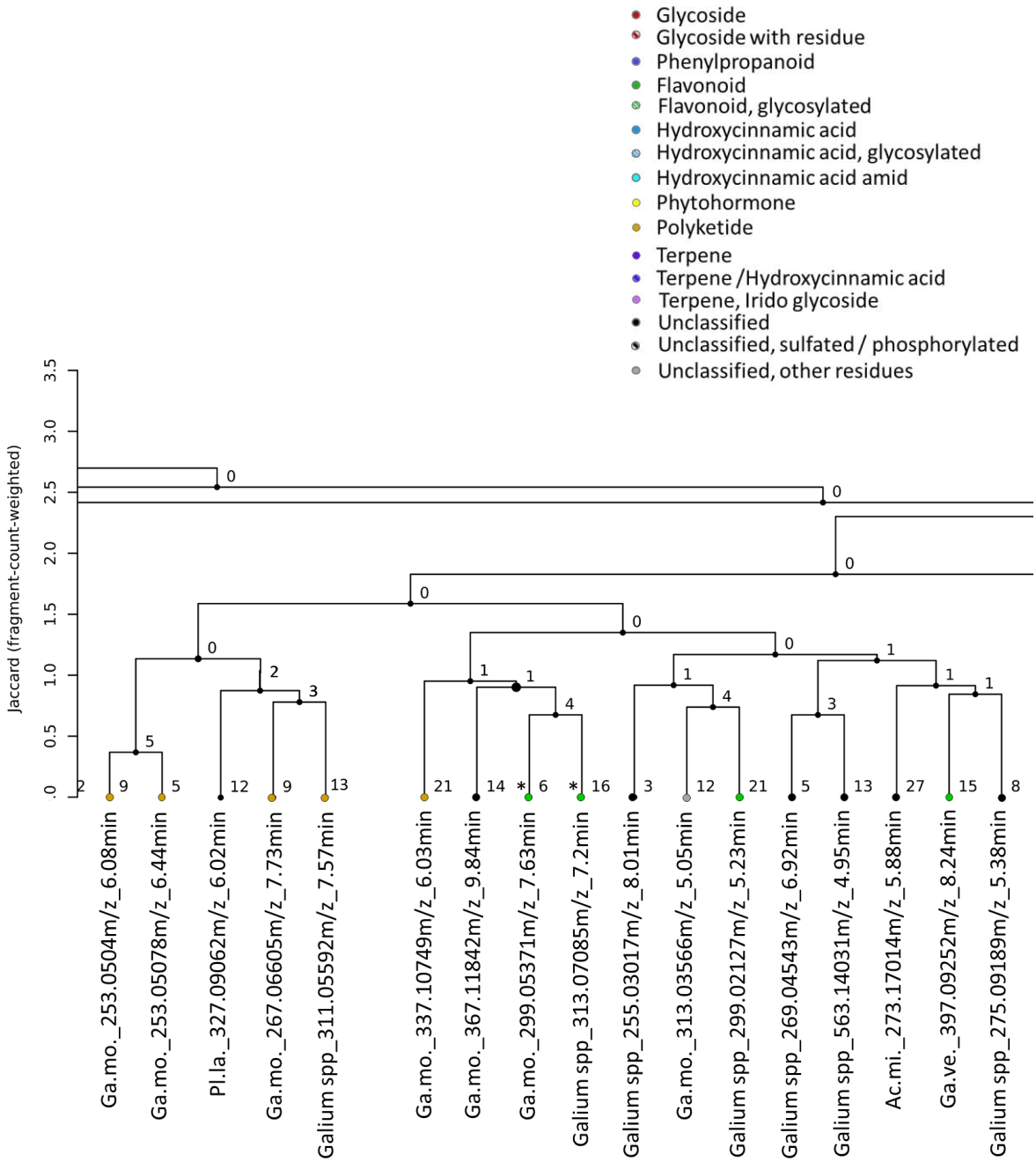
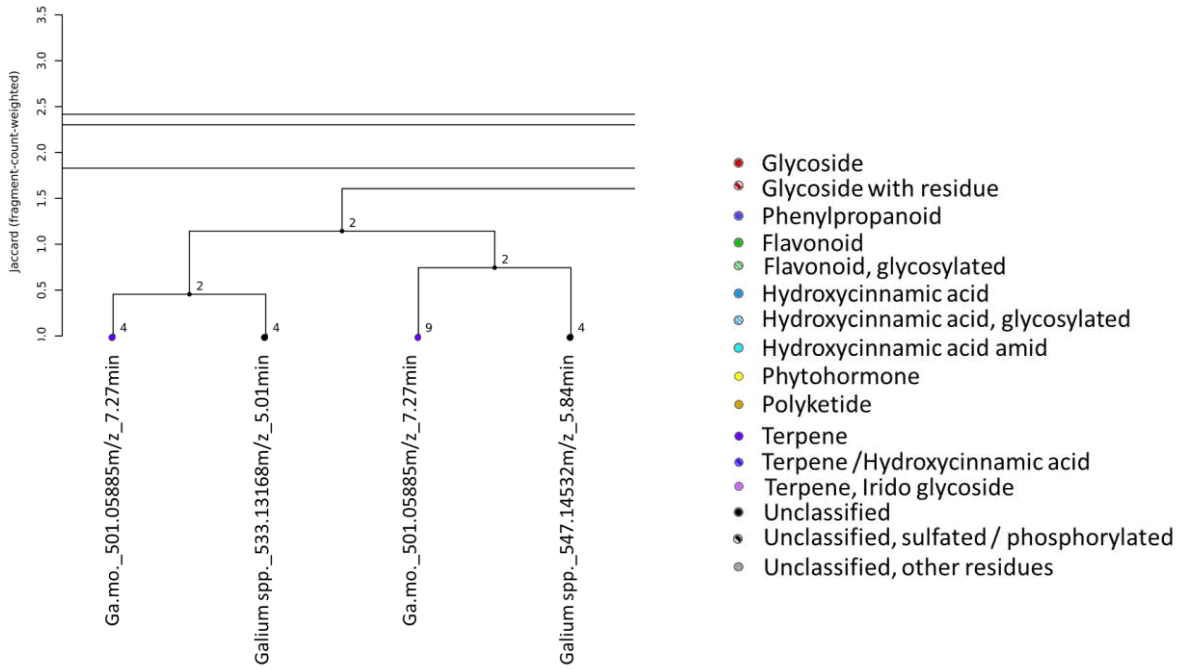
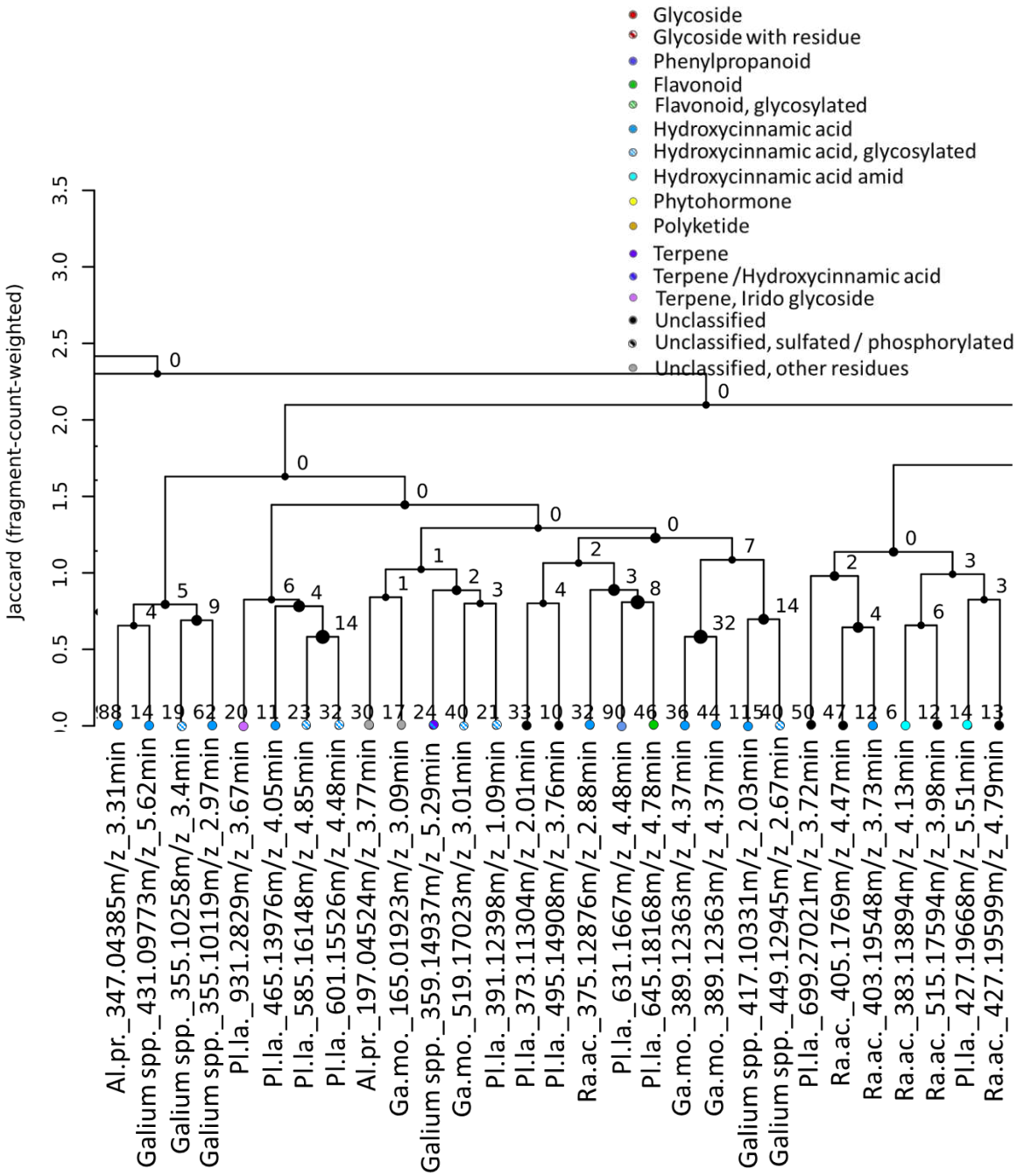


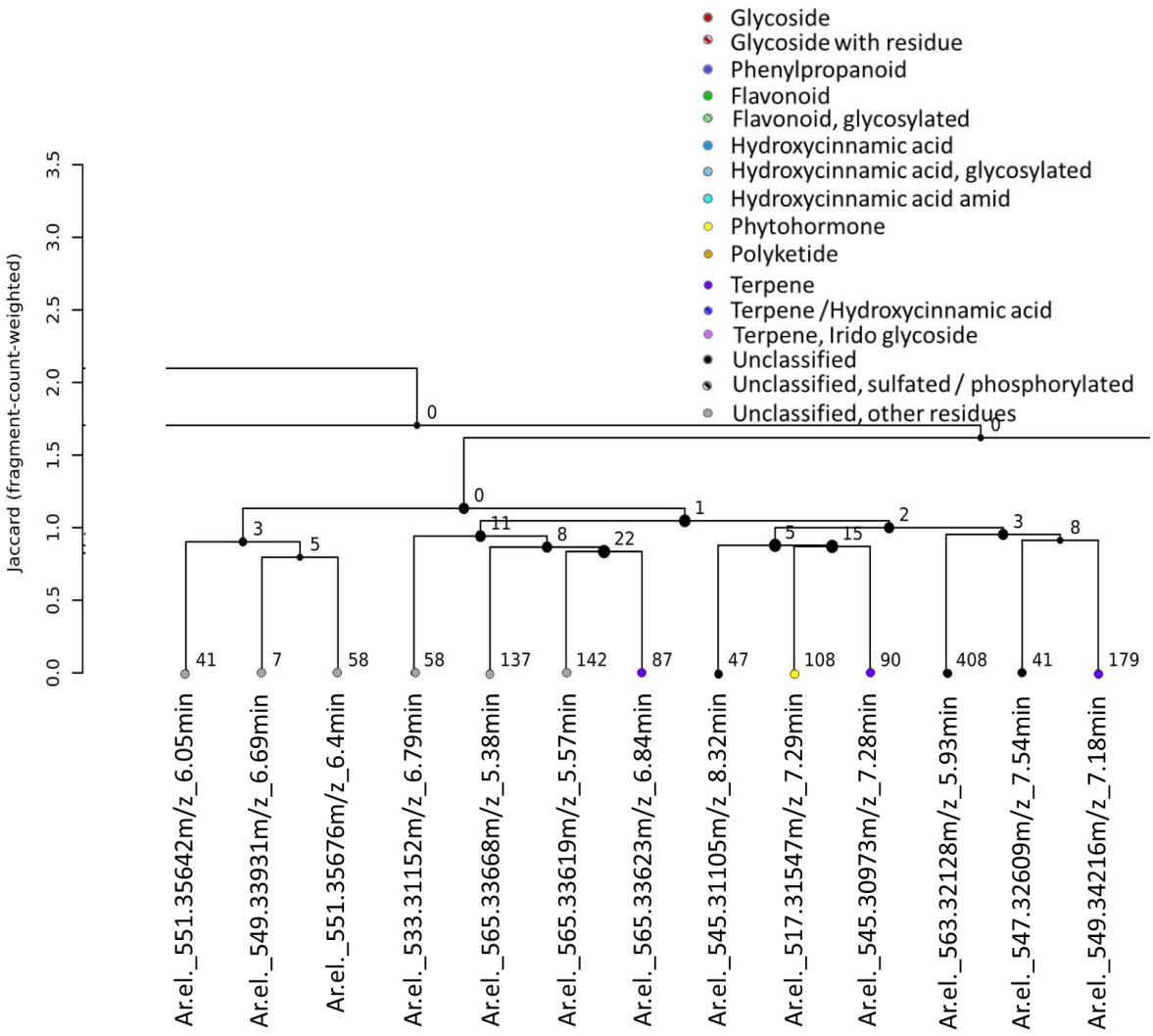
Supplementary Fig. 8: Detailed view of branch 4 of hierarchical clustering of species specific semi-polar compounds. Hierarchical clustering was performed on the tandem-mass spectra (CID and DDA) of the significant species specific compounds. Spectra cluster are based on fragment similarity by jaccard algorithm and fragment-count-weighted value rating.



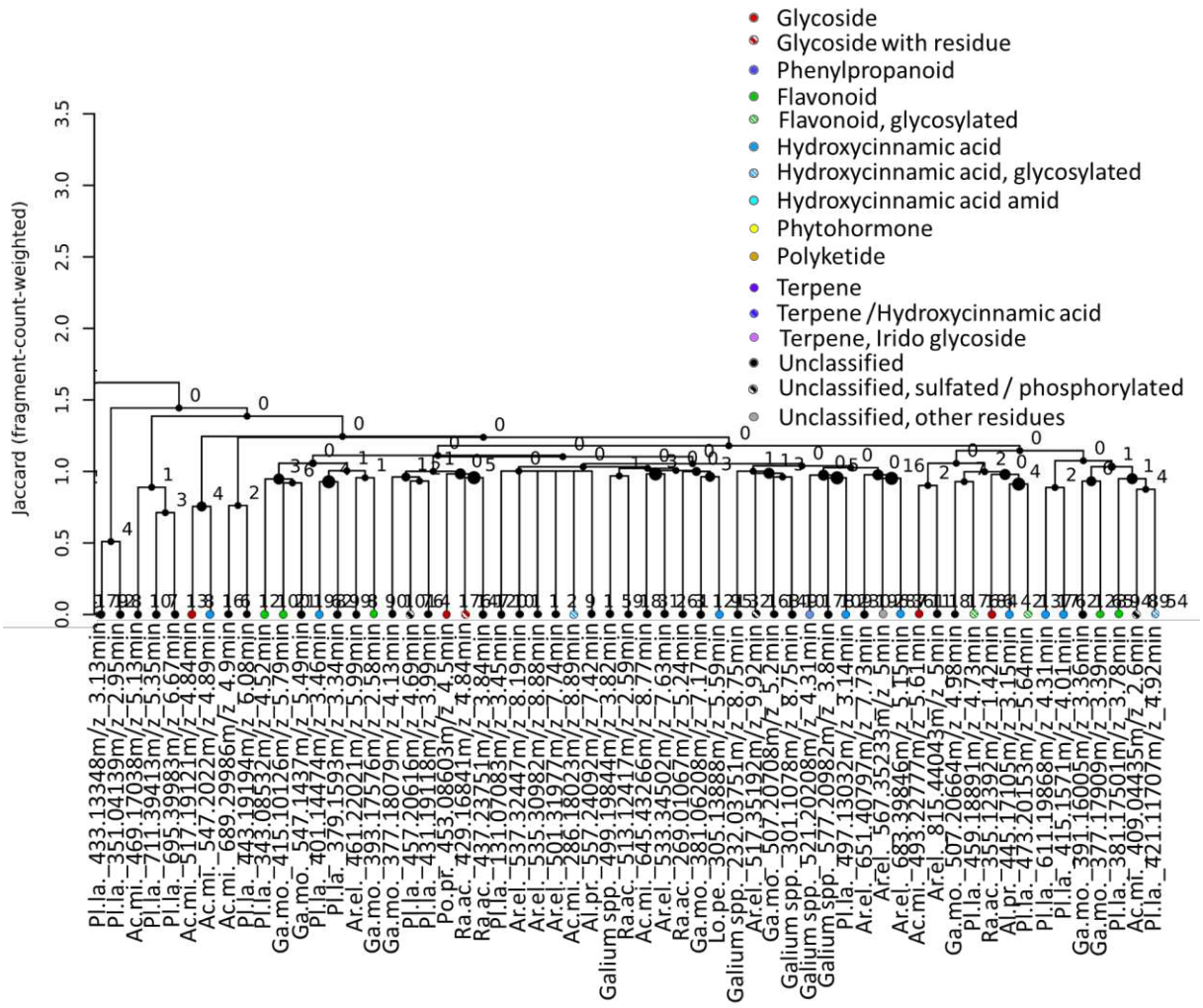
Supplementary Fig. 9: Detailed view of branch 5 of hierarchical clustering of species specific semi-polar compounds. Hierarchical clustering was performed on the tandem-mass spectra (CID and DDA) of the significant species specific compounds. Spectra cluster are based on fragment similarity by jaccard algorithm and fragment-count-weighted value rating.



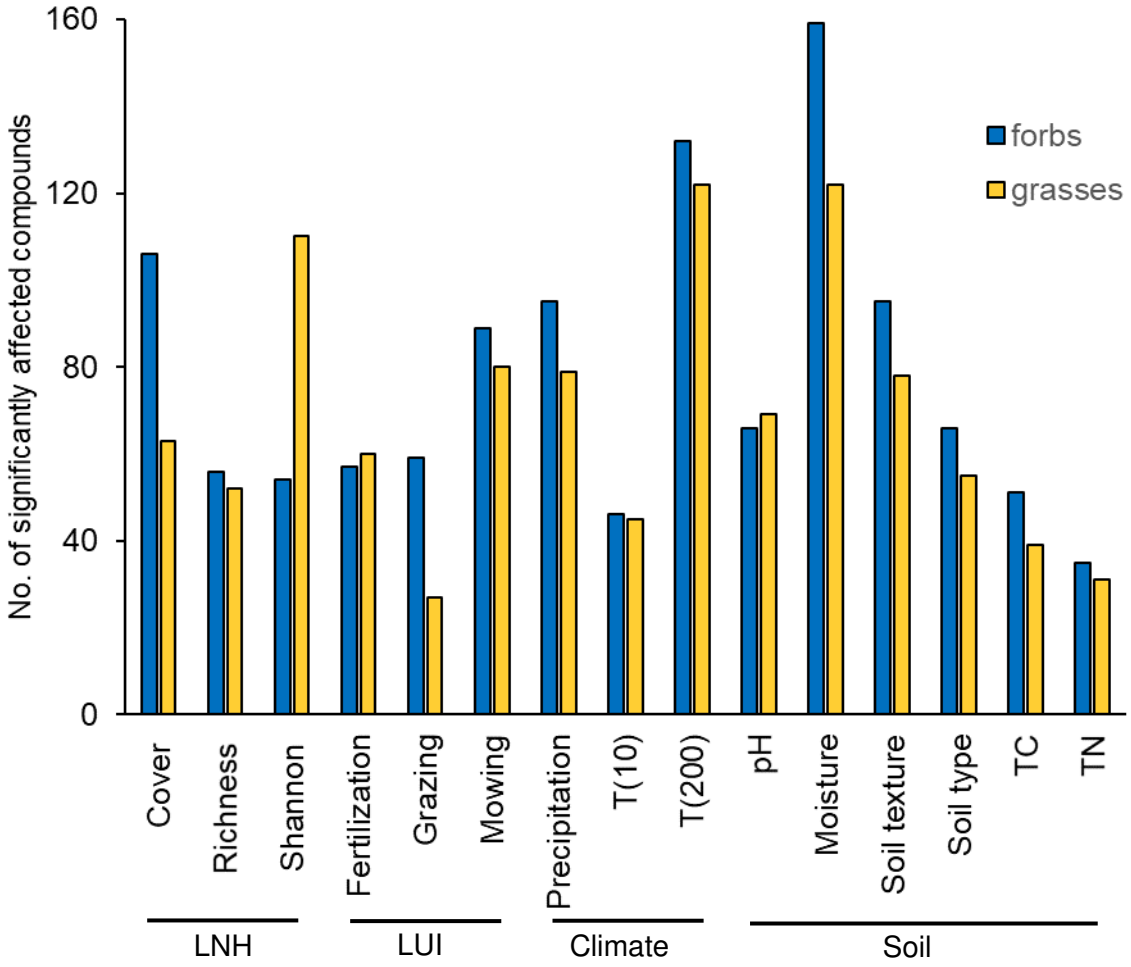
Supplementary Fig. 10: Detailed view of branch 6 and 7 of hierarchical clustering of species specific semi-polar compounds. Hierarchical clustering was performed on the tandem-mass spectra (CID and DDA) of the significant species specific compounds. Spectra cluster are based on fragment similarity by jaccard algorithm and fragment-count-weighted value rating.



Supplementary Fig. 11: Detailed view of branch 8 of hierarchical clustering of species specific semi-polar compounds. Hierarchical clustering was performed on the tandem-mass spectra (CID and DDA) of the significant species specific compounds. Spectra cluster are based on fragment similarity by jaccard algorithm and fragment-count-weighted value rating.



Supplementary Fig. 12: Detailed view of branch 9 of hierarchical clustering of species specific semi-polar compounds. Hierarchical clustering was performed on the tandem-mass spectra (CID and DDA) of the significant species specific compounds. Spectra cluster are based on fragment similarity by jaccard algorithm and fragment-count-weighted value rating.



Supplementary Fig. 13: Environmental characteristics affecting semi-polar compounds. Bar plot presents the number of significantly affected semi-polar compounds correlated to the different single variables of the environmental factors LNH, LUI, Climate and Soil. The numbers were calculated for each growth form (see legend). A detailed description of the abbreviation is given in Supplementary Table 10.

Supplementary Table 1: List of all polar metabolites. All annotated and identified polar metabolites are given with their quantifier ion (m/z), retention indices (Ri) and retention time (RT).

Compound Name	class	Quanti-fier ion	Ri	RT [min]
Noradrenalin (174)	alkaloid	174	1759.7	19.38
myo-Inositol (305)	alcohol	305	2133.3	24.23
Pinitol (260)	alcohol	260	1869.4	20.45
scyllo-inositol (204)	alcohol	204	2060	23.33
Sorbitol (217)	alcohol	217	1315.4	11.48
Threitol (217)	alcohol	217	1525.4	15.33
Xylitol (307)	alcohol	307	1735.6	18.59
Benzaldehyde (257)	aldehyde	257	1664.4	17.29
N-Acetylglucosamine (156)	amine	156	1796.5	19.62
Tyramine (174)	amine	174	1926.5	21.36
Alanine (116)	amino acid	116	1113.1	8.01
Asparagine (231)	amino acid	231	1697	18.07
Aspartate (232)	amino acid	232	1540.6	15.32
beta-Alanine (248)	amino acid	248	1436.7	13.63
Glutamate (246)	amino acid	246	1643.3	16.97
Glutamine (155)	amino acid	155	1484	19.36
Homoserine (218)	amino acid	218	1464.4	14.02
Isoleucine (158)	amino acid	158	1302.3	11.28
Leucine (158)	amino acid	158	1279.8	10.98
Lysine (156)	amino acid	156	1942.9	21.55
Methionine (176)	amino acid	176	1533.4	15.21
Ornithine / Citrullin (142)	amino acid	142	1843.3	20.05
Phenylalanine (192)	amino acid	192	1650.6	17.12
Proline (142)	amino acid	142	1304.4	11.32
Serine (204)	amino acid	204	1373.2	12.52
Threonine (218)	amino acid	218	1401.7	13.01
Tryptophan (202)	amino acid	202	2244.5	25.66
Tyrosine (218)	amino acid	218	1961.1	21.89
Valine (144)	amino acid	144	1222.4	9.89
Glycerol 3-phosphatee (357)	lipid	357	1799.3	19.67

Octadecadienoic acid (337)	lipid	337	2218.9	25.30
Octadecatrienoic acid (335)	lipid	335	2226.4	25.21
Octadecenoic acid (339)	lipid	339	2223.9	25.45
Adenine (264)	nuclic base/nucleotide	264	1879.6	21.31
Adenosine (236)	nuclic base/nucleotide	236	2680.6	22.92
Uracil (241)	nuclic base/nucleotide	241	1344.9	12.14
2-Aminoadipate (260)	organic acid	260	1742.3	23.67
2-Isopropylmalate (275)	organic acid	275	1599.2	20.56
2-Oxoglutarate (129)	organic acid	129	1597.1	16.29
4-Aminobutanoate [GABA] (174)	organic acid	174	1544.7	15.41
5-Indolecarboxylic acid (305)	organic acid	305	2033.7	22.84
Adipic acid (111)	organic acid	111	1515.5	15.02
Aminomalonic acid (218)	organic acid	218	1483.7	14.38
Azelaic acid (317)	organic acid	317	1806.9	19.64
Benzoic acid (267)	organic acid	267	1250.5	16.99
cis-Aconitate (229)	organic acid	229	1770.5	18.98
Coumaric acid (308)	organic acid	308	1807.4	19.60
Erythronic acid (292)	organic acid	292	1571.3	15.82
Gluconate (333)	organic acid	333	2037.5	23.02
Lactic acid (191)	organic acid	191	1076.6	7.32
Salicylic acid (267)	organic acid	267	1518.7	15.03
Shikimate (204)	organic acid	204	1834.8	19.99
Succinate (147)	organic acid	147	1316.3	11.56
Tartaric acid (292)	organic acid	292	1671.9	27.43
3-Caffeoyl-trans-quinic acid (345)	phenylpropanoid	345	3179	47.03
Fructose (217)	carbohydrates	217	1913.5/	
1924.8	21.23			
Glucose-6-phosphatee (387)	carbohydrates	387	2392.9	27.06
Lactose (361)	carbohydrates	361	2738.3/	
2754/				
2768.6	31.54			
Melibiose (361)	carbohydrates	361	2905.3/	
2917.9/				
2931.6	32.28			
Myo-Inositol-1-phosphatee (318)	carbohydrates	318	2486.6	16.89
Phosphoenolpyruvate (247)	carbohydrates	247	1624.7	21.11

Rhamnose (117)	carbohydrates	117	1756.3	18.74
Ribose (217)	carbohydrates	217	1709.9	18.14
Sucrose (361)	carbohydrates	361	2716.8	30.82
Xylose (217)	carbohydrates	217	1685.4/	
1694.4	17.67			
fatty acid (339) RT1518	unidentified lipid	339	-	25.30
carbohydrate (319) RT1314	unidentified carbohydrates	319	-	21.90
carbohydrate (319) RT1321	unidentified carbohydrates	319	-	22.02
carbohydrate (204) RT1781	unidentified carbohydrates	204	-	29.68
carbohydrate (204) RT1913	unidentified carbohydrates	204	-	31.88
compound (174) RT433	unidentified	174	-	7.22
compound (191) RT435	unidentified	191	-	7.25
compound (75) RT435	unidentified	75	-	7.25
compound (87) RT435	unidentified	87	-	7.25
compound (207) RT436	unidentified	207	-	7.27
compound (89) RT439	unidentified	89	-	7.32
compound (89) RT440	unidentified	89	-	7.33
compound (117) RT443	unidentified	117	-	7.38
compound (173) RT443	unidentified	173	-	7.38
compound (112) RT451	unidentified	112	-	7.52
compound (207) RT454	unidentified	207	-	7.57
compound (117) RT459	unidentified	117	-	7.65
compound (77) RT466	unidentified	77	-	7.77
compound (127) RT470	unidentified	127	-	7.83
compound (58) RT470	unidentified	58	-	7.83
compound (116) RT474	unidentified	116	-	7.90
compound (258) RT494	unidentified	258	-	8.23
compound (125) RT508	unidentified	125	-	8.47
compound (355) RT515	unidentified	355	-	8.58
compound (117) RT529	unidentified	117	-	8.82
compound (158) RT533	unidentified	158	-	8.88
compound (281) RT548	unidentified	281	-	9.13
compound (241) RT553	unidentified	241	-	9.22
compound (89) RT575	unidentified	89	-	9.58

compound (169) RT581	unidentified	169	-	9.68
compound (288) RT590	unidentified	288	-	9.83
compound (169) RT592	unidentified	169	-	9.87
compound (117) RT605	unidentified	117	-	10.08
compound (281) RT610	unidentified	281	-	10.17
compound (219) RT616	unidentified	219	-	10.27
compound (179) RT621	unidentified	179	-	10.35
compound (284) RT632	unidentified	284	-	10.53
compound (74) RT632	unidentified	74	-	10.53
compound (186) RT642	unidentified	186	-	10.70
compound (192) RT643	unidentified	192	-	10.72
compound (158) RT649	unidentified	158	-	10.82
compound (159) RT650	unidentified	159	-	10.83
compound (205) RT653	unidentified	205	-	10.88
compound (173) RT665	unidentified	173	-	11.08
compound (280) RT669	unidentified	280	-	11.15
compound (126) RT670	unidentified	126	-	11.17
compound (75) RT690	unidentified	75	-	11.50
compound (89) RT706	unidentified	89	-	11.77
compound (75) RT722	unidentified	75	-	12.03
compound (278) RT789	unidentified	278	-	13.15
compound (103) RT803	unidentified	103	-	13.38
compound (75) RT824	unidentified	75	-	13.73
compound (172) RT825	unidentified	172	-	13.75
compound (243) RT830	unidentified	243	-	13.83
compound (306) RT832	unidentified	306	-	13.87
compound (237) RT851	unidentified	237	-	14.18
compound (179) RT867	unidentified	179	-	14.45
compound (191) RT908	unidentified	191	-	15.13
compound (306) RT913	unidentified	306	-	15.22
compound (174) RT924	unidentified	174	-	15.40
compound (263) RT932	unidentified	263	-	15.53
compound (71) RT934	unidentified	71	-	15.57
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compound (227) RT945	unidentified	227	-	15.75

compound (174) RT956	unidentified	174	-	15.93
compound (217) RT964	unidentified	217	-	16.07
compound (142) RT973	unidentified	142	-	16.22
compound (103) RT974	unidentified	103	-	16.23
compound (103) RT982	unidentified	103	-	16.37
compound (223) RT983	unidentified	223	-	16.38
compound (245) RT987	unidentified	245	-	16.45
compound (342) RT996	unidentified	342	-	16.60
compound (117) RT1037	unidentified	117	-	17.28
compound (245) RT1049	unidentified	245	-	17.48
compound (103) RT1055	unidentified	103	-	17.58
compound (245) RT1065	unidentified	245	-	17.75
compound (277) RT1082	unidentified	277	-	18.03
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compound (217) RT1123	unidentified	217	-	18.72
compound (57) RT1127	unidentified	57	-	18.78
compound (93) RT1132	unidentified	93	-	18.87
compound (103) RT1133	unidentified	103	-	18.88
compound (69) RT1140	unidentified	69	-	19.00
compound (174) RT1143	unidentified	174	-	19.05
compound (69) RT1150	unidentified	69	-	19.17
compound (217) RT1155	unidentified	217	-	19.25
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compound (217) RT1166	unidentified	217	-	19.43
compound (292) RT1166	unidentified	292	-	19.43
compound (295) RT1171	unidentified	295	-	19.52
compound (69) RT1171	unidentified	69	-	19.52
compound (57) RT1173	unidentified	57	-	19.55
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compound (75) RT1218	unidentified	75	-	20.30
compound (156) RT1226	unidentified	156	-	20.43
compound (149) RT1233	unidentified	149	-	20.55

compound (160) RT1240	unidentified	160	-	20.67
compound (103) RT1243	unidentified	103	-	20.72
compound (71) RT1257	unidentified	71	-	20.95
compound (295) RT1259	unidentified	295	-	20.98
compound (344) RT1259	unidentified	344	-	20.98
compound (103) RT1264	unidentified	103	-	21.07
compound (319) RT1268	unidentified	319	-	21.13
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compound (319) RT1286	unidentified	319	-	21.43
compound (273) RT1289	unidentified	273	-	21.48
compound (273) RT1297	unidentified	273	-	21.62
compound (299) RT1298	unidentified	299	-	21.63
compound (132) RT1300	unidentified	132	-	21.67
compound (299) RT1309	unidentified	299	-	21.82
compound (285) RT1310	unidentified	285	-	21.83
compound (57) RT1314	unidentified	57	-	21.90
compound (319) RT1316	unidentified	319	-	21.93
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compound (174) RT1327	unidentified	174	-	22.12
compound (361) RT1330	unidentified	361	-	22.17
compound (318) RT1334	unidentified	318	-	22.23
compound (204) RT1340	unidentified	204	-	22.33
compound (205) RT1347	unidentified	205	-	22.45
compound (293) RT1357	unidentified	293	-	22.62
compound (311) RT1362	unidentified	311	-	22.70
compound (155) RT1366	unidentified	155	-	22.77
compound (297) RT1372	unidentified	297	-	22.87
compound (335) RT1385	unidentified	335	-	23.08
compound (331) RT1441	unidentified	331	-	24.02
compound (217) RT1443	unidentified	217	-	24.05
compound (331) RT1443	unidentified	331	-	24.05

compound (324) RT1456	unidentified	324	-	24.27
compound (327) RT1456	unidentified	327	-	24.27
compound (319) RT1465	unidentified	319	-	24.42
compound (319) RT1466	unidentified	319	-	24.43
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compound (204) RT1517	unidentified	204	-	25.28
compound (357) RT1526	unidentified	357	-	25.43
compound (204) RT1603	unidentified	204	-	26.72
compound (167) RT1609	unidentified	167	-	26.82
compound (204) RT1615	unidentified	204	-	26.92
compound (197) RT1637	unidentified	197	-	27.28
compound (239) RT1643	unidentified	239	-	27.38
compound (83) RT1643	unidentified	83	-	27.38
compound (255) RT1654	unidentified	255	-	27.57
compound (204) RT1655	unidentified	204	-	27.58
compound (57) RT1655	unidentified	57	-	27.58
compound (259) RT1657	unidentified	259	-	27.62
compound (91) RT1660	unidentified	91	-	27.67
compound (260) RT1676	unidentified	260	-	27.93
compound (204) RT1683	unidentified	204	-	28.05
compound (82) RT1700	unidentified	82	-	28.33
compound (204) RT1731	unidentified	204	-	28.85
compound (149) RT1745	unidentified	149	-	29.08
compound (204) RT1749	unidentified	204	-	29.15
compound (217) RT1751	unidentified	217	-	29.18
compound (204) RT1756	unidentified	204	-	29.27
compound (283) RT1774	unidentified	283	-	29.57
compound (219) RT1776	unidentified	219	-	29.60
compound (127) RT1780	unidentified	127	-	29.67
compound (204) RT1780	unidentified	204	-	29.67
compound (204) RT1788	unidentified	204	-	29.80
compound (216) RT1788	unidentified	216	-	29.80
compound (261) RT1791	unidentified	261	-	29.85
compound (204) RT1796	unidentified	204	-	29.93

compound (204) RT1798	unidentified	204	-	29.97
compound (117) RT1803	unidentified	117	-	30.05
compound (173) RT1809	unidentified	173	-	30.15
compound (191) RT1825	unidentified	191	-	30.42
compound (204) RT1834	unidentified	204	-	30.57
compound (204) RT1853	unidentified	204	-	30.88
compound (319) RT1860	unidentified	319	-	31.00
compound (356) RT1864	unidentified	356	-	31.07
compound (306) RT1865	unidentified	306	-	31.08
compound (217) RT1877	unidentified	217	-	31.28
compound (204) RT1902	unidentified	204	-	31.70
compound (259) RT1906	unidentified	259	-	31.77
compound (160) RT1909	unidentified	160	-	31.82
compound (361) RT1909	unidentified	361	-	31.82
compound (204) RT1914	unidentified	204	-	31.90
compound (361) RT1919	unidentified	361	-	31.98
compound (259) RT1920	unidentified	259	-	32.00
compound (361) RT1926	unidentified	361	-	32.10
compound (217) RT1936	unidentified	217	-	32.27
compound (223) RT1937	unidentified	223	-	32.28
compound (204) RT1939	unidentified	204	-	32.32
compound (361) RT1948	unidentified	361	-	32.47
compound (204) RT1958	unidentified	204	-	32.63
compound (319) RT1968	unidentified	319	-	32.80
compound (204) RT1971	unidentified	204	-	32.85
compound (83) RT1974	unidentified	83	-	32.90
compound (362) RT1978	unidentified	362	-	32.97
compound (204) RT1986	unidentified	204	-	33.10
compound (525) RT1992	unidentified	525	-	33.20
compound (201) RT1994	unidentified	201	-	33.23
compound (217) RT1994	unidentified	217	-	33.23
compound (361) RT2003	unidentified	361	-	33.38
compound (204) RT2011	unidentified	204	-	33.52
compound (119) RT2019	unidentified	119	-	33.65
compound (297) RT2022	unidentified	297	-	33.70
compound (217) RT2026	unidentified	217	-	33.77

compound (361) RT2053	unidentified	361	-	34.22
compound (91) RT2055	unidentified	91	-	34.25
compound (327) RT2065	unidentified	327	-	34.42
compound (204) RT2068	unidentified	204	-	34.47

Supplementary Table 2: Chemical richness as function of site, species and growth form. Supplementary Table shows the A) analysis of variance (ANOVA) and the B) parameter estimates of linear mixed effect models for chemical richness of polar and semi-polar metabolites. Fixed factors were either species, site and their interaction (model 1) or growth form, site and their interaction (model 2). In both models, plot was included as random factors. Note that the intercept in model 1 refers to species Achillea millefolium and the site ALB (Swabian Alb), and in model 2 to the growth form forb and site ALB.

a) ANOVA

	Predictor	Polar metabolites *p*	Semi polar metabolites *p*
Model 1	Species	0.023	<0.001
	Site	<0.001	<0.001
	Site × species	0.004	0.001
Model 2	Growth form	0.260	0.630
	Site	<0.001	<0.001
	Site × growth form	<0.001	<0.001

b) linear mixed effect models

Predictor		Polar metabolites		Semi-polar metabolites	
		Estimate	***p***	**Estimate**	***p***
Model 1	Intercept	38.653	**<0.001**	92.246	**<0.001**
	G. mollugo	9.634	0.267	36.365	0.144
	G. verum	4.672	0.590	-16.707	0.502
	P. lanceolata	11.859	0.184	0.248	0.992
	R. acris	-4.749	0.618	-36.648	0.177
	A. pratensis	0.035	0.997	0.0348	0.997
	A. elatius	16.950	**0.039**	8.790	0.708
	D. glomerata	-0.269	0.974	-30.566	0.194
	L. perenne	-2.774	0.753	-41.355	0.105
	P. pratensis	-2.678	0.772	-37.423	0.154
	HAI	12.891	0.159	30.971	0.197
	SCH	20.522	0.109	-19.523	0.568
	G. mollugo HAI	5.887	0.619	78.095	**0.023**
	G. verum HAI	-5.547	0.633	21.942	0.512
	P. lanceolata HAI	-0.919	0.941	48.305	0.173
	R. acris HAI	7.867	0.529	-1.461	0.967
	A. pratensis HAI	0.148	0.992	2.580	0.950
	A. elatius HAI	-28.357	**0.030**	-47.266	0.204
	D. glomerata HAI	2.465	0.834	15.383	0.650
	L. perenne HAI	6.714	0.605	21.875	0.558
	P. pratensis HAI	-5.469	0.667	-0.914	0.980
	G. mollugo SCH	17.573	0.257	97.729	**0.028**
	G. verum SCH	14.908	0.363	139.798	**0.003**
	P. lanceolata SCH	14.081	0.389	65.797	0.159
	R. acris SCH	55.602	**0.006**	96.651	**0.093**
	A. pratensis SCH	35.197	0.058	145.843	**0.005**
	A. elatius SCH	42.217	**0.009**	164.544	**<0.001**
	D. glomerata SCH	43.128	**0.004**	134.429	**0.002**
	L. perenne SCH	57.209	**<0.001**	151.172	**0.001**
	P. pratensis SCH	34.557	**0.037**	98.084	**0.038**
Model 2	Intercept	43.269	**<0.001**	88.58	**0.001**
	grass	-1.749	0.729	-19.95	0.392
	HAI	13.748	**0.014**	59.540	**<0.001**
	SCH	37.333	**<0.001**	60.210	**<0.001**
	grass: HAI	-5.493	0.353	-28.410	0.095
	grass: SCH	25.192	**<0.001**	57.330	**0.005**

Supplementary Table 3: Probability of occurrence of polar metabolite. Values represent the ratio of the percentage of occurrence of the sample groups, A) growth forms, the sites and their interaction, B) the species, and C) the interaction between species and sites. Metabolites occurring with an at least to times higher percentage in one of the groups are colored green. Asterisk marks all metabolites, which occur only in the corresponding sample group. Metabolites being not preferentially exuded by at least one sample group are left out.

a) Growth form, site, interaction of site and growth form

	growth form		site			site x growth form					
Metabolites	**forb**	**grass**	**ALB**	**HAI**	**SCH**	**ALB forb**	**ALB grass**	**HAI forb**	**HAI grass**	**SCH forb**	**SCH grass**
Noradrenalin (174)	0	2 *	2 *	0	0	0	2 *	0	0	0	0
Threitol (217)	0.19	5.19	0.25	1.06	0.4	0	0.22	0.04	1.05	0.24	0.08
N-Acetylglucosamine (156)	0	2 *	2 *	0	0	0	2 *	0	0	0	0
Tyramine (174)	0.45	2.23	0.34	0.35	0.94	0.33	0	0	0.48	0	0.75
Asparagine (231)	2 *	0	2 *	0	0	2 *	0	0	0	0	0
Aspartate (232)	0.51	1.95	0.23	0.38	1.16	0.08	0.13	0.1	0.26	0.23	0.49
beta-Alanine (248)	0.04	22.26	0.19	0.2	2.07	0.03	0.16	0	0.27	0	1.63
Glutamate (246)	0.49	2.04	0.24	0.32	1.32	0.06	0.16	0.1	0.2	0.25	0.54
Glutamine (155)	1.8	0.56	0.49	2.06	0	0	0.59	1.7	0	0	0
Homoserine (218)	0.05	18.92	0.13	0.31	1.87	0	0.13	0.03	0.33	0	1.48
Lysine (156)	0.36	2.78	0.26	0.27	1.41	0	0.3	0.24	0	0	1.36
Methionine (176)	0.12	8.68	0.13	0.16	2.99	0.01	0.12	0	0.21	0.15	1.37
Phenylalanine (192)	0.26	3.9	0.18	0.25	1.82	0.02	0.16	0.06	0.21	0.17	0.89
Tyrosine (218)	0.08	12.98	0.11	0.21	2.58	0	0.12	0.02	0.26	0.08	1.48
Glycerol 3-phosphate (357)	1.3	0.77	0.09	0.13	4.02	0.06	0.03	0.11	0	0.77	0.62

Octadecadienoic acid (337)	0.25	4.08	0.04	0	24.82	0	0.04	0	0	0.37	2.24
Octadecatrienoic acid (335)	3.59	0.28	0	0.81	1.24	0	0	0.67	0	0.51	0.36
Octadecenoic acid (339)	0.47	2.14	0.23	0.18	1.94	0.05	0.16	0.05	0.14	0.4	0.57
Uracil (241)	0.34	2.98	0.21	0.33	1.38	0.02	0.17	0.07	0.26	0.25	0.54
2-Aminoadipate (260)	0.15	6.68	0.26	0.27	1.41	0	0.28	0.1	0.16	0	1.22
2-Isopropylmalate (275)	2.92	0.34	0	2 *	0	0	0	2.18	0.46	0	0
Aminomalonic acid (218)	0	2 *	0	0	2 *	0	0	0	0	0	2 *
Azelaic acid (317)	7.19	0.14	0	4.31	0.23	0	0	1.74	0.16	0.3	0
Coumaric acid (308)	0	2 *	0	0.13	7.42	0	0	0	0.19	0	5.29
Lactic acid (191)	2.1	0.48	0.11	9.26	0	0.11	0	1.07	0.62	0	0
Succinate (147)	0	2 *	2 *	0	0	0	2 *	0	0	0	0
Tartaric acid (292)	0.9	1.11	0	0.13	7.42	0	0	0.11	0	0.64	1.02
3-Caffeoyl-trans-quinic acid (345)	2 *	0	0	2 *	0	0	0	2 *	0	0	0
Glucose-6-phosphate (387)	1.35	0.74	0	0.36	2.78	0	0	0.31	0	0.43	0.87
Lactose (361)	3.74	0.27	0.54	0.34	0.66	0.38	0.09	0.28	0	0.41	0.16
Melibiose (361)	4.94	0.2	0.08	3.89	0.15	0.08	0	1.35	0.23	0.19	0
Myo-Inositol-1-phosphate (318)	0.54	1.85	0	0.08	12.98	0	0	0	0.1	0.81	0.86
Phosphoenolpyruvate (247)	0.16	6.23	0.36	0.04	2.28	0.07	0.27	0	0.06	0.1	1.35
Sucrose (361)	0.9	1.11	2 *	0	0	0.98	1.02	0	0	0	0
Xylose (217)	1.71	0.59	0.03	5.55	0.14	0.03	0	1.06	0.52	0	0.13

unknown compound (174) RT433	0	2 *	2 *	0	0	0	2 *	0	0	0	0
unknown compound (191) RT435	1.35	0.74	0.08	0	12.41	0	0.08	0	0	2.24	0.31
unknown compound (75) RT435	0	2 *	2 *	0	0	0	2 *	0	0	0	0
unknown compound (89) RT439	4.49	0.22	0.11	2.48	0.23	0.12	0	1.23	0.16	0.13	0.1
unknown compound (117) RT443	0.78	1.28	0.02	0.36	2.58	0	0.02	0.14	0.17	0.53	0.59
unknown compound (173) RT443	2 *	0	0	0	2 *	0	0	0	0	2 *	0
unknown compound (112) RT451	0	2 *	2 *	0	0	0	2 *	0	0	0	0
unknown compound (117) RT459	0.82	1.21	5.5	0	0.18	0.81	0.71	0	0	0	0.16
unknown compound (77) RT466	0	2 *	0.08	0	12.41	0	0.09	0	0	0	10.9
unknown compound (127) RT470	0	2 *	2 *	0	0	0	2 *	0	0	0	0
unknown compound (58) RT470	0	2 *	2 *	0	0	0	2 *	0	0	0	0
unknown compound (258) RT494	0	2 *	2 *	0	0	0	2 *	0	0	0	0
unknown compound (125) RT508	2 *	0	2 *	0	0	2 *	0	0	0	0	0
unknown compound (117) RT529	0.08	12.8	0	0.1	9.74	0	0	0.02	0.1	0.06	4.92
unknown compound (158) RT533	0.78	1.28	0.13	0.13	3.39	0.05	0.07	0.07	0.05	0.59	0.67
unknown compound (241) RT553	1.2	0.83	0	0.4	2.47	0	0	0.26	0.07	0.47	0.7

unknown compound (89) RT575	0.67	1.48	0	0	2 *	0	0	0	0	0.97	1.03
unknown compound (288) RT590	0	2 *	2 *	0	0	0	2 *	0	0	0	0
unknown compound (169) RT592	4.04	0.25	0.07	0.49	1.55	0.07	0	0.39	0	0.76	0.29
unknown compound (179) RT621	0.9	1.11	0.07	0.34	2.1	0.03	0.04	0.17	0.11	0.44	0.6
unknown compound (284) RT632	2 *	0	0	2 *	0	0	0	2 *	0	0	0
unknown compound (74) RT632	0	2 *	0	2 *	0	0	0	0	2 *	0	0
unknown compound (186) RT642	0.78	1.28	0.03	0.27	3.14	0	0.03	0.15	0.08	0.49	0.77
unknown compound (192) RT643	2 *	0	0	2 *	0	0	0	2 *	0	0	0
unknown compound (158) RT649	0.22	4.45	1.46	0.69	0	0.22	0.57	0	0.85	0	0
unknown compound (159) RT650	0.48	2.09	0.13	2.72	0.18	0.04	0.08	0.29	1.13	0	0.15
unknown compound (173) RT665	0	2 *	2 *	0	0	0	2 *	0	0	0	0
unknown compound (280) RT669	0.82	1.21	0.04	0.33	2.42	0.01	0.03	0.16	0.13	0.46	0.64
unknown compound (126) RT670	2 *	0	0	0.54	1.85	0	0	0.39	0	2.54	0
unknown compound (75) RT690	0.74	1.36	0.05	0.12	5.61	0	0.05	0.07	0.04	0.74	0.75
unknown compound (89) RT706	0.64	1.56	0.12	3.95	0.11	0.05	0.05	0.38	1.17	0	0.09
unknown compound (278) RT789	0.26	3.9	0.07	0.17	3.74	0.07	0	0	0.22	0.18	1.48

unknown compound (306) RT832	2 *	0	0	0.36	2.78	0	0	0.26	0	3.81	0
unknown compound (179) RT867	1.11	0.9	0.14	0.24	2.18	0.08	0.04	0.1	0.11	0.63	0.43
unknown compound (191) RT908	0.45	2.23	1.94	0.51	0	0.43	0.44	0	0.64	0	0
unknown compound (306) RT913	0	2 *	0.26	0	3.82	0	0.3	0	0	0	3.35
unknown compound (174) RT924	3.77	0.26	0.17	0.66	0.83	0.14	0.03	0.46	0.04	0.5	0.18
unknown compound (71) RT934	0.07	14.47	0	0.04	24.11	0	0	0	0.06	0.1	5.86
unknown compound (158) RT944	1.04	0.96	0.09	0.21	2.89	0.05	0.04	0.14	0.05	0.56	0.65
unknown compound (227) RT945	1.32	0.76	0.04	9.21	0.06	0.02	0.02	0.86	0.79	0.02	0.04
unknown compound (174) RT956	0.13	7.79	0.31	0	3.18	0	0.34	0	0	0.22	1.3
unknown compound (217) RT964	0.46	2.15	0.18	0.24	1.88	0.08	0.08	0.04	0.22	0.31	0.64
unknown compound (142) RT973	0.68	1.47	0.1	0.31	2.02	0.03	0.08	0.16	0.11	0.33	0.71
unknown compound (103) RT974	0.3	3.34	0.08	0.08	5.64	0	0.08	0	0.11	0.48	1.02
unknown compound (103) RT982	0.6	1.67	0.02	0.29	3.15	0	0.02	0.1	0.16	0.47	0.76
unknown compound (223) RT983	0.1	9.57	0.12	0.16	3.13	0	0.12	0.01	0.19	0.14	1.51
unknown compound (245) RT987	0.1	10.02	0.06	0	17.18	0	0.06	0	0	0.15	4.24
unknown compound (342) RT996	1.7	0.59	0.08	12.35	0	0	0.09	1.33	0.54	0	0

unknown compound (245) RT1049	1.57	0.64	0.1	10.29	0	0.1	0	0.84	0.83	0	0
unknown compound (103) RT1055	1.25	0.8	0.04	5.06	0.14	0.04	0	0.68	0.77	0.05	0.08
unknown compound (245) RT1065	0	2 *	2 *	0	0	0	2 *	0	0	0	0
unknown compound (217) RT1123	2 *	0	2 *	0	0	2 *	0	0	0	0	0
unknown compound (57) RT1127	0.61	1.63	0	0.37	2.72	0	0	0.19	0.13	0.25	1.11
unknown compound (93) RT1132	2 *	0	0	2 *	0	0	0	2 *	0	0	0
unknown compound (69) RT1140	0.28	3.56	0.21	0.11	2.69	0.03	0.18	0.03	0.09	0.26	1.01
unknown compound (174) RT1143	1.8	0.56	0.26	0	3.82	0.2	0.04	0	0	1.06	0.4
unknown compound (217) RT1155	0	2 *	0.07	0.07	7.06	0	0.07	0	0.09	0	5.55
unknown compound (295) RT1171	0.58	1.73	0.13	0.03	6.04	0.05	0.07	0	0.03	0.65	0.84
unknown compound (69) RT1171	0.36	2.78	0	2 *	0	0	0	0.27	3.72	0	0
unknown compound (217) RT1180	2.95	0.34	0.2	0.32	1.44	0.17	0.02	0.23	0.03	0.72	0.25
unknown compound (292) RT1203	0.49	2.05	0.25	0.24	1.54	0.08	0.16	0.11	0.12	0.19	0.77
unknown compound (174) RT1212	0.9	1.11	0.52	0	1.91	0	0.46	0	0	2.17	0
unknown compound (103) RT1218	0	2 *	2 *	0	0	0	2 *	0	0	0	0
unknown compound (75) RT1218	0.16	6.2	0.36	0.17	1.45	0.07	0.26	0.01	0.19	0.07	0.94

unknown compound (156) RT1226	0.49	2.05	0.18	0.08	3.49	0.04	0.14	0.05	0.02	0.44	0.89
unknown compound (149) RT1233	0.84	1.19	0.24	0.12	2.29	0.09	0.12	0.08	0.03	0.5	0.58
unknown compound (160) RT1240	0.39	2.55	0.41	0.21	1.16	0.13	0.22	0.06	0.16	0.12	0.68
unknown compound (71) RT1257	2 *	0	0.42	0.58	0.51	0.42	0	0.45	0	0.65	0
unknown compound (295) RT1259	0.45	2.23	2 *	0	0	0.49	2.04	0	0	0	0
unknown compound (344) RT1259	2 *	0	0	2 *	0	0	0	2 *	0	0	0
unknown compound (103) RT1264	2.37	0.42	0.85	0.03	1.04	0.39	0.19	0.03	0	0.87	0.08
unknown compound (319) RT1268	0.25	4.08	0	0.04	24.11	0	0	0	0.05	0.36	2.16
unknown compound (179) RT1272	0	2 *	2 *	0	0	0	2 *	0	0	0	0
unknown compound (204) RT1272	0.9	1.11	0	2 *	0	0	0	0.67	1.49	0	0
unknown compound (217) RT1272	0.06	16.69	0.07	0	13.36	0	0.08	0	0	0.09	5.23
unknown compound (273) RT1297	0.83	1.2	0.08	0.31	2.22	0.03	0.04	0.12	0.16	0.55	0.49
unknown compound (299) RT1298	2 *	0	0	2 *	0	0	0	2 *	0	0	0
unknown compound (132) RT1300	0.71	1.4	0.1	0.29	2.2	0.02	0.07	0.11	0.14	0.5	0.54
unknown compound (299) RT1309	3.95	0.25	0.41	0.37	0.79	0.42	0	0.26	0.04	0.35	0.25
unknown compound (285) RT1310	0	2 *	2 *	0	0	0	2 *	0	0	0	0

unknown compound (57) RT1314	0.9	1.11	0.21	2.52	0.12	0.06	0.13	0.54	0.6	0	0.11
unknown compound (319) RT1316	0.73	1.37	0.24	0.15	2.1	0.1	0.12	0.08	0.05	0.38	0.66
unknown compound (71) RT1316	2.7	0.37	0.32	3.09	0	0.33	0	0.76	0.47	0	0
unknown compound (160) RT1319	2.4	0.42	0.3	0.26	1.3	0.29	0	0.13	0.09	0.55	0.28
unknown compound (333) RT1325	0.58	1.73	0.24	0.06	2.95	0.06	0.16	0.02	0.04	0.58	0.61
unknown compound (318) RT1334	1.2	0.83	0.04	0	24.82	0.04	0	0	0	1.37	0.62
unknown compound (204) RT1340	0.04	27.82	0.16	0.02	5.16	0	0.18	0	0.03	0.06	3.2
unknown compound (293) RT1357	0.1	10.02	0.03	0.03	16.93	0	0.03	0	0.04	0.15	4.07
unknown compound (311) RT1362	0.7	1.43	0.12	0.21	2.54	0.05	0.07	0.09	0.1	0.46	0.66
unknown compound (155) RT1366	1.8	0.56	0	0.27	3.71	0	0	0	0.29	3.42	0
unknown compound (297) RT1372	0	2 *	0	0	2 *	0	0	0	0	0	2 *
unknown compound (335) RT1385	2.9	0.35	0.24	0.12	2.33	0.23	0	0.1	0	0.84	0.36
unknown compound (331) RT1441	0.45	2.23	1.05	0	0.95	0.37	0.38	0	0	0	0.85
unknown compound (331) RT1443	0.63	1.59	0.21	4.8	0	0	0.2	0.5	0.99	0	0
unknown compound (324) RT1456	0.4	2.53	0.13	0	7.91	0.02	0.11	0	0	0.56	1.11
unknown compound (327) RT1456	0.86	1.17	0.13	0.06	4.88	0.03	0.09	0.04	0.02	0.94	0.54

unknown compound (319) RT1465	2.4	0.42	0.36	2.75	0	0.11	0.24	1.4	0.14	0	0
unknown compound (319) RT1466	0.57	1.74	0.17	0.21	2.08	0.08	0.09	0.1	0.1	0.26	0.85
unknown compound (319) RT1487	0.74	1.35	0.02	0.19	4.55	0	0.02	0.06	0.12	0.78	0.59
unknown compound (128) RT1504	0.11	8.9	0	1.89	0.53	0	0	0.08	1.81	0	0.4
unknown compound (185) RT1512	0	2 *	0	2 *	0	0	0	0	2 *	0	0
unknown compound (204) RT1603	1.05	0.95	0.08	0.23	2.81	0.01	0.07	0.08	0.12	1.05	0.3
unknown compound (204) RT1615	0.07	13.35	0	0.1	10.2	0	0	0	0.13	0.11	3.57
unknown compound (197) RT1637	0.6	1.67	0.03	0.3	2.79	0	0.03	0.13	0.14	0.4	0.79
unknown compound (239) RT1643	0	2 *	2 *	0	0	0	2 *	0	0	0	0
unknown compound (83) RT1643	0	2 *	2 *	0	0	0	2 *	0	0	0	0
unknown compound (204) RT1655	0.9	1.11	0.24	0.06	2.95	0.14	0.07	0.01	0.06	0.65	0.54
unknown compound (57) RT1655	1.62	0.62	0.21	0	4.77	0.2	0	0	0	0.88	0.57
unknown compound (259) RT1657	0.63	1.59	0.23	0.06	3.06	0.08	0.13	0.03	0.02	0.5	0.72
unknown compound (91) RT1660	0	2 *	2 *	0	0	0	2 *	0	0	0	0
unknown compound (260) RT1676	1.11	0.9	0.32	0.53	0.7	0.15	0.13	0.22	0.19	0.28	0.25
unknown compound (204) RT1683	0	2 *	2 *	0	0	0	2 *	0	0	0	0

unknown compound (204) RT1731	0.8	1.25	0.06	0.25	2.8	0.02	0.05	0.11	0.12	0.61	0.56
unknown compound (204) RT1749	2.52	0.4	1.01	0.31	0.35	0.61	0.17	0.26	0	0.12	0.19
unknown compound (217) RT1751	20.67	0.05	0.26	0.86	0.49	0.27	0	0.68	0	0.46	0.07
unknown compound (204) RT1756	8.99	0.11	1.19	0.19	0.42	0.84	0.08	0.15	0	0.5	0
unknown compound (283) RT1774	0.78	1.28	0	0.22	4.64	0	0	0.13	0.06	0.51	0.96
unknown compound (219) RT1776	2.44	0.41	0.25	0.43	0.99	0.22	0.03	0.3	0.04	0.36	0.34
unknown compound (127) RT1780	7.19	0.14	3.4	0.29	0	2.18	0.13	0.25	0	0	0
unknown compound (204) RT1780	0	2 *	2 *	0	0	0	2 *	0	0	0	0
unknown compound (204) RT1788	2 *	0	0	0	2 *	0	0	0	0	2 *	0
unknown compound (216) RT1788	2 *	0	0.23	0.4	1.13	0.22	0	0.3	0	1.44	0
unknown compound (261) RT1791	4.35	0.23	0.2	0.64	0.81	0.16	0.03	0.52	0	0.44	0.22
unknown compound (204) RT1796	2 *	0	1.7	0.15	0.32	1.62	0	0.12	0	0.38	0
unknown compound (204) RT1798	1.38	0.72	0.16	0.2	2.26	0.14	0.02	0.12	0.06	0.55	0.53
unknown compound (117) RT1803	0.77	1.3	0.03	0.23	3.52	0.01	0.02	0.1	0.11	0.62	0.65
unknown compound (173) RT1809	9.52	0.1	0.3	0.5	0.78	0.25	0.03	0.36	0.02	0.79	0.05
unknown compound (191) RT1825	0.81	1.24	0.17	0.22	2.09	0.08	0.08	0.15	0.05	0.32	0.78

unknown compound (204) RT1834	1.28	0.78	0.06	5.31	0.12	0.06	0	0.75	0.74	0	0.1
unknown compound (204) RT1853	36.84	0.03	0.32	0.83	0.44	0.29	0.02	0.64	0	0.57	0
unknown compound (319) RT1860	0	2 *	1.05	0	0.95	0	1.19	0	0	0	0.84
unknown compound (356) RT1864	2 *	0	0	2 *	0	0	0	2 *	0	0	0
unknown compound (204) RT1902	17.07	0.06	0.34	0.43	0.81	0.3	0.02	0.33	0	0.88	0.03
unknown compound (204) RT1914	0.66	1.52	0.21	2.22	0.16	0.04	0.16	0.43	0.68	0	0.13
unknown compound (361) RT1919	0.95	1.05	0.24	0.08	2.78	0.16	0.06	0.03	0.05	0.53	0.63
unknown compound (217) RT1936	2 *	0	0	2 *	0	0	0	2 *	0	0	0
unknown compound (223) RT1937	1.47	0.68	0.03	3.83	0.21	0.03	0	0.76	0.57	0.07	0.12
unknown compound (204) RT1939	3.14	0.32	0.12	8.24	0	0.13	0	1.45	0.42	0	0
unknown compound (204) RT1958	2.7	0.37	0.03	5.34	0.14	0.04	0	1.3	0.36	0.08	0.06
unknown compound (204) RT1971	0.85	1.17	0.03	0.21	3.88	0.03	0	0.09	0.09	0.58	0.74
unknown compound (83) RT1974	4.49	0.22	0.4	0.3	0.94	0.41	0	0.25	0	0.4	0.29
unknown compound (204) RT1986	7.19	0.14	0.28	3.6	0	0.14	0.14	3.01	0	0	0
unknown compound (525) RT1992	2 *	0	0.49	2.06	0	0.58	0	1.74	0	0	0
unknown compound (201) RT1994	3.77	0.26	0.49	0.27	0.84	0.34	0.06	0.14	0.08	0.79	0.05

unknown compound (217) RT1994	0.97	1.03	0.28	0.13	2.05	0.2	0.06	0.08	0.04	0.32	0.74
unknown compound (361) RT2003	2 *	0	0	0	2 *	0	0	0	0	2 *	0
unknown compound (297) RT2022	2.88	0.35	0.31	1.49	0.2	0.32	0	0.57	0.29	0.11	0.08
unknown compound (217) RT2026	4.59	0.22	0.3	0.31	1.16	0.28	0.01	0.25	0	0.63	0.23
unknown compound (361) RT2053	2 *	0	0.32	3.09	0	0.38	0	2.61	0	0	0
unknown compound (91) RT2055	2.25	0.45	0	2 *	0	0	0	1.68	0.6	0	0
unknown compound (204) RT2068	2 *	0	0.46	1	0.23	0.5	0	0.8	0	0.29	0
unknown compound (362) RT2071	2 *	0	0.19	5.15	0	0.23	0	4.34	0	0	0
unknown compound (91) RT2097	6.29	0.16	0.06	2.78	0.27	0.06	0	1.68	0.08	0.15	0.11
unknown compound (204) RT2147	2 *	0	0.2	0.88	0.58	0.2	0	0.67	0	0.75	0
unknown compound (153) RT2157	41.33	0.02	0.31	0.59	0.64	0.28	0.02	0.45	0	0.82	0
unknown compound (191) RT2175	0.85	1.17	0.07	0.23	2.99	0.07	0	0.18	0.02	0.24	1.3
unknown compound (204) RT2329	0	2 *	2 *	0	0	0	2 *	0	0	0	0

b) Species

	Species									
	Ac.mi.	Ga.mo.	Ga.ve.	Pl.la.	Ra.ac.	Al.pr.	Ar.el.	Da.gl.	Lo.pe.	Po.pr.
Noradrenalin (174)	0	0	0	0	0	0	2*	0	0	0
N-Acetylglucosamine (156)	0	0	0	0	0	0	0	0	0	2*
Asparagine (231)	2*	0	0	0	0	0	0	0	0	0
Octadecatrienoic acid (335)	0	0	0	5.44	0	0	0	0.18	0	0
2-Aminoadipate (260)	0	0.16	0	0	0	0	2.16	0	0.22	0
3-Caffeoyl-trans-quinic acid (345)	0	2*	0	0	0	0	0	0	0	0
Succinate (147)	0	0	0	0	0	0	0	0	2*	0
unknown compound (306) RT832	0	0	0	0	2*	0	0	0	0	0
unknown compound (174) RT433	0	0	0	0	0	2	0	0.5	0	0
unknown compound (75) RT435	0	0	0	0	0	2	0	0.5	0	0
unknown compound (112) RT451	0	0	0	0	0	2	0	0.5	0	0
unknown compound (127) RT470	0	0	0	0	0	2	0	0.5	0	0
unknown compound (58) RT470	0	0	0	0	0	2	0	0.5	0	0
unknown compound (116) RT474	0	0	0	2*	0	0	0	0	0	0
unknown compound (258) RT494	0	0	0	0	0	0	0	0	0	2*
unknown compound (125) RT508	0	0	0	0	2*	0	0	0	0	0

unknown compound (288) RT590	0	0	0	0	0	2*	0	0	0	0
unknown compound (169) RT592	0	0	0	0	5.91	0	0	0.17	0	0
unknown compound (284) RT632	0	0	0	0	2*	0	0	0	0	0
unknown compound (74) RT632	0	0	0	0	0	0	0	0	2*	0
unknown compound (192) RT643	0	0	0	0	2*	0	0	0	0	0
unknown compound (173) RT665	0	0	0	0	0	2	0	0.5	0	0
unknown compound (126) RT670	0	0	0	0	2*	0	0	0	0	0
unknown compound (191) RT908	0.43	0	0	0	0	0	0	0	0	2.33
unknown compound (174) RT956	0	0	0	0.14	0	0	7	0	0	0
unknown compound (245) RT1065	0	0	0	0	0	2	0	0.5	0	0
unknown compound (217) RT1123	0	2*	0	0	0	0	0	0	0	0
unknown compound (93) RT1132	2*	0	0	0	0	0	0	0	0	0
unknown compound (174) RT1212	0	0	0	0	0	0	2*	0	0	0
unknown compound (103) RT1218	0	0	0	0	0	2	0	0.5	0	0
unknown compound (71) RT1257	10.68	0	0	0	0.09	0	0	0	0	0
unknown compound (344) RT1259	0	0	0	2*	0	0	0	0	0	0

unknown compound (179) RT1272	0	0	0	0	0	0	0	2*	0	0
unknown compound (299) RT1298	0	0.27	0	3.72	0	0	0	0	0	0
unknown compound (299) RT1309	0	0	0.07	3.41	0	0	0.04	0.09	0.04	0
unknown compound (285) RT1310	0	0	0	0	0	2	0	0.5	0	0
unknown compound (185) RT1512	0	0	0	0	0	0	2*	0	0	0
unknown compound (239) RT1643	0	0	0	0	0	2	0	0.5	0	0
unknown compound (83) RT1643	0	0	0	0	0	2	0	0.5	0	0
unknown compound (91) RT1660	0	0	0	0	0	2	0	0.5	0	0
unknown compound (204) RT1683	0	0	0	0	0	2	0	0.5	0	0
unknown compound (204) RT1780	0	0	0	0	0	2	0	0.5	0	0
unknown compound (204) RT1788	0	0	0	0	2*	0	0	0	0	0
unknown compound (216) RT1788	0	0	0	0	2*	0	0	0	0	0
unknown compound (319) RT1860	0	0	0	0	0	0	2.72	0.37	0	0
unknown compound (356) RT1864	0	0	0	2*	0	0	0	0	0	0
unknown compound (217) RT1936	0	0	0	2*	0	0	0	0	0	0
unknown compound (83) RT1974	0	0	0.12	2.73	0	0	0.04	0.03	0.05	0.05

unknown compound (525) RT1992	0	0	0.4	2.48	0	0	0	0	0	0
unknown compound (201) RT1994	0	0.03	0	2.48	0.09	0	0	0.1	0	0.09
unknown compound (361) RT2003	0	0	2*	0	0	0	0	0	0	0
unknown compound (361) RT2053	0	0	0.27	3.72	0	0	0	0	0	0
unknown compound (362) RT2071	0	0	0.4	2.48	0	0	0	0	0	0
unknown compound (204) RT2329	0	0	0	0	0	2	0	0.5	0	0

c) Interaction of site and species

Site x species

	AEG_Ac.mi.	AEG_Ga.mo.	AEG_Ga.ve.	AEG_Pl.la.	AEG_Ra.ac.	AEG_Al.pr.	AEG_Ar.el.	AEG_Da.gl.	AEG_Lo.pe.	AEG_Po.pr.	HEG_Ac.mi.	HEG_Ga.mo.	HEG_Ga.ve.	HEG_Pl.la.	HEG_Ra.ac.	HEG_Al.pr.	HEG_Ar.el.	HEG_Da.gl.	HEG_Lo.pe.	HEG_Po.pr.
Noradrenalin (174)	0	0	0	0	0	0	2*	0	0	0	0	0	0	0	0	0	0	0	0	0
N-Acetylglucosamine (156)	0	0	0	0	0	0	0	0	0	2*	0	0	0	0	0	0	0	0	0	0
Asparagine (231)	2*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3-Caffeoyl-trans-quinic acid (345)	0	0	0	0	0	0	0	0	0	0	0	2*	0	0	0	0	0	0	0	0
Succinate (147)	0	0	0	0	0	0	0	0	2*	0	0	0	0	0	0	0	0	0	0	0
unknown compound (174) RT433	0	0	0	0	0	2.8	0	0.36	0	0	0	0	0	0	0	0	0	0	0	0

unknown compound (75) RT435	0	0	0	0	0	2.8	0	0.36	0	0	0	0	0	0	0	0	0	0	0	0
unknown compound (112) RT451	0	0	0	0	0	2.8	0	0.36	0	0	0	0	0	0	0	0	0	0	0	0
unknown compound (127) RT470	0	0	0	0	0	2.8	0	0.36	0	0	0	0	0	0	0	0	0	0	0	0
unknown compound (58) RT470	0	0	0	0	0	2.8	0	0.36	0	0	0	0	0	0	0	0	0	0	0	0
unknown compound (116) RT474	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0
unknown compound (258) RT494	0	0	0	0	0	0	0	0	0	2*	0	0	0	0	0	0	0	0	0	0
unknown compound (125) RT508	0	0	0	0	2*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

unknown compound RT590	(288)	0	0	0	0	0	2*	0	0	0	0	0	0	0	0	0	0	0	0	0
unknown compound RT632	(284)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2*	0	0	0	0
unknown compound RT632	(74)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2*	0
unknown compound RT643	(192)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2*	0	0	0	0
unknown compound RT665	(173)	0	0	0	0	0	2.8	0	0.36	0	0	0	0	0	0	0	0	0	0	0
unknown compound RT987	(245)	0	0	0	0	0	0	0	0.06	0	0	0	0	0	0	0	0	0	0	0
unknown compound RT1065	(245)	0	0	0	0	0	2.8	0	0.36	0	0	0	0	0	0	0	0	0	0	0

unknown compound RT1123	(217)	0	2*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
unknown compound RT1132	(93)	0	0	0	0	0	0	0	0	0	0	2*	0	0	0	0	0	0	0	0
unknown compound RT1218	(103)	0	0	0	0	0	2.8	0	0.36	0	0	0	0	0	0	0	0	0	0	0
unknown compound RT1259	(344)	0	0	0	0	0	0	0	0	0	0	0	0	0	2*	0	0	0	0	0
unknown compound RT1272	(179)	0	0	0	0	0	0	0	2*	0	0	0	0	0	0	0	0	0	0	0
unknown compound RT1298	(299)	0	0	0	0	0	0	0	0	0	0	0	0.28	0	3.6	0	0	0	0	0
unknown compound RT1310	(285)	0	0	0	0	0	2.8	0	0.36	0	0	0	0	0	0	0	0	0	0	0

unknown compound RT1366	(155)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.22
unknown compound RT1512	(185)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2*	0	0	0
unknown compound RT1643	(239)	0	0	0	0	0	2.8	0	0.36	0	0	0	0	0	0	0	0	0	0	0	0
unknown compound RT1643	(83)	0	0	0	0	0	2.8	0	0.36	0	0	0	0	0	0	0	0	0	0	0	0
unknown compound RT1660	(91)	0	0	0	0	0	2.8	0	0.36	0	0	0	0	0	0	0	0	0	0	0	0
unknown compound RT1683	(204)	0	0	0	0	0	2.8	0	0.36	0	0	0	0	0	0	0	0	0	0	0	0
unknown compound RT1774	(283)	0	0	0	0	0	0	0	0	0	0	0.04	0.02	0.06	0	0	0	0.04	0	0.04	0

unknown compound RT1780	(204)	0	0	0	0	0	2.8	0	0.36	0	0	0	0	0	0	0	0	0	0	0
unknown compound RT1864	(356)	0	0	0	0	0	0	0	0	0	0	0	0	0	2*	0	0	0	0	0
unknown compound RT1936	(217)	0	0	0	0	0	0	0	0	0	0	0	0	0	2*	0	0	0	0	0
unknown compound RT1992	(525)	0	0	0.42	0	0	0	0	0	0	0	0	0	0	2.4	0	0	0	0	0
unknown compound RT2003	(361)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
unknown compound RT2053	(361)	0	0	0.28	0	0	0	0	0	0	0	0	0	0	3.6	0	0	0	0	0
unknown compound RT2071	(362)	0	0	0.18	0	0	0	0	0	0	0	0	0	0.15	2.58	0	0	0	0	0

unknown compound (204) RT2329	0	0	0	0	0	2.8	0	0.36	0	0	0	0	0	0	0	0	0	0	0	0	0

Site x species

	SEG_Ac.mi.	SEG_Ga.mo.	SEG_Ga.ve.	SEG_Pl.la.	SEG_Ra.ac.	SEG_Al.pr.	SEG_Ar.el.	SEG_Da.gl.	SEG_Lo.pe.	SEG_Po.pr.
Noradrenalin (174)	0	0	0	0	0	0	0	0	0	0
N-Acetylglucosamine (156)	0	0	0	0	0	0	0	0	0	0
Asparagine (231)	0	0	0	0	0	0	0	0	0	0
3-Caffeoyl-trans-quinic acid (345)	0	0	0	0	0	0	0	0	0	0
Succinate (147)	0	0	0	0	0	0	0	0	0	0
unknown compound (174) RT433	0	0	0	0	0.23	0	0	0	0	0

unknown compound RT435	(75)	0	0	0	0	0	0	0	0	0	0
unknown compound RT451	(112)	0	0	0	0	0.8	0	0	0	0	0
unknown compound RT470	(127)	0	0	0	0	0	0	0	0	0	0
unknown compound RT470	(58)	0	0	0	0	2*	0	0	0	0	0
unknown compound RT474	(116)	0	0	0	0	2	0	0	0	1.43	0
unknown compound RT494	(258)	0	0	0	0	0.04	0	0	0	0	0
unknown compound RT508	(125)	0	0	0	0	0	0	0	0	0	0

unknown compound RT590	(288)	0	0	0	0	0	0	0	0	0	0
unknown compound RT632	(284)	0	0	0	0	0.05	0	0	0	0	0
unknown compound RT632	(74)	0	0	0	0	0	0	0	0	0	0
unknown compound RT643	(192)	0	0	0	0	0.07	0	0	0	0	0
unknown compound RT665	(173)	0	0	0	0	0	0	0	0	0	0
unknown compound RT987	(245)	0	0.12	0	0	6	0	0.18	0.51	0.28	0.15
unknown compound RT1065	(245)	0	0	0	0	0	0	0	0	0	0

unknown compound RT1123	(217)	0	0	0	0	0	0	0	0	0	0
unknown compound RT1132	(93)	0	0	0	0	0	0	0	0	0	0
unknown compound RT1218	(103)	0	0	0	0	0.08	0	0	0	0	0
unknown compound RT1259	(344)	0	0	0	0	0.12	0	0	0	0	0
unknown compound RT1272	(179)	0	0	0	0	0	0	0	0	0	0
unknown compound RT1298	(299)	0	0	0	0	4	0	0	0	0	0
unknown compound RT1310	(285)	0	0	0	0	0	0	0	0	0	0

unknown compound RT1366	(155)	4.5	0	0	0	0.03	0	0	0	0	0
unknown compound RT1512	(185)	0	0	0	0	0	0	0	0	0	0
unknown compound RT1643	(239)	0	0	0	0	0.07	0	0	0	0	0
unknown compound RT1643	(83)	0	0	0	0	0	0	0	0	0	0
unknown compound RT1660	(91)	0	0	0	0	0	0	0	0	0	0
unknown compound RT1683	(204)	0	0	0	0	0.06	0	0	0	0	0
unknown compound RT1774	(283)	0	0.12	0.05	0.18	4.67	0.07	0.11	0.13	0.17	0.09

unknown compound RT1780	(204)	0	0	0	0	0.06	0	0	0	0	0
unknown compound RT1864	(356)	0	0	0	0	0	0	0	0	0	0
unknown compound RT1936	(217)	0	0	0	0	0.14	0	0	0	0	0
unknown compound RT1992	(525)	0	0	0	0	0	0	0	0	0	0
unknown compound RT2003	(361)	0	0	2*	0	0	0	0	0	0	0
unknown compound RT2053	(361)	0	0	0	0	0	0	0	0	0	0
unknown compound RT2071	(362)	0	0	0	0	0	0	0	0	0	0

unknown compound RT2329	(204)	0	0	0	0	0	0	0	0	0	0

Supplementary Table 4: Individual explained variance of the polar metabolite composition of exudates by single variables of the environmental factors of LNH, Soil and Climate. Table contains the explained amount of variance (in %) of polar metabolites by different impacting factors and included variables. A detailed description of the abbreviation is given in Supplementary Table 10. Residuals = remaining unexplained variation.

	Environ-mental factor and included single variables (SV)	Species	Plot	SV	Species +Plot	Plot +SV	SV +Species	Species +Plot +SV	Residuals
forb	LNH	8.75	24.09	0	0	1.03	0	0	68.41
	- Cover	8.79	23.06	0	0	2.05	0	0	68.61
	- Richness	8.76	24.62	0	0	0.49	0	0	68.68
	- Shannon	8.76	24.62	0	0	0.49	0	0	68.68
	LUI	8.75	24.64	0	0	0.48	0	0	68.41
	- fertilization	8.75	24.18	0	0	0.93	0	0	68.41
	- grazing	8.75	24.18	0	0	0.93	0	0	68.41
	- mowing	8.53	23.74	0	0	1.37	0.22	0	68.61
	Soil	8.84	23.29	0	0	1.82	0	0.01	68.61
	- pH	8.83	23.83	0	0	1.28	0	0	68.5
	- TC	8.75	22.26	0	0	2.85	0	0	68.41
	- TN	8.75	22.65	0	0	2.46	0	0	68.42
	- moisture	8.78	24.61	0	0	0.5	0	0	68.68
	- soil texture	8.83	23.83	0	0	1.28	0	0	68.5
	- soil type	8.75	24.83	0	0	0.28	0	0	68.41
	Climate	8.75	23.38	0	0	1.73	0	0	68.41
	- precipitation	8.75	24.09	0	0	1.03	0	0	68.41
	- T(10)	8.79	23.06	0	0	2.05	0	0	68.61
	- T(200)	8.76	24.62	0	0	0.49	0	0	68.68
grass	LNH	0.74	30.72	0	0	0.65	0	0	68.78
	- Cover	0.86	24.82	0.23	0.08	6.54	0	0	68.55
	- Richness	0.69	29.51	0	0	1.85	0.05	0	68.95
	- Shannon	0.69	29.51	0	0	1.85	0.05	0	68.95
	LUI	0.74	31.47	0	0	0	0	0.03	68.78
	- fertilization	0.74	31.26	0	0	0.1	0	0.02	68.78
	- grazing	0.74	31.26	0	0	0.1	0	0.02	68.78
	- mowing	0.85	30.43	0.16	0	0.93	0	0.16	68.62
	Soil	0.73	29.92	0.09	0	1.45	0.01	0.04	68.69
	- pH	0.61	30.37	0.04	0	1	0.13	0	68.74
	- TC	0.74	23.99	0	0	7.38	0	0	68.78
	- TN	0.86	24.52	0.14	0	6.85	0	0	68.64
	- moisture	0.72	29.5	0	0	1.87	0.02	0	68.99
	- soil texture	0.61	30.37	0.04	0	1	0.13	0	68.74
	- soil type	0.74	30.25	0	0	1.11	0	0.04	68.78
	Climate	0.74	24.94	0	0	6.43	0	0	68.78

- precipitation	0.74	30.72	0	0	0.65	0	0	68.78
- T(10)	0.86	24.82	0.23	0.08	6.54	0	0	68.55
- T(200)	0.69	29.51	0	0	1.85	0.05	0	68.95

Supplementary Table 5: Correlation of exuded compounds and environmental variables. Tables display the significantly correlated compound profiles of A) polar metabolite and B) semi-polar metabolite. Correlations between metabolites and variables were investigated by logistic regression in which metabolites were the dependent variable and environmental variables the independent variable. Compounds coloured in black represent all compounds correlated to the specific variable in highly significant ($p<0.001$) manner. Compounds coloured in orange represent all additional compounds being also highly significant ($p<0.01$) correlated to the specific variable. Compounds coloured in grey represent all additional compounds being significant ($p<0.05$) correlated to the specific variable. The abbreviations are listed in Supplementary Table 10.

a) polar metabolites

	Forbs	Grasses
Fertilization		
forbs *p<0.001* = 0 *p<0.01* = 1 *p<0.05* = 8 total = 9	carbohydrate (319) RT1314, compound (117) RT1121, compound (75) RT824, compound (204) RT2068, compound (71) RT1316, Tyramine (174), compound (127) RT1780, compound (160) RT1909, Adenosine (236)	carbohydrate (319) RT1314, compound (117) RT1121, compound (75) RT824, compound (71) RT1316, Tyramine (174), compound (127) RT1780, compound (160) RT1909, Adenosine (236)
grasses *p<0.001* = 1 *p<0.01* = 7 *p<0.05* = 8 total = 41		
Grazing		
forbs *p<0.001* = 6 *p<0.01* = 15 *p<0.05* = 20 total = 41	compound (75) RT1218, compound (160) RT1240, compound (333) RT1325, Methionine (176), Phenylalanine (192), Phosphoenolpyruvate (247),	compound (75) RT1218, compound (160) RT1240, compound (333) RT1325, Phosphoenolpyruvate (247), Methionine (176), Phenylalanine (192),
grasses *p<0.001* = 6 *p<0.01* = 14 *p<0.05* = 34 total = 20	Asparagine (231), compound (280) RT669, compound (223) RT983, compound (335) RT1385, compound (217) RT1994, compound (204) RT1340, compound (179) RT621, compound (319) RT1466, compound (362) RT1978, compound (361) RT1919, Uracil (241), compound (69) RT1140, Shikimate (204), compound (149) RT1233, compound (204) RT1798, compound (207) RT436, Benzoic acid (267), Tyrosine (218), compound (57) RT1655, compound (204) RT2011, Glutamate (246), Aspartate (232), compound (319) RT1968, compound (103)	compound (280) RT669, compound (223) RT983, compound (335) RT1385, compound (179) RT621, compound (204) RT1340, compound (217) RT1994, compound (319) RT1466, compound (362) RT1978, compound (361) RT1919, Uracil (241), Shikimate (204), compound (69) RT1140, compound (149) RT1233, compound (204) RT1798, compound (207) RT436, Benzoic acid (267), Tyrosine (218), compound (57) RT1655, compound (204) RT2011, Glutamate (246), Aspartate (232), compound (319) RT1968, compound (103)

	RT1133, compound (361) RT1948, compound (217) RT964, compound (255) RT1654, compound (285) RT1213, compound (319) RT1316, compound (169) RT581, compound (205) RT653, compound (156) RT1226, Gluconate (333), compound (292) RT1203, Homoserine (218)	RT1133, compound (217) RT964, compound (361) RT1948, compound (255) RT1654, compound (285) RT1213, compound (319) RT1316, compound (169) RT581, compound (205) RT653, compound (156) RT1226, Gluconate (333), compound (292) RT1203, Homoserine (218)
Mowing <u>forbs</u> *p<0.001* = 0 *p<0.01* = 3 *p<0.05* = 4 total = 7	Proline (142), Tyramine (174), Isoleucine (158), compound (75) RT824, Salicylic acid (267), Serine (204), 5-Indolecarboxylic acid (305)	Proline (142), Tyramine (174), Isoleucine (158), compound (174) RT433, compound (75) RT435, compound (112) RT451, compound (127) RT470, compound (58) RT470, compound (173) RT665, compound (245) RT1065, compound (103) RT1218, compound (285) RT1310, compound (239) RT1643, compound (83) RT1643, compound (91) RT1660, compound (204) RT1683, compound (204) RT1780, compound (204) RT2329, compound (75) RT824, Salicylic acid (267), Serine (204), 5-Indolecarboxylic acid (305)
<u>grasses</u> *p<0.001* = 0 *p<0.01* = 3 *p<0.05* = 19 total = 22		

soil texture		
forbs *p<0.001* = 4 *p<0.01* = 17 *p<0.05* = 11 total = 32	Leucine (158), compound (89) RT440, compound (91) RT2097, 2-Isopropylmalate (275), compound (204) RT1958, compound (158) RT533, Xylose (217), compound (103) RT1055, compound (204) RT1834, compound (89) RT439, compound (158) RT944, Melibiose (361), compound (117) RT1803, compound (223) RT1937, compound (281) RT548, 4-Aminobutanoate [GABA] (174), compound (204) RT1749, compound (204) RT1655, compound (319) RT1487, compound (362) RT2071, compound (117) RT529, compound (342) RT996, Lactic acid (191), compound (197) RT1637, Adipic acid (111), compound (75) RT824, Benzoic acid (267), compound (261) RT1791, compound (204) RT1939, Lactose (361), compound (132) RT1300, compound (273) RT1297	Leucine (158), compound (89) RT440, compound (91) RT2097, 2-Isopropylmalate (275), compound (204) RT1958, compound (158) RT533, Xylose (217), compound (103) RT1055, compound (204) RT1834, compound (89) RT439, compound (158) RT944, Melibiose (361), compound (117) RT1803, compound (223) RT1937, compound (281) RT548, 4-Aminobutanoate [GABA] (174), compound (204) RT1749, compound (204) RT1655, compound (319) RT1487, compound (117) RT529, compound (342) RT996, Lactic acid (191), compound (197) RT1637, Adipic acid (111), compound (75) RT824, Benzoic acid (267), compound (261) RT1791, compound (204) RT1939, Lactose (361), compound (132) RT1300, compound (217) RT1155, compound (273) RT1297
pH		
forbs *p<0.001* = 3 *p<0.01* = 7 *p<0.05* = 9 total = 19	compound (261) RT1791, compound (174) RT924, compound (197) RT1637, compound (149) RT1745, compound (132) RT1300, compound (295) RT1171, compound (69) RT1140, Glycerol 3-phosphate (357), compound (172) RT825, compound (204) RT1731, compound (319) RT1487, compound (117) RT1803, compound (173) RT1809, compound (219) RT1776, compound (204) RT1655,	compound (174) RT924, compound (197) RT1637, compound (261) RT1791, compound (149) RT1745, compound (132) RT1300, compound (295) RT1171, compound (69) RT1140, Glycerol 3-phosphate (357), compound (172) RT825, compound (204) RT1731, compound (319) RT1487, compound (117) RT1803, compound (173) RT1809, compound (219) RT1776, compound (204) RT1655,
grasses *p<0.001* = 3 *p<0.01* = 7 *p<0.05* = 9 total = 19		

	Adipic acid (111), compound (186) RT642, compound (237) RT851, Octadecenoic acid (339)	Adipic acid (111), compound (186) RT642, compound (237) RT851, Octadecenoic acid (339)
moisture forbs *p<0.001* = 35 *p<0.01* = 38 *p<0.05* = 28 total = 101 grasses *p<0.001* = 34 *p<0.01* = 38 *p<0.05* = 29 total = 101	Adipic acid (111), Alanine (116), Leucine (158), Octadecenoic acid (339) , Threonine (218), Uracil (241), compound (319) RT1316, compound (204) RT1340, Aspartate (232), Glutamate (246), Glycerol 3-phosphate (357), Homoserine (218), Methionine (176), compound (280) RT669, compound (223) RT983, compound (217) RT1166, compound (57) RT1173, compound (167) RT1609, compound (283) RT1774, compound (204) RT1798, compound (361) RT1948, compound (57) RT2144, compound (204) RT2147, compound (75) RT722, compound (69) RT1140, compound (149) RT1233, compound (335) RT1385, compound (204) RT1603, fatty acid (339) RT1518, compound (281) RT548, compound (103) RT803, compound (243) RT830, compound (117) RT529, compound (205) RT653, compound (204) RT1615, compound (174) RT1143, compound (191) RT2175, Glucose-6-phosphate (387), Myo-Inositol-1-phosphate (318), Tyrosine (218), compound (158) RT533, compound (361) RT1919, 2-Aminoadipate (260), compound (207) RT436, compound (179) RT867, compound (273) RT1297, compound (174) RT1327, compound (103) RT982, Phosphoenolpyruvate (247), Phenylalanine (192), compound (311) RT1362, Rhamnose (117), compound (75) RT690, compound (217) RT964, compound (319) RT1466, cis-Aconitate (229), compound (260) RT1676, Tryptophan (202), compound (103) RT1243, compound (204) RT1731,	Adipic acid (111), Alanine (116), Leucine (158), Octadecenoic acid (339) , Threonine (218), Uracil (241), compound (319) RT1316, compound (204) RT1340, Aspartate (232), Glutamate (246), Glycerol 3-phosphate (357), Homoserine (218), Methionine (176), compound (280) RT669, compound (223) RT983, compound (217) RT1166, compound (57) RT1173, compound (167) RT1609, compound (283) RT1774, compound (204) RT1798, compound (361) RT1948, compound (57) RT2144, compound (75) RT722, compound (69) RT1140, compound (149) RT1233, compound (335) RT1385, compound (204) RT1603, fatty acid (339) RT1518, compound (281) RT548, compound (103) RT803, compound (243) RT830, compound (117) RT529, compound (205) RT653, compound (204) RT1615, compound (191) RT2175, compound (174) RT1143, Glucose-6-phosphate (387), compound (204) RT2011, compound (117) RT1803, compound (299) RT1309, Gluconate (333), Myo-Inositol-1-phosphate (318), Tyrosine (218), compound (158) RT533, compound (361) RT1919, 2-Aminoadipate (260), compound (207) RT436, compound (179) RT867, compound (273) RT1297, compound (174) RT1327, compound (103) RT982, Phosphoenolpyruvate (247), Phenylalanine (192), compound (311) RT1362, Rhamnose (117), compound (75) RT690, compound (217) RT964, compound (319) RT1466, cis-Aconitate (229),

	compound (204) RT1517, compound (319) RT1968, compound (217) RT1180, Tartaric acid (292), compound (119) RT2019, compound (259) RT1920, Adenine (264), compound (217) RT2026, compound (217) RT1994, compound (204) RT2011, compound (117) RT1803, compound (299) RT1309, Gluconate (333), compound (89) RT575, Xylitol (307), compound (204) RT1971, compound (117) RT1037, Salicylic acid (267), Serine (204), compound (142) RT973, compound (295) RT1259, compound (361) RT1330, compound (75) RT1218, compound (103) RT1133, compound (333) RT1325, Ornithine / Citrullin (142), compound (255) RT1654, compound (156) RT1226, Sorbitol (217), compound (69) RT1150, compound (362) RT1978, compound (149) RT1745, compound (292) RT1203, compound (217) RT1877, Valine (144), compound (172) RT825, scyllo-inositol (204) , compound (261) RT1791, compound (160) RT1319, compound (217) RT1443, compound (160) RT1240	compound (260) RT1676, Tryptophan (202), compound (103) RT1243, compound (204) RT1731, compound (204) RT1517, compound (319) RT1968, compound (217) RT1180, Tartaric acid (292), compound (119) RT2019, compound (259) RT1920, Adenine (264), compound (217) RT2026, compound (217) RT1994, compound (89) RT575, Xylitol (307), compound (204) RT1971, compound (117) RT1037, Salicylic acid (267), Serine (204), compound (142) RT973, compound (295) RT1259, compound (361) RT1330, compound (75) RT1218, compound (103) RT1133, compound (333) RT1325, Ornithine / Citrullin (142), compound (255) RT1654, compound (156) RT1226, Aminomalonic acid (218) , Sorbitol (217), compound (69) RT1150, compound (362) RT1978, compound (149) RT1745, compound (292) RT1203, compound (217) RT1877, Valine (144), compound (172) RT825, scyllo-inositol (204) , compound (261) RT1791, compound (160) RT1319, compound (217) RT1443, compound (160) RT1240
soil type		
<u>forbs</u> *p<0.001* = 2 *p<0.01* = 3 *p<0.05* = 7 total = 12	compound (89) RT440, compound (216) RT1788, compound (160) RT1909, 2-Isopropylmalate (275), compound (89) RT439, compound (91) RT2097, compound (204) RT2011, 4-Aminobutanoate [GABA] (174), compound (103) RT1264, compound (204) RT1902, Phosphoenolpyruvate (247), compound (342) RT996	compound (89) RT440, compound (160) RT1909, 2-Isopropylmalate (275), compound (89) RT439, compound (91) RT2097, compound (204) RT2011, 4-Aminobutanoate [GABA] (174), compound (103) RT1264, compound (204) RT1902, Phosphoenolpyruvate (247), compound (342) RT996
<u>grasses</u> *p<0.001* = 1 *p<0.01* = 3 *p<0.05* = 7		

total = 11		
TC		
<u>forbs</u> *p<0.001* = 6 *p<0.01* = 7 *p<0.05* = 16 total = 29	compound (120) RT940, compound (295) RT1171, Glycerol 3-phosphate (357), compound (158) RT944, compound (324) RT1456, compound (207) RT436,	compound (120) RT940, compound (295) RT1171, Glycerol 3-phosphate (357), compound (158) RT944, compound (324) RT1456, compound (207) RT436,
<u>grasses</u> *p<0.001* = 0 *p<0.01* = 6 *p<0.05* = 7 total = 15	compound (191) RT1825, compound (273) RT1289, Myo-Inositol-1-phosphate (318), compound (319) RT1487, compound (263) RT932, compound (299) RT1309, compound (103) RT982, 2-Oxoglutarate (129), compound (361) RT1909, compound (132) RT1300, compound (204) RT2068, compound (281) RT610, compound (172) RT825, compound (160) RT1319, compound (327) RT1456, carbohydrate (319) RT1321, Tartaric acid (292), compound (204) RT2011, compound (297) RT2022, compound (204) RT1731, compound (174) RT924, compound (292) RT1203, compound (204) RT1655	compound (191) RT1825, compound (273) RT1289, Myo-Inositol-1-phosphate (318), compound (319) RT1487, compound (263) RT932, compound (299) RT1309, compound (103) RT982, 2-Oxoglutarate (129), compound (361) RT1909, compound (132) RT1300, compound (281) RT610, compound (172) RT825, compound (160) RT1319, compound (327) RT1456, carbohydrate (319) RT1321, Tartaric acid (292), compound (204) RT2011, compound (297) RT2022, compound (204) RT1731, compound (174) RT924, compound (292) RT1203, compound (204) RT1655
TN		
<u>forbs</u> *p<0.001* = 3 *p<0.01* = 9 *p<0.05* = 13 total = 25	compound (207) RT436, compound (117) RT605, compound (324) RT1456,	compound (207) RT436, compound (117) RT605, compound (324) RT1456,
<u>grasses</u> *p<0.001* = 3 *p<0.01* = 8 *p<0.05* = 13 total = 24	compound (295) RT1171, compound (158) RT944, Glycerol 3-phosphate (357), compound (120) RT940, 2-Oxoglutarate (129), Myo-Inositol-1-phosphate (318), compound (299) RT1309, compound (319) RT1487, compound (204) RT2068, compound (273) RT1289, compound (361) RT1909, compound (132) RT1300, compound (197) RT1637, compound (263) RT932, compound (117) RT1803, compound (191) RT1825, carbohydrate	compound (295) RT1171, compound (158) RT944, Glycerol 3-phosphate (357), compound (120) RT940, 2-Oxoglutarate (129), Myo-Inositol-1-phosphate (318), compound (299) RT1309, compound (319) RT1487, compound (273) RT1289, compound (361) RT1909, compound (132) RT1300, compound (197) RT1637, compound (263) RT932, compound (117) RT1803, compound (191) RT1825, carbohydrate

	(319) RT1321, compound (172) RT825, compound (297) RT2022, compound (318) RT1334, compound (281) RT610, Tyramine (174)	(319) RT1321, compound (172) RT825, compound (297) RT2022, compound (318) RT1334, compound (281) RT610, Tyramine (174)
Precipitation		
<u>forbs</u> *p<0.001* = 0 *p<0.01* = 0 *p<0.05* = 7 total = 7	compound (223) RT983, compound (204) RT1749, Glycerol 3-phosphate (357), compound (156) RT1226, compound (285) RT1213, Octadecenoic acid (339) , compound (87) RT435	Succinate (147), Glycerol 3-phosphate (357), compound (223) RT983, compound (204) RT1749, compound (156) RT1226, compound (285) RT1213, Octadecenoic acid (339) , compound (87) RT435
<u>grasses</u> *p<0.001* = 0 *p<0.01* = 1 *p<0.05* = 7 total = 8		
T(10)		
<u>forbs</u> *p<0.001* = 0 *p<0.01* = 3 *p<0.05* = 10 total = 13	compound (204) RT2011, compound (159) RT650, compound (205) RT653, Tyrosine (218), carbohydrate (319) RT1321, compound (227) RT945, compound (281) RT548, compound (201) RT1994, Methionine (176), Leucine (158), compound (297) RT2022, Pinitol (260) , compound (327) RT2065	compound (204) RT2011, compound (159) RT650, compound (205) RT653, Tyrosine (218), carbohydrate (319) RT1321, compound (227) RT945, compound (281) RT548, compound (201) RT1994, Methionine (176), Leucine (158), compound (297) RT2022, Pinitol (260) , compound (327) RT2065
<u>grasses</u> *p<0.001* = 0 *p<0.01* = 3 *p<0.05* = 10 total = 13		

T(200) forbs *p<0.001* = 20 *p<0.01* = 31 *p<0.05* = 28 total = 79 grasses *p<0.001* = 21 *p<0.01* = 29 *p<0.05* = 28 total = 78	Adipic acid (111), compound (117) RT443, compound (281) RT548, compound (243) RT830, compound (273) RT1297, compound (204) RT1731, Leucine (158), compound (142) RT973, compound (283) RT1774, compound (57) RT2144, compound (327) RT2065, compound (204) RT1615, compound (103) RT982, compound (319) RT1487, Erythronic acid (292), compound (205) RT653, compound (117) RT1803, compound (75) RT722, compound (186) RT642, compound (117) RT529, Asparagine (231), Aspartate (232), Tryptophan (202), compound (280) RT669, compound (103) RT803, compound (361) RT1926, Methionine (176), compound (204) RT1603, Tyrosine (218), Uracil (241), Threonine (218), compound (204) RT1971, compound (103) RT1264, compound (174) RT1327, compound (217) RT1166, compound (167) RT1609, compound (260) RT1676, compound (179) RT867, compound (149) RT1745, Glutamate (246), compound (119) RT2019, compound (158) RT533, Glycerol 3-phosphate (357), compound (197) RT1637, compound (204) RT2011, compound (204) RT1796, scyllo-inositol (204) , compound (69) RT1150, compound (299) RT1309, compound (191) RT2175, Phenylalanine (192), compound (57) RT1127, compound (179) RT621, beta-Alanine (248), compound (311) RT1362, compound (227) RT945, compound (57) RT1173, Ornithine / Citrullin (142), compound (69) RT1140, compound (204) RT1517, Octadecenoic acid (339) , compound (223) RT983, Homoserine (218), compound (117)	Adipic acid (111), compound (117) RT443, compound (77) RT466, compound (281) RT548, compound (243) RT830, compound (273) RT1297, compound (204) RT1731, Leucine (158), compound (142) RT973, compound (283) RT1774, compound (57) RT2144, compound (327) RT2065, compound (204) RT1615, compound (103) RT982, compound (319) RT1487, Erythronic acid (292), compound (205) RT653, compound (117) RT1803, compound (75) RT722, compound (186) RT642, compound (117) RT529, Aspartate (232), Tryptophan (202), compound (280) RT669, compound (103) RT803, compound (361) RT1926, Methionine (176), compound (204) RT1603, Tyrosine (218), Uracil (241), Threonine (218), compound (204) RT1971, compound (103) RT1264, compound (174) RT1327, compound (217) RT1166, compound (167) RT1609, compound (260) RT1676, compound (179) RT867, compound (149) RT1745, Glutamate (246), compound (119) RT2019, compound (158) RT533, Glycerol 3-phosphate (357), compound (197) RT1637, compound (204) RT2011, scyllo-inositol (204) , compound (69) RT1150, compound (299) RT1309, compound (191) RT2175, Phenylalanine (192), compound (57) RT1127, compound (179) RT621, beta-Alanine (248), compound (311) RT1362, compound (227) RT945, compound (57) RT1173, Ornithine / Citrullin (142), compound (69) RT1140, compound (204) RT1517, Octadecenoic acid (339) , compound (223) RT983, Homoserine (218), compound (117) RT1037, compound (204) RT1340, fatty acid (339) RT1518,

	RT1037, compound (204) RT1340, fatty acid (339) RT1518, compound (156) RT1226, Pinitol (260) , compound (292) RT1203, compound (91) RT2097, Adenine (264), compound (319) RT1968, compound (259) RT1920, compound (172) RT825, compound (241) RT553, compound (217) RT964, compound (319) RT1466, Rhamnose (117), compound (355) RT515	compound (156) RT1226, Pinitol (260) , compound (292) RT1203, compound (91) RT2097, Adenine (264), compound (319) RT1968, compound (259) RT1920, compound (172) RT825, compound (241) RT553, compound (217) RT964, compound (319) RT1466, Rhamnose (117), compound (355) RT515
Cover		
<u>forbs</u> *p<0.001* = 0 *p<0.01* = 5 *p<0.05* = 19 total = 24	compound (311) RT1362, compound (197) RT1637, compound (174) RT1212, Erythronic acid (292), compound (319) RT1466, compound (283) RT1774, compound (172) RT825, compound (223) RT983, compound (179) RT867, compound (117) RT1803, Benzoic acid (267), compound (217) RT1166, Glutamate (246), compound (207) RT454, compound (160) RT1909, compound (237) RT851, compound (158) RT944, compound (243) RT830, compound (117) RT529, compound (156) RT1226, Uracil (241), compound (57) RT1173, compound (186) RT642, compound (319) RT1487	compound (311) RT1362, compound (197) RT1637, compound (174) RT1212, Erythronic acid (292), compound (319) RT1466, compound (283) RT1774, compound (172) RT825, compound (223) RT983, compound (179) RT867, compound (117) RT1803, Benzoic acid (267), compound (217) RT1166, Glutamate (246), compound (207) RT454, compound (160) RT1909, compound (237) RT851, compound (158) RT944, compound (243) RT830, compound (117) RT529, compound (156) RT1226, Uracil (241), compound (57) RT1173, compound (186) RT642, compound (319) RT1487
<u>grasses</u> *p<0.001* = 0 *p<0.01* = 5 *p<0.05* = 19 total = 24		
Richness		

forbs *p<0.001* = 23 *p<0.01* = 23 *p<0.05* = 35 total = 81	Adipic acid (111), Erythronic acid (292), compound (117) RT529, compound (324) RT1456, compound (283) RT1774, compound (361) RT1926, Leucine (158), Phenylalanine (192), Uracil (241), compound (186) RT642, compound (103) RT982, compound (223) RT983, compound (311) RT1362, compound (319) RT1466, compound (319) RT1487, compound (197) RT1637, compound (57) RT2144, compound (243) RT830, compound (217) RT1166, compound (57) RT1173, Tyrosine (218), compound (327) RT1456, Methionine (176),	Adipic acid (111), Erythronic acid (292), compound (117) RT529, compound (324) RT1456, compound (283) RT1774, compound (361) RT1926, Leucine (158), Phenylalanine (192), Uracil (241), compound (186) RT642, compound (103) RT982, compound (223) RT983, compound (311) RT1362, compound (319) RT1466, compound (319) RT1487, compound (197) RT1637, compound (57) RT2144, compound (243) RT830, compound (217) RT1166, compound (57) RT1173, Tyrosine (218), compound (327) RT1456, Methionine (176),
grasses *p<0.001* = 23 *p<0.01* = 23 *p<0.05* = 36 total = 82	Threonine (218), compound (117) RT1803, compound (117) RT443, compound (89) RT440, compound (132) RT1300, compound (259) RT1920, compound (217) RT1994, compound (204) RT1517, compound (172) RT825, compound (295) RT1171, Octadecenoic acid (339) , compound (204) RT1731, compound (204) RT1971, compound (263) RT932, Aspartate (232), compound (156) RT1226, compound (117) RT1037, Glutamate (246), compound (204) RT1340, compound (117) RT605, Xylitol (307), compound (292) RT1203, compound (75) RT722, compound (167) RT1609, 2-Oxoglutarate (129), compound (119) RT2019, compound (281) RT548, compound (75) RT690, compound (204) RT1603, compound (191) RT2175, compound (158) RT944, fatty acid (339) RT1518, beta-Alanine (248), carbohydrate (204) RT1781, compound (142) RT973, Gluconate (333), compound (333) RT1325, compound (259) RT1657, Adenosine (236), compound (335) RT1385, compound (361) RT1919, compound (160) RT1319, compound (204) RT1756,	Threonine (218), compound (117) RT1803, compound (117) RT443, compound (89) RT440, compound (132) RT1300, compound (259) RT1920, compound (217) RT1994, compound (204) RT1517, compound (172) RT825, compound (295) RT1171, Octadecenoic acid (339) , compound (204) RT1731, compound (204) RT1971, compound (263) RT932, Aspartate (232), compound (156) RT1226, compound (117) RT1037, Glutamate (246), compound (204) RT1340, compound (117) RT605, Xylitol (307), compound (292) RT1203, compound (75) RT722, compound (167) RT1609, 2-Oxoglutarate (129), compound (119) RT2019, compound (281) RT548, compound (75) RT690, compound (204) RT1603, compound (191) RT2175, compound (158) RT944, fatty acid (339) RT1518, compound (77) RT466, beta-Alanine (248), carbohydrate (204) RT1781, compound (142) RT973, Gluconate (333), compound (333) RT1325, compound (259) RT1657, Adenosine (236), compound (335) RT1385, compound (361) RT1919, compound (160) RT1319, compound

	compound (273) RT1297, compound (306) RT1865, compound (205) RT653, compound (255) RT1654, compound (319) RT1316, compound (285) RT1213, compound (204) RT1798, compound (69) RT1140, compound (57) RT1127, compound (179) RT867, Shikimate (204), compound (260) RT1676, compound (327) RT2065, compound (160) RT1909, compound (117) RT459	(204) RT1756, compound (273) RT1297, compound (306) RT1865, compound (205) RT653, compound (255) RT1654, compound (319) RT1316, compound (285) RT1213, compound (204) RT1798, compound (69) RT1140, compound (57) RT1127, compound (179) RT867, Shikimate (204), compound (260) RT1676, compound (327) RT2065, compound (160) RT1909, compound (117) RT459
Shannon forbs *p<0.001* = 22 *p<0.01* = 22 *p<0.05* = 34 total = 78 grasses *p<0.001* = 22 *p<0.01* = 22 *p<0.05* = 36 total = 80	Uracil (241), compound (117) RT529, compound (217) RT1994, Adipic acid (111), Leucine (158), Phenylalanine (192), Threonine (218), compound (186) RT642, compound (103) RT982, compound (217) RT1166, compound (57) RT1173, compound (311) RT1362, compound (324) RT1456, compound (319) RT1466, compound (319) RT1487, compound (57) RT2144, compound (197) RT1637, compound (283) RT1774, compound (259) RT1920, compound (191) RT2175, compound (259) RT1657, compound (318) RT1334, compound (223) RT983, compound (191) RT1825, Erythronic acid (292), compound (361) RT1926, compound (75) RT690, compound (292) RT1203, Xylitol (307), compound (327) RT1456, Tyrosine (218), compound (255) RT1654, Methionine (176), compound (167) RT1609, compound (204) RT1340, Gluconate (333), carbohydrate (204) RT1781, 2-Oxoglutarate (129), fatty acid (339)	Uracil (241), compound (117) RT529, compound (217) RT1994, Adipic acid (111), Leucine (158), Phenylalanine (192), Threonine (218), compound (186) RT642, compound (103) RT982, compound (217) RT1166, compound (57) RT1173, compound (311) RT1362, compound (324) RT1456, compound (319) RT1466, compound (319) RT1487, compound (57) RT2144, compound (197) RT1637, compound (283) RT1774, compound (259) RT1920, compound (191) RT2175, compound (259) RT1657, compound (318) RT1334, compound (223) RT983, Erythronic acid (292), compound (361) RT1926, compound (75) RT690, compound (292) RT1203, Xylitol (307), compound (327) RT1456, Tyrosine (218), compound (255) RT1654, Methionine (176), compound (167) RT1609, compound (204) RT1340, Gluconate (333), carbohydrate (204) RT1781, 2-Oxoglutarate (129), fatty acid (339) RT1518, compound (263)

RT1518, compound (263) RT932, Aspartate (232), compound (75) RT722, Benzoic acid (267), compound (243) RT830,
compound (204) RT1971, compound (204) RT1731, compound (117) RT1803, compound (119) RT2019, compound (204) RT1517, compound (361) RT1948, compound (158) RT533, compound (204) RT1603, compound (361) RT1919, compound (333) RT1325, compound (319) RT1316, compound (285) RT1213, Octadecenoic acid (339) , compound (173) RT1809, compound (158) RT944, compound (281) RT548, compound (362) RT1978, compound (319) RT1968, compound (69) RT1140, compound (156) RT1226, compound (295) RT1171, Glutamate (246), compound (273) RT1297, compound (142) RT973, compound (306) RT1865, compound (132) RT1300, compound (217) RT964, compound (204) RT1655, compound (207) RT436, Adenosine (236), compound (172) RT825, compound (160) RT1319, compound (149) RT1233, compound (261) RT1791

RT932, Aspartate (232), compound (75) RT722, Benzoic acid (267), compound (243) RT830, compound (191) RT1825,
compound (204) RT1971, compound (204) RT1731, compound (117) RT1803, compound (119) RT2019, compound (217) RT1155, compound (204) RT1517, compound (361) RT1948, compound (158) RT533, compound (204) RT1603, compound (361) RT1919, compound (333) RT1325, compound (319) RT1316, compound (285) RT1213, Octadecenoic acid (339) , compound (173) RT1809, compound (158) RT944, compound (281) RT548, compound (362) RT1978, compound (319) RT1968, compound (69) RT1140, compound (156) RT1226, compound (295) RT1171, compound (77) RT466, Glutamate (246), compound (273) RT1297, compound (142) RT973, compound (306) RT1865, compound (132) RT1300, compound (217) RT964, compound (204) RT1655, compound (207) RT436, Adenosine (236), compound (172) RT825, compound (160) RT1319, compound (149) RT1233, compound (261) RT1791

b) semi-polar metabolites

	Forbs	grasses
Fertilization		
<u>forbs</u> *p<0.001* = 23 *p<0.01* = 7 *p<0.05* = 27 total = 57 <u>grasses</u> *p<0.001* = 16 *p<0.01* = 10 *p<0.05* = 34 total = 60	329.0882m/z_1.56min, 517.1154m/z_2.3min, 299.0775m/z_2.82min, 643.2024m/z_3.15min, 737.1919m/z_4.17min, 405.2103m/z_4.62min, 615.1831m/z_4.89min, 741.2561m/z_5.17min, 557.1862m/z_5.1min, 443.1919m/z_6.08min, 571.4201m/z_7.01min, 347.243m/z_8.19min, 351.1857m/z_13.79min, 176.0113m/z_4.29min, 375.1282m/z_2.37min, 469.1666m/z_4.27min, 391.1066m/z_5.34min, 355.2122m/z_9.24min, 385.0547m/z_3.97min, 555.1304m/z_2.86min, 415.1494m/z_5.09min, 563.1684m/z_5.75min, 601.1761m/z_5.04min, 388.9898m/z_3.61min, 385.1129m/z_2.46min, 283.0368m/z_5.63min, 309.2791m/z_15.05min, 191.0197m/z_1.08min, 369.3m/z_13.34min, 619.348m/z_8.36min, 339.072m/z_3.61min, 527.3192m/z_2.73min, 321.2047m/z_12.98min, 593.2503m/z_3.92min, 287.2218m/z_8.17min, 441.2519m/z_11.41min, 243.1958m/z_8.64min, 365.1172m/z_4.25min, 358.1975m/z_2.29min, 549.123m/z_3.55min, 385.1118m/z_4.15min, 563.2333m/z_3.62min, 451.1265m/z_3.63min, 323.1677m/z_10.06min, 432.2342m/z_2.43min, 401.1809m/z_4.27min, 259.1287m/z_2.42min, 271.2274m/z_12.35min, 477.1801m/z_5.17min, 441.2022m/z_14.72min,	299.0775m/z_2.82min, 405.2103m/z_4.62min, 352.1748m/z_5.29min, 797.4304m/z_5.43min, 661.3633m/z_6.01min, 443.1919m/z_6.08min, 567.3523m/z_6.11min, 571.4201m/z_7.01min, 221.0812m/z_7.35min, 505.3511m/z_8.25min, 381.2249m/z_9.47min, 351.1857m/z_13.79min, 469.1666m/z_4.27min, 391.1066m/z_5.34min, 355.2122m/z_9.24min, 563.1684m/z_5.75min, 557.2409m/z_7.42min, 388.9898m/z_3.61min, 385.1129m/z_2.46min, 283.0368m/z_5.63min, 309.2791m/z_15.05min, 191.0197m/z_1.08min, 369.3m/z_13.34min, 341.1426m/z_7.06min, 355.1583m/z_7.59min, 619.348m/z_8.36min, 417.2274m/z_5.68min, 519.3296m/z_5.76min, 399.1082m/z_6.77min, 339.072m/z_3.61min, 527.3192m/z_2.73min, 321.2047m/z_12.98min, 311.2218m/z_8.92min, 593.2503m/z_3.92min, 577.2292m/z_4.29min, 287.2218m/z_8.17min, 441.2519m/z_11.41min, 243.1958m/z_8.64min, 365.1172m/z_4.25min, 358.1975m/z_2.29min, 549.123m/z_3.55min, 385.1118m/z_4.15min, 563.2333m/z_3.62min, 451.1265m/z_3.63min, 432.2342m/z_2.43min, 401.1809m/z_4.27min, 329.2316m/z_7.29min, 259.1287m/z_2.42min, 271.2274m/z_12.35min, 297.243m/z_11.79min, 477.1801m/z_5.17min, 441.2022m/z_14.72min, 303.217m/z_6.74min, 395.1192m/z_3.12min, 446.2484m/z_3.19min, 197.0447m/z_3.71min, 315.2171m/z_6.35min, 315.2171m/z_6.97min, 533.1656m/z_4.43min, 241.1127m/z_4.78min

	303.217m/z_6.74min, 395.1192m/z_3.12min, 446.2484m/z_3.19min, 197.0447m/z_3.71min, 407.1863m/z_12.36min, 533.1656m/z_4.43min, 241.1127m/z_4.78min	
Grazing		
forbs *p<0.001* = 25 *p<0.01* = 13 *p<0.05* = 21 total = 59	485.1296m/z_2.2min, 563.2302m/z_3.2min, 539.2308m/z_3.86min, 561.2156m/z_3.96min, 345.1546m/z_4.27min, 523.2246m/z_4.77min, 585.1615m/z_4.85min, 523.056m/z_4.92min, 621.2369m/z_5.41min, 193.0748m/z_5.49min, 211.0247m/z_3.29min, 551.1823m/z_3.89min, 579.1277m/z_4.04min, 379.0093m/z_4.17min, 607.1315m/z_5.01min, 569.1659m/z_5.7min, 451.1596m/z_6.15min, 609.1806m/z_5.16min, 529.1547m/z_3.6min, 313.0709m/z_7.2min, 947.2767m/z_3.64min, 593.1499m/z_4.69min, 591.2266m/z_4.92min, 423.1283m/z_4.39min, 237.0557m/z_8.07min,	345.1546m/z_4.27min, 229.0774m/z_5.35min, 211.0247m/z_3.29min, 551.1823m/z_3.89min, 607.1315m/z_5.01min, 635.1963m/z_7.23min, 241.0826m/z_1.06min, 609.4118m/z_10.79min, 281.0597m/z_6.97min, 193.0501m/z_4.31min, 469.1666m/z_4.27min, 301.2006m/z_6.67min, 255.2321m/z_13.83min, 347.2424m/z_7.85min, 357.2133m/z_1.3min, 527.3192m/z_2.73min, 325.1832m/z_11.33min, 125.0963m/z_4.9min, 565.2156m/z_3.84min, 295.2269m/z_10.1min, 421.2255m/z_11.51min, 242.1757m/z_6.64min, 297.0401m/z_7.58min, 309.1708m/z_8.13min, 387.1124m/z_1.09min, 491.1744m/z_3.52min, 343.2116m/z_8.84min
grasses *p<0.001* = 6 *p<0.01* = 12 *p<0.05* = 9 total = 27	193.0501m/z_4.31min, 241.0826m/z_1.06min, 565.2156m/z_3.84min, 401.141m/z_5.86min, 469.1666m/z_4.27min, 301.2006m/z_6.67min, 255.2321m/z_13.83min, 456.2459m/z_2.74min, 281.14m/z_8.95min, 347.2424m/z_7.85min, 357.2133m/z_1.3min, 563.1408m/z_3.76min, 527.3192m/z_2.73min, 325.1832m/z_11.33min, 219.0869m/z_3.63min, 295.2269m/z_10.1min, 421.2255m/z_11.51min, 351.0414m/z_2.95min, 253.0504m/z_6.08min, 242.1757m/z_6.64min, 309.1708m/z_8.13min, 307.1382m/z_3.91min, 591.2079m/z_4.35min, 413.1614m/z_4.64min, 569.1719m/z_1.1min, 421.1269m/z_2.78min, 581.1645m/z_2.81min, 463.1384m/z_4.6min, 463.2154m/z_3.23min, 387.1124m/z_1.09min, 743.2203m/z_2.82min, 491.1744m/z_3.52min, 373.0696m/z_4.21min, 343.2116m/z_8.84min	

Mowing

<u>forbs</u>	171.1022m/z_5.75min, 356.0987m/z_2.52min, 455.1174m/z_3.44min, 469.1666m/z_4.27min,
p<0.001 = 23	530.1862m/z_3.25min, 363.1637m/z_3.31min, 365.192m/z_5.75min, 563.1684m/z_5.75min,
p<0.01 = 20	455.1174m/z_3.44min, 291.0857m/z_3.88min, 477.2808m/z_6.33min, 531.3197m/z_7.46min,
p<0.05 = 46	465.1397m/z_4.16min, 469.1666m/z_4.27min, 279.2323m/z_13.23min, 171.1022m/z_5.75min,
total = 89	629.1719m/z_4.73min, 243.0295m/z_4.75min, 351.1857m/z_13.79min, 563.2333m/z_3.62min,
	671.1596m/z_4.91min, 899.2243m/z_4.93min, 355.1583m/z_7.59min, 563.3213m/z_5.93min,
<u>grasses</u>	599.1631m/z_4.95min, 803.2387m/z_4.9min, 533.1656m/z_4.43min, 432.2342m/z_2.43min,
p<0.001 = 12	741.2561m/z_5.17min, 785.3102m/z_5.21min, 651.1202m/z_4.23min, 395.203m/z_7.41min,
p<0.01 = 18	443.2121m/z_5.45min, 359.1691m/z_5.75min, 179.1074m/z_6.19min, 271.2274m/z_12.35min,
p<0.05 = 50	563.1684m/z_5.75min, 605.2019m/z_6.51min, 341.1426m/z_7.06min, 309.2039m/z_9.76min,
total = 80	279.2323m/z_13.23min, 351.1857m/z_13.79min, 421.2255m/z_11.51min, 173.1117m/z_5.03min,
	563.2333m/z_3.62min, 295.2269m/z_11.31min, 441.2519m/z_11.41min,
	565.2302m/z_3.65min, 445.1888m/z_3.73min, 309.2791m/z_15.05min, 681.1298m/z_4.27min,
	441.1166m/z_4.51min, 533.1656m/z_4.43min, 191.0197m/z_1.08min, 283.0368m/z_5.63min,
	432.2342m/z_2.43min, 651.1202m/z_4.23min, 385.259m/z_10.16min, 301.2006m/z_6.67min,
	395.203m/z_7.41min, 179.1074m/z_6.19min, 581.1739m/z_4.26min, 565.2156m/z_3.84min,
	271.2274m/z_12.35min, 309.2039m/z_9.76min, 311.2218m/z_8.92min, 649.1848m/z_7.89min,
	421.2255m/z_11.51min, 173.1117m/z_5.03min, 611.185m/z_5.05min, 125.0963m/z_4.9min, 413.1498m/z_4.9min,
	295.2269m/z_11.31min, 441.2519m/z_11.41min, 491.2088m/z_2.75min, 319.138m/z_3.38min,
	309.2791m/z_15.05min, 681.1298m/z_4.27min, 389.1087m/z_1.15min, 413.2898m/z_11.29min,
	191.0197m/z_1.08min, 283.0368m/z_5.63min, 615.2247m/z_4.38min, 253.2167m/z_13min,
	385.259m/z_10.16min, 301.2006m/z_6.67min, 397.2129m/z_7.49min, 393.1711m/z_11.42min,
	293.1782m/z_11.32min, 565.2156m/z_3.84min, 439.3414m/z_13.97min, 365.0792m/z_8.4min,
	611.185m/z_5.05min, 491.2088m/z_2.75min, 285.0797m/z_4.86min, 295.2269m/z_10.1min,
	319.138m/z_3.38min, 389.1087m/z_1.15min, 385.1129m/z_2.46min, 431.2259m/z_7.21min,
	413.2898m/z_11.29min, 611.1828m/z_2.64min, 477.1801m/z_5.17min, 327.2161m/z_7min, 377.1578m/z_8.36min,
	615.2247m/z_4.38min, 253.2167m/z_13min, 327.2169m/z_8.34min, 242.1757m/z_6.64min,
	452.1914m/z_5.37min, 397.2129m/z_7.49min, 527.3192m/z_2.73min, 401.1809m/z_4.27min,
	393.1711m/z_11.42min, 439.3414m/z_13.97min, 381.2311m/z_13.44min, 243.1958m/z_8.64min,
	365.0792m/z_8.4min, 295.2269m/z_10.1min, 535.3598m/z_7.81min, 365.1172m/z_4.25min,
	385.1129m/z_2.46min, 431.2259m/z_7.21min, 613.2764m/z_6.08min, 339.1993m/z_12.63min,
	477.1801m/z_5.17min, 327.2161m/z_7min, 358.1975m/z_2.29min, 327.2168m/z_6.47min,
	377.1578m/z_8.36min, 327.2169m/z_8.34min, 173.0816m/z_4.1min, 505.1133m/z_4.94min,
	242.1757m/z_6.64min, 527.3192m/z_2.73min, 561.2172m/z_3.79min, 283.1909m/z_7.59min,
	401.1809m/z_4.27min, 381.2311m/z_13.44min, 293.2111m/z_10.79min, 227.2013m/z_12.53min,
	243.1958m/z_8.64min, 365.1172m/z_4.25min, 397.1813m/z_4.9min, 351.1807m/z_7.58min,
	613.2764m/z_6.08min, 339.1993m/z_12.63min, 311.2219m/z_7.72min, 379.1578m/z_10.2min,

	358.1975m/z_2.29min, 327.2168m/z_6.47min, 173.0816m/z_4.1min, 505.1133m/z_4.94min, 561.2172m/z_3.79min, 283.1909m/z_7.59min, 293.2111m/z_10.79min, 227.2013m/z_12.53min, 397.1813m/z_4.9min, 351.1807m/z_7.58min, 323.1677m/z_10.06min, 311.2219m/z_7.72min, 379.1578m/z_10.2min, 347.0439m/z_3.31min, 241.1127m/z_4.78min, 629.2697m/z_5.24min	347.0439m/z_3.31min, 241.1127m/z_4.78min, 311.201m/z_10.14min, 629.2697m/z_5.24min
soil texture		
<u>forbs</u> *p<0.001* = 38 *p<0.01* = 20 *p<0.05* = 37 total = 95	485.1296m/z_2.2min, 241.0716m/z_2.67min, 137.0244m/z_2.74min, 525.2182m/z_2.76min, 530.1862m/z_3.25min, 523.2316m/z_3.3min, 655.2221m/z_3.4min, 339.1023m/z_3.55min, 623.1629m/z_3.92min, 340.1031m/z_3.99min, 379.0093m/z_4.17min, 631.1667m/z_4.48min, 761.2136m/z_4.49min, 615.2199m/z_4.54min, 409.1094m/z_4.63min, 475.2175m/z_4.6min, 645.1817m/z_4.78min, 653.149m/z_4.83min, 441.1079m/z_5.08min, 443.2276m/z_5.16min, 313.0358m/z_5.21min, 366.0968m/z_5.33min, 179.0708m/z_5.71min, 709.3784m/z_5.89min, 347.243m/z_8.19min, 591.2266m/z_4.92min, 447.1655m/z_7.29min, 337.2032m/z_9.3min, 655.2235m/z_4.42min, 509.0895m/z_2.04min, 299.0775m/z_2.82min, 461.1306m/z_4.02min, 507.1528m/z_4.71min, 741.2561m/z_5.17min, 267.0871m/z_3.62min, 669.2214m/z_3.33min, 213.0121m/z_4.88min, 549.123m/z_3.55min, 493.1571m/z_2.1min, 501.32m/z_9.34min, 241.0826m/z_1.06min, 521.1852m/z_4.12min, 279.2323m/z_13.23min, 389.1117m/z_3.09min, 393.1711m/z_11.42min, 547.2386m/z_4.38min, 329.2324m/z_7.49min, 469.1666m/z_4.27min, 389.1087m/z_1.15min, 379.2096m/z_9.07min, 456.2459m/z_2.74min, 281.14m/z_8.95min, 671.291m/z_6.56min, 193.0504m/z_3.75min, 357.2133m/z_1.3min, 381.2311m/z_13.44min, 288.9479m/z_7.1min, 283.0461m/z_6.61min,	337.2032m/z_9.3min, 339.1023m/z_3.55min, 623.1629m/z_3.92min, 667.1503m/z_4.39min, 229.0774m/z_5.35min, 305.1396m/z_5.8min, 353.2327m/z_7.33min, 533.3441m/z_8.44min, 655.2235m/z_4.42min, 299.0775m/z_2.82min, 549.123m/z_3.55min, 267.0871m/z_3.62min, 669.2214m/z_3.33min, 213.0121m/z_4.88min, 241.0826m/z_1.06min, 609.4118m/z_10.79min, 521.1852m/z_4.12min, 365.2304m/z_10.77min, 279.2323m/z_13.23min, 393.1711m/z_11.42min, 547.2386m/z_4.38min, 329.2324m/z_7.49min, 469.1666m/z_4.27min, 357.2133m/z_1.3min, 381.2311m/z_13.44min, 671.291m/z_6.56min, 193.0504m/z_3.75min, 389.1087m/z_1.15min, 379.2096m/z_9.07min, 288.9479m/z_7.1min, 309.1955m/z_9.15min, 861.2448m/z_5.31min, 367.0868m/z_3.48min, 329.2324m/z_6.83min, 385.1118m/z_4.15min, 395.2428m/z_8.5min, 429.1377m/z_3.26min, 467.1615m/z_3.73min, 451.1952m/z_4.57min, 553.3379m/z_4.68min, 231.1216m/z_4.76min, 659.3782m/z_4.89min, 315.0521m/z_4.97min, 381.0967m/z_5.34min, 617.2243m/z_5.73min, 297.04m/z_6.95min, 521.3466m/z_7.03min, 649.3931m/z_7.35min, 295.0239m/z_8.07min, 305.1741m/z_8.2min, 331.205m/z_8.51min, 295.2269m/z_10.1min, 293.2109m/z_9.69min, 275.0919m/z_5.38min, 242.1757m/z_6.64min, 323.219m/z_13.85min, 431.1703m/z_8.05min, 365.1495m/z_9.47min, 311.2219m/z_7.72min,
<u>grasses</u> *p<0.001* = 14 *p<0.01* = 17 *p<0.05* = 47 total = 78		

	367.0868m/z_3.48min, 329.2324m/z_6.83min, 385.1118m/z_4.15min, 395.2428m/z_8.5min, 555.1466m/z_5.49min, 429.1377m/z_3.26min, 467.1615m/z_3.73min, 331.205m/z_8.51min, 581.182m/z_3.62min, 295.2269m/z_10.1min, 293.2109m/z_9.69min, 275.0919m/z_5.38min, 861.2448m/z_5.31min, 242.1757m/z_6.64min, 323.219m/z_13.85min, 431.1703m/z_8.05min, 365.1495m/z_9.47min, 311.2219m/z_7.72min, 355.2085m/z_8.17min, 200.9961m/z_4.08min, 589.0386m/z_6.18min, 293.2111m/z_9.42min, 227.2013m/z_12.53min, 343.0515m/z_4.34min, 465.1179m/z_2.92min, 480.9715m/z_6.3min, 297.2426m/z_10.81min, 327.2168m/z_6.47min, 295.227m/z_10.35min, 239.1284m/z_4.91min, 467.0597m/z_3.97min, 201.1129m/z_5.67min, 440.9281m/z_7.05min, 361.1622m/z_8.15min, 388.9898m/z_3.61min, 327.2111m/z_7.58min, 731.14m/z_5.5min	355.2085m/z_8.17min, 200.9961m/z_4.08min, 293.2111m/z_9.42min, 227.2013m/z_12.53min, 343.0515m/z_4.34min, 480.9715m/z_6.3min, 297.2426m/z_10.81min, 599.1424m/z_4.41min, 327.2168m/z_6.47min, 295.227m/z_10.35min, 239.1284m/z_4.91min, 467.0597m/z_3.97min, 201.1129m/z_5.67min, 285.0797m/z_4.86min, 440.9281m/z_7.05min, 361.1622m/z_8.15min, 463.2347m/z_6.62min, 388.9898m/z_3.61min, 327.2111m/z_7.58min
pH		
<u>forbs</u> *p<0.001* = 14 *p<0.01* = 18 *p<0.05* = 34 total = 66	437.2375m/z_3.84min, 307.1382m/z_3.91min, 259.1079m/z_3.96min, 523.056m/z_4.92min, 601.1761m/z_5.04min, 311.2217m/z_9.26min, 293.2111m/z_10.79min, 453.0888m/z_4.61min, 313.2378m/z_8.24min, 613.2764m/z_6.08min, 507.2064m/z_3.57min, 629.2697m/z_5.24min,	293.2111m/z_10.79min, 437.2375m/z_3.84min, 311.2217m/z_9.26min, 453.0888m/z_4.61min, 313.2378m/z_8.24min, 613.2764m/z_6.08min, 487.3407m/z_9.7min, 551.3568m/z_6.4min, 629.2697m/z_5.24min, 567.3523m/z_6.11min, 365.2676m/z_9.63min, 607.2289m/z_4.26min, 415.1679m/z_5.92min,
<u>grasses</u> *p<0.001* = 11 *p<0.01* = 18 *p<0.05* = 40 total = 69	631.1667m/z_4.48min, 645.1817m/z_4.78min, 607.2289m/z_4.26min, 579.1351m/z_4.88min, 415.1679m/z_5.92min, 285.2065m/z_7.82min, 283.1909m/z_7.59min, 278.0661m/z_4.87min, 387.0694m/z_4.3min, 377.1808m/z_4.13min, 803.3671m/z_4.26min, 611.2253m/z_4.12min, 491.182m/z_4.63min, 171.1022m/z_5.75min, 581.1858m/z_4.06min, 313.2376m/z_8.94min, 585.1615m/z_4.85min, 327.2169m/z_8.34min, 395.2037m/z_6.47min, 293.2109m/z_9.69min, 381.1805m/z_3.94min, 279.2323m/z_13.23min, 467.2166m/z_6.08min, 481.1372m/z_3.29min,	285.2065m/z_7.82min, 283.1909m/z_7.59min, 549.3422m/z_7.18min, 269.2112m/z_11.31min, 387.0694m/z_4.3min, 567.3523m/z_5min, 803.3671m/z_4.26min, 611.2253m/z_4.12min, 491.182m/z_4.63min, 171.1022m/z_5.75min, 581.1858m/z_4.06min, 313.2376m/z_8.94min, 327.2169m/z_8.34min, 561.4099m/z_9.3min, 395.2037m/z_6.47min, 293.2109m/z_9.69min, 381.1805m/z_3.94min, 419.2319m/z_6.48min, 319.2275m/z_10.24min, 279.2323m/z_13.23min, 467.2166m/z_6.08min, 267.1249m/z_5.53min, 469.1666m/z_4.27min, 563.2333m/z_3.62min,

	469.1666m/z_4.27min, 563.2333m/z_3.62min, 179.1074m/z_6.19min, 683.1849m/z_4.88min, 711.3953m/z_5.59min, 409.1002m/z_3.12min, 197.0447m/z_3.71min, 565.1658m/z_4.92min, 431.192m/z_4.36min, 347.2424m/z_7.85min, 563.2564m/z_6.68min, 439.0564m/z_1.79min, 421.0906m/z_3.42min, 533.1656m/z_4.43min, 307.1914m/z_8.98min, 337.2032m/z_9.3min, 559.1652m/z_3.15min, 513.1242m/z_2.59min, 243.1235m/z_5.01min, 273.0959m/z_5.67min, 267.196m/z_9.82min, 561.2172m/z_3.79min, 287.1194m/z_4.84min, 327.2168m/z_6.47min, 277.067m/z_4.92min, 325.1288m/z_6.05min, 391.0338m/z_3.5min, 561.1753m/z_3.3min, 271.05m/z_4.91min, 297.2431m/z_12.02min	179.1074m/z_6.19min, 311.2216m/z_8.27min, 409.1002m/z_3.12min, 197.0447m/z_3.71min, 565.1658m/z_4.92min, 431.192m/z_4.36min, 295.2272m/z_11.84min, 347.2424m/z_7.85min, 563.2564m/z_6.68min, 439.0564m/z_1.79min, 533.1656m/z_4.43min, 525.2404m/z_5.35min, 307.1914m/z_8.98min, 337.2032m/z_9.3min, 559.1652m/z_3.15min, 469.1839m/z_5.89min, 243.1235m/z_5.01min, 273.0959m/z_5.67min, 267.196m/z_9.82min, 561.2172m/z_3.79min, 295.2273m/z_9.82min, 287.1194m/z_4.84min, 171.1024m/z_5.36min, 327.2168m/z_6.47min, 277.067m/z_4.92min, 325.1288m/z_6.05min, 391.0338m/z_3.5min, 540.221m/z_6.61min, 561.1753m/z_3.3min, 125.0963m/z_4.9min, 271.05m/z_4.91min, 297.2431m/z_12.02min
Soil moisture		
<u>forbs</u> *p<0.001* = 51 *p<0.01* = 72 *p<0.05* = 36 total = 159	581.1858m/z_4.06min, 427.1959m/z_4.52min, 239.1284m/z_4.91min, 285.2064m/z_7.01min, 269.2116m/z_10.42min, 351.0414m/z_2.95min, 545.1494m/z_3.06min, 445.1907m/z_3.28min, 579.1308m/z_3.64min, 637.1419m/z_3.67min, 607.2195m/z_3.71min, 563.1408m/z_3.76min, 664.1887m/z_3.76min, 678.2013m/z_3.81min, 379.0093m/z_4.17min, 469.1666m/z_4.27min, 581.1881m/z_4.72min, 633.2376m/z_4.8min, 206.984m/z_5.11min, 609.1806m/z_5.16min, 385.0918m/z_5.96min, 463.1626m/z_6.08min, 327.2168m/z_6.47min, 242.1757m/z_6.64min, 329.2324m/z_6.83min, 283.1909m/z_7.59min, 267.196m/z_9.82min, 401.1809m/z_4.27min, 547.2386m/z_4.38min, 255.0302m/z_6.51min, 505.1345m/z_6.43min, 819.4185m/z_6.81min, 253.0503m/z_7.02min, 243.1235m/z_5.01min, 255.0302m/z_8.01min, 585.2387m/z_5.72min, 285.2065m/z_8.13min, 467.2166m/z_6.08min, 283.0534m/z_8.43min, 563.2333m/z_3.62min, 369.0952m/z_8.75min, 273.0959m/z_5.67min, 511.1454m/z_4.08min, 607.1697m/z_3.94min,	637.1419m/z_3.67min, 664.1887m/z_3.76min, 678.2013m/z_3.81min, 469.1666m/z_4.27min, 607.1664m/z_4.67min, 797.4304m/z_5.43min, 535.2907m/z_5.69min, 461.2202m/z_5.99min, 535.3267m/z_6.08min, 327.2168m/z_6.47min, 242.1757m/z_6.64min, 329.2324m/z_6.83min, 519.3298m/z_7.55min, 283.1909m/z_7.59min, 379.2098m/z_7.72min, 505.3511m/z_8.25min, 523.0414m/z_9.61min, 267.196m/z_9.82min, 521.028m/z_10.01min, 557.0016m/z_10.25min, 401.1809m/z_4.27min, 547.2386m/z_4.38min, 581.1858m/z_4.06min, 427.1959m/z_4.52min, 239.1284m/z_4.91min, 285.2064m/z_7.01min, 269.2116m/z_10.42min, 243.1235m/z_5.01min, 585.2387m/z_5.72min, 331.1632m/z_8.91min, 285.2065m/z_8.13min, 467.2166m/z_6.08min, 283.0534m/z_8.43min, 563.2333m/z_3.62min, 273.0959m/z_5.67min, 365.2676m/z_9.63min, 266.0188m/z_3.72min, 651.408m/z_7.73min, 423.0583m/z_3.65min, 371.1708m/z_4.64min, 463.2347m/z_6.62min, 431.1703m/z_8.05min, 421.2255m/z_11.51min,
<u>grasses</u> *p<0.001* = 39 *p<0.01* = 44 *p<0.05* = 39 total = 122		

431.0977m/z_5.62min, 266.0188m/z_3.72min, 259.1079m/z_3.96min, 299.0213m/z_5.23min, 507.2071m/z_5.2min, 267.0304m/z_9.64min, 423.0583m/z_3.65min, 456.2459m/z_2.74min, 281.14m/z_8.95min, 431.1703m/z_8.05min, 421.2255m/z_11.51min, 433.1873m/z_3.58min, 551.1765m/z_3.6min, 295.2269m/z_10.1min, 397.2129m/z_7.49min, 311.2219m/z_7.72min, 293.2111m/z_9.42min, 293.2111m/z_10.79min, 329.2324m/z_7.49min, 401.1808m/z_4.04min, 253.2167m/z_13min, 291.196m/z_10.14min, 255.2321m/z_13.83min, 327.2166m/z_7.41min, 313.2277m/z_9.69min, 165.0192m/z_3.09min, 373.1125m/z_1.64min, 467.1439m/z_2.45min, 681.223m/z_2.93min, 173.0236m/z_3.17min, 300.0873m/z_3.33min, 567.2278m/z_3.62min, 625.219m/z_3.65min, 683.2633m/z_3.97min, 788.2898m/z_3.97min, 665.2427m/z_3.98min, 829.3392m/z_4.25min, 551.1554m/z_4.26min, 531.1447m/z_4.2min, 541.1143m/z_4.53min, 429.1638m/z_4.59min, 481.0991m/z_5.08min, 745.3056m/z_5.2min, 388.1394m/z_5.64min, 481.1394m/z_5.69min, 269.0463m/z_5.97min, 481.1333m/z_5.98min, 381.1684m/z_5.99min, 343.1714m/z_5min, 607.2495m/z_6.04min, 637.1892m/z_6.63min, 179.1074m/z_6.19min, 239.0351m/z_7.94min, 803.3671m/z_4.26min, 239.1283m/z_4.7min, 563.2564m/z_6.68min, 267.1312m/z_5.99min, 409.0444m/z_2.6min, 327.2169m/z_8.34min, 293.2109m/z_9.69min, 533.1656m/z_4.43min, 553.2367m/z_3.8min, 515.1521m/z_6.25min, 611.2253m/z_4.12min, 241.0799m/z_1.39min, 337.2032m/z_9.3min, 613.2764m/z_6.08min, 389.1236m/z_4.37min, 589.0386m/z_6.18min, 457.2062m/z_4.69min, 397.0925m/z_8.24min, 295.2269m/z_11.31min, 631.1637m/z_3.93min, 383.3513m/z_15min, 395.203m/z_7.41min, 433.1873m/z_3.58min, 551.1765m/z_3.6min, 295.2269m/z_10.1min, 397.2129m/z_7.49min, 311.2219m/z_7.72min, 293.2111m/z_9.42min, 293.2111m/z_10.79min, 329.2324m/z_7.49min, 401.1808m/z_4.04min, 253.2167m/z_13min, 291.196m/z_10.14min, 255.2321m/z_13.83min, 327.2166m/z_7.41min, 313.2277m/z_9.69min, 179.1074m/z_6.19min, 803.3671m/z_4.26min, 239.1283m/z_4.7min, 295.2273m/z_9.82min, 563.2564m/z_6.68min, 267.1312m/z_5.99min, 561.4099m/z_9.3min, 409.0444m/z_2.6min, 327.2169m/z_8.34min, 293.2109m/z_9.69min, 533.1656m/z_4.43min, 553.2367m/z_3.8min, 611.2253m/z_4.12min, 241.0799m/z_1.39min, 337.2032m/z_9.3min, 613.2764m/z_6.08min, 389.1236m/z_4.37min, 457.2062m/z_4.69min, 351.2155m/z_7.02min, 295.2269m/z_11.31min, 631.1637m/z_3.93min, 383.3513m/z_15min, 395.203m/z_7.41min, 521.1852m/z_4.12min, 339.1988m/z_12.17min, 563.2179m/z_3.98min, 319.2275m/z_10.24min, 295.227m/z_10.35min, 499.1984m/z_3.82min, 389.1232m/z_4.43min, 671.291m/z_6.56min, 171.1022m/z_5.75min, 125.0963m/z_4.9min, 271.2274m/z_12.35min, 285.2065m/z_7.82min, 311.2217m/z_9.26min, 313.2378m/z_8.24min, 439.0564m/z_1.79min, 255.1034m/z_6.19min, 525.2404m/z_5.35min, 309.2051m/z_8min, 269.2099m/z_9.3min, 173.1117m/z_5.03min, 279.2323m/z_13.23min, 325.1288m/z_6.05min, 433.2051m/z_3.99min, 476.2757m/z_9.66min, 431.2638m/z_7.55min, 297.1522m/z_9.69min, 411.1901m/z_5.02min, 424.9673m/z_5.14min, 574.958m/z_6.85min, 563.1406m/z_3.88min, 309.2038m/z_8.66min, 197.0452m/z_3.77min, 363.2138m/z_10.35min, 593.1603m/z_4.02min, 297.2426m/z_10.81min, 299.1757m/z_9.54min, 293.2101m/z_10.65min, 295.2263m/z_11.17min, 431.2259m/z_7.21min, 348.9199m/z_7.09min, 397.2192m/z_6.83min, 180.9897m/z_4.28min

	239.0349m/z_6.21min, 521.1852m/z_4.12min, 339.1988m/z_12.17min, 563.2179m/z_3.98min, 625.1763m/z_5.07min, 295.227m/z_10.35min, 499.1984m/z_3.82min, 389.1232m/z_4.43min, 671.291m/z_6.56min, 171.1022m/z_5.75min, 565.1771m/z_3.45min, 271.2274m/z_12.35min, 285.2065m/z_7.82min, 311.2217m/z_9.26min, 313.2378m/z_8.24min, 439.0564m/z_1.79min, 255.1034m/z_6.19min, 269.2099m/z_9.3min, 173.1117m/z_5.03min, 279.2323m/z_13.23min, 325.1288m/z_6.05min, 433.2051m/z_3.99min, 431.2638m/z_7.55min, 299.0209m/z_6.2min, 297.1522m/z_9.69min, 373.0696m/z_4.21min, 411.1901m/z_5.02min, 563.1406m/z_3.88min, 309.2038m/z_8.66min, 197.0452m/z_3.77min, 363.2138m/z_10.35min, 593.1603m/z_4.02min, 297.2426m/z_10.81min, 299.1757m/z_9.54min, 293.2101m/z_10.65min, 295.2263m/z_11.17min, 431.2259m/z_7.21min, 348.9199m/z_7.09min, 397.2192m/z_6.83min, 180.9897m/z_4.28min	
soil type		
<u>forbs</u>	525.2182m/z_2.76min, 375.1311m/z_3.29min,	345.1546m/z_4.27min, 699.3939m/z_4.6min,
p<0.001 = 19	505.1898m/z_3.45min, 607.2195m/z_3.71min,	815.4389m/z_4.83min, 813.4231m/z_5.11min,
p<0.01 = 13	527.136m/z_3.93min, 345.1546m/z_4.27min,	321.1701m/z_6.94min, 593.403m/z_7min, 431.1703m/z_8.05min,
p<0.05 = 34	507.2066m/z_4.98min, 473.1653m/z_5.31min,	317.1389m/z_8.15min, 355.249m/z_10.36min,
total = 66	709.3784m/z_5.89min, 385.0918m/z_5.96min,	547.2386m/z_4.38min, 603.3586m/z_5.56min,
	431.1703m/z_8.05min, 423.1283m/z_4.39min,	573.3478m/z_5.85min, 587.3618m/z_6.31min,
<u>grasses</u>	547.2386m/z_4.38min, 461.1318m/z_2.82min,	733.4564m/z_6.96min, 221.1538m/z_8.15min,
p<0.001 = 15	221.1538m/z_8.15min, 351.0115m/z_6.24min,	285.2064m/z_7.01min, 353.1941m/z_7.01min,
p<0.01 = 10	485.1296m/z_2.2min, 655.2799m/z_4.97min,	325.1835m/z_11.45min, 303.0909m/z_4.04min,
p<0.05 = 30	297.0398m/z_7.29min,	267.0871m/z_3.62min, 511.1875m/z_4.49min,
total = 55	507.2071m/z_5.2min, 269.2481m/z_14.22min,	417.1743m/z_5.35min, 313.2374m/z_8.53min,
	353.1941m/z_7.01min, 285.2064m/z_7.01min,	487.1812m/z_4.84min, 329.2323m/z_7.63min,
	389.1117m/z_3.09min, 325.1835m/z_11.45min,	469.1666m/z_4.27min, 367.1042m/z_3.25min,
	511.1875m/z_4.49min, 417.1743m/z_5.35min,	317.2119m/z_10.6min, 236.105m/z_8.15min,
	359.0985m/z_1.6min, 389.1117m/z_3.09min,	293.1751m/z_8.15min, 369.1718m/z_8.13min,
	325.1835m/z_11.45min, 313.2374m/z_8.53min,	329.2324m/z_7.49min, 191.0346m/z_4.29min,
	487.1812m/z_4.84min,	357.2133m/z_1.3min, 243.1597m/z_7.72min, 171.066m/z_4.24min,

	367.1042m/z_3.25min, 236.105m/z_8.15min, 293.1751m/z_8.15min, 329.2324m/z_7.49min, 191.0346m/z_4.29min, 231.0301m/z_7.39min, 357.2133m/z_1.3min, 469.1666m/z_4.27min, 243.1597m/z_7.72min, 171.066m/z_4.24min, 625.1776m/z_5.21min, 297.2426m/z_10.81min, 239.1284m/z_4.91min, 269.2116m/z_10.42min, 325.1833m/z_11.51min, 429.1762m/z_4.96min, 507.2076m/z_3.72min, 369.0952m/z_8.75min, 995.2638m/z_4.25min, 361.1622m/z_8.15min, 285.2065m/z_7.82min, 379.2096m/z_9.07min, 397.1813m/z_4.9min, 269.0851m/z_9.66min, 371.0972m/z_3.8min, 397.2129m/z_7.49min, 381.2311m/z_13.44min, 431.2259m/z_7.21min, 427.1959m/z_4.52min, 339.199m/z_12.23min, 433.1873m/z_3.58min, 291.196m/z_9.42min, 473.3262m/z_12.65min, 327.2169m/z_8.34min	297.2426m/z_10.81min, 239.1284m/z_4.91min, 269.2116m/z_10.42min, 325.1833m/z_11.51min, 429.1762m/z_4.96min, 361.1622m/z_8.15min, 285.2065m/z_7.82min, 379.2096m/z_9.07min, 397.1813m/z_4.9min, 371.0972m/z_3.8min, 397.2129m/z_7.49min, 381.2311m/z_13.44min, 431.2259m/z_7.21min, 427.1959m/z_4.52min, 339.199m/z_12.23min, 433.1873m/z_3.58min, 291.196m/z_9.42min, 473.3262m/z_12.65min, 327.2169m/z_8.34min
TC forbs *p<0.001* = 15 *p<0.01* = 21 *p<0.05* = 15 total = 51 grasses *p<0.001* = 19 *p<0.01* = 5 *p<0.05* = 15 total = 39	329.0882m/z_1.56min, 461.1301m/z_2.57min, 643.2213m/z_3.44min, 637.1419m/z_3.67min, 664.1887m/z_3.76min, 525.1612m/z_3.78min, 678.2013m/z_3.81min, 340.1031m/z_3.99min, 761.2136m/z_4.49min, 615.2199m/z_4.54min, 433.0282m/z_4.99min, 441.1079m/z_5.08min, 295.1364m/z_8.45min, 625.2039m/z_8.75min, 251.0559m/z_3.64min, 377.1792m/z_2.89min, 591.1649m/z_3.07min, 319.0859m/z_3.13min, 275.0856m/z_3.51min, 535.2379m/z_3.59min, 493.1943m/z_3.61min, 445.1185m/z_3.86min, 455.1035m/z_4.11min, 635.1613m/z_4.38min, 345.1539m/z_4.68min, 489.197m/z_5.48min, 517.2283m/z_5.85min, 259.0824m/z_6.02min, 445.2418m/z_6.06min, 257.1397m/z_6.29min, 401.124m/z_6.55min, 311.1681m/z_10.05min, 421.0906m/z_3.42min, 215.1284m/z_6.38min, 507.2064m/z_3.57min, 358.1975m/z_2.29min,	461.1301m/z_2.57min, 637.1419m/z_3.67min, 664.1887m/z_3.76min, 678.2013m/z_3.81min, 667.1503m/z_4.39min, 607.1664m/z_4.67min, 433.0282m/z_4.99min, 975.5116m/z_6.73min, 533.3303m/z_7.16min, 329.2316m/z_7.29min, 353.2327m/z_7.33min, 325.1998m/z_7.78min, 505.3511m/z_8.25min, 547.3252m/z_6.98min, 797.4304m/z_5.43min, 533.3441m/z_8.44min, 573.2494m/z_4.26min, 545.2227m/z_4.89min, 251.0559m/z_3.64min, 311.1681m/z_10.05min, 215.1284m/z_6.38min, 268.116m/z_4.81min, 491.2121m/z_4.98min, 358.1975m/z_2.29min, 293.1751m/z_8.15min, 423.0583m/z_3.65min, 239.1284m/z_4.91min, 385.1129m/z_2.46min, 266.0188m/z_3.72min, 293.1787m/z_11.2min, 297.2423m/z_10.74min, 519.186m/z_4.64min, 335.1889m/z_11.52min, 529.1855m/z_4.56min, 393.171m/z_10.92min, 311.2214m/z_9.06min,

	293.1751m/z_8.15min, 633.1408m/z_3.41min, 423.0583m/z_3.65min, 239.1284m/z_4.91min, 385.1129m/z_2.46min, 266.0188m/z_3.72min, 293.1787m/z_11.2min, 351.0414m/z_2.95min, 297.2423m/z_10.74min, 519.186m/z_4.64min, 335.1889m/z_11.52min, 529.1855m/z_4.56min, 311.2214m/z_9.06min, 269.0454m/z_6.92min, 229.1804m/z_7.96min	269.0454m/z_6.92min, 229.1804m/z_7.96min, 653.0978m/z_7.03min
TN		
<u>forbs</u>	455.1174m/z_3.44min, 555.1466m/z_5.49min,	455.1174m/z_3.44min, 200.1286m/z_4.83min, 327.2161m/z_7min,
p<0.001 = 4	200.1286m/z_4.83min, 327.2161m/z_7min,	311.1681m/z_10.05min, 239.1284m/z_4.91min,
p<0.01 = 9	311.1681m/z_10.05min, 239.1284m/z_4.91min,	297.2423m/z_10.74min, 295.2269m/z_10.1min,
p<0.05 = 22	297.2423m/z_10.74min, 295.2269m/z_10.1min,	293.1787m/z_11.2min, 585.1836m/z_3.67min,
total = 35	293.1787m/z_11.2min, 617.1089m/z_3.54min,	215.1284m/z_6.38min, 423.0583m/z_3.65min,
	215.1284m/z_6.38min, 423.0583m/z_3.65min,	329.2324m/z_7.49min, 563.2898m/z_5.57min,
<u>grasses</u>	585.1836m/z_3.67min,	311.2214m/z_9.06min, 229.1804m/z_7.96min,
p<0.001 = 3	456.2459m/z_2.74min, 281.14m/z_8.95min,	311.201m/z_10.14min, 313.2376m/z_8.94min,
p<0.01 = 8	329.2324m/z_7.49min, 563.2898m/z_5.57min,	266.0188m/z_3.72min, 341.1073m/z_5.74min,
p<0.05 = 20	311.2214m/z_9.06min, 229.1804m/z_7.96min,	651.2143m/z_3.82min, 611.185m/z_5.05min,
total = 31	313.2376m/z_8.94min, 266.0188m/z_3.72min,	529.1855m/z_4.56min, 585.2387m/z_5.72min,
	341.1073m/z_5.74min, 589.1887m/z_4.35min,	269.0454m/z_6.92min, 335.1889m/z_11.52min,
	651.2143m/z_3.82min, 611.185m/z_5.05min,	547.2386m/z_4.38min, 393.171m/z_10.92min,
	529.1855m/z_4.56min, 585.2387m/z_5.72min,	201.1129m/z_5.67min, 293.2111m/z_10.79min,
	269.0454m/z_6.92min, 335.1889m/z_11.52min,	144.0458m/z_4.62min, 551.1823m/z_3.89min
	547.2386m/z_4.38min, 372.123m/z_8.35min,	
	201.1129m/z_5.67min, 293.2111m/z_10.79min,	
	529.0899m/z_8.99min, 551.1823m/z_3.89min	
Precipitation		
<u>forbs</u>	517.1154m/z_2.3min, 595.2582m/z_3.65min,	475.1251m/z_7.03min, 321.2095m/z_13.8min,
p<0.001 = 12	213.0121m/z_4.88min, 683.1849m/z_4.88min,	213.0121m/z_4.88min, 567.3263m/z_5.22min,
p<0.01 = 31	557.1862m/z_5.1min, 567.3263m/z_5.22min,	331.1632m/z_8.91min, 533.345m/z_7.63min, 479.269m/z_6.24min,
p<0.05 = 52	711.3953m/z_5.59min, 321.2095m/z_13.8min,	328.212m/z_8.92min, 365.2676m/z_9.63min,
total = 95	479.269m/z_6.24min, 381.0621m/z_7.17min,	631.1637m/z_3.93min, 401.1809m/z_4.27min,
	328.212m/z_8.92min, 338.1388m/z_8.53min,	323.219m/z_13.85min, 337.2032m/z_9.3min,
<u>grasses</u>	479.269m/z_6.24min, 381.0621m/z_7.17min,	321.0538m/z_4.14min, 350.9167m/z_7.05min,
p<0.001 = 9	328.212m/z_8.92min, 338.1388m/z_8.53min,	543.1993m/z_4.16min, 303.1195m/z_4.19min,
p<0.01 = 22	587.2182m/z_5.35min, 267.1597m/z_5.66min,	251.0917m/z_4.74min, 273.1695m/z_5.1min,

p<0.05 = 48 total = 79	613.2667m/z_5.66min, 463.1453m/z_1.05min, 197.0453m/z_1.42min, 387.0977m/z_2.06min, 491.1753m/z_3.39min, 343.137m/z_3.8min, 413.2169m/z_5.37min, 325.0916m/z_5.56min, 421.1158m/z_5.6min, 311.1681m/z_10.63min, 631.1637m/z_3.93min, 401.1809m/z_4.27min, 323.219m/z_13.85min, 337.2032m/z_9.3min, 441.1166m/z_4.51min, 321.0538m/z_4.14min, 350.9167m/z_7.05min, 507.2071m/z_5.2min, 324.1236m/z_7.94min, 537.1978m/z_3.9min, 659.4715m/z_6.83min, 411.1901m/z_5.02min, 973.4448m/z_3.79min, 561.4127m/z_9.51min, 273.0959m/z_5.67min, 389.1117m/z_3.09min, 271.2274m/z_12.35min, 377.1776m/z_4.31min, 295.2263m/z_11.17min, 293.1751m/z_8.15min, 283.0719m/z_5.8min, 283.0534m/z_8.43min, 365.1495m/z_9.47min, 447.1152m/z_2.45min, 643.2079m/z_4.22min, 269.0483m/z_7.72min, 269.2116m/z_10.42min, 563.2564m/z_6.68min, 547.2386m/z_4.38min, 313.2376m/z_8.94min, 463.1818m/z_4.3min, 239.1284m/z_4.91min, 391.1601m/z_3.36min, 529.0899m/z_8.99min, 507.2356m/z_6.17min, 277.0712m/z_1.4min, 231.0297m/z_4.3min, 377.1806m/z_3.7min, 313.2378m/z_8.24min, 565.1771m/z_3.45min, 489.1246m/z_2.58min, 311.2219m/z_7.72min, 363.2138m/z_10.35min, 431.1338m/z_5.86min, 481.1363m/z_3.57min, 655.4203m/z_6.48min, 455.3366m/z_12.92min, 393.1711m/z_11.42min, 327.2169m/z_8.34min, 329.0875m/z_1.86min, 423.1636m/z_4.01min, 291.196m/z_10.14min, 745.2468m/z_4.93min, 293.2109m/z_9.69min, 365.1394m/z_9.54min, 327.2111m/z_7.58min, 551.1765m/z_3.6min, 283.0359m/z_6.15min, 297.2426m/z_10.81min, 563.1449m/z_5.35min, 519.186m/z_4.64min, 371.1121m/z_5.59min, 373.113m/z_2.01min, 259.0999m/z_7.07min, 619.1862m/z_3.12min, 393.2488m/z_8.98min, 163.04m/z_4min, 487.3414m/z_8.82min, 356.2425m/z_10.21min, 525.2404m/z_5.35min, 351.2143m/z_5.19min, 531.2955m/z_6.62min, 659.4715m/z_6.83min, 411.1901m/z_5.02min, 561.4127m/z_9.51min, 144.0458m/z_4.62min, 273.0959m/z_5.67min, 271.2274m/z_12.35min, 377.1776m/z_4.31min, 295.2263m/z_11.17min, 293.1751m/z_8.15min, 339.1987m/z_11.79min, 283.0719m/z_5.8min, 283.0534m/z_8.43min, 365.1495m/z_9.47min, 447.1152m/z_2.45min, 269.0483m/z_7.72min, 269.2116m/z_10.42min, 563.2564m/z_6.68min, 547.2386m/z_4.38min, 319.2275m/z_10.24min, 313.2376m/z_8.94min, 239.1284m/z_4.91min, 277.0712m/z_1.4min, 313.2378m/z_8.24min, 433.1855m/z_5.14min, 311.2219m/z_7.72min, 363.2138m/z_10.35min, 481.1363m/z_3.57min, 655.4203m/z_6.48min, 455.3366m/z_12.92min, 393.1711m/z_11.42min, 327.2169m/z_8.34min, 329.0875m/z_1.86min, 423.1636m/z_4.01min, 291.196m/z_10.14min, 337.1945m/z_10.53min, 293.2109m/z_9.69min, 365.1394m/z_9.54min, 327.2111m/z_7.58min, 295.2273m/z_9.82min, 551.1765m/z_3.6min, 297.2426m/z_10.81min, 563.1449m/z_5.35min, 353.2317m/z_7.98min, 519.186m/z_4.64min, 371.1121m/z_5.59min, 373.113m/z_2.01min, 259.0999m/z_7.07min, 619.1862m/z_3.12min, 393.2488m/z_8.98min, 163.04m/z_4min, 399.1662m/z_4.4min, 487.3414m/z_8.82min, 395.1667m/z_4.76min, 295.2269m/z_11.31min, 557.3813m/z_9.82min, 593.1416m/z_3.6min

	395.1667m/z_4.76min, 295.2269m/z_11.31min, 593.1416m/z_3.6min	
T(10)		
<u>forbs</u>	563.2302m/z_3.2min, 947.2767m/z_3.64min,	663.2823m/z_6.72min, 461.1301m/z_2.57min,
p<0.001 = 66	340.1031m/z_3.99min, 415.1494m/z_5.09min,	455.1174m/z_3.44min, 441.1403m/z_4.66min,
p<0.01 = 13	663.2823m/z_6.72min, 461.1301m/z_2.57min,	627.1898m/z_3.99min, 397.2192m/z_6.83min, 643.26m/z_5.59min,
p<0.05 = 25	455.1174m/z_3.44min, 441.1403m/z_4.66min,	337.2043m/z_12.49min, 577.1735m/z_5min,
total = 46	627.1898m/z_3.99min, 397.2192m/z_6.83min,	317.1233m/z_3.26min, 443.1915m/z_3.3min,
	643.26m/z_5.59min, 337.2043m/z_12.49min,	475.2179m/z_3.74min, 457.2059m/z_4.37min,
<u>grasses</u>	577.1735m/z_5min, 317.1233m/z_3.26min,	441.1741m/z_4.4min, 553.1935m/z_5.14min,
p<0.001 = 4	443.1915m/z_3.3min, 475.2179m/z_3.74min,	263.1283m/z_6.06min, 277.2167m/z_12.49min,
p<0.01 = 14	317.1026m/z_5.33min, 481.1372m/z_3.29min,	313.2376m/z_8.94min,
p<0.05 = 27	277.2167m/z_12.49min, 313.2376m/z_8.94min,	204.1236m/z_1.7min, 373.1393m/z_4.65min,
total = 45	421.0732m/z_2.91min,	487.1502m/z_5.44min, 377.2061m/z_6.05min,
	204.1236m/z_1.7min, 507.1571m/z_4.11min,	507.1571m/z_4.11min, 217.123m/z_6.31min,
	431.1703m/z_8.05min, 659.4715m/z_6.83min,	431.1703m/z_8.05min, 659.4715m/z_6.83min,
	372.123m/z_8.35min, 327.2168m/z_6.47min,	285.0797m/z_4.86min, 327.2168m/z_6.47min,
	311.2217m/z_9.26min, 529.0899m/z_8.99min,	311.2217m/z_9.26min, 293.2109m/z_9.69min,
	293.2109m/z_9.69min, 259.1287m/z_2.42min,	259.1287m/z_2.42min, 381.1805m/z_3.94min,
	381.1805m/z_3.94min, 593.1603m/z_4.02min,	419.2319m/z_6.48min, 593.1603m/z_4.02min,
	297.2431m/z_12.02min, 221.0456m/z_4.46min,	297.2431m/z_12.02min, 242.1757m/z_6.64min,
	242.1757m/z_6.64min, 393.1709m/z_11.22min,	393.1709m/z_11.22min, 287.2221m/z_11.9min,
	287.2221m/z_11.9min, 311.1679m/z_10.51min,	311.1679m/z_10.51min, 397.0525m/z_3.84min,
	397.0525m/z_3.84min, 283.1378m/z_8.91min,	283.1378m/z_8.91min, 329.1063m/z_5.71min,
	329.1063m/z_5.71min, 369.0981m/z_6.53min,	385.1159m/z_2.35min, 361.0723m/z_3.61min,
	485.1091m/z_1.62min, 432.1255m/z_2.84min,	415.143m/z_4.85min
	461.2192m/z_3.52min, 375.1215m/z_3.73min,	
	501.1525m/z_3.77min, 539.2298m/z_4.37min,	
	641.2347m/z_4.64min, 466.7693m/z_4.78min,	
	535.106m/z_4.84min, 447.1122m/z_5.22min,	
	385.1159m/z_2.35min, 361.0723m/z_3.61min,	
	415.143m/z_4.85min	
T(200)		
<u>forbs</u>	397.2129m/z_7.49min, 547.2386m/z_4.38min,	547.2386m/z_4.38min, 239.1284m/z_4.91min,
p<0.001 = 23	487.1991m/z_4.39min, 391.1278m/z_4.66min,	327.2168m/z_6.47min, 283.1909m/z_7.59min,
p<0.01 = 47	477.1979m/z_4.69min, 239.1284m/z_4.91min,	431.1703m/z_8.05min, 277.2167m/z_12.49min,
p<0.05 = 62	655.2799m/z_4.97min, 475.2169m/z_5.13min,	397.2129m/z_7.49min, 327.2169m/z_8.34min,

total = 132	327.2168m/z_6.47min, 283.1909m/z_7.59min, 293.2109m/z_9.69min, 487.1812m/z_4.84min,
	431.1703m/z_8.05min, 277.2167m/z_12.49min, 295.2269m/z_10.1min, 511.1875m/z_4.49min,
<u>grasses</u>	487.1812m/z_4.84min, 295.2269m/z_10.1min, 311.2217m/z_9.26min, 253.2167m/z_12.85min,
p<0.001 = 17	327.2169m/z_8.34min, 293.2109m/z_9.69min, 266.0188m/z_3.72min, 291.196m/z_9.42min,
p<0.01 = 52	182.989m/z_15.14min, 511.1875m/z_4.49min, 265.0581m/z_4.21min,
p<0.05 = 53	311.2217m/z_9.26min, 253.2167m/z_12.85min, 469.1666m/z_4.27min, 329.2324m/z_6.83min,
total = 122	266.0188m/z_3.72min, 291.196m/z_9.42min, 177.0549m/z_3.37min, 365.2676m/z_9.63min,
	265.0581m/z_4.21min, 445.2057m/z_3.4min, 417.212m/z_4.85min, 329.2324m/z_7.49min,
	469.1666m/z_4.27min, 329.2324m/z_6.83min, 313.2376m/z_8.94min, 611.2253m/z_4.12min,
	177.0549m/z_3.37min, 239.0199m/z_3.81min, 361.1987m/z_10.79min, 303.2321m/z_13.11min,
	445.2057m/z_3.4min, 417.212m/z_4.85min, 125.0963m/z_4.9min, 493.2279m/z_5.48min,
	329.2324m/z_7.49min, 313.2376m/z_8.94min, 242.1757m/z_6.64min, 397.1813m/z_4.9min,
	611.2253m/z_4.12min, 361.1987m/z_10.79min, 291.196m/z_10.14min, 563.2333m/z_3.62min, 643.26m/z_5.59min,
	493.2279m/z_5.48min, 242.1757m/z_6.64min, 401.1808m/z_4.04min, 309.2038m/z_8.66min,
	397.1813m/z_4.9min, 291.196m/z_10.14min, 201.1129m/z_5.67min, 311.2219m/z_7.72min,
	563.2333m/z_3.62min, 643.26m/z_5.59min, 297.2426m/z_10.81min, 509.223m/z_3.78min,
	401.1808m/z_4.04min, 309.2038m/z_8.66min, 397.2192m/z_6.83min, 311.2214m/z_9.06min,
	201.1129m/z_5.67min, 311.2219m/z_7.72min, 363.2138m/z_10.35min, 295.2269m/z_11.31min,
	297.2426m/z_10.81min, 509.223m/z_3.78min, 241.0799m/z_1.39min, 431.192m/z_4.36min,
	397.2192m/z_6.83min, 311.2214m/z_9.06min, 803.3671m/z_4.26min, 455.317m/z_13.87min,
	363.2138m/z_10.35min, 295.2269m/z_11.31min, 255.1034m/z_6.19min, 327.2161m/z_7min, 309.2064m/z_7.25min,
	241.0799m/z_1.39min, 803.3671m/z_4.26min, 267.1249m/z_5.53min, 295.2272m/z_11.84min,
	255.1034m/z_6.19min, 317.1026m/z_5.33min, 239.1283m/z_4.7min, 277.0712m/z_1.4min, 447.1669m/z_5.95min,
	327.2161m/z_7min, 309.2064m/z_7.25min, 613.2764m/z_6.08min, 379.2096m/z_9.07min,
	239.1283m/z_4.7min, 277.0712m/z_1.4min, 565.3362m/z_5.57min, 381.2311m/z_13.44min,
	447.1669m/z_5.95min, 613.2764m/z_6.08min, 435.2223m/z_3.89min, 299.1757m/z_9.54min,
	379.2096m/z_9.07min, 513.1825m/z_3.41min, 631.1637m/z_3.93min, 537.1675m/z_4.87min,
	381.2311m/z_13.44min, 435.2223m/z_3.89min, 467.2166m/z_6.08min, 537.3211m/z_5.45min,
	299.1757m/z_9.54min, 631.1637m/z_3.93min, 449.2105m/z_4.9min, 511.1694m/z_4.64min,
	537.1675m/z_4.87min, 431.192m/z_4.36min, 563.1449m/z_5.35min, 293.1751m/z_8.15min,
	467.2166m/z_6.08min, 511.1694m/z_4.64min, 321.2095m/z_13.8min, 401.1809m/z_4.27min,
	369.0952m/z_8.75min, 389.1232m/z_4.43min, 243.1235m/z_5.01min,
	393.1758m/z_2.58min, 293.1751m/z_8.15min, 273.0959m/z_5.67min, 563.2179m/z_3.98min,
	563.1449m/z_5.35min, 401.1809m/z_4.27min, 313.2378m/z_8.24min, 427.1959m/z_4.52min,
	321.2095m/z_13.8min, 389.1232m/z_4.43min, 615.2247m/z_4.38min, 311.0771m/z_4.13min,
	243.1235m/z_5.01min, 507.2013m/z_3.87min, 293.2111m/z_9.42min, 283.0534m/z_8.43min,
	273.0959m/z_5.67min, 463.1818m/z_4.3min, 267.0871m/z_3.62min, 217.123m/z_6.31min, 309.2051m/z_8min,
	563.2179m/z_3.98min, 509.218m/z_3.25min, 611.185m/z_5.05min, 348.9199m/z_7.09min,
	313.2378m/z_8.24min, 427.1959m/z_4.52min, 279.2323m/z_13.23min, 449.2022m/z_4.42min,

	615.2247m/z_4.38min, 311.0771m/z_4.13min, 293.2111m/z_9.42min, 283.0534m/z_8.43min, 267.0871m/z_3.62min, 507.2356m/z_6.17min, 269.0851m/z_9.66min, 611.185m/z_5.05min, 348.9199m/z_7.09min, 499.181m/z_4.39min, 279.2323m/z_13.23min, 449.2022m/z_4.42min, 311.1679m/z_10.51min, 625.1776m/z_5.21min, 431.2638m/z_7.55min, 271.2274m/z_12.35min, 297.0398m/z_7.29min, 333.1368m/z_9.45min, 403.1956m/z_4.2min, 389.1087m/z_1.15min, 481.1372m/z_3.29min, 337.2043m/z_12.49min, 309.1949m/z_9.45min, 309.2039m/z_9.76min, 439.0564m/z_1.79min, 447.2224m/z_5.5min, 293.2111m/z_10.79min, 173.0816m/z_4.1min, 307.1907m/z_7.68min, 441.2519m/z_11.41min, 209.1179m/z_6.32min, 231.0297m/z_4.3min, 395.2428m/z_8.5min, 267.0661m/z_7.73min, 425.1805m/z_4.99min, 337.2032m/z_9.3min, 383.3513m/z_15min, 221.1538m/z_8.15min, 269.2116m/z_10.42min, 327.2166m/z_7.41min, 533.1656m/z_4.43min, 351.1807m/z_7.58min, 395.2037m/z_6.47min, 561.1753m/z_3.3min, 285.2065m/z_7.82min, 297.2423m/z_10.74min, 285.2065m/z_8.13min, 551.1765m/z_3.6min	311.1679m/z_10.51min, 431.2638m/z_7.55min, 271.2274m/z_12.35min, 333.1368m/z_9.45min, 403.1956m/z_4.2min, 389.1087m/z_1.15min, 337.2043m/z_12.49min, 309.1949m/z_9.45min, 309.2039m/z_9.76min, 439.0564m/z_1.79min, 271.2271m/z_10min, 293.2111m/z_10.79min, 173.0816m/z_4.1min, 307.1907m/z_7.68min, 441.2519m/z_11.41min, 209.1179m/z_6.32min, 395.2428m/z_8.5min, 267.0661m/z_7.73min, 425.1805m/z_4.99min, 337.2032m/z_9.3min, 383.3513m/z_15min, 221.1538m/z_8.15min, 269.2116m/z_10.42min, 327.2166m/z_7.41min, 533.1656m/z_4.43min, 351.1807m/z_7.58min, 395.2037m/z_6.47min, 561.1753m/z_3.3min, 285.2065m/z_7.82min, 297.2423m/z_10.74min, 285.2065m/z_8.13min, 551.1765m/z_3.6min
Cover		
<u>forbs</u> *p<0.001* = 26 *p<0.01* = 33 *p<0.05* = 47 total = 106 <u>grasses</u> *p<0.001* = 14 *p<0.01* = 12 *p<0.05* = 37 total = 63	445.1907m/z_3.28min, 291.0874m/z_3.49min, 579.1308m/z_3.64min, 261.0434m/z_3.7min, 623.1629m/z_3.92min, 385.0547m/z_3.97min, 475.1722m/z_4.08min, 615.2199m/z_4.54min, 200.9959m/z_4.77min, 633.2376m/z_4.8min, 441.1079m/z_5.08min, 443.2276m/z_5.16min, 366.0968m/z_5.33min, 351.0115m/z_6.24min, 272.0338m/z_8.4min, 337.2032m/z_9.3min, 511.1454m/z_4.08min, 279.2323m/z_13.23min, 461.1306m/z_4.02min, 393.1711m/z_11.42min, 509.0895m/z_2.04min, 259.1079m/z_3.96min, 737.1919m/z_4.17min, 821.2118m/z_3.6min, 165.0184m/z_3.01min, 219.0869m/z_3.63min,	623.1629m/z_3.92min, 549.3402m/z_5.25min, 681.3819m/z_5.25min, 461.2202m/z_5.99min, 813.428m/z_5min, 377.2265m/z_6.45min, 689.4298m/z_6.88min, 329.2316m/z_7.29min, 325.1998m/z_7.78min, 337.2032m/z_9.3min, 279.2323m/z_13.23min, 393.1711m/z_11.42min, 229.0774m/z_5.35min, 533.3115m/z_6.79min, 303.1513m/z_6.42min, 547.2386m/z_4.38min, 267.0871m/z_3.62min, 469.1666m/z_4.27min, 365.1495m/z_9.47min, 329.2324m/z_7.49min, 671.291m/z_6.56min, 379.2096m/z_9.07min, 365.2304m/z_10.77min, 357.2133m/z_1.3min, 288.9479m/z_7.1min, 311.2219m/z_7.72min,

377.1792m/z_2.89min, 591.1649m/z_3.07min, 319.0859m/z_3.13min, 275.0856m/z_3.51min, 535.2379m/z_3.59min, 493.1943m/z_3.61min, 445.1185m/z_3.86min, 455.1035m/z_4.11min, 635.1613m/z_4.38min, 345.1539m/z_4.68min, 489.197m/z_5.48min, 517.2283m/z_5.85min, 259.0824m/z_6.02min, 445.2418m/z_6.06min, 257.1397m/z_6.29min, 401.124m/z_6.55min, 509.2225m/z_2.72min, 295.0134m/z_6min, 303.1513m/z_6.42min, 547.2386m/z_4.38min, 389.1117m/z_3.09min, 267.0871m/z_3.62min, 469.1666m/z_4.27min, 365.1495m/z_9.47min, 329.2324m/z_7.49min, 339.1991m/z_11.9min, 671.291m/z_6.56min, 379.2096m/z_9.07min, 357.2133m/z_1.3min, 456.2459m/z_2.74min, 281.14m/z_8.95min, 288.9479m/z_7.1min, 311.2219m/z_7.72min, 283.0368m/z_5.63min, 209.0792m/z_4.9min, 403.1956m/z_4.2min, 611.1828m/z_2.64min, 599.1941m/z_8.39min, 295.2269m/z_10.1min, 293.2111m/z_10.79min, 429.1762m/z_4.96min, 381.0951m/z_1.08min, 273.1701m/z_7.68min, 193.0504m/z_3.75min, 237.0557m/z_8.07min, 493.1749m/z_4.4min, 200.9961m/z_4.08min, 295.227m/z_10.35min, 293.2109m/z_9.69min, 283.0461m/z_6.61min, 327.2111m/z_7.58min, 242.1757m/z_6.64min, 355.2085m/z_8.17min, 239.1284m/z_4.91min, 339.1993m/z_12.63min, 227.2013m/z_12.53min, 580.965m/z_7.42min, 343.0515m/z_4.34min, 389.1087m/z_1.15min, 475.0883m/z_4.96min, 480.9715m/z_6.3min, 381.2311m/z_13.44min, 329.2324m/z_6.83min, 201.1129m/z_5.67min, 465.1179m/z_2.92min, 293.2111m/z_9.42min, 525.2182m/z_2.76min, 361.1622m/z_8.15min, 379.1578m/z_10.2min, 585.1615m/z_4.85min, 431.1703m/z_8.05min, 287.2221m/z_11.9min, 438.9672m/z_4.47min, 327.2169m/z_8.34min, 297.1522m/z_9.69min, 339.1995m/z_12.42min, 440.9281m/z_7.05min, 209.0792m/z_4.9min, 403.1956m/z_4.2min, 295.2269m/z_10.1min, 293.2111m/z_10.79min, 429.1762m/z_4.96min, 273.1701m/z_7.68min, 283.0368m/z_5.63min, 193.0504m/z_3.75min, 200.9961m/z_4.08min, 295.227m/z_10.35min, 293.2109m/z_9.69min, 327.2111m/z_7.58min, 242.1757m/z_6.64min, 355.2085m/z_8.17min, 239.1284m/z_4.91min, 339.1993m/z_12.63min, 227.2013m/z_12.53min, 580.965m/z_7.42min, 343.0515m/z_4.34min, 389.1087m/z_1.15min, 480.9715m/z_6.3min, 381.2311m/z_13.44min, 329.2324m/z_6.83min, 201.1129m/z_5.67min, 293.2111m/z_9.42min, 361.1622m/z_8.15min, 379.1578m/z_10.2min, 431.1703m/z_8.05min, 287.2221m/z_11.9min, 438.9672m/z_4.47min, 327.2169m/z_8.34min, 297.1522m/z_9.69min, 339.1995m/z_12.42min, 440.9281m/z_7.05min, 467.0597m/z_3.97min, 327.2168m/z_6.47min, 593.1416m/z_3.6min

	467.0597m/z_3.97min, 327.2168m/z_6.47min, 593.1416m/z_3.6min	
Richness		
forbs	537.1584m/z_4.22min, 137.0244m/z_2.74min,	637.1419m/z_3.67min, 664.1887m/z_3.76min,
p<0.001 = 18	637.1419m/z_3.67min, 821.2118m/z_3.6min,	678.2013m/z_3.81min, 547.2386m/z_4.38min,
p<0.01 = 8	664.1887m/z_3.76min, 525.1612m/z_3.78min,	607.1664m/z_4.67min, 699.3939m/z_4.6min,
p<0.05 = 30	678.2013m/z_3.81min, 379.0093m/z_4.17min,	815.4389m/z_4.83min, 813.4231m/z_5.11min,
total = 56	737.1919m/z_4.17min, 547.2386m/z_4.38min,	567.3263m/z_5.22min, 329.2316m/z_7.29min,
	581.1881m/z_4.72min, 827.3337m/z_4.91min,	431.1703m/z_8.05min, 537.1584m/z_4.22min,
grasses	567.3263m/z_5.22min, 431.1703m/z_8.05min,	359.2433m/z_7.84min, 448.1236m/z_5.1min,
p<0.001 = 18	347.243m/z_8.19min, 461.1318m/z_2.82min,	239.1284m/z_4.91min, 661.3633m/z_6.01min,
p<0.01 = 4	239.1284m/z_4.91min, 267.0871m/z_3.62min,	465.2486m/z_4.88min, 267.0871m/z_3.62min,
p<0.05 = 30	401.1081m/z_2.92min, 253.0503m/z_7.02min,	269.0632m/z_4.06min, 221.0812m/z_7.35min,
total = 52	389.1117m/z_3.09min, 397.0925m/z_8.24min,	588.2609m/z_6.15min, 611.185m/z_5.05min,
	297.0398m/z_7.29min, 611.185m/z_5.05min,	651.2143m/z_3.82min, 129.0553m/z_2.75min,
	597.2157m/z_5.97min, 463.1626m/z_6.08min,	285.2064m/z_7.01min, 353.1941m/z_7.01min,
	651.2143m/z_3.82min, 129.0553m/z_2.75min,	221.1538m/z_8.15min, 277.067m/z_4.92min,
	507.2356m/z_6.17min, 285.2064m/z_7.01min,	329.2324m/z_6.83min, 429.1377m/z_3.26min,
	231.0301m/z_7.39min, 255.0302m/z_8.01min,	467.1615m/z_3.73min, 451.1952m/z_4.57min,
	353.1941m/z_7.01min, 221.1538m/z_8.15min,	553.3379m/z_4.68min, 231.1216m/z_4.76min,
	277.067m/z_4.92min, 403.1874m/z_4.13min,	659.3782m/z_4.89min, 315.0521m/z_4.97min,
	329.2324m/z_6.83min, 369.0952m/z_8.75min,	381.0967m/z_5.34min, 617.2243m/z_5.73min, 297.04m/z_6.95min,
	269.0851m/z_9.66min, 429.1377m/z_3.26min,	521.3466m/z_7.03min, 649.3931m/z_7.35min,
	467.1615m/z_3.73min, 267.0304m/z_9.64min,	295.0239m/z_8.07min, 305.1741m/z_8.2min,
	449.1299m/z_2.3min, 469.1666m/z_4.27min,	469.1666m/z_4.27min, 249.1136m/z_6.6min,
	242.1757m/z_6.64min, 653.2065m/z_6.17min,	242.1757m/z_6.64min, 339.1993m/z_12.63min,
	627.1571m/z_5.51min, 299.0213m/z_5.23min,	281.0665m/z_3.75min, 359.2095m/z_9.76min,
	493.1756m/z_4.14min, 589.0386m/z_6.18min,	449.1298m/z_2.23min, 439.2531m/z_6.15min,
	507.2076m/z_3.72min, 339.1993m/z_12.63min,	361.1622m/z_8.15min
	281.0665m/z_3.75min, 449.1298m/z_2.23min,	
	439.2531m/z_6.15min, 361.1622m/z_8.15min	
Shannon		
forbs	547.2386m/z_4.38min, 415.1494m/z_5.09min,	547.2386m/z_4.38min, 643.3536m/z_5.41min,
p<0.001 = 9	431.1703m/z_8.05min, 537.1584m/z_4.22min,	549.3397m/z_5.78min, 547.3225m/z_6.19min,
p<0.01 = 15	213.0121m/z_4.88min, 709.3784m/z_5.89min,	377.2265m/z_6.45min, 547.3261m/z_7.39min,
p<0.05 = 30	461.1318m/z_2.82min, 297.0398m/z_7.29min,	867.4731m/z_7.86min, 431.1703m/z_8.05min,
total = 54	267.0871m/z_3.62min,	

<u>grasses</u> *p<0.001* = 12 *p<0.01* = 66 *p<0.05* = 32 total = 110	325.1835m/z_11.45min, 221.1538m/z_8.15min, 597.2157m/z_5.97min, 239.1284m/z_4.91min, 353.1941m/z_7.01min, 473.1962m/z_5.42min, 397.0925m/z_8.24min, 489.16m/z_3.23min, 206.0218m/z_4.44min, 739.2449m/z_5.16min, 559.1567m/z_5.66min, 525.2182m/z_2.76min, 511.1875m/z_4.49min, 417.1743m/z_5.35min, 625.1776m/z_5.21min, 361.1622m/z_8.15min, 469.1666m/z_4.27min, 487.1812m/z_4.84min, 995.2638m/z_4.25min, 285.2064m/z_7.01min, 313.2374m/z_8.53min, 480.9715m/z_6.3min, 297.2426m/z_10.81min, 580.965m/z_7.42min, 525.3063m/z_4.42min, 243.1597m/z_7.72min, 191.0346m/z_4.29min, 449.1298m/z_2.23min, 269.0851m/z_9.66min, 507.2076m/z_3.72min, 204.1236m/z_1.7min, 429.1762m/z_4.96min, 171.066m/z_4.24min, 242.1757m/z_6.64min, 236.105m/z_8.15min, 524.9613m/z_6.3min, 389.1117m/z_3.09min, 439.2531m/z_6.15min, 269.2116m/z_10.42min, 417.1432m/z_3.12min, 401.1447m/z_3.46min, 296.9923m/z_3.29min, 339.199m/z_12.23min, 451.2167m/z_5.73min, 351.1662m/z_5.88min	537.1584m/z_4.22min, 213.0121m/z_4.88min, 325.1997m/z_7.09min, 267.0871m/z_3.62min, 325.1835m/z_11.45min, 221.1538m/z_8.15min, 239.1284m/z_4.91min, 353.1941m/z_7.01min, 511.1875m/z_4.49min, 417.1743m/z_5.35min, 285.206m/z_8.94min, 361.1622m/z_8.15min, 469.1666m/z_4.27min, 487.1812m/z_4.84min, 285.2064m/z_7.01min, 313.2374m/z_8.53min, 480.9715m/z_6.3min, 297.2426m/z_10.81min, 580.965m/z_7.42min, 525.3063m/z_4.42min, 243.1597m/z_7.72min, 191.0346m/z_4.29min, 249.1136m/z_6.6min, 449.1298m/z_2.23min, 204.1236m/z_1.7min, 373.1393m/z_4.65min, 487.1502m/z_5.44min, 377.2061m/z_6.05min, 429.1762m/z_4.96min, 171.066m/z_4.24min, 242.1757m/z_6.64min, 236.105m/z_8.15min, 329.2323m/z_7.63min, 524.9613m/z_6.3min, 439.2531m/z_6.15min, 269.2116m/z_10.42min, 417.1432m/z_3.12min, 401.1447m/z_3.46min, 296.9923m/z_3.29min, 339.199m/z_12.23min, 451.2167m/z_5.73min, 351.1662m/z_5.88min

c) Percentage of correlated compounds per predictor

Metabolite polarity	Growth form	Predictor	LUI	soil	climate	LNH	Total
Semi-polar	forb	significantly affected compounds [%]	19.37	52.82	30.28	34.15	65.14
	grass		23.94	52.11	30.28	34.86	69.72
polar	forb	significantly affected compounds [%]	6.05	12.20	8.27	6.15	21.90
	grass		4.68	9.94	7.14	4.68	17.49

Supplementary Table 6: Putative classification of the significantly species specific semi-polar metabolites. The table contains no. of metabolites, species, retention time (RT), m/z value, p value for significance of occurrence in single species, type of adduct, putative elemental composition, putative classification, observed neutral losses and fragment ions upon CID. The identifier ions (see Supplementary Table 7) were marked in bold. * = precursor ion in addition to fragment ions and neutral losses used for annotation of compound. All measurements were obtained in negative ionisation mode.

No.	Species	RT [min]	m/z	p -value	Type of adduct	Putative Elemental composition	Putative Class	Fragment ions and neutral losses detected in CID (identifier ions in bold)	
								neutral losses	fragment ion
1	*A. millfefolium*	2.6	409.0444	$1.62x10^{-14}$	[M-H]-	C14H18O12S	Unclassified, sulfate/phosphate residue[1]	212.0004, 198.054, 227.0276, 312.0816, 210.9981, 218.9993, 312.1053, 145.0984, 284.0586, 262.9787, 339.2814, 208.0553, 197.0461, 32.1573, 50.9537	197.0453, 210.9946, 182.0217, **96.9568**, 198.0467, 190.0527, 96.9336, 263.9458, 124.9758, 146.063, 69.7617, 200.9865, 74.724, 167.0497, 185.071

2	*A. millfefolium*	3.12	619.1862	$1.05x10^{-08}$	[M-H]-	C26H36O17	Hydroxycinnamic acid, glycosylated	474.1642, 502.1571, 278.0764, 506.1669, 473.1586, 500.1504, 488.145, 440.1207, 476.1574, 357.1224, 296.1033	**145.0209**, 117.0335, 341.1108, **113.0231**, 146.0322, 119.0359, 131.0378, **179.0672**, 143.0333, 262.0666, **323.0905**
3	*A. millfefolium*	3.46	515.139	$1.11x10^{-14}$	[M-H]-	C22H28O14	Phenylpropanoid, Coumarin derivative[1]	309.1171, 294.0955, 324.1371, 308.1123	206.0189, 221.0502, 190.996, **207.0349**
4	*A. millfefolium*	3.72	577.2099	$6.40x10^{-12}$			Unclassified		
5	*A. millfefolium*	3.94	607.1697	$1.12x10^{-07}$	[M-H]-	C20H36N2O17S	Unclassified	382.1073, 397.132, 381.1031	225.0533, 210.031, 226.0558, 223.0388
6	*A. millfefolium*	4.06	581.1858	$1.44x10^{-06}$			Unclassified		
7	*A. millfefolium*	4.46	221.0456	$1.12x10^{-07}$			Unclassified		
8	*A. millfefolium*	4.6	475.2175	$1.10x10^{-06}$			Unclassified		

9	*A. millfefolium*	4.77	509.2194	$6.54x10^{-15}$	[M-H]-	C22H38O13	Glycoside, Hydroxycarbonic acid residue	178.0517, 276.154, 396.1957, 360.1758, 318.1651, 378.185, 348.1771, 177.0455, 384.1958, 46.0095, 366.1882, 408.1954, 258.148	331.1765, 233.0658, **113.0231**, 149.0453, **191.0543**, 131.0378, 161.0446, 332.1799, 125.0251, 463.2163, 143.0333, **101.0229**, 251.0699
10	*A. millfefolium*	4.82	503.1812	$5.56x10^{-17}$	[M-H]-	C22H32O13	Glycoside	355.1187, 340.095, 378.1496, 390.151, 354.1195, 270.1076	148.043, **163.0702**, 125.0251, **113.023**1, 149.0602, 233.0658
11	*A. millfefolium*	4.84	517.1912	$3.15x10^{-22}$	[M-H]-	C22H32O11	Glycoside[1]	369.1435, 354.1195, 270.1182, 374.1608	148.043, **163.0702**, 247.0788, 143.0333
12	*A. millfefolium*	4.89	547.2022	$4.70x10^{-24}$	[M-H]-	C24H36O14	Hydroxycinnamic acid	354.1195, 369.1435, 422.1791, 384.1565, 353.1128, 300.1209	193.0852, 178.061, 125.0251, **163.0379**, 194.0828, 247.0788, **179.0672**

13	*A. millfefolium*	4.9	689.2999	$2.15x10^{-16}$	[M-H]-	C30H48N3O15	Unclassified	502.2034, 564.2056, 501.2007	187.1029, 125.0948, 188.1097
14	*A. millfefolium*	5.02	487.1807	$1.30x10^{-16}$	[M-H]-	C22H32O12	Glycoside[1]	374.1504, 372.1714	113.0231, 115.0009, *116.0109,* ***175.0257***
15	*A. millfefolium*	5.13	469.1704	$1.34x10^{-18}$	[M-H]-	C17H30N2O13	Unclassified	252.0464, 208.0553, 251.0431	217.1273, 261.1183, 218.127
16	*A. millfefolium*	5.37	452.1914	$1.26x10^{-14}$	[M-H]-	C23H33O7S	Glycoside, sulfated/phosphorylated	355.2365, 93.7395, 339.1698, 185.1303, 272.2962, 240.113, 232.1912, 322.1442, 99.0917, 172.1712, 279.1086, 119.1266, 197.1374, 80.4742, 133.0596	**96.9568**, **113.0231**, 358.4508, 267.0669, 179.8941, 212.0831, 130.0557, 219.9961, 173.0896, 280.0169, 353.0986, 333.0583, 255.0523, 157.0404, 223.0388
17	*A. millfefolium*	5.39	505.225	$3.31x10^{-12}$	[M-H]-	C21H42O9S	Glycoside, sulfated, hydroxycarbonic acid	392.2027, 272.1498, 380.2012, 344.178, 346.1952, 404.2041, 314.1683,	**113.0231**, 233.0658, 125.0251, **161.0446**, 159.0277, **101.0229**, **191.0543**,

								354.1195, 362.2023, 342.1676, 376.2111	151.1072, 143.0333, 163.0568, 129.0167, *119.0359*
18	*A. millfefolium*	5.61	493.2278	$1.42x10^{-06}$	[M-H]-	C22H38O12	Glycoside[1]	178.0517, 46.0095, 332.1813, 177.0455	315.1787, 447.221, **161.0446**, 316.1873
19	*A. millfefolium*	5.88	273.1701	$7.27x10^{-10}$	[M-H]-	C9H26N2O7	Unclassified	158.1232, 18.0104, 144.1132, 128.1194, 80.009, 134.0531, 146.0581, 98.0194, 118.0644, 36.0189, 162.1357	273.1682, 115.039, 255.1592, 129.0568, 145.0603, 193.1583, 139.1142, 127.1175, 175.1505, 155.1104, 237.1528, 111.0432
20	*A. millfefolium*	5.91	177.0552	$1.30x10^{-10}$			Unclassified		
21	*A. millfefolium*	6.1	508.2429	$1.30x10^{-10}$	[M-H]-	C49H78O22	Glycoside	395.2192, 100.9131, 299.1167, 255.1396, 301.1845, 323.1252	**113.0231**, 407.3307, 209.1159, 253.1134, **207.0689**, 185.1131
22	*A. millfefolium*	8.77	645.4327	$1.10x10^{-06}$	[M-H]-	C36H60N3O7	Unclassified	452.3543, 334.2151, 438.3361, 444.3181,	193.0852, 311.228, 207.0908, 201.1212, 293.2057,

								352.2271, 370.2336, 474.3344, 460.3196	275.2032, 171.1069, 185.1131
23	*A. millfefolium*	8.89	286.1802	$1.26x10^{-14}$	[M-H]-	C18H25NO2	Unclassified	120.0572, 150.1077	286.1903, 166.1256, 136.0773
24	*G. mollugo*	2.58	393.1758	$5.26x10^{-15}$	[M-H]-	C16H28O8	Flavonoid	164.255, 283.5116, 43.1881, 292.5814, 242.1361, 245.0963, 256.1353, 192.1184, 204.23, 183.1512, 263.3766, 216.165, 103.2068, 154.241, 222.5841	228.9224, 109.6651, 349.9885, 100.5952, **151.0413**, **137.0259,** 201.0544, 148.0824, 188.9466, 210.031, 129.8001, 289.9826, […], 170.5926, 209.1159
25	*G. mollugo*	3.01	519.1702	$5.86x10^{-13}$	[M-H]-	C29H28O9	Hydroxycinnamic acid	340.095, 398.1402, 355.1187, 373.1277, 354.1518, 358.1025, 397.132	**179.0672**, 121.0303, **164.0454**, 146.0322, 165.0216, 161.0601, 122.0342, **113.0231**

26	*G. mollugo*	3.05	391.1576	$1.48x10^{-13}$	[M-H]-	C17H28O10	Hydroxycinnamic acid, glycosylated	198.1164, 190.171, 168.1308, 272.1252, 280.0771, 76.1027, 131.0851, 160.0235, 62.0868, 75.0328, 343.5843, 189.0801, 278.1277, 174.0308, 144.1132	193.0497, 200.9865, 223.0388, 119.0359, 111.0818, 315.0499, 260.0687, 231.1328, 47.5766, 316.1281, 329.0818, **113.0231**, […], 217.1273, 199.017, **178.0276**
27	*G. mollugo*	3.09	165.0192	$7.81x10^{-14}$	[M-H]-	C6H4N3O3	Unclassified, aromatic acid[1]	**43.989**, 23.0548	121.0303, 141.9658
28	*G. mollugo*	3.36	391.1601	$8.05x10^{-16}$	[M-H]-	C24H26NO2S	Unclassified	194.1111, 193.1121, 238.1003, 189.1605, 190.171, 136.0447, 154.9775, 156.0962, 209.1341, 314.9471, 163.0483, 239.2411, 216.0961, 37.0474, 289.9756	197.0453, 198.0467, 153.0575, 202.0021, 200.9865, 255.1176, 236.1812, 235.0632, 76.2137, 182.0217, 228.1068, 151.9285, 354.1048, 101.1812, 175.0619

29	*G. mollugo*	3.39	377.1791	$1.83x10^{-08}$	[M-H]-	C17H30O9	Flavonoid, Kaempferol derivative[1]	311.2672, 78.0308, 146.1246, 230.1554, 162.1357, 176.1981, 151.1389, 176.1042, 161.9119, 229.146, 222.4788, 186.1652, 103.1389, 226.2397, 183.1034	65.9136, 299.1498, 231.0557, 147.0334, 215.0327, 200.9865, 226.0323, 201.0772, 148.043, 215.2684, 154.7016, 191.0163, 274.0467, 150.9364, 136.0447, 194.0828, […], **255.0294**
30	*G. mollugo*	3.4	581.1875	$2.57x10^{-10}$	[M-H]-	C30H32NO11	Unclassified	369.1011, 368.097, 381.1328	212.0831, 213.0898, 200.0544
31	*G. mollugo*	3.57	507.2064	$4.60x10^{-11}$			Unclassified		
32	*G. mollugo*	3.73	401.111	$9.32x10^{-11}$			Unclassified		
33	*G. mollugo*	3.93	527.136	$9.38x10^{-19}$	[M-H]-	C21H26N3O13	Unclassified	325.1092, 324.1021, 314.0802	202.0256, 203.0356, 213.0539

34	*G. mollugo*	4.13	377.1808	$1.49x10^{-15}$	[M-H]-	C18H26N4O5	Unclassified	160.0532, 120.1355, 89.0959, 70.1055, 133.0881, 161.1321, 190.1113, 146.0843, 265.0856, 162.0547, 132.0699, 176.1981, 258.1007, 88.0964, 183.0566	217.1273, 257.0462, 288.0859, 244.0925, 307.0801, 216.0419, 231.1006, 187.0653, 112.096, 215.124, 245.1126, […], 93.0304, […], 205.1235
35	*G. mollugo*	4.15	527.104	$4.69x10^{-14}$	[M-H]-	C23H20N4O11	Unclassified	325.0858, 324.0765	202.0256, 203.0356
36	*G. mollugo*	4.37	389.1236	$1.24x10^{-12}$	[M-H]-	C20H22O8	Hydroxycinnamic acid	224.0607, 196.0708, 239.0878, 107.0383, 255.0854, 211.087, 209.0788, 194.0539, 150.0549, 267.0877, 225.0701, 139.0619, 195.0678, 254.0869, 135.0641	165.0558, **193.0497,** 150.038, 282.0876, 134.0357, 178.0276, 180.042, 195.0709, 239.0709, 122.0342, **164.0454**, 250.0566, 194.0551, **135.0437**, 254.0558, 121.0303, 149.0142, […],**151.0413**

37	*G. mollugo*	4.37	389.1236	$1.24x10^{-12}$	[M-H]-		C20H22O8	Hydroxycinnamic acid	224.0723, 255.0854, 196.0708, 239.0878, 107.0383, 211.0984, 240.0705, 150.0549, 268.096, 106.0398, 122.0631, 195.0678, 139.0619, 209.0788, 225.0701	165.0558, 134.0357, **193.0497**, 282.0876, **178.0276**, 149.0602, 239.0709, 121.0303, 267.0669, 194.0551, 250.0566, **164.0454**	150.038, 283.1, 180.042,
38	*G. mollugo*	4.98	507.2066	$1.74x10^{-07}$	[M-H]-, [M-H-H2O]-		C22H36O13	Flavonoid, glycosylated	305.1819, 270.1357, 223.1699, 208.1579, 305.1145, 346.166, 270.2334, 360.1568, 140.1467, 276.1678, 296.0789, 284.2023, 268.167, 376.1676, 149.2726	202.0256, 237.0561, **284.0313**, 299.0483, 202.0964, **161.0446**, 147.0459, 236.9701, 211.1362, 231.0299, 367.0587, 239.0357, 357.9329	[…],

39	*G. mollugo*	5.05	313.0357	$1.26x10^{-06}$	[M-H]-	C14H8N3O6	Unclassified, imin	87.0185, **43.9987**, **59.0156**	226.0323, 269.044, 254.0167, 227.0341
40	*G. mollugo*	5.15	577.1552	$2.01x10^{-14}$	[M-H]-	C27H30O14	Hydroxycinnamic acid, glycosylated	356.0863325.1092 324.1021340.0954 16.1043464.13224 46.1132	221.065, 252.0408, 253.0499, 237.0561, **161.0446**, **113.0231**, 236.0486, 131.0378, *222.0664,* ***101.0229****,* *224.0455,* ***179.0672****,* ***193.0497***
41	*G. mollugo*	5.2	507.2071	$9.38x10^{-19}$	[M-H]-	C17H38N3O12S	Unclassified	305.1819, 304.1743, 338.0845, 306.1887, 252.1182, 58.9521, 375.1782, 315.1467, 376.2111, 289.1956, 310.1393, 219.0976, 192.1629, 294.1511, 379.193	202.0256, 203.0356, 169.1139, 201.0119, 255.0892, 448.2562, 132.0295, 192.0649, 131.0014, 197.0713, 218.0128, 213.0539, 58.0232, 315.0499, 128.0193

42	*G. mollugo*	5.49	547.1437	$1.40x10^{-17}$	[M-H]-	C24H26N3O12	Unclassified	295.1038, 294.0955, 297.0948, 310.0917, 278.1277, 323.1025, 282.1465, 282.0569, 334.062, 309.0735, 292.1415, 222.0919, 244.1754, 328.0758, 308.0726	252.0408, 253.0499, 250.0566, 237.0561, 269.0075, 224.0455, 264.9976, 265.092, 213.0898, 238.0726, 255.0025, 325.0584, 219.072, 302.9707, 239.0709
43	*G. mollugo*	5.74	419.134	$1.82x10^{-13}$	[M-H]-	C21H24O9	Flavonoid[1]	207.0486, 163.0633, 206.04, 192.0948, 213.0769	212.0831, 256.0748, 213.0898, 227.0341, 206.0671, 200.0544, **255.0294**
44	*G. mollugo*	5.79	415.1013	$1.88x10^{-08}$	[M-H]-	C21H20O9	Flavonoid[1]	120.0772, 94.0907, 178.0425, 163.0633, 160.0735, 119.072, 161.0716	295.0236, 321.0029, 237.0561, 252.0408, **255.0294**, 254.0316, 209.1159, 268.0375, 253.0499

45	*G. mollugo*	5.89	669.2385	$2.02x10^{-12}$	[M-H]-	C18H44N2O21	Unclassified	385.1305, 384.1321, 370.1145	284.1041, 285.1101, 299.1222
46	*G. mollugo*	6.03	337.1075	$1.49x10^{-11}$	[M-H]-	C20H20O6	Polyketide[1]	30.0455, 58.0429, 46.0391, 29.0407, 15.0225, 31.0211, 45.0353, 71.0265, 86.0358, 43.0172, **42.0168**	307.0609, 279.0696, 291.0655, 322.0769, 306.0859, 292.0747, 266.083, 251.0699, 294.0872, 295.1001
47	*G. mollugo*	6.08	253.0504	$1.73x10^{-21}$	[M-H]-	C20H20O6	Polyketide[1]	43.0172, **42.0168**	210.031, 253.0499, *211.0398, 223.0388*
48	*G. mollugo*	6.13	269.0452	$2.57x10^{-10}$			Unclassified		
49	*G. mollugo*	6.25	515.1521	$9.38x10^{-19}$	[M-H]-	C14H31N5O13	Unclassified, methoxylated	231.0401, 286.0994, 290.0612, 308.0726, 230.0483, 274.095	284.1041, 229.0505, 225.0892, 207.0908, 285.1101, 241.0482
50	*G. mollugo*	6.44	253.0508	$2.38x10^{-11}$	[M-H]-	C15H10O4	Polyketide[1]	43.0172, **42.0168**	210.031, 253.0499, 211.0398
51	*G. mollugo*	6.45	267.0298	$1.51x10^{-09}$	[M-H]-	C6H14N5OS3	Unclassified	**43.989**, 71.9859, 42.9854	223.0388, 195.0451, 224.0455

52	*G. mollugo*	6.8	285.0405	$9.11x10^{-08}$	[M-H]-	C15H10O6	Flavonoid	44.9997, **46.0007**, 72.9917, **43.9987**, **27.9962**, 88.9936, 15.0225	**285.0426**, 240.0384, **239.0357**, 212.0454, 241.0482, 257.0462, 196.0531, 270.0217
53	*G. mollugo*	6.81	489.1752	$5.86x10^{-13}$	[M-H]-	C12H34N4O14S	Unclassified	205.0691, 260.13, 264.0868, 282.0933, 248.1248, 204.066, 263.0795, 220.0928, 281.0887	284.1041, 229.0505, 225.0892, 207.0908, 241.0482, 285.1101, 226.0911, 269.0837, 208.0806
54	*G. mollugo*	6.9	283.0257	$1.83x10^{-08}$			Unclassified		
55	*G. mollugo*	7.17	381.0621	$9.11x10^{-08}$	[M-H]-	C9H22N2O10S2	Unclassified	**43.989**, 42.9854, 61.9988, 71.9859, 18.0104, 99.0465, 139.0329, 87.9783, 70.9819, 127.041, 149.9638, 94.8676, 143.0218, 71.897, 18.1036	337.0782, 338.0735, 319.0581, 309.0732, 363.0423, 282.0119, 242.0326, 293.077, 310.0785, 254.0167, 231.1006, 286.1903, 238.0404, 309.1665, 362.9718

56	*G. mollugo*	7.17	491.1549	$1.14x10^{-20}$	[M-H]-	C23H26O9	Hydroxycinnamic acid[1]	208.0553, 267.0696, 268.0747, 209.0668, 240.0845, 282.0933, 266.0616, 284.0706, 207.0486	283.1, 224.0745, **223.0655**, 282.0876, 251.0699, 209.0613, 225.0892, 207.0908, 284.1041
57	*G. mollugo*	7.27	239.0351	$6.32x10^{-16}$	[M-H]-	C6H14N2O7S	Unclassified	**27.9962**, 29.0025, 81.0227, 77.9057, 26.991, 81.4106, 42.9854	211.0398, 210.031, 238.0273, 158.0214, 161.1349, 238.1167
58	*G. mollugo*	7.27	501.0589	$2.20x10^{-10}$	[M-H]-	C20H14N4O12	Terpene, Sesqueterpene[1]	262.0255, 261.0287	**239.0357**, 240.0384
59	*G. mollugo*	7.63	299.0537	$2.37x10^{-11}$	[M-H]-	C16H12O6	Flavonoid, Kaempferol derivative	15.0225, **44.0226**, 14.0188	**284.0313**, **255.0294**, **285.0426**
60	*G. mollugo*	7.73	267.0661	$4.26x10^{-22}$	[M-H]-	C16H12O4	Polyketide	43.0172, 15.0225, **42.0168**	224.0455, 267.0669, 252.0408, 225.0533
61	*G. mollugo*	7.74	224.047	$1.88x10^{-08}$			Unclassified		
62	*G. mollugo*	8.57	253.0481	$2.57x10^{-10}$			Unclassified		
63	*G. mollugo*	9.67	283.0247	$1.38x10^{-13}$	[M-H]-	C16H4N4O2	Terpene, Sesqueterpene	**43.989**, 87.9783, 42.9854, 71.9859	**239.0357**, 195.0451, 240.0384, 211.0398

64	*G. mollugo*	9.84	367.1184	$9.11x10^{-08}$	[M-H]-	C21H20O6	Unclassified	70.0755, 58.0759, 57.074, 15.0225, 69.0738	297.0412, 309.0401, 310.0321, 352.0935, 298.047
65	*G. verum*	8.24	397.0925	1.02x10-06	[M-H]-	C21H18O8	Flavonoid	102.0702, 114.0704, 76.1027, **59.0156**, 101.0738, 18.0104	295.0236, **283.0318,** 321.0029, 338.0735, 379.0867, **284.0313**
66	*Galium spp.*	2.03	417.1033	$4.12x10^{-08}$	[M-H]-	C17H22O12	Hydroxycinnamic acid	224.052, 213.0956, 256.0736, 284.0706, 294.0542, 268.0376, 198.0706, 290.0612, 212.0888, 206.04, 223.0476, 316.078, 266.0616, 197.0673, 255.074	**193.0497**, 204.0101, **161.0265**, 133.0335, 123.0439, 149.0602, 219.0272, 127.0411, 205.0178, 211.0615, 194.0551, **101.0229**, **151.0413**, 220.0415, 162.035
67	*Galium spp.*	2.67	449.1295	$6.13x10^{-31}$	[M-H]-	C17H24O11	Hydroxycinnamic acid, glycosylated	310.0799, 208.0553, 240.0705, 348.0897, 206.04, 258.0849, 322.0782, 238.0638, 254.0532,	139.0405, 241.072, 209.0456, **101.0229**, 243.0919, 191.0368, 127.0411, 211.0615, 195.0709,

								226.0665, 324.1021, 328.0912, 338.1138, 309.0735	**223.0655**, 125.0251, 121.0303, 111.0098
68	*Galium spp.*	2.97	355.1012	$7.48x10^{-14}$	[M-H]-	C16H20O9	Hydroxycinnamic acid	162.0547, 221.062, 206.04, 177.0753, 220.0571, 188.062	**193.0497**, 134.0357, 149.0602, **178.0276**, **135.0437**, 167.0339
69	*Galium spp.*	3.25	509.218	$2.80x10^{-12}$			Unclassified		
70	*Galium spp.*	3.4	355.1026	$4.79x10^{-09}$	[M-H]-	C23H16O4	Hydroxycinnamic acid, glycosylated	**162.0418**, 221.062, 177.0753, 206.04, 161.0481, 241.0424, 59.9545, 247.0824	**193.0497**, 134.0357, **178.0276**, 149.0602, 194.0551, 114.0562, 295.1493, 108.0202, 249.0584, […], 151.1072
71	*Galium spp.*	3.8	577.2098	$2.61x10^{-35}$	[M-H]-	C17H40NO20	Terpene, Sesqueterpene[1]	464.2245, 338.1698, 339.1795, 275.9658, 460.2774, 338.097, 378.167, 143.0172, 361.1723, 380.1574,	112.9827, **239.0357**, 238.0273, 301.245, 116.9292, 239.1014, 199.043, 216.0419, 534.193, 197.0453, 170.8584, 335.089,

								406.3482, 242.1173, 380.078, 66.462, 643.989	197.1306, 510.744, 153.0575
72	*Galium spp.*	3.82	499.1984	$4.79x10^{-09}$	[M-H]-	C13H34N5O15	Unclassified	258.1194, 281.1192, 440.1816, 168.0139, 128.2059, 127.9336, 259.9124, 404.9913, 298.1503, 355.1484, 258.9818, 254.841, 277.1299, 167.7446, 337.269	241.072, 218.0722, 59.0104, 331.1765, 370.9866, 94.1978, 371.2544, 239.2731, 201.0544, 144.0525, 222.0664, 240.2049, 244.3515, 497.2539, 161.9298
73	*Galium spp.*	4.09	509.223	$5.55x10^{-11}$	[M-H]-	C22H38O13	Glycoside	178.0425, 348.1771, 276.154, 177.0455, 396.1957, **46.0007**	331.1765, **161.0446**, 233.0658, 332.1799, **113.0231**, 463.2163, *149.0453*

74	*Galium spp.*	4.31	521.2021	$2.08x10^{-13}$	[M-H]-	C26H34O11	Hydroxycinnamic acid	192.0659, 346.1264, 191.0724, 322.1811, 282.1649, 299.1693, 318.1812, 319.2001, 319.1666, 251.0431, 402.1568, 256.3123, 185.0876, 231.7082, 306.1406	329.1413, 175.0776, 330.1251, 199.017, 239.0357, […], **207.0689**, […], **151.0413**, […], **179.0368**
75	*Galium spp.*	4.42	655.2235	$2.04x10^{-35}$	[M-H]-	C16H43N4O18P	Unclassified	371.1161, 370.1145, 277.1299	284.1041, 285.1101, 378.0999
76	*Galium spp.*	4.53	547.2385	$3.86x10^{-12}$	[M-H]-	C25H40O13	Glycoside	434.2131, 338.0845, 337.0776, 176.0262, 118.0197, 372.2099	**113.0231**, 209.1516, 371.2055, 429.2187, **175.0257**
77	*Galium spp.*	4.77	535.1165	$1.19x10^{-14}$	[M-H]-	C12H32N4O13S3	Unclassified	333.0777, 332.0725, 322.0542	202.0256, 203.0356, 213.0539
78	*Galium spp.*	4.78	467.122	$2.26x10^{-16}$	[M-H]-	C11H26N5O13S	Unclassified	265.0953, 264.0868	202.0256, 203.0356

79	*Galium spp.*	4.95	563.1403	$5.13x10^{-34}$	[M-H]-	C14H36N4O13S3	Unclassified	312.1053, 294.0955, 311.1003, 326.0809, 298.0884, 293.0893	251.0354, 269.044, 252.0408, 237.0561, 265.051
80	*Galium spp.*	5.01	533.1317	$4.79x10^{-09}$	[M-H]-	C12H32N5O14S2	Terpene, Sesqueterpene[1]	294.0955, 293.0893	**239.0357**, 240.0384
81	*Galium spp.*	5.07	625.1763	$1.59x10^{-19}$	[M-H]-	C25H36N6O7P2S	Unclassified	369.1011, 413.0935, 412.0863, 368.097	256.0748, 212.0831, 213.0898, 257.0826, *227.0341*
82	*Galium spp.*	5.16	609.1806	$3.99x10^{-10}$	[M-H]-	C28H34O15	Glycoside	488.145, 340.1301, 478.1305, 460.1193, 342.1139, 358.1453, 496.161, 418.1303, 487.1496, 495.1459, 402.1869, 353.1021, 356.0971, 338.0845, 381.1031	121.0303, 269.044, 131.0378, 149.0602, 267.0669, 251.0354, ***113.0231,*** *[…], 271.1001, […], 97.0278, […],* ***161.0446***
83	*Galium spp.*	5.23	299.0213	$7.46x10^{-19}$	[M-H]-	C15H8O7	Flavonoid	**43.9987**, 18.0104, 42.9854, 17.0123, 29.9765	**255.0294**, 281.0195,

									256.0349, 282.0119, 269.044
84	*Galium spp.*	5.29	359.1494	$1.49x10^{-23}$	[M-H]-	C20H24O6	Terpene / Hydroxycinnamic acid	184.072, 199.0918, 167.0673, 30.0082, 181.0848, 166.0672, 182.0937, 151.0681, 108.1096	175.0776, 160.051, 192.084, 329.1413, 178.061, 193.0852, 177.0541, 208.0806, 251.0354, […], *137.0619, 159.0451,* ***239.0357****, 281.0676,* ***179.0672***
85	*Galium spp.*	5.29	595.1663	$3.17x10^{-24}$			Unclassified		
86	*Galium spp.*	5.38	275.0919	$1.09x10^{-32}$	[M-H]-	C15H16O5	Unclassified	88.0516, 87.0518, 116.0505, 18.0104	187.0368, 188.0465
87	*Galium spp.*	5.47	595.1663	$2.93x10^{-36}$	[M-H]-	C33H28N2O9	Unclassified	339.0913, 383.0826, 338.0845, 368.1348	256.0748, 212.0831, 257.0826, 227.0341
88	*Galium spp.*	5.62	431.0977	$8.50x10^{-11}$	[M-H]-	C21H20O10	Hydroxycinnamic acid	266.0421, 238.0464, 282.0413, 297.0632, 324.0476, 284.0586, 265.0419	165.0558, **193.0497**, 149.0602, 134.0357, 107.0499, 147.0459, *112.9827,* ***178.0276***

89	*Galium spp.*	5.73	463.1281	$9.23x10^{-32}$	[M-H]-	C20H22N3O10	Unclassified	207.0486, 251.0431, 250.0348, 206.04, 236.0892	256.0748, 212.0831, 213.0898, 257.0826, 227.0341
90	*Galium spp.*	5.84	547.1453	$6.99x10^{-23}$	[M-H]-	C26H28O13	Unclassified	294.0955, 293.0893	253.0499, 254.0558
91	*Galium spp.*	6.17	653.2065	$1.75x10^{-13}$	[M-H]-	C25H38N2O18	Unclassified	370.1145, 369.1113	283.1, 284.1041
92	*Galium spp.*	6.21	239.0349	$7.46x10^{-19}$	[M-H]-	C15H4N4	Terpene, Sesqueterpene	**27.9962**, **43.989**, 26.991, 29.0025	**239.0357**, 211.0398, 195.0451
93	*Galium spp.*	6.24	447.1654	$1.18x10^{-34}$	[M-H]-	C20H27N5O5P	Unclassified	163.0633, 222.0789, 218.1138, 240.0845, 206.122, 162.0547	284.1041, 225.0892, 229.0505, 207.0908, 241.0482, 285.1101
94	*Galium spp.*	6.6	267.0297	$6.76x10^{-25}$	[M-H]-	C15H8O5	Unclassified		
95	*Galium spp.*	6.92	269.0454	$9.75x10^{-29}$	[M-H]-	C15H10O5	Unclassified	18.0104, 17.0123	251.0354, 252.0408
96	*Galium spp.*	7.02	253.0503	$1.39x10^{-27}$			Unclassified		
97	*Galium spp.*	7.2	313.0709	$4.12x10^{-08}$	[M-H]-	C17H14O6	Flavonoid, Kaempferol derivative	**30.0455**, 15.0225, 58.0429, 29.0407	**283.0318**, 298.047, **255.0294**, **284.0313**, 313.0576
98	*Galium spp.*	7.29	447.1655	$3.07x10^{-26}$	[M-H]-	C24H24N4O5	Unclassified	163.0633, 218.1138,	284.1041, 229.0505,

								240.0845, 222.0789, 206.1074, 162.0547,	239.075	207.0908, 225.0892, 241.0482, 285.1101, 208.0806
99	*Galium spp.*	7.57	311.0559	$3.99x10^{-10}$	[M-H]-	C17H12O6	Polyketide[1]	43.0172, 73.0329	**42.0168**,	268.0375, 311.0516, 238.0273 269.044,
100	*Galium spp.*	7.72	269.0483	$6.25x10^{-13}$	[M-H]-	C16H6N4O	Unclassified, imin, aliphatic acid	15.0225, **46.0007**, **43.0172**, 44.9997, 58.0101, 29.0769, 29.0025, 73.9989	43.989, **27.9962**, 18.0104, **59.0156**, 71.9859, 72.9917, 26.991,	254.0167, 225.0533, 223.0388, 241.0482, 226.0323, 251.0354, 224.0455, 211.0398, 197.0573, 239.9687, 196.0531, 240.0608, 242.0578, 210.031, 267.0371
101	*Galium spp.*	7.85	269.0458	$2.79x10^{-32}$			Unclassified			
102	*Galium spp.*	7.94	239.0351	$1.09x10^{-32}$			Unclassified			
103	*Galium spp.*	8.01	255.0302	$3.62x10^{-16}$	[M-H]-	C9H8N2O7	Unclassified	255.0294, 227.0341		-27.9962

104	*Galium spp.*	8.75	232.0375	$7.96x10^{-15}$	[M-H]-	C12H9O5	Unclassified	29.0025, 124.0171, 89.1289, 88.0516, 90.2506, 111.4848, 134.0749, 131.6607, 153.5161, 69.4696, 174.1558, 149.4254, 40.7212, 79.4318, 174.6441	203.0356, 108.0202, 142.9091, 231.0299, 231.1478, 143.9826, 141.7874, 120.5484, 97.9631, 100.3686, 78.5219, 57.876, 162.5684, 82.6126, 57.4019
105	*Galium spp.*	8.75	301.1078	$4.86x10^{-30}$	[M-H]-	C15H16N3O4	Unclassified	101.0954, 113.0963, 69.0738	200.0144, 188.0014, 232.0384
106	*Galium spp.*	9.64	267.0304	$3.28x10^{-22}$	[M-H]-	C16H4N4O	Terpene, Sesqueterpene	**27.9962**, 55.9997, 71.9859, 26.991	**239.0357**, 211.0398, 195.0451, 267.0371, 240.0384
107	*P. lanceolata*	1.08	381.0951	$5.02x10^{-11}$	[M-H]-	C13H34S6	Unclassified, sulfate/phosphate residue[1]	140.0882, 284.1351	241.0025, **96.9568**

108	*P. lanceolata*	1.09	391.124	$1.35x10^{-18}$	[M+HCOOH-H]-, [M-H]-	C15H22O9	Hydroxycinnamic acid, glycosylated	226.0665, 252.082, 254.0657, 208.0553, 270.0957, 272.0794, 244.0786, 182.0774, 256.0736, 238.0638, 278.0952	165.0558, 139.0405, 137.0619, 183.0547, 121.0303, **119.0474**, 147.0459, 209.0456, **135.0437**, 153.0575, **113.0231**
109	*P. lanceolata*	1.44	409.0453	$1.54x10^{-12}$	[M-H]-	C13H19N2O9PS	Unclassified, sulfate/phosphate residue[1]	168.0443, 312.0922	241.0025, **96.9568**
110	*P. lanceolata*	1.79	439.0564	$6.60x10^{-12}$	[M-H]-	C14H21N2O10PS	Unclassified, sulfate/phosphate residue[1]	198.054, 342.0947	241.0025, **96.9568**
111	*P. lanceolata*	2.01	373.113	$2.81x10^{-13}$	[M-H]-	C16H22O10	Unclassified	206.0835, 250.074, 265.0953, 221.0989	167.0339, 123.0439, 108.0202, 152.0105, *149.0602*
112	*P. lanceolata*	2.48	463.1445	$2.62x10^{-07}$			Unclassified		
113	*P. lanceolata*	2.95	351.0414	$9.20x10^{-13}$	[M-H]-	C13H12N4O6S	Unclassified	153.9984, 169.0232, 152.9927	197.0453, 182.0217, 198.0467
114	*P. lanceolata*	3.13	433.1335	$8.67x10^{-22}$	[M-H]-	C16H24N3O11	Unclassified	236.0892, 251.1142,	197.0453, 182.0217, 138.0261, 198.0467

								295.1038, 235.0862		
115	*P. lanceolata*	3.14	497.1303	$3.45x10^{-09}$	[M-H]-	C22H26O13	Hydroxycinnamic acid	344.1063, 302.1003, 242.0719, 334.0936, 300.0852, 200.0688, 256.1095, 388.097, 362.0796, 360.0663, 332.1016, 259.0787, 278.026, 237.9732, 171.9826	153.0188, 195.0281, 255.0523, **163.0379**, 197.0453, 241.0208, 109.0304, **135.0437**, 137.0619, 165.0216, 259.1539, […], **151.0413**, […], 259.0083, […], *313.1123, 305.1755*	297.065,

116	*P. lanceolata*	3.2	563.2302	$3.01x10^{-08}$	[M-H]-	C32H36O9	Glycoside	450.2024, 358.1025, 356.0863, 440.1816, 396.1957, 374.1001, 388.203, 270.2028, 352.2007, 404.1653, 366.1882, 357.2167, 370.1786, 329.1172, 516.9181		1**13.0231**, 205.1235, 207.1389, 123.0439, 167.0339, 206.1272, 189.1289, **175.0257**, 153.0918, 223.1375, **161.0446**, **101.0229**	[…], […], […],	
117	*P. lanceolata*	3.34	379.1593	$6.06x10^{-11}$	[M+Na-H]-, [M+HCOOH-H]-	C18H23N5O3	Unclassified	192.1316, 154.0811, 176.1637, 147.1295, 234.1001, 168.1636, 111.0893, 188.062, 140.1033, 159.0669, 73.3389, 173.0712, 196.1066, 223.0476	34.818,	187.0368, 225.0892, 202.9933, 232.0384, 145.0603, 210.9946, 344.3371, 268.0732, 191.1059, 220.1006, 239.0535, 149.0602,	245.1126	

118	*P. lanceolata*	3.4	445.2057	$1.43x10^{-15}$	[M-H]-	C28H30O5	Hydroxycinnamic acid, glycosylated	208.0767, 206.04, 344.178, 314.1323, 162.0547, 180.0629, 222.0653, 143.8394, 284.1621, 207.082, 132.1511, 224.0723, 354.9964, 178.0425, 385.0764	237.1127, 239.1632, **101.0229**, 131.0749, 283.1466, 265.1399, 223.1375, 301.3603, **161.0265**, 238.1167, 313.0576, 221.1291, 90.2033, 267.1609, 269.1412
119	*P. lanceolata*	3.45	131.0708	$3.96x10^{-10}$	[M-H]-	C17H18N3	Unclassified	11.8495, 40.1688, 62.2914, 32.8592, 78.4634	90.9014, 119.2207, 68.7789, 98.211, 52.6068
120	*P. lanceolata*	3.46	401.1447	$8.37x10^{-23}$	[M-H]-	C18H26O10	Hydroxycinnamic acid	208.0934, 267.1058, 222.1065	**193.0497,** 134.0357, **179.0368**, 149.0602, 251.0354, *214.0609, 199.017, 209.1159*
121	*P. lanceolata*	3.47	467.155	$4.54x10^{-11}$	[M-H]-	C22H28O11	Hydroxycinnamic acid	330.1244, 228.1005, 288.1134, 186.0789, 258.1007, 272.0794	**137.0259**, 239.0535, **179.0368**, 281.0676, 209.0456, 195.0709

122	*P. lanceolata*	3.55	549.123	$6.05x10^{-22}$			Unclassified		
123	*P. lanceolata*	3.57	481.1363	$8.47x10^{-16}$	[M-H]-	C21H27N2O9P	Unclassified, sulfate/phosphate residue[1]	222.124, 260.0223, 282.1465, 278.026, 240.134	259.0083, 221.1134, 198.9932, 203.1113, 241.0025, *239.1276,* ***96.9568***
124	*P. lanceolata*	3.64	511.1454	$1.89x10^{-06}$			Unclassified		
125	*P. lanceolata*	3.67	931.2829	$5.98x10^{-13}$	[M-H]-	C42H50N3O21	Terpene, Iridio glycoside	466.1461, 650.2194, 465.1435, 692.23, 794.262	**465.1352**, 281.0676, 239.0535, **137.0259**
126	*P. lanceolata*	3.7	261.0434	$3.01x10^{-08}$	[M-H]-	C14H14OS2	Unclassified, sulfate/phosphate residue[1]	164.08, 18.010, 443.989	**96.9568**, 261.0392, 243.0397

127	*P. lanceolata*	3.72	699.2702	$4.53x10^{-10}$	[M-H]-	C29H48O19	Unclassified	476.1707, 386.1521, 478.1494, 180.0629, 520.1596, 404.1653, 548.2029, 434.1592, 564.1513, 506.1483, 418.2034, 475.1701, 374.1337, 433.1501, 494.1764	223.0949, 313.1123, 221.1134, 519.206, 179.1051, 295.1001, 151.0714, 265.1089, 135.1155, 193.1226, 281.0676, 224.0989, 325.1246, 266.1096, 205.0896
128	*P. lanceolata*	3.76	495.1491	$3.12x10^{-24}$	[M-H]-	C21H26N3O11	Unclassified	286.0994, 328.1099, 184.0599, 226.0759, 199.0918, 240.0845, 372.0902	209.0456, 167.0339, 311.0789, 269.0666, 296.0601, 255.0523, 123.0439, *151.0045, 108.0202, 195.0281, 152.0105, 210.053, 139.0405, 137.0619*

129	*P. lanceolata*	3.78	381.175	$3.01x10^{-08}$	[M-H]-	C16H30O10	Flavonoid[1]	184.1287, 132.0413, 187.1729, 220.0928, 188.1116, 171.1144, 189.1036, 210.0648, 175.1943, 246.091, 209.1341, 152.8221, 266.8692, 288.6575	27.3037,	197.0453, 194.0057, 193.0668, 192.0649, 171.1069, 135.0848, 205.9717, 353.8729, 172.0542, 114.3074, 228.3545, **151.0413**, 317.2094	249.135, 161.076, 210.053, […], […],
130	*P. lanceolata*	3.9	415.1597	$1.13x10^{-13}$			Unclassified				
131	*P. lanceolata*	3.94	565.2126	$4.53x10^{-10}$	[M-H]-	C24H38O15	Hydroxycinnamic acid, glycosylated	342.1255, 300.1104, 428.1936, 341.1179, 386.117, 299.1167,		223.0949, 265.1089, **137.0259**, 224.0989, 179.1051, 266.1096, *281.0549,* ***193.0497,*** *221.1134, 239.0535,* ***119.0474****,* ***179.0368****,* ***113.0231,*** *241.0025, 191.0368*	

132	*P. lanceolata*	3.99	431.1912	$4.41x10^{-09}$	[M-H]-	C17H31N5O6P	Unclassified	226.0665, 225.0701	205.1235, 206.1272
133	*P. lanceolata*	4.01	415.1571	$2.93x10^{-18}$	[M-H]-	C19H28O10	Hydroxycinnamic acid	**44.0226**, 266.1134, 319.5587, 236.0892, 368.3196, 369.0683, 213.0769, 192.0948, 370.3739, 193.9941, 326.7262, 220.0928, 86.1686, 123.0615, 244.0786	371.1343, 149.0453, 95.6, **179.0672**, 46.8418, 46.0904, 202.0768, **223.0655**, 44.7848, 221.165, 88.4325, 195.0709, 328.9901, 292.1031, 255.0892
134	*P. lanceolata*	4.05	465.1398	$9.20x10^{-13}$	[M-H]-	C25H24NO8	Hydroxycinnamic acid amid	180.0629, 328.1099, 329.1172, 224.052, 344.1063, **179.0589**, 300.0852, 184.072	285.0724, **137.0259**, 136.0176, 241.0859, 121.0303, 165.0558, 281.0676

135	*P. lanceolata*	4.07	501.1253	$3.45x10^{-09}$	[M-H]-	C38H16NO	Terpene, Iridio glycoside	216.0379, 364.0792, 215.029, 307.0846, 259.9977, 220.0378, 366.0745, 304.0484, 363.0765, 300.0149, 293.0068, 277.9911, 336.0541, 308.7639, 384.0738	285.0724, **137.0259**, 136.0176, 194.0207, 241.1218, 281.0676, 135.0276, […], 205.0896, […], **113.0231,** […], **465.1352**, […], **291.1985**, **239.0357**
136	*P. lanceolata*	4.18	495.1239	$3.01x10^{-08}$	[M-H]-	C33H20O5	Glycoside, sulfated/phosphorylated	374.1001, 348.0897, 372.0902, 328.0912, 360.1005, 373.0879, 344.1229, 256.0736, **180.0515**, 358.1025, 239.0569, 378.1099, 398.1749, 306.0736, 284.0706	121.0303, 147.0334, 123.0439, 167.0339, 135.0276, 151.0045, 122.0342, 239.0535, 315.0784, 166.0261, **137.0259**, […], **96.9568**

137	*P. lanceolata*	4.31	611.1987	$3.71x10^{-09}$	[M-H]-	C35H32O10	Hydroxycinnamic acid[1]	388.1334, 416.1293, 403.16, 386.117, 401.141, 387.1241, 402.115	**223.0655**, 195.0709, 208.0414, 225.0737, 210.053, 224.0745, 209.0792, *241.0025*
138	*P. lanceolata*	4.48	601.1553	$3.96x10^{-10}$	[M-H]-	C36H26O9	Hydroxycinnamic acid, glycosylated	464.1322, 320.0893, 362.1029, 346.1053, **120.023**, 406.1256, 304.0964, 319.0831, 422.1189	**137.0259**, 281.0676, 239.0535, 255.0523, 481.1337, 195.0281, 297.065, 282.0647, **179.0368**, […], 209.0456, 241.0025, […], 285.0724,
139	*P. lanceolata*	4.48	631.1667	$2.62x10^{-07}$	[M-H]-	C30H32O15	Hydroxycinnamic acid	422.1189, 494.1412, 320.0893, 464.1322, 362.1029, 434.1186, 421.1157, 319.0831, 334.0936	209.0456, 137.0259, 311.0789, 167.0339, 269.0666, 197.0453, 210.053, 312.0897, 297.065, **135.0437**, […], **151.0413**

140	*P. lanceolata*	4.52	343.0853	$3.96x10^{-10}$	[M-H]-	C18H16O7	Flavonoid[1]	133.0131, 234.0514, 150.0187, 283.4155, 122.0194, 125.0103, 22.1424, 90.0397, 14.5222, 109.3641, 129.9858, 45.0353, 166.5308, 147.054, 138.0736	109.0304, 193.0668, 218.0722, 320.9433, 253.0499, **283.0318**, 315.2312,	59.6702, 221.065,
141	*P. lanceolata*	4.55	595.203	$1.19x10^{-17}$	[M-H]-	C35H32O9	Hydroxycinnamic acid, glycosylated	386.117, 372.1338, 401.141, 388.1334, 387.1603, 392.1254, 195.0872, 385.1101, 390.0676, 464.1632, 482.1704, 371.1442, 402.1744, 346.084, 256.0736	209.0792, **223.0655**, 194.0551, **207.0689**, 208.0414, 203.0717, 400.1042, 210.0895, 131.0378, 205.1235, **113.0231**, **101.0229**, 285.0724, 137.0259	[…], […],

142	*P. lanceolata*	4.62	405.2103	$5.02x10^{-11}$	[M-H]-	C26H30O4	Glycoside	206.04, 129.0568, 229.2584, 240.1509, 135.0641, 339.344, 123.1261, 182.1555, 192.1316, 120.0369, 140.0205, 143.166, 291.1697, 190.1113, 194.1305	199.1759, 276.1589, 165.0679, 270.1502, 282.0876, 285.1612, **113.0231**, **207.0689**, 253.0499	175.951, 65.8654, 223.052, […], […], [...],
143	*P. lanceolata*	4.69	457.2062	$2.60x10^{-10}$	[M-H]-	C29H32NO2S	Unclassified, sulfate/phosphate residue [1]	238.0638, 226.0665, 256.0736, 130.0646, 225.0553, 237.0681, 252.082, 254.0657, 360.244, 296.1033	219.1376, 231.1328, 201.1212, 327.1386, 232.1484, 220.1396, 205.1235, 203.1425, *161.102, 223.0388*	**96.9568**, *285.0724,*
144	*P. lanceolata*	4.7	235.0292	$4.23x10^{-20}$	[M-H-H]2-, [M-H]-	C31H12N4S	Unclassified	138.0736	96.9568	

145	*P. lanceolata*	4.73	459.1889	$3.01x10^{-08}$	[M-H]-	C21H32O11	Flavonoid, glycosylated	240.0587, 160.1377, 258.0849, 219.0598, 220.0378, 262.0611, 328.1672, 190.0562, 218.2008, 318.1317, 227.096, 268.2253, 156.074, 114.0704	262.17, 219.1376, 299.0483, 201.1034, 197.0247, 240.1344, 239.1632, 131.0378, 197.1306, 269.1412, 241.0025, **101.0229**, **284.0313**
146	*P. lanceolata*	4.78	645.1817	$2.62x10^{-07}$	[M-H]-	C38H30O10	Flavonoid	448.1318, 406.1256, 360.1005, 420.1363, 508.1587, 462.1513, 405.1185, 434.1186, 346.1053, 524.1493, 447.135, 404.0961, 494.1412	197.0453, 239.0535, 285.0724, 225.0533, **137.0259**, 183.0269, 240.0608, 211.0615, 299.0802, 121.0303, 198.0467, 241.072, **151.0413**
147	*P. lanceolata*	4.84	487.1812	$1.17x10^{-22}$			Unclassified		
148	*P. lanceolata*	4.85	417.212	$2.37x10^{-08}$			Unclassified		

149	*P. lanceolata*	4.85	585.1615	$6.06x10^{-11}$	[M-H]-	C29H30O13	Hydroxycinnamic acid, glycosylated	300.0852, 448.1401, 304.0964, 346.1053, 406.1256, **120.023**, 299.0826, 344.0761, 303.0885	285.0724, **137.0259**, 281.0676, 239.0535, **179.0368**, **465.1352**, 241.0859, 136.0176, 282.0647, *303.0888, 240.0608, 209.0456*
150	*P. lanceolata*	4.91	467.2123	$4.54x10^{-11}$			Unclassified		
151	*P. lanceolata*	4.92	421.1171	$3.96x10^{-10}$	[M-H]-	C13H26O15	Hydroxycinnamic acid, glycosylated	224.0723, 213.0769, 239.1095, 198.054, 324.1594, 282.0802, 268.0644, 214.046, 219.0598	197.0453, 208.0414, 182.0217, **223.0655**, **96.9568**, 139.0405, 153.0575, **207.0689**, […], *241.0025*,
152	*P. lanceolata*	4.96	429.1762	$1.93x10^{-13}$	[M-H]-	C18H31N4O4PS	Unclassified	190.043, 224.052, 234.0315, 180.0629, 204.066, 233.0387, 189.0382, 230.1554, 268.0376, 186.0566, 223.0476,	239.1276, 205.1235, 195.1405, 249.111, 225.11, 240.1344, 199.017, 161.1349, 243.1191, 206.1272, 207.0908

								222.0789, 316.1603	
153	*P. lanceolata*	4.99	236.0371	$9.04x10^{-16}$	[M-H-H]2-	C16H26O12S2	Unclassified	139.0782	96.9568
154	*P. lanceolata*	5.22	517.1903	$5.21x10^{-12}$	[M-H]-	C23H34O13	Glycoside	404.1653, 240.0135, 183.9854, 356.1414, 239.0144, 380.1701, 368.1802, 297.1105, 406.1852, 388.1716, 317.1711	**113.0231**, 277.1859, 333.2139, **161.0446**, **137.0259**, 149.0142, 220.0741, 111.0098, 129.0167, 200.0144, *241.0025*
155	*P. lanceolata*	5.28	443.1913	$2.43x10^{-17}$	[M-H]-	C21H32O10	Hydroxycinnamic acid, glycosylated	330.1763, 312.1657, 206.3121, 327.1493, 308.1546, 346.166, 299.1693, 398.0877, 132.0413, 270.2668, 240.0587, 380.1226, 198.054, 180.1778, 332.1922	**113.0231**, 131.0378, 236.8825, 116.0436, **135.0437**, 97.0278, 144.0211, 45.1113, 311.153, 172.9278, 203.1425, 63.0727, 263.0289, 111.0098, 245.1308

156	*P. lanceolata*	5.35	711.3941	$2.93x10^{-18}$	[M-H]-	C38H56N4O9	Unclassified	208.0553, 207.0486	503.3365
157	*P. lanceolata*	5.39	303.0538	$5.98x10^{-13}$	[M-H]-	C12H16O7S	Unclassified, sulfate/phosphate residue[1]	206.1074	**96.9568**, 303.063
158	*P. lanceolata*	5.51	427.1967	$1.42x10^{-15}$	[M-H]-	C21H32O9	Hydroxycinnamic acid amid[1]	224.052, 180.0629, 292.1142, 226.0665, 223.0476, **179.0589**	203.1425, 247.1363, 135.0848, 201.1212, 248.1343
159	*P. lanceolata*	5.64	473.2015	$2.62x10^{-07}$	[M-H]-	C22H34O11	Hydroxycinnamic acid, glycosylated	270.0571, 226.0665, 338.1138, 376.2461, 208.0553, 225.0701, 270.1182, 272.0649, 354.1518, 372.6861, 352.1338, 297.6142, 395.5417, 342.1565, 269.0468	203.1425, 247.1363, 135.0848, **96.9568**, 265.1399, 248.1343, 203.0854, 201.1212, **119.0474**, 100.5144, 121.0666, ***161.0446***
160	*P. lanceolata*	5.65	393.1218	$3.45x10^{-09}$	[M-H]-	C23H24NOS2	Unclassified, sulfate/phosphate residue[1]	152.1228, 136.1242, 296.1629, 154.127, 44.0731	241.0025, 393.1141, 257.0019, **96.9568**, 239.0009, 349.0557

161	*P. lanceolata*	5.89	499.2544	$3.90x10^{-09}$	[M-H]-	C25H40O10	Jasmonate derivative, glycosylated	208.0553, 263.1739, 335.1664, 236.0404, 123.1576, 286.2019, 362.2213, 270.0957, 200.3785, 307.2391, 328.958, 306.1887, 385.2589, 296.2336, 328.1997	**291.1985,** 236.0884, 164.0758, 263.2105, 376.081, 137.0259, 213.0539, 192.0115, 229.1565, 298.8654, 170.2973, 193.0668, 203.0174, **113.9943**, 113.0231
162	*P. lanceolata*	6.02	327.0906	$1.37x10^{-14}$	[M-H]-	C12H24O6S2	Unclassified	104.0434, 103.0463, 118.0644, 87.0185	239.0709, 223.0388, 224.0455, 209.0222, 240.0608
163	*P. lanceolata*	6.08	443.1919	$3.79x10^{-10}$	[M-H]-	C19H30N3O9	Unclassified	256.0868, 180.0722, 224.0607	187.1029, 263.1313, 219.1376
164	*P. lanceolata*	6.67	695.3998	$4.54x10^{-11}$	[M-H]-	C30H58N5O13	Unclassified	208.0553, 207.0486	487.3394, 207.1389
165	*R. acris*	1.42	355.1239	$7.93x10^{-15}$	[M-H]-	C13H24O11	Glycoside[1]	240.0845, 78.0308, 254.1077	115.039, 277.0888, **101.0229**
166	*R. acris*	2.11	315.1074	$7.93x10^{-15}$			Unclassified		

167	*R. acris*	2.59	513.1242	$8.91x10^{-08}$	[M-H]-	C21H24O12	Unclassified	314.0478, 270.0571, 296.0371, 313.0423, 298.011	358.04,	
168	*R. acris*	2.74	137.0244	$6.51x10^{-07}$	[M-H]-	C4H10O3S	Unclassified	43.989	93.0304	
169	*R. acris*	2.88	375.1288	$8.91x10^{-08}$	[M-H]-	C23H20O5	Hydroxycinnamic acid	178.0813, 166.0885, 241.0909, 164.0621, 177.0753, 252.082, 268.0747, 179.0983, 182.0937, 165.0762, 176.0582, 224.1105, 222.0377, 208.0553, 254.1748	197.0453, 209.0456, 134.0357, 211.0615, 198.0467, 123.0439, 107.0499, 196.0308, **193.0497**, 153.0918, **137.0259**, 207.0908	[…], […],
170	*R. acris*	2.99	477.1606	$1.02x10^{-09}$	[M-H]-	C27H26O8	Hydroxycinnamic acid, glycosylated	364.1365, 208.0553, 316.1063, 232.092, 352.1338, 334.1267, 376.1352, 207.069, 276.1007,	**113.0231**, 269.1031, **161.0446**, 245.0666, 125.0251, 143.0333, **101.0229**, 270.0875, 201.0544,	263.0875

								214.0638, 332.1016, 233.1578, 265.0149, 385.0764, 273.2598	, *[…],* ***355.0771,*** *[…], 191.0368, […]*
171	*R. acris*	3.4	327.1082	$1.90x10^{-23}$			Unclassified		
172	*R. acris*	3.65	405.2115	$6.51x10^{-07}$			Unclassified		
173	*R. acris*	3.73	403.1955	$9.10x10^{-11}$	[M-H]-	C19H32O9	Hydroxycinnamic acid[1]	180.0629, **179.0589**	223.1375, 403.2004
174	*R. acris*	3.84	437.2375	$2.80x10^{-10}$	[M-H]-	C13H36N5O11	Unclassified	311.2297, 245.0832, 232.1056, 299.1693, 262.1517, 244.163, 279.1605, 199.1304, 180.1089, 309.3672, 294.1511, 300.1765, 155.0775, 258.1194, 214.1384	126.0036, 192.1501, 205.1235, 138.0614, 175.0776, 193.0668, 158.068, 257.1197, 238.1, 127.8661, 137.0619, […], 332.1799, 285.1101

175	*R. acris*	3.89	435.2223	$5.30x10^{-14}$	[M-H]-	C20H36O10	Glycoside, acidified	189.134, 322.1988, 216.1147, 246.1423, 166.2978, 250.1509, 248.1118, 268.1292, 210.0648, 298.1878, 123.0935, 169.1362, 286.2019, 232.2052, 296.2336	**113.0231**, 246.0869, 219.1092, 189.0853, 268.9209, 185.071, 187.1029, 167.0902, 225.1551, **137.0259**
176	*R. acris*	3.98	515.1759	$8.34x10^{-12}$	[M-H]-	C13H34N5O14S	Unclassified	312.1053, 294.0955, 324.1021, 311.1003	203.0717, 221.0818, 191.0681, 204.0741
177	*R. acris*	4.13	383.1389	$6.17x10^{-11}$	[M-H]-	C16H22N3O8	Hydroxycinnamic acid amid[1]	162.0547, 180.0629, 161.0481, **179.0589**	221.0818, 203.0717, 222.0885, 204.0741, *191.0681*
178	*R. acris*	4.2	403.1956	$1.45x10^{-21}$			Unclassified		
179	*R. acris*	4.47	405.1769	$6.17x10^{-11}$	[M-H]-	C18H30O10	Unclassified	180.0629, 182.0445, 224.052, 181.0362	225.11, 223.1375, 181.1207, *403.2004, 207.0908*

180	*R. acris*	4.79	427.196	$6.51x10^{-07}$	[M-H]-	C11H34N5O10S	Unclassified	206.1074, 224.1315, 224.052, 266.0616, 223.1161, 223.0476	221.0818, 203.0717, 203.1425, 161.1349
181	*R. acris*	4.83	383.1283	$6.51x10^{-07}$			Unclassified		
182	*R. acris*	4.84	429.1684	$3.87x10^{-07}$	[M-H]-	C27H26O5	Glycoside, acidified	190.043, 234.0315, 186.1345, 224.0393, 188.0227, 226.0217, **180.0515**, 208.0767, 242.0555, 189.9641, 322.1142, 268.0376, 44.0413, 189.0382, 316.1716	239.1276, 195.1405, 243.0397, 205.1235, 241.1415, 203.1425, 249.111 , […], 119.4981, **137.0259**
183	*R. acris*	5.24	269.0107	$1.39x10^{-20}$	[M-H]-	C8H7N4O5P	Unclassified	*71.9859*	269.0075
184	*A. pratensis*	3.31	347.0439	$1.30x10^{-08}$	[M-H]-	C16H12O9	Hydroxycinnamic acid	153.9984, 169.0232, 152.9927, 213.0146	**193.0497**, **178.0276**, 194.0551, 134.0357, 149.0602

185	*A. pratensis*	3.77	197.0452	$4.74x10^{-07}$	[M-H]-	C7H8N3O4	Unclassified, methoxylated	74.0398, **30.0455**, 76.0075, 91.0479, 15.0225	123.009, 166.9979, 121.0303, 105.9976
186	*A. pratensis*	4.43	389.1232	$6.05x10^{-06}$			Unclassified		
187	*A. pratensis*	5.17	477.1801	$7.31x10^{-07}$	[M-H]-	C24H30O10	Flavonoid, glycosylated	236.177, 380.2189, 176.1637, 228.1218, 252.1681, 192.1447, 278.1856, 235.1754, 140.1033, 254.1907	241.0025, **96.9568**, 301.0129, 249.0584, 225.0151, **285.0426**, 198.9932, 242.003, […], **207.0689**, […], 259.0083, **113.0231**
188	*A. pratensis*	7.42	557.2409	$1.55x10^{-06}$	[M-H]-	C25H40N3O9S	Unclassified		-
189	*A. pratensis*	3.15	445.1711	$7.15x10^{-07}$	[M-H]-, [M+HCOOH-H]-	C20H30O11	Hydroxycinnamic acid	240.098, 326.1145, 314.1323, 330.1244, 260.1647, 222.0653, 204.0467, 344.1475, 248.1248, 232.1343, 238.1944, 322.124, 202.0427,	205.0667, **119.0474**, 131.0378, 115.039, 185.0035, 223.1113, 241.1218, **101.0229**, 197.0453, 213.0379, 206.9821, 123.0439,

							264.0997, 252.1182		243.1191, **193.0497**, 96.9568,
190	*A. elatius*	5	567.3523	$2.03x10^{-11}$	[M-H]-	C28H50N5O5S	Unclassified, aliphatic acid[1]	**46.0007**, 324.2067, 212.1099, 342.2376, 330.2911, 371.258, 160.1377, 351.2545, 234.1441, 426.2234, 346.2339, 296.2557, 412.2794, 232.2547, 268.2253, 374.2232, 397.284, 239.2619, 251.2875, 322.2388	44.986, 419.27, […],	521.3444, 243.1355, 355.2324, 225.11, 522.3602, 237.0561, 196.0895, 407.2, […], 271.1001, […], 193.1226, […], 277.1859, […], 335.222, 86.7158, 203.0356, 223.1375
191	*A. elatius*	5	815.4404	$3.27x10^{-08}$	[M-H]-	C42H64N4O12	Unclassified	178.0517, 310.0917, 177.0455, 309.0887		637.3967, 505.3441

192	*A. elatius*	5.15	683.3985	$2.79x10^{-09}$	[M-H]-	C36H60O12	Hydroxycinnamic acid	**46.0007**, 366.1566, 552.3563, 440.2834, 44.986, 414.3207, 514.3099, 462.298, 458.288, 262.13, 526.3577, 412.3319, 534.3425, 468.3587, 354.2501	637.3967, 317.2382, 131.0378, 243.1191, 269.0666, 169.0875, 221.0969, 225.11, 421.2697, 157.0404, **271.0641**, 149.0453, […], 280.0651, 119.0359, 195.0709, 161.011, 356.2882, 507.3065, 202.0964, 215.124, 373.2316, 217.1001, 111.0098, 199.1314, 127.0411, 64.5409, 188.0663, […], 414.2356, 553.2325, 505.3441, 104.5848, 195.2681, 330.0706, 339.1384, 638.301, **223.0655**, 205.1235, 68.0812

193	*A. elatius*	5.25	681.3819	$3.84x10^{-07}$	[M-H]-	C36H58O12	Glycoside	324.2403, 467.8713, 568.3635, 384.2952, 461.3946, 458.288, 454.3821, 472.2283, 484.3326, 448.4163, 468.3058, 552.3361, 474.309, 527.3238, 564.3278, 567.3276, 234.196	357.148, 213.5164, **113.0231**, 297.0916, 219.9961, 209.1516, 197.0573, 223.0949, 227.0132, 129.0568, 213.0765, 232.9686, **207.0689**, 117.054, 114.0562
194	*A. elatius*	5.38	565.3367	$3.84x10^{-07}$	[M-H]-	C27H54N2O6S2	Unclassified, aliphatic acid[1]	292.1493, 234.1118, 76.0075, 291.1441, 248.1248, 242.0909, 124.039, 344.2492, 178.0674, 396.2479, 166.0885, 344.1991, 75.0144, **46.0007**	273.1844, 331.2252, 489.3266, 274.1883, 317.2094, 323.2398, […], 329.2154, 369.2372, 121.0666, 272.0955, 271.1689, […], 521.3444, […] 149.0602, […], 223.1375

195	*A. elatius*	5.57	565.3362	$2.32x10^{-08}$	[M-H]-	C29H48N3O8	Unclassified, aliphatic acid	292.1493, 160.0532, 130.0401, 234.1001, **46.0007**, 78.0162, 76.0075, 225.2359, 352.2007, 142.0402, 291.1441, 210.2094, 204.0774, 262.13, 94.0213	273.1844, 405.2783, 435.2854, 331.242, 519.335, 487.3098, 489.3266, 340.087, 213.13, 423.29, 274.1883, 355.1211, 361.245, 303.1995, […], 323.2398, 317.2094, 329.2154
196	*A. elatius*	5.74	341.1073	$1.01x10^{-06}$			Unclassified		
197	*A. elatius*	5.93	563.3213	$3.27x10^{-08}$	[M-H]-	C33H46N3O3S	Unclassified	76.0075, 248.1118, 75.0009, 43.9742, 41.965, 106.0016, 46.0233, 260.1117, 424.2433, 234.0893, 62.0082, 138.0285, 246.1031, 186.0789, 290.1257	487.3098, 315.2042, 519.335, 521.3444, 457.3035, 517.2826, 518.3384, 303.1995, 139.0677, 329.2154, 501.2964, 425.2881, 317.2094, […], 316.1873, 239.1276, 205.1235, [...], 245.1126

198	*A. elatius*	5.99	461.2202	$2.49x10^{-06}$	[M-H]-	C22H30N3O5	Unclassified	329.1921, 252.1023, 204.0774, 264.1511	132.0295, 209.1159, 257.1437, 197.0713
199	*A. elatius*	6.05	551.3564	$1.07x10^{-15}$	[M-H]-	C29H50N3O7	Unclassified, aliphatic acid	**46.0007**, 44.986, 110.046, 212.1099, 218.1015, **43.9987**	505.3441, 441.3025, 339.2311, 333.2504, *507.3547, 287.2074, 261.1478, 327.218, 289.2173, 417.2452, 315.2312, 101.0535, 419.2563, 506.4809, 277.1309*
200	*A. elatius*	6.4	551.3568	$1.07x10^{-15}$	[M-H]-	C28H48N3O5	Unclassified, aliphatic acid	130.0401, **46.0007**, 44.9997, 129.0391, 236.1211, 286.1751, 78.0308, 48.0118, 338.2234, 268.167, 148.0624, 266.1499, 285.1717, 296.1994, 64.0136	421.3025, 505.3441, 315.2312, 265.1819, 473.3278, 503.3365, 213.13, 283.1942, 403.2943, 285.2004, 255.1592, 487.3394, 284.1947, 355.261, 298.1401

201	*A. elatius*	6.69	549.3393	$1.34x10^{-15}$	[M-H]-	C30H52N3O2S2	Unclassified, aliphatic acid	**46.0007**, **43.989**, 44.9997, 234.1118	503.3365, 505.3441, 315.2312
202	*A. elatius*	6.79	533.3115	$3.84x10^{-07}$	[M-H]-	C24H48N5O4S2	Unclassified, aliphatic acid[1]	216.0961, 106.0686, **46.0007**, 232.1175, 268.1292, 318.2188, 410.2298, 286.1751, 122.0631, 242.1361, 163.0633, 15.9992, 170.0707, 209.0958, 187.0831	317.2094, 427.243, 487.3098, 301.1902, 265.1819, 215.1011, 123.0819, 247.1363, 411.2511, 291.1644, 370.244, 517.3189, […], 149.0602, […], *153.0918*
203	*A. elatius*	6.84	565.3362	$1.47x10^{-13}$	[M-H]-	C29H48N3O8	Terpene, Sesqueterpene[1]	262.13, 76.0075, **46.0007**, 267.1899, 222.1065, 94.0213, 75.0144, 112.0342, 261.1299, 44.9997, 248.1248, 380.2189, 210.1013, 314.1683, 106.0239	303.1995, 489.3266, 519.335, 298.1401, 343.2291, 471.3123, 453.305, 304.2075, 317.2094, 185.1131, 355.2324, […], 329.2154, […], 383.2587, 413.2626, 521.3444

204	*A. elatius*	7.18	549.3422	$1.26x10^{-14}$	[M-H]-	C28H46N3O5	Terpene, Sesqueterpene, methoxylated[1]	130.0401, 45.9861, 78.0308, 262.1167, 306.1887, 44.9997, 47.9903, 220.1206, 210.1013, 222.124, 364.1855, 390.9772, 308.1939, 488.6523, 273.1702	419.2905, 503.3365, 471.3123, 287.2074, 243.15, 501.3325, 329.2154, […], 315.2312,, […], 401.2742, **383.2587**
205	*A. elatius*	7.28	545.3097	$2.79x10^{-09}$	[M-H]-	C21H46N4O12	Terpene, Sesqueterpene[1]	41.965, 43.9742, 144.0263, 126.0133, 202.0772, 258.1007, 76.0075, 234.1118, 216.0961, 384.3476, 192.0659, 292.1493, 232.092, 218.0786, 344.1991	503.3365, 501.3325, 401.2742, 419.2905, […], 469.3009, 311.1974, 329.2154, 160.9552, 353.2457, 253.1635, 313.2146, 327.218, 201.1212, […], 239.1276, […], 153.1273, **383.2587**

206	*A. elatius*	7.29	517.3155	$2.32x10^{-08}$	[M-H]-	C30H46O7	Jasmonate derivative[1]	204.0979, 186.0789, 98.0194, 230.1057, 214.0824, 304.1743, 284.1494, 61.9988, 252.1182, 242.1054, 188.0931, 96.0272, 302.1653,	80.009, 18.0104	313.2146, 437.3018, 331.2252, 419.2905, 287.2074, 303.2249, 233.1619, 455.3146, 265.1819, 275.2032, 329.2154, 421.2697, 215.1512,	213.13, **263.1705**
207	*A. elatius*	7.54	547.3261	$2.19x10^{-09}$	[M-H]-	C30H48N2O5S	Unclassified	**43.989**, 42.9854, 260.1117, 230.1057, 128.023, 258.1007, 312.183, 203.0903, 350.1924, 274.1389, 246.091, 248.1475,	338.1971, 44.9997	503.3365, 209.1159, 287.2074, 317.2094, 419.2905, 289.2173, 344.2309, 197.1306, 273.1844, 301.2162, 329.2154,	235.134,

208	*A. elatius*	7.63	533.345	$2.32x10^{-08}$	[M-H]-	C30H48N2O5S	Unclassified	190.1113, 242.1054, 189.1171, 164.1048, 264.133, 280.1259, 272.1921, 107.0383, 46.0095, 121.0477, 132.1096, 314.1179, 126.0566, 391.234, 108.0228	343.2291, 291.2381, 344.2309, 369.2372, 269.2125, 253.2208, 261.1478, 426.3014, 487.3394, 412.3063, 401.229, 219.2272, 142.118, 407.2874, 425.3276
209	*A. elatius*	7.73	651.408	$2.79x10^{-09}$	[M-H]-	C36H54N4O4	Unclassified	438.3059, 442.2545, 342.1982, 348.1771, 389.242, 316.1886, 386.2276, 368.1802, 434.2931, 292.2296, 320.2811, 359.2915, 439.2593, 184.1096, 120.1707	213.1007, 209.1516, 309.1961, 303.2249, 262.1675, 335.222, 265.1819, 283.2213, 217.1001, […], 119.0359, 239.1632, 284.1947, 313.2146, […], 235.1667, 205.1235, […], 305.1755
210	*A. elatius*	7.74	501.3198	$2.32x10^{-08}$	[M-H]-	C23H54N2O3S3	Unclassified	304.1572	197.1636

211	*A. elatius*	8.19	537.3245	$3.27x10^{-08}$	[M-H]-	C30H50O6S	Unclassified		-	
212	*A. elatius*	8.32	545.3111	$9.23x10^{-06}$	[M-H]-	C32H50O3S2	Unclassified	392.2177, 232.092, 334.1379, 216.0961, 89.9852, 306.1406, 204.0979, 258.1007, 350.1707, 290.1547, **43.9987**, 202.0772, 234.0893, 328.2884, 408.2147	153.0918, 313.2146, 211.1654, 329.2154, 455.3146, 239.1632, 341.2086, 287.2074, 328.2169, 195.1405, 255.1592, 501.2964, 343.2291, 217.0273	311.228,
213	*A. elatius*	8.88	535.3098	$3.84x10^{-07}$	[M-H]-	C30H48O6S	Unclassified		-	
214	*A. elatius*	9.18	529.3155	$2.32x10^{-08}$			Unclassified			
215	*A. elatius*	9.92	517.3519	$3.84x10^{-07}$	[M-H]-	C30H50N2O3S	Unclassified, sulfate/phosphate residue, aliphatic acid	220.2027, 247.2371, 421.3033, 298.3552, 206.1554, 45.0353, 242.2199, 424.1264, 258.1731, 294.1686,	297.1417, 270.1163, 218.9983, 311.1974, 472.3191, 200.1165, 223.1853, 259.1748, 273.1844,	96.0502, 275.123, 93.2271, **96.9568**,

								317.2381, 244.163, **46.0007**	[...],	245.1126, 201.0119,
216	*L. perenne*	5.59	305.1389	$9.06x10^{-07}$	[M-H]-	C17H22O5	Hydroxycinnamic acid[1]	96.0272, 198.0828, 103.0652, 102.0227, 18.0104, 194.0539, 142.1069, 144.0754, 113.1488, 100.0532, 102.0702, 156.1176, 230.9023,	80.009, 90.0052	209.1159, 305.1357, 107.0499, 202.0768, 225.1254, 203.1113, 287.1273, 111.0818, **163.0379**, 161.0601, 191.9791, 205.0896, 303.2521, 203.0717, 149.0142

217	*P. pratensis*	4.5	453.086	$5.13x10^{-06}$	[M-H]-	C23H18O10	Glycoside[1]	123.9555, 168.9858, 241.9507, 326.0192, 216.0104, 364.5956, 235.015, 339.0741, 337.0241, 167.9776, 168.032, 317.0434, 256.0736, 234.9089, 344.0579	329.1413, 284.1041, 211.1362, 127.0707, 237.0779, 218.0722, 114.0154, 136.0447, 285.1101, 313.1123, **163.0702**	88.5034, 116.07, […], […],

[1] annotation bases on one characteristic fragment or neutral loss

Supplementary Table 7: Identifier fragments and characteristic masses. The table contains all neutral losses and fragment ions which were used for classification of compounds in Supplementary Table 6.

identifier fragments	masses (m/z)
neutral losses	
Mehtyl residue 15.0225	15.0225
CO residue 27.9912	27.9912
Methoxylated aromatic compounds 30.0455	30.0455
Polyketides 42.0168	42.0168
CO2 residue 43.9890	43.989
CO2 residue 43.9987	43.9987
2-hydroxylated (Propyl)Chromones 44.0226	44.0226
Aliphatic acid 46.0007	46.0007
Glycoside C-Glycoside 120.023	120.023
Hexose 162.0418	162.0418
Hydroxycinnamic acid amid (C9H9NO3) 179.0589	179.0589
Hexose 180.0515	180.0515
Fragment ions	
Sulfate fragment (HSO4-)/Phosporous group (H2PO4-) 96.9568	96.9568
Carbohydrates fragment 101.0229	101.0229
Carbohydrates fragment 113.0231	113.0231
Coumaroyl fragment 119.0474	119.0474
Caffeoyl fragment 135.0437	135.0437
Salicylate fragment 137.0259	137.0259
Caffeoyl fragment 145.0209	145.0209

Myricetin fragment 151.0413	151.0413
Coumarine fragment 161.0265	161.0265
Desoxyglycosylated fragment 161.0446	161.0446
Coumaroyl fragment 163.0379	163.0379
Rhamnoside fragment 163.0702	163.0702
Sinapoyl fragment 164.0454	164.0454
Ferulic acid fragment 178.0276	178.0276
Caffeoyl fragment 179.0368	179.0368
Sinapoyl fragment 179.0672	179.0672
Quinic acid fragment 191.0543	191.0543
Ferulic acid fragment 193.0497	193.0497
Lauric- acid 199.1759	199.1759
Fraxetin fragment 207.0349	207.0349
1-O-methyl-β-D-glucuronate fragment 207.0689	207.0689
Sinapic acid fragment 223.0655	223.0655
Aglycon of sesquiterpene glycosides 239.0357	239.0357
Kaempferol backbone fragment 255.0294	255.0294
Jasmonate fragment 263.1705	263.1705
Naringenine fragment 271.0641	271.0641
Cyanidin fragment 283.0318	283.0318
Kaempferol fragment 284.0313	284.0313
Kaempferol fragment, Anthocyanidine-backbone 285.0426	285.0426
Jasmonate fragment 291.1985	291.1985
Disaccharide fragment 323.0905	323.0905
Esculetin(4-O-8)G fragment 355.0771	355.0771

Sesqueterpene fragment 383.2587	383.2587
Iridoid glycoside fragment 465.1352	465.1353

Supplementary Table 8: Individual explained variance of the semi-polar metabolite composition of exudates by single variables of the factors LNH, Soil and Climate. Table contains the explained amount of variance (in %) of semi-polar metabolites by different environmental variables. A detailed description of the abbreviation is given in Supplementary Table 10. Residuals = remaining unexplained variation.

	Environ-mental factor and included single variables (SV)	Species	Plot	SV	Species +Plot	Plot +SV	SV +Species	Species +Plot +SV	Residuals
forb	LNH	19.60	9.22	0.03	0.00	0.28	0.03	0.10	75.10
	- Cover	19.79	9.22	0.12	0.00	0.28	0.00	0.06	75.01
	- Richness	19.60	9.22	0.03	0.00	0.28	0.03	0.10	75.10
	- Shannon	19.76	9.59	0.19	0.00	0.00	0.00	0.08	74.94
	LUI	19.64	9.74	0.34	0.00	0.00	0.00	0.00	74.79
	- fertilization	19.68	9.77	0.37	0.00	0.00	0.00	0.00	74.76
	- grazing	19.64	9.74	0.34	0.00	0.00	0.00	0.00	74.79
	- mowing	19.77	9.53	0.01	0.00	0.00	0.00	0.11	75.12
	Soil	19.67	9.34	0.00	0.00	0.15	0.00	0.07	75.36
	- pH	19.73	9.39	0.00	0.00	0.11	0.00	0.03	75.37
	- TC	19.74	8.95	0.00	0.00	0.54	0.00	0.21	75.16
	- TN	19.50	8.69	0.00	0.00	0.80	0.13	0.00	75.22
	- moisture	19.57	9.21	0.05	0.00	0.29	0.06	0.04	75.08
	- soil texture	19.73	9.39	0.00	0.00	0.11	0.00	0.03	75.37
	- soil type	19.47	9.54	0.15	0.00	0.00	0.16	0.00	74.98
	Climate	19.66	9.09	0.05	0.00	0.41	0.00	0.02	75.08
	- precipitation	19.60	9.22	0.03	0.00	0.28	0.03	0.10	75.10
	- T(10)	19.79	9.22	0.12	0.00	0.28	0.00	0.06	75.01
	- T(200)	19.60	9.22	0.03	0.00	0.28	0.03	0.10	75.10
grass	LNH	2.89	13.13	0.10	0.00	0.35	0.00	0.09	84.25
	- Cover	2.78	12.96	0.00	0.00	0.51	0.04	0.00	84.45
	- Richness	2.89	13.13	0.10	0.00	0.35	0.00	0.09	84.25
	- Shannon	2.60	13.84	0.52	0.00	0.00	0.21	0.00	83.83
	LUI	2.71	13.90	0.96	0.00	0.00	0.11	0.00	83.39
	- fertilization	2.69	13.87	1.12	0.00	0.00	0.12	0.00	83.23
	- grazing	2.71	13.90	0.96	0.00	0.00	0.11	0.00	83.39
	- mowing	2.76	13.29	0.11	0.00	0.19	0.05	0.00	84.24
	Soil	2.89	13.90	0.39	0.00	0.00	0.00	0.06	83.96
	- pH	2.82	13.88	0.42	0.00	0.00	0.00	0.00	83.94
	- TC	2.51	13.49	0.57	0.00	0.00	0.31	0.00	83.78
	- TN	2.78	12.71	0.00	0.00	0.76	0.04	0.00	84.42
	- moisture	2.83	13.11	0.00	0.00	0.37	0.00	0.05	84.37
	- soil texture	2.82	13.88	0.42	0.00	0.00	0.00	0.00	83.94
	- soil type	2.85	13.44	0.00	0.00	0.04	0.00	0.06	84.37
	Climate	2.86	11.62	0.00	0.00	1.85	0.00	0.05	84.66
	- precipitation	2.89	13.13	0.10	0.00	0.35	0.00	0.09	84.25
	- T(10)	2.78	12.96	0.00	0.00	0.51	0.04	0.00	84.45

├ T(200)	2.89	13.13	0.10	0.00	0.35	0.00	0.09	84.25

Supplementary Table 9: List of samples. Table lists the number of samples per growth form or species and site, respectively.

	ALB	HAI	SCH
forb	53	61	24
grass	52	41	31
A.millefolium	11	13	4
G.mollugo	12	12	7
G.verum	12	14	5
P.lanceolata	10	10	5
R.acris	8	12	3
A.pratensis	5	8	4
A.elatius	14	6	5
D.glomerata	14	11	9
L.perenne	10	7	7
P.pratensis	9	9	6

Supplementary Table 10: List of used environmental factors and included variables including abbreviations, category, unit and description.

Environmental factors and variable	Abbreviation	category	Unit
Local neighbouring plant community	LNH		
Covered area by neighbouring plants	Cover		%
Richness of each species occurring around the target plant	Richness		
Species diversity of neighbouring plant community	Shannon	LNH	
Land use intensity index	LUI		-
Annual grazing frequency	Grazing		livestock units days of grazing*ha^{-1}*year^{-1}
Annual mowing frequency	Mowing		times* year^{-1}
Annual fertilization intensity	Fertilization	LUI	kg N*ha $^{-1}$ *yr^{-1}
Relative annual precipitation	precipitation		%
Annual temperature in 10 cm height	T (10)		°C
Annual temperature in 200 cm height	T (200)	Climate	°C
pH of the soil	pH		-
Soil moisture	moisture		%
Total soil carbon content	TC		%
Total soil nitrogen content	TN		%
Soil textures of WRB database	Soil texture		-
Soil type	Soil type	Soil	-

Discussion:

Table 1: Overview of the polar metabolites observed in samples of both harvest campaigns (2014, 2015).

2014				2015			
Metabolite	Metabo-lite class	qun at ion	Retent-ion index [RI]	Metabolite	Metabo-lite class	qun at ion	Retent-ion index [RI]
Pinitol (260)	Alcohol	260	1868	Pinitol (260)	Alcohol	260	1868
scyllo-inositol (204)	Alcohol	204	2072	scyllo-inositol (204)	Alcohol	204	2072
Xylitol (307)	Alcohol	307	1735	Xylitol (307)	Alcohol	307	1735
Asparagine (245)	Amino acid	245	1697	Asparagine (231)	Amino acid	231	1697
beta-Alanine (248)	Amino acid	248	1473	beta-Alanine (248)	Amino acid	248	1473
Glutamine (155)	Amino acid	155	1485	Glutamine (155)	Amino acid	155	1485
Homoserine (218)	Amino acid	218	1463	Homoserine (218)	Amino acid	218	1463
Lysine (156)	Amino acid	156	1939	Lysine (156)	Amino acid	156	1939
Methionine (176)	Amino acid	176	1532	Methionine (176)	Amino acid	176	1532
Ornithine / Citrullin (142)	Amino acid	142	1840	Ornithine / Citrullin (142)	Amino acid	142	1840
Tryptophan (202)	Amino acid	202	2250	Tryptophan (202)	Amino acid	202	2250
Tyrosine (218)	Amino acid	218	1960	Tyrosine (218)	Amino acid	218	1960
Octadecadienoic acid (337)	Lipid	337	2218	Octadecadienoic acid (337)	Lipid	337	2218
Octadecatrienoic acid (335)	Lipid	335	2230	Octadecatrienoic acid (335)	Lipid	335	2230
Adenine (264)	Nuclic base	264	1883	Adenine (264)	Nuclic base	264	1883
Adenosine (236)	Nucliotid e	236	2679	Adenosine (236)	Nucliotid e	236	2679
2-Aminoadipate (260)	Organic acid	260	1739	2-Aminoadipate (260)	Organic acid	260	1739
2-Isopropylmalate (275)	Organic acid	275	1600	2-Isopropylmalate (275)	Organic acid	275	1600

3-Caffeoyl-trans-Quinic acid (345)	Organic acid	345	3166
4-Aminobutanoate [GABA] (174)	Organic acid	174	1543
Aminomalonic acid (218)	Organic acid	218	1483
Gluconate (333)	Organic acid	333	2049
Phosphoenolpyruvate (247)	Organic acid	247	1623
Salicylic acid (267)	Organic acid	267	1533
Shikimate (204)	Organic acid	204	1841
Glucose-6-phosphate (387)	Sugar	387	2393
Lactose (361)	Sugar	361	2734
Melibiose (361)	Sugar	361	2946
Myo-Inositol-1-phosphate (318)	Sugar	318	2487
Rhamnose (117)	Sugar	117	1756
Unknown_sugar (204) RT1781	Sugar	204	1781
unknown compound (167) RT1517	uniden-tified	167	1517

3-Caffeoyl-trans-quinic acid (345)	Organic acid	345	3166
4-Aminobutanoate [GABA] (174)	Organic acid	174	1543
Aminomalonic acid (218)	Organic acid	218	1483
Gluconate (333)	Organic acid	333	2049
Phosphoenolpyruvate (247)	Organic acid	247	1623
Salicylic acid (267)	Organic acid	267	1533
Shikimate (204)	Organic acid	204	1841
Glucose-6-phosphate (387)	Sugar	387	2393
Lactose (361)	Sugar	361	2734
Melibiose (361)	Sugar	361	2946
Myo-Inositol-1-phosphate (318)	Sugar	318	2487
Rhamnose (117)	Sugar	117	1756
Unknown_sugar (204) RT1781	Sugar	204	1781
unkown fatty acid (339) RT1518	uniden-tified	339	1517

Table 2: Overview of the putatively classified semi-polar metabolites observed in samples of both harvest campaigns (2014, 2015).

2014					2015				
No.	Species	RT [min]	m/z	Classification	No.	Species	RT [min]	m/z	Classification
1	*A. millefolium*	2.62	409.04451	Unclassified, sulfated	1	*A. millfefolium*	2.6	409.0444	Unclassified, sulfated/phosphorylated
2	*A. millefolium*	3.11	619.18815	Flavonoid, glycosylated	2	*A. millfefolium*	3.12	619.1862	Hydroxycinnamic acid, glycosylated
7	*A. millefolium*	4.77	509.22072	Glycoside	9	*A. millfefolium*	4.77	509.2194	Glycoside, hydroxycarbonic acid
8	*A. millefolium*	4.82	503.17797	Glycoside	10	*A. millfefolium*	4.82	503.1812	Glycoside
11	*A. millefolium*	4.89	547.20258	Phenylpropa-noid	12	*A. millfefolium*	4.89	547.2022	Hydroxycinnamic acid
13	*A. millefolium*	4.9	689.29637	Unclassified	13	*A. millfefolium*	4.9	689.2999	Unclassified
15	*A. millefolium*	5.02	487.18126	Glycoside	14	*A. millfefolium*	5.02	487.1807	Glycoside
17	*A. millefolium*	5.13	469.1706	Unclassified	15	*A. millfefolium*	5.13	469.1704	Unclassified
19	*A. millefolium*	5.36	452.19105	Unclassified	16	*A. millfefolium*	5.37	452.1914	Glycoside, sulfated/phosphorylated
24	*A. millefolium*	6.1	508.24348	Unclassified	21	*A. millfefolium*	6.1	508.2429	Glycoside
28	*A. millefolium*	8.94	286.18069	Unclassified	23	*A. millfefolium*	8.89	286.1802	Unclassified
43	*G. mollugo*	4.13	377.17599	Flavonoid, glycosylated	29	*G. mollugo*	3.39	377.1791	Flavonoid

44	*G. mollugo*	4.78	467.11897	Unclassified	78	*Galium spp.*	4.78	467.122	Unclassified
45	*G. mollugo*	5.73	419.13406	Flavonoid	43	*G. mollugo*	5.74	419.134	Flavonoid
48	*G. mollugo*	6.1	253.05029	Polyketide, Aromatic acetate	62	*G. mollugo*	6.08	253.0504	Polyketide
51	*G. mollugo*	6.83	489.17542	Unclassified	53	*G. mollugo*	6.81	489.1752	Unclassified
52	*G. mollugo*	7.74	269.0503	Unclassified	100	*Galium spp.*	7.72	269.0483	Unclassified, imin, aliphatic acid
56	*Galium spp.*	2.68	449.12732	Phenylpropa-noid, glycosylated	67	*Galium spp.*	2.67	449.1295	Hydroxycinnamic acid, glycosylated
57	*Galium spp.*	2.96	355.09956	Polyphenole, Hydroxycinnamic acid	68	*Galium spp.*	2.97	355.1012	Hydroxycinnamic acid
58	*Galium spp.*	3.05	391.15882	Flavonoid, glycosylated	26	*G. mollugo*	3.05	391.1576	Hydroxycinnamic acid, glycosylated
69	*Galium spp.*	4.18	509.22311	Glycoside	73	*Galium spp.*	4.09	509.223	Glycoside
71	*Galium spp.*	4.36	389.12107	Unclassified	187	*A. pratensis*	4.43	389.1232	Unclassified
75	*Galium spp.*	4.94	563.13922	Unclassified	79	*Galium spp.*	4.95	563.1403	Unclassified
76	*Galium spp.*	5.07	625.17515	Unclassified	81	*Galium spp.*	5.07	625.1763	Unclassified
77	*Galium spp.*	5.15	609.1758	Phenylpropa-noid, glycosylated	82	*Galium spp.*	5.16	609.1806	Glycoside
79	*Galium spp.*	5.38	275.0922	Unclassified	86	*Galium spp.*	5.38	275.0919	Unclassified

80	*Galium spp.*	5.47	595.16624	Phenylpropa-noid, glycosylated	87	*Galium spp.*	5.47	595.1663	Unclassified
81	*Galium spp.*	5.48	547.14532	Unclassified	90	*Galium spp.*	5.84	547.1453	Unclassified
82	*Galium spp.*	5.73	463.12566	Unclassified	89	*Galium spp.*	5.73	463.1281	Unclassified
83	*Galium spp.*	5.84	547.14493	Unclassified	42	*G. mollugo*	5.49	547.1437	Unclassified
89	*Galium spp.*	6.62	267.02998	Unclassified	94	*Galium spp.*	6.6	267.0297	Unclassified
91	*Galium spp.*	6.94	269.04756	Unclassified	95	*Galium spp.*	6.92	269.0454	Unclassified
92	*Galium spp.*	7.03	253.05071	Polyketide	96	*Galium spp.*	7.02	253.0503	Unclassified
94	*Galium spp.*	7.22	313.0712	Flavonoid	97	*Galium spp.*	7.2	313.0709	Flavonoid, (Kaempferol)
96	*Galium spp.*	7.87	269.04591	Unclassified	101	*Galium spp.*	7.85	269.0458	Unclassified
97	*Galium spp.*	8.05	255.03005	Flavonoid	103	*Galium spp.*	8.01	255.0302	Unclassified
99	*Galium spp.*	8.79	301.10803	Unclassified	105	*Galium spp.*	8.75	301.1078	Unclassified
100	*Galium spp.*	8.8	232.03736	Unclassified	104	*Galium spp.*	8.75	232.0375	Unclassified
101	*Galium spp.*	9.87	367.11863	Unclassified	64	*G. mollugo*	9.84	367.1184	Unclassified
106	*P. lanceolata*	1.09	381.09511	Unclassified, sulfated	108	*P. lanceolata*	1.08	381.0951	Unclassified, sulfated/phosphorylated
107	*P. lanceolata*	1.45	409.04766	Unclassified, sulfated	110	*P. lanceolata*	1.44	409.0453	Unclassified, sulfated/phosphorylated
108	*P. lanceolata*	1.83	439.05513	Unclassified, sulfated	111	*P. lanceolata*	1.79	439.0564	Unclassified, sulfated/phosphorylated
114	*P. lanceolata*	3.42	401.14426	Polyphenole, Hydroxycinnamic acid	121	*P. lanceolata*	3.46	401.1447	Hydroxycinnamic acid

122	*P. lanceolata*	3.68	261.0437	Unclassified, sulfated	127	*P. lanceolata*	3.7	261.0434	Unclassified, sulfated/phosphorylated
127	*P. lanceolata*	4.03	931.28229	Iridoglycoside	126	*P. lanceolata*	3.67	931.2829	Terpene, Iridio glycoside
139	*P. lanceolata*	4.3	611.19774	Phenylpropa-noid	138	*P. lanceolata*	4.31	611.1987	Hydroxycinnamic acid
144	*P. lanceolata*	4.55	595.20091	Phenylpropa-noid, glycosylated	142	*P. lanceolata*	4.55	595.203	Hydroxycinnamic acid, glycosylated
153	*P. lanceolata*	4.72	235.02909	Unclassified, sulfated	145	*P. lanceolata*	4.7	235.0292	Unclassified
154	*P. lanceolata*	4.72	459.19136	Flavonoid, glycosylated	146	*P. lanceolata*	4.73	459.1889	Flavonoid, glycosylated
156	*P. lanceolata*	4.77	645.18102	Phenylpropa-noid	147	*P. lanceolata*	4.78	645.1817	Flavonoid
158	*P. lanceolata*	4.83	487.18138	Glycoside	148	*P. lanceolata*	4.84	487.1812	Unclassified
159	*P. lanceolata*	4.84	585.16223	Iridoglycoside	150	*P. lanceolata*	4.85	585.1615	Hydroxycinnamic acid, glycosylated
161	*P. lanceolata*	4.96	429.17621	Unclassified	153	*P. lanceolata*	4.96	429.1762	Unclassified
178	*P. lanceolata*	6.08	443.19255	Glycoside	164	*P. lanceolata*	6.08	443.1919	Unclassified
181	*R. acris*	3	477.16081	Unclassified	171	*R. acris*	2.99	477.1606	Hydroxycinnamic acid, glycosylated
190	*R. acris*	4.47	405.17841	unclassified, glycosylated	180	*R. acris*	4.47	405.1769	Unclassified
192	*R. acris*	4.78	427.19573	Unclassified	181	*R. acris*	4.79	427.196	Unclassified
197	*R. acris*	5.24	269.00934	Unclassified	184	*R. acris*	5.24	269.0107	Unclassified
29	*A. pratensis*	3.3	347.04423	Polyphenole, Hydroxy-cinnamic acid	185	*A. pratensis*	3.31	347.0439	Hydroxycinnamic acid
36	*A. elatius*	5.92	563.31879	Terpene, glycosylated	198	*A. elatius*	5.93	563.3213	Unclassified
38	*A. elatius*	7.57	547.32609	Unclassified, Aromatic acid	208	*A. elatius*	7.54	547.3261	Unclassified

104	*L. perenne*	5.6	305.13879	Phenylpropa-noid	107	*L. perenne*	5.59	305.1389	Hydroxycinnamic acid