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Responses of native and invasive woody seedlings to combined competition and drought are species-specific

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Running title: Tree invasions: Competition under drought

Abstract

Woody species invasions are a major threat to native communities with intensified consequences during increased periods of summer drought as predicted for the future. Competition for growth-limiting nitrogen (N) between native and invasive tree species might represent a key mechanism underlying the invasion process, because soil water availability and N acquisition of plants are closely linked. To study whether the traits of invasive species provide an advantage over natives in Central Europe in the competition for N under drought, we conducted a greenhouse experiment. We analysed the responses of three native (i.e. *Fagus sylvatica*, *Quercus robur*, and *Pinus sylvestris*) and two invasive woody species (i.e. *Prunus serotina* and *Robinia pseudoacacia*) to competition in terms of their organic and inorganic N acquisition, as well as allocation of N to N pools in the leaves and fine roots. In our study, competition resulted in reduced growth and changes in internal N pools in both native and invasive species mediated by the physiological characteristics of the target species, the competitor, as well as soil water supply. N acquisition, however, was not affected by competition indicating that changes in growth and N pools were rather linked to the remobilization of stored N. Drought led to reduced N acquisition, growth and total soluble protein-N levels, while total soluble amino acid-N levels increased, most likely as osmoprotectants as an adaptation to the reduced water supply. Generally, the consequences of drought were enhanced with competition across all species. Comparing the invasive competitors, *P. serotina* was a greater threat to the native species than *R. pseudoacacia*. Furthermore, deciduous and coniferous native species affected the invasives differently, with the species-specific responses being mediated by soil water supply.

1. Introduction

Invasion of natural habitats by exotic species is considered a major aspect of anthropogenic global change (Vitousek et al. 1997, Ricciardi 2007). In forest ecosystems, successful plant invasions have a negative impact on the structure and composition of plant and microbial communities, as well as forest biogeochemical processes (Castro-Díez et al. 2006, Corbin and D'Antonio 2012). For example, invasives can suppress the recruitment and growth of native plant species, affect carbon pools and nutrient fluxes, and modify litter quality and decomposition (Ehrenfeld et al. 2001, Ehrenfeld 2003, Vilà et al. 2011). As a result, forest functioning is altered and the provision of ecosystem services may be impaired (Holmes et al. 2009, Vilà et al. 2011, Wardle and Peltzer 2017). Invasive plant species commonly display functional traits and growth strategies that improve resource capture and favour reproduction including high seed production (Mason et al. 2008), specific leaf area (Grotkopp and Rejmánek 2007, Leishman et al. 2007), photosynthetic rates (Pattison et al. 1998, McDowell 2002), relative growth rates (Grotkopp and Rejmánek 2007), root biomass (Broadbent et al. 2018) and/or specific root length (Dawson 2015). These traits contribute to the successful establishment and dispersal of invasive species in new habitats by enhancing their competitive ability. For example, invasive *Prunus serotina* and *Robinia pseudoacacia* produce more biomass to the detriment of slower growing of native *Quercus robur* and *Carpinus betulus* when grown in competition (Kawaletz et al. 2013).

A key resource in the competition between native and invasive plant species is plant-growth limiting nitrogen (N) (Littschwager et al. 2010, Eller and Oliveira 2017). The competitive ability of plants for acquiring N is determined by plant morphological (e.g. density and length of root hairs) and physiological (e.g. N uptake capacity, expression and activity of transporters in root cells) traits, as well as species-specific plasticity in these traits (Casper and Jackson 1997), but also the support of symbionts such as mycorrhiza (e.g. Näsholm et al. 2009) and/or N_2-fixing bacteria (Bueno et al. 2019). Previous studies investigating the effects of competition for N found plasticity in the N uptake capacity of trees with increases or decreases in response to interspecific competition which, however, depended on the competing species, environmental conditions, and available N sources (e.g. inorganic vs organic N) (Simon et al. 2010, Simon et al. 2014, Li et al. 2015, Bueno et al. 2019). For example, *Fagus sylvatica* increased organic N acquisition in competition with *Acer pseudoplatanus* compared to intraspecific competition at high but not low soil N availability (Li et al. 2015): With ambient but not reduced light, organic N acquisition decreased in competition with *A. pseudoplatanus* reflecting a better adaptation of *F. sylvatica* to low light conditions compared to *A. pseudoplatanus* (Simon et al. 2014). The utilization of different N sources likely provides an advantage when competing for N (McKane et al. 2002, Simon et al. 2014) with a high potential to drive niche differentiation and species coexistence (McKane et al. 2002, Ashton et al. 2010, Boudsocq et al. 2012). In the context of competition between native and invasive species, the preference of different N forms in competition (i.e. one species favouring organic N, whereas the other prefers inorganic N sources) might provide an important mechanism to effectively avoid competition for N. To our knowledge, this theory has mostly been tested for non-woody species (Fraterrigo et al. 2011, Huangfu et al. 2016) and only recently for tree species (but see Bueno et al. 2019).

The threat of invasive species to European forests might become even more severe in light of the predicted climate changes for Central Europe. In Bueno et al. (2019), the responses of three native and two invasive tree species (i.e. the same species as used here) to high soil N availability were investigated due to the expected increase in atmospheric N deposition (Rennenberg et al. 2009). In the present study, responses of native and invasive tree species to competition for N are investigated under drought conditions. Drought periods in summer are expected to increase in frequency and severity (Spinoni et al. 2017). Thus, understanding the consequences of drought on the outcome of competition for N between native and invasive woody plant species is crucial. N dynamics in both plant and soil are tightly linked to water availability (Gessler et al. 2017): For example, drought negatively affects soil N mineralization processes resulting in reduced plant available N in the rhizosphere (Simon et al. 2017), soil N diffusion and mass flow (Rennenberg et al. 2006), and the activity of root proteins related to N acquisition (Bista et al. 2018). Reduced mycorrhizal colonization might additionally lead to less N transfer to plants (Nilsen et al. 1998). Therefore, also the competition for N between plants is altered with drought. For example, in studies by Fotelli et al. (2001, 2002) the combination of drought and competition with fast-growing *Rubus fruticosus* resulted in impaired inorganic N acquisition and water status for *F. sylvatica* seedlings, as well as increased amino acid levels due to protein degradation in the leaves to act as osmoprotectants. However, whether invasive species have an advantage over native species in the competition for N under drought scenarios is still unknown, despite woody species invasions becoming a major concern in forests around the globe (Lamarque et al. 2011).

We conducted a greenhouse experiment to study the responses of three native and two invasive woody plant species to different competitors and drought in terms of organic and inorganic N acquisition as well as allocation of N to N pools in the leaves and fine roots. As native species we chose some of the most abundant and widespread species of Central European forests (Ellenberg and Leuschner 2010, Eaton et al. 2016, Houston et al. 2016), which differ in physiological traits and growth strategies: (i) European beech (*Fagus sylvatica* L., Fagaceae), a drought-sensitive slow growing species (Houston et al. 2016), (ii) pedunculated oak (*Quercus robur* L., Fagaceae), a drought-tolerant slow growing species (Eaton et al. 2016), and (iii) Scots pine (*Pinus sylvestris* L., Pinaceae), a drought-tolerant fast growing conifer (Kuster et al. 2013, Sohn et al. 2016). The two invasive tree species are originally from North America, were first introduced in Europe in the 17th century and are now widely distributed due to their use in reforestation programs and considered two of the most frequent and important woody invaders in Central European forests (Campagnaro et al. 2018): (i) black cherry (*Prunus serotina* Ehrh., Rosaceae), a fast growing species producing cyanogenic compounds (Csiszár 2009), and (ii) black locust (*Robinia pseudoacacia* L., Leguminosae), a fast growing, N_2-fixing species (Robakowski et al. 2016, Vítková et al. 2017). Both, the remobilization of N from cyanogenic compounds and the ability to symbiotically fix N_2 allow these two invasive species to acquire additional N independently from soil N supply, which could thus serve as alternate sources of N. From here on, species used in this study will be referred to using their genus, i.e. *Fagus*, *Quercus*, *Pinus*, *Prunus* and *Robinia*.

Our general research aim was to evaluate whether N acquisition and allocation of N to N pools of native and invasive species shift in response to drought and different competitors reflecting their functional traits, i.e. growth rate (slow-growing vs. fast-growing), drought tolerance (drought-tolerant vs. drought-sensitive), and/or the ability to access alternative sources of N (e.g. via remobilization of N stored in cyanogenic compounds or symbiotic N_2 fixation). Our specific hypotheses were: (1) Drought generally reduces inorganic and organic N acquisition because N uptake from the soil strongly depends on water availability (Gessler et al. 2004, Rennenberg et al. 2006). (2) Species-specific coping mechanisms (related to their functional traits) lead to changes in the allocation of N to N pools in the leaves and fine roots, because generally with drought less N is acquired from the soil (Gessler et al. 2017). (3) N acquisition and allocation of N to N pools of a given species change depending on the species' functional traits and the competitor. For example, competition between a slow and a fast grower leads to a decrease in N acquisition and allocation to N pools in the slow growing species because of the fast grower's higher N demand (Reich 2014). (4) Native and invasive species differ in their preference for organic and inorganic N sources resulting in avoidance of competition for N (Fraterrigo et al. 2011, Huangfu et al. 2016).

2. Materials and Methods

2.1. Plant material and growth conditions

One-year-old seedlings of all species were purchased from Müller Münchehof Pflanzen GmbH (Seesen/Münchehof, Germany) and planted in different combinations (i.e. two seedlings per pot, see 2.2 "Experimental design") in a 1:1 mixture of sand and vermiculite in 3 L plastic pots (25 cm x 12 cm) at the end of November 2015. Pots stayed outdoors over winter and spring and were watered regularly. They were brought into the greenhouse on June 20th 2016 after leaf development. For the following 7 days, all pots were watered regularly with sufficient tap water, and received on two occasions 100 ml of an artificial low N nutrient solution containing 100 µM KNO_3, 90 µM $CaCl_2*2H_2O$, 70 µM $MgCl_2*6H_2O$, 50 µM KCl, 24 µM $MnCl_2*4H_2O$, 20 µM NaCl, 10 µM $AlCl_3$, 7 µM $FeSO_4*7H_2O$, 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). The pots were subjected to natural light conditions and day length regime (16/8, day/night). Air temperature was 23.5 ± 2.3 °C / 21.0 ± 2.4 °C (day/night, mean ± standard deviation), and relative humidity was 63.1 ± 9.3 % / 71.3 ± 8.8 % (day/night, mean ± standard deviation) for the duration of the experiment. With increasing duration of the drought treatment, some individuals started to show signs of wilting. To ensure sufficient replication for each species and treatment, we did the ^{15}N uptake experiments followed by the harvest after four weeks.

2.2. Experimental design

The experiment was conducted in a fully orthogonal design with two factors, “water availability” (i.e. control or drought) and “competitor” (interspecific competition with 2 or 3 different competitors). Seedlings were planted in native-invasive interspecific competition (i.e. one seedling of a native species and one seedling of an invasive species per pot). Pots were established for every combination of native species and invasive species. For each species, a total of 24 pots was setup for each combination of native-invasive, summing up to a total of 144 pots. Pots were assigned to either the control or drought treatment (i.e. n = 12 per combination of species, competitor, and soil water availability). For the drought treatment, irrigation was fully stopped starting June 27th until the final harvest from July 21st to 27th, while for the control treatment irrigation continued with sufficient tap water supply every second day. To confirm that water availability was significantly reduced in the respective treatment, we measured soil water content every second day for 3 pots per combination of species, soil water availability treatment and competitor, by inserting a probe into the soil in three different locations in each pot (HH2 Moisture Meter, Delta-T Devices, Cambridge). Soil water content in the drought treatment was 7.2 ± 2.4 % (mean ± standard deviation) and significantly lower than in the control 24.0 ± 2.5% (mean ± standard deviation) at the end of the experiment. $\delta^{13}C$ values in the leaves (Suppl. Table 6) were higher in the drought treatment compared to the control at the time of harvest indicating drought stress for *Fagus*, *Quercus*, and *Robinia* depending on the competitor, while for *Pinus* and *Prunus* no differences were found.

2.3. ^{15}N uptake experiments

To quantify inorganic (i.e. ammonium and nitrate) and organic (i.e. glutamine and arginine) net N uptake capacity of the seedlings' fine roots, the ^{15}N enrichment technique was used as described by Gessler et al. (1998) and modified by Simon et al. (2010). Seedlings were carefully removed from the pots and the roots washed thoroughly with tap water to remove any remaining substrate. Then, fine roots still attached to the seedlings were incubated in the same artificial low N solution as used during plant growth (see above) containing all four N sources but only one labelled as either $^{15}NH_4^+$, $^{15}NO_3^-$, $^{13}C/^{15}N$-glutamine, or $^{13}C/^{15}N$-arginine. Controls with no label were included to account for natural abundance in the fine roots (n = 4 to 6 per N source including controls with no label, per species, competitor, and soil water availability treatment). The roots not used for incubation were wrapped in wet tissue to prevent desiccation for the duration of the ^{15}N uptake experiment. After 2 hours of incubation, the fine roots were cut from the seedling and washed twice in 0.5 M $CaCl_2$ solution to remove the incubation solution from the root surface. The fresh weight of the fine roots was determined, and after oven-drying for 48 h at 60 °C, their dry weight was determined. Amino acids were $^{13}C/^{15}N$-labelled 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 ^{13}C compared to that on ^{15}N incorporation indicating 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). Incubation took place between 10 am and 2 pm to avoid diurnal variation in net N uptake capacity (Gessler et al. 2002).

2.4. Harvest and quantification of growth and biomass indices

After the ^{15}N uptake experiment, seedlings were separated into leaves, stems, and roots. Their fresh weight was determined, then all tissues were oven-dried for 48 h at 60 °C and their dry weight was determined. Before oven-drying, a subsample of 8 to 10 representative leaves was collected from each seedling and their leaf area measured (LI-3100C Area Meter, LI-COR, Lincoln, USA) to calculate specific leaf area (SLA). Likewise, a subsample of fine roots was collected from each seedling, stained, scanned and total length measured (WinRhizo 2012, Regent Instruments Inc., Quebec, Canada) to calculate specific root length (SRL) based on Liu and van Kleunen (2017). Furthermore, samples of leaves and fine roots were collected from each seedling to quantify total soluble amino acid-N and total soluble protein-N contents. These samples were shock- frozen in liquid N_2 immediately after sampling and determining their fresh weight, and then stored at -80 °C until further analyses. Root:shoot ratio was calculated as the relation between total belowground biomass (i.e. root biomass) and total aboveground biomass (i.e. leaves and stem biomass). For each seedling, RGR was calculated according to the equation: $RGR = (\ln b_2 - \ln b_1) * t^{-1}$, where b_1 is total seedling biomass (g dw) at the initial harvest, b_2 is total seedling biomass (g dw) at the final harvest, and t is the time period in days between the initial and the final harvest (Grubb et al. 1996). Initial seedling biomass was determined on 3 to 4 pots per species, competitor, and soil water availability treatment that were harvested immediately before commencing the drought treatment.

2.5. Quantification of total N and C, ^{15}N, and ^{13}C in fine roots and $\delta^{13}C$ in leaves

Dried fine root samples from the ^{15}N uptake experiment were ground using a ball mill (TissueLyser, Retsch, Haan, Germany) to a fine homogeneous powder. Aliquots of 1.2 to 2.4 mg were weighed into 4x6 mm tin capsules (IVA Analysentechnik, Meerbusch, Germany) for analyses with an isotope ratio mass spectrometer (Delta V Advantage, Thermo Electron, Dreieich, Germany) coupled to an elemental analyzer (Euro EA, Eurovector, Milano, Italy). Δ values were calculated using a laboratory standard (acetanilide) that was part of every sequence in intervals also used in different weights to determine isotope linearity of the system. The laboratory standard was calibrated against several suitable international isotope standards (IAEA, Vienna). Final correction of isotope values was done with several international isotope standards and other suitable laboratory standards which cover the range of ^{15}N and ^{13}C results. Inorganic and organic N net uptake capacity (nmol N g^{-1} fw h^{-1}) was calculated based on the incorporation of ^{15}N into the fine roots according to Kreuzwieser et al. (2002): Net N uptake capacity = $((^{15}N_l - ^{15}N_n) * N_{tot} * dw * 10^5) / (MW * fw * t)^{-1}$, where $^{15}N_l$ and $^{15}N_n$ are the atom% of ^{15}N in labeled (N_l) and unlabeled control plants (N_n, natural abundance), respectively, N_{tot} is the total N percentage, MW is the molecular weight (^{15}N g mol^{-1}), and t is the incubation time.

2.6. Quantification of total soluble protein and total soluble amino acid levels in leaves and fine roots

To extract total soluble proteins from the leaves and fine roots (Dannenmann et al. 2009), ~50 mg aliquots of finely ground frozen samples were incubated in 1.5 ml extraction buffer (50 mM Tris-HCl pH 8.0, 1 mM EDTA, 15% (v/v) glycerol, 0.6 mM dithiothreitol, 1% Triton X-100, 2 EDTA-free protease inhibitor cocktail tablets per 100 ml buffer) at 4 °C for 30 min followed by centrifugation for 10 min at 14,000 rpm and 4 °C. The extraction was done twice to increase the yield. Subsequently, 500 µL of the combined supernatant from both extractions were incubated with 1 ml 10 % (v/v) trichloroacetic acid for 10 min at room temperature and then centrifuged for 10 min at 14,000 rpm and 4 °C. The resulting protein pellet was dissolved in 1 ml 1 M KOH. Next, total soluble proteins were quantified following Simon et al. (2010) by adding 1 ml of Bradford reagent to 50 µL of extract. Following a 10 min incubation at room temperature in the dark, the absorbance was measured at 595 nm in a spectrophotometer (Ultrospec 3100pro, Amersham Biosciences). Bovine serum albumin (BSA) was used as standard.

Total soluble amino acid-N content in the leaves and fine roots were extracted according to Winter et al. (1992): 200 µL Hepes buffer (5 mM EGTA, 20 mM HEPES, 10 mM NaF) and 1 ml 3.5:1.5 (v:v) methanol/chloroform were added to ~50 mg aliquots of finely ground frozen sample and incubated for 30 min on ice, followed by the addition of 600 µL of distilled water and centrifugation for 5 min at 14,000 rpm and 4 °C. The addition of distilled water and centrifugation was performed twice to increase the yield. Total soluble amino acid-N content in the leaves and fine roots were quantified according to Liu et al. (2005): 50 µL ninhydrin solution was added to a 50 µL aliquot of the combined extract and boiled for 30 min. The ninhydrin solution was composed of an equal parts mixture of solution A (i.e. 3.84 g citric acid, 0.134 g $SnCl_2$, and 40 ml 1 M NaOH, filled to 100 ml with distilled water at pH 5) and solution B (i.e. 4 g ninhydrin in 100 ml ethylene-glycol-monomethyl-ether). Subsequently, the extracts were cooled to room temperature and 1 ml 50% isopropanol was added, followed by a 15 min incubation. The absorption was measured at 570 nm in a spectrophotometer (Ultrospec 3100pro, Amersham Biosciences). L-glutamine was used as standard.

2.7. Statistical analyses

Two-way permutational ANOVAs (PERMANOVA) based on a Euclidean resemblance matrix between samples (Anderson et al. 2008) were performed for each species to test for differences between water availability and competitor levels using as variables inorganic and organic net N uptake capacity, total soluble amino acid-N, and total soluble protein-N contents in the leaves and fine roots, as well as total biomass, root:shoot ratio, SLA, SRL, and RGR. PERMANOVAs were performed using “water availability” (i.e. drought and control) and “competitor” as fixed orthogonal factors. “Competitor” consisted of two levels for the native species (i.e. competition with *Prunus* or *Robinia*) or three levels for the invasive species (i.e. competition with *Fagus*, *Quercus*, or *Pinus*). For significant interactions between factors, *post hoc* PERMANOVA pair-wise comparisons were performed. To test for differences in $\delta^{13}C$ in leaves between drought and control for each species grown with different competitors, Mann-Whitney U-tests were performed. Finally, to test for species preferences in net N uptake capacity of the different N forms, one-way PERMANOVAs were performed for each combination of species and competitor using "N source" as factor at both levels of soil water availability. All PERMANOVA analyses were performed using PRIMER 6.0 with the PERMANOVA+ add-on (PRIMER-E Ltd, Plymouth, UK), while Mann-Whitney-U tests were performed using SigmaPlot 14.0 (Systat Software, San Jose, USA).

3. Results

3.1. Drought effects on N acquisition and allocation to N pools in the leaves and fine roots of native and invasive tree seedlings

For all species (both native and invasive), net N uptake capacity was lower with drought compared to sufficient water supply, but with differences among species regarding which specific N form was significantly affected and with which competitor (native species: Fig. 1, invasive species: Fig. 2). Ammonium acquisition, already very low, was reduced further with drought for *Prunus* and *Robinia* (Fig. 2) regardless of competitor, whereas nitrate acquisition was lower with drought for *Fagus*, *Quercus*, *Pinus* (Fig. 1) and *Prunus* regardless of competitor, and for *Robinia* only in competition with *Quercus* and *Pinus* (Fig. 2). Glutamine acquisition was reduced by drought for *Pinus* (Fig. 1), *Prunus* and *Robinia* (Fig. 2) regardless of competitor, and for *Quercus* (Fig. 1) only in competition with *Robinia*, while arginine acquisition was lower with drought for *Fagus*, *Quercus* (both Fig. 1) and *Robinia* (Fig. 2) regardless of competitor (Table 1, Suppl. Table 1).

Drought also led to changes in N allocation to N pools in leaves and fine roots. With drought compared to the controls, total soluble protein-N content was reduced regardless of competitor in the leaves of *Fagus* and *Quercus*, and in the fine roots of *Prunus*, as well as in the fine roots of *Robinia* in competition with *Pinus*, while it increased in the fine roots of *Robinia* in competition with *Fagus* with drought (Table 1, Suppl. Table 2, 3, 4). Total soluble amino acid-N content was increased with drought in the fine roots of *Pinus* and the leaves of *Robinia* regardless of competitor, as well as in the leaves and fine roots of *Quercus* in competition with *Prunus* (Table 1, Suppl. Tables 2, 3, 4). However, total soluble amino acid-N levels were lower with drought in the fine roots of *Prunus* grown in competition with *Quercus* or *Pinus* (Table 1, Suppl. Tables 2, 3, 4).

Finally, drought resulted in higher δ^{13}C values in the leaves of *Fagus* and *Quercus* grown in competition with *Prunus*, and in the leaves of *Robinia* grown in competition with *Quercus* or *Pinus* (Suppl. Tables 5, 6). Drought affected also the total biomass, root:shoot ratio, RGR, SLA and SRL of native and invasive species depending on both the target species and the competitor (Table 1, Suppl. Tables 7, 8, 9, Suppl. Figure 1).

3.2. Competitor effects on N acquisition and allocation to N pools in the leaves and fine roots of native and invasive tree species

For native species, N acquisition did not change with different invasive competitors, but there were different responses regarding allocation to N pools (Fig. 1). In competition with *Prunus* compared to *Robinia* and regardless of soil water availability, *Quercus* had lower total soluble protein-N content but higher total soluble amino acid-N content in the fine roots, whereas *Pinus* had lower total soluble amino acid-N content and higher total soluble protein-N content in the leaves, and higher total soluble amino acid-N content in the fine roots (Table 2, Suppl. Tables 2, 3,4). For *Fagus*, N allocation to N pools did not vary with competitor (Table 2, Suppl. Tables 2, 3, 4). All native species responded differently to the invasive competitors regarding their biomass and growth indices (Table 2, Suppl. Tables 7, 8, 9).

Similarly, responses of the invasive species depended on competitor and were partly also mediated by soil water availability. Under drought, *Prunus* seedlings had lower total soluble amino acid-N contents in the fine roots when competing with *Quercus* compared to *Pinus* (Table 2, Suppl. Table 2, 3, 4). With sufficient soil water supply, total soluble amino acid-N content in the fine roots of *Prunus* was lower competing with *Fagus* than with *Quercus* or *Pinus* (Table 2, Suppl. Table 2, 3, 4). *Prunus* seedlings had a lower total soluble amino acid-N content in the leaves when competing with *Fagus* than with *Quercus*, and a lower total soluble protein-N content in the leaves when competing with *Fagus* or *Quercus* than with *Pinus* (Table 2, Suppl. Tables 2, 4, 10). *Robinia* seedlings responded to competitor with changes in other parameters than *Prunus*. With drought, *Robinia* seedlings had higher total soluble protein-N content in the fine roots when competing with *Fagus* than with *Quercus*, and higher total soluble protein-N content in the fine roots competing with *Quercus* than with *Pinus* (Table 2, Suppl. Tables 2, 3, 4). Under control conditions, *Robinia* competing with *Fagus* had a lower nitrate net uptake capacity than when competing with *Pinus* (Fig. 2, Table 2). Both *Prunus* and *Robinia* responded differently with regard to their biomass and growth indices (Table 2, Suppl. Tables 7, 8, 9, 11).

3.3. N acquisition preferences for different N sources of native and invasive species

Preferences for certain N sources were found among species depending on the competitor and soil water availability. Generally, organic N, especially arginine-N, was favoured over inorganic N sources. *Quercus* and *Prunus* did not change N preferences with different competitors or drought (Tables 3, 4). *Fagus* preferred organic N over inorganic N with drought (Table 3). *Pinus* did not prefer specific N sources in competition with *Robinia* with sufficient water supply, but preferred organic N over inorganic N with drought and in competition with *Prunus* regardless of water availability (Table 3). *Robinia* showed a distinct pattern depending on the competitor: With drought, seedlings preferred arginine-N over inorganic N but only in competition with *Quercus*, but not in competition with *Fagus* or *Pinus* (Table 4). With sufficient water supply, *Robinia* preferred organic over inorganic N when grown in competition with *Fagus*, but not with *Quercus* or *Pinus* (Table 4).

4. Discussion

4.1. Drought reduces N acquisition among species, but allocation of N to N pools varies with species and competitor

In accordance with our hypothesis, inorganic and organic N acquisition of both native and invasive tree seedlings overall decreased in response to drought, confirming that tree N acquisition and soil water availability are strongly linked (e.g. Fotelli et al. 2002, 2004, Rennenberg et al. 2006, Gessler et al. 2017). The influence on organic N acquisition for tree species in competition is a novel insight provided by our work, while also confirming previous studies in which drought reduced the inorganic N acquisition of *F. sylvatica* seedlings growing both in intra- and interspecific competition (Fotelli et al. 2002). In plant communities, when soil water availability is reduced, microbial activity is decreased negatively impacting soil N mineralization processes, thus resulting in reduced soil N availability (Schimel et al. 2007, Hueso et al. 2012). Furthermore, soil N diffusion and mass flow are reduced (Rennenberg et al. 2009) and the composition of mycorrhizal communities that symbiotically provide plants with N is changed (e.g. Gessler et al. 2005, Leberecht et al. 2016).

Our studied tree species appear to show four different responses to drought regarding the allocation of N to N pools in the leaves and fine roots depending on the species and competitor: (1) Total soluble protein levels were reduced (in the leaves of *Fagus* and *Quercus* regardless of competitor, and in the fine roots of *Prunus* and *Robinia* competing with *Pinus*) as a consequence of reduced N acquisition and thus, N assimilation (Gessler et al. 2017). Storage proteins from vegetative tissue are degraded and remobilized leading to lower N in storage (e.g. Millard 1988, Staswick 1994, Millard and Grelet 2010). With potential leaf shedding as a consequence of drought and, consequently, N stored in the leaves being lost by the plant, the roots become an important tissue for N storage (Millard and Grelet 2010). In our study, the leaves were not (yet) shed at the time of the harvest, suggesting that N was likely still remobilized. (2) In the fine roots of *Robinia* in competition with *Fagus*, the levels of total soluble proteins increased in response to drought likely due to the synthesis of protective proteins (Brunner et al. 2015), proteins with a role in dehydration tolerance (Close 1996, Kozlowski and Pallardy 2002), and/or proteins required for the development of root nodules for N_2-fixing species (Verma et al. 1992) such as *Robinia*. (3) Soluble amino acid levels decreased in the fine roots of *Prunus* when grown in competition with *Quercus* or *Pinus* in response to drought. This is likely due to amino acid degradation, their translocation to other plant tissues, their usage for protein synthesis, and/or the usage of their carbon skeletons to produce alternative non-N-containing osmotic compounds which not only decrease osmotic potential but can also function as cell membrane and metabolic protectants (Chaves et al. 2003). (4) In contrast, total soluble amino acid levels increased in the leaves of *Robinia* and the fine roots of *Pinus* regardless of competitor, as well as in the leaves and fine roots of *Quercus* in competition with *Prunus* via protein degradation to serve as osmoprotectants, thus improving the overall plant water status (Hu et al. 2013, 2013b). Overall, our results indicate that the study species show different responses to drought conditions in this short-term experiment with regards to N allocation to N pools in leaves and fine roots in combination with changes in biomass allocation.

Some species showed changes in biomass allocation in response to drought while others did not. A higher root:shoot ratio resulting in a larger soil volume to be exploited and a parallel decrease in aboveground biomass as well as SLA reduces water loss via the leaves further improving a plant's water status (Fotelli et al. 2005, Mantovani et al. 2014, Duan et al. 2018). This strategy was found in our study for *Robinia*. On the other hand, drought sensitivity of a species might be reflected by reduced root growth, eventually leading to a decrease in root:shoot ratio (Ostonen et al. 2007, Brunner et al. 2015), root hydraulic failure (Mao et al. 2018), and higher root mortality (Zhou et al. 2018). In our study, drought conditions were severe enough to cause a decrease in root:shoot ratio for *Fagus* indicating the drought-sensitivity of this species, but not the other native or the invasive species.

In general, all study species were negatively affected by drought with no clear distinction between native and invasive species. Native *Fagus* and – to a lesser extent – invasive *Robinia* were most sensitive showing several changes in above- and belowground traits. For *Fagus*, the strong response to drought generally prevailed over the effects of the competitor, while for

Robinia it was mediated by the competitor. The ability to fix N_2 allows *Robinia* to obtain external N and thus be less affected by the negative effect of drought on N acquisition from the soil (Wurzburger and Miniat 2014, Mantovani et al. 2014, 2015). For *Quercus* and invasive *Prunus*, responses to drought also varied according to competitor. In contrast, we found no interaction between drought and competitor for *Pinus* which indicates that the two invasive species did not influence its response to drought. Furthermore, coniferous *Pinus* was generally less responsive to drought than the native and invasive deciduous species most likely due to its isohydric behaviour, i.e. the closing of stomata early during a drought event, thereby minimizing water losses via the needles (Irvine et al. 1998). These results imply that under future scenarios of global change, *Pinus* can withstand simultaneous short-term drought stress and an invasion by exotic woody species better than *Fagus* and *Quercus*.

4.2. Species-specific responses of native and invasive species in response to competitor and soil water availability

Our hypothesis that N acquisition from the soil and its allocation to plant internal N pools vary for a given species depending on its functional traits and the competing species could partly be confirmed. None of the native species and only one of the two invasives responded to different competitors with changes in N acquisition, thus contrasting previous studies (Simon et al. 2010, 2014, Bueno et al. 2019). This suggests that soil water availability was a stronger driver of N uptake than competition. The allocation of N to different N pools of our studied species depended on the competitor and varied with soil water availability, thus confirming our hypothesis that responses to different competitors are species-specific according to the physiological characteristics of the study species.

Both invasive species in our study, show functional traits commonly linked to fast growth (Grotkopp and Reimánek 2007), such as higher biomass (*Prunus*) or higher SLA, and in turn, RGR (*Robinia*), higher N allocation to N pools in leaves and fine roots thus enhancing resource acquisition above- and belowground, and overall increasing their competitive advantage under high resource availabilty (e.g. Li et al. 2015). A negative influence of *Robinia* on the growth of competing tree seedlings has been reported before via the depletion of soil resources due to *Robinia*'s fast growth and occupation of rooting space (Kawaletz et al. 2013, 2014). In our study *Robinia* was the only species with increased root:shoot ratio under drought suggesting a strong potential for below-ground competition and resource gain for metabolic processes, which apparently had a stronger effect on drought-sensitive *Fagus* than the drought-tolerant natives. N acquisition of all three native species did not differ with competitor, and the higher allocation of N to N pools combined with slower growth of *Quercus* and *Pinus* when competing with *Prunus* than *Robinia* suggests an increased storage of N metabolites rather than a use for biomass production (Reich et al. 1997, Millet et al. 2005, Millard and Grelet 2010). Moreover, the negative effects on growth and biomass indices when competing with *Prunus* rather than *Robinia* further indicate negative consequences of competition with *Prunus* for overall plant development.

Similar to the native species, the competitor had no effect on N acquisition of *Prunus* indicating that the observed changes in biomass and growth indices as well as N metabolites content occurred in relation to internal N dynamics in the seedlings. This is likely due to remobilization and *de novo* synthesis of N metabolites (Simon et al. 2010, Li et al. 2015), and/or the reliance on N stored in plant tissues as N-based defense compounds (Gleadow and Woodrow 2002). The other responses of invasive *Prunus* to native species depended on soil water availability. For example, drought-sensitive *Fagus* was a stronger competitor for *Prunus* only with sufficient soil water availability whereas this effect was absent with drought, reflecting the drought-sensitivity of *Fagus*. With drought, N pools of *Prunus* were higher in competition with *Pinus* compared to deciduous natives. This indicates a similar mechanism of metabolic adaptation to stress as in the slow growing deciduous *Fagus* and *Quercus* (Millard and Grelet 2010). This might be a differential response of *Prunus* to specific competitors with drought conditions, possibly related to the drought tolerance of such competitors. This would be in accordance with studies indicating that evergreen conifers are on average more drought-tolerant due to their more conservative resource use than broadleaved deciduous temperate woody species (Hallik et al. 2009), though further studies considering a greater number of species are needed to provide additional insights into this.

Robinia responded to competition with native deciduous *Fagus* and *Quercus* stronger than with coniferous *Pinus*, although the affected specific growth and physiological parameters depended on soil water availability. In contrast to all other study species, nitrate acquisition of *Robinia* was reduced in its competition with *Fagus* compared to competition with *Pinus* suggesting the release of active compounds that potentially impair N acquisition as suggested for *Acer pseudoplatanus* in a previous study (Simon et al. 2010). This would negatively affect nutrition and development of *Robinia* seedlings compared to competition with other natives because although *Robinia* can fix N_2, N acquisition from the soil is preferred over N_2 fixation when soil

N is not limiting (Pfautsch et al. 2009).These differences in the responses to competitors became more apparent when *Robinia* was additionally affected by drought and may be explained by *de novo* synthesis of proteins as an adaptation to competition (Simon et al. 2010, 2014). Additionally, *Robinia* seedlings grew slower in competition with *Fagus* or *Quercus* compared to *Pinus* regardless of soil water availability further highlighting their negative competitive effects on *Robinia*.

Overall, native and invasive species responded to different competitors in accordance with their physiological and life history traits such as drought tolerance and leaf habit. For example, drought-sensitive *Fagus* responded negatively to different competitors and affected invasive species only with sufficient water supply, indicating that drought effects override those of the two invasive competitors. Moreover, invasive *Prunus* was generally a stronger competitor for the native species. With competition, tissue nutrient content plays an important role in plant performance. For example, seedlings of black spruce (*Picea mariana*) with high levels of N (and other nutrients) had higher biomass than seedlings with low tissue N content after growing in competition with natural vegetation (Malik and Timmer 1998) indicating the importance of plant internal N reserves in competitive interactions.

4.3. Organic N was generally preferred by all species regardless of drought

We hypothesized that native and invasive species differ in their preference for organic and inorganic N sources. However, organic N forms, especially arginine, were generally preferred over inorganic N by all study species confirming results from studies in the field and under controlled settings (Simon et al. 2017) using the same technique and artificial soil solution as here (e.g. for *F. sylvatica*: Dannenmann et al. 2009, Simon et al. 2010, Simon et al. 2011, Simon et al. 2014, for *R. pseudoacacia*: Hu et al. 2017), as well as the results found at low soil N availability in our previous study using the same species (Bueno et al. 2019). The preferred acquisition of specific N sources is generally driven by their higher abundance in the soil (e.g. Kuster et al. 2013b, Song et al. 2015, Simon et al. 2017). For native *Fagus* and *Pinus*, as well as invasive *Robinia* preferences shifted depending on the competitor (see Tables 3, 4) indicating plasticity in resource use induced by the interaction with other species which might influence the plant's competitive ability (Ashton et al. 2010). Such shifts were not found for native *Quercus* and invasive *Prunus* indicating that competition does not affect their N form preference. Furthermore, to our knowledge, no previous studies have directly measured the effect of drought on the preference for different N forms. We found no changes in the N form preference with drought suggesting that changes in N preference for the study species does not play a role for adapting to drought, at least under these experimental conditions.

Conclusions

In our study, drought generally reduced inorganic and also – shown for the first time – organic N acquisition among both native and invasive study species, because reduced water availability leads to less N available in the soil for plants. Overall, N acquisition was driven by water supply rather than competition. The allocation of N to internal N pools in the leaves and fine roots, however, varied with study species and competitor in relation to their physiological characteristics showing their respective species-specific coping mechanisms (e.g. the remobilization of N from storage and/or synthesis of osmoprotectants). For example, within the native tree species, for drought-sensitive *Fagus*, reduced water availability overrode the influence of competition, whereas the responses of drought-tolerant *Pinus* were entirely independent of water supply. Deciduous and coniferous species might respond differently to competition with other plants because of their differences in, for example, tissue concentrations of nutrients (Calder et al. 2011, Wang et al. 2016). Organic N was generally favoured by both native and invasive species regardless of drought suggesting that competition for N was not avoided. Moreover, our results suggest that *Prunus* as an invasive species is a stronger competitor than *Robinia* for most native tree species (which is in accordance with a previous study (Bueno et al. 2019)), possibly due to its higher biomass. In contrast, for *Robinia*, the deciduous natives *Fagus* and *Quercus* had more negative effects than the conifer *Pinus*. In conclusion, our results suggest that species-specific mechanisms to cope with drought related to their physiological characteristics might play a role for the competitive ability of the studied species. However, further studies investigating a larger number of species are necessary to confirm this. Because water availability strongly affects plant and soil N dynamics (e.g. Gessler et al. 2017, Simon et al. 2017), longer term competition (> 1 year) might further pronounce the effects of competition seen in our study.

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

AB and JS conceived and designed the study. AB conducted the ^{15}N uptake experiments, N metabolite analyses, and evaluated all data. KP contributed the IRMS analyses. AB and JS led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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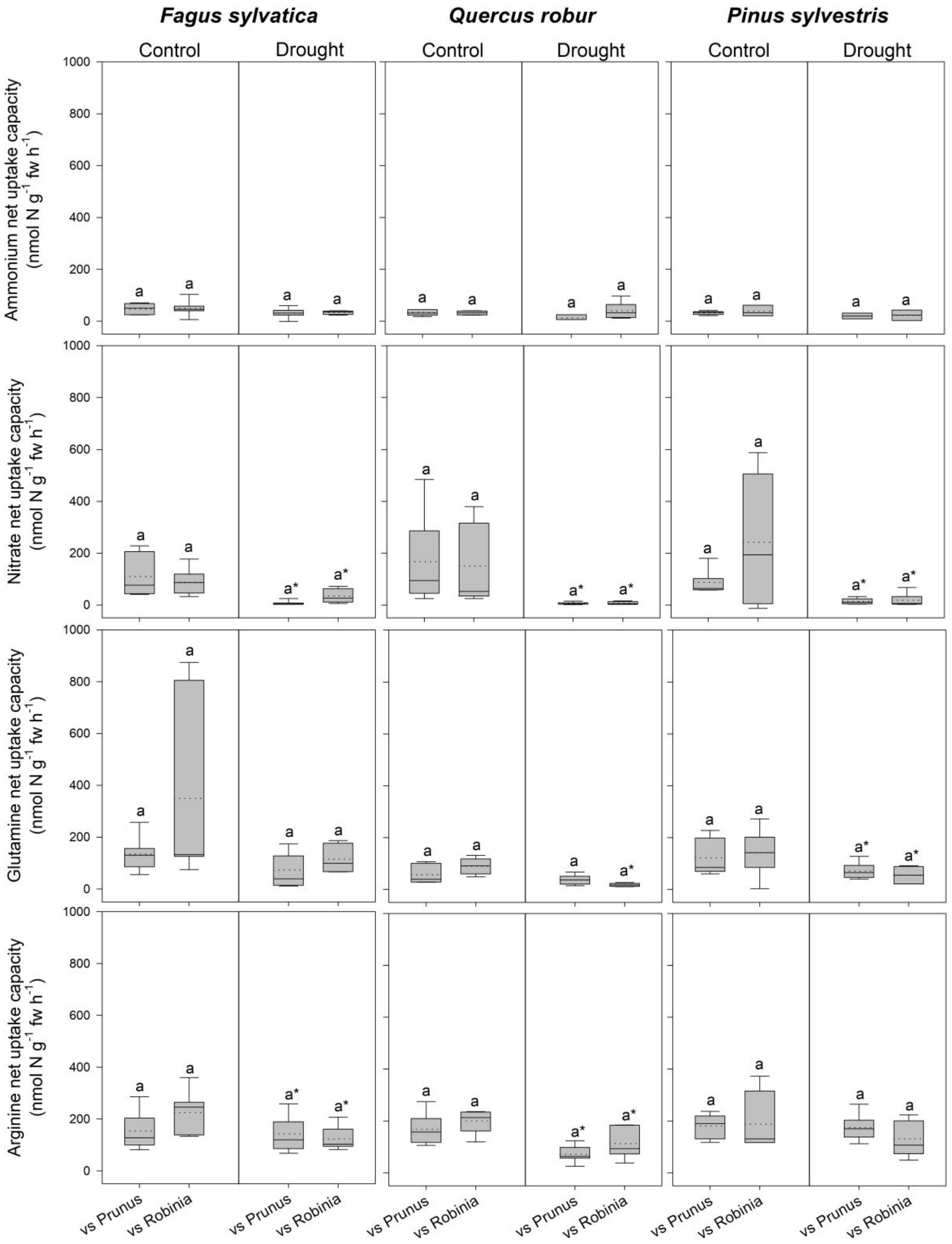


Figure 1. Ammonium, nitrate, glutamine-N, and arginine-N net uptake capacity (nmol N g^{-1} fw h^{-1}) by fine roots of *Fagus sylvatica*, *Quercus robur* and *Pinus sylvestris* seedlings at control and drought conditions under different competition regimes. vs *Prunus* = competition with *Prunus serotina*; vs *Robinia* = competition with *Robinia pseudoacacia*. Box plots show mean (dotted line) and median (continuous line). Different letters indicate significant differences between competition regimes within a specific soil water availability treatment, and asterisks indicate significant differences between control and drought detected using permutational analysis of variance ($p < 0.05$).

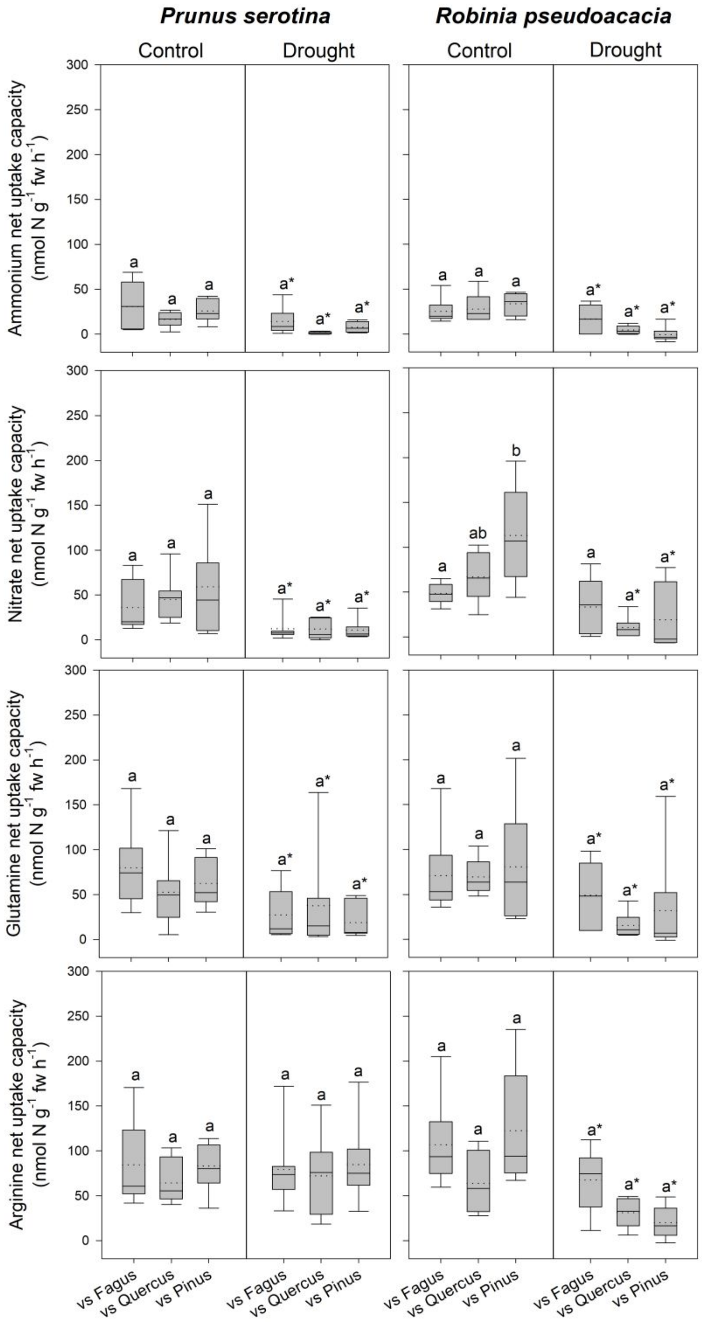


Figure 2. Ammonium, nitrate, glutamine-N, and arginine-N net uptake capacity (nmol N g^{-1} fw h^{-1}) by fine roots of *Prunus serotina* and *Robinia pseudoacacia* seedlings at control and drought conditions under different competition regimes. vs *Fagus* = competition with *Fagus sylvatica*; vs *Quercus* = competition with *Quercus robur*; vs *Pinus* = competition with *Pinus sylvestris*. Box plots show mean (dotted line) and median (continuous line). Different letters indicate significant differences between competition regimes within a specific soil water availability treatment, and asterisks indicate significant differences between control and drought detected using permutational analysis of variance ($p < 0.05$).

Table 1. Effects of drought on total biomass, growth indices, inorganic and organic N net uptake capacity, and N metabolite levels on seedlings of *Fagus sylvatica*, *Quercus robur*, *Pinus sylvestris*, *Prunus serotina*, and *Robinia pseudoacacia* growing in competition. ↑ = significant increase with drought, ↓ = significant decrease with drought, n.s. = no significant differences between drought and control. Total biomass (g dw), root:shoot ratio: ratio of belowground to aboveground biomass, RGR: relative growth rate (g dw g^{-1} dw d^{-1}), SLA: specific leaf area (cm^2 g^{-1} dw), SRL: specific root length (cm g^{-1} dw), N net uptake capacity (nmol N g^{-1} fw h^{-1}), NH_4^+: ammonium, NO_3^-: nitrate, Gln-N: glutamine-N, Arg-N: arginine-N, total soluble amino acid-N (mg g^{-1} dw), total soluble protein-N (mg g^{-1} dw).

	Biomass and growth indices					**N net uptake capacity**				**N metabolites**			
										Total soluble amino acid-N		**Total soluble protein-N**	
	Total biomass	**Root:shoot ratio**	**RGR**	**SLA**	**SRL**	**NH_4^+**	**NO_3^-**	**Gln-N**	**Arg-N**	**Leaves**	**Fine roots**	**Leaves**	**Fine roots**
Fagus sylvatica	n.s.	↓	↑(b)	↓	n.s.	n.s.	↓	n.s.	↓	n.s.	n.s.	↓	n.s.
Quercus robur	n.s.	n.s.	n.s.	n.s.	↓(b)	n.s.	↓	↓(b)	↓	↑(d)	↑(d)	↓	n.s.
Pinus sylvestris	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	↓	↓	n.s.	n.s.	↑	n.s.	n.s.
Prunus serotina	↓(a)	n.s.	n.s.	↑	n.s.	↓	↓	↓	n.s.	n.s.	↓(c)	n.s.	↓
Robinia pseudoacacia	n.s.	↑	n.s.	n.s.	n.s.	↓	↓(c)	↓	↓	↑	n.s.	n.s.	↑(e) - ↓(f)

(a): only in competition with *Quercus*
(b): only in competition with *Robinia*
(c): only in competition with *Quercus* or *Pinus*
(d): only in competition with *Prunus*
(e): only in competition with *Fagus*
(f): only in competition with *Pinus*
No letter: effect of drought regardless of competitor

Table 2. Effects of competitor on total biomass, growth indices, inorganic and organic N net uptake capacity, and N metabolite levels of *Fagus sylvatica*, *Quercus robur*, *Pinus sylvestris*, *Prunus serotina*, and *Robinia pseudoacacia* seedlings. Species A < species B = seedlings competing with species A had significantly lower values than seedlings competing with species B, n.s. = no significant differences between competitor identities. Total biomass (g dw), root:shoot ratio: ratio of belowground to aboveground biomass, RGR: relative growth rate (g dw g^{-1} dw d^{-1}), SLA: specific leaf area (cm^2 g^{-1} dw), SRL: specific root length (cm g^{-1} dw), N net uptake capacity (nmol N g^{-1} fw h^{-1}), NH_4^+: ammonium, NO_3^-: nitrate, Gln-N: glutamine-N, Arg-N: arginine-N, total soluble amino acid-N (mg g^{-1} dw), total soluble protein-N (mg g^{-1} dw).

	Biomass and growth indices					**N net uptake capacity**				**N metabolites**			
										Total soluble amino acid-N		**Total soluble protein-N**	
	Total biomass	**Root:shoot ratio**	**RGR**	**SLA**	**SRL**	**NH_4^+**	**NO_3^-**	**Gln-N**	**Arg-N**	**Leaves**	**Fine roots**	**Leaves**	**Fine roots**
Fagus sylvatica	n.s.	n.s.	*Robinia* < *Prunus* (a)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Quercus robur	n.s.	*Prunus* < *Robinia*	*Prunus* < *Robinia*	n.s.	*Robinia* < *Prunus* (b)	n.s.	n.s.	n.s.	n.s.	n.s.	*Robinia* < *Prunus*	n.s.	*Prunus* < *Robinia* (a)
Pinus sylvestris	*Prunus* < *Robinia*	n.s.	n.s.	-	n.s.	n.s.	n.s.	n.s.	n.s.	*Prunus* < *Robinia*	*Robinia* < *Prunus*	*Robinia* < *Prunus*	n.s.
Prunus serotina	*Fagus*, *Pinus* < *Quercus* (a)	n.s.	*Quercus*, *Pinus* < *Fagus*	*Quercus* < *Fagus* < *Pinus*	*Quercus* < *Fagus* (a)	n.s.	n.s.	n.s.	n.s.	*Fagus* < *Quercus*	*Fagus* < *Quercus*, *Pinus* (a) *Quercus* < *Pinus* (b)	*Fagus*, *Quercus* < *Pinus* (b)	n.s.
Robinia pseudoacacia	n.s.	n.s.	*Fagus*, *Quercus* < *Pinus*	n.s.	n.s.	n.s.	*Fagus* < *Pinus* (a)	n.s.	n.s.	n.s.	n.s.	n.s.	*Pinus* < *Quercus* < *Fagus* (b)

(a): only under control conditions

(b): only with drought

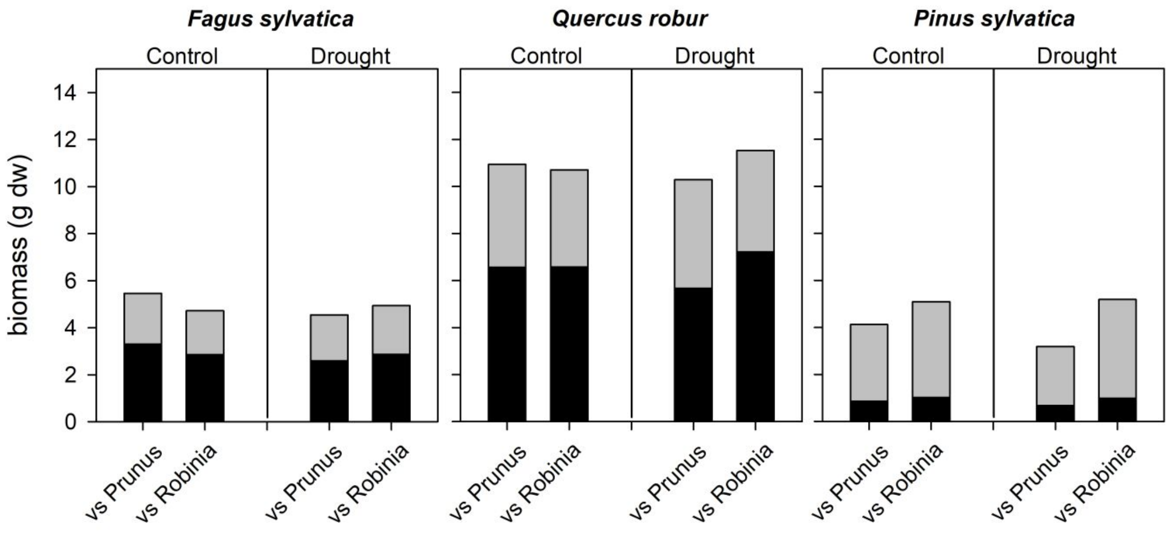
No letter: effect of competitor regardless of drought

Table 3. Differences between ammonium (NH_4^+), nitrate (NO_3^-), glutamine-N (Gln-N), and arginine-N (Arg-N) net uptake capacity of the fine roots of *Fagus sylvatica*, *Quercus robur*, and *Pinus sylvestris* seedlings grown in competition under drought and control conditions. Only significant differences are presented. n.s. = no significant differences between net uptake capacity of different N forms.

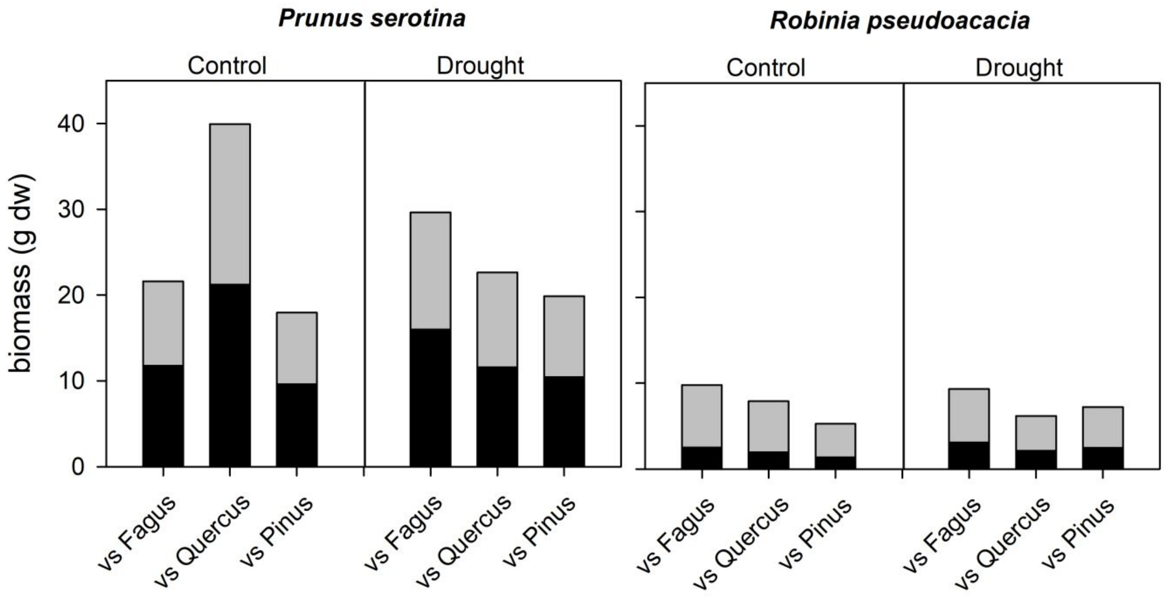
Competitor	**Water availability**	***Fagus sylvatica***	***Quercus robur***	***Pinus sylvestris***
Prunus serotina	Control	Gln-N, Arg-N > NH_4^+	Arg-N > NH_4^+, Gln-N	NO_3^-, Gln-N, Arg-N > NH_4^+ Arg > NO_3^-
	Drought	NH_4^+, Gln-N, Arg-N > NO_3^- Arg-N > NH_4^+	Arg-N > NH_4^+, NO_3^-, Gln-N Gln-N > NO_3^-	Arg-N > NH_4^+, NO_3^-, Gln-N Gln-N > NO_3^-
Robinia pseudoacacia	Control	Gln-N, Arg-N > NH_4^+, NO_3^-	Arg-N > NH_4^+, Gln-N Gln-N > NH_4^+	n.s.
	Drought	Gln-N, Arg-N > NH_4^+, NO_3^-	Arg-N > NH_4^+, NO_3^-, Gln-N Gln-N > NO_3^-	Arg-N > NO_3^-

Table 4. Differences between ammonium (NH_4^+), nitrate (NO_3^-), glutamine-N (Gln-N), and arginine-N (Arg-N) net uptake capacity of the fine roots of *Prunus serotina* and *Robinia pseudoacacia* seedlings grown in competition under drought and control conditions. Only significant differences are presented. n.s. = no significant differences between net uptake capacity of different N forms.

Competitor	**Water availability**	***Prunus serotina***	***Robinia pseudoacacia***
Fagus sylvatica	Drought	Arg-N > NH_4^+, NO_3^-, Gln-N	n.s.
	Control	Gln-N, Arg-N > NH_4^+, NO_3^-	NO_3^-, Gln-N, Arg-N > NH_4^+ Arg-N > NO_3^-
Quercus robur	Drought	Arg-N > NH_4^+, NO_3^-	Arg-N > NH_4^+, NO_3^-
	Control	NO_3^-, Arg-N > NH_4^+ Arg-N > Gln-N	n.s.
Pinus sylvestris	Drought	Arg-N > NH_4^+, NO_3^-, Gln-N	n.s.
	Control	Gln-N, Arg-N > NH_4^+	n.s.



Supplemental Figure 1a. Root (black) and shoot (gray) biomass (g dw) of *Fagus sylvatica*, *Quercus robur* and *Pinus sylvestris* seedlings at control and drought conditions under different competition regimes. vs *Prunus* = competition with *Prunus serotina*; vs *Robinia* = competition with *Robinia pseudoacacia*.



Supplemental Figure 1b. Root (black) and shoot (gray) biomass (g dw) of *Prunus serotina* and *Robinia pseudoacacia* seedlings at control and drought conditions under different competition regimes. vs *Fagus* = competition with *Fagus sylvatica*; vs *Quercus* = competition with *Quercus robur*; vs *Pinus* = competition with *Pinus sylvestris*.

Responses of native and invasive woody seedlings to combined competition and drought are species-specific

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Running title: Tree invasions: Competition under drought

Abstract

Woody species invasions are a major threat to native communities with intensified consequences during increased periods of summer drought as predicted for the future. Competition for growth-limiting nitrogen (N) between native and invasive tree species might represent a key mechanism underlying the invasion process, because soil water availability and N acquisition of plants are closely linked. To study whether the traits of invasive species provide an advantage over natives in Central Europe in the competition for N under drought, we conducted a greenhouse experiment. We analysed the responses of three native (i.e. *Fagus sylvatica*, *Quercus robur*, and *Pinus sylvestris*) and two invasive woody species (i.e. *Prunus serotina* and *Robinia pseudoacacia*) to competition in terms of their organic and inorganic N acquisition, as well as allocation of N to N pools in the leaves and fine roots. In our study, competition resulted in reduced growth and changes in internal N pools in both native and invasive species mediated by the physiological characteristics of the target species, the competitor, as well as soil water supply. N acquisition, however, was not affected by competition indicating that changes in growth and N pools were rather linked to the remobilization of stored N. Drought le~~a~~d to reduced N acquisition, growth and total soluble protein-N levels, while total soluble amino acid-N levels increased, most likely as osmoprotectants as an adaptation to the reduced water supply. Generally, the consequences of drought were enhanced with competition across all species. Comparing the invasive competitors, *P. serotina* was a greater threat to the native species than *R. pseudoacacia*. Furthermore, deciduous and coniferous native species affected the invasives differently, with the species-specific responses being mediated by soil water supply.

1. Introduction

Invasion of natural habitats by exotic species is considered a major aspect of anthropogenic global change (Vitousek et al. 1997, Ricciardi 2007). In forest ecosystems, successful plant invasions have a negative impact on the structure and composition of plant and microbial communities, as well as forest biogeochemical processes (Castro-Díez et al. 2006, Corbin and D'Antonio 2012). For example, invasives can suppress the recruitment and growth of native plant species, affect carbon pools and nutrient fluxes, and modify litter quality and decomposition (Ehrenfeld et al. 2001, Ehrenfeld 2003, Vilà et al. 2011). As a result, forest functioning is altered and the provision of ecosystem services may be~~is~~ impaired (Holmes et al. 2009, Vilà et al. 2011, Wardle and Peltzer 2017). Invasive plant species commonly display functional traits and growth strategies that improve resource capture and favour reproduction including high seed production (Mason et al. 2008), specific leaf area (Grotkopp and Rejmánek 2007, Leishman et al. 2007), photosynthetic rates (Pattison et al. 1998, McDowell 2002), relative growth rates (Grotkopp and Rejmánek 2007), root biomass (Broadbent et al. 2018) and/or specific root length (Dawson 2015). These traits contribute to the successful establishment and dispersal of invasive species in new habitats by enhancing their competitive ability~~ over native species~~. For example, invasive *Prunus serotina* and *Robinia pseudoacacia* produce more biomass ~~at the cost~~to the detriment of slower growing~~th~~ of native *Quercus robur* and *Carpinus betulus* when grown in competition (Kawaletz et al. 2013).

A key resource in the competition between native and invasive plant species is plant-growth limiting nitrogen (N) (Littschwager et al. 2010, Eller and Oliveira 2017). The competitive ability of plants for acquiring N is determined by plant morphological (e.g. density and length of root hairs) and physiological (e.g. N uptake capacity, expression and activity of transporters in root cells) traits, as well as species-specific plasticity in these traits (Casper and Jackson 1997), but also the support of symbionts such as mycorrhiza (e.g. Näsholm et al. 2009) and/or N_2-fixing bacteria (Bueno et al. 2019). Previous studies investigating the effects of competition for N found plasticity in the N uptake capacity of trees with increases or decreases in response to interspecific competition which, however, depended on the competing species, environmental conditions, and available N sources (e.g. inorganic vs organic N) (Simon et al. 2010, Simon et al. 2014, Li et al. 2015, Bueno et al. 2019). For example, *Fagus sylvatica* increased organic N acquisition in competition with *Acer pseudoplatanus* compared to intraspecific competition at high but not low soil N availability (Li et al. 2015): With ambient but not reduced light, organic N acquisition decreased in competition with *A. pseudoplatanus* reflecting a better adaptation of *F. sylvatica* to low light conditions compared to *A. pseudoplatanus* (Simon et al. 2014). The utilization of different N sources likely provides an advantage when competing for N (McKane et al. 2002, Simon et al. 2014) with a high potential to drive niche differentiation and species coexistence (McKane et al. 2002, Ashton et al. 2010, Boudsocq et al. 2012). In the context of competition between native and invasive species, the preference of different N forms in competition (i.e. one species favouring organic N, whereas the other prefers inorganic N sources) might provide an important mechanism to effectively avoid competition for N. To our knowledge, this theory has mostly been tested for non-woody species (Fraterrigo et al. 2011, Huangfu et al. 2016) and only recently for tree species (but see Bueno et al. 2019).

The threat of invasive species to European forests might become even more severe in ~~the view~~ light of the predicted climate changes for Central Europe. In Bueno et al. (2019), the responses of three native and two invasive tree species (i.e. the same species as used here) to high soil N availability were investigated due to the expected increase in atmospheric N deposition (Rennenberg et al. 2009). In the present study, responses of native and invasive tree species to competition for N are investigated under drought conditions. Drought periods in summer are expected to increase in frequency and severity (Spinoni et al. 2017). Thus, understanding the consequences of drought on the outcome of competition for N between native and invasive woody plant species is crucial. N dynamics in both plant and soil are tightly linked to water availability (Gessler et al. 2017): For example, drought negatively affects soil N mineralization processes resulting in reduced plant available N in the rhizosphere (Simon et al. 2017), soil N diffusion and mass flow (Rennenberg et al. 2006), and the activity of root proteins related to N acquisition (Bista et al. 2018). Reduced mycorrhizal colonization might additionally lead to less N transfer to plants (Nilsen et al. 1998). Therefore, also the competition for N between plants is altered with drought. For example, in studies by Fotelli et al. (2001, 2002) the combination of drought and competition with fast-growing *Rubus fruticosus* resulted in impaired inorganic N acquisition and water status for *F. sylvatica* seedlings, as well as increased amino acid levels due to protein degradation in the leaves to act as osmoprotectants. However, whether invasive species have an advantage over native species in the competition for N under drought scenarios is still unknown, despite woody species invasions becoming a major concern in forests around the globe (Lamarque et al. 2011).

We conducted a greenhouse experiment to study the responses of three native and two invasive woody plant species to different competitors and drought in terms of organic and inorganic N acquisition as well as allocation of N to N pools in the leaves and fine roots. As native species we chose some of the most abundant and widespread species of Central European forests (Ellenberg and Leuschner 2010, Eaton et al. 2016, Houston et al. 2016), which differ in physiological traits and growth strategies: (i) European beech (*Fagus sylvatica* L., Fagaceae), a drought-sensitive slow growing species (Houston et al. 2016), (ii) pedunculated oak (*Quercus robur* L., Fagaceae), a drought-tolerant slow growing species (Eaton et al. 2016), and (iii) Scots pine (*Pinus sylvestris* L., Pinaceae), a drought-tolerant fast growing conifer (Kuster et al. 2013, Sohn et al. 2016). The two invasive tree species are originally from North America, were first introduced in Europe in the 17th century and are now widely distributed due to their use in reforestation programs and considered two of the most frequent and important woody invaders in Central European forests (Campagnaro et al. 2018): (i) black cherry (*Prunus serotina* Ehrh., Rosaceae), a fast growing species producing cyanogenic compounds (Csiszár 2009), and (ii) black locust (*Robinia pseudoacacia* L., Leguminosae), a fast growing, N_2-fixing species (Robakowski et al. 2016, Vítková et al. 2017). Both, the remobilization of N from cyanogenic compounds and the ability to symbiotically fix N_2 allow these two invasive species to acquire additional N independently from soil N supply, which could thus serve as alternate sources of N. From here on, species used in this study will be referred to using their genus, i.e. *Fagus*, *Quercus*, *Pinus*, *Prunus* and *Robinia*.

Our general research aim was to evaluate whether N acquisition and allocation of N to N pools of native and invasive species shift in response to drought and different competitors reflecting their functional traits, i.e. growth rate (slow-growing vs. fast-growing), drought tolerance (drought-tolerant vs. drought-sensitive), and/or the ability to access alternative sources of N (e.g. via remobilization of N stored in cyanogenic compounds or symbiotic N_2 fixation). Our specific hypotheses were: (1) Drought generally reduces inorganic and organic N acquisition because N uptake from the soil strongly depends on water availability (Gessler et al. 2004, Rennenberg et al. 2006). (2) Species-specific coping mechanisms (related to their functional traits) lead to changes in the allocation of N to N pools in the leaves and fine roots, because generally with drought less N is acquired from the soil (Gessler et al. 2017). (3) N acquisition and allocation of N to N pools of a given species change depending on the species' functional traits and the competitor. For example, competition between a slow and a fast grower leads to a decrease in N acquisition and allocation to N pools in the slow growing species because of the fast grower's higher N demand (Reich 2014). (4) Native and invasive species differ in their preference for organic and inorganic N sources resulting in avoidance of competition for N (Fraterrigo et al. 2011, Huangfu et al. 2016).

2. Materials and Methods

2.1. Plant material and growth conditions

One-year-old seedlings of all species were purchased from Müller Münchehof Pflanzen GmbH (Seesen/Münchehof, Germany) and planted in different combinations (i.e. ~~one or~~ two seedlings per pot, see 2.2 "Experimental design") in a 1:1 mixture of sand and vermiculite in 3 L plastic pots (25 cm x 12 cm) at the end of November 2015. Pots stayed outdoors over winter and spring and were watered regularly. They were brought into the greenhouse on June 20th 2016 after leaf development. For the following 7 days, all pots were watered regularly with sufficient tap water, and received on two occasions 100 ml of an artificial low N nutrient solution containing 100 μM KNO_3, 90 μM $CaCl_2*2H_2O$, 70 μM $MgCl_2*6H_2O$, 50 μM KCl, 24 μM $MnCl_2*4H_2O$, 20 μM NaCl, 10 μM $AlCl_3$, 7 μM $FeSO_4*7H_2O$, 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). The pots were subjected to natural light conditions and day length regime (16/8, day/night). Air temperature was 23.5 ± 2.3 °C / 21.0 ± 2.4 °C (day/night, mean ± standard deviation), and relative humidity was 63.1 ± 9.3 % / 71.3 ± 8.8 % (day/night, mean ± standard deviation) for the duration of the experiment. With increasing duration of the drought treatment, some individuals started to show signs of wilting. To ensure sufficient replication for each species and treatment, we did the ^{15}N uptake experiments followed by the harvest after four weeks.

2.2. Experimental design

The experiment was conducted in a fully orthogonal design with two factors, “water availability” (i.e. control or drought) and “competitor” (interspecific competition with 2 or 3 different competitors). Seedlings were planted in native-invasive interspecific competition (i.e. one seedling of a native species and one seedling of an invasive species per pot). Pots were established for every combination of native species and invasive species. For each species, a total of 24 pots was setup for each combination of native-invasive, summing up to a total of 144 pots. Pots were assigned to either the control or drought treatment (i.e. n = 12 per combination of species, competitor, and soil water availability). For the drought treatment, irrigation was fully stopped starting June 27th until the final harvest from July 21st to 27th, while for the control treatment irrigation continued with sufficient tap water supply every second day. To confirm that water availability was significantly reduced in the respective treatment, we measured soil water content every second day for 3 pots per combination of species, soil water availability treatment and competitor, by inserting a probe into the soil in three different locations in each pot (HH2 Moisture Meter, Delta-T Devices, Cambridge). Soil water content in the drought treatment was ~~with~~ 7.2 ± 2.4 % (mean ± standard deviation) and significantly lower ~~l~~than in the control 24.0 ± 2.5% (mean ± standard deviation) at the end of the experiment. δ~~Δ~~^{13}C values in the leaves (Suppl. Table 6) were higher in the drought treatment compared to the control at the time of harvest indicating drought stress for *Fagus*, *Quercus*, and *Robinia* depending on the competitor, while for *Pinus* and *Prunus* no differences were found.

2.3. ^{15}N uptake experiments

To quantify inorganic (i.e. ammonium and nitrate) and organic (i.e. glutamine and arginine) net N uptake capacity of the seedlings' fine roots, the ^{15}N enrichment technique was used as described by Gessler et al. (1998) and modified by Simon et al. (2010). Seedlings were carefully removed from the pots and the roots washed thoroughly with tap water to remove any remaining substrate. Then, fine roots still attached to the seedlings were incubated in the same artificial low N solution as used during plant growth (see above) containing all four N sources but only one labelled as either $^{15}NH_4^+$, $^{15}NO_3^-$, $^{13}C/^{15}N$-glutamine, or $^{13}C/^{15}N$-arginine. Controls with no label were included to account for natural abundance in the fine roots (n = 4 to 6 per N source including controls with no label, per species, competitor, and soil water availability treatment). The roots not used for incubation were wrapped in wet tissue to prevent desiccation for the duration of the ^{15}N uptake experiment. After 2 hours of incubation, the fine roots were cut from the seedling and washed twice in 0.5 M $CaCl_2$ solution to remove the incubation solution from the root surface. The fresh weight of the fine roots was determined, and after oven-drying for 48 h at 60 °C, their dry weight was determined. Amino acids were $^{13}C/^{15}N$-labelled 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 ^{13}C compared to that on ^{15}N incorporation indicating 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). Incubation took place between 10 am and 2 pm to avoid diurnal variation in net N uptake capacity (Gessler et al. 2002).

2.4. Harvest and quantification of growth and biomass indices

After the ^{15}N uptake experiment, seedlings were separated into leaves, stems, and roots. Their fresh weight was determined, then all tissues were oven-dried for 48 h at 60 °C and their dry weight was determined. Before oven-drying, a subsample of 8 to 10 representative leaves was collected from each seedling and their leaf area measured (LI-3100C Area Meter, LI-COR, Lincoln, USA) to calculate specific leaf area (SLA). Likewise, a subsample of fine roots was collected from each seedling, stained, scanned and total length measured (WinRhizo 2012, Regent Instruments Inc., Quebec, Canada) to calculate specific root length (SRL) based on Liu and van Kleunen (2017). Furthermore, samples of leaves and fine roots were collected from each seedling to quantify total soluble amino acid-N and total soluble protein-N contents. These samples were shock- frozen in liquid N_2 immediately after sampling and determining their fresh weight, and then stored at -80 °C until further analyses. Root:shoot ratio was calculated as the relation between total belowground biomass (i.e. root biomass) and total aboveground biomass (i.e. leaves and stem biomass). For each seedling, RGR was calculated according to the equation: RGR = (ln b_2 - ln b_1) * t^{-1}, where b_1 is total seedling biomass (g dw) at the initial harvest, b_2 is total seedling biomass (g dw) at the final harvest, and t is the time period in days between the initial and the final harvest (Grubb et al. 1996). Initial seedling biomass was determined on 3 to 4 pots per species, competitor, and soil water availability treatment that were harvested immediately before commencing the drought treatment.

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

Dried fine root samples from the ^{15}N uptake experiment were ground using a ball mill (TissueLyser, Retsch, Haan, Germany) to a fine homogeneous powder. Aliquots of 1.2 to 2.4 mg were weighed into 4x6 mm tin capsules (IVA Analysentechnik, Meerbusch, Germany) for analyses with an isotope ratio mass spectrometer (Delta V Advantage, Thermo Electron, Dreieich, Germany) coupled to an elemental analyzer (Euro EA, Eurovector, Milano, Italy). Δ values were calculated using a laboratory standard (acetanilide) that was part of every sequence in intervals also used in different weights to determine isotope linearity of the system. The laboratory standard was calibrated against several suitable international isotope standards (IAEA, Vienna). Final correction of isotope values was done with several international isotope standards and other suitable laboratory standards which cover the range of ^{15}N and ^{13}C results. Inorganic and organic N net uptake capacity (nmol N g^{-1} fw h^{-1}) was calculated based on the incorporation of ^{15}N into the fine roots according to Kreuzwieser et al. (2002): Net N uptake capacity = $((^{15}N_l - ^{15}N_n) * N_{tot} * dw * 10^5) / (MW * fw * t)^{-1}$, where $^{15}N_l$ and $^{15}N_n$ are the atom% of ^{15}N in labeled (N_l) and unlabeled control plants (N_n, natural abundance), respectively, N_{tot} is the total N percentage, MW is the molecular weight (^{15}N g mol^{-1}), and t is the incubation time.

2.6. Quantification of total soluble protein and total soluble amino acid levels in leaves and fine roots

To extract total soluble proteins from the leaves and fine roots (Dannenmann et al. 2009), ~50 mg aliquots of finely ground frozen samples were incubated in 1.5 ml extraction buffer (50 mM Tris-HCl pH 8.0, 1 mM EDTA, 15% (v/v) glycerol, 0.6 mM dithiothreitol, 1% Triton X-100, 2 EDTA-free protease inhibitor cocktail tablets per 100 ml buffer) at 4 °C for 30 min followed by centrifugation for 10 min at 14,000 rpm and 4 °C. The extraction was done twice to increase the yield. Subsequently, 500 µL of the combined supernatant from both extractions were incubated with 1 ml 10 % (v/v) trichloroacetic acid for 10 min at room temperature and then centrifuged for 10 min at 14,000 rpm and 4 °C. The resulting protein pellet was dissolved in 1 ml 1 M KOH. Next, total soluble proteins were quantified following Simon et al. (2010) by adding 1 ml of Bradford reagent to 50 µL of extract. Following a 10 min incubation at room temperature in the dark, the absorbance was measured at 595 nm in a spectrophotometer (Ultrospec 3100pro, Amersham Biosciences). Bovine serum albumin (BSA) was used as standard.

Total soluble amino acid-N content in the leaves and fine roots were extracted according to Winter et al. (1992): 200 µL Hepes buffer (5 mM EGTA, 20 mM HEPES, 10 mM NaF) and 1 ml 3.5:1.5 (v:v) methanol/chloroform were added to ~50 mg aliquots of finely ground frozen sample and incubated for 30 min on ice, followed by the addition of 600 µL of distilled water and centrifugation for 5 min at 14,000 rpm and 4 °C. The addition of distilled water and centrifugation was performed twice to increase the yield. Total soluble amino acid-N content in the leaves and fine roots were quantified according to Liu et al. (2005): 50 µL ninhydrin solution was added to a 50 µL aliquot of the combined extract and boiled for 30 min. The ninhydrin solution was composed of an equal parts mixture of solution A (i.e. 3.84 g citric acid, 0.134 g $SnCl_2$, and 40 ml 1 M NaOH, filled ~~up~~ to 100 ml with distilled water at pH 5) and solution B (i.e. 4 g ninhydrin in 100 ml ethylene-glycol-monomethyl-ether). Subsequently, the extracts were cooled to room temperature and 1 ml 50% isopropanol was added, followed by a 15 min incubation. The absorption was measured at 570 nm in a spectrophotometer (Ultrospec 3100pro, Amersham Biosciences). L-glutamine was used as standard.

2.7. Statistical analyses

Two-way permutational ANOVAs (PERMANOVA) based on a Euclidean resemblance matrix between samples (Anderson et al. 2008) were performed for each species to test for differences between water availability and competitor levels using as variables inorganic and organic net N uptake capacity, total soluble amino acid-N, and total soluble protein-N contents in the leaves and fine roots, as well as total biomass, root:shoot ratio, SLA, SRL, and RGR. PERMANOVAs were performed using "water availability" (i.e. drought and control) and "competitor" as fixed orthogonal factors. "Competitor" consisted of two levels for the native species (i.e. competition with *Prunus* or *Robinia*) or three levels for the invasive species (i.e. competition with *Fagus*, *Quercus*, or *Pinus*). For significant interactions between factors, *post hoc* PERMANOVA pair-wise comparisons were performed. To test for differences in $\delta^{13}C$ in leaves between drought and control for each species grown with different competitors, Mann-Whitney U-tests were performed. Finally, to test for species preferences in net N uptake capacity of the different N forms, one-way PERMANOVAs were performed for each combination of species and competitor using “N source” as factor at both levels of soil water availability. All PERMANOVA analyses were performed using PRIMER 6.0 with the PERMANOVA+ add-on (PRIMER-E Ltd, Plymouth, UK), while Mann-Whitney-U tests were performed using SigmaPlot 14.0 (Systat Software, San Jose, USA).

3. Results

3.1. Drought effects on N acquisition and allocation to N pools in the leaves and fine roots of native and invasive tree seedlings

For all species (both native and invasive), net N uptake capacity was lower with drought compared to sufficient water supply, but with differences among species regarding which specific N form was significantly affected and with which competitor (native species: Fig. 1, invasive species: Fig. 2). Ammonium acquisition, already very low, was reduced further with drought for *Prunus* and *Robinia* (Fig. 2) regardless of competitor, whereas nitrate acquisition was lower with drought for *Fagus*, *Quercus*, *Pinus* (Fig. 1) and *Prunus* regardless of competitor, and for *Robinia* only in competition with *Quercus* and *Pinus* (Fig. 2). Glutamine acquisition was reduced by drought for *Pinus* (Fig. 1), *Prunus* and *Robinia* (Fig. 2) regardless of competitor, and for *Quercus* (Fig. 1) only in competition with *Robinia*, while arginine acquisition was lower with drought for *Fagus*, *Quercus* (both Fig. 1) and *Robinia* (Fig. 2) regardless of competitor (~~Fig. 1, Fig. 2,~~ Table 1, Suppl. Table 1).

Drought also led to changes in N allocation to N pools in leaves and fine roots. With drought compared to the controls, total soluble protein-N content was reduced regardless of competitor in the leaves of *Fagus* and *Quercus*, and in the fine roots of *Prunus*, as well as in the fine roots of *Robinia* in competition with *Pinus*, while it increased in the fine roots of *Robinia* in competition with *Fagus* with drought (Table 1, Suppl. Table 2, 3, 4). Total soluble amino acid-N content was increased with drought in the fine roots of *Pinus* and the leaves of *Robinia* regardless of competitor, as well as in the leaves and fine roots of *Quercus* in competition with *Prunus* (Table 1, Suppl. Tables 2, 3, 4). However, total soluble amino acid-N levels were lower with drought in the fine roots of *Prunus* grown in competition with *Quercus* or *Pinus* (Table 1, Suppl. Tables 2, 3, 4).

Finally, drought resulted in higher $\delta^{13}C$ values in the leaves of *Fagus* and *Quercus* grown in competition with *Prunus*, and in the leaves of *Robinia* grown in competition with *Quercus* or *Pinus* (Suppl. Tables 5, 6). Drought affected also the total biomass, root:shoot ratio, RGR, SLA and SRL of native and invasive species depending on both the target species and the competitor (Table 1, Suppl. Tables 7, 8, 9, Suppl. Figure 1).

3.2. Competitor effects on N acquisition and allocation to N pools in the leaves and fine roots of native and invasive tree species

For native species, N acquisition did not change with different invasive competitors, but there were different responses regarding allocation to N pools (Fig. 1). In competition with *Prunus* compared to *Robinia* and regardless of soil water availability, *Quercus* had lower total soluble protein-N content but higher total soluble amino acid-N content in the fine roots, whereas *Pinus* had lower total soluble amino acid-N content and higher total soluble protein-N content in the leaves, and higher total soluble amino acid-N content in the fine roots (Table 2, Suppl. Tables 2, 3,4). For *Fagus*, N allocation to N pools did not vary with competitor (Table 2, Suppl. Tables 2, 3, 4). All native species responded differently to the invasive competitors regarding their biomass and growth indices (Table 2, Suppl. Tables 7, 8, 9).

Similarly, responses of the invasive species depended on competitor and were partly also mediated by soil water availability. Under drought, *Prunus* seedlings had lower total soluble amino acid-N contents in the fine roots when competing with *Quercus* compared to *Pinus* (Table 2, Suppl. Table 2, 3, 4). With sufficient soil water supply, total soluble amino acid-N content in the fine roots of *Prunus* was lower competing with *Fagus* than with *Quercus* or *Pinus* (Table 2, Suppl. Table 2, 3, 4). *Prunus* seedlings had a lower total soluble amino acid-N content in the leaves when competing with *Fagus* than with *Quercus*, and a lower total soluble protein-N content in the leaves when competing with *Fagus* or *Quercus* than with *Pinus* (Table 2, Suppl. Tables 2, 4, 10). *Robinia* seedlings responded to competitor with changes in other parameters than *Prunus*. With drought, *Robinia* seedlings had higher total soluble protein-N content in the fine roots when competing with *Fagus* than with *Quercus*, and higher total soluble protein-N content in the fine roots competing with *Quercus* than with *Pinus* (Table 2, Suppl. Tables 2, 3, 4). Under control conditions, *Robinia* competing with *Fagus* had a lower nitrate net uptake capacity than when competing with *Pinus* (Fig. 2, Table 2). Both *Prunus* and *Robinia* responded differently with regard to their biomass and growth indices (Table 2, Suppl. Tables 7, 8, 9, 11).

3.3. N acquisition preferences for different N sources of native and invasive species

Preferences for certain N sources were found among species depending on the competitor and soil water availability. Generally, organic N, especially arginine-N, was favoured over inorganic N sources. *Quercus* and *Prunus* did not change N preferences with different competitors or drought (Tables 3, 4). *Fagus* preferred organic N over inorganic N with drought (Table 3). *Pinus* did not prefer specific N sources in competition with *Robinia* with sufficient water supply, but preferred organic N over inorganic N with drought and in competition with *Prunus* regardless of water availability (Table 3). *Robinia* showed a distinct pattern depending on the competitor: With drought, seedlings preferred arginine-N over inorganic N but only in competition with *Quercus*, but not in competition with *Fagus* or *Pinus* (Table 4). With sufficient water supply, *Robinia* preferred organic over inorganic N when grown in competition with *Fagus*, but not with *Quercus* or *Pinus* (Table 4).

4. Discussion

4.1. Drought reduces N acquisition among species, but allocation of N to N pools varies with species and competitor

In accordance with our hypothesis, inorganic and organic N acquisition of both native and invasive tree seedlings overall decreased in response to drought, confirming that tree N acquisition and soil water availability are strongly linked (e.g. Fotelli et al. 2002, 2004, Rennenberg et al. 2006, Gessler et al. 2017). The influence on organic N acquisition for tree species in competition is a novel insight provided by our work, while also confirming previous studies in which drought reduced the inorganic N acquisition of *F. sylvatica* seedlings growing both in intra- and interspecific competition (Fotelli et al. 2002). In plant communities, when soil water availability is reduced, microbial activity is decreased negatively impacting ~~on~~ soil N mineralization processes, thus resulting in reduced soil N availability (Schimel et al. 2007, Hueso et al. 2012). Furthermore, soil N diffusion and mass flow are reduced (Rennenberg et al. 2009) and the composition of mycorrhizal communities that symbiotically provide plants with N is changed (e.g. Gessler et al. 2005, Leberecht et al. 2016).

Our studied tree species appear to show~~ed~~ four different ~~mechanisms to cope~~responses to ~~with~~ drought regarding the~~ir~~ allocation of N to N pools in the leaves and fine roots depending on the species and competitor: (1) Total soluble protein levels were reduced (in the leaves of *Fagus* and *Quercus* regardless of competitor, and in the fine roots of *Prunus* and *Robinia* competing with *Pinus*) as a consequence of reduced N acquisition and thus, N assimilation (Gessler et al. 2017). Storage proteins from vegetative tissue are degraded and remobilized leading to lower N in storage (e.g. Millard 1988, Staswick 1994, Millard and Grelet 2010). With potential leaf shedding as a consequence of drought and, consequently, N stored in the leaves being lost by the plant, the roots become an important tissue for N storage (Millard and Grelet 2010). In our study, the leaves were not (yet) shed at the time of the harvest, suggesting that N was likely still remobilized. (2) In the fine roots of *Robinia* in competition with *Fagus*, the levels of total soluble proteins increased in response to drought likely due to the synthesis of protective proteins (Brunner et al. 2015), proteins with a role in dehydration tolerance (Close 1996, Kozlowski and Pallardy 2002), and/or proteins required for the development of root nodules for N_2-fixing species (Verma et al. 1992) such as *Robinia*. (3) Soluble amino acid levels decreased in the fine roots of *Prunus* when grown in competition with *Quercus* or *Pinus* in response to drought. This is likely due to amino acid degradation, their translocation to other plant tissues, their usage for protein synthesis, and/or the usage of their carbon skeletons to produce alternative non-N-containing osmotic compounds which not only decrease osmotic potential but can also function as cell membrane and metabolic protectants (Chaves et al. 2003). (4) In contrast, total soluble amino acid levels increased in the leaves of *Robinia* and the fine roots of *Pinus* regardless of competitor, as well as in the leaves and fine roots of *Quercus* in competition with *Prunus* via protein degradation to serve as osmoprotectants, thus improving the overall plant water status (Hu et al. 2013, 2013b). Overall, our results indicate that the study ~~different~~ species ~~use~~ show ~~diverse~~ different ~~coping mechanisms in~~ responses to drought conditions in this short-term experiment with regards to N allocation to N pools in leaves and fine roots ~~. These responses were found~~ in combination with changes in biomass allocation~~,~~ ~~further highlighting the species-specific responses to drought~~.

Some species showed changes in biomass allocation in response to drought while others did not. A higher root:shoot ratio resulting in a larger soil volume to be exploited and ~~in~~ a parallel decrease in aboveground biomass as well as SLA reduces~~d~~ water loss via the leaves further improving~~es~~ a plant's water status (Fotelli et al. 2005, Mantovani et al. 2014, Duan et al. 2018). This strategy was found in our study for *Robinia*. On the other hand, drought sensitivity of a species might be reflected by reduced root growth, eventually leading to a decrease in root:shoot ratio (Ostonen et al. 2007, Brunner et al. 2015), root hydraulic failure (Mao et al. 2018), and higher root mortality (Zhou et al. 2018). In our study, drought conditions were severe enough to cause a decrease in root:shoot ratio for *Fagus* indicating the drought-sensitivity of this species, but not the other native or the invasive species.

In general, all study species were negatively affected by drought with no clear distinction between native and invasive species. Native *Fagus* and – to a lesser extent – invasive *Robinia* were most sensitive showing several changes in above- and belowground traits. For *Fagus*, the strong response to drought generally prevailed over the effects of the competitor, while for *Robinia* it was mediated by the competitor. The ability to fix N_2 allows *Robinia* to obtain external N and thus be less affected by the negative effect of drought on N acquisition from the soil (Wurzburger and Miniat 2014, Mantovani et al. 2014, 2015). For *Quercus* and invasive *Prunus*, responses to drought also varied according to competitor. In contrast, we found no interaction between drought and competitor for *Pinus* which indicates that the two invasive species did not influence its response to drought. Furthermore, coniferous *Pinus* was generally less responsive to drought than the native and invasive deciduous species most likely due to its isohydric behaviour, i.e. the closing of stomata early during a drought event, thereby minimizing water losses via the needles (Irvine et al. 1998). These results imply that under future scenarios of global change, *Pinus* can withstand simultaneous short-term drought stress and an invasion by exotic woody species better than *Fagus* and *Quercus*. ~~However, *Quercus* appears to have an advantage over *Prunus* under drought, again highlighting the specific nature of the species-species responses.~~

4.2. Species-specific responses of native and invasive species in response to competitor and soil water availability

Our hypothesis that N acquisition from the soil and its allocation to plant internal N pools vary for a given species depending on its functional traits and the competing species could partly be confirmed. None of the native species and only one of the two invasives responded to different competitors with changes in N acquisition, thus contrasting previous studies (Simon et al. 2010, 2014, Bueno et al. 2019). This suggests that soil water availability was a stronger driver of N uptake than competition. The allocation of N to different N pools of our studied species depended on the competitor and varied with soil water availability, thus confirming our hypothesis that responses to different competitors are species-specific according to the physiological characteristics of the study species**.**

Both invasive species in our study, show functional traits commonly linked to fast growth (Grotkopp and Reimánek 2007), such as higher biomass (*Prunus*) or higher SLA, and in turn, RGR (*Robinia*), higher N allocation to N pools in leaves and fine roots thus enhancing resource acquisition above- and belowground, and overall increasing their competitive advantage under high resource availabilty (e.g. Li et al. 2015). A negative influence of *Robinia* on the growth of competing tree seedlings has been reported before via the depletion of soil resources due to

Robinia's fast growth and occupation of rooting space (Kawaletz et al. 2013, 2014). In our study *Robinia* was the only species with increased root:shoot ratio under drought suggesting a strong potential for below-ground competition and resource gain for metabolic processes, which apparently had a stronger effect on drought-sensitive *Fagus* than the drought-tolerant natives. N acquisition of all three native species did not differ with competitor, and the higher allocation of N to N pools combined with slower growth of *Quercus* and *Pinus* when competing with *Prunus* than *Robinia* suggests an increased storage of N metabolites rather than a use for biomass production (Reich et al. 1997, Millet et al. 2005, Millard and Grelet 2010). Moreover, the negative effects on growth and biomass indices when competing with *Prunus* rather than *Robinia* further indicate negative consequences of competition with *Prunus* for overall plant development.

Similar to the native species, the competitor had no effect on N acquisition of *Prunus* indicating that the observed changes in biomass and growth indices as well as N metabolites content occurred in relation to internal N dynamics in the seedlings. This is likely due to remobilization and *de novo* synthesis of N metabolites (Simon et al. 2010, Li et al. 2015), and/or the reliance on N stored in plant tissues as N-based defense compounds (Gleadow and Woodrow 2002). The other responses of invasive *Prunus* to native species depended on soil water availability. For example, drought-sensitive *Fagus* was a stronger competitor for *Prunus* only with sufficient soil water availability whereas this effect was absent with drought, reflecting the drought-sensitivity of *Fagus*. With drought, N pools of *Prunus* were higher in competition with *Pinus* compared to deciduous natives. This indicates a similar mechanism of metabolic adaptation to stress as in the slow growing deciduous *Fagus* and *Quercus* (Millard and Grelet 2010). This might be a differential response of *Prunus* to specific competitors with drought conditions, possibly related to the drought tolerance of such competitors. This would be in accordance with studies indicating that evergreen conifers are on average more drought-tolerant due to their more conservative resource use than broadleaved deciduous temperate woody species (Hallik et al. 2009), though further studies considering a greater number of species are needed to provide additional insights into this.

Robinia responded to competition with native deciduous *Fagus* and *Quercus* stronger than with coniferous *Pinus*, although the affected specific growth and physiological parameters depended on soil water availability. In contrast to all other study species, nitrate acquisition of *Robinia* was reduced in its competition with *Fagus* compared to competition with *Pinus* suggesting the release of active compounds that potentially impair N acquisition as suggested for *Acer pseudoplatanus* in a previous study (Simon et al. 2010). This would negatively affect nutrition and development of *Robinia* seedlings compared to competition with other natives because although *Robinia* can fix N_2, N acquisition from the soil is preferred over N_2 fixation when soil N is not limiting (Pfautsch et al. 2009).These differences in the responses to competitors became more apparent when *Robinia* was additionally affected by drought and may be explained by *de novo* synthesis of proteins as an adaptation to competition (Simon et al. 2010, 2014). Additionally, *Robinia* seedlings grew slower in competition with *Fagus* or *Quercus* compared to *Pinus* regardless of soil water availability further highlighting their negative competitive effects on *Robinia*.

~~For the natives, *Fagus* was more affected by *Robinia*, while *Quercus* and *Pinus* responded strongest to invasive *Prunus*. For drought-sensitive *Fagus* competition only mattered when soil water availability was sufficient indicating that drought effects overrode the different influence of the two invasive competitors. When water was sufficiently available, the overall growth of *Fagus* was more affected in competition with *Robinia* than *Prunus*, while N acquisition and allocation of N to N pools in the leaves and fine roots were similar between competitors. A negative influence of *Robinia* on the growth of competing tree seedlings has been reported before via the depletion of soil resources due to *Robinia*'s fast growth and occupation of rooting space (Kawaletz et al. 2013, 2014)~~. ~~Similar to *Fagus*, N acquisition of *Quercus* and *Pinus* did not differ with competitor. However, the~~Their ~~higher allocation of N to N pools~~ in less competitive species ~~combined with slower growth when competing with *Prunus* than *Robinia* for *Quercus* and *Pinus* suggests an increased storage of N metabolites rather than a use for biomass production with different competitors (Reich et al. 1997, Millet et al. 2005, Millard and Grelet 2010).~~

~~Furthermore, the negative effects on growth and biomass indices when competing with *Prunus* rather than *Robinia* indicates negative consequences for overall plant development. Despite the relative common responses of *Quercus* and *Pinus* to competition with *Prunus* regardless of water supply, some responses of *Quercus* were mediated by soil water availability (e.g. higher SRL with *Prunus* than *Robinia* under drought, and higher total soluble protein-N content in fine roots with *Robinia* than *Prunus* under sufficient water), whereas the responses of *Pinus* were entirely independent of soil water availability.~~

~~Similar to the native species, inorganic and organic N acquisition of *Prunus* did not change with competitor indicating that the changes in biomass and growth indices as well as N metabolites content occurred in relation to internal N dynamics in the seedlings, probably involving processes such as remobilization and *de novo* synthesis of N metabolites (Simon et al. 2010, Li et al. 2015), and/or the reliance on N stored in plant tissues as N-based defense compounds (Gleadow and Woodrow 2002). The other responses of invasive *Prunus* to native species depended on soil water availability, reflecting the physiological characteristics of the native competitors related to their drought tolerance. For example, drought-sensitive *Fagus* was a stronger competitor for *Prunus* only with sufficient soil water availability whereas this effect was absent with drought. In contrast, drought-tolerant *Quercus* and *Pinus* negatively affected *Prunus* relative to other competitors regardless of soil water availability. With sufficient water availability, *Prunus* reduced growth in competition with all native species. This overall competitor effect was no longer found for *Prunus* in competition with *Fagus* with drought, because drought was a stronger driver of *Fagus* metabolism than competition. With drought, N metabolite levels in leaves and fine roots of *Prunus* were higher in competition with *Pinus* compared to deciduous tree species indicating that this metabolic adaptation to stress (Millard and Grelet 2010) might be a differential response of *Prunus* to specific competitors with drought conditions. This would be in accordance with studies indicating that evergreen conifers are on average more drought-tolerant than broadleaved deciduous temperate woody species (Hallik et al. 2009), though further studies considering a greater number of species are needed to provide additional insights into this. In plant communities, coniferous and deciduous species might differ in their competitive effects on other plant species due to their differences in nutrient concentration in plant tissues, litter quality, and their differential impact on soil biogeochemistry (Calder et al. 2011, Wang et al. 2016).~~

~~*Robinia* responded to competition with deciduous *Fagus* and *Quercus* stronger than with coniferous *Pinus*, although the affected specific growth and physiological parameters depended on soil water availability. In contrast to all other study species, nitrate acquisition of *Robinia* was reduced in its competition with *Fagus* compared to *Pinus* with sufficient soil water availability suggesting that *Fagus* is a stronger competitor potentially impairing N acquisition via the release of active compounds as suggested for *Acer pseudoplatanus* in a previous study (Simon et al. 2010). This would negatively affect nutrition and development of *Robinia* seedlings compared to competition with other natives. Although *Robinia* can fix N_2, N acquisition from the soil is preferred over N_2 fixation when soil N is not limiting (Pfautsch et~~

~~al. 2009). Thus, the reduced nitrate acquisition in competition with *Fagus* compared to *Pinus* might be disadvantageous for *Robinia*. This gradient between competitors became more apparent when *Robinia* was additionally affected by drought: Total soluble protein-N levels in the fine roots were higher in competition with *Fagus* than *Quercus* or *Pinus* indicating *de novo* synthesis of proteins as an adaptation to competition (Simon et al. 2010, 2014). Additionally, *Robinia* seedlings grew slower in competition with *Fagus* or *Quercus* compared to *Pinus* regardless of soil water availability further highlighting their negative competitive effects on *Robinia*.~~

Overall, native and invasive species responded to different competitors in accordance with their physiological and life history traits such as drought tolerance and leaf habit. For example, drought-sensitive *Fagus* responded negatively to different competitors and affected invasive species only with sufficient water supply, indicating that drought effects override those of the two invasive competitors. Moreover, invasive *Prunus* was generally a stronger competitor for the native species ~~inducing higher N metabolite levels in leaves and roots among other effects~~. With competition, t~~T~~issue nutrient content plays an important role in plant performance ~~with competition~~. For example, seedlings of black spruce (*Picea mariana*) with high levels of N (and other nutrients) had higher biomass than seedlings with low tissue N content after growing in competition with natural vegetation (Malik and Timmer 1998) indicating the importance of plant internal N reserves in competitive interactions.

4.3. Organic N was generally preferred by all species regardless of drought

We hypothesized that native and invasive species differ in their preference for organic and inorganic N sources. However, organic N forms, especially arginine, were generally preferred over inorganic N by all study species confirming results from studies in the field and under controlled settings (Simon et al. 2017) using the same technique and artificial soil solution as here (e.g. for *F. sylvatica*: Dannenmann et al. 2009, Simon et al. 2010, Simon et al. 2011, Simon et al. 2014, for *R. pseudoacacia*: Hu et al. 2017), as well as the results found at low soil N availability in our previous study using the same species (Bueno et al. 2019). The preferred acquisition of specific N sources is generally driven by their higher abundance in the soil (e.g. Kuster et al. 2013b, Song et al. 2015, Simon et al. 2017). For native *Fagus* and *Pinus*, as well as invasive *Robinia* preferences shifted depending on the competitor (see Tables 3, 4) indicating plasticity in resource use induced by the interaction with other species which might influence the plant's competitive ability (Ashton et al. 2010). Such shifts were not found for native

Quercus and invasive *Prunus* indicating that competition does not affect their N form preference. Furthermore, to our knowledge, no previous studies have directly measured the effect of drought on the preference for different N forms. We found no changes in the N form preference with drought suggesting that changes in N preference for the study species does not play a role for adapting to drought, at least under these experimental conditions.

Conclusions

In our study, drought generally reduced inorganic and also – shown for the first time – organic N acquisition among both native and invasive study species, because reduced water availability leads to less N available in the soil for plants. Overall, N acquisition was driven by water supply rather than competition. The allocation of N to internal N pools in the leaves and fine roots, however, varied with study species and competitor in relation to their physiological characteristics (i.e. drought sensitive vs. drought tolerant tree species) showing their respective species-specific coping mechanisms (e.g. the remobilization of N from storage and/or synthesis of osmoprotectants). For example, within the native tree species, for drought-sensitive *Fagus*, reduced water availability overrode the influence of competition, whereas the responses of drought-tolerant *Pinus* were entirely independent of water supply. Deciduous and coniferous species might respond differently to competition with other plants because of their differences in, for example, tissue concentrations of nutrients (Calder et al. 2011, Wang et al. 2016). Organic N was generally favoured by both native and invasive species regardless of drought suggesting that competition for N was not avoided. Moreover, our results suggest that *Prunus* as an invasive species is a stronger competitor than *Robinia* for most native tree species (which is in accordance with a previous study (Bueno et al. 2019)), possibly due to its higher biomass. In contrast, for *Robinia*, the deciduous natives *Fagus* and *Quercus* had more negative effects than the conifer *Pinus*. In conclusion, our results suggest that species-specific mechanisms to cope with drought related to their physiological characteristics might play a role for the competitive ability of the studied species. However, further studies investigating a larger number of species are necessary to confirm this. Because water availability strongly affects plant and soil N dynamics (e.g. Gessler et al. 2017, Simon et al. 2017), longer term competition (> 1 year) might pronounce the effects of competition seen in our study further.

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

AB and JS conceived and designed the study. AB conducted the ^{15}N uptake experiments, N metabolite analyses, and evaluated all data. KP contributed the IRMS analyses. AB and JS led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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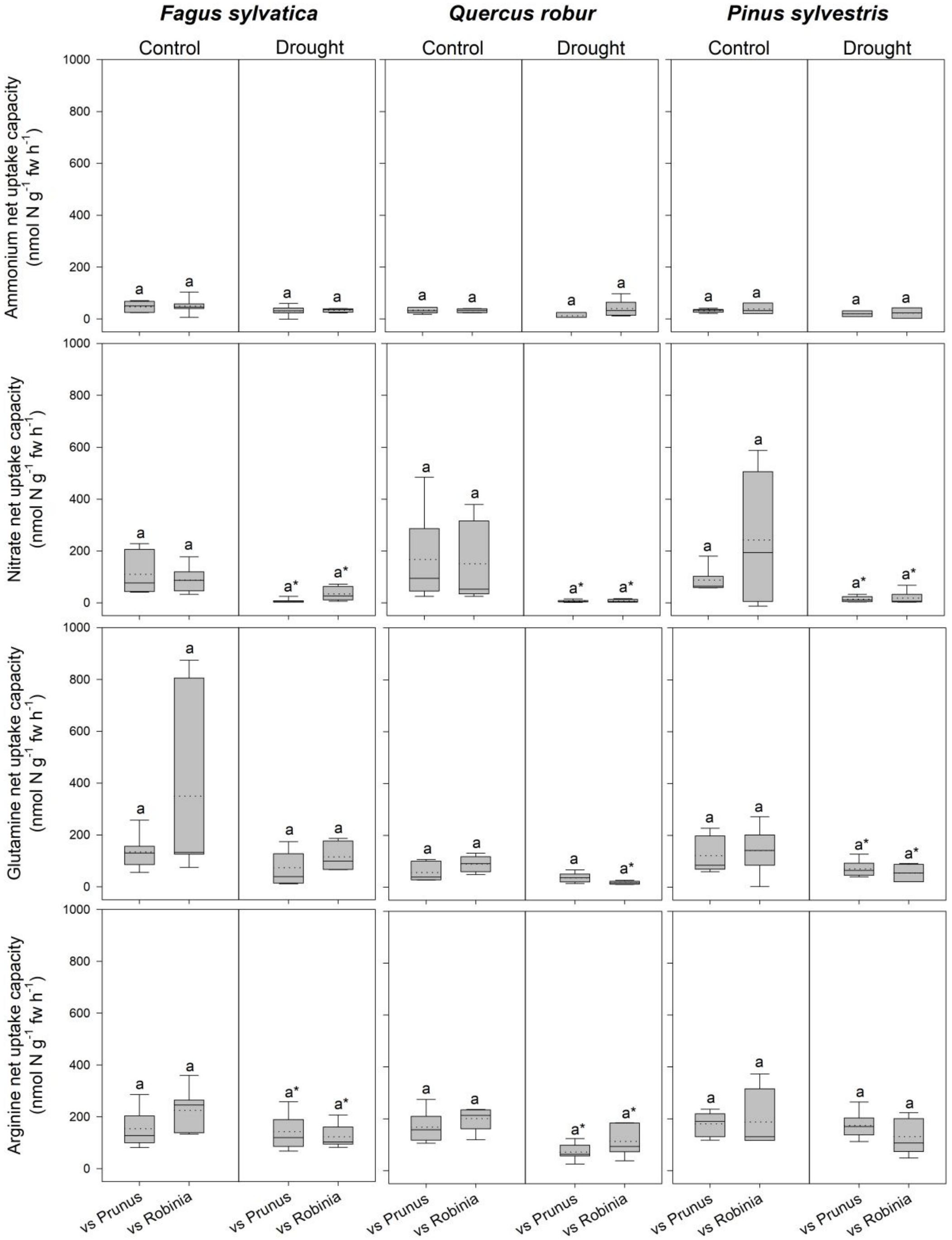


Figure 1. Ammonium, nitrate, glutamine-N, and arginine-N net uptake capacity (nmol N g^{-1} fw h^{-1}) by fine roots of *Fagus sylvatica*, *Quercus robur* and *Pinus sylvestris* seedlings at control and drought conditions under different competition regimes. vs *Prunus* = competition with *Prunus serotina*; vs *Robinia* = competition with *Robinia pseudoacacia*. Box plots show mean (dotted line) and median (continuous line). Different letters indicate significant differences between competition regimes within a specific soil water availability treatment, and asterisks indicate significant differences between control and drought detected using permutational analysis of variance ($p < 0.05$).

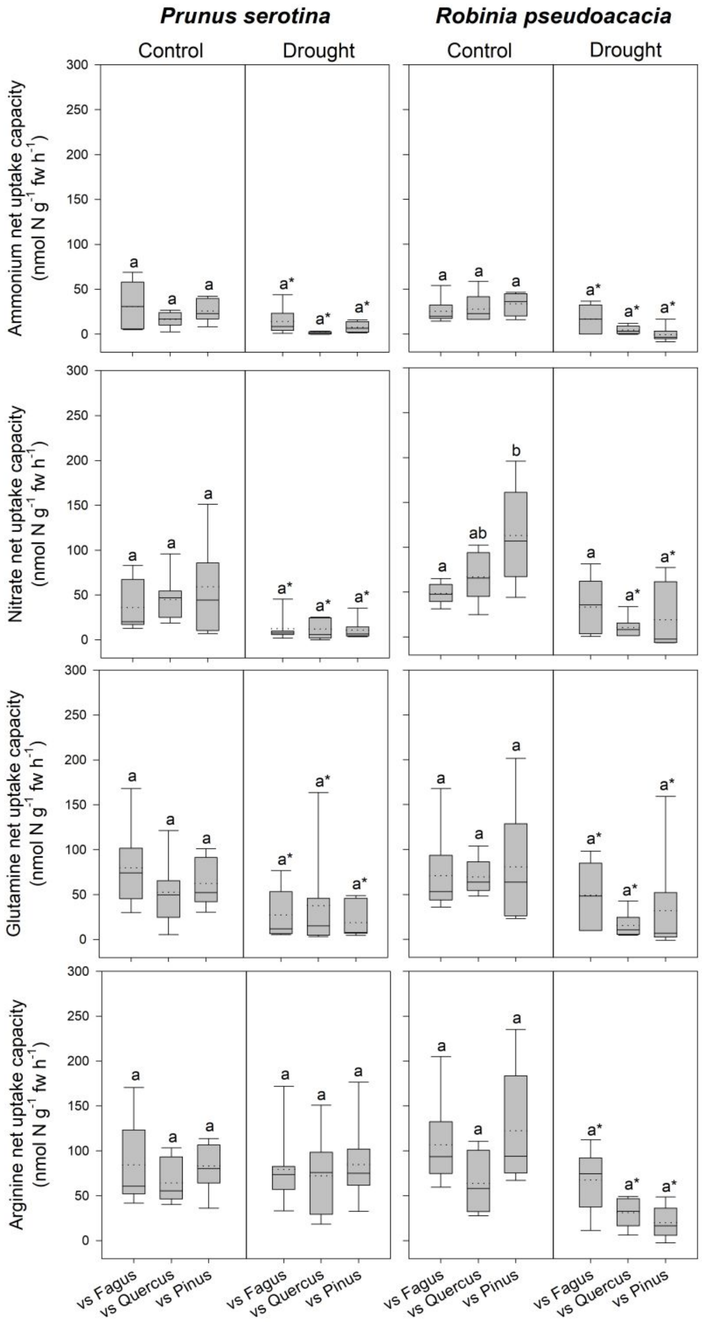


Figure 2. Ammonium, nitrate, glutamine-N, and arginine-N net uptake capacity (nmol N g^{-1} fw h^{-1}) by fine roots of *Prunus serotina* and *Robinia pseudoacacia* seedlings at control and drought conditions under different competition regimes. vs *Fagus* = competition with *Fagus sylvatica*; vs *Quercus* = competition with *Quercus robur*; vs *Pinus* = competition with *Pinus sylvestris*. Box plots show mean (dotted line) and median (continuous line). Different letters indicate significant differences between competition regimes within a specific soil water availability treatment, and asterisks indicate significant differences between control and drought detected using permutational analysis of variance ($p < 0.05$).

Table 1. Effects of drought on total biomass, growth indices, inorganic and organic N net uptake capacity, and N metabolite levels on seedlings of *Fagus sylvatica*, *Quercus robur*, *Pinus sylvestris*, *Prunus serotina*, and *Robinia pseudoacacia* growing in competition. ↑ = significant increase with drought, ↓ = significant decrease with drought, n.s. = no significant differences between drought and control. Total biomass (g dw), root:shoot ratio: ratio of belowground to aboveground biomass, RGR: relative growth rate (g dw g^{-1} dw d^{-1}), SLA: specific leaf area (cm^2 g^{-1} dw), SRL: specific root length (cm g^{-1} dw), N net uptake capacity (nmol N g^{-1} fw h^{-1}), NH_4^+: ammonium, NO_3^-: nitrate, Gln-N: glutamine-N, Arg-N: arginine-N, total soluble amino acid-N (mg g^{-1} dw), total soluble protein-N (mg g^{-1} dw).

	Biomass and growth indices					**N net uptake capacity**				**N metabolites**			
										Total soluble amino acid-N		**Total soluble protein-N**	
	Total biomass	**Root:shoot ratio**	**RGR**	**SLA**	**SRL**	**NH_4^+**	**NO_3^-**	**Gln-N**	**Arg-N**	**Leaves**	**Fine roots**	**Leaves**	**Fine roots**
Fagus sylvatica	n.s.	↓	↑(b)	↓	n.s.	n.s.	↓	n.s.	↓	n.s.	n.s.	↓	n.s.
Quercus robur	n.s.	n.s.	n.s.	n.s.	↓(b)	n.s.	↓	↓(b)	↓	↑(d)	↑(d)	↓	n.s.
Pinus sylvestris	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	↓	↓	n.s.	n.s.	↑	n.s.	n.s.
Prunus serotina	↓(a)	n.s.	n.s.	↑	n.s.	↓	↓	↓	n.s.	n.s.	↓(c)	n.s.	↓
Robinia pseudoacacia	n.s.	↑	n.s.	n.s.	n.s.	↓	↓(c)	↓	↓	↑	n.s.	n.s.	↑(e) - ↓(f)

(a): only in competition with *Quercus*
(b): only in competition with *Robinia*
(c): only in competition with *Quercus* or *Pinus*
(d): only in competition with *Prunus*
(e): only in competition with *Fagus*
(f): only in competition with *Pinus*
No letter: effect of drought regardless of competitor

Table 2. Effects of competitor on total biomass, growth indices, inorganic and organic N net uptake capacity, and N metabolite levels of *Fagus sylvatica*, *Quercus robur*, *Pinus sylvestris*, *Prunus serotina*, and *Robinia pseudoacacia* seedlings. Species A < species B = seedlings competing with species A had significantly lower values than seedlings competing with species B, n.s. = no significant differences between competitor identities. Total biomass (g dw), root:shoot ratio: ratio of belowground to aboveground biomass, RGR: relative growth rate (g dw g^{-1} dw d^{-1}), SLA: specific leaf area (cm^2 g^{-1} dw), SRL: specific root length (cm g^{-1} dw), N net uptake capacity (nmol N g^{-1} fw h^{-1}), NH_4^+: ammonium, NO_3^-: nitrate, Gln-N: glutamine-N, Arg-N: arginine-N, total soluble amino acid-N (mg g^{-1} dw), total soluble protein-N (mg g^{-1} dw).

	Biomass and growth indices					**N net uptake capacity**				**N metabolites**			
										Total soluble amino acid-N		**Total soluble protein-N**	
	Total biomass	**Root:shoot ratio**	**RGR**	**SLA**	**SRL**	**NH_4^+**	**NO_3^-**	**Gln-N**	**Arg-N**	**Leaves**	**Fine roots**	**Leaves**	**Fine roots**
Fagus sylvatica	n.s.	n.s.	*Robinia* < *Prunus* (a)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Quercus robur	n.s.	*Prunus* < *Robinia*	*Prunus* < *Robinia*	n.s.	*Robinia* < *Prunus* (b)	n.s.	n.s.	n.s.	n.s.	n.s.	*Robinia* < *Prunus*	n.s.	*Prunus* < *Robinia* (a)
Pinus sylvestris	*Prunus* < *Robinia*	n.s.	n.s.	-	n.s.	n.s.	n.s.	n.s.	n.s.	*Prunus* < *Robinia*	*Robinia* < *Prunus*	*Robinia* < *Prunus*	n.s.
Prunus serotina	*Fagus*, *Pinus* < *Quercus* (a)	n.s.	*Quercus*, *Pinus* < *Fagus*	*Quercus* < *Fagus* < *Pinus*	*Quercus* < *Fagus* (a)	n.s.	n.s.	n.s.	n.s.	*Fagus* < *Quercus*	*Fagus* < *Quercus*, *Pinus* (a) *Quercus* < *Pinus* (b)	*Fagus*, *Quercus* < *Pinus* (b)	n.s.
Robinia pseudoacacia	n.s.	n.s.	*Fagus*, *Quercus* < *Pinus*	n.s.	n.s.	n.s.	*Fagus* < *Pinus* (a)	n.s.	n.s.	n.s.	n.s.	n.s.	*Pinus* < *Quercus* < *Fagus* (b)

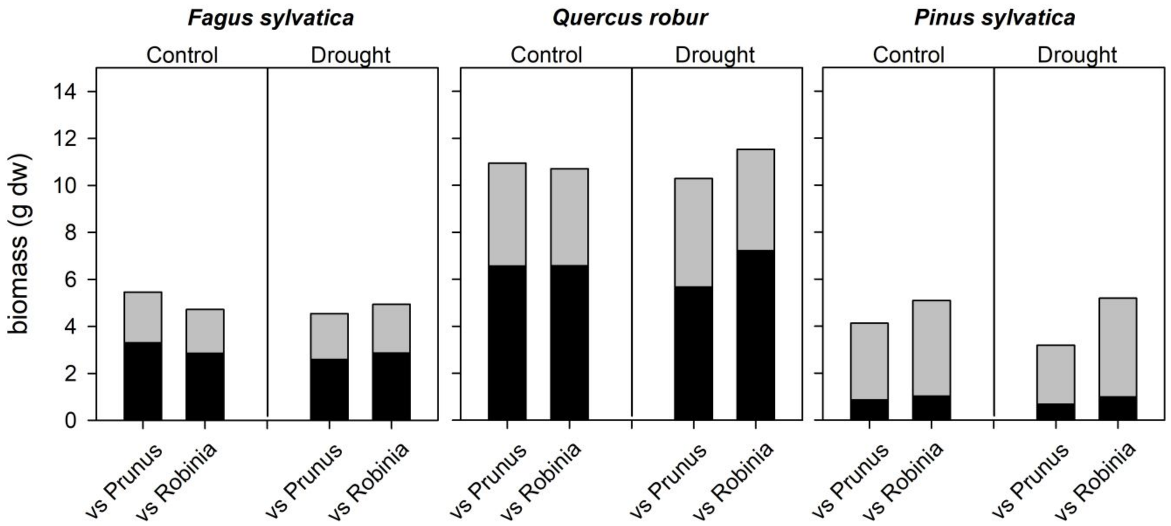
(a): only under control conditions
(b): only with drought
No letter: effect of competitor regardless of drought

Table 3. Differences between ammonium (NH_4^+), nitrate (NO_3^-), glutamine-N (Gln-N), and arginine-N (Arg-N) net uptake capacity of the fine roots of *Fagus sylvatica*, *Quercus robur*, and *Pinus sylvestris* seedlings grown in competition under drought and control conditions. Only significant differences are presented. n.s. = no significant differences between net uptake capacity of different N forms.

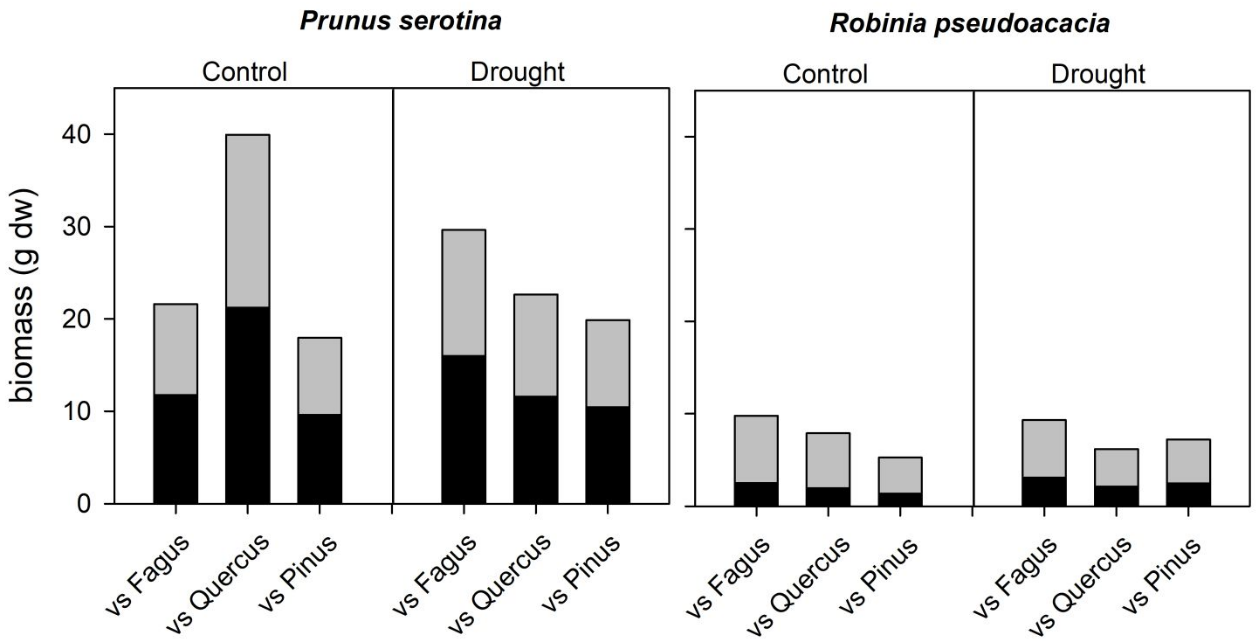
Competitor	**Water availability**	***Fagus sylvatica***	***Quercus robur***	***Pinus sylvestris***
Prunus serotina	Control	Gln-N, Arg-N > NH_4^+	Arg-N > NH_4^+, Gln-N	NO_3^-, Gln-N, Arg-N > NH_4^+ Arg > NO_3^-
	Drought	NH_4^+, Gln-N, Arg-N > NO_3^- Arg-N > NH_4^+	Arg-N > NH_4^+, NO_3^-, Gln-N Gln-N > NO_3^-	Arg-N > NH_4^+, NO_3^-, Gln-N Gln-N > NO_3^-
Robinia pseudoacacia	Control	Gln-N, Arg-N > NH_4^+, NO_3^-	Arg-N > NH_4^+, Gln-N Gln-N > NH_4^+	n.s.
	Drought	Gln-N, Arg-N > NH_4^+, NO_3^-	Arg-N > NH_4^+, NO_3^-, Gln-N Gln-N > NO_3^-	Arg-N > NO_3^-

Table 4. Differences between ammonium (NH_4^+), nitrate (NO_3^-), glutamine-N (Gln-N), and arginine-N (Arg-N) net uptake capacity of the fine roots of *Prunus serotina* and *Robinia pseudoacacia* seedlings grown in competition under drought and control conditions. Only significant differences are presented. n.s. = no significant differences between net uptake capacity of different N forms.

Competitor	**Water availability**	***Prunus serotina***	***Robinia pseudoacacia***
Fagus sylvatica	Drought	Arg-N > NH_4^+, NO_3^-, Gln-N	n.s.
	Control	Gln-N, Arg-N > NH_4^+, NO_3^-	NO_3^-, Gln-N, Arg-N > NH_4^+ Arg-N > NO_3^-
Quercus robur	Drought	Arg-N > NH_4^+, NO_3^-	Arg-N > NH_4^+, NO_3^-
	Control	NO_3^-, Arg-N > NH_4^+ Arg-N > Gln-N	n.s.
Pinus sylvestris	Drought	Arg-N > NH_4^+, NO_3^-, Gln-N	n.s.
	Control	Gln-N, Arg-N > NH_4^+	n.s.



Supplemental Figure 1a. Root (black) and shoot (gray) biomass (g dw) of *Fagus sylvatica*, *Quercus robur* and *Pinus sylvestris* seedlings at control and drought conditions under different competition regimes. vs *Prunus* = competition with *Prunus serotina*; vs *Robinia* = competition with *Robinia pseudoacacia.*



Supplemental Figure 1b. Root (black) and shoot (gray) biomass (g dw) of *Prunus serotina* and *Robinia pseudoacacia* seedlings at control and drought conditions under different competition regimes. vs *Fagus* = competition with *Fagus sylvatica*; vs *Quercus* = competition with *Quercus robur*; vs *Pinus* = competition with *Pinus sylvestris*.