

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

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3 **1 Responses of native and invasive woody seedlings to combined competition and drought**
4 **are species-specific**
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27 forests, nitrogen metabolites
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35 **Abstract**

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

58 **1. Introduction**

59 Invasion of natural habitats by exotic species is considered a major aspect of anthropogenic
60 global change (Vitousek et al. 1997, Ricciardi 2007). In forest ecosystems, successful plant
61 invasions have a negative impact on the structure and composition of plant and microbial
62 communities, as well as forest biogeochemical processes (Castro-Diez et al. 2006, Corbin and
63 D'Antonio 2012). For example, invasives can suppress the recruitment and growth of native
64 plant species, affect carbon pools and nutrient fluxes, and modify litter quality and
65 decomposition (Ehrenfeld et al. 2001, Ehrenfeld 2003, Vilà et al. 2011). As a result, forest
66 functioning is altered and the provision of ecosystem services may be impaired (Holmes et al.
67 2009, Vilà et al. 2011, Wardle and Peltzer 2017). Invasive plant species commonly display
68 functional traits and growth strategies that improve resource capture and favour reproduction

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3 69 including high seed production (Mason et al. 2008), specific leaf area (Grotkopp and Rejmánek
4 70 2007, Leishman et al. 2007), photosynthetic rates (Pattison et al. 1998, McDowell 2002),
5 71 relative growth rates (Grotkopp and Rejmánek 2007), root biomass (Broadbent et al. 2018)
6 72 and/or specific root length (Dawson 2015). These traits contribute to the successful
7 73 establishment and dispersal of invasive species in new habitats by enhancing their competitive
8 74 ability. For example, invasive *Prunus serotina* and *Robinia pseudoacacia* produce more
9 75 biomass to the detriment of slower growing of native *Quercus robur* and *Carpinus betulus* when
10 76 grown in competition (Kawaletz et al. 2013).
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19 78 A key resource in the competition between native and invasive plant species is plant-growth
20 79 limiting nitrogen (N) (Littschwager et al. 2010, Eller and Oliveira 2017). The competitive
21 80 ability of plants for acquiring N is determined by plant morphological (e.g. density and length
22 81 of root hairs) and physiological (e.g. N uptake capacity, expression and activity of transporters
23 82 in root cells) traits, as well as species-specific plasticity in these traits (Casper and Jackson
24 83 1997), but also the support of symbionts such as mycorrhiza (e.g. Näsholm et al. 2009) and/or
25 84 N₂-fixing bacteria (Bueno et al. 2019). Previous studies investigating the effects of competition
26 85 for N found plasticity in the N uptake capacity of trees with increases or decreases in response
27 86 to interspecific competition which, however, depended on the competing species,
28 87 environmental conditions, and available N sources (e.g. inorganic vs organic N) (Simon et al.
29 88 2010, Simon et al. 2014, Li et al. 2015, Bueno et al. 2019). For example, *Fagus sylvatica*
30 89 increased organic N acquisition in competition with *Acer pseudoplatanus* compared to
31 90 intraspecific competition at high but not low soil N availability (Li et al. 2015): With ambient
32 91 but not reduced light, organic N acquisition decreased in competition with *A. pseudoplatanus*
33 92 reflecting a better adaptation of *F. sylvatica* to low light conditions compared to *A.*
34 93 *pseudoplatanus* (Simon et al. 2014). The utilization of different N sources likely provides an
35 94 advantage when competing for N (McKane et al. 2002, Simon et al. 2014) with a high potential
36 95 to drive niche differentiation and species coexistence (McKane et al. 2002, Ashton et al. 2010,
37 96 Boudsocq et al. 2012). In the context of competition between native and invasive species, the
38 97 preference of different N forms in competition (i.e. one species favouring organic N, whereas
39 98 the other prefers inorganic N sources) might provide an important mechanism to effectively
40 99 avoid competition for N. To our knowledge, this theory has mostly been tested for non-woody
41 100 species (Fraterrigo et al. 2011, Huangfu et al. 2016) and only recently for tree species (but see
42 101 Bueno et al. 2019).
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3 103 The threat of invasive species to European forests might become even more severe in light of
4 104 the predicted climate changes for Central Europe. In Bueno et al. (2019), the responses of three
5 105 native and two invasive tree species (i.e. the same species as used here) to high soil N
6 106 availability were investigated due to the expected increase in atmospheric N deposition
7 107 (Rennenberg et al. 2009). In the present study, responses of native and invasive tree species to
8 108 competition for N are investigated under drought conditions. Drought periods in summer are
9 109 expected to increase in frequency and severity (Spinoni et al. 2017). Thus, understanding the
10 110 consequences of drought on the outcome of competition for N between native and invasive
11 111 woody plant species is crucial. N dynamics in both plant and soil are tightly linked to water
12 112 availability (Gessler et al. 2017): For example, drought negatively affects soil N mineralization
13 113 processes resulting in reduced plant available N in the rhizosphere (Simon et al. 2017), soil N
14 114 diffusion and mass flow (Rennenberg et al. 2006), and the activity of root proteins related to N
15 115 acquisition (Bista et al. 2018). Reduced mycorrhizal colonization might additionally lead to less
16 116 N transfer to plants (Nilsen et al. 1998). Therefore, also the competition for N between plants
17 117 is altered with drought. For example, in studies by Fotelli et al. (2001, 2002) the combination
18 118 of drought and competition with fast-growing *Rubus fruticosus* resulted in impaired inorganic
19 119 N acquisition and water status for *F. sylvatica* seedlings, as well as increased amino acid levels
20 120 due to protein degradation in the leaves to act as osmoprotectants. However, whether invasive
21 121 species have an advantage over native species in the competition for N under drought scenarios
22 122 is still unknown, despite woody species invasions becoming a major concern in forests around
23 123 the globe (Lamarque et al. 2011).

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25 125 We conducted a greenhouse experiment to study the responses of three native and two invasive
26 126 woody plant species to different competitors and drought in terms of organic and inorganic N
27 127 acquisition as well as allocation of N to N pools in the leaves and fine roots. As native species
28 128 we chose some of the most abundant and widespread species of Central European forests
29 129 (Ellenberg and Leuschner 2010, Eaton et al. 2016, Houston et al. 2016), which differ in
30 130 physiological traits and growth strategies: (i) European beech (*Fagus sylvatica* L., Fagaceae),
31 131 a drought-sensitive slow growing species (Houston et al. 2016), (ii) pedunculated oak (*Quercus*
32 132 *robur* L., Fagaceae), a drought-tolerant slow growing species (Eaton et al. 2016), and (iii) Scots
33 133 pine (*Pinus sylvestris* L., Pinaceae), a drought-tolerant fast growing conifer (Kuster et al. 2013,
34 134 Sohn et al. 2016). The two invasive tree species are originally from North America, were first
35 135 introduced in Europe in the 17th century and are now widely distributed due to their use in
36 136 reforestation programs and considered two of the most frequent and important woody invaders

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3 137 in Central European forests (Campagnaro et al. 2018): (i) black cherry (*Prunus serotina* Ehrh.,
4 138 Rosaceae), a fast growing species producing cyanogenic compounds (Csiszár 2009), and (ii)
5 139 black locust (*Robinia pseudoacacia* L., Leguminosae), a fast growing, N₂-fixing species
6 140 (Robakowski et al. 2016, Vítková et al. 2017). Both, the remobilization of N from cyanogenic
7 141 compounds and the ability to symbiotically fix N₂ allow these two invasive species to acquire
8 142 additional N independently from soil N supply, which could thus serve as alternate sources of
9 143 N. From here on, species used in this study will be referred to using their genus, i.e. *Fagus*,
10 144 *Quercus*, *Pinus*, *Prunus* and *Robinia*.

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146 Our general research aim was to evaluate whether N acquisition and allocation of N to N pools
147 of native and invasive species shift in response to drought and different competitors reflecting
148 their functional traits, i.e. growth rate (slow-growing vs. fast-growing), drought tolerance
149 (drought-tolerant vs. drought-sensitive), and/or the ability to access alternative sources of N
150 (e.g. via remobilization of N stored in cyanogenic compounds or symbiotic N₂ fixation). Our
151 specific hypotheses were: (1) Drought generally reduces inorganic and organic N acquisition
152 because N uptake from the soil strongly depends on water availability (Gessler et al. 2004,
153 Rennenberg et al. 2006). (2) Species-specific coping mechanisms (related to their functional
154 traits) lead to changes in the allocation of N to N pools in the leaves and fine roots, because
155 generally with drought less N is acquired from the soil (Gessler et al. 2017). (3) N acquisition
156 and allocation of N to N pools of a given species change depending on the species' functional
157 traits and the competitor. For example, competition between a slow and a fast grower leads to
158 a decrease in N acquisition and allocation to N pools in the slow growing species because of
159 the fast grower's higher N demand (Reich 2014). (4) Native and invasive species differ in their
160 preference for organic and inorganic N sources resulting in avoidance of competition for N
161 (Fraterrigo et al. 2011, Huangfu et al. 2016).

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163 2. Materials and Methods

164 2.1. Plant material and growth conditions

165 One-year-old seedlings of all species were purchased from Müller Münchhof Pflanzen GmbH
166 (Seesen/Münchhof, Germany) and planted in different combinations (i.e. two seedlings per
167 pot, see 2.2 "Experimental design") in a 1:1 mixture of sand and vermiculite in 3 L plastic pots
168 (25 cm x 12 cm) at the end of November 2015. Pots stayed outdoors over winter and spring and
169 were watered regularly. They were brought into the greenhouse on June 20th 2016 after leaf
170 development. For the following 7 days, all pots were watered regularly with sufficient tap water,

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3 171 and received on two occasions 100 ml of an artificial low N nutrient solution containing 100
4 172 μM KNO_3 , 90 μM $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 70 μM $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 50 μM KCl , 24 μM $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 20
5 173 μM NaCl , 10 μM AlCl_3 , 7 μM $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 6 μM K_2HPO_4 , 1 μM NH_4Cl , 25 μM glutamine,
6 174 and 25 μM arginine mimicking the soil solution of a low N field site (Dannenmann et al. 2009).
7 175 The pots were subjected to natural light conditions and day length regime (16/8, day/night). Air
8 176 temperature was 23.5 ± 2.3 °C / 21.0 ± 2.4 °C (day/night, mean \pm standard deviation), and
9 177 relative humidity was 63.1 ± 9.3 % / 71.3 ± 8.8 % (day/night, mean \pm standard deviation) for
10 178 the duration of the experiment. With increasing duration of the drought treatment, some
11 179 individuals started to show signs of wilting. To ensure sufficient replication for each species
12 180 and treatment, we did the ^{15}N uptake experiments followed by the harvest after four weeks.
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22 182 **2.2. Experimental design**

23 183 The experiment was conducted in a fully orthogonal design with two factors, “water
24 184 availability” (i.e. control or drought) and “competitor” (interspecific competition with 2 or 3
25 185 different competitors). Seedlings were planted in native-invasive interspecific competition (i.e.
26 186 one seedling of a native species and one seedling of an invasive species per pot). Pots were
27 187 established for every combination of native species and invasive species. For each species, a
28 188 total of 24 pots was setup for each combination of native-invasive, summing up to a total of
29 189 144 pots. Pots were assigned to either the control or drought treatment (i.e. $n = 12$ per
30 190 combination of species, competitor, and soil water availability). For the drought treatment,
31 191 irrigation was fully stopped starting June 27th until the final harvest from July 21st to 27th, while
32 192 for the control treatment irrigation continued with sufficient tap water supply every second day.
33 193 To confirm that water availability was significantly reduced in the respective treatment, we
34 194 measured soil water content every second day for 3 pots per combination of species, soil water
35 195 availability treatment and competitor, by inserting a probe into the soil in three different
36 196 locations in each pot (HH2 Moisture Meter, Delta-T Devices, Cambridge). Soil water content
37 197 in the drought treatment was 7.2 ± 2.4 % (mean \pm standard deviation) and significantly lower
38 198 than in the control 24.0 ± 2.5 % (mean \pm standard deviation) at the end of the experiment. $\delta^{13}\text{C}$
39 199 values in the leaves (Suppl. Table 6) were higher in the drought treatment compared to the
40 200 control at the time of harvest indicating drought stress for *Fagus*, *Quercus*, and *Robinia*
41 201 depending on the competitor, while for *Pinus* and *Prunus* no differences were found.
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58 203 **2.3. ^{15}N uptake experiments**

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3 204 To quantify inorganic (i.e. ammonium and nitrate) and organic (i.e. glutamine and arginine) net
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5 205 N uptake capacity of the seedlings' fine roots, the ^{15}N enrichment technique was used as
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7 206 described by Gessler et al. (1998) and modified by Simon et al. (2010). Seedlings were carefully
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9 207 removed from the pots and the roots washed thoroughly with tap water to remove any remaining
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11 208 substrate. Then, fine roots still attached to the seedlings were incubated in the same artificial
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13 209 low N solution as used during plant growth (see above) containing all four N sources but only
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15 210 one labelled as either $^{15}\text{NH}_4^+$, $^{15}\text{NO}_3^-$, $^{13}\text{C}/^{15}\text{N}$ -glutamine, or $^{13}\text{C}/^{15}\text{N}$ -arginine. Controls with no
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17 211 label were included to account for natural abundance in the fine roots ($n = 4$ to 6 per N source
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19 212 including controls with no label, per species, competitor, and soil water availability treatment).
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21 213 The roots not used for incubation were wrapped in wet tissue to prevent desiccation for the
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23 214 duration of the ^{15}N uptake experiment. After 2 hours of incubation, the fine roots were cut from
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25 215 the seedling and washed twice in 0.5 M CaCl_2 solution to remove the incubation solution from
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27 216 the root surface. The fresh weight of the fine roots was determined, and after oven-drying for
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29 217 48 h at $60\text{ }^\circ\text{C}$, their dry weight was determined. Amino acids were $^{13}\text{C}/^{15}\text{N}$ -labelled to determine
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31 218 whether they were taken up as intact molecules (Simon et al. 2011). Net uptake capacity of
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33 219 glutamine and arginine was lower based on ^{13}C compared to that on ^{15}N incorporation indicating
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35 220 that amino acids degraded in the solution or on the surface of the roots, and/or the respiration
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37 221 of amino acid-derived C inside the roots (Simon et al. 2011). Incubation took place between 10
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39 222 am and 2 pm to avoid diurnal variation in net N uptake capacity (Gessler et al. 2002).
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224 **2.4. Harvest and quantification of growth and biomass indices**

225 After the ^{15}N uptake experiment, seedlings were separated into leaves, stems, and roots. Their
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227 fresh weight was determined, then all tissues were oven-dried for 48 h at $60\text{ }^\circ\text{C}$ and their dry
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229 weight was determined. Before oven-drying, a subsample of 8 to 10 representative leaves was
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231 collected from each seedling and their leaf area measured (LI-3100C Area Meter, LI-COR,
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233 Lincoln, USA) to calculate specific leaf area (SLA). Likewise, a subsample of fine roots was
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235 collected from each seedling, stained, scanned and total length measured (WinRhizo 2012,
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237 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 $-80\text{ }^\circ\text{C}$ 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

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3 238 equation: $RGR = (\ln b_2 - \ln b_1) * t^{-1}$, where b_1 is total seedling biomass (g dw) at the initial
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5 239 harvest, b_2 is total seedling biomass (g dw) at the final harvest, and t is the time period in days
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7 240 between the initial and the final harvest (Grubb et al. 1996). Initial seedling biomass was
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9 241 determined on 3 to 4 pots per species, competitor, and soil water availability treatment that were
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11 242 harvested immediately before commencing the drought treatment.
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13 244 **2.5. Quantification of total N and C, ^{15}N , and ^{13}C in fine roots and $\delta^{13}\text{C}$ in leaves**

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15 245 Dried fine root samples from the ^{15}N uptake experiment were ground using a ball mill
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17 246 (TissueLyser, Retsch, Haan, Germany) to a fine homogeneous powder. Aliquots of 1.2 to 2.4
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19 247 mg were weighed into 4x6 mm tin capsules (IVA Analysentechnik, Meerbusch, Germany) for
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21 248 analyses with an isotope ratio mass spectrometer (Delta V Advantage, Thermo Electron,
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23 249 Dreieich, Germany) coupled to an elemental analyzer (Euro EA, Eurovector, Milano, Italy). Δ
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25 250 values were calculated using a laboratory standard (acetanilide) that was part of every sequence
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27 251 in intervals also used in different weights to determine isotope linearity of the system. The
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29 252 laboratory standard was calibrated against several suitable international isotope standards
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31 253 (IAEA, Vienna). Final correction of isotope values was done with several international isotope
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33 254 standards and other suitable laboratory standards which cover the range of ^{15}N and ^{13}C results.
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35 255 Inorganic and organic N net uptake capacity ($\text{nmol N g}^{-1} \text{fw h}^{-1}$) was calculated based on the
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37 256 incorporation of ^{15}N into the fine roots according to Kreuzwieser et al. (2002): Net N uptake
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39 257 capacity = $((^{15}\text{N}_l - ^{15}\text{N}_n) * N_{\text{tot}} * \text{dw} * 10^5) / (\text{MW} * \text{fw} * t)^{-1}$, where $^{15}\text{N}_l$ and $^{15}\text{N}_n$ are the atom% of ^{15}N
40
41 258 in labeled (N_l) and unlabeled control plants (N_n , natural abundance), respectively, N_{tot} is the
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43 259 total N percentage, MW is the molecular weight ($^{15}\text{N g mol}^{-1}$), and t is the incubation time.
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45 261 **2.6. Quantification of total soluble protein and total soluble amino acid levels in leaves** 46 47 262 **and fine roots**

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49 263 To extract total soluble proteins from the leaves and fine roots (Dannenmann et al. 2009), ~50
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51 264 mg aliquots of finely ground frozen samples were incubated in 1.5 ml extraction buffer (50 mM
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53 265 Tris-HCl pH 8.0, 1 mM EDTA, 15% (v/v) glycerol, 0.6 mM dithiothreitol, 1% Triton X-100, 2
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55 266 EDTA-free protease inhibitor cocktail tablets per 100 ml buffer) at 4 °C for 30 min followed
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57 267 by centrifugation for 10 min at 14,000 rpm and 4 °C. The extraction was done twice to increase
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59 268 the yield. Subsequently, 500 μL of the combined supernatant from both extractions were
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269 incubated with 1 ml 10 % (v/v) trichloroacetic acid for 10 min at room temperature and then
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271 270 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

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3 272 adding 1 ml of Bradford reagent to 50 μ L of extract. Following a 10 min incubation at room
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5 273 temperature in the dark, the absorbance was measured at 595 nm in a spectrophotometer
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7 274 (Ultrospec 3100pro, Amersham Biosciences). Bovine serum albumin (BSA) was used as
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9 275 standard.

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11 277 Total soluble amino acid-N content in the leaves and fine roots were extracted according to
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13 278 Winter et al. (1992): 200 μ L Hepes buffer (5 mM EGTA, 20 mM HEPES, 10 mM NaF) and 1
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15 279 ml 3.5:1.5 (v:v) methanol/chloroform were added to ~50 mg aliquots of finely ground frozen
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17 280 sample and incubated for 30 min on ice, followed by the addition of 600 μ L of distilled water
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19 281 and centrifugation for 5 min at 14,000 rpm and 4 °C. The addition of distilled water and
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21 282 centrifugation was performed twice to increase the yield. Total soluble amino acid-N content
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23 283 in the leaves and fine roots were quantified according to Liu et al. (2005): 50 μ L ninhydrin
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25 284 solution was added to a 50 μ L aliquot of the combined extract and boiled for 30 min. The
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27 285 ninhydrin solution was composed of an equal parts mixture of solution A (i.e. 3.84 g citric acid,
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29 286 0.134 g SnCl₂, and 40 ml 1 M NaOH, filled to 100 ml with distilled water at pH 5) and solution
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31 287 B (i.e. 4 g ninhydrin in 100 ml ethylene-glycol-monomethyl-ether). Subsequently, the extracts
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33 288 were cooled to room temperature and 1 ml 50% isopropanol was added, followed by a 15 min
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35 289 incubation. The absorption was measured at 570 nm in a spectrophotometer (Ultrospec
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37 290 3100pro, Amersham Biosciences). L-glutamine was used as standard.

36 291

37 292 **2.7. Statistical analyses**

39 293 Two-way permutational ANOVAs (PERMANOVA) based on a Euclidean resemblance matrix
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41 294 between samples (Anderson et al. 2008) were performed for each species to test for differences
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43 295 between water availability and competitor levels using as variables inorganic and organic net
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45 296 N uptake capacity, total soluble amino acid-N, and total soluble protein-N contents in the leaves
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47 297 and fine roots, as well as total biomass, root:shoot ratio, SLA, SRL, and RGR. PERMANOVAs
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49 298 were performed using “water availability” (i.e. drought and control) and “competitor” as fixed
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51 299 orthogonal factors. “Competitor” consisted of two levels for the native species (i.e. competition
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53 300 with *Prunus* or *Robinia*) or three levels for the invasive species (i.e. competition with *Fagus*,
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55 301 *Quercus*, or *Pinus*). For significant interactions between factors, *post hoc* PERMANOVA pair-
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57 302 wise comparisons were performed. To test for differences in $\delta^{13}\text{C}$ in leaves between drought
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59 303 and control for each species grown with different competitors, Mann-Whitney U-tests were
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304 performed. Finally, to test for species preferences in net N uptake capacity of the different N
305 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 $\delta^{13}\text{C}$ 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

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3 340 and SRL of native and invasive species depending on both the target species and the competitor
4 (Table 1, Suppl. Tables 7, 8, 9, Suppl. Figure 1).
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8 343 **3.2. Competitor effects on N acquisition and allocation to N pools in the leaves and fine** 9 **344 roots of native and invasive tree species**

10 345 For native species, N acquisition did not change with different invasive competitors, but there
11 were different responses regarding allocation to N pools (Fig. 1). In competition with *Prunus*
12 346 compared to *Robinia* and regardless of soil water availability, *Quercus* had lower total soluble
13 347 protein-N content but higher total soluble amino acid-N content in the fine roots, whereas *Pinus*
14 348 had lower total soluble amino acid-N content and higher total soluble protein-N content in the
15 349 leaves, and higher total soluble amino acid-N content in the fine roots (Table 2, Suppl. Tables
16 350 2, 3,4). For *Fagus*, N allocation to N pools did not vary with competitor (Table 2, Suppl. Tables
17 351 2, 3, 4). All native species responded differently to the invasive competitors regarding their
18 352 biomass and growth indices (Table 2, Suppl. Tables 7, 8, 9).
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21 355 Similarly, responses of the invasive species depended on competitor and were partly also
22 356 mediated by soil water availability. Under drought, *Prunus* seedlings had lower total soluble
23 357 amino acid-N contents in the fine roots when competing with *Quercus* compared to *Pinus*
24 358 (Table 2, Suppl. Table 2, 3, 4). With sufficient soil water supply, total soluble amino acid-N
25 359 content in the fine roots of *Prunus* was lower competing with *Fagus* than with *Quercus* or *Pinus*
26 360 (Table 2, Suppl. Table 2, 3, 4). *Prunus* seedlings had a lower total soluble amino acid-N content
27 361 in the leaves when competing with *Fagus* than with *Quercus*, and a lower total soluble protein-
28 362 N content in the leaves when competing with *Fagus* or *Quercus* than with *Pinus* (Table 2, Suppl.
29 363 Tables 2, 4, 10). *Robinia* seedlings responded to competitor with changes in other parameters
30 364 than *Prunus*. With drought, *Robinia* seedlings had higher total soluble protein-N content in the
31 365 fine roots when competing with *Fagus* than with *Quercus*, and higher total soluble protein-N
32 366 content in the fine roots competing with *Quercus* than with *Pinus* (Table 2, Suppl. Tables 2, 3,
33 367 4). Under control conditions, *Robinia* competing with *Fagus* had a lower nitrate net uptake
34 368 capacity than when competing with *Pinus* (Fig. 2, Table 2). Both *Prunus* and *Robinia* responded
35 369 differently with regard to their biomass and growth indices (Table 2, Suppl. Tables 7, 8, 9, 11).
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37 371 **3.3. N acquisition preferences for different N sources of native and invasive species**

38 372 Preferences for certain N sources were found among species depending on the competitor and
39 373 soil water availability. Generally, organic N, especially arginine-N, was favoured over

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3 374 inorganic N sources. *Quercus* and *Prunus* did not change N preferences with different
4 375 competitors or drought (Tables 3, 4). *Fagus* preferred organic N over inorganic N with drought
5 376 (Table 3). *Pinus* did not prefer specific N sources in competition with *Robinia* with sufficient
6 377 water supply, but preferred organic N over inorganic N with drought and in competition with
7 378 *Prunus* regardless of water availability (Table 3). *Robinia* showed a distinct pattern depending
8 379 on the competitor: With drought, seedlings preferred arginine-N over inorganic N but only in
9 380 competition with *Quercus*, but not in competition with *Fagus* or *Pinus* (Table 4). With sufficient
10 381 water supply, *Robinia* preferred organic over inorganic N when grown in competition with
11 382 *Fagus*, but not with *Quercus* or *Pinus* (Table 4).
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20 384 **4. Discussion**

21 385 **4.1. Drought reduces N acquisition among species, but allocation of N to N pools varies** 22 386 **with species and competitor**

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24 387 In accordance with our hypothesis, inorganic and organic N acquisition of both native and
25 388 invasive tree seedlings overall decreased in response to drought, confirming that tree N
26 389 acquisition and soil water availability are strongly linked (e.g. Fotelli et al. 2002, 2004,
27 390 Rennenberg et al. 2006, Gessler et al. 2017). The influence on organic N acquisition for tree
28 391 species in competition is a novel insight provided by our work, while also confirming previous
29 392 studies in which drought reduced the inorganic N acquisition of *F. sylvatica* seedlings growing
30 393 both in intra- and interspecific competition (Fotelli et al. 2002). In plant communities, when
31 394 soil water availability is reduced, microbial activity is decreased negatively impacting soil N
32 395 mineralization processes, thus resulting in reduced soil N availability (Schimel et al. 2007,
33 396 Hueso et al. 2012). Furthermore, soil N diffusion and mass flow are reduced (Rennenberg et al.
34 397 2009) and the composition of mycorrhizal communities that symbiotically provide plants with
35 398 N is changed (e.g. Gessler et al. 2005, Leberecht et al. 2016).
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48 400 Our studied tree species appear to show four different responses to drought regarding the
49 401 allocation of N to N pools in the leaves and fine roots depending on the species and competitor:
50 402 (1) Total soluble protein levels were reduced (in the leaves of *Fagus* and *Quercus* regardless of
51 403 competitor, and in the fine roots of *Prunus* and *Robinia* competing with *Pinus*) as a consequence
52 404 of reduced N acquisition and thus, N assimilation (Gessler et al. 2017). Storage proteins from
53 405 vegetative tissue are degraded and remobilized leading to lower N in storage (e.g. Millard 1988,
54 406 Staswick 1994, Millard and Grelet 2010). With potential leaf shedding as a consequence of
55 407 drought and, consequently, N stored in the leaves being lost by the plant, the roots become an

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3 408 important tissue for N storage (Millard and Grelet 2010). In our study, the leaves were not (yet)
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5 409 shed at the time of the harvest, suggesting that N was likely still remobilized. (2) In the fine
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7 410 roots of *Robinia* in competition with *Fagus*, the levels of total soluble proteins increased in
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9 411 response to drought likely due to the synthesis of protective proteins (Brunner et al. 2015),
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11 412 proteins with a role in dehydration tolerance (Close 1996, Kozłowski and Pallardy 2002), and/or
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13 413 proteins required for the development of root nodules for N₂-fixing species (Verma et al. 1992)
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15 414 such as *Robinia*. (3) Soluble amino acid levels decreased in the fine roots of *Prunus* when grown
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17 415 in competition with *Quercus* or *Pinus* in response to drought. This is likely due to amino acid
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19 416 degradation, their translocation to other plant tissues, their usage for protein synthesis, and/or
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21 417 the usage of their carbon skeletons to produce alternative non-N-containing osmotic compounds
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23 418 which not only decrease osmotic potential but can also function as cell membrane and metabolic
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25 419 protectants (Chaves et al. 2003). (4) In contrast, total soluble amino acid levels increased in the
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27 420 leaves of *Robinia* and the fine roots of *Pinus* regardless of competitor, as well as in the leaves
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29 421 and fine roots of *Quercus* in competition with *Prunus* via protein degradation to serve as
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31 422 osmoprotectants, thus improving the overall plant water status (Hu et al. 2013, 2013b). Overall,
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33 423 our results indicate that the study species show different responses to drought conditions in this
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35 424 short-term experiment with regards to N allocation to N pools in leaves and fine roots in
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37 425 combination with changes in biomass allocation.

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41 427 Some species showed changes in biomass allocation in response to drought while others did
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43 428 not. A higher root:shoot ratio resulting in a larger soil volume to be exploited and a parallel
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45 429 decrease in aboveground biomass as well as SLA reduces water loss via the leaves further
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47 430 improving a plant's water status (Fotelli et al. 2005, Mantovani et al. 2014, Duan et al. 2018).
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49 431 This strategy was found in our study for *Robinia*. On the other hand, drought sensitivity of a
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51 432 species might be reflected by reduced root growth, eventually leading to a decrease in root:shoot
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53 433 ratio (Ostonen et al. 2007, Brunner et al. 2015), root hydraulic failure (Mao et al. 2018), and
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55 434 higher root mortality (Zhou et al. 2018). In our study, drought conditions were severe enough
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57 435 to cause a decrease in root:shoot ratio for *Fagus* indicating the drought-sensitivity of this
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59 436 species, but not the other native or the invasive species.

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63 438 In general, all study species were negatively affected by drought with no clear distinction
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65 439 between native and invasive species. Native *Fagus* and – to a lesser extent – invasive *Robinia*
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67 440 were most sensitive showing several changes in above- and belowground traits. For *Fagus*, the
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69 441 strong response to drought generally prevailed over the effects of the competitor, while for

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3 442 *Robinia* it was mediated by the competitor. The ability to fix N₂ allows *Robinia* to obtain
4 443 external N and thus be less affected by the negative effect of drought on N acquisition from the
5 444 soil (Wurzburger and Miniat 2014, Mantovani et al. 2014, 2015). For *Quercus* and invasive
6 445 *Prunus*, responses to drought also varied according to competitor. In contrast, we found no
7 446 interaction between drought and competitor for *Pinus* which indicates that the two invasive
8 447 species did not influence its response to drought. Furthermore, coniferous *Pinus* was generally
9 448 less responsive to drought than the native and invasive deciduous species most likely due to its
10 449 isohydric behaviour, i.e. the closing of stomata early during a drought event, thereby
11 450 minimizing water losses via the needles (Irvine et al. 1998). These results imply that under
12 451 future scenarios of global change, *Pinus* can withstand simultaneous short-term drought stress
13 452 and an invasion by exotic woody species better than *Fagus* and *Quercus*.
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24 454 **4.2. Species-specific responses of native and invasive species in response to competitor** 25 455 **and soil water availability**

26 456 Our hypothesis that N acquisition from the soil and its allocation to plant internal N pools vary
27 457 for a given species depending on its functional traits and the competing species could partly be
28 458 confirmed. None of the native species and only one of the two invasives responded to different
29 459 competitors with changes in N acquisition, thus contrasting previous studies (Simon et al. 2010,
30 460 2014, Bueno et al. 2019). This suggests that soil water availability was a stronger driver of N
31 461 uptake than competition. The allocation of N to different N pools of our studied species
32 462 depended on the competitor and varied with soil water availability, thus confirming our
33 463 hypothesis that responses to different competitors are species-specific according to the
34 464 physiological characteristics of the study species.
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44 466 Both invasive species in our study, show functional traits commonly linked to fast growth
45 467 (Grotkopp and Reimánek 2007), such as higher biomass (*Prunus*) or higher SLA, and in turn,
46 468 RGR (*Robinia*), higher N allocation to N pools in leaves and fine roots thus enhancing resource
47 469 acquisition above- and belowground, and overall increasing their competitive advantage under
48 470 high resource availability (e.g. Li et al. 2015). A negative influence of *Robinia* on the growth of
49 471 competing tree seedlings has been reported before via the depletion of soil resources due to
50 472 *Robinia*'s fast growth and occupation of rooting space (Kawaletz et al. 2013, 2014). In our
51 473 study *Robinia* was the only species with increased root:shoot ratio under drought suggesting a
52 474 strong potential for below-ground competition and resource gain for metabolic processes,
53 475 which apparently had a stronger effect on drought-sensitive *Fagus* than the drought-tolerant

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3 476 natives. N acquisition of all three native species did not differ with competitor, and the higher
4 477 allocation of N to N pools combined with slower growth of *Quercus* and *Pinus* when competing
5 478 with *Prunus* than *Robinia* suggests an increased storage of N metabolites rather than a use for
6 479 biomass production (Reich et al. 1997, Millet et al. 2005, Millard and Grelet 2010). Moreover,
7 480 the negative effects on growth and biomass indices when competing with *Prunus* rather than
8 481 *Robinia* further indicate negative consequences of competition with *Prunus* for overall plant
9 482 development.

10 483
11 484 Similar to the native species, the competitor had no effect on N acquisition of *Prunus* indicating
12 485 that the observed changes in biomass and growth indices as well as N metabolites content
13 486 occurred in relation to internal N dynamics in the seedlings. This is likely due to remobilization
14 487 and *de novo* synthesis of N metabolites (Simon et al. 2010, Li et al. 2015), and/or the reliance
15 488 on N stored in plant tissues as N-based defense compounds (Gleadow and Woodrow 2002).
16 489 The other responses of invasive *Prunus* to native species depended on soil water availability.
17 490 For example, drought-sensitive *Fagus* was a stronger competitor for *Prunus* only with sufficient
18 491 soil water availability whereas this effect was absent with drought, reflecting the drought-
19 492 sensitivity of *Fagus*. With drought, N pools of *Prunus* were higher in competition with *Pinus*
20 493 compared to deciduous natives. This indicates a similar mechanism of metabolic adaptation to
21 494 stress as in the slow growing deciduous *Fagus* and *Quercus* (Millard and Grelet 2010). This
22 495 might be a differential response of *Prunus* to specific competitors with drought conditions,
23 496 possibly related to the drought tolerance of such competitors. This would be in accordance with
24 497 studies indicating that evergreen conifers are on average more drought-tolerant due to their
25 498 more conservative resource use than broadleaved deciduous temperate woody species (Hallik
26 499 et al. 2009), though further studies considering a greater number of species are needed to
27 500 provide additional insights into this.

28 501
29 502 *Robinia* responded to competition with native deciduous *Fagus* and *Quercus* stronger than with
30 503 coniferous *Pinus*, although the affected specific growth and physiological parameters depended
31 504 on soil water availability. In contrast to all other study species, nitrate acquisition of *Robinia*
32 505 was reduced in its competition with *Fagus* compared to competition with *Pinus* suggesting the
33 506 release of active compounds that potentially impair N acquisition as suggested for *Acer*
34 507 *pseudoplatanus* in a previous study (Simon et al. 2010). This would negatively affect nutrition
35 508 and development of *Robinia* seedlings compared to competition with other natives because
36 509 although *Robinia* can fix N₂, N acquisition from the soil is preferred over N₂ fixation when soil

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3 510 N is not limiting (Pfautsch et al. 2009). These differences in the responses to competitors became
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5 511 more apparent when *Robinia* was additionally affected by drought and may be explained by *de*
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7 512 *novo* synthesis of proteins as an adaptation to competition (Simon et al. 2010, 2014).
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9 513 Additionally, *Robinia* seedlings grew slower in competition with *Fagus* or *Quercus* compared
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11 514 to *Pinus* regardless of soil water availability further highlighting their negative competitive
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13 515 effects on *Robinia*.

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15 517 Overall, native and invasive species responded to different competitors in accordance with their
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17 518 physiological and life history traits such as drought tolerance and leaf habit. For example,
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19 519 drought-sensitive *Fagus* responded negatively to different competitors and affected invasive
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21 520 species only with sufficient water supply, indicating that drought effects override those of the
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23 521 two invasive competitors. Moreover, invasive *Prunus* was generally a stronger competitor for
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25 522 the native species. With competition, tissue nutrient content plays an important role in plant
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27 523 performance. For example, seedlings of black spruce (*Picea mariana*) with high levels of N
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29 524 (and other nutrients) had higher biomass than seedlings with low tissue N content after growing
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31 525 in competition with natural vegetation (Malik and Timmer 1998) indicating the importance of
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33 526 plant internal N reserves in competitive interactions.

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35 528 **4.3. Organic N was generally preferred by all species regardless of drought**
36 529 We hypothesized that native and invasive species differ in their preference for organic and
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38 530 inorganic N sources. However, organic N forms, especially arginine, were generally preferred
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40 531 over inorganic N by all study species confirming results from studies in the field and under
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42 532 controlled settings (Simon et al. 2017) using the same technique and artificial soil solution as
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44 533 here (e.g. for *F. sylvatica*: Dannenmann et al. 2009, Simon et al. 2010, Simon et al. 2011, Simon
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46 534 et al. 2014, for *R. pseudoacacia*: Hu et al. 2017), as well as the results found at low soil N
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48 535 availability in our previous study using the same species (Bueno et al. 2019). The preferred
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50 536 acquisition of specific N sources is generally driven by their higher abundance in the soil (e.g.
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52 537 Kuster et al. 2013b, Song et al. 2015, Simon et al. 2017). For native *Fagus* and *Pinus*, as well
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54 538 as invasive *Robinia* preferences shifted depending on the competitor (see Tables 3, 4) indicating
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56 539 plasticity in resource use induced by the interaction with other species which might influence
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58 540 the plant's competitive ability (Ashton et al. 2010). Such shifts were not found for native
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60 541 *Quercus* and invasive *Prunus* indicating that competition does not affect their N form
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543 542 preference. Furthermore, to our knowledge, no previous studies have directly measured the
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544 543 effect of drought on the preference for different N forms. We found no changes in the N form

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3 544 preference with drought suggesting that changes in N preference for the study species does not
4 545 play a role for adapting to drought, at least under these experimental conditions.
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8 547 **Conclusions**

9
10 548 In our study, drought generally reduced inorganic and also – shown for the first time – organic
11 549 N acquisition among both native and invasive study species, because reduced water availability
12 550 leads to less N available in the soil for plants. Overall, N acquisition was driven by water supply
13 551 rather than competition. The allocation of N to internal N pools in the leaves and fine roots,
14 552 however, varied with study species and competitor in relation to their physiological
15 553 characteristics showing their respective species-specific coping mechanisms (e.g. the
16 554 remobilization of N from storage and/or synthesis of osmoprotectants). For example, within the
17 555 native tree species, for drought-sensitive *Fagus*, reduced water availability overrode the
18 556 influence of competition, whereas the responses of drought-tolerant *Pinus* were entirely
19 557 independent of water supply. Deciduous and coniferous species might respond differently to
20 558 competition with other plants because of their differences in, for example, tissue concentrations
21 559 of nutrients (Calder et al. 2011, Wang et al. 2016). Organic N was generally favoured by both
22 560 native and invasive species regardless of drought suggesting that competition for N was not
23 561 avoided. Moreover, our results suggest that *Prunus* as an invasive species is a stronger
24 562 competitor than *Robinia* for most native tree species (which is in accordance with a previous
25 563 study (Bueno et al. 2019)), possibly due to its higher biomass. In contrast, for *Robinia*, the
26 564 deciduous natives *Fagus* and *Quercus* had more negative effects than the conifer *Pinus*. In
27 565 conclusion, our results suggest that species-specific mechanisms to cope with drought related
28 566 to their physiological characteristics might play a role for the competitive ability of the studied
29 567 species. However, further studies investigating a larger number of species are necessary to
30 568 confirm this. Because water availability strongly affects plant and soil N dynamics (e.g. Gessler
31 569 et al. 2017, Simon et al. 2017), longer term competition (> 1 year) might further pronounce the
32 570 effects of competition seen in our study.
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51 572 **Data and Materials Accessibility**

52 573 Data will be available from the Dryad Digital Repository upon acceptance of the manuscript.
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56 575 **Supplementary Data**

57 576 Supplementary Data is available online.
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3 578 **Conflict of Interest**

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5 579 The authors declare no conflict of interest.

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9
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26 595

27 596 **Author's contributions**

28 597 AB and JS conceived and designed the study. AB conducted the ¹⁵N uptake experiments, N
29 598 metabolite analyses, and evaluated all data. KP contributed the IRMS analyses. AB and JS led
30 599 the writing of the manuscript. All authors contributed critically to the drafts and gave final
31 600 approval for publication.

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34 602 **References**

- 35
36 603 Anderson M, Gorley R, Clarke K (2008) PERMANOVA+ for PRIMER: Guide to Software and
37 604 Statistical Methods. Plymouth: PRIMER-E.
- 38 605 Ashton IW, Miller AE, Bowman WD, Suding KN (2010) Niche complementarity due to
39 606 plasticity in resource use: plant partitioning of chemical N forms. *Ecology* 91: 3252-3260.
40 607 doi: 10.1890/09-1849.1
- 41 608 Bista DR, Heckathorn SA, Jayawardena DM, Mishra S, Boldt JK (2018) Effects of drought on
42 609 nutrient uptake and the levels of nutrient-uptake proteins in roots of drought-sensitive and
43 610 tolerant grasses. *Plants* 7: 28. doi: 10.3390/plants7020028

- 1
2
3 611 Boudsocq S, Niboyet A, Lata JC, Raynaud X, Loeuille N, Mathieu J, Blouin M, Abbadie L,
4 612 Barot S (2012) Plant preference for ammonium versus nitrate: a neglected determinant of
5 613 ecosystem functioning? *The American Naturalist* 180: 60-69. doi: 10.1086/665997
6
7
8 614 Broadbent A, Stevens CJ, Peltzer DA, Ostle NJ, Orwin KH (2018) Belowground competition
9 615 drives invasive plant impact on native species regardless of nitrogen availability. *Oecologia*
10 616 186:577-587. doi: 10.1007/s00442-017-4039-5
11
12
13 617 Brunner I, Herzog C, Dawes MA, Arend M, Sperisen C (2015) How tree roots respond to
14 618 drought. *Front. Plant Sci.* 6:547. doi: 10.3389/fpls.2015.00547
15
16
17 619 Bueno A, Pritsch K, Simon J (2019) Species-specific outcome in the competition for nitrogen
18 620 between invasive and native tree seedlings. *Frontiers in Plant Science* 10:337. doi:
19 621 10.3389/fpls.2019.00337
20
21
22 622 Calder W, Horn K, St. Clair S (2011) Conifer expansion reduces the competitive ability and
23 623 herbivore defense of aspen by modifying light environment and soil chemistry. *Tree*
24 624 *Physiology* 31:582-591.
25
26
27 625 Campagnaro T, Brundub G, Sitzia T (2018) Five major invasive alien tree species in European
28 626 Union forest habitat types of the Alpine and Continental biogeographical regions. *Journal*
29 627 *for Nature Conservation* 43: 227-238. doi: 10.1016/j.jnc.2017.07.007
30
31
32 628 Casper BB, Jackson RB (1997) Plant competition underground. *Annual Review of Ecology,*
33 629 *Evolution, and Systematics* 28: 545-570. doi: 10.1146/annurev.ecolsys.28.1.545
34
35
36 630 Castro-Díez P, Pauchard A, Traveset A, Vilà M (2006) Linking the impacts of plant invasion
37 631 on community functional structure and ecosystem properties. *Journal of Vegetation Science*
38 632 27: 1233-1242. doi: 10.1111/jvs.12429
39
40
41 633 Chaves MM, Maroco JP, Pereira JS (2003) Understanding plant responses to drought – from
42 634 genes to the whole plant. *Functional Plant Biology* 30: 239-264. doi: 10.1071/FP02076
43
44
45 635 Close TJ (1996) Dehydrins: Emergence of a biochemical role of a family of plant dehydration
46 636 proteins. *Physiologia Plantarum* 97: 795-803.
47
48
49 637 Corbin JD, D'Antonio CM (2012) Gone but not forgotten? Invasive plants' legacies on
50 638 community and ecosystem properties. *Invasive Plant Science and Management* 5:117-124.
51 639 doi: 10.1614/IPSM-D-11-00005.1
52
53
54 640 Csiszár A (2009) Allelopathic effects of invasive woody plant species in Hungary. *Acta*
55 641 *Silvatica et Lignaria Hungarica* 5: 9-17.
56
57
58 642 Dannenmann M, Simon J, Gasche R, Holst J, Naumann PS, Koegel-Knabner I et al. (2009)
59 643 Tree girdling provides insight on the role of labile carbon in nitrogen partitioning between
60

- 1
2
3 644 soil microorganisms and adult European beech. *Soil Biology and Biochemistry* 41: 1622-
4 1631. doi: 10.1016/j.soilbio.2009.04.024
5 645
6 646 Dawson W (2015) Release from belowground enemies and shifts in root traits as interrelated
7 drivers of alien plant invasion success: a hypothesis. *Ecology and Evolution* 5: 4505-4516.
8 647 doi: 10.1002/ece3.1725
9 648
10 649 Duan H, Huang G, Zhou S, Tissue D (2018) Dry mass production, allocation patterns and water
11 use efficiency of two conifers with different water use strategies under elevated [CO₂],
12 650 warming and drought conditions. *European Journal of Forest Research* 137: 605-618. doi:
13 651 10.1007/s10342-018-1128-x
14 652
15 653 Eaton E, Caudullo G, Oliveira S, de Rigo D (2016) “*Quercus robur* and *Quercus petraea* in
16 Europe: distribution, habitat, usage and threats”, in *European Atlas of Forest Tree Species*,
17 654 ed. San-Miguel-Ayanz J, de Rigo D, Caudullo G, Houston Durrant T, Mauri A
18 655 (Luxembourg: Publ. Off. EU), e01c6df+
19 656
20 657 Ehrenfeld JG (2003) Effects of exotic plant invasions on soil nutrient cycling processes.
21 *Ecosystems* 6: 503-523.
22 658
23 659 Ehrenfeld JG, Kourtev P, Huang W (2001) Changes in soil functions following invasions of
24 exotic understory plants in deciduous forests. *Ecological Applications* 11: 1287-1300. doi:
25 660 10.2307/3060920
26 661
27 662 Ellenberg H, Leuschner C (2010) *Vegetation Mitteleuropas mit den Alpen*, Vol 6. Ulmer,
28 Stuttgart, Germany.
29 663
30 664 Eller CB, Oliveira RS (2017) Effects of nitrogen availability on the competitive interactions
31 between an invasive and a native grass from Brazilian cerrado. *Plant and Soil* 410: 63-72.
32 665 doi: 10.1007/s11104-016-2984-0
33 666
34 667 Fotelli MN, Gessler A, Peuke AD, Rennenberg H (2001) Drought affects the competitive
35 interactions between *Fagus sylvatica* seedlings and an early successional species, *Rubus*
36 668 *fruticosus*: responses of growth, water status and $\delta^{13}\text{C}$ composition. *New Phytologist* 151:
37 669 427-435. doi: 10.1046/j.1469-8137.2001.00186.x
38 670
39 671 Fotelli MN, Rennenberg H, Gessler A (2002) Effects of drought on the competitive interference
40 of an early successional species (*Rubus fruticosus*) on *Fagus sylvatica* L. seedlings: ¹⁵N
41 672 uptake and partitioning, responses of amino acids and other N compounds. *Plant Biology* 4:
42 673 311-320. doi: 10.1055/s-2002-32334
43 674
44 675 Fotelli MN, Rienks M, Rennenberg H, Gessler A (2004) Climate and forest management affect
45 ¹⁵N-uptake, N balance and biomass of European beech seedlings. *Trees* 18: 157-166. doi:
46 676 10.1007/s00468-003-0289-4
47 677

- 1
2
3 678 Fotelli MN, Rudolph P, Rennenberg H, Gessler A (2005) Irradiance and temperature affect the
4 competitive interference of blackberry on the physiology of European beech seedlings. *New*
5 679 *Phytologist* 165: 453-462. doi: 10.1111/j.1469-8137.2004.01255.x
6
7 680
8
9 681 Fraterrigo JM, Strickland MS, Keiser AD, Bradford MA (2011) Nitrogen uptake and preference
10 682 in a forest understory following invasion by an exotic grass. *Oecologia* 167: 781-791.
11
12 683 Gessler A, Schneider S, von Sengbusch D, Weber P, Hanemann U, Huber C, Rothe A, Kreuzer
13 684 K, Rennenberg H (1998) Field and laboratory experiments on net uptake of nitrate and
14 685 ammonium by the roots of spruce (*Picea abies*) and beech (*Fagus sylvatica*) trees. *New*
15 686 *Phytologist* 138: 275-285.
16
17
18 687 Gessler A, Kreuzwieser J, Dopatka T, Rennenberg H (2002) Diurnal courses of ammonium net
19 688 uptake by the roots of adult beech (*Fagus sylvatica*) and spruce (*Picea abies*) trees. *Plant*
20 689 *and Soil* 240: 23-32. doi: 10.1023/A:1015831304911
21
22
23 690 Gessler A, Keitel C, Nahm M, Rennenberg H (2004) Water shortage affects the water and
24 691 nitrogen balance in Central European beech forests. *Plant Biology* 6: 289-298. doi:
25 692 10.1055/s-2004-820878
26
27
28 693 Gessler A, Jung K, Gasche R, Papen H, Heidenfelder A, Börner E, Metzler B, Augustin S,
29 694 Hildebrand E, Rennenberg H (2005) Climate and forest management influence nitrogen
30 695 balance of European beech forests: microbial N transformations and inorganic N net uptake
31 696 capacity of mycorrhizal roots. *European Journal of Forest Research* 124: 95-111. doi:
32 697 10.1007/s10342-005-0055-9
33
34
35 698 Gessler A, Schaub M, McDowell NG (2017) The role of nutrients in drought-induced tree
36 699 mortality and recovery. *New Phytologist* 214: 513-520. doi: 10.1111/nph.14340
37
38
39 700 Gleadow RM, Woodrow IE (2002) Constraints on effectiveness of cyanogenic glycosides in
40 701 herbivore defense. *Journal of Chemical Ecology* 28: 1297-1309.
41
42
43 702 Grotkopp E, Rejmánek M (2007) High seedling relative growth rate and specific leaf area are
44 703 traits of invasive species: phylogenetically independent contrasts of woody angiosperms.
45 704 *American Journal of Botany* 94: 526-532. doi: 10.3732/ajb.94.4.526
46
47
48 705 Grubb PJ, Lee WG, Kollmann J, Wilson JB (1996) Interaction of irradiance and soil nutrient
49 706 supply on growth of seedlings of ten European tall-shrub species and *Fagus sylvatica*.
50 707 *Journal of Ecology* 84: 827-840. doi:10.2307/2960555
51
52
53 708 Hallik L, Niinemets Ü, Wright IJ (2009) Are species shade and drought tolerance reflected in
54 709 leaf-level structural and functional differentiation in Northern Hemisphere temperate woody
55 710 flora? *New Phytologist* 184: 257-274. doi: 10.1111/j.1469-8137.2009.02918.x
56
57
58
59
60

- 1
2
3 711 Holmes TP, Aukema JE, Von Holle B, Liebhold A, Sills E (2009) Economic impacts of
4 712 invasive species in forests - past, present, and future. *Annals of the New York Academy of*
5 713 *Sciences* 1162: 18-38. doi: 10.1111/j.1749-6632.2009.04446.x
6
7
8 714 Houston T, de Rigo D, Caudullo G (2016) “*Fagus sylvatica* and other beeches in Europe:
9 715 distribution, habitat, usage and threats”, in *European Atlas of Forest Tree Species*, ed. San-
10 716 Miguel-Ayanz J, de Rigo D, Caudullo G, Houston T, Mauri A (Luxembourg: Publ. Off. EU),
11 717 e012b90+
12
13 718 Hu B, Simon J, Rennenberg H (2013) Drought and air warming affect the species-specific levels
14 719 of stress-related foliar metabolites of three oak species on acidic and calcareous soil. *Tree*
15 720 *Physiology* 33 (5), 489-504, doi: 10.1093/treephys/tpt025
16
17 721 Hu B, Simon J, Kuster TM, Arend M, Siegwolf R, Rennenberg H (2013b) Nitrogen partitioning
18 722 in oak leaves depends on species, provenance, climate conditions, and soil type. *Plant*
19 723 *Biology* 15 (Suppl. 1): 198-209. doi: 10.1111/j.1438-8677.2012.00658.x.
20
21 724 Hu B, Zhou M, Dannenmann M, Saiz G, Simon J, Bilela S, Liu X, Hou L, Chen H, Zhang S,
22 725 Butterbach-Bahl K, Rennenberg H (2017) Comparison of nitrogen nutrition and soil carbon
23 726 status of afforested stands established in degraded soil of the Loess Plateau, China. *Forest*
24 727 *Ecology and Management* 389: 46-58.
25
26 728 Huangfu C, Li H, Chen X, Liu H, Wang H, Yang D (2016) Response of an invasive plant,
27 729 *Flaveria bidentis*, to nitrogen addition: a test of form-preference uptake. *Biological*
28 730 *Invasions* 18: 3365-3380.
29
30 731 Hueso S, García C, Hernández T (2012) Severe drought conditions modify the microbial
31 732 community structure, size and activity in amended and unamended soils. *Soil Biology and*
32 733 *Biochemistry* 50: 167e173.
33
34 734 Irvine J, Perks MP, Magnani F, Grace J (1998) The response of *Pinus sylvestris* to drought:
35 735 stomatal control of transpiration and hydraulic conductance. *Tree Physiology* 18: 393-402.
36
37 736 Kawaletz H, Mölder I, Zerbe S, Annighöfer P, Terwei A, Ammer C (2013) Exotic tree seedlings
38 737 are much more competitive than natives but show underyielding when growing together.
39 738 *Plant Ecology* 6: 305-315. doi: 10.1093/jpe/rts044
40
41 739 Kawaletz H, Mölder I, Annighöfer P, Terwei A, Zerbe S, Ammer C (2014) Back to the roots:
42 740 how do seedlings of native tree species react to the competition by exotic species? *Annals*
43 741 *of Forest Science* 71: 337-347. doi: 10.1007/s13595-013-0347-z
44
45 742 (2002) Acclimation and adaptive responses of woody plants to environmental stresses. *The*
46 743 *Botanical Review* 68: 270-334.
47
48
49
50
51
52
53
54
55
56
57
58
59
60

- 1
2
3 744 Kreuzwieser J, Furniss S, Rennenberg H (2002) Impact of waterlogging on the N-metabolism
4 of flood tolerant and non-tolerant tree species. *Plant, Cell and Environment* 25: 1039-1049.
5 745
6 746 Kuster TM, Arend M, Günthardt-Goerg MS, Schulin R (2013) Root growth of different oak
7 provenances in two soils under drought stress and air warming conditions. *Plant and Soil*
8 747 369: 61-71. doi: 10.1007/s11104-012-1541-8
9 748
10 749 Kuster TM, Schleppi P, Hu B, Schulin R, Günthardt-Goerg MS (2013b) Nitrogen dynamics in
11 oak model ecosystems subjected to air warming and drought on two different soils. *Plant*
12 750 *Biology* 15 (Suppl. 1): 220-229. doi:10.1111/j.1438-8677.2012.00686.x
13 751
14 752 Lamarque LJ, Delzon S, Lortie CJ (2011) Tree invasions: a comparative test of the dominant
15 753 hypotheses and functional traits. *Biological Invasions* 13:1969-1989. doi: 10.1007/s10530-
16 754 011-0015-x
17 755
18 756 Leberecht M, Tu J, Polle A (2016) Acid and calcareous soils affect nitrogen nutrition and
19 757 organic nitrogen uptake by beech seedlings (*Fagus sylvatica* L.) under drought, and their
20 758 ectomycorrhizal community structure. *Plant and Soil* 409:143-157. doi: 10.1007/s11104-
21 759 016-2956-4
22 760
23 761 Leishman MR, Haslehurst T, Ares A, Baruch Z (2007) Leaf trait relationships of native and
24 762 invasive plants: community- and global-scale comparisons. *New Phytologist* 176: 635-643.
25 763
26 764 Li X, Rennenberg H, Simon J (2015) Competition for nitrogen between *Fagus sylvatica* and
27 765 *Acer pseudoplatanus* seedlings depends on soil nitrogen availability. *Frontiers in Plant*
28 766 *Science* 6: 302. doi: 10.3389/fpls.2015.00302
29 767
30 768 Littschwager J, Lauerer M, Blagodatskaya E, Kuzyakov Y (2010) Nitrogen uptake and
31 769 utilisation as a competition factor between invasive *Duchesnea indica* and native *Fragaria*
32 770 *vesca*. *Plant and Soil* 331: 105-114. doi: 10.1007/s11104-009-0236-2
33 771
34 772 Liu Y, van Kleunen M (2017) Responses of common and rare aliens and natives to nutrient
35 773 availability and fluctuations. *Journal of Ecology* 105: 1111-1122. doi: 10.1111/1365-
36 774 2745.12733
37 775
38 776 Liu XP, Grams T, Matyssek R, Rennenberg H (2005) Effects of elevated pCO₂ and/or pO₃ on
39 777 C-, N-, and S-metabolites in the leaves of juvenile beech and spruce differ between trees
40 778 grown in monoculture and mixed culture. *Plant Physiology and Biochemistry* 43: 147-154.
41 779
42 780 Malik V, Timmer VR (1998) Biomass partitioning and nitrogen retranslocation in black spruce
43 781 seedlings on competitive mixedwood sites: a bioassay study. *Canadian Journal of Forest*
44 782 *Research* 28: 206-215. doi: 10.1139/x97-207
45 783
46 784
47 785
48 786
49 787
50 788
51 789
52 790
53 791
54 792
55 793
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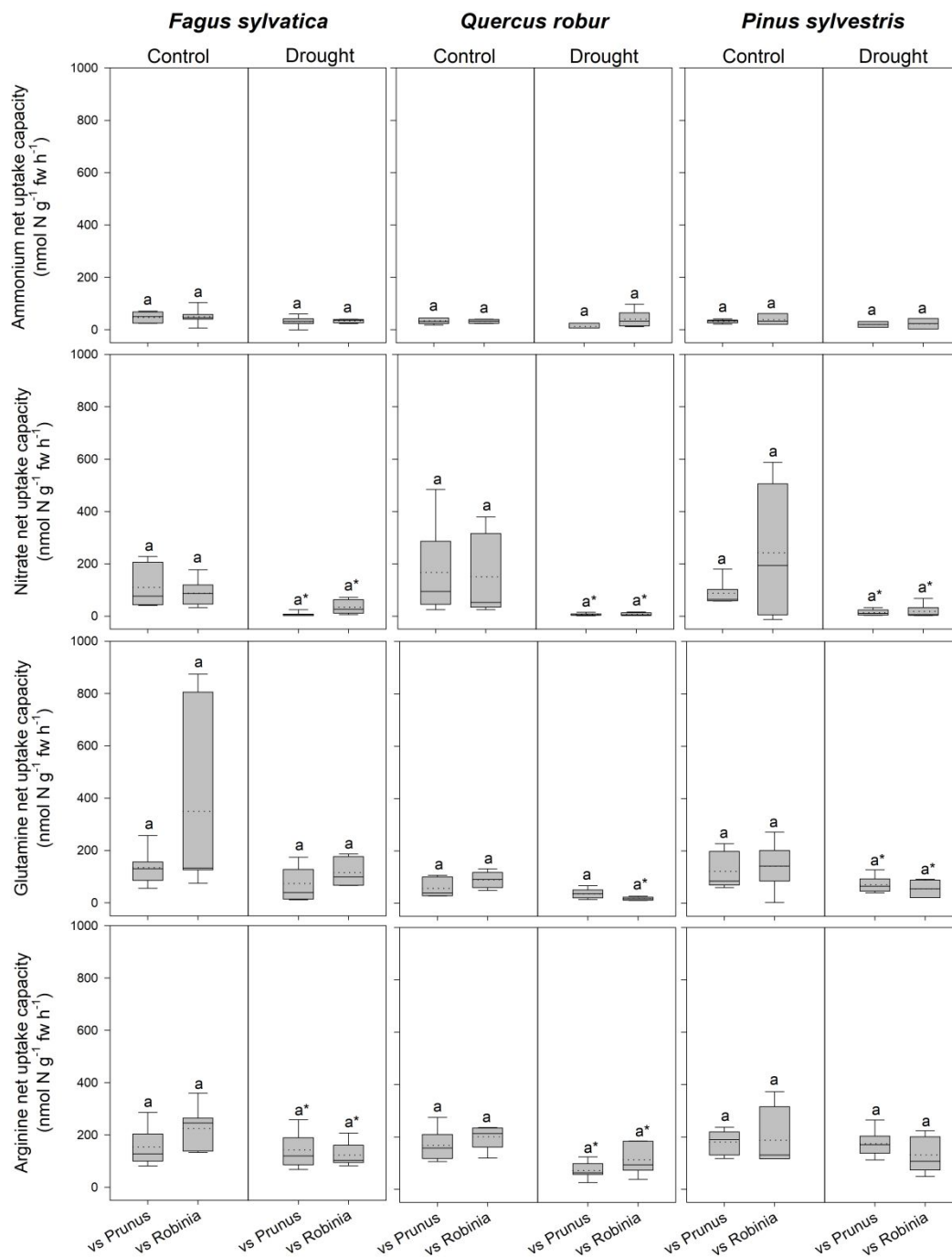
- 1
2
3 776 Mantovani D, Veste M, Freese D (2014) Effects of drought frequency on growth performance
4 and transpiration of young black locust (*Robinia pseudoacacia* L.). International Journal of
5 Forestry Research 821891. doi: 10.1155/2014/821891
6
7 778
8 779 Mantovani D, Veste M, Boldt-Burisch K, Fritsch S, Koning LA, Freese D (2015) Carbon
9 allocation, nodulation, and biological nitrogen fixation of black locust (*Robinia*
10 780 *pseudoacacia* L.) under soil water limitation. Annals of Forest Research 58: 259-274. doi:
11 781 10.15287/afr.2015.420
12 782
13 783 Mao W, Felton AJ, Ma Y, Zhang T, Sun Z, Zhao X, Smith MD (2018) Relationships between
14 784 aboveground and belowground trait responses of a dominant plant species to alterations in
15 785 watertable depth. Land Degradation and Development 29: 4015-4024.
16 786
17 786 Mason RAB, Cooke J, Moles AT, Leishman MR (2008) Reproductive output of invasive versus
18 787 native plants. Global Ecology and Biogeography 17: 633-640.
19 788
20 788 McDowell SCL (2002) Photosynthetic characteristics of invasive and noninvasive species of
21 789 *Rubus* (Rosaceae). American Journal of Botany 89: 1431-1438.
22 790
23 790 McKane R, Johnson L, Shaver G, Nadelhoffer K, Rastetter E, Fry B, Giblin A, Kielland K,
24 791 Kwiatowski B, Laundre J, Murray G (2002) Resource-based niches provide a basis for plant
25 792 species diversity and dominance in arctic tundra. Nature 412: 68-71.
26 793
27 793 Millard P (1988) The accumulation and storage of nitrogen by herbaceous plants. Plant, Cell
28 794 and Environment 11: 1-8. doi: 10.1111/j.1365-3040.1988.tb01769.x
29 795
30 795 Millard P, Grelet GA (2010) Nitrogen storage and remobilization by trees: ecophysiological
31 796 relevance in a changing world. Tree Physiology 30: 1083-1095. doi:
32 797 10.1093/treephys/tpq042
33 798
34 798 Millet J, Millard P, Hester AJ, McDonald AJS (2005) Do competition and herbivory alter the
35 799 internal nitrogen dynamics of birch saplings? New Phytologist 168: 413-422.
36 800
37 800 Näsholm T, Kielland K, Ganeteg U (200) Uptake of organic nitrogen by plants. New
38 801 Phytologist 182: 31-48. doi: 10.1111/j.1469-8137.2008.02751.x
39 802
40 802 Nilsen P, Børja I, Knutsen H, Brean R (1998) Nitrogen and drought effects on ectomycorrhizae
41 803 of Norway spruce [*Picea abies* L.(Karst.)]. Plant and Soil 198: 179-184.
42 804
43 804 Ostonen I, Püttsepp Ü, Biel C, Alberton O, Bakker MR, Lõhmus K, Majdi H, Metcalfe D,
44 805 Olsthoorn AFM, Pronk A, Vanguelova E, Weih M, Brunner I (2007) Specific root length as
45 806 an indicator of environmental change. Plant Biosystems 141: 426-442. doi:
46 807 10.1080/11263500701626069
47 808
48 808 Pattison RR, Goldstein G, Ares A (1998) Growth, biomass allocation and photosynthesis of
49 809 invasive and native Hawaiian rainforest species. Oecologia 117:449-459.
50
51
52
53
54
55
56
57
58
59
60

- 1
2
3 810 Pfautsch S, Rennenberg H, Bell TL, Adams MA (2009) Nitrogen uptake by *Eucalyptus regnans*
4 and *Acacia* spp. – preferences, resource overlap and energetic costs. *Tree Physiology* 29:
5 811 389-399.
6 812
7
8 813 Reich PB (2014) The world-wide ‘fast–slow’ plant economics spectrum: a traits manifesto.
9 *Journal of Ecology* 102: 275-301. doi: 10.1111/1365-2745.12211
10 814
11 815 Reich PB, Walters MB, Ellsworth DS (1997) From tropics to tundra: Global convergence in
12 plant functioning. *Proceedings of the National Academy of Sciences* 94: 13730-13734. doi:
13 816 10.1073/pnas.94.25.13730
14 817
15 818 Rennenberg H, Loreto F, Polle A, Brilli F, Fares S, Beniwal RS, Gessler A (2006) Physiological
16 responses of forest trees to heat and drought. *Plant Biology* 8: 556-571. doi: 10.1055/s-2006-
17 819 924084
18 820
19 821 Rennenberg H, Dannenmann M, Gessler A, Kreuzwieser J, Simon J, Papen H (2009) Nitrogen
20 balance in forest soils: nutritional limitation of plants under climate change stresses. *Plant*
21 *Biology* 11: 4-23. doi: 10.1111/j.1438-8677.2009.00241.x
22 822
23 823 Ricciardi A (2007) Are modern biological invasions an unprecedented form of global change?
24 *Conservation Biology* 21: 329-336. doi: 10.1111/j.1523-1739.2006.00615.x
25 825
26 826 Robakowski P, Bielinis E, Stachowiak J, Mejza I, Bułaj B (2016) Seasonal changes affect root
27 prunasin concentration in *Prunus serotina* and override species interactions between *P.*
28 *serotina* and *Quercus petraea*. *Journal of Chemical Ecology* 42: 202-214. doi:
29 829 10.1007/s10886-016-0678-y
30 830
31 831 Schimel J, Balsler TC, Wallenstein M (2007) Microbial stress-response physiology and its
32 implications for ecosystem function. *Ecology* 88: 1386-1394.
33 832
34 833 Simon J, Waldhecker P, Brüggemann N, Rennenberg H (2010) Competition for nitrogen
35 sources between European beech (*Fagus sylvatica*) and sycamore maple (*Acer*
36 *pseudoplatanus*) seedlings. *Plant Biology* 12: 453-458. doi: doi.org/10.1111/j.1438-
37 834 8677.2009.00225.x
38 835
39 836 Simon J, Dannenmann M, Gasche R, Holst J, Mayer H, Papen H, et al. (2011) Competition for
40 nitrogen between adult European beech and its offspring is reduced by avoidance strategy.
41 *Forest Ecology and Management* 262: 105-114. doi: 10.1016/j.foreco.2011.01.035
42 837
43 838 Simon J, Li X, Rennenberg H (2014) Competition for nitrogen between European beech and
44 sycamore maple shifts in favour of beech with decreasing light availability. *Tree Physiology*
45 840 34: 49-60. doi: 10.1093/treephys/tpt112
46 841
47
48
49
50
51
52
53
54
55
56
57
58
59
60

- 1
2
3 842 Simon J, Dannenmann M, Pena R, Gessler A, Rennenberg H (2017) Nitrogen nutrition of beech
4 843 forests in a changing climate: importance of plant-soil-microbe water, carbon, and nitrogen
5 844 interactions. *Plant and Soil* 418: 89-114. doi: 10.1007/s11104-017-3293-y
6
7
8 845 Sohn JA, Hartig F, Kohler M, Huss J, Bauhus J (2016) Heavy and frequent thinning promotes
9 846 drought adaptation in *Pinus sylvestris* forests. *Ecological Applications* 26: 2190-2205.
10
11 847 Song M, Zheng L, Suding KN, Yin T, Yu F (2015) Plasticity in nitrogen form uptake and
12 848 preference in response to long-term nitrogen fertilization. *Plant and Soil* 394: 215-224. doi:
13 849 10.1007/s11104-015-2532-3
14
15
16
17 850 Spinoni J, Vogt JV, Naumann G, Barbosa P, Dosio A (2017) Will drought events become more
18 851 frequent and severe in Europe? *International Journal of Climatology* 38: 1718-1736. doi:
19 852 10.1002/joc.5291
20
21
22 853 Staswick PE (1994) Storage proteins of vegetative plant tissues. *Annual Review of Plant*
23 854 *Physiology and Plant Molecular Biology* 45: 303-22.
24
25 855 Verma DPS, Hu CA, Zhang M (1992) Root nodule development: origin, function and regulation
26 856 of nodulin genes. *Physiologia Plantarum* 85: 253-265.
27
28
29 857 Vilà M, Espinar JL, Hejda M, Hulme PE, Jarosik V, Maron JL, et al. (2011) Ecological impacts
30 858 of invasive alien plants: a meta-analysis of their effects on species, communities and
31 859 ecosystems. *Ecology Letters* 14: 702-708. doi: 10.1111/j.1461-0248.2011.01628.x
32
33
34 860 Vítková M, Müllerová J, Sádlo J, Pergl J, Pyšek P (2017) Black locust (*Robinia pseudoacacia*)
35 861 beloved and despised: a story of an invasive tree in Central Europe. *Forest Ecology and*
36 862 *Management* 384: 287-302. doi: 10.1016/j.foreco.2016.10.057
37
38
39 863 Vitousek PM, Aber JD, Howarth RW, Likens GE, Matson PA, Schindler DW, Schlesinger WH,
40 864 Tilman DG (1997) Human alteration of the global nitrogen cycle: sources and consequences.
41 865 *Ecological Applications* 7: 737-750.
42
43
44 866 Wang H, Liu S, Wang J, Shi Z, Xu J, Hong P, Ming A, Yu H, Chen L, Lu L, Cai D (2016)
45 867 Differential effects of conifer and broadleaf litter inputs on soil organic carbon chemical
46 868 composition through altered soil microbial community composition. *Scientific Reports* 6:
47 869 27097.
48
49
50
51 870 Wardle DA, Peltzer DA (2017) Impacts of invasive biota in forest ecosystems in an
52 871 aboveground–belowground context. *Biological Invasions* 19: 3301-3316. doi:
53 872 10.1007/s10530-017-1372-x
54
55
56
57 873 Winter H, Lohaus G, Heldt HW (1992) Phloem transport of aminoacids in relation to their
58 874 cytosolic levels in barley leaves. *Plant Physiology* 99: 996-1004. doi: 10.1104/pp.99.3.996
59
60

1
2
3 875 Wurzburger N, Miniat CF (2014) Drought enhances symbiotic dinitrogen fixation and
4 876 competitive ability of a temperate forest tree. *Oecologia* 174: 1117-1126. doi:
5 877 10.1007/s00442-013-2851-0

6
7
8 878 Zhou G, Zhou X, Nie Y, Bai SH, Zhou L, Shao J, Cheng W, Wang J, Hu F, Fu Y (2018)
9 879 Drought-induced changes in root biomass largely result from altered root morphological
10 880 traits: Evidence from a synthesis of global field trials. *Plant, Cell and Environment* 41: 2589-
11 881 2599. doi: 10.1111/pce.13356
12
13
14
15 882

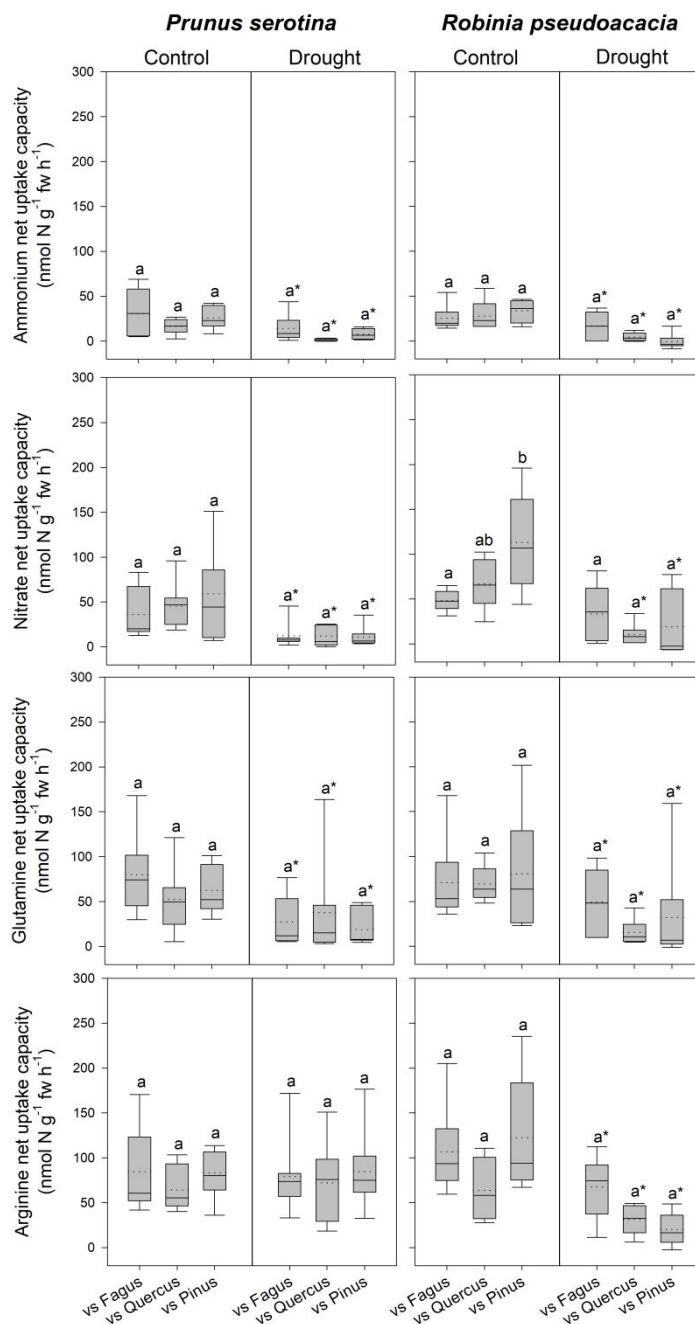


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3 884 Figure 1. Ammonium, nitrate, glutamine-N, and arginine-N net uptake capacity (nmol N g⁻¹ fw
4 885 h⁻¹) by fine roots of *Fagus sylvatica*, *Quercus robur* and *Pinus sylvestris* seedlings at control
5 886 and drought conditions under different competition regimes. vs *Prunus* = competition with
6 887 *Prunus serotina*; vs *Robinia* = competition with *Robinia pseudoacacia*. Box plots show mean
7 888 (dotted line) and median (continuous line). Different letters indicate significant differences
8 889 between competition regimes within a specific soil water availability treatment, and asterisks
9 890 indicate significant differences between control and drought detected using permutational
10 891 analysis of variance ($p < 0.05$).

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For Peer Review



892
 893 Figure 2. Ammonium, nitrate, glutamine-N, and arginine-N net uptake capacity (nmol N g⁻¹ fw
 894 h⁻¹) by fine roots of *Prunus serotina* and *Robinia pseudoacacia* seedlings at control and drought
 895 conditions under different competition regimes. vs *Fagus* = competition with *Fagus sylvatica*;
 896 vs *Quercus* = competition with *Quercus robur*; vs *Pinus* = competition with *Pinus sylvestris*.
 897 Box plots show mean (dotted line) and median (continuous line). Different letters indicate
 898 significant differences between competition regimes within a specific soil water availability
 899 treatment, and asterisks indicate significant differences between control and drought detected
 900 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 (cm² g⁻¹ dw), SRL: specific root length (cm g⁻¹ dw), N net uptake capacity (nmol N g⁻¹ fw h⁻¹), NH₄⁺: ammonium, NO₃⁻: 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 biomass	Root:shoot ratio	RGR	SLA	SRL	NH ₄ ⁺	NO ₃ ⁻	Gln-N	Arg-N	Total soluble amino acid-N		Total soluble protein-N	
										Leaves	Fine roots	Leaves	Fine roots
<i>Fagus sylvatica</i>	n.s.	↓	↑(b)	↓	n.s.	n.s.	↓	n.s.	↓	n.s.	n.s.	↓	n.s.
<i>Quercus robur</i>	n.s.	n.s.	n.s.	n.s.	↓(b)	n.s.	↓	↓(b)	↓	↑(d)	↑(d)	↓	n.s.
<i>Pinus sylvestris</i>	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	↓	↓	n.s.	n.s.	↑	n.s.	n.s.
<i>Prunus serotina</i>	↓(a)	n.s.	n.s.	↑	n.s.	↓	↓	↓	n.s.	n.s.	↓(c)	n.s.	↓
<i>Robinia pseudoacacia</i>	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

917 Table 2. Effects of competitor on total biomass, growth indices, inorganic and organic N net uptake capacity, and N metabolite levels of *Fagus*
 918 *sylvatica*, *Quercus robur*, *Pinus sylvestris*, *Prunus serotina*, and *Robinia pseudoacacia* seedlings. Species A < species B = seedlings competing with
 919 species A had significantly lower values than seedlings competing with species B, n.s. = no significant differences between competitor identities.
 920 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
 921 area (cm² g⁻¹ dw), SRL: specific root length (cm g⁻¹ dw), N net uptake capacity (nmol N g⁻¹ fw h⁻¹), NH₄⁺: ammonium, NO₃⁻: nitrate, Gln-N: glutamine-
 922 N, Arg-N: arginine-N, total soluble amino acid-N (mg g⁻¹ dw), total soluble protein-N (mg g⁻¹ dw).

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	Biomass and growth indices					N net uptake capacity				N metabolites			
	Total biomass	Root:shoot ratio	RGR	SLA	SRL	NH ₄ ⁺	NO ₃ ⁻	Gln-N	Arg-N	Total soluble amino acid-N		Total soluble protein-N	
										Leaves	Fine roots	Leaves	Fine roots
<i>Fagus sylvatica</i>	n.s.	n.s.	<i>Robinia</i> < <i>Prunus</i> (a)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
<i>Quercus robur</i>	n.s.	<i>Prunus</i> < <i>Robinia</i>	<i>Prunus</i> < <i>Robinia</i>	n.s.	<i>Robinia</i> < <i>Prunus</i> (b)	n.s.	n.s.	n.s.	n.s.	n.s.	<i>Robinia</i> < <i>Prunus</i>	n.s.	<i>Prunus</i> < <i>Robinia</i> (a)
<i>Pinus sylvestris</i>	<i>Prunus</i> < <i>Robinia</i>	n.s.	n.s.	-	n.s.	n.s.	n.s.	n.s.	n.s.	<i>Prunus</i> < <i>Robinia</i>	<i>Robinia</i> < <i>Prunus</i>	<i>Robinia</i> < <i>Prunus</i>	n.s.
<i>Prunus serotina</i>	<i>Fagus</i> , <i>Pinus</i> < <i>Quercus</i> (a)	n.s.	<i>Quercus</i> , <i>Pinus</i> < <i>Fagus</i>	<i>Quercus</i> < <i>Fagus</i> < <i>Pinus</i>	<i>Quercus</i> < <i>Fagus</i> (a)	n.s.	n.s.	n.s.	n.s.	<i>Fagus</i> < <i>Quercus</i>	<i>Fagus</i> < <i>Quercus</i> , <i>Pinus</i> (a) <i>Quercus</i> < <i>Pinus</i> (b)	<i>Fagus</i> , <i>Quercus</i> < <i>Pinus</i> (b)	n.s.
<i>Robinia pseudoacacia</i>	n.s.	n.s.	<i>Fagus</i> , <i>Quercus</i> < <i>Pinus</i>	n.s.	n.s.	n.s.	<i>Fagus</i> < <i>Pinus</i> (a)	n.s.	n.s.	n.s.	n.s.	n.s.	<i>Pinus</i> < <i>Quercus</i> < <i>Fagus</i> (b)

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925 (a): only under control conditions

926 (b): only with drought

927 No letter: effect of competitor regardless of drought

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929 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
 930 of *Fagus sylvatica*, *Quercus robur*, and *Pinus sylvestris* seedlings grown in competition under drought and control conditions. Only significant
 931 differences are presented. n.s. = no significant differences between net uptake capacity of different N forms.

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Competitor	Water availability	<i>Fagus sylvatica</i>	<i>Quercus robur</i>	<i>Pinus sylvestris</i>
<i>Prunus serotina</i>	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^-
<i>Robinia pseudoacacia</i>	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^-

933

934 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
 935 of *Prunus serotina* and *Robinia pseudoacacia* seedlings grown in competition under drought and control conditions. Only significant differences are
 936 presented. n.s. = no significant differences between net uptake capacity of different N forms.

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

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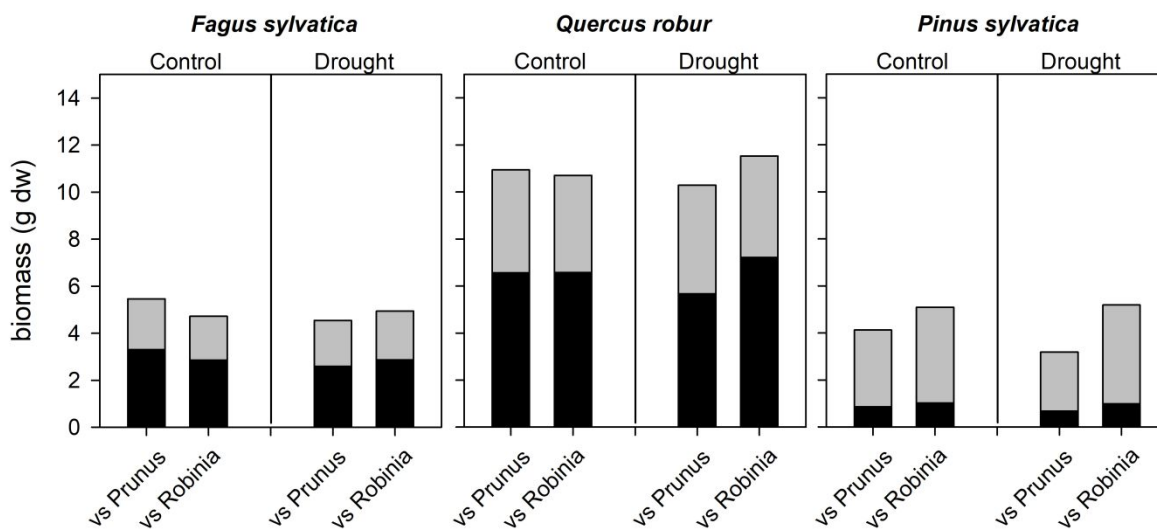
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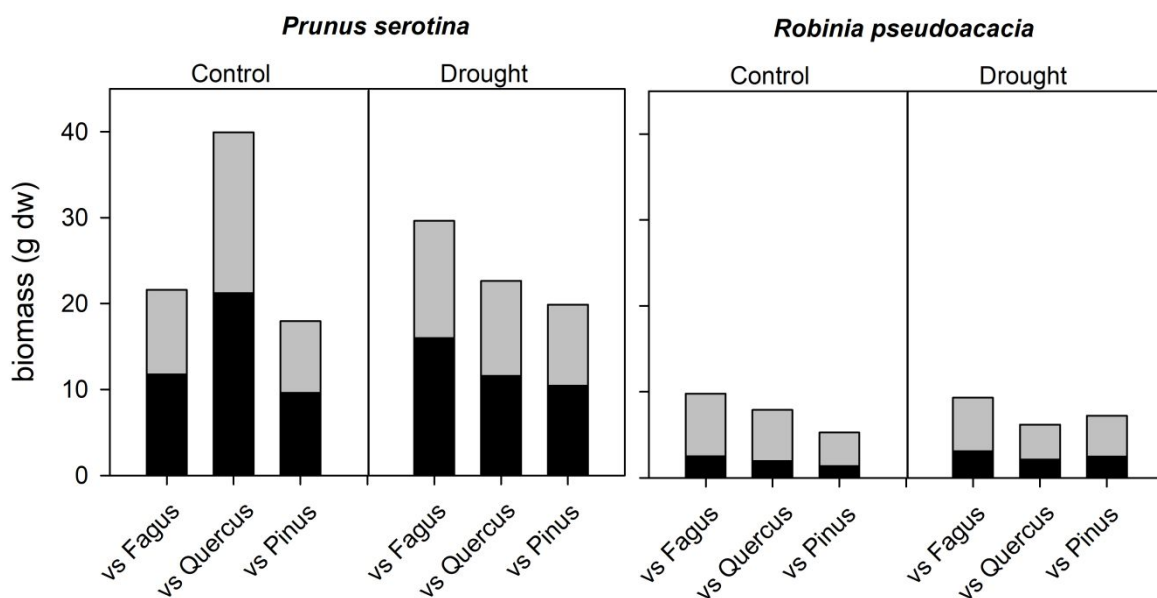


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947 Supplemental Figure 1a. Root (black) and shoot (gray) biomass (g dw) of *Fagus sylvatica*,
 948 *Quercus robur* and *Pinus sylvestris* seedlings at control and drought conditions under different
 949 competition regimes. vs *Prunus* = competition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

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

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3 1 **Responses of native and invasive woody seedlings to combined competition and drought**
4 **are species-specific**
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8 4 **Andrea Bueno¹, Karin Pritsch², Judy Simon^{1*}**
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21 11
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25 14 **Keywords:** reduced soil water availability, inorganic nitrogen, nitrogen pools, nitrogen
26 14
27 15 uptake, nitrogen acquisition, organic nitrogen, plant competition, woody invaders, temperate
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29 16 forests, nitrogen metabolites
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32 18 **Running title:** Tree invasions: Competition under drought
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35 Abstract

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

58 1. Introduction

59 Invasion of natural habitats by exotic species is considered a major aspect of anthropogenic
60 global change (Vitousek et al. 1997, Ricciardi 2007). In forest ecosystems, successful plant
61 invasions have a negative impact on the structure and composition of plant and microbial
62 communities, as well as forest biogeochemical processes (Castro-Diez et al. 2006, Corbin and
63 D'Antonio 2012). For example, invasives can suppress the recruitment and growth of native
64 plant species, affect carbon pools and nutrient fluxes, and modify litter quality and
65 decomposition (Ehrenfeld et al. 2001, Ehrenfeld 2003, Vilà et al. 2011). As a result, forest
66 functioning is altered and the provision of ecosystem services may be impaired (Holmes et al.
67 2009, Vilà et al. 2011, Wardle and Peltzer 2017). Invasive plant species commonly display
68 functional traits and growth strategies that improve resource capture and favour reproduction

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3 69 including high seed production (Mason et al. 2008), specific leaf area (Grotkopp and Rejmánek
4 70 2007, Leishman et al. 2007), photosynthetic rates (Pattison et al. 1998, McDowell 2002),
5 71 relative growth rates (Grotkopp and Rejmánek 2007), root biomass (Broadbent et al. 2018)
6 72 and/or specific root length (Dawson 2015). These traits contribute to the successful
7 73 establishment and dispersal of invasive species in new habitats by enhancing their competitive
8 74 ability ~~over native species~~. For example, invasive *Prunus serotina* and *Robinia pseudoacacia*
9 75 produce more biomass ~~at the cost to the detriment~~ of slower growing ~~ingth~~ of native *Quercus robur*
10 76 and *Carpinus betulus* when grown in competition (Kawaletz et al. 2013).
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19 78 A key resource in the competition between native and invasive plant species is plant-growth
20 79 limiting nitrogen (N) (Littschwager et al. 2010, Eller and Oliveira 2017). The competitive
21 80 ability of plants for acquiring N is determined by plant morphological (e.g. density and length
22 81 of root hairs) and physiological (e.g. N uptake capacity, expression and activity of transporters
23 82 in root cells) traits, as well as species-specific plasticity in these traits (Casper and Jackson
24 83 1997), ~~but also the support of symbionts such as mycorrhiza (e.g. Näsholm et al. 2009) and/or~~
25 84 ~~N₂-fixing bacteria (Bueno et al. 2019)~~. Previous studies investigating the effects of competition
26 85 for N found plasticity in the N uptake capacity of trees with increases or decreases in response
27 86 to interspecific competition which, however, depended on the competing species,
28 87 environmental conditions, and available N sources (e.g. inorganic vs organic N) (Simon et al.
29 88 2010, Simon et al. 2014, Li et al. 2015, Bueno et al. 2019). For example, *Fagus sylvatica*
30 89 increased organic N acquisition in competition with *Acer pseudoplatanus* compared to
31 90 intraspecific competition at high but not low soil N availability (Li et al. 2015): With ambient
32 91 but not reduced light, organic N acquisition decreased in competition with *A. pseudoplatanus*
33 92 reflecting a better adaptation of *F. sylvatica* to low light conditions compared to *A.*
34 93 *pseudoplatanus* (Simon et al. 2014). The utilization of different N sources likely provides an
35 94 advantage when competing for N (McKane et al. 2002, Simon et al. 2014) with a high potential
36 95 to drive niche differentiation and species coexistence (McKane et al. 2002, Ashton et al. 2010,
37 96 Boudsocq et al. 2012). In the context of competition between native and invasive species, the
38 97 preference of different N forms in competition (i.e. one species favouring organic N, whereas
39 98 the other prefers inorganic N sources) might provide an important mechanism to effectively
40 99 avoid competition for N. To our knowledge, this theory has mostly been tested for non-woody
41 100 species (Fraterrigo et al. 2011, Huangfu et al. 2016) and only recently for tree species (but see
42 101 Bueno et al. 2019).
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3 103 The threat of invasive species to European forests might become even more severe in ~~the view~~
4 ~~light~~ of the predicted climate changes for Central Europe. In Bueno et al. (2019), the responses
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6 104
7 105 of three native and two invasive tree species (i.e. the same species as used here) to high soil N
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9 106 availability were investigated due to the expected increase in atmospheric N deposition
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11 107 (Rennenberg et al. 2009). In the present study, responses of native and invasive tree species to
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13 108 competition for N are investigated under drought conditions. Drought periods in summer are
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15 109 expected to increase in frequency and severity (Spinoni et al. 2017). Thus, understanding the
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17 110 consequences of drought on the outcome of competition for N between native and invasive
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19 111 woody plant species is crucial. N dynamics in both plant and soil are tightly linked to water
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21 112 availability (Gessler et al. 2017): For example, drought negatively affects soil N mineralization
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23 113 processes resulting in reduced plant available N in the rhizosphere (Simon et al. 2017), soil N
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25 114 diffusion and mass flow (Rennenberg et al. 2006), and the activity of root proteins related to N
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27 115 acquisition (Bista et al. 2018). Reduced mycorrhizal colonization might additionally lead to less
28
29 116 N transfer to plants (Nilsen et al. 1998). Therefore, also the competition for N between plants
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31 117 is altered with drought. For example, in studies by Fotelli et al. (2001, 2002) the combination
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33 118 of drought and competition with fast-growing *Rubus fruticosus* resulted in impaired inorganic
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35 119 N acquisition and water status for *F. sylvatica* seedlings, as well as increased amino acid levels
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37 120 due to protein degradation in the leaves to act as osmoprotectants. However, whether invasive
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39 121 species have an advantage over native species in the competition for N under drought scenarios
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41 122 is still unknown, despite woody species invasions becoming a major concern in forests around
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43 123 the globe (Lamarque et al. 2011).

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46 125 We conducted a greenhouse experiment to study the responses of three native and two invasive
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48 126 woody plant species to different competitors and drought in terms of organic and inorganic N
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50 127 acquisition as well as allocation of N to N pools in the leaves and fine roots. As native species
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52 128 we chose some of the most abundant and widespread species of Central European forests
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54 129 (Ellenberg and Leuschner 2010, Eaton et al. 2016, Houston et al. 2016), which differ in
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56 130 physiological traits and growth strategies: (i) European beech (*Fagus sylvatica* L., Fagaceae),
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58 131 a drought-sensitive slow growing species (Houston et al. 2016), (ii) pedunculated oak (*Quercus*
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60 132 *robur* L., Fagaceae), a drought-tolerant slow growing species (Eaton et al. 2016), and (iii) Scots
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134 pine (*Pinus sylvestris* L., Pinaceae), a drought-tolerant fast growing conifer (Kuster et al. 2013,
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136 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

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3 137 in Central European forests (Campagnaro et al. 2018): (i) black cherry (*Prunus serotina* Ehrh.,
4 138 Rosaceae), a fast growing species producing cyanogenic compounds (Csiszár 2009), and (ii)
5 139 black locust (*Robinia pseudoacacia* L., Leguminosae), a fast growing, N₂-fixing species
6 140 (Robakowski et al. 2016, Vítková et al. 2017). Both, the remobilization of N from cyanogenic
7 141 compounds and the ability to symbiotically fix N₂ allow these two invasive species to acquire
8 142 additional N independently from soil N supply, which could thus serve as alternate sources of
9 143 N. From here on, species used in this study will be referred to using their genus, i.e. *Fagus*,
10 144 *Quercus*, *Pinus*, *Prunus* and *Robinia*.

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19 146 Our general research aim was to evaluate whether N acquisition and allocation of N to N pools
20 147 of native and invasive species shift in response to drought and different competitors reflecting
21 148 their functional traits, i.e. growth rate (slow-growing vs. fast-growing), drought tolerance
22 149 (drought-tolerant vs. drought-sensitive), and/or the ability to access alternative sources of N
23 150 (e.g. via remobilization of N stored in cyanogenic compounds or symbiotic N₂ fixation). Our
24 151 specific hypotheses were: (1) Drought generally reduces inorganic and organic N acquisition
25 152 because N uptake from the soil strongly depends on water availability (Gessler et al. 2004,
26 153 Rennenberg et al. 2006). (2) Species-specific coping mechanisms (related to their functional
27 154 traits) lead to changes in the allocation of N to N pools in the leaves and fine roots, because
28 155 generally with drought less N is acquired from the soil (Gessler et al. 2017). (3) N acquisition
29 156 and allocation of N to N pools of a given species change depending on the species' functional
30 157 traits and the competitor. For example, competition between a slow and a fast grower leads to
31 158 a decrease in N acquisition and allocation to N pools in the slow growing species because of
32 159 the fast grower's higher N demand (Reich 2014). (4) Native and invasive species differ in their
33 160 preference for organic and inorganic N sources resulting in avoidance of competition for N
34 161 (Fraterrigo et al. 2011, Huangfu et al. 2016).

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47 163 **2. Materials and Methods**

48 164 **2.1. Plant material and growth conditions**

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50 165 One-year-old seedlings of all species were purchased from Müller Münchhof Pflanzen GmbH
51 166 (Seesen/Münchhof, Germany) and planted in different combinations (i.e. ~~one or~~ two seedlings
52 167 per pot, see 2.2 "Experimental design") in a 1:1 mixture of sand and vermiculite in 3 L plastic
53 168 pots (25 cm x 12 cm) at the end of November 2015. Pots stayed outdoors over winter and spring
54 169 and were watered regularly. They were brought into the greenhouse on June 20th 2016 after leaf
55 170 development. For the following 7 days, all pots were watered regularly with sufficient tap water,

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3 171 and received on two occasions 100 ml of an artificial low N nutrient solution containing 100
4 172 μM KNO_3 , 90 μM $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 70 μM $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 50 μM KCl , 24 μM $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 20
5 173 μM NaCl , 10 μM AlCl_3 , 7 μM $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 6 μM K_2HPO_4 , 1 μM NH_4Cl , 25 μM glutamine,
6 174 and 25 μM arginine mimicking the soil solution of a low N field site (Dannenmann et al. 2009).
7
8 175 The pots were subjected to natural light conditions and day length regime (16/8, day/night). Air
9 176 temperature was 23.5 ± 2.3 °C / 21.0 ± 2.4 °C (day/night, mean \pm standard deviation), and
10 177 relative humidity was 63.1 ± 9.3 % / 71.3 ± 8.8 % (day/night, mean \pm standard deviation) for
11 178 the duration of the experiment. With increasing duration of the drought treatment, some
12 179 individuals started to show signs of wilting. To ensure sufficient replication for each species
13 180 and treatment, we did the ^{15}N uptake experiments followed by the harvest after four weeks.
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22 182 2.2. Experimental design

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24 183 The experiment was conducted in a fully orthogonal design with two factors, “water
25 184 availability” (i.e. control or drought) and “competitor” (interspecific competition with 2 or 3
26 185 different competitors). Seedlings were planted in native-invasive interspecific competition (i.e.
27 186 one seedling of a native species and one seedling of an invasive species per pot). Pots were
28 187 established for every combination of native species and invasive species. For each species, a
29 188 total of 24 pots was setup for each combination of native-invasive, summing up to a total of
30 189 144 pots. Pots were assigned to either the control or drought treatment (i.e. $n = 12$ per
31 190 combination of species, competitor, and soil water availability). For the drought treatment,
32 191 irrigation was fully stopped starting June 27th until the final harvest from July 21st to 27th, while
33 192 for the control treatment irrigation continued with sufficient tap water supply every second day.
34 193 To confirm that water availability was significantly reduced in the respective treatment, we
35 194 measured soil water content every second day for 3 pots per combination of species, soil water
36 195 availability treatment and competitor, by inserting a probe into the soil in three different
37 196 locations in each pot (HH2 Moisture Meter, Delta-T Devices, Cambridge). Soil water content
38 197 in the drought treatment was ~~with~~ 7.2 ± 2.4 % (mean \pm standard deviation) and significantly
39 198 lower ~~than~~ in the control 24.0 ± 2.5 % (mean \pm standard deviation) at the end of the experiment.
40 199 $\delta\Delta^{13}\text{C}$ values in the leaves (Suppl. Table 6) were higher in the drought treatment compared to
41 200 the control at the time of harvest indicating drought stress for *Fagus*, *Quercus*, and *Robinia*
42 201 depending on the competitor, while for *Pinus* and *Prunus* no differences were found.
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203 2.3. ^{15}N uptake experiments

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3 204 To quantify inorganic (i.e. ammonium and nitrate) and organic (i.e. glutamine and arginine) net
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5 205 N uptake capacity of the seedlings' fine roots, the ^{15}N enrichment technique was used as
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7 206 described by Gessler et al. (1998) and modified by Simon et al. (2010). Seedlings were carefully
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9 207 removed from the pots and the roots washed thoroughly with tap water to remove any remaining
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11 208 substrate. Then, fine roots still attached to the seedlings were incubated in the same artificial
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13 209 low N solution as used during plant growth (see above) containing all four N sources but only
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15 210 one labelled as either $^{15}\text{NH}_4^+$, $^{15}\text{NO}_3^-$, $^{13}\text{C}/^{15}\text{N}$ -glutamine, or $^{13}\text{C}/^{15}\text{N}$ -arginine. Controls with no
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17 211 label were included to account for natural abundance in the fine roots ($n = 4$ to 6 per N source
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19 212 including controls with no label, per species, competitor, and soil water availability treatment).
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21 213 The roots not used for incubation were wrapped in wet tissue to prevent desiccation for the
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23 214 duration of the ^{15}N uptake experiment. After 2 hours of incubation, the fine roots were cut from
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25 215 the seedling and washed twice in 0.5 M CaCl_2 solution to remove the incubation solution from
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27 216 the root surface. The fresh weight of the fine roots was determined, and after oven-drying for
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29 217 48 h at $60\text{ }^\circ\text{C}$, their dry weight was determined. Amino acids were $^{13}\text{C}/^{15}\text{N}$ -labelled to determine
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31 218 whether they were taken up as intact molecules (Simon et al. 2011). Net uptake capacity of
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33 219 glutamine and arginine was lower based on ^{13}C compared to that on ^{15}N incorporation indicating
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35 220 that amino acids degraded in the solution or on the surface of the roots, and/or the respiration
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37 221 of amino acid-derived C inside the roots (Simon et al. 2011). Incubation took place between 10
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39 222 am and 2 pm to avoid diurnal variation in net N uptake capacity (Gessler et al. 2002).
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224 **2.4. Harvest and quantification of growth and biomass indices**

225 After the ^{15}N uptake experiment, seedlings were separated into leaves, stems, and roots. Their
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227 fresh weight was determined, then all tissues were oven-dried for 48 h at $60\text{ }^\circ\text{C}$ and their dry
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229 weight was determined. Before oven-drying, a subsample of 8 to 10 representative leaves was
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231 collected from each seedling and their leaf area measured (LI-3100C Area Meter, LI-COR,
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233 Lincoln, USA) to calculate specific leaf area (SLA). Likewise, a subsample of fine roots was
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235 collected from each seedling, stained, scanned and total length measured (WinRhizo 2012,
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237 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 $-80\text{ }^\circ\text{C}$ 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

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3 238 equation: $RGR = (\ln b_2 - \ln b_1) * t^{-1}$, where b_1 is total seedling biomass (g dw) at the initial
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5 239 harvest, b_2 is total seedling biomass (g dw) at the final harvest, and t is the time period in days
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7 240 between the initial and the final harvest (Grubb et al. 1996). Initial seedling biomass was
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9 241 determined on 3 to 4 pots per species, competitor, and soil water availability treatment that were
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11 242 harvested immediately before commencing the drought treatment.

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13 244 **2.5. Quantification of total N and C, ^{15}N , and ^{13}C in fine roots and $\delta^{13}\text{C}$ in leaves**

14 245 Dried fine root samples from the ^{15}N uptake experiment were ground using a ball mill
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16 246 (TissueLyser, Retsch, Haan, Germany) to a fine homogeneous powder. Aliquots of 1.2 to 2.4
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18 247 mg were weighed into 4x6 mm tin capsules (IVA Analysentechnik, Meerbusch, Germany) for
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20 248 analyses with an isotope ratio mass spectrometer (Delta V Advantage, Thermo Electron,
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22 249 Dreieich, Germany) coupled to an elemental analyzer (Euro EA, Eurovector, Milano, Italy). Δ
23
24 250 values were calculated using a laboratory standard (acetanilide) that was part of every sequence
25
26 251 in intervals also used in different weights to determine isotope linearity of the system. The
27
28 252 laboratory standard was calibrated against several suitable international isotope standards
29
30 253 (IAEA, Vienna). Final correction of isotope values was done with several international isotope
31
32 254 standards and other suitable laboratory standards which cover the range of ^{15}N and ^{13}C results.
33
34 255 Inorganic and organic N net uptake capacity ($\text{nmol N g}^{-1} \text{fw h}^{-1}$) was calculated based on the
35
36 256 incorporation of ^{15}N into the fine roots according to Kreuzwieser et al. (2002): Net N uptake
37
38 257 capacity = $((^{15}\text{N}_l - ^{15}\text{N}_n) * N_{\text{tot}} * \text{dw} * 10^5) / (\text{MW} * \text{fw} * t)^{-1}$, where $^{15}\text{N}_l$ and $^{15}\text{N}_n$ are the atom% of ^{15}N
39
40 258 in labeled (N_l) and unlabeled control plants (N_n , natural abundance), respectively, N_{tot} is the
41
42 259 total N percentage, MW is the molecular weight ($^{15}\text{N g mol}^{-1}$), and t is the incubation time.

43 260

44 261 **2.6. Quantification of total soluble protein and total soluble amino acid levels in leaves** 45 262 **and fine roots**

46 263 To extract total soluble proteins from the leaves and fine roots (Dannenmann et al. 2009), ~50
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48 264 mg aliquots of finely ground frozen samples were incubated in 1.5 ml extraction buffer (50 mM
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50 265 Tris-HCl pH 8.0, 1 mM EDTA, 15% (v/v) glycerol, 0.6 mM dithiothreitol, 1% Triton X-100, 2
51
52 266 EDTA-free protease inhibitor cocktail tablets per 100 ml buffer) at 4 °C for 30 min followed
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54 267 by centrifugation for 10 min at 14,000 rpm and 4 °C. The extraction was done twice to increase
55
56 268 the yield. Subsequently, 500 μL of the combined supernatant from both extractions were
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58 269 incubated with 1 ml 10 % (v/v) trichloroacetic acid for 10 min at room temperature and then
59
60 270 centrifuged for 10 min at 14,000 rpm and 4 °C. The resulting protein pellet was dissolved in 1
271 271 ml 1 M KOH. Next, total soluble proteins were quantified following Simon et al. (2010) by

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2
3 272 adding 1 ml of Bradford reagent to 50 μ L of extract. Following a 10 min incubation at room
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5 273 temperature in the dark, the absorbance was measured at 595 nm in a spectrophotometer
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7 274 (Ultrospec 3100pro, Amersham Biosciences). Bovine serum albumin (BSA) was used as
8
9 275 standard.

10 276
11 277 Total soluble amino acid-N content in the leaves and fine roots were extracted according to
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13 278 Winter et al. (1992): 200 μ L Hepes buffer (5 mM EGTA, 20 mM HEPES, 10 mM NaF) and 1
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15 279 ml 3.5:1.5 (v:v) methanol/chloroform were added to ~50 mg aliquots of finely ground frozen
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17 280 sample and incubated for 30 min on ice, followed by the addition of 600 μ L of distilled water
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19 281 and centrifugation for 5 min at 14,000 rpm and 4 °C. The addition of distilled water and
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21 282 centrifugation was performed twice to increase the yield. Total soluble amino acid-N content
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23 283 in the leaves and fine roots were quantified according to Liu et al. (2005): 50 μ L ninhydrin
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25 284 solution was added to a 50 μ L aliquot of the combined extract and boiled for 30 min. The
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27 285 ninhydrin solution was composed of an equal parts mixture of solution A (i.e. 3.84 g citric acid,
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29 286 0.134 g SnCl₂, and 40 ml 1 M NaOH, filled up to 100 ml with distilled water at pH 5) and
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31 287 solution B (i.e. 4 g ninhydrin in 100 ml ethylene-glycol-monomethyl-ether). Subsequently, the
32
33 288 extracts were cooled to room temperature and 1 ml 50% isopropanol was added, followed by a
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35 289 15 min incubation. The absorption was measured at 570 nm in a spectrophotometer (Ultrospec
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37 290 3100pro, Amersham Biosciences). L-glutamine was used as standard.

36 291

37 292 **2.7. Statistical analyses**

39 293 Two-way permutational ANOVAs (PERMANOVA) based on a Euclidean resemblance matrix
40
41 294 between samples (Anderson et al. 2008) were performed for each species to test for differences
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43 295 between water availability and competitor levels using as variables inorganic and organic net
44
45 296 N uptake capacity, total soluble amino acid-N, and total soluble protein-N contents in the leaves
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47 297 and fine roots, as well as total biomass, root:shoot ratio, SLA, SRL, and RGR. PERMANOVAs
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49 298 were performed using “water availability” (i.e. drought and control) and “competitor” as fixed
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51 299 orthogonal factors. “Competitor” consisted of two levels for the native species (i.e. competition
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53 300 with *Prunus* or *Robinia*) or three levels for the invasive species (i.e. competition with *Fagus*,
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55 301 *Quercus*, or *Pinus*). For significant interactions between factors, *post hoc* PERMANOVA pair-
56
57 302 wise comparisons were performed. To test for differences in $\delta^{13}\text{C}$ in leaves between drought
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59 303 and control for each species grown with different competitors, Mann-Whitney U-tests were
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304 performed. Finally, to test for species preferences in net N uptake capacity of the different N
305 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).

310

311 3. Results

312 3.1. Drought effects on N acquisition and allocation to N pools in the leaves and fine roots of native and invasive tree seedlings

314 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).

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326 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).

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337 Finally, drought resulted in higher $\delta^{13}\text{C}$ 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

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3 340 and SRL of native and invasive species depending on both the target species and the competitor
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5 341 (Table 1, Suppl. Tables 7, 8, 9, [Suppl. Figure 1](#)).

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8 343 **3.2. Competitor effects on N acquisition and allocation to N pools in the leaves and fine**
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10 344 **roots of native and invasive tree species**

11 345 For native species, N acquisition did not change with different invasive competitors, but there
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13 346 were different responses regarding allocation to N pools ([Fig. 1](#)). In competition with *Prunus*
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15 347 compared to *Robinia* and regardless of soil water availability, *Quercus* had lower total soluble
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17 348 protein-N content but higher total soluble amino acid-N content in the fine roots, whereas *Pinus*
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19 349 had lower total soluble amino acid-N content and higher total soluble protein-N content in the
20
21 350 leaves, and higher total soluble amino acid-N content in the fine roots ([Table 2, Suppl. Tables](#)
22
23 351 [2, 3, 4](#)). For *Fagus*, N allocation to N pools did not vary with competitor (Table 2, Suppl. Tables
24
25 352 2, 3, 4). All native species responded differently to the invasive competitors regarding their
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27 353 biomass and growth indices (Table 2, Suppl. Tables 7, 8, 9).

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29 355 Similarly, responses of the invasive species depended on competitor and were partly also
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31 356 mediated by soil water availability. Under drought, *Prunus* seedlings had lower total soluble
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33 357 amino acid-N contents in the fine roots when competing with *Quercus* compared to *Pinus*
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35 358 (Table 2, Suppl. Table 2, 3, 4). With sufficient soil water supply, total soluble amino acid-N
36
37 359 content in the fine roots of *Prunus* was lower competing with *Fagus* than with *Quercus* or *Pinus*
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39 360 (Table 2, Suppl. Table 2, 3, 4). *Prunus* seedlings had a lower total soluble amino acid-N content
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41 361 in the leaves when competing with *Fagus* than with *Quercus*, and a lower total soluble protein-
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43 362 N content in the leaves when competing with *Fagus* or *Quercus* than with *Pinus* (Table 2, Suppl.
44
45 363 Tables 2, 4, 10). *Robinia* seedlings responded to competitor with changes in other parameters
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47 364 than *Prunus*. With drought, *Robinia* seedlings had higher total soluble protein-N content in the
48
49 365 fine roots when competing with *Fagus* than with *Quercus*, and higher total soluble protein-N
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51 366 content in the fine roots competing with *Quercus* than with *Pinus* (Table 2, Suppl. Tables 2, 3,
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53 367 4). Under control conditions, *Robinia* competing with *Fagus* had a lower nitrate net uptake
54
55 368 capacity than when competing with *Pinus* (Fig. 2, Table 2). Both *Prunus* and *Robinia* responded
56
57 369 differently with regard to their biomass and growth indices (Table 2, Suppl. Tables 7, 8, 9, 11).

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59 371 **3.3. N acquisition preferences for different N sources of native and invasive species**

60 372 Preferences for certain N sources were found among species depending on the competitor and
373 373 soil water availability. Generally, organic N, especially arginine-N, was favoured over

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3 374 inorganic N sources. *Quercus* and *Prunus* did not change N preferences with different
4 375 competitors or drought (Tables 3, 4). *Fagus* preferred organic N over inorganic N with drought
5 376 (Table 3). *Pinus* did not prefer specific N sources in competition with *Robinia* with sufficient
6 377 water supply, but preferred organic N over inorganic N with drought and in competition with
7 378 *Prunus* regardless of water availability (Table 3). *Robinia* showed a distinct pattern depending
8 379 on the competitor: With drought, seedlings preferred arginine-N over inorganic N but only in
9 380 competition with *Quercus*, but not in competition with *Fagus* or *Pinus* (Table 4). With sufficient
10 381 water supply, *Robinia* preferred organic over inorganic N when grown in competition with
11 382 *Fagus*, but not with *Quercus* or *Pinus* (Table 4).
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20 384 4. Discussion

21 385 4.1. Drought reduces N acquisition among species, but allocation of N to N pools varies 22 386 with species and competitor

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24 387 In accordance with our hypothesis, inorganic and organic N acquisition of both native and
25 388 invasive tree seedlings overall decreased in response to drought, confirming that tree N
26 389 acquisition and soil water availability are strongly linked (e.g. Fotelli et al. 2002, 2004,
27 390 Rennenberg et al. 2006, Gessler et al. 2017). The influence on organic N acquisition for tree
28 391 species in competition is a novel insight provided by our work, while also confirming previous
29 392 studies in which drought reduced the inorganic N acquisition of *F. sylvatica* seedlings growing
30 393 both in intra- and interspecific competition (Fotelli et al. 2002). In plant communities, when
31 394 soil water availability is reduced, microbial activity is decreased negatively impacting on soil
32 395 N mineralization processes, thus resulting in reduced soil N availability (Schimel et al. 2007,
33 396 Hueso et al. 2012). Furthermore, soil N diffusion and mass flow are reduced (Rennenberg et al.
34 397 2009) and the composition of mycorrhizal communities that symbiotically provide plants with
35 398 N is changed (e.g. Gessler et al. 2005, Leberecht et al. 2016).
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48 400 Our studied tree species ~~appear to showed~~ four different ~~mechanisms to eoperesponses to with~~
49 401 drought regarding the ~~ir~~ allocation of N to N pools in the leaves and fine roots depending on the
50 402 species and competitor: (1) Total soluble protein levels were reduced (in the leaves of *Fagus*
51 403 and *Quercus* regardless of competitor, and in the fine roots of *Prunus* and *Robinia* competing
52 404 with *Pinus*) as a consequence of reduced N acquisition and thus, N assimilation (Gessler et al.
53 405 2017). Storage proteins from vegetative tissue are degraded and remobilized leading to lower
54 406 N in storage (e.g. Millard 1988, Staswick 1994, Millard and Grelet 2010). With potential leaf
55 407 shedding as a consequence of drought and, consequently, N stored in the leaves being lost by
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3 408 the plant, the roots become an important tissue for N storage (Millard and Grelet 2010). In our
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5 409 study, the leaves were not (yet) shed at the time of the harvest, suggesting that N was likely still
6
7 410 remobilized. (2) In the fine roots of *Robinia* in competition with *Fagus*, the levels of total
8
9 411 soluble proteins increased in response to drought likely due to the synthesis of protective
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11 412 proteins (Brunner et al. 2015), proteins with a role in dehydration tolerance (Close 1996,
12
13 413 Kozłowski and Pallardy 2002), and/or proteins required for the development of root nodules
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15 414 for N₂-fixing species (Verma et al. 1992) such as *Robinia*. (3) Soluble amino acid levels
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17 415 decreased in the fine roots of *Prunus* when grown in competition with *Quercus* or *Pinus* in
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19 416 response to drought. This is likely due to amino acid degradation, their translocation to other
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21 417 plant tissues, their usage for protein synthesis, and/or the usage of their carbon skeletons to
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23 418 produce alternative non-N-containing osmotic compounds which not only decrease osmotic
24
25 419 potential but can also function as cell membrane and metabolic protectants (Chaves et al. 2003).
26
27 420 (4) In contrast, total soluble amino acid levels increased in the leaves of *Robinia* and the fine
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29 421 roots of *Pinus* regardless of competitor, as well as in the leaves and fine roots of *Quercus* in
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31 422 competition with *Prunus* via protein degradation to serve as osmoprotectants, thus improving
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33 423 the overall plant water status (Hu et al. 2013, 2013b). Overall, our results indicate that the study
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35 424 ~~different species use show diverse different coping mechanisms in responses~~ to drought
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37 425 conditions in this short-term experiment with regards to N allocation to N pools in leaves and
38
39 426 fine roots. ~~These responses were found~~ in combination with changes in biomass allocation,
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41 427 ~~further highlighting the species-specific responses to drought.~~

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429 Some species showed changes in biomass allocation in response to drought while others did
430
431 not. A higher root:shoot ratio resulting in a larger soil volume to be exploited and ~~in~~ a parallel
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433 decrease in aboveground biomass as well as SLA ~~reduces~~ water loss via the leaves further
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435 improvings a plant's water status (Fotelli et al. 2005, Mantovani et al. 2014, Duan et al. 2018).
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437 This strategy was found in our study for *Robinia*. On the other hand, drought sensitivity of a
438
439 species might be reflected by reduced root growth, eventually leading to a decrease in root:shoot
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441 ratio (Ostonen et al. 2007, Brunner et al. 2015), root hydraulic failure (Mao et al. 2018), and
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443 higher root mortality (Zhou et al. 2018). In our study, drought conditions were severe enough
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445 to cause a decrease in root:shoot ratio for *Fagus* indicating the drought-sensitivity of this
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447 species, but not the other native or the invasive species.

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461 In general, all study species were negatively affected by drought with no clear distinction
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463 between native and invasive species. Native *Fagus* and – to a lesser extent – invasive *Robinia*

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3 442 were most sensitive showing several changes in above- and belowground traits. For *Fagus*, the
4 443 strong response to drought generally prevailed over the effects of the competitor, while for
5 444 *Robinia* it was mediated by the competitor. The ability to fix N₂ allows *Robinia* to obtain
6 445 external N and thus be less affected by the negative effect of drought on N acquisition from the
7 446 soil (Wurzburger and Miniat 2014, Mantovani et al. 2014, 2015). For *Quercus* and invasive
8 447 *Prunus*, responses to drought also varied according to competitor. In contrast, we found no
9 448 interaction between drought and competitor for *Pinus* which indicates that the two invasive
10 449 species did not influence its response to drought. Furthermore, coniferous *Pinus* was generally
11 450 less responsive to drought than the native and invasive deciduous species most likely due to its
12 451 isohydric behaviour, i.e. the closing of stomata early during a drought event, thereby
13 452 minimizing water losses via the needles (Irvine et al. 1998). These results imply that under
14 453 future scenarios of global change, *Pinus* can withstand simultaneous short-term drought stress
15 454 and an invasion by exotic woody species better than *Fagus* and *Quercus*. ~~However, *Quercus*~~
16 455 ~~appears to have an advantage over *Prunus* under drought, again highlighting the specific nature~~
17 456 ~~of the species-species responses.~~

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

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460 Our hypothesis that N acquisition from the soil and its allocation to plant internal N pools vary
461 for a given species depending on its functional traits and the competing species could partly be
462 confirmed. None of the native species and only one of the two invasives responded to different
463 competitors with changes in N acquisition, thus contrasting previous studies (Simon et al. 2010,
464 2014, Bueno et al. 2019). This suggests that soil water availability was a stronger driver of N
465 uptake than competition. The allocation of N to different N pools of our studied species
466 depended on the competitor and varied with soil water availability, thus confirming our
467 hypothesis that responses to different competitors are species-specific according to the
468 physiological characteristics of the study species.

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

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3 476 *Robinia*'s fast growth and occupation of rooting space (Kawaletz et al. 2013, 2014). In our
4 study *Robinia* was the only species with increased root:shoot ratio under drought suggesting a
5 strong potential for below-ground competition and resource gain for metabolic processes,
6 which apparently had a stronger effect on drought-sensitive *Fagus* than the drought-tolerant
7 natives. N acquisition of all three native species did not differ with competitor, and the higher
8 allocation of N to N pools combined with slower growth of *Quercus* and *Pinus* when competing
9 with *Prunus* than *Robinia* suggests an increased storage of N metabolites rather than a use for
10 biomass production (Reich et al. 1997, Millet et al. 2005, Millard and Grelet 2010). Moreover,
11 the negative effects on growth and biomass indices when competing with *Prunus* rather than
12 *Robinia* further indicate negative consequences of competition with *Prunus* for overall plant
13 development.

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24 488 Similar to the native species, the competitor had no effect on N acquisition of *Prunus* indicating
25 that the observed changes in biomass and growth indices as well as N metabolites content
26 occurred in relation to internal N dynamics in the seedlings. This is likely due to remobilization
27 and *de novo* synthesis of N metabolites (Simon et al. 2010, Li et al. 2015), and/or the reliance
28 on N stored in plant tissues as N-based defense compounds (Gleadow and Woodrow 2002).
29 The other responses of invasive *Prunus* to native species depended on soil water availability.
30 For example, drought-sensitive *Fagus* was a stronger competitor for *Prunus* only with sufficient
31 soil water availability whereas this effect was absent with drought, reflecting the drought-
32 sensitivity of *Fagus*. With drought, N pools of *Prunus* were higher in competition with *Pinus*
33 compared to deciduous natives. This indicates a similar mechanism of metabolic adaptation to
34 stress as in the slow growing deciduous *Fagus* and *Quercus* (Millard and Grelet 2010). This
35 might be a differential response of *Prunus* to specific competitors with drought conditions,
36 possibly related to the drought tolerance of such competitors. This would be in accordance with
37 studies indicating that evergreen conifers are on average more drought-tolerant due to their
38 more conservative resource use than broadleaved deciduous temperate woody species (Hallik
39 et al. 2009), though further studies considering a greater number of species are needed to
40 provide additional insights into this.

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55 506 *Robinia* responded to competition with native deciduous *Fagus* and *Quercus* stronger than with
56 coniferous *Pinus*, although the affected specific growth and physiological parameters depended
57 on soil water availability. In contrast to all other study species, nitrate acquisition of *Robinia*
58 was reduced in its competition with *Fagus* compared to competition with *Pinus* suggesting the
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3 510 release of active compounds that potentially impair N acquisition as suggested for *Acer*
4 *pseudoplatanus* in a previous study (Simon et al. 2010). This would negatively affect nutrition
5 511 and development of *Robinia* seedlings compared to competition with other natives because
6 512 although *Robinia* can fix N₂, N acquisition from the soil is preferred over N₂ fixation when soil
7 513 N is not limiting (Pfautsch et al. 2009). These differences in the responses to competitors became
8 514 more apparent when *Robinia* was additionally affected by drought and may be explained by *de*
9 515 *novo* synthesis of proteins as an adaptation to competition (Simon et al. 2010, 2014).
10 516 Additionally, *Robinia* seedlings grew slower in competition with *Fagus* or *Quercus* compared
11 517 to *Pinus* regardless of soil water availability further highlighting their negative competitive
12 518 effects on *Robinia*.
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22 521 ~~For the natives, *Fagus* was more affected by *Robinia*, while *Quercus* and *Pinus* responded~~
23 ~~strongest to invasive *Prunus*. For drought-sensitive *Fagus* competition only mattered when soil~~
24 ~~water availability was sufficient indicating that drought effects overrode the different influence~~
25 ~~of the two invasive competitors. When water was sufficiently available, the overall growth of~~
26 ~~*Fagus* was more affected in competition with *Robinia* than *Prunus*, while N acquisition and~~
27 ~~allocation of N to N-pools in the leaves and fine roots were similar between competitors. A~~
28 ~~negative influence of *Robinia* on the growth of competing tree seedlings has been reported~~
29 ~~before via the depletion of soil resources due to *Robinia*'s fast growth and occupation of rooting~~
30 ~~space (Kawaletz et al. 2013, 2014). Similar to *Fagus*, N acquisition of *Quercus* and *Pinus* did~~
31 ~~not differ with competitor. However, the Their higher allocation of N to N-pools in less~~
32 ~~competitive species combined with slower growth when competing with *Prunus* than *Robinia*~~
33 ~~for *Quercus* and *Pinus* suggests an increased storage of N metabolites rather than a use for~~
34 ~~biomass production with different competitors (Reich et al. 1997, Millet et al. 2005, Millard~~
35 ~~and Grelet 2010).~~
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48 536 ~~Furthermore, the negative effects on growth and biomass indices when competing with *Prunus*~~
49 ~~rather than *Robinia* indicates negative consequences for overall plant development. Despite the~~
50 ~~relative common responses of *Quercus* and *Pinus* to competition with *Prunus* regardless of~~
51 ~~water supply, some responses of *Quercus* were mediated by soil water availability (e.g. higher~~
52 ~~SRL with *Prunus* than *Robinia* under drought, and higher total soluble protein-N content in fine~~
53 ~~roots with *Robinia* than *Prunus* under sufficient water), whereas the responses of *Pinus* were~~
54 ~~entirely independent of soil water availability.~~
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3 544 Similar to the native species, inorganic and organic N acquisition of *Prunus* did not change with
4 competitor indicating that the changes in biomass and growth indices as well as N metabolites
5 545 content occurred in relation to internal N dynamics in the seedlings, probably involving
6 546 processes such as remobilization and *de novo* synthesis of N metabolites (Simon et al. 2010, Li
7 547 et al. 2015), and/or the reliance on N stored in plant tissues as N-based defense compounds
8 548 (Gleadow and Woodrow 2002). The other responses of invasive *Prunus* to native species
9 549 depended on soil water availability, reflecting the physiological characteristics of the native
10 550 competitors related to their drought tolerance. For example, drought sensitive *Fagus* was a
11 551 stronger competitor for *Prunus* only with sufficient soil water availability whereas this effect
12 552 was absent with drought. In contrast, drought-tolerant *Quercus* and *Pinus* negatively affected
13 553 *Prunus* relative to other competitors regardless of soil water availability. With sufficient water
14 554 availability, *Prunus* reduced growth in competition with all native species. This overall
15 555 competitor effect was no longer found for *Prunus* in competition with *Fagus* with drought,
16 556 because drought was a stronger driver of *Fagus* metabolism than competition. With drought, N
17 557 metabolite levels in leaves and fine roots of *Prunus* were higher in competition with *Pinus*
18 558 compared to deciduous tree species indicating that this metabolic adaptation to stress (Millard
19 559 and Grelet 2010) might be a differential response of *Prunus* to specific competitors with drought
20 560 conditions. This would be in accordance with studies indicating that evergreen conifers are on
21 561 average more drought-tolerant than broadleaved deciduous temperate woody species (Hallik et
22 562 al. 2009), though further studies considering a greater number of species are needed to provide
23 563 additional insights into this. In plant communities, coniferous and deciduous species might
24 564 differ in their competitive effects on other plant species due to their differences in nutrient
25 565 concentration in plant tissues, litter quality, and their differential impact on soil
26 566 biogeochemistry (Calder et al. 2011, Wang et al. 2016).

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46 569 *Robinia* responded to competition with deciduous *Fagus* and *Quercus* stronger than with
47 570 coniferous *Pinus*, although the affected specific growth and physiological parameters depended
48 571 on soil water availability. In contrast to all other study species, nitrate acquisition of *Robinia*
49 572 was reduced in its competition with *Fagus* compared to *Pinus* with sufficient soil water
50 573 availability suggesting that *Fagus* is a stronger competitor potentially impairing N acquisition
51 574 via the release of active compounds as suggested for *Acer pseudoplatanus* in a previous study
52 575 (Simon et al. 2010). This would negatively affect nutrition and development of *Robinia*
53 576 seedlings compared to competition with other natives. Although *Robinia* can fix N₂, N
54 577 acquisition from the soil is preferred over N₂ fixation when soil N is not limiting (Pfausch et

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3 578 ~~al. 2009). Thus, the reduced nitrate acquisition in competition with *Fagus* compared to *Pinus*~~
4 ~~might be disadvantageous for *Robinia*. This gradient between competitors became more~~
5 579 ~~apparent when *Robinia* was additionally affected by drought. Total soluble protein-N levels in~~
6 ~~the fine roots were higher in competition with *Fagus* than *Quercus* or *Pinus* indicating *de novo*~~
7 580 ~~synthesis of proteins as an adaptation to competition (Simon et al. 2010, 2014). Additionally,~~
8 581 ~~*Robinia* seedlings grew slower in competition with *Fagus* or *Quercus* compared to *Pinus*~~
9 ~~regardless of soil water availability further highlighting their negative competitive effects on~~
10 582 ~~*Robinia*.~~

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14 586
15 587 Overall, native and invasive species responded to different competitors in accordance with their
16 588 physiological and life history traits such as drought tolerance and leaf habit. For example,
17 589 drought-sensitive *Fagus* responded negatively to different competitors and affected invasive
18 590 species only with sufficient water supply, indicating that drought effects override those of the
19 591 two invasive competitors. Moreover, invasive *Prunus* was generally a stronger competitor for
20 592 the native species ~~inducing higher N metabolite levels in leaves and roots among other effects.~~
21 593 With competition, tissue nutrient content plays an important role in plant performance
22 594 ~~with~~ competition. For example, seedlings of black spruce (*Picea mariana*) with high levels of N (and
23 595 other nutrients) had higher biomass than seedlings with low tissue N content after growing in
24 596 competition with natural vegetation (Malik and Timmer 1998) indicating the importance of
25 597 plant internal N reserves in competitive interactions.

26 598
27 599 **4.3. Organic N was generally preferred by all species regardless of drought**
28 600 We hypothesized that native and invasive species differ in their preference for organic and
29 601 inorganic N sources. However, organic N forms, especially arginine, were generally preferred
30 602 over inorganic N by all study species confirming results from studies in the field and under
31 603 controlled settings (Simon et al. 2017) using the same technique and artificial soil solution as
32 604 here (e.g. for *F. sylvatica*: Dannenmann et al. 2009, Simon et al. 2010, Simon et al. 2011, Simon
33 605 et al. 2014, for *R. pseudoacacia*: Hu et al. 2017), as well as the results found at low soil N
34 606 availability in our previous study using the same species (Bueno et al. 2019). The preferred
35 607 acquisition of specific N sources is generally driven by their higher abundance in the soil (e.g.
36 608 Kuster et al. 2013b, Song et al. 2015, Simon et al. 2017). For native *Fagus* and *Pinus*, as well
37 609 as invasive *Robinia* preferences shifted depending on the competitor (see Tables 3, 4) indicating
38 610 plasticity in resource use induced by the interaction with other species which might influence
39 611 the plant's competitive ability (Ashton et al. 2010). Such shifts were not found for native

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3 612 *Quercus* and invasive *Prunus* indicating that competition does not affect their N form
4 preference. Furthermore, to our knowledge, no previous studies have directly measured the
5 613 effect of drought on the preference for different N forms. We found no changes in the N form
6 614 preference with drought suggesting that changes in N preference for the study species does not
7 615 play a role for adapting to drought, at least under these experimental conditions.
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13 618 **Conclusions**

15 619 In our study, drought generally reduced inorganic and also – shown for the first time – organic
16 620 N acquisition among both native and invasive study species, because reduced water availability
17 621 leads to less N available in the soil for plants. Overall, N acquisition was driven by water supply
18 622 rather than competition. The allocation of N to internal N pools in the leaves and fine roots,
19 623 however, varied with study species and competitor in relation to their physiological
20 624 characteristics (i.e. drought sensitive vs. drought tolerant tree species) showing their respective
21 625 species-specific coping mechanisms (e.g. the remobilization of N from storage and/or synthesis
22 626 of osmoprotectants). For example, within the native tree species, for drought-sensitive *Fagus*,
23 627 reduced water availability overrode the influence of competition, whereas the responses of
24 628 drought-tolerant *Pinus* were entirely independent of water supply. Deciduous and coniferous
25 629 species might respond differently to competition with other plants because of their differences
26 630 in, for example, tissue concentrations of nutrients (Calder et al. 2011, Wang et al. 2016).
27 631 Organic N was generally favoured by both native and invasive species regardless of drought
28 632 suggesting that competition for N was not avoided. Moreover, our results suggest that *Prunus*
29 633 as an invasive species is a stronger competitor than *Robinia* for most native tree species (which
30 634 is in accordance with a previous study (Bueno et al. 2019)), possibly due to its higher biomass.
31 635 In contrast, for *Robinia*, the deciduous natives *Fagus* and *Quercus* had more negative effects
32 636 than the conifer *Pinus*. In conclusion, our results suggest that species-specific mechanisms to
33 637 cope with drought related to their physiological characteristics might play a role for the
34 638 competitive ability of the studied species. However, further studies investigating a larger
35 639 number of species are necessary to confirm this. Because water availability strongly affects
36 640 plant and soil N dynamics (e.g. Gessler et al. 2017, Simon et al. 2017), longer term competition
37 641 (> 1 year) might pronounce the effects of competition seen in our study further.
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643 **Data and Materials Accessibility**

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

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3 646 **Supplementary Data**

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5 647 Supplementary Data is available online.
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7 648

8 649 **Conflict of Interest**

9
10 650 The authors declare no conflict of interest.
11
12 651

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39
40 666

41 667 **Author's contributions**

42
43 668 AB and JS conceived and designed the study. AB conducted the ¹⁵N uptake experiments, N
44
45 669 metabolite analyses, and evaluated all data. KP contributed the IRMS analyses. AB and JS led
46
47 670 the writing of the manuscript. All authors contributed critically to the drafts and gave final
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49 671 approval for publication.
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52 673 **References**

- 53 674 Anderson M, Gorley R, Clarke K (2008) PERMANOVA+ for PRIMER: Guide to Software and
54
55 675 Statistical Methods. Plymouth: PRIMER-E.
56
57 676 Ashton IW, Miller AE, Bowman WD, Suding KN (2010) Niche complementarity due to
58
59 677 plasticity in resource use: plant partitioning of chemical N forms. *Ecology* 91: 3252-3260.
60
678 doi: 10.1890/09-1849.1

- 1
2
3 679 Bista DR, Heckathorn SA, Jayawardena DM, Mishra S, Boldt JK (2018) Effects of drought on
4 nutrient uptake and the levels of nutrient-uptake proteins in roots of drought-sensitive and
5 680 tolerant grasses. *Plants* 7: 28. doi: 10.3390/plants7020028
6 681
7
8 682 Boudsocq S, Niboyet A, Lata JC, Raynaud X, Loeuille N, Mathieu J, Blouin M, Abbadie L,
9 Barot S (2012) Plant preference for ammonium versus nitrate: a neglected determinant of
10 683 ecosystem functioning? *The American Naturalist* 180: 60-69. doi: 10.1086/665997
11 684
12
13 685 Broadbent A, Stevens CJ, Peltzer DA, Ostle NJ, Orwin KH (2018) Belowground competition
14 686 drives invasive plant impact on native species regardless of nitrogen availability. *Oecologia*
15 687 186:577-587. doi: 10.1007/s00442-017-4039-5
16
17
18 688 Brunner I, Herzog C, Dawes MA, Arend M, Sperisen C (2015) How tree roots respond to
19 689 drought. *Front. Plant Sci.* 6:547. doi: 10.3389/fpls.2015.00547
20
21
22 690 Bueno A, Pritsch K, Simon J (2019) Species-specific outcome in the competition for nitrogen
23 691 between invasive and native tree seedlings. *Frontiers in Plant Science* 10:337. doi:
24 692 10.3389/fpls.2019.00337
25
26
27 693 Calder W, Horn K, St. Clair S (2011) Conifer expansion reduces the competitive ability and
28 694 herbivore defense of aspen by modifying light environment and soil chemistry. *Tree*
29 695 *Physiology* 31:582-591.
30
31
32 696 Campagnaro T, Brundub G, Sitzia T (2018) Five major invasive alien tree species in European
33 697 Union forest habitat types of the Alpine and Continental biogeographical regions. *Journal*
34 698 *for Nature Conservation* 43: 227-238. doi: 10.1016/j.jnc.2017.07.007
35
36
37 699 Casper BB, Jackson RB (1997) Plant competition underground. *Annual Review of Ecology,*
38 700 *Evolution, and Systematics* 28: 545-570. doi: 10.1146/annurev.ecolsys.28.1.545
39
40
41 701 Castro-Díez P, Pauchard A, Traveset A, Vilà M (2006) Linking the impacts of plant invasion
42 702 on community functional structure and ecosystem properties. *Journal of Vegetation Science*
43 703 27: 1233-1242. doi: 10.1111/jvs.12429
44
45
46 704 Chaves MM, Maroco JP, Pereira JS (2003) Understanding plant responses to drought – from
47 705 genes to the whole plant. *Functional Plant Biology* 30: 239-264. doi: 10.1071/FP02076
48
49
50 706 Close TJ (1996) Dehydrins: Emergence of a biochemical role of a family of plant dehydration
51 707 proteins. *Physiologia Plantarum* 97: 795-803.
52
53
54 708 Corbin JD, D'Antonio CM (2012) Gone but not forgotten? Invasive plants' legacies on
55 709 community and ecosystem properties. *Invasive Plant Science and Management* 5:117-124.
56 710 doi: 10.1614/IPSM-D-11-00005.1
57
58
59 711 Csiszár A (2009) Allelopathic effects of invasive woody plant species in Hungary. *Acta*
60 712 *Silvatica et Lignaria Hungarica* 5: 9-17.

- 1
2
3 713 Dannenmann M, Simon J, Gasche R, Holst J, Naumann PS, Koegel-Knabner I et al. (2009)
4 714 Tree girdling provides insight on the role of labile carbon in nitrogen partitioning between
5 715 soil microorganisms and adult European beech. *Soil Biology and Biochemistry* 41: 1622-
6 716 1631. doi: 10.1016/j.soilbio.2009.04.024
7
8
9 717 Dawson W (2015) Release from belowground enemies and shifts in root traits as interrelated
10 718 drivers of alien plant invasion success: a hypothesis. *Ecology and Evolution* 5: 4505-4516.
11 719 doi: 10.1002/ece3.1725
12
13 720 Duan H, Huang G, Zhou S, Tissue D (2018) Dry mass production, allocation patterns and water
14 721 use efficiency of two conifers with different water use strategies under elevated [CO₂],
15 722 warming and drought conditions. *European Journal of Forest Research* 137: 605-618. doi:
16 723 10.1007/s10342-018-1128-x
17
18 724 Eaton E, Caudullo G, Oliveira S, de Rigo D (2016) “*Quercus robur* and *Quercus petraea* in
19 725 Europe: distribution, habitat, usage and threats”, in *European Atlas of Forest Tree Species*,
20 726 ed. San-Miguel-Ayanz J, de Rigo D, Caudullo G, Houston Durrant T, Mauri A
21 727 (Luxembourg: Publ. Off. EU), e01c6df+
22
23 728 Ehrenfeld JG (2003) Effects of exotic plant invasions on soil nutrient cycling processes.
24 729 *Ecosystems* 6: 503-523.
25
26 730 Ehrenfeld JG, Kourtev P, Huang W (2001) Changes in soil functions following invasions of
27 731 exotic understory plants in deciduous forests. *Ecological Applications* 11: 1287-1300. doi:
28 732 10.2307/3060920
29
30 733 Ellenberg H, Leuschner C (2010) *Vegetation Mitteleuropas mit den Alpen*, Vol 6. Ulmer,
31 734 Stuttgart, Germany.
32
33 735 Eller CB, Oliveira RS (2017) Effects of nitrogen availability on the competitive interactions
34 736 between an invasive and a native grass from Brazilian cerrado. *Plant and Soil* 410: 63-72.
35 737 doi: 10.1007/s11104-016-2984-0
36
37 738 Fotelli MN, Gessler A, Peuke AD, Rennenberg H (2001) Drought affects the competitive
38 739 interactions between *Fagus sylvatica* seedlings and an early successional species, *Rubus*
39 740 *fruticosus*: responses of growth, water status and $\delta^{13}\text{C}$ composition. *New Phytologist* 151:
40 741 427-435. doi: 10.1046/j.1469-8137.2001.00186.x
41
42 742 Fotelli MN, Rennenberg H, Gessler A (2002) Effects of drought on the competitive interference
43 743 of an early successional species (*Rubus fruticosus*) on *Fagus sylvatica* L. seedlings: ¹⁵N
44 744 uptake and partitioning, responses of amino acids and other N compounds. *Plant Biology* 4:
45 745 311-320. doi: 10.1055/s-2002-32334
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

- 1
2
3 746 Fotelli MN, Rienks M, Rennenberg H, Gessler A (2004) Climate and forest management affect
4 747 ¹⁵N-uptake, N balance and biomass of European beech seedlings. *Trees* 18: 157-166. doi:
5 748 10.1007/s00468-003-0289-4
6
7
8 749 Fotelli MN, Rudolph P, Rennenberg H, Gessler A (2005) Irradiance and temperature affect the
9 750 competitive interference of blackberry on the physiology of European beech seedlings. *New*
10 751 *Phytologist* 165: 453-462. doi: 10.1111/j.1469-8137.2004.01255.x
11
12 752 Fraterrigo JM, Strickland MS, Keiser AD, Bradford MA (2011) Nitrogen uptake and preference
13 753 in a forest understory following invasion by an exotic grass. *Oecologia* 167: 781-791.
14
15 754 Gessler A, Schneider S, von Sengbusch D, Weber P, Hanemann U, Huber C, Rothe A, Kreuzer
16 755 K, Rennenberg H (1998) Field and laboratory experiments on net uptake of nitrate and
17 756 ammonium by the roots of spruce (*Picea abies*) and beech (*Fagus sylvatica*) trees. *New*
18 757 *Phytologist* 138: 275-285.
19
20 758 Gessler A, Kreuzwieser J, Dopatka T, Rennenberg H (2002) Diurnal courses of ammonium net
21 759 uptake by the roots of adult beech (*Fagus sylvatica*) and spruce (*Picea abies*) trees. *Plant*
22 760 *and Soil* 240: 23-32. doi: 10.1023/A:1015831304911
23
24 761 Gessler A, Keitel C, Nahm M, Rennenberg H (2004) Water shortage affects the water and
25 762 nitrogen balance in Central European beech forests. *Plant Biology* 6: 289-298. doi:
26 763 10.1055/s-2004-820878
27
28 764 Gessler A, Jung K, Gasche R, Papen H, Heidenfelder A, Börner E, Metzler B, Augustin S,
29 765 Hildebrand E, Rennenberg H (2005) Climate and forest management influence nitrogen
30 766 balance of European beech forests: microbial N transformations and inorganic N net uptake
31 767 capacity of mycorrhizal roots. *European Journal of Forest Research* 124: 95-111. doi:
32 768 10.1007/s10342-005-0055-9
33
34 769 Gessler A, Schaub M, McDowell NG (2017) The role of nutrients in drought-induced tree
35 770 mortality and recovery. *New Phytologist* 214: 513-520. doi: 10.1111/nph.14340
36
37 771 Gleadow RM, Woodrow IE (2002) Constraints on effectiveness of cyanogenic glycosides in
38 772 herbivore defense. *Journal of Chemical Ecology* 28: 1297-1309.
39
40 773 Grotkopp E, Rejmánek M (2007) High seedling relative growth rate and specific leaf area are
41 774 traits of invasive species: phylogenetically independent contrasts of woody angiosperms.
42 775 *American Journal of Botany* 94: 526-532. doi: 10.3732/ajb.94.4.526
43
44 776 Grubb PJ, Lee WG, Kollmann J, Wilson JB (1996) Interaction of irradiance and soil nutrient
45 777 supply on growth of seedlings of ten European tall-shrub species and *Fagus sylvatica*.
46 778 *Journal of Ecology* 84: 827-840. doi:10.2307/2960555
47
48
49
50
51
52
53
54
55
56
57
58
59
60

- 1
2
3 779 Hallik L, Niinemets Ü, Wright IJ (2009) Are species shade and drought tolerance reflected in
4 780 leaf-level structural and functional differentiation in Northern Hemisphere temperate woody
5 781 flora? *New Phytologist* 184: 257-274. doi: 10.1111/j.1469-8137.2009.02918.x
6
7
8 782 Holmes TP, Aukema JE, Von Holle B, Liebhold A, Sills E (2009) Economic impacts of
9 783 invasive species in forests - past, present, and future. *Annals of the New York Academy of*
10 784 *Sciences* 1162: 18-38. doi: 10.1111/j.1749-6632.2009.04446.x
11
12
13 785 Houston T, de Rigo D, Caudullo G (2016) “*Fagus sylvatica* and other beeches in Europe:
14 786 distribution, habitat, usage and threats”, in *European Atlas of Forest Tree Species*, ed. San-
15 787 Miguel-Ayanz J, de Rigo D, Caudullo G, Houston T, Mauri A (Luxembourg: Publ. Off. EU),
16 788 e012b90+
17
18
19
20 789 Hu B, Simon J, Rennenberg H (2013) Drought and air warming affect the species-specific levels
21 790 of stress-related foliar metabolites of three oak species on acidic and calcareous soil. *Tree*
22 791 *Physiology* 33 (5), 489-504, doi: 10.1093/treephys/tpt025
23
24
25 792 Hu B, Simon J, Kuster TM, Arend M, Siegwolf R, Rennenberg H (2013b) Nitrogen partitioning
26 793 in oak leaves depends on species, provenance, climate conditions, and soil type. *Plant*
27 794 *Biology* 15 (Suppl. 1): 198-209. doi: 10.1111/j.1438-8677.2012.00658.x.
28
29
30 795 Hu B, Zhou M, Dannenmann M, Saiz G, Simon J, Bilela S, Liu X, Hou L, Chen H, Zhang S,
31 796 Butterbach-Bahl K, Rennenberg H (2017) Comparison of nitrogen nutrition and soil carbon
32 797 status of afforested stands established in degraded soil of the Loess Plateau, China. *Forest*
33 798 *Ecology and Management* 389: 46-58.
34
35
36
37 799 Huangfu C, Li H, Chen X, Liu H, Wang H, Yang D (2016) Response of an invasive plant,
38 800 *Flaveria bidentis*, to nitrogen addition: a test of form-preference uptake. *Biological*
39 801 *Invasions* 18: 3365-3380.
40
41
42
43 802 Hueso S, García C, Hernández T (2012) Severe drought conditions modify the microbial
44 803 community structure, size and activity in amended and unamended soils. *Soil Biology and*
45 804 *Biochemistry* 50: 167e173.
46
47
48 805 Irvine J, Perks MP, Magnani F, Grace J (1998) The response of *Pinus sylvestris* to drought:
49 806 stomatal control of transpiration and hydraulic conductance. *Tree Physiology* 18: 393-402.
50
51
52 807 Kawaletz H, Mölder I, Zerbe S, Annighöfer P, Terwei A, Ammer C (2013) Exotic tree seedlings
53 808 are much more competitive than natives but show underyielding when growing together.
54 809 *Plant Ecology* 6: 305-315. doi: 10.1093/jpe/rts044
55
56
57 810 Kawaletz H, Mölder I, Annighöfer P, Terwei A, Zerbe S, Ammer C (2014) Back to the roots:
58 811 how do seedlings of native tree species react to the competition by exotic species? *Annals*
59 812 *of Forest Science* 71: 337-347. doi: 10.1007/s13595-013-0347-z

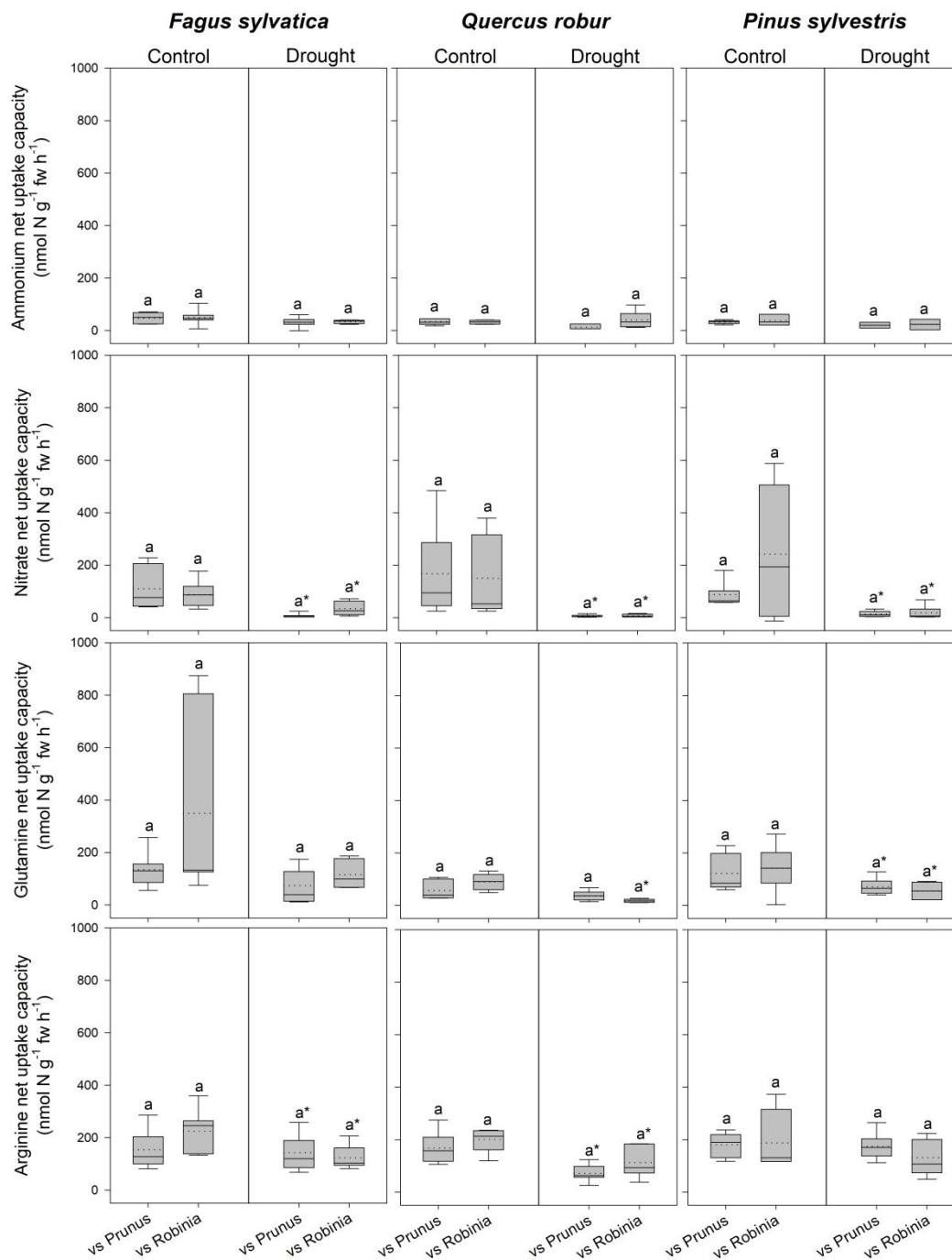
- 1
2
3 813 [Kitajima K \(1994\) Relative importance of photosynthetic traits and allocation patterns as](#)
4 [correlates of seedling shade tolerance of 13 tropical trees. *Oecologia* 98: 419-428.](#)
5
6 815 Kozłowski TT, Pallardy SG (2002) Acclimation and adaptive responses of woody plants to
7
8 816 environmental stresses. *The Botanical Review* 68: 270-334.
9
10 817 Kreuzwieser J, Furniss S, Rennenberg H (2002) Impact of waterlogging on the N-metabolism
11
12 818 of flood tolerant and non-tolerant tree species. *Plant, Cell and Environment* 25: 1039-1049.
13
14 819 Kuster TM, Arend M, Günthardt-Goerg MS, Schulin R (2013) Root growth of different oak
15
16 820 provenances in two soils under drought stress and air warming conditions. *Plant and Soil*
17
18 821 369: 61-71. doi: 10.1007/s11104-012-1541-8
19
20 822 Kuster TM, Schleppei P, Hu B, Schulin R, Günthardt-Goerg MS (2013b) Nitrogen dynamics in
21
22 823 oak model ecosystems subjected to air warming and drought on two different soils. *Plant*
23
24 824 *Biology* 15 (Suppl. 1): 220-229. doi:10.1111/j.1438-8677.2012.00686.x
25
26 825 Lamarque LJ, Delzon S, Lortie CJ (2011) Tree invasions: a comparative test of the dominant
27
28 826 hypotheses and functional traits. *Biological Invasions* 13:1969-1989. doi: 10.1007/s10530-
29
30 827 011-0015-x
31
32 828 [Lambers H, Poorter H \(1992\) Inherent variation in growth rate between higher plants: a search](#)
33
34 829 [for physiological causes and ecological consequences. *Advances in Ecological Research* 23:](#)
35
36 830 [187-261. doi: 10.1016/S0065-2504\(03\)34004-8](#)
37
38 831 Leberecht M, Tu J, Polle A (2016) Acid and calcareous soils affect nitrogen nutrition and
39
40 832 organic nitrogen uptake by beech seedlings (*Fagus sylvatica* L.) under drought, and their
41
42 833 ectomycorrhizal community structure. *Plant and Soil* 409:143-157. doi: 10.1007/s11104-
43
44 834 016-2956-4
45
46 835 Leishman MR, Haslehurst T, Ares A, Baruch Z (2007) Leaf trait relationships of native and
47
48 836 invasive plants: community- and global-scale comparisons. *New Phytologist* 176: 635-643.
49
50 837 Li X, Rennenberg H, Simon J (2015) Competition for nitrogen between *Fagus sylvatica* and
51
52 838 *Acer pseudoplatanus* seedlings depends on soil nitrogen availability. *Frontiers in Plant*
53
54 839 *Science* 6: 302. doi: 10.3389/fpls.2015.00302
55
56 840 Littschwager J, Lauerer M, Blagodatskaya E, Kuzyakov Y (2010) Nitrogen uptake and
57
58 841 utilisation as a competition factor between invasive *Duchesnea indica* and native *Fragaria*
59
60 842 *vesca*. *Plant and Soil* 331: 105-114. doi: 10.1007/s11104-009-0236-2
843 Liu Y, van Kleunen M (2017) Responses of common and rare aliens and natives to nutrient
844 availability and fluctuations. *Journal of Ecology* 105: 1111-1122. doi: 10.1111/1365-
845 2745.12733

- 1
2
3 846 Liu XP, Grams T, Matyssek R, Rennenberg H (2005) Effects of elevated pCO₂ and/or pO₃ on
4 847 C-, N-, and S-metabolites in the leaves of juvenile beech and spruce differ between trees
5 848 grown in monoculture and mixed culture. *Plant Physiology and Biochemistry* 43: 147-154.
6
7
8 849 Malik V, Timmer VR (1998) Biomass partitioning and nitrogen retranslocation in black spruce
9 850 seedlings on competitive mixedwood sites: a bioassay study. *Canadian Journal of Forest*
10 851 *Research* 28: 206-215. doi: 10.1139/x97-207
11
12
13 852 Mantovani D, Veste M, Freese D (2014) Effects of drought frequency on growth performance
14 853 and transpiration of young black locust (*Robinia pseudoacacia* L.). *International Journal of*
15 854 *Forestry Research* 821891. doi: 10.1155/2014/821891
16
17
18 855 Mantovani D, Veste M, Boldt-Burisch K, Fritsch S, Koning LA, Freese D (2015) Carbon
19 856 allocation, nodulation, and biological nitrogen fixation of black locust (*Robinia*
20 857 *pseudoacacia* L.) under soil water limitation. *Annals of Forest Research* 58: 259-274. doi:
21 858 10.15287/afr.2015.420
22
23
24 859 Mao W, Felton AJ, Ma Y, Zhang T, Sun Z, Zhao X, Smith MD (2018) Relationships between
25 860 aboveground and belowground trait responses of a dominant plant species to alterations in
26 861 watertable depth. *Land Degradation and Development* 29: 4015-4024.
27
28
29 862 Mason RAB, Cooke J, Moles AT, Leishman MR (2008) Reproductive output of invasive versus
30 863 native plants. *Global Ecology and Biogeography* 17: 633-640.
31
32
33 864 McDowell SCL (2002) Photosynthetic characteristics of invasive and noninvasive species of
34 865 *Rubus* (Rosaceae). *American Journal of Botany* 89: 1431-1438.
35
36
37 866 McKane R, Johnson L, Shaver G, Nadelhoffer K, Rastetter E, Fry B, Giblin A, Kielland K,
38 867 Kwiatowski B, Laundre J, Murray G (2002) Resource-based niches provide a basis for plant
39 868 species diversity and dominance in arctic tundra. *Nature* 412: 68-71.
40
41
42 869 Millard P (1988) The accumulation and storage of nitrogen by herbaceous plants. *Plant, Cell*
43 870 *and Environment* 11: 1-8. doi: 10.1111/j.1365-3040.1988.tb01769.x
44
45
46 871 Millard P, Grelet GA (2010) Nitrogen storage and remobilization by trees: ecophysiological
47 872 relevance in a changing world. *Tree Physiology* 30: 1083-1095. doi:
48 873 10.1093/treephys/tpq042
49
50
51 874 Millet J, Millard P, Hester AJ, McDonald AJS (2005) Do competition and herbivory alter the
52 875 internal nitrogen dynamics of birch saplings? *New Phytologist* 168: 413-422.
53
54
55 876 Näsholm T, Kielland K, Ganeteg U (200) Uptake of organic nitrogen by plants. *New*
56 877 *Phytologist* 182: 31-48. doi: 10.1111/j.1469-8137.2008.02751.x
57
58
59 878 Nilsen P, Børja I, Knutsen H, Brean R (1998) Nitrogen and drought effects on ectomycorrhizae
60 879 of Norway spruce [*Picea abies* L.(Karst.)]. *Plant and Soil* 198: 179-184.

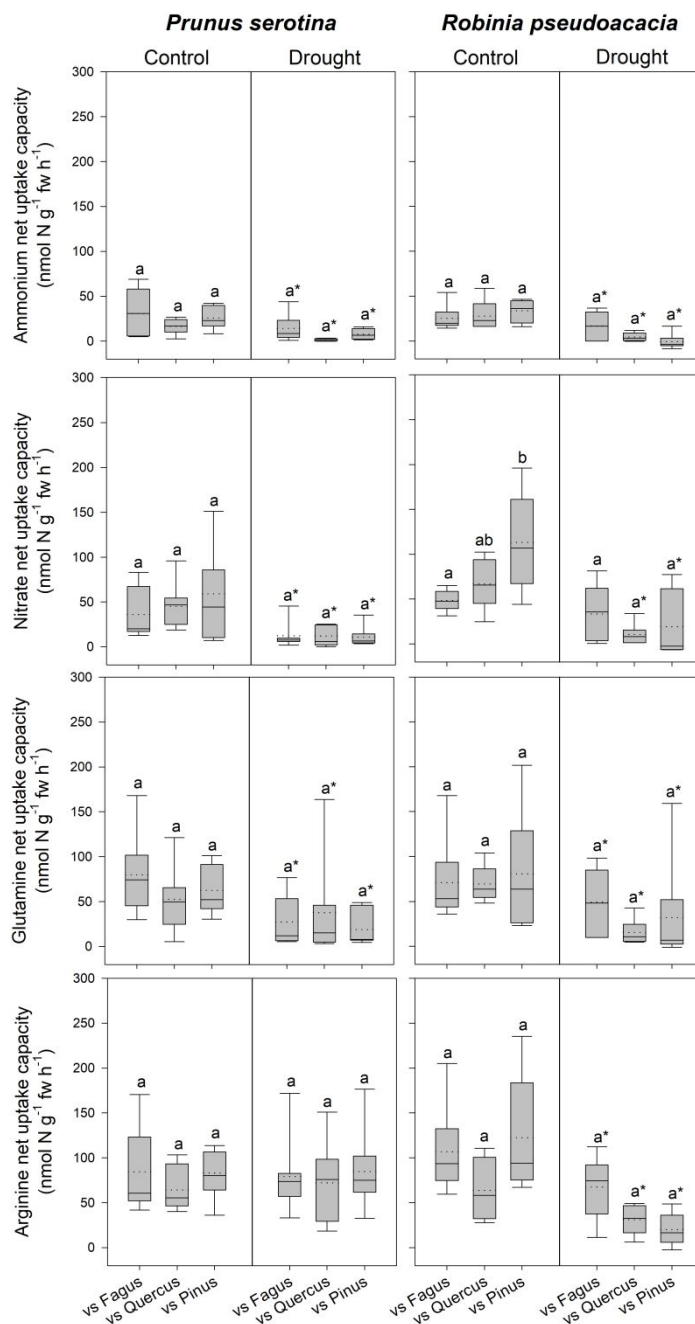
- 1
2
3 880 Ostonen I, Püttsepp Ü, Biel C, Alberton O, Bakker MR, Lõhmus K, Majdi H, Metcalfe D,
4
5 881 Olsthoorn AFM, Pronk A, Vanguelova E, Weih M, Brunner I (2007) Specific root length as
6
7 882 an indicator of environmental change. *Plant Biosystems* 141: 426-442. doi:
8
9 883 10.1080/11263500701626069
- 10 884 Pattison RR, Goldstein G, Ares A (1998) Growth, biomass allocation and photosynthesis of
11
12 885 invasive and native Hawaiian rainforest species. *Oecologia* 117:449-459.
- 13 886 Pfautsch S, Rennenberg H, Bell TL, Adams MA (2009) Nitrogen uptake by *Eucalyptus regnans*
14
15 887 and *Acacia* spp. – preferences, resource overlap and energetic costs. *Tree Physiology* 29:
16
17 888 389-399.
- 18 889 Reich PB (2014) The world-wide ‘fast–slow’ plant economics spectrum: a traits manifesto.
19
20 890 *Journal of Ecology* 102: 275-301. doi: 10.1111/1365-2745.12211
- 21 891 Reich PB, Walters MB, Ellsworth DS (1997) From tropics to tundra: Global convergence in
22
23 892 plant functioning. *Proceedings of the National Academy of Sciences* 94: 13730-13734. doi:
24
25 893 10.1073/pnas.94.25.13730
- 26 894 Rennenberg H, Loreto F, Polle A, Brilli F, Fares S, Beniwal RS, Gessler A (2006) Physiological
27
28 895 responses of forest trees to heat and drought. *Plant Biology* 8: 556-571. doi: 10.1055/s-2006-
29
30 896 924084
- 31 897 Rennenberg H, Dannenmann M, Gessler A, Kreuzwieser J, Simon J, Papen H (2009) Nitrogen
32
33 898 balance in forest soils: nutritional limitation of plants under climate change stresses. *Plant*
34
35 899 *Biology* 11: 4-23. doi: 10.1111/j.1438-8677.2009.00241.x
- 36 900 Ricciardi A (2007) Are modern biological invasions an unprecedented form of global change?
37
38 901 *Conservation Biology* 21: 329-336. doi: 10.1111/j.1523-1739.2006.00615.x
- 39 902 Robakowski P, Bielinis E, Stachowiak J, Mejza I, Bułaj B (2016) Seasonal changes affect root
40
41 903 prunasin concentration in *Prunus serotina* and override species interactions between *P.*
42
43 904 *serotina* and *Quercus petraea*. *Journal of Chemical Ecology* 42: 202-214. doi:
44
45 905 10.1007/s10886-016-0678-y
- 46 906 Schimel J, Balsler TC, Wallenstein M (2007) Microbial stress-response physiology and its
47
48 907 implications for ecosystem function. *Ecology* 88: 1386-1394.
- 49 908 Simon J, Waldhecker P, Brüggemann N, Rennenberg H (2010) Competition for nitrogen
50
51 909 sources between European beech (*Fagus sylvatica*) and sycamore maple (*Acer*
52
53 910 *pseudoplatanus*) seedlings. *Plant Biology* 12: 453-458. doi: doi.org/10.1111/j.1438-
54
55 911 8677.2009.00225.x

- 1
2
3 912 Simon J, Dannenmann M, Gasche R, Holst J, Mayer H, Papen H, et al. (2011) Competition for
4 nitrogen between adult European beech and its offspring is reduced by avoidance strategy.
5 913 Forest Ecology and Management 262: 105-114. doi: 10.1016/j.foreco.2011.01.035
6 914
7
8 915 Simon J, Li X, Rennenberg H (2014) Competition for nitrogen between European beech and
9 sycamore maple shifts in favour of beech with decreasing light availability. Tree Physiology
10 916 34: 49-60. doi: 10.1093/treephys/tpt112
11 917
12
13 918 Simon J, Dannenmann M, Pena R, Gessler A, Rennenberg H (2017) Nitrogen nutrition of beech
14 forests in a changing climate: importance of plant-soil-microbe water, carbon, and nitrogen
15 919 interactions. Plant and Soil 418: 89-114. doi: 10.1007/s11104-017-3293-y
16 920
17 921 Sohn JA, Hartig F, Kohler M, Huss J, Bauhus J (2016) Heavy and frequent thinning promotes
18 drought adaptation in *Pinus sylvestris* forests. Ecological Applications 26: 2190-2205.
19 922
20 923 Song M, Zheng L, Suding KN, Yin T, Yu F (2015) Plasticity in nitrogen form uptake and
21 preference in response to long-term nitrogen fertilization. Plant and Soil 394: 215-224. doi:
22 924 10.1007/s11104-015-2532-3
23 925
24 926 Spinoni J, Vogt JV, Naumann G, Barbosa P, Dosio A (2017) Will drought events become more
25 frequent and severe in Europe? International Journal of Climatology 38: 1718-1736. doi:
26 927 10.1002/joc.5291
27 928
28 929 Staswick PE (1994) Storage proteins of vegetative plant tissues. Annual Review of Plant
29 Physiology and Plant Molecular Biology 45: 303-22.
30 930
31 931 Verma DPS, Hu CA, Zhang M (1992) Root nodule development: origin, function and regulation
32 of nodulin genes. Physiologia Plantarum 85: 253-265.
33 932
34 933 Vilà M, Espinar JL, Hejda M, Hulme PE, Jarosik V, Maron JL, et al. (2011) Ecological impacts
35 of invasive alien plants: a meta-analysis of their effects on species, communities and
36 934 ecosystems. Ecology Letters 14: 702-708. doi: 10.1111/j.1461-0248.2011.01628.x
37 935
38 936 Vítková M, Müllerová J, Sádlo J, Pergl J, Pyšek P (2017) Black locust (*Robinia pseudoacacia*)
39 beloved and despised: a story of an invasive tree in Central Europe. Forest Ecology and
40 937 Management 384: 287-302. doi: 10.1016/j.foreco.2016.10.057
41 938
42 939 Vitousek PM, Aber JD, Howarth RW, Likens GE, Matson PA, Schindler DW, Schlesinger WH,
43 940 Tilman DG (1997) Human alteration of the global nitrogen cycle: sources and consequences.
44 941 Ecological Applications 7: 737-750.
45 942
46 943 Wang H, Liu S, Wang J, Shi Z, Xu J, Hong P, Ming A, Yu H, Chen L, Lu L, Cai D (2016)
47 Differential effects of conifer and broadleaf litter inputs on soil organic carbon chemical
48 944 composition through altered soil microbial community composition. Scientific Reports 6:
49 945 27097.
50
51
52
53
54
55
56
57
58
59
60

- 1
2
3 946 Wardle DA, Peltzer DA (2017) Impacts of invasive biota in forest ecosystems in an
4 aboveground–belowground context. *Biological Invasions* 19: 3301-3316. doi:
5 947 10.1007/s10530-017-1372-x
6 948
7
8 949 Winter H, Lohaus G, Heldt HW (1992) Phloem transport of aminoacids in relation to their
9 cytosolic levels in barley leaves. *Plant Physiology* 99: 996-1004. doi: 10.1104/pp.99.3.996
10 950
11 951 Wurzbürger N, Miniat CF (2014) Drought enhances symbiotic dinitrogen fixation and
12 competitive ability of a temperate forest tree. *Oecologia* 174: 1117-1126. doi:
13 952 10.1007/s00442-013-2851-0
14 953
15 954 Zhou G, Zhou X, Nie Y, Bai SH, Zhou L, Shao J, Cheng W, Wang J, Hu F, Fu Y (2018)
16 Drought-induced changes in root biomass largely result from altered root morphological
17 traits: Evidence from a synthesis of global field trials. *Plant, Cell and Environment* 41: 2589-
18 955 2599. doi: 10.1111/pce.13356
19 956
20 957
21
22
23
24 958



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



968

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

977 Table 1. Effects of drought on total biomass, growth indices, inorganic and organic N net uptake capacity, and N metabolite levels on seedlings of
 978 *Fagus sylvatica*, *Quercus robur*, *Pinus sylvestris*, *Prunus serotina*, and *Robinia pseudoacacia* growing in competition. ↑ = significant increase with
 979 drought, ↓ = significant decrease with drought, n.s. = no significant differences between drought and control. Total biomass (g dw), root:shoot ratio:
 980 ratio of belowground to aboveground biomass, RGR: relative growth rate (g dw g⁻¹ dw d⁻¹), SLA: specific leaf area (cm² g⁻¹ dw), SRL: specific root
 981 length (cm g⁻¹ dw), N net uptake capacity (nmol N g⁻¹ fw h⁻¹), NH₄⁺: ammonium, NO₃⁻: nitrate, Gln-N: glutamine-N, Arg-N: arginine-N, total soluble
 982 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 biomass	Root:shoot ratio	RGR	SLA	SRL	NH ₄ ⁺	NO ₃ ⁻	Gln-N	Arg-N	Total soluble amino acid-N		Total soluble protein-N	
										Leaves	Fine roots	Leaves	Fine roots
<i>Fagus sylvatica</i>	n.s.	↓	↑(b)	↓	n.s.	n.s.	↓	n.s.	↓	n.s.	n.s.	↓	n.s.
<i>Quercus robur</i>	n.s.	n.s.	n.s.	n.s.	↓(b)	n.s.	↓	↓(b)	↓	↑(d)	↑(d)	↓	n.s.
<i>Pinus sylvestris</i>	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	↓	↓	n.s.	n.s.	↑	n.s.	n.s.
<i>Prunus serotina</i>	↓(a)	n.s.	n.s.	↑	n.s.	↓	↓	↓	n.s.	n.s.	↓(c)	n.s.	↓
<i>Robinia pseudoacacia</i>	n.s.	↑	n.s.	n.s.	n.s.	↓	↓(c)	↓	↓	↑	n.s.	n.s.	↑(e) - ↓(f)

984 (a): only in competition with *Quercus*

985 (b): only in competition with *Robinia*

986 (c): only in competition with *Quercus* or *Pinus*

987 (d): only in competition with *Prunus*

988 (e): only in competition with *Fagus*

989 (f): only in competition with *Pinus*

990 No letter: effect of drought regardless of competitor

993 Table 2. Effects of competitor on total biomass, growth indices, inorganic and organic N net uptake capacity, and N metabolite levels of *Fagus*
 994 *sylvatica*, *Quercus robur*, *Pinus sylvestris*, *Prunus serotina*, and *Robinia pseudoacacia* seedlings. Species A < species B = seedlings competing with
 995 species A had significantly lower values than seedlings competing with species B, n.s. = no significant differences between competitor identities.
 996 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
 997 area (cm² g⁻¹ dw), SRL: specific root length (cm g⁻¹ dw), N net uptake capacity (nmol N g⁻¹ fw h⁻¹), NH₄⁺: ammonium, NO₃⁻: nitrate, Gln-N: glutamine-
 998 N, Arg-N: arginine-N, total soluble amino acid-N (mg g⁻¹ dw), total soluble protein-N (mg g⁻¹ dw).

999

	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
<i>Fagus sylvatica</i>	n.s.	n.s.	<i>Robinia</i> < <i>Prunus</i> (a)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
<i>Quercus robur</i>	n.s.	<i>Prunus</i> < <i>Robinia</i>	<i>Prunus</i> < <i>Robinia</i>	n.s.	<i>Robinia</i> < <i>Prunus</i> (b)	n.s.	n.s.	n.s.	n.s.	n.s.	<i>Robinia</i> < <i>Prunus</i>	n.s.	<i>Prunus</i> < <i>Robinia</i> (a)
<i>Pinus sylvestris</i>	<i>Prunus</i> < <i>Robinia</i>	n.s.	n.s.	-	n.s.	n.s.	n.s.	n.s.	n.s.	<i>Prunus</i> < <i>Robinia</i>	<i>Robinia</i> < <i>Prunus</i>	<i>Robinia</i> < <i>Prunus</i>	n.s.
<i>Prunus serotina</i>	<i>Fagus</i> , <i>Pinus</i> < <i>Quercus</i> (a)	n.s.	<i>Quercus</i> , <i>Pinus</i> < <i>Fagus</i>	<i>Quercus</i> < <i>Fagus</i> < <i>Pinus</i>	<i>Quercus</i> < <i>Fagus</i> (a)	n.s.	n.s.	n.s.	n.s.	<i>Fagus</i> < <i>Quercus</i>	<i>Fagus</i> < <i>Quercus</i> , <i>Pinus</i> (a) <i>Quercus</i> < <i>Pinus</i> (b)	<i>Fagus</i> , <i>Quercus</i> < <i>Pinus</i> (b)	n.s.
<i>Robinia pseudoacacia</i>	n.s.	n.s.	<i>Fagus</i> , <i>Quercus</i> < <i>Pinus</i>	n.s.	n.s.	n.s.	<i>Fagus</i> < <i>Pinus</i> (a)	n.s.	n.s.	n.s.	n.s.	n.s.	<i>Pinus</i> < <i>Quercus</i> < <i>Fagus</i> (b)

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1001 (a): only under control conditions

1002 (b): only with drought

1003 No letter: effect of competitor regardless of drought

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1005 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
 1006 of *Fagus sylvatica*, *Quercus robur*, and *Pinus sylvestris* seedlings grown in competition under drought and control conditions. Only significant
 1007 differences are presented. n.s. = no significant differences between net uptake capacity of different N forms.

Competitor	Water availability	<i>Fagus sylvatica</i>	<i>Quercus robur</i>	<i>Pinus sylvestris</i>
<i>Prunus serotina</i>	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^-
<i>Robinia pseudoacacia</i>	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^-

1009

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2
3 1010 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
4 1011 of *Prunus serotina* and *Robinia pseudoacacia* seedlings grown in competition under drought and control conditions. Only significant differences are
5 1012 presented. n.s. = no significant differences between net uptake capacity of different N forms.
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Competitor	Water availability	<i>Prunus serotina</i>	<i>Robinia pseudoacacia</i>
<i>Fagus sylvatica</i>	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^-
<i>Quercus robur</i>	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.
<i>Pinus sylvestris</i>	Drought	Arg-N > NH_4^+ , NO_3^- , Gln-N	n.s.
	Control	Gln-N, Arg-N > NH_4^+	n.s.

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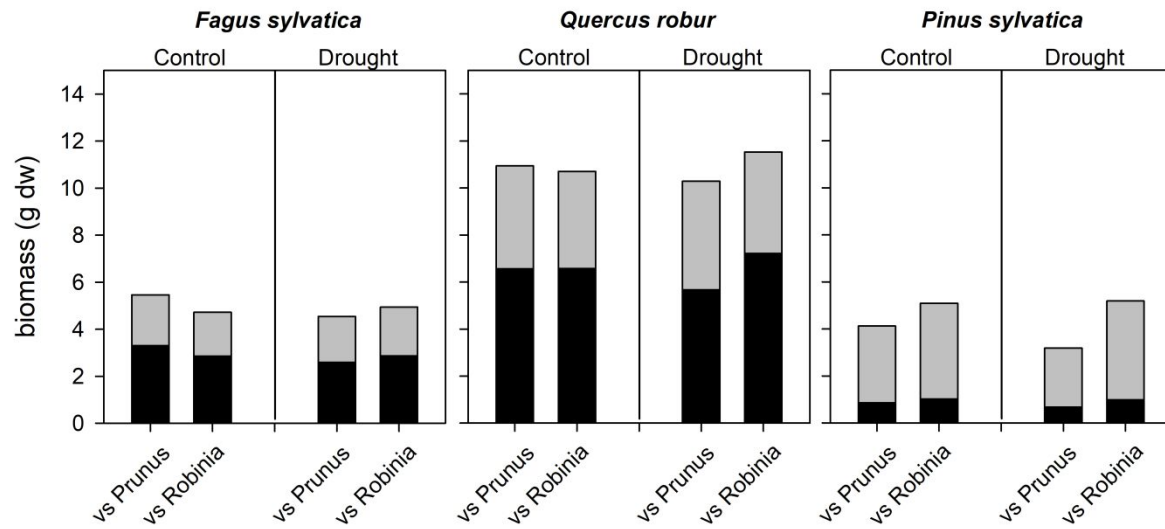
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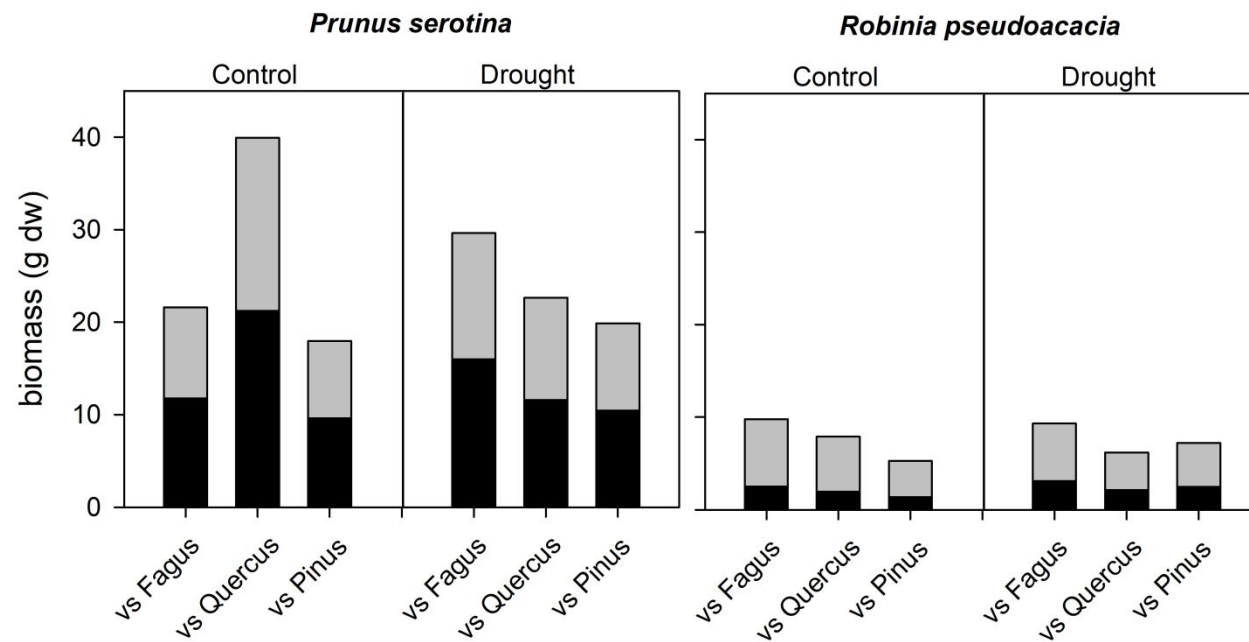
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1022 Supplemental Figure 1a. Root (black) and shoot (gray) biomass (g dw) of *Fagus sylvatica*, *Quercus robur* and *Pinus sylvestris* seedlings at control
 1023 and drought conditions under different competition regimes. vs *Prunus* = competition with *Prunus serotina*; vs *Robinia* = competition with *Robinia*
 1024 *pseudoacacia*.

1025



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*.