Spatio-temporal plant-pollinator interactions and floral nectar quality in a plant diversity experiment

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Summary

The worldwide decline of plant and insect species during the last decades has far-reaching consequences for the functionality of ecosystems and their inherent processes. Pollination as one of them is an indispensable ecosystem service for human wellbeing. More than 85% of the worldwide flowering-plant species depend to some degree on pollination by insects (pollinators). Similarly, many pollinators depend on the flowers of the plants, as they need nectar and pollen as food resources for themselves and their offspring. However, an increasing number of pollinator and plant species are threatened by multiple, interacting, and sometimes synergistic causes (habitat loss, fragmentation, diseases, parasites, pesticides, monocultures) that are becoming a growing threat to ecosystem functioning. Given the loss of plant species diversity, it is increasingly difficult for pollinators to find food throughout the year. Therefore, this study analyses the influence of plant diversity on pollinators. The study was conducted in the course of the Jena Experiment, which is a long-term biodiversity experiment (since 2002) with 60 plant species, common to Central European Arrhenatherum grasslands. With a plant diversity gradient of 1, 2, 4, 8, 16, and 60 plant species per plot, time-series data resulted from a wide range of ecosystem processes, ranging from productivity, decomposition, C-storage, and N-storage to herbivory, and pollination. These were studied to investigate the mechanisms underlying the relationships between biodiversity and ecosystem processes.

Chapter 2 studies the spatio-temporal distribution of pollinators on flowers along an experimental plant diversity gradient. For this purpose, the pollinators were divided into four different functional groups, i.e. honeybees, bumblebees, solitary bees and hoverflies. In particular, the spatial pollinator behaviour was examined, that is, in which flowering height the flowers were visited within the plant community. In order to study the temporal component, pollinator visits were observed over the course of the day and the season. As a result, an unprecedented high resolution of plant-pollinator interactions was found. For the first time it was possible to demonstrate that the different pollinator functional groups can complementarily use different spatio-temporal niches which was most pronounced in species-rich plant mixtures. This leads to the conclusion that species-rich plant mixtures provide sufficient resources that can be used by generalists, such as honeybees and bumblebees, as well as other pollinator functional groups, such as hoverflies and solitary bees.

Chapters 3 and 4 continues on the chemical composition of flower nectar (nectar) of various plant species. Nectar is used as food resource for adult pollinators, but is also largely used

as a supply for their offspring, making it the most important pollinator reward. The chemical composition of the nectar was analysed for the two most important macronutrients, carbohydrates (C) and amino acids (AA), using high performance liquid chromatography (HPLC). Subsequently, their contents were analysed in terms of concentration, proportional content and the ratio of carbohydrates to amino acids (C:AA).

In Chapter 3, the nectar of 34 plant species from the grasslands of the Jena Experiment was compared. In doing so, similarities and/or differences of the nectar compositions were investigated with respect to the most important macronutrients carbohydrates and amino acids between the individual species but also between the most representative plant families. This should lead to a better understanding about how plant diversity influences consuming pollinators and which factors, e.g. phylogenetics, morphology or ecology, can lead to different nectar compositions. We could show that each plant species differs in terms of carbohydrate content, amino acid content and C:AA-ratio. In addition, there were clear differences between the four representative plant families Apiaceae, Asteraceae, Fabaceae and Lamiaceae regarding the proportions of essential amino acids. The proportions of the individual sugars and the C:AA-ratios also differed greatly between the four plant families. Therefore, it can be assumed that these nectar contents are family-specific. The need for differences in carbohydrate content are probably due to the different morphology of the flowers, as plants with open flowers and exposed nectar, as in Apiaceae and Asteraceae, can protect their nectar from evaporation if the nectar has a higher osmolality, which can be achieved by a higher hexose (fructose and glucose) content. Thus, the nectar can remain dilute for a longer time and consequently remain consumable for pollinators, which in turn can contribute to the pollination of plants. Fabaceae and Lamiaceae showed different results. Here the nectar was probably protected from evaporation by closed flowers, which explains the high proportion of sucrose, leading to a lower osmolality that would enhance evaporation for exposed nectar. The metabolic pathways controlling the family-specific C:AA-ratios are yet to be explored. In conclusion, it can be suggested that this study contributes to elucidating the morphological and phylogenetic characteristics that control each plant species' nectar composition.

In Chapter 4, nectar was investigated in the context of diversity effects on the example of the plant species Field Scabious, *Knautia arvensis*. It was analysed to what extent the nectar quality (nutrient content) differs between plant individuals of one species. The underlying factors causing these differences in nectar composition have never been studied before. In order to investigate these coherences, plant communities in the Jena Experiment of different plant species richness levels containing the target plant species *K. arvensis* were used. In particular, we examined whether the nectar of *K. arvensis* is influenced by other

neighbouring plant species, e.g. through competition for pollinators. The carbohydrate and amino acid content in nectar varied both between individuals of *K. arvensis* and between the different plant species richness levels. However, there were significant non-linear differences in the proportions of certain essential and phagostimulatory amino acids, which were produced proportionally more in the nectar of *K. arvensis* plants in species-rich plant communities, while histidine, one of the generally inhibiting amino acids tended to be less present. Our findings therefore suggest that the nectar of *K. arvensis* is more palatable when the plants grow in species-rich plant communities.

Overall, these studies indicate how fragile plant-pollinator interactions are but also how important plant species-rich grasslands are to support plant-pollinator interactions. Increased plant species diversity is essential to ensure the availability of flowering resources throughout the year. Pollinators, such as honeybees, bumblebees, solitary bees, and hoverflies can use the niches in time and in vertical space complementarily. However, in plant species-poor grasslands there may be more niche overlaps, which is probably due to a reduced availability of resources. This points to the need to include different plant species belonging to different plant families, whose nectar may have evolved in response to morphological flower traits and metabolic pathways. Therefore plant species diversity can supply pollinators with nectar differing in carbohydrate and amino acid content and thus differing in quality. Also C-AA ratios have proven to be a useful measurement to reveal differences between plant species. In addition, C:AA ratios were not differing in nectar of *K. arvensis* individuals growing in different plant species richness levels, although their nectar seemed to be more attractive in mixtures with 16 plant species, likely due to higher content of essential and phagostimulatory amino acids than in plant species-poor mixtures. Thus further research investigating diversified farming systems, including pollinator-friendly practices to reveal the attractiveness of different plant species. More diversified field margins and grasslands, for the maintenance of pollinator services for sustainable provision of crop pollination.

Zusammenfassung

Der weltweite Rückgang der Pflanzen- und Insektenarten während der letzten Jahrzehnte hat weitreichende Folgen für die Funktionalität von Ökosystemen und deren immanenten Prozesse. Bestäubung von Pflanzen ist eine auch für den Menschen unverzichtbare Ökosystem-Dienstleistung. Hierbei sind mehr als 85% aller Blütenpflanzen zu einem gewissen Grad auf eine Bestäubung durch Insekten (Bestäuber) angewiesen. Ebenso sind viele Bestäuber abhängig von den Blüten der Pflanzen, da sie Nektar und Pollen als Nahrungsquellen für sich und ihren Nachwuchs benötigen. Immer mehr Bestäuber- und Pflanzenarten geraten jedoch in Bedrängnis durch vielfältige, wechselwirkende und manchmal synergistisch wirkende Ursachen (Habitatverlust, Fragmentierung, Krankheiten, Parasiten, Pestizide, Monokulturen), die eine stärker werdende Bedrohung für funktionsfähige Ökosysteme werden. In Anbetracht des Verlusts der Pflanzenartenvielfalt können Bestäuber über das ganze Jahr gesehen kaum genügend Nahrung finden. Darum wird in dieser Studie analysiert, welchen Einfluss die Pflanzenartenvielfalt auf Bestäuber hat. Die Studie wurde im Rahmen des Jena-Experiments durchgeführt, bei dem es sich um ein Biodiversitäts-Langzeitexperiment (seit 2002) mit insgesamt 60 Pflanzenarten handelt, die typisch für mitteleuropäische Glatthafer-Wiesen sind. Mit einem Pflanzendiversitätsgradienten von 1, 2, 4, 8, 16 und 60 Pflanzenarten pro Fläche konnten Zeitreihen für ein breites Spektrum an Ökosystemprozessen wie z. B. der Produktivität, dem Stoffabbau, der Kohlenstoff- und Stickstoffspeicherung, Schädigung von Pflanzen durch Fraßfeinde sowie der Bestäubung aufgenommen werden. Diese wurden analysiert, um die Mechanismen zu untersuchen, die der Beziehung zwischen Biodiversität und Ökosystemprozessen zugrunde liegen.

Im Kapitel 2 wird die räumlich-zeitliche Verteilung von Bestäubern auf Blüten entlang eines experimentellen Pflanzendiversitätsgradienten untersucht. Hierfür wurden die Bestäuber in die vier funktionellen Bestäubergruppen Honigbienen, Hummeln, Solitärbienen und Schwebfliegen unterteilt. Insbesondere wurde das räumliche Bestäuberverhalten betrachtet, das heißt, in welcher Höhe die Blüten innerhalb der Pflanzengemeinschaft besucht wurden. Zur Untersuchung der zeitlichen Komponente wurden zum anderen die Bestäuberbesuche über den tages- und jahreszeitlichen Verlauf hinweg untersucht. Dies führte dazu, dass eine nie zuvor dagewesene hohe Auflösung der Pflanzen-Bestäuber-Interaktionen aufgenommen wurde. Hiermit konnte erstmals nachgewiesen werden, dass in besonders artenreichen Pflanzenmischungen die unterschiedlichen funktionellen Bestäubergruppen verschiedene räumlich-zeitliche Nischen komplementär nutzen können. Dies lässt wiederum den Rückschluss zu, dass artenreiche Pflanzenmischungen genügend Ressourcen bieten, die

sowohl Generalisten, wie Honigbienen und Hummeln, als auch andere funktionelle Bestäubergruppen, wie Schwebfliegen und Solitärbienen, nutzen können.

Die Kapitel 3 und 4 beschäftigen sich mit der chemischen Zusammensetzung von Blütennektar (Nektar) verschiedener Pflanzenarten. Nektar dient adulten Bestäubern als Nahrung, wird aber auch vielfach als Versorgung für ihre Nachkommen gesammelt und stellt damit die wichtigste Belohnung für Bestäuber dar. Die chemische Zusammensetzung des Blütennektars wurde auf die beiden wichtigsten Makronährstoffe Kohlenhydrate (C) und Aminosäuren (AA) mit Hilfe von Hochleistungsflüssigkeitschromatographie (HPLC) analysiert. Anschließend wurde Nektar in Bezug auf Konzentration, prozentualen Gehalt und dem Verhältnis von Kohlenhydraten zu Aminosäuren (C:AA) untersucht.

In Kapitel 3 wurde der Nektar von 34 Pflanzenarten der Graslandflächen aus dem Jena Experiment miteinander verglichen. Dabei sollten Ähnlichkeiten bzw. Unterschiede der Nektarzusammensetzungen in Hinblick auf die wichtigsten Makronährstoffe (Kohlenhydrate und Aminosäuren) zwischen den einzelnen Arten aber auch zwischen den repräsentativsten Pflanzenfamilien untersucht werden. Dies soll zu einem besseren Verständnis darüber beitragen, welchen Einfluss Pflanzendiversität auf konsumierende Bestäuber hat und welche Faktoren, z.B. Phylogenie, Morphology oder Ökologie, zu unterschiedlichem Nektargehalt führen können. Es hat sich gezeigt, dass sich alle Pflanzenarten hinsichtlich der Gehalte an Kohlenhydraten, Aminosäuren und der C:AA-Verhältnisse signifikant voneinander unterscheiden. Außerdem gab es klare Unterschiede zwischen den vier repräsentativen Pflanzenfamilien der Doldenblütler (Apiaceae), Korbblütler (Asteraceae), Schmetterlingsblütler (Fabaceae) und Lippenblütler (Lamiaceae) hinsichtlich der prozentualen Anteile der essentiellen Aminosäuren. Auch bei den prozentualen Anteilen der einzelnen Zucker und den C:AA-Verhältnissen waren starke Unterschiede zwischen den vier Pflanzenfamilien vorhanden. Daher ist anzunehmen, dass diese Nektargehalte familienspezifisch sind. Die Notwendigkeit für Unterschiede in den Kohlenhydrat-Anteilen ließen sich durch die unterschiedliche Morphologie der Blüten erklären, da Pflanzen mit offenen Blüten und damit exponiertem Nektar, wie bei Apiaceae und Asteraceae, ihren Nektar vor Verdunstung schützen können, wenn der Nektar eine höhere Osmolalität aufweist, was durch einen höheren Hexose-Anteil (Fruktose und Glukose) erreicht werden kann. Somit bleibt der Nektar länger flüssig und in der Konsequenz für potenzielle Bestäuber länger konsumierbar, was wiederum zum Bestäubungserfolg der Pflanzen beitragen kann. Fabaceae und Lamiaceae zeigten gänzlich andere Ergebnisse. Hier wurde der Nektar wahrscheinlich durch geschlossene Blüten vor Verdunstung geschützt, was den hohen Saccharose-Anteil erklärt, der eine niedrigere Osmolalität bedingt und bei einer Exposition des Nektars höhere Verdunstungsraten verursachen würde. Die Stoffwechselwege, durch

die die familienspezifischen C:AA-Verhältnisse bedingt werden, müssen noch weitergehend untersucht werden. Rückschließend lässt sich aber sagen, dass diese Studie dazu beiträgt, dass die Nektarzusammensetzung sowohl von morphologischen als auch phylogenetischen Merkmalen gesteuert ist.

In Kapitel 4 wurde der Einfluss von Diversitätseffekten auf den Nektar am Beispiel der Ackerwitwenblume, *Knautia arvensis*, untersucht. Hierbei wurde betrachtet, inwiefern sich die Nektarqualität (Nährstoffgehalt) zwischen Pflanzenindividuen einer Art unterscheidet. Die zugrundeliegenden Faktoren, die die Unterschiede im Nektargehalt steuern, waren bisher noch nicht näher untersucht worden. Es wurden daher im Jena Experiment Pflanzengemeinschaften unterschiedlicher Diversität genutzt, die zusammen mit der Zielpflanze *K. arvensis* wuchsen. Dabei sollte vor allem beleuchtet werden, ob der Nektar von *K. arvensis* durch andere benachbarte Pflanzenarten beeinflusst wird, z. B. durch den Wettbewerb um Bestäuber. Der Kohlenhydrat- und Aminosäuregehalt im Nektar variierte sowohl zwischen den *K. arvensis*-Individuen als auch zwischen den verschiedenen Diversitätsstufen. Jedoch gab es in den Proportionen signifikante, nicht lineare Unterschiede bei einigen essentiellen und phagostimulatorischen Aminosäuren, die höher konzentriert im Nektar von *K. arvensis*-Pflanzen artenreicher Pflanzengemeinschaften gefunden wurden, während die hemmende Aminosäure Histidin tendenziell weniger enthalten war. Dies lässt darauf schließen, dass der Nektar von *K. arvensis* für Insekten attraktiver ist, wenn die Pflanzen in artenreicheren Pflanzengemeinschaften wachsen.

Insgesamt zeigt diese Studie, wie anfällig Pflanzen-Bestäuber-Interaktionen sind, aber auch, wie wichtig pflanzenartenreiche Wiesen sind für den Erhalt von Wechselwirkungen zwischen Pflanzen und Bestäubern sind. Eine erhöhte Artenvielfalt der Pflanzen ist wichtig, damit es genügend blühende Ressourcen über das ganze Jahr durchgehend geben kann. Bestäuber, wie Honigbienen, Hummeln, Solitärbienen und Schwebfliegen, können die räumlich-zeitlichen Nischen komplementärer nutzen, während in pflanzenartenarmen Wiesen mehr Nischenüberschneidungen vorkommen, was auf weniger verfügbare Ressourcen schließen lässt. Meine Forschung weist auf die Notwendigkeit hin, Pflanzenarten einzubeziehen, die zu verschiedenen Pflanzenfamilien gehören, deren Nektar sich möglicherweise als Reaktion auf morphologische Blütenmerkmale und Stoffwechselwege entwickelt hat. Daher ist erhöhte Artenvielfalt der Pflanzen notwendig, um Bestäuber mit Nektar versorgen zu können, der sich im Kohlenhydrat- und Aminosäuregehalt unterscheidet und sich somit in der Qualität unterscheidet. Auch C-AA-Verhältnisse haben sich als ein nützliches Maß erwiesen, um Unterschiede zwischen Pflanzenarten aufzudecken. Darüber hinaus unterschieden sich die C:AA-Verhältinisse im Nektar von *K. arvensis*-Individuen, die in unterschiedlichen Diversitätsstufen von Pflanzenarten wuchsen, nicht. Dennoch schien Nektar aus Mischungen

mit 16 Pflanzenarten wegen des höheren Gehalts an essentiellen und phagostimulatorischen Aminosäuren attraktiver zu sein als aus artenärmeren Mischungen. Daher möchte ich dazu anregen, weitere Studien hinsichtlich diversifizierte Landwirtschaftssysteme zu betreiben, einschließlich bestäubungsfreundlicher Praktiken, um die Attraktivität verschiedener Pflanzenarten aufzuzeigen. Ich empfehle auch, breite Feldränder und artenreiches Grünland zu erhalten, um eine nachhaltige Bestäubung von Nutzpflanzen in der Zukunft aufrecht zu sichern.

Chapter 1

Spatio-temporal plant-pollinator interactions and floral nectar quality in a plant diversity experiment

1.1 General Introduction

1.1.1 Endangered biodiversity and pollination

Biodiversity is the variety of life, encompassing the diversity of genes, taxa, ecosystems, habitats, and functions (Hillebrand & Matthiessen 2009; Potts *et al.* 2010; Cardinale *et al.* 2012; Weisser *et al.* 2017). At present, global biodiversity is facing a human-induced continuous decline (Sala 2000; Hooper *et al.* 2005; Rands *et al.* 2010). Important drivers of biodiversity loss include, for example, land-use intensification (herein Tscharntke *et al.* 2005), pesticides use (Kevan *et al.* 1997; Goulson *et al.* 2015) and global warming. Several studies have shown that such drivers can cause mismatches in species-interactions, such as plant-pollinator mutualisms (Memmott *et al.* 2007), and evidence exists that this may in turn reduce ecosystem stability (Hautier *et al.* 2015; Isbell *et al.* 2015). One of the most important ecosystem functions is animal-mediated pollination, which constitutes a key ecosystem service to humanity (Klein *et al.* 2007; Kremen *et al.* 2007; Gallai *et al.* 2009; Potts *et al.* 2010; Leonhardt *et al.* 2013). However, the recent decline in global biodiversity is also substantially affecting plant-pollinator interactions (Biesmeijer *et al.* 2006; Pimm *et al.* 2014): Over the last 27 years insect biomass declined by 76% (Hallmann *et al.* 2017) in protected areas in some parts of Germany. As an estimated 87,5% of worldwide flowering plant species are pollinator dependent (Ollerton *et al.* 2011), an ongoing of this trend will without doubt cause massive upheavals in most terrestrial ecosystem nexuses.

The proportion of global food crops relying on the plant-pollinator interaction to a variable extend is estimated at about 75%, which account for 35% of our global crop production volume (Klein *et al.* 2007). Pollinator services for food production were estimated to cause an economic benefit of 153 billion € per year (reference year 2005), accounting for 9.5% of the annual agricultural production value at that time (Gallai *et al.* 2009; Garratt *et al.* 2016; Potts *et al.* 2016; Klein *et al.* 2018).

The most important insect pollinators worldwide are bees (Klein *et al.* 2007, 2018). This is mainly due to their dense hair structure, which easily picks up and transports pollen and unintentionally leaves it on the flowers' stigmata (Michener 2007). Another reason why bees are outstandingly valuable for pollination is the fact, that true social bees, such as honey bees (*Apis mellifera* L.), as well as other eu-social and semi-social wild bees, appear in high numbers (Klein *et al.* 2007, 2018; Aizen & Harder 2009; Ollerton *et al.* 2011; Dicks *et al.* 2016; Potts *et al.* 2016). Bees on the other site are fully reliant on flowers as nutrition (Michener 2007). Thus, changes in abundance of one of them will cause changes in the abundance and diversity of the other (Ebeling *et al.* 2008).

Thus, there is a mutual dependency between plants and pollinators linking biodiversity and food production. It is thus important to understand the consequences of biodiversity changes for ecosystem services (Biesmeijer *et al.* 2006; Goulson 2013; Goulson *et al.* 2015), especially pollination, and to find the key mechanisms underlying the biodiversity-ecosystem functioning relationship (Balvanera *et al.* 2013; Weisser *et al.* 2017).

1.1.2 Biodiversity research in the Jena Experiment

The study of plant-pollinator interactions can be conducted in real world studies across natural plant diversity gradients (Kühsel & Blüthgen 2015; Kämper *et al.* 2017) or in biodiversity experiments, in which the plant species richness is experimentally manipulated, such as Jena Experiment (Ebeling *et al.* 2008, 2011), which is a large scale multidisciplinary research project that investigates in the biodiversity-ecosystem functioning relationship and their underlying mechanisms (Roscher *et al.* 2004). An advantage of real world studies is, that natural plant communities have already established for a long time, but these studies cannot regulate plant community compositions. This lacking control makes it difficult to reveal the causes of a response in, for example, plant-pollinator interactions.

The Jena Experiment, as a representative of the biodiversity experiments, is a manipulated plant diversity experiment with a large target plant species pool of 60 plant species, which belong to the Central European *Arrhenatherum* meadows (Molinio-Arrhenatheretea meadows, Arrhenatherion community (Ellenberg 1996)) (see Table 1.1). The advantage to study plant-pollinator interactions in a framework like the Jena Experiment, is the use of a manipulated plant species richness gradient to control for plant species richness *per se* as an independent variable which is not confounded with an effect of the surrounding landscape. The use of artificial plant communities allows to analyse the response of plant-pollinator interactions to extremes like monocultures or highly diverse mixtures, which rarely exist in nature.

In the Jena Experiment, many measurements that are focusing on ecosystem processes are taken simultaneously on the same plots, resulting in time-series data, which widely ranges from productivity, decomposition, C-storage, and N-storage to herbivory, and pollination. Therefore the Jena Experiment is one of the longest lasting and biggest biodiversity experiments of Europe (16 years to date, 10 ha), contributing to a better understanding of biodiversity effects on ecosystem functioning (Weisser *et al.* 2017).

The Jena Experiment is located in the north of Jena (Jena-Loebstedt, Thuringia), Germany, on the flood plain of the river Saale (50°55´N, 11°35´E; 130 m a.s.l.) and was established in

May 2002. Before, it was used as an arable field for about 40 years and highly fertilized for the cultivation of wheat and vegetable.

The sown plant mixtures form a plant diversity gradient, each mixture consisting of either 1, 2, 4, 8, 16, or 60 plant species, respectively. The plant species were randomly selected from the plant species pool. Further, the plant species were divided into four functional groups accounting for differences in their traits: morphological (e.g. growth form, clonal growth, above- and below-ground species performances), phenological (e.g. start and duration of flowering period), and physiological (Nitrogen fixation). All traits were analysed using multivariate statistics which resulted in four different functional groups: grasses, small herbs, tall herbs, and legumes (for more details see Roscher *et al.* (2004)) (see Figure 1.1). Furthermore, the number of replicates in each diversity level including the different functional groups resulted in total of 82 mixtures (plots) (see Fig. 1.1). Plant species richness levels with 1 – 8 plant species per plot were replicated 16 times and plant species richness levels with 16 and 60 plant species 14 and four times, respectively. Due to the abiotic gradient of the soil-sand content (distance to the river Saale), four blocks each with about 22 plots are organized perpendicular to this gradient (randomized complete block design). Each block was set up with the same number of replicates of plant species richness levels. For maintenance of the sown species mixtures, all plots were weeded twice per year until 2009 and afterwards weeded three times (April, July and October).

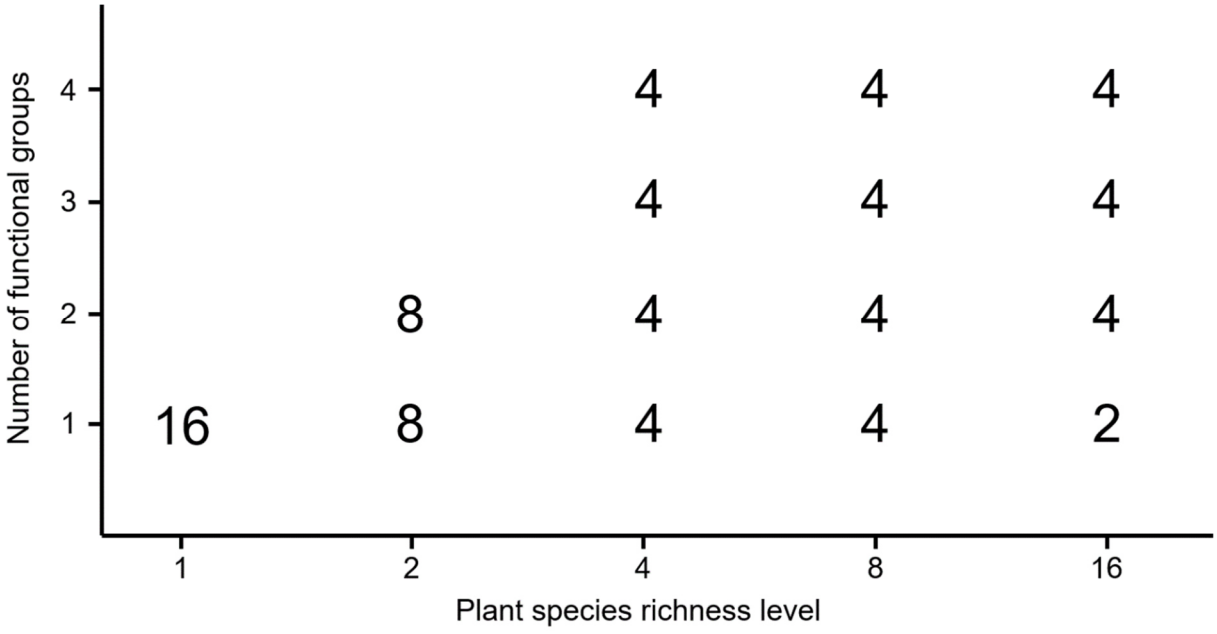

Figure 1.1: Number of plant species mixtures regarding possible combinations of functional groups in different plant species richness levels. .

Table 1.1: Plant species pool of 60 plant species used in the Jena Experiment, belonging to the the Central European *Arrhenatherum* meadows and divided into four functional groups (Ellenberg 1996; herein Roscher *et al.* 2004), supplemented with an indication if plant species were included in the different studies.

Functional Group	Plant species	Family	German common name	Investigated in studies: 1, 2, and 3
Grasses (16 species)	*Alopecurus pratensis*	Poaceae	Wiesen-Fuchsschwanz	-
	Anthoxanthum odoratum	Poaceae	Gewöhnliches Ruchgras	-
	Arrhenatherum elatius	Poaceae	Glatthafer	-
	Avenula pubescens	Poaceae	Flaumiger Wiesenhafer	-
	Bromus erectus	Poaceae	Aufrechte Trespe	-
	Bromus hordeaceus	Poaceae	Weiche Trespe	-
	Cynosurus cristatus	Poaceae	Wiesen-Kammgras	-
	Dactylis glomerata	Poaceae	Wiesen-Knäuelgras	-
	Festuca pratensis	Poaceae	Wiesen-Schwingel	-
	Festuca rubra	Poaceae	Gewöhnlicher Rot-Schwingel	-
	Holcus lanatus	Poaceae	Wolliges Honiggras	-
	Luzula campestris	Juncaceae	Feld-Hainsimse	-
	Phleum pratense	Poaceae	Wiesen-Lieschgras	-
	Poa pratensis	Poaceae	Gewöhnliches Wiesen-Rispengras	-
	Poa trivialis	Poaceae	Gewöhnliches Rispengras	-
	Trisetum flavescens	Poaceae	Wiesen-Goldhafer	-
Small herbs (12 species)	*Ajuga reptans*	Lamiaceae	Kriechender Günsel	1 & 2
	Bellis perennis	Asteraceae	Gänseblümchen	1
	Glechoma hederacea	Lamiaceae	Gundermann	1 & 2
	Leontodon autumnalis	Asteraceae	Herbst-Löwenzahn	1 & 2
	Leontodon hispidus	Asteraceae	Rauher Löwenzahn	1 & 2
	Plantago lanceolata	Plantaginaceae	Spitzwegerich	1
	Plantago media	Plantaginaceae	Mittlerer Wegerich	1
	Primula veris	Primulaceae	Wiesen-Schlüsselblume	1 & 2
	Prunella vulgaris	Lamiaceae	Kleine Braunelle	1 & 2
	Ranunculus repens	Ranunculaceae	Kriechender Hahnenfuß	1 & 2
	Taraxacum officinale	Asteraceae	Wiesen-Löwenzahn	1 & 2
	Veronica chamaedrys	Scrophulariaceae	Gamander-Ehrenpreis	1 & 2
Tall herbs (20 species)	*Achillea millefolium*	Asteraceae	Gewöhnliche Wiesen-Schafgarbe	1
	Anthriscus sylvestris	Apiaceae	Wiesen-Kerbel	1 & 2
	Campanula patula	Campanulaceae	Wiesen-Glockenblume	1 & 2
	Cardamine pratensis	Brassiacaceae	Wiesen-Schaumkraut	1 & 2
	Carum carvi	Apiaceae	Wiesen-Kümmel	1
	Centaurea jacea	Asteraceae	Wiesen-Flockenblume	1 & 2
	Cirsium oleraceum	Asteraceae	Kohl-Kratzdistel	1 & 2
	Crepis biennis	Asteraceae	Wiesen-Pippau	1 & 2
	Daucus carota	Apiaceae	Wilde Möhre	1 & 2
	Galium mollugo	Rubiaceae	Wiesen-Labkraut	1
	Geranium pratense	Geraniaceae	Wiesen-Storchschnabel	1 & 2
	Heracleum sphondylium	Apiaceae	Wiesen-Bärenklau	1 & 2
	Knautia arvensis	Dipsacaceae	Wiesen-Witwenblume	1 & 2 & 3
	Leucanthemum vulgare	Asteraceae	Gewöhnliche Margerite	1
	Pastinaca sativa	Apiaceae	Pastinak	1 & 2
	Pimpinella major	Apiaceae	Große Bibernelle	1 & 2
	Ranunculus acris	Ranunculaceae	Scharfer Hahnenfuß	1 & 2
	Rumex acetosa	Polygonaceae	Großer Sauerampfer	1
	Sanguisorba officinalis	Rosaceae	Großer Wiesenknopf	1 & 2
	Tragopogon pratensis	Asteraceae	Wiesen-Bocksbart	1 & 2
Legumes (12 Species)	*Lathyrus pratensis*	Fabaceae	Wiesen-Platterbse	1 & 2
	Lotus corniculatus	Fabaceae	Gewöhnlicher Hornklee	1 & 2
	Medicago lupulina	Fabaceae	Hopfenklee	1
	Medicago varia	Fabaceae	Luzerne	1 & 2
	Onobrychis viciifolia	Fabaceae	Esparsette	1 & 2
	Trifolium campestre	Fabaceae	Feld-Klee	1 & 2
	Trifolium fragiferum	Fabaceae	Erdbeer-Klee	1
	Trifolium dubium	Fabaceae	Gewöhnlicher Kleiner Klee	1
	Trifolium hybridum	Fabaceae	Schweden-Klee	1 & 2
	Trifolium pratense	Fabaceae	Wiesen-Klee	1 & 2
	Trifolium repens	Fabaceae	Weiß-Klee	1 & 2
	Vicia cracca	Fabaceae	Gewöhnliche Vogel-Wicke	1 & 2

1.1.3 Niche complementarity

Coexistence of different plant species can be positively influenced by niche partitioning, therefore plant species, which grow in competition may use narrower niches (von Felten *et al.* 2009). This indirectly also affects the structure of pollinator communities (Potts *et al.* 2003; Ebeling *et al.* 2008) through the associated change of floral traits (Binkenstein *et al.* 2013).
Niche partitioning of pollinators has not been investigated yet. The classical niche concept (Hutchinson 1957; modified by Holt 2009) implies demographic parameters as niche-influencing factor. This has not yet been analysed at a pollinator community level, which is due to the fact that the measurement of these parameters is not feasible if it is applied to small organisms, such as pollinating insects. Another way to investigate niche overlap in pollinator communities is to focus on foraging ranges of the pollinators, thereby involving spatio-temporal niches, which is part of the concept of environmental niches (Tracy & Christian 1986; Chesson *et al.* 2001). The biodiversity-niche hypothesis is also applicable to pollinators, since they can use different spatio-temporal niches, which may be influenced by plant diversity (MacArthur 1955; Loreau & Hector 2001; Rosenfeld 2002; Blüthgen & Klein 2011).

There are different possibilities how plant diversity can influence the spatio-temporal niches of pollinators. On the one hand, plant species numbers can influence spatio-temporal use of resources due to differing species-specific resource use. On the other hand plant diversity could cause changes in vegetation parameters via vegetation structure, floral abundance, or in abiotic conditions, such as microclimate, which has an effect on the resource use of the pollinators, e.g. by changing traits like nectar viscosity. Also phenological and architectural niche complementarity could play a role for different functional pollinator groups (Blüthgen & Klein 2011).

Spatial complementarity was observed for pollinator communities on flowering pumpkin plants (Hoehn *et al.* 2008) and on almond trees (Brittain *et al.* 2013a). For a better understanding of biodiversity-ecosystem functioning relationship it is substantial to focus on pollinators with a main emphasis on spatio-temporal resource diversity. Using an experimentally manipulated plant species diversity gradient allows to aim at a higher resolution in space, time and resources.

1.1.4 Nectar diversity and production

Among angiosperms, floral nectaries have convergently evolved at different times (Fahn 1988; Lee 2005). Thus the origin, i.e. from which tissue they emerge, and position within the

flower can vary in different plant species. Nevertheless, most nectaries are situated at the base of the flower (Pacini *et al.* 2003; Bernardello 2007). Though pollen for pollinators is an important supplier for protein, which is crucial for reproduction and larval nutrition (Heinrich 1981), it has been shown that nectar is less expensive to produce than pollen to attract pollinators (Willmer 2011b). In order to gather enough pollen to rear one offspring, bees have to visit a high number of flowers, ranging from 20 to over 1,000, depending on the bee and plant species (Müller *et al.* 2006). Thus, bees have to compensate the energy costs that emerge with each flower visit for pollen by visiting more plants to feed on nectar (Willmer 2011a). Nectar is recognised as the most attractive reward for pollinators (Simpson & Neff 1983), since the nutrition of many adult pollinators, e.g. butterflies, entirely relies on nectar to outbalance malnutrition at the time of larval development (Erhardt & Rusterholz 1998). Some pollinators, e.g. bees, collect nectar for their offspring or even as reserves for winter, as in the case of honey bees (Heinrich 1981). Bees collect nectar for direct energy uptake, since the contained carbohydrates can easily be metabolized (Willmer 2011b), and also to extend flight range/time (Beenakkers *et al.* 1984; Micheu *et al.* 2000). Pollinators were already suspected to drive the selective pressure on floral adaptations by Darwin (1877) and recent studies could confirm this idea of coevolution (Whittall & Hodges 2007; van der Niet & Johnson 2012; Schiestl & Johnson 2013; Newman *et al.* 2015).

The main ingredients or macronutrients of nectar are carbohydrates, which are substantial for adult insects, such as bees and butterflies. Furthermore it can contain a diversity of amino acids (Heinrich 1981; Wcislo & Cane 1996; Carter *et al.* 2006) and some have been shown to be essential and non-essential for nutritional requirements of honeybees (see Table 1.2) (sensu De Groot 1953). Among carbohydrates, disaccharide sucrose and two hexoses, glucose and fructose, are the most common sugars contained in nectar. The sucrose to hexose ratio varies and is supposed to be plant species-specific and similar between species of the same family or tribe (Percival 1961; Bernardello *et al.* 1994; Wolff 2006; Witt *et al.* 2013), which was also suggested by Baker & Baker (1986) for amino acid profiles.

Floral nectar profiles in terms of carbohydrate and amino acid composition are assumed to be similar between plant species of the same family due to shared morphological traits, i.e. shape of flowers or occurrence of hair, that both can help to reduce evaporation of water from the nectar (Witt *et al.* 2013). Another option could be similarity in composition as a consequence of close genetic relatedness (Van Wyk *et al.* 1993; Perret *et al.* 2001; Kaczorowski *et al.* 2005). This may act via similar metabolic pathways and excretion systems. Long floral tubes as found in Caryophyllaceae (Witt *et al.* 2013) produce sucrose-rich nectar, proving that nectar with low osmolality is protected from evaporation by flower morphology. This is in contrast to open flowers with exposed nectaries, which contain hexose-rich nectar,

having thereby a higher osmolality which prevents evaporation, too (Bernardello 2007; Witt *et al.* 2013).

Table 1.2: Essential and non-essential amino acids (AA) (sensu De Groot 1953) are grouped in different taste classes for two fly species *Boettcherisca peregrina* and *Phormia regina* (Shiraishi & Kuwabara 1970)

	Class 1 - no effect –	**Class 2** - inhibitory -	**Class 3** - salt cell stimulatory -	**Class 4** - sugar cell stimulatory -
NON - ESSENTIAL AA	Alanine Cystine Glycine Serine Tyrosine	Aspartic acid Glutamic acid	Proline	
ESSENTIAL AA	Threonine	Arginine Histidine Lysine		Isoleucine Leucine Methionine Phenylalanine Valine

In some plant families (e.g. Brassicaceae, Asterids, and Legumes) a nectar-specific efflux transporter (SWEET9) was found, which is supposed to transport sucrose into extracellular space (Lin *et al.* 2014). Other metabolic pathways involve phytohormones (Radhika *et al.* 2010; Bender *et al.* 2013; Wang *et al.* 2014) that induce nectar secretion and may be species-specific. Phloem is the most common supplier of nectar carbohydrates and amino acids (De la Barrera & Nobel 2004; Bertazzini & Forlani 2016). Lohaus & Schwerdtfeger (2014) found clear differences between phloem and nectar concentrations, showing that phloem concentrations are higher than in nectar, which is due to selectively driven secretion, resorption, and/or different metabolic pathways of the nectary cells. They compared nectar and phloem, indicating that phloem supplies nectar with all nutrients (e.g. sucrose and amino acids) except hexose carbohydrates, which are the product of sucrose hydrolysis catalysed by invertases in the nectariferous tissue. Cell-wall bound enzymes (invertases) hydrolyse sucrose to fructose and glucose, also in order to prevent sucrose from being transported back to the phloem, thereby forming a source-sink relationship (Escalante-Pérez & Heil

2012). One of the metabolic pathways in the nectary cells was found to result in deviating hexose-sucrose ratios, although expectations were that the transport of sucrose from phloem into the nectary cell and further processing via hydrolysis would result in a one-to-one ratio (Wenzler *et al.* 2008). But due to further intermediary metabolic pathways (i.e. gluconeogenesis, glycolysis, and pentose phosphate pathways) sucrose will be hydrolysed into imbalanced ratios, which can differ from plant species to plant species (Wenzler *et al.* 2008).

The formation of amino acids is complex as well. Some amino acids may be available in the soil and can be taken up by the roots. Plants are also able to synthesise all 20 proteinogenic amino acids by themselves, which most likely happens in chloroplasts (Sonnewald 2014). For the biosynthesis of amino acids, the carbon skeleton is derived from photosynthesis and some other components are taken up from the soil, such as minerals and nitrogen in form of ammonia or nitrate. A special feature allows Leguminosae to accumulate nitrogen from the air via rhizobia in nodules at the roots (Taiz & Zeiger 2000). Subsequently, plants process the collected nitrogen to gain the different amino acids needed. These pathways have developed within the plant kingdom approximately 1.5 billion years ago together with the engulfment of proto-eukaryotic cells, which are preserved as plastids (Wise 2006). Some amino acids function as precursors in cascading pathways with other amino acids as side products in between. For example asparagine is used as a storage molecule for nitrogen, since it is stable and has a very good nitrogen-carbon-ratio in comparison to glutamine (2-4 to 2-5 respectively) (Taiz & Zeiger 2000). But it is also in a competitive interaction with aspartic acid, which is a precursor for other amino acids (threonine, isoleucine, lysine, and methionine) (Lancien *et al.* 2006; Sonnewald 2014).

Other amino acid biosynthesis pathways encompass amino acid families with different origins of the carbon skeleton. This includes different products from the Calvin cycle, such as ribose 5-phosphates (for histidine), 2-phosphoglycolate (for serine, glycine, and cysteine), erythrose 4-phosphate and shikimate and/or, 3-phosphoglycerates and phosphoenolpyruvate (for phenylalanine, tyrosine, and tryptophan). Further pathways include processed pyruvates (for alanine, valine, and leucine), oxaloacetate (for aspartic acid, asparagine, threonine isoleucine, lysine, and methionine), and/or processed to 2-oxoglutarate (glutamic acid, glutamine, arginine, and proline) (for more details Sonnewald 2014).

Regarding the variety of metabolic pathways, it is assumed that closely related plant species due to phylogenetic constraints show more similar nectar compositions in terms of amino acids and carbohydrates.

1.2 General approaches and main research questions

For a better understanding of plant-pollinator interactions I focused my dissertation on three different approaches. The first approach involved comprehensive observations of plant-pollinator interactions along a plant diversity gradient to in order to find out whether the species of the pollinator community use different niches. The second approach involved nectar analyses of 34 flowering plant species (including *K. arvensis*; all growing in the Jena Experiment), which were visited by pollinators from the first approach a year before sampling. The third approach concerned one plant species, Field Scabious, *Knautia arvensis*, which has been shown to be more attractive to pollinators than other flowering grassland plant species (Ebeling *et al.* 2008) and thus was chosen as a model plant species. In this context, nectar was sampled along a plant diversity gradient in the Jena Experiment and chemical compositions were analysed.

Thus, this thesis focuses on the following main questions:

1. Spatio-temporal niches of plant-pollinator interactions (Chapter 2)
    a. Does plant species richness change pollinator flower visitation height and timing within a day either directly or indirectly?
    b. Do individual pollinator functional groups visit different flowering heights in the vegetation and at different times of day, depending on plant species richness?
    c. Are species-rich plant communities characterized by higher complementarity in spatio-temporal resource use?
2. Nectar amino acid and carbohydrate composition, flower morphology and phylogeny between 34 different plant species (Chapter 4)
    a. Do plant species differ from each other regarding nectar amino acid and carbohydrate composition?
    b. Do related plant species have more similar nectar amino acid and carbohydrate compositions than plant species outside the plant family or tribe?
3. Inter-individual differences in nectar quality of Field Scabious (*Knautia arvensis*) (Chapter 3)
    a. Does nectar of plant individuals in species-rich plant mixtures have a higher concentration of carbohydrates, overall amino acids and essential amino acids than nectar from plant individuals from monocultures?
    b. Is the ratio of carbohydrates to overall amino acids and the ratio of carbohydrates to essential amino acids the same across the plant species richness gradient because the ratio is species-specific?

Chapter 2 deals with visitation patterns of pollinator functional groups in plant species mixtures across a plant diversity gradient. I conducted flower-visitation surveys of study plots in the Jena Experiment, which is located in the north of Jena (Jena-Löbstedt, Thuringia, Germany), on the flood plain of the river Saale (50°550N, 11°350E; 130 m a.s.l.). The study was conducted throughout the year 2011 on seven different days, each day from morning to evening, up to seven times per day, the observations lasted 15 min each and were conducted in 19 plant mixtures (plant species mixtures: 5 plots x 4 plant species, 5x8, 5x16, 4x60), with an observation square (0.8 m x 0.8 m) on each mixture, using three categories of flowering heights to locate the visitation of the pollinator (to address questions 1a and 1b). I found that four pollinator functional groups (honeybees, bumblebees, solitary bees, and hoverflies) were influenced by plant species richness with time and space differently (addressing questions 1a and 1 b).

In species-poor mixtures honeybees and bumblebees visited mainly flowers that ranged from the intermediate to the upper flowering height, with a visitation peak at midday, whereas in the species-rich mixtures honeybees limited their visits to the upper flowering height. In the species-rich mixtures bumblebees still visited the intermediate flowering height, but with the number of visits increasing toward the top and shifting the visitation peak towards the evening. For solitary bees flower visitation was confined to species-rich mixtures with a visitation peak at the lower flowering height at midday. Hoverflies preferred to start their visitations early in the morning at all flowering heights in all plant species mixtures. During the course of the day hoverflies shifted progressively towards the upper part of the vegetation. They had their highest numbers of visits early in the morning and in the species-rich mixtures. All four pollinator functional groups showed highest niche complementarity in space and in time in the species-rich mixtures (addressing question 1c).

Chapter 3 deals with differences and/or similarities of nectar nutrient compositions (carbohydrates: sucrose, fructose, glucose; amino acids: essential and non-essential) between 34 plant species with further regard to flower morphology and phylogeny as potential drivers in four abundant plant families (addressing questions 3a and 3b). The selected plant species are typical for mesophilic Arrhenatherion grasslands in Central Europe and were sown in the Jena Experiment. Of each plant species nectar was sampled from plant individuals whose flower heads were protected from feeding pollinators by wrapping the flower heads in gauze overnight beforehand. Nectar sampling was conducted with microcapillaries (1µl). The sampled plant species have been shown to be attractive for pollinators (see chapter 2); for each plant species it was aimed to collect a minimum of seven samples each with a volume of 1µl. After nectar sampling, the microcapillaries were stored in

a freezer (-20 °C), then the nectar content was prepared for chemical analysis and finally analysed via HPLC.

Nectar analysis showed that all plant species had species-specific nectar nutrient compositions (addressing question 3a). Concentration of the total sum of amino acids and of non-essential amino acids differed between the 34 plant species (addressing question 3a). Also the proportion of essential amino acids differed significantly. Highest concentrations of amino acids and essential amino acids were found in nectar of *Crepis biennis* L., *Tragopogon pratensis* L., *Leontodon hispidus* L. (all Asteraceae), and *Sanguisorba officinalis* L. (Rosaceae) (order of decreasing concentration). Highest concentrations of non-essential amino acids were found in *T. pratensis* (Asteraceae)*, Trifolium campestre* Schreb.*, Trifolium hybridum* L.*, Trifolium pratense* L. (all Fabaceae), and *P. veris* (Primulaceae). The highest proportion of essential amino acids was found in *C. biennis* (Asteraceae), *Ajuga reptans* L.*,* and *Prunella vulgaris* L. (both Lamiaceae), but these were low in Fabaceae (*T. campestre*, *T. hybridum*, *T. pratense*, *T. repens*, and *Vicia cracca*) and in *P. veris* (Primulaceae).

Regarding carbohydrates, all analysed plant species differed significantly in total carbohydrate nectar concentration: highest concentrations found in *T. campestre, Lotus corniculatus* L. (both Fabaceae)*, A. reptans* (Lamiaceae)*,* and *T. pratensis* (Asteraceae) and lowest in *Daucus carota* L. and *Pimpinella major* (L.) Huds. (both Apiaceae).

Ratios were generally carbohydrate biased and differed between plant species. The carbohydrate to amino acid (C:AA) ratios ranged from mean ratios of C:AA = 1:1 (for *A. sylvestris* (Apiaceae) and *L. hispidus* (Asteraceae)) to mean ratios largely dominated by carbohydrates C:AA = >20:1 (for *Geranium pratense* L. (Geraniaceae), *A. reptans* and *P. vulgaris*). Also the ratio of carbohydrates to essential amino acids (C:EAA) differed between plant species (addressing question 3a).

With regard to the differences between plant families, the most representative plant families (≥ 3 plant species per plant family), i.e. Apiaceae (5 plant species), Asteraceae (7), Fabaceae (10) and Lamiaceae (3), differed in the composition of all amino acids when concentrations were considered (addressing question 3b). Further, a difference was found in the proportion of all essential amino acids. Solely the essential amino acid histidine tended to differ between the four plant families.

Considering carbohydrate concentrations, differences in compound concentration were found as well as in proportions (addressing question 3b). Plant species belonging to the Apiaceae and Asteraceae produced nectar with low concentrations of sucrose, while Fabaceae and especially Lamiaceae had nectar with high concentrations of sucrose. Also proportions

differed, having low sucrose proportions in nectar of Apiaceae and Asteraceae and high proportions of glucose and fructose, respectively. An almost reversed pattern was found for Fabaceae and Lamiaceae. The nectar of Lamiaceae contained a more pronounced proportion of sucrose and less fructose and glucose compared to Fabaceae. As a contextual result (addressing question 3b), nectar osmolality seems to differ across plant species, which is due to different floral adaptations (morphology). For example, the floral length positively correlates with sucrose concentration. I therefore conclude that there are different adaptations for the four plant families, which support a reduction of nectar evaporation at lowest costs and with the available species-specific metabolic pathways. Thus, plants with more open flowers, such as Apiaceae and Asteraceae, which are exposing nectar open and upwards, may need to decelerate nectar evaporation, which is possible by downregulating sucrose and upregulating hexose, i.e. glucose and fructose, thereby increasing osmolality. The opposite applies to Fabaceae and Lamiaceae. Due to their gullet-shaped (Lamiaceae) or flag-shaped (Fabaceae) flowers, nectar is less exposed, thus comparatively lower amounts of sucrose need to be hydrolysed to hexoses. Finally, I conclude that these four plant families use different ways to decelerate nectar evaporation and thus extend the time to reward pollinators with nectar, which is species-specific in composition, but also shares family-specific similarities.

Chapter 4 aims at answering the questions whether and how floral nectar composition changes if the plant species grow in mixtures with different plant species richness levels (monoculture, four, eight and 16 plant species mixtures). For this I conducted nectar sampling via microcapillaries with a volume of 1µl on flowers of the study plant species *Knautia arvensis* (addressing questions 2a and 2b). Every evening before sampling, flower heads of *K. arvensis* were covered with gauze bags (mesh size 0.8-1.00 mm) to prevent nectar depletion by pollinators. After collecting the nectar, the microcapillaries were stored in a freezer and prepared for chemical analysis. Finally, the nectar contents were analysed by High Liquid Performance Chromatography (HPLC).

The nectar of *K. arvensis* showed non-linear changes of amino acid and carbohydrate concentrations. Nectar amino acid and carbohydrate concentration varied among individuals within the plots, but also with the surrounding plant species mixtures, thus with species richness (addressing question 2a). However, nectar composition of amino acids did not increase with increasing plant species richness (addressing question 2a). Total carbohydrate concentration and individual carbohydrate (i.e. sucrose, glucose, and fructose) concentrations were highest in the mixtures with four plant species and lowest in the mixtures with 16 plant species (addressing question 2a). Regarding amino acids, only proportions of some individual essential amino acids (i.e. valine, isoleucine, leucine, and

lysine) differed significantly and showed highest proportions in nectar of plants in mixtures with 16 plant species, which is also in contrast to what was expected (addressing question 2a). Neither carbohydrate to amino acid (C:AA) ratios, nor carbohydrates to essential amino acids (C:EAA) ratios showed clear differences across the plant species richness gradient. Thus nectar ratio of *K. arvensis* can still be considered species-specific (addressing question 2b). I conclude that although concentrations of carbohydrates and amino acids were lowest from nectar of plant individuals in mixtures with 16 plant species, which seems to contradict expectations (addressing question 2a), nectar quality may be improved due to higher proportions of the four essential amino acids in nectar of plant individuals in mixtures with 16 plant species.

The proportional increase in essential amino acids (valine, isoleucine, leucine, and lysine) in nectar of *K. arvensis* from mixtures with 16 plant species are supposed to act phagostimulatory and may explain why most honeybees (*Apis mellifera* Linnaeus, 1758) were observed on *K. arvensis* plants in the mixtures with 16 species (Supplementary table S2 and S3 of the Appendix C).

1.3 General Discussion

This thesis investigates three topics: First, I studied the response of different pollinator functional groups to plant species richness, by capitalizing on a dataset with unprecedented resolution in space and time. This led to the key insight that changes in plant species richness alter spatial and temporal complementarity in flower visitation of globally important pollinator groups. Second, I used a dataset comprising carbohydrate and amino acid measurements for 34 grassland plant species to investigate between-species and within-family variability of floral nectar composition (carbohydrates and amino acids). I show that nectar is species-specific and that nectar compositions within families are similar. The key drivers of nectar composition seem to be morphological traits and phylogenetic relatedness. Third, I showed that the floral nectar composition of an important nectar source, the Field Scabious (*Knautia arvensis* (L.) Coult. Dipsacaceae), depends on the species richness of the surrounding plant community. In particular, although overall concentrations of carbohydrates and amino acids decreased with plant richness, essential amino acids showed the opposite trends, thus probably increasing overall nectar quality in species-rich plots.

All studies were conducted in the framework of the Jena Experiment. With regard to the experimental plant communities, the manipulation of the plant species richness gradient has been shown to be substantial to unravel the effects of plant species richness from other effects of the habitat, thus giving insights in the complex functioning of ecosystems (Eisenhauer *et al.* 2016; Weisser *et al.* 2017). Although one could argue that the selected plots were not representing natural plant communities, it is important to note, that all species belonged to the same pool of plant species that are generally found in semi-natural mesophilic grasslands (Arrhenatherion grassland) (Roscher *et al.* 2004). Furthermore, the large set of plant combinations in the Jena Experiment allowed us to select a subset of plots that provided floral resources over a longer period and were therefore optimal for repeated surveys of the temporal dynamics of plant-pollinator interactions and the analysis of plant nectar. Finally, the highly replicated plot design of the Jena Experiment gave us the opportunity to cover all measurements on one plot with plant species richness as an independent variable, to gain insight into the complex effects of plant species richness on plant-pollinator interactions.

In summary, my study contributes to a better understanding of the relationship between biodiversity and ecosystem functioning and highlights the need for sustainable agriculture that establishes and maintains plant species-rich habitats.

1.3.1 Three dimensions of niche complementarity

This study shows that plant species richness drives spatial and temporal niche complementarity in plant-pollinator interactions. In particular, I found that the foraging niches of the four most important pollinator functional groups (honeybees, bumblebees, solitary bees, and hoverflies) changed across the experimental plant species richness gradient. When considering the entire pollinator community, the available spatio-temporal niche space was exploited to a higher extent in time (time of day) and space (vertical distribution within the vegetation) in the species-rich communities than in the plant species-poor communities. While niches of the four pollinator groups showed a high spatio-temporal overlap in plant species-poor plots, pollinator niches were more segregated under high plant diversity. However, how and to what degree pollinator groups altered their niche use as a function of plant species richness varied between pollinator groups. For example, honeybees and bumblebees shared a similar spatio-temporal niche, and showed little change with increasing plant species richness. In contrast, hoverflies and solitary bees occupied separate spatio-temporal niches, and both groups also exhibited substantial niche use changes with plant species richness.

Interestingly, temporal and spatial flower visitation patterns were highly dynamic in species-rich plant mixtures, which suggests some stability of pollination services against pollinator-diversity loss (insurance hypothesis). In combination with space and time, plant species richness was a better predictor of niche use than flower cover alone. Resource distribution in species-rich mixtures was more multi-layered than in species-poor mixtures, resulting in a diversity of available niches (Elmqvist *et al.* 2003; Finke & Snyder 2008; Cardinale 2011). Limitations in flower resources due to species-poor plant mixtures resemble crop monocultures, such as strawberry and blueberry fields or orchards leading to a small volume of niche space (Carré *et al.* 2009; Holzschuh *et al.* 2012; Blaauw & Isaacs 2014; Rosa García & Miñarro 2014). Thus, food availability may be low outside the peak flowering period of a few weeks (Rosa García & Miñarro 2014). Additionally, such species-poor mixtures will only offer few niches for pollinators, potentially reducing attractiveness for pollinators.

Overall, these results demonstrate that decreased plant species richness is a critical factor for pollinator communities. Plant species richness indirectly structures resource use and partitioning of pollinators in space and time.

1.3.2 Increased plant diversity promotes nectar diversity

In all 34 analysed plant species of the Jena Experiment it could be shown that nectar compositions of carbohydrates and amino acids differed significantly from each other, indicating that nectar composition is species-specific. But when comparing the most representative plant families with each other, it becomes apparent that nectar between plant family members is more similar to each other than to plant species outside the family. This is made evident by the significant differences in sucrose concentration and proportion, as well as the significant differences in the proportions of hexoses, i.e. glucose and fructose.

The main solute in nectar is represented by carbohydrates (Bernardello *et al.* 1994). Carbohydrate composition is influencing osmolality, which is supposed to change with floral morphology, i.e. the positive correlation of floral tube length with sucrose content (Witt *et al.* 2013). This can be considered as an adaptation, which is directly or indirectly linked to attraction of pollinators (Nicolson 2007). Witt *et al.* (2013) also found that plant species belonging to the family of Caryophyllaceae are similar in terms of morphological traits, and carbohydrate composition.

The different plant families assessed differed in their morphology which in turn influenced their sucrose concentrations. For example, Apiaceae were shown to present their nectar openly (see Fig. 1.2 A, E) (Knuth 1898; Erbar & Leins 2010). Asteraceae have small tubular flowers that are assembled in a pseudanthium (a radially symmetrical flower head, see Fig. 1.2 B, F), which is parabolic-shaped and thus can collect solar radiance, which increases the temperature of the surrounding tissue (Kevan 1989). Fabaceae and Lamiaceae have different floral morphologies compared to Apiaceae and Asteraceae (see Fig. 1.2 A-H). They are either flag-shaped (Fabaceae) (see Fig. 1.2 C, G) or gullet-shaped (Lamiaceae) (see Fig. 1.2 D, H) (Bernardello 2007), which enables them to protect nectar from evaporation (Corbet *et al.* 1979; Petanidou 2005). Apiaceae and Asteraceae have lower sucrose concentrations in their open flower heads, while Fabaceae and Lamiaceae can afford to produce higher concentrations of sucrose, due to the stable humidity inside their protected flowers. With regard to less viscous nectar, the higher proportions of hexoses in the nectar of Apiaceae and Asteraceae may serve the purpose of decelerating evaporation and to reward pollinators with attractive dilute nectar. Fabaceae and Lamiaceae do not need to produce more osmolytes via substituting sucrose with hexose due to their protective flowers.

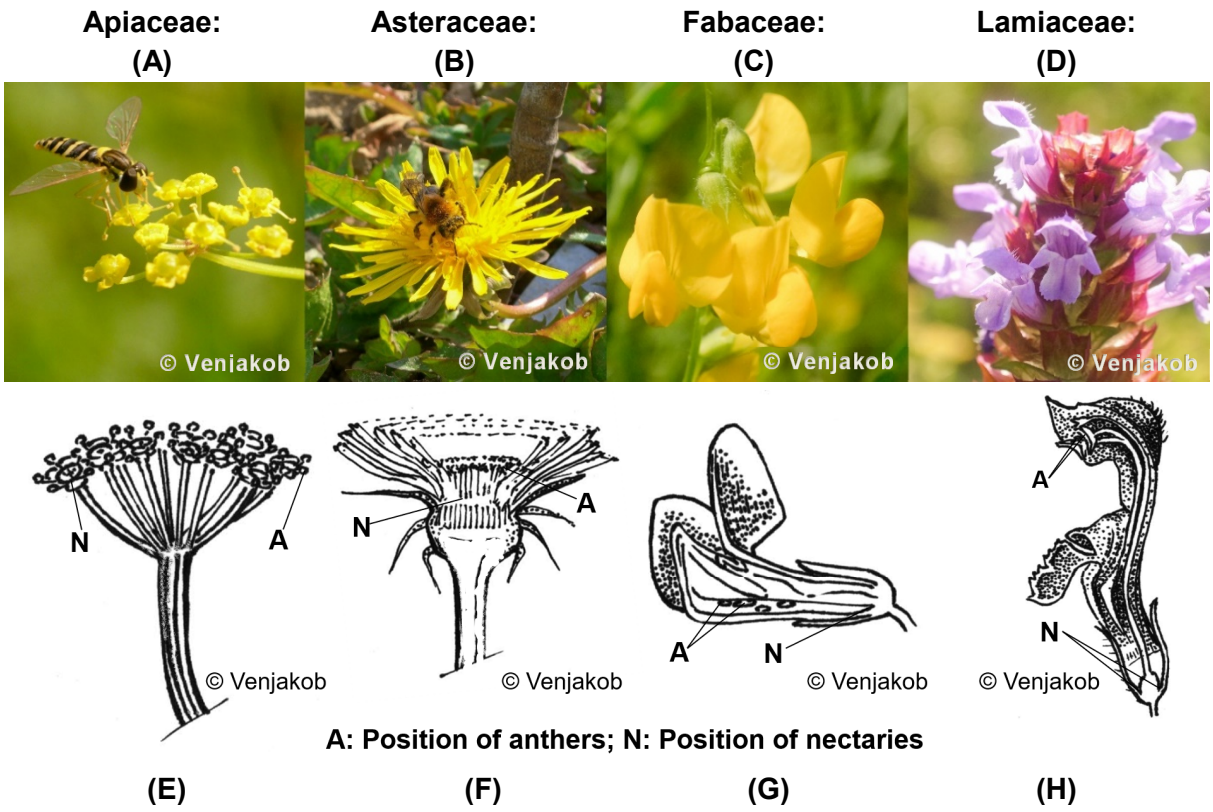

Figure 1.1: Four families are representing different morphological adaptations to present or protect their nectar as a reward for pollinators: **(A,E)** exposed nectar of *Pastinaca sativa* (Apiaceae), and **(B,F)** of flowers of *Taraxacum* cf. *officinale* (Asteraceae), which were visited by a solitary bee (*Anthophora* cf. *plumipes*), **(C,G)** flag-shaped flowers of *Lathyrus pratensis* (Fabaceae) and **(D,H)** of gullet-shaped flowers of *Prunella vulgaris* (Lamiaceae). (The copyrights of the drawings and photographs are with C. Venjakob).

Regarding amino acid composition in all 34 plant species, I found that they all differ from each other significantly. Though no single amino acid differed between families, a trend was found for the concentration of histidine in the nectar of Asteraceae, which belongs to the essential amino acids. It was found to act as a deterrent in taste experiments with flies (Shiraishi & Kuwabara 1970). But as shown in chapter 2, flowers of Asteraceae were frequently visited by many pollinators (see Appendix B, Supplementary of chapter 3, table S2; also Venjakob et al. 2016; Ebeling et al. 2008). Some amino acids can act as repellents or phagostimulants and many nectars contain toxic secondary plant compounds (Adler 2000; Escalante-Pérez & Heil 2012), which suggests that plants do not necessarily produce a nectar composition that has the purpose of meeting their pollinators' needs, but to both attract and repel flower-visitors (Adler 2000; Gardener & Gillman 2002; herein Hendriksma *et al.* 2014). Phenylalanine is an essential amino acid and was found in the nectar of Lamiaceae in a Mediterranean plant community by Petanidou et al. (2006), who suggested that this, in combination with dilute sucrose-rich nectar, is a result of coevolutionary specialization with Megachilidae (long-tongued bees). Proline is a non-essential amino acid,

known for its capability to provide energy for pollinators at the start of their flight and in combination with carbohydrates to prolong flights (Carter *et al.* 2006). Although proline was not found to differ between families, Fabaceae produced the largest amounts of it. Therefore, some pollinators may warm up in the morning on the parabolic-shaped flower heads of Asteraceae or get a starter help due to proline from the nectar of plant species belonging to the family of Fabaceae. The only family-specific amino acid pattern found was in the ratio of carbohydrates to amino acids. Therefore, this measure should be used as a tool for describing the quality of nectar, which may be a result of constrained metabolic pathways. Furthermore, this study indicates that nectar chemical composition is controlled by morphological traits and phylogenetic relatedness.

1.3.3 Species richness influences nectar quality

Nectar chemical compositions of Field Scabious, *Knautia arvensis*, showed differences when compared across a plant species richness gradient. With regard to compositions of proportional amino acids and compositions of carbohydrates in concentrations both differed in a non-linear way. Although proportions of all essential amino acids did not differ between mixtures, four individual essential amino acids (valine, isoleucine, leucine, and lysine) occurred in higher proportions in mixtures with 16 plant species. It has been shown that honeybees prefer sugar solutions with essential amino acids over sugar solutions with non-essential amino acids (Hendriksma *et al.* 2014), but they can also be deterred by specific amino acids (e.g. alanine (Bertazzini *et al.* 2010) or glycine (Hendriksma *et al.* 2014)). The occurrence of the essential amino acids, combined with the lowest carbohydrate concentrations and the fact that honeybees showed the highest visitation rate on flower heads of *K. arvensis* in the mixtures with 16 plant species indicates that the taste of essential amino acids might be attractive to honeybees (see Appendix C, Suppelementary of chapter 4, table S2 and S3). This effect could be a plant species richness effect due to competition for pollinators. In contrast, the nectar ratio of carbohydrates to amino acids was not significantly different, thus confirming the hypothesis that nectar quality of *K. arvensis* is species-specific.

1.3.4 Conclusions

Four important pollinator groups, i.e. honeybees, bumblebees, solitary bees, and hoverflies, used more segregated niches in time and vertical space in plant species-rich grassland mixtures than in plant species-poor mixtures. To meet the requirements of each pollinator species, an elevated plant species richness in different habitats is vital. For pollinators, it is substantial that the nectar requirements are met throughout their entire life-cycle. While

some species only live for a short time, colonies of eu-social bees require flower supplies throughout the vegetation period, and are thus likely to suffer from a flowerless environment.

In summary, study one showed that niche complementarity and thus reduced competition between different pollinator functional groups resulted from plant species-rich habitats. Although pollinator species differ in their habitat and nutritional requirements, my study indicates that pollinator visitation patterns covered more of the available niche volume in species-rich plots. Therefore, nutritional needs may be met best by plant communities that cover a broad variety of plant families. My results suggest that this may be due to at least two mechanisms: Study two shows that a high variation exists in nectar composition between plant families. Thus, diverse plant communities are more likely to include plant families that complement each other's nectar composition. In addition, study three shows that nectar quality of an individual plant species (*K. arvensis*) can change with the species richness of the surrounding plant community, such that flowers from species-rich communities (16 plant species) produced qualitatively superior nectar compared to flowers from species-poor communities.

Empirical evidence (including this thesis) has shown that some pollinators share the same functional niche (Fontaine *et al.* 2006; Blüthgen & Klein 2011; Fründ *et al.* 2013). This suggests that different pollinator species may be redundant (insurance hypothesis (Yachi & Loreau 1999). Thus, the loss of individual pollinator species may therefore not decrease pollination function. However, it has been shown that the presence of multiple pollinator species can improve fruit quality (Brittain et al. 2013b, 2014; Klatt et al. 2014; Wietzke et al. 2018). Further, Winfree *et al.* (2018) recently documented that "to provide crop pollination in natural systems, the number of bee species must increase by at least one order of magnitude compared with that in field experiments" due to species turnover and the change of dominant pollinator species across large spatial scales.

However, the continuing pollinator species loss may result in a reduction of reproductive success of single plant species (Brosi & Briggs 2013), or even in ecosystem collapse if key generalist pollinators disappear (Pauw 2007). Thus, maintaining pollinators' nutritional needs must be of high priority to decision makers worldwide. This study suggests that this may be achieved by species-rich plant communities. However, further investigations are clearly needed to incorporate plant species diversity in intercropping systems and diversified farming systems for pollinator-friendly practices to reveal the attractiveness of different plant species. Diversified field margins and grasslands are highly recommended for the maintenance of pollinator services for the sustainable provision of crop pollination for the future.

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1.5 Article overview

(In accordance with the guideline for cumulative dissertations in Sustainability Science [January 2012], in the following termed “the guideline”.)

Title of the PhD thesis: Spatio-temporal plant-pollinator interactions and floral nectar quality in a plant diversity experiment

Papers included

1. Venjakob, C., Klein, A.-M., Ebeling, A., Tscharntke, T. & Scherber, C. (2016). Plant diversity increases spatio-temporal niche complementarity in plant-pollinator interactions. Ecology and Evolution, 6, 2249–2261, DOI: 10.1002/ece3.2026

2. Venjakob, C., F. A. Ruedenauer, Klein, A.-M., Leonhardt, S. (2022). Variation in nectar quality across 34 grassland plant species. Published in Plant Biology, 24(1), DOI: 10.1111/plb.13343 [1]

3. Venjakob, C., Leonhardt, S., Klein, A.-M., (2020). Inter-Individual Nectar Chemistry Changes of Field Scabious *Knautia arvensis*. Published in Insects, 11(2), 75; DOI: 10.3390/insects11020075 [2]

[1] Im Zuge des Veröffentlichungsprozesses wurde das hier abgedruckte Manuskript gegenüber dem Stand der Ersteinreichung der Dissertation umgeschrieben und um statistische Analysen erweitert, die für eine Verbesserung der Aussagekraft erforderlich waren. In Absprache mit der Erstbetreuerin Alexandra-Maria Klein ist Felix A. Ruedenauer zudem in die Koautorenschaft aufgenommen worden.
In the course of the publication process, the manuscript printed here was rewritten compared to the status of the initial submission of the dissertation and expanded to include statistical analyses that were necessary to improve its validity. In consultation with the first supervisor Alexandra-Maria Klein, Felix A. Ruedenauer has also been included in the co-authorship.

[2] Im Zuge des Veröffentlichungsprozesses wurde das hier abgedruckte Manuskript gegenüber dem Stand der Ersteinreichung der Dissertation umgeschrieben und um statistische Analysen erweitert, die für eine Verbesserung der Aussagekraft erforderlich waren.
In the course of the publication process, the manuscript printed here was rewritten compared to the status of the initial submission of the dissertation and expanded to include statistical analyses that were necessary to improve its validity.

Table 1.3: Authors' contribution to articles and article publication status (according to §16 of the guideline)

Article	Short title	Specific contributions of all authors	Author Status	Weigh-ing Factor	Publication Status	Conference Contri-butions
#1	Plant diversity and pollinators niche comple-mentarity	CV, AE: data collection CV, CS: analysis CV: literature review, CV, AMK, AE, TT, CS: question of the paper, writing of the paper, research design	Co-author with pre-dominant contribution	1.0	Published in Ecology and Evolution (2016), IF=2.44 DOI: 10.1002/ece3.2026	
#2	Variation in nectar quality across 34 grass-land plant species	CV, AMK, and SL designed the experiment. CV collected data; SL and FAR per-formed statistical analyses. CV developed the first draft of the manuscript, and all authors contributed substantially to revisions.	Co-author with pre-dominant contribution	1.0	Published in Plant Biology (2022) IF= 3.081 DOI: 10.1111/plb.13343	
#3	Inter-Individual Nectar Chemis-try Changes of Field Scabi-ous, *Knautia arvensis*	CV: data collection SL: analysis CV: literature review, CV, AMK, SL: question of the paper writing of the paper, research design	Co-author with pre-dominant contribution	1.0	Published in Insects (2020) IF= 2.769 DOI: 10.3390/insects11020075	GfÖ 2012
#4	Function-al flower traits and their diversity drive pollinator visitation	CV, AE: data collection, FF: analysis, literature review, FF, AMK, AE, FH, GB, CV, MHS: question of the paper writing of the paper, research design	Co-author with important contribution (Wichtiger Anteil) 0.5	0.5	Published in Oikos (2017) IF=4.03 DOI: 10.1111/oik.03869	
			sum	3.5		

Publication status

IF = ISI Web of Science - Impact Factor

Specific contributions of all authors (order depends on the order of appearance)

CV Christine Venjakob

AE Anne Ebeling

CS Christoph Scherber

AMK Alexandra-Maria Klein

TT Teja Tscharntke

SL Sara Leonhardt

FAR Fabian A. Ruedenauer

FF Felix Fornoff

FH Florian Hartig

GB Gita Benadi

MHS Martin H. Schaefer

Author status

According to §12b of the guideline:

Single author (Allein-Autorenschaft): Own contribution amounts to 100%.

Co-author with predominant contribution (Überwiegender Anteil): Own contribution is greater than the individual share of all other co-authors and is at least 35%.

Co-author with equal contribution (Gleicher Anteil): (1) own contribution is as high as the share of other co-authors, (2) no other co-author has a contribution higher than the own contribution, and (3) the own contribution is at least 25%.

Co-author with important contribution (Wichtiger Anteil): own contribution is at least 25%, but is insufficient to qualify as single authorship, predominant or equal contribution.

Co-author with small contribution (Geringer Anteil): own contribution is less than 20%.

Weighting factor

According to §14 of the guideline:

Single author (Allein-Autorenschaft)	1.0
Co-author with predominant contribution (Überwiegender Anteil)	1.0
Co-author with equal contribution (Gleicher Anteil)	1.0
Co-author with important contribution (Wichtiger Anteil)	0.5
Co-author with small contribution (Geringer Anteil)	0

Conference contributions (acronym, society, date, venue, website)

GfÖ 2012 Annual Meeting of the Ecological Society of Germany, Switzerland and Austria, 10th-14th Sept 2012, Lüneburg, Germany, www.gfoe-2012.de. Talk.

Chapter 2

Plant diversity increases spatio-temporal niche complementarity in plant-pollinator interactions

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Plant diversity increases spatio-temporal niche complementarity in plant-pollinator interactions

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Keywords
Environmental niche, floral resource use, functional pollinator diversity, generalized additive models, Jena Experiment, niche overlap.

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Abstract

Ongoing biodiversity decline impairs ecosystem processes, including pollination. Flower visitation, an important indicator of pollination services, is influenced by plant species richness. However, the spatio-temporal responses of different pollinator groups to plant species richness have not yet been analyzed experimentally. Here, we used an experimental plant species richness gradient to analyze plant–pollinator interactions with an unprecedented spatio-temporal resolution. We observed four pollinator functional groups (honeybees, bumblebees, solitary bees, and hoverflies) in experimental plots at three different vegetation strata between sunrise and sunset. Visits were modified by plant species richness interacting with time and space. Furthermore, the complementarity of pollinator functional groups in space and time was stronger in species-rich mixtures. We conclude that high plant diversity should ensure stable pollination services, mediated via spatio-temporal niche complementarity in flower visitation.

Introduction

Declining biodiversity has been shown to affect many ecosystem processes, including pollination services (Biesmeijer et al. 2006; Garibaldi et al. 2013). Grassland biodiversity experiments have demonstrated that plant diversity increases pollinator abundance and species richness (Ebeling et al. 2008; Scherber et al. 2010). However, the mechanisms structuring pollinator community structure have remained unclear, and spatio-temporal pollinator behavior (including foraging behavior; e.g., Rusterholz and Baur 2010) in response to plant diversity has only rarely been addressed.

Plant species richness has been shown to reduce niche overlap among coexisting plant species (von Felten et al. 2009), affecting floral traits (Binkenstein et al. 2013), thereby indirectly structuring pollinator communities (Potts et al. 2003; Ebeling et al. 2008). However, niche overlap has so far rarely been studied from a pollinator's perspective. One reason may be that the classical Hutchinsonian niche concept (Hutchinson 1957; modified by Holt 2009) involves demographic parameters that are usually not measurable for whole pollinator communities. An alternative approach to study niche overlap in pollinator communities is the concept of environmental niches (Tracy and Christian 1986; Chesson et al. 2001), which includes spatio-temporal niches, where the focus is on an organism's foraging range. Small organisms, such as pollinators, can differ in spatio-temporal niches that are potentially modified by plant diversity, as predicted by

the biodiversity-niche hypothesis (MacArthur 1955; Loreau et al. 2001; Rosenfeld 2002; Blüthgen and Klein 2011).

Plant diversity may modify the spatio-temporal niches of pollinators in two ways: first, the number of plant species itself may affect pollinators' spatio-temporal resource use; second, plant diversity may affect vegetation parameters (e.g., floral abundance, vegetation structure) or abiotic conditions (e.g., microclimate) that then indirectly affect resource use.

Individual functional groups of pollinators may express phenological and/or architectural niche complementarity (Blüthgen and Klein 2011). Hoehn et al. (2008) observed spatial complementarity of pollinators within small plots of flowering herbaceous pumpkin plants; similar results were reported by Brittain et al. (2013) for woody almond trees. However, studies are lacking where resource diversity (here: plant diversity) has been experimentally manipulated to study pollinators' environmental niches in space and time at sufficient spatio-temporal resolution.

Previous studies have used experimental manipulations of plant species richness (Roscher et al. 2004) to investigate plant–pollinator interaction networks in response to plant species richness on a coarse time scale (months or years; Ebeling et al. 2008). These studies demonstrate temporal stability of flower visitation, indicating higher complementarity in time, due to increasing flower cover provided by high richness of flowering plant species.

However, the effects of declining plant species richness on spatio-temporal dynamics of plant–pollinator interactions have so far never been investigated. While studies investigating plant–pollinator interactions along plant diversity gradients exist (Ebeling et al. 2008; Scherber et al. 2010; Hudewenz et al. 2012), the spatial and/or temporal resolution of these datasets has not allowed deeper insights into spatial complementarity (vegetation strata) or temporal complementarity (time of day) in floral resource use.

Here, we use a large-scale, long-term biodiversity experiment (Roscher et al. 2004) to study the effects of plant species richness on spatio-temporal complementarity of pollinators. Our study provides new insights into the timing of flower visitation, its spatial stratification, and how spatio-temporal niches are modified by declining plant species richness. The dataset we analyze has an unprecedented spatio-temporal resolution: almost 12 h of observation in three vegetation strata on $N = 19$ plots across a whole flowering season.

The overall aim of our study was to understand the complexity of niche differentiation in three dimensions (space, time, and plant species richness), across four pollinator functional groups (bumblebees, honeybees, solitary bees, and hoverflies). In particular, we test the following hypotheses:

1 Plant species richness modifies pollinator flower visitation height and timing within a day either directly or indirectly (i.e., modified by vegetation characteristics such as flower cover).
2 Individual pollinator functional groups visit different flowering heights in the vegetation and at different times of day, depending on plant species richness.
3 Species-rich plant communities are characterized by higher complementarity in spatio-temporal resource use because of higher resource diversity and higher specialization of pollinators (Blüthgen and Klein 2011).

Materials and Methods

Experimental design

The study was conducted as part of the Jena Experiment that was established in 2002 to investigate how plant biodiversity affects ecosystem functioning. The field site (Fig. 1A) comprises 10 ha and is located in the north of Jena (Jena-Löbstedt, Thuringia, Germany), on the flood plain of the river Saale (50°55′N, 11°35′E; 130 m a.s.l.). There are 82 plots divided into two adjacent subplots comprising 6×5.5 m and 3×3.5 m, respectively (43.5 m^2 in total). Plant species richness was manipulated by establishing a gradient from 1 to 60 plant species per plot, containing either one, two, four, eight, 16, or 60 plant species from a total pool of 60 plant species (Roscher et al. 2004). Plots with a plant species richness of one, two, four, or eight plant species were replicated 16 times, whereas plots with a plant species richness level of 16 and 60 plant species were replicated 14 and four times, respectively. The 60 plant species were divided into four functional groups (small herbs, tall herbs, legumes, and grasses) using morphological, phenological, and physiological traits (for further details see Roscher et al. 2004). Species within plots were originally sown in equal proportions. Plots were arranged in four blocks, accounting for changes in soil abiotic conditions perpendicular to the river Saale (Roscher et al. 2004). All plots were mown every June and September according to common extensive grassland management. Plots were weeded twice per year until 2009 and afterward three times per year (April, July, and October) to maintain the sown target plant species mixtures.

From the experimental pool of 60 plant species, including 16 grass species that are not insect-pollinated, 32 entomophilous plant species were previously observed to be visited by functionally relevant insect pollinators (Ebeling et al. 2008). A crucial trait of plant species visited by insects is the development of flowers that produce pollen

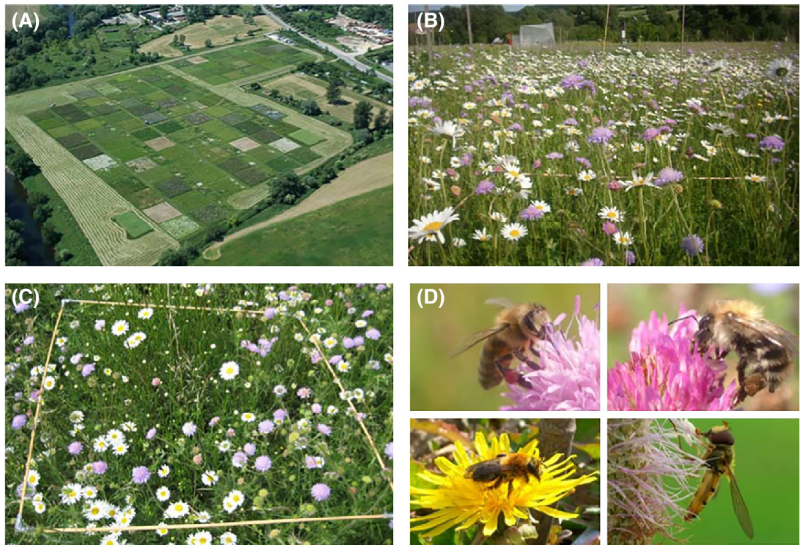

Figure 1. (A) Overview of the Jena Experiment (photograph by The Jena Experiment/C. Scherber/A. Weigelt/W. Voigt), (B) profile view from an example study plot with *Leucanthemum vulgare* and *Knautia arvensis* (photograph by C. Venjakob), (C) Square frame (0.8 m × 0.8 m) representing the sampling area where flower visitors were observed (photograph by C. Venjakob), (D) examples of four different pollinator functional groups, clockwise: Honeybee (*Apis mellifera*), bumblebee (*Bombus pascuorum*), solitary bee (*Anthophora* cf. *plumipes*), hoverfly (*Episyrphus* cf. *balteatus*) (photographs by C. Venjakob).

and nectar. We divided all flowering plant species into three functional groups: small herbs, tall herbs, or legumes (see Table S1).

Because we aimed at detailed, long-time pollinator observations (resulting in almost 12 h per plot in total), we restricted our observations to a subset of plots defined as follows: (i) no grasses present (except 60-species mixtures and plots B3A22, B4A11); (ii) >2 plant species present, and (iii) 4 or 5 replicates in each block. This resulted in $N = 19$ plots used in this study (see Fig. S1).

Pollinator observations

Pollinator observations were conducted from 11 April until 23 August in 2011, with approximately 3 weeks between observations, depending on flower cover and weather conditions. Observation time was from early morning (7–9 AM) until evening (6.30–8.30 PM). Sunny conditions with a minimum temperature of 18°C with windless to slightly windy conditions (<2 m/sec) were chosen for the observations.

All 19 plots were observed together on every sampling day according to a synchronized scheme by one observer per block and plot with an additional observer on the observation days 12 July and 2 August.

The observations were performed at the same location for each observation day. A square frame of 0.8 m × 0.8 m was systematically placed inside the central core area of each plot to observe flower-visiting pollinators (see Fig. 1B,C), on either the eastern or western side of the plot, facing away from main pathways. Distance between plot centroids ranged from 28 to 405 m. Each plot was observed for 15 min, representing a "single observation period". A fully observed block of five single-observation periods plus walking time between the plots plus a buffer time of 15 min (5 plots 15 min + 6 · 3 min walking + 15 min buffer = 1 h 48 min) was considered a "run". After each run, each observer switched to the next block (clockwise). Observation order of plots within a block changed (clockwise) for each new run. Each of the 19 plots was observed up to seven times per day. In total, we observed each plot 47 times on 7 days (total observation time: 11.75 h per plot).

During the observations, all pollinator visits on flowers to the plot were recorded as well as the identity of the visited plant species. Additional to our focal groups (bumblebees, honeybees, solitary bees, and hoverflies), we observed other groups of pollinators such as beetles, wasps (Hymenoptera: Vespidae), flies (Diptera), and ants (Hymenoptera: Formicidae). While we recorded all visitations by these groups, we decided to exclude them from analyses as their abundances were very low and models (e.g., for wasps) did not converge due to lack of sufficient observations. Flower visitation rate was defined as the number of flower visits per plot during a single observation period. Pollinators were identified in the field to genus, morphospecies, or species level, or caught with a sweep net for subsequent identification in the laboratory. Honeybees, bumblebees, solitary bees, and hoverflies (see Fig. 1D) were grouped for further analyses. Solitary bees were defined as non-Apis bees sensu Brittain et al. (2013).

For each pollinator observation, we documented time of day. Insects clearly not feeding on pollen or nectar were not considered.

Flower visitation height (flowering height) was recorded using three categories: ground level (1–10 cm), intermediate (11–25 cm), and upper vegetation (≥26 cm).

Strata were defined following Lorentzen et al. (2008) with a focus on lower vegetation layers (flowering height of small herbs, *reptantia* [sensu Ellenberg and Mueller-Dombois 1967]: *c.* 10 cm and *rosulata*: *c.* 25 cm).

Percentage flower cover of each plant species was recorded within the frame by estimating percentage of open flowers in relation to the total observed area. These measurements were used to calculate flower cover and realized species richness of flowering plants (see Figs. S2, S3).

Statistical analyses

Calculation of standardized daytime

We calculated a standardized daytime (SDT) ranging from zero to one, based on sunrise and sunset, separately for each day, because day length changed over the year. The full code for this is in the Supporting Information (Appendix S1). SDT was aggregated to 1 significant digit, resulting in nine time steps.

Generalized additive mixed models for flower visitation rates

The number of visits for each pollinator group was summed for each plot, time of day, and flowering height, resulting in a sample size of 309. Time of day was aggregated on the basis of hours and minutes. We analyzed the effect of (i) plant species richness (which was the main explanatory variable) and (ii) flower cover (to test for potential effects of resource abundance) on flower visitation rate (number of flower visits; count data) of pollinators using generalized additive mixed models (GAMMs) with negative binomial errors (R, package: mgcv, version 1.7-28 [Wood 2006]). Additive models allowed us to model three-way nonlinear interactions, while accounting for spatio-temporal nonindependence in the data both with smooth terms and random effects. Initial models included either plant species richness or flower cover, and time of day and flowering height as fixed effects, fitted sequentially using smooth terms defined by tensor product interactions as implemented in the ti() function in GAMM. Random effects for plot and height stratum were used. We added stratum as a random effect to account for spatio-temporal nonindependence of observations taken within the height strata of a particular plot. This is similar to a split-plot design, where the height strata are the "subplots" within a plot. Fixed-effects terms were modeled using a basis dimension of $k = 3$ with option "select = T". The theta parameter of the negative binomial distribution was estimated during model fitting by specifying a starting interval within [0;10]. For further model simplification, we performed backward selection, which was performed by manually removing each term with the highest *P*-value sequentially (as indicated in summary.gam). We continued refitting the model until all terms were either significant or were part of a higher-order significant interaction term according to the principle of marginality (see R documentation on gam.selection [Wood 2014]). To compare among different sets of explanatory variables (e.g., flower cover vs. plant species richness), we used Akaike's information criterion with a correction for finite sample sizes (AICc; Scherber et al. 2014; Wood 2014). Explained deviance for each model was calculated as described in the Supporting Information, Appendix S2.

Multinomial models for pollinator community composition

Changes in pollinator community composition and spatio-temporal niche complementarity were assessed using multinomial models with plant species richness, flowering height, and time of day as explanatory variables. This was carried out using a data frame containing the flower visitation rates of each pollinator group as a response matrix (La Rosa et al. 2012; Qian et al. 2012). Explanatory variables were the same as for the GAMMs, but these were fitted as polynomial splines using the bs() function in the splines package in R. Model fitting was performed with the function "multinom" (R, package: nnet, version 7.3-7 [Venables and Ripley 2002]). Models were simplified using stepAIC (MASS library), and significance of terms was assessed using likelihood ratio tests. Differences in pollinator community composition were assessed by dividing the model coefficients by their standard errors; we then used two-tailed Wald *z*-tests to test significance of these coefficients. We additionally refitted multinomial models with random effects using function "BayesX" in R package R2BayesX, version 1.0.0 (Belitz et al. 2015; Umlauf et al. 2015). However, in these models, only linear three-way interactions were fitted as three-dimensional smooth terms are not yet implemented.

Analysis of niche complementarity versus overlap

To assess whether pollinator groups differed in spatio-temporal niche complementarity versus overlap, we used the predictions of the GAMM models (see II.) and transformed these into presence/absence data for each group (setting singletons to zero). This resulted in four vectors containing the presences/absences for each pollinator group i (coded as 0 or 1). Using bumblebees as our reference category, we calculated pairwise sums of these vectors to arrive at three resource use categories: 0 (neither bumblebee nor group i present); 1 (either bumblebee or group i present; indicating complementarity); 2 (both bumblebee and group i present; indicating overlap). The

resulting vector (consisting of 0's, 1's, and 2's) was then entered into a multinomial model as described above (see III.) to explicitly test for resource use. In these models, plant diversity was entered as a factor, and other terms were entered using natural splines. All analyses were performed using R, version 3.0.1 (R Development Core Team 2013).

Results

Flower visitor community

We recorded 59 flower-visiting species with a total of 10,653 individual flower visits on 34 different flowering plant species. Comprising 31 solitary bee species (including semisocial species) with 236 visits, ten bumblebee species with 3,059 visits, 17 hoverfly species with 676 visits, and the European honey bee (*Apis mellifera* L.) with 6,682 flower visits (see Table S2). Common sainfoin (*Onobrychis viciifolia* Scop.) was the most frequently visited plant species, although it was not more abundant than other flowering species (see Table S3 and Fig. S2); it was mainly visited by honeybees and the red-tailed bumblebee (*Bombus lapidarius* L.; see Table S3). Other frequently visited plants, mainly visited by honeybees, were field scabious (*Knautia arvensis* (L.) Coult.) and bastard medic (*Medicago* × *varia* Martyn). Meadow crane (*Geranium pratense* L.) and bird's-foot trefoil (*Lotus corniculatus* L.) were mainly visited by bumblebees, especially by the red-tailed bumblebee (see Table S3). The most common solitary bee species were *Halictus tumulorum* L., *Lasioglossum calceatum* Scop., *Lasioglossum pauxillum* A. Schenck, which visited mostly meadow crane. Other species frequently visited by solitary bees were germander speedwell (*Veronica chamaedrys* L.) and field scabious; these were visited often either by *Andrena viridescens* Viereck or by *Lasioglossum leucozonium* Schrank, see Table S3.

Hoverflies visited different plant species to bumblebees, honeybees, and solitary bees. They especially favoured hogweed (*Heracleum sphondylium* L.) that was predominantly visited by *Sphaerophoria scripta* L. A sister species of this order (*Sphaerophoria interrupta* Jones) most often visited ribgrass (*Plantago lanceolata* L.). Parsnip (*Pastinaca sativa* L.) was mostly visited by *Melanostoma mellinum* L. (see Table S3).

Effects of plant species richness, time of day, and flowering height on flower visitation

Overall flower visitation (i.e., across all pollinator functional groups) showed separate nonlinear (approximately quadratic) effects with time of day and flowering height, resulting in peaks in visitation rates at midday (1–3 PM, i.e., 0.6 standardized time) and in the taller flowers, regardless of plant species richness (Table 1, Fig. 2A,B). When all pollinators, except honeybees, were analyzed, flower visitation rate was not significantly influenced by plant species richness, but there were main effects of flowering height and time of day (Table S4 and Fig. S4).

Honeybee visits were influenced by a three-way, nonlinear interaction of all three explanatory variables (Table 1, Fig. 2C,D): if plant species richness was low, flower visitation ranged spatially from intermediate flowering height to the upper flowering height and started temporally at about 0.2 standardized time, with a peak at midday (0.5 standardized time, from approx. 11 AM–2 PM, depending on the season) and ending in the evening (1 standardized time, from approx. 18–20 PM, depending on the season). By contrast, if plant species richness was high, honeybees limited their spatio-temporal visitation pattern to the upper flowers, while intermediate flowers were not visited any more. The highest visitation rate was in plant species mixtures with only four plant species, at around midday and in the tallest flowers (Fig. 2D).

Bumblebee visits were influenced by a linear two-way interaction between plant species richness and time of day (Table 1, Fig. 2E,F): while visitation to species-poor mixtures showed a peak around midday (0.55 standardized time), visitation shifted toward the evening (0.7 standardized time) in species-rich mixtures. In addition, there was a separate quadratic effect of flowering height. The interaction and main effects resulted in a height stratification of flower visitation (Fig. 2E,F) with visits increasing toward the top of the vegetation.

Visits of solitary bees were influenced by a nonlinear three-way interaction among time of day, flowering height, and plant species richness (Table 1, Fig. 2G,H). In species-poor mixtures, visits were not influenced by time or flowering height, while in species-rich mixtures visits concentrated near the ground and around midday (depending on the season, approx. 11 AM–2 PM; Fig. 2G,H).

Finally, hoverfly visits were influenced by two two-way interactions between time of day and plant species richness, and by time of day and flowering height (Table 1, Fig. 2I,J). Hoverfly visits occurred preferably early and at high plant species richness; in addition, early visits occurred preferably in the upper vegetation (Fig. 2I,J). Across all plant species richness levels and flowering heights, hoverflies were always the first pollinators that visited the flowers. Independent of plant species richness, hoverflies started visiting flowers equally across all flowering heights in the very early morning. During the course of the day, they concentrated their activity to the intermediate and upper vegetation and later on only to the tallest flowers. Visiting time of the flowers in the bottom and

Table 1. Flower visitation rate of the pollinator functional groups: all pollinators, honeybees, bumblebees, solitary bees, and hoverflies. Summary of terms for generalized additive mixed models.

Response variable (flower visitation rate)	Parameter	Est. df (est. pp)	Effect[1]	Ref. df (SE)	*F*-value (*t*-value)	*P*	Deviance explained [%]
Overall pollinators	(Intercept)	(2.86)	–	(0.11)	(25.51)	<0.001	57
	Time of day[2]	1.88	Quadratic	1.88	47.31	<0.001	
	Flowering height[2]	2.00	Quadratic	2.00	45.49	<0.001	
	Plant species richness[2]	2.00	Quadratic	2.00	1.15	0.319	
	Time of day*Flowering height[2]	1.00	Linear	1.00	2.68	0.103	
Honeybees	(Intercept)	(1.7038)	–	(0.17)	(9.95)	<0.001	66
	Time of day[2]	1.99	Quadratic	1.99	54.67	<0.001	
	Flowering height[2]	2.00	Quadratic	2.00	31.69	<0.001	
	Plant species richness[2]	2.00	Quadratic	2.00	0.89	0.413	
	Time of day*Flowering height[2]	1.00	Linear	1.00	5.82	0.016	
	Time of day*Plant species richness[2]	1.90	Quadratic	1.90	5.00	0.009	
	Flowering height*Plant species richness[2]	1.00	Linear	1.00	1.01	0.316	
	Time of day*Flowering height*Plant species richness[2]	1.87	Quadratic	1.87	4.42	0.015	
Bumblebees	(Intercept)	(1.4597)	–	(0.23)	(6.41)	<0.001	21
	Time of day[2]	1.98	Quadratic	1.98	5.27	0.006	
	Flowering height[2]	2.00	Quadratic	2.00	6.11	0.003	
	Plant species richness[2]	2.00	Quadratic	2.00	0.16	0.851	
	Time of day*Plant species richness[2]	1.00	Linear	1.00	3.94	0.048	
Solitary bees	(Intercept)	(−1.0879)	–	(0.15)	(−7.38)	<0.001	27
	Time of day[2]	1.10	Linear	1.10	50.85	<0.001	
	Flowering height[2]	1.97	Quadratic	1.97	1.70	0.184	
	Plant species richness[2]	2.00	Quadratic	2.00	0.09	0.914	
	Time of day*Flowering height[2]	2.00	Quadratic	2.00	0.05	0.955	
	Time of day*Plant species richness[2]	1.69	Quadratic	1.69	1.55	0.207	
	Flowering height*Plant species richness[2]	2.73	Cubic	2.73	4.66	0.005	
	Time of day*Flowering height*Plant species richness[2]	2.02	Quadratic	2.02	4.37	0.013	
Hoverflies	(Intercept)	(−0.3084)	–	(0.17)	(−1.802)	0.073	41
	Time of day[2]	1.92	Quadratic	1.92	40.85	<0.001	
	Flowering height[2]	2.00	Quadratic	2.00	12.15	<0.001	
	Plant species richness[2]	2.00	Quadratic	2.00	0.08	0.927	
	Time of day*Flowering height[2]	1.00	Linear	1.00	8.73	0.003	
	Time of day*Plant species richness[2]	1.59	Quadratic	1.59	3.84	0.033	

Est.df, estimated degrees of freedom of term; est.pp, estimated parameter value.
[1]Interpretation of smooth term.
[2]Term was fitted using ti() function in generalized additive mixed models. $n = 309$.

intermediate flowering heights ended about 2 h before midday (approx. 10 AM–12 PM, depending on the season).

When flower cover was used as an explanatory variable instead of plant species richness, the model fits were generally poorer for all pollinator groups except solitary bees, as indicated by strong increases in AICc (see Table S5).

Shifts in pollinator community composition

To analyze the proportional composition of the pollinator community, we additionally analyzed all groups combined as a multinomially distributed response variable, because responses of pollinator functional groups are likely nonindependent. This resulted in relative data on flower visitation (Fig. 3) analyzed using multinomial models.

These models showed a significant three-way interaction between plant species richness, flowering height, and time of day (Fig. 3; likelihood ratio = 23.058, $P < 0.001$). There was a strong significant shift in pollinator niches; for example, hoverflies dominated in the morning and at low height, making up almost 100% of the community in species-rich grassland. However, later in the day, hoverflies became subdominant and were replaced by bumblebees close to ground level. In the tallest vegetation, completely different groups of pollinators dominated the community and they also responded

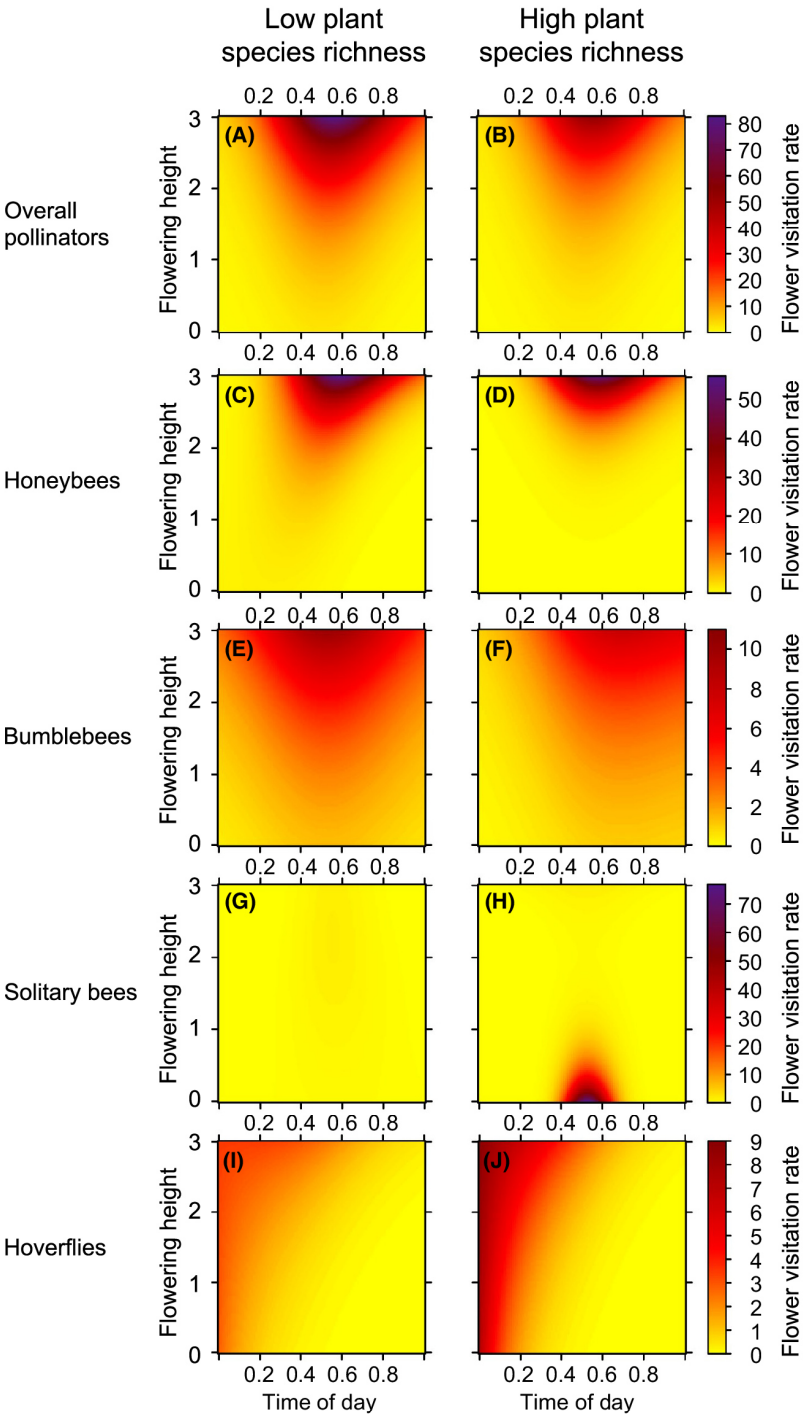

Figure 2. Effects of plant species richness, time of day, and flowering height on flower visitation rate. Shown are the results of minimal adequate generalized additive mixed models, for all pollinator functional groups. (A, B) Overall pollinators (honeybees + bumblebees + solitary bees + hoverflies), and each pollinator group separately (C, D): honeybees, (E, F): bumblebees, (G, H): solitary bees, (I, J): hoverflies. Flower visitation rate is influenced by plant species richness (low = 4, high = 60 plant species), time of day (0–1; range representing the observation time, between the onset of sunrise and sunset), and three different flowering heights (A–J: 1 = 1–10 cm, 2 = 11–25 cm, 3 = ≥26 cm).

differently to plant species richness. In comparison with the other pollinator functional groups, honeybees were the most frequent flower visitors, making up approximately 60–85% during midday and evening and in the taller vegetation, across all plant species richness levels (Fig. 3). All pollinator groups differed significantly in spatio-temporal resource use and in their response to plant species richness, as indicated by two-tailed Wald tests with bumblebees as a reference level (see Table S6).

Niche complementarity versus overlap

When analysing resource use (categories 0, 1, or 2), we found significant interactions among plant species richness, flowering height, and time of day for numbers of groups present (Table 2, Fig. 4) for all pollinator groups, indicating significant spatio-temporal niche shifts. When comparing bumblebees with honeybees (Fig. 4A) and bumblebees with hoverflies (Fig. 4C), we found highly significant three-way interactions between plant species richness, flowering height, and time of day, indicating that the presence or absence of these groups was modified by space, time, and plant diversity. For solitary bees (Fig. 4B), there were two-way interactions between time, plant species richness, and flowering height, again indicating strong spatio-temporal niche shifts.

Discussion

This study demonstrates that experimentally manipulated plant species richness influences spatial and temporal complementarity of flower visitation. We have shown that plant species richness, combined with time and space or with either time or space, drives spatio-temporal niche complementarity in different and globally relevant pollinator functional groups such as honeybees, bumblebees, solitary bees, and hoverflies (Fig. 5). In our study, overall flower visitation rate was strongly influenced by time or flowering height, but not by plant species richness per se. The pattern remained after excluding honeybees from the model, which corroborates that honeybees were not the main driver of overall flower visitation, despite their high abundance (see Table S4 and Fig. S4). Previous studies (e.g., Ebeling et al. 2008) reported significant main effects of plant species richness on overall flower visitation. Differences between both results could be due to (i) sample sizes/plot selection, (ii) different observation times, or (iii) higher spatio-temporal resolution. For (i), we reanalyzed data from Ebeling et al. (2008) and restricted these to the $N = 19$ plots used in our study (see Fig. S5). These analyses showed that even with $N = 19$ plots, positive plant species richness effects could have been found. For (ii), we restricted analyses of our data to times of day before 5 PM (as in Ebeling et al. 2008) and again found no differences. For (iii), we excluded all spatio-temporal information, also resulting in a positive effect of plant species richness. We therefore conclude that differences are due to a higher spatio-temporal resolution in the current dataset. In addition, competition between pollinators for floral resources is another possible mechanism of resource partitioning (Fründ et al. 2013). While we only indirectly manipulated the availability of floral resources (via plant diversity), our findings indicate that changes in resource availability (flower cover, see Fig. S3) influence spatio-temporal niche partitioning in different pollinator functional groups (Fontaine et al. 2008; Fründ et al. 2013). Overall flower visitation covered almost the full volume of spatio-temporal niche space (Fig. 2A,B) (Blüthgen and Klein 2011). In contrast, individual functional groups of pollinators used space and time differently, depending on plant species richness. Other studies have shown that

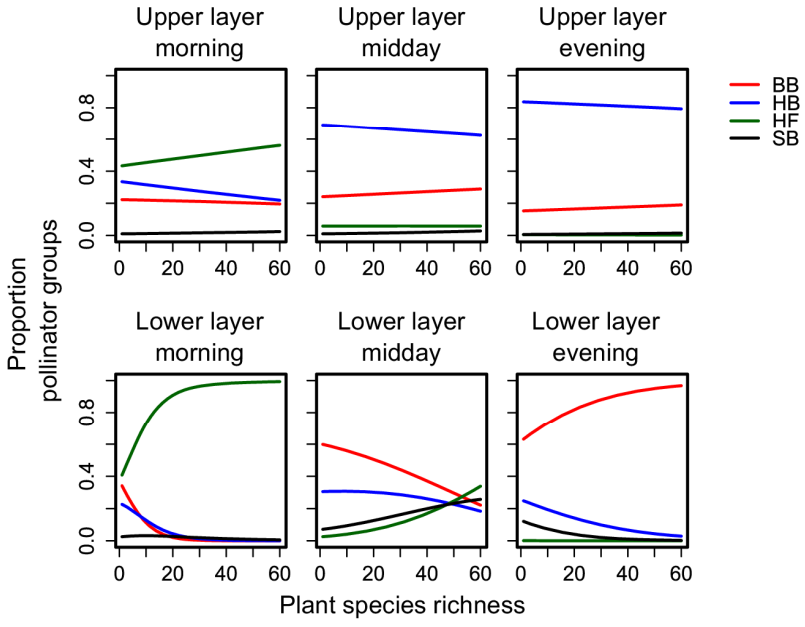

Figure 3. Effects of time of day (morning, midday, and evening), plant species richness (plant species richness: 1–60 plant species), and flowering height (3 = ≥26 cm and 1 = 1–10 cm) on proportions (%) of each flower-visiting pollinator group [bumblebees [BB = red], honeybees [HB = blue], hoverflies [HF = green], and solitary bees [SB = black]); lines show the predicted values of a minimal adequate multinomial model (likelihood ratio test model with three-way interactions versus model containing all pairwise two-way interactions, $\chi^2 = 23.058$, $P < 0.001$).

Table 2. Interactions among plant species richness, flowering height, and time of day across all pollinator functional groups (honeybees, bumblebees, solitary bees, and hoverflies). We analyzed pairwise (bumblebees as reference group) presence of all pollinator groups (see text). The response variable was an ordered categorical taking values of 0, 1, or 2 (0 = no pollinator group present, 1 = one pollinator group present, 2 = both pollinator groups present).

Response variable (categorical)	Parameter	LR Chisq	df	Pr (>Chisq)
Bumblebees and honeybees	Flowering height[1]	484.23	2	<0.001
	Time of day[1]	108.32	2	<0.001
	Plant species richness	103.00	2	<0.001
	Flowering height[1] : Time of day[1]	62.76	2	<0.001
	Flowering height[1] : Plant species richness	15.86	2	<0.001
	Time of day[1]: Plant species richness	17.40	2	<0.001
	Flowering height[1] : Time of day[1]: Plant species richness	34.95	2	<0.001
Bumblebees and solitary bees	Flowering height[1]	244.42	2	<0.001
	Time of day[1]	96.31	2	<0.001
	Plant species richness	5.33	2	0.070
	Flowering height[1]: Time of day[1]	62.80	2	<0.001
	Flowering height[1]: Plant species richness	41.87	2	<0.001
	Time of day[1]: Plant species richness	10.29	2	0.006
	Flowering height[1]*Time of day[1]*Plant species richness	1.80	2	0.406
Bumblebees and hoverflies	Flowering height[1]	570.19	2	<0.001
	Time of day[1]	129.63	2	<0.001
	Plant species richness	12.26	2	0.002
	Flowering height[1]: Time of day[1]	38.44	2	<0.001
	Flowering height[1]: Plant species richness	6.48	2	0.039
	Time of day[1]: Plant species richness	29.81	2	<0.001
	Flowering height[1]*Time of day[1]*Plant species richness	25.13	2	<0.001

The results are shown as an analysis of deviance table (Type II tests; LR: likelihood ratio calculated using chi-square test, df: unused degrees of freedom, Pr(>chi-square test): *P*-value).

[1]Term was fitted in a multinomial model using natural splines.

crop monocultures, such as orchards and strawberry or blueberry fields (Carré et al. 2009; Holzschuh et al. 2012; Blaauw and Isaacs 2014; Rosa García and Miñarro 2014), are comparable with species-poor mixtures that offer limited flower resources in a small volume of niche space. This potentially causes limitations in food supply, for example, because peak flowering time is often limited to a few weeks (Rosa García and Miñarro 2014). Furthermore, the dynamically changing spatio-temporal flower visitation patterns of some pollinator functional groups indicate stability against biodiversity loss (insurance hypothesis) in species-rich mixtures. A superposition of pollinator niches (Fig. 5) shows that hoverflies and solitary bees occupied separate spatio-temporal niches when compared to all other functional groups, while bumblebees and honeybees showed higher niche overlap. Based on the analysis using flower cover as a potential explanatory variable (see Table S5), it is clearly shown that except for solitary bees all pollinator functional groups strongly alter their spatio-temporal niches in response to plant diversity and in addition to that, plant species richness is a better predictor in combination with time and space than flower cover. Spatio-temporal niche overlaps are decreasing with increasing plant species richness (Fig. 4). But niche complementarity and partitioning is increasing in the species-rich mixtures. Even without considering overlapping niches of hoverflies and solitary bees in the species-poor mixtures (never more than three visits, see Fig. 5), overall niche overlap was always higher in species-poor mixtures. In species-rich mixtures, however, resource distribution in space and time is more complex than in species-poor plant communities, allowing for greater niche diversity (Elmqvist et al. 2003; Finke and Snyder 2008; Cardinale 2011). Our study shows that declining plant species richness is an important factor influencing functional pollinator composition, thereby decreasing complementarity. Changes in the diet breadth of inferior pollinators in response to the loss of dominant pollinators have been reported by Brosi and Briggs (2013).

In our study, pollinator functional groups largely facilitate each other and show increasing complementarity with plant species richness, thus ensuring stable provision of pollination services. However, spatio-temporal niches in low plant species mixtures were less well covered than in the species-rich mixtures. Our analyses showed that this was not caused by a lack of available floral resources, because plant species richness was a better predictor for pollinator visitation than flower cover. Hence, our results support the hypothesis that plant species richness

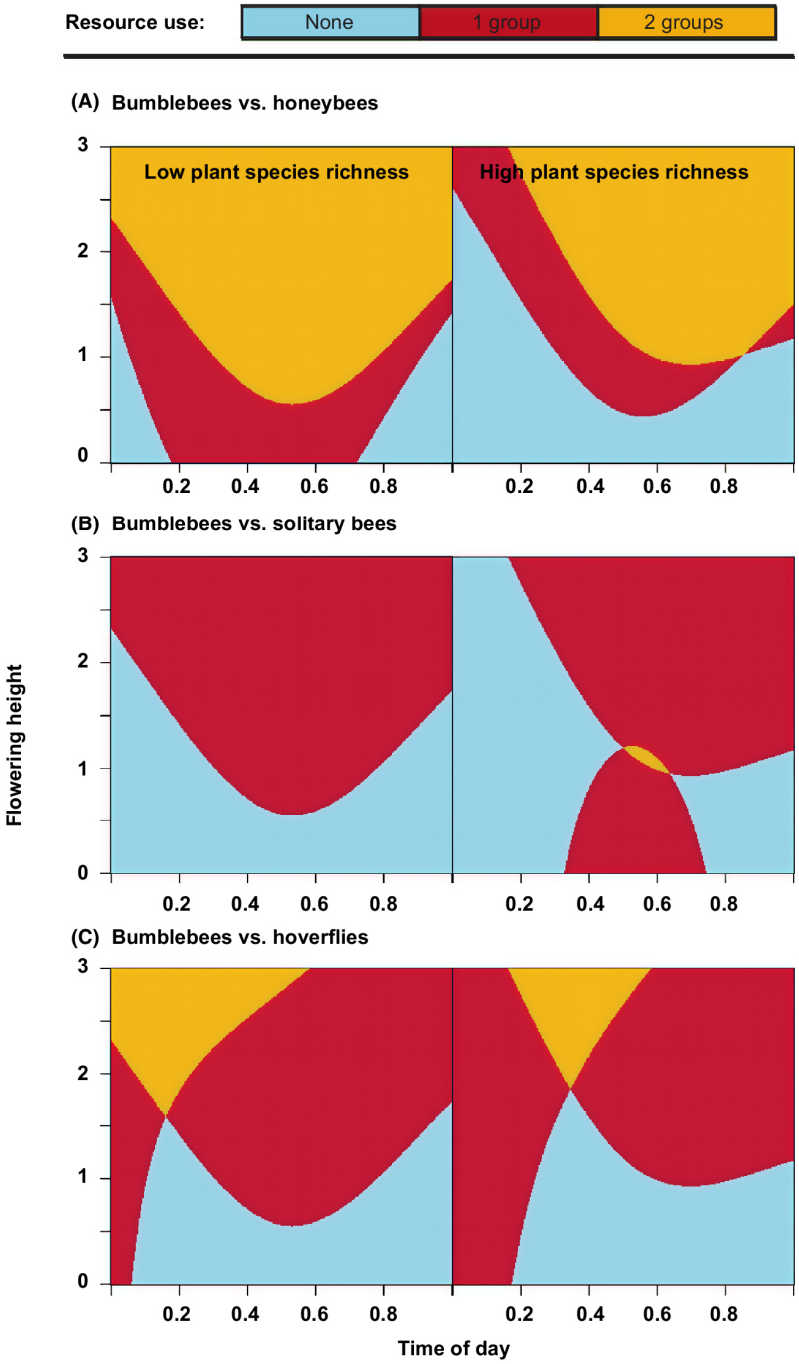

Figure 4. An explicit test of resource use overlap among different functional groups of pollinators. The *x*-axis represents the standardized time of day (range: 0–1), the *y*-axis indicates three levels of flowering height (1–3), and the panels show low (4) versus high (60) plant species richness. Colors represent the number of pollinator groups visiting the available plant resources. The Figure shows predictions from multinomial models fitted to categorical response variables, comparing bumblebee presence with the presence of (A) honeybees, (B) solitary bees, and (C) hoverflies.

indirectly structures spatio-temporal resource use of pollinators.

Under field conditions, host plants situated in plant species-poor locations may face extinction in the long term due to the absence of pollinators (Scheper et al. 2014). In this context, future studies should also investigate plant reproductive success accounting for differences in pollination efficiency (Jauker et al. 2012), thereby looking at the effects of pollinator complementarity on plant reproductive success.

In summary, our study shows that declining plant species richness may alter spatio-temporal resource use of pollinators, leading to higher complementarity of flower visitation in space and time. This has important implica-

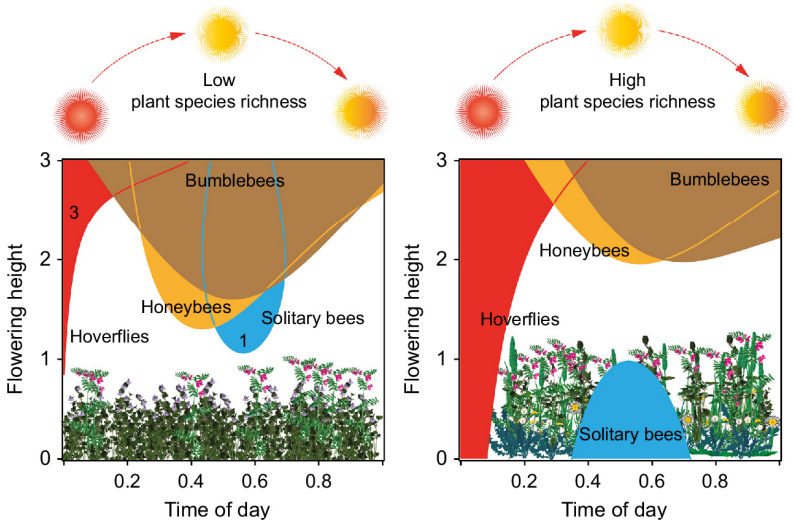

Figure 5. A summary of niche complementarity and overlap in functional groups of pollinators, showing effects of plant species richness, time of day, and flowering height on flower visitation rate of each group. Flower visitation patterns of honeybees (orange), bumblebees (brown), solitary bees (blue), and hoverflies (red) along a plant species richness gradient. The *x*-axis is standardized time of day (range: 0–1), the *y*-axis is flowering height (1–3), and the panels show low (4) versus high (60) plant species richness. Pronounced niche complementarity of all pollinator functional groups in species-rich mixtures (visitation patterns start at four visits, except in low plant species richness mixtures where solitary bees visited only once and hoverflies up to three times [see Fig. 2]). Patterns are predictions from minimal adequate generalized additive mixed models models, created using the ContourPlot function in R (package: lattice, version 0.20-15 [Sarkar 2008]).

tions for plant communities in general where a more efficient use of three-dimensional space and sufficient temporal coverage is important, not only for plant reproduction, but also for crop pollination.

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Conflict of Interest

None declared.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Plant species used in the experiment, with an indication of the flowering phenology during the course of a year.
Figure S1. Location of study plots.
Figure S2. Top 20 highest flower cover per plant species.
Figure S3. The number of flowering plant species and flower cover in relation to the number of identified pollinator species and on number of pollinator visits.
Appendix S1. R-code for the calculation of the SDT.
Appendix S2. R-code for the calculation of the proportion of the deviance.
Table S2. List of identified pollinators.
Table S3. List of visited plant species by all pollinator functional groups, with pollinator species.
Table S4. GAMM model of flower visitation rate of all pollinators (bumblebees, solitary bees, hoverflies) excluding honeybees.
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Table S6. All pollinator groups differed significantly in spatio-temporal resource use and in their response to plant species richness.
Figure S5. Effects of plant species richness on the flower visitation of all pollinators (honeybees, bumblebees, solitary bees, hoverflies), (a) based on data from Ebeling et al. (2008) and (b) based on our data.

Chapter 3

Variation in nectar quality across 34 grassland plant species

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RESEARCH PAPER

Variation in nectar quality across 34 grassland plant species

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ABSTRACT

- Floral nectar is considered *the* most important floral reward for attracting pollinators. It contains large amounts of carbohydrates besides variable concentrations of amino acids and thus represents an important food source for many pollinators. Its nutrient content and composition can, however, strongly vary within and between plant species. The factors driving this variation in nectar quality are still largely unclear.
- We investigated factors underlying interspecific variation in macronutrient composition of floral nectar in 34 different grassland plant species. Specifically, we tested for correlations between the phylogenetic relatedness and morphology of plants and the carbohydrate (C) and total amino acid (AA) composition and C:AA ratios of nectar.
- We found that compositions of carbohydrates and (essential) amino acids as well as C:AA ratios in nectar varied significantly within and between plant species. They showed no clear phylogenetic signal. Moreover, variation in carbohydrate composition was related to family-specific structural characteristics and combinations of morphological traits. Plants with nectar-exposing flowers, bowl- or parabolic-shaped flowers, as often found in the Apiaceae and Asteraceae, had nectar with higher proportions of hexoses, indicating a selective pressure to decelerate evaporation by increasing nectar osmolality.
- Our study suggests that variation in nectar nutrient composition is, among others, affected by family-specific combinations of morphological traits. However, even within species, variation in nectar quality is high. As nectar quality can strongly affect visitation patterns of pollinators and thus pollination success, this intra- and interspecific variation requires more studies to fully elucidate the underlying causes and the consequences for pollinator behaviour.

INTRODUCTION

Many flowering plant species need flower-visiting insects for pollination. They often provide rewards (mostly nectar and occasionally pollen) to attract pollinators (Agthe 1951; Baker 1963; Kearns *et al.* 1998; Klein *et al.* 2007; Ollerton *et al.* 2011). Although most macronutrients, which are essential for organisms, are located in pollen (in particular protein and fat) (De Groot 1953; Roulston & Cane 2000; Keller *et al.* 2005; Weiner *et al.* 2010), nectar represents the main source of carbohydrates and additionally contains variable concentrations of amino acids (Heinrich 1981; Wcislo & Cane 1996; Carter *et al.* 2006; Venjakob *et al.* 2020). In fact, some pollinators, *e.g.* butterflies, even entirely depend on nectar as sole nutrient source (Erhardt & Rusterholz 1998) and rely on its amino acid content to compensate for nitrogen deprivation during larval development (Mevi-Schütz & Erhardt 2005). In other groups, *e.g.* parasitoid wasps, nectar can increase longevity, fecundity and mobility (Winkler *et al.* 2006). Nectar was therefore considered *the* most important floral reward for attracting pollinators (Simpson & Neff 1983; Somme *et al.* 2015; Parachnowitsch *et al.* 2019).

From the pollinators' perspective, nectar needs to meet nutritional requirements, outbalance foraging costs (Pyke *et al.* 1977; Waddington 1982) and provide sufficient energy for mobility and thermoregulation (herein McCallum *et al.* 2013; Hendriksma *et al.* 2014). From the plants' perspective, nectar needs to attract pollinators at minimum production costs (Pyke 1991; Nepi & Stpiczyńska 2008), deter non-mutualists, *e.g.* nectar robbers and micro-organisms, from stealing or degrading nectar (Adler 2000; González-Teuber & Heil 2009; Escalante-Pérez & Heil 2012) and ideally manipulate pollinator behaviour to their advantage (Pyke 2016). These different requirements render the nutritional composition of nectar (henceforth referred to as nectar quality) a multifunctional trait, which can be subject to conflicting interests (Parachnowitsch *et al.* 2019; van der Kooi *et al.* 2021). For example, different flower-visiting and pollinating species (henceforth all referred to as pollinators) prefer different sugar concentrations, *i.e.* bees prefer nectar with 50–60% sugar concentration and butterflies and birds prefer nectar with 35% sugar concentration, likely due to different ways of consumption (*i.e.* dipping *versus* suction) (Kim *et al.* 2011). Also, nectar sugar

concentration negatively correlates with yeast abundance (Herrera *et al.* 2009) which may in turn affect pollinator preferences (Vannette & Fukami 2016; Schaeffer *et al.* 2017). Consequently, nectar quality can directly and indirectly affect visitation patterns of pollinators.

Several biotic and abiotic factors were found to be related to variation in nectar quality, including age and damage to flowers (Gottsberger *et al.* 1990), soil conditions (Baude *et al.* 2011; Becklin *et al.* 2011), genetic relatedness (*i.e.* phylogeny) (Baker & Baker 1976; Nicolson & Van Wyk 1998; Perret *et al.* 2001), flower morphology (Gusman & Gottsberger 1996; Torres & Galetto 2002), microbial communities (Vannette *et al.* 2013), selection by specific pollinators (Petanidou *et al.* 2006; Willmer 2011; Chalcoff *et al.* 2017; Tiedge & Lohaus 2017; Silva *et al.* 2020) and various interactive effects [see Parachnowitsch *et al.* (2019) for a complete review of factors affecting nectar quality]. For example, phylogenetically related plant species, *i.e.* belonging to the same tribe or family, can show similar chemical characteristics of nectar, such as a clear dominance of hexose or sucrose (Percival, 1961; Bernardello *et al.* 1994; Wolff 2006) and similar amino acid profiles (Baker & Baker 1986). However, so far, only a few studies have investigated heritability and evidence for selection in nectar traits, such as sugar concentration and content (Parachnowitsch *et al.* 2019). Similarities in nectar quality may alternatively be due to similar morphological traits, *e.g.* shape of flower tubes, and thus similar requirements for protection against evaporation (Witt *et al.* 2013). For example, plant species with long tubular flowers typically contain sucrose-rich nectar (high sucrose proportion), whereas plant species with more open flowers typically contain hexose-rich nectar (high hexose proportion) to reduce evaporation *via* increased osmolality (Percival 1961; Bernardello 2007; Witt *et al.* 2013), because the loss of water in nectar leads to increased viscosity, which decelerates nectar uptake or even prevents pollinators from consumption (Köhler *et al.* 2010; Kim *et al.* 2011). In fact, several studies found a strong preference of long-tongued pollinators, such as butterflies, moths and long-tongued bees, for sucrose-rich nectar, and of short-tongued bees and flies for hexose-rich nectar (Baker & Baker 1983; Petanidou *et al.* 2006; González-Teuber & Heil 2009), indicating that floral morphology and nectar quality interact with pollinator preferences. Despite its importance as floral reward and source of nutrients for pollinators, the precise factors driving variation in nectar quality, such as the partial roles of phylogenetic relatedness, pollinator preferences and floral morphology, are still largely unclear (Parachnowitsch *et al.* 2019).

To contribute to a better understanding of the parameters which affect variation in nectar quality, we analysed potential correlations between the phylogenetic relatedness and morphology of plants and the amino acid and carbohydrate composition of their nectar for 34 grassland species. We hypothesized that: (i) plant species generally differed both qualitatively and quantitatively in their amino acid and carbohydrate composition, but that (ii) related plant species (*i.e.* species within the same families) would show a more similar nectar composition (with regard to concentrations and proportions of sugars and amino acids as well as their ratios) than plant species from different families due to phylogenetic and/or morphological constraints.

MATERIAL AND METHODS

Experimental field site

The study was conducted within the framework of the Jena Experiment, which is a grassland biodiversity experiment in Thuringia, Germany (50°55′ N, 11°35′ E; 130 m a.s.l.) established in 2002, comprising 60 native plant species of the plant association Arrhenatherion, with 16 grass species and 44 flowering herb species (Roscher *et al.* 2004). The study field is situated near the river Saale and encompasses 82 plots containing different plant species mixtures, forming a plant species richness gradient. We collected nectar of 34 flowering plant species from 22 of those plots, preferentially from species' monocultures, but also from plots with higher levels of plant species richness, when monocultures comprised insufficient numbers of flowering individuals. We selected all flowering plant species for which we could access a minimum of 1 µl, *i.e.* 34 out of 41 species. For more details on the design of the Jena Experiment, see Roscher *et al.* (2004).

Nectar sampling

In 2011, we sampled nectar of a total of 34 flowering plant species on sunny to cloudy days. Flowers were bagged in cotton gauze 1 day before nectar sampling (mesh size 0.80–1.00 mm) to exclude pollinators (Klein *et al.* 2003) and ensure that nectar was not depleted by insects prior to sampling. Accumulated nectar was collected in the morning using a microcapillary pipette (1 µl holding volume) equipped with a pipetting aid (for more details see Corbet 2003; Venjakob *et al.* 2020). We aimed to collect a minimum of 1 µl nectar per sample. When 1 µl could not be obtained from one flower, additional flowers were sampled. We collected samples from at least seven plant individuals per species. Exceptions with less samples for carbohydrate analysis were *Geranium pratense* L. (6 samples), *Trifolium fragiferum* L. (6), and *Trifolium pratense* L. (3). Six plant species (*Anthriscus sylvestris* Hoffm., *Campanula patula* L., *Glechoma hederacea* L., *Primula veris* L., *Ranunculus repens* L. and *Veronica chamaedrys* L.) were re-sampled in 2012 because nectar was too dilute due to heavy rain during the flowering period or because of insufficient amounts of nectar. Microcapillaries for nectar sampling were stored in Eppendorf tubes (see Venjakob *et al.* 2020) and kept at −20 °C until analyses.

Nectar preparation

Prior to analysis, microcapillaries (32 mm) with nectar were rinsed with 100 µl 99.8% ethanol and centrifuged for 5 min to remove nectar from capillaries into 2-ml Eppendorf tubes (see Venjakob *et al.* 2020). Eppendorf tubes with nectar were kept in a DURAN® desiccator to evaporate ethanol, and nectar was subsequently re-dissolved in 50 µl ultra-pure water and centrifuged for 3 min (Venjakob *et al.* 2020). Finally, 48 µl of the supernatant were transferred into 1-ml glass vials equipped with 250 µl pulled-point glass inserts and stored at −20 °C (for details see Venjakob *et al.* 2020).

Amino acid and carbohydrate analysis

Analyses of amino acids and carbohydrates were performed with high performance liquid chromatography (HPLC: Agilent

Technologies 1260 Series; Agilent, Böblingen, Germany). The LC was equipped with an Agilent 1260 Infinity Quaternary Pump (G1311C, Agilent), an Agilent 1260 Infinity Standard Autosampler (G1329B) and an Agilent 1260 Infinity Thermostatted Column Compartment (G1316A). Oven temperature was 40 °C for amino acids and 30 °C for carbohydrates.

Of each nectar extract, 8 µl (amino acids) and 30 µl (carbohydrates) were injected into the system. Prior to analysis, amino acids were derivatized using either ortho-phthalaldehyde (OPA; Agilent, for primary amino acids) or 9-fluorenylmethyl chloroformate (FMOC; Agilent, for secondary amino acids, *e.g.* proline). Separation of amino acids was achieved on an Extend-C18 column (Zorbax: 3.0 × 150 mm, 3.5 µm; Agilent) preceded by an Extend-C18 guard column (Zorbax: 2.1 × 12.5 mm, 5 µm; Agilent). We used a solvent gradient to separate amino acids with a buffer (1 l ultra-pure water, 10 mM Na_2HPO_4, 10 mM $Na_2B_4O_7$, 0.5 mM NaN_3, pH 8.2) as polar and acetonitrile–methanol–water (45%, 45%, 10% [vol/vol], all CHROMASOLV®; Sigma-Aldrich, Taufkirchen, Germany) as non-polar phase. A Diode Array Detector (DAD; Agilent 1260 Infinity System, G4212B) was used for detection. Each run started with a 2–98% non-polar to polar phase. The ratio was then gradually changed to 57% to 43% over 13 min and finally to 100% non-polar phase, which was kept for about 2 min. Solvent flow rate was constant at 0.75 ml min^{-1}.

Carbohydrates were eluted on a NH2 column (Zorbax: 4.6 × 250 mm, 5 µm; Agilent) preceded by a NH2 guard column (Zorbax: 4.6 × 12.5 mm, 5 µm; Agilent) under isocratic conditions. We used an elution buffer containing 78% [vol/vol] acetonitrile and 22% [vol/vol] ultra-pure water at a constant flow rate of 1.5 ml min^{-1}. Detection was achieved with a Refractive Index Detector (RID; Agilent 1260 Infinity, G1362 A).

After every five samples, we ran an external standard containing 17 amino acids (Amino Acid Standard solution; Sigma-Aldrich) or three carbohydrates (sucrose, fructose and glucose, HPLC grade; Sigma-Aldrich) in four different concentrations. Using the Agilent ChemStation software for LC 3D systems (Agilent), amino acids and carbohydrates were identified and quantified by matching retention times and areas of calibrated standard compounds with retention times and areas of compounds found in nectar samples. Note that we cannot measure glutamine, arginine and tryptophan with our method due to either their destruction during acid hydrolysis (tryptophan) or a lack of standards (asparagine, glutamine).

Statistical analysis

We investigated whether nectar from the 34 plant species and the four most abundant plant families differed in the composition of carbohydrates and/or amino acids using permutation tests (PerMANOVA, using 10,000 permutations), which were based on Bray-Curtis distances between substances (R package: vegan; Adonis command). Separate permutation tests were performed for concentrations (in mg ml^{-1}) and proportions of all carbohydrates and amino acids. We obtained proportions of individual compounds by dividing the concentration of each individual carbohydrate/amino acid by the total concentration of all carbohydrates/amino acids analysed.

We tested for plant species-specific differences in total carbohydrate and amino acid concentrations (*i.e.* the sum of all individual compounds), the total concentration and proportion of all essential amino acids and the total concentrations of all non-essential amino acids, as well as the ratio of all carbohydrates to all amino acids. Due to the nested plot design from which the samples were taken, we always tested first whether sample plot influenced the explained variance by composing both a generalized linear model (GLM) and generalized linear mixed effect models (GLMMs), with plant species entered as fixed factor and the plot from which the sample was taken as random factor. Due to the lack of a phylogenetic signal for all nutrient groups (see Table 1) we did not use GLMMs corrected for phylogenetic relatedness. Models were compared using the Akaike information criterion (AIC) and likelihood ratio tests (R package: lme4; anova command; following Zuur *et al.* 2009). We always provide results for the GLM if it did not have a significantly higher AIC value than the GLMM. For tests on plant species-specific differences, GLMs were always better than GLMMs, which renders the application of permutation tests for plant species-specific differences in compound compositions valid despite the nested plot design.

All analyses were repeated for plant family-specific differences between Asteraceae, Apiaceae, Fabaceae and Lamiaceae, as well as for different morphological flower traits. We confined family analyses to these four plant families, because they were the only families with sufficient numbers of plant species sampled per family ($N \geq 3$). Information on floral traits composed for the same plots was obtained from Fornoff *et al.* (2017) and included flower symmetry (binomial: actinomorphic or bilateral), nectar access (binomial: open or hidden), inflorescence area (mm^2) and flower height (mm). All morphological traits were obtained as mean per plant species. We therefore also calculated mean per plant species for amino acid and carbohydrate concentrations and proportions when analysing the effect of family and traits on nectar chemistry and for the equivalent permutation tests. We again used generalized linear (mixed effect) models (GL(M)Ms) and compared GLMs with GLMMs, with plot included as random factor. GLMMs often had significantly higher AIC values than GLMs, indicating that a high proportion of the observed variance was explained by plot identity. We additionally extracted marginal R^2 values for the different models to compare the variance explained by morphological traits and by plant family.

Following Junker *et al.* (2017) and Ruedenauer *et al.* (2019), we tested for a phylogenetic signal in the total, essential and non-essential content of amino acids, as well as the content of sugars and the carbohydrate (C) to total, essential and non-essential amino acid (AA) ratios (*i.e.* C:AA ratios) using

Table 1. Results of Blomberg's *K* tests for a phylogenetic signal within total/essential/non-essential amino acid and carbohydrates content, as well as the carbohydrate to amino acid ratios of 34 plant species.

nutrient	*K*	*P*
Total amino acids	0.101	0.325
Essential amino acids	0.094	0.476
Non-essential amino acids	0.132	0.177
Carbohydrates	0.112	0.331
Carbohydrate to amino acid ratio	0.304	0.107
Carbohydrate to essential amino acid ratio	0.306	0.097
Carbohydrate to non-essential amino acids ratio	0.160	0.265

Blomberg's *K*. The underlying phylogenetic tree was based on the phylogeny of Zanne *et al.* (2014).

Response variables were always tested for normality and homogeneity of variances using graphical tools, as suggested by Zuur *et al.* (2009) and either log- or square root- (concentrations, ratios) or arcsine square root (proportions)-transformed when these requirements were not met. Due to multiple use of the same dataset, we only considered $P < 0.01$ as being significant. We finally used the mean carbohydrate and amino acid proportions and concentrations of each plant species to visually assess similarities within plant families using cluster dendrograms (R package vegan), also based on Bray-Curtis distances between substances. All analyses were performed in R version 3.1.3 (R Development Core Team 2015).

RESULTS

Differences between plant species

The 34 plant species sampled showed species-specific compositions for both carbohydrates (PerMANOVA: concentrations: $R^2 = 0.69$, $P < 0.001$; proportions: $R^2 = 0.75$, $P < 0.001$) and amino acids (concentrations: $R^2 = 0.65$, $P < 0.001$; proportions: $R^2 = 0.82$, $P < 0.001$) with significant differences between species in the concentrations and proportions of all individual carbohydrates and amino acids (GLMs: *P*-values always ≤0.001; Tables S1, S2, Fig. 1). Intraspecific variation was also pronounced, particularly for amino acids (see standard variations in Table S1). Interestingly, related species (*i.e.* species within the same family) could be either chemically similar (*e.g.* Apiaceae species with regard to amino acid compositions, except *Pimpinella major*) or chemically distinct (*e.g.* Lamiaceae species with regard to amino acid compositions) or both (Asteraceae species with regard to amino acid compositions) as assessed by dendrograms and chemical distances between species (see Table S2a–d, Fig. S1). Also, some plant species (*e.g. Crepis biennis* or *Ajuga reptans* with regard to amino acid compositions, *Ranunculus acris* and *Sanguisorba officinalis* with regard to carbohydrate compositions) showed very distinct nutritional profiles which strongly deviated from most other plant species (Table S2a–d, Fig. S1). None of the nutrient contents, proportions and ratios showed a phylogenetic signal (Table 1).

Nectar of the 34 plant species also differed in the concentrations of the sum of all amino acids (GLM: $F = 18.93$, $P < 0.001$), all essential amino acids (GLM: $F = 17.15$, $P < 0.001$) and all non-essential amino acids (GLM: $F = 25.69$, $P < 0.001$), as well as in the proportion of all essential amino acids (GLM: $F = 35.44$, $P < 0.001$; Table S1, Fig. 1). *Crepis biennis* L., *Tragopogon pratensis* L., *Leontodon hispidus* L. (all Asteraceae) and *Sanguisorba officinalis* L. (Rosaceae) had nectar with the highest overall amino acid concentrations (all mean values >20 mg ml^{-1}; Table S1, Fig. 1). Nectar of these plant species also contained most essential amino acids, while highest concentrations of all non-essential amino acids were found in *Tr. pratensis* (Asteraceae), *T. campestre* Schreb., *T. hybridum* L., *T. pratense* L. (all Fabaceae) and *P. veris* (Primulaceae) (all mean values >10 mg ml^{-1}; Table S1, Fig. 1). Essential amino acids made up the highest proportion of all amino acids in *C. biennis* (Asteraceae), *Ajuga reptans* L, and *Prunella vulgaris* L. (both Lamiaceae) (all mean values ≥80%; Table S1, Fig. 1), while they were generally low in Fabaceae (*T. campestre*, *T. hybridum*, *T. pratense*, *T. repens* and *Vicia cracca*) and in *P. veris* (all mean values <40%; Table S1, Fig. 1).

The total carbohydrate amount in nectar also differed between plant species ($F = 18.95$, $P < 0.001$), with highest overall carbohydrate concentrations in *T. campestre*, *Lotus corniculatus* L. (both Fabaceae), *A. reptans* (Lamiaceae) and *Tr. pratensis* (Asteraceae) (all mean values >100 mg ml^{-1}; Table S1, Fig. 1). Overall carbohydrate concentrations were low in *Daucus carota* L. and *Pimpinella major* (L.) Huds. (both Apiaceae) (all mean values <10 mg ml^{-1}; Table S1, Fig. 1). Individual amino acids and carbohydrates followed similar trends (Table S1, Fig. 1).

The C:AA ratios in nectar of the 34 plant species were generally carbohydrate-biased, but ranged from equal mean ratios of C:AA = 1:1 [for *A. sylvestris* (Apiaceae) and *L. hispidus* (Asteraceae)] to mean ratios largely dominated by carbohydrates, C:AA = >20:1 [for *Geranium pratense* L. (Geraniaceae), *A. reptans* and *P. vulgaris*] and differed between plant species (GLM: $F = 19.17$, $P < 0.001$; Fig. S1, Fig. 2). The same was true for the ratio of all carbohydrates to all essential amino acids (henceforth referred to as C:EAA) (GLM: $F = 17.83$, $P < 0.001$).

Differences between plant families and related to morphological traits

Asteraceae, Apiaceae, Fabaceae and Lamiaceae differed in the composition of all amino acids when concentrations were considered (PerMANOVA: $P^2 = 0.27$, $P = 0.007$; Fig. S2a), and marginally when proportions were considered ($R^2 = 0.25$, $P = 0.02$; Fig. S2b). Essential amino acids also tended to show family-specific profiles (concentrations: $R^2 = 0.27$, $P = 0.01$; Fig. S2c; proportions: $R^2 = 0.27$, $P = 0.02$; Fig. S2d). However, visual inspection of chemical similarities showed that separation by family was not strict and mostly driven by differences between Fabaceae and Asteraceae (Fig. S2). Moreover, some species of different families (*e.g. Centaurea* and *Anthriscus*) were more similar to each other than to other species within their respective plant families (Fig. S2, Table S2a–d). The proportion of all essential amino acids (proportion: GLMM: $F = 4.090$, $P = 0.003$; Fig. 3c) also differed between the four plant families, whereas the overall amino acid concentrations did not (GLMM: $F = 1.425$, $P = 0.241$; Fig. 3a). Nectar of Asteraceae and Lamiaceae generally contained the highest concentrations and proportions of essential amino acids (Fig. 3b, c). Concentrations and proportions of 11 out of 17 single amino acids (*e.g.* glutamic acid, histidine, arginine. . .) also differed or tended to differ between the four plant families (Table S3).

When examining the effect of morphological traits of flowers, we found the concentration and proportion of lysine differed between symmetric and non-symmetric flowers (concentration: GLMM: $F = 11.329$, $P = 0.007$; proportion: $F = 38.488$, $P < 0.001$). The proportions of three other amino acids also tended to differ between the two flower types, while the remaining species were not affected by any floral traits (Table S3).

However, variation in nectar amino acid concentrations and proportions was always better explained by plant family than by flower morphology ($0.1 < R^2 \leq 0.92$ for 32 out of 36 models on the effect of plant family on variation in amino acid concentrations and proportions; $0.1 < R^2 \leq 0.13$ for 3 out of 144

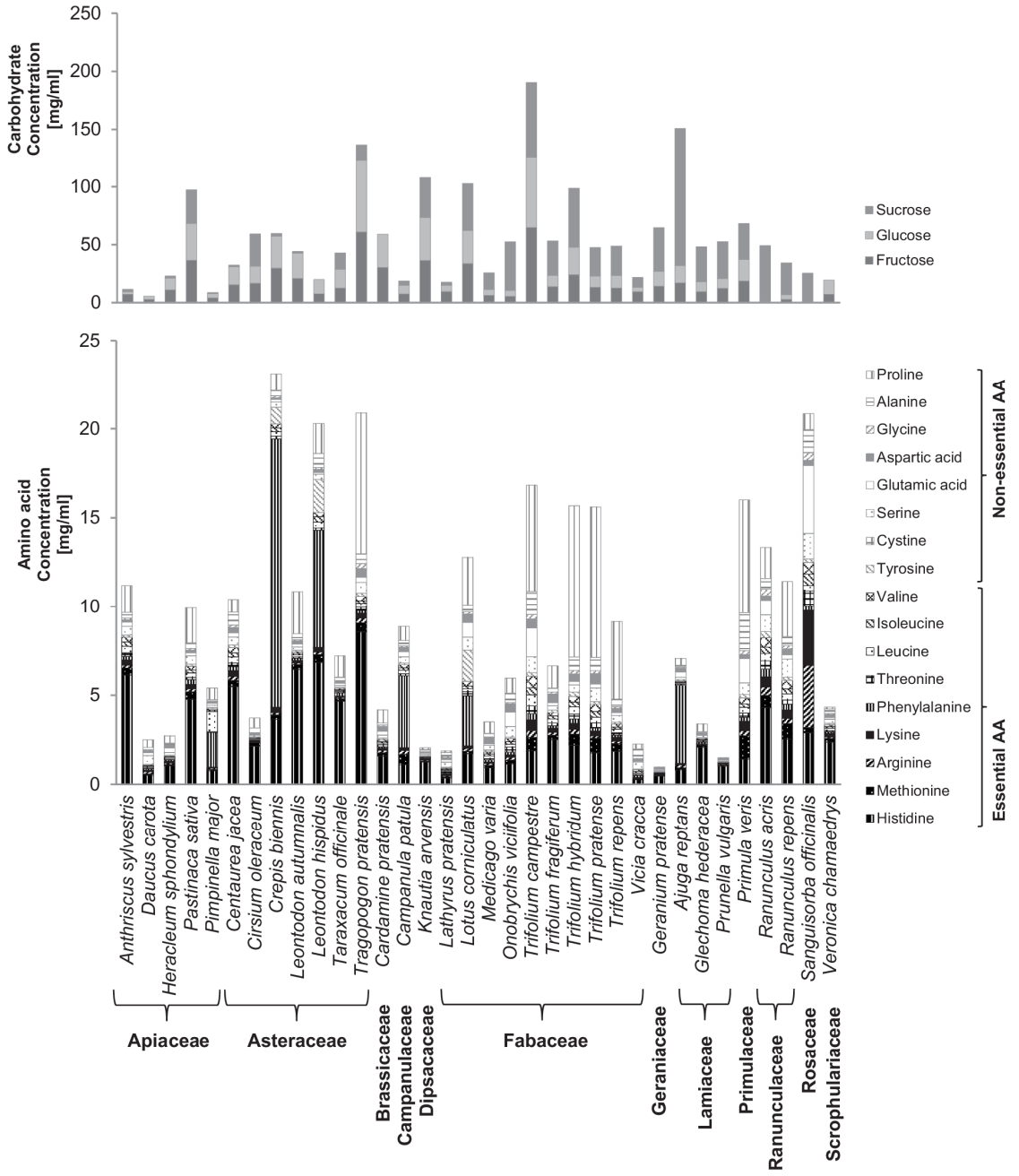

Fig. 1. Nectar carbohydrate and amino acid concentrations in mg ml^{-1} of 34 plant species, grouped by family in alphabetical order. Grey amino acids are non-essential amino acids, while black amino acids are amino acids considered essential for honeybees (following De Groot 1953).

models on the effect of morphology on variation in amino acid concentrations and proportions; Table S3).

The four plant families also had specific nectar carbohydrate profiles with regard to compound concentrations (PerMANOVA: $R^2 = 0.32$, $P = 0.003$; Fig. 4) and particularly proportions ($R^2 = 0.61$, $P < 0.001$; Fig. 5). Sucrose proportions were generally higher in Lamiaceae and Fabaceae and lower in Asteraceae and Apiaceae (GLMM: $F = 7.580$, $P < 0.001$; Fig. 5a). This pattern was reversed for glucose (GLMM: $F = 5.805$, $P < 0.001$; Fig. 5b) and fructose (GLMM: $F = 3.813$, $P = 0.004$; Fig. 5c). Single carbohydrate concentrations did not significantly differ between plant families (Fig. 4a–c), and neither did

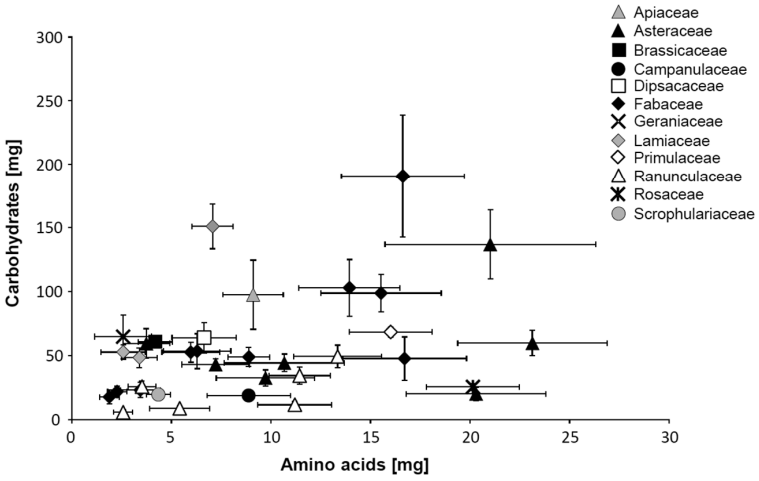

Fig. 2. Mean amounts [± SE] of total carbohydrates and amino acids in nectar of 34 plant species. Each symbol represents one plant species, with plant species having the same symbol belonging to the same plant family.

the total carbohydrate concentration (GLMM: $F = 0.585$, $P = 0.820$; Fig. 4d).

The concentrations of fructose (GLMM: $F = 4.347$, $P = 0.045$) and glucose (GLMM: $F = 4.537$, $P = 0.041$), as well as the total carbohydrate concentration (GLMM: $F = 4.629$, $P = 0.039$), also tended to differ between plants with open nectar access and plants with restricted nectar access. Proportions of glucose (GLMM: $F = 4.325$, $P = 0.046$) and sucrose (GLMM: $F = 4.276$, $P = 0.047$) also tended to increase with inflorescence area.

As found for amino acids, plant family explained most of the variance in nectar carbohydrate concentrations and proportions ($0.1 < R^2 \leq 0.67$ for all models on the effect of plant family on variation in carbohydrate concentrations and proportions; Table S4). Some variation was also explained by nectar access, *i.e.* variation in fructose ($R^2 = 0.12$), glucose ($R^2 = 0.13$) and total carbohydrate ($R^2 = 0.12$) concentrations, and by inflorescence area, *i.e.* variation in glucose ($R^2 = 0.12$) and sucrose ($R^2 = 0.12$) proportions (Table S4).

Interestingly, all plant species within a specific family, *i.e.* Asteraceae, Apiaceae, Fabaceae and Lamiaceae, showed similar C:AA ratios (Fig. 2), although total amounts of carbohydrates and amino acids per ml nectar differed between species (Table S1). Thus, C:AA ratios were family-specific, with significant differences between families (GLMM: $F = 9.16$, $P < 0.001$; Fig. 2). These differences could be attributed mainly to the Lamiaceae, whose nectar contained two to four times more carbohydrates (mean C:AA = 19:1) than nectar of the other plant families (Asteraceae 6:1, Apiaceae 5:1, Fabaceae 8:1; Fig. 2).

DISCUSSION

As expected, nectar of the 34 studied plant species and four most abundant plant families of a mesotrophic grassland community differed in carbohydrate and amino acid composition. However, despite significant differences between families, nectar composition was not significantly influenced by phylogenetic relatedness, indicating that additional factors affected variation in floral nectar quality.

Interestingly, when comparing the explanatory power of floral morphology and plant family, family continuously explained a larger proportion of the variation in nectar chemistry, *e.g.* up to 92% for the concentration of lysine, than the investigated morphological parameters. Differences between Apiaceae, Asteraceae, Fabaceae and Lamiaceae (all very attractive for pollinators: Ebeling *et al.* 2008; Venjakob *et al.* 2016) were particularly pronounced for carbohydrates, with higher hexose concentrations and proportions in Apiaceae (open flowers) and Asteraceae, and higher sucrose concentrations and proportions in Fabaceae and Lamiaceae (closed/tubular flowers). This finding agrees with previous studies indicating that nectar carbohydrate composition largely correlates with floral morphology (Percival 1961; Bernardello 2007; Witt *et al.* 2013) and thus likely with constraints imposed through osmolality. However, Asteraceae also have tubular flowers (ray or disc flowers) composed in radially symmetrical flower heads (Jäger *et al.* 2013), often formed as bowl- or even parabola-shaped flower heads (Kevan 1989). They nevertheless had mostly hexose-rich nectar, except for three species, which had relatively similar proportions of sucrose, fructose and glucose (Fig. 1). The hexose-rich nectar in most Asteraceae may be explained by the stronger tendency of the flowers to heat up as a consequence of the bowl- or parabola-shaped flower heads (Percival 1961). Different combinations of family-specific morphological aspects of flowers determining overall inflorescence shape and structure therefore need to be taken into account when addressing variations in nectar chemistry. The importance of such family-specific structural characteristics and combinations of morphological traits may also explain why 'nectar access' (a parameter based on the morphology of individual flowers in our study) had overall less explanatory power than 'plant family', and why we found strong family-specific differences in nectar chemistry but no in the phylogenetic signal. In fact, the two differently related plant families, Fabaceae and Lamiaceae, showed similarities in nectar carbohydrate composition, likely due to structural similarities of the flowers. The two more closely related plant families, Asteraceae and Apiaceae, also showed similarities in nectar carbohydrate composition, despite different flower structures. Moreover, within plant families, the nectar composition of individual species still varied independent of their relatedness.

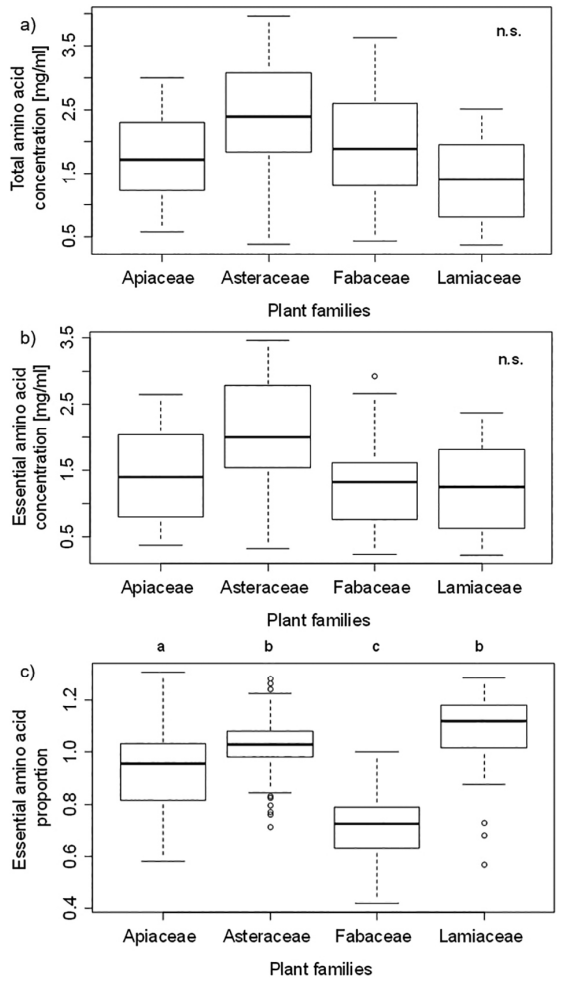

Fig. 3. Differences in (a) total concentrations (mg ml^{-1}) of amino acids, (b) concentrations (mg ml^{-1}) of all essential amino acids (both datasets were log-transformed) and (c) proportions of all essential amino acids (data were (arcsin(sqrt(x))-transformed)).

Alternatively or additionally, the observed differences in nectar chemistry may be related to different pollination syndromes. In fact, morphological and/or functional traits of specific pollinators can reliably predict the spectrum of flowering plants visited and thus specific links in pollination networks (Rosas-Guerrero *et al.* 2014). Besides preferences for specific flower colours or shapes, pollinators can also show preferences for specific nectar profiles. For example, many flower visitors prefer sugar solutions with amino acids (*i.e.* nectar) over pure sugar solutions, *e.g.* butterflies (Alm *et al.* 1990; Mevi-Schütz & Erhardt 2005; Beck 2007), flies (Shiraishi & Kuwabara 1970; Potter & Bertin 1988), honeybees (Inouye & Waller 1984; Alm *et al.* 1990; Carter *et al.* 2006; Bertazzini *et al.* 2010) and solitary bees (Petanidou *et al.* 2006). Honeybees further prefer sugar solutions with essential amino acids over sugar solutions with non-essential amino acids (Hendriksma *et al.* 2014). Moreover, essential amino acids significantly support their feeding gland and flight muscle development (Hendriksma *et al.* 2019). Increasing concentrations and proportions of (essential) amino acids or of amino acids known to be perceived by pollinators (Ruedenauer *et al.* 2020) may consequently be a useful tool to increase attractiveness to potential pollinators and/or distract them from collecting pollen. In fact, Asteraceae plants in our study tended to have the highest concentrations and proportions of all (essential) amino acids and of the essential amino acid histidine, which may (among others) explain why Asteraceae were frequently visited by many different pollinators and other flower-visiting insects (Table S5; also Ebeling *et al.* 2008; Venjakob *et al.* 2016). However, some amino acids can also deter insects (Bell *et al.* 1996; Toshima & Tanimura 2012), as can several plant secondary metabolites (Stevenson *et al.* 2017). Moreover, nectar often contains additional substances in relatively low amounts, such as minerals or fatty acids (Nicolson & Thornburg 2007), which may also affect nectar preferences (Parachnowitsch *et al.* 2019). Notably, nectar of the Lamiaceae species investigated in our study did not show as high concentrations of the essential amino acid phenylalanine as Lamiaceae nectar of plants from the Mediterranean phryganic community (Petanidou *et al.* 2006), suggesting that different selection pressures may act on the nectar amino acid composition of related plant species growing in different habitats.

Besides individual compounds and compound groups (*e.g.* carbohydrates, C, or amino acids, AA), ratios (e.g. C:AA) between different compound groups were recently found to correlate with pollen foraging preferences, *e.g.* in bumblebees (Vaudo *et al.* 2016). Ratios have, to our knowledge, hitherto not been related to phylogeny or other floral traits. We found C:AA and C:EAA ratios in general to be carbohydrate-biased and variable, but also to show some plant species and family specificity. The carbohydrate-biased ratios meet the nutritional needs of most flower visitors, *e.g.* adult honeybee and bumblebee workers, which typically prioritize carbohydrate over (essential) amino acid intake, even over-consuming amino acids to obtain sufficient carbohydrates, and perform generally better on carbohydrate-rich diets (Paoli *et al.* 2014; Stabler *et al.* 2015; Austin & Gilbert 2021). However, (in particular female) butterflies prefer nectars rich in amino acids, and thus likely having lower C:AA ratios, because amino acids increase their fecundity (Mevi-Schütz & Erhardt 2005). Nutritional requirements of different pollinator groups (*e.g.* butterflies *versus* bees) might thus exert different selection pressures on the nectar chemistry of insect-pollinated plants (Jervis & Boggs 2005), and might affect C:AA ratios (*e.g.* towards amino acids/proteins in flowering plant species pollinated by butterflies). In our dataset, C:AA ratios were lowest in Apiaceae and Fabaceae and high in Lamiaceae, which might explain (among others) why Lamiaceae (with their proportionally lower amino acid content) were most frequently visited by bees, while Apiaceae and Fabaceae (with their proportionally higher amino acid content) attracted all sorts of flower visitors (including butterflies; see Tables S3, S4).

However, studies investigating the importance of various plant traits in predicting niche partitioning in temperate interaction networks between plants and insect flower visitors found floral phenology, morphology (*e.g.* tube length), scent and colour, but not nectar chemistry, to most strongly determine choices of insect visitors (Junker *et al.* 2013; Rafferty & Ives

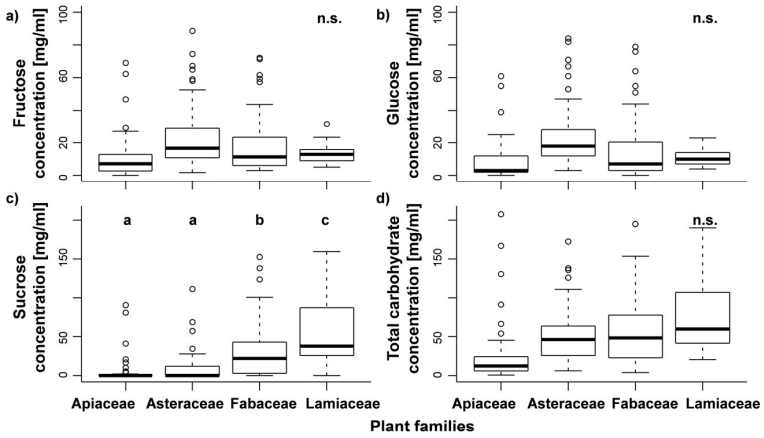

Fig. 4. Concentrations of fructose (a), glucose (b), sucrose (c) and all three (total carbohydrates) (d) (in mg ml^{-1}) in nectar of the most abundant plant families, Apiaceae, Asteraceae, Fabaceae and Lamiaceae. Different lowercase letters within each diagram indicate significant differences between the plant families, n.s. indicates no significance.

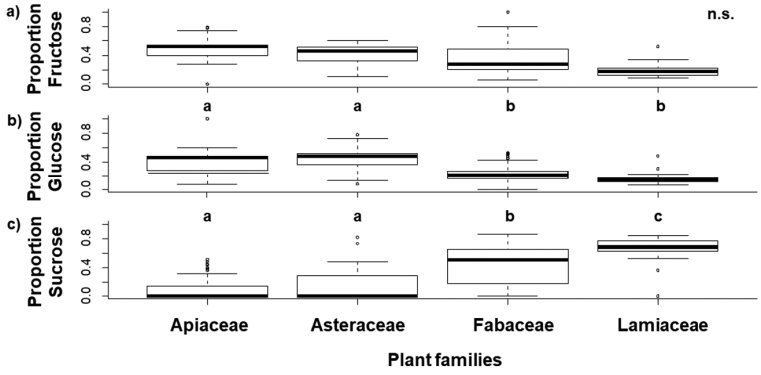

Fig. 5. Proportions of fructose (a), glucose (b) and sucrose (c) in nectar of the most abundant plant families, Apiaceae, Asteraceae, Fabaceae and Lamiaceae. Different lowercase letters within each diagram indicate significant differences between the plant families.

2013; Kantsa *et al.* 2018). Nevertheless, interestingly, different floral traits can interact. For example, bee-pollinated flowers often have a blue or yellow colour and UV marks (Wilson *et al.* 2004), known to be correlated with a comparatively high sugar content (Chittka & Menzel 1992) and to match the visual capacities of bees (Chittka & Menzel 1992). In fact, local variation in nectar sugar content might even drive selection for innate colour preferences (Raine & Chittka 2007). In turn, nectar volume in the bumblebee-pollinated *Aconitum gymnandrum* was found to be under strong selection pressure by pollinators (Zhao *et al.* 2016). These studies indicate that the plant–pollinator mutualism can affect nectar chemistry, but that directional outcomes are variable and likely depend on specific interactions. Our results suggest that these additionally depend on plant family-specific morphological constraints. Finally, an increasing number of studies highlight the role of specific microbiota in nectar (Fridman *et al.* 2012) and even of the foraging behaviour of pollinators themselves (Bogo *et al.* 2021) in affecting nectar chemistry, providing additional factors that may determine variation in floral nectar chemistry.

More thorough investigations of nutrient amounts and ratios found in floral resources, *i.e.* pollen and nectar, of plant species differing in phylogenetic relatedness, morphological characters, pollinator dependency and reward strategy, as well as their effect on pollinator preferences, should enable us to better disentangle the contributions of these various factors impacting floral resource chemistry. This knowledge is essential for better understanding the mechanisms underlying plant–pollinator interactions (Kantsa *et al.* 2018; van der Kooi *et al.* 2021).

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CONFLICTS OF INTEREST/COMPETING INTERESTS

The authors declare no conflict of interest.

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AUTHOR CONTRIBUTIONS

CV, AMK and SDL designed the experiment. CV collected data; SDL and FAR performed statistical analyses. CV developed the first draft of the manuscript, and all authors contributed substantially to revisions.

AVAILABILITY OF DATA AND MATERIAL

All original data is submitted with this manuscript and will be deposited at the Jena Experiment upon publication.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Fig. S1. Ratio of total amino acids (AA) in mg to total sugar in mg.

Fig. S2. Cluster dendrograms of the four most abundant plant families (Apiaceae, Asteraceae, Fabaceae and Lamiaceae).

Table S1. Nectar concentration and proportion of chemical components, such as amino acids and carbohydrates, measured *via* HPLC.

Table S2. Chemical distance matrix between 34 plant species based on Bray-Curtis distances between either: the (a,c) proportions (%) or (b,d) concentrations (conc) of amino acids and carbohydrates.

Table S3. Statistical results of generalized linear (mixed) models (GL(M)Ms) testing for effects of morphological factors (flower symmetry, nectar access, inflorescence area, flower height) and family for different single and total amino acid concentrations and proportions.

Table S4. Statistical results of generalized linear (mixed) models (GL(M)Ms) testing for effects of morphological factors (flower symmetry, nectar access, inflorescence area, flower height) and family for different single and total carbohydrate concentrations and proportions.

Table S5. Plant species studied (Roscher *et al*. 2004) for their nectar amino acid and carbohydrate composition (plant species in italic and families in bold letters) with flower visitors recorded at different plots of the Jena Experiment in 2011 (for further details see Venjakob *et al*. 2016).

Table S6. Morphological traits of flowers and accessibility of pollen and/or nectar.

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Chapter 4

Inter-Individual Nectar Chemistry Changes of Field Scabious *Knautia arvensis*

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Inter-Individual Nectar Chemistry Changes of Field Scabious, *Knautia arvensis*

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Abstract: Nectar is crucial to maintain plant-pollinator mutualism. Nectar quality (nutritional composition) can vary strongly between individuals of the same plant species. The factors driving such inter-individual variation have however not been investigated closer. We investigated nectar quality of field scabious, *Knautia arvensis* in different grassland plant communities varying in species composition and richness to assess whether nectar quality can be affected by the surrounding plant community. We analyzed (with high performance liquid chromatography) the content of carbohydrates, overall amino acids, and essential amino acids. Amino acid and carbohydrate concentrations and proportions varied among plant individuals and with the surrounding plant community but were not related to the surrounding plant species richness. Total and individual carbohydrate concentrations were lowest, while proportions of the essential amino acids, valine, isoleucine, leucine (all phagostimulatory), and lysine were highest in plant species communities of the highest diversity. Our results show that *K. arvensis* nectar chemistry varies with the composition of the surrounding plant community, which may alter the taste and nutritional value and thus affect the plant's visitor spectrum and visitation rate. However, the strong inter-individual variation in nectar quality requires additional studies (e.g., in semi-field studies) to disentangle different biotic and abiotic factors contributing to inter-individual nectar chemistry in a plant-community context.

Keywords: amino acids; carbohydrates; flower-visiting insects; insect nutrition; Jena Experiment

1. Introduction

Plants are important bottom up partners of multitrophic interactions [1–3]. Interactions between plants and animals further drive important ecosystem functions and processes, such as herbivory, pollination or seed dispersal [1,4–6], because plants provide a habitat and resources for many animals [7,8]. These interactions in turn depend on the composition and diversity of the surrounding plant community [1]. Plant community composition directly determines the spectrum of interaction partners, as different animals require different spectra of plant species for resource acquisition [9] (pp. 190–200), while plant species diversity typically determines the diversity of higher trophic levels [10–12].

In general, resources essentially link plants and their interaction partners. For example, flower resources such as pollen and nectar, attract flower-visiting and pollinating insects, as they provide

essential nutrients and thus represent the currency for their pollination success. Resource quality (i.e., the chemical nutritional composition of resources, such as nectar, pollen or leaves) varies between different plant species [13–15], and even between individuals of the same species and flowers of the same individual [16,17]. Resource quality can also change in relation to specific plant—insect interactions, likely to adjust to a specific interaction partner (e.g., specific flower visitor or herbivore species), as shown for vegetative tissue (e.g., leaves) [18–22]. The factors driving such inter-individual variation in resource quality are however still unclear, particularly for floral resources.

In plant-pollinator interactions, nectar plays a pivotal role; unlike pollen, it is consumed by most, if not all, flower visitors. Thus, pollinators visit flowers more frequently to collect nectar than pollen [23,24]. Nectar is produced in floral nectaries [25], but not in high volumes as it is costly for plants [26]. Nectar predominantly contains carbohydrates, namely two hexose monosaccharides (fructose and glucose) and a disaccharide (sucrose) [27,28] (pp. 215–264). Other components, such as amino acids, lipids [29], antioxidants, and alkaloids, are also present, albeit in much lower quantities, and can play an important nutritional (e.g., amino acids) role for pollinators [28,30,31]. After carbohydrates, amino acids are the most abundant nectar nutrient and encompass a wide range of different essential and non-essential amino acids sensu De Groot [32], which may vary in composition and concentration depending on the plant species [13]. Nectar amino acid and carbohydrate content can differ even between different cultivars (e.g., in rapeseed) [33]. Ratios of different macro-nutrient groups (e.g., of amino acids to carbohydrates) may also differ for floral nectar, but have been little investigated [30]. This is surprising, as nutrient ratios can be more important than overall content of different nutrients in determining nutritional quality for consumers in general [34,35] and flower visitors in particular [36–39].

Given the large inter- and intraspecific variation in nectar nutritional composition (henceforth termed nectar quality), differences in nectar quality likely contribute to pollinator community partitioning, as different flower visitors differ in taste preferences [14] and their nutritional requirements [18,37]. In fact, Garratt et al. [40] found that different apple varieties were pollinated by different pollinator communities likely due to variety-specific differences in nectar quantity and quality. Again, the factors underlying such inter-individual variation in nectar quality remain to be determined. They may comprise both biotic factors (e.g., community composition, species interactions) and abiotic factors (e.g., soil composition or pH).

Here, we investigate whether inter-individual variation in nectar quality (and thus potentially nectar attractiveness) varies with the surrounding plant community by investigating nectar quality of a common grassland (Arrhenatherion) plant species, the field scabious, *Knautia arvensis* (L.) Coult. Dipsacaceae, which typically attracts many flower-visiting insects [41] (pp. 557–562) or [42,43]. The study species was sown in 2002 in various plant communities differing in species richness and community composition (more details on the Jena Experiment [44]). We analyzed the composition of amino acids and carbohydrates as well as the ratios of carbohydrates to amino acids in nectar of *K. arvensis*, to relate nectar quality to changes in plant species richness and thus community composition.

We hypothesized that concentrations of carbohydrates, overall amino acids (AA), and essential amino acids (EAA) in nectar of *K. arvensis* will increase with increasing plant species richness, while their proportions should remain constant, because plants may be competing more strongly for pollinators in communities with more plant species, and thus pollinators present [11,45] compared to communities with less plant species. At least in some plant species, nectar composition can be phenotypically plastic and thus change following exposure to pollinators, as shown in *Helleborus foetidus* [46]. Such phenotypic plasticity in nectar chemistry could enable plants to adjust their nectar composition in response to an increased pollinator visitation frequency as likely found in species-rich plant communities. With regard to carbohydrate to amino acid ratios (henceforth referred to as C:AA and C:EAA ratios), we expected them to be constant across different communities, because different plant species typically have species-specific ratios of carbohydrates to amino acids [47].

2. Materials and Methods

2.1. Experimental Field Site

We collected nectar from *K. arvensis* grown in 7 different plots (i.e., from six 6 × 5.5 m + 3 × 3.5 m = 43.5 m^2 community plots and one 1 × 1 m monoculture), in August 2010 (three out of seven plots) and June 2012 (all seven plots) (Figure S1 and Table S1). The community plots had different stabilized plant communities and were part of the Jena Experiment [44], which is a grassland biodiversity experiment located in Thuringia, Germany (50°55′ N, 11°35′ E; 130 meters above sea level (m a.s.l.)). Started in 2002, it comprises 82 plots, which were sown with 1, 2, 4, 8, 16 or 60 plant species from a 60-plant species pool, common in Central European mesophilic Arrhenatherion grasslands [44]. Specifically, 80 plots of 43.5 m^2 (hereafter large plots) are located within 20 × 20 m squares comprising all diversity mixtures [48]. Monocultures of all species are either grown in large or small (1 × 1 m) plots (i.e., one monoculture per species) [48]. In June and September, all plots were mown, simulating traditional extensive hay meadow management. Plots were weeded three times during the year and all non-target plant species were removed. More detailed description of the experiment is given in a study by Roscher et al. [44].

We collected *K. arvensis* nectar from all plots comprising sufficient (i.e., a minimum of five plants) *K. arvensis* individuals, namely from one small monoculture, one 4-species, three 8-species, and two 16-species plots (see Figure S1 for plot distribution and distances between plots). As plot size of the monoculture (8 replicates) was smaller than of the mixtures (47 replicates), we tested if plot size affected any of our nectar response variables. As plot size did not explain the nectar variables, we did not consider it in our analyses described below.

All plant species, including *K. arvensis*, were sown in 2002 and since then maintained by regular mowing and weeding of non-target plant species three times per year. At plots with plant species mixtures, other plant species typically flowered simultaneously and thus likely competed for pollinators with *K. arvensis* (see Table S1 for a detailed list of plant species flowering at each plot). We considered one plant individual as one sample and one inflorescence typically consists of approximately 55–100 flowers [49], which are arranged in a dense flower head and mostly provide enough nectar for one sample (>1 µL). We took great care not to scratch flowers or inner tissue or to contaminate samples with pollen [50] during nectar sampling. However, even if slight contamination with pollen grains occurred, it unlikely affected the amino acid composition of nectar as shown for *Aloe marlothii* nectar [51] (p. 206). We consequently collected a minimum of five samples per plot (7.86 ± 4.02).

2.2. Nectar Sampling

For standardized nectar sampling, nectar was collected from at least five *K. arvensis* individuals per plot between 10 a.m. and 2 p.m. on sunny or light cloudy days. Five was the minimum number of samples that we aimed for. Where possible, we collected more samples to obtain a more robust dataset. The day before sampling, we placed gauze bags (mesh size 0.8–1.00 mm) around at least five inflorescences (8.14 ± 3.93) to prevent early foraging pollinators from depleting nectar standing crop [52]. Since nectar secretion is typically dynamic and highly variable over the course of one day, we rapidly assessed nectar production, prior to the actual sampling, through repeated sampling and measuring at the site using a hand-held refractometer. We found *Knautia arvensis* to produce nectar all day long. Nectar was collected from several florets using microcapillaries with a minimum volume of 1 µL (pipetting aid and a disposable capillary; minicaps®, Hirschmann Laborgeräte GmbH & Co. KG, Eberstadt, Germany) [53]. We chose and sampled florets of all ages to average across age-specific differences for each sampled plant individual. Nectar samples of each plant individual were stored in clean and autoclaved 1.5 mL Eppendorf tubes (Safe-Lock Tubes, Eppendorf AG, Hamburg, Germany) and kept in a cool box in the field before freezing at −20 °C.

2.3. Sample Preparation

To analyze the amino acid and carbohydrate composition in nectar, samples were re-dissolved in 100 µL of 99.8% ethanol (CHROMASOLV®, Sigma-Aldrich Laborchemikalien GmbH, Hannover, Germany), centrifuged for 5 min (158 g, Mikro 22 R, Hettich Lab Technology, Schwerin, Germany), and transferred from capillaries into Eppendorf tubes using a pipetting aid. Samples were kept in a DURAN®-desiccator (CARL ROTH GMBH + CO. KG, Karlsruhe, Germany) to completely evaporate alcohol at 20 °C, before adding 50 µL ultra-pure water (Siemens AG, Barsbüttel, Germany) for centrifuging (3 min) to remove potential left-over precipitates. From the supernatant, 48 µL were pipetted into 1 mL glass vials for HPLC analytics (Agilent Technologies, Böblingen, Germany), equipped with 250 µL pulled-point glass inserts (Agilent Technologies, Böblingen, Germany), and frozen at −20 °C prior to chemical analyses. Before analysis, we waited until the samples adjusted to the ambient temperature and ensured that no precipitation occurred after taking the prepared samples out of the freezer.

2.4. Amino Acid and Carbohydrate Analysis

Amino acids and carbohydrates were analyzed using high performance liquid chromatography (HPLC) from Agilent Technologies 1260 Series provided with an Agilent 1260 Infinity Quaternary Pump (G1311C, Agilent Technologies, Böblingen, Germany), an Agilent 1260 Infinity Standard Autosampler (G1329B), and an Agilent 1260 Infinity Thermostatted Column Compartment (G1316A), to maintain the temperature for amino acids at 40 °C and for carbohydrates at 30 °C.

Amino acids were separated on a Zorbax Extend-C18 column (3.0 × 150 mm, 3.5 µm, Agilent Technologies, Böblingen, Germany), preceded by a guard column Zorbax Extend-C18 (2.1 × 12.5 mm, 5 µm, Agilent Technologies, Böblingen, Germany), and were detected by an Agilent 1260 Infinity System Diode Array Detector (DAD, G4212B) with a flow rate of 1 mL min^{-1}. Prior to injection, amino acids were derivatized with either ortho-phthalaldehyde (OPA, Agilent Technologies, Böblingen, Germany, for primary amino acids: alanine, arginine, aspartic acid, cystine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, serine, threonine, tyrosine, and valine) or 9-fluorenylmethyl chloroformate (FMOC, Agilent Technologies, Böblingen, Germany, for proline) [54–56]. Amino acids were separated by a solvent gradient with a buffer (1 L ultra-pure water, 10 mM Na_2HPO_4, 10 mM $Na_2B_4O_7$, 0.5 mM NaN_3, pH 8.2) used as polar phase and acetonitrile-methanol-water (45%:45%:10% (*v*/*v*), all CHROMASOLV®, Sigma-Aldrich Chemie GmbH, Munich, Germany) used as non-polar phase [54,55]. We started with a 2%:98% non-polar to polar phase, then gradually changed the ratio to 57%:43% for 13 min, until finally increasing the non-polar phase to 100% for a period of 2 min, followed by a re-equilibration to 2%:98% non-polar to polar phase for about 9 min [54]. Solvent flow rate was 0.750 mL min^{-1} [54].

Carbohydrates were separated on a NH2 column (Zorbax: 4.6 × 250 mm, 5 µm, Agilent Technologies) preceded by a NH2 guard column (Zorbax: 4.6 × 12.5 mm, 5 µm, Agilent Technologies) under isocratic conditions using an elution buffer with 78%:22% (*v*/*v*) acetonitrile and ultra-pure water and a flow rate of 1.5 mL min^{-1}. Carbohydrates were detected by a refractive index detector (RID, Agilent 1260 Infinity, G1362 A) [57].

Four different concentrations of a standard comprising 17 amino acids (Amino Acid Standard solution, Sigma-Aldrich Laborchemikalien GmbH, Hannover, Germany) or three carbohydrates (sucrose, fructose, and glucose, HPLC grade, Sigma-Aldrich Laborchemikalien GmbH, Hannover, Germany) were run every five samples as an external reference. All amino acids and carbohydrates in nectar samples were identified based on standard reference compounds. HPLC control and compound quantification was carried out with Agilent ChemStation for LC 3D systems (Agilent Technologies, Böblingen, Germany).

2.5. Observations of Flower Visitors

Flower-visiting insects, such as honeybees, bumblebees, solitary bees, and hoverflies, were surveyed within the framework of the Jena Experiment on a subset of plots between May and August 2011 (see [58] for details on how flower visitors were observed). Thus, nectar sampling and flower-visitor observations were performed at different years. We extracted all observations on flower visitors to *K. arvensis* for those plots for which we also had collected nectar (i.e., two 8-species plots and one 16-species plot). Flower visitors were grouped as honeybees, bumblebees, solitary bees, and hoverflies for subsequent analyses, and we defined all solitary bees as non-eusocial Apidae [59]. We finally summed all flower visitors across all observations performed in 2011 and calculated per plot the Shannon diversity index, total number of species, total number of individuals, and number of individuals for the different flower-visiting groups for each plant community.

2.6. Statistical Analyses

We investigated whether nectar from *K. arvensis* plants grown in plant communities that differed in species richness (i.e., *K. arvensis* monocultures and communities with 4, 8, and 16 plant species) and composition of plants differed in the composition of carbohydrates and/or amino acids as well as the ratio of all carbohydrates to all amino acids. We used permutation tests based on Bray–Curtis distances between substances (i.e., Adonis in the vegan R package) to test for an effect of community richness. Separate permutation tests were performed for concentrations (in mg/mL) and proportions of individual carbohydrates and amino acids. Proportions of individual compounds were obtained by dividing the concentration of each individual carbohydrate/amino acid by the total concentration of all carbohydrates/amino acids analyzed. When significant differences between communities were found, we subsequently analyzed differences between plant species in the concentrations and/or proportions of all individual carbohydrates/amino acids. We further assessed community-specific differences for total carbohydrate and amino acid concentrations, the concentration and proportion of essential amino acids (EAA), and non-essential amino acids (nEAA) and the C:AA, C:EAA, and C:nEAA ratios. Three outliers were excluded from the original dataset for the amino acid analyses.

Due to the nested plot design, from which the samples were taken, we always tested first whether the sample plot significantly affected the explained variance by composing both generalized linear models (GLMs) and generalized linear mixed effect models (GLMMs) with plant species richness level entered as a categorical fixed factor and the plot from which the sample was taken as a random factor. Models were compared using the Akaike information criterion (AIC) and likelihood ratio tests (Adonis command in the lme4 R package). AIC values were always similar for GLMMs and GLMs, which renders the application of permutation tests and GLMs (not accounting for random plot effects) for richness level-specific differences in compound compositions as valid. The lack of a plot effect further indicates that plot, and thus the corresponding plot-specific plant community composition, did not significantly explain the observed variation in nectar chemistry. Significant variation in nectar quality between communities as revealed by GLMs was subsequently analyzed with Tukey's post hoc tests. Preliminary data screening revealed that total carbohydrate concentrations as well as concentrations of all individual carbohydrates (i.e., glucose, fructose, and sucrose) significantly increased over the course of the day (all $r > 0.3$, $p < 0.03$, Spearman correlation). We therefore included "time of day" as a random factor in all models including carbohydrate concentrations (using the lmer function in the lme4 R package). Due to unequal plot numbers between years and different sampling months (August in 2010 and June in 2012), we did not include year as random factor in the models. Moreover, differences between years were relatively low (i.e., total sugars: 61.4 ± 47.1 mg/mL in 2010 and 66.0 ± 95.8 mg/mL in 2012; total AA: 1.9 ± 1.6 mg/mL in 2010 and 2.3 ± 2.8 mg/mL in 2012), indicating that variation was better explained by factors other than the year.

Response variables were always tested for normality and homogeneity of variances using the Shapiro–Wilk normality test (stats R package, version 4.0.0) and graphical tools as suggested by [60]. We log or square root (concentrations, ratios) or arcsine square root (proportions) transformed the data

when these requirements were not met. Due to multiple usage of the same dataset, we only considered *p*-values below 0.01 as significant.

We finally used the carbohydrate and amino acid proportions and concentrations of each plant sample to visually display differences between richness levels using non-metrical dimensional scaling (NMDS, R package vegan) also based on Bray–Curtis distances between substances. All analyses were performed in R, version 3.1.3 [61].

3. Results

Overall, nectar amino acid and carbohydrate concentrations and proportions varied among *K. arvensis* individuals, but also with community composition and thus species richness (Table 1, Figure S2). Nectar generally contained similar concentrations/proportions of the three major carbohydrates (glucose, fructose, and sucrose), but different concentrations/proportions of amino acids (Table 1, Figures 1 and S2). Histidine was most prominent and accounted for more than 50% of all amino acids in most samples (see Table 1, Figures 1c,d and S2), with proline and alanine representing the second most prominent amino acids (each present in more than 10 times lower concentrations than histidine; Table 1, Figures 1c,d and S2).

K. arvensis individuals from communities differing in plant species richness had specific compositions of amino acids when proportions were considered (Adonis: $r^2 = 0.12$, $p < 0.01$; Table 1, Figures 2a and S2) and of carbohydrates when concentrations were considered ($r^2 = 0.29$, $p < 0.001$; Table 1, Figures 2b and S2). With regard to concentrations of amino acids their composition tended to show community-specific profiles ($r^2 = 0.11$, $p = 0.02$), as did proportions of carbohydrates ($r^2 = 0.18$, $p = 0.02$).

Concentrations of all amino acids tended to differ between communities (GLM: $F = 2.56$, $p = 0.06$) and were highest in the four-species community (Figure 1c, Table 1, Figure S2). Proportions of all essential amino acids did not differ between communities ($F = 1.94$, $p = 0.13$) (Table 1, Figures 1d and S2), but the individual essential amino acids valine ($F = 5.16$, $p < 0.01$), isoleucine ($F = 6.16$, $p < 0.001$), leucine ($F = 5.56$, $p < 0.01$), and lysine ($F = 4.71$, $p < 0.01$) were all found in significantly higher proportions in the nectar of plants of the 16, rather than the eight, species community (Tukey's test: all $p < 0.01$). There were no significant differences when comparing with the other plant species communities (monoculture and four plant species community) (Tukey's test: all $p > 0.01$) (Table 1, Figures 1 and S2).

The only non-essential amino acids that tended to proportionally differ between communities was aspartic acid (GLM: $F = 3.64$, $p = 0.02$) with the highest proportions in the *K. arvensis* monoculture and the 16 plant community (Tukey's test: $p = 0.01$; Table 1, Figures 1d and S2).

With regard to carbohydrates, both total carbohydrate concentration ($\chi^2 = 24.84$, $p < 0.001$) and the concentrations of individual carbohydrates (glucose: $\chi^2 = 23.58$, $p < 0.001$; fructose: $\chi^2 = 23.07$, $p < 0.001$; sucrose: $\chi^2 = 26.40$, $p < 0.001$) were lowest in the 16 species community and highest in the four species community (Table 1, Figures 1a and S2). Trends were the same for sucrose proportions ($F = 3.40$, $p = 0.02$), but reversed for proportions of glucose ($F = 3.40$, $p = 0.02$) and fructose ($F = 3.40$, $p = 0.01$) (Table 1, Figures 1b and S2).

Nectar generally contained more carbohydrates than amino acids (Figures 1 and 3), but the ratio of carbohydrates to amino acids (C:AA) varied strongly between individual plants (Table 1, Figures 3 and S2) and ranged from C:AA ratios of 4:1 (16 species community) to 170:1 (four species community) with a mean value (±standard deviation) of C:AA 49 (±37):1 (Figure 3). However, C:AA ratios did not show clear community-specific differences ($F = 1.96$, $p = 0.13$), and neither did C:EAA ($F = 0.27$, $p = 0.85$) and C:nEAA ($F = 0.97$, $p = 0.41$) ratios (Table 1, Figures 3 and S2).

Though the Shannon index of flower-visiting insects was slightly higher in one of the eight plant species communities than in the 16 plant species community (Tables 2 and S2), the number of individuals of flower-visiting guilds increased with plant species community (from eight to 16 plant species community) as did the total numbers and species richness of flower visitors (Table 2, for more details Table S2).

Table 1. Nectar volume, concentration (mg/mL), proportion, and ratios of chemical components, such as amino acids and carbohydrates shown here as mean per plant species level with standard deviation (SD).

Plant Species Community [1]			**1**		**4**		**8**		**16**	
N Samples [2]			**8**		**5**		**24**		**18**	
			Mean	**±SD**	**Mean**	**±SD**	**Mean**	**±SD**	**Mean**	**±SD**
Volume Nectar [3]	µL		**3**	**4.11**	**3.31**	**2.71**	**2.73**	**2.73**	**4.35**	**2.76**
Amino Acids										
Asp [4]	**C**		0.05	0.03	0.08	0.09	0.07	0.07	0.03	0.03
Glu	**O**		0.08	0.08	0.11	0.09	0.07	0.08	0.04	0.03
Ser	N		0.07	0.07	0.09	0.07	0.07	0.08	0.03	0.02
His	**C**		1.26	1	3.06	3.38	1.55	1.65	0.5	0.31
Gly	**E**		0.04	0.04	0.06	0.04	0.04	0.05	0.02	0.01
Thr	**N**		0.03	0.03	0.04	0.03	0.03	0.02	0.02	0.01
Arg	**T**		0.06	0.09	0.06	0.04	0.04	0.03	0.03	0.02
Ala	**R**		0.11	0.09	0.35	0.31	0.17	0.14	0.08	0.07
Tyr	**A**		0.04	0.04	0.04	0.02	0.03	0.03	0.02	0.02
Cystine	**T**	mg/mL	0	0	0.01	0.01	0	0	0	0
Val	**I**		0.03	0.03	0.05	0.04	0.03	0.02	0.02	0.02
Met	**O**		0.04	0.1	0.01	0.02	0.02	0.05	0.01	0.01
Phe	**N**		0.03	0.03	0.03	0.02	0.03	0.03	0.02	0.02
Ile			0.03	0.02	0.03	0.02	0.01	0.01	0.02	0.01
Leu			0.04	0.04	0.06	0.03	0.04	0.03	0.03	0.03
Lys			0.04	0.04	0.07	0.04	0.04	0.02	0.03	0.03
Pro			0.12	0.09	0.45	0.52	0.35	0.37	0.12	0.17
Total AA [5]			2.07	1.66	4.59	4.71	2.59	2.4	1.03	0.74
EAA [6]			1.57	1.31	3.41	3.59	1.79	1.75	0.69	0.43
nEAA [7]			0.49	0.4	1.18	1.12	0.8	0.69	0.34	0.33
Asp [4]	**P**		0.03	0.01	0.02	0.01	0.03	0.01	0.03	0.01
Glu	**R**		0.04	0.01	0.03	0.02	0.03	0.02	0.05	0.02
Ser	**O**		0.04	0.02	0.03	0.02	0.03	0.02	0.04	0.02
His	**P**		0.58	0.13	0.58	0.12	0.56	0.09	0.5	0.13
Gly	**O**		0.02	0.01	0.02	0.01	0.02	0.01	0.02	0.01
Thr	**R**		0.02	0.01	0.01	0.01	0.02	0.01	0.02	0.01
Arg	**T**		0.03	0.01	0.02	0.02	0.02	0.01	0.03	0.01
Ala	**I**		0.06	0.02	0.1	0.03	0.08	0.03	0.08	0.02
Tyr	**O**		0.02	0.01	0.01	0.01	0.01	0.01	0.02	0.01
Cystine	**N**		0	0	0	0	0	0	0	0
Val			0.02	0.01	0.02	0.01	0.02	0.01	0.02	0.01
Met			0.01	0.02	0.01	0.02	0.01	0.01	0.01	0.02
Phe			0.02	0.01	0.01	0.01	0.01	0.01	0.02	0.01
Ile			0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.01
Leu			0.02	0.01	0.02	0.01	0.02	0.01	0.03	0.01
Lys			0.02	0.01	0.02	0.01	0.02	0.01	0.03	0.01
Pro			0.08	0.06	0.08	0.03	0.12	0.07	0.09	0.05
Total AA [5]			1	-	1	-	1	-	1	-
EAA [6]			0.73	0.09	0.71	0.04	0.68	0.07	0.69	0.08
nEAA [7]			0.27	0.09	0.29	0.04	0.32	0.07	0.31	0.08
Carbohydrates										
Fructose	**C**		36.44	25.51	57.25	22.67	23.95	12.76	11.21	7.39
Glucose	**O**	mg/mL	37.12	27.73	57.04	22.2	24.6	12.65	11.25	7.16
Sucrose	**N**		34.41	27.43	91.14	44.25	26.54	23.9	8.12	11.42
Total C [5]	**C.**		107.98	77.02	205.43	85.82	75.09	41.62	30.58	24.39
	P									
Fructose	**R**		0.36	0.08	0.29	0.06	0.34	0.1	0.42	0.09
Glucose	**O**		0.37	0.1	0.29	0.06	0.35	0.11	0.43	0.1
Sucrose	**P**		0.27	0.18	0.42	0.13	0.31	0.21	0.15	0.18
Carbohydrate and Amino Acid Ratios	**R A**									
mean C:AA [8]	**T**		50.96	22.97	89.41	62.48	45.55	28.95	42.03	37.72
mean C:EAA [9]	**I**		61.64	47.66	100	89.38	89.37	145.87	103.17	137.14
mean C:nEAA [10]	**O**		422.54	761.96	286.99	282.55	732.98	1882.35	726.34	1120.32

[1] Plot number and plant species richness level of the sown plant community including *Knautia arvensis*. [2] For each plant species the minimum sampling number was five samples per plant species for both analyses, N gives the number of analyzed samples for amino acids and carbohydrates, respectively. [3] Volume nectar gives the mean value per inflorescence in µL ± SD (standard deviation). [4] Abbreviations: Ala—alanine, Arg—arginine, Asp—aspartic acid, cystine, Glu—glutamic acid, Gly—glycine, His—histidine, Ile—isoleucine, Leu—leucine, Lys—lysine, Met—methionine, Phe—phenylalanine, Pro—proline, Ser—serine, Thr—threonine, Tyr—tyrosine, Val—valine. Order of displayed amino acids reflects the order of appearance in the chromatogram. [5] Total AA (amino acids) are the mean sum of all single amino acids ± SD (standard deviation) in mg per mL, followed by individual amino acids. Total C (carbohydrates) are the mean sum of the three main carbohydrates (fructose, glucose, sucrose) in mg/mL ± SD (standard deviation). [6] EAA: essential amino acids (His, Thr, Arg, Val, Met, Phe, Ile, Leu, Lys). [7] nEAA: non-essential amino acids (Asp, Glu, Ser, Gly, Ala, Tyr, cysteine measured as cystine, Pro). [8] Ratio C:AA: ratio of total carbohydrates to total amino acids. [9] Ratio C:EAA: ratio of total carbohydrates to essential amino acids. [10] Ratio C:nEAA: ratio of total carbohydrates to non-essential amino acids.

Table 2. Shannon diversity index [62], total numbers, species richness of flower visitors, number of individuals per flower-visiting guild of *K. arvensis* growing in plant species communities of eight and 16 plant species.

Plant Species Community	8 (B3A20)	8 (B2A12)	16 (B1A20)
Shannon Index	0.72	0.97	0.96
Total Numbers	232	300	383
Species Richness of Flower Visitors	9	12	15
Beetles	-	3	9
Bumblebees	28	49	36
Butterflies	-	2	10
Flies	-	5	2
Honeybees	195	230	303
Hoverflies	6	8	17
Solitary bees	3	2	6
Wasps	-	1	-

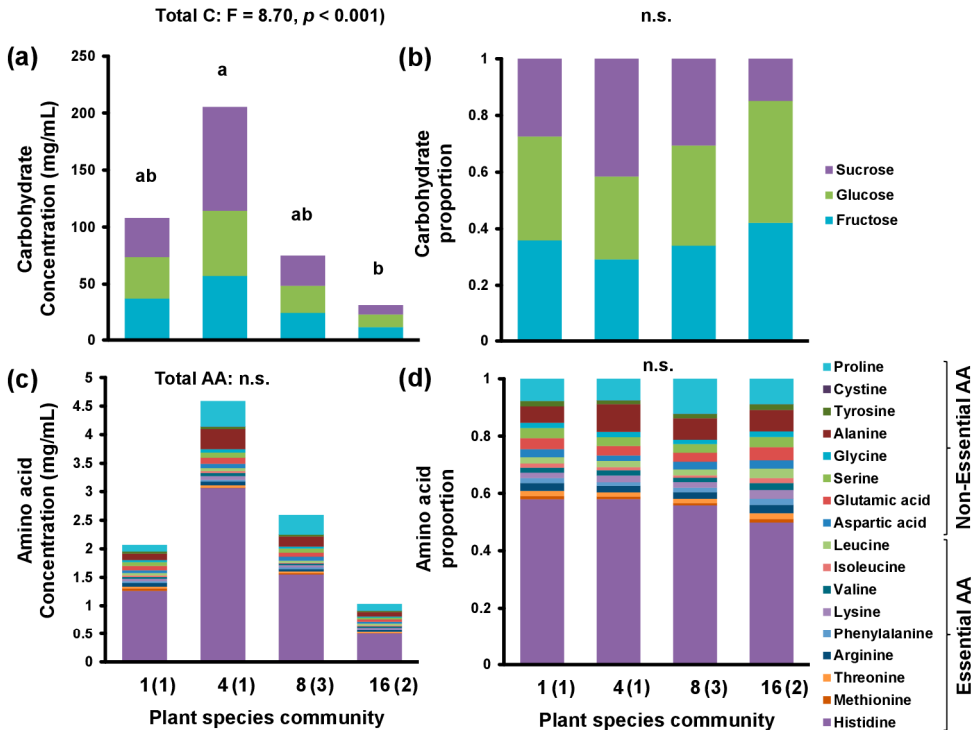

Figure 1. Carbohydrate (**a**) concentrations and (**b**) proportions as well as amino acid (**c**) concentrations and (**d**) proportions in nectar of *Knautia arvensis* growing in different plant communities with one, four, eight, and 16 different plant species. Concentration is given in mg/mL. Amino acids are divided into non-essential and essential amino acids (sensu [32]). Numbers in parentheses give the number of plots sampled per plant community. Letters above the bars indicate the significance of differences between plant species communities, while n.s. indicates no significance.

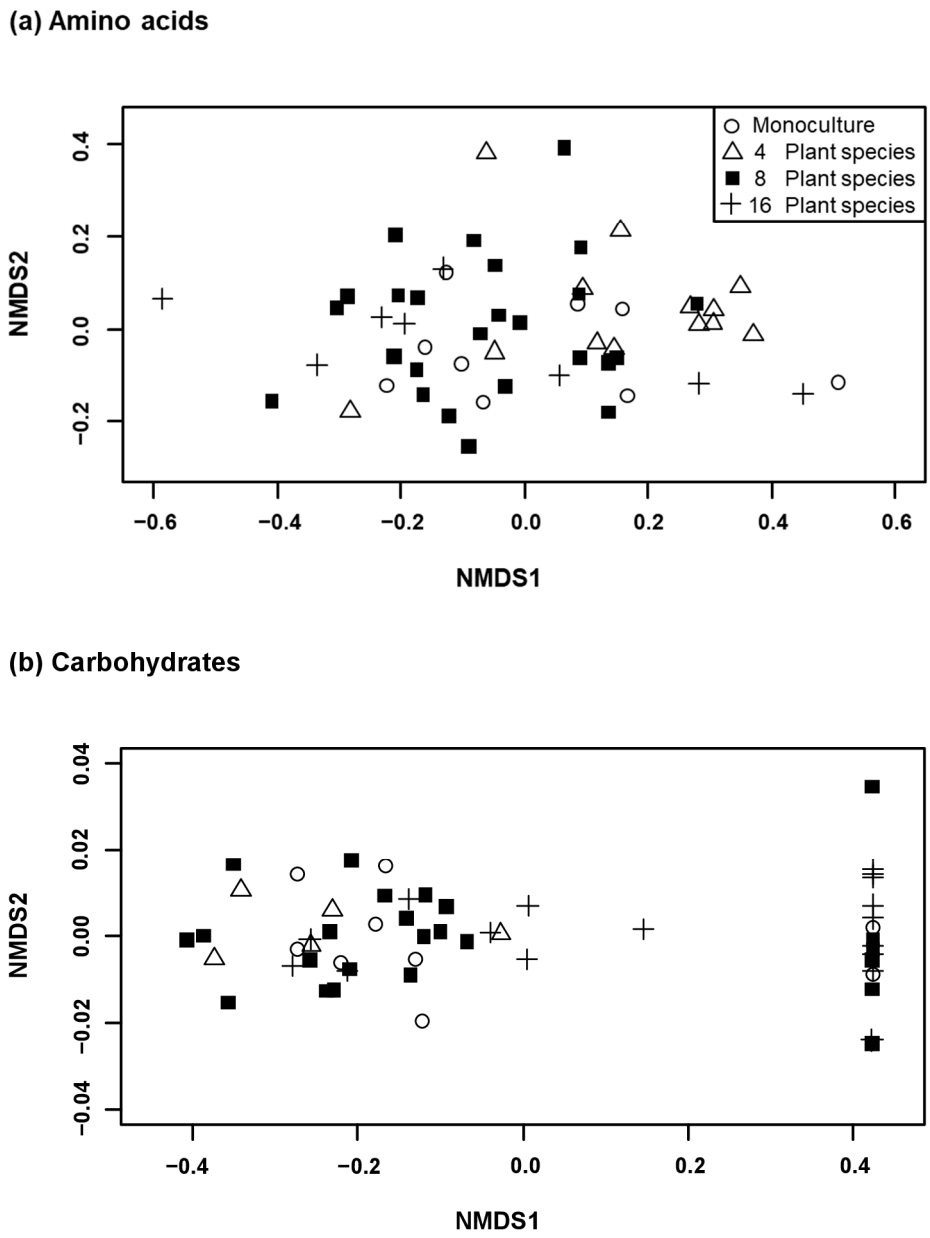

Figure 2. Non-metrical dimensional scaling (NMDS) based on (**a**) proportions of amino acids and (**b**) concentrations of carbohydrates in floral nectar of *Knautia arvensis*. Each symbol represents a sample of different plant communities: open circles = monoculture, open triangles = four plant species communities, filled black squares = eight plant species communities, and black crosses = 16 plant species communities. Note that all samples accumulating at the right side of the graph lack sucrose, likely because sucrose had already been hydrolyzed to fructose and glucose by yeast and/or bacteria which naturally occur in nectar [63].

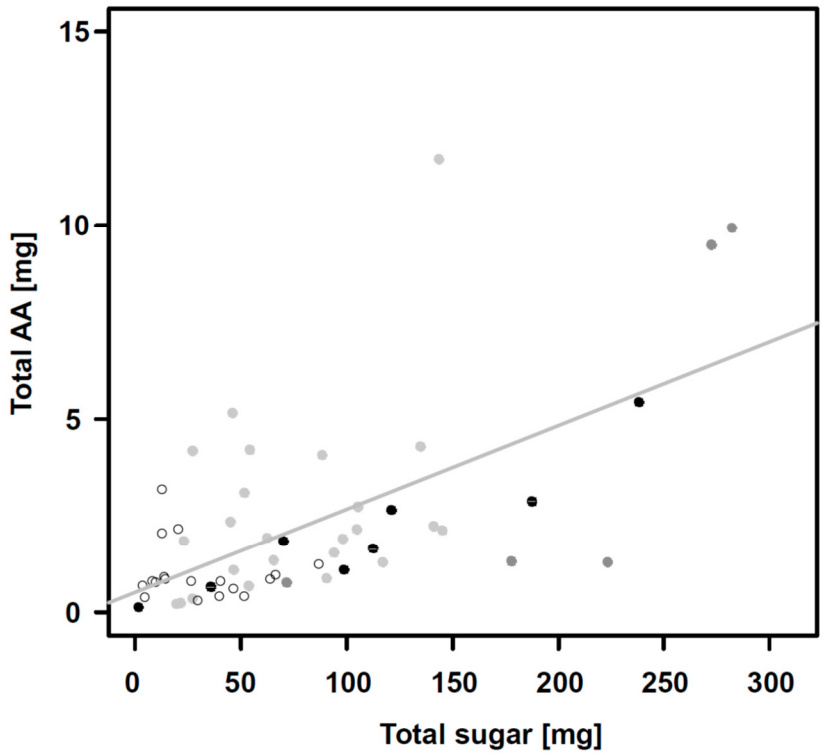

Figure 3. Ratio of total amino acids to total carbohydrates in mg (C:AA) in nectar of *Knautia arvensis* grown in different plant species richness communities. Each dot represents one sample with different colors indicating different plant species communities: black dots = monoculture, dark grey = 4, light grey = 8, white = 16 plant species community.

4. Discussion

Our results confirm strong inter-individual variation in nectar chemistry and further show that the nutritional composition of floral nectar of *K. arvensis* can vary strongly with the surrounding plant species community. For example, proportions of the essential amino acids, valine, isoleucine, leucine, and lysine and the non-essential amino acid, aspartic acid, differed between communities and were all highest in the 16 species community. However, contrary to our hypothesis, we found the observed variation in nutrient concentrations to be independent of the plant species richness in the surrounding plant community. Inter-individual variation in *K. arvensis* nectar chemistry therefore appeared to be affected by genetic differences between individuals, by abiotic factors or by the composition of the surrounding community rather than by its species richness. In fact, the spectrum of plant species co-occurring and in particular co-flowering with *K. arvensis* differed between plots (see Table S1). As the competitiveness of a specific plant species can differ with the surrounding plant community [63,64], it is possible that *K. arvensis* experienced different, and community-dependent, levels of competition at different plots, which may have indirectly affected its nectar chemistry. In interaction with subtle, potentially also plant-community mediated, differences in soil quality (i.e., concentration and composition of soil nutrients, microbial communities), such community-dependent competition may (at least partly) explain the considerable variation in nectar chemistry both within and between plant communities [58,65–67]. Community-dependent competition can also be caused by different intensities of wind-pollinated plant species [65]. It does, however, not explain the large variation in nectar chemistry observed for different individuals even within the same plot. Nutrient concentrations in nectar can vary due to water evaporation over the course of a day and in relation to ambient relative humidity [68], resulting in nectar viscosity increasing with increasing temperatures and/or decreasing humidity [53]. Although nectar sampling was confined to a period of 4 h (i.e., took place between 10 a.m. and 2 p.m.) in our study, the total carbohydrate concentration in nectar significantly increased

over this period (Spearman rank correlation test: $r = 0.33$, $p = 0.02$) and ranged from mean 37.78 (± 20.01) mg/mL at 10 a.m. to mean 103.62 (± 92.60) mg/mL at 2 p.m. (data pooled for both years). This significant effect of sampling time indicates that abiotic factors can determine nectar chemistry more strongly than biotic factors, such as the surrounding plant community. However, sampling time did not affect total amino acid concentration ($r = 0.03$, $p = 0.86$). It therefore remains unclear which alternative factors caused the variation in nectar amino acid content (coefficient of variation (CV): 1.09) which was even slightly higher than nectar carbohydrate content (CV: 0.88). Additional variation may have been caused by differences in the biomass and/or density of *K. arvensis* plants between plots, differences in pollinator communities and thus visitation frequencies between plots [11,58], differences in plot sizes, and/or by flower handling and sample collection, although we took extreme caution to standardize sampling.

The observed variation in nectar chemistry may in turn have had strong effects on flower visitors. For example, honeybees typically prefer sugar solutions with essential amino acids over sugar solutions with non-essential amino acids [69], but can be deterred by specific amino acids (e.g., alanine [68] or glycine [67]), while other amino acids (e.g., isoleucine) appear to act as a feeding stimulant and increase nectar consumption [70]. Differences in the concentration or proportion of specific amino acids can consequently attract or deter specific flower visitors and differences in amino acid proportions and ratios may thus structure visitation patterns. In fact, the proportional increase in phagostimulatory attractive amino acids (i.e., essential amino acids and isoleucine) in *K. arvensis* nectar at 16 species plots may (among others) explain why most honeybees (*Apis mellifera* Linnaeus, 1758) were observed on *K. arvensis* plants in the 16 species plot (Tables 2 and S2) [59,62].

The general prevalence of histidine in *K. arvensis* nectar (which could account for <50% of total amino acids) is intriguing and differs from other plant species where histidine proportions commonly lie between 12% and 16% (for different *Brassica napus* cultivars [33]) or below 3% (for *Maurandya barclayana*, *Lophospermum erubescens*, and *Brassica napus* [69]). Consumers of *K. arvensis* nectar will thus likely over-eat histidine if they aim to obtain sufficient amounts of the other amino acids (or a balanced C:AA ratio), with unknown consequences for their health or behavior. However, histidine may act as a repellent to honeybees as shown by Hendriksma et al. [67], where nectar with histidine was less frequently consumed than nectar with glycine and cysteine.

In contrast to proportions of specific amino acids, carbohydrate to amino acid ratios (C:AA, Table S2) showed, as expected, no community-specific pattern, but also varied strongly between plant individuals. The carbohydrate to amino acid ratio was generally carbohydrate biased, which agrees with nectar's major role as a carbohydrate source [71] (pp. 142–159). It also meets the nutritional needs of most flower visitors, such as honeybee and bumblebee workers, which typically prioritize carbohydrate over (essential) amino acid intake, even over-eat amino acids to obtain sufficient carbohydrates, and perform generally better on carbohydrate-rich diets [36,72]. Carbohydrates are important for flight performance in flower visitors [73].

For future work, we propose to repeat similar investigations and analyses, ideally under more controlled conditions (e.g., in greenhouses) to reduce sources of variation. It would further be worthwhile expanding nectar sampling and pollinator observations to more plant communities and species to directly relate nectar quality, flower visitor spectra, visitation rates, and floral constancy of pollinators to the composition of the direct and wider surrounding community. This is essential for understanding which factors drive inter-individual variation in nectar quality and how interactions between resource (nutritional) characteristics and the environment structure flower visitor interactions.

5. Conclusions

Both carbohydrate and amino acid content in nectar varied between *K. arvensis* individuals as well as between the different plant species richness levels of plant communities. However, there were significant differences in proportions in some essential and phagostimulatory amino acids in nectar of *K. arvensis* plants in plant species-rich communities, while the inhibiting amino acid histidine tended

to be less available. This suggests that *K. arvensis* nectar is more palatable to insects when plants grow in plant communities with high plant species richness. However, the strong inter-individual variation in nectar quality requires additional studies (e.g., in semi-field conditions).

Supplementary Materials: The following are available online at http://www.mdpi.com/2075-4450/11/2/75/s1. Figure S1: Map displaying sizes and locations of plots sampled in this experiment (modified after [48]). Table S1: List of plant species communities with one, four, eight, and 16 plant species, providing plot IDs, sampling year, and sucrose concentration (%) as measured for one sample with a hand-held refractometer, and the numbers and actual species of other plant species flowering and not flowering when *Knautia arvensis* was flowering in 2011. Table S2: List of individual flower visitors to all *Knautia arvensis* in plant species communities with eight and 16 plant species observed in 2011. Figure S2: List of figures presenting the mean (±SD) concentrations and proportions of individual amino acids and carbohydrates as well as total amino acids (AA), all essential amino acids (EAA), and all non-essential amino acids (nEAA) as found in floral nectar of *Knautia arvensis* from different plant species mixtures (i.e., monoculture, four, eight, and 16 plant species mixtures).

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Appendix A

Plant diversity increases spatio-temporal niche complementarity in plant-pollinator interactions

Table S1: Plant species used in the experiment, with an indication of the flowering phenology during the course of a year

Figure S1: Location of study plots

Figure S2: Top 20 highest flower cover per plant species. Flower cover was measured as proportion of the observed area (0.8 x 0.8 m) for each plant species and plant species level respectively; shown are the calculated flower cover as mean, maximum and minimum.

Figure S3: The number of flowering plant species and flower cover in relation to the number of identified pollinator species and on number of pollinator visits

Appendix S1: R-code for the calculation of the SDT

Appendix S2: R-code for the calculation of the proportion of the deviance

Table S2: List of identified pollinators

Table S3: List of visited plant species by all pollinator functional groups, with pollinator species

Table S4: GAMM model of flower visitation rate of all pollinators (bumblebees, solitary bees, hoverflies) excluding honeybees

Figure S4: Effects of plant species richness, time of day and flowering height on flower visitation rate of the pollinator community without honeybees

Table S5: Plant species richness vs. flower cover as explanatory variable

Table S6: All pollinator groups differed significantly in spatio-temporal resource use and in their response to plant species richness

Figure S5: Effects of plant species richness on the flower visitation of all pollinators (honeybees, bumblebees, solitary bees, hoverflies), (a) based on data from Ebeling et al. (2008) and (b) based on our data

Location of study plots

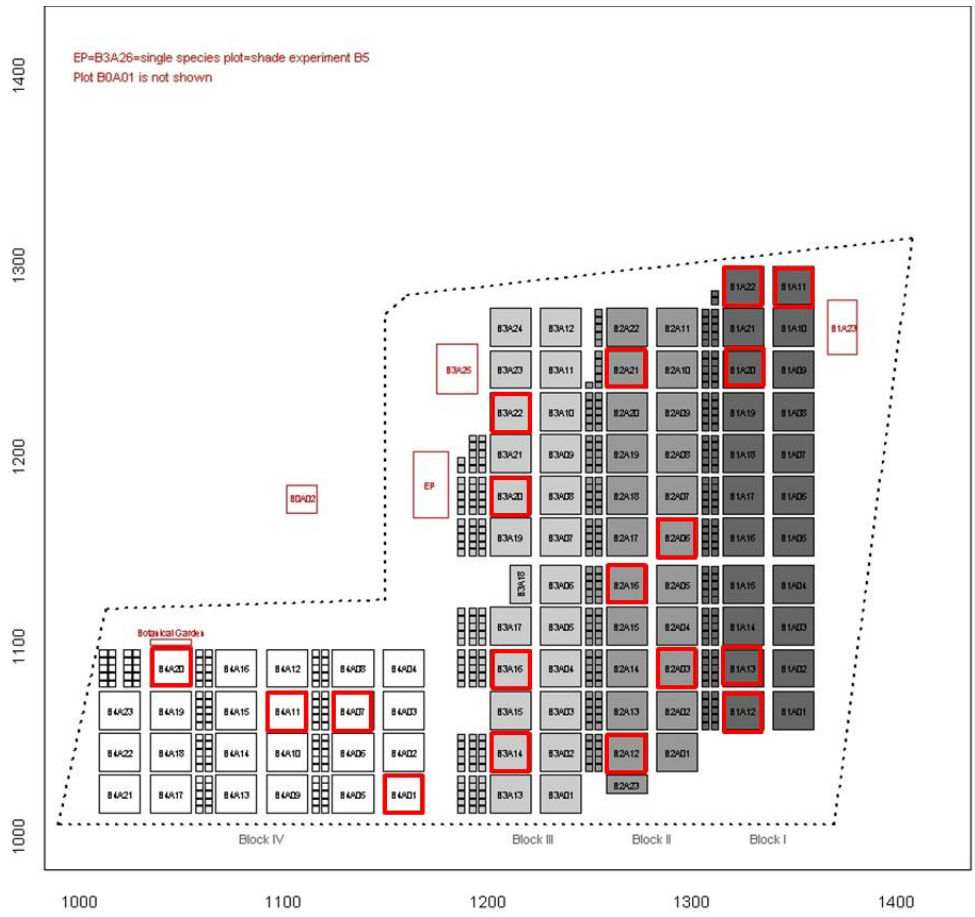


Figure S2: Location of individual study plots at the experimental field site. Each block contained up to five plots, except block 3 which contained four plots; red frames mark the plots.

Table S1: Plant species used in the experiment, with an indication of the flowering phenology over the year. Different functional groups are indicated as leg= legume, therb=tall herbs and sherb=small herbs. Flowering periods for each plant species are marked in orange color. Pollinator observations were performed for a total of seven days in 2011 (14 April, 5 and 23 May, 23 June, 12 July, 2 and 23 August).

Plant species	Family	Funct. Groups (leg, therb, sherb)	January	Febru-ary	March	April	May	June	July	August	Sep-tember	October	Novem-ber	Decem-ber
Achillea millefolium	Asteraceae	therb												
Ajuga reptans	Lamiaceae	sherb												
Anthriscus sylvestris	Apiaceae	therb												
Bellis perennis	Asteraceae	sherb												
Campanula patula	Campanulaceae	therb												
Cardamine pratensis	Brassicaceae	therb												
Carum carvi	Apiaceae	therb												
Centaurea jacea	Asteraceae	therb												
Cirsium oleraceum	Asteraceae	therb												
Crepis biennis	Asteraceae	therb												
Daucus carota	Apiaceae	therb												

(continued)

Table S1. (continued)

Plant species	Family	Funct. Groups (leg, therb, sherb)	January	Febru-ary	March	April	May	June	July	August	Sep-tember	October	Novem-ber	Decem-ber
Galium mollugo	Rubiaceae	therb												
Geranium pratense	Geraniaceae	therb												
Glechoma hederacea	Lamiaceae	sherb												
Heracleum sphondylium	Apiaceae	therb												
Knautia arvensis	Dipsacaceae	therb												
Lathyrus pratensis	Fabaceae	leg												
Leontodon autumnalis	Asteraceae	sherb												
Leontodon hispidus	Asteraceae	sherb												
Leucanthemum vulgare	Asteraceae	therb												
Lotus corniculatus	Fabaceae	leg												
Medicago lupulina	Fabaceae	leg												

(continued)

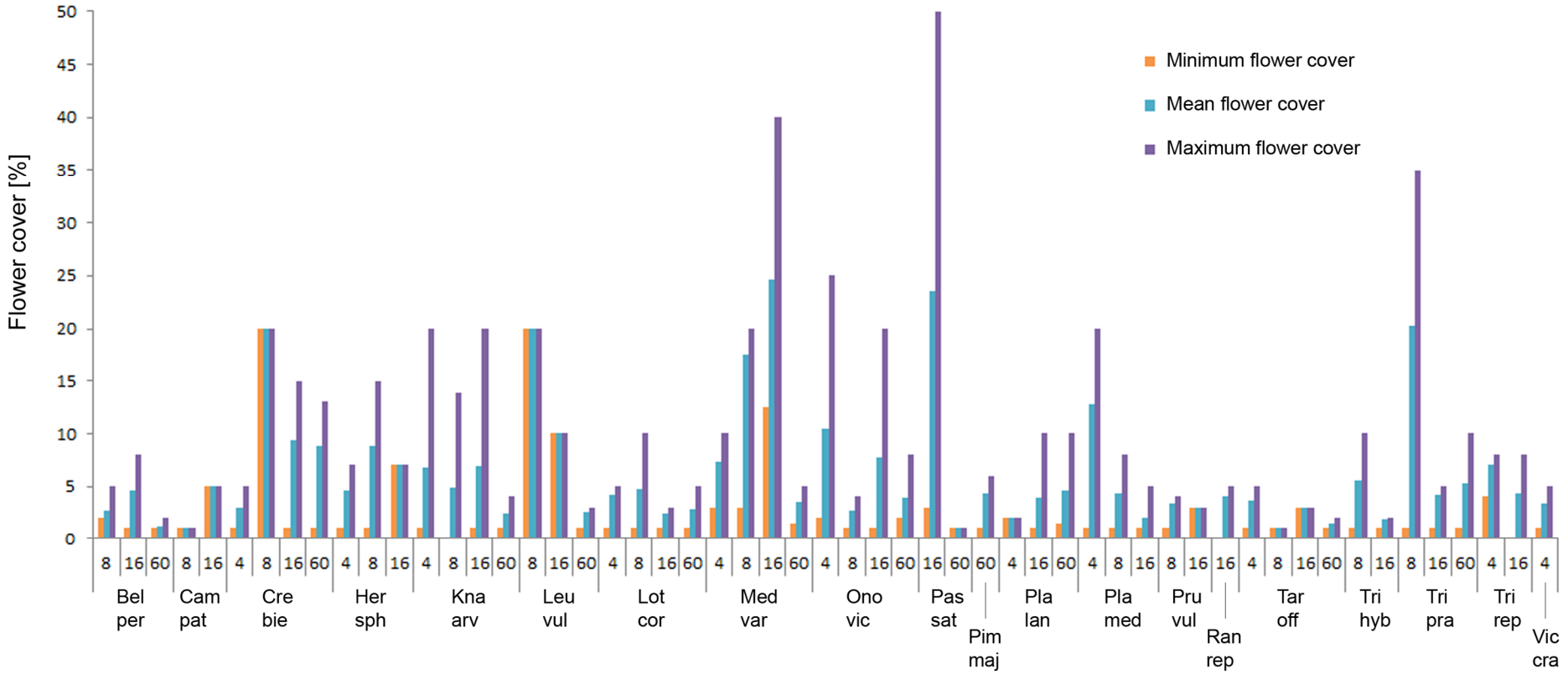
Table S1. (continued)

Plant species	Family	Funct. Groups (leg, therb, sherb)	January	Febru-ary	March	April	May	June	July	August	Sep-tember	October	Novem-ber	Decem-ber
Medicago varia	Fabaceae	leg												
Onobrychis vicifolia	Fabaceae	leg												
Pastinaca sativa	Apiaceae	therb												
Pimpinella major	Apiaceae	therb												
Plantago lanceolata	Plantaginaceae	sherb												
Plantago media	Plantaginaceae	sherb												
Primula veris	Primulaceae	sherb												
Prunella vulgaris	Lamiaceae	sherb												
Ranunculus acris	Ranunculaceae	therb												
Ranunculus repens	Ranunculaceae	sherb												
Rumex acetosa	Polygonaceae	therb												

(continued)

Table S1. (continued)

Plant species	Family	Funct. Groups (leg, therb, sherb)	January	Febru-ary	March	April	May	June	July	August	Sep-tember	October	Novem-ber	Decem-ber
Sanguisorba officinalis	Rosaceae	therb												
Taraxacum officinale	Asteraceae	sherb												
Tragopogon pratensis	Asteraceae	therb												
Trifolium campestre	Fabaceae	leg												
Trifolium dubium	Fabaceae	leg												
Trifolium fragiferum	Fabaceae	leg												
Trifolium hybridum	Fabaceae	leg												
Trifolium pratense	Fabaceae	leg												
Trifolium repens	Fabaceae	leg												
Veronica chamaedrys	Scrophulariaceae	sherb												
Vicia cracca	Fabaceae	leg												



Top 20 plant species with highest mean flower cover

Figure S2: Top 20 plant species with the highest mean flower cover (blue) (proportion of observed area) with maximum (lilac) and minimum (orange) flower cover for each plant species (first three letters of the species name were used to form the short version: *Bellis perennis, Campanula patula, Crepis biennis, Heracleum sphondylium, Knautia arvensis, Leucanthemum vulgare, Lotus corniculatus, Medicago varia, Onobrychis vicifolia, Pastinaca sativa, Pimpinella major, Plantago lanceolata, Plantago media, Prunella vulgaris, Ranunculus repens, Taraxacum officinale, Trifolium hybridum, Trifolium pratense, Trifolium repens, Vicia cracca*) and plant species richness level (4, 8, 16, 60) respectively

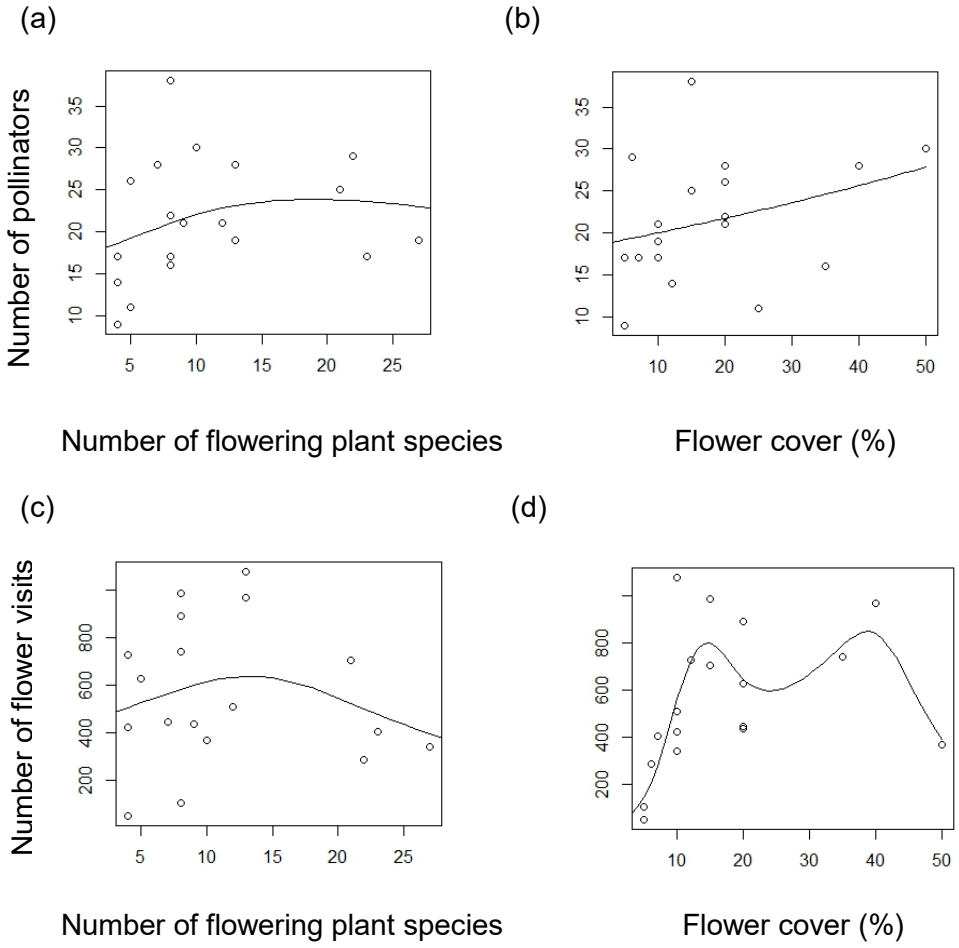


Figure S3: Number of pollinator species (a, b) and number of pollinator visits (c, d) as a function of (realized) number of plant species richness and flower cover.

Appendix S1: R-code for the calculation of STD (standardized time)

(R package maptools, version 0.8-34, (Bivand & Lewin-Koh 2015))

```
library(maptools)


sunrise.set <- function(lat, long, date, timezone="CET", num.days=1){
  lat.long <- matrix(c(long, lat), nrow=1)
  day <- as.POSIXct(date, tz=timezone)
  sequence <- seq(from=day, length.out=num.days , by="days")
  sunrise <- sunriset(lat.long, sequence, direction="sunrise",
                      POSIXct=TRUE)
  sunset <- sunriset(lat.long, sequence, direction="sunset", POSIXct=TRUE)
  ss <- data.frame(sunrise, sunset)
  ss <- ss[,-c(1,3)]
  colnames(ss)<-c("sunrise", "sunset")
  return(ss)
}


sunrise.set <- function(lat, long, date, timezone="CET", num.days=1){
  #this needs to be long lat#
  lat.long <- matrix(c(long, lat), nrow=1)
  day <- as.POSIXct(date, tz=timezone)
  sequence <- seq(from=day, length.out=num.days , by="days")
  sunrise <- sunriset(lat.long, sequence, direction="sunrise",
                      POSIXct=TRUE)
  sunset <- sunriset(lat.long, sequence, direction="sunset", POSIXct=TRUE)
  ss <- list(sunrise=sunrise, sunset=sunset)
  return(ss)
}



mysunset=function(x)
  as.POSIXct(
    unlist(sunriset(matrix(c(11.624744,50.950989),nrow=1),       as.POSIXct(x,       tz="CET"),
direction="sunset", POSIXct.out=T)[2]),origin="1970-01-01")



mysunrise=function(x)


  as.POSIXct(
    unlist(sunriset(matrix(c(11.624744,50.950989),nrow=1),       as.POSIXct(x,       tz="CET"),
direction="sunrise", POSIXct.out=T)[2]),origin="1970-01-01")


mysunrises=sapply(B2a$DATE,function(x)mysunrise(x))
mysunsets=sapply(B2a$DATE,function(x)mysunset(x))


sunrise.hour=sapply(mysunrises,function(x)as.POSIXlt(x,origin="1970-01-01")$hour)
```

```
sunrise.minute=sapply(mysunrises,function(x)as.POSIXlt(x,origin="1970-01-01")$min)


sunset.hour=sapply(mysunsets,function(x)as.POSIXlt(x,origin="1970-01-01")$hour)
sunset.minute=sapply(mysunsets,function(x)as.POSIXlt(x,origin="1970-01-01")$min)
# convert times to angles using circular distributions


circletime=function(x)(360*x)/24


myradians=function(x)(pi/180)*x


B2a$TIME.decimal=hours(B2a$TIME)+minutes(B2a$TIME)/60

sunrise.dec=sunrise.hour+sunrise.minute/60

sunset.dec=sunset.hour+sunset.minute/60

measured.time=B2a$TIME.decimal

prop.time=function(t1,t2,t3)(t2-t1)/(t3-t1)
```

Appendix S2: R-code for the calculation of the proportion of explained deviance (Bolker 2013; Robinson 2013)

The following R code was used (DN=null deviance, DR=residual deviance, DE=explained deviance and DE%= proportion of DE):

```
DN <- sum(residuals(GAMM-null-model$gam, type="deviance")^2)

DR <- sum(residuals(GAMM-best-model$gam, type="deviance")^2)

DE% <- (DN-DR)*100/DN
```

Table S2: Total number of species and flower visits for all functional groups investigated

Pollinator groups	Total number of species	Total number of flower visits
bumblebees	10	3059
honeybees	1	6682
hoverflies	17	676
solitary bees	31	236

Table S2: List of plant species visited by all pollinator functional groups, with pollinator species.

Plant species visited for each pollinator functional group and pollinator species	Sum of visits
Ajuga reptans	**20**
bumblebees	**20**
Bombus lapidarius	5
Bombus pascuorum	9
Bombus terrestris	6
Bellis perennis	**10**
bumblebees	**2**
Bombus lapidarius	2
hoverflies	**4**
Sphaerophoria scripta	4
solitary bees	**4**
Andrena nigroaenea	2
Andrena viridescens	1
Lasioglossum calceatum	1
Campanula patula	**11**
bumblebees	**4**
Bombus lapidarius	4
hoverflies	**1**
syrphidae, unidentified	1
solitary bees	**6**
Lasioglossum calceatum	1
Lasioglossum lativentre	3
Lasioglossum pauxillum	1
solitary bee, unidentified	1
Cardamine pratensis	**1**
honeybees	**1**
Apis mellifera	1
Centaurea jacea	**27**
bumblebees	**2**
Bombus lapidarius	1
Bombus terrestris	1
honeybees	**23**
Apis mellifera	23
solitary bees	**2**
Halictus langobardicus	1
Lasioglossum leucozonium	1

(continued)

Table S3. (continued)

Plant species visited for each pollinator functional group and pollinator species	Sum of visits
Cirsium oleraceum	**1**
bumblebees	**1**
Bombus quadricolor	1
Crepis biennis	**321**
bumblebees	**177**
Bombus lapidarius	177
honeybees	**129**
Apis mellifera	129
hoverflies	**3**
Episyrphus balteatus	1
Sphaerophoria scripta	2
solitary bees	**12**
Anthophora aestivalis	2
Lasioglossum calceatum	1
Lasioglossum lativentre	2
Lasioglossum pauxillum	1
Lasioglossum villosulum	2
solitary bee, unidentified	4
Galium mollugo	**19**
honeybees	**1**
Apis mellifera	1
hoverflies	**17**
Episyrphus balteatus	8
Sphaerophoria scripta	1
syrphidae, unidentified	8
solitary bees	**1**
Halictus tumulorum	1
Geranium pratense	**896**
bumblebees	**432**
Bombus lapidarius	355
Bombus ruderarius	6
Bombus sp.	1
Bombus terrestris	68
Bombus veteranus	2
honeybees	**413**
Apis mellifera	413
hoverflies	**18**
Episyrphus balteatus	2

(continued)

Table S3. (continued)

Plant species visited for each pollinator functional group and pollinator species	Sum of visits
Eupeodes corollae	1
Melanostoma mellinum	4
Sphaerophoria interrupta	1
Sphaerophoria scripta	1
syrphidae, unidentified	9
solitary bees	**33**
Halictus confusus	1
Halictus tumulorum	6
Hylaeus hyalinatus	1
Hylaeus paulus	2
Lasioglossum albipes	1
Lasioglossum calceatum	4
Lasioglossum fulvicorne	1
Lasioglossum laticeps	2
Lasioglossum leucozonium	1
Lasioglossum pauxillum	4
Melitta leporina	1
solitary bee, unidentified	9
Glechoma hederacea	**11**
bumblebees	**1**
Bombus lapidarius	1
honeybees	**1**
Apis mellifera	1
solitary bees	**9**
Andrena haemorrhoa	1
Andrena mitis	1
Anthophora crinipes	2
Anthophora plumipes	3
Eucera nigrescens	2
Heracleum sphondylium	**213**
honeybees	**22**
Apis mellifera	22
hoverflies	**185**
Episyrphus balteatus	20
Eristalis arbustorum	1
Eristalis tenax	1
Eupeodes corollae	1
Melanostoma mellinum	36

(continued)

Table S3. (continued)

Plant species visited for each pollinator functional group and pollinator species	Sum of visits
Myathropa florea	2
Scaeva pyrastri	4
Sphaerophoria interrupta	34
Sphaerophoria scripta	53
Syritta pipiens	2
syrphidae, unidentified	30
Syrphus ribesii	1
solitary bees	**6**
Lasioglossum leucozonium	3
solitary bee, unidentified	3
Knautia arvensis	**1128**
bumblebees	**174**
Bombus lapidarius	131
Bombus pascuorum	4
Bombus pratorum	9
Bombus soroeensis	2
Bombus sp.	3
Bombus sylvarum	1
Bombus terrestris	23
Bombus veteranus	1
honeybees	**878**
Apis mellifera	878
hoverflies	**52**
Episyrphus balteatus	16
Eristalis jugorum	2
Eristalis tenax	4
Melanostoma mellinum	8
Scaeva pyrastri	2
Sphaerophoria interrupta	1
Syritta pipiens	1
syrphidae, unidentified	18
solitary bees	**24**
Andrena cineraria	1
Andrena hattorfiana	3
Halictus scabiosae	1
Lasioglossum calceatum	2
Lasioglossum leucozonium	8
Lasioglossum villosulum	1

(continued)

Table S3. (continued)

Plant species visited for each pollinator functional group and pollinator species	Sum of visits
solitary bee, unidentified	8
Lathyrus pratensis	**75**
bumblebees	**17**
Bombus lapidarius	10
Bombus sylvarum	7
honeybees	**50**
Apis mellifera	50
solitary bees	**8**
Halictus confusus	2
solitary bee, unidentified	6
Leontodon autumnalis	**5**
honeybees	**3**
Apis mellifera	3
hoverflies	**2**
Eristalis tenax	1
Sphaerophoria scripta	1
Leontodon hispidus	**7**
bumblebees	**1**
Bombus lapidarius	1
honeybees	**2**
Apis mellifera	2
hoverflies	**4**
Eupeodes corollae	1
Sphaerophoria scripta	2
syrphidae, unidentified	1
Leucanthemum vulgare	**9**
bumblebees	**4**
Bombus lapidarius	4
honeybees	**2**
Apis mellifera	2
hoverflies	**2**
Episyrphus balteatus	2
solitary bees	**1**
Andrena flavipes	1
Lotus corniculatus	**538**
bumblebees	**391**
Bombus lapidarius	382
Bombus pascuorum	3

(continued)

Table S3. (continued)

Plant species visited for each pollinator functional group and pollinator species	Sum of visits
Bombus sp.	1
Bombus terrestris	3
Bombus veteranus	2
honeybees	**116**
Apis mellifera	116
hoverflies	**13**
Episyrphus balteatus	3
Sphaerophoria scripta	5
syrphidae, unidentified	5
solitary bees	**18**
Halictus tumulorum	8
solitary bee, unidentified	10
Medicago lupulina	**5**
hoverflies	**4**
Sphaerophoria scripta	3
syrphidae, unidentified	1
solitary bees	**1**
Lasioglossum fulvicorne	1
Medicago varia	**787**
bumblebees	**2**
Bombus pascuorum	2
honeybees	**763**
Apis mellifera	763
hoverflies	**4**
Episyrphus balteatus	1
syrphidae, unidentified	3
solitary bees	**18**
Melitta leporina	14
solitary bee, unidentified	4
Onobrychis vicifolia	**3947**
bumblebees	**970**
Bombus lapidarius	943
Bombus pascuorum	8
Bombus ruderarius	3
Bombus sp.	8
Bombus sylvarum	1
Bombus terrestris	6
Bombus veteranus	1

(continued)

Table S3. (continued)

Plant species visited for each pollinator functional group and pollinator species	Sum of visits
honeybees	**2934**
Apis mellifera	2934
hoverflies	**29**
Episyrphus balteatus	12
Eristalis arbustorum	1
Melanostoma mellinum	1
Sphaerophoria scripta	4
syrphidae, unidentified	11
solitary bees	**14**
Chelostoma sp.	2
Halictus tumulorum	2
Lasioglossum pauxillum	3
Megachile sp.	1
Melitta leporina	2
solitary bee, unidentified	4
Pastinaca sativa	**77**
honeybees	**4**
Apis mellifera	4
hoverflies	**71**
Chrysotoxum bicinctum	1
Episyrphus balteatus	12
Melanostoma mellinum	22
Melanostoma scalare	1
Scaeva pyrastri	2
Sphaerophoria interrupta	1
Sphaerophoria scripta	18
syrphidae, unidentified	14
solitary bees	**2**
Andrena minutuloides	1
Lasioglossum interruptum	1
Pimpinella major	**51**
hoverflies	**43**
Chrysotoxum bicinctum	2
Episyrphus balteatus	9
Melanostoma mellinum	4
Melanostoma scalare	1
Sphaerophoria interrupta	15
Sphaerophoria scripta	6

(continued)

Table S3. (continued)

Plant species visited for each pollinator functional group and pollinator species	Sum of visits
Syritta pipiens	1
syrphidae, unidentified	5
solitary bees	**8**
Andrena minutula	3
Lasioglossum laticeps	2
solitary bee, unidentified	3
Plantago lanceolata	**112**
bumblebees	**1**
Bombus lapidarius	1
honeybees	**2**
Apis mellifera	2
hoverflies	**107**
Episyrphus balteatus	18
Melanostoma mellinum	16
Melanostoma scalare	1
Platycheirus parmatus	3
Scaeva pyrastri	4
Sphaerophoria interrupta	26
Sphaerophoria scripta	6
syrphidae, unidentified	32
Syrphus ribesii	1
solitary bees	**2**
Lasioglossum calceatum	1
solitary bee, unidentified	1
Plantago media	**311**
bumblebees	**43**
Bombus lapidarius	7
Bombus terrestris	36
honeybees	**202**
Apis mellifera	202
hoverflies	**60**
Chrysotoxum bicinctum	1
Chrysotoxum cautum	2
Episyrphus balteatus	7
Eristalis tenax	1
Melanostoma mellinum	18
Scaeva pyrastri	12
Sphaerophoria interrupta	4

(continued)

Table S3. (continued)

Plant species visited for each pollinator functional group and pollinator species	Sum of visits
Sphaerophoria scripta	5
syrphidae, unidentified	10
solitary bees	**6**
Andrena hattorfiana	1
Halictus tumulorum	1
Lasioglossum pauxillum	4
Primula veris	**9**
bumblebees	**4**
Bombus terrestris	4
solitary bees	**5**
Anthophora plumipes	3
Anthophora spec	2
Prunella vulgaris	**73**
bumblebees	**66**
Bombus lapidarius	64
Bombus pascuorum	1
Bombus sp.	1
hoverflies	**7**
Sphaerophoria interrupta	1
Sphaerophoria scripta	1
Syritta pipiens	1
syrphidae, unidentified	4
Ranunculus acris	**5**
honeybees	**1**
Apis mellifera	1
hoverflies	**3**
Episyrphus balteatus	1
syrphidae, unidentified	2
solitary bees	**1**
Lasioglossum calceatum	1
Ranunculus repens	**2**
hoverflies	**1**
syrphidae, unidentified	1
solitary bees	**1**
Andrena viridescens	1
Rumex acetosa	**5**
hoverflies	**5**
Episyrphus balteatus	1

(continued)

Table S3. (continued)

Plant species visited for each pollinator functional group and pollinator species	Sum of visits
Melanostoma mellinum	1
Sphaerophoria scripta	1
syrphidae, unidentified	2
Taraxacum officinale	**8**
honeybees	**8**
Apis mellifera	8
Trifolium hybridum	**256**
bumblebees	**37**
Bombus lapidarius	37
honeybees	**210**
Apis mellifera	210
hoverflies	**8**
Episyrphus balteatus	1
Eristalis interrupta	2
syrphidae, unidentified	5
solitary bees	**1**
Halictus eurygnathus	1
Trifolium pratense	**646**
bumblebees	**312**
Bombus humilis	2
Bombus lapidarius	287
Bombus pascuorum	11
Bombus sylvarum	7
Bombus terrestris	5
honeybees	**313**
Apis mellifera	313
hoverflies	**14**
Episyrphus balteatus	2
Melanostoma mellinum	5
Sphaerophoria scripta	1
syrphidae, unidentified	6
solitary bees	**7**
Andrena wilkella	2
Halictus eurygnathus	2
Lasioglossum lativentre	1
solitary bee, unidentified	2
Trifolium repens	**837**
bumblebees	**376**

(continued)

Table S3. (continued)

Plant species visited for each pollinator functional group and pollinator species	Sum of visits
Bombus humilis	1
Bombus lapidarius	373
Bombus sylvarum	1
Bombus terrestris	1
honeybees	**435**
Apis mellifera	435
hoverflies	**17**
Sphaerophoria interrupta	5
Sphaerophoria scripta	8
syrphidae, unidentified	4
solitary bees	**9**
Andrena flavipes	3
solitary bee, unidentified	6
Veronica chamaedrys	**27**
hoverflies	**1**
syrphidae, unidentified	1
solitary bees	**26**
Andrena viridescens	16
Halictus tumulorum	1
solitary bee, unidentified	9
Vicia cracca	**203**
bumblebees	**22**
Bombus humilis	2
Bombus lapidarius	4
Bombus pascuorum	15
Bombus sp.	1
honeybees	**169**
Apis mellifera	169
hoverflies	**1**
Episyrphus balteatus	1
solitary bees	**11**
Lasioglossum fulvicorne	10
solitary bee, unidentified	1
Total	**10653**

Table S4: GAMM model of flower visitation rate of all pollinators (bumblebees, solitary bees, hoverflies) excluding honeybees. Summary of terms for generalized additive mixed models. ‡ Term was fitted using ti() function in GAMM. n=309.

Response variable (Flower visitation rate)	**Parameter**	**Est. *df* (est. pp)**	**Effect[a]**	**Ref. df (SE)**	**F-value (t-value)**	**P**	**Deviance explained [%]**
All	(Intercept)	(2.03)	-	(0.13)	(15.69)	<0.001	26
pollinators	Time of day‡	1.89	quadratic	1.89	12.42	<0.001	
without honeybees	Flowering height‡	2.00	quadratic	2.00	10.05	<0.001	
	Plant species richness‡	2.00	quadratic	2.00	0.22	0.805	
	Time of day * Flowering height‡	2.00	quadratic	2.00	0.86	0.426	
	Time of day * Plant species richness	1.00	linear	1.00	0.26	0.613	
	Flowering height * Plant species richness	1.00	linear	1.00	0.34	0.561	
	Time of day * Flowering height * Plant species richness‡	1.00	linear	1.00	0.13	0.721	

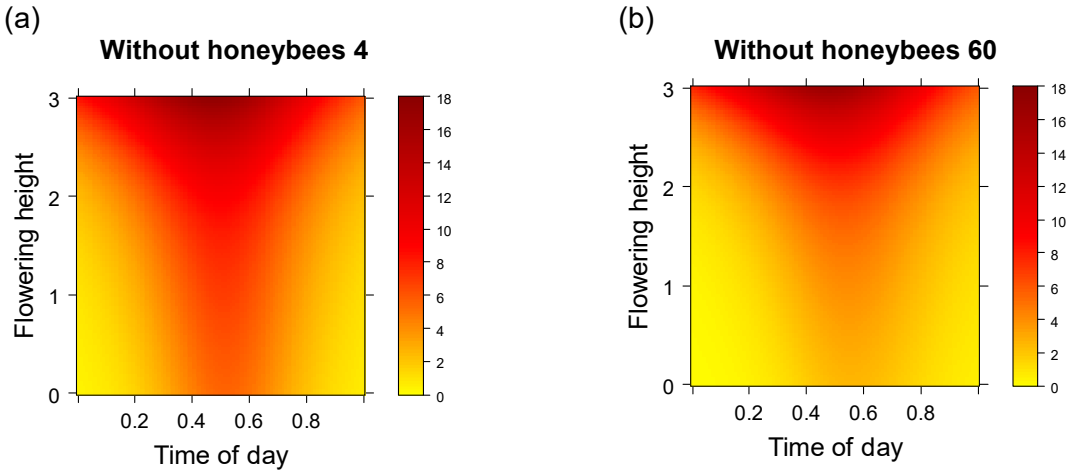


Figure S4: Effects of plant species richness, time of day and flowering height on flower visitation rate of the pollinator community without honeybees. Shown are the results of minimal adequate generalized additive mixed models for all pollinator functional groups (bumblebees, solitary bees, hoverflies) without honeybees in mixtures with (a) four plant species and (b) 60 plant species: Flower visitation rate is not siginificantly influenced by plant species richness (4=low, 60=high plant species richness), but by time of day (0-1; range representing the observation time, between the onset of sunrise and sunset), and three different flowering heights (a-j: 1= 1-10 cm, 2= 11-25 cm, 3= ≥26 cm).

Table S5: Plant species richness vs. flower cover as explanatory variable. Shown are the best models for all pollinator functional groups with either plant species richness (PSR) or flower cover (FC) as explanatory variable. Better model is indicated by Akaike information criterion with a correction for finite sample sizes (AICc) and numbers of degrees of freedom (df).

Modell	df	AICc
honeybees PSR	23	1154.522
honeybees FC	23	1201.246
bumblebees PSR	13	1091.863
bumblebees FC	23	1142.916
solitary bees PSR	23	1475.208
solitary bees FC	23	1443.503
hoverflies PSR	16	1258.816
hoverflies FC	23	1275.898

Table S6: All pollinator groups differed significantly in spatio-temporal (Flowering height and Time of day) resource use and in their response to plant species richness, as indicated by two-tailed Wald tests with bumblebees as a reference level; shown are p-values. The values 1, 2, 3 correspond to the knots; parameters in the table are weights of the B-spline basis functions, evaluated at knots 1, 2 or 3.

(Intercept)	Honey bees	Hoverflies	Solitary bees
bs(Plant species richness)1	0.408	0.04	0.036
bs(Plant species richness)2	0	0.739	0.152
bs(Plant species richness)3	0	0.004	0.001
bs(Flowering height)1	0	0	0
bs(Flowering height)2	0.819	0.001	0.015
bs(Flowering height)3	0.819	0.001	0.015
bs(Time of day)1	0.554	0.015	0.432
bs(Time of day)2	0.706	0.053	0.119
bs(Time of day)3	0.003	0.538	0.203
bs(Plant species richness)1:bs(Flowering height)1	0.253	0.033	0.764
bs(Plant species richness)2:bs(Flowering height)1	0	0.003	0.39
bs(Plant species richness)3:bs(Flowering height)1	0.019	0	0.008
bs(Plant species richness)1:bs(Flowering height)2	0	0	0
bs(Plant species richness)2:bs(Flowering height)2	0	0.003	0.39
bs(Plant species richness)3:bs(Flowering height)2	0.019	0	0.008
bs(Plant species richness)1:bs(Flowering height)3	0	0	0
bs(Plant species richness)2:bs(Flowering height)3	0	0.506	0.001
bs(Plant species richness)3:bs(Flowering height)3	0	0.012	0
bs(Plant species richness)1:bs(Time of day)1	0	0	0.002
bs(Plant species richness)2:bs(Time of day)1	0	0.115	0.602
bs(Plant species richness)3:bs(Time of day)1	0	0	0
bs(Plant species richness)1:bs(Time of day)2	0	0	0
bs(Plant species richness)2:bs(Time of day)2	0	0.314	0.416
bs(Plant species richness)3:bs(Time of day)2	0.001	0.553	0.028
bs(Plant species richness)1:bs(Time of day)3	0.86	0.244	0.044
bs(Plant species richness)2:bs(Time of day)3	0	0	0
bs(Plant species richness)3:bs(Time of day)3	0	0	0
bs(Flowering height)1:bs(Time of day)1	0.029	0	0.004
bs(Flowering height)2:bs(Time of day)1	0.996	0.001	0.016
bs(Flowering height)3:bs(Time of day)1	0.996	0.001	0.016
bs(Flowering height)1:bs(Time of day)2	0.955	0.015	0.72
bs(Flowering height)2:bs(Time of day)2	0.006	0.174	0.062
bs(Flowering height)3:bs(Time of day)2	0.006	0.174	0.062
bs(Flowering height)1:bs(Time of day)3	0	0.334	0.001
bs(Flowering height)2:bs(Time of day)3	0.487	0.181	0.873
bs(Flowering height)3:bs(Time of day)3	0.487	0.181	0.873

(continued)

Table S6. (continued)

(Intercept)	Honey bees	Hoverflies	Solitary bees
bs(Plant species richness)1:bs(Flowering height)1:bs(Time of day)1	0.343	0.084	0.661
bs(Plant species richness)2:bs(Flowering height)1:bs(Time of day)1	0	0.004	0.351
bs(Plant species richness)3:bs(Flowering height)1:bs(Time of day)1	0	0	0
bs(Plant species richness)1:bs(Flowering height)2:bs(Time of day)1	0.054	0	0
bs(Plant species richness)3:bs(Flowering height)3:bs(Time of day)1	0	0.006	0.027
bs(Plant species richness)1:bs(Flowering height)1:bs(Time of day)2	0.001	0	0.001
bs(Plant species richness)2:bs(Flowering height)1:bs(Time of day)2	0	0.063	0
bs(Plant species richness)3:bs(Flowering height)1:bs(Time of day)2	0	0.287	0.023
bs(Plant species richness)1:bs(Flowering height)2:bs(Time of day)2	0	0.414	0
bs(Plant species richness)2:bs(Flowering height)2:bs(Time of day)2	0	0.063	0
bs(Plant species richness)3:bs(Flowering height)2:bs(Time of day)2	0	0.287	0.023
bs(Plant species richness)1:bs(Flowering height)3:bs(Time of day)2	0	0.414	0
bs(Plant species richness)2:bs(Flowering height)3:bs(Time of day)2	0	0.707	0
bs(Plant species richness)3:bs(Flowering height)3:bs(Time of day)2	0	0.005	0
bs(Plant species richness)1:bs(Flowering height)1:bs(Time of day)3	0.26	0.25	0.962
bs(Plant species richness)2:bs(Flowering height)1:bs(Time of day)3	0	0	0
bs(Plant species richness)3:bs(Flowering height)1:bs(Time of day)3	0	0	0
bs(Plant species richness)1:bs(Flowering height)2:bs(Time of day)3	0	0	0
bs(Plant species richness)2:bs(Flowering height)2:bs(Time of day)3	0	0	0
bs(Plant species richness)3:bs(Flowering height)2:bs(Time of day)3	0	0	0
bs(Plant species richness)1:bs(Flowering height)3:bs(Time of day)3	0	0	0
bs(Plant species richness)2:bs(Flowering height)3:bs(Time of day)3	0	0	0.414
bs(Plant species richness)3:bs(Flowering height)3:bs(Time of day)3	0.006	0	0.018

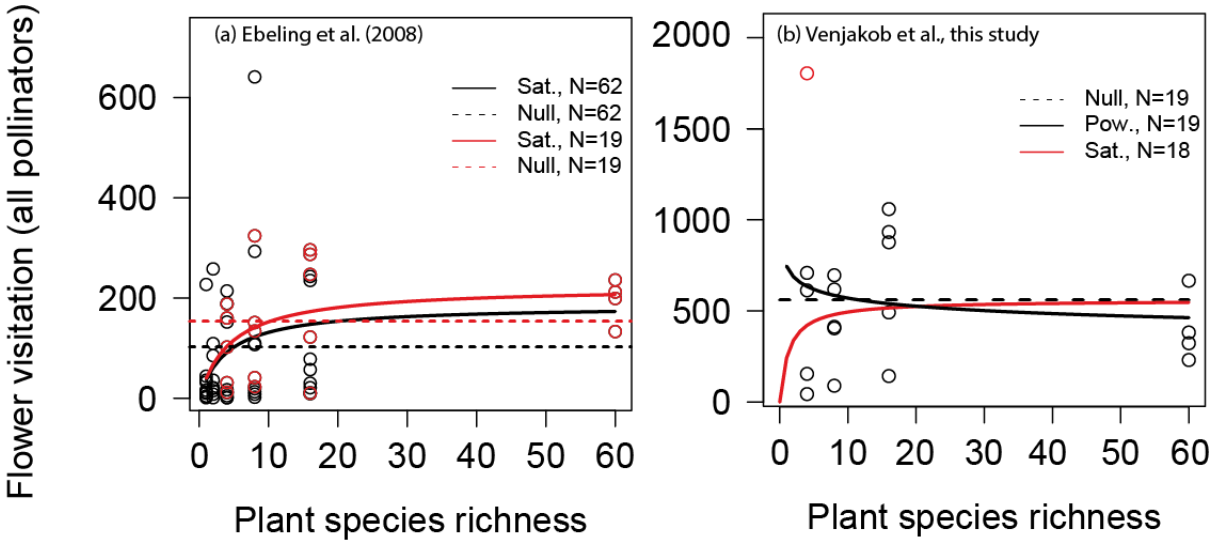


Figure S5: Effects of plant species richness on the flower visitation of all pollinators (honeybees, bumblebees, solitary bees, hoverflies), (a) based on data from Ebeling *et al.* (2008) and (b) based on our data. (a) solid lines are the predicted values of the saturated model (Sat.) and dotted lines show predictions from the null model (Null); red color indicates the number of observed plots (black = 62 plots; red = 19 plots, exactly the same plots as used in our experiment). (b) black solid line shows predicted values of the power model (Pow., 19 plots) with an outlier (red circle), red solid line shows prediction of the saturated model (Sat., 18 plots) without the outlier, dotted line shows predictions from the null model (Null, 19 plots).

Figure (a) shows that effects of plant species richness on flower visitation were not fundamentally altered by our sampling design with N=19 plots. Figure (b) shows that plant species richness had a positive effect on the flower visitation of all pollinators resulting in a saturation curve as found in Ebeling *et al.* (2008).

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Appendix B
Variation in nectar quality across 34 grassland plant species

Supporting Information

Variation in nectar quality across 34 grassland plant species

Running title: Interspecific variation in floral nectar quality

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Table S1 Nectar concentration and proportion of chemical components, such as amino acids and carbohydrates, were measured via HPLC. All values are mean and standard deviation (SD), either in mg/ml or dimensionless, N gives number of specimens sampled for nectar analysis, Volume gives sampled nectar volumes.

				Amino acids																	
Plant family/ *species*[1]	**N AA[2]**	**Volume AA mean ±SD[3]**	**Total AA[4]**	**Asp[5]**	**Glu**	**Ser**	**His**	**Gly**	**Thr**	**Arg**	**Ala**	**Tyr**	**Cystine**	**Val**	**Met**	**Phe**	**Ile**	**Leu**	**Lys**	**Pro**	**EAA[6]**	**Non-EAA[7]**
			Concentration mg/ml																		
Apiaceae																					
Anthriscus sylvestris	7	2.2 ±1.06	11.2 ±4.65	0.13 ±0.09	0.24 ±0.09	0.47 ±0.17	6.14 ±2.32	0.09 ±0.03	0.19 ±0.07	0.16 ±0.06	0.32 ±0.07	0.13 ±0.04	0 ±0.00	0.27 ±0.09	0.39 ±0.16	0.2 ±0.07	0.25 ±0.09	0.37 ±0.12	0.29 ±0.13	1.54 ±1.33	8.26 ±2.96	2.93 ±1.73
Daucus carota	9	2.55 ±1.23	2.51 ±1.07	0.12 ±0.06	0.17 ±0.07	0.48 ±0.26	0.52 ±0.31	0.04 ±0.01	0.05 ±0.02	0.13 ±0.10	0.14 ±0.04	0.03 ±0.01	0 ±0.00	0.05 ±0.02	0.06 ±0.03	0.08 ±0.04	0.04 ±0.01	0.08 ±0.03	0.08 ±0.03	0.44 ±0.46	1.1 ±0.46	1.41 ±0.65
Heracleum sphondylium	9	1.47 ±0.84	2.72 ±1.93	0.15 ±0.08	0.26 ±0.23	0.14 ±0.09	1.05 ±0.89	0.04 ±0.02	0.05 ±0.03	0.09 ±0.04	0.08 ±0.05	0.05 ±0.03	0 ±0.00	0.05 ±0.03	0.07 ±0.08	0.08 ±0.05	0.03 ±0.02	0.08 ±0.04	0.1 ±0.06	0.4 ±0.40	1.6 ±1.21	1.12 ±0.79
Pastinaca sativa	8	1.8 ±1.31	9.92 ±3.33	0.14 ±0.05	0.18 ±0.08	0.46 ±0.23	4.78 ±2.04	0.1 ±0.03	0.13 ±0.04	0.16 ±0.07	0.31 ±0.17	0.11 ±0.06	0 ±0.00	0.22 ±0.10	0.42 ±0.23	0.27 ±0.12	0.16 ±0.07	0.24 ±0.12	0.25 ±0.12	1.98 ±0.54	6.64 ±2.70	3.28 ±0.84
Pimpinella major	10	2.69 ±1.81	5.41 ±3.89	0.07 ±0.05	0.11 ±0.07	0.13 ±0.10	0.77 ±0.56	0.03 ±0.02	0.04 ±0.03	0.07 ±0.04	0.08 ±0.05	0.06 ±0.03	0 ±0.00	0.08 ±0.06	0.07 ±0.05	1.95 ±1.78	0.09 ±0.07	1.13 ±1.21	0.06 ±0.03	0.66 ±0.59	4.26 ±3.27	1.15 ±0.88
Asteraceae																					
Centaurea jacea	8	1.43 ±1.07	10.42 ±8.22	0.3 ±0.21	0.24 ±0.13	0.43 ±0.33	5.5 ±4.89	0.15 ±0.09	0.21 ±0.14	0.23 ±0.12	0.72 ±0.58	0.15 ±0.11	0 ±0.00	0.3 ±0.21	0.33 ±0.20	0.22 ±0.16	0.23 ±0.15	0.34 ±0.19	0.32 ±0.18	0.74 ±0.79	7.68 ±6.11	2.74 ±2.16
Cirsium oleraceum	9	3.97 ±3.87	3.74 ±3.37	0.06 ±0.04	0.03 ±0.02	0.1 ±0.06	2.15 ±2.06	0.05 ±0.02	0.04 ±0.02	0.04 ±0.02	0.27 ±0.24	0.02 ±0.01	0 ±0.00	0.05 ±0.03	0.18 ±0.14	0.04 ±0.02	0.03 ±0.02	0.06 ±0.03	0.08 ±0.06	0.57 ±0.73	2.65 ±2.39	1.08 ±1.05
Crepis biennis	10	0.65 ±0.32	23.13 ±9.57	0.09 ±0.13	0.14 ±0.10	0.3 ±0.15	3.64 ±1.97	0.1 ±0.03	0.16 ±0.07	0.15 ±0.06	0.33 ±0.19	0.94 ±0.49	0 ±0.00	0.22 ±0.10	0.25 ±0.12	15.11 ±7.68	0.22 ±0.17	0.25 ±0.12	0.28 ±0.10	0.95 ±0.40	20.28 ±8.84	2.85 ±0.93
Leontodon autumnalis	9	0.68 ±0.33	10.86 ±9.94	0.18 ±0.14	0.18 ±0.10	0.18 ±0.13	6.45 ±6.18	0.07 ±0.04	0.07 ±0.04	0.1 ±0.06	0.33 ±0.27	0.07 ±0.04	0 ±0.00	0.1 ±0.07	0.26 ±0.21	0.13 ±0.09	0.09 ±0.05	0.12 ±0.09	0.14 ±0.10	2.4 ±2.48	7.45 ±6.82	3.41 ±3.15
Leontodon hispidus	10	0.73 ±0.36	20.3 ±10.19	0.18 ±0.07	0.1 ±0.04	0.28 ±0.11	6.84 ±3.67	0.11 ±0.04	0.13 ±0.05	0.15 ±0.05	0.78 ±0.40	1.86 ±1.19	0 ±0.00	0.23 ±0.10	0.44 ±0.19	6.62 ±3.85	0.31 ±0.15	0.33 ±0.15	0.26 ±0.11	1.68 ±0.92	15.3 ±7.88	5 ±2.36
Taraxacum officinale	10	0.85 ±0.26	7.21 ±5.12	0.1 ±0.04	0.12 ±0.03	0.11 ±0.03	4.67 ±3.84	0.05 ±0.01	0.06 ±0.02	0.08 ±0.02	0.19 ±0.14	0.06 ±0.02	0 ±0.00	0.07 ±0.02	0.13 ±0.06	0.17 ±0.04	0.05 ±0.02	0.07 ±0.03	0.09 ±0.04	1.19 ±0.97	5.39 ±3.94	1.82 ±1.19
Tragopogon pratensis	8	0.25 ±0.09	20.89 ±14.20	0.49 ±0.24	0.29 ±0.08	0.6 ±0.41	8.56 ±5.89	0.28 ±0.17	0.2 ±0.11	0.27 ±0.22	0.55 ±0.39	0.18 ±0.08	0 ±0.00	0.24 ±0.16	0.51 ±0.38	0.15 ±0.07	0.16 ±0.10	0.22 ±0.16	0.28 ±0.15	7.9 ±6.39	10.59 ±6.95	10.3 ±7.35
Brassicaceae																					
Cardamine pratensis	9	0.82 ±0.36	4.18 ±2.43	0.26 ±0.11	0.22 ±0.07	0.16 ±0.05	1.59 ±1.03	0.09 ±0.05	0.12 ±0.04	0.15 ±0.09	0.11 ±0.06	0.08 ±0.04	0 ±0.00	0.12 ±0.04	0.18 ±0.13	0.07 ±0.04	0.08 ±0.04	0.09 ±0.04	0.13 ±0.07	0.73 ±0.67	2.52 ±1.49	1.66 ±0.95

Table S1 (continued)

Plant family/ *species*[1]	N AA[2]	Volume AA mean ±SD[3]	Amino acids																	
			Asp[5]	Glu	Ser	His	Gly	Thr	Arg	Ala	Tyr	Cystine	Val	Met	Phe	Ile	Leu	Lys	Pro	EAA[6]	Non-EAA[7]
			Proportion																	
Apiaceae																				
Anthriscus sylvestris	7	2.2 ±1.06	0.01 ±0.01	0.02 ±0.01	0.04 ±0.01	0.56 ±0.06	0.01 ±0.00	0.02 ±0.00	0.01 ±0.00	0.03 ±0.01	0.01 ±0.00	0 ±0.00	0.02 ±0.01	0.04 ±0.00	0.02 ±0.00	0.02 ±0.01	0.03 ±0.01	0.03 ±0.00	0.12 ±0.05	0.75 ±0.05	0.25 ±0.05
Daucus carota	9	2.55 ±1.23	0.05 ±0.02	0.07 ±0.02	0.2 ±0.10	0.2 ±0.06	0.02 ±0.01	0.02 ±0.00	0.06 ±0.04	0.06 ±0.02	0.01 ±0.00	0 ±0.00	0.02 ±0.01	0.03 ±0.00	0.04 ±0.02	0.02 ±0.00	0.03 ±0.00	0.04 ±0.01	0.15 ±0.10	0.44 ±0.06	0.56 ±0.06
Heracleum sphondylium	9	1.47 ±0.84	0.06 ±0.01	0.09 ±0.05	0.06 ±0.02	0.37 ±0.08	0.02 ±0.01	0.02 ±0.00	0.04 ±0.01	0.03 ±0.01	0.02 ±0.01	0 ±0.00	0.02 ±0.01	0.02 ±0.02	0.03 ±0.01	0.01 ±0.00	0.03 ±0.01	0.04 ±0.01	0.13 ±0.04	0.59 ±0.08	0.41 ±0.08
Pastinaca sativa	8	1.8 ±1.31	0.02 ±0.01	0.02 ±0.01	0.04 ±0.01	0.47 ±0.08	0.01 ±0.00	0.01 ±0.00	0.02 ±0.00	0.03 ±0.01	0.01 ±0.01	0 ±0.00	0.02 ±0.00	0.04 ±0.01	0.03 ±0.01	0.02 ±0.00	0.02 ±0.01	0.03 ±0.01	0.21 ±0.06	0.65 ±0.08	0.35 ±0.08
Pimpinella major	10	2.69 ±1.81	0.02 ±0.01	0.02 ±0.01	0.03 ±0.01	0.15 ±0.07	0.01 ±0.00	0.01 ±0.00	0.02 ±0.01	0.02 ±0.01	0.01 ±0.01	0 ±0.00	0.02 ±0.01	0.01 ±0.01	0.36 ±0.22	0.02 ±0.01	0.18 ±0.11	0.01 ±0.00	0.12 ±0.07	0.78 ±0.10	0.22 ±0.10
Asteraceae																				
Centaurea jacea	8	1.43 ±1.07	0.03 ±0.01	0.03 ±0.01	0.04 ±0.00	0.52 ±0.09	0.02 ±0.00	0.02 ±0.00	0.02 ±0.01	0.07 ±0.01	0.01 ±0.00	0 ±0.00	0.03 ±0.01	0.03 ±0.01	0.02 ±0.00	0.02 ±0.01	0.03 ±0.01	0.03 ±0.01	0.07 ±0.05	0.74 ±0.06	0.26 ±0.06
Cirsium oleraceum	9	3.97 ±3.87	0.02 ±0.00	0.01 ±0.00	0.03 ±0.01	0.57 ±0.06	0.02 ±0.01	0.01 ±0.00	0.01 ±0.01	0.07 ±0.02	0.01 ±0.00	0 ±0.00	0.02 ±0.01	0.06 ±0.02	0.01 ±0.00	0.01 ±0.00	0.02 ±0.01	0.02 ±0.01	0.11 ±0.09	0.74 ±0.08	0.26 ±0.08
Crepis biennis	10	0.65 ±0.32	0.01 ±0.01	0.01 ±0.00	0.01 ±0.00	0.17 ±0.07	0 ±0.00	0.01 ±0.00	0.01 ±0.00	0.02 ±0.01	0.04 ±0.01	0 ±0.00	0.01 ±0.00	0.01 ±0.00	0.63 ±0.12	0.01 ±0.01	0.01 ±0.00	0.01 ±0.00	0.05 ±0.03	0.86 ±0.04	0.14 ±0.04
Leontodon autumnalis	9	0.68 ±0.33	0.02 ±0.00	0.02 ±0.01	0.02 ±0.00	0.59 ±0.05	0.01 ±0.00	0.01 ±0.00	0.01 ±0.01	0.03 ±0.01	0.01 ±0.00	0 ±0.00	0.01 ±0.00	0.03 ±0.00	0.01 ±0.01	0.01 ±0.00	0.01 ±0.00	0.01 ±0.00	0.2 ±0.05	0.69 ±0.05	0.31 ±0.05
Leontodon hispidus	10	0.73 ±0.36	0.01 ±0.00	0.01 ±0.00	0.02 ±0.01	0.33 ±0.07	0.01 ±0.00	0.01 ±0.00	0.01 ±0.00	0.04 ±0.01	0.09 ±0.03	0 ±0.00	0.01 ±0.01	0.02 ±0.01	0.32 ±0.05	0.02 ±0.01	0.02 ±0.01	0.01 ±0.00	0.08 ±0.02	0.75 ±0.03	0.25 ±0.03
Taraxacum officinale	10	0.85 ±0.26	0.02 ±0.01	0.02 ±0.01	0.02 ±0.02	0.59 ±0.14	0.01 ±0.01	0.01 ±0.01	0.01 ±0.01	0.03 ±0.01	0.01 ±0.01	0 ±0.00	0.02 ±0.01	0.02 ±0.01	0.04 ±0.03	0.01 ±0.01	0.02 ±0.02	0.02 ±0.01	0.16 ±0.02	0.73 ±0.05	0.27 ±0.05
Tragopogon pratensis	8	0.25 ±0.09	0.03 ±0.00	0.02 ±0.01	0.03 ±0.01	0.41 ±0.06	0.02 ±0.02	0.01 ±0.00	0.02 ±0.02	0.02 ±0.01	0.01 ±0.00	0 ±0.00	0.01 ±0.00	0.02 ±0.00	0.01 ±0.00	0.01 ±0.00	0.01 ±0.00	0.01 ±0.00	0.36 ±0.06	0.51 ±0.05	0.49 ±0.05
Brassicaceae																				
Cardamine pratensis	9	0.82 ±0.36	0.07 ±0.02	0.06 ±0.03	0.04 ±0.01	0.37 ±0.03	0.02 ±0.00	0.03 ±0.01	0.03 ±0.01	0.03 ±0.01	0.02 ±0.00	0 ±0.00	0.03 ±0.01	0.04 ±0.01	0.02 ±0.00	0.02 ±0.00	0.02 ±0.00	0.03 ±0.00	0.15 ±0.06	0.6 ±0.02	0.4 ±0.02

Table S1 (continued)

Plant family/ *species*[1]	N C[2]	Volume C mean ±SD[3]	Carbohydrates						
			Total C[4]	Fructose	Glucose	Sucrose	Fructose	Glucose	Sucrose
			Concentration mg/ml				Proportion		
Apiaceae									
Anthriscus		2.2	11.48	7.2	2.13	2.15	0.66	0.21	0.13
sylvestris	7	±1.06	±4.43	±2.10	±0.62	±3.92	±0.16	±0.07	±0.22
Daucus		2.55	5.65	2.7	2.96	0	0.4	0.6	0
carota	9	±1.23	±6.06	±2.88	±3.19	±0.00	±0.23	±0.23	±0.00
Heracleum		1.47	23.06	11.02	10.04	2	0.48	0.47	0.05
sphondylium	9	±0.84	±13.99	±6.61	±5.34	±5.50	±0.08	±0.08	±0.13
Pastinaca		1.8	97.45	36.58	31.71	29.16	0.42	0.37	0.21
sativa	8	±1.31	±65.10	±20.41	±18.08	±37.89	±0.12	±0.11	±0.23
Pimpinella		2.69	8.7	4.18	3.42	1.1	0.48	0.38	0.14
major	10	±1.81	±3.73	±2.27	±1.95	±1.65	±0.13	±0.10	±0.19
Asteraceae									
Centaurea		1.43	32.4	15.49	15.54	1.37	0.48	0.47	0.05
jacea	8	±1.07	±12.78	±6.87	±7.32	±3.86	±0.10	±0.06	±0.15
Cirsium		3.97	59.31	16.72	14.6	27.99	0.31	0.26	0.43
oleraceum	9	±3.87	±22.25	±6.62	±6.61	±21.25	±0.12	±0.10	±0.20
Crepis		0.65	59.68	29.78	27.48	2.42	0.5	0.47	0.03
biennis	10	±0.32	±25.02	±12.57	±11.16	±7.64	±0.05	±0.05	±0.09
Leontodon		0.68	44.17	20.85	21.84	1.48	0.46	0.51	0.02
autumnalis	9	±0.33	±17.37	±10.09	±7.21	±3.40	±0.10	±0.10	±0.06
Leontodon		0.73	20.04	7.69	12.36	0	0.38	0.62	0
hispidus	10	±0.36	±16.18	±6.83	±9.69	±0.00	±0.12	±0.12	±0.00
Taraxacum		0.85	42.92	12.69	16.13	14.09	0.31	0.39	0.31
officinale	10	±0.26	±9.08	±2.58	±2.41	±9.30	±0.09	±0.09	±0.18
Tragopogon		0.25	136.68	60.96	61.82	13.9	0.47	0.47	0.05
pratensis	8	±0.09	±59.89	±18.13	±18.87	±39.32	±0.09	±0.07	±0.15
Brassicaceae									
Cardamine		0.82	59.15	30.34	28.81	0	0.51	0.49	0
pratensis	9	±0.36	±13.92	±7.14	±6.83	±0.00	±0.01	±0.01	±0.00

Table S1 (continued)

Plant family/ *species*[1]	N AA[2]	Volume AA mean ±SD[3]	Amino acids																		
			Total AA[4]	Asp[5]	Glu	Ser	His	Gly	Thr	Arg	Ala	Tyr	Cystine	Val	Met	Phe	Ile	Leu	Lys	Pro	EAA[6]	Non-EAA[7]
			Concentration mg/ml																		
Campanulaceae																					
Campanula patula	11	1.25 ±0.23	8.88 ±5.54	0.24 ±0.18	0.3 ±0.17	0.35 ±0.21	1.11 ±0.69	0.07 ±0.06	0.12 ±0.07	0.22 ±0.15	0.31 ±0.20	0.1 ±0.06	0 ±0.00	0.16 ±0.08	0.58 ±0.45	4.03 ±4.13	0.14 ±0.09	0.2 ±0.10	0.16 ±0.10	0.8 ±0.21	6.71 ±5.00	2.16 ±0.79
Dipsacaceae																					
Knautia arvensis	8	1.65 ±0.98	5.51 ±3.97	0.22 ±0.17	0.18 ±0.12	0.17 ±0.10	2.12 ±1.49	0.09 ±0.06	0.11 ±0.09	0.18 ±0.14	0.4 ±0.31	0.13 ±0.11	0 ±0.00	0.13 ±0.11	0.18 ±0.14	0.14 ±0.12	0.11 ±0.09	0.2 ±0.18	0.15 ±0.12	1 ±0.84	3.33 ±2.39	2.19 ±1.62
Fabaceae																					
Lathyrus pratensis	10	3.9 ±1.47	1.87 ±1.50	0.12 ±0.08	0.14 ±0.09	0.29 ±0.23	0.36 ±0.32	0.06 ±0.06	0.1 ±0.07	0.05 ±0.06	0.22 ±0.17	0.05 ±0.04	0 ±0.00	0.06 ±0.05	0.08 ±0.08	0.05 ±0.04	0.06 ±0.06	0.08 ±0.06	0.08 ±0.07	0.08 ±0.06	0.92 ±0.80	0.95 ±0.71
Lotus corniculatus	7	0.68 ±0.21	12.78 ±4.31	0.47 ±0.19	0.83 ±0.37	0.72 ±0.36	1.72 ±0.57	0.12 ±0.04	0.22 ±0.08	0.16 ±0.05	0.42 ±0.25	1.76 ±0.77	0 ±0.00	0.27 ±0.14	0.14 ±0.07	2.81 ±2.17	0.15 ±0.08	0.16 ±0.08	0.15 ±0.06	2.68 ±1.32	5.78 ±2.97	7 ±2.21
Medicago varia	10	1.19 ±0.56	3.52 ±1.84	0.33 ±0.30	0.1 ±0.06	0.24 ±0.16	0.94 ±0.52	0.05 ±0.03	0.07 ±0.07	0.12 ±0.07	0.2 ±0.12	0.09 ±0.05	0.02 ±0.02	0.12 ±0.07	0.13 ±0.06	0.11 ±0.06	0.1 ±0.06	0.16 ±0.09	0.08 ±0.05	0.65 ±0.39	1.83 ±0.98	1.69 ±0.90
Onobrychis viciifolia	10	1.47 ±1.06	5.97 ±3.98	0.46 ±0.39	0.81 ±0.93	0.6 ±0.40	1.14 ±0.72	0.07 ±0.03	0.28 ±0.13	0.17 ±0.11	0.53 ±0.47	0.08 ±0.04	0 ±0.00	0.19 ±0.10	0.22 ±0.15	0.16 ±0.11	0.13 ±0.07	0.16 ±0.10	0.13 ±0.11	0.85 ±0.71	2.58 ±1.44	3.39 ±2.59
Trifolium campestre	8	0.18 ±0.07	16.84 ±8.88	0.51 ±0.22	1.61 ±1.19	0.9 ±0.50	1.89 ±1.56	0.25 ±0.25	0.43 ±0.25	0.39 ±0.57	1.34 ±0.66	0.2 ±0.21	0 ±0.00	0.65 ±0.36	0.75 ±0.48	0.35 ±0.24	0.4 ±0.23	0.62 ±0.54	0.6 ±0.63	5.96 ±2.57	6.08 ±4.80	10.76 ±4.47
Trifolium fragiferum	6	0.97 ±0.38	6.65 ±6.34	0.47 ±0.43	0.19 ±0.09	0.23 ±0.23	2.52 ±1.47	0.11 ±0.14	0.15 ±0.20	0.16 ±0.20	0.23 ±0.34	0.14 ±0.14	0 ±0.00	0.19 ±0.21	0.24 ±0.32	0.13 ±0.15	0.17 ±0.19	0.23 ±0.29	0.23 ±0.27	1.25 ±1.89	4.03 ±3.23	2.62 ±3.13
Trifolium hybridum	9	0.77 ±0.28	15.69 ±8.93	0.41 ±0.23	0.19 ±0.10	0.41 ±0.22	2.2 ±1.19	0.2 ±0.17	0.27 ±0.25	0.28 ±0.17	0.76 ±0.64	0.24 ±0.22	0 ±0.00	0.32 ±0.33	0.63 ±0.43	0.25 ±0.24	0.26 ±0.26	0.42 ±0.44	0.32 ±0.19	8.54 ±4.97	4.95 ±3.38	10.74 ±6.00
Trifolium pratense	3	0.38 ±0.15	15.62 ±13.68	0.59 ±0.56	0.21 ±0.10	0.76 ±0.74	1.77 ±0.94	0.16 ±0.12	0.28 ±0.29	0.15 ±0.09	0.75 ±0.83	0.16 ±0.11	0 ±0.00	0.39 ±0.45	0.81 ±0.68	0.18 ±0.18	0.33 ±0.36	0.32 ±0.31	0.26 ±0.19	8.49 ±7.85	4.49 ±3.45	11.13 ±10.24
Trifolium repens	8	0.87 ±0.22	9.14 ±2.86	0.24 ±0.14	0.15 ±0.05	0.38 ±0.09	1.86 ±0.57	0.09 ±0.04	0.13 ±0.05	0.14 ±0.08	0.43 ±0.13	0.1 ±0.05	0 ±0.00	0.17 ±0.06	0.43 ±0.15	0.11 ±0.05	0.12 ±0.06	0.21 ±0.09	0.22 ±0.11	4.36 ±1.89	3.39 ±1.11	5.75 ±2.06
Vicia cracca	9	3.56 ±2.10	2.27 ±1.27	0.1 ±0.07	0.06 ±0.04	0.39 ±0.28	0.27 ±0.09	0.04 ±0.03	0.06 ±0.04	0.03 ±0.02	0.54 ±0.47	0.03 ±0.02	0 ±0.00	0.1 ±0.06	0.05 ±0.02	0.13 ±0.08	0.08 ±0.04	0.08 ±0.04	0.03 ±0.02	0.27 ±0.15	0.83 ±0.36	1.44 ±0.94

Table S1 (continued)

Plant family/ *species*[1]	N AA[2]	Volume AA mean ±SD[3]	Amino acids																	
			Asp[5]	Glu	Ser	His	Gly	Thr	Arg	Ala	Tyr	Cystine	Val	Met	Phe	Ile	Leu	Lys	Pro	EAA[6]	Non-EAA[7]
			Proportion																		
Campanulaceae																					
Campanula		1.25	0.03	0.04	0.05	0.14	0.01	0.01	0.03	0.04	0.01	0	0.02	0.07	0.36	0.02	0.03	0.02	0.12	0.7	0.3
patula	11	±0.23	±0.02	±0.01	±0.03	±0.06	±0.01	±0.01	±0.01	±0.02	±0.00	±0.00	±0.01	±0.03	±0.24	±0.01	±0.01	±0.01	±0.06	±0.13	±0.13
Dipsacaceae																					
Knautia		1.65	0.04	0.04	0.03	0.39	0.02	0.02	0.03	0.07	0.02	0	0.02	0.03	0.03	0.02	0.04	0.03	0.16	0.61	0.39
arvensis	8	±0.98	±0.01	±0.02	±0.01	±0.06	±0.00	±0.00	±0.01	±0.01	±0.01	±0.00	±0.00	±0.00	±0.01	±0.00	±0.01	±0.01	±0.05	±0.05	±0.05
Fabaceae																					
Lathyrus		3.9	0.07	0.08	0.15	0.2	0.03	0.05	0.02	0.12	0.02	0	0.03	0.04	0.03	0.03	0.04	0.04	0.05	0.48	0.52
pratensis	10	±1.47	±0.03	±0.03	±0.04	±0.03	±0.01	±0.01	±0.01	±0.03	±0.00	±0.00	±0.01	±0.01	±0.00	±0.01	±0.01	±0.01	±0.02	±0.04	±0.04
Lotus		0.68	0.04	0.07	0.06	0.14	0.01	0.02	0.01	0.03	0.14	0	0.02	0.01	0.21	0.01	0.01	0.01	0.21	0.44	0.56
corniculatus	7	±0.21	±0.02	±0.03	±0.02	±0.02	±0.00	±0.01	±0.00	±0.01	±0.06	±0.00	±0.01	±0.00	±0.11	±0.00	±0.00	±0.00	±0.09	±0.10	±0.10
Medicago		1.19	0.09	0.03	0.07	0.27	0.01	0.02	0.03	0.05	0.03	0.01	0.03	0.04	0.03	0.03	0.04	0.03	0.19	0.52	0.48
varia	10	±0.56	±0.06	±0.01	±0.02	±0.06	±0.00	±0.01	±0.01	±0.02	±0.01	±0.00	±0.01	±0.01	±0.00	±0.00	±0.01	±0.01	±0.04	±0.06	±0.06
Onobrychis		1.47	0.07	0.11	0.1	0.21	0.01	0.05	0.03	0.09	0.01	0	0.04	0.04	0.03	0.02	0.03	0.02	0.14	0.46	0.54
viciifolia	10	±1.06	±0.02	±0.06	±0.02	±0.06	±0.00	±0.02	±0.01	±0.05	±0.00	±0.00	±0.01	±0.01	±0.01	±0.01	±0.01	±0.01	±0.06	±0.07	±0.07
Trifolium		0.18	0.03	0.09	0.05	0.1	0.01	0.03	0.02	0.09	0.01	0	0.04	0.04	0.02	0.02	0.03	0.03	0.37	0.34	0.66
campestre	8	±0.07	±0.01	±0.03	±0.01	±0.04	±0.00	±0.01	±0.01	±0.04	±0.01	±0.00	±0.01	±0.01	±0.00	±0.01	±0.01	±0.01	±0.09	±0.07	±0.07
Trifolium		0.97	0.07	0.04	0.03	0.45	0.01	0.02	0.02	0.03	0.02	0	0.03	0.03	0.02	0.02	0.03	0.03	0.14	0.65	0.35
fragiferum	6	±0.38	±0.03	±0.02	±0.01	±0.10	±0.00	±0.01	±0.01	±0.01	±0.00	±0.00	±0.00	±0.01	±0.00	±0.00	±0.01	±0.01	±0.06	±0.06	±0.06
Trifolium		0.77	0.03	0.01	0.03	0.15	0.01	0.02	0.02	0.05	0.01	0	0.02	0.04	0.02	0.02	0.02	0.02	0.53	0.32	0.68
hybridum	9	±0.28	±0.01	±0.01	±0.02	±0.05	±0.00	±0.01	±0.01	±0.01	±0.01	±0.00	±0.01	±0.01	±0.01	±0.01	±0.01	±0.01	±0.12	±0.09	±0.09
Trifolium		0.38	0.04	0.02	0.05	0.14	0.01	0.02	0.01	0.04	0.01	0	0.02	0.05	0.01	0.02	0.02	0.02	0.53	0.31	0.69
pratense	3	±0.15	±0.01	±0.01	±0.01	±0.04	±0.00	±0.00	±0.00	±0.01	±0.00	±0.00	±0.01	±0.00	±0.00	±0.01	±0.00	±0.01	±0.03	±0.04	±0.04
Trifolium		0.87	0.03	0.02	0.04	0.21	0.01	0.01	0.01	0.05	0.01	0	0.02	0.05	0.01	0.01	0.02	0.02	0.47	0.38	0.62
repens	8	±0.22	±0.01	±0.01	±0.00	±0.04	±0.00	±0.00	±0.01	±0.01	±0.00	±0.00	±0.00	±0.01	±0.00	±0.00	±0.01	±0.01	±0.09	±0.06	±0.06
Vicia		3.56	0.04	0.03	0.16	0.14	0.02	0.02	0.02	0.21	0.01	0	0.05	0.03	0.06	0.04	0.04	0.02	0.13	0.39	0.61
cracca	9	±2.10	±0.02	±0.01	±0.04	±0.04	±0.00	±0.00	±0.00	±0.08	±0.00	±0.00	±0.01	±0.01	±0.02	±0.01	±0.01	±0.00	±0.09	±0.07	±0.07

Table S1 (continued)

Plant family/ *species*[1]	N C[2]	Volume C mean ±SD[3]	Carbohydrates						
			Total C[4]	Fructose	Glucose	Sucrose	Fructose	Glucose	Sucrose
			Concentration mg/ml				Proportion		
Campanulaceae									
Campanula		1.25	18.64	7.52	7.46	3.66	0.42	0.42	0.15
patula	11	±0.23	±5.47	±1.51	±1.64	±4.66	±0.10	±0.10	±0.19
Dipsacaceae									
Knautia		1.65	63.93	30.83	33.09	0	0.49	0.51	0
arvensis	8	±0.98	±33.41	±15.66	±17.79	±0.00	±0.02	±0.02	±0.00
Fabaceae									
Lathyrus		3.9	17.64	9.61	5.37	2.66	0.57	0.27	0.16
pratensis	10	±1.47	±13.40	±8.06	±5.84	±3.50	±0.17	±0.09	±0.20
Lotus		0.68	102.81	33.8	28.44	40.58	0.37	0.31	0.32
corniculatus	7	±0.21	±38.03	±5.87	±13.98	±38.66	±0.14	±0.19	±0.30
Medicago		1.19	25.69	6.28	5.08	14.33	0.28	0.24	0.49
varia	10	±0.56	±8.64	±1.74	±2.29	±9.80	±0.14	±0.16	±0.28
Onobrychis		1.47	52.5	5.55	4.97	41.97	0.11	0.1	0.79
viciifolia	10	±1.06	±23.42	±2.15	±2.00	±20.61	±0.04	±0.04	±0.08
Trifolium		0.18	190.5	64.9	61.26	64.34	0.43	0.32	0.25
campestre	8	±0.07	±97.48	±27.33	±38.41	±70.31	±0.26	±0.18	±0.27
Trifolium		0.97	53.31	13.82	9.66	29.82	0.29	0.19	0.52
fragiferum	6	±0.38	±27.46	±5.61	±9.46	±18.33	±0.12	±0.19	±0.26
Trifolium		0.77	98.73	24.05	23.74	50.95	0.25	0.25	0.5
hybridum	9	±0.28	±26.32	±7.21	±8.64	±27.78	±0.09	±0.11	±0.20
Trifolium		0.38	47.58	13.25	9.71	24.61	0.35	0.25	0.41
pratense	3	±0.15	±26.39	±2.46	±1.92	±24.95	±0.22	±0.14	±0.36
Trifolium		0.87	48.82	12.7	10.7	25.42	0.29	0.24	0.46
repens	8	±0.22	±17.46	±4.48	±4.67	±11.78	±0.11	±0.10	±0.21
Vicia		3.56	21.73	9.5	3.77	8.47	0.48	0.17	0.35
cracca	9	±2.10	±9.70	±3.50	±1.82	±7.64	±0.16	±0.05	±0.19

Table S1 (continued)

Plant family/ *species*[1]	N AA[2]	Volume AA mean ±SD[3]	Amino acids																		
			Total AA[4]	Asp[5]	Glu	Ser	His	Gly	Thr	Arg	Ala	Tyr	Cystine	Val	Met	Phe	Ile	Leu	Lys	Pro	EAA[6]	Non-EAA[7]
			Concentration mg/ml																		
Geraniaceae																					
Geranium		3.38	0.97	0.05	0.07	0.05	0.47	0.03	0.03	0.03	0.03	0.03	0.02	0.02	0.03	0.04	0.02	0.03	0.01	0.01	0.69	0.28
pratense	6	±2.17	±0.91	±0.05	±0.06	±0.04	±0.46	±0.03	±0.03	±0.03	±0.03	±0.02	±0.02	±0.02	±0.03	±0.04	±0.02	±0.03	±0.02	±0.01	±0.67	±0.24
Lamiaceae																					
Ajuga		0.92	7.07	0.21	0.3	0.12	0.81	0.05	0.07	0.17	0.11	0.07	0	0.06	0.12	4.39	0.05	0.07	0.08	0.38	5.82	1.25
reptans	10	±0.28	±2.89	±0.14	±0.25	±0.06	±0.22	±0.02	±0.03	±0.12	±0.09	±0.02	±0.00	±0.03	±0.08	±1.97	±0.02	±0.03	±0.03	±0.16	±2.39	±0.66
Glechoma		1.39	3.39	0.04	0.05	0.13	2.09	0.02	0.04	0.02	0.09	0.03	0	0.07	0.13	0.08	0.06	0.07	0.04	0.44	2.59	0.79
hederacea	10	±0.52	±2.53	±0.03	±0.04	±0.09	±2.07	±0.03	±0.02	±0.01	±0.07	±0.02	±0.00	±0.04	±0.11	±0.03	±0.03	±0.04	±0.02	±0.14	±2.34	±0.27
Prunella		2.43	1.5	0.05	0.07	0.04	1.02	0.02	0.03	0.03	0.03	0.02	0	0.03	0.03	0.02	0.02	0.03	0.04	0.03	1.25	0.26
vulgaris	10	±1.71	±0.84	±0.02	±0.03	±0.02	±0.78	±0.01	±0.01	±0.01	±0.02	±0.01	±0.00	±0.02	±0.02	±0.01	±0.01	±0.03	±0.02	±0.02	±0.81	±0.12
Primulaceae																					
Primula		1.27	16.01	0.23	1.37	0.64	1.42	0.22	0.24	0.29	2.13	0.19	0	0.33	1.31	0.22	0.2	0.32	0.53	6.37	4.85	11.16
veris	10	±0.14	±5.82	±0.08	±0.67	±0.31	±0.52	±0.10	±0.10	±0.10	±0.79	±0.09	±0.00	±0.15	±0.63	±0.09	±0.09	±0.16	±0.30	±2.38	±1.87	±4.10
Ranuncu-laceae																					
Ranunculus		0.82	13.35	0.25	0.86	0.94	4.31	0.38	0.46	0.48	0.6	0.33	0	0.55	0.68	0.44	0.34	0.42	0.57	1.74	8.24	5.1
acris	10	±0.15	±6.29	±0.17	±0.43	±0.57	±2.03	±0.23	±0.24	±0.27	±0.37	±0.17	±0.00	±0.23	±0.36	±0.20	±0.19	±0.29	±0.49	±0.73	±4.22	±2.15
Ranunculus		1.15	11.43	0.34	0.25	1.02	2.54	0.18	0.23	0.31	0.5	0.18	0	0.47	0.87	0.33	0.28	0.34	0.46	3.14	5.83	5.6
repens	13	±0.26	±4.10	±0.48	±0.32	±0.43	±0.94	±0.07	±0.08	±0.11	±0.34	±0.04	±0.00	±0.23	±0.47	±0.13	±0.10	±0.09	±0.20	±1.52	±1.85	±2.57
Rosaceae																					
Sanguisorba		0.99	20.85	0.29	3.8	1.44	2.9	0.43	0.88	3.49	1.3	0.2	0	0.66	0.29	0.25	0.64	0.29	3.1	0.9	12.5	8.35
officinalis	13	±0.28	±4.95	±0.12	±1.31	±0.30	±0.86	±0.21	±0.33	±1.96	±0.33	±0.04	±0.00	±0.22	±0.07	±0.08	±0.74	±0.10	±1.35	±0.36	±4.06	±2.02
Scrophu-lariaceae																					
Veronica		1.01	4.34	0.17	0.1	0.23	2.37	0.06	0.07	0.09	0.26	0.08	0	0.11	0.2	0.09	0.08	0.14	0.2	0.1	3.35	0.99
chamaedrys	7	±0.21	±1.42	±0.08	±0.03	±0.08	±0.90	±0.03	±0.03	±0.02	±0.09	±0.03	±0.00	±0.04	±0.08	±0.05	±0.03	±0.05	±0.04	±0.04	±1.15	±0.29

Table S1 (continued)

Plant family/ *species*[1]	N AA[2]	Volume AA mean ±SD[3]	Amino acids																	
			Asp[5]	Glu	Ser	His	Gly	Thr	Arg	Ala	Tyr	Cystine	Val	Met	Phe	Ile	Leu	Lys	Pro	EAA[6]	Non-EAA[7]
			Proportion																	
Geraniaceae																				
Geranium		3.38	0.05	0.07	0.06	0.46	0.03	0.04	0.03	0.03	0.02	0.02	0.03	0.03	0.04	0.02	0.04	0.01	0.02	0.69	0.31
pratense	6	±2.17	±0.02	±0.01	±0.01	±0.04	±0.00	±0.00	±0.01	±0.00	±0.00	±0.00	±0.00	±0.01	±0.01	±0.00	±0.00	±0.01	±0.02	±0.04	±0.04
Lamiaceae																				
Ajuga		0.92	0.03	0.04	0.02	0.13	0.01	0.01	0.02	0.01	0.01	0	0.01	0.02	0.61	0.01	0.01	0.01	0.06	0.82	0.18
reptans	10	±0.28	±0.01	±0.02	±0.00	±0.04	±0.00	±0.00	±0.01	±0.01	±0.01	±0.00	±0.00	±0.01	±0.10	±0.00	±0.00	±0.00	±0.02	±0.05	±0.05
Glechoma		1.39	0.02	0.02	0.05	0.5	0.01	0.01	0.01	0.02	0.01	0	0.02	0.04	0.03	0.02	0.02	0.02	0.2	0.66	0.34
hederacea	10	±0.52	±0.02	±0.01	±0.06	±0.24	±0.02	±0.01	±0.00	±0.01	±0.01	±0.00	±0.01	±0.01	±0.01	±0.01	±0.01	±0.01	±0.15	±0.21	±0.21
Prunella		2.43	0.03	0.05	0.03	0.62	0.01	0.02	0.02	0.03	0.02	0	0.02	0.03	0.01	0.01	0.03	0.03	0.02	0.8	0.2
vulgaris	10	±1.71	±0.01	±0.03	±0.02	±0.20	±0.01	±0.01	±0.01	±0.02	±0.02	±0.00	±0.01	±0.02	±0.01	±0.01	±0.02	±0.01	±0.02	±0.11	±0.11
Primulaceae																				
Primula		1.27	0.01	0.08	0.04	0.09	0.01	0.02	0.02	0.13	0.01	0	0.02	0.08	0.01	0.01	0.02	0.03	0.4	0.3	0.7
veris	10	±0.14	±0.00	±0.02	±0.02	±0.02	±0.00	±0.01	±0.00	±0.02	±0.00	±0.00	±0.01	±0.02	±0.01	±0.00	±0.01	±0.01	±0.05	±0.04	±0.04
Ranunculaceae																				
Ranunculus		0.82	0.02	0.07	0.07	0.32	0.03	0.03	0.04	0.04	0.02	0	0.04	0.05	0.03	0.02	0.03	0.04	0.13	0.61	0.39
acris	10	±0.15	±0.01	±0.04	±0.01	±0.03	±0.00	±0.00	±0.01	±0.01	±0.00	±0.00	±0.01	±0.01	±0.00	±0.00	±0.01	±0.01	±0.02	±0.04	±0.04
Ranunculus		1.15	0.03	0.02	0.09	0.23	0.02	0.02	0.03	0.04	0.02	0	0.04	0.07	0.03	0.03	0.03	0.04	0.27	0.52	0.48
repens	13	±0.26	±0.02	±0.02	±0.02	±0.04	±0.00	±0.01	±0.01	±0.02	±0.00	±0.00	±0.03	±0.02	±0.01	±0.01	±0.01	±0.01	±0.07	±0.06	±0.06
Rosaceae																				
Sanguisorba		0.99	0.01	0.19	0.07	0.14	0.02	0.04	0.16	0.06	0.01	0	0.03	0.01	0.01	0.03	0.01	0.14	0.05	0.59	0.41
officinalis	13	±0.28	±0.01	±0.06	±0.02	±0.03	±0.01	±0.01	±0.07	±0.01	±0.00	±0.00	±0.01	±0.00	±0.00	±0.03	±0.00	±0.04	±0.02	±0.08	±0.08
Scrophulariaceae																				
Veronica		1.01	0.04	0.02	0.05	0.54	0.01	0.02	0.02	0.06	0.02	0	0.02	0.05	0.02	0.02	0.03	0.05	0.02	0.77	0.23
chamaedrys	7	±0.21	±0.01	±0.01	±0.01	±0.05	±0.01	±0.00	±0.00	±0.01	±0.00	±0.00	±0.01	±0.00	±0.01	±0.01	±0.01	±0.01	±0.01	±0.02	±0.02

Table S1 (continued)

Plant family/ *species*[1]	N C[2]	Volume C mean ±SD[3]	Carbohydrates						
			Total C[4]	Fructose	Glucose	Sucrose	Fructose	Glucose	Sucrose
			Concentration mg/ml				Proportion		
Geraniaceae									
Geranium		3.38	64.7	14.24	12.73	37.73	0.21	0.19	0.6
pratense	6	±2.17	±41.33	±10.18	±9.12	±22.07	±0.01	±0.02	±0.03
Lamiaceae									
Ajuga		0.92	150.9	17.11	14.92	118.87	0.12	0.1	0.78
reptans	10	±0.28	±50.77	±6.21	±4.41	±44.57	±0.03	±0.02	±0.05
Glechoma		1.39	48.29	9.5	8.5	30.29	0.21	0.19	0.6
hederacea	10	±0.52	±14.69	±3.99	±3.52	±16.97	±0.13	±0.11	±0.24
Prunella		2.43	52.64	12.4	8.4	31.84	0.24	0.16	0.6
vulgaris	10	±1.71	±16.47	±3.29	±2.60	±11.97	±0.03	±0.04	±0.06
Primulaceae									
Primula		1.27	68.33	18.64	18.6	31.1	0.27	0.27	0.46
veris	10	±0.14	±11.17	±3.38	±3.21	±5.55	±0.02	±0.01	±0.03
Ranunculaceae									
Ranunculus		0.82	49.26	0.86	0.48	47.92	0.03	0.02	0.95
acris	10	±0.15	±23.79	±1.41	±1.52	±24.66	±0.05	±0.07	±0.12
Ranunculus		1.15	34.22	2.82	4.01	27.4	0.14	0.2	0.66
repens	13	±0.26	±19.69	±2.15	±3.08	±19.51	±0.15	±0.19	±0.34
Rosaceae									
Sanguisorba		0.99	25.45	0	0	25.45	0	0	1
officinalis	13	±0.28	±9.54	±0.00	±0.00	±9.54	±0.00	±0.00	±0.00
Scrophulariaceae									
Veronica		1.01	19.45	7.19	12.26	0	0.35	0.65	0
chamaedrys	7	±0.21	±6.13	±3.91	±2.92	±0.00	±0.15	±0.15	±0.00

1. Plant families (bold) are in alphabetic order, also plant species within each family.
2. For each plant species minimum sampling number was aimed at 7 samples per plant species for both analyses (only exception were: *G. pratense, T. fragiferum,* and *T. pratense*), N is the number of samples analysed for AA (amino acids) and C (carbohydrates), respectively.
3. Volume is the mean value per plant species in µl ± SD (standard deviation).
4. Total amino acids are the mean sum of all single amino acids ± standard deviation in mg per ml, followed by individual amino acids. Order of displayed amino acids reflects the order of appearance in the chromatogram. Total carbohydrates are the mean sum of the three main carbohydrates (fructose, glucose, sucrose) in mg/ml ± SD (standard deviation).
5. Abbreviations: Ala - alanine, Arg - arginine, Asp - aspartic acid, Cystine, Glu - glutamic acid, Gly - glycine, His - histidine, Ile – isoleucine, Leu - leucine, Lys - lysine, Met - methionine,Phe - phenylalanine, Pro - proline, Ser - serine, Thr - threonine, Tyr - tyrosine, Val - valine. EAA: essential AA (His, Gly, Thr, Arg, Ala, Tyr, Cystine, Val, Met, Phe, Ile, Leu, Lys)
6. Non-EAA: non-essential AA (Asp, Glu, Ser, Gly, Ala, Tyr, cystine, Pro)

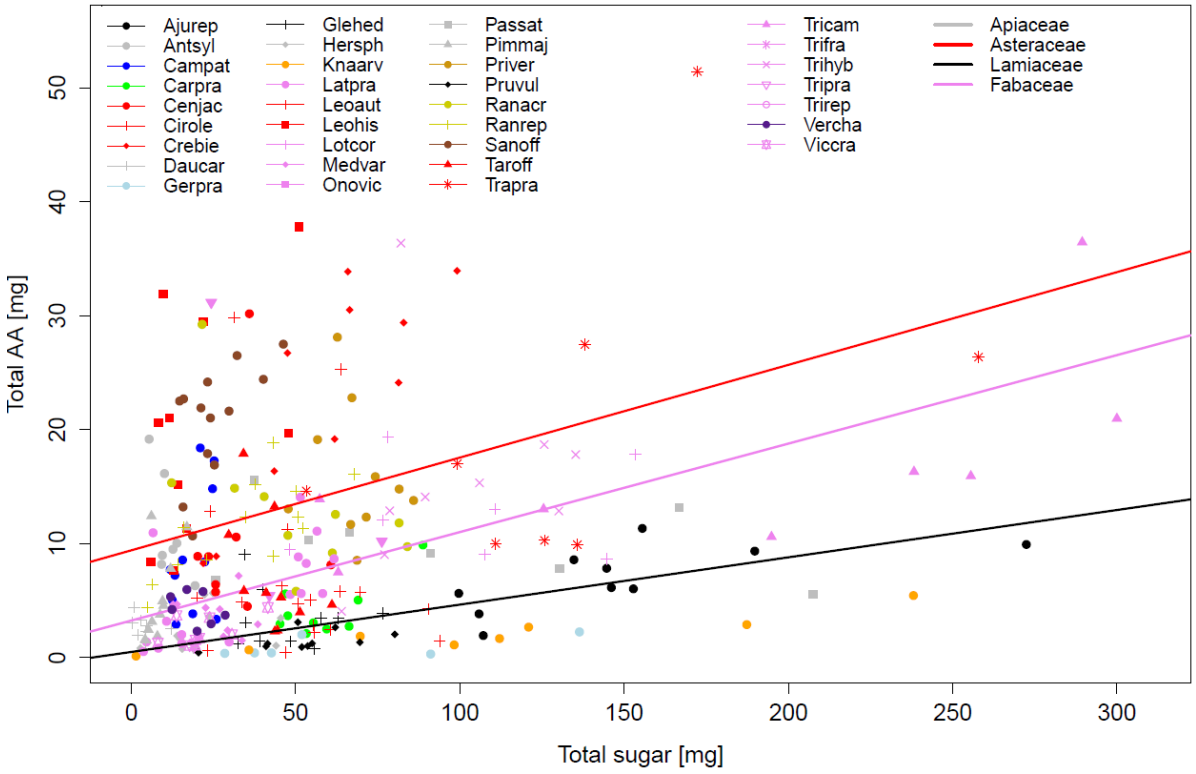

Fig. S1 Ratio of total amino acids (AA) in mg against total sugar in mg. Different symbols indicate different plant species (in alphabetical order), with species of the same family depicted by the same colour. Lines added for the four most abundant families (i.e. Apiaceae (grey), Asteraceae (red), Fabaceae (violet), and Lamiaceae (black)). Depicted plant species: Ajurep = *Ajuga reptans*, Antsyl = *Anthriscus sylvestris*, Campat = *Campanula patula*, Carpra = *Cardamine pratensis*, Cenjac = *Centaurea jacea*, Cirole = *Cirsium oleraceum*, Crebie = *Crepis biennis*, Daucar = *Daucus carota*, Gerpra = *Geranium pratense*, Glehed = *Glechoma hederacea*, Hersph = *Heracleum sphondylium*, Knaarv = *Knautia arvensis*, Latpra = *Lathyrus pratensis*, Leoaut = *Leontodon autumnalis*, Leohis = *Leontodon hispidus*, Lotcor = *Lotus corniculatus*, Medvar = *Medicago varia*, Onovic = *Onobrychis viciifolia*, Passat = *Pastinaca sativa*, Pimmaj = *Pimpinella major*, Priver = *Primula veris*, Pruvul = *Prunella vulgaris*, Ranacr = *Ranunculus acris*, Ranrep = *Ranunculus repens*, Sanoff = *Sanguisorba officinalis*, Taroff = *Taraxacum officinale*, Trapra = *Tragopogon pratensis*, Tricam = *Trifolium campestre*, Trifra = *Trifolium fragiferum*, Trihyb = *Trifolium hybridum*, Tripra = *Trifolium pratense*, Trirep = *Trifolium repens*, Vercha = *Veronica chamaedrys*, Viccra = *Vicia cracca*

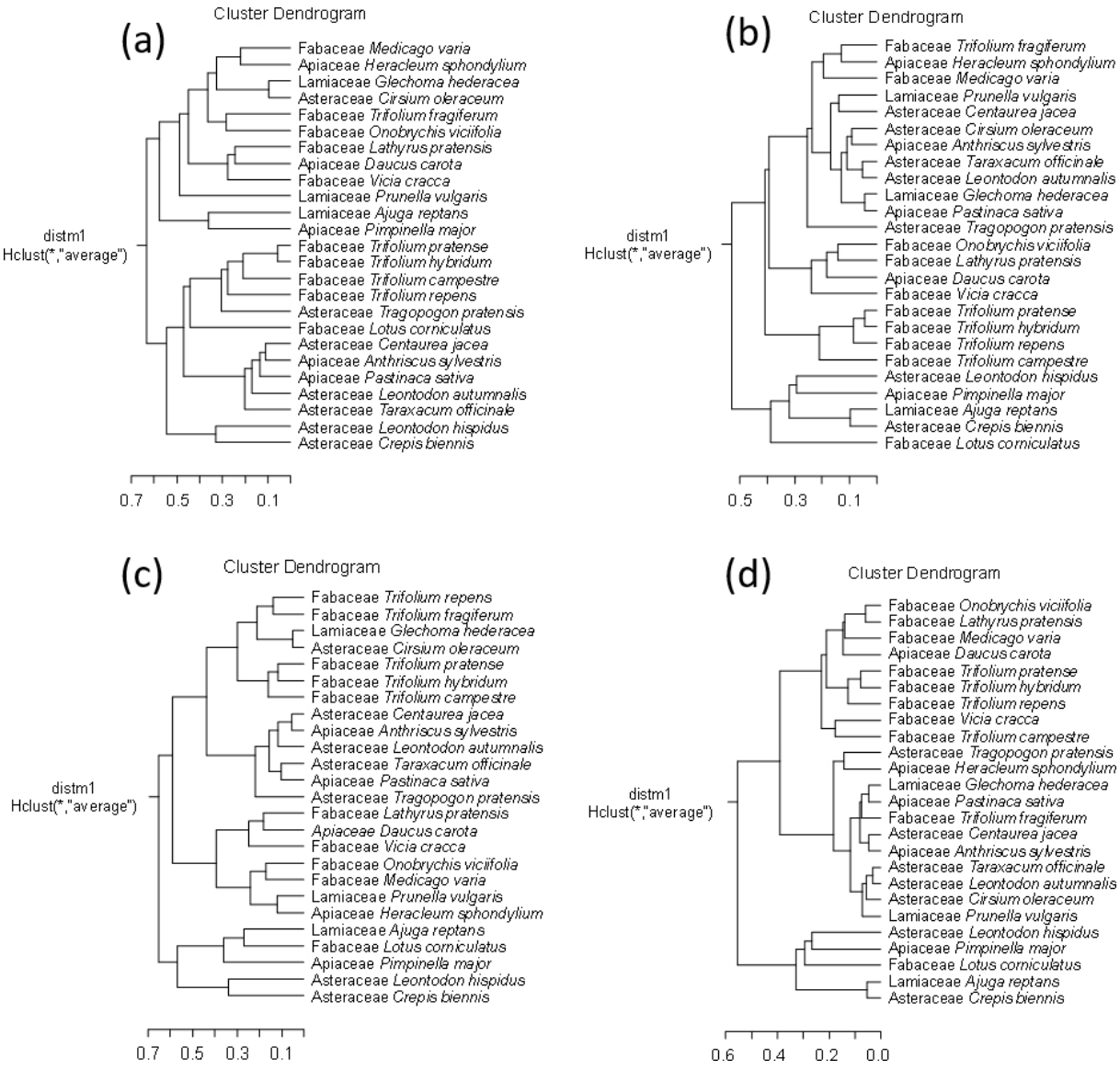

Fig. S2 Cluster dendrograms of the four most abundant plant families (Apiaceae, Asteraceae, Fabaceae, and Lamiaceae). Based on a) mean concentrations, b) mean proportions of the sum of all amino acids, and c) mean concentrations and d) mean proportions of the sum of essential amino acids in the nectar.

Table S2 is in a separate Excel file.

Table S2 Chemical distance matrix between 34 plant species based on Bray-Curtis distances between either the a,c) proportions (%) or b,d) concentrations (conc) of amino acids and carbohydrates.

a) Amino acid %

Chemical distance matrix between 34 plant species based on Bray-Curtis distances between either the proportions (%) or concentrations (conc) of amino acids and carbohydrates.

high chemical similarity

intermediate chemical similarity

low chemical similarity

		Anthriscus sylvestris	*Daucus carota*	*Heracleum sphondylium*	*Pastinaca sativa*	*Pimpinella major*
Apiaceae	*Anthriscus sylvestris*	0	0,24	0,16	0,23	0,57
Apiaceae	*Daucus carota*	0,24	0	0,25	0,33	0,64
Apiaceae	*Heracleum sphondylium*	0,16	0,25	0	0,27	0,58
Apiaceae	*Pastinaca sativa*	0,23	0,33	0,27	0	0,56
Apiaceae	*Pimpinella major*	0,57	0,64	0,58	0,56	0
Asteraceae	*Centaurea jacea*	0,3	0,43	0,32	0,19	0,55
Asteraceae	*Cirsium oleraceum*	0,19	0,35	0,26	0,29	0,62
Asteraceae	*Crepis biennis*	0,71	0,75	0,7	0,7	0,45
Asteraceae	*Leontodon autumnalis*	0,14	0,26	0,25	0,33	0,6
Asteraceae	*Leontodon hispidus*	0,55	0,61	0,55	0,51	0,35
Asteraceae	*Taraxacum officinale*	0,18	0,28	0,27	0,33	0,61
Asteraceae	*Tragopogon pratensis*	0,2	0,28	0,27	0,32	0,61
Brassicaceae	*Cardamine pratensis*	0,15	0,28	0,09	0,25	0,59
Campanulaceae	*Campanula patula*	0,57	0,61	0,56	0,51	0,4
Dipsacaceae	*Knautia arvensis*	0,28	0,37	0,23	0,17	0,54
Fabaceae	*Lathyrus pratensis*	0,49	0,44	0,47	0,33	0,7
Fabaceae	*Lotus corniculatus*	0,28	0,34	0,25	0,26	0,47
Fabaceae	*Medicago varia*	0,29	0,35	0,26	0,14	0,56
Fabaceae	*Onobrychis vicifolia*	0,42	0,4	0,35	0,31	0,6
Fabaceae	*Trifolium campestre*	0,24	0,34	0,22	0,18	0,56
Fabaceae	*Trifolium fragiferum*	0,18	0,33	0,15	0,19	0,56
Fabaceae	*Trifolium hybridum*	0,19	0,28	0,26	0,3	0,6
Fabaceae	*Trifolium pratense*	0,16	0,21	0,22	0,31	0,6
Fabaceae	*Trifolium repens*	0,21	0,27	0,27	0,37	0,61
Fabaceae	*Vicia cracca*	0,54	0,48	0,56	0,41	0,65
Geraniaceae	*Geranium pratense*	0,35	0,46	0,29	0,36	0,54
Lamiaceae	*Ajuga reptans*	0,67	0,66	0,59	0,65	0,44
Lamiaceae	*Glechoma hederacea*	0,34	0,44	0,39	0,13	0,56
Lamiaceae	*Prunella vulgaris*	0,44	0,54	0,4	0,37	0,68
Primulaceae	*Primula veris*	0,25	0,32	0,2	0,25	0,6
Ranunculaceae	*Ranunculus acris*	0,37	0,4	0,32	0,19	0,58
Ranunculaceae	*Ranunculus repens*	0,21	0,24	0,2	0,14	0,61
Rosaceae	*Sanguisorba officinalis*	0,59	0,58	0,53	0,5	0,73
Scrophulariaceae	*Veronica chamaedrys*	0,44	0,52	0,46	0,24	0,68

a) Amino acid %

		Centaurea jacea	*Cirsium oleraceum*	*Crepis biennis*	*Leontodon autumnalis*	*Leontodon hispidus*
Apiaceae	*Anthriscus sylvestris*	0,3	0,19	0,71	0,14	0,55
Apiaceae	*Daucus carota*	0,43	0,35	0,75	0,26	0,61
Apiaceae	*Heracleum sphondylium*	0,32	0,26	0,7	0,25	0,55
Apiaceae	*Pastinaca sativa*	0,19	0,29	0,7	0,33	0,51
Apiaceae	*Pimpinella major*	0,55	0,62	0,45	0,6	0,35
Asteraceae	*Centaurea jacea*	0	0,31	0,7	0,4	0,49
Asteraceae	*Cirsium oleraceum*	0,31	0	0,74	0,15	0,53
Asteraceae	*Crepis biennis*	0,7	0,74	0	0,72	0,31
Asteraceae	*Leontodon autumnalis*	0,4	0,15	0,72	0	0,51
Asteraceae	*Leontodon hispidus*	0,49	0,53	0,31	0,51	0
Asteraceae	*Taraxacum officinale*	0,41	0,18	0,73	0,07	0,51
Asteraceae	*Tragopogon pratensis*	0,39	0,27	0,75	0,17	0,57
Brassicaceae	*Cardamine pratensis*	0,33	0,21	0,71	0,22	0,54
Campanulaceae	*Campanula patula*	0,53	0,61	0,29	0,61	0,26
Dipsacaceae	*Knautia arvensis*	0,15	0,35	0,68	0,37	0,47
Fabaceae	*Lathyrus pratensis*	0,25	0,5	0,71	0,6	0,63
Fabaceae	*Lotus corniculatus*	0,34	0,37	0,57	0,35	0,34
Fabaceae	*Medicago varia*	0,18	0,36	0,69	0,38	0,51
Fabaceae	*Onobrychis vicifolia*	0,23	0,38	0,73	0,46	0,55
Fabaceae	*Trifolium campestre*	0,26	0,29	0,72	0,33	0,55
Fabaceae	*Trifolium fragiferum*	0,22	0,25	0,71	0,25	0,5
Fabaceae	*Trifolium hybridum*	0,39	0,23	0,73	0,13	0,55
Fabaceae	*Trifolium pratense*	0,4	0,22	0,73	0,11	0,55
Fabaceae	*Trifolium repens*	0,46	0,26	0,74	0,16	0,58
Fabaceae	*Vicia cracca*	0,34	0,52	0,71	0,6	0,61
Geraniaceae	*Geranium pratense*	0,31	0,44	0,67	0,41	0,51
Lamiaceae	*Ajuga reptans*	0,64	0,7	0,16	0,67	0,33
Lamiaceae	*Glechoma hederacea*	0,18	0,36	0,71	0,41	0,51
Lamiaceae	*Prunella vulgaris*	0,28	0,54	0,7	0,52	0,59
Primulaceae	*Primula veris*	0,32	0,15	0,74	0,27	0,56
Ranunculaceae	*Ranunculus acris*	0,2	0,44	0,69	0,48	0,49
Ranunculaceae	*Ranunculus repens*	0,28	0,23	0,73	0,28	0,56
Rosaceae	*Sanguisorba officinalis*	0,45	0,64	0,76	0,66	0,68
Scrophulariaceae	*Veronica chamaedrys*	0,18	0,44	0,7	0,55	0,59

a) Amino acid %

		Taraxacum officinale	*Tragopogon pratensis*	*Cardamine pratensis*	*Campanula patula*	*Knautia arvensis*
Apiaceae	*Anthriscus sylvestris*	0,18	0,2	0,15	0,57	0,28
Apiaceae	*Daucus carota*	0,28	0,28	0,28	0,61	0,37
Apiaceae	*Heracleum sphondylium*	0,27	0,27	0,09	0,56	0,23
Apiaceae	*Pastinaca sativa*	0,33	0,32	0,25	0,51	0,17
Apiaceae	*Pimpinella major*	0,61	0,61	0,59	0,4	0,54
Asteraceae	*Centaurea jacea*	0,41	0,39	0,33	0,53	0,15
Asteraceae	*Cirsium oleraceum*	0,18	0,27	0,21	0,61	0,35
Asteraceae	*Crepis biennis*	0,73	0,75	0,71	0,29	0,68
Asteraceae	*Leontodon autumnalis*	0,07	0,17	0,22	0,61	0,37
Asteraceae	*Leontodon hispidus*	0,51	0,57	0,54	0,26	0,47
Asteraceae	*Taraxacum officinale*	0	0,2	0,26	0,64	0,38
Asteraceae	*Tragopogon pratensis*	0,2	0	0,24	0,63	0,33
Brassicaceae	*Cardamine pratensis*	0,26	0,24	0	0,55	0,24
Campanulaceae	*Campanula patula*	0,64	0,63	0,55	0	0,53
Dipsacaceae	*Knautia arvensis*	0,38	0,33	0,24	0,53	0
Fabaceae	*Lathyrus pratensis*	0,61	0,55	0,46	0,52	0,29
Fabaceae	*Lotus corniculatus*	0,37	0,33	0,3	0,45	0,27
Fabaceae	*Medicago varia*	0,39	0,34	0,26	0,54	0,11
Fabaceae	*Onobrychis vicifolia*	0,48	0,48	0,38	0,56	0,27
Fabaceae	*Trifolium campestre*	0,35	0,3	0,24	0,55	0,16
Fabaceae	*Trifolium fragiferum*	0,29	0,24	0,12	0,54	0,15
Fabaceae	*Trifolium hybridum*	0,17	0,11	0,24	0,63	0,33
Fabaceae	*Trifolium pratense*	0,16	0,15	0,2	0,55	0,33
Fabaceae	*Trifolium repens*	0,21	0,08	0,24	0,61	0,41
Fabaceae	*Vicia cracca*	0,6	0,58	0,57	0,59	0,44
Geraniaceae	*Geranium pratense*	0,4	0,45	0,29	0,56	0,27
Lamiaceae	*Ajuga reptans*	0,67	0,71	0,64	0,23	0,61
Lamiaceae	*Glechoma hederacea*	0,42	0,42	0,36	0,52	0,21
Lamiaceae	*Prunella vulgaris*	0,51	0,49	0,43	0,52	0,26
Primulaceae	*Primula veris*	0,3	0,31	0,21	0,54	0,29
Ranunculaceae	*Ranunculus acris*	0,48	0,43	0,33	0,51	0,16
Ranunculaceae	*Ranunculus repens*	0,29	0,29	0,21	0,52	0,25
Rosaceae	*Sanguisorba officinalis*	0,66	0,62	0,57	0,64	0,43
Scrophulariaceae	*Veronica chamaedrys*	0,55	0,52	0,42	0,5	0,27

a) Amino acid %

		Lathyrus pratensis	*Lotus corniculatus*	*Medicago varia*	*Onobrychis vicifolia*	*Trifolium campestre*
Apiaceae	*Anthriscus sylvestris*	0,49	0,28	0,29	0,42	0,24
Apiaceae	*Daucus carota*	0,44	0,34	0,35	0,4	0,34
Apiaceae	*Heracleum sphondylium*	0,47	0,25	0,26	0,35	0,22
Apiaceae	*Pastinaca sativa*	0,33	0,26	0,14	0,31	0,18
Apiaceae	*Pimpinella major*	0,7	0,47	0,56	0,6	0,56
Asteraceae	*Centaurea jacea*	0,25	0,34	0,18	0,23	0,26
Asteraceae	*Cirsium oleraceum*	0,5	0,37	0,36	0,38	0,29
Asteraceae	*Crepis biennis*	0,71	0,57	0,69	0,73	0,72
Asteraceae	*Leontodon autumnalis*	0,6	0,35	0,38	0,46	0,33
Asteraceae	*Leontodon hispidus*	0,63	0,34	0,51	0,55	0,55
Asteraceae	*Taraxacum officinale*	0,61	0,37	0,39	0,48	0,35
Asteraceae	*Tragopogon pratensis*	0,55	0,33	0,34	0,48	0,3
Brassicaceae	*Cardamine pratensis*	0,46	0,3	0,26	0,38	0,24
Campanulaceae	*Campanula patula*	0,52	0,45	0,54	0,56	0,55
Dipsacaceae	*Knautia arvensis*	0,29	0,27	0,11	0,27	0,16
Fabaceae	*Lathyrus pratensis*	0	0,45	0,29	0,3	0,35
Fabaceae	*Lotus corniculatus*	0,45	0	0,26	0,36	0,22
Fabaceae	*Medicago varia*	0,29	0,26	0	0,24	0,19
Fabaceae	*Onobrychis vicifolia*	0,3	0,36	0,24	0	0,26
Fabaceae	*Trifolium campestre*	0,35	0,22	0,19	0,26	0
Fabaceae	*Trifolium fragiferum*	0,38	0,24	0,17	0,33	0,16
Fabaceae	*Trifolium hybridum*	0,52	0,31	0,32	0,44	0,25
Fabaceae	*Trifolium pratense*	0,52	0,29	0,32	0,4	0,26
Fabaceae	*Trifolium repens*	0,59	0,36	0,4	0,47	0,33
Fabaceae	*Vicia cracca*	0,31	0,49	0,41	0,37	0,47
Geraniaceae	*Geranium pratense*	0,51	0,41	0,29	0,38	0,41
Lamiaceae	*Ajuga reptans*	0,64	0,51	0,64	0,61	0,62
Lamiaceae	*Glechoma hederacea*	0,29	0,35	0,22	0,29	0,26
Lamiaceae	*Prunella vulgaris*	0,3	0,45	0,34	0,44	0,37
Primulaceae	*Primula veris*	0,41	0,27	0,31	0,28	0,16
Ranunculaceae	*Ranunculus acris*	0,21	0,33	0,18	0,29	0,23
Ranunculaceae	*Ranunculus repens*	0,36	0,23	0,22	0,28	0,17
Rosaceae	*Sanguisorba officinalis*	0,42	0,53	0,46	0,37	0,42
Scrophulariaceae	*Veronica chamaedrys*	0,18	0,47	0,27	0,34	0,37

a) Amino acid %

		Trifolium fragiferum	*Trifolium hybridum*	*Trifolium pratense*	*Trifolium repens*	*Vicia cracca*
Apiaceae	*Anthriscus sylvestris*	0,18	0,19	0,16	0,21	0,54
Apiaceae	*Daucus carota*	0,33	0,28	0,21	0,27	0,48
Apiaceae	*Heracleum sphondylium*	0,15	0,26	0,22	0,27	0,56
Apiaceae	*Pastinaca sativa*	0,19	0,3	0,31	0,37	0,41
Apiaceae	*Pimpinella major*	0,56	0,6	0,6	0,61	0,65
Asteraceae	*Centaurea jacea*	0,22	0,39	0,4	0,46	0,34
Asteraceae	*Cirsium oleraceum*	0,25	0,23	0,22	0,26	0,52
Asteraceae	*Crepis biennis*	0,71	0,73	0,73	0,74	0,71
Asteraceae	*Leontodon autumnalis*	0,25	0,13	0,11	0,16	0,6
Asteraceae	*Leontodon hispidus*	0,5	0,55	0,55	0,58	0,61
Asteraceae	*Taraxacum officinale*	0,29	0,17	0,16	0,21	0,6
Asteraceae	*Tragopogon pratensis*	0,24	0,11	0,15	0,08	0,58
Brassicaceae	*Cardamine pratensis*	0,12	0,24	0,2	0,24	0,57
Campanulaceae	*Campanula patula*	0,54	0,63	0,55	0,61	0,59
Dipsacaceae	*Knautia arvensis*	0,15	0,33	0,33	0,41	0,44
Fabaceae	*Lathyrus pratensis*	0,38	0,52	0,52	0,59	0,31
Fabaceae	*Lotus corniculatus*	0,24	0,31	0,29	0,36	0,49
Fabaceae	*Medicago varia*	0,17	0,32	0,32	0,4	0,41
Fabaceae	*Onobrychis vicifolia*	0,33	0,44	0,4	0,47	0,37
Fabaceae	*Trifolium campestre*	0,16	0,25	0,26	0,33	0,47
Fabaceae	*Trifolium fragiferum*	0	0,23	0,23	0,29	0,5
Fabaceae	*Trifolium hybridum*	0,23	0	0,1	0,11	0,54
Fabaceae	*Trifolium pratense*	0,23	0,1	0	0,12	0,54
Fabaceae	*Trifolium repens*	0,29	0,11	0,12	0	0,6
Fabaceae	*Vicia cracca*	0,5	0,54	0,54	0,6	0
Geraniaceae	*Geranium pratense*	0,31	0,48	0,45	0,48	0,55
Lamiaceae	*Ajuga reptans*	0,66	0,7	0,66	0,7	0,68
Lamiaceae	*Glechoma hederacea*	0,31	0,41	0,42	0,48	0,39
Lamiaceae	*Prunella vulgaris*	0,32	0,53	0,53	0,59	0,53
Primulaceae	*Primula veris*	0,24	0,24	0,2	0,27	0,47
Ranunculaceae	*Ranunculus acris*	0,27	0,42	0,43	0,49	0,4
Ranunculaceae	*Ranunculus repens*	0,22	0,26	0,22	0,3	0,43
Rosaceae	*Sanguisorba officinalis*	0,51	0,6	0,59	0,65	0,55
Scrophulariaceae	*Veronica chamaedrys*	0,35	0,53	0,54	0,6	0,39

a) Amino acid %

		Geranium pratense	*Ajuga reptans*	*Glechoma hederacea*	*Prunella vulgaris*	*Primula veris*
Apiaceae	*Anthriscus sylvestris*	0,35	0,67	0,34	0,44	0,25
Apiaceae	*Daucus carota*	0,46	0,66	0,44	0,54	0,32
Apiaceae	*Heracleum sphondylium*	0,29	0,59	0,39	0,4	0,2
Apiaceae	*Pastinaca sativa*	0,36	0,65	0,13	0,37	0,25
Apiaceae	*Pimpinella major*	0,54	0,44	0,56	0,68	0,6
Asteraceae	*Centaurea jacea*	0,31	0,64	0,18	0,28	0,32
Asteraceae	*Cirsium oleraceum*	0,44	0,7	0,36	0,54	0,15
Asteraceae	*Crepis biennis*	0,67	0,16	0,71	0,7	0,74
Asteraceae	*Leontodon autumnalis*	0,41	0,67	0,41	0,52	0,27
Asteraceae	*Leontodon hispidus*	0,51	0,33	0,51	0,59	0,56
Asteraceae	*Taraxacum officinale*	0,4	0,67	0,42	0,51	0,3
Asteraceae	*Tragopogon pratensis*	0,45	0,71	0,42	0,49	0,31
Brassicaceae	*Cardamine pratensis*	0,29	0,64	0,36	0,43	0,21
Campanulaceae	*Campanula patula*	0,56	0,23	0,52	0,52	0,54
Dipsacaceae	*Knautia arvensis*	0,27	0,61	0,21	0,26	0,29
Fabaceae	*Lathyrus pratensis*	0,51	0,64	0,29	0,3	0,41
Fabaceae	*Lotus corniculatus*	0,41	0,51	0,35	0,45	0,27
Fabaceae	*Medicago varia*	0,29	0,64	0,22	0,34	0,31
Fabaceae	*Onobrychis vicifolia*	0,38	0,61	0,29	0,44	0,28
Fabaceae	*Trifolium campestre*	0,41	0,62	0,26	0,37	0,16
Fabaceae	*Trifolium fragiferum*	0,31	0,66	0,31	0,32	0,24
Fabaceae	*Trifolium hybridum*	0,48	0,7	0,41	0,53	0,24
Fabaceae	*Trifolium pratense*	0,45	0,66	0,42	0,53	0,2
Fabaceae	*Trifolium repens*	0,48	0,7	0,48	0,59	0,27
Fabaceae	*Vicia cracca*	0,55	0,68	0,39	0,53	0,47
Geraniaceae	*Geranium pratense*	0	0,63	0,33	0,38	0,45
Lamiaceae	*Ajuga reptans*	0,63	0	0,65	0,61	0,64
Lamiaceae	*Glechoma hederacea*	0,33	0,65	0	0,34	0,33
Lamiaceae	*Prunella vulgaris*	0,38	0,61	0,34	0	0,49
Primulaceae	*Primula veris*	0,45	0,64	0,33	0,49	0
Ranunculaceae	*Ranunculus acris*	0,38	0,6	0,17	0,27	0,35
Ranunculaceae	*Ranunculus repens*	0,45	0,63	0,23	0,45	0,13
Rosaceae	*Sanguisorba officinalis*	0,5	0,68	0,48	0,46	0,52
Scrophulariaceae	*Veronica chamaedrys*	0,43	0,67	0,19	0,28	0,42

a) Amino acid %

		Ranun-culus acris	*Ranun-culus repens*	*Sangui-sorba offici-nalis*	*Vero-nica chama-edrys*
Apiaceae	*Anthriscus sylvestris*	0,37	0,21	0,59	0,44
Apiaceae	*Daucus carota*	0,4	0,24	0,58	0,52
Apiaceae	*Heracleum sphondylium*	0,32	0,2	0,53	0,46
Apiaceae	*Pastinaca sativa*	0,19	0,14	0,5	0,24
Apiaceae	*Pimpinella major*	0,58	0,61	0,73	0,68
Asteraceae	*Centaurea jacea*	0,2	0,28	0,45	0,18
Asteraceae	*Cirsium oleraceum*	0,44	0,23	0,64	0,44
Asteraceae	*Crepis biennis*	0,69	0,73	0,76	0,7
Asteraceae	*Leontodon autumnalis*	0,48	0,28	0,66	0,55
Asteraceae	*Leontodon hispidus*	0,49	0,56	0,68	0,59
Asteraceae	*Taraxacum officinale*	0,48	0,29	0,66	0,55
Asteraceae	*Tragopogon pratensis*	0,43	0,29	0,62	0,52
Brassicaceae	*Cardamine pratensis*	0,33	0,21	0,57	0,42
Campanulaceae	*Campanula patula*	0,51	0,52	0,64	0,5
Dipsacaceae	*Knautia arvensis*	0,16	0,25	0,43	0,27
Fabaceae	*Lathyrus pratensis*	0,21	0,36	0,42	0,18
Fabaceae	*Lotus corniculatus*	0,33	0,23	0,53	0,47
Fabaceae	*Medicago varia*	0,18	0,22	0,46	0,27
Fabaceae	*Onobrychis vicifolia*	0,29	0,28	0,37	0,34
Fabaceae	*Trifolium campestre*	0,23	0,17	0,42	0,37
Fabaceae	*Trifolium fragiferum*	0,27	0,22	0,51	0,35
Fabaceae	*Trifolium hybridum*	0,42	0,26	0,6	0,53
Fabaceae	*Trifolium pratense*	0,43	0,22	0,59	0,54
Fabaceae	*Trifolium repens*	0,49	0,3	0,65	0,6
Fabaceae	*Vicia cracca*	0,4	0,43	0,55	0,39
Geraniaceae	*Geranium pratense*	0,38	0,45	0,5	0,43
Lamiaceae	*Ajuga reptans*	0,6	0,63	0,68	0,67
Lamiaceae	*Glechoma hederacea*	0,17	0,23	0,48	0,19
Lamiaceae	*Prunella vulgaris*	0,27	0,45	0,46	0,28
Primulaceae	*Primula veris*	0,35	0,13	0,52	0,42
Ranunculaceae	*Ranunculus acris*	0	0,26	0,35	0,19
Ranunculaceae	*Ranunculus repens*	0,26	0	0,49	0,34
Rosaceae	*Sanguisorba officinalis*	0,35	0,49	0	0,43
Scrophulariaceae	*Veronica chamaedrys*	0,19	0,34	0,43	0

b) Amino acid concentration

Chemical distance matrix between 34 plant species based on Bray-Curtis distances between either the proportions (%) or concentrations (conc) of amino acids and carbohydrates.

high chemical similarity

intermediate chemical similarity

low chemical similarity

		Anthriscus sylvestris	*Daucus carota*	*Heracleum sphondylium*	*Pastinaca sativa*	*Pimpinella major*
Apiaceae	*Anthriscus sylvestris*	0	0,48	0,33	0,11	0,37
Apiaceae	*Daucus carota*	0,48	0	0,24	0,42	0,54
Apiaceae	*Heracleum sphondylium*	0,33	0,24	0	0,3	0,44
Apiaceae	*Pastinaca sativa*	0,11	0,42	0,3	0	0,39
Apiaceae	*Pimpinella major*	0,37	0,54	0,44	0,39	0
Asteraceae	*Centaurea jacea*	0,15	0,5	0,36	0,18	0,34
Asteraceae	*Cirsium oleraceum*	0,42	0,24	0,18	0,36	0,52
Asteraceae	*Crepis biennis*	0,59	0,8	0,73	0,61	0,56
Asteraceae	*Leontodon autumnalis*	0,19	0,53	0,37	0,21	0,45
Asteraceae	*Leontodon hispidus*	0,42	0,74	0,64	0,47	0,45
Asteraceae	*Taraxacum officinale*	0,3	0,29	0,18	0,25	0,47
Asteraceae	*Tragopogon pratensis*	0,48	0,78	0,69	0,53	0,57
Brassicaceae	*Cardamine pratensis*	0,34	0,21	0,13	0,29	0,45
Campanulaceae	*Campanula patula*	0,38	0,64	0,54	0,39	0,31
Dipsacaceae	*Knautia arvensis*	0,16	0,45	0,28	0,17	0,33
Fabaceae	*Lathyrus pratensis*	0,5	0,21	0,27	0,44	0,56
Fabaceae	*Lotus corniculatus*	0,35	0,69	0,57	0,4	0,38
Fabaceae	*Medicago varia*	0,36	0,2	0,16	0,3	0,45
Fabaceae	*Onobrychis vicifolia*	0,19	0,47	0,33	0,2	0,41
Fabaceae	*Trifolium campestre*	0,46	0,76	0,67	0,51	0,52
Fabaceae	*Trifolium fragiferum*	0,18	0,49	0,32	0,21	0,36
Fabaceae	*Trifolium hybridum*	0,45	0,76	0,66	0,5	0,56
Fabaceae	*Trifolium pratense*	0,45	0,76	0,67	0,5	0,6
Fabaceae	*Trifolium repens*	0,21	0,53	0,39	0,23	0,49
Fabaceae	*Vicia cracca*	0,45	0,19	0,25	0,39	0,55
Geraniaceae	*Geranium pratense*	0,57	0,35	0,36	0,52	0,6
Lamiaceae	*Ajuga reptans*	0,41	0,55	0,46	0,4	0,25
Lamiaceae	*Glechoma hederacea*	0,56	0,27	0,34	0,51	0,62
Lamiaceae	*Prunella vulgaris*	0,7	0,46	0,53	0,66	0,74
Primulaceae	*Primula veris*	0,42	0,74	0,64	0,47	0,58
Ranunculaceae	*Ranunculus acris*	0,29	0,65	0,54	0,33	0,39
Ranunculaceae	*Ranunculus repens*	0,23	0,62	0,49	0,29	0,44
Rosaceae	*Sanguisorba officinalis*	0,52	0,77	0,69	0,56	0,54
Scrophulariaceae	*Veronica chamaedrys*	0,49	0,25	0,3	0,43	0,57

b) Amino acid concentration

		Centaurea jacea	*Cirsium oleraceum*	*Crepis biennis*	*Leontodon autumnalis*	*Leontodon hispidus*
Apiaceae	*Anthriscus sylvestris*	0,15	0,42	0,59	0,19	0,42
Apiaceae	*Daucus carota*	0,5	0,24	0,8	0,53	0,74
Apiaceae	*Heracleum sphondylium*	0,36	0,18	0,73	0,37	0,64
Apiaceae	*Pastinaca sativa*	0,18	0,36	0,61	0,21	0,47
Apiaceae	*Pimpinella major*	0,34	0,52	0,56	0,45	0,45
Asteraceae	*Centaurea jacea*	0	0,43	0,57	0,21	0,41
Asteraceae	*Cirsium oleraceum*	0,43	0	0,77	0,44	0,69
Asteraceae	*Crepis biennis*	0,57	0,77	0	0,62	0,26
Asteraceae	*Leontodon autumnalis*	0,21	0,44	0,62	0	0,44
Asteraceae	*Leontodon hispidus*	0,41	0,69	0,26	0,44	0
Asteraceae	*Taraxacum officinale*	0,35	0,17	0,72	0,33	0,61
Asteraceae	*Tragopogon pratensis*	0,45	0,74	0,52	0,44	0,39
Brassicaceae	*Cardamine pratensis*	0,37	0,17	0,74	0,39	0,65
Campanulaceae	*Campanula patula*	0,34	0,61	0,36	0,42	0,23
Dipsacaceae	*Knautia arvensis*	0,11	0,36	0,6	0,22	0,46
Fabaceae	*Lathyrus pratensis*	0,51	0,24	0,8	0,54	0,75
Fabaceae	*Lotus corniculatus*	0,34	0,64	0,43	0,34	0,23
Fabaceae	*Medicago varia*	0,38	0,21	0,73	0,41	0,66
Fabaceae	*Onobrychis vicifolia*	0,16	0,42	0,62	0,2	0,47
Fabaceae	*Trifolium campestre*	0,43	0,72	0,47	0,43	0,35
Fabaceae	*Trifolium fragiferum*	0,13	0,41	0,6	0,17	0,45
Fabaceae	*Trifolium hybridum*	0,42	0,72	0,51	0,42	0,38
Fabaceae	*Trifolium pratense*	0,43	0,72	0,52	0,42	0,4
Fabaceae	*Trifolium repens*	0,3	0,47	0,63	0,22	0,45
Fabaceae	*Vicia cracca*	0,43	0,23	0,78	0,49	0,7
Geraniaceae	*Geranium pratense*	0,57	0,34	0,83	0,6	0,78
Lamiaceae	*Ajuga reptans*	0,41	0,53	0,53	0,49	0,42
Lamiaceae	*Glechoma hederacea*	0,59	0,24	0,84	0,59	0,78
Lamiaceae	*Prunella vulgaris*	0,71	0,43	0,89	0,72	0,86
Primulaceae	*Primula veris*	0,4	0,7	0,51	0,39	0,38
Ranunculaceae	*Ranunculus acris*	0,25	0,6	0,49	0,32	0,28
Ranunculaceae	*Ranunculus repens*	0,24	0,57	0,55	0,25	0,34
Rosaceae	*Sanguisorba officinalis*	0,48	0,75	0,38	0,54	0,29
Scrophulariaceae	*Veronica chamaedrys*	0,51	0,29	0,8	0,53	0,74

b) Amino acid concentration

		Taraxacum officinale	*Tragopogon pratensis*	*Cardamine pratensis*	*Campanula patula*	*Knautia arvensis*
Apiaceae	*Anthriscus sylvestris*	0,3	0,48	0,34	0,38	0,16
Apiaceae	*Daucus carota*	0,29	0,78	0,21	0,64	0,45
Apiaceae	*Heracleum sphondylium*	0,18	0,69	0,13	0,54	0,28
Apiaceae	*Pastinaca sativa*	0,25	0,53	0,29	0,39	0,17
Apiaceae	*Pimpinella major*	0,47	0,57	0,45	0,31	0,33
Asteraceae	*Centaurea jacea*	0,35	0,45	0,37	0,34	0,11
Asteraceae	*Cirsium oleraceum*	0,17	0,74	0,17	0,61	0,36
Asteraceae	*Crepis biennis*	0,72	0,52	0,74	0,36	0,6
Asteraceae	*Leontodon autumnalis*	0,33	0,44	0,39	0,42	0,22
Asteraceae	*Leontodon hispidus*	0,61	0,39	0,65	0,23	0,46
Asteraceae	*Taraxacum officinale*	0	0,67	0,15	0,54	0,28
Asteraceae	*Tragopogon pratensis*	0,67	0	0,7	0,52	0,52
Brassicaceae	*Cardamine pratensis*	0,15	0,7	0	0,54	0,29
Campanulaceae	*Campanula patula*	0,54	0,52	0,54	0	0,38
Dipsacaceae	*Knautia arvensis*	0,28	0,52	0,29	0,38	0
Fabaceae	*Lathyrus pratensis*	0,34	0,78	0,25	0,63	0,45
Fabaceae	*Lotus corniculatus*	0,57	0,32	0,59	0,29	0,37
Fabaceae	*Medicago varia*	0,19	0,71	0,1	0,53	0,3
Fabaceae	*Onobrychis vicifolia*	0,36	0,5	0,34	0,41	0,14
Fabaceae	*Trifolium campestre*	0,66	0,15	0,68	0,47	0,49
Fabaceae	*Trifolium fragiferum*	0,3	0,48	0,33	0,38	0,09
Fabaceae	*Trifolium hybridum*	0,65	0,08	0,67	0,51	0,48
Fabaceae	*Trifolium pratense*	0,65	0,09	0,68	0,53	0,49
Fabaceae	*Trifolium repens*	0,37	0,43	0,39	0,45	0,27
Fabaceae	*Vicia cracca*	0,28	0,74	0,23	0,6	0,4
Geraniaceae	*Geranium pratense*	0,41	0,81	0,36	0,69	0,52
Lamiaceae	*Ajuga reptans*	0,49	0,65	0,47	0,24	0,41
Lamiaceae	*Glechoma hederacea*	0,36	0,82	0,31	0,7	0,53
Lamiaceae	*Prunella vulgaris*	0,52	0,88	0,5	0,8	0,68
Primulaceae	*Primula veris*	0,63	0,19	0,66	0,5	0,46
Ranunculaceae	*Ranunculus acris*	0,52	0,32	0,55	0,33	0,31
Ranunculaceae	*Ranunculus repens*	0,48	0,32	0,51	0,38	0,28
Rosaceae	*Sanguisorba officinalis*	0,69	0,39	0,7	0,4	0,52
Scrophulariaceae	*Veronica chamaedrys*	0,32	0,78	0,24	0,64	0,45

b) Amino acid concentration

		Lathyrus pratensis	*Lotus corniculatus*	*Medicago varia*	*Onobrychis vicifolia*	*Trifolium campestre*
Apiaceae	*Anthriscus sylvestris*	0,5	0,35	0,36	0,19	0,46
Apiaceae	*Daucus carota*	0,21	0,69	0,2	0,47	0,76
Apiaceae	*Heracleum sphondylium*	0,27	0,57	0,16	0,33	0,67
Apiaceae	*Pastinaca sativa*	0,44	0,4	0,3	0,2	0,51
Apiaceae	*Pimpinella major*	0,56	0,38	0,45	0,41	0,52
Asteraceae	*Centaurea jacea*	0,51	0,34	0,38	0,16	0,43
Asteraceae	*Cirsium oleraceum*	0,24	0,64	0,21	0,42	0,72
Asteraceae	*Crepis biennis*	0,8	0,43	0,73	0,62	0,47
Asteraceae	*Leontodon autumnalis*	0,54	0,34	0,41	0,2	0,43
Asteraceae	*Leontodon hispidus*	0,75	0,23	0,66	0,47	0,35
Asteraceae	*Taraxacum officinale*	0,34	0,57	0,19	0,36	0,66
Asteraceae	*Tragopogon pratensis*	0,78	0,32	0,71	0,5	0,15
Brassicaceae	*Cardamine pratensis*	0,25	0,59	0,1	0,34	0,68
Campanulaceae	*Campanula patula*	0,63	0,29	0,53	0,41	0,47
Dipsacaceae	*Knautia arvensis*	0,45	0,37	0,3	0,14	0,49
Fabaceae	*Lathyrus pratensis*	0	0,7	0,21	0,49	0,77
Fabaceae	*Lotus corniculatus*	0,7	0	0,6	0,35	0,25
Fabaceae	*Medicago varia*	0,21	0,6	0	0,34	0,69
Fabaceae	*Onobrychis vicifolia*	0,49	0,35	0,34	0	0,45
Fabaceae	*Trifolium campestre*	0,77	0,25	0,69	0,45	0
Fabaceae	*Trifolium fragiferum*	0,49	0,34	0,34	0,17	0,46
Fabaceae	*Trifolium hybridum*	0,76	0,3	0,68	0,48	0,12
Fabaceae	*Trifolium pratense*	0,76	0,3	0,68	0,46	0,13
Fabaceae	*Trifolium repens*	0,55	0,31	0,41	0,27	0,41
Fabaceae	*Vicia cracca*	0,21	0,65	0,19	0,4	0,72
Geraniaceae	*Geranium pratense*	0,29	0,74	0,37	0,57	0,8
Lamiaceae	*Ajuga reptans*	0,55	0,39	0,48	0,44	0,59
Lamiaceae	*Glechoma hederacea*	0,25	0,75	0,32	0,58	0,81
Lamiaceae	*Prunella vulgaris*	0,41	0,84	0,52	0,72	0,88
Primulaceae	*Primula veris*	0,74	0,27	0,66	0,43	0,11
Ranunculaceae	*Ranunculus acris*	0,66	0,18	0,56	0,29	0,24
Ranunculaceae	*Ranunculus repens*	0,63	0,18	0,52	0,26	0,28
Rosaceae	*Sanguisorba officinalis*	0,77	0,31	0,7	0,49	0,28
Scrophulariaceae	*Veronica chamaedrys*	0,22	0,7	0,24	0,5	0,77

b) Amino acid concentration

		Trifolium fragiferum	*Trifolium hybridum*	*Trifolium pratense*	*Trifolium repens*	*Vicia cracca*
Apiaceae	*Anthriscus sylvestris*	0,18	0,45	0,45	0,21	0,45
Apiaceae	*Daucus carota*	0,49	0,76	0,76	0,53	0,19
Apiaceae	*Heracleum sphondylium*	0,32	0,66	0,67	0,39	0,25
Apiaceae	*Pastinaca sativa*	0,21	0,5	0,5	0,23	0,39
Apiaceae	*Pimpinella major*	0,36	0,56	0,6	0,49	0,55
Asteraceae	*Centaurea jacea*	0,13	0,42	0,43	0,3	0,43
Asteraceae	*Cirsium oleraceum*	0,41	0,72	0,72	0,47	0,23
Asteraceae	*Crepis biennis*	0,6	0,51	0,52	0,63	0,78
Asteraceae	*Leontodon autumnalis*	0,17	0,42	0,42	0,22	0,49
Asteraceae	*Leontodon hispidus*	0,45	0,38	0,4	0,45	0,7
Asteraceae	*Taraxacum officinale*	0,3	0,65	0,65	0,37	0,28
Asteraceae	*Tragopogon pratensis*	0,48	0,08	0,09	0,43	0,74
Brassicaceae	*Cardamine pratensis*	0,33	0,67	0,68	0,39	0,23
Campanulaceae	*Campanula patula*	0,38	0,51	0,53	0,45	0,6
Dipsacaceae	*Knautia arvensis*	0,09	0,48	0,49	0,27	0,4
Fabaceae	*Lathyrus pratensis*	0,49	0,76	0,76	0,55	0,21
Fabaceae	*Lotus corniculatus*	0,34	0,3	0,3	0,31	0,65
Fabaceae	*Medicago varia*	0,34	0,68	0,68	0,41	0,19
Fabaceae	*Onobrychis vicifolia*	0,17	0,48	0,46	0,27	0,4
Fabaceae	*Trifolium campestre*	0,46	0,12	0,13	0,41	0,72
Fabaceae	*Trifolium fragiferum*	0	0,45	0,45	0,28	0,45
Fabaceae	*Trifolium hybridum*	0,45	0	0,05	0,39	0,71
Fabaceae	*Trifolium pratense*	0,45	0,05	0	0,4	0,72
Fabaceae	*Trifolium repens*	0,28	0,39	0,4	0	0,49
Fabaceae	*Vicia cracca*	0,45	0,71	0,72	0,49	0
Geraniaceae	*Geranium pratense*	0,55	0,8	0,8	0,64	0,41
Lamiaceae	*Ajuga reptans*	0,45	0,63	0,64	0,5	0,55
Lamiaceae	*Glechoma hederacea*	0,56	0,81	0,81	0,61	0,35
Lamiaceae	*Prunella vulgaris*	0,7	0,87	0,87	0,74	0,53
Primulaceae	*Primula veris*	0,43	0,15	0,13	0,36	0,69
Ranunculaceae	*Ranunculus acris*	0,3	0,31	0,31	0,31	0,6
Ranunculaceae	*Ranunculus repens*	0,26	0,3	0,28	0,19	0,57
Rosaceae	*Sanguisorba officinalis*	0,52	0,39	0,39	0,51	0,73
Scrophulariaceae	*Veronica chamaedrys*	0,49	0,76	0,77	0,53	0,29

b) Amino acid concentration

		Geranium pratense	*Ajuga reptans*	*Glechoma hederacea*	*Prunella vulgaris*	*Primula veris*
Apiaceae	*Anthriscus sylvestris*	0,57	0,41	0,56	0,7	0,42
Apiaceae	*Daucus carota*	0,35	0,55	0,27	0,46	0,74
Apiaceae	*Heracleum sphondylium*	0,36	0,46	0,34	0,53	0,64
Apiaceae	*Pastinaca sativa*	0,52	0,4	0,51	0,66	0,47
Apiaceae	*Pimpinella major*	0,6	0,25	0,62	0,74	0,58
Asteraceae	*Centaurea jacea*	0,57	0,41	0,59	0,71	0,4
Asteraceae	*Cirsium oleraceum*	0,34	0,53	0,24	0,43	0,7
Asteraceae	*Crepis biennis*	0,83	0,53	0,84	0,89	0,51
Asteraceae	*Leontodon autumnalis*	0,6	0,49	0,59	0,72	0,39
Asteraceae	*Leontodon hispidus*	0,78	0,42	0,78	0,86	0,38
Asteraceae	*Taraxacum officinale*	0,41	0,49	0,36	0,52	0,63
Asteraceae	*Tragopogon pratensis*	0,81	0,65	0,82	0,88	0,19
Brassicaceae	*Cardamine pratensis*	0,36	0,47	0,31	0,5	0,66
Campanulaceae	*Campanula patula*	0,69	0,24	0,7	0,8	0,5
Dipsacaceae	*Knautia arvensis*	0,52	0,41	0,53	0,68	0,46
Fabaceae	*Lathyrus pratensis*	0,29	0,55	0,25	0,41	0,74
Fabaceae	*Lotus corniculatus*	0,74	0,39	0,75	0,84	0,27
Fabaceae	*Medicago varia*	0,37	0,48	0,32	0,52	0,66
Fabaceae	*Onobrychis vicifolia*	0,57	0,44	0,58	0,72	0,43
Fabaceae	*Trifolium campestre*	0,8	0,59	0,81	0,88	0,11
Fabaceae	*Trifolium fragiferum*	0,55	0,45	0,56	0,7	0,43
Fabaceae	*Trifolium hybridum*	0,8	0,63	0,81	0,87	0,15
Fabaceae	*Trifolium pratense*	0,8	0,64	0,81	0,87	0,13
Fabaceae	*Trifolium repens*	0,64	0,5	0,61	0,74	0,36
Fabaceae	*Vicia cracca*	0,41	0,55	0,35	0,53	0,69
Geraniaceae	*Geranium pratense*	0	0,58	0,28	0,29	0,79
Lamiaceae	*Ajuga reptans*	0,58	0	0,6	0,73	0,61
Lamiaceae	*Glechoma hederacea*	0,28	0,6	0	0,25	0,79
Lamiaceae	*Prunella vulgaris*	0,29	0,73	0,25	0	0,87
Primulaceae	*Primula veris*	0,79	0,61	0,79	0,87	0
Ranunculaceae	*Ranunculus acris*	0,71	0,45	0,72	0,81	0,28
Ranunculaceae	*Ranunculus repens*	0,69	0,48	0,7	0,8	0,25
Rosaceae	*Sanguisorba officinalis*	0,81	0,56	0,82	0,88	0,34
Scrophulariaceae	*Veronica chamaedrys*	0,36	0,55	0,2	0,38	0,75

b) Amino acid concentration

		Ranun-culus acris	*Ranun-culus repens*	*Sangui-sorba offici-nalis*	*Vero-nica chama-edrys*
Apiaceae	*Anthriscus sylvestris*	0,29	0,23	0,52	0,49
Apiaceae	*Daucus carota*	0,65	0,62	0,77	0,25
Apiaceae	*Heracleum sphondylium*	0,54	0,49	0,69	0,3
Apiaceae	*Pastinaca sativa*	0,33	0,29	0,56	0,43
Apiaceae	*Pimpinella major*	0,39	0,44	0,54	0,57
Asteraceae	*Centaurea jacea*	0,25	0,24	0,48	0,51
Asteraceae	*Cirsium oleraceum*	0,6	0,57	0,75	0,29
Asteraceae	*Crepis biennis*	0,49	0,55	0,38	0,8
Asteraceae	*Leontodon autumnalis*	0,32	0,25	0,54	0,53
Asteraceae	*Leontodon hispidus*	0,28	0,34	0,29	0,74
Asteraceae	*Taraxacum officinale*	0,52	0,48	0,69	0,32
Asteraceae	*Tragopogon pratensis*	0,32	0,32	0,39	0,78
Brassicaceae	*Cardamine pratensis*	0,55	0,51	0,7	0,24
Campanulaceae	*Campanula patula*	0,33	0,38	0,4	0,64
Dipsacaceae	*Knautia arvensis*	0,31	0,28	0,52	0,45
Fabaceae	*Lathyrus pratensis*	0,66	0,63	0,77	0,22
Fabaceae	*Lotus corniculatus*	0,18	0,18	0,31	0,7
Fabaceae	*Medicago varia*	0,56	0,52	0,7	0,24
Fabaceae	*Onobrychis vicifolia*	0,29	0,26	0,49	0,5
Fabaceae	*Trifolium campestre*	0,24	0,28	0,28	0,77
Fabaceae	*Trifolium fragiferum*	0,3	0,26	0,52	0,49
Fabaceae	*Trifolium hybridum*	0,31	0,3	0,39	0,76
Fabaceae	*Trifolium pratense*	0,31	0,28	0,39	0,77
Fabaceae	*Trifolium repens*	0,31	0,19	0,51	0,53
Fabaceae	*Vicia cracca*	0,6	0,57	0,73	0,29
Geraniaceae	*Geranium pratense*	0,71	0,69	0,81	0,36
Lamiaceae	*Ajuga reptans*	0,45	0,48	0,56	0,55
Lamiaceae	*Glechoma hederacea*	0,72	0,7	0,82	0,2
Lamiaceae	*Prunella vulgaris*	0,81	0,8	0,88	0,38
Primulaceae	*Primula veris*	0,28	0,25	0,34	0,75
Ranunculaceae	*Ranunculus acris*	0	0,14	0,3	0,66
Ranunculaceae	*Ranunculus repens*	0,14	0	0,38	0,63
Rosaceae	*Sanguisorba officinalis*	0,3	0,38	0	0,77
Scrophulariaceae	*Veronica chamaedrys*	0,66	0,63	0,77	0

c) Carbohydrates %

Chemical distance matrix between 34 plant species based on Bray-Curtis distances between either the proportions (%) or concentrations (conc) of amino acids and carbohydrates.

high chemical similarity
intermediate chemical similarity
low chemical similarity

		Anthriscus sylvestris	*Daucus carota*	*Heracleum sphondylium*	*Pastinaca sativa*	*Pimpinella major*
Apiaceae	*Anthriscus sylvestris*	0	0,39	0,27	0,24	0,19
Apiaceae	*Daucus carota*	0,39	0	0,12	0,2	0,2
Apiaceae	*Heracleum sphondylium*	0,27	0,12	0	0,11	0,08
Apiaceae	*Pastinaca sativa*	0,24	0,2	0,11	0	0,05
Apiaceae	*Pimpinella major*	0,19	0,2	0,08	0,05	0
Asteraceae	*Centaurea jacea*	0,27	0,12	0,01	0,1	0,08
Asteraceae	*Cirsium oleraceum*	0,32	0,31	0,28	0,17	0,22
Asteraceae	*Crepis biennis*	0,26	0,13	0,02	0,12	0,07
Asteraceae	*Leontodon autumnalis*	0,31	0,08	0,04	0,13	0,12
Asteraceae	*Leontodon hispidus*	0,41	0,02	0,14	0,22	0,22
Asteraceae	*Taraxacum officinale*	0,35	0,2	0,17	0,11	0,16
Asteraceae	*Tragopogon pratensis*	0,27	0,12	0,01	0,1	0,08
Brassicaceae	*Cardamine pratensis*	0,28	0,11	0,03	0,14	0,09
Campanulaceae	*Campanula patula*	0,25	0,15	0,07	0,05	0,06
Dipsacaceae	*Knautia arvensis*	0,3	0,09	0,03	0,14	0,11
Fabaceae	*Lathyrus pratensis*	0,1	0,31	0,19	0,14	0,11
Fabaceae	*Lotus corniculatus*	0,28	0,25	0,2	0,09	0,14
Fabaceae	*Medicago varia*	0,36	0,37	0,34	0,23	0,28
Fabaceae	*Onobrychis vicifolia*	0,57	0,66	0,63	0,52	0,57
Fabaceae	*Trifolium campestre*	0,24	0,24	0,15	0,04	0,09
Fabaceae	*Trifolium fragiferum*	0,32	0,39	0,36	0,25	0,3
Fabaceae	*Trifolium hybridum*	0,38	0,36	0,33	0,22	0,27
Fabaceae	*Trifolium pratense*	0,29	0,31	0,27	0,16	0,21
Fabaceae	*Trifolium repens*	0,34	0,34	0,31	0,2	0,25
Fabaceae	*Vicia cracca*	0,14	0,39	0,27	0,19	0,19
Geraniaceae	*Geranium pratense*	0,4	0,44	0,41	0,3	0,35
Lamiaceae	*Ajuga reptans*	0,57	0,66	0,63	0,52	0,57
Lamiaceae	*Glechoma hederacea*	0,42	0,47	0,44	0,33	0,38
Lamiaceae	*Prunella vulgaris*	0,37	0,44	0,41	0,3	0,35
Primulaceae	*Primula veris*	0,35	0,3	0,27	0,16	0,21
Ranunculaceae	*Ranunculus acris*	0,84	0,93	0,9	0,79	0,84
Ranunculaceae	*Ranunculus repens*	0,52	0,57	0,54	0,43	0,48
Rosaceae	*Sanguisorba officinalis*	0,91	1	0,97	0,86	0,91
Scrophulariaceae	*Veronica chamaedrys*	0,44	0,05	0,17	0,25	0,25

c) Carbohydrates %

		Centaurea jacea	*Cirsium oleraceum*	*Crepis biennis*	*Leontodon autumnalis*	*Leontodon hispidus*
Apiaceae	*Anthriscus sylvestris*	0,27	0,32	0,26	0,31	0,41
Apiaceae	*Daucus carota*	0,12	0,31	0,13	0,08	0,02
Apiaceae	*Heracleum sphondylium*	0,01	0,28	0,02	0,04	0,14
Apiaceae	*Pastinaca sativa*	0,1	0,17	0,12	0,13	0,22
Apiaceae	*Pimpinella major*	0,08	0,22	0,07	0,12	0,22
Asteraceae	*Centaurea jacea*	0	0,27	0,03	0,04	0,14
Asteraceae	*Cirsium oleraceum*	0,27	0	0,29	0,3	0,31
Asteraceae	*Crepis biennis*	0,03	0,29	0	0,05	0,15
Asteraceae	*Leontodon autumnalis*	0,04	0,3	0,05	0	0,1
Asteraceae	*Leontodon hispidus*	0,14	0,31	0,15	0,1	0
Asteraceae	*Taraxacum officinale*	0,16	0,14	0,18	0,19	0,2
Asteraceae	*Tragopogon pratensis*	0	0,27	0,03	0,04	0,14
Brassicaceae	*Cardamine pratensis*	0,04	0,31	0,02	0,04	0,13
Campanulaceae	*Campanula patula*	0,06	0,21	0,08	0,09	0,17
Dipsacaceae	*Knautia arvensis*	0,04	0,31	0,04	0,02	0,11
Fabaceae	*Lathyrus pratensis*	0,19	0,22	0,18	0,23	0,33
Fabaceae	*Lotus corniculatus*	0,19	0,08	0,21	0,22	0,27
Fabaceae	*Medicago varia*	0,33	0,06	0,35	0,36	0,37
Fabaceae	*Onobrychis vicifolia*	0,62	0,35	0,64	0,65	0,66
Fabaceae	*Trifolium campestre*	0,14	0,14	0,16	0,17	0,26
Fabaceae	*Trifolium fragiferum*	0,35	0,08	0,37	0,38	0,39
Fabaceae	*Trifolium hybridum*	0,32	0,06	0,34	0,35	0,36
Fabaceae	*Trifolium pratense*	0,26	0,03	0,28	0,29	0,33
Fabaceae	*Trifolium repens*	0,3	0,03	0,32	0,33	0,34
Fabaceae	*Vicia cracca*	0,27	0,17	0,26	0,31	0,41
Geraniaceae	*Geranium pratense*	0,4	0,13	0,42	0,43	0,44
Lamiaceae	*Ajuga reptans*	0,62	0,35	0,64	0,65	0,66
Lamiaceae	*Glechoma hederacea*	0,43	0,16	0,45	0,46	0,47
Lamiaceae	*Prunella vulgaris*	0,4	0,13	0,42	0,43	0,44
Primulaceae	*Primula veris*	0,26	0,03	0,28	0,29	0,3
Ranunculaceae	*Ranunculus acris*	0,89	0,62	0,91	0,92	0,93
Ranunculaceae	*Ranunculus repens*	0,53	0,26	0,55	0,56	0,57
Rosaceae	*Sanguisorba officinalis*	0,96	0,69	0,98	0,99	1
Scrophulariaceae	*Veronica chamaedrys*	0,17	0,34	0,18	0,13	0,03

c) Carbohydrates %

		Taraxacum officinale	*Tragopogon pratensis*	*Cardamine pratensis*	*Campanula patula*	*Knautia arvensis*
Apiaceae	*Anthriscus sylvestris*	0,35	0,27	0,28	0,25	0,3
Apiaceae	*Daucus carota*	0,2	0,12	0,11	0,15	0,09
Apiaceae	*Heracleum sphondylium*	0,17	0,01	0,03	0,07	0,03
Apiaceae	*Pastinaca sativa*	0,11	0,1	0,14	0,05	0,14
Apiaceae	*Pimpinella major*	0,16	0,08	0,09	0,06	0,11
Asteraceae	*Centaurea jacea*	0,16	0	0,04	0,06	0,04
Asteraceae	*Cirsium oleraceum*	0,14	0,27	0,31	0,21	0,31
Asteraceae	*Crepis biennis*	0,18	0,03	0,02	0,08	0,04
Asteraceae	*Leontodon autumnalis*	0,19	0,04	0,04	0,09	0,02
Asteraceae	*Leontodon hispidus*	0,2	0,14	0,13	0,17	0,11
Asteraceae	*Taraxacum officinale*	0	0,16	0,2	0,1	0,2
Asteraceae	*Tragopogon pratensis*	0,16	0	0,04	0,06	0,04
Brassicaceae	*Cardamine pratensis*	0,2	0,04	0	0,1	0,02
Campanulaceae	*Campanula patula*	0,1	0,06	0,1	0	0,1
Dipsacaceae	*Knautia arvensis*	0,2	0,04	0,02	0,1	0
Fabaceae	*Lathyrus pratensis*	0,25	0,19	0,2	0,16	0,22
Fabaceae	*Lotus corniculatus*	0,1	0,19	0,23	0,13	0,23
Fabaceae	*Medicago varia*	0,17	0,33	0,37	0,27	0,37
Fabaceae	*Onobrychis vicifolia*	0,46	0,62	0,66	0,56	0,66
Fabaceae	*Trifolium campestre*	0,12	0,14	0,18	0,09	0,18
Fabaceae	*Trifolium fragiferum*	0,22	0,35	0,39	0,29	0,39
Fabaceae	*Trifolium hybridum*	0,16	0,32	0,36	0,26	0,36
Fabaceae	*Trifolium pratense*	0,16	0,26	0,3	0,2	0,3
Fabaceae	*Trifolium repens*	0,15	0,3	0,34	0,24	0,34
Fabaceae	*Vicia cracca*	0,24	0,27	0,28	0,24	0,3
Geraniaceae	*Geranium pratense*	0,24	0,4	0,44	0,34	0,44
Lamiaceae	*Ajuga reptans*	0,46	0,62	0,66	0,56	0,66
Lamiaceae	*Glechoma hederacea*	0,27	0,43	0,47	0,37	0,47
Lamiaceae	*Prunella vulgaris*	0,24	0,4	0,44	0,34	0,44
Primulaceae	*Primula veris*	0,1	0,26	0,3	0,2	0,3
Ranunculaceae	*Ranunculus acris*	0,73	0,89	0,93	0,83	0,93
Ranunculaceae	*Ranunculus repens*	0,37	0,53	0,57	0,47	0,57
Rosaceae	*Sanguisorba officinalis*	0,8	0,96	1	0,9	1
Scrophulariaceae	*Veronica chamaedrys*	0,2	0,17	0,16	0,2	0,14

c) Carbohydrates %

		Lathyrus pratensis	*Lotus corniculatus*	*Medicago varia*	*Onobrychis vicifolia*	*Trifolium campestre*
Apiaceae	*Anthriscus sylvestris*	0,1	0,28	0,36	0,57	0,24
Apiaceae	*Daucus carota*	0,31	0,25	0,37	0,66	0,24
Apiaceae	*Heracleum sphondylium*	0,19	0,2	0,34	0,63	0,15
Apiaceae	*Pastinaca sativa*	0,14	0,09	0,23	0,52	0,04
Apiaceae	*Pimpinella major*	0,11	0,14	0,28	0,57	0,09
Asteraceae	*Centaurea jacea*	0,19	0,19	0,33	0,62	0,14
Asteraceae	*Cirsium oleraceum*	0,22	0,08	0,06	0,35	0,14
Asteraceae	*Crepis biennis*	0,18	0,21	0,35	0,64	0,16
Asteraceae	*Leontodon autumnalis*	0,23	0,22	0,36	0,65	0,17
Asteraceae	*Leontodon hispidus*	0,33	0,27	0,37	0,66	0,26
Asteraceae	*Taraxacum officinale*	0,25	0,1	0,17	0,46	0,12
Asteraceae	*Tragopogon pratensis*	0,19	0,19	0,33	0,62	0,14
Brassicaceae	*Cardamine pratensis*	0,2	0,23	0,37	0,66	0,18
Campanulaceae	*Campanula patula*	0,16	0,13	0,27	0,56	0,09
Dipsacaceae	*Knautia arvensis*	0,22	0,23	0,37	0,66	0,18
Fabaceae	*Lathyrus pratensis*	0	0,18	0,26	0,55	0,14
Fabaceae	*Lotus corniculatus*	0,18	0	0,14	0,43	0,06
Fabaceae	*Medicago varia*	0,26	0,14	0	0,29	0,2
Fabaceae	*Onobrychis vicifolia*	0,55	0,43	0,29	0	0,49
Fabaceae	*Trifolium campestre*	0,14	0,06	0,2	0,49	0
Fabaceae	*Trifolium fragiferum*	0,28	0,16	0,06	0,27	0,22
Fabaceae	*Trifolium hybridum*	0,28	0,13	0,03	0,3	0,19
Fabaceae	*Trifolium pratense*	0,19	0,07	0,07	0,36	0,13
Fabaceae	*Trifolium repens*	0,24	0,11	0,04	0,33	0,16
Fabaceae	*Vicia cracca*	0,12	0,14	0,21	0,42	0,16
Geraniaceae	*Geranium pratense*	0,33	0,21	0,07	0,22	0,27
Lamiaceae	*Ajuga reptans*	0,55	0,43	0,29	0	0,49
Lamiaceae	*Glechoma hederacea*	0,36	0,24	0,1	0,2	0,29
Lamiaceae	*Prunella vulgaris*	0,33	0,21	0,07	0,22	0,27
Primulaceae	*Primula veris*	0,25	0,07	0,06	0,35	0,13
Ranunculaceae	*Ranunculus acris*	0,82	0,7	0,56	0,27	0,76
Ranunculaceae	*Ranunculus repens*	0,46	0,34	0,2	0,09	0,4
Rosaceae	*Sanguisorba officinalis*	0,89	0,77	0,63	0,34	0,83
Scrophulariaceae	*Veronica chamaedrys*	0,36	0,3	0,37	0,66	0,29

c) Carbohydrates %

		Trifolium fragiferum	*Trifolium hybridum*	*Trifolium pratense*	*Trifolium repens*	*Vicia cracca*
Apiaceae	*Anthriscus sylvestris*	0,32	0,38	0,29	0,34	0,14
Apiaceae	*Daucus carota*	0,39	0,36	0,31	0,34	0,39
Apiaceae	*Heracleum sphondylium*	0,36	0,33	0,27	0,31	0,27
Apiaceae	*Pastinaca sativa*	0,25	0,22	0,16	0,2	0,19
Apiaceae	*Pimpinella major*	0,3	0,27	0,21	0,25	0,19
Asteraceae	*Centaurea jacea*	0,35	0,32	0,26	0,3	0,27
Asteraceae	*Cirsium oleraceum*	0,08	0,06	0,03	0,03	0,17
Asteraceae	*Crepis biennis*	0,37	0,34	0,28	0,32	0,26
Asteraceae	*Leontodon autumnalis*	0,38	0,35	0,29	0,33	0,31
Asteraceae	*Leontodon hispidus*	0,39	0,36	0,33	0,34	0,41
Asteraceae	*Taraxacum officinale*	0,22	0,16	0,16	0,15	0,24
Asteraceae	*Tragopogon pratensis*	0,35	0,32	0,26	0,3	0,27
Brassicaceae	*Cardamine pratensis*	0,39	0,36	0,3	0,34	0,28
Campanulaceae	*Campanula patula*	0,29	0,26	0,2	0,24	0,24
Dipsacaceae	*Knautia arvensis*	0,39	0,36	0,3	0,34	0,3
Fabaceae	*Lathyrus pratensis*	0,28	0,28	0,19	0,24	0,12
Fabaceae	*Lotus corniculatus*	0,16	0,13	0,07	0,11	0,14
Fabaceae	*Medicago varia*	0,06	0,03	0,07	0,04	0,21
Fabaceae	*Onobrychis vicifolia*	0,27	0,3	0,36	0,33	0,42
Fabaceae	*Trifolium campestre*	0,22	0,19	0,13	0,16	0,16
Fabaceae	*Trifolium fragiferum*	0	0,09	0,09	0,08	0,17
Fabaceae	*Trifolium hybridum*	0,09	0	0,09	0,05	0,23
Fabaceae	*Trifolium pratense*	0,09	0,09	0	0,05	0,14
Fabaceae	*Trifolium repens*	0,08	0,05	0,05	0	0,19
Fabaceae	*Vicia cracca*	0,17	0,23	0,14	0,19	0
Geraniaceae	*Geranium pratense*	0,08	0,08	0,14	0,11	0,25
Lamiaceae	*Ajuga reptans*	0,27	0,3	0,36	0,33	0,42
Lamiaceae	*Glechoma hederacea*	0,1	0,11	0,17	0,13	0,27
Lamiaceae	*Prunella vulgaris*	0,05	0,09	0,14	0,11	0,22
Primulaceae	*Primula veris*	0,11	0,05	0,06	0,04	0,21
Ranunculaceae	*Ranunculus acris*	0,54	0,57	0,63	0,6	0,69
Ranunculaceae	*Ranunculus repens*	0,2	0,21	0,27	0,24	0,37
Rosaceae	*Sanguisorba officinalis*	0,61	0,64	0,7	0,67	0,76
Scrophulariaceae	*Veronica chamaedrys*	0,42	0,36	0,36	0,35	0,44

c) Carbohydrates %

		Geranium pratense	*Ajuga reptans*	*Glechoma hederacea*	*Prunella vulgaris*	*Primula veris*
Apiaceae	*Anthriscus sylvestris*	0,4	0,57	0,42	0,37	0,35
Apiaceae	*Daucus carota*	0,44	0,66	0,47	0,44	0,3
Apiaceae	*Heracleum sphondylium*	0,41	0,63	0,44	0,41	0,27
Apiaceae	*Pastinaca sativa*	0,3	0,52	0,33	0,3	0,16
Apiaceae	*Pimpinella major*	0,35	0,57	0,38	0,35	0,21
Asteraceae	*Centaurea jacea*	0,4	0,62	0,43	0,4	0,26
Asteraceae	*Cirsium oleraceum*	0,13	0,35	0,16	0,13	0,03
Asteraceae	*Crepis biennis*	0,42	0,64	0,45	0,42	0,28
Asteraceae	*Leontodon autumnalis*	0,43	0,65	0,46	0,43	0,29
Asteraceae	*Leontodon hispidus*	0,44	0,66	0,47	0,44	0,3
Asteraceae	*Taraxacum officinale*	0,24	0,46	0,27	0,24	0,1
Asteraceae	*Tragopogon pratensis*	0,4	0,62	0,43	0,4	0,26
Brassicaceae	*Cardamine pratensis*	0,44	0,66	0,47	0,44	0,3
Campanulaceae	*Campanula patula*	0,34	0,56	0,37	0,34	0,2
Dipsacaceae	*Knautia arvensis*	0,44	0,66	0,47	0,44	0,3
Fabaceae	*Lathyrus pratensis*	0,33	0,55	0,36	0,33	0,25
Fabaceae	*Lotus corniculatus*	0,21	0,43	0,24	0,21	0,07
Fabaceae	*Medicago varia*	0,07	0,29	0,1	0,07	0,06
Fabaceae	*Onobrychis vicifolia*	0,22	0	0,2	0,22	0,35
Fabaceae	*Trifolium campestre*	0,27	0,49	0,29	0,27	0,13
Fabaceae	*Trifolium fragiferum*	0,08	0,27	0,1	0,05	0,11
Fabaceae	*Trifolium hybridum*	0,08	0,3	0,11	0,09	0,05
Fabaceae	*Trifolium pratense*	0,14	0,36	0,17	0,14	0,06
Fabaceae	*Trifolium repens*	0,11	0,33	0,13	0,11	0,04
Fabaceae	*Vicia cracca*	0,25	0,42	0,27	0,22	0,21
Geraniaceae	*Geranium pratense*	0	0,22	0,03	0,03	0,13
Lamiaceae	*Ajuga reptans*	0,22	0	0,2	0,22	0,35
Lamiaceae	*Glechoma hederacea*	0,03	0,2	0	0,05	0,16
Lamiaceae	*Prunella vulgaris*	0,03	0,22	0,05	0	0,13
Primulaceae	*Primula veris*	0,13	0,35	0,16	0,13	0
Ranunculaceae	*Ranunculus acris*	0,49	0,27	0,47	0,49	0,62
Ranunculaceae	*Ranunculus repens*	0,13	0,09	0,11	0,15	0,26
Rosaceae	*Sanguisorba officinalis*	0,56	0,34	0,54	0,56	0,69
Scrophulariaceae	*Veronica chamaedrys*	0,44	0,66	0,47	0,44	0,3

c) Carbohydrates %

		Ranun-culus acris	*Ranun-culus repens*	*Sangui-sorba offici-nalis*	*Vero-nica chama-edrys*
Apiaceae	*Anthriscus sylvestris*	0,84	0,52	0,91	0,44
Apiaceae	*Daucus carota*	0,93	0,57	1	0,05
Apiaceae	*Heracleum sphondylium*	0,9	0,54	0,97	0,17
Apiaceae	*Pastinaca sativa*	0,79	0,43	0,86	0,25
Apiaceae	*Pimpinella major*	0,84	0,48	0,91	0,25
Asteraceae	*Centaurea jacea*	0,89	0,53	0,96	0,17
Asteraceae	*Cirsium oleraceum*	0,62	0,26	0,69	0,34
Asteraceae	*Crepis biennis*	0,91	0,55	0,98	0,18
Asteraceae	*Leontodon autumnalis*	0,92	0,56	0,99	0,13
Asteraceae	*Leontodon hispidus*	0,93	0,57	1	0,03
Asteraceae	*Taraxacum officinale*	0,73	0,37	0,8	0,2
Asteraceae	*Tragopogon pratensis*	0,89	0,53	0,96	0,17
Brassicaceae	*Cardamine pratensis*	0,93	0,57	1	0,16
Campanulaceae	*Campanula patula*	0,83	0,47	0,9	0,2
Dipsacaceae	*Knautia arvensis*	0,93	0,57	1	0,14
Fabaceae	*Lathyrus pratensis*	0,82	0,46	0,89	0,36
Fabaceae	*Lotus corniculatus*	0,7	0,34	0,77	0,3
Fabaceae	*Medicago varia*	0,56	0,2	0,63	0,37
Fabaceae	*Onobrychis vicifolia*	0,27	0,09	0,34	0,66
Fabaceae	*Trifolium campestre*	0,76	0,4	0,83	0,29
Fabaceae	*Trifolium fragiferum*	0,54	0,2	0,61	0,42
Fabaceae	*Trifolium hybridum*	0,57	0,21	0,64	0,36
Fabaceae	*Trifolium pratense*	0,63	0,27	0,7	0,36
Fabaceae	*Trifolium repens*	0,6	0,24	0,67	0,35
Fabaceae	*Vicia cracca*	0,69	0,37	0,76	0,44
Geraniaceae	*Geranium pratense*	0,49	0,13	0,56	0,44
Lamiaceae	*Ajuga reptans*	0,27	0,09	0,34	0,66
Lamiaceae	*Glechoma hederacea*	0,47	0,11	0,54	0,47
Lamiaceae	*Prunella vulgaris*	0,49	0,15	0,56	0,44
Primulaceae	*Primula veris*	0,62	0,26	0,69	0,3
Ranunculaceae	*Ranunculus acris*	0	0,36	0,07	0,93
Ranunculaceae	*Ranunculus repens*	0,36	0	0,43	0,57
Rosaceae	*Sanguisorba officinalis*	0,07	0,43	0	1
Scrophulariaceae	*Veronica chamaedrys*	0,93	0,57	1	0

d) Carbohydrates concentration

Chemical distance matrix between 34 plant species based on Bray-Curtis distances between either the proportions (%) or concentrations (conc) of amino acids and carbohydrates.

high chemical similarity

intermediate chemical similarity

low chemical similarity

		Anthriscus sylvestris	*Daucus carota*	*Heracleum sphondylium*	*Pastinaca sativa*	*Pimpinella major*
Apiaceae	*Anthriscus sylvestris*	0	0,4	0,36	0,78	0,26
Apiaceae	*Daucus carota*	0,4	0	0,59	0,87	0,18
Apiaceae	*Heracleum sphondylium*	0,36	0,59	0	0,58	0,46
Apiaceae	*Pastinaca sativa*	0,78	0,87	0,58	0	0,82
Apiaceae	*Pimpinella major*	0,26	0,18	0,46	0,82	0
Asteraceae	*Centaurea jacea*	0,52	0,7	0,19	0,45	0,59
Asteraceae	*Cirsium oleraceum*	0,63	0,78	0,35	0,29	0,7
Asteraceae	*Crepis biennis*	0,7	0,82	0,45	0,18	0,75
Asteraceae	*Leontodon autumnalis*	0,62	0,77	0,33	0,32	0,68
Asteraceae	*Leontodon hispidus*	0,39	0,56	0,16	0,61	0,46
Asteraceae	*Taraxacum officinale*	0,55	0,73	0,24	0,4	0,63
Asteraceae	*Tragopogon pratensis*	0,85	0,92	0,71	0,29	0,88
Brassicaceae	*Cardamine pratensis*	0,74	0,83	0,49	0,16	0,78
Campanulaceae	*Campanula patula*	0,24	0,5	0,18	0,66	0,35
Dipsacaceae	*Knautia arvensis*	0,75	0,84	0,51	0,15	0,79
Fabaceae	*Lathyrus pratensis*	0,22	0,49	0,17	0,67	0,33
Fabaceae	*Lotus corniculatus*	0,78	0,87	0,58	0,07	0,82
Fabaceae	*Medicago varia*	0,35	0,54	0,39	0,63	0,4
Fabaceae	*Onobrychis vicifolia*	0,59	0,7	0,58	0,55	0,6
Fabaceae	*Trifolium campestre*	0,88	0,93	0,76	0,31	0,9
Fabaceae	*Trifolium fragiferum*	0,58	0,75	0,29	0,37	0,65
Fabaceae	*Trifolium hybridum*	0,75	0,86	0,54	0,2	0,8
Fabaceae	*Trifolium pratense*	0,55	0,73	0,25	0,4	0,63
Fabaceae	*Trifolium repens*	0,56	0,73	0,25	0,39	0,64
Fabaceae	*Vicia cracca*	0,26	0,52	0,28	0,65	0,37
Geraniaceae	*Geranium pratense*	0,63	0,78	0,36	0,35	0,7
Lamiaceae	*Ajuga reptans*	0,8	0,89	0,62	0,47	0,84
Lamiaceae	*Glechoma hederacea*	0,53	0,71	0,32	0,43	0,61
Lamiaceae	*Prunella vulgaris*	0,56	0,74	0,31	0,4	0,64
Primulaceae	*Primula veris*	0,67	0,81	0,42	0,23	0,74
Ranunculaceae	*Ranunculus acris*	0,87	0,92	0,9	0,7	0,89
Ranunculaceae	*Ranunculus repens*	0,62	0,58	0,64	0,59	0,54
Rosaceae	*Sanguisorba officinalis*	0,91	1	0,94	0,72	0,95
Scrophulariaceae	*Veronica chamaedrys*	0,38	0,55	0,17	0,62	0,45

d) Carbohydrates concentration

		Centaurea jacea	*Cirsium oleraceum*	*Crepis biennis*	*Leontodon autumnalis*	*Leontodon hispidus*
Apiaceae	*Anthriscus sylvestris*	0,52	0,63	0,7	0,62	0,39
Apiaceae	*Daucus carota*	0,7	0,78	0,82	0,77	0,56
Apiaceae	*Heracleum sphondylium*	0,19	0,35	0,45	0,33	0,16
Apiaceae	*Pastinaca sativa*	0,45	0,29	0,18	0,32	0,61
Apiaceae	*Pimpinella major*	0,59	0,7	0,75	0,68	0,46
Asteraceae	*Centaurea jacea*	0	0,21	0,3	0,16	0,23
Asteraceae	*Cirsium oleraceum*	0,21	0	0,38	0,28	0,39
Asteraceae	*Crepis biennis*	0,3	0,38	0	0,15	0,49
Asteraceae	*Leontodon autumnalis*	0,16	0,28	0,15	0	0,37
Asteraceae	*Leontodon hispidus*	0,23	0,39	0,49	0,37	0
Asteraceae	*Taraxacum officinale*	0,15	0,16	0,36	0,26	0,29
Asteraceae	*Tragopogon pratensis*	0,61	0,56	0,38	0,5	0,73
Brassicaceae	*Cardamine pratensis*	0,33	0,41	0,04	0,18	0,5
Campanulaceae	*Campanula patula*	0,35	0,46	0,57	0,48	0,19
Dipsacaceae	*Knautia arvensis*	0,35	0,43	0,06	0,21	0,52
Fabaceae	*Lathyrus pratensis*	0,35	0,48	0,57	0,47	0,28
Fabaceae	*Lotus corniculatus*	0,45	0,29	0,18	0,32	0,61
Fabaceae	*Medicago varia*	0,52	0,42	0,67	0,61	0,42
Fabaceae	*Onobrychis vicifolia*	0,65	0,36	0,74	0,7	0,6
Fabaceae	*Trifolium campestre*	0,67	0,55	0,46	0,57	0,78
Fabaceae	*Trifolium fragiferum*	0,32	0,1	0,49	0,41	0,41
Fabaceae	*Trifolium hybridum*	0,4	0,24	0,26	0,26	0,58
Fabaceae	*Trifolium pratense*	0,3	0,12	0,49	0,4	0,38
Fabaceae	*Trifolium repens*	0,3	0,11	0,48	0,4	0,35
Fabaceae	*Vicia cracca*	0,43	0,44	0,62	0,54	0,39
Geraniaceae	*Geranium pratense*	0,3	0,1	0,46	0,39	0,4
Lamiaceae	*Ajuga reptans*	0,51	0,35	0,57	0,52	0,65
Lamiaceae	*Glechoma hederacea*	0,43	0,18	0,58	0,51	0,4
Lamiaceae	*Prunella vulgaris*	0,38	0,15	0,54	0,47	0,44
Primulaceae	*Primula veris*	0,26	0,08	0,31	0,22	0,46
Ranunculaceae	*Ranunculus acris*	0,93	0,56	0,94	0,94	0,94
Ranunculaceae	*Ranunculus repens*	0,72	0,37	0,8	0,76	0,67
Rosaceae	*Sanguisorba officinalis*	0,97	0,55	0,96	0,97	1
Scrophulariaceae	*Veronica chamaedrys*	0,24	0,41	0,5	0,38	0,02

d) Carbohydrates concentration

		Taraxacum officinale	*Tragopogon pratensis*	*Cardamine pratensis*	*Campanula patula*	*Knautia arvensis*
Apiaceae	*Anthriscus sylvestris*	0,55	0,85	0,74	0,24	0,75
Apiaceae	*Daucus carota*	0,73	0,92	0,83	0,5	0,84
Apiaceae	*Heracleum sphondylium*	0,24	0,71	0,49	0,18	0,51
Apiaceae	*Pastinaca sativa*	0,4	0,29	0,16	0,66	0,15
Apiaceae	*Pimpinella major*	0,63	0,88	0,78	0,35	0,79
Asteraceae	*Centaurea jacea*	0,15	0,61	0,33	0,35	0,35
Asteraceae	*Cirsium oleraceum*	0,16	0,56	0,41	0,46	0,43
Asteraceae	*Crepis biennis*	0,36	0,38	0,04	0,57	0,06
Asteraceae	*Leontodon autumnalis*	0,26	0,5	0,18	0,48	0,21
Asteraceae	*Leontodon hispidus*	0,29	0,73	0,5	0,19	0,52
Asteraceae	*Taraxacum officinale*	0	0,57	0,4	0,36	0,42
Asteraceae	*Tragopogon pratensis*	0,57	0	0,37	0,77	0,34
Brassicaceae	*Cardamine pratensis*	0,4	0,37	0	0,61	0,03
Campanulaceae	*Campanula patula*	0,36	0,77	0,61	0	0,63
Dipsacaceae	*Knautia arvensis*	0,42	0,34	0,03	0,63	0
Fabaceae	*Lathyrus pratensis*	0,38	0,78	0,61	0,14	0,63
Fabaceae	*Lotus corniculatus*	0,4	0,35	0,17	0,66	0,2
Fabaceae	*Medicago varia*	0,32	0,75	0,71	0,26	0,73
Fabaceae	*Onobrychis vicifolia*	0,48	0,78	0,77	0,5	0,78
Fabaceae	*Trifolium campestre*	0,63	0,11	0,45	0,81	0,43
Fabaceae	*Trifolium fragiferum*	0,21	0,64	0,53	0,4	0,54
Fabaceae	*Trifolium hybridum*	0,35	0,46	0,29	0,63	0,31
Fabaceae	*Trifolium pratense*	0,17	0,64	0,52	0,36	0,54
Fabaceae	*Trifolium repens*	0,16	0,63	0,52	0,37	0,54
Fabaceae	*Vicia cracca*	0,34	0,76	0,66	0,24	0,68
Geraniaceae	*Geranium pratense*	0,21	0,61	0,5	0,47	0,51
Lamiaceae	*Ajuga reptans*	0,46	0,65	0,59	0,7	0,6
Lamiaceae	*Glechoma hederacea*	0,28	0,69	0,62	0,34	0,63
Lamiaceae	*Prunella vulgaris*	0,24	0,66	0,58	0,38	0,59
Primulaceae	*Primula veris*	0,19	0,51	0,35	0,52	0,37
Ranunculaceae	*Ranunculus acris*	0,72	0,89	0,97	0,85	0,97
Ranunculaceae	*Ranunculus repens*	0,5	0,81	0,83	0,54	0,84
Rosaceae	*Sanguisorba officinalis*	0,7	0,9	1	0,87	1
Scrophulariaceae	*Veronica chamaedrys*	0,3	0,74	0,51	0,19	0,53

d) Carbohydrates concentration

		Lathyrus pratensis	*Lotus corniculatus*	*Medicago varia*	*Onobrychis vicifolia*	*Trifolium campestre*
Apiaceae	*Anthriscus sylvestris*	0,22	0,78	0,35	0,59	0,88
Apiaceae	*Daucus carota*	0,49	0,87	0,54	0,7	0,93
Apiaceae	*Heracleum sphondylium*	0,17	0,58	0,39	0,58	0,76
Apiaceae	*Pastinaca sativa*	0,67	0,07	0,63	0,55	0,31
Apiaceae	*Pimpinella major*	0,33	0,82	0,4	0,6	0,9
Asteraceae	*Centaurea jacea*	0,35	0,45	0,52	0,65	0,67
Asteraceae	*Cirsium oleraceum*	0,48	0,29	0,42	0,36	0,55
Asteraceae	*Crepis biennis*	0,57	0,18	0,67	0,74	0,46
Asteraceae	*Leontodon autumnalis*	0,47	0,32	0,61	0,7	0,57
Asteraceae	*Leontodon hispidus*	0,28	0,61	0,42	0,6	0,78
Asteraceae	*Taraxacum officinale*	0,38	0,4	0,32	0,48	0,63
Asteraceae	*Tragopogon pratensis*	0,78	0,35	0,75	0,78	0,11
Brassicaceae	*Cardamine pratensis*	0,61	0,17	0,71	0,77	0,45
Campanulaceae	*Campanula patula*	0,14	0,66	0,26	0,5	0,81
Dipsacaceae	*Knautia arvensis*	0,63	0,2	0,73	0,78	0,43
Fabaceae	*Lathyrus pratensis*	0	0,67	0,28	0,51	0,81
Fabaceae	*Lotus corniculatus*	0,67	0	0,63	0,45	0,31
Fabaceae	*Medicago varia*	0,28	0,63	0	0,3	0,79
Fabaceae	*Onobrychis vicifolia*	0,51	0,45	0,3	0	0,66
Fabaceae	*Trifolium campestre*	0,81	0,31	0,79	0,66	0
Fabaceae	*Trifolium fragiferum*	0,41	0,36	0,35	0,27	0,61
Fabaceae	*Trifolium hybridum*	0,64	0,13	0,6	0,39	0,36
Fabaceae	*Trifolium pratense*	0,37	0,4	0,31	0,31	0,63
Fabaceae	*Trifolium repens*	0,38	0,39	0,32	0,31	0,63
Fabaceae	*Vicia cracca*	0,14	0,65	0,21	0,45	0,8
Geraniaceae	*Geranium pratense*	0,48	0,28	0,42	0,24	0,55
Lamiaceae	*Ajuga reptans*	0,7	0,4	0,67	0,49	0,48
Lamiaceae	*Glechoma hederacea*	0,35	0,42	0,28	0,2	0,65
Lamiaceae	*Prunella vulgaris*	0,39	0,38	0,33	0,22	0,62
Primulaceae	*Primula veris*	0,53	0,22	0,48	0,38	0,5
Ranunculaceae	*Ranunculus acris*	0,87	0,59	0,61	0,21	0,72
Ranunculaceae	*Ranunculus repens*	0,56	0,59	0,28	0,21	0,77
Rosaceae	*Sanguisorba officinalis*	0,91	0,72	0,53	0,42	0,85
Scrophulariaceae	*Veronica chamaedrys*	0,3	0,62	0,41	0,6	0,78

d) Carbohydrates concentration

		Trifolium fragiferum	*Trifolium hybridum*	*Trifolium pratense*	*Trifolium repens*	*Vicia cracca*
Apiaceae	*Anthriscus sylvestris*	0,58	0,75	0,55	0,56	0,26
Apiaceae	*Daucus carota*	0,75	0,86	0,73	0,73	0,52
Apiaceae	*Heracleum sphondylium*	0,29	0,54	0,25	0,25	0,28
Apiaceae	*Pastinaca sativa*	0,37	0,2	0,4	0,39	0,65
Apiaceae	*Pimpinella major*	0,65	0,8	0,63	0,64	0,37
Asteraceae	*Centaurea jacea*	0,32	0,4	0,3	0,3	0,43
Asteraceae	*Cirsium oleraceum*	0,1	0,24	0,12	0,11	0,44
Asteraceae	*Crepis biennis*	0,49	0,26	0,49	0,48	0,62
Asteraceae	*Leontodon autumnalis*	0,41	0,26	0,4	0,4	0,54
Asteraceae	*Leontodon hispidus*	0,41	0,58	0,38	0,35	0,39
Asteraceae	*Taraxacum officinale*	0,21	0,35	0,17	0,16	0,34
Asteraceae	*Tragopogon pratensis*	0,64	0,46	0,64	0,63	0,76
Brassicaceae	*Cardamine pratensis*	0,53	0,29	0,52	0,52	0,66
Campanulaceae	*Campanula patula*	0,4	0,63	0,36	0,37	0,24
Dipsacaceae	*Knautia arvensis*	0,54	0,31	0,54	0,54	0,68
Fabaceae	*Lathyrus pratensis*	0,41	0,64	0,37	0,38	0,14
Fabaceae	*Lotus corniculatus*	0,36	0,13	0,4	0,39	0,65
Fabaceae	*Medicago varia*	0,35	0,6	0,31	0,32	0,21
Fabaceae	*Onobrychis vicifolia*	0,27	0,39	0,31	0,31	0,45
Fabaceae	*Trifolium campestre*	0,61	0,36	0,63	0,63	0,8
Fabaceae	*Trifolium fragiferum*	0	0,31	0,04	0,06	0,38
Fabaceae	*Trifolium hybridum*	0,31	0	0,35	0,34	0,62
Fabaceae	*Trifolium pratense*	0,04	0,35	0	0,03	0,34
Fabaceae	*Trifolium repens*	0,06	0,34	0,03	0	0,35
Fabaceae	*Vicia cracca*	0,38	0,62	0,34	0,35	0
Geraniaceae	*Geranium pratense*	0,09	0,23	0,13	0,12	0,45
Lamiaceae	*Ajuga reptans*	0,41	0,3	0,45	0,44	0,68
Lamiaceae	*Glechoma hederacea*	0,08	0,37	0,11	0,11	0,31
Lamiaceae	*Prunella vulgaris*	0,05	0,33	0,08	0,08	0,36
Primulaceae	*Primula veris*	0,16	0,16	0,2	0,19	0,5
Ranunculaceae	*Ranunculus acris*	0,48	0,47	0,54	0,54	0,74
Ranunculaceae	*Ranunculus repens*	0,3	0,56	0,31	0,3	0,43
Rosaceae	*Sanguisorba officinalis*	0,49	0,7	0,47	0,47	0,71
Scrophulariaceae	*Veronica chamaedrys*	0,43	0,59	0,39	0,36	0,41

d) Carbohydrates concentration

		Geranium pratense	*Ajuga reptans*	*Glechoma hederacea*	*Prunella vulgaris*	*Primula veris*
Apiaceae	*Anthriscus sylvestris*	0,63	0,8	0,53	0,56	0,67
Apiaceae	*Daucus carota*	0,78	0,89	0,71	0,74	0,81
Apiaceae	*Heracleum sphondylium*	0,36	0,62	0,32	0,31	0,42
Apiaceae	*Pastinaca sativa*	0,35	0,47	0,43	0,4	0,23
Apiaceae	*Pimpinella major*	0,7	0,84	0,61	0,64	0,74
Asteraceae	*Centaurea jacea*	0,3	0,51	0,43	0,38	0,26
Asteraceae	*Cirsium oleraceum*	0,1	0,35	0,18	0,15	0,08
Asteraceae	*Crepis biennis*	0,46	0,57	0,58	0,54	0,31
Asteraceae	*Leontodon autumnalis*	0,39	0,52	0,51	0,47	0,22
Asteraceae	*Leontodon hispidus*	0,4	0,65	0,4	0,44	0,46
Asteraceae	*Taraxacum officinale*	0,21	0,46	0,28	0,24	0,19
Asteraceae	*Tragopogon pratensis*	0,61	0,65	0,69	0,66	0,51
Brassicaceae	*Cardamine pratensis*	0,5	0,59	0,62	0,58	0,35
Campanulaceae	*Campanula patula*	0,47	0,7	0,34	0,38	0,52
Dipsacaceae	*Knautia arvensis*	0,51	0,6	0,63	0,59	0,37
Fabaceae	*Lathyrus pratensis*	0,48	0,7	0,35	0,39	0,53
Fabaceae	*Lotus corniculatus*	0,28	0,4	0,42	0,38	0,22
Fabaceae	*Medicago varia*	0,42	0,67	0,28	0,33	0,48
Fabaceae	*Onobrychis vicifolia*	0,24	0,49	0,2	0,22	0,38
Fabaceae	*Trifolium campestre*	0,55	0,48	0,65	0,62	0,5
Fabaceae	*Trifolium fragiferum*	0,09	0,41	0,08	0,05	0,16
Fabaceae	*Trifolium hybridum*	0,23	0,3	0,37	0,33	0,16
Fabaceae	*Trifolium pratense*	0,13	0,45	0,11	0,08	0,2
Fabaceae	*Trifolium repens*	0,12	0,44	0,11	0,08	0,19
Fabaceae	*Vicia cracca*	0,45	0,68	0,31	0,36	0,5
Geraniaceae	*Geranium pratense*	0	0,34	0,16	0,11	0,14
Lamiaceae	*Ajuga reptans*	0,34	0	0,47	0,43	0,35
Lamiaceae	*Glechoma hederacea*	0,16	0,47	0	0,05	0,22
Lamiaceae	*Prunella vulgaris*	0,11	0,43	0,05	0	0,18
Primulaceae	*Primula veris*	0,14	0,35	0,22	0,18	0
Ranunculaceae	*Ranunculus acris*	0,42	0,56	0,43	0,44	0,56
Ranunculaceae	*Ranunculus repens*	0,38	0,63	0,23	0,28	0,43
Rosaceae	*Sanguisorba officinalis*	0,56	0,75	0,43	0,47	0,6
Scrophulariaceae	*Veronica chamaedrys*	0,41	0,66	0,41	0,45	0,47

d) Carbohydrates concentration

		Ranun-culus acris	*Ranun-culus repens*	*Sangui-sorba offici-nalis*	*Vero-nica chama-edrys*
Apiaceae	*Anthriscus sylvestris*	0,87	0,62	0,91	0,38
Apiaceae	*Daucus carota*	0,92	0,58	1	0,55
Apiaceae	*Heracleum sphondylium*	0,9	0,64	0,94	0,17
Apiaceae	*Pastinaca sativa*	0,7	0,59	0,72	0,62
Apiaceae	*Pimpinella major*	0,89	0,54	0,95	0,45
Asteraceae	*Centaurea jacea*	0,93	0,72	0,97	0,24
Asteraceae	*Cirsium oleraceum*	0,56	0,37	0,55	0,41
Asteraceae	*Crepis biennis*	0,94	0,8	0,96	0,5
Asteraceae	*Leontodon autumnalis*	0,94	0,76	0,97	0,38
Asteraceae	*Leontodon hispidus*	0,94	0,67	1	0,02
Asteraceae	*Taraxacum officinale*	0,72	0,5	0,7	0,3
Asteraceae	*Tragopogon pratensis*	0,89	0,81	0,9	0,74
Brassicaceae	*Cardamine pratensis*	0,97	0,83	1	0,51
Campanulaceae	*Campanula patula*	0,85	0,54	0,87	0,19
Dipsacaceae	*Knautia arvensis*	0,97	0,84	1	0,53
Fabaceae	*Lathyrus pratensis*	0,87	0,56	0,91	0,3
Fabaceae	*Lotus corniculatus*	0,59	0,59	0,72	0,62
Fabaceae	*Medicago varia*	0,61	0,28	0,53	0,41
Fabaceae	*Onobrychis vicifolia*	0,21	0,21	0,42	0,6
Fabaceae	*Trifolium campestre*	0,72	0,77	0,85	0,78
Fabaceae	*Trifolium fragiferum*	0,48	0,3	0,49	0,43
Fabaceae	*Trifolium hybridum*	0,47	0,56	0,7	0,59
Fabaceae	*Trifolium pratense*	0,54	0,31	0,47	0,39
Fabaceae	*Trifolium repens*	0,54	0,3	0,47	0,36
Fabaceae	*Vicia cracca*	0,74	0,43	0,71	0,41
Geraniaceae	*Geranium pratense*	0,42	0,38	0,56	0,41
Lamiaceae	*Ajuga reptans*	0,56	0,63	0,75	0,66
Lamiaceae	*Glechoma hederacea*	0,43	0,23	0,43	0,41
Lamiaceae	*Prunella vulgaris*	0,44	0,28	0,47	0,45
Primulaceae	*Primula veris*	0,56	0,43	0,6	0,47
Ranunculaceae	*Ranunculus acris*	0	0,34	0,33	0,94
Ranunculaceae	*Ranunculus repens*	0,34	0	0,23	0,66
Rosaceae	*Sanguisorba officinalis*	0,33	0,23	0	1
Scrophulariaceae	*Veronica chamaedrys*	0,94	0,66	1	0

Table S3 Statistical results of generalized linear (mixed) models (GL(M)Ms) testing for effects of morphological factors (flower symmetry, nectar access, inflorescence area, flower height) and family on variation in individual and total amino acid concentrations and proportions. Given are the *F*- and *p*-values as well as the R^2-values for each model. *P*-values below 0.05 are marked in italics. *P*-values below the significance level of 0.01 are additionally marked in bold. R^2-values above 0.1 are marked in bold as well. If GLMMs were used, plot nested in plant species was used as random factor.

Amino acid	Flower symmetry	Nectar access	Inflorescence area	Flower height	Family
Aspartic acid concentration	$F = 0.668$ $p = 0.420$ $R^2 = 0.02$	$F = 2.317$ $p = 0.138$ $R^2 = 0.06$	$F = 2.386$ $p = 0.133$ $R^2 = 0.07$	$F = 2.273$ $p = 0.144$ $R^2 = 0.06$	$F = 1.491$ $p = 0.212$ **$R^2 = 0.34$**
Aspartic acid proportion	$F = 1.159$ $p = 0.290$ $R^2 = 0.03$	$F = 4.076$ $p = 0.052$ **$R^2 = 0.10$**	$F = 0.049$ $p = 0.826$ $R^2 < 0.01$	$F = 0.347$ $p = 0.560$ $R^2 = 0.01$	$F = 2.266$ $p = 0.062$ **$R^2 = 0.39$**
Glutamic acid concentration	$F = 3.842$ $p = 0.068$ $R^2 = 0.05$	$F = 0.848$ $p = 0.367$ $R^2 = 0.02$	$F = 0.471$ $p = 0.498$ $R^2 = 0.01$	$F = 0.199$ $p = 0.662$ $R^2 < 0.01$	$F = 10.943$ ***p < 0.001*** **$R^2 = 0.74$**
Glutamic acid proportion	$F = 1.638$ $p = 0.210$ $R^2 = 0.05$	$F = 1.139$ $p = 0.294$ $R^2 = 0.03$	$F = 0.199$ $p = 0.659$ $R^2 < 0.01$	$F = 0.152$ $p = 0.699$ $R^2 < 0.01$	$F = 3.325$ *p = 0.011* **$R^2 = 0.52$**
Serine concentration	$F = 2.231$ $p = 0.146$ $R^2 = 0.06$	$F = 4.929$ $p = 0.034$ **$R^2 = 0.13$**	$F = 0.211$ $p = 0.649$ $R^2 = 0.01$	$F = 0.218$ $p = 0.643$ $R^2 = 0.01$	$F = 5.690$ ***p < 0.001*** **$R^2 = 0.66$**
Serine proportion	$F = 0.093$ $p = 0.763$ $R^2 < 0.01$	$F = 1.722$ $p = 0.199$ $R^2 = 0.05$	$F = 2.912$ $p = 0.098$ $R^2 = 0.08$	$F = 0.116$ $p = 0.736$ $R^2 < 0.01$	$F = 0.979$ $p = 0.501$ **$R^2 = 0.22$**
Histidine concentration	$F = 0.144$ $p = 0.707$ $R^2 < 0.01$	$F = 0.026$ $p = 0.874$ $R^2 < 0.01$	$F = 0.186$ $p = 0.669$ $R^2 = 0.01$	$F = 2.833$ $p = 0.102$ $R^2 = 0.09$	$F = 2.444$ *p = 0.037* **$R^2 = 0.48$**
Histidine proportion	$F = 0.011$ $p = 0.916$ $R^2 < 0.01$	$F = 0.503$ $p = 0.484$ $R^2 = 0.02$	$F = 0.381$ $p = 0.542$ $R^2 = 0.01$	$F = 0.978$ $p = 0.330$ $R^2 = 0.03$	$F = 2.024$ $p = 0.079$ **$R^2 = 0.43$**
Glycine concentration	$F = 2.732$ $p = 0.109$ $R^2 = 0.07$	$F = 1.830$ $p = 0.186$ $R^2 = 0.05$	$F = 2.283$ $p = 0.141$ $R^2 = 0.07$	$F = 0.185$ $p = 0.671$ $R^2 = 0.01$	$F = 3.780$ ***p = 0.004*** **$R^2 = 0.57$**
Glycine proportion	$F = 6.326$ *p = 0.024* $R^2 = 0.07$	$F = 0.278$ $p = 0.605$ $R^2 < 0.01$	$F = 0.369$ $p = 0.548$ $R^2 = 0.01$	$F = 0.456$ $p = 0.510$ $R^2 = 0.01$	$F = 1.555$ $p = 0.213$ **$R^2 = 0.32$**
Threonine concentration	$F = 2.955$ $p = 0.107$ $R^2 = 0.03$	$F = 0.923$ $p = 0.349$ $R^2 = 0.01$	$F = 0.929$ $p = 0.342$ $R^2 = 0.02$	$F = 0.097$ $p = 0.760$ $R^2 < 0.01$	$F = 6.371$ ***p < 0.001*** **$R^2 = 0.68$**
Threonine proportion	$F = 0.512$ $p = 0.479$ $R^2 = 0.02$	$F = 0.034$ $p = 0.856$ $R^2 < 0.01$	$F = 0.108$ $p = 0.745$ $R^2 < 0.01$	$F = 0.051$ $p = 0.823$ $R^2 < 0.01$	$F = 1.738$ $p = 0.133$ **$R^2 = 0.39$**
Arginine concentration	$F = 4.661$ $p = 0.051$ $R^2 = 0.02$	$F = 0.838$ $p = 0.378$ $R^2 < 0.01$	$F = 0.288$ $p = 0.598$ $R^2 < 0.01$	$F = 0.160$ $p = 0.697$ $R^2 < 0.01$	$F = 15.983$ ***p < 0.001*** **$R^2 = 0.85$**
Arginine proportion	$F = 4.673$ $p = 0.051$ $R^2 = 0.02$	$F = 0.032$ $p = 0.893$ $R^2 < 0.01$	$F = 0.288$ $p = 0.598$ $R^2 < 0.01$	$F = 0.132$ $p = 0.732$ $R^2 < 0.01$	$F = 14.392$ ***p < 0.001*** **$R^2 = 0.39$**
Alanine concentration	$F = 0.776$ $p = 0.385$ $R^2 = 0.02$	$F = 0.097$ $p = 0.758$ $R^2 < 0.01$	$F = 1.122$ $p = 0.298$ $R^2 = 0.03$	$F = 0.344$ $p = 0.562$ $R^2 = 0.01$	$F = 7.200$ ***p < 0.001*** **$R^2 = 0.69$**
Alanine proportion	$F = 0.041$ $p = 0.840$ $R^2 < 0.01$	$F = 1.198$ $p = 0.282$ $R^2 = 0.03$	$F = 0.345$ $p = 0.561$ $R^2 = 0.01$	$F = 0.374$ $p = 0.545$ $R^2 = 0.01$	$F = 1.073$ $p = 0.426$ **$R^2 = 0.29$**
Tyrosine concentration	$F = 0.257$ $p = 0.617$ $R^2 = 0.01$	$F = 0.256$ $p = 0.617$ $R^2 = 0.01$	$F = 0.348$ $p = 0.560$ $R^2 = 0.01$	$F = 0.637$ $p = 0.435$ $R^2 = 0.01$	$F = 0.124$ $p = 0.999$ $R^2 = 0.04$
Tyrosine proportion	$F = 0.466$ $p = 0.503$ $R^2 = 0.01$	$F = 0.546$ $p = 0.468$ $R^2 = 0.01$	$F = 0.143$ $p = 0.708$ $R^2 < 0.01$	$F = 0.819$ $p = 0.562$ $R^2 = 0.01$	$F = 0.087$ $p = 1$ $R^2 = 0.02$
Cystine concentration	NA	NA	NA	NA	NA
Cystine proportion	NA	NA	NA	NA	NA

Table S3 (continued)

Valine concentration	$F = 1.643$ $p = 0.209$ $R^2 = 0.05$	$F = 1.087$ $p = 0.305$ $R^2 = 0.03$	$F = 0.371$ $p = 0.547$ $R^2 = 0.01$	$F = 0.018$ $p = 0.893$ $R^2 < 0.01$	$F = 2.671$ *$p = 0.025$* **$R^2 = 0.50$**
Valine proportion	$F = 1.535$ $p = 0.213$ $R^2 = 0.05$	$F = 1.974$ $p = 0.252$ $R^2 = 0.03$	$F = 0.785$ $p = 0.501$ $R^2 = 0.01$	$F = 0.198$ $p = 0.818$ $R^2 < 0.01$	$F = 1.984$ $p = 0.121$ **$R^2 = 0.50$**
Methionine concentration	$F = 2.018$ $p = 0.165$ $R^2 = 0.06$	$F = 0.453$ $p = 0.506$ $R^2 = 0.01$	$F = 0.634$ $p = 0.432$ $R^2 = 0.02$	$F = 0.527$ $p = 0.473$ $R^2 = 0.02$	$F = 3.838$ ***$p < 0.001$*** **$R^2 = 0.58$**
Methionine proportion	$F = 4.339$ *$p = 0.045$* **$R^2 = 0.12$**	$F = 0.289$ $p = 0.595$ $R^2 = 0.01$	$F = 0.745$ $p = 0.395$ $R^2 = 0.02$	$F = 0.943$ $p = 0.339$ $R^2 = 0.03$	$F = 4.544$ ***$p = 0.002$*** **$R^2 = 0.58$**
Phenylalanine concentration	$F = 0.033$ $p = 0.858$ $R^2 < 0.01$	$F = 0.078$ $p = 0.782$ $R^2 < 0.01$	$F = 0.203$ $p = 0.656$ $R^2 < 0.01$	$F = 0.003$ $p = 0.956$ $R^2 < 0.01$	$F = 0.490$ $p = 0.887$ **$R^2 = 0.15$**
Phenylalanine proportion	$F = 0.035$ $p = 0.843$ $R^2 < 0.01$	$F = 0.013$ $p = 0.921$ $R^2 < 0.01$	$F = 0.222$ $p = 0.612$ $R^2 < 0.01$	$F = 0.019$ $p = 0.910$ $R^2 < 0.01$	$F = 0.864$ $p = 0.784$ **$R^2 = 0.18$**
Isoleucine concentration	$F = 2.094$ $p = 0.163$ $R^2 = 0.04$	$F = 2.450$ $p = 0.130$ $R^2 = 0.05$	$F = 0.898$ $p = 0.351$ $R^2 = 0.03$	$F = 0.258$ $p = 0.617$ $R^2 = 0.01$	$F = 3.311$ *$p = 0.012$* **$R^2 = 0.53$**
Isoleucine proportion	$F = 0.319$ $p = 0.576$ $R^2 = 0.01$	$F = 0.631$ $p = 0.433$ $R^2 = 0.02$	$F = 0.948$ $p = 0.338$ $R^2 = 0.03$	$F = 0.261$ $p = 0.614$ $R^2 = 0.01$	$F = 1.718$ $p = 0.137$ **$R^2 = 0.39$**
Leucine concentration	$F = 4.515$ $p = 0.051$ $R^2 = 0.05$	$F = 3.324$ $p = 0.084$ $R^2 = 0.05$	$F = 0.091$ $p = 0.764$ $R^2 < 0.01$	$F = 0.062$ $p = 0.807$ $R^2 < 0.01$	$F = 0.894$ $p = 0.569$ $R^2 = 0.09$
Leucine proportion	$F = 2.055$ $p = 0.178$ $R^2 = 0.01$	$F = 2.410$ $p = 0.146$ $R^2 = 0.02$	$F = 7.539$ *$p = 0.014$* $R^2 = 0.07$	$F = 4.458$ $p = 0.060$ $R^2 = 0.01$	$F = 0.406$ $p = 0.927$ $R^2 = 0.05$
Lysine concentration	$F = 11.329$ ***$p = 0.007$*** $R^2 = 0.03$	$F = 1.925$ $p = 0.191$ $R^2 = 0.01$	$F = 0.526$ $p = 0.476$ $R^2 = 0.01$	$F = 0.138$ $p = 0.718$ $R^2 < 0.01$	$F = 39.530$ ***$p < 0.001$*** **$R^2 = 0.92$**
Lysine proportion	$F = 38.488$ ***$p < 0.001$*** $R^2 = 0.04$	$F = 1.876$ $p = 0.195$ $R^2 = 0.01$	$F = 0.218$ $p = 0.645$ $R^2 < 0.01$	$F = 0.800$ $p = 0.391$ $R^2 < 0.01$	$F = 13.941$ ***$p < 0.001$*** **$R^2 = 0.83$**
Proline concentration	$F = 0.579$ $p = 0.452$ $R^2 = 0.02$	$F = 0.921$ $p = 0.344$ $R^2 = 0.03$	$F = 0.827$ $p = 0.370$ $R^2 = 0.03$	$F = 1.474$ $p = 0.234$ $R^2 = 0.05$	$F = 0.779$ $p = 0.657$ **$R^2 = 0.23$**
Proline proportion	$F = 0.655$ $p = 0.427$ $R^2 = 0.02$	$F = 0.655$ $p = 0.424$ $R^2 = 0.02$	$F = 0.364$ $p = 0.551$ $R^2 = 0.01$	$F = 2.399$ $p = 0.131$ $R^2 = 0.07$	$F = 1.091$ $p = 0.413$ **$R^2 = 0.29$**
Total AA concentration	$F = 0.136$ $p = 0.715$ $R^2 < 0.01$	$F = 0.193$ $p = 0.664$ $R^2 = 0.01$	$F = 1.281$ $p = 0.267$ $R^2 = 0.04$	$F = 0.026$ $p = 0.873$ $R^2 < 0.01$	$F = 1.425$ $p = 0.241$ **$R^2 = 0.34$**
Total EAA concentration	$F = 0.372$ $p = 0.547$ $R^2 = 0.01$	$F = 1.653$ $p = 0.209$ $R^2 = 0.04$	$F = 0.087$ $p = 0.770$ $R^2 < 0.01$	$F = 2.270$ $p = 0.146$ $R^2 = 0.05$	$F = 1.718$ $p = 0.158$ **$R^2 = 0.30$**
Total non-EAA concentration	$F = 0.028$ $p = 0.869$ $R^2 < 0.01$	$F = 0.126$ $p = 0.725$ $R^2 < 0.01$	$F = 1.360$ $p = 0.252$ $R^2 = 0.04$	$F = 0.699$ $p = 0.410$ $R^2 = 0.02$	$F = 1.589$ $p = 0.174$ **$R^2 = 0.37$**
Total EAA proportion	$F = 0.484$ $p = 0.492$ $R^2 = 0.02$	$F = 0.350$ $p = 0.558$ $R^2 = 0.01$	$F = 0.018$ $p = 0.893$ $R^2 < 0.01$	$F = 1.924$ $p = 0.175$ $R^2 = 0.06$	$F = 4.091$ ***$p = 0.003$*** **$R^2 = 0.62$**

Table S4 Statistical results of generalized linear (mixed) models (GL(M)Ms) testing for effects of morphological factors (flower symmetry, nectar access, inflorescence area, flower height) and family on variation in individual and total carbohydrate concentrations and proportions. Given are the *F*- and *p*-values as well as the R^2-values for each model. *P*-values below 0.05 are marked in italics. *P*-values below the significance level of 0.01 are additionally marked in bold. R^2-values above 0.1 are marked in bold as well. If GLMMs were used, plot nested in plant species was used as random factor.

Carbohydrate	Flower symmetry	Nectar access	Inflorescence area	Flower height	Family
Fructose concentration	$F = 1.042$ $p = 0.315$ $R^2 = 0.03$	$F = 4.347$ *p = 0.045* **$R^2 = 0.12$**	$F = 0.077$ $p = 0.783$ $R^2 < 0.01$	$F = 0.033$ $p = 0.858$ $R^2 < 0.01$	$F = 0.661$ $p = 0.758$ **$R^2 = 0.19$**
Fructose proportion	$F = 0.601$ $p = 0.444$ $R^2 = 0.02$	$F = 0.011$ $p = 0.919$ $R^2 < 0.01$	$F = 2.533$ $p = 0.121$ $R^2 = 0.07$	$F = 0.980$ $p = 0.330$ $R^2 = 0.03$	$F = 3.813$ ***p = 0.004*** **$R^2 = 0.58$**
Glucose concentration	$F = 0.929$ $p = 0.343$ $R^2 = 0.03$	$F = 4.537$ *p = 0.041* **$R^2 = 0.13$**	$F = 0.074$ $p = 0.787$ $R^2 < 0.01$	$F = 0.014$ $p = 0.907$ $R^2 < 0.01$	$F = 0.738$ $p = 0.692$ **$R^2 = 0.21$**
Glucose proportion	$F = 0.204$ $p = 0.655$ $R^2 = 0.01$	$F = 0.108$ $p = 0.744$ $R^2 < 0.01$	$F = 4.325$ *p = 0.046* **$R^2 = 0.12$**	$F = 0.059$ $p = 0.809$ $R^2 < 0.01$	$F = 5.805$ ***p < 0.001*** **$R^2 = 0.67$**
Sucrose concentration	$F = 0.365$ $p = 0.550$ $R^2 = 0.01$	$F = 1.179$ $p = 0.286$ $R^2 = 0.04$	$F = 2.626$ $p = 0.115$ $R^2 = 0.08$	$F = 1.710$ $p = 0.200$ $R^2 = 0.05$	$F = 2.006$ $p = 0.085$ **$R^2 = 0.42$**
Sucrose proportion	$F = 0.454$ $p = 0.506$ $R^2 = 0.01$	$F = 0.060$ $p = 0.808$ $R^2 < 0.01$	$F = 4.276$ *p = 0.047* **$R^2 = 0.12$**	$F = 0.059$ $p = 0.809$ $R^2 < 0.01$	$F = 5.805$ ***p < 0.001*** **$R^2 = 0.67$**
Total carboyhdrate concentration	$F = 1.112$ $p = 0.300$ $R^2 = 0.03$	$F = 4.629$ *p = 0.039* **$R^2 = 0.13$**	$F = 1.224$ $p = 0.277$ $R^2 = 0.04$	$F = 0.391$ $p = 0.536$ $R^2 = 0.01$	$F = 0.585$ $p = 0.820$ **$R^2 = 0.17$**

Table S5 Plant species studied (Roscher *et al*. 2004) for their nectar amino acid and carbohydrate composition (plant species in italic and families in bold letters) with flower visitors recorded at different plots of the Jena Experiment in 2011 (for further details see Venjakob *et al*. 2016). Flower visitors were grouped as: B=beetles, BB=bumblebees, BF=butterflies, F=flies, HB=honeybees, HF=hoverflies, SB=solitary bees, W=wasps. Na (not available) indicates that a plant species was not flowering when visitor observations took place.

Plant family	**Observed flower visitor groups**							
Plant species	**HB**	**BB**	**SB**	**W**	**HF**	**F**	**BF**	**B**
Apiaceae								
Anthriscus sylvestris					na			
Daucus carota	-	-	-	-	-	F	-	-
Heracleum sphondylium	HB	-	SB	W	HF	F	BF	B
Pastinaca sativa	HB	-	SB	W	HF	F	-	B
Pimpinella major		-	SB	W	HF	F	-	B
Asteraceae								
Centaurea jacea	HB	BB	SB	-	-	-	-	-
Cirsium oleraceum		BB	-	-	-	-	-	-
Crepis biennis	HB	BB	SB	-	HF	F	-	B
Leontodon autumnalis	HB	-	-	-	HF	F	BF	-
Leontodon hispidus	HB	BB	-	-	HF	F	-	B
Taraxacum officinale	HB	-	-	-	-	F	-	B
Tragopogon pratensis					na			
Brassicaceae								
Cardamine pratensis	HB	-	-	-	-	F	-	-
Campanulaceae								
Campanula patula	-	BB	SB	-	HF	F	-	B
Dipsacaceae								
Knautia arvensis	HB	BB	SB	W	HF	F	BF	B
Fabaceae								
Lathyrus pratensis	HB	BB	SB					
Lotus corniculatus	HB	BB	SB	-	HF	F	BF	-
Medicago varia	HB	BB	SB	W	HF	F	BF	-
Onobrychis viciifolia	HB	BB	SB	-	HF	F	-	B
Trifolium campestre					na			
Trifolium fragiferum					na			
Trifolium hybridum	HB	BB	SB	W	HF	F	-	-
Trifolium pratense	HB	BB	SB	W	HF	F	BF	B
Trifolium repens	HB	BB	SB	-	HF	F	-	-
Vicia cracca	HB	BB	SB	-	HF	F	BF	-
Geraniaceae								
Geranium pratense	HB	BB	SB	W	HF	F	-	B
Lamiaceae								
Ajuga reptans	-	BB	-	-	-	-	-	B
Glechoma hederacea	HB	BB	SB	-	-	F	-	-
Prunella vulgaris	-	BB	-	-	HF	-	-	-
Primulaceae								
Primula veris	-	BB	SB	-	-	F	-	-
Ranunculaceae								
Ranunculus acris	HB	-	SB	-	HF	F	-	B
Ranunculus repens	-	-	SB	-	HF	F	-	-
Rosaceae								
Sanguisorba officinalis	-	-	-	-	-	F	-	-
Scrophulariaceae								
Veronica chamaedrys	-	-	SB	-	HF	F	-	-

Table S6 Morphological traits of flowers and accessibility of pollen and/or nectar. Classified either by flower type (after Kugler 1970) or flower class (Mueller 1881); with typical pollinator guilds (Mueller 1881) listed following (Mueller 1881 supplemented with personal notes for Fabaceae, Lamiaceae, and Scrophulariaceae; Kugler 1970, data obtained from "BiolFlor - a new plant-trait database as a tool for plant invasion ecology" 2004).

Plant family	Plant species	Flower type after Kugler (1970)	Flower class after Mueller (1881)	Typical pollinators after Mueller (1881)
Apiaceae	*Anthriscus sylvestris*	disk flowers with nectar open	flowers with open nectar	beetles, flies, syrphids, wasps, medium tongued bees
Apiaceae	*Daucus carota*	disk flowers with nectar open	flowers with open nectar	beetles, flies, syrphids, wasps, medium tongued bees
Apiaceae	*Heracleum sphondylium*	disk flowers with nectar open	flowers with open nectar	beetles, flies, syrphids, wasps, medium tongued bees
Apiaceae	*Pastinaca sativa*	disk flowers with nectar open	flowers with open nectar	beetles, flies, syrphids, wasps, medium tongued bees
Apiaceae	*Pimpinella major*	disk flowers with nectar open	flowers with open nectar	beetles, flies, syrphids, wasps, medium tongued bees
Asteraceae	*Centaurea jacea*	flower heads, Asteraceae, only disk flowers	flower associations with totally hidden nectar	bees, bumblebees, wasps, bombylides, syrphids
Asteraceae	*Cirsium oleraceum*	flower heads, Asteraceae, only disk flowers	flower associations with totally hidden nectar	bees, bumblebees, wasps, bombylides, syrphids
Asteraceae	*Crepis biennis*	flower heads, Asteraceae, only ray flowers	flower associations with totally hidden nectar	bees, bumblebees, wasps, bombylides, syrphids
Asteraceae	*Leontodon autumnalis*	flower heads, Asteraceae, only ray flowers	flower associations with totally hidden nectar	bees, bumblebees, wasps, bombylides, syrphids
Asteraceae	*Leontodon hispidus*	flower heads, Asteraceae, only ray flowers	flower associations with totally hidden nectar	bees, bumblebees, wasps, bombylides, syrphids
Asteraceae	*Taraxacum officinale*	flower heads, Asteraceae, only ray flowers	flower associations with totally hidden nectar	bees, bumblebees, wasps, bombylides, syrphids
Asteraceae	*Tragopogon pratensis*	flower heads, Asteraceae, only ray flowers	flower associations with totally hidden nectar	bees, bumblebees, wasps, bombylides, syrphids
Brassicaceae	*Cardamine pratensis*	disk flowers with nectar ± hidden nectaries at base of stamens	flowers with totally hidden nectar	bees, bumblebees, wasps, bombylides, syrphids
Campanulaceae	*Campanula patula*	bell shaped flowers with sticky pollen	bee flowers; hidden nectar (personal note)	bees
Dipsacaceae	*Knautia arvensis*	flower heads, non-Asteraceae	flower associations with totally hidden nectar	bees, bumblebees, wasps, bombylides, syrphids
Fabaceae	*Lathyrus pratensis*	flag blossom, Fabaceae type, explosive mechanism	hymenoptere flowers; hidden nectar (personal note)	hymenopteres
Fabaceae	*Lotus corniculatus*	flag blossom, Fabaceae type, brush mechanism	bee flowers; hidden nectar (personal note)	bees
Fabaceae	*Medicago varia*	flag blossom, Fabaceae type, explosive mechanism	hymenoptere flowers; hidden nectar (personal note)	hymenopteres
Fabaceae	*Onobrychis viciifolia*	flag blossom, Fabaceae type, valvular mechanism	hymenoptere flowers; hidden nectar (personal note)	hymenopteres
Fabaceae	*Trifolium campestre*	flag blossom, Fabaceae type, valvular mechanism	hymenoptere flowers; hidden nectar (personal note)	hymenopteres
Fabaceae	*Trifolium fragiferum*	flag blossom, Fabaceae type, valvular mechanism	hymenoptere flowers; hidden nectar (personal note)	hymenopteres
Fabaceae	*Trifolium hybridum*	flag blossom, Fabaceae type, valvular mechanism	hymenoptere flowers; hidden nectar (personal note)	hymenopteres
Fabaceae	*Trifolium pratense*	flag blossom, Fabaceae type, valvular mechanism	bumble bee flowers; hidden nectar (personal note)	bumblebees
Fabaceae	*Trifolium repens*	flag blossom, Fabaceae type, valvular mechanism	bee flowers; hidden nectar (personal note)	bees
Fabaceae	*Vicia cracca*	flag blossom, Fabaceae type, explosive mechanism	hymenoptere flowers; hidden nectar (personal note)	hymenopteres
Geraniaceae	*Geranium pratense*	disk flowers with nectar ± hidden nectaries at base of stamens	flowers with totally hidden nectar	bees, bumblebees, wasps, bombylides, syrphids
Lamiaceae	*Ajuga reptans*	true lip flowers	bumble bee flowers; hidden nectar (personal note)	bumblebees
Lamiaceae	*Glechoma hederacea*	true lip flowers	bumble bee flowers; hidden nectar (personal note)	bumblebees
Lamiaceae	*Prunella vulgaris*	true lip flowers	hymenoptere flowers; hidden nectar (personal note)	hymenopteres
Primulaceae	*Primula veris*	stalk disc flowers, stamina and pistil within tube	transition type bumble bee flowers - butterfly flowers	bumblebees, butterflies
Ranunculaceae	*Ranunculus acris*	disk flowers with nectar ± hidden nectaries at base of petals	flowers with partly hidden nectar	syrphids, bees, butterflies
Ranunculaceae	*Ranunculus repens*	disk flowers with nectar ± hidden nectaries at base of petals	flowers with partly hidden nectar	syrphids, bees, butterflies
Rosaceae	*Sanguisorba officinalis*	disk flowers with nectar ± hidden in centre of flower	flowers with partly hidden nectar	syrphids, bees, butterflies
Scrophulariaceae	*Veronica chamaedrys*	lip flowers, Verbascum type	syrphid flowers; open nectar (personal note)	syrphids

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Mueller H. (1881) *Alpenblumen: ihre Befruchtung durch Insekten und ihre Anpassung an dieselben*. Verlag von Wilhelm Engelmann; Leipzig: pp. 611.

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Appendix C

Inter-individual nectar quality changes of Field Scabious *Knautia arvensis*

Supplementary table S1: List of plant species communities with one, four, eight, and 16 plant species.

Methods of flower-visitor observation (for Table S2 and Table S3)

Supplementary table S2: List of individual flower visitors to all *Knautia arvensis* in plant species communities with eight and 16 plant species observed in 2011.

Supplementary table S3. Total numbers, species richness, guild numbers and Shannon diversity of flower visitors to *Knautia arvensis* growing in plant species communities of four, eight, and 16 plant species.

Supplementary table S4: List of figures presenting the mean [± SD] concentrations and proportions of individual amino acids and carbohydrates as well as total amino acids (AA), all essential amino acids (EAA) and all non-essential amino acids (nEAA) as found in floral nectar of *Knautia arvensis* from different plant species mixtures.

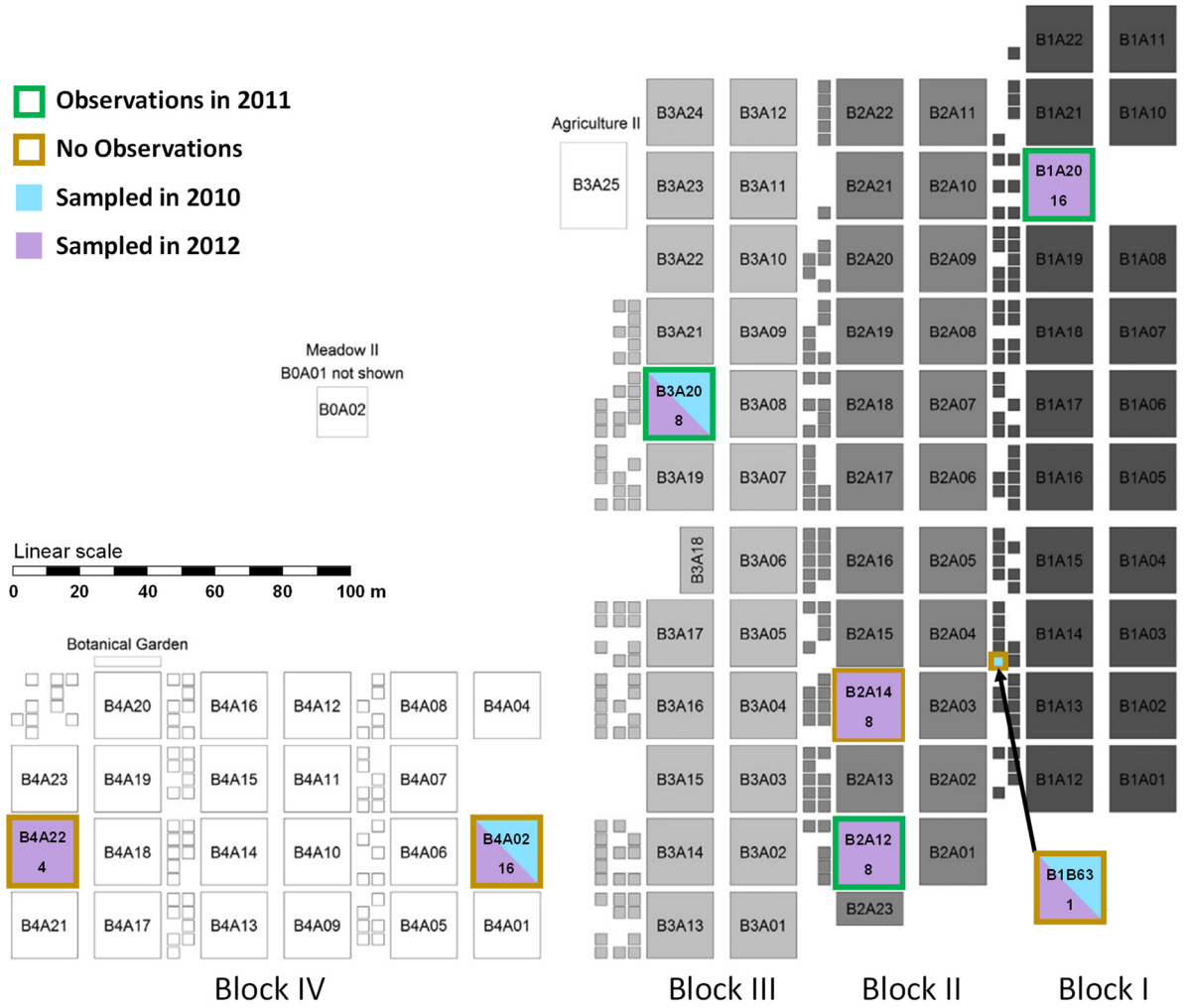


Figure S1. Map displaying sizes and locations of plots sampled in this experiment (modified after [1]). Plot are distributed in four blocks which are perpendicular to the river Saale (to the right). Plots used for pollinator observations in 2011 are indicated by a green frame, no observations by a brown frame. Year of nectar sampling is depicted by colour of the plot filling (blue indicate plots sampled in 2010, violet plots in 2012); note that three plots (B3A20, B1B63, B4A02) were sampled in 2010 and 2012. Numbers below plot IDs in sampled/marked plots give the number of plant species grown in community.

Table S1. List of plant species communities with one, four, eight, and 16 plant species, providing plot IDs, sampling year, sucrose concentration [%] as measured for one sample with a hand-held refractometer, and the numbers and actual species of other plant species flowering and not flowering when *Knautia arvensis* was flowering in 2011. Nectar sampling was performed in 2010 and /or 2012. n.a. indicates that there was no data available.

Plant species mixtures	Plot ID	Year of sampling	Sucrose [%]	Number of flowering plant species competing with *K. arvensis*	Flowering plant species	Not flowering plant species
1				0	-	-
	B1B063	2010	27			
		2012	32			
4				2	*Campanula patula*	*Cardamine pratensis*
	B4A22	2012	70			
					Geranium pratense	
8				5	*Anthriscus sylvestris*	*Heracleum sphondylium*
	B2A12	2012	60			
					Galium mollugo	*Sanguisorba officinalis*
					Geranium pratense	
					Leucanthemum vulgare	
					Ranunculus acris	
8				2	*Leontodon hispidus*	*Luzula campestris*
	B2A14	2012	41			
					Veronica chamaedrys	*Phleum pratense*
						Sanguisorba officinalis
						Trifolium dubium
						Trifolium hybridum
8				4	*Heracleum sphondylium*	*Campanula patula*
	B3A20	2010	n. a.			
					Lotus corniculatus	*Cardamine pratensis*
		2012	71			
					Trifolium fragiferum	*Trifolium campestre*
					Trifolium hybridum	
16				10	*Achillea millefolium*	*Ajuga reptans*
	B1A20	2012	57			
					Bellis perennis	*Leontodon autumnalis*
					Geranium pratense	*Medicago varia*
					Leontodon hispidus	*Sanguisorba officinalis*
					Lotus corniculatus	*Trifolium hybridum*
					Onobrychis viciifolia	
					Plantago lanceolata	
					Ranunculus acris	
					Trifolium repens	
					Veronica chamaedrys	
16				5	*Galium mollugo*	*Anthriscus sylvestris*
	B4A02	2010	30			
		2012	30		*Heracleum sphondylium*	*Arrhenatherum elatius*
					Leontodon hispidus	*Cynosurus cristatus*

	Pastinaca sativa	*Glechoma hederacea*
	Plantago media	
		Luzula campestris
		Phleum pratense
		Poa pratensis
		Ranunculus acris
		Ranunculus repens
		Taraxacum officinale

Observations of flower visitors (for Table S2 and Table S3)

We related plant species richness to the diversity and abundance of specific flower visitor guilds and the entire flower-visiting community of *Knautia arvensis* (L.) Coult.(Asteraceae) growing in different plant communities, because *K. arvensis* typically attracts many different flower visitors, including solitary bees, bumblebees, honeybees (*Apis mellifera* Linnaeus, 1758) and hoverflies [2–4].

Flower-visiting insects, e.g. honeybees, bumblebees, solitary bees and hoverflies, had been surveyed within the framework of the Jena Experiment on a subset of plots between May and August 2011 (see [5] for details on flower-visitor observations). Thus, nectar sampling and flower-visitor observations were performed at different years. We extracted all observations on flower visitors to *K. arvensis* for those plots for which we also had collected nectar, i.e. two 8-species plots and one 16–species plot. Flower visitors were grouped to honeybees, bumblebees, solitary bees and hoverflies for subsequent analyses, and we defined all solitary bees as non-eusocial Apidae [6]. We summed all flower visitors across all observations performed in 2011 and calculated per plot overall abundance and abundances for different groups as well as overall visitor species richness and the visitor species richness in each group, resulting in a sample size of three.

Notably, the observed and presented visitation patterns should not be considered representative for *Knautia arvensis* in general, as they only provide a snapshot for a largely artificial set-up and for only one year. This is also why this data is only provided as supplementary material.

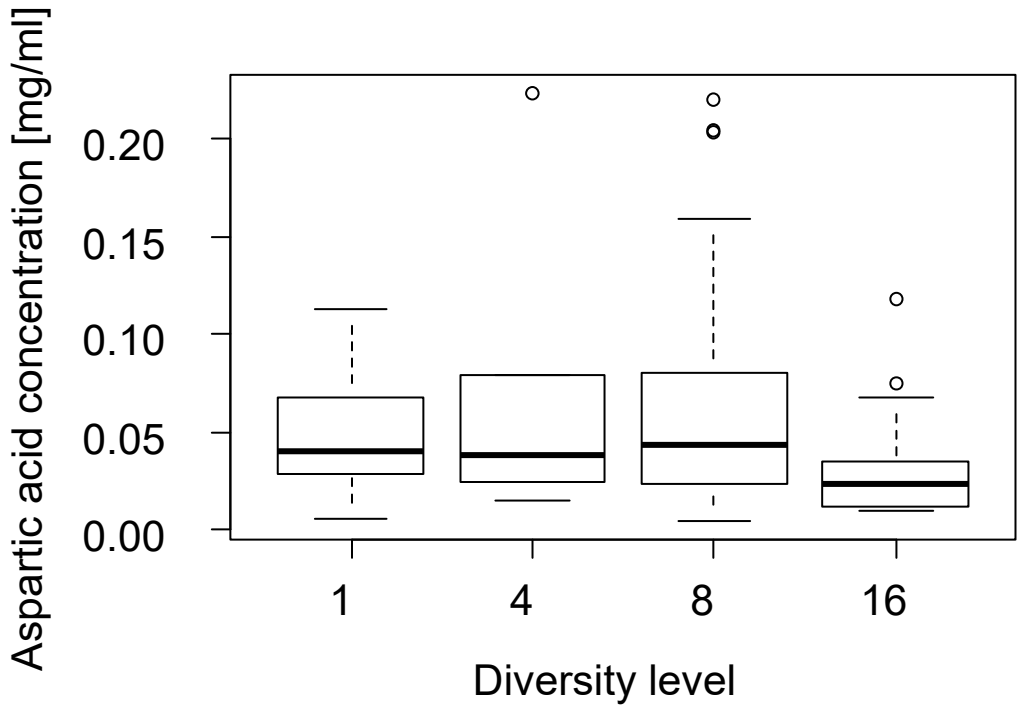
Table S2. List of individual flower visitors to all *Knautia arvensis* in plant species communities with eight and 16 plant species observed in 2011 (one plot per community with plot number in brackets for further details see [5]. Flower visitors are grouped into beetles, bumblebees, butterflies, flies, honeybees, hoverflies, solitary bees, and wasps (bold letters, alphabetical order). Note that nectar sampling was performed on the same plots, but in different years (2010 and 2012). Observations were performed on the same plots in 2011 from which nectar were taken in 2010 and 2012 for eight and 16 plant species communities.

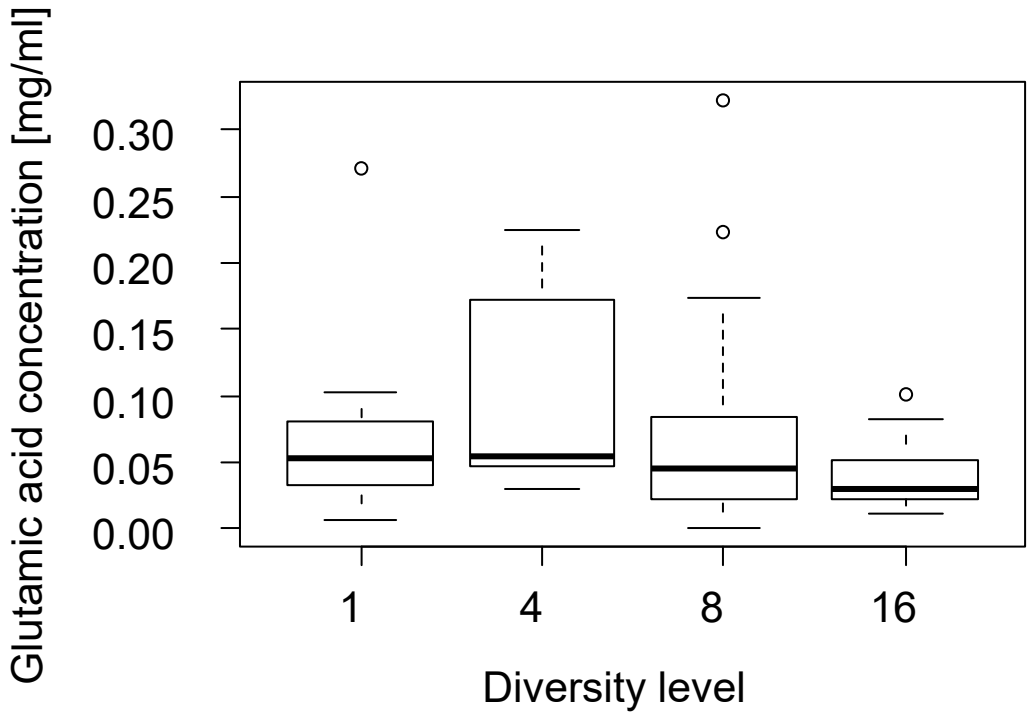
Flower visitors of *Knautia arvensis* per species community	**8 (B3A20)**	**8 (B2A12)**	**16 (B1A20)**
Beetles	-	-	-
Coleoptera unidentified	-	3	9
Bumblebees			
Bombus lapidarius (Linnaeus, 1758)	14	39	31
Bombus pascuorum (Scopoli, 1763)	-	1	1
Bombus pratorum (Linnaeus, 1761)	8	-	1
Bombus soroeensis (Fabricius, 1776)	-	2	-
Bombus sylvarum (Linnaeus, 1761)	1	-	-
Bombus terrestris (Linnaeus, 1758)	5	4	2
Bombus veteranus (Fabricius, 1793)	-	-	1
Bombus spec.	-	3	-
Butterflies			
Araschnia levana (Linnaeus, 1758)	-	-	2
Pieris spec.	-	1	1
Lepidoptera unidentified	-	1	7
Flies			
Bombylius venosus (Mikan, 1796)	-	3	-
Brachycera unidentified	-	2	2
Honeybees			
Apis mellifera (Linnaeus, 1758)	195	230	303
Hoverflies			
Episyrphus balteatus (Dujardin, 1842)	-	3	6
Eristalis jugorum (Egger, 1858)	-	-	-
Eristalis tenax (Linnaeus, 1758)	-	2	-
Melanostoma mellinum (Linnaeus, 1758)	1	-	2
Scaeva pyrastri (Linnaeus, 1758)	2	-	-
Syritta pipiens (Linnaeus, 1758)	1	-	-
Syrphidae unidentified	2	3	9
Solitary bees			
Andrena cineraria (Linnaeus, 1758)	-	-	-
Andrena hattorfiana (Fabricius, 1775)	-	-	-
Halictus scabiosae (Rossi, 1790)	-	-	1
Lasioglossum calceatum (Scopoli, 1763)	-	-	1
Lasioglossum leucozonium (Schrank, 1781)	-	2	-
Lasioglossum villosulum (Kirby, 1802)	-	-	1
Solitary bees unidentified	3	-	3
Wasps			
Lindenius spec.	-	1	-

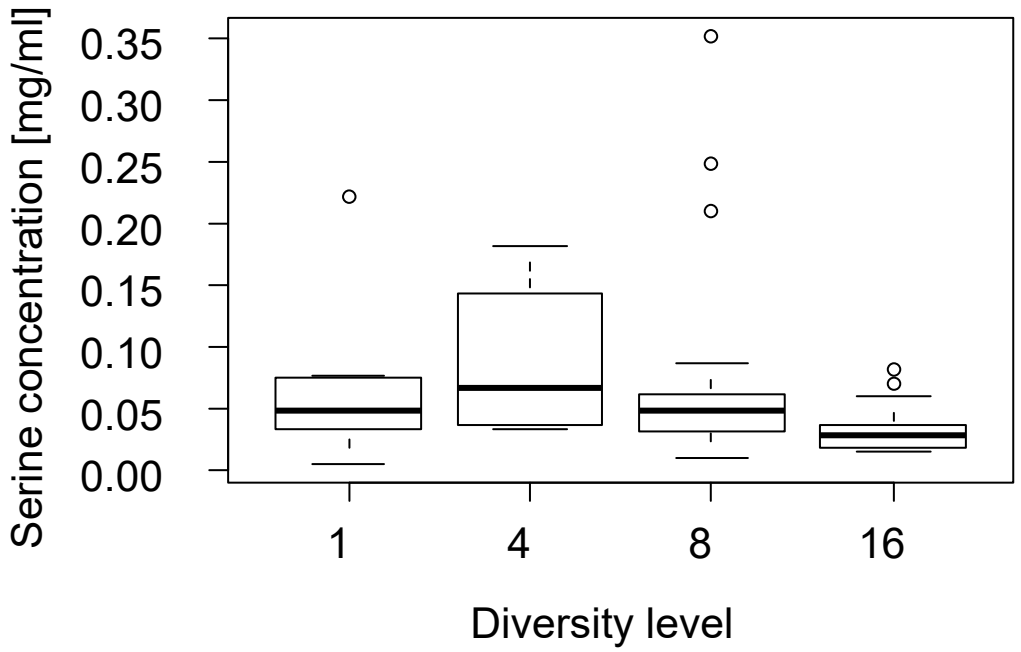
Table S3. Total numbers, species richness, guild numbers and Shannon diversity [7] of flower visitors to *Knautia arvensis* growing in plant species communities of four, eight, and 16 plant species.

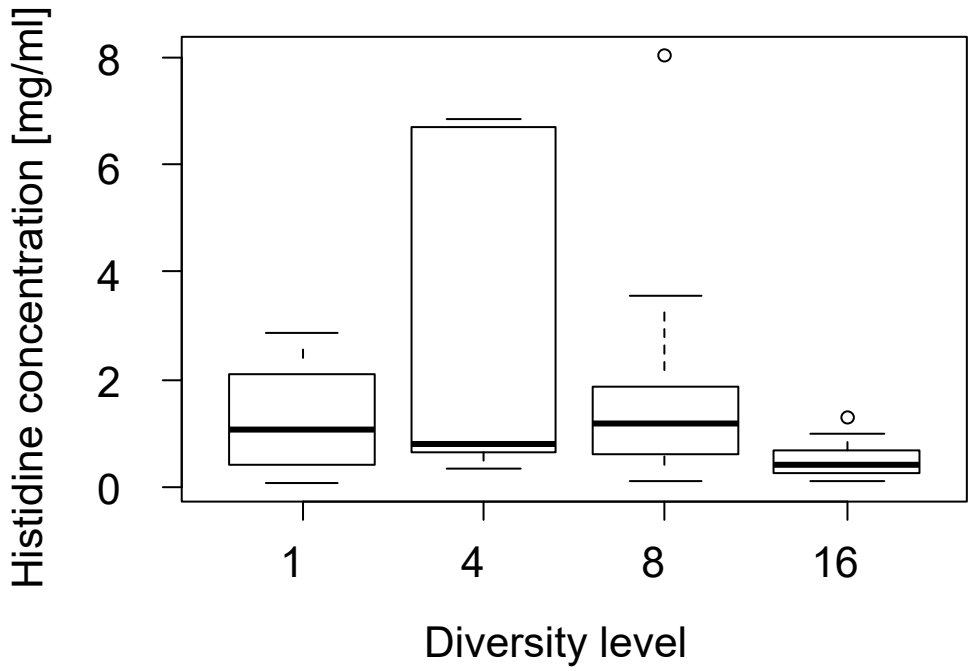
Plant species community	**8**	**8**	**16**
Shannon Index	**0.72**	**0.97**	**0.96**
Total numbers	**232**	**300**	**383**
Species richness of flower visitors	**9**	**12**	**15**
Beetles	-	3	9
Bumblebees	28	49	36
Butterflies	-	2	10
Flies	-	5	2
Honeybees	195	230	303
Hoverflies	6	8	17
Solitary bees	3	2	6
Wasps	-	1	-

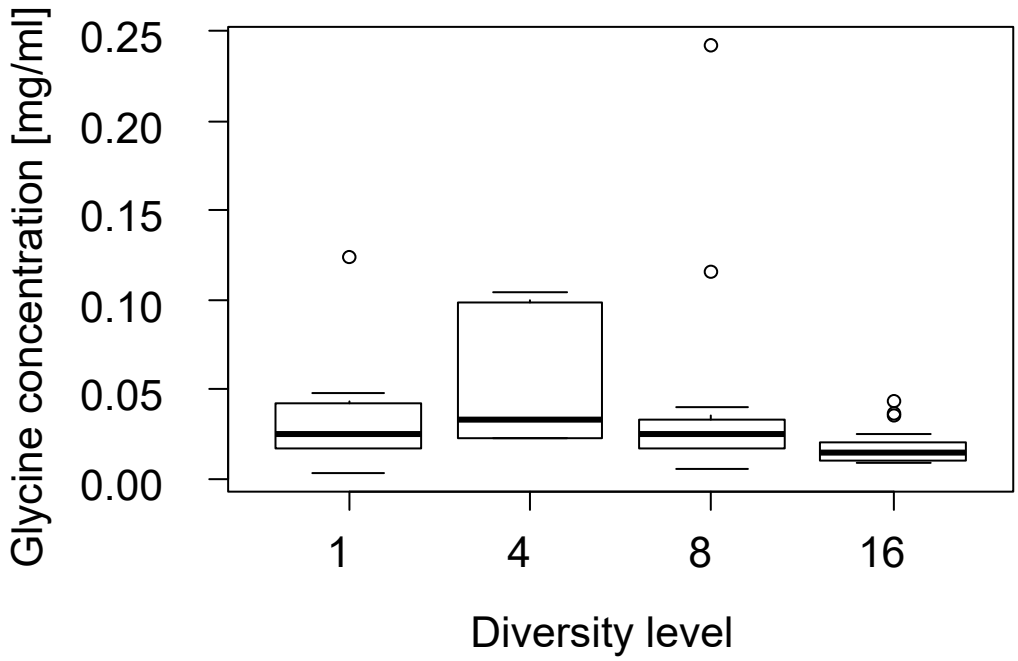
Figure S2. List of figures presenting the mean [± SD] concentrations and proportions of individual amino acids and carbohydrates as well as total amino acids (AA), all essential amino acids (EAA) and all non-essential amino acids (nEAA) as found in floral nectar of *Knautia arvensis* from different plant species mixtures, i.e. monoculture, four, eight, and 16 plant species mixtures. The figure correspond to Table 1.

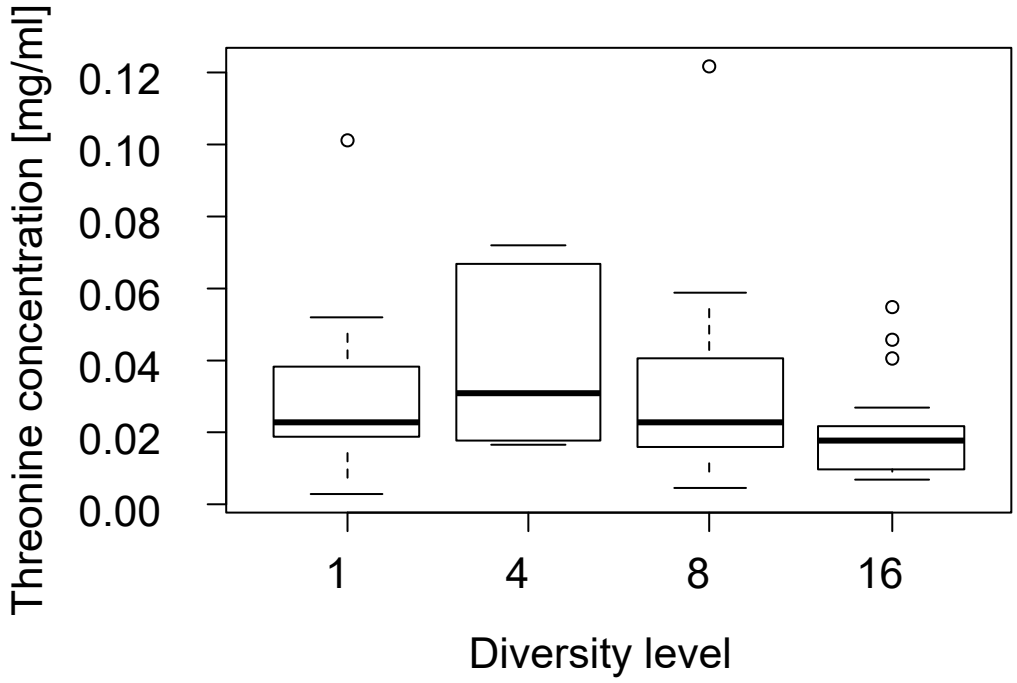


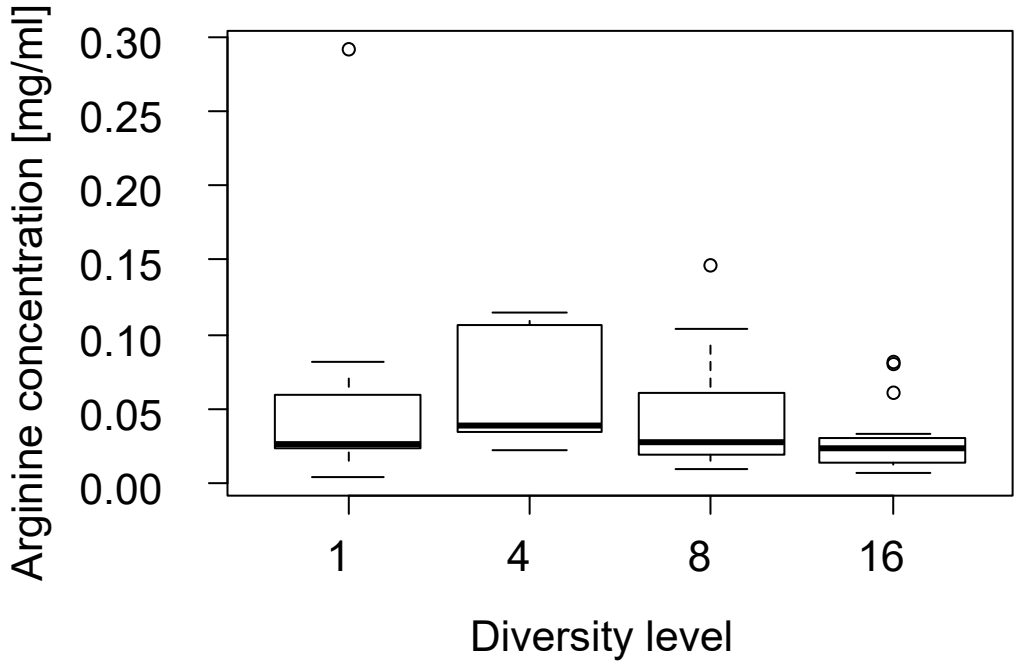

Glutamic acid concentration [mg/ml]
0.30
0.25
0.20
0.15
0.10
0.05
0.00
1
4
8
16
Diversity level

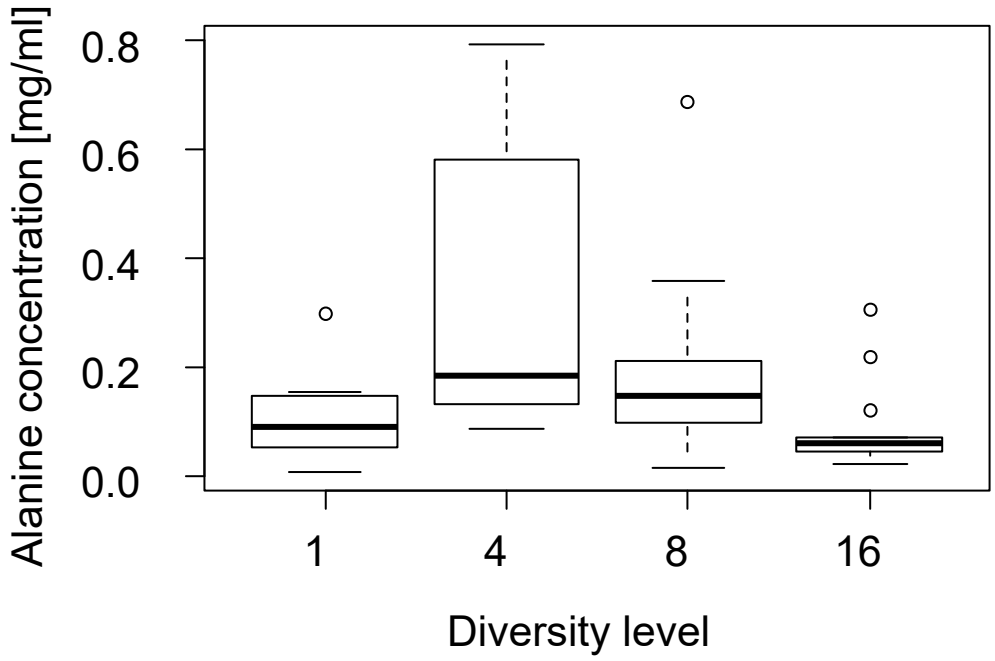

Serine concentration [mg/ml]
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
1
4
8
16
Diversity level

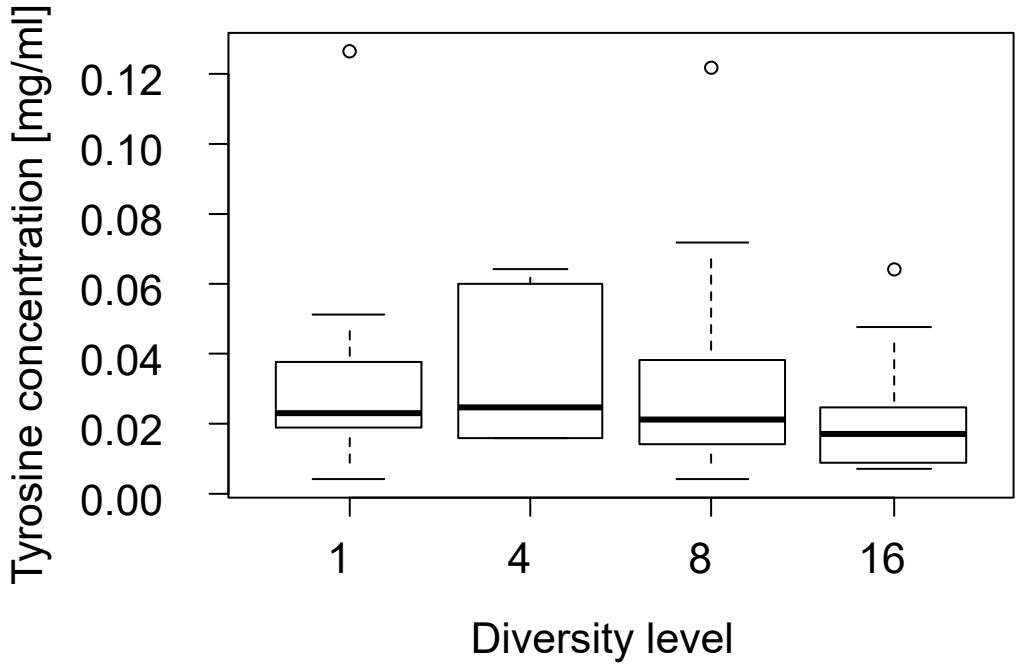

Histidine concentration [mg/ml]
0
2
4
6
8
1
4
8
16
Diversity level

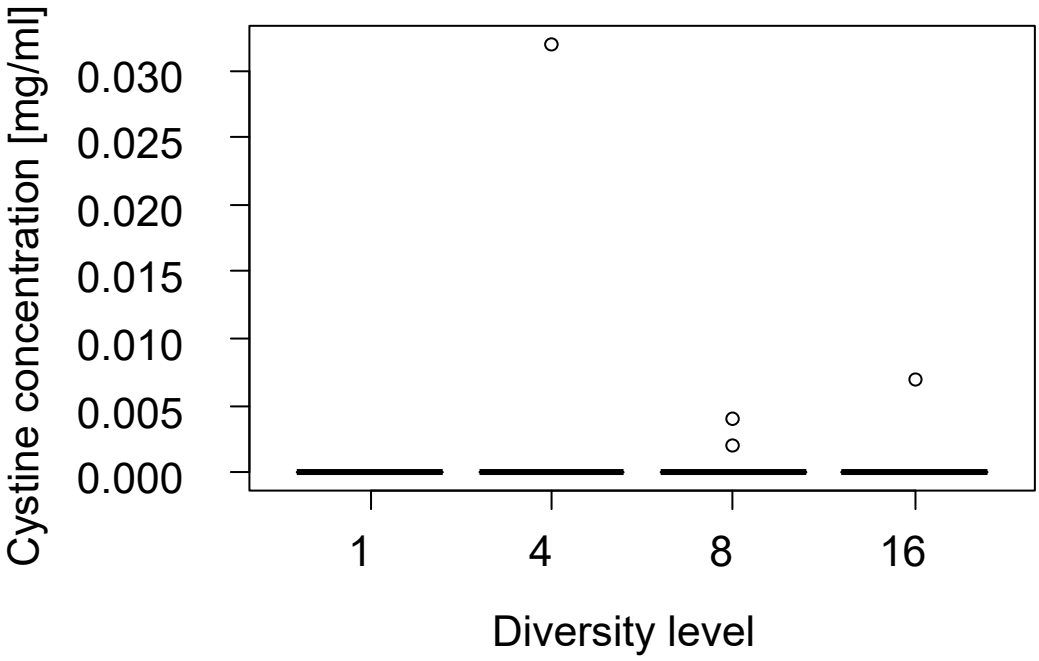

Glycine concentration [mg/ml]
0.00
0.05
0.10
0.15
0.20
0.25
1
4
8
16
Diversity level

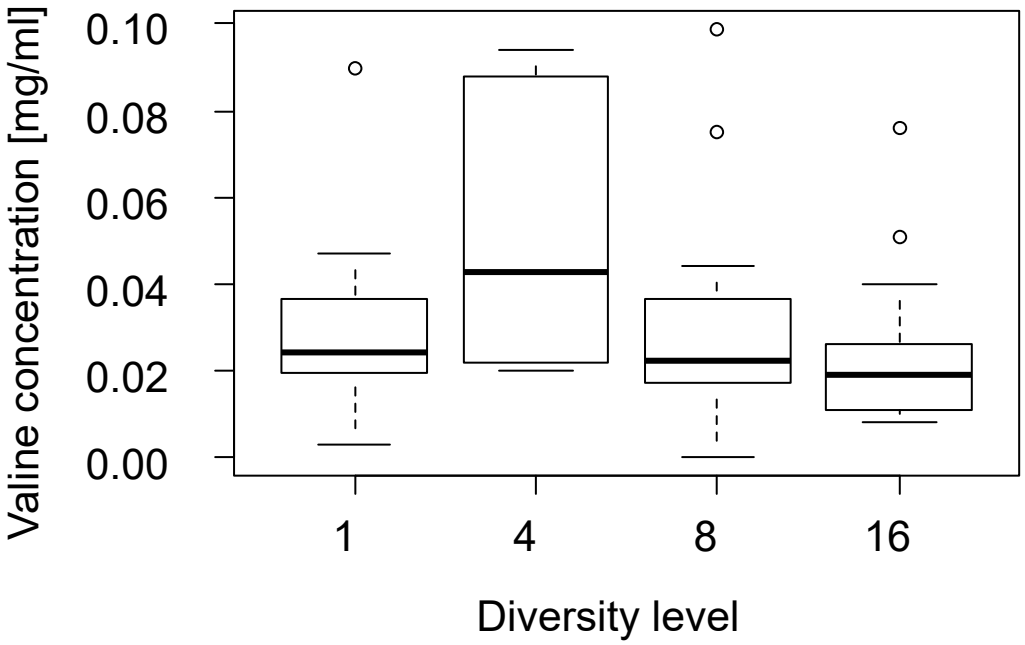

Threonine concentration [mg/ml]
0.12
0.10
0.08
0.06
0.04
0.02
0.00
1
4
8
16
Diversity level

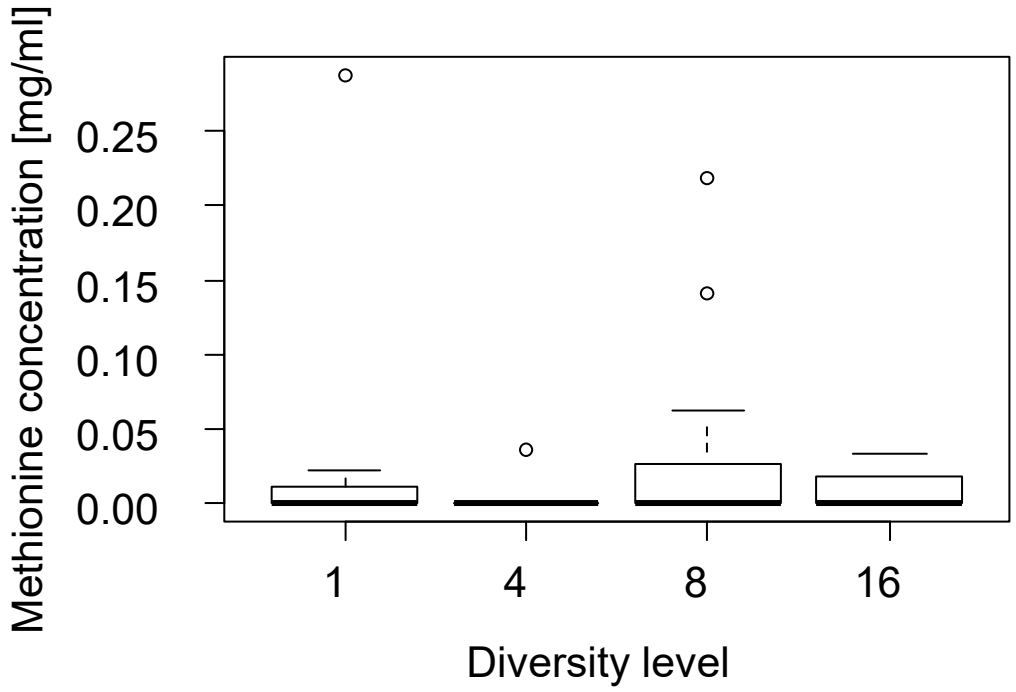

Arginine concentration [mg/ml]
0.30
0.25
0.20
0.15
0.10
0.05
0.00
1
4
8
16
Diversity level

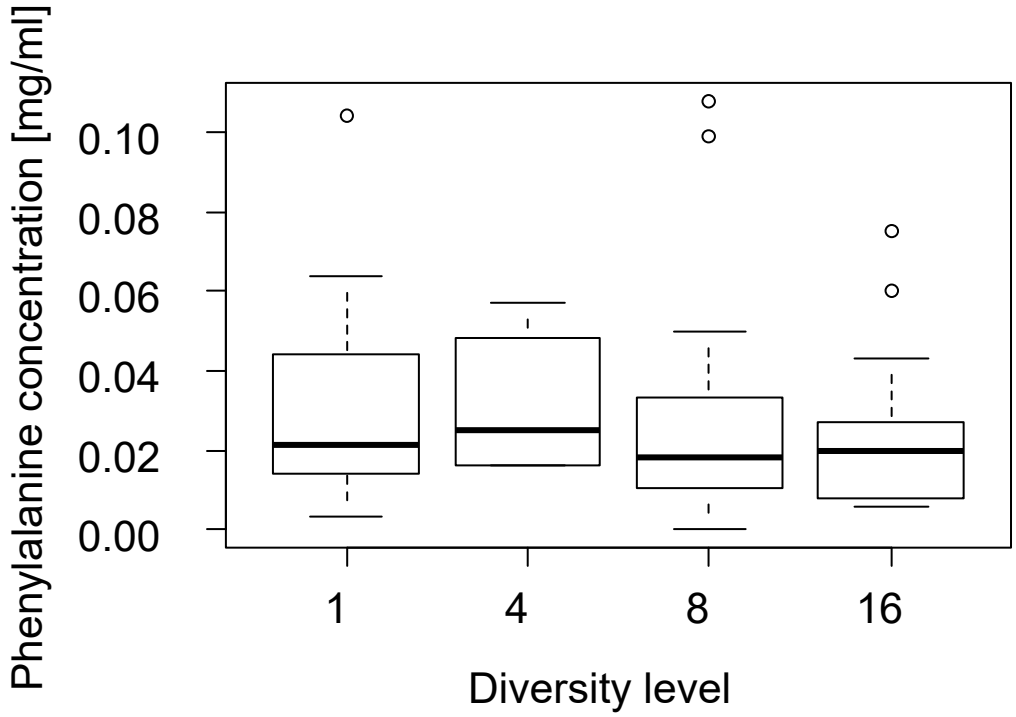

Alanine concentration [mg/ml]
0.0
0.2
0.4
0.6
0.8
1
4
8
16
Diversity level

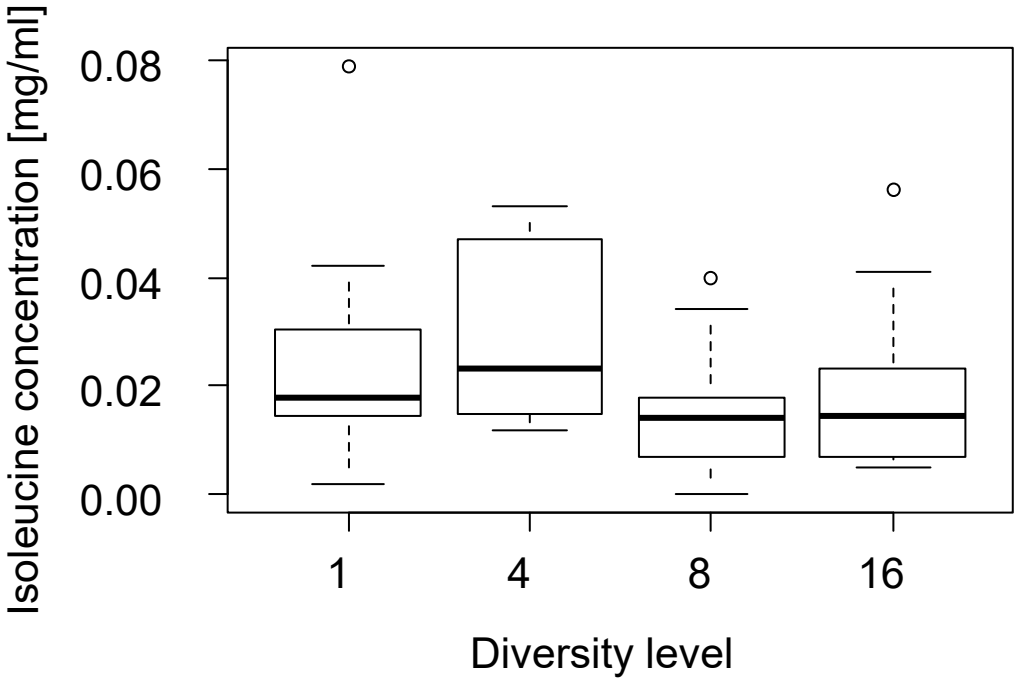

Tyrosine concentration [mg/ml]
0.00
0.02
0.04
0.06
0.08
0.10
0.12
1
4
8
16
Diversity level

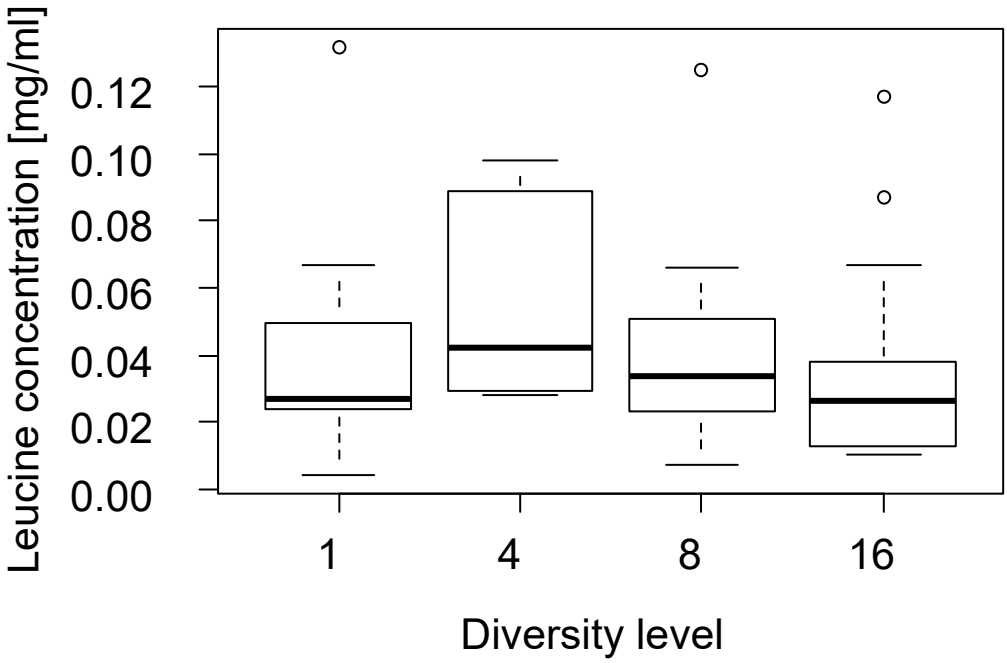

Cystine concentration [mg/ml]
0.030
0.025
0.020
0.015
0.010
0.005
0.000
1
4
8
16
Diversity level

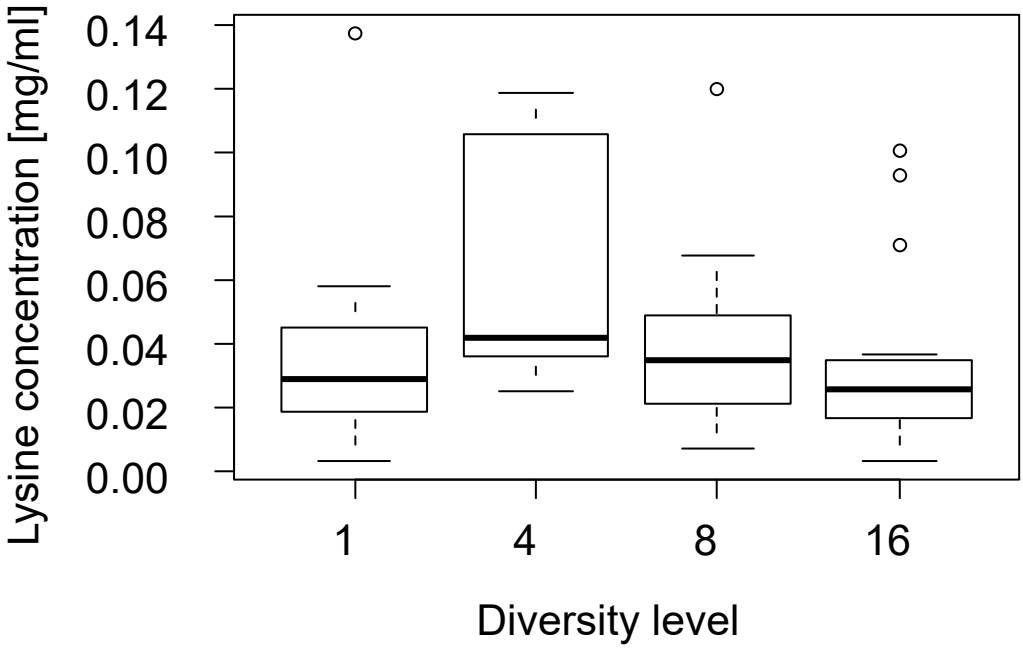

Valine concentration [mg/ml]
0.10
0.08
0.06
0.04
0.02
0.00
1
4
8
16
Diversity level

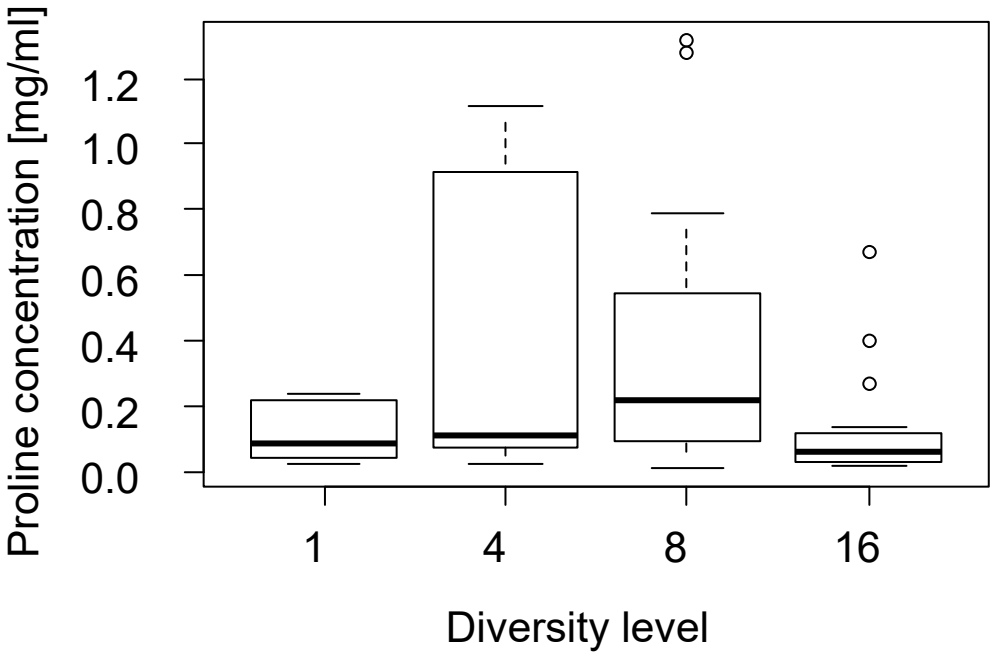

Methionine concentration [mg/ml]
0.25
0.20
0.15
0.10
0.05
0.00
1
4
8
16
Diversity level

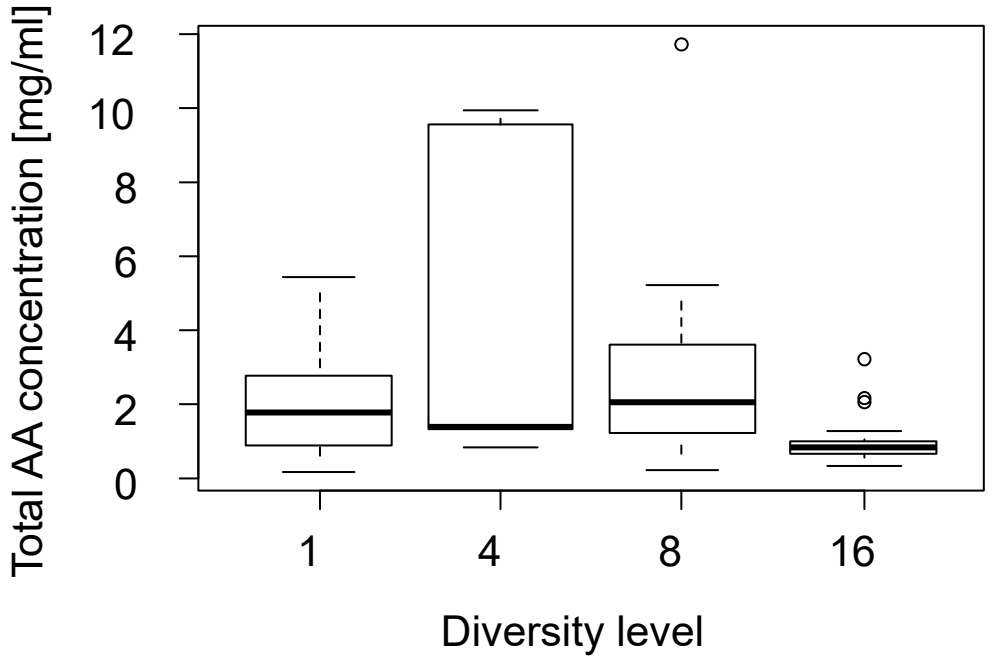

Phenylalanine concentration [mg/ml]
0.10
0.08
0.06
0.04
0.02
0.00
1
4
8
16
Diversity level

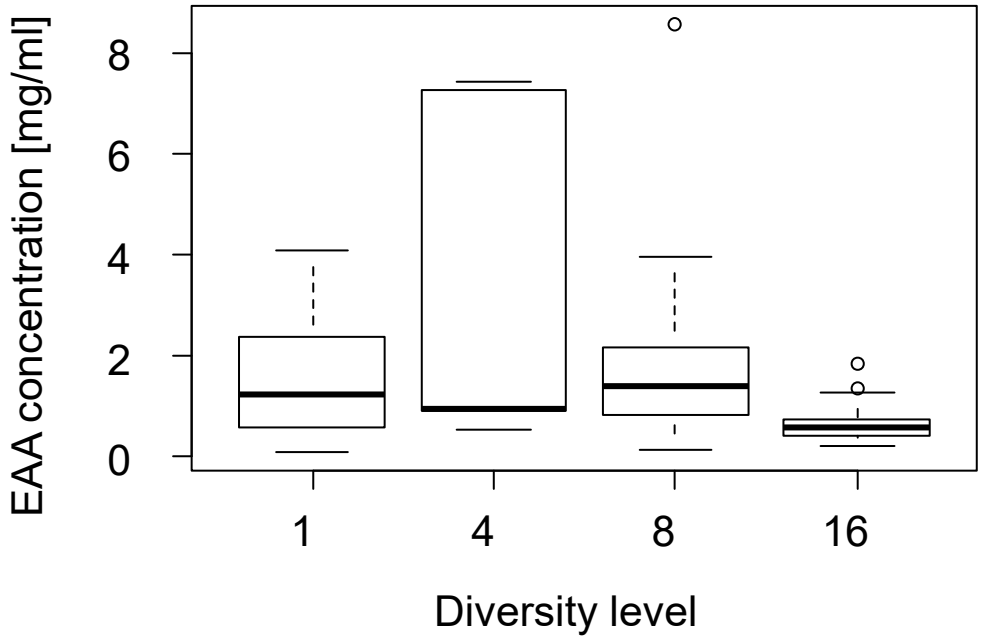

Isoleucine concentration [mg/ml]
0.08
0.06
0.04
0.02
0.00
1
4
8
16
Diversity level

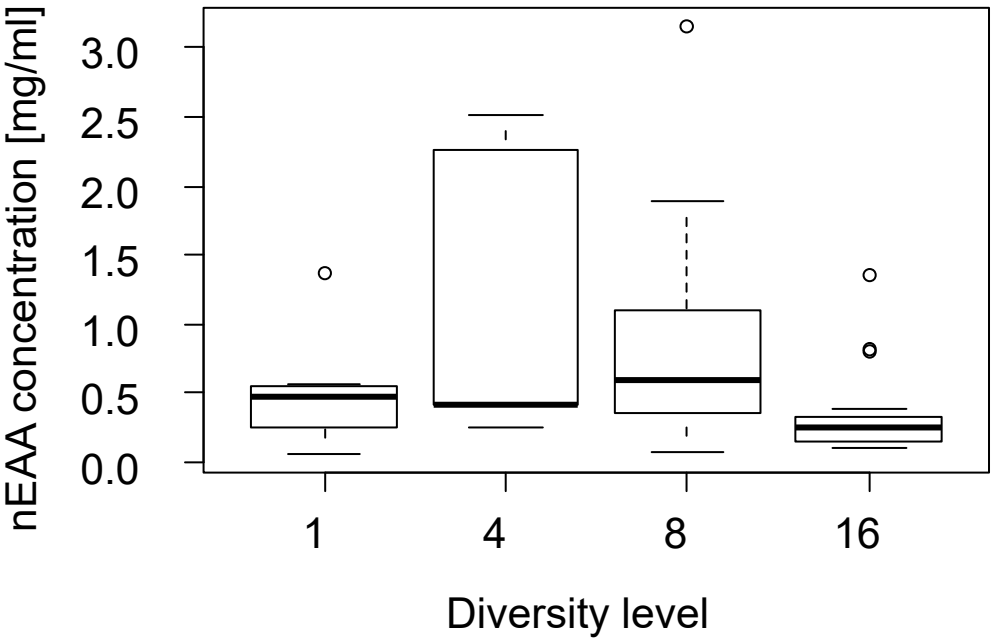

Leucine concentration [mg/ml]
0.12
0.10
0.08
0.06
0.04
0.02
0.00
1
4
8
16
Diversity level

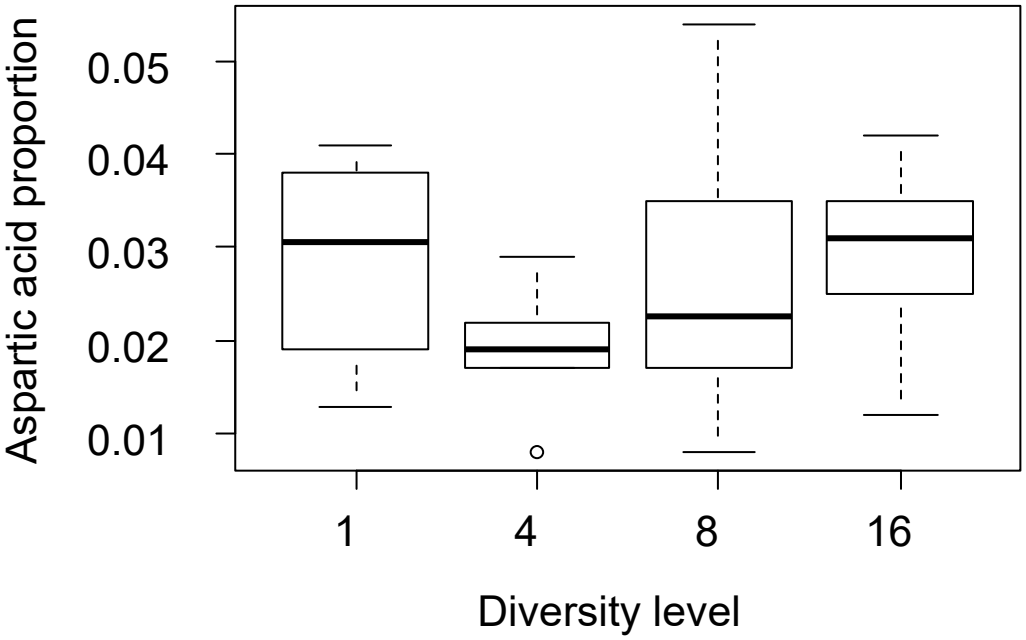

Lysine concentration [mg/ml]
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
1
4
8
16
Diversity level

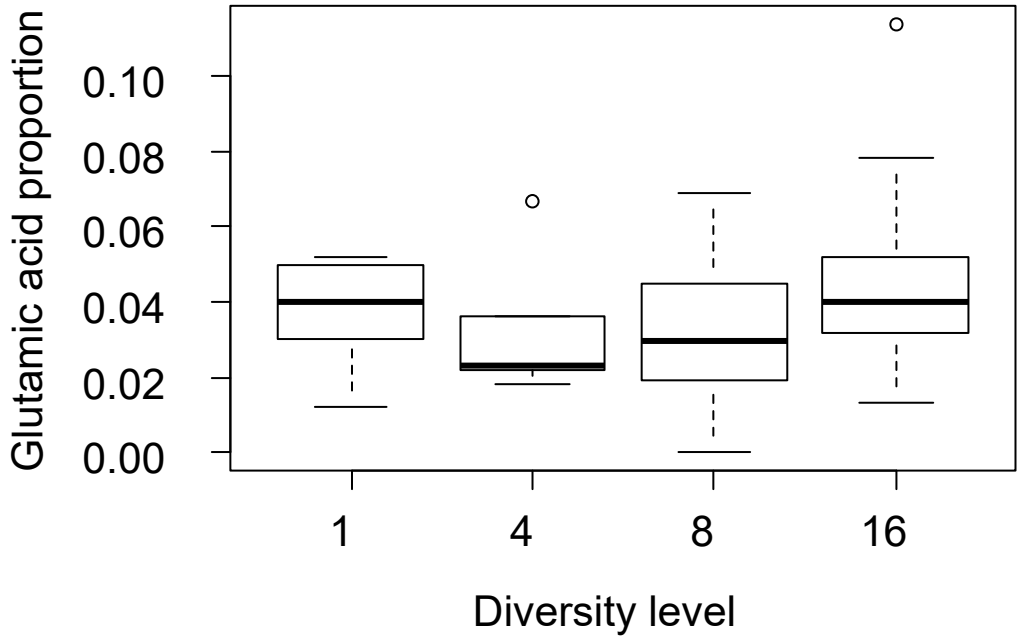

Proline concentration [mg/ml]
1.2
1.0
0.8
0.6
0.4
0.2
0.0
1
4
8
16
Diversity level

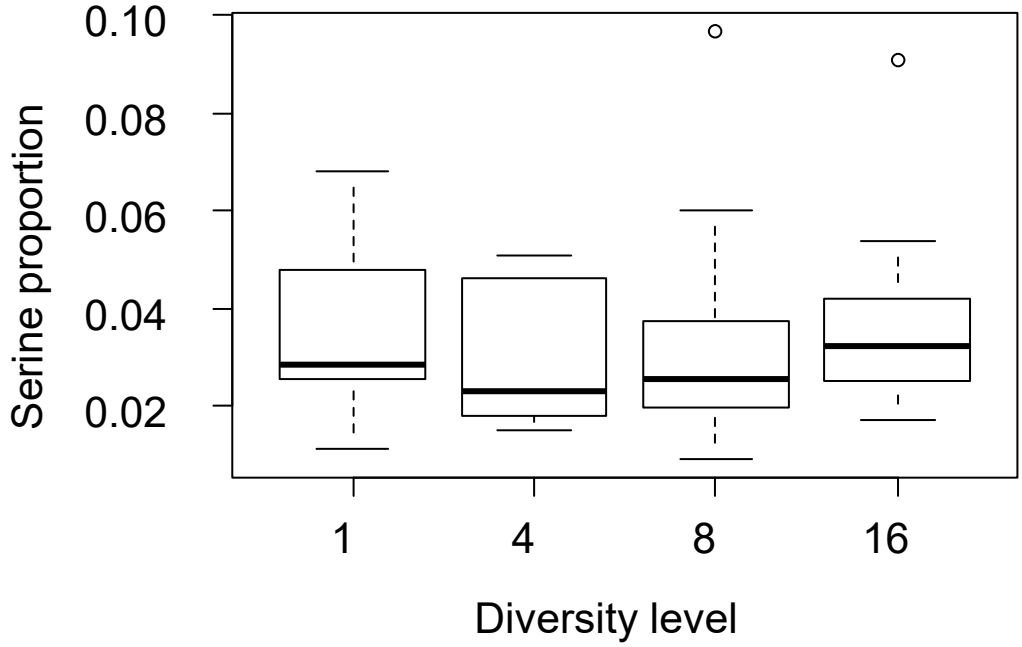

Total AA concentration [mg/ml]
12
10
8
6
4
2
0
1
4
8
16
Diversity level

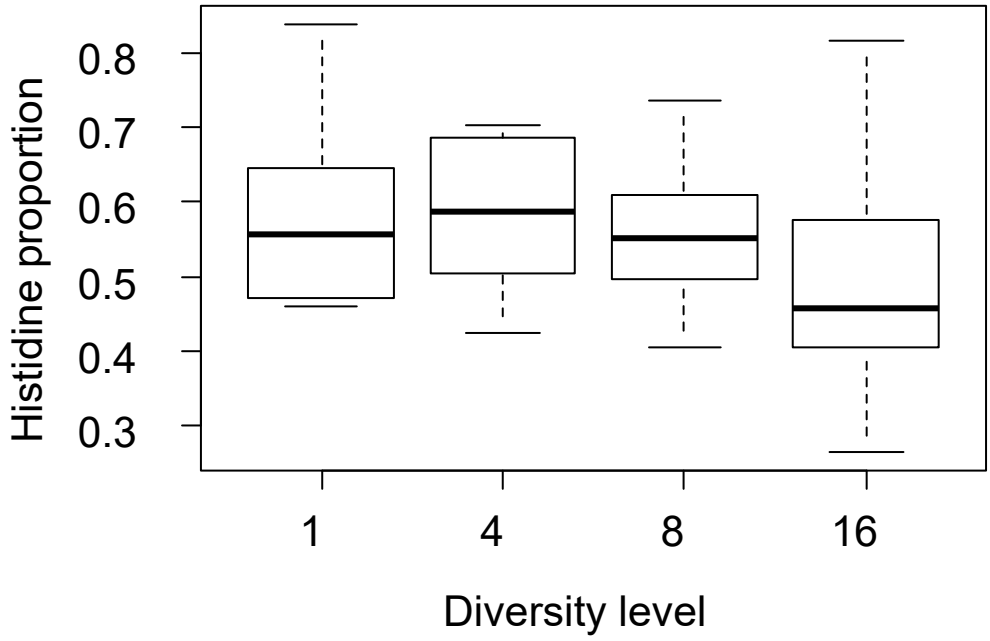

EAA concentration [mg/ml]
8
6
4
2
0
1
4
8
16
Diversity level

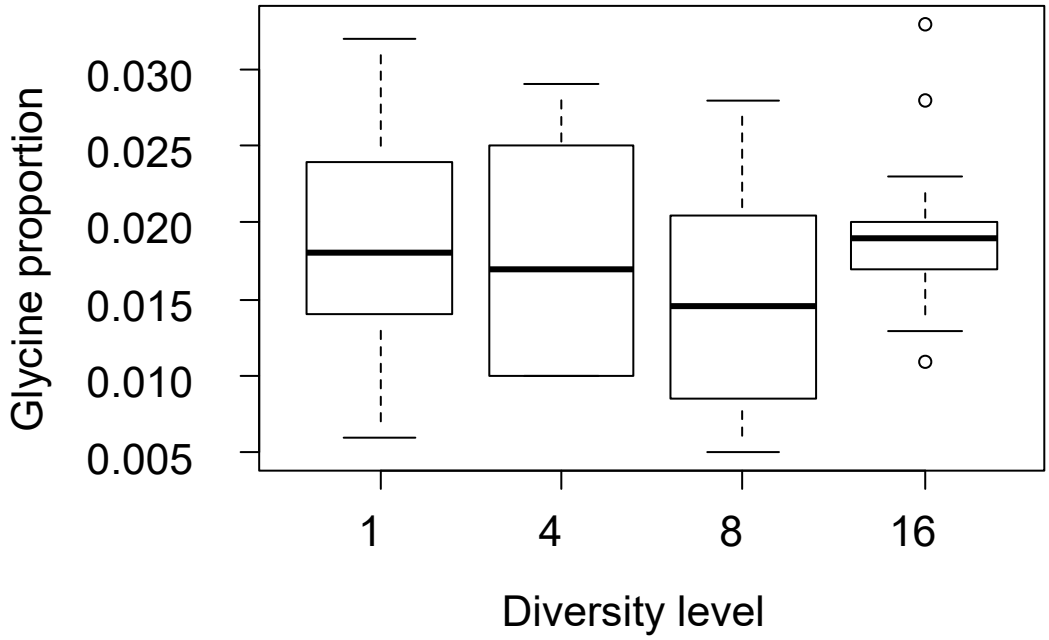

nEAA concentration [mg/ml]
3.0
2.5
2.0
1.5
1.0
0.5
0.0
1
4
8
16
Diversity level

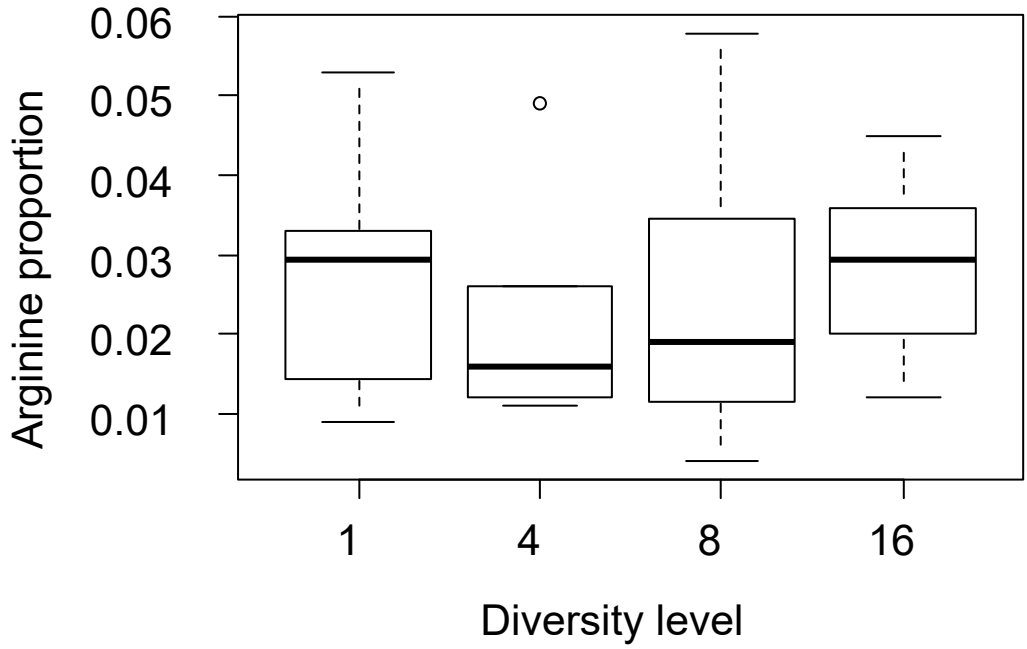

Aspartic acid proportion
0.05
0.04
0.03
0.02
0.01
1
4
8
16
Diversity level

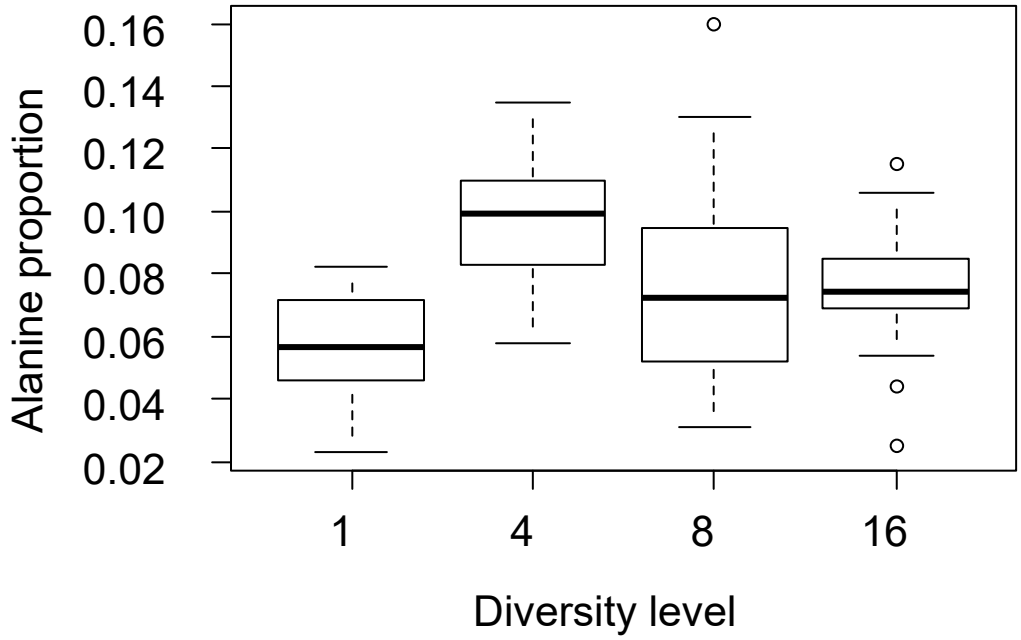

Glutamic acid proportion
0.10
0.08
0.06
0.04
0.02
0.00
1
4
8
16
Diversity level

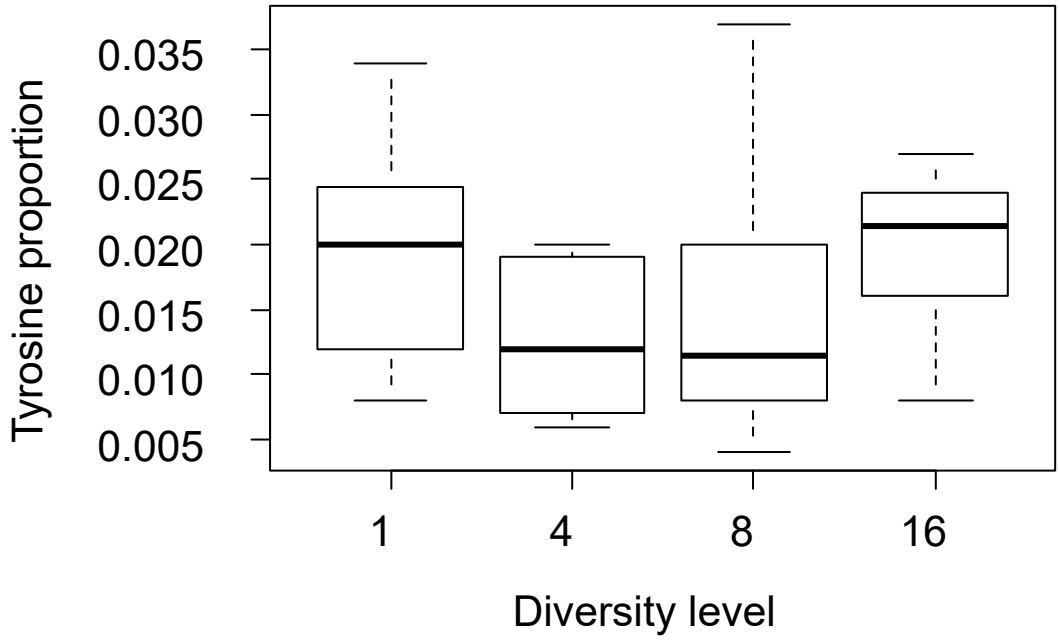

Serine proportion
0.10
0.08
0.06
0.04
0.02
1
4
8
16
Diversity level

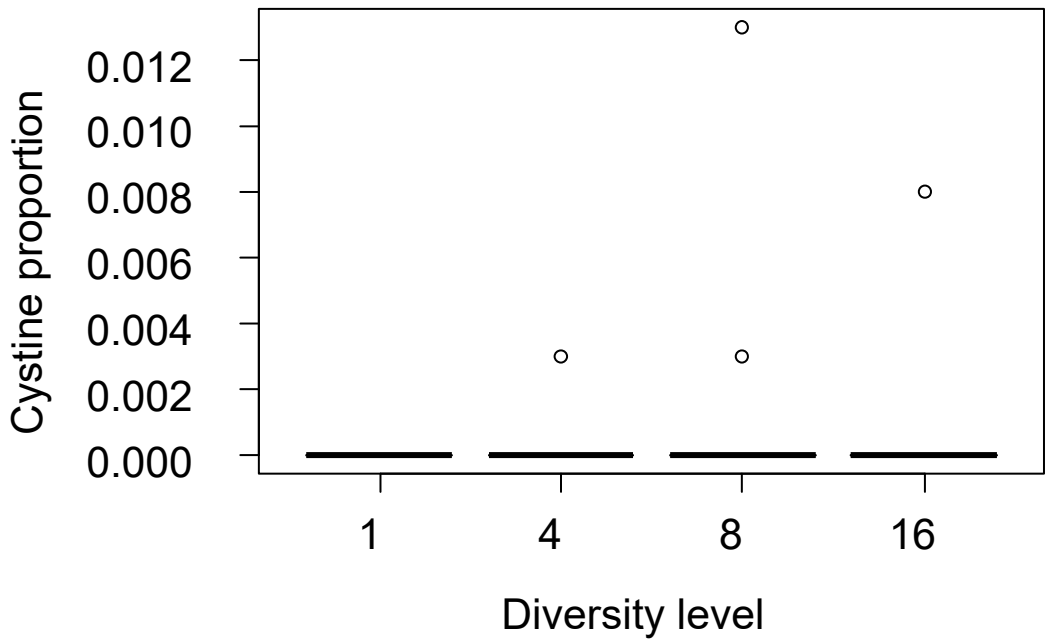

Histidine proportion
0.8
0.7
0.6
0.5
0.4
0.3
1
4
8
16
Diversity level

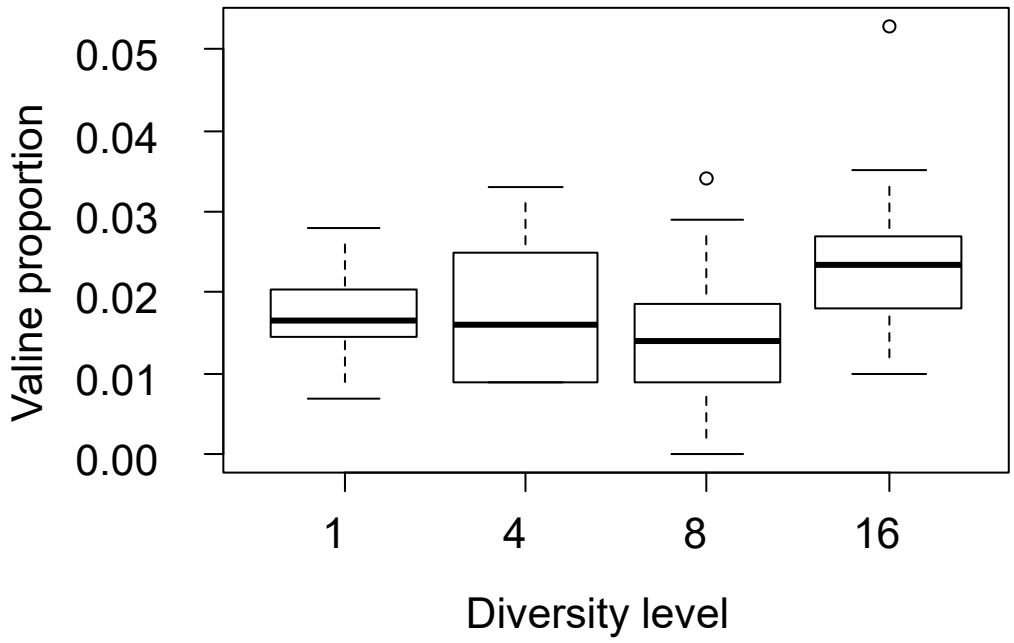

Glycine proportion
0.030
0.025
0.020
0.015
0.010
0.005
1
4
8
16
Diversity level

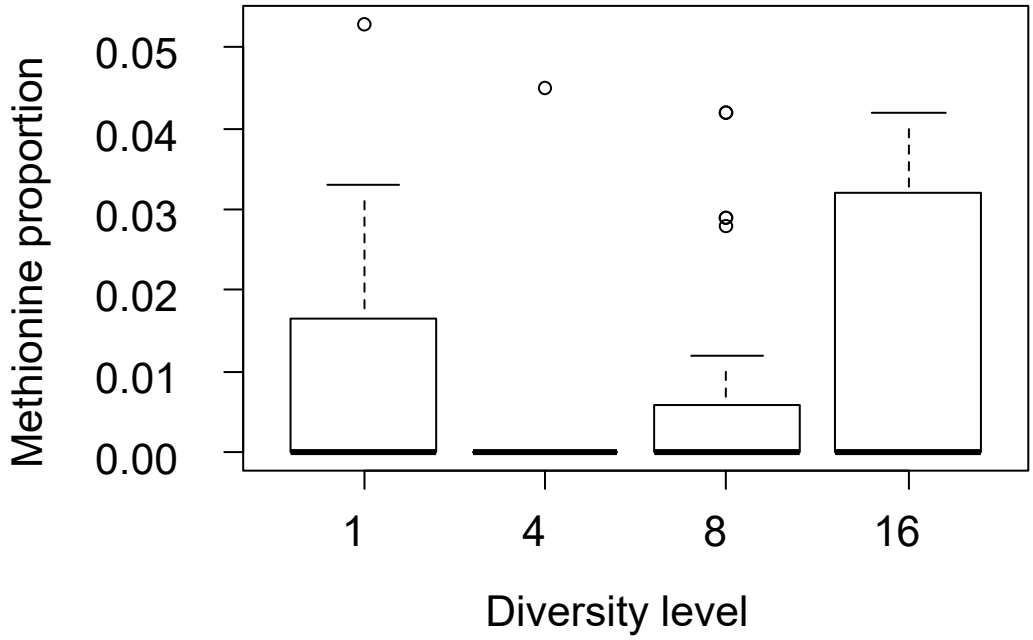

0.06
0.05
0.04
0.03
0.02
0.01
Arginine proportion
1
4
8
16
Diversity level

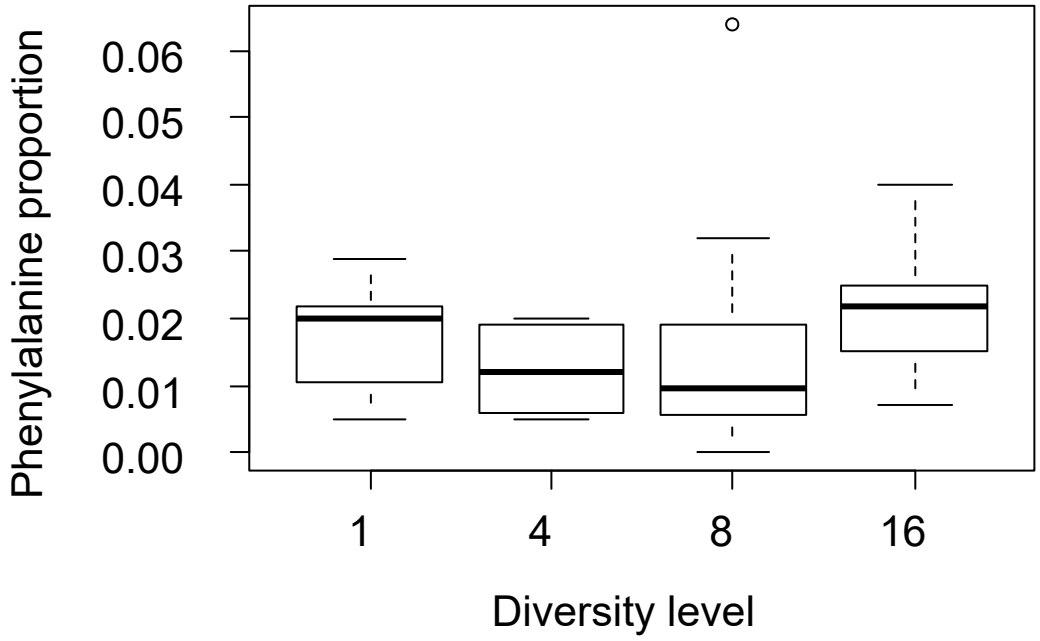

0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
Alanine proportion
1
4
8
16
Diversity level

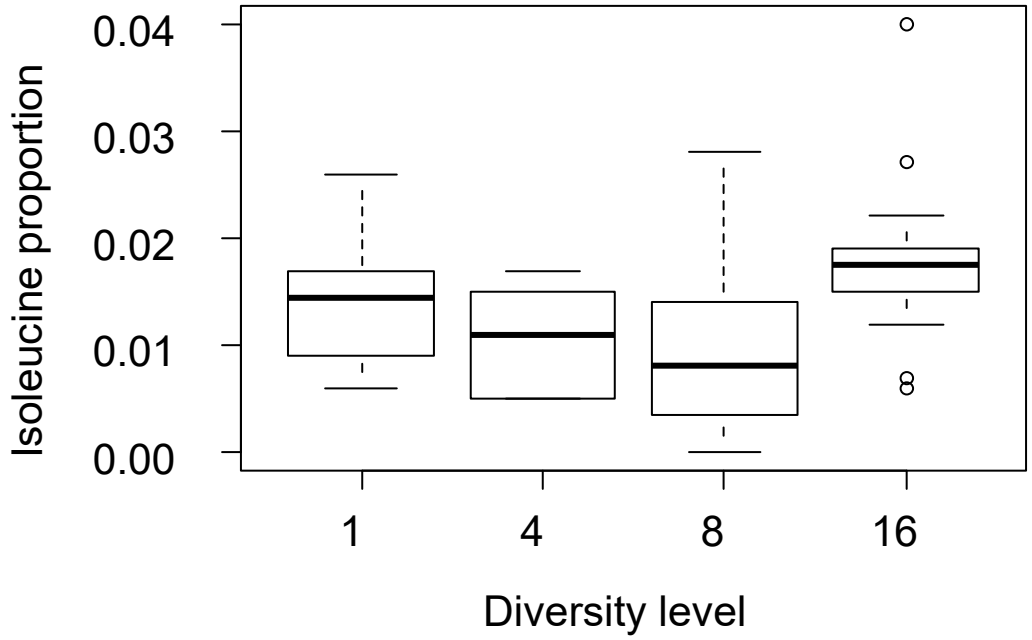

Tyrosine proportion
0.035
0.030
0.025
0.020
0.015
0.010
0.005
1
4
8
16
Diversity level

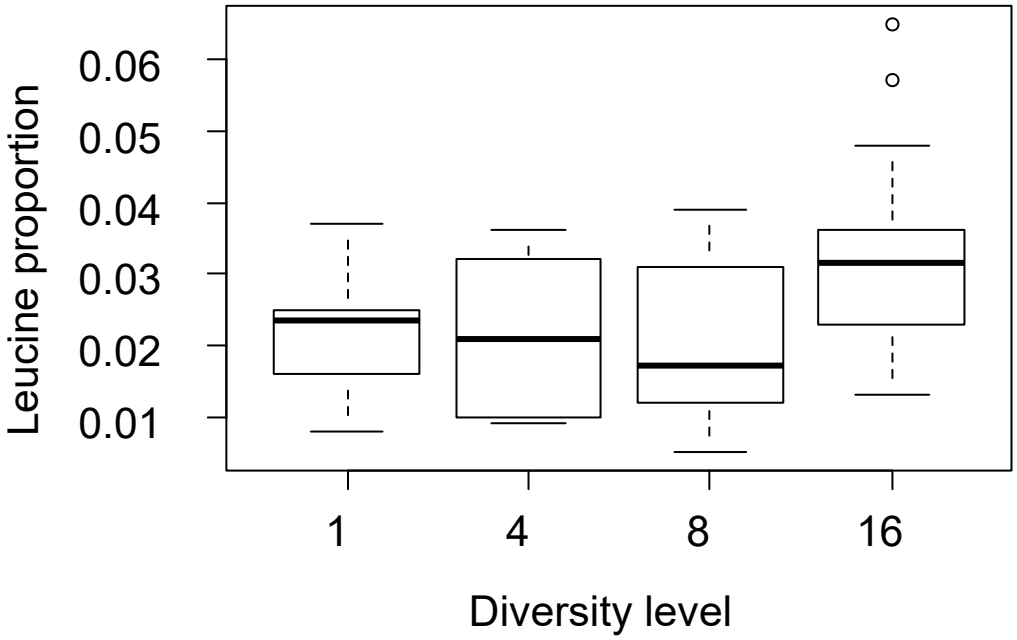

Cystine proportion
0.012
0.010
0.008
0.006
0.004
0.002
0.000
1
4
8
16
Diversity level

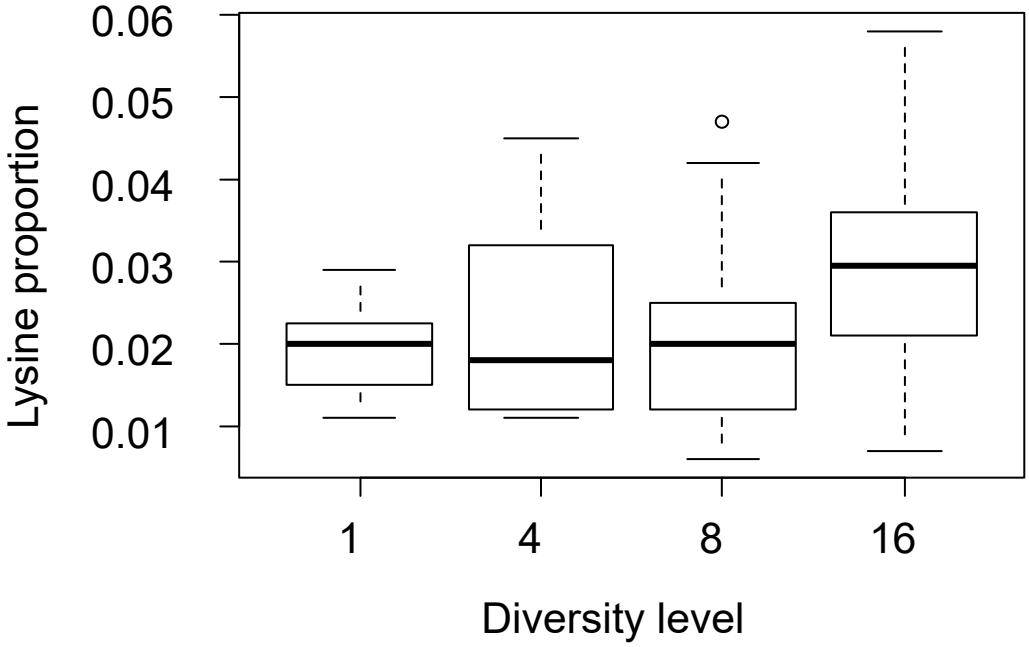

Valine proportion
0.05
0.04
0.03
0.02
0.01
0.00
1
4
8
16
Diversity level

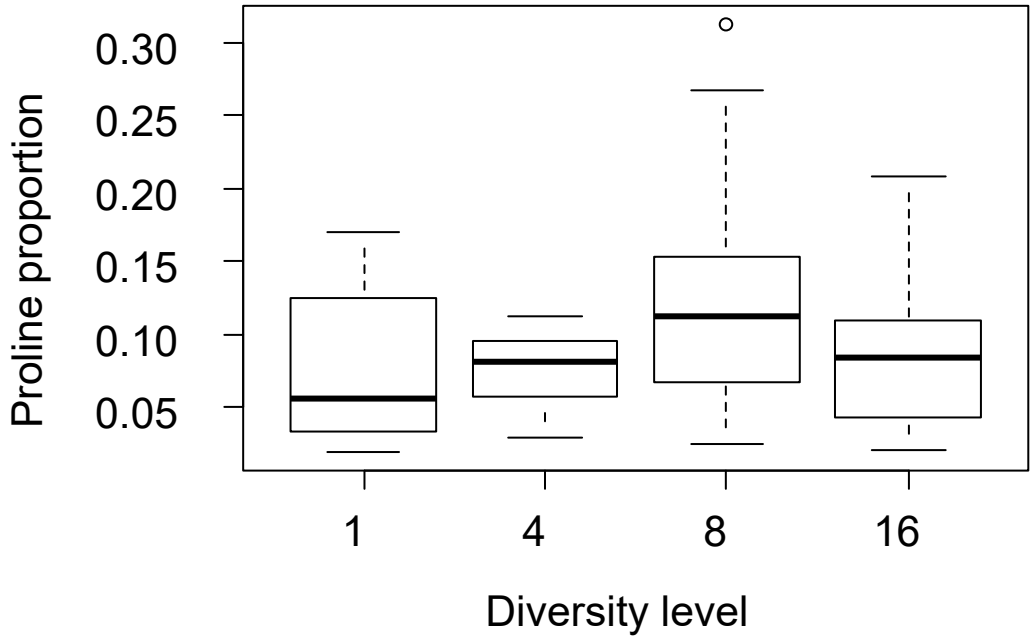

Methionine proportion
0.05
0.04
0.03
0.02
0.01
0.00
1
4
8
16
Diversity level

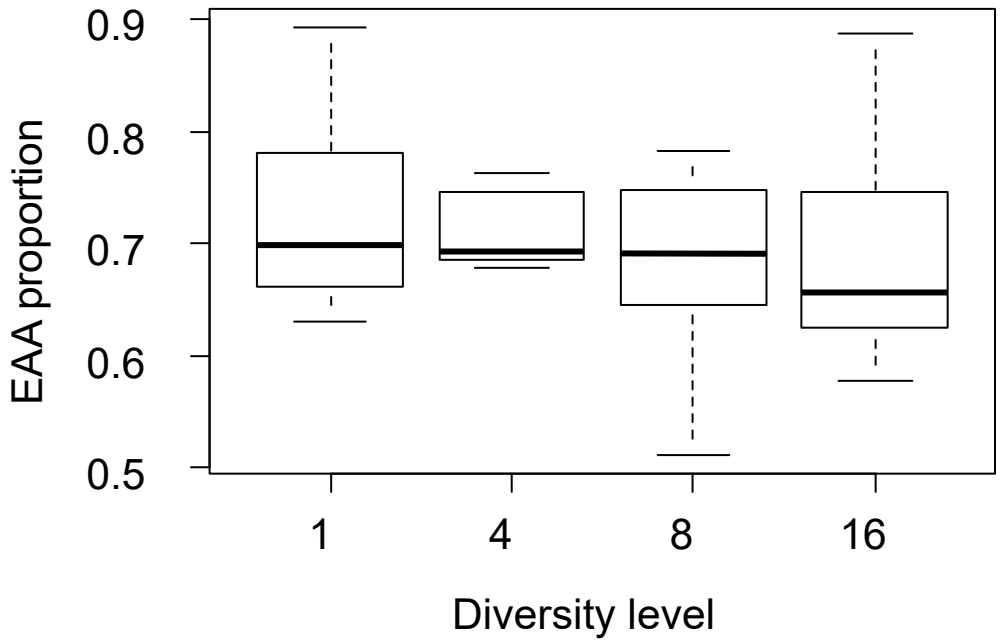

Phenylalanine proportion
0.06
0.05
0.04
0.03
0.02
0.01
0.00
1
4
8
16
Diversity level

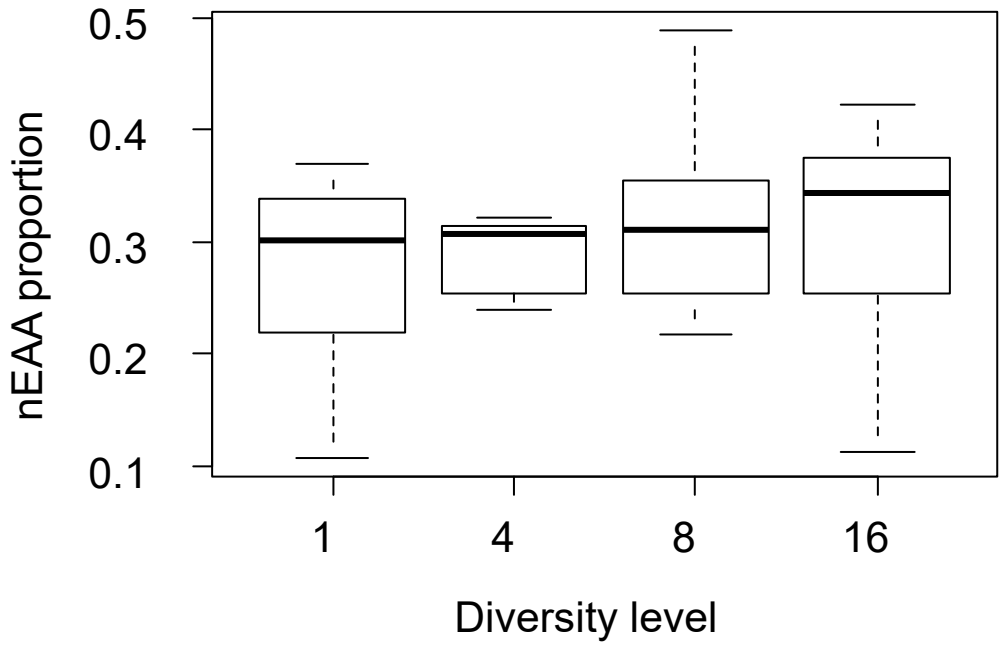

Isoleucine proportion
0.04
0.03
0.02
0.01
0.00
1
4
8
16
Diversity level

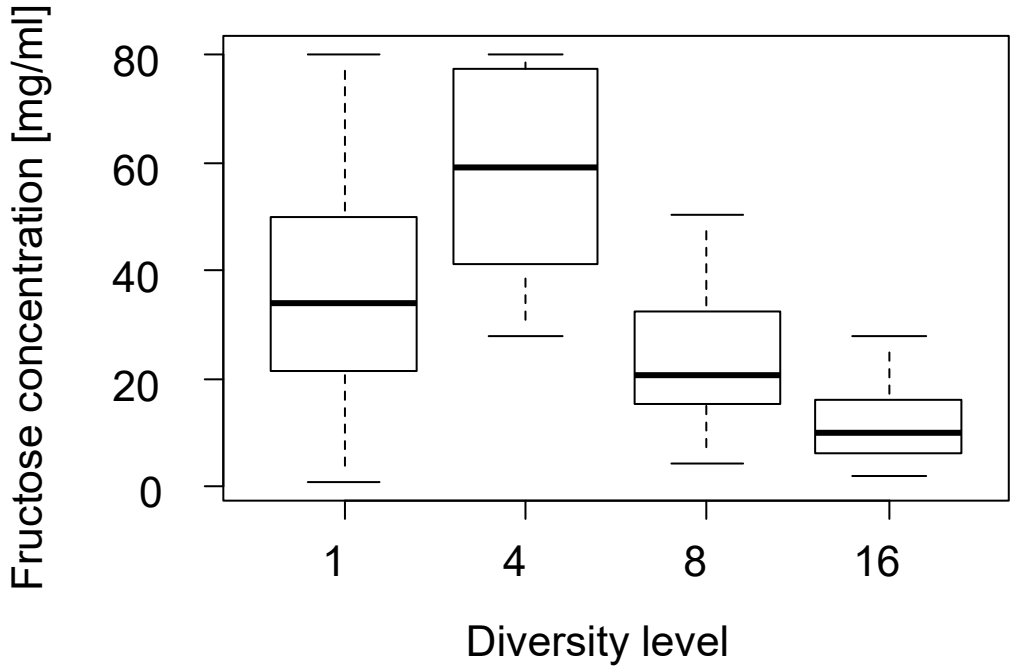

Leucine proportion
0.06
0.05
0.04
0.03
0.02
0.01
1
4
8
16
Diversity level

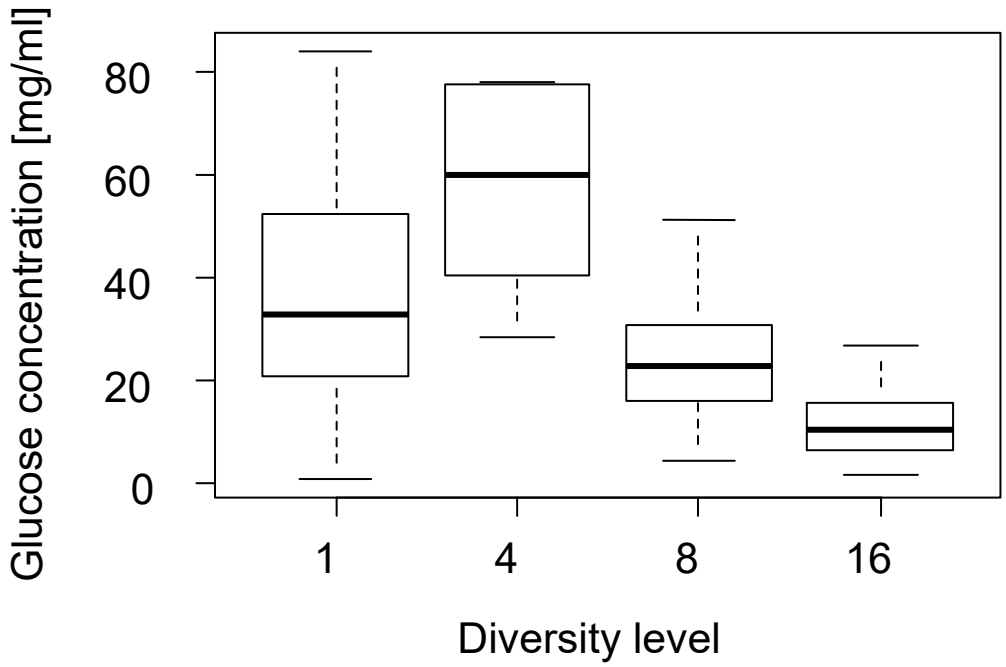

Lysine proportion
0.06
0.05
0.04
0.03
0.02
0.01
1
4
8
16
Diversity level

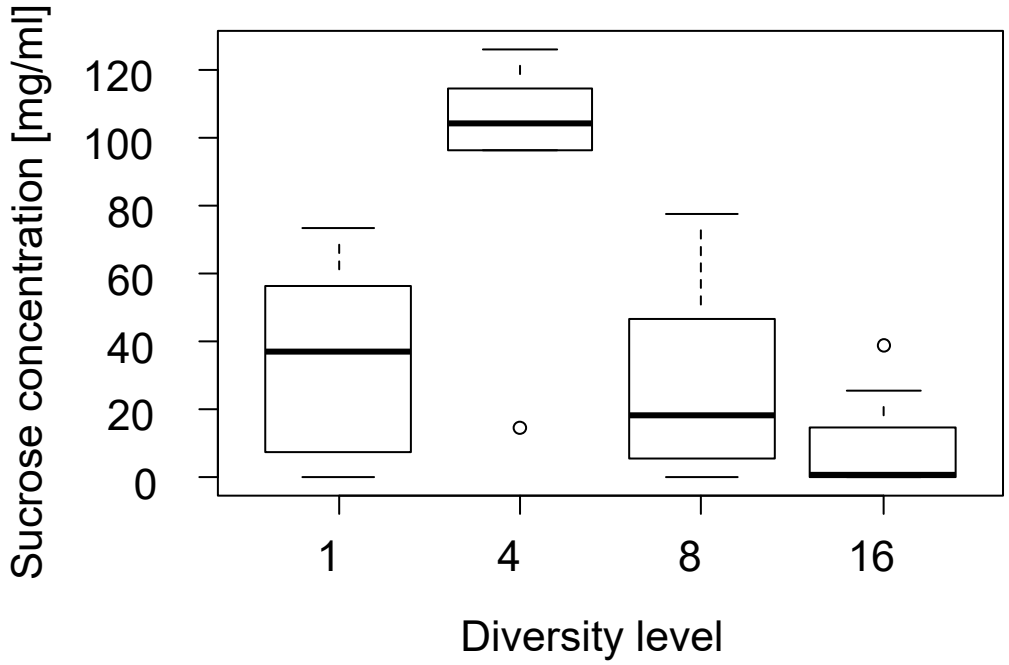

Proline proportion
0.30
0.25
0.20
0.15
0.10
0.05
1
4
8
16
Diversity level

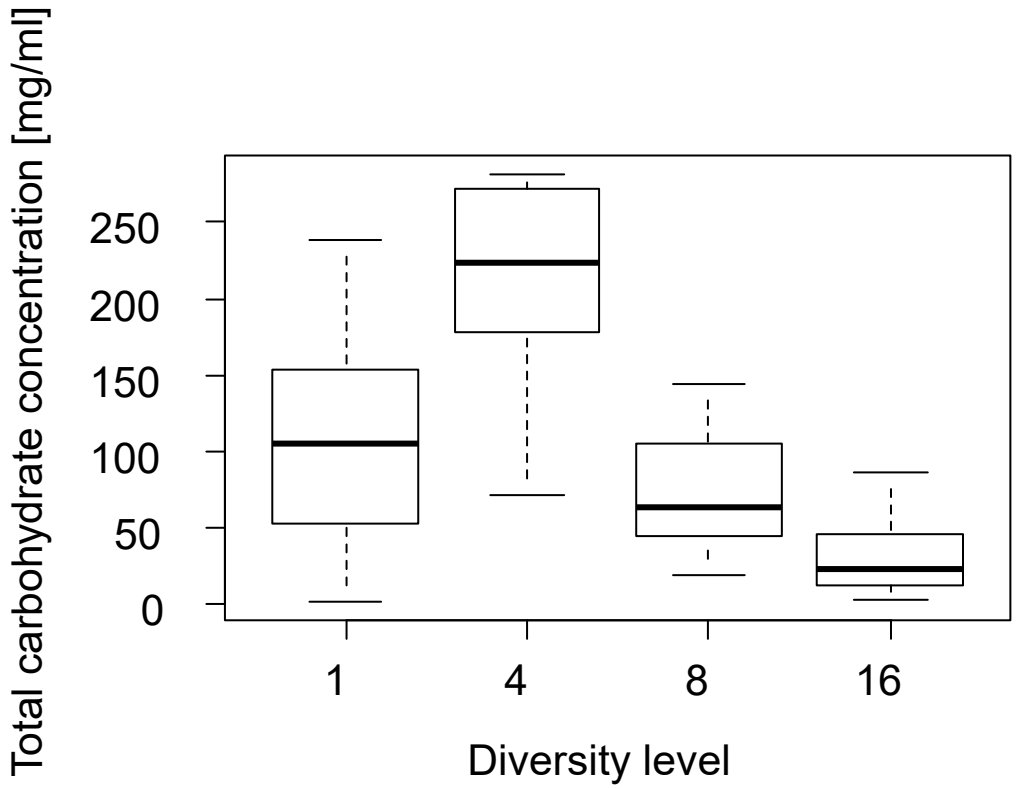

EAA proportion
0.9
0.8
0.7
0.6
0.5
1
4
8
16
Diversity level

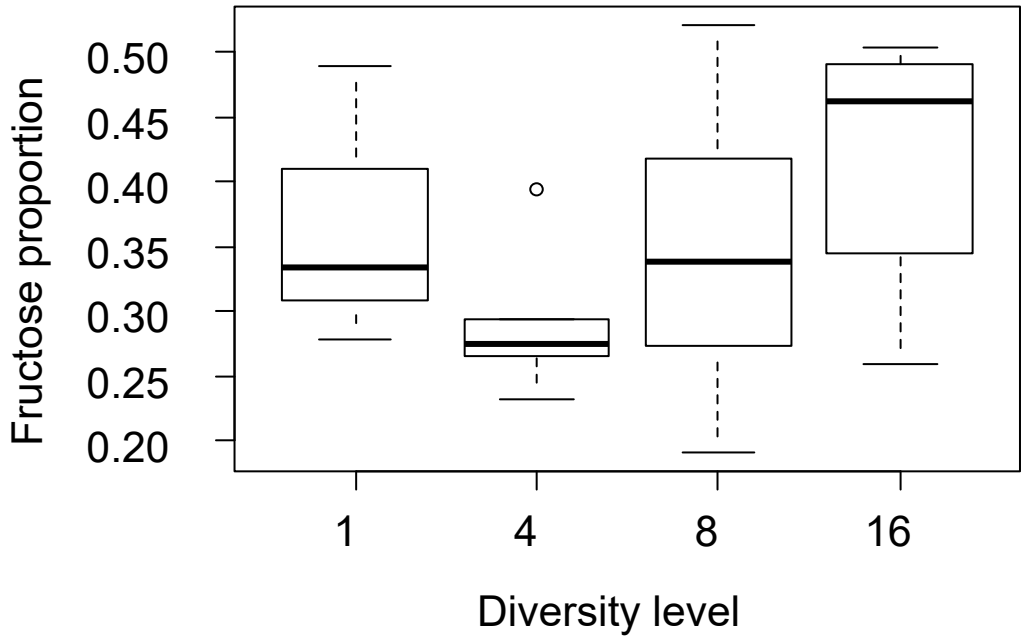

0.5
0.4
0.3
0.2
0.1
nEAA proportion
1
4
8
16
Diversity level

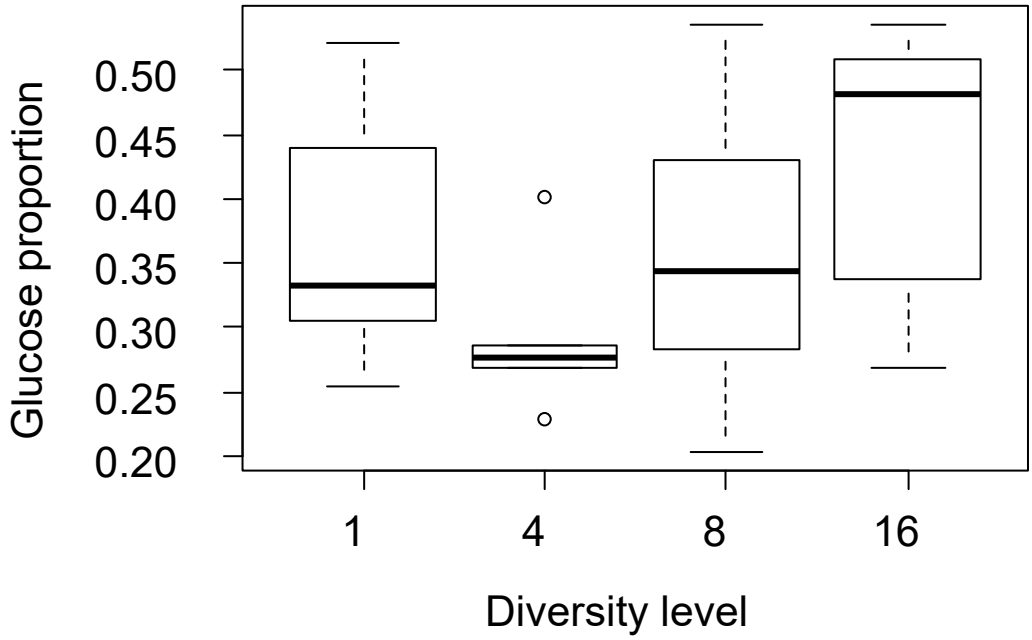

80
60
40
20
0
Fructose concentration [mg/ml]
1
4
8
16
Diversity level

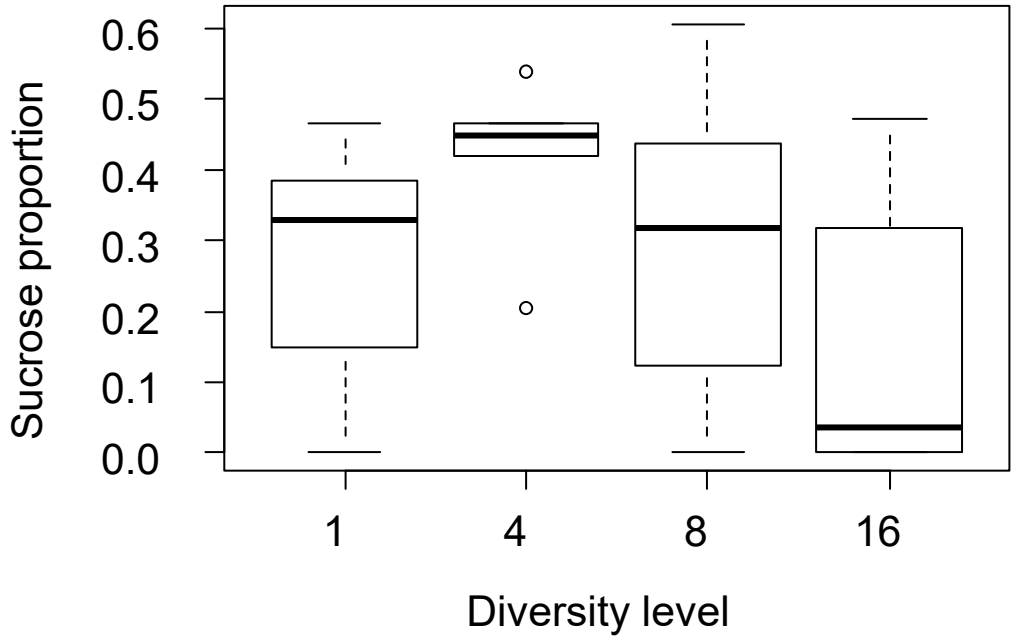

Glucose concentration [mg/ml]
0
20
40
60
80
1
4
8
16
Diversity level


Sucrose concentration [mg/ml]
0
20
40
60
80
100
120
1
4
8
16
Diversity level


Total carbohydrate concentration [mg/ml]
250
200
150
100
50
0
1
4
8
16
Diversity level


Fructose proportion
0.50
0.45
0.40
0.35
0.30
0.25
0.20
1
4
8
16
Diversity level


Glucose proportion
0.50
0.45
0.40
0.35
0.30
0.25
0.20
1
4
8
16
Diversity level


Sucrose proportion
0.6
0.5
0.4
0.3
0.2
0.1
0.0
1
4
8
16
Diversity level

References

1. Weisser, W.W.; Roscher, C.; Meyer, S.T.; Ebeling, A.; Luo, G.; Allan, E.; Beßler, H.; Barnard, R.L.; Buchmann, N.; Buscot, F.; et al. Biodiversity effects on ecosystem functioning in a 15-year grassland experiment: Patterns, mechanisms, and open questions. *Basic Appl. Ecol.* **2017**, *23*, 1–73.

2. Ebeling, A.; Klein, A.-M.; Tscharntke, T. Plant–flower visitor interaction webs: Temporal stability and pollinator specialization increases along an experimental plant diversity gradient. *Basic Appl. Ecol.* **2011**, *12*, 300–309.

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