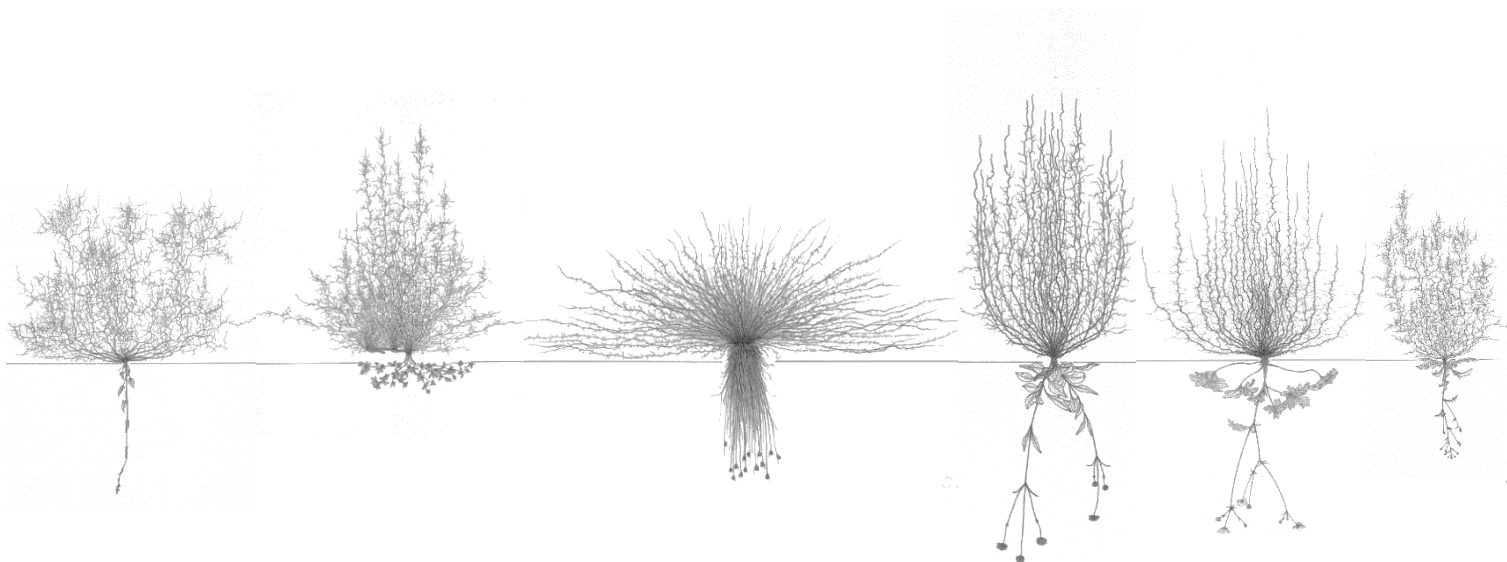


BACK TO THE ROOTS

Belowground nutrient acquisition strategies of herbaceous plants along an N:P gradient



Master's Thesis

by

Daniil Scheifes



Utrecht University

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Utrecht

Daniil Jesse Peter Scheifes (422488)

d.j.p.scheifes@uu.nl

Utrecht University

45 ECTS

Supervisor: Prof. Martin J. Wassen

Second reader: Dr. Ir. Mariska te Beest

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Cover image: root system drawings from left to right: (1) *Rumex acetosa*, (2) *Potentilla erecta*, (3) *Juncus articulatus*, (4) *Succisa pratensis*,
(5) *Ranunculus acris*, (6) *Crepis biennis*.

1, 2, 4, 5, 6 from Kutschera & Lichtenegger, (1992); 3 from Kutschera & Lichtenegger, (1982).

Summary

Since modern days, anthropogenic activity significantly alters the nitrogen (N) and phosphorus (P) cycle, and these changes are substantially affecting terrestrial plant communities. Along with potassium (K), N and P are key nutrients limiting plant growth. Plants occupy distinct niches along N:P ratio gradients, and their physiological adaptation to these environments could potentially help us understand current and future species composition within N- and P-limited soils. This is especially important due to the high concentration of threatened P-limited areas. It has been suggested that belowground rooting behaviour is oriented along a slow to fast growing spectrum. However, such one-dimensional view has not been able to explain the variation in root traits adequately. An improved two-dimensional conceptual framework was introduced to understand resource acquisition by incorporating plants' ability to cooperate with fungi. This framework discerned two largely independent gradients using four core traits: a conservation and a collaboration gradient. This framework has not been assessed from a nutrient stoichiometric perspective, neither has it been used to better understand the threatened status of species. For this, we retrieved twelve belowground traits from trait databases and linked them to a European-wide field dataset of 991 vegetation recordings with species composition, site productivity and biomass nutrient contents of herbaceous plant communities. First, our results confirm that threatened species tend to be concentrated in P-limited sites and species richness was highest at low productivity. Second, threatened species compared to non-threatened species do not possess different belowground traits. Third, using four core traits we demonstrate that a conservation and collaboration gradient can be identified on plant community level. Finally, we show that plant communities adopt a fast and do-it-yourself to a slow and outsourcing belowground strategy along an increasing N:P gradient. However, mycorrhizal traits provide contrasting results, questioning present understanding of fungal importance for belowground nutrient acquisition. Our results imply that, in addition to the benefit for fast-growing species in a nutrient-enriching world, anthropogenic alterations in the nutrient balance may also heavily affect species fitness and survival due to their nutrient-specific rooting strategies. The biggest remaining question is whether species will be able to adapt to changes in nutrient stoichiometry and if they can, how fast this adaptation process will be. Our analysis on threatened species seem to indicate that the answer on this question is not to be found belowground, but much more research is needed.

Preface

One year ago, I was strolling through Periyar national park in India looking forward to my master's thesis starting in March 2020. I arranged to write my thesis at the research group of Jake Alexander, ETH Zurich, on plant trait variation along a climate gradient. I was ignorant at that time, not willing to see what was coming. Alongside the climate crisis, one of the largest humanitarian crisis hit our world. Covid-19 shattered future paths, where academic ecological-oriented research was fated to be mostly indoors behind a desk. Although these disappointing circumstances, Martin and Mariska were very supportive in finding a new topic. I obtained some knowledge on ecohydrology in my studies, but the belowground behavior of plants was new to me. As environmental scientist, it has always fascinated me how the environment shapes diversity in all its forms. While hidden under a layer of soil, plants belowground part is no exemption. Hopefully, my master's thesis can spark new academic research on plants nutrient acquisition strategies. I would like to thank Martin by taking me out in the open air when possible and helping me structuring my thoughts. Furthermore, my thesis relies heavily on previous work of Yuki Fujita. She has assisted me with some difficult concepts and provided me the statistical and programming skills to properly address my research questions. Finally, the university provided students with a study place. I owe a lot to this lucky opportunity, where number 82 was my second home.

Daniil Scheifes, Utrecht, 25th of February 2021

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Introduction

Since modern days, anthropogenic activity significantly alters the nitrogen (N) and phosphorus (P) cycle and these changes are substantially affecting terrestrial plant communities (Vitousek et al., 2008). At this point, anthropogenic N fixation in terrestrial ecosystems exceeds fixation of all natural sources (Vitousek et al., 1997) and this large deposition of N has already shown to negatively affect species richness in these ecosystems (Stevens et al., 2004). While N has an abundant atmospheric pool, P can only be extracted limitary in a selected number of mines and is transported around the globe for fertilizer production (Elser, 2012). It is estimated that since pre-industrial times, humans have accelerated global P-input with 400% (Falkowski et al., 2000) and P pollution can seriously threaten biodiversity in terrestrial ecosystems (Ceulemans et al., 2014). Following Steffen et al. (2015), these alterations on the N and P cycle are momentarily exceeding the planetary boundaries allowing a safe human environment.

Along with potassium (K), N and P are key nutrients limiting plant growth (Elser et al., 2007). Plants need N and P for essential functions such as metabolism, energy storage, light absorption, and expression of genetic information (Sturner & Elser, 2002). The increase in availability of both nutrients could stimulate plant growth and productivity. The hump-backed model already indicated that high rates of productivity drastically reduce species diversity while an optimum is found at intermediate biomass production (Al-Mufti et al., 1977; Grime, 1973, 2001). Besides overall nutrient availability, the relative availability of N and P in plants biomass, also called nutrient stoichiometry and hereafter named N:P ratio, can be used as indicator to assess which nutrient is limiting for plant communities (Verhoeven et al., 1996). Concerning species richness, the highest number of species were observed in sites where N and P are co-limiting (Fujita et al., 2014) which can be explained by the resource balance hypothesis (Braakhekke & Hooftman, 1999). Threatened species tend to be mostly present in P-limited sites, indicating the importance of P fertilization in conservation management (Lannes et al., 2012; Wassen et al., 2005, 2021). One of the targets of the Sustainable Development Goal Life on Land is to prevent biodiversity loss and protect threatened species (UNDP, 2013). To achieve this target, it is crucial to improve knowledge on plant communities under varying N:P ratios.

Plants occupy distinct niches along N:P ratio gradient (Roeling et al., 2018), and must have, from an evolutionary perspective, responded physiologically to differences in nutrient availability to sustain fitness within these different environments. To understand how species have responded to these differences, the concept of functional traits could provide an insight. Functional traits are considered relevant to the response of such organisms to changes in their environment for their growth, reproduction and survival and are defined as morphological, physiological and phenological characteristics (Violle et al., 2007).

First, using the concept of traits, threatened species were smaller than non-threatened species and therefore poor competitors for light. Furthermore, they invest little in sexual reproduction traits and therefore have a low seed number and seed investment, short flowering period and a late flowering start

(Fujita et al., 2014). Second, P-limited plant communities also invest little in sexual reproduction traits (Fujita et al., 2014). This reduced sexual investment of plants in P-limited environments can be explained by the lack of P which is needed for reproductive material such as DNA. Furthermore, they had conservative leaf-economic traits: a low specific leaf area and high leaf dry-matter content (Fujita et al., 2014). Following the leaf economics spectrum, these leaf characteristics are associated with slow-growing species, which invest in a late resource return, compared to fast-growing species, which focus on quick resource acquisition (Wright et al., 2004). Increasing presence of slow-growing species in P-limited environments is in line with the Growth Rate Hypothesis (GRH). This states that species with a slow growth rate have a relatively low content of P-rich RNA, which is needed for growth, and therefore show low P concentrations and high N:P ratios (Main et al., 1997; Sterner & Elser, 2002). For this reason, there is a relationship between plants productivity and biomass N:P ratio.

Besides aboveground observed plant characteristics, the belowground part of the plant plays a key role in our understanding of plants fitness under different N:P ratios. This is especially the case because the primary function of the terrestrial belowground root system is to acquire soil-based resources such as N and P (Fitter, 2002). Nutrient acquisition by plant roots is the ability to mine the soil for these essential nutrients. Plants have evolved a wide range of belowground strategies to take up limiting nutrients by their roots (Bardgett et al., 2014).

As with leaf economic traits discussed earlier, it has been hypothesized that belowground traits can be categorized along a slow-fast growing spectrum (Reich, 2014). A belowground slow strategy is associated with long-lived thick roots, while a fast strategy shows long narrow roots with high metabolic rate. This one-dimensional framework, however, has not been able to explain the variation in root traits adequately, and a multidimensional framework is suggested (McCormack & Iversen, 2019; Weemstra et al., 2016).

Under P-limited conditions, plants fungal associations, also called mycorrhiza, are deemed to be of importance for P uptake (Ceulemans et al., 2011; Lambers et al., 2008; S. E. Smith et al., 2003) and enhances plants belowground nutrient-absorbing surface via their hyphae (S. E. Smith & Read, 2002). Fungal associations have been identified as an key evolutionary adaption for P-uptake by land plants (Smith et al., 2000), next to the availability of cluster roots (Lambers et al., 2008), and it is expected that 85 percent of vascular plants have gained this ability (Brundrett & Tedersoo, 2018). Plants under N limitation seem to rely less on fungal collaboration for their N uptake (Hawkins et al., 2000; Hodge, 2001) and show a high investment in their own root system (Minden & Olde Venterink, 2019; Olde Venterink & Gusewell, 2010; Ryser & Lambers, 1995), although contrasting results exist (Hodge et al., 2001). N fixation via microbial association is an example of a collaborative trait predominantly useful under low N availabilities (Rastetter et al., 2001). Besides a slow-fast growing spectrum, species seem to orient themselves along a second dimension focusing on the ability to cooperate with fungi or choose a self-oriented belowground strategy for nutrient acquisition.

Such a two-dimensional framework was introduced by discerning two belowground strategy gradients. (Bergmann et al., 2020). A conservation and a collaboration gradient were retrieved using only four core root traits (Fig. 1). The conservation gradient differentiates between species with a slow strategy, which have a high root tissue density (RTD) implying slow resource uptake and investment in long-living roots, and species with a fast strategy, that show high root nitrogen concentration (RN) implying high resource uptake but a short lifespan. The collaboration gradient ranges from an outsourcing strategy with a high root diameter (D) allowing carbon investment in fungal partners versus a do-it-yourself soil exploration strategy which requires a high specific root length (SRL). These two gradients can be found independently of plant growth form or biome.

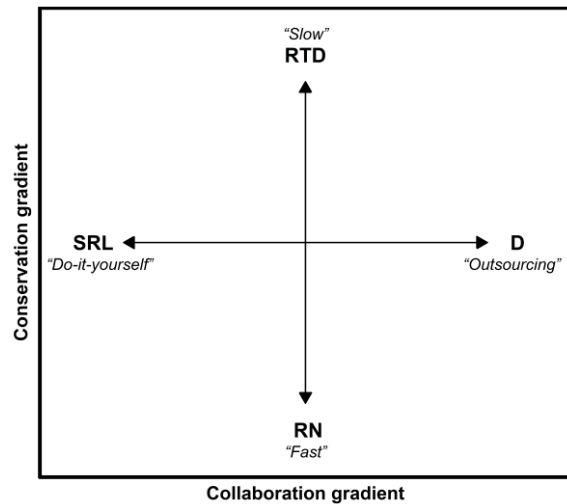


Figure 1 | Conceptual reproduction of the framework of Bergmann et al. (2020) on resource acquisition strategies. The conservation gradient ranges from a fast, with a high root nitrogen content (RN), to a slow strategy with high root tissue density (RTD). The collaboration gradient ranges from a do-it-yourself, with a high specific root length (SRL), till an outsourcing strategy with a high mean root diameter (D).

Problem definition

This thesis will respond to three knowledge gaps. First, the effect of N:P ratio and productivity on species richness and threatened species have been studied with a limited dataset (Wassen et al., 2005). Although the effect of N:P ratio, corrected for productivity, on species richness has been re-examined (Fujita et al., 2014), it is unclear whether the effects of N:P ratio and productivity combined reproduce the same results. Second, aboveground traits were able to shed light on the possible explanation of the threatened status of species (Fujita et al., 2014). However, the effect of belowground traits for threatened species has not yet been assessed. It is unclear whether their threatened status could be explained by a weak belowground nutrient acquisition strategy making them poor competitors belowground.

Third, belowground traits capture multiple key dimensions of plants, but have been mostly underrepresented in large-scale comparative studies (Guerrero-Ramirez et al., 2020). Although, several large-scale studies have focussed on the role of nutrient availability on plant traits (Fujita et al., 2014; Ordoñez et al., 2009), such an analysis is missing for belowground traits. Studies on belowground traits under an N:P gradient, were either experimental studies with a limited number of species (Minden & Olde Venterink, 2019; Olde Venterink & Güsewell, 2010), or field studies with a limited number of sites (Treseder & Vitousek, 2001). More importantly, these studies have not been able to capture the influence of solely N:P, corrected for plants productivity, on belowground nutrient acquisition strategies with a focus on conservation and collaboration. Furthermore, although the conservation and collaboration gradients

have been demarcated on species level, it is unclear whether these gradients can also be observed on plant community level.

Research aims

In this study it was aimed to:

- (1) re-identify species richness along an N:P and productivity gradient by incorporating the red list status within the dataset. This repeated analysis provides a solid basis to better understand the next research aims.
- (2) examine the importance of belowground traits as explanation for the threatened status of species by linking twelve belowground functional traits to threatened and non-threatened species and compare them.
- (3) investigate whether the conservation and collaboration gradient are present on plant community level by assessing four core community-mean belowground traits using a principal component analysis.
- (4) understand how N:P ratio shape belowground strategies within herbaceous plant communities by assessing twelve community-mean belowground functional traits related to a conservation and collaboration gradient and compare them to N:P ratio, corrected for productivity.

This thesis had access to an extensive field vegetation dataset of 991 plots with species composition, site productivity and plants nutrient contents of herbaceous ecosystems.

Research questions

To achieve these four aims, four research questions are formulated.

- (1) How does species richness vary with productivity and N:P ratio?
- (2) Can belowground traits explain the threatened status of species?
- (3) Is a conservation and collaboration gradient observed in our species dataset and can we demarcate these gradients on plant community level?
- (4) What is the relationship between N:P ratio, corrected for productivity, and twelve community-mean belowground traits related to a conservation and collaboration gradient?

Hypotheses

First, it is hypothesized that a larger dataset produces the same results as Wassen et al. (2005) considering the relationship between N:P ratio, productivity, and species richness. The highest number of species is found at intermediate N:P ratio while the number and percentage of threatened species is highest in P-limited sites. Furthermore, highest number of species, threatened species and percentage of threatened species is retrieved at low productivity and declines at higher productivity.

Second, there is no knowledge on specific belowground behaviour of threatened species. However, as indicated by Fujita et al. (2014), the threatened status of species could be assigned to their low height, making them poor competitors for light. We hypothesise that threatened species are also weak competitors belowground which could explain their low canopy height, due to a general lower nutrient uptake. We expect that non-threatened species have a variety of belowground traits, while threatened species show less diversity. This should result in threatened species scoring significantly different for several belowground traits than non-threatened species.

Thirdly, the sampled plots in the dataset mostly originate from a temperate biome. A conservation and collaboration gradient have already been identified in a principal component analysis (PCA) for species from a temperate biome (Bergmann et al., 2020), therefore a similar result is expected. Next, it is hypothesized that these gradients are also present on plant community level because nutrient conditions within site will provide survival for plants with similar successful belowground strategies. We expect that the direction and length of the eigenvectors within the PCA for the community-mean trait variables are relatively conserved. The eigenvectors should be organized in the form of a cross, where RN to RTD is in a 90 degrees angle of D to SRL.

Fourthly, plant communities adopted conservative leaf-economic traits under P-limited conditions (Fujita et al., 2014). Following this aboveground reasoning, in addition to the GRH, it is expected that with increasing N:P gradient belowground strategy would shift from fast to slow. This would mean that traits such as root tissue density, root carbon concentration, root carbon-nitrogen ratio and root dry matter content, would increase, being associated with a slow belowground growth. In comparison, root N concentration and root mass fraction would decrease, which are associated with a fast belowground growth (literature supporting this hypothesis is provided in table 1).

Plants collaboration with fungi provides a key role for plants P uptake under P limitation. Therefore, it is expected that a high N:P gradient is associated with an outsourcing strategy for P uptake, where mean root diameter, mycorrhizal associations and mycorrhizal colonization intensity would increase. In contrast, specific root length, as indicator for a do it yourself strategy, would be decrease. Furthermore, N fixing is hypothesized to decrease along an N:P gradient due to its importance for N uptake, while cluster roots would increase due to its importance for P uptake (literature supporting this hypothesis is provided in table 1).

Table 1 | Traits, categorization within the conservation and collaboration gradient, its rationale and proposed relationship. It is hypothesized that traits associated with conservation and outsourcing strategies are increasing with N:P ratio. Cluster roots and N fixing being two exemption on this expectation.

Trait	Gradient	Rationale	N:P
Root tissue density	Conservation	Proxy for long lasting tissue indicating a slow acquisition strategy (Kramer-Walter et al., 2016; Wahl & Ryser, 2000).	↗
Root nitrogen concentration	Conservation	Is high under high metabolic rate, indicating fast growth (Reich et al., 2008).	↘
Root carbon concentration	Conservation	Relate to root longevity and enables longer life span (McCormack et al., 2012).	↗
Root carbon-to-nitrogen ratio	Conservation	Is negatively correlated to fast plant growth and respiration (McCormack et al., 2012).	↘
Root mass fraction	Conservation	Low investment in belowground root system (Minden & Olde Venterink, 2019; Olde Venterink & Güsewell, 2010)	↘
Root dry matter content	Conservation	Used as proxy for RTD (Birouste et al., 2014).	↗
Mean root diameter	Collaboration	Provides intraradical habitat for arbuscular mycorrhizal (Kong et al., 2014).	↗
Specific root length	Collaboration	Investment in thin roots that explore the soil (Ma et al., 2018). Hypothesized to be important for N-limited soils (Ryser & Lambers, 1995).	↘
N Fixing	Collaboration	Useful under low N-availability (Rastetter et al., 2001).	↘
Mycorrhizal association	Collaboration	Key evolutionary adaptation for P-uptake (F. W. Smith et al., 2000).	↗
Mycorrhizal colonization intensity	Collaboration	Is suppressed under high P-availability (Treseder & Vitousek, 2001).	↗
Cluster roots	Collaboration	Specific adaptation to p-limited environments (Lambers et al., 2008).	↗

Methods

The methodology of this study, based on Fujita et al. (2014), consisted of several phases. First, sample collection within plant communities and plot selection is explained. Second, species trait data plus associated red list status supplementation and community-mean trait value calculation will be elaborated on. Third, the data analysis and statistics will be clarified.

Sample collection plant communities

Dataset

This study builds on a dataset consisting of vegetation data on 643 herbaceous vascular plant communities. This data was sampled between 1992 and 2008 from a variety of countries. The samples contained herbaceous grasslands, fens, marshes, reed bed and dune-slack vegetation. This dataset was enriched by vegetation data of 151 plots which were collected between 1989 and 2017 in the Netherlands, Poland, Italy and France. In the summer of 2020, another 197 plots were sampled by vegetation field experts across The Netherlands and added to the dataset. This resulted in a total of 991 plots across 11 countries (Fig. 1). Due to the diverse characteristics of the dataset in time and space, the sampling methodology within the dataset can be slightly different. For this study, the methodology used this summer will be explained.

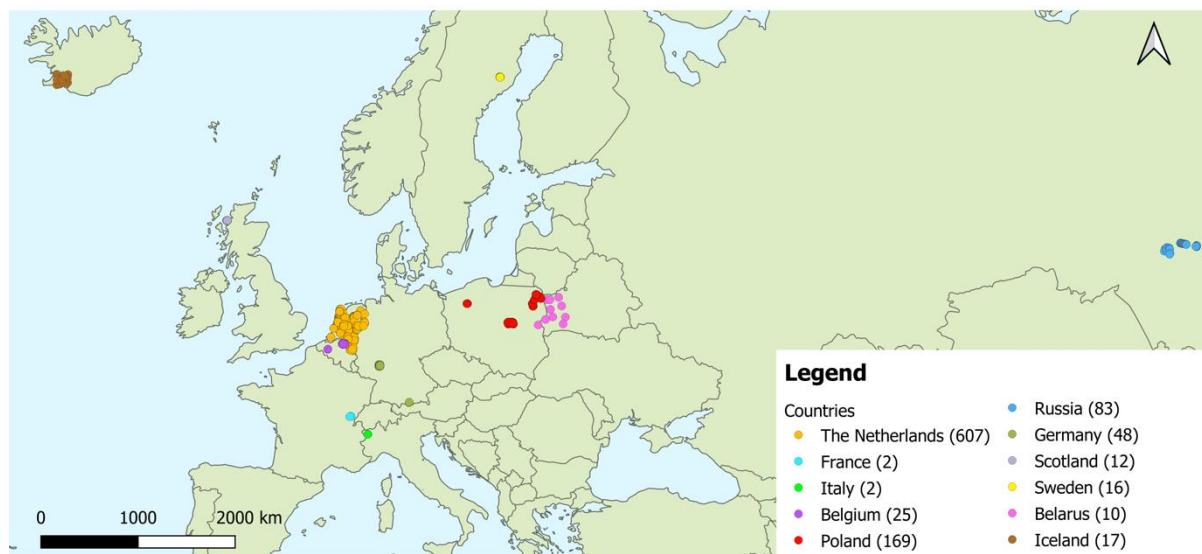


Figure 2 | Map showing locations of data-collection for 991 plots. The points are coloured by country. The number of plots per country are provided between brackets in the legend. Map was produced with QGIS and Natural Earth.

Sampling data

In every plot, species occurrence and abundance was noted. The sample size for species identification varied between 0.06 m² and 25 m², and using experts' site-specific knowledge, plot size was determined to best capture site-specific species composition. The abundance was noted using different methods, such as Van der Maarel, Braun-blauquet, Londo and Tansley. If available, abundance conversion tables were used to convert these scales to numeric percentage cover estimates (Londo, 1976; van der Maarel, 1979). For

several methods, abundance conversion tables were not available and species abundance was estimated combining multiple scale descriptions.

Aboveground living plant biomass was used as proxy for plot nutrient availability and harvested following Wassen et al. (2005) by cutting 20x20 cm just above ground level. Woody plant parts or biomass from previous years were removed from the harvest. These samples were air-dried for several days in an open cardboard or plastic bag to prevent fungal infection. Afterwards, the samples were transferred to a standardized cardboard bag and dried for 48 hours on 70 degrees. The oven-dried samples were immediately measured on a Sartorius BP4100 to determine dry weight.

The dried samples were treated to determine N, P, and K values. The dried plant material was cut by Fritsch Cutting Mill Pulverisette 15 to prevent loss during crushing. A Herzog HP-Ma automatic pulverizing machine grounded the material to fine particles. The N concentration was determined in a subsample between 5 and 20 mg using a Fisons Instruments NA 1500 NCS analyser. The P and K content was calculated using an aqua regia digestion method. A subsample of 125 mg was digested in Teflon Jars for 24 hours using a mixture of 4,5 ml hydrochloric acid (HCL) and 1,5 ml nitric acid (HNO₃) at 90 degrees. Afterwards P and K were determined using an ICP-OES spectrometer (PerkinElmer Avio 500).

Plots omitted

We are interested in herbaceous plants species and N:P ratio. Therefore, any influence of K limitation or woody species on trait values was prevented by omitting plots when:

- (1) woody species abundance made up 50 percent or more.
- (2) they showed signs of K limitation. Plots were considered K-limited if $N:K > 2.1$ and $K:P < 3.4$ (critical values of Olde Venterink et al., 2003).

90 plots were assessed as K-limited, resulting in 872 valid plots.

Species and community-mean traits

Plant synonyms

Next, all species were appointed a trait value from a variety of trait databases. Species can have several synonyms and therefore misidentification must be prevented. A unique species number provided by *NDFD verspreidingsatlas* (Tamis et al., 2004) was appointed to species within our species data and trait databases to assure correct trait supplementation. In addition, extra synonyms were determined using *The Taxonomic Name Resolution of the Iplant Collaborative* (Boyle et al., 2013; see appendix A, table A1 for an overview). Within 872 plots, 632 unique species and subspecies were identified.

Threatened species

National red lists for vascular plants of the designated countries were combined by Fujita et al. (2014) to create a new study-related red list. The list of Belgium was not included due to the position of the Belgium plots: they were close to the Dutch border, having comparable vegetation as the Dutch plots. Also, the list of Belarus was not included due to its relatively small number of plots and its resemblance to Polish vegetation. Due to the high proportion of Dutch plots in the database, the study-related red list was again updated with the most recent Dutch red list (Sparrius et al., 2014).

The reason for this non-regional approach was to eliminate regional habitat characteristics and focus on the fragile characteristics of these species susceptible to environmental change. Scarcity status was not considered to exclude species which are rare despite environmental change. Species were acknowledged to be threatened if their red list status was 'vulnerable', 'threatened' or 'critically threatened'.

Trait supplementation

Twelve functional belowground traits were collected from four databases (Table 2). Types of mycorrhiza on genus-level were determined by Soudzilovskaia et al. (2020). Species having a proven undisputed association such as arbuscular mycorrhiza (AM), ectomycorrhizal (EcM), orchid mycorrhiza (OM), and ericoid mycorrhiza (ErM) were transformed to 1; whereas no mycorrhiza (NM) or disputed mycorrhiza (NM-AM) were transformed to 0. Mycorrhizal colonization intensity represents the percentage of roots infected by AM and is only taken into consideration when infection was present. Therefore, species with a trait value of zero were excluded. Dauciform roots were included in the trait cluster roots due to its analogous function (Shane et al., 2006). Only a small number of species possessed this trait (n=10). For continuous traits, we assigned median values to species. Compared to the mean, this would be less sensitive to outliers or false measurements which are more common in datasets with low observations. For in-text abbreviation of traits, see table 2.

Table 2 | List of functional belowground herbaceous plant traits. Twelve belowground traits were retrieved from four trait databases. Abbreviation after trait in brackets is provided for in-text reference. The traits are retrieved for a dataset with 632 herbaceous species. Due to low availability of trait data, nine traits were only covered by a limited number of species (between 18 and 34 percent). A: Guerrero-Ramirez et al., 2020; B: Tedersoo et al., 2018; C: Kattge et al., 2020; D: Soudzilovskaia et al., 2020

Trait	Scale	Unit	% species	Source
Mean root diameter (D)	Continuous	mm	28	A
Specific root length (SRL)	Continuous	m g ⁻¹	28	A
Root tissue density (RTD)	Continuous	g cm ⁻³	24	A
Root nitrogen concentration (RN)	Continuous	mg g ⁻¹	27	A
Root carbon concentration (C)	Continuous	mg g ⁻¹	23	A
Root carbon-to-nitrogen ratio (C:N ratio)	Continuous	mg g ⁻¹ / mg g ⁻¹	18	A
Root mass fraction (RMF)	Continuous	g g ⁻¹	33	A
Root dry matter content (RDMC)	Continuous	g g ⁻¹	18	A
N fixing	Binary	1: nodulated species, 0: others	99	B
Mycorrhizal association	Binary	1: AM/EcM/ErM/OM, 0: NM/NM-AM	99	C
Mycorrhizal colonization intensity (M%)	Continuous	%	34	D
Cluster roots	Binary	1: ability cluster roots, 0: others	67	D

Community-mean trait value

Combining species trait with plot data, an unweighted community-mean trait value (CMTV) was calculated using:

$$\text{CMTV} = \sum_{j=0}^n \frac{t_j}{a_j}$$

t_j is the specific trait value for species j and a_j is the availability of this trait in the dataset. We assigned $a = 1$ to species with available data and $a = 0$ if data was unavailable. The number of species within a plot is represented by n . This formula calculates CMTV for all plots, where t_j reflects the trait assessed. Woody species were excluded from the calculation.

To ensure normal distribution, D, C, RN, SRL, RMF, RTD and RDMC were log-transformed prior to CMTV calculations. Plots were omitted following two rules to ensure accurate calculations.

1. There were fewer than 3 species with available trait data:

$$\sum_{j=0}^n a_j < 3$$

2. The cover of herbaceous species with available trait value did not exceed 50 percent:

$$\frac{\sum_{j=0}^n a_j}{n} < 0.5$$

Data analysis and statistics

Species richness

Firstly, we would like to reproduce the analysis of Wassen et al. (2005). Species richness is approached using three biodiversity indices: total species count, number of threatened species and number of threatened species per total species (%). Fujita et al. (2014) used a quantile regression analysis to test the effect of N:P ratio corrected for productivity effects. In contrary, in this study N:P ratio was not corrected for productivity when assessing species richness. Furthermore, the relationship of productivity, provided by site biomass productivity, on species richness was assessed. Afterwards a LOESS curve was fitted to the data to assess the trend. In addition, the relationship between the percentage of threatened species and N:P ratio was tested with a linear regression model. To determine nutrient limitation for plots, critical values of Wassen et al. (2005) were used.

Belowground traits of threatened and non-threatened species

Secondly, it was aimed to compare the belowground traits of threatened species with non-threatened species. The relative difference between traits of threatened and non-threatened were quantified by calculating the effect size. For continuous traits, Cohen's *d* was used as effect size measure (see Appendix C for formula and 95% confidence interval). Before analysis, D, RN, RTD, and RDMC were log-transformed; SRL, C:N ratio and RMF were square root transformed. All traits were normally distributed ($p > 0.05$ with Kolmogorov-Smirnov test). For binary traits, log-odds ratio was used as effect size measure (see Appendix C for formula and 95% confidence interval). Positive values indicate that threatened species have a higher trait value than non-threatened species.

Conservation and collaboration gradient on species and plant community level

Thirdly, we are interested in the existence of a conservation and collaboration gradient for four core CMTV traits (RTD, D, SRL, RN), corrected for productivity. First, a PCA was used to assess both the conservation and collaboration gradient within the species dataset. A PCA allows us to reduce multidimensional data to just two dimensions, with the arrows, or eigenvectors, of the variables pointing in the directions of increasing values. Four core traits, RTD, D, SRL and RN, were log- and Z-transformed with a mean of 0 and SD of 1 to sustain variance homogeneity. Subspecies were not included, resulting in 97 species with complete trait data. Traits were not corrected for a phylogenetic signal. Bergmann et al. (2020, see supplementary Figure 2) showed the same outcome when a phylogenetic signal was not included and is therefore, for simplicity, left out. Second, the four community-mean traits regressed for productivity were also Z-transformed with a mean of 0 and SD of 1. There were 279 complete cases.

Community- mean traits along an N:P gradient

Fourthly, it was aimed to determine the significance and explained variation of N:P on several community-mean traits. First, there is a relationship between the N:P ratio in plants biomass and the rate of growth of plants, also called the Growth Rate Hypothesis (GRH). The GRH states that fast-growing species are low in N:P biomass because fast growth requires P-rich RNA. Fast-growing species are mostly present under high productivity and therefore there is a correlation between the aboveground N:P biomass ratio and site productivity measured by aboveground productivity of biomass (g/m²) (see Appendix B, Fig. B1 for linear regression model). Because the interest of this thesis lies in the solely effect of N:P on CMTV (arrow *b* in Fig. 3), irrespectively of the growth rate, the effects of site productivity on N:P will be removed (arrow *a* in Fig. 3). Furthermore, site productivity could influence CMTV directly by a high nutrient availability, and therefore must be corrected for (arrow *c* in Fig. 3). The proposed relationship has been tested by Fujita et al. (2014) and was assumed to be correct.

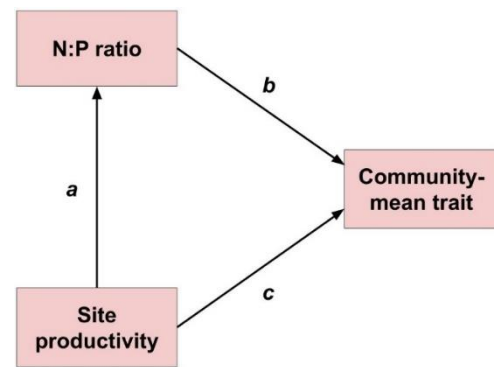


Figure 3 | Data analysis approach for relationship N:P ratio and community-mean trait. This schematic overview is based on Fujita et al. (2014). To assess the effect of N:P ratio on community-mean traits (arrow *b*), the effects of site productivity on community-mean traits and N:P ratio must be removed (arrow *a* and *c*). This achieved by comparing the residual values of N:P ratio and community-mean traits.

The relationship of the residuals of N:P regressed by productivity ($res_{NP,p}$) and the residuals of community-mean traits regressed by productivity ($res_{tr,p}$) were tested using a linear regression model. If the coefficient was significantly ($p < 0.05$) different than zero, a relationship between N:P ratio and a community-mean trait was assumed to be present. $res_{tr,p}$ was derived using a generalized linear model for continuous traits and a logistic model with negative binomial distribution for binary traits. Prior to analysis, N fixing was log-transformed and cluster roots was square-root-transformed. The residuals of the linear regression models were tested on normal distribution ($p < 0.05$ with Kolmogorov-Smirnov test). This was not the case for D, RMF, cluster roots, N fixing, %M and RTD, and for these traits the $res_{tr,p} - res_{NP,p}$ relationship was again tested with Spearman's correlation analysis. To quantify the explained variation of N:P on community-mean traits, taking into consideration the effect of productivity on N:P and shared effect on community-mean traits, the measure of goodness-of-fit of the regression models was calculated. Log likelihood was used for logistic models (deviance) while R^2 was used for linear models (variance). Data analysis was performed in R, figures were made with ggplot2 (R Core Team, 2020).

Results

Species richness

N:P ratio within sites varied between 3.13 and 52.9 and most of the sampled sites were N-limited (67%), followed by P-limited (21%) and N/P co-limited (12%). 72% of the sites were low-productive, having a biomass between 2.25 and 500 g/m². Species richness declines at high productivity, where optimum values for species richness indicators are found around 250 g/m². The number of species and threatened species within sites is highest at low productivity, where both N, P and co-limitation follow the same trend (Fig. 4a, c). Further, the highest percentage of threatened species is found at low biomass where P-limited sites are especially sensitive to changes in productivity by steeply decreasing with biomass (Fig. 4e).

The highest number of species is found at intermediate N:P ratio between the transition from N-limitation to N/P co-limitation and declines when sites become more P-limited (Fig. 4b). The number of threatened species shows a hump-shaped figure and first increases, having its peak at high N:P ratio, but declines afterwards (Fig. 4d). The percentage of threatened species increases with N:P ratio (Fig. 4f) and approaches a linear function ($y = -0.86 + 2.08x$, $p < 0.001$, $R^2 = 0.36$).

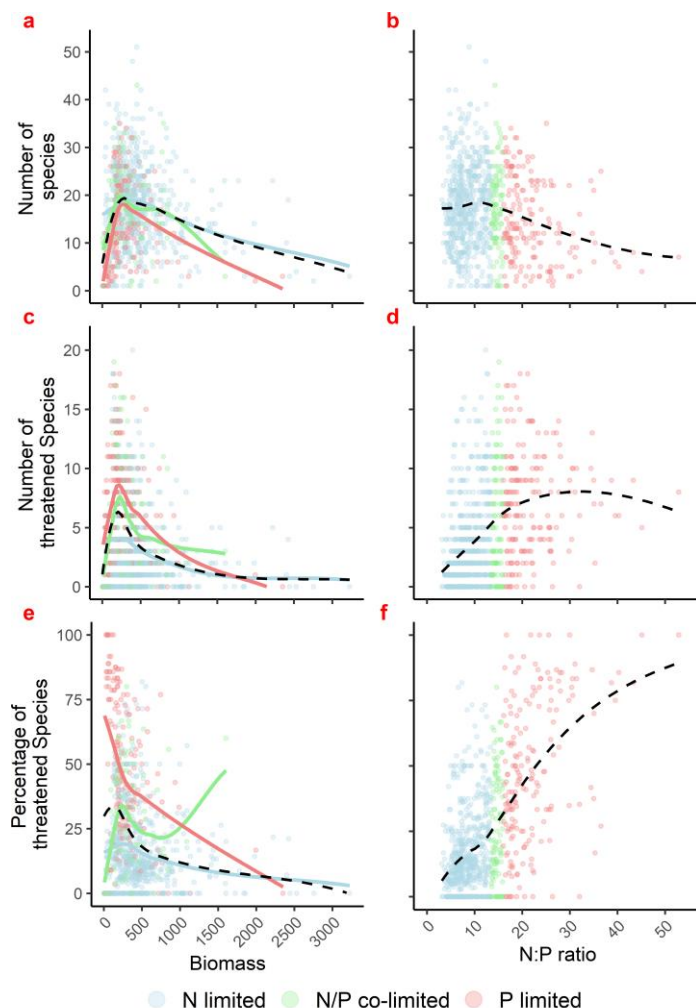


Figure 4 | Species richness affected by productivity and N:P ratio. Total number of herbaceous plants (a and b), number of threatened species (c and d) and percentage of threatened species (e and f) for 872 sites are plotted against biomass (g/m²) and N:P ratio. N-limited in blue (n=586), N/P co-limited in green (n=102) and P-limited in red (n=184). Coloured lines (a, c, e) represent trends along nutrient limited sites in corresponding colour; dotted line (a-f) represents trend of all sites combined (loess regression, span 2/3, degree 1).

Belowground traits of threatened and non-threatened species

From 632 unique identified species, 226 species were categorised as threatened (36%) versus 406 categorised as non-threatened (64%). Threatened species do not possess different belowground traits than non-threatened species (Whiskers do not exceed zero, Fig. 5a, b). Cohen's d and log-odds ratio for RTD and mycorrhizal association scored close to zero (0.02 and -0.04) showing identical trait values between threatened and non-threatened species. SRL, RN, C, RMF and N fixing score lower while D, %M, C:N ratio and cluster roots score higher.

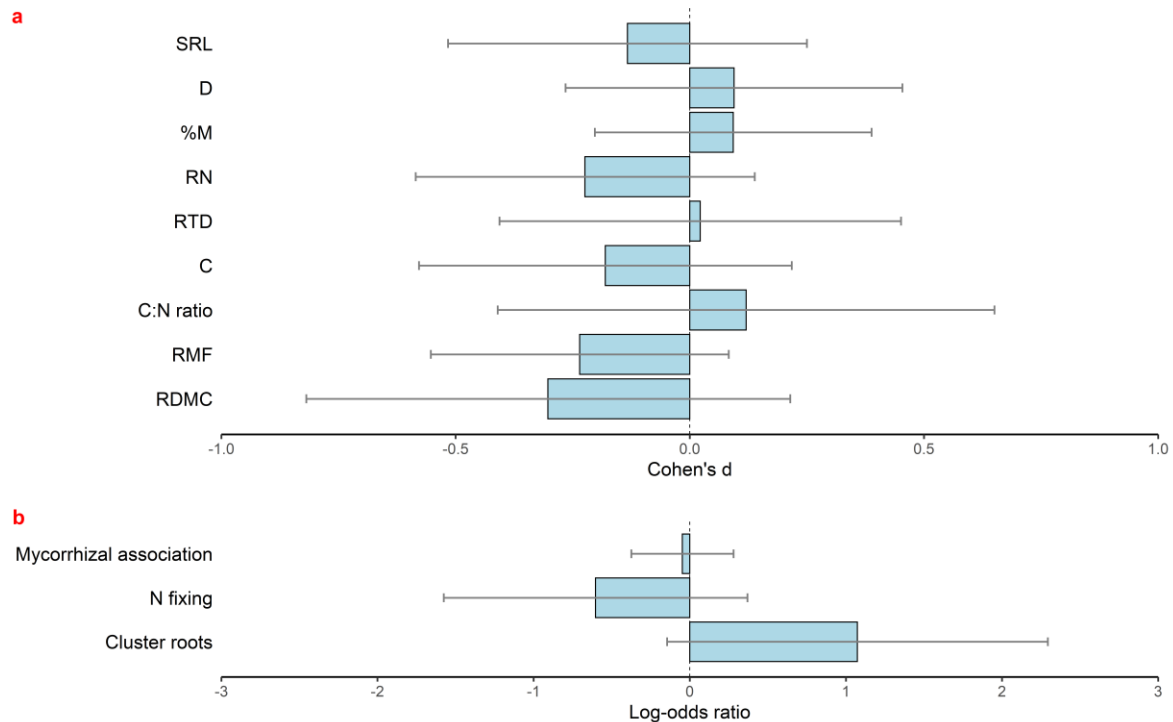


Figure 5 | Difference in belowground trait values between threatened and non-threatened species. (a) Continuous traits were assessed with Cohen's d and (b) binary traits with log-odds ratio. Distribution of threatened vs non-threatened species is from top to bottom: (a) 35/145, 38/137, 63/147, 38/131, 25/128, 31/113, 16/94, 50/162, 17/98 and (b) 225/403, 226/402, 141/278. Positive values indicate higher trait values for threatened species. Whiskers show a 95% confidence interval.

Conservation and collaboration gradient on species and plant community level

On a subset of 97 species with complete data for the D, RN, SRL and RTD, a conservation and collaboration gradient can be determined (Fig. 6a). The collaboration gradient explained 43% of the variation while conservation gradient explained 35%. Both gradients together have a cumulative explanatory power of 77%. A plant community approach shows the eigenvectors being slightly tilted in comparison with the axes (Fig. 6b). Thus, principal components 1 and 2 were not able to reflect the gradients. However, the relative position of the variable eigenvectors is conserved, and therefore a collaboration gradient from SRL till D and a conservation gradient from RTD to RN can be distilled. These gradients are not aligned with the principal components but rotated 45° counter clockwise. This PCA is quite powerful to explain community-mean trait variation, with pc1 and pc2 having almost similar explanatory power (42% and 31%) as species' PCA.

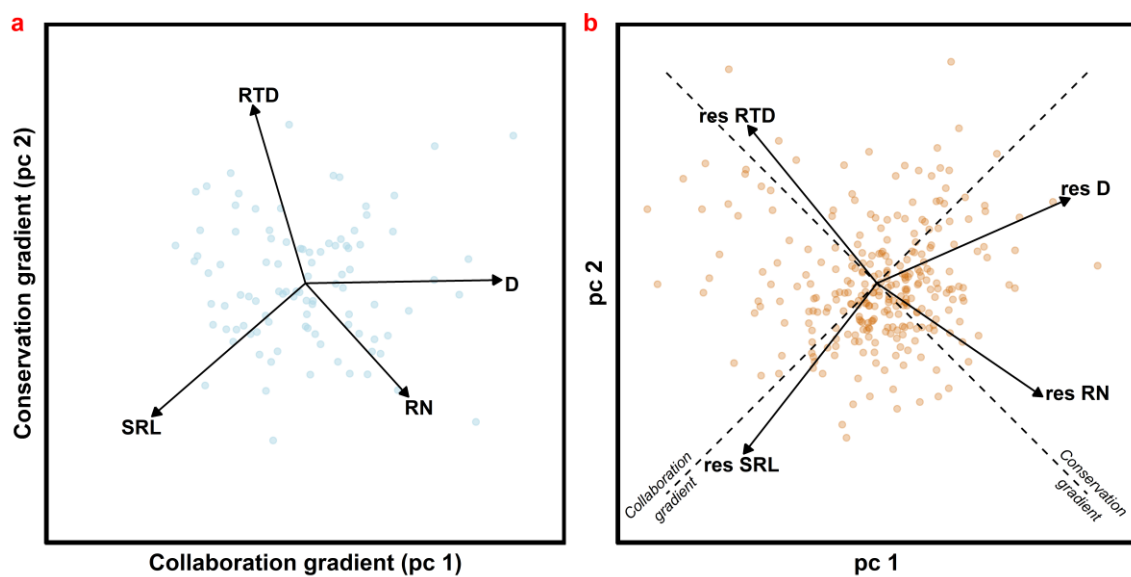


Figure 6 | Conservation and collaboration gradient using four key core traits represented in species and community data. (a) Principal component analysis using four core species traits: mean root diameter (D), root nitrogen concentration (RN), specific root length (SRL) and root tissue density (RTD). For 96 species the collaboration gradient (pc1) explained 42.6% of trait variation, the conservation gradient (pc2) explained 35.1%. (b) Principal component analysis using four community-mean core traits corrected for influence of productivity indicated with “res”. For 279 sites, pc1 explained 42.3% and pc2 explained 31.5% of community-mean trait variation. The conservation gradient and collaboration gradient on community level do not follow both principal components but are rotated 45° counter clockwise, indicated by the dashed lines.

Community-mean traits along an N:P gradient

Concerning community-mean traits associated with a conservation gradient, RTD (Fig. 7b), C (Fig. 7c), C:N ratio (Fig. 7d), and RDMC (Fig. 7f) increase significantly with N:P ratio while RN (Fig. 7a) decreases. RMF shows no significant relationship with N:P (Fig. 7e). Residuals from the model of RTD and RMF were not normally distributed ($p < 0.05$ with Kolmogorov-Smirnov test) and again assessed using Spearman's correlation analysis. For RTD, the difference between both tests were negligible. However, in comparison with the linear regression model, a significant negative relationship was found for RMF ($r = -0.13$, $p < 0.001$). Productivity and N:P ratio explain a relatively large part of the variation for C (30%) and RN (21%). For RTD, C:N ratio and RDMC, N:P ratio could only explain a small part of the variation (3%, 7% and 2%).

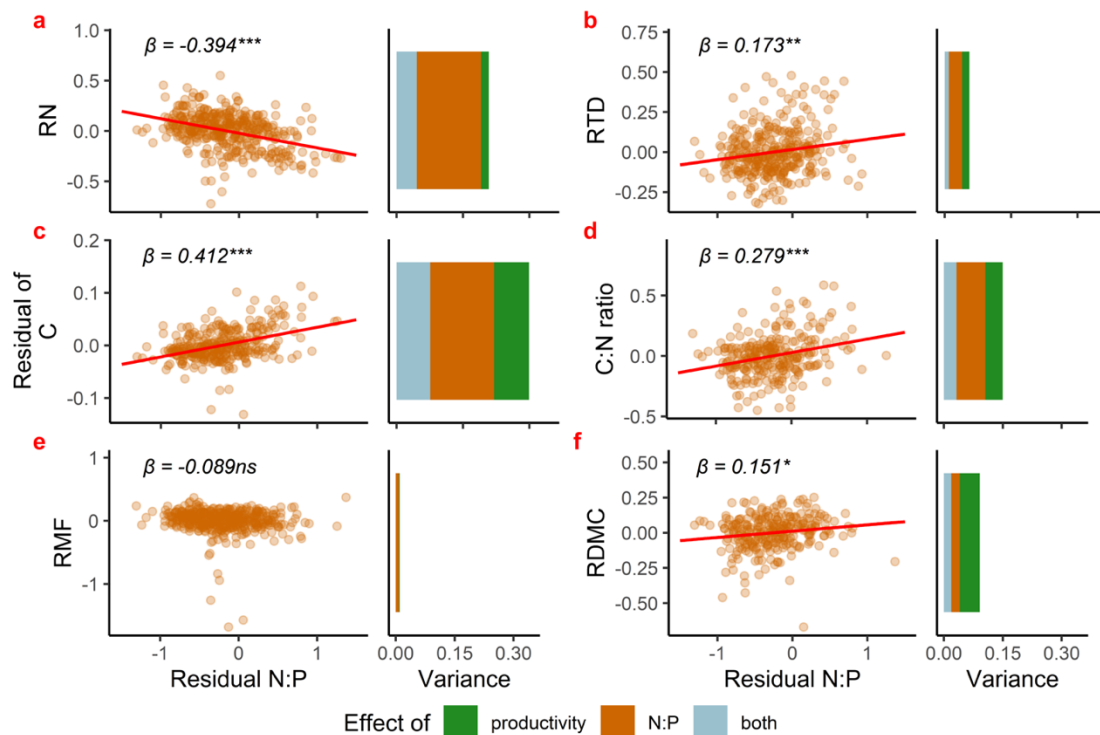


Figure 7 | Community-mean trait values associated with a conservation gradient versus N:P ratio corrected for productivity. Residual values regressed by productivity of six community-mean trait values are on they-axis and N:P ratio on the x-axis. The six traits associated to a conservation gradient are: root nitrogen concentration (**a**, $n=402$), root tissue density (**b**, $n=335$), root carbon concentration (**c**, $n=321$), root carbon-to-nitrogen ratio (**d**, $n=262$), root mass fraction (**e**, $n=416$) and root dry matter content (**f**, $n=262$). For the linear regression models, the standardized Beta coefficient is provided (β) and its two tailed p-value (*** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; NS, Not Significant). The bars indicate the fraction of variance (**a-f**) for community-mean trait values explained by productivity and N:P ratio.

Concerning community-mean traits associated with a collaboration gradient, D (Fig. 8c) and cluster roots (Fig. 8f) increase significantly with N:P ratio, while SRL (Fig. 8a), mycorrhizal association (Fig. 8b), N fixing (Fig. 8d) and %M (Fig. 8e) significantly decrease. Residuals from the model of D, N fixing, %M and cluster roots were not normally distributed ($p < 0.05$ with Kolmogorov-Smirnov test) and again assessed using Spearman's correlation analysis. This did not alter the outcome. The explained variation by productivity and N:P ratio for these traits were all below 20%, with D and SRL being 9% and 6%. D, mycorrhizal association, N fixing, %M and cluster roots show almost no explained variation by productivity and were solely explained by N:P ratio. N:P ratio could only explain 1% of variation in SRL.

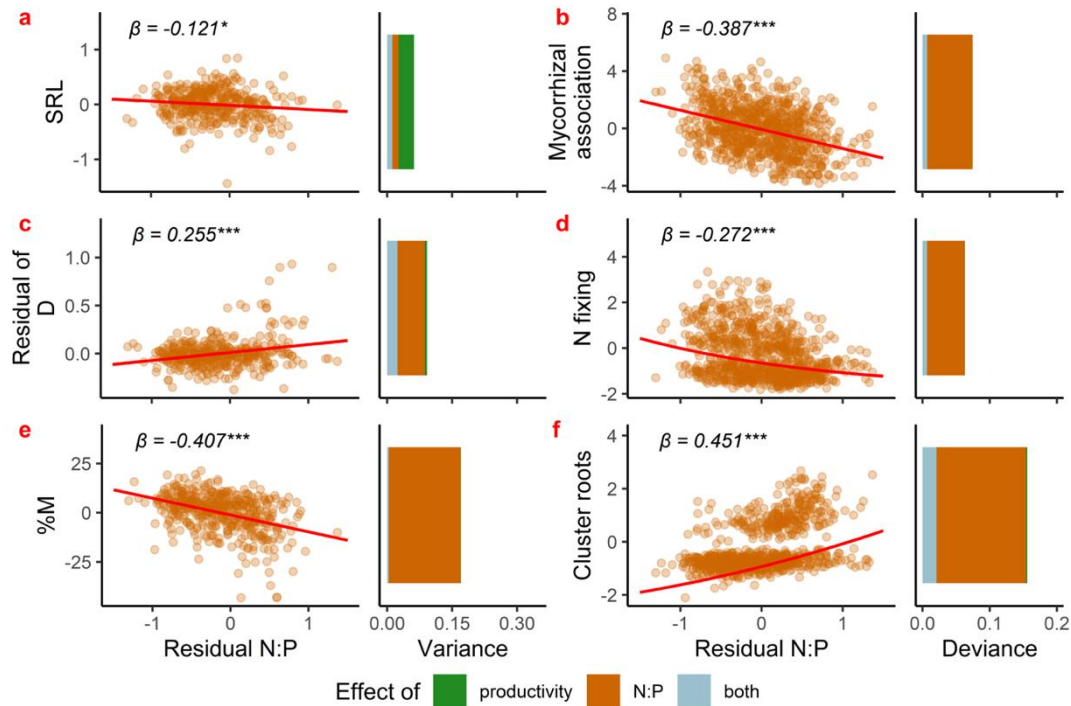


Figure 8 | Community-mean trait values associated with a collaboration gradient versus N:P ratio corrected for productivity. Residual values regressed by productivity of six community-mean trait values are on they-axis and N:P ratio on the x-axis. The six traits associated to a collaboration gradient are: specific root length (**a**, $n=379$), mycorrhizal association (**b**, $n=841$), mean root diameter (**c**, $n=404$), N fixing (**d**, $n=841$), percentage root colonized by fungi (**e**, $n=450$) and cluster roots (**f**, $n=828$). for the linear regression models, the standardized Beta coefficient is provided (β) and its two tailed p-value ($^{***}p < 0.001$; $^*p < 0.05$). The bars indicate the fraction of variance (**a**, **c**, **e** for continuous traits) or deviance (**b**, **d**, **f** for binary traits) for community-mean trait values explained by productivity and N:P ratio.

Discussion

Although belowground traits have gained more interest, its influence on threatened species and nutrient acquisition strategies along an N:P ratio have not yet been fully understood. The aim of this thesis was to understand how N:P ratio shapes species richness, threatened status of species and belowground strategies within herbaceous communities. First, reproduction of the analysis of Wassen et al. (2005) showed similar results. Second, threatened species show no difference in belowground traits compared to non-threatened species. Third, both on species and plant community level, a conservation and collaboration gradient could be discerned. Fourth, our results indicate that herbaceous plant communities along an increasing N:P ratio, corrected for productivity, show a trend from a slow and outsourcing to a fast and do-it-yourself belowground strategy, although mycorrhizal traits show conflicting results.

Species richness

Species richness along an N:P gradient was in line with the first hypothesis and so with previous studies (Fujita et al., 2014; Wassen et al., 2005, 2021). The total number of species is highest at intermediate N:P ratios, which was expected following the resource balance hypothesis (Braakhekke & Hooftman, 1999). The number and percentage of threatened species shows to be highest in P-limited areas. Compared to Wassen et al. (2005), the number of threatened species in sites is higher, especially in N-limited sites. Furthermore, a linear regression model of N:P ratio versus percentage of threatened species shows a tripling of the coefficient (from 0.77 to 2.08). This could be explained by the broader definitions for threatening status in this study or the increased knowledge on threatened status of several species. Overall, our results confirm that threatened species tend to be concentrated in P-limited sites. Besides managing overall nutrient availability, these results again indicate the importance of phosphor fertilization measures in present nature management to better protect threatened species.

Belowground traits threatened species

The absence of belowground trait differences between threatened and non-threatened species did not follow the second hypothesis. While Fujita et al. (2014) has indicated that threatened species are smaller making them poor competitors, the results do not show any difference in belowground traits which could point to a poor belowground competitive position. This would indicate that, in comparison with several aboveground traits, both threatened and non-threatened species seem to compete equally for nutrients. If we would therefore assume that nutrient uptake is not different along species, an explanation of their threatened status seems to be found in the allocation of these nutrients. Fujita et al. (2014) has shown that threatened species invest little in their sexual reproduction, but this low investment seems not to be the consequence of a lower nutrient uptake. This would leave us with two explanations. Firstly, a low nutrient efficient use can be assigned to an inefficient sexual reproduction, where threatened species allocate the same nutrients to sexual production but yield lower effectively sexual traits for its fitness. Secondly, threatened species could allocate more nutrients to other functional traits compared to sexual reproduction traits but due to an inefficient nutritional use this does not improve its fitness. Both

explanations demand further research to understand the threatened status of species in P-limited sites and would help to improve threatened species protection management.

Conservation and collaboration gradients

PCA

The PCA of the four core community-mean traits reveal a conservation and collaboration gradient on plant community level. Bergmann et al. (2020) already showed that species possess an individual position for belowground nutrient acquisition strategies. This study shows this for our species dataset but also for plant communities. This is irrespectively of any effects of productivity, but the same can be concluded when these effects were included (see appendix D). The configuration of these four traits is conserved within a plant community perspective. This finding is important for community ecology, where community traits are identified as a good approach to understand ecological communities (McGill et al., 2006). It is therefore encouraged to further investigate both gradients within a plant community perspective for several environmental factors.

Conservation gradient

Nutrient acquisition strategies shift from fast to slow under increasing N:P ratio, irrespectively of productivity. This is confirmed by the relationship of RTD and RN with N:P ratio. This slow-fast trend along an N:P ratio has already been observed in leaf-economic traits associated with slow-growing species, such as specific leaf area and leaf dry matter content (Busch et al., 2018), regardless of nutrient availability (Fujita et al., 2014), and is now observed in belowground plant traits. This slow-to-fast shift is also confirmed by the relationship of C, C:N ratio and RDMC with N:P ratio. It must be again emphasized that this effect is present when corrected for confounding effects of nutrient availability. This means that a slow or fast leaf- and root-economic strategies is influenced by N:P gradient irrespectively of nutrient availability. More remarkable, fast-growing species are associated with a high metabolic rate which requires high N-input, and it is RN which is limited under low N:P. Reasons why N-limited plant communities seem to mostly rely on fast nutrient acquisition strategies, while this is not the case for P-limited plant communities, is not well understood. A possible explanation could lie in the GRH where fast-growing species are favored under low N:P ratios due to the demand of P-rich RNA (Main et al., 1997; Sterner & Elser, 2002).

Collaboration gradient

Nutrient acquisition strategies shift from a do-it-yourself to an outsourcing strategy under increasing N:P ratio, irrespectively of biomass. The importance of fungal collaboration for P-uptake has already been suggested (Lambers et al., 2008; Lynch & Brown, 2008; S. E. Smith et al., 2003). By using D and SRL as proxy, this study shows an increasing outsourcing strategy in plant communities under increasing N:P ratio. At P-limitation, the cost of a do-it-yourself strategy via investment in a large root system, seems to not outweigh the merits of P acquisition. Increasing D indicates a symbiosis with fungi, and with this association plants can increase its absorbing rooting surface. It must be noticed that our results show that cluster roots and N fixing as specialized strategy are counter examples on both sides of the gradients: plant communities'

ability for symbiosis with N fixing bacteria is highest under low N:P ratios while the ability for specialized do-it-yourself cluster roots increases with N:P ratios.

Mycorrhizal traits

However not in line with the hypothesis is a declining %M with N:P. If fungal associations are more important for sufficient P uptake under high N:P ratio, increasing %M along N:P ratio was expected. In fertilization experiments, %M have mostly been higher under increasing N availability (Blanke et al., 2012; Furlan & Bernier-Cardou, 1989; Treseder & Vitousek, 2001). Nevertheless, several studies show comparable results as ours (Blanke et al., 2005, 2011), indicating the importance of mycorrhiza for N-uptake (Hodge & Storer, 2015; S. E. Smith & Read, 2002). It has been hypothesized by Treseder and Allen (2002) that when N or P is limited for plants, fungal collaboration is maintained. This would indicate %M to be highest at both N:P extremes. This is not the case in our data, hinting at high fungal collaboration solely at N-limitation.

Furthermore, plants' communal ability for mycorrhizal associations is declining with N:P gradient. First, it was tested if the scoring of NM and NM-AM was the underlying reason by assigning 1 point to NM-AM associations instead of 0. This did however not change the declining trend (see appendix E, Fig. E1). NM and NM-AM are overrepresented in specialized environments as habitat and nutritional specialists (Brundrett & Tedersoo, 2018) and plants with specialized alternative strategies such as cluster roots stop interaction with AM fungi (Werner et al., 2018). Therefore, second, it could be argued that P-limited species are more likely to be nutritional specialists, where, for example, cluster roots are a specific adaptation (Lambers et al., 2008; Neumann & Martinoia, 2002). We expect that NM and NM-AM species are more likely to be P-limited in comparison with species having undisputed mycorrhiza, such as AM, EcM, ErM and OM. To check this, the N:P niche of the species was compared to its mycorrhizal association. Species with NM and NM-AM have a significantly higher N:P niche position than species with undisputed mycorrhiza (moderate effect size, see Appendix E). Combining this with a declining plants' communal ability for mycorrhizal association along an N:P gradient, it would suggest that P-limited sites can be considered specialized habitats, where most species lose their mycorrhizal association and gain specialized alternative belowground strategies. Besides cluster roots, it is unclear what specific adaptations are common for P-limited species. One example is the investment in root exudates, such as phosphatases and carboxylates, which allows species to enhance chemical soil properties for P-uptake (Lambers et al., 2006). Overall, these mycorrhizal traits questions present understanding of fungal importance for nutrient acquisition under P-limitation.

Explained variation

The relationship of several core traits, D, RTD and SRL, with N:P ratio seem to follow the hypotheses, but the explained variation by N:P is low (between 1% and 6%). We think this low explained variation could be assigned to, first, the incoherent data sampling nature of the GRoot database. This database possesses trait data from a variety of sources having distinct factors such as soil conditions, plant age or research

design (situ or in pots). This is especially important for root nutrient concentration and biomass allocation, which are most plastic under nutrient limitation (Kramer-Walter & Laughlin, 2017). It is expected that in an experimental design with controlling factors, N:P ratio should explain a larger variation than is presented in this study. Similar to Olde Venterink and Minden (2019), we recommend an experiment under different nutrient ratios for a variety of species to assess the importance of belowground strategies on species fitness, with a focus on a conservation and collaboration gradient.

Second, the low explained variation could be allocated to the sparse availability of root traits which only partially supplemented our species dataset (between 18 and 33 percent for nine traits). Furthermore, for a considerable proportion of species only a few observations were included in the databases, making species trait values less accurate. For example, this could explain the large outliers for several community-mean traits such as D (Fig. 8c) and RMF (Fig. 7f). We therefore strongly recommend increasing belowground trait sampling especially for D, RTD, SRL and RN to increase knowledge on belowground nutrient acquisition strategies. This is most pressing for P-limited species, which are underrepresented in root trait databases. This would increase complete cases for the four core traits, allowing to calculate corresponding principal component values. These values could function as a proxy for nutrient acquisition strategies, assisting further belowground analysis within plant communities.

Our results imply that, in addition to the benefit for fast-growing species in a nutrient-enriching world, anthropogenic alterations in the nutrient balance may also affect species fitness and survival due to their nutrient-specific rooting strategies. Relationships between community-mean traits and N:P ratio is also observed without any correction for productivity (appendix F). This denotes the importance of belowground plant behaviour for conservational practices within nature, where N:P ratio and productivity areas are inseparable. The biggest remaining question is whether species will be able to adapt to changes in nutrient stoichiometry and if they can, how fast this adaptation process will be. Our analysis on threatened species seems to indicate that the answer on this question is not to be found belowground, but much more research is needed.

Conclusion

First, our results confirm that threatened species tend to be concentrated in P-limited sites and species richness was highest at low productivity. Second, threatened species compared to non-threatened species do not possess different belowground traits. Third, using four core traits we demonstrate that a conservation and collaboration gradient can be identified on plant community level. Finally, we show that plant communities in P-limited sites have adopted a slow and outsourcing belowground strategy, whereas N-limited plant communities show a fast and do-it-yourself belowground strategy. However, mycorrhizal traits provide contrasting results, questioning present understanding of fungal influence on belowground nutrient acquisition. Anthropogenic alterations in the nutrient balance may affect species fitness and survival due to their nutrient-specific rooting strategies. Our analysis on threatened species seem to indicate that species are able to adapt belowground, but much more research is needed.

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Appendix A: Plant synonyms

Table A1 | List of 47 plant synonyms assessed using iPlant collaborative (Boyle et al., 2013).

1	<i>Agrostis capillaris</i>	17	<i>Anisantha sterilis</i>	<i>Polygonum mite</i>
	<i>Agrostis tenuis</i>		<i>Bromus sterilis</i>	35
2	<i>Pilosella officinarum</i>	18	<i>Caltha palustris</i> subsp. <i>palustris</i>	<i>Leontodon hispidus</i>
	<i>Hieracium pilosella</i>		<i>Caltha palustris</i>	<i>Leontodon saxatilis</i>
3	<i>Betula pendula</i>	19	<i>Odontites vernus</i> subsp. <i>serotinus</i>	36
	<i>Betula verrucosa</i>		<i>Odontites vulgaris</i>	<i>Euphrasia officinalis</i>
4	<i>Leucanthemum vulgare</i>	20	<i>Galium x pomeranicum</i>	<i>Euphrasia rostkoviana</i>
	<i>Chrysanthemum leucanthemum</i>		<i>Galium pomeranicum</i>	37
5	<i>Lotus pedunculatus</i>	21	<i>Lotus glaber</i>	<i>Calamagrostis canadensis</i>
	<i>Lotus uliginosus</i>		<i>Lotus tenuis</i>	<i>Calamagrostis langsdorffii</i>
6	<i>Myosotis scorpioides</i> subsp. <i>scorpioides</i>	22	<i>Fallopia convolvulus</i>	38
	<i>Myosotis palustris</i>		<i>Polygonum convolvulus</i>	<i>Cnidium dubium</i>
7	<i>Mentha x verticillata</i>	23	<i>Persicaria hydropiper</i>	<i>Selinum dubium</i>
	<i>Mentha verticillata</i>		<i>Polygonum hydropiper</i>	39
8	<i>Dactylorhiza praetermissa</i>	24	<i>Persicaria minor</i>	<i>Aster bellidiastrum</i>
	<i>Dactylorhiza majalis</i> subsp. <i>praetermissa</i>		<i>Polygonum minus</i>	<i>Bellidiastrum michelii</i>
	<i>Orchis praetermissa</i>	25	<i>Persicaria maculosa</i>	40
9	<i>Bolboschoenus maritimus</i>		<i>Polygonum persicaria</i>	<i>Allium suaveolens</i>
	<i>Scirpus maritimus</i>	26	<i>Rhinanthus alectorolophus</i>	<i>Allium ericetorum</i>
			<i>Rhinanthus major</i>	41
10	<i>Brachypodium pinnatum</i>		<i>Rhinanthus angustifolius</i>	<i>Allium schoenoprasum</i>
	<i>Bromus pinnatum</i>	27	<i>Rhinanthus serotinus</i>	<i>Allium montanum</i>
11	<i>Taraxacum officinale</i>		<i>Rumex sanguineus</i>	42
	<i>Taraxacum vulgare</i>	28	<i>Rumex nemorosus</i>	<i>Conyza sumatrensis</i>
12	<i>Rumex triangulivalvis</i>		<i>Isolepis setacea</i>	<i>Conyza daveauana</i>
	<i>Rumex salicifolius</i>	29	<i>Scirpus setaceus</i>	<i>Erigeron sumatrensis</i>
13	<i>Juncus alpinoarticulatus</i>	30	<i>Utricularia intermedia</i>	43
	<i>Juncus alpinoarticulatus</i> subsp. <i>alpinoarticulatus</i>		<i>Utricularia media</i>	<i>Heracleum sphondylium</i> subsp. <i>sibiricum</i>
	<i>Juncus alpinus</i>	31	<i>Dactylorhiza maculata</i>	<i>Heracleum sibiricum</i>
	<i>Juncus alpino-articulatus</i>		<i>Dactylorhiza maculata</i> subsp. <i>maculata</i>	44
14	<i>Bromopsis erecta</i>		<i>Orchis maculata</i>	<i>Bromopsis inermis</i> subsp. <i>inermis</i>
	<i>Bromus erectus</i>	32	<i>Persicaria amphibia</i>	<i>Bromopsis inermis</i>
15	<i>Galium mollugo</i> subsp. <i>erectum</i>		<i>Polygonum amphibium</i>	45
	<i>Galium mollugo</i>	33	<i>Persicaria lapathifolia</i>	<i>Galium palustre</i>
	<i>Galium album</i>		<i>Polygonum lapathifolium</i>	<i>Galium elongatum</i>
16	<i>Bromus hordeaceus</i>	34	<i>Persicaria mitis</i>	46
	<i>Bromus hordeaceus</i> subsp. <i>hordeaceus</i>			<i>Gnaphalium luteoalbum</i>
				<i>Gnaphalium luteo-album</i>
				47
				<i>Ceratocarpus claviculata</i>
				<i>Corydalis claviculata</i>

Appendix B: N:P and site productivity

The relationship between N:P and site productivity in the dataset of 872 plots is provided in Figure B1. As depicted by the growth rate hypothesis, N:P ratios significantly ($p < 0.001$) decreases with site productivity. To assess the effect of solely N:P ratio on CMTV, the productivity effect on N:P ratio is removed.

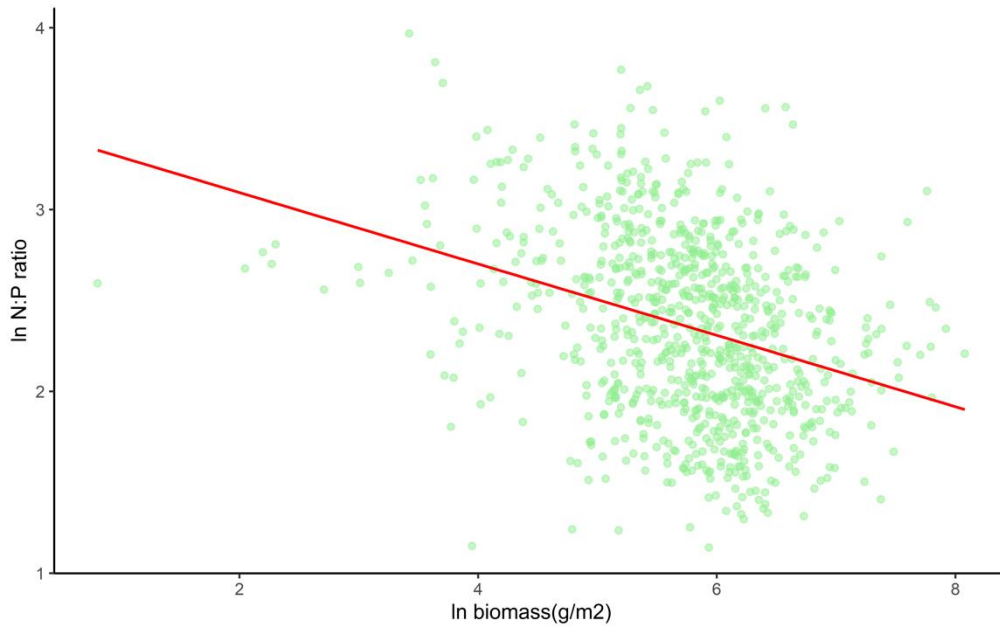


Figure B1 | Relationship between site productivity (biomass in g/m^2) and N:P ratio for 872 plots. Linear regression model ($y = -0.20x + 3.48$) shows to be significant ($p < 0.001$) with $R^2 = 0.23$.

Appendix C: Formulas Cohen's d and log-odds ratio

The equations and descriptions are a direct copy of Fujita et al. (2014) and were unchanged for this study. See their extended methods for further information and in-text citation.

Cohen's d was computed as:

$$d = \frac{\mu_e - \mu_n}{\sqrt{\frac{N_e - 1 \cdot s_e^2 + N_n - 1 \cdot s_n^2}{N_e + N_n}}}$$

where μ_e and μ_n are the mean trait values of threatened and non-threatened species respectively, N_e and N_n are the numbers of threatened and non-threatened species respectively, and s_e and s_n are the standard deviations of threatened and non-threatened species respectively. 95% confidence intervals of Cohen's d were calculated by means of non-centrality parameters (*ncp*) for which the t-value is at the lower or upper 0.025 level (*ncp*_{.025} and *ncp*_{.975}). Lower and upper values of *d*, $d^*_{.025}$ and $d^*_{.975}$, were computed as:

$$d^*_{.025} = ncp_{.025} \cdot \sqrt{\frac{1}{N_e} + \frac{1}{N_n}}$$

$$d^*_{.975} = ncp_{.975} \cdot \sqrt{\frac{1}{N_e} + \frac{1}{N_n}}$$

Log-odds ratio, $\ln\omega$, was computed as:

$$\ln\omega = \ln\left(\frac{a_e + 1/2}{b_e + 1/2}\right) - \ln\left(\frac{a_n + 1/2}{b_n + 1/2}\right)$$

where a_e and b_e are the numbers of threatened species with binary values 1 and 0, respectively, and a_n and b_n are the numbers of non-threatened species with binary values 1 and 0, respectively. Standard error of the $\ln\omega$ was calculated as:

$$s_{\ln\omega} = \sqrt{\frac{1}{a_e + 1/2} + \frac{1}{b_e + 1/2} + \frac{1}{a_n + 1/2} + \frac{1}{b_n + 1/2}}$$

95% confidence intervals of log-odds ratios were computed as $\ln\omega \pm 1.96 s_{\ln\omega}$.

Appendix D: Gradients including productivity

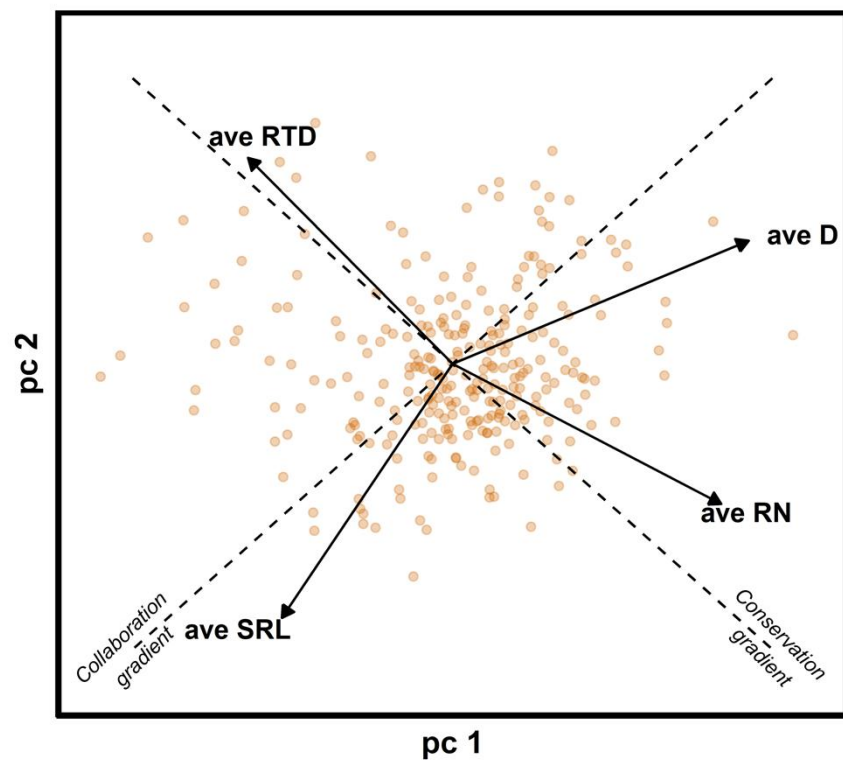


Figure D1 | Conservation and collaboration gradient using four key core traits represented in community data not corrected for productivity. Principal component analysis (PCA) using four community-mean core traits: average mean root diameter (ave D), average root nitrogen concentration (ave RN), average specific root length (ave SRL) and average root tissue density (ave RTD), not corrected for influence of productivity. For 279 sites, pc1 explained 42.6% and pc2 explained 32.8% of community-mean trait variation. The conservation gradient and collaboration gradient do not follow both principal components but are rotated 45% counter clockwise, indicated by the dashed lines. These results are similar to PCA of community-mean trait values corrected for productivity.

Appendix E: Disputed and undisputed mycorrhiza

The regression model was again executed with NM-AM scoring 1 instead of 0. Despite a higher beta-value (from -0.387 to -0.165) and lower explained variation by N:P (from 6.7% to 1.3%), a significant negative trend is still present (Fig. E1). This would indicate that NM and especially NM-AM species are more present at higher N:P ratios.

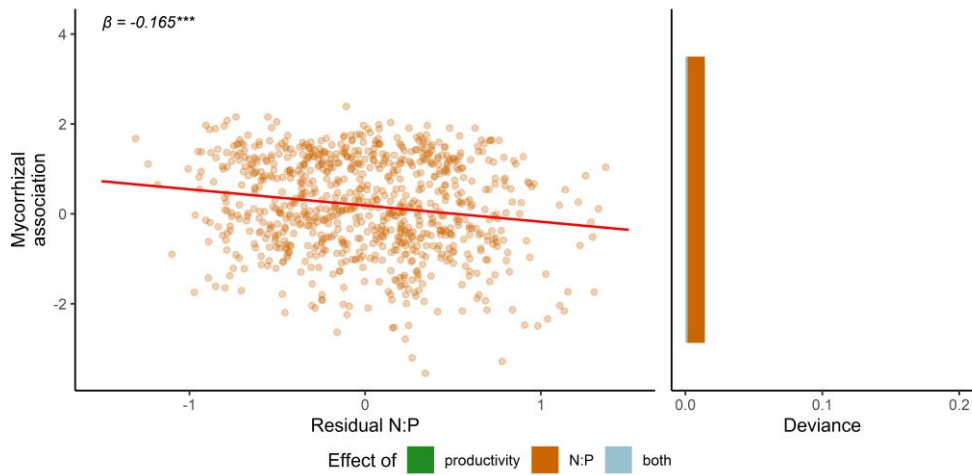


Figure E1 | Reanalysis of mycorrhizal association versus N:P ratio corrected for productivity. Residual values regressed by productivity of mycorrhizal association (n=841) on the y-axis and N:P ratio on the x-axis. Mycorrhizal association is reanalysed where species with NM-AM score 1 and NM score 0. For the linear regression models, the standardized Beta coefficient is provided (β) and its two tailed p-value (***) $p < 0.001$). The bars indicate the fraction of deviance for mycorrhizal association values explained by productivity and N:P ratio.

Figure E2 shows “Undisputed mycorrhiza” (AM, EcM, OM, and EM) or “No or disputed mycorrhiza” (NM and NM-AM) along species’ N:P niche position. It is hypothesized that “No or disputed mycorrhiza” has a higher N:P niche position than “Undisputed mycorrhiza”. Following Roeling et al. (2018), we calculated the N:P niche position of species by the mean of all plots in which it was present. Only species which were present in more than 10 sites were included. N:P niche position was log-transformed and checked on normal distribution ($p > 0.05$ with Kolmogorov-Smirnov test) and equality of variances ($p < 0.05$ with F-test). An unpaired two samples t-test confirms the hypothesis ($p < 0.001$), with a moderate effect size ($d = 0.5$).

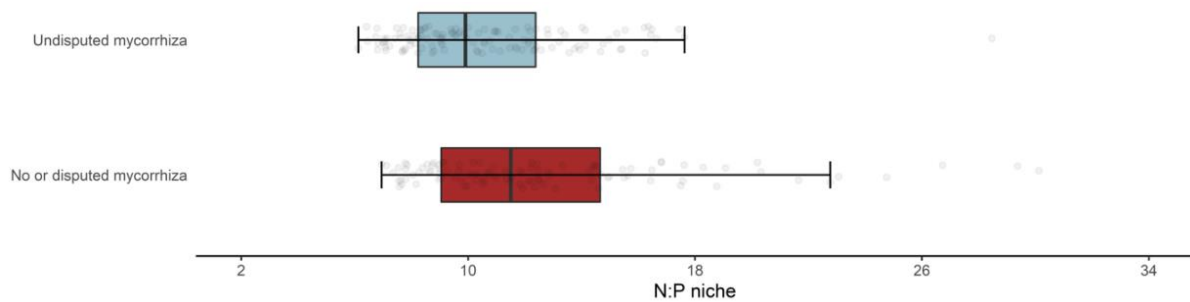


Figure E2 | Species mycorrhizal type along their N:P niche. AM, EcM, OM, and EM were categorized as “Undisputed mycorrhiza” (n=126), NM and NM-AM as “No or disputed mycorrhiza” (n=102). We calculated the N:P niche position of species by the mean of all plots in which it was present. Only species which were present in more than 10 sites were included

Appendix F: CMTV and N:P ratio including effects of productivity

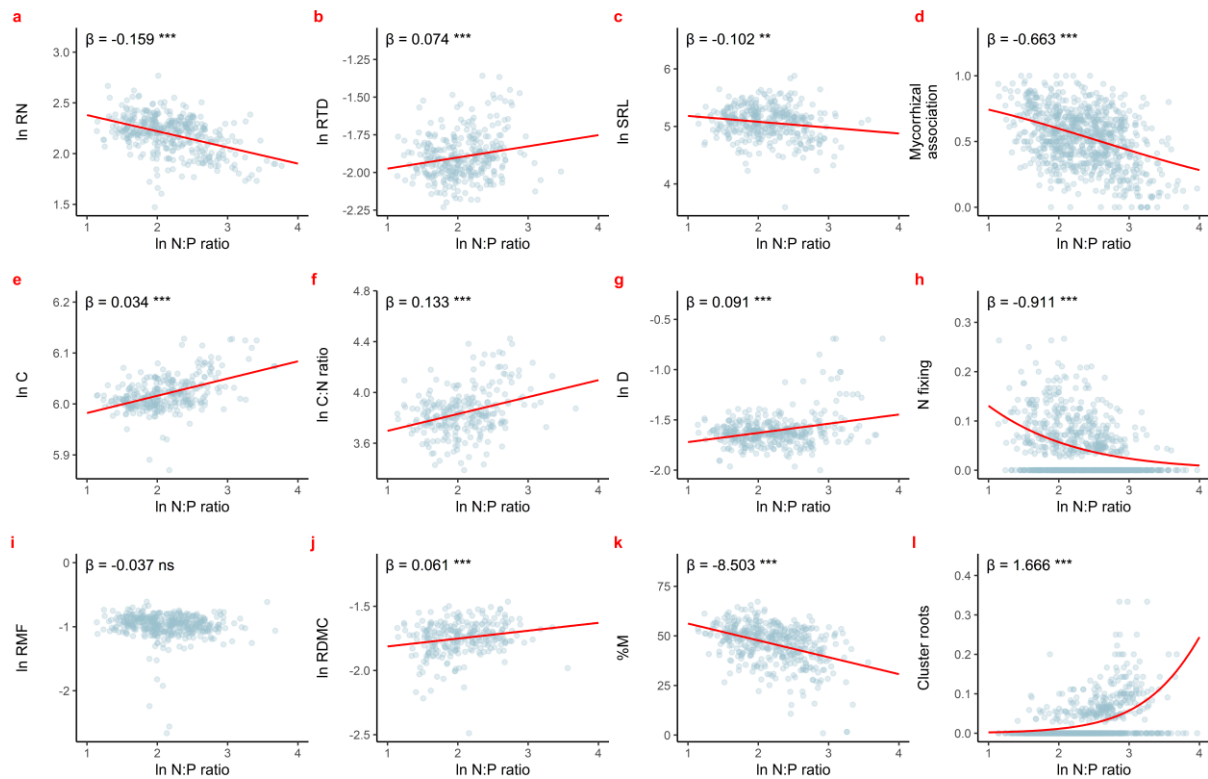


Figure F1 | Community-mean trait values versus N:P ratio, not corrected for biomass. Community-mean trait values are on they-axis and N:P ratio on the x-axis. The tested traits are root nitrogen concentration (**a**, n=402), root tissue density (**b**, n=335), specific root length (**c**, n=379), mycorrhizal association (**d**, n=841), root carbon concentration (**e**, n=321), root carbon-to-nitrogen ratio (**f**, n=262), mean root diameter (**g**, n=404), N fixing (**h**, n=841), root mass fraction (**i**, n=416) and root dry matter content (**j**, n=262), percentage root colonized by fungi (**k**, n=450) and cluster roots (**l**, n=828). RN, RTD, SRL, C, C:N ratio, D, RMF and RDMC were log-transformed prior to community-mean trait calculation. For the generalized linear regression model, the standardized Beta coefficient is provided (β) and its two tailed p-value (***p<0.001; **p<0.01; *p<0.05; NS, Not Significant).