The functional significance of tree diversity for nutrient acquisition in a tropical tree plantation

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Publication Date: 2010

Permanent Link: https://doi.org/10.3929/ethz-a-006132761

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The functional significance of tree diversity for nutrient acquisition in a tropical tree plantation

A dissertation submitted to
ETH Zurich
for the degree of
Doctor of Sciences

presented by
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2010
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Abstract

At present, monocultures of fast growing, mostly exotic tree species predominate plantation forestry in the tropics. This has been seen as a problem for long-term nutrient availability and associated biodiversity. An increase of tree species richness in such plantations has been claimed to provide multiple benefits, e.g. stand stability. However, there is little experimental evidence on how tree diversity may affect ecosystem processes as stand productivity and nutrient cycling. Belowground resource complementarity due to niche differentiation could be one important mechanism of positive biodiversity effects in tropical tree plantations.

The main part of the thesis at hand was carried out in a large-scale experimental tree plantation in Sardinilla, Panama. The plantation was established in 2001 and consisted of six native tree species from three functional groups, planted as monocultures, three-species or six-species mixtures. The aim of this thesis was to test whether communities with higher tree diversity show complementary nitrogen (N) and phosphorus (P) acquisition, either because of nutrient uptake from different soil depths, from different chemical sources and/or through association with mycorrhizal fungi.

In Chapter 2, we quantified the amount of N and P stored in the aboveground biomass of trees in the Sardinilla plantation after five and six years of growth and tested the effect of tree diversity and environmental growing conditions on the nutrient storage. Small scale heterogeneity in topography and nutrient availability was the predominant factor explaining 58% of the variation in the N and P pools. A positive biodiversity effect was found on tree N and P storage at the intermediate tree diversity level indicating complementarity as the underlying mechanism.

In Chapter 3, a \(^{33}\text{P}\) and \(^{32}\text{P}\) soil labeling was carried out in combination with root-excluding mesh-bags to study the contribution of arbuscular mycorrhizal fungi for the plant P uptake. Further, species-specific molecular markers were developed to quantify the proportion of each species in the root biomass of mixed communities. Species-specific differences in biomass accumulation and P uptake were strongly enhanced under interspecific competition. The tree species depended to a large degree on the extraradical mycelium for P uptake and showed a similar extent of colonization by Glomus intraradices. Further, differences in aboveground biomass were not necessarily reflected belowground in mixture pots.

In Chapter 4, a \(^{15}\text{N}\) labeling experiment was conducted in the Sardinilla plantation to
test for the impact of heterospecific tree neighbors on the species-specific N uptake from different soil depths. In addition, we carried out a $^{15}$N labeling with different chemical N sources to test for species-specific N preferences in a pot experiment. Two of three tested tree species acquired the labeled N mainly from the topsoil whereas one species was capable to take up comparable amounts of N from both soil depths. Organic N as a potential N source was of little relevance for the tropical tree species, and trees grown in mixtures showed significantly reduced $^{15}$N uptakes compared to plants in monoculture pots.

To our knowledge, this is one of the few studies conducted in an experimental diversity plantation which assessed the significance of species interactions for tree nutrition as well as the importance of mycorrhizal fungi for the P acquisition of different tropical tree species. The species-specific uptake strategies for N and P did not differ substantially in mixed, more diverse communities. Hence, belowground nutrient partitioning did not seem to be the main mechanism behind the observed biodiversity effect in aboveground N and P pools. As a consequence, a more integrative approach is needed, combining belowground- and aboveground functional plant traits, to gain insight into these synergistic diversity effects. However, information on nutrient requirements and differences in nutrient acquisition strategies might serve as an additional and relevant criterion for the successful design of mixed species plantation.

2|Abstract
Zusammenfassung


In Kapitel 2 wurde die Grösse der N- und P-Vorräte in der oberirdischen Baumbiomasse errechnet und getestet, welchen Einfluss die Diversität und die Umwelt auf die Nährstoffspeicherung haben. Kleinräumige Unterschiede in der Geländetopographie und in der Nährstoffverfügbarkeit erklärten über 58% der Varianz in den N- und P-Vorräten. Zudem wurde ein positiver Effekt der Diversität auf die N- und P-Speicherung in den 3-Artenmischungen festgestellt. Es konnte auf die Komplementarität als diesem Effekt zugrunde liegender Mechanismus geschlossen werden.

In Kapitel 3 wurde die Bedeutung der Mykorrhizapilze für die pflanzliche P-Aufnahme unter dem Einfluss intra- und interspezifischer Konkurrenz in einem Topfsystem untersucht. Der Einsatz spezieller Beutel aus Nylongewebe mit unterschiedlichen Maschenweiten und eine unterschiedliche Markierung des darin enthaltenen Bodens mit Radioisotopen ($^{33}$P oder $^{32}$P) ermöglichten die direkte Quantifizierung der P-Aufnahme über das Pilzmyzel. Zudem wurden artspezifische molekulare Marker entwickelt, um den Anteil


1 General introduction

1.1 The importance of forest plantations

The world’s forests are under heavy pressure due to intensive exploitation. Deforestation and encroachment, especially in the tropics, occur at an alarmingly high rate and contribute to the tremendous loss of global biodiversity (FAO 2005, Millennium Ecosystem Assessment 2005). Since humanity depends on ecosystem services such as water and nutrient cycling, control of regional climate, generation of soils and their maintenance, pollination for agriculture and much more (Daily 1997), changes in these services are very likely to affect human beings and the global economy.

According to FAO (2005), 13 million hectares of forest cover disappeared every year between 2000 and 2005 with the highest deforestation rates reported in South America (4.3 mio ha year\(^{-1}\)) and Africa (4.0 mio ha year\(^{-1}\)). Compared to earlier assessment periods, the net change rate (i.e. the difference between deforestation and forest expansion) slightly decreased from -8.9 million hectares per year between 1990–2000 to -7.3 million hectares between 2000 and 2005 (FAO 2005). This is mainly due to natural expansion of forests (e.g. in Europe), large landscape restoration (e.g. in China) and an increase in forest planting. Forest plantations include both afforestation (planting trees on areas which were not forested before) and reforestation (planting trees in areas previously covered by natural forests). Although forest plantations account only for 3.8% of the total forest cover (~140 million hectares, FAO 2005), they provide about 35% of global roundwood (FAO 2001). Especially in the tropics where the supply of high-value hardwood from natural forest is decreasing due to loss and forest protection measures, forest plantations are expected to play a significant role in the future (Varmola and Carle 2002).
Central America is one of the subregions with the highest deforestation rates in the world, exceeding 1% per year (FAO 2009). This loss is mainly caused by converting natural forests into pasture and agriculture land as in many tropical regions (FAO 2005). The Republic of Panama, where the data of the present thesis were collected, has lost over 30% of its natural forests during the last 50 years (Romero et al. 1999, FAO 2005). Subsequently, unsustainable land use has led to large areas of abandoned farmland due to soil degradation and reduction of soil fertility after logging (Bruijnzeel 2004, McGrath et al. 2001, Murty et al. 2002, Reiners et al. 1994). As a consequence, these disturbed lands become now available for planting (Evans and Turnbull 2004). Whether future plantations on those lands will have a pure productive or as well a protective function, such as the conservation of biodiversity, soils and water, largely depends on the planting design and the selected tree species. At present, the majority of forest plantations in Latin America are monocultures of only a few well known exotic tree species such as Tectona grandis, Eucalyptus sp. and Acacia sp., representing about the half of all neotropical plantations (Evans and Turnbull 2004, Wishnie et al. 2007). Monocultures are traditionally preferred over mixed-species plantation as they allow to allocate all resources to one promising tree species and keep plantation management at a simple level thus leading to reduced economic costs (Evans and Turnbull 2004). However, several studies reported adverse effects of such exotic monocultures for system stability, associated biodiversity and long-term nutrient availability (see the review by Hartley 2002).

1.2 Biodiversity and its implication for plantation forestry

In line with ecological theory, a combination of several species has the potential for higher productivity compared to a pure stand due to complementary resource use (Ewel 1986, Haggar and Ewel 1997) or good “ecological combining abilities” (Harper 1977). The basis for complementarity is rooted in the niche concept claiming that two or more species must use resources differently if they are to coexist on a site (Hutchinson 1957). If a mixture of species is able to exploit the resources of a site more completely than a single species, then this mixture should be more productive. The relationship between biodiversity and ecosystem functioning, especially productivity and nutrient cycling, has been intensively studied in grasslands. This research has repeatedly shown positive biodiversity effects and has brought new insights on the underlying mechanisms (see reviews by Balvanera et al. 2006, Cardinale et al. 2006 and Hooper et al. 2005). In contrast, significant experimental efforts in tropical forestry including appropriate planting designs have not been made until the 1980s (Kelty 2006). The first plantation experiments comparing pure to mixed stands mainly concentrated on the optimal species proportion at a constant overall spacing, canopy stratification and the potential advantages of including nitrogen (N) fixing tree species (e.g. DeBell et al. 1997, Khanna 1998 and Menalled et al. 1998 but also see reviews on these subjects by Forrester et al. 2006 and Kelty 2006).
However, most of these early manipulative experiments were not designed to study the interaction of species richness and ecosystem functioning per se. Comparisons were usually done between pure stands and assemblages of two or three species of economically important trees. Tree diversity gradients were generally lacking, and therefore it was not possible to determine the species richness level where most effects would occur (Scherer-Lorenzen et al. 2005a). Tree plantations that also include intermediate and high diversity treatments were only recently established (Scherer-Lorenzen et al. 2005b).

So far, several studies made in the tropics and using native tree species reported positive effects of mixed plantations on biomass production and tree growth (Erskine et al. 2006, Redondo-Brenes and Montagnini 2006, Potvin and Gotelli 2008), tree regeneration (Carnevale and Montagnini 2002) or nutrient storage (Montagnini 2002). However, like in other ecosystems (see review by Srivastava and Vellend 2005), neutral or negative effects of tree species diversity on ecosystem functioning were also documented (e.g. Butler et al. 2008, Firn et al. 2007 and Scherer-Lorenzen et al. 2007), highlighting the fact that not all species combinations result in complementary resource use and that species identity and functional traits also matter. While such traits (e.g. high light-demand or shade-tolerance) are well known for a wide range of boreal and temperate tree species, little information is available on the myriad of tropical tree species. Several attempts were made in the last years to screen native tree species in Central America for their suitability for reforestation and plantation forestry (e.g. Hooper et al. 2002, Wishnie et al. 2007). Yet, most of these studies were restricted to aboveground growth parameters such as tree height or basal diameter.

To fully understand why a particular species combination is successful and another not, we need to know more about the belowground interactions. For example, several studies from agroforestry systems showed that mixtures of trees and crops resulted in increase of total resource capture and plant biomass because of different rooting patterns (see review by Schroth et al. 2001). Species in mixtures were able to exploit the soil volume more completely than monospecific stands of either the tree or the crop species. Other studies in natural rainforests found evidence that tropical tree species from different successional guilds acquired different chemical forms of N (Bazzaz 1984, Freedan et al. 1991, Stewart and Hegarty 1988). Pioneer species generally showed a preference for nitrate whereas late-successional species tended to take up more ammonium. It seems thus reasonable that such additional information on differences in belowground nutrient partitioning could improve the methods to design mixed species plantations. It would allow to combine complementary species which use different belowground resources or the same resource at different spatial or temporal scales and hence reduce intra- and interspecific competition. However, this gap in knowledge currently limits the capability to successfully design mixed species plantations in the tropics (Jose et al. 2006). Moreover, we have little information on the functional significance of soil microorganism for belowground plant to plant interactions. Mycorrhizal associations are known to modulate the
availability of resources such as phosphate and are likely to affect competitive interactions between plants (Smith and Read 2008).

1.3 Mycorrhizal symbiosis in the tropics

Whereas the bulk of literature on the mineral nutrition of trees concentrates on temperate and boreal species, much less is known on tropical tree species. The majority of tropical trees grows on relatively nutrient-poor soils and fertilization experiments in natural forests and plantations provide evidence that tree growth is limited by nutrient supply (see Baker et al. 2003 for a review), and phosphorus (P) in particular (Vitousek 1984). However, most tropical rainforest tree species are colonized by arbuscular mycorrhizal fungi (AMF), known to improve the uptake of nutrients by the trees (Alexander and Lee 2005, Janos 1980).

AMF are very ancient organisms that originate more than 450 million years ago (Redecker et al. 2000) and form associations with over 80% of vascular plants (Smith and Read 2008). They provide several benefits to their hosts including improved mineral nutrition and plant growth, plant resistance to diseases and pathogens, and enhanced tolerance against stress such as drought, salinity or metal toxicity (Smith and Read 2008). Moreover, in grasslands AMF were shown to exert a significant influence on plant community structures (Hartnett and Wilson 2002). On the other hand, they may also cause growth depressions in less responsive plant species or under luxurious mineral nutrition, hence moving from mutualistic to parasitic effects on its host (see recent review by Johnson 2010). However, most of this evidence comes from highly artificial model systems or temperate ecosystems. As a comparison, only about 3% of all research papers on mycorrhizas published since 2000 explicitly dealt with tropical rainforests (Alexander and Selosse 2009).

Both molecular data from intact rainforest and data from a seedling experiment in Panama showed pronounced spatial and temporal heterogeneity in AMF communities and significant non-random associations between AMF and host tree species (Husband et al. 2002a, b and Kiers et al. 2000). Seedlings of early-successional species seemed to depend more on mycorrhizal association than late-successional species, and AMF community structures in roots of different seedlings clearly shifted over time. Some experiments in tropical managed systems have shown mycorrhizal association change and diversity loss along with an intensification of land use (Boddington and Dodd 2000, Cuenca et al. 1998, Johnson and Wedin 1997). In contrast, a study conducted in Costa Rica and Nicaragua showed that pastures (formerly covered by natural forests) contained a surprising abundance and diversity of AMF spores compared to natural forests and suggested that plant succession during reforestation or restoration projects are not limited by lack of mycorrhizal colonization (Picone 2000). Aldrich-Wolfe (2007) planted forest seedlings of Terminalia amazonia in a pasture dominated by Urochloa sp., testing whether
the seedlings were either colonized by the AM fungal community of the grass species or of the canopy tree species. Colonization by the former would suggest the absence of host specificity and high impact of the local environment whereas colonization by the AMF community of *T. amazonia* would indicate host specificity. After two years of growth, the seedlings were colonized by neither community but by an AMF species that was rarely observed in other plants.

Altogether, it seems that interactions between tree species and AMF are very complex. How important these interactions are for forest productivity and diversity, and what consequences they have for practical applications as in plantation forestry, remains an open question (Alexander and Selosse 2009). To investigate the functional significance of mycorrhizal fungi for the mineral nutrition of tropical trees, especially with regard to P, experiments to quantify the contribution of AMF to the nutrient uptake of the trees are needed.

### 1.4 The Sardinilla Project

The Sardinilla Project in Panama, Central America, is one of few large-scale experimental plantations in the tropics which aim to understand the complex relationship between tree biodiversity, land use and ecosystem functioning. It is part of a new global network of forest diversity experiments which are unique in size and experimental design and include gradients of biodiversity to study the diversity-functioning relationships with trees (Scherer-Lorenzen et al. 2005b). Other sites of this network are located in Thuringia (Germany), Pori (Finland), Borneo (Malaysia) and Zhejiang (China). The Sardinilla Project is a collaborative research effort lead by Prof. Dr. Catherine Potvin (McGill University, Montréal, Canada) and the Smithsonian Tropical Research Institute (Panama). Research at the Sardinilla site is interdisciplinary and international, addressing several aspects of the relationship between biodiversity and ecosystem functioning and services, such as carbon sequestration, stand productivity, water use, nutrient cycling and plant health and herbivory.

The Sardinilla Project comprises three different types of plantations: 1) a low diversity plantation established in 2001, consisting of pure stands, three-species and six-species, 2) a high diversity plantation with plots of six, nine and eighteen tree species planted in 2003 and 3) a new agroforestry plantation established in 2006. The data for the thesis at hand were collected in the low diversity plantation. The layout of this plantation experiment is a substitutive randomized block design in which the three diversity levels (monocultures, triplets and six-species plots) were randomly allocated to twenty-four plots. In addition, tree species are classified into functional groups referring to the successional statuts of the species in natural forests. The different functional groups are equally represented in each diversity level and, moreover, species richness was manipulated independent of species identity (Scherer-Lorenzen et al. 2005b).
The low diversity plantation contains six native tree species from three functional groups (Fig 1.1): two pioneer (*Luehea seemannii* and *Cordia alliodora*), two light-intermediate (*Anacardium excelsum* and *Hura crepitans*), and two shade-tolerant species (*Cedrela odorata* and *Tabebuia rosea*). The classification into functional groups was based on the relative growth rates (9.1 and 7.0%; 5.9 and 4.9%, 2.3 and 3.4%, respectively) reported from the 50 ha permanent plot of Barro Colorado Island (Scherer-Lorenzen et al. 2005b). The wood of *Cedrela odorata, Cordia alliodora, Luehea seemannii* and *Tabebuia rosea* is of high economic value and is traditionally used for furniture, housing, flooring and ornaments. The wood of *Hura crepitans* is of medium timber quality and logs are frequently used for heavy constructions, whereas the wood of *Anacardium excelsum* is good timber quality, used to build canoes or for furniture (Delagrange et al. 2008).

1.5 Objectives and thesis outline

In this thesis, we focus on the belowground nutrient acquisition of different tree species and try to understand how tree diversity affects the nutrient uptake of the different species. We concentrate on the two macronutrients N and P as they are the key limiting resources to primary productivity in tropical ecosystems (Vitousek 1984). Moreover, we account for the importance of mycorrhizal fungi for tree nutrition by assessing their contribution to the total P uptake of the trees.

Our main hypothesis is that, in contrast to monocultures, communities with higher tree diversity show complementary N and P acquisition, either because of nutrient uptake by different soil depths, by different chemical forms and/or through association with specific mycorrhizal fungi. We expect that resource partitioning in the mixed, more diverse communities will result in enhanced aboveground nutrient storage and biomass production.

To test this hypothesis, we conducted several experiments both in the Sardinilla plantation as well as in a pot experiment using a subset of the species from the Sardinilla experiment as described in the following:

In Chapter 2, the amount of N and P stored (N and P pools) in the aboveground tree biomass was quantified after five and six years of growth. By applying the additive partitioning method of Loreau and Hector (2001), we tested whether biodiversity affects the N and P acquisition of the different tree species and if so, whether this effect is due to complementarity or selection. We expected that species mixtures would yield higher N and P pools compared to monocultures of the component species due differences in N and P acquisition strategies and thus complementary resource use. Further, we assessed the importance of the environmental growing conditions in Sardinilla on the N and P acquisition of the trees.
Figure 1.1: Tree species used in the low diversity plantation in Sardinilla, Panama. a) Cordia alliodora, b) Luehea seemannii, c) Anacardium excelsum, d) Hura crepitans, e) Tabebuia rosea and f) Cedrela odorata. Figure a, b, c, e and f are from Vozzo (2003) and figure d from Francis and Lowe (2000).
In Chapter 3, we focused on the P uptake strategies of the most productive species combination in the plantation. To control for environment heterogeneity and to get permission for radioisotope labeling, we grew seedlings of the three species in pots for two years. We used soil $^{32}$P and $^{33}$P labeling in combination with root-excluding mesh-bags to assess the dependence of the tree species on mycorrhizal fungi for their P uptake. Molecular tools were used to estimate the mycorrhizal colonization of tree roots, and to test for differences in biomass allocation to the roots. The dependence on mycorrhizal association for P uptake as well as plant growth were measured in the presence of conspecific and heterospecific neighbors. Thereby, we aimed to test whether interspecific competition results in resource partitioning.

In Chapter 4, we conducted first a $^{15}$N labeling experiment in the Sardinilla plantation to test the influence of conspecific and heterospecific neighbors on the N uptake from two different soil depths. Here, the same tree species as in the pot experiment were studied. We expected that interspecific competition would lead to complementary resource uptake and hence to higher aboveground tree biomass and N storage of trees in mixture. Second, we labeled different chemical sources of soil N with $^{15}$N to assess potential N uptake preferences of the trees grown in the pot experiment with regard to chemical form.


Is tree diversity an important driver for phosphorus and nitrogen acquisition of a young tropical plantation?

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accepted for publication in Forest Ecology and Management on July 12, 2010

Abstract

Many tropical plantations in Central America are monocultures of fast growing, mostly exotic species such as a teak, eucalypts and pines. This has been perceived as a problem for ecosystem stability, pest control, local biodiversity and long-term nutrient availability. In our study, we followed the effects of increasing tree diversity (1, 3 and 6 native species) on aboveground nitrogen (N) and phosphorus (P) pools in a young experimental biodiversity plantation (central Panama) over two subsequent years. Our results show a positive but not consistent net effect of biodiversity on the N and P pools, mainly explained by the complementarity effect. N and P use efficiencies strongly varied among the investigated tree species and the species richness gradient. *Anacardium excelsum* and *Luehea seemannii* were associated with higher N and P use efficiencies while *Hura crepitans* and *Tabebuia rosea* were less efficient in aboveground biomass production per unit N or P. Tree species tended to have lower P use efficiencies in the intermediate diversity level compared to monocultures and six-species mixtures. Although the environmental conditions explained a large part of the variation in the N and P pools (58%) in our experiment, we argue that incorporating tree mixtures in the management can bring additional benefits and improve tree growth and nutrient uptake as compared to the monocultures.
2.1 Introduction

As the cover of natural tropical forests has declined over the last decades and the demand for wood products continues to grow, tree plantations have become increasingly important. The conversion of grasslands into forest plantations is currently a wide-spread land use in tropical regions and contributes to the production of timber, fuelwood and could serve as carbon-sinks under the Clean Development Mechanisms (UNEP 2008) within the Kyoto Protocol. According to FAO (2005), the area of forest plantations increased at a rate of 2.8 million ha yr\(^{-1}\) between 2000 and 2005. However, many of the tropical plantations are monocultures of fast growing, mostly exotic species as teak (Tectona grandis), eucalypts or pines. This has been seen as a problem for ecosystem stability, long-term nutrient availability and associated biodiversity (Aweto 2001, Haggar et al. 1998, Piotto et al. 2003, Spangenberg et al. 1996 and references in Hartley 2002).

Thus, both the tremendous loss of biodiversity due to disappearance of the tropical rainforest and the predominance of monocultures in plantation forestry have raised concerns about the significance of tree diversity for ecosystem functioning and the delivery of goods and services (Millennium Ecosystem Assessment 2005). Although some of the experimental and observational studies from the tropics suggested positive effects of mixed plantations on tree growth and productivity (Erskine et al. 2006, Potvin and Gotelli 2008, Petit and Montagnini 2004, Piotto et al. 2004, Redondo-Brenes and Montagnini 2006), tree regeneration (Carnevale and Montagnini 2002) and nutrient storage (Montagnini 2000), no or negative effects were found for litter decomposition (Scherer-Lorenzen et al. 2007), soil nutrient concentrations (Firn et al. 2007, Stanley and Montagnini 1999) and woody understory diversity (Butler et al. 2008). As a result, several of these studies highlighted the importance of tree species identities in mixed stands rather than species richness per se. Positive effects of diversity were often observed in mixtures including N\(_2\)-fixing tree species. This has led to intensive research on facilitative tree interactions (cf. Forrester et al. 2006, and Kelty 2006). In contrast, less information is available about other positive plant interactions such as complementary resource use. Two important aspects of complementary resource use in tropical managed systems are light partitioning through canopy stratification and water and/or nutrient partitioning, e.g. through root stratification. While there is substantial evidence from plantation forestry on the first aspect (Ewel and Mazzarino 2008, Menalled et al. 1998), information on belowground mechanisms largely comes from tropical agroforestry studies (e.g Dinkelmeyer et al. 2003, Rowe et al. 2001, Schroth et al. 2001). Nevertheless, the implications for tree nutrition and hence tree productivity are large, if species with complementary resource use can be identified for plantation purposes (Richards et al. 2010).

A possible way to statistically assess the importance of tree species complementarity in mixtures is by applying the additive partitioning method of Loreau and Hector (2001). This method allows to partition the net effect of biodiversity (NE) into a complementarity effect (CE) and a selection effect (SE). SE represents the changes in resource uptake and/or
biomass production of a mixture due to the dominance of a particular species with a disproportionate effect on these traits (also referred to as sampling effect). In contrast, CE represents the changes in NE which cannot be attributed to any single species in the mixture and is often interpreted as evidence for niche separation of facilitative species interactions. However, Cardinale et al. (2007) concluded in their study that CE rather includes all forms of resource partitioning, i.e. also indirect and non additive species interactions, thus making it impossible to identify a single biological mechanism for positive mixture effects. Nevertheless, applying the additive partitioning method in plantation forestry still could be useful to identify combinations of tree species resulting in a positive biodiversity effect through multiple species processes. In addition, site characteristics such as soil nutrient availability and topographical heterogeneity must be included in studies on diversity effects as they can have a strong influence on tree nutrition and may enhance or hide effects of species richness (Healy et al. 2008, Hiremath and Ewel 2001).

The quantification of nutrients stocks in the aboveground biomass is an important issue in sustainable plantation management. Depending on rotation length and harvest practices, the amount of nutrients lost through biomass removal can crucially determine the future success of productive plantations (Montagnini and Jordan 2005). Nutrient use efficiency, i.e. the amount of biomass produced per unit of a certain macro- or micronutrient, is a useful measure to assess the nutrient demand and the productivity of a tree species on a site. Especially, the use efficiencies of the two macronutrients nitrogen (N) and phosphorus (P) by different tree species need to be considered for sustainable site management, since the two nutrients are pivotal in many metabolic plant processes and are known to limit plant growth (Marschner 1995, Niklas 2008).

The Sardinilla plantation in Panama is a tree biodiversity experiment designed to test the relationship between biodiversity and ecosystem functioning. In contrast to many other research plantations in the tropics, its experimental setup does not include N$_2$-fixing tree species due to their known and strong effects in mixed forest experiments. Therefore, the design allows to address other, more subtle mechanisms than N$_2$-fixation, as for example complementary soil resource use or changes in nutrient use efficiencies (Richards et al. 2010). The aim of this study was to address the importance of tree diversity for acquisition of P and N by the trees in a native tree species plantation. By estimating the amount of the two macronutrients stored in the standing biomass, henceforth referred as N and P pools, we intended to answer the following questions:

1. Does species richness and species composition affect the size of N and P pools in the trees and if so, is this caused by selection or complementarity?

2. To what extent do the environmental variables contribute to the explanation of the N and P pool patterns in the plantation?

3. Are N and P use efficiencies affected by the tree species and/or the species richness?
2.2 Material and method

2.2.1 Study site

The study was conducted during 2006 and 2007 in an experimental tree plantation in Sardinilla (9°19'30"N, 79°38'00"W), central Panama, approximately 50 km north of Panama City. The study site has an elevation of 70 m a.s.l. and extends over a slightly undulated terrain. Mean annual precipitation is around 2350 mm with a prominent dry season from January to March, and mean annual temperature is 25.1°C with a daily minimum of 21.7°C and a maximum of 33.1°C. The soils belong to the order of Alfisol with Typic Tropudalfs on the ridges shifting to Aquic Tropudalfs in the depressions. They contain a high content of expanding clays (up to 65%), causing deep cracks during the dry season. The bedrock is composed of Tertiary limestone and other sedimentary rocks (Potvin et al. 2004). The site was originally covered with semideciduous tropical lowland forest until it was logged in 1952/53, subsequently used for cropping (2-3 years) and eventually converted into a pasture. The plantation was established in 2001; it consists of 24 plots (45 x 45 m), each divided into 4 subplots of equal size (Healy et al. 2008), with an initial tree density of 1111 trees ha⁻¹. The six tree species planted are all native to Panama and include two fast-growing species (*Luehea seemanii* (Tiliaceae) and *Cordia alliodora* (Boraginaceae), two moderately fast growing species *Anacardium excelsum* (Anacardiaceae) and *Hura crepitans* (Euphorbiaceae) and two slow growing species *Cedrela odorata* (Meliaceae) and *Tabebuia rosea* (Bignoniaceae). The design included 12 monocultures (2 replicate plots per species), six replicates of three-species and six replicates of six-species mixture plots (see Appendix Fig. 2.7). Three-species mixtures differed in their species composition, whereas six-species mixtures did originally include all six tree species and were thus identical in composition (Scherer-Lorenzen et al. 2007). *Cordia alliodora* suffered such high mortality rates after planting that it almost completely failed to establish in the plantation. The two monoculture plots of *C. alliodora* had to be abandoned. Therefore, we excluded this species from the analyses. In extension to a previous study by Oelmann et al. (2010) that used an individual tree approach, our investigation expanded over two years and included the collection of plant and soil samples in all relevant plots of the experimental design. In particular, the terrain elevation differences and information on the slope inclination were integrated in the analyses.

2.2.2 Sampling

At the end of the growing season (December to January) in 2006 and 2007, every tree in the plantation was measured in order to estimate aboveground tree biomass. Tree height was assessed using a hypsometer (Vertex III, Haglå, Sweden). Tree basal diameter (BD), taken at 10 cm aboveground, and tree diameter at breast height (DBH) were measured with a circumference chain.

For the determination of nitrogen and phosphorus concentrations, leaves and branches
were collected during the rainy season between 4 and 7 July 2006 and 2 and 6 July 2007. In each plot, 3 individuals (within ± 2 m of mean tree height) were semi-randomly selected for each species. Border trees were omitted to avoid edge effects. From each tree, 5 to 10 sun and shade leaves each, and 3 terminal branches were collected. From each of the branches, one disc of about 1 cm thickness, including both bark and wood, was processed. All samples were dried for 3 days at 65 °C to constant mass and ground. Nutrient data of stems were kindly provided by Y. Oelmann (Oelmann et al. 2010).

Soil samples for the quantification of pH, total, organic and microbial phosphorus concentrations were collected at the early rainy season in June 2007. Soil samples were collected at 4 equally distributed locations per subplot at a depth of 0-10 cm and pooled to one sample per subplot. Samples were taken with an soil core sampler (ø 2 cm, Eijkelkamp Agrisearch Equipment, The Netherlands). Coarse plant debris and stones were manually removed from the samples since the moist soil was too sticky to pass through any sieve.

For the analysis of total soil C and N, inorganic N (nitrate and ammonium) and plant available P concentrations, soil samples were collected in a second sampling campaign during the wet season between the 9 and 27 August 2007. Samples were taken from six locations per subplot with the constraint that all species were represented equally near the sampling locations. We collected soil from 3 different soil depths: 0-5 cm, 5-15 cm and 25-35 cm. The six soil samples from a subplot were pooled to one sample per subplot per depth, resulting in 12 samples per plot (4 subplots × 3 depths).

N mineralization rates (ammonification and nitrification) in the field were estimated using in-situ aerobic incubation (Hart et al. 1994). The first incubation experiment was done during the transition from the dry to the wet season (15 - 23 May 2007), the second campaign was carried out in the wet season (15 - 23 August 2007). In one subplot per plot, we randomly chose two locations in monocultures, 3 locations in 3-species mixtures and 6 locations in the six-species mixtures (locations were equally distributed to the different tree species). Two plastic tubes with a diameter of 7.5 cm were inserted 15 cm into the soil at each location. One cylinder was removed immediately and taken to the laboratory for analysis. The other tube was capped with a styrofoam cover permitting gas exchange with the atmosphere and remained in the field for a period of 8 days. In addition, we visually estimated the cover of the herbaceous understorey in the area, where tubes were inserted.

All soil samples were divided into different subsamples. One subsample was dried at 105 °C for 48 h to determine water content gravimetrically. Another subsample was dried at 65 °C for 72 h and pulverized in a ball mill for nutrient analyses. For the determination of inorganic N, field-moist soils were kept in cool darkness and were processed within two days after the field sampling.
2.2.3 Chemical analyses

Total C and N concentrations in plant and soil samples were measured with an elemental analyzer (Euro EA, HEKAtech GmbH, Germany). P concentrations in plant samples were determined according to Ohno and Zibiliske (1991). In brief, 100 mg of plant material were incinerated for 8 h at 550°C, ashes dissolved in 2ml HNO₃ (65%), filtered through a paper filter (Whatman No 40) and made up to 25 ml. Soil microbial P and plant available P was extracted from soil by anion exchange resin adsorption according to Kouno et al. (1995) and Saggar et al. (1990), organic and total P in the soils were assessed after ignition according to Saunders and Williams (1955). The concentration of P in the soil extracts was measured colometrically using malachite green according to Ohno and Zibiliske (1991). Mineral N (NO₃⁻ and NH₄⁺) was extracted from soils according to Faithfull (2002), filtered and kept frozen at -20°C until analysis. Extracts were then analyzed photometrically using a Flow Injection Analyzer (San⁺, SKALAR Analytical B.V., The Netherlands). Soil pH was measured with an Orion pH meter (720A) in water extracts (1:5; w:v).

2.2.4 Calculations and statistical analyses

Aboveground tree biomass was estimated by species-specific allometric equations based on tree height, BD and/or DBH (Oelmann et al. 2010). For each species, the allometric equation was derived from the data of 10 trees per diversity level harvested during December 2006 and January 2007. Further, this data was used to define the biomass allocation to different aboveground plant compartments (leaves, branches and stems) for each species separately. We applied the same set of allometric equations and allocation patterns for both years of our study as species-specific allometric equations were not available for 2007.

Aboveground tree biomass in Mg ha⁻¹ at the plot-level was calculated by summing across all individuals of all species measured in the plot.

N and P pools (kg N or P ha⁻¹) at the stand level were calculated as the sum of products of mean nutrient concentrations in leaves, branches and stems times the plot biomass of each compartment (leaves, branches or stems). Biomass and pool calculations were done for each species, diversity level and year separately.

As a measure of nutrient use efficiencies, we determined the amount of tree biomass produced per unit of aboveground N or P (NUE and PUE, respectively, in kg DM kg⁻¹N or P) of each tree species for both years.

Soil nutrient concentrations were averaged over the different soil depths and subplots resulting in a mean concentration per plot.

N mineralisation rates were calculated according to Hart et al. (1994):

\[
N_{\text{Min}} = \frac{(NH_4^+ + NO_3^-)_{t_1} - (NH_4^+ + NO_3^-)_{t_0}}{\Delta t}
\]  

(2.1)
where N_{Min} is the net mineralization rate (mg N kg^{-1} soil day^{-1}), NH_{4}^{+} and NO_{3}^{-} are the ammonium and nitrate concentrations (mg N kg^{-1} soil), respectively, at the beginning (t_{0}) and the end (t_{1}) of the incubation period Δt (8 days).

To compare the productivity of the different tree species, we tested the effects of diversity, species and time on the aboveground biomass normalized for species richness. Therefore, we applied a linear mixed effect model with repeated measurements (year), where planted diversity (=species richness 1, 3 or 6), species and sampling years were defined as fixed effects and species within plots was defined as random effect. We chose mixed effect because they are recommended for unbalanced datasets (Pinheiro and Bates 2000). In our design, the imbalance is the number of replicates per diversity level. Estimates of variance parameters were calculated by Restricted Maximum Likelihood, which takes into account the degrees of freedom for fixed effects. Effect of the different treatments on aboveground biomass, N and P pools in the different plant compartments and on N and P use efficiencies were tested with similar models. Where necessary, data were log-transformed for analysis. Linear mixed effect models were fitted using the nlme package (Pinheiro et al. 2008) of R version 2.8 (R Development Core Team 2007).

As a second approach to determine effects of tree diversity on aboveground N and P pools, we applied the additive partitioning method of Loreau and Hector (2001) to the nutrient pool data to unravel any biodiversity effect. This method defines an overall net effect (NE) of biodiversity composed of two additive components: a selection effect (SE) and a complementarity effect (CE). Therefore, we calculated ΔRY_{i}, which is the deviation of the observed from the expected relative N or P pool of species i in the mixture, according to the following equation:

\[ \Delta RY_{i} = RY_{Oi} - RY_{Ei} \]  \hspace{1cm} (2.2)

where RY_{Oi} is the observed N or P pool of species i in the mixture divided by the N or P pool of its monoculture (mean of two monoculture plots) and RY_{Ei} is the expected relative N or P pool, which corresponds to the proportion of the species i planted in the mixture. Tree species, which are not affected by other species in mixtures, will show Δ RY_{i} ≈ 0. Effects of competition on the N and P pool size would lead to Δ RY_{i} < 0, whereas complementarity effects will be indicated by Δ RY_{i} > 0 according to Loreau and Hector (2001).

Similar to Healy et al. (2008), we performed a partial redundancy analysis (RDA) to evaluate the influence of soil nutrient parameters on the variability of the N and P pools in the plants. We reduced the set of environmental variables by conducting stepwise regression (forward selection) of the following fourteen parameters: Total soil C and N, nitrate, ammonium, plant available phosphate, microbial, organic and total P concentrations, soil pH, N mineralization rates in May and August 2007, herbaceous understory cover, plot elevation and slope inclination. Variables used were those statistically significant at the 10% level (Monte Carlo permutation test, n=999). The reduced set of environmental variables
was then used for unraveling the interaction between plant and soil data. The response matrix consisted of N and P pools in the trees at the plot-level, whereas the diversity matrix included the species richness and the realized species composition of each plot. Response and explanatory variables were centered and standardized for the analysis. In a first step, we employed RDA to estimate the total variation in the response data by both the diversity and environment variables. In a second step, we conducted a RDA for each of the two explanatory matrices solely with no covariables. In a third step, RDA was done with the environment matrix as explanatory variable and including the diversity matrix as a covariable and vice versa. As a result, total variation was decomposed into the pure effects of each one of the explanatory matrices and their joint effect (Legendre and Legendre 1998). The significance of pure effects were tested by the Monte Carlo permutations \((n=999)\). For the multivariate analysis, the software application CANOCO Version 4.5 (ter Braak and Smilauer 2002) was used.

### 2.3 Results

#### 2.3.1 Aboveground biomass, nitrogen and phosphorus pools

Comparing the productivity of the different species, trees species had no effect on the normalized aboveground biomass in 2006 but in 2007 (species × year, \(F_{4,40} = 35.3, P < 0.0001\), Fig. 2.1). In 2007, \(A. excelsum, L. seemannii\) and \(T. rosea\) had more standing biomass than \(H. crepitans\). Differences between the species across diversity were not significant (diversity × species, \(F_{8,21} = 1.14, P = 0.127\)).

From 2006 to 2007, average standing biomass at the stand level nearly doubled from 7.47 to 15.1 Mg ha\(^{-1}\) (see Fig. 2.2a and b), which proved highly significant (Table 2.1). The largest fraction of freshly produced biomass appeared in the stems of trees (4.08 Mg ha\(^{-1}\)), followed by branches (2.86 Mg ha\(^{-1}\)) and leaves (2.17 Mg ha\(^{-1}\)). Species richness had no significant effect on any of the three plant compartments (Table 2.1). In both years, three-species mixtures tended to have higher standing biomass (9.15 and 17.5 Mg ha\(^{-1}\) for 2006 and 2007, respectively) than six-species mixtures (7.58 and 15.7 Mg ha\(^{-1}\)) or monocultures (6.41 and 13.3 Mg ha\(^{-1}\)), but the effect of species richness was not significant (\(F_{2,19} = 0.83, P = 0.449\), Fig. 2.2a and b).

The total N pool in the standing biomass significantly increased by 35.9 kg N ha\(^{-1}\) from 2006 to 2007. Most of the N taken up or remobilized was stored in the leaves (42%), whereas the proportions in stems and branches were similar, 32% and 26%, respectively (see Fig. 2.2c and d). Again, there was no significant effect of species richness on either the stem, branch or leaf compartment (Table 2.1).

P pools in the standing biomass increased from 10.6 ± 1.29 to 16.8 ± 1.93 kg P ha\(^{-1}\) between 2006 and 2007 with a significant increase in the stem and leaf compartments (see Fig. 2.2e and f, Table 2.1). The largest fraction of P was allocated to stems (70.9%) and leaves (22.5%) and only a small fraction was stored in the branches (6.5%).
richness had a marginally significant effect on the P pools in stems ($F_{2,19} = 2.74, P = 0.090$, Table 2.1), but not on the P pool in branches or leaves. In addition, interactions between species richness and years were not significant for any of the three plant compartments.

The additive partitioning of the aboveground N pools revealed a marginally significant net effect of species richness in 2006 and 2007 for the three-species mixtures (2006: $P = 0.052$; 2007: $P = 0.052$), but not for the six-species mixtures (Fig. 2.3, left panels). The net effect was actually not different between the three-species and six-species mixtures for any of the two years (Species richness: % SS = 0.15, $P = 0.857$; Year: % SS = 1.65, $P = 0.558$). In 2007, the negative selection effect in the six-species mixtures was significantly different from zero.

Tree diversity had a significantly positive net effect on the aboveground P pools in the three-species mixtures in both measured years, but not in the six-species mixtures (Fig. 2.3, right panels, 7.96 ± 2.65 vs. 3.15 ± 2.56 in 2006 and 10.9 ± 3.90 vs. 4.57 ± 3.49 in 2007). The difference between the two mixture types were only marginally significant (% SS = 13.0, $P = 0.088$). The selection effect on the P uptake was negligible.

Analysis of the deviation from the expected relative nutrient pools showed that though the N and P pools of the five tree species were mostly positively affected in the three-species mixtures on average ($\Delta$ RY > 0), e.g. *A. excelsum*, *C. odorata*, *H. crepitans* and *L. seemannii*, none of the deviations were actually significantly different from zero (see Appendix Table 2.3). Only the six-species mixtures showed a synergistic effect on the N and P pools of *A. excelsum* ($P<0.05$), whereas the nutrient pools of the other four tree species did not differ from the respective monocultures.

### 2.3.2 Soil characteristics

We applied partial RDA to separate the variation in aboveground N and P pools into percentages explained by the environmental growing conditions vs the tree diversity. By forward selection, we reduced the set of fourteen environmental variables (Appendix Table 2.4) to a set of five variables, consisting of elevation (m a.s.l.), slope inclination (°), soil NO$_3^-$ and NH$_4^+$ concentrations (mg N kg soil$^{-1}$) and plant available PO$_4^{3-}$ concentration (mg P kg soil$^{-1}$) (Fig. 2.4). The five most productive plots, in terms of aboveground biomass, N and P pools (Ls1, T2, T4, A3 and A4), were located on the top of the small ridges and on the adjacent slopes facing the north-west direction, while the least productive plots (Ae1, Hc1, Ls2, T6 and A2) were found on less steep slopes close to the depressions (see Fig. 2.7 in the Appendix). P pools in trees depended on plant available P concentration (Fig. 2.5a), whereas N pools correlated significantly with the soil NO$_3^-$ concentration (Fig. 2.5b), but not with the NH$_4^+$ concentration (Fig. 2.5c). Slope inclination was positively correlated with the tree P pools ($r=0.48, P < 0.05$) but no relationship was found between slope and plant available P ($r = 0.02, P = 0.46$). However, the different soil nutrient concentrations were not significantly affected by diversity (Fig. 2.5).

As resulted from the partial RDA, this set of environmental variables and the tree...
species diversity explained 88.5% of the variance in the N and P pool data (Monte Carlo permutation test, $F = 82.2$, significance of the first canonical axis $P = 0.001$). The pure effect of the environmental variables covered 57.5% ($F = 71.2$, $P = 0.001$), tree diversity alone explained 17.3% of the total variance ($F = 20.2$, $P = 0.004$). The part of variation shared by the environmental variables and tree diversity was 13.7%. The explained variation by tree diversity was only significant after removing the effect of the environment, but the effect explained by environment was significant both with and without tree diversity as covariate.

### 2.3.3 Nitrogen and phosphorus use efficiencies

Nitrogen use efficiencies (NUE) of the five tree species were significantly affected by species richness (SR $\times$ species interaction, Table 2.2 and Fig. 2.6a). Trees of *A. excelsum*, *L. seemannii* and *T. rosea* showed no change in NUE along the species richness gradient, whereas *C. odorata* had significantly higher NUE in monocultures and six-species mixtures compared to the three-species mixture. The opposite pattern was observed for *H. crepitans*, which showed a significant increase in NUE in the intermediate diversity plots. Within the monoculture and six-species mixtures, *A. excelsum*, *C. odorata* and *L. seemannii* were significantly more efficient in terms of biomass production per unit N than *T. rosea* and *H. crepitans*, the latter being the least efficient tree species. NUE significantly increased from 2006 to 2007 (160 vs. 177 kg DM kg$^{-1}$ N (Table 2.2).

The general linear mixed effect model also revealed a significant effect of species richness on the phosphorus use efficiencies (PUE) of our five species (SR $\times$ species interaction, Table 2.2 and Fig. 2.6b). No significant changes in PUE along the species richness gradient were observed for *H. crepitans* and *T. rosea*. However, *A. excelsum* showed a significant linear increase in PUE with increasing species richness, whereas *C. odorata* and *L. seemannii* had significantly lower biomass production per unit P stored in the three-species mixtures compared to the six-species plots and corresponding monocultures. When planted with conspecific neighbours, *C. odorata* and *L. seemannii* were significantly more efficient than *A. excelsum*, *H. crepitans* and *T. rosea*. At the intermediate and high diversity level, the species patterns for PUE were similar. *A. excelsum* and *L. seemannii* showed the highest PUE, while *C. odorata* and *H. crepitans* were the least efficient species. Again, PUE increased over time from 757 to 908 (kg DM kg$^{-1}$ P) between 2006 and 2007, respectively, revealing no significant interactions with species or species richness (Table 2.2).

### 2.4 Discussion

#### 2.4.1 Tree diversity effects on standing biomass and N and P pools

This research attempted to assess the importance of tree species diversity on aboveground biomass production and N and P acquisition on a per hectare basis. We originally
assumed that higher tree diversity leads to an increase of the standing biomass and the N and P pools therein, based on either local deterministic mechanisms such as niche differentiation or complementary resource use (Loreau et al. 2001) or on local/regional stochastic processes such as the sampling or selection effect (Huston 1997, Tilman et al. 1997).

When accounting for differences in tree density and tree age, standing biomass and macronutrient pools were within values reported from other tropical plantations (Hiremath et al. 2002, Lugo 1992, Montagnini 2000, Parrotta 1999). The two approaches applied to detect possible effects of species richness on the N and P pools revealed, however, no linear or increasing relationship. This supports some other studies on the relationship between biodiversity and ecosystem functioning (Cardinale et al. 2006, Potvin and Gotelli 2008). In our first analysis, species richness had only a marginally positive effect on the P pools in the stem compartment of the three-species mixtures. Since the P concentrations in stems did not differ within the different diversity levels (Oelmann et al. 2010), the increase in the P pools within the three-species mixtures was probably due to slightly higher biomass allocation to the stems and significantly lower PUE in three of the five three species.

In contrast to absence of clear effects in the first analysis, interesting results were obtained by the additive partitioning method (Loreau and Hector 2001). We found a distinct positive net effect of biodiversity (NE), due to complementarity (CE), on the aboveground P pools in the three-species mixtures (Fig. 2.3). Similarly, we found a positive trend for NE and CE on the N pools in the three-species mixtures, although the results were not as obvious as for the P pools. However, we were not able to assign this effect to some specific tree species although one species, *A. excelsum* had higher than expected N and P pools in most mixtures (see Appendix Table 2.3). Obviously, some species performed well in particular three-species combinations but not in others. It strengths the observations made in other studies that not only species richness but also the species identity is crucial for the type of species interaction and the performance in the mixture (Potvin and Gotelli 2008, Redondo-Brenes and Montagnini 2006, Scherer-Lorenzen et al. 2007). Further, it is worth noting that we found an increasing negative selection effect (SE) over time in the partitioning of the N pools, suggesting the dominance of a species with small N pools in monocultures. From the comparison between monoculture and mixture performance (ΔRY), *A. excelsum* was the only species with significantly higher N and P pools in species rich plots than in pure stands. We ascribe this finding to the position of the two pure stands of *A. excelsum* in waterlogged areas of the plantation (Appendix Fig. 2.7), where tree growth was probably suppressed. Healy et al. (2008) in their study also observed low productivity and high mortality rates for *A. excelsum* in the Sardinilla plantation but were not able to relate it to a specific cause. However, the example of *A. excelsum* points out the difficulty in interpreting the effect size calculated by the additive partitioning method. According to the method of Loreau and Hector (2001), the ability of
a species to compete in a mixture is predicted on its performance in monoculture. As in the case of *A. excelsum*, however, the monoculture of a species might not necessarily be a good reference point for such a prediction (Schmid et al. 2008).

### 2.4.2 Tree diversity and environmental effects on NUE and PUE

The nutrient use efficiencies calculated for our five tree species were within the range reported for other tropical tree species (Hiremath et al. 2002, Wang et al. 1991), although our calculation did not account for the amount of N and P in the litter and belowground biomass. In contrast to the findings of van Ruijven and Berendse (2005), we did not detect an increasing relationship between diversity and NUE and PUE. In their experiment with four grasses and four dicot species, they showed an increase of NUE with increasing species richness for five of their eight species. They argue that, in their N-limited system, this effect was a result of changes in the biomass allocation and represents another important mechanism contributing to the often observed positive relationship between diversity and productivity. A recent review by Richards et al. (2010) also showed that in 65% of tree mixture studies, where resource use efficiencies could be calculated, trees growing in mixtures had different use efficiencies than in monoculture. However, both increases and decreases in these efficiencies were observed, and a variety of potential mechanisms for such changes have been discussed. In our study, changes in NUE were not consistent with increasing biodiversity. This goes in line with the fact that there are yet no general diversity effects on ecosystem processes in the Sardinilla plantation. For specific species mixtures, especially at the three-species level, some positive effects of mixing species have been documented, e.g. on biomass production, litter fall and nutrient pools (Potvin and Gotelli 2008, Scherer-Lorenzen et al. 2007, Oelmann et al. 2010) as discussed below.

Interestingly, we observed significantly lower PUE in the three-species mixtures compared to monoculture or the high diverse plots, which is counter intuitive giving the above mentioned positive diversity effects at this diversity level. Richards et al. (2010) in their review reported that 47% of the studies where shifts in P use efficiency could be calculated showed a decrease of > 10% for species grown in mixtures compared to monocultures. In some of those studies, productivity of the plantation increased although NUE and PUE of individual species decreased. As discussed in Richards et al. (2010), the productivity in mixed stands may not necessarily be determined by the use efficiency of a single nutrient but rather how the individual species use all limiting above- and belowground resources.

Further, lower nutrient use efficiencies have often been related to higher nutrient availabilities (Aerts 1990, Boerner 1984, Hidaka and Kitayama 2009, Silver 1994, Vitousek 1982). For example, values given by Hiremath et al. (2002) for *C. odorata* were lower (145 and 469, for NUE and PUE, respectively) than in our study (206 and 798), which might be due to the relatively nutrient rich soils at La Selva Biological Station in Costa Rica compared to our site. Other authors suggested that mixed species stands have higher
nutrient availabilities than monocultures, even in the absence of N\textsubscript{2}-fixing species (Rothe and Binkley 2001, Binkley and Giardina 1998). Likewise, we found a linkage between resource use efficiency, diversity and nutrient availability in the Sardinilla experiment. On the one hand, there was for both N and P, respectively, a positive relationship between nutrient availability and the corresponding nutrient pools in the trees but no effect of diversity on the N or P availability (Fig. 2.5). On the other hand, tree nutrient pools themselves tended to be higher in three-species mixtures as shown with the additive partitioning method, supporting previous results by Oelmann et al. (2010). Although nutrient availability is an important determinant of tree nutrient pools, some patterns can apparently only be explained by species interactions. This suggestion is confirmed by the decomposition of variation in the N and P pool data by the RDA. Soil nutrient availability, scale and topographic heterogeneity have often been mentioned to mask or enhance biodiversity effects (Vila et al. 2005, Mittelbach et al. 2001). In our study, the part of variation explained by the environment was three times larger than the explanatory power of tree diversity. This finding is in good agreement with the results of a previous study of Healy et al. (2008) on the effect of environmental heterogeneity on tree growth and mortality. The effects of tree diversity became only significant after the effect of the environment was removed, indicating that the influence of diversity was hidden by the effect of environment. However, to examine the feedback mechanisms of tree diversity and tree species on soil nutrient availability and vice versa, repeated measures of soil nutrient availability soil would be needed on the long-term. Nevertheless, the significant increase in N and P use efficiencies from 2006 to 2007 in the Sardinilla plantation could indicate that N and P availability in the soil is gradually decreasing.

Other researchers in the Sardinilla project reported similar prominent effects of the three-species mixture. For example, Scherer-Lorenzen et al. (2007) found higher litter production in plots of intermediate diversity as compared to monocultures and six-species mixtures, five years after planting, and Potvin and Gotelli (2008) found significant over-yielding of three-species mixtures in that year. Oelmann et al. (2010), following an individual tree approach, found also increased N and P storage in the tree biomass in 2007 in the intermediate diversity treatment. In our study, two out of the six three-species mixture plots showed transgressive overyielding in N acquisition and even four out of the six three-species mixtures had higher P acquisition than expected from their best monoculture in both years (see Table 2.5 in the Appendix), but only one six-species mixture out-performed the respective monoculture. Since the three-species mixtures all differed in their species composition, there was not a particular set of species causing this effect. The observed pattern might perhaps be explained by a combination of positive species interactions and small scale heterogeneity in nutrient availability, that eventually resulted in resource use complementarity. Furthermore, we suppose that positive species effects found in certain three-species combinations were “diluted” in the highest diversity level. In the six-species mixtures, species with similar growth rates (Scherer-Lorenzen et al. 2005)
were planted in direct adjacencies. This probably led to an adverse increase in interspecific competition (Scherer-Lorenzen et al. 2007, Oelmann et al. 2010). In addition, the lower inter- and intraspecific competition and the increased P acquisition in the three-species mixture could also be related to association with mycorrhizal fungi. As Klironomos et al. (2000) found in an old grassland field, the presence of arbuscular mycorrhizal fungi led to a positive but asymptotic response curve between plant species richness and productivity, reaching the maximum biomass not at the highest species richness level but at a lower to intermediate level of species richness.

2.5 Conclusion

The Sardinilla plantation is still at an early stage of development. Large amounts of N and P are being taken up by the trees from the soil every year. We expect that effects of tree diversity on ecosystem processes, such as nutrient accumulation, will change over the time course as it has been shown for other tropical plantations (Ewel and Mazzarino 2008). Though the outcome of a plantation might indeed depend predominantly on the given environmental conditions, our data clearly indicate that incorporating tree mixtures, and particularly the selection of matching tree species in the tropical forest management, has the potential to bring additional benefits as compared to monocultures and improve tree growth and nutrient uptake per unit of land surface. Further, information on aboveground nutrient pools and nutrient use efficiencies might also help selecting tree species for restoration of degraded lands. Planting species with high nutrient demands and low nutrient use efficiencies (e.g. *H. crepitans* and *T. rosea*) might result in adverse effects on soil fertility on the long run. Under such circumstances, sustainable management should include less nutrient demanding species, which are able to increase their nutrient use efficiencies when grown in mixtures (e.g. *A. excelsum*).

2.6 Acknowledgement

We are grateful to Iliana & Jose Monteza, Felipe Rodriguez and all the field workers in Sardinilla for great help in the field. We would like to thank Ben Turner (Smithsonian Tropical Research Institute, Panama), Karin Sörgel, Annika Lenz and Thomas Flura (ETH Zurich, Switzerland) for help with the lab analyses. Information on the elevation and the slope inclination of each plot and the plantation map were kindly provided by Sebastian Wolf (ETH Zurich, Switzerland). Authors are indebted to Yvonne Oelmann (Johannes Gutenberg University Mainz, Germany) who accepted to share raw data on stem N and P concentrations with us, and to Else Bünemann and Thomas Seitlinger (ETH Zurich and University Zurich, Switzerland), who kindly provided part of the soil phosphorus data. We also like to thank Christian Schöb and Petr Šmilauer for their advices on the statistical analyses. We are grateful to the Smithsonian Tropical Research Institute, Panama, and especially to Raineldo Urriola, for constant support. This research was made possible by a grant of the Swiss National Science Foundation (3100A0-110031/1).
Bibliography


| Tables and Figures |
Table 2.1: Linear mixed effect model for effects of species richness and sampling years on standing biomass, nitrogen pools and phosphorus pools in three compartments.

<table>
<thead>
<tr>
<th>Variable</th>
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<th>Mean (± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standing biomass (Mg ha⁻¹)</td>
<td>Species richness</td>
<td>2; 19</td>
<td>0.77</td>
<td>0.476</td>
<td>0.85 (± 0.443)</td>
<td>1.25</td>
<td>0.310</td>
<td>6.00 (± 0.18)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Species richness</td>
<td></td>
<td></td>
<td>2; 19</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Species richness</td>
<td>Year</td>
<td>1; 19</td>
<td>193</td>
<td>&lt;0.0001</td>
<td>190 (± 0.58)</td>
<td>197</td>
<td>&lt;0.0001</td>
<td>6.00 (± 0.94)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Species richness</td>
<td>Year</td>
<td>2006</td>
<td>3.91 (± 0.459)</td>
<td>2.84 (± 0.306)</td>
<td>1.25 (± 0.310)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Species richness</td>
<td>Year</td>
<td>2007</td>
<td>7.99 (± 1.02)</td>
<td>5.70 (± 0.64)</td>
<td>1.25 (± 0.24)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Species richness × Year</td>
<td></td>
<td>2; 19</td>
<td>0.40</td>
<td>0.673</td>
<td>0.39 (± 0.684)</td>
<td>0.48</td>
<td>0.627</td>
<td>6.00 (± 1.10)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nitrogen pool (kg ha⁻¹)</td>
<td>Species richness</td>
<td>2; 19</td>
<td>0.04</td>
<td>0.959</td>
<td>0.82 (± 0.457)</td>
<td>1.15</td>
<td>0.399</td>
<td>6.00 (± 0.94)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Species richness × Year</td>
<td></td>
<td>2; 19</td>
<td>0.81</td>
<td>0.459</td>
<td>0.01 (± 0.992)</td>
<td>0.95</td>
<td>0.405</td>
<td>6.00 (± 1.00)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phosphorus pool (kg ha⁻¹)</td>
<td>Species richness</td>
<td>2; 19</td>
<td>2.74</td>
<td>0.090</td>
<td>0.81 (± 0.459)</td>
<td>0.95</td>
<td>0.405</td>
<td>6.00 (± 1.10)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Species richness × Year</td>
<td></td>
<td>2; 19</td>
<td>0.32</td>
<td>0.727</td>
<td>0.26 (± 0.770)</td>
<td>0.74</td>
<td>0.490</td>
<td>6.00 (± 1.10)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a Data were log-transformed for analysis, except the P pool in branches. Within-group means ± standard errors are presented for categorical variables. Values for the three plant compartments are averages of the two sampling years. Different letters denote significantly different means according to Tukey-Kramer multiple comparison test. n=22.
Table 2.2: Linear mixed effects model for effects of species richness, species and sampling years on nitrogen (NUE) and phosphorus use efficiencies (PUE).

<table>
<thead>
<tr>
<th>Effect</th>
<th>df</th>
<th>F</th>
<th>P</th>
<th>Mean (± SE)</th>
<th>F</th>
<th>P</th>
<th>Mean (± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species richness (SR)</td>
<td>2; 19</td>
<td>0.48</td>
<td>0.627</td>
<td>17.4</td>
<td>0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td>171 ± 13.1</td>
<td></td>
<td></td>
<td>870 ± 59.9a</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td>170 ± 8.61</td>
<td></td>
<td></td>
<td>710 ± 35.9b</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td>167 ± 7.47</td>
<td></td>
<td></td>
<td>881 ± 32.8a</td>
</tr>
<tr>
<td>Species</td>
<td>4; 21</td>
<td>&lt;0.0001</td>
<td></td>
<td>90.8</td>
<td>&lt;0.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. exc.</td>
<td></td>
<td>203 ± 3.66a</td>
<td></td>
<td>983 ± 52.5a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. odo.</td>
<td></td>
<td>206 ± 8.20a</td>
<td></td>
<td>798 ± 46.9b</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H. cre.</td>
<td></td>
<td>83.9 ± 3.86c</td>
<td></td>
<td>570 ± 22.7c</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. see.</td>
<td></td>
<td>210 ± 4.31a</td>
<td></td>
<td>1066 ± 34.6c</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T. ros.</td>
<td></td>
<td>139 ± 2.47b</td>
<td></td>
<td>746 ± 31.0b</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Year</td>
<td>1; 40</td>
<td>26.6</td>
<td>&lt;0.0001</td>
<td>28.8</td>
<td>&lt;0.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2006</td>
<td></td>
<td>160 ± 6.93b</td>
<td></td>
<td>757 ± 32.2b</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2007</td>
<td></td>
<td>177 ± 7.71a</td>
<td></td>
<td>908 ± 33.0a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SR × Species</td>
<td>8; 21</td>
<td>2.87</td>
<td>0.025</td>
<td>10.5</td>
<td>&lt;0.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SR × Year</td>
<td>2; 40</td>
<td>0.12</td>
<td>0.884</td>
<td></td>
<td>0.43</td>
<td>0.657</td>
<td></td>
</tr>
<tr>
<td>Species × Year</td>
<td>4; 40</td>
<td>2.21</td>
<td>0.085</td>
<td></td>
<td>0.25</td>
<td>0.907</td>
<td></td>
</tr>
<tr>
<td>SR × Species × Year</td>
<td>8; 40</td>
<td>0.70</td>
<td>0.687</td>
<td></td>
<td>0.68</td>
<td>0.707</td>
<td></td>
</tr>
</tbody>
</table>

Within-group means ± standard errors are presented for categorical variables, different letters denote different means according to a Tukey-Kramer multiple comparison test. n = 55. Species abbreviations are A.exc. = Anacardium excelsum, C. odo. = Cedrela odorata, H. cre. = Hura crepitans, L. see. = Luehea seemannii, T. ros. = Tabebuia rosea.
Figure 2.1: Normalized aboveground biomass (AGB) in Mg ha\(^{-1}\) for each species across the species richness gradient. Values are means ± standard errors. Species had no effect on AGB in 2006 but in 2007 with A. exc. > H. cre.; L. see. > C. odo.; L. see. > H. cre. and T. ros. > H. cre. Species richness had no significant effect on normalized AGB in none of the two years. Species abbreviations are given in Table 2.2.
Figure 2.2: Aboveground biomass (AGB), N and P pools in stems, branches and leaves along the species richness gradient at the treatment level. Values are means ± standard errors with $n = 10$ for monocultures and $n = 6$ for three-species and six-species mixtures.
Figure 2.3: Additive partitioning of nitrogen pool (left) and phosphorus pool (right) by species richness, calculated for each year separately. The net effect (NE) is the sum of the complementarity effect (CE) and the selection effect (SE). Means ± standard errors are given (n=6). All effects in all diversity treatments and years were tested against zero (Student’s t-test). Significant effects are indicated as followed: · P < 0.1; * P < 0.05.
Figure 2.4: Effect of soil nutrient availability and tree diversity on the aboveground nitrogen and phosphorus pools. Bold arrows represent response variables, dashed vectors represent explanatory variables, nominal variables are given as filled triangle, circles are given for the different plots. Vector are labeled as follows: Npool = N pool in standing biomass, Ppool = P pool in standing biomass, NO3- = Soil nitrate concentration, NH4+ = Soil ammonium concentration, PO43- = Plant available phosphorus, Elev. = plot elevation, Slope = slope inclination of a plot, A.exc. = Anacardium excelsum, C.odo. = Cedrela odorata, H.cre. = Hura crepitans, L.see. = Luehea seemannii, T.ros. = Tabebuia rosea, SR = Species richness.
Figure 2.5: Correlation between aboveground nutrient pools and soil nutrient concentrations. Different symbols indicate different diversity treatments. Species richness had no significant effect on plant available phosphorus ($F_{2,19} = 1.66, P = 0.22$), soil nitrate concentrations ($F_{2,19} = 0.37, P > 0.5$) and soil ammonium concentrations ($F_{2,19} = 0.36, P > 0.5$).
Figure 2.6: Nitrogen and phosphorus use efficiencies (NUE and PUE, respectively) in monocultures, three-species and six-species mixtures for the five tree species. Given are means ± standard errors for the two years combined. Letters denote significant differences between diversity treatments for each individual species. The two study years were significantly different from each for both NUE ($F_{1,40} = 26.6, P < 0.0001$) and PUE ($F_{1,40} = 28.8, P < 0.0001$). Species abbreviations are given in Table 2.2.
APPENDIX A
Table 2.3: Deviation $\Delta RY_i$ from expected N and P pools of the five tree species.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>3 species</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. exc.</td>
<td>$0.70 \pm 0.67$</td>
<td>$0.43 \pm 0.24$</td>
<td>$0.21 \pm 0.18$</td>
<td></td>
</tr>
<tr>
<td>C. odo.</td>
<td>$0.27 \pm 0.37$</td>
<td>$0.32 \pm 0.55$</td>
<td>$0.70 \pm 0.89$</td>
<td></td>
</tr>
<tr>
<td>H. cre.</td>
<td>$0.19 \pm 0.18$</td>
<td>$0.13 \pm 0.19$</td>
<td>$0.40 \pm 0.30$</td>
<td></td>
</tr>
<tr>
<td>L. see.</td>
<td>$0.18 \pm 0.25$</td>
<td>$0.19 \pm 0.19$</td>
<td>$0.40 \pm 0.33$</td>
<td></td>
</tr>
<tr>
<td>T. ros.</td>
<td>$0.04 \pm 0.17$</td>
<td>$-0.01 \pm 0.12$</td>
<td>$0.07 \pm 0.20$</td>
<td></td>
</tr>
<tr>
<td>6 species</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. exc.</td>
<td>$0.40 \pm 0.26$</td>
<td>$0.26 \pm 0.25$</td>
<td>$0.35 \pm 0.13$</td>
<td>$0.13 \pm 0.12$</td>
</tr>
<tr>
<td>C. odo.</td>
<td>$0.03 \pm 0.12$</td>
<td>$0.08 \pm 0.16$</td>
<td>$0.05 \pm 0.13$</td>
<td>$0.14 \pm 0.19$</td>
</tr>
<tr>
<td>H. cre.</td>
<td>$0.15 \pm 0.29$</td>
<td>$0.08 \pm 0.20$</td>
<td>$0.08 \pm 0.18$</td>
<td>$0.05 \pm 0.17$</td>
</tr>
<tr>
<td>L. see.</td>
<td>$0.00 \pm 0.14$</td>
<td>$0.00 \pm 0.14$</td>
<td>$0.03 \pm 0.13$</td>
<td>$0.02 \pm 0.12$</td>
</tr>
<tr>
<td>T. ros.</td>
<td>$0.01 \pm 0.08$</td>
<td>$0.03 \pm 0.10$</td>
<td>$0.02 \pm 0.09$</td>
<td>$0.04 \pm 0.09$</td>
</tr>
</tbody>
</table>

$\Delta RY_i$ uses the average monoculture N or P pool of the corresponding species to compare it with the observed N or P pool in the mixture. Bold numbers indicate a significant difference from zero ($P<0.05$), italics stand for marginal significant differences ($P<0.1$). Means ± standard deviation are showed.
Table 2.4: Mean soil and plot characteristics of the experimental plantation in Sardinilla in 2007.

<table>
<thead>
<tr>
<th>Site variable</th>
<th>Specification</th>
<th>Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slope inclination °</td>
<td>Plot center</td>
<td>3.92 ± 0.32</td>
</tr>
<tr>
<td>Elevation m a.s.l.</td>
<td>Plot center</td>
<td>74.7 ± 0.36</td>
</tr>
<tr>
<td>pH</td>
<td>0-10 cm</td>
<td>5.85 ± 0.05</td>
</tr>
<tr>
<td>Organic P (mg P kg⁻¹ soil)</td>
<td>0-10 cm</td>
<td>302 ± 8.60</td>
</tr>
<tr>
<td>Total P (mg P kg⁻¹ soil)</td>
<td>0-10 cm</td>
<td>432 ± 12.1</td>
</tr>
<tr>
<td>Microbial P (mg P kg⁻¹ soil)</td>
<td>0-10 cm</td>
<td>49.9 ± 2.20</td>
</tr>
<tr>
<td>Plant available P (mg P kg⁻¹ soil)</td>
<td>0-5, 5-15, 25-35 cm</td>
<td>3.53 ± 0.38</td>
</tr>
<tr>
<td>Carbon (%)</td>
<td>0-5, 5-15, 25-35 cm</td>
<td>3.63 ± 0.11</td>
</tr>
<tr>
<td>Nitrogen (%)</td>
<td>0-5, 5-15, 25-35 cm</td>
<td>0.34 ± 0.01</td>
</tr>
<tr>
<td>Nitrate (mg N kg⁻¹ soil)</td>
<td>0-5, 5-15, 25-35 cm</td>
<td>5.46 ± 0.49</td>
</tr>
<tr>
<td>Ammonium (mg N kg⁻¹ soil)</td>
<td>0-5, 5-15, 25-35 cm</td>
<td>10.1 ± 0.83</td>
</tr>
<tr>
<td>N mineralization (mg N kg⁻¹ soil day⁻¹) a</td>
<td>0-15, May</td>
<td>1.23 ± 0.11</td>
</tr>
<tr>
<td>N mineralization (mg N kg⁻¹ soil day⁻¹) a</td>
<td>0-15, August</td>
<td>-1.44 ± 0.14</td>
</tr>
<tr>
<td>Herbaceous understorey (%) a</td>
<td></td>
<td>48.0 ± 2.95</td>
</tr>
</tbody>
</table>

a Mean and standard error for N mineralization rates in May and August and understorey cover are based on n=20.
The index $D_T$ uses the average monoculture N and P pool sizes of the component species for comparison with the observed N and P pools, while $D_{max}$ is based on the largest monoculture pool. In plots with *Cordia alliodora*, the indices were calculated excluding this species because of failure in monoculture.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
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<th></th>
<th></th>
<th></th>
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<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>AM 6</td>
<td>A. exc., C. odo., H. cre., L. see., T. ros.</td>
<td>0.63</td>
<td>-0.14</td>
<td>0.38</td>
<td>0.07</td>
<td>0.24</td>
<td>-0.09</td>
<td>0.09</td>
<td>-0.08</td>
</tr>
<tr>
<td>AN 6</td>
<td>A. exc., C. odo., H. cre., L. see., T. ros.</td>
<td>1.37</td>
<td>0.43</td>
<td>2.01</td>
<td>0.93</td>
<td>0.85</td>
<td>1.27</td>
<td>0.07</td>
<td>0.49</td>
</tr>
<tr>
<td>AS 3</td>
<td>A. exc., C. all., C. odo., H. cre., L. see., T. ros.</td>
<td>0.03</td>
<td>-0.17</td>
<td>0.34</td>
<td>1.14</td>
<td>0.24</td>
<td>-0.09</td>
<td>0.09</td>
<td>-0.08</td>
</tr>
<tr>
<td>AT 5</td>
<td>A. exc., C. all., C. odo., H. cre., L. see., T. ros.</td>
<td>0.76</td>
<td>0.17</td>
<td>1.47</td>
<td>1.14</td>
<td>1.27</td>
<td>1.09</td>
<td>0.11</td>
<td>0.11</td>
</tr>
<tr>
<td>AX 6</td>
<td>A. exc., C. all., C. odo., H. cre., L. see., T. ros.</td>
<td>0.12</td>
<td>-0.37</td>
<td>-0.49</td>
<td>-0.42</td>
<td>-0.07</td>
<td>0.04</td>
<td>-0.49</td>
<td>-0.39</td>
</tr>
<tr>
<td>AY 4</td>
<td>A. exc., C. all., C. odo., H. cre., L. see., T. ros.</td>
<td>-0.48</td>
<td>-0.71</td>
<td>-0.35</td>
<td>-0.51</td>
<td>-0.35</td>
<td>-0.51</td>
<td>-0.48</td>
<td>-0.71</td>
</tr>
<tr>
<td>AZ 2</td>
<td>A. exc., C. all., C. odo., H. cre., L. see., T. ros.</td>
<td>0.53</td>
<td>0.14</td>
<td>0.62</td>
<td>0.94</td>
<td>0.99</td>
<td>-0.10</td>
<td>0.17</td>
<td>0.17</td>
</tr>
<tr>
<td>A1 6</td>
<td>A. exc., C. all., C. odo., H. cre., L. see., T. ros.</td>
<td>2.44</td>
<td>0.94</td>
<td>2.17</td>
<td>1.54</td>
<td>0.76</td>
<td>0.49</td>
<td>2.17</td>
<td>1.54</td>
</tr>
<tr>
<td>A2 6</td>
<td>A. exc., C. all., C. odo., H. cre., L. see., T. ros.</td>
<td>0.28</td>
<td>-0.28</td>
<td>0.41</td>
<td>0.25</td>
<td>-0.22</td>
<td>-0.27</td>
<td>0.41</td>
<td>0.25</td>
</tr>
<tr>
<td>A3 6</td>
<td>A. exc., C. all., C. odo., H. cre., L. see., T. ros.</td>
<td>0.18</td>
<td>-0.33</td>
<td>-0.46</td>
<td>-0.38</td>
<td>-0.03</td>
<td>0.06</td>
<td>-0.46</td>
<td>-0.38</td>
</tr>
</tbody>
</table>

Table 2.5: Deviation from expected N and P pools for each mixture in the two study years.
Figure 2.7: Experimental design of the young plantation in Sardinilla, Panama, showing the monoculture (Ae1-2, Co1-2, Hc1-2, Ls1-2 and Tr1-2), three-species (T1-T6) and six-species (A1-A6) plots. The two plots at the bottom represent the abandoned monocultures of Cordia alliodora (Ca1-2). Note that in this study, only the large plots with 1, 3 and 6 species mixtures were used. Abbreviation for the monoculture plots are: Ae = Anacardium excelsum, Ca = Cordia alliodora, Co = Cedrela odorata, Hc = Hura crepitans, Ls = Luehea seemannii and Tr = Tabebuia rosea.
Phosphorus uptake strategies of three tropical tree species under intra- and interspecific competition

Zeugin F., Scherer-Lorenzen M. and Jansa, J.

Abstract

As native tree species in Latin America are more frequently used in sustainable plantation management or for reforestation of degraded land, not only information on their performance in terms of growth and survival is needed but also on their nutrient uptake strategies under intra- and interspecific competition. We planted three tropical tree species (*Anacardium excelsum*, *Luehea seemannii* and *Tabebuia rosea*), which are currently investigated in a tropical tree diversity plantation in the Republic of Panama, in large pots filled with soil from the plantation site. After two years of growth, we conducted soil phosphorus labeling. We combined $^{32}$P and $^{33}$P radioisotopes and root excluding mesh-bags to quantify the dependency of the tree species on mycorrhizal fungi for their P uptake under the influence of conspecific versus heterospecific neighbors. In addition, we developed species-specific molecular markers to quantify the contribution of each species to the root biomass in the mixture pots. Our data show that species specific differences in biomass production and P uptake are strongly enhanced under interspecific competition and that the tree species depended on a large degree on the hyphal pathway for P uptake. Patterns observed in the aboveground biomass were not necessarily reflected belowground. We suggest that information on the P demand of tree species and differences in P acquisition might serve as an additional selection criterion for the design of mixed species plantations.
3.1 Introduction

Information about the ecology of native tree species becomes increasingly important for sustainable plantation forestry in Latin America (Evans and Turnbull 2004, Hartley 2002). Besides producing the same desired goods for the market, native tree species are considered to be better adapted to local conditions and more sustainable concerning local biodiversity compared to exotic species, such as *Tectona grandis* or *Eucalyptus* spp. (Montagnini and Jordan 2005, Wishnie et al. 2007, Healy and Gara 2003, Haggar et al. 1998). Tree plantations in the tropics are traditionally planted as monocultures (Evans and Turnbull 2004). However, a growing body of evidence indicates that plantation systems including several local tree species can provide more diverse goods and environmental services as compared to pure stands, for example through improving the soil quality or restoration of degraded farmland, through efficient resource use (Kelty 2006, Grant et al. 2006, Hartley 2002) or even through enhanced productivity (Piotto 2008, Erskine et al. 2006).

While there is good knowledge about mixture effects on productivity related to canopy stratification (Ewel and Mazzarino 2008), relatively little is known about the importance of belowground niche complementarity for the performance of tree species and nutrient dynamics in mixtures (Jose et al. 2006, Rothe and Binkley 2001). Interspecific competition may force plant species to use different chemical forms of the same nutrient, for example nitrogen in form of nitrate, ammonium or organic N, or through spatiotemporal differences in resource uptake (Miller et al. 2007, Kahmen et al. 2006, McKane et al. 1990). Such belowground resource partitioning may result in higher nutrient retention and increasing productivity of the system (Scherer-Lorenzen et al. 2003). Most studies in neotropical plantations, however, remained on a phenomenological level with focus on plant establishment, growth and survival (e.g. Erskine et al. 2006, Montagnini 2000). In contrast, mechanistically driven research into belowground resource use was mostly performed in agroforestry (Dinkelmeyer et al. 2003, Lehmann et al. 2001).

Moreover, not only belowground interactions between different tree species can affect productivity and nutrient uptake in plantation systems. Interactions between plant species and soil microorganism, such as mycorrhizal fungi, have the potential to alter aboveground productivity, inter- and intraspecific plant competition, plant resistance to diseases and pathogens and the composition of plant communities (Herre et al. 2007, Klironomos 2003, Hartnett and Wilson 2002, Facelli et al. 1999, van der Heijden et al. 1998). Mycorrhizas are common associations between the majority of terrestrial plant species and some fungal species (Smith and Read 2008). Mycorrhizal fungi gather soil nutrients such as phosphorus (P) beyond the depletion zones of roots and deliver them via hyphae to the host plants in exchange for organic carbon (Smith et al. 2001, George et al. 1995, Marschner and Dell 1994). While associations with ectomycorrhizal fungi (EMF) are mainly found with plant species from alpine, boreal or tundra ecosystems and play an important role in the mobilization and uptake of organic N forms (Smith and Read 2008),
the mycorrhizal status of a great majority of neotropical trees is still unknown. However, tropical tree species studied so far are often associated with arbuscular mycorrhizal fungi (AMF) (Zangaro et al. 2003, Siqueira and Saggin-Junior 2001, Janos 1980b), although symbiosis with EMF also occurs on wide range of tropical soils (Alexander and Lee 2005, Newbery et al. 1997). As low P availability is one of major constraints for plant growth in tropical forests (Vitousek and Sanford 1986) and hence, is crucial for tree establishment in plantation systems, the role of AMF in the P nutrition of tropical tree species is still poorly understood and should be addressed more in detail.

Here, we present data from a pot experiment on the P acquisition strategies of three tropical tree species native to Panama, Central America. We applied a combination of soil labeling with $^{32}$P and $^{33}$P radioisotopes and root-excluding mesh-bags to quantify the contribution of root and mycorrhizal pathways to the P uptake of plants. The selected tree species are part of an experimental tree diversity plantation in Sardinilla, Panama, where several aspects of the relationship between tree diversity and ecosystem functioning are currently investigated (Potvin and Gotelli 2008, Scherer-Lorenzen et al. 2005). We chose this particular combination of species because this mixture, compared to others, showed the highest productivity after five years of growth in the field experiment. Specifically, we aimed to answer the following question:

- Do different tree species vary in their dependency on mycorrhizal associations for their P uptake, i.e. by taking up different proportions of P via the root and hyphal pathways?

### 3.2 Materials and Methods

#### 3.2.1 Study site and plant material

This study was conducted at Santa Cruz, the Experimental Field Facility of the Smithsonian Tropical Research Institute in Gamboa (9°06′N, 79°41′W), Panama. The site, 28 m a.s.l., has a mean annual temperature of 26 °C and rainfall of 2100 mm (Cernusak et al. 2009) with a pronounced dry season from December to April. The three tree species used are native to Panama and included the fast-growing species *Luehea seemannii* (*L. seemannii*), Tiliaceae, the moderately fast growing species *Anacardium excelsum* (*A. excelsum*), Anacardiaceae, and the slow growing species *Tabebuia rosea* (*T. rosea*), Bignoniaceae (Condit et al. 1993). Seedlings were provided by a nearby nursery (PRORENA - Proyecto de Reforestacion con Especies Nativas). Mean individual height ± standard deviation at planting was 21.4 cm ± 4.1 for *A. excelsum*, 16.8 ± 4.6 for *L. seemannii* and 12.8 ± 2.6 for *T. rosea*.

Tree seedlings were grown in 48 pots with a volume of 750 L each (120 cm diameter and 70 cm height). The containers were filled with soil from a nearby experimental plantation in Sardinilla, Panama, where the same three tree species were included in a larger tree diversity project (Scherer-Lorenzen et al. 2005). The soil at the Sardinilla site has been described as clayey *Typic Tropudalfs* (Potvin et al. 2004) with a pH of 5.85 ± 0.05. Plant
available phosphorus extracted by anion exchange resin adsorption (Saggar et al. 1990) was 3.53 ± 0.38 (mg P kg\(^{-1}\) soil), total soil nitrogen had a mean (± standard error) of 0.34 ± 0.01 %, and total soil carbon was on average 3.63 ± 0.01 % in the upper 30 cm (Zeugin et al., unpublished). We included a layer of gravel (5 cm) at the bottom of the pots to improve drainage. The soil profile in the container was kept as close as possible to the soil profile in the plantation. Seedlings were planted on the 14 August 2006 and harvested two years later on the 15 July 2008. Two seedlings, which died after planting, were immediately replaced, but the establishment of both plants failed during the first year of growth. Hence, they were not substituted again. All plants were watered twice a week during the dry season to assure plant survival. They did not receive any fertilizer, and commercial herbicides were only applied in September 2007 to avoid plant death by plagues.

We planted three seedlings either from the same tree species (i.e. monocultures) or as three-species mixtures in each container, yielding in 12 replicate pots per treatment. Since the area was surrounded by tall trees in the south-east and north-west direction, pots were arranged in two blocks to account for shading. Each block contained 6 replicates per treatment, which were randomly assigned to the containers. The spacing between the seedlings in the pot was 60 cm with a 30 cm distance towards the pot wall (Fig. 3.1).

### 3.2.2 32P and 33P labeling

24 days before harvest, we buried a set of two in-growth mesh-bags (Schweiger et al. 1999) in the center of each pot (Fig. 3.1). Each mesh-bag contained 300 g of a soil-sand mixture (2:1, w:w), labeled with either 3 ml 33P (2.82 MBq ml\(^{-1}\)) or 32P (3.25 MBq ml\(^{-1}\)) in form of orthophosphate (aqueous PO\(_4^{3-}\), carrier-free, Hartmann Analytic Gmb, Braunschweig Germany). The mesh-bag filled with 33P-labeled soil was made of 1 mm nylon mesh (Sefar, Thal, Switzerland), which allowed both roots and AMF hyphae to grow in. The second mesh-bag filled with 32P-labeled soil was only accessible to AMF hyphae due to the mesh size of 30 \(\mu\)m. Mesh-bags were buried in the center between the three saplings at \(\sim\)15 cm depth. Unlabeled soil-sand mixture was spread under and around the bags to ensure close contact between the soil in the pot and the mesh-bags.

In order to have a non-mycorrhizal control, we applied the fungicide benomyl inside the mesh-bags in half of the sets. Benomyl was successfully tested in earlier studies and is known to suppress AMF (Merryweather and Fitter 1996 and references therein). Each mesh-bag for the non-mycorrhizal control was treated with 2.5 g benomyl (Benlate, 50%, DuPont, wettable powder). We chose this high dose because we expected a rapid degradation of the fungicide under tropical conditions. In this way, the contribution of the root and mycorrhizal pathways to P uptake of plants could be quantified separately. Furthermore, plants grown in containers where no mesh-bags were buried (“control”), served for the determination of natural background activities of 32P and 33P. Each labeling treatment (“label”, “label + benomyl” and “control”) was randomly assigned to four pots.
of each species treatment (Table 4.1).

3.2.3 Plant harvest and measurements

Prior to harvest, total plant height and stem diameter at 10 cm above ground were assessed. On 15 July 2008, all plants were cut at the stem base, and total aboveground biomass of each plant was divided into a foliar and wooden sections to measure fresh weight. Two subsamples of each section were collected; one for the determination of the dry weight (72 h at 65 °C), and the second one for the phosphorus analyses. In addition, we collected three soil samples per container and pooled them to one sample per pot to determine root biomass and mycorrhizal colonization rates. Samples were taken in the interspace between the saplings from 0-15 cm soil depth. Roots were washed from the soil under tap water, dried for 48 h at 65 °C, cut into small pieces of 1 cm length and thoroughly mixed before analysis. The presented results always relate to amount of roots found in one kilogram fresh soil.

The activity of both $^{32}$P and $^{33}$P and the total P concentration in plant material were measured in acidic extracts. Approximately 1-2 g plant material was incinerated at 550 °C for 6 h, extracted with 3 ml boiling HNO$_3$ (70%), made up to 25 ml with deionized water and filtered through a paper filter (Whatman No. 40). P concentrations were determined by molybdate reaction with continuous flow analysis. The radioactivities of $^{32}$P and $^{33}$P in the extracts were assessed by liquid scintillation counting (Beckman Coulter, Inc., LS 6500, California, USA) using a Ultima Gold AB scintillation cocktail (Perkin Elmer, Boston MA, USA) in a 1:5 (v:v) sample/cocktail ratio. Samples were counted for 30 min each, and dual $^{32}$P and $^{33}$P measurements were performed using energy separation (lower energy window 0-480 keV, upper energy window 481-2000 keV). Results were corrected for chemical quenching based on single radioisotope solutions added with different amounts of acetone (C$_3$H$_6$O) and dichloromethane (CH$_2$Cl$_2$) as quencher. In addition, we used the H number (a quenching indicator) of the measurements of the P extracts from control plants to correct for color quenching. Background activities of HNO$_3$ blanks were subtracted from the sample activities. To avoid negative values for isotope activities due to the energy separation, the results (Bq g$^{-1}$) were subjected to linear transformation (+16).

3.2.4 Molecular marker development and quantitative real-time PCR

To obtain information about the belowground interspecific interactions between the three species, we designed species-specific molecular markers for the identification and quantification of fine roots. This allowed us to determine the relative contribution of each species to the root biomass in the mixture pots. We collected fine roots from five year old trees in the Sardinilla Plantation (Panama) in April 2006 to establish the molecular markers. Fine roots were stored in 50 % ethanol for 6 months.
A first crude DNA extraction was done using a commercial extraction kit (DNeasy Plant Mini Kit, Qiagen, Hombrechtikon, Switzerland) and PCR amplifications with eukaryotic universal primers ITS3 and NDL22 (van Tuinen et al. 1998, White et al. 1990) for nuclear ribosomal DNA. Resulted DNA fragments were cloned (pGEM-T Easy Plasmid, Promega, Dübendorf, Switzerland) and sequenced (Microsynth, Balgach, Switzerland). The design of primers and TaqMan probes for quantitative real-time PCR was undertaken with AlleleID version 4 software (Premier Biosoft International, California, USA) based on the cloned tree species sequences. Primers and probes labeled with fluorescein and BHQ-1 were synthesized and HPLC-purified in Microsynth and tested for cross-specificity and amplification efficiency (data not shown). To assure that number of DNA copies were correlated with fine root biomass, we did a calibration experiment with different root weights (Fig. 3.7 in the Appendix). Finally, we extracted DNA from the 48 roots samples of the experimental pots, following the protocol of the DNeasy Plant Mini Kit (Qiagen). Each sample was diluted 20 times and quantified with a LightCycler 2.0 (Roche Diagnostics, Rotkreuz, Switzerland) and Roche chemistry (LightCycler TaqMan Master, Roche). The cycling conditions are briefly described here: Pre-incubation was at 95 °C for 15 min, followed by 45 cycles with an initial denaturation at 95 °C for 5 sec. The annealing was performed at 50 °C for 40 sec and amplification at 72 °C for 10 sec was followed by fluorescence measurement at 530 nm. The reaction was finished with a cooling down at 40 °C for 30 sec.

We used the same root DNA extracts to check for the presence of different AMF species by quantitative real-time PCR. In contrast to other approaches, this is the only known method, at the moment, which allows the quick detection and quantification of several AMF species in one root sample. Moreover, a recent study by Thonar (2009) demonstrated that quantitative real-time PCR is a useful tool for the assessment of root colonization by the different AMF species. Molecular markers were designed for the large ribosomal subunit (LSU) genes (Thonar 2009), and currently they are available for *Glomus intraradices, Glomus claroidum, Glomus mossae, Gigaspora margarita* and *Scutellospora pellucida*. We followed the quantification protocols according to Thonar (2009) and Jansa et al. (2008). For *Glomus intraradices* (short G. intraradices), two sets of species-specific primers and TaqMan probes were available: one for the amplification of nuclear DNA and a second set for the mitochondrial DNA in this fungus. For the real-time PCR analyses, DNA extracts were diluted 20 times.

### 3.2.5 Calculations and statistical analyses

Total aboveground biomass per plant was calculated by summing the dry weight of the foliar and wooden plant material. In terms of fine roots, we calculated dry root biomass per kilogram fresh soil. We decided not to scale up for the total container volume as we did not have an estimate of the coarse or tap roots in the pot and because we expected uneven root distributions at the walls and at the bottom of the pots.
P concentrations in leaves, wood and roots were multiplied with the corresponding biomass to calculate the P pools in plants.

The amount of the two radioisotopes $^{32}$P and $^{33}$P taken up by the plants (% recovery) was calculated as the ratio of the specific isotope in the aboveground biomass (corrected for isotope decay) divided by the activity of $^{32}$P and $^{33}$P label, respectively, applied to the mesh-bags. For fine roots, we only present the radioisotope activity (Bq g$^{-1}$, corrected for the isotope decay), since we do not have the total root biomass per pot to calculate the belowground recovery.

The number of DNA copies per $\mu$l DNA extract was assessed by the following equation:

$$\text{DNA copies} = \frac{10^{b-Cp} \times \text{ev} \times \text{df}}{\text{DM}_{\text{root}}}$$  \hspace{1cm} (3.1)

where $a$ and $b$ are species-specific parameters (see Thonar 2009 for AMF parameters and Table 3.3 in the Appendix for tree species parameters), $C_p$ are the cross-point values from the real-time PCR, $ev$ gives the end volume of the DNA extract, $df$ is the dilution factor and $DM_{\text{root}}$ represents the dry matter of the root subsample from which the DNA was extracted.

In general, aboveground response variables were analyzed on the species level ($n=72$), using analysis of variance (ANOVA). For monocultures, we averaged the three plant values per pot and compared the means ($n=36$) with the single plant measurements in the mixtures ($n=36$), as proposed by von Felten et al. (2009). Results are presented according to Schmid et al. (2002). To analyse plant growth, nutrient concentrations and nutrient pools, terms were entered in the order: (1) block, (2) species richness, (3) species in monocultures and (4) species in mixtures. Factors 1-3 were tested against the pot residuals, whereas species in mixtures was tested against the residual variation. Results from the $^{32}$P and $^{33}$P labeling were analyzed likewise, entering the terms as follows: (1) block, (2) P treatment, (3) species richness, (4) species in monocultures, (5) species in mixtures and (6) interactions. Terms 1-4 and possible interactions were tested against the between pot variation, whereas term 5 and possible interactions were tested against species residuals. Analyses of belowground variables were performed on the pot level ($n=48$) without testing for species differences in the mixtures. Data were either log or square-root transformed, if necessary, to meet the assumptions of ANOVA. The differences in means of significant terms were tested using the Tukey-Kramer method for multiple comparison test ($P < 0.05$).

### 3.3 Results

#### 3.3.1 Plant growth, phosphorus concentrations and pools

After two years of growth, total plant height ranged from 0.69 m to 4.90 m with a mean and standard deviation of $2.29 \pm 0.77$ m, hence trees have increased their height approximately
tenfold during the two years of growth. In monoculture, species differed significantly in height, in the order *A. excelsum*, *T. rosea* and *L. seemannii* (Table 3.2). In mixture, this order changed with *A. excelsum* still being the tallest species, but with *L. seemannii* outperforming *T. rosea*. Mean stem diameter was 5.80 ± 2.41 cm with clear differences between the species. In both monoculture and mixture, *A. excelsum* and *L. seemannii* had greater stem diameters than *T. rosea* (Table 3.2).

Species richness had no significant effect on total aboveground biomass, but biomass differed between the species in each group (Fig. 3.2a and b, Table 3.4 in the Appendix). *A. excelsum* and *L. seemannii* accumulated significantly more biomass than *T. rosea* in both monoculture and mixture pots, with more pronounced interspecific differences in the latter case. Mean foliar biomass was 17 ± 7% (standard deviation) of total biomass in monoculture, with *A. excelsum* allocating 22% into the foliar tissue, followed by *T. rosea* (16%) and *L. seemannii* (11%). When planted in mixture, *A. excelsum* and *T. rosea* had similar allocations to the leaves (25% and 17%, respectively) as monoculture plants. Only *L. see* showed a decrease in relative foliar biomass (6%) and allocated more biomass into stems and branches (88% in monoculture, 93% in mixture).

No species richness effect was observed for the P concentrations (Table 3.4 in the Appendix). *T. rosea* showed the highest P concentrations in leaves and roots when planted as monoculture (Table 3.2). In the three-species mixtures, foliar P concentrations did not significantly differ between the three species. However, *A. excelsum* showed a higher P concentration in woody tissue compared to the other two species in mixtures. Species richness had no significant effect on the aboveground P pools (Table 3.4 in the Appendix). No differences were detected between the species in monoculture, irrespective of the plant tissue (Fig. 3.2c). However, species in mixture differed significantly. Similar to total biomass, *A. excelsum* had the largest P pools (foliar and woody pools combined), followed by *L. seemannii* and *T. rosea* (Fig. 3.2d). On average, plants stored ~42% of P in the leaf compartment.

In the topsoil, we found the highest root biomass in pots of *A. excelsum* (Fig. 3.6a and Table 3.5 in the Appendix) with three-times more root biomass compared to *L. seemannii* and *T. rosea*. Based on the high P concentration, *T. rosea* stored more P in the root tissue than *L. seemannii* (Fig. 3.6b in the Appendix). We did not find any effect of species richness on either the biomass or the P pools of roots.

Based on the results of the real-time PCR, we calculated the relative proportion of each species in the roots samples collected in mixture pots. This was then compared to the relative proportions in the aboveground biomass. Although *A. excelsum* produced relatively more shoot biomass than the other species in mixture (*A. excelsum*: 69.3 ± 3.73%, *L. seemannii*: 28.4 ± 3.56% and *T. rosea*: 4.67 ± 0.84%), its relative contribution of 22.0 ± 6.38% (mean ± standard error) to the root biomass was lower compared to *L. seemannii* (79.8 ± 4.96 %). Only *T. rosea* had similar proportions in belowground (5.25 ± 2.00%) and aboveground biomass. Both *A. excelsum* and *T. rosea* showed a positive linear trend
between aboveground and belowground fractions (Fig. 3.3). No trend was observed for *L. seemannii*.

### 3.3.2 Phosphorus uptake via root and hyphal pathway

Three weeks after burying the $^{32}$P and $^{33}$P labeled mesh-bags, a network of newly produced fine roots was found underneath and around each mesh-bag, a good indication that the timespan was sufficient for roots to grow towards the new P sources. The $^{33}$P activity in leaves, a measure for the P amount transferred to the plant by hyphae and roots, increased on average to $31 \pm 5$ Bq g$^{-1}$ (standard error) and was significantly higher than the foliar $^{33}$P activity in control plants, where the values ranged from 14 to 16 Bq g$^{-1}$ (Table 3.6 in the Appendix). Non-mycorrhizal control plants, which were treated with the fungicide benomyl, did not take up any $^{32}$P by roots either and showed the same activity as unlabeled plants (17 versus 16 Bq g$^{-1}$). Pooled over all treatments, the effect of the species in monoculture on the $^{33}$P activity was not significant (Fig. 3.4a and Table 3.6 in the Appendix). However, if grown with heterospecific neighbors, trees of *T. rosea* showed higher foliar $^{33}$P activities than the other two species (Fig. 3.4c). The $^{32}$P uptake by AMF hyphae from the hyphal-only mesh-bags was very small ($\sim$20 Bq g$^{-1}$) but still higher than in the control plants (Fig. 3.4b and d). As expected, saplings treated with the fungicide showed no $^{32}$P uptake at all. No significant effect of the species on the $^{32}$P uptake was found, regardless whether they were grown with conspecific- or heterospecific neighbors (Table 3.6 in the Appendix).

The assessment of the $^{32}$P and $^{33}$P recoveries in the foliar and shoot (foliar and wood combined) biomass revealed that only a small portion of the offered $^{32}$P and $^{33}$P appeared in the aboveground tissues (Fig. 3.4e-l). Species richness and species treatments combined, labeled plants showed a significant higher foliar $^{33}$P recovery than control plants or plants treated with benomyl, which in turn were not different from each other. In monoculture and mixture pots, trees of *A. excelsum* allocated significantly more $^{33}$P into the leaves than trees of *T. rosea* (Fig. 3.4e and g). For the hyphal pathway, we found only a marginal effect of the treatments on the $^{32}$P recovery in leaves. Similar to the results observed for the foliar $^{33}$P recovery, *A. excelsum* trees had higher $^{32}$P allocation to leaves compared to saplings of *L. seemannii* and *T. rosea*, both in monoculture and mixture pots (Fig. 3.4f and h).

If we look at the total aboveground $^{33}$P recovery, the labeled plants were still significantly different from the control plants, but not from the benomyl treated saplings anymore (Table 3.6 in the Appendix and Fig. 3.4i and l). In monoculture, $^{33}$P recovery for *A. excelsum* and *L. seemannii* was similar, and the uptake only differed between *A. excelsum* and *T. rosea*. Differences between species were found in mixture pots in the $^{33}$P uptake with highest values found in *A. excelsum*, followed by *L. seemannii* and *T. rosea*. The same species patterns were also observed for the $^{32}$P recovery, but no differences between labeled, benomyl treated and control plants were found at all.
The activity of $^{33}$P (Bq g$^{-1}$) measured in root samples (Fig. 3.5a) varied strongly, and we did not observe any treatment effects (Table 3.7 in the Appendix). However, $^{32}$P activities in labeled plants were on average five times higher than in control plants (Fig. 3.5b). Especially the $^{32}$P activity measured in roots of labeled L. seemannii and T. rosea were high compared to follar activities (L. seemannii: 253 versus 18 Bq g$^{-1}$, T. rosea: 166 versus 23 Bq g$^{-1}$). We did not find any elevated $^{32}$P in roots of A. excelsum or species mixtures, which corresponds to trends observed in the leaves. In addition, a marginal significant interaction between the P treatment and species richness was detected (Table 3.7 in the Appendix), showing a trend towards increased $^{32}$P activities in labeled monoculture pots compared to mixture pots. Testing the three species grown in monoculture, L. seemannii had significantly higher $^{32}$P activities in roots than trees of A. excelsum, whereas T. rosea was not different from neither of them.

### 3.3.3 Quantification of mycorrhizal DNA in roots

Within roots, no amplification with the molecular markers for Glomus claroidum, Glomus mossae and Scutellosopora pellucida was obtained. In one sample, we detected a low DNA concentration of Gigaspora margarita (37 LSU gene copies per mg root), and several samples contained detectable amounts of gene copies from Glomus intraradices (mitochondrial DNA in 38 samples, nuclear DNA in 26 samples). Number of DNA copies, a measure for root colonization intensity, strongly varied between root samples (Fig. 3.8 in the Appendix) and therefore, no relationship with tree species, species richness, nutrient treatments or plant responses could be substantiated (analyses not shown).

### 3.4 Discussion

#### 3.4.1 Plant growth and species interactions

In this study, we aimed to understand the interspecific differences in plant growth and P acquisition strategies of three tropical tree species grown either with conspecific or heterospecific neighbors. After two years of growth, surprisingly it was not the pioneer species L. seemannii which tended to grow best in terms of aboveground and belowground biomass but the mid-successional species A. excelsum. As expected, T. rosea, the late-successional species, lagged behind the two other species in terms of biomass production. Because the classification of these species to successional stages derives from occurrence in treefall gaps in natural forests, growing conditions in the pots might have been very different than in natural forests, despite their large size. However, we were not able to detect an increase in plant biomass per pot with increasing species richness, which is in contrast to the findings of Scherer-Lorenzen et al. (2007) and Potvin and Gotelli (2008), who found significant transgressive overyielding of this specific three-species mixture in the Sardinilla biodiversity experiment. There, those species developed a stratified
canopy with *L. seemannii* in the upper, *A. excelsum* in the middle, and *T. rosea* in the lower canopy, indicating the potential for complementary light use. Based on these results we had expected diversity effects in this particular species combination also in our pot experiment due to potential root stratification and hence differences in nutrient uptake. While *L. seemannii* was not affected by heterospecific neighbors, interspecific competition enhanced the biomass production of *A. excelsum* and hampered growth of *T. rosea*. This in return resulted in a zero net increase in the biomass of mixture pots. Thus, although species interactions led to differentiated growth responses, which is a prerequisite of diversity effects on biomass production, this specific three-species mixture did not result in overyielding (i.e. a higher production in mixture than in monoculture). We relate these differences in growth to the different phenologies of the species. Whereas *L. seemannii* and *T. rosea* shed all their leaves and were completely leafless during the dry season, *A. excelsum*, a brevideciduous species (Newell et al. 2002), only lost part of the foliage and was most likely able to continue photosynthesis, since we watered the plants regularly in the dry season. We assume that this led to an additional advantage in growth of *A. excelsum*. Similar to the aboveground pattern, root biomass production of *A. excelsum* was as well higher than that of the other two species in monoculture (Fig. 3.6). Nonetheless, observed root patterns must be regarded with care. They relate only to the amount of roots found in a small sample of surface substrate and might not be representative for the whole root biomass in the container, as coarse or tap roots were not represented in those samples.

Comparing the contributions of the different species to the shoot and root biomass in mixture pots, we were surprised by the large proportion of *L. seemannii* in the root samples. As *A. excelsum* seems to be more efficient in terms of P use efficiency (high productivity, low nutrient concentrations) and, therefore, more competitive in mixture, trees of *L. seemannii* had obviously allocated more biomass to belowground parts to acquire sufficient amount of nutrients and/or water (Craine 2006, Paz 2003). In sum, our results did not support the hypothesis of enhanced biomass production in mixture compared to monoculture but showed that interspecific interaction do change growing patterns of the constituent species. Nevertheless, these differences of growth might in the long run still lead to partitioning of resources (light, nutrients or water), which could result in different biomass production in mixed versus pure communities. In most biodiversity experiments done with grassland species, diversity effects usually develop with time (Fargione et al. 2007, Marquard et al. 2009). Similarly, diversity effects also developed only after three years of growth in the Sardinilla Experiment (Potvin and Gotelli 2008). Thus, both the short duration of our experiment, but maybe also the special situation of a pot experiment, might explain the lack of any diversity effect on biomass at this stage.
3.4.2 Species-specific differences in P uptake

To our knowledge, this is the first study presenting results about the contribution of the hyphal pathway to total plant P uptake of tropical tree species grown in an open-air pot experiment over two years. Twentyfour days after insertion of the mesh-bags, total uptake of $^{33}$P and $^{32}$P, measured as P recovery (%), was very low (usually less than 0.5% of the applied radioisotope amount) in both total shoot biomass and in the foliage of the plants. This indicates that most of the $^{33}$P and $^{32}$P was immobilized and/or absorbed to soil minerals (Gillman and Uehara 1980, Uehara and Gillman 1980). The differences between the labeling treatments detected in the foliar activity (Bq g$^{-1}$) suffered from high variability between experimental units and reflected rather differences in plant growth when upscaled to the whole shoot or foliage level. Therefore, we will focus on the foliar $^{33}$P and $^{32}$P activities in terms of P uptake during the discussion. The species × P treatment interaction was not significant for foliar or root $^{33}$P activity, we, therefore, can only follow trends in the species-specific P uptake. The T. rosea tended to hold higher foliar $^{33}$P activities than the other two species which is congruent with the higher foliar P concentration. Again, we think this might be partly related to the phenology of T. rosea (Borchert 1983). The species lagged behind in leaf flush and leaf expansion compared to the A. excelsum and L. seemannii (F. Zeugin, personal observation) and probably reached full activity only shortly before the labeling began. A higher need for P in saplings of T. rosea during leaf expansion seems therefore comprehensible.

Even though foliar $^{33}$P and $^{32}$P activities indicate that the high P uptake in trees of T. rosea was not affected by heterospecific neighbors, belowground $^{33}$P and $^{32}$P activities are not consistent with this finding. We found actually no increased uptake of $^{33}$P and $^{32}$P from the labeled mesh-bags into the roots of this species. This inconsistency is probably connected to the way of root sampling. We were not able to visually distinguish the fine roots in mixture pots according to the three species. Consequently, the roots samples from the mixture represent a combination of all species. Based on the outcome of the quantitative real-time PCR, the fraction of T. rosea in the root biomass was not more than ~5%. Potential uptake of $^{33}$P and $^{32}$P by this species was obviously diluted by the large biomass fraction of the other two species.

Of all three species, A. excelsum seemed to be one with a very small or negligible P uptake from the labeled soil (Fig. 3.4 and 3.5). This is consistent with the low P concentrations in all three tissues. However, A. excelsum is more efficient in terms of biomass production per unit P than L. seemannii or T. rosea. As a plausible mechanism, we refer to the above mentioned differences in phenology among the species. We speculate that A. excelsum has its peak in P uptake early in the rainy season, probably right after the first rainfalls, and benefits from the newly mineralized phosphate while the other two tree species have only started to produce new leaves.

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3.4.3 Direct plant versus hyphal pathway

Searching for differences in P uptake strategies, our results indicate that plants, which transferred $^{33}\text{P}$ to their leaves, depended on their associated AMF for the P uptake. We did not detect any $^{33}\text{P}$ uptake by root hairs or root epidermis in pots treated with benomyl, which served us as a control for the direct plant P uptake. Regarding this outcome, the question raised whether benomyl could have affected the root growth negatively in our experiment. No evidence supports this notion, however. First, we removed the mesh-bags after the harvest and visually checked whether fine roots grew towards and into the mesh-bags. We did not observe a lack of fine roots in the close vicinity of the benomyl treated mesh-bags. Second, the fungicide has been widely used in the AMF research and no or only low phytotoxic effects have been reported so far (Helgason et al. 2007, Smith et al. 1999, Merryweather and Fitter 1996). In the study of Kiers et al. (2000) where a similar high dose of benomyl ($4.4\text{ g L}^{-1}$) was applied, seedlings of $L. \text{ seemannii}$ grown on benomyl treated soil experienced indeed a high mortality rate (82%), but the investigators related this to the dependence of $L. \text{ seemannii}$ on AMF for survival and growth rather than on benomyl toxicity. Furthermore, we did not have any practical alternative to the fungicide benomyl. Sterilization was not feasible due to the large soil volume and would neither have guaranteed absence of colonization by AMF during the two study years. Genetic mutants of our tree species, such as reported for some legumes (e.g. $P. \text{ sativum}$ L. and $V. \text{ faba}$ L., Duc et al. 1989), unable to undergo the association with AMF, were neither available. Alternative fungicides were neither available (A. Fitter, personal communication). We thus suggest other mechanisms explaining the absence of the direct P uptake by the plants than due to phytotoxic effects of benomyl. As it has been shown for medic $M. \text{ trunculata}$ and tomato $L. \text{ esculentum}$ plants (Burleigh et al. 2002, Rausch et al. 2001), plants can respond differently to AMF even on the level of gene expression. Following the suggestions of Jansa et al. (2005) and Smith et al. (2010, 2004), we assume that the lack of evidence for a direct P uptake by roots must be related to some down-regulation of the expression of genes encoding for P transporters. In contrast the earlier studies using a physical barrier (compartments or mesh-bags) to measure the contribution of the hyphae to total P uptake (for example Smith et al. 2004, Jansa et al. 2003, Jakobsen et al. 1992), seedlings in our experiment were allowed to grow in association with their AM fungal partners from the beginning. The activity of the AMF was not suppressed until the $^{32}\text{P}$ and $^{33}\text{P}$ labeled mesh-bags were buried, and in turn, the suppression only applied to AMF mycelium extending into the mesh-bags but not the residual soil volume. Plants in the benomyl treatment obviously did not have sufficient time to adapt to the new situation in terms of gene expression or the P transporter expression was not so strictly localized. This means that the plants as whole did not rely at all on the offered $^{33}\text{P}$ in the mesh-bags, because they were still able to receive enough P through the AMF outside the mesh-bag.

The results from the foliar $^{32}\text{P}$ activity support the idea of an inactive root pathway
for P acquisition. Moreover, plants with high $^{33}$P activities in leaves also showed high $^{32}$P activities reinforcing the notion that these plants took up most of their P via AMF. This is because $^{32}$P must have been taken up by the AMF hyphae as roots were physically excluded from the mesh-bags by the mesh size of 30 µm. Surprisingly, the $^{32}$P activities in leaves were generally lower than $^{33}$P activities but drastically higher in the roots (Fig. 3.5). One possibility to explain these findings is a delay in the $^{32}$P transport to the shoot. We suppose that mycorrhizal roots were able to grow quickly into the $^{33}$P labeled mesh-bags and that the mycelium associated with those roots had an rapid access to the $^{33}$P sources. On the other hand, AMF that spread into the $^{32}$P labeled mesh-bags probably needed more time to cover the distance without the help of a transport vehicle (root). This may explain why the $^{32}$P found in mycorrhizal roots was not yet transferred to the leaves at the time of harvest. G. intraradices, the AMF species detected in the majority of root samples, is known to transfer P over long distances (up to 10 cm in association with maize, Jansa et al. 2005, 2003) and to contribute up to 100% of the P when colonizing flax, medic or tomato (Smith et al. 2004, 2003). Other authors observed high activities of P radioisotopes in the extraradical mycelium and/or roots colonized by AMF species from the family Gigasporaceae and related the retention of phosphate to the life-cycle of Gigaspora (Thonar 2009, Boddington and Dodd 1999, Jakobsen et al. 1992). The need for plant-derived carbohydrates to produce spores and mycelium is thought to be higher for Gigaspora compared to Glomus ssp. and the P retention by the fungus has been interpreted as a mechanism to control the carbon supply by the plant. However, we detected structures of Gigaspora margarita in only one root sample. This does not necessarily mean that our plants were not colonized by other Gigaspora species, as will be discussed below, but it seems rather unlikely that only the $^{32}$P but not the $^{33}$P would have been stored in the intraradical hyphae.

### 3.4.4 Differences in root colonization by AMF

Using a molecular approach to measure AMF colonization in roots, we were able to prove the occurrence of G. intraradices in 75% and of Gigaspora margarita in only 1% of all root samples. The intensity of colonization varied strongly between different pots for each AMF species. We did not detect presence of Glomus claroidum, Glomus mossae or Scutellospora pellucida in the roots. However, abundance of AMF biomass in these tree roots is not known and might fall below the detection limit of the real-time PCR. The results further suggest that other molecular species-specific markers did not match the DNA sequence of AMF species being present in the roots. For example, the same set of markers, which were originally designed based on AMF species isolated from a single field in Switzerland (Jansa et al. 2002), were also applied to mycorrhizal roots of Salix ssp. collected from a glacier forefield in Switzerland. Although AMF structures in Salix were observed by traditional staining-microscopy, no amplifications with these molecular markers were obtained similarly to this study (Monika Welc, ETH Zurich,
personal communication). We speculate that different ecosystems (such as an arable field vs. glacier forefield vs. tropical soil) probably harbor very distinct AMF communities although other scientists suggested little difference in AMF flora between tropical and temperate ecosystems (Herre et al. 2005). However, though AMF show a relatively poor diversity in terms of morphospecies, genetic variation within AMF species or even populations is expected to be high and to affect both the phenotypic plasticity of AMF and plant-fungus interactions (Ehinger et al. 2009, Koch et al. 2006). This high diversity of genotypes makes it obviously rather difficult to develop a set of species-specific molecular markers applicable for samples collected in very distinct geographic regions.

All three tree species were colonized by AMF (at least by *G. intraradices*) as it has been reported earlier for *A. excelsum* and *L. seemannii* (Husband et al. 2002a, Kiers et al. 2000) and other species members of the Bignoniaceae (e.g. *Tabebuia roseo-alba*, Zangaro et al. 2003). The findings are also in agreement with a root and spore survey from samples collected in April 2006 at the Sardinilla plantation from where the soil for the pot experiment was taken (F. Zeugin, unpublished data). Colonization rates according to the approach by McGonigle et al. (1990) varied between 42 and 56% for the three species, however, trees in Sardinilla were five years old at the time of sampling. In the soil, we found a high spore density (∼73 spores per g fresh soil) and 13 different AMF morphotypes including the genera of *Acaulospora*, *Glomus*, *Entrophospora* and *Scutellospora*, whereas *Glomus* spp dominated the AMF community in Sardinilla. However, tissue structures of *Acaulospora* are difficult to stain, thus colonization rates might even be higher in roots at the Sardinilla field site. Infection patterns related to the successional status of a species (pioneer, mid-successional or late-successional), as demonstrated by Janos (1980a, b) or Zangaro et al. (2003), were not found. It is possible that such differences in AM response and colonization are more important at early stage of seedling establishment and become less significant with time. For example, Husband et al. (2002b), using AM-specific primers and ribosomal RNA gene sequences, were able to show that the fungal diversity decreased as seedlings of *Tetragastris panamensis* mature and that the developmental stage of the plant affects the AM fungal community.

In summary, we successfully applied a combination of molecular tools and radioactive isotope labeling here to bring new insights into the P acquisition strategies of three tropical tree species. Our data show that species specific differences in biomass accumulation were strongly affected by heterospecific neighbours. Differences in P acquisition, mirrored in biomass differences, are probably related to the phenology of the species. Further, we were able to demonstrate that all the three species associated with arbuscular mycorrhizal fungi (*G. intraradices*) and depended to a large degree on the P uptake by the extraradical mycelium. However, we were not able to unequivocally detect differences in the P delivered by the AMF among the tree species, which is probably related to the short time span of the experiment and to the large pot volume.
3.4.5 Conclusion

Where different tree species are considered for mixed species plantation, combinations of species are often based on criteria such as relative growth rates or successional categories (e.g. in the Sardinilla plantation). These attributes might be of little ecological relevance on sites where trees species are competing for belowground resources. Information on their nutrient requirements and differences in nutrient acquisition strategies, as provided by this pot experiment, might serve as an additional and more relevant criterion for the successful selection of tree species for mixed plantation designs.

Acknowledgements

Authors are indebted to Ben Turner, Jose Ramon Perurena, Jose Barahona, Raineldo Urriola, Klaus Winter and Jorge Aranda from the Smithsonian Tropical Research Institute in Panama who provided substantial support in many stages during the experiment. We are very grateful to Jose Alberto Maynard for his green thumb and assistance over the two years and to Iliana Cabrera Phillips, Jose Monteza, Felipe Rodriguez and David Sarco for their great help at the harvest. We thank Annika Lenz (ETH Zurich, Switzerland) for the stable isotope measurements and Stefanie von Felten and Hans-Rudolf Roth for advice on the statistical analyses. This project was funded by a grant of the Swiss National Science Foundation (3100A0-110031/1) to Michael Scherer-Lorenzen and Jan Jansa.
Bibliography


Tables and Figures
Table 3.1: Number of replicates (pots) per treatment for the dual $^{32}$P and $^{33}$P labeling

<table>
<thead>
<tr>
<th>Species $^a$</th>
<th>control</th>
<th>label</th>
<th>label + benomyl</th>
</tr>
</thead>
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<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>L. see.</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>T. ros.</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>3 spec.</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>

Table 3.2: Mean plant height, stem diameter and P concentrations in foliar, woody and root tissue of the three species, grown in monoculture or mixture.

<table>
<thead>
<tr>
<th>Species †</th>
<th>Height (m)</th>
<th>Diameter (cm)</th>
<th>P concentration (mg g⁻¹)</th>
</tr>
</thead>
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<tr>
<td></td>
<td></td>
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<td><strong>Monoculture §</strong></td>
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<td>2.86&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.76&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.51&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>2.14&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.54&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.25&lt;sup&gt;b&lt;/sup&gt;</td>
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<td><em>T. ros.</em></td>
<td>2.46&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>4.50&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.48&lt;sup&gt;a&lt;/sup&gt;</td>
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<td><strong>3 species mixture §</strong></td>
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<td><em>A. exc.</em></td>
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<td>7.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.41&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
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<td>2.71&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.40&lt;sup&gt;a&lt;/sup&gt;</td>
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</tbody>
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† Species abbreviations are listed in Table 4.1.
§ Different letters denote different within-group means according to a post-hoc Tukey-Kramer multiple comparison test (P < 0.05).
Figure 3.1: Schematic pot design. The two mesh-bag labeled with $^{32}\text{P}$ and $^{33}\text{P}$ were placed in the center between the three saplings at 15 cm depth and remained there for 24 days.
Figure 3.2: Foliar and wood biomass and P pools for monocultures and three-species mixtures. Given are means ± standard errors on the species level. Different letters denote different within-group means for total biomass or nutrient pools, respectively, according to a post-hoc Tukey-Kramer multiple comparison test ($P < 0.05$). Species abbreviations are listed in Table 4.1.
Figure 3.3: Linear regression on the percentages of aboveground biomass (shoot) and belowground biomass (root) in mixture pots. Root proportions were derived from the number of DNA copies of the tree species in a root sample. Plotted are regression lines and 95% confidence intervals. Species abbreviations are listed in Table 4.1.
Figure 3.4: Activity (Bq g\(^{-1}\)) and recovery (%) of the radioisotopes \(^{33}\)P and \(^{32}\)P in the foliar and shoot biomass. Presented are means ± standard errors on the species level. Species abbreviations are listed in Table 4.1.
Figure 3.5: Activity (Bq g\(^{-1}\)) of the radioisotopes \(^{33}\)P and \(^{32}\)P in roots. Given are means ± standard errors on the plot level (n=4). Species abbreviations are listed in Table 4.1.
APPENDIX B
Table 3.3: Primer and probe sequences developed for the species-specific quantification of root DNA by quantitative real-time PCR.

<table>
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<th>Species</th>
<th>Primer 1</th>
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<th>$b$</th>
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<td>T. ros.</td>
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† Species abbreviations are listed in Table 4.1.

§ $a$ and $b$ were derived by a calibration with cloned DNA fragments of the corresponding species in different concentrations.

Table 3.3: Primer and probe sequences developed for the species-specific quantification of root DNA by quantitative real-time PCR.
Table 3.4: Analysis of variance for foliar, wood and aboveground biomass, P concentrations (conc.) and pools.

<table>
<thead>
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<th>Source of variation</th>
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† a refers to residuals at the pot level, b to the species (lowest) level. Species residuals for foliar responses differ from woody and aboveground residuals, since two saplings in mixtures were leafless at the time of harvest.

Significant effects are indicated as followed: · P < 0.1; * P < 0.05; ** * P < 0.001.
Table 3.5: Analysis of variance for biomass and P concentrations (conc.) in roots.

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<td>38.8</td>
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Significant effects are indicated as followed: · $P < 0.1$; * * * $P < 0.001$. 
Table 3.6: Analysis of variance for the recovery and activity of $^{33}$P and $^{32}$P in foliar and aboveground biomass.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f.</th>
<th>Error†</th>
<th>$^{33}$P</th>
<th>$^{32}$P</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>activity (Bq g$^{-1}$)</td>
<td>recovery (%)</td>
<td>recovery (%)</td>
<td>recovery (%)</td>
<td>recovery (%)</td>
<td>recovery (%)</td>
</tr>
<tr>
<td>Block</td>
<td>1</td>
<td>a</td>
<td>0.51</td>
<td>0.45</td>
<td>0.35</td>
<td>0.51</td>
<td>0.71</td>
<td>0.87</td>
</tr>
<tr>
<td>P treatment (P)</td>
<td>2</td>
<td>a</td>
<td>22.0 **</td>
<td>12.7 *</td>
<td>11.2 **</td>
<td>3.17 *</td>
<td>2.45 *</td>
<td>0.96</td>
</tr>
<tr>
<td>Species richness (sr)</td>
<td>1</td>
<td>a</td>
<td>0.29</td>
<td>1.35</td>
<td>0.11</td>
<td>0.17</td>
<td>0.21</td>
<td>0.25</td>
</tr>
<tr>
<td>Monoculture species (mono)</td>
<td>2</td>
<td>a</td>
<td>5.09</td>
<td>5.08</td>
<td>3.84 *</td>
<td>7.13 **</td>
<td>3.28 *</td>
<td>4.11 **</td>
</tr>
<tr>
<td>Mixture species (mix)</td>
<td>2</td>
<td>b</td>
<td>3.09 *</td>
<td>3.11</td>
<td>61.0 ***</td>
<td>66.3 ***</td>
<td>69.2 ***</td>
<td>69.3 ***</td>
</tr>
<tr>
<td>P × sr</td>
<td>2</td>
<td>a</td>
<td>0.77</td>
<td>1.91</td>
<td>0.27</td>
<td>0.30</td>
<td>1.10</td>
<td>1.23</td>
</tr>
<tr>
<td>P × mono</td>
<td>4</td>
<td>a</td>
<td>8.34</td>
<td>6.07</td>
<td>0.79</td>
<td>0.25</td>
<td>0.42</td>
<td>0.55</td>
</tr>
<tr>
<td>P × mix</td>
<td>4</td>
<td>b</td>
<td>4.91</td>
<td>2.92</td>
<td>2.63 *</td>
<td>1.29</td>
<td>0.95</td>
<td>0.86</td>
</tr>
<tr>
<td>Pot residuals (a)</td>
<td>35</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Species residuals (b)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

† Degrees of freedom for species residuals differ between foliar and aboveground (foliar and wood combined) response variables as two plants in mixtures did not have leaves at the time of harvest.

Significant effects are indicated as followed: · $P < 0.1$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. 
Table 3.7: Analysis of variance for the activity of $^{32}$P and $^{33}$P in roots (Bq g$^{-1}$).

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f.</th>
<th>$^{33}$P SS%</th>
<th>$^{32}$P SS%</th>
</tr>
</thead>
<tbody>
<tr>
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<td>0.78</td>
<td>1.49</td>
</tr>
<tr>
<td>P treatment (P)</td>
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<td>12.8*</td>
</tr>
<tr>
<td>Species richness (sr)</td>
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<td>0.54</td>
<td>4.66·</td>
</tr>
<tr>
<td>Monoculture species (mono)</td>
<td>2</td>
<td>3.47</td>
<td>20.8**</td>
</tr>
<tr>
<td>$P \times$ sr</td>
<td>2</td>
<td>1.09</td>
<td>6.82·</td>
</tr>
<tr>
<td>$P \times$ mono</td>
<td>4</td>
<td>14.5</td>
<td>5.48</td>
</tr>
<tr>
<td>Residuals</td>
<td>35</td>
<td>75.2</td>
<td>48.0</td>
</tr>
</tbody>
</table>

Significant effects are indicated as followed: · $P < 0.1$; * $P < 0.05$; ** $P < 0.01$. 
Figure 3.6: Root biomass and P pools for species monocultures (black bars) and three-species mixtures (gray bar). Values are given per kg fresh weight of soil. Presented are means ± standard errors on the pot level. Quantities of root biomass and nutrient pools always refer to the amount of roots found in one kilogram fresh soil. Different letters denote different within-group (monoculture species) means according to a post-hoc Tukey-Kramer multiple comparison test (p<0.05). Species abbreviations are listed in Table 4.1.
Figure 3.7: Calibration of real-time PCR assay for plant DNA with different amount of root biomass. Presented are regression lines and 95% confidence intervals. Species abbreviations are listed in Table 4.1.
Figure 3.8: Copy numbers of LSU genes of *Glomus intraradices*, as a measure of root colonization, detected either as nuclear or as mitochondrial DNA. Means ± standard errors are shown for each species. Species abbreviations are listed in Table 4.1.
Species-specific nitrogen uptake of three tropical tree species under intra- and interspecific competition

Zeugin, F., Jansa J. and Scherer-Lorenzen, M.

Abstract

Resource complementarity is one plausible biological mechanism of biodiversity effects on ecosystem functions and services, with belowground niche partitioning being one import aspect. $^{15}$N labeling experiments represent an alternative to extensive root architecture examinations to get information on soil-depth specific nitrogen (N) uptake of tree species and further allow to study the preferences of species for different chemical N sources. In this study, we conducted two independent soil labelings with the aim to test the influence of conspecific and heterospecific neighbors on the N uptake of three tropical tree species. The first $^{15}$N labeling was conducted in a neotropical tree plantation to study the differences in N uptake from two different soil depths. In a second labeling, we added $^{15}$N in form of nitrate, ammonium or an amino-acid (glycine) to trees growing in a pot experiment to detect potential resource partitioning among the same three species under intra- and interspecific competition. The three native species investigated showed significant difference in N uptake from 10 cm and 40 cm soil depth and in form of different inorganic N sources. While Anacardium excelsum and Luehea seemannii relied to 90% on N from the topsoil, Tabebuia rosea acquired about 55% of the $^{15}$N from the deep soil layer. In addition, Luehea seemannii showed a clear preference for nitrate whereas Anacardium excelsum and Tabebuia rosea took up comparable amounts of nitrate and ammonium. The presence of heterospecific neigh-
bours had no effect on $^{15}$N uptake strategies from different soil depths nor on the uptake from different chemical sources. We suggest that differences in N acquisition strategies might be one mechanism to avoid interspecific competition but does not necessarily lead to positive mixture effects.

4.1 Introduction

The last decades brought a wealth of information on the functional significance of biodiversity, i.e. how changes in the richness, composition and abundance of species and their specific functional traits affect ecological processes such as ecosystem productivity or nutrient cycling. It became clear that interactions between co-occurring species are responsible for such biodiversity effects. Most of these studies were made with fast-growing model system such as microcosms or grasslands (see reviews by Balvanera et al. 2006, Cardinale et al. 2006, Hooper et al. 2005), and knowledge about biodiversity effects in woody ecosystems is still scarce (Scherer-Lorenzen et al. 2005a). However, such insights would allow designing mixed species tree plantations that could capitalize on these biodiversity effects to increase wood production and the ecological sustainability.

To understand biodiversity effects on productivity, nutrient cycling and related ecosystem processes in mixed tree plantations, it is necessary to study the ecological interactions between tree species in detail as well as under relevant conditions and through relevant time scales. Theoretically, there are three main interactions which can occur in mixed stands: competition, facilitation and complementarity (Ewel 1986, Kelty 1992, Vandermeer 1989). The first, interspecific competition, occurs if one species negatively affects another species leading, for example, to suppression in growth or high mortality in the inferior competitor (Pretzsch 2005, Vandermeer 1989). The opposite mechanism, facilitation, occurs when one species has a positive influence on another. This has been demonstrated for example by including N-fixing species into the planting design (DeBell et al. 1997, Forrester et al. 2006). Complementarity takes place if species use different resources, or the same resource at a different time or at different point in space and therefore reduce interspecific competition (Ewel 1986, Harper 1977, Vandermeer 1989). This is based on niche differentiation and can lead to resource partitioning as in stratified mixtures of early and late successional tree species where different species have different demands for light and belowground resources (Haggar and Ewel 1997, Ewel and Mazzarino 2008).

While belowground nutrient partitioning through space, time and/or regarding different chemical forms has mainly been investigated in grassland communities (von Felten et al. 2009, Kahmen et al. 2006, McKane et al. 1990), in arctic tundra (McKane et al. 2002) or agroforestry systems (Cannell et al. 1996, Dinkelmeier et al. 2003, Lehmann et al. 2001), little attention has been paid so far to mixed-species forest plantations (Jose et al. 2006, Rothe and Binkley 2001), especially in the tropics. However, from indirect approaches such as examinations of root systems (Bouillet et al. 2002, Coll et al. 2008) and rooting
depths (Canadell et al. 1996, Jackson et al. 1996), it seems evident that different tree species explore the soil volume differently and that belowground complementarity in mixed stands may result in higher total nutrient uptake and thus lower nutrient losses, higher nutrient retention in the system, and higher productivity, as shown for grassland communities (Scherer-Lorenzen et al. 2003).

Here, we present data on the N uptake of three tropical tree species growing both in a plantation and in a pot experiment in Panama, Central America. The two experimental systems include pure and mixed communities of the three species which allows to investigate species interactions. In the plantation, we measured the $^{15}$N uptake of the species from two different soil depths. Inorganic N, especially the highly mobile nitrate ($\text{NO}_3^{-}$) is one of the main N sources of tropical tree species (Aidar et al. 2003, Schimann et al. 2008) and is prone to leaching in tropical soils (Lehmann 2003, Silver et al. 2005), leading to potential accumulation in subsoils. In the pot experiment, we applied three different chemical N forms to the soil to test for differences in N acquisition under intraspecific and interspecific competition.

In detail, we wanted to answer the following question:

- Do different tree species show belowground resource partitioning for N uptake by accessing N at different soil depths or as different chemical form?
- Does interspecific competition amplify such niche differentiation and hence, lead to belowground complementary N uptake?

### 4.2 Materials and Methods

#### 4.2.1 Study site and experimental design

**The Sardinilla plantation**

The first part of this study was carried out in Sardinilla, (9°19’30”N, 79°38’00”W), Central Panama, in a large-scale experimental biodiversity plantation. The facility was established in 2001 to study the relationship between tree diversity and ecosystem functioning (Scherer-Lorenzen et al. 2005b) and is maintained by the Smithsonian Tropical Research Institute (STRI) in Panama. The site has a slightly undulating terrain with an elevation around 70 m a.s.l. and soils with a high clay content (~ 65%, *Typic Tropudalfs* and *Aquic Tropudalfs*). The topsoil (0-30 cm) is characterized by a pH of 5.85, a mean nitrogen content of 0.34% and a mean carbon content of 3.63% (F. Zeugin, unpublished data). Mean annual rainfall is 2350 mm with a prominent dry season from January to March. Mean daily temperature ranges from 21.7°C to 33.1°C with an annual mean of 25.1°C. Further details on the early land-use in Sardinilla are described in Wilsey et al. (2002).

The plantation includes six tree species native to Panama belonging to three growth categories: *Luehea seemanii* (Tiliaceae) and *Cordia alliodora* (Boraginaceae) as fast-growing
species, *Anacardium excelsum* (Anacardiaceae) and *Hura crepitans* (Euphorbiaceae) as moderately fast growing species, and *Cedrela odorata* (Meliaceae) and *Tabebuia rosea* (Bignoniaceae) as slow growing tree species (Condit et al. 1993). Trees were either planted as monocultures (two replicated plots per species), as three-species mixtures (six replicates with different species composition) or six-species mixture (six replicates), resulting in a total of 24 plots, each with a size of 45 × 45 m and an initial tree density of 1111 trees ha⁻¹. Species richness and species composition were randomly assigned to the plots. In the three-species mixture, species were randomly combined with the constraints that each growth category was represented and each species occurred three times. A scheme of the planting design can be found in Potvin and Dutilleul (2009).

For the ¹⁵N soil labeling in the plantation, we focused on the following tree species: *A. excelsum, L. seemannii* and *T. rosea*. These three species were chosen because their corresponding three-mixture plot was the most productive mixture plot after the first five years of growth (Scherer-Lorenzen et al. 2007). To study the nitrogen partitioning by soil depth among these three species, we followed an individual tree approach. From each species, we selected randomly twelve replicate trees, six individuals in monocultures and six in three-species mixtures, with the constraint that they were surrounded by at least two living neighbor trees. Since all three-species mixtures differed in their species composition, we could only test for the generic effect of species richness on the N uptake of the target trees but not for the effect of particular neighbor species. Further, target trees had to be at least 6 m (two rows) apart from each other to avoid horizontal transfer of the ¹⁵N tracer. In addition, we tried to avoid extreme small or tall trees.

**The pot experiment**

The second part of this study was conducted at the Experimental Field Facility Santa Cruz of the Smithsonian Tropical Research Institute in Gamboa (9°06′N, 79°41′W), Panama. The site is approximately 28 m a.s.l., and has a mean annual temperature of 26 °C, and a mean annual rainfall of 2100 mm (Cernusak et al. 2009) with a pronounced dry season from December to April. The following three tree species were planted in the pot experiment: *A. excelsum, L. seemannii* and *T. rosea*. Seedlings were provided by a nearby nursery (PRORENA - Proyecto de Reforestacion con Especies Nativas). Mean individual height ± standard deviation at planting was 21.4 cm ± 4.1 for *A. excelsum*, 16.8 ± 4.6 for *L. seemannii* and 12.8 ± 2.6 for *T. rosea*.

Tree seedlings were grown in 48 containers, each of 70 cm height and 120 cm diameter (~750 L volume). The pots were filled with soil from the experimental plantation in Sardinilla. We included a layer of gravel (5 cm) at the bottom of the pots to improve drainage. The soil profile in the container was kept as close as possible to the soil profile in the plantation. Seedlings were planted on the 14 August 2006 and harvested after two years of growth on the 15 July 2008. Two seedlings died after planting. To assure survival of the seedlings, all plants were watered twice a week during the dry season. No fertilizer
was applied, and commercial herbicides were only applied once in September 2007 to avoid plant death due to an insect plague.

We planted three seedlings either of the same tree species (i.e. monocultures) or as three-species mixtures in each pot, yielding in 12 replicate pots per treatment. Since the area was surrounded by tall trees in the south-east and north-west direction, pots were arranged in two blocks to account for shading. Each block contained 6 replicates per treatment, which were randomly assigned to the containers. The spacing between the seedlings in the pot was 60 cm with a 30 cm distance towards the pot wall.

### 4.2.2 $^{15}$N soil labeling

The $^{15}$N tracer experiment in the Sardinilla plantation was conducted during June and July 2008 at the early wet season after leaf flush and leaf expansion of the three species. The $^{15}$N labeling technique was adapted from McKane et al. (1990) and further developed for our purposes by Grob (2006) in a pilot-study near the site. The soil around each target tree was labeled with 787 mg $^{15}$N in form of K$^{15}$NO$_3$ (10% enriched). To apply the $^{15}$N label, a ring was laid out around each tree with 1 m radius to the stem, consisting of $\sim$120 holes (see Fig. 4.1b). In order to drill the holes into the soil, it was necessary to remove a band of the herbaceous layer (white area in Fig. 4.1b). Holes of 1 cm diameter were drilled in 5 cm distance in either 10 cm or 40 cm soil depth. In each injection point, we inserted 5 ml tracer solution using a dispenser (Eppendorf Multipette 4780 with Combitips plus 50 ml, Eppendorf, Germany) attached to a tube of corresponding length fitted to a 3 mm thick four-side port needle. Hereby, we avoided the spreading of the $^{15}$N label along the inside of the holes and applied it to the correct soil depth. To prevent dilution of the $^{15}$N label by afternoon rainfall, we manually closed the openings after insertion of the tracer. Based on a dye-tracer injection by Grob (2006), a diffusion of the tracer solution through the soil by c. 2 cm in each direction has to be expected. Nevertheless, we will refer to the soil depths of 10 cm and 40 cm throughout the paper. Each soil depth treatment was randomly assigned to three replicate trees per tree species and species richness level. To determine the natural $^{15}$N background in the foliage, a mixture of sun and shade leaves ($n \approx 10$) from different parts of the canopy was collected with pruning shears attached to a telescope bar.

The $^{15}$N soil labeling in the pot experiment was conducted in June 2008. Each container received 88.5 mg $^{15}$N, either in form of highly enriched (99%) K$^{15}$NO$_3$, $^{15}$NH$_4$Cl or dual-labeled U-$^{13}$C$_2$-$^{15}$N-glycine (99% $^{13}$C, 99% $^{15}$N). The $^{15}$N tracer was injected in 32 points per pot, laid-out as a ring in the inner spacing of the tree saplings (Fig. 4.1a). Distance between the injection points was 5 cm, and the tracer was inserted at 10 cm soil depth. Six days before harvest, 5 ml of the tracer solution were inserted in each point. We added dicystandiamide (DCD, 26 mg per pot) as nitrification inhibitor to the tracer solution to assure that $^{15}$NH$_4^+$ was not oxidized rapidly in the soil. DCD was added to all three tracers due to its function as an additional N source. The number of replicates per species
treatment is summarized in Table 4.1. To determine natural $^{15}$N abundance, we collected leaves of each plant a few hours before adding the tracer.

### 4.2.3 Plant and soil sampling and chemical analysis

Prior to the $^{15}$N tracer application in the plantation, we measured total plant height using a hypsometer (Vertex III, Haglö, Sweden). Stem girth at breast height (1.3 m aboveground) was assessed with a circumference chain. Six days after the $^{15}$N soil labeling, we harvested sun and shade leaves from each target tree to measure the $^{15}$N acquisition from the different soil depths. We chose this time span because the three species showed significant tracer uptake after six days in the study of Grob (2006).

Prior to the harvest of the pot experiment, total plant height and stem diameter at 10 cm above ground were assessed. On 15 July 2008, all plants were cut at the stem base, and total aboveground biomass of each plant was divided into a foliar and wooden sections to measure fresh weight. In addition, we collected three soil samples per container and pooled them to one sample per pot to determine root biomass and $^{15}$N content. Samples were taken in the interspace between the saplings from 0-15 cm soil depth. Roots were washed from the soil under tap water, dried for 48 h at 65 °C, cut into small pieces of 1 cm length and thoroughly mixed before analysis. The presented results always relate to amount of roots found in one kilogram fresh soil.

All plant samples were dried at 65°C for 48h to constant mass and ground in a ball mill to a fine powder. Foliar $\delta^{15}$N and N concentrations of samples were analyzed with an isotope ratio mass spectrometer (Delta plus XP, Finnigan MAT, Germany) connected to an elemental analyzer (Flash EA 1112 NC, CE Instruments, Italy). Plants labeled with dual-labeled U-$^{13}$C$_2$-$^{15}$N-glycine were analyzed for $\delta^{13}$C as well.

As we applied the $^{15}$N tracer in equal quantities into the different soil depths or as different chemical sources, dilution and uptake from different N pool sizes in the soil had to be taken into account. Therefore, we used data on plant available NO$_3^-$ and NH$_4^+$ measured in August 2007 in the Sardinilla plantation (Zeugin et al., Chapter 2). Samples were collected from six locations in each quadrant per plot with the constraint that all species were represented equally near the sampling locations. Two different soil depths were sampled: 5–15 cm and 25–35 cm, shortly referred as “shallow” and “deep” from now on. The six soil samples were pooled per depth and quadrant to give one sample, resulting in 8 samples per plot (4 quadrants × 2 depths).

Plant available NO$_3^-$ and NH$_4^+$ were extracted from field-moist soils according to Faithfull (2002), filtered and kept frozen at -20 °C until analysis. Subsamples were dried at 105 °C for 48 h to determine the water content gravimetrically. Extracts were analyzed photometrically using a Flow Injection Analyzer (San+, SKALAR Analytical B.V., The Netherlands). Concentrations of plant available NO$_3^-$ and NH$_4^+$ are given in Table 4.2. Plant available glycine concentrations could not be measured here.
4.2.4 Calculations and statistical analyses

Total aboveground biomass and foliar biomass of each tree in the plantation were estimated using tree height and girth, respectively, and the species-specific allometric equations and allocation patterns developed by Oelmann et al. (2010). Data of basal diameter needed to assess the aboveground biomass of *A. excelsum* and *T. rosea* were kindly provided C. Potvin (McGill University, Montréal, Canada).

Total aboveground biomass of each sapling in the pot experiment was calculated by summing the dry weight of the foliar and wooden plant material. In terms of fine roots, we calculated dry root biomass per kilogram fresh soil. We decided not to scale up for the total container volume as we did not have an estimate of the coarse or tap roots in the pot and because we expected uneven root distributions at the walls and at the bottom of the pots.

N concentrations in the different tissues (leaves or wood) were multiplied with the corresponding biomass to calculate the N pools in the plants.

Natural $^{15}$N in leaves or wood is expressed in $\delta^{15}$N (‰), calculated as:

$$\delta^{15}\text{N}_{\text{tissue}} = 1000 \times \frac{\text{atom}\%_{\text{natural}} - \text{atom}\%_{\text{reference}}}{\text{atom}\%_{\text{reference}}}$$

(4.1)

where $\text{atom}\%_{\text{natural}}$ is the fraction of $^{15}$N in the total N prior to the $^{15}$N labeling and $\text{atom}\%_{\text{reference}}$ is defined as 0.3662%. The enrichment in $^{15}$N was then calculated as $^{15}$N excess (mg) by following equation:

$$\text{excess}^{15}\text{N}_{\text{tissue}} = \frac{\text{atom}\%_{\text{labeled}} - \text{atom}\%_{\text{natural}}}{100} \times \text{N pool}_{\text{tissue}}$$

(4.2)

where $\text{excess}^{15}\text{N}_{\text{tissue}}$ is the total amount (mg) of $^{15}$N acquired by the plant in a particular treatment and transferred to this tissue after the labeling, $\text{atom}\%_{\text{labeled}}$ is the atom% in the tissue six days after the $^{15}$N soil labeling, $\text{atom}\%_{\text{natural}}$ is the natural $^{15}$N abundance in the tissue and N pool ist the amount of N stored in the tissue. For trees in the plantation, we only calculated the $^{15}$N excess in the foliage. For plants in the pot experiment, we used $\delta^{15}$N of the control plants to calculate the excess in $^{15}$N in wood in addition to the foliar $^{15}$N excess and summed them up to $^{15}$N enrichment in the plant shoot. The recovery of the $^{15}$N tracer was calculated for each tree by dividing the $^{15}$N excess in the foliage or shoot, respectively, by the applied $^{15}$N amount. To account for the dilution of the $^{15}$N tracer in the different soil N pools, we calculated a conservative estimate of $^{15}$N uptake by the plant by multiplying the $^{15}$N excess retained after each treatment with the corresponding dilution factors (see Table 4.2). Since measurements on plant available glycine concentrations were not available, these calculation were only done for $\text{NH}_4^+$ and $\text{NO}_3^-$. To compare the estimated $^{15}$N uptake between the different treatments, we defined the sum of estimated $^{15}$N taken up from different depths or from different sources, respectively, as 1 and calculated the fraction for each source, for each species and
each diversity level separately. Note that glycin was not applied to the mixture pots, so that these fractions can only be calculated for nitrate and ammonium.

Because the experimental setup differed between the Sardinilla plantation and the pot experiment, different statistical analyses were used. For response variables retained from the $^{15}$N labeling in the Sardinilla plantation, the statistical analyses included analyses of variance (ANOVA) with treatment, species richness and species as the main factors and all possible interactions. Differences among means of significant terms and interactions were tested using Tukey’s HSD test for multiple comparisons ($P < 0.05$). Estimated $^{15}$N excess data were log transformed and fraction values were square root transformed to improve normality of error distribution.

For data obtained from the pot experiment, the response variables were analyzed on the species level (n=72), using ANOVA. For monocultures, we averaged the three plant values per pot and compared the means (n=36) with the single plant measurements in the mixtures (n=36), as proposed by von Felten et al. (2009). Results are presented according to Schmid et al. (2002). To analyse plant growth, N concentrations and N pools, terms were entered in the order: (1) block, (2) treatment, (3) species richness, (4) species in monocultures and (5) species in mixtures and (6) interactions. Terms 1–4 and possible interactions were tested against the pot residuals, whereas species in mixtures and possible interactions were tested against species residuals. Analyses of belowground variables were performed on the pot level (n=48) without testing for species differences in the mixtures. Data were either log or square-root transformed, if necessary, to meet the assumptions of ANOVA. The differences in means of significant terms were tested using the Tukey-Kramer method for multiple comparison test ($P < 0.05$).

To test whether the dual-labeled glycine (U-$^{13}$C$_2-^{15}$N-glycine) was taken up as intact amino-acid, we used linear regression as proposed by Naesholm et al. (1998). A regression slope of 2 would support the hypothesis that $^{13}$C and $^{15}$N were taken as up as one molecule. All calculations and statistical analysis were done with the statistical software R (version 2.8, R Development Core Team 2007).

### 4.3 Results

#### 4.3.1 Tree growth

Tree growth in the plantation was significantly affected by the different species as shown in Table 4.3. Individuals of *L. seemannii* were significantly taller than trees of *T. rosea*, and girth at breast height decreased in the order *A. excelsum*, *L. seemannii* and *T. rosea*. The model explained 42% and 39% of the total variance (adjusted $R^2$) for tree height and girth, respectively. In contrast to these two growth characteristics, aboveground biomass of the seven years old target trees did not differ among the three species. Standing biomass varied from 5.03 kg to 134 kg per tree and had a mean and a standard error of 54.6 ± 5.67 kg. *T. rosea* tended to have higher tree biomass compared to *L. seemannii*, however,
the difference was not significant (Table 4.8 in the Appendix). Likewise, the biomass of the foliage varied strongly among the selected trees ranging from 0.35 to 20 kg per tree with no significant difference among the species. Species richness had a marginal positive influence on tree height ($P = 0.068$) but no significant effect on the other growth parameters (Table 4.8 in the Appendix).

Tree biomass of two years old tree saplings grown in the pot experiment was significantly affected by species but not by species richness (Table 4.4 and Table 4.7). *T. rosea* showed significantly lower shoot biomass than *A. excelsum* and *L. seemannii* in both monoculture and mixture pots though differences were more pronounced in the mixtures. *A. excelsum* allocated on average 22% of aboveground biomass into the leaves when planted with conspecific neighbours, followed by *T. rosea* with 16% and *L. seemannii* with 11%. In mixtures, allocation of biomass into the foliar tissue was similar for *A. excelsum* and *T. rosea* (25% and 17%, respectively) compared to the monoculture plants (see Table 4.4). Biomass allocation to leaves of *L. seemannii* decreased to 6% in mixtures pots but allocation to woody tissue increased (88% in monoculture, 93% in mixture). The highest root biomass was found in containers of *A. excelsum* with three-times more root biomass compared to *L. seemannii* and *T. rosea* (Table 4.4 and Table 4.7 in the Appendix). This, however, only relates to the roots found in 1 kilogram fresh soil at the top 15 cm of the pots.

### 4.3.2 Foliar N parameters

In the tree plantation, N concentrations in leaves of seven year old trees significantly differed among the species with *T. rosea* having a foliar N content almost twice as high as *A. excelsum* (see Table 4.3 and Table 4.8 in the Appendix). Similarly, the size of the N pool in the foliage of *T. rosea* was the twofold of the N pools in leaves of *A. excelsum* and *L. seemannii*. While the model explained a large percentage of the variance in foliar N concentrations (71%, adjusted $R^2$), it only explained 13% of the variance for the foliar N pools. $\delta^{15}$N values measured in leaves prior to the labeling varied between 1.50‰ and 6.68‰ with a mean and standard error of 3.33 ($\pm$ 0.19‰). Natural $\delta^{15}$N in leaves was not affected by species ($F_{2,30} = 2.04, P = 0.147$), however, species richness had a marginal effect on foliar natural $\delta^{15}$N ($F_{1,30} = 2.89, P = 0.099$), indicating a trend towards higher foliar $\delta^{15}$N values in monocultures relative to mixture trees ($3.63\%$ vs $3.03\%$).

In the pot experiment, N concentrations were generally higher in monocultures than in mixtures (effect of species richness on foliar N: 14.3% SS (sum of squares), $P < 0.001$; effect of species richness on wood N: 8.51% SS, $P < 0.001$). Similar to the plants grown in the plantation, *T. rosea* in the pots showed the highest N concentrations in all three tissues when planted as monoculture (Table 4.4). In the three-species mixtures, *L. seemannii* and *T. rosea* showed similar N concentrations. Species richness had no significant effect on the N pools in the shoots or roots of plants grown in the containers (Table 4.7 in the Appendix). No differences across the species were detected in N pools of monoculture plants except in roots where *A. excelsum* held significantly higher N pools compared to *L. seemannii*.
(see Table 4.4). However, species in mixtures differed significantly. Similar to the shoot biomass, *A. excelsum* had the largest shoot N pools, followed by *L. seemannii* and *T. rosea* (Fig. 4.4). On average, plants stored ~55% of aboveground N in the leaf compartment.

Natural $\delta^{15}N$ values in leaves differed significantly between the three species grown in pots (ANOVA, $F = 10.2, P<0.001$), with values of $1.21 \pm 0.23^\circ\mathrm{C}$ (mean $\pm$ standard error) for *T. rosea*, $1.07 \pm 0.19^\circ\mathrm{C}$ for *L. seemannii*, and $0.06 \pm 0.17^\circ\mathrm{C}$ for *A. excelsum*.

### 4.3.3 Species-specific $^{15}N$ uptake from different soil depths

Foliar $^{15}N$ enrichment, estimated as the amount of $^{15}N$ taken from the soil after dilution and delivered to the leaves, was significantly affected by tree species and labeled soil depths in the Sardinilla plantation (Table 4.5). However, whether target trees were surrounded by conspecific or heterospecific neighbors had no influence on the estimated $^{15}N$ acquisition as indicated in Fig. 4.2a and b. *L. seemannii* acquired significantly more $^{15}N$ compared to the other two species, which were not statistically different from each other. In total, $^{15}N$ uptake corrected for depth-specific dilution in the soil N pools was significantly higher from the topsoil as compared to the uptake from the deep soil. The species $\times$ depth interaction was found to be marginally significant (see Table 4.5). *T. rosea* tended to take up similar amounts of $^{15}N$ from 10 cm and 40 cm soil depth while *A. excelsum* and *L. seemannii* rather showed higher $^{15}N$ acquisition from the topsoil (Fig. 4.2a and b). Fifty-nine percent of the variance in the estimated foliar $^{15}N$ enrichment were explained by the three-factorial model. Comparing the fractions of total $^{15}N$ uptake across the two soil depths, significant differences were found (Table 4.5 and Fig. 4.2c and d). Plant took up 79% of $^{15}N$ from the topsoil and 21% from the deep soil layer. The species $\times$ depth interaction was found to be marginally significant showing again that *T. rosea* tended to absorb similar amount of $^{15}N$ from the two labeled soil depths. The mean tracer recovery after six days was low in the Sardinilla plantation with 0.33% for *A. excelsum*, 0.45% for *T. rosea* and 4.30% for *L. seemannii*. However, $^{15}N$ recovery rates were significantly different from zero for all three species (analyses not shown).

### 4.3.4 Species-specific $^{15}N$ uptake from different chemical N sources

To test whether glycine was absorbed as intact molecule by the plants grown in the pot experiment, we compared the $^{13}C$ and $^{15}N$ excess (atom%) for each species in monoculture separately. Natural background $\delta^{13}C$ values in leaves did not differ between the three species (analysis not shown) and showed a mean and standard error of $-30.38 \pm 0.42^\circ\mathrm{C}$. Six days after glycine had been applied, mean $\delta^{13}C$ values were not significantly different from the background values ($-30.25 \pm 0.24^\circ\mathrm{C}, F = 2.68, P = 0.101$). None of the three species had a $^{13}C:\!^{15}N$ ratio close to 2:1 (*A. excelsum* = -0.03, *L. seemannii* = 0.01, *T. rosea* = 0.03). No trends were observed for $^{13}C$ and $^{15}N$ in root samples either (analysis not shown). Consequently, the glycine treatment was omitted from further analyses.
The estimated $^{15}$N uptake into shoots differed significantly between the three species, the species richness levels and the treatment with different chemical N sources (Table 4.7). In general, plants grown in monocultures took up more $^{15}$N compared to plants in the mixture communities (4.33 mg vs 2.05 mg $^{15}$N after correcting for dilution in the different soil pools). Within monoculture and mixture communities, *L. seemannii* showed a significantly higher $^{15}$N enrichment in the shoots compared to the other two species after nitrogen was offered in form of nitrate (Fig. 4.3a and b) whereas *A. excelsum* and *T. rosea* showed similar $^{15}$N enrichments in both treatment levels. The fraction of $^{15}$N taken up from the two mineral N sources differed significantly between the species in monocultures and marginally in the species in mixtures (Table 4.7 and Fig. 4.3c and d). Across the two species richness levels, *L. seemannii* took up about 77% of $^{15}$N in form of $^{15}$NO$_3^-$ and only 23% in form of $^{15}$NH$_4^+$. No differences in $^{15}$N uptake from NO$_3^-$ and NH$_4^+$ were found in *A. excelsum* (45% vs 55%) or *T. rosea* (59% vs 41%). The average recovery of the $^{15}$N tracer in the pot experiment was 3.61% of total applied $^{15}$N with the highest value found in monoculture plants of *L. seemannii* (9.38%) and lowest recovery detected in *T. rosea* grown in mixture communities (0.56%).

4.4 Discussion

4.4.1 Tree species effect on N uptake from different soil depths

The three species significantly differed in their N acquisition from two different soil depths. *L. seemannii* showed a higher $^{15}$N uptake capacity from both soil depths relative to the other two species. However, if we compare the fraction of $^{15}$N taken up from a particular depth among the species, the pioneer *L. seemannii* and the intermediately growing species *A. excelsum* obtained their N primarily from the topsoil whereas the late-successional *T. rosea* had relatively similar root activities in the top and deep soil layers. These findings fit well to those of Coll et al. (2008) who measured root architecture and allocation patterns of three year old saplings, including the species *L. seemannii* and *T. rosea*, at a nearby site. Despite the lack of a clear trend in belowground biomass production between pioneer and non-pioneer tree species, Coll et al. (2008) found significant morphological differences in roots among these functional groups. While *T. rosea* allocated three times more biomass into the taproot and therefore invested more into the storage than *L. seemannii*, the latter showed a thinner and more branched root system with a higher number of root apices indicating a higher investment in soil exploration. The authors related these differences in belowground strategies to the aboveground architectural traits and differences in light demand. As known from many studies (e.g. Poorter et al. 2005, Menalled and Kelty 2001, King et al. 1997), pioneer species tend to allocate more biomass into height growth to avoid shading while non-pioneers in shaded environments invest less biomass in primary growth but more into foliage for efficient light interception. In the first part of our study, the selected trees showed clear species-specific differences in height and
girth growth according to their categorization in successional groups. Although we did not detect a significant difference in aboveground or foliar biomass, individuals of *T. rosea* tended to have higher biomass allocation to leaves compared to the fast growing *L. seemannii*. This suggests that the pioneer *L. seemannii* has to sustain its primary growth by taking up large amounts of nutrients, as shown by the estimated $^{15}$N uptake. We expect that this is achieved by an extended root system in the soil surface (Coll et al. 2008), where for example total soil N content is found to be higher relative to deep soil layers (0.55 % versus 0.15%, F. Zeugin, unpublished data). Dinkelmeyer et al. (2003) carried out a $^{15}$N fertilizer study in a mixed tree crop system in Brazil and found that Brazil nut (*Bertholletia excelsa*), the tallest species with the highest aboveground biomass and N pools in that experiment, primarily did not take up the $^{15}$N which was applied directly around its stem but the N fertilizer which was applied to heterospecific neighbor trees. This shows further that species with a high N demand actively explore the soil through an extensive root system and compete with other tree species.

In contrast to the pioneer species, *T. rosea* as a late successional species showed a relatively small $^{15}$N uptake from both of the soil depths but high foliar N concentrations and N pools (Table 4.3). As this deciduous species only started to flush and produce new leaves about two weeks prior to the $^{15}$N labeling (Kunert et al. 2010), we suppose that *T. rosea* still relied on the N stored in other organs (e.g. taproot) for internal N recycling and therefore showed an exiguous N uptake activity (Lal et al. 2001, Millard 1996). In the study of Oelmann et al. (2010) using the same tree species at Sardinilla in 2007, foliar N concentrations of *T. rosea* were not significantly different from those of *A. excelsum* and *L. seemannii*. However, as they collected the leaves for assessment of nutrient concentrations at the end of the dry season, substantial amounts of N might already have been remobilized from the leaves of *T. rosea* and relocated to other storage organs, supporting our hypothesis of internal N recycling. Since we have no information on the root architecture of *A. excelsum*, we are not able to explain the small amount of $^{15}$N taken up by this semi-deciduous species. The species is known for its high nutrient use efficiency (Oelmann et al. 2010) and recalcitrant litter (Scherer-Lorenzen et al. 2007) and probably has a lower N requirement relative to *L. seemannii*. However, the generally small tracer recovery might as well be related to some caveats in our experimental design. We cannot completely exclude the possibility that the chosen soil depths, the time span and/or the distance to the stem at which the tracer was injected were of limited relevance for some of the investigated species though the field study of Grob (2006) do not suggest that. Our trees were on average 4.5 m taller than the trees in the study of Grob (2006) and might have needed more time to take up the $^{15}$N by the roots and transport it to the leaves. However, a later sampling of some of our target trees in September 2008, i.e. two and a half months after the labeling (M. Mohns, personal communication), showed a further increase in the foliar $\delta^{15}$N values for *L. seemannii* (+204‰) compared to a relatively small increase in *A. excelsum* and *T. rosea*, respectively (+12% and +24‰). Altogether *L.
seemannii seems thus to be more active in foraging for N compared to the other two species.

In the plantation system, we did not find a change in growth, aboveground or foliar biomass nor in the depth-specific $^{15}$N uptake of the selected trees along the increasing species richness. Since our target trees were located in mixture plots with unique species combinations, the effect of neighbor identity could not be tested here, which might be as important as the generic effect of species richness (Potvin and Dutilleul 2009). Here, it seems that increasing species richness per se had no effect on the depth-specific N uptake of the three species.

4.4.2 Tree species effect on N uptake from different chemical N sources

The three tree species revealed significant differences in their ability to take up nitrogen in form of NO$_3^-$, NH$_4^+$ or glycine. Our results showed that the species relied more on mineral N than on organic N. Moreover, we found no evidence that glycine was acquired as intact molecule by the plants and/or their associated mycorrhizas. The lack of an increase in plant $^{13}$C and the missing significant relationship between $^{13}$C and $^{15}$N indicates that glycine was probably immobilized in the soil and mineralized prior to the uptake by the plant (Jones et al. 2005, Naesholm and Persson 2001). There is some uncertainty to it as we have no information about the isotopic dilution of the tracers in the ambient soil organic N pool nor did we measure the $^{13}$C:$^{15}$N ratio in the soluble fraction of the roots (Naesholm et al. 2009, Naesholm and Persson 2001, von Felten et al. 2008). We thus cannot completely exclude the possibility that glycine had been acquired by the plant and/or their mycorrhizas but in amounts below the detection limit or that the molar $^{13}$C:$^{15}$N ratio has largely changed during the six days due to rapid metabolic conversions (Naesholm et al. 2009). According to the concept of Schimel and Bennett (2004), the relevance of organic N as a source for plants is considered to be low in ecosystems with intermediate or high soil N availability (e.g. many tropical forests, Vitousek and Sanford 1986, Vitousek 1984). It is because competition between microbes and plants for N-containing monomers would decrease under such conditions and substrate availability for mineralization and nitrification should increase (Hall and Matson 2003, 1999). This in return would lead to higher soil NH$_4^+$ and NO$_3^-$ concentrations accessible to the plants and/or their mycorrhizas. For example, Kahmen et al. (2009) and Pfautsch et al. (2009), both studying the uptake of organic N by woody plants in N-intermediate ecosystems in Australia, found that organic N played a minor role in the overall N acquisition and that NH$_4^+$ was the preferred N source of the different tree species, thus supporting the concept of Schimel and Bennett (2004). Although the interpretation of the glycine uptake in our study is biased by methodological constraints, we suggest that the preferences of our tree species also are towards mineral N sources.

When comparing the two mineral N forms, NO$_3^-$ was clearly preferred over NH$_4^+$ as N source by the pioneer species L. seemannii whereas the intermediate- and late-successional
A. excelsum and T. rosea showed no preferences for one of the two sources (Fig. 4.3). This was unexpected as we assumed some ammonium fixation due to the high clay content of our soil (~ 65%) (Kowalenko and Ross 1980) and thus higher $^{15}$N enrichment in pots labeled with $^{15}$NO$_3^-$−. However, our results are in good agreement with other studies from natural rainforests (Bazzaz 1984, Freeden et al. 1991, Stewart and Hegarty 1988, Aidar et al. 2003). They provided evidence that tropical tree species from different successional guilds acquired different chemical forms of nitrogen. Pioneer species generally showed a preference for nitrate whereas late-successional species tended to take up more ammonium.

Interestingly, we found that the $^{15}$N uptake of plants grown with heterospecific neighbors was significantly reduced compared to plants grown with conspecific neighbors. In an alpine N-limited meadow, Miller et al. (2007) conducted a neighbor removal experiment combined with a $^{15}$N-labeling using $^{15}$NO$_3^-$−, $^{15}$NH$_4^+$ and glycine as N sources. They found that the presence of a heterospecific neighbor reduced the uptake of a particular N form in the target species by up to 50%. The absolute $^{15}$NO$_3^-$− and $^{15}$NH$_4^+$ uptake by L. seemannii and T. rosea was also largely reduced in the mixtures of our study. Since we applied the same amount of $^{15}$N to each container and corrected for the dilution of the $^{15}$N tracer according to species and inorganic soil pool, we speculate that this decrease in N uptake is a result of species interactions and not primarily a result of N availability (Nordin et al. 2004). The root data from the mixtures showed that L. seemannii allocated more to the belowground biomass in mixtures, at least in the soil surface layer, than the other two species, probably as response to the interspecific competition. We thus suggest that a relevant proportion of the adsorbed $^{15}$N was not transported to the shoots of L. seemannii but was directly allocated belowground due to a high fine root turnover (Nadelhoffer et al. 1985). This would explain the decrease in $^{15}$NO$_3^-$− uptake observed in the shoots of this species. We found some support for this notion in the high δ $^{15}$N values of roots samples from the mixtures (data not shown): roots collected in pots which were labeled with $^{15}$NO$_3^-$− had mean δ $^{15}$N and standard error of 2426 ± 232‰ compared to roots from $^{15}$NH$_4^+$ labeled pots (972 ± 325‰). Even with the correction for the different dilutions in the ambient N soil pools, the δ $^{15}$N values in roots of L. seemannii in $^{15}$NO$_3^-$− labeled pots would be higher than those of $^{15}$NH$_4^+$ labeled pots.

4.4.3 Conclusions

We conclude that tropical tree species avoid interspecific competition for N to some extent by exploring different soil depths for N acquisition or different chemical sources of N. However, this complementary resource use does not necessarily lead to higher aboveground biomass production and N uptake in mixture plants as it has been the case in several grassland studies (e.g. Hector et al. 1999, Oelmann et al. 2007, Tilman et al. 2001) but probably facilitates coexistence of those species. In addition, competitive relationships among the long-lived plant species are expected to shift with time as shown by Ewel and
Mazzarino (2008) in another experimental plantation in Costa Rica. Therefore, a further
$^{15}$N soil labeling including additional soil depths, a sampling regime over several time
intervals and the N uptake by neighbor trees would provide more information on the
dynamics of tree species interactions for nutrient acquisition. Together with information
on N turnover in indigenous microbial communities, such additional knowledge would
then also help to combine species with different N uptake strategies in mixed plantations
to enhance total N use.

Acknowledgements

Authors are indebted to Iliana Cabrera Phillips, Jose Monteza, Jose Alberto Maynard, David Sarco,
Marc Schneebeli, Verena Hammes, Felipe Rodriguez and the field workers in Sardinilla for their
assistance during field work. We are grateful to Nina Buchmann for useful discussions on our
results and her advice on the isotope dilution calculations. We would like to thank Annika Lenz
(ETH Zurich, Switzerland) for isotope analyses. We are indebted to Catherine Potvin (McGill
University, Canada) who accepted to share data on tree basal diameter with us and Melanie
Mohn (Johannes Gutenberg University Mainz, Germany) for information on the $\delta^{15}$N values in
September 2008. We are grateful to the Smithsonian Tropical Research Institute in Panama and
especially to Raineldo Urriola for help with logistics. We thank Stefanie von Felten and Hans-
Rudolf Roth for advice on the statistical analyses and Marlen Gubsch for comments on a previous
version of the manuscript. This research was made possible by a grant of the Swiss National
Science Foundation (3100A0-110031/1) to Michael Scherer-Lorenzen and Jan Jansa.


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Tables and Figures
Table 4.1: Number of replicates (trees or pots) per treatment for the two $^{15}$N labelings.

<table>
<thead>
<tr>
<th>Species $^a$</th>
<th>Plantation</th>
<th>Pot experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10 cm depth</td>
<td>40 cm depth</td>
</tr>
<tr>
<td>A. exc.</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>L. see.</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>T. ros.</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>3 spec.</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

$^a$ Species abbreviations: A. exc. Anacardium excelsum; L. see. Luehea seemannii; T. ros. Tabebuia rosea; 3 spec. 3-species mixture.
Table 4.2: Ratio of plant available NH$_4^+$ and NO$_3^-$ concentrations in the shallow soil and ratio of NO$_3^-$ concentrations in shallow and deep soils in the Sardinilla plantation.

<table>
<thead>
<tr>
<th>Species</th>
<th>NO$_3^-$ (mg N kg$^{-1}$soil)</th>
<th>shallow soil layer (mg N kg$^{-1}$soil)</th>
<th>dilution factor</th>
<th>NH$_4^+$</th>
<th>NO$_3^-$</th>
<th>dilution factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. exc.</td>
<td>3.27 ± 0.67</td>
<td>5.49 ± 1.76</td>
<td>0.59 : 1</td>
<td>7.46 ± 1.52</td>
<td>3.27 ± 0.67</td>
<td>2.28 : 1</td>
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<tr>
<td>L. see.</td>
<td>7.21 ± 1.74</td>
<td>6.98 ± 2.18</td>
<td>1.03 : 1</td>
<td>6.70 ± 1.57</td>
<td>7.21 ± 1.74</td>
<td>0.93 : 1</td>
</tr>
<tr>
<td>T. ros.</td>
<td>4.80 ± 0.73</td>
<td>1.80 ± 0.48</td>
<td>2.66 : 1</td>
<td>17.5 ± 2.70</td>
<td>4.80 ± 0.73</td>
<td>3.65 : 1</td>
</tr>
<tr>
<td>3 spec.</td>
<td>7.42 ± 1.12</td>
<td>4.09 ± 0.30</td>
<td>1.81 : 1</td>
<td>11.7 ± 3.55</td>
<td>7.42 ± 1.12</td>
<td>1.58 : 1</td>
</tr>
</tbody>
</table>

Soil samples were collected in August 2007 in the Sardinilla plantation. Means and standard errors ($n = 4$) are presented. Species abbreviations: A. exc. *Anacardium excelsum*; L. see. *Luehea seemannii*; T. ros. *Tabebuia rosea*; 3 spec. 3-species mixture.
Table 4.3: Tree growth, N concentrations and N pools of different tree species grown in a tropical tree plantation in Panama.

<table>
<thead>
<tr>
<th>Species</th>
<th>Height (m)</th>
<th>Girth (cm)</th>
<th>Biomass (kg)</th>
<th>Tree</th>
<th></th>
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<th></th>
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<tbody>
<tr>
<td>Monoculture</td>
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<td></td>
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</tr>
<tr>
<td>A. exc.</td>
<td>7.13 b</td>
<td>46.9 ab</td>
<td>36.5 a</td>
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<tr>
<td>L. see.</td>
<td>9.47 a</td>
<td>67.2 a</td>
<td>61.0 a</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>T. ros.</td>
<td>7.16 b</td>
<td>36.0 b</td>
<td>47.8 a</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 species mixture</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>A. exc.</td>
<td>9.45 ab</td>
<td>63.8 a</td>
<td>48.9 a</td>
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<td>L. see.</td>
<td>10.4 a</td>
<td>69.6 a</td>
<td>74.7 a</td>
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<tr>
<td>T. ros.</td>
<td>6.79 b</td>
<td>35.1 b</td>
<td>58.5 a</td>
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<table>
<thead>
<tr>
<th>Species</th>
<th>Biomass (kg tree⁻¹)</th>
<th>N concentration (mg N kg⁻¹)</th>
<th>N pool (g tree⁻¹)</th>
<th>Foliar</th>
<th></th>
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</thead>
<tbody>
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<td>Monoculture</td>
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<td>A. exc.</td>
<td>5.84 a</td>
<td>12.1 b</td>
<td>70.4 b</td>
<td></td>
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<tr>
<td>L. see.</td>
<td>4.27 a</td>
<td>21.1 a</td>
<td>89.4 b</td>
<td></td>
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<tr>
<td>T. ros.</td>
<td>7.17 a</td>
<td>23.1 a</td>
<td>154 a</td>
<td></td>
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<td>3 species mixture</td>
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<tr>
<td>A. exc.</td>
<td>7.82 a</td>
<td>13.8 c</td>
<td>106 b</td>
<td></td>
<td></td>
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<tr>
<td>L. see.</td>
<td>5.23 a</td>
<td>20.6 b</td>
<td>105 b</td>
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<td>T. ros.</td>
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<td>27.0 a</td>
<td>253 a</td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

Species abbreviations: A. exc. *Anacardium excelsum*; L. see. *Luehea seemannii*; T. ros. *Tabebuia rosea*. Different letters denote significantly different within-group means to a post-hoc Tukey-Kramer multiple comparison test ($P < 0.05, n = 3$).
Table 4.4: Biomass, N concentrations and N pools in different plant compartments of three trees species grown in a pot experiment in Panama.

<table>
<thead>
<tr>
<th>Species</th>
<th>Biomass (g tree$^{-1}$)</th>
<th>N concentration (mg N kg$^{-1}$)</th>
<th>N pool (g tree$^{-1}$)</th>
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<tr>
<td></td>
<td>Foliar</td>
<td>Wood</td>
<td>Shoot</td>
</tr>
<tr>
<td>Monoculture</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. exc.</td>
<td>281$^{a}$</td>
<td>936$^{a}$</td>
<td>1217$^{a}$</td>
</tr>
<tr>
<td>L. see.</td>
<td>122$^{b}$</td>
<td>886$^{a}$</td>
<td>1008$^{b}$</td>
</tr>
<tr>
<td>T. ros.</td>
<td>113$^{b}$</td>
<td>578$^{b}$</td>
<td>676$^{c}$</td>
</tr>
<tr>
<td>3 species mixture</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. exc.</td>
<td>547$^{a}$</td>
<td>1776$^{a}$</td>
<td>2323$^{a}$</td>
</tr>
<tr>
<td>L. see.</td>
<td>68.9$^{b}$</td>
<td>893$^{b}$</td>
<td>962$^{b}$</td>
</tr>
<tr>
<td>T. ros.</td>
<td>26.0$^{b}$</td>
<td>113$^{c}$</td>
<td>139$^{c}$</td>
</tr>
</tbody>
</table>

† Species abbreviations: A. exc. Anacardium excelsum; L. see. Luehea seemannii; T. ros. Tabebuia rosea. Means and standard errors ($n = 12$) are presented. Different letters denote significantly different within-group means to a post-hoc Tukey-Kramer multiple comparison test ($P < 0.05$).

‡ Root biomass, N concentration and N pools refer to the amount of roots found in 1 kilogram fresh soil.
Table 4.5: Analysis of variance for effects of soil depth, species richness and tree species on the estimated $^{15}$N uptake from soil N pool into the leaves (excess in mg per tree) and on the estimated fraction of total $^{15}$N tracer taken up from 10 and 40 cm soil depth.

<table>
<thead>
<tr>
<th>Source</th>
<th>d.f.</th>
<th>$^{15}$N excess $^a$</th>
<th>Fraction $^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MS</td>
<td>F</td>
<td>$P$</td>
</tr>
<tr>
<td>Depth (D)</td>
<td>1</td>
<td>25.5</td>
<td>16.8</td>
</tr>
<tr>
<td>Species richness (SR)</td>
<td>1</td>
<td>0.52</td>
<td>0.34</td>
</tr>
<tr>
<td>Species (S)</td>
<td>2</td>
<td>25.8</td>
<td>17.1</td>
</tr>
<tr>
<td>D $\times$ SR</td>
<td>1</td>
<td>0.60</td>
<td>0.39</td>
</tr>
<tr>
<td>D $\times$ S</td>
<td>2</td>
<td>4.18</td>
<td>2.76</td>
</tr>
<tr>
<td>SR $\times$ S</td>
<td>2</td>
<td>2.66</td>
<td>1.76</td>
</tr>
<tr>
<td>D $\times$ SR $\times$ S</td>
<td>2</td>
<td>1.10</td>
<td>0.73</td>
</tr>
<tr>
<td>Residual</td>
<td>24</td>
<td>1.15</td>
<td></td>
</tr>
</tbody>
</table>

$^a$ Foliar $^{15}$N excess data have been log transformed and fraction data have been square root transformed to meet assumptions of normal errors. Significant effects at $P<0.05$ are in bold, marginally significant effects at $0.1>P<0.05$ are in italics.
Table 4.6: Analysis of variance for effects of different chemical N sources, species richness and tree species on the estimated $^{15}$N uptake from soil N pools into the shoot (excess in mg per tree) and on the estimated fraction of total $^{15}$N tracer taken up from the two mineral N sources.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Error</th>
<th>d.f</th>
<th>SS%</th>
<th>d.f</th>
<th>SS%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Block</td>
<td></td>
<td></td>
<td></td>
<td>0.17</td>
<td></td>
</tr>
<tr>
<td>N treatment (N)</td>
<td>a</td>
<td>2</td>
<td>5.24</td>
<td>1</td>
<td>14.7</td>
</tr>
<tr>
<td>Species richness (sr)</td>
<td>a</td>
<td>1</td>
<td>25.1</td>
<td>1</td>
<td>0.00</td>
</tr>
<tr>
<td>Monoculture species (mono)</td>
<td>a</td>
<td>2</td>
<td>8.05</td>
<td>2</td>
<td>0.00</td>
</tr>
<tr>
<td>Mixture species (mix)</td>
<td>b</td>
<td>2</td>
<td>27.0</td>
<td>2</td>
<td>0.35</td>
</tr>
<tr>
<td>N $\times$ sr</td>
<td></td>
<td>2</td>
<td>0.86</td>
<td></td>
<td>0.11</td>
</tr>
<tr>
<td>N $\times$ mono</td>
<td>a</td>
<td>4</td>
<td>10.5</td>
<td>2</td>
<td>14.8</td>
</tr>
<tr>
<td>N $\times$ mix</td>
<td>b</td>
<td>4</td>
<td>4.02</td>
<td>2</td>
<td>15.2</td>
</tr>
<tr>
<td>Pot residuals (a)</td>
<td></td>
<td>29</td>
<td>11.9</td>
<td>23</td>
<td>32.3</td>
</tr>
<tr>
<td>Species residuals (b)</td>
<td></td>
<td>15</td>
<td>7.14</td>
<td>11</td>
<td>22.5</td>
</tr>
</tbody>
</table>

$^a$ Data from the glycine treatment were excluded from the analysis because glycine was not applied to the mixture pots.

$^b$ Degrees of freedoms differ from $^{15}$N excess because “control” plants were excluded from the analysis of the fractions.

$^{a,b}$ Data have been square-root transformed to meet assumptions of normal errors. Significant effects are indicated as followed: · $P < 0.1$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. 
Figure 4.1: Scheme of the two $^{15}$N soil labelings. a) Position of the three saplings in the pot and layout of the $^{15}$N injection ring. Per pot, 88.5 mg $^{15}$N were inserted into 32 holes. b) Layout of a $^{15}$N-injection ring around a tree in the plantation. The white band along the injection holes indicates where the herbaceous layer was removed to apply the tracer. 787 mg $^{15}$N were injected per tree in a total of 120 holes.
Figure 4.2: Estimated foliar $^{15}\text{N}$ excess in mg (a and b) and fraction (c and d) of $^{15}\text{N}$ taken up from 10 cm and 40 cm soil depth in the plantation during one week. Values are means ± standard errors for each species in monoculture and mixture ($n = 3$). Estimated $^{15}\text{N}$ uptake from soil $\text{NO}_3^-$ pools into the tree leaves accounts for dilution in the soil solution (see Table 4.2). Species abbreviation are listed in Table 4.1.
Figure 4.3: Estimated $^{15}$N excess in shoots (a and b) and fraction of total $^{15}$N uptake from the two mineral N sources $^{15}$NO$_3^-$ and $^{15}$NH$_4^+$ (c and d) for the three species in monoculture and mixture pots. Estimated $^{15}$N uptake from soil NO$_3^-$ pools into the tree shoots accounts for dilution in the soil solution (see Table 4.2). Shown are means ± standard errors on the species level. Species abbreviations are listed in Table 4.1.
Table 4.7: Analysis of variance for foliar, wood, root and shoot biomass, N concentrations and N pools of three different tropical trees species grown in a pot experiment in Panama.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f</th>
<th>Error †</th>
<th>Foliar SS%</th>
<th>Wood SS%</th>
<th>Shoot SS%</th>
<th>Root SS%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Block</td>
<td>1</td>
<td>a</td>
<td>0.33</td>
<td>2.52</td>
<td>1.78</td>
<td>0.33</td>
</tr>
<tr>
<td>Species richness</td>
<td>1</td>
<td>a</td>
<td>1.63</td>
<td>0.91</td>
<td>1.10</td>
<td>2.90</td>
</tr>
<tr>
<td>Monoculture species</td>
<td>2</td>
<td>a</td>
<td>5.97</td>
<td>*</td>
<td>2.94</td>
<td>*</td>
</tr>
<tr>
<td>Mixture species</td>
<td>2</td>
<td>b</td>
<td>53.1</td>
<td>* * *</td>
<td>54.3</td>
<td>* * *</td>
</tr>
<tr>
<td>Pot residuals (a)</td>
<td>43</td>
<td></td>
<td>26.0</td>
<td>19.6</td>
<td>17.7</td>
<td>57.8</td>
</tr>
<tr>
<td>Species residuals (b)</td>
<td>19/23</td>
<td></td>
<td>19.0</td>
<td>19.8</td>
<td>16.1</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f</th>
<th>Error †</th>
<th>Foliar SS%</th>
<th>Wood SS%</th>
<th>Root SS%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Block</td>
<td>1</td>
<td>a</td>
<td>2.20</td>
<td>1.97</td>
<td>0.41</td>
</tr>
<tr>
<td>Species richness</td>
<td>1</td>
<td>a</td>
<td>14.3</td>
<td>* * *</td>
<td>8.51</td>
</tr>
<tr>
<td>Monoculture species</td>
<td>2</td>
<td>a</td>
<td>41.2</td>
<td>* * *</td>
<td>28.7</td>
</tr>
<tr>
<td>Mixture species</td>
<td>2</td>
<td>b</td>
<td>7.45</td>
<td>**</td>
<td>9.53</td>
</tr>
<tr>
<td>Pot residuals (a)</td>
<td>43</td>
<td></td>
<td>25.1</td>
<td>29.4</td>
<td>47.1</td>
</tr>
<tr>
<td>Species residuals (b)</td>
<td>19/23</td>
<td></td>
<td>9.76</td>
<td>21.9</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f</th>
<th>Error †</th>
<th>Foliar SS%</th>
<th>Wood SS%</th>
<th>Shoot SS%</th>
<th>Root SS%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Block</td>
<td>1</td>
<td>a</td>
<td>0.91</td>
<td>2.04</td>
<td>0.85</td>
<td>0.73</td>
</tr>
<tr>
<td>Species richness</td>
<td>1</td>
<td>a</td>
<td>0.03</td>
<td>3.70</td>
<td>*</td>
<td>0.32</td>
</tr>
<tr>
<td>Monoculture species</td>
<td>2</td>
<td>a</td>
<td>1.80</td>
<td>1.64</td>
<td>0.51</td>
<td>25.9</td>
</tr>
<tr>
<td>Mixture species</td>
<td>2</td>
<td>b</td>
<td>49.2</td>
<td>* * *</td>
<td>33.3</td>
<td>* * *</td>
</tr>
<tr>
<td>Pot residuals (a)</td>
<td>43</td>
<td></td>
<td>36.8</td>
<td>46.2</td>
<td>32.7</td>
<td>72.9</td>
</tr>
<tr>
<td>Species residuals (b)</td>
<td>19/23</td>
<td></td>
<td>11.3</td>
<td>13.2</td>
<td>15.0</td>
<td></td>
</tr>
</tbody>
</table>

† refers to residuals at the pot level, b to the species (lowest) level. Species residuals for foliar responses differ from woody and aboveground residuals, since two saplings in mixtures were leafless at the time of harvest. Significant effects are indicated as followed: · $P < 0.1$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. 
Table 4.8: Analysis of variance for tree growth and foliar biomass, N concentration and N pools of three different tropical trees species grown in the Sardinilla plantation in Panama.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f.</th>
<th>Tree</th>
<th>Height</th>
<th>Girth</th>
<th>Biomass</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>F</td>
<td>P</td>
<td>F</td>
</tr>
<tr>
<td>Species richness (SR)</td>
<td>1; 30</td>
<td>3.57</td>
<td>*</td>
<td>1.25</td>
<td>n.s.</td>
</tr>
<tr>
<td>Species (S)</td>
<td>2; 30</td>
<td>11.3</td>
<td>***</td>
<td>11.9</td>
<td>***</td>
</tr>
<tr>
<td>SR × S</td>
<td>2; 30</td>
<td>2.31</td>
<td>0.98</td>
<td>n.s.</td>
<td>0.01</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f.</th>
<th>Foliar</th>
<th>Biomass</th>
<th>N concentration</th>
<th>N pool</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>F</td>
<td>P</td>
<td>F</td>
</tr>
<tr>
<td>Species richness (SR)</td>
<td>1; 30</td>
<td>0.92</td>
<td>n.s.</td>
<td>2.42</td>
<td>n.s.</td>
</tr>
<tr>
<td>Species (S)</td>
<td>2; 30</td>
<td>1.43</td>
<td>n.s.</td>
<td>43.9</td>
<td>***</td>
</tr>
<tr>
<td>SR × S</td>
<td>2; 30</td>
<td>0.04</td>
<td>n.s.</td>
<td>0.86</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

Significant effects are indicated as followed: · $P < 0.1$; * $P < 0.05$; *** $P < 0.001$. 

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Complementary resource use due to niche differentiation has often been proposed to be one plausible mechanism that leads to enhanced productivity and nutrient storage in mixed species stands (Ewel 1986, Harper 1977, Vandermeer 1989). While there is some evidence for complementary light use in stratified canopies of early and late successional tree species, little information is still available on belowground resource partitioning (Jose et al. 2006).

The aim of the present thesis was to test whether increased tree diversity, i.e. species richness, leads to complementary nitrogen (N) and phosphorus (P) acquisition due to different uptake strategies, and if so, whether this is because of nutrient uptake at different spatial scales, by different chemical forms and/or through association with mycorrhizal fungi. We tested this hypothesis with different experimental approaches such as stable isotope and radioisotope labeling in the field plantation and in the pot experiment.

Tree diversity had obviously an effect on the N and P acquisition of the trees in the Sardinilla plantation as well as in the pot experiment although the results did not meet our expectation. First, we did not detect a consistent overall effect along the species richness gradient on the aboveground N and P pools as described in Chapter 2 and second, we found only limited evidence for complementary N and P uptake strategies both in the pot experiment and in the field (Chapter 3 and 4).
5.1 Environmental heterogeneity and biodiversity

Resource availability, scale and topographic heterogeneity are often considered to be a major source of variation in the relationship between biodiversity and ecosystem functioning (Mittelbach et al. 2001, Vila et al. 2005). The environmental growing conditions in Sardinilla obviously exerted a strong influence on the N and P acquisition of the trees as revealed by the partial redundancy analysis (Chapter 2). This has previously been shown for tree productivity and mortality at the same site by Healy et al. (2008), but also for productivity and soil nutrient availability in Australian tree plantations (Firn et al. 2007, Vila et al. 2005). Thus, this findings emphasize the need to account for site-specific sources of variation on stand productivity and nutrient acquisition as this impedes generalizations of biodiversity effects on ecosystem functioning.

In their extensive literature review, Hooper et al. (2005) suggested that increasing nutrient availability allows for stronger complementarity, at least in the short-term as shown e.g. by Fridley (2003) and recently by Roscher et al. (2008) in a grassland experiment. Similarly, Schenk (2006) referred to higher interspecific competition at nutrient-poor or water-limited forest sites. These suggestions are corroborated by our findings in Chapter 2. We found a positive trend in aboveground N pools in more diverse stands compared to monocultures as well as a strong positive complementarity effect (CE) on the P pools in three-species mixtures. Both aboveground N and P pools correlated well with inorganic N and P availability. Apparently, subtle differences in soil N and P availability and small-scale changes in topography reduced interspecific root competition for resources to a level which improved tree growth and nutrient storage at the intermediate diversity level. However, the question whether the difference in soil N and P availability is a consequence of attributes of the component species in mixtures (Firn et al. 2007), for instance through litter input, was not adressed in the present thesis. Nevertheless, there is evidence for higher litter production in the three-species mixtures, and decomposition rates were found to be species-specific (Scherer-Lorenzen et al. 2007) which in return might affect the amount of N and P released through litter decomposition and mineralization.

Biodiversity effects on plant productivity and nutrient storage at low or intermediate levels have been discussed in detail for grassland studies (e.g. Tilman et al. 1997, Loreau 2000). However, as most experimental mixed species plantations in the tropics lack a tree diversity gradient, it is difficult to compare the findings with other sites. Montagnini (2000) reported an increase in N but not in P accumulation in a four-species mixtures consisting of fast- and slow-growing tree species. However, this increase was mainly due to the N-fixing tree species (*Albizia guachapele*) which affected the N uptake of the other tree species. Other studies reported similar effects of N-fixing tree species on productivity (i.e. facilitation) but those stands did not include more than two tree species. Nevertheless, previous studies from the Sardinilla plantation reported pronounced effects of three-species mixtures on nutrient storage, tree growth and litter production (Oelmann et al. 2010, Potvin and Gotelli 2008, Scherer-Lorenzen et al. 2007). Yet, no clear mechanism could
be identified to explain these patterns. It seems that complementarity and facilitation outweigh interspecific competitive interactions at the intermediate diversity level whereas interspecific competition, linked with less favorable environmental conditions, dominates productivity and nutrient retention in six-species mixtures. A plausible explanation for the higher interspecific competition at the most diverse level might be the fact that species with similar functional traits (i.e. growth rates) were planted in direct neighborhood (Scherer-Lorenzen et al. 2005).

5.1.1 Nutrient uptake strategies and complementarity

The two experiments on the uptake strategies provided interesting results on species’ responses to interspecific competition (Chapter 3 and 4). Organic N as a source was of little relevance for the three investigated species. This is in line with studies from other woody ecosystems where N apparently was not a limiting macronutrient (Kahmen et al. 2009, Pfautsch et al. 2009). One out of three species clearly preferred NO$_3^-$ over NH$_4^+$ and showed higher nitrate uptake from the topsoil than from the subsoil. Interspecific competition enhanced the differences among the species in biomass accumulation as well as in N and P uptake. However, the species-specific strategies were too similar to be complementary. Such limited evidence for chemical or spatial N partitioning has been reported from other ecosystems such as grasslands (von Felten et al. 2009, Kahmen et al. 2006). Besides, one important aspect of complementary nutrient uptake has not been directly addressed in the thesis at hand, i.e. the temporal variation in nutrient uptake. Results from Chapter 3 and 4 suggest that species-specific nutrient acquisition might vary on a temporal scale and is probably driven by seasonal variation in nutrient availability and phenology (Lucash et al. 2007, Van Schaik et al. 1993).

In addition, the CE in aboveground N and P pools (Chapter 2) could also be caused by differences in nutrient use (e.g. nutrient allocation to different plant organs). For example, differences in internal nutrient recycling between the species could explain why nutrient storage and nutrient uptake patterns are not consistent (Mead and Preston 1994, Millard 1996, Lal et al. 2001). Consequently, an integrative approach is needed to understand synergistic diversity effects on tree growth and nutrient storage, e.g. by linking various functional plant traits below- and aboveground (Ewel and Mazzarino 2008).

A prime example of a successful species mixture in Sardinilla is the combination of *Anacardium excelsum*, *Luehea seemannii* and *Tabebuia rosea*. This mixture probably exhibits resource partitioning through small differences occurring in various traits. The results from this thesis and findings of several other studies from the same site indicate such an overall complementarity albeit the studies were conducted at different stages of tree growth. The three species differed to some extent in growth, root system, phenology, tree water use, transpiration, nutrient requirement and uptake strategy (Coll et al. 2008, Kunert et al. 2010, Oelmann et al. 2010, Potvin and Gotelli 2008). These differences in functional plant traits in combination with favorable environmental growing conditions
(Chapter 2) resulted in a synergistic diversity effect. This effect can be explained by less interspecific competition for resources in the mixture compared to stronger intraspecific competition in monocultures (Harper 1977).

Moreover, arbuscular mycorrhizal fungi (AMF) might affect various processes and plant interactions as outlined by Smith and Read (2008). Several studies have shown the effect of AMF on productivity, inter- and intraspecific plant competition, plant resistance to diseases and pathogens, and stress tolerance (e.g. Herre et al. 2007, Klironomos et al. 2000, Facelli et al. 1999). As demonstrated in Chapter 3, the association with AMF was crucial for the P uptake of the tree species. Trees were similarly colonized by Glomus intraradices and depended to a large degree on the P uptake by the extraradical mycelium. Due to the short duration, no conclusions can be made on the effect of the colonization by AMF on tree growth in the pot experiment. However, establishment and growth of tree seedlings were mostly improved through AMF as reported in several studies from the tropics (Siqueira and Saggin-Junior 2001, Urgiles et al. 2009, Zangaro et al. 2003).

5.2 Outlook

Information on nutrient requirements, nutrient uptake strategies and belowground interactions of different tree species is surprisingly rare for mixed species plantations. However, this kind of data is needed since only an integrative approach, combining below- and aboveground plant characteristics, can ultimately unravel the mechanisms behind synergistic diversity effects and improve the design of mixed species plantations.

The tools used in the present thesis proved to be useful and their application could be extended for future experiments. For instance, the excavation of a root system is usually a difficult task, especially when the tree has reached a certain size. Stable isotope labeling in combination with species-specific molecular markers, as developed in this thesis, would represent a valuable alternative to such extensive examinations. With a root sampling (e.g. by soil coring) at different temporal and spatial scales, new insights could be gained into the dynamics of root growth, their distribution and their metabolic activities. Moreover, species-specific molecular AMF markers, as developed by Jansa et al. (2008) and Thonar (2009) and applied in this thesis, could provide additional information on the colonization of the roots and the importance of AMF for tree growth. However, such AMF markers need to be designed specifically for the Sardinilla site since different ecosystems are likely to harbor very distinct AMF communities.

Information on aboveground nutrient pools and nutrient use efficiencies might help to select tree species for restoration of degraded lands. Planting species with high nutrient demands and low nutrient use efficiencies (e.g. Hura crepitans and Tabebuia rosea) might result in adverse effects on soil fertility. Under such circumstances, sustainable management should include less nutrient demanding species (e.g. Anacardium excelsum or Luehea seemannii).
Whether the successful species mixture consisting of *Anacardium excelsum*, *Luehea seemannii* and *Tabebuia rosea* should be recommended (e.g. to smallholders) cannot be concluded from the results in this thesis alone. Replication at the stand level is missing. It seems that the good growth of this three-species mixture is linked to the environmental growing conditions in Sardinilla. Planting the same species combination at a different site would thus confirm or disprove its suitability for upcoming mixed species plantations in the tropics.

In conclusion, the findings from the experimental tree plantation in Sardinilla highlight how important tree diversity gradients are to study the link between biodiversity and ecosystem functioning. “The better the more” in terms of species richness, does not apply for stand productivity and nutrient storage in Sardinilla. This emphasizes the importance of species identity and species composition for ecosystem functioning.


Acknowledgments

The work presented in this thesis is anything else but the outcome of an one man show. Many people put a lot of effort and time into this project, and I received help from many directions during the last four years. Here, I would like to express my gratitude to those who gave me the possibility to complete this thesis at ETH Zurich and the Smithsonian Tropical Research Institute (STRI) in Panama and supported me in various ways:

Michael Scherer-Lorenzen for initiating this project and hence sending me to a beautiful and exciting country, for excellent remote supervision, especially during the field trips in Panama, and for very constructive remarks on the manuscripts.

Jan Jansa for excellent guidance and sharing his profound knowledge, for his substantial help in Eschikon as well as in Panama, for carefully reading my manuscripts and for drawing my attention to dirty side of ecology.

Nina Buchmann, head of the Grasland Science Group and my doctoral mother, for giving me the opportunity to conduct this project in her lively group, for constant support and advice on science and non-science related questions, and for additional funding at the end of the thesis.

Emmanuel Frossard, head of the Plant Nutrition Group, for welcoming me in his group, for the nice working atmosphere in Eschikon, for visiting the Sardinilla field site and for constructive remarks and lively discussions during the last four years.

Catherine Potvin (STRI), head of the Sardinilla project, for supporting my project in Sardinilla, for comments on the first manuscript and external evaluation of this thesis and for doing a lot of pioneer work in the tropics.

Ben Turner (STRI) and the soil lab team for all the help, especially with this nightmare of doing a radioisotope experiment in Panama, for sharing lab facilities and analyzing samples, and finally for dedicating an award to one of my diploma students.

Iliana Cabrera Phillips & José Monteza and family for making this project possible. For constant advice and help in the field, for sacrificing so many weekends, for
giving me shelter, for unpayable friendship and for showing me the manifoldness of Panama.

Alberto “Beto” Maynard, Felipe Rodriguez, Mello, Sr Daniel and his sons, Sr Benicio, Izzy, Xiomara Pasquale and all the people in Sardinilla and Gamboa for great support and countless hours of hard fieldwork.

The STRI staff, especially Raineldo Urriola, José Barahona, José Ramon Perurena, Allen Herre, Klaus Winter, Jorge Aranda and José Déago for their incredible help at various stages of the project. My requests were often not easy but there was always a solution.

Claudine Hostettler and Nilka Tejeira for their great help with administrative issues.

The former diploma students Carole Grob und Thomas Seitlinger for their interest in the project and sharing their data. I am very grateful to Else Bünemann for the supervision of Thomy and the help with the soil analyses.

Annika Lenz, Karin Sörgel and Roland Werner for C/N and isotope ratio measurements, Thomas Flura for help with the FIA, Cecile Thonar and Carolin Schwer for various support with the molecular work and Lea Bona and Tuija Waldvogel for helping with the sample preparations.

Rebecca Hiller, Susanne Burri, Stefan Trogisch, Nadine Rühr and Stefanie von Felten for the relaxed atmosphere in A2 and for being such nice office mates.

Marlen Gubsch, Stefanie von Felten and Nadine Rühr for their constructive remarks on various chapters.

Christian Schöb for familiarizing me with CANOCO.

All my colleagues from the Grasland Group and the Plant Nutrition Group in Eschikon for four years of excellent company, constructive input on the science and the other life, for nice apéros, group excursions, group retreats and the good time.

Mirco Plath, Sebastian Wolf, Carole Grob and all my colleagues from Sardinilla for adventurous moments out there in the field, for afterwork recreation and lively conversations in the evenings. A big thank-you to Mirco who became a close friend and somehow managed to stand all my moods and tics during several months of flat sharing.

All my friends for their support and encouragement during the last years and for reminding me that there is a life beyond science. A special thank goes to Corinne and Sabina who decided to visit me in Panama and had to pay it in return with several days of fieldwork. I also want to thank the both and Julia for the January and February survival packages.
My family, especially my parents, for their interest in my work, for always believing in me and supporting my decisions and for regular home-food supply during my field trips to Panama.

Last but not least, my beloved husband Marc Schneebeli for huge support, confidence and encouragement - I owe you one.

The financial support of the Swiss National Science Foundation (grant no. 3100A0-110031/1) is gratefully acknowledged.
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