

**Stefan Trogisch**

**The functional significance of tree diversity for soil N-pools,  
leaf litter decomposition and N-uptake complementarity in  
subtropical forests in China**

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subtropical forests in China**

A dissertation submitted to  
ETH ZURICH  
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presented by  
STEFAN TROGISCH  
Dipl.-Biol., University of Leipzig  
born May 17, 1983  
Germany

accepted on the recommendation of

Prof. Nina Buchmann  
Prof. Michael Scherer-Lorenzen  
Prof. Andrew Hector

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# CONTENTS

<b>SUMMARY .....</b>	<b>1</b>
<b>ZUSAMMENFASSUNG.....</b>	<b>3</b>
<b>GENERAL INTRODUCTION.....</b>	<b>5</b>
<b>CHAPTER 1 .....</b>	<b>19</b>
Manuscript	
<b>What drives the soil nitrogen status in a subtropical evergreen broad-leaved forest, South-East China: tree diversity, stand age or environmental factors?</b>	
(with Jin-Sheng He, Andy Hector, Christian Geißler, Peter Kühn, Yinlei Ma, Karin Nadrowski & Michael Scherer-Lorenzen)	
<b>CHAPTER 2 .....</b>	<b>49</b>
Manuscript	
<b>Impact of tree diversity, successional stage, and environmental factors on leaf litter decomposition in an evergreen broad-leaved forest in subtropical China</b>	
(with Jin-Sheng He, Andy Hector & Michael Scherer-Lorenzen)	
<b>CHAPTER 3 .....</b>	<b>85</b>
Manuscript	
<b>Spatio-temporal and chemical <sup>15</sup>N uptake patterns of four subtropical tree species in monoculture and 4-species mixtures</b>	
(with Jin-Sheng He, Andy Hector & Michael Scherer-Lorenzen)	
<b>GENERAL DISCUSSION .....</b>	<b>123</b>
<b>ACKNOWLEDGEMENTS.....</b>	<b>133</b>
<b>CURRICULUM VITAE .....</b>	<b>135</b>



# SUMMARY

One of the most lively and important current discussions in ecology is on the significance of biodiversity for ecosystem functioning and the provision of goods and services. Most of the knowledge in biodiversity-ecosystem functioning (BEF) research has been acquired from experimental studies conducted along controlled levels of herbaceous plant species richness. These studies have accumulated evidence for a general positive relationship between plant diversity and several ecosystem processes and services. So far, however, there has been little discussion on the importance of tree diversity for primary productivity, nutrient cycling and other important services provided by forest ecosystems. To date, the relationship between tree diversity and the cycling of nitrogen, a key element in plant nutrition that most often limits primary production, has not been sufficiently evaluated. Therefore, this thesis has addressed key aspects of ecosystem nitrogen cycling with focus on belowground processes in forest ecosystems. The aim of this thesis was to identify the importance of tree diversity for the soil N status and leaf litter decomposition in a subtropical forest ecosystem in China, and to test for N uptake complementarity among subtropical tree species in an additional experimental approach.

In **Chapter 1**, the soil and forest floor leaf litter N status were studied along gradients of tree diversity and successional stage comprising 27 forest stands. I aimed to identify the importance of tree diversity, forest stand age and environmental factors for the soil and litter N status. Soil physical properties such as soil moisture as a function of soil texture were more important in determining the N status in the mineral soil than tree diversity and forest stand age. Only the N pool of the litter layer accumulated significantly during succession. It is concluded that abiotic edaphic factors strongly control the soil N status in the subtropical forest studied, while effects of tree species composition and diversity are of only minor importance.

In **Chapter 2**, the significance of tree species diversity for leaf litter decomposition was assessed in three complementary decomposition experiments. First, the decomposition constant of 26 tree species was determined under homogenous physical and biological site conditions. Second, decomposition rates of leaf litter derived by the common species *Schima superba* were related to environmental factors changing along gradients of diversity and successional stage. And third, the influence of litter bag species richness on decomposition of 27 plot-specific litter mixtures comprising 7 to 17 species was assessed. Decomposition rate constants of single litter species varied largely and decomposition rate of *Schima superba* leaf litter decreased along the successional gradient but was not related to tree diversity. Two thirds of plot-specific litter mixtures showed a positive non-additive mixture effect but litter bag species richness did only marginally positively influence litter mixture effects.

In **Chapter 3**, N-uptake complementarity among tree species was tested in an additional mixture experiment with tree saplings of two deciduous (*Castanea henryi* and *Quercus serrata*) and two evergreen species (*Elaeocarpus decipiens* and *Schima suberba*) planted in short distances to induce competitive interactions quickly after planting. I studied spatio-temporal and chemical N uptake patterns of the four subtropical tree species growing in monoculture and mixture by injection of  $^{15}\text{N}$  tracers (ammonium, nitrate, glycine) at two soil depths (5 cm, 20 cm) in four seasons. Species differed slightly in spatial and temporal  $^{15}\text{N}$  uptake, and evergreen species clearly preferred nitrate whereas deciduous trees showed a marginal preference for ammonium. Niche breadth and niche overlap among species remained unaffected by tree species richness.

In conclusion, the results of this thesis suggest that soil physical properties have a strong control on the soil N status and the determination of the soil N storage capacity. Hence, the identification of tree diversity effects at the ecosystem level is complicated due to large environmental heterogeneity. However, tree diversity could promote more rapid N cycling by increasing the rate of leaf litter decomposition, although litter species richness did only marginally influence decomposition of litter mixtures. But especially the decomposition of recalcitrant coniferous litter could be accelerated when mixed with broad-leaved litter. The risk of N limitation might be further reduced by combining tree species showing complementary patterns in N acquisition. Complementary N uptake might also decrease interspecific competition. While this thesis has mainly focussed on the soil N status, leaf litter decomposition and plant N uptake, there is a definite need for integrating tree diversity effects on litter production and changes in plant nutrient stocks during forest stand development.

# ZUSAMMENFASSUNG

Eine der derzeit wichtigsten und am lebhaftesten diskutierten Fragen der Ökologie ist die Bedeutung der Artenvielfalt für Ökosystemfunktionen und -dienstleistungen. Experimentelle Untersuchungen in Graslandökosystemen, in denen die Artenvielfalt krautiger Pflanzen künstlich manipuliert wurde, haben wichtige Erkenntnisse innerhalb der Biodiversitätsforschung erbracht. Die Ergebnisse dieser Experimente zeigen, dass Biodiversität im Allgemeinen einen positiven Einfluss auf verschiedene Ökosystemprozesse und -dienstleistungen besitzt. Allerdings wurde bisher dieser Zusammenhang in Waldökosystemen nur unzureichend untersucht. Besonders die Frage welchen Einfluss die Baumartenvielfalt auf den Stickstoffkreislauf hat, ist weitestgehend ungeklärt. Dabei spielt Stickstoff, eines der wichtigsten Makronährelemente für Pflanzen, eine bedeutsame Rolle, da seine Verfügbarkeit oft die Primärproduktion von Ökosystemen limitiert. Die vorliegende Arbeit konzentriert sich deshalb auf wichtige, vor allem den Boden betreffende Prozesse innerhalb des Stickstoffkreislaufs. Das Ziel der Untersuchung war es, die Bedeutung der Baumartenvielfalt für den Bodenstickstoffzustand und die Laubstreuzersetzung in einem subtropischen Waldökosystem in China zu identifizieren. Des Weiteren wurde die komplementäre Stickstoffaufnahme von vier subtropischen Baumarten in einem Diversitätsexperiment untersucht.

**Kapitel 1** befasst sich mit der Bestimmung des Stickstoff-Status des Bodens und der Streuschicht in 27 Untersuchungsflächen entlang eines Diversitäts- und Sukzessionsgradienten. Hierbei sollte die Bedeutung der Gehölzartenvielfalt, des Alters des Waldbestandes und der Umweltfaktoren untersucht werden. Der Stickstoff-Status des Bodens und der Streuschicht wurden wesentlich von edaphischen Faktoren, wie Bodenfeuchtigkeit und Bodentextur, beeinflusst. Nur der Stickstoff-Pool der Streuschicht zeigte eine signifikante Akkumulation entlang des Sukzessionsgradienten. Es kann geschlossen werden, dass abiotische edaphische Faktoren hauptsächlich den Bodenstickstoff-Status bestimmen und somit die Identifikation von Diversitätseffekten in komplexen und heterogenen Ökosystemen erschwert wird.

**Kapitel 2** analysiert die Bedeutung der Baumartendiversität für die Zersetzung von Laubstreu mittels drei sich ergänzenden Zersetzungsexperimenten. Zuerst wurde die Streuzersetzungsrate von 26 Baumarten unter homogenen Umweltbedingungen bestimmt. In einem zweiten Experiment wurde die Abbaurate einer einheitlichen Streuart (*Schima suberba*) in den 27 Waldbeständen entlang des Diversitäts- und Sukzessionsgradienten untersucht. Welchen Einfluss die Streuartenvielfalt in 27 verschiedenen spezifischen Streumischungen (7 – 17 Arten) hat, wurde in einem weiteren Experiment evaluiert. Die Abbauraten der einzelnen Arten unter einheitlichen Umweltbedingungen zeigten große Unterschiede. Das Bestandsalter der 27 Untersuchungsflächen

beeinflusste die Abbaurate der Laubstreu von *Schima superba* negativ. Zwei Drittel der 27 Streumischungen zeigten eine höhere Abbaurate als erwartet aber die Anzahl der Arten in der Mischung beeinflusste die Rate der Streuzersetzung nur geringfügig.

In **Kapitel 3** wurde getestet, ob Baumarten zweier wechselgrüner (*Castanea henryi* und *Quercus serrata*) und zweier immergrüner Arten (*Elaeocarpus decipiens* und *Schima superba*) eine komplementäre Stickstoffaufnahme zeigen. Die Baumarten wurden dafür in einem zusätzlichen Diversitätsexperiment (Monokulturen und 4-Arten-Mischungen) mit geringem Abstand, der eine schnelle Ausbildung von intra- und interspezifischen Interaktionen sichern sollte, angepflanzt. Zwei Bodenschichten (5 cm, 20 cm) wurden in allen vier Jahreszeiten mit drei unterschiedlichen chemischen Stickstoffverbindungen (Ammonium, Nitrat, Glyzin) mit Hilfe des stabilen <sup>15</sup>N-Isotops markiert, um räumlich-zeitliche und chemische Muster in der Stickstoffaufnahme der Baumarten zu identifizieren. Die Baumarten unterschieden sich geringfügig in der Stickstoffaufnahme aus unterschiedlichen Bodentiefen und Jahreszeiten. Immergrüne Arten zeigten eine höhere Aufnahme von Nitrat, während wechselgrüne Arten Ammonium geringfügig bevorzugten. Diversität hatte keinen Einfluss auf Nischenbreite einzelner Arten oder auf die Nischenüberlappung zwischen zwei Arten.

Die Ergebnisse dieser Arbeit zeigen, dass abiotische edaphische Faktoren einen großen Einfluss auf den Bodenstickstoff-Status ausüben. Dabei erschwert die große Heterogenität in den Umweltbedingungen die Identifikation von Diversitätseffekten. Durch positive Mischungseffekte während der Streuzersetzung könnte aber eine höhere Baumartendiversität die Stickstoffverfügbarkeit erhöhen, obwohl die Artenzahl innerhalb der Streumischung nur einen geringfügigen Einfluss hatte. Aber besonders die Zersetzung von schwer abbaubarer Nadelblattstreu könnte in Mischung mit schnell zersetzender Laubstreu gesteigert werden. Die vier untersuchten Baumarten unterschieden sich hinsichtlich ihres Stickstoffaufnahmемusters, wobei eine komplementäre Stickstoffnutzung das Potential hat, interspezifische Konkurrenz zu verringern. Allerdings konnten keine Diversitätseffekte hinsichtlich einer höheren Stickstoffnutzung festgestellt werden. Es bedarf weiterer Studien, die zusätzlich Diversitätseffekte auf Streuproduktion und Pflanzennährstoffgehalte entlang von Sukzessionsgradienten betrachten, um wichtige Wechselwirkungen zwischen Boden- und Pflanzenstickstoffpools einbeziehen zu können.

# GENERAL INTRODUCTION

## **Relationship between forest diversity and ecosystem functioning**

Forests have become a focal point on the political agenda due to their importance for global climate, protection of biodiversity and the provision of goods and services for human well-being (Agrawal et al. 2008; Canadell and Raupach 2008; FAO 2010; Zhang et al. 2000). Forests are subject to increasing demands and are utilised to satisfy numerous tasks such as supporting the international timber industry, carbon offset initiatives or biodiversity conservation programs. Ecosystem services, i.e. “all benefits that people derive from ecosystems” (Lamb 2011) are indispensable for human existence, and the functioning of ecosystems determines the quality and quantity of provided goods and services (Díaz et al. 2006). Ecosystem services provided by forests are versatile and have been classified into provisioning services (e.g. fibre, fresh water), regulating services (e.g. climate regulation, water purification), cultural services (e.g. cultural heritage, recreation) and supporting services (e.g. nutrient cycling, primary production) according to the Millennium Ecosystem Assessment report (2005).

Ecosystems function thanks to the numerous species and their countless interactions embedded in a complex trophic network. However, species loss continues at high rate and land use change, climate change, nitrogen deposition, biotic exchange and elevated CO<sub>2</sub> concentration have been identified as the five most important drivers for global decline in biodiversity (Butchart et al. 2010; Sala et al. 2000; Stork 2010). This perception has led to a growing concern about the future performance and stability of ecosystems under reduced species richness (Chapin et al. 2000; Duffy 2009; McCann 2000). The question of how many species are necessary to maintain ecosystem functioning and services has become of central importance in ecology and has triggered a large body of research since the early 1990s (Loreau et al. 2001; Naeem et al. 2009; Schulze and Mooney 1993).

There is consensus about a general positive relationship between biodiversity and ecosystem processes with resource-use complementarity, facilitation and the sampling or selection effect identified as underlying mechanisms (Cardinale et al. 2007; Hooper et al. 2005). Furthermore, species-rich ecosystems are considered to be less vulnerable to environmental perturbation (McCann 2000). According to the insurance hypothesis, there is a higher probability that functioning and services provided by an ecosystem will remain unchanged or are less affected if high species redundancy is present (Yachi and Loreau 1999). As a result of species redundancy, potential consequences of local extinction events would be ameliorated by functional equivalence as well as functional overlap among species. Moreover, species-rich ecosystems possess a greater resistance to invasion – a finding explained by higher occupation of available niche space in more

diverse communities that hamper the establishment of exotics (Hector et al. 2001; Fargione and Tilman 2005; Kennedy et al. 2002).

Most biodiversity – ecosystem functioning (BEF) studies have been conducted in grasslands (Hector et al. 1999; Marquard et al. 2009; Tilman et al. 1996) and microcosms (Cardinale et al. 2002) under rather homogenous environmental site conditions. The same mechanisms identified in herbaceous plant communities might also be applicable to forest ecosystems. But the relationship between diversity and ecosystem functioning in forests remains poorly understood due to the high level of complexity and environmental heterogeneity (Scherer-Lorenzen et al. 2005).

A good starting point was provided by silvicultural research aiming to increase forest productivity by establishing tree species mixtures (Pretzsch 2009). Higher productivity in multi-species plantations was revealed by several studies (Forrester et al. 2006, Kelty 2006, Piotta 2008). But the observed biodiversity effects are often attributed to N fixing tree species leading to reduced interspecific competition for N in polycultures. In another study by Erksine et al. (2006) diverse plantations comprising up to 8 tree species tended to be more productive than monocultures. However, the low number of species involved in silvicultural studies does not reflect diverse real-world forest ecosystems. Only a few studies are based on forest inventory data and have identified a positive relationship between tree diversity and productivity (Caspersen and Pacala 2001; Liang et al. 2007; Vilà et al. 2007). Previous studies have mainly focussed on how tree species richness might affect productivity as the single response variable. Less is known about the influence of forest diversity on other vital ecosystem functions and services such as nutrient cycling, belowground carbon sequestration or erosion control.

After reviewing recent literature Nadrowski et al. (2010) concluded that tree diversity has the potential to significantly influence ecosystem functions and services but that confounding environmental variables often makes it difficult to isolate diversity effects. The recognized lack of knowledge about the biodiversity - ecosystem functioning relationship in forests has therefore motivated the establishment of nine larger tree diversity experiments in tropical, subtropical, temperate and boreal regions since 1999 ([www.treedivnet.ugent.be](http://www.treedivnet.ugent.be)). These experiments offer the opportunity to study all important response variables and associated environmental parameters along a controlled tree species gradient. The common goal of all these experiments is to examine the importance of tree diversity for versatile ecosystem processes and services and to identify the underlying mechanisms. Combining such large tree diversity experiments with comparative studies in natural forests will provide valuable insights on how abiotic factors and successional stage influence the biodiversity-ecosystem functioning relationship in real ecosystems.

## Effects of tree diversity and successional stage on forest N cycling

Nitrogen (N) is a key element in biogeochemical processes and often limits net primary productivity in major terrestrial ecosystems (LeBauer and Treseder 2008; Vitousek and Howarth 1991). The atmosphere (78 %) and sedimentary rocks (20 %) are the Earth's main N pools, but only roughly 2 % are directly available to organisms in form of reactive N (Galloway 1998). Under natural conditions, N is transformed from its inert triple-bonded form ( $N_2$ ) into reactive and therefore plant-available forms by biological N fixation and lightening. Biological N fixation is the most important pathway of how N enters the soil-plant system. Internal ecosystem N cycling comprises the decomposition of dead organic matter including the transformation of complex N compounds into dissolved organic N (DON), the mineralization of DON into ammonium ( $NH_4^+$ ) and nitrate ( $NO_3^-$ ) by microbes, and finally the uptake of mineralized N and DON by plants to build up new biomass. Ammonia volatilization, fire events, denitrification and leaching are pathways of how N is lost from ecosystems (Galloway et al. 2004).

Tree species effects on forest N cycling are well known and are caused by species-specific differences in N acquisition, N-use efficiency, litter production and litter quality or interaction with microbes (Binkley and Giardina 1998; Hobbie 1992; Knops et al. 2002; Rhoades 1996). According to Knops et al. (2002) ecosystem N cycling is most importantly affected by plant species impacts on N inputs and losses. The most obvious example of species effects on soil N availability are plant species living in symbiosis with N fixing prokaryotes (Hughes and Denslow 2005; Vitousek and Walker 1989). In this case, N input by biological fixation can exceed by far inputs via atmospheric N deposition and leads to a substantial increase in soil fertility. However, non-fixing tree species also differ largely with respect to their influence on forest floor and soil N properties (Lovett et al. 2004; Trum et al. 2011; Vesterdal et al. 2008; Zeller et al. 2007). For example dissimilarities in N mineralization rates are often explained by different litter characteristics in particular by the lignin:N ratio that negatively affects net N mineralization across forest sites in North America (Scott and Binkley 1997).

Generalization of tree species mixture effects on N pool sizes and fluxes at the stand level is impeded by the low number of studies. Rothe & Binkley (2001) have reviewed mixed-species effects on nutritional interactions in forest and proposed that the strongest interactions will occur when a species significantly improves the availability of a limiting resource. In case of N, this has been demonstrated by several studies (Forrester et al. 2006) which indicated higher yields in mixtures that include a N-fixing tree species. Although supporting data is rare, complementarity in N-uptake might significantly contribute to a more efficient use of given N resources even in the absence of N fixing trees in the community. In a meta-analysis by Richard et al (2010), more than 50 % of mixtures had higher plant nutrient contents than monocultures, suggesting higher

resource utilization. Niche differentiation is a requisite of N use complementarity and it was demonstrated that tree species differ in their N acquisition pattern and therefore occupying distinct niches. Theoretically there is the possibility for chemical, spatial and temporal niche differentiation (McKane et al. 2002). For example, tree species differ in their preference for nitrate and ammonium (Stewart et al. 1988, Templer and Dawson 2004) or uptake of N from different soil depths (Jose et al. 2006). But species-specific N acquisition patterns as well as N uptake dynamics of tree species are often unidentified.

Besides diversity effects resulting from N uptake dynamics and complementary resource utilization, tree diversity can influence N dynamics and availability during leaf litter decomposition. Litter decomposition is the main process replenishing the pool of available N in the soil by break-down of dead organic matter (Swift et al. 1979). In mixed forests litter species differing in physicochemical characteristics decompose not isolated but rather interact with each other mediated by microorganisms and the physical environment (Gessner et al. 2010). Interactions of litter types lead often to non-additive litter diversity effects, i.e. the litter mixture shows a different decomposition rate than predicted from individual rates of involved species. As reviewed by Gartner and Cardon (2004) 67 % of litter mixtures show non-additive effects for mass loss and 76 % of mixtures possess non-additive dynamics of nutrient concentrations. Synergistic litter mixtures effects can be attributed to several mechanisms according to Hättenschwiler et al. (2005): (1) translocation of nutrients from high-quality to low quality litter, (2) influence of litter-specific chemical compounds on detritivore activity, (3) changes in microclimatic conditions due to specific structural litter traits, and (4) higher habitat diversity for decomposer species showing functional complementary in degradation abilities in litter mixtures.

During forest succession, profound changes in species composition, tree diversity and forest structure are observed (Bruehlheide et al. 2011; Yamada et al. 2011). Accumulation of plant biomass is generally fast due to the large percentage of rapid growing pioneer species in early secondary forests (Lamb 2011). Similar, litter production follows a logarithmic increase and reaches a steady state after about 15 years (Brown and Lugo 1990). Above- and belowground litter input increases soil organic matter (SOM) that reaches quantities comparable to those measured in mature forests within 50 years. Brown and Lugo (1990) conclude that nutrient cycling and rate of nutrient accumulation in biomass is most rapid during the first 20 years of secondary forest succession. Subsequently, the rate of N cycling might slow down concurrent with reduced nutrient losses and a closing of major biogeochemical cycles as stated by Odum (1969). As aboveground plant biomass increases for example at a rate of  $2.9 \text{ Mg ha}^{-1} \text{ yr}^{-1}$  over the first 80 years in tropical forest regrowth (Silver et al. 2000), the living vegetation can be regarded as a strong sink for N over decades in general (Yang et al. 2011). The fast increase in plant N pools

through succession should lead to a depletion of soil N. However, input of N via atmospheric N deposition or biological N fixation might prevent N limitation for plant productivity if exceeding N losses via leaching. In contrast, phosphorous limitation is often identified as a major driver of ecosystem development, especially in tropical regions (Vitousek 1984). But as shown by Hooker and Compton (2003) for a temperate forest chronosequence comprising 115 years, N accumulation in plant biomass can cause significant decline in mineral soil N while total ecosystem N remained unchanged. During forest maturation N retention increases due to growing amounts of detrital biomass and faster rates of microbial N immobilization (Fisk et al. 2002). Taking such considerable stand age related changes in N cycling into account, I also focused on the successional effects on soil N pools, net N mineralization and decomposition.

### **Evergreen broad-leaved forest in China – characteristics, importance and current status**

Covering roughly 25 % of China's land area, the evergreen broad-leaved forest (EBLF) belt stretches from eastern Tibet to the most eastern borders of continental China in Zhejiang Province. Located in a warm-temperate monsoon climate, it is often referred as a "subtropical evergreen broad-leaved forest" and is split into a Western semi-moist and an Eastern moist EBLF (Wang et al. 2007). Dai et al. (2011) breaks down subtropical EBLF into three major types. The typical EBLF is the most widespread, but becomes a subtropical mixed evergreen-deciduous broad-leaved forest in more northern regions, whereas subtropical sclerophyllous EBLF has developed in western and southwestern parts of the subtropical zone on the foothills of the Tibetan Plateau and Hengduan Mountains.

Mean annual temperature ranges from 15 to 20 °C, but distinct seasonality with cool winters and hot summers is present. Annual precipitation is about 1000 to 2000 mm, of which most occurs in summer, whereas the driest months are typically in winter. Acidic soils (pH 4.0 to 5.5) often referred as "red and yellow earths" with a poorly developed humus layer are the predominant results of pedogenesis over sandstones, shale or granite. Tree species richness of the EBLF is high with Fagaceae, Lauraceae, Theaceae, Magnoliaceae and Hamamelidaceae being floristically prominent plant families. Evergreen broad-leaved trees are predominant but coniferous and especially deciduous trees contribute substantially to total tree diversity. The herb layer is often sparsely developed with fern species dominating. Typical features of tropical rainforests such as tree buttresses, epiphytic angiosperms, drip-tips or cauliflory are nearly absent or are missing completely (Richardson 1990; Song 1995).

The forests of subtropical China have been intensively managed and exploited for centuries. Most of the EBLF has been replaced with secondary vegetation or was transformed into agricultural

land by anthropogenic interference (Wang et al. 2007). Along degradation gradients the typical sequence of secondary vegetation comprises coniferous broad-leaved mixed forest, coniferous forest and shrubland that is further degraded into shrub-grassland and pure grassland often dominated by *Miscanthus spp.* (Ren et al. 2012). Nowadays, only remnants of EBLF on steeper slopes or remote areas remain. Besides timber, fuelwood for cooking and heating is one the most important economically valued ecosystem services provided by subtropical forests. Especially in rural areas, a large percentage of residents depend heavily on fuelwood collection from community forests (Cai and Jiang 2010). But also non-wood forest products contribute significantly to household revenue. For example, the seeds of *Camellia oleifera* are harvested to produce tea oil for cooking and *Pinus massoniana* resin, collected by local farmers, has been widely used in turpentine production.

As the demand for forests products increases, the need for more sustainable forest management is recognized. The importance of Chinese forests for carbon sequestration (Zhang et al. 2010), nitrogen retention (Huang et al. 2011), erosion control, flood protection and species conservation (Huang 2011) is reflected by the establishment of large numbers of forest reserves and initiation of extended afforestation campaigns in the last decades (Li 2004; Zhang et al. 2000). However, ecosystem functioning of EBLF including its stability and resilience, might change due to increasing atmospheric N deposition in China (Liu et al. 2011). Elevated levels of plant-available N might also have negative consequences on forest diversity (Lu et al. 2008). Therefore, studying the relationship between tree diversity and ecosystem N cycling is of high priority in order to understand and project the effects of global change on forest functioning.

### **The BEF-China Project**

Entitled “The role of tree and shrub diversity for production, erosion control, element cycling, and species conservation in Chinese subtropical forest ecosystems” the BEF-China Project (DFG Forschergruppe 891) is the first tree diversity experiment established in the humid subtropics. China is, owing to its vast area and topographical complexity, a “megadiversity country” (Tang et al. 2006). Southern China in particular represents a biodiversity hotspot in the northern hemisphere. Located in south-eastern China, the study site is situated in the evergreen broad-leaved forest zone, where exceptional high woody plant species richness has developed. The project seeks to answer the overarching question of how plant diversity may maintain vital services in forest ecosystems. According to Wang et al. (2010), 29 % of the worlds’ plantation area is located in China, of which 63 % can be found in the subtropics. However, coniferous species such as *Pinus massoniana* and *Cunninghamia lanceolata* constitute 72% of the subtropical plantation area despite high local tree species richness. Identifying the role of tree diversity for

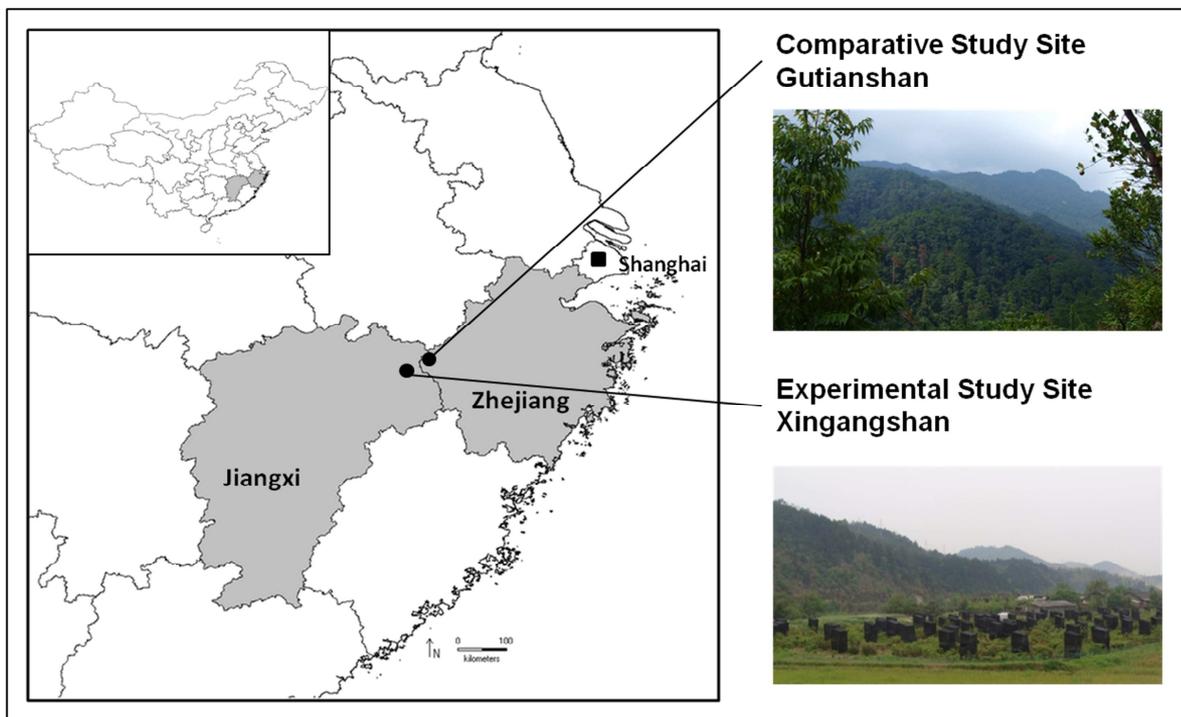
forest ecosystem functioning and services will therefore contribute key knowledge for forest restoration efforts, species conservation and plantation management. The importance of forests for human well-being has been also recognized by government leaders and serious attempts have been made to reduce environmental degradation and foster sustainable forestry (Dai et al. 2011). The subprojects of BEF-China focus on specific aspects of the biodiversity – ecosystem functioning relationship such as primary production, nutrient cycling, soil erosion, functional diversity, invasibility or microbial diversity. Each European subproject is complemented by a Chinese counterpart emphasizing the multilateral research structure.

The BEF-China project follows an observational and an experimental approach to study ecosystem functioning in relation to plant diversity. In the observational approach, 27 comparative study plots (CSPs) have been established along gradients of woody plant diversity and successional stage in secondary forests stands of the Gutianshan National Nature Reserve located in western Zhejiang Province. In order to preserve the last remaining old forests of Gutianshan, an area of about 8100 ha was put under protection in 1975. The steep mountain slopes of the reserve have prevented complete deforestation and transformation into agricultural land. However, there is ample evidence that especially slope toes have been terraced to grow crops. Historic terrace remnants and considerable amounts of charcoal are clear indications for the former land use. As a consequence, a mosaic of different aged forest stands can be found in the reserve today. The established CSPs comprise forests stands of 22 years to 116 years with woody plant species richness ranging from 25 to 69 species (Bruehlheide et al. 2011). The primary objective of the observational study that was the focus of the first project phase (2008 to 2011), but will continue in the next years, was to disentangle the effects of plant diversity, stand age and environmental factors on ecosystem processes in a natural evergreen broad-leaved forest. The results of the observational study will be compared later on with those gained from the newly established tree diversity experiment.

The two experimental sites representing the Main Experiment of BEF-China are located near the village of Xingangshan, Jiangxi Province, (about 60 km from Gutianshan) and were established in 2009 and 2010. In the experimental approach, roughly 300,000 samplings of 42 tree species and 10 shrub species were planted on a total area of 50 ha divided into two sites of equal size. The diversity gradient comprises 1, 2, 8, 16 and 24 tree species that were planted on 566 plots in total. The Main Experiment in Xingangshan will be the focus of BEF-China in the second phase of the project (2011 – 2014) and therefore does not contribute to this thesis.

In order to study competition among tree saplings and to project species interactions during the initiation phase of the Main Experiment, a Pilot Experiment was set up in 2009 allowing destructive harvesting of tree saplings. On a former agricultural field in Xingangshan, tree saplings

were planted as a common garden experiment in short-neighbour distances to induce fast formation of below- and aboveground interactions. Each plant community comprised 16 tree individuals planted on 1 m<sup>2</sup> sized plots, respectively, in monocultures, 2 species and 4 species mixtures. Additionally, treatments, for example, light intensity or planting distance, were included to enable the respective subprojects of BEF-China to study the effects of species diversity and species functional traits under different environmental conditions on multiple processes such as productivity, nutrient cycling or herb-layer invasibility. In the Pilot Experiment in Xingangshan, I tested for N uptake complementarity using <sup>15</sup>N stable isotopic tracers among four subtropical tree species.



**Fig. 1** Location of the BEF China Project's comparative and experimental study sites in China.

## Objectives and thesis outline

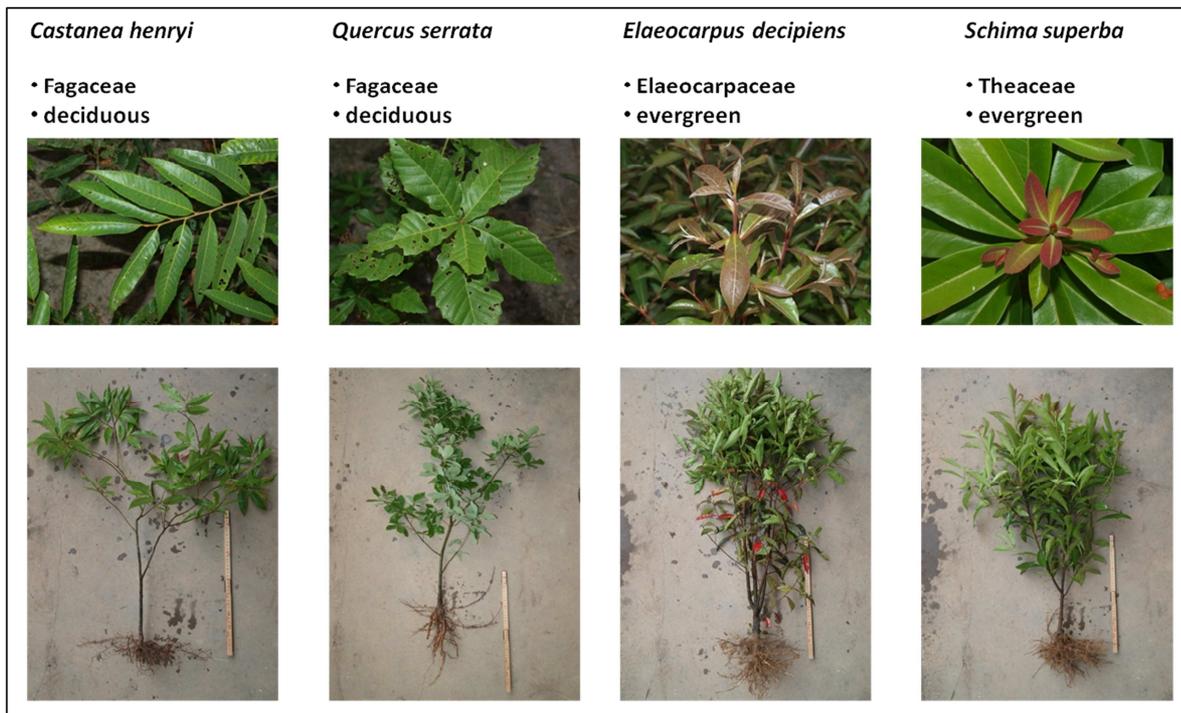
Within the framework of BEF-China, this thesis aims to identify the significance of tree diversity for soil N pools, leaf litter decomposition and N uptake complementarity in a subtropical forest ecosystem in Southeast China. I primarily address belowground processes of N cycling including mass loss and N release during decomposition, soil N storage and net N mineralization, and N uptake by tree species. The influence of tree diversity on soil N pools (Chapter 1) and leaf litter decomposition (Chapter 2) was studied with an observational approach using undisturbed forest stands of different species richness and stand age in Gutianshan. N acquisition patterns and uptake complementarity of four native tree species were investigated with the  $^{15}\text{N}$  tracer technique in the Pilot Experiment located in Xingangshan (Chapter 3).

In **Chapter 1**, I examined the influence of tree diversity and forest stand age on major soil-related N pools including N stocks of the litter layer, soil total N as well as mineral N in different soil layers. Furthermore, net-N mineralization was measured in the topsoil layer to estimate plant-available N. As N dynamics and pool sizes were expected to vary temporally throughout the year, measurements were conducted seasonally. I hypothesized that tree diversity and forest stand age positively affect N pool sizes. Moreover, the importance of confounding site factors such as, for example, soil moisture, soil texture and topographical variables was assessed.

In **Chapter 2**, I conducted three complementary litter decomposition experiments using the litter bag technique. First, I assessed mass loss and N dynamics of 26 canopy and subcanopy tree species under homogenous site conditions during a decomposition period of 383 days. Second, mass loss of leaf litter of the dominant canopy species *Schima superba* was studied along the tree diversity and successional gradient. Third, decomposition of plot-specific litter mixtures comprising 7 to 17 species was followed in the corresponding comparative plots. My main objectives were to identify diversity effects both at the stand level of trees and at the number of litter species in plot-specific mixtures on leaf litter decomposition. It was hypothesized that high litter diversity induces predominantly positive non-additive mixture effects on decomposition rates.

In **Chapter 3**, I studied spatio-temporal and chemical N uptake complementarity among four native tree species differing in phenology using  $^{15}\text{N}$  stable isotopes. Saplings of two deciduous species and two evergreen tree species were planted in monoculture and in 4-species mixtures. Three chemical N forms were injected at two soil depth into the soil in four seasons to trace N uptake by tree saplings under intra- and interspecific competition. I aimed to identify species preferences in N uptake, assess the influence of heterospecific competition on N-use strategies and quantify species niche breadth and niche overlap of tree species in monoculture and mixture.

I hypothesized that the mixture of all four species exploits N resources more efficiently compared to monocultures because of complementarity in species-specific N uptake patterns.



**Fig. 2** Tree species used to test for N uptake complementarity in the additional mixture experiment (Chapter 3) in Xingangshan.

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# CHAPTER 1

Manuscript

## **What drives the soil nitrogen status in a subtropical evergreen broad-leaved forest, South-East China: tree diversity, stand age or environmental factors?**

Stefan Trogisch<sup>1,2</sup>, Jin-Sheng He<sup>3</sup>, Andy Hector<sup>4</sup>, Christian Geißler<sup>5</sup>, Peter Kühn<sup>5</sup>, Yinlei Ma<sup>3</sup>, Karin Nadrowski<sup>6</sup> & Michael Scherer-Lorenzen<sup>2</sup>

<sup>1</sup> Institute of Agricultural Sciences, ETH Zurich, Switzerland

<sup>2</sup> Institute of Biology II, Geobotany, University of Freiburg, Germany

<sup>3</sup> Department of Ecology, Peking University, Beijing, China

<sup>4</sup> Institute of Evolutionary Biology and Environmental Studies, University of Zurich, Switzerland

<sup>5</sup> Institute of Geography, University of Tübingen, Germany

<sup>6</sup> Institute of Biology I, Special Botany and Functional Ecology, University of Leipzig, Germany

## Abstract

*Background and aims* In addition to environmental factors, tree diversity can influence ecosystem processes such as nitrogen cycling. However, little is known about the strength of this control, the underlying mechanisms and the interactions between biodiversity, ecosystem processes and environmental parameters in forest ecosystems. In this study we aim to determine the importance of tree diversity, stand age and environmental factors for the soil N status of an evergreen broad-leaved forest in South-East China.

*Methods* The soil and forest floor leaf litter N status were studied along gradients of tree diversity and successional stage comprising 27 forest stands. Causal relationships of species richness, environmental parameters and N pool sizes (soil total N, soil mineral N, forest floor litter N) were assessed using Structural Equation Modelling (SEM).

*Results* Pool sizes of soil total N and mineral N ranged from 3 Mg ha<sup>-1</sup> to 12 Mg ha<sup>-1</sup> and 11 kg ha<sup>-1</sup> to 68 kg ha<sup>-1</sup> in the top 50 cm of the mineral soil. Mineral N pool and net N mineralization showed large seasonal fluctuations and were highest in summer and generally lower in the cooler seasons. Tree diversity exerted no direct control on the soil and forest floor litter N pools. Soil total N and mineral N pools did not accumulate along the successional forest gradient, but were rather influenced by site-specific factors such as soil moisture. Standing litter N pool ranged from 58.14 kg ha<sup>-1</sup> to 163.50 kg ha<sup>-1</sup> and accumulated significantly with forest stand age. In the top soil layer (0 -10 cm), SEM revealed a strong direct effect of soil moisture on soil N pools whereas the forest floor litter N pool was equally influenced by soil moisture and forest stand age.

*Conclusions* In the subtropical forest studied the soil and forest floor litter N status were strongly affected by abiotic environmental factors. The accumulation of forest floor litter N through succession indicates the importance of the litter layer for nitrogen retention during forest development. We conclude that potential effects of tree diversity or forest stand age on the soil N status are masked by high spatial variation of abiotic factors, i.e. soil moisture as a function of soil texture, and probably by soil-plant-soil associations. Therefore, the strength of potential tree diversity effects on N cycling or productivity may differ largely depending on small-scale variation of environmental conditions especially in heterogeneous mountainous areas.

**Keywords:** BEF China, forest biodiversity, Gutianshan National Nature Reserve (GNNR), nitrogen cycling, soil moisture, soil texture, secondary forest succession

## Introduction

Forests account for 80 % of the world's plant biomass and are therefore a main driver and component of the Earth's biogeochemical cycles (Watson et al. 2000). Their versatile services such as climate regulation and protection of soil resources, denotes them as one of the most important terrestrial ecosystems for human wellbeing (Scherer-Lorenzen et al. 2005a). Forest ecosystems show a high net primary productivity that is generally limited by the availability of nitrogen (N) (Gruber and Galloway 2008; LeBauer and Treseder 2008; Vitousek and Howarth 1991). Thus, nitrogen cycling is a key factor for understand the structure and functioning of ecosystems.

The relationship between diversity and ecosystem functioning, e.g. N cycling and productivity, is not well understood in forests (Scherer-Lorenzen et al. 2005a). Studies conducted in grasslands have shown that species richness positively influences ecosystem performance with niche complementarity, facilitation and selection effects identified as underlying mechanisms (Loreau and Hector 2001). For instance, species-rich grassland communities possess a higher exploitation of nitrate leading to a reduced N leaching rate than in monocultures (Scherer-Lorenzen et al. 2003; Tilman et al. 1996). Species-rich forests might perform better than monospecific stands in providing numerous services; but supporting data are currently insufficient to draw general conclusions (Firn et al. 2007; Nadrowski et al. 2010; Vilà et al. 2007). Therefore, a better understanding of how tree diversity is influencing ecosystem performance with a focus on N pool sizes and N fluxes is needed.

So far, we have good insights of how single tree species can affect soil nitrogen cycling and soil properties (Augusto et al. 2002; Binkley and Valentine 1991; Knops et al. 2002; Rhoades 1996; Trum et al. 2011). For example, forest floor litter mass and litterfall N can be quite different among single species stands growing under the same environmental conditions (Binkley and Giardina 1998). Lovett et al. (2004) studied the impact of five temperate tree species showing large differences in litter quality on N cycling and found that tree species identity significantly affected soil C:N ratio and N mineralization rates. Tree species exert a strong control on nutrient availability by quantity, quality and nutrient concentrations of litter produced (Prescott 2002; Reich et al. 2005) Species-specific traits can have therefore great implications for nitrogen cycling (Hobbie 1992), but few studies have addressed mixture effects in forest ecosystems (e.g. Rothe and Binkley 2001). Most of the results have been received from studies conducted in tree plantations with a rather limited number of species involved (Kelty 2006). In studies focusing on resource use, the majority (65%) showed a shift in N- and P-use efficiency of a given species growing in mixture compared to monoculture (Richards et al. 2010), indicating biodiversity effects on tree nutrition. The processes leading to such shifts can be manifold. For example decomposition dynamics in mixed leaf litter often show non-additive effects so that N is released

at a faster rate than predicted from decomposition rates of corresponding single-species leaf litter (Chapman and Koch 2007; Gartner and Cardon 2004). Such litter diversity effects during decomposition can lead to a feedback reaction positively influencing plant productivity (Hättenschwiler et al. 2005). Other studies have shown that tree species richness affects litter production emphasizing multiple process-specific effects of diversity on ecosystem functioning (Scherer-Lorenzen et al. 2007).

The interactions among numerous tree species and their compound effects on ecosystem functioning are difficult to predict because of the strong influence of site characteristics on species performance. As a consequence, biodiversity effects are less pronounced at the ecosystem but more at the community level (Balvanera et al. 2006). Loreau (2000) has stated that describing the interaction between biodiversity, ecosystem processes and abiotic factors is a major future challenge, underpinned recently by Cardinale et al. (2011). Forests usually cover large areas varying in abiotic parameters so that the strength of species interactions might depend on environmental conditions such as nitrogen or phosphorous supply and availability (Boyden et al. 2005; Pretzsch 2009). Furthermore, species interactions mediated by competition, facilitation and complementarity are supposed to change strongly during forest succession. In the course of secondary forest succession, plants alter environmental factors and the supply of resources they rely on in multiple ways. For example, changes in the amount and quality of leaf litter have direct impact on soil N cycling and nutrient availability (Prescott 2002). Fisk et al. (2002) observed higher detrital N and microbial N immobilization in old-growth than in secondary-growth northern hardwood forests and soil total organic N is observed to accumulate in the course of succession (De Kovel et al. 2000). Clearly, quantifying such complex spatio-temporal dynamics within forest ecosystems and their influence on ecosystem functions and services ask for complementary research approaches (Scherer-Lorenzen et al. 2005b). Tree diversity experiments focusing on diversity effects while keeping environmental conditions as constant as possible is one promising approach. However, in order to scale up such effects to real ecosystems, there is a need for better insights on how abiotic factors and their spatial and temporal heterogeneity, successional stage and tree diversity are inter-related (Loreau 2000). This might best be achieved by comparative studies in natural forests.

In this study, we investigated the soil N status along a tree diversity and successional gradient in a subtropical forest in South-East China. Subtropical evergreen broad-leaved forests are highly diverse in terms of woody plant species with changing species composition and richness through forest succession (Bruehlheide et al. 2011). Large areas of this forest type have been transformed into tree plantations (Wang et al. 2007b) leading often to land degradation with declining

productivity over time. Thus, several studies have investigated how N pools and fluxes as well as N availability are changing along forest degradation gradients or between different vegetation types (Mo et al. 2003; Yan et al. 2008; Yan et al. 2009; Yang et al. 2005). However, knowledge about how tree diversity and environmental factors affect N pools and fluxes is very limited. Therefore, our main objectives were to study the influence of woody plant diversity, forest stand age, and abiotic factors on soil total N and mineral N pools, net N mineralization rate as well as on N pool size of the forest floor litter layer. Our particular hypotheses were: (H1) species diversity and successional stage influence N pool sizes in the soil and in the litter layer as well as net N mineralization rate. (H2) Changes in the soil N status are explained by modified environmental factors such as litter turnover or soil moisture along the diversity and successional gradient.

## **Materials and Methods**

### **Site characteristics**

This study was conducted in the Gutianshan National Nature Reserve (GNNR) located in the mountainous area of western Zhejiang Province, South-East China (29°8'18"–29°17'29" N, 118°2'14"–118°11'12" E; Fig. 1). The reserve covers an area of about 8100 ha and comprises an altitudinal range from 305 to 1258 m above sea level. The region encounters a warm-temperate climate with distinct seasonality: a hot-humid season from April to September and a cool-dry season from October to March. Monthly mean temperature is lowest in January (4.3°C) and highest in July (27.9°C). Annual mean temperature amounts to 15.3°C and annual precipitation is 1964 mm of which most occurs between March and September (Hu and Yu 2008). The geological bedrock is mainly comprised of granite covered with relatively shallow soils (average soil depth: 67 cm). Sandy-loamy and silty-loamy textured acidic Cambisols with pH values ranging from 4 to 5 are the predominant soil type. The vegetation is classified as monsoon subtropical evergreen broad-leaved forest characterized by a high diversity of woody plant species. This forest type was formerly widespread in Eastern China, covering around 25% of the country, but has experienced strong conversion into farmland, tree plantations and shrub land in the last decades (Wang et al. 2007b). To protect the remaining less disturbed forest resources, the Gutianshan Forest Reserve was established in 1975 and was declared as National Nature Reserve in 2001. Within the GNNR 1426 species of seed plants belonging to 648 genera and 149 plant families are documented (Lou and Jin 2000). During secondary forest succession the fraction of deciduous and conifer species decreases whereas evergreen broad-leaved species become more dominant (Bruelheide et al. 2011; Hu and Yu 2008). Further site characteristics and species lists are provided by Legendre et al. (2009) and Bruelheide et al. (2011).

**Study design**

Between May and July 2008, 27 study plots (30 x 30 m), hereafter named “comparative study plots” (CSPs, see Bruelheide et al. 2011), were established along a tree diversity (25 – 69 species) and successional gradient (22 – 116 yrs). Our original design aimed at sampling three plots each of three diversity levels and three successional stages. In the field, this design could not be fully realized and thus was not fully orthogonal. However, richness and age classes were not correlated (Chi square = 3.17, df = 4, p = 0.53), neither were richness and age as continuous variables (t = 1.25, df = 25, p=0.222, r<sup>2</sup>=0.059). Some areas of the GNNR had to be excluded for plot selection due to inaccessibility (inclination > 50°), inhomogeneity (rocks, streams, clearings) or recent anthropogenic disturbances. Forest stand age of each plot was defined by the age of the fifth-oldest tree which showed a high correlation with successional stage (r = 0.88, p < 0.001). Relying on the oldest tree individual for stand age determination would have led to overestimation because younger plots also contained several older trees left for logging before the forest protection status had been declared (Bruelheide et al. 2011). All shrub and tree species including their respective frequency and dominance were recorded as described in Bruelheide et al. (2011).

**Soil sampling and chemical analyses**

Soil total N and mineral N concentration were determined seasonally: at peak growth in summer 2008 (May to July), at the beginning of leaf shedding in autumn 2008 (October, November), at the time of bud burst in spring 2009 (March) and in winter 2010 (January, February). In all four seasonal field campaigns soil samples were taken from 5 depth increments (0 - 10, 10 - 20, 20 - 30, 30 - 40, 40 - 50 cm) using a standard soil corer (diameter of 1.3 cm, Eijkelpamp, Netherlands). Soil samples were collected in the four corners of the central subplot (10 m x 10 m) and were pooled per depth increment and plot for analysis. Soil samples were transported in a cooling box to the field laboratory and were processed within 24 h to minimize any N transformation. After sieving through a 2 mm sieve, 5 g of field moist soil was extracted for ammonium (NH<sub>4</sub><sup>+</sup>) and nitrate (NO<sub>3</sub><sup>-</sup>) with 50 mL of 1 mol L<sup>-1</sup> KCl solution. Samples were shaken for 30 min and filtered through KCl pre-rinsed ashfree filter paper (Whatman 589/1). Soil extracts were stored at -15 °C and analyzed photometrically for ammonium-N and nitrate-N using a Flow Injection Analyzer (FIAstar 500 Analyzer, FOSS, Hillerød, Denmark). Soil moisture, expressed as % water per gram dry soil, was determined gravimetrically by drying an aliquot of 5 g at 105 °C for 24 h. Total N and C concentration of air dried soil samples was measured by an Elemental Analyzer (0 - 10 cm: vario EL cube, Elementar, Germany; 10 - 50 cm: PE 2400 II CHN elemental analyser, Perkin-Elmer, Boston, MA, USA). As we expected to observe only slight seasonal changes in total soil N, only the depth increment 0 - 10 cm was analyzed for total N concentration in each season. In order to

convert concentrations into pool sizes, soil bulk density (mass of oven dried soil divided by core volume) was determined in each soil horizon with three replicated measurements using one soil profile per plot. We then computed bulk density for each depth increment assuming constant density within the horizon.

In parallel to soil sampling for ammonium and nitrate analysis, we determined net N mineralization, defined as the sum of net ammonification and net nitrification, following the in situ incubation method (Boer et al. 1993; Hart et al. 1994). Field incubation of undisturbed soil cores is regarded to reflect N transformation rates in the best way as samples remain subject to natural diurnal temperature fluxes (Arnold et al. 2008). Next to each soil sampling location a PVC tube of 7 cm diameter and 25 cm length was carefully driven into the soil to sample intact cores consisting of the litter layer and the upper 10 cm of the mineral soil. Tubes were closed with a lid to prevent leaching of inorganic N to deeper depth increments. However, aeration was possible through two holes (5 mm in diameter) in the upper parts of the tube to avoid anaerobic conditions. By using in situ cores, plant uptake of inorganic N was avoided, soil structure was maintained and microbial activity continued under natural environmental conditions. After an incubation period of 14 days we analyzed the samples for extractable ammonium and nitrate as described above. Net N mineralization rate was calculated by subtracting the pre-incubation quantities of inorganic N from the extractable amounts of inorganic N in the incubated soil cores divided by number of incubation days (Hart et al. 1994).

Soil samples for grain size analyses and pH measurements were collected systematically in each of 9 subplots of the CSPs from six depth increments (0 - 5, 5 - 10, 10 - 20, 20 - 30, 30 - 40, 40 - 50 cm) using a 5 cm diameter auger. For each depth increment, the 9 subsamples were then bulked into one composite sample per CSP. Homogenized samples were immediately hand-sieved in the field ( $\leq 2$  mm) and then air dried. Grain size analyses followed the combined sieve and pipette method (Pansu and Gautheyrou 2006) and pH was determined potentiometrically in a 1:2.5 soil-water suspension.

The litter layer (L horizon) was defined as dead loose and only slightly decomposed plant material including twigs up to 0.6 cm in diameter. A moderately to highly decomposed organic layer (Of and Oh) was inconsistently developed in all plots and therefore not included in our analyses. Four litter cores were taken seasonally (spring 2009, summer 2009, autumn 2009, winter 2010) with a PVC ring (19 cm in diameter) driven into the undisturbed litter layer at randomly selected sites within each plot. Thickness of the litter layer of each core was recorded. Additionally, we measured the thickness of the litter layer at 12 points equally distributed along the outer plot border (avoiding disturbance by plot access) to account for spatial variability of litter thickness. Dry weight of litter cores (48 h at 70 °C) pooled per plot and litter thickness were then used to

calculate litter pool sizes. To determine litter N pools, subsamples from all pooled cores ( $n = 108$ ) were taken, ground to powder and analyzed for concentration of total N and C.

Litter production was recorded using five litter traps installed in each CSP from January to December 2009. Four traps were placed in a distance of 10 m to each other around the central subplot with the fifth one positioned in the plot centre point. All 135 traps with a height of 1 m above ground and an area of 0.75 m x 0.75 m were emptied on a monthly basis. After drying at 70 °C until constant mass, plant litter was weighed excluding twigs > 0.6 cm in diameter. We then calculated turnover rate of the forest floor litter layer by dividing annual litter fall by the standing forest floor litter pool averaged over the four seasons (Olson 1963).

### **Statistical analyses**

All statistical analyses were performed using R 2.11.1 (R Development Core Team 2010). First, linear mixed effects models were applied to test for fixed effects (tree diversity, forest stand age, soil depth and season) on the response variables soil total N pool, mineral N pool, net N mineralization rate and forest floor litter N pool using the package “nlme” (Pinheiro et al. 2009). CSP identity or depth increment nested within CSP were treated as random effect terms influencing only the model’s variance. The response variables mineral N pool and net N mineralization have been  $\log_{10}$  or  $\log_{10}(x+1)$ -transformed to meet the requirements of normal distribution and homoscedasticity. Seasonal differences in response variables were examined using Tukey post-hoc tests (Hothorn et al. 2008).

Univariate relationships between variables were assessed using Pearson correlation coefficients for the top soil layer (0 - 10 cm) as well as for the complete soil column (0 -50 cm). We then applied Structural Equation Modelling (SEM) to analyze causal relationships among species richness, forest stand age, selected environmental variables and respective N pool sizes (Malaeb et al. 2000). As environmental effects are supposed to be more pronounced in the topsoil (Augusto et al. 2003), all subsequent analyses focussed on the upper depth increments (0 - 10 cm) with averaged values across seasons. Compared to traditional methods, e.g. multiple regression analysis, SEM is more effective because each structural equation coefficient is calculated while simultaneously accounting for all other variances in the model (Grace and Bollen 2005). Furthermore SEM allows differentiating between direct and indirect effects. The packages “sem” (Fox 2006) and “lavaan” (Rosseel 2011) in R were used for parameter estimation. Prior to analyses, data were standardized (mean of zero and standard deviation of one). Model fit was assessed by maximum likelihood estimation with robust standard errors and a Satorra-Bentler scaled test statistic (estimator = “MLM”), that accounted for non-normal data (Shapiro-Wilks Test for Multivariate Normality,  $p < 0.01$ ). Models differing in number of paths were compared using

Akaike's Information Criteria (AICc) corrected for sample size and simplified by stepwise reduction of paths that did not improve model fit.

## Results

### Total soil N pool

Soil total N pool size reached 5.7 Mg ha<sup>-1</sup> with a mean total N concentration of 0.12 % across all plots in the top 50 cm of the mineral soil. Among CSPs soil total N ranged from 3 to 12 Mg ha<sup>-1</sup> (Table 1). N pool and N concentration decreased strongly while bulk density increased logarithmically with soil depth (data not shown). Average total N concentration declined from 0.24 % to 0.06 % with soil depth (data not shown). N pool size was highly correlated with total N concentration in the upper 10 cm of the mineral soil ( $p < 0.001$ ,  $r = 0.75$ , data averaged across seasons) despite relatively large among-plot variation of bulk density (0.37 to 1.24 g cm<sup>-3</sup> in the upper depth increment). The upper 10 cm of the mineral soil accounted for a fraction of 35 % of total soil N whereas the lowest depth increment (40 - 50 cm) still contained 11 %. Soil total N pool was not influenced by tree diversity or forest stand age whereas the fixed effects of season and soil depth were significant (Table 2, Fig. 2). We found the largest soil total N pool in the upper depth increment in summer while the other seasons showed similar N stocks (Fig. 4a).

### Mineral N pool and net N mineralization

The soil mineral N pool amounted to 29 kg ha<sup>-1</sup> in the top 50 cm in the annual average (Table 1). The fraction of mineral N was below 1 % of total N in all depth increments. NH<sub>4</sub><sup>+</sup>-N was the dominant inorganic N form in all seasons and depth increments along the successional gradient accounting for 93 % in the upper 10 cm on average. In autumn proportion of NO<sub>3</sub><sup>-</sup>-N on total mineral N was highest with 21 % whereas in the other seasons it ranged from 2 % to 4 %. Therefore, we decided not to split mineral N into NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N but using total mineral N (NH<sub>4</sub><sup>+</sup>-N + NO<sub>3</sub><sup>-</sup>-N) as single variable in our quantitative analyses focusing on soil N stocks. Significant interactions between species richness x season, stand age x soil depth, stand age x season and N pools were found (Table 2). The relationships between species richness and mineral N pool as well as between forest stand age and mineral N pool did not follow a uniform pattern from season to season therefore leading to significant interaction terms. In contrast, the mineral N pool was significantly affected by season and soil depth. Mineral N pool was highest in summer and lower in spring and autumn (Fig. 4b). Along the soil profile mineral N decreased significantly with soil depth in spring, autumn and winter while a more homogenous distribution of inorganic N among depth increments was found in summer (data not shown).

Similar to the mineral N pool, net N mineralization was only influenced by season whereas no significant changes were found along the diversity or successional gradient (Table 3). Averaged across seasons, net N mineralization rate reached  $0.59 \text{ mg-N kg}^{-1} \text{ d}^{-1}$  with a minimum of  $-0.25 \text{ mg N kg}^{-1} \text{ d}^{-1}$  and a maximum rate of  $2.26 \text{ mg N kg}^{-1} \text{ d}^{-1}$ . Net N mineralization clearly followed temperature dynamics with highest rates in the hot-humid season and lower rates in spring and winter when soil temperature is lowest (Fig. 4d).

### **Forest floor litter N pool**

Species richness had no effect on the forest floor litter N pool size as revealed by mixed effects models (Table 3). In contrast to soil N pools, forest floor litter N stocks were significantly affected by forest stand age with N accumulating in the litter layer through succession (Table 3, Fig. 3). In each season this relationship was tested being significant and in the annual mean nitrogen accumulated by a rate of  $5.77 \text{ kg ha}^{-1}$  per decade. Seasonal dynamics of forest floor litter N pools were relatively low with the largest N pool observed in summer and the lowest occurring in spring (Fig. 4c). Average annual N pool of the litter layer varied about 3-fold ( $58.1$  to  $163.5 \text{ kg ha}^{-1}$ ) among plots (Table 4). Mean annual forest floor litter biomass fluctuated largely among plots and ranged from  $5.16 \text{ Mg ha}^{-1}$  to  $12.56 \text{ Mg ha}^{-1}$  along the successional gradient (mean:  $8.17 \text{ Mg ha}^{-1}$ ). Regarding litter layer N concentration ( $0.86 \%$  to  $1.49 \%$ ) and C:N ratio ( $32$  to  $60$ ), pronounced differences in forest floor litter quality between forest stands were observed.

### **Univariate relationships between response and environmental factors**

Multiple significant relationships between response and environmental variables were revealed using data averaged across seasons from the upper 10 cm of the soil and aggregated over all soil increments ( $0 - 50 \text{ cm}$ ; Table 5). As already shown, species richness was not related to any of the four response variables. Forest stand age and tree basal area (a surrogate for tree biomass) was positively correlated with forest floor litter N pool ( $r = 0.56$ ,  $p = 0.002$ ). Similar, plot tree basal area was positively associated with mineral N pool in the top soil layer ( $r = 0.41$ ,  $p = 0.04$ ). Among all recorded non-nitrogen related environmental parameters gravimetric soil water content was identified to be a main driver of N pool sizes in the soil and in the litter layer. Further soil related factors influencing soil total N pool were pH and the fraction of clay and silt. However, none of the recorded variables were significantly related to net N mineralization. C:N ratio of the litter layer was negatively correlated with soil total N pool ( $r = -0.44$ ,  $p = 0.02$ ), mineral N pool ( $r = -0.47$ ,  $p = 0.02$ ) and forest floor litter N pool ( $r = -0.57$ ,  $p = 0.002$ ). Elevation was the only topographic factor that was significant related to changes in the mineral N and litter N pool sizes. The three N pool sizes were positively correlated with each other.

### Structural equation models

Based on mechanistic understanding of processes influencing the soil and litter N status, we developed empirical models for each of our target variables (soil total N pool, mineral N pool, forest floor litter N pool; Fig. 5). The covariance patterns predicted by the simplified models did not differ from the observed covariance, meaning that the models fitted our observed data well (total soil N pool:  $\chi^2 = 4.7$ ,  $df = 5$ ,  $p = 0.46$ ; soil mineral pool:  $\chi^2 = 4.5$ ,  $df = 5$ ,  $p = 0.48$ ; litter N pool:  $\chi^2 = 4.5$ ,  $df = 5$ ,  $p = 0.48$ ). The path coefficient for the direct effect of species richness on N pool sizes was not significant. However, a significant path was found between species richness and soil moisture (standardized coefficient estimate = 0.28,  $p = 0.004$ ), suggesting that the number of woody plant species may indirectly influence the soil N status to a certain extent via changes in soil water status. Soil moisture was identified to affect most strongly total and mineral N pools whereas the litter N pool was equally directly influenced by soil moisture (standardized coefficient = 0.45,  $p = 0.001$ ) and forest stand age (standardized coefficient = 0.47,  $p = 0.01$ ). The content of clay and silt and elevation positively affected soil moisture and thereby indirectly N pools in the soil and litter layer. Proportion of variance explained by the models for each of the response variables soil total N pool, mineral N pool and forest floor litter N pool was 0.46, 0.35 and 0.46, respectively.

### Discussion

The determined soil N pool in Gutianshan was similar to those reported in other studies conducted in Chinese subtropical evergreen broad-leaved forests (Chen and Mulder 2007; Yang et al. 2005). For example, Zhang et al. (2010) reported soil total N pools for 0 - 10 cm of 1934 kg ha<sup>-1</sup> and 2208 kg ha<sup>-1</sup> for a young and a premature forest stand in eastern China, respectively. In general, the red earths of South-East China are among the three most important soil types for N storage in the country with highest soil N density found in forest ecosystems (Tian et al. 2006). Comparing N pool sizes within a 60 years old evergreen subtropical forest ecosystem, the mineral soil represents the largest reservoir for N (77 %) whereas the living vegetation (21 %) and the forest floor including deadwood (2 %) is of minor importance (Zhang et al. 2010). This is opposite to the relationship for carbon which is predominantly stored in plant biomass (Yang et al. 2005; Zhang et al. 2010). The observed seasonal variation in the topsoil, with a higher soil total N pool in summer, can be probably explained by rapid leaf litter and fine-root decomposition in the rainy season.

In our study, the mineral N pool was dominated by ammonium whereas nitrate occurred in smaller quantities along the diversity and successional gradient. The predominance of ammonium in acidic forest soil is a common feature reported by many studies conducted in the humid

subtropics (Maithani et al. 1998; Mo et al. 2003; Yan et al. 2008; Yan et al. 2009). We found smaller mineral N pools in spring and autumn which can be explained by high N demand by plants during leaf flush at the beginning of the growing season. In autumn, evergreen species might maintain a relatively high N demand leading to depleted soil mineral N levels while net N mineralization already declines. As a microbial process, net N mineralization is directly dependent on environmental conditions and we could observe strong seasonality with maximum values in summer. In a study by Yan et al. (2009) net N mineralization was highest in early summer but declined strongly in middle-summer when soil moisture reached its annual minimum. In a subtropical reforestation in South China, net N mineralization was highest in March and decreased throughout the year (Wang et al. 2010), and in subtropical India, net N mineralization peaked during the rainy season in summer and was lower in the cooler seasons (Maithani et al. 1998). These studies reflect the seasonal variation in net N mineralization and its dependence on climatic factors such as temperature and the soil moisture regime.

Biomass and N pool size of the standing litter layer was comparable to those reported in other studies in subtropical China. For example, forest floor N pool size of a secondary broad-leaved forest was 78 kg ha<sup>-1</sup> whereas an adjacent coniferous forest had only 35 kg ha<sup>-1</sup> (Yang et al. 2005). Observed seasonal differences in forest floor N stocks can be attributed to different monthly litter production with two annual maxima in April and November.

### **Tree diversity effects**

Tree diversity exerted no direct control on the soil total N and forest floor litter N status. The mineral N pool followed opposing seasonal trends along the diversity gradient. Based on theoretical models of species richness effects on nutrient cycling (Loreau 2001; Tilman et al. 1997), and empirical results from biodiversity experiments (Oelmann et al. 2007; Oelmann et al. 2011; Scherer-Lorenzen et al. 2003) tree diversity could be expected to have a detectable influence on soil N.

As shown by experiments in grassland systems plant diversity can affect N pools and fluxes by N use complementarity and facilitative interactions (Fornara and Tilman 2008; Hooper et al. 2005; Loreau and Hector 2001). Complementarity in resource use leads to more efficient exploitation of plant-available N and reduced leaching (Scherer-Lorenzen et al. 2003; Tilman et al. 1996). As a consequence of the system's greater N utilization and interception, aboveground N pools are higher in species rich communities (Oelmann et al. 2007; Oelmann et al. 2010) with positive feedback to N fluxes via litter fall (Scherer-Lorenzen et al. 2007). Complementarity effects promoting tree productivity including litter production might be an important mechanism regulating N pools and fluxes in Gutianshan with plot species richness ranging from 25 to 69

species (Bruehlheide et al. 2011). However, most insights into the tree diversity - productivity or N cycling relationship comes from studies conducted in forest polycultures with relatively few species involved (Kelty 2006). As reviewed by Richards et al. (2010) more than 50 % of these studies demonstrated a higher nutrient content in species aboveground biomass in mixture than compared to monoculture. There is a growing consensus on a positive relationship between tree diversity and productivity in multi-species plantations (Erskine et al. 2006; Piotta 2008) and several studies support this result also for unmanaged forests (Caspersen and Pacala 2001; Vilà et al. 2007). Furthermore, the importance of species complementarity for ecosystem functioning has been recently emphasized by a mechanistic forest model for the temperate region (Morin et al. 2011).

As a second mechanism, facilitation processes would be prevalent for example when N-fixing species are present in the community and legumes have been shown to affect strongly N pools and fluxes in species mixtures (Binkley et al. 1992; Forrester et al. 2006; Khanna 1997; Richards et al. 2010). Rothe and Binkley (2001) concluded that without N-fixing tree species no larger soil N pools in mixed stands compared to pure stands can be observed. Facilitation by N-fixing trees or effects on soil N pool sizes might be applicable for Gutianshan to a limited extent as the average plot dominance of two potential N fixing tree species (*Myrica rubra*, *Albizia kalkora*) reached 2.4 %. Beside N fixation, facilitation is mediated by other effective plant traits affecting for example microclimatic conditions, hydrological processes which in turn influence litter decomposability and hence N cycling (Chapin 2003; Prescott 2002). As a consequence, the frequency and composition of specific effective traits in a forest stand would then determine the intensity how strong site factors are changed by the vegetation.

Both, resource use complementarity and facilitation are also effective during decomposition. Synergistic effects during litter decomposition, i.e. litter mixtures decompose faster than expected from single-species rates, are often attributed to functional niche complementary and facilitative interactions (Gartner and Cardon 2004; Gessner et al. 2010; Hättenschwiler et al. 2005; Schimel and Hättenschwiler 2007). As a result of positive non-additive decomposition effects several studies have reported higher N availability in tree species mixtures than in monocultures. For example in a study by Firn et al. (2007) woody canopy species diversity affected positively soil N availability and differences in litter quality were identified as one potential explanation.

Several reasons must be considered in order to explain that neither the determined N pools nor net N mineralization could be related to tree diversity in our study. First, uncertainties in linking the determined total and mineral N pool and net N mineralization rate to plot species richness arise probably from high spatial and temporal variation of mineral N and environmental conditions in the soil (Wang et al. 2007a). As we needed several days to visit all 27 CSPs this could

have generated further uncertainty because mineral N pools and net N mineralization are temporally highly variable. Second, SEM did not indicate direct effects of species richness on soil and litter N pools whereas other factors such as soil moisture and forest stand age were identified as primary drivers of the N status. The strong between-plot heterogeneity in environmental factors even with the same successional stage might have therefore masked any direct diversity effect. The predominance of environmental factors in determining ecosystem functioning is underlined by a study of Healy et al. (2008). Here, variation in tree productivity was influenced by site conditions twice as much as by tree diversity despite relatively homogenous environmental conditions in the experimental tree plantation. In the natural evergreen broad-leaved forest with its complex topography and large environmental heterogeneity, the explainable power of tree diversity for observed N pools and fluxes might be even more reduced. And lastly, it remains questionable if clear diversity effects are still observable after species richness and functional species equivalence have reached a certain threshold. In general, effects of diversity on single ecosystem processes show strongly diminishing returns with the strongest effects at low levels of diversity. In contrast, even our most species poor plots had 25 species. However, species redundancy might substantially contribute to ecosystem stability on the long run (Díaz and Cabido 2001)

### **Stand age effects**

Soil total N and mineral N pools did not accumulate along the successional forest gradient, but were rather influenced by site-specific factors such as soil moisture. Only forest floor litter N stocks accumulated through secondary forest succession. Although basal area as an indicator of plant N pool size was highly related to forest stand age, this was not the case for soil total N pool. However, most studies suggest soil N accumulation through forest succession. For example, Zhang (2010) found that soil total N content was 23.8 % higher in a premature subtropical broad-leaved forest (60 years) compared to a nearby young stand (18 years). Such increase in soil total N pools during succession is generally explained by a closer internal N cycle in older forest stands leading to higher N retention. Therefore, N accumulates with time if the amount of N entering the ecosystem by biological fixation or atmospheric deposition exceeds N losses by leaching or denitrification (Aber 1992; Currie 1999; Odum 1969). In contrast, we demonstrated that the soil N pool is independent from forest age and that soil characteristics such as soil texture and soil moisture were more important in determining the soil N status. Especially in mountainous terrain with complex topography, soil characteristics can be highly variable due to the hydrologic drainage regime and erosion processes. The resulting differences in soil texture not only influence water-holding capacity but also N accumulation rate. Soils with high content of negatively charged

clay minerals possess a higher chemical stabilization and a larger binding capacity for organic compounds compared to more sandy soils (Chapin et al. 2002). Further heterogeneity can be generated by uprooting of trees with deeper N-poor soils being exposed to re-colonization by vegetation. Through time this processes is expected to cause severe soil mixing and the formation of patch-specific differences in the soil N status (Šamonil et al. 2010).

The mineral N pool was not affected by forest stand age but as SEM revealed directly by soil moisture. Studies on soil N dynamics along continuous successional forest gradients are rare in the subtropics. Most often, few different forest types are compared. For example, Zhang et al. (2011a) observed higher ammonium concentration in the top 20 cm of the mineral soil in a natural secondary broad-leaved forest ( $45.1 \text{ mg N kg}^{-1}$ ) compared to a nearby secondary coniferous forest ( $18.8 \text{ mg N kg}^{-1}$ ). In another study, Yan et al. (2008) found the lowest ammonium concentration in a climax evergreen broad-leaved forest but higher concentrations in other vegetation types (secondary shrubs, Chinese fir plantations, bamboo plantations, waxberry groves). In contrast, ammonium concentration showed a hump-shaped pattern along a successional series comprising five successional forests (secondary shrubs, conifer forests, mixed forest, sub-climax forest, climax forest) in subtropical China (Yan et al. 2009). In general, forest succession theory predicts lower availability of mineral N in later phases due to higher N immobilization (Odum 1969). Growing pools of coarse woody debris along the successional gradient can lead to an increasing N immobilisation and simultaneously to higher N retention. But as shown in our study, site-specific factors might be more important than successional stage in determining the soil mineral N status.

Net N mineralization was unrelated to stand age or site conditions. As mentioned above, high spatial or temporal variability of net N mineralization might be an explanation for this finding (Wang et al. 2007a). Other studies suggest that vegetation types or single species can exert a great influence on N mineralization (Knoepp and Swank 1998; Trum et al. 2011). In a subtropical reforestation for example, Wang et al. (2010) compared N mineralization between six planting treatments. They found that N mineralization was 30-60 % lower in an exotic Eucalyptus monoculture compared to a native mixture plantation and unplanted shrubland. Hence, single species can slow down internal nitrogen cycling if their leaf litter fosters N immobilization, i.e. acting as a sink for mineral N. Zhang et al. (2011b) found lower gross mineralization in coniferous than in broad-leaved forest soils across a climate gradient (tropical to temperate) in Eastern China comprising thirteen forest soils. Similar, net N mineralization was highest in a climax evergreen broad-leaved forest and lowest in a Chinese fir plantation (Yan et al. 2008). These studies underline the importance of vegetation traits, especially litter characteristics, for turnover rate of N in forest ecosystems.

N pool size of the standing litter layer increased through forest succession. Similar, the standing litter N pool rose from 59 kg ha<sup>-1</sup> in a young forest (18 years) to 121 kg ha<sup>-1</sup> in a 60 years old premature stand (Zhang et al. 2010). In general, forest floor litter N stocks are influenced by amount of litterfall, decomposition rate, and litter chemistry which are subjected to changes during stand development. Even in an old-growth forest Li et al (2010) showed that litter input still increases whereas litter quality (C:N and lignin:N ratios) decreases during a study period of 27 years. In Gutianshan, leaf litter production did not increase considerably with forest stand age or species richness and the tree canopy was already closed in early successional forest stands. Therefore it is likely that higher litter N pool sizes are mainly attributed to lower decomposition rates in older stands of the evergreen broad-leaved forest studied.

## **Conclusions**

This study has shown strong effects of abiotic environmental factors on soil N pool sizes of an evergreen broad-leaved forest in China. Our results indicate that physical soil properties (soil texture determining water-holding capacity) are more important for determining soil N pools than tree diversity or forest stand age. Contrary to our hypothesis, forest stand age was not an indicator for soil total and mineral N pool size - a result which complicates the extrapolation of soil N and probably C storage based on successional stage. However, forest floor litter became more important for N storage during forest succession. The identification of diversity effects on soil and litter N pools and fluxes at the ecosystem scale is challenging due to large environmental complexity, even in a relatively small area.

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## **Tables and Figures**

**Table 1** Soil total N and mineral N pool sizes averaged from seasonal measurements for each depth increment. Mean  $\pm$  standard errors, maximum and minimum pool sizes are shown respectively.

Layer (cm)	Total N pool (Mg ha <sup>-1</sup> )			Mineral N pool (kg ha <sup>-1</sup> )		
	Mean	Min	Max	Mean	Min	Max
0-10	2.02 $\pm$ 0.14	1.04	4.09	6.88 $\pm$ 1.08	1.43	31.48
10-20	1.36 $\pm$ 0.12	0.62	2.75	6.55 $\pm$ 0.71	1.66	17.54
20-30	1.03 $\pm$ 0.09	0.36	2.51	6.22 $\pm$ 0.67	1.96	16.16
30-40	0.78 $\pm$ 0.07	0.27	1.70	5.47 $\pm$ 0.73	1.60	19.88
40-50	0.61 $\pm$ 0.06	0.09	1.21	5.71 $\pm$ 0.70	1.82	16.59
0-50	5.73 $\pm$ 0.41	3.03	12.02	29.13 $\pm$ 2.71	10.80	67.93

**Table 2** Linear mixed effects model for fixed effects of species richness, forest stand age, soil depth and season on soil total N pool and mineral N pool sizes. 3-way interaction terms did not improve model fit and were excluded. The 2-way interaction depth x season is not applicable for the total N pool because only the depth increment (0-10 cm) was analyzed for total N in all seasons. Soil mineral N pool has been log<sub>10</sub>(x+1)-transformed.

Fixed effect	Total N pool				Mineral N pool			
	DF <sub>n</sub>	DF <sub>d</sub>	F	p	DF <sub>n</sub>	DF <sub>d</sub>	F	p
Species richness (SR)	1	23	0.997	0.329	1	23	0.078	0.783
Age	1	23	0.020	0.888	1	23	0.017	0.899
Depth	1	104	157.798	<b>&lt;0.001</b>	1	104	11.423	<b>&lt;0.001</b>
Season	3	72	12.752	<b>&lt;0.001</b>	3	390	45.402	<b>&lt;0.001</b>
SR x Age	1	23	0.945	0.341	1	23	0.718	0.406
SR x Depth	1	104	0.038	0.845	1	104	0.574	0.451
SR x Season	3	72	1.606	0.196	3	390	13.086	<b>&lt;0.001</b>
Age x Depth	1	104	1.347	0.249	1	104	4.316	<b>0.040</b>
Age x Season	3	72	0.693	0.559	3	390	4.128	<b>0.007</b>
Depth x Season	-	-	-	-	3	390	0.571	0.634

**Table 3** Linear mixed effects model for fixed effects of species richness, forest stand age and season on net N mineralization rate and forest floor litter N pool. Net N mineralization has been  $\log_{10}(x+1)$ -transformed. The 3-way interaction term did not improve model fit and was removed.

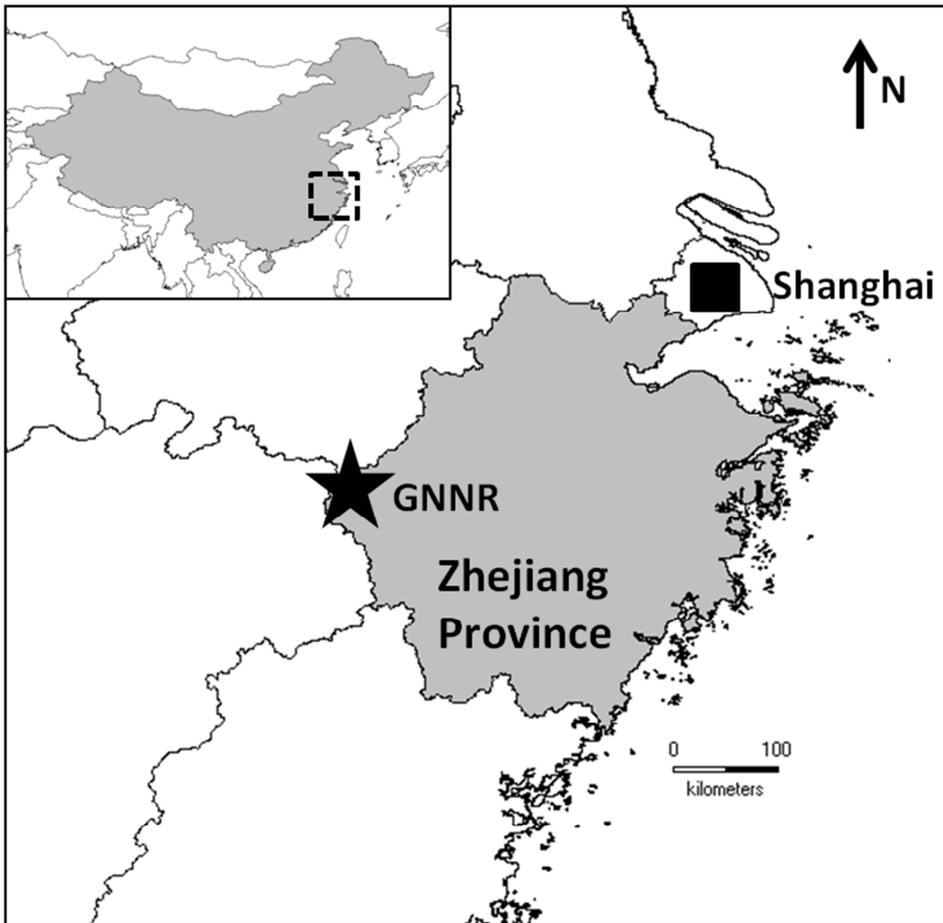
Fixed effect	Net N mineralization				Forest floor litter N pool			
	DF <sub>n</sub>	DF <sub>d</sub>	F	p	DF <sub>n</sub>	DF <sub>d</sub>	F	p
Species richness (SR)	1	23	0.624	0.438	1	23	1.631	0.214
Age	1	23	0.529	0.474	1	23	9.728	<b>0.005</b>
Season	3	72	10.841	<b>&lt;0.001</b>	3	72	5.688	<b>0.001</b>
SR x Age	1	23	0.700	0.411	1	23	0.306	0.585
SR x Season	3	72	0.860	0.466	3	72	0.110	0.954
Age x Season	3	72	0.157	0.925	3	72	1.638	0.188

**Table 4** Forest floor litter properties averaged across all studied forest stands (n=27) and seasons. Mean ( $\pm$ SE), minimum and maximum values are presented.

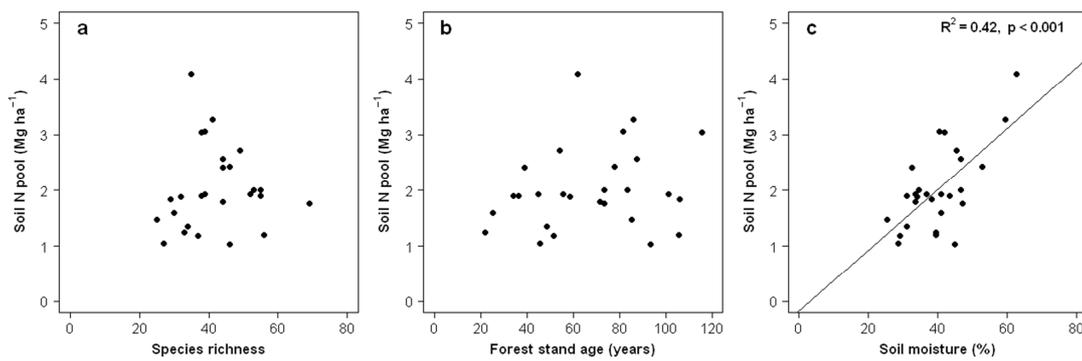
	mean	min	max
Biomass (kg ha <sup>-1</sup> )	8166 $\pm$ 334	5160	12560
N pool (kg ha <sup>-1</sup> )	92.67 $\pm$ 5.16	58.14	163.50
N (%)	1.13 $\pm$ 0.03	0.86	1.49
C (%)	48.69 $\pm$ 0.22	47.22	51.39
C:N ratio	44.75 $\pm$ 1.27	32.30	59.75

**Table 5** Univariate relationships between response variables (soil total N pool, mineral N pool, forest floor litter N pool, net N mineralization) and environmental predictors. Pearson correlation coefficient and p-value are given (n=27). For soil total N pool (0 – 50 cm) and mineral N pool (0 – 50 cm): n = 26 because sampling was not possible in the deepest soil layer (40 – 50 cm) in CSP 15. One outlier was removed in the mineral N pool dataset (n=26).

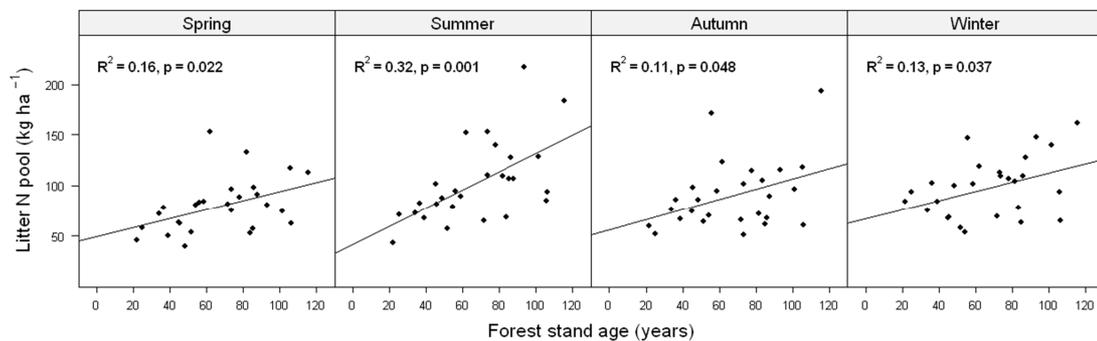
	Soil total N pool (0 - 10 cm)		Soil total N pool (0 - 50 cm)		Mineral N pool (0 - 10 cm)		Mineral N pool (0 - 50 cm)		Litter N pool		Net N mineralization (0 - 10 cm)	
	r	p	r	p	r	p	r	p	r	p	r	p
<b>Vegetation</b>												
Species richness	0.051	0.802	0.222	0.276	0.026	0.900	-0.185	0.365	0.215	0.283	0.191	0.340
Stand age	0.209	0.296	0.030	0.882	0.120	0.558	-0.233	0.253	0.561	<b>0.002</b>	0.284	0.151
Basalarea	0.347	0.076	0.062	0.765	0.408	<b>0.038</b>	-0.037	0.856	0.624	<b>&lt;0.001</b>	0.211	0.291
<b>Mineral soil</b>												
Soil total N pool	-	-	-	-	0.593	<b>0.001</b>	0.357	0.073	0.438	<b>0.022</b>	0.184	0.359
Soil mineral N pool	0.593	<b>0.001</b>	0.357	0.073	-	-	-	-	0.472	<b>0.015</b>	0.104	0.612
Net N mineralization	0.184	0.359	0.285	0.159	0.104	0.612	-0.213	0.296	0.246	0.215	-	-
Soil moisture	0.667	<b>&lt;0.001</b>	0.671	<b>&lt;0.001</b>	0.575	<b>0.002</b>	0.244	0.229	0.540	<b>0.004</b>	0.238	0.231
C:N ratio	-0.241	0.225	-0.268	0.186	-0.140	0.496	-0.192	0.348	0.009	0.964	-0.304	0.123
Clay+Silt	0.494	<b>0.009</b>	0.417	<b>0.034</b>	0.347	0.083	0.070	0.734	0.248	0.211	0.118	0.556
pH	-0.529	<b>0.005</b>	-0.087	0.672	-0.357	0.074	0.047	0.819	-0.523	<b>0.005</b>	-0.262	0.186
<b>Forest floor litter</b>												
Litter N pool	0.438	<b>0.022</b>	0.312	0.120	0.472	<b>0.015</b>	0.109	0.597	-	-	0.246	0.215
Litter turnover	-0.192	0.336	-0.109	0.598	-0.107	0.601	0.176	0.389	-0.405	<b>0.036</b>	0.031	0.879
C:N ratio	-0.441	<b>0.021</b>	-0.049	<b>0.011</b>	-0.466	<b>0.016</b>	-0.248	0.222	-0.573	<b>0.002</b>	-0.046	0.821
Litter production	0.023	0.091	-0.051	0.804	0.097	0.639	0.123	0.549	0.327	0.095	0.302	0.126
<b>Topography</b>												
Elevation	0.314	0.111	-0.100	0.627	0.445	<b>0.023</b>	-0.103	0.615	0.461	<b>0.016</b>	0.034	0.865
Inclination	0.235	0.237	0.366	0.066	0.147	0.478	0.146	0.476	-0.038	0.853	-0.027	0.893
Aspect east	-0.120	0.552	-0.093	0.653	-0.112	0.587	0.129	0.531	-0.247	0.215	-0.032	0.875
Aspect north	-0.129	0.523	0.056	0.786	0.108	0.599	0.211	0.300	-0.254	0.200	-0.266	0.181



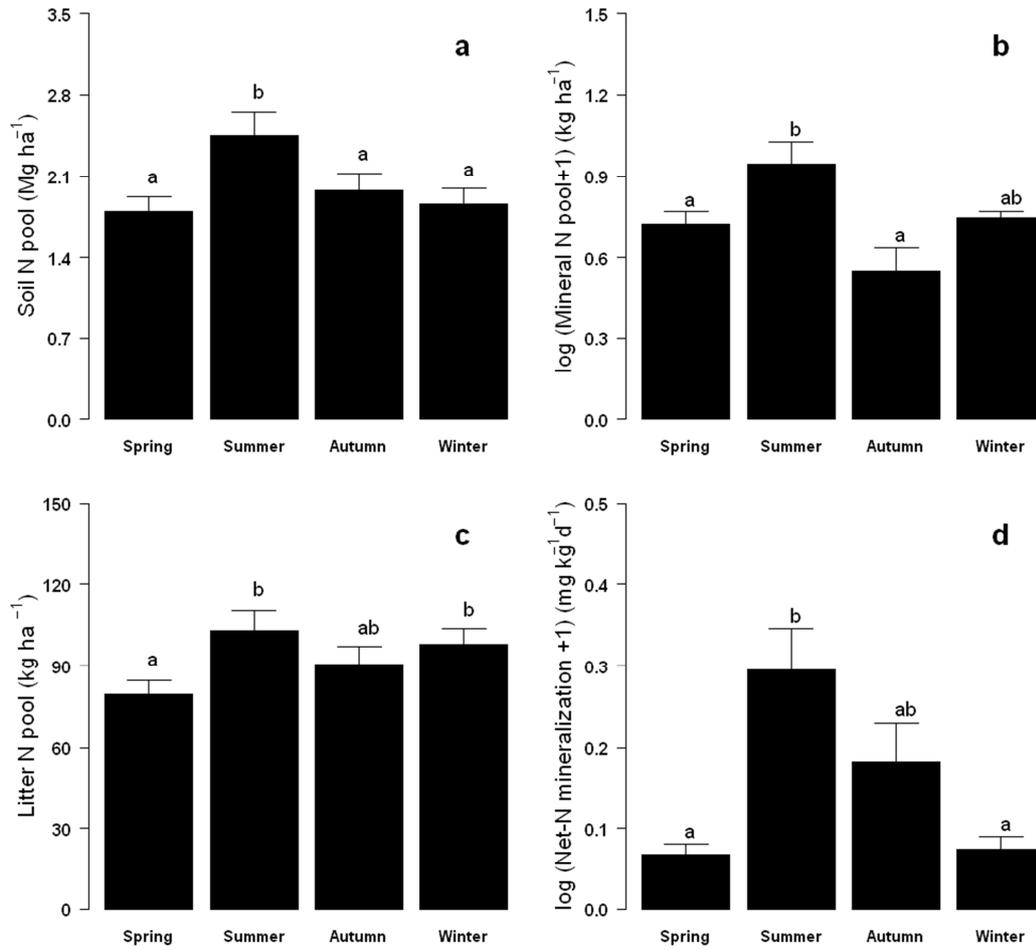
**Fig. 1** Location of the study area Gutianshan National Nature Reserve (GNNR) in western Zhejiang Province, South-East China.



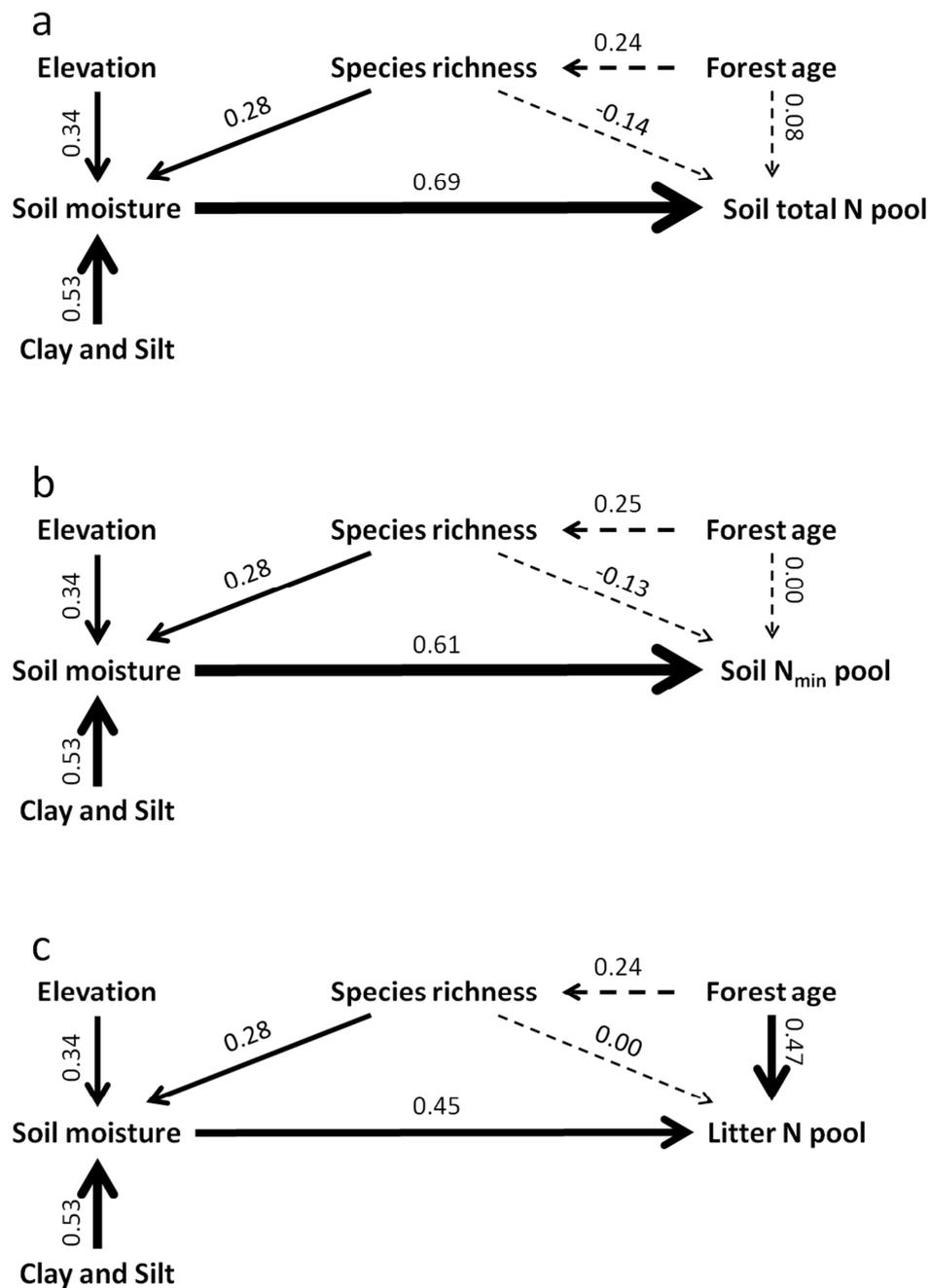
**Fig. 2** Soil total N pool as a function of woody plant species richness (a), forest stand age (b) and gravimetric soil water content (c). Data represent the top 10 cm of mineral soil and were averaged across the four seasons.



**Fig. 3** Relationship between forest floor litter N pool and forest stand age in each season. Adjusted  $R^2$  and significance levels are given.



**Fig. 4** Seasonal variation of the total (a) and mineral N pool (b), forest floor litter N pool (c) and net N mineralization (d) averaged across all 27 CSPs, respectively. Means ( $\pm$ SE) are presented for top depth increment (0-10 cm). Different letters indicate significant differences between seasons.



**Fig. 5** Structural equation models to test for specific hypotheses on causal relationships among biotic (species richness, forest stand age), abiotic (elevation, fraction of clay and silt, elevation) and soil N pool (a), mineral N pool (b) and standing litter N pool (c), respectively. Solid arrows indicate significant ( $p < 0.05$ ) whereas dashed lines represent non significant paths. Increasing line width specifies strength of causal relationship between variables. Positive and negative values are standardized coefficient estimates and indicate either a positive or a negative unidirectional relationship. One outlier was removed in the mineral N pool dataset.



# CHAPTER 2

Manuscript

## **Impact of tree diversity, successional stage, and environmental factors on leaf litter decomposition in an evergreen broad-leaved forest in subtropical China**

Stefan Trogisch<sup>1,2</sup>, Jin-Sheng He<sup>3</sup>, Andy Hector<sup>4</sup> & Michael Scherer-Lorenzen<sup>2</sup>

<sup>1</sup> Institute of Agricultural Sciences, ETH Zurich, Switzerland

<sup>2</sup> Institute of Biology II, Geobotany, University of Freiburg, Germany

<sup>3</sup> Department of Ecology, Peking University, Beijing, China

<sup>4</sup> Institute of Evolutionary Biology and Environmental Studies, University of Zurich, Switzerland

## Abstract

Litter decomposition replenishes the pool of essential nutrients for plant biomass production and is therefore a major process of ecosystem functioning. Tree diversity might influence decomposition processes either by changing environmental conditions or by the occurrence of interactive litter mixture effects. Using the litterbag-technique, we studied leaf litter decomposition along a tree diversity and successional gradient in a Chinese subtropical forest ecosystem. Three complementary decomposition trials were performed. First, the decomposition constant of 26 tree species was determined under homogenous physical and biological site conditions. Second, decomposition rate of leaf litter derived by a common species *Schima superba* was related to environmental factors changing along the diversity and successional gradient. And third, the influence of litter bag species richness on decomposition of 27 plot-specific litter mixtures comprising 7 to 17 species was assessed. Decomposition constants of single litter species ranged from 0.23 to 0.95 yr<sup>-1</sup> and were negatively related to initial C:N ratio. N concentration significantly increased in 16 species during the experiment. Decomposition rate of *Schima superba* leaf litter decreased along the successional gradient but was not related to standing tree diversity. Two thirds of plot-specific litter mixtures showed a positive non-additive mixture effect whose strength was marginally positively influenced by litter bag species richness. Coniferous litter decomposed significantly faster when mixed with broad-leaved litter. Our results indicate that litter species differ greatly in decomposition dynamics with the initial C:N ratio explaining only a small fraction of this variation. Declining decomposition rates along the successional gradient support the scenario that nutrient cycling slows down during forest maturation. Furthermore our study suggests that synergistic effects are predominant in multi-species litter mixtures in the first year of litter decomposition.

**Keywords:** BEF-China, Gutianshan National Nature Reserve, forest biodiversity, litter quality, non-additive mixture effects, nitrogen dynamics, secondary forest succession, subtropical broad-leaved forest

## Introduction

Litter decomposition is an integral component of the global cycles of nitrogen, phosphorous and carbon and connects above- and belowground biotic processes. Most of terrestrial net primary production enters decomposition as plant litter. During the process of decomposition and subsequent mineralization, organic matter breaks down into CO<sub>2</sub>, inorganic nutrients and a more recalcitrant humus fraction as a result of physical, chemical and biological degradation. Thus, litter decomposition replenishes the pools of essential mineral nutrients, especially nitrogen and phosphorous, for net primary production and creates soil organic matter (Swift et al. 1979).

Much research has focused on the quantification of the most important determinants of decomposition rate, which are the physical environment (e.g. moisture, temperature), physicochemical features of the leaf litter (e.g. leaf toughness, C:N ratio, phenolic compounds) and composition and functional complexity of the decomposer community (Coûteaux et al. 1995; Melillo et al. 1982; Swift et al. 1979). Despite several decades of litter decomposition research (Falconer et al. 1933; Gustafson 1943) and first application of litter bags by Bock and Gilbert (1957), the biodiversity-decomposition relationship has been more intensively assessed since about 20 years (Blair et al. 1990; Gartner and Cardon 2004; Gessner et al. 2010; Knops et al. 2001; Srivastava et al. 2009). As different litter species do not decompose isolated but rather interacting with each other mediated by microorganisms and the physical environment, decomposition dynamics of litter mixtures cannot always be predicted from single-species decay rates (Gartner and Cardon 2004; Hättenschwiler et al. 2005). These non-additive litter diversity effects, i.e. the litter mixture shows a different decomposition rate than predicted from individual rates of involved species, have been attributed to several mechanisms as reviewed by Hättenschwiler et al. (2005): (1) translocation of nutrients from high-quality to low quality litter, (2) influence of litter-specific chemical compounds on detritivore activity, (3) changes in microclimatic conditions due to specific structural litter traits and (4) higher habitat diversity for decomposer species showing functional complementary in degradation abilities in litter mixtures. As reviewed by Gardner and Cardon (2004), in 67 % of litter mixtures tested, non-additive effects for litter mass loss were revealed with the majority being synergistic rather than antagonistic. In 76 % of litter mixtures nutrient dynamics could not be predicted from single species decomposition patterns. Most of the studies (60 %) have been conducted in temperate forest ecosystems, thus generalization must be interpreted with caution (Hättenschwiler et al. 2005). Another caveat is the often low number of species involved which does not reflect natural conditions only to a limited extent, especially for forest ecosystems at lower latitudes with high species richness and large variety of functional litter types. Therefore, more data are needed from various ecosystems to understand the impact of litter diversity on decomposition processes.

Another challenge is to disentangle the interactions between biodiversity and environmental heterogeneity on single ecosystem functions (Balvanera et al. 2006; Loreau and Hector 2001; Solan et al. 2009) such as decay of plant litter and nutrient cycling. In natural forests varying in tree diversity, species composition and successional stage, the strength of non-additive mixture effects might therefore depend to a large extent on environmental factors like microclimatic conditions, edaphic factors or the presence and abundance of particular decomposer organisms. Broadening our understanding of biotic controls on ecosystem functioning derived from rather environmentally homogenous study plots to the more complex ecosystem and landscape level is the next step to integrate ecosystem functions and services on a larger spatial scale (Balvanera et al. 2006).

Subtropical evergreen broad-leaved forest (EBLF) in Southeast China is highly diverse in terms of woody plant species (Bruehlheide et al. 2011; Legendre et al. 2009). As this forest type is stocking on the most productive soils of the country, the majority of EBLF has been converted into agricultural land (Richardson 1990). In the last decade, forest area has been increasing in China but largely due to the establishment of short-rotation tree plantations planted often as coniferous monocultures aiming to satisfy increasing timber demand (Zhang et al. 2000). Thereby, other important ecosystem services such as maintenance of site fertility and carbon sequestration are not specifically accounted for. Combining tree species differing in functional litter traits might have synergistic effects on nutrient cycling and soil fertility (Kelty 2006) and could reduce soil degradation and yield decline over time. Hence, knowledge about litter mixture effects also has a large applied potential for the consideration of multiple ecosystem services in tree plantations (Bauhus et al. 2010).

Using the litterbag-technique we studied leaf litter decomposition and N dynamics during decomposition in 27 subtropical forest stands varying in tree species richness and successional stage. We conducted three complementary decomposition trials to test the following hypotheses: (i) Coexisting tree species show large differences in mass loss and N dynamics during leaf litter decomposition due to species-specific litter traits. Here, decomposition rates of foliar litter derived from 26 sub-canopy and canopy tree species were determined under the same environmental conditions, thus focusing on species-identity effects on decomposition processes. (ii) Plot tree species richness and forest stand age positively affect leaf litter decomposition. Beneficial effects can result from altered decomposer diversity or modified microclimatic conditions. Therefore, effects of forest stand diversity and habitat heterogeneity were examined by decomposing leaf litter from the abundant tree species *Schima superba* as a common substrate in each plot. (iii) High litter diversity induces predominantly positive non-additive mixture effects on decomposition rates. To test for such diversity effects, decomposition of forest stand-specific

litter mixtures varying in species richness and composition was studied in the respective forest stands.

## Materials and Methods

### Study site

This study was conducted in the Gutianshan National Nature Reserve (GNNR) located in the mountainous area of western Zhejiang Province, East China (29°8'18"–29°17'29" N, 118°2'14"–118°11'12" E). The region encounters a warm-temperate climate with distinct seasonality: a hot-humid season from April to September and a cool-dry season from October to March. Monthly mean temperature is lowest in January (4.3 °C) and highest in July (27.9 °C). Annual mean temperature amounts to 15.3 °C and annual precipitation sums to 1964 mm of which most occurs between March and September (Hu and Yu 2008). The geological bedrock is comprised of granite and gneiss. Sandy-loamy and silty-loamy textured acidic Cambisol with pH ranging from 4 to 5 is the predominant soil type. The vegetation is classified as evergreen broad-leaved forest (EBLF) which is characterized by a high diversity of woody plant species (Wang et al. 2007b). Within the GNNR 1426 species of seed plants belonging to 648 genera and 149 plant families have been documented (Lou and Jin 2000). Species most frequently represented in the upper canopy are the evergreen broad-leaved species *Castanopsis eyrei* (Champ. ex Benth) Tutch. (Fagaceae) and *Schima superba* Gardn. et Champ. (Theaceae). Further site characteristics and species lists are provided by Bruelheide et al. (2011) and Legendre et al. (2009).

### Study design and environmental predictors

We established 27 plots (30 m x 30 m), hereafter named “comparative study plots” (CSPs), differing in woody plant diversity (25 – 69 species) and forest stand age (22 - 116 yrs) in the GNNR (see Bruelheide et al. 2011 and Schuldt et al. 2011). Our original design aimed at sampling three plots each of three diversity levels and three successional stages. In the field, orthogonality could not be fully realized because young successional stages did not reach tree species richness recorded in older forest stands. However, richness and age classes were not correlated ( $r = 0.25$ ), neither were richness and age as continuous variables ( $r = 0.24$ ). Based on tree ring analysis, CSPs were reclassified into 5 different successional stages (1, < 20 yr; 2, < 40 yr; 3, < 60 yr; 4, < 80 yr; 5,  $\geq 80$  yr) with five, four, five, six and seven replicates, respectively. The age of the fifth largest tree individual was taken as a measure of forest stand age (Bruelheide et al. 2011). Areas of the GNNR showing stand heterogeneity (rocks, streams, clearings) or inaccessibility (inclination > 55°) were excluded for plot selection. CSPs were marked permanently and all woody species and their respective frequency and dominance were recorded as described by Bruelheide et al. (2011). In

addition to total plot species richness, the Shannon index and species richness per 100 tree individuals (rarefied species richness) were used to study the tree diversity - decomposition relationship. Besides litter production, we measured seasonally the characteristics of the forest floor litter layer (N and C concentration, C:N ratio, biomass and N stocks) as well as mineral soil related parameters (e.g. N and C concentration, gravimetric soil water content) in the topsoil layer (0 - 10 cm). Seasonally recorded variables were averaged per plot over all dates. Litter turnover, the quotient of annual litter production and standing forest floor litter pool (Olson 1963) was compared with decomposition constants determined with litter bags. Furthermore, the leaf area index (LAI) was optically measured by digital hemispheric photography at 1.3 m height on all plots in summer 2009 and analyzed with Hemisfer (Schleppi et al. 2007).

### **Litter collection and preparation of litter bags**

In each CSP, five litter traps (135 in total) made of plastic tubes and nylon mesh (mesh wide = 1 mm) were set up in autumn 2008. Litter traps were 1 m in height and comprised an area of 0.75 m x 0.75 m, respectively. Four litter traps were arranged around the central subplot (10 m x 10 m) in equal distance to each other with the fifth one erected in the plot centre point of each CSP. Monthly collected leaf litter was dried at 60 °C and separated into species. In order to increase the amount of leaf litter and species number, we collected additional freshly fallen litter from the forest floor in spring 2009. Two annual maxima of litter production of equal magnitude do occur in Gutianshan (Yinlei Ma, personal communication). Besides increased litter production in late autumn many evergreen species shed their older leaves at the time of bud burst and leaf flush in spring.

Litter bags (15 cm x 15 cm) were made of nylon with 1 mm mesh size and filled with 5 g of dried leaf litter. After carefully removing loose forest floor litter, bags were slightly anchored in 5 cm spacing at the ground with 10 cm long metal stakes puncturing the hem in spring 2009. Because an organic humus layer (Oh) was often absent, litter bags were placed directly on the mineral soil in most cases. Gaps between litter bags were refilled with the removed forest floor litter. Replicated litter bags were retrieved in six time steps after 92, 146, 205, 272, 333 and 383 days of decomposition in the field. Because CSPs were spread over the entire GNNR, litter bag retrieval took about seven days to finish. Thus, the given time intervals represent the mean number of days calculated for each sampling period after the start of the experiment. After retrieval, remaining litter was carefully cleaned from adherent mineral soil, ingrown roots and fecal matter, dried at 60 °C until constant mass and weighed after cooling down. We used three complementary approaches to study leaf litter decomposition:

**(i) Decomposition of single-species leaf litter**

We determined litter decomposition rate of 26 sub-canopy and canopy tree species occurring in the CSPs under the same microclimatic and edaphic conditions. For each species, 12 litter bags were filled with dried leaf litter, and two replicated bags per species were retrieved at each time step. Owing to the large coverage (several square meters of forest floor) by litter bags, we decided not to conduct the experiment within a selected CSP but rather outside on undisturbed forest floor providing enough space. Hence, litter bags were placed on the ground a few metres next to one plot (CSP 9) in two blocks containing one replicated bag for each time step, respectively. Random arrangement of the 156 bags in each block ensured that species-specific decomposition rates were not affected by spatial variation of microclimatic conditions.

**(ii) Decomposition of *Schima superba* leaf litter**

Leaf litter collected from one of the most dominant and abundant tree species (*Schima superba*) was used to study plot-specific litter decomposition along the tree diversity and successional forest gradient using a total number of 324 litter bags. 12 bags filled with *Schima superba* leaf litter were placed in every plot with 2 bags to be sampled at each of the 6 retrieval events. Bags were randomly positioned within an area of 1 m by 2 m in the central subplot of the corresponding CSP.

**(iii) Decomposition of mixed-species leaf litter**

In the litter mixture approach, the number of litter species per bag equalled the number of species found in the different CSPs based on 24 species for which sufficient quantities of litter could be collected. Hence, species number in plot-specific litter mixtures ranged from 7 to 17 with 0.294 g to 0.714 g of leaf litter per species contained in each bag, respectively (Supplementary material Appendix 1, Table A1). Litter bag species richness was significantly related to plot species richness ( $R^2 = 0.64$ ,  $p < 0.001$ ; Supplementary material Appendix 1, Fig. A1). For each plot, 12 bags filled with a plot-specific mixture were prepared and placed next to the *Schima superba* litter bags in each CSP covering together an area of about 2 m<sup>2</sup>. After retrieval, species separation was not possible due to the large number of species involved and fragmentation during decomposition. However, we differentiated between broad-leaved and coniferous litter (*Pinus massoniana*, *Pinus taiwanensis* and *Cunninghamia lanceolata*) and measured separately remaining dry mass for the two functional litter types.

**Chemical analysis**

Initial carbon and nitrogen concentration was measured for all litter species with an Elemental Analyzer (PE 2400 II CHN Elemental Analyser, Perkin-Elmer, Boston, MA, USA). These data were also used to calculate the initial carbon and nitrogen concentration of mixed-species leaf litter. To

follow nitrogen dynamics during decomposition, the remaining content of all 960 litter bags were analyzed for carbon and nitrogen concentration.

### Calculations and statistical analysis

All analyses were performed using R 2.11.1 (R Development Core Team 2010). We calculated the annual exponential decay coefficient  $k$  (decomposition constant) for each of the 80 decomposition series (26 mono-specific, 27 *Schima superba* and 27 mixed-species series) based on the single exponential decomposition model by Olson (1963) where  $X$  is the proportion of litter mass remaining at time  $t$ ;  $k$  is the exponential decay coefficient (decomposition constant), and  $t$  is the time in days (1).

$$X = 100e^{-kt} \quad (1)$$

This model reflects well the observed exponential decline of litter mass with time (see Results section below): high mass loss during the initial phase of leaf litter decomposition when soluble compounds are removed easily by leaching to a more retarded decay of accumulating recalcitrant substances. The non-linear model was fitted via least-squares estimation with 100 % of litter present at time = 0. We used decay coefficients of the 26 species determined at CSP 9 to calculate the predicted decay coefficient of all 27 plot-specific litter mixtures comprising 7 to 17 species. As litter species did occur with identical initial mass, the average of species-specific decay coefficients ( $k$ ) of all species present in the litter mix ( $n$ ) equals the mixture's predicted decay coefficient  $k_{\text{predicted}}$  (2).

$$k_{\text{predicted}} = \frac{1}{n} \sum_{i=1}^n k_i \quad (2)$$

In order to account for differences in environmental conditions between plots, the observed decay coefficients ( $k_{\text{observed CSPX}}$ ) of the 27 litter mixtures were standardized ( $k_{\text{standardized observed}}$ ) using the plot-specific decay constants of *Schima superba* leaf litter (3).

$$k_{\text{standardized observed}} = k_{\text{observed CSPX}} \times \frac{k_{\text{Schima CSP9}}}{k_{\text{Schima CSPX}}} \quad (3)$$

By this means the effect of litter species richness and identity on decomposition dynamics could be separated from other environmental factors. A positive difference ( $\Delta k > 0$ ) between standardized observed and predicted  $k$ -values of a mixture indicates a synergistic litter mixture

effect. In contrast, if the standardized observed decay coefficient is lower than predicted ( $\Delta k < 0$ ), an antagonistic non-additive effect would be prevalent. Accordingly, equation 2 and 3 were also applied to calculate predicted and standardized observed values for remaining litter mass and remaining N at each retrieval date. In the mass approach, a synergistic mixture effect, i.e. higher mass loss than predicted, was defined as a positive difference between predicted remaining mass and standardized observed remaining mass.

To test for significant changes in N concentration during decomposition we performed linear regression analyses with the regression line fitted through the initial N concentration at time = 0. In order to relate plot-specific decomposition rates of *Schima superba* litter to environmental factors Pearson's correlation was used to reveal significant single predictor variables.

## Results

### Decomposition of single-species leaf litter

Observed mass loss over time was in accordance with the applied single exponential decomposition model (mean  $R^2 = 0.82$ ). Decomposition constant was lowest for coniferous tree species (*Pinus massoniana*, *Pinus taiwanensis*, *Cunninghamia lanceolata*) and one ericaous species (*Rhododendron latouchea*). Highest decomposition constants were observed for the sub-canopy species *Loropetalum chinensis* and the deciduous canopy tree species *Castanea henryi* (Table 1, Fig. 1). Mass loss after 383 days of decomposition ranged from 21.6 % (*Pinus massoniana*) to 63.9 % (*Castanea henryi*). Accordingly, half life time of leaf litter ranged from 0.7 to 3 years and duration until 95 % of litter mass loss was calculated as 3.2 to 13 years.

Initial C:N ratio of the litter varied from 27 (*Castanopsis tibetana*) to 97 (*Rhododendron latoucheae*) and affected negatively decomposition rate ( $R^2 = 0.27$ ,  $p = 0.006$ , Fig 2). Initial litter N concentration ranged from 0.52 % (*Rhododendron latoucheae*) to 1.82 % (*Castanopsis tibetana*) and was positively related to decomposition rate ( $R^2 = 0.24$ ,  $p = 0.01$ ). During decomposition a significant increase in N concentration was observed for 16 species and followed often a linear N accumulation pattern (Table 1, Fig. 3). The slope of the linear model was highest for leaf litter of the evergreen species *Daphniphyllum oldhammii* showing an increase in N concentration from initial 0.92 % to 1.97 % at the end of the experiment.

### Decomposition of *Schima superba* leaf litter along environmental gradients

Decomposition constant of *Schima superba* leaf litter varied by factor 2 (minimum:  $0.32 \text{ yr}^{-1}$ , maximum:  $0.67 \text{ yr}^{-1}$ ) among the 27 plots. Mass loss ranged from 27.5 % to 51.3 % after 383 days of decomposition. The two decomposition series of *Schima superba* with one conducted inside and the second one in two blocks arranged outside of CSP 9 yielded nearly identical k-values:

0.547 yr<sup>-1</sup> and 0.545 yr<sup>-1</sup>, respectively. When all 27 CSPs were combined, decomposition constant for *Schima superba* was calculated as 0.45 yr<sup>-1</sup> ( $R^2 = 0.77$ ,  $p < 0.001$ ) and N concentration increased with time of decomposition ( $R^2 = 0.64$ ,  $p < 0.001$ ).

None of the three measures of plot tree diversity (species richness, rarefied species richness, Shannon index) was related to decomposition rate in the common substrate approach. In contrast, forest stand age, successional stage and tree basal area were all negatively correlated with *Schima superba* plot specific k-values (Table 2, Fig. 4). The relationship was found to be strongest for tree basal area ( $r = -0.6$ ,  $p < 0.001$ ) which was in turn highly correlated with forest stand age ( $r = 0.8$ ,  $p < 0.001$ ). Forest stands showing high decomposition of *Schima superba* litter had a lower forest floor litter biomass ( $r = -0.4$ ,  $p = 0.03$ ) but no significant lower forest floor litter N pool size ( $r = -0.2$ ,  $p = 0.14$ ). However, plot-specific differences in quality of the forest floor litter layer expressed as C:N ratio and N concentration did not affect litter decomposition of the common *Schima superba* litter substrate. Calculated litter turnover (the ratio of annual litter production divided by the average standing forest floor litter biomass) was positively related with plot-specific decomposition rates determined with the litterbag-technique ( $r = 0.6$ ,  $p = 0.002$ ). Recorded topsoil characteristics (0 - 10 cm) were unrelated to determined decomposition rates.

### **Decomposition of mixed-species leaf litter**

Standardized observed and predicted decomposition rate of the plot-specific mixtures ranged from 0.43 yr<sup>-1</sup> to 0.92 yr<sup>-1</sup> and from 0.42 yr<sup>-1</sup> to 0.66 yr<sup>-1</sup>, respectively. Standardized observed decomposition rate was significantly affected by initial litter N concentration ( $R^2 = 0.22$ ,  $p = 0.008$ ) and C:N ratio ( $R^2 = 0.16$ ,  $p = 0.02$ ). Although the fraction of coniferous litter did not exceed 30 %, initial C:N ratio was positively affected by percentage of needle-leaved litter in the mixture ( $R^2 = 0.31$ ,  $p = 0.001$ ). A higher standardized observed than predicted decomposition rate was shown by two thirds of all mixtures (Fig 5). Mean  $\Delta k$  values (difference between standardized observed and predicted k values) of positive effects ( $n = 18$ ) and antagonistic ( $n = 9$ ) effects reached 0.085 and -0.073, respectively. However, strengths of synergistic and antagonistic effects were balanced. The influence of litter bag species richness on standardized litter decomposition was weak but significant (Fig. 6B). After removing one litter mixture showing a particular high decomposition rate ( $k = 0.92$ ), species richness still explained 13 % of variation ( $p = 0.04$ ). Furthermore we found that more species-rich litter mixtures had slightly higher predicted k-values, suggesting that the fraction of more rapidly decomposing litter species was increasing (Fig. 6A). Nevertheless, the positive relationship between  $\Delta k$ , indicating the strength of the litter mixture effect, and litter bag species richness was marginally significant ( $p = 0.061$ ; Fig. 6C).

Standardized observed decomposition constant of coniferous litter as well as conifer  $\Delta k$  values were not related to litter bag species richness (Supplementary material, Appendix 1, Fig. A2). However, in general the decomposition constant was higher for coniferous litter decomposing in mixture with broad-leaved species than in monospecific litter bags. Based on all litter mixtures containing litter of conifers ( $n = 24$ ), the decomposition constant of coniferous litter increased by 67 % (standardized observed  $k$ : 0.516, predicted  $k$ : 0.309).

Considering mass loss separately at each litter bag retrieval date, we found that deviation from predicted remaining litter mass and litter bag species richness was unrelated after 92 and 146 days of decomposition (Fig. 7). After 205 days, litter bag species richness positively influenced deviation from predicted remaining mass and this trend was maintained until the end of the experiment although not anymore significant. Regarding remaining N, the difference between predicted and standardized observed remaining N was not uniformly influenced by litter bag species richness at the six litter bag retrieval dates, respectively (Supplementary material Appendix 1, Fig. A3). However, the majority of litter mixtures showed higher N release than predicted.

## Discussion

### Decomposition of single-species leaf litter

In line with our first hypothesis, we found pronounced differences in litter mass loss and N dynamics among the 26 tree species. Decomposition constants ranged below  $1 \text{ yr}^{-1}$  and were similar to  $k$ -values reported in other studies conducted in the subtropical forest zone (Guo et al. 2009; Xu and Hirata 2005). The single exponential model of litter mass loss fitted well our observed data with mean  $R^2$  across species of 0.82. However, the mesh size used here (1 mm) could not completely prevent the intrusion of litter-feeding macrofauna such as caterpillars at an early larval stage and termites to a subset of our litter bags. The resulting higher variation in mass loss between replicated bags decreased therefore the model fit to some extent. Mesh size is an important factor when comparing decomposition rates among studies. For example, Huang et al. (2007) used litter bags made of 2 mm mesh in a comparable subtropical forest and calculated significantly higher  $k$ -values. Typically, remaining litter mass is higher in fine than in coarse mesh bags with the latter ones being more accessible to macro-detrivores (Mayer 2008; Yang and Chen 2009). It has been argued that the litterbag-technique is not an effective tool to model decomposition processes as the influence of soil fauna is mostly disregarded (Prescott 2005). This is true as especially soil fauna can exert a strong control on decomposition mainly by transformation processes such as consumption or fragmentation and thereby facilitating access for microorganisms to plant litter (Hättenschwiler et al. 2011). However, differences in

decomposition rates among species attributed to specific litter traits can be properly revealed using litter bags.

The decomposition rates of the 26 studied species varied by factor 4 which proves the expected high variability, based on large chemical and morphological differences among litter species. Initial litter quality (C:N ratio, N concentration) significantly affected decomposition rate but the explained variation was rather low. Other studies have described a strong relationship between initial litter quality and decomposition rate (Aerts 1997; Melillo et al. 1982; Taylor et al. 1989; Vitousek et al. 1994). The reason for the low explainable power of our litter quality data might be that decomposition is simultaneously affected by multiple factors such as lignin:N ratio, phosphorous concentration, leaf toughness or content of inhibitory phenolic compounds (Berg and McClaugherty 2008). Especially in the species-rich Chinese subtropical forest zone with deciduous, evergreen broad-leaved and coniferous species co-existing, litter species are highly variable for instance in litter chemistry, leaf toughness or occurrence of a waxy litter surface. Therefore, it can be concluded that initial C:N ratio or N concentration alone are not good predictors for decomposition rate when analysing highly diverse litter species combined.

Pronounced differences in N dynamics among litter species could be revealed. The majority showed an increase in N concentration throughout the decomposition process, while in some species N concentration remained unchanged (e.g. *Pinus massoniana*). Most likely, the often observed increase in N concentration during decomposition is attributed to microbial carbon respiration leading to a relative N enrichment of the litter substrate. Especially mass loss resulting from fast degradation of non-lignified carbohydrates increases litter N concentration during early decomposition (Berg and Laskowki 2005). The increase in N concentration is further supported by the formation of covalent bonds between N and recalcitrant macromolecules such as protein-binding phenolics (Berg and McClaugherty 2008). As an additional mechanism the active import of N into decomposing leaf litter by ingrown fungal mycelia has been identified (Frey et al. 2003). Therefore, the observed species differences in N dynamics during decomposition might be explained by different decomposer colonization conditioned by species-specific litter traits and the initial litter N status (Berg and Laskowki 2005; Parton et al. 2007).

### **Decomposition of *Schima suberba* leaf litter along environmental gradients**

The decomposition constant of *Schima suberba* leaf litter varied considerably among CSPs confirming the existence of strong environmental heterogeneity affecting decomposition processes (hypothesis 2). Which factors could have been responsible for the observed variability in decomposition of our common substrate? First, plot tree species richness could influence microenvironmental conditions or modify the decomposer composition leading to diversity

effects. For example, in experimental grassland studies, increasing decomposition rates of common litter substrates with increasing plant species richness or functional diversity were shown (Hector et al. 2000; Scherer-Lorenzen 2008). In contrast, standing woody plant diversity as single predictor had no effect on decomposition of *Schima superba* leaf litter in our study.

Second, we found that decomposition rates declined with forest stand age. This relationship was even more pronounced when regarding tree basal area as an indirect measure of plant biomass. Microclimatic conditions mediated by plant structure or composition are likely to change strongly along successional gradients (Lebrija-Trejos et al. 2011). Although detailed microclimatic data for the litter layer are missing for our study plots, it can be argued that microclimatic site conditions such as temperature or air humidity are more favourable for litter decomposition in younger forest stands. As decomposition rate was positively related to LAI, microclimatic conditions might also be more favourable under a dense canopy. Variation in mean temperature (21.1 °C - 24.2 °C) and mean relative air humidity (84.3 % - 95.6 %) among plots compiled from data recorded every 15 minutes between June and October 2011 at 1 m height above ground do support the occurrence of distinct microclimatic gradients. Taken these data, air humidity decreased with stand age ( $r = -0.43$ ,  $p = 0.02$ ) but was positively correlated with decomposition rate of the common litter substrate ( $r = 0.49$ ,  $p = 0.01$ ). Temperature data measured over the relatively short time period could not be related to decomposition although its importance is demonstrated by studies along altitudinal gradients, latitudinal transects or soil-warming experiments (Berg and McClaugherty 2008; Hobbie et al. 2006; Vitousek et al. 1994; Wang et al. 2010).

Third, it is conceivable that exogenous soil and litter variables such as nutrient availability and content influence decomposition within litter bags (Berg and McClaugherty 2008; Chadwick et al. 1998). For example, leaching processes and fungi-driven translocation of nutrients are possible mechanisms how the endogenous and exogenous litter environment do interact (Lummer et al. 2012; Schimel and Hättenschwiler 2007). However, neither C:N ratio nor N concentration of the mineral soil and forest floor litter did directly affect the decomposition constant of our common litter substrate. Interestingly, fertilization experiments indicate no uniform effect of exogenous nitrogen availability on litter decomposition (Fang et al. 2007; Knorr et al. 2005; Prescott 1995) questioning the general importance of the surrounding soil and litter nutrient status. Similarly, decomposition was not influenced by soil moisture as single predictor variable.

### **Decomposition of mixed-species leaf litter**

We found that two thirds of our plot-specific litter mixtures varying in species richness showed synergistic and one third antagonistic decomposition effects, thus generally confirming our third hypothesis. It has been shown that synergistic responses are twice as frequently observed than

antagonistic mixture effects (Gartner and Cardon 2004; Hättenschwiler et al. 2005) which is in line with our results. However, the small number of studies, diverse methodologies and the low number of species involved question the predominance of positive non-additive litter mixture effects (Hättenschwiler et al. 2005). In most decomposition experiments conducted in the humid subtropics that are underrepresented in decomposition research, mixtures with relatively few species have been used (Liu et al. 2005; Wang et al. 2009; Zhang et al. 2008). Our results are based on litter mixtures containing 7 to 17 species which is more likely to reflect natural conditions considering plot species richness ranging from 25 to 69 woody plant species in Gutianshan (Bruehlheide et al. 2011). By combining our decomposition study with a common substrate approach we were able to account for differences in microclimatic conditions but without disregarding potential effects of the local soil biota community on litter mixtures (Ayres et al. 2009).

The mixture effect calculated for k-values was marginally positively related to litter bag species richness. A similar trend was found for remaining litter mass after 205 days of decomposition. Theoretically, it is more likely to detect potential diversity effects in mixtures comprising less species. This is because high functional species equivalence could also translate into high redundancy in litter species. Another reason is that functional litter traits and their relative abundance in a litter mixture might be more important than number of species (Wardle et al. 1997). For instance, in a subtropical tree plantation decomposition of coniferous leaf litter was either promoted or retarded when mixed with certain broad-leaved litter species (Wang et al. 2007a; Wang et al. 2009). Similarly, Hättenschwiler (2005) also documented strong species-specific responses within litter mixtures, arguing that effective litter traits and interaction with decomposers remain often unrevealed making it difficult to identify the role of litter species diversity for decomposition. Likewise, knowledge about specific decomposer traits important for decomposition processes such as colonization sequence, capability of nutrient transfer, and exclusion of other microbial organisms is very limited.

Species-specific responses within litter mixtures could not be resolved in our experiment because high similarity of litter fragments of broad-leaved trees did not allow species separation. Instead, we followed decomposition of coniferous litter in mixture and could show a 67 % higher decay rate than predicted from single-species decomposition rates. Such positive effects by broad-leaved species on coniferous litter decomposition have been often observed (e.g. Salamanca et al. 1998; Wang et al. 2007a). But other studies could not prove such synergistic effects (Prescott et al. 2000) or conclude that differences in initial litter chemistry cannot resolve observed mixture effects (Hoorens et al. 2003). However, nutrient translocation is an important process how litter species do interact in mixtures (Lummer et al. 2012; Schimel and Hättenschwiler 2007).

## Conclusions

The present study is one of the few that accounted explicitly for the high tree species richness found in subtropical forests when examining litter mixture effects. Single-species decomposition constants varied largely reflecting the high litter trait diversity in tree species communities assembled by temperate, subtropical and tropical floristic components. The observed decrease in decomposition of a common litter substrate through forest succession supports the idea that nutrient cycling slows down during forest maturation with negative consequences for nutrient availability and net primary productivity (Gower et al 1996). Our study suggests that positive non-additive mixture effects are predominant during early leaf litter decomposition in species-rich evergreen broad-leaved forests. Although litter species richness did only marginally significantly increase the strength of the observed mixture effects, decomposition may be faster in more diverse forests. Especially the observed accelerated decomposition of needle-leaved litter when mixed with broad-leaved litter indicates that the integration of broad-leaved tree species with high litter quality in monospecific conifer stands might have beneficial effects on nutrient turnover. Thereby, the occurrence of antagonistic effects might be less likely in a species-rich litter environment. It will be an issue of future studies to identify species combinations offering greatest synergy and to elucidate the interplay between litter trait diversity and the decomposer subsystem.

## Acknowledgements

We wish to record our gratitude to the staff of the Gutianshan National Nature Reserve, especially to Fang Teng, for the received support. We thank the members of the BEF China project for help in plot establishment and Andreas Kundela and Katherina Pietsch for sharing LAI and microclimate data. Nina Buchmann contributed to the improvement of the manuscript by providing valuable comments. This study was funded by the German Science Foundation (DFG FOR 891/1).



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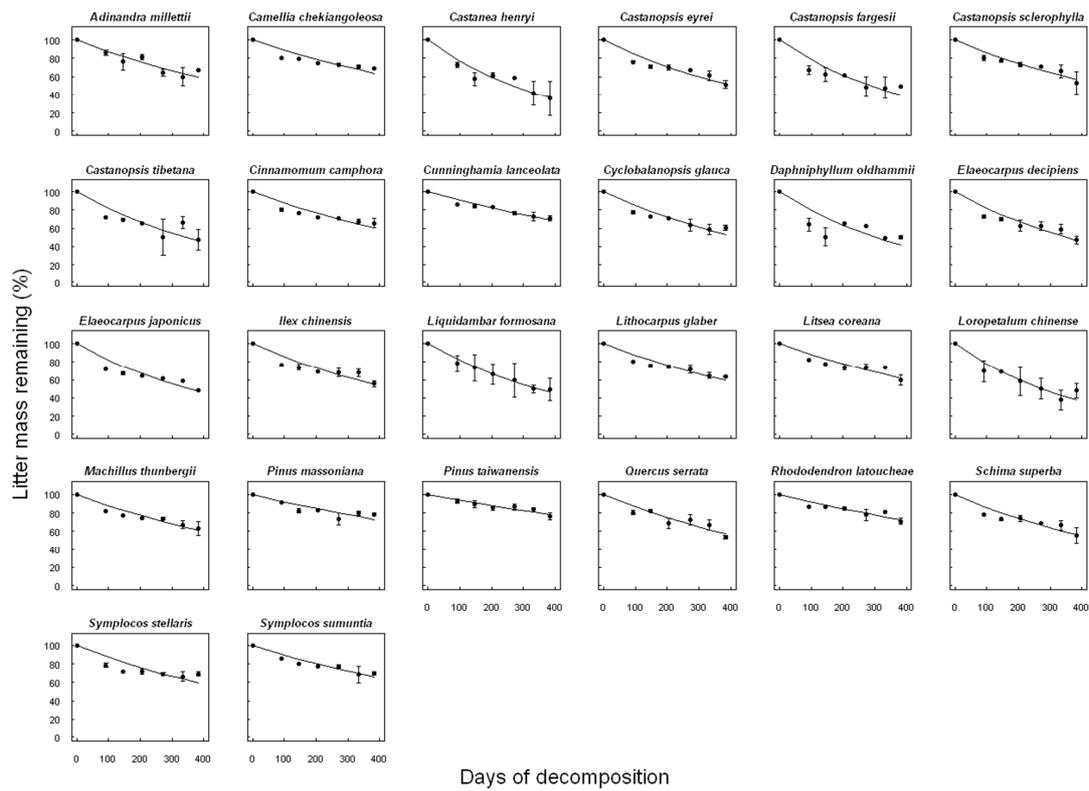
## Tables and Figures

**Table 1** Initial litter chemical characteristics (total N, C:N ratio), mass loss at the end of the experiment, decomposition constant (k), model fit of the exponential decomposition model ( $R^2$ ), increase in total N concentration during decomposition (significance levels are ‘\*\*\*’ < 0.001, ‘\*\*’ < 0.01, ‘\*’ < 0.05), half-life time (T50 = 0.693/k) and time until 95 % mass loss (T95 = 3/k) for the 26 species studied. Classification into functional groups according to canopy height (canopy tree: c; sub-canopy tree: s), phenology (evergreen: e; deciduous: d) and leaf morphology (broad-leaved: b; needle-leaved: n) follows Lang et al. (2010).

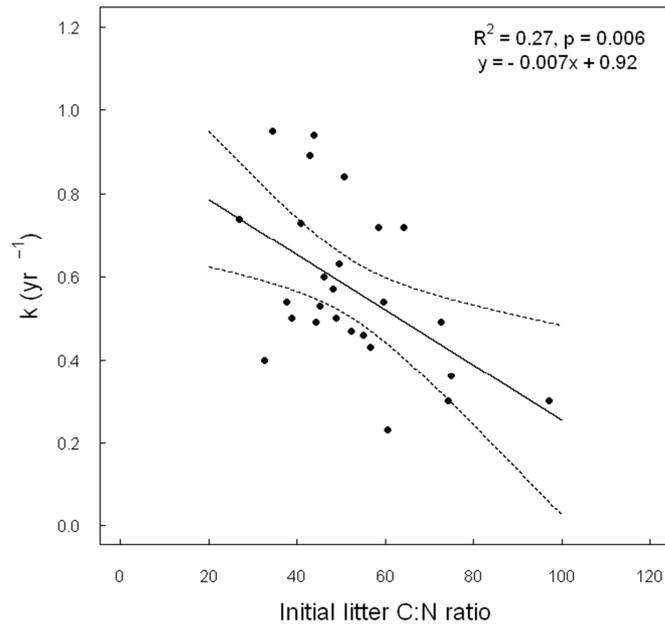
Species (Functional Group)	Total N (%)	C:N ratio	Mass loss (%)	k (yr <sup>-1</sup> )	R <sup>2</sup>	N increase (%)	T50 (yr)	T95 (yr)
<i>Adinandra millettii</i> (ceb)	1.21	38.80	33.19	0.50	0.86	59.09***	1.40	6.06
<i>Camellia chekiangoleosa</i> (seb)	0.81	56.69	31.22	0.43	0.78	22.82***	1.62	7.03
<i>Castanea henryi</i> (cdb)	1.44	34.34	63.90	0.95	0.91	8.33	0.73	3.15
<i>Castanopsis eyrei</i> (ceb)	0.98	49.47	48.46	0.63	0.87	47.96***	1.11	4.79
<i>Castanopsis fargesii</i> (ceb)	1.16	42.97	50.26	0.89	0.83	62.93**	0.78	3.39
<i>Castanopsis sclerophylla</i> (ceb)	1.29	37.67	46.82	0.54	0.91	7.75	1.28	5.55
<i>Castanopsis tibetana</i> (ceb)	1.82	26.98	52.74	0.74	0.76	3.02	0.94	4.08
<i>Cinnamomum camphora</i> (ceb)	0.68	72.63	34.44	0.49	0.81	50.74***	1.43	6.18
<i>Cunninghamia lanceolata</i> (cen)	0.66	74.91	29.38	0.36	0.93	58.33**	1.95	8.45
<i>Cyclobalanopsis glauca</i> (ceb)	1.06	46.16	39.42	0.60	0.87	34.91***	1.16	5.02
<i>Daphniphyllum oldhamii</i> (ceb)	0.92	50.68	49.47	0.84	0.54	113.59***	0.83	3.58
<i>Elaeocarpus decipiens</i> (ceb)	0.83	58.37	52.77	0.72	0.87	71.08***	0.96	4.16
<i>Elaeocarpus japonicus</i> (ceb)s	0.71	64.08	51.32	0.72	0.84	79.58***	0.96	4.16
<i>Ilex chinensis</i> (seb)	1.04	48.25	44.30	0.57	0.81	53.85***	1.22	5.29
<i>Liquidambar formosana</i> (cdb)	1.04	40.75	50.84	0.73	0.98	38.46**	0.95	4.09
<i>Lithocarpus glaber</i> (ceb)	1.06	48.91	36.84	0.50	0.87	36.32**	1.40	6.04
<i>Litsea coreana</i> (ceb)	0.94	55.12	40.25	0.46	0.82	46.28***	1.51	6.52
<i>Loropetalum chinense</i> (seb)	1.04	43.84	52.22	0.94	0.91	65.87***	0.74	3.20
<i>Machilus thunbergii</i> (ceb)	0.97	52.25	36.57	0.47	0.88	-1.55	1.48	6.41
<i>Pinus massoniana</i> (cen)	0.70	74.30	21.59	0.30	0.73	7.14	2.30	9.95
<i>Pinus taiwanensis</i> (cen)	0.86	60.50	23.43	0.23	0.92	0.00	2.99	12.96
<i>Quercus serrata</i> (cdb)	1.07	45.19	46.04	0.53	0.89	14.95	1.31	5.68
<i>Rhododendron latoucheae</i> (seb)	0.52	97.12	28.95	0.30	0.85	76.92***	2.28	9.86
<i>Schima superba</i> (ceb)	0.87	59.62	44.21	0.54	0.86	28.16	1.27	5.51
<i>Symplocos stellaris</i> (seb)	1.00	44.30	30.77	0.49	0.63	-18.00*	1.40	6.08
<i>Symplocos sumuntia</i> (seb)	1.17	32.68	29.99	0.40	0.89	11.11	1.73	7.49

**Table 2** Univariate relationships between plot-specific decomposition rate of *Schima superba* leaf litter and environmental factors. Pearson's correlation coefficient (*r*) and *p*-value are given.

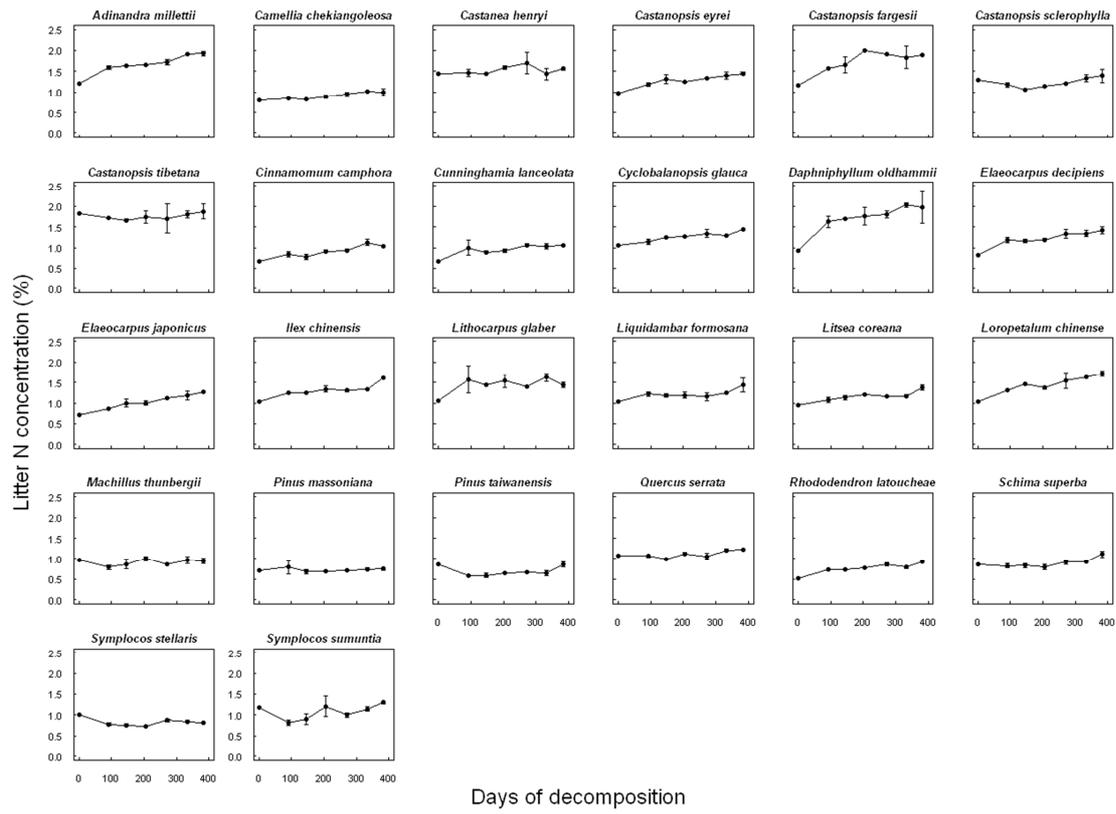
Predictor variables	<i>r</i>	<i>p</i>
<b>Topographic factors</b>		
Elevation [m]	-0.263	0.184
Inclination [°]	0.125	0.535
Northness = cos(aspect [°])	0.082	0.686
Eastness = sin(aspect [°])	<b>0.414</b>	<b>0.032</b>
<b>Stand characteristics</b>		
Stand age [yr]	<b>-0.450</b>	<b>0.018</b>
Successional stage	<b>-0.482</b>	<b>0.011</b>
Basal area [m <sup>2</sup> ]	<b>-0.641</b>	<b>&lt;0.001</b>
Leaf area index	<b>0.442</b>	<b>0.021</b>
Species richness	0.166	0.407
Rarefied species richness	-0.035	0.861
Shannon index	-0.092	0.647
Litter production [kg ha <sup>-1</sup> ]	-0.025	0.900
<b>Forest floor litter</b>		
Biomass [kg ha <sup>-1</sup> ]	<b>-0.419</b>	<b>0.029</b>
Total N concentration [%]	0.214	0.283
N pool [kg ha <sup>-1</sup> ]	-0.225	0.260
Total C concentration [%]	-0.289	0.144
C pool [kg ha <sup>-1</sup> ]	<b>-0.426</b>	<b>0.027</b>
C:N ratio	-0.242	0.225
Litter turnover	<b>0.578</b>	<b>0.002</b>
<b>Mineral soil (0-10 cm)</b>		
Total N concentration [%]	0.077	0.704
N pool [kg ha <sup>-1</sup> ]	0.077	0.704
Total C concentration [%]	-0.048	0.813
C pool [kg ha <sup>-1</sup> ]	-0.043	0.831
C:N ratio	-0.359	0.066
Moisture (g H <sub>2</sub> O g <sup>-1</sup> dry soil)	0.193	0.335
pH measured in H <sub>2</sub> O	0.063	0.757



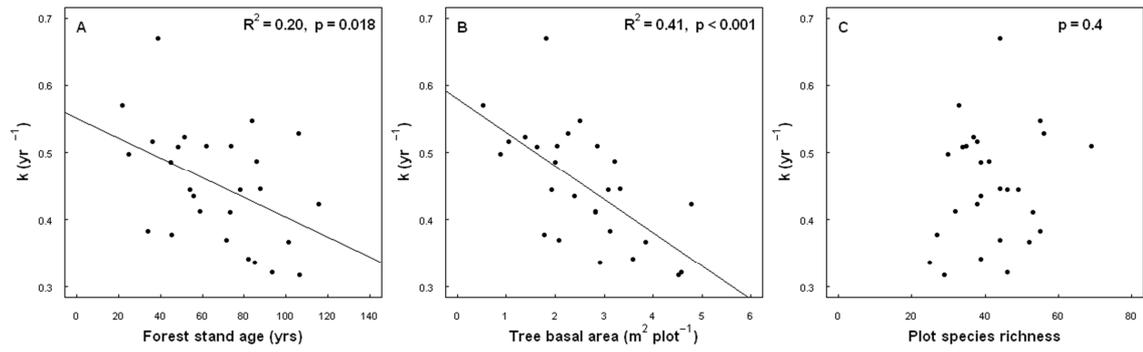
**Fig. 1** Species-specific change in litter mass loss during 383 days of decomposition for the 26 tree species studied. Means  $\pm$  SE are shown based on two replicated bags per species and retrieval date. Lines represent single exponential decay functions.



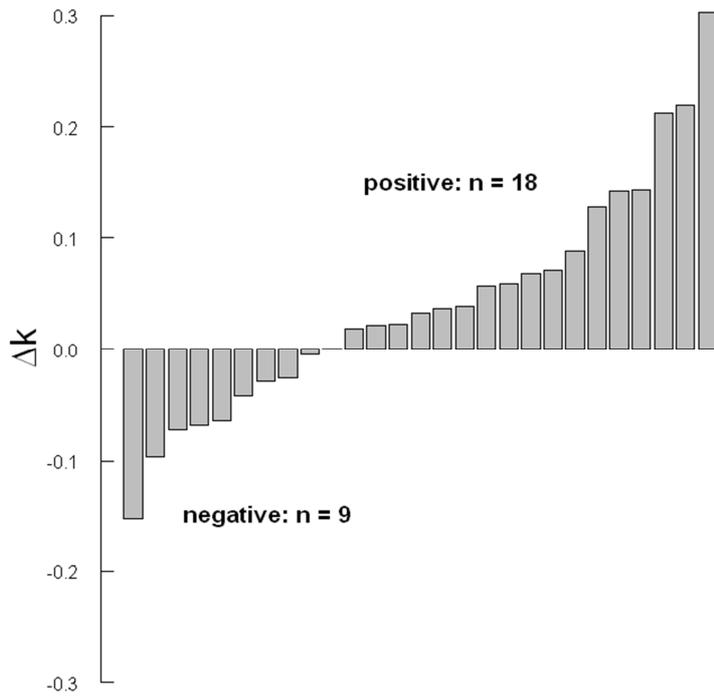
**Fig. 2** Influence of initial litter C:N ratio on decomposition constant (k) based on 26 litter species. Dashed lines indicate 95 % confidence interval of the regression line.



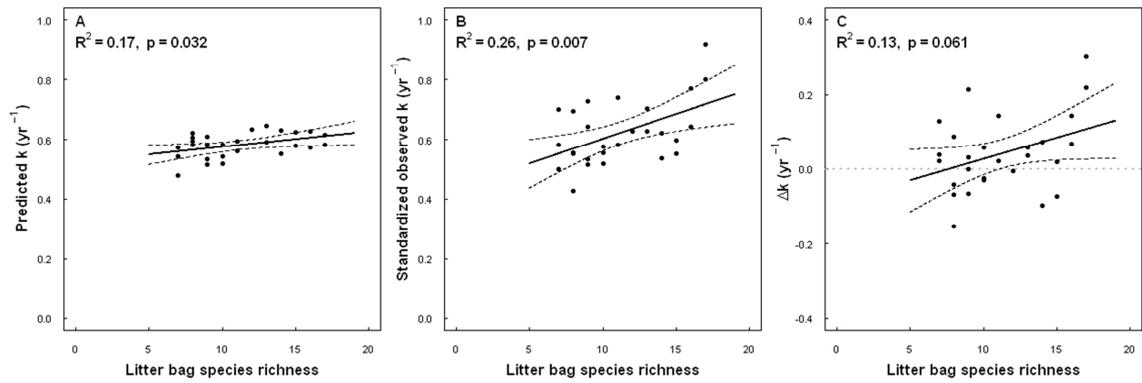
**Fig. 3** Species-specific changes in litter N concentration during 383 days of decomposition. Means  $\pm$  SE are shown based on two replicated bags per species and retrieval date.



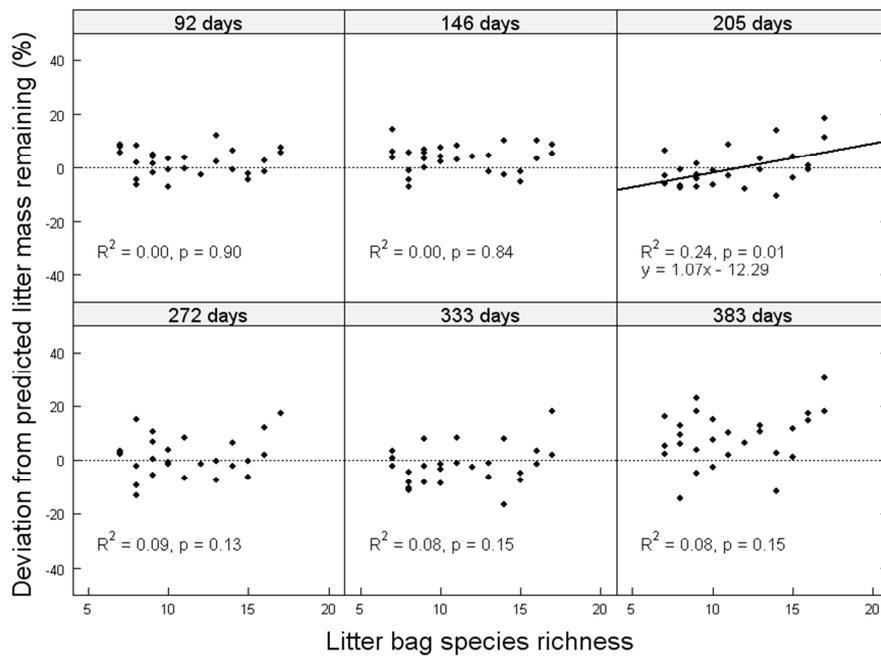
**Fig. 4** Relationship between plot-specific decomposition rate of *Schima superba* leaf litter and forest stand age (A), tree basal area (B) and plot species richness of woody plants (C), respectively.



**Fig. 5** Difference between standardized observed and predicted decomposition rate ( $\Delta k$ ) for each of the 27 litter mixtures varying in species richness. Negative values ( $n = 9$ ) indicate slower decay than predicted from single decomposition rates whereas mixtures with a positive difference ( $n = 18$ ) showed faster mass loss than expected.



**Fig. 6** Relationship between litter bag species richness and predicted decomposition rate (A) as well as standardized observed k-values of plot-specific litter mixtures (B). The 95 % confidence interval (dashed lines) of the regression line is given. The difference between standardized observed and predicted k-values ( $\Delta k$ ) was marginally related to litter bag species richness ( $p = 0.061$ ; C). Litter mixtures with  $\Delta k > 0$  decomposed faster whereas mixtures with  $\Delta k < 0$  had a lower mass loss than predicted from single-species decomposition rates.



**Fig. 7** Relationship between deviation from predicted remaining litter mass and litter bag species richness at each retrieval date (92, 146, 205, 272, 333, 383 days of decomposition). If deviation > 0, a positive mixture effect, i.e. lower remaining litter mass than predicted was observed.

## Supplementary material, Appendix 1

**Table A1.** Species composition and richness of plot-specific litter mixtures (CSP 1 – CSP 27). The number of litter species per bag equalled the number of tree species found in the different CSPs based on 24 species for which sufficient quantities of litter could be collected.

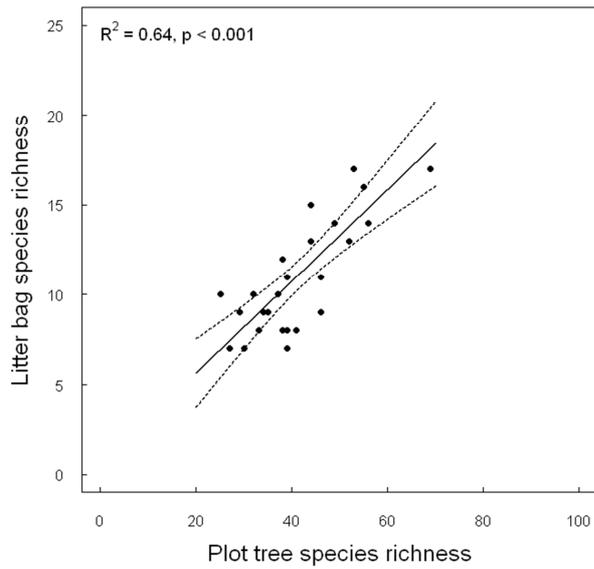
Species	CSP 1	CSP 2	CSP 3	CSP 4	CSP 5	CSP 6	CSP 7	CSP 8	CSP 9
<i>Adinandra millettii</i>	0	1	0	1	1	0	1	1	1
<i>Castanea henryi</i>	0	1	1	0	0	0	1	0	0
<i>Castanopsis eyrei</i>	1	1	1	1	1	1	1	1	1
<i>Castanopsis fargesii</i>	1	1	1	1	0	0	0	1	1
<i>Castanopsis sclerophylla</i>	0	0	0	0	0	0	0	1	1
<i>Castanopsis tibetana</i>	0	1	0	1	0	0	0	1	0
<i>Cinnamomum camphora</i>	0	0	0	0	0	0	0	0	0
<i>Cunninghamia lanceolata</i>	1	1	1	0	1	0	0	1	1
<i>Cyclobalanopsis glauca</i>	1	1	0	1	0	0	0	1	1
<i>Daphniphyllum oldhamii</i>	1	1	1	1	0	0	0	1	1
<i>Elaeocarpus decipiens</i>	0	0	0	1	0	0	0	0	0
<i>Elaeocarpus japonicus</i>	0	1	1	0	0	0	0	1	0
<i>Ilex chinensis</i>	0	0	0	1	0	0	0	0	0
<i>Liquidambar formosana</i>	1	0	1	0	0	0	0	0	1
<i>Lithocarpus glaber</i>	1	1	0	1	1	0	0	1	1
<i>Litsea coreana</i>	1	1	1	0	0	1	1	1	1
<i>Loropetalum chinense</i>	1	1	1	1	1	0	0	1	1
<i>Machilus thunbergii</i>	1	1	1	1	0	1	1	1	1
<i>Pinus massoniana</i>	1	1	1	0	1	0	0	1	1
<i>Pinus taiwanensis</i>	0	0	0	0	0	1	1	0	0
<i>Quercus serrata</i>	1	1	1	0	1	1	1	0	0
<i>Schima superba</i>	1	1	1	1	1	1	1	1	1
<i>Symplocos stellaris</i>	1	1	1	1	1	1	1	1	1
<i>Symplocos sumuntia</i>	1	0	0	0	1	0	0	1	1
Number of species	15	17	14	13	10	7	9	17	16
Litter mass per species (g)	0.333	0.294	0.357	0.385	0.500	0.714	0.556	0.294	0.313

Table A1. (continued)

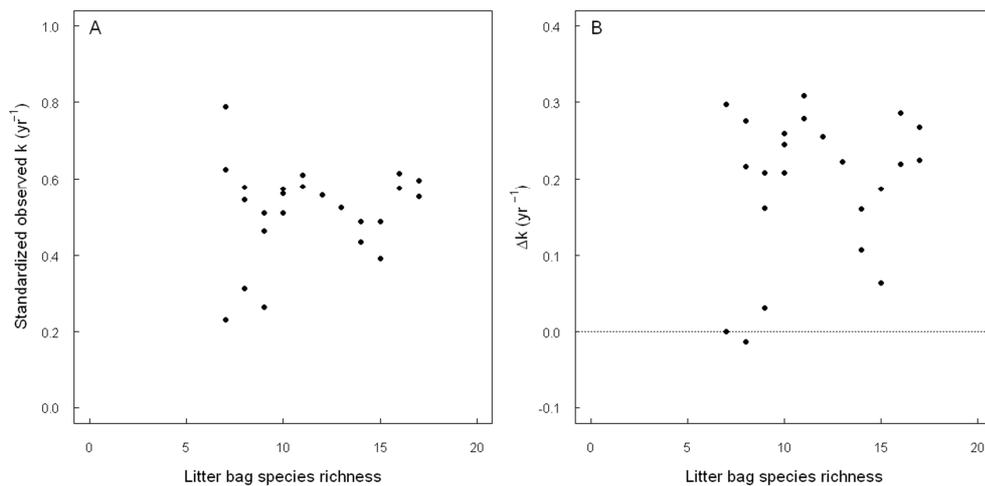
Species	CSP 10	CSP 11	CSP 12	CSP 13	CSP 14	CSP 15	CSP 16	CSP 17	CSP 18
<i>Adinandra millettii</i>	1	0	0	1	1	0	1	1	1
<i>Castanea henryi</i>	1	1	0	0	1	0	0	0	0
<i>Castanopsis eyrei</i>	1	1	1	1	1	1	1	1	1
<i>Castanopsis fargesii</i>	0	1	0	0	0	0	0	0	1
<i>Castanopsis sclerophylla</i>	0	0	0	0	0	0	0	0	0
<i>Castanopsis tibetana</i>	0	0	0	0	1	0	0	0	0
<i>Cinnamomum camphora</i>	0	0	0	0	0	0	0	0	0
<i>Cunninghamia lanceolata</i>	0	0	0	0	0	0	0	0	0
<i>Cyclobalanopsis glauca</i>	0	0	0	0	0	0	1	1	1
<i>Daphniphyllum oldhamii</i>	1	0	1	1	1	1	0	0	1
<i>Elaeocarpus decipiens</i>	0	0	0	1	0	0	0	0	0
<i>Elaeocarpus japonicus</i>	0	0	0	0	0	0	0	0	0
<i>Ilex chinensis</i>	0	1	0	0	0	0	0	0	0
<i>Liquidambar formosana</i>	0	0	0	0	1	0	0	1	0
<i>Lithocarpus glaber</i>	1	1	1	1	1	1	1	1	1
<i>Litsea coreana</i>	0	1	1	0	1	0	0	0	1
<i>Loropetalum chinense</i>	0	0	0	1	1	1	1	1	1
<i>Machilus thunbergii</i>	0	1	1	1	1	1	0	0	1
<i>Pinus massoniana</i>	0	0	1	1	1	1	1	1	1
<i>Pinus taiwanensis</i>	0	0	0	0	0	0	0	0	0
<i>Quercus serrata</i>	0	0	0	0	0	1	1	1	1
<i>Schima superba</i>	1	1	1	1	1	1	1	1	1
<i>Symplocos stellaris</i>	1	1	1	0	0	0	1	1	1
<i>Symplocos sumuntia</i>	1	0	1	1	0	0	1	1	0
Number of species	8	9	9	10	12	8	10	11	13
Litter mass per species (g)	0.625	0.556	0.556	0.500	0.417	0.625	0.500	0.455	0.385

Table A1 (continued)

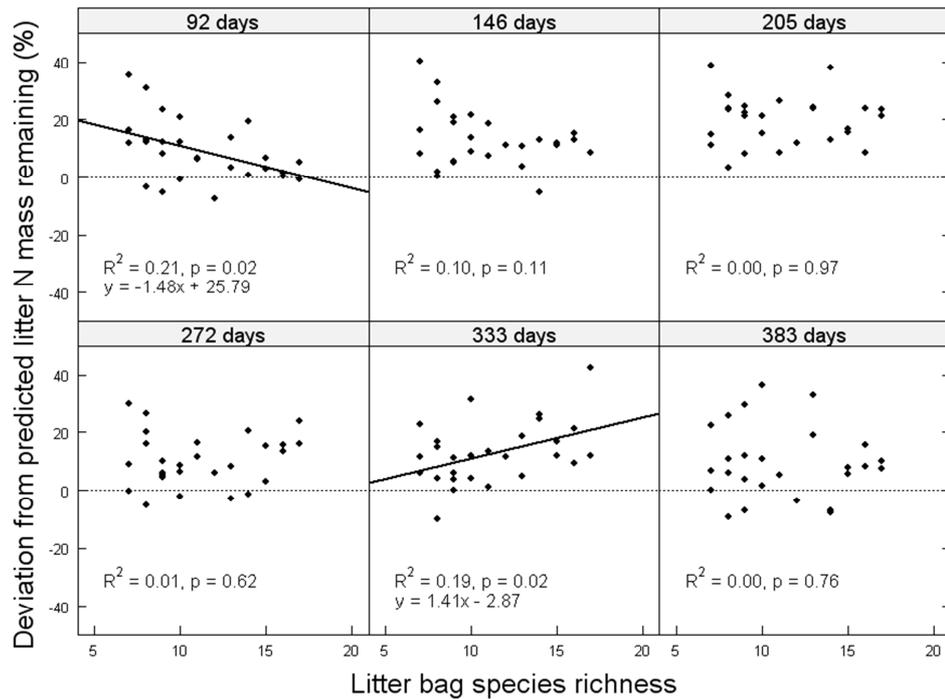
Species	CSP 19	CSP 20	CSP 21	CSP 22	CSP 23	CSP 24	CSP 25	CSP 26	CSP 27
<i>Adinandra millettii</i>	0	0	0	0	1	1	1	1	0
<i>Castanea henryi</i>	0	1	1	1	1	0	0	1	1
<i>Castanopsis eyrei</i>	0	1	1	1	1	1	1	1	1
<i>Castanopsis fargesii</i>	0	0	0	0	1	0	0	1	0
<i>Castanopsis sclerophylla</i>	0	0	1	0	0	0	0	1	0
<i>Castanopsis tibetana</i>	0	0	0	0	1	0	0	0	1
<i>Cinnamomum camphora</i>	0	0	0	0	1	0	0	0	0
<i>Cunninghamia lanceolata</i>	1	1	1	1	1	0	0	0	0
<i>Cyclobalanopsis glauca</i>	0	0	1	0	1	1	1	1	1
<i>Daphniphyllum oldhamii</i>	0	0	0	0	1	0	0	1	1
<i>Elaeocarpus decipiens</i>	0	0	0	0	0	0	0	0	0
<i>Elaeocarpus japonicus</i>	0	0	0	0	1	0	0	0	0
<i>Ilex chinensis</i>	0	0	0	0	0	0	0	0	0
<i>Liquidambar formosana</i>	1	1	0	0	0	1	0	1	0
<i>Lithocarpus glaber</i>	0	0	1	0	1	1	1	1	0
<i>Litsea coreana</i>	0	0	1	0	0	0	0	0	1
<i>Loropetalum chinense</i>	1	1	1	1	1	1	1	1	0
<i>Machilus thunbergii</i>	0	0	1	0	1	0	0	1	1
<i>Pinus massoniana</i>	1	1	1	1	0	1	1	1	1
<i>Pinus taiwanensis</i>	0	0	0	0	0	0	0	0	0
<i>Quercus serrata</i>	1	1	1	1	0	0	0	1	1
<i>Schima superba</i>	1	1	1	1	1	1	1	1	1
<i>Symplocos stellaris</i>	0	0	1	0	1	1	0	0	1
<i>Symplocos sumuntia</i>	1	0	1	1	1	0	0	1	0
Number of species	7	8	14	8	16	9	7	15	11
Litter mass per species (g)	0.714	0.625	0.357	0.625	0.313	0.556	0.714	0.333	0.455



**Fig. A1** Species richness in litter bags as a function of plot tree species richness. Dashed lines indicate 95 % confidence interval of the regression line.



**Fig. A2** Decomposition constant  $k$  determined for coniferous leaf litter in 24 plot-specific litter mixtures as a function of litter bag species richness ( $p = 0.60$ ; A). The difference between standardized observed and predicted conifer  $k$ -values ( $\Delta k$ ) was unrelated to litter bag species richness ( $p = 0.98$ ; B). Higher  $k$ -values than expected from single-coniferous species determined decomposition rates ( $\Delta k > 0$ ) were calculated for nearly all mixtures.



**Fig. A3** Relationship between deviation from predicted remaining litter N mass and litter bag species richness at each retrieval date (92, 146, 205, 272, 333, 383 days of decomposition). If deviation  $> 0$ , a positive mixture effect, i.e. lower remaining litter N mass than predicted was observed.



# CHAPTER 3

Manuscript

## **Spatio-temporal and chemical $^{15}\text{N}$ uptake patterns of four subtropical tree species in monoculture and 4-species mixtures**

Stefan Trogisch<sup>1,2</sup>, Jin-Sheng He<sup>3</sup>, Andy Hector<sup>4</sup> & Michael Scherer-Lorenzen<sup>1</sup>

<sup>1</sup> Institute of Agricultural Sciences, ETH Zurich, Switzerland

<sup>2</sup> Institute of Biology II, Geobotany, University of Freiburg, Germany

<sup>3</sup> Department of Ecology, Peking University, Beijing, China

<sup>4</sup> Institute of Evolutionary Biology and Environmental Studies, University of Zurich, Switzerland

**Abstract**

Overyielding effects in biodiversity-ecosystem functioning experiments have been often attributed to belowground niche differentiation leading to higher resource exploitation in mixture than observed in corresponding monocultures. Theoretically, coexisting plant species can reduce interspecific competition for limiting soil resources such as nitrogen by spatial, temporal and chemical N use complementarity. Yet, direct quantification of this potential underlying mechanism determining the relationship between tree diversity and forest ecosystem functioning is scarce.

We studied N uptake patterns of four subtropical tree species growing in monoculture and mixture by injection of  $^{15}\text{N}$  tracers ( $^{15}\text{NH}_4\text{Cl}$ ,  $\text{K}^{15}\text{NO}_3$ , dual-labelled glycine) at two soil depths (5 cm, 20 cm) in four seasons. Tree saplings of two deciduous (*Castanea henryi* and *Quercus serrata*) and two evergreen species (*Elaeocarpus decipiens* and *Schima suberba*) were planted in short distances to induce competitive interactions quickly after planting. Evergreen species preferred nitrate whereas deciduous species relied on different chemical N forms more equally with consistent pattern in each season. Species differed slightly in temporal and spatial  $^{15}\text{N}$  uptake with most of their N demand in summer (46 %) and spring (24 %) followed by autumn (15 %) and winter (15 %). Fraction of  $^{15}\text{N}$  uptake from the deep soil layer was lowest for *Castanea* (10 %) whereas the other species acquired in average more  $^{15}\text{N}$  from 20 cm soil depth (*Schima*: 19 %, *Quercus*: 17 %, *Elaeocarpus*: 15 %). Niche breadth and niche overlap among species remained unaffected by tree species richness, and community niche breadth calculated for the 4-species mixture only exceeded niche breadth of the *Castanea* monoculture. We conclude that multidimensional N-use complementarity can facilitate species coexistence but does not necessarily support greater community utilization of resources.

**Keywords:** BEF China, biodiversity-ecosystem functioning, niche differentiation, nitrogen uptake complementarity, resource partitioning, subtropical tree species

## Introduction

Plant diversity has been recognized to influence ecosystem functioning and services (Balvanera et al. 2006; Hooper et al. 2005). Besides the selection or sampling effect, facilitation and complementary resource use have been proposed as underlying biological mechanisms leading to a positive biodiversity-ecosystem functioning relationship (Fridley 2001; Loreau 2000; Loreau and Hector 2001; Tilman et al. 1997).

Facilitation occurs if one species positively influences the performance of another species either directly or indirectly (Cardinale et al. 2002; Vandermeer 1992). For example, the combination of N-fixing with non-fixing tree species can increase nitrogen availability and therefore total stand productivity (DeBell et al. 1997, Forrester et al 2006). Complementarity in resource use occurs when species use different types of resources, or when they use the same resource but partition it in space, time or both (Loreau and Hector 2001). For instance, light interception can increase in a stratified canopy structured by shade tolerant species in the understory and sun-adapted species in the upper canopy (Kelty 2006). Belowground, niche differentiation in N use might lead to higher soil nitrate depletion and lower N leaching in species-rich than in species-poor communities (Oelmann et al. 2007; Scherer-Lorenzen et al. 2003; Tilman et al. 1996). Combining species with complementary resource acquisition patterns can therefore enlarge total community niche breadth and reduce interspecific competition for limiting resources.

From all nutrients, N is regarded to be most limiting for plant productivity in many terrestrial ecosystems (LeBauer and Treseder 2008; Vitousek and Howarth 1991) resulting in high interspecific competition for plant-available N. Therefore, belowground niche differentiation might lead to reduced competition for N and thereby foster species coexistence and community N uptake through complementary N use (Lambers et al. 2004). Theoretically, N use complementarity among coexisting species could be the result of a one-dimensional niche differentiation, or by a multi-dimensional one: species could be differentiated by spatial, temporal or chemical uptake patterns representing distinct niche axes. Combining species with complementary patterns of vertical root distribution and activity (Ewel and Mazzarino 2008; Gale and Grigal 1987; Mamolos et al. 1995) would allow greater nutrient exploration of the soil column, thus increasing total soil N utilization. In the long run, N taken up from deeper soil layers will be accessible to shallow rooting species via internal nutrient cycling. Furthermore, owing to morphological root plasticity, rooting patterns could be adapted to a certain degree in order to reduce root overlap and consequently interspecific competition (Mou et al. 1997; Rothe and Binkley 2001).

Temporal niche differentiation is a result of seasonal variation in N-uptake. For example, McKane et al. (1990) showed that five herbaceous species in an old-field community not only take up N

from different soil layers but also possess different temporal N uptake patterns during the growing season.

Besides spatial (root extension, rooting depth) and temporal (seasonal variation in N uptake activity) N use complementarity there is the potential for chemical niche differentiation. Plant species often differ in their preference for ammonium and nitrate (Garnett and Smethurst 1999; Templer and Dawson 2004; Pfautsch et al. 2009) and, as shown by several studies, have access to dissolved organic N either taken up directly or mediated by ectomycorrhizal associations (Finzi and Berthrong 2005; McKane et al. 2002; Näsholm and Persson 2001; Warren and Adams 2007).

Given these possibilities for differentiation in N uptake, the occupied niche space can be defined as a n-dimensional hypervolume according to Hutchinson (1957). However, species niche space is not static but can adapt to changing environmental conditions and different competitive scenarios. The fundamental niche characterizes the maximum niche space that a species can occupy without interspecific competition. In contrast, the realized niche indicates the actual niche space under interspecific competition (Silvertown 2004; Vandermeer 1992). Therefore, the niche breadth of a species, e.g. calculated as Levins' B (Levins 1968), as well as niche overlap between species (e.g. Schoener 1970; Parrish and Bazzaz 1976) should decline if interspecific competition increases. Finally, a larger niche breadth of the community than the one of corresponding monocultures would indicate N-uptake complementarity among species (von Felten et al. 2009).

Using  $^{15}\text{N}$  tracers we studied spatio-temporal and chemical N use complementarity in experimental tree sapling communities planted in monocultures or mixtures. This study was embedded in a larger biodiversity experiment ("BEF-China", Bruehlheide et al. 2011) which aims to examine the influence of tree species diversity on ecosystem functioning and services in subtropical China. We used four natural tree species co-occurring in the surrounding subtropical broad-leaved forest. Species which differed in phenology (evergreen and deciduous) and natural abundance (dominant and sub-ordinate) were planted in short distances to induce below- and aboveground interactions shortly after planting. Three chemical N forms were injected into the soil at two depths and in four seasons to trace N uptake by tree saplings under intra- and interspecific competition. We aimed to characterize the multidimensional niche differentiation for the selected species in monoculture (fundamental niche) and 4-species mixture (realized niche) by calculating niche breadth as Levins' B (Levins 1968) and niche overlap between species as proportional similarity (Schoener 1970). We hypothesized:

- (1) Species differ in their N acquisition in space, time and chemical form
- (2) Niche breadth and niche overlap between species decrease under interspecific competition (realized niche).

- (3) The full species mixture has a higher niche breadth than the individual monocultures of the species involved.

## Materials and Methods

### Study site

This study was conducted in Jiangxi Province, East China (N29°06.293 E117°55.286). The region encounters a warm-temperate climate with distinct seasonality: a hot-humid season from April to September and a cool-dry season from October to March. Mean temperature is about 15 °C and mean annual precipitation is 1964 mm of which most occurs between March and September (Hu and Yu 2008). The region belongs to the subtropical evergreen-broad-leaved forest (EBLF) belt, one of the most important vegetation formations of eastern Asia, ranging from eastern Tibet to the southeastern coastline on mainland China (Richardson 1990; Wang et al. 2007). EBLF is characterized by a high diversity of woody plant species with evergreen species dominating in terms of number of individuals (Bruehlheide et al. 2011; Wang et al. 2007).

### Experimental design

The experiment was set up on a former agricultural field with rice, rape and vegetables grown in a double-cropping system. In March 2009, the field with a gross area of 7900 m<sup>2</sup> was ploughed and 4 blocks of equal size were established. Each block was divided into 1 m<sup>2</sup> plots separated by about 20 cm deep and wide trenches. Around blocks, 50 cm deep channels connected to trenches were arranged allowing drainage of excess rain water. For our experiment we used a subset of totally 480 plots with 120 plots assigned to each block. In late March 2009, each plot was planted with 16 tree saplings (4 x 4) belonging to four species (Fig. 1). Two deciduous species (*Castanea henryi* and *Quercus serrata*) and two evergreen species (*Elaeocarpus decipiens* and *Schima superba*) were planted in monocultures or in 4-species mixtures with equal proportion of each species. Hereafter, genus names are used to refer to all species. Planting distance was about 25 cm thus allowing occurrence of above- and belowground interactions shortly after planting. To test for spatio-temporal and chemical preferences in nitrogen uptake, we injected either <sup>15</sup>NH<sub>4</sub>Cl (99 % <sup>15</sup>N) solution, K<sup>15</sup>NO<sub>3</sub> (99 % <sup>15</sup>N) solution or double-labeled glycine (97-99 % U-<sup>13</sup>C<sub>2</sub>; 97-99 % <sup>15</sup>N) solution into the mineral soil, at two depths (5 cm and 20 cm), and at four times during the year (in summer when temperature is highest; before leaf shedding in autumn; in winter when temperature is lowest and during leaf flush in spring). The full factorial design yielded a total of 480 plots (5 species arrays x 3 N forms x 2 depths x 4 times x 4 replicates).

### **<sup>15</sup>N tracer application**

<sup>15</sup>N tracer solutions were applied only to the central quadrant containing four target individuals from which basal diameter and height measurements were seasonally taken (Fig. 1). Tracer solution was injected in a regular grid (49 single injections spaced by 7 cm) with a dispenser (Eppendorf Multipette 4780 with Combitips plus 50 ml, Eppendorf, Hamburg, Germany) connected to a 3 mm thick four site-port needle. By this means, the <sup>15</sup>N solution could be released more accurately in the respective soil layer, thus minimizing downward movement of tracers within the soil matrix. Each injection hole, pre-drilled using a screwdriver (4 mm in diameter), received 2 ml of tracer solution. The amount of applied <sup>15</sup>N was calculated in order to reach a detectable threshold of <sup>15</sup>N enrichment in the plant without inducing any fertilizer effect. Therefore, we accounted for a higher dilution of <sup>15</sup>N within the plant N pool during the course of the experiment and adjusted the amount of applied <sup>15</sup>N to the estimated tree sapling biomass in each season. Thus, each plot received 1.92 mg in summer 2009, 3.21 mg in autumn 2009 and winter 2010, and 4.56 mg of <sup>15</sup>N in spring 2010. The nitrification inhibitor dicyandiamide (DCD, 8.28 mg per plot) was added to all tracer solutions in order to minimize oxidation of ammonium by *Nitrosomas* bacteria. Labeled plot areas were covered by transparent plastic tarps for six days until plant harvest to minimize <sup>15</sup>N leaching to deeper soil layers and tracer dilution in case of rain events.

### **Plant sampling and <sup>15</sup>N analysis**

One day prior to tracer application, root, stem and leaf samples were taken from one randomly selected outer individual per species and block to measure natural <sup>15</sup>N and <sup>13</sup>C abundance. Six days after tracer application one randomly selected target individual from the mono-specific plots and all four target trees in the mixtures were harvested. From each individual, fine roots (< 2 mm in diameter), parts of the lower stem and 4 to 8 preferably fully developed leaves from the upper canopy were sampled. Because evergreen species shed most of their older leaves at the time of leaf flush in spring, we sampled last year's and fresh leaves to account for the presence of two leaf generations. Roots were thoroughly washed under tap water to remove any adherent soil. Plant samples were dried at 70 °C for 48 h. Plant organ specific  $\delta^{15}\text{N}$  and N concentration as well as  $\delta^{13}\text{C}$  and C concentration (glycine treatments) were analyzed at the laboratory of the Grassland Sciences Group at ETH Zurich, Switzerland, using an "EA-IRMS" system consisting of an isotope ratio mass spectrometer (IRMS Delta<sup>plus</sup> XP, Finnigan MAT, Bremen, Germany) coupled to an elemental analyzer (Flash EA 1112 NC, CE Instruments, Milan Italy).

### Allometric equations

In each season we randomly sampled 20 individuals per species (5 individuals per species in each block) and measured basal diameter at 3 cm above soil surface. Roots, stem and leaves from harvested individuals were separated and dried until constant weight at 70 °C. Here, stem refers to all woody aboveground parts including twigs. In spring 2010 leaves of the two evergreen species were additionally separated in young recently developed and older last year's leaves to account for two simultaneously occurring leaf generations of different N sink capacity. Species-specific allometric equations for total tree biomass were developed with basal diameter (D) as single predictor variable following the equation:  $\ln(\text{biomass}) = A + B(\ln D)$  (Chave et al. 2001, Appendix Fig. 7). The exponential models explained 76 % to 90 % of variation in total tree biomass based on 80 tree individuals for the evergreen species (saplings harvested in all four seasons) and 60 individuals for deciduous species (saplings harvested in summer, autumn and spring), respectively. As the fraction of plant organs to total biomass was not supposed to change considerably in a few months, allocation of biomass to plant organs (roots, stem and leaves) was calculated for each species across seasons. This means that total plant biomass was multiplied by the average of the relative fraction of the plant organs, calculated across seasons, to obtain biomass of roots, stems and leaves.

### Soil related factors

Important soil related factors were determined for the two soil layers (0-10 cm and 15-25 cm) which received  $^{15}\text{N}$  tracers in order to evaluate inter-block variability in soil characteristics (Appendix Table 7). Measured parameters included mineral N concentration and gravimetric water content (Summer 2009), pH (autumn 2009) as well as bulk density, and total carbon and nitrogen concentration (winter 2010). Glycine concentrations could not be determined.

### Calculations and statistical analyses

Plant  $^{15}\text{N}$  uptake

The sample  $^{15}\text{N}/^{14}\text{N}$  ratio ( $R_{\text{sample}}$ ) was calculated using equation (1) where  $\delta^{15}\text{N}$  refers to the difference in  $^{15}\text{N}$  enrichment between sample and atmospheric  $\text{N}_2$  as standard ( $R_{\text{standard}} = 0.0036765$ ,  $\delta^{15}\text{N} = 0$ ).

$$R_{\text{sample}} = \left[ \left( \frac{\delta^{15}\text{N}}{1000} \right) + 1 \right] \times R_{\text{standard}} \quad (1)$$

Using atomic abundances (atom%; equation 2), excess  $^{15}\text{N}$  in the plant tissues was calculated as shown in equation 3, where excess  $^{15}\text{N}$  tissue is the amount of allocated  $^{15}\text{N}$  ( $\mu\text{g}$ ) to the respective

plant tissue within 6 days,  $atom\%_{\text{labeled}}$  and  $atom\%_{\text{natural}}$  are the atomic abundances of  $^{15}\text{N}$  measured in the tissue after and before tracer application and  $N\ pool_{\text{tissue}}$  is the amount of N in the tissue. The sum of excess  $^{15}\text{N}$  of all plant tissues equals the total amount of  $^{15}\text{N}$  (excess  $^{15}\text{N}_{\text{plant}}$ ) taken up by a plant individual (equation 4).

$$atom\%_{\text{sample}} = 100 \left( \frac{R_{\text{sample}}}{R_{\text{sample}} + 1} \right) \quad (2)$$

$$excess\ ^{15}\text{N}\ tissue = \frac{atom\%_{\text{labeled}} - atom\%_{\text{natural}}}{100} \times N\ pool_{\text{tissue}} \quad (3)$$

$$excess\ ^{15}\text{N}_{\text{plant}} = excess\ ^{15}\text{N}_{\text{roots}} + excess\ ^{15}\text{N}_{\text{stem}} + excess\ ^{15}\text{N}_{\text{leaves}} \quad (4)$$

In order to account for different amounts of  $^{15}\text{N}$  tracer applied in each season we then calculated total plant  $^{15}\text{N}$  uptake per 1 g of plant dry weight biomass and 1 mg  $^{15}\text{N}$  tracer applied.

To test for intact uptake of doubled labeled glycine ( $^{15}\text{N}$ ,  $^{13}\text{C}$ ) we applied linear regression of excess  $^{13}\text{C}$  and excess  $^{15}\text{N}$  in plant tissues, as suggested by Näsholm et al. (1998). A regression slope of 2 would reflect uptake of glycine as intact molecule.

#### Niche breadth and niche overlap

Species niche breadth in monoculture and mixture was calculated as Levins' normalized B (Levins 1968) from single fractions ( $p_i$ ) of N uptake by the respective species from the 24 treatments (4 times x 3 N forms x 2 soil depths):

$$B_n = \frac{1}{24 \sum_{i=1}^{24} p_i^2} \quad (5)$$

Theoretically, the broadest niche breadth ( $B_n = 1$ ) is achieved if N uptake from each treatment is exactly the same. The more a plant species is specialized in its N acquisition, the lower becomes its niche breadth. In case a species only uses one out of 24 offered N resources its niche breadth would be 1/24.

Niche overlap between pairs of species was calculated as proportional similarity (PS) based on Parrish and Bazzaz (1976). Niche overlap is highest (PS = 1) when N acquisition patterns of two species are identical and decreases as treatment fractions ( $p_1$ ,  $p_2$ ) of N uptake become more different.

$$PS = 1 - 0.5 \sum_{i=1}^{24} |p_{1i} - p_{2i}| \quad (6)$$

Analyses were performed using R 2.11.1 (R Development Core Team 2010). First, we tested for treatment effects on  $^{15}\text{N}$  uptake by fitting a full mixed-effects model including diversity, species identity, N source, soil depth, seasons and all interaction terms as fixed effects and block as random effect. Stepwise model selection by AIC was performed to remove variables and interaction terms not improving model fit using the stepAIC () function in the MASS package.  $^{15}\text{N}$  uptake data were square-root transformed to meet assumptions of normality and homoscedasticity.

In order to detect species-specific  $^{15}\text{N}$  uptake preferences for chemical N forms, soil depths and seasons, mixed effect models were fitted for each species separately using fractional data of  $^{15}\text{N}$  uptake. Models were fitted with N source, soil depth and season including interaction with diversity (monoculture and 4-species mixture) as fixed effects and block as random effect. Post-hoc Tukey tests, implemented in the “multcomp” package (Hothorn et al. 2008), were used for multiple comparisons of treatment means.

To test for significant effects of diversity and species identity on niche breadth and niche overlap two-way factorial ANOVA were performed and community niche breadth was compared with niche breadth of monocultures using independent two-sample t-tests.

## Results

### Biomass and N status

Total tree biomass (Table 1, Fig. 2) and plant N stocks (Appendix Fig. 10) of plant individuals sampled for  $^{15}\text{N}$  analysis varied largely between the four species in each sampling period. In general, the two evergreen species *Elaeocarpus* and *Schima* had a higher biomass than *Castanea* and *Quercus*. Biomass of the evergreen species continuously increased throughout the experiment whereas biomass of *Castanea* and *Quercus* showed a maximum in autumn 2009 before leaf shedding but with fast recovery in spring during bud burst followed by leaf flush. Pronounced leaf flush was also observed for *Elaeocarpus* and *Schima* with simultaneously shedding of the previous year's leaves at the beginning of the new growing season. Based on the sampled individuals in each season evergreen species doubled (*Elaeocarpus*) or even tripled (*Schima*) total tree biomass whereas biomass of deciduous species increased about 50 % within our sampling period. Tree species richness significantly affected total community biomass between monocultures and mixtures only at the first harvest in summer 2009, with lower biomass in mixture than in monoculture. In the other three seasons, monoculture and mixture did not differ in tree biomass averaged across species.

Species showed different biomass allocation patterns (Table 2). *Schima* allocated most of its biomass to leaves (44 %) whereas *Quercus* invested most biomass into roots (41 %). Allocation of total biomass to plant organs was most similar between *Castanea* and *Elaeocarpus* with woody shoots accounting for 43 % and 44 % of total biomass, respectively.

Typically, N concentration was highest in leaves, intermediate in roots and lowest in stem tissue in all species (Table 3). The highest N concentration was observed in leaf tissue sampled in spring for the two deciduous species and *Elaeocarpus* but not for *Schima*. The previous year's leaves of *Elaeocarpus* and *Schima* had a significant lower N concentration than new leaves. Root and stem tissue of the deciduous species *Castanea* and *Quercus* showed pronounced seasonal N dynamics with the highest N concentration measured in winter.

### **Treatment effects on $^{15}\text{N}$ uptake**

Highly elevated  $\delta^{15}\text{N}$  values relative to natural background values (average 1 ‰) in plant tissues indicated N uptake from injected  $^{15}\text{N}$  sources. Across all treatments and plant organs  $\delta^{15}\text{N}$  values ranged from -4 ‰ to 1648 ‰ with an average of 80 ‰. Mean recovery of  $^{15}\text{N}$  related to total tree biomass was 6.5 % across species and treatments. Regression analysis showed no significant relation between  $^{13}\text{C}$  excess and  $^{15}\text{N}$  excess in plant tissues, indicating that glycine was presumably not taken up as intact molecules. Following the argumentation of von Felten et al. (2009), we nevertheless included the glycine treatments in the calculation of niche breadth and overlap because microbial transformations of added N sources in the soil prior to plant uptake cannot be ruled out for ammonium and nitrate either. In addition, rapid metabolism of absorbed tracers could also lead to loss of  $^{13}\text{CO}_2$  before detection in plant tissues sampled (Näsholm and Persson 2001).

Across all species and treatments,  $^{15}\text{N}$  uptake per unit DW and amount of  $^{15}\text{N}$  tracer applied was highest in summer ( $2.02 \pm 0.13 \mu\text{g g}^{-1}_{\text{DW}} \text{mg}^{-1}_{\text{tracer}}$ ) and spring ( $1.04 \pm 0.09 \mu\text{g g}^{-1}_{\text{DW}} \text{mg}^{-1}_{\text{tracer}}$ ) and lowest in autumn ( $0.63 \pm 0.08 \mu\text{g g}^{-1}_{\text{DW}} \text{mg}^{-1}_{\text{tracer}}$ ) and winter ( $0.62 \pm 0.07 \mu\text{g g}^{-1}_{\text{DW}} \text{mg}^{-1}_{\text{tracer}}$ ). Injection of  $^{15}\text{N}$  tracers in the shallow soil layer led to higher plant  $^{15}\text{N}$  uptake ( $1.82 \pm 0.08 \mu\text{g g}^{-1}_{\text{DW}} \text{mg}^{-1}_{\text{tracer}}$ ) than in the deep soil layer ( $0.33 \pm 0.03 \mu\text{g g}^{-1}_{\text{DW}} \text{mg}^{-1}_{\text{tracer}}$ ). Plant  $^{15}\text{N}$  uptake was largest for nitrate ( $1.36 \pm 0.11 \mu\text{g g}^{-1}_{\text{DW}} \text{mg}^{-1}_{\text{tracer}}$ ) followed by ammonium ( $1.04 \pm 0.09 \mu\text{g g}^{-1}_{\text{DW}} \text{mg}^{-1}_{\text{tracer}}$ ) and glycine ( $0.80 \pm 0.06 \mu\text{g g}^{-1}_{\text{DW}} \text{mg}^{-1}_{\text{tracer}}$ ) averaged across all species. Species average  $^{15}\text{N}$  uptake across treatments declined in the order *Schima* ( $1.35 \pm 0.13 \mu\text{g g}^{-1}_{\text{DW}} \text{mg}^{-1}_{\text{tracer}}$ ), *Elaeocarpus* ( $1.27 \pm 0.10 \mu\text{g g}^{-1}_{\text{DW}} \text{mg}^{-1}_{\text{tracer}}$ ), *Quercus* ( $0.94 \pm 0.09 \mu\text{g g}^{-1}_{\text{DW}} \text{mg}^{-1}_{\text{tracer}}$ ) and *Castanea* ( $0.70 \pm 0.07 \mu\text{g g}^{-1}_{\text{DW}} \text{mg}^{-1}_{\text{tracer}}$ ). Allocation of  $^{15}\text{N}$  to plant organs varied significantly among species. For example in summer 2009 *Schima* incorporated most the  $^{15}\text{N}$  taken up into leaves

(64 %) whereas in *Quercus* the roots were the most important sink for  $^{15}\text{N}$  (77 %) in the first 6 days after tracer application (Appendix Fig. 8).

Plant  $^{15}\text{N}$  uptake was significantly affected by species identity, N source, soil depth, season and also marginally by plot diversity (monoculture and 4-species mixture, Fig. 3, Table 4). However, the significant species x diversity interaction indicates that  $^{15}\text{N}$  uptake of each species was differently affected by diversity. Similarly, the significant interaction terms species x N form, species x depth and species x season demonstrate strong species-specific effects on spatial-temporal and chemical  $^{15}\text{N}$  uptake patterns.

### Fractions of $^{15}\text{N}$ uptake from different treatments

$^{15}\text{N}$  uptake from applied  $^{15}\text{N}$  sources (glycine,  $\text{NH}_4^+$  and  $\text{NO}_3^-$ ) differed significantly in *Castanea*, *Elaeocarpus* and *Schima* indicating species-specific preferences for N forms (Table 5, Fig. 4). *Castanea* preferred  $\text{NH}_4^+$  (42 %) over  $\text{NO}_3^-$  (30 %) and glycine (28 %) whereas *Quercus* used  $\text{NH}_4^+$  (39 %),  $\text{NO}_3^-$  (34 %) and glycine (27 %) in more equal proportions. Evergreen species clearly showed a preference for  $\text{NO}_3^-$  (49 % in each species respectively) over  $\text{NH}_4^+$  (*Elaeocarpus*: 26 %, *Schima*: 28 %) and glycine (*Elaeocarpus*: 25 %, *Schima*: 23 %). As shown in Fig. 3 this trend was observed to be stable in every season despite temporal variation in pool sizes of soil plant-available N. Plot diversity had no influence on fractions of used N sources.

Tree species satisfied most of their N demand in summer (*Castanea*: 45 %, *Quercus*: 43 %, *Elaeocarpus*: 42 %, *Schima*: 55 %) followed by spring (*Castanea*: 24 %, *Quercus*: 27 %, *Elaeocarpus*: 24 %, *Schima*: 22 %). But a significant fraction of N was taken up also in autumn (*Castanea*: 14 %, *Quercus*: 22 %, *Elaeocarpus*: 13 %, *Schima*: 10 %) and winter (*Castanea*: 17 %, *Quercus*: 8 %, *Elaeocarpus*: 21 %, *Schima*: 13 %). Nevertheless, the significant species x season interaction (Tab. 4) indicates that the four species indeed differed in their temporal uptake patterns. Except for *Quercus*, tree diversity did not affect the observed species-specific seasonal patterns.

The predominant fraction of  $^{15}\text{N}$  taken up was acquired from the shallow soil layer. In average the uptake of  $^{15}\text{N}$  from the shallow soil layer covered 85 % of total  $^{15}\text{N}$  uptake across species. However, species differed slightly but significantly (species x depth interaction, Tab. 4) in their vertical N uptake pattern. Fraction of  $^{15}\text{N}$  uptake from the deep soil layer was lowest for *Castanea* (10 %) whereas the other species acquired in average more  $^{15}\text{N}$  from 20 cm soil depth (*Schima*: 19 %, *Quercus*: 17 %, *Elaeocarpus*: 15 %). The fractions of  $^{15}\text{N}$  uptake from different soil layers remained unaffected by diversity.

### Niche breadth and niche overlap

Tree species diversity did not influence niche breadth of the species studied here. Niche breadth of the four species calculated across diversity levels ranged from 0.39 in *Castanea* to 0.46 in *Elaeocarpus* but species did not differ significantly (Table 6, Fig. 5). Community niche breadth calculated for the 4-species mixture was 0.51 and significantly larger than niche breadth of the *Castanea* monoculture (Welch's t test:  $p = 0.01$ ).

Whether the species were grown under intra- or interspecific competition did not affect niche overlap between species (Table 6, Fig. 6). Niche overlap calculated across diversity levels was highest between the two evergreens *Elaeocarpus* and *Schima* and rather similar for the other five species combinations but differences were not significant.

## Discussion

### Chemical N uptake patterns

We have shown that the four species studied here differed in their spatial, temporal and chemical N uptake patterns, supporting hypothesis 1. Thus, due to such differences in N acquisition, one precondition for complementary resource use might be fulfilled (see von Felten et al. 2009). More specifically, we found that evergreen and deciduous species use chemical N sources differently. Evergreen species clearly preferred  $\text{NO}_3^-$  whereas deciduous species slightly favoured  $\text{NH}_4^+$  (*Castanea*) or followed an undifferentiated uptake (*Quercus*). Currently, there are no general rules regarding chemical preferences of N forms in tree species, as shown by the following examples: Templer and Dawson (2004) identified contrasting preference patterns in seedlings of temperate tree species: whereas sugar maple (*Acer saccharum*) and eastern hemlock (*Tsuga canadensis*) favoured  $\text{NH}_4^+$  over  $\text{NO}_3^-$ , American beech (*Fagus grandifolia*) took up more  $\text{NO}_3^-$  than  $\text{NH}_4^+$  per unit biomass. In a pot experiment using three tropical tree species, higher  $\text{NO}_3^-$  uptake was shown by the pioneer species *Luehea seemannii* while fractions of  $\text{NO}_3^-$  and  $\text{NH}_4^+$  uptake did not differ in the mid and late successional species *Anacardium excelsum* and *Tabebuia rosea* (Zeugin 2010). In contrast, in a wet eucalypt forest coexisting species of dominant *Eucalyptus* and two other sub-canopy species showed a uniform pattern with highest uptake of  $\text{NH}_4^+$  followed by glycine and  $\text{NO}_3^-$  (Paulding et al. 2010; Warren and Adams 2007). Theoretically, it is more likely that our tree species prefer  $\text{NH}_4^+$  for two reasons. First,  $\text{NH}_4^+$  uptake is more cost-efficient as  $\text{NO}_3^-$  uptake requires a higher amount of energy than  $\text{NH}_4^+$  assimilation. This is because two additional enzymes (nitrate reductase and nitrite reductase) are involved in the reduction of  $\text{NO}_3^-$  to  $\text{NH}_4^+$  which is finally incorporated into glutamine (Marschner and Rengel 2007; Miller and Cramer 2005). And second,  $\text{NH}_4^+$  dominates usually over  $\text{NO}_3^-$  in forest soil in subtropical China (Mo et al. 2003; Yan et al. 2009), and it has been suggested that species preference reflects the relative

abundance of plant-available N forms (Kronzucker et al. 1997). However, local availability of  $\text{NH}_4^+$  is constricted by its relatively low immobility and small diffusion coefficient (Miller and Cramer 2005) whereas  $\text{NO}_3^-$  is more mobile in the soil and dominated the mineral N pool on our former agricultural site. Interestingly, several studies have shown that early successional species prefer  $\text{NO}_3^-$  whereas in late successional species  $\text{NH}_4^+$  becomes more important as N source (Aidar et al. 2003; Stewart et al. 1989; Zeugin 2010). Although the fraction of deciduous species decreases and evergreen species become more dominant during forest succession of EBLF in subtropical China (Bruehlheide et al. 2011), we could not relate chemical N use to successional status of the species studied. Instead, high plasticity in chemical N use pattern would allow tree species to adapt quickly to predominant chemical N forms and mycorrhizal symbionts after planting. Although we did not study mycorrhizal associations in our experiment, we frequently observed the occurrence of ectomycorrhizal colonization of roots of *Castanea* and *Quercus*. As ectomycorrhiza increase plant uptake of  $\text{NH}_4^+$  (Buscot et al. 2000) this finding might explain differences in chemical N use between deciduous and evergreen species and support the idea of microbe-mediated niche differentiation (Reynolds et al. 2003).

One caveat of our study was that we did not determine temporal variation and plot-specific  $\text{NO}_3^-$ ,  $\text{NH}_4^+$  and glycine concentration in the soil. However, the relative differences in  $^{15}\text{N}$  uptake from all three N forms were consistent from season to season in each species despite expected temporal changes in plant-available N. Furthermore, we detected pronounced differences in natural  $\delta^{15}\text{N}$  between deciduous and evergreen species indicating that the two functional groups follow different strategies in N acquisition or N metabolic pathways. But it has to be emphasized that natural plant  $\delta^{15}\text{N}$  is not a tracer of N sources taken up by the plant (Evans 2001). Although nitrification leaves  $\text{NH}_4^+$  more enriched in  $^{15}\text{N}$  than  $\text{NO}_3^-$  - which would explain why the evergreen species are naturally more depleted in  $^{15}\text{N}$  if preferring  $\text{NO}_3^-$  (Appendix Fig. 9) - the natural  $\delta^{15}\text{N}$  signature of plant tissues is an integrator of multiple processes (Kahmen et al. 2008). For example discrimination against the heavier  $^{15}\text{N}$  isotope does not only occur during nitrification but also during  $\text{NH}_3$  volatilization, denitrification and mycorrhizal transfer of N (Evans 2001; Garten 1993). We have applied dual-labelled glycine ( $^{15}\text{N}$ ,  $^{13}\text{C}$ ) to test if the plant species studied have direct access to organic N. Several studies have proven that glycine and other amino acids are directly accessible by plants. (Näsholm et al. 1998; Persson and Näsholm 2001; Warren and Adams 2007). Theoretically, intact uptake of doubled-labelled glycine ( $^{15}\text{N}$ ,  $^{13}\text{C}$ ) would lead to a strong positive relationship between  $^{15}\text{N}$  excess and  $^{13}\text{C}$  excess in root extracts (Näsholm et al. 1998) and dried biomass samples (Warren 2009). In our study, injection of doubled-labelled glycine led to a significant enrichment of  $^{15}\text{N}$  in plant tissues that was not related to the measured excess of  $^{13}\text{C}$ . On the one hand detection of the  $^{13}\text{C}$  label in the plant is impeded by the high dilution factor of

$^{13}\text{C}$  in the plant C pool (Näsholm and Persson 2001) and on the other hand by C respiration losses (Hodge et al. 2000).  $^{15}\text{N}$  tracer studies by Warren (2009) and Warren and Adams (2007) demonstrated clearly that plants have the physiological capacity to access organic N compounds directly from a sterile hydrosolution. But given the very short half-life time of amino acids of about 4 h in the soil (Miller and Cramer 2005), it is most likely that also in our case injected glycine was mineralized by soil microbes prior to uptake by plants (Jones et al. 2005). However, we followed the argumentation of von Felten (2009) and decided to take  $^{15}\text{N}$  uptake from applied glycine into account as an additional niche axis (see above).

### **Seasonal N uptake patterns**

The species studied showed similar seasonal N uptake patterns reflecting large N demand during times of high biomass increment and lower N demand in winter. It has to be emphasized that our punctual measurements do not allow integrating total seasonal N uptake. Owing to low soil water content during tracer application in autumn, measured N uptake might be underestimated for example. However, species differed slightly in temporal N uptake dynamics. For instance, *Quercus* maintained relatively high N uptake in autumn compared to summer whereas N acquisition of *Castanea* decreased clearly. Rapid leaf development after bud burst needs great amounts of N that originates to a large fraction from remobilized resources (Chapin 1980; Dyckmans and Flessa 2001; Millard and Proe 1991). In case of evergreen species, N in root, stem and senescing leaves is remobilized and transported to new developing leaf tissue before rapid N uptake by roots in spring. (Aerts 1996; Wendler et al. 1995). Observed time-delayed increase in N uptake in spring could reduce competition between the two evergreen species *Elaeocarpus* and *Schima*. In deciduous species, the role of stem and roots for N storage is even more pronounced indicated by highly elevated N levels that were measured in these tissues in winter. According to Ueda et al. (2009) remobilized N from storage tissues contributes more than 75 % of total N in expanding leaves of *Quercus serrata*. By combining species showing temporal shifts in N remobilization and retranslocation, interspecific competition for N could become reduced at times of high N demand. In this respect, contrasting patterns in phenology are not only important for light interception in stratified canopies but could also foster N partitioning among species (Ewel and Mazzarino 2008).

### **Spatial N uptake patterns**

All species clearly preferred  $^{15}\text{N}$  uptake from the topsoil whereas the deep soil layer was less important as N source, although differences between species were nevertheless evident. Regarding fractions of N uptake from different soil layers, significant differences between species were mainly caused by root architectural traits. For example, *Castanea* developed a lateral root

system in our plots (data not shown), which could explain the highest fraction of N uptake from the shallow soil layer. In contrast, tap-rooted *Quercus* and the evergreens *Elaeocarpus* and *Schima*, characterized by a more diffuse non-specialized root system at sapling stage, had similar spatial N uptake patterns. Application of  $^{15}\text{N}$  tracers at different soil depth is an appropriate tool to study root uptake activity along the soil column (Rowe et al. 2001; Zeugin 2010). Root excavation and determination of vertical distribution of root mass and root length can contribute further important insights into belowground space partitioning among species (Toky and Bisht 1992). For example, the overstory tree species *Hyeronima alchorneoides* and the palm *Euterpe oleracea* were well differentiated in their vertical root distribution contributing to complementary resource use in a study by Ewel and Mazzarino (2008). In a temperate tree diversity experiment with 5-6 year old trees, Lei et al. (2012b) also observed changes in vertical fine root distribution between conifers and hardwood species when grown in mixture, which at least initially might have contributed to higher root production with increasing species richness.

#### **Niche breadth and niche overlap**

We could not confirm our second hypothesis stating that species N uptake becomes more specialized under interspecific competition, thereby decreasing niche breadth and niche overlap. The fundamental niche (under intraspecific competition in monocultures) and realized niche (under interspecific competition in 4-species mixtures) differed not significantly: species preferences and fractions of N uptake from chemical N sources, seasons and soil layers remained mostly unaffected by diversity. One reason might be that belowground competition for space and N resources among trees had not sufficiently developed yet, despite the narrow planting design. Although we have observed high biomass increment over the course of the experiment, root overlap might have not been large enough to induce detectable belowground interactions in the rooting zone. In addition, we also did not observe any diversity effects on aboveground biomass production, except a reduction in mixture at the first harvest. In most grassland biodiversity experiments, belowground responses to changing diversity lag behind aboveground ones (e.g. Bessler et al. 2009). Hence, this experiment simply might have been conducted too early, i.e. before the emergence of species interactions leading to diversity effects on above- or belowground processes. However, in the temperate tree diversity experiment mentioned above, belowground interactions were well detectable at an very early stage of growth, despite larger planting distances than applied here, although after 5-6 years of growth (Lei et al. 2012a). As a second reason, it is likely that plant-available nitrogen was not a limiting factor for tree growth in our experiment as trees were planted on a relatively fertile agricultural field. It has often been argued that complementarity for soil resources should be more pronounced under conditions of

limiting availability of these resources, while competitive exclusion should dominate in more fertile and productive environments (Paquette and Messier 2011; Warren et al. 2009). Although not measured, there is little evidence that N accumulation in trees would have the potential to cause N limitation within one growing season after planting. This is supported by another study conducted at the same experimental site in Xingangshan but on different plots, showing no effect of N fertilization on tree height increment (Both et al. 2012). In N-limited subalpine and tundra ecosystems, however, facilitation of species coexistence through complementarity and plasticity in N use has been demonstrated (Ashton et al. 2010; Pornon et al. 2007). In another alpine study by Miller et al. (2007), plant neighbour species identity did not only affect total  $^{15}\text{N}$  uptake but also fractions of chemical N forms used by a target species. For forest ecosystems, to our knowledge similar studies are not available, but Paquette and Messier (2010) could also show stronger positive tree diversity effects on productivity in less productive boreal forests than in more productive temperate ones. On the contrary, if ecosystem productivity is not limited by soil nutrients, interspecific competition for light becomes more important (Aerts 1999). Given the fast growth rate of the evergreens *Elaeocarpus* and *Schima*, overtopping and shading of deciduous species could have compensated any biodiversity effect on total community biomass despite species-specific differences in N acquisition. First indication of increasing competitiveness of evergreen species was shown in spring as both deciduous species took up less  $^{15}\text{N}$  in mixture than in monoculture, although differences were not significant. Taken these considerations into account, low root overlap and lack of N limitation could have prevented the induction of belowground interspecific interactions and influence of tree diversity on niche breadth of the species studied.

The largest niche overlap in N use was identified between the two evergreen species *Elaeocarpus* and *Schima*. Therefore, we would expect that interspecific competition for N would be most intensive between these two species in the long run. Although niche overlap differed not significantly from other species combinations in mixtures, the observed trend supports the idea that the combination of ecologically similar tree species provokes high niche overlap. Deciduous species had smaller niche overlap than evergreen species because *Castanea* and *Quercus* additionally differed in root architecture and could therefore use N resources more differently than the two evergreens.

Community niche breadth provides a measure for how equally resources are taken up by a plant community along niche axes. It simply describes the potential for resource use complementarity in different plant communities but cannot be directly related to total N uptake. In our experiment, community niche breadth of the 4-species mixture was not significantly larger than niche breadths of the involved monocultures of *Quercus*, *Elaeocarpus* and *Schima*, respectively, thus not

supporting hypothesis 3. This finding can be explained by the relatively large similarity of observed species N use patterns that did not substantially contribute to broadening of the community niche breadth. Furthermore, *Elaeocarpus*, might have dominated N uptake pattern of the community, simply by its large biomass. Similarly, in a grassland study conducted by von Felten et al. (2009), community niche breadth did not increase along a diversity gradient comprising monocultures, three and six species mixtures. However, community niche breadth of the mixture was significantly larger than niche breadth of the *Castanea* monoculture indicating that N uptake was divided more equally among treatments in mixture.

### **Critical assessment of methodology**

One caveat of our study was that we did not account for different pool sizes of plant-available N forms and therefore disregarded  $^{15}\text{N}$  dilution effects in the soil. Our initial measurement conducted in summer 2009 indicated that  $\text{NO}_3^-$  is the dominant mineral N-form in the topsoil layer of the former agricultural field. High nitrate accumulation in agricultural soil is commonly observed in subtropical China due to intensive vegetable cultivation and large amounts of fertilizers applied (Zhu et al. 2011). Although we did not measure seasonal variability of plant-available mineral N, it is likely that the dominance of  $\text{NO}_3^-$  was maintained during the course of our labeling experiment. Therefore the dilution of  $^{15}\text{N}$  in different soil N pools might have affected our results regarding the relative preference for chemical N forms observed. In summer 2009 the  $\text{N-NO}_3^-$  -  $\text{N-NH}_4^+$  ratio was 5.3 : 1 in the top 10 cm of the mineral soil. Taken this dilution factor into account we would have underestimated  $^{15}\text{N}$  uptake from  $\text{N-NO}_3^-$  in all species. However, there remain a number of uncertainties beyond the consideration of dilution effects.

First, we cannot rule out that  $^{15}\text{N}$  was taken up as N form that was applied to our plots. Although we used a nitrification inhibitor some of the  $^{15}\text{N-NH}_4^+$  injected could have been further diluted by microbial transformation into  $\text{NO}_3^-$ . Similar, it is likely that glycine with its short half-life time of a couple of hours in the soil was taken up and mineralized by microorganisms prior to uptake by plants (Miller and Cramer 2005). Furthermore our study did not aim at determining the availability of low complex organic molecules such as glycine in the soil so that we were not able to consider any dilution of  $^{15}\text{N}$ -glycine as well.

Second, a further uncertainty is the unknown immobilization of applied  $^{15}\text{N}$  by the microbial biomass. Large quantities of  $^{15}\text{N}$  tracer might be taken up by bacteria or are incorporated in the fungal mycelium with unknown turnover rate. For example Harrison et al. (2007) recovered most of the  $^{15}\text{N}$  applied in the microbial biomass revealing strong competition between soil microbes and plant for applied  $^{15}\text{N}$  in the first days of their experiment. Therefore, further studies need to take into account such immobilization processes and to consider not only  $^{15}\text{N}$  dilution in plant-

available N pools but also in the microbial biomass. This has to be done plot-specifically because different tree species and tree species combinations might promote or suppress certain types of soil microorganisms.

Third, diffusion coefficients of chemical N forms vary greatly leading to different soil volumes enriched with  $^{15}\text{N}$  (Jones et al. 2005). This leads to uncertainties when calculating the amount of plant-available N in a labeled soil volume. In addition, soil water content affecting especially the mobility of  $\text{NO}_3^-$  in the soil solution, might be influenced by tree species-specific canopy interception and transpiration rates. Furthermore, we cannot rule out that  $\text{NO}_3^-$  was leached to deeper soil layers or was carried away by lateral transport during strong rain events although plots were covered with plastic tarps.

Fourth, strong lateral root growth was observed in some species (e.g. *Elaeocarpus*) that enabled tree individuals to access N from unlabeled soil. This would have led to further dilution of  $^{15}\text{N}$  in the plant compared to tree individuals rooting exclusively in the labeled soil column. Moreover, spacing of tracer injections may be an influential factor for  $^{15}\text{N}$  uptake (McKane et al. 2002) because species forming a lateral and dense rooting system might have a greater probability to reach more injection points than tap-rooted species.

Against this background we clearly recommend the consideration of  $^{15}\text{N}$  dilution effects to account for different soil N pool sizes. In addition, the quantification of  $^{15}\text{N}$  turnover rates as well as the recovery of  $^{15}\text{N}$  in different soil N pools at the end of experiment would further improve our understanding of the fate of  $^{15}\text{N}$  during tracer experiments. Thereby, all analyses have to be done on the plot level as tree species might affect transformation and fluxes of injected  $^{15}\text{N}$  tracers differently.

## Conclusions

We conclude that differences in N acquisition patterns can facilitate species coexistence by reducing interspecific competition between the investigated species. In this context, we expect that differences in plant traits e.g. phenology and root architecture promote niche differentiation in space, time and chemical N form. Although niche breadth and niche overlap was not influenced by tree diversity in our study, N partitioning and plasticity in resource use could become more important during stand development. Therefore, in designing sustainable tree plantations or multifunctional forests one needs to consider species-specific differences in multidimensional resource use in order to combine those species yielding the greatest exploitation of available nutrients. However, this requires detailed knowledge about plant nutrient acquisition and nutritional interactions among tree species as well the microbial subsystem. Taken these considerations into account, future studies should focus on compound effects of complementary

use of several resources such as N, water, phosphorous and light, together with the spatial, temporal and chemical partitioning of resources studied here, to address community niche breadth and resource exploitation along diversity gradients.

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## **Tables and Figures**

**Table 1** Calculated total biomass expressed as dry weight (g) of sampled tree individuals harvested six days after tracer application. Means and standard errors are presented (n = 24 for monoculture and mixture, respectively, n = 48 for overall mean).

Species	Monoculture	Mixture	Overall
<b><i>Castanea henryi</i></b>			
Summer 09	32.9 ± 6.1	26.2 ± 4.9	30.0 ± 4.0
Autumn 09	50.1 ± 5.5	50.4 ± 13.8	50.2 ± 6.3
Winter 10	36.0 ± 7.0	28.4 ± 4.6	33.0 ± 4.6
Spring 10	47.6 ± 4.7	37.0 ± 6.1	43.2 ± 3.8
<b><i>Quercus serrata</i></b>			
Summer 09	31.4 ± 3.3	21.6 ± 2.2	26.6 ± 2.1
Autumn 09	49.3 ± 3.9	41.7 ± 5.7	45.5 ± 3.5
Winter 10	32.5 ± 3.2	33.9 ± 5.0	33.2 ± 2.9
Spring 10	41.4 ± 2.7	42.6 ± 4.5	42.0 ± 2.6
<b><i>Elaeocarpus decipiens</i></b>			
Summer 09	75.4 ± 7.2	49.1 ± 6.3	62.8 ± 5.2
Autumn 09	93.7 ± 9.1	99.2 ± 8.5	96.5 ± 6.2
Winter 10	117.1 ± 10.4	111.5 ± 13.4	114.4 ± 8.3
Spring 10	125.2 ± 11.7	158.5 ± 18.8	141.8 ± 11.2
<b><i>Schima superba</i></b>			
Summer 09	31.6 ± 3.3	23.9 ± 3.9	28.2 ± 2.6
Autumn 09	62.1 ± 5.5	63.5 ± 7.6	62.7 ± 4.6
Winter 10	95.0 ± 10.5	52.5 ± 5.6	76.2 ± 7.1
Spring 10	88.8 ± 9.5	75.7 ± 11.5	83.0 ± 7.3

**Table 2** Species-specific allocation (%) of total tree biomass to plant organs.

Species	Roots	Stem	Leaves
<i>Castanea henryi</i>	31.74	42.81	25.45
<i>Quercus serrata</i>	41.28	30.11	28.61
<i>Elaeocarpus decipiens</i>	27.75	43.88	28.37
<i>Schima superba</i>	25.13	31.14	43.73

**Table 3** N concentration (mg N g<sup>-1</sup>) of plant tissues (roots, stem, leaves) of sampled tree individuals harvested six days after tracer application. Means and standard errors are presented (n = 48).

Species	Roots	Stem	Leaves	Leaves (old)
<b><i>Castanea henryi</i></b>				
Summer 09	10.5 ± 0.3	5.7 ± 0.1	19.7 ± 0.2	
Autumn 09	10.6 ± 0.3	7.3 ± 0.2	20.9 ± 0.3	
Winter 10	16.6 ± 0.3	10.4 ± 0.8		
Spring 10	10.7 ± 0.3	5.1 ± 0.2	22.6 ± 0.6	
<b><i>Quercus serrata</i></b>				
Summer 09	11.9 ± 0.3	6.0 ± 0.1	18.9 ± 0.3	
Autumn 09	11.5 ± 0.3	5.9 ± 0.1	20.5 ± 0.3	
Winter 10	15.7 ± 0.3	8.6 ± 0.2		
Spring 10	10.8 ± 0.3	5.6 ± 0.2	22.7 ± 0.4	
<b><i>Elaeocarpus decipiens</i></b>				
Summer 09	11.5 ± 0.2	4.5 ± 0.1	18.7 ± 0.4	
Autumn 09	11.6 ± 0.2	4.7 ± 0.1	18.1 ± 0.3	
Winter 10	12.6 ± 0.2	5.6 ± 0.2	17.8 ± 0.5	
Spring 10	12.0 ± 0.2	5.5 ± 0.2	22.7 ± 0.6	15.4 ± 0.3
<b><i>Schima superba</i></b>				
Summer 09	9.9 ± 0.3	3.7 ± 0.2	20.3 ± 0.5	
Autumn 09	9.7 ± 0.2	4.0 ± 0.1	15.7 ± 0.4	
Winter 10	10.2 ± 0.3	5.3 ± 0.2	16.1 ± 0.5	
Spring 10	9.7 ± 0.2	4.0 ± 0.1	16.4 ± 0.4	12.1 ± 0.3

**Table 4** Results of a simplified mixed-effects model for treatment effects on <sup>15</sup>N uptake per unit DW and <sup>15</sup>N tracer applied.

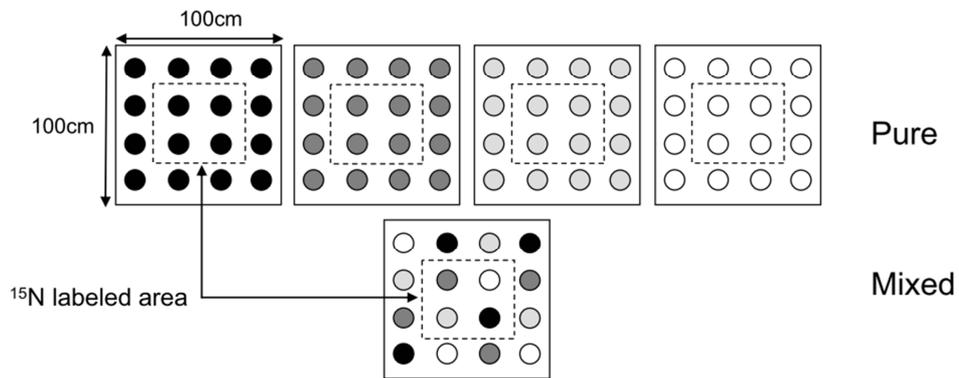
	DF <sub>n</sub>	DF <sub>d</sub>	F-value	p-value
Diversity (Div)	1	660	4	0.061
Species	3	660	28	<0.001
N source	2	660	33	<0.001
Depth	1	660	1223	<0.001
Season	3	660	196	<0.001
Species : Div	3	660	3	0.026
Species : N source	6	660	10	<0.001
Species : Depth	3	660	4	0.009
N source : Depth	2	660	3	0.031
Species : Season	9	660	10	<0.001
Species : N source : Depth	6	660	5	<0.001
Species : Depth : Season	12	660	3	0.001

**Table 5** Results of mixed effects models calculated on fractional  $^{15}\text{N}$  uptake from each treatment. Fixed effects of N source, season and soil depth and interaction terms including diversity are shown for each species. Fractional  $^{15}\text{N}$  uptake was square root transformed prior to analysis.

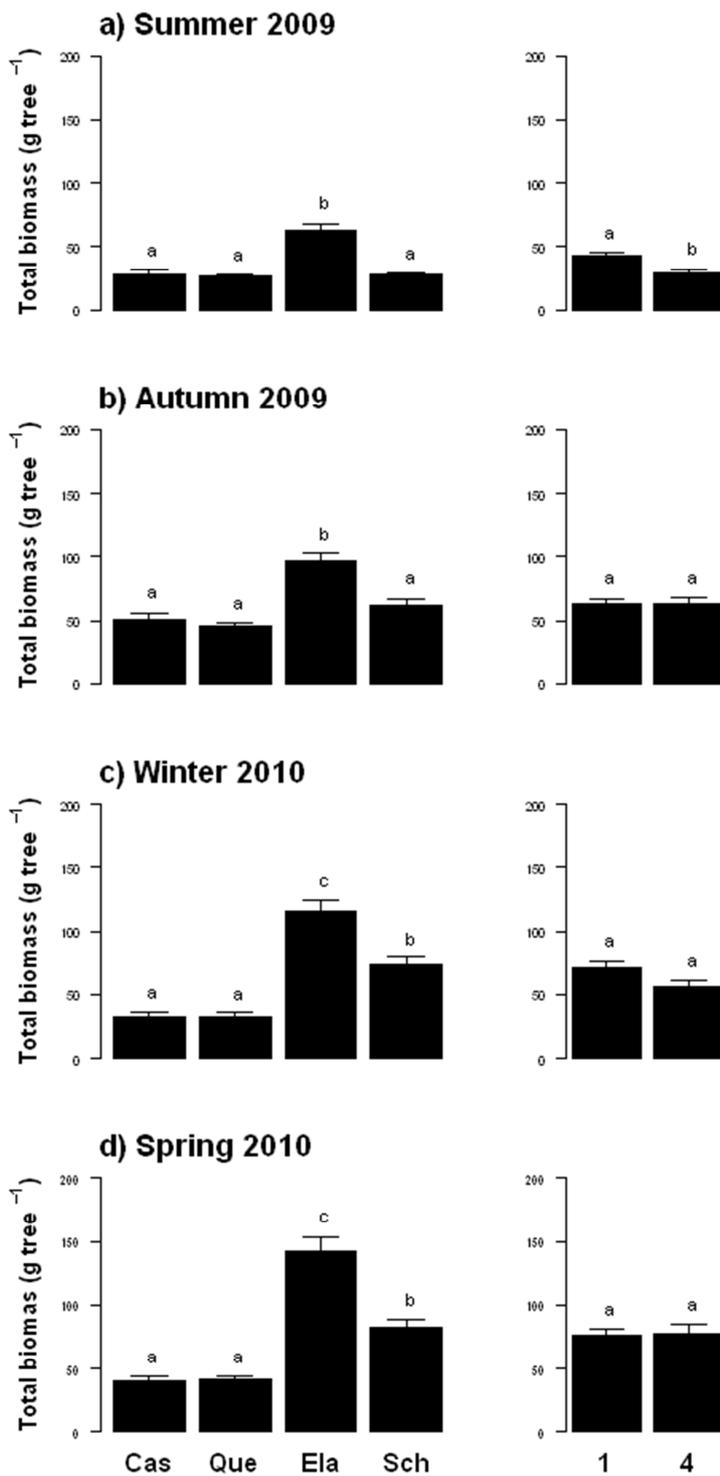
<b>Fixed effects</b>	<b>DF<sub>n</sub></b>	<b>F-value</b>	<b>p-value</b>
<b><i>Castanea henryi</i></b>			
N source	2	5.53	<b>0.005</b>
Season	3	37.74	<b>&lt;0.001</b>
Depth	1	422.43	<b>&lt;0.001</b>
N source : Diversity	3	0.93	0.425
Season : Diversity	3	1.79	0.150
Depth : Diversity	1	0.40	0.529
<b><i>Quercus serrata</i></b>			
N source	2	1.72	0.182
Season	3	39.96	<b>&lt;0.001</b>
Depth	1	204.31	<b>&lt;0.001</b>
N source : Diversity	3	0.17	0.916
Season : Diversity	3	3.07	<b>0.029</b>
Depth : Diversity	1	0.23	0.635
<b><i>Elaeocarpus decipiens</i></b>			
N source	2	35.77	<b>&lt;0.001</b>
Season	3	61.24	<b>&lt;0.001</b>
Depth	1	426.81	<b>&lt;0.001</b>
N source : Diversity	3	0.57	0.636
Season : Diversity	3	0.26	0.852
Depth : Diversity	1	0.55	0.461
<b><i>Schima suberba</i></b>			
N source	2	26.38	<b>&lt;0.001</b>
Season	3	108.84	<b>&lt;0.001</b>
Depth	1	348.51	<b>&lt;0.001</b>
N source : Diversity	3	0.73	0.538
Season : Diversity	3	0.53	0.661
Depth : Diversity	1	1.19	0.278

**Table 6** Analysis of variance for effects of plot diversity and species identity on niche breadth and niche overlap.

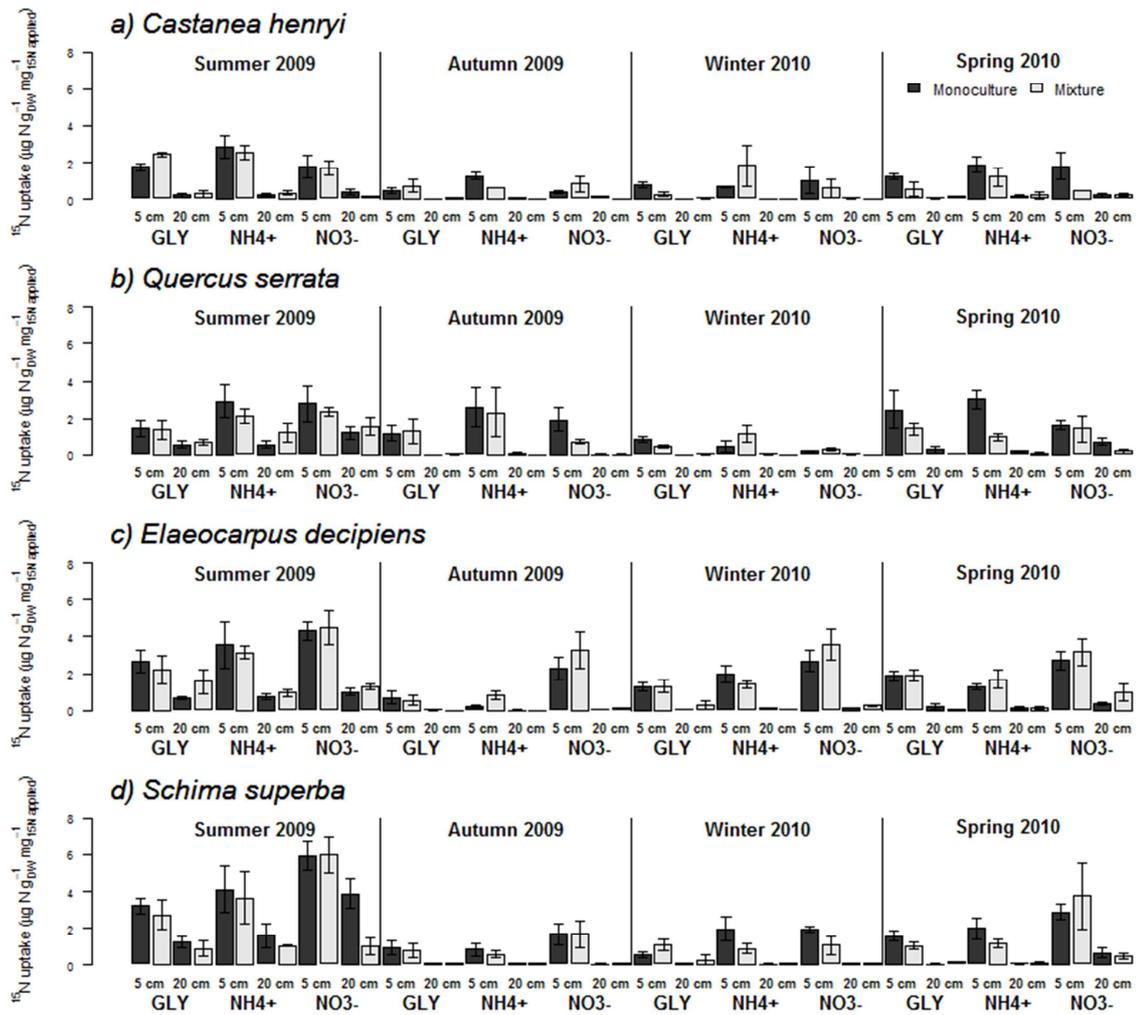
<b>Niche breadth</b>	DF	Sum Sq	Mean Sq	F value	P value
Diversity	1	0.001	0.001	0.247	0.624
Species	3	0.020	0.007	1.232	0.320
Diversity : Species	3	0.013	0.004	0.811	0.500
Residuals	24	0.128	0.005		
<b>Niche overlap</b>					
Diversity	1	0.000	0.000	0.021	0.886
Species Pair	5	0.037	0.007	1.214	0.322
Diversity : Species Pair	5	0.019	0.004	0.612	0.691
Residuals	36	0.218	0.006		



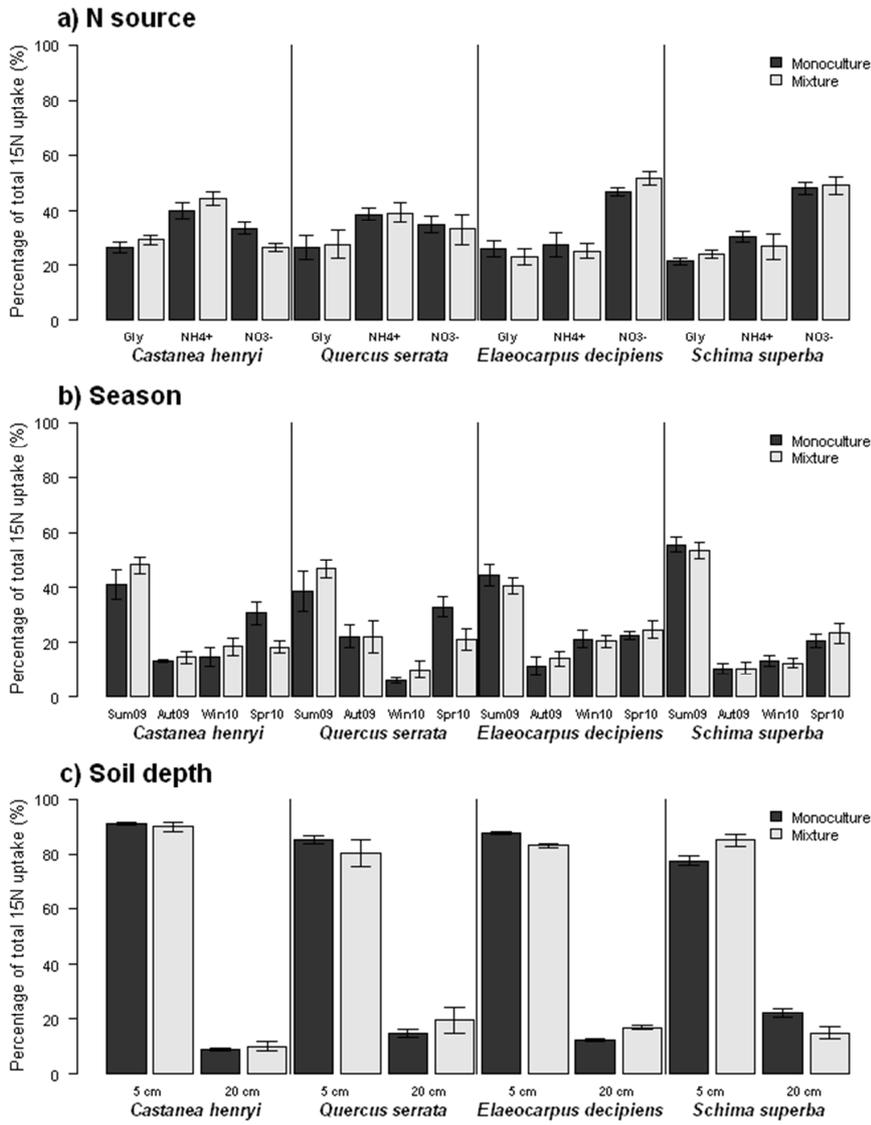
**Fig. 1** Tree saplings were planted in monoculture and in 4-species mixtures in plots containing 16 tree individuals, respectively. Planting distance was about 25 cm to induce inter- and intraspecific competition shortly after planting. Different grey scales of circles represent four different tree species. <sup>15</sup>N tracers were injected in 49 single equally-spaced injections (7 x 7) covering the inner square in each plot.



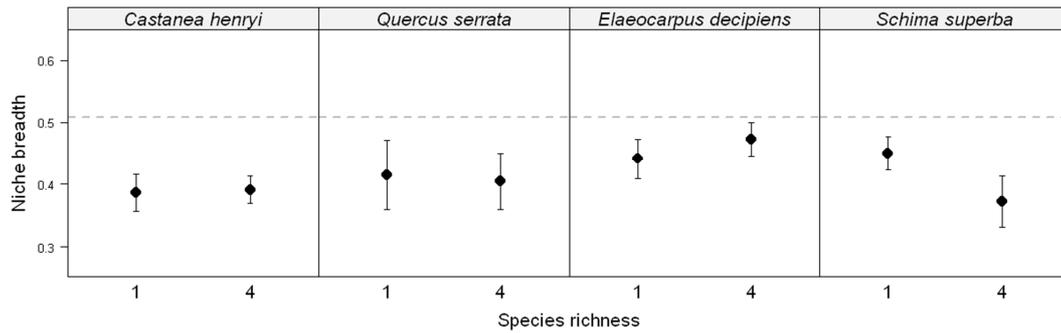
**Fig. 2** Total tree biomass expressed as dry weight (g) calculated as average of all harvested target individuals across diversity levels (left panels) and across species for monoculture and 4-species mixture (right panels) in each season (Cas = *Castanea henryi*, Que = *Quercus serrata*, Ela = *Elaeocarpus decipiens*, Sch = *Schima superba*). Differing letters indicate significant differences ( $p < 0.05$ ) between the four species and between diversity levels, respectively.



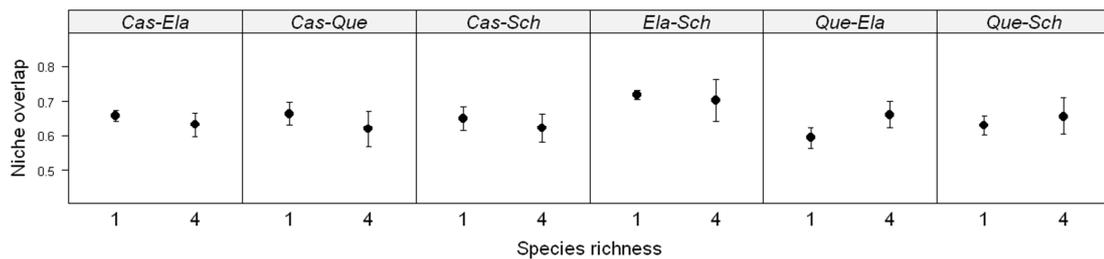
**Fig. 3** Total plant  $^{15}\text{N}$  uptake per unit DW and  $^{15}\text{N}$  tracer applied in monoculture and 4-species mixture for all 4 species and 24 treatments, respectively.



**Fig. 4** Fraction of <sup>15</sup>N uptake from N sources (a), seasons (b) and soil depths (c) accumulated across all treatment combinations. Error bars represent standard error of the mean (n = 4).



**Fig. 5** Niche breadth calculated as Levins' normalized B of the four studied species in monoculture and in mixture. Error bars are stand errors of the mean based on 4 replicates. Dashed grey line indicates community niche breadth calculated from treatment fractions of  $^{15}\text{N}$  uptake per unit biomass by all four species in mixture.

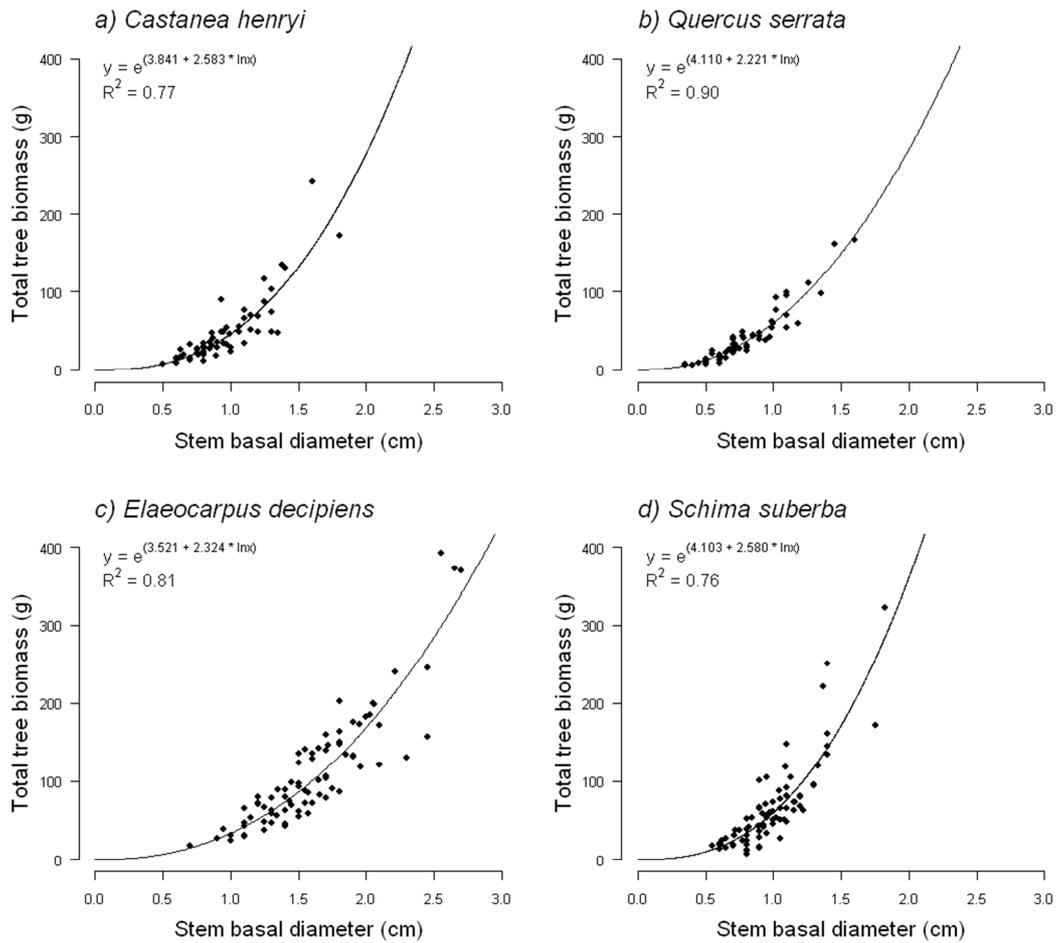


**Fig. 6** Niche overlap between species grown in monoculture or mixture. Error bars are standard errors of the mean based on 4 replicates. Species abbreviations: Cas = *Castanea henryi*, Que = *Quercus serrata*, Ela = *Elaeocarpus decipiens*, Sch = *Schima superba*.

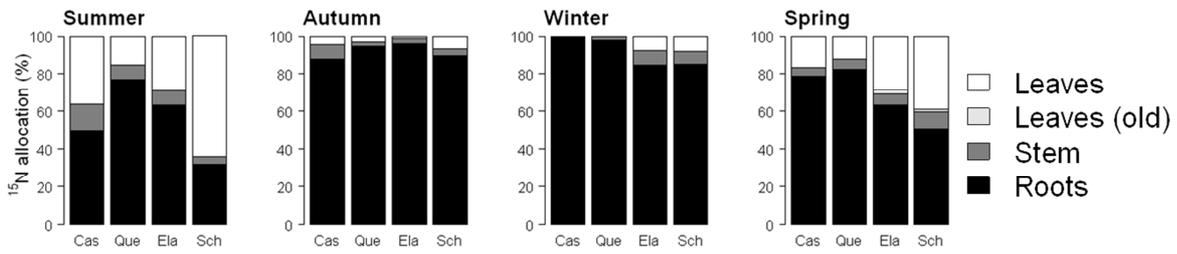
## Appendix

**Table 7** Soil characteristics for the two soil layers that received  $^{15}\text{N}$  tracer solutions. Note that mineral N concentration and soil moisture were measured in summer 2009. All other variables were determined in autumn 2009 and winter 2010.

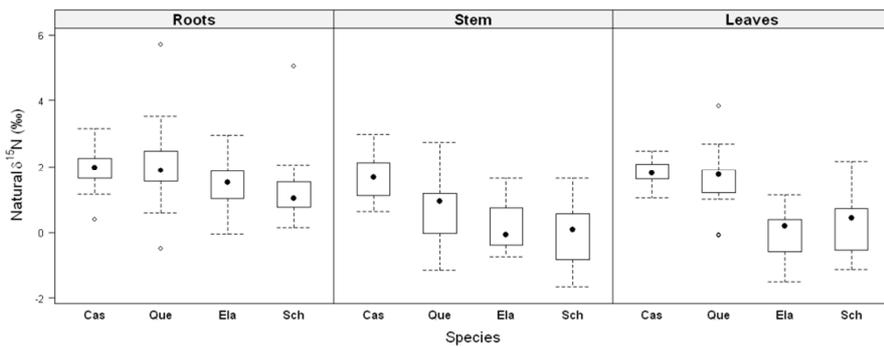
Soil characteristics	0 - 10 cm	15 - 25 cm
N (%)	$0.37 \pm 0.01$	$0.26 \pm 0.01$
C (%)	$1.80 \pm 0.04$	$0.80 \pm 0.07$
C:N ratio	$4.95 \pm 0.15$	$3.09 \pm 0.22$
$\text{NO}_3^-$ (mg N $\text{kg}^{-1}$ soil)	$19.40 \pm 3.27$	$0.68 \pm 0.39$
$\text{NH}_4^+$ (mg N $\text{kg}^{-1}$ soil)	$3.64 \pm 0.39$	$2.18 \pm 0.18$
pH (measured in $\text{H}_2\text{O}$ )	$4.92 \pm 0.07$	$5.43 \pm 0.17$
Soil moisture (g $\text{H}_2\text{O}$ $\text{g}^{-1}$ dry soil)	$25.07 \pm 1.07$	$19.65 \pm 0.47$
Bulk density ( $\text{g cm}^{-3}$ )	$1.09 \pm 0.04$	$1.64 \pm 0.03$



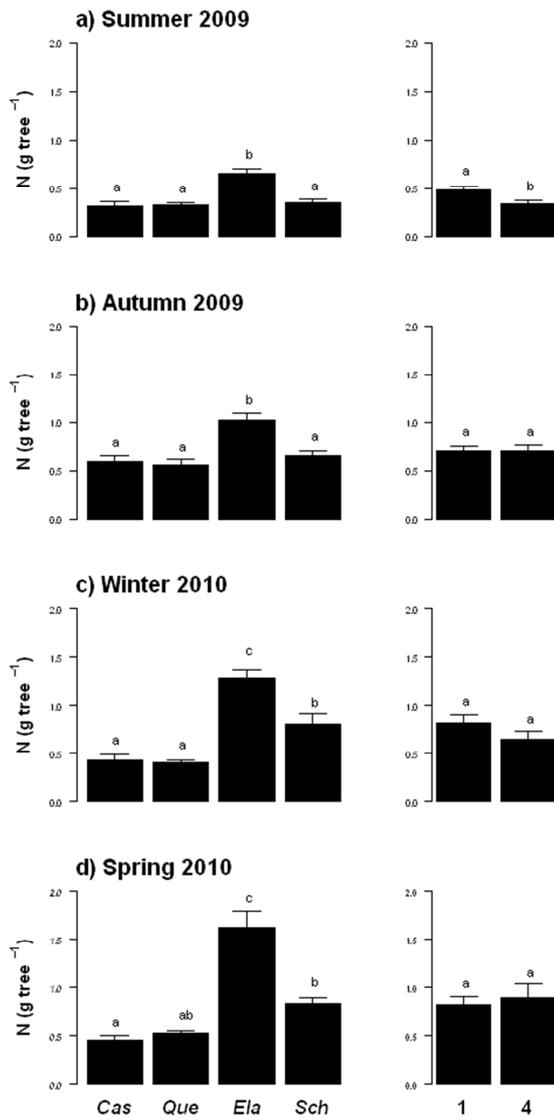
**Fig. 7** Allometric equations for the relationship between total tree biomass and stem basal diameter determined for each species. Equations of deciduous species are based on 60 individuals whereas for evergreen species 80 individuals were sampled.



**Fig. 8** Allocation of <sup>15</sup>N to plant tissues in each season. Species abbreviation: Cas = *Castanea henryi*, Que = *Quercus serrata*, Ela = *Elaeocarpus decipiens*, Sch = *Schima superba*.



**Fig. 9** Natural  $\delta^{15}\text{N}$  values in roots, stem and leaves for all species averaged across seasons (n=16). Species abbreviation: Cas = *Castanea henryi*, Que = *Quercus serrata*, Ela = *Elaeocarpus decipiens*, Sch = *Schima superba*.



**Fig. 10** Amount of N stored in tree species calculated as average of all harvested target individuals across diversity levels. Furthermore, mean N storage of all trees in monoculture and 4-species mixtures are shown. Species abbreviation: Cas = *Castanea henryi*, Que = *Quercus serrata*, Ela = *Elaeocarpus decipiens*, Sch = *Schima superba*.



# GENERAL DISCUSSION

In recent years, questions have been raised how plant diversity may maintain vital services in forest ecosystems, reflected by the establishment of several larger forest diversity experiments, among other scientific approaches (Scherer-Lorenzen et al. 2005). Forests are of paramount importance as they play a central role in global biogeochemical cycles and provide multiple ecosystem services. Despite the long history in silvicultural research the significance of tree diversity for forest functioning remains largely unidentified (Nadrowski et al. 2010). The scope of this thesis was to identify the significance of tree diversity for soil N pools, leaf litter decomposition and N uptake-complementarity in subtropical forests in South-East China. I focused on N as it is a key element in plant nutrition and limits most often ecosystem productivity (LeBauer and Treseder 2008).

In an observational and comparative approach, I determined total soil and litter total N stocks, the soil mineral N pool as well as net N mineralization along gradients of species richness and forest stand age (Chapter 1). Here, I hypothesized that tree diversity, forest stand age and environmental factors all have – inter alia – important effects on the soil N status of secondary forest stands of a species-rich subtropical forest ecosystem. Effects of woody plant diversity on leaf litter decomposition, an important process replenishing the pools of plant-available N, were studied in three complementary decomposition experiments (Chapter 2). This study investigated the hypothesis that plant diversity at the stand level and number of species in decomposing litter positively affect decomposition processes. Finally, I tested for spatio-temporal and chemical N uptake complementarity in a tree mixture experiment using  $^{15}\text{N}$  tracers (Chapter 3). Belowground niche complementarity was hypothesized to be an important process leading to higher resource exploitation and facilitation of species coexistence. Beyond that, this work has contributed to two studies focusing on plant community assembly during forest succession (Bruehlheide et al. 2011) and the influence of diversity and successional stage on the interaction between the tree and herb layer (Both et al. 2011).

The three independent, but thematically interlinked projects of this PhD thesis were conducted in subtropical South-East China, one of the World's biodiversity hotspots of terrestrial plants (Bruehlheide et al. 2011). The two observational and comparative studies in secondary forests were carried out in the Gutianshan National Nature Reserve (Zhejiang Province), while the experimental study on N complementarity was done in Xingangshan (Jiangxi Province).

My first study conducted in Gutianshan provided good insights on how soil and litter N pools are related to tree diversity, forest stand age and environmental factors (Chapter 1). I found that soil physical properties were more important in determining the N status in the mineral soil than tree

diversity and forest stand age. So far, many studies have addressed plant diversity effects on N dynamics in herbaceous experimental model systems under homogenous site conditions (e.g. Hooper and Vitousek 1998; Oelmann et al. 2007). However experimental derived results encounter limitations when applied to natural ecosystems with different species composition and environmental factors (Kahmen et al. 2005). Several studies have demonstrated decreasing levels of soil mineral N in the rooting zone with increasing plant species richness or functional diversity in grassland experiments (Scherer-Lorenzen et al. 2003; Tilman et al. 1996). These findings suggest that plant diversity has clear effects on the soil N status because of higher N exploitation and retention in the soil-plant system at higher levels of plant species richness. However, due to large environmental heterogeneity the identification of direct effects of tree diversity on the soil N status was impeded in our study. This is because soil physical properties strongly determine N storage potential and accumulation (Chapin et al. 2002). Strong dependency of the N status and aboveground net primary production of forest stands on soil properties was also confirmed by Reich et al. (1997). Similarly, abiotic site conditions had a higher control on tree productivity than species richness in a tropical tree plantation (Healy et al. 2008).

Results derived from observational studies have to be interpreted with caution as the separation of cause and effect of biodiversity – ecosystem functioning relationships is difficult in natural ecosystems. For example, the distribution of 88.2 % of examined species in Gutianshan show strong associations with soil properties (Zhang et al. 2011). Thus, soil properties could have influenced spatial patterns in species composition in the evergreen broad-leaved forest studied, although species richness was not related to abiotic site conditions (Bruehlheide et al. 2011).

Along the successional gradient, nitrogen accumulation was only observed in the litter layer emphasizing the importance of detrital matter for N retention during forest development in forest ecosystems (Fisk et al. 2002). Therefore, nitrogen retention can be regarded as primarily based on the accumulation of detrital matter and tree biomass, indicated by an increase in tree basal area during succession, in this evergreen broad-leaved forest. During forest succession large amounts of N accumulate in the plant and forest floor (Yang et al. 2011) and it might therefore be possible that changes in tree species composition and diversity may also have effects on litter decomposition, an aspect studied in detail here, too (Chapter 2). Tree species richness could theoretically increase soil N interception and reduce leaching losses, therefore leading to higher N availability for tree growth and larger plant N pool sizes: a more efficient N exploitation in species-rich forest stands could be either attributed to higher occupation of belowground space or by complementary N use. Therefore, I studied this potential mechanism of tree diversity effects on N dynamics experimentally with young tree saplings (Chapter 3).

Changes in plant diversity can also influence litter decomposition, a key process in nutrient cycling (Gessner et al. 2010; Hättenschwiler et al. 2005; Song et al. 2010). Leaf litter differing in physico-chemical traits do not decompose isolated but interact with each other, and antagonistic as well as synergistic effects on decomposition processes have been observed in litter mixtures (Gartner and Cardon 2004). In Chapter 2, I have shown that litter of 26 coexisting tree species varied profoundly in decomposition rate that was negatively affected by the initial litter C:N ratio. Typically, litter of the coniferous species possessed the lowest decomposition rate constant but also some broad-leaved species produced comparable recalcitrant litter. Plants can have therefore strong species-specific effects on biogeochemical cycles via litter quality that determines the rate of decomposition (Cornwell et al. 2008; Melillo et al. 1982). For example, the observed accumulation of the forest floor litter N pool (Chapter 1) might be also a result of changes in species composition during succession with the proportion of species producing a more recalcitrant litter becoming more dominant.

In a litter mixture experiment, I could show that the majority of plot-specific litter mixtures decomposed faster than predicted from single decomposition rates and that litter species richness marginally positively influenced decomposition. The identification of underlying mechanisms leading to the demonstrated positive mixture effects during decomposition was beyond the scope of this study. However, it is most likely that several mechanisms (nutrient transfer, effects of specific compounds, improved microenvironmental conditions and interaction across trophic levels), reviewed by Hättenschwiler et al. (2005), have concurred. The relative importance of each single mechanism is difficult to quantify. For example, our results indicated that coniferous leaf litter significantly decomposed faster when mixed with broad-leaved litter. Such interactive litter mixture effects could have resulted from nutrient transfer either by passive diffusion or mediated by fungal hyphae between litter species differing in N content as shown by several studies (Briones and Ineson 1996; Lummer et al. 2012; Salamanca et al. 1998; Schimel and Hättenschwiler 2007). Tree diversity mediated effects on the microbial community could have also played a role: plant diversity can have a positive effect on microbial activity and functional diversity (Stephan et al. 2000; Zak et al. 2003) and microbial diversity could also increase the rate of decomposition if complementary enzymatic activities lead to higher decay rates. Interestingly, a recent study shows that decomposition is more affected by detritivore diversity than by litter diversity (Srivastava et al. 2009). Our results support the idea that decomposition of mixed leaf litter could reduce N limitation by higher nutrient turnover rate. However, strong species-specific effects during decomposition have been observed in other experiments (Chapman and Koch 2007; Wang et al. 2009) which makes it difficult to predict interactive litter mixture effects. This

also provides an argument that leaf litter identity is more important than leaf litter richness in determining decomposition rates. (Gießelmann et al. 2010).

Tree diversity and tree species composition at the stand level might also affect decomposition via changes in microclimatic conditions (Hättenschwiler et al. 2005; Prescott 2002) and activity of the decomposer community (De Deyn and van der Putten 2005). I could not confirm this hypothesis along the gradient of species richness in Gutianshan. Instead, I found a decrease in litter decomposition of *Schima superba* leaf litter with forest stand age suggesting that N turnover slows down during forest succession (Odum 1969).

Whereas I have addressed the significance of tree diversity for the soil N status and leaf litter decomposition in secondary forests stands varying in tree species richness and successional stage, spatio-temporal and chemical N use complementarity of coexisting subtropical tree species was evaluated under controlled levels of species richness in an additional mixture experiment (Chapter 3). Resource use complementarity is an important mechanism leading to a positive relationship between biodiversity and ecosystem functioning, characterized by reduced interspecific competition and higher resource exploitation and biomass production in high versus low diversity systems (Eisenhauer 2012; Loreau and Hector 2001). Previous research on belowground niche differentiation have focused on grasslands (Ashton et al. 2010; Kahmen et al. 2006; McKane et al. 1990; Pornon et al. 2007; von Felten et al. 2009) and only a few studies have considered spatio-temporal and chemical N use in a single experiment (McKane et al. 2002). The  $^{15}\text{N}$  tracer injection technique, successfully tested in a tropical tree plantation by Zeugin et al. (2010), was used to reveal species-specific N uptake patterns of tree saplings planted in monocultures and 4-species mixtures. Similar to other experiments, I found significant differences in multidimensional N acquisition patterns among species and functional groups. For example, evergreen species preferred nitrate whereas deciduous species slightly preferred ammonium. Besides plant-physiological differences in N uptake among species (Miller and Cramer 2005), a further explanation for species-specific preferences in chemical N form may be microbe-mediated niche differentiation (Reynolds et al. 2003). Especially ectomycorrhiza, also associated with the two deciduous species *Castanea henryi* and *Quercus serrata* in our experiment, was shown to increase plant uptake of ammonium (Buscot et al. 2000). Although several studies have proven uptake of glycine as intact molecule (Näsholm and Persson 2001; Warren 2009), we could not confirm this finding in our study. A possible explanation for this might be that glycine was rapidly mineralized or that the detection of the  $^{13}\text{C}$  signal was hampered by high dilution in the plant C pool or by respiration losses (Näsholm and Persson 2001).

The observed differences in N uptake preferences have the potential to decrease interspecific competition to some extent. However, higher N exploitation or a significant broader community

niche breadth in the 4-species mixtures could not be revealed. It can be argued that species-specific differences in N uptake were too similar in order to significantly extend the community niche breadth and therefore the potential for higher N exploitation. Similarly, the community niche breadth did not increase with species richness in a grassland experiment (von Felten et al. 2009). Another study has reported plasticity and shifts in resource use depending on the strength of competitive interactions (Ashton et al. 2010) suggesting highly dynamic ecological niches. Instead, we found that the preference in N resource use of a species remained unaffected across diversity levels. While our findings are important at the sapling stage, there remains uncertainty on how N uptake patterns will change if interspecific competition intensifies during stand development.

In conclusion, this thesis has shown that tree diversity effects revealed by experimental studies are difficult to isolate in an observational approach due to large environmental heterogeneity (Chapter 1). Especially, stable soil physical properties were more important in determining the N status in the mineral soil than tree diversity and forest stand age. The significance of tree diversity for leaf litter decomposition was shown by predominant synergistic effects in multi-species litter mixtures, although litter species richness did not substantially increase decomposition in this study (Chapter 2). Hence, species-specific effects largely dominate decomposition processes. Regarding the mechanistic understanding of potential tree diversity effects on N dynamics, my experimental approach showed that coexisting tree species may differ in their spatio-temporal and chemical N uptake pattern, but that these differences in resource use did not support greater community N utilization (Chapter 3). Future studies should therefore focus on compound effects of complementary use of multiple resources such as N, water, phosphorous and light to address community niche breadth and resource exploitation along diversity gradients.

The findings of this thesis highlight the importance of studying tree diversity effects on ecosystem N dynamics in an integrated approach as the soil N status, leaf litter decomposition and plant N uptake are intimately linked. Clearly, more observational studies along gradients of species richness and stand age in various forest ecosystems are needed, combined with experimental approaches that allow the manipulation of tree species diversity. A better understanding of the significance of tree diversity for key processes in the nitrogen cycle is essential to predict potential changes in ecosystem functioning as a consequence of declining species richness. In this respect, the importance of N is highlighted by its ambivalent impact: on the one hand, N promotes growth and biomass production, but on the other hand, excess plant-available N is one of the most important threats to global biodiversity (Sala et al. 2000). Therefore, considerably more work will

need to be done to disentangle these interactions and to evaluate the biodiversity – ecosystem functioning relationship at the ecosystem level.

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# CURRICULUM VITAE

## Stefan Trogisch

(Dipl.-Biol.)

**Date of birth:** 17th May 1983  
**Place of birth:** Leipzig, Germany

### Education

- 2008 - 2012** **PhD Candidate, ETH Zurich, Switzerland**
- Thesis:** "The functional significance of tree diversity for soil N-pools, leaf litter decomposition and N-uptake complementarity in subtropical forests in China"
- 2002 - 2008** **Studies in Biology, University of Leipzig, Germany**
- Major subjects: Ecology, Botany, Genetics, Biochemistry
- Diploma thesis:** "Diversität und indigene Nutzung der Pflanzenfamilien Araceae, Gesneriaceae und Piperaceae in Abhängigkeit von verschiedenen Standortparametern im Biosphärenreservat Sumaco, Ecuador"
- Semester thesis:** "Vegetationsanalyse eines afro-montanen Regenwaldes und Einfluss des Baumbestandes auf Volumen und Verteilung der Kronentraufe"
- 2001** **Abitur, Johann-Gottfried-Seume Gymnasium Grimma**

### Field work

- 2008 – 2010** **China**, Gutianshan National Forest Reserve (15 months)  
**2006** **Ecuador**, Sumaco Napo-Galeras National Park (3 months)  
**2005** **Kenya**, Kakamega Forest Reserve (3 months)  
**Brazil**, Excursion, Mata Atlântica and cerrados (3 weeks)

## Other work experience

2003 –2006                      Student assistant, System Ecotoxicology, Helmholtz Centre for Environmental Research - UFZ Leipzig (6 months)

## Conference contributions

Trogisch S, He JS, Hector A, Scherer-Lorenzen M (2011) Testing for N-use complementarity among four subtropical tree species using <sup>15</sup>N tracers, Gesellschaft für Ökologie (GfÖ), 41st Annual Conference 2011, Oldenburg/Germany, talk

Trogisch S & Scherer-Lorenzen M (2011) <sup>15</sup>N tracer technique as effective tool to study N-use complementarity in experimental plant communities COST Action Meeting "Advances in N tracer experiments and 15N methods", Gothenburg/Sweden, invited talk

Trogisch S, He JS, Hector A, Geißler C, Ma Y, Scherer-Lorenzen M (2011) Soil nitrogen storage potential along a successional forest gradient in subtropical China, Nitrogen and Global Change, Edinburgh/UK, talk

Trogisch S, Moritz S, Homeier J, Leuschner C (2008) Diversity and indigenous use of five important plant families in the Sumaco Biosphere Reserve, Ecuador. gtö (Society for Tropical Ecology) conference, Hohenheim/Germany, poster

## Publications (peer-reviewed)

Both S, Fang T, Boehnke M, Bruelheide H, Geissler C, Kuehn P, Scholten T, Trogisch S, Erfmeier A (2011) Lack of tree layer control on herb layer characteristics in a subtropical forest, China. *Journal of Vegetation Science* 22: 1120–1131

Bruelheide H, Böhnke M, Both S, Fang T, Assmann T, Baruffol M, Bauhus J, Buscot F, Chen X-Y, Ding B.-Y, Durka W, Erfmeier A., Fischer M, Geißler C, Guo D, Guo L-D, Härdtle W, He, J-S, Hector, A, Kröber, W, Kühn, P, Lang, AC, Nadrowski K, Pei KQ, Scherer-Lorenzen M, Shi XZ, Scholten T, Schuldt A, Trogisch S, von Oheimb G, Welk E, Wirth C, Wu, Y-T, Yang XF, Yu MJ, Zeng XQ, Zhang SR, Zhou HZ, Ma KP & Schmid B (2011) Community assembly during secondary forest succession in a Chinese subtropical forest. *Ecological Monographs* 81: 25-41