



Relevance of organic vs. inorganic nitrogen in intra- and interspecific competition of seven central European tree species

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Received: 29 October 2022 / Accepted: 15 April 2023 / Published online: 23 June 2023
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Abstract

Key message When competing for nitrogen with other tree species, organic N is more important than inorganic N for most species

Abstract Competition for nitrogen (N) in the rhizosphere is a key process regulating a seedling's chances for growth and survival. However, only few studies have investigated this at the individual species' level when using a community approach. In this study, ¹⁵N incubation experiments were conducted to quantify inorganic (i.e. ammonium and nitrate) and organic (i.e. glutamine-N and arginine-N) net N-uptake capacity of seven temperate woody species co-occurring on calcareous substrate and to investigate the consequences of intra- vs. interspecific competition in a woody seedling community. The results showed that short-term net inorganic and organic N uptake capacity was unrelated to a seedling's fast or slow growth. Furthermore, competition with other tree species did not change a seedlings' overall capacity to take up inorganic and organic N sources. Organic N was preferred over inorganic N for most species and the preferences for specific N source shifted with competition regime and tree species. Overall, this study indicates the importance of organic N sources for N uptake in woody seedlings.

Keywords Amino acids · Glutamine · Arginine · Inorganic nitrogen · Nitrogen uptake · Nitrogen acquisition

Introduction

Forest ecosystems play a crucial role for the future of human society, not only for mitigating climate change via the capture and long-term storage of carbon (C), but also via the provision of fundamental ecosystem services including resources (timber, food) and recreation (MEA 2005; FAO 2015; Sabatini et al. 2019; Simon and Adamczyk 2019). Thus, a sustainable management of forests is vital when facing today's ecological challenges. Currently discussed strategies for a sustainable management of forest ecosystems in the future include the transition of forest monocultures to heterogenous mixed-species stands (Pretzsch 2020)

because of their enhanced resilience to abiotic stressors (e.g. Fares et al. 2015; Mina et al. 2018). However, studies using multi-species approaches focus on the overall effect in plant communities, whereas the role of individual species in the game of competition and facilitation might be masked (e.g. Rewald and Leuschner 2009; Tobner et al. 2016; Fichtner et al. 2018; Trogisch et al. 2021). Furthermore, key processes in the rhizosphere are still not fully understood (Trinder et al. 2013; Weemstra et al. 2016; Pommerening and Sánchez Meador 2018), especially related to the acquisition of tree-growth limiting nitrogen (N) (Körner 2003; Millard et al. 2007; Millard and Grelet 2010).

Tree N acquisition from the soil is a key aspect when it comes to competition for N in the rhizosphere and is influenced by a variety of factors including the availability of different N forms (e.g. Näsholm et al. 2009; Stoelken et al. 2010; Hodge and Fitter 2013; Simon et al. 2017; Bueno et al. 2019) and/or a species' functional traits—for example fast vs. slow growth (Trinder et al. 2013; Li et al. 2015; Simon et al. 2017; Freschet et al. 2021). Growth strategy and N acquisition might be linked. For example, when competing with a fast-growing pioneer species slow growing *Fagus sylvatica* seedlings showed a reduced uptake

Communicated by Lee Kalcsits.

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of inorganic N, whereas inorganic N uptake increased in the pioneer species (Fotelli et al. 2002, 2005). In addition, preferring certain N forms is a mechanism with the potential to avoid competition (Simon et al. 2014, 2017). However, most competition studies only included inorganic N sources. Furthermore, only few studies have investigated the competition for N between trees at the species level (Simon et al. 2010, 2014; Li et al. 2015; Bueno et al. 2020; Reuter et al. 2021). Hence, the relevance of inorganic vs. organic N forms for the interactions between woody species still needs to be further elucidated (Tegeer and Perchlik 2018; Moreau et al. 2019). N acquisition is particularly crucial for woody seedlings when competing for limiting soil N (e.g. Körner 2003; Millard et al. 2007) due to their limited storage capacities for N (Millard and Grelet 2010).

Thus, the overall aim of this study was to investigate the consequences of competition in tree seedling communities including seven temperate woody species—co-occurring on calcareous soil—on inorganic and organic N acquisition. The specific hypotheses were: (1) Inorganic and organic N acquisition by the roots differs among tree species based on their physiological and morphological properties, such as growth rate and/or nutrient demand (e.g. Miller et al. 2007; Andersen et al. 2017; Simon et al. 2017; Liese et al. 2018; Bueno et al. 2019). For example, fast-growing species have a higher N demand and thus take up more N from the soil compared to slow-growing species, especially at the seedling stage (Millard and Grelet 2010). (2) Inorganic and organic N acquisition changes from intra- and interspecific competition depending on the competing species (Miller et al. 2007; Simon et al. 2010; Li et al. 2015; Bueno et al. 2019). For example, inorganic and organic N acquisition in European beech was significantly reduced when competing for N with sycamore maple (Simon et al. 2010). (3) Within a tree species', its preference for certain N sources changes when competing for N with other tree species (e.g. Ashton et al. 2008; Simon et al. 2010, 2017; Bueno et al. 2019) which could be a means to avoid competition with other species. For example, when grown in competition, seedlings of

European beech prefer organic N, whereas sycamore maple favours inorganic N (Simon et al. 2010, 2017).

Materials and methods

Plant material

One-year-old mycorrhizal seedlings of seven temperate tree species (provenance southwestern Germany) were purchased from a commercial tree nursery (Müller Münchhof Pflanzen GmbH (Seesen/Münchhof, Germany). Species included *Fagus sylvatica* L. (Fagaceae, ectomycorrhizal—EM), *Acer pseudoplatanus* L. (Sapindaceae, arbuscular mycorrhizal—AM), *Carpinus betulus* L. (Betulaceae, EM), *Fraxinus excelsior* L. (Oleaceae, AM), *Prunus avium* L. (Rosaceae, AM), *Quercus robur* L. (Fagaceae, EM), and *Tilia cordata* Mill. (Malvaceae, EM). *F. sylvatica* was chosen as a model species because it represents the dominant tree species of the potential natural vegetation in moist to moderately dry areas of the sub-mountainous altitude range in Central Europe (Ellenberg and Leuschner 2014; Simon et al. 2021). Although beech can grow on different soils, it commonly occurs on soil derived from limestone which is highly susceptible to water deprivation; the other tree species were chosen because they co-occur in beech forest ecosystems on calcareous substrate (Ellenberg and Leuschner 2014). Furthermore, the species used here display different growth strategies and nutrient requirements, as well as tolerance to shade and drought (see Table 1), thus providing ideal target species to study the consequences of plant interactions on inorganic and organic N acquisition in woody seedlings. From here on, species used in this study will be referred to by their corresponding genus names, i.e. *Fagus*, *Acer*, *Carpinus*, *Fraxinus*, *Prunus*, *Quercus*, and *Tilia*.

Experimental design

In a multi-species community approach, individuals were grown in sand/vermiculite (1:1 mixture) in mesocosms (30

Table 1 Description of target tree species regarding their growth strategies, nutrient requirements, shade tolerance and drought sensitivity at the seedling stage

	Growth ^a	Nutrient requirements ^a	Shade tolerance ^b	Drought ^b
<i>Fagus sylvatica</i>	Slow	Low-Medium	Very high	High
<i>Acer pseudoplatanus</i>	Fast	High	High	Medium
<i>Carpinus betulus</i>	Slow	Medium-High	High	Medium
<i>Fraxinus excelsior</i>	Slow	Medium-High	High	High
<i>Prunus avium</i>	Fast	Medium-High	High	Medium
<i>Quercus robur</i>	Fast	Low	Very low	Very low
<i>Tilia cordata</i>	Slow	Medium	Medium	Low

^aAccording to Professur für Waldbau und Professur für Forstschutz & Dendrologie der ETH Zürich (2002)^bAccording to Ellenberg and Leuschner (2014)

L) either in interspecific competition (i.e. one individual of each species) or in intraspecific competition (i.e. seven individuals of one species as a control) to quantify the inorganic and organic net N uptake capacity for each species. For each mesocosm (5–8 replicates per target species and competition regime), the target species was planted in the center surrounded by 6 other individuals at equal distance. Mesocosms were planted in October and stayed outside under a shaded roof with 30% shading (H. Nitsch & Sohn GmbH & Co. KG, Kreuztal, Germany) over winter until the end of the experiment in July the following year. Mesocosms were irrigated with tap water depending on the weather conditions to ensure a sufficient water supply. From April onwards, mesocosms received additionally 100 ml of an artificial low N nutrient solution (pH 6.5) once a week: 100 μM KNO_3 , 90 μM $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 70 μM $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 50 μM KCl , 24 μM $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 20 μM NaCl , 10 μM AlCl_3 , 7 μM $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 6 μM K_2HPO_4 , 1 μM NH_4Cl , 25 μM glutamine, and 25 μM arginine mimicking the soil solution of a low N field site (Dannenmann et al. 2009). Glutamine and arginine were chosen as the dominant amino acids in forest soil and the concentration used in this study was within the range of previously reported estimates (Inselsbacher et al. 2011). Mean annual temperature and precipitation were 9.8 °C and 845 mm, respectively, at the weather station Konstanz (#2712, 47.6774, 9.1901, 443 m above sea level; 1981–2010, Deutscher Wetterdienst DWD). During the experiment (Oct–Jul), mean air temperature was 9.3 °C.

¹⁵N uptake experiments and harvest

For quantification of inorganic (i.e. ammonium, nitrate) and organic (i.e. glutamine-N, arginine-N) net N uptake capacity of the target seedlings in the center of the mesocosms, the ¹⁵N enrichment technique described by Gessler et al. (1998) and modified by Simon et al. (2010) was used. Seedlings were carefully removed from the mesocosms. The root system was thoroughly washed with tap water to remove any adhering substrate particles. Fine roots still attached to the seedlings were then incubated for 2 h (between 10 am and 2 pm to avoid diurnal variation in N uptake (Gessler et al. 2002) in 4 ml of the artificial low N soil solution (Dannenmann et al. 2009; see above). The artificial soil solution contained all four N sources, with only one labelled as either ¹⁵NH₄⁺, ¹⁵NO₃⁻, ¹³C/¹⁵N-glutamine, or ¹³C/¹⁵N-arginine. Natural abundance was accounted for by non-labelled controls. After incubating for 2 h, the submersed root tips and moistened upper parts (~8–10 cm) were cut off, washed twice with 0.5 μM CaCl_2 to remove excess ¹⁵N on the root surface, dried with cellulose paper and oven-dried for 48 h at 60 °C. Fresh and dry weight was determined. The roots not incubating during the ¹⁵N uptake experiments were wrapped in wet tissue to prevent desiccation. Following the ¹⁵N uptake experiments, seedlings

were separated into leaves, stems, and roots. Leaf area was measured for each seedling (LI-3100 C Area Meter, LI-COR, Lincoln, USA) to calculate specific leaf area (SLA). Fresh and dry weight (after 48 h at 60 °C) were determined for all plant tissues. Root:shoot ratio was calculated as the ratio between total belowground biomass (i.e. root biomass) and total aboveground biomass (i.e. leaves and stem biomass).

Quantification of ¹⁵N, ¹³C, and total N and C in the fine roots

For the quantification of ¹⁵N, ¹³C, and total N and C, the fine roots were dried (48 h, 60 °C) and ground into a fine homogenous powder using a ball mill. Aliquots of 1.2–2 mg were transferred into tin capsules (IVA Analysentechnik, Meerbusch, Germany). Samples were sent to Agroisotop GmbH (Jülich, Germany) where they were analysed using an elemental analyser (EA; Carlo Erba Instruments NA 1500 series 2, CE Instruments, Milan, Italy) coupled to an isotope ratio mass spectrometer (IRMS; Nu Horizon, Nu Instruments Ltd., Wrexham, UK). Working standards (L-leucine) calibrated against the primary standards IAEA-CH-6 (sucrose, $\delta^{13}\text{C}_{\text{PDB}} = -1.0449$), IAEA-CH-7 (polyethylene, $\delta^{13}\text{C}_{\text{PDB}} = -32.151$), IAEA-N-1 (ammonium sulfate, $\delta^{15}\text{N}_{\text{air}} = +0.4$), and IAEA-N-2 (ammonium sulfate, $\delta^{15}\text{N}_{\text{air}} = +20.3$) were analysed after every 12th sample to detect a potential instrument drift over time. Inorganic and organic net N uptake capacity (nmol / g fw / h) was calculated based on the incorporation of ¹⁵N into the fine roots and the respective plant biomass according to Gessler et al. (1998): $\text{Net N uptake capacity} = ((^{15}\text{N}_i - ^{15}\text{N}_c) \times \text{N}_{\text{tot}} \times \text{dw} \times 10^5) / (MW \times \text{fw} \times t)^{-1}$ where ¹⁵N_i and ¹⁵N_c are the atom % of ¹⁵N in labeled (N_i) and unlabeled control plants (N_c, natural abundance), respectively, N_{tot} is the total N percentage, MW is the molecular weight (¹⁵N g mol⁻¹), dw is the dry weight, fw is the fresh weight, and t is the incubation time. Amino acids were double-labelled with ¹³C and ¹⁵N to determine whether they were taken up as intact molecules (Simon et al. 2011). Net uptake capacity of glutamine and arginine was lower based on ¹³C compared to that based on ¹⁵N incorporation suggesting that amino acids degraded in the solution or on the surface of the roots, and/or the respiration of amino acid-derived C inside the roots (Simon et al. 2011).

Statistical analyses

Data were tested for normality and homogeneity of variance. Differences among species as well as preferences of N sources within a species were tested using ANOVA on Ranks followed by Dunn's test. Differences between competition regimes within a given species were tested using Rank Sum test. Significant differences ($p \leq 0.050$) were tested using Sigmaplot 14 (Systat Software GmbH, Erkrath,

Table 2 Inorganic and organic N uptake (nmol N/g fw h)—mean and standard error of different species grown in intra- and interspecific competition

	Ammonium	Nitrate	Glutamine-N	Arginine-N
<i>Acer pseudoplatanus</i>				
Intraspecific	6.137 ± 5.234	104.338 ± 58.099	40.110 ± 12.958	6.227 ± 20.448
Interspecific	8.353 ± 5.763	74.705 ± 79.766	31.354 ± 16.631	29.742 ± 12.305
<i>Carpinus betulus</i>				
Intraspecific	18.543 ± 13.247	62.052 ± 29.373	78.638 ± 30.898	129.003 ± 51.847
Interspecific	20.932 ± 6.038	47.078 ± 13.755	93.030 ± 44.957	107.333 ± 47.744
<i>Fagus sylvatica</i>				
Intraspecific	14.384 ± 14.771	61.280 ± 28.308	70.892 ± 43.874	70.396 ± 24.685
Interspecific	12.630 ± 7.625	36.849 ± 8.412	63.881 ± 30.133	83.107 ± 84.929
<i>Fraxinus excelsior</i>				
Intraspecific	10.541 ± 6.055	116.650 ± 104.883	18.171 ± 6.009	14.106 ± 4.151
Interspecific	11.245 ± 8.754	75.903 ± 35.788	16.672 ± 7.798	16.786 ± 6.472
<i>Prunus avium</i>				
Intraspecific	9.824 ± 4.404	55.240 ± 26.445	68.326 ± 32.742	61.926 ± 11.149
Interspecific	13.986 ± 9.377	47.415 ± 24.730	47.823 ± 25.332	72.582 ± 32.894
<i>Quercus robur</i>				
Intraspecific	15.517 ± 8.339	54.404 ± 49.470	77.336 ± 58.079	124.332 ± 58.326
Interspecific	13.757 ± 10.322	71.112 ± 73.868	41.759 ± 26.601	93.067 ± 23.644
<i>Tilia cordata</i>				
Intraspecific	10.822 ± 3.877	55.139 ± 33.119	90.566 ± 58.391	63.269 ± 25.311
Interspecific	13.257 ± 12.977	37.182 ± 33.038	83.754 ± 16.515	85.378 ± 48.523

Germany) for all statistical analysis. In addition, principal component analysis (PCA) was conducted to expose potential differences in the combination of the four N sources taken up by the roots of the different species using MetaboAnalyst (Chong et al. 2018, 2019; Xia et al. 2009, 2011a, b, 2012, 2015, 2016). Before PCA, data were pre-processed by log transformation.

Results and discussion

In a tree community, net inorganic and organic N uptake capacity is unrelated to a seedling's fast or slow growth

Inorganic and organic N acquisition strategies differ among tree species based on their physiological and morphological properties (e.g. Miller et al. 2007; Andersen et al. 2017; Simon et al. 2017; Liese et al. 2018; Bueno et al. 2019). For example, fast-growing species need more N because of their higher investment in new growth compared to slow-growing species (e.g. Reich et al. 1997; Miller and Hawkins 2007). Especially higher amounts of fine roots could explore more soil volume (Comas and Eissenstat 2004) and in turn lead to an increased N uptake (e.g. Ryser 1996; Craine et al. 2001; Reuter et al. 2021). In the present study, the tree species differed in their growth and biomass indices when grown in intraspecific

competition (Supplemental Table 1). More specifically, *Fraxinus* had higher leaf, stem, and total biomass than *Fagus*, higher stem and total biomass than *Carpinus*, higher root biomass than that of *Acer* and *Quercus* ($p < 0.001$), and higher total biomass than *Prunus* ($p \leq 0.003$). For *Acer*, root biomass and root:shoot ratio were higher compared to *Carpinus* ($p < 0.001$). Root:shoot ratio in *Quercus* and *Fagus* was higher compared to that of *Carpinus* when grown in intraspecific competition ($p < 0.001$). However, these differences in biomass allocation were only partly reflected in differences in organic but not inorganic net N uptake capacity (Fig. 1; Table 2): Glutamine-N net uptake capacity was lower in *Fraxinus* compared to *Carpinus* and *Tilia* ($p \leq 0.002$) and arginine-N net uptake capacity was lower in *Fraxinus* than in *Carpinus* and *Quercus* ($p < 0.001$) when seedlings were grown in intraspecific competition. Thus, there is no evidence that supports the hypothesis that a species' growth rate or N demand is directly related to its short-term N acquisition strategies. However, as seedlings used in this experiment were 1-year old and species grow at different rates, certain biomass effects cannot be excluded. Furthermore, seedlings of *Fraxinus excelsior* are classified slow-growing by the Professur für Waldbau und Professur für Forstschutz & Dendrologie der ETH Zürich (Zürich 2002) which was the basis in this study, whereas Schulz et al. (2011) considers them as fast-growing.

The lower uptake of organic N by *Fraxinus* been reported previously (Reuter et al. 2021) and could be explained by a

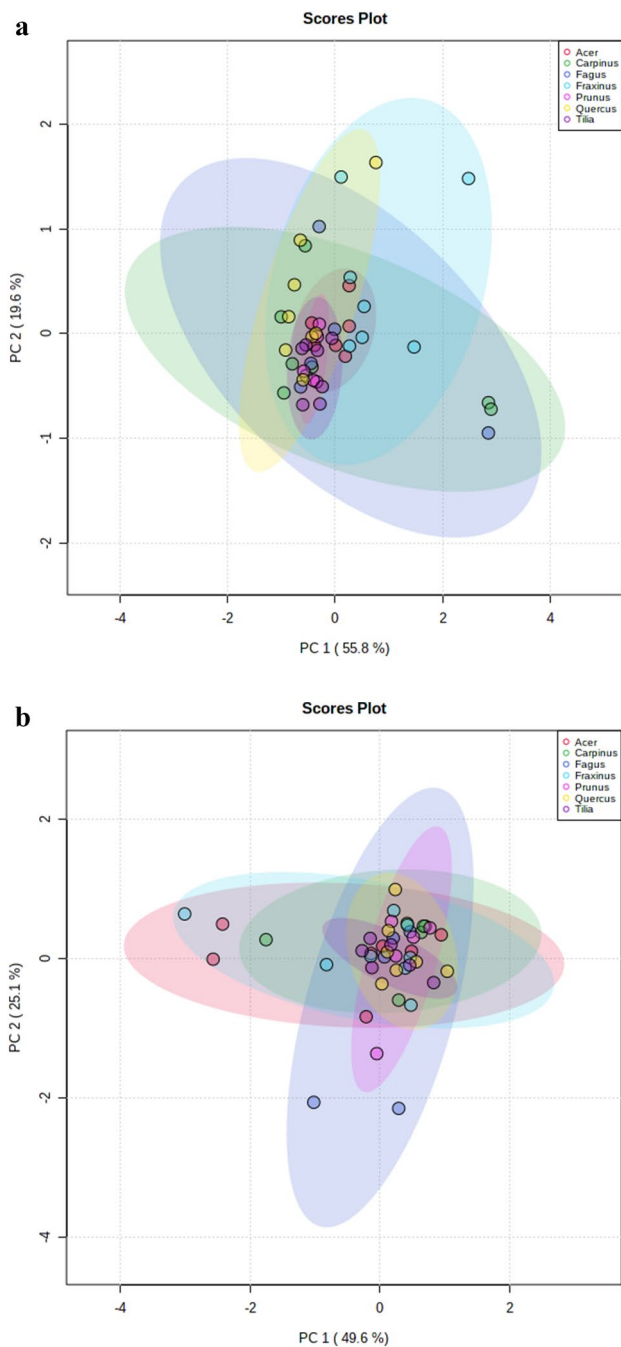


Fig. 1 **a**. Two-dimensional score plot of principal component analysis computed with net N uptake capacity of ammonium, nitrate, glutamine-N, and arginine-N for tree seedlings grown in intra-specific competition. Species are shown in different colours: *Acer*—*A. pseudoplatanus*, *Carpinus*—*C. betulus*, *Fagus*—*F. sylvatica*, *Fraxinus*—*F. excelsior*, *Prunus*—*P. avium*, *Quercus*—*Q. robur*, *Tilia*—*T. cordata*. The explained variances (in percentage) are shown in x- and y-axes in the plot **b**. Two-dimensional score plot of principal component analysis computed with net N uptake capacity of ammonium, nitrate, glutamine-N, and arginine-N for tree seedlings grown in inter-specific competition. Species are shown in different colours: *Acer*—*A. pseudoplatanus*, *Carpinus*—*C. betulus*, *Fagus*—*F. sylvatica*, *Fraxinus*—*F. excelsior*, *Prunus*—*P. avium*, *Quercus*—*Q. robur*, *Tilia*—*T. cordata*. The explained variances (in percentage) are shown in x- and y-axes in the plot

higher density of nitrate transporters in the root membranes and chemical properties (Jacob and Leuschner 2015) as well as a positive interaction between arbuscular mycorrhiza and the uptake of nitrate (Liu et al. 2018; Reuter et al. 2021). Furthermore, N acquisition is related to species-specific root traits such as root hair length and density, root branching density, and/or specific root length (Freschet et al. 2021). These results are similar to those by Reuter et al. (2021) studying six of the seven species used here in the field who found that tree species and not mycorrhization type influenced net N uptake capacity overall.

Similarly, with interspecific competition, tree species differed partly in their growth and biomass indices (Supplemental Table 1) but only in organic and not inorganic net N uptake capacity (Table 2): Glutamine-N net uptake capacity was lower in *Fraxinus* compared to *Carpinus*, *Tilia*, and *Fagus* grown in interspecific competition ($p < 0.001$). Arginine-N net uptake capacity was lower in *Fraxinus* compared to *Carpinus*, *Tilia*, and *Quercus* grown in interspecific competition and in *Acer* compared to *Carpinus* ($p < 0.001$). These results suggest that regardless of competition regime, differences among species are consistent with regard to the specific N sources they take up (e.g. Simon et al. 2017; Bueno et al. 2019; 2020; Reuter et al. 2021). Differences among species were found only for organic but not inorganic N sources highlighting the relevance of organic N as sources of N for trees.

Considering the different N sources, principal component analysis (Figs. 1a and b; Table 3) indicated that net uptake of ammonium, nitrate, glutamine-N, and arginine-N differed between species. When grown in intraspecific competition, 55.8% of the variation was explained by nitrate and 19.6% by arginine-N, whereas when grown in interspecific competition, 49.6% of the variation was explained by nitrate and 25.1% by ammonium. Inorganic N uptake differentiated species more than organic N uptake. Furthermore, comparing among species when grown in intra- vs. interspecific competition (Fig. 1a and b), the results indicate the relevance of specific N sources for tree N acquisition depending on their biotic environment.

In a community, a seedling's capacity to take up inorganic and organic N sources does not change in competition with other tree species

Competition for N in the rhizosphere leads to a shift in inorganic and organic N acquisition strategies in trees (e.g. Simon et al. 2010; Li et al. 2015; Bueno et al. 2019). For example, when grown in competition, *Fagus sylvatica* had a generally lower organic and inorganic N uptake, whereas inorganic, but not organic N acquisition of *Acer pseudoplatanus* increased (Simon et al. 2010). In the present study, growth and biomass indices did not differ significantly

Table 3 Principal Component Analysis—factor loadings for the measured parameters. Levels of net N uptake capacity of arginine, glutamine, nitrate, and ammonium via the roots of seven temperate woody species grown in intra- and interspecific competition

N source	Intraspecific competition		Interspecific competition	
	Loadings 1	Loadings 2	Loadings 1	Loadings 2
Arginine-N	− 0.9755	0.11443	0.21488	− 0.04388
Glutamine-N	− 0.16825	− 0.92486	0.22122	− 0.17491
Nitrate	0.12124	− 0.3627	0.94872	− 0.02120
Ammonium	− 0.07342	0.00017	0.06939	0.98338

The parameter explaining most of the variation is highlighted in bold

between competition regimes for most species, except for a lower SLA in *Fraxinus* in inter- compared to intraspecific competition ($p=0.040$). Similarly, inorganic and organic net N uptake capacity did not change from intra to interspecific competition for any of the study species which might be due to the high variation in net N uptake capacity displayed by the individuals in this study. Only trends were found for a higher glutamine-N (*Quercus*, $p=0.073$) and nitrate (*Fagus*, $p=0.056$) net uptake capacity when grown in intra- compared to interspecific competition. These results are in contrast with previous studies investigating the consequences of competition for N for inorganic and organic N acquisition using two-species approaches (e.g. Fotelli et al. 2004; Simon et al. 2010, 2014; Li et al. 2015). Liese et al. (2018) used a community approach with four species grown together in a mesocosm; however, they only measured total N uptake and not that of the different tree species. Overall, the results of the present study suggest that the interactions between seedlings in a community and with regard to N uptake are more complex. In the multi-species approach used here, the consequences of potential competition and/or facilitation between certain individual species—such as a negative effect with one species, but a positive effect of another—might have canceled each other out and thus might have masked any

individual species' effects. However, the trends of reduced inorganic or organic N acquisition found in the present study for certain N sources and species suggests a negative effect of competition with certain species.

N source preferences shift with competition regime and tree species

Tree species prefer different N sources when competing for N (e.g. Ashton et al. 2008; Simon et al. 2010; 2017; Bueno et al. 2019; Reuter et al. 2021). This hypothesis was confirmed in the present study. When seedlings were grown in intraspecific competition, the preferred N sources in short-term uptake experiments varied depending on the species (Table 4): *Fagus* showed no preferences, whereas *Carpinus*, *Quercus*, *Prunus*, and *Tilia* preferred organic N over ammonium ($p \leq 0.011$). *Acer* preferred nitrate over ammonium and *Fraxinus* nitrate over ammonium and arginine-N ($p < 0.001$). Preferences for N also differed among species when grown in interspecific competition: Organic N was favoured over ammonium for *Carpinus*, *Acer*, and *Tilia*; glutamine-N was preferred over ammonium for *Fagus*, arginine-N over ammonium for *Quercus* and *Prunus*, and nitrate over ammonium for *Acer* ($p \leq 0.017$). *Fraxinus* took up more nitrate than glutamine-N or ammonium ($p < 0.001$). These results highlight the significance of organic N for tree N acquisition for most of the studied species, except for *Fraxinus*.

Looking at how N source preferences of the study species shifted when seedlings were from intra- to interspecific competition treatments, five patterns were found (Table 4): (1) *Acer* included organic N as preferred N sources over ammonium; (2) *Fagus* preferred glutamine-N compared to no preferences in intraspecific competition; (3) *Fraxinus* switched between amino acids, but still preferred nitrate; (4) *Prunus* and *Quercus* no longer preferred glutamine-N over ammonium, and (5) *Carpinus* and *Tilia* showed no differences between competition regimes. Thus, the preference to take up specific N sources depends on the species and is regulated by underlying physiological traits, such as, the

Table 4 Preferences of ammonium (NH_4^+), nitrate (NO_3^-), glutamine-N (Gln), and arginine-N (Arg) net uptake capacity (nmol N / g fw h) of seven temperate tree species

Species	Intraspecific competition		Interspecific competition	
	N preferences	P-values	N preferences	P-values
<i>Acer pseudoplatanus</i>	$\text{NO}_3^- > \text{NH}_4^+$	<0.001	$\text{NO}_3^- / \text{orgN} > \text{NH}_4^+$	0.004
<i>Carpinus betulus</i>	$\text{orgN} > \text{NH}_4^+$	<0.001	$\text{orgN} > \text{NH}_4^+$	0.002
<i>Fagus sylvatica</i>	n.a.	0.085	$\text{Gln-N} > \text{NH}_4^+$	0.017
<i>Fraxinus excelsior</i>	$\text{NO}_3^- > \text{NH}_4^+ / \text{Arg-N}$	0.002	$\text{NO}_3^- > \text{NH}_4^+ / \text{Gln-N}$	<0.001
<i>Prunus avium</i>	$\text{orgN} > \text{NH}_4^+$	0.011	$\text{Arg-N} > \text{NH}_4^+$	0.013
<i>Quercus robur</i>	$\text{orgN} > \text{NH}_4^+$	<0.001	$\text{Arg-N} > \text{NH}_4^+$	0.002
<i>Tilia cordata</i>	$\text{orgN} > \text{NH}_4^+$	0.002	$\text{orgN} > \text{NH}_4^+$	0.004

Significant differences are marked in bold. n.a.: not applicable. Results are based on ANOVA on Ranks with N source as main factor followed by posthoc Dunn's test for each species combination ($p \leq 0.050$)

density of specific transporters (Näsholm et al. 2009; Jacob and Leuschner 2015), as well as free amino acid synthesised when ammonium is assimilated (e.g. Imsande and Touraine 1994; Kreuzwieser et al. 1997; Collier et al. 2003; Reuter et al. 2021). Organic N uptake is an important N source for certain tree species, particularly when N is limiting. As the assimilation process can be bypassed, organic N acquisition requires less energy (Moreau et al. 2019).

In conclusion, short-term net inorganic and organic N uptake capacity are not related to a seedling's inherent growth rate when grown in a tree community and do not shift in response to competing species, but the preferences for certain N sources can change.

Author contributions Statement JS conceived and designed the study, conducted the experiment, analysed the data, and wrote the manuscript.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s00468-023-02418-0>.

Acknowledgement I gratefully thank Leonard Schink and Iljas Müller for their help with the ¹⁵N incubation experiments. Furthermore, I thank Silvia Kuhn, Rosi Miller, and Regina Grimm for their help with the biomass sampling. I also thank Oliver Hartmann for his help with sample processing. Moreover, I thank the gardeners at the Botanical Garden of the University of Konstanz for their help with the experimental set up.

Funding Open Access funding enabled and organized by Projekt DEAL. This work was supported by the Young Scholar Fund at the University of Konstanz, Germany. JS is currently supported by a Heisenberg Fellowship (no. SI 1556/4-1) of the German Research Foundation (DFG).

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

Declarations

Conflicts of interest The authors declare no conflicts of interest.

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References

- Andersen KM, Mayor JR, Turner BL (2017) Plasticity in nitrogen uptake among plant species with contrasting nutrient acquisition strategies in tropical forests. *Ecology* 98:1388–1398. <https://doi.org/10.1002/ecy.1793>
- Ashton IW, Miller AE, Bowman WD et al (2008) Nitrogen preferences and plant-soil feedbacks as influenced by neighbors in the alpine tundra. *Oecologia* 156:625–636. <https://doi.org/10.1007/s00442-008-1006-1>
- Bueno A, Pritsch K, Simon J (2019) Species-specific outcome in the competition for nitrogen between invasive and native tree seedlings. *Front Plant Sci* 10:337. <https://doi.org/10.3389/fpls.2019.0337>
- Bueno A, Pritsch K, Simon J (2020) Responses of native and invasive woody seedlings to combined competition and drought are species-specific. *Tree Physiol* 41:343–357. <https://doi.org/10.1093/treephys/tpaa134>
- Chong J, Soufan O, Li C, Caraus I, Li S, Bourque G, Wishart DS, Xia J (2018) MetaboAnalyst 4.0: towards more transparent and integrative metabolomics analysis. *Nucleic Acids Res* 46:W486–494. <https://doi.org/10.1093/nar/gky310>
- Chong J, Wishart DS, Xia J (2019) Using MetaboAnalyst 4.0 für comprehensive and integrative metabolomics data analysis. *Curr Protoc Bioinform* 68:e86. <https://doi.org/10.1002/cpbi.86>
- Collier MD, Fotelli MN, Nahm M et al (2003) Regulation of nitrogen uptake by *Fagus sylvatica* on a whole plant level—interactions between cytokinins and soluble N compounds. *Plant Cell Environ* 26:1540–1560. <https://doi.org/10.1046/j.1365-3040.2003.01079.x>
- Comas LH, Eissenstat DM (2004) Linking fine root traits to maximum potential growth rate among 11 mature temperate tree species. *Func Ecol* 18:388–397. <https://doi.org/10.1111/j.0269-8463.2004.00835.x>
- Craine JM, Froehle J, Tilman DG et al (2001) The relationship among root and leaf traits of 76 grassland species and relative abundance along fertility and disturbance gradients. *Oikos* 93:274–285. <https://doi.org/10.1034/j.1600-0706.2001.930210.x>
- Dannenmann M, Simon J, Gasche R et al (2009) Tree girdling provides insight on the role of labile carbon in nitrogen partitioning between soil microorganisms and adult european beech. *Soil Biol Biochem* 41:1622–1631. <https://doi.org/10.1016/j.soilbio.2009.04.024>
- Ellenberg H, Leuschner C (2014) *Vegetation Mitteleuropas mit den Alpen in ökologischer, dynamischer und historischer sicht*, 6th edn. Ulmer Verlag, Stuttgart, Germany
- FAO (2015) *Global Forest Resources Assessment 2015*. Desk reference. FAO, Rome, Italy
- Fares S, Mugnoz GS, Corona P et al (2015) Sustainability: five steps for managing Europe's forests. *Nature* 519:407–409. <https://doi.org/10.1038/519407a>
- Fichtner A, Härdtle W, Bruelheide H et al (2018) Neighbourhood interactions drive overyielding in mixed-species tree communities. *Nat Comm* 9:1144. <https://doi.org/10.1038/s41467-018-03529-w>
- Fotelli MN, Rennenberg H, Geßler A (2002) Effects of drought on the competitive interference of an early successional species (*Rubus fruticosus*) on *Fagus sylvatica* seedlings: N uptake and partitioning, responses of amino acids and other N compounds. *Plant Biol* 4:311–320. <https://doi.org/10.1055/s-2002-32334>
- Fotelli MN, Rienks M, Rennenberg H et al (2004) Climate and forest management affect ¹⁵N-uptake, N balance and biomass of european beech seedlings. *Trees* 18:157–166. <https://doi.org/10.1007/s00468-003-0289-4>
- Fotelli MN, Rudolph P, Rennenberg H et al (2005) Irradiance and temperature affect the competitive interference of blackberry on the

- physiology of european beech seedlings. *New Phytol* 165:453–462. <https://doi.org/10.1111/j.1469-8137.2004.01255.x>
- Freschet GT, Roumet C, Comas LH et al (2021) Root traits as drivers of plant and ecosystem functioning: current understanding, pitfalls and future research needs. *New Phytol* 232:1123–1158. <https://doi.org/10.1111/nph.17072>
- Gessler A, Schneider S, von Sengbusch D et al (1998) Field and laboratory experiments on net uptake of nitrate and ammonium by the roots of spruce (*Picea abies*) and beech (*Fagus sylvatica*) trees. *New Phytol* 138:275–285. <https://doi.org/10.1046/j.1469-8137>
- Gessler A, Kreuzwieser J, Dopatka T et al (2002) Diurnal courses of ammonium net uptake by the roots of adult beech (*Fagus sylvatica*) and spruce (*Picea abies*) trees. *Plant Soil* 240:23–32. <https://doi.org/10.1023/A:1015831304911>
- Hodge A, Fitter A (2013) Microbial mediation of plant competition and community structure. *Func Ecol* 27:865–875. <https://doi.org/10.1111/1365-2435.12002>
- Imساند H, Touraine B (1994) N demand and the regulation of nitrate uptake. *Plant Physiol* 105:3–7. <https://doi.org/10.1104/pp.105.1.3>
- Inselsbacher E, Öhlund J, Jämtgard S et al (2011) The potential of microdialysis to monitor organic and inorganic nitrogen compounds in soil. *Soil Biol Biochem* 43:1321–1332. <https://doi.org/10.1016/j.soilbio.2011.03.003>
- Jacob A, Leuschner C (2015) Complementarity in the use of nitrogen forms in a temperate broad-leaved mixed forest. *Plant Ecol Divers* 8:243–258. <https://doi.org/10.1080/17550874.898166>
- Körner C (2003) Carbon limitation in trees. *J Ecol* 91:4–17. <https://doi.org/10.1046/j.1365-2745.2003.00742.x>
- Kreuzwieser J, Herschbach C, Stulen I et al (1997) Interactions of NH_4^+ and L-glutamate with NO_3^- transport processes of non-mycorrhizal *Fagus sylvatica* roots. *J Exp Bot* 48:1431–1438. <https://doi.org/10.1093/jxb/48.7.1431>
- Li X, Rennenberg H, Simon J (2015) Competition for nitrogen between *Fagus sylvatica* and *Acer pseudoplatanus* seedlings depends on soil nitrogen availability. *Front Plant Sci* 6:302. <https://doi.org/10.3389/fpls.2015.00302>
- Liese R, Lübke T, Albers NW et al (2018) The mycorrhizal type governs root exudation and nitrogen uptake of temperate tree species. *Tree Physiol* 38:83–95. <https://doi.org/10.1093/treephys/tpx131>
- Liu M, Xu F, Xu X et al (2018) Age alters uptake pattern of organic and inorganic nitrogen by rubber trees. *Tree Physiol* 38:1685–1693. <https://doi.org/10.1093/treephys/tpy031>
- MEA (2005) Millennium ecosystem assessment. Ecosystems and human well-being. Synthesis. Island Press, Washington, DC, USA
- Millard P, Grelet G-A (2010) Nitrogen storage and remobilization by trees: ecophysiological relevance in a changing world. *Tree Physiol* 30:1083–1095. <https://doi.org/10.1093/treephys/tpq042>
- Millard P, Sommerkorn M, Grelet G-A (2007) Environmental change and carbon limitation in trees: a biochemical, ecophysiological and ecosystem appraisal. *New Phytol* 175:11–28. <https://doi.org/10.1111/j.1469-8137.2007.02079.x>
- Miller BD, Hawkins BJ (2007) Ammonium and nitrate uptake, nitrogen productivity and biomass allocation in interior spruce families with contrasting growth rates and mineral nutrient preconditioning. *Tree Physiol* 27:901–909. <https://doi.org/10.1093/treephys/27.6.901>
- Miller AE, Boman WD, Suding KN (2007) Plant uptake of inorganic and organic nitrogen: neighbor identity matters. *Ecology* 88:1832–1840. <https://doi.org/10.1890/06-0946.1>
- Mina M, del Rio M, Huber MO et al (2018) The symmetry of competitive interactions in mixed Norway spruce, silver fir and european beech forests. *J Veg Science* 29:775–787. <https://doi.org/10.1111/jvs.12664>
- Moreau D, Bardgett RD, Finlay RD et al (2019) A plant perspective on nitrogen cycling in the rhizosphere. *Func Ecol* 33:540–552. <https://doi.org/10.1111/1365-2435.13303>
- Näsholm T, Kielland K, Ganeteg U (2009) Uptake of organic nitrogen by plants. *New Phytol* 182:31–48. <https://doi.org/10.1111/j.1469-8137.2008.02751.x>
- Pommerening A, Sánchez Meador AJ (2018) Tamm review: Tree interactions between myth and reality. *For Ecol Manage* 424:164–176. <https://doi.org/10.1016/j.foreco.2018.04.051>
- Pretzsch H (2020) Transitioning monocultures to complex forest stands in Central Europe: principles and practise. In: Stanturf JA (ed) Achieving sustainable management of boreal and temperate forests. Burleigh Dodds Science Publishing, UK. <https://doi.org/10.19103/AS.2019.0057.14>
- Professur für Waldbau und Professur für Forstschutz, Dendrologie der ETH Zürich (2002) Mitteleuropäische Waldbaumarten. In: Artbeschreibung unter besonderer Berücksichtigung der Schweiz, ETH Zürich, Zürich
- Reich PB, Walters MB, Ellsworth DS (1997) From tropics to tundra: global convergence in plant functioning. *Proc Natl Acad Sci USA* 94:13730–13734. <https://doi.org/10.1073/pnas.94.25.13730>
- Reuter R, Ferlian O, Tarkka M et al (2021) Tree species rather than type of mycorrhizal association drive inorganic and organic nitrogen acquisition in tree-tree interactions. *Tree Physiol* 41:2096–2108. <https://doi.org/10.1093/treephys/tpab059>
- Rewald B, Leuschner C (2009) Belowground competition in a broad-leaved temperate mixed forest: pattern analysis and experiments in a four-species stand. *Eur J For Res* 128:387–398. <https://doi.org/10.1007/s10342-009-0276-4>
- Ryser P (1996) The importance of tissue density for growth and life span of leaves and roots: a comparison of five ecologically contrasting grasses. *Func Ecol* 10:717–723. <https://doi.org/10.2307/2390506>
- Sabatini FM, Barreto de Andrade R, Paillet Y et al (2019) Trade-off between carbon stocks and biodiversity in european temperate forests. *Glob Change Biol* 25:536–548. <https://doi.org/10.1111/gcb.14503>
- Schulz H, Härtling S, Stange CF (2011) Species-specific differences in nitrogen uptake and utilization by six European tree species. *J Plant Nutr Soil Sci* 174:28–37
- Simon J, Adamczyk B (2019) Plant secondary compounds in forest ecosystems under global change: from defense to carbon sequestration. *Front Plant Sci*. <https://doi.org/10.3389/fpls.2019-00831>
- Simon J, Waldhecker P, Brüggemann N et al (2010) Competition for nitrogen sources between european beech (*Fagus sylvatica*) and sycamore maple (*Acer pseudoplatanus*) seedlings. *Plant Biol* 12:453–458. <https://doi.org/10.1111/j.1438-8677.2009.00225.x>
- Simon J, Dannenmann M, Gasche R et al (2011) Competition for nitrogen between adult european beech and its offspring is reduced by avoidance strategy. *For Ecol Manage* 252:105–114. <https://doi.org/10.1016/j.foreco.2011.01.035>
- Simon J, Li X, Rennenberg H (2014) Competition for nitrogen between european beech and sycamore maple shifts in favour of beech with decreasing light availability. *Tree Physiol* 34:49–60. <https://doi.org/10.1105/tpc.114.125849>
- Simon J, Dannenmann M, Pena R et al (2017) Nitrogen nutrition of beech forests in a changing climate: importance of plant-soil-microbe water, carbon, and nitrogen interactions. *Plant Soil* 148:89–114. <https://doi.org/10.1007/s11104-017-3293-y>
- Simon J, Bilela S, Rennenberg H (2021) Nitrogen uptake capacity of European beech (*Fagus sylvatica* L.) only partially depends on tree age. *Tree* 35:1739–1745. <https://doi.org/10.1007/s00468-021-02190-z>
- Stoelken G, Simon J, Ehrling B et al (2010) The presence of amino acids affects inorganic N uptake in non-mycorrhizal seedlings of

- European beech (*Fagus sylvatica*). *Tree Physiol* 30:1118–1128. <https://doi.org/10.1093/treephys/tpq050>
- Tegeder M, Perchlik M (2018) The importance of organic nitrogen transport processes for plant productivity and nitrogen use efficiency. In: Shrawat A, Zayed A, Lightfoot DA (eds) *Engineering nitrogen utilization in crop plants*. Springer, Heidelberg, pp 233–253
- Tobner CM, Paquette A, Gravel D et al (2016) Functional identity is the main driver of diversity effects in young tree communities. *Ecol Lett* 19:638–647. <https://doi.org/10.1111/ele.12600>
- Trinder CJ, Brooker RW, Robinson D (2013) Plant ecology's guilty little secret: understanding the dynamics of plant competition. *Func Ecol* 27:918–929. <https://doi.org/10.1111/1365-2435.12078>
- Trogisch S, Liu X, Rutten G et al (2021) The significance of tree-tree interactions for forest ecosystem functioning. *Basic Appl Ecol* 55:33–52. <https://doi.org/10.1016/j.baee.2021.02.003>
- Weemstra M, Mommer L, Visser EJW et al (2016) Towards a multi-dimensional root trait framework: a tree root review. *New Phytol* 211:1159–1169. <https://doi.org/10.1111/nph.14003>
- Xia J, Sinelnikov I, Han B, Wishart DS (2015) MetaboAnalyst 3.0 - making metabolomics more meaningful. *Nucl Acids Res* 43:W251–W257.
- Xia J, Wishart DS (2011) Metabolomic data processing, analysis, and interpretation using metaboanalyst. *Curr Protoc Bioinform* 34(1):14–10
- Xia J, Wishart DS (2011) Web-based inference of biological patterns, functions and pathways from metabolomic data using metaboanalyst. *Nat Protoc* 6:743–760. <https://doi.org/10.1038/nprot.2011.319>
- Xia J, Wishart DS (2016) Using MetaboAnalyst 3.0 for comprehensive metabolomics data analysis. *Curr Protoc Bioinform* 55(1):14–10
- Xia J, Psychogios N, Young N, Wishart DS (2009) MetaboAnalyst: a web server for metabolomic data analysis and interpretation. *Nucleic Acids Res* 37:W652–W660. <https://doi.org/10.1093/nar/gkp356>
- Xia J, Mandal R, Sinelnikov I, Broadhurst D, Wishart DS (2012) MetaboAnalyst 2.0—A comprehensive server for metabolomic data analysis. *Nucleic Acids Res* 40:W127–W133. <https://doi.org/10.1093/nar/gks374>
- Xia J, Sinelnikov I, Han B, Wishart DS (2015) MetaboAnalyst 3.0—making metabolomics more meaningful. *Nucleic Acids Res* 43:W251–W257. <https://doi.org/10.1093/nar/gkv380>

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