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Research

Ectomycorrhizal diversity, taxon-specific traits and root N uptake in temperate beech forests

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Summary

- Roots of forest trees are colonized by a diverse spectrum of ectomycorrhizal (EM) fungal species differing in their nitrogen (N) acquisition abilities. Here, we hypothesized that root N gain is the result of EM fungal diversity or related to taxon-specific traits for N uptake.
- To test our hypotheses, we traced ^{15}N enrichment in fine roots, coarse roots and taxon-specific ectomycorrhizas in temperate beech forests in two regions and three seasons, feeding 1 mM NH_4NO_3 labelled with either $^{15}NH_4^+$ or $^{15}NO_3^-$.
- We morphotyped > 45 000 vital root tips and identified 51 of 53 detected EM species by sequencing. EM root tips exhibited strong, fungal taxon-specific variation in ^{15}N enrichment with higher NH_4^+ than NO_3^- enrichment. The translocation of N into the upper parts of the root system increased with increasing EM fungal diversity. Across the growth season, influential EM species predicting root N gain were not identified, probably due to high temporal dynamics of the species composition of EM assemblages.
- Our results support that root N acquisition is related to EM fungal community-level traits and highlight the importance of EM diversity for tree N nutrition.

Introduction

Ectomycorrhizal (EM) fungi are key components of nitrogen (N) dynamics in forest ecosystems since they mediate nutrient fluxes at the soil–plant interface (Hobbie & Högberg, 2012; Tedersoo & Bahram, 2019). EM fungi engage in tight interactions with the root tips of trees, forming feeding structures (ectomycorrhizas) composed of interwoven hyphae and root cells for nutrient provision to the host in exchange for plant-derived carbohydrates (Smith & Read, 2010). External EM hyphae enwrap the colonized root tips with a tight mycelium (mantle), contribute to N mobilization from soil by enzymatic activities and thus, facilitate plant N supply (van der Heijden *et al.*, 2015; Baldrian, 2017). The association of EM fungi with roots is considered as a plant strategy to improve access to N (Read & Perez-Moreno, 2003; Tedersoo *et al.*, 2020). This is especially important under N-limiting conditions found in many terrestrial ecosystems (Elser *et al.*, 2007).

Forest soils contain soluble forms of inorganic (ammonium (NH_4^+), nitrate (NO_3^-)) and organic N (amino acids), which are readily accessible to mycorrhizal fungi and whose importance for tree nutrition varies with stand tree species composition (Read & Perez-Moreno, 2003; Chalot & Plassard, 2011; Phillips *et al.*, 2013). The availabilities of these N compounds show strong temporal and spatial fluctuations, for example, with season, soil pH and drought (Dannenmann *et al.*, 2009; Nacry *et al.*, 2013; Schröter *et al.*, 2019; Gao *et al.*, 2020; Nguyen *et al.*, 2020). Trees can use both organic and mineral N (e.g. Gallet-Budynek *et al.*, 2009; Averill & Finzi, 2011; Vadeboncoeur *et al.*, 2015), but most studies conducted with tree species from temperate European forests show higher uptake of NH_4^+ than of other N compounds (Finlay *et al.*, 1989; Gessler *et al.*, 1998; Geßler *et al.*, 2005; Stoelken *et al.*, 2010; Pena *et al.*, 2013a; Gruffman *et al.*, 2014; Jacob & Leuschner, 2015; Leberecht *et al.*, 2015, 2016b; Liu *et al.*, 2017; Nguyen *et al.*, 2017; Reuter *et al.*, 2021). Ectomycorrhizas also accumulate more N from NH_4^+ than from NO_3^- when supplied with mixed N sources (Leberecht *et al.*, 2016a,b), and N fluxes at the EM mantle surface are higher for NH_4^+ than for NO_3^- (Kranabetter *et al.*, 2015), suggesting that EM fungal traits may directly control host N supply.

A further characteristic of EM associations with the roots in forest ecosystems is the high diversity of fungal species at the root tips (Tedersoo & Bahram, 2019). Since EM symbioses evolved polyphyletically, their genetic differences are large and form the basis for a wide range of functional differences among EM species (Kohler *et al.*, 2015; Miyauchi *et al.*, 2020). Physiological studies recognized taxon-specific variation in N utilization among EM fungi for a long time (Pena, 2016), for instance, by growth effects in culture experiments with isolated fungi (France & Reid, 1984; Abuzinadah & Read, 1986; Ahmad *et al.*, 1990; Finlay *et al.*, 1992; Lilleskov *et al.*, 2002b; Nygren *et al.*, 2008) or after feeding different N forms labelled with ^{15}N to young trees colonized with single or composed mixtures of mycorrhizal fungi (e.g. Finlay *et al.*, 1989). Furthermore, the application of ^{15}N revealed strong taxon-specific differences for N absorption in naturally diverse EM fungal communities (Albarracín *et al.*, 2013; Pena *et al.*, 2013b; Pena & Polle, 2014; Valtanen

et al., 2014). Interspecific variation in N uptake was also confirmed by N flux measurements on the mantle surface of different EM taxa from temperate forests (Kranabetter *et al.*, 2015). It has, therefore, been suggested that plant N uptake capacity should be considered an exogenous trait related to the functional diversity of EM fungal species or communities (Hawkins & Kranabetter, 2017). Laboratory experiments with experimentally composited EM communities suggest that diverse EM communities increase plant resource acquisition (Baxter & Dighton, 2001; Booth & Hoeksema, 2010; Hazard *et al.*, 2017; Köhler *et al.*, 2018) but support for this proposal is lacking for natural EM assemblages. Thus, field experiments clarifying the role of taxon-specific traits of EM fungi in plant N acquisition are necessary to understand mycorrhizal-associated soil–plant N fluxes and inform the debate on benefits of distinct EM fungal species or natural EM fungal communities for plant nutrition (Franklin *et al.*, 2014).

In this study, we asked whether EM fungal diversity or specific EM species with high affinities for NH_4^+ or NO_3^- control mineral N uptake and plant allocation in temperate forests. To answer these questions, we determined the diversity of EM fungal communities in beech forests and exposed the attached roots to artificial soil solutions, in which either NH_4^+ or NO_3^- of NH_4NO_3 was ^{15}N-labelled. We measured ^{15}N enrichment in EM root tips, fine and coarse roots and in nonexposed roots along the transport path to the stem and determined ^{15}N enrichment in ectomycorrhizas formed with distinct EM taxa. We conducted our investigations in different seasons (spring, summer and autumn) and in two regions, which differ in soil and climatic conditions (Fischer *et al.*, 2010), to include spatial and temporal variations in EM fungi and beech root N uptake. Based on the results of previous studies, we expected higher N uptake from NH_4^+ than from NO_3^- and taxon-specific ^{15}N enrichment in EM root tips. Here, we hypothesized that specific fungal N traits drive root N acquisition; therefore, distinct EM taxa can be identified, which explain root N acquisition. The alternative hypothesis was that EM diversity drives beech N uptake. In this case, the transport of N would not be dependent on specific fungal traits but would increase with increasing diversity of the root EM fungal assemblage as an emergent community feature. Lastly, if neither EM diversity nor their N traits were important, N acquisition may entirely be related to the size of the active root system.

Materials and Methods

Field plots

We conducted our study in beech (*Fagus sylvatica* L.) forests using selected research plots (100 m × 100 m) of the Biodiversity Exploratories (http://www.biodiversity-exploratories.de/; Fischer *et al.*, 2010). Three plots were located in the Hainich-Dün (HAI) region in central Germany and three plots were located in the Schorfheide-Chorin (SCH) region in the northeastern part of Germany (Supporting Information Table S1). The SCH region is drier and warmer than the HAI region and both regions differ in soil types (Table S1). The HAI plots contained mean NH_4^+ contents of 90–136 μmol kg^{-1} dry soil and NO_3^- contents of 52–166 μmol kg^{-1} dry soil (Table S1). The SCH plots contained mean NH_4^+ contents of 35–45 μmol kg^{-1} dry soil and NO_3^- contents of 29–47 μmol kg^{-1} dry soil (Table S1). The amino acid contents were more than a factor of 100 lower than the inorganic N contents (HAI: 0.7–1 μmol kg^{-1} dry soil and SCH: 0.3–0.4 μmol kg^{-1} dry soil; Pena *et al.*, 2017). In each plot, we established five subplots (5 m × 5 m) containing beech trees of *c.* 5–6 m height.

Stable isotope labelling (^{15}N) and harvesting

Roots of the young beech trees were ^{15}N-labelled and harvested during the growing season in spring before the leaves were fully developed (mid-April 2019), in summer (mid-July 2018) and autumn (mid-October 2018). For root N uptake studies, a single attached beech root was gently removed from the soil without any damage. Severing of the emanating hyphae cannot be avoided. The root was washed with water and incubated in an artificial soil solution for 4 h (Fig. S1). The artificial soil solution was prepared with some modifications as described elsewhere (Geßler *et al.*, 2005; Simon *et al.*, 2011): 90 μM $CaCl_2{\cdot}2H_2O$, 70 μM $MgCl_2{\cdot}6H_2O$, 50 μM KCl, 24 μM $MnCl_2{\cdot}4H_2O$, 20 μM NaCl, 7 μM $FeSO_4{\cdot}7H_2O$, 6 μM K_2HPO_4, 2 μM glycine and 1 mM NH_4NO_3. The pH was adjusted to that of the prevailing soil conditions of the plot (Table S1). The inorganic N concentration in the artificial soil solution was slightly above those present in mineral soils from HAI and SCH (HAI: 0.36–0.78 mM N, SCH: 0.63–0.91 mM N, concentrations calculated with the sum of the NO_3^- and NH_4^+ concentrations according to Table S1 and mean soil water dry-to-fresh weight ratios of 0.72 in HAI and 0.91 in SCH).

To trace N enrichment, we used $^{15}NH_4NO_3$ and $NH_4{}^{15}NO_3$ solutions (98% ^{15}N; Cambridge Isotope Laboratories Inc., Andover, MA, USA). Controls were performed with 1 mM $^{14}NH_4{}^{14}NO_3$. The experiments were conducted between 8:00 and 14:00 h time of day. The selected root was partially submersed in 50 ml of ^{15}N or ^{14}N-containing artificial soil solution (Fig. S1). After 4 h of exposure, the attached root was cut and separated into two segments: the incubated root system and *c.* 7.7 ± 2.4 cm long piece of the nonexposed main root, which connected the incubated root system with the stem (Fig. S1). The incubated root segment was briefly washed with non-labelled soil solution to remove ^{15}N from the root surface. The incubated root system consisted of EM root tips, fine roots (diameter < 2 mm) and coarse roots (diameter > 2 mm). The root segment not in contact with the nutrient solution was defined as the transport segment (Fig. S1). The enrichment of ^{15}N in the transport root segment reflects transport from the root tips towards the stem. In each season, five replicates per ^{15}N form, each from a different tree and five non-labelled samples (^{14}N), were harvested in each plot. In each season, the same trees were used. Since we observed different mycorrhizal assemblages in different seasons, the seasonal samples from the same tree were considered as independent samples. On return from the field to the laboratory, samples were transported in an icebox, stored at 4°C

wrapped in moist tissue paper and immediately used for mycorrhizal analyses. In total, 270 beech roots (15 trees × 3 plots × 2 regions × 3 seasons) were harvested in both regions across all seasons. Since the non-labelled samples were used to determine the natural ^{15}N abundance for the correction of ^{15}N-labelled samples (as described below), the number of ^{15}N-enriched roots was $n = 180$. Aliquots of each root were used to determine ^{15}N in the following tissues: EM root tips (a mixed sample of EM root tips), fine roots, coarse roots and the transport segment. Furthermore, subsamples of 3 or 4 roots per plot and season corresponding to 62 of the $^{15}NH_4^+$ and 60 of the $^{15}NO_3^-$-labelled roots were used for the collection of the individual EM species per root system. This resulted in *c.* 4–5 additional samples per root system and therefore, handling of a larger number of roots was not possible.

EM morphological analyses

A total of 122 labelled beech roots were used for morphological assessment. The collected fine roots were inspected under a dissecting microscope (Leica M205 FA; Leica Microsystems GmbH, Wetzlar, Germany) and used to determine the abundance of the vital EM root tips as well as dead and vital non-mycorrhizal (NM) root tips (Valtanen *et al.*, 2014). Dead root tips were distinguished from vital root tips by their shrunken and dry appearance. A total of 450 root tips were inspected per ^{15}N-labelled root system. If the available root system contained < 450 root tips, all root tips were counted. The EM root tips were assigned to a morphotype based on distinct morphological traits such as mantle colour and structure, type of branching, abundance of external hyphae, shape of unramified ends and rhizomorphs (Agerer, 2001). All morphotypes were photographed at ×10–40 magnifications using a digital camera (Leica DFC 420 C) attached to the microscope. The documentation is shown in Table S2. In total, in each region, *c.* 40–100 root tips from each distinct morphotype were cut using fine forceps. A total of 8–10 root tips per morphotype were used for molecular analysis, and the remaining pools of each morphotype were stored at −80°C and used for ^{15}N measurements. All remaining EM root tips of a sample were cut and stored as a mixed EM sample. Two different sets of equipment were used to process the labelled and non-labelled root tips to avoid cross-contamination.

DNA extraction and molecular identification of the EM fungal species

Ectomycorrhizal fungal species were identified by sequencing the internal transcribed spacer (ITS) region of each MT. The root tips of a distinct morphotype were ground with a pellet mixer (VWR 431-01009; Avantor, Darmstadt, Germany). DNA was extracted using the innu PREP Plant DNA isolation Kit following the SLS protocol as recommended by the manufacturer (845-KS-1060250; Analytikjena GmbH, Jena, Germany). For EM identification, the fungal rDNA ITS region was amplified by PCR tag polymerase using the primer pair ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) and ITS1F (5′-CTTGGTCATTTAGAGGAAGTAA-3′) (White *et al.*, 1990; Gardes & Bruns, 1993) as described previously (Pena *et al.*, 2017). Sanger sequencing was performed by a sequencing service (Microsynth, Göttingen, Germany). The STADEN package (v.2.0.0b11) was used to assemble the sequences. For fungal identification, the Basic Local Alignment Search Tool (BLAST) was used to search the sequences against the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/) and UNITE (http://unite.ut.ee) open-access databases. A species name was assigned to a morphotype when BLAST matches showed sequence identity > 99% (except four species with 97% sequence identities: *Russula integriformis*, *Pezizales* sp. 2, *Helotiales* sp. 3 and *Sebacina incrustans*). Details of the assignment of morphotypes to sequences are shown in Table S2.

Determination of ^{15}N, ^{14}N and C

Samples of labelled and non-labelled EM root tips (mixture from the remaining root tips), fine, coarse and transport roots were oven-dried at 60°C for 1 wk and ground to a fine homogeneous powder with a ball mill (Type MM400; Retsch, Haan, Germany). To measure ^{15}N enrichment in distinct EM species, each sample of the collected morphotypes was freeze-dried for 1 wk and crushed with a spatula inside an Eppendorf reaction vessel. Then, aliquots of the samples were weighed into 4 mm × mm tin capsules (IVA Analysentechnik, Meerbusch, Germany) using a super-micro balance (S4; Sartorius, Göttingen, Germany). The following amounts of samples were used for the determination of ^{15}N, ^{14}N and C: 0.29–1.50 mg for individual morphotypes, 1.0–1.5 mg for mixed EM root tips, 2–5 mg for fine roots, coarse roots and the transport root segment. The amounts of ^{14}N, ^{15}N, ^{12}C and ^{13}C were measured at the Centre for Stable Isotope Research and Analysis (KOSI, Georg-August-Universität Göttingen, Germany) using separate devices for the measurements of labelled and non-labelled samples (labelled – Isotope mass spectrometer: Delta C, Finnigan MAT, Bremen, Germany; Interface: Conflo III; Thermo Electron Corp., Bremen, Germany; element analyser: NA1108; Fisons-Instruments, Rodano, Milano, Italy and non-labelled – mass spectrometer: Delta V Plus, Finnigan MAT; Interface: Conflo III; Finnigan MAT; element analyzer: NA1110; Fisons-Instruments). Acetanilide was used as the calibration standard (71.09% C, 10.36% N).

Calculations of ^{15}N uptake

The relative ^{15}N contents were expressed as the ratio:

$$^{15}N \text{ atom } \% = \left(\frac{^{15}N}{^{14}N + ^{15}N}\right) \times 100 \qquad \text{Eqn 1}$$

where ^{15}N and ^{14}N are the contents (μg) of these isotopes in a sample.

$$^{15}N \text{ atom } \% \text{ excess} = {}^{15}N \text{ atom } \%_{(\text{labelled tissue})} - {}^{15}N \text{ atom } \%_{(\text{non-labelled tissue})} \qquad \text{Eqn 2}$$

For the determination of ^{15}N atom % excess (APE), labelled and non-labelled tissues refer to corresponding samples, for example, ^{15}N-labelled and non-labelled fine roots, collected in the same plot and season. For the determination of APE in morphotypes, we used mixed, non-labelled EM samples (per forest and per season) as controls. It is known that the natural ^{15}N abundance varies among different EM species (Lilleskov *et al.*, 2002b; Hobbie & Agerer, 2010; Hobbie & Högberg, 2012), but these variations are much lower than the enrichment found here (Table S3) and therefore should not affect our results.

^{15}N enrichment in particular tissues (mixed EM root tips, fine roots, coarse roots and transport roots) and in distinct EM species was calculated using the following equations:

$$^{15}\text{N enrichment}_{\text{tissue}}\ (\mu g\ g^{-1}DW) = APE/100 \times N \quad \text{Eqn 3}$$

$$^{15}\text{N enrichment}_{\text{EM species}}\ (\mu g\ g^{-1}DW) = APE/100 \times N \quad \text{Eqn 4}$$

where N is the concentration of nitrogen in the sample (μg g^{-1} DW) and 100 the conversion factor between % and a fraction of ^{15}N.

The newly taken up ^{15}N of the labelled root system was calculated as:

$$\begin{aligned} ^{15}\text{N}_{\text{content new}}(\mu g\ \text{root}^{-1}) = {} & {}^{15}\text{N enrichment}_{\text{fine roots}} \\ & \times DW_{\text{fine roots}} \\ & + {}^{15}\text{N enrichment}_{\text{coarse roots}} \\ & \times DW_{\text{coarse roots}} \\ & + {}^{15}\text{N enrichment}_{\text{transport root}} \\ & \times DW_{\text{transport root}} \end{aligned} \quad \text{Eqn 5}$$

where DW refers to the total dry mass (g) of each root fraction.

$$^{15}\text{N enrichment}_{\text{whole root}}\ (\mu g\ g^{-1}\ DW) = \frac{^{15}\text{N}_{\text{content new}}\ (\mu g\ \text{root}^{-1})}{\text{Total root dry mass}} \quad \text{Eqn 6}$$

The contribution of a distinct EM species to ^{15}N enrichment in an EM assemblage was calculated as:

$$^{15}\text{N contribution of EM species } i\ (\mu g\ g^{-1}\ DW) = \frac{\text{Abundance of EM species } i \times {}^{15}\text{N enrichment of EM species } i}{\text{Abundance of all EM root tips in the sample}} \quad \text{Eqn 7}$$

where the abundance of EM species *i* refers to the number of root tips colonized by EM species *i* in a root sample and the abundance of all EM species refers to the number of vital mycorrhizal root tips in that root sample. Since the same EM species were not present in all plots, the number of replicates per EM species pool varied from 2 to 6 (Table S3). The ^{15}N contribution of each EM species was used to determine the weighted ^{15}N enrichment of the EM assemblage:

$$\text{Weighted}^{15}\text{N enrichment of EM assemblages}\ (\mu g\ g^{-1}\ DW) = \sum_{i=1}^{n} (^{15}\text{N contribution of EM species } i) \quad \text{Eqn 8}$$

To control the validity of the weighted ^{15}N enrichment of EM assemblages, we fitted the measured ^{15}N enrichment $_{\text{mixed EM species}}$ (Eqn 3) to the calculated ^{15}N enrichment of the EM assemblage (Eqn 8) and tested the deviation from the expected slope = 1 from a linear model using the function 'compare regression curves' in Statgraphics CENTURION XVIII (2017 by Statgraphics Technologies Inc., The Plains, VA, USA).

We calculated EM colonization and root vitality as:

$$\text{EM colonization}\ (\%) = \frac{\text{EM root tips}}{(\text{EM} + \text{NM root tips})} \times 100 \quad \text{Eqn 9}$$

$$\text{Root vitality}\ (\%) = \frac{\text{EM} + \text{NM root tips}}{\text{Total number of root tips}} \times 100 \quad \text{Eqn 10}$$

Statistical analyses

The statistical analyses were performed in R v.4.0.2 (R Development Core Team, 2020). Data were visualized with R using the GGPLOT2 package (Wickham, 2009). The data distribution and homogeneity of variances were inspected visually using histograms and residual plots. Data were logarithmically transformed to meet the criteria of normal distribution and homogeneity of variances when necessary. EM species richness, Shannon diversity (H') and evenness were calculated using the VEGAN package (Oksanen *et al.*, 2013). Rarefaction curves were conducted for each sample, indicating sufficient sampling depth (Fig. S2). Generalized linear mixed effect models (*glmer*, Poisson regression and χ^2-test) were used with the function *glmer()* from the LME4 package to analyse the count data such as EM species richness (Bates *et al.*, 2015). Linear mixed-effect models (lmer) with the function *lmer()* from the LME4 package were used to compare means of continuous data such as C, N, C : N ratio, ^{15}N enrichment and $\delta^{13}C$ (Bates *et al.*, 2015). Random factors used in *glmer* and *lmer* models are mentioned in figure and table legends. The Beta regression test was used with the function *betareg()* from BETAREG package to compare the means of percentage data (EM colonization and root vitality; Cribari-Neto & Zeileis, 2010). Differences among the variables were calculated using the *Anova()* function from the CAR package (Fox & Weisberg, 2011). Effects of region and season on the composition of the EM assemblages were tested by analysis of similarity (ANOSIM with 999 permutation steps) using the function *anosim()* in the VEGAN package (Oksanen *et al.*, 2013).

To determine the preferred N source (NH_4^+ or NO_3^-), we fitted linear mixed-effect models with plot and season as random factors. Pairwise differences between groups (root fractions,

season and region) were compared with a *post hoc* test (Tukey's honestly significant difference, HSD) using the function *glht()* from the MULTCOMP package (Hothorn *et al.*, 2008). To detect significant differences in ^{15}N enrichment among EM species in different seasons, we fitted linear mixed effect models with plot and region as a random factor (Bates *et al.*, 2015). Significant differences in ^{15}N enrichment among different parts of the root system and among different EM species were calculated using the *Anova()* function from the CAR package (Fox & Weisberg, 2011).

We fitted linear mixed-effects models with the *lmer()* function from the STATS package (Bates *et al.*, 2015) using ^{15}N enrichment in the transport root segment (Eqn 3) as the dependent variable, and EM species richness, Shannon diversity, fine root biomass, season and root vitality as predictors. Plot and region were set as random factor. The *vif()* function from the CAR package (Fox & Weisberg, 2011) was used to check for collinearity among the predictors. Predictors with variance inflation factors (VIF) ≥ 2.5 were excluded from the model, because this threshold is considered to indicate that there is still considerable collinearity among the predictors, which would violate the model assumptions (Johnston *et al.*, 2018). This threshold resulted in removing EM species richness for both nitrogen treatments (VIF = 4.41 for $^{15}NH_4^+$, VIF = 9.25 for $^{15}NO_3^-$). The models were further developed with stepwise backward elimination using the *step()* function from the STAT package (Bates *et al.*, 2015). The model with the lowest Akaike information criterion was chosen. The relationship between Shannon diversity and ^{15}N enrichment in the transport segment was visualized by Pearson correlation using the *cor.test()* function from the STATS package (R Development Core Team, 2020). The same approach was applied to predict the best model for ^{15}N enrichment in the whole root system (Eqn 6).

We used the function *glmnet()* from the GLMNET package (Friedman *et al.*, 2010) and conducted Least Absolute Shrinkage and Selection Operator (LASSO) regression to detect predictive EM species for ^{15}N enrichment in the transport root. LASSO regression was used to estimate the contribution of each predictor against the dependent variables (Warton *et al.*, 2015). The LASSO approach provides regression coefficients for all predictors to measure their stability scores. Predictors with zero coefficients are nonsignificant, and a coefficient above 0.6 is usually a significant predictor (Meinshausen & Bühlmann, 2006). Here, we used ^{15}N enrichment in the transport root segment as the dependent variable to fit the LASSO regression model. As independent variables, the ^{15}N contributions of each EM species (Eqn 7) were used as the species community matrix. The same approach was used to detect predictive EM taxa for ^{15}N enrichment in whole root systems. We run the LASSO models (1) for all seasons and plots together and (2) for each region and each season individually. The second approach resulted in warning messages because the number of observations per EM species was not sufficiently high because of the large variation of EM species. Therefore, we could only use the complete data set to investigate predictive EM species for N uptake.

Results

Characterization of EM fungal species colonizing root tips in beech forests

The morphological assessment of a total number of 63 487 root tips revealed 45 360 vital EM (71.4%), 18 019 dead (28.4%) and 108 NM (0.2%) root tips across all seasons and plots. Ectomycorrhizal colonization of the vital root tips was > 99% and did not vary among the plots and seasons (Table 1). Ectomycorrhizal species richness and Shannon diversity were higher in SCH than in HAI and higher in spring than in summer and fall (Table 1). Root vitality was also higher in spring than in other seasons and varied slightly between the regions (Table 1).

Molecular investigations of all detected morphotypes revealed 53 different EM fungal taxa forming vital associations with root tips (Table S2). Of these, 43 were identified at the species level, eight at the order or genus level and two morphotypes could not be amplified by PCR, and therefore maintained their original numbers, MTSU6 and MTAU37. *Lactarius subdulcis* was the most abundant EM species (colonizing *c.* 20–30% of the root tips) and was present across all plots and seasons (Fig. S3a). *Lactarius blennius* (*c.* 10% of the root tips) and *Tomentella stuposa* (*c.* 5% of the root tips) were found in all studied seasons. Other EM species varied with season or region (Fig. S3a). These variations resulted in significant differences in the composition of the EM assemblages among the seasons (ANOSIM: HAI, $r = 0.31$, $P < 0.001$ and SCH, $r = 0.43$, $P < 0.001$) but only in small differences among the regions (ANOSIM: $r = 0.04$, $P = 0.01$) and no differences between the N treatments (Fig. S3b).

Enrichment of ^{15}N along the root transport path and interspecific differences among EM taxa

The enrichment of ^{15}N (as defined in Eqn 3) after 4 h of exposure to labelled NH_4NO_3 solution (Fig. S1) was highest in EM root tips and declined significantly through the root system with fine roots > coarse roots > transport root (Fig. 1), that is with increasing distance from the root tips. In each tissue, ^{15}N enrichment from NH_4^+ was significantly higher than from NO_3^- (EM root tips, $F = 440.74$, $P < 0.001$; fine roots, $F = 523.37$, $P < 0.001$; coarse roots, $F = 272.52$, $P < 0.001$ and transport roots, $F = 25.24$, $P < 0.001$; Fig. 1). The ^{15}N enrichment was higher in spring and autumn than in summer, with two exceptions (coarse roots at SCH, transport root at SCH; Table S4).

We determined taxon-specific ^{15}N enrichment in EM root tips (Eqn 4) per plot and season. ^{15}N enrichment in the root tips colonized with different EM species differed significantly between the N forms and among the seasons (Fig. 2; Table S3). The EM species showed higher ^{15}N enrichment from NH_4^+ than from NO_3^- (median: 534 vs 77 $\mu g\, g^{-1}$ DW ^{15}N enrichment in 4 h, Wilcoxon test, $W = 149.5$, $P < 0.001$), except *Helotiales* sp. 1 (Fig. 2). The ^{15}N enrichment from NH_4^+ varied among root tips colonized by different EM species by *c.* 5- to 7-fold, while the ^{15}N enrichment from NO_3^- varied more strongly (22- to 86-fold; Fig. 2; Table S3). The majority of EM species showed low

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Table 1 Characteristics of beech root tips in beech (*Fagus sylvatica*) forests located in the Hainich-Dun (HAI) region and in the Schorfheide-Chorin (SCH) region in three seasons.

	HAI			SCH			(HAI – SCH)	
	Spring	Summer	Autumn	Spring	Summer	Autumn	χ^2	*P*
Root tip vitality	77.54 ± 1.92b,c	63.58 ± 1.69a	68.39 ± 1.57a	83.01 ± 2.02c	71.63 ± 2.14a,b	65.99 ± 2.67a	4.73	**0.03**
EM colonization	99.88 ± 0.06a	99.71 ± 0.10a	99.74 ± 0.10a	99.81 ± 0.10a	99.85 ± 0.07a	99.58 ± 0.15a	0.05	0.82
Species richness	9.22 ± 0.57c,d	4.61 ± 0.47a	6.00 ± 0.49a,b	11.33 ± 0.94d	7.50 ± 0.59b,c	6.00 ± 0.58a,b	10.05	**0.00**
Shannon diversity	1.76 ± 0.09b,c	1.24 ± 0.07a	1.43 ± 0.07a,b	1.87 ± 0.11b	1.57 ± 0.10a,b	1.48 ± 0.10a,c	5.08	**0.02**
Evenness	0.67 ± 0.04a	0.81 ± 0.03b	0.74 ± 0.04a,b	0.64 ± 0.04a	0.70 ± 0.03a,b	0.81 ± 0.02b	1.07	0.28

Data show means ± SE ($n = 18$ per forest and season) for root tip vitality (%), ectomycorrhizal (EM) colonization (%), EM species richness, EM Shannon diversity and evenness. The analyses included ^{15}N-labelled roots (treatment: $^{15}NH_4^+$ and $^{15}NO_3^-$). Generalized linear mixed effect models (*glmer*, Poisson regression and χ^2-test) were used to compare means of species richness. Linear mixed effect models (*lmer*) were used to compare the means of Shannon diversity and evenness. In both glmer and lmer models, plot was used as a random factor when comparing HAI (season) and SCH (season), and season as random factor when comparing regions (HAI-SCH). Beta regression was used to compare the means of root tip vitality and EM colonization. Pairwise differences of each variable were compared using a *post hoc* test (Tukey's honestly significant differences, HSD). Significant differences of the means at $P < 0.05$ among the regions are shown in bold. Different letters denote significant differences of the means at $P < 0.05$.

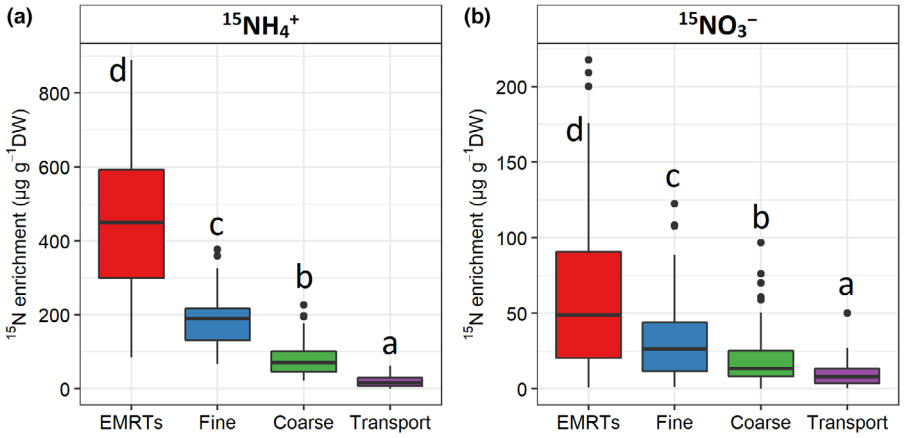

Fig. 1 ^{15}N enrichment in ectomycorrhizal root tips (EMRTs), fine roots (fine), coarse roots (coarse) and the transport root (transport) of beech (*Fagus sylvatica*) after 4 h exposure to $^{15}NH_4NO_3$ (a) or $NH_4{}^{15}NO_3$ (b). Box plots show the range of the first to the third quantile with the horizontal line indicating the median. Whiskers represent the variability of data, expressed as the 1.5-fold interquartile range. Linear mixed-effects models were used to compare the means of ^{15}N enrichment in different root segments with tree as random factor. Pairwise comparisons between different root segments were conducted with a *post hoc* test (HSD Tukey's honestly significant difference). Different letters indicate significant differences of the means ($n = 90$ per root segment and per treatment) at $P \leq 0.05$.

NO_3^- (< 50 µg g^{-1} DW 4 h^{-1}) but high potential for NH_4^+ absorption (400–600 µg g^{-1} DW 4 h^{-1}; Fig. S4). There was no relationship between the ability of the EM taxa to accumulate NH_4^+ and NO_3^- (linear regression model: $r = 0.14$, $P = 0.25$). Increasing dissimilarity of the EM assemblages was unrelated to ^{15}N enrichment (Fig. S5).

Across all seasons, EM species with high ^{15}N enrichment (> 1000 µg g^{-1} DW 4 h^{-1}) from NH_4^+ were *Clavulina cristata*, *Tomentella ramosissima*, *Inosperma maculatum* (previously known as *Inocybe maculata*) and *Xerocomus chrysenteron*, and those with low ^{15}N enrichment (< 200 µg g^{-1} DW 4 h^{-1}) from NH_4^+ were *Tomentella coerulea*, *Byssocorticium* sp., *Pezizales* sp. 2 and *Helotiales* sp. 3 (Fig. 2). Fungal species with high ^{15}N enrichment from NO_3^- (> 250 µg g^{-1} DW 4 h^{-1}) were Helotiales sp. 1, *Tomentella stuposa* and *Humaria hemisphaerica* and those with low ^{15}N enrichment from NO_3^- (< 15 µg g^{-1} DW 4 h^{-1}) were *Tomentella viridula*, *Russula integriformis* and *Pezizales* sp. 2 and *Helotiales* sp. 3 (Fig. 2). Analysis of functional traits showed that nine of the EM taxa detected here were classified as nitrophilic and three as nitrophobic but for the majority no information was available (Table S5). The majority of root tips was colonized by EM species of the contact-medium smooth exploration type (Fig. S6), whereas medium-distance fringe and long-distance EM species contributed together < 10% to total EM abundance (Fig. S6).

There were also significant differences in the C and N contents, C : N ratios, δ^{13}C and ^{15}N-NH_4^+ : ^{15}N–NO_3^- ratios among the EM species (Table S3) and the roots (Table S4).

N uptake by beech roots in relation to EM fungal diversity

To test drivers for N uptake, we modelled ^{15}N enrichment in the root transport segment (Eqn 3) and the entire root system (Eqn 6) with the following predictor variables: EM Shannon

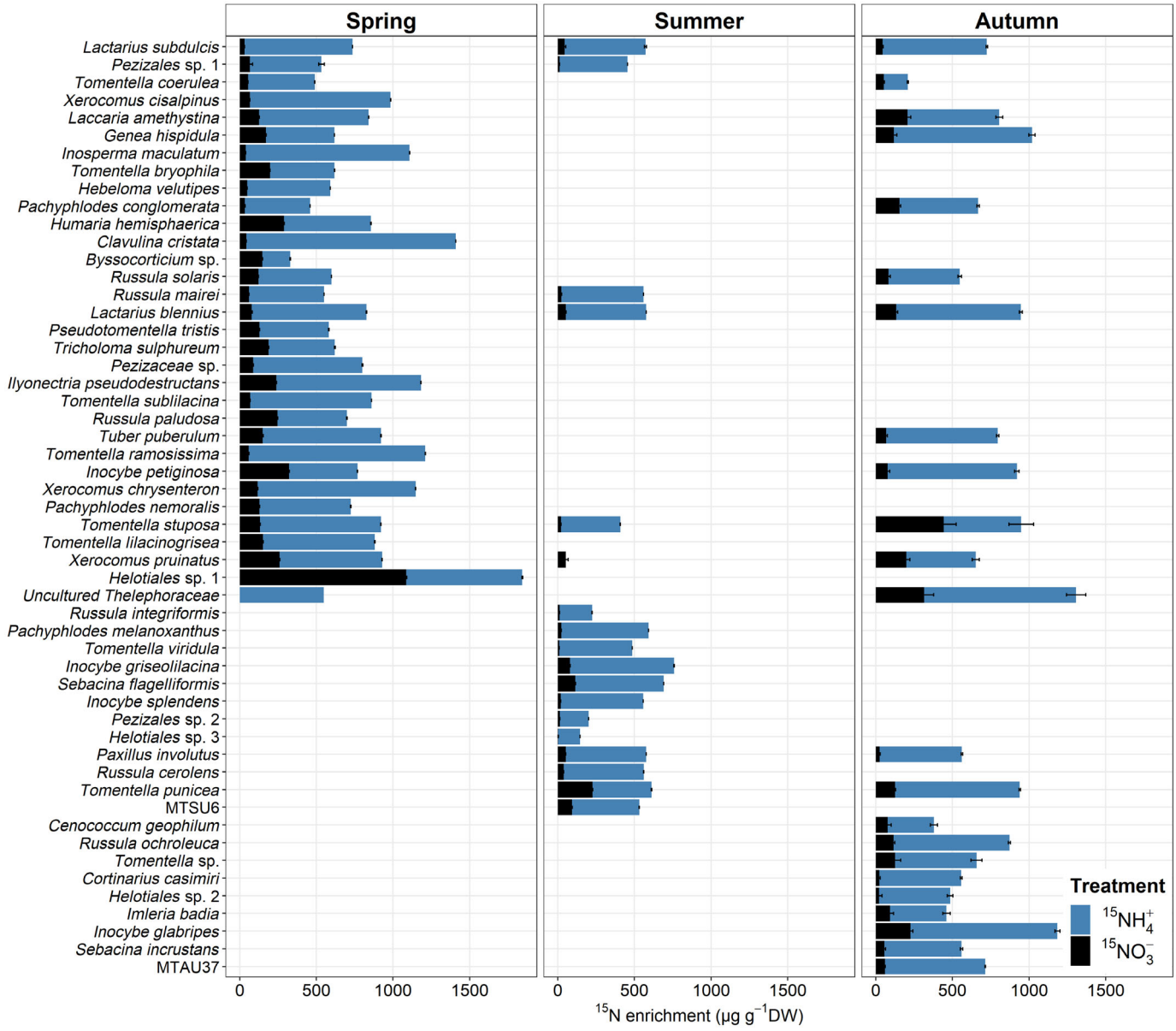

Fig. 2 ^{15}N enrichment in ectomycorrhizas formed with different fungal species after 4 h-exposure to $^{15}NH_4NO_3$ or $NH_4{}^{15}NO_3$. Fungal species were determined on beech (*Fagus sylvatica*) roots. The stacked bars indicate ^{15}N enrichment from NH_4^+ (blue) and from NO_3^- (black). Data show means ($n = 2$–6, ± SE) of samples collected on six plots per season. Since the ectomycorrhizal (EM) species were not found in all plots or not in sufficient amounts for analyses in all plots, the number of replicates varies. Linear mixed-effects model was used to compare the means of ^{15}N enrichment in EM fungal species with plot and region as a random factors. ($^{15}NH_4^+$: spring, $F = 54.79$, $P < 0.001$, summer, $F = 254.92$, $P < 0.001$, autumn, $F = 17.12$, $P < 0.001$; $^{15}NO_3^-$: spring, $F = 43.12$, $P = 0.02$, summer, $F = 526.18$, $P < 0.001$, autumn, $F = 7.94$, $P < 0.001$).

diversity, biomass of fine roots, season and root vitality index. EM species richness had to be removed from the analyses because of significant collinearity with EM Shannon diversity. In the transport segment of the root, EM Shannon diversity was the only significant predictor for ^{15}N enrichment from NH_4^+ (Table 2). The best model for ^{15}N uptake from NO_3^- in the transport segment included EM Shannon diversity and season (Table 2). The slope of the curve for ^{15}N enrichment from NH_4^+ was similar to that from NO_3^- ($F = 0.98$, $P = 0.324$; Fig. 3a).

We also developed regression models for N enrichment in the whole root system, expecting to find the same predictor variables as for the transport root and perhaps additional factors due to the direct contact of the roots with the labelled solutions. We found that root tip vitality, season and EM Shannon diversity were significant predictors for ^{15}N uptake of the whole root system, irrespective the N form (Table 2). Season was identified as an additional important factor to explain the variation of NO_3^- uptake in the transport segment (Table 2). Altogether, the models consistently showed relationships between N uptake and EM diversity and demonstrated that NO_3^- is more heavily influenced by season than NH_4^+ transport.

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Table 2 Multiple linear regression models for beech N uptake in the transport root and whole root systems.

Treatment	Root segments	Predictors	AIC-values	Residual sum sq	Marginal r^2	conditional r^2	*F*-value	*P*-value
$^{15}NH_4^+$	Transport	EM diversity (*H*′)	79.13	8.97	0.25	0.38	21.40	< **0.001**
$^{15}NO_3^-$	Transport	EM diversity (*H*′)	29.65	3.39	0.51	0.57	9.52	**0.003**
		Season					26.12	< **0.001**
$^{15}NH_4^+$	Whole root	EM diversity (*H*′)	−70.14	0.59	0.75	0.78	25.31	< **0.001**
		Season					19.15	< **0.001**
		Root tip vitality					20.72	< **0.001**
$^{15}NO_3^-$	Whole root	EM diversity (*H*′)	−7.38	1.76	0.70	0.71	10.12	**0.002**
		Season					8.94	**0.000**
		Root tip vitality					17.99	< **0.001**

Linear mixed-effects models (*lmer*) were used to predict ^{15}N uptake derived from NH_4^+ or NO_3^- in the transport root or the entire root system used for labelling. Region and plot were used as random factors in lmer models. Ectomycorrhizal (EM) Shannon diversity (*H*′), season, fine root biomass (g dry mass) and root vitality index (%) were used as predictor variables. The best model was chosen by backward selection on the basis of AIC values. Significant effects at $P \leq 0.05$ are indicated with bold letters. AIC, Akaike information criterion.

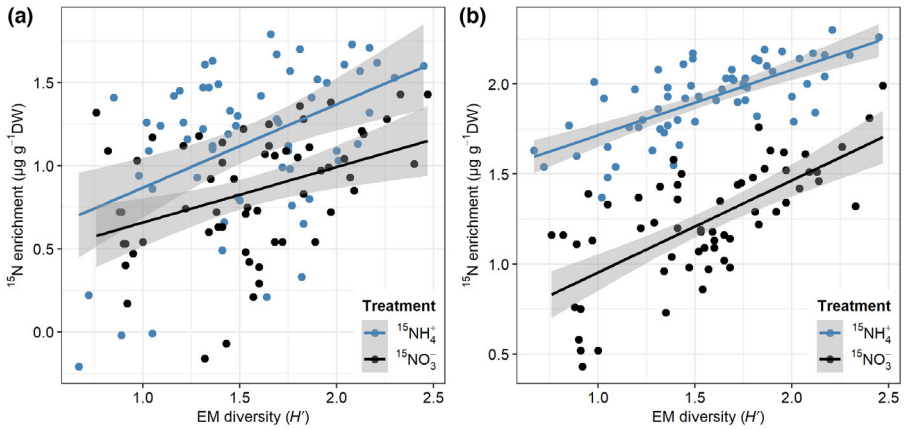

Fig. 3 Relationship between ectomycorrhizal (EM) fungal Shannon diversity *H*′ and beech root N uptake in the transport root (a) and the whole root system (b). Pearson correlation analysis was conducted with log-transformed data for ^{15}N enrichment obtained after 4-h exposure to $^{15}NH_4NO_3$ (blue) or $NH_4{}^{15}NO_3$ (black). Linear curves for the transport root: ^{15}N–NH_4^+: $y = 0.50x + 0.37$, $r^2 = 0.20$, $P < 0.001$, ^{15}N–NO_3: $y = 0.34x + 0.32$, $r^2 = 0.15$, $P = 0.002$ and for the entire root system: ^{15}N–NH_4^+: $y = 0.39x + 1.49$, $r^2 = 0.50$, $P \leq 0.001$, and ^{15}N–NO_3: $y = 0.51x + 0.49$, $r^2 = 0.46$, $P < 0.001$. Shading refers to the SE of the regression.

Contributions of EM fungal taxa and assemblages to root N uptake

We asked if the contributions of EM species to ^{15}N enrichment of the EM assemblage could be upscaled to estimate the ^{15}N enrichment in the mixed EM sample. We found a highly significant positive linear relationship between the measured ^{15}N enrichment in the EM mixture and the weighted ^{15}N enrichment in EM assemblages ($y = 0.89x + 86.24$, $r_{adj}{}^2 = 0.78$, $P < 0.001$; Fig. S7) and no significant deviation of the slope from 1 ($F = 0.65$, $P = 0.527$). The tight regression coefficient linked ^{15}N enrichment due to taxon-specific traits with that of the entire EM community at the roots. It should be noted that this approach has limitations due to the use of the relative abundance of the EM species (in Eqn 8 since fungal biomass was not available) and removal of a number of root tips from the mixed EM sample for ^{15}N and species analyses. However, our result suggests that the potential bias caused by these limitation was small.

We further found that the pool of ^{15}N present in total EM biomass of the root system was related to the pool of ^{15}N in whole root system ($\log_e(y) = 2.199 + 0.718 \times \log_e(x)$, $r^2_{adj} = 65.6\%$, $P < 0.001$; Fig. S8). The best model was not linear (linear regression model: $r^2_{adj} = 27.8\%$, $P < 0.001$) since root N uptake levelled off at high N enrichment in EM biomass.

We then asked if distinct, influential EM taxa in the diverse EM communities could be identified as major drivers of root N uptake applying LASSO regression. LASSO regression is a statistical method to identify significant drivers, when the statistical assumption underlying linear models is not met, as in our case, where linear regression models were flawed by a high number of zero values. A limitation was that this approach could only be applied to the whole data set because of sparse data availability for the taxon-specific traits at individual sampling time points. Irrespective of the N form, the optimum models for the root transport segment and the whole root system did not predict any influential EM species for root ^{15}N uptake (Fig. S9).

Discussion

EM fungal diversity drives root N uptake

Our study reports two key results: first, the diversity of the EM fungal assemblages is a major factor for root N acquisition and second, ectomycorrhizas show taxon-specific differences for N absorption in active forest assemblages. It was unexpected that neither the EM species with superior capacity for N acquisition (NH_4^+: *Clavulina cristata*, *Tomentella ramisissima*, *Inosperma maculatum* and *Xerocomus chrysenteron*, NO_3^-: *Helotiales* sp. 1)

nor abundant species present in all three seasons (*Lactarius subdulcis* 27.7% and *Lactarius blennius* 10.9% of all root tips) could be identified as key species for root N uptake under our experimental conditions. Obviously, these fungi were never dominant enough in their multi-species context to consistently affect whole root N dynamics. A limitation of our study is that we could not discern contributions of taxon-specific traits to plant N acquisition in a given seasonal or regional context. Therefore, potential benefits of individual traits to plant nutrition might be masked by the dynamic nature of EM species composition. Nevertheless, our results are important since they show that physiological differences among fungal species are present in forest ecosystems and highlight the importance of community-level traits for plant N nutrition.

A widely accepted concept for arbuscular mycorrhizal fungi suggests that mutualistic partners directly trade for nutrients (N from the fungus for carbohydrates from the plants) with the consequence that more beneficial symbionts obtain more rewards (Bever *et al.*, 2009; Kiers *et al.*, 2011; Fellbaum *et al.*, 2014). Despite various different experimental approaches (stable isotope labelling, split root experiments, nanoSIMS imaging), clear-cut evidence for direct, reciprocal C–N trade has not been demonstrated for EM symbioses (Valtanen *et al.*, 2014; Hortal *et al.*, 2017; Plett *et al.*, 2020; Mayerhofer *et al.*, 2021). Therefore, the idea that enhanced plant-derived C flux to roots directly entails enhanced N flux in roots with EM diversity being a result of enhanced C flux and thus, a covariant of N flux appears unlikely. On the contrary, increased tree carbon allocation to the roots enhanced N entrapment in fungal biomass, thereby withholding rather than stimulating plant N supply (Hasselquist *et al.*, 2016; Högberg *et al.*, 2017). In light of these findings, we suggest that a diverse EM community of a root provides multiple, spatially scattered opportunities for the plant to access N. Hence, taking the temporal trajectory into account, plant N supply across a growth season would depend on context-dependent properties of the EM assemblages but would not be dominated by traits of individual taxa. In a similar vein, a recent study showed a significant positive relationship of tree growth with the genetic potential of the metagenome for energy and nutrient metabolism of the root-colonizing EM community (Anthony *et al.*, 2022). Together, these results may imply that at the level of the tree, the collective properties of the root-associated EM assemblages and their interspecific variations play a major role for plant nutrition. We propose that this mechanism enables the maintenance of high EM diversity and secures the benefits of EM-assisted N provision. We are aware that the underlying physiological processes that could explain the relationship between diversity and root supply are speculative and require further investigation. However, our proposed concept opens new avenues for understanding mycorrhizal functions for N dynamics in forest ecosystems.

EM community characteristics and taxon-specific traits for NO_3^- and NH_4^+ acquisition

In the forest regions studied here, total atmospheric N inputs (13.8–16.6 kg N ha^{-1} yr^{-1}) were in the range of those present in many temperate European forests (10–20 kg N ha^{-1} yr^{-1}; Schwede *et al.*, 2018). NH_4^+ was the major inorganic N compound in soil (Gao *et al.*, 2020). Accordingly, our fungal communities were dominated by species with high net NH_4^+ acquisition rates (400–800 μmol N g^{-1} 4 h^{-1}). For example, typical nitrophilic species such as *Russula ochroleuca* and *Lactarius subdulcis* (Suz *et al.*, 2014; Kranabetter *et al.*, 2015; de Witte *et al.*, 2017; Lilleskov *et al.*, 2019) present in our forests have previously been identified as indicator species for high N throughfall deposition (van der Linde *et al.*, 2018). Furthermore, the fungal communities were mainly composed of contact-smooth medium distance EM species, which are characteristic for N_{min}-rich forests (Lilleskov *et al.*, 2019), whereas long-distance and fringe exploration types were not abundant. These results support that the site conditions favoured co-occurring EM fungal species that can utilize the most abundant soil N source (Tibbett *et al.*, 1998; Lilleskov *et al.*, 2002a). Therefore, it may not be surprising that we detected only one species (*Helotiales* sp. 1) with outstanding abilities for NO_3^- acquisition. High NO_3^- acquisition might be a specialized trait of some Helotiales since culture studies showed that several members of this order grew better on NO_3^- than NH_4^+-containing media (France & Reid, 1984). Given the high mobility of NO_3^- in soil, ammonification by soil microbes (Chalot & Plassard, 2011) and higher energy costs for metabolization (Marschner & Marschner, 2012), it is uncertain whether soil NO_3^- resources may select EM assemblages with high NO_3^- uptake capacities. However, NO_3^--adapted fungal communities could play a role in NO_3^--rich ecosystems such as alder forests, which are known for their specific EM composition (Bogar & Kennedy, 2013). Therefore, future studies on the N-related traits of EM fungi should include sites with high soil NO_3^- stocks to clarify whether there was a wider range of EM fungi with a preference for NO_3^-.

Although the general NH_4^+ preference of the present EM communities concurs with previous studies (e.g. Hawkins & Kranabetter, 2017), our study has limitations. Root unearthing inevitably led to hyphal damage and, thus, may have affected apparent fungal traits. However, the negative effects are probably small because preferential NH_4^+ enrichment was also detected in native EM assemblages after soil labelling, where impairment of hyphae prior to the N treatment was avoided (e.g. Leberecht *et al.*, 2016a). Furthermore, the present EM assemblages had relatively short hyphae, suggesting that damage was probably restricted.

In controlled experiments with only one plant and one EM species, biochemical regulation such as upregulation of nitrate transporters within short periods of time (< 2 h; Jargeat *et al.*, 2000, 2003; Montanini *et al.*, 2006; Willmann *et al.*, 2014) as well as suppression of nitrate transporter activity in the presence of NH_4^+ was found (Nehls & Plassard, 2018). However, soil labelling with a high dose of either NH_4^+ or NO_3^- for 2 d induced no detectable transcriptional responses to these N forms in EM assemblages composed of different species but the EM metatranscriptomes revealed taxon-specific transcriptional profiles of genes involved in N uptake and metabolism (Rivera Pérez *et al.*, 2022). In our study, five (out of 19) EM

fungal genera (*Clavulina*, *Russula*, *Lactarius*, *Tomentella*, and *Thelephora*) did not possess a single nitrate transporter gene (identified as IPR004737; Romero-Olivares *et al.*, 2021). Hence, part of the fungal community may not engage in active NO_3^- uptake. After long-term NO_3^- treatment (> 1 yr) of pine trees, root-associated EM species still showed higher NH_4^+ than NO_3^- fluxes and moreover, prevented NH_4^+ loss in contrast to non-mycorrhizal root tips (Hawkins & Robbins, 2022). It is also notable that NO_3^- loss was only partially prevented by the EM species. Collectively, genome sequencing, metatranscriptome analyses (Kohler *et al.*, 2015; Miyauchi *et al.*, 2020; Romero-Olivares *et al.*, 2021; Rivera Pérez *et al.*, 2022) and physiological studies underpin strong genetic signature in fungal traits for N handling.

Seasonal changes in N uptake

In agreement with other studies (e.g. Koide *et al.*, 2007; Courty *et al.*, 2008), we found significant seasonal changes in EM species richness and diversity. The observed reductions in summer might be related to changes in environmental conditions (higher temperatures, drought), which are known to be important drivers of EM fungal communities at local and global scales (Tedersoo *et al.*, 2014; Nickel *et al.*, 2018; van der Linde *et al.*, 2018). In addition, seasonal variation in N acquisition could be related to changes in tree demand for N, which is particularly high in spring (Rennenberg & Dannenmann, 2015). However, several studies showed higher N uptake in summer than in spring (Gessler *et al.*, 1998; Geßler *et al.*, 2005; Li *et al.*, 2016), suggesting that N demand in spring cannot be met by uptake but relies on internal N storage. Whether temporal uncoupling of tree demand and N uptake also occurs in N_{min}-rich ecosystems requires further inspection. In our study, decreased root vitality in summer and fall implies that in addition to EM diversity and seasonal tree N demand, environmental constraints affected net N enrichment in the whole root system. However, once taken up, further translocation of NH_4^+-derived N was only related to EM diversity, whereas NO_3^--derived N was additionally affected by season. It is feasible that physiological plant traits play a stronger role in NO_3^- than in NH_4^+ uptake for beech forests. NO_3^- translocation may – at least partly – be independent from ectomycorrhizas. For example, application of NO_3^- to forest soil resulted in strong upregulation of marker genes for NO_3^- assimilation in beech roots but not in the associated EM assemblage (Rivera Pérez *et al.*, 2022), suggesting that roots may have directly acquired NO_3^-. Thus, direct plant NO_3^- uptake may compensate for the low efficiencies of fungal communities for NO_3^- uptake. We speculate that different handling of NO_3^- and NH_4^+ may be ecologically advantageous for sustained resource access.

In conclusion, we found that the N uptake of roots is positively related to EM fungal diversity. Environmental conditions that negatively affect EM fungal diversity show negative consequences for tree N supply. We demonstrated high taxon-specific differences in N enrichment in ectomycorrhizas. These physiological traits can be modelled as a composite community trait of the EM species assemblage for field-grown trees, in agreement with a previous study with seedlings (Pena & Polle, 2014). We provided evidence that plant N acquisition across a growth season may be viewed as the result of EM community traits. The ecological implications of our results are that maintenance of high EM fungal diversity is important to secure tree N nutrition and suggest that resource penalties would be unfavourable to uphold root N supply.

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Competing interests

None declared.

Author contributions

AMK organized the research, led the field experiments, conducted the laboratory analyses, performed the statistical analyses and data visualization, and wrote the first draft of the manuscript. DJ conducted statistical analyses. AP secured funding, designed and supervised the study, and drafted the final manuscript. All authors commented and agreed on the final version of this manuscript.

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Data availability

All data are available in the BExIS database (https://www.bexis.uni-jena.de) under the following accession nos. (data owner): Soil chemistry – 26228 and 26229 (Polle), EM fungal species – 31078

(Polle), Fine root tips dynamics – 31079 (Polle), Ammonium and nitrate uptake by root segments – 31080 (Polle) and ammonium and nitrate enrichment in EM fungal species – 31082 (Polle). The sequences were deposited in GenBank under the accession numbers MN947338–MN947405. The R scripts were deposited in Github under: https://github.com/Khokon130990/15N_Beech_EMF_N_Uptake.

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Supporting Information

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

Fig. S1 Schematic overview of ^{15}N labelling of beech roots in the forests.

Fig. S2 Rarefaction curves of ectomycorrhizal species for each root system.

Fig. S3 Relative abundance and ordination of ectomycorrhizal (EM) fungal species in different seasons and regions.

Fig. S4 Classification of ectomycorrhizal species according to their N absorption rates.

Fig. S5 Relationship between EM fungal community dissimilarity with ^{15}N enrichment distance in the transport and in the whole root systems.

Fig. S6 Relative abundances of different exploration types in different seasons.

Fig. S7 Relationship between ^{15}N enrichment in mixed EM assemblages and weighted ^{15}N enrichment in EM assemblages based on abundance and taxon-specific enrichment in ectomycorrhizal root tips.

Fig. S8 Relationship between ^{15}N pool in the ectomycorrhizal root tips of the whole root system and ^{15}N pool in the whole root system.

Fig. S9 Ectomycorrhizal fungal taxon-specific influence on N uptake in the root transport segment and in the entire root system from NH_4^+ and NO_3^-.

Table S1 Key characteristics of the study plots.

Table S2 Morphological characterization and molecular identification of ectomycorrhizal fungal species associated with European beech (*Fagus sylvatica*).

Table S3 ^{15}N atom% excess (APE), ratio of APE from $^{15}NH_4^+$: $^{15}NO_3^-$, δ^{13}C, carbon (C), nitrogen (N) and C : N ratio (C : N) of ectomycorrhizas colonized with different fungal species in spring, summer and fall.

Table S4 Carbon, nitrogen, C : N ratio, δ^{13}C and ^{15}N enrichment from $^{15}NH_4NO_3$ or $NH_4{}^{15}NO_3$ in ectomycorrhizal root tips, fine roots, coarse roots and the transport root.

Table S5 Literature survey on ectomycorrhizal fungal traits.

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