

Dominance of legume trees alters nutrient relations in mixed species forest restoration plantings within seven years

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Abstract Failures in reforestation are often attributed to nutrient limitation for tree growth. We compared tree performance and nitrogen and phosphorus relations in adjacent mixed-species plantings of contrasting composition, established for forest restoration on Ultisol soil, originally covered by tropical semi-deciduous Atlantic Forest in Southeast Brazil. Nutrient relations of four tree species occurring in both planting mixtures were compared between a legume-dominated, species-poor direct seeding mixture of early-successional species (“legume mixture”), and a species-diverse, legume-poor mixture of all successional groups (“diverse mixture”). After 7 years, the legume mixture had 6-fold higher abundance of N₂-fixing trees, 177% higher total tree basal area, 22% lower litter C/N, six-fold higher in situ soil resin-nitrate, and 40% lower in

situ soil resin-P, compared to the diverse mixture. In the legume mixture, non-N₂-fixing legume *Schizolobium parahyba* (Fabaceae-Caesalpinioideae) had significantly lower proportional N resorption, and both naturally regenerating non-legume trees had significantly higher leaf N concentrations, and higher proportional P resorption, than in the diverse mixture. This demonstrates forms of plastic adjustment in all three non-N₂-fixing species to diverged nutrient relations between mixtures. By contrast, leaf nutrient relations in N₂-fixing *Enterolobium contortisiliquum* (Fabaceae-Mimosoideae) did not respond to planting mixtures. Rapid N accumulation in the legume mixture caused excess soil nitrification over nitrate immobilization and tighter P recycling compared with the diverse mixture. The legume mixture succeeded in accelerating tree growth and canopy closure, but may imply periods of N losses and possibly P limitation. Incorporation of species with efficient nitrate uptake and P mobilization from resistant soil pools offers potential to optimize these tradeoffs.

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Introduction

Secondary succession in tropical deforested areas can be delayed or even arrested in grass-dominated

vegetation over large areas after severe anthropogenic disturbance (Uhl et al. 1988; Otsamo et al. 1995; Nepstad et al. 1996; Fearnside and Guimarães 1996). To overcome such barriers to forest recovery, several objectives have been driving recent forest restoration, including: (1) production of timber and non-timber forest products, (2) ecological restoration for biodiversity conservation, (3) catchment protection and other ecological services, and/or (4) facilitating forest fallow to restore soil fertility for subsequent agriculture. To achieve any of these objectives, tree growth is of central importance, but to date we have little understanding of the mechanisms controlling tree productivity in mixed plantings (Rothe and Binkley 2001).

Secondary succession can be delayed for a range of reasons, including recurrent fires, competition with grasses, limitations to seed dispersal, herbivory, harsh microclimate and/or unfavorable soil conditions (Chazdon 2003; Davidson et al. 2004). Land use disturbances have widely caused substantial redistribution and losses from site, especially of nitrogen, but also of phosphorus (Kauffman et al. 1995; Markewitz et al. 2004). Subsequent natural forest regrowth has frequently been delayed by exacerbated nutrient limitation, particularly of nitrogen, even on strongly weathered old soils (Buschbacher et al. 1988; Herbert et al. 2003; Davidson et al. 2004, 2007). Although N_2 -fixing leguminous trees are widespread in most tropical secondary forests, their N_2 -fixation rates can be sensitive to low soil P availability, which in turn may thus limit ecosystem productivity and N accumulation (Vitousek et al. 2002; Güsewell 2004). Tree species within secondary forests have a variety of strategies of P and N acquisition and use, including low or high tissue nutrient concentrations (Denich 1991), i.e., with low or high maximum photosynthetic capacity (Wright et al. 2005), which may be termed nutrient-intensive versus nutrient-efficient characteristics, respectively. However, it remains unclear whether or not it is beneficial to incorporate different nutritional types in restoration plantings, or whether functionally homogeneous plantings of species with high nutrient use efficiencies (i.e., large biomass production per nutrient consumed), are more successful (Hiremath and Ewel 2001).

It has been suggested that more efficient nutrient accrual and use at the plant community level may in some circumstances be achieved by including higher plant functional diversity (Hiremath and Ewel 2001),

especially on infertile sites where functionally diverse mixtures may be more likely to attain higher degrees of acquisition and retention of ecosystem nutrients than homogeneous plant communities (Rothe and Binkley 2001; Güsewell 2004; Forrester et al. 2005a). However, our understanding of the underlying mechanisms is limited, since this notion is difficult to test in generalizable terms (Rothe and Binkley 2001). Most replicated experimental plantings have been small and/or involve few inter-planted species. Currently, we have poor knowledge of which combinations of plant functional types are likely to survive under nutrient constraints, and, in turn, how plant species combinations are likely to affect nutrient utilization and cycling processes within the ecosystem. Surprisingly few studies have compared nutrient concentrations of the same species in different mixtures of tree species (Parrotta 1999; Rothe and Binkley 2001). However, such an approach would help to elucidate the effects of community composition on plant nutrient relations, without being confounded by species with different nutritional characteristics dominating the samples from the communities being compared.

In this study, we aimed to determine how contrasting restoration plantings of ‘species-poor, N_2 -fixer-rich’ versus ‘species-rich, N_2 -fixer-poor’ mixtures affect (1) the establishment success of N_2 -fixing and non-fixing trees and growth of tree species common to both mixtures; (2) N and P relations in these species; and (3) soil N and P availability. We compared adjacent experimental plantings, which had been established on grasslands persisting after agricultural abandonment, where natural forest succession had been arrested for unknown reasons for more than 15 years. All four tree species which survived in both plantings were examined for their specific leaf areas (SLA), nutrient concentrations and $\delta^{15}N$ in mature and freshly abscised leaves and litter layer. We estimated proportional N and P resorption, nitrate, ammonium and phosphate availability in the soil using in situ ion exchange resin, as well as total soil C and N.

Materials and methods

Site description

The study was conducted in experimental plantings on Edgardia Experimental Farm within the campus of

São Paulo State University at Botucatu (UNESP-FCA) at 574 m altitude in the south-central region of São Paulo state (22°50' S; 48°24' W). The area was originally covered in seasonal semi-deciduous tropical forest within the Atlantic Forest biome. Annual rainfall averages around 1,300 mm, the majority of which falls between October and March. Mean annual temperature is 20.3°C, with a monthly minimum of 16.5°C in June and maximum of 23.4°C in January. The soil is an Ultisol (Red-Yellow Argisol; Embrapa-Solos 1999), derived from sandstone and basic igneous rocks from the Cretaceous and Jurassic, with an acid, loamy sand topsoil (pH 4.8 in CaCl₂; ion exchange resin-extractable P: 6.3 mg P l⁻¹ soil), dominated by low-activity clays in the subsoil (Engel and Parrotta 2001).

The experimental site had been under pasture for about 50 years prior to conversion to a citrus orchard in 1971, which was abandoned in the early 1980s. Before establishment of the restoration plantings in 1997/1998, the site was dominated by the grass *Brachiaria decumbens* Stapf, which had invaded from nearby pastures, and relict citrus trees. The site lacked woody re-colonization since the citrus plantation had been abandoned more than 15 years earlier, despite the absence of fire and proximity of remnant seasonal semi-deciduous tropical Atlantic Forest in good state of conservation within 50 m of the experimental plots.

Experimental layout

The experimental area was protected against fire and fenced, though some cattle entered the area during the experimental period. Relict citrus trees were removed mechanically, and a post-emergent herbicide (glyphosate) was applied initially to suppress grasses and increase the survival of tree seedlings. During the first 4–5 years of plantation establishment, mechanical and chemical mowing, and traps for leaf-cutting ants were applied as necessary to increase tree survival.

We compared two planting mixtures of contrasting functional composition, which are part of a large-scale experiment involving a randomized complete block design with four planting designs and an unplanted control (details in Engel and Parrotta 2001). The two mixtures were selected, because they had four tree species in common, all of which are native to the local Atlantic Forest. These included

two deciduous legumes *Enterolobium contortisiliquum* (Vell.) Morong (Leguminosae-Mimosoideae) and *Schizolobium parahyba* (Vell.) S.F. Blake (Leguminosae-Caesalpinioideae). The two most abundant lower canopy tree species had established from natural regeneration (i.e., not planted) in both mixtures, and were also sampled, i.e., guava, *Psidium guajava* L. (Myrtaceae) and *Peschiera fuchsiaefolia* Miers. (Apocynaceae). Subsequently, these four species are referred to by their genus only.

Among three replicate blocks planted in 1997/1998, one block exhibited very high mortality across all four planting mixtures, and could therefore not be sampled. Planting failure across this third block may have been associated with its different land use history, i.e., continuous pasture use for more than seven decades, without a subsequent period of orchard prior to establishment of the present experiment (Fig. 1). Furthermore, several cattle invasions from the nearby pastures have also contributed to this poor development. By contrast, the remaining two replicate blocks have had the same land use history and exhibited uniform tree survival among the four planting mixtures in both blocks. Plot sizes of 50 × 50 m and randomized location ensured that confounding effects within the successful two blocks were highly unlikely, in the absence of detectable environmental gradients or boundaries.

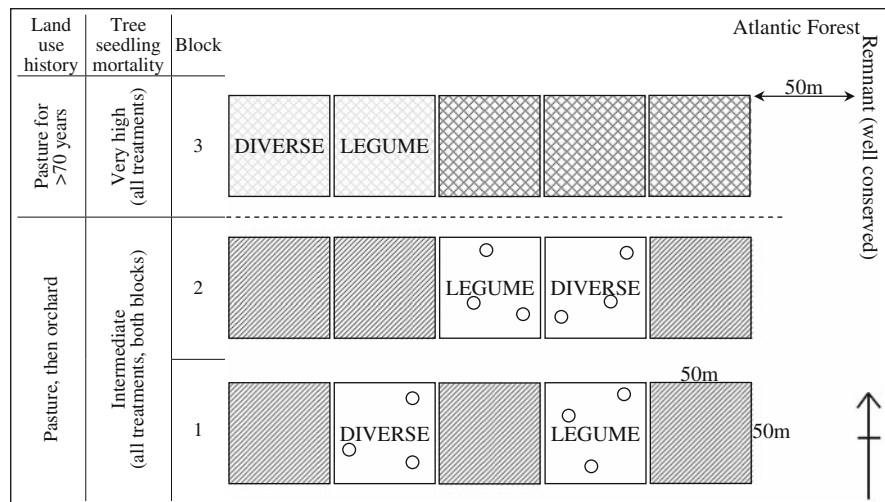
Planting mixtures

The unplanted control plots of the same size had been subjected to the same site preparation and weed control (Engel and Parrotta 2001). The two planting mixtures compared in this study were:

Legume-dominated (“legume”) mixture: Planting lines were prepared to 40 cm depth with a soil ripper, and five early-successional tree species were directly seeded in mono-specific rows at a spacing of 1 × 1 m (equivalent to 10,000 individuals ha⁻¹) in March 1997. Only two legumes established to seedlings, *Enterolobium* and *Schizolobium*. Within 7 years of establishment these two legumes comprised 97% of total basal area, attaining a largely closed canopy. A discontinuous cover of relict grasses (mainly *B. decumbens*) remained in the understorey.

Diverse mixture: After conventional plowing to 30 cm depth, a mixture of about 1-year old nursery-grown

Fig. 1 Schematic layout of experimental plantings. All planting treatments in Block 3 (cross-hatched plots) failed due to very high tree mortality, and could therefore not be sampled. *Open circles* in plots represent subplots ($n = 6$) within which all sample types were retrieved. Sampled planting treatments: *LEGUME*-dominated mixture; *DIVERSE* mixture



seedlings of 18 early-successional tree species were planted at higher densities, and of 22 late-successional tree species at lower densities (total of 3,054 individuals ha^{-1}) in January to March 1998. Despite a high initial mortality, a diverse range of early-, mid- and late-successional species formed a discontinuous canopy within less than 7 years of establishment, interrupted by dense grass patches of mainly *B. decumbens*. The overall proportion of N_2 -fixing, as well as non- N_2 -fixing legumes in the mixture was low (25% of all stems), and the four most abundant tree species of the legume mixture were present at low densities in the diverse mixture.

Plant community structure

To calculate tree abundance, all stems above 1.3 m height were counted. Total basal areas for all species per plot were estimated in February 2006 with a Spiegel relascope, using the Bitterlich method (Nascimento et al. 2004). Readings were taken at five systematic points per plot with a factor of 1. For the four studied species, specific basal area estimates were calculated as the product of their total abundance per plot and their average basal area in nine random circular subplots of 6 m diameter.

To estimate proportional abundance of putative N_2 -fixers per plot, published reports assessed by Sprent (2001) were used to distinguish putative N_2 -fixers, i.e., *Enterolobium contortisiliquum* (Vell) Morong, *Machaerium stipitatum* (DC.) Vogel, *Centrolobium tomentosum* Benth., *Poecilante parviflora*

Benth., *Ormosia arborea* (Vell) Harms, *Parapiptadenia rigida* (Benth.) Brenan—from putative non- N_2 -fixers, i.e., *Dipteryx alata* Vog, *Pterogyne nitens* Tul., *Hymenaea courbaril* L. var. *stilbocarpa*, *Copaifera langsdorffii* Desf., *Schizolobium parahyba* (Vell) Blake (order of decreasing abundance in the diverse mixture).

Plant sample collection and preparation

In each treatment six healthy individual trees of each of the four species were randomly selected within the same subplots established for species-specific basal area estimation. All three surviving individuals of *Schizolobium* in the diverse mixture were sampled.

The youngest, fully expanded ('mature') sun leaves were sampled from different branches in external parts of the upper canopy at least 3 h after sunrise and at least 2 h before sunset during the transition between wet and dry seasons in May–June 2004. Freshly abscised leaves were collected beneath the same individuals within a few days of spreading shade cloth in June–August in 2004. Approximately 30–300 leaves or leaflets (depending on species leaf size) without obvious symptoms of pathogen or herbivore damage and without substantial cover of epiphylls were selected for both mature and abscised leaves. Samples were homogenized before sub-sampling. Blades of growing and abscised leaves or leaflets were separated from petioles, rachis, petioles and oven-dried at 60°C for 48 h, and ground to powder.

Estimated proportional N and P resorption (PR) were calculated from nutrient concentrations in mature (nt.mature) and abscised (nt.abscised) leaves. Mass-based calculations of proportional resorption of elements are generally underestimations, since besides the nutrient in question, other elements are also retranslocated from senescing leaves into surviving plant parts (van Heerwaarden et al. 2003). Consequently, leaf mass and nutrient concentration decline simultaneously during leaf senescence. Since mass resorption data were unavailable, we applied a correction factor ‘Fractional Change of Measurement Basis’, based on a standardized, weighted average of mass loss of 21% compiled by van Heerwaarden et al. (2003) from studies on species from a range of ecosystems and phenological groups ($n = 126$):

$$\text{Corrected PR} = 100 * [1 - [1 - (1 - \text{nt.abscised}/\text{nt.mature})] * [1 - 0.21]]$$

Thus, proportional resorption reported here cannot be directly compared with values published elsewhere. However, the purpose of the data is to compare relative resorption in the same four species co-occurring in adjacent plots of different planting mixtures. Luyssaert et al. (2005) judged estimated proportional resorption to be a useful approximation for this purpose.

Litter and soil sample collection and preparation

Litter layer samples (including discrete and amorphous litter above the A horizon) were collected in three 25 × 25 cm quadrats, homogenized and subsampled by cone-quartering below each of the sampled trees. Litter layer samples were oven-dried at 60°C for 7 days. All C/N and N/P-ratios were expressed as mass ratios.

Soil samples were taken by auger from 0 to 5, 5 to 10, 10 to 20, 20 to 30 cm depths at four random points at 40 cm distance from the tree base beneath each sampled tree, bulked and subsampled by cone-quartering. Soil samples were sieved to 2 mm and oven-dried at 105°C for 48 h, and ground to powder. Field nodulation status of three individuals of both legumes species in both treatments was assessed by verifying a continuous connection between the parent trunk and all fine roots examined for the presence of nodules.

In situ mixed-bed ion exchange resin was used as a measure of potentially plant-available soil nutrients in the field. Five ml (≈ 4.8 g fresh weight) of densely packed resin (Dowex Monosphere MR-3 UPW, >99.7% conversion to H^+ and OH^-) were filled in 5 × 5 cm nylon mesh bags (430 μm mesh aperture). Resin bags were kept in a sealed, moist container until installation on a day with high soil moisture levels and left in situ for 6 weeks in June/July 2004. Resin bags were installed at two opposite points at 40 cm distance from the tree trunk below the canopies of each of the four target tree species. Resin bags were installed below 12 individual trees per species in the legume plots and below nine individuals per species in the diverse plots, including the same individuals from which other samples were retrieved. Locations of resin bags were chosen so as to ensure full canopy shading, in order to minimize potential treatment differences in irradiation related to canopy cover, and associated topsoil drying which affect ion adsorption dynamics in exchange resins (Kjønnaas 1999). For resin bag installation, soil was lifted with a spade at 45° angle and the resin bag positioned at 10 cm depth of the A horizon, and maximum physical contact with the soil matrix was ensured to establish hydraulic contact. On retrieval, resin bags were sealed in polythene bags and refrigerated until extraction.

Chemical analyses

Sub-samples of 2 mg of ground leaf or litter material, or 20 mg of pounded soil were analyzed in a ThermoQuest-Finnigan Delta Plus isotope ratio mass spectrometer interfaced with a Carlo Erba 1110 Elemental Analyzer at CENA-USP, Brazil. Stable isotope proportions are reported as $\delta^{15}\text{N}$ (‰). The precision of isotope ratio measurements was $\pm 0.3\text{‰}$ for $\delta^{15}\text{N}$, and of N and C concentrations were 0.15% and 0.01%, respectively, as validated by internal and international standards.

All analyses of plant and soil P, and resin pools were carried out at the Natural Resources Department of UNESP-FCA, Brazil. For determination of total P concentrations in leaves and litter, samples were extracted in a digest of concentrated nitric and perchloric acid (6:1) for 2:30 h at 225°C, and determined by metavanadate colorimetry in a UV/Vis spectrophotometer (Malavolta et al. 1997). For soil

extractable P determination, sieved soil samples were extracted for 16 h on a shaker with ex situ mixed-bed ion exchange resin in 1 M NaCl, and determined by colorimetry with the molybdate/ascorbic acid method (Raij et al. 2001).

In situ ion-exchange resins were removed from the mesh bags and briefly rinsed with a wash bottle of deionized water to remove any illuviated clay and silt. Resin from the two mesh bags from below the same individual tree were bulked (except for resin bags from below *Schizolobium* in the diverse mixture, where only three individuals of *Schizolobium* had survived). Bulk resin samples were shaken five times in fresh 20 ml 2 M KCl for 30 min and the supernatant extracts were decanted for analysis. Extracts were made up to 100 ml and frozen until P was determined in resin extracts by colorimetry with the molybdate/ascorbic acid method (Raij et al. 2001). Resin ammonium and nitrate were determined by distilling in the presence of MgO (for ammonium) or Devarda's alloy (for nitrate) using a steam-distillation apparatus, trapped with boric acid and subsequently titrated with 0.01 M HCl (Rayment and Higginson 1992).

Statistical analysis

Factorial two-way analysis of variance (ANOVA) was carried out on nutrient concentrations and resin nutrient pools for planting mixture versus species and on soil nutrient concentrations for treatment versus soil depth, using STATISTICA 7.1 series 0205b (StatSoft 2005). Fisher LSDs were computed for comparisons of >2 levels. Nutrient concentrations were log-transformed for ANOVA if variables showed heterogeneous variances. Where transformations did not homogenize variances, pairwise *t*-tests with separate variance estimates were carried out on untransformed data and the confidence threshold adjusted to $p < 0.01$ (six comparisons among species) or $p < 0.001$ (28 comparisons among species * treatments), to further reduce the probability of Type I error.

Results

Development of plant communities

Nine years after germination both reforestation treatments had established a tree canopy with

variable degrees of closure. Average basal area in the legume mixture was more than double that of the diverse mixture (Table 2). In turn, the diverse plantings had a basal area more than 16 times larger than the unplanted control plots ($0.66 \text{ m}^2 \text{ ha}^{-1}$; cf. Table 2), which showed only sparse *Psidium* colonization with slow growth, despite having been subjected to the same initial site preparation. Densities of nursery-grown seedlings planted out in the diverse mixture were substantially higher than seedling densities in the legume mixture 2 years after direct seeding in the field, but this pattern was reversed 7 years later due to lower mortality of trees, and higher recruitment of naturally colonizing trees in the legume mixture (Fig. 2).

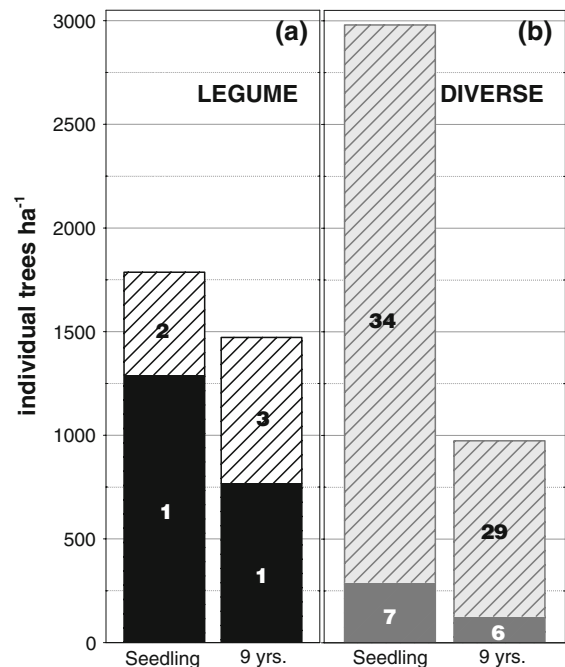


Fig. 2 Density of individuals >1 m tall of all putatively N₂-fixing (filled) and non-N₂-fixing (hatched) trees in adjacent planting mixtures, including naturally colonizing trees: **(a)** LEGUME-dominated mixture: seedling establishment 2 years after direct seeding (*Seedling*) (data from Engel and Parrotta 2001), and survival 9 years after direct seeding (*9 years*); **(b)** DIVERSE mixture: total 1-year old nursery-grown seedling numbers transplanted into field plots (*Seedling*), and survival 9 years after germination in the nursery (*9 years*). **Bold numbers** within bars indicate species numbers comprising the graphed numbers of individuals (**white numbers**: N₂-fixing, **black numbers**: non-N₂-fixing; putative N₂-fixing status based on Sprent 2001)

While all four tree species studied here occurred in both planting mixtures, their basal area share varied greatly between the two plant communities 7 years after germination (Table 1). N₂-fixing *Enterolobium* attained ~68% and 12% of the estimated total basal area in the legume and diverse mixtures, respectively. Non-N₂-fixing legume *Schizolobium* constituted ~29% and 2% of total basal area in the legume

and diverse treatments, respectively. The naturally colonizing non-legume trees *Psidium* and *Peschiera* attained basal areas in the legume mixture on average more than double those in the diverse mixture. These differences were due to higher densities as well as faster growth of all four species in the legume mixture, although it was field-established 1 year before the diverse mixture.

Table 1 Main effects of species (2-way ANOVA; means $\pm$ s.e.m.), 7 years after germination, with data for two planting mixtures pooled within species, unless specified otherwise

	<i>Enterolobium contortisiliquum</i>			<i>Schizolobium parahyba</i>			<i>Peschiera fuchsiaeifolia</i>			<i>Psidium guajava</i>			<i>p</i>
Family/sub-family	Fabaceae/Mimosoideae			Fabaceae/Caesalpinioideae			Apocynaceae			Myrtaceae			
Leaf phenology	Deciduous			Brevideciduous			Evergreen			Evergreen			
Root nodules observed in field	Abundant			None			None			None			
Observed nutritional characteristic	N ₂ -fixing legume			Non-N ₂ -fixing legume			Nutrient-intensive			Nutrient-efficient			
Basal area (m ² ha ⁻¹) in LEGUME ^a	13.28	(± 3.28)		5.78	(± 1.91)		0.18	(± 0.03)		0.41	(± 0.11)		–
Basal area (m ² ha ⁻¹) in DIVERSE ^a	0.87	(± 0.49)		0.15	(± 0.15)		0.11	(± 0.01)		0.13	(± 0.02)		–
Specific leaf area, SLA (cm ² g ⁻¹)	116	± 3	a	119	± 5	a	134	± 3	b	87	± 3	c	**
$\delta^{15}\text{N}$ (‰) in mature leaves	-0.22	± 0.35	a	1.92	± 0.24	b	4.10	± 0.46	c	1.27	± 0.23	b	***
N conc. in mature leaves (g m ⁻²)	2.81	± 0.10	a	2.38	± 0.12	b	2.71	± 0.09	a	2.26	± 0.07	b	*
N conc. in mature leaves (mg g ⁻¹)	32.2	± 0.6	a	27.9	± 0.5	b	36.2	± 1.4	c	19.7	± 1.0	d	**
Proportional N resorption (%)	41.0	± 1.4	a	51.7	± 1.9	b	54.0	± 1.5	b	44.6	± 1.4	a	**
P conc. in mature leaves (g m ⁻²) ^b	0.128	± 0.004	a	0.136	± 0.007	a	0.290	± 0.049	b	0.192	± 0.010	b	*
P conc. in mature leaves (mg g ⁻¹) ^b	1.47	± 0.03	a	1.58	± 0.05	ab	4.00	± 0.70	c	1.65	± 0.05	b	**
Proportional P resorption (%) ^b	53.0	± 1.6	a	40.2	± 3.3	b	30.5	± 7.3	b	22.6	± 5.8	b	**
N/P ratio in mature leaves ^b	22.0	± 0.4	a	17.9	± 0.8	b	11.3	± 1.5	c	12.0	± 0.6	c	**
C/N-ratios in litter layer below canopy	21.7	± 0.8	a	31.8	± 2.6	b	24.2	± 2.1	a	23.4	± 1.5	a	***
C/P-ratios in litter layer below canopy	441	± 29	(a)	563	± 42	(b)	461	± 27	(a)	428	± 22	(a)	0.051

Under *p*, *, **, *** denote significant differences at the 0.05, 0.01, 0.001 levels, respectively. Means differ from one another significantly ($p < 0.05$) if they have no lower case letters in common (Fisher LSD, unless specified otherwise). Leaf attributes $n \geq 8$ (in *Schizolobium*) or $n \geq 11$ (in all other species)

^a Means of two plots (50 $\times$ 50 m each); (difference between plots)/2 is given instead of s.e.m.; estimated at 7 years after germination; Planting treatments: *LEGUME*-dominated mixture; *DIVERSE* mixture; Stem density data for *LEGUME* from A.M. Martins and V.L. Engel (unpublished)

^b Pairwise *t*-test with separate variance estimates and adjusted *p*

During the 7 years since the first census, the proportion of the only N₂-fixing species in the legume mixture (*Enterolobium*) among all tree stems declined from 72% to 52%, due to colonization by the non-legumes *Psidium* and *Peschiera* (Fig. 2a). In the diverse mixture the proportion of all N₂-fixing trees, comprising six surviving species, was low, i.e., increasing from 10% to 13% of all stems during 8 years since transplanting into the field (Fig. 2b).

Main effects of tree species

The factorial ANOVA revealed marked inter-specific differences in plant attributes that were significant despite differences between planting mixtures (Table 1). Both legumes had similar, intermediate SLA, while *Psidium* had lower and *Peschiera* had very high SLA. *Peschiera* also had the highest mass-based leaf N concentrations (3.6%), while the legumes *Enterolobium* and *Schizolobium* had intermediate (3.2% and 2.8%) and *Psidium* had the lowest (2%) leaf N concentrations. N concentrations expressed on leaf area basis were similar in *Peschiera* and *Enterolobium*, and significantly higher than in *Schizolobium* and *Psidium*. *Peschiera* and *Schizolobium* showed, on average, about 10% higher proportional N resorption than *Enterolobium* and *Psidium* (Table 1). The litter layer beneath the canopies of *Schizolobium* had higher C/N-ratios than beneath all other species ($p < 0.001$), with a similar trend for C/P-ratios, and was characterized by a large mass of abscised woody petioles and petiolules of *Schizolobium*.

Abundant root nodules were observed on all examined individuals of *Enterolobium* in both treatments, whereas no nodule was found on any of six individuals examined of *Schizolobium*. *Enterolobium* leaves were depleted in ¹⁵N (−0.2‰), whereas leaves of non-N₂-fixing species were enriched in ¹⁵N (1.3–4.1‰).

Mass-based leaf P concentrations in *Peschiera* varied from 0.19% to 0.87% and were on average (0.40%) significantly higher than in the other species (0.16%) (Table 1). Leaf area-based P concentrations in the legumes were about half of those observed in the non-legumes. Leaf N/P-ratios were highest in *Enterolobium*, intermediate in *Schizolobium*, and lowest in the non-legumes. *Enterolobium* showed higher proportional P resorption than all non-N₂-fixing species.

Main effects of planting mixtures

Factorial ANOVA identified marked differences in plant and soil attributes between the legume-dominated and the diverse mixtures (Table 2), despite data pooling from all four species with contrasting inherent nutrient relations (see previous section). Although leaf N concentrations were significantly higher in the legume mixture, these differences were small. Leaf ¹⁵N of *Enterolobium* was enriched in the legume plantings (0.9‰), and depleted in the diverse plantings (−1.3‰) ($p < 0.001$). By contrast, consistent topsoil $\delta^{15}\text{N}$ (6.1‰) was observed in both planting mixtures (Table 2).

Litter layer N concentrations across all four studied species in the legume were on average 49% higher than in the diverse mixture, which resulted in 28% higher litter C/N-ratios (Table 2). Ion exchange resin incubated in soil in situ showed six-fold higher levels of nitrate in the legume than in the diverse mixture ($p < 0.001$). In situ resin ammonium levels were low in both mixtures compared to nitrate, but levels in the legume plantings were approximately half of those in the diverse plantings.

While leaf P concentrations were similar in both planting mixtures, proportional P resorption in the legume mixture was approximately 60% higher than in the diverse mixture (Table 2). N/P-ratios in mature leaves, and especially in abscised leaves and litter layer in the legume plantings were markedly higher than in the diverse mixture. In situ resin-P in the legume was 40% lower than in the diverse mixture.

Interactions of species and planting mixtures

Significant interactions were found between factors of species and planting mixture, indicating that planting mixtures had different effects on some species than on others (Figs. 3 and 4). While leaf N concentrations in legumes showed no difference between planting mixtures, both non-legumes had higher mass-based leaf N in the legume mixture (only leaf area-based data shown; Fig. 3a). *Schizolobium* was the only species that had lower proportional N resorption in the legume ($49\% \pm 2 \text{ SE}$) than in the diverse mixture ($57\% \pm 2 \text{ SE}$; $p < 0.05$; data not shown).

Litter layer C/N-ratios beneath the canopies of all three non-N₂-fixing species were substantially lower in the legume than in the diverse plantings, whereas litter C/N-ratios beneath *Enterolobium* did not differ

Table 2 Main effects of planting mixture (2-way ANOVA) 7 years after germination, with data for four species pooled within planting mixtures, unless specified otherwise

	Legume-dominated mixture (‘legume’)			Diverse mixture (‘diverse’) <i>p</i>			
	Mean	±s.e.m.	<i>n</i>	Mean	±s.e.m.	<i>n</i>	
Total basal area (m ² ha ⁻¹) ^a	19.7	(±5.1)	2	7.1	(±1.7)	2	–
N conc. in mature leaves (g m ⁻²)	2.65	±0.07	24	2.44	±0.08	21	**
N conc. in mature leaves (mg g ⁻¹)	30.6	±1.5	24	27.3	±1.5	21	***
N conc. in litter layer (mg g ⁻¹)	20.5	±0.7	24	13.9	±0.7	21	***
δ ¹⁵ N (‰) in topsoil (0–5 cm depth) ^b	6.0	±0.1	6	6.2	±0.1	6	ns
δ ¹⁵ N (‰) in mature leaves of <i>Enterolobium</i> (‰) ^c	0.86	±0.28	6	-1.30	±0.12	6	***
δ ¹⁵ N (‰) in litter layer ^d	1.22	±0.13	24	0.63	±0.26	21	*
P concentrations in mature leaves (g m ⁻²) ^d	0.16	±0.01	24	0.22	±0.03	21	ns
P concentrations in mature leaves (mg g ⁻¹) ^d	1.83	±0.11	24	2.56	±0.47	21	ns
Proportional P resorption (%) ^d	44.9	±2.1	24	27.5	±5.3	21	**
C/N-ratios in mature leaves	16.7	±0.8	24	19.2	±1.3	21	***
C/N-ratios in abscised leaves	25.2	±1.1	24	30.3	±1.8	21	***
C/N-ratios in litter layer	21.8	±0.9	24	27.8	±1.6	21	***
C/P-ratios in mature leaves	280	±15	24	267	±23	21	ns
C/P-ratios in abscised leaves	423	±31	24	350	±45	21	**
C/P-ratios in litter layer	491	±21	24	437	±22	21	ns
N/P-ratios in mature leaves	17.0	±0.8	24	14.3	±1.4	21	**
N/P-ratios in abscised leaves	17.3	±1.4	24	13.2	±2.3	21	***
N/P-ratios in litter layer	22.7	±0.6	24	16.2	±0.9	21	***
In situ resin-NH ₄ ⁺ -N (μg g ⁻¹ FW resin d ⁻¹)	18.5	±3.6	47	33.1	±4.4	35	*
In situ resin-NO ₃ ⁻ -N + NO ₂ ⁻ -N (μg g ⁻¹ FW resin d ⁻¹) ^d	164.8	±14.0	47	27.3	±4.2	35	***
In situ resin-PO ₄ ³⁻ -P (μg g ⁻¹ FW resin d ⁻¹) ^d	1.96	±0.24	47	3.26	±0.56	35	*

Numbers of replicate subplots (*n*) samples were derived from. Under *p*, *, **, *** denote significant differences at the 0.05, 0.01, 0.001 levels, respectively; ns, non-significant

^a Means of two plots (50 × 50 m each); (difference between plots)/2 is given instead of s.e.; estimated at 9 years after germination (Bitterlich-method)

^b Two-way ANOVA (planting mixture * soil depth); depths below 5 cm not shown (Fisher LSD non-significant at all depths)

^c One-way ANOVA

^d *t*-Test with separate variance estimates and adjusted *p*

between mixtures (Fig. 3b). Resin nitrate levels accumulating in soil beneath the canopies of *Peschiera* (238 μg g⁻¹ FW resin d⁻¹ ± 33 s.e.) were more than 60% higher than beneath all other species (142 μg g⁻¹ FW resin d⁻¹ ± 13 s.e.) within the legume plantings (*p* < 0.01; data not shown).

Both non-legumes pooled, tripled their proportional P resorption in the legume (41% ± 3 s.e.) relative to the diverse mixture (13% ± 6 s.e.; *p* < 0.001; data not shown). N/P-ratios in mature leaves of *Peschiera* in the legume were almost double those found in the diverse plantings (Fig. 4a). N/P-ratios in freshly abscised leaves showed the same

response to planting mixture in all non-N₂-fixing species (Fig. 4b). By contrast, N₂-fixing *Enterolobium* had more than two-thirds higher N/P-ratios in abscised leaves than any non-N₂-fixer, regardless of treatment (Fig. 4b).

Discussion

Establishment of planting mixtures and N₂-fixers

Within 9 years of germination, both planting mixtures had attained more than 16-fold higher tree basal areas

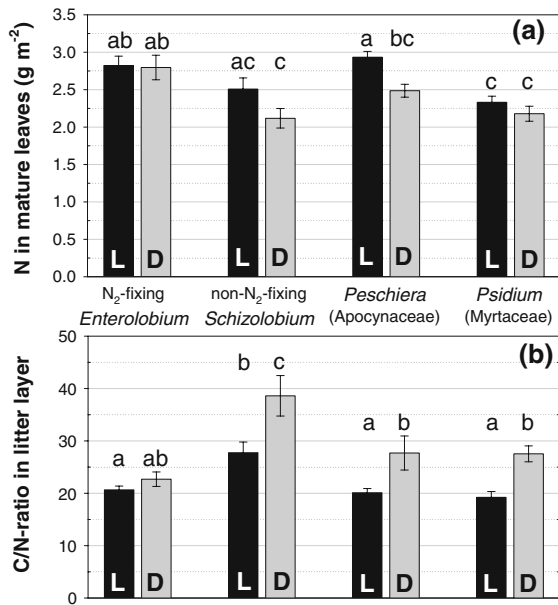


Fig. 3 Leaf N concentrations (a), and C/N-ratios in the litter layer (b) below the same individual trees 7 years after germination. L, Legume-dominated mixture; D, Diverse mixture. Error bars denote $\pm$ s.e.m. Means differ significantly from one another if they have no lower case letters in common (Fisher LSD *p* < 0.01)

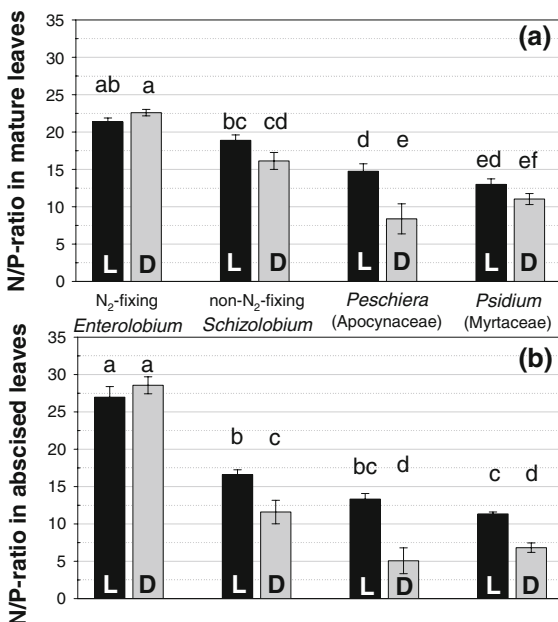


Fig. 4 N/P-ratios in mature leaves (a), and freshly abscised leaves (b) 7 years after germination. L, Legume-dominated mixture; D, Diverse mixture. Error bars denote $\pm$ s.e.m. Means differ significantly from one another if they have no lower case letters in common (Fisher LSD *p* < 0.05)

than in unplanted controls, despite the same initial site preparation and proximity of remnant semi-deciduous Atlantic Forest. This demonstrates that pre-planting site conditions had prevented natural forest regrowth, which could instead be initiated by planting or direct-seeding different species mixtures. In the legume-dominated mixture, substantially higher survival and growth of all four tree species occurring in both planting mixtures indicate better environmental conditions for these species of different nutritional characteristics (Table 1), than in the diverse mixture.

Differences in basal area estimates between planting mixtures indicate several-fold larger total biomass of N₂-fixing trees in the legume than in the diverse mixture. *Enterolobium* was the only N₂-fixing tree in the legume plantings, and the one with the highest biomass share in the diverse mixture amongst five other N₂-fixing tree species. Abundant active root nodules, high leaf N concentrations and low leaf proportional N resorption in both planting mixtures confirmed that *Enterolobium* had been actively fixing atmospheric N₂, irrespective of planting mixtures. However, the ¹⁵N-enriched leaves of *Enterolobium* in the legume mixture are inconclusive, since δ^{15} N values of non-N₂-fixing (reference) species were variable and similar to those of *Enterolobium*.

Nitrogen accumulation in planting mixtures

The legume mixture had consistently higher N concentrations in leaves, litter and available soil pools, and a much larger tree biomass, which demonstrates more rapid ecosystem N accumulation than in the diverse plantings. For instance, the legume mixture showed significantly higher mass-based N concentrations in mature leaves of both non-legumes, and especially in the litter layer below all studied species. Similarly, Binkley and Giardina (1997) reported 20–30% higher leaf N concentrations in 8-year-old *Eucalyptus saligna*, when planted in mixture with N₂-fixing *Albizia falcataria*, compared to its monoculture. However, in field experiments conducted in Puerto Rico, Parrotta (1999) reported no significant differences in foliar N concentrations in 3.5-year-old *Eucalyptus robusta* between single-species and 1:1 mixed stands with either of the N₂-fixing tree species *Leucaena leucocephala* or *Casuarina equisetifolia*. These contrasting results suggest that effects of N₂-fixing trees on leaf N status of inter-planted non-fixers depend on other

factors, potentially including species combinations, plantation age, edaphic or climatic conditions.

Approximately 6-fold higher levels of available soil nitrate detected in the legume mixture, compared to the diverse mixture, reflect high nitrification rates, probably due to lower ammonium immobilization capacity relative to the apparent high N mineralization in the legume mixture. Higher rates of N mineralization in the legume mixture are likely to be caused by the observed, markedly lower litter layer C/N-ratios than in the diverse mixture. Similarly, Forrester et al. (2005b) found highest rates of N release from litter in *Acacia mearnsii* monocultures, intermediate rates in mixture with *Eucalyptus globulus*, and lowest rates in *Eucalyptus* monocultures, which were accompanied by an equivalent pattern of N input from litterfall.

We observed an average ratio of resin- NO_3^- -N to resin- NH_4^+ -N of 8.9 in the legume mixture, contrasting with 0.8 in the diverse mixture. However, it remains to be investigated whether or not this reflected prolonged high gross nitrification and/or soil solution nitrate concentrations in the legume mixture, which could cause gaseous and/or leaching losses of N (Bigelow et al. 2004; Silver et al. 2005), with potential off-site environmental damage.

Resin-nitrate levels beneath *Peschiera* were significantly higher than beneath all other studied species within the legume mixture. Similarly, *Peschiera* leaves were more ^{15}N -enriched than all other species within the legume mixture. It remains to be investigated, whether this reflects preferential uptake of ammonium over nitrate by *Peschiera*, as the high enrichment factor of $^{15}\text{NH}_4^+$ relative to $^{15}\text{NO}_3^-$, demonstrated in similar soil type by Pérez et al. (2006), may suggest. However, this requires further research since the scope for interpreting plant N sources from $\delta^{15}\text{N}$ in leaves, litter and total soil alone is limited, due to ^{15}N fractionation processes occurring by several mechanisms at several levels (Robinson 2001; Evans 2001; Schmidt et al. 2006).

The observed high soil N availability in the legume mixture apparently down-regulated proportional N resorption in *Schizolobium*, a phenomenon reported only for few species (Güsewell 2004). However, consistent with our findings, Khanna (1997) reported higher N concentrations in senescent foliage of *Eucalyptus globulus* already after

25 months, when grown in mixture with *Acacia mearnsii*, compared to *Eucalyptus* monocultures.

Phosphorus relations in planting mixtures

Leaf and litter P concentrations did not differ significantly between planting mixtures, due to high variability in *Peschiera* in the diverse mixture. However, despite this high variability, both non-legumes tripled proportional P resorption in the legume mixture, indicating increased internal P conservation, relative to the diverse plantings. This may suggest that both non-legumes down-regulate P resorption in the diverse mixture, presumably associated with adequate P supply (Güsewell 2004). Substantially higher N/P-ratios in abscised leaves of all non- N_2 -fixers in the legume mixture indicate adjustment of internal P relative to N recycling, which reflects the contrasting supply ratios observed between the planting mixtures.

By contrast, Aerts (1996) argued that proportional nutrient resorption is only weakly controlled by nutrient availability, and is often similar for N and P. Yet, the present data indicate that proportional P resorption in the non-legumes, and proportional N resorption in the non- N_2 -fixing legume, may indeed respond to variation in competition for these nutrients. These patterns suggest that proportional resorption of N versus P may indeed diverge within a species in response to N accumulation and associated increasing competition for P, even in the absence of soil fertility differences related to pedology or land use history. These differences were of a similar magnitude as inherent inter-specific differences.

Consistency among species with contrasting nutritional characteristics

The divergence in nutrient relations between planting mixtures is supported by the consistency in responses of litter and soil nutrients beneath all four species shared by both mixtures, although significant inter-specific differences in nutrient concentrations and SLA were observed. This consistent divergence between mixtures also extended to leaf nutrient relations in the three non- N_2 -fixing species that showed plasticity in leaf nutrient relations.

N_2 -fixing *Enterolobium contortisiliquum*, on the other hand, was characterized by a lack of plasticity

in leaf nutrient relations, with consistently high leaf N and low P concentrations and the highest proportional P resorption. This supports the notion that this species (Moreira and Siqueira 2002), and more generally N₂-fixing trees have a P-limited physiology (Vitousek et al. 2002).

Non-N₂-fixing *Schizolobium parahyba* had consistently high leaf N concentrations and N/P-ratios, which reflects the high inherent N use physiology of legumes (McKey 1994). Yet, its complete reliance on combined soil N is reflected by its plasticity in proportional N resorption in response to contrasting soil N availability between planting mixtures.

Peschiera fuchsiaefolia showed very high leaf N and P concentrations and proportional resorption, which reflect its nutrient-intensive, yet conservative, evergreen physiology. Its pronounced responses to planting mixtures in leaf N (and P) concentrations, N/P-ratios, and relative proportional resorption indicate highly plastic adjustment to available nutrient ratios. The competitive P acquisition of *Peschiera* is likely to negatively impact on the P supply to other species, if it attains substantial biomass proportions in a N₂-fixer-dominated community. By contrast, P mineralization and availability to adjacent species could be enhanced where species mixtures are nutritionally complementary (Forrester et al. 2005b).

Psidium guajava had relatively low leaf nutrient concentrations and SLA, suggesting a 'nutrient-efficient', evergreen physiology, although proportional N and P resorption were also low. Increased mass-based, but not area-based leaf N concentrations in the legume mixture suggest that this species has some nutritional plasticity.

Conclusions

Rapid forest recovery was initiated by direct seeding of trees or planting of seedlings, where natural forest regrowth had been inhibited for over 15 years. We show that different tree species mixtures markedly diverged in N and P relations. Although these results are based on a single study area, they demonstrate the potential for rapid changes caused by plantings with contrasting relative tree species composition.

Within 7 years, dominance of a single N₂-fixing tree species in the legume mixture caused faster biomass accumulation as well as higher N

concentrations in biomass and litter, than the diverse mixture with a low density of N₂-fixers. In the legume mixture, several-fold higher soil nitrate and reduced soil P availabilities were associated with consistently higher leaf N concentrations and more conservative leaf P relations in responsive species. This suggests that the legume mixture has rapidly increased its N/P-supply ratio relative to the diverse mixture.

We propose that incorporation of species with complementary nutritional characteristics may assist early tree survival and optimize biogeochemical effects of mixed plantings. For instance, species which efficiently mobilize P from resistant soil pools could reduce competition for soil P. Furthermore, species with the capacity to take up soil nitrate at high rates could enhance N use and retention on-site in N₂-fixer-dominated plantings.

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