Belowground functional traits of plants as drivers of biodiversity and plant strategies

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academic degree

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Summary

Plant roots and belowground organs have been overlooked in ecology, despite their importance for plant functioning and their potential for explaining ecological patterns. The 'hidden half' of plants determine their ability to forage for soil nutrients and water, serve as an anchor and interact directly or indirectly with soil organisms such as the root microbiota, pathogens, pests, and decomposers. Recent advances allowed identifying traits of interest (functional traits) that could explain how root systems are organised and how their diversity relates to the distribution of plants across spatial and environmental scales. However, most of the insights come from small experiments with low sample sizes or their compilation in databases, where differences in methodology can add significant biases. We still lack extensive belowground trait screenings obtained for different habitats necessary to ascertain the robustness and generality of the findings. Despite increasing inequalities in species distribution, notably with plant invasion and extinction of rare species, the importance of belowground traits in determining abundance and dominance has not yet been assessed. Though consensually viewed as important structures for resource uptake, root hairs have not yet been empirically integrated into a conceptual view of the root functional space. The existence of a bidimensional root trait space with a 'collaboration' and a 'conservation' gradient has not been tested in plant communities, nor its variation across environmental conditions. This thesis endeavours to participate in the discussion about these four frontiers of belowground ecology.

I conducted three trait screening multispecies experiments in semi-controlled conditions on individual plants in pots to unravel these issues. I tried to include the maximum number of species that occur in German grasslands to encompass most of the vegetation cover occurring in the field and a large spectrum of phylogenetic differences. Traits measured

included root morphology on fine roots and root system, rooting depth, root nitrogen content and uptake rates, and aboveground traits. I used databases and collaborative work to add root hairs, mycorrhizal colonization, belowground clonal traits, and additional aboveground traits. I related the traits between them and with metrics related to plant distribution and environmental conditions.

In the first study, ten above- and belowground traits from 241 central European grassland species were used as predictors of the species abundance and occurrence frequency at seven spatial scales, from 16 m^2 grassland plots to their naturalization success. Species success was always associated with at least three traits, and the explanatory power of traits ranged from 2 to 42%. Low root tissue density was a predictor of species success at every scale, whereas other traits showed directional changes depending on spatial resolution. Local abundance was characterized by traits usually associated with a 'competitive' strategy and global distribution with a 'ruderal' strategy. Belowground traits were at least as important and sometimes more than aboveground traits.

In the second study, root hair abundance and incidence, mycorrhizal colonization rate, and root morphology were assessed on 82 central European grassland species with fluorescence and compound microscopy. We found a phylogenetically conserved trade-off between investment in root hairs and colonization by mycorrhiza. The trade-off also occurred within species and was highest in species that rely less on root hairs. These findings suggest that the 'do-it-yourself' side of the 'collaboration' plant strategy gradient might compensate for the absence of root hyphae for nutrient foraging by using root hairs instead.

In the third study, nine belowground traits and specific leaf area were used to calculate Community Weighted Means for 150 grassland plots surveyed 11 years for vegetation cover. We identified principal components (dimensions) and related the scores of the communities

along the dimensions to ten environmental variables relating to soil parameters and land use. Two main dimensions corresponding to a 'collaboration' and a 'conservation' gradients were enough to capture functionally relevant variability for the ten traits included. Overlooked traits did not load on additional functionally relevant dimensions: bud-bank size was associated with the 'slow', branching intensity with the 'do-it-yourself', rooting depth, and, surprisingly, root nitrogen content with the 'outsourcing' strategies. Land-use intensity and soil parameters indicative of high soil fertility were associated with the 'fast' and 'outsourcing' communities but with different indicators and a contrasting effect of ammonium and nitrate.

This thesis provides evidence that belowground traits provide additional information on plant functioning compared to aboveground traits and explain ecological patterns. Both the integrated above-belowground 'fast-slow' gradient and a bidimensional root functional space explain the functional structure of traits in herbaceous species and communities. I provide indirect evidence that particular trait values of belowground traits provide fitness advantages that result in differences in species distribution, with and without considering their adaptive values in different environmental conditions.

Zusammenfassung

Pflanzenwurzeln und unterirdische Organe wurden, trotz ihrer Bedeutung für das Funktionieren der Pflanze und ihres Potenzials zur Erklärung ökologischer Muster, in der Ökologie übersehen. Die "verborgene Hälfte" der Pflanzen bestimmt ihre Fähigkeit, nach Bodennährstoffen und Wasser zu suchen, dient als Anker und interagiert direkt oder indirekt mit Bodenorganismen wie Wurzelmikrobiota, Pathogenen, Schädlingen und Zersetzern. Jüngste Fortschritte ermöglichten die Identifizierung von interessanten Merkmalen (funktionalen Merkmalen), die erklären könnten, wie Wurzelsysteme organisiert sind und wie ihre Vielfalt mit der Verteilung von Pflanzen über räumliche und ökologische Größenordnungen zusammenhängt. Die meisten Erkenntnisse stammen jedoch aus kleinen Experimenten mit geringen Stichprobenumfängen oder ihrer Erfassung in Datenbanken, wo Unterschiede in der Methodik zu erheblichen Verzerrungen führen können. Uns fehlen immer noch umfangreiche Untersuchungen von unterirdischen Merkmalen, die für verschiedene Lebensräume erhoben wurden und die notwendig sind, um die Robustheit und Allgemeingültigkeit der Ergebnisse zu überprüfen. Trotz zunehmender Ungleichheiten in der Artenverteilung, insbesondere durch die Invasion von Pflanzen und das Aussterben seltener Arten, wurde die Bedeutung von unterirdischen Merkmalen bei der Bestimmung von Häufigkeit und Dominanz bisher nicht untersucht. Obwohl Wurzelhaare als wichtige Strukturen für die Ressourcenaufnahme angesehen werden, wurden sie empirisch noch nicht in eine konzeptionelle Betrachtungsweise des Wurzelfunktionsraums integriert. Die Existenz eines zweidimensionalen Wurzel-Funktionsraumes mit einem "Kollaborations"- und einem "Erhaltungs"-Gradienten wurde in Pflanzengemeinschaften noch nicht untersucht, ebenso wenig wie seine Variation über unterschiedliche Umweltbedingungen hinweg. Diese Arbeit versucht, sich an der Diskussion über diese vier Grenzbereiche der unterirdischen Ökologie zu beteiligen.

Um diese Fragen zu klären, habe ich drei Trait-Screening-Multispezies-Experimente unter halb-kontrollierten Bedingungen an einzelnen Pflanzen in Töpfen durchgeführt. Ich habe versucht, die maximale Anzahl von Arten einzubeziehen, die im deutschen Grünland vorkommen, um den größten Teil der im Feld vorkommenden Vegetationsdecke und ein großes Spektrum an phylogenetischen Unterschieden abzudecken. Zu den gemessenen Merkmalen gehörten die Wurzelmorphologie an Feinwurzeln und Wurzelsystem, die Durchwurzelungstiefe, der Stickstoffgehalt und die Aufnahmeraten der Wurzeln sowie oberirdische Merkmale. Mithilfe von Datenbanken und kollaborativer Arbeit fügte ich Wurzelhaare, Mykorrhiza-Besiedlung, unterirdische klonale Merkmale und zusätzliche oberirdische Merkmale hinzu. Ich verglich die Merkmale untereinander und mit Metriken, die sich auf die Pflanzenverteilung und die Umweltbedingungen bezogen.

In der ersten Studie wurden zehn ober- und unterirdische Merkmale von 241 mitteleuropäischen Grünlandarten als Prädiktoren für die Abundanz und die Häufigkeit des Vorkommens der Arten auf sieben räumlichen Skalen verwendet, von 16 m² großen Grünlandparzellen bis hin zu ihrem Naturalisierungserfolg. Der Erfolg der Arten war immer mit mindestens drei Merkmalen verbunden, und die Erklärungskraft der Merkmale reichte von 2 bis 42 %. Eine niedrige Wurzelgewebedichte war auf jeder Skala ein Prädiktor für den Arterfolg, während andere Merkmale in Abhängigkeit von der räumlichen Auflösung gerichtete Veränderungen zeigten. Lokale Häufigkeit wurde durch Merkmale charakterisiert, die üblicherweise mit einer "kompetitiven" Strategie in Verbindung gebracht werden,die globale Verbreitung dagegen mit einer "ruderalen" Strategie. Unterirdische Merkmale waren mindestens so wichtig und manchmal wichtiger als oberirdische Merkmale.

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General Introduction

Belowground functional ecology as an emerging field

At the edge of our planet, six thousand kilometres away from the mineral layers that separate us from its scorching core, a thin and curious mixture of mineral and organic particles we call soil is home to one of the most fundamental organs allowing terrestrial life: the plant root. Not content with interacting with the millions of species that dwell underground: bacteria, nematodes, insects, earthworms, fungi, to cite a few, roots are also crucial in absorbing and transporting the soil water and nutrients necessary to build and maintain plant tissues. These plant tissues will then constitute, directly or indirectly, almost 99% of human energy intake (White *et al.* 2013), and aerial plant tissues will produce the majority of the atmospheric oxygen (Huang *et al.* 2018). Despite being so crucial for our survival, soil and its inhabitants were long depreciated and synonym to dirt (Bardgett 2016). Soil is now globally threatened by erosion, notably because of the increase in human land-use and other global changes characteristic of the Anthropocene (Wuepper *et al.* 2020), thereby threatening terrestrial life and food security. The raised awareness of the importance of soil and its inhabitants, notably plant roots, is spurring a new era of belowground research. Genetic tools and metabolomics have seen considerable development these last decades and inform us on which genes are activated and regulated and which metabolites are produced in different parts of the roots, at different developmental stages, and in other environmental conditions (Giehl & Wirén 2014). Next-Generation Sequencing enabled the exploration of the diversity of the microorganisms inhabiting the roots and the rhizosphere, interacting with the plant and impacting its fitness (Vandenkoornhuyse *et al.* 2015). 3D-imaging combined with nanometric tracer methods allows the observation and quantification of the flux of matter and energy at the root-soil interface (Vidal *et al.* 2018).

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Despite these tremendous research efforts, our knowledge of plant roots' functioning is still considered rudimentary (Ryan *et al.* 2016). A missing piece of the root puzzle is how form follows function, or function follows form (Woodward 2008). The focus on the processes happening at the microscopic and smaller scales in roots failed to acknowledge that, contrary to aboveground organs, we lack a basic understanding of the function performed by different root morphologies and architectures. This is reflected by the lack of appropriate botanical terminology to describe the shape and configuration of root systems (Rewald *et al.* 2014). In contrast, phyllotaxis, floral formulae, and other aboveground descriptors have been widely adopted, notably for taxonomy (Rouhan & Gaudeul 2021).

Belowground traits dimensionality

Comparative ecology focuses on the differences between organisms and identifies the main constraints that act on plant construction (Grime *et al.* 1996). We are now in an era where combining many observations contributes to an exploratory approach to these constraints. "In plant ecology as in golf there is a time for precision and a time for progression" (Grime 1985). The use of simple descriptors, or traits, such as the height, surface and mass of leaves, stems, and seeds, measured on a large diversity of plant species permitted to identify a global spectrum of plant shape and function (Díaz *et al.* 2016). Accordingly, each plant species has a position on two main axes or dimensions: the size axis, which ranges from a few centimetres of plant height for the model plant *Arabidopsis thaliana* to several dozens of meters for tropical trees like *Berthollettia excelsa*, and the 'leaf economics spectrum' axis that distinguish hardy, resistant-leaved plants (e.g., most conifers) and flimsy, cheap-leaved plants (e.g., the herbaceous *Sinapis arvensis*, *Arctium tomentosum*). The leaf economics spectrum is a plant strategy gradient that integrates a set of traits that covary and reflect a trade-off between growth rate and life span of tissues. Some species adopt the 'fast' or 'resource-acquisitive' strategy with low-cost, cheap tissues rich in nitrogen, allowing rapid acquisition of resources at the

detriment of durability. Some other species adopt the 'slow' or 'resource-conservative' strategy with high-cost, expensive tissues allowing a slower acquisition of resources but a greater efficiency in resource use, and producing more durable tissues, with investment in carbon-rich structural components that provide resistance against stressors like pathogens, herbivores, drought, freezing (Reich 2014; Wright *et al.* 2017). Because different trait values have different adaptive values based on the environmental context, there is a correspondence between the traits observed in the vegetation, and the abiotic and biotic conditions of the ecosystem (Kraft *et al.* 2015). For example, at the global scale, low rainfall is associated with species with high leaf mass area, a 'resource-conservative' strategy, with structural tissues limiting evapotranspiration (Wright *et al.* 2004). High soil fertility is associated with species with high specific leaf area, a 'resource-acquisitive' strategy, that maximises light surface interception per unit of carbon cost, thus allowing fast growth and higher productivity (Poorter & de Jong 1999). These trait responses to the environment have cascading consequences at the ecosystem level. For example, plant communities dominated with 'resource-conservative' plant species will produce litter at a slower rate than with 'resource-acquisitive' plant species. This litter will also be slower to decompose because of the structural tissues with a high C:N ratio, and will offer less palatable resources to herbivores (Lavorel *et al.* 2007). The promise of scaling-up measurements of plants to the functioning of whole ecosystems (the 'Holy grail' of functional ecology) is now an active research field. Belowground trait diversity is thought to be mediating many ecosystem processes related to ecological and biogeochemical cycles (Bardgett *et al.* 2014; Bardgett & van der Putten 2014). For example, low soil moisture is associated with high root length density (Gross *et al.* 2008). Wood production is higher in forests, with trees differing in rooting depth after accounting for species richness and differences in aboveground traits (Jing *et al.* 2021). A current lack of data on belowground traits impedes the investigation of belowground trait diversity, root traits being far less available than plant aboveground traits,

with 1% of trait records related to roots in TRY, the biggest trait database (Kattge *et al.* 2020). Indeed, trait relationships are still poorly known belowground compared to aboveground. The leading theory until the second half of the 2010s was that root traits are analogous to leaf traits for plant economics, with a 'fast-slow' continuum. The 'fast' strategy would be characterized by a small root diameter and root tissue density (resulting in high specific root length) to maximize the surface area per unit of carbon cost for belowground resource (water and nutrients) acquisition (Reich 2014). This should correspond to the 'fast' leaf strategy characterized by low leaf thickness and tissue density (resulting in high specific leaf area) to maximize the surface area per unit of carbon cost for aboveground resources (light and CO_2). However, findings can diverge from these assumptions. Several studies show a tendency for root diameter and specific root length to be independent of root-tissue density and leaf-economics spectrum traits (Kramer-Walter *et al.* 2016; Weemstra *et al.* 2016), at least in trees. The first large-scale analyses from a compilation of root-traits data indicate that root diameter is positively associated with mycorrhization because of the colonization potential offered by thickened cortical tissues (Bergmann *et al.* 2020). But, root diameter also changed idiosyncratically during evolutionary history. Strong differences exist between main plant clades (i.e., Asterids, Rosids, Monocots, Magnoliids, Gymnosperms) independently of mycorrhizal associations (Valverde-Barrantes *et al.* 2020). Rather than solely decreasing over evolutionary time (Ma *et al.* 2018), fine root diameters rather diversified (increased and decreased in the 0.1 to 1 mm range) these last 100 million years compared to a mean of 0.5 mm during -300 to -100 million years. Root traits are also varying at the global scale with climate. Cold mean temperatures are associated with thin, high specific root length, and high precipitation with high root tissue density (Freschet *et al.* 2017; Valverde-Barrantes *et al.* 2017). The strength of the correlation between the above and belowground traits depends on the clades considered, indicating a phylogenetic dependence of the traits relationships. In

summary, the relationships between aboveground traits and belowground traits are still unclear, and different selective pressures in roots contribute to their independent evolution in terms of traits.

Research gaps

Belowground traits will prove crucial in understanding the changes associated with the Anthropocene. An increase in plant invasion and decrease in biodiversity with homogenization of vegetation (McKinney & Lockwood 1999) lead to negative economic impacts (Diagne *et al.* 2021), notably through reduced crop yield, damaged infrastructure, and reduction in ecosystem-services provision. Invasive plants have different aboveground traits than native, non-invasive plants (van Kleunen *et al.* 2010), but their differences in belowground traits are still poorly known. Food production is threatened by drought and resource scarcity, and root traits should be leveraged in breeding programs to select adaptations to limited water (Comas *et al.* 2013) and nutrient (Kong *et al.* 2014b) availability. However, the syndromes of traits of interest (ideotypes) are often based on small-scale experiments and modeling (Del Bianco & Kepinski 2018; Koevoets *et al.* 2016), rather than trying to harness the patterns found in biodiversity, shaped by the millions of years of plant evolutionary adaptations to harsh conditions. Mycorrhizal colonization is associated with protection against several stresses, notably drought and pests, and breeding for effective symbiosis is considered (Thirkell *et al.* 2017). Root hairs are important in phosphorus acquisition (Zhu *et al.* 2010), and have structural and functional similitudes with fungal hyphae (Zou *et al.* 2018). The interplay between mycorrhizal fungi and root hair is still poorly known but could be considered as a breeding target for the unique benefits they confer to the plants. Grasslands constitute a significant habitat representing 40% of the terrestrial surface (Suttie *et al.* 2005), hosting many herbaceous species. Their environmental conditions are changing rapidly due to climate change, nitrogen deposition, changes in land-use (Gibson & Newman 2019). These changes modify their

vegetation and the provided ecosystem services in a way that is still poorly characterized and predicted (Lemaire *et al.* 2011).

Six areas of research, or belowground frontiers, will help us predict future changes in plant biodiversity and their consequences on ecosystem functioning (Laliberté 2017).

1) Redefining fine roots by using an order or diameter-based classification of roots will improve our precision, as root segments can be more implied in transport (coarse, low-order roots) or absorption and mycorrhizal interaction (McCormack *et al.* 2015). 2) Quantifying trait dimensionality by identifying how traits covary or are independent of each other. A two-dimensional space has been identified at the inter-specific level for commonly measured traits of fine roots (Bergmann *et al.* 2020). The position of other important traits is unknown, as they may represent additional dimensions of trait variation. 3) Integrating mycorrhizas, as the main microbial partner of plant roots, but often overlooked as their trait characterization is tedious beyond the mycorrhizal status. 4) Broadening the suite of belowground traits by integrating physiological (i.e., respiration, metabolites), clonal (bud-bank and clonal traits) and root hair traits. 5) Determining trait-environment linkages, by evaluating which trait values are more prevalent in which environment, we can infer the adaptive values of traits and the changes in vegetation that will occur with changes in the environment. 6) Understanding ecosystem-level consequences by characterizing how particular trait values influence biogeochemical cycles and their effects on the soil.

Contribution of this thesis

In this thesis, I partake in the discussion of these five first frontiers. The three chapters attempt to provide answers to the following questions:

Chapter I: Which above- and belowground traits are associated with the commonness and rarity of grassland species, from the plot to the naturalization success?

Chapter II: Is there a trade-off between root hair development and mycorrhizal colonization

in grassland species?

Chapter III: What are the main dimensions of belowground trait diversity in German grasslands, and how do they relate to environmental conditions?

To do so, with my colleagues, I conducted multi-species experiments in controlled or semi-controlled conditions to measure traits on a variety of herbaceous species (82 to 241) found in German grasslands. Traits were measured both on entire root systems and fine roots. I added data from databases for traits that couldn't be measured directly. I investigated the relationships between the traits (at the species and community levels, Chapter I, II, III), between traits and occurrence data (at the species and community levels, Chapter I, III) and between traits, occurrence, and environmental data (at the community level, Chapter III). Multi-species experiments were chosen to increase the generality of findings (van Kleunen *et al.* 2014) and to achieve a sample size that allows finding robust patterns that can be replicated (Nakagawa & Parker 2015). This work is part of the Biodiversity Exploratories project (Fischer *et al.* 2010) and is leveraging its unique large-scale data collection and insights. In **Chapter I,** we selected ten above- and belowground traits of 241 species and related the trait values to the distribution (abundance and occurrence) at seven spatial scales, from local abundance to global naturalization success. In **Chapter II,** we characterized, for fine roots, the root hair length and density (fluorescence microscopy) and the mycorrhizal colonization rate (dissecting microscope) of 82 species and explored the relationships between root traits and mycorrhizal traits. In **Chapter III,** we used species' trait data to calculate community-level metrics (community weighted means) for ten above- and belowground traits in 150 German grassland vegetation plots. We then used PCAs to investigate the dimensionality of traits and related PCs and community weighted means to ten land-use and soil variables. The three main theses defended here are 1) Belowground traits are determinants for species commonness and invasiveness independently of their relationships with aboveground traits. 2) There is a trade-

off between root hair and mycorrhizal reliance. 3) A bi-dimensional trait space made of a ‘collaboration’ and a ‘conservation’ gradient exist at the community scale, includes rooting depth, clonal and branching traits, and vary with soil parameters.

Chapter I : Below- and aboveground traits explain success of German grassland plants from plot to global scales

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Abstract

1. Plants vary widely in how common or rare they are, but whether commonness of species is associated with functional traits is still debated. This might partly be because commonness can be measured at different spatial scales, and because most studies focus solely on aboveground functional traits.

2. We measured five root traits and seed mass on 241 Central European grassland species, and extracted their specific leaf area, height, mycorrhizal status and bud-bank size from databases. Then we tested if trait values are associated with commonness at seven spatial scales, ranging from abundance in 16-m² grassland plots, via regional and European-wide occurrence frequencies, to worldwide naturalization success.

3. At every spatial scale, commonness was associated with at least three traits. The traits explained the greatest proportions of variance for abundance in grassland plots (42%) and naturalization success (41%), and the least for occurrence frequencies in Europe and the Mediterranean (2%). Low root tissue density characterized common species at every scale, whereas other traits showed directional changes depending on the scale. We also found that many of the effects had significant non-linear effects, in most cases with the highest commonness-metric value at intermediate trait values. Across scales, belowground traits explained overall more variance in species commonness (19.4%) than aboveground traits (12.6%).

4. The changes we found in the relationships between traits and commonness, when going from one spatial scale to another, could at least partly explain the maintenance of trait variation in nature. Most importantly, our study shows that within grasslands, belowground traits are at least as important as aboveground traits for species commonness. Therefore, belowground traits should be more frequently considered in studies on plant functional ecology.

Introduction

Flowering plants are estimated to have diversified into an extant global flora of about 369.000 species (Willis 2017). Most of those species have small ranges and maintain low population densities, and are thus considered rare (Enquist *et al.* 2019). On the other side, a few species are considered common or dominant, as they achieve high population densities and have colonized large stretches of land and many regions around the world (Pyšek *et al.* 2017; Ulrich *et al.* 2010). Although this pattern has been recognized as early as the 19th century (Darwin 1859), the reasons why most species are rare and a few are common or dominant are still unclear (Gaston 2011).

Plant functional traits have been successfully used to explain species occurrence patterns in relation to environmental conditions (Violle *et al.* 2007). It has also been shown that species with particular trait values increase in abundance when filtered by the environment (Lavorel & Garnier 2002). About two decades ago, Murray *et al.* (2002) reviewed studies that compared traits between common and rare species. They found 54 such studies, but most of those included very few species, which made it difficult to draw any general conclusions. Since then more and larger studies have compared traits of common and rare species (e.g. (Arellano *et al.* 2015; Cadotte & Lovett-Doust 2002; Cornwell & Ackerly 2010; Gabrielová *et al.* 2013). Nevertheless, drawing general conclusions is still difficult as the studies vary in the traits included and in how common and rare plants were defined. The latter is not surprising as commonness (or rarity) has multiple dimensions (Rabinowitz 1981) and can apply to different spatial scales. For example, while a species may be locally abundant in a certain habitat type (i.e. be common), it might have a restricted distribution globally (i.e. be rare). As a consequence, the importance of traits for commonness may depend on the spatial scale considered. For example, it is conceivable that large-seeded species have a competitive advantage resulting in high local abundances, whereas small-seeded species might disperse

more widely resulting in higher occurrence frequencies at larger spatial scales (Westoby *et al.* 1992).

The vast majority of studies relating traits to ecological parameters are focussing on aboveground traits, and consequently such traits are overrepresented in trait databases (Laliberté 2017). Although leaf traits, plant height and seed weight are considered to capture most variation in plant form and function (Díaz *et al.* 2016; Westoby 1998), it has been shown that inclusion of belowground traits can substantially increase predictive power for species distributions (Klimešová *et al.* 2016). Indeed, as roots are important for anchorage, storage and the uptake of water and nutrients, belowground functional traits might actually be crucial drivers of a species' abundance and occurrence frequency. Nevertheless, to the best of our knowledge, so far none of the studies comparing rare and common species has included belowground functional traits (Arellano *et al.* 2015; Cadotte & Lovett-Doust 2002; Cornwell & Ackerly 2010; Gabrielová *et al.* 2013). One reason why belowground traits are only rarely considered in plant functional trait analysis is that their measurement is technically challenging and labour intensive (Freschet & Roumet 2017). Another reason is that it is frequently assumed that belowground traits are correlated with aboveground trait (Pérez-Ramos *et al.* 2012; Reich 2014; Shen *et al.* 2019). For example, leaf manganese has been shown to correlate with root traits related to phosphorus acquisition strategy (Lambers *et al.* 2015), and shoot blumenols with the interaction of roots with arbuscular-mycorrhizal fungi (Wang *et al.* 2018a). However, several recent studies show that correlations between above and belowground traits vary tremendously among clades (Valverde-Barrantes *et al.* 2017) and depend on the traits considered (Bergmann *et al.* 2017; Kembel & Cahill 2011; Kramer-Walter *et al.* 2016; Ma *et al.* 2018; Weemstra *et al.* 2016) as some evolutionary drivers are unique to roots (Bergmann *et al.* 2020).

We tested if plant functional traits explain the commonness of species across spatial scales

from local abundance in grassland plots to their worldwide distribution. To do so, we measured root traits and seed weight on 241 grassland species grown in two large common-environment experiments. In addition, we extracted aboveground traits, bud-bank traits and mycorrhizal status from databases. We then tested how the traits relate to the abundance and occurrence in grassland plots of the German Biodiversity Exploratories (Fischer *et al.* 2010), the occurrence frequency across Germany, the occurrence frequency across the European and Mediterranean native region, and their global occurrence as naturalized alien species. We aimed to answer the following questions:

1) Do plant functional traits explain species commonness, i.e. abundance and occurrence frequency across spatial scales?

2) Do the contributions of traits to species commonness vary with the spatial scale considered?

3) Do above- and belowground traits contribute differently to explaining species commonness?

Methods

Species traits

Species selection, seed material and precultivation

The species used are herbaceous angiosperms occurring in the grassland plots of the German "Biodiversity Exploratories" (Fischer *et al.* 2010; Socher *et al.* 2012). In each of three regions of Germany, the Schwäbische-Alb (south-western Germany), Hainich-Dün (central Germany), and Schorfheide-Chorin (north-eastern Germany), 50 plots (4 m × 4 m) were selected in grassland habitats covering a wide range of land-use intensities. From 2008 to 2016, the vegetation composition of each of the 150 plots was assessed annually in late spring by estimating the cover of each species. We standardized the species names according to the accepted names in http://www.theplantlist.org, accessed on 15th June 2019, using the *Taxonstand* package (Cayuela *et al.* 2012) to allow us to align the species names between different distribution and trait datasets (see below). In total, 363 vascular plant species have been identified in the plots of the "Biodiversity Exploratories". For 311 of those species, we were able to obtain seeds from commercial seed suppliers or botanical gardens for our experiments (see Appendix S1 in Supporting Information). For *Alchemilla vulgaris agg.*, which also includes taxa that are difficult to distinguish from *A. vulgaris*, we used seeds from *A. vulgaris*. For *Leucanthemum vulgare agg.*, which includes both *L. vulgare* and *L. ircutianum*, we used seeds from both species and the trait values were averaged.

In two experiments, we measured functional traits on those species. Before the first experiment, we individually weighed 10 seeds, randomly chosen from the supplier's bag, for each of the 311 species. Then we did an indoor pot experiment to determine root morphology of the species, and an outdoor pot experiment to determine rooting depth. For both experiments, seeds were first sown in plastic pots (7 cm × 7 cm × 6.5 cm) filled with peat soil. The pots were then placed in a growth chamber for two to three weeks (night/day 9/15 h; 18/21 ± 1.5°C; relative

humidity 90 ± 5%) before transplanting the seedlings into the pots used for the experiments (for cultivation times, see Appendix S1). In addition to the traits measured in the experiments, we obtained data on aboveground traits (specific leaf area, height), bud-bank size and mycorrhizal status from databases (see the section "Traits from databases and data imputation" below).

Experiment on root-system morphology

From May 1 to October 6, 2017, we performed a glasshouse experiment to measure root-system morphological traits of the study species. As root morphology might depend on nutrient availability, we grew half of the plants per species at intermediate nutrient levels and the other half at high nutrient levels, after which we averaged the trait values per species. Because of the large number of species and the time-consuming measurements, we grew the plants in four temporally shifted (4-6 weeks) batches. We aimed to have each species represented in each batch, and to have a total of seven replicates per species and nutrient level across all batches (Appendix S1). The seedlings of the species that had germinated (N=233) were transplanted individually into plastic pots (1.3 L) filled with a mixture of sand and vermiculite (1:1 volume ratio). The pots were then randomly allocated to positions in two glasshouse compartments, and allowed to grow for four weeks (night/day 10/14 h; 22/28 ± 1.5°C; relative humidity 80 ± 15%). Plants were fertilized three times a week with either an intermediate nutrient solution (40 ml with 1500 µM KNO_3) or a high nutrient solution (40 ml with 12000 µM KNO_3). The fertilizer was a modified version of the Hoagland recipe (see Appendix S2).

We grew the plants for four weeks only to avoid roots becoming pot-bound, to be able to analyse the entire root systems, and to ensure that all the belowground biomass was formed by roots, excluding rhizomes. After washing off the substrate, the root system was cut below the collar and stored for <1 week in a plastic tube filled with distilled water at 4°C. Then, root

systems were spread individually in a thin layer of water in transparent trays (11 cm × 11 cm) and scanned at 800 dpi with a flatbed scanner modified for root scanning (Epson Expression 10000 XL and 11000 XL). The images were analysed using the software WinRHIZO™ 2017a (Regent Instruments, Quebec, Canada) to obtain the total root length and root volume. Root systems were then oven-dried for >48 hours at 65°C and weighed. We calculated specific root length by dividing the total root length by the belowground dry biomass, and root tissue density by dividing the belowground dry biomass by the sum of the root volumes according to Rose (2017). The diameter of fine roots (i.e. distal roots), thought to be the most important roots for nutrient uptake (Freschet & Roumet 2017), was determined by randomly sampling a distal root branch (or a portion of it) for each root system and calculating the mean of the external-internal links diameter obtained with the "Link analysis" function in WinRHIZO™. This subsampling avoided the inclusion of thicker transport roots and allowed us to obtain values that were representative for first order roots. We also dried and weighed the aboveground biomass of each plant, and calculated the root weight ratio (i.e. root biomass divided by total biomass).

Experiment on rooting depth

From the 15th of May to the 10th of October 2018, we performed an outdoor pot experiment to measure the maximum rooting depth of the species. Up to five seedlings of the species that had germinated (n=196; Appendix S1) were transplanted individually into 120 cm tall plastic tree shelter tubes (Tubex ® Standard Plus, http://www.tubex.com/products/tree-shelters/tubex-standard-treeshelters/specification.php), which are normally used in forestry to protect young trees against animals and the elements. We closed the bottoms of these tubes with thick pieces of cotton tissue to be able to use them as pots. The tubes were filled with a mixture of sand and vermiculite (1:1 volume ratio) up to a height of 115 cm. This substrate can be easily penetrated by the roots, and therefore allows each plant to reach its maximum rooting depth quickly. The tree shelter tubes were delivered in packages of five tubes stacked into each other, and they

therefore came in five diameter classes (8.0, 8.4, 10.0, 10.8 and 12.0 cm). To avoid that tube diameter would be confounded with species identity, each of the five seedlings per species was planted in a different tube-diameter class. We placed the tubes upright in a randomized design in the Botanical Garden of the University of Konstanz (47°41'24.0"N 9°10'48.0"E; see Appendix S11 for pictures).

We planted 734 plants but, due to early mortality, we had to replace 126 of them within the next three weeks. The growth period therefore ranged from 16 to 19 weeks. The experiment took place during the summer of 2018 (mean temperature: 19.5°C, min/max 2.5/37.4°C; relative humidity: mean 74%, min/max 22.7/100%). All plants were fertilized once a week with 60 ml of a standard nutrient solution (1‰ Universol® Blue, Nordhorn, Germany), and watered regularly from above. We harvested the plants in October 2018. Each tube was sliced open, and we measured the distance from the top of the substrate to the deepest root.

Traits from databases and data imputation

Data on the aboveground traits specific leaf area (230 species) and height (228 species) were obtained from the LEDA database (Kleyer *et al.* 2008). Data on bud-bank size (230 species) including stem and root-derived buds occurring belowground or at the soil surface was obtained from Klimešová *et al.* (2017). In addition, mycorrhizal status was extracted from the FungalRoot database (Soudzilovskaia *et al.* 2020). We assigned the corresponding genus-level mycorrhizal status for each of our species (241 species) included in the analysis. Though most of our species are considered either obligatorily arbuscular-mycorrhizal (167 species), facultatively arbuscular-mycorrhizal (57 species) or non-mycorrhizal (16 species), *Helianthemum nummularium* is considered ectomycorrhizal. Therefore, it was grouped with the obligatorily arbuscular-mycorrhizal species to form the obligate-mycorrhizal category (168 species).

Although for each of the traits we had data for 196 (rooting depth) to 311 (seed weight) species,

the number of species with complete data for all traits was 170. Therefore, we did phylogenetically informed imputation of missing data for the 241 species that germinated and survived until trait measurement in at least one of our two experiments. Data imputation is a powerful but still underutilized tool that increases sample size —and thus statistical power— and reduces potential biases that might occur if the species with missing data are a non-random subset (Nakagawa 2015). Imputation can perform well with up to 50% of missing data (Graham 2009). In our case, 4.6% of the trait values were missing and had to be imputed (see Appendix S4 for details on the imputation procedure). We also ran all analyses for the subset of 170 species with complete data (i.e. without imputed data), and the results were largely similar to the analyses of the 241 species with partly imputed data (Appendix S9). Because the analyses with the imputed data allowed us to include more species (i.e. increase statistical power and generality), we present only those results in the main text. The phylogenetic tree of the species used, their standardized trait values and phylogenetic signal can be found in Appendix S5, S6 and S7.

Species abundance and occurrence frequency

To quantify each species' commonness from local scale abundance to global naturalization success, we used four different data sources.

The Biodiversity Exploratories

To obtain information on local abundance and occurrence frequency of our study species in German grasslands, we used data from the Biodiversity Exploratories grassland-composition surveys. In each of the three regions, c. 500 so-called grid plots (GPs) and a subset of those, the 50 so-called experimental plots (EPs), have been monitored for biodiversity measures. The plots are 50 m × 50 m, and in each of those there is a subplot of 4 m × 4 m, in which the relative abundance of each plant species has been determined. In the 1494 GPs, vegetation was sampled once from 25 May to 15 August 2007. In May 2009, 138 plots were re-assessed and earlier

relevés were discarded, because they were considered unreliable as the vegetation had been recorded too late in the season of 2007 (Socher *et al.* 2013). Of our 241 study species, 213 were present in that census of the GPs (Appendix S1), and, when present in a plot, they covered on average 2.8% of the plot (min: 0.27%; median: 1.45%; max. 17.16%). For the 150 EPs, the vegetation data were collected annually between mid-May and mid-June from 2008 to 2016, and we averaged the data across years. Of our 241 study species, 239 were present in the EPs vegetation survey, and, when present in a plot, they covered on average 1.05% of the plot (min.: 0.01%; median: 0.34%; max.: 13.05%). Two study species, *Spergula arvensis* and *Taraxacum campylodes*, had been excluded because their names were included in an earlier version of the vegetation survey due to misidentification. While there are 10 times more GPs than EPs, the latter include data over a longer period. For both the GPs and EPs, we used two distribution metrics for each species: the occurrence frequency defined as the number of plots in which a species is present divided by the total number of plots, and the local abundance defined as the mean cover of a species across all the plots where it is present. Because it is based on the presence-absence only, the occurrence frequency estimates how frequent a species is within grasslands in Germany. The average abundance, on the other hand, which is calculated using abundance data for only those plots where the species occurs, estimates how dense the populations of the species are on average.

FloraWeb

For information on the occurrence frequency in all of Germany, irrespective of habitat type, we obtained data from the German plant distribution atlas of NetPhyD and BfN through the FloraWeb data portal (Bundesamt für Naturschutz 2013). For each species, we extracted the number of grid cells in which the species has been reported. Each grid cell is about 130 km², and there are 2995 grid cells in total. Of our 241 study species, 235 had grid-cell data available (Appendix S1).

Euro+Med PlantBase

To obtain information on the extent of the native distribution in all of Europe and its adjacent Mediterranean regions, we used Euro+Med PlantBase (PESI 2015). This on-line database provides information on the presence of vascular plant taxa in 117 regions (mostly countries) covering all of Europe and the Mediterranean regions of North Africa and the Near East. Of our 241 study species, 237 species were found in Euro+Med PlantBase, and for those we extracted the total number of regions with native occurrences (Appendix S1). The four remaining species, *Cerastium nutans*, *Erigeron canadensis*, *Matricaria discoidea* and *Medicago* × *varia*, are not native to the region.

GloNAF

As 237 of our 241 study species are native to Europe, we also assessed the extent of their global occurrence as naturalized alien species, using the Global Naturalized Alien Flora (GloNAF) database, version 1.2 (van Kleunen *et al.* 2019). GloNAF is a compendium of lists of naturalized alien plant species for 1029 regions covering >80% of the terrestrial ice-free surface. Of our 241 study species, 221 species had at least one record in GloNAF. For those species, we extracted the number of regions in which they are naturalized, and for the 20 species without GloNAF records, we set the number of GloNAF regions equal to zero.

Statistical analyses

All statistical analyses were performed in R version 3.6.1 (R Core Team 2019). To test whether more abundant and more widespread species have particular trait values, we used generalized linear models in which the response variables were the different measures of species commonness and the predictors were a selection of trait mean values. For number of occurrences in GloNAF regions, and in grassland GPs and EPs, we used negative binomial error distributions (with a log-link function). As the number of occurrences in FloraWeb grid

cells and Euro+Med regions did not follow negative binomial or Poisson error distributions, we instead analysed the proportion of FloraWeb grid cells and Euro+Med regions in which a species had been recorded, with a binomial error distribution. To account for overdispersion, we used the 'quasibinomial' setting. For analyses of the mean local abundance (i.e. the cover proportion) of the species in the GPs and EPs, we used a gamma conditional distribution (with log-link function).

For each commonness measure, we used a multivariate model with ten traits as predictors. We *a priori* chose traits that represent different aspects of plant functioning and that had relatively low pairwise correlations (all $r \leq |0.49|$, Appendix S3) to minimize multicolinearity (the maximum generalized variance-inflation factor of a model was 3.32). We used the following traits: individual seed weight (measured on seeds ordered for the experiments), specific root length, root tissue density and fine roots diameter (measured in the root-system morphology experiment), maximum rooting depth (measured in the rooting-depth experiment), and bud-bank size, height, specific leaf area and mycorrhizal status (from trait databases). Seed weight was log transformed. To facilitate interpretation of and comparison between model coefficients, each trait was scaled to a mean of zero and a standard deviation of one (Schielzeth 2010). To test for potential non-linear effects of traits, orthogonal polynomial terms of second degree (i.e. quadratic terms) were also included for each trait, using the *poly* function. To estimate the proportion of variance explained by the models, we calculated delta R^2 values, applicable to all distributions and link functions, according to Nakagawa *et al.* (2017) using the package MuMIn (Barton & Barton 2015). To assess whether belowground traits explained more variance in commonness measures than aboveground traits, we also extracted delta R^2 values for models using only the three aboveground predictors and for models using only the three belowground predictors with the highest standardized coefficients. To account for phylogenetic non-independence of the study species, the models were also run using

phylogenetic relatedness of species as a variance-covariance matrix (for details, see Appendix S4). Although the significances of the trait effects differed in some instances between the non-phylogenetic models and the phylogenetic ones, the directions of the effects were largely the same in both types of models (compare Fig. 1 and Appendix S8). Therefore, we only present the results of the non-phylogenetic analyses in the main text.

Results

All of our species commonness metrics were significantly related to at least three of the ten traits considered, including both above- and belowground traits (Fig. 1). The abundance measures in the grassland EPs and the occurrence in the EPs and GPs were associated with the largest number of traits (Fig. 1). The delta R^2 values ranged from 0.02 for the model on occurrence frequency in the native range (i.e. the proportion of occurrences in Euro+Med) to 0.42 for the model on abundance in the EPs (i.e. the mean cover of a species when present in a grassland plot; Fig. 1; Table 1). When we reduced the models to either include only the three aboveground traits or the three best belowground traits, the variation explained by the belowground-trait models was equal or greater than the variation explained by the aboveground-trait models for all species commonness metrics, except frequency of occurrence in Germany and in the grassland EPs (Table 1).

Root-tissue density was a significant predictor in all models (Fig. 1). Species with low root-tissue densities were consistently more common than species with high root-tissue densities across all spatial scales considered (i.e. all linear coefficients were negative and significant; Fig. 1). For occurrence frequency outside the native range, the coefficient of the quadratic term was also significantly negative (Fig. 1), indicating that this commonness metric was highest for species with intermediate root-tissue densities (Fig. 1, Appendix S10).

Specific leaf area (SLA) was positively associated with species commonness in most large spatial scale models (Fig. 1). Occurrence frequencies outside their native range, in Germany, and in the grassland GPs and EPs increased with SLA, and sometimes slightly decreased again at higher SLA values. However, SLA had no significant effect on the abundance within GPs and EPs (Fig. 1, Appendix S10).

Bud-bank size was also a significant predictor in most models, but its effects on species commonness varied with spatial scale (Fig. 1). At large spatial scales, occurrence frequencies

were highest for species with either small or large bud-bank sizes and lowest for species with intermediate ones (marginally and significantly positive coefficients of the quadratic terms for the numbers of GloNAF and Euro+Med regions, respectively; Fig. 1, Appendix S10). On the other hand, occurrence frequencies and abundances in the grassland GPs and EPs increased linearly or asymptotically with bud-bank size (Fig. 1, Appendix S10).

Obligate-mycorrhizal and facultative-mycorrhizal species were consistently more frequent and more abundant than non-mycorrhizal species, although not always significantly so for occurrence frequencies at larger spatial scales (Fig. 1, Appendix S10).

Fine root diameter had a negative effect on the occurrence frequencies outside the native range, in Germany, in the EPs and on abundance in the GPs and EPs (Fig. 1, Appendix S10).

The other traits were only significant predictors in a few of the models of species commonness (Fig. 1). Maximum rooting depth was non-linearly positively associated with occurrence frequency outside the native range, and was positively associated with occurrence frequencies in the native range and Germany, but not significantly in the EPs and GPs (Fig. 1, Appendix S10). Plant height was not significantly associated with commonness at the larger spatial scales, but was negatively associated with occurrence frequency in the GPs and EPs and positively with abundance in the GPs (Fig. 1, Appendix S10). Seed weight tended to have negative but non-significant associations with commonness at the larger spatial scales, but positive significant associations with abundances in the GPs and EPs (Fig. 1, Appendix S10). Root-weight ratio was negatively associated with occurrence frequency outside the native range, with an optimum in the mid-lower range of the trait, which was also the case for abundance in EPs (Fig. 1, Appendix S10). Specific root length was not significantly associated with any measure of commonness (Fig. 1). However, it tended to be negatively associated with occurrence frequency in Germany and the highest native occurrence frequency was associated with intermediate values (marginally significant effects in Fig. 1, Appendix S10).

Figure 1 Estimates of trait effects on different commonness metrics of German grassland species from generalized linear models. On the y-axis are the 10 traits used as predictors, with a linear term (white rows) and a quadratic (non-linear) term (grey rows) for each trait. The errors bars around the estimates are standard errors. Significant negative and positive estimates are marked in red and blue, respectively. In addition, estimates with $p < 0.001$ are indicated with ***, estimates with $p < 0.01$ with **, and estimates with $p < 0.05$ with *. Marginally significant estimates ($p <$ 0. 1) are indicated with †. The spatial scale of the commonness metric decreases from left to right. GloNAF: number of regions in which a species is naturalized (number of species, N=241); Euro+Med: number of regions in Europe and the Mediterranean basin in which a species is native (N=237); FloraWeb: number of grid cells in Germany in which a species is present (N=235); GPs Frequency: number of grassland grid plots in which a species is present (N=213); EPs Frequency: number of grassland experimental plots in which a species is present (N=239); GPs Abundance: mean species cover in grassland grid plots in which the species is present (N=213); EPs Abundance: mean species cover in grassland experimental plots in which a species is present (N=239). Delta R^2 was calculated according to Nakagawa *et al.* (2017).

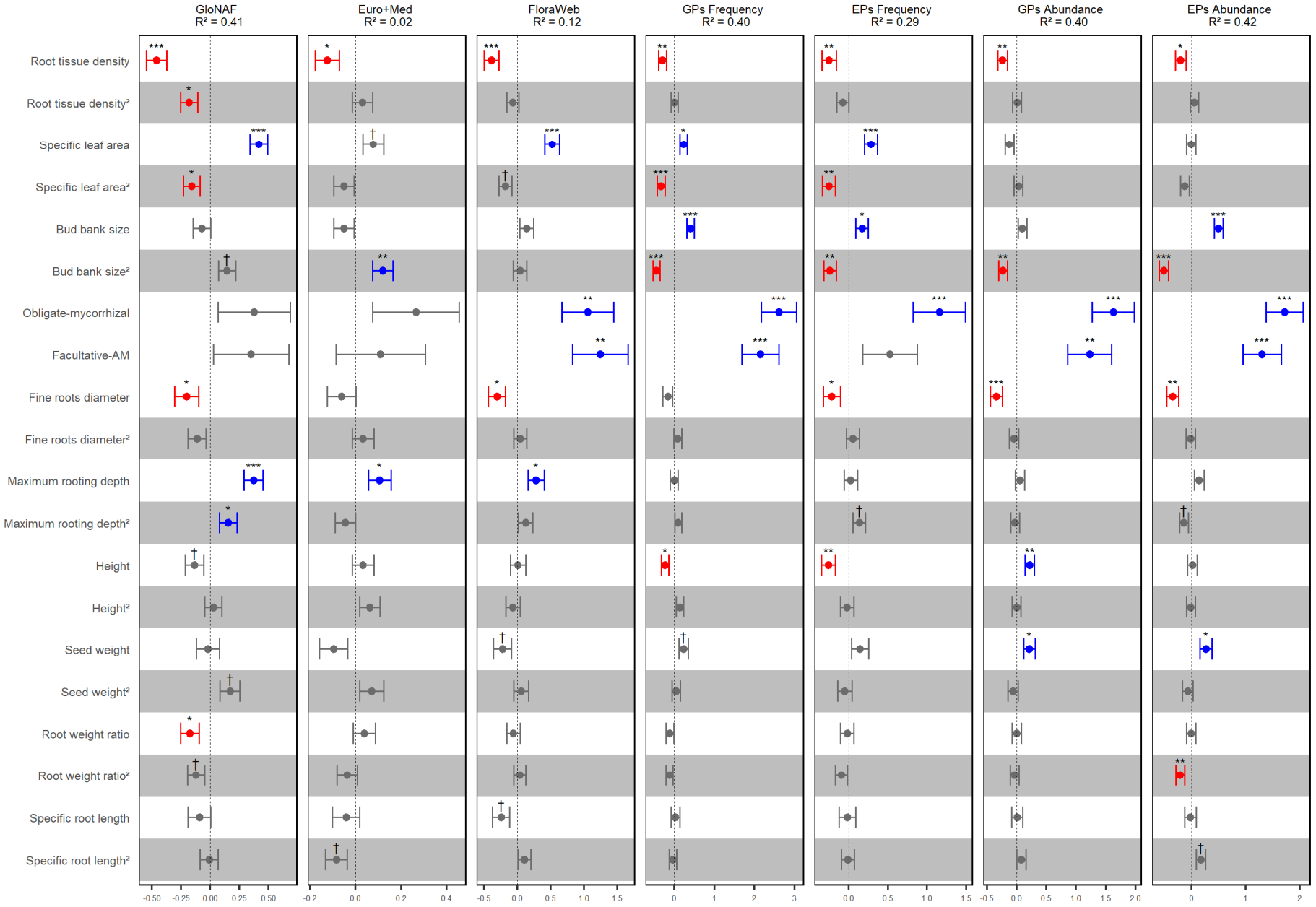
GloNAF
R² = 0.41
Euro+Med
R² = 0.02
FloraWeb
R² = 0.12
GPs Frequency
R² = 0.40
EPs Frequency
R² = 0.29
GPs Abundance
R² = 0.40
EPs Abundance
R² = 0.42
Root tissue density
Root tissue density²
Specific leaf area
Specific leaf area²
Bud bank size
Bud bank size²
Obligate-mycorrhizal
Facultative-AM
Fine roots diameter
Fine roots diameter²
Maximum rooting depth
Maximum rooting depth²
Height
Height²
Seed weight
Seed weight²
Root weight ratio
Root weight ratio²
Specific root length
Specific root length²

Table 1 Delta R^2 of models explaining species commonness using a different set of predictors. On the two first lines are the R^2 values for models with the three predictors with highest explanatory power aboveground and belowground. On the last line are the R^2 values for models with all 10 traits used as predictors.

	GloNAF	Euro+Med	FloraWeb	GPs Frequency	EPs Frequency	GPs Abundance	EPs Abundance
Specific leaf area, seed size, height	0.18	0.01	0.06	0.16	0.16	0.15	0.16
Root tissue density, bud-bank size, mycorrhizal status	0.24	0.01	0.04	0.31	0.13	0.28	0.35
All the predictors (10 traits)	0.41	0.02	0.12	0.40	0.29	0.40	0.42

Discussion

We tested how above- and belowground functional traits of grassland species related to their commonness across multiple spatial scales. Among the 241 study species, low root-tissue density was identified as important for all commonness metrics. However, the effects of the other traits were frequently dependent on the commonness metric considered. They notably differed between the occurrence frequency metrics, capturing how widespread a species is, and the abundance metrics, capturing the mean density of individuals of a species where they occur. For example, while specific leaf area was positively and asymptotically related to the large-scale commonness metrics, it was not related to the local abundance in German grasslands. This illustrates that some traits may have different effects on different dimensions of species rarity and commonness. Moreover, we found that many of the effects had significant non-linear effects, in most cases with the highest commonness-metric value at intermediate trait values. While all previous studies on the importance of functional traits for commonness focussed on aboveground traits that are easy to measure or available in databases (Arellano *et al.* 2015; Cadotte & Lovett-Doust 2002; Cornwell & Ackerly 2010; Gabrielová *et al.* 2013), our study shows that belowground traits can also explain a significant amount of variation in species commonness.

The spatial scale of commonness ranged from local abundance in 16-m² grassland plots in Germany (GPs and EPs) to the global occurrence outside the native range (number of GloNAF regions). While the local scale abundance data are restricted to a single habitat type, the occurrence at larger spatial scales also covers other habitat types (e.g. 86% of the area in Germany is not used as grassland; DESTATIS 2019). Each habitat type might select for different values of a trait (Lososová *et al.* 2006; Shipley *et al.* 2017), and species that can occur in many different habitat types (i.e. are habitat generalists) may differ in their trait values from

those of habitat specialists. Future studies should therefore try to separate the effects of functional traits on range size from those on habitat generalism.

We found varying degrees of consistency in the trait values of common species across spatial scales. Root-tissue density was the only trait with a consistent effect on all commonness metrics. Probably, a low root-tissue density, which is indicative of a high resource-acquisition-rate strategy (Kramer-Walter *et al.* 2016) is beneficial in nutrient-rich habitats, which have locally and globally become more widespread as a consequences of agriculture and atmospheric nitrogen deposition. On the other hand, the effect of specific leaf area, an aboveground trait associated with the resource-acquisition strategy (Lambers & Poorter 1992; Onoda & Wright 2018) depended on the spatial scale of the commonness metric. Occurrence frequencies at all spatial scales tended to asymptotically increase with specific leaf area, which is in line with the frequent observation that high specific leaf area promotes invasion success (Pyšek & Richardson 2008; van Kleunen *et al.* 2010). However, abundance in the grassland plots was not related to specific leaf area, possibly reflecting that persistence under highly competitive pressures in dense grasslands could require a more conservative growth strategy.

Bud-bank size had an effect on most commonness metrics, but the direction and shape of the relationship varied a lot. Species with intermediate or large bud-banks had the highest abundance and occurrence frequency in the grassland plots. A large bud-bank is essential for regrowth of long-lived perennials after e.g. grazing or mowing (Ott *et al.* 2019). On the other hand, species with small bud-banks, as well as those with large bud banks, tended to have larger naturalized ranges and higher occurrence frequencies in the native range than species with intermediate bud-bank sizes. Although buds themselves are not very costly (Vesk & Westoby 2004), they require bud-bearing organs and nutrient reserves, which may trade-off with seed production (Herben *et al.* 2012; Herben *et al.* 2015). Thus, species with small bud banks, which are more likely to be short-lived, may invest more in seed production, resulting in a higher

dispersal ability and larger native and naturalized distributions.

The obligate or facultative interaction with mycorrhizal fungi, mostly arbuscular mycorrhiza, had a positive effect on commonness metrics in the grassland plots and occurrence frequency in Germany. Mycorrhizal plants also tended to be more widely naturalized around the globe, confirming the results of a recent global analysis (Pyšek *et al.* 2019), although this effect was not statistically significant. In return for carbon, mycorrhizal fungi provide plants with nutrients and improve their resistance against various stresses. These benefits of the interaction for plants could allow them to survive and reproduce in a greater variety of habitats and environmental conditions than non-mycorrhizal species. Mycorrhizal plants are found in a large variety of habitats (van der Heijden *et al.* 2015), but could take particular advantage of the minimally disturbed soil in managed grasslands where the mycelium networks can be preserved over several years (Read & Birch 1988). On the other hand, Brassicaceae, which are predominant in our non-mycorrhizal species set (see Appendix S6), are more characteristic of disturbed habitats, typical for where these species frequently grow outside their native range. As a consequence of the strong phylogenetic signal in mycorrhizal status (Appendix S7) and the small number of non-mycorrhizal plants in our dataset (16), none of the mycorrhizal effects on commonness remained significant after accounting for phylogenetic non-independence of the species (Appendix S8). The results on the effects of mycorrhizal status on commonness should thus be interpreted with caution.

Thin roots generally characterized common species in our study at both large and small spatial scales. Furthermore, root-tissue density was lower in common species, indicating the tendency to develop 'cheap' root systems, in terms of carbon. Thin, but especially low-density roots potentially have shorter lifespans than thick roots or roots with dense tissues (Ma *et al.* 2018; Ryser 1996), though the functional mechanisms underlying these relationships differ between these two traits (Bergmann *et al.* 2020; Weemstra *et al.* 2016). They could potentially exploit

more soil volume per unit of carbon invested. This could be an advantage in both grasslands and anthropogenic habitats, which are generally fertile, and thus could explain why thin roots are more common among both highly naturalized species and dominant species in German grasslands. The specific costs and benefits of root diameter and root tissue density in different environments are however still poorly known (Laliberté 2017).

Maximum rooting depth was positively related to commonness metrics at the largest spatial scales. Deep roots allow a plant to take up water with nutrients from deeper soil layers, increasing survival and growth, particularly when the surface soil regularly dries out (Comas *et al.* 2013). As most of the agriculturally used grasslands in Germany are mesic (European Environment Agency 2019), this could explain why rooting depth was not significantly associated with occurrence frequency and abundance in the grassland plots. At the larger spatial scales, which also cover other habitat types, species with deep roots might be more persistent. For naturalization success, however, there was a significant non-linear effect of rooting depth as both deep-rooting and superficially rooting species were most successful. This could indicate that the alternative strategy of lateral spread to acquire resources and avoid competition with deeper rooting species (Fitter 1986) might also be beneficial at the global scale.

Height of the plants was not significantly related to commonness of the species at the largest spatial scales. However, it tended to be positively (marginally significantly) associated with naturalization success, which corroborates numerous studies on naturalization and invasion success that found that tall species were more successful (Bucharova & van Kleunen 2009; Pyšek & Richardson 2008). Interestingly, while plant height increased abundance in the German grassland plots (at least in the GPs), it decreased the occurrence frequency in those grasslands. On the one hand, tall plants, when they occur somewhere, might be competitively superior and become dominant, whereas, on the other hand, small plants might be less at risk of losing reproductive organs due to mowing or grazing.

The effect of seed weight on species commonness metrics was positive at the plot scale and tended to be negative or absent at larger spatial scales. The finding that species with heavy seeds tended to be more frequent and abundant in the grassland plots, most likely reflects that large amounts of resources stored in seeds increase seedling survival under the strong competition in grasslands (Kempel *et al.* 2013; Moles & Westoby 2004). Species with light seeds, on the other hand, might have a higher reproductive output (Moles & Westoby 2006), could potentially disperse over longer distances (Tackenberg *et al.* 2003; Thomson *et al.* 2011) and could persist longer in the seed bank (Garnier & Navas 2012). At the larger spatial scales, this benefit of small seeds could have compensated or overcompensated the reduced seedling survival chances.

We found that species that are abundant in grasslands are typically characterized by thin, mycorrhizal, low-density roots, which promote the uptake of belowground resources. In grasslands, belowground organs are of particular importance, as net primary production allocated belowground could be more than 80% (Lauenroth & Gill 2003). Rhizomes, lignotubers and belowground stems can allow plants to resprout and survive disturbances, such as mowing and grazing, and promote regrowth after unfavourable seasons. The importance of bud-bank size in grasslands emphasizes the need to integrate these different belowground organs in the study of functional traits linked to species dominance (Ottaviani *et al.* 2020). The patterns for commonness metrics at larger spatial scales, at least those in the native ranges, were less clear. Indeed, plant traits explained large proportions of the variation in local abundance and occurrence frequency in the grasslands (>25%; Table 1), whereas the proportion of variation in occurrence frequency in the native range and in Germany was very low (2% and 12%, respectively; Table 1). This suggests that plant traits could be good predictors of species commonness if one considers a single habitat type, but that this is less the case for commonness metrics at large spatial scales that are not habitat specific. However, a notable exception is the

global naturalization success of the species, as 41% of the variation in occurrence frequency outside the native range was explained by the plant functional traits. Possibly, this reflects that most naturalizations happen in anthropogenic environments (Chytrý *et al.* 2009), and thus largely in a single habitat type.

The plant economics spectrum postulates that the high specific leaf area typical for "acquisitive" plants should be mirrored belowground by a high specific root length, low root-tissue density and a low root diameter (Prieto *et al.* 2015). Indeed, specific leaf area was negatively correlated with root-tissue density, but it was not significantly correlated with specific root length and diameter of the fine roots (Appendix S3). Although the traits were not strongly correlated at the species level, species common at the larger scales were more likely to have a high specific leaf area, a low root tissue density and thin roots. This decoupling from the plant economic spectrum at the species level has previously been found for grassland plants (Bergmann *et al.* 2017) as well as tree seedlings (Kramer-Walter *et al.* 2016) and supports recent findings emphasizing that specific root length and diameter vary independently from the fast-slow economics spectrum (Bergmann *et al.* 2020). Seed weight and plant height, the other aboveground traits frequently used in studies on functional ecology of plants, were also not strongly correlated with the belowground traits in our study. We measured the diameter of fine roots, which has been proposed to be a proxy of multiple physiological and anatomical traits related to the resource-acquisition strategy (Guo *et al.* 2008; Wen *et al.* 2019) and notably of mycorrhizal colonization (Bergmann *et al.* 2020; Ma *et al.* 2018). Abundant species in grasslands were characterized with having thin roots but also an obligate mycorrhizal status, suggesting that direct measurements of mycorrhizal colonization and physiological traits would improve our understanding of the links between traits and functions (Laliberté 2017). These belowground traits explained a considerable proportion of variation in the commonness metrics, in addition to the variation explained by the three aboveground traits (Table 1). Indeed,

for all commonness metrics, except occurrence frequency in the EPs, the belowground traits explained at least as much of the variation in commonness metrics as the three aboveground traits did. Therefore, our results show that aboveground traits cannot always substitute for belowground traits in studies on plant functional ecology.

Conclusions

We here showed that functional traits of common grassland species differed from those of less common ones, but that the pattern depended on the spatial scale of the commonness metric it applies to. Low root-tissue density was the only trait that characterized common species at every spatial scale, from being abundant in German grassland plots to being widely naturalized around the world. We showed that belowground traits are at least as important as the aboveground traits in explaining species commonness at the different spatial scales. The variation in importance and the sometimes-opposing directions of the effects of traits on species commonness at different spatial scales can explain why trait variation is maintained. Our study shows that, for Central European grassland species, variation in commonness is related not only to aboveground traits, but also to belowground traits. Therefore, belowground traits should be more frequently considered in studies on plant functional ecology.

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Data availability

The data supporting the paper will be archived in Dryad, and the data DOI will be included at the end of the article.

Supporting Information

Appendix S1 Information on the 241 study species used in the analyses

The five species marked with an asterisk (*) were present under several names in the Biodiversity Exploratories vegetation records. The upper name in the row is the accepted name, according to The Plant List, and the lower name is a synonym name. Some of those species had initially been included as separate species in the root-morphology and rooting-depth experiments, and these were later merged (resulting in more than 14 and 5 replicates, respectively). The seedling cultivation time is the time between sowing and transplanting of seedlings in the root-morphology experiment. For the rooting-depth experiment, one more week of was added to reduce seedlings mortality after transplanting. Abbreviations used for "Traits imputed" column: BB = bud-bank size, FRD = fine roots diameter, MRD = maximum rooting depth, RTD = root tissue density, RWR = root weight ratio, SLA = specific leaf area, SRL = specific root length.

The Plant List 1.1 binomial name accessed: 15 June 2019	Family According to TPL 1.1	Seed Source City / Company	Seedling cultivation time W = Week	Cold stratification -20°C in freezer	Sample size Morphology Exp. Intermediate Nutrients	Sample size Morphology Exp. High Nutrients	Sample size Rooting depth Exp.	Traits Imputed	EPs Abundance	GPs Abundance	EPs Frequency	GPs Frequency	FloraWeb	Euro+Med	GloNAF
Achillea millefolium	Asteraceae	Rieger-Hofmann	3W	No	6	7	5		2.04	2.47	100	683	2991	60	137
Agrimonia eupatoria	Rosaceae	Rieger-Hofmann	3W	Yes	1	1	3		0.83	1.60	60	266	2647	21	21
Agrostis capillaris	Poaceae	Rieger-Hofmann	2W	No	7	6	4		0.89	6.11	36	151	2974	50	131
Agrostis stolonifera	Poaceae	Konstanz ; Goettingen	2W	No	7	6	5	Height	1.66	12.31	43	199	2979	74	228
Alchemilla monticola	Rosaceae	Bormio Rezia. I	3W	Yes	7	5	3	BB	0.21	0.95	27	94	1256	3	16
Alchemilla vulgaris agg.	Rosaceae	Rostock	3W	Yes	4	5	0	MRD	0.12	NA	31	NA	2421	36	3
Allium schoenoprasum	Amaryllidaceae	Rieger-Hofmann	2W	No	7	7	5		0.05	NA	10	NA	1035	32	22
Allium scorodoprasum	Amaryllidaceae	Halle (Matthias Stolle)	2W	No	7	6	0	MRD	0.06	NA	1	NA	970	26	6
Alopecurus geniculatus	Poaceae	Rieger-Hofmann	2W	No	5	4	4		2.50	10.68	7	14	2599	45	107
Alopecurus pratensis	Poaceae	Rieger-Hofmann	2W	No	6	6	5		9.48	8.29	95	494	2965	55	104

The Plant List 1.1 binomial name accessed: 15 June 2019	Family According to TPL 1.1	Seed Source City / Company	Seedling cultivation time W = Week	Cold stratification -20°C in freezer	Sample size Morphology Exp. Intermediate Nutrients	Sample size Morphology Exp. High Nutrients	Sample size Rooting depth Exp.	Traits Imputed	EPs Abundance	GPs Abundance	EPs Frequency	GPs Frequency	FloraWeb	Euro+Med	GloNAF
Anchusa officinalis	Boraginaceae	Rieger-Hofmann	2W	No	6	7	1		0.01	NA	1	NA	1260	42	39
Anthoxanthum odoratum	Poaceae	Rieger-Hofmann	2W	No	6	7	5		3.07	2.18	35	131	2937	71	197
Anthyllis vulneraria	Fabaceae	Rieger-Hofmann	3W	Yes	7	4	0	MRD	0.93	3.83	2	19	2119	61	24
Aphanes arvensis	Rosaceae	Nantes	3W	Yes	6	6	2		0.08	0.56	8	3	2519	4	32
Arabidopsis arenosa	Brassicaceae	Konstanz	2W	No	7	6	5		0.54	0.39	4	10	1741	31	15
Arabidopsis thaliana	Brassicaceae	Rieger-Hofmann	2W	No	6	6	1		0.02	0.38	5	2	2807	60	84
Arabis sagittata	Brassicaceae	Eötvös Uni Budapest	3W	Yes	5	7	4	BB	0.02	NA	2	NA	133	34	0
Arctium lappa	Asteraceae	RH ; Konstanz	2W	No	7	7	5		0.35	3.01	1	45	2535	59	76
Arctium minus	Asteraceae	Konstanz	2W	No	7	7	5		1.03	2.30	24	70	2725	67	98
Arctium nemorosum	Asteraceae	RH ; Konstanz	3W	No	7	7	5	SLA	0.01	5.02	1	2	1464	39	0
Arctium tomentosum	Asteraceae	RH ; Konstanz	2W	No	7	7	0	MRD	0.06	1.47	11	43	2031	44	39
Arenaria serpyllifolia	Caryophyllaceae	Rieger-Hofmann	2W	No	6	6	5		0.06	0.61	9	16	2932	64	111
Arrhenatherum elatius	Poaceae	Rieger-Hofmann	2W	No	6	6	5		5.97	12.72	98	577	2967	80	149
Asperula cynanchica	Rubiaceae	Rieger-Hofmann	3W	Yes	6	2	1		0.42	0.65	7	37	862	44	1
Barbarea vulgaris	Brassicaceae	Rieger-Hofmann	2W	No	7	7	0	MRD	0.06	0.66	1	2	2737	53	74
Bellis perennis	Asteraceae	Rieger-Hofmann	2W	No	6	7	5		0.69	1.55	59	209	2984	72	110
Berula erecta	Apiaceae	Konstanz	3W	No	4	3	0	MRD	0.13	1.44	1	2	2338	70	38
Brachypodium pinnatum	Poaceae	RH ; Konstanz	3W	No	7	7	5		7.16	10.95	19	104	1956	66	11
Brassica rapa	Brassicaceae	Rieger-Hofmann	2W	No	7	6	2	BB	0.02	NA	1	NA	612	33	345
Briza media	Poaceae	Rieger-Hofmann	3W	No	6	7	0	MRD	2.05	4.20	19	119	2611	55	24
Bromus erectus	Poaceae	Rieger-Hofmann	2W	No	7	5	5		13.06	15.90	23	121	1822	48	48
Bromus hordeaceus	Poaceae	Rieger-Hofmann	2W	No	6	7	5		2.34	3.37	96	377	2928	85	191
Bromus inermis	Poaceae	Mattias Stolle	2W	No	7	6	5		7.14	8.71	6	18	2815	38	120
Bromus sterilis	Poaceae	Konstanz	2W	No	7	6	5		0.08	7.14	1	19	2651	60	88
Calamagrostis canescens	Poaceae	Hohenheim	3W	No	7	7	5		1.84	2.84	1	2	2077	40	0
Campanula glomerata	Campanulaceae	Rieger-Hofmann	3W	No	6	7	0	MRD	0.03	0.43	4	4	1380	49	25

The Plant List 1.1 binomial name accessed: 15 June 2019	Family According to TPL 1.1	Seed Source City / Company	Seedling cultivation time W = Week	Cold stratification -20°C in freezer	Sample size Morphology Exp. Intermediate Nutrients	Sample size Morphology Exp. High Nutrients	Sample size Rooting depth Exp.	Traits Imputed	EPs Abundance	GPs Abundance	EPs Frequency	GPs Frequency	FloraWeb	Euro+Med	GloNAF
Campanula patula	Campanulaceae	Rieger-Hofmann	3W	No	6	5	0	MRD	0.21	0.36	9	22	2117	42	13
Campanula persicifolia	Campanulaceae	Rieger-Hofmann	3W	No	6	5	2		0.41	NA	1	NA	1894	50	22
Campanula rapunculoides	Campanulaceae	Rieger-Hofmann	3W	No	7	7	4		0.04	1.24	3	15	2580	47	81
Campanula rotundifolia	Campanulaceae	Rieger-Hofmann	3W	No	6	4	4		0.26	0.50	15	80	2914	51	6
Capsella bursa-pastoris	Brassicaceae	Konstanz	2W	No	6	6	5		0.23	NA	53	NA	2981	63	348
Cardamine pratensis	Brassicaceae	Rieger-Hofmann	3W	No	3	1	0	MRD	0.62	0.93	13	41	2923	52	7
Carduus acanthoides	Asteraceae	Köln	3W	Yes	4	1	0	MRD	0.06	0.45	1	8	1270	41	85
Carex acutiformis	Cyperaceae	Rieger-Hofmann	3W	No	3	3	0	MRD	5.20	17.16	4	67	2724	55	25
Carex flacca	Cyperaceae	RH ; Cry d'Er	3W	No	3	4	0	MRD	3.05	3.10	13	74	2326	71	15
Carex leporina * *Carex ovalis*	Cyperaceae	Rieger-Hofmann	3W	No	11	11	9		0.15	NA	4	NA	2748	55	31
Carex muricata	Cyperaceae	Rieger-Hofmann	3W	No	7	7	2		0.28	1.90	10	13	2682	65	21
Carex ornithopoda	Cyperaceae	Alpino Rezia	3W	No	5	4	0	BB MRD	0.09	NA	2	NA	668	34	0
Carex praecox	Cyperaceae	Regensbourg	3W	No	7	6	5		0.07	NA	1	NA	703	40	0
Carex vulpina	Cyperaceae	RH ; Hohenheim	3W	No	6	7	4	Height	0.08	2.29	2	6	2060	42	0
Carlina acaulis	Asteraceae	Klaipeda ; Salzburg ; Rezia	2W	No	6	7	0	MRD	0.37	0.73	9	49	749	31	3
Carlina vulgaris	Asteraceae	Rieger-Hofmann	3W	No	7	7	4		0.14	0.45	10	35	2089	52	3
Carum carvi	Apiaceae	Rieger-Hofmann	3W	No	6	3	0	MRD	0.98	1.43	50	175	2093	53	72
Centaurea jacea	Asteraceae	Rieger-Hofmann	3W	Yes	5	4	3		1.35	1.33	23	82	2892	55	65
Centaurium erythraea	Gentianaceae	Rieger-Hofmann	3W	Yes	6	4	1		0.12	0.56	2	15	2367	64	101
Cerastium arvense	Caryophyllaceae	Rieger-Hofmann	3W	Yes	5	5	1		0.32	0.47	14	35	2814	35	66
Cerastium glomeratum	Caryophyllaceae	.002906 Uni KN (Angers. F)	3W	Yes	6	6	2		0.01	NA	8	NA	2516	63	196
Cerastium nutans	Caryophyllaceae	Rieger-Hofmann	3W	Yes	7	4	0	SLA BB MRD Height	0.01	0.31	1	3	NA	NA	0
Cerastium pumilum	Caryophyllaceae	Siena	3W	Yes	6	7	0	MRD	0.01	NA	5	NA	1324	49	44
Cerastium semidecandrum	Caryophyllaceae	Göttingen	3W	Yes	5	5	1		0.15	0.43	2	1	2124	53	63
Chaerophyllum aureum	Apiaceae	Rieger-Hofmann	3W	No	NA	NA	1	RTD RWR FRD SRL	0.18	1.43	13	27	1032	37	14
Chenopodium album	Amaranthaceae	Rieger-Hofmann	3W	No	7	6	4		0.08	0.56	17	18	2974	15	416

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Cichorium intybus	Asteraceae	Rieger-Hofmann	3W	Yes	7	7	5		0.70	1.32	45	35	2611	64	221
Cirsium acaule	Asteraceae	Rieger-Hofmann	3W	Yes	7	6	2		1.54	1.95	18	93	15	25	0
Cirsium eriophorum	Asteraceae	Rieger-Hofmann	3W	Yes	3	6	0	MRD	0.12	0.64	6	27	561	30	0
Clinopodium acinos	Lamiaceae	UfA	2W	No	7	7	5		0.05	0.78	3	8	1815	41	28
Convolvulus arvensis	Convolvulaceae	Aigues Vives	2W	No	6	7	5		0.78	2.36	53	268	2887	86	249
Cota tinctoria	Asteraceae	Rieger-Hofmann	2W	No	6	7	5		0.28	0.43	1	3	1863	55	64
Crepis biennis	Asteraceae	Rieger-Hofmann	3W	Yes	1	NA	0	MRD	1.16	2.17	69	325	2527	43	10
Cruciata laevipes	Rubiaceae	Stuttgart-Hohenheim	3W	Yes	NA	NA	1	RTD RWR FRD SRL	1.39	2.41	5	16	1651	44	14
Cynosurus cristatus	Poaceae	Rieger-Hofmann	2W	No	7	6	5		1.52	4.12	45	241	2710	55	75
Dactylis glomerata	Poaceae	Rieger-Hofmann	2W	No	6	7	5		6.34	10.10	138	1088	2990	83	285
Daucus carota	Apiaceae	Rieger-Hofmann	2W	No	7	6	5		0.34	1.25	56	206	2942	88	200
*Deschampsia cespitosa * Deschampsia littoralis*	Poaceae	Rieger-Hofmann	2W	No	8	8	5		3.12	6.19	27	195	2955	54	25
Dianthus carthusianorum	Caryophyllaceae	Rieger-Hofmann	2W	No	6	7	5		0.01	1.72	1	9	1656	28	6
Dipsacus fullonum	Dipsacaceae	Rieger-Hofmann	2W	No	7	7	5		0.04	0.76	2	11	2236	44	80
Elymus repens	Poaceae	Rezia ; Kew	3W	No	7	6	2		3.69	8.11	91	369	2975	70	129
Epilobium hirsutum	Onagraceae	Rieger-Hofmann	2W	No	7	7	4		0.11	0.48	1	5	2914	81	32
Epilobium parviflorum	Onagraceae	Rieger-Hofmann	2W	No	7	7	5		0.28	0.73	2	7	2794	79	16
Erigeron acris	Asteraceae	Konstanz ; Oldersburg	2W	No	7	4	0	SLA MRD Height	0.05	0.76	1	5	2441	63	3
Erigeron canadensis	Asteraceae	Konstanz	3W	No	3	3	5		0.03	0.79	2	7	2898	0	370
Erophila verna	Brassicaceae	Rieger-Hofmann	3W	Yes	7	6	0	MRD	0.14	0.44	32	15	2826	62	81
Eryngium campestre	Apiaceae	Rieger-Hofmann	3W	Yes	5	3	0	MRD	0.10	3.24	2	14	592	61	12
Euphorbia cyparissias	Euphorbiaceae	Rieger-Hofmann	3W	Yes	7	6	0	MRD	1.39	1.82	18	136	2590	31	79
Euphorbia helioscopia	Euphorbiaceae	Nantes. F (011944)	3W	Yes	3	1	0	MRD	0.09	NA	1	NA	2881	51	143
Euphrasia officinalis	Orobanchaceae	Rieger-Hofmann	3W	Yes	1	NA	0	BB MRD	0.10	NA	11	NA	1675	18	0
Falcaria vulgaris	Apiaceae	Rieger-Hofmann	3W	Yes	2	1	0	MRD	1.79	0.61	4	13	1324	46	29
Fallopia convolvulus	Polygonaceae	Münster. D	3W	Yes	1	2	0	MRD	0.04	0.46	1	4	2950	1	184

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Festuca arundinacea	Poaceae	Rieger-Hofmann	2W	No	7	7	5		0.50	9.18	7	37	2726	70	120
Festuca guestfalica	Poaceae	Rieger-Hofmann	2W	No	7	7	5	SLA BB	1.75	7.58	24	86	684	14	0
Festuca nigrescens	Poaceae	Rieger-Hofmann	2W	No	7	7	5	SLA BB	1.18	5.29	5	14	523	26	10
Festuca ovina	Poaceae	Rieger-Hofmann	3W	No	1	1	1	BB	2.38	12.37	31	29	2951	43	23
Festuca pratensis	Poaceae	Rieger-Hofmann	2W	No	7	7	5		4.39	5.67	115	638	2961	62	98
Festuca rubra	Poaceae	Rieger-Hofmann	2W	No	6	7	5		5.65	8.48	97	446	2983	56	136
Fragaria vesca	Rosaceae	Rieger-Hofmann	2W	No	6	6	5		0.22	0.57	2	9	2744	24	70
Fragaria viridis	Rosaceae	Konstanz ; Budapest	2W	No	7	6	5		1.09	3.67	3	16	1183	50	0
Galium album	Rubiaceae	Rieger-Hofmann	2W	No	7	7	4	BB	0.38	NA	68	NA	2463	51	3
Galium aparine	Rubiaceae	Rieger-Hofmann	3W	No	7	6	5		0.35	4.70	19	87	2967	61	142
Galium pumilum	Rubiaceae	Konstanz ; Angers	3W	No	6	7	4		0.17	0.56	11	35	1270	30	5
Galium verum	Rubiaceae	Rieger-Hofmann	3W	No	6	6	5		1.25	1.56	17	108	2621	58	58
Genista sagittalis	Fabaceae	Göttingen	3W	Yes	NA	NA	2	RTD BB RWR FRD SRL	3.42	2.59	3	14	580	17	3
Genista tinctoria	Fabaceae	Rieger-Hofmann	3W	Yes	4	4	5	BB	0.71	0.86	2	4	2118	39	29
Geranium columbinum	Geraniaceae	Caen. F	3W	Yes	7	6	3		0.04	0.38	32	9	1982	60	26
Geranium dissectum	Geraniaceae	Altenburg	3W	Yes	NA	NA	2	RTD RWR FRD SRL	0.20	0.74	47	135	2451	79	97
Geranium molle	Geraniaceae	Nantes. F	3W	Yes	7	2	0	BB MRD	0.20	1.30	28	48	2534	83	124
Geranium pratense	Geraniaceae	Rieger-Hofmann	3W	Yes	2	NA	2		0.74	2.97	22	50	1840	42	33
Geranium pusillum	Geraniaceae	.977408 Uni KN (Osnabrück. D)	3W	Yes	4	2	0	MRD	0.13	1.01	40	65	2839	58	71
Geum rivale	Rosaceae	Rieger-Hofmann	3W	No	3	1	3		0.20	4.86	1	3	2231	7	2
Geum urbanum	Rosaceae	Rieger-Hofmann	2W	No	6	6	1		0.06	1.20	27	58	2930	12	22
Glechoma hederacea	Lamiaceae	Rieger-Hofmann	2W	No	7	6	4		0.38	2.32	68	299	2960	38	84
Glyceria fluitans	Poaceae	Konstanz	2W	No	6	6	5		1.34	10.11	5	7	2956	62	46
Helianthemum nummularium	Cistaceae	Rieger-Hofmann	3W	No	5	3	1		2.73	3.94	2	14	1427	70	0
Helictotrichon pratense	Poaceae	RH ; Regensburg	3W	No	7	6	5		1.21	3.10	24	72	1377	24	0
Helictotrichon pubescens	Poaceae	RH ; Konstanz	2W	No	6	7	1		3.17	3.60	44	95	2362	47	12

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Holcus lanatus	Poaceae	Rieger-Hofmann	2W	No	7	7	4		2.61	7.88	66	372	2975	79	226
Hypericum hirsutum	Hypericaceae	RH ; UfA	3W	No	3	2	1		0.08	1.26	2	7	1578	45	0
Hypericum perforatum	Hypericaceae	Rieger-Hofmann	3W	No	7	7	4		0.23	0.64	22	121	2975	71	117
Hypochaeris radicata	Asteraceae	Rieger-Hofmann	2W	No	7	5	5	SLA Height	0.82	1.54	18	29	2917	66	231
Juncus effusus	Juncaceae	Rieger-Hofmann	3W	Yes	NA	NA	1	RTD RWR FRD SRL	2.10	3.18	5	23	2966	53	46
Juncus inflexus	Juncaceae	Rieger-Hofmann	3W	Yes	NA	NA	1	RTD RWR FRD SRL	0.14	2.96	4	24	2355	50	13
Knautia arvensis	Caprifoliaceae	Rieger-Hofmann	3W	No	6	5	2		0.44	1.28	17	87	2855	48	38
Koeleria macrantha	Poaceae	Rieger-Hofmann	3W	No	7	7	1		0.26	0.45	5	16	856	45	20
Koeleria pyramidata	Poaceae	Rieger-Hofmann	3W	No	7	5	5		0.75	1.42	12	70	1617	34	4
Lactuca serriola	Asteraceae	Rieger-Hofmann	2W	No	7	6	5		0.04	2.96	1	4	2552	75	259
Lamium album	Lamiaceae	Muenster ; Regensburg	3W	No	6	3	0	MRD	1.42	1.87	9	48	2920	34	37
Lamium purpureum	Lamiaceae	Rieger-Hofmann	3W	No	1	1	2		0.11	1.11	22	17	2960	49	104
Lathyrus nissolia	Fabaceae	UfA	3W	No	7	6	2	BB	0.10	NA	1	NA	298	42	11
Lathyrus pratensis	Fabaceae	Rieger-Hofmann	2W	No	7	5	5		0.26	1.90	26	143	2955	58	33
Lathyrus tuberosus	Fabaceae	Rieger-Hofmann	3W	No	5	7	5		0.52	0.58	4	12	1742	39	46
Leontodon hispidus	Asteraceae	Rieger-Hofmann	3W	No	7	6	5		1.97	1.60	32	99	2446	58	11
Lepidium campestre	Brassicaceae	Konstanz	2W	No	7	6	5		0.10	NA	1	NA	1928	40	98
Leucanthemum vulgare agg. (L. vulgare and L.ircutianum)	Asteraceae	Rieger-Hofmann	2W	No	12	12	6		0.45	1.04	43	120	2946	40	205
Linaria vulgaris	Lamiaceae	Rieger-Hofmann	3W	Yes	6	5	3		1.87	1.04	1	11	2923	46	107
Linum catharticum	Linaceae	Münster. D	3W	Yes	1	NA	1		0.19	0.50	23	94	2410	62	21
Lolium multiflorum	Poaceae	Rieger-Hofmann	2W	No	7	6	5		1.60	12.60	40	59	2639	66	260
Lolium perenne	Poaceae	Rieger-Hofmann	2W	No	7	7	5		6.95	13.45	114	777	2981	74	365
Lotus corniculatus	Fabaceae	Rieger-Hofmann	2W	No	7	7	5		1.29	1.46	49	218	2944	1	140
Luzula campestris	Juncaceae	Rieger-Hofmann	3W	No	3	5	1		0.99	1.00	24	57	2927	38	19
*Matricaria chamomilla * Matricaria recutita*	Asteraceae	Rieger-Hofmann	3W	Yes	5	4	1		0.02	0.78	10	4	2829	65	127
Matricaria discoidea	Asteraceae	Münster. D	3W	Yes	7	6	5		0.04	0.64	5	1	2968	0	91

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Medicago falcata	Fabaceae	Rieger-Hofmann	2W	No	6	7	5	Height	0.30	1.66	2	10	1732	54	15
Medicago lupulina	Fabaceae	Rieger-Hofmann	2W	No	7	7	1		0.81	1.87	65	286	2969	68	242
Medicago varia	Fabaceae	Konstanz ; RH	2W	No	7	7	5	SLA BB Height	0.96	6.47	10	26	NA	NA	25
Myosotis arvensis	Boraginaceae	Konstanz	2W	No	6	6	5		0.22	0.52	45	117	2959	61	81
Myosotis discolor	Boraginaceae	Konstanz	2W	No	6	6	1		0.05	NA	4	NA	1603	60	81
Myosotis ramosissima	Boraginaceae	La Rochelle	2W	No	6	7	3		0.05	0.37	5	21	1949	66	6
Myosurus minimus	Ranunculaceae	Hohenheim ; Goettingen	2W	No	7	7	3		0.05	0.68	1	1	1742	66	18
Ononis spinosa * *Ononis repens*	Fabaceae	UfA ; RH	2W	No	12	14	6		1.26	2.14	11	62	2552	62	10
Origanum vulgare	Lamiaceae	BNT ; RH	2W	No	6	6	5		0.43	2.36	5	35	2029	51	47
Persicaria lapathifolia	Polygonaceae	Konstanz, Osnabrück. D	3W	Yes	NA	NA	1	RTD RWR FRD SRL	0.01	0.58	1	1	2926	84	151
Phalaris arundinacea	Poaceae	Rieger-Hofmann	2W	No	6	7	5		5.20	9.45	18	138	2969	55	94
Phleum bertolonii	Poaceae	Goettingen	3W	No	4	3	5	SLA Height	0.11	2.72	3	33	NA	34	1
Phleum pratense	Poaceae	Rieger-Hofmann	2W	No	7	7	4		1.09	3.12	87	470	2978	63	158
Phragmites australis	Poaceae	Rieger-Hofmann	3W	No	5	6	4		0.60	11.35	1	46	2887	81	98
Picris hieracioides	Asteraceae	Rieger-Hofmann	3W	Yes	6	6	0	MRD	1.87	6.24	14	32	2073	62	31
Pilosella officinarum	Asteraceae	Rieger-Hofmann	2W	No	4	7	5		2.64	3.46	17	97	2954	56	45
Plantago lanceolata	Plantaginaceae	Rieger-Hofmann	3W	No	5	7	3		1.86	2.27	122	754	2986	66	331
Plantago major	Plantaginaceae	Rieger-Hofmann	2W	No	7	7	5		0.43	1.32	78	299	2988	70	364
Plantago media	Plantaginaceae	Rieger-Hofmann	2W	No	7	5	3		1.35	2.15	55	263	2327	47	22
Poa angustifolia	Poaceae	Rieger-Hofmann	2W	No	7	7	5	BB	1.58	4.03	123	141	2050	56	11
Poa annua	Poaceae	Rieger-Hofmann	2W	No	6	7	5		0.29	1.03	10	22	2982	91	370
Poa pratensis	Poaceae	Rieger-Hofmann	2W	No	7	6	5		4.89	8.35	144	769	2980	64	237
Poa trivialis	Poaceae	Rieger-Hofmann	2W	No	4	7	5	Height	8.42	15.37	131	953	2968	82	127
Polygala vulgaris	Polygalaceae	Tübingen	3W	Yes	6	4	0	MRD	0.15	0.43	3	4	2241	56	9
Potentilla anserina	Rosaceae	Mailand. I	3W	Yes	4	4	2		1.78	4.45	17	129	2959	55	35
Potentilla argentea	Rosaceae	Rieger-Hofmann	3W	Yes	6	7	3		0.18	1.57	2	8	2403	18	50

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Potentilla collina	Rosaceae	Göttingen. D	3W	Yes	5	6	3	SLA BB Height	0.01	NA	1	NA	126	4	2
Potentilla erecta	Rosaceae	Rieger-Hofmann	3W	Yes	5	1	1		0.28	0.98	1	16	2849	66	5
Potentilla heptaphylla	Rosaceae	Göttingen. D	3W	Yes	5	5	2		0.44	0.60	14	53	888	10	0
Potentilla recta	Rosaceae	Rieger-Hofmann	3W	Yes	6	7	5		0.04	NA	2	NA	1558	40	91
Potentilla tabernaemontani	Rosaceae	Rieger-Hofmann	3W	Yes	4	4	2		0.47	1.35	13	76	NA	9	0
Primula veris	Primulaceae	Rieger-Hofmann	3W	Yes	3	3	0	MRD	0.60	2.73	7	27	2037	49	15
Prunella grandiflora	Lamiaceae	Rieger-Hofmann	2W	No	6	6	5		1.26	6.47	14	41	935	29	3
Prunella vulgaris	Lamiaceae	Rieger-Hofmann	3W	No	6	7	5		0.32	1.42	42	186	2974	55	169
Ranunculus acris	Ranunculaceae	Rieger-Hofmann	3W	Yes	4	2	4		2.80	2.76	76	524	2985	57	74
Ranunculus bulbosus	Ranunculaceae	Rieger-Hofmann	3W	Yes	7	4	5		0.74	1.40	80	219	2495	71	51
Ranunculus repens	Ranunculaceae	Rieger-Hofmann	3W	Yes	5	3	4		2.72	5.50	88	531	2989	71	124
Rumex acetosa	Polygonaceae	Rieger-Hofmann	2W	No	6	7	5		1.41	1.15	66	312	2981	13	41
Rumex acetosella	Polygonaceae	Rieger-Hofmann	3W	No	7	5	4		0.66	0.57	5	13	2915	33	245
Rumex obtusifolius	Polygonaceae	Colisses	3W	No	6	6	1		1.27	2.42	21	147	2980	26	225
Rumex thyrsiflorus	Polygonaceae	Goettingen	2W	No	7	6	0	MRD	0.23	1.54	3	12	1380	5	15
Salvia pratensis	Lamiaceae	Rieger-Hofmann	3W	No	7	6	0	MRD	0.64	1.45	5	36	1694	28	32
Sanguisorba minor	Rosaceae	Rieger-Hofmann	3W	No	6	5	5		1.35	1.94	23	112	2366	45	73
Sanguisorba officinalis	Rosaceae	Rieger-Hofmann	3W	No	5	4	0	MRD	0.35	NA	1	NA	1998	30	5
Saxifraga granulata	Saxifragaceae	Rieger-Hofmann	3W	Yes	6	5	0	MRD	0.51	NA	1	NA	2147	39	3
Saxifraga tridactylites	Saxifragaceae	Salzburg. A	3W	Yes	6	6	0	MRD	0.07	NA	2	NA	1703	64	4
Scabiosa columbaria	Dipsacaceae	Rieger-Hofmann	3W	No	3	NA	1		0.73	0.86	15	61	1749	46	6
Scorzoneroides autumnalis	Asteraceae	Rieger-Hofmann	2W	No	7	6	5		0.73	1.17	66	128	2964	53	49
Sedum spurium	Crassulaceae	Rieger-Hofmann	2W	No	6	7	3	BB	0.01	NA	1	NA	1625	3	70
Sedum telephium	Crassulaceae	Rieger-Hofmann	2W	No	7	7	5	Height	0.02	NA	1	NA	2801	37	47
Senecio erucifolius	Asteraceae	Munster	3W	No	5	1	0	BB MRD	0.16	0.63	13	15	1659	54	4
Sesleria albicans	Poaceae	Goettingen	3W	No	1	NA	5	BB	0.06	0.45	1	2	NA	3	1

The Plant List 1.1 binomial name accessed: 15 June 2019	Family According to TPL 1.1	Seed Source City / Company	Seedling cultivation time W = Week	Cold stratification -20°C in freezer	Sample size Morphology Exp. Intermediate Nutrients	Sample size Morphology Exp. High Nutrients	Sample size Rooting depth Exp.	Traits Imputed	EPs Abundance	GPs Abundance	EPs Frequency	GPs Frequency	FloraWeb	Euro+Med	GloNAF
Sherardia arvensis	Rubiaceae	Konstanz	2W	No	7	5	5		0.07	NA	3	NA	1786	55	148
Silene dioica	Caryophyllaceae	Rieger-Hofmann	2W	No	6	6	5		0.18	1.18	8	21	2601	32	50
Silene flos-cuculi	Caryophyllaceae	Rieger-Hofmann	2W	No	5	5	3		0.02	NA	4	NA	2901	43	31
Silene vulgaris	Caryophyllaceae	Rieger-Hofmann	2W	No	6	7	5		0.64	0.51	1	5	2754	62	111
Sinapis alba	Brassicaceae	Konstanz	2W	No	7	7	5	BB	0.06	NA	3	NA	1156	81	113
Sinapis arvensis	Brassicaceae	Rieger-Hofmann	2W	No	7	6	5		0.06	0.27	3	1	2835	52	205
Sisymbrium officinale	Brassicaceae	Krefeld	3W	Yes	7	6	1		0.11	0.45	1	4	2877	56	242
Spergula arvensis	Caryophyllaceae	Rieger-Hofmann	3W	Yes	5	6	0	MRD	0.00	NA	0	NA	2741	51	271
Stachys arvensis	Lamiaceae	Paris	2W	No	7	6	5	BB	0.02	NA	3	NA	1293	32	131
*Stachys officinalis * Betonica officinalis*	Lamiaceae	Rieger-Hofmann	3W	Yes	7	7	0		0.15	1.97	1	6	1974	37	5
Stellaria graminea	Caryophyllaceae	Rieger-Hofmann	3W	No	7	6	1		0.25	0.69	28	94	2951	41	73
Stellaria media	Caryophyllaceae	Rieger-Hofmann	2W	No	7	6	5		0.24	1.31	52	108	2984	66	404
Symphytum officinale	Boraginaceae	Rieger-Hofmann	3W	Yes	3	2	5		3.26	4.50	11	123	2799	45	84
Taraxacum campylodes	Asteraceae	RH ; Stolle	2W	No	7	7	2	BB Height	0.00	NA	0	NA	NA	2	300
Taraxacum erythrospermum	Asteraceae	Paris ; Nantes	2W	No	7	6	0	SLA BB MRD	0.10	0.43	5	1	119	4	69
Teucrium montanum	Lamiaceae	Mailand. I	3W	Yes	5	6	1		1.49	2.44	4	20	324	27	0
Thlaspi arvense	Brassicaceae	Rieger-Hofmann	3W	No	2	NA	5		0.04	0.43	13	3	2848	42	135
Thlaspi perfoliatum	Brassicaceae	Paris	2W	No	7	7	5	SLA	0.10	0.42	29	20	1094	55	27
Thymus pulegioides	Lamiaceae	UfA ; RH	3W	No	6	7	5		4.02	5.44	18	108	2674	34	39
Tragopogon pratensis	Asteraceae	Rieger-Hofmann	3W	No	7	7	5		0.39	0.69	64	131	2851	51	64
Trifolium alpestre	Fabaceae	Konstanz ; Goettingen	2W	No	7	7	5		0.82	1.71	1	2	1091	35	0
Trifolium arvense	Fabaceae	Rieger-Hofmann	2W	No	7	6	4		0.08	3.31	2	14	2635	73	152
Trifolium campestre	Fabaceae	Rieger-Hofmann	2W	No	6	6	5		0.29	1.25	41	85	2782	76	173
Trifolium dubium	Fabaceae	Rieger-Hofmann	2W	No	7	6	5		0.81	2.53	63	137	2920	49	200
Trifolium medium	Fabaceae	Rieger-Hofmann	2W	No	6	7	5		0.02	1.14	5	8	2663	49	30
Trifolium montanum	Fabaceae	Rieger-Hofmann	2W	No	6	6	5		0.06	0.62	5	16	1257	36	6

The Plant List 1.1 binomial name accessed: 15 June 2019	Family According to TPL 1.1	Seed Source City / Company	Seedling cultivation time W = Week	Cold stratification -20°C in freezer	Sample size Morphology Exp. Intermediate Nutrients	Sample size Morphology Exp. High Nutrients	Sample size Rooting depth Exp.	Traits Imputed	EPs Abundance	GPs Abundance	EPs Frequency	GPs Frequency	FloraWeb	Euro+Med	GloNAF
Trifolium pratense	Fabaceae	Rieger-Hofmann	2W	No	7	7	5		2.82	4.20	123	630	2989	34	221
Trifolium repens	Fabaceae	Rieger-Hofmann	2W	No	5	7	4		5.92	7.63	135	931	2991	69	367
Tripleurospermum inodorum	Asteraceae	Rieger-Hofmann	2W	No	6	6	4	Height	0.33	0.46	36	18	2783	56	54
Trisetum flavescens	Poaceae	Konstanz ; RH	2W	No	6	6	5		4.64	7.96	83	500	2303	55	41
Urtica dioica	Urticaceae	Rieger-Hofmann	3W	Yes	6	6	5		1.51	6.19	39	237	2991	30	93
Valeriana officinalis	Caprifoliaceae	Rieger-Hofmann	3W	No	6	4	0	MRD	0.08	1.06	3	9	2890	61	36
Valerianella locusta	Caprifoliaceae	Rieger-Hofmann	2W	No	5	5	2		0.13	0.57	49	24	2197	66	60
Veronica arvensis	Plantaginaceae	Konstanz ; RH	2W	No	6	7	4		0.27	0.44	116	340	2954	68	192
Veronica austriaca	Plantaginaceae	Rieger-Hofmann	3W	No	6	5	1		0.34	0.81	9	24	1135	38	26
Veronica chamaedrys	Plantaginaceae	Konstanz	2W	No	6	5	5		0.95	1.30	106	524	2937	49	48
Veronica hederifolia	Plantaginaceae	Aigues Vive ; BondT747	3W	No	3	3	0	MRD	0.14	0.39	11	8	2822	61	66
Veronica officinalis	Plantaginaceae	Rieger-Hofmann	3W	No	6	5	2		0.01	1.36	1	5	2827	61	54
Veronica persica	Plantaginaceae	Konstanz	2W	No	5	5	5		0.10	0.38	20	13	2863	33	314
Veronica serpyllifolia	Plantaginaceae	Salzburg ; Hohenheim	3W	No	6	5	4		0.14	0.50	71	128	2789	65	129
Veronica spicata	Plantaginaceae	Rieger-Hofmann	3W	No	7	7	3		0.07	2.28	5	19	551	42	4
Vicia cracca	Fabaceae	Rieger-Hofmann	2W	No	6	7	4		0.40	1.14	37	132	2980	55	70
Vicia hirsuta	Fabaceae	Goettingen	2W	No	7	6	5		0.68	4.85	19	65	2900	61	147
Vicia lathyroides	Fabaceae	Paris (MNHN)	3W	No	7	7	4		0.10	0.43	4	2	1105	58	21
Vicia sativa	Fabaceae	Salzburg ; RH	2W	No	6	7	5		0.20	0.96	48	95	2882	67	246
Vicia sepium	Fabaceae	Rieger-Hofmann	3W	No	3	5	4		0.51	0.85	69	313	2859	47	19
Vicia tenuifolia	Fabaceae	Budapest	3W	No	6	7	5		0.02	NA	3	NA	894	53	9
Vicia tetrasperma	Fabaceae	Halle (Matthias Stolle)	3W	No	6	6	5		0.39	2.92	29	96	2673	53	96
Viola arvensis	Violaceae	Rieger-Hofmann	3W	No	2	NA	1		0.04	0.45	9	4	2926	67	99

Appendix S2 Modified Hoagland solution

For the modified Hoagland solution used in the root-morphology experiment, we prepared six stock solutions:

1. 1 M potassium nitrate (KNO_3)
2. 1 M calcium nitrate ($Ca(NO_3)_2$)
3. 1 M monopotassium phosphate (KH_2PO_4)
4. 1 M magnesium sulfate ($MgSO_4$)
5. Micronutrients stock solution (see recipe below)
6. Iron solution from iron chelate (Fe-EDTA) (see recipe below)

Micronutrients stock:

2.86 g boric acid

1.81 g manganese chloride – 4 hydrate

0.22 g zinc sulfate – 7 hydrate

0.08 g copper sulfate – 5 hydrate

0.02 g 85% molybdic acid

We added these chemicals one by one to 500 mL distilled water while stirring. Then we added more distilled water until we had a final volume of 1 L.

Iron stock:

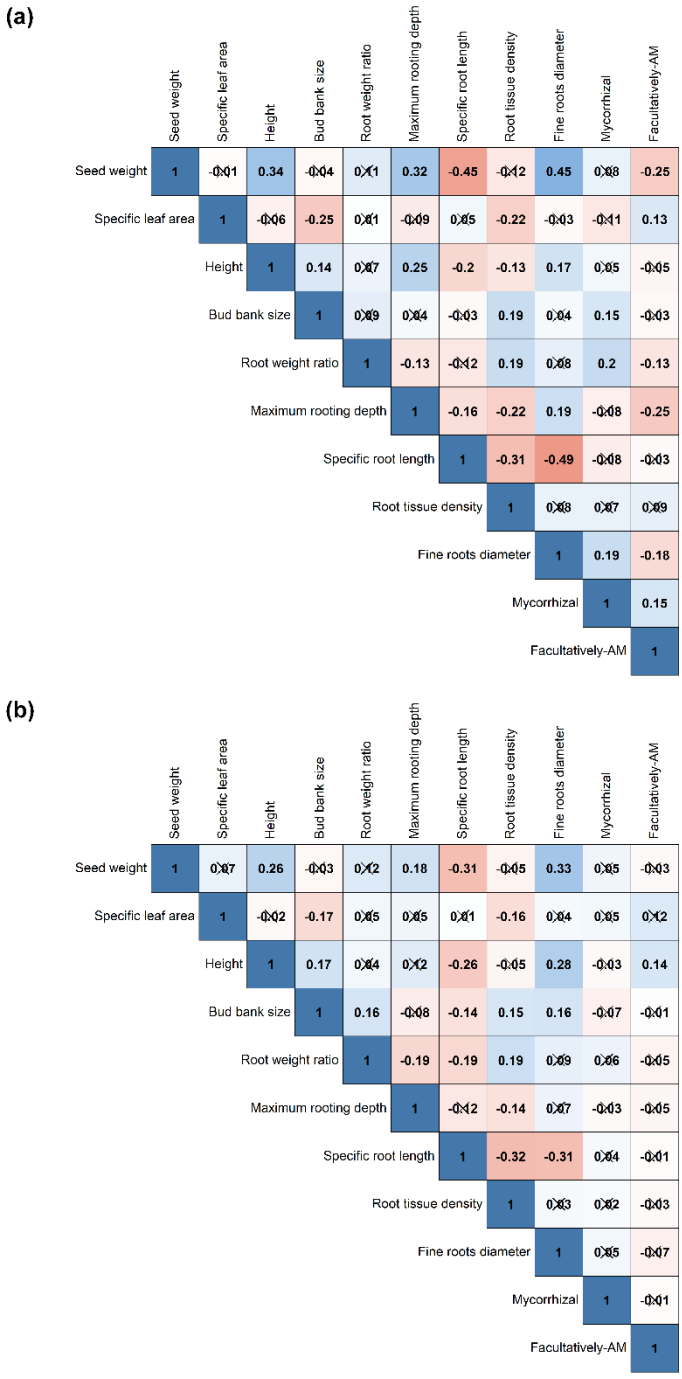
To make up the iron stock, we took 26.1 g EDTA and dissolved it in 286 ml distilled water that has ~19 g KOH in it. Then we dissolved 24.9 g FeSO4.7H2O in 500 ml distilled water. After that we slowly added the iron sulfate solution to the potassium EDTA solution and aerated this solution overnight by stirring. The pH rose to about 7.1 while the solution turned wine red and stirring was continued until no precipitation occurred. Distilled water was added to get a final volume of 1 L. The solution was stored in a bottle covered with aluminum foil.

We used the following volumes (mL) of stock solutions to make 1L of the intermediate and high fertilizer solutions. We added deionized water to get a total of 1L.

Stock solution	**Intermediate**	**High**
KNO_3	1.5	12
$Ca(NO_3)_2$	1	8
KH_2PO_4	0.25	2
$MgSO_4$	0.5	4
Micronutrients	0.25	2
Iron	0.25	2

Appendix S3 Trait relationships

Correlation matrix of (a) raw trait means for the 241 species of the study and (b) phylogenetically independent contrasts. Spearman's correlation coefficients are displayed. Negative correlations are in red, positive correlations are in blue and non-significant correlations are indicated with a cross.



Appendix S4 Methods for the phylogenetically informed analysis

Phylogenetic imputation of trait data

For the phylogenetically informed imputation of missing trait data (4.6% of total trait data), we used PhyloPars (Bruggeman *et al.* 2009). The phylogeny was derived by pruning Daphne, a dated phylogeny of the European flora (Durka & Michalski 2012) to our set of study species using the picante package (Kembel *et al.* 2010). Imputation was done for root tissue density (8 species, error = 0.211, bias = -0.00685), specific leaf area (11 species. error = 6.29 mm^2/mg. bias = 0.269), bud-bank size (11 species. error = 5.64 buds. bias = 0.00535), root weight ratio (8 species. error = 0.0431. bias = -0.000939), maximum rooting depth (45 species. error = 20.2 cm. bias = -0.234), fine roots diameter (8 species. error = 0.0535. bias = 0.000193), specific root length (8 species. error = 0.171. bias = 0.00413) and height (13 species. error = 0.205 m. bias = -0.00243).

Phylogenetic signal metrics

We computed the phylogenetic signal statistics Blomberg's K and K*, Abouheif's Cmean, Moran's I, and Pagel's Lambda (Appendix S7) using the phylosignal package in R (Keck *et al.* 2016).

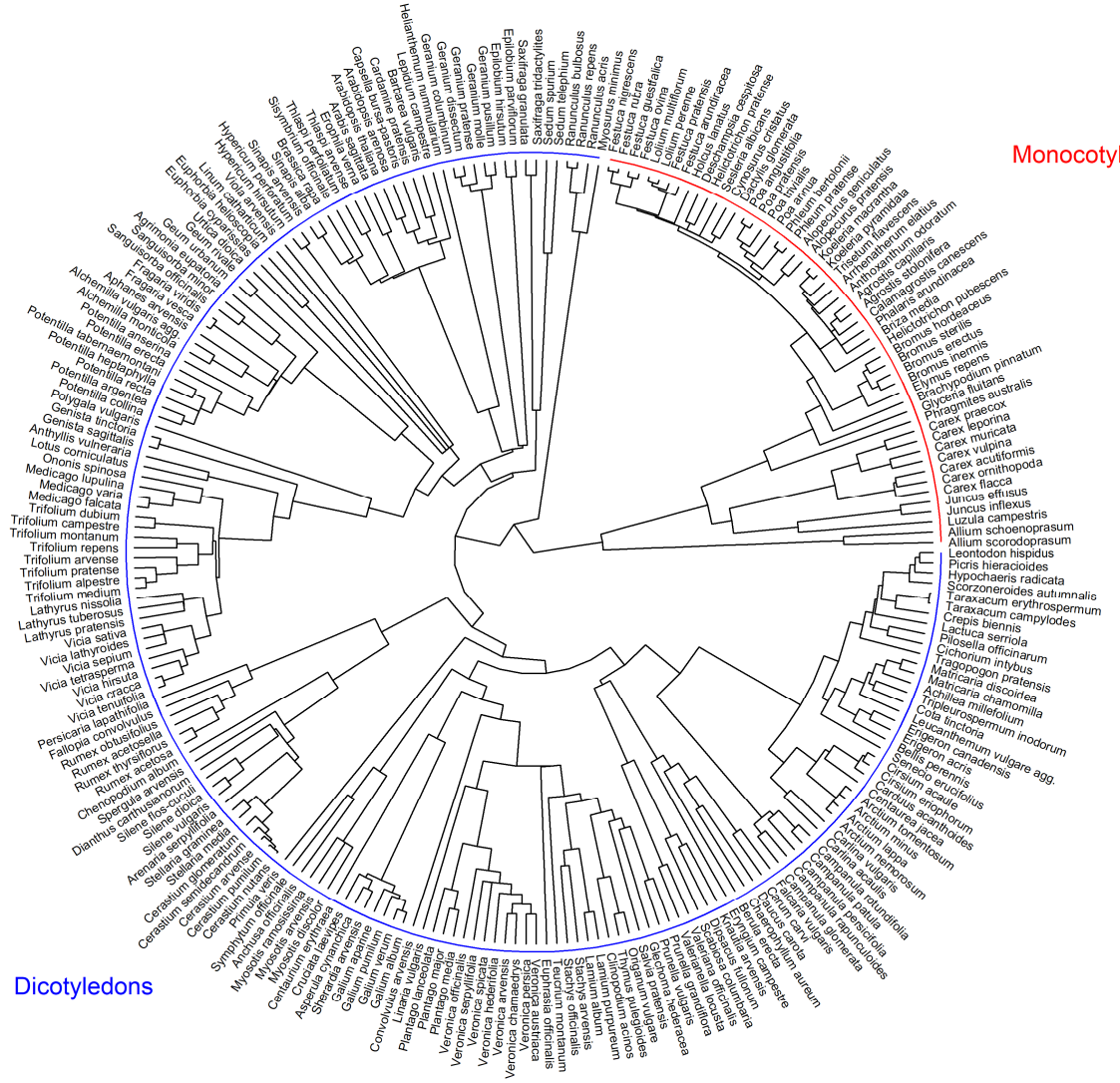
Phylogenetically informed models of species success explained by traits

To build the phylogenetic distance covariance matrix, we used the tree pruned from Daphne (Durka & Michalski 2012). Bayesian models were fitted using the *brms* package (Bürkner 2017) using the traits as predictors and the phylogenetic tree as covariance matrix. We estimated the trait coefficients using the posterior median and 89% credible intervals (highest density intervals). Negative binomial distributions of errors were used for Euro+Med and FloraWeb occurrence as the 'quasibinomial' method is not available in *brms*. For the other spatial scales, the same distributions of errors were used as in the frequentist models. Priors

were specified conservatively, drawn from a normal distribution with a mean of zero and standard deviation of 0.5, both for the coefficients and their standard deviations. Intercept priors were also drawn from a normal distribution, with the mean being the rounded estimate from the frequentist models and a standard deviation of 2. Two chains were run with 20,000 iterations each and the delta was set to 0.98 to achieve model convergence ($\hat{R} = 1$). Bayesian R^2 values were calculated in brms, defined as the variance of the predicted values divided by the variance of predicted values plus the expected variance of the errors, according to Gelman *et al.* (2019). The estimates for the phylogenetically informed models are displayed in Appendix S8.

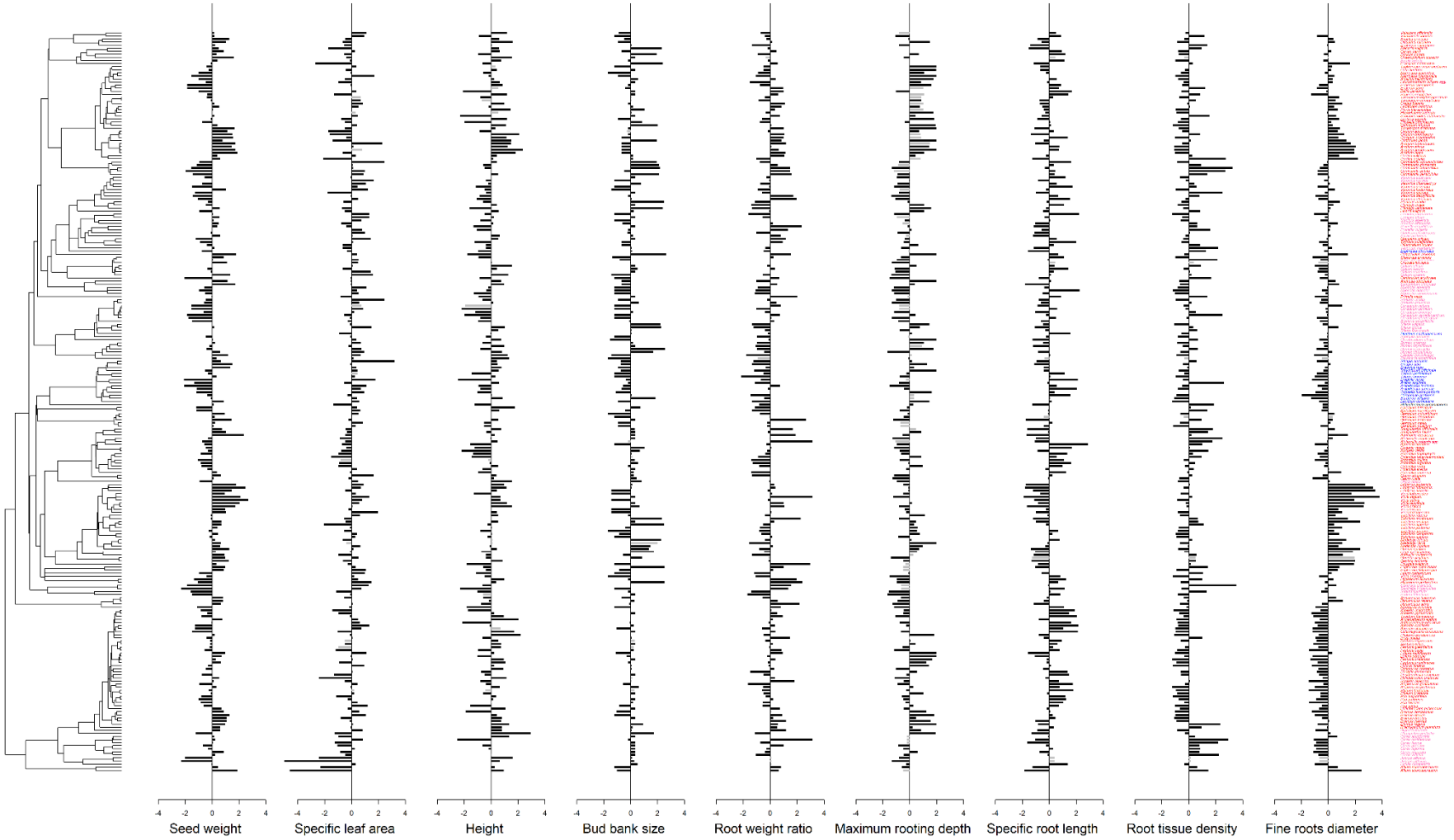
Appendix S5 Phylogenetic tree of the study species

Circular representation of a phylogenetic tree of the 241 study species derived by pruning the Daphne meta tree (Durka & Michalski 2012) using the *ape* package (Paradis *et al.* 2004).



Appendix S6 Trait means distribution across the phylogeny

Barplots with standardized mean trait values (centered and scaled) for the 241 species and the 10 traits used in the study. The black bars represent trait means obtained during the experiments or extracted from databases and the grey bars represent imputed values. Seed weight, height and specific leaf area where $\log_{10}$ transformed before standardization. The colours of the species names correspond to their mycorrhizal status. Red = obligately arbuscular mycorrhizal; Pink = facultatively arbuscular mycorrhizal; Blue = non-mycorrhizal; Black = obligately ectomycorrhizal. In the analyses, *Helianthemum nummularium*, the only ectomycorrhizal species is combined with obligately arbuscular-mycorrhizal species to form the obligately mycorrhizal group.


-4 -2 0 2 4
Seed weight
Specific leaf area
Height
Bud bank size
Root weight ratio
Maximum rooting depth
Specific root length
Root tissue density
Fine roots diameter

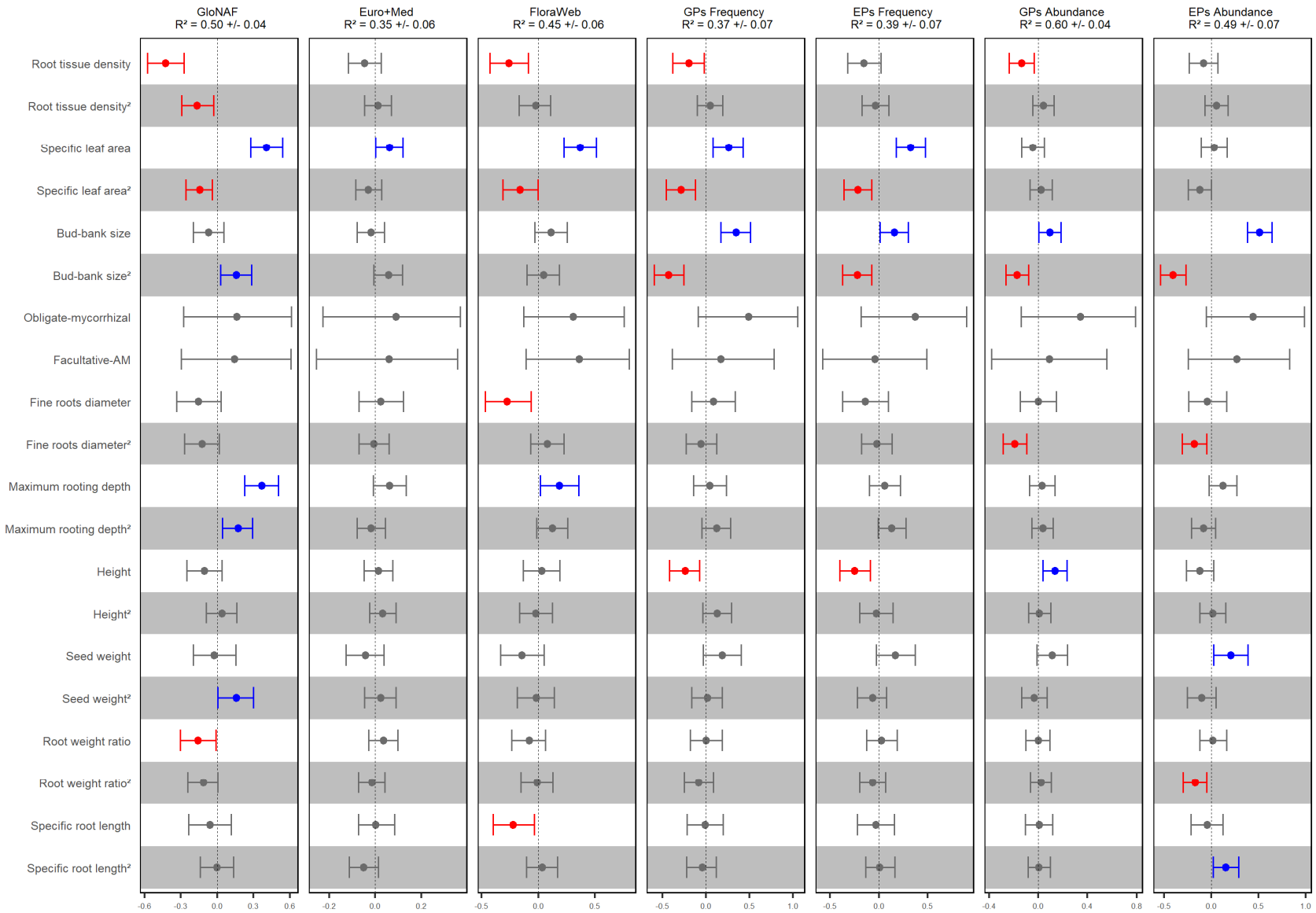
Appendix S7 Phylogenetic signal for trait means

Phylogenetic signal tests for the 10 traits used as predictors in the models. The indices were calculated using the measured traits only (i.e. without imputed data). All the indices are significant ($p<0.05$).

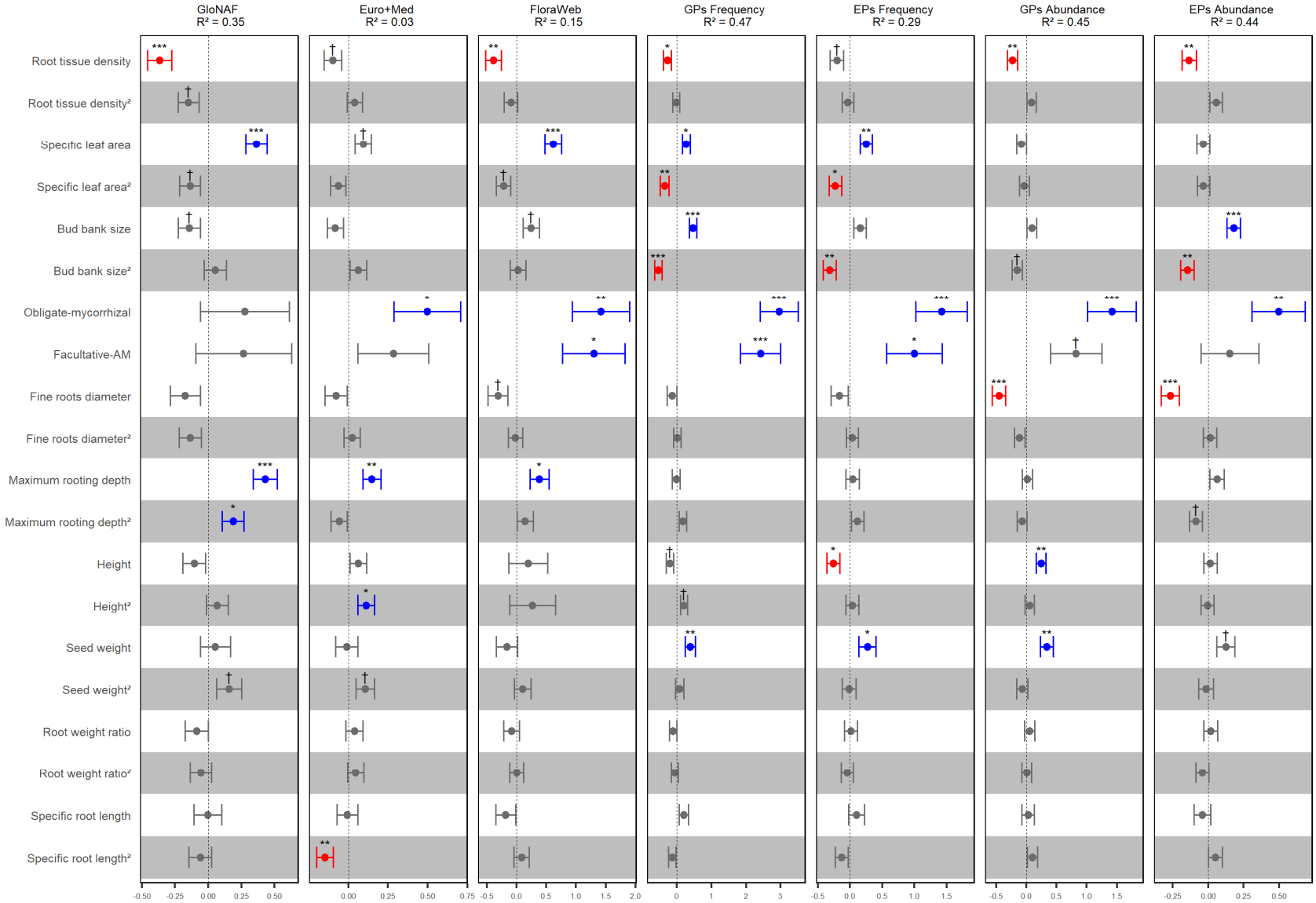
	Cmean	I	K	K*	Lambda	Number of species
Seed weight	0.54	0.10	0.36	0.38	0.99	241
Specific leaf area	0.26	0.03	0.19	0.16	0.57	230
Height	0.15	0.06	0.11	0.12	0.25	228
Bud-bank size	0.20	0.05	0.14	0.15	0.30	230
Root weight ratio	0.18	0.02	0.13	0.13	0.63	233
Maximum rooting depth	0.42	0.1	0.25	0.24	0.82	196
Specific root length	0.22	0.05	0.12	0.12	0.38	233
Root tissue density	0.21	0.05	0.14	0.14	0.44	233
Fine roots diameter	0.64	0.20	0.26	0.28	0.83	233
Obligate-mycorrhizal	0.80	0.14	2.35	2.48	1.00	241
Facultative-AM	0.74	0.15	1.15	1.15	1.00	241

Appendix S8 Estimates of trait effects on different success metrics from phylogenetically-informed generalized linear models

Estimates of trait effects on different success metrics of German grassland species from phylogenetic generalized linear models. On the y-axis are the 10 traits used as predictors with a linear term (white rows) and a quadratic (non-linear) term (grey rows) for each trait. The error bars around the estimates are 89% credible intervals. Red points indicate significant (89% credible interval do not overlap with zero) negative model estimates. Blue points indicate significant (89% credible interval do not overlap with zero) positive estimates and grey points indicate non-significant estimates (89% credible interval overlaps with zero). Bayesian R^2 and their standard errors are presented under the spatial scale label. The spatial scale of the success metric decreases from left to right. GloNAF: number of regions in which a species is naturalized (number of species. N=241); Euro+Med: number of regions in Europe and the Mediterranean basin in which a species is native (N=237); FloraWeb: number of grid cells in Germany in which a species is present (N=235); GPs Frequency: number of grassland grid plots in which a species is present (N=213); EPs Frequency: number of grassland experimental plots in which a species is present (N=239); GPs Abundance: mean species cover in grassland grid plots in which the species is present (N=213); EPs Abundance: mean species cover in grassland experimental plots in which a species is present (N=239).


GloNAF
R² = 0.50 +/- 0.04
Euro+Med
R² = 0.35 +/- 0.06
FloraWeb
R² = 0.45 +/- 0.06
GPs Frequency
R² = 0.37 +/- 0.07
EPs Frequency
R² = 0.39 +/- 0.07
GPs Abundance
R² = 0.60 +/- 0.04
EPs Abundance
R² = 0.49 +/- 0.07
Root tissue density
Root tissue density²
Specific leaf area
Specific leaf area²
Bud-bank size
Bud-bank size²
Obligate-mycorrhizal
Facultative-AM
Fine roots diameter
Fine roots diameter²
Maximum rooting depth
Maximum rooting depth²
Height
Height²
Seed weight
Seed weight²
Root weight ratio
Root weight ratio²
Specific root length
Specific root length²
-0.6 -0.3 0.0 0.3 0.6
-0.2 0.0 0.2
-0.5 0.0 0.5
-0.5 0.0 0.5 1.0
-0.5 0.0 0.5
-0.4 0.0 0.4 0.8
-0.5 0.0 0.5 1.0

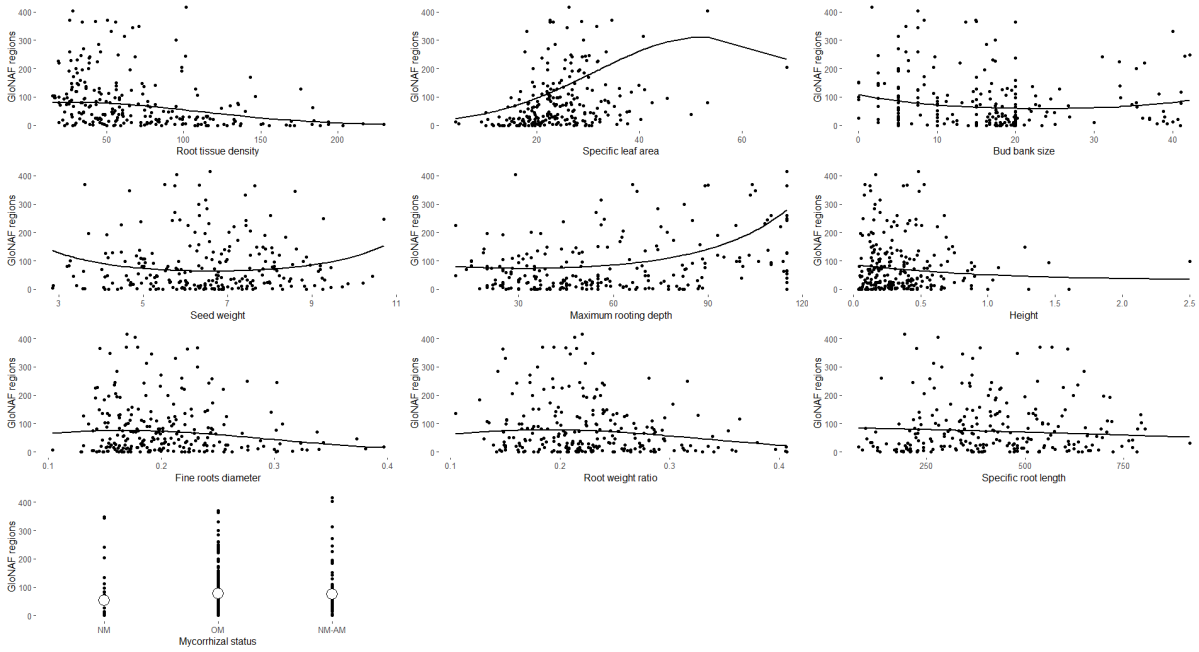
Appendix S9 Estimates of trait effects on different commonness metrics of German grassland species from generalized linear models for non-imputed trait data. On the y-axis are the 10 traits used as predictors, with a linear term (white rows) and a quadratic (non-linear) term (grey rows) for each trait. The errors bars around the estimates are standard errors. Significant negative and positive estimates are marked in red and blue, respectively. In addition, estimates with $p < 0.001$ are indicated with ***, estimates with $p < 0.01$ with **, and estimates with $p < 0.05$ with *. Marginally significant estimates ($p < 0.1$) are indicated with †. The spatial scale of the commonness metric decreases from left to right. GloNAF: number of regions in which a species is naturalized (number of species, N=170); Euro+Med: number of regions in Europe and the Mediterranean basin in which a species is native (N=170); FloraWeb: number of grid cells in Germany in which a species is present (N=170); GPs Frequency: number of grassland grid plots in which a species is present (N=150); EPs Frequency: number of grassland experimental plots in which a species is present (N=170); GPs Abundance: mean species cover in grassland grid plots in which the species is present (N=150); EPs Abundance: mean species cover in grassland experimental plots in which a species is present (N=170). Delta R^2 were calculated according to Nakagawa *et al.* (2017).


GloNAF
R² = 0.35
Euro+Med
R² = 0.03
FloraWeb
R² = 0.15
GPs Frequency
R² = 0.47
EPs Frequency
R² = 0.29
GPs Abundance
R² = 0.45
EPs Abundance
R² = 0.44
Root tissue density
Root tissue density²
Specific leaf area
Specific leaf area²
Bud bank size
Bud bank size²
Obligate-mycorrhizal
Facultative-AM
Fine roots diameter
Fine roots diameter²
Maximum rooting depth
Maximum rooting depth²
Height
Height²
Seed weight
Seed weight²
Root weight ratio
Root weight ratio²
Specific root length
Specific root length²

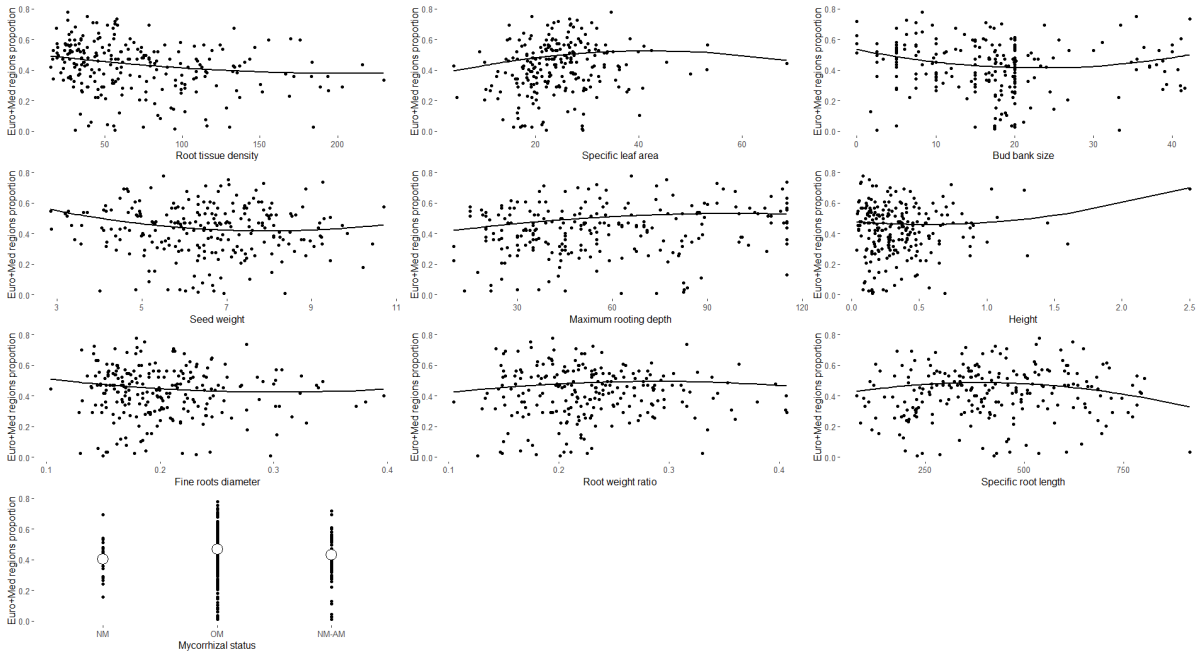
Appendix S10 Scatterplots of species success metrics for each trait and modelled relationships

Displayed are 10 traits predicting species success metrics of different spatial scales in generalized linear models. Each point corresponds to a species and the fitted lines for continuous variables and the filled white points for mycorrhizal status show the model predictions. Units: seed weight in µg, specific leaf area in m² per kg, height in meters, bud-bank size in number of buds per individual, maximum rooting depth in cm, root weight ratio is unitless (ratio of root biomass to total biomass), specific root length in m per g, root tissue density in mg per cm^3, fine roots diameter in mm. Seed weight was log transformed. For mycorrhizal status: NM = non-mycorrhizal; OM = obligately mycorrhizal; NM-AM = facultatively mycorrhizal. GloNAF: number of regions (1029 regions) in which a species is naturalized (number of species. N=241); Euro+Med: proportion of regions (117 regions) in Europe and the Mediterranean basin in which a species is native (N=237); FloraWeb: proportion of grid plots (2995 grid plots) in Germany in which a species is present (N=235); GPs Frequency: number of grassland grid plots (1494 plots) in which a species is present (N=213); EPs Frequency: number of grassland experimental plots (150 plots) in which a species is present (N=239); GPs Abundance: mean species cover in grassland grid plots in which a species is present (N=213); EPs Abundance: mean species cover in grassland experimental plots in which a species is present (N=239).

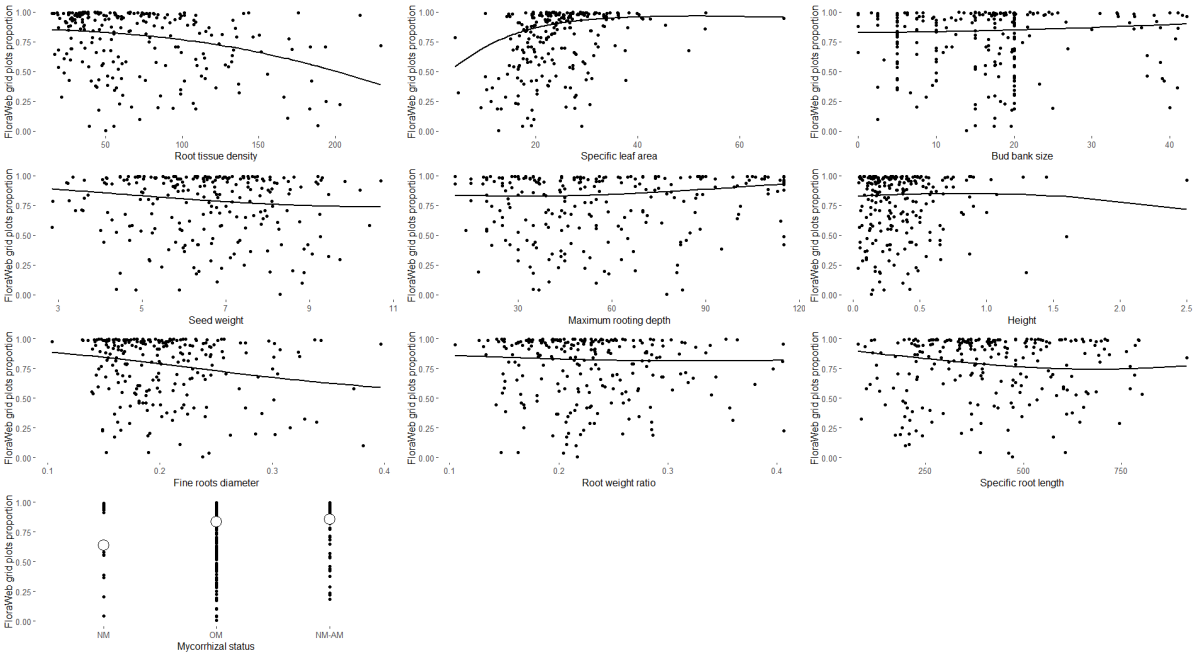
GloNAF



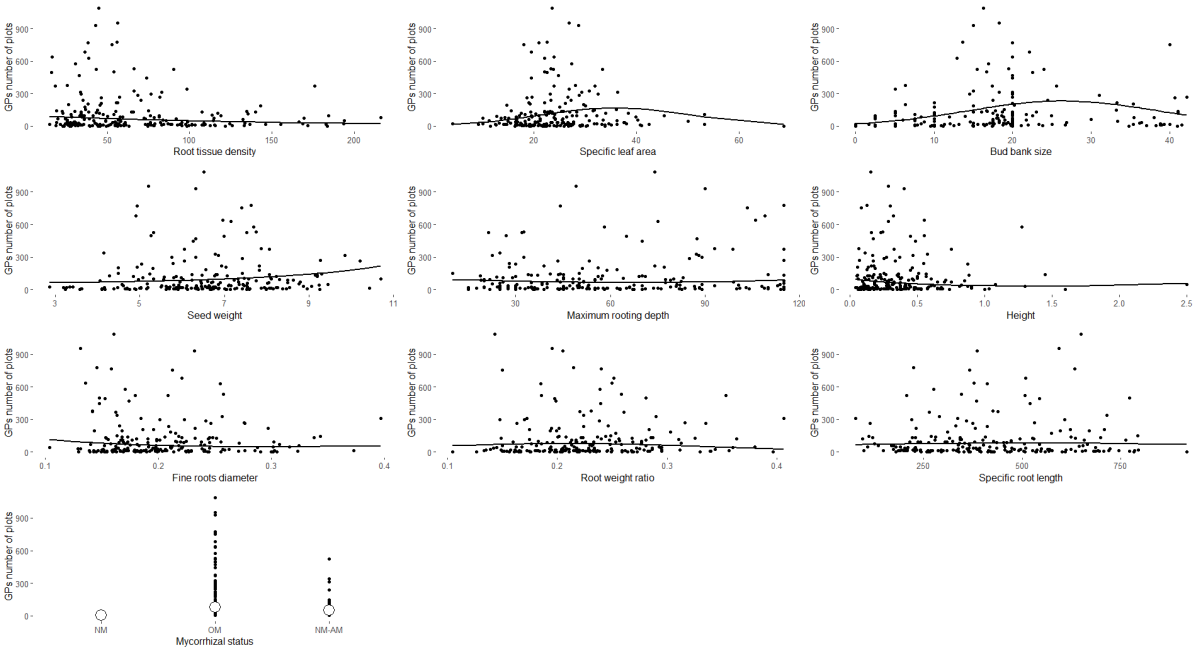
Euro+Med



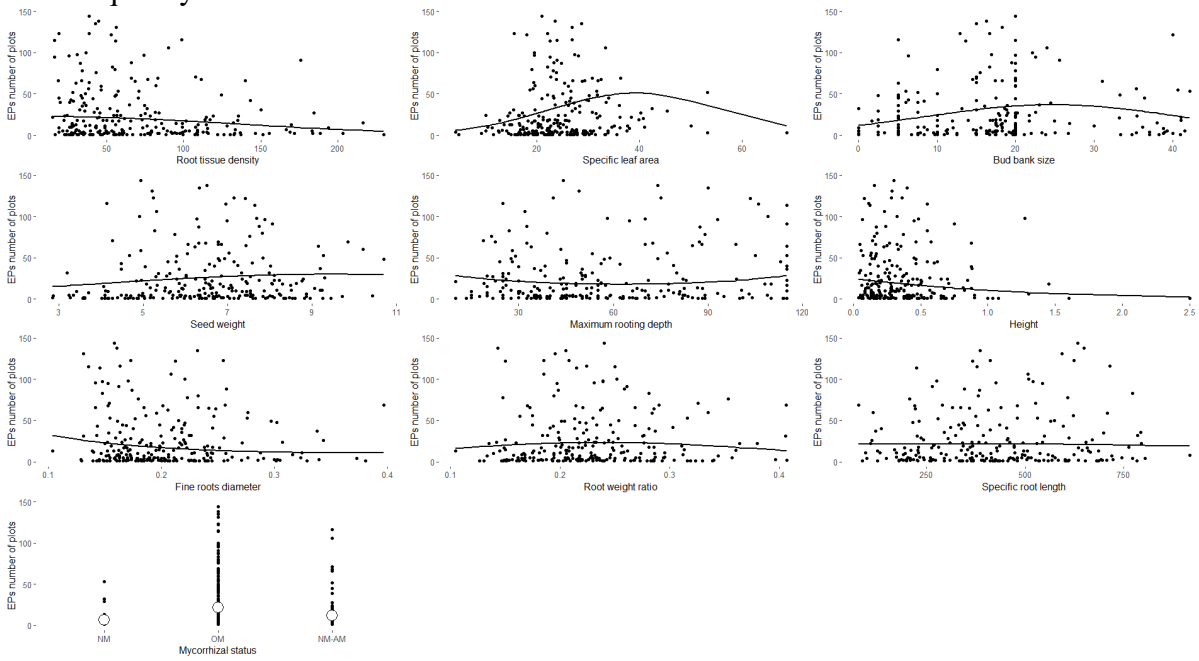
FloraWeb



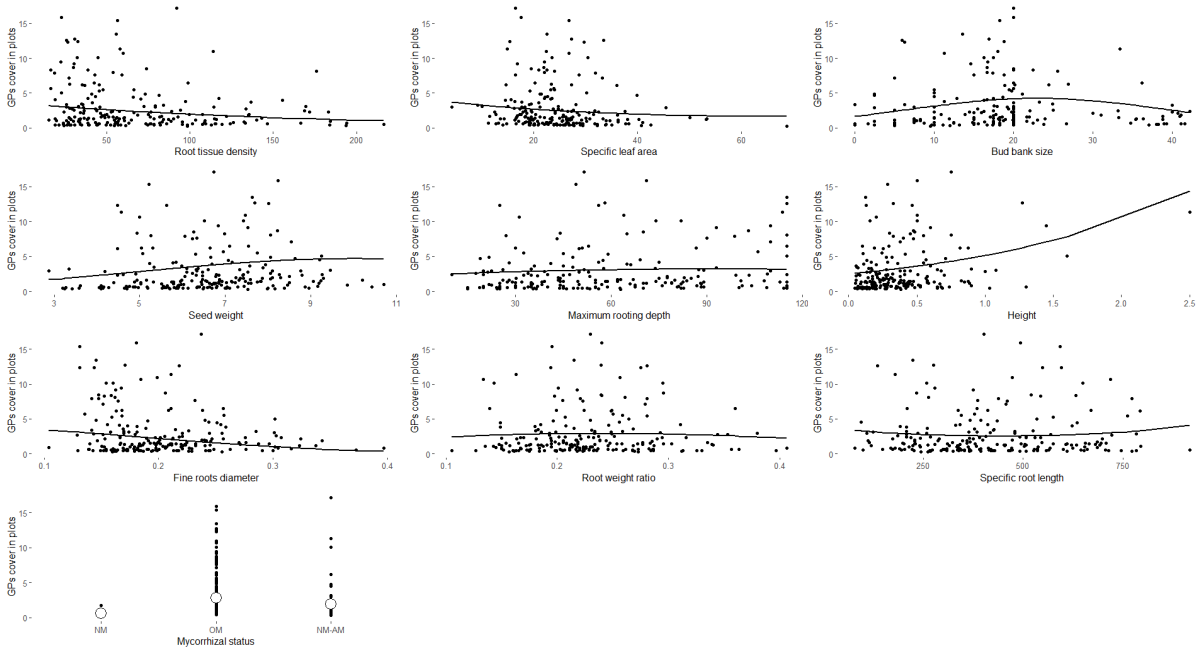
GPs Frequency



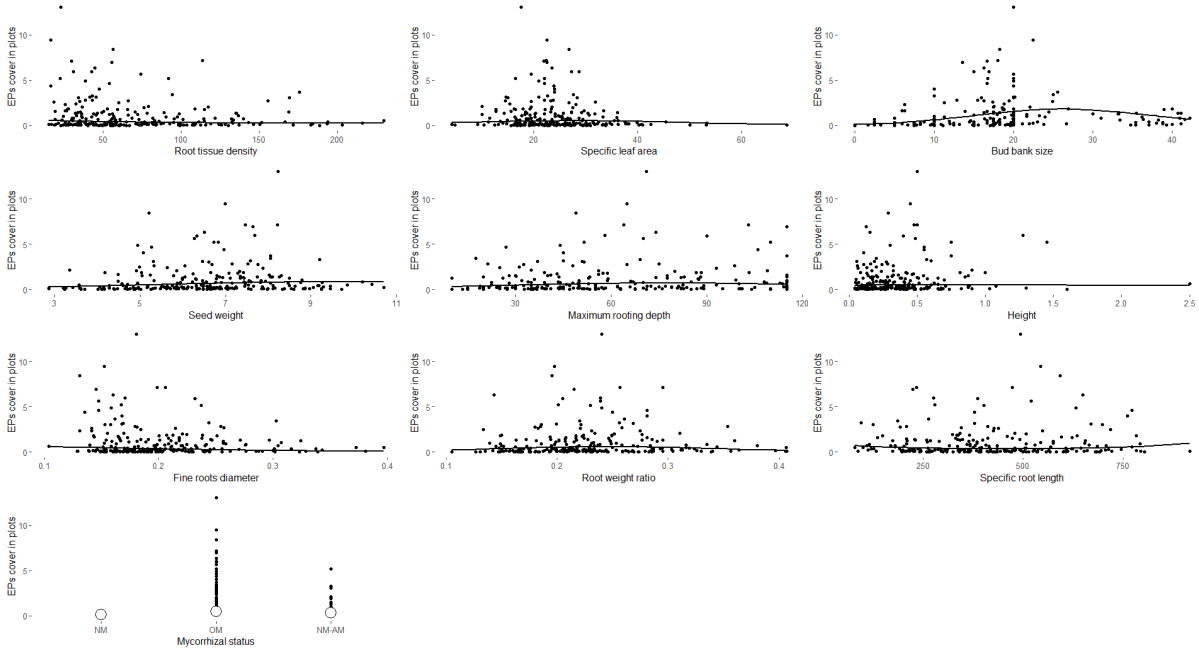
EPs Frequency



GPs Abundance



EPs Abundance



Appendix S11 Pictures of the experiment on rooting depth

The 120 cm tall tubes were filled with a sand-vermiculite mixture up to 115 cm. A single seedling was planted into each of them. The tubes were placed outside in the Botanical Garden of the University of Konstanz (Germany) and held upright with metal grids fixated on top of wooden pallets.

Chapter II: Root hairs and mycorrhiza represent alternative phylogenetically conserved strategies for belowground absorptive surface maximization

Joana Bergmann, Tom Lachaise, Karla Barfuss, Emma Bretherick, Elsa Matthus, Mark van Kleunen, Matthias Rillig

in preparation

Abstract

Plants have to forage the soil to ensure sufficient resource uptake facing a trade-off to invest in absorptive root surface or mycorrhizal partners. Root hairs - known to be a major structure for nutrient uptake and cheap to build - increase the absorptive root surface. As such they are a powerful component of a do-it-yourself strategy but neglected in root economic concepts so far. This is mainly due to data scarcity, which we set out to overcome by measuring root hair traits on 82 European grassland species in a greenhouse experiment. Using fluorescence and light microscopy, root hair length and incidence was measured along with mycorrhizal colonization. We found a phylogenetically conserved trade-off between plant investment into root hairs and the mycorrhizal colonization rate. A similar trade-off between root hair incidence and mycorrhizal colonization also occurred within species, while species with high colonization rates showed highest variability in root hair incidence. We conclude that plant species either invest into root hairs as part of a do-it-yourself strategy, or collaborate with mycorrhizal fungi while showing intraspecific variation in root hair incidence. These findings demonstrate that root hairs play a fundamental role in fine root trait variation and need to be considered when studying belowground plant strategies and plant soil interactions.

Introduction

The resource economy of plants has been a focal area of studies investigating plant functional traits (Bergmann *et al.* 2020; Freschet *et al.* 2013a; Freschet *et al.* 2013b; Kong *et al.* 2017; Reich 2014; Wright *et al.* 2004). The general idea is that plants have to invest in construction and conservation of tissue to ensure the uptake and transport of resources. For aboveground organs - mainly leaves - an economic spectrum of plant strategies has been described and confirmed on a global basis (Díaz *et al.* 2016; Reich 2014; Wright *et al.* 2004). This spectrum ranges from fast growth and resource acquisition of short-lived organs to slow but steady resource acquisition of organs constructed for longevity.

During the last years, ecologists have used morphological, anatomical and biochemical traits of fine roots to also describe belowground patterns of the plant nutrient economy. Aspects of a fast-slow economic spectrum have been detected for roots as well (Roumet *et al.* 2016) specifically at the community level (Prieto *et al.* 2015). Interestingly, evidence points towards root traits showing additional dimensions of variation due to evolutionary drivers solely acting belowground (Bergmann *et al.* 2017; Comas *et al.* 2014; Kramer-Walter *et al.* 2016; Ma *et al.* 2018; Wang *et al.* 2018b; Weemstra *et al.* 2016). Recently, the concept of a collaboration gradient in root trait variation that is independent from the conservation gradient - the analogue of the aboveground economic spectrum - and unique to belowground economy has been proposed (Bergmann *et al.* 2020). This collaboration gradient describes plant strategies in soil exploration ranging gradually from do-it-yourself investment in specific root length (SRL) to outsourcing to mycorrhizal fungal partners with the consequence of higher fine root diameter and cortex fraction.

Arbuscular mycorrhizal (AM) fungi that collaborate with about 80% of all land plants thereby colonize the root's cortex and explore the soil with extraradical hyphae (Smith & Read 2009). AM fungi can take up limiting nutrients like immobile phosphorus and nitrogen, supplying

them to the roots in exchange for carbon synthesized by the plant partner´s aboveground photosynthesis (Smith & Read 2009). Besides the exploration of a certain volume of soil, the actual surface and the soil contact of an absorptive plant or fungal structure determines the rate of return on investment of a plant (McCormack & Iversen 2019). Little is known about traits of fungal extraradical hyphae, but a large body of literature reveals that for the plant itself an effective way to maximize absorptive surface and soil exploration is the production of root hairs (Bates & Lynch 2000a, 2000b; Brown *et al.* 2013b; Haling *et al.* 2013; Singh Gahoonia *et al.* 1997) . Yet, most likely because of the effort involved in data collection the coverage of root hair traits in databases is poor (Guerrero-Ramírez *et al.* 2021; Iversen *et al.* 2017; Kattge *et al.* 2020), and their integration into broader plant economics concepts lacking.

Root hairs are unicellular epidermal extensions on living fine roots of most land plants (Peterson & Farquhar 1996). They enhance nutrient and water uptake of fine roots (Carminati *et al.* 2017; Freschet *et al.* 2020; Gilroy & Jones 2000; Haling *et al.* 2013) contributing to >60% of the plant´s phosphorus-demand (Gahoonia & Nielsen 1998). Root-hair traits are known to widely vary between species and along environmental gradients of soil fertility (Holdaway *et al.* 2011; Lambers *et al.* 2008), while a large root hair surface can be realized with long (Haling *et al.* 2016; Yang *et al.* 2015) or many (Brown *et al.* 2013a; Marzec *et al.* 2015) root hairs. Furthermore, they have been described to be comparably responsive to soil P availability, in part because of their dynamic growth and life-span (Bates & Lynch 1996; Nestler & Wissuwa 2016; Zhu *et al.* 2010). Cheap and dynamic in construction, root hairs could therefore resemble a ‘fast’ economic strategy component within the conservation gradient.

In addition to soil water and nutrient levels, the mycorrhizal symbiosis has also been identified as a main mediator of intraspecific plasticity in root hair length, even though patterns vary between plant species (Sun & Tang 2013; Wu *et al.* 2016). Furthermore, it has long been known that the plant species specific beneficial effect of being mycorrhizal is related to root hair length

(Schweiger *et al.* 1995). Therefore, it has been hypothesized that the investment into root hairs might be an alternative strategy to the mycorrhizal symbiosis on an interspecific (Hafiz Maherali 2017) and intraspecific level (Kumar *et al.* 2019) for acquiring soil resources. If this pattern were to be verified for a larger set of species, it would imply that root hairs represent another aspect of a do-it-yourself strategy of plant economics within the collaboration gradient. Here, we measured root hair length (HL) and hair incidence (HI) as well as mycorrhizal colonization on a large set of grassland species grown under common conditions to 1) test the hypothesis of an interspecific trade-off between the investment in root hairs and the mycorrhizal symbiosis and to 2) explore the intraspecific variation of root hair traits. Furthermore, we aimed to 3) integrate HL and HI into the concept of the root economics space, hypothesizing that root hair investment represents an alternative do-it-yourself strategy to the overall increase of SRL.

Methods

Species set

The experiment was conducted in the framework of the Biodiversity Exploratories (Fischer *et al.* 2010), a large scale and long term land-use experiment with 150 grassland plots located in three areas in northern, central and southern Germany. From the vegetation records of the Exploratories, we chose a set of 94 grassland species which could be purchased from the commercial seed supplier Rieger-Hofmann GmbH (Blaufelden-Raboldshausen, Germany).

Greenhouse experiment

All data presented here originate from one pot experiment conducted under controlled greenhouse conditions, at the facilities of Freie Universität Berlin, between February and June 2018 (16 h day at 22°C, 8 h night at 15°C). We set up the experiment with 94 initial species, two treatments (with and without mycorrhizal inoculation), and 8 replicates distributed over 4 overlapping time blocks of 6 weeks growing time each. The entire experiment therefore consisted of 4 time blocks x 94 species x 2 treatments x 2 replicates = 1504 experimental units. Whenever a replicate did not survive, we tried to substitute it in the next time block. Nevertheless, some species did not reach a replication of 8 per treatment and some did not germinate at all. In the final analysis, we only included species with a minimum of 3 successful replicates per treatment leading to a total of 1151 experimental units of 82 species from 20 families.

Prior to the first time block all seeds were surface sterilized in paper tea bags for 3 min in 7% bleach followed by washing in de-ionized (DI) water until the smell of bleach was gone. The seeds were dried at 20°C and stored until sowing for subsequent time blocks. Seeds germinated in plastic boxes filled with 1:1 steamed sand and vermiculite (1-3 mm, ISOLA Vermiculite GmbH; Sprockhövel, Germany). Based on germination times recorded in pre-experiments, we

sowed the seeds so as to assure that all seedlings were in the cotyledon stage or had their first leaves developed at time of transplanting. Seedlings were transplanted into plastic cones (410 ml 0.41 L; Stueve & Sons; USA) filled with the same substrate as for germination.

The mycorrhizal treatment was realized as follows: after filling the cone to c. ¾ we added a 30 ml horizon of a 1:1 mixture of steamed sand and mycorrhizal inoculum in vermiculite (INOQ Agri, Inoq GmbH, Schnega, Germany). According to the supplier, the inoculum contains 145 spores/ml of *Rhizophagus irregularis* propagated on vermiculite (1-2 mm) under non sterile greenhouse conditions. *Rhizophagus irregularis* is a generalist AM fungus associating with almost all mycorrhizal plants (van der Heijden *et al.* 2015). To account for other soil microbes, we prepared a microbial wash from the *Rhizophagus* inoculum (20 µm mesh, soil:water-ratio: 1:2) for the non-mycorrhizal treatment. We carefully adjusted the amount of inoculum as well as the amount of DI water used to prepare the microbial wash to make sure that each pot received the approximate same number of microbial units irrespective of the treatment. To control for nutrients and physical structure of the AM inoculum, we autoclaved the solid inoculum used to prepare the microbial wash and added a 1:1 mixture with steamed sand as a horizon to the –AM treatment. For both treatments the added horizons were covered with another layer of ~30 ml substrate to avoid cross contamination. During transplanting, seedlings of the non-mycorrhizal treatment received 30 ml of the microbial wash while seedlings of the mycorrhizal treatment received 30 ml of DI water. We replaced seedlings that died shortly after transplanting during the first week.

Within each time block, plants grew for 6 weeks in the cones before harvest. All cones were fully randomized at time of transplanting and were rearranged every two weeks. Plants received 25 ml of DI water 3 times a week; two weeks and four weeks after transplanting they received 25 ml of a ¼ strength Hoagland solution instead.

At time of harvest, aboveground and belowground biomass of the plants were separated. Roots

were first rinsed with water. Three first order roots per plant were carefully cut, transferred to 10% formalin at pH 7 (ROTI Histofix, Carl Roth, Karlsruhe, Germany) in Phosphate Buffered Saline (PBS) buffer and kept at 4 °C for overnight fixation. The next day, the formalin solution was first replaced by PBS buffer twice for approx. 2 hours each and finally by a solution of 70% ethanol, 5% glycerin and 25% DI water for long term preservation. The remaining roots were carefully washed by hand during harvest, transferred to cold DI water and kept at 4 °C. Within a week they were scanned in water-filled plastic trays using an Epson perfection 8000 Photo scanner at a resolution of 800 dpi. As root systems were small and young, we decided to measure traits on the entire root system. This included mainly first to third order roots and a small fraction of higher order roots. 99.98 % of root length within the entire experiment belonged to roots with a diameter < 2 mm.

Trait measurements

Total root length and volume as well as the average root diameter (D [mm]) were measured using WinRhizo 2017 software (Regent Instruments Inc., Québec, Canada). Aboveground and belowground dry biomass was determined after drying at 60°C for at least three days. The mycorrhizal growth response (MGR) of each plant species was calculated as MGR= ln[total dry biomass inoculated / total dry biomass non-inoculated] (Hoeksema *et al.* 2010; Maherali 2014). All other root traits were measured within the mycorrhizal treatment assuming that this resembles the natural soil biotic condition. Root dry biomass was used to calculate the specific root length (SRL – root length/dry biomass [m/g]) and root tissue density (RTD – dry biomass/root volume [g/cm³]) by calculating the volume as the sum of 0.2 mm diameter size classes according to Rose (2017).

Root hair length (HL [µm]), cortex fraction (CF [%]) and first order root diameter (D_{first} [mm]) were measured on the preserved first order root tips of three randomly chosen replicates per

species from the mycorrhizal treatment using a fluorescence microscope (Zeiss Axio Imager 2, Carl Zeiss AG, Oberkochen, Germany). One root per replicate was randomly picked and dyed in 0.01% Calcofluor-white (Thermo Fisher Scientific, Waltham, USA) for 5-10 seconds. Subsequently, it was rinsed in DI water for a few seconds, mounted on a slide and carefully covered with a cover slip without applying pressure. As Calcofluor-white binds to cellulose, it helps distinguish plant cell walls including those of fine root hairs. As all roots were small and translucent there was no need for cross sectioning to measure stele and cortex diameter. Microscopic images were taken with a Zeiss AxioCam at a magnification of x5 using a 430 nm fluorescence filter. For each replicate, several images were taken using the functions "Z-Stacks" and "Tiles" to display a continuous segment of 5 mm within the mature root hair zone. The "Tiles" function merges several images along the root while the "Z-Stacks" function combines images vertically, thereby producing an in-focus image throughout the entire range of depths. We defined the Z-Stacks to range from the middle of the stele to the upper epidermal layer of the root just beneath the cover slip. The first order root diameter (D_{first}) as well as the stele diameter were measured at three positions along the image. We calculated the cortex fraction (CF) as the percent area of a first order root cross section that is occupied by tissue outside the stele (Freschet *et al.* 2020). Mean values of D_{first} and CF were first calculated on replicate level and subsequently on the species level. RHL was measured according to Delhaize *et al.* (2012). In brief, we divided the 5 mm root segments into 5 sub-segments of 1 mm each and measured the length of the longest root hair on each side of the root in each sub-segment. All 10 measurements per 5 mm root segment were averaged to calculate the mean RHL per replicate.

For the determination of the percentage of mycorrhizal colonization (%M) and the root hair incidence (HI [%]), we used representative subsamples of the dried root systems of the three replicates from the mycorrhizal treatment of each species. Furthermore, one replicate per

species in the non-mycorrhizal treatment was randomly chosen and checked for AM colonization. Roots were first cleared in 10% KOH for 15 min at 80°C and then stained in 0.05% Trypan Blue in lactoglycerol for another 15 min at 80°C. Mycorrhizal colonization was determined with the magnified intersection method (McGonigle *et al.* 1990) at a magnification of x200, using a minimum of 30 root pieces on a slide to count presence or absence of mycorrhizal structures in 50-100 intersects. For the non-mycorrhizal treatment, mycorrhizal colonization rates between 1% and 6% were detected for 6 out of 82 replicates suggesting very limited contamination. Within the mycorrhizal treatment colonization rates of up to 86% and rates of arbuscular colonization up to 72% clearly confirmed a successful inoculation. RHI was determined simultaneously and analogously to %M, recorded as presence or absence at each intersect (Siqueira & Saggin-Júnior 2001) giving a proxy of how much percent of the root length was covered by root hairs. The coefficients of variation of HI and HL (cvHI, cvHL) were calculated by using the general R function cv(x) that computes the sample coefficient of variation as (SD/mean)*100. To display the within-species correlation between %M and HI while accounting for overall between-species differences in both traits (Figure 4), we coded the intraspecific median of the three trait records per species as 0, the lower value as = lower value – median value and the higher value as = higher value – median value.

Root nitrogen content (N [%]) was measured on the three replicates after drying and milling the roots using an Elemental Analyzer (EuroEA, HekaTech, Germany). Mycorrhizal status (obligate mycorrhizal, facultative mycorrhizal, non-mycorrhizal) was assigned according to the FungalRoot database (Soudzilovskaia *et al.* 2020).

Analysis

All analyses were carried out in R version 3.6.3 (R Core Team 2019). We used the function drop.tip() from the package ape (Paradis *et al.* 2004) to prune the DaPhnE phylogeny (Durka

& Michalski 2012) for our species set and the function phylosig() as well as phylo.heatmap() from the package phytools (Revell 2012) to calculate the phylogenetic signal of all traits and to display trait variation along the tree by using color palettes from the package viridis (Garnier 2018). The package ggplot2 (Wickham 2016) was used to display the violin plots (Figure 2), the pairwise correlation heatmap (figure S1) and the correlation between %M and HI at intraspecific level (Figure 4).

To meet model assumptions, prior to the calculation of pairwise correlations and principal component analyses (PCA), we log transformed all traits except CF, HI and %M, which we transformed using the function logit() from the gtools (Warnes 2015) package, since these traits varied between 0 and 1. The function rcorr() from the package Hmisc (Harrell 2018) was used to calculate Pearson's correlations of all trait pairs, and the functions comparative.data() and pgls() from the package caper (Orme 2013) were used to calculate phylogenetically corrected pairwise correlations. We determined the phylogenetically corrected correlation coefficient by taking the square root from the adjusted r^2 of the model multiplied by -1 in case of a negative regression coefficient while assigning $r=0$ in case of negative adjusted r^2 values. The phylogenetically informed PCAs were calculated using phylpca() from the package phytools (Revell 2012) and displayed using functions from the package shape (Soetaert 2012). Euclidean pairwise distances in PCA space among species were calculated using the pairwise.adonis() function from the package pairwise.adonis.

Results

Root hair traits show a phylogenetically conserved pattern

Root-hair length and incidence showed strong phylogenetic signals (table S1) and were highly positively correlated (figure S1). Monocotyledons showed many long root hairs, with the hairless *Allium schoenoprasum* being the only exception, while legumes (Fabaceae) had few and short root hairs (Figure 1). Within the other dicotyledonous families, Asteraceae showed low values while Polygonaceae, Caryophyllaceae and Brassicaceae showed high values for both root hair length and incidence. Throughout the entire set of species, mycorrhizal colonization showed a completely opposite pattern which was strongly phylogenetically conserved as well (Figure 1, Table S1). Clades with long root hairs and high root hair incidence were poorly colonized by mycorrhiza while clades with short and few root hairs showed high colonization rates. Root-hair length and incidence where both negatively correlated with mycorrhizal colonization. This pattern disappeared for root hair length after phylogenetic correction (Figure S1).

Examining plant functional types, root hair length was lower in legumes as compared to grasses and forbs, with grasses having the longest root hairs overall (Figure 2a). The coefficient of variation in root hair length did not differ significantly among these plant functional groups, even though grasses tended to have the least variation (Figure 2e). Root-hair incidence was higher and its coefficient of variation was lower in grasses compared to both legumes and forbs (Figure 2b,f).

Mycorrhizal status is a weak predictor of root hair traits

Of the 82 grassland species tested in the experiment, 62 were obligate mycorrhizal, 14 facultative mycorrhizal and 6 non-mycorrhizal as classified according to the FungalRoot database. Mycorrhizal status did not predict species root hair length or its coefficient of

variation well, even though obligate mycorrhizal species tended to have shorter root hairs (Figure 2c). Root-hair incidence instead was lower in obligate mycorrhizal species than in non-mycorrhizal species, while facultative mycorrhizal species had intermediate values (Figure 2d). The coefficient of variation of root-hair incidence showed the opposite pattern with obligate mycorrhizal species being more variable than non-mycorrhizal species, and facultative mycorrhizal species showing intermediate values again (Figure 2h).

An ecological trade-off between root hairs and mycorrhization

The phylogenetically informed PCA revealed a strong trade-off (PC 1=45%) between high root-hair incidence and length on one end, and high mycorrhizal colonization rates on the other, accompanied by an increase in variation in root hair incidence (Figure 3, Table S2). PC 2 explained 23% of variation, with the coefficient of variation of root-hair length influencing this axis most strongly.

Mycorrhizal status as well as plant functional types affected species locations within the PCA (Table S3). Non-mycorrhizal species differed from obligate mycorrhizal species by being closely aggregated at high values of root-hair incidence on PC 1. Grasses differed from both forbs and legumes by showing high root-hair incidence as well, even though considerable variation occurred within each of the functional types. Legumes were located at high values of mycorrhizal colonization and variation in root-hair incidence on PC 1 while spanning the entire range of PC 2.

At intraspecific level, root-hair incidence but not root-hair length correlates with mycorrhizal colonization rate

Despite low within-species replication (n=3 individual plants per species scored for root hair traits), we could detect an overall association between root hair incidence and mycorrhizal

colonization rate (Figure 4). Individual plants with higher colonization rates had lower root hair incidence than less-colonized individuals within the same species. Despite considerable variation among species, the overall pattern had small confidence intervals and a slight negative correlation (slope=-0.29, $p<0.001$). No intraspecific correlation was found between the mycorrhizal colonization rate and root-hair length.

Root-hair traits add to the root economics space

The inclusion of root-hair traits introduced multidimensionality into the root economics space. The first axis (22%) of the extended PCA (Figure S3 and S4, Table S4) was dominated by specific root length on one end and root average diameter and diameter of first order roots on the other, accompanied by variation in cortex fraction but also root tissue density. The trade-off between the investment in root-hair length and incidence on one end and the coefficient of variation of root-hair incidence on the other dominated the second axis (20%). Mycorrhizal colonization intensity and mycorrhizal growth response were associated with variation in root-hair incidence. Root tissue density loaded strongest on the third axis (14%) together with the intraspecific variation in root-hair length and antagonistically to root nitrogen content and cortex fraction.

Plants of different mycorrhizal status as well as different plant functional types differed in their root economic strategies within the space (Table S5). Non-mycorrhizal plants showed high specific root length on PC1 and high root-hair length and incidence on PC2, and there was no general pattern along PC3. Obligate mycorrhizal plants spanned the entire space but clearly showed the highest values for root diameter on PC1, lowest root-hair incidence on PC2 and highest root-nitrogen content on PC3. Legumes were located at high root diameter, cortex fraction and colonization rates as well as high root-nitrogen content. Grasses showed a clear trend towards high root-hair length and incidence as well as specific root length. As such,

grasses and legumes formed distinct groups almost without overlap, while forbs spanned the entire root economics space.

Figure 1: The phylogenetically conserved trade-off between the investment in root hairs and mycorrhization. Colour-coded are the species mean values of root hair length and incidence as well as percent mycorrhizal colonization to the right and the corresponding phylogenetic tree with broader taxonomic groups to the left. Trait values are standardized to the same range, colour-coded from yellow (low) via green (medium) to blue (high). Phylogenetic signal of each trait is displayed as Pagel´s lambda. Grasses are shaded in medium grey, non-leguminous forbs in light grey and leguminous forbs in dark grey. The grass with the lowest hair incidence had no root hairs in the samples for determination of hair length leading to a missing value.

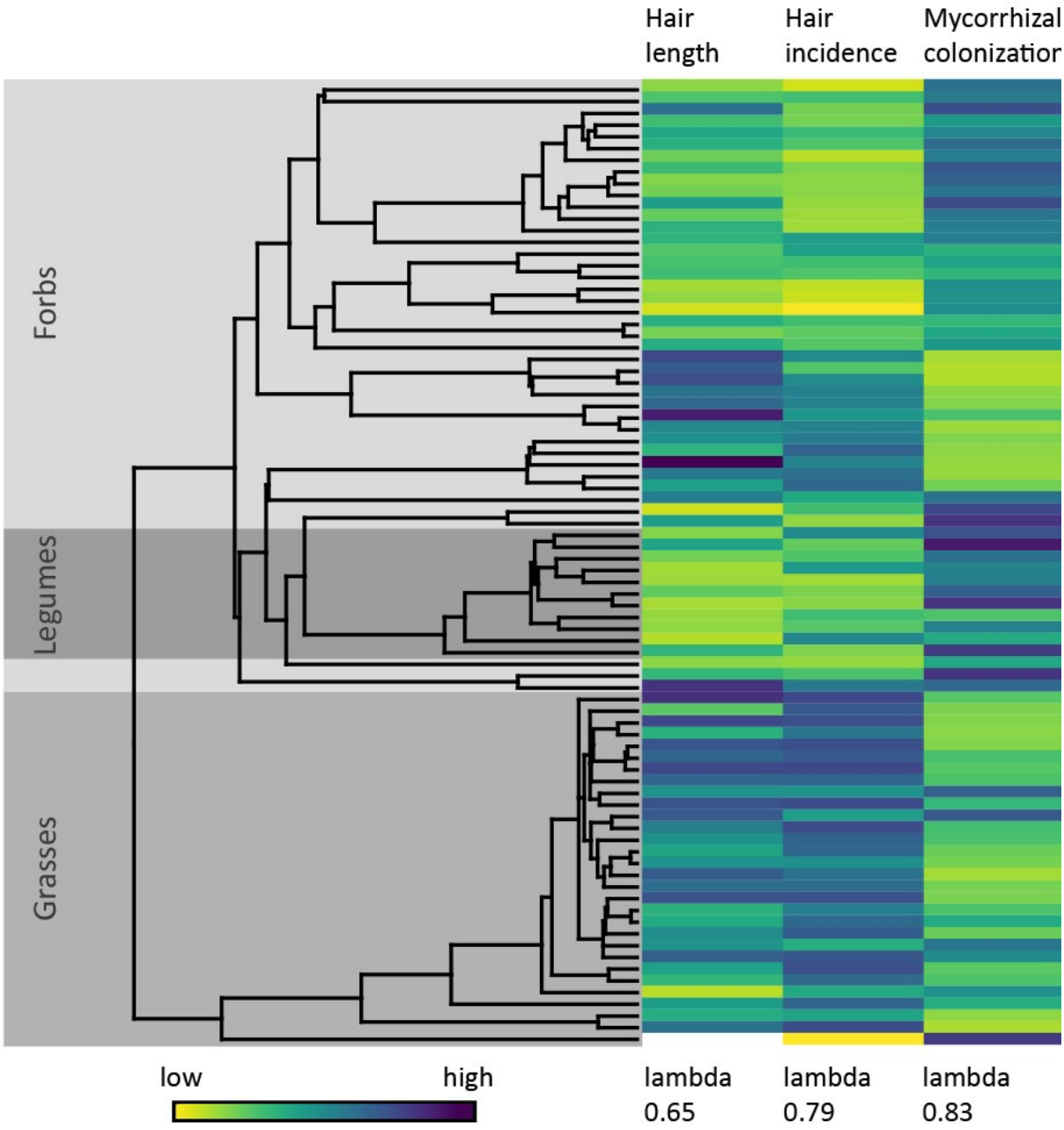

Hair length
Hair incidence
Mycorrhizal colonizatior
Forbs
Legumes
Grasses
low
high
lambda 0.65
lambda 0.79
lambda 0.83

Figure 2: Variation in root hair traits according to plant functional type and mycorrhizal status. Displayed are species mean root-hair length (HL, panel A, C) and incidence (HI, panel B, D) as well as the coefficient of variation in root-hair length (cvHL, panel E, G) and incidence (cvHI, panel F, H), displayed are kernel density distributions and group means (black dots) with 95% confidence interval. Non-overlapping confidence intervals are highlighted by coloured ribbon to visualize group differences. Plant functional types: grasses, forbs, legumes; mycorrhizal status: obligate mycorrhizal (AM), facultative mycorrhizal (AM-NM), non-mycorrhizal (NM).

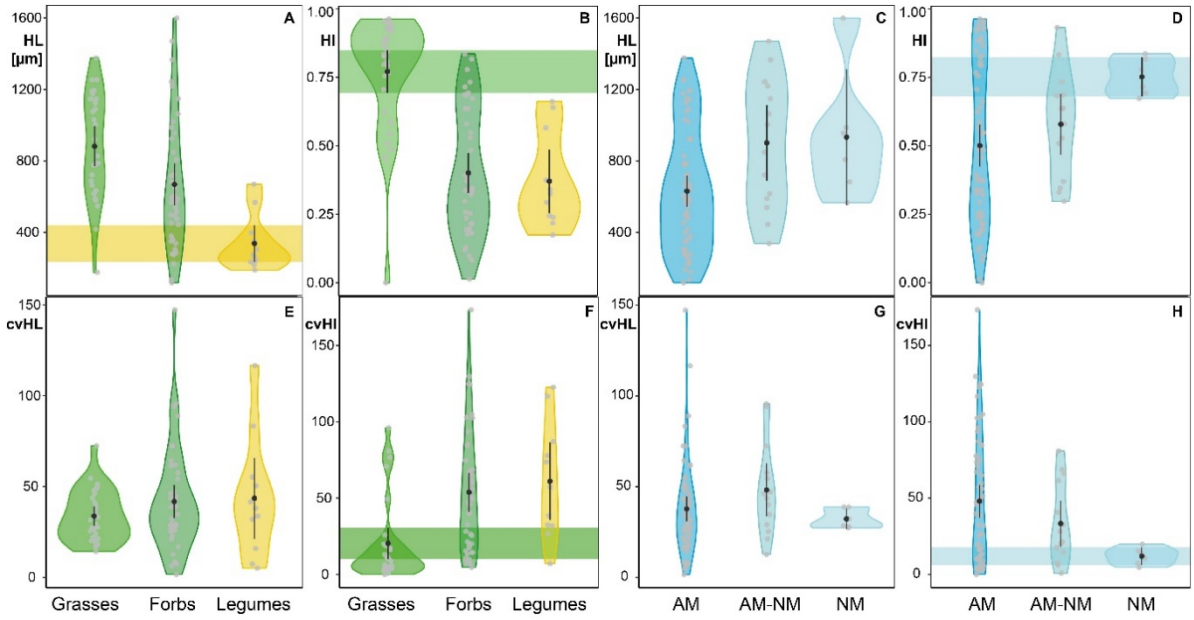


Figure 3: Phylogenetically informed principal component analysis of root hair traits and mycorrhizal colonization rate. Panel A displays species based on their mycorrhizal type (dark blue – obligate mycorrhizal, light blue – facultative mycorrhizal, orange – non-mycorrhizal) while panel B displays species based on their taxonomic group (dark green – forbs, light green – grasses, yellow – legumes). PCA results can be found in table S2. HL – hair length, HI – hair incidence, cvHL – coefficient of variation in hair length, cvHI – coefficient of variation in hair incidence, %M - percent mycorrhizal colonization.

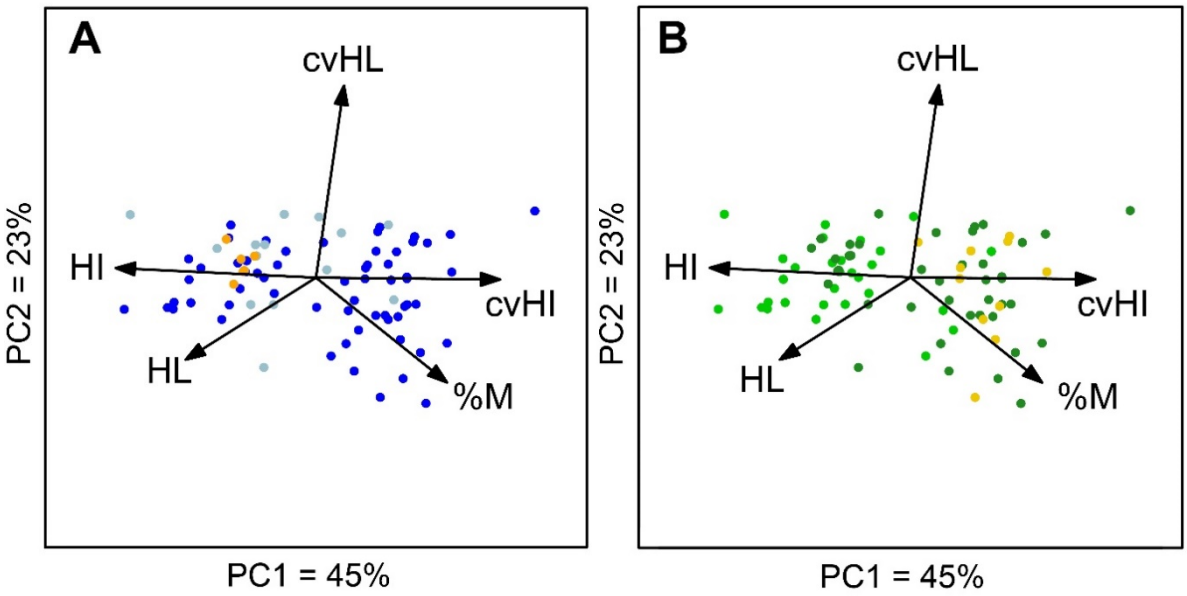
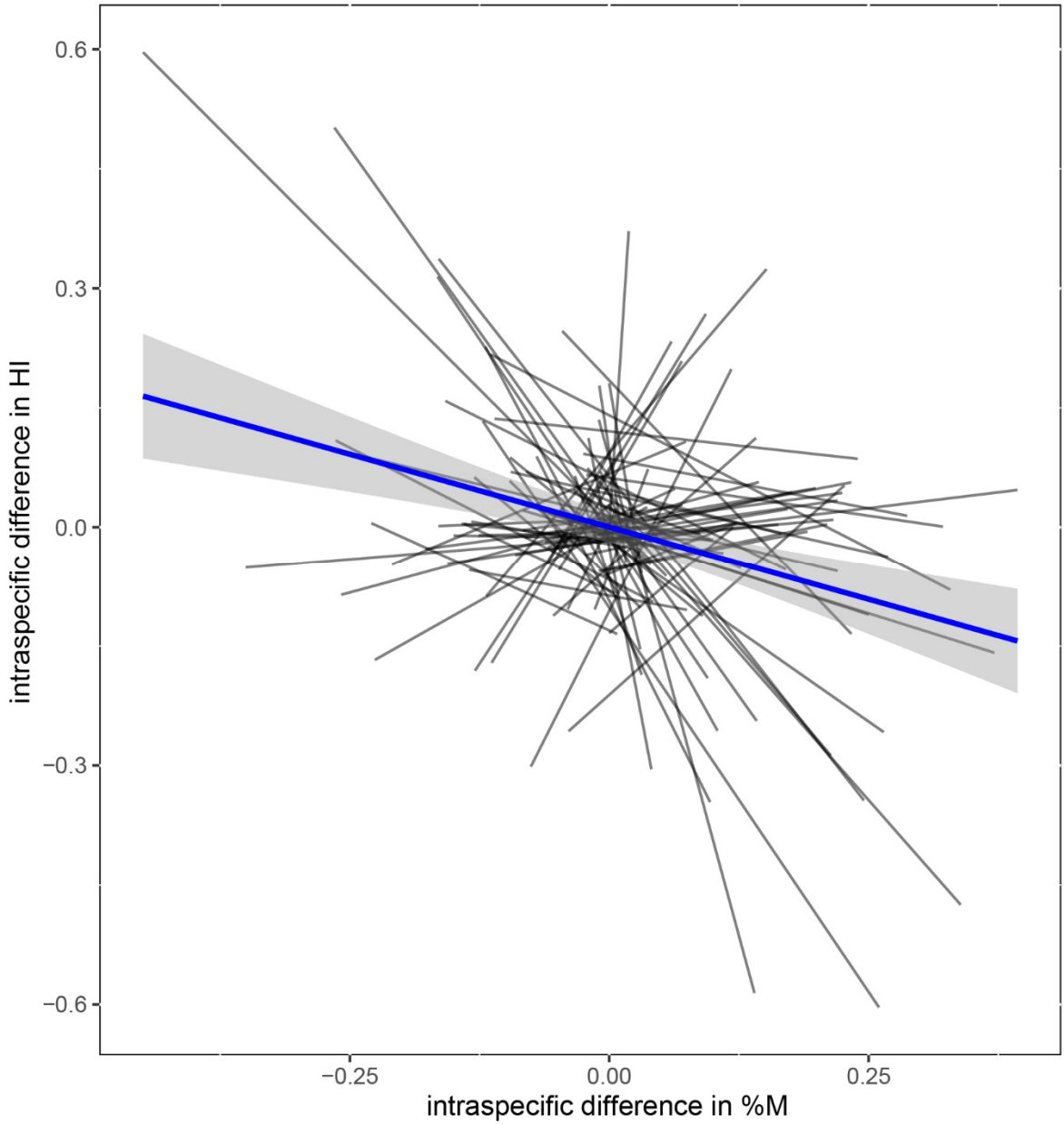


Figure 4: Intraspecific correlation of root hair incidence and mycorrhizal colonization. Displayed is the relative difference in mycorrhizal colonization (%M) and root hair incidence (HI) within each species as well as the overall correlation with 95% confidence interval.



Discussion

Plants can either invest in root hairs or rely on mycorrhizal partners while still keeping some flexibility

We found a striking pattern of an evolutionarily conserved trade-off between plant investment in root hairs – specifically their incidence – and mycorrhizal symbiosis. The phylogenetic conservation occurred at high taxonomic levels; grasses showing high root-hair incidence and length, paired with low mycorrhizal colonization rates, while legumes exhibited the opposite pattern (Figures 1 and 2). Non-leguminous forbs exhibited a range of strategies along the entire gradient of variation. This evolutionarily deep rooted phylogenetic signal might be the reason, why many correlations between raw trait data disappeared after phylogenetic correction.

We found mycorrhizal status to be a weak predictor of root hair traits. The traditional classification of species as being obligate, facultative or non-mycorrhizal and the respective definitions have been discussed lately (Cosme *et al.* 2018; Brundrett & Tedersoo 2019). Cosme *et al.* (2018) argue that species classified as being non-mycorrhizal can have low levels of colonization and even a few arbuscules. We found the same pattern in our non-mycorrhizal species with low colonization rates and no or very few arbuscules. Moreover, we found that these non-mycorrhizal species showed high root-hair length and incidence. The classification of facultative mycorrhizal species includes both species that are mycorrhizal only under specific circumstances and species that always have low colonization rates; hence plants with different ecological strategies (Brundrett & Tedersoo 2019; Soudzilovskaia *et al.* 2020). Accordingly, we found strong overlap in root hair traits between obligate and facultative mycorrhizal species, even though the latter tended to have more and longer root hairs as we would have expected given the fact that they have lower colonization rates (Figure S2). However, non-mycorrhizal plants differed strongly from obligate mycorrhizal plants by having higher root-hair incidence and lower variation therein. This pattern also dominated the overall

trade-off between the investment in root hairs and mycorrhiza: a strong investment in root-hair incidence was accompanied by low variation of the same trait. Species with high mycorrhizal colonization rates – mainly obligate mycorrhizal ones – produce fewer root hairs but encompass more intraspecific variation.

Although this experiment was not designed to test for intraspecific variation, we could show that within species, root-hair incidence was higher at lower colonization rates. We cannot determine if this variation originates from a plastic response of the plant to different colonization levels of the AM fungus or from genetic variation between plant individuals. Further research is needed to evaluate this question and to determine cause and effect. Nevertheless, our results suggest an intraspecific trade-off between root hair incidence and mycorrhizal colonization mirroring the interspecific pattern and leading to stronger variation in root hair incidence in obligate mycorrhizal species with high colonization rates.

Root hairs add to the do-it-yourself strategy of plants

The trade-off between root hairs and mycorrhizal colonization rate defined the second axis of the principal component analysis on all traits, with the root traits of the collaboration gradient dominating the first and those of the construction gradient the third axis.

Root-hair length and incidence behaved antagonistically to the variation in root-hair incidence accompanied by mycorrhizal colonization rate and growth response. With 20% variance, the trade-off explained a considerable amount of variation within the entire trait space. The first axis resembled the collaboration gradient with a trade-off between 'do-it-yourself' with high SRL and 'outsourcing' with high root diameter as expected within the framework of the root economics space (Bergmann *et al.* 2020; Ding *et al.* 2020). Mycorrhizal colonization was less strongly associated with the first axis than with the root-hair dominated second axis though pairwise correlation with cortex fraction was strong as expected in the concept of the

collaboration gradient. We suggest that this might originate from the fact, that mycorrhizal colonization was measured at the same subsamples than root hair incidence, creating a stronger dependence of the data. As for pairwise correlations with %M, both traits of the first and the second axis link to the concept of collaboration.

Unexpectedly, RTD also loaded on axis one with a considerable amount of variation, being negatively correlated to SRL. This correlation has been reported before (Eissenstat 1992; Reich 2014) and might originate from the fact that at a given diameter SRL has to increase with decreasing RTD (Ostonen *et al.* 2007). This might be the most important driver behind detections of a one dimensional root economics spectrum that parallels leaf economics (Freschet *et al.* 2010; Reich 2014).

The third axis resembled the conservation gradient proposed as the belowground analogue of the fast-slow economic spectrum in leaves (Bergmann *et al.* 2020) with RTD representing the 'slow' and root N representing the 'fast' strategy. Unexpectedly, variation in root hair length was also associated with the 'slow' strategy on axis three. Cortex fraction loaded on axis one where it was correlated with mycorrhizal colonization rate as expected but even more strongly on the 'fast' side of axis three. We hypothesize that this unexpected link might occur because AMF also enhance species N uptake under limiting conditions (Govindarajulu *et al.* 2005; Hodge & Fitter 2010). As root N, cortex fraction and colonization rate were measured on the same replicates, the effect of mycorrhizal colonization rate on root N and a resulting positive correlation (r=0.3) might be overestimated by our data that were measured on plants growing under relatively low nutrient conditions. Furthermore, our dataset was restricted to a single AMF species and excludes plant mycorrhizal types others than arbuscular mycorrhiza. Ectomycorrhizal species tend to occupy areas of the 'slow' strategy in the root economics space (Bergmann *et al.* 2020), hence adding variation to the conservation gradient that is not covered in the present experiment. Even more importantly, due to the nature of the ectomycorrhizal

symbiosis with fungal hyphae covering entire fine roots and leading to fast degradation of root hairs (Peterson & Farquhar 1996) the importance of root-hair traits might change in a global dataset.

Conclusions

We show that the investment into root hairs and mycorrhizal partnerships are alternative ecological strategies for soil exploration and resource uptake with a strong evolutionary history. This interspecific ecological trade-off is mirrored at the intraspecific level with plants showing more root hairs at lower mycorrhizal colonization rates. High variation in root-hair incidence is associated with high mycorrhizal colonization rates and growth response at the species level. The ecological trade-off between the investment in root hairs and the variation in incidence being interspecifically correlated with mycorrhizal colonization rates dominates the second axis of the root economics space. We conclude that variation in root hair patterns is neither fully aligned with the conservation nor the existing concept of the collaboration gradient but rather introduces a new dimension of variation into the picture. Still, regarding the strong trade-off with mycorrhizal colonization, we consider root hairs, and specifically their incidence, to add to the ecological strategy of ‘do-it-yourself. Hence, we find the concept of collaboration to span the first and second and the construction gradient to represent the third axis of variation in the root economics space.

Acknowledgements

We thank Erik Faltin, Cathrin Schierenbeck, Anja Wulf, Max Fussan, Maxi Bergmann and many others for help with root washing and scanning. We further thank Julien Bachelier for use of the fluorescence microscope and Maria Schauer for sharing her knowledge about fixation and microscopy of plant material.

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Supporting Information

Figure S1: Pairwise correlations of all traits. Upper triangle (solid line) represents phylogenetically informed correlations from PGLS (phylogenetic generalized least square) models while lower triangle (dashed line) represents correlations of raw traits. Displayed is the strength of the Pearson correlation while significant negative values ($P < 0.05$) appear in shades of blue and significant positive values appear in shades of red. HL – hair length, HI – hair incidence, cvHL – coefficient of variation in hair length, cvHI – coefficient of variation in hair incidence, %M - % mycorrhizal colonization, SRL – specific root length, AD – average diameter, D_{first} – diameter of first order roots, CF – cortex fraction, RTD – root tissue density, N – root nitrogen content, MGR – mycorrhizal growth response.

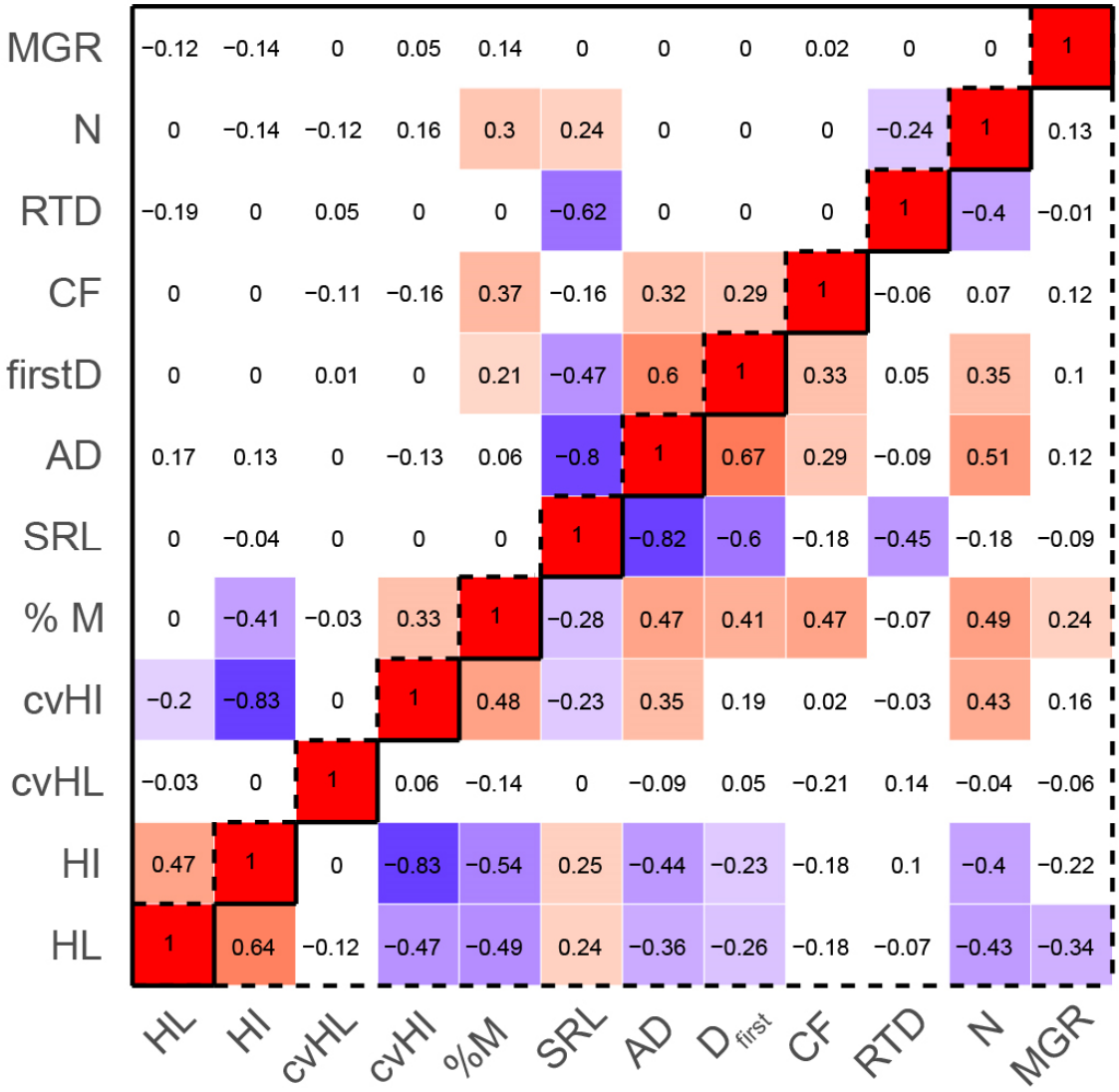

MGR −0.12 −0.14 0 0.05 0.14 0 0 0 0.02 0 0 1
N 0 −0.14 −0.12 0.16 0.3 0.24 0 0 0 −0.24 1 0.13
RTD −0.19 0 0.05 0 0 −0.62 0 0 0 1 −0.4 −0.01
CF 0 0 −0.11 −0.16 0.37 −0.16 0.32 0.29 1 −0.06 0.07 0.12
firstD 0 0 0.01 0 0.21 −0.47 0.6 1 0.33 0.05 0.35 0.1
AD 0.17 0.13 0 −0.13 0.06 −0.8 1 0.67 0.29 −0.09 0.51 0.12
SRL 0 −0.04 0 0 0 1 −0.82 −0.6 −0.18 −0.45 −0.18 −0.09
% M 0 −0.41 −0.03 0.33 1 −0.28 0.47 0.41 0.47 −0.07 0.49 0.24
cvHI −0.2 −0.83 0 1 0.48 −0.23 0.35 0.19 0.02 −0.03 0.43 0.16
cvHL −0.03 0 1 0.06 −0.14 0 −0.09 0.05 −0.21 0.14 −0.04 −0.06
HI 0.47 1 0 −0.83 −0.54 0.25 −0.44 −0.23 −0.18 0.1 −0.4 −0.22
HL 1 0.64 −0.12 −0.47 −0.49 0.24 −0.36 −0.26 −0.18 −0.07 −0.43 −0.34
HL HI cvHL cvHI %M SRL AD D first CF RTD N MGR

Figure S2: Variation in mycorrhizal colonization (%M) between plants of different mycorrhizal status. Displayed are single species values with kernel density plots as well as mean (black dot) and 95% confidence interval per group. AM – obligate mycorrhizal, AM+NM – facultative mycorrhizal, NM – non-mycorrhizal.

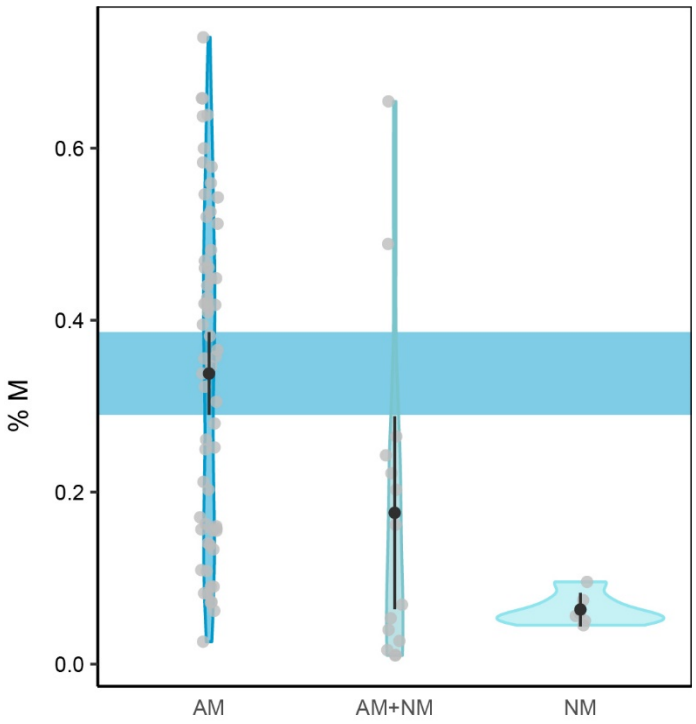


Figure S3: Extended phylogenetically informed principal component analysis. Displayed are species based on their taxonomic group (dark green – forbs, light green – grasses, yellow – legumes). PCA results can be found in table S4. HL – hair length, HI – hair incidence, cvHL – coefficient of variation in hair length, cvHI – coefficient of variation in hair incidence, %M - % mycorrhizal colonization, SRL – specific root length, AD – average diameter, D_{first} – diameter of first order roots, CF – cortex fraction, RTD – root tissue density, N – root nitrogen content, MGR – mycorrhizal growth response.

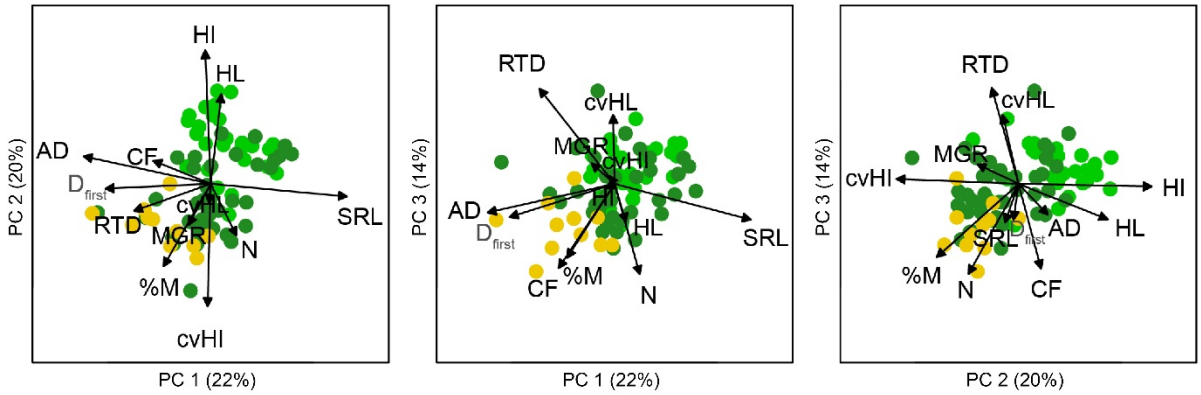


Figure S4: Extended phylogenetically informed principal component analysis. Displayed are species based on their mycorrhizal type (dark blue – obligate mycorrhizal, light blue – facultative mycorrhizal, green – non-mycorrhizal). PCA results can be found in table S4. HL – hair length, HI – hair incidence, cvHL – coefficient of variation in hair length, cvHI – coefficient of variation in hair incidence, %M - % mycorrhizal colonization, SRL – specific root length, AD – average diameter, D_{first} – diameter of first order roots, CF – cortex fraction, RTD – root tissue density, N – root nitrogen content, MGR – mycorrhizal growth response.

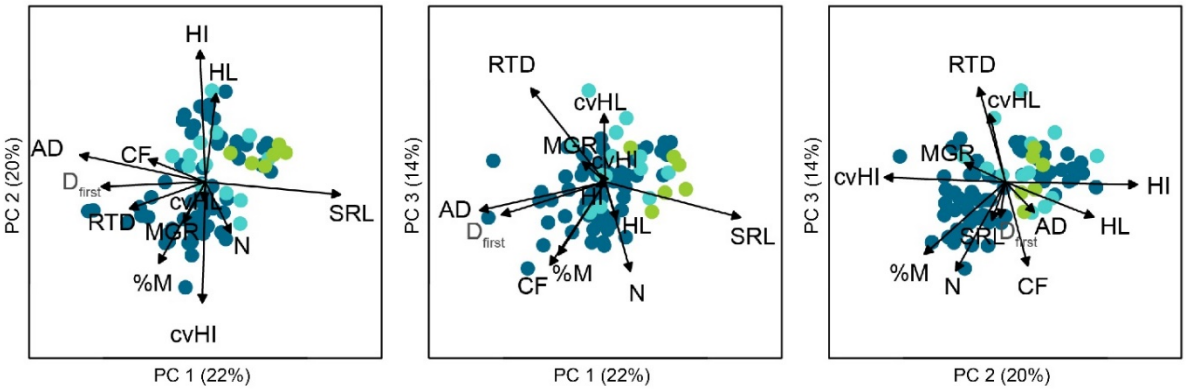


Table S1: Phylogenetic signal of all traits. Displayed is Pagel's lambda. HL – hair length, HI – hair incidence, cvHL – coefficient of variation in hair length, cvHI – coefficient of variation in hair incidence, %M - % mycorrhizal colonization, SRL – specific root length, AD – average diameter, D_{first} – diameter of first order roots, CF – cortex fraction, RTD – root tissue density, N – root nitrogen content, MGR – mycorrhizal growth response.

	lambda	***P***
%M	0.831191	1.45E-12
HL	0.653663	2.78E-08
HI	0.789602	3.97E-18
SRL	0.733511	1.63E-05
AD	0.988914	2.86E-15
Dfirst	0.774926	1.95E-07
CF	0.656625	1.18E-05
RTD	0.220441	7.72E-02
N	0.995809	8.55E-22
cvHI	0.682502	3.36E-07
cvHL	0.768836	1.77E-02
MGR	0.687188	5.87E-04

Table S2: Phylogenetically informed principal component analysis of root hair traits and %M. lambda = 0.606. HL – hair length, HI – hair incidence, cvHL – coefficient of variation in hair length, cvHI – coefficient of variation in hair incidence, %M - percent mycorrhizal colonization.

	PC1	PC2	PC3	PC4	PC5
SD	1.496	1.065	0.871	0.812	0.456
Variance	0.448	0.227	0.152	0.132	0.042
HL	0.584	-0.370	0.712	0.063	0.105
%M	-0.591	-0.475	-0.052	0.649	-0.028
HI	0.912	0.044	-0.078	0.213	-0.338
cvHI	-0.835	-0.008	0.407	-0.238	-0.283
cvHL	-0.128	0.878	0.280	0.365	0.044

Table S3: Pairwise dissimilarities of plant functional types and mycorrhizal types within the principal component analysis displayed in table S2. AM – obligate mycorrhizal, AM+NM – facultative mycorrhizal, NM – non-mycorrhizal.

			Sums of Squares	*F*	R^2	*P*
AM+NM	**vs**	**AM**	2014.093	3.698	0.048	0.054
AM+NM	**vs**	**NM**	441.490	1.565	0.080	0.227
AM	**vs**	**NM**	2741.708	5.110	0.073	0.042
grasses	**vs**	**forbs**	10561.737	26.129	0.278	0.002
grasses	**vs**	**legumes**	9796.955	34.155	0.473	0.002
forbs	**vs**	**legumes**	887.421	2.139	0.041	0.126

Table S4: Extended phylogenetically informed principal component analysis, lambda = 0.588. HL – hair length, HI – hair incidence, cvHL – coefficient of variation in hair length, cvHI – coefficient of variation in hair incidence, %M - % mycorrhizal colonization, SRL – specific root length, AD – average diameter, D_{first} – diameter of first order roots, CF – cortex fraction, RTD – root tissue density, N – root nitrogen content, MGR – mycorrhizal growth response.

	PC1	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**	**PC7**	**PC8**	**PC9**	**PC10**	**PC11**	**PC12**
SD	1.614	1.550	1.316	1.079	0.988	0.863	0.835	0.778	0.675	0.639	0.437	0.134
Var.	0.217	0.200	0.144	0.097	0.081	0.062	0.058	0.050	0.038	0.034	0.016	0.001
SRL	0.918	-0.085	-0.241	0.020	-0.191	0.049	0.085	-0.008	0.191	0.010	0.012	0.095
AD	-0.852	0.184	-0.192	-0.136	0.099	0.086	-0.070	0.251	-0.246	0.181	0.014	0.076
D_{first}	-0.700	-0.031	-0.221	-0.320	-0.228	0.167	-0.085	0.096	0.503	-0.078	-0.032	-0.006
HL	0.071	0.592	-0.235	-0.194	0.421	0.446	-0.002	-0.397	-0.023	-0.070	-0.111	0.006
CF	-0.372	0.149	-0.559	0.336	-0.396	-0.011	0.346	-0.076	-0.163	-0.323	-0.030	0.002
%M	-0.313	-0.546	-0.487	0.069	0.056	-0.167	0.180	-0.413	0.072	0.343	0.073	-0.005
HI	-0.037	0.895	-0.018	0.033	-0.138	-0.118	-0.188	-0.151	0.050	-0.012	0.319	0.000
RTD	-0.504	-0.177	0.637	0.129	0.169	-0.317	-0.056	-0.311	0.089	-0.226	-0.044	0.054
N	0.167	-0.333	-0.598	-0.226	-0.036	-0.242	-0.584	-0.084	-0.135	-0.154	-0.051	-0.001
cvHI	-0.021	-0.818	0.032	-0.217	0.213	0.324	0.084	0.021	-0.080	-0.233	0.253	0.004
cvHL	-0.009	-0.104	0.449	-0.482	-0.643	0.168	-0.012	-0.259	-0.194	0.088	-0.031	0.002
MGR	-0.139	-0.260	0.123	0.734	-0.183	0.418	-0.372	-0.072	0.020	0.077	-0.002	0.003

Table S5: Pairwise dissimilarities of plant functional types and mycorrhizal types within the extended principal component analysis displayed in table S4. AM – obligate mycorrhizal, AM+NM – facultative mycorrhizal, NM – non-mycorrhizal.

			Sums of Squares	*F*	R^2	*P*
AM+NM	**vs**	**AM**	6479.329	5.274	0.068	0.003
AM+NM	**vs**	**NM**	2678.570	2.896	0.146	0.020
AM	**vs**	**NM**	7806.784	6.654	0.093	0.003
grasses	**vs**	**forbs**	13089.700	13.567	0.168	0.001
grasses	**vs**	**legumes**	26578.000	39.506	0.510	0.001
forbs	**vs**	**legumes**	10684.200	9.732	0.166	0.001

Chapter III: Soil conditions determine the position of temperate grassland communities in a two-dimensional belowground-trait space

Tom Lachaise, Joana Bergmann, Matthias C. Rillig & Mark van Kleunen

submitted

Abstract

1. Plant belowground organs perform essential functions, including water and nutrient uptake, anchorage, vegetative regeneration and recruitment of mutualistic soil microbiota. Determining how belowground traits jointly determine dimensions of plant functioning and how these dimensions are linked to environmental conditions would further advance our understanding of plant functioning and community assembly.
2. Here, we investigated belowground plant traits dimensionality and their variation along 10 soil and land-use parameters in 150 temperate grasslands plots. We used eight belowground traits collected in glasshouse and garden experiments, as well as bud-bank size and specific leaf area from databases, for a total of 313 species, and we calculated community weighted means (CWMs).
3. Using PCA, we found that about 55% of the variance in CWMs was explained by two main dimensions, corresponding to a mycorrhizal 'collaboration' and a resource 'conservation' gradient. Frequently overlooked traits such as rooting depth, bud-bank size and root branching intensity were largely integrated in this bidimensional trait space. The two plant strategy gradients were partially dependent, with 'outsourcing' communities being more likely to be 'slow'. 'Outsourcing' communities were more likely to be deep-rooting, and associated with low moisture and sand content, high top soil pH, high C:N and low delta-N-15. 'Slow' communities had large bud-banks and were associated with low land-use intensity, high top soil pH, and a low nitrate content but a high ammonium content. We did not find a substantial role of phosphorus-availability indicators on the 'collaboration' gradient.

4. In conclusion, the ‘collaboration’ and ‘conservation’ gradients previously identified based on species mean values scale up to the community scale in grasslands, encompass more traits than previously described, and vary with the environment.

Introduction

Plant traits are of major interest as they determine plant functioning (Solbrig 1993), covary with environmental conditions (Garnier *et al.* 2016), and influence ecosystem functions (de Bello *et al.* 2010; Hanisch *et al.* 2020). Nevertheless, traits still appear to have limited predictive power (Klimešová *et al.* 2016; van der Plas *et al.* 2020), possibly due to a lack of understanding of which and how many traits to focus on (Shipley *et al.* 2016). An important step forward has been the grouping of multiple traits into a limited number of syndromes, with continuous variation in the form of gradients of plant strategies (Bergmann *et al.* 2020; Chave *et al.* 2009; Díaz *et al.* 2016; Klimešová *et al.* 2018a; Pierce *et al.* 2013; Roddy *et al.* 2020; Westoby *et al.* 2002; Wright *et al.* 2004). For example, (Díaz *et al.* 2016) showed that the variation in aboveground traits can be captured by a 'size' gradient representing the size of whole plants and their parts, and an 'economic' gradient representing the leaf economics spectrum. A similar effort has recently addressed root traits, and identified a 'conservation' gradient and a 'collaboration' gradient as two independent aspects of belowground plant economy (Weemstra et al. 2016; Kramer-Walter et al. 2016; Bergmann et al. 2020).

Bergmann *et al.* 2020 suggested that in the root economic space the 'conservation' gradient, ranging from 'slow' to 'fast', is related to carbon conservation and determined by root tissue density and nitrogen content, while the 'collaboration' gradient, ranging from 'do-it-yourself' to 'outsourcing' of resource uptake to fungal partners, is determined by specific root length and root diameter along with mycorrhizal colonization (Fig. 1a). Despite the recent developments in understanding of trait dimensionality, several root traits that could be important for plant functioning (Laliberté 2017) have not yet been fully integrated into existing frameworks. For example, high root-branching intensity could resemble an alternative to the reliance on mycorrhiza, and be associated with specific root length for local soil exploitation (Ding *et al.* 2020; Freschet *et al.* 2020; Kong *et al.* 2014a), thus being indicative of a 'do-it-

yourself' strategy. Furthermore, rooting depth is also likely to be an important source of interspecific variation as it varies considerably across biomes (Schenk & Jackson 2002), and could explain overyielding in grasslands (Mommer *et al.* 2010; Mueller *et al.* 2013). As deep-rooting species are able to take up nutrients and water from deeper soil layers, rooting depth might be part of the 'fast' strategy of the conservation gradient (Fig. 1a). Alternatively, rooting depth could also be rather independent of the conservation gradient, as it generally correlates with aboveground plant size (Díaz *et al.* 2016).

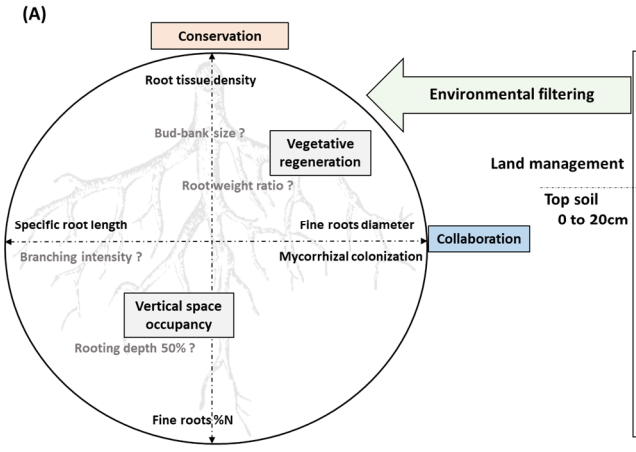
Belowground organs other than roots add another layer of complexity in terms of form and function. Plant structures such as rhizomes, belowground buds and tubers play important roles in storage and vegetative regeneration (Klimešová *et al.* 2018b). Species with large bud-bank sizes are more likely to be perennial and 'slow' growing (E-Vojtkó et al. 2017). Furthermore, although not strictly a belowground trait, root-weight ratio (i.e. the proportion of biomass allocated to roots) is a useful indicator of how plants invest in the uptake of different resources (Reynolds & D'Antonio 1996), and may be linked to rooting depth (Schenk and Jackson 2002a) and bud-bank size. It remains to be tested, whether these belowground traits are aligned with the 'conservation' or 'collaboration' gradient, or represent independent plant strategies.

Furthermore, as communities consist of plants with diverse strategies, it remains to be tested whether variation between plant community means mirrors the patterns known from interspecific variation in plant functioning. In grassland communities, which cover about 40% of the terrestrial surface excluding Greenland and Antarctica (Suttie *et al.* 2005), the few studies of belowground traits and their variation along environmental variables are generally limited to root morphological traits and use a limited set of coarse environmental descriptors (Craine *et al.* 2001; Erktan *et al.* 2018; Prieto *et al.* 2015). Analysing the relationships between various types of environmental variables, such as climate, soil fertility and land-use intensity, and aboveground traits has already improved our understanding of drivers of trait variation in

grasslands (Garnier *et al.* 2007), and should also be applied to belowground traits. Soil fertility is defined by a range of parameters (Abbott & Murphy 2007), and plants may have various strategies to deal with those. For example, it is likely that mycorrhizal collaboration becomes more important in sites with limited phosphorus (P) availability (Ma *et al.* 2020). Furthermore, the form of plant-available nitrogen in the soil could also select for different belowground traits, as species vary in the form of N they prefer (Maire *et al.* 2009; Pornon *et al.* 2007; Weigelt *et al.* 2005).

To better understand how belowground plant traits relate to environmental variation, we investigated 1) how mean community values of different belowground traits align along known plant-strategy gradients, and 2) how the shift of community means along these strategy gradients depends on environmental variables. We complemented traits defining the gradients of the root economics space with additional traits that might represent independent strategies of plant functioning. Therefore, we measured traits in pot experiments and combined them with data extracted from databases and vegetation relevés to calculate community weighted means of belowground traits for 150 grassland plots in Germany. We then assessed the dimensionality of the variation in community weighted means of ten traits with principal component analysis (PCA), and related the PCA axes to ten land-use intensity and soil variables. We present a priori hypotheses of the relationships between traits, plant strategy dimensions and environmental variables in Fig. 1.

Figure 1 Hypothesized relationships between (A) community weighted means (CWMs) of belowground traits in grasslands potentially aligned on two plant strategy gradients. A 'conservation' and a 'collaboration' gradient are expected as the main dimensions of plant variation, as our trait selection contains mostly traits of the root economics space. A 'vegetative regeneration' and 'vertical space occupancy' aspect could represent additional plant strategy gradients, or be embedded within the 'conservation' and 'collaboration' gradients. The two known belowground dimensions are here represented as two orthogonal axes ('Conservation' and 'Collaboration') with the traits that have previously shown to be associated with them in black. The positions of four other traits, bud-bank size, root weight ratio, branching intensity and rooting depth 50% (in grey), are yet unknown. (B) As a result of environmental filtering, each plant-strategy gradient or aspect could be associated with different or overlapping environmental variables. The signs and colors indicate the hypothesized direction of the relationships. The references on which these hypothesized relationships are based can be found in Appendix S4.



(B)

Environment	Belowground traits			
	Conservation	Collaboration	Vegetative regeneration	Vertical space occupancy
Land-use-intensity: Integrates Mowing Fertilisation and Grazing	-	-	?	?
Water availability: *Moisture*	-	+	-	?
Texture: *Sand content*	+	?	?	?
Nitrogen availability indicators: *pH C:N NO3 NH4 delta-15-N*	-	-	?	+
Phosphorus availability indicators: *pH N:P Phosphorus*	-	-	?	-

Methods

Data on grassland vegetation composition

The plant-community data used are from the grassland plots of the German 'Biodiversity Exploratories' (Fischer *et al.* 2010). In each of three regions of Germany, the Schwäbische-Alb (south-western Germany), Hainich-Dün (central Germany), and Schorfheide-Chorin (north-eastern Germany), 50 plots of 4 m × 4 m were selected in grasslands covering a wide range of land-use intensities. From 2008 to 2019, the vegetation composition of each of the 150 plots was assessed annually in late spring by identifying each vascular plant species and estimating its cover. To allow us to align the species names between the vegetation and trait datasets, we standardized the species names according to the accepted names in The Plant List (www.theplantlist.org, accessed 15 June 2019, using the Taxonstand R package (Cayuela *et al.* 2012). In total, 319 vascular plant species have been identified in the grassland plots of the 'Biodiversity Exploratories'.

Plant species traits

We obtained mean species values for eight traits from four pot experiments that we did, and for two further traits from databases. For 291 of the 319 grassland species, we were able to obtain seeds from commercial seed suppliers or botanical gardens. We then performed four pot experiments to measure species traits. *Taraxacum* spp. are abundant in the grassland plots, though, due to their complex taxonomy, rarely identified at the species level. We here used trait values of *Taraxacum campylodes* for *Taraxacum* spp. The trait values are part of a previously published dataset (Lachaise *et al.* 2020) and an unpublished dataset (Bergmann et al. *unpublished manuscript*), and comprehensive descriptions of the experiments are provided in Appendix S1. In brief, we did one greenhouse experiment in which we grew 2659 individual plants, representing 216 species, for four weeks after which we weighed the roots and analysed scanned images of the roots with WinRHIZO 2017a software (Regent Instruments Inc.,

Canada) to determine root-tissue density, specific root length, fine-root diameter, root weight ratio and root-branching intensity (Lachaise *et al.* 2020). We did a second greenhouse experiment using 2007 plants, representing 196 species, to determine the nitrogen content of fine roots (Fine roots %N) using isotope-ratio mass spectrometry. In a third greenhouse pot experiment, we determined mycorrhizal colonization rate for 225 plants, representing 75 species that are among the most common ones in the grasslands plots (mean cover of 65%, Appendix S3). Six weeks after inoculation with spores of *Rhizophagus irregularis* (see Bergmann et al. *unpublished manuscript*), roots were harvested and washed, and the percentage of mycorrhizal colonization was determined using the line-intersect method (McGonigle *et al.* 1990). In a fourth experiment, we grew 752 plants, representing 183 species, in outdoors growth-tubes to determine the depth above and below which plants have 50% of their root biomass (Rooting depth 50%, see Appendix S1 or Schenk & Jackson 2002 for the calculation method). In addition, to have an estimate of the belowground regeneration potential, we extracted bud-bank size, including stem and root-derived buds occurring belowground or at the soil surface, from the CLO-PLA database (Klimešová *et al.* 2017) for 313 of the 319 species. Finally, to also have a reliable indicator of the plant communities' acquisitive side of the plant economics spectrum (Allan *et al.* 2015; Kleinebecker *et al.* 2018), we extracted specific leaf area, the one and only aboveground trait in our analyses, for 279 of the 319 species from the LEDA database (Kleyer *et al.* 2008).

Environmental variables of grassland plots

To relate the different axes of variation in community weighted trait means of the grassland plots to the abiotic environment, we used ten environmental variables related to land-use intensity and soil conditions. The goal was to capture a relatively independent set of descriptors likely to drive the belowground functioning of plants. Each of the environmental variables has already been used in studies within the Biodiversity Exploratories (Allan *et al.* 2015; Herz *et*

al. 2017; Kleinebecker *et al.* 2018; Solly *et al.* 2014). We only give a brief description of each variable and refer for more details to Appendix S2. We used the land-use-intensity index (Blüthgen *et al.* 2012), which aggregates the intensity of mowing, fertilization and grazing, and is a major driver of ecosystem properties (Allan *et al.* 2015). We used a variety of physicochemical indicators related to fertility and mechanical properties of the topsoil (0-20 cm). We used soil-moisture content and sand content to capture soil water availability and texture, respectively. We used soil pH because it determines the degree to which the nutrients present in the soil are available for plant growth. We used soil extractable NO_3, NH_4 and delta-N-15 as indicators of soil nitrogen content (Kleinebecker *et al.* 2014; Robinson 2001), and the C:N ratio as a commonly used coarse indicator of soil stoichiometry (Schachtschabel *et al.* 1998). We further used resin-bags-adsorbed phosphorus and the N:P ratio to capture phosphorus availability (Güsewell 2004). Because soil volume is a central element in soil fertility and root-system distribution, we used data on soil bulk density to convert per-mass nutrient concentrations to per-volume concentrations. Few of the grassland-site descriptors were measured for each of the years for which we had vegetation-composition data (i.e. for the period 2008-2019). However, we tried to maximize the coverage for this period by using all available census dates for these variables (see Appendix S2 for years covered) and averaging the values per plot.

Statistical analyses

Community weighted trait means

To characterize the plant communities of each of the 150 grassland plots based on values of functional traits of their species, we calculated community weighted means (CWMs) as

$$_{CWM}Trait = \sum_{j=1}^{S} p_j \, Trait_j.$$

Here p_j is the relative cover of species *j* in the community, $Trait_j$ is the trait value of species *j,* and *S* is the number of species in the community with available trait data. Because some plots

had patches of bare soil in some of the annual vegetation surveys, and because for some species trait data were missing, we normalized plant cover to cumulate to 100% for all species with available trait data in each plot before calculating the CWM. As we have trait data for most of the dominant grassland species, we have data for about 90% of the total cover in most plots for most traits (Appendix S3). The only exception is mycorrhizal colonization, which was only available for 78 species, but, even for that trait, the average cover was 65% (range 32 - 87%, Appendix S3).

Principal axes of CWMs variation with principal component analysis

As the CWMs of several traits were correlated (Appendix S10), we performed principal component analyses (PCA) to reduce the dimensionality of the CWMs. To assess how robust the resulting dimensions are to the inclusion of additional information, we performed four separate PCAs. Each of these PCAs included all nine belowground traits, but they differed in that we also included or excluded $_{CWM}$*Specific leaf area*, as one of the major traits associated with the aboveground 'fast' side of the plant economics spectrum, and that we included or excluded plant-functional-type information (i.e. the percentages cover of graminoids, N-fixing forbs and non-N-fixing forbs). So, one PCA included CWMs of belowground traits only ("Belowground PCA"), one additionally included $_{CWM}$*Specific leaf area* ("Above-Belowground PCA"), one additionally included the proportions of Poales, Fabaceae and non-Fabaceae forbs, and one included all. To increase the separation of the variable loadings (the trait CWMs) on the two first axes, we performed an 'oblimin' rotation on these axes for the Belowground PCA and the Above-Belowground PCA. We $\log_{10}$ transformed $_{CWM}$*Root tissue density* to conform to the multinormality requirement of the PCAs. To complement the information provided on taxonomic or phylogenetic influence on community trait values, we also looked at the ten most dominant species or taxa in the trait space formed by PC1 and PC2 and the indicator species or taxa that associated with each quadrant of the two-dimensional

space formed by PC1 and PC2 (Appendix S15). As the proportions of plant functional types were shown to be significantly related to specific plant strategies in PC1 and PC2 (Appendix S6, Appendix S10), we also did three additional versions of the Above-Belowground PCA, removing each plant functional type in the CWMs calculation once, to evaluate how much the trait relationships are affected by the presence of the respective plant functional type (Appendix S7). To compare the relationships observed at the community level and at the species level, we also did the Above-Belowground PCA using trait means of the species instead of CWMs (Appendix S16).

Associations of the principal components of CWMs with environmental variables

To test for associations between the principal components of CWMs of the grassland plots and the environmental variables, we performed multiple regression. The PC1 and PC2 scores from each of the four PCAs on CWMs of the functional traits were used as response variables, and the environmental variables were used as predictors. C:N, N:P, sand content, NH_4, NO_3, and delta-N-15 were log-transformed before analysis to get a more regular (less clumped) distribution of the predictor values. To account for the fact that the grassland plots are in three different regions of Germany, we also included region as a predictor in the models. For model reduction, backward stepwise model selection based on AIC was performed using the function step(). This procedure selects a parsimonious set of predictors while minimizing the variance inflation factor (max VIF = 3.6 for Above-Belowground PCA). Because the two first axes (PC1 and PC2) of the four PCAs produced similar scores for the CWMs of the grassland plots (all pairwise correlations of the PC1s were >0.98 and those of the PC2s were >0.67), we present the results of the analysis of the "Above-Belowground PCA" in the main text (Fig. 3 based on the PC axes of Fig. 2), and the results for the other three PCAs in Appendix S8. We did the same for the PC3 to PC6 scores from the Above-Belowground PCA (Appendix S13), and for each of the ten $_{CWM}Traits$ (Appendix S14). We further tested if the proportions of the three

plant functional types, as related to the trait dimensions, responded to environmental variables in a similar way, and ran the same models with the proportions of plant functional types as the response variables (Appendix S12).

Results

Dimensionality of CWMs

The Above-Belowground PCA (Fig. 2, Appendix S5), as well as the other three PCAs (Appendix S5, S6), revealed that the two first axes generally explained about 55-60% of the total trait variance, and that each of the 10 traits had intermediate to strong loadings on at least one of these two axes (Appendix S9). PC1 had strong negative loadings of *CWM Specific root length* and *CWM Branching intensity*, and strong positive loadings of *CWM Mycorrhizal colonization*, *CWM Fine roots %N*, and *CWM Fine roots diameter*. PC2 had strong positive loadings of *CWM Bud-bank size*, *CWM Root weight ratio*, and *CWM Root tissue density*, and strong negative loadings of *CWM Specific leaf area*. PC1 thus overall captured the mycorrhizal 'collaboration' gradient of the root economic space, and PC2 captured the resource 'conservation' gradient. The traits characteristic of the two gradients of plant functioning, the 'collaboration' and the 'conservation' gradients, were, however, only partially independent (see CWMs correlations in Appendix S10). The 'fast' strategy tended to associate with the 'do-it-yourself' strategy. There was a rather strong loading of *CWM Rooting depth 50%* on both of these two PCs (Fig 2A; Appendix S9), suggesting that deep-rooting communities were associated with the 'outsourcing' side of the 'collaboration' gradient as well as the 'fast' side of the 'conservation' gradient.

Associations of the dimensions of CWMs with environmental variables

The position of grassland communities along the 'collaboration gradient' (PC1) and the 'conservation' gradient (PC2) was significantly related to several environmental variables (Fig. 3). The delta-N-15 fraction, sand content and moisture of the top soil were associated with the 'do-it-yourself' side of the 'collaboration' gradient (*i.e.* had negative effects on PC1). Land-use intensity and NO_3 content were retained by the model-selection procedure, as being associated with the 'do-it-yourself' side, but their effects were not significant (Fig. 3a). The pH

and C:N ratio, on the other hand, were associated with the 'outsourcing' side of the 'collaboration' gradient (*i.e.* had positive effects on PC1; Fig. 3a).

Among the environmental variables, NO_3 content and land-use intensity were significantly associated with 'fast' communities (*i.e.* had negative effects on PC2; Fig. 3b). Phosphorus content was also associated with 'fast' communities, but this effect was only marginally significant (Fig. 3b). NH_4 content and pH, on the other hand, were significantly associated with the 'slow' communities (*i.e*. had positive effects on PC2; Fig. 3b). The effects and the variance explained by the different models are comparable for the four PCAs, with and without $_{CWM}$*Specific leaf area* and with and without the plant functional types (Appendix S6).

Figure 2 The two first PCs of the Above-belowground PCA, explaining 56.4% of the total variance in community weighted means (CMWs). Every $_{CWM}$*Trait* has a strong loading on either PC (Appendix S9). PC3 representing 14.8% of the total variance (Appendix S5), separated the three regions of Germany but did not strongly relate to any environmental parameters (Appendix S13), probably capturing differences in regional species pool. The sole aboveground trait that we included, $_{CWM}$*Specific leaf area*, is shown in green. The scores of the 150 grassland plots used for the PCA are shown in different colors for each of the three regions (red for the Schwäbische Alb, brown for Hainich, blue for Schorfheide, each with N=50). PC1 is mostly characterized by CWMs of traits related to the mycorrhizal 'collaboration' gradient of the root economic space, with on the left, the 'do-it-yourself' strategy and on the right, the 'outsourcing' strategy. PC2 is more strongly characterized by CWMs of traits related to the 'conservation' gradient of a 'root and leaf economic spectrum', with on the top, the 'slow' strategy and on the bottom, the 'fast' strategy. Bud-bank size, as a surrogate of the vegetative regeneration potential, is associated with the 'slow' strategy. Correlation coefficients between the CWMs are provided in Appendix S10 and corroborate the relationships observed on PC1 and PC2. The loadings onto the PC1 to PC6 (90% of variance explained) are in Appendix S9. To maximize the loadings of the traits characteristic of the 'collaboration' and 'conservation' gradients on PC1 and PC2, an "oblimin" rotation was performed on the plot scores.

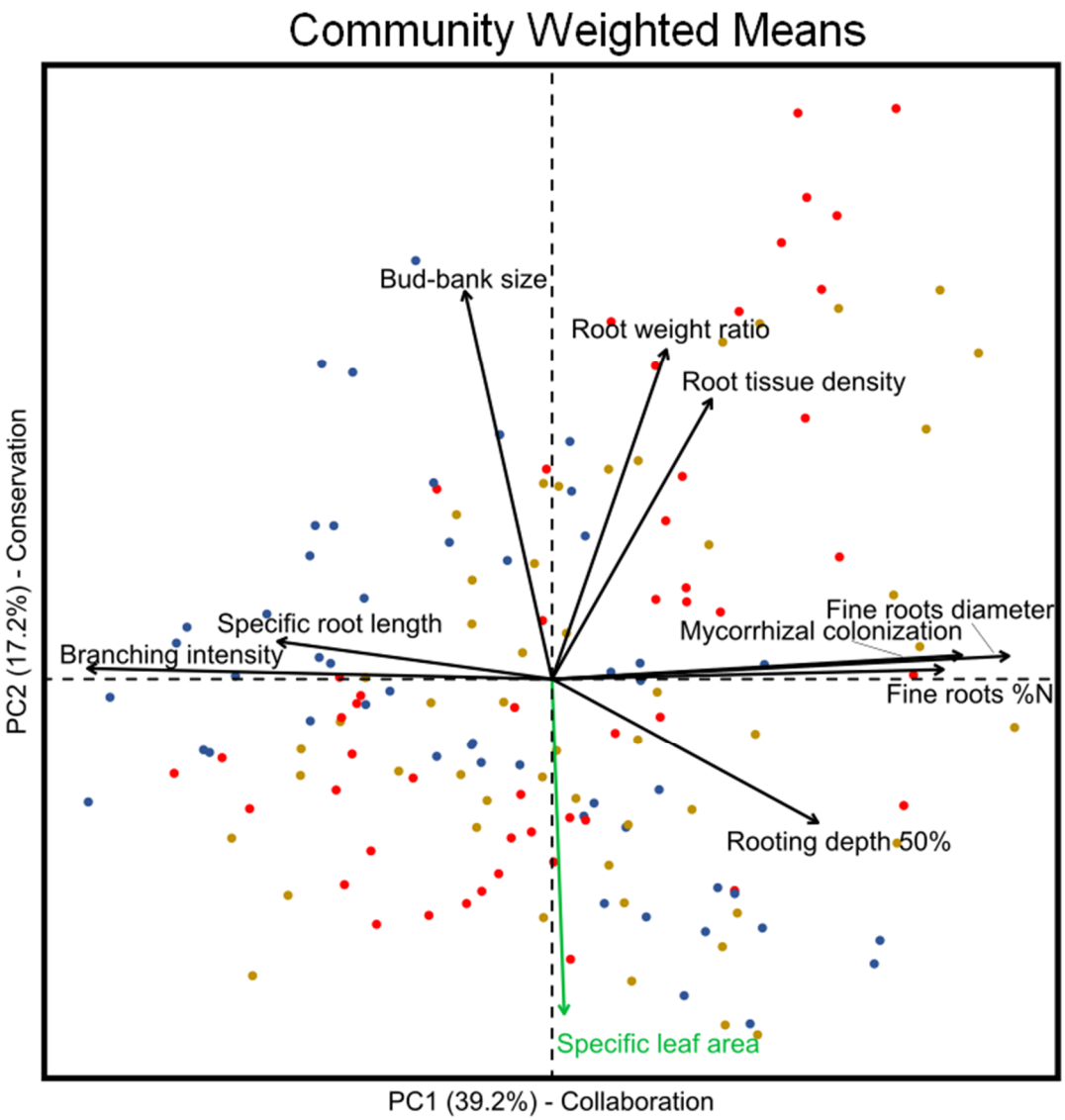
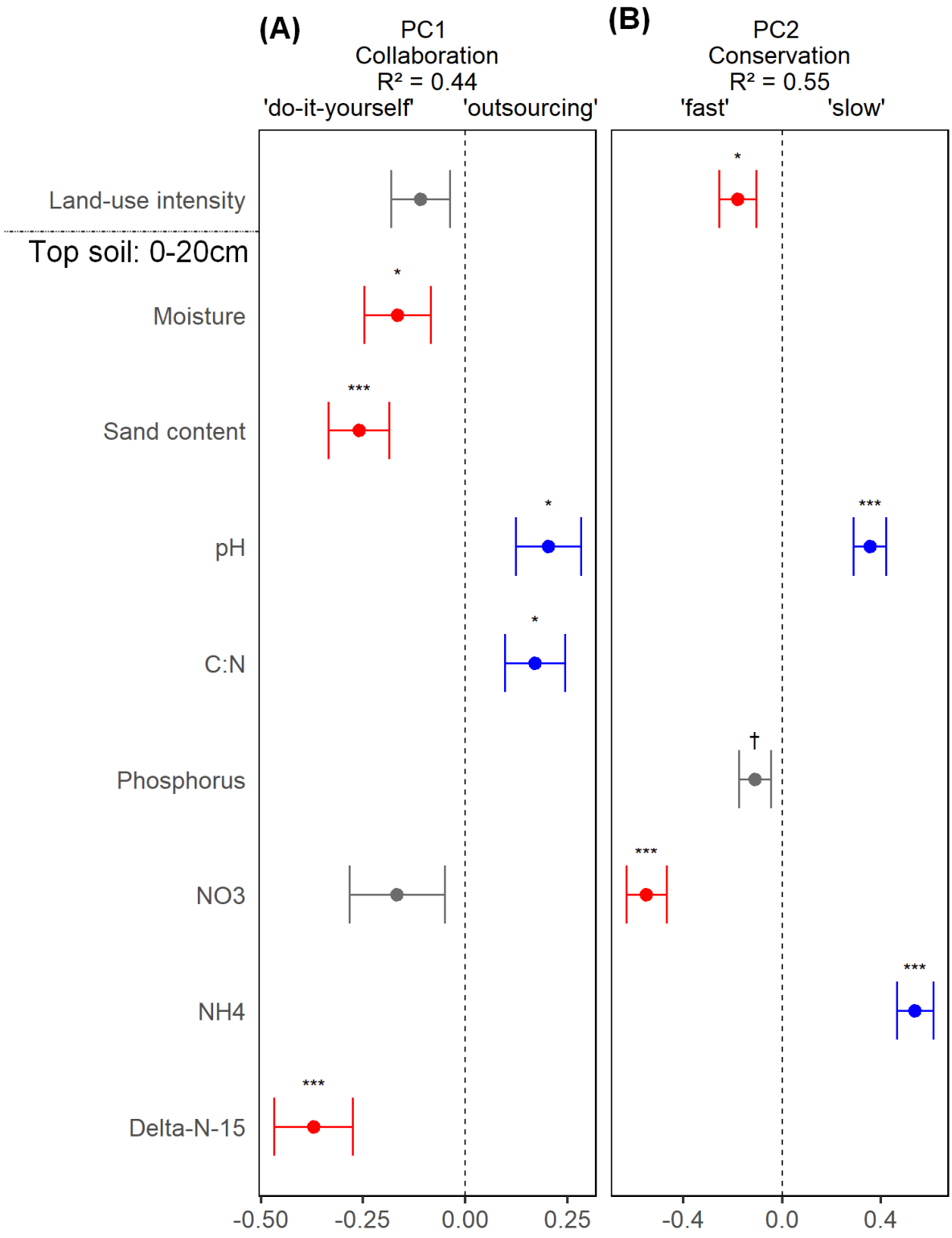

Community Weighted Means
Bud-bank size
Root weight ratio
Root tissue density
Specific root length
Branching intensity
Mycorrhizal colonization
Fine roots diameter
Fine roots %N
Rooting depth 50%
Specific leaf area
PC2 (17.2%) - Conservation
PC1 (39.2%) - Collaboration

Figure 3 Estimates from linear models of the effects of environmental variables on PCA scores for (A) PC1 -'Collaboration' gradient and (B) PC2 - 'Conservation' gradient from the Above-Belowground PCA on community weighted means of traits. On the y-axis are the nine environmental variables that were retained in the most parsimonious models (Region and N:P were not retained). The error bars around the estimates are standard errors. Significant (* for $p < 0.05$; ** for $p < 0.01$; *** for $p < 0.001$) negative and positive estimates are marked in red and blue, respectively. Non-significant ($p > 0.05$) estimates are marked in grey. Marginally significant ($p < 0.10$) estimates are marked with †.


(A)
PC1
Collaboration
R² = 0.44
'do-it-yourself'
'outsourcing'
(B)
PC2
Conservation
R² = 0.55
'fast'
'slow'
Land-use intensity
Top soil: 0-20cm
Moisture
Sand content
pH
C:N
Phosphorus
NO3
NH4
Delta-N-15
*
***
*
*
†
***
***
***
-0.50
-0.25
0.00
0.25
-0.4
0.0
0.4

Discussion

We investigated belowground trait dimensionality of grassland communities and found that a 'collaboration' and a 'conservation' gradient (*sensu* Bergmann et al. 2020) explained most of the variation in community-weighted means of belowground traits. Three traits that were not considered previously in the belowground trait space were largely part of these two dimensions. $_{CWM}$*Rooting depth 50%* was associated with the 'outsourcing' and 'fast' strategies, $_{CWM}$*Branching intensity* with the 'do-it-yourself' strategy, and $_{CWM}$*Bud-bank size* with the 'slow' strategy. $_{CWM}$*Fine root %N* was surprisingly associated with the 'outsourcing' strategy. Both gradients responded to environmental variables related to soil conditions, and more fertile soils where generally associated with the 'fast' and the 'do-it-yourself" strategies. In line with this, we also found that land-use intensity was associated with the 'fast' strategy and tended to be associated with the 'do-it-yourself' strategy.

Traits relationships and dimensionality of belowground traits

For the grasslands in our study, variation in community weighed means of belowground traits tended to separate along two dimensions that largely correspond to the two ecological root-trait gradients recently identified for species. PC1 related to the collaboration of plants with mycorrhizal fungi. This 'collaboration' gradient (Bergmann *et al.* 2020) ranged from 'outsourcing' communities with a high mycorrhizal colonization rate and thick roots but also, surprisingly, high root nitrogen content, to 'do-it-yourself' communities with high specific root length and a high root branching intensity. PC2 related to the construction cost of roots and leaves and the vegetative regeneration potential. This 'conservation' gradient ranged from 'slow' communities with high root tissue density, high root weight ratio and large bud-banks to 'fast' communities with high specific leaf area. $_{CWM}$*Rooting depth 50%* relates to both of these PCs, with deep-rooting communities being 'outsourcing' and 'fast'. To maximize the loadings of the traits onto one of the PC axes, we used an 'oblimin' rotation, and as a

consequence the PCs are not orthogonal to each other. This also shows that the two plant-strategy gradients(i.e. PC1 and PC2) were not entirely independent, as the 'fast' strategy and 'do-it-yourself' strategy partly coincide, both with PC1 and PC2 scores, and with the traits that are associated with each strategy (Appendix S10).

At the community level, belowground trait relationships can differ from the one found at the inter-specific level (Craine *et al.* 2001; Delpiano *et al.* 2020; Erktan *et al.* 2018; Prieto *et al.* 2015; Roumet *et al.* 2006; Schroeder-Georgi *et al.* 2016; Zhou *et al.* 2018). The trait clustering we found for CWMs was generally in accordance with previous findings of trait clustering among species, both for trees and herbaceous plants (Bergmann *et al.* 2020; Kramer-Walter *et al.* 2016; Weemstra *et al.* 2016). The main exception was $_{CWM}$*Fine roots %N*, which in our study associated with the 'outsourcing' side of the collaboration gradient instead of the 'fast' side of the conservation gradient. This might be particular to our study using CWMs, as root nitrogen content relates to both the 'fast' and the 'outsourcing' strategies when we do the PCA at the species level (Appendix S16a), but only with the 'outsourcing' strategy when scaling up to the community level (Fig. 2). It could be that the actual trait values of the plants in the field sites vary and deviate from the ones that we calculated from traits of plants grown in pots under common environmental conditions. Indeed, trait-performance relationships have already been shown to differ between common garden and field condition in the Biodiversity Exploratories grasslands (Breitschwerdt *et al.* 2019). The differences in relationships among species traits and among community weighted means of traits could thus reflect the multiple constraints exerted by environmental filtering on the trait values selected in a field context. More effort will be required to disentangle filtering effects and phenotypic changes when traits are assessed in controlled versus field conditions.

We found that communities with large bud-banks were on the 'slow' side of the 'conservation' gradient. Previously, bud-bank size was shown to be rather independent of the plant economics

spectrum, as specific leaf area — a key trait in this spectrum — explained less than 2% of variation in bud-bank size among 1359 herbaceous species (Klimešová *et al.* 2016). In our study, the correlation between bud-bank size and specific leaf area of species mean values was significantly negative (-0.17, $p < 0.01$; Appendix S16b), though still weaker than between the corresponding CWMs (-0.34; Appendix S10). Because all of our species were selected based on their presence in permanent grasslands, it could be that the association between bud-bank size and 'conservation' traits is a feature of this specific habitat. Nevertheless, inclusion of other traits linked to clonality, like clonal lateral spread, shoot persistence or bud-bank depth could reveal specific clonal strategies (Herben & Klimešová 2020), potentially increasing the dimensionality of belowground trait space (Ladouceur *et al.* 2019). The smaller bud-bank size we observed in communities with the 'fast' strategy, typical for resource rich grasslands, where competition for light might be more intense (Hautier *et al.* 2009) could indicate that those plants invest more in immediate aboveground light-harvesting structures at a cost of future regrowth ability. In line with this we also found that low root weight ratios are indicative of 'fast' communities.

Variation in community-trait dimensions explained by the environment

The 'conservation' and 'collaboration' gradients were associated with several environmental variables, partly in overlapping and partly in unique ways. About half of the variation in PC1 and PC2 scores was explained by environmental variables. Along the 'collaboration' gradient, the 'outsourcing' strategy was found on dry, non-acidic soils with a low sand content and low N availability (i.e. high C:N, low delta-N-15 and marginally low NO_3), and tended to be associated with a low land-use intensity (although not significantly). Along the 'conservation' gradient, the 'slow' strategy was found on non-acidic soils with low P and NO_3 but high NH_4 availabilities, and in sites with low land-use intensities. Hence, although the 'outsourcing' and 'slow' strategies correspond to two different plant-strategy gradients, they both tend to be

associated with less fertile sites with low land-use intensities.

The relationships we found between the 'collaboration' gradient and environmental variables are generally in accordance with the current knowledge in mycorrhizal ecology. For example, (Hempel *et al.* 2013) found, that among 1752 species of the German flora, obligatorily mycorrhizal species tended to be positively associated with dry, non-acidic, infertile habitats. In line with this, we found that drier top soils were associated with deeper rooting, more mycorrhizal-associating communities (Fig. 3, Appendix S14). While mycorrhiza have a well-known positive effect under water limited conditions (Augé 2001), deeper roots allow the uptake of water from deeper soil layers (Fan *et al.* 2017). We found that 'outsourcing' communities were also linked with lower delta-15N isotopic ratios of the soil. The value of delta-15-N in the soil is the result of multiple processes implicated in the nitrogen cycle (Robinson 2001). In the grassland plots of the Biodiversity Exploratories, a high delta-15-N has been linked to higher plant productivity and lower species richness, potentially indicating the dominance of one form of nitrogen which is taken up by species with a 'fast' strategy (Kleinebecker *et al.* 2014). Indeed, delta-15-N is strongly positively correlated with NO_3 concentration in the soil and moderately with NH_4 concentration, moisture and land-use intensity (Appendix S11). If interpreted as an indicator of more plant-available nitrogen, the negative relationship between delta-15-N and the 'outsourcing' strategy is in line with the finding of reduced mycorrhizal colonization in response to nitrogen addition (Ma *et al.* 2020) and with our finding that 'outsourcing' communities tend to be on the 'slow' side of the 'conservation' gradient.

As arbuscular mycorrhizal fungi are known to help plants with the uptake of phosphorus, we expected that communities on soils with low phosphorus content would score high on the 'collaboration' gradient. Nitrogen addition generally decreases the degree of mycorrhizal colonization in conditions of high P availability and increases it under low P availability at the

plot level (Ma *et al.* 2020). Arbuscular mycorrhizal fungi could also help with nitrogen uptake in conditions of high phosphorus concentrations, with a possible negative relationship between N:P and mycorrhizal colonization rates (Blanke *et al.* 2005; Blanke *et al.* 2011). Soils depleted in P or in which P is not plant-available are also selecting for root systems with little reliance on mycorrhiza, for example by having cluster roots and carboxylate exudation to mobilize inorganic phosphorus (Lambers *et al.* 2012). Reliance on mycorrhiza could depend on how much phosphorus is available, but also on the balance between phosphorus and nitrogen (i.e. the N:P ratio). Neither the anion-exchange-resin data we used as our indicator of soil phosphorus availability nor the N:P ratio related to the 'collaboration' gradient (though N:P is marginally positively associated with $_{CWM}$*Mycorrhizal colonization*, Appendix S14). However, low resin-phosphorus was marginally related to the 'slow' side of the 'conservation' gradient. The nature of plant-available phosphorus in soil is still debated (Barrow 2021). Phosphorus is also more available in slightly acidic soils (Alt *et al.* 2011). The pH of our soils ranged from acidic to slightly alkaline (min. 4.5, max 7.5), with a mean of 6.5, and only 37 out of 150 plots have a pH between 6.5 and 7, which is often used as an optimum pH to assess phosphorus availability (Penn & Camberato 2019). So, the positive effect of pH on the 'collaboration' gradient could indicate a lower P availability at high pH values. Furthermore, organic fertiliser, which is applied in many of the plots with high land-use intensity, is usually rich in P, and our land-use-intensity variable thus could capture part of the P supply that is not captured by resin-P (r=0.49 between resin-P and land-use intensity, Appendix S11). In conclusion, we did not find a decrease of mycorrhizal colonization when P is more available through the effects of resin-P on 'collaboration' gradient (Fig. 3), but we cannot rule out an effect of P availability, because of the potential changes in phosphorus availability through fertilisation and pH changes.

The relationships we found between the 'conservation' gradient and environmental variables

are overall in line with expectations about how soil fertility should relate to the plant economic spectrum. Accordingly, high land-use-intensity and acidic soils with high phosphorus and nitrate concentrations were associated with the 'fast' strategy. The decrease in bud-bank size at higher soil fertility (Fig. 3, Appendix S14) is congruent with recent findings that land-use intensity and nitrogen addition decrease total bud density and rhizome biomass in temperate perennial grasslands (Ottaviani *et al.* 2021; Qian *et al.* 2021). Disturbance and habitat-productivity indices of species have been associated with an increase in specific leaf area and a decrease in bud-bank size (Herben *et al.* 2018). In contrast to the negative effect of nitrate concentration on the 'conservation' gradient, we found a positive effect of soil ammonium concentration. Species preferences for specific nitrogen forms vary with ecological strategies. Early successional species, which are usually on the 'fast' side of the 'conservation' gradient, generally prefer nitrate, whereas late successional species, which are usually on the 'slow' side, generally prefer ammonium (Britto & Kronzucker 2002; Warren 2009). It has also been shown that there might be a trade-off between nitrate and ammonium uptake in grassland species (Maire *et al.* 2009). Some plants can also inhibit nitrification, thereby retaining NH_4, which is less prone to leaching than NO_3 (Boudsocq *et al.* 2012). High rates of nitrification in fertile soil with high microbial activity could lead to a stronger dominance of nitrogen in form of nitrate. Increase in ammonia oxidation with land-use intensity (and therefore fertilisation) has already been shown in our grassland system (Stempfhuber *et al.* 2014). So, the positive association between 'fast' communities and NO_3 could reflect an overall higher nitrifying activity of microbial communities in fertile, nitrogen-rich soils. In conclusion, the form of nitrogen available in the soil has contrasting effects on belowground traits, with ammonium more related to the 'slow' strategy and nitrate more related to the 'fast' strategy.

Conclusions

The dimensionality of trait syndromes and their associations with environmental variables are

central questions in ecology. We found an integration at the grassland community level of root branching intensity, root-weight ratio, bud-bank size and rooting depth within the bidimensional 'conservation' and 'collaboration' trait space previously observed at the species level. The variation of the 'conservation' and 'collaboration' gradients with environmental variables was partly overlapping, partly independent. Indicators of soil fertility were generally associated with both the 'fast' and the 'do-it-yourself' strategies.

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Data availability

The trait data, owned by the authors and supporting the paper will be archived in Dryad, and the data DOI will be included at the end of the article. The environmental variable data is partly

publicly available on BExIS and partly under an embargo period of three years. It can be requested directly to the authors of the data.

Supporting Information

Appendix S1 Extended methods for the four experiments on root-trait measurements.

Experiment on root-system morphology

From May 1 to October 6, 2017, we performed a glasshouse experiment to measure root-system morphological traits of the study species. As root morphology might depend on nutrient availability, we grew half of the plants per species at an intermediate nutrient level and the other half at a high nutrient level. As plasticity was not of interest for this study, we averaged the trait values per species. Because of the large number of species and the time-consuming measurements, we grew the plants in four temporally shifted (4-6 weeks) batches. We aimed to have each species represented in each batch, and to have a total of seven replicates per species and nutrient level across all batches. Out of the 291 species sown, the seedlings of the species that had germinated (2659 seedlings of 216 species) were transplanted individually into plastic pots (1.3 L) filled with a mixture of sand and vermiculite (1:1 volume ratio). The pots were then randomly allocated to positions in two glasshouse compartments, and allowed to grow for four weeks (night/day 10/14 h; 22/28 ± 1.5 °C; relative humidity 80 ± 15%). Plants were fertilized three times a week with either a low nutrient solution (40 mL with 1500 µM KNO3) or a high nutrient solution (40 mL with 12000 µM KNO3). The fertilizer was a modified version of the Hoagland recipe (see online). We grew the plants for four weeks only to avoid roots becoming pot-bound, and to be able to analyze the entire root systems. After carefully washing off the substrate, each root system was cut below the collar and stored separately for <1 week in a plastic tube filled with distilled water at 4°C. Then, root systems were spread individually in a thin layer of water in transparent trays (11 cm × 11 cm) and scanned at 800 dpi with a flatbed scanner modified for root scanning (Epson Expression 10000 XL and 11000 XL). The images were analyzed using the software WinRHIZO™ 2017a (Regent Instruments, Quebec, Canada) to obtain the total root length and root volume. Root

systems were then oven-dried for >48 hours at 65 °C and weighed. We calculated specific root length by dividing the total root length by the belowground dry biomass, and root tissue density by dividing the belowground dry biomass by the sum of the root volumes according to Rose 2017). We estimated branching intensity by dividing the total number of root tips by the total length of the root system. The diameter of fine roots (i.e. distal roots), thought to be the most important roots for nutrient uptake (Freschet & Roumet 2017) was determined by randomly sampling a distal root branch (or a portion of it) for each root system and calculating the mean of the diameter of the external-internal link obtained with the "Link analysis" function in WinRHIZO. This subsampling excluded the thicker transport roots and allowed to obtain a mean value mostly measured on first order roots. We also dried and weighed the aboveground biomass of each plant, and calculated the root weight ratio (i.e. root biomass divided by total plant biomass).

Experiment on root nitrogen content (Fine roots %N)

From 15 January to 8 June 2018, we performed a glasshouse experiment to measure root nitrogen content and ammonium and nitrate uptake rate of the study species. Because of the large number of species and the time-consuming measurements, we grew the plants in three temporally shifted (4–6 weeks) batches. We aimed to have each species represented in each batch, and to have a total of six replicates per species and for each of the three modalities of the N source treatment across all batches. The 2007 seedlings of the species that had germinated (n = 196) were transplanted individually into plastic pots (2 L) filled with a mixture of sand and vermiculite (1:1 volume ratio). The pots were then randomly allocated to positions in two glasshouse compartments, and allowed to grow for six weeks (night/day 10/14 h; 22/28 ± 1.5°C; relative humidity 80 ± 15%). Plants were fertilized three times a week with an intermediate nutrient solution (40 mL with 6000 µM KNO3). The fertilizer was a modified version of the Hoagland recipe (see online). We grew the plants for six weeks to get enough

root material for the nitrogen content and uptake analysis. After washing off the substrate between 7.30 and 8.00 am, the root systems were split into three bundles of fine roots. Each bundle was immersed for 120 minutes between 8.00 and 12.00 am in an 8 mL tube containing 4 mL of the following nutrient solutions, which differ only in the form of label used: 1) ^{15}N-NO3 labeled 2) ^{15}N-NH4 labeled or 3) control solution without ^{15}N-label (see online). After 120 minutes of incubation, immersed fine root bundles were cut off, washed twice with 0.5µM CaCl2, dried with cellulose paper, and stored at −20°C until their fresh weight determination. They were then oven-dried for >48 h at 65°C, weighed, and finely ground. Fine root materials (0.5–1.5mg aliquots) were processed in an Elemental Analyser (Elementar, Analysensysteme, Germany) to determine total nitrogen content (Fine roots %N).

Experiment on mycorrhizal colonization rate

All data on mycorrhizal colonization originate from a pot experiment that took place in a controlled greenhouse in Berlin in 2018 (16 h daylight at 22°C, 8 h night at 15 °C). The study included a larger species set but only 75 of them were part of the vegetation in our grasslands. 8 replicates were set up per species distributed over 4 time blocks. Dead replicates were substituted in the following time block if necessary. Seeds were surface sterilized once before the experiment (3 min in 7% bleach, washing in de-ionized (DI) water), dried at 20 °C and stored for 2-12 weeks until sowing. Germination took place in plastic boxes on 1:1 steamed sand and vermiculite (1-3 mm, ISOLA Vermiculite GmbH; Sprockhövel, Germany). Seeds were sown consecutively based on pre-findings to assure that seedlings were between the cotyledon stage and the stage of first leaves at time of transplanting. The actual experiment took place in plastic cones (410 mL) filled with the same substrate as for germination.

Cones first got filled to c. ¾. Subsequently we added a 30 mL horizon of a 1:1 mixture of steamed sand and mycorrhizal inoculum in 1-2 mm vermiculite (INOQ Agri, Inoq GmbH, Schnega, Germany). According to supplier information, the inoculum contains 145 spores/mL

of *Rhizophagus irregularis*, a mycorrhizal fungus commonly used in agricultural and scientific approaches in temperate habitats (Lenoir et al. 2016). The horizons were further covered with ~30 mL substrate. Seedlings received 30 mL of DI water during transplantation. We allowed dead seedlings to be replaced during the first week. Plants grew for 6 weeks within each time block. At time of transplanting all pots were fully randomized and got rearranged every two weeks. Plants received 25 mL of DI water 3 times a week, plants were watered with 25 mL of DI water while instead 25 mL of a ¼ strength Hoagland solution was applied two weeks and four weeks after transplanting. At time of harvest, roots were carefully washed by hand and kept in water at 4°C for less than a week before they got processed and dried at 60°C for three days. For the determination of the mycorrhizal colonization, we used representative subsamples of the total root system of three random replicates per species. Dry roots were cut into small pieces and cleared in 10% KOH for 15 min at 80°C followed by staining in 0.05% Trypan Blue in Lactoglycerol for another 15 min at 80°C. We determined mycorrhizal colonization with the 200× magnified intersection method (McGonigle *et al.*, 1990). Each slide contained a minimum of 30 root pieces to count presence or absence of mycorrhizal structures in 50-100 intersects. Mycorrhizal colonization rates of up to 86% confirmed a successful inoculation.

Experiment on rooting depth

From the 15th of May to the 10th of October 2018, we performed an outdoor pot experiment to measure the maximum rooting depth of the species. Up to five seedlings of the species that had germinated (N=183 out of the 291 species sown), totaling 752 plants, were transplanted individually into 120 cm high plastic tree shelter tubes (Tubex ® Standard Plus, http://www.tubex.com/products/tree-shelters/tubex-standard-treeshelters/specification.php), which are normally used in forestry to protect young trees against animals and the elements. We closed the bottoms of these tubes with thick pieces of cotton tissue to be able to use them

as pots. The tubes were filled with a mixture of sand and vermiculite (1:1 volume ratio) up to a height of 115 cm. This substrate can be easily penetrated by the roots, and therefore allows each species to reach its maximum rooting depth quickly. The tree shelter tubes were delivered in packages of five tubes stacked into one another, and they, therefore, came in five diameter classes (8.0, 8.4, 10.0, 10.8, and 12.0 cm). To avoid that tube diameter would be confounded with species identity, each of the five seedlings per species was planted in a different tube-diameter class. We placed the tubes upright in a randomized design in the Botanical Garden of the University of Konstanz (47° 41' 24.0" N 9° 10' 48.0").

We planted 752 plants but, due to early mortality, we had to replace 126 of them within the next three weeks. The growth period, therefore, ranged from 16 to 19 weeks. The experiment took place during the summer of 2018 (mean temperature: 19.5 °C, min/max 2.5/37.4 °C; relative humidity: mean 74%, min/max 22.7/100%). All the plants were fertilized once a week with 60 mL of a standard nutrient solution (1‰ Universol® Blue, Nordhorn, Germany), and watered regularly from above. We harvested the plants in October 2018. Each tube was carefully sliced open, and we measured the distance from the top of the substrate to the deepest root. During the harvest of each plant root system, four slices of different depths (0-15 cm; 15-30 cm; 30-60 cm; 60-115 cm) were performed, and the root biomass contained in each slice was separately weighted. To adjust for individual plant maximum rooting depth, the depth of the last slice was stopped at the deepest observed root. For example, if the maximum rooting depth of a plant was 50 cm, then the corresponding last slice of the plant was assigned to the depth of 30-50 cm. Then for each plant, a model of exponential decrease of biomass with depth was fitted (Schenk & Jackson 2002). As the whole root system was sampled, we could interpolate the depth at which 50% of the total root system biomass was located. Compared to the maximum rooting depth, which is the distance from the top of the substrate to the deepest root, the rooting depth 50% takes into account the difference in biomass investment at different

depths. This should better capture the depth at which most water and nutrient uptake take place. The two metrics are however strongly correlated (Pearson's r = 0.78).

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Appendix S2 Information about the environmental variables of the Biodiversity Exploratories grassland plots used in the linear regression models presented in Fig. 3, Appendix S8, S12, S13, and S14. The data were collected in the period 2008-2019, corresponding to the period covered by the CWMs calculation from vegetation surveys. The datasets are present in the BExIS database https://www.bexis.uni-jena.de/ (http://doi.org/10.17616/R32P9Q). Some of the datasets are already public and some are still under the three years embargo period. A description of the metadata provided by their authors of each dataset is available below. The land-use-intensity (LUI) was calculated as global mean of grassland management for the three regions overall according to (Blüthgen *et al.* 2012), based on information from the land owners on mowing, grazing and fertilization (Vogt *et al.* 2019) using the LUI calculation tool (Ostrowski *et al.* 2020). It was visually assessed that most of the variation in soil variables for which several years of data are available comes from differences among plots rather than differences over time. Soil depth and bulk density were not used directly in our models and are indicated in grey. Soil-depth measurements were performed in only 144 plots and were constrained to a maximum depth of 100 cm, leading to underestimation of its variance and considerable bias in the estimation of its true value in the Schorfheide region. Bulk density was related to many other soil variables and was used to convert the soil variables originally expressed per unit of soil mass to the following units of volume: sand content and phosphorus in g/m3 and mg/m3 of soil respectively, C:N, N:P, NH4, NO3, delta-N-15 per cm3 of soil instead of per g of soil.

						Mean +/- standard error		
Variable	**Dataset(s) ID on BExIS**	**Year(s)**	**Soil depth**	**Original unit**	**Used unit**	**Alb**	**Hainich**	**Schorfheide**
Land use intensity	25086 with LUI tool	2008-2019		unitless	unitless	1.73 +/- 0.67	1.65 +/- 0.61	1.55 +/- 0.32
Moisture	19007 with Climate tool	2008-2019	10 cm	% volumetric	% volumetric	34.00 +/- 4.60	31.13 +/- 3.12	29.05 +/- 9.98
Sand content	14686	2011	0-10 cm	g/kg of soil	g/m^3 of soil	72.38 +/- 58.72	68.45 +/- 28.36	509.08 +/- 201.74
pH	14447	2011	0-10 cm	unitless	unitless	6.26 +/- 0.55	6.89 +/- 0.51	6.39 +/- 0.91
C:N	18787 ; 23846	2014; 2017	0-10 cm	unitless	unitless	12.06 +/- 1.74	11.25 +/- 1.51	15.31 +/- 4.66
Phosphorus	19009 ; 20447	2011; 2015	~10-20 cm	mg/kg	mg/m^3	0.31 +/- 1.39	-0.12 +/- 0.77	-0.45 +/- 0.73
N:P	18787 ; 23846 ; 19009 ; 20447	2011; 2014; 2015; 2017	0-20 cm	unitless	unitless	4.81 +/- 1.85	4.05 +/- 1.22	6.75 +/- 4.67
NH4	20248 ; 20249	2011; 2014	0-10 cm	µg N/g soil DM	µg N/cm^3 soil DM	23.76 +/- 10.11	14.94 +/- 6.54	25.98 +/- 28.23
NO3	20248 ; 20249	2011; 2014	0-10 cm	µg N/g soil DM	µg N/cm^3 soil DM	14.03 +/- 13.10	13.77 +/- 9.43	29.05 +/- 34.22
delta-N-15	15306	2011	0-10 cm	unitless	unitless	4.43 +/- 1.53	4.08 +/- 1.26	5.12 +/- 2.18
Soil depth	5340	2009	0-100 cm	cm	cm	29.00 +/- 2.00	47.00 +/- 2.60	99.00 +/- 0.60
Bulk density	20248 ; 20249	2011 ; 2014	0-10 cm	g/cm3	g/cm3	0.81 +/- 0.11	0.85 +/- 0.09	0.92 +/- 0.41

Dataset ID in BExIS	Used for variable(s)	Description provided by authors in metadata as of 25 February 2021
25086	Land use intensity	The data derive from calculated values of the land use survey (annual interviews with all grassland land owners / users of the three Exploratories -basic Dataset 23746). These data are the land management components (mowing, grazing, fertilization) for the LUI Calculation Tool. Calibration: Livestock unit conversion factor: cattle: <0.5 years = 0.3, 0.5-2y =0.6, >2y = 1 sheep/goats: <1y = 0.05, >1y = 0.1 ponies/small horses: 0.7 horses: <3y = 0.7, > 3y = 1.1 Conversion factor for total Nitrogen input: Manure [t/ha] with conversion factor [kg/t]: cattle: 5.6, horse: 4.9, sheep: 8.13 Slurry [m³/ha] with conversion factor [kg/m³]: cattle: 3.85, pig 5.4, mixed: 4.45, biogas/digestate: 4.4 Procedure: Formulae calculated per year and plot: mowing = number of cuts grazing = livestock units*days/ha summed up for all grazing periods livestock units consist of the number of livestock multiplied by a conversion factor, which is species and age dependent. Check detailed information above in "calibration" fertilization = amount of applied nitrogen within the fertilizer per ha. The nitrogen content depends on the type of fertilizer. For further information see above in "calibration"
19007	Moisture	NEW Climate Data Set - Time Series Web Interface - a new climate data base system, includes all raw values from Advanced environmental monitoring units (AEMUs) + enhanced EMUs (EEMUs) + core EMUs (CEMU)+ Tower - Automated environmental monitoring by including air pressure, wind, precipitation and radiation at 10 min resolution - aggregated to 1hour + 1day + 1week + 1month + 1year. Instruments: ADL-MX Datalogger System; DeltaT ML2X Soil Humidity Probe; MNT FExtension 2010 Soil and Ground Surface Temperature Sensor; MELA KPC1/5-ME & #IAK1.00.F137.520.CS8 Humidity and Air Temperature Sensor; OTT Pluvio2; T.Friedrich Wind sensors

		4035+4123; THIES Barometric pressure sensor 3.1157.10.000; Kipp & Zonen CNR4 Net Radiometer; Procedure: quality 0 - no quality check quality 1 - just physical range check quality 2 - physical range + step range check quality 3 - physical range + step + empirical check
14686	Sand content	The core project 'Soil' provides essential information on soil properties and ecosystem functions across all 300 Experimental Plots (EPs) of the Biodiversity Exploratories. Soil texture is one of the most fundamental properties of a soil. It is a major control on the effective surface area, pore size distribution, water and nutrient holding capacity of a soil. Soil texture focuses on the soil particles that are less than 2 mm in diameter. It indicates the relative content of sand (2-0.063 mm), silt (0.063-0.002 mm) and clay (<0.002 mm). Size separation of particles is based on sieving (particles >0.063 mm) and on the principle that a solution settles out at a rate that depends on the size of the particles. The larger the particle size, the faster particles settle. The settling rate is given by Stokes Law. Soil texture tends to be relatively stable over time since weathering only very slowly changes particle size distribution in soil. Texture analysis consists of three main steps: (i) distruction of soil organic matter with hydrogen peroxide, (ii) dispersion of soil aggregates into discrete units, and (iii) separation of soil particles of different size by sieving and sedimentation (DIN-ISO 11277). Instruments: 0.63, 0,2, 0.063 mm sieves for isolation of sand fractions (Retsch, Haan, Germany) Instrumentation for pipette method including Atterberg cylinders to separate silt and clay fractions Calibration: Reference soil of VDLUFA/LUFA for quality control (LUFA, Speyer,

		Germany) Procedure: In each of the 300 experimental plots of the biodiversity exploratories we collected 14 soil cores with a split tube sampler (diameter of 5 cm) along two 20 m transects in grasslands and 40 m transects in forests in May 2011. Organic layers in forests and aboveground plant parts in grasslands were removed before coring. We then prepared a composite sample from the 14 soil cores by mixing the upper 10 cm of the soil. A subsample of the composite sample was sieved to <2 mm, dried and subsequently used for soil texture analysis.
14447	pH	The core project 'Soil' provides essential information on soil properties and ecosystem functions across all 300 Experimental Plots (EPs) of the Biodiversity Exploratories. Soil pH is considered a master variable in soil science as it affects many chemical processes in soils. Most importantly, the plant nutrient availability is greatly affected by the soil pH as it controlls the chemical forms of the different nutrients and influences the chemical reactions they undergo. The acidity or alkalinity of soil is measured in terms of pH. It is a measurement of the hydronium ion activity in a soil suspension. pH is defined as the negative logarithm (base 10) of the activity of hydronium ions. Acid soils have a pH <7 and alkaline soils have a pH >7. The soil pH was measured in a weak (0.01 M) calcium chloride solution using a pH meter. Instruments: WTW pH meter 538 (WTW, Weilheim, Germany) WTW pH glass electrode SenTix 61 (WTW, Weilheim, Germany) Calibration: The pH meter was calibrated using buffer solutions with a pH of 4 and 7. Procedure: In each of the 300 experimental plots of the biodiversity exploratories we

		collected 14 soil cores with a split tube sampler (diameter of 5 cm) along two 20 m transects in grasslands and 40 m transects in forests in May 2011. Organic layers in forests and aboveground plant parts in grasslands were removed before coring. We then prepared a composite sample from the 14 soil cores by mixing the upper 10 cm of the soil. Soil samples were sieved to <2 mm and air-dried. Subsequently, 10 g of sieved and air-dried soil were mixed with 25 mL 0.01 M CaCl2 solution and shook for 2 hours. Afterwards the pH of the soil suspension was measured using a glass electrode. The pH of each sample was measured twice (pH 1 and pH 2).
18787	C:N ; N:P	The core project 'Soil' provides essential information on soil properties and ecosystem functions across all 300 Experimental Plots (EPs) of the Biodiversity Exploratories. Soil organic carbon and nitrogen concentrations are master variables in soil science. Soil organic carbon and nitrogen are the measureable components of soil organic matter (SOM). SOM exerts positive effects on numerous physical, chemical and biological soil properties. An increase in SOM generally results in an improved soil structure, soil moisture retention, soil buffering capacity, retention of pollutants, cycling and storage of plant nutrients and biodiversity. SOM also acts as a sink and source for atmospheric carbon dioxide. Carbon in soil is present in two forms: organic carbon (OC) and inorganic carbon (IC). In soils, residues of animals, plants, or microorganisms in various stages of decomposition are considered to form the OC. Calcite and dolomite, are the predominant forms of IC in soils. Carbonates have a great influence on soils because of their alkalinity and their pH-buffering properties. Nitrogen also occurs as organic and inorganic N in soils. With the CN analysis, however, only the total N is determined. The C:N ratio in soil is calculated as the mass of organic carbon to the mass of total nitrogen. The C:N ratio is as well-established indicator for the nitrogen availability in soil. Total C (TC) and total N (TN) concentrations in soil were determined using an elemental

		analyser. After removal of organic carbon (OC) by ignition of soil samples at 450 °C for 16 h, inorganic C (IC) was determined with the same elemental analyzer. OC concentrations were calculated as the difference between TC and IC. Instruments: Elemental Analyser (VarioMax, Elementar, Hanau, Germany) Calibration: The C and N analyser was calibrated with glutamic acid. Procedure: In each of the 300 experimental plots of the biodiversity exploratories we collected 14 soil cores with a split tube sampler (diameter of 5 cm) along two 20 m transects in grasslands and 40 m transects in forests in May 2014. Organic layers in forests and aboveground plant parts in grasslands were removed before coring. We then prepared a composite sample from the 14 soil cores by mixing the upper 10 cm of the soil. Soil samples were sieved to <2 mm and air-dried. Subsequently, subsamples of sieved soil were ground in a ball mill. Total C and N concentrations were determined by dry combustion in an elemental analyser at a temperature of 1100°C. The evolving CO2 and N2 was determined with a Thermal Conductivity Detector (TCD).
23846	C:N ; N:P	The core project 'Soil' provides essential information on soil properties and ecosystem functions across all 300 Experimental Plots (EPs) of the Biodiversity Exploratories. Soil organic carbon and nitrogen concentrations are master variables in soil science. Soil organic carbon and nitrogen are the measureable components of soil organic matter (SOM). SOM exerts positive effects on numerous physical, chemical and biological soil properties. An increase in SOM generally results in an improved soil structure, soil moisture retention, soil buffering capacity, retention of pollutants, cycling and storage of plant nutrients and biodiversity. SOM also acts as a sink and source for atmospheric carbon dioxide. Carbon in soil is present in two forms: organic carbon (OC) and inorganic carbon (IC). In soils, residues of animals, plants, or microorganisms in various stages of decomposition

		are considered to form the OC. Calcite and dolomite, are the predominant forms of IC in soils. Carbonates have a great influence on soils because of their alkalinity and their pH-buffering properties. Nitrogen also occurs as organic and inorganic N in soils. With the CN analysis, however, only the total N is determined. The C:N ratio in soil is calculated as the mass of organic carbon to the mass of total nitrogen. The C:N ratio is as well-established indicator for the nitrogen availability in soil. Total C (TC) and total N (TN) concentrations in soil were determined using an elemental analyser. After removal of organic carbon (OC) by ignition of soil samples at 450 °C for 16 h, inorganic C (IC) was determined with the same elemental analyzer. OC concentrations were calculated as the difference between TC and IC. Instruments: Elemental Analyser (VarioMax, Elementar, Hanau, Germany) Calibration: The C and N analyser was calibrated with glutamic acid. Procedure: In each of the 300 experimental plots of the biodiversity exploratories we collected 14 soil cores with a split tube sampler (diameter of 5 cm) along two 20 m transects in grasslands and 40 m transects in forests in May 2017. Organic layers in forests and aboveground plant parts in grasslands were removed before coring. We then prepared a composite sample from the 14 soil cores by mixing the upper 10 cm of the soil. Soil samples were sieved to <2 mm and air-dried. Subsequently, subsamples of sieved soil were ground in a ball mill. Total C and N concentrations were determined by dry combustion in an elemental analyser at a temperature of 1100°C. The evolving CO2 and N2 was determined with a Thermal Conductivity Detector (TCD).
19009	Phosphorus ; N:P	Resin P extraction with anion exchange resin membranes. Combination of methods proposed by Kouno et al. (1995) and McLaughlinet al. (1986) Instruments: Continuous Flow Analyzer (SEAL Analytical, Norderstedt, Germany)

		Murphy J & Riley JP (1962): A modified single solution method for the determination of phosphate in natural waters. Anal. Chim. Acta, 27, 31-36. McLaughlin MJ, Alston AM & Martin JK (1986): Measurement of phosphorus in the soil microbial biomass: A modified procedure for field soils. Soil Biol. Biochem., 18, 437-443. Kuo S (1996): Phosphorus. In: eds. Sparks DL, Page AL, Helmke PA, Loeppert R, Soltanpour PN, Tabatabai MA, Johnston AE, Sumner ME, Methods of Soil Analysis. Part 3 - Chemical Methods, 5, 869-919 pp. Madison, Wisconsin: SSSA.
20447	Phosphorus ; N:P	Small bags with filled with resin: a granular material onto which nutrients are adsorbed that comes into contact with the material. These bags are incubated at a certain depth in order that all nutrients that are in solution around the bags is caught in the bag. After incubation, nitrate is detached from the resin and the concentrations are measured. Number of plot based repetitions (information from the old BExIS schema): 3 Small bags with filled with resin: a granular material onto which nutrients are adsorbed that comes into contact with the material. These bags are incubated at a certain depth in order that all nutrients that are in solution around the bags is caught in the bag. After incubation, nitrate is detached from the resin and the concentrations are measured. Resin bags, extracted in lab. nstruments: resin bags (TerrAquat GmbH, Nürtingen; Mischbettaustauscher, insbes. für Nitrat und Ammonium plus Austauscher für anionisch vorliegende Schwermetalle und Phosphat) Calibration: ask owners for details Owners: Norbert Hoelzel; Till Kleinebecker; Valentin Klaus; Ute Hamer Procedure: Bag have been buried at 20 cm depth where possible, accessed from above by taking out a soil core. In Alb and Hainich, at some shallow soils, bags could be put to 10-15 cm depth only.

		Bags have been in soil approximately from mid March to beginning of August 2015 (a remarkably dry summer). So, resin bag remained in soil for 140 to 145 days. Resin bag extraction: Resin bags speciefied as “Mischbettaustauscher” plus resin for anionic heavy metals and phosphate (Firma TerrAquat, Nürtingen, Germany) Extraction of 15 g resin with H2SO4. Measurement have been done with a Continuous Flow Analysator (Skalar Analytic GmbH, The Netherlands)
20248	NH4 ; NO3	The study focussed on regional biogeography of soil microorganisms over time, i.e. how below ground microbial communities are organised and differentiated along geographic, climatic and environmental variables. To address this question we characterised soil microbial communities at three levels of resolution (gene abundance, enzyme activity, and community level). The three exploratories will allow us to up-scale the biogeography of soil microorganisms from plot to regional scale. Land-use intensity, climate and environmental variables shape soil microbial communities, functions and biomass. Here, data on physico-chemical soil properties of the project are enlisted. Within the joint soil sampling campaign 2011 all samples are taken in the beginning of May 2011 as a mixed sample from 14 soil cores of the top horizon (0-10 cm) of the all 50 grassland experimental plots of all three exploratories (AEG1–AEG50; HEG1-HEG50; SEG1-SEG50). Procedure: Bulk density was determined as g per cm³ using the volume of the 14 soil cores per site and the dry soil weight (without stone). Soil water content (SWC) was determined gravimetrically on approximately 5 g of soil; the soil samples were dried at 60 °C until constant weight was reached, minimum 72 hours.

		Based on the non-fumigated samples of the Chloroform-fumigation-extraction method (CFE), according to Vance et al. (1987) "An extraction method for measuring soil microbial biomass C" Soil Biology and Biochemistry and Keil et al. (2011) "Influence of land-use intensity on the spatial distribution of N-cycling microorganisms in grassland soils" FEMS Microbiology Ecology, the extractable organic carbon (EOC) and extractable nitrogen (EN) were determined. In brief, C and N were extracted from each non-fumigated replicate (5 g) with 40 mL 0.5 M K2SO4. The suspension was horizontally shaken (30 Min, 150 rpm) and centrifuged (30 Min, 4400 x g). C and N concentrations in dissolved (1:4, extract:deion. H2O) extracts were measured with a TOC/TN analyzer (Multi N/C 2100S, Analytik Jena AG, Jena, Germany). Mineral nitrogen in form of ammonium (NH4+) and nitrate (NO3-) was determined by DIN ISO 14256-2 (2006) with an AutoAnalyzer 3 (Bran & Luebbe, Norderstedt, Germany) on the undiluted extracts from CFE analysis.
20249	NH4 ; NO3	The study focussed on regional biogeography of soil microorganisms over time, i.e. how below ground microbial communities are organised and differentiated along geographic, climatic and environmental variables. To address this question we characterised soil microbial communities at three levels of resolution (gene abundance, enzyme activity, and community level). The three exploratories will allow us to up-scale the biogeography of soil microorganisms from plot to regional scale. Land-use intensity, climate and environmental variables shape soil microbial communities, functions and biomass. Here, data on physico-chemical soil properties of the project are enlisted. Within the joint soil sampling campaign 2014 all samples are taken in the beginning of May 2014 as a mixed sample from 14 soil cores of the top horizon (0-10 cm) of the all 50 grassland experimental plots of all three exploratories (AEG1–AEG50; HEG1-HEG50; SEG1-SEG50). Procedure: Bulk density was determined as g per cm³ using the volume of the 14 soil cores

		per site and the dry soil weight (without stone). Soil water content (SWC) was determined gravimetrically on approximately 5 g of soil; the soil samples were dried at 60 °C until constant weight was reached, minimum 72 hours. Based on the non-fumigated samples of the Chloroform-fumigation-extraction method (CFE), according to Vance et al. (1987) "An extraction method for measuring soil microbial biomass C" Soil Biology and Biochemistry and Keil et al. (2011) "Influence of land-use intensity on the spatial distribution of N-cycling microorganisms in grassland soils" FEMS Microbiology Ecology, the extractable organic carbon (EOC) and extractable nitrogen (EN) were determined. In brief, C and N were extracted from each non-fumigated replicate (5 g) with 40 mL 0.5 M K2SO4. The suspension was horizontally shaken (30 Min, 150 rpm) and centrifuged (30 Min, 4400 x g). C and N concentrations in dissolved (1:4, extract:deion. H2O) extracts were measured with a TOC/TN analyzer (Multi N/C 2100S, Analytik Jena AG, Jena, Germany). Mineral nitrogen in form of ammonium (NH4+) and nitrate (NO3-) was determined by DIN ISO 14256-2 (2006) with an AutoAnalyzer 3 (Bran & Luebbe, Norderstedt, Germany) on the undiluted extracts from CFE analysis.
15306	delta-N-15	d15N as a marker for ecosystem processes and land use Common wet chemical lab analyses Instruments: Finnigan mass spectrometer Calibration: Atropin IAEA N-1 IAEA N-2 Procedure: for details ask authors Authors: Valentin Klaus ; Norbert Hoelzel ; Till Kleinebecker ; Martin Freitag delta 15 N ratio of topsoil

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Appendix S3 Number of species out of 319 used for the calculation of community weighted means (CWMs) for traits and proportions for plant groups, and mean proportions of cover for CWMs and plant groups across the 150 grassland plots.

Community parameter	**Number of species**	**Mean cover**
CWM Specific leaf area	279	95%
CWM Root weight ratio	216	92%
CWM Bud-bank size	313	91%
CWM Root tissue density	216	92%
CWM Specific root length	216	92%
CWM Root branching intensity	216	92%
CWM Fine roots diameter	216	92%
CWM Mycorrhizal colonization	75	65%
CWM Fine roots %N	196	89%
CWM Rooting depth 50%	183	90%
Proportion of Poales	40	57%
Proportion of Forbs	254	30%
Proportion of Fabaceae	31	9%
Proportion of Annuals	80	3%
Proportion of Multiannuals	246	95%

Appendix S4 References for the expected relationships between potential dimensions of belowground economic space and environmental variables. We combined results from meta-analysis, field and greenhouse experiments, and plant nutrition manuals to formulate hypotheses on the existence and the direction of a relationship between a belowground functional aspect and an environmental factor. The direction of the relationship is indicated with a "+" when positive, with a "-" when negative, and with "+/-" for mixed expectations regarding the direction. Directional relationships with a strong degree of confidence are shown in red, with low degree of confidence in orange, and with mixed results regarding the direction in grey. The studies included focussed overwhelmingly though not exclusively on herbaceous communities. This table does not intend to provide a comprehensive review of the relationships between belowground traits and environment, but rather to emphasize that different plant-strategy gradients or aspects could be influenced by the same or different environmental drivers.

		Root conservation	Mycorrhizal collaboration	Vegetative regeneration	Vertical space occupancy
Land Management	Land-use intensity (Mowing Fertilisation and Grazing)	- [4][7][5][9]	- [1][2][4][5][8]	+/- [7][13]	
Top soil	Moisture	- [16]	+ [16]	- [13]	+/- [3][10][12]
	Texture (Sand content)	+ [14]			
	Nitrogen availability indicators: pH C:N NO3 NH4 delta-15-N	- [3][6][9]	- [1][2][3]		+ [11]
	Phosphorus availability indicators: N:P Phosphorus	- [3]	- [15]		- [11]

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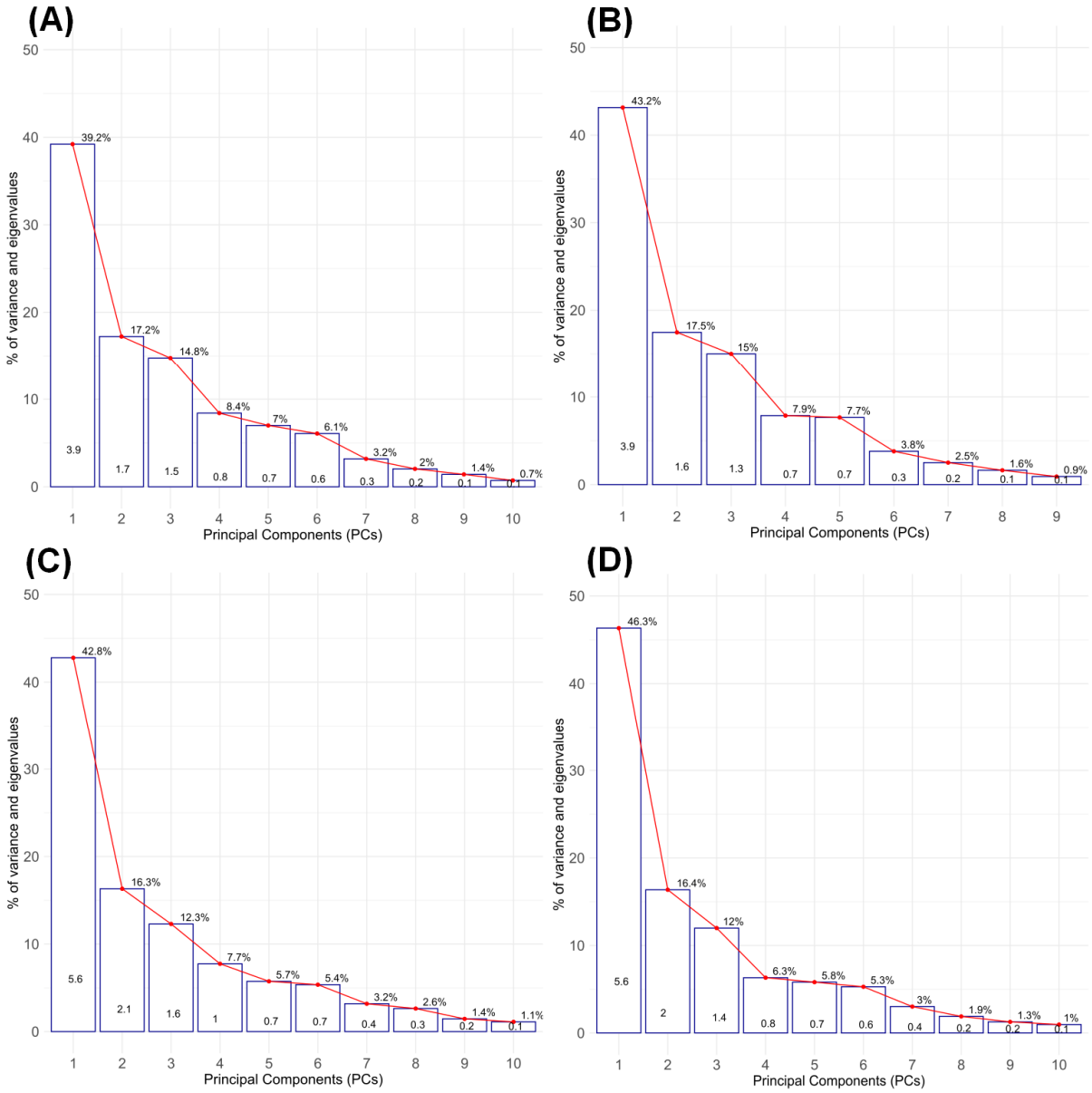
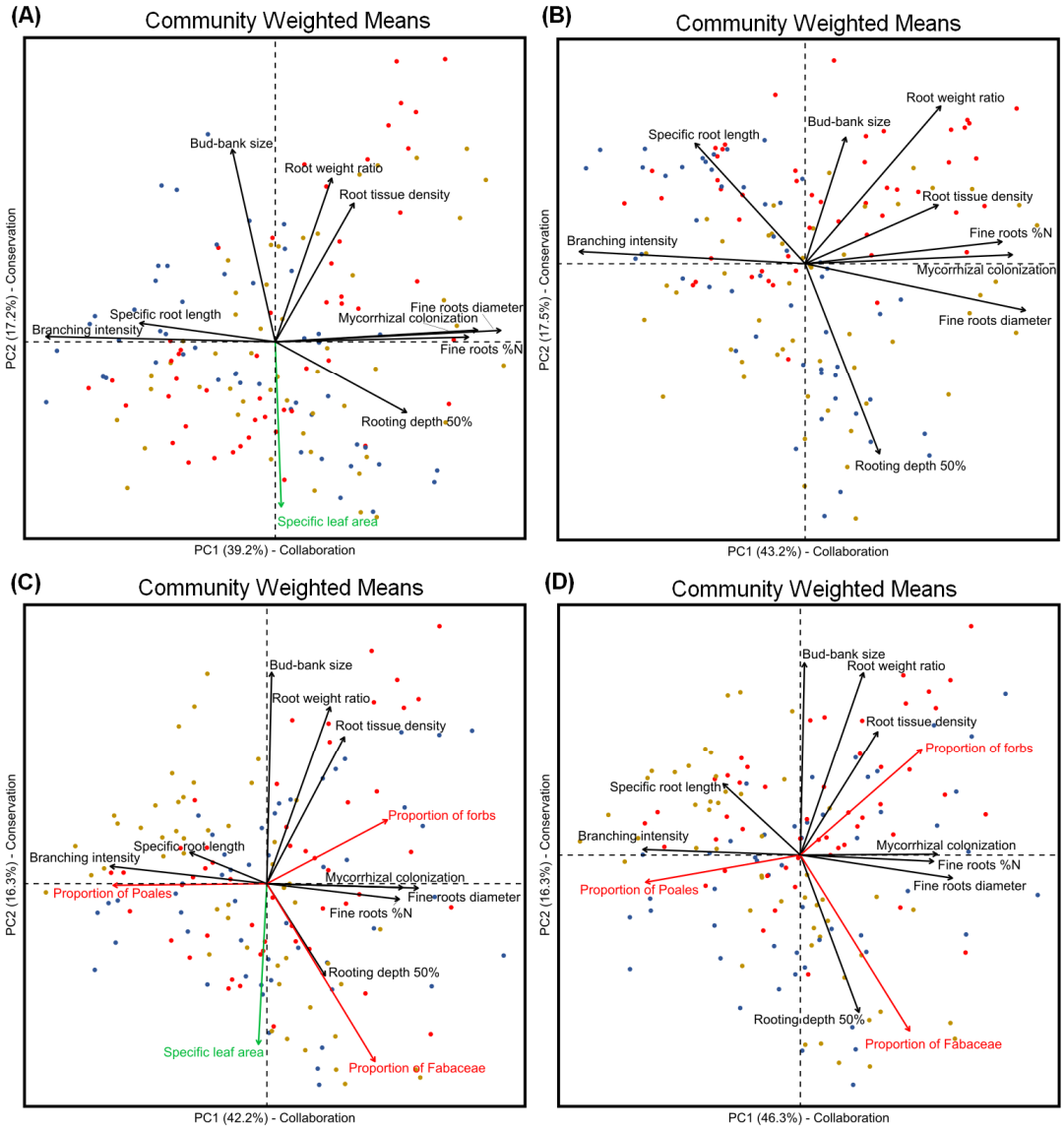
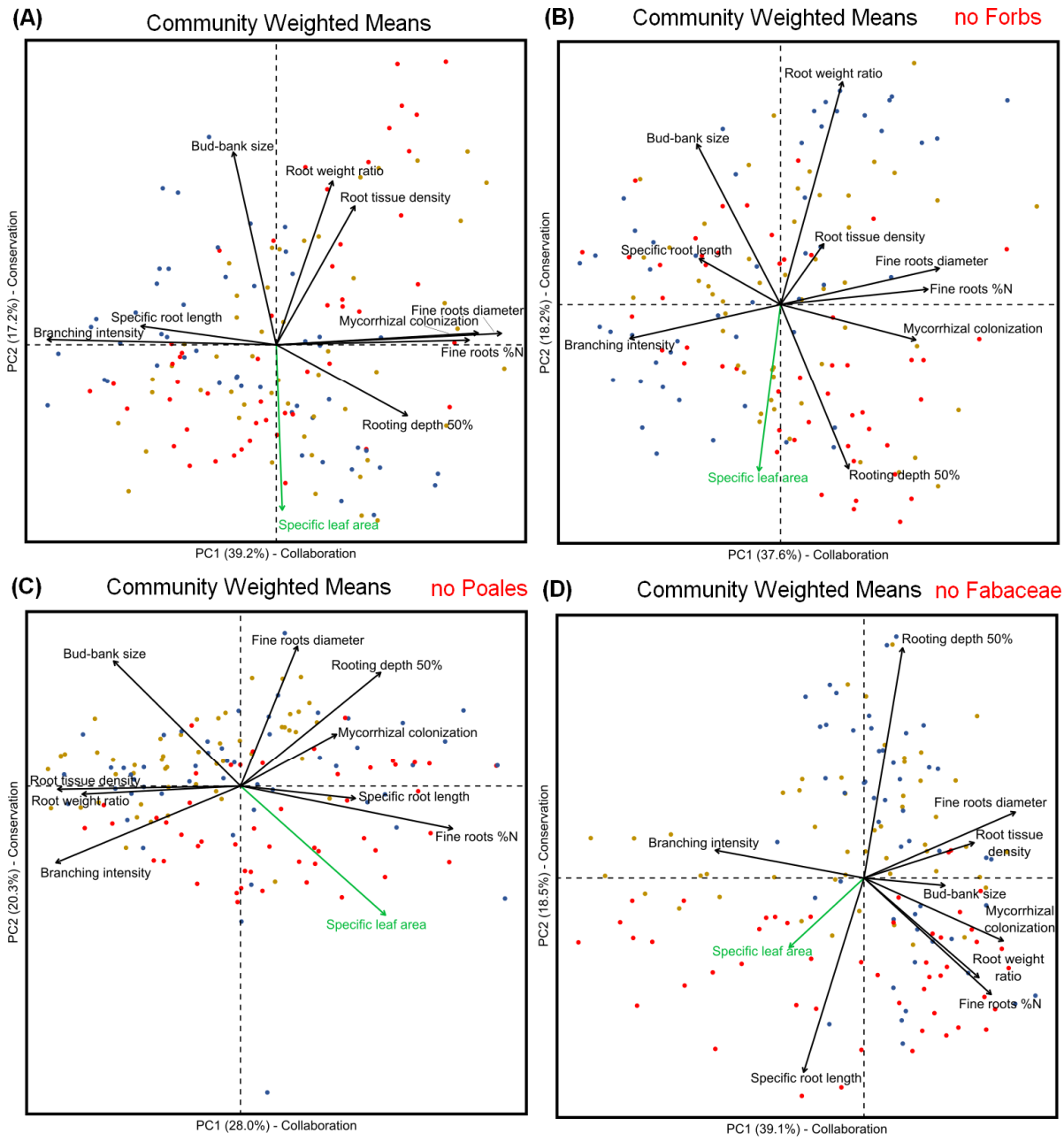
Appendix S5 Percentage of variance explained and eigenvalues (printed inside the bars) for the four principal component analyses we did: (A) Above-belowground; (B) Belowground; (C) Above-belowground with plant functional type; (D) Belowground with plant functional type. The two first PCs explain about 55-60% of the total variance in each analysis.



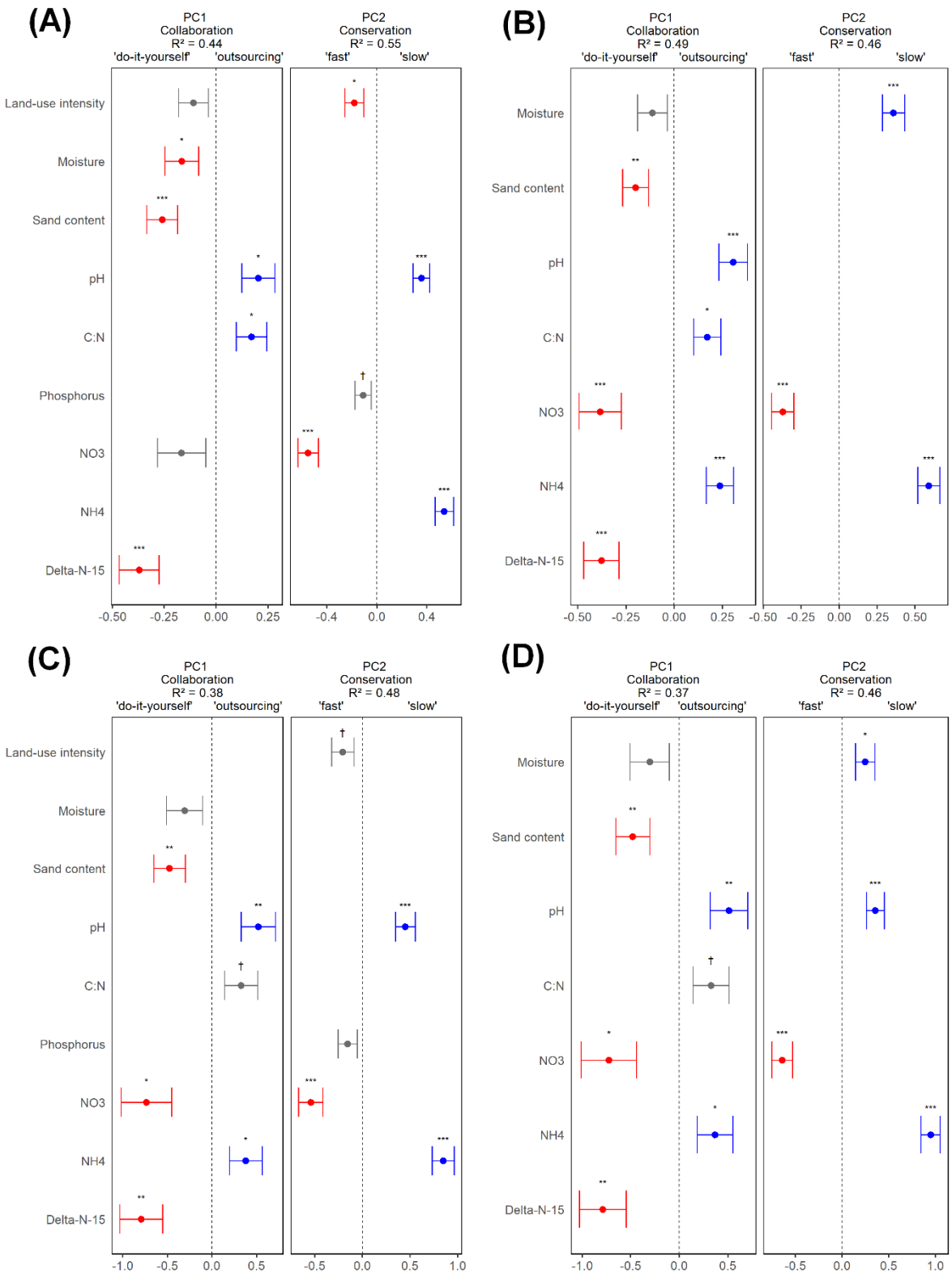
Appendix S6 Two-dimensional projections of the two first PCs resulting from the four PCAs on (A) Above-belowground (also shown in Fig. 2); (B) Belowground; (C) Above-belowground with plant functional type; (D) Belowground with plant functional type. The two first PCs explain about 55% of the total variance in each configuration. $_{CWM}$*Specific leaf area*, as the sole leaf trait is shown in green. The three plant functional types Poales, forbs (non-Fabaceae dicotyledons) and Fabaceae are shown in red. The scores of the 150 grassland plots used for the PCA are shown in red (Alb region, N=50), brown (Hainich region, N=50) and blue (Schorfheide region, N=50). The first PC ('Collaboration') is more characterized by CWMs of traits related to the 'collaboration' gradient. It associates thick fine roots with mycorrhizal colonization ('outsourcing'), opposed to highly branched roots with high specific root length ('do-it-yourself'). High proportion of Poales are characteristic of the 'do-it-yourself' strategy. The PC2 ('Conservation') is more strongly characterized by CWMs of traits related to the plant economic spectrum and vegetative regeneration potential. It associates high root weight ratio with large bud banks and high root tissue density ('slow'), opposed to high specific leaf area ('fast'). High proportions of forbs and Fabaceae are characteristic of the 'outsourcing' strategy. A high proportion of Fabaceae is also associated with 'fast', deep rooting communities, whereas a high proportion of forbs is associated with more 'slow', regenerative communities.


(A)
Community Weighted Means
Bud-bank size
Root weight ratio
Root tissue density
Specific root length
Branching intensity
Fine roots diameter
Mycorrhizal colonization
Fine roots %N
Rooting depth 50%
Specific leaf area
PC2 (17.2%) - Conservation
PC1 (39.2%) - Collaboration
(B)
Community Weighted Means
Root weight ratio
Bud-bank size
Specific root length
Root tissue density
Fine roots %N
Branching intensity
Mycorrhizal colonization
Fine roots diameter
Rooting depth 50%
PC2 (17.5%) - Conservation
PC1 (43.2%) - Collaboration
(C)
Community Weighted Means
Bud-bank size
Root weight ratio
Root tissue density
Proportion of forbs
Specific root length
Branching intensity
Mycorrhizal colonization
Proportion of Poales
Fine roots diameter
Fine roots %N
Rooting depth 50%
Specific leaf area
Proportion of Fabaceae
PC2 (16.3%) - Conservation
PC1 (42.2%) - Collaboration
(D)
Community Weighted Means
Bud-bank size
Root weight ratio
Root tissue density
Proportion of forbs
Specific root length
Branching intensity
Mycorrhizal colonization
Fine roots %N
Proportion of Poales
Fine roots diameter
Rooting depth 50%
Proportion of Fabaceae
PC2 (16.3%) - Conservation
PC1 (46.3%) - Collaboration

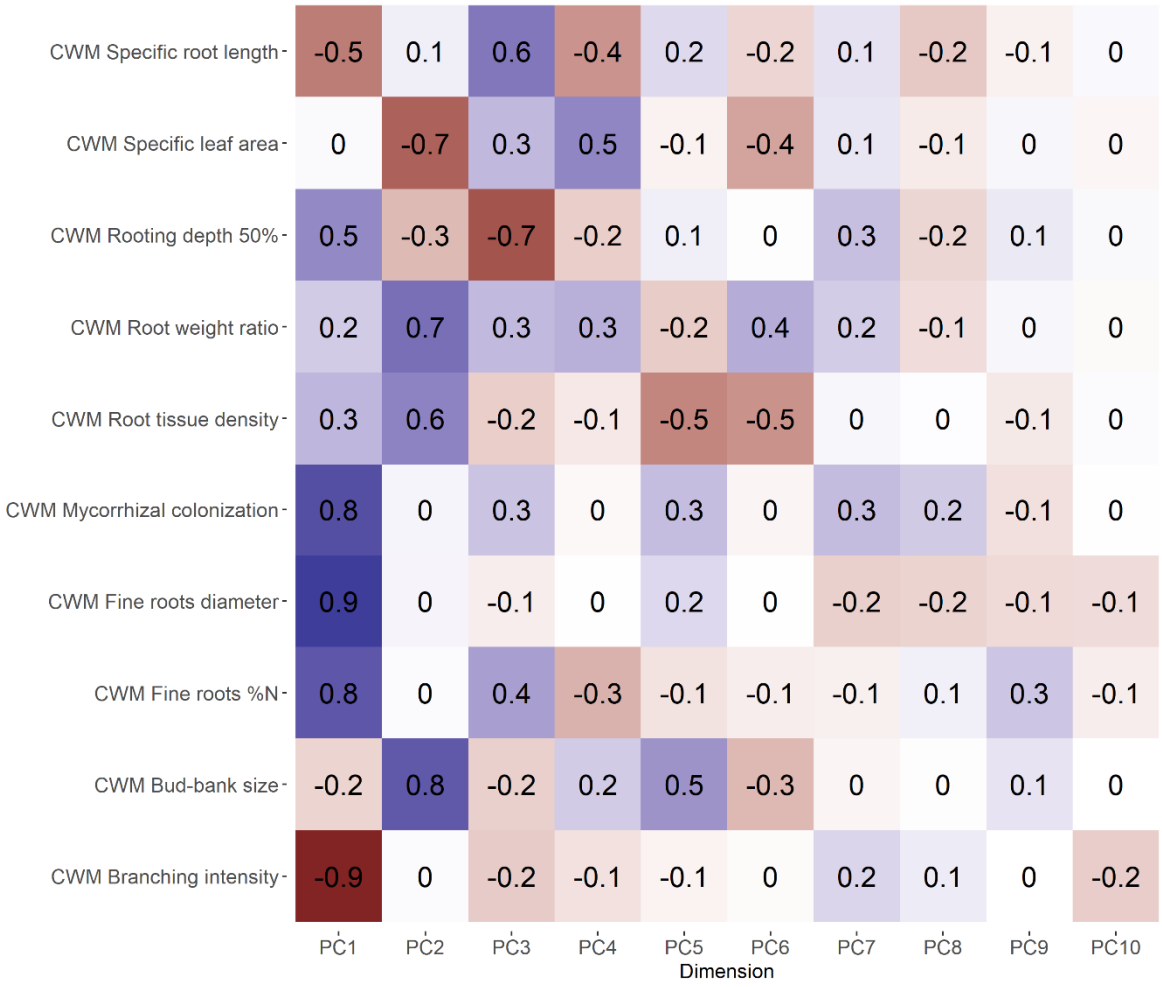
Appendix S7 Two-dimensional projections of the two first PCs resulting from the four PCAs on (A) Above-belowground with the entire set of species included for CWMs calculations (also shown in Fig. 2); (B) Above-belowground excluding non-Fabaceae forbs for CWMs calculations; (C) Above-belowground excluding Poales for CWMs calculations; (D) Above-belowground excluding Fabaceae in CWMs calculations. $_{CWM}$*Specific leaf area*, as the sole leaf trait is shown in green. The scores of the 150 grassland plots used for the PCA are shown in red (Alb region, N=50), brown (Hainich region, N=50) and blue (Schorfheide region, N=50). Removing Fabaceae or Poales for CWMs calculation has significant effects in modifying CWMs loadings on the two first PC, indicating that belowground traits relationships depend on plant functional types when considered at the community scale.


(A)
Community Weighted Means
Bud-bank size
Root weight ratio
Root tissue density
Specific root length
Branching intensity
Fine roots diameter
Mycorrhizal colonization
Fine roots %N
Rooting depth 50%
Specific leaf area
PC2 (17.2%) - Conservation
PC1 (39.2%) - Collaboration
(B)
Community Weighted Means
no Forbs
Root weight ratio
Bud-bank size
Root tissue density
Specific root length
Fine roots diameter
Fine roots %N
Mycorrhizal colonization
Branching intensity
Specific leaf area
Rooting depth 50%
PC2 (18.2%) - Conservation
PC1 (37.6%) - Collaboration
(C)
Community Weighted Means
no Poales
Fine roots diameter
Bud-bank size
Rooting depth 50%
Mycorrhizal colonization
Root tissue density
Root weight ratio
Specific root length
Fine roots %N
Branching intensity
Specific leaf area
PC2 (20.3%) - Conservation
PC1 (28.0%) - Collaboration
(D)
Community Weighted Means
no Fabaceae
Rooting depth 50%
Fine roots diameter
Branching intensity
Root tissue density
Bud-bank size
Mycorrhizal colonization
Specific leaf area
Root weight ratio
Fine roots %N
Specific root length
PC2 (18.5%) - Conservation
PC1 (39.1%) - Collaboration

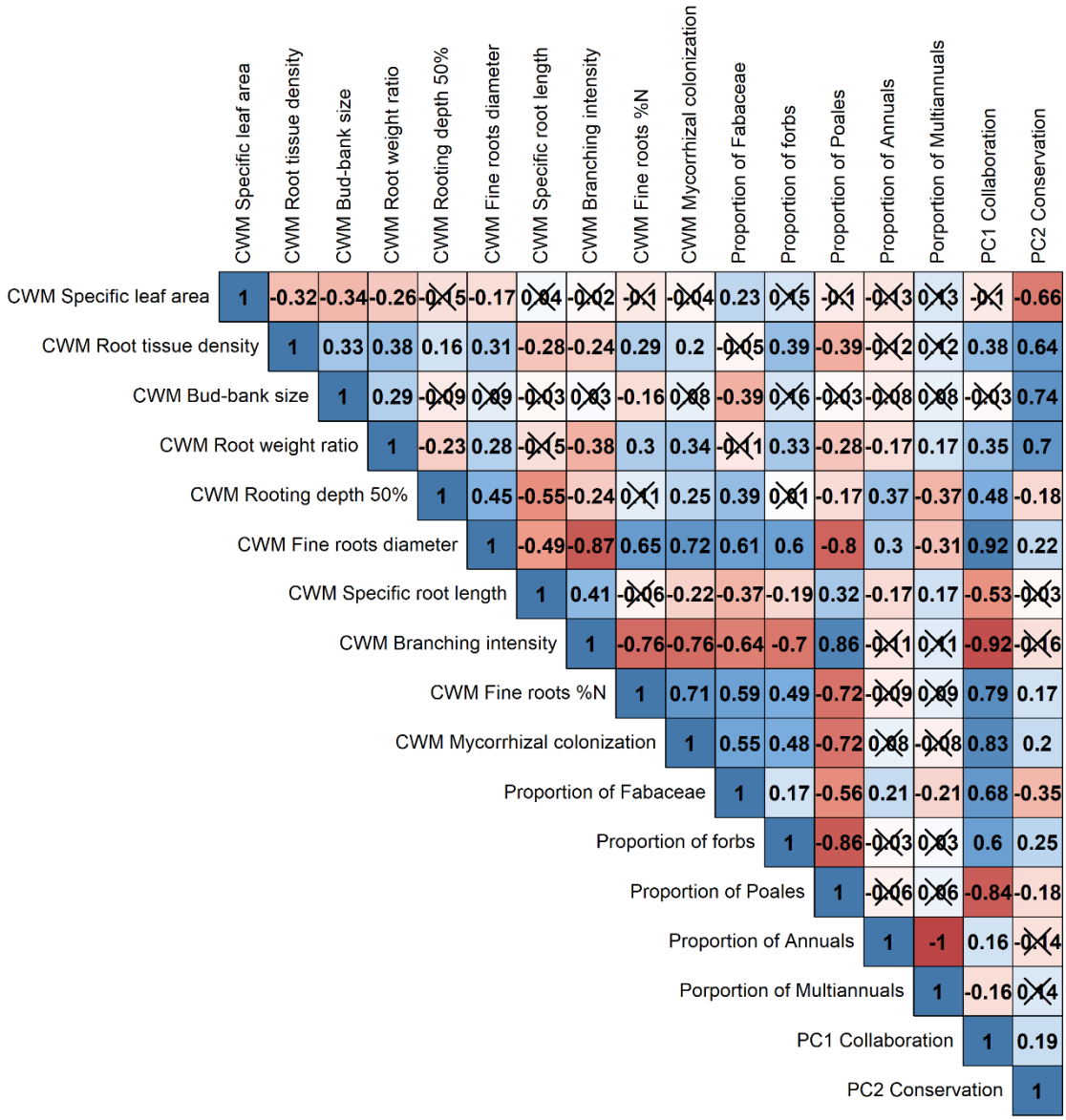
Appendix S8 Estimates from linear models of environmental variables effects on the grassland plots functional identity, as captured by their scores for PC1 - Collaboration and PC2 - Conservation-regeneration, of the four PCAs on (A) Above-belowground (also shown in Fig. 2); (B) Belowground (corresponding to Appendix S6b); (C) Above-belowground with plant functional type (corresponding to Appendix S6c); (D) Belowground with plant functional type (corresponding to Appendix S6d). On the y-axis are the 9 environmental variables retained as predictors. The error bars around the estimates are standard errors. Significant (* for $p < 0.05$; ** for $p < 0.01$; *** for $p < 0.001$) negative and positive estimates are marked in red and blue, respectively. Non-significant ($p > 0.05$) estimates are marked in grey. Marginally significant ($p < 0.10$) estimates are marked with †.


(A)
PC1
Collaboration
R² = 0.44
'do-it-yourself'
'outsourcing'
PC2
Conservation
R² = 0.55
'fast'
'slow'
Land-use intensity
Moisture
Sand content
pH
C:N
Phosphorus
NO3
NH4
Delta-N-15
-0.50
-0.25
0.00
0.25
-0.4
0.0
0.4
(B)
PC1
Collaboration
R² = 0.49
'do-it-yourself'
'outsourcing'
PC2
Conservation
R² = 0.46
'fast'
'slow'
Moisture
Sand content
pH
C:N
NO3
NH4
Delta-N-15
-0.50
-0.25
0.00
0.25
0.50
(C)
PC1
Collaboration
R² = 0.38
'do-it-yourself'
'outsourcing'
PC2
Conservation
R² = 0.48
'fast'
'slow'
Land-use intensity
Moisture
Sand content
pH
C:N
Phosphorus
NO3
NH4
Delta-N-15
-1.0
-0.5
0.0
0.5
1.0
(D)
PC1
Collaboration
R² = 0.37
'do-it-yourself'
'outsourcing'
PC2
Conservation
R² = 0.46
'fast'
'slow'
Moisture
Sand content
pH
C:N
NO3
NH4
Delta-N-15

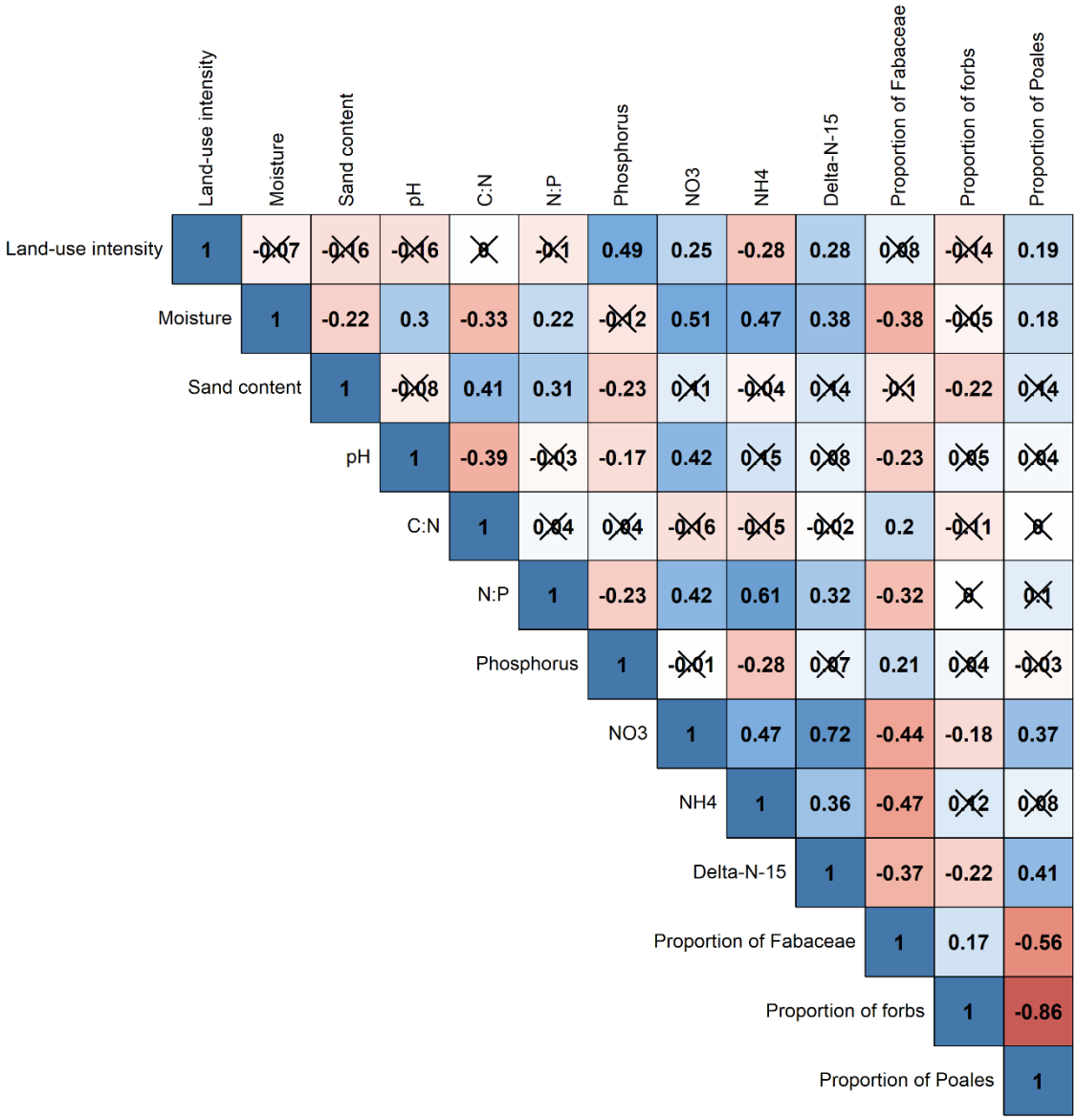
Appendix S9 Loadings on the ten PCs of the Above-belowground PCA (as shown in Fig. 2 for PC1 and PC2 only). All the traits have strong loadings either on PC1 or PC2, representing 55-60% of the total variance. PC3 has a strong loading of $_{CWM}$*Rooting depth 50%* and captures the differences in soil depth across the three regions (see Appendix S2 and Appendix S13). 'Oblimin' rotation was used on PC1 and PC2 to accentuate the loading of CWMs on each PC.



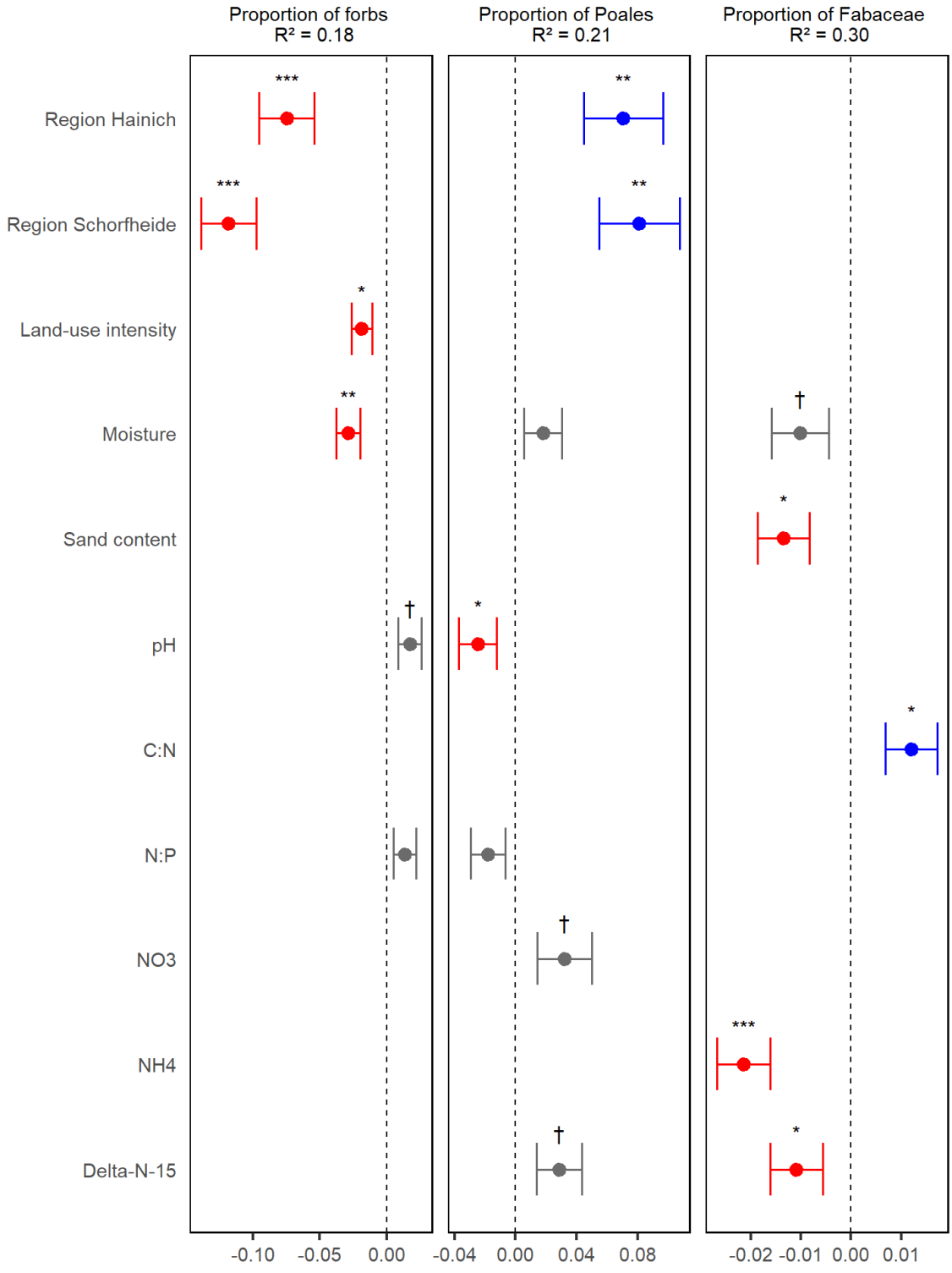
Appendix S10 Correlation matrix of community weighted means (CWMs), proportions of three plant functional types, and PC scores for the two first PCs of the Above-Belowground PCA (as shown in Fig. 2). Pearson's correlation coefficients are displayed. Negative correlations are in red, positive correlations are in blue, and non-significant correlations are indicated with a cross. Forbs = non-Fabaceae dicots. Multiannuals = biennials plus perennials.



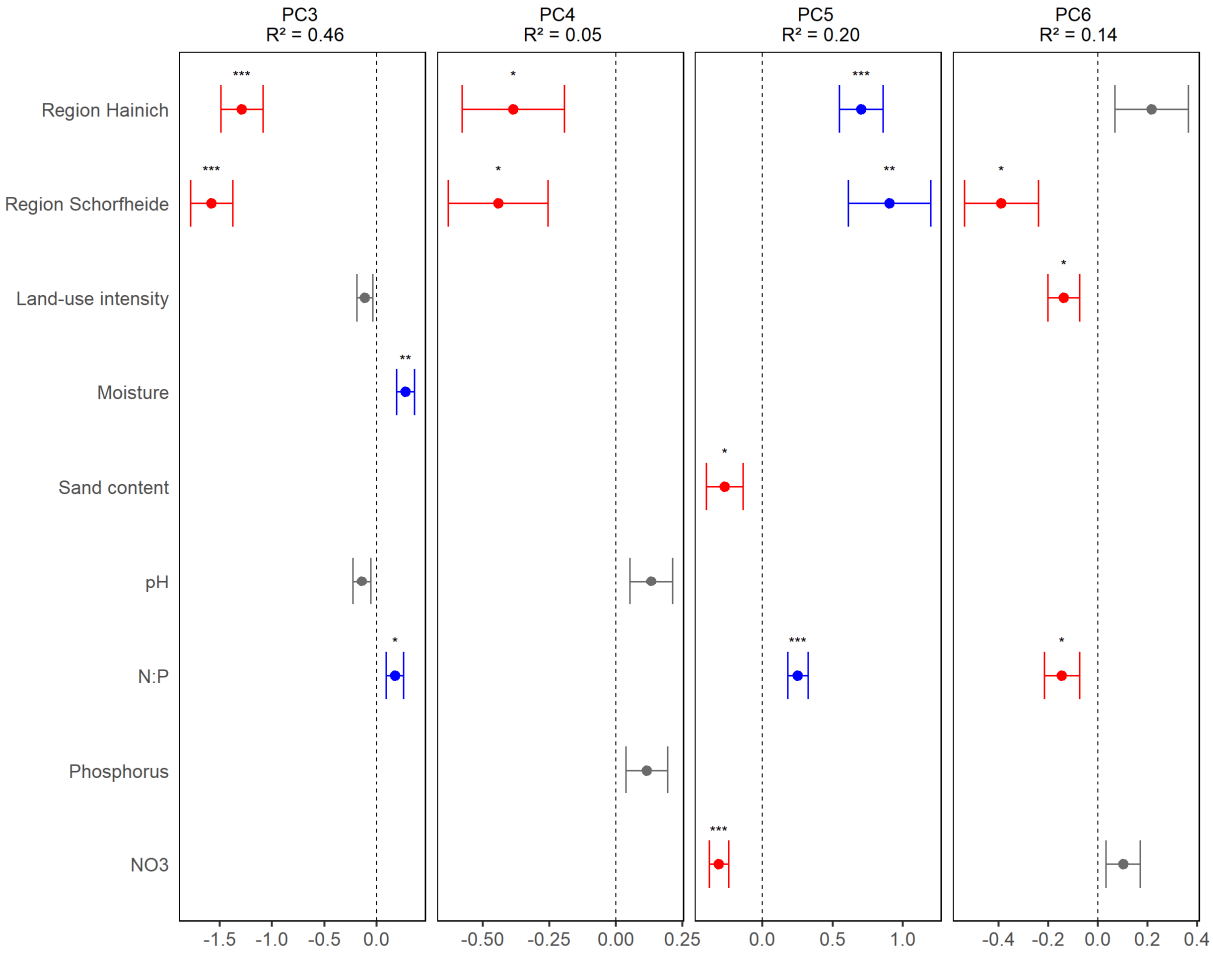
Appendix S11 Correlation matrix of the environmental variables and proportions of three plant functional types. Bulk density wasn't used directly as a predictor in models because of its strong correlation with several other soil parameters, resulting in large variance inflation factors (VIF), and was used indirectly to express soil parameters as unit of volume instead of mass when possible. Soil depth wasn't used in models because of its insufficient sampling (N=144 and limited to 100 cm sampling, whereas for all the other predictors, N=150 and sampling values were not censored). Pearson's correlation coefficients are displayed. Negative correlations are in red, positive correlations are in blue, and non-significant correlations are indicated with a cross.


Land-use intensity
Moisture
Sand content
pH
C:N
N:P
Phosphorus
NO3
NH4
Delta-N-15
Proportion of Fabaceae
Proportion of forbs
Proportion of Poales
Land-use intensity 1 -0.07 -0.16 -0.16 0 -0.1 0.49 0.25 -0.28 0.28 0.08 -0.14 0.19
Moisture 1 -0.22 0.3 -0.33 0.22 -0.12 0.51 0.47 0.38 -0.38 -0.05 0.18
Sand content 1 -0.08 0.41 0.31 -0.23 0.11 -0.04 0.14 -0.1 -0.22 0.14
pH 1 -0.39 -0.03 -0.17 0.42 0.15 0.08 -0.23 0.05 0.04
C:N 1 0.04 0.04 -0.16 -0.15 -0.02 0.2 -0.11 0
N:P 1 -0.23 0.42 0.61 0.32 -0.32 0 0.1
Phosphorus 1 -0.01 -0.28 0.07 0.21 0.04 -0.03
NO3 1 0.47 0.72 -0.44 -0.18 0.37
NH4 1 0.36 -0.47 0.12 0.08
Delta-N-15 1 -0.37 -0.22 0.41
Proportion of Fabaceae 1 0.17 -0.56
Proportion of forbs 1 -0.86
Proportion of Poales 1

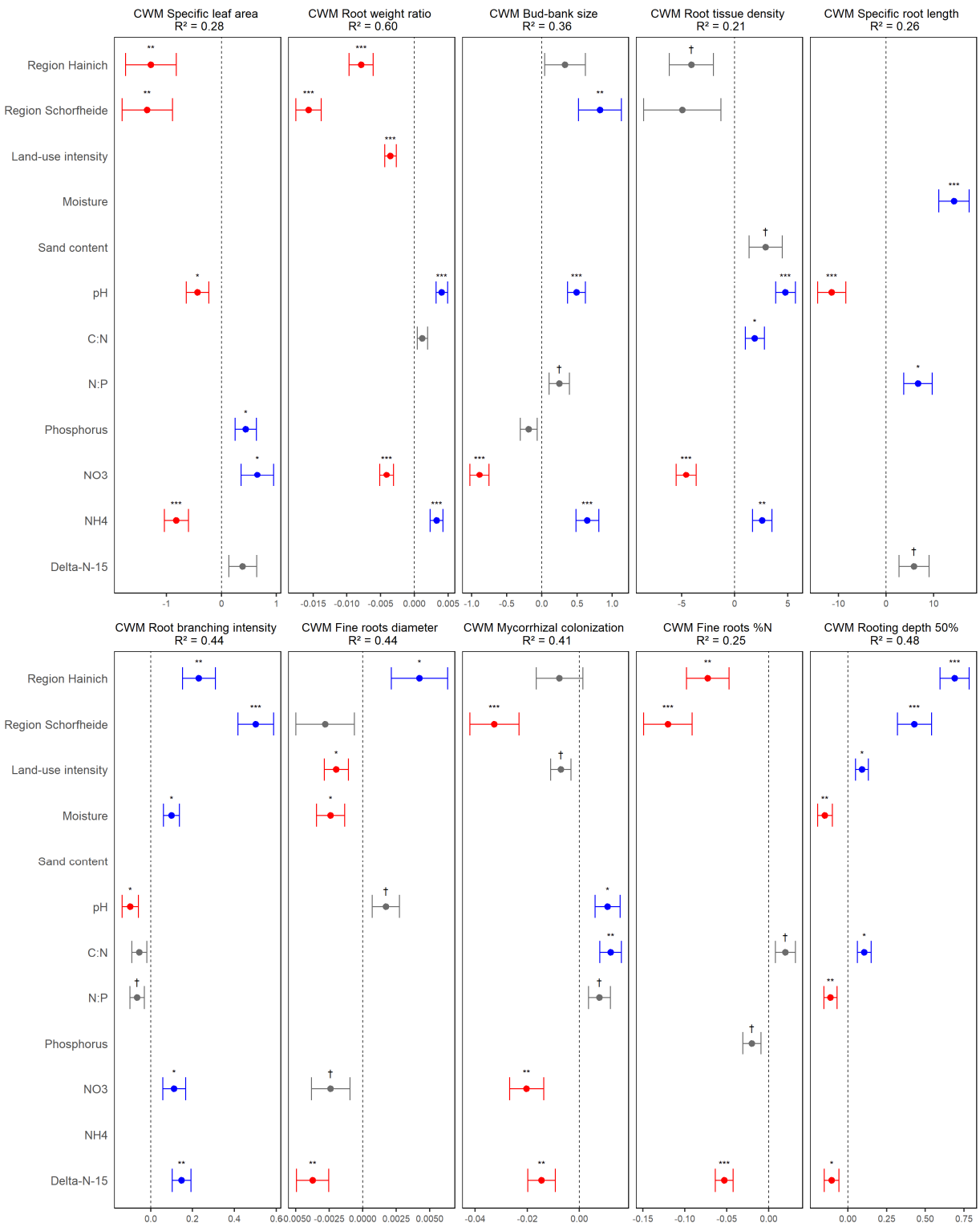
Appendix S12 Estimates from linear models of environmental variables effects on the proportion of plant functional types. On the y-axis are the 9 environmental variables and the two regions retained by the models as predictors. The error bars around the estimates are standard errors. Significant (* for $p < 0.05$; ** for $p < 0.01$; *** for $p < 0.001$) negative and positive estimates are marked in red and blue, respectively. Non-significant ($p > 0.05$) estimates are marked in grey. Marginally significant ($p < 0.10$) estimates are marked with †. Forbs = non-Fabaceae dicots.


Proportion of forbs
R² = 0.18
Proportion of Poales
R² = 0.21
Proportion of Fabaceae
R² = 0.30
Region Hainich
Region Schorfheide
Land-use intensity
Moisture
Sand content
pH
C:N
N:P
NO3
NH4
Delta-N-15
***
***
*
**
†
*
†
*
*
†
†
**
**
†
*
*
***
*
-0.10
-0.05
0.00
-0.04
0.00
0.04
0.08
-0.02
-0.01
0.00
0.01

Appendix S13 Estimates from linear models of environmental variables effects on the scores for the third to sixth PC of the Above-belowground PCA (as in Fig. 3 but for subsequent PC). Combined with PC1 and PC2, they together explain more than 90% of the total variance (see Appendix S5). On the y-axis are the 7 environmental variables and the two regions retained by the models as predictors. The error bars around the estimates are standard errors. Significant (* for $p < 0.05$; ** for $p < 0.01$; *** for $p < 0.001$) negative and positive estimates are marked in red and blue, respectively. Non-significant ($p > 0.05$) estimates are marked in grey. Marginally significant ($p < 0.10$) estimates are marked with †.



Appendix S14 Estimates from linear models of environmental variables effects on the grassland plots community weighted means (CWMs) for each of the ten traits used in the four PCAs. On the y-axis are the environmental variables and regions retained by the models as predictors. The error bars around the estimates are standard errors. Significant (* for $p < 0.05$; ** for $p < 0.01$; *** for $p < 0.001$) negative and positive estimates are marked in red and blue, respectively. Non-significant ($p > 0.05$) estimates are marked in grey. Marginally significant ($p < 0.10$) estimates are marked with †.


CWM Specific leaf area
R² = 0.28
CWM Root weight ratio
R² = 0.60
CWM Bud-bank size
R² = 0.36
CWM Root tissue density
R² = 0.21
CWM Specific root length
R² = 0.26
Region Hainich
Region Schorfheide
Land-use intensity
Moisture
Sand content
pH
C:N
N:P
Phosphorus
NO3
NH4
Delta-N-15
CWM Root branching intensity
R² = 0.44
CWM Fine roots diameter
R² = 0.44
CWM Mycorrhizal colonization
R² = 0.41
CWM Fine roots %N
R² = 0.25
CWM Rooting depth 50%
R² = 0.48

Appendix S15 Indicator species or taxa for combinations of the dimensions of CWMs

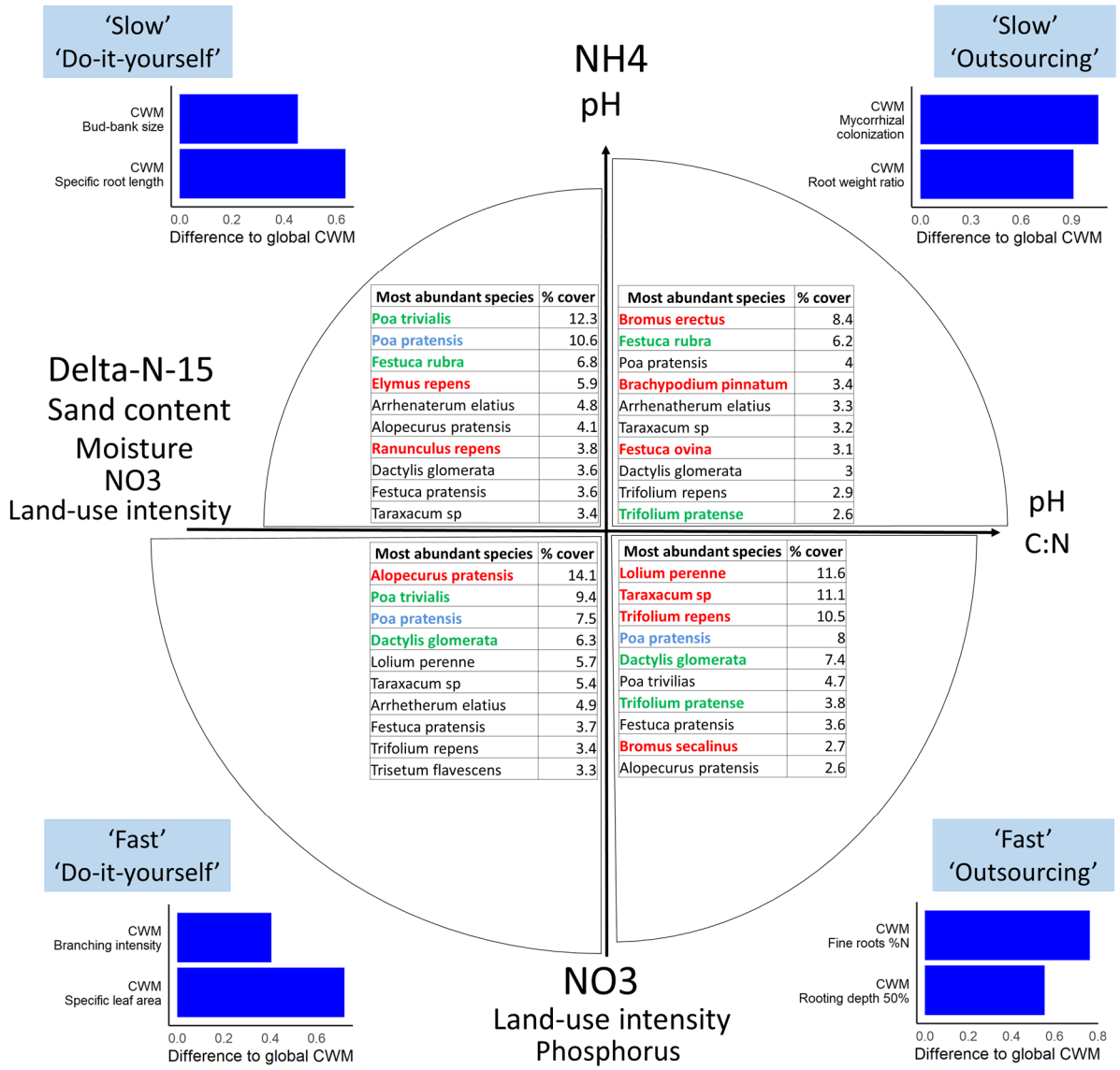
Methods

To identify which species or taxa were associated with particular combinations of the dimensions of the CWMs of traits, we subsetted the grassland plots based on their scores on PC1 and PC2 of the Above-Belowground PCA (Fig. 2) into four quadrants, corresponding to the four combinations of positive and negative coordinates of the two PCs. For each quadrant, we calculated the percentage of cover represented by the ten most common species or taxa in the plots. We then calculated Pearson's phi coefficient of association between species or taxa and the four PCA quadrants, used as sites (Cáceres & Legendre 2009), with the function multipatt() from the package 'indicspecies' (Cáceres & Jansen 2015).

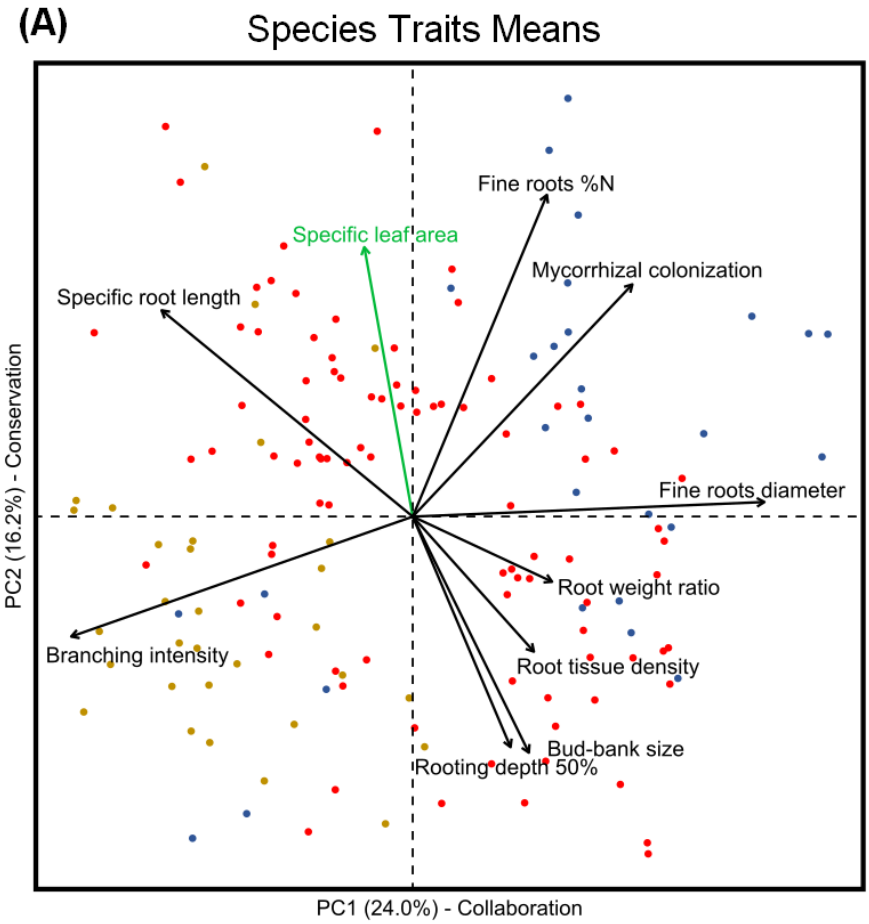
Summary diagram of the two main dimensions of the CWMs of belowground traits in German grasslands and their variation along environmental gradients.

PC1 of the "Above-belowground PCA" corresponds to a 'conservation' gradient (vertical) with 'fast' and 'slow' strategies. PC2 corresponds to a 'collaboration' gradient (horizontal) with 'do-it-yourself' and 'outsourcing' strategies. Therefore, communities can be classified into four categories, according to their position in one of the four quadrants of the projection system of PC1 and PC2. The size of the quadrants reflects the number of plots in each quadrant ('Slow & Do-it-yourself': N = 28 plots; 'Slow & Outsourcing': N = 37 plots; 'Fast & Do-it-yourself': N = 46 plots; 'Fast & Outsourcing': N = 39 plots. Inside each quadrant, we indicate the ten dominant species or taxa with the highest cover in the plots. They contributed from 40.1% of the total cover for 'Slow & Outsourcing' to 66% for 'Fast & Outsourcing'. Highlighted in different colors are the indicator species or taxa for one or several quadrants. Red = one quadrant; Green = two quadrants; Blue = three quadrants. The largely overlapping list of dominant species or taxa indicates that variation in CWMs relies on the turnover of the same set of dominant species. It is worth mentioning that although Poales are overall characterized

by lower mycorrhizal colonization than forbs, grasses with high mycorrhizal colonization are dominant species in the ‘Slow & Outsourcing‘ quadrant. This indicates that the gradient from ‘Do-it-yourself’ to ‘Outsourcing’ communities is not only explained by replacement of Poales by forbs, but also by changes within the Poales group itself. The barplots are highlighting, for two traits characteristic of each quadrant, their difference between their (scaled) CWMs and the CWMs for all communities. At the four extremities of the two-dimensional space, we highlighted which environmental gradients are associated with which kind of communities, and the font size is scaled with the coefficients from regression models as shown in Fig. 3.



Appendix S16 (A) The two first PCs of the Above-belowground PCA at the species level (instead of the community level), explaining 40.2% of the total variance in species-level trait means. The sole aboveground trait that we included, specific leaf area, is shown in green. The scores of the 218 species, covered by the morphology experiment are shown as dots, and imputation by the mean are used for the other traits to fill the gaps between their sample size and the sample size of the morphology experiment for the following number of species (specific leaf area: N=21; fine roots %N: N=20; mycorrhizal colonization: N=143; bud-bank size: N=7, rooting depth 50%: N=39). The plant functional types of the species are indicated in blue: Fabaceae (N=23); red: non-Fabaceae forbs (N=151); brown: Poales (N=44). (B) Pearson's correlation coefficients between the traits at the species level. Negative correlations are in red, positive correlations are in blue, and non-significant correlations are indicated with a cross.



(B)

	Specific leaf area	Root tissue density	Bud-bank size	Root weight ratio	Rooting depth 50%	Fine roots diameter	Specific root length	Fine roots %N	Branching intensity	Mycorrhizal colonization
Specific leaf area	1	-0.18	-0.17	-0.04	-0.12	-0.02	0.1	0.05	0.02	0.21
Root tissue density		1	0.19	0.24	-0.16	0.02	-0.27	0.1	-0.06	0.13
Bud-bank size			1	0.06	0.17	0.13	-0.11	-0.04	-0.13	-0.05
Root weight ratio				1	-0.01	0.15	-0.13	-0.01	-0.15	0.2
Rooting depth 50%					1	0.26	-0.18	-0.25	-0.11	0.01
Fine roots diameter						1	-0.49	0.22	-0.61	0.54
Specific root length							1	0.17	0.3	-0.11
Fine roots %N								1	-0.37	0.43
Branching intensity									1	-0.62
Mycorrhizal colonization										1

General Discussion

The belowground 'hidden half' of plants is starting to reveal its secrets, impacting our understanding of plant functioning, their ecology and potentially helping us to better prevent and manage plant invasions, to conserve biodiversity, and to secure food supply. Furthermore, comparative and functional ecology allow us to identify patterns in nature, that have been shaped by millions of years of evolution, to determine the structure and the functioning of ecosystems. In this thesis, I applied methods from comparative ecology by using the differences in above- and belowground traits between a large number of grassland species to identify predictors of species rarity and commonness (Chapter I). Low root tissue density was a consistent predictor of plant abundance at seven spatial scales ranging from grassland plot to naturalization success. Deep rooting depth was a significant predictor for success at larger scales (regional, continental) where climate and soil condition variability is high. Large bud-bank sizes were important for success in temperate grasslands where competition for resources and seasonality play filtering roles. The relationships between traits we found with our collaborators in Berlin indicate belowground trade-offs, emphasizing the impossibility for a single plant to invest in all aspects of plant functioning, and thus resulting in differentiated functional strategies (Chapter II & III). A two-dimensional trait space encompasses belowground diversity in the traits we measured in grasslands (Chapter III).

The first dimension corresponds to a 'conservation' gradient characterized by a 'fast' side: low root tissue density, low belowground biomass investment (root-weight-ratio), and small bud-bank size, and a 'slow' side, with opposite trait values to the ones for the 'fast' side. The second dimension corresponds to a mycorrhizal 'collaboration' gradient, with an 'outsourcing' side: high mycorrhizal colonization, thick root diameter, and a 'do-it-yourself' side: high specific root length and high branching intensity. Interestingly, root nitrogen content tended to relate to both the 'outsourcing' and the 'fast' strategies, depending on whether the traits were

studied at the community scale or at the interspecific scales. This indicates an interplay between mycorrhiza and root nitrogen. We also show that at the interspecific scale, 'do-it-yourself' can be achieved by high reliance on root hair (both length and incidence, Chapter II). In 150 grassland plots, the plant communities with a high proportion of species with 'fast' and 'do-it-yourself' strategies were associated with high land-use intensities and soil parameters related to soil fertility, probably representing some competitive advantages provided by the traits underlying these strategies, and thus allowing for a fast capture of nutrients in this competitive habitat (Chapter III).

Additional dimensions to plant functioning provided by belowground traits and plant strategies

Beyond the two main trait dimensions described aboveground in plants, one related to the overall size of the plants (size axis) and one related to the leaf resource economics (leaf economics spectrum), up to four other dimensions are suspected to exist in plants and describe trait relationships (Laughlin 2014). Additional dimensionality might be provided by different organs that perform different functions in the plant life cycle. A seed-number/seed-size trade-off exists (Gallagher 2014). A wood economic spectrum seems to emerge from large-scale analysis, as the relationships between wood traits do not correlate linearly with the plant size and the leaf dimensions (Chave *et al.* 2009). A flower economics spectrum has been suggested but still lacks a body of empirical data to support it (Roddy *et al.* 2020). The relationships between traits are complex because some dimensionality might arise at a regional scale (Wang *et al.* 2021) or for a portion of the plant phylogeny only (Silvertown *et al.* 2006). Furthermore, the relationships can be blurred when considering higher scales of organization (e.g. the seed-number/seed-size trade-off becomes quantitatively insignificant when interpreting a PCA when we consider a high number of variedly sized species, as in Díaz *et al.* 2016). Alternatively, functional trade-offs might not appear when considering the filtering effect of only one

extremity of an environmental gradient (Laughlin *et al.* 2021). Thus, it is necessary to consider several scales of organization in functional ecology if we want to go beyond the finding that size differences in plants is a main organizer of plant diversity. We notably found that a bi-dimensional root space, characterized by a 'conservation' and a 'collaboration' gradient, and that was recently described across plant functional types (e.g. trees and herbs) from different biomes (Bergmann et al. 2020) does exist in the temperate grassland habitat.

In recent years, the ongoing theory is that a 'fast-slow' gradient is conserved across organ systems in plants (Reich 2014). The belowground implication for this theory is that plants with leaf traits characteristic of a fast acquisition rate of light and CO_2 (i.e. high specific leaf area, high photosynthetic rate, high nitrogen concentration) must be sustained by corresponding fast acquisition rates of water and nutrient from belowground structures. The traits contributing to this 'fast' strategy imply a maximization of root surface by carbon cost, through fine root diameter and low root tissue density, resulting in high specific root length. However, the results for these assumptions are inconsistent , as root and leaf tissue density can be more integrated into a construction-cost gradient that resembles the leaf economic spectrum, whereas root diameter can be partly independent of this construction-cost gradient (Kramer-Walter *et al.* 2016; Weemstra *et al.* 2016). This independence could be a result of the strong evolutionary conservatism of the mycorrhizal infection resulting from a co-evolution between mycorrhizal fungi and plants. Furthermore, as a biological marker, this co-evolutionary process is captured by a correlation between root diameter (proxy of cortical tissues diameter) and intensity of mycorrhizal colonization. Plants with or without mycorrhizal infection would have independently developed the ability to uptake sufficient resources. This would result in an independence of the mycorrhizal aspect of plant functioning with the 'fast-slow' gradient. It is unclear if this pattern is general to vascular plants or specific to trees, as indicators of high fertility (land-use, soil parameters) select herbaceous communities that display both thin fine

roots and low root tissue density (Craine *et al.* 2001; Erktan *et al.* 2018; Prieto *et al.* 2015).

We also found that high soil fertility selected for communities with both low root tissue density ('fast' side of the 'conservation' gradient') and thin diameter ('do-it-yourself' side of the 'collaboration' gradient), indicating that the maximization of root surface by carbon cost hypothesis might hold in herbaceous communities. It is important to note that the filtering of communities was still quantitatively stronger towards the 'fast' side than towards the 'do-it-yourself' side. So, rather than complete independence of the 'collaboration' axis from the leaf economic spectrum, our results suggest a stronger dependence on the root 'conservation' axis and a weaker dependence of the root 'collaboration' axis to the leaf economic spectrum. I hypothesize three mechanisms that could explain this pattern:

1) The mycorrhizal symbiosis has unique benefits for plants to acclimatize to stress. In our grassland communities, soil fertility is associated with the overall harshness of the habitat. Grasslands with an overall low plant productivity potential because of soil texture, slope, climate, low base soil fertility are used as pastures instead of meadows. Thus soil fertility is confounded with other stress factors that impact plant growth and survival like water availability, frost risk, pathogens and herbivores, and soil density. In these habitats, mycorrhiza can provide fitness benefits due to their stress-protection effects, as they add genes in the toolbox of the plant holobiont (Vandenkoornhuyse *et al.* 2015).

2) The unique cost of maintaining the mycorrhizal symbiosis results in an overall decrease in plant nutrient efficiency. Despite the benefits of mycorrhizal infection, each symbiotic partner (plant and fungi) tries to maximize its profit and derive more from the symbiotic interaction from the partner than from its investment. A tightly controlled chemical communication ensures the fairness of the interaction (Kiers *et al.* 2011; van't Padje *et al.* 2020). Because the maintenance of this

communication is costly, mycorrhizal colonization could be suboptimal in situations where their added functionality becomes irrelevant, such as when nutrients are easily forageable by the plants' own roots. Indeed, more pathogenic than mutualistic fungi have been found to be selected with nitrogen and phosphorus fertilization in grasslands (Lekberg *et al.* 2021).

3) The short-lived nature of herbaceous plants render mycorrhizal interaction more flexible compared to trees. Mycorrhizal infection takes several weeks before being widespread in a root system. Compared to trees, these weeks can represent a large proportion of the total lifespan of herbaceous plants. Therefore, mycorrhizal colonization could be less likely to occur in herbaceous, short-lived plants than in long-lived trees, and the co-evolutionary pressure would be lower in herbaceous species. These relaxed constraints could result in herbaceous plants being closer to an idiotype of a root system which maximizes root surface by carbon cost by reducing both root diameter and tissue density.

We observed non-linear patterns in the effects of traits on plant success, often with intermediate optimum (Chapter I). The idea that a quantitatively measurable trait should contribute linearly to fitness has no good theoretical and empirical support (Shipley *et al.* 2016). In analysing root morphological traits, allometric relationships between parameters are unavoidable and can be parameterized when roots are considered cylindrical (Kong *et al.* 2019). Considering that root tissue density and specific root length change non-linearly with root diameter, they can influence model selection and the interpretation of results (Ma *et al.* 2018; Mao *et al.* 2018). These a priori relationships can be integrated in statistical analysis and a framework can be made usable by functional ecology researchers for future research. Dimensionality analysis like PCA and correlation methods usually either do not detect these non-linear patterns or make them readily interpretable. Other clustering methods such as trait

correlation networks (Kleyer *et al.* 2019) should be considered to explore relationships between traits further. For example, the correlation between root diameter and mycorrhizal colonization is usually stronger when comparing root diameters of below 2 mm and above, but more variability exists within the 0-2 mm class. It is primarily low mycorrhizal colonization that becomes unlikely when roots thicken, whereas both low and high mycorrhizal colonization is possible in thin roots (McCormack & Iversen 2019).

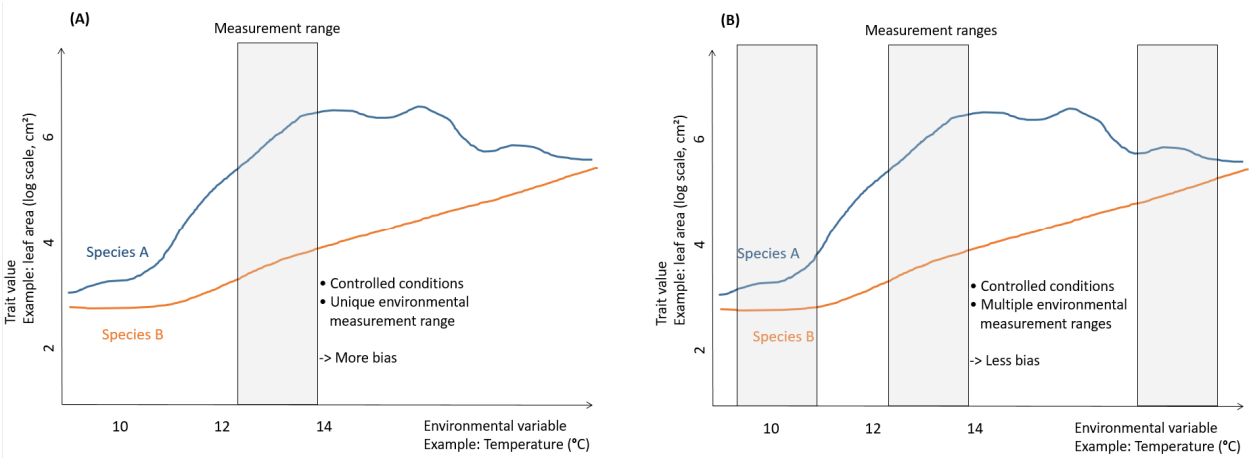
From pot to plot: scaling-up observational greenhouse experiments to understand observational natural experiments

The original data we produced for the needs of this thesis is exclusively trait data collected from controlled or semi-controlled experiments. Note that the distinction between these two terms is not clear. The idea was to measure traits on young plants grown in the same environmental conditions: substrate, climate, duration of growth, size at transplanting time. Thus the difference observed should be mainly due to genetic differences between species that are conserved when comparing different populations from the same set of species. We could then use this glasshouse and semi-field data to infer processes happening to other plants that were not directly observed, given they are from the same species, and given that the hierarchy in trait values is conserved. This approach was previously successfully used for root traits (Schroeder-Georgi *et al.* 2016), and indicates that root trait values could be more important for plant productivity in the context of competition (population scale) than for individual growth.

Criticism has been rightfully raised that this approach ignores both plasticity and intraspecific variation. Unfortunately, this is the case for most plant functional research, even when traits are measured directly on the plants of interest, because of the statistical practicability of focussing on single mean values per species. Scaling-up observation is a central problem in biology, be it when translating yeast and mice results to human health or lab-grown plants to patterns in natural communities (Birch *et al.* 2007; Milinkovitch & Tzika

2007). We could find numerous significant patterns between the traits measured in lab conditions and the occurrence of other individuals of the respective species measured independently, with up to half of the variance explained (Chapter I, III). Thus, this approach is getting a solid empirical validation based on the assumption of the overall conservation in the hierarchy of trait values (Kramer-Walter *et al.* 2016; Yin *et al.* 2021). The approach could be optimised by adding more variability to the environmental conditions in which the replicates are grown, while keeping a balanced design across the entity of interest (here, the species). The resulting mean value should then be closer to the mean trait values observed in field conditions of the species (Figure 1).

Figure 1 Relationship how increased environmental variability during trait measurement can improve scaling-up to natural communities. Each species will express different trait values given a set of environmental conditions in a specific manner. (A) The measurements are done on replicates grown in a unique set of conditions. In that case, the differences between species can be distorted, reflecting more the environmental conditions than inherent inter-specific phenotypical differences. (B) The measurements are aggregated from replicates grown in different conditions; the mean value will be closer to the mean differences in natural communities. For this experimental method, replicates grown in the same environmental conditions could represent a particular case of pseudo-replication, as they become non-independent. To obtain a more realistic proxy of the mean value of a species for a given trait, environmental variability and a balanced number of replicates in the different environmental conditions could add ecologically relevant variability to the species metrics instead of noise.



Future directions

To advance in our understanding of belowground plant functioning, we need a more explicit differentiation of dimensionality analysis (i.e. how many independent axes explain the observed diversity (Laughlin 2014), and mechanistic analysis (i.e finding an explanation for the existence of the axes through adaptive value, e.g. McCormack & Iversen 2019). The dimensionality analysis would make bigger progress when applied in belowground ecology by integrating a maximum number of traits without *a priori* selection from the part of the investigator, while the mechanistic analysis would need careful selection of traits to build conceptual and heuristic models describing the relationships between traits. Both are limited by the fact that few belowground traits are investigated, and their selection is more based on practical reasons like the easiness of measurement (typically root morphology with WinRHIZO), or compilation from databases (Guerrero-Ramírez *et al.* 2021) than for conceptual reasons. We showed that fluoroscopy microscopy, while being less available than other methods, provides crucial insights by quantifying root hair length and incidence. While having the unique opportunity to spend hundreds of hours in the botanical garden to study thousands of plants from hundreds of species, I wondered how much of the variability I could experience was captured by the metrics we end up using. Like stems and leaves, roots have distinct transport and absorptive functions that are more easily separated in tree species than in herbaceous species because of their diameter differences (McCormack *et al.* 2015). By either

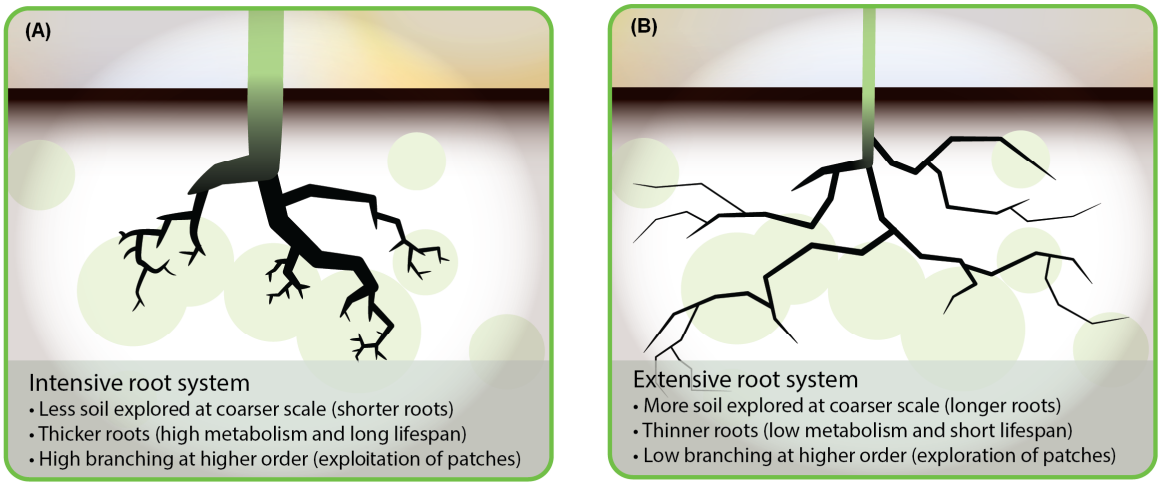
measuring whole root systems or a subset of fine, absorptive roots, we lack the bigger picture of how much of each type of roots exist within a root system. I think root system architecture should be measured and will provide insights into the biomass allocation to different roots. The usual root system classification we find in textbooks that opposes taproots and fibrous roots didn't strike me that much during the observation of root systems by root scanning and rooting depth measurement. I noticed the diversity within tap and fibrous root systems could pinpoint a degree of dominance of main root axes (i.e. long roots mainly serving transport and exploration purposes). In both forbs and grasses, main axes with strong gravitropism could be observed and were thicker than their daughter roots. Quantification of the number and length of these main axes could serve as a basis for investigating the allocation to transport and absorptive roots. Given the sheer length and number of roots, it is vain to separate them by hand. The use of 3D-scanning in transparent substrate could help describe the in-depth root system architecture and yield high, qualitative data (Downie *et al.* 2012). More operable could be using 2-D (flat) high-quality pictures of a whole extracted root system on a uniform background, associated with computer vision and deep learning to identify features of the root system architecture (Voulodimos *et al.* 2018). Classification methods based on root topological data are already bringing us closer to an objective root system clustering system (Bodner *et al.* 2013).

Future research on root systems needs to consider the size of root systems. Again because of methodological limitations, it is challenging to estimate or measure the entirety of the root system. However, this can have implications for the exploration of root economics. A 'fast' strategy could be theoretically achieved through an extensive or an intensive root system, or both (Figure 2). A plant can intensively exploit nutrient-rich patches through high branching intensity of thick, long-lived, metabolically active, fine roots (Figure 2A). Root system plasticity to nutrient-rich patches generally includes an increase in root growth rate, branching,

and diameter. However, these responses are not universal across species (up to 30% of species do not show this response), suggesting that an overarching foraging strategy might be genetically determined at the species level (M. J. Hutchings & E. A. John 2003). Some species show an increase in root diameter with an increase in soil nutrient concentration, whereas others show a decrease (Zobel *et al.* 2007). The investigation of whether maximizing root surface area per carbon cost through a decrease in diameter and root tissue density is a hallmark of a 'fast' strategy might be insufficient if the focus is on the first-order, fine roots only, and does not include the whole root system too. The root system shows a coordinated growth depending on the number, size and distance of soil patches which are highly heterogeneous in nutrients (Jackson & Caldwell 1993). Additionally, root systems could show specialisation in root morphology depending on the spatial distribution and physicochemical (Maire *et al.* 2009). We measured fine and whole root system diameter, root system total root length, and nitrogen uptake rate for ammonium and nitrate separately for 220 species. The data are not available at the moment of writing this thesis. Correlations between fine roots and root system morphology and nitrate and ammonium uptake rates might provide insights into a possible extensive vs intensive root system gradient.

Figure 2 How intensive (A) and extensive (B) root systems can lead result from different trait values measured on fine root-scale, but yielding comparable resource uptake rates at the plant scale. (A) Intensive root systems, characterized by high branching of thick fine roots with high metabolic activity and carbon cost in nutrient-rich patches (pale green circles), could provide a high rate of resource uptake per cm of root. (B) Extensive root systems, characterized by long, thin roots from different root orders with low metabolic activity and carbon cost, could explore a larger volume of soil and more nutrient patches of various quality. The consideration of both whole root systems and fine roots could help reconcile the discrepancies observed in the plasticity of root traits to nutrient availability and alternative strategies for the same set of

environmental conditions.



It is important that we move towards a mechanistic view of the relationships between traits instead of a flat view where all traits are equally important. For example, specific root length is often considered independently of root tissue density and root diameter, despite its calculation generally and directly being derived from these two traits (Rose 2017). As a result, specific root length should invariably be negatively related to root tissue density and root diameter. The point of interest should then be whether most variation in specific root length comes from differences in diameter or differences in root tissue density. This point is rarely explored, and instead, specific root length is simply referred as 'opposite' of one of the two other traits. A mechanistic view would take into account that some traits result from other traits, implying a causal structure. Finding structural relationships would allow building models where assumptions are made on the importance of root traits and their adaptive values can be tested with real-life data (Cabal *et al.* 2020). We could then have a better assessment of the fitness advantages procured by belowground trait syndromes.

Invasion management and crop breeding considerations

The importance of belowground traits we found, added to the previously described importance of aboveground traits for plant naturalization, and could offer an additional screening tool to identify species at risk of becoming invasive. Low root tissue density was a

consistent predictor of abundance across the scale for the 241 species we studied. Routine root morphology assessment could be done for the about 14.000 taxa currently naturalized to confirm this pattern and for alien species sold in nurseries to better assess their naturalization risk. Low root tissue density might also be a marker of vulnerability to pathogens, pests and herbicides and strategies of control based on root traits could be designed.

In agronomy, breeding crops that develop both root hair and mycorrhiza or that have high plasticity for these traits might offer unique advantages in terms of function and reap the unique benefits of both types of structures. A better assessment of the unique benefits of the presence of root hair or mycorrhiza should, however, be investigated. The relationships between environmental variables and root traits we found at the community level can inform the direction of these potential adaptive benefits. Finally, the use of multi-cropping based on the complementarity of root traits could be considered. Rows of 'slow' strategists with high root tissue density might deter soil pests and protect the 'fast' growing cash crops.

Conclusion

This thesis highlights the importance of root traits diversity to understand plant functioning and the transformation in vegetation with global changes. It shows that root traits are both integrated within the leaf economics spectrum, as part of a 'fast-slow' gradient, but also have their own drivers of diversity. By adding root traits, the explanatory power of models about the abundance of plants generally doubled. A fundamental trade-off between 'do-it-yourself' and 'outsourcing' root strategies was further supported by replacing mycorrhiza hyphae with root hairs in 'do-it-yourself'. High soil fertility was associated with both 'fast' and 'do-it-yourself' communities in grassland. As we start to decipher the organisation of belowground traits and their diversity, a significant next step will be to estimate the adaptive values and the fitness advantages provided by the suite of traits.

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Author Contributions

Chapter I

Tom Lachaise performed the experiments, ran the analyses and wrote the paper. Joana Bergmann collected data and participated in the experiments. Matthias Rillig contributed to the design of the experiments. Mark van Kleunen designed the experiments, advised on data analysis and extensively revised the paper. All authors contributed substantially to revisions.

Chapter II

Joana Bergmann designed and performed the experiment, ran the analyses and wrote the paper. Tom Lachaise contributed to the analysis and the conceptual development of the study and revised the paper. Karla Barfuss and Emma Bretherick participated in the experiment and data exploration. Elsa Matthus revised the paper. Mark van Kleunen and Matthias Rillig contributed to the study design and revised the paper.

Chapter III

Tom Lachaise performed the experiments, ran the analyses and wrote the paper. Joana Bergmann collected data, participated in the experiments and in the study design. Till Kleinebecker, Klaus Valentin and Norbert Hölzel collected environmental data. Matthias Rillig contributed to the design of the experiments. Mark van Kleunen designed the experiments, advised on data analysis and extensively revised the paper. All authors contributed to revisions.

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