The phosphorus and nitrogen nutrition of European beech under a future warmer and drier climate: climate chamber experiments and transect studies

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SUMMARY

Productivity of temperate forests may increasingly be limited by phosphorus (P) shortage, as indicated by decreasing P concentrations and increasing nitrogen (N):P ratios in leaves and fine roots and reports on recent growth reductions that appear to be caused by P limitation. The predicted increases in temperatures and a shift in seasonal precipitation patterns toward higher winter and lower summer precipitation with a higher frequency of drought events in combination with continued high atmospheric N deposition are likely to further alter soil nutrient dynamics, especially the cycling of the two main limiting nutrients, P and N. A future warmer and drier climate combined with higher soil N availability is also thought to influence biochemical cycles, with potential consequences for the amount of carbon (C) sequestered in forest soils and the quantity of mineralized N. At the same time, there is a lack of knowledge about the response of ectomycorrhizal fungi (ECMF), which are the most important fungal symbionts for temperate forest ecosystems, to global change.

In order to improve predictions on the P and N nutrition of temperate forest ecosystems under global change, a combined observational and experimental study with mature trees and saplings of European beech (*Fagus sylvatica* L.) was conducted. European beech is the dominant native tree species of Central Europe's temperate forest biome, with high economic importance in many countries.

To investigate the effect of climatic drought and N deposition on soil C and N dynamics, notably soil respiration rate and net N mineralization rate in their seasonal change, a transect study with 11 mature beech forests along a precipitation (855–576 mm yr^{-1}) and temperature gradient (8.7–9.4°C) on sandy to sandy-loamy glacial substrates in northern Germany was conducted.

To enable sound predictions about the P nutrition of European beech under the conditions of altered climate and elevated N deposition at different levels of soil P availability, a four-factorial climate chamber experiment (2 temperature x 3 soil moisture x 2 N supply x 3 P supply levels; 36 treatments in total) was conducted to investigate their effect on various morphological and physiological parameters of beech saplings. A focus of this experiment in highly controllable walk-in chambers was on the morphological and physiological adaptations of beech to decreasing plant-available P in the course of increasing N fertilization, i.e., an increasing P limitation, and to increasing soil desiccation. Furthermore, the purpose of this climate chamber experiment was to identify the role of ECMF community composition and diversity on the P and N nutrition of European beech saplings at ambient climatic conditions and expected global change conditions.

SUMMARY

In mature beech forests, decreasing precipitation and increasing temperature enhanced soil respiration in summer but decreased net N mineralization. Here, increasing temperatures combined with an increasing summer drought frequency and intensity have the potential to increase soil C loss and decrease C sequestration potential and soil N availability in beech forests on sandy to sandy-loamy soils. In a future warmer and drier climate, sandy soils are more prone to C losses than loam-richer soils. Further results suggest that N deposition might attenuate these effects by enhancing N cycling and curtailing C cycling.

Beech saplings could increase their efficiencies in P uptake and use in response to low soil P availability. This demonstrates that under current environmental conditions, the physiological adaptations of beech saplings to P-limiting conditions are sufficient to circumvent negative P-limitation effects and allow maximum growth even when photosynthetic capacity and plant tissue P concentrations are low. Furthermore, beech saplings are capable of adjusting their N uptake efficiency to soil N availability, thereby stabilizing plant tissue N concentrations.

ECMF symbiosis enhances the P nutrition of beech saplings. Higher ECMF diversity and species richness have been shown to increase the efficiencies of P uptake as well as N use under ambient environmental conditions. By contrast, P use efficiencies were a direct (negative) function of soil P availability and not of ECMF diversity.

Higher temperatures combined with reduced soil water availability deteriorated the physiological adaptations of beech saplings to P limitation, i.e., disturbed the adjustment of plant stoichiometry in response to increased soil N availability. This increased plant tissue N:P ratios and induced P imbalances, which had negative effects on beech sapling productivity and the P and N economy. Drought reduced ECMF colonization and diversity and shifted the ECMF community from a higher portion of fungi with contact (and some long-distance) exploration towards a higher portion of fungi with short- or medium-distance soil exploration. This likely reduces the complementarity of P uptake by ECMF species. As a consequence of decreases in ECMF diversity, P uptake efficiency decreased when soil moisture was limited. Independent of the soil N:P ratios, the N use efficiency in dry soil was then significantly reduced due to a combination of limited P uptake efficiency, i.e., a soil drought-induced relative P deficiency, limited N uptake efficiency, and reduced photosynthetic C fixation.

These results emphasize that decreased summer precipitation and increased temperature in combination with continued high N deposition in the course of global change will result in nutrient imbalances, alter the P and N nutrition of European beech in the next decades, and have the potential to disturb positive mycorrhiza-plant interactions, with negative consequences for the future growth and productivity of *F. sylvatica*. This study highlights the importance of ECMF for the P and N nutrition of European beech and the need to further advance our mechanistic understanding of the interactions between ECMF and root functioning.

ZUSAMMENFASSUNG

Die Produktivität temperater Wälder wird in Zukunft wahrscheinlich zunehmend durch Phosphor(P)-Mangel limitiert werden. Hierauf deuten abnehmende P-Konzentrationen und zunehmende Stickstoff (N):P-Verhältnisse in Blättern und Feinwurzeln sowie Berichte über aktuelle Wachstumsabnahmen, die durch P-Mangel verursacht scheinen, hin. Der prognostizierte Temperaturanstieg, die Verschiebung von saisonalen Niederschlagsmustern hin zu höheren Winter- und niedrigeren Sommerniederschlägen zusammen mit einer gesteigerten Häufigkeit von Dürreereignissen werden, in Kombination mit einer anhaltend hohen N-Deposition, wahrscheinlich zu weiteren Veränderungen in der Nährstoffversorgung temperater Waldbäume in den nächsten Jahrzehnten führen. Dies betrifft vor allem den Kreislauf der beiden wichtigsten, für die Produktivität von Waldbäumen limitierenden Nährstoffe P und N. Es wird angenommen, dass ein zukünftig wärmeres und trockeneres Klima in Kombination mit einer höheren N-Verfügbarkeit im Boden auch biochemische Kreisläufe beeinflusst, was möglicherweise Folgen auf den im Boden gespeicherten Kohlenstoff (C) und auf die Menge des mineralisierten N im Boden hat. Zugleich fehlen Erkenntnisse zu der Reaktion von Ektomykorrhiza-Pilzen (ECMF), welche die bedeutendsten Symbionten für temperate Waldökosysteme darstellen, auf den globalen Wandel.

Um Vorhersagen zur P- und N-Ernährung von temperaten Waldökosystemen unter Bedingungen des globalen Wandels zu verbessern, wurden in der vorliegenden Studie sowohl junge als auch alte Bäume der Rotbuche (*Fagus sylvatica* L.) untersucht. Die Rotbuche ist die dominierende Baumart in den temperaten Wäldern Zentraleuropas und hat in vielen Ländern eine hohe ökonomische Bedeutung.

Um den Effekt von Trockenheit und N-Deposition auf die Kohlenstoff- und Stickstoffdynamik im Boden, hier vor allem Bodenatmungsrate und Netto-Stickstoffmineralisationsrate im Laufe der jahreszeitlichen Veränderung, zu untersuchen, wurde eine Transektstudie entlang eines Niederschlags- und Temperaturgradienten (855 bis 576 mm yr^{-1}; 8.7 bis 9.4°C) mit 11 Buchenaltbeständen, die auf sandigen bis sandig-lehmigen Böden mit einheitlichem geologischen Substrat wachsen, in Norddeutschland durchgeführt.

Um belastbare Vorhersagen über die P-Ernährung der Rotbuche bei verändertem Klima und erhöhter N-Deposition sowie unterschiedlicher P-Versorgung durch den Boden treffen zu können, wurde ein vier-faktorielles Klimakammer-Experiment (3 Bodenfeuchten, 2 Temperaturstufen, 2 N-Versorgungsstufen und 3 P-Versorgungsstufen; insgesamt 36 Behandlungsvarianten) durchgeführt, um

die Einzel- und Interaktionseffekte dieser Faktoren auf morphologische und physiologische Parameter von Jungbuchen zu untersuchen. Ein Fokus dieses Experimentes in präzise kontrollierbaren Klimakammern lag auf den morphologischen und physiologischen Anpassungsreaktionen der Rotbuche in Abhängigkeit von steigendem P-Mangel im Zusammenhang mit einer gesteigerten N-Verfügbarkeit sowie einer zunehmenden Bodenaustrocknung.

Das Klimakammer-Experiment hatte zudem das Ziel, die Bedeutung der Zusammensetzung und Diversität der ECMF-Gemeinschaft auf die P- und N-Ernährung der Jungbuchen unter gegenwärtigen klimatischen Bedingungen und unter Bedingungen des globalen Wandels zu bestimmen.

In Altbuchenbeständen verstärkten eine abnehmende Niederschlagsmenge und eine höhere Temperatur die Bodenatmung im Sommer, aber verringerten die Netto-Stickstoffmineralisation. Folglich haben höhere Temperaturen zusammen mit einer zunehmenden Häufigkeit und Intensität von Sommertrockenheit das Potential den Verlust von im Boden gespeicherten C zu erhöhen sowie die N-Verfügbarkeit in Buchenwäldern auf sandigen bis sandig-lehmigen Böden zu reduzieren. Zudem sind in einem zukünftig wärmeren und trockeneren Klima eher sandige als sandig-lehmige Böden anfällig für einen C-Verlust. Die weiteren Ergebnisse der Transektstudie lassen jedoch vermuten, dass durch die N-Deposition diese Effekte abgemildert werden, da die N-Deposition den N-Kreislauf fördert und den C-Kreislauf einschränkt.

Die Jungbuchen des Klimakammer-Experimentes steigerten ihre P-Aufnahme und P-Nutzungseffizienz als Antwort auf eine geringe P-Verfügbarkeit im Boden. Dies verdeutlicht, dass unter den derzeitigen Umweltbedingungen die physiologischen Anpassungen der Jungbuchen ausreichend sind, um negative Effekte durch P-Mangel zu verhindern und ein maximales Wachstum selbst bei geringer Photosyntheseleistung und geringen P-Gewebekonzentrationen zu gewährleisten. Des Weiteren sind Jungbuchen in der Lage ihre N-Aufnahmeeffizienz auf die N-Verfügbarkeit im Boden anzupassen, wodurch der N-Gehalt im Pflanzengewebe stabilisiert wird.

Die Symbiose mit ECMF fördert die P-Ernährung der Jungbuchen. Unter derzeitigen Umweltverhältnissen steigert eine höhere ECMF-Diversität und ein höherer ECMF-Artenreichtum die Effizienz der P-Aufnahme sowie der N-Nutzung. Die P-Nutzungseffizienten hingegen waren direkt (negativ) abhängig von der P-Verfügbarkeit im Boden und nicht von der ECMF-Diversität.

Höhere Temperaturen in Kombination mit einer reduzierten Bodenwasserverfügbarkeit verschlechterten jedoch die physiologischen Anpassungen der Jungbuchen an P-Mangel, d. h. störten die Anpassung der Pflanzenstöchiometrie als Reaktion auf eine höhere N-Verfügbarkeit. Dies hatte zur

Folge, dass sich das N:P-Verhältnis im Pflanzengewebe erhöhte und ein P-Ungleichgewicht auslöste, was negative Folgen auf die Produktivität und den P- und N-Nährstoffhaushalt der Jungbuchen hatte. Starke Trockenheit verringerte die ECMF-Diversität sowie die ECMF-Besiedlung und veränderte die Zusammensetzung der ECMF-Gemeinschaft, wobei sich der Anteil von ECMF mit Contact- und Long-Distance Explorationstyp zugunsten von ECMF mit Short- oder Medium-Distance-Explorationstyp verschob. Dies verringerte die Komplementarität der P-Aufnahme durch die ECMF-Arten. Durch den trockenheitsbedingten Rückgang der ECMF-Diversität verringerte sich die P-Aufnahmeeffizienz. Bei Trockenheit wurde die N-Nutzungseffizienz durch eine Kombination von einer begrenzten P- und N-Aufnahmeeffizienz und einer verringerten photosynthetischen C-Fixierung signifikant reduziert.

Diese Ergebnisse verdeutlichen, dass verringerte Sommerniederschläge und erhöhte Temperaturen in Kombination mit einer anhaltend hohen N-Deposition im Zuge des globalen Wandels in den nächsten Jahrzehnten zu Nährstoff-Ungleichgewichten und zu Veränderungen der P- und N-Ernährung der Rotbuche führen können. Dies kann die positiven Mykorrhiza-Pflanze-Interaktionen stören, was in Zukunft wiederum negative Auswirkungen auf das Wachstum und die Produktivität von *F. sylvatica* hat. Diese Studie unterstreicht die Bedeutung von ECMF für die P- und N-Ernährung der Rotbuche und die Notwendigkeit, unser mechanistisches Verständnis der Wechselwirkungen zwischen ECMF und Wurzelfunktion weiterzuentwickeln.

CHAPTER 1

GENERAL INTRODUCTION

Phosphorus – importance, availability and limitation

Phosphorus (P) is one of the most essential elements required for plant growth and metabolism but one of the least available ones in the soil (Raghothama, 1999; Elser and Bennett, 2011). P, which is used in energy-dependent metabolic processes such as photosynthesis, respiration, or glycolysis, is a component of nucleic acids, DNA, phospholipids, ATP, and ADP (Vance et al., 2003). Beside the deposition of mineral aerosols, almost all P is originally derived from rock weathering (Yang and Post, 2011; Peñuelas et al., 2013). In contrast to nitrogen (N), P cannot be obtained from the air, and ecosystem losses of P cannot be replenished from other sources (Elser and Bennett, 2011).

Plants can access soil P in the form of inorganic orthophosphate ($H_2PO_4^-$ and $H_2PO_4^{2-}$), which has a very low concentration in soil solution (0.1 to 10 µM; Hinsinger, 2001; Vance et al., 2003) and has its uptake optimum at a pH of 4.5 to 5.0 (Raghothama, 1999; Vance et al., 2003). Up to 70% of total soil P consists of orthophosphate, which occurs in very stable primary minerals, less stable secondary P minerals such as Al-, Ca-, or Fe-phosphates, and P that is mainly occluded by Al and Fe oxides (Shen et al., 2011). Furthermore, P can be present in soil in its organic form bound in mono- and di-phosphate esters such as phospholipids or nucleic acids (Leake and Miles 1996; Plassard and Dell, 2010). During pedogenesis, total soil P concentration decreases, plant-available P-fractions decrease but the portion of occluded P increases (Walker and Syers, 1976; Yang and Post, 2011). Here, inorganic P is mainly occluded by Al and Fe oxides (Herbert and Fownes, 1995). P is immobile in soil as the orthophosphate ions are characterized by slow diffusion (Plassard and Dell, 2010), and P is very fast sorbed to soil particles or is part of complex formation (Vance et al., 2003). Thus, the proportion of plant-available orthophosphate is very low in the soil and might be further impaired in highly acidic soil (Hinsinger, 2001), so that P is one of the most limiting nutrients for plant growth (Vance et al., 2003).

The net primary production of temperate forests is primarily limited by nitrogen (N) availability (LeBauer and Treseder, 2008). But due to increased N deposition from fossil fuel combustion and intensive agriculture, N availability has rapidly increased in most areas of the globe in recent decades (Galloway et al., 2008). These increases in N availability have the potential to change nutrient stoichiometry, i.e., cause rises in the N:P ratio of ecosystems (Peñuelas et al., 2013; 2020). As a result, the degree of relative P deficiency increases and potentially shifts temperate forests from N limitation to P limitation (Talkner et al., 2015; Sardans et al., 2016; Hedwall et al., 2017). According to the co-limitation hypothesis, the efficiency of N acquisition and use depends on P availability and vice versa (Ågren et al., 2012). According to this theory, a P deficiency increases the efficiency of P uptake while decreasing the efficiency of N uptake. Yet there is currently no mechanistic understanding of whether (at all) plants achieve simultaneous co-limitation by N and P via modifications in root uptake kinetics (Rothstein et al., 2000) or via changes in root morphology and biomass partitioning (Zhang et al., 2018) or both. However, the undersupply of plants with P is already indicated by decreasing P concentrations and increasing N:P ratios in leaves and fine roots, as well as by reports on recent growth reductions due to P limitation for various forest regions of central and southern Europe and elsewhere (Elser et al., 2007; Peñuelas et al., 2012).

Plant responses to P limitation

In response to low nutrient availability, plants can enhance nutrient uptake and acquisition as well as the conservation of nutrient use, thereby showing adaptations on the morphological, physiological, biochemical, and molecular level (Raghothama et al., 1999; Vance et al., 2003). One strategy to be able to cope with low P availability and P limitation is to conserve the use of P (Vance et al., 2003). This comprises a more efficient internal P-cycling, P-storage, P-reuse, P- mobilization, and P-translocation (Netzer et al., 2017).

Increased conservation of P use can be achieved, for example, by (re)mobilization of Pi from the vacuole to the cytoplasm (Veneklaas et al., 2012), or from P stored in stem tissues and roots (Netzer et al., 2017), from senescing leaves (Hofman et al., 2016; Netzer et al., 2017), or by reductions of no longer needed RNA and replacement of phospholipids by sulfolipids and galactolipids (Lambers et al., 2012). A more efficient increase in plant growth per unit P, i.e., a higher P use efficiency, was found for poplar under P limitation and indicated an important link between plant productivity, photosynthesis, and P use efficiency (Gan et al., 2005). Furthermore, a more efficient P use with respect to photosynthesis was found under P limitation or low P availability for the Mediterranean tree species *Quercus calliprinos* (Dirks et al., 2019), for tropical montane forests in Borneo (Hidaka and Kitayama, 2009), and for Proteaceae species in south-western Australia (Lambers et al., 2012). On the level of forest ecosystems, a tight P recycling system at P-poor sites might be of high importance (Lang et al., 2016).

Another strategy to cope with low P availability and/or P limitation is to enhance the acquisition and uptake of P (Vance et al., 2003). For this, plants can increase the expression of high affinity P_i transporters of the *Pht1* gene family, resulting in an altered membrane structure (Smith et al., 2000; Kavka and Polle, 2016). Furthermore, plants might respond by shifting in C allocation to compensate for low P availability. Here, the allocation of carbohydrates and nutrients to the root system is increased as a response to limiting soil resource availability (optimal partitioning theory; Bloom, 1985). According to this theory, relative C allocation to roots and the root:shoot ratio and root proliferation are predicted to increase with a decrease in P availability. Additionally, trees can change their root morphology and functioning through a higher intensity in root branching or the production of short and thin, fibrous roots with a high frequency of passage cells for the absorption of nutrients and water in response to resource-rich soil spots in P-poor soil (Vance et al., 2003; Zadworny and Eissenstat, 2011; Tückmantel et al., 2017).

Beside this, plants can enhance P acquisition by exuding a complex mixture of low molecular weight carbohydrates, amino acids, organic acids, secondary metabolites like phenolics, or enzymes like phosphatases (Hinsinger, 2001). Under P- or N-limiting conditions, plants increase root exudation (Phillips et al., 2011; Yin et al., 2014). This enhanced rhizodeposition is directed to soil spots with a high soil organic matter (SOM) content, where positive priming effects are likely (Tückmantel et al., 2017). Due to enhanced carboxylate exudation, soil P mobilization via ligand exchange, dissolution, and occupation of P sorption sites can be improved (Ding et al., 2021). Some carboxylates. i.e., malate, citrate, and oxalate, and phenolic acids are discussed as being more efficient for P mobilization than other compounds (Hinsinger, 2001; Ding et al., 2021). Due to the enhanced acquisition and solubilization in the rhizosphere, combined with the fast uptake of P and the slow diffusion of orthophosphate, a P depletion zone around the root surface arises (Mikkelsen, 2005).

Furthermore, mycorrhizal symbiosis plays a major role in the P acquisition (Ferlian et al., 2018; Raven et al., 2018) and (to some extent) in the N acquisition of plants (Gobert and Plassard, 2008; Pena and Polle, 2014). Here, a more efficient acquisition and uptake of P can be achieved by the ectomycorrhizal fungi (ECMF) symbiosis, which greatly increases the absorptive surface area through the production of small-diameter hyphae that contain many high-affinity transporters and can access smaller soil pores than fine roots can (Richardson et al., 2009; Cairney, 2011). ECMF species increase the solubility of mineral and occluded P through enhanced exudation of protons or organic acids (Hinsinger, 2001). Furthermore, ECMF species support the mineralization of organic P through the release of phospholytic enzymes (Neumann and Römheld, 2007). It is expected that more diverse ECMF communities can access a greater diversity of P pools and organic matter (Baxter and Dighton, 2001; Jones et al., 2010; Velmala et al., 2014), which results in an increase in P availability for plants. Beside this, the composition of the ECMF community might also be an important factor for functional diversity and, thus, for nutrient acquisition (Pena and Polle, 2014). However, there is only little knowledge about the mechanisms that control these strategies. It has to be noted that these strategies for a more efficient P acquisition, uptake, and use may also change N and C cycling due to their effects on SOM decomposition (Ding et al., 2021).

Climate change – from global to regional scale

Global scale

Human activities like the burning of fossil fuels such as coal, oil, and gas have increased atmospheric greenhouse gas concentrations, which contribute considerably to the observed warming of the atmosphere, oceans, and land (Al-Ghussain, 2019; Intergovernmental Panel on Climate Change (ICCP), 2021). According to the most recent ICCP report, the last few decades have become increasingly warmer, reaching levels of warming unprecedented in more than 2000 years. Global surface temperature increased by 1.09°C between 2011 and 2020 compared to 1850 to 1900, while global average precipitation over land increased (IPCC, 2021; **Figure 1.1**; **Figure 1.2**). This human-induced climate change already causes a higher frequency and intensity of weather and climate extremes, such as heatwaves, drought events, and heavy precipitation (ICCP, 2021). Models that simulate future precipitation patterns predict severe and widespread droughts in the next 20 to 80 years, especially over land areas, due to decreases in precipitation and increases in evapotranspiration (Dai, 2012). Depending on the emission scenarios considered, global surface temperature is expected to increase in the range of 1.2°C to 3.0°C in the mid-term (time period from 2041 to 2060) and in the range of 1.0°C to 5.7°C in the long term (time period from 2081 to 2100) compared to the average global surface temperature of the period 1850-1900 (IPCC, 2021).

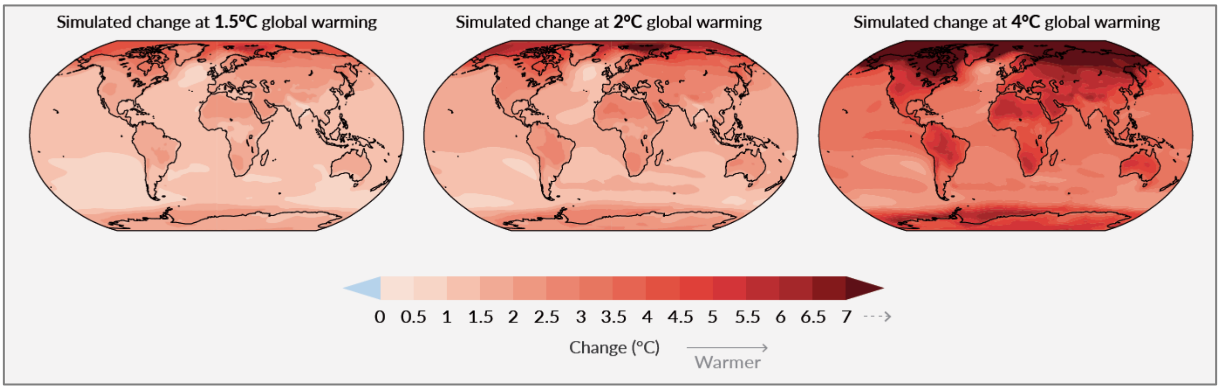


Figure 1.1 Simulated annual mean temperature change (°C) relative to the period from 1850 to 1900 at different global warming levels (modified; IPCC, 2021).

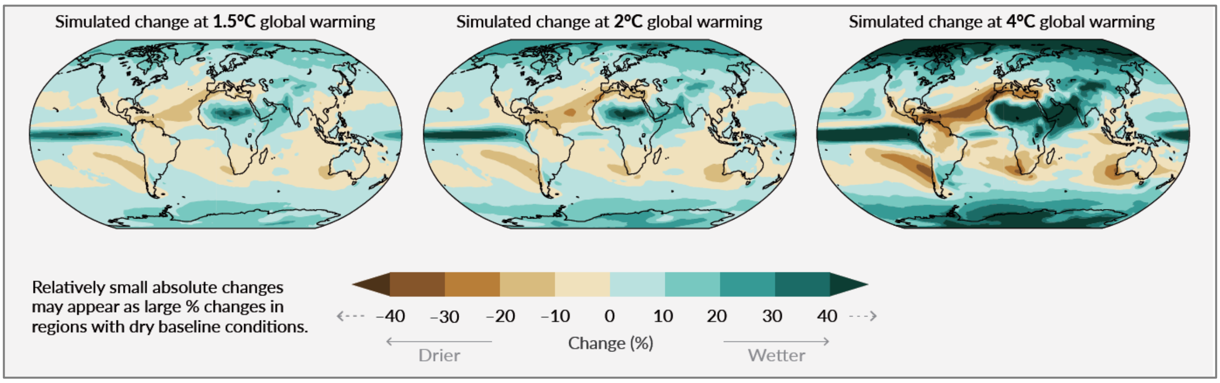


Figure 1.2 Simulated annual mean precipitation changes (%) relative to the period from 1850 to 1900 at different global warming levels (modified; IPCC, 2021).

Central Europe and Germany

In Central Europe, higher mean annual temperatures and a shift in seasonal precipitation patterns towards higher winter and lower summer precipitation, with a higher risk and frequency of drought events, are the biologically most relevant consequences of climate change (Christensen et al., 2007). From 1881 to 2014, measurements at climate stations in Germany show that mean annual temperatures (MAT) increased by 1.3°C, mean summer temperatures by 1.2°C, and mean winter temperatures by 1.1°C. These results indicate that warming is more pronounced in Germany than on the earth on average (Kaspar and Mächel, 2017). This is in accordance with the results of several measurements by the German Weather Service (**Figure 1.3**).

Additionally, in the same period (1881–2014), mean annual precipitation increased by 10.2% compared to mean precipitation from 1961 to 1990, which was mainly caused by the increase in winter precipitation by 26%. At the same time, summer precipitation decreased by 0.6% (Kaspar and Mächel, 2017). Future climate projections predict further temperature increases for Germany (for example, climate models by the German Weather Service; see **Figure 1.4**). Here, the number of heat waves could increase by up to five events per year in northern Germany and by up to 30 events per year in southern Germany until the end of the 21st century (Deutschländer and Mächel, 2017). However, the results of climate model-based analyses of future precipitation patterns in Germany are rather uncertain, with some significant regional differences (Kunz et al., 2017). The higher amount of water vapor will likely result in a higher risk for severe thunderstorms and hail (Kunz et al., 2017).

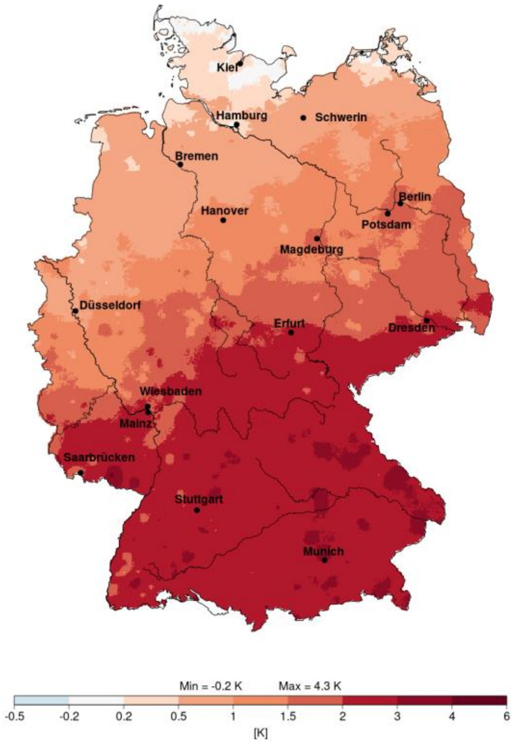


Figure 1.3 Anomaly of mean summer air temperature in 2015 with respect to mean annual summer air temperature in the period from 1971 to 2000 (modified; German Weather Service (DWD) - German Climate Atlas, 2022). Darker red tones indicate higher (positive) anomaly of mean annual summer temperature.

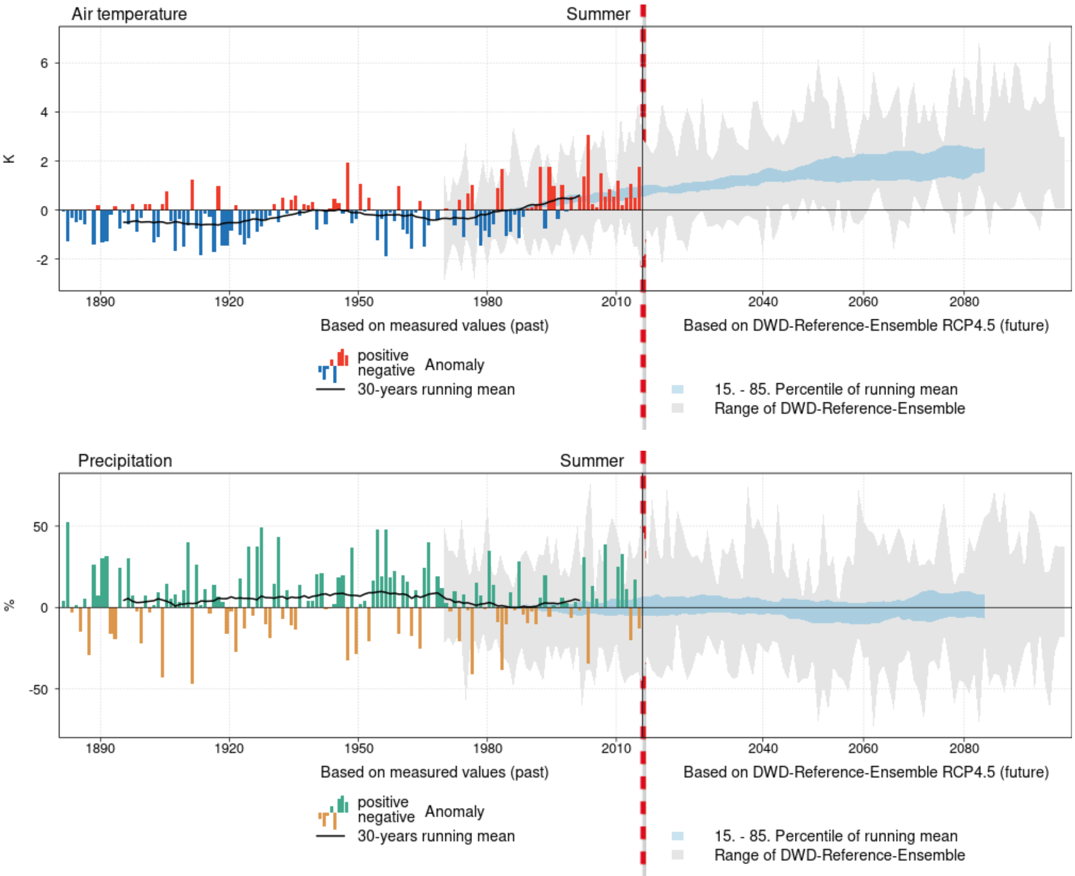


Figure 1.4 Time series plots of anomaly of mean annual summer air temperature with respect to mean annual summer temperature in the period from 1971 to 2000, and mean annual summer precipitation with respect to mean annual summer precipitation in the period from 1971 to 2000 (modified; German Weather Service (DWD) - German Climate Atlas, 2022). Shown are measured values and predicted values for the intermediate emission scenario RCP 4.5 (representative concentration pathway; Meinshausen et al., 2011).

The effect of global change on phosphorus and nitrogen nutrition of temperate forests

Continued high atmospheric N deposition in combination with decreased summer precipitation and increased temperature in the course of global change is likely to alter plant-soil interactions, with consequences for the cycling of the two main limiting nutrients, P and N, as well as for key components of the C cycle.

Increasing temperature

As warming significantly affects the activity of soil microbes (Jansson and Hofmockel, 2020), higher temperatures have been found to speed up soil respiration (Borken et al., 2002; Søe and Buchmann, 2005; Lu et al., 2013) and stimulate N cycling, which might result in higher N availability due to increased net N mineralization (Rustad et al., 2001; Lu et al., 2013). As higher temperatures can enhance mineralization rates (Withers and Jarvie, 2008), warming should also positively affect soil P mineralization. Higher temperatures increase the soil microorganism enzyme activity and, thus, should enhance the mineralization of SOM, especially in colder climates (Rustad et al., 2001; Lu et al., 2013), which should result in increased nutrient uptake. This holds true as long as the availability of soil water and SOM is sufficient, i.e., there is a trade-off between water and temperature (Zuccarini et al., 2020). Furthermore, higher temperatures should affect ECMF activity and the composition of the ECMF community (Fernandez et al., 2017). Yuan et al. (2011) could show decreasing P concentrations and increasing N:P ratios in fine roots with increasing temperatures.

Reduced precipitation and soil moisture

Drought has been shown to slow down C, P, and N mineralization (Sardans and Peñuelas, 2004). Furthermore, decreasing soil moisture reduces the diffusion of N and P in the rhizosphere (Gessler et al., 2005; Kreuzwieser and Gessler, 2010) and changes nutrient uptake capacity (Gessler et al., 2004). Hence, drought impairs plant nutrition by affecting both soil nutrient availability and the kinetics of the uptake system of the mycorrhizal tree roots (Gessler et al., 2005; Kreuzwieser and Gessler, 2010). Additionally, drought can change plants' P and N uptake capacities, causing imbalances in plant tissue C/N/P stoichiometry and further nutritional limitations with negative effects on plant growth (Sardans and Peñuelas, 2005; Dannenmann et al., 2016; Dirks et al., 2019).

Drought can also reduce fine root biomass due to increased root mortality and decreased C allocation to the root system to produce new roots (Meier and Leuschner, 2008), which reduces the ability of the root system to respond via morphological changes to low soil moisture availability.

Furthermore, drought may disturb the plant-soil interaction due to a negative effect on the photosynthetic capacity and a reduction in the supply of photosynthates to the root symbionts (Ruehr et al., 2009; Courty et al., 2010). This, in turn, causes the premature death of C-demanding (e.g., long-distance ECMF; Pena et al., 2010) or drought-sensitive (Shi et al., 2002; Leberecht et al., 2016) ECMF species, which results in changes in ECMF diversity and ECMF community composition (Pena et al., 2010). Drought has been found to reduce the growth, biomass, and activity of ECMF hyphae (Ekblad et al., 2013; Teste et al., 2016). Furthermore, the reduced allocation of photosynthates to belowground structures results in a decrease in soil respiration (Schindelbacher et al., 2012). However, Preece and Peñuelas (2016) could show that low-moderate drought increases root exudation, while the response was more variable under extreme water stress.

Increased soil N availability

Increased soil N availability as a result of N deposition has been found to either increase or decrease decomposition rates, and thus nutrient mineralization (Hyvönen et al., 2007; Vitousek et al., 2010; Lladó et al., 2017); to decrease ECMF diversity and abundance (Kjøller et al., 2012; de Witte et al., 2017); to promote plant productivity; to decrease root:shoot ratio (Lambers et al., 2008) and to decrease soil respiration (Janssens et al., 2010). Increasing acidification due to increased N deposition has the potential to reduce the activity of soil microorganisms and the functioning of SOM degrading enzymes (Janssens et al., 2010; Cheng et al., 2019; Zuccarini et al., 2021) and to further reduce soil P availability. However, N deposition often also reduces the standing fine root biomass of trees, while root turnover increases (e.g., Leuschner and Hertel, 2003; Majdi and Andersson, 2005). Higher N availability induces nutritional imbalances due to a large-scale undersupply of P in various temperate tree species (Prietzel et al., 2008; Talkner et al., 2015), likely caused by atmospheric N deposition from anthropogenic sources (Duquesnay et al., 2000; Braun et al., 2010; Sardans et al., 2016).

The consequences of global change on the growth and productivity of European beech

European beech (*Fagus sylvatica* L.; hereafter referred to as beech) is the dominant native tree species of Central Europe's temperate forest biome, with high economic importance in many countries (Leuschner and Ellenberg, 2017). Beech grows on a wide range of soils, from poor and highly acidic to neutral, and soil moisture conditions, and would naturally dominate forest in Central Europe (Ellenberg and Leuschner 2010). At present, European beech covers up to 16% of the cultivated wood ground area in Germany (BMEL, 2021). The temperate climate zone contains the majority of the beech distribution range, which extends from southern Italy to south Norway and from northern Spain to eastern Romania (Bohn and Gollub, 2007; Leuschner, 2020).

Recent studies have shown that beech is relatively drought-sensitive (Knutzen et al., 2017; Leuschner and Meier, 2018; Leuschner, 2020; Schuldt et al., 2020). N deposition might enhance the species' sensitivity to water deficits and elevated temperatures (Hess et al., 2018; Mausolf et al., 2020). Jump et al. (2006) observed a decline in beech growth in northeast Spain in response to increasing temperatures and a simultaneously constant amount of precipitation. These results suggest a retreat of beech in its southern distribution range. The results by Dulamsuren et al. (2017) demonstrate that a warmer and drier climate causes a reduction in the productivity of beech in lowland and lower montane regions of Central Europe in the center of beech's distribution range. In their comparative dendrochronological study in mature beech forest along a precipitation gradient in northwest Germany, Knutzen et al. (2017) found that the following precipitation amounts are at least needed to secure vital beech growth: 660 mm of mean annual precipitation, 350 mm of growing season precipitation (April to September), and 200 mm (Zimmermann et al., 2015: 190 mm) of summer precipitation (June to August). The results of Stolz et al. (2021) indicate that beech has a relatively low resistance to extreme drought and shows the highest sensitivity to summer droughts in early summer. Thus, the expected warmer and drier climate likely results in an increasing decline in productivity or even an increase in mortality rates, which was already shown for beech in the context of the successive 2018 to 2019 hot droughts in central Germany (Obladen et al., 2021).

Study objectives

In Central Europe, higher mean annual temperatures and a shift in seasonal precipitation patterns toward higher winter and lower summer precipitation with a higher frequency of drought events are the biologically most relevant consequences of climate change (IPCC, 2021), with substantial effects on temperate forest ecosystems (Jump et al., 2006; Zang et al., 2014; Martin et al., 2015; Leuschner, 2020). Combined with continued high atmospheric N deposition, these changes likely will alter soil nutrient dynamics, especially the cycling of the two main limiting nutrients, P and N. Beside N and P dynamics, climate change might also affect various key components of the C cycle, including soil respiration, which is the second largest C flux between the atmosphere and terrestrial ecosystems (Davidson and Janssens, 2006; Zhou et al., 2016).

So far, knowledge about the effect of a warmer and drier climate in combination with continued elevated soil N availability on the P and N nutrition of temperate forest species like *F. sylvatica* L. is scarce. Furthermore, it is not well known how global change affects ectomycorrhizal fungi (ECMF), which are the most important fungal symbionts for temperate forest ecosystems, and mycorrhiza-plant interactions (Bennett and Classen, 2020).

Major study aims were to

- investigate the morphological and physiological adaptations of beech to decreasing plant-available P in the course of increasing N fertilization, i.e., an increasing P limitation,
- evaluate the effect of increasing climatic drought, i.e., decreasing precipitation and increasing temperatures, and N deposition on soil C and N dynamics as well as on the P and N nutrition of European beech,
- identify the impact of ECMF community composition and diversity on the P and N nutrition of European beech at ambient climatic conditions and expected global change conditions.

The results of this study are expected to identify efficient P and N uptake and use strategies in European beech and to enable sound predictions about the P and N nutrition and, thus, growth and productivity of European beech under the conditions of an altered climate and elevated N deposition. Furthermore, the findings of this study will contribute to our understanding of the role of ECMF symbiosis in the P and N nutrition of European beech, as well as how the mycorrhiza-plant interaction will respond to the expected global change conditions.

Methodical approach

In order to improve predictions on the P and N nutrition of temperate forest ecosystems under global change, a combined observational and experimental study with mature trees and saplings of European beech (*Fagus sylvatica* L.) was conducted, which was subdivided as follows:

- A transect study with eleven mature beech stands growing on sandy and sandy-loamy soil (closed stands, 85-140 years old) along a precipitation (576–855 mm yr^{-1}) and temperature (8.7–9.4°C) gradient in eastern Lower Saxony/Saxony Anhalt (**CHAPTER 2**).

- A four-factorial climate chamber experiment with two-year-old European beech saplings that originated from a mature beech forest in northwest Germany, simulating the expected global change conditions, i.e., an increase in temperature and N deposition and a decrease in soil moisture and P availability (**CHAPTER 3**, **CHAPTER 4**).

I.) Transect study

Study area, climate, forest stands, and experimental design

The study was conducted between June 2014 and August 2015 in eleven mature beech forests in the Pleistocene lowlands of north-west Germany along a precipitation and temperature gradient. Stands were located at five study sites in the federal states Lower Saxony (Lüneburg Heath region) and Saxony-Anhalt (Altmark) in the forest districts Sellhorn, Unterlüß, Göhrde, Klötze, and Calvörde along a 130-km long north-west to south-east transect. The transect represents the transition from an oceanic to a subcontinental climate (Hertel et al., 2013; Müller-Haubold et al., 2013). Mean annual precipitation (MAP) decreases from 855 to 576 mm yr^{-1} along the gradient, while mean annual temperature (MAT) increases slightly from 8.7 to 9.4 °C from north-west to south-east (**Figure 1.5**).

All stands are characterized by (i) the dominance of beech, (ii) mature age (85–140 years), and (iii) a closed canopy without larger gaps. All stands were fenced, originate from natural regeneration, and are not affected by recent forest management activities. Modeled mean annual N deposition for the time period 2013–2015 ranges from 15 to 19 kg ha^{-1} yr^{-1} (Schaap et al., 2018). The studied mature beech forests grow on nutrient-poor, highly acidic, sandy soils. Soils have a variable silt content (dystric or umbric Arenosols or Podzols), a relatively low water storage capacity (WSC), and are covered by thick (4–9 cm) organic layers. Soils had a pH(H_2O) range of 4.1–4.4, a mean C:N of 25.4 mol mol^{-1} in the organic layer and 26.1 mol mol^{-1} in the mineral soil (0-10 cm) layer, a mean total soil N (N_{tot}) content of 1.13 mmol g^{-1} in the organic layer and of 0.13 mmol g^{-1} in the mineral soil layer, a mean total soil P (P_{tot}) content of 21.1 µmol g^{-1} in the organic layer and of 3.7 µmol g^{-1} in the mineral soil layer, a mean plant-available P (P_a) content of 0.11 µmol g^{-1} in the organic layer and of 0.02 µmol g^{-1} in the mineral soil layer, a mean base saturation of 9.8, and 4.7–53.5% fine-grained (< 63 µm) soil particles in the mineral topsoil (Hertel et al., 2013; Müller-Haubold et al., 2013; **CHAPTER 2**).

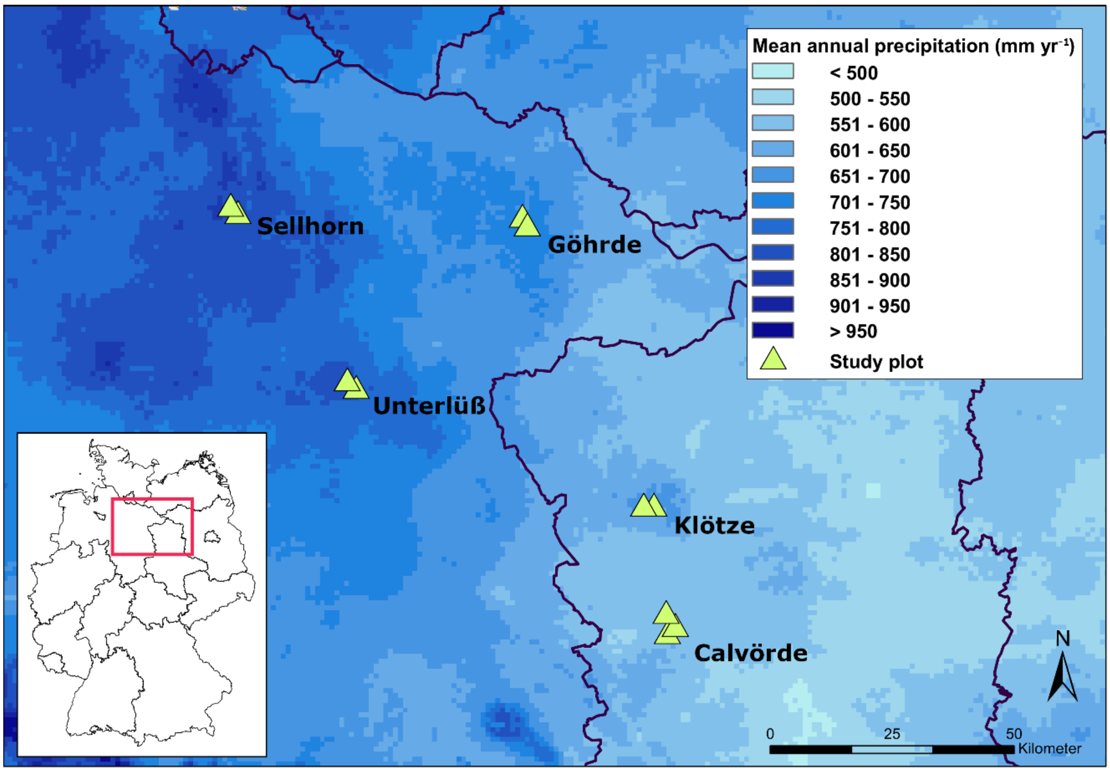


Figure 1.5 Study area with the location of the five study sites along the precipitation gradient from north-west to south-east. Triangles visualize the location of the eleven study plots. Climate data refer to the period 1981–2010 and were obtained from the Climate Data Center of the German Weather Service (Deutscher Wetterdienst, DWD). The differing amounts of mean annual precipitation are visualized by graded shades of blue.

To account for considerable edaphic heterogeneity, at each site two plots of 30 x 30 m differing in soil texture were established, i.e., sandy-loamy and sandy (**Figure 1.6**). One additional plot with sandy soil texture was installed in the forest district Calvörde.

Figure 1.6 Mature beech forest stand in Göhrde growing on sandy-loamy soil.

Measured parameters

The aim of this transect study was to investigate the effect of climatic drought and N deposition on soil C and N dynamics for beech stands growing on differing soil textures during their seasonal change. For soil textures, a comparison between sandy and sandy-loams conditions was performed. Parameters were measured for a total of five seasons, i.e., three seasons in 2014 (June/July to August; August to October; October to December) and two seasons in 2015 (April/May to June; July to August). The following parameters were measured:

A) Parameters characterizing N cycling and supply:

- net N mineralization rate
- net ammonification rate
- net nitrification rate
- soil free amino acid concentration

B) Parameters characterizing C dynamics:

- soil respiration rate
- release of rhizodeposits by roots

To account for soil chemistry and nutrient availability, the results of soil C, N, and P concentrations were used in further analyses. These soil samples were collected at the end of April and in the middle of June 2013 (Eder, 2014).

II.) Climate chamber experiment

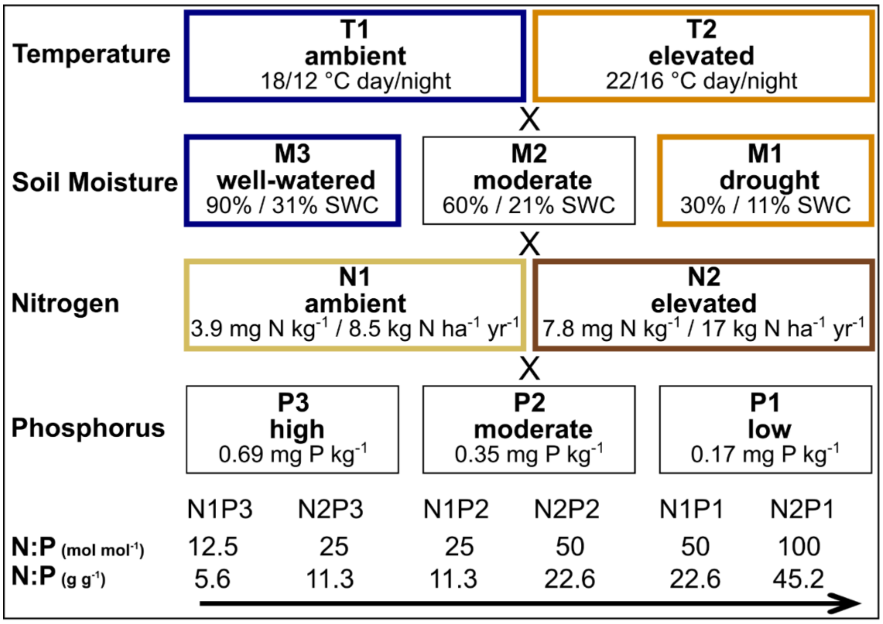
Plant material

This experiment was conducted with 288 European beech saplings that were collected from a mature beech forest in the lowlands of northwest Germany (Unterlüß; close to the study plots that were used in the field study). The saplings were growing on highly acidic (pH(H_2O) of 4.3) nutrient-poor sandy soil with a relatively low water storage capacity (79 mm 120 cm^{-1} at a mean annual precipitation of 816 mm and mean annual temperature of 8.7°C; Hertel et al., 2013)). At the forest district in Unterlüß, on average, 75% of adult trees were colonized by ECMF (Hertel et al., 2013). The ECMF communities were comparably species-poor. The average number of ECMF species was nine on adult trees (Zavišić et al., 2016) and three to six on beech saplings (Leberecht et al., 2016). In May 2013, circa 500 beech seedlings of similar size were selected that originated from the tree masting in 2012. Seedlings were carefully excavated and transported to the botanical garden in Goettingen. The adhering soil was removed, and the beech seedlings and their associated indigenous ECMF communities cultivated in a sterilized 2:1 sand:peat mixture outdoors for 1 year. In May 2014, saplings were carefully removed from their substrate, rinsed with bi-distilled water, and planted in 1.5-liter pots filled with sieved and homogenized mineral topsoil. The topsoil originated from the site of origin, which contained the indigenous ECMF. This procedure aimed to maximize the ECMF colonization rate of beech saplings. Pots were randomly arranged in a controlled growth chamber. Experimental treatments started in July 2014.

Experimental set-up

To simulate a gradient from current environmental conditions to the expected future global change conditions (elevated temperature and N deposition as well as reduced soil moisture and P availability), a full-factorial experiment with four factors was established in July 2014. Two temperature levels, three soil moisture levels, two N supply levels, and three P supply levels were chosen as factors. Each single factor and their interactions were tested. This resulted in 36 treatments in total (factors and combinations described in **Table 1.1**). Each treatment was replicated eight times.

Table 1.1 Study design of the full-factorial climate chamber experiment with two temperature (T), three soil moisture (M), two nitrogen (N) supply and three phosphorus (P) supply levels (*n* = 36 treatments in total; *cf.* Köhler et al., 2021). A gradient from current environmental conditions to the expected future global change conditions (elevated temperature and N deposition and reduced soil moisture and P availability) was simulated. Temperature: T1, ambient; T2, elevated. Soil moisture: M1, drought; M2, moderately moist; M3, well-watered. N supply: N1, ambient; N2, elevated. P supply: P1, low; P2, moderate; P3, high. SWC = soil water content.



Temperature	**T1** **ambient** 18/12 °C day/night	**T2** **elevated** 22/16 °C day/night	
	X		
Soil Moisture	**M3** **well-watered** 90% / 31% SWC	**M2** **moderate** 60% / 21% SWC	**M1** **drought** 30% / 11% SWC
	X		
Nitrogen	**N1** **ambient** 3.9 mg N kg^{-1} / 8.5 kg N ha^{-1} yr^{-1}	**N2** **elevated** 7.8 mg N kg^{-1} / 17 kg N ha^{-1} yr^{-1}	
	X		
Phosphorus	**P3** **high** 0.69 mg P kg^{-1}	**P2** **moderate** 0.35 mg P kg^{-1}	**P1** **low** 0.17 mg P kg^{-1}

	N1P3	N2P3	N1P2	N2P2	N1P1	N2P1
N:P (mol mol^{-1})	12.5	25	25	50	50	100
N:P (g g^{-1})	5.6	11.3	11.3	22.6	22.6	45.2

Beech saplings were assigned to their experimental treatment at random, were set up in a randomized design in two highly controllable walk-in climate chambers, and were re-randomized biweekly for two growing seasons (**Figure 1.7**). Temperatures were reduced to 10°C by day and 8°C by night during the non-growing season (November 2014 to March 2015), and day length was reduced from 14 to 7.5 hours. Drought and fertilization treatments were paused, and the photosynthetic photon flux density was reduced from 190 to 30 µmol photons m^{-2} s^{-1}. Treatment conditions were reestablished in April 2015 and maintained until the plants were harvested in September 2015. Thus, two subsequent growing seasons with altered climatic conditions for a total of 456 days were simulated.

Figure 1.7 European beech saplings in climate chamber experiment.

^{33}P labeling experiment

To measure the instantaneous P uptake, a radioisotope labeling experiment with ^{33}P was performed. Thirty minutes after the even application of 20 ml of $H_3{}^{33}PO_4$ tracer solution to three soil depths per pot (**Figure 1.8**), saplings were immediately excavated, separated into plant compartments, and inserted into liquid N_2 to halt any further ^{33}P transport. The radioactivity in the plant compartments was determined using a low-activity liquid scintillation analyzer, and the relative P uptake was estimated from the ^{33}P concentration in the biomass.

The labeling experiment was conducted in the Laboratory for Radio-Isotopes (LARI) at the University of Goettingen (in cooperation with Prof. A. Polle, Forest Botany and Tree Physiology) at the beginning of September 2015 (i.e., 2 weeks before harvest). The ^{33}P uptake capacity was measured for the following eight selected treatment combinations: elevated temperature, well-watered and drought treatments, ambient and elevated N deposition, and high and low P availability (three replicates per treatment; $n = 24$ saplings in total).

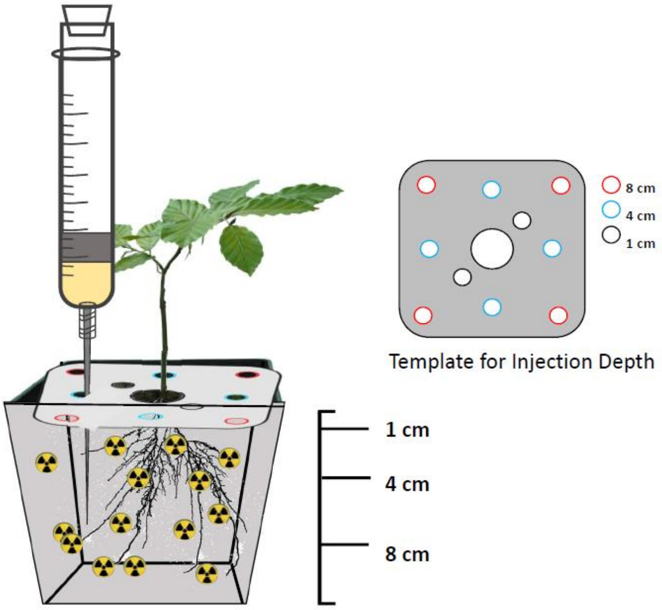


Figure 1.8 Schematic illustration of the ^{33}P tracer solution application to three soil depths per pot.

Ectomycorrhizal fungal colonization

In mid-September 2015, the remaining 264 beech saplings not subjected to the ^{33}P labeling experiment were also fractionated. For each sapling, a representative fine root section was transferred to a 5% glycerol solution and kept frozen at 18°C to later analyze the ECMF colonization with a dissecting microscope ($n = 8$ replicates per treatment). All root tips were counted, classified after Agerer (1987–2012; *cf*. Pena et al., 2010), and their respective ECMF morphotypes characterized.

A total of 10–20 tips of each distinct ECMF morphotype were collected from different samples, stored at 20°C, and used for molecular identification of fungal species by ribosomal DNA internal transcribed spacer (ITS) sequencing (*cf.* Pena et al., 2010; Zavišić et al., 2016).

Measured parameters

The aim of the climate chamber experiment was to investigate the consequences of different global change scenarios on the P and N economies of European beech saplings as well as on the colonization and composition of ECMF communities. Furthermore, this experiment aimed to explore the consequences of these likely changes in ECMF communities on beech saplings' root functioning. The following parameters were measured to determine this:

A) Parameters characterizing P/N economy:

- P/N uptake efficiency
- P/N use efficiency
- photosynthetic P/N use efficiency
- instantaneous ^{33}P uptake efficiency

B) Physiological and morphological parameters:

- Photosynthetic capacity of the uppermost leaves at light saturation (390 µmol CO_2 mol^{-1} air)
- P, N, C concentrations and N:P ratios and C:N ratios in plant compartments
- specific root area, specific root length

C) Parameters characterizing productivity and allocation patterns:

- total plant biomass and plant compartment specific biomass
- root:shoot ratio

D) Parameters characterizing ECMF and symbiosis:

- root vitality
- molecular ECMF species identification
- ECMF colonization, ECMF species richness, ECMF diversity

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CHAPTER 2

Soil respiration and net N mineralization in mature European beech forests across a precipitation gradient

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Summary

Increasing summer droughts under global climate change and increasing nitrogen (N) deposition are thought to influence biochemical cycles with potential consequences for the amount of carbon (C) sequestered in forest soils and the quantity of mineralized N; yet key biogeochemical processes determining the sink strength of mature forests under drought remain poorly quantified. In our study, we examined *in situ* soil respiration rate (closed-path gas exchange analysis) and net N mineralization rate (net N_{min}) in 11 mature European beech (*Fagus sylvatica* L.) forests along a precipitation gradient (855 to 576 mm yr^{-1}; located in northwest Germany) on sandy to loamy glacial substrates in northern Germany. We aimed at testing the hypotheses that (H1) decreasing mean annual precipitation (MAP) and increasing mean annual temperature (MAT), i.e., increasing climatic aridity, are impairing both soil respiration rate and net N_{min}, with (H2) a stronger decrease taking place in forest stands on sandy soil than on loam-richer soil. Furthermore, we hypothesized that N deposition speeds up net N_{min} but reduces soil respiration rate (H3). Surprisingly, we found a significant negative relationship between soil respiration rate and MAP in the summer season, which contradicts H1 and seems to be driven by the positive effect of increasing temperature. While this relationship in principle occurred on both soil types, it had a steeper slope in stands on sandy soil, which contradicts H2. Season had a greater influence on soil respiration rate, soil amino acid N (AA-N), and net N_{min} than soil texture. Soil texture only influenced soil respiration rate, with beech stands growing on sandy soil showing higher CO_2 loss. In the mid-growing season (July to August) 2015, stands on sandy soil had lower and even negative net N_{min}, a higher soil respiration rate, and a higher soil AA-N content than stands on sandy-loamy soil. In the late growing season (end of August to October) 2014, net N_{min} was reduced by higher temperatures in forest stands on sandy-loamy soil but increased by N deposition on sandy stands. Regardless of soil texture, N deposition reduced soil respiration rate in the middle of the growing season in 2015, partially confirming H3. Our results suggest that increasing temperatures combined with increasing summer drought frequency and severity have the potential to increase C loss and, thus, decrease C sequestration potential in beech forests on sandy to sandy-loamy soils, while net N_{min} likely will be reduced in a future warmer and drier climate, suggesting negative effects on beech growth and productivity in the future. However, N deposition might attenuate these effects by enhancing net N_{min} and curtailing soil respiration rates.

Keywords: CO_2 loss, drought, *Fagus sylvatica*, global change, nitrogen (N) deposition, sandy soil, soil amino acids

Introduction

In Central Europe, higher mean annual temperatures and a shift in seasonal precipitation patterns towards higher winter and lower summer precipitation with a higher frequency of drought events are the biologically most relevant consequences of climate change (IPCC, 2021), with substantial effects on temperate forest ecosystems (Jump et al., 2006; Zang et al., 2014; Martin et al., 2015; Leuschner, 2020). Combined with continued high atmospheric nitrogen (N) deposition, these changes likely will alter soil nutrient dynamics, especially the cycling of the two main limiting nutrients, N and phosphorus (P).

Decreasing soil moisture in summer with rising temperatures and extending droughts negatively affects the nitrogen supply of temperate tree species, largely because of a reduced nitrogen uptake capacity and nitrogen uptake efficiency of mycorrhizal roots (Gessler et al., 2004; Köhler et al., 2021). In addition, decreasing soil moisture reduces the diffusion of N and P in the rhizosphere (Gessler et al., 2005; Kreuzwieser and Gessler, 2010). Thus, drought impairs plant nutrition by affecting both soil nutrient availability and the kinetics of the uptake system of the mycorrhizal tree roots (Gessler et al., 2005; Nahm et al., 2006; Kreuzwieser and Gessler, 2010). Drought also reduces the ability of the root system to respond via morphological changes, as it may reduce fine root biomass due to increased root mortality and decreased C allocation to the root system to produce new roots (Meier and Leuschner, 2008). On the other hand, higher temperatures might increase inorganic N availability by speeding up processes like litter decomposition and net N mineralization rate (net N_{min}), which is the net sum of gross N mineralization and soil microbial N uptake, resulting in N immobilization (Rustad et al., 2001; Schimel and Bennet, 2004; Lu et al., 2013), among others, due to enhanced activity of soil microorganisms, given that soil moisture is favorable and sufficient labile soil organic matter (SOM) is available (Zuccarini et al., 2020).

Soil moisture, and in turn the amount of N mineralization, depends on the climate regime but also on edaphic factors, like soil texture, which modulates overall nutrient availability through nutrient adsorption to minerals and the storage capacity for plant-available water. Soils with a higher clay content store more plant-available water than sandy soils, resulting in a higher risk for sandy soils to suffer from edaphic drought (Bouma and Bryla, 2000; Dilustro et al., 2005). Furthermore, the interplay of climatic change in the different seasons and seasonal variation in the nutritional needs of the tree species likely shapes the nutrient cycling in temperate forest ecosystems. Beside inorganic N in the form of ammonium (NH_4^+) and nitrate (NO_3^-), many trees are capable of taking up organic N compounds like amino acids, which can make an important contribution to the N nutrition of temperate forests (Lipson and Näsholm, 2001; Näsholm et al., 2009; Rothstein, 2009).

Several studies have found evidence for nutritional imbalances due to a large-scale undersupply of P in various temperate tree species (Duquesnay et al., 2000; Prietzel et al., 2008; Talkner et al., 2015), likely caused by atmospheric N deposition from anthropogenic sources (Duquesnay et al., 2000; Braun et al., 2010; Sardans et al., 2016). Increased availability of soil N due to N deposition has been found either to increase or to decrease decomposition rates, and thus nutrient mineralization (Hyvönen et al., 2007; Vitousek et al., 2010), to decrease ectomycorrhizal fungi (ECMF) diversity and abundance (Kjøller et al., 2012; de Witte et al., 2017), to promote plant productivity, and to decrease the root:shoot ratio (Lambers et al., 2008). However, N deposition often also reduces the standing fine root biomass of trees, while root turnover increases (e.g., Leuschner and Hertel, 2003; Majdi and Andersson, 2005).

Beside N and P dynamics, climate change is also affecting various key components of the carbon (C) cycle, including soil respiration, which is the second largest C flux between the atmosphere and terrestrial ecosystems (Davidson and Janssens, 2006; Zhou et al., 2016). Soil respiration results from the metabolic activity of all organisms present in the soil and thus mainly consists of the respiration of plant roots and their associated microorganisms in the rhizosphere (autotrophic respiration) and free-living soil microbes, which decompose litter and soil organic matter (heterotrophic respiration) (Rodeghiero and Cescatti, 2008; Hopkins et al., 2013).

The physiological processes underlying soil respiration are influenced by a variety of abiotic factors, notably soil temperature (Davidson et al., 1998; Buchmann, 2000) and soil water content (Poblador et al., 2017), thereby showing considerable spatiotemporal variability (Hereş et al., 2021). Soil respiration varies seasonally and interannually due to factors such as photosynthetic carbon gain and assimilate allocation to roots (Ekblad and Högbert, 2001; Ruehr and Buchmann, 2009). Spatial variation in soil respiration is caused by small-scale gradients in soil texture (Bouma and Bryla, 2000; Dilustro et al., 2005) or soil nutrient content (carbon: Søe and Buchmann, 2005; phosphorus: Borken et al., 2002; N deposition: Janssens et al., 2010; Zhou et al., 2014). In general, higher temperatures have been found to speed up soil respiration (Buchmann, 2000; Borken et al., 2002; Søe and Buchmann, 2005; Lu et al., 2013). However, soil respiration is also influenced by biotic factors like forest stand structure and root or microbial parameters closely linked to stand structure, such as fine root turnover, root exudation, or the specific activity of soil microbes (Søe and Buchmann, 2005; Cai et al., 2021). Plant phenology highly influences root respiration and its temperature sensitivity through the seasonal dynamics of photosynthetic activity and belowground C allocation (Ruehr and Buchmann, 2009; Kuptz et al., 2011).

European beech (*Fagus sylvatica* L.) is the dominant native tree species of Central Europe's temperate forest biome, with high economic importance in many countries (Leuschner and Ellenberg, 2017). Recent studies have shown that this species is relatively drought sensitive (Knutzen et al., 2017; Leuschner and Meier, 2018; Leuschner, 2020; Schuldt et al., 2020), and N deposition might enhance the species' sensitivity to water deficits and elevated temperatures (Hess et al., 2018; Mausolf et al., 2020). However, it is not well known how climate warming is influencing soil N cycling and soil respiration in beech forest ecosystems.

In this study, we aimed to investigate the effect of climatic drought and N deposition on soil C and N dynamics, notably soil respiration rate and N mineralization in their seasonal changes, in a transect study with 11 mature beech forests along a precipitation and temperature gradient on sandy to loamy glacial substrates in northern Germany. We hypothesized that (H1) decreasing mean annual precipitation (MAP) and increasing mean annual temperature (MAT), i.e., increasing climatic aridity, are impairing both soil respiration rate and net N_{min}, with (H2) a stronger decrease taking place in forest stands on sandy soil than on loam-richer soil. We further hypothesized that N deposition speeds up net N_{min} but reduces soil respiration rate (H3).

Material and Methods

Study area, climate, and forest stands

The study was conducted between June 2014 and August 2015 in eleven mature beech forests (*F. sylvatica* L.) in the Pleistocene lowlands of north-west Germany along a precipitation and temperature gradient. Stands are located at five study sites in the states of Lower Saxony (Lüneburg Heath region) and Saxony-Anhalt (Altmark) in the forest districts Sellhorn (Se), Unterlüß (Un), Göhrde (Go), Klötze (Kl), and Calvörde (Ca) along a 130-km long NW-SE transect that represents the transition from an oceanic to a subcontinental climate (Hertel et al., 2013; Müller-Haubold et al., 2013). Along the gradient, MAP decreases from 855 to 576 mm yr^{-1} and MAT increases slightly from 8.7 to 9.4°C from the north-west to the south-east (**Table 2.1**). All stands are characterized by (i) dominance of beech, (ii) mature age (85–140 years), and (iii) closed canopy without larger gaps (> ~10 m in diameter). Forests grow on nutrient-poor, highly acidic sandy soils with variable silt content (dystric or umbric Arenosols or Podzols) and relatively low water storage capacity (WSC), which are covered by thick (4–9 cm) organic layers. Soils had a pH(H_2O) of 4.1-4.4, a C:N of 23.6-

27.5 mol mol^{-1} (mean 25.4 mol mol^{-1}) in the organic layer and of 20.3-33.8 mol mol^{-1} (mean 26.1 mol mol^{-1}) in the mineral soil layer, a total soil N (N_{tot}) content of 1.0-1.3 mmol g^{-1} (mean: 1.13 mmol g^{-1}) in the organic layer and 0.1-0.2 mmol g^{-1} (mean: 0.13 mmol g^{-1}) in the mineral soil layer, a total soil P (P_{tot}) content of 15.8-24.8 µmol g^{-1} (mean 21.1 µmol g^{-1}) in the organic layer and of 2.5-5.5 µmol g^{-1} (mean 3.7 µmol g^{-1}) in the mineral soil layer, a plant-available P (P_a) content of 0.08-0.13 µmol g^{-1} (mean 0.11 µmol g^{-1}) in the organic layer and of 0.02-0.03 µmol g^{-1} (mean 0.02 µmol g^{-1}) in the mineral soil layer, a base saturation of 2.8- 24.3% (mean: 9.8), and 4.7–53.5% fine-grained (< 63 µm) soil particles in the mineral topsoil (**Table 2.1**; Hertel et al., 2013; Müller-Haubold et al., 2013). To account for considerable edaphic heterogeneity, at each site two plots of 30 x 30 m differing in soil texture were established, i.e., sandy-loamy (L) and sandy (S). In the forest district of Calvörde, one additional plot with sandy soil texture (SII) was installed. All stands originate from natural regeneration and are not affected by recent forest management activities.

Table 2.1 Location, climatic data, elevation and annual N deposition of the 11 investigated beech stands on loamy-sandy (L) and sandy (S) soil in northwestern Germany. Study sites from west to east: Sellhorn (Se), Unterlüss (Un), Göhrde (Go), Klötze (Kl) and Calvörde (Ca). MAP: mean annual precipitation; MGSP = mean growing season precipitation (April - September); MSP = mean summer precipitation (June – August); MAT = mean annual temperature. Climate data refer to the period 1981–2010 and were derived from weather station data provided by the National Climate Monitoring Centre of the German Weather Service (Deutscher Wetterdienst DWD), which were corrected for elevation. Mean annual N deposition data for the time period 2013-2015 according to Schaap et al. (2018).

Site	**Soil texture**	**Code**	**Latitude (N)**	**Longitude (E)**	**Elevation (m a. s. l.)**	**MAP (mm)**	**MGSP (mm)**	**MSP (mm)**	**MAT (°C)**	**N deposition (kg ha^{-1} yr^{-1})**
Se	Loamy	Se_L	53°10'	09°57'	127	855	419	237	8.7	19
	Sandy	Se_S	53°10'	09°57'	130	855	419	237	8.7	19
Un	Loamy	Un_L	52°50'	10°19'	120	816	394	223	8.7	18
	Sandy	Un_S	52°50'	10°19'	117	816	394	223	8.7	18
Go	Loamy	Go_L	53°07'	10°49'	85	718	370	215	8.9	17
	Sandy	Go_S	53°09'	10°52'	85	707	365	212	9.0	17
Kl	Loamy	Kl_L	52°37'	11°14'	102	656	343	195	9.0	15
	Sandy	Kl_S	52°37'	11°15'	85	655	344	196	9.1	15
Ca	Loamy	Ca_L	52°24'	11°16'	72	577	308	175	9.3	14
	Sandy	Ca_S	52°23'	11°17'	75	576	307	174	9.4	15
	Sandy	Ca_S II	52°22'	11°16'	105	594	315	179	9.3	15

Climatic data and N deposition

Precipitation and temperature data were obtained from the 1 x 1 km^2 grid data set of the German Weather Service (Deutscher Wetterdienst, Offenbach, Germany). To regionalize the climatic data for the eleven study plots, data from the nine nearest grid fields was interpolated for each location and corrected for altitude. MAP, mean growing season precipitation (MGSP; April–September), mean summer precipitation (MSP; June -August) and MAT as factors characterizing the long-term mean climate were averaged from climate data for the years 1981–2010. Short-term thermal and hydrometeorological weather conditions were characterized by extracting monthly precipitation and temperature data one or three months before the dates of sample collection ($Precip_{1\ month\ before}$, $Precip_{3\ month\ before}$; $T_{1\ month\ before}$, $T_{3\ month\ before}$) in the study years 2014 and 2015, respectively. The Standardized Precipitation-Evapotranspiration Index (SPEI) was used as a measure of drought intensity, which calculates the climatic water balance referenced to the local mean (Vicente-Serrano et al., 2010). SPEI data with a one-month timescale and 0.5° spatial resolution was obtained from the global SPEI database (http://SPEI.csic.es/database.html), showing variation in drought intensity over short timescales. The classification of SPEI values according to McKee et al. (1993) and Ye et al. (2015) is as follows: SPEI $\geq$ 2.0 extremely wet, SPEI < 2.0 to $\geq$ 1.5 severely wet, SPEI < 1.5 to $\geq$ 1.0 moderately wet, SPEI < 1.0 to > -1.0 normal, SPEI $\leq$ -1.0 to > -1.5 moderately dry, SPEI $\leq$ -1.5 to > -2.0 severely dry, and SPEI $\leq$ -2.0 extremely dry. It has to be noted that the SPEI cannot identify droughts with a duration of less than one month (Vicente-Serrano et al., 2010; Van der Schrier et al., 2011). To analyze the effect of atmospheric N deposition on soil respiration and net N_{min}, mean annual total deposition data for the vegetation class “deciduous forest” was derived from the PINETI-3 project (Pollutant INput and EcosysTem Impact; Schaap et al., 2018), which models dry, moist, and wet N deposition, i.e., in sum the total N deposition, in Germany with a spatial resolution of 1 km x 1 km for the time period 2013–2015.

Soil respiration rate

Soil respiration rate measurements were conducted around noon (between 11 a.m. and 2 p.m.) in two campaigns in the growing season 2015, i.e., the end of April/May (early season) and July 2015 (mid-season). We used the closed chamber system with continuous air circulation and CO_2 concentration measurement through infrared gas analysis (IRGA) supplied by Li-Cor (LI-6400-09 Soil CO_2 Flux Chamber, Li-Cor, Lincoln, NE; Madsen et al. 2010). Ambient CO_2 concentration was measured, and subsequently, the chamber was placed 1 cm above the soil surface while being stabilized by soil collars (PVC, 10 cm in diameter and 5 cm in height, with stainless steel legs for further stabilization). Ten soil collars were already installed in 2014 in each of the eleven study plots to minimize disturbance effects on the measurements in 2015 caused by fixing the chamber head and to allow for repeated measurements at the same location over time. To minimize leaks and to avoid the disturbing effects of wind, a fine foam gasket ring was placed between the soil collar and the chamber (**Figure 2.1**). CO_2 was scrubbed below the ambient CO_2 concentration and then allowed to rise. The rate of CO_2 efflux from the soil was estimated from the rate of CO_2 concentration increase inside the chamber, which was related to the soil area included in the chamber (Madsen et al., 2010; in µmol CO_2 m^{-2} s^{-1}). While the measurement cycle was repeated four times at each soil collar, the CO_2 efflux, i.e., soil respiration rate, was calculated only for the last two cycles. Hence, overall plot means were calculated by considering 20 measurement cycles (10 soil collars x 2 cycles). Soil temperature was measured simultaneously with a temperature probe outside the chamber, close to each soil collar, at a depth of about 5 cm.

Figure 2.1 Soil respiration measurements with a LI-6400 and a soil CO_2 flux chamber (LI-6400-09). Chamber was stabilized by PVC soil collars and placed 1 cm above soil surface. A (blue) fine foam gasket ring was placed between soil collar and chamber to avoid disturbance and air leakage. Simultaneous measurement of soil temperature was performed via temperature probe (violet cable).

Net N mineralization and microbial immobilization of N

Nitrogen mineralization rate, mineral N concentration in the soil, and microbial N uptake (immobilization) were all measured using the *in situ* buried bag method developed by Eno (1960). This method allows measuring the net release of NH_4^+ and NO_3^- in root-free soil enclosed in polyethylene (PE) bags under natural soil temperature conditions while soil water content is kept constant. The bag impedes the uptake of mineral N by plant roots, but does not hamper N acquisition by soil microbes. PE is permeable to oxygen and carbon dioxide but not to nitrate (Eno 1960). In 2014, field work was conducted in three campaigns, i.e., June/July to August (mid-season 2014; June 18 to August 22), August to October (late season 2014; August 4 to October 2), and October to December (non-growing season 2014; October 2 to December 9), and in two campaigns in 2015, i.e., the end of April/May to June (early season 2015; April 28 to June 29) and July to August (mid-season 2015; July 21 to August 26), with the exact exposure periods varying slightly between sites.

Close to each soil collar (see section *Soil respiration rate*, 10 soil collars per plot), two soil cores were dug to a depth of 10 cm depth close to each other and extracted with the litter layer on top kept intact. The first soil core was carefully placed in a PE bag, tightly sealed, replaced into the hole and covered with litter (*cf.* Eno, 1960; Schmidt et al., 1999). The second soil core was separated into the organic layer, i.e., the visible plant material, and the mineral soil layer, and immediately brought back to the institute and stored at -20°C until laboratory analyses. After a mean incubation time of 39 ± 8 days, the incubated soil cores were collected in the field, the two layers were separated, and stored in the freezer. The organic layer and mineral soil material were sieved at 2- and 4-mm mesh widths, respectively, for the subsequent analysis of NH_4^+ and NO_3^- concentrations, and 5 g of the soil material was mixed with 20 ml of 0.5 M K_2SO_4 and shaken overhead for 2 hours to extract mineral N compounds. The suspension was filtered through Whatman no. 2 filters and frozen at -18°C until further analysis. Samples were diluted with aqua dest. at a ratio of 1:2 and analyzed for mineral N concentrations through continuous flow injection colorimetry (Cenco/Skalar Instruments, Breda, Netherlands). Taking the soil dry mass in the layers into account, the NH_4^+ and NO_3^- concentrations of the second soil core were subtracted from the concentrations of the incubated core. Net ammonification rate (µmol NH_4^+ m^{-2} 10 cm^{-1} d^{-1}) and net nitrification rate (µmol NO_3^- m^{-2} cm^{-1} d^{-1}) per ground area were calculated by relating mass-specific mineral N net release to the cross-sectional area of the core and dividing by the incubation period length. The sum of measured ammonification and nitrification rates is net N_{min} (in µmol N m^{-2} d^{-1}), which is the gross N mineralization rate minus the immobilization in microbial biomass.

Due to the sample collection procedure used here, the soil surface in the incubated cores might have been increased somewhat, especially in the loamy-sandy samples with higher aggregation of soil particles. Hence, all calculated net N_{min} rates must be regarded as approximates. To estimate the cumulated net N_{min} for the whole growing season, we summed up the figures of the mid-growing season 2014, the late season 2014, the early season 2015, and the mid-growing season 2015. For the sake of comparability, we normalized each season to 45 days (mid 2014: 01.07.–15.08.14; late 2014: 16.08.–30.09.2014; early 2015: 01.05.–15.06.2015; mid 2015: 01.07.–15.08.2015) and multiplied the respective net N_{min} with 45 to obtain the seasonal total. The figures for the non-growing season of 2014 were not included in this total (**Figure 2.2**).

As a short-term indicator for water availability, we measured gravimetric water content (GWC; in g g^{-1}) in 10 cm depth for each sampling date by calculating the mass difference between fresh and dried soil (48 h at 105°C).

Amino acid-N

In order to quantify organic N availability in the soil, amino acid-N (AA-N) concentration in the organic layer was determined with the colorimetric detection method after Rosen (1957). An aliquot of 200 µl of a K_2SO_4 soil extract, which was also used for measuring the concentration of mineral N compounds in the soil, was mixed with a cyanide-acetate buffer, and the contained amino acids were reduced with a 3% ninhydrin solution in 2-methoxyethanol. The solution was heated for 15 minutes in a 100°C-heating block and subsequently diluted with an isopropyl alcohol-water (1:1) solution. The resulting violet coloration was measured photometrically at 570 nm (GENESYS 20 Visible Spectrophotometer, Thermo Fisher Scientific, Waltham, MA, USA), and amino acid concentrations were calculated based on a reference curve established with glycine concentrations varying between 0 and 0.75 mM. AA-N concentrations were related to soil dry mass (mmol AA-N kg^{-1}, $n = 8$ samples per study plot and season in 2014; $n = 10$ in 2015).

Characterizing soil chemistry and nutrient availability

The collection of soil samples for nutrient analyses took place at the study sites at the end of April and in mid-June 2013 and was completed within four days to ensure similarity in terms of temperature and moisture conditions. Soil cores were collected at each site at five randomly chosen locations within a 30 m x 30 m plot placed in a representative section of the stand. In stands with presence of other tree species than beech, sufficient distance to other tree species was ensured. Samples were taken with a soil corer of 5.5 cm in diameter from the organic layer and the first 10 centimeters of the mineral topsoil. The border between the two horizons was defined as the transition zone from the organic layer horizon with visible plant debris to the layer below, where plant material was either not discernable or mineral particles were present. Each mineral soil sample consisted of two subsamples extracted at 0–10 cm depth, while the organic layer samples consisted of three subsamples each. All subsamples were taken within a one-meter radius around the respective sampling point to represent possible small-scale spatial variability of soil properties and to obtain enough material for the analyses. Fine root samples were extracted from three soil subsamples taken in the organic layer and the mineral soil at 0–10 cm depth. After collection, all samples were immediately cooled to 4°C until they were sieved in the lab within three days at the most (mineral soil: mesh size 2 mm; organic layer: mesh size 5 mm). Subsamples used for analyzing plant-available phosphorus (resin-extractable P) were stored at 4°C. Subsamples for the determination of total contents of carbon, nitrogen, and phosphorus were dried at 70°C for 48 hours and ground to powder. As the soils of the study region are highly acidic and contain no carbonate, total C was assumed to be organic C (C_{org}).

C_{org} and total soil nitrogen content (N_{tot}) were analyzed in the dried (70°C, 48 h) and ground mineral soil, organic layer, or fine root material. Five mg of mineral soil or 20 mg of organic layer or fine root material were analyzed for their C and N content through gas chromatography in a C/N-analyzer (Model: Vario EL III, elementar, Hanau, Germany) using acetanilid as a standard. Plant-available phosphorus (P_a) was quantified by extraction with resin bags (Sibbesen, 1978).

In a 100 ml PE-flask, one gram of field-moist mineral soil or 0.5 g of field-moist organic layer material suspended in 30 ml of deionized water was shaken overhead with a resin bag for 16 hours. After removal of the bag from the flasks, adhering soil was washed off with deionized water, and the adsorbed P was desorbed by adding 25 ml of a 10%-NaCl solution. After shaking for 30 minutes and filtration through blue-ribbon filter paper, a second desorption procedure with 25 ml of 2%-NaOH solution followed. P in both extracts was colored with the molybdenum-blue method (Murphy and Riley, 1962) and analyzed photometrically at 712 nm against deionized water (Model: Libra S21, Biochrom, UK). The total phosphorus (P_{tot}) content of organic layer and mineral soil was analyzed after acid pressure digestion by Heinrichs et al. (1986) by incubating 200 mg of dried, ground mineral soil or 50 mg of organic layer or roots, respectively, with 2 ml of 65%-HNO_3 at 185°C for twelve (mineral soil) or seven hours (organic layer and roots). The extracts were filtered through pre-rinsed MN 640w black ribbon filter paper, diluted with deionized water, and analyzed with inductively coupled plasma-optical emission spectrometry (ICP-OES) (Model: Optima 5300 DV, Perkin Elmer, USA).

Fine root production and turnover

Fine root production and fine root turnover (roots < 2 mm in diameter) were studied in the 11 plots in 2010 by Hertel et al. (2013). To measure fine root growth and estimate annual fine root production in the plots, the ingrowth core approach was employed (Persson 1980; Powell and Day 1991; Majdi 1996). Per plot, 12 ingrowth cores were installed at random locations to a depth of 20 cm in the mineral soil in May/June 2009, and re-sampled in March 2011, that is, after 22 months. Fine roots started to grow into the bulk of ingrowth cores around June 2010 after the initial soil disturbance with core installation, i.e., an active root growth period of 9 months was assumed. Soil cores were extracted in March 2011, and the dry mass of larger fine root branches (> 10 mm; living and dead fine roots) in each core was quantified by washing the soil over sieves, collecting the roots quantitatively, and drying them at 70°C for 48 h. The data were expressed as fine root growth per soil volume and 9 months, extrapolated to a complete year, and related to m^2 ground area, which gave an estimate of annual fine root production (in g m^{-2} yr^{-1}). Fine root turnover in the study plots was estimated by relating annual root production to the mean standing fine root biomass at the site (Aber et al., 1985; Aerts et al., 1992; Gill and Jackson, 2000). For further details, see Hertel et al. (2013).

Statistical analyses

All statistical analyses were conducted with R, version 4.0.3 (R Development Core Team). Significance is assumed at $P \leq 0.05$ throughout the paper. The probability of fit to a normal distribution was tested with a Shapiro-Wilk test. Non-normally distributed data were transformed to achieve normality and homogeneity of variances. Means, standard errors, and coefficients of variance (CV in %) were calculated for net ammonification, net nitrification, net N_{min}, and soil respiration rates, as well as soil AA-N concentration. A Kruskal-Wallis multiple comparison test was used to compare stand means of the respective response variables. Pearson correlation analysis was used to characterize the relationships between N flux and respiration variables and climatic and edaphic parameters. For significant correlations, we further conducted linear regression analyses. We conducted a linear mixed-effects model analysis with restricted maximum likelihood estimation of variance components using the ‘lmer’ command of the R-package *lme4* (Bates et al., 2015) to examine the significance and importance of the two fixed factors “Soil texture” (sandy-loamy vs. sandy) and “Season” (mid 2014, late 2014, non-grow 2014, early 2015, mid 2015) and their interaction on the response variables net N mineralization, soil respiration, and AA-N, accounting for the effect of (non-independent) samples taken at the same sites using “Site” as a random factor. We assumed that the intercept and the slope differ (y ~ texture + (texture|site)). The R-package *lsmeans* (Lenth, 2016) was used to conduct a post-hoc test on fixed-effects interactions with Bonferroni correction. Meteorological data was visualized in the form of Walter-Lieth climate diagrams using the ‘daigwl’ function in the *climatol* R package (Guijarro, 2019).

Results

Weather conditions in the study years

The studied transect represents, in the long-term mean (1981–2010), a precipitation gradient with a 279 mm difference, ranging from 855 mm in Sellhorn at the wetter end to 576 mm in Calvörde at the drier end. This gradient is associated with a temperature increase of 0.7°C from west to east

(**Table 2.1**). Compared to the long-term mean, the study year 2014 was drier and warmer, with a mean temperature increase of 1.4°C over all study sites and a mean precipitation decrease of -10%. The precipitation gradient was reduced to 185 mm in this year due to the precipitation decline being especially pronounced for the study sites in the wetter north-west (-15.1% in Sellhorn or 13.9% in Unterlüß, compared to -6.0% in Calvörde at the south-eastern dry end). By contrast, in 2015, the amount of precipitation was on average 2.6% higher than MAP, varying from an increase in rainfall of +5.8% and +6.5% for Sellhorn and Unterlüß, respectively, on the wetter end to nearly no deviation from MAP at the drier end of the transect (+0.6 % for Klötze or +0.2% Calvörde), resulting in a precipitation gradient of 279 mm for our study transect. The temperature in 2015 was on average 0.9°C higher compared to MAT (see also the climate diagrams in **Figure S2.1**).

SPEI, a drought intensity metric, indicated a severe drought in February and March 2014, with values around 1.9 and 1.7, respectively, with the lowest values at the dry end of the studied precipitation gradient (**Figure S2.2**). In contrast, in May 2014, study sites experienced severe to moderately wet conditions, with SPEI values around +1.5. SPEI values in the mid- and late-growing seasons of 2014 indicated normal wet conditions. In November 2014 and February 2015, SPEI dropped under -1.5. In spring 2015, SPEI returned to normal values, which lasted the whole growing season of 2015, albeit with some monthly variation. In consequence of these SPEI data, in the study period of this paper (June 2014 to August 2015), study sites predominantly experienced normal conditions in regard to precipitation-evapotranspiration, with the exception of severe drought in both November 2014 and February 2015 and moderate drought in April 2015.

The precipitation gradient in the year 2010, when fine root production and fine root turnover were determined, ranged from 817 mm in the wetter north-west (in Sellhorn; -4.4% compared to MAP) to 672 mm at the south-eastern dry end (Calvörde; +18.7% compared to MAP), with an overall difference of +9.5% compared to MAP. However, growing season precipitation (April to September) in 2010 was 24% higher compared to mean growing season precipitation, while summer precipitation (June to August) in 2010 was -3.6% of mean summer precipitation. In contrast, the average temperature in 2010 was 1.2°C lower than the MAT.

The GWC as a short-term indicator for water availability showed rather minor fluctuations during the study period between June 2014 and August 2015 (**Figure S2.3**).

N mineralization

The sum of net N_{min} in the period from July 2014 to mid-August 2015 did not significantly differ between plots along the precipitation gradient (**Figure 2.2**A), ranging from 75.3 mmol N m^{-2} for Se_L as the wettest plot to -1.27 mmol N m^{-2} in Kl_S on the drier end of the gradient (**Figure 2.2**A). The relative proportion of net nitrification in net N_{min} was highest for Un_S with 59.5% and lowest for all plots in Calvörde (Ca_SII, Ca_S, Ca_L) as well as Go_S with a proportion close to zero (**Figure 2.2**B). For detailed values of net nitrification rate, net ammonification rate, and net N_{min} for each season see **Figure S2.4**, and for each site averaged over all five seasons, see **Table S2.2**.

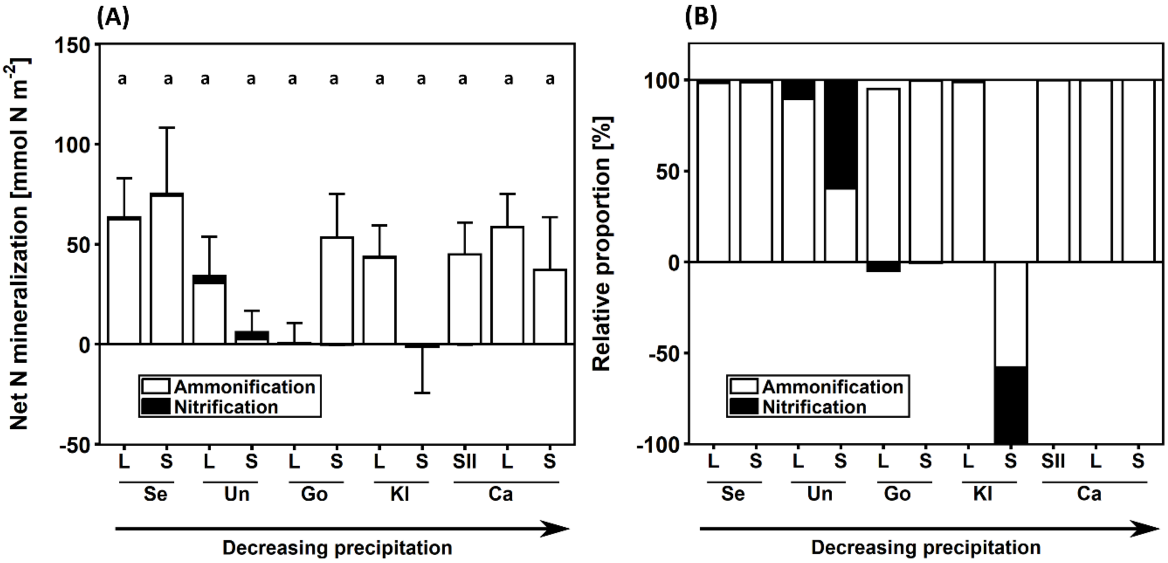

Figure 2.2 (A) Sums and standard errors and **(B)** the relative proportion of net ammonification rate and net nitrification rate in net N mineralization rate (in 10 cm depth) at the 11 study sites along a precipitation gradient (from high to low precipitation; Se: Sellhorn, Un: Unterlüss, Go: Göhrde, Kl: Klötze, Ca: Calvörde) on loamy-sandy (L) and sandy (S) soil in the period June 2014 to August 2015. Values of mid-growing season 2014, late season 2014, early season 2015 and mid-growing season 2015 were summed up. For the sake of comparability, each season was normalized to 45 days (mid 2014: 01.07-15.08.14; late 2014: 16.08.-30.09.2014; early 2015: 01.05.-15.06.2015; mid 2015: 01.07.-15.08.2015), and the net N mineralization rate multiplied by 45 to obtain the seasonal total. The data of the non-growing season 2014 were not included in this total. Kruskall-Wallis test at $P \leq 0.05$ showed no significant differences (indicated by same lower-case letters) between sites or plots, respectively.

Higher temperature had–on the long term and short-term scale–a significant negative effect on net N_{min} on loamy plots in the late growing season of 2014 (loamy, late 2014: MAT: $R = -0.95$, $T_{1\ \mathrm{month\ before}}$: $R = -0.90$, $T_{3\ \mathrm{month\ before}}$: $R = -0.93$; $P \leq 0.05$) (**Figure 2.3**A; **Table S2.3**). Here, an increase of 1°C in MAT resulted in a decrease of net N_{min} of 0.68 mmol N m^{-2} d^{-1} (loamy texture, late 2014), while an increase of 1°C in mean temperature one month before sample collection resulted in a decrease of net N_{min} of 0.48 mmol N m^{-2} d^{-1} (data not shown). Net N_{min} on sandy plots was significantly increased by N deposition in the late growing season of 2014 (sandy, late 2014: $R = 0.82$; $P \leq 0.05$; **Figure 2.3**B; **Table S2.3**), higher MSP (sandy, late 2014: $R = 0.81$; $P \leq 0.05$; **Table S2.3**), a higher water storage capacity (sandy, late 2014: $R = 0.87$; $P \leq 0.05$; **Table S2.3**) and a higher content of C_{org} and N_{tot} in the organic layer (sandy, late 2014: $R = 0.93$ for C_{org} and $R = 0.94$ for N_{tot}; $P \leq 0.01$; **Table S2.3**).

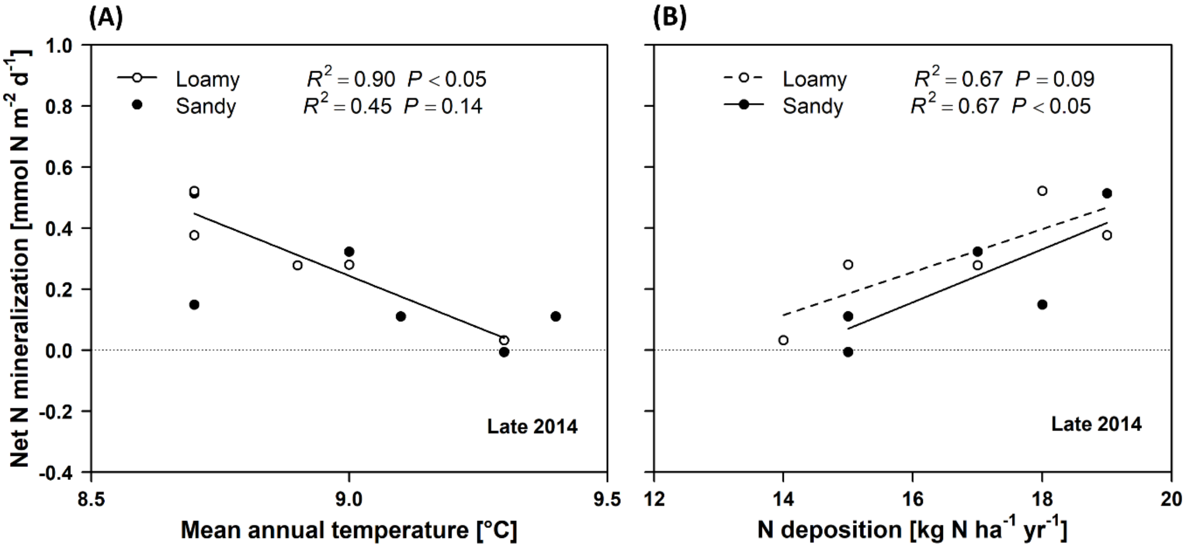

Figure 2.3 Relationship between net N mineralization rate in 10 cm depth and **(A)** mean annual temperature (period 1981-2010) or **(B)** annual N deposition. Mean values are shown for each beech stand with sandy-loamy (n = 5) or sandy (n = 6) soil texture, respectively, for late growing season (August – October 2014). Significant ($P \leq 0.05$) relationships are indicated by continuous lines. Marginally significant ($P \leq 0.1$) relationship is indicated by a dashed line. Equations: (A) loamy: $y = -0.68x + 6.3$, (B) loamy: $y = 0.07x - 0.9$, sandy: $y = 0.09x - 1.2$.

MAP had a marginally significant positive effect on net N_{min} for the late growing season, independent of soil texture, but short-term precipitation was not affecting net N_{min} (**Table S2.3**; **Figure 2.4**A,B). In the non-growing season, samples collected on sandy plots showed lower net N_{min} with higher temperature and higher precipitation three months before sample collection (sandy, non-grow 2014: $P_{3\ \text{months before}}$: $R = -0.94$, $P \leq 0.01$; $T_{3\ \text{months before}}$: $R = -0.84$, $P \leq 0.05$; **Table S2.3**). For sandy plots, net N_{min} was negatively related to an increasing P_a concentration in the organic layer in the early growing season but not in the mid-growing season of 2015 (sandy, early 2015: $R = -0.84$, $P \leq 0.05$; **Table S2.4**, **Figure S2.5**A, B). In contrast, for loamy plots in the early and mid-growing seasons of 2015, net N_{min} was negatively correlated with P_a of the mineral soil (loamy, early and mid-growing seasons of 2015: $R = -0.83$, $P = 0.09$ for the early season, $R = -0.90$, $P \leq 0.05$ for the mid- growing season; **Table S2.4**; **Figure S2.5**C, D).

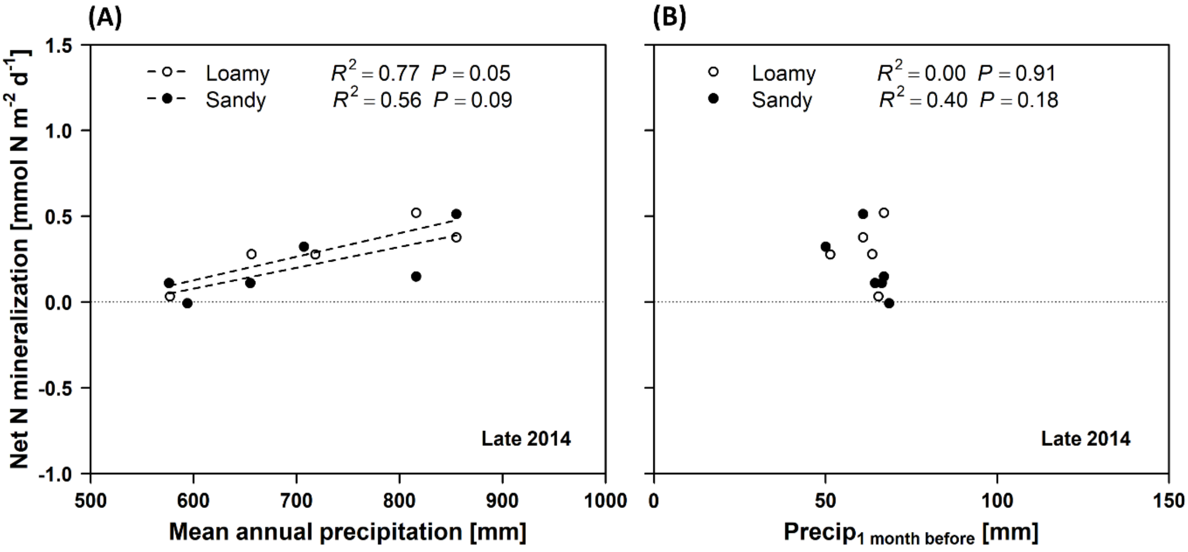

Figure 2.4 Relationship between net N mineralization rate and **(A)** mean annual precipitation (period 1981-2010) or **(B)** precipitation one month before sampling (Precip$_{1 \text{ month before}}$). Mean values are shown for each beech stand with sandy-loamy (n = 5) or sandy (n = 6) soil texture, respectively, for late growing season (August – October) 2014. Marginally significant ($P \leq 0.1$) relationships are indicated by dashed lines. Equations: (A) loamy: y = 0.001x – 0.70, sandy: y = 0.001x – 0.65; (B) n. a..

Fine root production positively correlated with net N$_{\text{min}}$ in mid-growing season 2015 on sandy plots but not on loamy plots (sandy, mid 2015: R = 0.81, $P \leq 0.05$; **Table S2.4**; **Figure S2.6**A, B). Fine root turnover showed a positive relationship with net N$_{\text{min}}$ in the early and mid-growing seasons for loamy plots, and in the mid-growing season for sandy plots (loamy, early 2015: R = 0.83, P = 0.09; loamy, mid 2015: R = 0.82, P = 0.09; sandy, mid 2015: R = 0.86, $P \leq 0.05$; **Table S2.4**, **Figure S2.6**C,D). Net N$_{\text{min}}$ showed a negative relationship with soil AA-N for sandy soils in early 2015 (marginally significant; R = -0.78, P = 0.07; **Table S2.4**). A linear mixed effects model with "site" as a random factor revealed that season had a significant effect on net N$_{\text{min}}$ but not soil texture or the interaction soil texture x season (**Table 2.2**).

Net N$_{\text{min}}$ differed marginally for loamy soil texture and significantly for sandy soil texture between the early and mid-growing seasons in 2015, and turned negative for sandy soils in the mid-growing season of 2015 (**Figure 2.5**A). In none of the seasons was Net N$_{\text{min}}$ significantly related to soil respiration rate or soil AA-N concentration (data not shown).

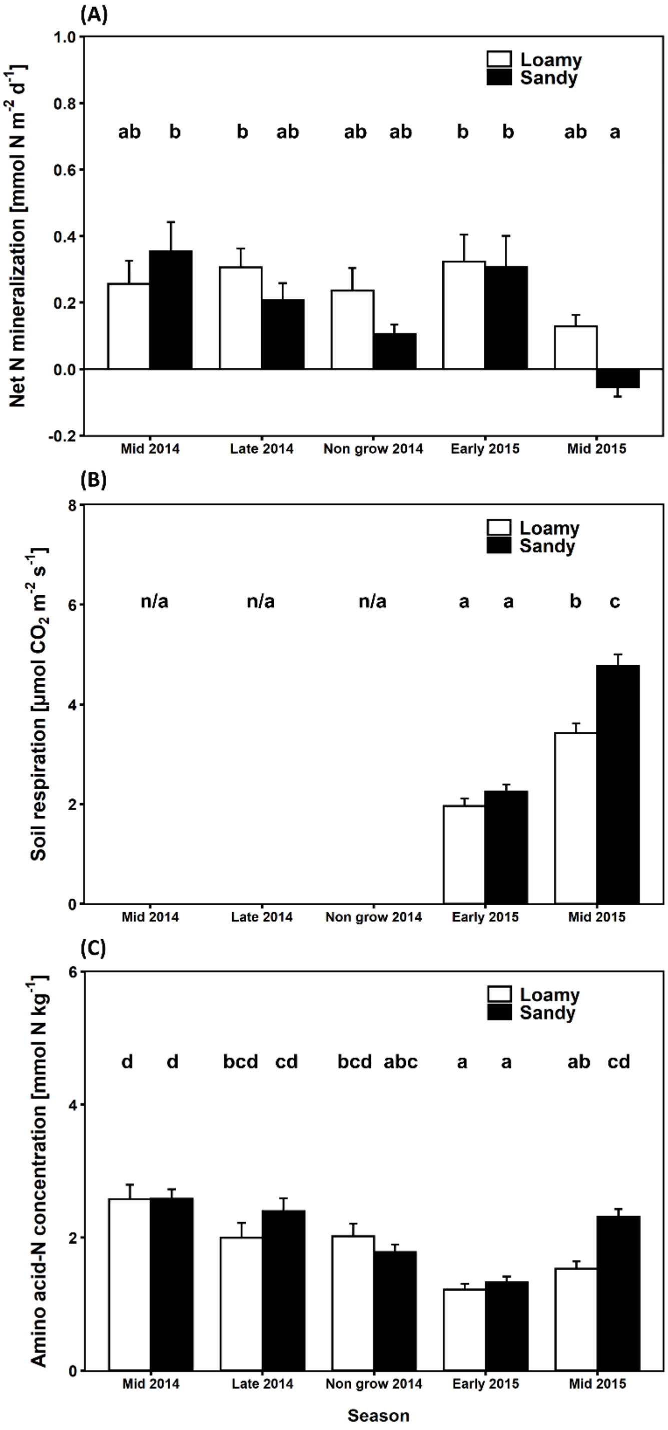

Figure 2.5 Means and standard errors of **(A)** soil respiration rate, **(B)** soil amino acid-N concentration, and **(C)** net N mineralization rate in 10 cm depth on sandy-loamy (Loamy; white) and sandy soils (Sandy; black) under beech stands for three seasons in 2014 (mid = June/July to August; late = August to October; non grow = October to December), and two seasons in 2015 (early = April/May to June; mid = July to August). For soil respiration and soil amino acid-N concentration in 2015: $n = 50$ (for loamy) and $n = 60$ (for sandy); for soil amino acid-N concentration in 2014: $n = 40$ (loamy) and $n = 48$ (sandy); for net N mineralization: $n = 30$. Bars with different lower-case letters indicate significant differences based on a Kruskall-Wallis test at $P \leq 0.05$. n/a = not applicable.

Table 2.2 Summary statistics for the linear mixed effects model of net N mineralization rate (net Nmin), soil respiration rate, and soil amino acid concentration (AA-N) with the fixed effects soil texture and season and the interaction between season and soil texture. "Site" (i.e., Sellhorn, Unterlüß, Göhrde, Klötze and Calvörde) was included as random factor. Seasons: three seasons in 2014 (mid = June/July to August; late = August to October; non-growing (non grow) = October to December), two seasons in 2015 (early = April/May to June; mid = July to August). For soil respiration the two seasons in 2015 are only considered. AA-N was square root transformed to achieve normality and homogeneity of variances. Given are *F* values and probabilities of error *P*. Soil texture: loamy or sandy. Significant effects are indicated by bold letters. The standard deviation of random factor "Site" was 0.06 (net N_{min}), 0.71 (soil respiration rate), 0.04 (AA-N), respectively. numDF, numerator degrees of freedom; denDF, denominator degrees of freedom.

Response	**Fixed effects**	**numDF**	**denDF**	***F***	***P***
Net N_{min}	Soil texture	1	5	0.90	0.39
	Season	4	290	5.72	**<0.001**
	Soil texture x season	4	290	1.22	0.30
Soil respiration rate	Soil texture	1	25	17.46	**<0.001**
	Season	1	205	152.64	**<0.001**
	Soil texture x season	1	205	10.81	**<0.01**
AA-N	Soil texture	1	5	1.61	0.26
	Season	4	447	27.75	**<0.001**
	Soil texture x season	4	447	4.14	**<0.01**

Soil respiration rate

Soil respiration rate significantly increased with decreasing MAP in the mid growing season of 2015 for both soil textures (loamy and sandy, mid 2015: for loamy plots: $R^2 = 0.98$, $P = 0.003$; for sandy plots: $R^2 = 0.98$, $P \leq 0.001$; **Figure 2.6**A; **Table S2.4**) with a steeper slope for sandy study plots.

For the early growing season 2015 in April/May, soil respiration rate was highest at the site Göhrde with 3.3 and 3.5 µmol CO_2 m^{-2} s^{-1} for loamy and sandy soil textures, respectively, and was lowest at the loamy plots in Sellhorn and Unterlüß (Se_L: 1.2 µmol CO_2 m^{-2} s^{-1}; Un_L: 1.3 µmol CO_2 m^{-2} s^{-1}) (data not shown). Thus, in spring 2015, respiratory CO_2 losses were neither affected by the long-term precipitation gradient nor by actual precipitation patterns (**Table S2.4**). This pattern changed to a significant effect of the precipitation gradient during the mid-growing season in July/August 2015 (**Table S2.4**).

While soil respiration rate was only moderate at the wetter end of the transect (2.3 and 3.2 mol CO2 m-2 s-1 (Se_L and Se_S; data not shown), it increased more than 2.5 times toward the drier end, which was especially pronounced for plots with sandy soil texture (6.1 and 6.4 mol CO2 m-2 s-1 for Ca_S and Ca_S II, but Ca_L with 4.5 mol CO2 m-2

While soil respiration rate was only moderate at the wetter end of the transect with 2.3 and 3.2 µmol CO_2 m^{-2} s^{-1} (Se_L and Se_S; data not shown), it increased more than 2.5 times toward the drier end, which was especially pronounced for plots with sandy soil texture (6.1 and 6.4 µmol CO_2 m^{-2} s^{-1} for Ca_S and Ca_S II, but Ca_L with 4.5 µmol CO_2 m^{-2} s^{-1}; data not shown; for means averaged over early and mid-growing season of 2015 see **Table S2.2**). The apparently stimulating effect of decreasing precipitation on soil respiration in mid-growing season 2015 was visible at the long-term and short-term scales, especially for sandy soil texture (**Figure 2.6**A, B; **Table S2.4**).

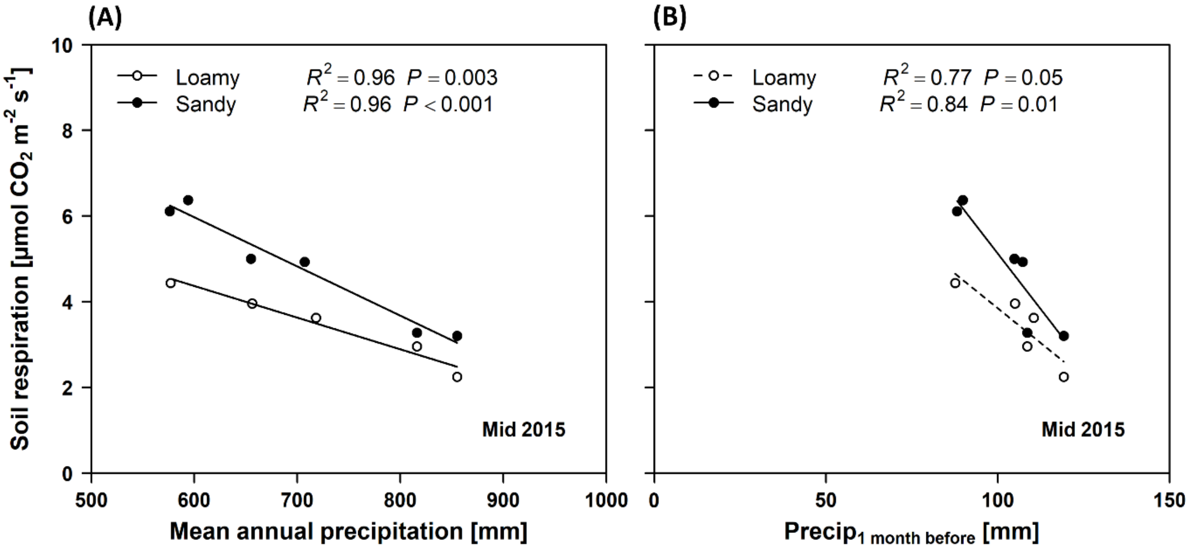

Figure 2.6 Relationship between soil respiration rate and **(A)** mean annual precipitation (period 1981-2010) or **(B)** precipitation one month before measurement ($Precip_{1\ month\ before}$) in the 11 beech stands in mid-growing season (July) 2015. Mean values are shown for each beech stand with sandy-loamy (*n* = 5) or sandy (*n* = 6) soil texture, respectively, for mid-growing season (July 2015). Significant ($P \leq 0.05$) relationships are indicated by continuous lines. Marginally significant ($P \leq 0.1$) relationship is indicated by a dashed line. Equations: (A) loamy: y = -0.01x + 8.8, sandy: y = -0.01x + 12.9; (B) loamy: y = -0.06x + 10.3, sandy: y = -0.10x + 15.5.

Furthermore, soil respiration rate was negatively correlated with increasing N deposition in mid-growing season 2015 (mid 2015: loamy: $R = -0.97$, $P \leq 0.01$; sandy: $R = -0.91$, $P \leq 0.05$; **Table S2.4**). As a consequence of the inherent relationship between MAP and MAT along our study transect, soil respiration rate was significantly enhanced by higher MAT in the mid-growing season of 2015 for both soil textures (mid 2015: for loamy plots: $R^2 = 0.84$, $P \leq 0.05$; for sandy plots: $R^2 = 0.96$, $P \leq 0.001$; **Figure S2.7**A; **Table S2.4**). However, we could find a comparable significant positive relationship between soil respiration rate and mean temperature 1–3 months before measurement, i.e., the actual thermal conditions in the mid-growing season of 2015 (mid 2015: for loamy plots: $R^2 = 0.92$, $P \leq 0.05$; for sandy plots: $R^2 = 0.94$, $P \leq 0.01$; **Figure S2.7**B; **Table S2.4**) with a steeper increase for sandy study plots. Furthermore, increasing soil temperature resulted in a significant linear increase in soil respiration rate at plots with sandy soil texture, for both investigated seasons (early: $R^2 = 0.73$, $P \leq 0.05$; mid: $R^2 = 0.70$, $P \leq 0.05$; **Figure S2.8**A; **Table S2.4**), but not for loamy plots (**Figure S2.8**B; **Table S2.4**). For sandy plots, soil respiration rate was enhanced by higher P_{tot} contents of the organic layer and mineral soil in mid-growing season 2015 (sandy, mid 2015: $R = 0.83$, $P \leq 0.05$; P_{tot} mineral soil: $R = 0.83$, $P \leq 0.05$; **Table S2.4**), whereas higher P_a of the mineral soil had a positive effect in early growing season 2015 for both soil textures (early 2015: marginally significant for loamy: $R = 0.85$, $P = 0.07$; sandy: $R = 0.96$, $P \leq 0.05$; **Table S2.4**). In mid-growing season 2015, soil respiration rate was positively correlated with fine root production for both soil textures (loamy, mid 2015: $R = 0.95$, $P \leq 0.05$; sandy: $R = 0.83$, $P \leq 0.05$; **Table S2.4**; **Figure 2.7**A). Furthermore, the soil respiration rate for stands on sandy soil in mid-growing season 2015 was enhanced by a higher fine root turnover (sandy, mid 2015: marginally significant, $R = 0.74$, $P = 0.08$; **Table S2.4**; **Figure 2.7**B). Soil respiration rate was not correlated to net NO_3^- or NH_4^+ release, net N mineralization rate, or soil amino acid-N concentration (data not shown). Soil respiration rate was significantly affected by soil texture, season, and their interaction, with plots with sandy soil texture having the highest mean values in mid-growing season (**Table 2.2**).

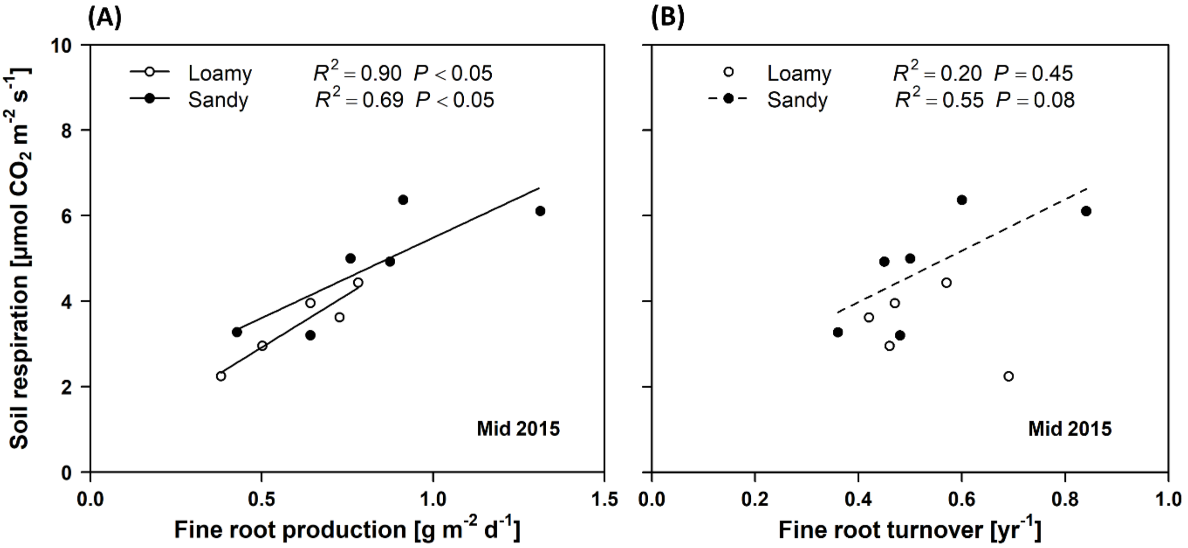

Figure 2.7 Relationship between soil respiration rate and **(A)** fine root production or **(B)** fine root turnover on sandy-loamy (Loamy; white) and sandy soils (Sandy; black) under beech stands for the mid-growing season (July) in 2015. Data for annual fine root production and fine root turnover (organic layer and 0-20 cm of mineral soil for the year 2010) were taken from Hertel et al. (2013). Mean values are shown for each stand with sandy-loamy ($n = 5$) and sandy ($n = 6$) soil texture. Significant ($P \leq 0.05$) relationships are indicated by continuous lines. Marginally significant ($P \leq 0.1$) relationships are indicated by dashed lines. Equations: (A) loamy: $y = 0.01x + 0.5$, sandy: $y = 0.01x + 1.7$; (B) sandy: $y = 6.00x + 1.6$.

AA-N concentration in the soil

Soil AA-N concentration was significantly influenced by the season and the interaction of soil texture with the season but not by soil texture alone (**Table 2.2**; for detailed information per study plot per season, see **Figure S2.9**).

In 2015, the mean soil AA-N concentration was highest in plots with sandy soil texture (**Figure 2.5**C). Averaged over all five seasons examined in 2014 and 2015, soil AA-N concentration was highest at Se_S with 2.48 mmol AA-N kg^{-1} but lowest at Go_L with 1.53 mmol AA-N kg^{-1} and Se_L with 1.58 mmol AA-N kg^{-1} (**Table 2.2**). A higher amount of plant-available P increased soil AA-N content in loamy soils in the late-growing season as well as in the non-growing season (loamy; late 2014: $R = 0.90$, $P \leq 0.05$, non-grow 2014: $R = 0.92$, $P \leq 0.05$; data not shown). Furthermore, in the non-growing season 2014, soil amino acid-N concentration in loamy soils was enhanced by higher precipitation one month before sampling ($R = 0.93$, $P \leq 0.05$; data not shown), whereas soil AA-N concentration on sandy soils increased with higher GWC ($R = 0.91$, $P \leq 0.05$; data not shown).

Discussion

Continued high atmospheric N deposition, combined with rising temperatures and a shift in seasonal precipitation patterns toward more winter but less summer precipitation, as well as increased frequency and duration of drought events, are likely to have an impact on biogeochemical cycles. The amount of C sequestered in forest soils and the rate of N mineralization are potentially affected (Rustad et al., 2001; Janssens et al., 2010; Lu et al., 2013; Liu et al., 2017; Gan et al., 2021), with either positive or negative effects on the productivity of temperate forests.

In our study with mature European beech forests along a precipitation and temperature gradient covering several seasons, we show that increasing summer droughts might enhance soil C losses and affect N availability and N cycling by reducing net N_{min} and increasing microbial N immobilization. Overall, season had a greater influence on soil respiration rate, net N_{min}, and AA-N than soil texture.

Seasonal variation in soil respiration rate

In our study, soil respiration rate increased with decreasing MAP and also with actual precipitation in the mid-growing season, with the relationship being more pronounced for sandy plots. Soil respiration rate varied considerably both with season and with soil texture, showing significant differences between the early and mid-growing seasons as well as between the two soil textures.

In the mid-growing season (July 2015), plots with sandy soil texture had the highest soil respiration rates (**Table 2.2**; **Figure 2.5**B).

The increased soil respiration rate at the drier end of our gradient is most likely explained by increased root respiration, which is caused by increased fine root growth and turnover in drier and warmer soils. Using the data for fine root production and turnover of the year 2010 by Hertel et al. (2013), the measured soil respiration rate in mid-season 2015 was positively related to fine root production in both soil texture types, and it apparently was enhanced by a higher fine root turnover on the sandy plots. Compared to beech saplings, adult beech trees show a higher flexibility in fine root system size under varying soil moisture levels, i.e., along a precipitation gradient (Leuschner, 2020). Hertel et al. (2013) found an increase in fine root biomass, total fine root surface area, and fine root production in our study plots with reduced MAP and precipitation. This relationship was more pronounced in stands on sandy soil, which generally had a higher fine root biomass and productivity in comparison to stands with sandy-loamy soil texture (Hertel et al., 2013). However, Meier et al. (2018) found no significant change in fine root biomass with a MAP decrease for a subsample of these plots. Fine root biomass even decreased with reduced MAP along a precipitation gradient on acid sedimentary rock in central Germany (Meier and Leuschner, 2008).

Higher autotrophic respiration in the drier plots of the precipitation gradient in our study could also be due to higher fine root mortality in drier stands in mid-growing season, and caused by the subsequent compensatory production of new fine roots that replaced shedded older fine roots and were physiologically more active with a higher respiration rate (Eissenstat et al., 2000; Brunner et al., 2015). In fact, new root growth is a strategy to increase water and nutrient uptake if sufficient carbohydrate resources are available, resulting in increased root turnover, that leads to a rejuvenation of the root population (Leuschner, 2020). This is in accordance with findings that beech produces thin, ephemeral, absorptive, non-mycorrhizal fine roots with a high root surface area and enhanced respiratory activity as a fast response to drought (Nikolova et al., 2020).

In the study years 2014 and 2015, our study sites predominantly experienced normal conditions with regard to precipitation and potential evapotranspiration, with the exception of November 2014 and February 2015 (pronounced precipitation deficits) and April 2015 (moderate drought). However, as the SPEI cannot identify droughts of less than one month duration (Vicente-Serrano et al., 2010; Van der Schrier et al., 2011), it cannot be ruled out that our study sites experienced short-term drought events. Borken et al. (2002) discovered that soil respiration was limited by soil moisture during relatively dry and warm periods on sandy sites in Unterlüß, and that interannual variation in soil respiration was caused more by varying seasonal precipitation patterns than by the annual amount of precipitation. Rewetting events subsequent to a drought can cause considerable CO_2 flushes. Even if those CO_2 flushes may just have a minor influence on the annual mean respiration rate in this study area (Borken et al., 2002; but see Borken et al., 1999), temporally limited, i.e., short-term, precipitation patterns may influence seasonal nutrient fluxes. The underlying cause are soil microorganisms that react to water availability within a few minutes through enhanced microbial respiration. The enhanced microbial activity can last for weeks, depending on the length of the drought, precipitation duration and quantity, as well as soil temperature (Borken et al., 1999). Because of a negative relationship between mean annual precipitation and temperature in the studied gradient, we cannot disentangle both effects. Buchmann (2000), Borken et al. (2002), and Knohl et al. (2008) could show that in temperate forest ecosystems with relatively high precipitation, soil temperature is the most important factor influencing temporal variation in soil respiration. Schindelbacher et al. (2012) found in a 125-year-old *Picea abies*-dominated forest in Austria that warming increased soil respiration, whereas soil respiration decreased considerably when drought was imposed through throughfall exclusion. This highlights that warming causes increased soil C loss as long as precipitation and thus soil water availability are sufficient. Consistent with these results, Knohl et al. (2008) could show for a European beech forest in Germany that negative effects on soil respiration occurred when soil moisture sank below 23 vol.%.

In our plots, soil respiration rate was highest in the mid-growing season (July/August). We assume that soil respiration rate was enhanced by higher soil temperatures at the drier end of the precipitation gradient, as long as water availability was sufficiently high. As the negative relationship between soil respiration rate and MAP was observed in mid-growing season 2015, we assume that unfavorable temperatures may have reduced autotrophic and heterotrophic respiration at the wettest sites in the warm season. Fine root growth in European beech is typically highest between May and August (Leuschner, 2020), i.e., when air and soil temperatures are at their highest, resulting in higher soil respiration rates (Borken et al., 2002). For a temperate mixed forest, Ruehr and Buchmann (2009) showed that plant phenology in the form of aboveground C assimilation and belowground C allocation largely influences root respiration and its temperature sensitivity. Kuptz et al. (2011) could show that the supply of recent photosynthates to beech roots was rather low in the spring but increased toward early summer (July). Thus, the increased soil respiration rates in midsummer 2015 can be explained by both a higher root respiration due to an abundance of carbohydrates from recent assimilation and increased root growth (Ekblad and Högberg, 2001; Hopkins et al., 2013), and a higher heterotrophic respiration due to enhanced activity of free-living soil microbes, which may profit from priming via root exudates (Kuzyakov et al., 2000).

Effects of seasonal climatic changes on N cycling

Our results from the drier year 2014 show that net N_{min} increases with higher MAP in sandy and sandy-loamy soils, even though the relationship is only marginally significant. However, in our study, net N_{min} decreased with increasing MAT and actual air temperature in the late season (August to October) of 2014 for forest stands growing on sandy-loamy soil.

These results highlight that warming has a stronger effect on net N_{min} than the precipitation regime *per se*. This is in accordance with the results of the analyses by Rustad et al. (2001) and Li et al. (2019), which show a positive relationship between net N mineralization and MAP and MGSP. However, in contrast to our results, meta-analyses by Rustad et al. (2001) and Lu et al. (2013) found a positive effect of MAT and higher soil temperature on net N_{min}. These results were explained by the positive effect of higher temperatures on soil microorganism enzyme activity and an enhanced mineralization of SOM, especially in colder climates. This relationship holds only if soil water and/or SOM are sufficient and none of these factors become limiting, i.e., a trade-off between water and temperature (Zuccarini et al., 2020). Our results from 2014 suggest that a warming and drying climate may lead to reduced N mineralization in sandy and sandy-loamy beech forest soils. This is likely caused either by the suppression of genes in soil microbial communities involved in denitrification and ammonification (Yu et al., 2018) or by an increase in N pool sizes in soil and plants. An increased N pool would have a negative effect on microbial decomposition rates (Dijkstra et al., 2010; Lladó et al., 2017), i.e., an overall negative effect on N cycling due to decreased soil microbial and enzyme activity. The resulting impaired N mineralization may cause N limitation with consequences for plant productivity (Gessler et al., 2005; Kreuzwieser and Gessler, 2010).

Additionally, drought can change plants’ N uptake capacity, causing imbalances in plant tissue C/N/P stoichiometry and further nutritional limitations with negative effects on plant growth (Sardans and Peñuelas, 2005; Sardans et al., 2008; Dannenmann et al., 2016). Köhler et al. (2021) demonstrated that soil drought indeed reduces both N uptake efficiency and N use efficiencies in beech saplings. This has consequences for the tissue N:P ratio, causing P limitations and imbalances, which in turn can further negatively affect plant N nutrition.

In contrast to 2014, no effect of long-term precipitation or temperature patterns on N_{min} was found in the moister study year 2015. N_{min} was also independent from the actual precipitation and temperature regimes prior to sample collection.

As the precipitation and temperature regime influences soil microbial growth and activity, it also affects the form of inorganic N produced by the mineralization of SOM. In our study, we could find a much higher relative proportion of net NH_4^+ compared to net NH_3^-, contributing to plant-available inorganic N. Similarly, net NH_4^+ was the main driver for net N_{min} in the examined nutrient-poor soils. The threshold for the inhibition of bacterial nitrifying activity is a pH of 3.5–3.8 (Ribbons et al. 2018). Therefore, the inhibition of nitrification was probably caused by the low, close to threshold pH (H_2O) values between pH 4.4 and pH 4.1 in the examined soils (Hertel et al., 2013). Our results fit with the preference of beech for NH_4^+ uptake (Gessler et al., 1998; BassiriRad, 2000; Stoelken et al., 2010). Additionally, low and high temperatures, anaerobic and acidic soil conditions, as well as low soil moisture content, hamper the activity of enzymes and microbes involved in N mineralization (Miller and Cramer, 2005). Consequently, mineralization to NH_4^+ dominates in regions or seasons that are wet and cold and in soils with low soil pH, which corresponds to the characteristics of our study sites.

According to the linear mixed-effects model, only season had a significant effect on net N_{min}. This was primarily caused by the negative value of net N_{min} in sandy soils in mid-growing season 2015, which likely indicates microbial N immobilization. However, there were no significant differences in net N_{min} across the other four seasons studied. This is in contrast to the results of Kaiser et al. (2011), who found a summer N mineralization phase between July and August and a winter N immobilization phase between November and February. As we did not take samples between January and the beginning of April, we cannot make any statements regarding the winter phase.

Factors that affect soil microbe activity, like temperature, moisture, pH, and quality of SOM, regulate gross mineralization and immobilization and, thus, net N_{min} (Lladó et al., 2017; Li et al., 2019; Mukai et al., 2020). Mooshammer et al. (2014) could show that N-limited soil microbes sequester N into microbial biomass but release only small or no amounts of N back to the environment. This results in low N mineralization or N immobilization. Furthermore, C-limited soil microbes release a large fraction of organic N taken up as NH_4^+, i.e., which results in high N mineralization. We suggest, that soil microbes on our sandy study sites were N limited in mid-growing season 2015, as organic C (assimilates) was likely sufficiently available as indicated by seasonal high production of fine roots, i.e. high autotrophic respiration, and excretion of root exudates, i.e. priming of microbes, and hence, a higher heterotrophic respiration). This probably resulted in the observed N immobilization.

N mineralization also includes the rate-limiting step of depolymerization of proteins to organic, N-containing polymers and bioavailable N monomers, like amino acids, by extracellular enzymes (proteases) excreted, e.g., by mycorrhizal fungi (Schimel and Bennet, 2004; Mooshammer et al., 2014). These amino acids can then be further mineralized to inorganic NH_4^+, as shown for our study sites in spring 2015. However, in addition to inorganic N such as NH_4^+ or NO_3^-, organic N in the form of amino acids can contribute significantly to the N nutrition of temperate forests (Lipson and Näsholm, 2001; Näsholm et al., 2009; Rothstein, 2009). Dannenmann et al. (2009) found that European beech even preferred organic N over inorganic N. Amino acids can be taken up by mycorrhizal fungi with subsequent transfer to the tree or by the tree itself directly from the soil (Dannenmann et al., 2009; Stoelken et al., 2010; Simon et al., 2017; Leberecht et al., 2018). Soil amino acids originate, e.g., from proteolysis, are components of root exudates, are exuded by microbes (Rothstein, 2009), or result from the lysis of microbial dead and live cells (Brödlin et al., 2019). On the other hand, labile C originating from root exudates has the potential to stimulate the production of microbial enzymes. This in turn might further enhance the decomposition of SOM and, thus, increase soil AA-N concentration (Meier et al., 2017), e.g., in the ECM rhizosphere of beech (Brzostek et al., 2013).

Although we could not detect additional seasonal differences for net N_{min} in our forest stands, the measured soil AA-N concentration showed significant seasonal and spatiotemporal variability, with highest values in mid growing season (June/July) 2014 for both soil textures and in 2015 for stands growing on sandy soils. Soil AA-N decreased subsequently towards the non-growing season and had the lowest concentration in spring 2015 (early season; April/May). These findings could point to the enhanced mineralization of amino acids into inorganic N in the spring. The concentration of free amino acids in soil might increase with enhanced root exudation as well as microbial growth, activity, and biomass as a result of higher substrate availability (proteins and peptides) as well as due to a higher concentration and activity of proteases in the course of the growing season (Schimel and Bennett, 2004; Rothstein, 2009).

Higher temperatures and less precipitation in the mid-growing season may also result in temporally and spatially limited drought events. Thus, forest stands have a higher risk of experiencing drying-rewetting events. Leitner et al. (2017) demonstrated for a mature beech forest in Austria that rewetting dry soil results in a short-lived N flush due to the mobilization of NO_3^- and amino acids, which was exacerbated by increased drought duration, so that amino acids were the dominant plant-available N form.

Effect of soil properties on soil respiration, net N_{min} and AA-N concentration

Our results show that both soil respiration rate and net N_{min} are influenced by soil nutrient concentrations as well as additional soil properties such as soil texture or water storage capacity, whereas AA-N was affected by soil texture alone. We demonstrate that sandy soils are prone to C losses in a future warmer and drier climate.

Independent of soil texture, higher P_{tot} in the organic and mineral soil layers for sandy soils in mid-season 2015 and higher P_a in mineral soil in early season 2015 increased C loss in our study sites. This is likely explained by the low P availability in our study sites, which might be further intensified by the measured low pH as well as by increasing N deposition and resulting soil acidification. Higher soil P_{tot} and P_a content may help soil microorganisms to overcome their P limitation, resulting in increased microbial activity (Cleveland and Townsend, 2006; but see Feng and Zhu, 2019 or Preece et al., 2020). Here, in mid-growing season, the assumed increased release of root exudates might stimulate microbial activity and abundance and, thus, P mobilization and soil P availability (Spohn et al., 2013; Clausing et al., 2021; Michas et al., 2021). Our results are in accordance with Borken et al. (2002), who also found a positive relationship between soil respiration and total P content in a mature beech forest in Unterlüß, suggesting an increased microbial decay of SOM with higher soil P content.

Furthermore, the highest values for soil respiration rate were measured in mid-growing season 2015 for sandy soils, indicating an interactive effect of season and soil texture. The negative relationship between soil respiration rate and MAP was especially pronounced for stands with sandy soils. Independent of season, only sandy soils responded to rising T_{soil} with increasing C losses, demonstrating the vulnerability of sandy soils to C losses in a future warmer and drier climate. Compared to the finer-textured loamy soils, sandy soils have in general a lower water storage capacity and show a higher porosity with large interconnecting pores, such that CO_2 and water can diffuse more easily through soil pores into and out of the soil (Bouma and Bryla, 2000). In comparison to sandy soils, loamy soils are characterized by a higher portion of clay minerals, which have a predominantly negative surface charge. Thus, loamy soils can adsorb cations like NH_4^+ more easily, resulting in sandy soils showing generally lower nutrient availability. Furthermore, sandy soils have a higher risk of suffering from edaphic drought and experiencing drying with subsequent rewetting events and CO_2 flushing. As a result, forest stands with a deficiency of water may experience a disruption of the soil water film, causing a stop of nutrient diffusion and the death or inactivity of soil micro-organisms. The resulting enhanced accumulation of substrate and soil nutrients is followed by its release due to rewetting. This in turn leads to the release of amino acids or other compounds through cell lysis (Schimel et al., 2007; Bimüller et al., 2014; Leitner et al., 2017; Brödlin et al., 2019; Gao et al., 2020). This likely explains the higher soil AA-N concentration in sandy soils in the mid-growing season of 2015. Here, finer-textured loamy soils might provide more suitable and sufficient soil moisture conditions for soil organisms in the organic layer over a longer time period (Cable et al., 2008).

Surprisingly, net N_{min} was not influenced by soil texture but by soil nutrient concentrations. In late season 2014, net N_{min} was enhanced by higher soil N_{tot} in the organic layer and increasing soil C_{org} but decreased with increasing P_{tot} in the organic layer (sandy soils) or mineral soil layer (sandy-loamy soils). We assume that soil C_{org} serves as an important energy source for N cycling (Booth et al., 2005), which is in line with the observed positive relationship between C and N. The negative relationship between N and P could, however, be explained by the occurrence of phosphatase enzymes, which are exuded by plants and soil microbes to mobilize P and make it bioavailable. Phosphatase contains a large amount of N (~15% N; Treseder and Vitousek, 2001). Hence, organisms have to invest N to gain P (Houlton et al., 2008; Marklein and Houlton, 2012). Furthermore, we found an influence of soil physics on net N_{min}. This positive effect of a higher water storage capacity on net N_{min} of the sandy soils in the late-growing season of 2014 likely shows the positive effect of soil water on soil microbes involved in N cycling.

Our results likely demonstrate that in terms of net N_{min} the resident soil microbial community responsible for N cycling is adapted to different soil textures and reacts very differently depending on seasonal shifts in soil nutrient concentrations.

Effect of N deposition on the C and N cycles

In our study, we could show that N deposition curtails soil respiration rate in mid-growing season (July to August) and enhances net N_{min} in late-growing season (August to October), but has no effect on soil AA-N concentration.

Our study sites experienced a mean annual N deposition (sum of dry, moist, and wet N deposition) of 14 kg ha^{-1} yr^{-1} at the drier end of the study gradient up to 19 kg ha^{-1} yr^{-1} in the wetter study sites for the time period 2013–2015 (Schaap et al., 2018). These values are in the range of critical N loads for beech (10–20 kg ha^{-1} yr^{-1}; Bobbink and Hettelingh, 2010). However, soil N availability can be assumed to be sufficient, with N not being an overall limiting nutrient for the growth of our beech stands. We found an increase in soil N_{tot} with increasing precipitation (N_{tot} ~ MAP; $R = 0.99$, $P \leq 0.001$), resulting in a positive relationship between N deposition and soil N_{tot} in the organic layer (only for sandy-loamy soils; N_{tot} ~ N deposition; $R = 0.87$, $P = 0.053$). Lower soil N_{tot} could be attributed to lower wet nitrogen deposition on the more continental sites at the drier end of our study gradient. However, N deposition effects may interfere with MAP effects, and the underlying mechanism cannot be clearly distinguished. According to Blume et al. (2010), N_{tot} is expected to range between 0.7 and 1.4 mmol g^{-1} in the organic layer and between 0.1 and 0.4 mmol g^{-1} in the mineral soil. Hence, the observed N_{tot} concentrations for the organic layer between 1.0 and 1.3 mmol g^{-1} were in the medium or upper medium range for forest soils, whereas the values for mineral soil between 0.1 and 0.2 mmol g^{-1} were in the lower margins (see **Table S2.1**). These results emphasize the importance of the organic layer for the nutrient supply of our studied beech stands. This might also be the result of the highly acidic soil conditions that hamper nutrient cycling and nutrient translocation into deeper soil layers. Hence, elevated N deposition in combination with a higher risk for drought due to increasing temperatures and a shift in seasonal precipitation patterns is assumed to change nutrient availability and stoichiometry (Peñuelas et al., 2013; Sardans et al., 2016; Köhler et al., 2021).

In accordance with our results, Janssens et al. (2010) and Zhou et al. (2014) showed in their meta-analyses that N deposition reduces soil respiration. This reduction in soil respiration can be explained by a decrease in belowground C allocation or root exudation rates with negative effects on the activity and growth of ECMF symbionts and other SOM degrading soil microorganisms, i.e., resulting in a reduction in autotrophic and heterotrophic respiration (Janssens et al., 2010; Zhang et al., 2018). Here, increasing acidification due to increased N deposition has the potential to reduce the activity of soil microorganisms and the functioning of SOM-degrading enzymes (Janssens et al., 2010; Tian and Niu, 2015; Cheng et al., 2019; Zuccarini et al., 2021).

Furthermore, with higher N availability, beech trees might reduce their C investment in below-ground organs responsible for N uptake at the expense of root production, which likely results in decreased autotrophic respiration (Janssens et al., 2010). This fits with the results of Hertel et al. (2013), who showed for the same study gradient a decreasing fine root biomass and production for stands characterized by higher precipitation and, thus, higher N deposition. This might also explain the observed negative relationship between soil respiration rate and MAP in our study.

However, the deposition of additional reactive N may stimulate the activity of soil microbes and thus net N_{min} in the late growing season due to positive effects on substrate quantity and quality (Lu et al., 2011; Cheng et al., 2019).

Conclusion

We conclude from this climate transect study that increasing temperatures in combination with increasing summer drought frequency and severity have the potential to significantly increase soil respiration rate in beech forests on sandy to sandy-loamy soils, while N mineralization likely will decrease in a future warmer and drier climate. However, N deposition might attenuate negative climate effects on N mineralization. While there is still a lack of knowledge about how the mycorrhizal symbiosis of beech and the soil microbiota in mature beech stands and their N demand are responding to a warmer and drier climate, a major change in the C and N dynamics in the soil is to be expected. Moreover, both increased root respiration and reduced soil N supply, as a possible outcome of future warming, will likely impair beech growth and productivity in the future, thereby further deteriorating the growth climate of beech in many regions of Central Europe, in addition to direct drought and heat effects. Warming and drying experiments in beech forests are needed to test this hypothesis on the belowground consequences of climate warming.

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Supplementary Material

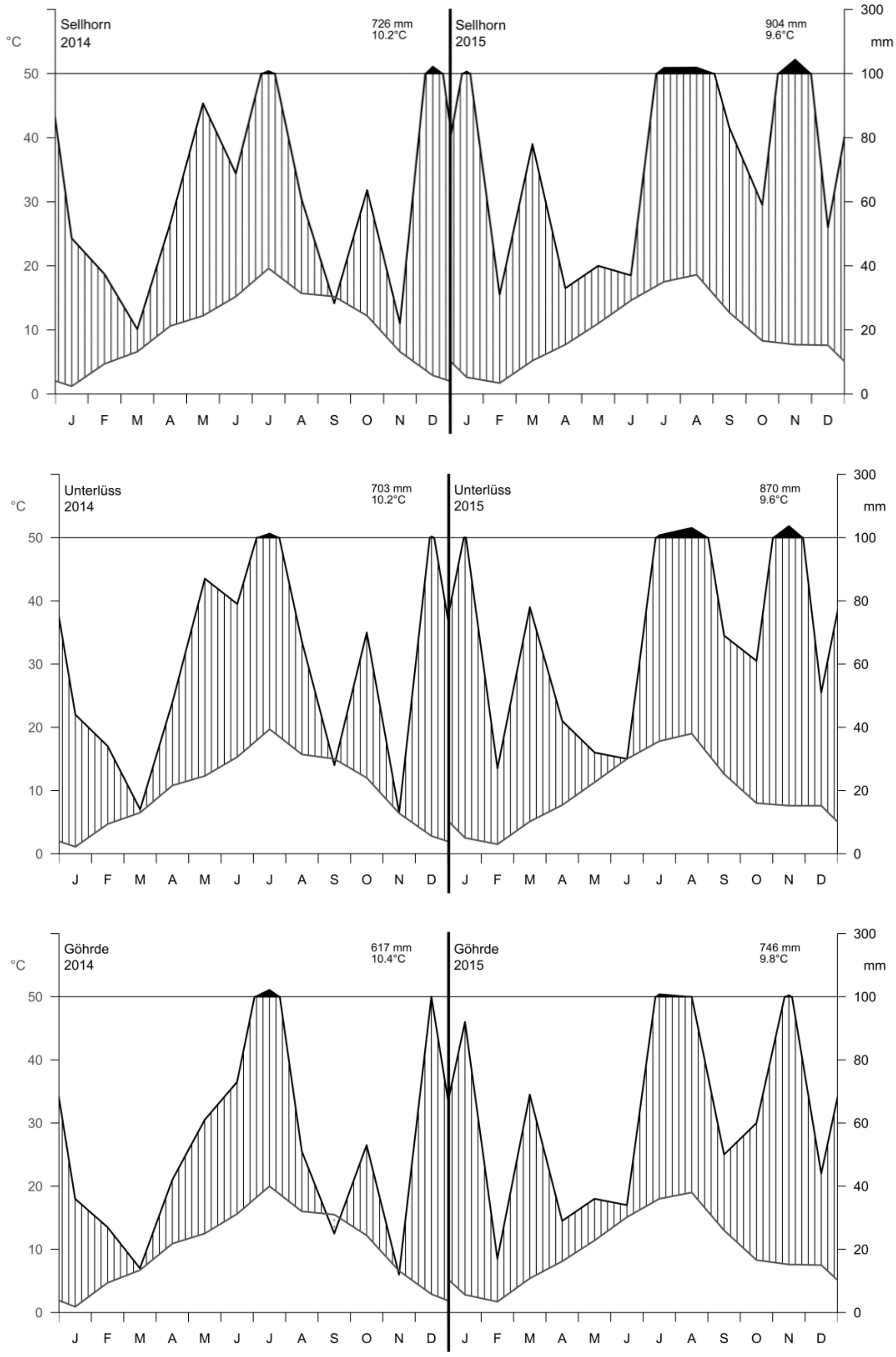
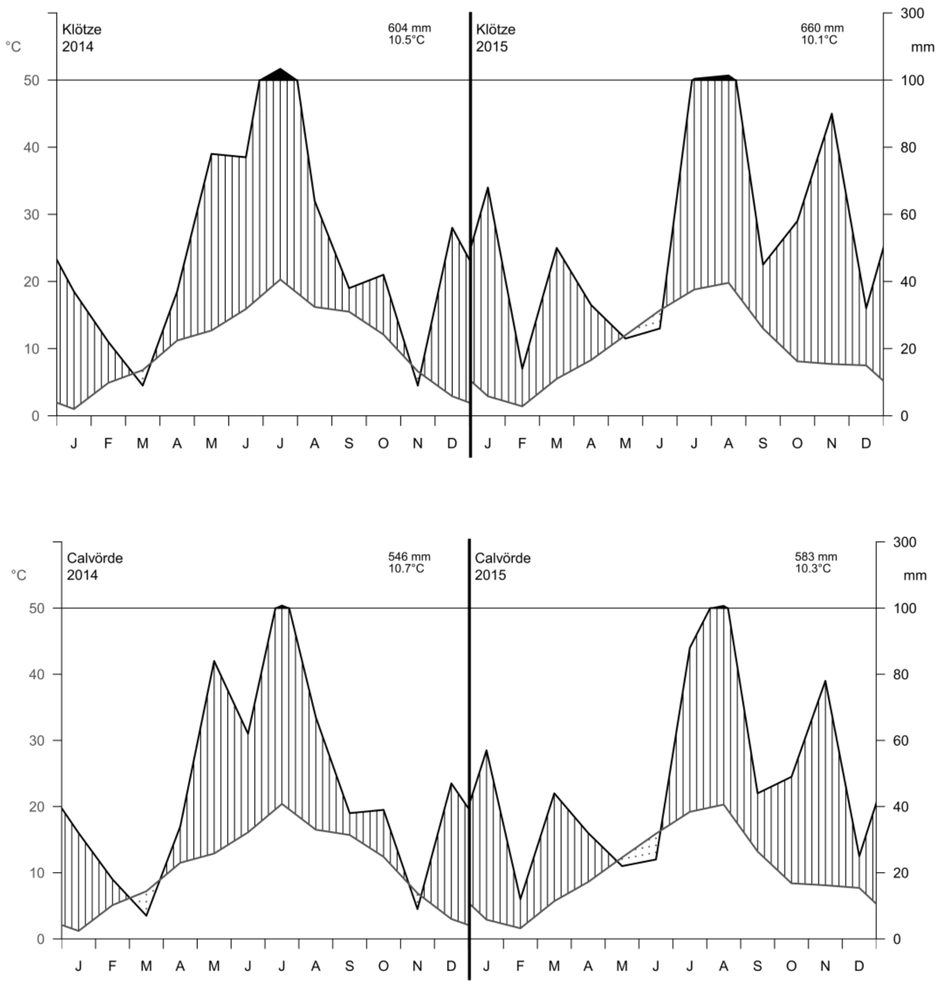


Figure S2.1 Climate diagrams for the five investigated study sites along the precipitation gradient in northwestern Germany (from west to east with decreasing precipitation: Sellhorn, Unterlüss, Göhrde, Klötze and Calvörde, respectively). Given are the annual precipitation, the mean annual temperature for the study years 2014 (left-hand) and 2015 (right-hand), respectively. Values are averaged over plots with different soil textures (loamy or sandy, respectively). Climate data obtained from the 1 x 1 km grid data set from of the German Weather Service (Deutscher Wetterdienst, Offenbach, Germany). Precipitation: black line, temperature: grey line.



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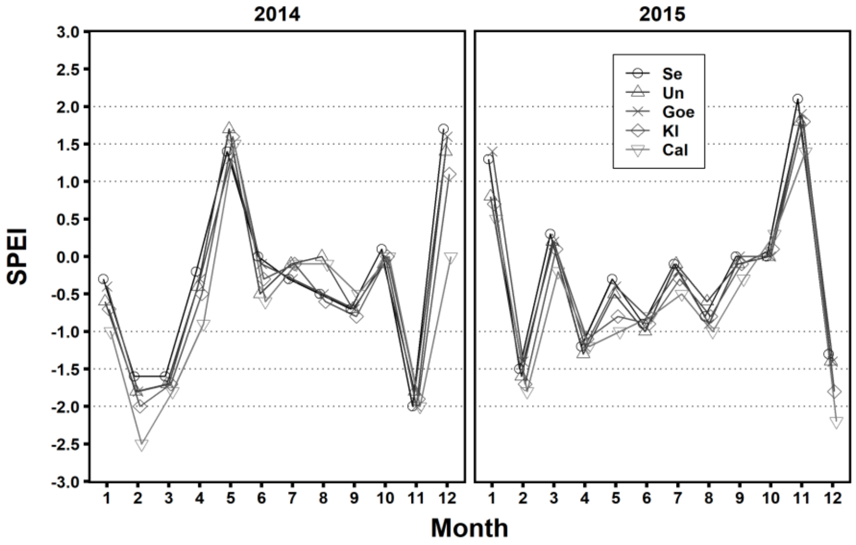


Figure S2.2 Standardized precipitation-evapotranspiration index (SPEI; Vicente-Serrano et al., 2010) for the five investigated study sites along the precipitation gradient in northwestern Germany (from west to east with decreasing precipitation: Se = Sellhorn, Un = Unterlüss, Goe = Göhrde, Kl = Klötze and Cal = Calvörde, respectively). Given are the monthly values for the years 2014 and 2015. Data obtained from the global SPEI database (http://SPEI.csic.es/database.html). Classification according to McKee et al. (1993) and Ye et al. (2015): SPEI ≥ 2.0 extreme wet, SPEI < 2.0 to ≥ 1.5 severe wet, SPEI < 1.5 to ≥ 1.0 moderate wet, SPEI < 1.0 to > -1.0 normal, SPEI ≤ -1.0 to > -1.5 moderate dry, SPEI ≤ -1.5 to > -2.0 severe dry, SPEI ≤ -2.0 extreme dry; see dotted lines.

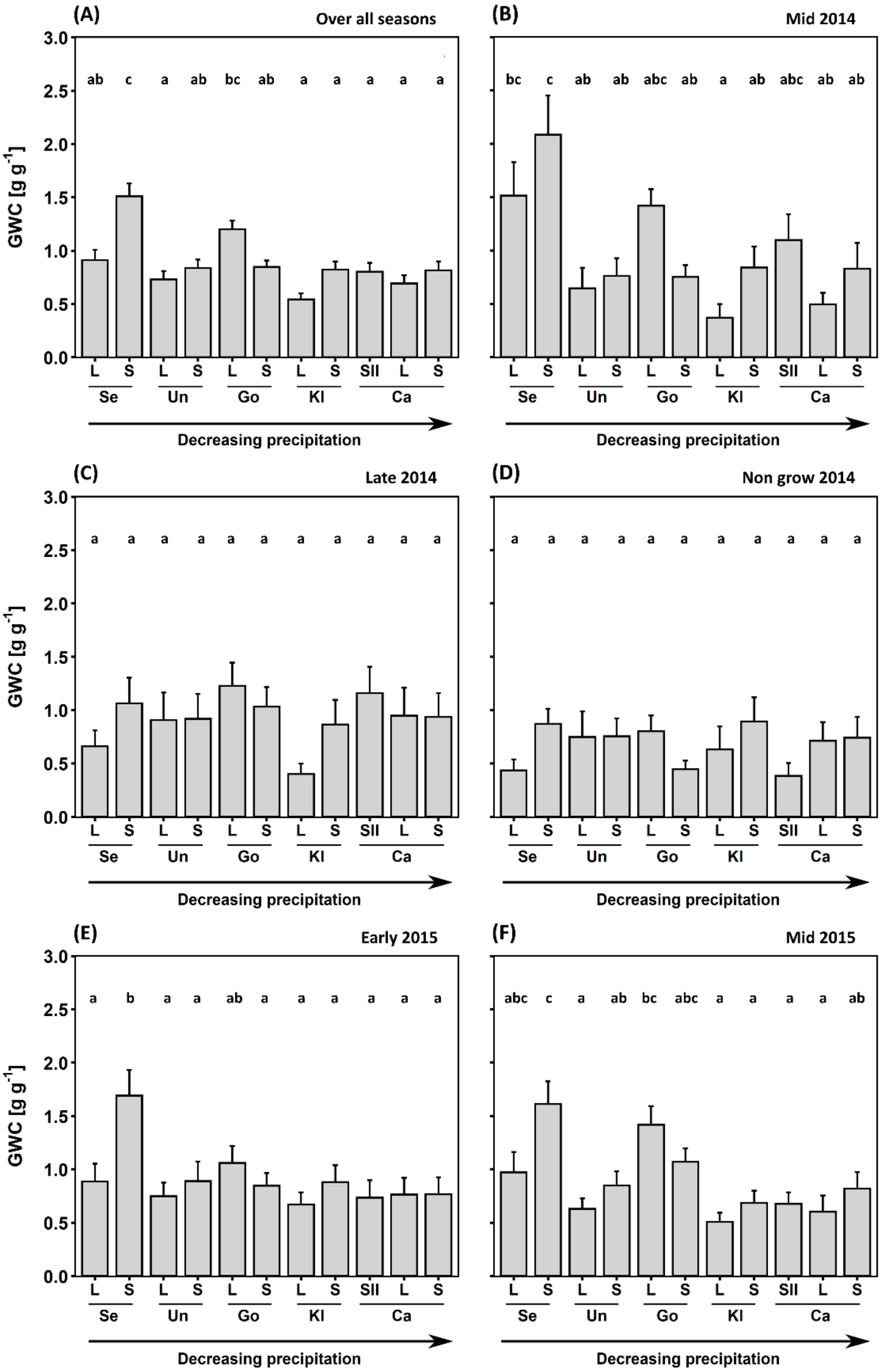


Figure S2.3 Means and standard errors of gravimetric water content (GWC) at the depth of 10 cm for the 11 study sites along the precipitation gradient (from high to low precipitation; Se: Sellhorn, Un: Unterlüss, Go: Göhrde, Kl: Klötze, Ca: Calvörde) on loamy-sandy (L) and sandy (S) soil **(A)** over all 5 analyzed seasons, three seasons in 2014 (**(B)** mid = June/July to August; **(C)** late = August to October; **(D)** non grow = October to December), and two seasons in 2015 (**(E)** early = April/May to June; **(F)** mid = July to August). Bars with different lower-case letters indicate significant differences based on a Kruskall-Wallis test at $P \leq 0.05$. 2014: $n = 12$; 2015: $n = 20$.

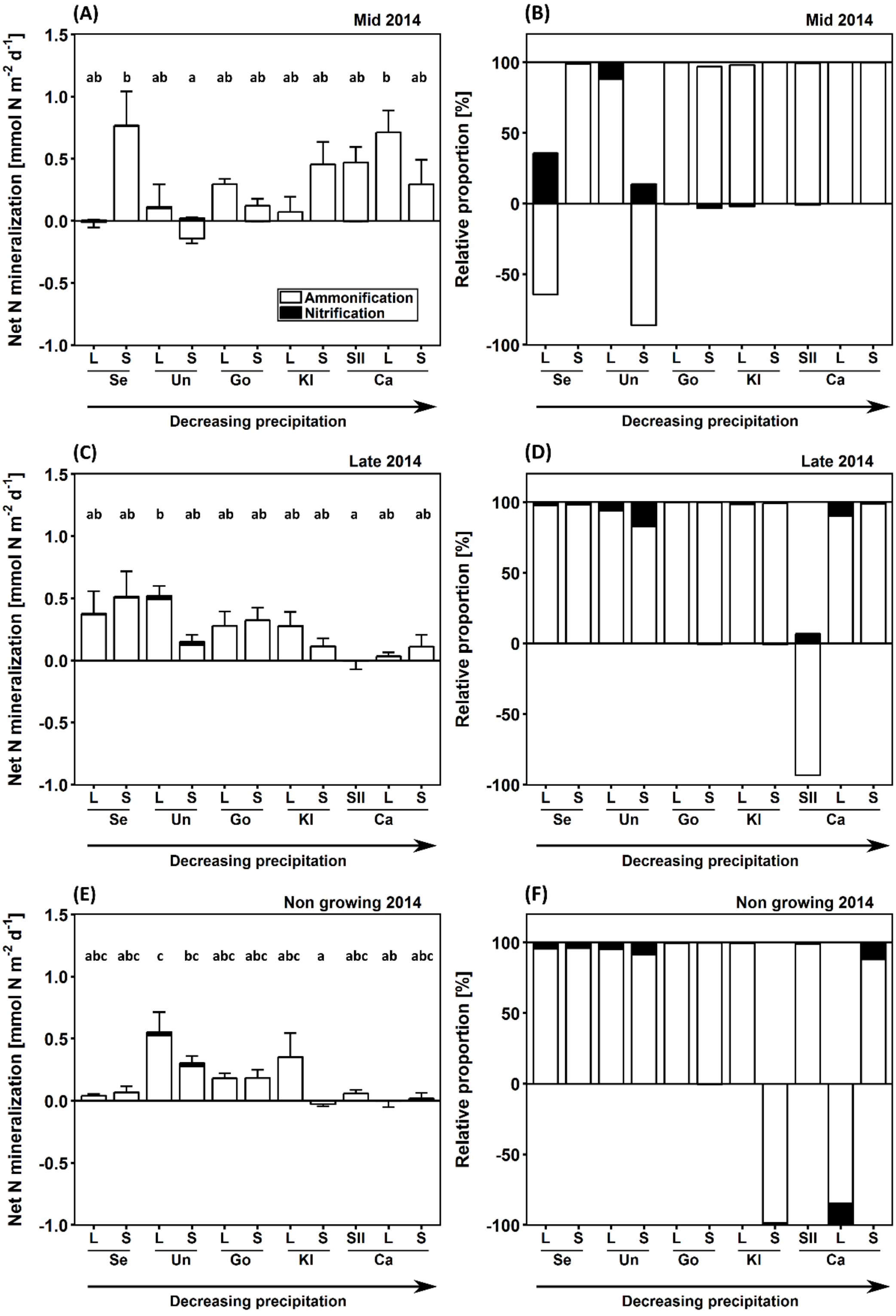
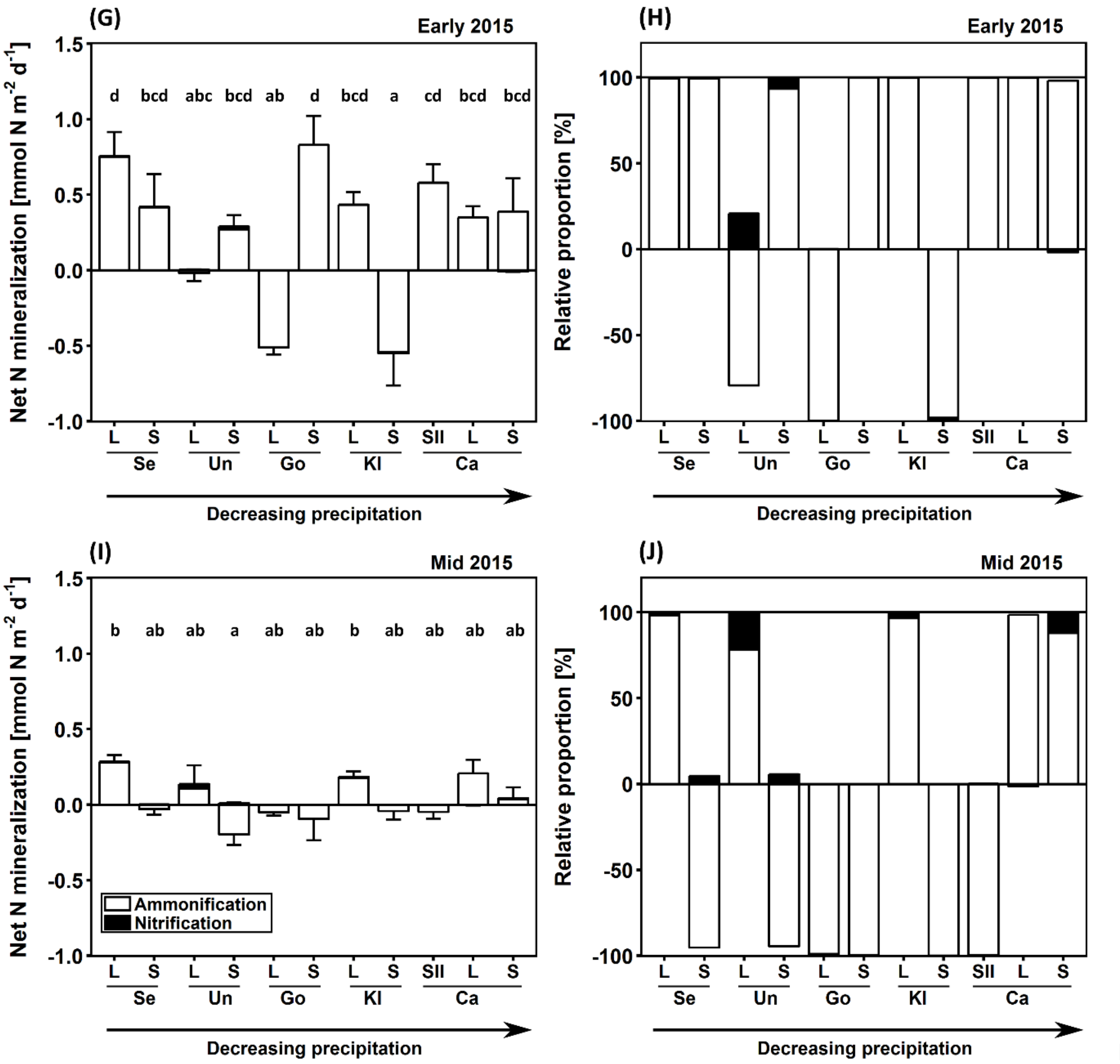


Figure S2.4 Means and standard errors of net ammonification rate and net nitrification rate (A, C, E, G, I), and their relative proportion (B, D, F, H, J) in the net N mineralization rate for a depth of 10 cm for the eleven study sites along a precipitation gradient (from high to low precipitation; Se: Sellhorn, Un: Unterlüss, Go: Göhrde, Kl: Klötze, Ca: Calvörde) on loamy-sandy (L) and sandy (S) soil for three seasons in 2014 (mid = June/July to August; late = August to October; non growing = October to December), and two seasons in 2015 (early = April/May to June; mid = July to August). White = net ammonification; black = net nitrification. Bars with different lower-case letters indicate significant differences based on Kruskall-Wallis test at $P \leq 0.05$.



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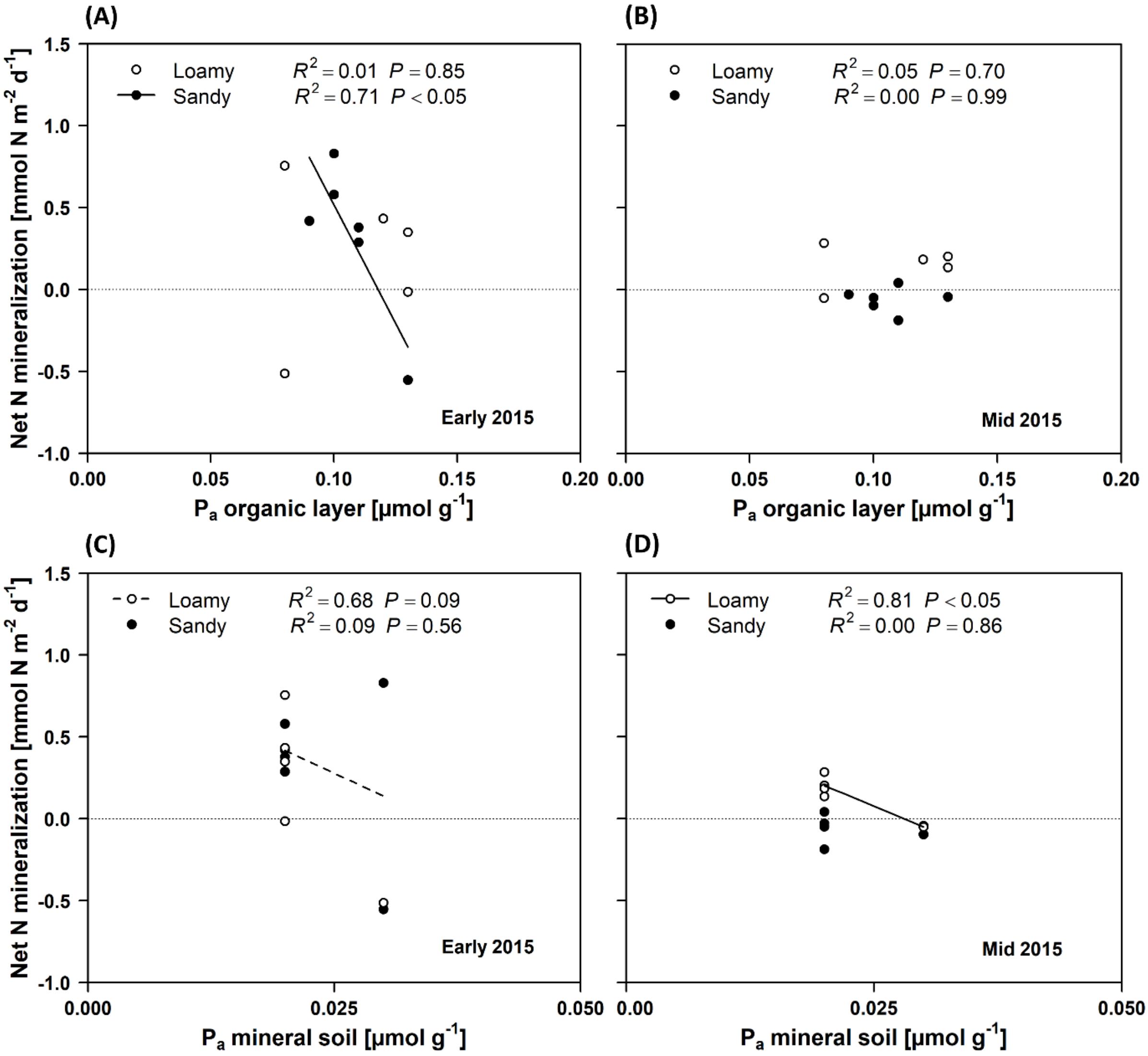

Figure S2.5 Relationship between net N mineralization rate in 10 cm depth and plant-available phosphorus (P_a) concentration in **(A, B)** organic layer or **(C, D)** mineral topsoil in sandy-loamy (Loamy; white) and sandy (Sandy; black) soil under beech stands for two seasons in 2015 (early = April/May to June; mid = July to August). Data for P_a in organic layer and mineral soil (0-10 cm) are from the year 2013. Mean values are shown for each stand with sandy-loamy (n = 5) and sandy (n = 6) soil texture. Significant ($P \leq 0.05$) relationships are indicated by continuous lines. Marginally significant ($P \leq 0.1$) relationships are indicated by dashed lines. Equations: (A) sandy: y = -28.94x + 3.4; (B) n. a.; (C) loamy: y = -89.32x + 2.2; (D) loamy: y = -25.15x + 0.7.

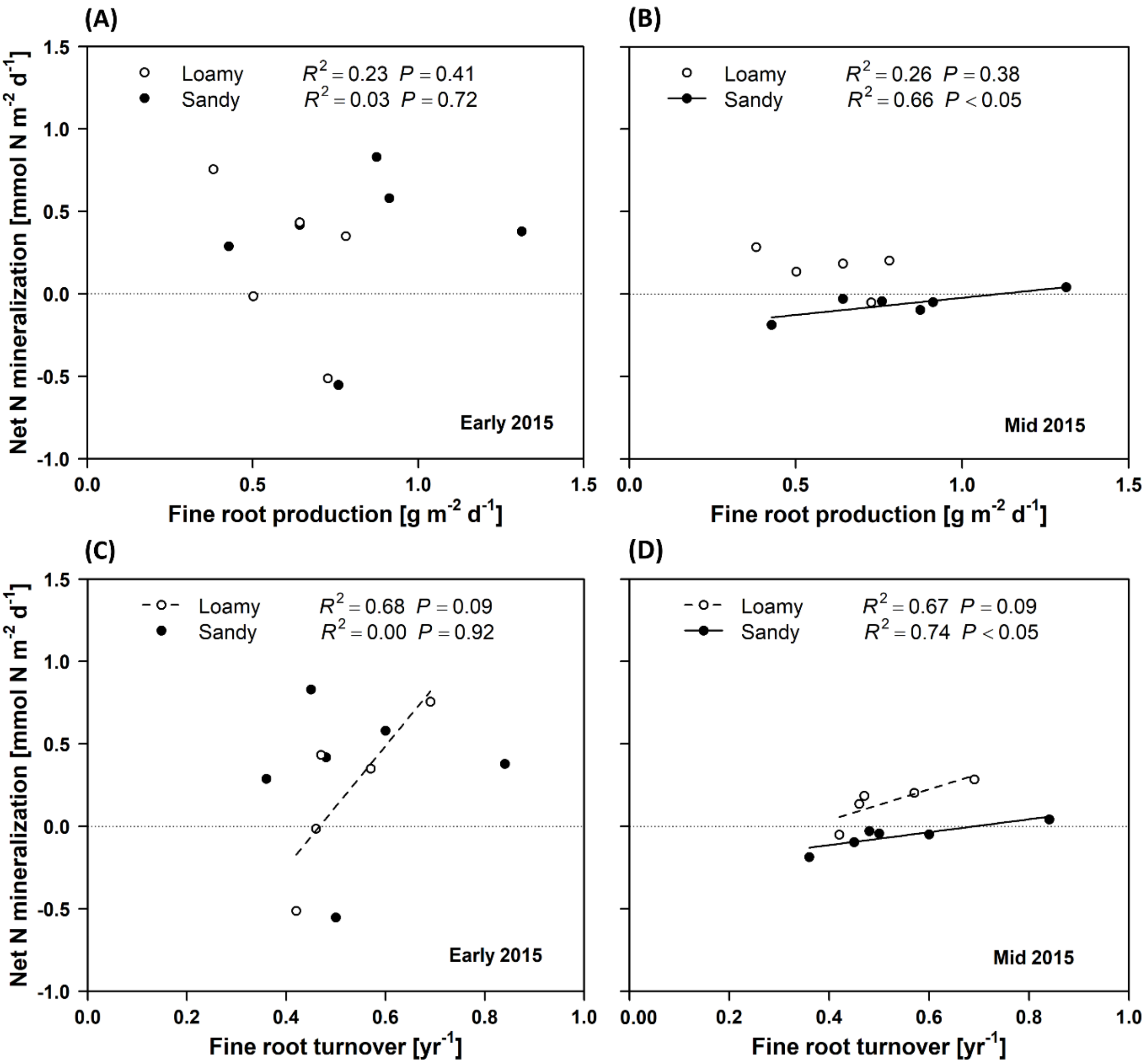

Figure S2.6 Relationship between net N mineralization rate in 10 cm depth and **(A, B)** fine root production or **(C, D)** fine root turnover in sandy-loamy (Loamy; white) and sandy soil (Sandy; black) under beech stands for two seasons in 2015 (early = April/May to June; mid = July to August). Data for annual fine root production and fine root turnover (organic layer and 0-20 cm of mineral soil measured in the year 2010) were taken from Hertel et al. (2013). Mean values are shown for each stand with sandy-loamy (*n* = 5) and sandy (*n* = 6) soil texture. Significant ($P \leq 0.05$) relationships are indicated by continuous lines. Marginally significant ($P \leq 0.1$) relationships are indicated by dashed lines. Equations: (A) n. a.; (B) sandy: y = 0.00x - 0.2; (C) loamy: y = 3.67x- 1.7; (D): loamy: y = 0.94x - 0.3, sandy: y = 0.39x - 0.3.

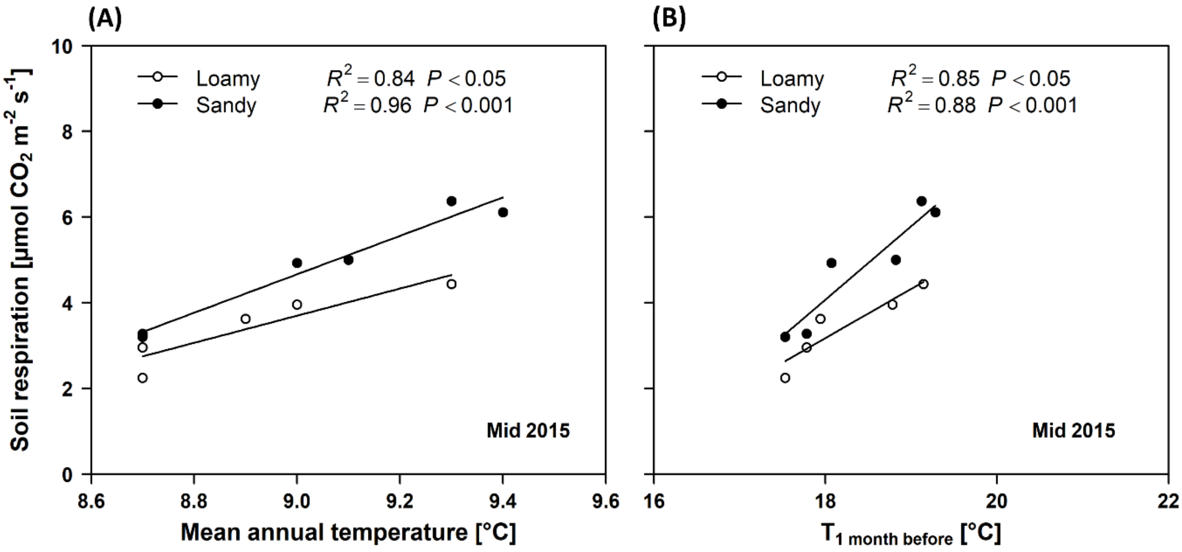

Figure S2.7 Relationship between soil respiration rate and **(A)** mean annual temperature (period 1981-2010) and **(B)** mean temperature 1 month before measurement ($T_{1\ \mathrm{month\ before}}$) at the 11 sites. Mean values are shown for each stand with sandy-loamy (n = 5) or sandy (n = 6) soil texture, respectively, for mid-growing season (July) 2015. Significant ($P \leq 0.05$) relationships are indicated by continuous lines. Equations: (A) loamy: $y = 3.17x - 24.8$; sandy: $y = 4.49x - 35.7$; (B) loamy: $y = 1.15x – 17.5$, sandy: $y = 1.71x – 26.8$.

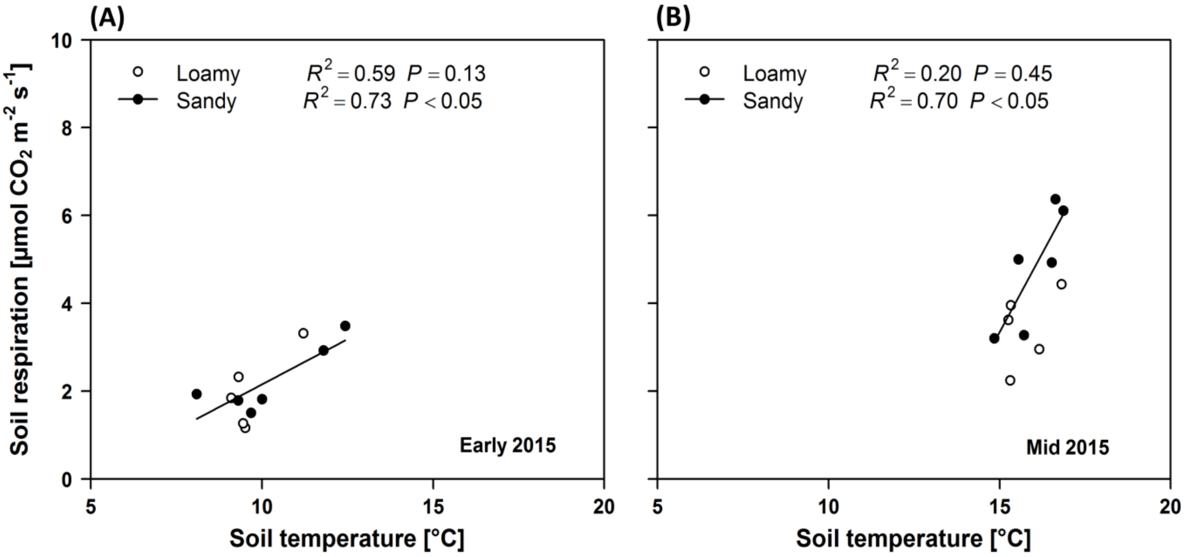

Figure S2.8 Relationship between soil respiration rate and soil temperature in the sample from 11 sites. Mean values are shown for each stand on sandy-loamy (n = 5) or sandy (n = 6) soil, respectively, for **(A)** early (April/May) or **(B)** mid-growing season (July) 2015. Significant ($P \leq 0.05$) relationships are indicated by continuous lines. Equations: sandy early 2015: $y = 0.41x - 2.0$; sandy mid 2015: $y = 1.44x - 18.3$.

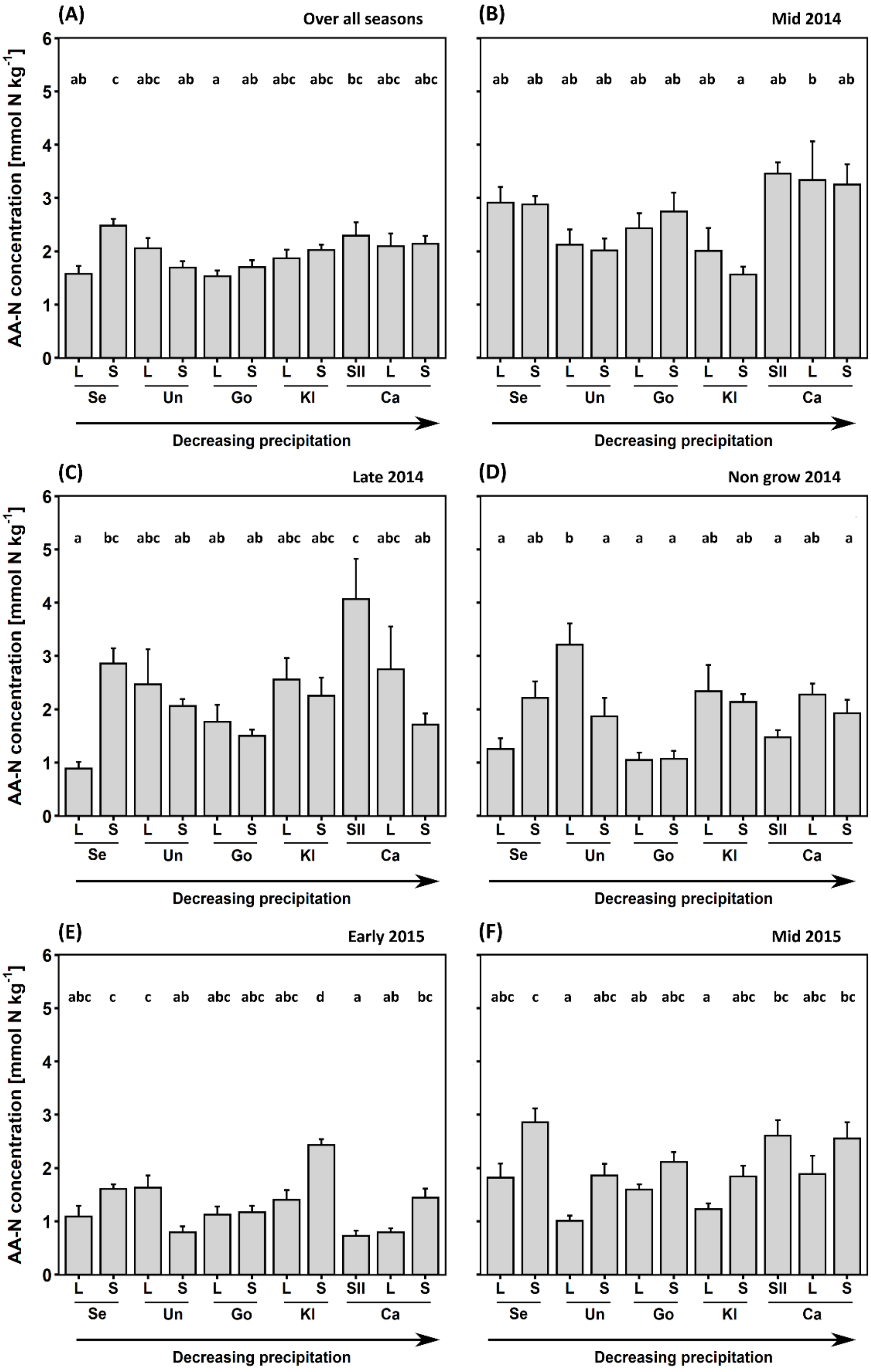

Figure S2.9 Means and standard errors of soil amino acid (AA)-N concentrations at the depth of 10 cm for the 11 study sites along the precipitation gradient (from high to low precipitation; Se: Sellhorn, Un: Unterlüss, Go: Göhrde, Kl: Klötze, Ca: Calvörde) on loamy-sandy (L) and sandy (S) soil **(A)** over all 5 analyzed seasons, three seasons in 2014 (**(B)** mid = June/July to August; **(C)** late = August to October; **(D)** non growing = October to December), and two seasons in 2015 (**(E)** early = April/May to June; **(F)** mid = July to August). Bars with different lower-case letters indicate significant differences based on a Kruskall-Wallis test at $P \leq 0.05$. 2014: $n = 8$; 2015: $n = 10$.

Table S2.1 Organic carbon(C_{org}), total nitrogen (N_{tot}), total phosphorus (P_{tot}) and plant-available phosphorus (P_a) content and N:P ratio (organic layer and 0-10 cm of mineral soil for the year 2013), annual fine root production and fine root turnover (organic layer and 0 - 20 cm of mineral soil for the year 2010; data taken from Hertel et al. (2013)) and water storage capacity (taken from Hertel et al. (2013)) of the 11 investigated beech stands on loamy-sandy (L) and sandy (S) soil in northwestern Germany. Study sites from west to east: Sellhorn (Se), Unterlüss (Un), Göhrde (Go), Klötze (Kl) and Calvörde (Ca). For additional information regarding stand structural properties of the investigated beech stands see Müller-Haubold et al. (2013).

Site Code	**C_{org} Organic layer** (mmol g^{-1})	**C_{org} Mineral soil** (mmol g^{-1})	**N_{tot} Organic layer** (mmol g^{-1})	**N_{tot} Mineral soil** (mmol g^{-1})	**Fine root production** (g m^{-2} yr^{-1})	**Fine root turnover** (yr^{-1})	**Water storage capacity** (mm 120 cm^{-1})
Se L	28.24	3.53	1.13	0.14	139	0.48	90
Se S	33.37	4.16	1.30	0.12	234	0.69	111
Un L	27.46	2.55	1.16	0.10	183	0.46	95
Un S	27.74	3.03	1.12	0.11	156	0.36	79
Go L	30.06	5.42	1.08	0.19	265	0.42	78
Go S	31.73	5.36	1.17	0.20	319	0.45	107
Kl L	25.08	2.04	1.04	0.10	234	0.47	124
Kl S	30.05	3.00	1.16	0.12	277	0.50	90
Ca L	27.63	3.54	1.05	0.13	285	0.57	140
Ca S	28.38	3.65	1.13	0.15	479	0.84	81
Ca S II	25.87	2.71	1.06	0.10	333	0.60	46

Table S2.1 Continued.

Site Code	**P_{tot} Organic layer** (µmol g^{-1})	**P_{tot} Mineral soil** (µmol g^{-1})	**P_a Organic layer** (µmol g^{-1})	**P_a Mineral soil** (µmol g^{-1})	**N:P Organic layer** (mol mol^{-1})	**N:P Mineral soil** (mol mol^{-1})
Se L	17.79	3.15	0.08	0.02	65.04	47.78
Se S	18.82	2.54	0.09	0.02	69.18	43.42
Un L	23.28	2.54	0.13	0.02	50.81	36.75
Un S	21.61	3.39	0.11	0.02	53.06	32.58
Go L	15.82	3.19	0.08	0.03	69.94	54.79
Go S	21.10	4.30	0.10	0.03	56.67	45.66
Kl L	20.46	3.99	0.12	0.02	51.41	25.09
Kl S	21.36	3.51	0.13	0.03	54.90	34.97
Ca L	24.78	4.38	0.13	0.02	43.39	29.58
Ca S	23.27	5.46	0.11	0.02	49.09	27.09
Ca S II	23.33	4.34	0.10	0.02	49.23	23.66

Table S2.2 Means, standard errors (SE) and coefficient of variation (CV in %) of net ammonification rate, net nitrification rate, net N mineralization rate, soil amino acid N concentration (AA-N) and soil respiration averaged over all five seasons (i.e. three seasons in 2014 (mid = June/July to August; late = August to October; non-growing = October to December), and two seasons in 2015 (early = April/May to June; mid = July to August) for the eleven study sites along the precipitation gradient (from high to low precipitation; Se: Sellhorn, Un: Unterlüss, Go: Göhrde, Kl: Klötze, Ca: Calvörde) on loamy-sandy (L) and sandy (S) soil, respectively; for soil respiration averaged over the two seasons in 2015 only. Within a column, different lowercase letters mark significant differences between study plots based on a Kruskall-Wallis test at $P \leq 0.05$.

		Net ammonification rate [µmol NH_4^+-N m^{-2} 10 cm^{-1} d^{-1}]					**Net nitrification rate [µmol NO_3^--N m^{-2} 10 cm^{-1} d^{-1}]**					**Net N mineralization rate [µmol N m^{-2} 10 cm^{-1} d^{-1}]**				
Site	**Soil texture**	**Mean**		**SE**		**CV [%]**	**Mean**		**SE**		**CV [%]**	**Mean**		**SE**		**CV [%]**
Se	**L**	361.51	±	84.81	^{b}	28.2	5.22	±	1.39	^{b}	27.7	366.73	±	85.32	^{b}	28.3
Se	**S**	356.32	±	97.28	^{b}	36.8	4.65	±	1.15	^{b}	26.6	360.97	±	97.35	^{b}	36.6
Un	**L**	264.31	±	67.84	^{ab}	23.2	20.87	±	4.19	^{c}	49.4	285.18	±	69.62	^{ab}	23.4
Un	**S**	79.31	±	49.21	^{ab}	20.6	20.59	±	2.70	^{c}	34.0	99.89	±	50.04	^{ab}	20.5
Go	**L**	98.69	±	55.26	^{ab}	23.6	-0.11	±	0.26	^{ab}	7.1	98.58	±	55.23	^{ab}	23.5
Go	**S**	296.41	±	72.89	^{b}	27.8	-1.18	±	0.49	^{ab}	14.3	295.23	±	73.03	^{b}	27.8
Kl	**L**	308.03	±	58.06	^{b}	20.1	2.96	±	0.89	^{ab}	19.5	310.99	±	58.09	^{b}	20.0
Kl	**S**	-66.46	±	97.44	^{a}	47.2	-3.41	±	1.08	^{a}	34.2	-69.87	±	97.35	^{a}	47.4
Ca	**L**	240.72	±	59.89	^{ab}	22.1	0.46	±	0.54	^{ab}	13.5	241.19	±	59.95	^{ab}	22.1
Ca	**S**	181.00	±	72.49	^{ab}	28.0	-0.11	±	1.48	^{ab}	38.2	180.89	±	72.32	^{ab}	27.9
Ca	**SII**	247.86	±	66.69	^{ab}	26.3	-0.05	±	0.41	^{ab}	11.3	247.81	±	66.69	^{ab}	26.3

Table S2.2 Continued.

		AA-N [mmol AA-N kg^{-1}]					**Soil respiration rate [µmol CO_2 m^{-2} s^{-1}]**				
Site	**Soil texture**	**Mean**		**SE**		**CV [%]**	**Mean**		**SE**		**CV [%]**
Se	**L**	1.58	±	0.15	^{ab}	61	1.71	±	0.29	^{a}	75.3
Se	**S**	2.48	±	0.13	^{c}	33.6	2.51	±	0.28	^{abc}	50.7
Un	**L**	2.05	±	0.19	^{abc}	59.5	2.07	±	0.24	^{ab}	50
Un	**S**	1.69	±	0.12	^{ab}	45.8	2.44	±	0.31	^{abc}	56.2
Go	**L**	1.53	±	0.11	^{a}	46.7	3.48	±	0.24	^{bcd}	29.8
Go	**S**	1.70	±	0.13	^{ab}	49.2	4.25	±	0.33	^{d}	34
Kl	**L**	1.87	±	0.16	^{abc}	57.5	3.10	±	0.27	^{abcd}	38.5
Kl	**S**	2.03	±	0.10	^{abc}	30.8	3.97	±	0.35	^{cd}	39.7
Ca	**L**	2.10	±	0.24	^{abc}	70.2	3.08	±	0.37	^{abcd}	51.7
Ca	**S**	2.14	±	0.15	^{abc}	46.0	3.95	±	0.6	^{cd}	67.5
Ca	**SII**	2.29	±	0.25	^{bc}	68.4	4.03	±	0.57	^{cd}	58.4

Table S2.3 Pearson correlation analysis for the relationships between mean net N mineralization rate (net N_{min}; 10 cm depth) and long-term means of climate factors (MAT = mean annual temperature; MAP = mean annual precipitation; MGSP = mean growing season precipitation (April - September); MSP = mean summer precipitation (June - August); for the period 1981-2010), and short-term climate factor means (temperature (T) or precipitation (Precip) one month or three month before sample collection; mean for T and cumulative value for P), mean soil temperature (T_{soil}), annual N deposition, water storage capacity (in the profile to 1.2 m depth; taken from Hertel et al. (2013)), mean gravimetric water content (GWC), elevation, organic carbon (C_{org}), total nitrogen (N_{tot}), total P (P_{tot}), and plant-available P (P_a) content, and N:P ratio (organic layer and 0-10 cm of mineral soil, data for the year 2013), annual fine root production and fine root turnover (organic layer and 0-20 cm of mineral soil for the year 2010; data taken from Hertel et al. (2013)) and soil amino acid concentration (AA-N) for the 11 investigated beech stands on loamy-sandy (L) and sandy (S) soil in northwestern Germany for three seasons in 2014 (mid = June/July to August; late = August to October; non growing (non-grow) = October to December). Given are the correlation coefficients *R* and the probabilities of error *P* (***, **,*, and (*) for $P \leq 0.001$, 0.01, 0.05, and 0.1). Significant correlations ($P \leq 0.05$) are indicated by bold letters.

		Loamy						**Sandy**					
		Mid		**Late**		**Non-grow**		**Mid**		**Late**		**Non-grow**	
		R	***P***	***R***	***P***	***R***	***P***	***R***	***P***	***R***	***P***	***R***	***P***
Net N_{min}	MAT	0.86	(*)	**-0.95**	*	-0.47		0.09		-0.67		-0.61	
	$T_{3\ \text{months before}}$	-0.12		**-0.90**	*	-0.77		-0.33		-0.70		**-0.84**	*
	$T_{1\ \text{month before}}$	0.26		**-0.93**	*	-0.79		-0.33		-0.65		**-0.83**	*
	MAP	-0.78		0.88	(*)	0.30		-0.01		0.75	(*)	0.57	
	MGSP	-0.80		0.85	(*)	0.24		0.05		0.80	(*)	0.51	
	MSP	-0.79		0.83	(*)	0.23		0.02		**0.81**	*	0.53	
	Precip $_{3\ \text{months before}}$	-0.29		0.36		-0.38		-0.34		-0.25		**-0.94**	**
	Precip $_{1\ \text{month before}}$	-0.06		0.07		0.79		-0.45		-0.63		0.58	
	SPEI $_{3\ \text{months before}}$	**-0.95**	*	0.50		0.11		0.09		-0.05		0.15	
	N deposition	-0.71		0.82	(*)	0.20		-0.01		**0.82**	*	0.60	
	Water storage capacity	0.57		-0.68		-0.21		0.14		**0.87**	*	0.08	
	GWC	-0.31		-0.12		0.38		0.80	(*)	0.11		-0.22	
	Elevation	**-0.88**	*	0.87	(*)	0.42		0.26		0.46		0.39	
	C_{org} organic layer	0.21		0.01		-0.37		0.39		**0.93**	**	-0.07	
	C_{org} mineral soil	0.33		-0.25		-0.50		-0.04		0.71		0.20	
	N_{tot} organic layer	-0.50		0.83	(*)	0.39		0.52		**0.94**	**	-0.10	
	N_{tot} mineral soil	0.23		-0.25		-0.53		-0.29		0.34		0.13	
	P_{tot} organic layer	0.49		-0.24		0.19		-0.38		**-0.91**	*	-0.13	
	P_{tot} mineral soil	0.54		**-0.89**	*	-0.67		-0.32		-0.58		-0.20	
	P_a organic layer	0.36		-0.15		0.43		-0.26		-0.57		-0.28	
	P_a mineral soil	0.11		-0.06		-0.11		-0.11		0.07		-0.15	
	N:P organic layer	-0.47		0.34		-0.17		0.54		**0.94**	**	0.01	
	N:P mineral soil	-0.15		0.27		-0.17		0.21		**0.93**	**	0.19	
	FR production	0.80		-0.77		-0.22		0.16		-0.31		-0.58	
	FR turnover	-0.06		-0.13		-0.64		0.26		-0.36		-0.63	
	AA-N	0.66		-0.34		0.69		0.28		-0.25		-0.38	
	pH	-0.46		0.55		**0.94**	*	-0.55		0.12		0.23	

Table S2.4 Pearson correlation analysis for the relationships between mean soil respiration rate or mean net N mineralization rate (Net N_{min}; 10 cm depth) and long-term means of climate factors (MAT = mean annual temperature; MAP = mean annual precipitation; MGSP = mean growing season precipitation (April - September); MSP = mean summer precipitation (June - August); for the period 1981-2010), and short-term climate factor means (temperature (T) or precipitation (Precip) one month or three month before sampling; mean for T and cumulative value for Precip), mean soil temperature (T_{soil}), annual N deposition, water storage capacity (in the profile to 1.2 m depth), mean gravimetric water content (GWC), elevation, total organic carbon (C_{org}), total nitrogen (N_{tot}), total phosphorus (P_{tot}), plant-available phosphorus (P_a) content and N:P ratio (organic layer and 0-10 cm of mineral soil from the year 2013), annual fine root production and fine root turnover (organic layer and 0-20 cm of mineral soil for the year 2010; data taken from Hertel et al. (2013)) and soil amino acid concentration (AA-N) for the 11 investigated beech stands on loamy-sandy (L) and sandy (S) soil in northwestern Germany for two seasons in 2015 (early = April/May to June; mid = July to August). Given are the correlation coefficients R and the probabilities of error *P* (***, **,* and (*) for $P \leq 0.001$, 0.01, 0.05, and 0.1). Significant correlations ($P \leq 0.05$) are indicated by bold letters.

		Loamy				**Sandy**			
		Early		**Mid**		**Early**		**Mid**	
		R	***P***	***R***	***P***	***R***	***P***	***R***	***P***
Net N_{min}	MAT	0.03		0.01		-0.01		0.66	
	$T_{3\ \text{months before}}$	-0.18		0.08		0.53		0.56	
	$T_{1\ \text{month before}}$	-0.16		0.11		0.53		0.60	
	T_{soil}	-0.79		0.23		-0.23		0.23	
	MAP	0.09		0.13		0.07		-0.56	
	MGSP	0.08		0.09		0.06		-0.53	
	MSP	0.00		0.00		0.09		-0.54	
	$Precip_{3\ \text{months before}}$	-0.18		-0.14		0.27		-0.40	
	$Precip_{1\ \text{month before}}$	-0.10		-0.03		0.08		-0.44	
	$SPEI_{3\ \text{months before}}$	-0.10		-0.22		0.31		-0.50	
	$SPEI_{1\ \text{months before}}$	-0.03		-0.09		0.39		-0.65	
	N deposition	0.00		0.03		0.31		-0.48	
	Water storage capacity	0.46		0.47		-0.01		0.00	
	GWC	-0.54		-0.64		0.02		0.06	
	Elevation	0.39		0.42		0.15		-0.42	
	C_{org} organic layer	-0.56		-0.55		0.01		0.11	
	C_{org} mineral soil	-0.59		-0.64		0.55		0.03	
	N_{tot} organic layer	-0.03		0.09		-0.04		0.16	
	N_{tot} mineral soil	-0.53		-0.61		0.43		0.09	
	P_{tot} organic layer	0.26		0.41		0.06		0.18	
	P_{tot}mineral soil	0.49		0.39		0.27		0.43	
	P_a organic layer	0.12		0.24		**-0.84**	*	-0.01	
	P_a mineral soil	-0.83	(*)	**-0.90**	*	-0.31		-0.09	
	N:P organic layer	-0.32		-0.43		0.03		-0.02	
	N:P mineral soil	-0.57		-0.60		0.15		-0.17	
	FR production	-0.48		-0.51		0.18		**0.81**	*
	FR turnover	0.83	(*)	0.82	(*)	0.06		**0.86**	*
	AA-N	-0.18		0.22		-0.78	(*)	0.62	
	pH	-0.35		-0.33		-0.31		-0.17	

Table S2.4 Continued.

		Loamy				**Sandy**			
		Early		**Mid**		**Early**		**Mid**	
		R	***P***	***R***	***P***	***R***	***P***	***R***	***P***
Soil respiration rate	MAT	0.33		**0.92**	*	0.12		**0.98**	***
	$T_{3\ \mathrm{months\ before}}$	0.35		**0.93**	*	0.47		**0.93**	**
	$T_{1\ \mathrm{months\ before}}$	0.37		**0.92**	*	0.49		**0.94**	**
	T_{soil}	0.77		0.45		**0.85**	*	**0.83**	*
	MAP	-0.46		**-0.98**	**	-0.19		**-0.98**	***
	MGSP	-0.38		**-0.98**	**	-0.10		**-0.97**	**
	MSP	-0.29		**-0.96**	**	-0.02		**-0.96**	**
	Precip $_{3\ \mathrm{months\ before}}$	-0.25		-0.87	(*)	-0.09		**-0.89**	*
	Precip $_{1\ \mathrm{month\ before}}$	-0.45		-0.88	(*)	-0.43		**-0.92**	*
	SPEI $_{3\ \mathrm{months\ before}}$	0.18		-0.86	(*)	0.39		**-0.85**	*
	SPEI $_{1\ \mathrm{months\ before}}$	0.26		**-0.90**	*	0.43		**-0.96**	**
	N deposition	-0.36		**-0.97**	**	-0.21		**-0.91**	*
	Water storage capacity	-0.16		0.71		0.41		-0.60	
	GWC	0.50		-0.28		-0.21		-0.65	
	Elevation	-0.64		**-0.90**	*	-0.53		-0.72	
	C_{org} organic layer	0.32		-0.25		0.41		-0.55	
	C_{org} mineral soil	0.60		0.02		0.57		-0.19	
	N_{tot} organic layer	-0.59		-0.84	(*)	0.08		-0.66	
	N_{tot} mineral soil	0.64		-0.01		0.69		0.13	
	P_{tot} organic layer	-0.51		0.41		-0.14		**0.83**	*
	P_{tot} mineral soil	0.13		0.63		0.09		**0.83**	*
	P_a organic layer	-0.32		0.51		0.24		0.21	
	P_a mineral soil	0.85	(*)	0.12		**0.96**	*	0.09	
	N:P organic layer	0.39		-0.55		0.06		-0.73	
	N:P mineral soil	0.38		-0.44		0.44		-0.66	
	FR production	0.70		**0.95**	*	0.12		**0.83**	*
	FR turnover	-0.66		-0.45		-0.21		0.74	(*)
	AA-N	-0.17		0.09		0.39		0.17	
	Net N min	-0.70		-0.28		-0.05		0.57	
	pH	0.32		0.07		0.16		-0.27	

CHAPTER 3

Ectomycorrhizal fungal diversity increases phosphorus uptake efficiency of European beech

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Summary

Increases in summer droughts and nitrogen (N) deposition have raised concerns of widespread biodiversity loss and nutrient imbalances, but our understanding of the ecological role of ectomycorrhizal fungal (ECMF) diversity in mediating root functions remains a major knowledge gap. We used different global change scenarios to experimentally alter the composition of ECMF communities colonizing European beech saplings and examined the consequences for phosphorus (P) uptake ($H_3{}^{33}PO_4$ feeding experiment) and use efficiencies of trees. Specifically, we simulated increases in temperature and N deposition and decreases in soil moisture and P availability in a factorial experiment. Here, we show that ECMF α diversity is a major factor contributing to root functioning under global change. P uptake efficiency of beech significantly increased with increasing ECMF species richness and diversity, as well as with decreasing P availability. As a consequence of decreases in ECMF diversity, P uptake efficiency decreased when soil moisture was limiting. By contrast, P use efficiencies were a direct (negative) function of P availability and not of ECMF diversity.

We conclude that increasing summer droughts may reduce ECMF diversity and the complementarity of P uptake by ECMF species, which will add to negative growth effects expected from nutrient imbalances under global change.

Keywords: biodiversity loss, drought, ectomycorrhizal fungi, *Fagus sylvatica*, global change, nitrogen (N) deposition, phosphorus (P) limitation, use efficiency

Introduction

Rapid and accelerating decreases in global biodiversity (Sala, 2000) have raised debates on the relationship between biodiversity and ecosystem functions (e.g., Loreau et al., 2001; Hooper et al., 2005). However, most studies have focused primarily on aboveground biodiversity–plant productivity relationships (e.g., Marquard et al., 2009; Tilman et al., 2014), neglecting the proposed key role of belowground biodiversity in important ecosystem functions and multifunctionality (Bardgett and van der Putten, 2014; Delgado-Baquerizo et al., 2016).

In particular, the understanding of the influence of the mycorrhizal fungal diversity on root functions and plant productivity remains poor, despite the contribution of mycorrhizal fungi to essential functions such as biogeochemical cycling, soil aggregation, and aboveground–belowground interactions (Cairney, 2011; Liese et al., 2017). This lack of knowledge is even more apparent for ectomycorrhizal (ECM) fungi (ECMF), which are the most important fungal symbionts for temperate and boreal forest ecosystems. ECM fungal taxa are physiologically heterogeneous both among and within species: ECMF species or genotypes differ in their colonization extent, mycelial growth rates and phosphorus (P) solubilization and uptake (Pena et al., 2013; Hazard et al., 2017). The few studies that investigated the effects of ECMF community diversity for the host tree showed mostly increases in host productivity (Baxter and Dighton, 2001; Hazard et al., 2017; but see Kipfer et al., 2012). However, it remains unknown if this was a consequence of spatial, temporal, or functional complementarity in resource exploitation and transfer to the host (*sensu* niche complementarity hypothesis; Tilman, 1997) or a sampling effect (Jonsson et al., 2001; Kipfer et al., 2012).

The diversity of ECMF increases with plant community development (Jumpponen et al., 2012; Dickie et al., 2013) as a function of the presence of soil organic matter (SOM; Hawkins et al., 2015). This influence of SOM on ECMF diversity is exerted, in parts, through changes in the availability of nitrogen (N) (Toljander et al., 2006; Lilleskov et al., 2011). The effect of N availability on the ECMF community contrasts markedly depending on whether the dominant N source is of natural or anthropogenic origin: in fertilization or N deposition studies, ECMF diversity and abundance decrease with increasing N availability (Kjøller et al., 2012; de Witte et al., 2017), whereas in observational field studies with a naturally low N range the ECMF colonization and diversity increase with increasing N availability (Soudzilovskaia et al., 2015). In mature ecosystems, ECMF diversity is thought to be promoted by spatial or temporal resource partitioning (complementarity) among ECMF species (Buée et al., 2007), but impaired by anthropogenic disturbance (e.g., by N deposition, irregular summer droughts under global change), which creates uncolonized patches on roots (Courty et al., 2010).

Increasing summer droughts and N deposition under global change affect environmental conditions that will likely reduce ECMF diversity, as well as constrain availability of soil resources, for which the ECM symbiosis plays a key acquisition role. Several studies predict nutritional imbalances and a large-scale undersupply of essential plant nutrients such as P for important temperate tree species in the near future (Prietzel et al., 2008; Talkner et al., 2015), which will be accelerated by atmospheric N deposition from anthropogenic sources (Sardans et al., 2016). This undersupply is already indicated by decreasing P concentrations and increasing N:P ratios in leaves and fine roots, as well as by reports on recent growth reductions due to P limitation for various forest regions of central and southern Europe and elsewhere (Elser et al., 2007; Peñuelas et al., 2012). In addition, decreasing summer precipitation and increasing temperatures in the course of climate change will likely further impair P nutrition due to decreased soil mobility (Schachtman et al., 1998) and diffusion rates (Kreuzwieser and Gessler, 2010), and a reduction in the growth and activity of mycorrhizal fungal hyphae in drier soil (Ekblad et al., 2013; Bakker et al., 2015). Reductions in ECMF hyphal growth and biomass (Teste et al., 2016) may further deteriorate P acquisition when soil P is already limiting P nutrition of forest trees.

Apart from the role of ECMF in P acquisition (Plassard and Dell, 2010; Cairney, 2011) and of the ECMF community in increasing soil exploration (Zavišić et al., 2016), plants have also evolved their own adaptive responses to enhance the efficiencies of P uptake and use: when P is limiting growth, plants respond by an increase in the root : shoot ratio and in root proliferation, a change in the root system architecture towards higher branching intensity, an increase in the expression of high-affinity inorganic phosphate transporters and in the secretion of phosphatases and tight internal nutrient cycling (Smith et al., 2000; Vance et al., 2003). These plant responses may alleviate decreases in ECMF colonization and diversity, but they may also be interdependent on the ECM symbiosis.

To better understand the importance of the relationship between ECMF diversity and root functioning in forest trees under global change, it is imperative, first, to investigate the response of the composition of ECMF communities to an increase in temperature and N deposition and a decrease in precipitation. As a second step, the consequences of these altered ECMF communities for root functioning under global change can be explored. Here, we experimentally altered the ECMF diversity on European beech saplings by exposing the trees to a four-factorial climate chamber experiment that simulated the expected global change conditions in central Europe and examined the consequences for P uptake and use efficiencies of trees. We hypothesized (H1) that the ECMF community composition of beech shifts towards ECMF species with enhanced soil exploration with a decrease in P availability and an increase in the soil N:P ratio, while the ECMF diversity decreases with decreasing soil moisture due to a decrease in ECMF species richness; (H2) that beech responds to decreasing plant availability of P with morphological adaptations to increase P uptake and P use; and (H3) that beech saplings colonized by a more diverse suite of mycorrhizal fungi have higher P uptake efficiency due to more complete resource exploitation (*sensu* niche complementarity hypothesis; Tilman, 1997).

Material and Methods

Plant material

Our experiment was conducted with 288 European beech (*Fagus sylvatica* L.) saplings that were collected from a mature beech forest in the lowlands of northwest Germany (N52°50', E10°19'). The forest site was located on spodic Dystrudept soil (IUSS et al., 2006), which developed from Pleistocene fluvioglacial sandy deposits from the penultimate Ice Age. The medium- to coarse-grained meltwater sands had relatively low water storage capacity (79 mm 120 cm^{-1} at a mean annual precipitation of 816 mm and mean annual temperature of 8.7°C) and were covered by an organic layer that was 7 cm thick (Hertel et al., 2013). The highly acidic, nutrient-poor sandy soil had a pH(H_2O) of 4.3, a carbon (C) : N ratio of 24.1 mol mol^{-1}, a P_{resin} content of 2.8 mg kg^{-1} and a base saturation of 8.3%. In May 2013, we selected c. 500 beech seedlings of similar size that originated from the 2012 tree masting. At this site, mycorrhizal fungal colonization of adult trees averaged at 75% (Hertel et al., 2013) and the ECMF communities were comparably species poor with, on average, nine ECMF species found on adult trees (Zavišić et al., 2016) and three to six ECMF species on beech saplings (Leberecht et al., 2016). Seedlings were carefully excavated and the adhering soil removed. Seedlings were kept moist and cool and were transported to the botanical garden in Goettingen, central Germany, where the beech seedlings and their associated indigenous ECMF communities were cultivated in a sterilized 2 : 1 sand : peat mixture outdoors for 1 yr. We refrained from ECMF characterization at this point of the experiment to keep the delicate ECMF root systems intact. In May 2014, saplings were carefully removed from their substrate, rinsed with bi-distilled water and planted in 1.5 l pots filled with sieved and homogenized mineral topsoil from the site of origin that contained the indigenous ECMF, with the aim to maximize the ECMF colonization rate of beech saplings. Pots were randomly arranged in a controlled growth chamber. Plants were kept well-watered until the start of the experimental treatments in July 2014. The selected saplings for the experiment had similar size (shoot length *c.* 20 cm, *c.* 13 leaves).

Experimental set-up

In July 2014, we established a full factorial experiment with four factors (two temperature levels x three soil moisture levels x two N supply levels x three P supply levels; 36 treatments in total; Supporting Information **Table S3.1**) and eight replicates per treatment. Beech saplings were assigned to their experimental treatment by random, were set up in randomized design in two highly controllable walk-in climate chambers (York, Johnson Control Systems, Essen, Germany) and were re-randomized biweekly for two growing seasons. We simulated a gradient from current environmental conditions to the expected future global change conditions (elevated temperature and N deposition and reduced soil moisture and P availability). We established (1) ambient (T1; temperature, 18°C : 12°C, day : night) and elevated temperature conditions (T2; 22°C : 16°C) in two separate climate chambers. We set up (2) three soil moisture levels: well-watered (M3; 90% field capacity (FC); 32% soil water content (SWC), v/v), moderately moist (M2; 60% FC; 21% SWC) and drought treatments (M1; 30% FC; 11% SWC). The FC of mineral topsoil from the site of sapling origin was calculated from the amount of water at field capacity (soil saturation with water for 48 h, draining for 48 h) per soil dry weight (105°C, 48 h). Water loss by evapotranspiration was replaced every second day by watering the pots up to the respective target weight with bidistilled water. We simulated (3) ambient (N1; 8.5 kg N ha^{-1} yr^{-1}) and elevated N deposition (N2; 17 kg N ha^{-1} yr^{-1}) on the basis of the N content of the nutrient-poor sandy soil used for the experiment (1.5 mg N g^{-1}). N was added as NH_4NO_3 by watering biweekly with a modified Hoagland solution. Finally, we established (4) three P availability levels – a high (P3; 0.69 mg P kg^{-1}), a moderate (P2; 0.34 mg P kg^{-1}) and a low P supply treatment (P1; 0.17 mg P kg^{-1}) – by adding NaH_2PO_4 as a component of the Hoagland solution biweekly. Owing to the interacting effects of N and P, this led to six N:P treatments: relative P availability decreased from N1P3 (P_{added} 22.4 µmol; N:P ratio 12.5 mol mol^{-1}) to N2P3 (22.4 µmol P; 25 mol mol^{-1}), N1P2 (11.2 µmol P; 25 mol mol^{-1}), N2P2 (11.2 µmol P; 50 mol mol^{-1}), N1P1 (5.6 µmol P; 50 mol mol^{-1}) and N2P1 (5.6 µmol P; 100 mol mol^{-1}). During the nongrowing season 2014–2015 (November 2014 to March 2015), temperatures were reduced to 10°C : 8°C, day : night, the day length was reduced from 14 to 7.5 h, and photosynthetic photon flux density was reduced from 190 to 30 µmol photons m^{-2} s^{-1}, while drought and fertilization treatments were paused. All conditions were brought back to the treatment conditions in April

2015 and maintained until the harvest of the experiment in September 2015. Thus, we simulated two subsequent growing seasons with altered climatic conditions for a total of 456 d.

Photosynthesis

Leaf gas exchange measurements were conducted for 16 selected treatment combinations: ambient and elevated temperature, the well-watered and drought treatments, ambient and elevated N deposition, and high and low P availability (five replicates per treatment; n = 80 saplings). Leaf gas exchange measurements were conducted in June 2015 on one canopy leaf per plant with an infrared CO_2 analyzer (LI-6400; LI-COR Biosciences, Lincoln, NE, USA) during the middle of a day. We measured maximum leaf photosynthesis (A_{max}; µmol m^{-2} s^{-1}) at high photosynthetically active radiation (1500 µmol m^{-2} s^{-1}). During the measurements, average leaf temperature was 18.2°C (T1) and 22.1°C (T2), relative humidity 58.8%, vapor pressure deficit 11.7 hPa and CO_2 concentrations ambient (390 µmol CO_2 mol^{-1} air). The photosynthetic P use efficiency (PPUE) was calculated according to PPUE = A_{max} x SLA/P_{Leaves} (mmol CO_2 g^{-1} P h^{-1}), where SLA is the specific leaf area (measured by using a flat-bed scanner and the computer program WinFOLIA, version 2014c; Régent Instruments Inc., Canada) and P_{Leaves} the foliar P concentration (*cf.* Li et al., 2012).

Phosphorus-33 labeling experiment

At the beginning of September 2015 (i.e., 2 wk before the end of the experiment), ^{33}P uptake capacity was measured for eight selected treatment combinations: elevated temperature, the well-watered and drought treatments, ambient and elevated N deposition, and high and low P availability (three replicates per treatment; n = 24 saplings). The radioisotope labeling experiment was conducted in the Laboratory for Radio-Isotopes (LARI), University of Goettingen. Thirty minutes after the even application of 20 ml $H_3{}^{33}PO_4$ tracer solution to three soil depths per pot (total ^{33}P activity: 1 MBq; i.e., 0.2 ng ^{33}P per sapling), saplings were immediately excavated, separated into leaves, shoots, coarse roots, and fine roots (≤ 2 mm in diameter) and inserted into liquid N_2 to halt any further ^{33}P transport. Subsequently, samples were freeze-dried for 5 d, ground, homogenized, and subjected to high-pressure chemical digestion (65% HNO_3 at 160°C; *cf.* Heinrichs et al., 1986). Digested samples were mixed with Rotiszint eco plus liquid scintillator (Roth, Karlsruhe, Germany) and the ^{33}P radioisotope signature determined with a low activity liquid scintillation analyzer (Tri-Carb 3180TR/SL, Perkin Elmer, MA, USA). The mass-specific rate of ^{33}P uptake (^{33}PUptake) was calculated from the whole-plant amount of ^{33}P after 30 min divided by the whole-plant biomass; and division of the quotient by the specific ^{33}P activity (g P g^{-1} biomass d^{-1}). The specific ^{33}P activity (i.e., isotope dilution of ^{33}P) was determined from the quotient of the amount of ^{33}P added and the amount of plant-available P (P_{resin}) in each pot. The fraction of plant-available P (P_{resin}) according to Bowman and Cole (1978) had previously been determined by resin bag extraction (anion exchange gel: Dowex 1 9 8–50; Dow Water & Process Solutions, USA). The resin was placed for 16 h in a solution of 1 g fieldmoist soil suspended in 30 ml water (Sibbesen, 1977). P was reexchanged by 10% NaCl and 2% NaOH solutions and analyzed by color reaction with 5 mM hexaammonium heptamolybdate (Murphy and Riley, 1962) and photometric measurement at 712 nm against water (spectrophotometer; Libra S 21, Biochrom, UK). The efficiency of ^{33}P uptake (^{33}PUptakeE; g P g^{-1} P_{resin} d^{-1}) was calculated by dividing ^{33}PUptake by the amount of P_{resin}.

Root and shoot biomass and ectomycorrhizal fungal colonization

In mid-September 2015, the remaining 264 beech saplings not subjected to the ^{33}P labeling experiment were also fractionated. For each sapling, a representative fine root section was transferred to 5% glycerol solution and kept frozen at 18°C for analysis of the mycorrhizal colonization with a dissecting microscope (*n* = 8 replicates per treatment). All root tips were counted and classified after Agerer (1987–2012; *cf*. Pena et al., 2010). For ECMF morphotype characterization, basic characters such as color and morphology of fungal structures (mantle type and extra-radical hyphae) were assessed. The root fractions (i.e., vital ECM, vital non-ECM, dead ECM and ECM root tips) colonized by each ECMF morphotype were counted. ECMF colonization was calculated as the percentage of the number of vital root tips colonized by ECMF.

A total of 10–20 tips of each distinct morphotype were collected from different samples, stored at 20°C and used for molecular identification of fungal species by ribosomal DNA internal transcribed spacer (ITS) sequencing (*cf*. Pena et al., 2010; Zavišić et al., 2016). Genomic DNA was isolated from each morphotype with Innu PREP Plant DNA kit (Analytik Jena, Germany). We used the primers ITS1-F and ITS4 (White et al., 1990) to amplify the ITS region. Single-band PCR products were purified (isopropanol precipitation), sequenced using BigDye Terminator, version 3.1, and screened on an Applied Biosystems 3730XL DNA Analyzer (Seqlab GmbH, Goettingen, Germany). When the PCR resulted in more than one amplification product, the PCR products with the expected sizes were cloned into a pGEM-Tvector (pGEM-T system I; Promega, Madison, WI, USA). At least eight clones were subsequently sequenced per morphotype sample. The sequences obtained were aligned with STADEN Package, v.4.10, and compared with fungal taxa in NCBI GenBank by BLAST analysis. Six sequences were assigned to fungal taxa and were deposited in NCBI GenBank (accession numbers KX545374–KX545379). The Shannon–Weaver index (Shannon H'; Shannon and Weaver, 1949) was calculated according to $H' = \sum p_i \log_e p_i$, where p_i is the relative proportion of the *i*th ECMF species in the total number of ECMF species of individual beech saplings (ECMF α diversity). Furthermore, the Shannon–Weaver H' was calculated as the relative proportion of the *i*th ECMF species in the total number of ECMF species in each treatment (ECMF β diversity).

A separate fine root section was used to determine root morphology (i.e., root length and surface area), by using a flat-bed scanner and WINRHIZO, v.2013e (Régent Instruments Inc., Canada) ($n = 8$ replicates per treatment). Subsequently, all leaves, stems, coarse roots and fine roots were dried (48 h, 70°C) and weighed. Specific root area ($cm^2 \ g^{-1}$), specific root length ($m \ g^{-1}$) and the root : shoot ratio ($g \ g^{-1}$) were calculated.

Phosphorus uptake and use efficiencies

All plant compartments were ground with a vibratory disc mill (TS-Siebtechnik GmbH; Mulheim, Germany) and analyzed for P by inductively coupled plasma optical emission spectroscopy (Optima 5300 DV; Perkin Elmer, Rodgau, Germany) after digestion with 65% HNO_3 at 195°C. P uptake efficiency (PUptakeE, mg $P_{BM} \ g^{-1} \ P_{added} \ d^{-1}$) was calculated for all plants – that is, for the plants subjected to the ^{33}P labeling experiment as well as all others ($n = 8$ replicates per treatment; 288 saplings in total) – by dividing the content of P in recent biomass (i.e., in fine roots, green and senescent leaves) by its amount added by fertilization during the 2015 growing season. The P use efficiency (PUE, g biomass $g^{-1} \ P_{BM} \ d^{-1}$) was calculated from the increment in total biomass for a given increase in P during a given period of time (*cf*. Koide, 1991). To analyze the relative effect by the extremes of our climate change gradient on P uptake and use efficiencies, we calculated a relative interaction intensity (RII) index (*cf*. Armas et al., 2004) according to RII = (CC - Amb)/ (CC + Amb), where CC represents the expected future climate change conditions (elevated temperature and reduced soil moisture, ElevT + RedM) and Amb represents the current environmental conditions (ambient temperature and soil moisture, AmbT + AmbM). The RII ranges between a maximum of 1 and +1.

Statistical analyses

Statistical analyses were conducted with R, v.3.3.1 (R Development Core Team). Significance was determined at $P < 0.05$. Means and standard errors were calculated from eight replicates per treatment (photosynthesis: $n = 5$, ^{33}P uptake: $n = 3$). Nonnormally distributed data were transformed to resemble normality and homogeneity of variances. The effects of P availability, N availability, temperature, soil moisture, and their interactions were analyzed by four-way ANOVAs, treating all effects as fixed effects. Tukey's HSD post-hoc tests were conducted to identify significant differences between individual factor levels. If data did not resemble normality after transformation (which was the case for root vitality and ECMF species richness), nonparametric Kruskal–Wallis tests were applied followed by Dunn's tests of multiple comparisons and Bonferroni adjustment of P-values. Owing to nonnormality of the residuals, we calculated simple linear Spearman rank correlations for treatment means to identify relationships between P uptake and use efficiencies and ECMF species richness. For significant correlations, we conducted linear regression analyses. We used fine root biomass, ECMF colonization, ECMF species richness, and ECMF α diversity as independent variables in a multiple regression analyses with backward variable elimination to test whether they were significant predictors for PUptakeE, PUE and PPUE.

To compare the similarities of the ECMF community compositions between the different treatments, analyses of similarity for the relative ECMF species abundance data were performed with PAST, v.3.15, using the Morisita similarity index (9999 permutations). Subsequently, we performed similarity percentage analyses to identify those ECMF species that contributed most to the significant Bray–Curtis dissimilarities. To identify potential linear relationships between the occurrence of individual ECMF species and PUptakeE and PUE, we calculated redundancy analyses with CANOCO, v.5.03 (Biometris; Wageningen University and Research Centre, Wageningen, Netherlands). The explained variation of PUptakeE and PUE by the occurrence of individual ECMF species was < 1% and the model for the relationship had to be rejected. To identify potential relationships between the occurrence of ECMF species groupings and PUptakeE and PUE, we calculated principal components for ECMF species occurrence by principal component analysis with SAS, v.9.3 (Statistical Analyses System, SAS Institute Inc., Cary, NC, USA), and used the first three principal components as independent variables in subsequent multiple regression analyses with backward variable elimination. The model became only significant when all variables were removed from the final model, and thus the model for the relationship between ECMF species groupings (principal components) and PUptakeE or PUE had to be rejected as well.

Results

Ectomycorrhizal fungal colonization and community diversity Across all treatments, 60% of the root tips were colonized by ECMF, 29% were nonmycorrhizal, and 11% were dead (n = 85 327 root tips analyzed). We identified six ECMF species (55% *Lactarius tabidus*, 27% *Cenococcum geophilum*, 11% *Genea hispidula*, 3.6% *Scleroderma* sp., 3.2% *Thelephora terrestris*, and 0.5% *Tomentellopsis submollis*; ordered in descending frequency of occurrence; **Table S3.2**, **Table**

S3.3). Higher ECMF species richness led to a more complete colonization of the root system (**Table S3.4**).

Soil P or N availability did not significantly influence root vitality or ECMF colonization and diversity, yet the portion of vital root tips significantly decreased with drought (95% in moist to 77% in dry soil) and increased with increasing temperature (**Figure S3.1**a). Similarly, ECMF colonization (71–56%), species richness (2.1–1.8), and α diversity (0.43–0.35) decreased with decreasing soil moisture, yet ECMF colonization also decreased with increasing temperature (**Figure S3.1**b; **Table S3.5**). It has to be noted that the number of ECMF species colonizing the beech saplings was overall very low, both at the site of origin and in the experiment. The decrease in ECMF species richness with drought was accompanied by an increase in the number of saplings colonized by only one ECMF species, whereas the majority of saplings remained colonized by three ECMF species (64%; data not shown). In addition, only saplings in the drought treatment were colonized by four ECMF species per saplings (3%). According to similarity percentage analyses, ECMF community composition was significantly influenced by both increasing drought and temperature (**Table 3.1**). In dry soil, the ECMF community shifted from its dominance by *L. tabidus* (contact exploration type; see Agerer, 2001) towards a higher portion of *C. geophilum* and *G. hispidula* (short-distance exploration) and the occurrence of T. submollis (medium-distance exploration with rhizomorphs). At elevated temperature and moderate soil moisture, the portions of *L. tabidus*, *T. terrestris* and *Scleroderma* sp. in the ECMF community were reduced in favor of the pioneer generalist *C. geophilum*. Despite these systematic and significant differences between treatments, a 5% probability of error remains that differences between ECMF communities had already existed before the start of the experiment and maintained or even propagated until the end of the experiment – notwithstanding the random assignment to the different treatments.

Plant biomass and root morphology

The P and N availabilities had no significant effect on total, aboveground and belowground biomass (**Figure S3.2**) or the root : shoot ratio (**Figure S3.3**b), despite a reduction of A_{max} by 23% when P availability decreased (**Figure S3.3**a). By contrast, the decrease of A_{max} by 33% with drought was accompanied by a reduction of total plant biomass by 50% (**Figure S3.2**; **Figure S3.3**a). The biomass reduction with soil drought was slightly stronger for aboveground (reduction from 3.4 to 1.5 g in dry soil) than for belowground biomass (reduction from 5.3 to 2.7 g; **Figure S3.2**). The root biomass allocation significantly increased in dry soil and decreased at elevated temperature (**Figure S3.3**b). The amount of root biomass in the different treatments was strongly positively influenced by root vitality and ECMF diversity and to a lesser extent by ECMF species richness (**Table S3.4**). In contrast to biomass and biomass allocation patterns, root morphology of beech saplings did not respond considerably to the experimental treatments: specific root length increased with elevated temperature, but was unaffected by the other experimental factors (**Figure S3.4**c). Specific root area did not change in any of the experimental treatments (**Figure S3.4**d).

Table 3.1 Similarity percentage analyses on the relative abundance of the investigated ectomycorrhizal fungal (ECMF) species associated with European beech saplings and their contribution to the Bray–Curtis dissimilarity between experimental treatments. Temperature: T1, ambient; T2, elevated. Soil moisture: M1, drought; M2, moderately moist. Similarity percentage analyses are given for those treatments only for which analyses of similarity indicate significant differences between ECMF communities. ECMF α diversity refers to ECMF species diversity at the individual beech sapling; ECMF β diversity refers to ECMF species diversity at the treatment level.

		Relative abundance [%]				**Contribution to dissimilarity [%]**	
	Exploration type	**T1M2**	**T2M2**	**M2**	**M1**	**T1M2 vs. T2M2**	**M2 vs. M1**
Lactarius tabidus	Contact	65.7	52.1	58.7	45.6	42.1	40.6
Cenococcum geophilum	Short distance	18.2	37.3	28.0	30.1	32.2	29.0
Genea hispidula	Short distance	4.7	6.9	5.9	16.1	11.3	17.2
Thelephora terrestris	Medium dist. (smooth)	5.9	2.0	3.9	3.9	7.5	6.5
Scleroderma sp.	Long distance	5.5	1.7	3.5	2.8	6.9	5.4
Tomentellopsis submollis	Medium dist. (smooth)	0.0	0.0	0.0	1.5	0.0	1.3
ECMF α diversity		0.34	0.52	0.43	0.35		
ECMF β diversity		1.06	1.04	1.08	1.30		

Phosphorus uptake efficiency

Both P availability and soil moisture had a significant effect on PUptakeE, while ^{33}PUptakeE was influenced by soil moisture availability only. The N availability, soil N:P ratio, and temperature were not influencing factors on ^{33}PUptakeE or PUptakeE. The ^{33}PUptakeE of beech saplings decreased distinctly by 54% with drought (**Figure 3.1**a). Soil drought also reduced PUptakeE by 60% in comparison with moderately moist and well-watered soil conditions. However, PUptakeE significantly increased from high to reduced soil P availability (P3: 88 mg P_{BM} g^{-1} P_{added} d^{-1}; P2 and P1: 125 mg P_{BM} g^{-1} P_{added} d^{-1}; **Figure 3.1**b). Consequently, there was a significant cross-effect of P availability and soil moisture on PUptakeE, which increased with decreasing P availability and increasing soil moisture (**Figure 3.1**b). The relative drought effect RII of our climate change treatment on PUptakeE increased with decreasing P availability to a positive effect at the highest N:P ratio (**Figure S3.5**). PUptakeE also related positively to root biomass and root vitality in the different treatments (**Figure 3.2**a; **Table S3.4**).

The P concentrations of both leaves and fine roots were reduced by about two-thirds when P was limiting (**Figure S3.3**c; **Figure S3.4**a), whereas their N:P ratios continuously increased by c. 2.5 times at low P availability (**Figure S3.3**d; **Figure S3.4**b). In dry soil, leaf P decreased by 30%, while fine root P was not significantly different. At the same time, foliar and fine root N:P ratios increased by a third. Elevated temperature had contrasting effects on leaf and fine root P: foliar P significantly decreased, while fine root P significantly increased with an increase in temperature.

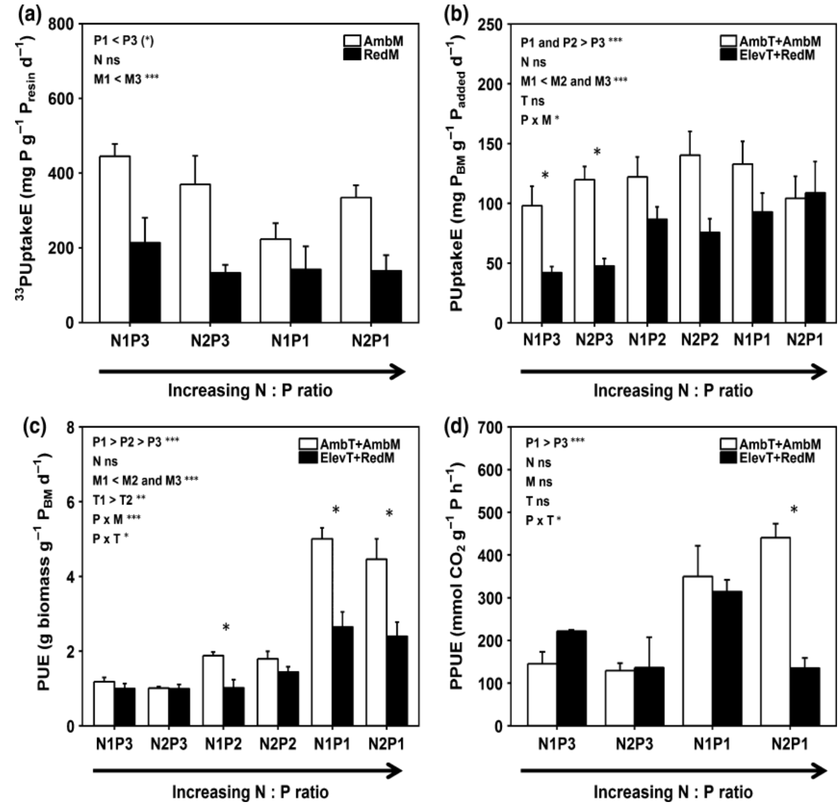

Figure 3.1 Means and standard errors of (a) ^{33}P uptake efficiency (^{33}PUptakeE), (b) phosphorus (P) uptake efficiency (PUptakeE), (c) P use efficiency (PUE) and (d) photosynthetic PUE (PPUE) of European beech saplings grown at increasing soil nitrogen (N) : P ratio and ambient (ambient temperature and soil moisture, AmbT + AmbM) or climate change conditions (elevated temperature and reduced soil moisture, ElevT + RedM) in climate chambers. The associated tables show the results of four-factorial ANOVAs (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, not significant; $n = 3$ replicates per treatment for ^{33}PUptakeE, $n = 8$ replicates per treatment for PUptakeE and PUE; $n = 5$ replicates per treatment for PPUE). P supply: P1, low; P2, moderate; P3, high. N supply: N1, ambient; N2, elevated. Temperature: T1, ambient; T2, elevated. Soil moisture: M1, drought; M2, moderately moist; M3, well-watered.

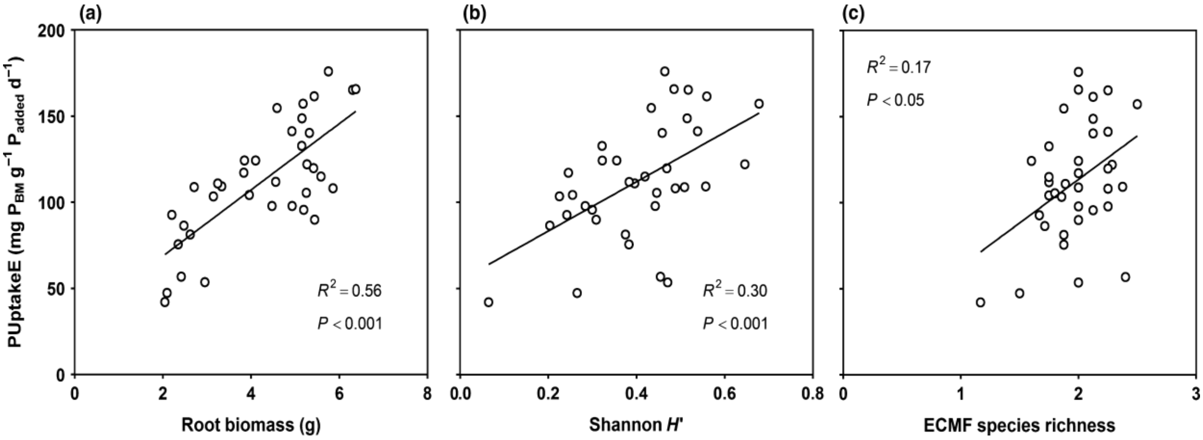

Figure 3.2 Linear regression analyses for the relationship between phosphorus (P) uptake efficiency (PUptakeE) and (a) root biomass, (b) ectomycorrhizal fungal (ECMF) α diversity (Shannon H') and (c) ECMF species richness of European beech saplings grown at increasing soil nitrogen (N) : P ratio and climate change (*n*= 36 treatments). ECMF α diversity refers to ECMF species diversity at the individual beech sapling level.

Phosphorus use efficiency

PUE increased with decreasing P availability, especially in interaction with the factor soil moisture: at ambient soil moisture, PUE increased by nearly fourfold with decreasing P availability, while it increased by only 2.5 times at moderate and low soil moisture conditions (**Figure 3.1**c). Drought itself decreased PUE by a fourth, while elevated temperature reduced PUE by only an eighth, resulting in the highest PUE at low P availability and ambient temperature (**Figure 3.1**c). The negative drought effect RII on PUE decreased with decreasing P availability (**Figure S3.5**). PPUE increased by twice with decreasing P availability (Figure 1d) and was positively correlated with PUE (**Table S3.4**). N availability had no significant effect on either PUE or PPUE (**Figure 3.1**; **Table 3.2**).

Relationships between the ectomycorrhizal fungal community and phosphorus uptake and use efficiencies

Linear regression analyses revealed a strong positive relationship between PUptakeE and ECMF α diversity (**Figure 3.2**b). Accordingly, PUptakeE also correlated positively with ECMF species richness (**Figure 3.2**c). This influence of ECMF diversity and species richness on PUptakeE could not be assigned to the occurrence of specific ECMF species (explained variance of redundancy analysis between ECMF species and P uptake or use efficiencies: 0.6%, not significant), but was an effect of the ECMF community instead. The multiple regression analysis showed that ECMF α diversity remained a significant predictor of PUptakeE even when fine root biomass entered the model, which had a strong positive effect (**Table 3.3**). In contrast to PUptakeE, neither PUE nor PPUE were significantly related to ECMF colonization, species richness, or diversity in simple linear regressions (**Table S3.6**), while multiple regressions revealed ECMF colonization as the only predictor of PUE (**Table 3.3**).

Table 3.2 Four-factorial ANOVAs on the significance of the effects of phosphorus (P) availability, nitrogen (N) availability, temperature (T), soil moisture (M), and their interactions on the variance of ^{33}P uptake effi-ciency (^{33}PUptakeE), P uptake efficiency (PUptakeE), P use efficiency (PUE) and photosynthetic PUE (PPUE) of European beech saplings grown at increasing soil N:P ratio and climate change. n/a, not applicable. Because of multiple comparisons, *P*-values are adjusted by Bonferroni correction. Statistically significant values are indicated in bold (n = 3 replicates per treatment for ^{33}PUptakeE, n = 8 replicates per treatment for PUptakeE and PUE, n = 5 replicates per treatment for PPUE).

	^{33}PUptakeE		**PUptakeE**		**PUE**		**PPUE**	
	F	***P***	***F***	***P***	***F***	***P***	***F***	***P***
P	3.2	0.09	**25.6**	**<0.001**	**349.1**	**<0.001**	**33.1**	**<0.001**
N	0.0	0.86	0.8	0.36	2.1	0.15	0.1	0.71
T	n/a		3.6	0.06	**7.1**	**<0.01**	1.4	0.25
M	**20.6**	**<0.001**	**39.8**	**<0.001**	**31.9**	**<0.001**	1.2	0.28
P x N	2.3	0.15	1.8	0.17	0.2	0.84	0.1	0.73
P x T	n/a		0.8	0.43	**3.3**	**<0.05**	**7.6**	**<0.01**
N x T	n/a		1.4	0.23	0.2	0.20	1.2	0.28
P x M	0.1	0.80	**2.7**	**<0.05**	**6.7**	**<0.001**	0.3	0.60
N x M	0.5	0.50	0.9	0.39	1.0	0.37	3.6	0.06
P x N x T	n/a		0.7	0.50	0.0	0.99	2.5	0.12
P x N x M	0.1	0.82	0.9	0.45	1.5	0.22	0.0	0.90

Table 3.3 Multiple regression analysis with backward variable elimination on the effects of fine root biomass, ectomycorrhizal fungal (ECMF) colonization, ECMF species richness, and ECMF α diversity on phosphorus (P) uptake efficiency (PUptakeE), P use efficiency (PUE) and photosynthetic PUE (PPUE) of European beech saplings grown at increasing soil nitrogen (N):P ratio and climate change. Values given are the determination coefficient R^2 and the probability of error *P* for the model and the *F* value and probability of error *P* for the selected predictors. ns = not significant. All significant relationships were positive.

	Model				
Y	***R*²**	***P***	**Predictor**	***F***	***P***
PUptakeE	0.62	**<0.001**	Fine root biomass	326.6	**<0.001**
			ECMF α diversity	6.3	0.01
PUE	0.02	0.02	ECMF colonization	6.0	0.02
PPUE		ns			

Discussion

Widespread biodiversity loss in the course of global change is assumed to hamper important ecosystem functions, but the role of ECMF diversity in root functioning and productivity of forest trees has yet to be clarified. Here, we show that summer drought induced a shift in the ECMF community associated with European beech roots and decreased the ECMF species richness and diversity. ECM fungal diversity of beech in turn related positively to the efficiency of P uptake and plant productivity – in contrast to P availability, which related negatively to ^{33}PUptakeE, PUptakeE and PUE. When combined, the direct negative drought effects on P mobility in soil and plant uptake were further aggravated by reduced ECMF diversity in dry soil and the close ECMF diversity–root function relationship.

Ectomycorrhizal beech counterbalances decreasing phosphorus availability

In contrast to hypothesis H1 (and in contrast to the response of mature beech ecosystems which were adapted to long-term, constant differences in P availabilities; Zavišić et al., 2016), the ECMF community composition did not shift in response to a decrease in P availability or an increase in the soil N:P ratio. This resilience of the ECMF community composition against changes in soil P and N may have been a consequence of the selection of a comparably low number of ECMF species both at the site of origin (Leberecht et al., 2016) and in the experiment. These ECMF species were probably already adapted to sandy, P poor soil conditions due to the spodic Dystrudept from which they originated. Yet, apart from the missing response of the ECM fungal symbiont, ECM host plants are also capable of their own adaptations to reduced nutrient availability through a multitude of modifications in morphology, physiology and biochemistry (Raghothama, 1999; Vance et al., 2003). Among the most effective plant strategies for acclimating or adapting to limiting soil resource availability is increased allocation of carbohydrates and nutrients to the root system (optimal partitioning theory; Bloom, 1985). According to this theory, relative C allocation to roots and the root:shoot ratios are predicted to increase with a decrease in P availability. In addition to the potential allocation shift, trees can also adapt their root morphology and functioning by producing short and thin, fibrous roots with a high frequency of passage cells for the absorption of nutrients and water in response to resource-rich soil spots in P-poor soil (Zadworny and Eissenstat, 2011; Tückmantel et al., 2017). They can likewise change the architecture of root systems to a higher portion of first-order roots when P is limiting (Vance et al., 2003). However, this study neither supports the optimal partitioning theory with respect to P limitation nor studies that found adaptations in root morphology or architecture, as we did not observe any changes in root biomass production, the root

: shoot ratio, or root morphology when beech trees were exposed to decreasing P availability. Therefore, we also have to reject H2.

Despite the absent biomass or morphology response, the European beech saplings investigated showed clear indications of P limitations in leaf photosynthesis and element concentrations in biomass when grown under P-limiting conditions. It seems that they mainly reacted to P limitation by adjusting their metabolism with respect to P uptake and use efficiencies. Plants can generally enhance PUptakeE by increasing the expression of high-affinity inorganic phosphate transporters and altering the membrane structure (Smith et al., 2000; Kavka and Polle, 2016). Increased PUptakeE can also be explained by the ECM symbiosis, which greatly increases the absorptive surface area through the production of small-diameter hyphae which contain many high-affinity transporters and can access smaller soil pores than fine roots can (Richardson et al., 2009; Cairney, 2011). The symbiosis with ECMF also supports the solubility of mineral and occluded P through enhanced exudation of protons or organic acids (Hinsinger, 2001), as well as the mineralization of organic P through the release of phospholytic enzymes (Neumann and Römheld, 2007). In contrast to PUptakeE, increased PUE and PPUE with P limitation have previously been explained by the reallocation of plant P to photosynthetic and growth functions, P recycling from waste RNA, and greater investment into P containing metabolites than into phospholipids for membrane structures (Lambers et al., 2012; Hidaka and Kitayama, 2013). Even though this study does not pinpoint the exact metabolic process that drives enhanced PUptakeE, PUE and PPUE under P-limiting conditions, it seems that the physiological adaptations are sufficient to circumvent negative P-limitation effects and allow maximum growth of beech even when maximum photosynthetic rates and P concentrations in leaves and fine roots are low.

Drought reduces resource use complementarity in the ectomycorrhizal fungal community

While there was no response of the comparably species-poor ECMF community investigated to P limitation (or to elevated N availability), drought induced a shift in the ECMF community from a higher portion of fungi with contact (and some long-distance) exploration towards a higher portion of fungi with short- or medium-distance soil exploration and production of rhizomorphs. A shift in the ECMF community with drought has previously been explained by a reduction in the supply of photosynthates to the root symbionts with drought (Ruehr et al., 2009; Courty et al., 2010), which causes premature death of C demanding (e.g., long-distance ECMF; Pena et al., 2010) or drought-sensitive (Shi et al., 2002; Leberecht et al., 2016) ECMF species. Suppression of photosynthate transport to the root system was a strong determinant of ECMF diversity, which decreased when C transport through the phloem was interrupted by girdling (Pena et al., 2010). This decrease in ECMF diversity was mainly caused by the replacement of subordinate by competitive species with low C demand. By contrast, the observed decrease in ECMF diversity with drought in our study was not accompanied by a community shift towards ECMF species with lower C demand, but instead induced an increase in the portion of ECMF species with higher C demand due to their production of hyphae with further soil exploration or even rhizomorphs (the portion of ECMF species with soil exploration increased from 40% in well-watered soil to 54% in dry soil). Rhizomorph forming fungi have especially high C demand (Trudell and Edmonds, 2004). This higher portion of C-demanding ECMF colonization may have even further strained the C balance of drought-exposed beech (*cf.* Nardini et al., 2000). Otherwise, the diversification of ECMF exploration types can also be considered as adaptation to reduced diffusion in dry soil (Kreuzwieser and Gessler, 2010), which increases the importance of soil exploration for limiting soil resources and spatial niche separation. Apart from the scavenging for soil moisture in dry soil, soil exploration is especially important in P uptake, since soil P occurs mainly in absorbed and not in dissolved forms and the rate of P diffusion to the absorbing hyphae and roots is slow. ECM fungal species with soil exploration via abundant external mycelium may, therefore, have a competitive advantage over species with contact exploration in the search for water and P.

In addition to the ECMF community shift to ECMF species with soil exploration via abundant external mycelia, the total ECMF colonization and diversity were reduced with drought, with negative effects on PUptakeE and tree biomass in support of hypothesis H3. The reduction of PUptakeE was a consequence of the ECMF diversity per se and not of ECMF species identity; thus, a sampling effect in the ECMF diversity–root functioning relationship of our study seems highly unlikely. In support of our result, a study with gray birch seedlings has previously shown that higher ECMF diversity – and not the ECMF community composition or ECMF colonization – increased whole-plant P (Baxter and Dighton, 2001), which may indicate a similar increase in PUptakeE with ECMF diversity as in our study. It has been suggested that diverse arbuscular mycorrhizal fungal communities are complementary in their ability to acquire and utilize inorganic and organic P (Koide, 2000). Diverse ECMF communities have high functional diversity with respect to their enzymatic activity profiles and are also assumed to be functionally complementary in their ecological niche: diverse ECMF communities can access a greater diversity of P pools and organic matter (Jones et al., 2010; Velmala et al., 2014). Reduced ECMF diversity in dry soil as a result of premature death of drought-sensitive and C-demanding ECMF species (in our study both contact and long-distance exploration types were affected) should accordingly lead to decreased and less complete resource exploitation of soil P and to colimitation of both water and P in dry soil. This effect of reduced ECMF diversity on the uptake of growth-limiting P by the host tree was stronger and could not be compensated for by a shift in the species-poor ECMF community investigated towards fungi with short- and medium-distance soil exploration.

Conclusion

We investigated interactive effects of the expected global change conditions on ECMF communities and the consequences of ECMF diversity for P uptake and use efficiency of European beech saplings. Using this approach, we found fundamentally different responses to decreasing P availability or increasing summer droughts. Notably, P limitation was mainly counterbalanced by physiological adaptations of the ECM host tree, while the species-poor ECMF community investigated remained unaltered. Summer droughts, in contrast, shifted the ECMF community composition and ECMF diversity, with negative effects on the efficiency of P uptake and tree biomass. Our findings indicate that the efficiency of P uptake is mediated by the ECMF symbiosis and (1) increases with decreasing P availability, but (2) decreases with increasing soil drought under global change, due to contrasting effects of the environments. When translating these results into a wider context, two facets have to be taken into account. First, since the study was conducted with saplings, the findings can be transferred to adult trees only with great care. Second, the low species richness of the ECMF communities investigated, which originated from nutrient-poor forest soil, may have limited the responsiveness to decreasing nutrient availability, and our results should be reconfirmed by field investigations across a natural range of nutrient availabilities and ECMF communities. Our current results thus highlight the need to further advance our mechanistic understanding of the interactions between ECMF diversity and root functioning.

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Supplementary Material

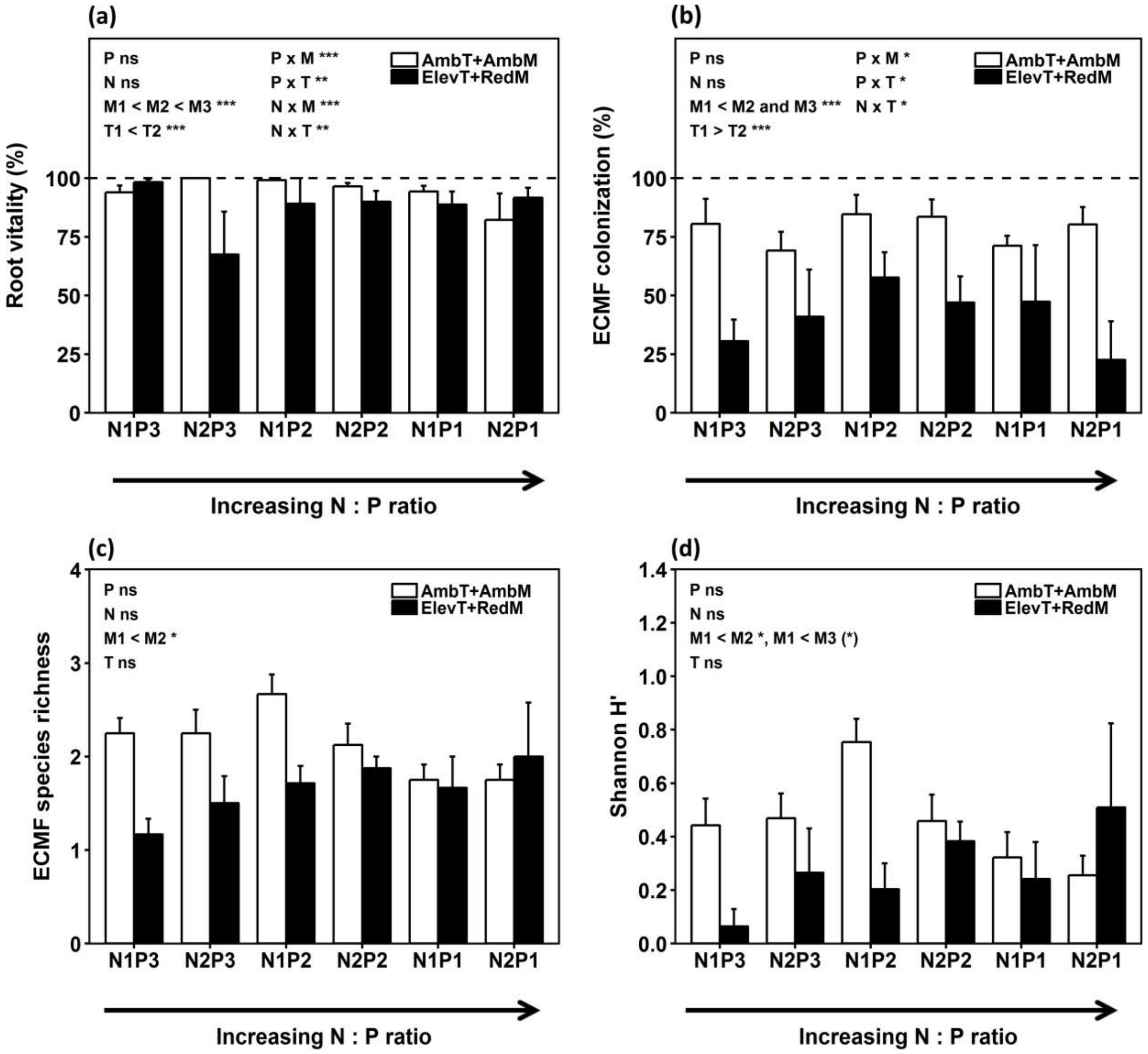


Figure S3.1 Means and SE of (a) root vitality, (b) ectomycorrhizal fungal (ECMF) colonization, (c) ECMF species richness and (d) ECMF α diversity (Shannon H') of European beech saplings grown at increasing soil nitrogen (N) : phosphorus (P) ratios and ambient climate (ambient temperature and soil moisture, AmbT+AmbM) or climate change conditions (elevated temperature and reduced soil moisture, ElevT+RedM) in climate chambers. The associated tables show the results of four-factorial ANOVAs ((*), P < 0.1; *, P < 0.05; **, P < 0.01; ***, P < 0.001; ns, not significant; *n* = eight replicates per treatment). P supply: P1, low; P2, moderate; P3, high. N supply: N1, ambient; N2, elevated. Temperature: T1, ambient; T2, elevated. Soil moisture: M1, drought; M2, moderately moist; M3, well-watered.

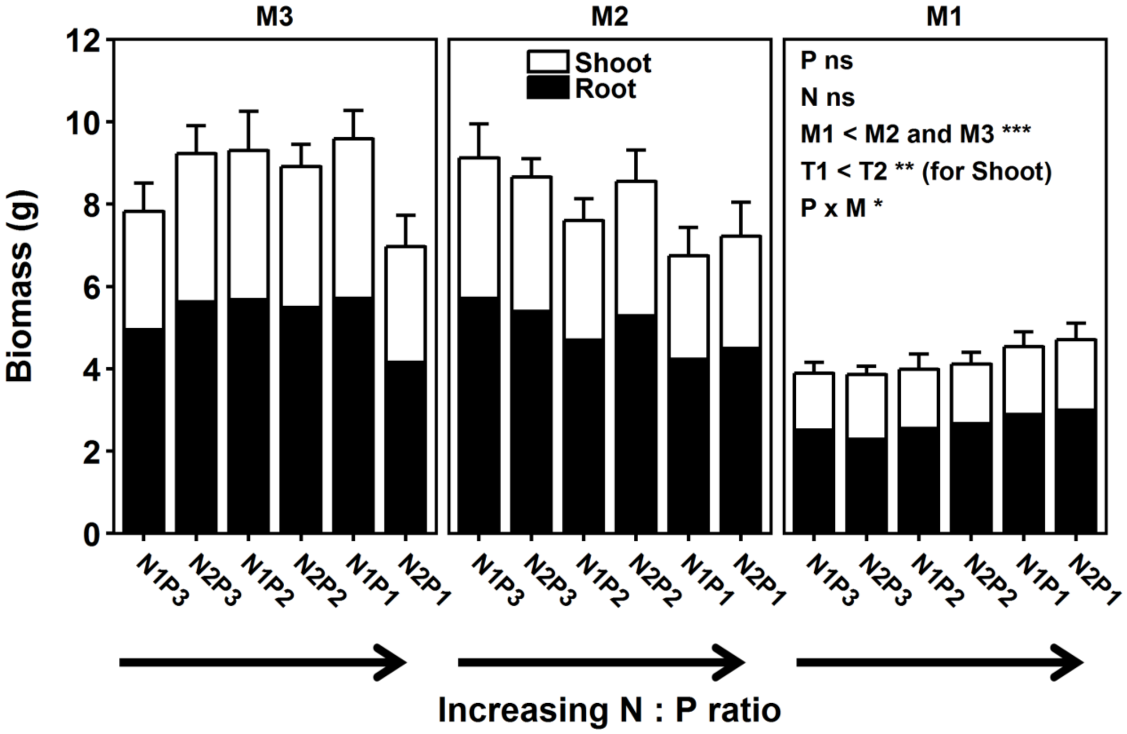


Figure S3.2 Means and SE of shoot (leaves and woody parts) and root (fine and coarse roots) biomass of European beech saplings grown at increasing soil nitrogen (N) : phosphorus (P) ratios and decreasing soil moisture in climate chambers. Shown are the results of four-factorial ANOVAs (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, not significant) for all investigated treatments (n = eight replicates per treatment). P supply: P1, low; P2, moderate; P3, high. N supply: N1, ambient; N2, elevated. Temperature: T1, ambient; T2, elevated. Soil moisture: M1, drought; M2, moderately moist; M3, well-watered.

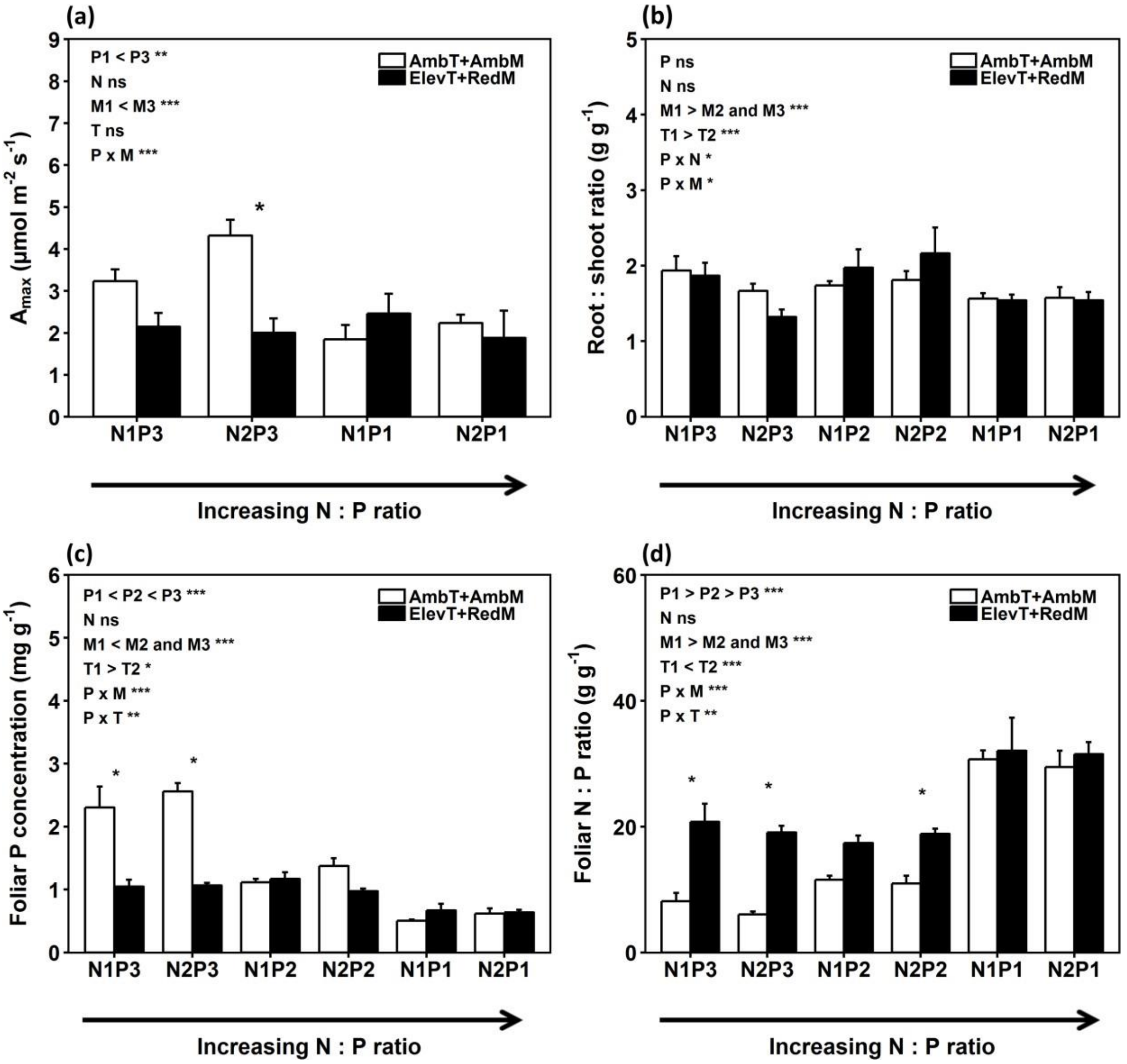


Figure S3.3 Means and SE of (a) A_{max}, (b) root : shoot ratio, (c) foliar phosphorus (P) concentration and (d) foliar nitrogen (N) : P ratio of European beech saplings grown at increasing soil N:P ratios and ambient climate (ambient temperature and soil moisture, AmbT+AmbM) or climate change conditions (elevated temperature and reduced soil moisture, ElevT+RedM) in climate chambers. The associated tables show the results of four-factorial ANOVAs (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, not significant; n = five replicates per treatment for A_{max}, n = eight replicates per treatment for the root : shoot ratio, foliar P concentration and foliar N:P ratio). P supply: P1, low; P2, moderate; P3, high. N supply: N1, ambient; N2, elevated. Temperature: T1, ambient; T2, elevated. Soil moisture: M1, drought; M2, moderately moist; M3, well-watered.

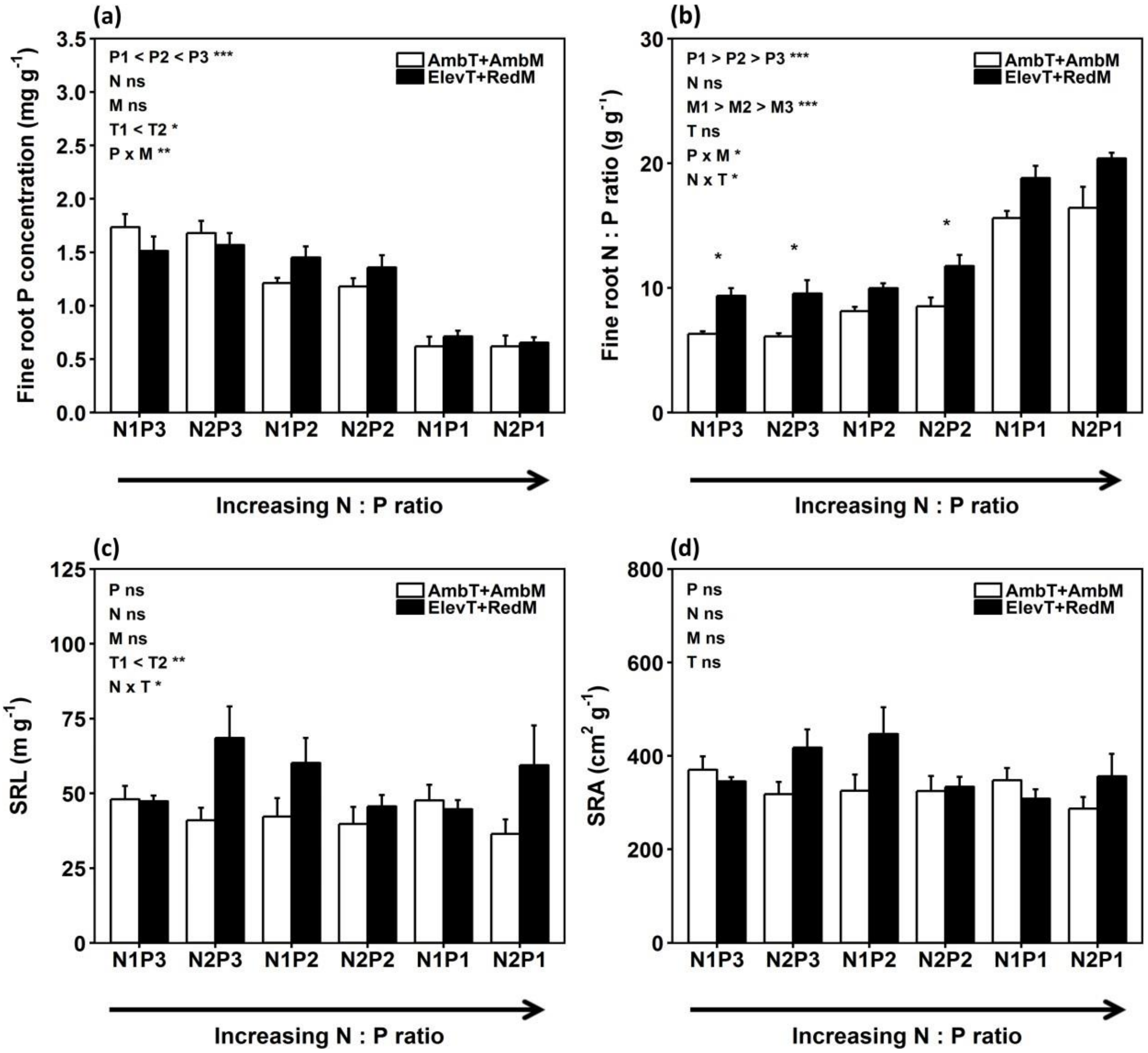


Figure S3.4 Means and SE of (a) fine root phosphorus (P) concentration, (b) fine root nitrogen (N) : P ratio, (c) specific root length (SRL) and (d) specific root area (SRA) of European beech saplings grown at increasing soil N:P ratios and ambient climate (ambient temperature and soil moisture, AmbT+AmbM) or climate change conditions (elevated temperature and reduced soil moisture, ElevT+RedM) in climate chambers. The associated tables show the results of four-factorial ANOVAs (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, not significant; n = eight replicates per treatment). P supply: P1, low; P2, moderate; P3, high. N supply: N1, ambient; N2, elevated. Temperature: T1, ambient; T2, elevated. Soil moisture: M1, drought; M2, moderately moist; M3, well-watered.

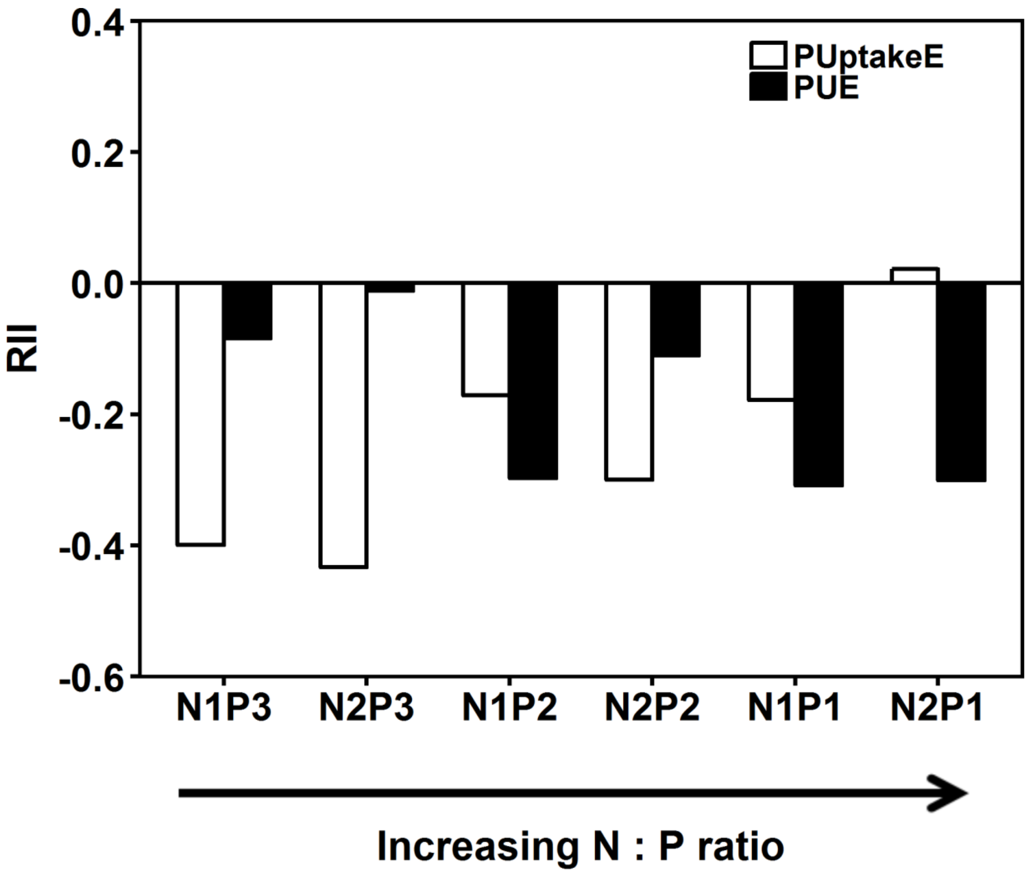
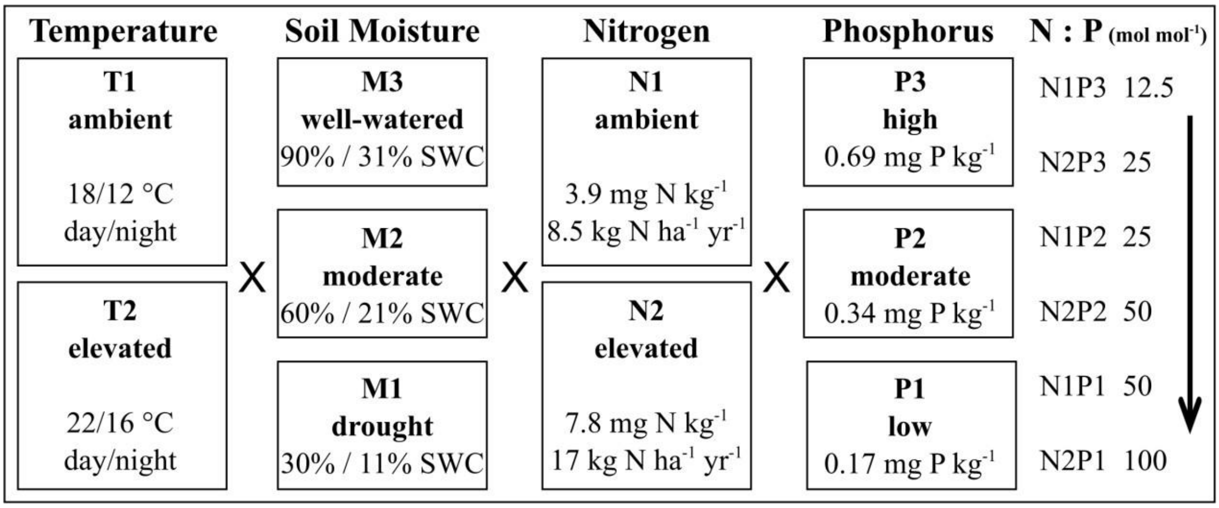


Figure S3.5 Relative interaction intensity (RII) for the drought effect on phosphorus (P) uptake (PUptakeE) and P use (PUE) efficiencies of European beech saplings grown at increasing soil N:P ratios in climate chambers (*n* = eight replicates per treatment). The relative drought effect on PUptakeE increased significantly with decreasing P availability (i.e., along the N:P ratio gradient; y = 0.004x - 0.44; R^2 = 0.61, $P < 0.05$). P supply: P1, low; P2, moderate; P3, high. N supply: N1, ambient; N2, elevated.

Table S3.1 Study design of the full-factorial experiment with two temperature, three soil moisture, two nitrogen (N) supply and three phosphorus (P) supply levels (*n* = 36 treatments in total). Temperature: T1, ambient; T2, elevated. Soil moisture: M1, drought; M2, moderately moist; M3, well-watered. N supply: N1, ambient; N2, elevated. P supply: P1, low; P2, moderate; P3, high. A gradient from current environmental conditions to the expected future global change conditions (elevated temperature and N deposition and reduced soil moisture and P availability) was simulated.



Temperature		**Soil Moisture**		**Nitrogen**		**Phosphorus**	**N : P** (mol mol^{-1})
T1 **ambient** 18/12 °C day/night		**M3** **well-watered** 90% / 31% SWC		**N1** **ambient** 3.9 mg N kg^{-1} 8.5 kg N ha^{-1} yr^{-1}		**P3** **high** 0.69 mg P kg^{-1}	N1P3 12.5 N2P3 25
	X	**M2** **moderate** 60% / 21% SWC	X		X	**P2** **moderate** 0.34 mg P kg^{-1}	N1P2 25 N2P2 50
T2 **elevated** 22/16 °C day/night		**M1** **drought** 30% / 11% SWC		**N2** **elevated** 7.8 mg N kg^{-1} 17 kg N ha^{-1} yr^{-1}		**P1** **low** 0.17 mg P kg^{-1}	N1P1 50 N2P1 100

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Table S3.2 Molecular identification and morphological characterization of ectomycorrhizal fungal species associated with European beech saplings. Given are species names according to the best BLAST match, the National Center for Biotechnology Information (NCBI) GenBank accession numbers, the length of the nucleotide sequences (identity), their similarity with the reference sequence, as well as a description of the morphotype.

Species	**Best BLAST matches**		**Morphotype**
	GenBank accession No.	Identity, % similarity	
Cenococcum geophilum FR.	KX545374	386/397 97%	 Single, black, black emanating hyphae, short-distance exploration type, no rhizomorphs
Genea hispidula BERK.& BROOME EX TUL.&C.TUL.	KX545376	690/690 100%	 Monopodial pinnate ramification, brown, smooth mantle surface, short-distance exploration type, no rhizomorphs
Lactarius tabidus FR.	KX545377	736/737 99%	 Irregularly pinnate ramification, beige, smooth mantle surface, contact exploration type, no rhizomorphs

Table S3.2 Continued.

Species	Best BLAST matches		Morphotype
	GenBank accession No.	Identity, % similarity	
Uncultured *Scleroderma* sp. PERS.	KX545378	709/715 99%	 Irregularly pinnate ramification, beige, cottony mantle surface with emanating white hyphae, long-distance exploration type, frequent rhizomorphs
Thelephora terrestris EHRH.:FR.	KX545375	687/689 99%	 Pinnate ramification, stringy mantle surface, medium-distance smooth exploration type, rhizomorphs
Tomentellopsis submollis (SVRČEK) HJORTSTAM	KX545379	561/562 99%	 Monopodial, brown, medium-distance smooth exploration type, rhizomorphs

Table S3.3 Relative abundances of ectomycorrhizal fungal (ECMF) species of individual beech saplings averaged for each treatment. Temperature: T1, ambient; T2, elevated. Soil moisture: M1, drought; M2, moderately moist; M3, well-watered. Nitrogen (N) supply: N1, ambient; N2, elevated. Phosphorus (P) supply: P1, low; P2, moderate; P3, high.

T	**M**	**N**	**P**	**NP**	***Cenoccocum geophilum***	***Genea hispidula***	***Lactarius tabidus***	***Scleroderma* sp.**	***Thelephora terrestris***	***Tomentellopsis submollis***
T1	**M1**	**N1**	**P1**	**N1P1**	0.22	0.09	0.52	0.00	0.17	0.00
			P2	**N1P2**	0.28	0.08	0.38	0.00	0.12	0.14
			P3	**N1P3**	0.33	0.28	0.39	0.00	0.00	0.00
		N2	**P1**	**N2P1**	0.28	0.13	0.30	0.24	0.05	0.00
			P2	**N2P2**	0.06	0.27	0.66	0.00	0.00	0.01
			P3	**N2P3**	0.26	0.46	0.24	0.00	0.04	0.00
	M2	**N1**	**P1**	**N1P1**	0.11	0.00	0.75	0.00	0.14	0.00
			P2	**N1P2**	0.15	0.00	0.84	0.00	0.01	0.00
			P3	**N1P3**	0.13	0.09	0.78	0.00	0.00	0.00
		N2	**P1**	**N2P1**	0.22	0.11	0.43	0.24	0.00	0.00
			P2	**N2P2**	0.29	0.10	0.55	0.06	0.00	0.00
			P3	**N2P3**	0.16	0.00	0.63	0.00	0.21	0.00
	M3	**N1**	**P1**	**N1P1**	0.14	0.00	0.86	0.00	0.00	0.00
			P2	**N1P2**	0.18	0.27	0.55	0.00	0.00	0.00
			P3	**N1P3**	0.13	0.27	0.60	0.00	0.00	0.00
		N2	**P1**	**N2P1**	0.22	0.00	0.32	0.46	0.00	0.00
			P2	**N2P2**	0.16	0.10	0.74	0.00	0.00	0.00
			P3	**N2P3**	0.15	0.05	0.79	0.00	0.01	0.00

Table S3.3 Continued.

T	**M**	**N**	**P**	**NP**	***Cenoccocum geophilum***	***Genea hispidula***	***Lactarius tabidus***	***Scleroderma* sp.**	***Thelephora terrestris***	***Tomentellopsis submollis***
T2	**M1**	**N1**	**P1**	**N1P1**	0.42	0.58	0.00	0.00	0.00	0.00
			P2	**N1P2**	0.38	0.00	0.62	0.00	0.00	0.00
			P3	**N1P3**	0.19	0.00	0.81	0.00	0.00	0.00
		N2	**P1**	**N2P1**	0.52	0.09	0.39	0.00	0.00	0.00
			P2	**N2P2**	0.25	0.10	0.65	0.00	0.00	0.00
			P3	**N2P3**	0.83	0.14	0.03	0.00	0.00	0.00
	M2	**N1**	**P1**	**N1P1**	0.23	0.05	0.72	0.00	0.00	0.00
			P2	**N1P2**	0.36	0.01	0.63	0.00	0.00	0.00
			P3	**N1P3**	0.32	0.02	0.54	0.00	0.12	0.00
		N2	**P1**	**N2P1**	0.29	0.08	0.63	0.00	0.00	0.00
			P2	**N2P2**	0.36	0.25	0.29	0.10	0.00	0.00
			P3	**N2P3**	0.68	0.00	0.32	0.00	0.00	0.00
	M3	**N1**	**P1**	**N1P1**	0.23	0.20	0.33	0.00	0.24	0.00
			P2	**N1P2**	0.40	0.23	0.31	0.00	0.06	0.00
			P3	**N1P3**	0.31	0.00	0.69	0.00	0.00	0.00
		N2	**P1**	**N2P1**	0.36	0.00	0.64	0.00	0.00	0.00
			P2	**N2P2**	0.24	0.00	0.76	0.00	0.00	0.00
			P3	**N2P3**	0.18	0.34	0.48	0.00	0.00	0.00

Table S3.4 Simple linear Spearman´s rank correlation analyses for the relationships between biomass, phosphorus (P) uptake and use efficiencies, root vitality and the ectomycorrhizal fungal (ECMF) community of European beech saplings grown at increasing soil N:P ratios and climate change PUptakeE, P uptake efficiency; PUE, P use efficiency; PPUE, photosynthetic P use efficiency. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. Statistically significant values are indicated in bold text.

	PUptakeE	**PUE**	**PPUE**	**Root vitality**	**ECMF coloni-zation**	**ECMF species richness**	**ECMF α diversity**
Total plant biomass	__0.73***__	0.18	0.34	__0.56***__	__0.37*__	__0.41*__	__0.54***__
Root biomass	__0.75***__	0.17	0.37	__0.55***__	__0.41*__	__0.44**__	__0.52***__
PUptakeE		__0.56***__	0.45	__0.42*__	0.28	__0.41*__	__0.55***__
PUE			__0.76***__	0.04	0.27	0.10	0.13
PPUE				0.04	0.33	-0.05	-0.01
Root vitality					0.09	0.10	0.20
ECMF colonization						__0.59***__	0.15
ECMF species richness							__0.75***__

Table S3.5 Four-factorial ANOVAs on the significance of the effects of phosphorus (P) availability, nitrogen (N) availability, temperature (T), soil moisture (M) and their interactions on the variance of ectomycorrhizal fungal (ECMF) colonization and ECMF α diversity of European beech saplings grown at increasing soil N:P ratios and climate change. Statistically significant values are indicated in bold text (n = eight replicates per treatment).

	ECMF colonization		**ECMF α diversity**	
	F	***P***	***F***	***P***
P	1.1	0.32	0.4	0.66
N	3.2	0.08	0.4	0.52
T	**20.9**	**<0.001**	0	0.85
M	**11.3**	**<0.001**	**3.6**	**<0.05**
P x N	2.4	0.09	0.1	0.89
P x T	**4.7**	**<0.05**	1.8	0.16
N x T	**6**	**<0.05**	1.4	0.24
P x M	**2.8**	**<0.05**	2	0.09
N x M	2.9	0.06	0.6	0.56
P x N x T	2.4	0.09	0	0.98
P x N x M	2.4	0.05	1	0.42

Table S3.6 Simple linear Spearman´s rank correlation analyses for the relationships between phosphorus (P) uptake or use efficiency and the ectomycorrhizal fungal (ECMF) community composition of European beech saplings grown at increasing soil nitrogen (N) : P ratios and climate change. PUptakeE, P uptake efficiency; PUE, P use efficiency; PPUE, photosynthetic P use efficiency. ECMF α diversity refers to ECMF species diversity at the individual beech sapling level, ECMF β diversity to ECMF species diversity at the treatment level. Statistically significant values are indicated in bold text (*n* = 36 treatments).

Y	**X**	***R***	***P***
PUptakeE	ECMF colonization	0.28	0.10
PUptakeE	ECMF species richness	**0.41**	**<0.05**
PUptakeE	ECMF α diversity	**0.55**	**<0.001**
PUptakeE	ECMF β diversity	0.11	0.53
PUE	ECMF colonization	0.27	0.11
PUE	ECMF species richness	0.10	0.55
PUE	ECMF α diversity	0.13	0.43
PUE	ECMF β diversity	0.17	0.33
PPUE	ECMF colonization	0.29	0.27
PPUE	ECMF species richness	-0.03	0.92
PPUE	ECMF α diversity	0.05	0.85
PPUE	ECMF β diversity	0.41	0.11

CHAPTER 4

Drought deteriorates the N stoichiometry of biomass production in European beech saplings under global change

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Ina C. Meier**

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Summary

Continuous nitrogen (N) deposition has raised concerns that temperate forest ecosystems shift from N limitation to progressing P limitation under global change. According to the multiple resource limitation theory, this will not only influence P economy, but also reduce N uptake and use efficiencies of trees such that growth is equally limited by N and P. We used different global change scenarios including summer drought to test the prediction of this hypothesis for the N economy of European beech (*Fagus sylvatica* L.) saplings. Our study demonstrates that the N uptake efficiency (NUptakeE) of beech adjusts to soil N availability and stabilizes leaf and fine root N concentrations. By contrast, both efficiencies of N use were curtailed by increasing phosphorus (P) limitation under elevated soil N. The photosynthetic N use efficiency (PNUE) was serially reduced with decreasing soil P availability and increasing foliar N:P ratios, while the N use efficiency (NUE) decreased with increasing fine root N:P ratios. Soil drought induced relative P deficiency alike and reduced NUptakeE, PNUE, and NUE independent from the soil N:P ratios.

We conclude that not only N deposition but also increasing summer droughts might affect N:P ratios, thereby inducing P imbalances and affecting the N economy of European beech saplings under global change.

Keywords: drought, *Fagus sylvatica*, nitrogen deposition, phosphorus limitation, uptake efficiency, use efficiency

Introduction

The net primary production of temperate forests is primarily limited by N availability (LeBauer and Treseder, 2008). In recent decades, N availability has rapidly increased in most areas of the globe due to increased N deposition from fossil fuel combustion and intensive agriculture (Galloway et al., 2008). Increased N availability changes nutrient stoichiometry, i.e., leads to rises in the N:P ratio of ecosystems (Peñuelas et al., 2013). As a result, the degree of relative P deficiency increases and potentially shifts temperate forests from N limitation to P limitation (Talkner et al., 2015; Sardans et al., 2016; Hedwall et al., 2017).

According to co-limitation hypothesis, N acquisition and use efficiencies depend on P availability and vice versa (Ågren et al., 2012). According to this theory, P deficiency will increase P uptake efficiency (PUptakeE, that is the P content in recent biomass relative to the amount of available soil P; Fujita et al., 2010), but simultaneously may also reduce N uptake efficiency (NUptakeE, that is the N content in recent biomass relative to the amount of soil N) and N use efficiency (NUE, i.e. the increment in biomass for a given increase in biomass N). Yet there is currently no mechanistic understanding if (at all) plants achieve simultaneous co-limitation by N and P via modifications in root uptake kinetics (Rothstein et al., 2000) or via changes in root morphology and biomass partitioning (Zhang et al., 2018) or both.

Mycorrhizal symbiosis plays a major role in the P acquisition (Ferlian et al., 2018; Raven et al., 2018) and (to some extent) in the N acquisition of plants (Gobert and Plassard, 2008; Pena and Polle, 2014). Ectomycorrhizal fungi (ECMF) species are functionally diverse and vary in their influence on N acquisition (Pena and Polle, 2014), which makes the ECMF community composition an important factor for nutrient acquisition. In addition, the degree of mycorrhizal colonization affects the portion of mycorrhizal control on resource acquisition. Yet mycorrhizal control may greatly differ between P and N acquisition: low mobility of orthophosphates causes depletion zones around roots and may raise the importance of ECMF hyphae for P access beyond the root depletion zone, while this effect seems less important for highly mobile nitrate.

Nutrient availability also depends on water availability (Gessler et al., 2017). In dry soil, nutrient transport to roots through mass flow and diffusion is slowed down, which can induce edaphic N limitation and reduce the N uptake capacity of trees even in N-rich soil. Soil drought impairs soil microbes and can shift the mycorrhizal community composition with potential consequences for the nutrient uptake of the host tree (Leberecht et al., 2016). This is likely the case in severely dry soil in which the allocation of photosynthates to belowground structures is inadequate (Ruehr et al., 2009). Finally, drought can also decrease root vitality (Burton et al., 1998; Huang et al., 2005; Köhler et al., 2018) and induce fine root dieback (Meier and Leuschner, 2008), which reduces the active root surface area for nutrient uptake. However, the dependence of nutrient stoichiometry and nutrient co-limitation of important temperate forest trees on soil drought as expected under global change are not well understood (Korell et al., 2019).

In previous research, we examined the consequences of different global change scenarios (i. e., elevated temperature, N deposition, reduced soil moisture, and P availability) for P uptake and use efficiencies of European beech saplings (Köhler et al., 2018). We demonstrated that P uptake efficiency of beech in dry soils significantly decreased with decreasing ECMF α diversity, i. e. the Shannon-Weaver index based on the total number of ECMF species of individual beech saplings, whereas the P use efficiency was negatively related to soil P availability. In the related current study, we aimed to investigate the effect of global change on the N economy of European beech saplings in a four-factorial climate chamber experiment that simulated the expected conditions for Central Europe. We hypothesized that (H1) beech achieves co-limitation by N and P under increasing soil N availability since NUptakeE and NUE decrease, (H2) increasing ECMF colonization rates and diversity increase NUptakeE; and (H3) summer drought strongly interferes with the acclimation of NUptakeE of beech to soil N availability and leads to shifts in the N:P stoichiometry and decreases in PNUE and NUE.

Material and Methods

Plant material and experimental set-up

The present study uses the same provenances and experimental set-up as the previous work on the effect of global change on the composition of ECMF communities and the consequences for P uptake and use efficiencies of beech trees (Köhler et al., 2018). Briefly, the experiment was conducted with two-year old European beech (*Fagus sylvatica* L.) saplings that originated from the 2012 tree masting of a mature beech forest in northwest Germany (N 52° 50’, E 10° 19’). The forest site was located on Pleistocene fluvioglacial sandy deposits with relatively low water storage capacity (79 mm 120 cm^{-1}) at a mean annual precipitation of 816 mm and with a mean annual temperature of 8.7°C (for the period 1981-2010; climate data were provided by Deutscher Wetterdienst and were corrected for altitude; *cf*. Knutzen et al., 2017). At this forest site, adult beech trees showed on average a fungal colonization rate of 75% (Hertel et al., 2013) and had comparably species poor ECMF communities with, on average, nine ECMF species in adult trees (Zavišić et al., 2016) and three to six ECMF species in beech saplings (Leberecht et al., 2016). Excavated beech seedlings and their associated indigenous ECMF communities were cultivated in a sterilized 2:1 sand:peat mixture outdoors for one year in the Botanical Garden Göttingen, Central Germany. In May 2014, beech saplings were planted in 1.5-l pots filled with sieved and homogenized sandy mineral topsoil from the provenance, which contained the indigenous ECMF community. Thus, we aimed for maximizing the ECMF colonization rate of the beech saplings. Saplings of similar size (mean shoot height 20 cm, thirteen leaves) were arranged in a randomized array in a greenhouse and were kept well-watered.

In July 2014, we established a full factorial experiment with four factors (two temperature x three soil moisture x two N supply x three P supply levels; 36 treatments in total; *n* = eight replicates per treatment; see **Table S4.1**). We simulated a gradient from current environmental conditions to the expected future global change conditions (elevated temperature and N deposition and reduced soil moisture and P availability), we established (i) ambient (T1; 18/12°C day/night) and elevated temperature conditions (T2; 22/16°C) in two separate climate chambers. Furthermore, we set-up (ii) three soil moisture levels, ambient, well-watered (M3; 90% field capacity (FC); 32% soil water content (SWC); v/v), moderately moist (M2; 60% FC; 21% SWC), and drought treatment (M1; 30% FC; 11% SWC). The field capacity of the sandy soil used in the experiment was determined according to Naeth et al. (1991). Water loss by evapotranspiration was replaced every second day by watering the pots to their target weight. We established (iii) three P availability levels, a high (P3; 0.69 mg P kg^{-1}), a moderate (P2; 0.35 mg P kg^{-1}), and a low P supply treatment (P1; 0.17 mg P kg^{-1}), by adding biweekly NaH_2PO_4 as component of the Hoagland solution. Finally, we simulated (iv) ambient (N1; 3.9 mg N pot^{-1} growing season^{-1}, representing 8.5 kg N ha^{-1} year^{-1}) and elevated N deposition (N2; 7.8 mg N pot^{-1} growing season^{-1}, representing 17 kg N ha^{-1} year^{-1}), estimated on the basis of the current N deposition rates occurring in deciduous forests of Northern Germany (Schaap et al., 2018). N was added biweekly as NH_4NO_3 as component of the Hoagland solution. Our fertilization regime led to six N:P treatments: relative P availability decreased from N1P3 (0.69 mg P; N:P 5.6 g g^{-1}) to N2P3 (0.69 mg P; N:P 11.3 g g^{-1}), N1P2 (0.35 mg P; N:P 11.3 g g^{-1}), N2P2 (0.35 mg P; N:P 22.6 g g^{-1}), N1P1 (0.17 mg P; N:P 22.6 g g^{-1}), and N2P1 (0.17 mg P; N:P 45.2 g g^{-1}). Beech saplings were randomly assigned to these experimental treatments, positioned in a randomized design in two highly controllable walk-in climate chambers (York, Johnson Control Systems, Essen, Germany), and re-randomized biweekly for two growing seasons. During the non-growing season 2014/2015 (i.e., November 2014 to March 2015) temperatures were reduced to 10/8°C day/night, the day length from 14 to 7.5 hrs, and photosynthetic photon flux density from 190 to 30 µmol photons $m^{-2} s^{-1}$, while drought and fertilization treatments were paused. All conditions were brought back to the experimental conditions in April 2015 and maintained until the harvest of the experiment in September 2015. In total, we simulated two subsequent growing seasons with altered climatic and nutrient conditions for a total of 456 days.

Photosynthetic capacity

At the end of June 2015, leaf gas exchange measurements were conducted for saplings grown at ambient and elevated temperature, well-watered and dry soil conditions, high and low P availability, and ambient and elevated N availability; each treatment combination being replicated five times (n = 80 saplings). Measurements were conducted on one canopy leaf per plant with an infrared CO_2 analyzer (LI-6400; LI-COR Biosciences, Lincoln, NE, USA) during the midday hours. We measured the photosynthetic capacity (A_{max}; µmol CO_2 $m^{-2} s^{-1}$) at high photosynthetically active radiation (PAR: 1500 µmol $m^{-2} s^{-1}$; see Supplementary Table S2). Saturating light conditions were previously determined from light curves measured for a subset of plants. During the measurements, average leaf temperature was 18.2°C (T1) and 22.1°C (T2), relative humidity 61.3% (T1) and 56.2% (T2), vapor pressure deficit 9.6 hPa (T1) and 13.9 hPa (T2), flow rate 400.03 µmol s^{-1} (T1) and 400.1 µmol s^{-1} (T2), block temperature 15.0°C (T1) and 18.6°C (T2) and CO_2 concentrations were ambient (390 µmol CO_2 mol^{-1} air). We calculated total C assimilation per plant (in µmol C h^{-1}) by multiplying A_{max} with total leaf area, which was determined from the product of specific leaf area (SLA in $m^2 kg^{-1}$; measured by using a flat-bed scanner and the computer program WinFOLIA 2014c; Régent Instruments Inc., Canada) and total dry biomass of green leaves. Photosynthetic N use efficiency was calculated according to PNUE = A_{max}*SLA/N_{Leaves} (in µmol CO_2 g^{-1} N s^{-1}), where N_{Leaves} is the foliar N concentration (*cf.* Li et al., 2012).

Above and Belowground Biomass and ECMF Colonization

All beech saplings were divided into senescent leaves, green leaves, shoots, coarse roots (diameter > 2 mm), and fine roots (diameter ≤ 2 mm) in mid-September 2015. For each sapling, we selected one representative, carefully washed, intact fine root section, transferred it to 5% glycerol solution, and kept it frozen at -18°C for subsequent analysis of the mycorrhizal colonization rate (in %; n = eight replicates per treatment). Fine root tips were counted (n = 85,327) and the ECMF morphotypes characterized (as per Agerer, 1987-2012). ECMF colonization rate was calculated as the percentage of the number of ECMF root tips in all vital root tips (see

Table S4.2). Root vitality was calculated as the percentage of vital root tips in all analyzed fine root tips (in %). Ten to twenty tips of each distinct morphotype were collected and used for molecular identification of fungal species (*cf*. Köhler et al., 2018). To describe ECMF diversity, we calculated the Shannon-Weaver index (Shannon and Weaver, 1949) from the relative proportion of an ECMF species in the total number of ECMF species per individual beech saplings (ECMF α diversity). Root length and root surface area were determined with WinRHIZO 2013e (Régent Instruments Inc., Canada; n = eight replicates per treatment). Subsequently, all leaves, stems, coarse roots, and fine roots were dried (48 h, 70°C) and weighed (see **Table S4.3**). Specific root area (SRA, in cm^2 g^{-1}) and specific root length (SRL, in m g^{-1}) were calculated.

N uptake and use efficiencies

All dried plant compartments were ground with a vibratory disc mill and were analyzed for total C and N content with an elemental analyzer (Vario EL III, elementar, Hanau, Germany). N uptake efficiency (NUptakeE; in g N_{DW} g^{-1} N_{added}) was calculated by dividing the N content in recent biomass, i.e. in fine roots, green and senescent leaves, by its amount added by fertilization during the 2015 growing season (n = eight replicates per treatment). N use efficiency (NUE, in g DW g^{-1} N_{DW}) was calculated from the increment in total dry biomass for a given increase in biomass N (*cf*. Koide, 1991).

Statistical analyses

Statistical analyses were conducted with R 3.3.1 (R Development Core Team). Means and SE were calculated from eight replicates per treatment (A_{max}: $n = 5$). Residuals of the models were tested for normal distribution and homogeneity of variances by Shapiro-Wilk tests. The effects of N availability, P availability, temperature, soil moisture, and their interactions (fixed effects) on the response variables NUptakeE, PNUE, NUE and plant nutrient concentrations(N concentration, C:N ratio and N:P ratio in leaves, fine roots and coarse roots) were analyzed by four-way ANOVAs type III using the *car* package (Fox and Weisberg, 2011). Tukey's honest significant difference post-hoc tests were conducted to identify significant differences between individual treatments. We calculated Pearson correlations for the relationships between NUptakeE, PNUE or NUE with root vitality, ECMF colonization rate, ECMF species richness, ECMF α diversity, A_{max}, C assimilation, leaf and root morphology, and plant nutrient concentrations. For significant correlations, we conducted linear regression analyses and tested the significances of the slopes and intercepts as well as of the entire correlation models. The slopes of all significant correlation models were significant as well, while the intercepts were significant in most cases (exceptions: the intercept of the relationships between NUptakeE and fine root C:N ratio and between PNUE and coarse root C:N). For the relationship between NUptakeE and fine root biomass or fine root C:N ratio, pairwise comparisons of the slopes of N1 and N2 were performed using the 'lstrends' function in the *lsmeans* R package (Lenth, 2016). We used SAS v 9.3 (SAS Institute Inc., Cary, NC, US) to conduct multiple regression analyses with backward variable elimination to test for significant independent predictors of NUptakeE, NUE, and PNUE. At each elimination step, the variable showing the smallest contribution to a model was deleted until all the variables remaining in the model produced significant *F* statistics even if their significant contribution was only small. Multicollinearity among variables was diagnosed: when (i) the significance of *t* tests for individual slopes differed from the *F* test of the model, (ii) pairs of predictor variables were highly correlated ($r > 0.7$), and (iii) collinearity diagnostics were critical (Belsley et al., 1980).

Results

Plant N concentrations and C:N ratios in leaves and fine roots

Despite the extended gradients in soil N availabilities (from 3.9 to 7.8 mg N kg^{-1}) and soil N:P ratios (from 5.6 to 45.2 g g^{-1}), N concentrations in beech leaves, fine roots, and coarse roots remained comparably constant across the range of soil nutrient conditions (see **Figure S4.1**A, C, E). The average N concentration of leaves was 15 mg N g^{-1} and the average N concentration of fine roots 10 mg N g^{-1} at ambient climate conditions. With elevated temperature and reduced soil moisture, N concentrations increased to 19 mg N g^{-1} in leaves and to 15 mg N g^{-1} in fine roots. Leaf and fine root C concentrations were similar and not influenced by any of the experimental treatments (leaves and fine roots: 0.5 g C g^{-1}). Due to the increase in N concentrations with elevated temperature and drought, fine root and foliar C:N ratios significantly decreased from ambient to climate change conditions (fine roots: from 48 to 33 g g^{-1}; leaves: from 31 to 25 g g^{-1}; see **Figure S4.1**B, F). Similarly, drought also strongly increased coarse root N concentrations by 83% (from 6 to 11 mg N g^{-1}; see **Figure S4.1**C), which led to a decrease in the coarse root C:N ratio by about a half (see **Figure S4.1**D).

Nitrogen uptake efficiency

NUptakeE related negatively to soil N availability (**Figure 4.1**A; see **Table 4.2** and **Table S4.4**). Elevated soil N availability reduced NUptakeE of beech saplings by almost half (from 9.5 to 4.9 g N_{DW} g^{-1} N_{added}). Elevated temperature slightly increased NUptakeE by 13% (from

6.8 to 7.7 g N_{DW} g^{-1} N_{added}), while soil drought reduced NUptakeE by 20% (from 7.7 to 6.2 g N_{DW} g^{-1} N_{added}). Soil P availability or the soil N:P ratio had no effect on NUptakeE.

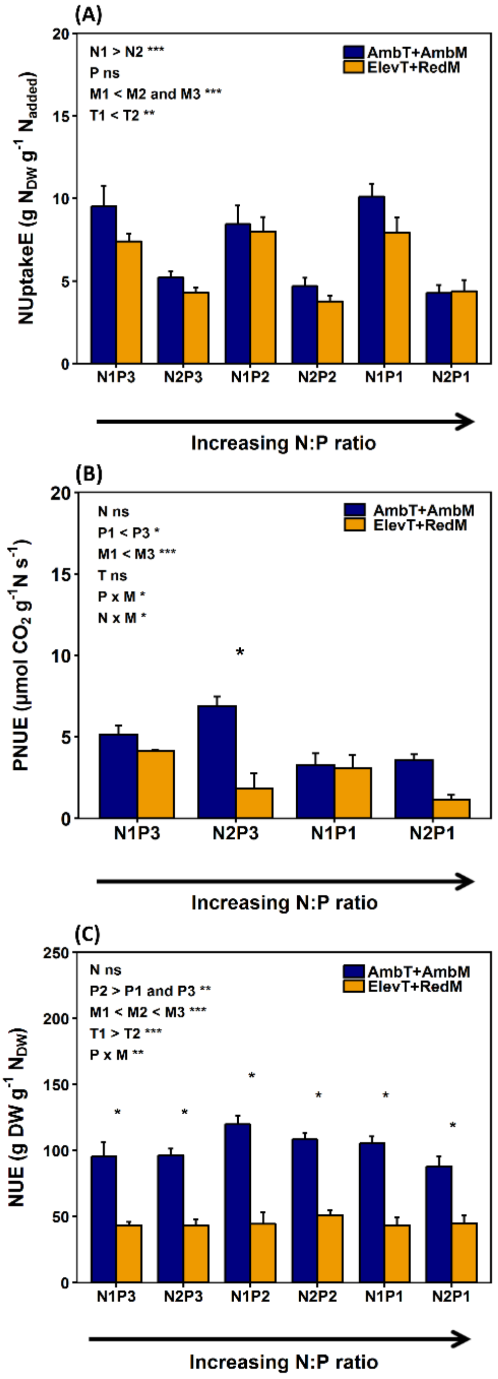

Figure 4.1 Means and SE of (A) N uptake efficiency (NUptakeE), (B) photosynthetic N use efficiency (PNUE) and (C) N use efficiency (NUE) of European beech saplings grown at increasing soil N:P ratios and ambient (dark bars; ambient temperature and soil moisture, AmbT+AmbM; treatment T1M3) or climate change conditions (light bars; elevated temperature and reduced soil moisture, ElevT+RedM; treatment T2M1) in climate chambers. The associated tables show the results of four-factorial ANOVAs and post-hoc multiple comparisons according to Tukey for all experimental treatments (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, not significant; n = eight replicates per treatment for NUptakeE and NUE; n = five replicates per treatment for PNUE). Asterisks indicate significant differences between ambient and climate change conditions (*, $P < 0.05$). N supply: N1, ambient; N2, elevated. P supply: P1, low; P2, moderate; P3, high. Temperature: T1, ambient; T2, elevated. Soil moisture: M1, drought; M2, moderately moist; M3, well-watered; see also Supplementary Table S4.1.

The main intrinsic influence on NUptakeE among the investigated influences had fine root and leaf biomass; that is, the larger the beech sapling and its fine root biomass the more efficient N uptake was (R^2 = 0.42; **Table 4.1** and **Table 4.3**). The relationship between fine root biomass and NUptakeE differed between soil N availabilities, though. The relationship had a greater intercept and slope at reduced soil N availability (**Figure 4.2**A), slopes of N1 and N2 were significantly different ($P < 0.001$). In contrast to fine root biomass, fine root morphology had no effect on NUptakeE (see **Table 4.3**). A secondary intrinsic influence on NUptakeE was exerted by root C:N stoichiometry, where NUptakeE increased with the C:N ratio in fine roots and coarse roots (**Figure 4.2**B; **Table 4.3**), but with increased scatter of data points around the regression lines (R^2 = 0.39 to 0.41) and non-significant intercepts. The relationship between NUptakeE and the fine root C:N ratio differed between soil N availability and had a greater intercept and slope at reduced soil N availability (**Figure 4.2**B). However, slopes of N1 and N2 were only significantly different if using single data points (P = 0.004; not shown), but not when using treatment means (P = 0.21).

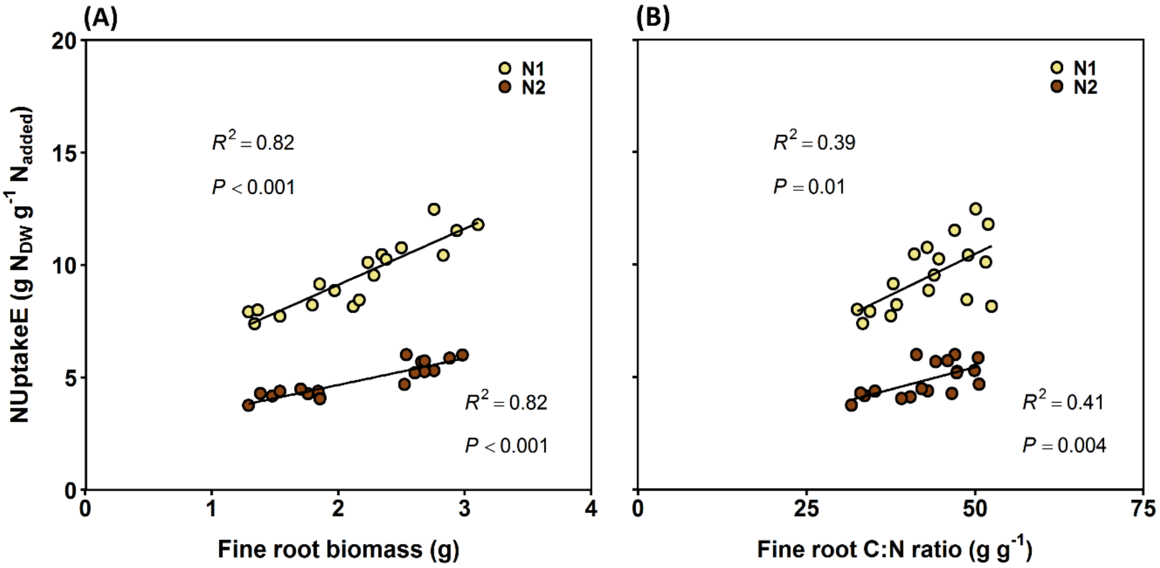

Figure 4.2 Linear regressions between nitrogen (N) uptake efficiency (NUptakeE) and (A) fine root biomass and (B) fine root carbon (C):N ratio of European beech saplings grown at increasing soil N: phosphorus (P) ratios and climate change conditions (n = 36 treatments; (A) N1: NUptakeE = 4.1 + 2.50 x fine root biomass; N2: NUptakeE = 2.3 + 1.18 x fine root biomass; (B) N1: NUptakeE = 3.2 + 0.15 x fine root C:N ratio; N2: NUptakeE = 1.5 + 0.08 x fine root C:N ratio). All slopes are significant, as well as the intercepts for the relationships between NUptakeE and fine root biomass. The intercepts for the relationships between NUptakeE and fine root C:N ratio are not significant (N1: P = 0.13, N2: P = 0.16). Slopes of N1 and N2 were significantly different only for the relationship between NUptakeE and fine root biomass ($P < 0.001$), but not for the relationship between NUptakeE and fine root C:N ratio (P = 0.21). N supply: N1, ambient; N2, elevated.

Nitrogen use efficiencies

Surprisingly, PNUE and NUE of the investigated beech saplings were not influenced by soil N availability, but by soil P availability and drought (**Figure 4.1**B, C; see **Table 4.2**). PNUE decreased by 45% with a reduction in P availability (from 5.5 to 3.8 µmol CO_2 g^{-1} N s^{-1} from high to low P availability) and by 57% with soil drought (from 5.4 to 3.4 µmol CO_2 g^{-1} N s^{-1} from the well-watered to the drought treatment when both temperature treatments were considered; **Figure 4.1**B; see **Table S4.5**). Hence, there was a significant interaction of P availability and soil moisture on PNUE, which increased with increasing P availability and increasing soil moisture (**Figure 4.1**B). PNUE declined with decreasing coarse and fine root C:N ratios and increased with fine root N:P ratios (intercept for the relationship between PNUE and coarse root C:N not significant; **Table 4.1**; **Figure 4.3**D; see **Table 4.3** and **Table S4.4** and **Table S4.6**), i.e., it was reduced with stoichiometric imbalance and relative deficiency of fine root P. Larger plants with greater and more vital root biomass had higher PNUE (see **Table 4.3**). Yet the low coefficient of determination in the multiple regression analysis (R^2 = 0.25) also indicates additional non-predicted influences on PNUE which did not enter our regression model.

Soil drought had a major impact on NUE (decrease by half from 102 to 53 g DW g^{-1} N_{DW} from the well-watered to the drought treatment when both temperature treatments were considered; **Figure 4.1**C; see **Table S4.5**). Further, NUE decreased with an increase in temperature (from 88 to 79 g DW g^{-1} N_{DW} from ambient to elevated temperature) and at limiting soil P availability (from 91 to 78 g DW g^{-1} N_{DW} from moderate to low P availability; see **Table S4.5**). Next to these extrinsic influences, the C:N ratios in fine and coarse roots or the degree of relative N deficiency strongly increased NUE of saplings (**Figure 4.3**A; **Table 4.1** and **Table 4.3**). The larger the leaf biomass of beech saplings was (and the higher the ECM colonization rate tended to be) the more efficient was their N use (**Table 4.1**; **Figure 4.3**B). NUE was also significantly enhanced by increasing ECMF species richness and ECMF diversity (see **Table 4.3**). When all experimental treatments were considered, it appeared that ECMF colonization rate related negatively to N concentrations in leaves, coarse roots, and fine roots (see **Table S4.7**), and positively to the corresponding C:N ratios (but with increased scatter of data points around the regression lines: R^2 = 0.23; **Figure 4.3**C).

Table 4.1 Three independent multiple regression analyses on the effects of multiple predictor plant traits on nitrogen (N) uptake efficiency (NUptakeE), photosynthetic N use efficiency (PNUE), or N use efficiency (NUE). Tested predictor plant traits were leaf and fine root biomass, coarse root C:N and N:P, leaf and fine root N, leaf and fine root C:N and N:P, root vitality, ECMF colonization rate, ECMF species richness, and ECMF α diversity. Values given are the determination coefficient R^2 and the probability of error *P* for the model and the *F*-value and probability of error *P* for the selected predictors. The '+' or '-' signs at the predictor variables indicate positive or negative relationships.

	Model					
Dependent variable	R^2	***P***		**Selected predictor variables**	***F***	***P***
NUptakeE	0.42	< 0.001	+	Fine root biomass	48.2	<0.001
			+	Leaf biomass	11.3	0.001
			-	Fine root C:N	13.5	0.002
PNUE	0.25	< 0.001	-	Leaf N:P	13.2	<0.001
			+	Plant biomass	5.8	0.02
NUE	0.84	< 0.001	+	Fine root C:N	91.7	<0.001
			+	Coarse root C:N	76.3	<0.001
			+	Leaf biomass	60.4	<0.001
			-	Fine root biomass	31.9	<0.001
			-	Fine root N:P	14.5	<0.001
			+	ECMF colonization rate	7.7	0.01

Table 4.2 Four-factorial ANOVAs (type III SS) on the effects of nitrogen availability (N), P availability (P), temperature (T), soil moisture (M), and their interactions on the variance of N uptake efficiency (NUptakeE), photosynthetic N use efficiency (PNUE), and N use efficiency (NUE) of European beech saplings grown at increasing soil N:P ratios and climate change. Efficiencies were square root transformed to resemble normality and homogeneity of variances. Given are *F* values and probabilities of error *P*. Significant effects are indicated by bold letters (*n* = eight replicates per treatment for NUptakeE and NUE; *n* = five replicates per treatment for PNUE).

	NUptakeE		**PNUE**		**NUE**	
	F	***P***	***F***	***P***	***F***	***P***
N	**299.4**	**<0.001**	1.7	0.20	1.4	0.24
P	0.5	0.60	**7.0**	**<0.05**	**7.0**	**<0.01**
T	**7.6**	**<0.01**	1.3	0.26	**13.2**	**<0.001**
M	**14.6**	**<0.001**	**17.3**	**<0.001**	**143.7**	**<0.001**
P x N	1.5	0.22	0.1	0.77	0.3	0.77
P x T	0.8	0.46	0.0	0.87	0.2	0.79
N x T	0.5	0.49	3.3	0.07	0.0	0.91
P x M	0.7	0.61	**6.2**	**<0.05**	**3.9**	**<0.01**
N x M	1.1	0.32	**4.0**	**<0.05**	2.5	0.08
P x N x T	1.0	0.36	0.0	0.89	0.0	0.97
P x N x M	1.1	0.38	0.7	0.39	1.2	0.32

Table 4.3 Pearson's correlations between nitrogen (N) uptake efficiency (NUptakeE), photosynthetic N use efficiency (PNUE), and N use efficiency (NUE) with plant biomass, leaf and root morphology, ectomycorrhizal fungal (ECMF) community, photosynthetic capacity, nutrient concentrations, and nutrient ratios of European beech saplings grown at increasing soil N:P ratios and climate change. Given are the correlation coefficients R and the probabilities of error P (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; $n = 36$ treatments). Significant correlations are indicated by bold letters. SLA, specific leaf area; SRA, specific root area; SRL, specific root length.

	NUptakeE	**PNUE**	**NUE**
Plant biomass	**0.39***	**0.72****	**0.90*****
Leaf biomass	**0.41***	**0.63****	**0.88*****
A_{max}	0.27	**0.94*****	**0.64****
SLA	-0.12	-0.40	**-0.66****
Total C assimilation	0.49	**0.88*****	**0.87*****
C_{leaves}	-0.16	**-0.69****	**-0.59*****
N_{leaves}	-0.24	**-0.70****	**-0.89*****
P_{leaves}	0.07	**0.67****	0.33
$C{:}N_{\text{leaves}}$	0.25	**0.69****	**0.86*****
$N{:}P_{\text{leaves}}$	-0.07	**-0.64****	**-0.43****
Coarse root biomass	**0.39***	**0.70****	**0.92*****
$C_{\text{coarse roots}}$	-0.03	-0.34	-0.06
$N_{\text{coarse roots}}$	-0.25	**-0.65****	**-0.92*****
$P_{\text{coarse roots}}$	-0.06	0.31	-0.14
$C{:}N_{\text{coarse roots}}$	**0.33***	**0.71****	**0.93*****
$N{:}P_{\text{coarse roots}}$	0.02	-0.49	-0.24
Fine root biomass	**0.37***	**0.77*****	**0.84*****
SRL	0.01	-0.24	**-0.36***
SRA	0.02	0.11	-0.26
Root vitality	0.23	**0.54***	**0.52****
ECMF colonization rate	0.25	0.04	**0.49****
ECMF species richness	0.16	0.10	**0.39***
ECMF α diversity	0.14	0.18	**0.38***
$C_{\text{fine roots}}$	0.23	**-0.51***	0.08
$N_{\text{fine roots}}$	-0.33	**-0.63****	**-0.96*****
$P_{\text{fine roots}}$	-0.03	0.44	0.01
$C{:}N_{\text{fine roots}}$	0.32	**0.58***	**0.96*****
$N{:}P_{\text{fine roots}}$	-0.08	**-0.60***	**-0.36***

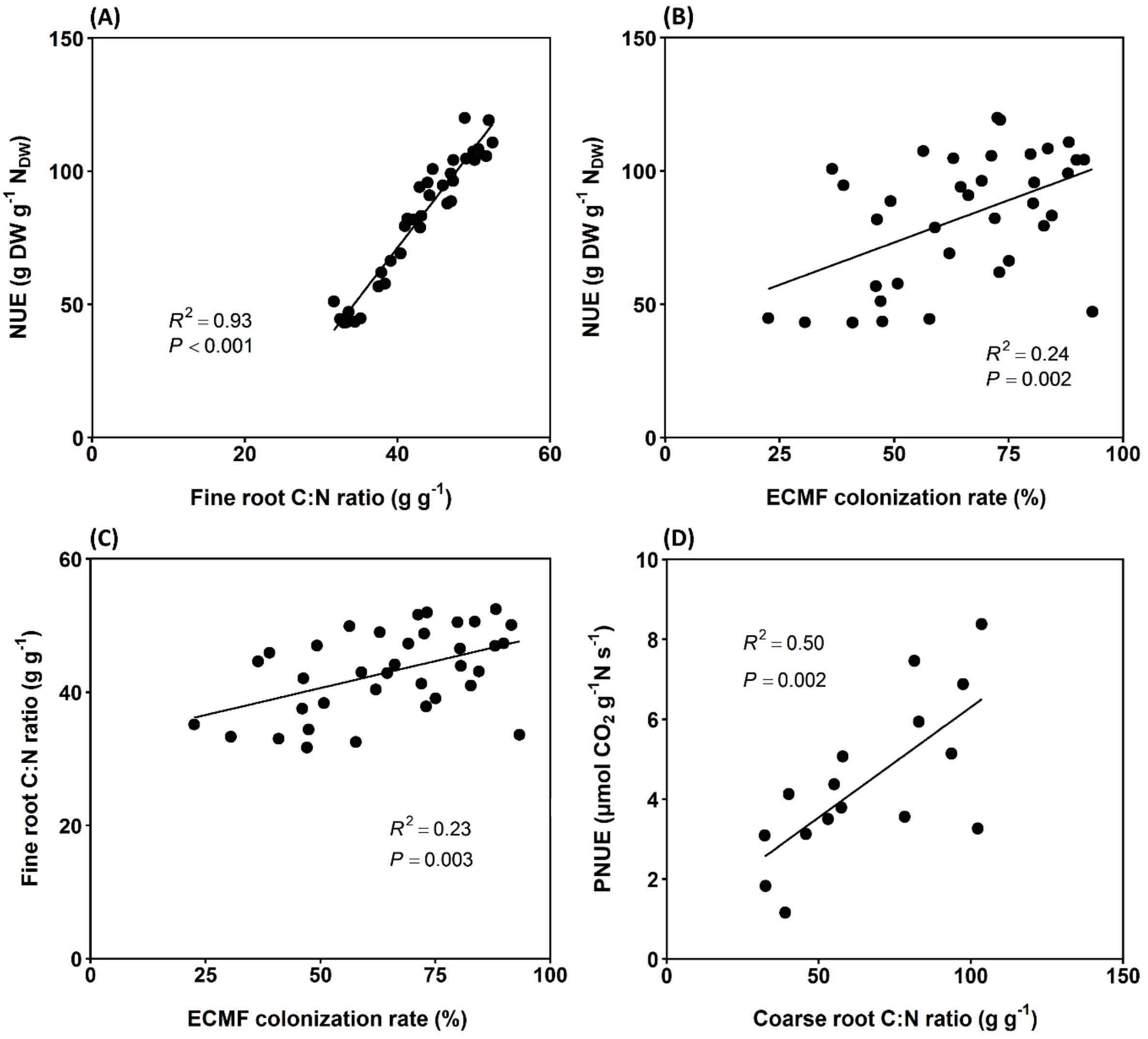

Figure 4.3 (A) Linear regressions between nitrogen (N) use efficiency (NUE) and fine root C:N ratio, (B) between NUE and ECMF colonization rate, (C) between fine root C:N ratio and ECMF colonization rate, and (D) between photosynthetic N use efficiency (PNUE) and coarse root C:N ratio of European beech saplings grown at increasing soil N:P ratios and climate change conditions (n = 36 treatments; (A) NUE = -77.7 + 3.72 x fine root C:N ratio; (B) NUE = 41.5 + 0.63 x ECMF colonization rate; (C) Fine root C:N ratio = 32.5 + 0.16 x ECMF colonization rate; (D) PNUE = 0.8 + 0.06 x coarse root C:N ratio). Shown are the means of all 36 treatments (for PNUE only 16 treatments). All slopes are significant, as well as the intercepts for the relationships between NUE and fine root C:N ratio, between NUE and ECMF colonization rate, and between fine root C:N ratio and ECMF colonization rate. The intercept for the relationship between PNUE and coarse root C:N is not significant (P = 0.46).

Discussion

Reduced N uptake efficiency compensates for elevated N availability

Elevated N deposition and summer droughts in the course of global change are assumed to affect nutrient stoichiometry (Vitousek et al., 2010; Peñuelas et al., 2013; Sardans et al., 2016), but it remains unclear to what degree plants counteract these changes by adjustments of the uptake efficiencies of individual nutrients. Here we demonstrate that European beech saplings respond to an increase in N availability by sufficient adjustments of NUptakeE (**Figure 4.1**A), with the consequence that tissue N concentrations and NUE remained comparably constant across the range of soil N availabilities and soil N:P ratios (**Figure 4.1**C; **Figure S4.1**) but at the cost of strong tissue N:P stoichiometric change (**Table S4.6**).

The capacity for N acquisition can be adjusted to N availability by changes in biomass partitioning, shifts in morphological or physiological traits, or by alterations at the cellular level (Kraiser et al., 2011) such as switching from low to high affinity nitrogen uptake (Gan et al., 2016). Previous fertilization experiments or N deposition studies showed that increases in N availability enhance total plant productivity, but they decrease fine root biomass partitioning (Nadelhoffer, 2000; Corrêa et al., 2011) and shift the ECMF community to nitrophilic taxa or even decrease ECMF colonization (Kjøller et al., 2012; de Witte et al., 2017). Yet in our fertilization study which simulated a naturally low to moderate N range, fine root biomass partitioning, ECMF colonization rate , and root morphology were unaffected by soil N availability (see also Köhler et al., 2018). A closer inspection of the regression between NUptakeE and fine root biomass illustrates that the relationship has similar coefficient of determination and is shifted to almost half the slope and to only a fraction of the intercept at elevated N availability. Theoretically, this bisection of the relationship can be caused by either a categorical shift in root architecture and functioning or by alterations in the membrane structure and transporter activity at elevated N availability. Plants can curtail NUptakeE by alterations in N uptake. Under high N availability, they exhibit a lower density and substrate affinity of nitrate transporters (Kraiser et al., 2011; Castro-Rodríguez et al., 2017). This shift in the relationship between NUptakeE and fine root biomass can stabilize tissue N at a given concentration optimal for plant functions and growth, as it was the case in the investigated European beech saplings.

The reduction of NUptakeE of beech by half when soil N availability was doubled reveals the negative relationship between NUptakeE and soil N availability. At the same time, the plant N:P ratio increased with increasing soil N:P, even though at a lower rate, which points at metastable tissue P concentrations and rising relative P limitation under elevated soil N:P. This rise in relative P limitation was attenuated in the investigated beech saplings by an increase in PUptakeE (Köhler et al., 2018), but not to the extent that no rise in tissue N:P occurred. Theoretically, plants may counteract an increase in tissue N:P ratio by both a (costly) increase in PUptakeE or a (cheaper) further restriction of NUptakeE when soil P is increasingly limiting. In our investigation the adjustment of NUptakeE was unresponsive to the availability of soil P and merely a function of N availability and fine root biomass. It seems that European beech saplings are only able to adjust their NUptakeE to the availability of N, but not to the availability of P. Biochemically dependent co-limitation (as defined by Saito et al., 2008) does not appear to play a role for N uptake.

Photosynthetic N use efficiency is curtailed by serial limitation and drought

In contrast to previous studies which focused on aboveground NUE in mature forests (e.g. Vitousek, 1982; Aerts and Chapin III, 1999; Tateno et al., 2017), PNUE and whole-plant NUE of beech saplings were not influenced by soil N availability but were related to soil P availability instead (**Figure 4.1**B and **Figure 4.1**C; **Table 4.2**). This points at stoichiometric constraints in PNUE and NUE, either from P or C limitation (Blanes et al., 2013). PNUE decreased with increasing P deficiency and decreasing A_{max} at high leaf N:P ratios. At a closer inspection it becomes clear that a response of PNUE to an increase in soil N availability occurs only *after* soil P availability increases (in the control climate; **Figure 4.1**B). This is an example of serially linked nutrient limitation as defined by Ågren et al. (2012), where the plant response to a second nutrient depends on the prior addition of another limiting resource. In the case of PNUE of beech, this response was synergistic and super-additive when both nutrients were added, but it was also dependent on the order of their addition. Serially linked nutrient limitation links to Liebig's law of the minimum (Von Liebig, 1855) and indicates no strict co-limitation of PNUE by P and N. As a consequence of this serial limitation, PNUE of beech will probably not respond to N deposition as long as soil P continues to be increasingly limiting for plants (Talkner et al., 2015; Sardans et al., 2016; Hedwall et al., 2017).

Soil drought reduced PNUE by more than one third and had an even stronger effect at high soil N availability, which emphasizes the essential and overriding role of C limitation in decreasing PNUE. At the same time, foliar N increased in dry soil independent from soil N availability or the soil N:P ratio while the foliar C:N ratio decreased. Increased leaf N contents with drought have previously been observed in plants around the world (Sardans et al., 2017). Assimilation under drought can be optimized by increased leaf N contents to sustain the photosynthetic capacity (Farquhar et al., 2002). Increased leaf N contents with drought were associated with better stress adaptation from cellular membrane stability, lower osmotic potentials, reduced lipid peroxidation, and increased osmoprotectant (e.g., proline) content (Ashraf and Foolad, 2007). In our study, the increase in absolute and relative leaf N content under drought was not a consequence of greater availability and uptake of soil N. Rather, it was probably related to missing investment opportunities of N when drought is leading to reductions in stomatal opening width and consequently in photosynthetic C assimilation, i.e. to stronger drought effect on biomass production than on N uptake. More N is then stored in vacuoles and not used for growth, which leads to a decrease in the foliar C:N ratio. Similarly, the N concentration of absorptive foraging fine roots of mature beech trees increased during a severe summer drought while their C:N ratio decreased, which was probably consequence of a relatively high proportion of ephemeral roots with a high N concentration (Nikolova et al., 2020). Nevertheless, even if this increase in foliar N in our study may have counteracted to some extent the most severe drought stress, it was not sufficient to avoid any stomatal closing and decreases in photosynthetic capacity in drought-exposed beech saplings.

In dry soil, photosynthetic C assimilation of beech saplings was also limited by rising foliar N:P ratios and relative P limitation (Köhler et al., 2018). The factorial addition of P and soil moisture revealed a super-additive independent co-limitation (as defined by Harpole et al., 2011) by the two factors on PNUE, yet with a dominating effect exerted by soil drought and a subordinate effect by P deficiency (see **Figure S4.2**). Previously, we have demonstrated that drought reduces the P uptake efficiency of beech from reductions in ECMF diversity and in the complementarity of P uptake by ECMF species (Köhler et al., 2018; see also Kreuzwieser and Gessler, 2010; Pena et al., 2010; Zavišić et al., 2016). This drought-induced P limitation probably also establishes the secondary role of P deficiency on PNUE. Limitations in plant P are reducing the ATP supply and thus the amount, regeneration, and specific activity of Rubisco (Reich et al., 2009; Yang et al., 2016), which decreases photosynthetic C assimilation and PNUE in addition to the direct stomatal control from soil drought. The observed significant decrease in PNUE in P-limited beech is in accordance with the results obtained from other tree species (Reich et al., 2009; Gan et al., 2016). Yet the dominating influence of soil moisture on PNUE will leave the response of European beech to P deficiency strongly dependent on future precipitation patterns.

Soil drought disturbs nutrient stoichiometry as a result of reductions in ECMF diversity

NUE decreased with decreasing fine and coarse root C:N ratios and decreasing leaf biomass, i.e. when less photosynthates were available for beech growth. This decrease in NUE occurred at both high and low soil P availabilities (that is NUE is, when all experimental treatments are considered, significantly higher at P2 than at P1 and P3, as shown in the four-factorial ANOVA of **Figure 4.1**C) probably due to different reasons: the decrease in NUE with low soil P availability can be a consequence of a decrease in photosynthetic capacity and a reduction in PNUE from P limitation, as described above. The decrease in NUE of beech at high soil P can be explained by a reduction of photosynthetic P use efficiency when P is excessively supplied (*cf.* Lambers et al., 2012; Hidaka and Kitayama 2013; Köhler et al., 2018). More P is then invested in non-photosynthetic membrane structures (Lambers et al., 2012) or accumulated in vacuoles (Elser et al., 2010; Gan et al., 2016) when surplus P cannot be used for growth. Previous studies found a decrease in NUE with increasing N availability in different deciduous tree species (e.g., Gan et al., 2016; Leberecht et al., 2016), but did not report on a decrease in NUE when the P supply was high. The observed close relationship of both PNUE and NUE with soil P availability in our study emphasizes the important role of the N:P stoichiometry for energy-dependent metabolic processes and, thus, beech NUE and growth.

The negative drought effect on the efficiencies for N uptake and N use intensified from NUptakeE (-20%) to PNUE (-37%) and NUE (-48%) (**Table S4.5**); that is downstream its metabolic pathway from uptake to photosynthetic processing and biomass production in beech saplings. In addition, drought adjusted the different levels of PNUE and NUE to a similar, low level across the range of soil N:P ratios (**Figure 4.1**). At the same time, tissue N concentrations increased under drought (**Figure S4.1**). The constant NUE with increasing tissue N in dry soil points at a counteracting effect by nutrient imbalances and P limitation (**Table S4.6**), which are causing growth declines of beech saplings. In a comparison of the nutrient uptake efficiencies, drought had an even stronger effect on PUptakeE (as reported in Köhler et al., 2018) than on NUptakeE (*cf.* Dirks et al., 2019). Drought strongly decreased PUptakeE of beech saplings as a consequence of decreased ECMF colonization rate and the loss of ECMF with long-distance exploration. Soil exploration is more important in P uptake than in N uptake, since soil P occurs mostly in adsorbed, undissolved forms and diffuses very slowly (Lambers et al., 2008). Due to the loss in soil exploration by ECMF in dry soil, the negative drought effect was three times higher for PUptakeE than for NUptakeE and led to a shift in uptake stoichiometry. In our multiple regression analysis, ECMF colonization rate was identified as a secondary influence on beech NUE. This likely reflects the indirect role of hampered ECMF colonization rate on NUE through decreases in PUptakeE and increases in P deficiency, which decreases PNUE (as described above) and beech sapling growth from deteriorated N:P stoichiometry. As a consequence of these water limitations and nutrient imbalances, leaf and coarse root biomass and NUE strongly decreased while the root:shoot biomass ratio increased in beech saplings exposed to drought. It seems that soil drought has the potential to disturb the adjustment of plant stoichiometry to elevated N deposition.

Conclusions

We conclude that elevated N availability aggravates growth reductions from nutrient imbalances and summer droughts in European beech saplings. Notably, the direct negative effect of experimental drought on N uptake efficiency of European beech saplings intensifies downstream the metabolic pathway (that is from uptake to use) from additional limitations by P and C. Decreases in the ECMF colonization rate and ECMF diversity reduce P uptake efficiency in dry soil, leading to reductions in photosynthetic N use efficiency. Therefore, N use efficiency in dry soil strongly decreases from a combination of limited P uptake efficiency (and N uptake efficiency) and reduced photosynthetic C fixation leading to deteriorated nutrient stoichiometry and reduced beech sapling growth when exposed to experimental global change conditions. If such an effect also occurs in European beech saplings under natural conditions will have to be tested in subsequent field trials.

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Supplementary Material

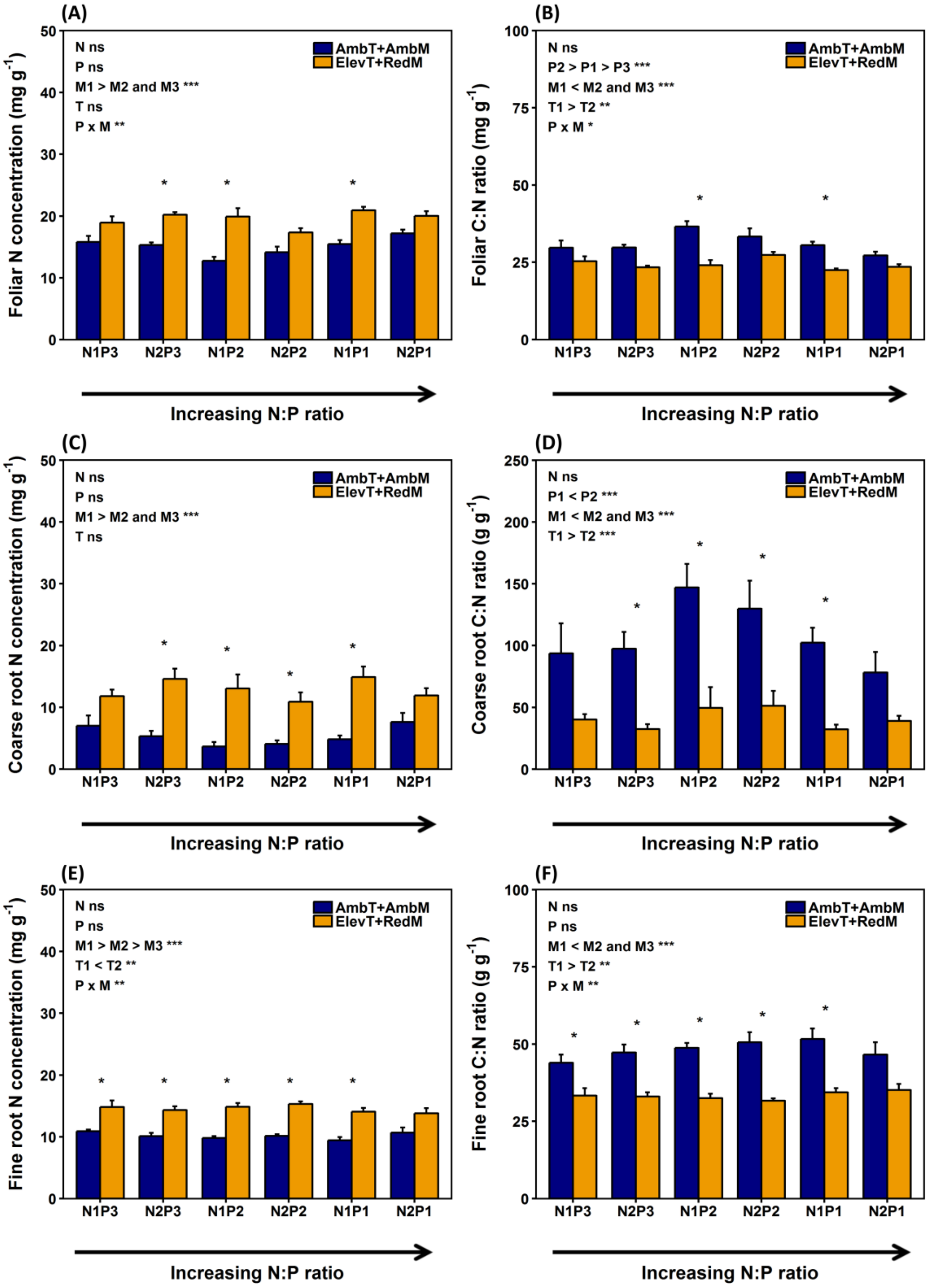


Figure S4.1 Means and SE of foliar, coarse root, and fine root (A, C, E) N concentration and (B, D, F) C:N ratios of European beech saplings grown at increasing soil N:P ratios and ambient (blue bars; ambient temperature and soil moisture, AmbT+AmbM) or climate change conditions (orange bars; elevated temperature and reduced soil moisture, ElevT+RedM) in climate chambers. The associated tables show the results of four-factorial ANOVAs and post-hoc multiple comparisons according to Tukey across all experimental treatments (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, not significant; n = eight replicates per treatment). Asterisks show significant differences between ambient and climate change conditions (*, $P < 0.05$). N supply: N1, ambient; N2, elevated. P supply: P1, low; P2, moderate; P3, high. Temperature: T1, ambient; T2, elevated. Soil moisture: M1, drought; M2, moderately moist; M3, well-watered.

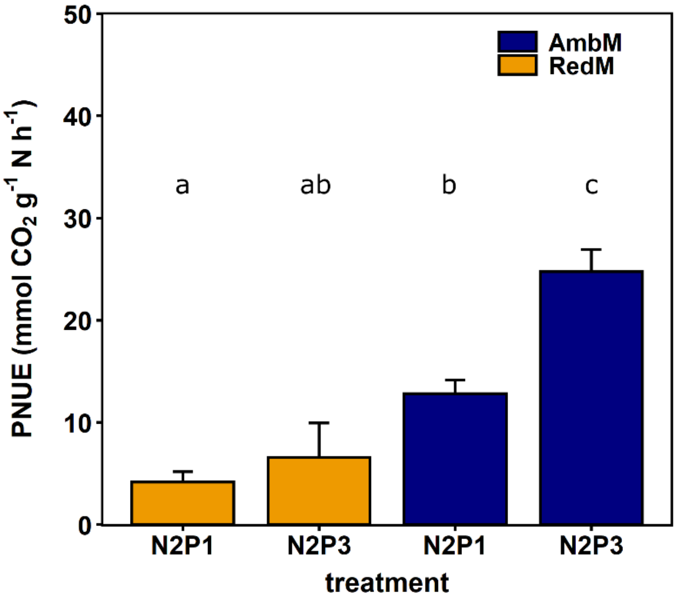
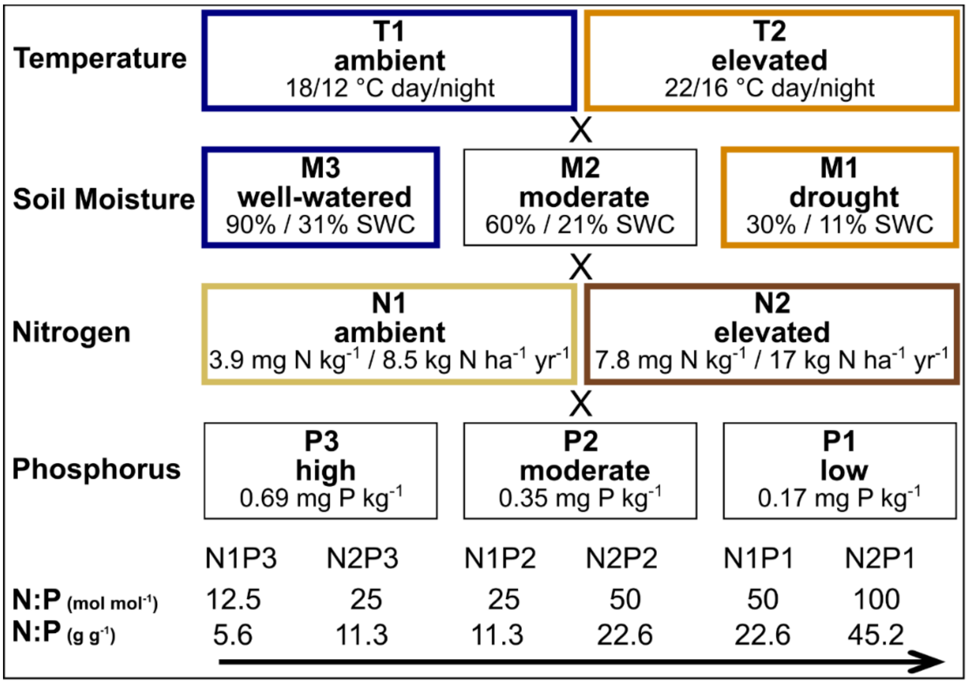


Figure S4.2 Response of photosynthetic N use efficiency (PNUE; means and SE) of European beech saplings to factorial addition of phosphorus and soil moisture (*n* = 16 replicates per treatment). The response represents super-additive independent co-limitation (as defined by Harpole et al., 2011), with a dominating effect exerted by soil drought and a subordinate effect by P deficiency. Significant differences between soil N:P treatments are indicated by different lower-case letters. N supply: N1, ambient; N2, elevated. P supply: P1, low; P3, high. Soil moisture: RedM, drought (orange bars; treatment M1); AmbM, ambient, well-watered (blue bars; treatment M3).

Table S4.1 Study design of the full-factorial climate chamber experiment with two temperature, three soil moisture, two N supply and three P supply levels (*n* = 36 treatments in total; after Köhler et al., 2018). A gradient from current environmental conditions to the expected future global change conditions (elevated temperature and N deposition and reduced soil moisture and P availability) was simulated. Temperature: T1, ambient; T2, elevated. Soil moisture: M1, drought; M2, moderately moist; M3, well-watered. N supply: N1, ambient; N2, elevated. P supply: P1, low; P2, moderate; P3, high.



Temperature	**T1** **ambient** 18/12 °C day/night			**T2** **elevated** 22/16 °C day/night		
			X			
Soil Moisture	**M3** **well-watered** 90% / 31% SWC		**M2** **moderate** 60% / 21% SWC		**M1** **drought** 30% / 11% SWC	
			X			
Nitrogen	**N1** **ambient** 3.9 mg N kg^{-1} / 8.5 kg N ha^{-1} yr^{-1}			**N2** **elevated** 7.8 mg N kg^{-1} / 17 kg N ha^{-1} yr^{-1}		
			X			
Phosphorus	**P3** **high** 0.69 mg P kg^{-1}		**P2** **moderate** 0.35 mg P kg^{-1}		**P1** **low** 0.17 mg P kg^{-1}	
	N1P3	N2P3	N1P2	N2P2	N1P1	N2P1
N:P (mol mol^{-1})	12.5	25	25	50	50	100
N:P (g g^{-1})	5.6	11.3	11.3	22.6	22.6	45.2

Table S4.2 Means and SE of photosynthetic capacity (A_{max}), total C assimilation, and ectomycorrhizal fungal (ECMF) colonization rate of European beech saplings grown at increasing soil N:P ratios and ambient (ambient temperature and soil moisture, AmbT+AmbM; treatment T1M3) or climate change conditions (elevated temperature and reduced soil moisture, ElevT+RedM; treatment T2M1) in climate chambers. Four-factorial ANOVAs and post-hoc multiple comparisons according to Tukey were calculated using a dataset limited to temperature and soil moisture treatments only. Significant differences between soil N:P treatments are indicated by different lower-case letters (n = eight replicates per treatment for ECMF colonization; n = five replicates per treatment for A_{max} and total C assimilation). N supply: N1, ambient; N2, elevated. P supply: P1, low; P2, moderate; P3, high. n/d = no data.

Treatment		**A_{max} (µmol CO_2 m^{-2} s^{-1})**	**Total C assimilation (µmol C h^{-1})**	**ECMF colonization rate (%)**
AmbT+AmbM	N1P3	3.2 (0.3) ^{ab}	60.4 (8.9) ^{bc}	80.6 (10.7) ^{ab}
	N2P3	4.3 (0.4) ^{b}	100.5 (9.4) ^{c}	69.1 (8.1) ^{ab}
	N1P2	n/d	n/d	72.6 (14.0) ^{ab}
	N2P2	n/d	n/d	83.6 (7.5) ^{b}
	N1P1	1.8 (0.3) ^{a}	54.9 (11.4) ^{bc}	71.3 (4.2) ^{ab}
	N2P1	2.2 (0.2) ^{a}	53.3 (9.2) ^{bc}	80.4 (7.3) ^{ab}
ElevT+RedM	N1P3	2.1 (0.3) ^{a}	13.2 (6.5) ^{ab}	30.5 (9.2) ^{a}
	N2P3	2.0 (0.3) ^{a}	5.7 (0.2) ^{a}	40.9 (20.2) ^{ab}
	N1P2	n/d	n/d	57.7 (10.7) ^{ab}
	N2P2	n/d	n/d	47.1 (11.0) ^{ab}
	N1P1	2.5 (0.5) ^{ab}	28.2 (10.4) ^{ab}	47.4 (24.1) ^{ab}
	N2P1	1.9 (0.6) ^{a}	1.6 (0.1) ^{a}	22.5 (16.5) ^{ab}

Table S4.3 Means and SE of total, leaf, coarse root, and fine root biomass of European beech saplings grown at increasing soil N:P ratios and ambient (ambient temperature and soil moisture, AmbT+AmbM; treatment T1M3) or climate change conditions (elevated temperature and reduced soil moisture, ElevT+RedM; treatment T2M1) in climate chambers. Four-factorial ANOVAs and post-hoc multiple comparisons according to Tukey were calculated using a dataset limited to temperature and soil moisture treatments only. Significant differences between soil N:P treatments are indicated by different lower-case letters (*n* = eight replicates per treatment). N supply: N1, ambient; N2, elevated. P supply: P1, low; P2, moderate; P3, high.

		Biomass (g)			
Treatment		**Total**	**Leaves**	**Coarse roots**	**Fine roots**
AmbT+AmbM	N1P3	7.6 (1.1) ^{cd}	0.9 (0.2) ^{bcd}	2.7 (0.4) ^{b}	2.3 (0.4) ^{ab}
	N2P3	8.7 (0.7) ^{d}	1.0 (0.1) ^{cd}	2.8 (0.3) ^{b}	2.6 (0.3) ^{b}
	N1P2	7.8 (1.1) ^{cd}	1.0 (0.2) ^{cd}	2.7 (0.4) ^{b}	2.2 (0.3) ^{ab}
	N2P2	8.3 (0.8) ^{d}	1.0 (0.1) ^{cd}	2.8 (0.2) ^{b}	2.5 (0.4) ^{ab}
	N1P1	8.5 (0.7) ^{d}	1.2 (0.1) ^{d}	2.9 (0.2) ^{b}	2.2 (0.3) ^{ab}
	N2P1	6.5 (0.5) ^{bcd}	0.9 (0.1) ^{bcd}	2.2 (0.3) ^{b}	1.8 (0.2) ^{ab}
ElevT+RedM	N1P3	3.4 (0.3) ^{a}	0.4 (0.1) ^{ab}	0.8 (0.1) ^{a}	1.3 (0.1) ^{a}
	N2P3	3.9 (0.3) ^{ab}	0.5 (0.1) ^{abc}	0.8 (0.1) ^{a}	1.4 (0.2) ^{ab}
	N1P2	3.8 (0.5) ^{ab}	0.5 (0.1) ^{abc}	1.1 (0.2) ^{a}	1.4 (0.2) ^{ab}
	N2P2	3.6 (0.3) ^{a}	0.3 (0.1) ^{a}	1.1 (0.1) ^{a}	1.3 (0.1) ^{a}
	N1P1	3.9 (0.4) ^{ab}	0.5 (0.1) ^{abc}	1.1 (0.1) ^{a}	1.3 (0.2) ^{a}
	N2P1	4.4 (0.6) ^{abc}	0.4 (0.2) ^{ab}	1.1 (0.1) ^{a}	1.5 (0.3) ^{ab}

Table S4.4 Means and SE of N uptake efficiency (NUptakeE), photosynthetic N use efficiency (PNUE), and N use efficiency (NUE) of European beech saplings grown at increasing soil N:P ratios and ambient temperature and reduced soil moisture (AmbT+RedM; treatment T1M1) or elevated temperature and ambient soil moisture (ElevT+AmbM; treatment T2M3) in climate chambers. Four-factorial ANOVAs and post-hoc multiple comparisons according to Tukey were calculated using a dataset limited to temperature and soil moisture treatments only. Significant differences between soil N:P treatments are indicated by different lower-case letters (*n* = eight replicates per treatment). N supply: N1, ambient; N2, elevated. P supply: P1, low; P2, moderate; P3, high. n/d = no data.

Treatment		**NUptakeE ($g\ N_{DW}\ g^{-1}\ N_{added}$)**	**PNUE ($\mu mol\ CO_2\ g^{-1}\ N\ s^{-1}$)**	**NUE ($g\ DW\ g^{-1}\ N_{DW}$)**
AmbT+RedM	N1P3	8.2 (0.7) ^{cde}	4.4 (1.0) ^{a}	57.8 (4.0) ^{a}
	N2P3	4.2 (0.2) ^{ab}	3.1 (0.4) ^{a}	47.2 (2.0) ^{a}
	N1P2	7.7 (1.0) ^{bcd}	n/d	56.8 (8.8) ^{a}
	N2P2	4.0 (0.3) ^{ab}	n/d	66.3 (3.6) ^{abc}
	N1P1	9.1 (0.6) ^{cde}	3.5 (0.2) ^{a}	62.0 (4.8) ^{ab}
	N2P1	4.1 (0.3) ^{a}	3.8 (0.7) ^{a}	69.1 (5.1) ^{abc}
ElevT+AmbM	N1P3	10.3 (1.1) ^{de}	8.4 (1.1) ^{a}	100.9 (8.9) ^{cd}
	N2P3	5.7 (0.6) ^{abc}	7.5 (0.5) ^{a}	94.7 (10.6) ^{bcd}
	N1P2	11.8 (1.2) ^{de}	n/d	119.1 (9.3) ^{d}
	N2P2	5.9 (0.4) ^{abc}	n/d	106.3 (8.4) ^{cd}
	N1P1	12.5 (1.1) ^{e}	5.9 (1.7) ^{a}	104.2 (11.5) ^{cd}
	N2P1	4.5 (0.8) ^{ab}	5.1 (1.0) ^{a}	81.8 (6.8) ^{abcd}

Table S4.5 Means and SE of N uptake efficiency (NUptakeE), photosynthetic N use efficiency (PNUE), and N use efficiency (NUE) of European beech saplings with varying temperature, soil moisture, P availability, and N availability in climate chambers. n/d = no data. Temperature: T1, ambient; T2, elevated. Soil moisture: M1, drought; M2, moderately moist; M3, well-watered. P supply: P1, low; P2, moderate; P3, high. N supply: N1, ambient; N2, elevated.

Effect	**Levels**	**NUptakeE** (g N_{DW} g^{-1} N_{added})	**PNUE** (µmol CO_2 g^{-1} N s^{-1})	**NUE** (g DW g^{-1} N_{DW})
Temperature	T1	6.8 (0.3)	4.3 (0.3)	87.7 (2.5)
	T2	7.6 (0.3)	5.2 (0.6)	79.2 (2.8)
Moisture	M1	6.2 (0.3)	3.4 (0.3)	53.0 (1.8)
	M2	7.7 (0.4)	n/d	93.5 (2.7)
	M3	7.7 (0.4)	5.4 (0.4)	101.9 (2.6)
P availability	P1	7.1 (0.4)	3.8 (0.3)	77.5 (2.9)
	P2	7.1 (0.3)	n/d	90.9 (3.4)
	P3	7.4 (0.3)	5.5 (0.4)	82.0 (3.3)
N availability	N1	9.5 (0.3)	4.5 (0.4)	85.1 (2.9)
	N2	4.9 (0.1)	4.6 (0.4)	81.9 (2.4)

Table S4.6 Means and SE of foliar, coarse root, and fine root N:P ratios of European beech saplings grown at increasing soil N:P ratios and ambient (ambient temperature and soil moisture, AmbT+AmbM; treatment T1M3) or climate change conditions (elevated temperature and reduced soil moisture, ElevT+RedM; treatment T2M1) in climate chambers. Four-factorial ANOVAs and post-hoc multiple comparisons according to Tukey were calculated using a dataset limited to temperature and soil moisture treatments only. Significant differences between soil N:P treatments are indicated by different lower-case letters (*n* = eight replicates per treatment). N supply: N1, ambient; N2, elevated. P supply: P1, low; P2, moderate; P3, high.

		N:P ratio (g g^{-1})		
Treatment		**Leaves**	**Coarse roots**	**Fine roots**
AmbT+AmbM	N1P3	8.2 (1.3) ^{ab}	4.8 (0.6) ^{ab}	6.3 (0.2) ^{a}
	N2P3	6.0 (0.5) ^{a}	3.9 (0.5) ^{a}	6.1 (0.3) ^{a}
	N1P2	11.6 (0.7) ^{bcd}	4.9 (0.6) ^{ab}	8.1 (0.3) ^{ab}
	N2P2	11.0 (1.2) ^{bc}	6.1 (1.2) ^{ab}	8.5 (0.7) ^{ab}
	N1P1	30.7 (1.4) ^{f}	70.0 (40.4) ^{def}	18.1 (3.6) ^{cd}
	N2P1	29.5 (2.6) ^{ef}	29.2 (6.9) ^{def}	19.9 (2.7) ^{d}
ElevT+RedM	N1P3	20.8 (2.9) ^{ef}	10.0 (2.3) ^{abc}	10.3 (1.1) ^{b}
	N2P3	19.1 (1.1) ^{def}	11.0 (2.1) ^{bcd}	9.6 (1.1) ^{ab}
	N1P2	17.4 (1.2) ^{cde}	9.6 (1.5) ^{abc}	10.4 (0.5) ^{b}
	N2P2	18.9 (0.8) ^{def}	18.0 (4.5) ^{cde}	11.8 (0.9) ^{bc}
	N1P1	32.1 (5.2) ^{ef}	41.8 (6.7) ^{f}	20.4 (1.8) ^{d}
	N2P1	31.5 (2.0) ^{f}	36.8 (4.6) ^{ef}	21.3 (0.9) ^{d}

Table S4.7 Pearson's correlations between the ectomycorrhizal fungal (ECMF) community and plant biomass, assimilation, nutrient concentrations, and nutrient ratios of European beech saplings grown at increasing soil N:P ratios and climate change. Given are the correlation coefficients *R* and the probabilities of error *P* (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; $n = 36$ treatments). Significant correlations are indicated by bold letters.

	ECMF colonization rate	**ECMF species richness**	**ECMF α diversity**
Leaf biomass	**0.39***	0.27	**0.36***
Total C assimilation	0.23	0.22	0.31
C_{leaves}	-0.30	**-0.52****	**-0.44****
N_{leaves}	**-0.42***	**-0.39***	**-0.37***
P_{leaves}	0.01	0.16	0.06
$C:N_{leaves}$	**0.40***	**0.36***	**0.36***
$N:P_{leaves}$	-0.12	-0.20	-0.15
Coarse root biomass	**0.42***	**0.37***	**0.44****
$C_{coarse\ roots}$	**0.51****	**0.34***	0.07
$N_{coarse\ roots}$	**-0.51****	**-0.43****	**-0.37***
$P_{coarse\ roots}$	-0.17	-0.13	-0.17
$C:N_{coarse\ roots}$	**0.49****	**0.38***	0.31
$N:P_{coarse\ roots}$	-0.05	-0.04	0.00
Fine root biomass	**0.40***	**0.44****	**0.49****
$C_{fine\ roots}$	0.26	**0.37***	0.28
$N_{fine\ roots}$	**-0.44****	**-0.34***	**-0.35***
$P_{fine\ roots}$	-0.12	-0.03	-0.04
$C:N_{fine\ roots}$	**0.48****	0.32	0.30
$N:P_{fine\ roots}$	-0.08	-0.11	-0.09

CHAPTER 5

Synopsis

CHAPTER 5

Physiological and morphological adaptations of European beech to P limitation

Phosphorus (P) is one of the most essential elements required for plant growth and metabolism but one of the least available ones in the soil (Raghothama, 1999). Several studies predict increasing growth limitations for important temperate tree species due to nutritional imbalances and a large-scale undersupply of P (Prietzel et al., 2008; Peñuelas et al., 2012; Talkner et al., 2015), which will likely be accelerated by atmospheric nitrogen (N) deposition from anthropogenic sources (Sardans et al., 2016). Furthermore, increasing acidification of forest soils due to rising N deposition may enhance P sorption, contributing to lower soil P concentrations and further curtail P supply to trees. Decreasing P concentrations and increasing N:P ratios in leaves and fine roots indicate an increasing undersupply of P. This, as well as recent tree growth reductions due to P limitation, have been observed in various forest regions of Central and Southern Europe (Peñuelas et al., 2012; Talkner et al., 2015; Braun et al., 2017; Braun et al., 2020).

In response to low nutrient availability, plants can enhance nutrient uptake and acquisition as well as the conservation of nutrient use, thereby showing adaptations on the morphological, physiological, biochemical, and molecular level (Raghothama et al., 1999; Vance et al., 2003). The aim of the present study was to investigate the morphological and physiological adaptations of beech to decreasing plant-available P in the course of increasing N fertilization, i.e., an increasing P limitation due to nutrient imbalances.

The results of the climate chamber study (**CHAPTER 3**) suggest that the physiological adaptations of beech saplings are sufficient to circumvent severe negative P limitation effects and allow for maximum growth of saplings. However, decreasing maximum photosynthetic rate (A_{max}) and P concentrations, as well as increasing N:P ratios in leaves and fine roots, clearly indicate P limitation (**Figure 5.1**). Beech saplings neither showed an increased allocation of carbohydrates and nutrients to the root system (*sensu* optimal portioning theory; Bloom, 1985) nor adaptations in root morphology or architecture in response to P limitation. Hence, beech saplings mainly reacted to P limitation with the adjustment of their metabolism, i.e., they increased their P uptake efficiency (PUptakeE; i.e., the P content in recent biomass relative to the amount of available soil P), P use efficiency (PUE; i.e., the increment in biomass for a given increase in biomass P) and photosynthetic P use efficiency (PPUE; i.e., photosynthetic capacity related to foliar P concentration) in response to reduced plant-available P. However, the recent study could show that PUptakeE is enhanced by a higher root biomass and mediated by the ectomycorrhizal fungi (ECMF) symbiosis (**Figure 5.1**). These findings are consistent with those of Paoli et al. (2005), who found an increasing P uptake efficiency with decreasing P in a Bornean lowland tropical rain forest, explaining their findings with the symbiotic relationship of canopy tree species with ECMF, which allows for an increase in explorative and absorptive surface area. Furthermore, Dirks et al. (2019) could also find an increase in instantaneous and long-term PUptakeE in response to an increasing soil N:P ratio for evergreen Palestine oak trees, which are representatives of the Eastern Mediterranean scrub woodland.

Beside a more efficient P uptake, the conservation of P can be a strategy to cope with P limitation (Vance et al., 2003). This can be accomplished through the (re)mobilization of P from the vacuole to cytoplasm (Veneklaas et al., 2012; Zavišić et al., 2018), or from P stored in stem tissues and roots (Netzer et al., 2017), from senescing leaves (Hofman et al., 2016; Netzer et al., 2017), or by reductions of no longer needed RNA and replacement of phospholipids by sulfolipids and galactolipids (Lambers et al., 2012). The increase of PUE in European beech saplings with decreasing soil P availability was likely the result of a decrease in plant P concentration at constant biomass. In accordance with the result of this study, Gan et al. (2016) found an increased PUE for poplar under P limitation, thereby emphasizing the important link between plant productivity, photosynthesis, and PUE. Here, PUE is determined by plants' ability to maximize A_{max} and C assimilation in relation to leaf nutrient content (Veneklaas et al., 2012, López-Arredondo et al., 2014; Waring et al., 2015). The found increase in PPUE of beech saplings growing under P-limiting conditions supports the findings of Hidaka and Kitayama (2009, 2013) for tropical montane forests in Borneo, and Lambers et al. (2010, 2012) for Proteaceae species in south-western Australia, who explain the high PPUE under low P availability with the greater investment of P into P-containing metabolites and a low relative investment in phospholipids (Lambers et al., 2012; Hidaka and Kitayama, 2013).

The recent study indicates that when soil N:P ratios increase, the N:P ratios in plant tissue also increase, but at a lower rate. This suggests (re)mobilization of P and metastable P concentrations in the tissue of the beech saplings and also hints at an increase in relative P limitation under elevated soil N:P ratios. To circumvent increasing nutritional N:P ratio imbalances, European beech saplings could increase PUptakeE or restrict N uptake efficiency (NUptakeE; i.e., the N content in recent biomass relative to the amount of available soil N).

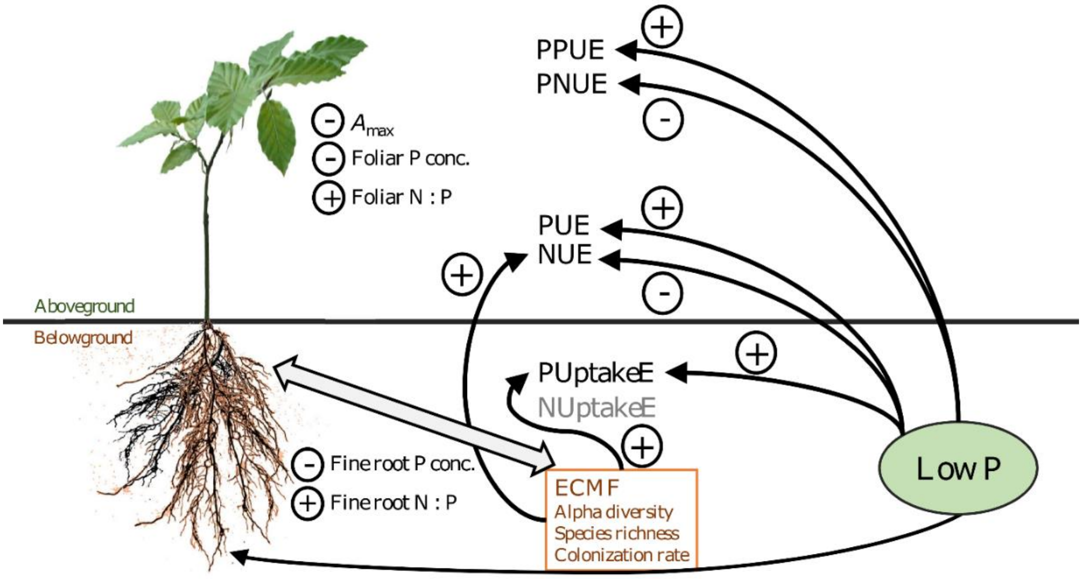


Figure 5.1 Schematic illustration of the main effects of decreasing soil phosphorus availability (low P) on parameters characterizing P and nitrogen (N) economy as well as physiology of European beech saplings in the climate chamber experiment. A_{max} = maximum photosynthetic rate. P economy: PUptakeE = P uptake efficiency, PUE = P use efficiency, PPUE = photosynthetic P use efficiency; N economy: NUptakeE = N uptake efficiency, NUE = N use efficiency, PNUE = photosynthetic N use efficiency. Thick grey arrow symbolizes the symbiosis between plant and ectomycorrhizal fungi (ECMF). The "+" and "-" signs indicate positive or negative relationships.

According to the co-limitation hypothesis, N acquisition and use efficiencies depend on P availability and vice versa (Ågren et al., 2012). Following this theory, P deficiency should increase PUptakeE, but simultaneously may reduce NUptakeE, whole-plant N use efficiency (NUE; i.e., the increment in biomass for a given increase in biomass N) and photosynthetic N use efficiency (PNUE; i.e., photosynthetic capacity related to foliar N concentration). In contrast, this study demonstrates that PUptakeE was responsive to increasing P availability but not to N availability (**Figure 5.2**).

Beech saplings, on the other hand, could adjust their NUptakeE to the availability of N but not to the availability of P (**CHAPTER 4)**. Thus, a biochemical co-limitation as defined by Saito et al. (2008), i.e., the uptake of one nutrient depends on the availability of another nutrient (Ågren et al., 2012), seems to be unimportant for P or N uptake. Nevertheless, the results indicate that both N use efficiencies increase with a decrease in soil P availability, whereas soil N availability has no effect (**Figure 5.1**). These results emphasize the important role of P for energy-dependent metabolic processes and, thus, for the maintenance of the internal N nutrient cycle. These findings are in contrast to several studies that indicated a decrease in NUE with increasing N availability in different deciduous tree species (Gan et al., 2016; Leberecht et al., 2016), but did not report on a decrease in NUE when the P supply was high.

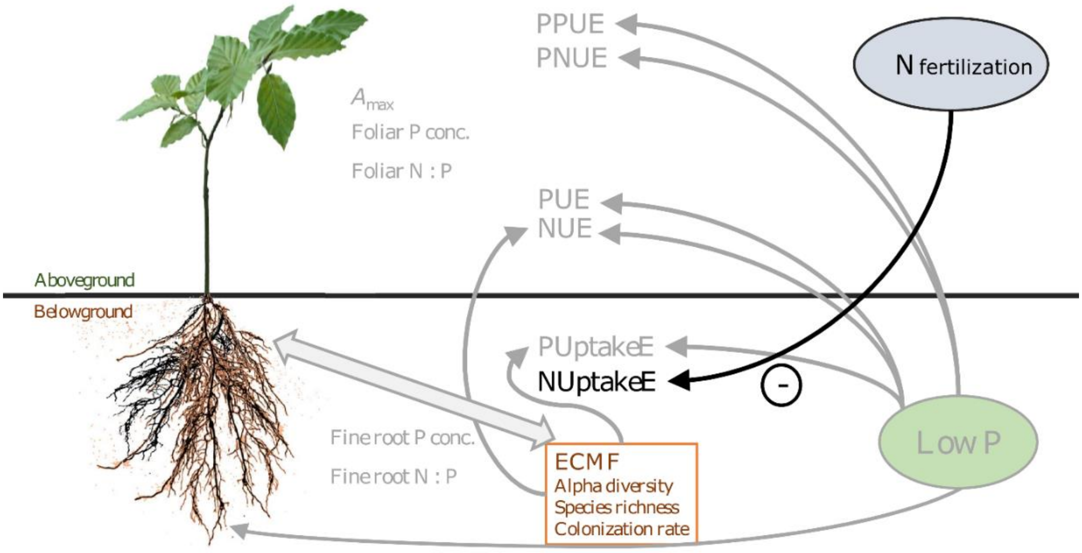


Figure 5.2 Schematic illustration of the main effects of increased soil N availability (N fertilization) on parameters characterizing P and nitrogen (N) economy as well as physiology of European beech saplings in the climate chamber experiment. A_{max} = maximum photosynthetic rate. P economy: PUptakeE = P uptake efficiency, PUE = P use efficiency, PPUE = photosynthetic P use efficiency; N economy: NUptakeE = N uptake efficiency, NUE = N use efficiency, PNUE = photosynthetic N use efficiency. Thick grey arrow symbolizes the symbiosis between plant and ectomycorrhizal fungi (ECMF). The “-“ sign indicates a negative relationship.

The results of the present study suggest that NUE and PNUE are constrained by nutritional imbalances, either due to P or C limitation (Blanes et al., 2013). Here, the reduced A_{max} and increasing P limitation at high foliar N:P ratios likely result in a decrease in PNUE and whole-plant NUE. Furthermore, the data of this study indicate a serially linked nutrient limitation for beech saplings’ PNUE, i.e., the plant response to a second nutrient is depending on the prior addition of another limiting resource (Ågren et al., 2012). In beech saplings, PNUE responds to an increase in soil N availability only after an increase in soil P availability. Hence, the results show that there is no strict co-limitation of N use efficiencies by P and N.

In a broader sense, the found serial P and N limitation suggests that the PNUE of beech will probably not respond to N deposition as long as soil P remains increasingly limiting for plants (Talkner et al., 2015; Sardans et al., 2016). This study highlights that European beech saplings are capable of adjusting their metabolism in response to P limitation resulting from decreasing P availability and/or increasing nutritional imbalances. These adjustments include a more efficient P uptake and internal P use, and are generally sufficient to circumvent severe negative P limitation effects and allow for maximum sapling growth. However, P limitation already showed negative effects on the carbon metabolism, i.e., photosynthetic C assimilation, of beech saplings. Additional increasing N fertilization, e.g., due to continued high N deposition, has the potential to cause nutritional imbalances which result in growth declines. From an ecosystem perspective, such efficient P uptake and use as part of a tight P recycling system is of crucial importance for forest ecosystems growing on P-poor soils (Lang et al., 2016; Lang et al., 2017; Netzer et al., 2017).

The effect of global change on P and N nutrition

In Central Europe, higher mean annual temperatures and a shift in seasonal precipitation patterns towards higher winter and lower summer precipitation with a higher frequency of drought events are the biologically most relevant consequences of climate change (IPCC, 2021), with substantial effects on temperate forest ecosystems (Jump et al., 2006; Zang et al., 2014; Martin et al., 2015; Leuschner, 2020). Climate change will likely alter soil nutrient dynamics, especially the cycling of the two main limiting nutrients N and P, and thus the nutrition of temperate forest trees in the next few decades, with either negative or positive consequences for productivity. Beside N and P dynamics, climate change is also affecting various key components of the carbon (C) cycle, including soil respiration (Schindelbacher et al., 2012; Lu et al., 2013; Wang et al., 2014), which is the second largest C flux between the atmosphere and terrestrial ecosystems (Davidson and Janssens, 2006; Zhou et al., 2016). Such a warmer and drier climate in combination with continued high N deposition might further aggravate nutritional imbalances and, thus, cause growth declines in European beech (Braun et al., 2017).

Decreasing mean annual precipitation (MAP) and increasing mean annual temperature (MAT) enhance the soil respiration rate in the summer season, which is especially pronounced for soils with sandy soil texture, i.e., low water storage capacity (**CHAPTER 2**). These results can be explained by a higher root respiration, i.e., autotrophic respiration, which results from enhanced fine root growth and turnover in the drier and warmer soil. This is in line with the findings by Hertel et al. (2013), who found an increase in fine root biomass, total fine root surface area, and fine root production in the study plots with reduced MAP and precipitation. Beech can produce new, thin ephemeral, absorptive, non-mycorrhizal fine roots with a high root surface area and enhanced respiratory activity as a fast response to drought-induced higher fine root mortality (Nikolova et al., 2020). This strategy is only applicable if sufficient carbohydrate resources are available (Leuschner, 2020). However, the response of fine root biomass to reduced MAP is variable; other studies have also shown no significant change (Meier et al., 2018) or even a decrease in fine root biomass (Meier and Leuschner, 2008). For European beech, fine root growth is in general highest between May and August, i.e., when air and soil temperatures are highest (Leuschner, 2020). Plant phenology in the form of aboveground C assimilation and belowground C allocation largely influences root respiration and its temperature sensitivity (Ruehr and Buchmann, 2009; but see Kuptz et al., 2011).

The found positive effect of higher soil temperatures on soil respiration rates is in accordance with several studies that demonstrate that in temperate forest ecosystems with relatively high precipitation, soil temperature is the most important factor that influences seasonal variation in soil respiration (Buchmann, 2000; Borken et al., 2002; Knohl et al., 2008). However, higher temperatures in the context of drought have the potential to decrease soil respiration considerably (Schindelbacher et al., 2012), but respiration can recover fast (Hagedorn et al., 2016). This highlights that warming causes an increasing soil C loss as long as precipitation occurs and, thus, soil water availability is sufficient. In the study years 2014 and 2015, the investigated study sites predominantly experienced normal conditions with regard to precipitation and potential evapotranspiration, but short-term drought events cannot be ruled out. Higher soil respiration rates in summer might also be caused by rewetting events subsequent to drought. Here, rewetting leads to considerable CO_2 flushes due to enhanced microbial activity and, thus, increases microbial respiration, i.e., heterotrophic respiration (Borken et al., 2002). The enhanced microbial activity can last for weeks, depending on the length of the drought, precipitation duration and quantity, as well as soil temperature (Borken et al., 1999). The recent study demonstrates that sandy soils are more prone to C losses than loam-richer soils in a future warmer and drier climate. Because sandy soils have a lower water storage capacity and higher porosity in general (Bouma and Bryla, 2000), they are more susceptible to edaphic drought and drying-rewetting events with subsequent C losses. The measured elevated soil respiration rate in summer 2015 can be explained by a higher abundance of carbohydrates from recent assimilation, an increase in root growth (Ekblad and Högberg, 2001; Hopkins et al., 2013), and a higher heterotrophic respiration due to enhanced activity of free-living soil microbes, which may profit from priming through root exudates (Kuzyakov et al., 2000).

Root exudation has been found to increase under low-moderate drought, while the response under extreme water stress is more variable (Preece and Peñuelas, 2016; Preece et al., 2018). In 2016, *in situ* root exudation of mature beech trees was investigated with a cuvette-based method (*cf.* Phillips et al., 2008) in the study plots along the previously described precipitation and temperature gradient. Here, root exudation was significantly higher in spring than in summer in forest stands growing on sandy soil (**Figure 5.3**; Habenicht, 2017). These results might indicate that the mature beech trees in sandy study plots experienced (climatic and edaphic) drought stress in the summer of 2016. Indeed, average summer precipitation (June to August) for study plots along the investigated gradient was nearly 20% lower in 2016 than the mean summer precipitation (for time period 1981-2010; data obtained from the German Weather Service (DWD)). Stomatal closure and the resulting decrease in photosynthetic C assimilation and, thus, a reduced allocation of photosynthates to belowground structures likely caused C-limitation for root exudation, resulting in the decline in root exudation (Ruehr et al., 2009; Adams et al., 2013; but see Prescott et al., 2020). In turn, this can result in a decrease in soil respiration (Schindelbacher et al., 2012). Hence, a decreased summer precipitation and an increased temperature in the course of climate change have the potential to significantly change the plant-soil interaction, future soil C dynamics, and further biochemical cycles (Sardans and Peñuelas, 2012; Preece and Peñuelas, 2016).

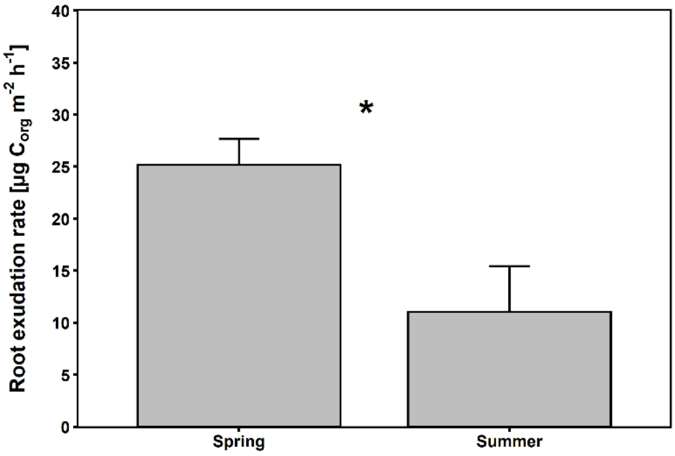


Figure 5.3 Means and standard errors of root-area-based exudation of mature European beech trees growing on sandy soil in spring and summer 2016. Asterisk indicates significant difference between seasons (*; $P < 0.05$).

The results of the recent study demonstrate that warming has a stronger effect on net N_{min} than the precipitation regime *per se*. In contrast to 2014, no effect of long-term precipitation or temperature patterns on N_{min} was found in the moister study year 2015. Other studies also demonstrated a positive relationship between net N_{min} and higher precipitation (Rustad et al., 2001; Li et al., 2019). However, the found negative relationship between net N_{min} and higher temperature contradicts the findings of Rustad et al. (2001) and Lu et al. (2013), who found a stimulating effect of higher temperature on N cycling due to increased soil microorganism enzyme activity and enhanced mineralization of soil organic matter (SOM), particularly in colder climates. This holds true as long as the availability of soil water and SOM is sufficient, i.e., there is a trade-off between water and temperature (Zuccarini et al., 2020). Factors which affect the growth and activity of soil microorganisms—like temperature, moisture, pH, and quality of SOM—regulate gross N mineralization and immobilization and, thus, net N_{min} (Lladó et al., 2017; Li et al., 2019; Mukai et al., 2020). Hence, a future warmer and drier climate will likely result in a decline in net N_{min} due to the suppression of genes in soil microbial communities involved in N cycling (Yu et al., 2018). Additionally, such a decline in net N_{min} can result from an overall negative effect of drought on N cycling caused by a decrease in soil microbial and enzyme activity (Borken and Matzner, 2009; Rennenberg et al., 2009; Deng et al., 2021). Furthermore, the negative values of net N_{min} found in the summer of 2015 might indicate microbial N immobilization in sandy soils. This is in contrast to the results of Kaiser et al. (2011), who found a summer N mineralization phase between July and August and a winter N immobilization phase between November and February. Mooshammer et al. (2014) could show that N-limited soil microbes sequester N into microbial biomass but release only small or no amounts of N back to the environment, resulting in low N mineralization or even N immobilization. On the other hand, C-limited soil microbes mineralize a large fraction of organic N to NH_4^+, i.e., this results in high N mineralization. The results of this study suggest that soil microbes in the sandy study sites were N-limited in the summer of 2015, as organic C (assimilates) can be assumed to be sufficiently available, as indicated by high soil respiration rate in summer 2015. This probably resulted in the observed N immobilization. However, the exact source of soil respiration, i.e., autotrophic respiration via root growth and/or heterotrophic respiration via excretion of root exudates and priming of soil microbes, was not determined in this study.

In addition to the expected changes in temperature and precipitation, continued high N deposition has the potential to alter nutrient cycling and, thus, the nutrition of European beech (Braun et al., 2017). For the time period 2013–2015, the study sites experienced a (modeled) mean annual N deposition of 14 kg ha^{-1} yr^{-1} at the drier end of the study gradient up to 19 kg ha^{-1} yr^{-1} at the wetter study sites (PINETI-3 project (Pollutant INput and EcosysTem Impact); sum of dry, moist and wet N deposition; Schaap et al., 2018). These values are in the range of critical N loads for beech (10–20 kg ha^{-1} yr^{-1}; Bobbink and Hettelingh, 2010). This pattern was also found for the total soil N content (N_{tot}) of the study sites (Eder, 2014). Here, N_{tot} increased with increasing MAP, which is likely caused by wet N deposition. The results of this study demonstrate that N deposition curtails soil respiration rate in summer and enhances net N_{min} in the late growing season (August to October). The reduced soil respiration rate under increasing N deposition is in accordance with previous research (Janssens et al., 2010; Zhou et al., 2014) and can be explained by a decrease in belowground C allocation or root exudation rates and an increase in soil acidification. This decreases the activity and growth of ECMF symbionts and

SOM-degrading soil microorganisms as well as the functioning of SOM degrading enzymes, which reduces autotrophic and heterotrophic respiration (Janssens et al., 2010; Tian and Niu, 2015; Zhang et al., 2018). Additionally, the reduced belowground C allocation under increased N availability due to higher N deposition can reduce root production and further decrease autotrophic respiration. This is in accordance with the results of Hertel et al. (2013), who showed for the same study gradient a decreasing fine root biomass and production for stands characterized by higher MAP and, thus, higher precipitation and (wet) N deposition. This might also explain the observed negative relationship between soil respiration rate and MAP in this study. Furthermore, this study suggests that net N_{min} is stimulated by higher N deposition and N_{tot}. Here, the additional N can have a positive effect on soil microbe activity due to enhanced substrate quantity and quality. But the effect of N deposition on N mineralization has been found to be highly variable (Lu et al., 2011; Cheng et al., 2019).

The results of the field study support the hypothesis that under a future warmer and drier climate, soil respiration rates in beech forests on sandy to sandy-loamy soils will increase, while net N_{min} will be reduced. However, N deposition might attenuate these effects by enhancing net N_{min} and curtailing soil respiration rates. Thus, increasing temperatures in combination with increasing summer drought frequency and severity are expected to decrease C sequestration potential and to reduce soil N supply in beech forests. This likely will alter C and N dynamics, impair beech growth and productivity in the future, and might even intensify the direct negative effects of drought and heat (Braun et al., 2017). In this context, the effect of changing climatic conditions on the activity and growth of ECMF symbionts and SOM degrading soil microorganisms and the resulting alterations in plant-soil interactions should be considered (Naylor et al. 2020). These results must be carefully interpreted as N deposition effects may interfere with MAP effects, and the underlying mechanism cannot be clearly distinguished in this study. Furthermore, because of the inherent negative relationship between MAP and MAT in the studied gradient, the effects of long-term precipitation and long-term temperature cannot be disentangled.

The findings of this study indicate that under (controlled) drought conditions, beech saplings show a significant decrease in almost all P and N uptake and use efficiencies (**Table S5.1**; **Figure 5.4**). This is especially pronounced for the PUptakeE (-60%), while the NUptakeE shows just a decrease of 20%. P occurs mostly in adsorbed, undissolved forms and is characterized by a low diffusion rate (Lambers et al., 2008; Kreuzwieser and Gessler, 2010). Here, ECMF play an important role in P acquisition and soil exploration (Plassard and Dell, 2010;

Zavišić et al., 2016). In contrast, ions like nitrate are highly mobile (Kreuzwieser and Gessler, 2010). Thus, in dry soil, exploration is more important for P uptake than for N uptake. The results found in this study indicate a negative effect of drought and higher temperatures on ECMF colonization and diversity (**Figure 5.4**; **Figure 5.5**), so that soil exploration by ECMF was impaired. This resulted in a three times higher negative drought effect for PUptakeE than for NUptakeE (*cf*. Dirks et al., 2019). However, N uptake kinetics are also negatively affected by reduced soil water availability due to decreased mass flow and diffusion (Gessler et al., 2005; Kreuzwieser and Gessler, 2010; but see Dirks et al., 2019). The found reduced nutrient uptake efficiencies and the decreasing fine root biomass under soil drought conditions impaired the N and P uptake capacities (Gessler et al., 2005; Kreuzwieser and Gessler, 2010). Even though the results of this study do not support the optimal portioning theory (OPT; Bloom, 1985) in regard to P limitation, the increased root:shoot ratio suggests enhanced C and nutrient allocation to the roots as a response to reduced soil water availability, i.e., supporting OPT in regard to drought. This is in contrast to Meier et al. (2018), who found that OPT does not apply for mature beech trees exposed to long-term drying. Even with this increase in root:shoot ratio, the observed negative drought effects cannot be mitigated. Due to impaired $A_{\max}$, overall C allocation to the root system likely decreased, which explains the observed reduction in fine root biomass due to root mortality (Meier and Leuschner, 2008). In accordance with Zang et al. (2021), root morphology did not respond to soil drought.

The recent study shows that drought, together with increasing temperature, shifts uptake stoichiometry and causes nutritional imbalances as plant compartments' N:P ratios increase but C:N ratios decrease (**Figure 5.4**; **Figure 5.5**). This mainly results from decreasing P and increasing N concentrations in plant tissue. According to Koerselman and Meuleman (1996) and Güsewell (2004), N:P ratios > 16 g g^{-1} in plant material indicate a pronounced P limitation. Thus, the results reveal a pronounced P limitation under drought with average N:P ratios of 13 g $g-^{1}$ (20 g g^{-1} for interaction drought x low soil P availability) for fine roots and of 18 g g^{-1} (25 g g^{-1} for interaction drought x low soil P availability) for leaves. Increased foliar N concentrations independent of soil N availability likely were the result of missing investment opportunities of N in biomass as drought reduced stomatal opening width and, thus, $A_{\max}$ and the production of assimilates. The storage of N in vacuoles is then enhanced, which results in increasing N:P ratios and decreasing C:N ratios. However, increased foliar N content has been shown to sustain the photosynthetic capacity (Farquhar et al., 2002) and allows for assimilation under drought.

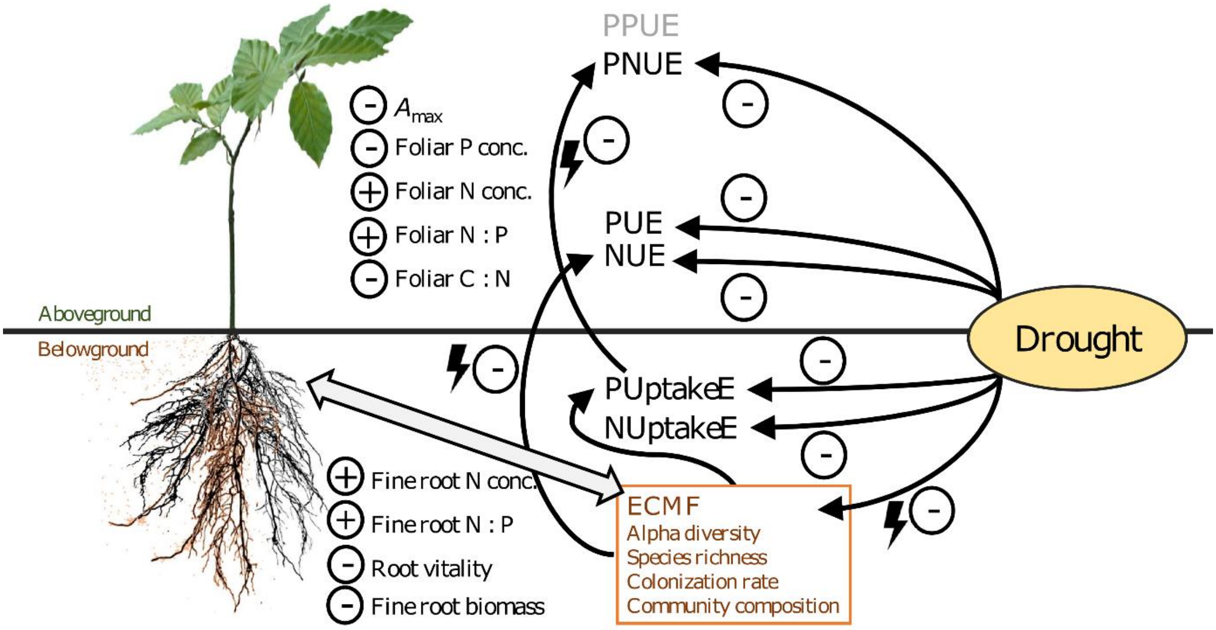


Figure 5.4 Schematic illustration of the main effects of decreasing soil moisture content (drought) on parameters characterizing P and nitrogen (N) economy as well as physiology and productivity of European beech saplings in the climate chamber experiment. A_{max} = maximum photosynthetic rate. P economy: PUptakeE = P uptake efficiency, PUE = P use efficiency, PPUE = photosynthetic P use efficiency; N economy: NUptakeE = N uptake efficiency, NUE = N use efficiency, PNUE = photosynthetic N use efficiency. Thick grey arrow symbolizes symbiosis between plant and ectomycorrhizal fungi (ECMF). The “+” and “-“ signs indicate positive or negative relationships.

Even though higher foliar N contents may have helped beech saplings to better cope with drought stress, they were not sufficient to avoid decreases in photosynthetic capacity under drought conditions. For example, the reduction in PNUE with drought was especially pronounced under high soil N availability, and NUE decreased with decreasing plant tissue C:N ratios. This further emphasizes the negative effect of C limitation on beech saplings’ N use. Under soil drought, rising foliar N:P ratios result in increasing relative P limitation. This likely causes an ATP undersupply with negative effects on the activity and regeneration of ribulose-1,5-bisphosphate, which in turn further aggravates A_{max} (Reich et al., 2009; Yang et al., 2016). This relationship between drought and P limitation becomes especially apparent for PNUE. Here, the results of this study indicate a super-additive independent co-limitation (as defined by Harpole et al., 2011), i.e., a dominating effect exerted by soil drought and a subordinate effect by P deficiency, for PNUE. The negative effect of drought on N uptake and use efficiencies intensifies downstream its metabolic pathway, i.e., from N uptake to photosynthesis and biomass production. Hence, increasing temperatures and decreasing soil water availability, as well as a drought-induced P limitation, can change C cycling and the P and N nutrition of European beech, which in turn might aggravate nutrient imbalances and, thus, further intensify P limitation.

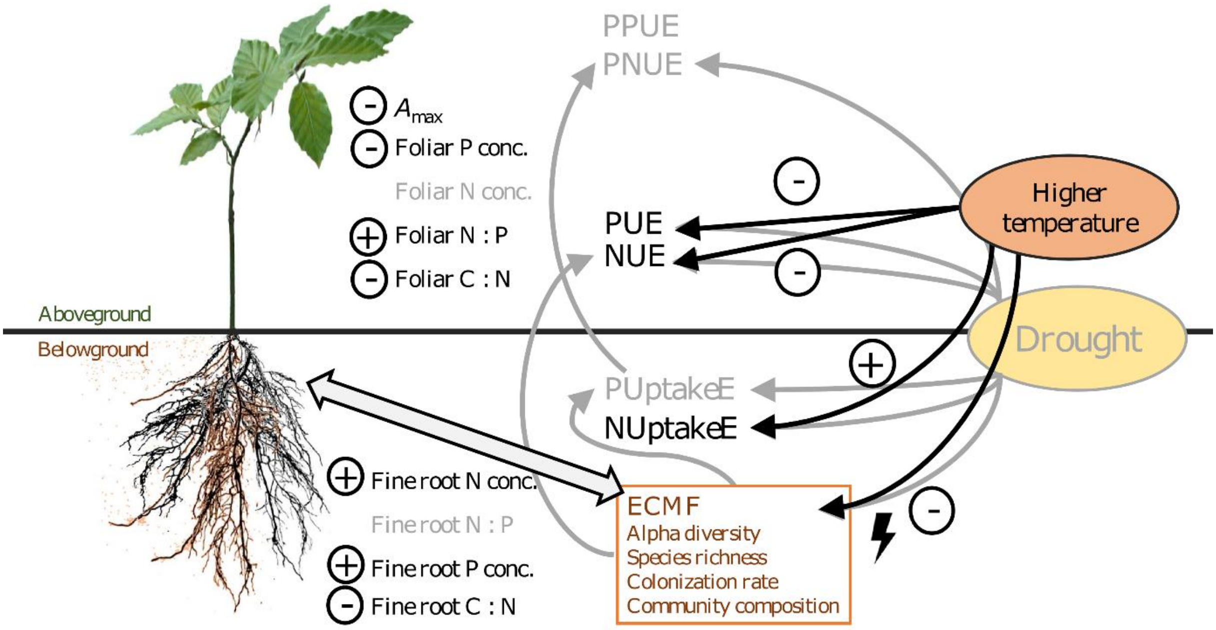


Figure 5.5 Schematic illustration of the main effects of increasing temperature (higher temperature) on parameters characterizing P and nitrogen (N) economy as well as physiology of European beech saplings in the climate chamber experiment. A_{max} = maximum photosynthetic rate. P economy: PUptakeE = P uptake efficiency, PUE = P use efficiency, PPUE = photosynthetic P use efficiency; N economy: NUptakeE = N uptake efficiency, NUE = N use efficiency, PNUE = photosynthetic N use efficiency. Thick grey arrow symbolizes symbiosis between plant and ectomycorrhizal fungi (ECMF). The “+” and “-“ signs indicate positive or negative relationships.

To improve P availability in the rhizosphere, plant roots can exude a complex mixture of low molecular weight carbohydrates, amino acids, organic acids and secondary metabolites like phenolics or enzymes (Hinsinger, 2001). An enhanced carboxylate exudation has been shown to mediate soil P mobilization via ligand exchange, dissolution, and occupation of P sorption sites (Ding et al., 2021). Some carboxylates. i.e., malate, citrate, oxalate, and phenolic acids, are discussed as being more efficient for P mobilization than other compounds. Furthermore, an enhanced exudation of organic acids has been found in plants adapted to dry soil (Song et al., 2012). However, knowledge about the metabolomic composition of root exudates excreted by trees exposed to a combination of drought and nutrient deficiency is scarce. From a subset of investigated beech saplings (n = 162) in the climate chamber experiment, the metabolomic composition of root exudates was investigated (Gargallo-Garriga et al., unpublished). Root exudates were collected with a culture-based cuvette method (*cf.* Phillips et al. 2008). The results show that P limitation is the strongest driver for root exudate composition, while drought is a rather subordinate factor (**Table 5.1**). The availability of P significantly affected metabolomics composition in terms of shifting the concentration of some carbohydrates, organic acids, and amino acids under P limitation. Under drought conditions, particularly, the concentration of specific carbohydrates and some amino acids is changing.

Table 5.1 Permutation-based non-parametric analysis of variance (PERMANOVA) on the significance of the effects of phosphorus (P) availability, nitrogen (N) availability, soil moisture (M), study year (Y), and their interactions on the variance of metabolites in root exudates of European beech saplings (*n* = 7 replicates per treatment). MS = mean sum of squares. Given are *F* values and probabilities of error *P*. The factor with strongest influence according to the *F* statistics is written in bold. Taken from Gargallo-Garriga et al. (unpublished).

	MS	***F***	***P***
P	**0.58**	**4.0**	**0.001**
N	0.15	1.0	0.41
M	0.27	1.9	0.02
Y	0.47	3.2	0.001
P x M	0.28	1.9	0.03
P x Y	0.60	4.1	0.001
N x M	0.22	1.5	0.09
M x Y	0.33	2.3	0.005
P x M x Y	0.50	3.4	0.001
Residuals	0.15		

Further analyses could show that plants growing under drought conditions combined with higher soil P availability show a greater metabolome shift towards a higher concentration of sugars and organic acid compounds (**Figure 5.6**). This change in metabolomic pattern suggests that improved P nutrition may help the studied beech saplings adapt their metabolism with regard to drought stress avoidance.

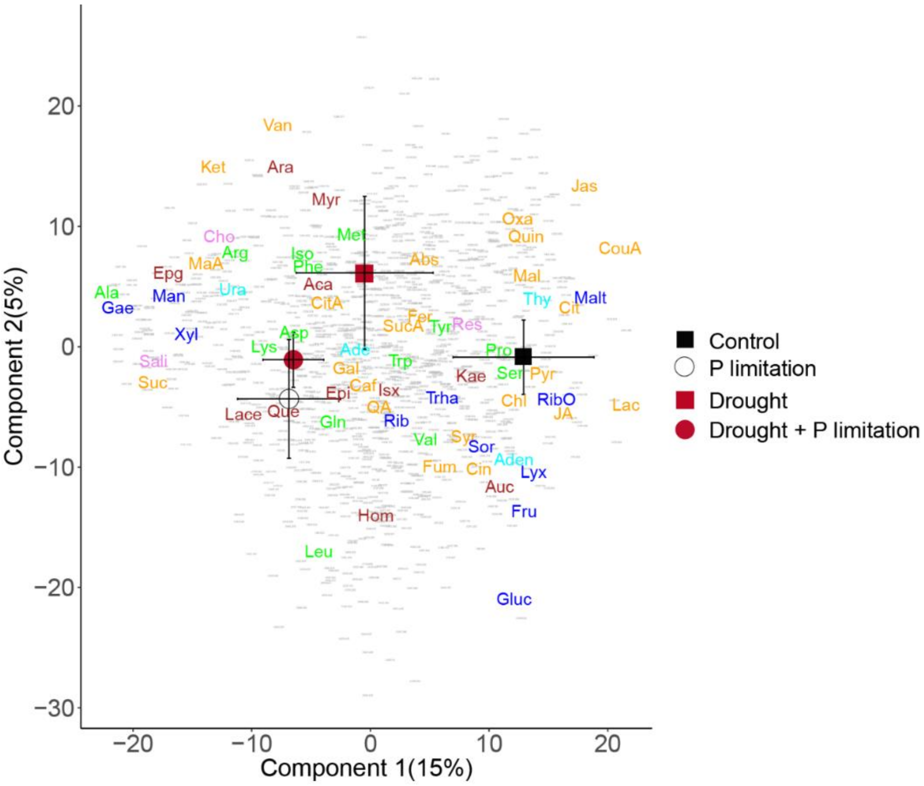


Figure 5.6 Partial least squares-discriminant analysis (PLS-DA) on the control of the soil P availability and soil moisture on the metabolome of root exudates of European beech saplings (*n* = 109). Taken from Gargallo-Garriga et al. (unpublished).

The various metabolite families are represented by colors: dark blue, sugars; green, amino acids; orange, compounds involved in the metabolism of amino acids and sugars; cyan, nucleotides; brown, phenolics; pink, others. Unidentified metabolites are written in grey. Metabolites: Abs, abscisic acid; Aca, acacetin; Ade, adenine; Aden, adenosine; Ala, alanine; Ara, arabitol; Arg, arginine; Asp, aspartic acid; Auc, aucubine; Chl, chlorogenic acid; Cho, choline; Cin, cinnic acid; Cit, citric acid; CouA, 3-coumaric acid; Epg, epigallocatechin; Epi, epicatechin; Fer, trans-ferulic acid; Fru, fructose; Fum, fumaric acid; Gae, D-galactose; Caf, trans-caffeic acid; Gal, gallic acid; Gln, glutamine; Gluc, glucose; Hom, homoorientin; Iso, isoleucine; Isx, isovitexin; JA, jasmonic acid; Jas, jasmone; Kae, kaempferol; Ket, ketoglutaric acid; Lac, lactic acid; Lace, lactate; Leu, leucine; Lys, lysine; Lyx, lyxose; MaA, malic acid; Mal, malate; Malt, maltose; Man, mannose; Met, methionine; Myr, myricetin; Oxa, oxaloglutarate; Phe, phenylalanine; Pro, proline; Pyr, pyruvate; QA, quinic acid; Que, quercetin; Quin, quinilonic acid; Res, resveratrol; Rib, ribose; RibO, 5-methylthio-D-ribose; Sali, sodium salicylate; Ser, serine; Sor, sorbitol; Suc, sucrose; SucA, succinic acid; Syr, syringic acid; Thy, thymine; Trha, threhalose; Trp, tryptophan; Tyr, tyrosine; Ura, uracil; Val, valine; Van, vanic acid; Xyl, xylose.

The recent study demonstrates that higher temperatures together with reduced soil water availability have the potential to deteriorate the physiological adaptations of beech saplings to P limitation, i.e., to disturb the adjustment of plant stoichiometry in response to elevated N deposition/fertilization, as described in the previous section. Furthermore, the plant-soil interaction, future soil C dynamics, and further biochemical cycles will likely be altered (Sardans and Peñuelas, 2012; Preece and Peñuelas, 2016). Hence, not only N deposition but also increasing summer droughts intensify P imbalances, which alter C cycling and have negative effects on the N and P nutrition of European beech. This will likely impair beech growth and productivity in future.

The role of ectomycorrhizal fungi for P and N nutrition of European beech under global change

ECMF play an important role in P acquisition (Zavišić et al., 2016; Raven et al., 2018) and, to some extent, in the N acquisition of plants (Gobert and Plassard, 2008). ECMF symbiosis has been shown to enhance P uptake kinetics and capacity (van Tichelen and Colpaert, 2000; Plassard and Dell, 2010). Pena and Polle (2014) found that under environmental stress, like drought, the root tips colonized by ECM show a higher inorganic N uptake than non-mycorrhizal root tips. This can be accomplished by the exploration of a higher soil volume by emanating fungal hyphae, which are very fine and can access smaller soil pores than fine roots and, thus, increase the absorptive surface area (Richardson et al., 2009). Furthermore, these fungal hyphae contain many high-affinity transporters and play a major role in foraging for and translocating nutrients and water (Cairney 2011). As a result, mycorrhizal fungi association can be considered a highly effective strategy for European beech to cope with nutrient limitation under drought, elevated temperature, and increasing N:P imbalance.

Across all experimental treatments, 60% of the beech saplings' root tips were colonized by a total of six ECMF species, with a higher ECMF species richness resulting in a more complete colonization of the saplings' root systems (**CHAPTER 3**). Here, soil moisture and temperature rather than P or N availability *per se* affect ECMF colonization and diversity. Drought and elevated temperatures can cause a shift in the ECMF community composition (**Figure 5.4**; **Figure 5.5**). An increase in N or P availability is expected to decrease mycorrhizal abundance due to host plants´ strategy to allocate carbohydrates to other plant tissues, which results in C-limitation of mycorrhizal fungi (Treseder, 2004; de Witte et al., 2017). Even though a direct effect of N and P availability was not detected, nutrient availability combined with altered soil moisture and temperature regimes might have affected ECMF colonization rate and diversity, which results in a shift of ECMF species occurrence and abundance (Agerer and Göttlein, 2003). Higher temperature and drought can change the physiological abilities of ECMF hyphae, i.e., growth rate, nutrient uptake, or translocation, which possibly reduces P transfer from the fungal partner to the host (for arbuscular mycorrhiza: Rillig et al., 2002). In contrast to the results of the recent study, Shi et al. (2002), who conducted a water-exclusion experiment with European beech in the Black Forest, Germany, could not find a significant effect of drought on colonization or the number of ECM fungi types per root system, but the composition of the ECM fungi community was changed. Indeed, in this recent study, the ECMF community composition shifted in response to decreased soil moisture availability and elevated temperatures. In dry soil, the ECMF community shifted from the contact exploration type (see Agerer, 2001) towards the short-distance exploration type (*Cenococcum geophilum* and *Genea hispidula*) and the medium-distance exploration type with rhizomorphs. A higher temperature increased the proportion of the pioneer generalist *C. geophilum*. Drought resistance of these pioneer ECMF species as the fungal partner might confer the ability to withstand drought or stressful environmental conditions on beech as the host tree (Pigott, 1982; Fernandez and Koide, 2013). As drought likely causes death of fine roots (Meier and Leuschner, 2008) and ectomycorrhizae (Courty et al., 2006; Danielsen and Polle, 2014), and fine root regeneration may require high amounts of C (Leuschner, 2020), those trees have the highest adaptive advantage, which are having an ECMF community with high survival and persistence to longer periods of drought. With such an ECMF community, the physiological integrity of beech roots facing drought stress can be maintained (Jany et al., 2003) and/or the immediate usage of moisture coming back after drought by trees can be ensured (di Pietro et al., 2007).

In accordance with the results of the recent study, Baxter and Dighton (2001) found in their study with *Betula populifolia* seedlings that higher ECMF diversity, not ECMF composition or colonization, increases P uptake, suggesting a more efficient P uptake. Higher ECM fungi diversity may result in increased and more complete resource exploitation (*sensu* niche complementarity hypothesis by Tilman et al., 2007; Koide, 2000). The enhanced PUptakeE with higher ECMF diversity might be explained by the different abilities of ECMF species for soil exploitation and nutrient acquisition, i.e., ECMF species of long-, medium-, and short-distance type (Agerer, 2001). In this case, factors such as resource partitioning, competition, or interaction with other organisms can shape fungal diversity (*sensu* niche differentiation; Bruns, 1995). Dickie et al. (2002) found that ECMF species differed in their frequencies of occurrence at different soil depths, thus showing a spatial partitioning of ECMF hyphae. Furthermore, ECMF can develop highly variable mycelial structures in soil and on roots (Agerer, 1996), which results in varying soil colonization and mobilization of SOM (Bending and Read, 1995; Agerer, 2001). Additionally, ECMF can show high functional diversity in its enzymatic activity profiles (Courty et al., 2005). The taxonomic and functional ECMF diversity is strongly influenced by the respective ecological niche, i.e., ECMF communities show functional complementarity (Buée et al., 2007). Both community structure and metabolic activity patterns of ECMF are influenced by environmental factors like season, temperature, and soil moisture (Buée et al., 2005). Especially in the context of global change, this might be of high importance—for the host tree and its fungal partner.

This is supported by the results of the recent study, which indicates that P and N uptake and use efficiencies decrease under drought as a consequence of drought-induced negative effects on ECMF parameters. The association with ECMF has been shown to alleviate drought stress for several tree species (Pena et al., 2013; Pena and Polle, 2014; Yin et al., 2017; Wang et al., 2021). Here, trees might favor the co-occurrence of a functionally complementary, more diverse ECMF community (Buée et al., 2007). In this way, the time in which trees can maintain photosynthesis and transfer C to the ECM fungi should be extended (Shi et al., 2002), which confers a higher level of resilience to environmental stress (Courty et al., 2010). In our study, water stress and the arising decrease in P nutrition due to limited P diffusion in soil (Kreuzwieser and Gessler, 2010) presumably exceeded this resilience. This likely caused stomatal closure (Courty et al. 2010) and, thus, a decrease in maximum leaf photosynthesis. Furthermore, the reductions in the growth of ECMF hyphae under drought (Teste et al., 2016) have the potential to further deteriorate P acquisition and, thus, further increase P limitation for beech trees. Drought affects the host tree–ECMF symbiosis on various levels, i.e., a direct effect on the host tree, a direct impact on ECMF species and communities, and most importantly, an indirect effect on ECM fungi by drought (**Figure 5.4**) changing the CO_2 assimilation and hence the C allocation from the host tree (Ruehr et al., 2009; Courty et al., 2010). A constrained photosynthetic capacity and decreased assimilate flux to belowground parts might then cause a fine root dieback (Meier and Leuschner, 2008). Here, a premature death of ECM fungi might occur as it might be too cost-intensive to supply a highly diverse ECM fungi community with C under drought conditions (Danielsen and Polle, 2014). This can decrease ECMF colonization rate and diversity (Courty et al., 2010) and shift the ECMF community composition to less demanding, more competitive ECMF species. Indeed, Pena et al. (2010), who suppressed the C allocation of mature beech trees to roots by girdling, could determine a strong decrease in ECMF diversity. Here, mainly subordinate species were negatively affected and probably outcompeted by highly competitive species like *C. geophilum* with low carbon.

Drought's negative effect on P uptake due to reductions in ECMF diversity, species richness and colonization rate results in additional P limitations, which negatively affect ATP supply and, thus, energy-dependent metabolic processes. This P limitation decreases photosynthetic capacity and, in the next steps, PNUE and NUE (**Figure 5.4**). Thus, a combination of limited PUptakeE (and NUptakeE) and reduced photosynthetic assimilation causes a decrease in NUE, which deteriorates beech sapling growth. Hence, the positive effects of ECMF are erased by the negative effects of drought due to the reduced coupling between the host tree and the fungi symbiont, which results in a reduced ECMF diversity–root functioning relationship. As a result, above- and belowground interactions, as well as plant-soil interactions, are disturbed. Even a shift in the relatively species-poor ECMF community toward fungi with short- and medium-distance soil exploration could not compensate for these negative effects. These results must be carefully interpreted, as the low ECMF species richness in this study (*cf.* Leberecht et al., 2016) might have limited the responsiveness to decreasing soil nutrient availability. Furthermore, as the climate chamber experiment was conducted with beech saplings, the results can only be limitedly transferred to mature beech trees. Here, the higher flexibility in the size of the fine root system of mature beech trees compared to saplings (Leuschner, 2020) might also influence ECMF parameters and, thus, its response to global change.

Conclusions

From this present study on the effect of a future warmer and drier climate in combination with a higher N availability on the P and N nutrition of mature and young *Fagus sylvatica* trees, the following conclusions can be drawn:

1. In mature beech forests, increasing temperatures combined with increasing summer drought reduce C sequestration potential and soil N availability. In a future warmer and drier climate, sandy soils are more prone to C losses than loam-richer soils. However, N deposition might attenuate these effects by enhancing N cycling and curtailing C cycling.
2. Under current environmental conditions, the physiological adaptations of beech saplings to P-limiting conditions are sufficient to circumvent negative P-limitation effects and allow maximum growth even when photosynthetic capacity and plant tissue P concentrations are low.
3. Higher temperatures combined with reduced soil water availability have the potential to deteriorate the physiological adaptations of beech saplings to P limitation, i.e., to disturb the adjustment of plant stoichiometry in response to increased soil N availability. This increases plant tissue N:P ratios, thereby inducing P imbalances and affecting the P and N economy of beech saplings.
4. Elevated N availability further aggravates growth reductions from nutrient imbalances and summer droughts in European beech saplings.

5. Under ambient environmental conditions, ECMF diversity and species richness increase the efficiency of P uptake as well as the efficiencies of P and N use.
6. Increasing summer droughts and increased temperatures reduce the ECMF colonization and diversity and shift the ECMF community. This reduces the complementarity of P uptake by ECMF species, which will likely impair the growth and productivity of European beech saplings.

These results emphasize the importance of plant-soil feedbacks and mycorrhiza-plant interactions for the nutrition of European beech under current and future warmer and drier conditions (van der Putten et al., 2016; Bennett and Classen, 2020).

Further research recommendations

The climate chamber experiment was conducted under highly controllable conditions and with beech saplings, which were colonized by only a relatively small number of ECMF species. Hence, the results of the climate chamber experiment should be reconfirmed not only for mature beech forests but also for beech saplings under natural conditions by field studies across different precipitation and temperature gradients across soils with differing nutrient availabilities and more diverse ECMF communities. To simulate drought, throughfall exclusion experiments could be established. To account for continued high N deposition and to investigate the effect of increasing N:P imbalances, these experiments could be combined with fertilizer experiments.

In this context, the effect of changing climatic conditions on the diversity, activity, and growth of free-living, SOM-degrading soil microorganisms and the resulting alterations in plant-soil interactions should be investigated (Naylor et al., 2020). For example, phospholipid fatty acid analyses could be used to explore the biomass and composition of soil fungal and soil bacterial communities. Furthermore, root exudation has the potential to regulate plant-soil interactions. Thus, future research should analyze the rhizodeposition in natural, mature tree ecosystems in response to a warmer and drier climate and continued elevated N deposition.

This study highlights the importance of ECMF diversity, colonization, and species richness for the P and N nutrition of European beech. However, further experiments and studies are needed to further advance our mechanistic understanding of relationships between ECMF and root functioning. Additionally, research on the response of the ECMF-plant interactions to global change is needed (Bennett and Classen, 2020) to make sound predictions about the P and N nutrition of temperate forest ecosystems.

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Supplementary Material

Table S5.1 Summary of the main results of climate chamber experiment. Described are the significant positive or negative relationships of parameters characterizing phosphorus (P) and nitrogen (N) economy, parameters characterizing physiology and root morphology and parameters characterizing productivity and allocation patterns with abiotic factors and their interaction and biotic factors (ectomycorrhizal fungal (ECMF) parameters). P limitation refers to low soil P availability treatment (P1). Drought limitation refers to low soil moisture treatment (M1). Elevated temperature refers to elevated temperature treatment (T2). N fertilization refers to elevated soil N availability treatment (N2). N x P stands for the general interaction of N and P treatments. P x M stands for general interaction of P and soil moisture (M) treatments. "-" no relationship, "yes" relationship is existing.

		Abiotic factors						**ECMF**		
		P limitation	**Drought**	**Elevated temperature**	**N fertilization**	**N x P**	**P x M**	**Colonization rate**	**Alpha diversity**	**Species richness**
Efficiencies	**PUptakeE**	positive	negative	-	-	-	yes	-	positive	positive
	NUptakeE	-	negative	positive	negative	-		-	-	-
	PUE	positive	negative	negative	-	-	yes	positive	-	-
	NUE	indifferent	negative	negative	-	-	yes	positive	positive	positive
	PPUE	positive	-	-	-	-		-	-	-
	PNUE	negativ	negative	-	-	-	yes	-	-	-
									-	
Biomass	**Total**	-	negative	-	-	-	yes	positive	positive	positive
	Leaves	-	negative	positive	-	-	yes	positive	positive	-
	Fine roots	-	negative	-	-	-		positive	positive	positive
Nutrient concentration	**Foliar P**	negative	negative	negative	-	-	yes	-	-	-
	Foliar N	-	positive	-	-	-	yes	negative	negative	negative
	Foliar N:P	positive	positive	positive	-	-	yes	-	-	-
	Foliar C:N	indifferent	negative	negative	-	-	yes	positive	positive	positive
	Fine root P	negative	-	positive	-	-	yes	-	-	-
	Fine root N	-	positive	positive	-	-	yes	negative	negative	negative
	Fine root N:P	positive	positive	-	-	-	yes	-	-	-

	Fine root C:N		negative	negative	-	-	yes	positive	-	-

Table S5.2 Continued. n/a not applicable.

		Abiotic factors						**ECMF**		
		P limitation	**Drought**	**Elevated temperature**	**N fertilization**	**N x P**	**P x M**	**Colonization rate**	**Alpha diversity**	**Species richness**
ECMF	**Colonization rate**	-	negative	negative	-	-	yes	n/a	-	positive
	Alpha diversity	-	negative	-	-	-	-	-	n/a	positive
	Species richness	-	negative	-	-	-	-	positive	-	n/a
	Community composition	-	yes	yes	-	-	-	/	/	/
Photosynthesis	A_{max}	negative	negative	negative	-	-	yes	-	-	-
	Total C assimilation	-	negative	-	-	-	-	-	-	-
Root parameters	**Root vitality**	-	negative	positive	-	-	yes	-	-	-
	SRA	-	-	-	-	-	-	negative	-	-
	SRL	-	-	positive	-	-	-	negative	-	-

Appendix

APPENDIX

INDEX OF FIGURES

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Ley, A. C., Dauby, G., *Köhler, J.*, Wypior, C., Röser, M., and Hardy, O. J. (2014). Comparative phylogeography of eight herbs and lianas (Marantaceae) in central African rainforests. *Front. Genet.* 5, 403. doi:10.3389/fgene.2014.00403.

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Presentations

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