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Tree species rather than type of mycorrhizal association drives inorganic and organic nitrogen acquisition in tree-tree interactions

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Key words: arbuscular mycorrhiza, competition, ectomycorrhiza, interspecific competition, intraspecific competition, nitrogen uptake

Running title: Neighbours drive N acquisition in tree interactions

Abstract

Mycorrhizal fungi play an important role for the nitrogen (N) supply of trees. The influence of different mycorrhizal types on N acquisition in tree-tree interactions is, however, not well understood, particularly with regard to the competition for growth-limiting N. We studied the effect of competition between temperate forest tree species on their inorganic and organic N acquisition in relation to their mycorrhizal type (i.e. arbuscular mycorrhiza or ectomycorrhiza). In a field experiment, we quantified net N uptake capacity from inorganic and organic N sources using $^{15}N/^{13}C$ stable isotopes for arbuscular mycorrhizal tree species (i.e. *Acer pseudoplatanus* L., *Fraxinus excelsior* L., and *Prunus avium* L.) as well as ectomycorrhizal tree species (i.e. *Carpinus betulus* L., *Fagus sylvatica* L., and *Tilia platyphyllos* Scop.). All species were grown in intra- and interspecific competition (i.e. monoculture or mixture). Our results showed that N sources were not used complementarily depending on a species´ mycorrhizal association, but their uptake rather depended on the competitor indicating species-specific effects. Generally, ammonium was preferred over glutamine and glutamine over nitrate. In conclusion, our findings suggest that inorganic and organic N acquisition of the studied temperate tree species is less regulated by mycorrhizal association, but rather by the availability of specific N sources in the soil as well as the competitive environment of different tree species.

1. Introduction

In temperate forest ecosystems, competition for growth limiting nitrogen (N) is high (Schenk 2006), thus, plants have developed different mechanisms to avoid competition for N. For example, plants can exude allelochemicals to inhibit root access to N by other plants (e.g. reviews by Schenk 2006, Trinder et al. 2013), forage for N via spatial variation (i.e. in different rooting depths, e.g. Berendse 1981, Schenk et al. 1999, Jumpponen et al. 2002, von Felten et al. 2012) and/or via temporal (seasonal) variation (e.g. Hodge et al. 1999, 2000, Simon et al. 2011, Hodge and Fitter 2013). Other mechanisms include the preferred uptake of certain N sources, such as inorganic or organic N (Näsholm et al. 2009, Inselsbacher and Näsholm 2012, Hodge and Fitter 2013, Simon et al. 2017) and/or a higher uptake of N by plants via mycorrhizal fungi (e.g. Helmisaari et al. 2009, Lankau et al. 2011, Schnitzer et al. 2011, Hodge and Fitter 2013, Simon et al. 2017). The mycorrhizal hyphae network increases the foraging capacities and N acquisition for roots (Pena 2016, Simon et al. 2017) via a larger absorbing surface area (e.g. van der Hejden and Kuyper 2003, Felten et al. 2009, Pena 2013a, Simon et al. 2017), particularly when N is limited. Thus, mycorrhizae play a key role in tree N acquisition and, consequently, for the competitive abilities of trees.

Mycorrhizal associations of trees occur in different types, such as arbuscular mycorrhiza (AM) or ectomycorrhiza (EM). Both mycorrhizal types take up inorganic and organic N sources (e.g. Bukovska et al. 2018, Liese et al. 2018) and their acquisition is regulated by the presence/absence of the specific N sources (Talbot and Treseder 2010) as well as environmental conditions, for example water availability (e.g. Simon et al. 2017). Many studies have investigated the effects of mycorrhizal type on nutrient uptake (Phillips et al. 2013), especially nitrogen and phosphorus; however, they were mainly conducted under controlled conditions (e.g. Jones et al. 1998, Schulz et al. 2011, Köhler et al. 2018, Liese et al. 2018) but rarely in the field (Jacob and Leuschner 2015, Li et al. 2016, Nave et al. 2013, Vadeboncoeur et al. 2015), both for single plant species or species combinations. For example, Liese et al. (2018) studied the effect of mycorrhizal type on root exudation and N uptake growing seedlings as mixtures of two AM and two EM tree species together in containers using ^{15}N tracer of single sources of inorganic N and glycine under controlled conditions: AM trees had higher inorganic N uptake than EM trees, whereas glycine uptake did not vary among trees of different mycorrhizal types (Liese et al. 2018). In their study, the overall effects of several species interacting were studied rather than the effects for a certain species (Liese et al. 2018). Furthermore, inorganic and glycine N uptake by the roots was both species- as well as N source-specific in a field study in mature stands of monocultures (i.e. *Acer pseudoplatanus*, *Carpinus betulus*, *Fagus sylvatica*, *Fraxinus excelsior*, *Tilia cordata*) using a ^{15}N tracer (Jacob and Leuschner 2015). In addition, with mild drought, beech seedlings benefitted from EM fungi colonization, whereas the presence of EM fungi led to a reduction in total plant N uptake with sufficient water supply, suggesting an effect of soil moisture on EM-dependent N acquisition (Pena et al. 2013a).

Mycorrhizal type directly and/or indirectly influences plant community composition by the provision of nutrients as well as the regulation of plant-soil microbe interactions and their effects on plant competition (Tedersoo et al. 2020). However, knowledge of species-specific tree-tree interactions is still limited. Especially at the seedling stage, competition for N can be expected to be highest because of higher levels of herbivory and limited N storage capacities compared to adult trees, thus resulting in a higher N demand of seedlings from external sources (Simon et al. 2011, 2017). Simon et al. (2010) showed in a 2-species experiment that the potential competition for N is avoided by preference of different N sources: *Fagus sylvatica* (EM-associated) favored organic N, whereas *Acer pseudoplatanus* (AM-associated) preferred inorganic N. However, whether this was regulated by mycorrhizal type and represents a general pattern across types of mycorrhizal association was not investigated. From previous studies it is therefore evident that the single and combined effects of tree species as well as mycorrhizal type (i.e. EM / AM) on inorganic and organic N sources need to be investigated to better understand N acquisition in tree species interactions. In addition, considering the influence of soil N availability on plant N acquisition (Li et al. 2015, Simon et al. 2017), increasing atmospheric N deposition due to global change is likely to alter the outcome of competition between plant species associated with different mycorrhiza types (deForest and Snell 2020).

Thus, the overall aim of this study was to investigate the influence of mycorrhizal type on N acquisition of tree species growing in competition. More specifically, our aims were to compare inorganic and organic N acquisition (1) among six temperate tree species dependent on their type of mycorrhizal association and (2) with different competing species, as well as (3) how the preferences for N source might shift. We hypothesized: (1) The acquisition of inorganic and organic N sources varies among different tree species depending on their physiological and morphological properties (e.g. Miller and Hawkins 2007, Liese et al. 2018, Simon et al. 2017). (2) The competitor affects N acquisition and leads to changes in the use of inorganic and organic N sources in the target tree species (e.g. Miller and Hawkins 2007, Simon et al. 2010, Li et al. 2015, Bueno et al. 2019). (3) The preferences for inorganic and organic N sources differ among tree species depending on their mycorrhizal association (e.g. Makarov 2019, deForest and Snell 2020). N sources are used complementarily by different mycorrhizal types with inorganic N uptake being higher in AM tree species, whereas more organic N is taken up by EM tree species.

2. Materials and methods

2.1 Study site characteristics

The study was conducted within the framework of the MyDiv tree diversity experiment established at the Bad Lauchstädt Experimental Research Station of the Helmholtz Centre for Environmental Research (UFZ) (Saxony-Anhalt, Germany) (51°23‘N, 11°53‘E, 114-116 m a. s. l.) in March 2015.

The natural vegetation of this area is mixed broad-leaved forest; however, it had been used as agricultural land since the beginning of human settlement due to the high fertility of the soil which was classified as Haplic Chernozem (pH 7.1) developed from loess with a silt loam texture with parent rock of silt over calcareous silt (Ferlian et al. 2018a). Chernozems are characterised by a thick humus horizon, stable aggregate structure, high bioturbation rates, water-retention capacity, as well as high base saturation (Altermann et al. 2005). The mean soil elemental concentrations at the study site are: C_{inorg}: 0.07%, C_{org}: 1.94%, N_{tot}: 0.17%, and P_{tot}: 513.13 mg/kg (Ferlian et al. 2018a).

Within the MyDiv experiment, the role of mycorrhizae in biodiversity-ecosystem functioning relationships is studied in deciduous trees (Ferlian et al. 2018ab) planted as two-year old seedlings (c. 50-80 cm height). Prior to planting at the field site, all study species were grown at a local tree nursery (P & P Dienstleistungs GmbH & Co KG, Eitelborn, Germany) with annually applied NPK fertiliser and no mycorrhiza application (see Ferlian et al. 2018a for further details on the overall MyDiv experimental setup). Meteorological data was collected from the Department of Soil System Science (UFZ): The climate of the study site is characterized as continental and summer-dry with a mean annual temperature of 9.9°C (1994-2017), a mean total annual precipitation of 507 mm (1994-2017), and a mean annual soil volumetric water content of 0.14 L/m^3 in 15 cm depth (2015-2017). During the vegetation period (i.e. May to October), mean temperature was 15.8 °C and mean total precipitation 330 mm (1994-2017). In 2017, mean annual temperature was 10.7 °C, total annual precipitation was 403 mm, and mean soil volumetric water content was 0.14 L/m^3. During the growing season 2017, mean temperature was 16.5 °C with a total precipitation of 262 mm.

2.2 Study species and experimental design

In our study, we used a subset of the MyDiv study species, three AM tree species (i.e. *Acer pseudoplatanus* L. (Sapindaceae), *Fraxinus excelsior* L. (Oleaceae), *Prunus avium* L. (Rosaceae)) and three EM tree species (i.e. *Carpinus betulus* L. (Betulaceae), *Tilia platyphyllos* Scop. (Malvaceae), *Fagus sylvatica* L. (Fagaceae)) growing either in monocultures or 2-species mixtures, either only ectomycorrhizal, only arbuscular mycorrhizal, or in a combination (see Table 1 for details). We studied five competition regimes: (1) intraspecific competition (i.e. monocultures) within AM tree species, (2) intraspecific competition within EM tree species, (3) interspecific competition (i.e. mixtures of two tree species) between two AM trees species, (4) interspecific competition between two EM trees species, and (5) interspecific competition between one AM and one EM trees species. Within these competition regimes, we had in total 16 different competition treatments (Table 1). Study areas for each competition treatment were 11 x 11 m (i.e. 121 m^2) with a planting distance of 1 m in a regular individual distribution pattern. Within each competition treatment, we studied 3 x 5 individuals per species (n = 15). The six tree species were chosen because they co-occur in forest ecosystems on calcareous substrate (Ellenberg and Leuschner 2014). In addition to differences with regard to mycorrhization, they vary in their growth strategies, nutrient requirements, shade tolerance, and drought sensitivity (Table 2) (Professur für Waldbau und Professur für Forstschutz & Dendrologie der ETH Zürich 2002, Ellenberg and Leuschner 2014). Total tree height and productivity (measured as change in basal area in m^2 of tree diameter measured at 5 cm above soil surface) differed among tree species and decreased in the order: *P. avium* (4.04 m and 0.029 m^2/year), *A. pseudoplatanus* (3.72 m and 0.025 m^2/year), *F. excelsior* (2.89 m and 0.016 m^2/year), *T. platyphyllos* (2.65 m and 0.016 m^2/year), *C. betulus* (2.53 m and 0.009 m^2/year), *F. sylvatica* (1.37 and 0.002 m^2/year). The setup of the MyDiv experiment did not allow to study all possible species combination pairs, while it provided a balanced design to the interactions between AM and EM tree species.

Total soil N amounts per study area were quantified in October 2015 prior to our sampling and in October 2017 after sampling. For this, five soil cores (2 cm diameter, 10 cm depth) were taken per study area considering an overall balanced proportion of competing tree species around the core. Cores per study area were pooled and sieved (2 mm grid) in the laboratory. A subsample was taken, dried at 60 °C for 72 h, ground with a ball mill, subsequently dried for another 24 h, and c. 50 mg aliquots were then transferred into tin capsules for analyses of total soil N (Vario EL II, Elementar Analysensysteme GmbH, Hanau, Germany). In both years, total soil N amounts did not vary significantly among AM, EM and AM+EM tree species study areas.

2.3 Quantification of arbuscular mycorrhizal and ectomycorrhizal colonization

In November 2019, rootlets with intact fine roots were excised (following the lateral roots from the tree stem to the surrounding soil) and stored at 4°C until further processing (n = 5 for *F. excelsior* and *T. platyphyllos*; n = 4 for *A. pseudoplatanus*, *C. betulus*, *F. sylvatica*, *P. avium*). Adherent soil was carefully removed under water using tweezers to maintain the integrity of the rootlets. The degree of AM colonization was determined according to Vierheilig et al. (2005) by bleaching the roots in 10 % KOH overnight at 60 °C and staining the roots in a solution of 10 % ink, 10 % concentrated acetic acid, and 80 % water. AM colonization of the roots was quantified by examining the abundances of vesicles, arbuscules, and internal hyphae using the gridline-intersect method (Giovannetti and Mosse 1980). AM-associated species *F. excelsior* had relatively more (23.7 ± 7.0 %, mean ± standard deviation) AM structures compared to *P. avium* (2.0 ± 1.3 %) and *A. pseudoplatanus* (1.1 ± 0.5 %) ($P < 0.001$). AM colonization rates in EM-associated tree species were overall low (*C. betulus*: 0.8 ± 0.7 %, *F. sylvatica*: 1.4 ± 1.1 %, and *T. platyphyllos*: 2.1 ± 1.8 %). EM colonization rates were determined from unstained roots under a preparatory microscope according to differences in fine root morphology, color, thickness, texture, and the branching patterns of rootlets. Average EM colonization rates for EM-associated trees were in descending order (mean ± standard deviation): *C. betulus* 78.0 ± 7.0%, T. *platyphyllos* 74.0 ± 25.6 %, and *F. sylvatica* 57.6 ± 25.5 %, and did not differ significantly among the species. AM-associated tree species had no EM colonization.

2.4 ^{15}N uptake experiments

The ^{15}N enrichment technique as described by Gessler et al. (1998) and modified by Simon et al. (2010) was used to quantify inorganic (i.e. ammonium and nitrate) and organic (i.e. glutamine) net N uptake capacity of the mycorrhizal fine roots of the six tree species. Fine roots (< 2 mm diameter) still attached to the individual trees were carefully dug out and adherent soil particles removed. Subsequently, roots were incubated in 4 mL of an artificial soil solution for 2 h, between 10 am and 2 pm to avoid diurnal variation (Gessler et al. 2002). The artificial soil solution was based on the soil solution composition of a high soil N field site in the Bavarian alpine upland containing 20 μM $Al_2(SO_4)_3$, 75 μM $CaCl_2{\cdot}2H_2O$, 4 μM $FeCl_3{\cdot}6H_2O$, 14 μM KCl, 10 μM $MnCl_2{\cdot}4H_2O$, 40 μM $MgCl_2{\cdot}6H_2O$, 4.5 μM Na_2HPO_4, 20 μM NaCl, including 50 μM NH_4Cl, 300 μM KNO_3, and 100 μM glutamine (Stoelken et al. 2010). Of the three N compounds, only one was labelled either as $^{15}NH_4^+$, $^{15}NO_3^-$, or $^{15}N/^{13}C$ double-labelled glutamine (all > 98 %) in the different solutions. Natural abundance of $^{15}N/^{13}C$ in the fine roots was accounted for with a control solution without label. After 2 h, the incubated roots (c. 5 cm) plus the moistened upper parts (c. 1 cm) were cut off, washed twice with 0.5 μM $CaCl_2$ to remove the artificial soil solution from the root surface, and dried with cellulose tissue. Samples were stored at 4 °C. Back in the laboratory, the fresh weight was determined followed 48 h oven drying at 65 °C, then the dry weight was determined.

Glutamine was chosen as amino acid because it is the dominant amino acid in forest soils (Inselbacher et al. 2011) as well as the most abundant amino compound in *F. sylvatica* and *A pseudoplatanus* roots and its important role as the main transport amino acid in plant N metabolism (Stoelken et al. 2010, Li et al. 2015).

2.5 Quantification of ^{15}N, ^{13}C, and total N and C amounts in fine roots

^{15}N and ^{13}C enrichment as well as total N and C in the fine roots were quantified in dried root samples ground to a fine homogenous powder. Aliquots of 1.5-2.0 mg were weighed into 4x6 mm tin capsules (IVA Analysentechnik, Meerbusch, Germany) and analysed with an isotope ratio mass spectrometer (Delta V Advantage, Thermo Electron, Dreieich, Germany) coupled to an elemental analyser (Euro EA, Eurovector, Milan, Italy). Acetanilide was used as standard to calculate δ values (i.e. included in every sequence in intervals and also used in different weights) to determine isotope linearity of the system, and was calibrated against different suitable international isotope standards (IAEA, Vienna). Isotope values were corrected using several international isotope and suitable laboratory standards covering the range of the ^{15}N and ^{13}C results. Inorganic and organic net N uptake capacity (µmol N / g fw h) was calculated based on the incorporation of ^{15}N into root fresh weight according to the equation by Gessler et al. (1998): net N uptake capacity = $((^{15}N_l - {}^{15}N_n) \times N_{tot} \times dw \times 10^5) / (MW \times fw \times t)$, where $^{15}N_l$ and $^{15}N_n$ are the atom% of ^{15}N in labelled (N_l) and control (N_n, natural abundance) roots, respectively, N_{tot} is the total N percentage in the roots, MW is the molecular weight of ^{15}N, and t represents the incubation time. Ratios of ^{13}C and ^{15}N incorporation for glutamine indicate (1) the degradation of glutamine in the solution or on the root surface, and/or (2) the respiration of glutamine-derived C inside the roots (Simon et al. 2011).

2.6 Statistical analyses

All analyses were carried out with R version 3.2.3 (R Development Core Team, 2018). Data was tested for normality and homogeneity of variance by quantile-quantile plot. To meet the assumptions of normal distribution and variance, all data were log-transformed prior to analyses. To test for differences between the tree seedlings with different mycorrhizal types (i.e. AM-AM vs AM-EM, EM-EM vs EM-AM tree species), t-tests were performed for each N source. To test for differences between tree species (regardless of competition), one-way ANOVAs were performed for each N source followed by posthoc Tukey tests. To test for the competitor effect on inorganic and organic N acquisition of the different species (i.e. 4 competitors for *Acer*, *Carpinus*, *Fagus*, and *Prunus*, as well as 5 competitors for *Fraxinus* and *Tilia*), one-way ANOVAs were performed for each species and N source followed by posthoc Tukey tests. For the preferences of the different N sources (i.e. ammonium, nitrate, and glutamine-N) for each mycorrhizal type or tree species, one-way ANOVAs were performed followed by posthoc Tukey tests.

3. Results

3.1 Effect of mycorrhizal type on N acquisition preferences in temperate tree species

Within AM or EM trees species, the overall preference was ammonium > glutamine-N > nitrate regardless of competition ($p \leq 0.018$, Fig. 1). N acquisition was affected by competition regime (i.e. AM-AM, AM-EM, EM-EM, EM-AM) depending on N source and mycorrhizal type: EM tree species in EM-EM interactions took up more glutamine-N than in EM-AM tree interactions, whereas ammonium net uptake capacity was higher in the AM-EM vs. AM-AM tree species ($p<0.001$) (Fig. 2). Glutamine-N net uptake capacity was higher in EM compared to AM tree species regardless of competition regime ($p = 0.005$), whereas inorganic N net uptake capacity did not differ between AM and EM tree species (Suppl. Table 1, 3a).

At the species level, inorganic and organic N acquisition differed among tree species regardless of the competitor ($p < 0.001$, Fig. 3, Suppl. Table 3b). *Fraxinus* (AM) took up more ammonium than *Acer* (AM), *Prunus* (AM), and *Tilia* (EM) ($p \leq 0.001$). *Prunus* had a lower ammonium net uptake capacity than *Carpinus* (EM) and *Fagus* (EM) ($p \leq 0.005$). Nitrate net uptake capacity was higher in *Fraxinus* compared to all other species ($p \leq 0.004$). *Fagus* took up more nitrate than *Acer* (AM) and *Prunus* (AM) ($p \leq 0.012$). Glutamine-N net uptake capacity was higher in all EM-associated tree species (i.e. *Carpinus*, *Fagus* and *Tilia*) compared to *Prunus* (AM) ($p \leq 0.006$) and higher in *Carpinus* (EM) compared to *Acer* (AM) and *Fraxinus* (AM) ($p \leq 0.021$), as well as *Tilia* (EM) compared to *Acer* (AM) ($p = 0.008$). Other species comparisons did not differ in inorganic and organic net N uptake capacity (Suppl. Table 3b).

3.2 Effect of competitor on N acquisition preferences in temperate tree species

N acquisition within tree species changed depending on the competitor (Table 3, Suppl. Table 1) and the investigated N source. Comparing between intra- and interspecific competition, changes were found for all tree species regardless of mycorrhizal association (Suppl. Table 2, 3c, d). Net N uptake capacity was higher in inter- compared to intraspecific competition: inorganic N for *Acer* with *Fraxinus*, for *Carpinus* with *Fagus* or *Fraxinus*, and for *Prunus* with *Tilia* ($p \leq 0.051$), ammonium for *Fagus* with *Carpinus* or *Fraxinus* ($p \leq 0.028$), as well as organic N for *Fagus* with *Tilia* ($p = 0.021$). For *Fraxinus*, net N uptake capacity was higher in intra- vs. interspecific competition with *Prunus* (i.e. both inorganic and organic N) and with *Fagus* (i.e. only nitrate) ($p \leq 0.005$). Similarly, organic net N uptake capacity was higher in intra- vs. interspecific competition in *Tilia* growing with *Acer* ($p = 0.021$). For the other combinations, no differences in inorganic and organic net N uptake capacity were found with intra- vs. interspecific competition (i.e. within AM tree species: *Acer* with *Prunus*, *Fraxinus* with *Acer*, *Prunus* with *Acer* or *Fraxinus*; within EM tree species: *Carpinus* with *Tilia*, *Tilia* with *Carpinus* or *Fagus*; between AM-EM tree species: *Acer* with *Tilia*, *Fraxinus* with *Carpinus*, *Tilia* with *Prunus*) (Suppl. Table 3c, d).

Comparing the species-specific interactions, preferences for different N sources showed some general patterns (Table 4, Suppl. Table 1): Ammonium was always the preferred N source over nitrate ($p \leq 0.044$) and favoured over glutamine for all species but only in certain species interactions ($p \leq 0.011$): Aps-Aps, Aps-Fex, Aps-Tpl, Cbe-Fex, Cbe-Tpl, Fsy-Cbe, Fsy-Fex, Fex-Fex, Fex-Aps, Fex-Cbe, Fex-Fsy, Fex-Pav, Pav-Tpl, and Tpl-Aps. Similarly, glutamine was preferred over nitrate in some interactions for all tree species (i.e. Aps-Aps, Aps-Pav, Aps-Tpl, Cbe-Cbe, Cbe-Fsy, Cbe-Tpl, Fsy-Cbe, Fsy-Tpl, Pav-Pav, Tpl-Tpl, Tpl-Aps, and Tpl-Cbe, except for *Fraxinus*) ($p \leq 0.020$). In contrast, *Fraxinus* – when grown in monoculture – preferred nitrate over glutamine ($p < 0.001$).

4. Discussion

4.1. Ammonium is the preferred N source in tree seedlings at high soil N availability regardless of mycorrhizal association

In our study, AM- and EM-associated trees species showed the same preferences for certain N sources, similar to a study by Keller et al. (2019). This contrasts our hypothesis that AM tree species acquire preferably inorganic N forms (Gallet-Budynek et al. 2009, Smith and Smith 2011) due to their dominant occurrence in habitats with high inorganic N availability (Liese et al. 2018), whereas EM tree species prefer organic N due to its higher concentrations in organic N rich habitats (Phillips et al. 2013, deForest and Snell 2020). In our study, ammonium was overall favoured over glutamine-N and nitrate regardless of mycorrhizal association or competition, although it was the least abundant of all included N sources. This pattern was also found in previous studies (Stoelken et al. 2010, Simon et al. 2010, Li et al. 2015) investigating inorganic and organic N acquisition in *Fagus sylvatica* and *Acer pseudoplatanus* that used the same composition of N sources. Mycorrhizal colonization rates in our study further confirmed distinct and respective mycorrhizal fungal communities between AM- and EM-associated tree species, thus suggesting that other factors drive the similarities regarding their N acquisition. Plant N uptake from the soil depends on the presence/absence of N sources as well as their individual concentrations (Näsholm et al. 2009, Stoelken et al. 2010). In studies using mixtures of different N sources, uptake rates of ammonium and nitrate are more reduced compared to those of amino acids than when only single N sources are present (e.g. Öhlund and Näsholm 2001, Thornton and Robinson 2004, Näsholm et al. 2009). The uptake of certain N compounds is regulated by feedback inhibitors (e.g. Siddiqi et al. 1989, King et al. 1993) and free amino acids as products of ammonium assimilation (e.g. Imsande and Touraine 1994, Kreuzwieser et al. 1997, Collier et al. 2003). For instance, in the presence of high levels of ammonium, the pool of cycling amino acids expands, and thus nitrate acquisition might be down-regulated, and also glutamine appears to be a specific inhibitor for nitrate uptake by trees (e.g. Kreuzwieser et al. 1997, Näsholm et al. 2009).

At the species level, the preferences for specific N sources might be related to a species' growth rate. For example, slow-growing *Fagus sylvatica* favoured organic N sources, while inorganic N was preferred by fast-growing *Acer pseudoplatanus* (e.g. Li et al. 2015, Simon et al. 2017). However, in our study, tree species had the same preferences for specific N sources despite their differences in growth rates. One exception in this study was *Fraxinus excelsior* (but only in monoculture) which favoured nitrate over glutamine. In general, nitrate uptake might be inhibited in the presence of ammonium and glutamine (Näsholm et al. 2009, Stoelken et al. 2010, Simon et al. 2013). However, a higher uptake of nitrate in *Fraxinus excelsior* might be explained by species-specific transporters in the root membranes and furthermore by kinetic constants of ion uptake in the roots which reflect an increased substrate affinity (Jacob and Leuschner 2015).

4.2 N acquisition among tree species depends on species-specific morphological and physiological properties

The acquisition of specific N sources among species might be related to their mycorrhizal association (Tedersoo et al. 2020). In our study, the EM-associated tree species (with comparable EM-colonization rates) took up more glutamine-N than AM-associated tree species, while inorganic N acquisition was species-specific. The higher uptake of glutamine in EM compared to AM tree seedlings can be explained by a higher production of extracellular enzymes by EM compared to AM fungi (Tedersoo and Bahram 2019, Makarov 2019) to exploit organic N sources (Smith and Smith 2011, Lindahl and Tunlid 2015), as an adaptation to low concentrations of inorganic N sources in their natural habitats (Phillips et al. 2013, deForest and Snell 2020). Liese et al. (2018) found no significant differences in the uptake of specific inorganic or organic N sources between AM and EM tree species. Organic N acquisition depends on the availability and concentration of specific N sources (e.g. amino acids) (Näsholm et al. 2009). We used glutamine in our study, whereas Liese et al. (2018) used glycine as organic N source. In forest soils, glycine is less common than other amino acids such as glutamine (Inselsbacher et al. 2011).

Differences in N acquisition strategies among species can be related to species' relative growth rate and/or N demand. Species with faster growth invest more into fine roots for increased soil exploration (Comas and Eissenstat 2004), and thus N uptake (Craine et al. 2001, Ryser 1996, Eissenstat et al. 2000, Tjoelker et al. 2005). In our study, slow-growing species took up more inorganic and organic N than fast-growing species (similar to Simon et al. 2010, 2014). In other studies, slow-growing *Fagus sylvatica* generally preferred organic N sources (Dannenmann et al. 2009, Li et al. 2015), whereas fast-growing *Acer pseudoplatanus* favoured inorganic N sources (Simon et al. 2011, Li et al. 2015). These contrasting results indicate that N uptake varies depending on environmental conditions (reviewed Simon et al. 2017), might shift depending on the age of the studied individuals (Simon et al. 2011), and also depends on the investigated species (Schulz et al. 2011, Bueno et al. 2019). Jacob and Leuschner (2015) found significant differences in the acquisition of inorganic N and glycine (provided as single N sources) among the same tree species as in our study, but as mature trees rather than seedlings. More specifically, mature *Fraxinus excelsior* took up more nitrate compared to *Fagus sylvatica*, *Carpinus betulus*, *Tilia cordata*, and *Acer pseudoplatanus* suggesting that differences in N uptake among species depend on root physiological (e.g. a higher density of transporters in the membranes) and chemical properties (Jacob and Leuschner 2015).

4.3 Tree N acquisition varies with the competitor

The differences in inorganic and organic N acquisition found in our study depending on the involved species were specific for competitor and N source. Three major patterns of N acquisition were found: (1) higher when competing with other tree species (i.e. interspecific competition), (2) higher when grown in monoculture (i.e. intraspecific competition), or (3) no change regardless of competitor. These inconsistent patterns in N uptake in monoculture vs. interaction with other species were found among both AM and EM tree species. Our results provide no consistent support for the theory that plant N acquisition depends on growth rate (e.g. Simon et al. 2010, 2017, Trinder et al. 2013, Li et al. 2015) or mycorrhizal association (e.g. Li et al. 2015, Liese et al. 2018). Furthermore, as all study individuals had the same age, were grown at the same field site, and were planted at the same time, variation due to developmental and environmental differences can be excluded. Thus, further investigations are required to test for the potential influence of other factors, such as root exudates or variation in microbial activity. For example, microbial activity can influence plant N uptake via the regulation of soil N processes like mineralisation, and in turn, soil N availability (e.g. Hodge and Fitter 2013). Furthermore, all studied tree species exude a highly diverse species-specific spectrum of amino acids and glucose that serves as energy source for rhizosphere microorganisms (Grayston et al. 1997). For example, roots of *Fagus sylvatica* saplings exuded higher levels of organic acids than those of *Fraxinus excelsior* in a greenhouse experiment (Fender et al. 2013). In addition, in our study only soluble low molecular weight N sources were tested, although in forest soils, the majority of organic N is present as polymers (e.g. chitin, proteins) and their complexes with phenolic compounds. As ectomycorrhizal fungi can break down and take up N from polymers (Pritsch and Garbaye 2011), this might be an important aspect to consider also in future studies.

4.4 N acquisition is affected by varying environmental conditions

Environmental variation also affects plant N acquisition from the soil and the potential gain via the support of mycorrhiza (e.g. Pena et al. 2013b, Pena and Polle 2014, Valtanen et al. 2014, Simon et al. 2017) which varies along the mutualism-antagonism continuum (Johnson et al. 1997, Jones and Smith 2004, de Mazancourt et al. 2005, Simon et al. 2017). The differences in local biotic and abiotic conditions could explain the different results between studies. N taken up by mycorrhizae is not necessarily transferred to the tree partner depending on abiotic stressors (Pena and Polle 2014, Leberecht et al. 2015), the availability of different N forms (Näsholm et al. 2013, Hasselquist et al. 2016, Makarov 2019), and EM community richness and composition (Simon et al. 2017). For example, when competing for ammonium as the single N source, *Fagus sylvatica* benefitted from the colonization with EM fungi under mild drought, but not with sufficient water availability (Pena et al. 2013a). Studies investigating N acquisition in plant-plant interactions at the species level are rare and mostly consider only the potential competition for inorganic N sources. Of the few studies including also organic N sources, the majority looked at non-woody plant species (e.g. Ashton et al. 2008, Robinson et al. 2010, but see Liese et al. 2018). The use of specific N sources as well as N composition in the soil are key factors to consider when evaluating N acquisition by trees. Overall, our results suggest that with excess soil N the availability of specific N sources is more important to avoid competition for N in tree-tree interactions than the type of mycorrhizal association.

In conclusion, our study suggests that inorganic and organic N acquisition of tree seedlings differs among tree species with considerable plasticity and depends on the investigated tree species and its competitor rather than the type of mycorrhizal association or relative growth rate. Competition for N is avoided in some species interactions by favoring certain N sources over others related to the morphological and/or physiological properties of an individual (Simon et al. 2014, 2017). Our study provides no evidence for niche differentiation based on the mycorrhizal association of trees. However, our results only consider one time point of the growing season and in the development of a tree. Thus, long-term studies are required to include potential shifts in N acquisition with seasonal and ontogenetic variation. Moreover, considering the predicted scenarios for changes in climate (IPCC 2013), exploring how tree-tree and tree-mycorrhizal interactions will change under future climate conditions, such as an increase in temperature and/or prolonged periods of drought will be crucial for a sustainable forest management.

Data and Materials Accessibility

Data will be available from the Dryad Digital Repository upon acceptance of the manuscript.

Supplementary Data

Supplementary Data is available online.

Conflict of Interest

The authors declare no conflict of interest.

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Author´s contributions

JS conceived and designed the study which was part of a larger experimental set up (MyDiv) that was conceived by NE and OF. RR and JS conducted the ^{15}N uptake experiments, collected and analysed the data. KP contributed the IRMS analyses. MT contributed the analyses of EM colonization rates, OF those of AM colonization. JS and RR led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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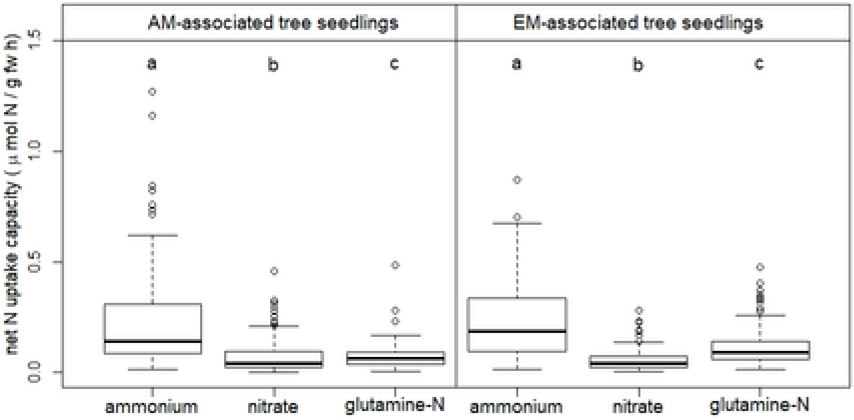
Figure legends

Figure 1. Differences in ammonium, nitrate, and glutamine-N net uptake capacity (µmol N / g fw h) of the fine roots of six temperate tree species associated with arbuscular mycorrhizal fungi (AM) or ectomycorrhizal fungi (EM). Different small letters indicate significant differences between N sources within tree seedlings of a given mycorrhiza type based on one-way ANOVAs followed by posthoc Tukey tests ($p \leq 0.050$). The horizontal line shows the median and the bottom and top of the box show the 25th and 75th percentiles. The whiskers show the data point that is less than 1.5 times the interquartile range above the 75th percentile.

Figure 2. Ammonium, nitrate, and glutamine-N net uptake capacity (µmol N / g fw h) of the fine roots of six temperate tree species associated with arbuscular mycorrhiza fungi (AM) or ectomycorrhizal fungi (EM) affected by competition regime (i.e. AM-AM vs AM-EM and EM-

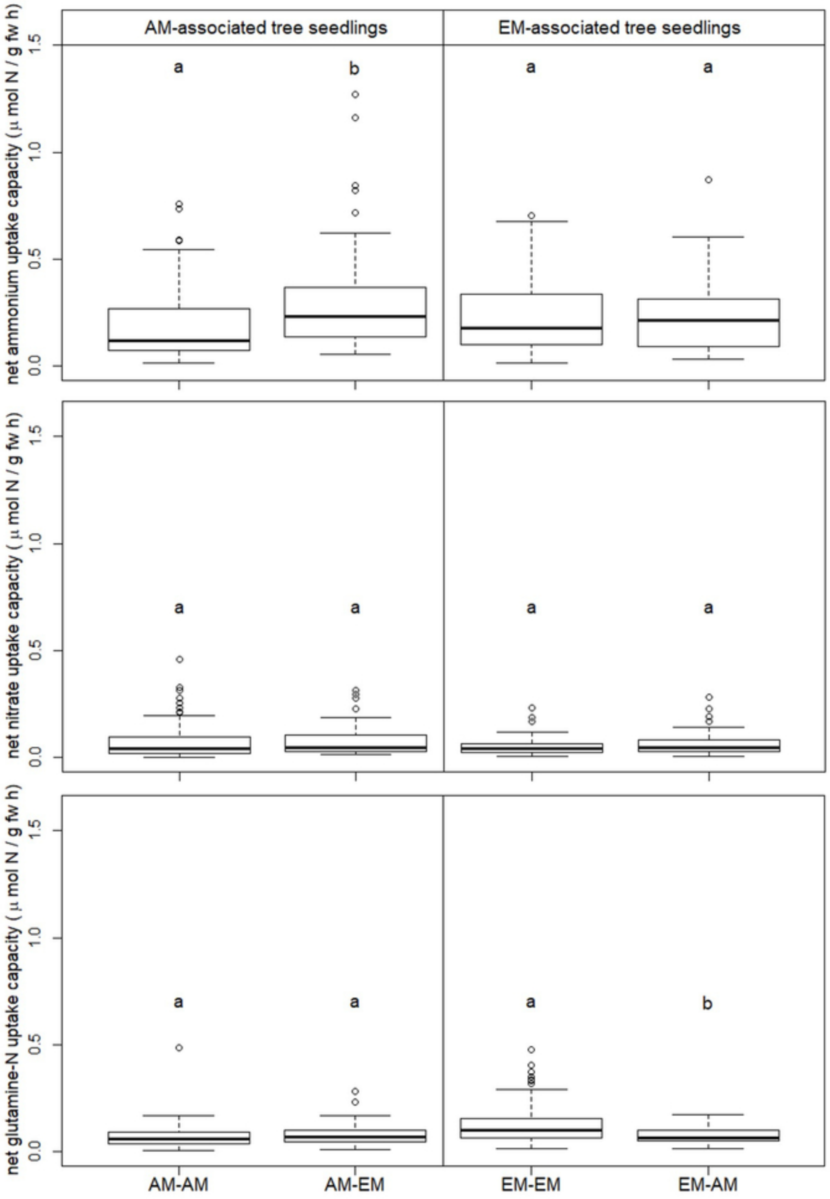
EM vs EM-AM). Different small letters indicate significant differences for each N source between competition regimes for a given mycorrhizal association of tree seedlings based on t-tests ($p \leq 0.050$). The horizontal line shows the median and the bottom and top of the box show the 25th and 75th percentiles. The whiskers show the data point that is less than 1.5 times the interquartile range above the 75th percentile.

Figure 3. Ammonium, nitrate, and glutamine-N net uptake capacity (µmol N / g fw h) of the fine roots of six temperate tree species associated with arbuscular mycorrhiza fungi (AM) (*Acer* = *Acer pseudoplatanus, Fraxinus* = *Fraxinus excelsior*, *Tilia* = *Tilia platyphyllos*) or ectomycorrhizal fungi (EM) (*Carpinus* = *Carpinus betulus*, *Fagus* = *Fagus sylvatica*, *Prunus* = *Prunus avium*). Different small letters indicate significant differences between species for each N source based on one-way ANOVAs followed by posthoc Tukey tests ($p \leq 0.050$). The horizontal line shows the median and the bottom and top of the box show the 25th and 75th percentiles. The whiskers show the data point that is less than 1.5 times the interquartile range above the 75th percentile.



Differences in ammonium, nitrate, and glutamine-N net uptake capacity (µmol N / g fw h) of the fine roots of six temperate tree species associated with arbuscular mycorrhizal fungi (AM) or ectomycorrhizal fungi (EM). Different small letters indicate significant differences between N sources within tree seedlings of a given mycorrhiza type based on one-way ANOVAs followed by posthoc Tukey tests ($p \leq 0.050$). The horizontal line shows the median and the bottom and top of the box show the 25th and 75th percentiles. The whiskers show the data point that is less than 1.5 times the interquartile range above the 75th percentile.

38x23mm (300 x 300 DPI)



Ammonium, nitrate, and glutamine-N net uptake capacity (μmol N / g fw h) of the fine roots of six temperate tree species associated with arbuscular mycorrhiza fungi (AM) or ectomycorrhizal fungi (EM) affected by competition regime (i.e. AM-AM vs AM-EM and EM-EM vs EM-AM). Different small letters indicate significant differences for each N source between competition regimes for a given mycorrhizal association of tree seedlings based on t-tests (p≤0.050). The horizontal line shows the median and the bottom and top of the box show the 25th and 75th percentiles. The whiskers show the data point that is less than 1.5 times the interquartile range above the 75th percentile.

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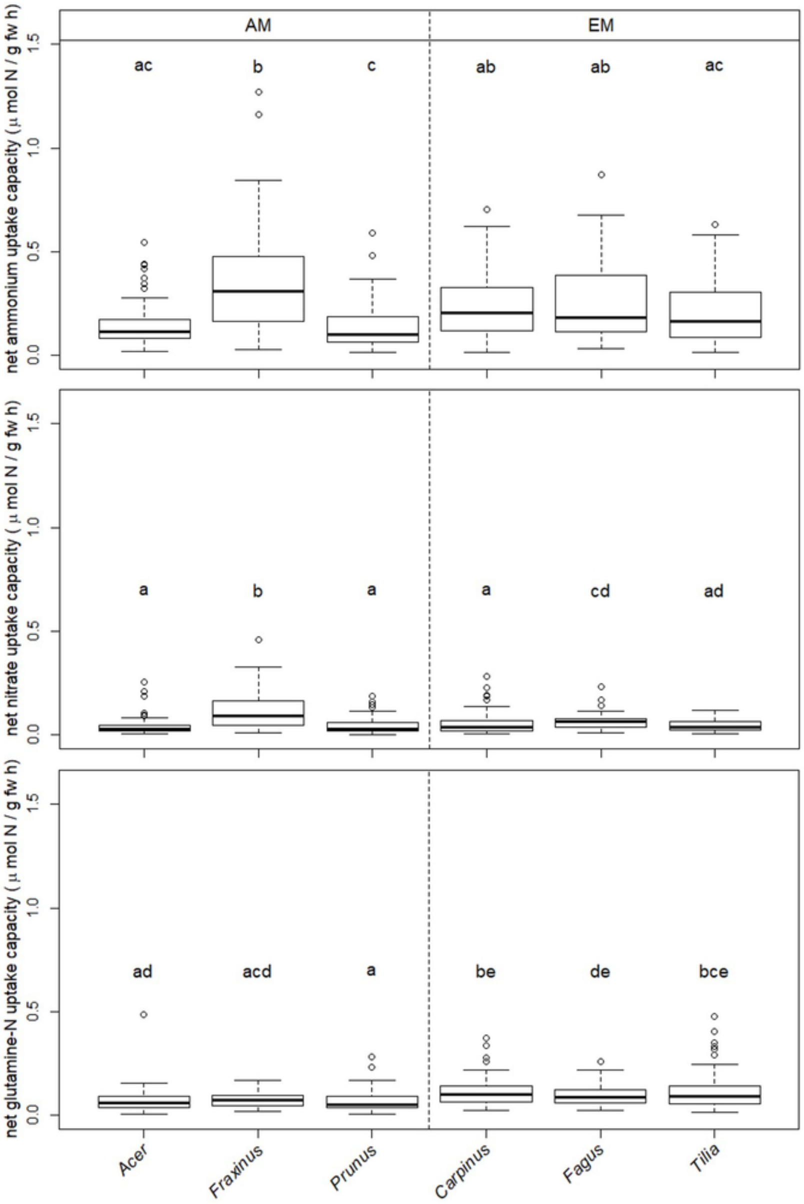

Ammonium, nitrate, and glutamine-N net uptake capacity (µmol N / g fw h) of the fine roots of six temperate tree species associated with arbuscular mycorrhiza fungi (AM) (Acer = Acer pseudoplatanus, Fraxinus = Fraxinus excelsior, Tilia = Tilia platyphyllos) or ectomycorrhizal fungi (EM) (Carpinus = Carpinus betulus, Fagus = Fagus sylvatica, Prunus = Prunus avium). Different small letters indicate significant differences between species for each N source based on one-way ANOVAs followed by posthoc Tukey tests ($p \leq 0.050$). The horizontal line shows the median and the bottom and top of the box show the 25th and 75th percentiles. The whiskers show the data point that is less than 1.5 times the interquartile range above the 75th percentile.

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Table 1: Investigated species combinations for intra- and interspecific competition. AM intra = intraspecific competition (i.e. monocultures) within AM tree species; AM-AM inter = interspecific competition between two AM tree species; EM intra = intraspecific competition (i.e. monocultures) within EM tree species; EM-EM inter = interspecific competition between two EM tree species; AM-EM inter = interspecific competition between an AM and an EM tree species. Aps = *Acer pseudoplatanus*, Cbe = *Carpinus betulus*, Fex = *Fraxinus excelsior*, Fsy = *Fagus sylvatica*, Pav = *Prunus avium*, Tpl = *Tilia platyphyllos*

AM intra	**AM-AM inter**	**AM-EM inter**	**EM-EM inter**	**EM intra**
Aps – Aps	Aps – Fex	Aps – Tpl	Cbe – Fsy	Cbe- Cbe
Fex – Fex	Aps – Pav	Fex – Cbe	Cbe – Tpl	Fsy - Fsy
Pav – Pav	Fex – Pav	Fex – Fsy	Fsy – Tpl	Tpl – Tpl
		Pav – Tpl		

Table 2: Description of the six temperate European tree species used in our study. Mycorrhizal type (i.e. AM – arbuscular mycorrhiza, EM – ectomycorrhiza), growth rate, nutrient requirements, shade tolerance, and drought sensitivity.

Species	**mycorrhizal type***	**growth rate****	**nutrient requirements****	**drought sensitivity*****	**shade tolerance*****
Acer pseudoplatanus	AM	fast	high	medium	high
Fraxinus excelsior	AM	slow	medium-high	high	high
Prunus avium	AM	fast	medium-high	medium	high
Carpinus betulus	EM	slow	medium-high	medium	high
Fagus sylvatica	EM	slow	low-medium	high	very high
Tilia platyphyllos	EM	slow	medium	low	medium

* according to Ferlian et al. (2018a)

** according to Professur für Waldbau und Professur für Forstschutz & Dendrologie der ETH Zürich (2002)

*** according to Ellenberg and Leuschner (2014)

Table 3: Differences in inorganic and organic net N uptake capacity of six tree species dependent on the neighbouring tree species. Results are based on one-way ANOVAs for each species with neighbouring identity as main factor followed by posthoc Tukey test comparing net N uptake capacity for each N source when grown in monoculture vs. with another species ($P \leq 0.050$), n.s. = not significant Aps = *Acer pseudoplatanus*, Cbe = *Carpinus betulus*, Fex = *Fraxinus excelsior*, Fsy = *Fagus sylvatica*, Pav = *Prunus avium*, Tpl = *Tilia platyphyllos*

	species	**NH_4^+**	**NO_3^-**	**Gln**	**species**	**NH_4^+**	**NO_3^-**	**Gln**
AM-AM tree species interactions								
Aps vs Fex	*Aps*	Fex > Aps	Fex > Aps	n.s.	*Fex*	n.s.	n.s.	n.s.
Aps vs Pav	*Aps*	n.s.	n.s.	n.s.	*Pav*	n.s.	n.s.	n.s.
Fex vs Pav	*Fex*	Fex > Pav	Fex > Pav	Fex > Pav	*Pav*	n.s.	n.s.	n.s.
EM-EM tree species interactions								
Cbe vs Fsy	*Cbe*	Fsy > Cbe	Fsy > Cbe	n.s.	*Fsy*	Cbe > Fsy	n.s.	n.s.
Cbe vs Tpl	*Cbe*	n.s.	n.s.	Tpl > Cbe	*Tpl*	n.s.	n.s.	n.s.
Fsy vs Tpl	*Fsy*	n.s.	n.s.	Tpl > Fsy	*Tpl*	n.s.	n.s.	n.s.
AM-EM tree species interactions								
Aps vs Tpl	*Aps*	n.s.	n.s.	n.s.	*Tpl*	n.s.	n.s.	Tpl > Aps
Fex vs Cbe	*Fex*	n.s.	n.s.	n.s.	*Cbe*	Fex > Cbe	Fex > Cbe	n.s.
Fex vs Fsy	*Fex*	n.s.	Fex > Fsy	n.s.	*Fsy*	Fex > Fsy	n.s.	n.s.
Pav vs Tpl	*Pav*	Tpl > Pav	Tpl > Pav	n.s.	*Tpl*	n.s.	n.s.	n.s.

Table 4: Preferences of ammonium (NH_4^+), nitrate (NO_3^-) and glutamine-N (Gln) net uptake capacity of six tree species differing in their mycorrhizal association (i.e. AM or EM) in intraspecific or interspecific competition. Results are based on one-way ANOVA with N source as main factor followed by posthoc Tukey test for each species combination ($P \leq 0.050$).
Aps = *Acer pseudoplatanus*, Cbe = *Carpinus betulus*, Fex = *Fraxinus excelsior*, Fsy = *Fagus sylvatica*, Pav = *Prunus avium*, Tpl = *Tilia platyphyllos*

AM tree species			***EM tree species***		
species	*N preferences*	*P-values*	*species*	*N preferences*	*P-values*
Aps – Aps	NH_4^+ > Gln > NO_3^-	≤0.012	*Cbe – Cbe*	NH_4^+/Gln > NO_3^-	<0.001
Aps – Fex	NH_4^+ > Gln/NO_3^-	≤0.001	*Cbe – Fsy*	NH_4^+/Gln > NO_3^-	<0.001
Aps – Pav	NH_4^+/Gln > NO_3^-	≤0.020	*Cbe – Tpl*	NH_4^+ > Gln > NO_3^-	≤0.007
Aps – Tpl	NH_4^+ > Gln > NO_3^-	<0.001	*Cbe – Fex*	NH_4^+ > Gln/NO_3^-	<0.001
Fex – Fex	NH_4^+ > NO_3^- > Gln	<0.001	*Fsy – Fsy*	NH_4^+ > NO_3^-	0.044
Fex – Aps	NH_4^+ > Gln/NO_3^-	≤0.003	*Fsy – Cbe*	NH_4^+ > Gln > NO_3^-	≤0.010
Fex – Pav	NH_4^+ > Gln/NO_3^-	≤0.003	*Fsy – Tpl*	NH_4^+/Gln > NO_3^-	<0.001
Fex – Cbe	NH_4^+ > Gln/NO_3^-	<0.001	*Fsy – Fex*	NH_4^+ > Gln/NO_3^-	<0.001
Fex – Fsy	NH_4^+ > Gln/NO_3^-	<0.001			
Pav – Pav	NH_4^+/Gln > NO_3^-	≤0.018	*Tpl – Tpl*	NH_4^+/Gln > NO_3^-	<0.001
Pav – Aps	NH_4^+ > NO_3^-	0.013	*Tpl – Cbe*	NH_4^+/Gln > NO_3^-	<0.001
Pav – Fex	NH_4^+ > NO_3^-	<0.001	*Tpl – Fsy*	NH_4^+ > NO_3^-	0.006
Pav – Tpl	NH_4^+ > Gln/NO_3^-	≤0.010	*Tpl – Aps*	NH_4^+ > Gln > NO_3^-	≤0.011
			Tpl – Pav	NH_4^+ > NO_3^-	<0.001