Competition for nitrogen between native woody seedlings and invasive plants under abiotic stress

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Summary

Global anthropogenic change components such as plant invasion, increased atmospheric N deposition and severer droughts are expected to affect plant community structure, composition and functioning. Thus, the effects of competition between native and invasive plant species and how it is modulated by the abiotic environment has been the focus of much research, mostly focusing on responses of plant growth but to a lesser degree on tree physiological performance. Specifically, the underlying mechanisms of competition in relation to growth limiting nitrogen (N) have so far been rarely explored.

I used greenhouse experiments to investigate the responses of native tree seedlings and invasive trees and grasses to competition, high soil N availability and drought with regards to plant growth, organic and inorganic N acquisition and allocation to N pools. In the first experiment, I considered Australian subtropical native tree species used in forest reforestation and invasive grasses which frequently hinder the restoration efforts, and investigated the effect of soil N availability in the competitive interaction. In the second and third experiments, I selected native tree species from Central European forests and two important woody invasives, and used the same set of species to evaluate separately the effects of soil N and soil water supply on the competition.

I found general responses to biotic and abiotic stressors across native and invasive species which could be linked to species functional traits and physiological characteristics, and with the life form of the invasive species playing an important role. Responses to competitors across species included reduced biomass, relative growth rates and root biomass relative to shoot biomass, as well as higher specific leaf area and specific root length, as well as changes in N acquisition and plant internal N pools, indicating negative effects on plant growth, N internal dynamics and responses of morphological traits aimed at increasing resource acquisition. Furthermore, higher soil N availability and drought had similar general effects on native and invasive species, which were in some cases enhanced by competition. Specifically, soil N and water availability were strong drivers of N intake, overriding the various physiological differences between species. Moreover, high N supply and drought induced various changes in N pools, in organ- and species-specific patterns, in general pointing at the accumulation of osmoprotectants under drought, but reduction in N pools under non-limiting N supply in the case of high soil N availability. Overall, my results highlight the species-specific nature of responses to combined biotic and abiotic stressors in native and invasive species, and the importance of species life form, life history and growth strategies, as well as their different susceptibilities to specific changes in the abiotic environment. Such species-specific strategies in response to biotic and abiotic stressors must be taken into account in the design of management practices aiming at the control of invasive species in forest ecosystems.

Zusammenfassung

Globale Veränderungen durch anthropogene Einflüsse, wie die Invasion von gebietsfremden Pflanzenarten, vermehrte Anreicherung von atmosphärischem N und das häufigere Auftreten von Dürreperioden, verändern mutmaßlich die Struktur, Zusammensetzung und Funktion von Pflanzengesellschaften. Auf Grund dessen, hat die Auswirkung von Konkurrenz zwischen nativen und invasiven Pflanzenarten und deren Anpassungsmechanismen, abhängig von abiotischen Umweltfaktoren, erhebliche Aufmerksamkeit erhalten. Diese Studien konzentrierten sich hauptsächlich auf deren Reaktionen in Hinblick auf das Pflanzenwachstum, wohingegen den zugrundeliegenden physiologischen Prozessen im Baum weniger Beachtung geschenkt wurde. Besonders die zugrundeliegenden Mechanismen, die den Zusammenhang von Konkurrenz und wachstumslimitierendem Stickstoff (N) erklären, wurden bislang nur unzureichend untersucht.

Ich habe verschiedene Gewächshausexperiment durchgeführt, um die Reaktion von nativen Baumsetzlingen und invasiven Bäumen und Gräsern auf Konkurrenz, höhere N-Verfügbarkeit im Boden und Trockenstress zu untersuchen. Das Hauptaugenmerk lag dabei auf dem Wachstum, der Aufnahme von organischen und anorganischen N-Quellen, sowie deren Verteilung in verschiedenen N-Pools innerhalb des Baumes. Im ersten Experiment habe ich untersucht, wie sich die N-Verfügbarkeit im Boden auf die Konkurrenzbeziehungen zwischen nativen subtropischen australischen Baumarten, welche für die Wiederaufforstung verwendet werden, und invasiven Gräsern, die den Wiederaufforstungserfolg vermindern, auswirken. Für das zweite und dritte Experiment habe ich native mitteleuropäische, sowie zwei wichtige invasive Baumarten ausgewählt, um die Auswirkungen von hohem N-Gehalt im Boden oder Trockenstress auf deren Konkurrenzverhalten zu untersuchen.

Ich konnte allgemeine Reaktionen auf biotische und abiotische Stressfaktoren bei einheimischen und invasiven Arten aufzeigen, die mit den funktionellen Merkmalen und physiologischen Eigenschaften der Arten und der Lebensform der invasiven Arten zusammenhängen. Die Reaktionen auf Konkurrenz zeigten sich durch reduzierte Biomasse, relative Wachstumsrate und geringeres Wurzel:Spross-Verhältnis, höhere spezifische Blattfläche und spezifische Wurzellänge. Des Weiteren konnten Veränderungen in der N-Aufnahme und –Verteilung in den verschiedenen N-Pools nachgewiesen werden. Diese Ergebnisse lassen darauf schließen, dass sich Konkurrenz negativ auf das Wachstum von Pflanzen und interne N-Dynamiken auswirkt und Veränderungen der morphologischen

Pflanzenmerkmale auf eine Verbesserung der Ressourcennutzung abzielen. Außerdem zeigen eine erhöhte N-Verfügbarkeit und Trockenstress denselben Effekt auf native und invasive Arten. Konkurrenz verstärkt diesen Effekt bei einigen Arten zusätzlich. Die N- und Wasserverfügbarkeit im Boden haben sich als besondere Einflussfaktoren auf die N-Aufnahme herausgestellt. Diese überdecken mögliche physiologische Unterschiede zwischen verschiedenen Arten. Darüber hinaus führten eine hohe N-Verfügbarkeit und Trockenstress zu organ- und artenspezifischen Veränderungen bei der N-Verteilung in die verschiedenen N-Pools. Im Allgemeinen zeigte sich bei Trockenstress eine Anreicherung von Kompatible Solute, aber eine verminderte Anreicherung von N in die verschiedenen N-Pools unter hoher N-Verfügbarkeit.

Zusammengefasst zeigt meine Forschung die artspezifische Strategien auf das Zusammenspiel von biotische und abiotische Stressfaktoren bei nativen und invasiven Arten, sowie die Bedeutung der Lebensform und der Wachstumsstrategien der Arten, sowie deren unterschiedliche Reaktion für spezifische Veränderungen der abiotischen Umwelt. Solche artspezifischen Strategien auf biotische und abiotische Stressfaktoren sollten zukünftig in Waldmanagementprogrammen, die auf die Bekämpfung invasiver Arten in Waldökosystemen abzielen, berücksichtigt werden.

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1. General Introduction

In forest ecosystems, plants compete for the available soil nitrogen (N) with soil microorganisms (e.g. reviewed in Kuzyakov and Xu 2013), as well as with neighboring plants (Simon et al. 2017), which is of key relevance because N is one of the key macronutrients required to sustain plant development and is frequently a limiting factor for plant growth (Gutierrez 2012, Marschner 1995, Krapp 2015, Castro-Rodriguez et al. 2017). Competition for N between neighboring plants may result in changes of morphological and physiological traits involved in plant N acquisition from the soil, plant N uptake capacity of different N forms, as well as the allocation of N to plant internal N pools. The outcome of plant-plant competition for N may have important consequences for plant community composition and structure, and therefore also impact on ecosystem stability and functioning (Aschehoug et al. 2016).

Competition for N and the associated plant responses are further influenced by biotic and abiotic factors (Bengtsson et al. 1994, Rennenberg et al. 2009). Understanding the environmental effects on the N dynamics of plant-plant interactions is of particular relevance in view of anthropogenic global changes expected to affect forest ecosystems, such as plant invasions (Liebhold et al. 2017, Wardle and Peltzer 2017), the potential intensification of atmospheric N deposition affecting forest N cycling processes (Rennenberg et al. 2009, Rennenberg and Dannenmann 2015) as well as the expected increase in the frequency and duration of summer droughts (Spinoni et al. 2017). Therefore, the focus of this work is on the impact of these biotic (i.e. plant invasions) and abiotic (i.e. increased N deposition or drought) components of global change on the competition for N in tree species and their responses in morphological and physiological traits, organic and inorganic N acquisition, and plant internal N pools.

1.1.Plant morphological and physiological traits involved in soil N uptake

Plasticity in morphological and physiological traits allows plant individuals to adjust to variations in soil N availability and either enhance plant access to soil N when necessary or invest in aboveground growth when soil N is not limiting (e.g. Hodge 2004, Lambers et al. 2008, Bardgett et al. 2014, Chen et al. 2017). When soil N availability is low, the response of functional traits includes, among others, (i) increased biomass allocation to the root system relative to the shoot (i.e. higher root:shoot ratio), allowing the plant to occupy and exploit larger volumes of soil, (ii) production of longer roots per unit of root mass that allows the exploration of a larger soil volume and enhances the absorption surface per unit of carbon invested (i.e.

higher specific root length, SRL), and (iii) increased expression and activity of N transporter proteins on the membrane of root cells maximizing the intake of N into the plant (Bloor et al. 2008a, Poorter et al. 2012, Bardgett et al. 2014, Castro-Rodriguez et al. 2017). Similar responses of morphological traits that maximize the acquisition of belowground resources occur as a consequence of low soil water supply, which also results in reduced soil N availability (Sardans and Peñuelas 2012, Gessler et al. 2017). For example, an increased root:shoot ratio enhances root access to both soil N and water while simultaneously reducing leaf mass at the plant level (Poorter et al. 2012, Larson and Funk 2016, Eziz et al. 2017), thus decreasing water losses through the leaves and enhancing the overall plant water status. Likewise, specific leaf area (SLA, ratio of leaf area to leaf mass) might be reduced as a coping mechanism to minimize water losses through transpiration via the reduction of transpiring area (Fonseca et al. 2000, Wellstein et al. 2017), although potentially enhancing the negative impact of drought on photosynthesis due to the positive correlation between SLA and photosynthetic rate (Poorter et al. 1990, Reich et al. 1997, Feng et al. 2008). Overall, plasticity in these morphological and physiological traits is an important aspect of plant development in relation to its biotic and abiotic environment, because it allows an optimized carbon allocation and adjustment to shifts in soil N and water supply.

When soil N availability is reduced, the ability to optimize the acquisition of N via morphological and physiological adjustments can determine the competitive ability of a plant individual (Craine 2006). For example, seedlings of *Picea asperata* increase SRL in response to intraspecific competition, suggesting an adaptation to alleviate competition by increasing soil exploitation and nutrient uptake (Li et al. 2017). Adult *Fagus sylvatica* trees growing in mixed forests show similar strategies compared to pure *F. sylvatica* stands (Bolte and Villanueva 2006), as well as *F. sylvatica* seedlings growing at plots with high herbaceous biomass compared to plots with sparse herbaceous cover (Curt et al. 2005). Nevertheless, in general not all traits have to be altered simultaneously to adapt to competition, as plants may modify a given trait and thus reduce the need to modify others: for example, as a result of an adjustment in root morphology it may become unnecessary to enhance the kinetics of N uptake (Bassirirad 2000), or the relative allocation of biomass to the root system (Aerts et al. 1991). Moreover, in addition to direct N uptake from the soil, plant species are able to sustain symbiotic interactions with soil microorganisms to improve their N acquisition. For instance, plant roots associate with mycorrhizal fungi, resulting in enhanced N uptake for the plant while the fungi acquires plant-assimilated carbon compounds (Bücking and Kafle 2015, Liese et al. 2018). The relevance of mycorrhizal associations is well established and it plays a key role in N nutrition for most land plants (McFarland et al. 2010, Nave et al. 2013, Bücking and Kafle 2015). Moreover, some plant species are able to further obtain N through the process of atmospheric N_2-fixation by symbiotic bacteria which colonize the plant roots and induce the formation of nodules where the N_2-fixation process occurs (Sprent 1992). The establishment of such symbiotic interactions provides an advantage to some plant species over their competitors with potential consequences on the outcome of the competition (Miller 2010).

In contrast, when soil N availability is high, the need to maximize soil exploration and resource capture by plant roots is alleviated, which is reflected in their morphology and biomass allocation due to the plasticity associated to these traits. For example, SRL is reduced in coniferous and deciduous European and Asian tree species as a response to mineral N additions indicating a reduced need for longer and thinner roots (e.g. Ostonen et al. 2007, Wang et al. 2013), leading to higher root longevity and root depth (Larson and Funk 2016). With respect to biomass allocation for adult *Pinus* spp., the root:shoot ratio and the size of the root system decrease with fertilization (Albaugh et al. 1998, Bakker et al. 2009) suggesting a higher investment in aboveground growth and maximization of photosynthetic activity, and thus an optimized plant productivity when N is not limiting. Nevertheless, the magnitude and direction of the morphological response to increased N availability is likely species-specific, with some species being more sensitive in their functional response to higher N supply than others (Mei et al. 2010). Such differential response to soil N supply among species would impact the outcome of plant-plant competition for N depending on the identity of the competing species and their specific responses regarding N uptake, with potential important consequences at the ecosystem level in the context of atmospheric N deposition.

1.2.Plant N uptake and plant-plant competition for N

Plants obtain N mainly via direct uptake from the soil, where N is available to plants in inorganic (i.e. ammonium and nitrate) as well as organic forms (e.g. proteins and amino acids) (Näsholm et al. 2009, McFarland et al. 2010). Much work has focused on inorganic N acquisition and plant preferential uptake of either ammonium or nitrate due to the generally higher concentrations of these forms in natural and cropland soils (Hachiya and Sakakibara 2016). Nevertheless, organic N is also recognized as an important N source for plants (e.g. McFarland et al. 2002, Persson et al. 2003, Dannenmann et al. 2009, Näsholm et al. 2009), and constitutes an alternative path for N uptake that would allow plants to bypass the mineralization step of the N cycle in terrestrial systems (e.g. Warren and Adams 2007, McFarland et al. 2010).

However, plant species differ in their acquisition and preference of ammonium *vs* nitrate, or inorganic *vs* organic N forms (e.g. Harrison et al. 2007, Schulz et al. 2011, Weigelt et al. 2005), and this is further influenced by factors related to the chemical characteristics of N sources (e.g. energetic costs associated to uptake, ion mobility in the soil influencing diffusion and leaching rates) (Näsholm et al. 2009, Boudsocq et al. 2012, Britto and Kronzucker 2013). The preferential uptake of the different forms of N is ecologically relevant because it can lead to competition avoidance (Boudsocq et al. 2012, Britto and Kronzucker 2013, Jacob and Leuschner 2015) and contributes to a plant species' adaptation to changes in its biotic (e.g. competition with neighboring plants) and abiotic (e.g. reduced soil water supply, increased soil N availability) environment that could influence the availability of the different N sources in the soil (Houlton et al. 2007).

Competition with neighboring plants modifies the acquisition of inorganic and organic N by roots, as well as the preference for specific N forms, although the extent and direction of this change may vary according to species, plant age, and season (Simon et al. 2017). In temperate trees, direct measures of net N uptake capacity of the roots have shown that interspecific competition can either increase or decrease N acquisition in a N form- and species-specific manner (Fotelli et al. 2002, Simon et al. 2010, Simon et al. 2014, Li et al. 2015). However, although differences among species in their preference for various N forms have been found in different ecosystems and life forms (Näsholm et al. 1998, McKane et al. 2002), how these preferences are modified in interspecific competition compared to intraspecific- or no-competition has rarely been tested, and only in herbaceous vegetation (e.g. Harrison et al. 2007, Ashton et al. 2008, Fraterrigo et al. 2011). For instance, grass-herb competition influenced N acquisition but did not drive a change in the pattern of N preferences in an alpine tundra (Ashton et al. 2008), but in alpine dry meadows competition modified the preferences of the target species for different N forms, for example by reducing the uptake of one form and inducing the uptake of a different form, and this effect was dependent on neighbor identity (Miller et al. 2007). To my knowledge, such analyses of preference shifts due to competition have not been conducted for tree species. A major implication of the plasticity in the preference for different N forms in competition is the potential for niche differentiation and competition avoidance. However, coexisting tree species also show preference for similar N forms (e.g. Warren and Adams 2007, Paulding et al. 2010, Jacob and Leuschner 2015), suggesting that niche differentiation via distinct preferences may be restricted to certain species and/or ecosystems. Furthermore, N uptake and preferences are modified not only by competition, but also by the abiotic environment.

1.2.1. Effects of high soil N availability on plant N uptake and competition for N

Increased soil N availability due to atmospheric N deposition may influence plant N acquisition as well as the preferences for different N forms (Song et al. 2015). For example, tree species such as *Fagus sylvatica*, *Acer pseudoplatanus* and *Pinus sylvestris* show a higher organic and inorganic N uptake capacity at high compared to low soil N supply (Jones et al. 1994, Li et al. 2015). Additionally, soil N availability overall, but also the relative abundances of the different N forms available in the soil N pool can determine the acquisition preference by plants (Britto and Kronzucker 2013). For example, seedlings of *F. sylvatica* takes up nitrate preferentially in soils with low N availability whereas ammonium is preferred in soils with high N availability (Stolken et al. 2010), while in early successional stages of taiga ecosystems amino acids and ammonium are taken up equally but in late successional stands amino acid uptake is four times higher than ammonium, reflecting the abundance of the different sources in the soil (Kielland et al. 2006). However, the effect of soil N availability might also interact with further factors such as plant age and identity, i.e. the observations made in taiga ecosystems refer to overall uptake in the ecosystem (Kielland et al. 2006), thus including plants of different ages and several species which could still vary among them in their N acquisition behavior.

Soil N supply might further modulate the overall plant response to competition, regarding both N uptake and plant growth. For example, *F. sylvatica* increases glutamine-N acquisition in response to competition with *A. pseudoplatanus* at high soil N supply but not at low, whereas *F. sylvatica*'s growth was reduced as a consequence of competition with *A. pseudoplatanus* when soil N was low, but not at high soil N availability (Li et al. 2015). On the contrary, nitrate uptake of *A. pseudoplatanus* was reduced by competition with *F. sylvatica* regardless of soil N supply (Li et al. 2015), highlighting the species-specific nature of the responses to combined competition and changes in soil N availability. Moreover, competition may in turn mediate the general response of tree seedlings to changes in soil N supply. For example, N additions enhanced the growth of seedlings of *Fraxinus mandshurica* and *Phellodendron amurense* when growing single, but when growing in competition the increase in N availability enhanced the growth of *F. mandshurica* while negatively affecting the growth *P. amurense* (Yi et al. 2015). In the field, such dynamics of differential responses to soil N supply could lead in the long term to the competitive exclusion of one species by the other if N availability increases with time, thereby modifying plant community composition.

1.2.2. Effects of drought on plant N uptake and competition for N

Drought influences organic and inorganic N acquisition and preference by tree roots with consequences for plant-plant competition for N, similarly to increased soil N availability. Drought impairs N acquisition by trees both by reducing N availability in the soil (via its negative effects on N mineralization, and N diffusion and mass flow) and by negatively impacting root N uptake capacity (Gessler et al. 2004, Rennenberg et al. 2006, Leberecht et al. 2016). For example, seedlings and adult individuals of *F. sylvatica* have a lower inorganic N uptake capacity under conditions of low soil water availability compared to sufficient water supply (Fotelli et al. 2002, Gessler et al. 2005), but these studies did not consider effects on organic N forms. Furthermore, drought may also impact which N form is taken up preferentially, but this effect has been less investigated than the influence of soil N availability. Using ^{15}N tracers, Wang and Macko (2011) found a relation between inorganic N preferences and climatic conditions in African savannas, with plants of the same grass species preferring nitrate in the drier environments and ammonium in wetter environments, which was attributed to possible differences in ammonium/nitrate ratios in the soil. For another grass species, the optimized growth (which might suggest preference) on ammonium compared to nitrate is no longer present under drought stress (Hessini et al. 2017). In trees in field conditions, drought reduced root exudation leading to reduced soil microbial biomass, which then was likely used as an N source by trees as suggested by the increase in root amino acid N uptake with drought (Dannenmann et al. 2009, Guo et al. 2012). Nevertheless, to my knowledge, how drought affects N preference patterns in tree species has not been directly measured, despite its potential influence on tree competitive ability.

Reduced soil water availability may also affect competition for N via its direct negative effects soil N supply, which might intensify the effects of plant-plant competition for N (Fotelli et al. 2001, Rennenberg et al. 2006). For instance, nitrate uptake of *F. sylvatica* seedlings is lowered when grown in competition with the shrub *Rubus fruticosus*, particularly when irrigation is reduced (Fotelli et al. 2002), but the effects on organic N were not considered in this study. The interplay between drought and competition can have effects on the structure of plant communities, and data from long term forest monitoring show that forest growth declines due to drought are intensified by competition, as well as that species responses to drought may influence competitive dominance over time (Cavin et al. 2013, Gleason et al. 2017). Therefore, understanding the role that drought plays in the competition for N between plants in forest ecosystems is of great relevance, in view of future increases in frequency and intensity of drought events (Rennenberg et al. 2009, Simon et al. 2017). Furthermore, drought and abiotic factors in general have an impact not only plant N uptake and preferences, but it can exert a considerable influence on internal plant allocation to N pools.

1.3.Plant N nutrition and effects of competition for N

N acquisition through the roots is followed by N metabolism and distribution to the rest of the plant (Masclaux-Daubresse et al. 2010). After uptake, N is loaded into the xylem system and transported from the roots to the aboveground organs. Such transport is driven by plant transpiration, and can be regulated by interactions with the phloem, by signaling related to whole-plant nutritional status as well as by environmental cues (Gruffman et al. 2014, Rennenberg and Dannenmann 2015, Tegeder and Masclaux-Daubresse 2018). The acquired N becomes part of the plant N pool that cycles throughout the plant providing N for growth, development and defense, or is stored (Rennenberg and Dannenmann 2015, Tegeder and Masclaux-Daubresse 2018). Short- and long-term storage of N compounds when the supply exceeds the current plant demand, or as a mean to regulate osmotic balance, can occur in a variety of organs, tissues and cell compartments, e.g. nitrate in the vacuoles, amino acids with high N content such as arginine in the leaves, proteins such as the photosynthetic Rubisco in the leaves, or vegetative storage proteins in the bark (Millard 1988, Warren et al. 2003, Millard and Grelet 2010, Tegeder and Masclaux-Daubresse 2018). The importance of stored N is related to its further remobilization as required to meet nutritional demands (Tegeder and Masclaux-Daubresse 2018). As plant internal N pools can be directly affected by plant-plant competition for N and by the abiotic environment, with potential consequences for plant development due to the reliance on N storage and remobilization, it is important to investigate them in the context of global abiotic changes and plant-plant competition.

Competition for N may influence tree N nutrition and N metabolites content of plant organs. The influence of competition for N on plant N nutrition is linked, firstly, to the competition-induced change in plant N uptake capacity with direct consequences on plant N status, and secondly to variations in plant internal N pools resulting from the degradation or synthesis of N compounds as part of the plant's competition response strategy (Li et al. 2015). Competition results in changes in total N content in trees (e.g. Chang and Preston 2000, Welker et al. 1991, Hangs et al. 2002, Duan et al. 2014), and other N pools such as nitrate, ammonium, total soluble amino acid-N and protein-N, and structural N may change as a response to competition as well (e.g. Simon et al. 2010, Simon et al. 2014, Li et al. 2015). For example, both *F. sylvatica* and

A. pseudoplatanus show increases in total soluble protein-N levels in the fine roots with competition, probably as a result of *de novo* synthesis of proteins related to their response to competition (Simon et al. 2010). Furthermore, competition can impair plant growth thereby reducing sink strength and N storage capacity, which might have consequences the following growing season as N remobilization is required for new growth (Millet et al. 2005, Trinder et al. 2012).

Besides competition, abiotic variables and specifically soil N supply can also affect plant N pools, as soil N availability and plant overall N status are generally directly related. Reduced intake of N consequently reduces N available for assimilation, and plant N pools are decreased as a result (Gessler et al. 2017). In contrast, increased soil N supply from N deposition may cause higher N content in various plant organs. For example, foliar N content increases with fertilization for several temperate (e.g. Fahey et al. 1998, Ding et al. 2012) and tropical tree species (e.g. Pasquini and Santiago 2011). Likewise, amino acid, protein and total N content increase with fertilization in the needles of *P. sylvestris* (e.g. Näsholm and Ericsson 1990, Warren et al. 2003), and similar results were found for the fine roots of *A. pseudoplatanus* (Li et al. 2015). Moreover, N content in trees increases along N deposition gradients (Högberg et al. 1998, Ferretti et al. 2014). An increase in N levels may result in enhanced photosynthetic activity (as most of the N accumulated in leaves is in photosynthetic protein Rubisco), and in higher storage of N that may be remobilized for future growth and development (Millard and Grelet 2010). However, when N exposure exceeds a given threshold, it may have adverse effects on photosynthesis due to N-induced reductions in stomatal conductance thereby affecting carbon assimilation (Amponsah et al. 2004, Fusaro et al. 2017).

Similarly to soil N supply, drought also provokes changes in plant N pools, either increasing or decreasing N contents in plant tissues. For example, drought induces the accumulation of total soluble amino acid-N and proteins in the leaves and fine roots, as an adaptive response that increases the solute content in plant tissues thus favoring the overall plant water status (Close 1996, Kozlowski and Pallardy 2002, Brunner et al. 2015). Moreover, amino acid content may also decrease under drought indicating, among other possibilities, the degradation of amino acids and the usage of their carbon skeletons to produce alternative non-N-containing osmotic compounds which not only decrease osmotic potential but can also function as cell membrane and metabolic protectants (Chaves et al. 2003). Furthermore, protein content can be reduced with drought stress due to protein degradation, with potential negative consequences for photosynthetic activity when it affects Rubisco content in the leaves (Millard 1988, Kreuzwieser and Gessler 2010, Masclaux-Daubresse et al. 2010, Gessler et al. 2017), or for N storage when it affects protein content in the roots (Millard 1988, Staswick 1994, Millard and Grelet 2010). Root N storage is of relevance to plants coping with drought stress, because in a drought event leaves are prone to shedding and thus leaf-stored N is likely to be lost (Silla and Escudero 2006). Therefore, changes in plant internal N pools due to drought can have significant consequences in plant performance and development.

1.4.Plant invasions in forest ecosystems and competition for N

Plant invasions constitute an important aspect of global anthropogenic change (Ricciardi 2007, Wardle and Peltzer 2017) which occur when alien plant species are introduced into a new location and successfully establish a population (Wagner et al. 2017). Under certain circumstances invasive plants outcompete the native flora and become dominant with time (Gioria and Osborne 2014). The significant effect of plant invasions in forest ecosystems has been widely recognized (Liebhold et al. 2017, Wardle and Peltzer 2017), ranging from effects on biodiversity and community structure (Castro-Díez et al. 2006, Vilà et al. 2011), to changes in soil properties and ecosystem functioning (Ehrenfeld et al. 2001, González-Muñoz et al. 2013), as well as economic impacts (Holmes et al. 2009). Such effects may persist over long time scales even after the removal of the invasive species (Strayer et al. 2006, Corbin and D’Antonio 2012). The success of invasive species is related to functional traits that grant them competitive advantages over native plants and contribute to their successful establishment (Gioria and Osborne 2014). In general, compared to native species, invasive plants have greater seed production which increases their establishment ability (Mason et al. 2008), higher relative growth rates leading to fast occupation of space (Poorter 1989, Grotkopp and Rejmánek 2007), higher SLA allowing for enhanced light interception per unit of leaf mass (Leishman et al. 2007, Feng et al. 2008), higher maximum photosynthetic rates allowing for more CO_2 assimilation at a lower respiratory cost (Pattison et al. 1998, McDowell 2002), and higher root density and biomass enabling an optimized acquisition of belowground resources (Lopez-Zamora et al. 2004), among numerous other functional traits that favor dominance in plant communities (Leishman et al. 2007). Once dispersed into the new ecosystem, the possession of such traits allows invasive species to maximize above- and belowground resource exploitation, resulting in increased productivity and reproductive ability, and a successful invasion process (Gioria and Osborne 2014).

1.5. Knowledge gaps and justification

Although much attention has been dedicated to competitive interactions between native and invasive plant species, important gaps remain regarding the mechanisms of competition. Most studies that have explored N dynamics between natives and invasives have focused on the effects of soil N availability on the native-invasive competition by measuring plant biomass in grasses and forbs (e.g. Badgery et al. 2005, Eller and Oliveira 2017, Broadbent et al. 2018), as well as tree species (e.g. Ding et al. 2012, Luo et al. 2014). However, as biomass can be the result of numerous processes acting simultaneously, it is necessary to measure the physiological processes underlying competition for N between native and invasive species, such as N acquisition (Trinder et al. 2013). Estimations of plant N uptake in the context of native-invasive interactions have been performed in herbs and forbs using ^{15}N tracers or by calculating N acquisition based on plant tissue N content (e.g. Littschwager et al. 2010, Verlinden et al. 2014), but to my knowledge such approaches, or direct measurements of N net uptake capacity, have not been employed so far to study the effects of invasive species of different life forms on native tree species. Moreover, whether there is an effect of abiotic variables expected to be affected by anthropogenic climatic changes, such as soil N and water availability, on the native-invasives competition remains to be explored for native trees.

Despite numerous studies assessing the negative impact of plant invasions on forest ecosystems, the underlying mechanisms and plant responses regarding N dynamics, such as changes in N acquisition, shifts in N form preference, and adjustments of internal plant N pools have not been explored in native tree species competing with invasive species. Moreover, investigating how tree species respond to competition with invasives according to their specific life history traits and physiological characteristics (e.g. growth rate, leaf habit, drought tolerance) and how such responses vary according to the identity and life form of the invasive competitor, would provide information that could inform the design of forest management plans aimed at minimizing the impacts of plant invasions.

Additionally, invasion risk can be expected to increase with anthropogenic changes in the global climatic conditions due to the higher ability of invasive species to adapt to new conditions and their capacity to thrive in high-resource environments, such as forests with increased N availability resulting from N deposition (Dukes and Mooney 1999, Bradley et al. 2010, Liu et al. 2017), although other factors such as future reductions in soil water supply might reduce invasions (Liu et al. 2017). Therefore, investigating the interplay between these abiotic changes and competition for N between native trees and invasive species provides insights into the potential future outcome of these processes in forest ecosystems.

1.6.Research questions and hypotheses

In this work, I conducted three greenhouse experiments to answer the following questions:

1. How does competition affect growth indices and inorganic and organic N acquisition (Chapters 2, 3 and 4), as well as N allocation to N pools (Chapters 3 and 4) of native tree seedlings, invasive grasses (Chapter 2) and invasive tree seedlings (Chapters 3 and 4)?
2. Do native and invasive tree species show different responses to competition
    a. depending on their specific life history, growth strategies and physiological traits? (Chapters 2, 3 and 4).
    b. depending on the competing species? (Chapters 2, 3 and 4).
3. What are the effects of abiotic variables related to climate change on the competition for N between native and invasive species?
    a. Effects of high vs. low soil N availability (Chapters 2 and 3).
    b. Effects of drought (Chapter 4).

I hypothesized that:

1. Biomass, growth indices, N acquisition and N nutrition of native tree seedlings are negatively affected by competition with invasive species.
2. Native and invasive species show different responses to competition and abiotic factors that reflect their life history, growth strategies and physiological traits, and the responses vary depending on the competitor. E.g. drought is more important than competition for drought-intolerant species, and species with enhanced N storage capacity are relatively independent from external N supply.
3. Responses to competition of native and invasive species change with different levels of soil N availability.
4. Competition enhances the effects of drought on native and invasive species.
5. Responses to high soil N availability and drought change according to competitor identity.
6. Native species are more affected by abiotic stressors than invasive species.

In the experiment described in Chapter 2, I investigated the responses of native tree species of subtropical Australian forests and invasive grass species to competition for N the competition for N between. The selected native tree species, *Acacia melanoxylon* R.Br. and *Podocarpus elatus* R.Br. ex Endl., are commonly used in reforestation programs in subtropical Queensland (Australia), where constant efforts are put in place by a variety of stakeholders to contribute to the restoration of riparian land due to the importance of the region as a water catchment (Gageler et al. 2014). However, competition between the native tree seedlings used for reforestation and exotic grasses growing in restoration sites, such as *Pennisetum clandestinum* Hochst. ex Chiov. and *Cynodon dactylon* (L.) Pers., is an issue frequently faced by the local practitioners and can severely affect the results of restoration efforts by hindering the establishment of planted seedlings (Kanowski et al. 2003, Erskine et al. 2005). Although evidence exists that these grass species compete for N with woody species (Linares et al. 2010), it is unknown whether competition for N plays a role in the competitive interactions between the native tree seedlings and the introduced grasses in the reforestation sites in subtropical Australia, as well as the potential influence of soil N availability on the competition outcome.

In the experiments described in Chapters 3 and 4, I studied the responses to competition for N of tree species native to Central Europe and invasive woody species. Invasion by woody plant species is a major threat for protected forests in the continental and alpine biogeographical regions of Europe, with North American species *Prunus serotina* Ehrh. and *Robinia pseudoacacia* L. considered among the top five invasive tree species in such areas (Campagnaro et al. 2018). Woody invasive species are of special concern because trees are considered key to the regulation of ecosystem functioning in forests, and invasion by tree species could result in profound changes in community biotic and abiotic processes (Lamarque et al 2011). Previous studies have shown negative effects of *P. serotina* and *R. pseudoacacia* on the biomass production of native European tree species (Robakowski and Bielinis 2011, Kawaletz et al. 2013, 2014). However, how these invasive species affect N acquisition and dynamics of native tree species has not been investigated so far, as well as what are the effects of climate change components such as variations in soil N availability (Chapter 3) and drought (Chapter 4) on these dynamics.

For all experiments (i.e. Chapters 2, 3 and 4) native tree species were selected based on their abundance and relevance in their respective contexts (e.g. species used in reforestation programs in subtropical Australia, most important tree species in Central European forests). Invasive species were chosen based on their prevalence and the level of threat they pose to the native vegetation (e.g. two grass species growing in reforestation sites in Australia, two most important woody invaders of Central European forests). Moreover, I selected native and invasive species differing in their leaf habit and life history traits (i.e. deciduous and conifers, early- and late-successional), growth strategies (i.e. slow- and fast-growing), as well as various physiological characteristics (i.e. drought sensitive and drought tolerant, cyanogenic, N_2-fixer), in order to assess the influence of such traits on species response strategies and competitive effects.

In Chapter 5 I present an overall discussion and concluding remarks, I discuss the limitations of this work and offer suggestions for future research.

2. Responses to competition for nitrogen between subtropical native tree seedlings and exotic grasses are species-specific and mediated by soil N availability

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2.1.Abstract

Competitive interactions between native tree seedlings and exotic grasses frequently hinder forest restoration. We investigated the consequences of competition with exotic grasses on the growth and net nitrogen (N) uptake capacity of native rainforest seedlings used for reforestation depending on soil N availability and N source. Tree seedlings and grasses were grown in the greenhouse in different competition regimes (one tree species vs one grass species) and controls (grass monocultures or single tree seedlings) at low and high soil N. After 8 weeks, we quantified net N uptake capacity using ^{15}N-labelled organic (i.e. glutamine and arginine) and inorganic (i.e. ammonium and nitrate) N sources and biomass indices. Depending on soil N availability, we observed different species-specific responses to growth and N acquisition. Tree seedlings generally increased their net N uptake capacity in response to competition with grasses, although overall seedling growth was unaffected. In contrast, the responses to competition by the grasses were species-specific and varied with soil N availability. The different N acquisition strategies suggest the avoidance of competition for N between trees and grasses. Overall, the results highlight that quantifying underlying mechanisms of N acquisition complements the information on biomass allocation as a measure of responses to competition, particularly with varying environmental conditions.

2.2.Introduction

Plant–plant interactions shape the structure of plant communities (Callaway and Walker 1997, Brooker 2006, Tylianakis et al. 2008). Their magnitude and direction are influenced by factors including life stage, physiological requirements as well as environmental conditions (Callaway and Walker 1997). The interplay between these biotic and abiotic factors determines the outcome of species interactions which in turn affects the coexistence and spatial distribution of species (Brooker et al. 2008, Soliveres and Maestre 2014). A main interaction is the competition for resources such as soil nutrients. The ability of plants to acquire nutrients is determined by morphological and physiological traits, such as uptake rates of nutrients (Casper and Jackson 1997). By modifying nutrient uptake in response to environmental cues, such as nutrient availability, plants can be more competitive but the extent of such response varies (Hodge 2004).

Competitive interactions between native and exotic plant species are relevant in the context of global environmental change, because there is evidence that the predicted changes in the abiotic environment will likely influence competition in favour of exotic species (Bradley et al. 2010, Verlinden et al. 2014). The superior competitive ability of exotic plant species is frequently a key factor promoting invasions, with exotic species able to better exploit limited resources, to the detriment of native species (D'Antonio and Vitousek 1992, Gioria and Osborne 2014). Changing nutrient status, such as increased nitrogen (N) availability with atmospheric deposition, can alter plant growth and biomass allocation in a species-specific manner, and consequently results in shifts in the outcome of interspecific competition (Rennenberg et al. 2009). Here, we investigate the competition between exotic and native plants comparing the response strategies of different species, and how resource availability modulates these interactions. A focus on N physiology is warranted because N is quantitatively the most important of soil-acquired nutrients (Marschner 1995), limits plant growth in most ecosystems (Agren et al. 2012), drives competition (Eller and Oliveira 2017), and N availability and uptake can vary considerably in response to environmental change (BassiriRad 2000, Kuster et al. 2016). Since most studies focus on biomass production as indicator of the magnitude and direction of competitive interactions, even though other factors besides competition may influence biomass, (Trinder et al. 2013), we also examined net N uptake as a process that underlies the interaction between native and exotic species, because exotic species might alter soil N availability, show strong preferences to certain N forms and/or interfere with the N acquisition of native species, therefore affecting their performance (Fraterrigo et al. 2011, Huangfu et al. 2016, Eller and Oliveira 2017).

While earlier studies have focused mainly on the acquisition of inorganic N, organic N uptake has received increasing attention due to its potential to drive niche differentiation and species coexistence, and therefore ecosystem stability (Schmidt and Stewart 1999, McKane et al. 2002, Kielland et al. 2006, Näsholm et al. 2009, Ashton et al. 2010). A plant's ability to use a wider array of N sources can result in competition avoidance (e.g. Miller et al. 2007, Simon et al. 2010, Simon et al. 2014, Li et al. 2015). Studies that have quantified the uptake of organic and inorganic N forms found species-specific preferences for different N forms (e.g. Persson et al. 2003, Weigelt et al. 2005, Simon et al. 2010, Li et al. 2015). For example, European beech (*Fagus sylvatica*) shows a preference for organic rather than inorganic N, whereas the opposite is true for sycamore maple (*Acer pseudoplatanus*) (Li et al. 2015). In a study on excised roots of native and exotic grasses of Australian savannas, a general preference for ammonium was found, but preferences for nitrate and the amino acid glycine were species-specific (Rossiter-Rachor et al. 2009). Furthermore, the uptake capacity of different N sources is influenced by interspecific competition (e.g. Miller et al. 2007, Simon et al. 2010, Simon et al. 2014, Li et al.

2015). For example, when grown in competition with sycamore maple, the capacity for organic N uptake of European beech seedlings was reduced (Simon et al. 2014). However, the responses of competition with regard to N acquisition are also influenced by environmental factors (Simon et al. 2014, Li et al. 2015). Overall, most studies that have examined the acquisition of different N forms in context of competition have focused either on herbaceous or woody species in temperate ecosystems, rather than the interactions between trees and grasses. These, however, are important in the context of conservation and ecosystem restoration, because the successful establishment of tree seedlings (commonly planted in order to restore a plant community) often depends on the outcome of their interaction with grasses (Erskine et al. 2005, Doust et al. 2008, Elgar et al. 2014), where N is likely to play a determinant role (Coll et al. 2004, Barbosa et al. 2014).

Tree–grass interactions have been broadly studied in savanna ecosystems measuring the influence of abiotic factors (e.g. light, water and nutrient availability) that mediate the interaction (e.g. Ludwig et al. 2001, van der Waal et al. 2009, Moustakas et al. 2013) and in studies investigating the long-term coexistence of these contrasting life forms (e.g. Jeltsch et al. 2000, Sankaran et al. 2004). With regard to forest regeneration, tree–grass interactions have been studied in the context of secondary succession, tree regeneration in forest gaps after disturbances, or meadows (e.g. Coll et al. 2004, Bloor et al. 2008a, Elgar et al. 2014), where tree seedlings interact with the already established plant community commonly composed of early successional life forms, including grasses. However, research on the effect of herbaceous vegetation on tree seedlings mainly focusses on seedling survival and growth (e.g. Davis et al. 1998, Bloor et al. 2008b, Meli and Dirzo 2013), physiological aspects such as water relations (e.g. Quinteros et al. 2017) or photosynthesis (e.g. Davis et al. 1999, Loik and Holl 2001), while competition for nutrients, specifically N, has received little attention despite the potential of belowground competition with grasses to influence tree growth and establishment (e.g. Cramer et al. 2010) and the influence of soil N availability on the competition outcome (e.g. Cramer et al. 2012).

Here, we studied the interactions between native tree seedlings and exotic grasses because ecological forest restoration efforts are frequently hindered by competition from exotic grasses (Kanowski et al. 2003, Erskine et al. 2005). In reforestation programs, grasses successfully establish even after the planting of seedlings without regular weeding. To mimic these conditions, we used nursery-grown tree seedlings and grasses grown from seed in our study. We selected tree species that are relevant in the context of restoration of riparian zones in subtropical Australia (Catteral and Harrison 2006, Gageler et al. 2014), including an N_2 fixer. Tree species with the capacity to fix atmospheric N_2 via symbiotic rhizobacteria in root nodules are commonly used in restoration programs with the intent to improve the overall N supply at a site (Erskine et al. 2005). Their N_2 fixing ability would make them largely independent from external soil N supply (Messina and Barton 1985) and potentially alleviate competition for N (Epron et al. 2006). We conducted a greenhouse experiment to advance the understanding of whether competition for N plays a role in the interaction between seedlings of two native tree species and two exotic grass species, and the potential effects of soil N availability. Our objectives were to (i) determine the effect of interspecific competition on inorganic and organic N acquisition and biomass indices as response strategies of tree seedlings and grasses, and (ii) evaluate the impact of low vs high soil N availability on the competition outcome in terms of N acquisition. We hypothesized that tree seedlings and grasses potentially avoid competition by using different N forms (i.e., fast-growing grasses using inorganic forms and relatively slow-growing trees using organic forms), that growth and net N uptake capacity of tree seedlings is negatively affected in the presence of competing exotic grasses, and that this negative effect is lessened at high soil N availability.

2.3.Materials and methods

2.3.1. Study species and plant material

We selected two native tree species used in riparian reforestation in the Maleny region (26.7° S, 152.8° E, Queensland, Australia), angiosperm *Acacia melanoxylon* R.Br (Fabaceae) and gymnosperm *Podocarpus elatus* R.Br. ex Endl. (Podocarpaceae). *Acacia melanoxylon* is an early successional, N_2 fixing species native to south-east Australia (Smith et al. 2008, Birnbaum et al. 2012) that occurs in rainforests and wet sclerophyll forests (Doran and Turnbull 1997). *Podocarpus elatus* is a late-successional endemic species that grows in subtropical rainforests (Smith et al. 2008). Seedlings of *A. melanoxylon* (5 months old, ~40 cm tall) and *P. elatus* (10 months old, ~30 cm tall) were obtained from a local nursery (Brush Turkey Enterprises, Maleny, Australia). We selected tree species with contrasting successional status to explore potential differences in their N acquisition strategies related to growth rate (Li et al. 2015), and species adaptation to N forms available in soils of different successional status (e.g. higher nitrate availability in early successional sites compared to late sites) (Kronzucker et al. 1997, Britto and Kronzucker 2013). The exotic grasses *Pennisetum clandestinum* Hochst. ex Chiov. (Poaceae) and *Cynodon dactylon* (L.) Pers. (Poaceae) are perennial, originate from Africa and have been introduced to tropical areas around the world (Judd 1979). Spreading via rhizomes, stolons and seeds, these grasses naturally occur in grasslands and are common in pastures and lawns in their introduced range (Judd 1979). In subtropical Australia, these two grass species are reported as a threat to restoration efforts, and their negative effects on the growth of native tree seedlings have been observed in the field (M. Amos, personal communication). Grass seeds were purchased from Royston Petrie Seeds (Mudgee, Australia). From here on, species will be referred to by their genus, i.e. *Acacia*, *Podocarpus*, *Pennisetum* and *Cynodon*.

2.3.2. Experimental setup

Tree seedlings were planted and grasses were sown from seed into 1l pots (125 mm diameter, Anova Solutions, Brisbane, Australia) with a 1:1 mixture of grade A pit sand (grain size: 0.5 mm) and G2 vermiculite (grain size: 1–2 mm) in interspecific competition or under control conditions at low and high soil N availability. Competition treatments consisted of one tree individual surrounded by 10–12 individual grasses that were uniformly distributed ~4 cm from the seedling stem. The four species combinations were *Acacia* vs *Pennisetum, Acacia* vs *Cynodon*, *Podocarpus* vs *Pennisetum* and *Podocarpus* vs *Cynodon*. Control treatments consisted either of a single tree seedling or 12 individuals of one grass species (i.e. monoculture). Tree seedlings were not large enough to result in shading for the grasses (A. Bueno, *pers. obs.*). Because competition for N is highest under conditions of N limitation (Wilson and Tilman 1991, Casper and Jackson 1997), all competition regimes received the low soil N treatment. To ensure sufficient replication, the high soil N treatment was only applied for tree species vs *Pennisetum* because *Cynodon* had poor germination rates. Therefore, it was excluded from the high soil N treatment to ensure sufficient replicates in the low soil N treatment. For each combination of competition regime and soil N availability treatment, ten replicates were established. To control for the variation in initial size of the seedlings, we recorded the initial aboveground size of all tree seedlings (i.e. stem length measured from root crown, number of leaves and length of largest leaf) before planting. Pots were watered with tap water every second day to field capacity until the grasses germinated which occurred within 2 weeks of planting. Soil N availability treatments therefore commenced 2 weeks after planting. Subsequently, once a week 100 ml of nutrient solution was applied, with low or high N concentrations, for low N: 1 µM NH_4Cl, 100 µM KNO_3, 25 µM glutamine and 25 µM arginine; high N: 40 µM NH_4Cl, 400 µM KNO_3, 100 µM glutamine and 100 µM arginine, based on soil N availability in previous low vs high soil N studies (Simon et al. 2013) based on a low and high N forest site (Dannenmann et al. 2009, Stoelken et al. 2010, respectively), and on the occurrence of glutamine and arginine in subtropical Queensland soils (Holst et al. 2012). Other nutrients applied at the same rate in both N treatments were: 10 µM $AlCl_3$, 90 µM $CaCl_2$, 7 µM $FeSO_4$, 6 µM K_2HPO_4, 50 µM KCl, 24 µM $MnCl_2$, 20 µM NaCl and 70 µM $MgCl_2$. The experiment was conducted in a naturally lit greenhouse at The University of Queensland, St. Lucia Campus (Brisbane, Australia), between April and June 2015. Average temperature recorded in the greenhouse was 24.7 ± 3.7 °C during the day and 20.3 ± 1.8 °C at night, reaching a minimum of 13.5 °C and a maximum of 35.3 °C.

2.3.3. ^{15}N uptake experiments and harvest

Eight weeks after commencing the N treatments (and 10 weeks after planting), the net inorganic (i.e. ammonium, nitrate) and organic (i.e. glutamine, arginine) N uptake capacity of fine roots of tree seedlings and grasses was quantified following the ^{15}N enrichment technique described by Gessler et al. (1998) as modified by Simon et al. (2010) using fine roots still attached to the individuals. For this, we carefully removed the plants from the pots and washed the roots to remove adherent soil particles. Fine roots were then incubated for a 2 h period in 4 ml artificial nutrient solution containing all four N sources (as described above), but with one of the sources labelled as either ^{15}N-ammonium, ^{15}N-nitrate, ^{13}C/^{15}N-glutamine or ^{13}C/^{15}N-arginine. Amino acids were ^{13}C/^{15}N double-labelled to determine whether they were taken up as intact molecules or degraded over the time of incubation (Simon et al. 2011). Additionally, we used artificial soil solution without ^{15}N-label to account for the natural abundance of ^{15}N in the roots of each species for all treatment levels. Plants were incubated in either low or high N artificial soil solution matching the soil N availability treatment to which they had been subjected. Root incubation occurred between 10 am and 2 pm to avoid diurnal variation in N uptake (Gessler et al. 2002), i.e. the incubation period of 2h for any given individual sample started between 10 a.m. and 12 p.m. and ended between 12 and 2 p.m. For the duration of the ^{15}N incubation experiments, the remaining seedling’s roots were carefully wrapped in wet tissue to prevent drying out. A total of six replicate root samples were set up for each of the four N sources (plus controls for natural abundance) per species, competition regime and soil N availability level. Depending on the size of the root system for an individual plant, one to four N sources plus control were tested per individual. After the incubation period, fine roots were carefully cut, washed twice in 0.5 M $CaCl_2$ solution to remove the incubation solution from the root surface, and dried with tissue. Following the ^{15}N uptake experiments, we separated the plants into above- and belowground organs, i.e. the tree seedlings into leaves, stem and roots, and the grasses into shoots and roots, since no stem had developed. We determined the fresh weight (fw) of all plant tissues and the incubated fine roots, oven dried the samples at 65 °C for 72h and determined their dry weight (dw) to obtain biomass indices.

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

To quantify ^{15}N and ^{13}C enrichment the dried fine roots were ground using a ball mill (TissueLyser, Retsch, Haan, Germany). Aliquots of 1.2-2.5 mg of the resulting homogeneous fine powder were weighed into 4x6 mm tin capsules (IVA Analysentechnik, Meerbusch, Germany), and analysed with an isotope ratio mass spectrometer (Delta V Advantage, Thermo Electron, Dreieich, Germany) coupled to an elemental analyser (Euro EA, Eurovector, Milan, Italy). Δ values were calculated using a laboratory standard (acetanilide) that was part of every sequence in intervals, and also used in different weights to determine isotope linearity of the system. The laboratory standard was calibrated against different international standards from IAEA (Vienna): for ^{15}N USGS 40 ($\delta^{15}N_{Air}$ = -4.5 ‰), IAEA 600 ($\delta^{15}N_{Air}$ = +1.0‰), IAEA N2 ($\delta^{15}N_{Air}$ = +20.3‰), USGS 41 ($\delta^{15}N_{Air}$ = +47.6 ‰), USGS 26 ($\delta^{15}N_{Air}$ = +53.7 ‰), and USGS 32 ($\delta^{15}N_{Air}$ = +180 ‰), and for ^{13}C IAEA 600 ($\delta^{13}C_{V\text{-}PDB}$ = -27.77‰), IAEA 3 ($\delta^{13}C_{V\text{-}PDB}$ = -24.72‰), and IAEA CH6 ($\delta^{13}C_{V\text{-}PDB}$ = -10.45). ^{15}N results of highly enriched samples were finally corrected with different enriched ^{15}N standards (from 0.437 to 0.734 at %) from Fischer Analysen Instrumente (Leipzig, Germany). Δ values are defined as δ [‰] = $(R_{SA} / R_{Std} - 1) * 1000$, where R is the ratio of heavy isotope against light isotope (i.e. $^{13}C/^{12}C$ and $^{15}N/^{14}N$), SA is sample and Std the international reference for the respective element (V-PDB for ^{13}C and air-N_2 for ^{15}N). For each of the four N sources, we then calculated net N uptake capacity (nmol N g^{-1} fw h^{-1}) based on the incorporation of ^{15}N into root fresh weight according to Gessler et al. (1998): net N uptake capacity = $((^{15}N_l - ^{15}N_c) * N_{tot} * dw * 10^5)\ (MW * fw * t)^{-1}$, where $^{15}N_l$ and $^{15}N_c$ are the atom% of ^{15}N in labelled (N_l) and control plants (N_c, natural abundance), respectively, N_{tot} is the total N percentage, MW is the molecular weight ($^{15}Ngmol^{-1}$), and t represents the incubation time (120 min). Based on ^{13}C incorporation, net uptake capacity of glutamine and arginine was ~60% and ~95% respectively, compared to that based on ^{15}N incorporation, which indicates (1) the degradation of amino acids in the solution or on the root surface, and/or (2) the respiration of amino acid-derived C inside the roots (Simon et al. 2011).

2.3.5. Statistical analysis

For all species, we tested for differences between treatments for total biomass and root:shoot ratio as well as net uptake capacity of the four N sources by performing Permutational

ANOVAs (PERMANOVA) based on a Euclidean resemblance matrix between samples (Anderson et al. 2008). PERMANOVA was chosen as a non-parametric univariate test equivalent to ANOVA (Anderson et al. 2008). We performed two-way PERMANOVAs using “competition regime” and “soil N availability” as orthogonal factors. For the tree species, the “competition regime” factor had three levels: control, competition with *Pennisetum,* and competition with *Cynodon*. Moreover, for the tree seedlings biomass and root:shoot ratio analyses, initial size measurements of the tree seedlings were included as a covariate. For the grass species, the three levels were: control, competition with *Acacia,* and competition with *Podocarpus*. The “soil N availability” factor included two levels: high N and low N for *Acacia*, *Podocarpus* and *Pennisetum*. For the grass *Cynodon*, we performed one-way PERMANOVAs, considering the factor “competition regime” with three levels: control, competition with *Acacia,* and competition with *Podocarpus,* all at low soil N availability as described above. When a significant interaction between factors was found, post hoc PERMANOVA pairwise comparisons were performed. To test for differences in net N uptake capacity between species, one-way PERMANOVAs were performed on the control data (i.e. no competition), using species as factor, for each N form. To test for preferences in net uptake capacity of the four N sources for a given species, one-way PERMANOVAs were performed, using N source as factor, for each combination of competition regime and soil N availability treatment. All analyses were performed using PRIMER 6.0 with the PERMANOVA+ add-on (PRIMER-E Ltd, Plymouth, UK), and figures were prepared in SigmaPlot 13.0 (Systat Software Inc., San Jose, USA).

2.4.Results

2.4.1. Effects of competition regime and soil N availability on biomass and root:shoot ratio

Native tree seedlings and exotic grasses responded differently in biomass production depending on competition regime and soil N availability (Figure 2.1). For the tree species, neither *Acacia* nor *Podocarpus* differed significantly in total biomass between single and competing seedlings regardless of soil N availability (Figure 2.1, Supplementary Table 2.1). Positive correlations were found for *Acacia* between total biomass and the initial leaf number, for *Podocarpus* between total biomass and both initial leaf number and initial stem length (both *p-perm* < 0.001, data not shown). This indicates that total biomass at the end of the experiment was a function of initial tree seedling size. For the grass *Pennisetum,* total biomass was significantly reduced in competition with *Acacia* or *Podocarpus* compared to the control at high soil N availability, and it was also significantly reduced at low compared to high soil N availability irrespective of the competition regime (Figure 2.1, Supplementary Table 2.2). For the grass *Cynodon*, competition with *Acacia* significantly reduced total biomass compared to competition with *Podocarpus* or control (at low soil N availability) (Figure 2.1, Supplementary Table 2.2).

Root:shoot ratio was not affected by soil N availability or competition regime for both tree species as well as competition regimes for the grass *Cynodon* at low soil N availability (Figure 2.1). For the grass *Pennisetum*, root:shoot ratio was significantly lower in competition with *Podocarpus* compared to competition with *Acacia* or controls irrespective of soil N availability (Figure 2.1).

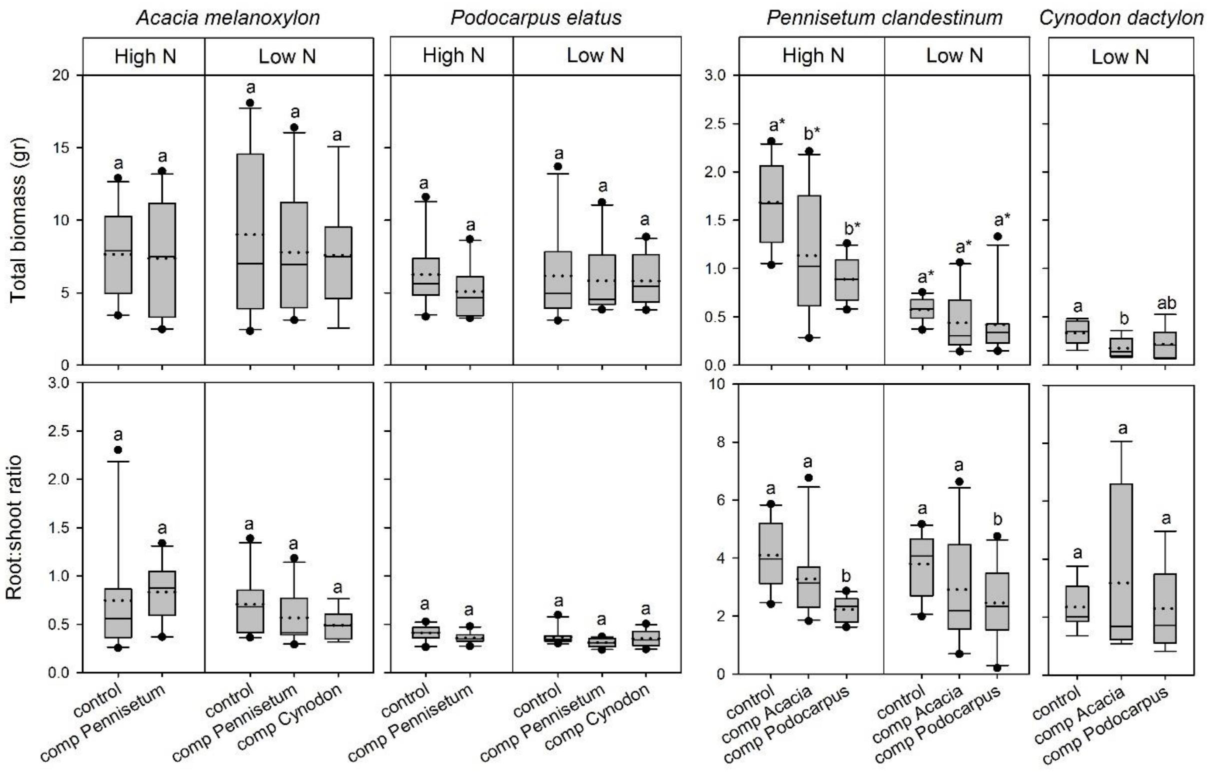

Figure 2.1. Total biomass and root:shoot ratio of *Acacia melanoxylon*, *Podocarpus elatus*, *Pennisetum clandestinum* and *Cynodon dactylon*, at low and high soil N availability under different competition regimes. For *C. dactylon*, only low soil N data is available. comp Pennisetum = competition with *P. clandestinum*; comp Cynodon = competition with *C. dactylon*; comp Acacia = competition with *A. melanoxylon*; comp Podocarpus = competition with *P. elatus*. Box plots show mean (dotted line) and median (straight line). Different letters indicate significant differences between competition regimes within a specific soil N availability treatment, and asterisks indicate significant differences between soil N availability treatments, detected using permutational analysis of variance ($P < 0.05$).

2.4.2. Effects of competition regime and soil N availability on inorganic and organic net N uptake capacity

Inorganic and organic net N uptake capacity of all species increased significantly with high compared to low soil N availability, whereas the responses to competition regime differed depending on the species (Figures 2.2 and 2.3, Supplementary Table 2.3). Inorganic and organic net N uptake capacity of *Acacia* seedlings increased with higher soil N availability regardless of competition regime. At high soil N availability, net ammonium uptake capacity of *Acacia* seedlings competing with the grass *Pennisetum* increased significantly compared to seedlings growing without competition (Figure 2.2, Supplementary Table 2.4), but no change for nitrate (Figure 2.2), glutamine-N or arginine-N (Figure 2.3) was found at either low or high soil N availability. Similarly, inorganic N (Figure 2.2) and arginine-N (Figure 2.3) net uptake capacity of *Podocarpus* increased with high compared to low soil N availability irrespective of competition regime. However, net glutamine-N uptake capacity increased with higher soil N availability only for *Podocarpus* seedlings grown in competition with *Pennisetum* (Figure 2.3, Supplementary Table 2.4). At high soil N availability, competition with *Pennisetum* increased net glutamine-N uptake capacity of *Podocarpus* seedlings compared to seedlings growing alone (Figure 2.3); however, net ammonium, nitrate (Figure 2.2), and arginine-N (Figure 2.3) uptake capacity were not affected. For the grass *Pennisetum*, net ammonium and organic N, but not nitrate uptake capacity were significantly higher at high compared to low soil N availability (Figures 2.2 and 2.3). For *Pennisetum*, competition regime had no significant effect on organic (Figure 2.3) and inorganic (Figure 2.2) net N uptake capacity at either low or high soil N availability. At low soil N availability, nitrate, but not ammonium or organic net N uptake capacity of the grass *Cynodon* was significantly higher when grown in competition with *Podocarpus* compared to that with *Acacia*, or control (Figures 2.2 and 2.3, Supplementary Table 2.4).

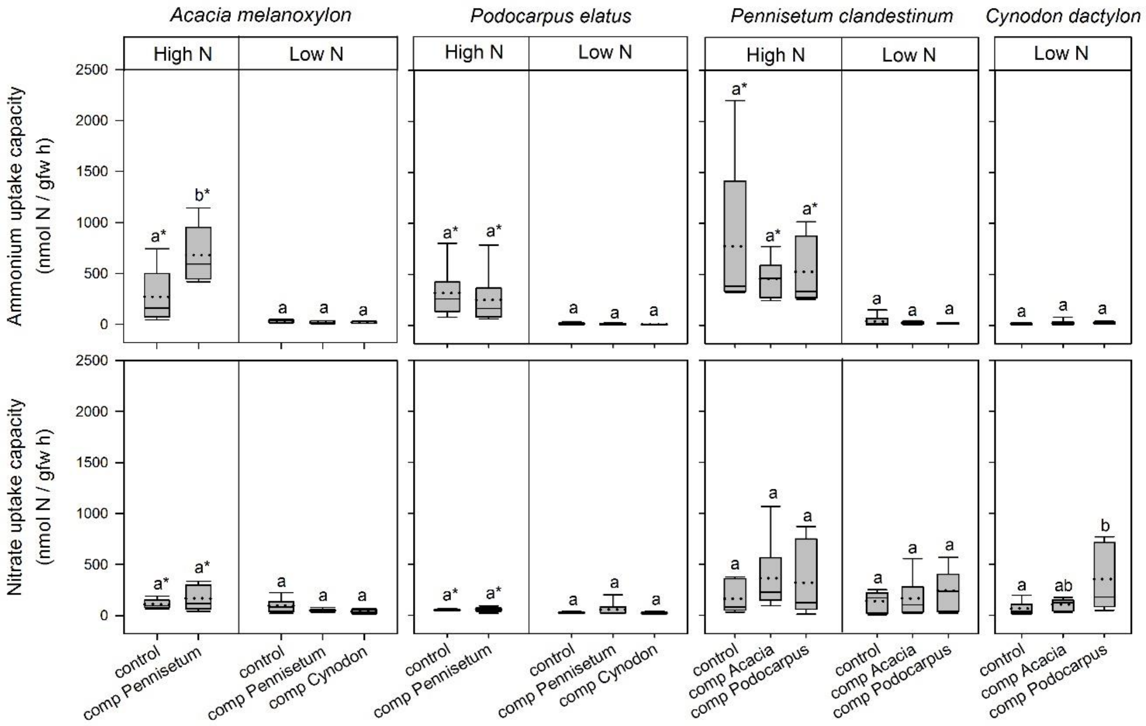

Figure 2.2. Ammonium (NH_4^+) and nitrate (NO_3^-) net uptake capacity (nmol N g^{-1} fw h^{-1}) by fine roots of *Acacia melanoxylon*, *Podocarpus elatus*, *Pennisetum clandestinum* and *Cynodon dactylon* at low and high soil N availability under different competition regimes. For *C. dactylon*, only low soil N data is available. comp Pennisetum = competition with *P. clandestinum*; comp Cynodon = competition with *C. dactylon*; comp Acacia = competition with *A. melanoxylon*; comp Podocarpus = competition with *P. elatus*. Box plots show mean (dotted line) and median (straight line). Different letters indicate significant differences between competition regimes within a specific soil N availability treatment, and asterisks indicate significant differences between soil N availability treatments detected using permutational analysis of variance ($P < 0.05$).

2.4.3. Differences between species with regard to inorganic and organic N acquisition

In the absence of interspecific competition (i.e. single grown tree seedlings or grasses growing in monocultures), species differed in their inorganic and organic N acquisition strategies depending on the N source: seedlings of *Podocarpus* took up significantly less nitrate than seedlings of *Acacia* and the grass *Pennisetum*, irrespective of soil N availability (Table 2.1). However, glutamine-N acquisition of *Pennisetum* was significantly lower than that of *Podocarpus*, which in turn was lower than that of *Acacia* seedlings at both low and high soil N availability (Table 2.1). No differences were found between any of the species with regard to ammonium and arginine-N acquisition (Table 2.1).

General preference patterns of the different N forms were found for each species within each competition regime depending on soil N availability: At low soil N availability, both tree species preferred nitrate and glutamine-N over ammonium and arginine-N (Supplementary Table 2.5). However, at high soil N availability, this pattern was reversed with ammonium and arginine-N being favoured over nitrate and glutamine-N for both tree species (Table 2.2, Supplementary Table 2.5). For the grasses, inorganic N sources, particularly nitrate, were preferred over organic N sources (regardless of soil N availability for *Pennisetum*) (Table 2.2, Supplementary Table 2.6).

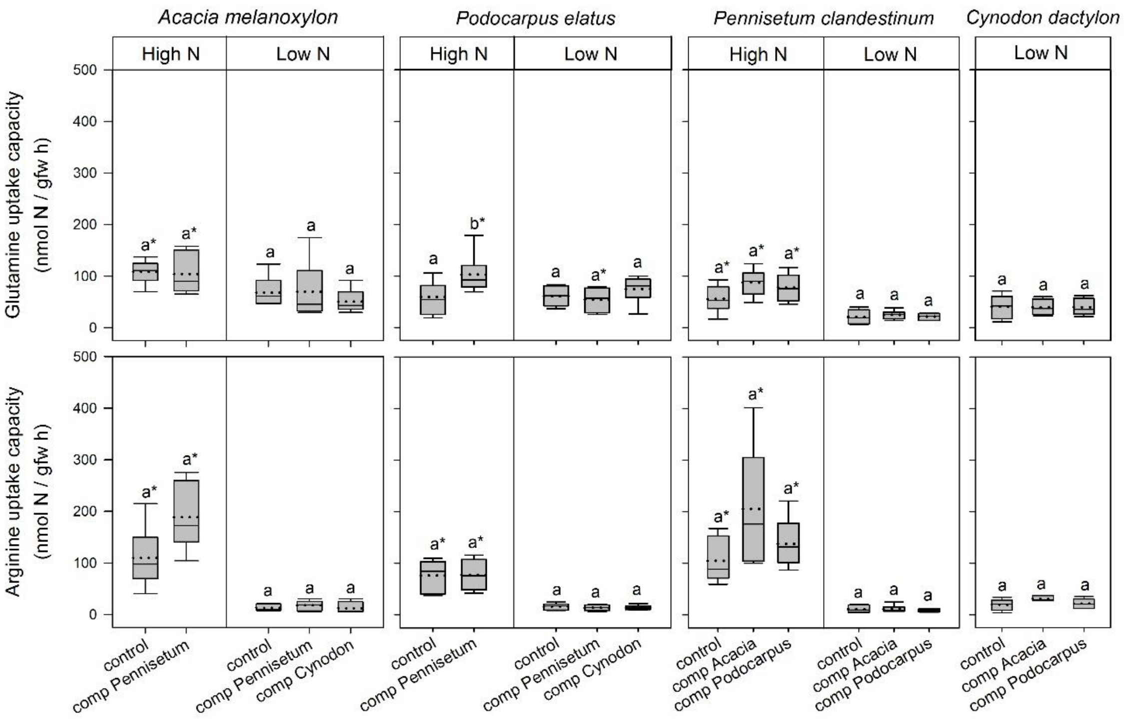

Figure 2.3. Glutamine-N (Gln-N) and arginine-N (Arg-N) net uptake capacity (nmol N g^{-1} fw h^{-1}) by fine roots of *Acacia melanoxylon*, *Podocarpus elatus*, *Pennisetum clandestinum* and *Cynodon dactylon* at low and high soil N availability under different competition regimes. For *C. dactylon*, only low soil N data is available. comp Pennisetum = competition with *P. clandestinum*; comp Cynodon = competition with *C. dactylon*; comp Acacia = competition with *A. melanoxylon*; comp Podocarpus = competition with *P. elatus*. Box plots show mean (dotted line) and median (straight line). Different letters indicate significant differences between competition regimes within a specific soil N availability treatment, and asterisks indicate significant differences between soil N availability treatments detected using permutational analysis of variance ($P < 0.05$).

Table 2.1. PERMANOVA results of the differences in ammonium (NH_4^+), nitrate (NO_3^-), glutamine-N (Gln-N) and arginine-N (Arg-N) net uptake capacity of fine roots between *Acacia melanoxylon*, *Podocarpus elatus*, *Pennisetum clandestinum* and *Cynodon dactylon*. Significant values are indicated in bold.

	NH_4^+		**NO_3^-**		**Gln-N**		**Arg-N**	
Main test	***Pseudo-F***	***P(perm)***	***Pseudo-F***	***P(perm)***	***Pseudo-F***	***P(perm)***	***Pseudo-F***	***P(perm)***
Species	1.630	0.170	4.248	**0.013**	7.469	**<0.001**	0.573	0.648
Soil N availability	16.749	**<0.001**	0.674	0.419	8.259	**0.006**	65.941	**<0.001**
Species x Soil N availability	2.184	0.098	0.011	0.991	2.545	0.092	1.307	0.295
Pairwise comparisons	***t***	***P(perm)***	***t***	***P(perm)***	***t***	***P(perm)***	***t***	***P(perm)***
Acacia vs *Podocarpus*	Not applicable		3.843	**<0.001**	2.493	**0.022**	Not applicable	
Acacia vs *Pennisetum*			1.180	0.251	4.766	**<0.001**		
Acacia vs *Cynodon*			0.803	0.441	1.771	0.096		
Podocarpus vs *Pennisetum*			3.0987	**0.005**	2.207	**0.041**		
Podocarpus vs *Cynodon*			1.760	0.066	1.401	0.179		
Pennisetum vs *Cynodon*			1.125	0.271	1.509	0.157		

Table 2.2. Differences between ammonium (NH_4^+), nitrate (NO_3^-), glutamine-N (Gln-N) and arginine-N (Arg-N) net uptake capacity of fine roots of *Acacia melanoxylon*, *Podocarpus elatus*, *Pennisetum clandestinum* and *Cynodon dactylon*, at low and high soil N availability. Only significant differences are presented.

	Acacia	***Podocarpus***
1) Low soil N availability		
Control	NO_3^-, Gln-N > NH_4^+ > Arg-N	Gln-N > NH_4^+, NO_3^-, Arg-N NO_3^- > Arg-N
Competition with *Pennisetum*	NO_3^-, Gln-N > NH_4^+, Arg-N	NO_3^-, Gln-N > NH_4^+, Arg-N
Competition with *Cynodon*	Gln-N > NH_4^+, Arg-N	Gln-N > NO_3^-, Arg-N > NH_4^+
2) High soil N availability		
Control	No differences	NH_4^+ > NO_3^-, Gln-N, Arg-N
Competition with *Pennisetum*	NH_4^+ > NO_3^-, Gln-N, Arg-N Arg-N > Gln-N	NH_4^+ > NO_3^-, Gln-N, Arg-N Gln-N > NO_3^-
	Pennisetum	***Cynodon***[1]
1) Low soil N availability		
Control	NO_3^- > NH_4^+, Gln-N, Arg-N	NO_3^-, Gln-N > NH_4^+
Competition with *Acacia*	NO_3^- > NH_4^+, Gln-N, Arg-N Gln-N > Arg-N	NO_3^- > NH_4^+
Competition with *Podocarpus*	NO_3^- > NH_4^+, Arg-N NH_4^+, Gln-N > Arg-N	NO_3^- > NH_4^+, Gln-N, Arg-N Gln-N > Arg-N
2) High soil N availability		
Control	NH_4^+ > NO_3^-, Gln-N, Arg-N Arg-N > Gln-N	
Competition with *Acacia*	NH_4^+ > Arg-N > Gln-N NO_3^- > Gln-N	
Competition with *Podocarpus*	NH_4^+ > Arg-N > Gln-N	

[1] At low soil N availability

2.5.Discussion

Our study provides novel insight into the species-specific growth and N acquisition patterns in native tree seedlings and exotic grasses competing for N. Depending on soil N availability, native tree seedlings responded to competition with increases in N acquisition only, whereas the responses of the grasses in terms of biomass and N acquisition varied with species and soil N availability. These results highlight that quantifying underlying mechanisms complements biomass indices to advance the understanding of interspecific competition (Trinder et al. 2013). Furthermore, the lack of response with regard to biomass in the tree seedlings compared to the grasses suggests that N storage capacity plays a role in the growth response as well.

2.5.1. Native tree seedlings increase N acquisition in competition with exotic grasses at high soil N availability

At high soil N availability, both tree species responded similarly to competition with the exotic grass *Pennisetum* by increasing N acquisition. However, growth and biomass allocation were unaffected, which indicates that they might allocate the newly acquired N to storage rather than investing into growth, thereby reducing N availability for their competitors (Millard and Grelet 2010). These similarities between *Acacia* and *Podocarpus* in terms of net N uptake capacity and growth responses to competition with grasses indicate that both tree species use a common strategy, which is rather unexpected given the different successional status, growth strategies and physiological characteristics of the two tree species. *Acacia* is an early successional, fast-growing and N_2 fixing species, while *Podocarpus* is a slow-growing, late-successional species (Smith et al. 2008). Thus, since fast-growing species would require higher amounts of N to meet their growth rate (Poorter et al. 2012), the observed increase in net N uptake capacity when growing in competition would be expected in *Acacia* and not in *Podocarpus*. However, previous studies have shown that *Acacia* seedlings are capable of N-fixation (Sun et al. 1992), and that root nodulation in *Acacia* species is stimulated by competition with grasses (Cramer et al. 2007). Therefore the increased requirements of *Acacia* due to competition would probably be mitigated by its ability to fix atmospheric N_2 (not quantified in our study) and thus reduce to an extent its dependency on external N supply. Moreover, the lack of response of *Acacia* to competition in terms of N uptake at low soil N availability in our study might also be attributed to a greater reliance on N_2 fixation under limiting N conditions (Pfautsch et al. 2009).

Tree species differed in the acquired N forms: in competition fast-growing *Acacia* acquired more ammonium compared to single grown individuals, while slow-growing *Podocarpus* used glutamine-N. This preference of inorganic vs organic N forms when competing might reflect different N acquisition strategies between fast- and slow-growers as reported for temperate tree seedlings (Simon et al. 2010, Simon et al. 2014, Li et al. 2015). The similar responses found here might be an early indication that, across biomes, N acquisition of trees is linked to growth strategy. These increases in net N uptake capacity as a response to competition were found only at high soil N availability suggesting mediation of N acquisition by soil N availability (e.g. Simon et al. 2013, Li et al. 2015). For *Acacia*, this could be related to a reduced nodule activity at high soil N concentrations, as it has been suggested that N acquisition from the soil, rather than N_2 fixation, is energetically more efficient for this species when inorganic N is readily available (Pfautsch et al. 2009). With regard to *Podocarpus*, an increase in organic N acquisition with competition was also reported for a temperate slow-growing tree species at high, but not low soil N (Li et al. 2015). Our results constitute a further example of increased N acquisition under competition with high N availability, but in a subtropical environment. Particularly in the view of increased atmospheric N deposition in the future (Rennenberg and Dannenmann 2015, Jia et al. 2016), the outcomes of competition in currently N-limited habitats are likely to change.

At low soil N availability, competition with grasses did not result in changes in N acquisition or biomass production and allocation for both tree species. Similarly, previous studies have reported a lack of effects of competition with the grass *Cynodon dactylon* on the growth (i.e. biomass, basal diameter and stem height) of a different *Acacia* species (*A. smallii* Isely) on non-supplemented native soil (Cohn et al. 1989). Nevertheless, this grass species hinders the growth of native tree seedlings in the field and is reported as a threat to restoration efforts in subtropical Australia (M. Amos, personal communication). Since we did not observe these negative effects in our study, it is likely that the impacts of the competition with *Cynodon* on tree seedling growth are evident at later developmental stages of both the grass and the tree species than the ones considered here. The lack of response to competition of the tree seedlings regarding total biomass could also be due to the short duration of our experiment, considering the relatively slow growth of tree seedlings compared to grasses. Previous studies reporting negative effects of grasses on *Acacia* measured responses over longer periods, e.g. 3 years (Messina and Barton 1985), and 8 years (Florentine and Westbrooke 2004). Furthermore, the effect of the grass on tree seedlings may be mediated by soil N availability: in our study, the effects of competition with *Cynodon* were not investigated at high soil N availability, but it has been reported that competition with *Cynodon* combined with an increase in soil nutrient availability results in 70–90% reduction in growth of *A. smallii* (Cohn et al. 1989).

2.5.2. Exotic grasses respond to competition for N depending on the competitor and soil N availability

For the exotic grasses, four strategies were identified which depended on the competing species and soil N availability: (1) *Pennisetum* biomass was reduced in competition with both tree species compared to the control at high, but not low soil N availability suggesting that the outcome of competitive interactions between native tree seedlings and *Pennisetum* depends on soil N availability. Moreover, the biomass of *Pennisetum* at high soil N availability, even if reduced by competition with the tree seedlings, was still higher than its biomass at low soil N in the absence of interspecific competition, suggesting that, at least in the short-term, the grass is more successful than the tree seedlings at high soil N availability, despite the observed negative effects of competition. (2) The grass *Cynodon* showed a similar strategy as *Pennisetum*, i.e. a reduction in biomass but no change in N acquisition, but only when competing with *Acacia*, but not *Podocarpus*, indicating that for both grass species the response to competition depends on the competitor. (3) Resource allocation shifted to aboveground at the expense of belowground tissue production (Aerts et al. 1991) for *Pennisetum* competing with *Podocarpus* at low and high soil N. This suggests that, despite the fact that shading from the tree seedlings was not apparent, the potential competition for light is more relevant for *Pennisetum* than competition for belowground resources (Poorter et al. 2012), thus not leading to an increase in N acquisition. However, these results were not observed when grown in competition with *Acacia* or for the grass *Cynodon* indicating that this biomass allocation strategy of *Pennisetum* also depends on the competing species, but not soil N availability. (4) Competition with *Podocarpus*, but not *Acacia*, resulted in increased net nitrate uptake in *Cynodon*, but not *Pennisetum* suggesting different N acquisition strategies between the two exotic grass species that vary with the competing tree species. Overall, our results suggest that the outcome of competitive interactions between native tree seedlings and exotic grasses are species-specific and mediated by soil N availability.

2.5.3. Differences in N acquisition strategies between species suggest potential for niche differentiation

All four species took up both inorganic and organic N forms, although specifics varied according to N forms. Nitrate acquisition was higher in *Pennisetum* and *Acacia* compared to *Podocarpus*, consistent with their successional status and the low abundance of nitrate in late-successional forest soils as well as a reduced nitrate use capacity in late-successional tree species (Kronzucker et al. 1997, Britto and Kronzucker 2013). At sites with higher soil nitrate availability, e.g. disturbed areas, the grass *Pennisetum* would have a competitive advantage over *Podocarpus,* but not *Acacia,* highlighting the importance of species-specific consideration for reforestation programs. Moreover, glutamine-N acquisition was higher for *Acacia* than *Podocarpus,* contrary to what we expected according to their successional status, since soil amino acid content generally increases as succession progresses (Werdin-Pfisterer et al. 2009). Nevertheless, glutamine-N acquisition was higher in both tree species compared to *Pennisetum* indicating a potential for avoidance of competition via the use of the different N forms (Simon et al. 2017). Overall, that plants take up organic N forms is now well established and has been reported for numerous plant species and ecosystems (Näsholm et al. 2009). Moreover, its ecological relevance is increasingly recognized due to the resulting niche differentiation and consequences in terms of species coexistence (Näsholm et al. 2009, Simon et al. 2014, Li et al. 2015, Andersen et al. 2017, Uscola et al. 2017).

Comparing within tree and grass species, higher soil N availability resulted in increased inorganic and organic N acquisition in general, which for *Pennisetum* was combined with increased biomass production (for *Cynodon* data is only available for low soil N). This increase in grass biomass production with increasing soil N availability while there was no short-term response of the tree seedlings suggests a potential advantage for the grass in habitats on N-rich soils or in future scenarios with increased atmospheric N deposition compared to the native trees. This link between high resource availability and success of invasive species has been explored in previous studies (e.g. Badgery et al. 2005, Gao et al. 2014, Yelenik et al. 2017), and has been linked to invader traits related to exploitation and efficient use of resources, which allows for better growth (Huangfu et al. 2016, Yelenik et al. 2017). In our study, although competition with seedlings of both tree species reduced the total biomass of *Pennisetum* compared to *Pennisetum* grown in controls, the increase in *Pennisetum* biomass in response to higher soil N availability was of greater magnitude. Increases in the productivity of *Pennisetum* with N fertilization resulting in its dominance at fertilized sites have been reported before (Mears 1970, Barton et al. 2008), and under such conditions, *Pennisetum* might be more competitive than tree seedlings. Moreover, the potential of *Acacia* to induce increases in soil N availability over time as a consequence of its N_2 fixing ability has been shown before in silvopastoral systems (Power et al. 2003). Thus, increased N supply as a consequence of the use of N_2 fixing tree species such as *Acacia* might favour the growth and potential dominance of *Pennisetum* at restoration sites.

2.5.4. Methodological considerations

Our experimental approach has several limitations. First, the competitor grasses were grown from seeds, whereas the trees were purchased as seedlings. This set up was selected because our aim was to mimic the actual timing in a restoration setting under field conditions for which tree species are planted as seedlings for reforestation and grasses arrive at a later time. Thus, a comparison between trees and grasses cannot exclude differences due to variation in developmental stages between tree species and grasses. Specifically, the lack of response in seedling biomass reported here might not persist with older or larger grasses. Second, tree seedlings differed in their age at the start of the experiment which might have influenced their responses to competition and/or soil N availability in our experiment. However, given the differences in growth rates between the tree species used in this study, seedlings of similar age would have likely differed in their initial above- and belowground dimensions, thereby introducing another variable. Third, the experiment ran for 8 weeks which might not be sufficient time for a response in tree seedling biomass, however they did respond with regard to N acquisition from the soil. Finally, the artificial N solution used in this study is based on a low and high N forest site (Dannenmann et al. 2009, Stoelken et al. 2010, respectively) as well as the occurrence of glutamine and arginine in soils of subtropical Queensland (Holst et al. 2012), but not actual measured concentrations at subtropical restoration sites.

2.6.Conclusions

In our study, native tree seedlings and exotic grasses responded to competition with two distinct response types: (1) a reduction in biomass, but no change in N acquisition, (2) an increase in N acquisition without a change in biomass. This outcome depended on the competing species and soil N availability. Thus, we suggest that these aspects be considered also for ecosystem restoration and management, with special attention to the potential feedbacks between native trees possessing traits such as N_2 fixing ability and exotic grasses that may benefit from the resulting increase in soil N availability. Exotic grasses such as *Pennisetum* and *Cynodon* are considered a high priority for control in Australia and their suppression can promote the reestablishment of woody vegetation, thus determining the success of subtropical rainforest restoration programs (Florentine and Westbrooke 2004, Downey et al. 2010, Shoo and Catterall 2013). We found no common N acquisition strategy across species in response to competition between native trees and invasive grasses indicating the need to consider species-specific approaches in restoration management plans.

3. Species-specific outcome in the competition for nitrogen between invasive and native tree seedlings

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3.1. Abstract

The outcome of competition for nitrogen (N) between native and invasive tree species is a major concern when considering increasing anthropogenic N deposition. Our study investigated whether three native (i.e. *Fagus sylvatica*, *Quercus robur*, and *Pinus sylvestris*) and two invasive woody species (i.e. *Prunus serotina* and *Robinia pseudoacacia*) showed different responses regarding morphological and physiological parameters (i.e. biomass and growth indices, inorganic vs. organic N acquisition strategies, and N allocation to N pools) depending on the identity of the competing species, and whether these responses were mediated by soil N availability. In a greenhouse experiment, tree seedlings were planted either single or in native-invasive competition at low and high soil N availability. We measured inorganic and organic N acquisition using ^{15}N labeling, total biomass, growth indices, as well as total soluble amino acid-N and protein-N levels in the leaves and fine roots of the seedlings. Our results indicate that invasive species have a competitive advantage via high growth rates, whereas native species could avoid competition with invasives via their higher organic N acquisition suggesting a better access to organic soil N sources. Moreover, native species responded to competition with distinct species- and parameter-specific strategies that were partly mediated by soil N availability. Native tree seedlings in general showed a stronger response to invasive *P. serotina* than *R. pseudoacacia*, and their strategies to cope with competition reflect the different species' life history strategies and physiological traits. Considering the responses of native and invasive species, our results suggest that specifically *Q. robur* seedlings have a competitive advantage over those of *R. pseudoacacia* but not *P. serotina*. Furthermore, native and invasive species show stronger responses to higher soil N availability under competition compared to when growing single. In conclusion, our study provides insights into the potential for niche differentiation between native and invasive species by using different N forms available in the soil, the combined effects of increased soil N availability and competition on tree seedling N nutrition, as well as the species-specific nature of competition between native and invasive tree seedlings which could be relevant for forest management strategies.

3.2. Introduction

Biological plant invasions have consequences on plant interactions in native communities, thus severely affecting ecosystems in the future given that plant community composition and structure are largely determined by the outcome of plant-plant interactions such as facilitation and/or competition for limiting resources (Goldberg and Barton 1992). Considering that nutrients, especially nitrogen (N), limit woody plant growth (Körner 2003, Miller et al. 2007, Millard and Grelet 2010) and that resource acquisition and internal allocation play an important role in achieving maximum growth and reproductive fitness in plants, particularly long-living woody species, competition for N between native and non-native woody species is of great relevance. Although evidence points in the direction that species in native plant communities have evolved different strategies to avoid competition for N via preference of different N sources to effectively avoid competition via niche differentiation (Näsholm et al. 2009, Hodge and Fitter 2013, Simon et al. 2017), via N acquisition at different times during the growing season (Simon et al. 2017), and/or via the interaction with soil microorganisms to enhance N acquisition (e.g. Hodge and Fitter 2013), these strategies might no longer be effective in competition with non-native plant species because of their higher competitive ability compared to native species (Gioria and Osborne 2014). Considering their higher capacity to exploit limited resources, better resource use efficiency, inhibition of growth, as well as establishment of potential competitors (D´Antonio and Vitousek 1992, Gioria and Osborne 2014), non-native plant species have a large potential to become invasive (Keller et al. 2011) and as a result induce profound changes in forest ecosystem structure and functioning (Holmes et al. 2009, Vilà et al. 2011, Aerts et al. 2017).

The outcome of plant interactions is not only determined by biotic interactions but also shifts depending on the environmental conditions (Aschehough et al. 2016). Abiotic factors, such as light availability (Simon et al. 2014), water availability (Fotelli et al. 2001, Fotelli et al. 2002), air temperature (Fotelli et al. 2005) as well as soil N availability (e.g. Li et al. 2015) influence the competition for N between plants, thereby shifting the outcome of plant-plant competition. For example, nitrate acquisition of sycamore maple (*Acer pseudoplatanus* L.) decreased when competing with European beech (*Fagus sylvatica* L.) compared to intraspecific competition at high but not low soil N availability suggesting that the response to competition for N is mediated by soil N availability (Li et al. 2015). Furthermore, plant-plant competition and soil N availability showed combined effects on plant N nutrition by inducing changes in N pools in the fine roots such as total soluble protein-N and total amino acid-N (Li et al. 2015). Overall, the interplay between interspecific competition for N and varying environmental conditions can impact on plant N acquisition and N nutrition with consequences for plant growth and survival, which in turn may affect plant community composition and structure (Aerts et al. 2017). However, in general, non-woody species are investigated preferably in plant interaction studies, thus the understanding of the mechanisms underlying tree interactions is rather scarce (Trinder et al. 2013, Pommerening and Sánchez Meador 2018), although resource acquisition and internal allocation are particularly important for resource-storing and -remobilizing in long-living trees.

Woody invasive species are a potential threat for forest habitats throughout Europe (Campagnaro et al. 2018), and studies indicate their highly competitive characteristics. For example, root competition with seedlings of *Prunus serotina* and *Robinia pseudoacacia* – the most important invaders in natural and semi-natural woodlands in Central Europe (Campagnaro et al. 2018, Halarewicz et al. 2017) – decreased total biomass and modified biomass allocation of seedlings of the native species *Quercus robur* and *Carpinus betulus* (i.e. by increased root biomass at the expense of aboveground production) (Kawaletz et al. 2013, Kawaletz et al. 2014). Furthermore, competition intensity increased more over time for the native species when competing with the two invasive species compared to intraspecific competition or competition among natives (Kawaletz et al. 2013, Kawaletz et al. 2014). On the other hand, competition with *P. serotina* stimulated the height growth of seedlings of *Q. petraea*, suggesting a potential for species-specific competitive effects (Robakowski and Bielinis 2011).

Although these studies stress the competitive effects of invasive *P. serotina* and *R. pseudoacacia* on the growth of native species, the understanding on the interactions between native and invasive woody species with regard to N cycling in forest ecosystems depending on abiotic stressors (i.e. soil N availability) is very limited. Soil N availability is expected to rise in terrestrial ecosystems of Central Europe as a result of increased atmospheric deposition (Rennenberg et al. 2009, Waldner et al. 2014), which will also impact on the outcome of invasion processes in plant communities (Bradley et al. 2010, Littschwager et al. 2010, Luo et al. 2014). Therefore, it is key to understand the processes of interspecific competition for N between native and invasive tree species, as well as the effects of soil N availability on the outcome of this competition with regard to plant N acquisition and N nutrition to predict the future dynamics of forest ecosystems. Thus, we conducted a greenhouse experiment using different native and invasive tree species to investigate whether native and invasive tree species show species-specific responses in terms of growth, N acquisition strategies and N allocation to N pools depending on the identity of their competitor, and whether those responses are influenced by increased soil N availability. The native species (i.e. *Fagus sylvatica* L., *Quercus robur* L., and *Pinus sylvestris* L.), represent the most abundant species in Central European forests (Ellenberg and Leuschner 2010). The two invasive species (i.e. *Prunus serotina* Ehrh. and *Robinia pseudoacacia* L.) are among the top five invasive tree species in Central European forests (Campagnaro et al. 2018). The selected species differ in their physiology and growth strategies: *F. sylvatica* and *Q. robur* are late successional, broadleaved species differing in their drought tolerance – i.e. *Q. robur* is drought tolerant, found on fertile, loamy-clay soils, whereas *F. sylvatica* is sensitive to drought, and is mostly found on moderately fertile, calcareous soils (Cavin et al. 2013, Houston et al. 2016a, Eaton et al. 2016). *P. sylvestris* is an evergreen conifer with needles present throughout the year functioning as N storage organs (Millard and Grelet 2010), as opposed to the broadleaved species which lose their leaves in the autumn season. Regarding the invasive species, *P. serotina* produces cyanogenic compounds that negatively affect other plant species (Csiszár 2009) by reducing their germination and growth rates (Robakowski et al. 2016), while *R. pseudoacacia* can fix atmospheric N_2 via symbiotic bacteria in root nodules and is potentially dominant at nutrient poor sites (Danso et al. 1995, Vítková et al. 2017). Moreover, we have chosen seedlings of uniform age to incorporate the aspect of the different demands for nitrogen between species according to their growth strategies (Reich 2014). These differences in physiological aspects between the species may result in different response strategies to interspecific competition and also shift with increasing soil N availability. We hypothesized that: (1) Invasive species have traits that allow them to successfully grow and establish compared to native species. (2) Higher soil N availability has a positive effect on the growth and N strategies of invasive species compared to native species. (3) Native tree species respond species-specific depending on the identity of the invasive competitor species. (4) Invasive species respond differently to different native competitor species. (5) Seedling responses to competition are mediated by soil N availability.

3.3.Material and methods

3.3.1. Plant material and growth conditions

We chose three tree species native to and widely found in Central European forests: (i) European beech (*Fagus sylvatica* L., Fagaceae), (ii) pedunculate oak (*Quercus robur* L., Fagaceae), and (iii) Scots pine (*Pinus sylvestris* L., Pinaceae). As invasive species we chose two tree species native to North America that were introduced to Europe in the 17th century as ornamental plants and later widely planted for restoration and reforestation purposes: (i) black cherry (*Prunus serotina* Ehrh., Rosaceae), and (ii) black locust (*Robinia pseudoacacia* L., Leguminosae) (Starfinger et al. 2003, Vítková et al. 2017). From here on, species used in this study will be referred to by their genus, i.e., *Fagus*, *Quercus*, *Pinus*, *Prunus* and *Robinia*. For all species, one-year-old seedlings were purchased from a commercial tree nursery (Müller Münchehof Pflanzen GmbH, Seesen/Münchehof, Germany) and planted in different competition regimes (one or two seedlings per pot, see 3.3.2. “Experimental design”) in a 1:1

mixture of sand and vermiculite in 3 L plastic pots (25 cm x 12 cm) at the end of November 2015. Pots overwintered outdoors and were brought into the greenhouse in early March 2016. For the next 10 days, pots were watered regularly and sufficiently with tap water. From mid-March, pots received 100 ml of a low N nutrient solution (see solution composition below) every second day as watering until the end of leaf development (early May) when the soil N availability treatments started (see 3.3.2. “Experimental design”). The pots were exposed to natural light conditions and day/night regime. The average air temperature was 19.3 ± 4.0 °C / 16.0 ± 3.8 °C (day/night, mean ± standard deviation). The average relative humidity was 54.7 ± 13.0 % / 63.4 ± 10.1 % (day/night, mean ± standard deviation).

3.3.2. Experimental design

The experiment was conducted in a fully orthogonal design with two factors, “soil N availability” (i.e. low or high) and “competitor identity” (i.e. native and invasive species in interspecific competition). Seedlings were planted in interspecific competition between native and invasive species (i.e. one seedling of a native species and one seedling of an invasive species per pot). Interspecific competition pots were established for every combination of native and invasive species. Furthermore, seedlings were planted without competition (i.e. one seedling per pot) to compare parameters between species and to determine the species general strategies and their responses to soil N availability without competition. For each species, a total of 24 pots were established as single seedlings as well as for each combination of competitor identity, summing up to a total of 264 pots. In early May, pots were assigned to either the low or high soil N availability treatment (i.e. n=12 per combination of species, competitor identity, and soil N availability treatment). Pots were irrigated every second day with 100 ml of either low N or high N artificial nutrient solution mimicking a low (Dannenmann et al. 2009) or high soil N field site (Stoelken et al. 2010) for 6 weeks. Tests prior to the experiment showed that 100 ml of water were sufficient under the moderate air temperatures that prevailed early in the experiment. When temperatures increased in late May, additional irrigation was provided (see below). The artificial low N nutrient solution consisted of 100 µM KNO_3, 90 µM $CaCl_2*2H_2O$, 70 µM $MgCl_2*6H_2O$, 50 µM KCl, 24 µM $MnCl_2*4H_2O$, 20 µM NaCl, 10 µM $AlCl_3$, 7 µM $FeSO_4*7H_2O$, 6 µM K_2HPO_4, 1 µM NH_4Cl, 25 µM glutamine, and 25 µM arginine. The artificial high N nutrient solution consisted of 20 µM $Al_2(SO_4)_3$, 75 µM $CaCl_2*2H_2O$, 4 µM $FeCl_3*6H_2O$, 14 µM KCl, 10 µM $MnCl_2*4H_2O$, 40 µM $MgCl_2*6H_2O$, 4.5 µM Na_2HPO_4, 20 µM NaCl, 50 µM NH_4Cl, 300 µM KNO_3, 100 µM glutamine, and 100 µM arginine. Additionally, from the end of May, pots were irrigated with tap water every second day (i.e. alternating with the days when the low/high soil N solutions were applied) to avoid drought stress related to increased air temperatures and solar radiation until the ^{15}N uptake experiments and final harvest commenced in mid-June.

3.3.3. ^{15}N uptake experiments

To quantify net inorganic (i.e. ammonium and nitrate) and organic (i.e. glutamine and arginine) N uptake capacity of the fine roots of the seedlings, the ^{15}N enrichment technique was used as described by Gessler et al. (1998) and modified by Simon et al. (2010) prior to the harvest of the seedlings. Seedlings were carefully removed from the pots, and their roots thoroughly washed with tap water to remove adherent substrate particles. Fine roots still attached to the seedlings were then incubated for 2 hours in the artificial soil solution (n = 4-6) according to their treatment (either low or high N as described above) containing all four N sources, but with only one of them labelled as either $^{15}NH_4^+$, $^{15}NO_3^-$, $^{13}C/^{15}N$-glutamine, or $^{13}C/^{15}N$-arginine. The remaining roots were carefully wrapped in wet tissue to avoid desiccation. After incubation, the fine roots were cut off and washed twice in 0.5 M $CaCl_2$ to remove the incubation solution from the root surface. The fresh weight was determined, followed by oven-drying at 60 °C for 48 h and determination of the dry weight. Amino acids were $^{13}C/^{15}N$-labelled to determine whether they are taken up as intact molecules (Simon et al., 2011). Controls with no ^{15}N or ^{13}C label were included to account for natural abundance of ^{15}N and ^{13}C in the fine roots. Incubation took place between 10 am and 2 pm to avoid diurnal variation in net N uptake capacity (Gessler et al. 2002).

3.3.4. Harvest and quantification of plant growth indices

To calculate relative growth rates (RGR), 3 to 4 pots per combination of species and competitor identity were harvested after leaf development and before commencing the soil N availability treatments to determine initial seedling biomass. Initial total seedling biomass (mean ± SD) was for *Fagus* 1.65 ± 0.53 g dw, for *Quercus* 6.43 ± 4.50 g dw, for *Pinus* 3.05 ± 1.16 g dw, for *Prunus* 10.62 ± 7.91 g dw, and for *Robinia* 4.23 ± 3.00 g dw (mean ± SD). Initial stem length was 21.7 ± 3.8 cm for *Fagus*, 33.4 ± 5.2 cm for *Quercus*, 29.2 ± 5.3 cm for *Pinus*, 63.8 ± 16.3 cm for *Prunus*, and 54.8 ± 8.4 cm for *Robinia*. Subsequent to the ^{15}N uptake experiments, all remaining seedlings were separated into leaves, stems, and roots. After determining their fresh weight, all organs were oven-dried at 60 °C for 48 h, and their dry weight was determined. On the final harvest, a subset of 8 to 10 representative leaves was collected from each seedling and leaf area was measured (LI-3100C Area Meter, LI-COR, Lincoln, USA) as well as fresh and dry weight determined to calculate specific leaf area (SLA). Based on Liu and van Kleunen (2017), a subset of fine roots was collected, stained, scanned and their total length measured (WinRhizo 2012, Regent Instruments Inc., Quebec, Canada) to calculate specific root length (SRL) before oven-drying and subsequent dry weight determination. Additionally, leaf and fine root samples were collected from each seedling for quantification of total soluble amino acid and total soluble protein levels, shock-frozen in liquid N_2 immediately after determining their fresh weight, and stored at -80 °C until further analyses. Root:shoot ratio was calculated as the ratio between total root biomass and the combined biomass of stem and leaves. RGR was calculated for each seedling following the formula: $RGR = (\ln b_2 – \ln b_1) * t^{-1}$, where b_1 is total seedling biomass in grams at initial harvest, b_2 is total seedling biomass in grams at the final harvest, and t is the time period in days between the initial harvest and the final harvest (Grubb et al. 1996).

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

To quantify total N and C as well as ^{15}N and ^{13}C enrichment, dried fine root samples were ground to a fine homogenous powder using a ball mill (TissueLyser, Retsch, Haan, Germany). Aliquots of 1.2 to 2.4 mg were weighed into 4x6 mm tin capsules (IVA Analysentechnik, Meerbusch, Germany) and analyzed with an isotope ratio mass spectrometer (Delta V Advantage, Thermo Electron, Dreieich, Germany) coupled to an elemental analyzer (Euro EA, Eurovector, Milano, Italy). Δ values were calculated using a laboratory standard (acetanilide) that was part of every sequence in intervals also used in different weights to determine isotope linearity of the system. The laboratory standard was calibrated against several suitable international isotope standards (IAEA, Vienna). Final correction of isotope values was done with several international isotope standards and other suitable laboratory standards which cover the range of ^{15}N and ^{13}C results. Net N uptake capacity (nmol N g^{-1} fw h^{-1}) was calculated based on the incorporation of ^{15}N into the fine root according to Gessler et al. (1998): N uptake capacity = $((^{15}N_l-^{15}N_c)*N_{tot}*dw*10^5)/(MW*fw*t)^{-1}$, where $^{15}N_l$ and $^{15}N_c$ are the atom% of ^{15}N in labelled (N_l) and control plants (N_c, natural abundance), respectively, N_{tot} is the total N percentage, MW is the molecular weight (^{15}N g mol^{-1}), and t represents the incubation time. Net uptake capacity of the amino acids glutamine and arginine was lower based on ^{13}C incorporation than on ^{15}N incorporation indicating either (1) the degradation of amino acids in the solution or on the root surface, and/or (2) the respiration of amino acid-derived C inside the roots (Simon et al. 2011).

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

Total soluble protein levels in the leaves and fine roots were extracted based on Dannenmann et al. (2009). Aliquots of ~50 mg ground frozen organ were incubated in 1.5 ml extraction buffer (50 mM Tris-HCl pH 8.0, 1 mM EDTA, 15% (v/v) glycerol, 0.6 mM dithiothreitol, 1% Triton X-100, 2 EDTA-free protease inhibitor cocktail tablets per 100 ml buffer solution) at 4 °C for 30 min and subsequently centrifuged for 10 min at 14,000 rpm and 4 °C. The extraction was repeated once to increase the yield. Then, 500 µL of the combined supernatant were incubated with 1 ml 10 % (v/v) trichloroacetic acid for 10 min at room temperature followed by centrifugation for 10 min at 14,000 rpm and 4 °C. The protein pellet was dissolved in 1 ml 1 M KOH. To quantify total soluble protein levels according to Simon et al. (2010), 1 ml of Bradford reagent was added to 50 µL of extract. After 10 min incubating in the dark at room temperature, the absorbance at 595 nm was measured in a spectrophotometer (Ultrospec 3100pro, Amersham Biosciences). Bovine serum albumin (BSA) was used as standard.

To quantify total soluble amino acid levels in the leaves and fine roots, aliquots of ~50 mg of frozen organ were extracted in 200 µL Hepes-buffer (5 mM EGTA, 20 mM HEPES, 10 mM NaF) and 1 ml 3.5:1.5 (v:v) methanol/chloroform, according to Winter et al. (1992). After 30 min incubation on ice, 600 µL of distilled water were added and the samples centrifuged for 5 min at 14,000 rpm and 4 °C. The addition of distilled water was repeated once to increase the yield. For the quantification, according to Liu et al. (2005), 50 µL ninhydrin solution was added to a 50 µL aliquot of the combined extract and boiled for 30 min. The ninhydrin solution consisted of a 1:1 mixture of solution A (i.e. 3.84 g citric acid, 0.134 g $SnCl_2$, and 40 ml 1 M NaOH, filled up to 100 ml with distilled water at pH 5), and solution B (i.e. 4 g ninhydrin in 100 ml ethylene-glycol-monomethyl-ether). After cooling to room temperature, 1 ml 50% isopropanol was added to the extract and incubated for 15 min. The absorption was measured at 570 nm in a spectrophotometer (Ultrospec 3100pro, Amersham Biosciences). L-glutamine was used as standard.

3.3.7. Statistical analyses

For all species, differences between treatment levels (i.e. competitor identity and low/high soil N availability) were tested (alpha level of 0.050) for total biomass, root:shoot ratio, SLA, SRL, RGR, total soluble amino acid and total soluble protein contents in the leaves and fine roots, as well as inorganic and organic net N uptake capacity by performing Permutational ANOVAs

(PERMANOVA) based on a Euclidean resemblance matrix between samples (Anderson et al. 2008). We performed two-way PERMANOVAs using “soil N availability” and “competitor identity” as orthogonal factors. The factor “soil N availability” had two levels: (i) low N and (ii) high N. The factor “competitor identity” had two levels for native species: (i) competition with *Prunus* and (ii) competition with *Robinia*; and three levels for the invasive species: (i) competition with *Fagus*, (ii) competition with *Quercus*, and (iii) competition with *Pinus*. When a significant interaction between factors was found, *post hoc* PERMANOVA pair-wise comparisons were performed. To test for differences between species in terms of SLA, SRL, RGR, total soluble amino acid and total soluble protein contents in the leaves and roots, as well as net uptake capacity of the four N sources, two-way PERMANOVAs were performed on the single seedlings data (i.e. no competition), using “species” and “soil N availability” as factors. To test for significant differences between low and high soil N availability for each species growing in absence of competition, Mann-Whitney U-tests were performed for all measured parameters. Furthermore, to test for preferences in net N uptake capacity, one-way PERMANOVAs were performed for each combination of species and competitor identity using “N source” as factor at both levels of soil N 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).

3.4.Results

3.4.1. Comparison between species and responses to soil N availability without competition

3.4.1.1.General differences in N acquisition and growth strategies between tree species

Seedlings of invasive species had a significantly higher total biomass and RGR than native seedlings, with *Prunus* having the largest total biomass among all species considered (Table 3.1 and Supplementary Table 3.1). Moreover, invasive *Robinia* had significantly higher SLA and SRL than both *Prunus* and the three native species (*Fagus*, *Quercus* and *Pinus*) (Table 3.1 and Supplementary Table 3.1). Similarly, *Robinia* had in general significantly higher levels of total soluble amino acid-N and protein-N in the leaves and fine roots than all other investigated species (Table 3.1 and Supplementary Table 3.2). Regarding N acquisition, inorganic N net uptake capacity did not differ significantly between species. However, seedlings of native tree species had significantly higher organic N net uptake capacity than seedlings of invasive tree species (Table 3.1 and Supplementary Table 3.3).

Table 3.1. Differences in total biomass, growth indices, inorganic and organic N net uptake capacity, and N metabolite levels between single grown seedlings of *Fagus sylvatica*, *Quercus robur*, *Pinus sylvestris*, *Prunus serotina*, and *Robinia pseudoacacia*. Species A > species B = seedlings of species A had significantly higher values than seedlings of species B, n.s. = no significant differences between species. Total biomass (g), root:shoot ratio: ratio of belowground biomass to aboveground biomass, RGR: relative growth rate (g dw g^{-1} dw d^{-1}), SLA: specific leaf area (cm^2 g^{-1} dw), SRL: specific root length (cm g^{-1} dw), N net uptake capacity (nmol N g^{-1} fw h^{-1}), NH_4^+: ammonium, NO_3^-: nitrate, Gln-N: glutamine, Arg-N: arginine, total soluble amino acid-N (mg amino acid-N g^{-1} dw), total soluble protein-N (mg protein-N g^{-1} dw).

Biomass and growth indices					**N net uptake capacity**				**N metabolites**			
									Total soluble amino acid-N		**Total soluble protein-N**	
Total biomass	**Root:shoot ratio**	**RGR**	**SLA**	**SRL**	**NH_4^+**	**NO_3^-**	**Gln-N**	**Arg-N**	**Leaves**	**Fine roots**	**Leaves**	**Fine roots**
Prunus > *Robinia* > *Quercus* > *Fagus*, *Pinus*	*Pinus* > *Quercus* > *Prunus* > *Fagus* > *Robinia*	*Prunus*, *Robinia* > *Fagus*, *Quercus*, *Pinus*	*Robinia* > *Fagus* > *Quercus* > *Prunus*	*Robinia* > *Fagus*, *Quercus*, *Pinus*, *Prunus*	n.s.	n.s.	*Fagus*, *Pinus* > *Prunus*, *Robinia*	*Fagus*, *Quercus*, *Pinus* > *Prunus*, *Robinia*	*Robinia* > *Prunus* > *Fagus*, *Quercus*, *Pinus*	*Prunus*, *Robinia* > *Quercus*, *Pinus*	*Robinia*, *Fagus*, *Pinus* > *Prunus*	*Robinia* > *Fagus*, *Quercus*, *Pinus*, *Prunus*

Table 3.2. Effects of increased soil N availability on total biomass, growth indices, inorganic and organic N net uptake capacity, and N metabolite levels on (A) seedlings growing single and (B) seedlings growing in competition of *Fagus sylvatica*, *Quercus robur*, *Pinus sylvestris*, *Prunus serotina*, and *Robinia pseudoacacia*. ↑ = significant increase with increased soil N availability, ↓ = significant decrease with increased soil N availability, n.s. = no significant differences between high and low soil N availability. Total biomass (g), root:shoot ratio: ratio of belowground biomass to aboveground biomass, RGR: relative growth rate (g dw g^{-1} dw d^{-1}), SLA: specific leaf area (cm^2 g^{-1} dw), SRL: specific root length (cm g^{-1} dw), N net uptake capacity (nmol N g^{-1} fw h^{-1}), NH_4^+: ammonium, NO_3^-: nitrate, Gln-N: glutamine, Arg-N: arginine, total soluble amino acid-N (mg amino acid-N g^{-1} dw), total soluble protein-N (mg protein-N g^{-1} dw).

	Biomass and growth indices					**N net uptake capacity**				**N metabolites**			
										Total soluble amino acid-N		**Total soluble protein-N**	
	Total biomass	**Root:shoot ratio**	**RGR**	**SLA**	**SRL**	**NH_4^+**	**NO_3^-**	**Gln-N**	**Arg-N**	**Leaves**	**Fine roots**	**Leaves**	**Fine roots**
(A) Seedlings growing single													
Fagus	n.s.	n.s.	n.s.	n.s.	n.s.	↑	↑	n.s.	↑	n.s.	n.s.	n.s.	↑
Quercus	n.s.	n.s.	n.s.	n.s.	n.s.	↑	n.s.	n.s.	↑	↓	n.s.	n.s.	n.s.
Pinus	n.s.	n.s.	n.s.	n.s.	n.s.	↑	↑	↑	↑	n.s.	n.s.	n.s.	n.s.
Prunus	n.s.	n.s.	n.s.	n.s.	n.s.	↑	n.s.	n.s.	↑	n.s.	n.s.	n.s.	n.s.
Robinia	n.s.	↓	n.s.	n.s.	n.s.	↑	n.s.	n.s.	↑	n.s.	n.s.	n.s.	n.s.
(B) Seedlings growing in competition													
Fagus	n.s.	n.s.	n.s.	n.s.	↓	↑	n.s.	n.s.	↑	↓	↓	n.s.	n.s.
Quercus	n.s.	n.s.	↓	n.s.	n.s.	↑	n.s.	↑	↑	n.s.	↓(b)	n.s.	↓
Pinus	n.s.	n.s.	↓	n.s.	n.s.	↑	n.s.	n.s.	↑	↓	n.s.	n.s.	n.s.
Prunus	n.s.	n.s.	n.s.	n.s.	↓	↑	n.s.	↑	↑	↓	↓	↓	n.s.
Robinia	n.s.	n.s.	n.s.	n.s.	n.s.	↑	↑	↑(a)	↑	↓	↓(c)	n.s.	↓

(a): only in competition with *Fagus* and *Pinus*, (b): only in competition with *Prunus*, (c): only in competition with *Fagus* and *Quercus*, no letter: effect of soil N availability regardless of competitor identity

Table 3.3. Effects of competitor identity on total biomass, growth indices, inorganic and organic N net uptake capacity, and N metabolite levels of *Fagus sylvatica*, *Quercus robur*, *Pinus sylvestris*, *Prunus serotina*, and *Robinia pseudoacacia* seedlings. Species A < species B = seedlings competing with species A had significantly lower values than seedlings competing with species B, n.s. = no significant differences between competitor identities. Total biomass (g), root:shoot ratio: ratio of belowground biomass to aboveground biomass, RGR: relative growth rate (g dw g^{-1} dw d^{-1}), SLA: specific leaf area (cm^2 g^{-1} dw), SRL: specific root length (cm g^{-1} dw), N net uptake capacity (nmol N g^{-1} fw h^{-1}), NH_4^+: ammonium, NO_3^-: nitrate, Gln-N: glutamine, Arg-N: arginine, total soluble amino acid-N (mg amino acid-N g^{-1} dw), total soluble protein-N (mg protein-N g^{-1} dw).

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

(a): only at high soil N availability, (b): only at low soil N availability, no letter: effect of competition regardless of soil N availability

3.4.1.2. Individual species responses to high compared to low soil N availability

Increased soil N availability had neither a significant effect on the total biomass nor the growth indices of the single grown species, except for a significantly lower root:shoot ratio of *Robinia* (Table 3.2A and Supplementary Table 3.4). However, inorganic and organic N net uptake capacity increased significantly for all species with high compared to low soil N availability following species-specific patterns. More specifically, an increase in net uptake capacity was shown for the native species for ammonium, nitrate, and arginine-N of *Fagus*, for ammonium and arginine-N of *Quercus*, and for all N forms of *Pinus*. Both invasive species had an increased ammonium and arginine-N net uptake capacity with higher soil N availability (Table 3.2A and Supplementary Table 3.5). Furthermore, the changes in N metabolites levels in the leaves and fine roots at high compared to low soil N availability were also species-specific: *Fagus* had higher total soluble protein-N content in the leaves, *Quercus* had lower total soluble amino acid-N content in the leaves, and *Prunus* had lower total soluble amino acid-N content in the leaves and fine roots, while for *Pinus* and *Robinia* the N metabolite content in the leaves and fine roots did not differ significantly between low and high soil N availability (Table 3.2A and Supplementary Table 3.6). No other significant differences were found between soil N availability treatments in the single grown seedlings.

3.4.2. Effects of competition on native and invasive tree species

3.4.2.1. Native tree species – Differences in the response to competitor identity regarding total biomass, growth indices, N acquisition, and N pools in the leaves and fine roots

Fagus seedlings grown in competition with *Prunus* had significantly lower nitrate and glutamine-N net uptake capacity as well as RGR, regardless of soil N availability than when grown with *Robinia*, while no other parameter differed significantly with different competitor species (Figure 3.1, Table 3.3 and Supplementary Tables 3.7, 3.8A). Unlike *Fagus*, for *Quercus* seedlings inorganic and organic N net uptake capacity did not differ between different competitor identities (Figure 3.1 and Table 3.3). However, seedlings of *Quercus* had significantly lower total biomass under competition with *Prunus* compared to under competition with *Robinia*, regardless of soil N availability (Table 3 and Supplementary Table 3.7). Moreover, total soluble amino acid-N content in the fine roots of *Quercus* seedlings was also significantly lower when grown in competition with *Prunus* compared to *Robinia* only at high soil N availability (Table 3.3 and Supplementary Tables 3.8A, 3.8B). For *Quercus* seedlings no differences were found between different competitor identities regarding all other parameters (Figure 3.1, Table 3.3 and Supplementary Tables 3.7, 3.8A). For *Pinus* seedlings the only difference between competitor identities was a significantly higher SRL for seedlings competing with *Prunus* compared to those competing with *Robinia* (Figure 3.1, Table 3.3 and Supplementary Tables 3.7, 3.8A).

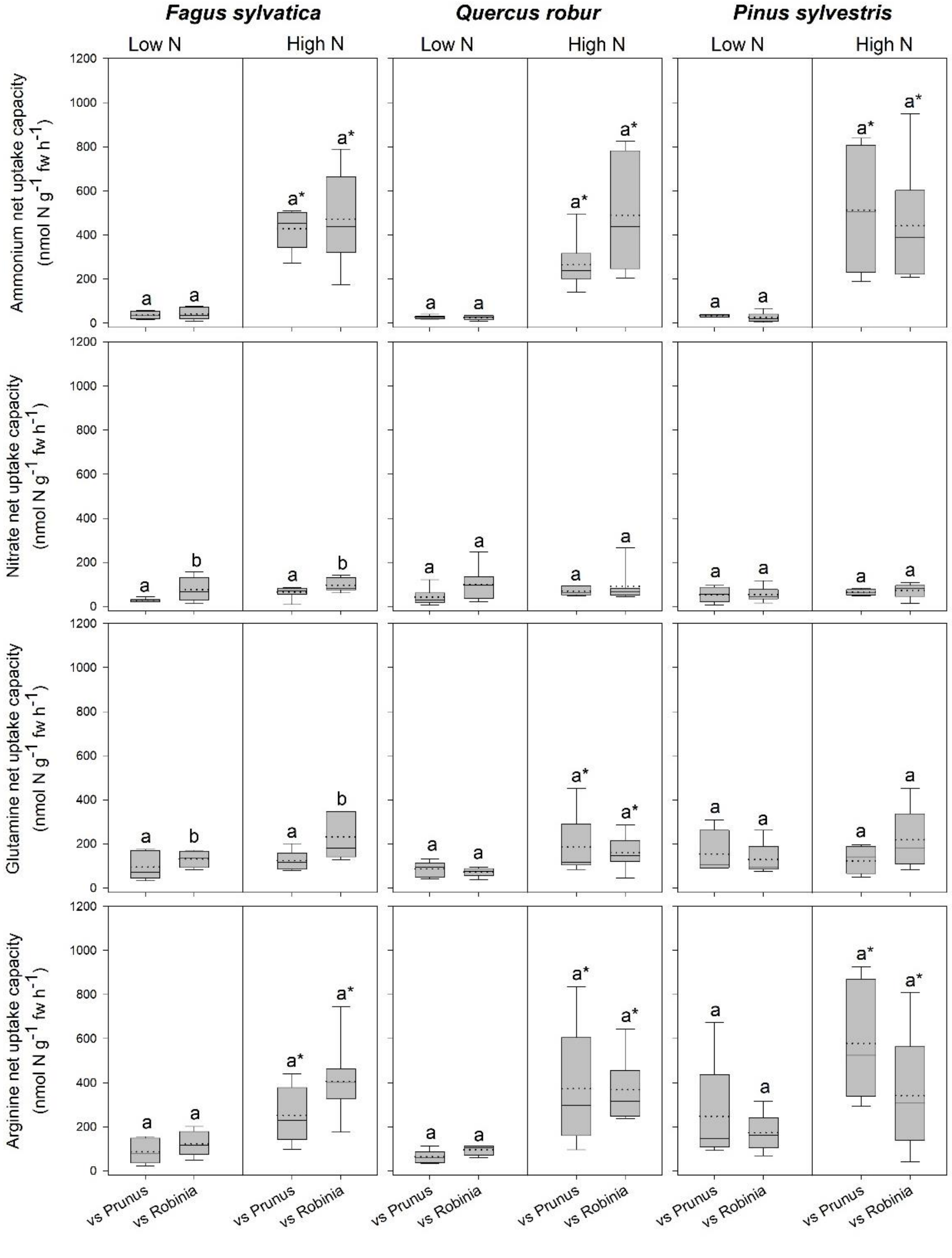

Figure 3.1. Ammonium, nitrate, glutamine-N, and arginine-N net uptake capacity (nmol N g^{-1} fw h^{-1}) by fine roots of *Fagus sylvatica*, *Quercus robur* and *P. sylvestris* seedlings at low and high soil N availability under different competition regimes. vs Prunus = competition with *Prunus serotina*; vs Robinia = competition with *Robinia pseudoacacia*. Box plots show mean (dotted line) and median (continuous line). Different letters indicate significant differences between competition regimes within a specific soil N availability treatment, and asterisks indicate significant differences between soil N availability treatments detected using permutational analysis of variance ($p < 0.05$).

3.4.2.2. Invasive tree species - Differences in the response to competitor identity regarding total biomass, growth indices, N acquisition, and N pools in the leaves and fine roots

Seedlings of *Prunus* had a significantly lower SLA when competing with *Fagus* than when competing with *Quercus* (Table 3.3 and Supplementary Table 3.7), while there were no differences between different competitor identities with respect to any of the other measured parameters (Figure 3.2, Table 3.3 and Supplementary Tables 3.7, 3.8A). Moreover, *Robinia* seedlings competing with *Fagus* had a significantly lower SLA (regardless of soil N availability), and higher total soluble amino acid-N content in the fine roots (only at low soil N availability) than seedlings competing with *Quercus* or *Pinus* (Table 3.3 and Supplementary Tables 3.7, 3.8A, 3.8B). Furthermore, seedlings of *Robinia* competing with *Quercus* had significantly lower glutamine-N net uptake capacity than those competing with either *Fagus* or *Pinus* (only at high soil N availability) (Figure 3.2). All other measured parameters did not differ significantly between different competitor identities for this species (Figure 3.2, Table 3.3 and Supplementary Tables 3.7, 3.8A).

3.4.3. Effects of high compared to low soil N availability on native and invasive tree seedlings growing in competition

3.4.3.1. Native tree species – Effects of high compared to low soil N availability on total biomass, growth indices, N acquisition, and N pools in the leaves and fine roots

For seedlings of *Fagus* a higher soil N availability resulted in significantly higher ammonium and arginine-N net uptake capacity (Figure 3.1), as well as a significantly lower SRL (Table 3.2B and Supplementary Table 3.7) and total soluble amino acid-N content in the leaves and fine roots (Table 3.2B and Supplementary Table 3.8A), regardless of competitor identity, while the rest of the measured parameters did not change significantly. For *Quercus*, ammonium and organic N net uptake capacity increased significantly (Figure 3.1), while RGR (Table 3.2B and Supplementary Table 3.7) and total soluble protein-N content in the fine roots (Table 3.2B and Supplementary Table 3.8A) decreased significantly at high compared to low soil N availability, regardless of competitor identity. Furthermore, higher soil N availability significantly reduced the total soluble amino acid-N content in the fine roots for *Quercus* seedlings when grown in competition with *Prunus* but not in seedlings grown in competition with *Robinia* (Table 3.2B and Supplementary Tables 3.8A, 3.8B). All other measured parameters remained unchanged between soil N availability treatments. For seedlings of *Pinus*, a high soil N availability significantly increased ammonium and arginine-N net uptake capacity (Figure 3.1), whereas RGR (Table 3.2 and Supplementary Table 3.7) and total soluble amino acid-N content in the leaves decreased (Table 3.2B and Supplementary Table 3.8A) compared to low soil N availability regardless of competitor identity, while the other measured parameters did not differ significantly.

3.4.3.2. Invasive tree species – Effects of high compared to low soil N availability on total biomass, growth indices, N acquisition, and N pools in the leaves and fine roots

Prunus seedlings at high compared to low soil N availability significantly increased their ammonium and organic N net uptake capacity (Figure 3.2), and decreased SRL (Table 3.2B and Supplementary Table 3.7), total soluble amino acid-N contents in the leaves and fine roots, as well as total soluble protein-N content in the leaves (Table 3.2B and Supplementary Table 3.8A) regardless of the competing species. There were no effects of soil N availability on any other measured parameter. For seedlings of *Robinia*, higher soil N availability resulted in significant increases in inorganic N and arginine-N net uptake capacity (Figure 3.2), as well as significantly decreased total soluble amino acid-N content in the leaves and total soluble protein-N content in the fine roots regardless of competition (Table 3.2B and Supplementary Table 3.8A). However, glutamine-N net uptake capacity increased significantly with higher soil N availability in seedlings competing with *Fagus* and *Pinus*, but not when competing with *Quercus* (Figure 3.2). Similarly, total soluble amino acid-N content in the fine roots decreased significantly with higher soil N availability in seedlings grown in competition with *Fagus* and *Quercus*, but not when grown in competition with *Pinus* (Table 3.2B and Supplementary Tables 3.8A, 3.8B). Soil N availability did not affect other parameters measured in *Robinia*.

3.4.4. N acquisition preferences of native and invasive species for specific N sources at low and high soil N availability

Comparing inorganic and organic N acquisition among N forms for each combination of species and competitor identity as well as for the single grown seedlings, we found general preference patterns at low and high soil N availability. At low soil N availability, organic N was preferred over inorganic N across species and competitor identities (Tables 3.4, 3.5), with a few exceptions that showed no preference for any N source: single seedlings of *Quercus* and *Prunus*, seedlings of *Quercus* competing with *Robinia*, and seedlings of *Prunus* and *Robinia* competing with *Fagus* (Tables 3.4, 3.5). At high soil N availability, a general pattern of preference for ammonium was found followed by organic N sources over nitrate. However, no preferences were found in single seedlings of *Prunus* and *Robinia*, seedlings of *Pinus* competing with *Robinia*, seedlings of *Robinia* competing with *Quercus*, and seedlings of *Prunus* and *Robinia* competing with *Pinus* (Tables 3.4, 3.5).

Table 3.4. Differences between ammonium (NH_4^+), nitrate (NO_3^-), glutamine-N (Gln-N), and arginine-N (Arg-N) net uptake capacity of the fine roots of *Fagus sylvatica*, *Quercus robur*, and *Pinus sylvestris* seedlings grown single and in competition at low and high soil N availability. Only significant differences are presented. n.s. = no significant differences between net uptake capacity of different N forms.

	Soil N availability	***Fagus***	***Quercus***	***Pinus***
Single grown seedlings	**Low**	Gln-N, Arg-N > NH_4^+ Gln-N > NO_3^-	n.s.	Gln-N, Arg-N > NH_4^+ Gln-N > NO_3^-
	High	NH_4^+, Arg-N > Gln-N > NO_3^-	n.s.	NH_4^+, Gln-N, Arg-N > NO_3^-
Seedlings in competition with *Prunus*	**Low**	Gln-N, Arg-N > NO_3^- Gln-N > NH_4^+	Gln-N, Arg-N > NH_4^+	Gln-N, Arg-N > NH_4^+, NO_3^-
	High	NH_4^+ > Arg-N > Gln-N > NO_3^-	NH_4^+, Gln-N, Arg-N > NO_3^-	NH_4^+, Arg-N > Gln-N > NO_3^-
Seedlings in competition with *Robinia*	**Low**	Gln-N, Arg-N > NH_4^+	n.s.	Gln-N, Arg-N > NH_4^+, NO_3^-
	High	NH_4^+ > Gln-N > NO_3^- Arg-N > NO_3^-	NH_4^+, Arg-N > NO_3^-, Gln-N	n.s.

Table 3.5. Differences between ammonium (NH_4^+), nitrate (NO_3^-), glutamine-N (Gln-N), and arginine-N (Arg-N) net uptake capacity of the fine roots of *Prunus serotina* and *Robinia pseudoacacia* seedlings grown single and in competition at low and high soil N availability. Only significant differences are presented. n.s. = no significant differences between net uptake capacity of different N forms.

	Soil N availability	***Prunus***	***Robinia***
Single grown seedlings	**Low**	n.s.	Gln-N, Arg-N > NH_4^+
	High	NH_4^+, Arg-N > Gln-N > NO_3^-	n.s.
Seedlings in competition with *Fagus*	**Low**	n.s.	n.s.
	High	NH_4^+, Arg-N > Gln-N, NO_3^-	NH_4^+, Gln-N, Arg-N > NO_3^-
Seedlings in competition with *Quercus*	**Low**	Arg-N > NH_4^+, NO_3^-	Gln-N, Arg-N > NH_4^+
	High	NH_4^+, Arg-N > NO_3^-, Gln-N	n.s.
Seedlings in competition with *Pinus*	**Low**	Arg-N > NH_4^+, Gln-N	Gln-N, Arg-N > NH_4^+, NO_3^-
	High	n.s.	n.s.

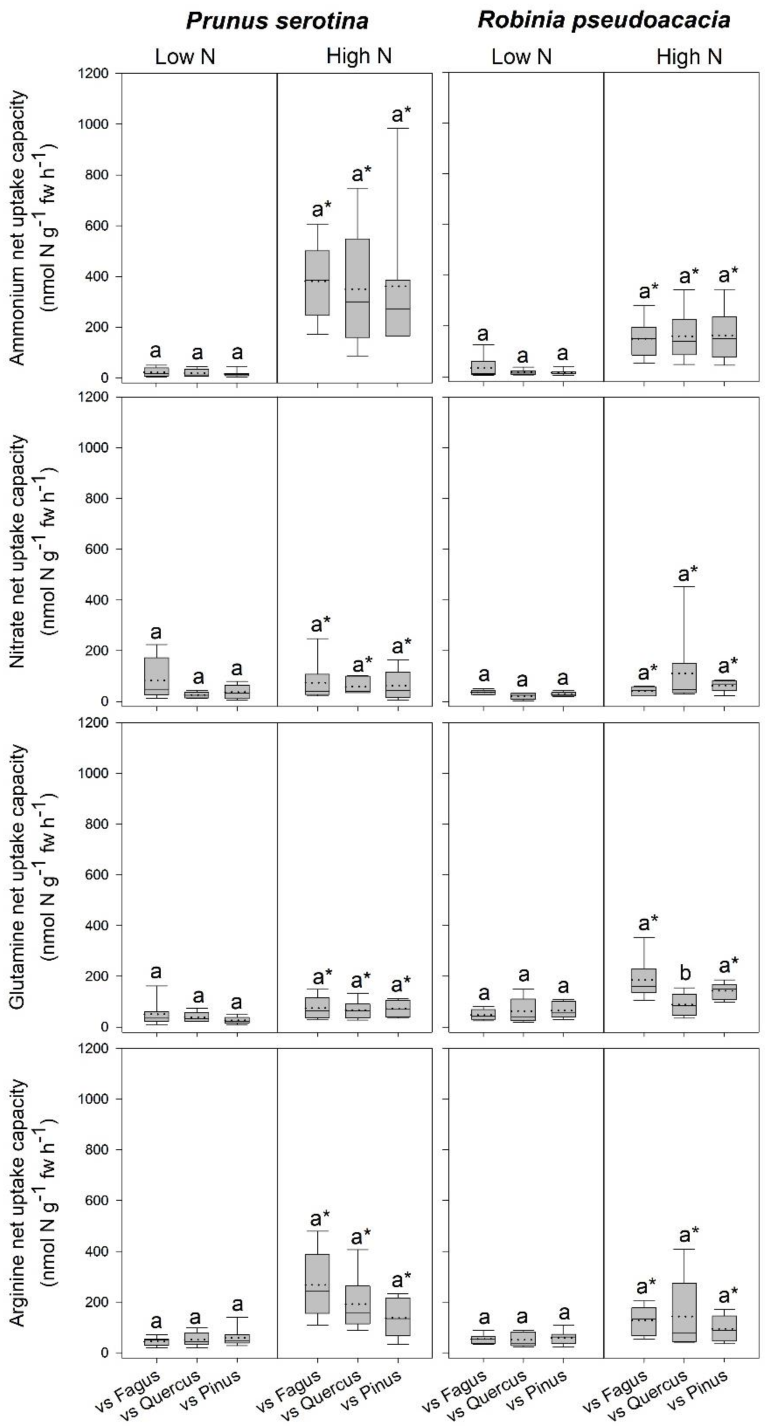

Figure 3.2. Ammonium, nitrate, glutamine-N, and arginine-N net uptake capacity (nmol N g^{-1} fw h^{-1}) by fine roots of *Prunus serotina* and *Robinia pseudoacacia* seedlings at low and high soil N availability under different competition regimes. vs Fagus = competition with *Fagus sylvatica*; vs Quercus = competition with *Quercus robur*; vs Pinus = competition with *Pinus sylvestris*. Box plots show mean (dotted line) and median (continuous line). Different letters indicate significant differences between competition regimes within a specific soil N availability treatment, and asterisks indicate significant differences between soil N availability treatments detected using permutational analysis of variance ($p < 0.05$).

With regard to the specific preferences of each species across different competitor identities and single grown seedlings, native *Fagus* and *Pinus* had in general similar preferences for N sources regardless of competitor identity or whether they grow single or in competition (Table 3.4). However, native *Quercus* showed no preference for any N source either when grown single or in competition with *Robinia*, but preferred organic N over ammonium when grown in competition with *Prunus* (at low soil N availability). Moreover, at high soil N availability, the preferences of *Quercus* shifted from no preference when grown single to preferring ammonium and organic N over nitrate when grown in competition with *Prunus* or *Robinia* (Table 3.4). For the invasive species, *Prunus* had no preference of N sources when grown single but generally preferred arginine-N over glutamine-N and inorganic N when competing with *Quercus* or *Pinus* at low soil N availability, while preferences for N sources did not shift across competitor identities at high soil N availability (Table 3.5). Seedlings of *Robinia* preferred organic over inorganic N sources regardless of competitor identity as well as when grown single at low soil N availability, except for seedlings competing with *Fagus* which showed no preference. However, at high soil N availability, *Robinia* seedlings preferred ammonium and organic N over nitrate when competing with *Fagus*, while seedlings competing with other native species or grown single took up all N forms without preference (Table 3.5).

3.5. Discussion

3.5.1. Strategies of native and invasive species growing without competition

3.5.1.1. Invasive species succeed with fast growth, but natives could counter with preference of organic N acquisition

Comparing single grown invasive and native species, invasive species showed traits characteristic for fast-growing species. The invasive species had higher total biomass, RGR, and SRL, as well as total soluble amino acid-N and protein-N levels in both leaves and fine roots compared to the natives. A fast RGR is related to a rapid increase in biomass as well as high leaf nutrient concentrations, whereas slow RGR is related to a higher investment of resources in defence (Reich et al. 1997, Aerts 1999). A higher SRL allows to forage larger soil volumes and is linked to a higher competitive ability for belowground resources (Aerts 1999). Our results suggest a potential of the studied invasive species to outcompete the native ones via rapid growth and exploitation of above- and belowground resources (Vilà and Weiner 2004, Gioria and Osborne 2014), which is in line with previous studies that found higher biomass (e.g. Closset-Kopp et al. 2007, Lee et al. 2004, Kawaletz et al. 2013, Ding et al. 2012), and monthly height increments (e.g. Ding et al. 2012) of *P. serotina* and *R. pseudoacacia* compared to plant species native to Europe and Asia. Moreover, the higher content of N metabolites in the fine roots is also linked to fast growing species compared to slow growers (e.g. *A. pseudoplatanus* compared to *F. sylvatica* in Li et al. 2015). For *Robinia*, a higher total N content in the leaves and fine roots compared to the other species is most likely associated with its N_2-fixing ability (McKey 1994, De Marco et al. 2013, Salpagarova et al. 2014). With regard to the N acquisition strategies, inorganic N acquisition was similar between invasive and native species. However, the lower organic N acquisition for the invasive compared to the native species suggests that native species could have a competitive advantage over the invasives via better exploitation of soil organic N. Although the invasives have faster growth, their species-specific N acquisition does not suggest a competitive advantage over natives.

3.5.1.2. With higher soil N availability N acquisition strategies of native and invasive seedlings are similar, whereas N allocation to N pools in the leaves and fine roots is species-specific

In our study, the responses of single growing native and invasive species to high compared to low soil N availability were similar for all species for N acquisition, biomass and growth indices, whereas the allocation of N to N pools in the fine roots and leaves depended on the species. More specifically, inorganic and organic N acquisition increased with rising soil N levels, while biomass and growth indices showed no response, except for an increased root:shoot ratio for *Robinia* indicating a greater allocation to root biomass with increasing soil N supply. The allocation of N to N pools in the leaves and fine roots was species-specific with one of three responses with higher compared to lower soil N availability: (1) an increase in total soluble protein-N levels in the leaves (i.e. for native *Fagus*), (2) a decrease in total soluble amino acid-N levels in the leaves (i.e. for native *Quercus*), and (3) no change of total soluble amino acid-N or protein-N levels in neither leaves nor fine roots (i.e. for native *Pinus* and both invasives *Prunus* and *Robinia*).

The increase in total soluble protein-N levels in the leaves of *Fagus* with higher soil N availability in combination with no change in overall biomass suggests that N is stored as Rubisco for later mobilization (Millard 1988, Masclaux-Daubresse et al. 2010). In contrast, *Quercus* had a reduced total soluble amino acid-N levels in the leaves which suggests that soluble amino acids are used to produce other compounds not quantified here, e.g. compounds related to defense. N allocation to N pools did not differ with increased soil N availability in

Pinus, *Prunus*, and *Robinia* which is likely related to life history traits resulting in a relative independence of external soil N supply: *Pinus* is a conifer with needles present throughout the year in which N is stored (Houston et al. 2016b), *Robinia* can fix atmospheric N_2 (Vítková et al. 2017), and *Prunus* allocates N to allelopathic compounds, such as cyanogenic glycosides (Csiszár 2009), thereby potentially inhibiting N uptake by competitors.

Overall, for all studied species, the increased inorganic and organic N acquisition as well as the reduced levels of N metabolites in leaves and fine roots with rising soil N availability indicate plant physiological adjustments to meet the N demands with different soil N supply (BassiriRad 2000). The lack of response with regard to biomass or growth indices suggests that the acquired N is either used for metabolism maintenance or assigned to storage (Millard and Grelet 2010). Plant N acquisition is directly related to soil N supply, thus, higher organic and inorganic N acquisition with higher soil N availability reflects the active regulation of N uptake (Kulmatiski et al. 2017) which has already been reported for seedlings of *F. sylvatica* (e.g. Li et al. 2015) and *P. sylvestris* (e.g. Simon et al. 2013).

3.5.2. Native species respond to competition with invasives with distinct species-specific strategies

The native species showed distinct species- and also parameter-specific strategies in their responses to competition with different invasive species that were partly mediated by soil N availability. When competing with *Prunus* compared to *Robinia*: (1) *Fagus* had lower RGR and N acquisition without changes in biomass or N metabolite levels, (2) *Quercus* had lower biomass and total soluble amino acid-N levels without changes in N acquisition, whereas (3) *Pinus* only had higher SRL, without changes in biomass, N acquisition or N metabolite levels. More specifically, the lower RGR combined with N acquisition in *Fagus* in competition with *Prunus* compared to *Robinia* suggests that the response strength to competition with an invasive species depends on the competitor. The slower growth combined with a reduced N acquisition from the soil indicates a remobilization of internal N in compensation for the impaired N acquisition from the soil assuming a similar N demand. This strategy of a reduced growth rate (Li et al. 2015) and N acquisition (Simon et al. 2010, Simon et al. 2014) was found also in response to competition with fast-growing *A. pseudoplatanus*, thus indicating a general response of *F. sylvatica* to fast-growing competitors. In contrast, *Quercus* had a decreased biomass as well as total soluble amino acid-N content in the fine roots (only at high soil N availability) in competition with *Prunus* compared to *Robinia*, with no change in N acquisition suggesting a shift in the allocation of N from growth to storage and/or the synthesis of defense compounds depending on the competing species (Millard and Grelet 2010). The lower total soluble amino acid-N levels in the fine roots when competing with *Prunus* compared to *Robinia* but only a high soil N availability indicates a mediation of the competitive response by soil N supply. Lower amino acid-N levels in the fine roots have been related to slower growth (Simon et al. 2010), whereas an increase in total soluble amino acid levels in the fine roots as a result of competition (Li et al. 2015) was found in fast-growing sycamore maple (*Acer pseudoplatanus* L.). However, *Fagus* showed a different response, despite also being a slow growing species which indicates that other species-specific factors regulate the response to competition in terms of total soluble amino acid-N content in the fine roots that were not considered in our study. For seedlings of *Pinus* the sole response to competition with *Prunus* compared to *Robinia* was a higher SRL which regulates the access to N (Andersen et al. 2017). Growing longer, thinner roots allows *Pinus* seedlings to maximize resource capture without changing N acquisition capacity per root, thereby being able to tolerate competition with no effects on productivity. Unlike both deciduous species *Fagus* and *Quercus*, coniferous *Pinus* did not show different responses to different invasive competitors regarding N acquisition and N pools in leaves and fine roots, likely because conifers maintain needles in which N is stored throughout the year (Millard and Grelet 2010) and can be utilized when needed. By drawing on these resources, *Pinus* can buffer the different competitive effects of the invasive species. Noticeably, none of our native study species responded differently depending on the competitor with regard to their total soluble protein-N levels in the leaves or fine roots. Previous studies on temperate tree seedlings including *F. sylvatica* (Simon et al. 2010, Simon et al. 2014, Li et al. 2015) found changes in total soluble protein-N contents in the fine roots when grown in intraspecific or interspecific competition indicating de novo protein synthesis as an adaptive response to competition. In the present study, we compare native species responses to different invasive species rather than intraspecific vs. interspecific competition within native species. Thus, it is possible that protein-N levels changed for the single species when grown in competition, but there the response was similar with different competitors.

Overall, the response of native seedlings in competition with invasive species is species-specific and reflects different coping mechanisms related to the species life history and growth strategies. For instance, coniferous species such as *Pinus* maintain their needles throughout the year resulting in higher N storage capacities compared to deciduous species like *Fagus* and *Quercus* (Millard and Grelet 2010). As a consequence, conifers might be less responsive to the different competitive effects of the invasive species in terms of the competition for N. All three native species responded stronger when competing with *Prunus* than *Robinia*.

3.5.2.1. Why is *P. serotina* a stronger competitor than *R. pseudoacacia* for the studied native species?

In our study, the native tree seedlings competing with *Prunus* responded with decreases in N acquisition, total biomass, RGR, and total soluble amino acid-N levels in the fine roots than when competing with *Robinia* which can be explained by its relatively high root biomass, and, in turn, a better exploitation of belowground resources of *Prunus* (Casper and Jackson 1997, Kawaletz et al. 2014) compared to all other species in our study which is linked to increased success rates of invasive plants (e.g. Grotkopp and Rejmánek 2007, Gioria and Osborne 2014, Kawaletz et al. 2013, Robakowski and Bielinis 2011). Moreover, *Prunus* produces cyanogenic compounds also in the roots, a strategy that might have had an allelopathic effect on the neighboring plants (Csiszár 2009, Robakowski et al. 2016) and contributed to the general negative influence of *Prunus* on the native species in our study. Overall, the higher competitive ability of *Prunus* in our study is in accordance with earlier work by others; however, previous studies investigated the effects of *Prunus* and *Robinia* when competing with native species mainly considering plant growth and biomass (e.g. Closset-Kopp et al. 2011, Robakowski and Bielinis 2011, Kawaletz et al. 2013, Kawaletz et al. 2014), whereas we found not only competition effects on growth and biomass but also on N acquisition and N allocation to different N pools depending on the species.

3.5.3. Invasive *R. pseudoacacia* reacts stronger to competition with native seedlings than *P. serotina*

The invasive species used in our study responded to competition depending on the native competitor species and soil N availability: the response of *Prunus* to competition with native seedlings was an increase in SLA when grown in competition with *Quercus* compared to competition with *Fagus*, but not *Pinus*. Usually, plants with a high SLA have a lower competitive effect related to a shorter leaf lifespan resulting in a lower leaf mass fraction (Kuster et al. 2016); which is, however mostly related to limiting resource conditions (Knops and Reinhart 2000). In contrast, having a higher SLA when belowground resources are sufficient becomes a competitive advantage as a strategy of fast resource acquisition (Liu et al. 2017). Therefore, given the higher root biomass of *Prunus* compared to our native study species, *Prunus* was a strong competitor for belowground resources, and enhanced its competitive ability and fast growth strategy by increasing light interception via a higher SLA (Liu et al. 2017).

In contrast, seedlings of *Robinia* responded to competition depending on the native species, with a decrease in organic N acquisition in competition with *Quercus* compared to *Pinus* and *Fagus,* as well as an increased SLA combined with a decrease in fine root total soluble amino acid-N content in competition with *Quercus* and *Pinus* compared to *Fagus*. Notably, the decrease in fine root total soluble amino acid-N content with competition was found only at low soil N availability, the decrease in organic N acquisition only at high soil N availability. This response dependency on soil N availability for *Robinia* but not *Prunus* indicates that *Robinia* shows a strong response to the combined effects of competition and soil N availability, whereas the response of *Prunus* is not mediated by soil N supply. The increase in SLA when competing with *Quercus* compared to *Fagus* was found regardless of soil N availability and suggests a potential reduced competitive ability of *Robinia* competing with *Quercus*, especially in combination with the reduced organic N acquisition and total soluble amino acid-N content in the fine roots indicating an impaired N nutrition. In general, leaves with a high SLA represent a disadvantage for plant growth under limiting nutrient conditions, because of the negative correlation with leaf lifespan, and therefore increased N losses through leaf senescence (Reich et al. 1997, Knops and Reinhart 2000).

Overall, the investigated invasive species responded at different levels (i.e. morphological and physiological) depending on the identity of the native competitor suggesting that *Robinia* represents a lesser threat than *Prunus* at the investigated time scale. However, the impact of *Robinia* might become more important in the long run as a result of its capacity to fix atmospheric N_2 and therefore potentially alter biogeochemical processes in invaded ecosystem (Strayer et al. 2006, Medina-Villar et al. 2016). By modifying soil N availability, for example, N_2-fixing plants might influence community composition and diversity (i.e. by favoring the establishment of nitrophilous species) (Staska et al. 2014), and consequently impact on plant-plant interactions such as competition.

3.5.4. Higher soil N availability results in increased N acquisition but no change in biomass or growth indices for all species in competition, while a reduction in N pool levels is species- and organ-specific

Seedlings of native and invasive species growing in competition in our study varied their responses with high soil N supply. Although, none of them increased in biomass with high soil

N supply seedlings of *Fagus* and *Prunus* had lower SRL indicating that foraging for nutrients via an investment in longer, thinner roots is only required when soil nutrients are limiting (Zhu et al. 2016). In all species, organic and inorganic N uptake increased with high compared to low soil N supply which is a key driver of N acquisition in woody species (Simon et al. 2017) as reported for *F. sylvatica* (Stoelken et al. 2010, Li et al. 2015), *P. sylvestris* (Simon et al. 2013), and *A. pseudoplatanus* (Li et al. 2015). However, reduced total soluble amino acid-N and total soluble protein-N levels with higher soil N availability were species- as well as organ-specific in our study. In general, plants have to rely less on internal N storage to maintain growth and metabolic functions when N is readily available in the soil (Millard and Grelet 2010). For *Fagus*, our results are in contrast with a previous study that found no changes in total soluble amino acid-N contents in the fine roots with increasing soil N availability neither in intraspecific competition nor in competition with *A. pseudoplatanus* (Li et al. 2015). However, in our study, the response of native species to competition with invasive species in terms of N strategies is evaluated for the first time and suggests a specific response of native species to combined soil N availability and competition with the invasive species *Prunus* and *Robinia*.

Species responded to soil N availability mostly regardless of competitor identity, however, for some species an interaction between soil N availability and competitor was detected. For example, the reduction in total soluble amino acid-N levels in the fine roots with at high compared to low soil N availability described above was found in some species only with specific competitors (i.e. for *Quercus* when competing with *Prunus*, and for *Robinia* when competing with *Fagus* or *Quercus*). Specifically, for *Quercus* this suggests that invasive species *Prunus* induces a stronger response than invasive *Robinia*, a result that is consistent with the general responses of *Quercus* to *Prunus* described in section 4.2. We found no effects of competition on seedling total soluble amino acid-N or total soluble protein-N levels in the leaves which contrasts with studies reporting the invasion by *Prunus* to affect foliar N levels of native mature tree species including the ones considered here (e.g. Aerts et al. 2017). This suggests an effect of plant age on the competition response, as well as the possibility that soil N availability in our study was a strong driver of foliar N levels, thereby overriding the potential effects of competition.

3.5.5. Native and invasive species share common preferences for N sources regardless of soil N availability, but there are species-specific patterns between competition regimes and competitor identities

The common N preference patterns across species in our study depended on soil N availability and species strategies in response to competition. At low soil N availability, organic N was taken up preferentially over inorganic N by seedlings of both native and invasive suggesting that tree seedlings maintain their metabolism and growth by drawing upon a wider variety of N sources. However, at high soil N availability, ammonium-N was the preferred N form followed by organic N, while nitrate-N was the least preferred N form taken up by tree seedlings, as also found for *F. sylvatica* (Stoelken et al. 2010) and *P. sylvestris* (Simon et al. 2010). Nitrate acquisition from the soil is inhibited by high concentrations of amino acids (particularly glutamine) and ammonium (Näsholm et al. 2009, Stoelken et al. 2010). Both native and invasive species preferred specific N sources linked to other aspects considered in our study. For example, *Quercus* preferred organic N forms over ammonium only when grown in competition with invasive *Prunus*. *Pinus* did not shift N source preferences. Both invasive species also lacked a preference for certain N forms when competing with *Fagus* (at low soil N availability) or *Pinus* (at high soil N availability) which corresponds with their general stronger reaction to competition with *Quercus*. Overall, N form preferences matched the general response strategies of native and invasive species to differing competitors that were found for growth, N acquisition and allocation of N to N pools.

3.6. Conclusion

In our study, we found that invasive species display traits that grant them competitive advantage, such as fast growth rates, which however did not result in a generally higher N acquisition of invasive species, because native species had a higher organic N net uptake capacity that would allow them to better utilize soil N sources, and thus potentially avoid competition with neighboring invasive species. When growing in competition, native tree seedlings showed a stronger response to competition with *Prunus* than *Robinia*, although the response variable changed between native species indicating the use of species-specific strategies of native seedlings to cope with the competition with invasive plants. These are further mediated by soil N availability in some cases. These strategies reflected the differences between native species in terms of life history and growth traits. The stronger response to *Prunus* could be related to the higher total biomass of this species and its ability to produce allelopathic compounds. Furthermore, when comparing the responses of invasive species, *Robinia* responded to different native competitors at different morphological and physiological levels, further influenced by soil N availability, while *Prunus* only showed changes in SLA, without interaction with soil N availability. This suggests that *Prunus* might have a stronger competitive advantage over native species than *Robinia* at least at the time scale of our study. Moreover, considering that native *Quercus* responded more negative to competition with *Prunus* than *Robinia*, as well as the several responses of *Robinia* to competition with *Quercus*, our results suggest that specifically *Quercus* could have a competitive advantage over invasive *Robinia*, but not over invasive *Prunus*. However, it is possible that the impact of *Robinia* becomes more important in the longer run at the plant community level, as a result of its capacity to fix atmospheric N_2 and therefore potentially alter biogeochemical processes in an invaded ecosystem. With regard to the effects of high compared to low soil N availability on competing seedlings, we found common patterns among species similar to those displayed by single grown seedlings regarding N acquisition (increased with soil N availability), but not regarding N allocation, i.e. in single seedlings N allocation was scarcely influenced by soil N availability, while in competing seedlings there was a general decrease in total soluble amino acid-N levels for most species, which suggests stronger responses to increased soil N availability when in combination with competition. Generally, our results highlight *Prunus* as a potential greater threat to seedlings of native species than *Robinia* in the time frame considered here. Overall, our results provide novel insights into the different species-specific effects of invasive species on native seedlings not only with regard to growth parameters, but also underlying physiological processes such as N acquisition and internal allocation. However, our study included only 5 species (3 natives, 2 invasives) differing in their ecological background, with a focus on the competition for N rather than other resources, such as light and/or space, thus more detailed studies are still required in the future. Furthermore, the results should be validated in long term studies conducted in the field. In conclusion, the species-specific nature of competition between native and invasive tree seedlings should be considered in forest management strategies in the future.

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

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4.1. Abstract

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

4.2. Introduction

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

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

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

We conducted a greenhouse experiment to study the responses of three native and two invasive woody plant species to competition and drought in terms of growth, organic and inorganic N acquisition, and allocation of N to N pools in the leaves and fine roots. As native species we chose the most representative species of Central European forests, which differ in physiological traits and growth strategies: (i) European beech (*Fagus sylvatica* L., Fagaceae), a drought-sensitive late successional species (Houston et al. 2016), (ii) pedunculated oak (*Quercus robur* L., Fagaceae), a drought-tolerant late successional species (Eaton et al. 2016), and (iii) Scots pine (*Pinus sylvestris* L., Pinaceae), a drought-tolerant, early successional conifer (Kuster et al. 2013, Sohn et al. 2016). The two invasive tree species are originally from North America, were first introduced in Europe in the 17th century and are now widely distributed due to their use in reforestation programs: (i) black cherry (*Prunus serotina* Ehrh., Rosaceae), an early successional species able to produce cyanogenic compounds (Csiszár 2009), and (ii) black locust (*Robinia pseudoacacia* L., Leguminosae), a N_2-fixing early successional species (Robakowski et al. 2016, Vítková et al. 2017). Both species are considered the two of the most frequent and important woody invaders in Central European forests (Campagnaro et al. 2018). Our hypotheses were: (1) Invasive *P. serotina* and *R. pseudoacacia* show traits that enhance their competitive ability compared to the native species. (2) Native and invasive species show responses to drought that reflect their life history and growth strategies. (3) Native and invasive species have species-specific responses to different competitors. (4) Drought enhances the effects of competition between native and invasive species. From here on, species used in this study will be referred to using their genus, i.e. *Fagus, Quercus, Pinus, Prunus* and *Robinia*.

4.3.Materials and Methods

4.3.1. Plant material and growth conditions

One-year-old seedlings of all species were purchased from Müller Münchehof Pflanzen GmbH (Seesen/Münchehof, Germany) and planted in different combinations (i.e. one or two seedlings per pot, see 4.3.2. “Experimental design”) in a 1:1 mixture of sand and vermiculite in 3 L plastic pots (25 cm x 12 cm) at the end of November 2015. Pots stayed outdoors over winter and spring and were watered regularly. They were brought into the greenhouse on June 20th 2016 after leaf development. For the following 7 days, all pots were watered regularly with sufficient tap water, and received on two occasions 100 ml of an artificial low N nutrient solution containing 100 µM KNO_3, 90 µM $CaCl_2*2H_2O$, 70 µM $MgCl_2*6H_2O$, 50 µM KCl, 24 µM $MnCl_2*4H_2O$, 20 µM NaCl, 10 µM $AlCl_3$, 7 µM $FeSO_4*7H_2O$, 6 µM K_2HPO_4, 1 µM NH_4Cl, 25 µM glutamine, and 25 µM arginine mimicking the soil solution of a low N field site (Dannenmann et al. 2009). The pots were subjected to natural light conditions and day length regime (16/8, day/night). Air temperature was 23.5 ± 2.3 °C / 21.0 ± 2.4 °C (day/night, mean ± standard deviation), and relative humidity was 63.1 ± 9.3 % / 71.3 ± 8.8 % (day/night, mean ± standard deviation) for the duration of the experiment. With increasing duration of the soil water availability treatment, some individuals started to show signs of wilting. To ensure sufficient replication for each species and treatment, we did the ^{15}N uptake experiments followed by the harvest after four weeks.

4.3.2. Experimental design

The experiment was conducted in a fully orthogonal design with two factors, “water availability” (i.e. control or drought) and “competitor identity” (interspecific competition with 2 or 3 different competing species). Furthermore, seedlings were planted without competition in order to compare among species regarding the measured parameters (see below), and to determine the species responses to drought in absence of competition. Seedlings were planted either as two seedlings in native-invasive interspecific competition (i.e. one seedling of a native species and one seedling of an invasive species per pot), or without competition (i.e. single seedling of each species per pot). Interspecific competition pots were established for every combination of native species and invasive species. For each species, a total of 24 pots was setup as single seedlings and for each combination of native-invasive, summing up to a total of 264 pots. Pots were assigned to either the control or drought treatment (i.e. n = 12 per combination of species, competitor identity and soil water availability). For the drought treatment, irrigation was fully stopped starting June 27th until the final harvest from July 21st to 27th, while for the control treatment irrigation continued with sufficient tap water supply every second day. To confirm that water availability was significantly reduced in the respective treatment, we measured soil water content every second day for 3 pots per combination of species, soil water availability treatment and competitor identity, by inserting a probe into the soil in three different locations in each pot (HH2 Moisture Meter, Delta-T Devices, Cambridge). Soil water content in the drought treatment was with 7.2 ± 2.4 % (mean ± standard deviation) significantly lower than in the control 24.0 ± 2.5% (mean ± standard deviation). $\Delta^{13}C$ values in the leaves were higher in the drought treatment compared to the control at the time of harvest indicating drought stress, for *Fagus, Quercus,* and *Robinia* depending on the competitor.

4.3.3. ^{15}N uptake experiments

To quantify inorganic (i.e. ammonium and nitrate) and organic (i.e. glutamine and arginine) net N uptake capacity of the seedlings' fine roots, the ^{15}N enrichment technique was used as described by Gessler et al. (1998) and modified by Simon et al. (2010). Seedlings were carefully removed from the pots and the roots washed thoroughly with tap water to remove any remaining substrate. Then, fine roots still attached to the seedlings were incubated in an artificial low N nutrient solution (composition described in 2.1 "Plant material and growth conditions") containing all four N sources but only one labelled as either $^{15}NH_4^+$, $^{15}NO_3^-$, $^{13}C/^{15}N$-glutamine, or $^{13}C/^{15}N$-arginine. Controls with no label were included to account for natural abundance in the fine roots (n = 4 to 6 per combination of N source including controls with no label, species, competitor identity including single seedlings, and soil water availability treatment). The roots not used for incubation were wrapped in wet tissue to prevent desiccation for the duration of the ^{15}N uptake experiment. After 2 hours of incubation, the fine roots were cut from the seedling and washed twice in 0.5 M $CaCl_2$ solution to remove the incubation solution from the root surface. The fresh weight of the fine roots was determined, and after oven-drying for 48 h at 60 °C, their dry weight was determined. Amino acids were $^{13}C/^{15}N$-labelled to determine whether they were taken up as intact molecules (Simon et al. 2011). Net uptake capacity of glutamine and arginine was lower based on ^{13}C compared to that on ^{15}N incorporation indicating that amino acids degraded in the solution or on the surface of the roots, and/or the respiration of amino acid-derived C inside the roots (Simon et al. 2011). Incubation took place between 10 am and 2 pm to avoid diurnal variation in net N uptake capacity (Gessler et al. 2002).

4.3.4. Harvest and quantification of growth and biomass indices

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

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

Dried fine root samples from the ^{15}N uptake experiment were ground using a ball mill (TissueLyser, Retsch, Haan, Germany) to a fine homogeneous powder. Aliquots of 1.2 to 2.4 mg were weighed into 4x6 mm tin capsules (IVA Analysentechnik, Meerbusch, Germany) for analyses with an isotope ratio mass spectrometer (Delta V Advantage, Thermo Electron, Dreieich, Germany) coupled to an elemental analyzer (Euro EA, Eurovector, Milano, Italy). Δ

values were calculated using a laboratory standard (acetanilide) that was part of every sequence in intervals also used in different weights to determine isotope linearity of the system. The laboratory standard was calibrated against several suitable international isotope standards (IAEA, Vienna). Final correction of isotope values was done with several international isotope standards and other suitable laboratory standards which cover the range of ^{15}N and ^{13}C results. Inorganic and organic N net uptake capacity (nmol N g^{-1} fw h^{-1}) was calculated based on the incorporation of ^{15}N into the fine roots according to Gessler et al. (1998): N uptake capacity = $((^{15}N_l - ^{15}N_c) * N_{tot} * dw * 10^5) / (MW * fw * t)^{-1}$, where $^{15}N_l$ and $^{15}N_c$ are the atom% of ^{15}N in labeled (N_l) and unlabeled control plants (N_c, natural abundance), respectively, N_{tot} is the total N percentage, MW is the molecular weight (^{15}N g mol^{-1}), and t is the incubation time.

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

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

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

4.3.7. Statistical analyses

Permutational ANOVAs (PERMANOVA) based on a Euclidean resemblance matrix between samples (Anderson et al. 2008) were performed for each species to test for differences between treatment levels using as variables inorganic and organic net N uptake capacity, total biomass, root:shoot ratio, SLA, SRL, RGR, as well as total soluble amino acid-N and total soluble protein-N contents in the leaves and fine roots. Two-way PERMANOVAs were performed using “water availability” and “competitor identity” as fixed orthogonal factors. “Water availability” consisted of two levels, i.e. drought and control. “Competitor identity” consisted of two levels for the native species, i.e. competition with *Prunus* or *Robinia*, and three levels for the invasive species, i.e. competition with *Fagus*, *Quercus*, or *Pinus*. For significant interactions between factors, *post hoc* PERMANOVA pair-wise comparisons were performed. To test for differences between species when seedlings were grown single, two-way PERMANOVAs were performed on the single seedlings data (i.e. no competition), using “species” and “water availability” as factors, for each variable mentioned above. To test for differences between drought and control for each species growing single, Mann-Whitney U-tests were performed for each of the variables mentioned above, with the addition of $\delta^{13}C$ as one more variable. Finally, to test for species preferences in net N uptake capacity of the different N forms, one-way PERMANOVAs were performed for each combination of species and competitor identity using “N source” as factor at both levels of 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).

4.4.Results

4.4.1. Strategies of single grown tree seedlings – comparisons between species and responses to drought

4.4.1.1.Differences in general strategies between single grown tree species

Species differed in their growth strategies in the absence of competition. *Prunus* had the highest total biomass followed by *Quercus* and *Robinia,* while *Fagus* and *Pinus* had the lowest (Table 4.1, Supplementary Table 4.1). RGR was highest in *Robinia*, *Quercus* had higher RGR than *Prunus,* while the rest of the species did not differ significantly (Table 4.1, Supplementary Table 4.1). Species ranked for SLA: *Fagus* / *Quercus* > *Prunus* > *Robinia* > *Pinus*, for root:shoot ratio: *Robinia* > *Fagus* > *Quercus* / *Prunus*, and for SRL: *Fagus* / *Quercus* / *Robinia* > *Pinus* > *Prunus* (Table 4.1, Supplementary Table 4.1). Native species had higher organic net N uptake capacity than the invasive species, while inorganic net N uptake capacity did not differ between species (Table 4.1, Supplementary Table 4.2). The invasive species had higher total soluble amino acid-N and protein-N contents in the leaves and fine roots than the native species (Table 4.1, Supplementary Table 4.3).

4.4.1.2.Drought responses of single grown native and invasive tree species

Species grown without competition responded to drought mainly with changes in N acquisition and N metabolite pools. With drought compared to sufficient water supply, *Fagus* had lower SRL and nitrate net uptake capacity. *Quercus* only showed lower nitrate net uptake capacity, without changes in the other three N sources for either species (Table 4.2, Supplementary Tables 4.4 and 4.5). Single grown *Pinus* showed no responses to drought in any of the measured parameters. Of the invasive species, *Prunus* had reduced ammonium and nitrate net uptake capacity with drought, as well as total soluble protein-N levels in the fine roots (Table 4.2, Supplementary Tables 4.5 and 4.6). *Robinia* had lower glutamine-N and arginine-N net uptake capacity with drought, as well as lower SLA and total soluble protein content in the leaves (Table 4.2, Supplementary Tables 4.4, 4.5, and 4.6). Drought did not affect the remaining parameters for all species.

Table 4.1. Differences in total biomass, growth indices, inorganic and organic N net uptake capacity, and N metabolite levels between single grown seedlings of *Fagus sylvatica*, *Quercus robur*, *Pinus sylvestris*, *Prunus serotina*, and *Robinia pseudoacacia*. Species A > species B = seedlings of species A had significantly higher values than seedlings of species B, n.s. = no significant differences between species. Total biomass (g dw), root:shoot ratio: ratio of belowground to aboveground biomass, RGR: relative growth rate (g dw g^{-1} dw d^{-1}), SLA: specific leaf area (cm^2 g^{-1} dw), SRL: specific root length (cm g^{-1} dw), N net uptake capacity (nmol N g^{-1} fw h^{-1}), NH_4^+: ammonium, NO_3^-: nitrate, Gln-N: glutamine-N, Arg-N: arginine-N, total soluble amino acid-N (mg g^{-1} dw), total soluble protein-N (mg g^{-1} dw).

Biomass and growth indices					**N net uptake capacity**				**N metabolites**			
									Total soluble amino acid-N		**Total soluble protein-N**	
Total biomass	**Root:shoot ratio**	**RGR**	**SLA**	**SRL**	**NH_4^+**	**NO_3^-**	**Gln-N**	**Arg-N**	**Leaves**	**Fine roots**	**Leaves**	**Fine roots**
Prunus > *Quercus*, *Robinia* > *Fagus*, *Pinus*	*Fagus*, *Quercus* > *Prunus* > *Robinia* > *Pinus*	*Robinia* > others	*Robinia* > *Fagus* > *Quercus*, *Prunus*	*Robinia*, *Fagus*, *Quercus* > *Pinus* > *Prunus*	n.s.	n.s.	*Fagus*, *Pinus* > *Prunus*,*Robinia*	*Fagus*, *Pinus* > *Robinia*	*Robinia* > *Prunus* > *Fagus*, *Quercus*, *Pinus*	*Prunus* > *Robinia*, *Quercus* > *Fagus* > *Pinus*	*Robinia*, *Pinus* > *Prunus* > *Fagus*, *Quercus*	*Prunus*, *Robinia* > *Fagus*, *Quercus*, *Pinus*

Table 4.2. Effects of drought on total biomass, growth indices, inorganic and organic N net uptake capacity, and N metabolite levels on (A) seedlings growing single and (B) seedlings growing in competition of *Fagus sylvatica*, *Quercus robur*, *Pinus sylvestris*, *Prunus serotina*, and *Robinia pseudoacacia*. ↑ = significant increase with drought, ↓ = significant decrease with drought, n.s. = no significant differences between drought and control. Total biomass (g dw), root:shoot ratio: ratio of belowground to aboveground biomass, RGR: relative growth rate (g dw g^{-1} dw d^{-1}), SLA: specific leaf area (cm^2 g^{-1} dw), SRL: specific root length (cm g^{-1} dw), N net uptake capacity (nmol N g^{-1} fw h^{-1}), NH_4^+: ammonium, NO_3^-: nitrate, Gln-N: glutamine-N, Arg-N: arginine-N, total soluble amino acid-N (mg g^{-1} dw), total soluble protein-N (mg g^{-1} dw).

	Biomass and growth indices					**N net uptake capacity**				**N metabolites**			
										Total soluble amino acid-N		**Total soluble protein-N**	
	Total biomass	**Root:shoot ratio**	**RGR**	**SLA**	**SRL**	**NH_4^+**	**NO_3^-**	**Gln-N**	**Arg-N**	**Leaves**	**Fine roots**	**Leaves**	**Fine roots**
A) Seedlings growing single													
Fagus	n.s.	n.s.	n.s.	n.s.	↓	n.s.	↓	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Quercus	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	↓	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Pinus	n.s.	n.s.	n.s.	-	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Prunus	n.s.	n.s.	n.s.	n.s.	n.s.	↓	↓	n.s.	n.s.	n.s.	n.s.	n.s.	↓
Robinia	n.s.	n.s.	n.s.	↓	n.s.	n.s.	n.s.	↓	↓	n.s.	n.s.	↓	n.s.
(B) Seedlings growing in competition													
Fagus	n.s.	↓	↑(b)	↓	n.s.	n.s.	↓	n.s.	↓	n.s.	n.s.	↓	n.s.
Quercus	n.s.	n.s.	n.s.	n.s.	↓(b)	n.s.	↓	↓(b)	↓	↑(d)	↑(d)	↓	n.s.
Pinus	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	↓	↓	n.s.	n.s.	↑	n.s.	n.s.
Prunus	↓(a)	n.s.	n.s.	↑	n.s.	↓	↓	↓	n.s.	n.s.	↓(c)	n.s.	↓
Robinia	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 identity

4.4.2. Effects of competition on native and invasive tree species

4.4.2.1. Native species - Effects of competitor identity on biomass and growth indices, N acquisition, and allocation to N pools in the leaves and fine roots

Native species showed different responses to competitor identity depending on soil water availability. *Fagus* competing with *Prunus* had significantly higher RGR compared to competition with *Robinia* with sufficient water supply, but not with drought (Table 4.3, Supplementary Table 4.7). *Quercus* grown in competition with *Prunus* compared to *Robinia* had higher SRL under drought conditions as well as lower RGR, lower root:shoot ratio, lower total soluble protein-N content in the fine roots, and higher total soluble amino acid-N content in the fine roots regardless of soil water availability (Table 4.3, Supplementary Tables 4.7 and 4.8). *Pinus* competing with *Prunus* had lower total biomass, lower total soluble amino acid-N content and higher total soluble protein-N content in the leaves, and higher total soluble amino acid-N content in the fine roots than when competing with *Robinia* regardless of soil water availability (Table 4.3, Supplementary Tables 4.7 and 4.8). No other parameter varied significantly with competitor identity (Figure 4.1, Table 4.3).

4.4.2.2. Invasive species - Effects of competitor identity on biomass and growth indices, N acquisition, and allocation to N pools in the leaves and fine roots

Similar to the native species, responses of the invasive species depended on competitor identity and were partly also mediated by soil water availability. Under drought, *Prunus* seedlings competing with *Quercus* had lower total soluble amino acid-N content in the fine roots than those competing with *Pinus* (Table 4.3, Supplementary Tables 4.10 and 4.11). With sufficient water supply, total biomass of *Prunus* was lower when grown in competition with *Fagus* or *Pinus* than with *Quercus*, (Table 4.3, Supplementary Tabled 4.7 and 4.8). Total soluble amino acid-N content in the fine roots of *Prunus* was lower competing with *Fagus* than with *Quercus* or *Pinus* (Table 4.3, Supplementary Tables 4.10 and 4.11). Irrespective of soil water availability, *Prunus* seedlings competing with *Quercus* had lower SLA than with *Fagus*. *Prunus* seedlings competing with *Fagus* had lower SLA than with *Pinus* (Table 4.3, Supplementary Tables 4.7 and 4.9). Moreover, *Prunus* seedlings had a lower RGR when competing with *Quercus* or *Pinus* than with *Fagus*, a lower total soluble amino acid-N content in the leaves when competing with *Fagus* than with *Quercus*, and a lower total soluble protein-N content in the leaves when competing with *Fagus* or *Quercus* than with *Pinus* (Table 4.3, Supplementary

Tables 4.7, 4.9, 4.10, and 4.12). The remaining parameters did not change significantly according to competitor identity for *Prunus*.

Robinia seedlings responded to competitor identity with changes in other parameters than *Prunus*. Under drought conditions, *Robinia* seedlings competing with *Fagus* had significantly higher total soluble protein-N content in the fine roots than with *Quercus*, and higher total soluble protein-N content in the fine roots competing with *Quercus* than with *Pinus* (Table 4.3, Supplementary Tables 4.10 and 4.11). Under control conditions, *Robinia* competing with *Fagus* had significantly lower nitrate net uptake capacity than with *Pinus* (Figure 4.2, Table 4.3). Regardless of soil water availability, RGR of *Robinia* was significantly lower when grown with *Fagus* or *Quercus* than with *Pinus* (Table 4.3, Supplementary Tables 4.7 and 4.9). The other measured parameters did not change significantly with competitor identity for *Robinia* (Table 4.3, Supplementary Tables 4.7 and 4.10).

Table 4.3. Effects of competitor identity on total biomass, growth indices, inorganic and organic N net uptake capacity, and N metabolite levels of *Fagus sylvatica*, *Quercus robur*, *Pinus sylvestris*, *Prunus serotina*, and *Robinia pseudoacacia* seedlings. Species A < species B = seedlings competing with species A had significantly lower values than seedlings competing with species B, n.s. = no significant differences between competitor identities. Total biomass (g dw), root:shoot ratio: ratio of belowground to aboveground biomass, RGR: relative growth rate (g dw g^{-1} dw d^{-1}), SLA: specific leaf area (cm^2 g^{-1} dw), SRL: specific root length (cm g^{-1} dw), N net uptake capacity (nmol N g^{-1} fw h^{-1}), NH_4^+: ammonium, NO_3^-: nitrate, Gln-N: glutamine-N, Arg-N: arginine-N, total soluble amino acid-N (mg g^{-1} dw), total soluble protein-N (mg g^{-1} dw).

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

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

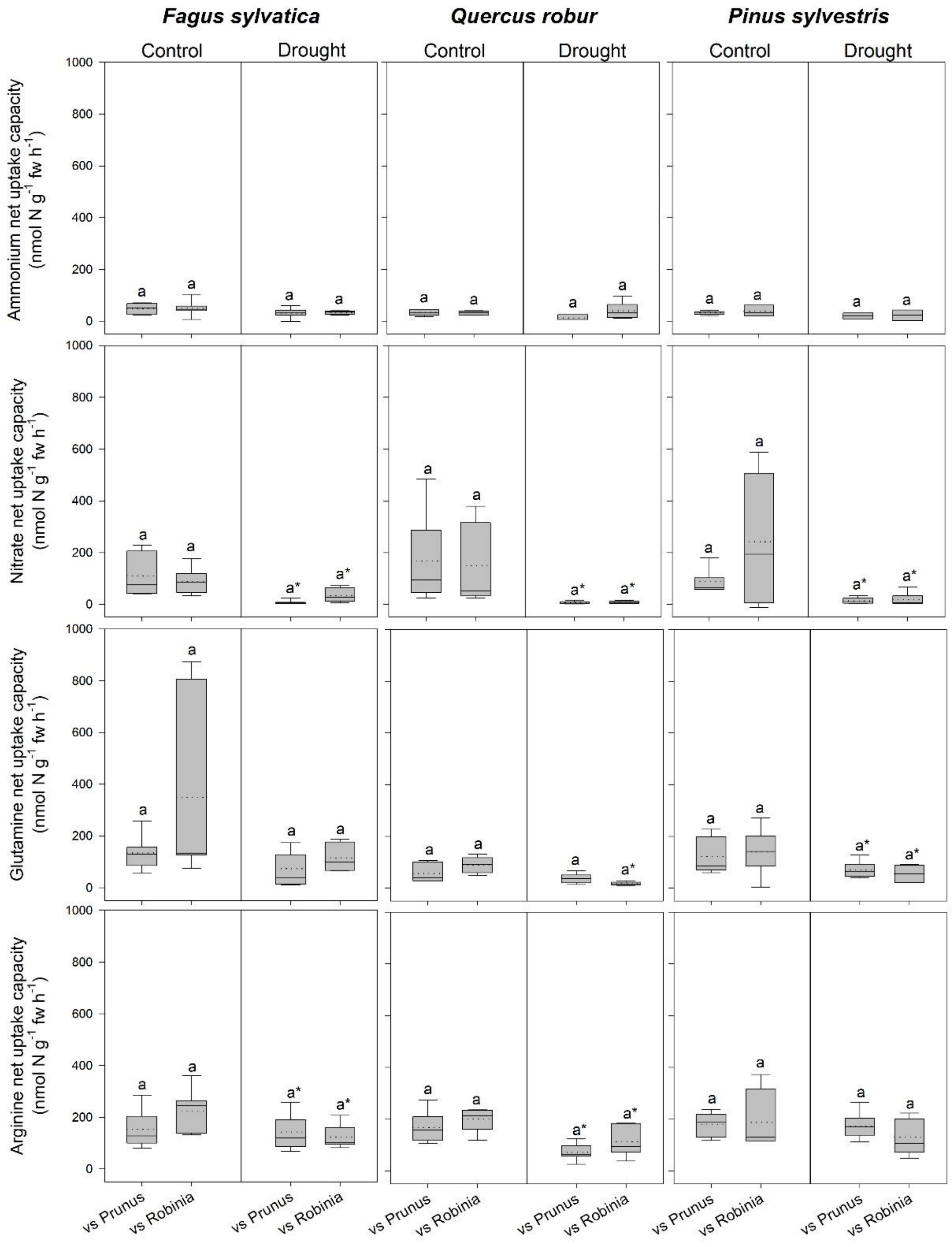

Figure 4.1. Ammonium, nitrate, glutamine-N, and arginine-N net uptake capacity (nmol N g^{-1} fw h^{-1}) by fine roots of *Fagus sylvatica*, *Quercus robur* and *Pinus sylvestris* seedlings at control and drought conditions under different competition regimes. vs *Prunus* = competition with *Prunus serotina*; vs *Robinia* = competition with *Robinia pseudoacacia*. Box plots show mean (dotted line) and median (continuous line). Different letters indicate significant differences between competition regimes within a specific soil water availability treatment, and asterisks indicate significant differences between control and drought detected using permutational analysis of variance ($p < 0.05$).

4.4.3. Effects of drought on native and invasive tree species growing in competition

4.4.3.1. Native species - Effects of drought on biomass and growth indices, N acquisition, and allocation to N pools in the leaves and fine roots of seedlings growing in competition

Native species showed different responses to drought in competition. *Fagus* responses to drought varied according to competitor identity. When grown in competition with *Prunus*, *Fagus* had higher $\delta^{13}C$ values in the leaves with drought compared to sufficient water supply (data not shown), whereas in competition with *Robinia*, RGR was increased with drought compared to sufficient water supply (Table 4.2, Supplementary Tables 4.7 and 4.8). Regardless of competitor identity, drought led to a significant reduction in root:shoot ratio, SLA, nitrate and arginine-N net uptake capacity, as well as total soluble protein-N content in the leaves of *Fagus* seedlings (Figure 4.1, Table 4.2, Supplementary Tables 4.7 and 4.10). The other measured parameters did not change significantly between drought and sufficient soil water availability for *Fagus.*

For *Quercus*, the effects of drought were mostly mediated by competitor identity. In competition with *Prunus, Quercus* had higher total soluble amino acid-N content in the leaves and fine roots, as well as higher $\delta^{13}C$ values in the leaves with drought compared to sufficient soil water availability (Table 4.2, Supplementary Tabled 4.10 and 4.11). When grown in competition with *Robinia*, *Quercus* seedlings had a significantly lower SRL and glutamine-N net uptake capacity with drought compared to the control conditions (Figure 4.1, Table 4.2, Supplementary Tables 4.7 and 4.8). Regardless of competitor identity, drought led to a reduced nitrate and arginine-N net uptake capacity, and total soluble protein-N content in the leaves in *Quercus* (Figure 4.1, Table 4.2, Supplementary Tables 4.8 and 4.10). No other parameter changed significantly with soil water availability for this species.

Pinus seedlings showed responses to drought independent of competitor identity. *Pinus* had reduced nitrate and glutamine-N net uptake capacity, as well as increased total soluble amino acid-N content in the fine roots with drought compared to control conditions (Figure 4.1, Table 4.2, Supplementary Table 4.10) regardless of competitor identity. Drought had no effect on the other measured parameters for seedlings of this species.

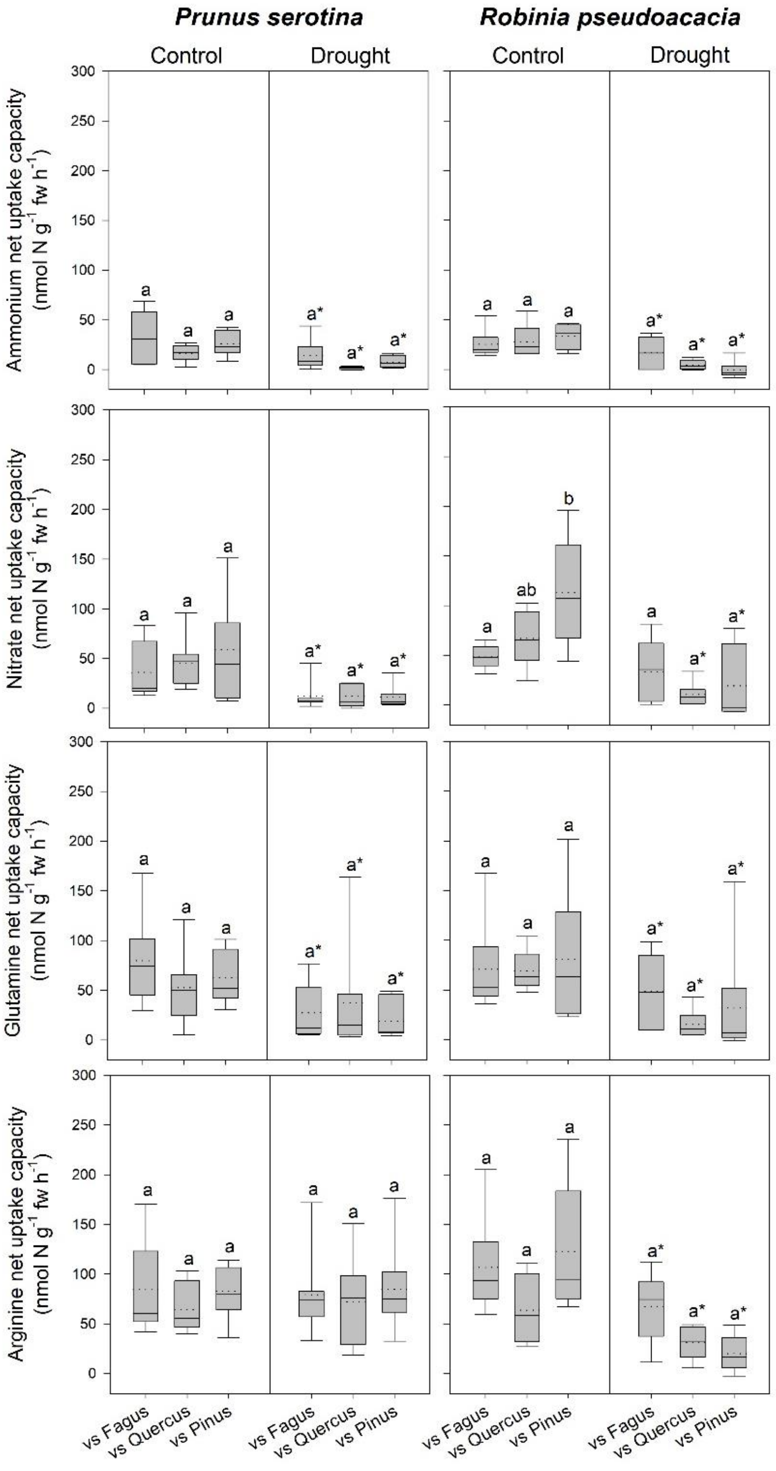

Figure 4.2. Ammonium, nitrate, glutamine-N, and arginine-N net uptake capacity (nmol N g^{-1} fw h^{-1}) by fine roots of *Prunus serotina* and *Robinia pseudoacacia* seedlings at control and drought conditions under different competition regimes. vs *Fagus* = competition with *Fagus sylvatica*; vs *Quercus* = competition with *Quercus robur*; vs *Pinus* = competition with *Pinus sylvestris*. Box plots show mean (dotted line) and median (continuous line). Different letters indicate significant differences between competition regimes within a specific soil water availability treatment, and asterisks indicate significant differences between control and drought detected using permutational analysis of variance ($p < 0.05$).

4.4.3.2. Invasive species - Effects of drought on biomass and growth indices, N acquisition, and allocation to N pools in the leaves and fine roots of seedlings grown in competition

Invasive species responded to drought depending on the competitor species. *Prunus* grown in competition with *Quercus* had lower total biomass and total soluble amino acid-N content in the fine roots with drought compared to sufficient water availability, whereas in competition with *Pinus* drought reduced total soluble amino acid-N content in the fine roots (Table 4.2, Supplementary Tables 4.7, 4.8, 4.10, and 4.11). Regardless of competitor identity, drought increased SLA, but decreased ammonium, nitrate, and glutamine-N net uptake capacity, as well as total soluble protein-N content in the fine roots (Figure 4.2, Table 4.2, Supplementary Tables 4.7 and 4.10). The remaining parameters did not change according to soil water availability for this species.

Similar to *Prunus*, responses of *Robinia* varied with competitor identity. When grown in competition with *Quercus* or *Pinus*, nitrate net uptake capacity was lower and δ^{13}C in the leaves was higher with drought compared to sufficient water supply (Figure 4.2, Table 4.2). Total soluble protein-N content in the fine roots increased with drought when grown in competition with *Fagus* but decreased with *Pinus* (Table 4.2, Supplementary Tables 4.10 and 4.11). Regardless of competitor identity, *Robinia* seedlings had higher root:shoot ratio and total soluble amino acid-N content in the leaves with drought, whereas ammonium, glutamine-N and arginine-N net uptake capacity were lower compared to sufficient water conditions (Figure 4.2, Table 4.2, Supplementary Tables 4.7 and 4.10). No significant effects of drought were found for the other measured parameters for this species.

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

Preferences for certain N sources were found across species depending on competitor identities and soil water availability. Generally, organic N, especially arginine-N, was favoured over inorganic N sources (Tables 4.4 and 4.5). *Quercus* and *Prunus* did not change N preferences with different competitors or drought (Tables 4.4 and 4.5). *Fagus* had no specific preference when grown single under control conditions, but preferred organic N over inorganic N both when grown in competition or single with drought (Table 4.4). *Pinus* did not prefer specific N sources when grown in competition with *Robinia* with sufficient water supply, but preferred organic N over inorganic N with drought and in competition with *Prunus* regardless of water availability (Table 4.4). *Robinia* showed a distinct pattern depending on competitor identity:

with drought, seedlings preferred arginine-N over inorganic N but only when grown in competition with *Quercus*, and showed no preference when grown single or in competition with *Fagus* or *Pinus* (Table 4.5). With sufficient water supply, *Robinia* preferred organic over inorganic N when grown single or in competition with *Fagus*, but not with *Quercus* or *Pinus* (Table 4.5).

Table 4.4. Differences between ammonium (NH_4^+), nitrate (NO_3^-), glutamine-N (Gln-N), and arginine-N (Arg-N) net uptake capacity of the fine roots of *Fagus sylvatica*, *Quercus robur*, and *Pinus sylvestris* seedlings grown single and in competition under drought and control conditions. Only significant differences are presented. n.s. = no significant differences between net uptake capacity of different N forms.

	Water availability	***Fagus***	***Quercus***	***Pinus***
Single grown seedlings	**Drought**	Arg-N > NH_4^+, NO_3^- Gln-N > NO_3^-	Arg-N > NH_4^+, NO_3^-, Gln-N	Arg-N > NH_4^+, NO_3^-
	Control	n.s.	Arg-N, NO_3^- > NH_4^+	Arg-N > NH_4^+, Gln-N
Seedlings in competition with *Prunus serotina*	**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^-
	Control	Gln-N, Arg-N > NH_4^+	Arg-N > NH_4^+, Gln-N	NO_3^-, Gln-N, Arg-N > NH_4^+ Arg > NO_3^-
Seedlings in competition with *Robinia pseudoacacia*	**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^-
	Control	Gln-N, Arg-N > NH_4^+, NO_3^-	Arg-N > NH_4^+, Gln-N Gln-N > NH_4^+	n.s.

Table 4.5. Differences between ammonium (NH_4^+), nitrate (NO_3^-), glutamine-N (Gln-N), and arginine-N (Arg-N) net uptake capacity of the fine roots of *Prunus serotina* and *Robinia pseudoacacia* seedlings grown single and in competition under drought and control conditions. Only significant differences are presented. n.s. = no significant differences between net uptake capacity of different N forms.

	Water availability	***Prunus***	***Robinia***
Single grown seedlings	**Drought**	Gln-N, Arg-N > NO_3^-	n.s.
	Control	NO_3^-, Gln-N, Arg-N > NH_4^+	Arg-N > NH_4^+, NO_3^-
Seedlings in competition with *Fagus sylvatica*	**Drought**	Arg-N > NH_4^+, NO_3^-, Gln-N	n.s.
	Control	Gln-N, Arg-N > NH_4^+, NO_3^-	NO_3^-, Gln-N, Arg-N > NH_4^+ Arg-N > NO_3^-
Seedlings in competition with *Quercus robur*	**Drought**	Arg-N > NH_4^+, NO_3^-	Arg-N > NH_4^+, NO_3^-
	Control	NO_3^-, Arg-N > NH_4^+ Arg-N > Gln-N	n.s.
Seedlings in competition with *Pinus sylvestris*	**Drought**	Arg-N > NH_4^+, NO_3^-, Gln-N	n.s.
	Control	Gln-N, Arg-N > NH_4^+	n.s.

4.5.Discussion

4.5.1. Comparison of growth and N acquisition strategies of single-grown native and invasive tree species

Plant functional traits vary widely across species representing different growth strategies adapted to the respective environmental conditions (Reich 2014). The native deciduous species in our study – *Fagus* and *Quercus* – showed functional traits associated with slow growth and shade tolerance (Kitajima 1994, Reich et al. 1997) such as a higher allocation of resources to the roots relative to the shoots than native coniferous *Pinus* or both invasive tree species. This strategy allows an enhanced acquisition of belowground resources for *Fagus* and *Quercus* related to their adaptation to resource-poor environments (Lambers and Poorter 1992, Reich et al. 1997) and suggest a potential advantage in the competition with the invasives due to higher organic N acquisition as found in our study. However, this higher relative investment in belowground growth and increased N acquisition did not result in higher biomass production indicating that the acquired N is rather allocated to the synthesis of defence compounds and/or invested in long-lived leaf tissue (Lambers and Poorter 1992, Reich et al. 1997) as indicated by the lower SLA also found in our study. A higher investment in defence mechanisms which also increases leaf longevity, and in turn reduces N losses through leaf turnover, is a key characteristic of slow-growing tree species that determines their long-term success (Wright et al. 2004). Compared to the native deciduous species and invasives, coniferous *Pinus* showed intermediate traits in the slow-fast growth spectrum: *Pinus* had the lowest investment in root biomass relative to shoots of all species (in contrast to the native deciduous species), and SRL of *Pinus* was lower than for the native deciduous species, but higher than for invasive *Prunus* indicating a morphological adaptation to low N availability of *Pinus* to maximise the acquisition of soil resources.

The invasive study species, in contrast, showed characteristics commonly linked to fast growth, such as a higher total biomass (*Prunus*) or higher SLA, and in turn, RGR (*Robinia*) than the native seedlings, thus enhancing resource acquisition above- and belowground, and overall increasing their competitive advantage. High SLA and high RGR are common traits in invasive species (Grotkopp and Rejmánek 2007). Additionally, both invasive species had higher total soluble amino acid-N and protein-N levels in the leaves and fine roots than the native species which is also related to a fast growth strategy (e.g. Li et al. 2015) and possibly due to the positive correlation between leaf N content and photosynthetic capacity (Lambers and Poorter 1992, Reich et al. 1998a). The N_2-fixing ability of *Robinia* is likely driving its higher N metabolite levels in the leaves and fine roots by increasing the N available for assimilation, potentially compensating for the lower organic N acquisition from the soil compared to the native species, and thus further supporting its faster growth. For *Prunus*, the synthesis of cyanogenic glycosides – that can be remobilized in times of need – might serve as an additional N source (Gleadow and Woodrow 2002) supporting its faster growth in absence of herbivory, while minimising the potential consequences of a lower organic N acquisition from the soil compared to the native species. In conclusion, our results show distinct strategies for natives and invasives in the absence of competition to cope with N limitation. The native deciduous species took up higher amounts of organic N, whereas the invasives can rely on individual coping mechanisms, such as N_2 fixation or N remobilisation from internal N storage pools.

4.5.2. Species-specific responses of native and invasive species depend on competitor identity and soil water availability

Competition between woody native and invasive species revealed three species-specific responses mediated by the physiological characteristics of the target species, the identity of the competitor as well as soil water supply, affecting (1) growth, (2) internal N pools, and/or (3) external N acquisition of tree seedlings. Because these responses were found in native and invasive species, invasive species might not have a general advantage over native species.

A slower growth and/or less biomass of a seedling grown in competition indicates a lower productivity as a consequence of competition for resources (e.g. Peltzer and Köchy 2001, Jensen et al. 2011, Aschehoug et al. 2016, Collins et al. 2018) (for *Fagus* competing with *Robinia* vs. *Prunus* with sufficient water supply; for *Quercus* and *Pinus* competing with *Prunus* vs. *Robinia* regardless of water supply; for *Prunus* competing with *Pinus*/*Quercus* vs. *Fagus* regardless of water supply (biomass) or only with sufficient water (RGR); for *Robinia* competing with *Fagus/Quercus* vs. *Pinus* regardless of water supply). Reduced growth in competing tree seedlings might be due to the fast growth and occupation of rooting space of a stronger competitor leading to the depletion of soil resources (e.g. Petritan et al. 2012, Zhang et al. 2012, Kawaletz et al. 2013, 2014). The changes in growth were partly due to a reduced specific leaf area (SLA; for *Prunus* competing with *Quercus*/*Fagus* vs. *Pinus* regardless of water supply) as a key driver of photosynthesis because of its direct effect on efficient light interception and carbon assimilation (e.g. Reich et al. 1997, 1998b). However, for the other study species and/or treatments other variables underlying RGR, such as net assimilation rate (e.g. Poorter et al. 1995, Reich et al. 1998b) might have been more important and/or have not been affected by competition. Decreases in growth as a response to interspecific competition are reported for *F. sylvatica*, *Q. robur* and *P. sylvestris* (e.g. Löf and Welander 2004, Picon-Cochard et al. 2006, Jensen and Löf 2017), as well as for *P. serotina* and *R. pseudoacacia* (e.g. Chen et al. 2012, Kawaletz et al. 2013). Effects of competition on plant biomass have been studied for decades, however, insights into the physiological mechanisms underlying competition outcomes, such as N acquisition and metabolism have received less attention (Trinder et al. 2013, but see Li et al. 2015, Bueno et al. 2019).

With regard to seedling N metabolism, we found overall four different response strategies to competitor identity. Two strategies were independent of soil water availability: (1) no change in N pools with competitor identity (for *Fagus*), or (2) higher total soluble protein levels with a simultaneous reduction in total soluble amino acid levels with one invasive competitor compared to the other (for *Quercus* and *Pinus*). This suggests the potential use of amino acids for protein synthesis as an adaptation to competition which has also been found in competing seedlings of *F. sylvatica* and *A. pseudoplatanus* (Simon et al. 2010, 2014). Furthermore, two more strategies regarding N pools were mediated by soil water supply: (3) higher total soluble amino acid content with *Quercus* compared to *Fagus* with sufficient water, but higher total soluble amino acid content with *Pinus* compared to *Quercus* with drought (for *Prunus*), and (4) higher total soluble protein content with deciduous natives than with conifer *Pinus* only with drought (for *Robinia*). These strategies suggest different responses to deciduous vs. conifer tree species with regard to the allocation of N to internal N pools, as well as linkage between the effects of competitor identity on plant N pools and soil water supply for the studied invasive species. Moreover, these responses indicate a general accumulation of N compounds in the leaves and fine roots which is a known response of plants to biotic and abiotic stress (Millard and Grelet 2010) and might play an important role in plant performance with competition. For example, seedlings of black spruce (*Picea mariana*) with higher levels of tissue N (and other nutrients) have higher biomass when grown in competition than seedlings with low tissue N content (Malik and Timmer 1998) indicating the importance of plant internal N reserves in competitive environments.

Acquisition of growth-limiting N from the soil did overall not vary with competition across native and invasive tree species. This suggests that the changes in biomass, growth, and N metabolites competing with different woody species were not a response to shifts in inorganic and organic N acquisition from the soil but rather related to remobilisation of stored N (Millard and Grelet 2010) or – for *Robinia* – N_2 fixation (Vítková et al. 2017). Only *Prunus* in competition with *Fagus* vs. *Pinus* had a lower nitrate uptake with sufficient water supply which indicates a stronger competition for specific N sources with certain competitors. *Prunus* compared to the native species produces cyanogenic glycosides as defence compounds which – in the absence of herbivory – can be remobilised in times of need (e.g. Gleadow and Woodrow 2002, Bueno et al. 2019). A reduced acquisition of N from the soil is a common response of woody seedlings to interspecific competition (Fotelli et al. 2002, 2005, Simon et al. 2010, 2014) which, however, might be more or less pronounced depending on alternative coping mechanisms of a certain species. Furthermore, N acquisition from the soil also depends on root morphology. For example, variation in SRL (for *Quercus* competing with *Prunus* compared to *Robinia* under drought) indicates a strategy to increase the root foraging capacity by having longer roots relative to the investment in root biomass, thus allowing seedlings to better exploit larger soil volumes and maximise the capture of limited belowground resources (Ostonen et al. 2007).

In general, competition between native and invasive tree seedlings lead to different responses depending on the identity of both competitors and soil water availability. *Prunus* was a stronger competitor than *Robinia* for the natives *Quercus* and *Pinus* in accordance with a previous study (Bueno et al. 2019) which is likely related to its higher biomass. However, for native *Fagus* grown with sufficient water supply, *Robinia* had stronger competitive effects than *Prunus*, in contrast with a previous study (Bueno et al. 2019) suggesting that the effects of drought on *Fagus* override the potential difference between the competitive effects of the two invasive species. The overall higher growth rate of *Robinia* (compared to all species) results in a fast occupation of space and resource depletion in detriment of *Fagus* (Kawaletz et al. 2013, 2014). For invasive *Prunus*, the effects of different native species highly depended on soil water availability: competition with native *Quercus* had stronger negative effects compared to *Fagus* regardless of soil water supply, but with drought *Prunus* had higher total soluble amino acid content competing with *Pinus* than with *Quercus*, indicating that the conifer was a worse competitor for *Prunus* than deciduous *Quercus*. Bueno et al. (2019) showed that *Prunus* scarcely responded to competitor identity, thus the present study suggests an enhanced response to the combined influence of competition and drought of *Prunus* to different native competitors. For *Robinia*, the deciduous natives *Fagus* and *Quercus* had more negative effects than the conifer *Pinus* confirming partially a previous study investigating the effects of soil N availability on the competition between the same species (Bueno et al. 2019).

Overall, our results suggest a difference in the competitive effect of native species over both invasives based on their leaf habit of the native species, though further studies considering a larger number of species are needed to provide insights into this suggestion. In plant communities, coniferous and deciduous species differ in their competitive effects on other plant species due to their differences in nutrient concentration in plant tissues, litter quality, and their differential impact on soil biogeochemistry (Calder et al. 2011, Wang et al. 2016). In conclusion, our results indicate that *Prunus* is generally a stronger competitor for the native species, as previously reported, and that native deciduous species have stronger competitive effects on both invasives considered here than the conifer, but under drought *Pinus* might have an advantage over invasive *Prunus*.

4.5.3. Responses to drought were similar across species but depended on the competitor identity

When in competition, the general responses to drought were similar for natives and invasives, but depended on the species and competitor identity. Drought led to significant changes in seedling growth and biomass indices, N acquisition as well as the internal allocation to N pools. Soil water availability is strongly linked to N uptake by plants (Gessler et al. 2004, Rennenberg et al. 2006, Gessler et al. 2017), thus seedlings improved the acquisition of water and N from the soil by allocating more resources to the roots than aboveground biomass (i.e. higher root:shoot ratio), so a larger soil volume can be exploited. The parallel decrease in aboveground biomass as well as SLA reduced water loss via the leaves further enhancing the plant's water status (Fotelli et al. 2005, Mantovani et al. 2014, Duan et al. 2018). However, with severe drought root growth cannot be maintained in the long run, eventually leading to a decrease in root:shoot ratio (Ostonen et al. 2007, Brunner et al. 2015), root hydraulic failure (Mao et al. 2018), and higher root mortality (Zhou et al. 2018). In our study, drought was severe enough to cause a decrease in root:shoot ratio only for *Fagus* indicating the drought-sensitivity of this species. Moreover, we found reductions in SRL for *Quercus* similar to studies reporting decreases in SRL with drought for woody species from dry habitats (i.e. a longer term reduction in soil water supply), and related this response to an adaptive strategy to increase root longevity, depth and thickness (Larson and Funk 2016, Phillips et al. 2016), with functional advantages such as the increased ability of thicker roots to grow through dry soil (Clark et al. 2003). Although this strategy excludes the advantage of exploring larger soil volumes with longer and thinner roots (i.e. higher SRL), it might be considered beneficial if drought persists over longer periods, because it promotes root longevity (Larson and Funk 2016), thereby still enhancing uptake of water and N.

Inorganic and organic N acquisition was decreased with drought with differences among species and competition regimes for specific N sources. The strong connection between tree N acquisition and soil water availability has been shown for woody species (Fotelli et al. 2002, 2004, Guo et al. 2013, Leberecht et al. 2016) and is due to the negative effects of low water availability on soil N mineralization processes via reduced microbial activity resulting in reduced N availability in the rhizosphere (Schimel et al. 2007, Hueso et al. 2012), on N diffusion and mass flow in the soil which depend on soil water status (Rennenberg et al. 2009), on the composition of mycorrhizal communities that symbiotically provide plants with N (e.g. Gessler et al. 2005, Leberecht et al. 2016).

As a consequence of reduced N acquisition, tree N assimilation decreases directly impacting on the levels of proteins (Gessler et al. 2017). Thus, storage proteins from vegetative tissue are degraded and remobilized leading to a lower storage capacity of N (Millard 1988, Staswick 1994, Millard and Grelet 2010). With drought, N storage in the roots becomes highly significant as a coping mechanism of plants, because drought leads to leaf shedding, thus N stored in the leaves is lost (Millard and Grelet 2010). Here, leaves were not (yet) shed at the time of the harvest, consequently N could still have been remobilized from its storage form as Rubisco. The combined loss of N stored in leaves and roots under drought removes potential N sources which could be of importance for plant recovery once the drought stress is alleviated (Azevedo Neto et al. 2009). Another key coping mechanism of plants under drought stress is the accumulation of soluble amino acids – also via degradation of proteins – serving as osmoprotectants, thus improving the overall plant water status (Hu et al. 2013a, 2013b). Moreover, fine root protein levels also increased in response to low soil water availability, likely due to synthesis of protective proteins (Brunner et al. 2015), proteins with a role in dehydration tolerance (Close 1996, Kozlowski and Pallardy 2002), and/or proteins required for the development of root nodules for N_2-fixing species (Verma et al. 1992), for example at the expense of total amino acid levels. However, amino acid pools also decrease as a response to drought due to the translocation of amino acids to other plant tissues, the usage of amino acids for protein synthesis, the degradation of amino acids, and/or the usage of their carbon skeletons to produce alternative non-N-containing osmotic compounds (which not only decrease osmotic potential but can also function as cell membrane and metabolic protectants) (Chaves et al.

2003). In our study, the mechanisms to cope with drought varied depending on the species and competitor.

Overall, interspecific competition enhanced the effects of drought for all species in the present study, as indicated by the changes in different above- and belowground variables in response to drought of the competing seedlings compared to the fewer responses seen in the single grown seedlings. For example, N acquisition was reduced by drought only for specific N forms and species in the single grown seedlings, but for all N forms and species when competing for N. Similar results were found for biomass and growth indices as well as plant internal N pools. Moreover, the expected difference in the drought response between drought-sensitive *Fagus* and drought-tolerant *Quercus* was only evident with combined drought and competition (i.e. single-grown seedlings of *Fagus* and *Quercus* did not differ in their drought response) further emphasising the enhancement of competition in addition to drought stress via intensified depletion of soil resources. In general, all species were negatively affected by drought, and no clear distinction was found between native and invasive species in their responses to drought. Native *Fagus* and – to a lesser extent – invasive *Robinia* were the most sensitive of the investigated species, showing several changes in above- and belowground traits in response to drought, both in competition and when grown single. For *Fagus*, the strong response to drought generally prevailed over the effects of competitor identity, while for *Robinia* it was mediated by competitor identity. However, the ability to fix N_2 represents an advantage under drought conditions for this species by allowing it to potentially overcome the drought-impaired acquisition of N from the soil (Wurzburger and Miniat 2014, Mantovani et al. 2014, 2015). For *Quercus* and invasive *Prunus*, responses to drought also varied according to competitor identity. In contrast, we found no interaction between drought and competitor identity for *Pinus* which indicates that the two invasive species did not influence the response to drought. Furthermore, coniferous *Pinus* (both growing single and in competition) was generally less responsive to drought than the native and invasive deciduous species most likely due to its isohydric behaviour, i.e. the closing of stomata early during a drought event, thereby minimizing water losses via the needles (Irvine et al. 1998). These results imply that under future scenarios of global change, *Pinus* will be more able than *Fagus* and *Quercus* to withstand simultaneous short-term drought stress and invasion by exotic woody species. However, *Quercus* appears to have an advantage over *Prunus* under drought, highlighting the species-specific nature of the responses.

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

Organic N forms, especially arginine, were generally preferred over inorganic N by all study species confirming the results from studies in the field and under controlled settings (Simon et al. 2017) using the same technique and artificial soil solution as here (e.g. for *F. sylvatica*: Dannenmann et al. 2009, Simon et al. 2010, Simon et al. 2011, Simon et al. 2014, for *R. pseudoacacia*: Hu et al. 2017), as well as the results found at low soil N availability in our previous study using the same species (Bueno et al. 2019). The preferred acquisition of specific N sources is generally driven by their higher abundance in the soil (e.g. Kuster et al. 2013b, Song et al. 2015, Simon et al. 2017). For native *Fagus* and *Pinus*, as well as invasive *Robinia* preferences shifted depending on competitor identity (see Table 4.4 and Table 4.5) indicating plasticity in resource use induced by the interaction with other species which might influence the plant's competitive ability (Ashton et al. 2010). Furthermore, we found no changes in the N form preference with drought suggesting that shifts in N preference would not play an important role in plant adaptation to global abiotic changes.

4.6.Conclusions

In our study, competition for N and drought between native and invasive species lead to general changes in growth, N acquisition and internal N pools, but specific responses depended both on the species and soil water availability. Our results suggest that invasive *Prunus* might be a stronger competitor for most native species than *Robinia*, possibly due to its higher biomass, which is in accordance with a previous study (Bueno et al. 2019). Moreover, the responses of *Prunus* to the different native species highly depended on soil water supply with *Pinus* potentially having an advantage over *Prunus* with drought. In contrast, for *Robinia*, the deciduous natives *Fagus* and *Quercus* had more negative effects than the conifer *Pinus*. Therefore, our results suggest that functional traits might play a role for the competitive ability over invasive species. However, further studies investigating a larger number of species are necessary to confirm this theory. Of the species studied here, native *Fagus* was the most sensitive to drought irrespective of competition. For the other deciduous species responses to drought were mediated by competitor identity, whereas coniferous *Pinus* showed no interaction suggesting that it can endure the combination of drought and invasion by exotic woody species better. Moreover, native *Quercus* apparently has a competitive advantage over invasive *Prunus* under drought conditions, indicating that the competition outcome between the two species is likely to be shaped by the abiotic environment. Overall, our study shows that native and invasive species respond to drought and the competition for N with species-specific strategies related to their physiological characteristics. Consequently, management plans aimed at controlling woody invasions should take into account species-specific responses.

5. General Discussion

5.1. Life form determines the general response of invasive species to biotic and abiotic factors

Plant life forms differ in a wide variety of morphological and physiological traits (Santiago and Wright 2006, Valverde-Barrantes 2017), and contrast in their responses to the biotic and abiotic environment (Díaz et al. 1998, McIntyre et al. 1999), with potential consequences for native-invasive competition (Ammondt and Litton 2012). For example, grass species with C_4 metabolism (studied here) are characterized by their higher photosynthetic N use efficiency (PNUE) compared to tree species (and C_3 grasses) (Ghannoum et al. 2011, Vogan and Sage 2011). Higher PNUE is achieved through a higher rate of CO_2 assimilation per unit of leaf N due to their smaller investment of N in photosynthetic carboxylation enzymes (Kumar et al. 2002). Therefore, grasses are able to rapidly assimilate N into new tissue resulting in greater productivity (Poorter et al. 1990). These physiological differences between life forms are relevant when considering invasive species, i.e. the invasive plant life form may influence invasion success (Funk et al. 2016). However, previous studies rarely compare N dynamics in invasive species of differing life forms, e.g. grasses and trees, although N is a key element limiting plant growth. According to N uptake calculations based on tissue N content invasive shrubs and grasses might interfere with the N acquisition of native species (e.g. Eller and Oliveira 2017, Hu et al. 2019), but N acquisition has rarely been considered in native-invasive interaction studies (e.g. Littschwager et al. 2010, Fraterrigo et al. 2011). To my knowledge, measurements of N acquisition have not been previously performed in invasive tree species, with most studies considering invasive tree seedlings growth either in competition with natives (e.g. Ding et al. 2012, Bao and Nilsen 2015), or in comparison with natives but not growing in direct competition (e.g. Rogers and Siemann 2002, Schumacher et al. 2009). Thus, my work provides first insights into organic and inorganic N acquisition in the context of native tree seedlings competing with invasive tree seedlings and invasive grasses under different N supply levels.

In my doctoral research, invasive grasses and invasive tree seedlings differed in their responses to short-term competition with native tree species and high soil N availability, most likely due to the functional dissimilarities between the grass and tree life form described above. Although both invasive grasses and invasive tree seedlings had higher organic and inorganic N acquisition with high compared to low soil N availability, for the grasses this further enhanced growth, likely via the positive impact on leaf N content and consequently on photosynthetic capacity. Therefore, the differences in competitive ability (understood as plant productivity) of invasive species with high soil N supply was probably not related to N uptake capacity –since all species increased N acquisition at high compared to low soil N– but rather to the N use efficiency after its acquisition, which is an attribute related to life form regardless of invasive status. Nevertheless, when compared to native species, invasive species generally have a higher PNUE (Parepa et al. 2019), even when life form is accounted for (Funk and Vitousek 2007), and also increase in biomass faster than competing native species of the same life form in response to high soil N supply (e.g. Badgery et al. 2005, Vasquez et al. 2008, Littschwager et al. 2010, Bao and Nilsen 2015, Tulloss and Cadenasso 2016). Therefore, life form is not the only factor determining the invasive potential of plant species, with underlying factors also coming into play.

The general differences in growth strategies and physiological requirements between trees and grasses suggest that the success and impact of invasive species of a given life form might be dependent on characteristics of the invaded habitat (Pysek et al. 2012, Sutherland 2004, Andreu and Vilà 2010, Fried et al. 2014). Specifically, the difference in PNUE and growth rates between tree seedlings and grasses described above suggests that at sites with high soil N availability invasive grasses can grow faster than invasive tree seedlings and potentially present a bigger threat. However, grasses have a stronger demand for light, which is more important for grass growth than soil N availability in forest understories (e.g. Strengbom et al. 2004). Thus, which functional traits e.g. growth rate, resource use efficiency, shade tolerance (which are closely linked to life form) determine the success of an invasive plant species depends on the habitat considered (Lamarque et al. 2011, Funk et al. 2016) and its specific environmental conditions. Considering the physiological requirements of invasive species is particularly relevant for the ecological restoration of forest ecosystems (Panetta and Gooden 2017). For example, the use of N_2-fixing species in reforestation programs and the subsequent increase in N input into the system may benefit natives but also invasive grasses in the long run and thus potentially hinder the restoration efforts. Moreover, the high light demand of grass species suggests that invasive grasses might pose a greater threat in open landscapes, agricultural systems, and disturbed areas with little canopy cover (Doust et al. 2008, Ortega-Pieck 2011, Ammondt and Litton 2012) as opposed to forest environments, where invasives with other life forms such as trees might be more significant.

Overall, my research indicates that invasive life form is an important aspect of the competitive effect of invasive species. The relevance of invasive life form should be considered in invasive management plans taking into consideration site-specific conditions of soil N supply, as well as other abiotic factors relevant to the success of the particular invasive life form. However, besides the general differences between life forms, many response strategies found here depended on the specific species considered, which are discussed in the next subsection(s).

5.2. Within a life form (i.e. trees), competition responses of native and invasive species are species-specific and related to functional traits

Functional traits determine species responses to the biotic and abiotic environment (Barbosa et al. 2014), and play an important role in determining competition responses for native-invasive plant competition (Funk et al. 2008, 2016). Functional traits associated with the superior performance of invasive plant species are related to high resource acquisition and include relative growth rate, photosynthetic capacity, leaf N content, and specific leaf area (e.g. Grotkopp and Rejmanek 2007, Luo et al. 2015). For native species, functional traits that determine their response to competition with invasives include relative biomass allocation to the roots, plant height, growth form, and root structure (Kawaletz et al. 2014, Byun and Lee 2017). In my studies, the responses of both native and invasive species to their competitors were species-specific across experiments and in general reflected the morphological and physiological traits of the target species. Therefore, my results suggest that functional traits play an important role in the response strategies for both native and invasive species. However, in order to make generalizations at the functional group level more species need to be investigated, particularly for specific traits found to be relevant here such as leaf habit (i.e. deciduous vs. coniferous).

Interspecific competition might affect the growth of deciduous species more strongly than that of evergreen coniferous species (Millard and Way 2011), probably due to inherent functional dissimilarities between evergreen conifers and deciduous species. For example, an important difference is the year-round availability of N stored in the needles of conifers (Millard 1996), in contrast with the deciduous plants. The ability to store N in the needles allows for a longer photosynthetic period and thus could give the individual a competitive advantage (Givnish 2002). Additionally, N stored in conifer needles as inactive Rubisco strongly supports regrowth early in the growing season (Warren et al. 2003, Wyka et al. 2016). Thus, the stored N confers relative independence from external N supply and consequently the further advantage of relative independence from external factors that affect such supply, such as competition and abiotic stressors (Nambiar and Fife 1991, Malik and Timmer 1998, Millard and Grelet 2010).

In my studies, the main difference in responses to competition within native species was between leaf habits, i.e. deciduous or coniferous. The native conifer showed scarce morphological and physiological differences between competition with one invasive compared to the second invasive species. Additionally, the responses were not influenced by abiotic factors (i.e. soil N and water availability) suggesting that the conifer in my studies can buffer the differential competitive effects of the invasives found for the native deciduous species. Therefore, my work suggests that plant traits such as leaf habit might be of relevance for the general response to competition with invasive plants, although more species should be investigated to confirm this pattern. Besides the difference in responses of coniferous compared to deciduous native species, the overall response of the target species (i.e. changes in specific physiological and morphological traits) was highly species-specific for both native and invasive species. Moreover, my work also shows that these species-specific responses further depend on the competing species, highlighting the need to take the species identity into account when designing forest management plans involving plant invasions.

In competitive interactions between native and invasive species, the identity of the competitor is a major factor determining the competition outcome and the invasion effect on community diversity and composition (Fried et al. 2014). The selected temperate native and invasive tree species in my work are of great relevance in Central European forests (Campagnaro et al. 2018), but thus far their competitive interactions with each other have not been evaluated, especially in relation to the underlying N dynamics. Although previous work had reported effects of *Prunus* on height increments, total biomass, and biomass allocation of native species (Robakowski and Bielinis 2011, Kawaletz et al. 2013, 2014), here I provide first insights on its effects on N acquisition, N allocation to plant internal pools and N preferences compared to *Robinia*, as well as on the effects of the native species on these invasives. In my experiments, native species –particularly deciduous trees – responded negatively to competition with invasive *Prunus* compared to *Robinia*, which is probably linked to the functional traits of *Prunus* such as higher total biomass and potential cyanogenic ability (Csiszár 2009), and the functional traits of *Robinia*, e.g. N_2-fixing ability which minimizes its need to compete for N. *Prunus* generally hindered growth and N acquisition, induced shifts in N source preference and affected plant internal N dynamics in the native species in comparison to *Robinia*, although the specific parameters that were affected were different for each native species. Similarly, the invasive species showed distinct responses to different native competitors. Native *Quercus* negatively affected the invasive species compared to the other natives across experiments and abiotic conditions, with invasive species generally having lower growth rates and N acquisition, changes in N pools, as well as showing preferences for specific N forms when competing with *Quercus* compared to the other natives, Although in general *Quercus* had negative effects on the invasives compared to the other native species, the specific responses (i.e. which trait or traits was affected) changed according to soil N and water supply, indicating that the abiotic environment also plays a role in the competitive interactions between native and invasive species. Overall, my research shows that the outcome of the competition between the selected temperate native and invasive tree species depends on the involved species. Furthermore, the results highlight the higher competitive ability of invasive *Prunus* compared to *Robinia*, as well as *Quercus* as a native species with potential to affect the invasives, though the abiotic environment plays an important role in the competitive responses.

5.3.Competition and abiotic stressors combined influence both native and invasive tree species

5.3.1 Single and competing seedlings have similar responses to abiotic factors, but specific competitors enhanced abiotic stress for a given species

Global anthropogenic changes such as increased atmospheric N deposition and frequency and intensity of drought events are expected to increase N availability and reduce water supply in forest ecosystems (Rennenberg et al. 2009, Rennenberg and Dannenmann 2015, Spinoni et al. 2017). Studies considering the simultaneous action of these two abiotic stressors or the effects of drought on plants previously exposed to high N loads found that increased N availability exacerbates drought stress on woody species (e.g. Nilsen 1995, Dziedek et al. 2016, Pivovaroff et al. 2016, Valliere et al. 2017). Drought combined with high soil N supply had negative effects on mortality, height and diameter growth, photosynthetic capacity, and hydraulic traits, which have been attributed to reduced water use efficiency as a consequence of increased productivity, transpiration and water demand caused by higher N availability (Valliere et al. 2017, González de Andrés 2019). Although N and water availability strongly influence N acquisition which is determinant for plant growth and development (Rennenberg et al. 2006, Rennenberg and Dannenmann 2015), studies performing direct measurements of organic and inorganic N acquisition under combined N deposition and drought are scarce (e.g. Zhou et al. 2018). The effects of these two abiotic stressors on morphological and physiological plant traits, including N acquisition, were investigated separately in my studies. Changes in functional traits like allocation to N pools in leaves and fine roots showed species-specific patterns, although responses of other traits such as N acquisition mostly occurred in the same direction across species. Generally, net N uptake capacity was increased at high compared to low soil N availability, and decreased with drought compared to sufficient water supply, suggesting that future N deposition (resulting in higher N availability) and drought events will have opposite effects on plant N acquisition across native and invasive species, with and without competition.

Responses to abiotic factors were similar across species, but seedlings in interspecific competition with specific species responded more to abiotic stressors compared to single-grown seedlings, indicating that some competitors can enhance the effects of abiotic stressors, especially drought. Such effect is likely a result of the increase in plant density in the competition treatment compared to single seedlings, which probably further intensified the resource depletion produced by the drought treatment (Davis et al. 1999, Baudis et al. 2014). These results highlight the impact of combined competition and changes in the abiotic environment on tree seedlings, which might ultimately lead to changes in species composition and dominance in plant communities (González de Andrés 2019). Whether such effects are attributed to an increase in plant density or to the specific competitor cannot be answered with my experimental set up. For this, an intraspecific control would be required. Nevertheless, the intensification of the abiotic stress due to increased plant density occurred only for specific competitors for native and invasive species, highlighting the species-specific nature of this effect and suggesting that some combinations of species might lead to one species having a competitive advantage over the other in view of expected global changes.

5.3.2 Tree species differ in their sensitivity to abiotic stressors

Tree species are affected differently by the combined competition and changes in the abiotic environment because they differ in their tolerance to abiotic stress and in their resource requirements. For plants of various life forms, abiotic factors such as the light environment, water availability, N availability, temperature, and irradiance influence plant-plant competition (e.g. Fotelli et al. 2002, 2005, Jiang et al. 2014, Simon et al. 2014, Yi et al. 2015, Kuster et al. 2016), but the effects of abiotic components on N acquisition of competing species have been measured only in few occasions (e.g. Simon et al. 2014, Li et al. 2015). Most work combining competition and changes in abiotic factors has been done on native species, and the rare studies considering invasive species have been performed on invasive grasses but not trees, without measuring N uptake (e.g. Verlinden et al. 2014). My studies contribute to further the understanding of the effects of the abiotic environment on native-invasive tree competition regarding N dynamics.

In my studies on native and invasive tree seedlings, responses to competition were mediated by high soil N availability and drought in a species-specific manner. The species considered here can be clustered in three distinct groups: (i) competition responses are fully independent from abiotic factors (*Pinus*), (ii) competition responses are mediated by both soil N and soil water availability (*Quercus*, *Robinia*), and (iii) competition responses are mediated by soil water but are independent from soil N availability (*Fagus*, *Prunus*). Such patterns can be related to functional traits (e.g. leaf habit, cyanogenesis), and to species-specific physiological characteristics related to their responses to abiotic conditions (e.g. Duan et al. 2014, Jiang et al. 2014, Yi et al. 2015, Fruleux et al. 2016). The few responses of the native conifer *Pinus* to competitor identity are fully independent from abiotic factors considered here, which is likely related to its capacity to draw upon stored N in needless and resulting ability to decouple its growth and functioning from outside factors (Nambiar and Fife 1991), as discussed in Chapter 5.2. In contrast, the responses of native deciduous *Quercus* and invasive deciduous *Robinia* are mediated by both soil N and soil water availability, suggesting that these two species are the most sensitive to combined biotic and abiotic stress. Finally, responses of *Fagus* and *Prunus* were mediated by soil water but independent from soil N supply. For the drought-sensitive native (*Fagus*), responses to competitor identity are found only with sufficient water supply, suggesting that drought effects override potential differences between competitor identities for this species, as found before for seedlings and adult trees of *Fagus* between interspecific competition and controls (e.g. Fotelli et al. 2001, Rötzer et al. 2017). However, for invasive *Prunus* the responses to competitor identity were largely variable according to water conditions, but not soil N availability. This is probably related to the ability of *Prunus* to produce cyanogenic compounds that can be stored and further remobilized thereby serving as N sources (Neilson et al. 2013, Gleadow and Moller 2014, Picmanová et al. 2015) and providing a certain degree of independence from soil N supply. The same degree of independence was not found for *Robinia*, despite its N_2-fixing capacity. These species-specific responses highlight the complexity of the consequences for forest communities of future scenarios of combined invasion by tree species and abiotic changes, and pose a challenge for forest conservation and management. My results suggest that management strategies aiming at controlling invasive species should take into account species identities and site abiotic conditions.

5.4. Concluding remarks

Plant invasions, increased atmospheric N deposition and droughts are important components of anthropogenic global changes with potential negative impact on forest ecosystems (Ricciardi et al. 2007, Bradley et al. 2010). Therefore, how invasive species affect native trees under increased soil N availability and drought is a significant question with relevance for the conservation and management of forest ecosystems. Most of the studies investigating the effects of invasive plants on trees focus on plant biomass, morphological traits and/or aboveground physiological responses such as photosynthetic capacity to measure invasives impact (e.g. Ammondt and Litton 2012, Kawaletz et al. 2013, Wheeler et al. 2017). However, responses related to N have been less considered, although N is a key element limiting plant growth (Marschner 1995), and there is evidence that invasive species may influence N-related processes at the individual and ecosystem levels (Castro-Díez et al. 2006, Vilà et al. 2011). Specifically, direct measurements of organic and inorganic N acquisition as well as allocation of N to N pools in leaves and roots have rarely been performed in native-invasive competition other than studies focused on grasses and forbs (e.g. Littschwager et al. 2010, Verlinden et al. 2014), but not on trees. My doctoral work provides first insights into competition for N between native trees and invasive grasses or trees and how this is further influenced by abiotic variables related to global changes, i.e. atmospheric N deposition and drought. My studies add to the understanding of how invasive species affect not only biomass and morphological traits but also the underlying physiological processes such as N uptake capacity and N allocation to plant internal N pools.

My studies show that biotic and abiotic stress generally lead to changes in growth, N acquisition and N pools of native trees and invasive species, and that the response strategies to combined competition and abiotic factors are species-specific and related to plant functional traits. Invasive species of different life forms (i.e. tree seedling and grasses) respond to competition and abiotic stress differently, likely reflecting functional differences in resource use efficiency and growth rates. However, life form is not the only determinant of species responses, as my studies show that within a given life form, i.e. tree seedlings, there are differences in the response strategies which can be related to functional traits of the competing species. The species-specific nature of these relations is also evident in the fact that some competitors enhance the effects of abiotic factors for a given species, as well as the differences between species in terms of responses to competition being dependent or independent from abiotic factors. Therefore, my work indicates that the ultimate outcome of the competition between native trees and invasive species under global abiotic change will depend on the species at hand, as well as the abiotic environment of the invaded habitat.

The results presented here have potential implications for forest conservation and management. My work suggests that specific invasives are a relatively more important threat for native tree species, and also that some natives can better withstand invasive competition. Additionally, such patterns can further be affected by the abiotic environment, and therefore restoration efforts aimed at controlling plant invasion should be designed taking these variables into account. Specifically for the Central European native tree species studied here, *Prunus* has the strongest effects on native species across experiments compared to *Robinia*. The effects of *Prunus* on native species has been reported before (e.g. Kawaletz et al. 2013, 2014), but here I provide novel evidence in relation to its effects on N dynamics. However, other aspects influencing the long-term impacts of invasive competition, for example the potential to alter N processes in the ecosystem via N_2 fixation (Vilà et al. 2011, Staska et al. 2014, Medina-Villar et al. 2016), or the influence of competition for other resources such as light and space, need to be considered to better predict invasive impact in forest ecosystems.

5.5. Limitations of these experiments and suggestions for future research

Three main limitations will be discussed: (i) the general limitations inherent to greenhouse studies, which also apply to my experiments, (ii) the selection of a limited number of species, and (iii) the decision to favor uniform age for the tree seedlings over uniform size. I suggest future research considering these limitations that would further the understanding of N dynamics in plant native-invasive competition under abiotic stress gained with my studies.

The suitability of greenhouse studies to answer ecological questions has been discussed extensively (e.g. Campbell et al. 1991, Freckleton and Watkinson 2000, Kawaletz et al. 2014). Although greenhouse conditions do not fully reflect the complexity of the field situation, greenhouses remain a valuable tool for the measurement of factors of interest in isolation from other variables. Studies under field conditions, or greenhouse studies with complex designs measuring various variables simultaneously are still required to complete the insights provided here as a first approach to native-invasive tree species competition for N in view of global anthropogenic change.

The generalizations of the patterns found here are not supported by a large number of species, e.g. the apparent link between functional traits and native and invasive species responses to combined plant competition and abiotic stress. The number of species considered in this work had to be limited because of logistical constraints and the time-consuming nature of the sample processing and measurements of N acquisition and N pools. Specifically in the studies on the temperate species, I aimed to investigate the effect of two different abiotic variables on the same set of species, and thus species numbers remained the same across experiments. Therefore, research projects using more species sharing similar functional traits could be very valuable to confirm the suggestions arising from my results.

In my experiments I had to decide whether to use seedlings of two given species of the same age which might differ in size due to contrasting growth strategies of the species (Reich et al. 1997) or seedlings of the same size that might then be at contrasting developmental stages. Here I have favored having uniform plant age to control for developmental stage and to take into account the fact that species have different growth strategies which result in specific N acquisition strategies at a given age. Consequently, the tree seedlings used in my experiments had the same age but not necessarily of the same size due to the variation in growth rate among species. Specifically, the invasives grew faster than the natives, and this is one of the several traits that enhances their competitive ability and therefore their capacity to become invasive (Grotkopp and Rejmánek 2007, van Kleunen et al. 2010, Lamarque et al. 2011). The growth rate (and therefore size) of the competitor might influence the response of the native species considered here, not only when the fast-growing competitor is an invasive species, but also when it is a native species (Simon et al. 2010, 2014, Li et al. 2015). However, in a forest understory, tree seedlings competing with each other might be of any age and size, and thus experiments that take into consideration a greater variety of seedling developmental stages would provide further insights into native-invasive competitive dynamics.

My studies answered research questions regarding the effects of competition combined with abiotic stress on native and invasive seedlings' growth, organic and inorganic N acquisition and N pools in leaves and fine roots. Nevertheless, considering other aspects in future research could create a more complete picture on the N dynamics in native-invasive competition under global change. For instance, a different experimental design including intraspecific competition treatments would be suited to investigate competition effects to complement my research about competitor identity effects. Moreover, measuring additional N pools (e.g. ammonium, nitrate, structural N), in a larger variety of plant tissues (e.g. coarse roots, stem, bark) and time points along the year (e.g. before leaf development, after leaf senescence) would allow to better understand the changes in tree N allocation to diverse functions such as structure, storage and defense and how this changes seasonally in response to competition with invasive plants and anthropogenic abiotic changes, as has been investigated for native plants and other plant life forms. Furthermore, the role of the interaction between soil N supply and light availability has not been investigated in invasive grasses-native tree seedlings competition as it has been for competition between native tree seedlings (e.g. Simon et al. 2014), and could provide important insights into the mechanisms of competition for N with invasive grasses. Moreover, considering additional physiological traits associated to plant N, such as photosynthetic activity as it relates directly to the leaf N pools (Millard and Grelet 2010), and N_2-fixation capacity as an alternative to external N acquisition for some species (Sprent 1992), would be of great importance as these are factors with great potential to influence native-invasive interactions. Finally, the long-term effects of competition should be studied under field conditions, considering plant individuals of different ages as well as variables related to the plant-soil interactions which are known to affect plant N dynamics, such as soil type and competition with the microbial community (Rennenberg and Dannenmann 2015, Simon et al. 2017).

Supplementary Data

A. Supplementary Tables, Chapter 2.

Supplementary Table 2.1. PERMANOVA results of the effects of soil N availability and competition regime on the total biomass and root:shoot ratio of *Acacia melanoxylon*, *Podocarpus elatus, Pennisetum clandestinum* and *Cynodon dactylon*.

	Total biomass		**Root:shoot ratio**	
	Pseudo-F	*P(perm)*	*Pseudo-F*	*P(perm)*
1) Acacia				
Soil N availability	0.429	0.515	1.709	0.199
Competition regime	0.264	0.770	0.525	0.619
Soil N availability x Competition regime	0.120	0.722	0.920	0.344
2) Podocarpus				
Soil N availability	0.185	0.676	3.732	0.061
Competition regime	0.508	0.612	2.961	0.061
Soil N availability x Competition regime	0.289	0.594	0.009	0.913
3) Pennisetum				
Soil N availability	57.563	<0.001	0.195	0.662
Competition regime	8.013	0.001	7.835	**0.001**
Soil N availability x Competition regime	3.509	**0.033**	0.330	0.716
4) Cynodon*				
Competition regime	3.488	**0.047**	0.548	0.608

Significant values are indicated in bold.

* at low soil N availability

Supplementary Table 2.2. Pairwise PERMANOVA comparisons of the effects of soil N availability and competition regime on the total biomass and root:shoot ratio of *Pennisetum clandestinum* and *Cynodon dactylon*, based on significant results from previous main tests.

	Pairwise comparison	*t*	*P(perm)*
1) Pennisetum			
Total biomass			
Competition regime			
Control	High N *vs* Low N	7.771	**<0.001**
Competition with *Acacia*	High N *vs* Low N	3.036	**0.006**
Competition with *Podocarpus*	High N *vs* Low N	3.609	**0.002**
Soil N availability			
Low N	Control *vs* Competition with *Acacia*	1.260	0.219
	Control *vs* Competition with *Podocarpus*	1.375	0.197
	Competition with *Acacia vs Podocarpus*	0.129	0.917
High N	Control *vs* Competition with *Acacia*	2.217	**0.039**
	Control *vs* Competition with *Podocarpus*	5.067	**<0.001**
	Competition with *Acacia vs Podocarpus*	1.122	0.281
Root:shoot ratio			
	Control *vs* Competition with *Acacia*	1.911	0.065
	Control *vs* Competition with *Podocarpus*	4.849	**<0.001**
	Competition with *Acacia vs Podocarpus*	1.748	0.088
2) Cynodon*			
Total biomass			
	Control *vs* Competition with *Acacia*	2.957	**0.014**
	Control *vs* Competition with *Podocarpus*	1.803	0.089
	Competition with *Acacia vs Podocarpus*	0.593	0.573

Significant values are indicated in bold.

* at low soil N availability

Supplementary Table 2.3. PERMANOVA results of the effects of soil N availability and competition regime on the ammonium (NH_4^+), nitrate (NO_3^-), glutamine-N (Gln-N) and arginine-N (Arg-N) net uptake capacity of fine roots of *Acacia melanoxylon*, *Podocarpus elatus*, *Pennisetum clandestinum* and *Cynodon dactylon.*

	NH_4^+		**NO_3^-**		**Gln-N**		**Arg-N**	
	Pseudo-F	*P(perm)*	*Pseudo-F*	*P(perm)*	*Pseudo-F*	*P(perm)*	*Pseudo-F*	*P(perm)*
1) Acacia								
Soil N availability	37.985	<0.001	6.185	**0.017**	5.570	**0.026**	62.242	<0.001
Competition regime	3.749	0.037	0.508	0.616	0.405	0.675	3.058	0.066
Soil N availability x Competition regime	8.475	**0.007**	3.597	0.072	0.035	0.852	4.753	**0.039**
2) Podocarpus								
Soil N availability	15.837	**<0.001**	24.976	**<0.001**	4.055	0.053	56.456	**<0.001**
Competition regime	0.143	0.883	0.266	0.772	1.910	0.172	0.012	0.989
Soil N availability x Competition regime	0.217	0.654	0.135	0.725	4.706	**0.036**	0.042	0.842
3) Pennisetum								
Soil N availability	59.970	**<0.001**	1.386	0.252	53.854	**<0.001**	56.747	**<0.001**
Competition regime	0.200	0.830	0.933	0.405	2.248	0.131	2.709	0.066
Soil N availability x Competition regime	0.414	0.690	0.369	0.706	1.344	0.268	2.621	0.075
4) Cynodon*								
Competition regime	0.453	0.749	3.797	**0.025**	0.006	0.993	1.469	0.269

Significant values are indicated in bold.

* at low soil N availability

Supplementary Table 2.4. Pairwise PERMANOVA comparisons of the effects of soil N availability and competition regime on the ammonium (NH_4^+), nitrate (NO_3^-), glutamine-N (Gln-N) and arginine-N (Arg-N) net uptake capacity of fine roots of *Acacia melanoxylon, Podocarpus elatus* and *Cynodon dactylon,* based on significant results from previous main tests.

	Pairwise comparison	*t*	*P(perm)*
1) Acacia			
NH_4^+			
Competition regime			
Control	High *vs* low N	2.160	**0.005**
Competition with *Pennisetum*	High *vs* low N	5.704	**0.002**
Soil N availability			
Low N	Control *vs* Competition with *Pennisetum*	1.467	0.167
	Control *vs* Competition with *Cynodon*	1.385	0.187
	Competition with *Pennisetum vs Cynodon*	0.141	0.898
High N	Control *vs* Competition with *Pennisetum*	2.583	**0.028**
Arg-N			
Competition regime			
Control	High N *vs* low N	3.998	**0.002**
Competition with *Pennisetum*	High N *vs* low N	6.358	**0.002**
Soil N availability			
Low N	Control *vs* Competition with *Pennisetum*	1.056	0.317
	Control *vs* Competition with *Cynodon*	0.098	0.951
	Competition with *Pennisetum vs Cynodon*	0.794	0.418
High N	Control *vs* Competition with *Pennisetum*	2.199	0.060
2) Podocarpus			
Gln-N			
Competition regime			
Control	High N *vs* low N	0.121	0.909
Competition with *Pennisetum*	High N *vs* low N	2.589	**0.011**
Soil N availability			
Low N	Control *vs* Competition with *Pennisetum*	0.551	0.575
	Control *vs* Competition with *Cynodon*	1.020	0.339
	Competition with *Pennisetum vs Cynodon*	1.415	0.188
High N	Control *vs* Competition with *Pennisetum*	2.223	**0.039**
3) Cynodon*			
NO_3^-			
	Control *vs* Competition with *Acacia*	1.142	0.270
	Control *vs* Competition with *Podocarpus*	2.106	**0.036**
	Competition with *Acacia vs Podocarpus*	1.820	0.082

Significant values are indicated in bold.

* at low soil N availability

Supplementary Table 2.5. PERMANOVA results of the differences between ammonium (NH_4^+), nitrate (NO_3^-), glutamine-N (Gln-N) and arginine-N (Arg-N) net uptake capacity of fine roots of *Acacia melanoxylon* and *Podocarpus elatus*.

		Acacia		***Podocarpus***	
1) *Low soil N availability*					
Control	*Main test*	*Pseudo-F*	*P(perm)*	*Pseudo-F*	*P(perm)*
	N form	4.855	**0.004**	18.023	**<0.001**
	Pairwise comparisons	*t*	*P(perm)*	*t*	*P(perm)*
	NH_4^+ *vs* NO_3^-	2.057	**0.033**	1.883	0.093
	NH_4^+ *vs* Gln-N	2.358	**0.017**	4.912	**0.005**
	NH_4^+ *vs* Arg-N	2.920	**0.018**	0.238	0.841
	NO_3^- *vs* Gln-N	0.762	0.548	4.135	**0.005**
	NO_3^- *vs* Arg-N	2.818	**0.004**	2.449	**0.042**
	Gln-N *vs* Arg-N	4.240	**0.002**	5.353	**0.001**
Competition with *Pennisetum*	*Main test*	*Pseudo-F*	*P(perm)*	*Pseudo-F*	*P(perm)*
	N form	4.003	**0.008**	12.086	**<0.001**
	Pairwise comparisons	*t*	*P(perm)*	*t*	*P(perm)*
	NH_4^+ *vs* NO_3^-	3.260	**0.006**	3.518	**0.006**
	NH_4^+ *vs* Gln-N	2.088	**0.015**	4.231	**0.003**
	NH_4^+ *vs* Arg-N	0.468	0.643	0.989	0.334
	NO_3^- *vs* Gln-N	0.898	0.501	2.313	0.056
	NO_3^- *vs* Arg-N	4.065	**0.002**	2.856	**0.012**
	Gln-N *vs* Arg-N	2.253	**0.003**	3.921	**0.002**
Competition with *Cynodon*	*Main test*	*Pseudo-F*	*P(perm)*	*Pseudo-F*	*P(perm)*
	N form	4.041	**0.023**	27.643	**<0.001**
	Pairwise comparisons	*t*	*P(perm)*	*t*	*P(perm)*
	NH_4^+ *vs* NO_3^-	1.445	0.154	3.359	**0.002**
	NH_4^+ *vs* Gln-N	2.523	**0.014**	6.269	**0.002**
	NH_4^+ *vs* Arg-N	1.307	0.232	2.473	**0.035**
	NO_3^- *vs* Gln-N	0.956	0.371	4.532	**0.007**
	NO_3^- *vs* Arg-N	2.057	0.053	2.017	0.070
	Gln-N *vs* Arg-N	2.941	**0.025**	5.697	**0.001**
2) *High soil N availability*					
Control	*Main test*	*Pseudo-F*	*P(perm)*	*Pseudo-F*	*P(perm)*
	N form	1.985	0.105	6.781	**<0.001**
	Pairwise comparisons	*t*	*P(perm)*	*t*	*P(perm)*
	NH_4^+ *vs* NO_3^-	Not applicable		2.820	**<0.001**
	NH_4^+ *vs* Gln-N			2.709	**0.002**
	NH_4^+ *vs* Arg-N			2.333	**0.018**
	NO_3^- *vs* Gln-N			0.689	0.508
	NO_3^- *vs* Arg-N			2.012	0.083
	Gln-N *vs* Arg-N			0.945	0.353
Competition with *Pennisetum*	*Main test*	*Pseudo-F*	*P(perm)*	*Pseudo-F*	*P(perm)*
	N form	16.238	**<0.001**	3.297	0.035
	Pairwise comparisons	*t*	*P(perm)*	*t*	*P(perm)*
	NH_4^+ *vs* NO_3^-	3.732	**0.005**	2.599	**0.012**
	NH_4^+ *vs* Gln-N	4.941	**0.002**	1.065	0.278
	NH_4^+ *vs* Arg-N	4.148	**0.002**	1.552	0.226
	NO_3^- *vs* Gln-N	1.228	0.291	2.539	**0.010**
	NO_3^- *vs* Arg-N	0.342	0.734	1.244	0.249
	Gln-N *vs* Arg-N	2.713	**0.019**	1.113	0.325

Supplementary Table 2.6. PERMANOVA results of the differences between ammonium (NH_4^+), nitrate (NO_3^-), glutamine-N (Gln-N) and arginine-N (Arg-N) net uptake capacity of fine roots of *Pennisetum clandestinum* and *Cynodon dactylon.*

		Pennisetum		***Cynodon****	
1) Low soil N availability					
Control	*Main test*	*Pseudo-F*	*P(perm)*	*Pseudo-F*	*P(perm)*
	N form	5.924	**0.006**	2.415	**0.044**
	Pairwise comparisons	*t*	*P(perm)*	*t*	*P(perm)*
	NH_4^+ *vs* NO_3^-	2.163	**0.046**	1.815	**0.021**
	NH_4^+ *vs* Gln-N	0.690	0.751	2.700	**0.025**
	NH_4^+ *vs* Arg-N	1.186	0.242	0.914	0.388
	NO_3^- *vs* Gln-N	2.637	**0.040**	0.836	0.536
	NO_3^- *vs* Arg-N	3.168	**0.013**	1.624	0.074
	Gln-N *vs* Arg-N	1.467	0.198	2.061	0.070
Competition with *Acacia*	*Main test*	*Pseudo-F*	*P(perm)*	*Pseudo-F*	*P(perm)*
	N form	3.252	**<0.001**	5.953	**0.008**
	Pairwise comparisons	*t*	*P(perm)*	*t*	*P(perm)*
	NH_4^+ *vs* NO_3^-	1.767	**0.019**	3.236	**0.016**
	NH_4^+ *vs* Gln-N	0.445	0.673	1.055	0.329
	NH_4^+ *vs* Arg-N	1.798	0.097	0.472	0.755
	NO_3^- *vs* Gln-N	1.735	**0.025**	2.270	0.076
	NO_3^- *vs* Arg-N	1.904	**0.004**	2.231	0.059
	Gln-N *vs* Arg-N	3.138	**0.010**	0.893	0.456
Competition with *Podocarpus*	*Main test*	*Pseudo-F*	*P(perm)*	*Pseudo-F*	*P(perm)*
	N form	7.070	**<0.001**	5.975	**<0.001**
	Pairwise comparisons	*t*	*P(perm)*	*t*	*P(perm)*
	NH_4^+ *vs* NO_3^-	2.641	**0.006**	2.471	**0.003**
	NH_4^+ *vs* Gln-N	1.242	0.238	2.086	0.064
	NH_4^+ *vs* Arg-N	3.151	**0.009**	0.241	0.800
	NO_3^- *vs* Gln-N	2.589	0.100	2.347	**0.006**
	NO_3^- *vs* Arg-N	2.743	**0.002**	2.482	**0.002**
	Gln-N *vs* Arg-N	4.643	**0.003**	2.403	**0.035**
2) High soil N availability					
Control	*Main test*	*Pseudo-F*	*P(perm)*		
	N form	10.764	**0.001**		
	Pairwise comparisons	*t*	*P(perm)*		
	NH_4^+ *vs* NO_3^-	2.614	**0.040**		
	NH_4^+ *vs* Gln-N	6.444	**0.005**		
	NH_4^+ *vs* Arg-N	5.311	**0.005**		
	NO_3^- *vs* Gln-N	1.652	0.163		
	NO_3^- *vs* Arg-N	0.884	0.435		
	Gln-N *vs* Arg-N	2.339	**0.041**		
Competition with *Acacia*	*Main test*	*Pseudo-F*	*P(perm)*		
	N form	3.637	**0.020**		
	Pairwise comparisons	*t*	*P(perm)*		
	NH_4^+ *vs* NO_3^-	0.565	0.585		
	NH_4^+ *vs* Gln-N	4.744	**0.002**		
	NH_4^+ *vs* Arg-N	2.792	**0.014**		
	NO_3^- *vs* Gln-N	1.880	**0.006**		
	NO_3^- *vs* Arg-N	1.031	0.403		
	Gln-N *vs* Arg-N	2.456	**0.014**		
Competition with *Podocarpus*	*Main test*	*Pseudo-F*	*P(perm)*		
	N form	3.380	**0.034**		
	Pairwise comparisons	*t*	*P(perm)*		
	NH_4^+ *vs* NO_3^-	0.958	0.372		
	NH_4^+ *vs* Gln-N	3.287	**0.003**		

	NH_4^+ *vs* Arg-N	2.552	**0.007**
	NO_3^- *vs* Gln-N	1.596	0.185
	NO_3^- *vs* Arg-N	1.080	0.426
	Gln-N *vs* Arg-N	2.498	**0.026**

Significant values are indicated in bold.

* at low soil N availability

B. Supplementary Tables, Chapter 3.

Supplementary Table 3.1. PERMANOVA results of the differences between species and the effect of high compared to low soil N availability on total biomass (g), root:shoot ratio, relative growth rate (RGR, g dw g^{-1} dw d^{-1}), specific leaf area (SLA, cm^2 g^{-1} dw), and specific root length (SRL, cm g^{-1} dw) of *Fagus sylvatica*, *Quercus robur*, *Pinus sylvestris*, *Prunus serotina*, and *Robinia pseudoacacia* single grown seedlings. SLA was not measured for *P. sylvestris*. Pairwise comparisons were performed only when a significant difference was detected in the main test. Significant values ($p \leq 0.05$) are indicated in bold.

	Total biomass		**Root:shoot**		**RGR**		**SLA**		**SRL**	
Main test	***Pseudo-F***	***P(perm)***	***Pseudo-F***	***P(perm)***	***Pseudo-F***	***P(perm)***	***Pseudo-F***	***P(perm)***	***Pseudo-F***	***P(perm)***
Soil N availability	0.065	0.794	0.095	0.753	0.048	0.829	1.263	0.265	8.497	**0.005**
Species	44.54	**<0.001**	122.83	**<0.001**	23.767	**<0.001**	32.559	**<0.001**	17.518	**<0.001**
Soil N availability x Species	1.922	0.1173	1.146	0.345	0.658	0.622	0.282	0.842	1.123	0.362
Pairwise comparisons	***t***	***P(perm)***	***t***	***P(perm)***	***t***	***P(perm)***	***t***	***P(perm)***	***t***	***P(perm)***
Fagus vs. *Prunus*	10.105	**<0.001**	5.089	**<0.001**	6.023	**<0.001**	8.897	**<0.001**	0.967	0.361
Quercus vs. *Prunus*	7.229	**<0.001**	7.024	**<0.001**	6.159	**<0.001**	5.165	**<0.001**	2.108	0.052
Pinus vs. *Prunus*	8.671	**<0.001**	19.831	**<0.001**	5.120	**<0.001**	-	-	1.082	0.294
Fagus vs. *Robinia*	6.823	**<0.001**	13.785	**<0.001**	6.801	**<0.001**	1.619	0.115	5.144	**<0.001**
Quercus vs. *Robinia*	3.435	**0.001**	13.449	**<0.001**	5.914	**<0.001**	4.788	**<0.001**	3.910	**0.002**
Pinus vs. *Robinia*	5.835	**<0.001**	3.456	**0.001**	5.676	**<0.001**	-	-	5.863	**<0.001**
Fagus vs. *Quercus*	4.323	**<0.001**	2.515	**0.014**	3.278	**0.002**	4.203	**<0.001**	0.936	0.353
Fagus vs. *Pinus*	0.309	0.765	14.878	**<0.001**	0.651	0.523	-	-	0.287	0.782
Quercus vs. *Pinus*	3.733	**<0.001**	14.185	**<0.001**	1.752	0.096	-	-	1.405	0.176
Prunus vs. *Robinia*	3.427	**0.002**	17.737	**<0.001**	1.709	0.095	8.042	**<0.001**	6.524	**<0.001**

Supplementary Table 3.2. PERMANOVA results of the differences between species and the effect of high compared to low soil N availability on total soluble amino acid-N (mg amino acid-N g^{-1} dw) and protein-N (mg protein-N g^{-1} dw) levels in leaves and fine roots of *Fagus sylvatica*, *Quercus robur*, *Pinus sylvestris*, *Prunus serotina*, and *Robinia pseudoacacia* single grown seedlings. Pairwise comparisons were performed only when a significant difference was detected in the main test. Significant values ($p \leq 0.05$) are indicated in bold.

	Leaves				**Fine roots**			
	Total soluble amino acid-N		**Total soluble protein-N**		**Total soluble amino acid-N**		**Total soluble protein-N**	
Main test	***Pseudo-F***	***P(perm)***	***Pseudo-F***	***P(perm)***	***Pseudo-F***	***P(perm)***	***Pseudo-F***	***P(perm)***
Soil N availability	10.605	**0.0012**	5.0134E-2	0.82	2.7574	0.1074	0.12312	0.7309
Species	27.046	**0.0001**	5.271	**0.0009**	5.0442	**0.0026**	6.465	**0.0005**
Soil N availability x Species	1.1957	0.3314	2.353	0.0689	2.1567	0.0864	2.0475	0.1037
Pairwise comparisons	***t***	***P(perm)***	***t***	***P(perm)***	***t***	***P(perm)***	***t***	***P(perm)***
Fagus vs. *Prunus*	3.576	**0.002**	2.560	**0.013**	2.087	0.051	1.693	0.087
Quercus vs. *Prunus*	4.405	**0.001**	1.992	0.056	2.755	**0.012**	0.183	0.872
Pinus vs. *Prunus*	3.199	**0.006**	4.221	**0.001**	3.997	**<0.001**	0.464	0.663
Fagus vs. *Robinia*	6.462	**<0.001**	1.881	0.074	1.569	0.127	4.963	**<0.001**
Quercus vs. *Robinia*	6.713	**<0.001**	2.005	0.055	2.207	**0.040**	3.154	**0.005**
Pinus vs. *Robinia*	6.468	**<0.001**	0.578	0.581	3.414	**0.004**	2.447	**0.022**
Fagus vs. *Quercus*	0.189	0.856	0.362	0.725	0.423	0.685	2.240	**0.039**
Fagus vs. *Pinus*	0.666	0.516	1.776	0.099	1.437	0.196	2.466	**0.017**
Quercus vs. *Pinus*	1.035	0.323	1.999	0.060	1.504	0.149	0.722	0.488
Prunus vs. *Robinia*	4.381	**<0.001**	3.580	**0.002**	0.558	0.578	2.775	0.014

Supplementary Table 3.3. PERMANOVA results of the differences between species and the effect of high compared to low soil N availability on the ammonium (NH_4^+), nitrate (NO_3^-), glutamine-N (Gln-N), and arginine-N (Arg-N) net uptake capacity (nmol N g^{-1} fw h^{-1}) of fine roots of *Fagus sylvatica*, *Quercus robur*, *Pinus sylvestris*, *Prunus serotina*, and *Robinia pseudoacacia* single grown seedlings. Pairwise comparisons were performed only when a significant difference was detected in the main test. Significant values ($p \leq 0.05$) are indicated in bold.

	NH_4^+		**NO_3^-**		**Gln-N**		**Arg-N**			
Main test	***Pseudo-F***	***P(perm)***	***Pseudo-F***	***P(perm)***	***Pseudo-F***	***P(perm)***	***Pseudo-F***		***P(perm)***	
Soil N availability	61.374	**<0.001**	3.754	**0.029**	22.543	**<0.001**	62.626		<0.001	
Species	2.052	0.098	1.361	0.219	9.868	**<0.001**	5.772		<0.001	
Soil N availability x Species	1.374	0.259	1.306	0.260	1.233	0.306	2.907		**0.028**	
Pairwise comparisons	***t***	***P(perm)***	***t***	***P(perm)***	***t***	***P(perm)***	***t***	***P(perm) (low N)***	***t***	***P(perm) (high N)***
Fagus vs. *Prunus*	*Not applicable*		*Not applicable*		5.710	**<0.001**	2.194	**0.045**	3.474	**0.001**
Quercus vs. *Prunus*					2.775	**0.006**	1.227	0.261	2.369	**0.030**
Pinus vs. *Prunus*					4.172	**<0.001**	2.356	**0.046**	2.403	**0.037**
Fagus vs. *Robinia*					4.861	**<0.001**	1.721	0.117	3.803	**0.001**
Quercus vs. *Robinia*					1.879	0.074	0.777	0.500	2.596	**0.016**
Pinus vs. *Robinia*					3.471	**0.002**	1.859	0.109	2.668	**0.029**
Fagus vs. *Quercus*					2.548	**0.019**	0.865	0.393	0.288	0.742
Fagus vs. *Pinus*					0.521	0.598	0.076	0.944	0.491	0.627
Quercus vs. *Pinus*					1.734	0.096	0.943	0.380	0.147	0.886
Prunus vs. *Robinia*					1.613	0.128	0.562	0.587	0.656	0.519

Supplementary Table 3.4. Mann-Whitney U-test results of the effect of high compared to low soil N availability on total biomass (g), root:shoot ratio, relative growth rate (RGR, g dw g^{-1} dw d^{-1}), specific leaf area (SLA, cm^2 g^{-1} dw), and specific root length (SRL, cm g^{-1} dw) of *Fagus sylvatica*, *Quercus robur*, *Pinus sylvestris*, *Prunus serotina*, and *Robinia pseudoacacia* single grown seedlings. SLA was not measured for *P. sylvestris*. Significant values ($p \leq 0.05$) are indicated in bold.

	Total biomass		**Root:shoot ratio**		**RGR**		**SLA**		**SRL**	
	Mann–Whitney U	***P***	***Mann–Whitney U***	***P***	***Mann–Whitney U***	***P***	***Mann–Whitney U***	***P***	***Mann–Whitney U***	***P***
Fagus	53.0	0.297	51.0	0.247	51.0	0.372	41.0	0.132	11.0	0.310
Quercus	54.0	0.201	52.0	0.165	3.0	0.800	68.0	0.605	4.0	0.114
Pinus	38.0	0.894	24.0	0.168	33.0	0.884	-	-	13.0	0.792
Prunus	49.0	0.121	60.0	0.341	54.0	0.201	58.0	0.289	12.0	0.662
Robinia	48.0	0.910	23.0	**0.045**	47.0	0.850	46.0	0.791	8.0	0.132

Supplementary Table 3.5. Mann-Whitney U-test results of the effect of high compared to low soil N availability on ammonium (NH_4^+), nitrate (NO_3^-), glutamine-N (Gln-N), and arginine-N (Arg-N) net uptake capacity (nmol N g^{-1} fw h^{-1}) of fine roots of *Fagus sylvatica*, *Quercus robur*, *Pinus sylvestris, Prunus serotina*, and *Robinia pseudoacacia* single grown seedlings. Significant values ($p \leq 0.05$) are indicated in bold.

	NH_4^+		**NO_3^-**		**Gln-N**		**Arg-N**	
	Mann–Whitney U	***P***	***Mann–Whitney U***	***P***	***Mann–Whitney U***	***P***	***Mann–Whitney U***	***P***
Fagus	0.0	**0.001**	6.0	**0.035**	10.0	0.138	0.0	**<0.001**
Quercus	1.0	**0.004**	21.0	0.279	9.0	0.053	0.0	**0.004**
Pinus	0.0	**0.004**	2.0	**0.032**	6.0	**0.035**	2.0	**0.005**
Prunus	0.0	**0.004**	9.0	0.180	7.0	0.051	1.0	**0.002**
Robinia	0.0	**0.001**	10.0	0.138	9.0	0.101	2.0	**0.017**

Supplementary Table 3.6. Mann-Whitney U-test results of the effect of high compared to low soil N availability on total soluble amino acid-N (mg amino acid-N g^{-1} dw) and protein-N (mg protein-N g^{-1} dw) levels in leaves and fine roots of *Fagus sylvatica*, *Quercus robur*, *Pinus sylvestris*, *Prunus serotina*, and *Robinia pseudoacacia* single grown seedlings. Significant values ($p \leq 0.05$) are indicated in bold.

	Leaves				**Fine roots**			
	Total soluble amino acid-N		**Total soluble protein-N**		**Total soluble amino acid-N**		**Total soluble protein-N**	
	Mann–Whitney U	***P***	***Mann–Whitney U***	***P***	***Mann–Whitney U***	***P***	***Mann–Whitney U***	***P***
Fagus	14.0	0.931	2.0	**0.017**	7.0	0.177	10.0	0.429
Quercus	0.0	**0.010**	6.0	0.257	8.0	0.476	11.0	0.914
Pinus	7.0	0.093	15.0	0.699	11.0	0.310	18.0	1.000
Prunus	6.0	0.065	6.0	0.065	7.0	0.093	10.0	0.240
Robinia	7.0	0.177	11.0	0.310	13.0	0.485	6.0	0.065

Supplementary Table 3.7. PERMANOVA results of the effects of high compared to low soil N availability and competitor identity on total biomass (g), root:shoot ratio, relative growth rate (RGR, g dw g^{-1} dw d^{-1}), specific leaf area (SLA, cm^{2} g^{-1} dw), and specific root length (SRL, cm g^{-1} dw) of *Fagus sylvatica*, *Quercus robur*, *Pinus sylvestris*, *Prunus serotina*, and *Robinia pseudoacacia* seedlings grown in competition. SLA was not measured for *P. sylvestris*. Significant values ($p \leq 0.05$) are indicated in bold.

	Total biomass		**Root:shoot ratio**		**RGR**		**SLA**		**SRL**	
	Pseudo-F	***P (perm)***	***Pseudo-F***	***P (perm)***	***Pseudo-F***	***P (perm)***	***Pseudo-F***	***P (perm)***	***Pseudo-F***	***P (perm)***
Fagus										
Soil N availability	2.530	0.123	1.878	0.178	0.799	0.377	0.663	0.419	12.226	**0.004**
Competitor identity	1.663	0.206	0.128	0.729	6.239	**0.019**	0.035	0.851	3.744	0.073
Soil N availability x Competitor identity	0.094	0.771	0.099	0.747	0.037	0.847	1.784	0.199	3.865	0.064
Quercus										
Soil N availability	1.511	0.244	0.025	0.890	4.138	**0.049**	0.127	0.723	0.123	0.729
Competitor identity	4.929	**0.017**	0.300	0.579	1.638	0.214	0.407	0.532	2.188	0.153
Soil N availability x Competitor identity	2.427	0.124	0.149	0.710	3.106	0.092	0.219	0.637	1.067	0.309
Pinus										
Soil N availability	0.628	0.446	0.484	0.522	5.325	**0.037**	-	-	0.469	0.501
Competitor identity	0.743	0.397	0.261	0.633	1.891	0.190	-	-	5.756	**0.026**
Soil N availability x Competitor identity	2.598	0.115	1.751	0.198	0.663	0.419	-	-	0.006	0.941
Prunus										
Soil N availability	0.189	0.657	0.813	0.589	2.610	0.119	<0.001	0.986	5.403	**0.025**
Competitor identity	0.659	0.528	0.855	0.644	3.221	0.053	3.299	**0.045**	1.198	0.312
Soil N availability x Competitor identity	0.435	0.649	0.855	0.635	0.218	0.804	0.440	0.641	0.345	0.713
Robinia										
Soil N availability	1.718	0.193	0.077	0.782	0.323	0.570	1.287	0.264	2.775	0.101
Competitor identity	1.132	0.340	0.366	0.691	0.672	0.512	5.915	**0.006**	0.632	0.542
Soil N availability x Competitor identity	0.237	0.790	0.005	0.994	0.195	0.831	1.984	0.149	0.007	0.993

Supplementary Table 3.8A. PERMANOVA results of the effects of high compared to low soil N availability and competitor identity on total soluble amino acid-N (mg amino acid-N g^{-1} dw) and protein-N (mg protein-N g^{-1} dw) levels in leaves and fine roots of *Fagus sylvatica*, *Quercus robur*, *Pinus sylvestris, Prunus serotina*, and *Robinia pseudoacacia* seedlings grown in competition. Significant values ($p \leq 0.05$) are indicated in bold.

	Leaves				**Fine roots**			
	Total soluble amino acid-N		**Total soluble protein-N**		**Total soluble amino acid-N**		**Total soluble protein-N**	
	Pseudo-F	***P (perm)***	***Pseudo-F***	***P (perm)***	***Pseudo-F***	***P (perm)***	***Pseudo-F***	***P (perm)***
Fagus								
Soil N availability	10.256	**0.005**	1.274	0.276	5.047	**0.035**	3.585	0.073
Competitor identity	0.005	0.949	0.184	0.676	1.711	0.212	1.005	0.334
Soil N availability x Competitor identity	0.031	0.871	1.656	0.208	1.569	0.234	3.317	0.083
Quercus								
Soil N availability	0.152	0.701	0.165	0.689	8.155	0.008	4.520	**0.046**
Competitor identity	0.016	0.901	1.789	0.197	0.843	0.375	1.044	0.325
Soil N availability x Competitor identity	1.365	0.264	0.615	0.442	4.525	**0.039**	0.434	0.525
Pinus								
Soil N availability	23.924	**<0.001**	0.606	0.439	2.420	0.134	1.957	0.177
Competitor identity	0.294	0.594	0.181	0.664	1.654	0.205	1.596	0.226
Soil N availability x Competitor identity	0.526	0.483	0.081	0.779	1.261	0.276	0.239	0.638
Prunus								
Soil N availability	5.951	**0.022**	4.614	**0.044**	8.319	**0.006**	1.698	0.203
Competitor identity	1.309	0.284	0.173	0.844	0.578	0.566	2.658	0.080
Soil N availability x Competitor identity	0.348	0.697	1.730	0.193	0.677	0.514	0.652	0.518
Robinia								
Soil N availability	8.542	**0.006**	0.580	0.460	31.308	<0.001	20.491	**<0.001**
Competitor identity	0.586	0.602	1.720	0.120	6.202	0.005	0.850	0.445
Soil N availability x Competitor identity	0.550	0.625	0.345	0.714	7.590	**0.001**	0.362	0.710

Supplementary Table 3.8B. Pairwise PERMANOVA comparisons of the effects of high compared to low soil N availability and competitor identity on the total amino acid-N (mg amino acid-N g^{-1} dw) levels in fine roots of *Quercus robur* and *Robinia pseudoacacia* seedlings grown in competition, based on significant results from previous main tests. Significant values ($p \leq 0.05$) are indicated in bold.

		t	***P (perm)***
Quercus			
Soil N availability			
Low N	*Quercus* vs. *Prunus* - *Quercus* vs. *Robinia*	1.411	0.208
High N	*Quercus* vs. *Prunus* - *Quercus* vs. *Robinia*	2.580	**0.007**
Competitor identity			
Q. robur vs. *P. serotina*	Low N - High N	3.120	**0.004**
Q. robur vs. *R. pseudoacacia*	Low N - High N	0.723	0.509
Robinia			
Soil N availability			
Low N	*Fagus* vs. *Robinia* - *Quercus* vs. *Robinia*	2.888	0.022
	Fagus vs. *Robinia* - *Pinus* vs. *Robinia*	3.003	**0.025**
	Quercus vs. *Robinia* - *Pinus* vs. *Robinia*	0.337	0.731
High N	*Fagus* vs. *Robinia* - *Quercus* vs. *Robinia*	0.181	0.861
	Fagus vs. *Robinia* - *Pinus* vs. *Robinia*	1.087	0.340
	Quercus vs. *Robinia* - *Pinus* vs. *Robinia*	0.935	0.447
Competitor identity			
Fagus vs. *Robinia*	Low N - High N	4.654	**0.002**
Quercus vs. *Robinia*	Low N - High N	3.016	**0.010**
Pinus vs. *Robinia*	Low N - High N	1.591	0.109

C. Supplementary Tables, Chapter 4.

Supplementary Table 4.2. PERMANOVA results of the differences between species and the effect of water availability on total biomass (g dw), root:shoot ratio, relative growth rate (RGR, g dw g^{-1} dw d^{-1}), specific leaf area (SLA, cm^{2} g^{-1} dw), and specific root length (SRL, cm g^{-1} dw) of *Fagus sylvatica, Quercus robur, Pinus sylvestris, Prunus serotina,* and *Robinia pseudoacacia* single grown seedlings. SLA was not measured for *Pinus*. Pairwise comparisons were performed only when a significant difference was detected in the main test. Significant values are indicated in bold.

	Total biomass		**Root:shoot**		**RGR**		**SLA**				**SRL**	
Main test	***Pseudo-***	***P(perm)***	***Pseudo-***	***P(perm)***	***Pseudo-***	***P(perm)***	***Pseudo-F***		***P(perm)***		***Pseudo-***	***P(perm)***
Water availability	0.598	0.448	0.144	0.7120	0.250	0.615	2.743		0.101		1.633	0.220
Species	21.253	**<0.001**	147.910	**<0.001**	6.434	**<0.001**	35.141		<0.001		8.479	**<0.001**
Water availability x Species	0.734	0.574	0.465	0.770	0.714	0.576	5.456		**<0.001**		0.305	0.889
Pairwise comparisons	***t***	***P(perm)***	***t***	***P(perm)***	***t***	***P(perm)***	***t***	***P(perm) drought***	***t***	***P(perm) control***	***t***	***P(perm)***
Fagus vs. *Prunus*	7.539	**<0.001**	7.678	**<0.001**	0.745	0.465	2.765	**<0.001**	6.622	**<0.001**	11.321	**<0.001**
Quercus vs. *Prunus*	2.829	**0.006**	5.773	**<0.001**	2.225	**0.034**	0.377	0.715	0.905	0.414	9.191	**<0.001**
Pinus vs. *Prunus*	7.026	**<0.001**	18.687	**<0.001**	0.257	0.795	-	-	-	-	2.828	0.006
Fagus vs. *Robinia*	5.451	**<0.001**	18.868	**<0.001**	3.729	**<0.001**	0.835	0.462	4.550	**<0.001**	1.669	0.108
Quercus vs. *Robinia*	0.445	0.662	12.677	**<0.001**	1.780	0.082	6.109	**<0.001**	6.076	**<0.001**	1.356	0.238
Pinus vs. *Robinia*	5.132	**<0.001**	3.075	**0.007**	3.278	**0.003**	-	-	-	-	3.174	**<0.001**
Fagus vs. *Quercus*	4.370	**<0.001**	0.372	0.709	1.798	0.081	3.116	**<0.001**	5.294	**<0.001**	1.292	0.208
Fagus vs. *Pinus*	0.514	0.598	22.535	**<0.001**	0.907	0.367	-	-	-	-	6.834	**<0.001**
Quercus vs. *Pinus*	4.161	**<0.001**	15.138	**<0.001**	1.874	0.072	-	-	-	-	6.778	**<0.001**
Prunus vs. *Robinia*	3.211	**0.002**	14.930	**<0.001**	4.016	**<0.001**	5.463	**<0.001**	5.870	**<0.001**	3.261	**<0.001**

Supplementary Table 4.2. PERMANOVA results of the differences between species and the effect of water availability on the ammonium (NH_4^+), nitrate (NO_3^-), glutamine-N (Gln-N), and arginine-N (Arg-N) net uptake capacity (nmol N g^{-1} fw h^{-1}) of fine roots of *Fagus sylvatica*, *Quercus robur*, *Pinus sylvestris*, *Prunus serotina*, and *Robinia pseudoacacia* single grown seedlings. Pairwise comparisons were performed only when a significant difference was detected in the main test. Significant values are indicated in bold.

	NH_4^+		**NO_3^-**		**Gln-N**		**Arg-N**	
Main test	***Pseudo-F***	***P(perm)***	***Pseudo-F***	***P(perm)***	***Pseudo-F***	***P(perm)***	***Pseudo-F***	***P(perm)***
Water availability	3.428	0.063	28.182	**<0.001**	4.777	**0.036**	2.181	0.147
Species	1.859	0.124	1.588	0.195	4.239	**0.005**	3.130	**0.020**
Water availability x Species	0.306	0.897	1.540	0.206	0.773	0.549	2.218	0.078
Pairwise comparisons	***t***	***P(perm)***	***t***	***P(perm)***	***t***	***P(perm)***	***t***	***P(perm)***
Fagus vs. *Prunus*	*Not applicable*		*Not applicable*		3.152	**0.003**	1.441	0.168
Quercus vs. *Prunus*					1.411	0.179	0.408	0.706
Pinus vs. *Prunus*					3.238	**0.002**	2.226	**0.032**
Fagus vs. *Robinia*					2.700	**0.013**	2.590	**0.016**
Quercus vs. *Robinia*					1.098	0.291	1.286	0.217
Pinus vs. *Robinia*					2.761	**0.008**	4.094	**<0.001**
Fagus vs. *Quercus*					1.418	0.167	1.251	0.222
Fagus vs. *Pinus*					0.052	0.961	1.166	0.251
Quercus vs. *Pinus*					1.427	0.179	2.496	**0.023**
Prunus vs. *Robinia*					0.257	0.804	0.537	0.651

Supplementary Table 4.3. PERMANOVA results of the differences between species and the effect of water availability on total soluble amino acid-N (mg amino acid-N g^{-1} dw) and protein-N (mg protein-N g^{-1} dw) content in the leaves and fine roots of *Fagus sylvatica*, *Quercus robur*, *Pinus sylvestris*, *Prunus serotina*, and *Robinia pseudoacacia* single grown seedlings. Pairwise comparisons were performed only when a significant difference was detected in the main test. Significant values are indicated in bold.

	Leaves						**Fine roots**			
	Total soluble amino acid-		**Total soluble protein-N**				**Total soluble amino acid-**		**Total soluble protein-**	
Main test	***Pseudo-F***	***P(perm)***	***Pseudo-F***		***P(perm)***		***Pseudo-F***	***P(perm)***	***Pseudo-F***	***P(perm)***
Water availability	2.383	0.118	16.269		**<0.001**		1.966	0.169	9.493	**0.003**
Species	8.590	**<0.001**	9.646		**<0.001**		74.386	**<0.001**	10.142	**<0.001**
Water availability x	1.946	0.079	6.286		**<0.001**		2.357	0.064	2.221	0.079
Pairwise comparisons	***t***	***P(perm)***	***t***	***P(perm)drought***	***t***	***P(perm)control***	***t***	***P(perm)***	***t***	***P(perm)***
Fagus vs. *Prunus*	3.615	**0.002**	5.605	**0.003**	2.357	**0.025**	19.422	**<0.001**	5.172	**<0.001**
Quercus vs. *Prunus*	4.705	**<0.001**	5.329	**0.002**	10.679	**0.003**	13.542	**<0.001**	3.344	**0.004**
Pinus vs. *Prunus*	2.260	**0.037**	1.614	0.139	3.258	**0.003**	24.646	**<0.001**	6.201	**<0.001**
Fagus vs. *Robinia*	3.164	**0.001**	0.017	0.985	4.222	**0.013**	3.436	**0.002**	3.209	**0.003**
Quercus vs. *Robinia*	3.301	**0.001**	0.161	0.866	9.969	**0.005**	1.196	0.251	2.219	**0.036**
Pinus vs. *Robinia*	2.715	**0.005**	0.556	0.580	2.280	0.057	5.129	**<0.001**	3.861	**<0.001**
Fagus vs. *Quercus*	0.717	0.489	0.414	0.647	1.344	0.209	4.909	**<0.001**	1.748	0.095
Fagus vs. *Pinus*	1.940	0.067	0.794	0.633	3.234	**0.015**	6.567	**<0.001**	1.353	0.194
Quercus vs. *Pinus*	3.278	**0.006**	0.922	0.493	8.931	**0.002**	10.159	**<0.001**	2.944	**0.007**
Prunus vs. *Robinia*	2.406	**0.011**	2.314	0.051	5.841	**0.004**	6.284	**<0.001**	0.171	0.870

Supplementary Table 4.4. Mann-Whitney U-test results of the effect of water availability on total biomass (g dw), root:shoot ratio, relative growth rate (RGR, g dw g^{-1} dw d^{-1}), specific leaf area (SLA, cm^2 g^{-1} dw), and specific root length (SRL, cm g^{-1} dw) of *Fagus sylvatica*, *Quercus robur*, *Pinus sylvestris*, *Prunus serotina*, and *Robinia pseudoacacia* single grown seedlings. SLA was not measured for *Pinus*. Significant values are indicated in bold.

	Total biomass		**Root:shoot ratio**		**RGR**		**SLA**		**SRL**	
	Mann–Whitney U	***P***	***Mann–Whitney U***	***P***	***Mann–Whitney U***	***P***	***Mann–Whitney U***	***P***	***Mann–Whitney U***	***P***
Fagus	76.0	0.700	70.0	0.487	38.0	0.403	70.0	0.487	4.0	**0.026**
Quercus	90.0	0.981	81.0	0.645	68.0	0.840	83.0	0.716	13.0	0.485
Pinus	50.0	0.511	49.0	0.470	6.0	0.413	-	-	19.0	0.377
Prunus	50.0	0.340	62.0	0.829	21.0	0.163	51.0	0.372	17.0	0.937
Robinia	26.0	0.216	23.0	0.133	26.0	0.361	16.0	**0.034**	9.0	0.180

Supplementary Table 4.5. Mann-Whitney U-test results of the effect of water availability on ammonium (NH_4^+), nitrate (NO_3^-), glutamine-N (Gln-N), and arginine-N (Arg-N) net uptake capacity (nmol N g^{-1} fw h^{-1}) of fine roots of *Fagus sylvatica*, *Quercus robur*, *Pinus sylvestris*, *Prunus serotina*, and *Robinia pseudoacacia* single grown seedlings. Significant values are indicated in bold.

	NH_4^+		**NO_3^-**		**Gln-N**		**Arg-N**	
	Mann–Whitney U	***P***	***Mann–Whitney U***	***P***	***Mann–Whitney U***	***P***	***Mann–Whitney U***	***P***
Fagus	7.0	0.352	7.0	**0.007**	27.0	0.955	22.0	0.536
Quercus	5.0	0.571	4.0	**0.030**	4.0	0.052	11.0	0.343
Pinus	7.0	0.352	9.0	0.101	13.0	0.530	13.0	0.530
Prunus	2.0	**0.038**	0.0	**<0.001**	14.0	0.209	24.0	1.000
Robinia	6.0	0.257	12.0	0.394	4.0	**0.026**	0.0	**0.004**

Supplementary Table 4.6. Mann-Whitney U-test results of the effect of water availability on total soluble amino acid-N (mg amino acid-N g^{-1} dw) and protein-N (mg protein-N g^{-1} dw) content in the leaves and fine roots of *Fagus sylvatica*, *Quercus robur*, *Pinus sylvestris*, *Prunus serotina*, and *Robinia pseudoacacia* single grown seedlings. Significant values are indicated in bold.

	Leaves				**Fine roots**			
	Total soluble amino acid-N		**Total soluble protein-N**		**Total soluble amino acid-N**		**Total soluble protein-N**	
	Mann–Whitney U	***P***	***Mann–Whitney U***	***P***	***Mann–Whitney U***	***P***	***Mann–Whitney U***	***P***
Fagus	15.0	0.699	10.0	0.240	14.0	0.589	13.0	0.485
Quercus	16.0	0.818	18.0	1.000	6.0	0.065	14.0	0.589
Pinus	11.0	0.537	5.0	0.082	11.0	0.537	12.0	0.394
Prunus	18.0	1.000	17.0	0.937	8.0	0.132	3.0	**0.015**
Robinia	10.0	0.429	1.0	**0.019**	11.0	0.310	7.0	0.093

Supplementary Table 4.7. PERMANOVA results of the effects of water availability and competitor identity on total biomass (g dw), root:shoot ratio, relative growth rate (RGR, g dw g^{-1} dw d^{-1}), specific leaf area (SLA, cm^2 g^{-1} dw), and specific root length (SRL, cm g^{-1} dw) of *Fagus sylvatica*, *Quercus robur*, *Pinus sylvestris*, *Prunus serotina*, and *Robinia pseudoacacia* seedlings grown in competition. SLA was not measured for *Pinus*. Significant values are indicated in bold.

	Total biomass		**Root:shoot ratio**		**RGR**		**SLA**		**SRL**	
	Pseudo-F	***P (perm)***	***Pseudo-F***	***P (perm)***	***Pseudo-F***	***P (perm)***	***Pseudo-F***	***P (perm)***	***Pseudo-F***	***P (perm)***
Fagus										
Water availability	0.424	0.528	5.521	**0.025**	0.639	0.434	5.098	**0.030**	0.338	0.564
Competitor identity	0.101	0.750	0.227	0.641	2.372	0.136	0.232	0.629	0.006	0.940
Water availability x Competitor identity	1.133	0.298	0.003	0.955	5.028	**0.036**	0.011	0.916	0.009	0.927
Quercus										
Water availability	0.001	0.972	0.811	0.386	0.464	0.494	2.484	0.120	1.220	0.277
Competitor identity	0.389	0.532	6.758	**0.014**	6.115	**0.033**	0.021	0.887	12.683	0.001
Water availability x Competitor identity	0.009	0.917	0.279	0.616	0.039	0.846	2.414	0.136	5.857	**0.022**
Pinus										
Water availability	0.454	0.506	0.002	0.965	0.103	0.754	-	-	0.745	0.399
Competitor identity	5.795	**0.025**	0.526	0.482	0.441	0.513	-	-	0.518	0.483
Water availability x Competitor identity	0.696	0.411	0.015	0.909	1.563	0.235	-	-	3.638	0.072
Prunus										
Water availability	0.506	0.481	0.027	0.866	0.040	0.843	10.300	**0.003**	0.744	0.392
Competitor identity	4.217	0.021	0.306	0.738	6.630	**0.004**	11.828	**<0.001**	1.586	0.218
Water availability x Competitor identity	4.997	**0.008**	0.281	0.759	0.643	0.537	0.185	0.830	2.823	0.075
Robinia										
Water availability	0.002	0.967	12.528	**<0.001**	0.043	0.833	0.776	0.384	0.076	0.892
Competitor identity	2.180	0.123	0.282	0.787	4.275	**0.027**	2.272	0.118	0.886	0.505
Water availability x Competitor identity	0.533	0.599	0.586	0.606	0.521	0.605	1.302	0.288	1.204	0.326

Supplementary Table 4.8. Pairwise PERMANOVA comparisons following a significant interaction of water availability and competitor identity in previous main tests. (A) Relative growth rate (RGR, g dw g^{-1} dw d^{-1}) of *Fagus sylvatica*. (B) Specific root length (SRL, cm g^{-1} dw) of *Quercus robur*. (C) Total biomass (g dw) of *Prunus serotina*. Significant values are indicated in bold.

(A)		**RGR**	
Fagus		***t***	***P(perm)***
Water availability – Control	*Fagus* vs. *Prunus* – *Fagus* vs. *Robinia*	2.782	**0.012**
Water availability – Drought	*Fagus* vs. *Prunus* – *Fagus* vs. *Robinia*	0.519	0.594
Competitor identity – *Fagus* vs. *Prunus*	Control – Drought	1.053	0.318
Competitor identity – *Fagus* vs. *Robinia*	Control – Drought	4.338	**0.014**

(B)		**SRL**	
Quercus		***t***	***P(perm)***
Water availability – Control	*Quercus* vs. *Prunus* – *Quercus* vs. *Robinia*	0.771	0.464
Water availability – Drought	*Quercus* vs. *Prunus* – *Quercus* vs. *Robinia*	4.450	**0.003**
Competitor identity – *Quercus* vs. *Prunus*	Control – Drought	0.787	0.465
Competitor identity – *Quercus* vs. *Robinia*	Control – Drought	3.206	**0.015**

(C)		**Total biomass**	
Prunus		***t***	***P(perm)***
Water availability – Control	*Prunus* vs. *Fagus* – *Prunus* vs. *Quercus*	2.721	**0.018**
	Prunus vs. *Fagus* – *Prunus* vs. *Pinus*	0.738	0.477
	Prunus vs. *Quercus* – *Prunus* vs. *Pinus*	3.681	**0.003**
Water availability – Drought	*Prunus* vs. *Fagus* – *Prunus* vs. *Quercus*	1.054	0.335
	Prunus vs. *Fagus* – *Prunus* vs. *Pinus*	1.465	0.174
	Prunus vs. *Quercus* – *Prunus* vs. *Pinus*	0.698	0.450
Competitor identity – *Prunus* vs. *Fagus*	Control – Drought	1.093	0.302
Competitor identity – *Prunus* vs. *Quercus*	Control – Drought	2.921	**0.010**
Competitor identity – *Prunus* vs. *Pinus*	Control – Drought	0.550	0.586

Supplementary Table 4.9. Pairwise PERMANOVA comparisons following a significant effect of competitor identity in previous main tests. (A) Relative growth rate (RGR, g dw g^{-1} dw d^{-1}) and specific leaf area (SLA, cm^{2} g^{-1} dw) of *Prunus serotina* (B) Relative growth rate (RGR, g dw g^{-1} dw d^{-1}) of *Robinia pseudoacacia*. Significant values are indicated in bold.

	RGR		***SLA***	
(A) *Prunus*	***t***	***P(perm)***	***t***	***P(perm)***
Prunus vs. *Fagus* – *Prunus* vs. *Quercus*	2.735	**0.012**	2.358	**0.025**
Prunus vs. *Fagus* – *Prunus* vs. *Pinus*	2.621	**0.015**	2.621	**0.016**
Prunus vs. *Quercus* – *Prunus* vs. *Pinus*	0.734	0.467	4.889	**<0.001**
(B) *Robinia*				
Robinia vs. *Fagus* – *Robinia* vs. *Quercus*	0.204	0.845	*Not applicable*	
Robinia vs. *Fagus* – *Robinia* vs. *Pinus*	2.267	**0.039**		
Robinia vs. *Quercus* – *Robinia* vs. *Pinus*	2.387	**0.031**		

Supplementary Table 4.10. PERMANOVA results of the effects of water availability and competitor identity on total soluble amino acid-N (mg amino acid-N g^{-1} dw) and protein-N (mg protein-N g^{-1} dw) content in the leaves and fine roots of *Fagus sylvatica*, *Quercus robur*, *Pinus sylvestris*, *Prunus serotina*, and *Robinia pseudoacacia* seedlings grown in competition. Significant values are indicated in bold.

	Leaves				**Fine roots**			
	Total soluble amino acid-N		**Total soluble protein-N**		**Total soluble amino acid-N**		**Total soluble protein-N**	
	Pseudo-F	***P (perm)***	***Pseudo-F***	***P (perm)***	***Pseudo-F***	***P (perm)***	***Pseudo-F***	***P (perm)***
Fagus								
Water availability	0.227	0.630	8.617	**0.010**	0.467	0.618	0.961	0.343
Competitor identity	0.787	0.381	0.183	0.674	2.425	0.117	0.055	0.821
Water availability x Competitor identity	1.619	0.220	3.236	0.086	0.111	0.840	0.264	0.608
Quercus								
Water availability	0.548	0.468	16.153	**<0.001**	45.017	<0.001	1.900	0.188
Competitor identity	0.788	0.383	0.223	0.645	69.644	<0.001	4.978	**0.035**
Water availability x Competitor identity	4.781	**0.038**	0.468	0.501	22.422	**<0.001**	2.057	0.171
Pinus								
Water availability	0.039	0.850	3.519	0.080	8.813	**0.002**	0.002	0.962
Competitor identity	10.133	**0.006**	11.804	**0.003**	7.391	**0.012**	3.320	0.080
Water availability x Competitor identity	0.576	0.469	2.918	0.096	0.039	0.847	1.191	0.286
Prunus								
Water availability	1.001	0.321	1.237	0.277	13.591	<0.001	23.047	**<0.001**
Competitor identity	5.546	**0.009**	8.045	**0.002**	2.889	0.070	1.356	0.274
Water availability x Competitor identity	1.158	0.328	3.000	0.065	5.165	**0.010**	0.653	0.530
Robinia								
Water availability	4.136	**0.026**	3.766	0.060	0.321	0.590	0.573	0.452
Competitor identity	1.809	0.153	1.259	0.300	0.177	0.852	3.934	0.025
Water availability x Competitor identity	0.263	0.863	0.847	0.438	0.416	0.689	10.028	**<0.001**

Supplementary Table 4.11. Pairwise PERMANOVA comparisons following a significant interaction of water availability and competitor identity in previous main tests. (A) Total soluble amino acid-N (mg amino acid-N g^{-1} dw) and protein-N (mg protein-N g^{-1} dw) content in leaves and fine roots of *Quercus robur*. (B) Total soluble amino acid-N (mg amino acid-N g^{-1} dw) content in fine roots of *Prunus serotina*. (C) Total soluble protein-N (mg protein-N g^{-1} dw) content in fine roots of *Robinia pseudoacacia*. Significant values are indicated in bold.

(A)		**Total soluble amino acid-N – leaves**		**Total soluble amino acid-N – fine roots**	
Quercus		***t***	***P(perm)***	***t***	***P(perm)***
Water availability – Control	*Quercus* vs. *Prunus* – *Quercus* vs. *Robinia*	1.227	0.249	3.494	**0.006**
Water availability – Drought	*Quercus* vs. *Prunus* – *Quercus* vs. *Robinia*	1.812	0.101	7.192	**0.008**
Competitor identity – *Quercus* vs. *Prunus*	Control – Drought	2.182	**0.035**	6.499	**0.002**
Competitor identity – *Quercus* vs. *Robinia*	Control – Drought	0.975	0.338	2.082	0.071

(B)		**Total soluble amino acid-N – fine roots**	
Prunus		***t***	***P(perm)***
Water availability – Control	*Prunus* vs. *Fagus* – *Prunus* vs. *Quercus*	2.432	**0.034**
	Prunus vs. *Fagus* – *Prunus* vs. *Pinus*	2.778	**0.027**
	Prunus vs. *Quercus* – *Prunus* vs. *Pinus*	0.273	0.806
Water availability – Drought	*Prunus* vs. *Fagus* – *Prunus* vs. *Quercus*	1.447	0.178
	Prunus vs. *Fagus* – *Prunus* vs. *Pinus*	0.672	0.513
	Prunus vs. *Quercus* – *Prunus* vs. *Pinus*	2.387	**0.018**
Competitor identity – *Prunus* vs. *Fagus*	Control – Drought	0.242	0.803
Competitor identity – *Prunus* vs. *Quercus*	Control – Drought	3.562	**0.002**
Competitor identity – *Prunus* vs. *Pinus*	Control – Drought	3.579	**0.003**

(C)		**Total soluble protein-N – fine roots**	
Robinia		***t***	***P(perm)***
Water availability – Control	*Robinia* vs. *Fagus* – *Robinia* vs. *Quercus*	0.117	0.909
	Robinia vs. *Fagus* – *Robinia* vs. *Pinus*	1.247	0.235
	Robinia vs. *Quercus* – *Robinia* vs. *Pinus*	1.505	0.186
Water availability – Drought	*Robinia* vs. *Fagus* – *Robinia* vs. *Quercus*	2.281	**0.042**
	Robinia vs. *Fagus* – *Robinia* vs. *Pinus*	4.363	**0.003**
	Robinia vs. *Quercus* – *Robinia* vs. *Pinus*	3.687	**0.010**
Competitor identity – *Robinia* vs. *Fagus*	Control – Drought	2.801	**0.020**
Competitor identity – *Robinia* vs. *Quercus*	Control – Drought	1.132	0.280
Competitor identity – *Robinia* vs. *Pinus*	Control – Drought	3.592	**0.002**

Supplementary Table 4.12. Pairwise PERMANOVA comparisons following a significant effect of competitor identity in previous main tests on the total soluble amino acid-N (mg amino acid-N g^{-1} dw) and protein-N (mg protein-N g^{-1} dw) content in leaves of *Prunus serotina*. Significant values are indicated in bold.

	Total soluble amino acid-N – leaves		**Total soluble protein-N – leaves**	
Prunus	***t***	***P(perm)***	***t***	***P(perm)***
Prunus vs. *Fagus* – *Prunus* vs. *Quercus*	2.965	**0.009**	0.260	0.800
Prunus vs. *Fagus* – *Prunus* vs. *Pinus*	1.787	0.092	3.172	**0.004**
Prunus vs. *Quercus* – *Prunus* vs. *Pinus*	1.872	0.075	3.332	**0.005**

Authors’ contributions

Chapter 2

AB and JS conceived the experimental design. AB and LG conducted the ^{15}N uptake experiments and harvest, and evaluated the data. Manuscript writing was performed by AB and JS. KP contributed stable isotope analyses and ideas to data interpretation. SS contributed ideas to the overall study and the manuscript. All authors contributed critically to the drafts and agreed on the final version of the manuscript.

Chapter 3

AB and JS conceived the experimental design and led manuscript writing. AB conducted the ^{15}N uptake experiments, N metabolite analyses, and evaluated the data. KP contributed stable isotope analyses and ideas to data interpretation. All authors contributed critically to the drafts and agreed on the final version of the manuscript.

Chapter 4

AB and JS conceived and designed the study. AB conducted the ^{15}N uptake experiments, N metabolite analyses, and evaluated all data. KP contributed the stable isotope analyses. AB and JS led the writing of the manuscript. All authors contributed critically to the drafts and agreed on the final version of the manuscript.

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