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Symbiotic nitrogen fixation in a tropical rainforest: ^{15}N natural abundance measurements supported by experimental isotopic enrichment

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Summary

• Leguminous trees are very common in the tropical rainforests of Guyana. Here, species-specific differences in N_2 fixation capability among nodulating legumes growing on different soils and a possible limitation of N_2 fixation by a relatively high nitrogen (N) and low phosphorus (P) availability in the forest were investigated.

• Leaves of 17 nodulating species and 17 non-nodulating reference trees were sampled and their $\delta^{15}N$ values measured. Estimates of N_2 fixation rates were calculated using the ^{15}N natural abundance method. Pot experiments were conducted on the effect of N and P availability on N_2 fixation using the ^{15}N-enriched isotope dilution method.

• Nine species showed estimates of > 33% leaf N derived from N_2 fixation, while the others had low or undetectable N_2 fixation rates. High N and low P availability reduced N_2 fixation substantially.

• The results suggest that a high N and low P availability in the forest limit N_2 fixation. At the forest ecosystem level, N_2 fixation was estimated at *c.* 6% of total N uptake by the tree community. We conclude that symbiotic N_2 fixation plays an important role in maintaining high amounts of soil available N in undisturbed forest.

Key words: leguminous trees, N_2 fixation, ^{15}N enrichment, ^{15}N natural abundance, nitrogen (N) availability, nodulation, phosphorus (P) availability, tropical rainforest.

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Introduction

Tropical forests are often growing on a substrate poor in mineral nutrients. However, nitrogen (N) is generally considered to be available in larger amounts in undisturbed systems (Vitousek & Sanford, 1986). An efficient cycling of N is thought to be responsible for the relatively high N status. A continued supply of N through biological N_2 fixation is another factor supposed to be involved in maintaining large N pools in undisturbed forests and rebuilding those after disturbance (Vitousek *et al.*, 2002). The Leguminosae that form a symbiosis with rhizobia in root nodules are effective in fixing N_2 in terrestrial systems. In addition to symbiotic N_2 fixation, phyllospheric cyanobacteria and lichens, and rhizospheric bacteria can also play an important role (Cleveland *et al.*, 1999; Vitousek *et al.*, 2002). The Leguminosae family is abundant in the forests of the Neotropics, particularly in the Guianas (ter Steege *et al.*, 2000a; Hammond, 2005). However, not all legumes are able to establish an effective symbiosis with rhizobia, or may not do so under natural conditions (de Souza Moreira *et al.*, 1992).

Several inventories have been carried out in Neotropical forests that have identified the nodulation of leguminous trees (de Souza Moreira *et al.*, 1992; de Faria & de Lima, 1998; Roggy & Prevost, 1999). Caesalpinioideae, when the Swartziae are not included in this subfamily, which have the largest

number of plant species in these forests, have the lowest percentage of nodulating species, whereas the Mimosoideae and the Papilionoideae subfamilies have larger percentages of nodulating species. Legumes, in particular the caesalpinioids, can make up > 50% of all trees (ter Steege *et al.*, 1993; ter Steege *et al.*, 2000a). Perreijn (2002) investigated the nodulation of a broad range of leguminous species occurring in a tropical forest in central Guyana. Large nodules were found on some caesalpinioid, several mimosoid and almost all papilionoid species. These species showed nitrogenase activity and contained rhizobial DNA, but smaller (< 2 mm) nodule-like structures found on several caesalpinioids failed to show nitrogenase activity (Perreijn, 2002). Although nodulating species are capable of N_2 fixation, the significance of N_2 fixation as a source of the N supply to the tree and ultimately the N economy of the forest ecosystem remains to be investigated.

The ^{15}N natural abundance method can be used to estimate the rate of the N_2 fixation process *in situ* (Shearer & Kohl, 1986; Bremer & van Kessel, 1990; Boddey *et al.*, 2000). However, this method can only be used when certain conditions are met. There should be a sufficiently large difference in the $\delta^{15}N$ values between soil available N and atmospheric N_2. Soil N is often slightly enriched in ^{15}N as a result of fractionation during microbial processes such as denitrification (Högberg, 1997). The method further relies on the identification of suitable non-N_2-fixing reference species that utilize the same source of soil N as the N_2-fixing species. The ^{15}N natural abundance method would be the most appropriate method to estimate N_2 fixation in mature trees when these conditions are met (Boddey *et al.*, 2000) and has been successfully used in similar forests in French Guiana and Brazil (Sprent *et al.*, 1996; Roggy *et al.*, 1999a; Kreibich *et al.*, 2006). Nevertheless, assumptions must be made when applying the method. Only semiquantitative estimates of N_2 fixation can thus be made rather than the measurement of exact rates.

N_2 fixation can be limited by environmental factors, and estimates may not reflect the capacity of a species to fix N_2. The supposedly high N availability in undisturbed forest can down-regulate N_2 fixation (van Kessel & Roskoski, 1983; Hartwig, 1998). Low availability of phosphorus (P) is prevailing in the Fe- and Al-oxide-rich soils found in central Guyana where the present study was carried out (Vitousek & Sanford, 1986; Brouwer, 1996; van Kekem *et al.*, 1996). As N_2-fixing species have often a high P requirement (Israel, 1987), N_2 fixation would be limited under low amounts of available P. However, little information is available on the significance of a putative high N and low P status of the soil for N_2 fixation in tropical rainforests.

The objectives of the study were to identify species-specific differences in N_2 fixation activity and possible differences between trees growing on different soil types. The questions as to whether the putatively low P and high N availability limits N_2 fixation rates in the forest and what the quantitative significance of the process is at the ecosystem level were also addressed. The study was carried out in an undisturbed tropical rainforest in central Guyana. Leaves of mature nodulating and non-nodulating leguminous and nonleguminous trees were sampled on different soil types and their $\delta^{15}N$ values were measured. These data were used to estimate N_2 fixation rates in four of the five soil types where conditions for the application of the ^{15}N natural abundance method were met. To investigate the effect of N and P availability on N_2 fixation, three nodulating tree species were grown on ^{15}N-enriched soil. The data on N_2 fixation rates, in combination with available data on tree leaf biomass and leaf turnover rates, were used to estimate the significance of symbiotic N_2 fixation at the forest ecosystem level.

Materials and Methods

Study area

The study was carried out in a 9 km^2 area of pristine tropical rainforest amidst a similar but selectively logged forest *c.* 20 km south of the Mabura Hill Township in central Guyana (5°13′N, 58°4′W). The site is locally known as the Mabura Hill forest reserve. Mean annual precipitation is 2700 mm and the mean annual temperature is 27°C (Jetten, 1994). Soil types in the area are sandy soils belonging to the Berbice formation, white sands (albic Arenosol) and brown (loamy) sands (ferralic Arenosol and haplic Ferralsol), lateritic gravelly clays (xanthic Ferralsol and dystric Leptosol) and alluvial creek deposits (dystric Fluvisol) (van Kekem *et al.*, 1996). The forest communities on the different soils were described by ter Steege *et al.* (1993, 2000b).

Leaf analysis of forest trees

Leaves were sampled in 1998 from mature trees of leguminous species of which nodulation status was known (Perreijn, 2002). Additional sampling was carried out for the most common nonleguminous species (Table 1). At least three mature leaves from the central part of the tree crown were collected by using a shotgun. Samples were cleaned and dried, and leaves from one crown were pooled for further analysis. Three soil samples were taken in the vicinity of sampled trees, pooled, dried and sieved. Sampling was to a depth of 12 cm after removal of coarse litter, since organic matter and nutrients are concentrated superficially (van Kekem *et al.*, 1996). This layer was considered most representative for characterizing nutrient availability for comparative purposes. The number of samples per species and soil type is given in Table 1. Analyses of P and N concentration, and ^{15}N isotopic composition were performed on ground leaf material. Soil was analysed for concentration of total N and its ^{15}N isotopic composition. Other soil parameters were derived from van Kekem *et al.* (1996).

To determine the N concentration and $\delta^{15}N$ value of the leaves, a subsample was ground to fine powder in a ball mill.

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Table 1 Species used in this study with annotation on the occurrence of nodules and nodule-like structures on the roots (Perreijn, 2002)

	Nodulation	Nodule-like structures	Number of samples				
			White sand	Brown sand	Alluvial	Laterite F	Laterite L
Leguminosae							
Caesalpinioideae							
Chamaecrista adiantifolia (Benth.) Irwin & Barneby var. *pteridophylla* (Sandw.) Irwin & Barneby	+	+	9	8	–	–	–
Chamaecrista apoucouita (Aubl.) Irwin & Barneby	+	–	–	13	7	3	7
Dicymbe altsonii Sandw.	–	–	3	10	–	–	–
Eperua falcata Aubl.	–	+	9	10	–	–	–
Eperua grandiflora (Aubl.) Benth. ssp. *guyanensis* R.S. Cowan	–	+	10	–	–	–	–
Eperua rubiginosa Miq.	–	+	–	–	6	–	–
Hymenaea courbaril L. var. *courbaril*	–	+	–	5	5	–	–
Mora excelsa Benth.	–	+	–	–	13	–	–
Mora gonggrijpii (Kleinh.) Sandw.	–	–	–	12	10	5	6
Sclerolobium guianense Benth. var. *guianense*	+	–	–	7	–	–	–
Vouacapoua macropetala Sandw.	–	+	–	7	–	3	8
Mimosoideae							
Abarema jupunba (Willd.) Briton & Killip var. *trapezifolia* (Vahl) Barneby & Grimes	+	–	–	4	–	–	–
Balizia pedicilaris (DC) Barneby & Grimes	+	–	–	5	–	–	–
Inga spp.	+	–	–	11	–	–	–
Parkia nitida Miq.	–	–	–	3	–	–	–
Pentaclethra macroloba (Willd.) Kuntze	+	+	–	18	9	4	7
Papilionoideae							
Clathropis brachypetala (Tul.) Kleinh.	+	–	–	17	–	3	7
Clathropis macrocarpa Ducke	+	–	–	–	6	3	7
Dioclea elliptica R.H. Maxwell	+	–	–	3	–	–	–
Diplotropis purpurea (Rich.) Amsh. var. *pupurea*	+	–	–	7	–	–	–
Ormosia coccinea (Aubl.) Jacks.	+	–	–	3	–	–	–
Ormosia coutinhoi Ducke	+	–	15	10	–	–	–
Pterocarpus officinalis Jacq. ssp. *officinalis*	+	–	–	–	5	–	–
Swartzia jenmanii Sandw. and *S. schomburgkii* Benth. var. *schomburgkii*	+	–	–	10	–	3	8
Swartzia leiocalycina Benth.	+	–	–	19	6	3	6
Swartzia oblanceolata Sandw.	+	–	–	6	7	–	–
Non-Leguminosae							
Aspidosperma excelsum Benth. Apocynaceae	–	–	8	14	–	–	–
Catostemma fragrans Benth. Bombaceae	–	–	5	–	–	–	–
Licania buxifolia Sandw. Chrysobalanacaea	–	–	–	11	–	–	–
Tovomita grata Sandw. Guttifereae	–	–	4	–	–	–	–
Chlorocardium rodiei (Schomb.) Rohwer, Richter & van de Werff, Lauraceae	–	–	–	21	–	4	8
Eschweilera sagotiana Miers Lecythidaceae	–	–	–	6	–	–	–
Carapa guianensis Aublet Meliaceae	–	–	–	–	8	3	7
Talisia sqarrosa Radlk. Sapindaceae	–	–	9	–	–	–	–
Soil			31	53	37	12	25

Number of samples for nitrogen (N) and phosphorus (P) concentration and $\delta^{15}N$ in leaves and soil are specified per soil type. Soil types were white sand (albic Arenosols), brown (loamy) sand (ferralic Arenosol and haplic Ferralsol), alluvial creek deposits (dystric Fluvisol), and two gravelly lateritic clays abbreviated as laterite F (xanthic Ferralsol) and laterite L (dystric Leptosol).

Dried soil samples were ground in a mortar. Samples were packed in tin capsules and analysed for N concentration and $\delta^{15}N$ on a CN analyser in tandem with a continuous-flow isotope ratio mass spectrometer (Europa ANCA-GSL, PDZ Europa Ltd, Sandbach, UK) at the UC Davis Stable Isotope Facility. The $\delta^{15}N$ values were expressed relative to atmospheric N_2.

To determine the P concentration in leaves, a subsample of approx. 300 mg was ashed at 550°C in porcelain vessels. Ash was treated with 5% HCl and dissolved in distilled H_2O at 90°C. Concentration of P was measured in a continuous-flow analyser (Skalar SA-40, Skalar, Breda, the Netherlands).

The percentage of plant N derived from atmospheric N_2 (%Ndfa) based on the natural variation in the abundance of ^{15}N in the forest was calculated according to Shearer & Kohl (1986):

$$Ndfa = (\delta^{15}N_{ref} - \delta^{15}N_{nod})/(\delta^{15}N_{ref} - \delta^{15}N_{fix})$$

($\delta^{15}N_{ref}$, $\delta^{15}N$ of non-fixing reference plants; $\delta^{15}N_{nod}$, $\delta^{15}N$ of the nodulating and potentially N_2-fixing plant; $\delta^{15}N_{fix}$, $\delta^{15}N$ of fixed N_2). For $\delta^{15}N_{fix}$ we used a value of −1.7‰, as reported earlier by Yoneyama *et al.* (1993) and Sprent *et al.* (1996) for Neotropical woody species. Nonnodulating Legumes and nonleguminous species were used as reference plants on each soil type.

Pot experiments

Two pot experiments were carried out on nodulation and N_2 fixation: the first (Expt 1) investigated the effect of N availability and the second (Expt 2) investigated the effect of P availability. The enriched ^{15}N isotope dilution method was used to quantify N_2 fixation. In Expt 1, three nodulating legumes were included (*Pentaclethra macroloba*, *Sclerolobium guianense* and *Chamaecrista apoucouita*) with *Dycimbe altsonii* and *Eperua falcata* as reference species. In Expt 2, *P. macroloba* and *S. guianense* were used with *Carapa guianensis* as a reference species. Treatments were replicated five times in Expt 1 and eight times in Expt 2.

Small saplings were excavated from the forest and nodulating plants were planted in 10 l pots. Sieved topsoil (brown sand) collected from the forest was used as a substrate. To prevent soil compaction, Perlite (20% by volume) was added to the soil in Expt 1. To create a low nutrient status in Expt 2, an additional 60% creek sand was added. Nutrients were supplied as slow-release fertilizers (Osmocote, Scotts Europe BV, De Meern, the Netherlands) and mixed throughout the upper soil layer following planting. All essential nutrients minus N in Expt 1 and minus N and P in Expt 2 were supplied in nonlimiting amounts. Calcium and magnesium carbonate were applied to prevent acidification. Plants received 9 g N in the high N treatment of Expt 1 and 0.5 g P in the high P treatment of Expt 2. The plants were grown in a nursery covered with transparent plastic and a shade screen that transmitted 20% of full daylight. Pots were irrigated daily as required.

Rates of N_2 fixation were estimated by the ^{15}N isotope dilution method (Parrotta *et al.*, 1994). A solution of 50.4 mg N applied as $(NH_4)_2SO_4$ at 10.01 atom% ^{15}N excess and 4 g sucrose was added to each pot. Sucrose was added to stimulate bacterial immobilization of ^{15}N followed by a gradual release. In Expt 1, the ^{15}N was applied 12 months after planting. In Expt 2, half of the ^{15}N was applied directly after planting and the other half 4 months later.

Five plants per species and treatment combination were harvested at 15.5 months after planting in Expt 1. A similar approach was adopted in Expt 2, where the first harvest was at the time of planting. Final harvest was at 8 months after planting for Expt 2. The $\delta^{15}N$ and plant N concentration in dry matter were measured as already described. The %Ndfa was calculated for the experimental period as described by Fried & Middleboe (1977) and Parrotta *et al.* (1994):

$$\%Ndfa = (1 - {}^{15}Nae_{nod}/{}^{15}Nae_{ref}) \times 100$$

($^{15}Nae_{nod}$, percentage ^{15}N atomic excess of N taken up by the nodulating legume between the start of the experiment and the end harvest; $^{15}Nae_{ref}$ is the same parameter for the nonnodulating reference plant).

Statistical analysis

Comparison of $\delta^{15}N$ values of nodulating species with the two reference groups was carried out with the nonparametric Mann–Whitney *U*-test. Estimates of %Ndfa calculated with the two reference groups were compared with an unpaired *t*-test. Soil and leaf N concentrations and log-transformed P concentrations were compared among groups with ANOVA. Means were compared *post hoc* with a Tukey HSD test in case of homogeneity of variance, otherwise the Tamhane T2 test was used. The factorial pot experiments were analysed by two-way ANOVA.

Results

N and P concentrations and $\delta^{15}N$ of forest tree leaves

Leaves of the leguminous species for which the nodulation was known from an earlier inventory (Table 1) and of a selection of nonleguminous species were sampled from 494 mature trees on the six soil types. As the $\delta^{15}N$ values of soil and the nonleguminous trees were not significantly different for the two types of brown sand (ferralic Arenosol and haplic Ferralsol), samples from these two types were combined for analysis. However, the $\delta^{15}N$ values were significantly different for the two types of laterite, i.e. xanthic Ferralsol and dystric Leptosol, and are treated as separate categories.

Leaf P concentrations were relatively low and there was a substantial variation within species (average CV = 2%), similar to the interspecific variation of means per species. When

Table 2 Phosphorus (P) and nitrogen (N) concentrations in leaf dry matter and the $\delta^{15}N$ of leaf N averaged by functional group and soil type

Soil type	Nodulating legumes	Non-nodulating legumes	Nonlegumes
[P] (mg g^{-1})			
White sand	0.47 ± 0.14 B a	0.55 ± 0.14 A a	0.46 ± 0.08 A a
Brown sand	0.60 ± 0.15 AB a	0.57 ± 0.20 A a	0.49 ± 0.15 A a
Alluvial	0.74 ± 0.40 A a	0.55 ± 0.12 A a	0.69 ± 0.25 A a
Laterite F	0.64 ± 0.22 AB a	0.56 ± 0.19 A a	0.52 ± 0.11 A a
Laterite L	0.59 ± 0.14 AB a	0.51 ± 0.09 A a	0.54 ± 0.15 A a
[N] (mg g^{-1})			
White sand	16.1 ± 2.6 B a	14.7 ± 1.8 B ab	13.8 ± 2.2 B b
Brown sand	22.4 ± 4.8 A a	16.2 ± 2.4 A c	17.9 ± 4.0 A b
Alluvial	25.5 ± 4.1 A a	14.3 ± 1.6 B b	13.9 ± 1.7 B b
Laterite F	23.5 ± 4.5 A a	16.5 ± 2.6 AB b	17.5 ± 2.9 AB b
Laterite L	24.1 ± 3.7 A a	17.1 ± 2.5 A b	18.6 ± 4.8 A b
$\delta^{15}N$ (‰)			
White sand	–2.45 ± 0.37 C a	–2.71 ± 1.23 D a	–2.96 ± 2.50 D a
Brown sand	0.68 ± 1.61 B c	1.87 ± 1.25 C b	2.91 ± 1.04 C a
Alluvial	0.84 ± 1.40 B c	2.03 ± 1.07 C b	3.93 ± 0.69 B a
Laterite F	1.17 ± 1.23 B b	2.87 ± 0.91 B a	3.23 ± 0.73 C a
Laterite L	3.91 ± 1.58 A b	4.77 ± 1.15 A ab	5.23 ± 0.73 A a

Values are mean of all individuals ± SD.
Different lower-case letters within soil types denote significant differences between functional groups; different upper-case letters within functional groups denote significant differences between soil types ($P < 0.05$).

Table 3 Chemical soil properties of the five soil types for the upper 12 cm

	Measured	Data from van Kekem *et al*. (1996)			
Soil type	Total N (mg g^{-1})	Total N (mg g^{-1})	Organic C (%)	Available P (mg kg^{-1})	CEC ($cmol^{+}$ kg^{-1})
White sand	0.59 ± 0.13	0.7	1.4	1.4	4.5
Brown sand	1.12 ± 0.27	1.2	1.7	1.3	5.3
Alluvial	2.93 ± 0.99	3.2	3.8	2.2	11.1
Laterite F	1.98 ± 0.28	1.1	1.7	2.4	4.8
Laterite L	2.83 ± 0.86	2.9	4.4	5.0	10.6

Total nitrogen (N) concentration presented in the first data column was measured on the samples used in the present study (mean ± SD). The other data of total N, organic C, available phosphorus (P) and cation exchange capacity (CEC) were adapted from the soil survey of van Kekem *et al*. (1996) for the area around the study site (south of Mabura Hill between the Demerara and Essequibo rivers).

averaged over soil type and functional group (nodulating legumes, non-nodulating legumes and nonlegumes), the differences were less pronounced, with a tendency towards lower concentrations of P in white sand tree leaves (mean for all species, 0.49 mg g^{-1}) relative to the leaves sampled on other soil types, particularly the alluvial soil (0.66 mg g^{-1}). The differences in the concentration of leaf P between these soil types were only significant for nodulating legumes, but not for the non-nodulating legumes and nonlegumes (Table 2). With some exceptions, such as *Pterocarpus officinalis* (1.28 mg g^{-1}), nodulating legumes did not show a higher P concentration compared with the non-nodulating legumes or nonlegumes (Table 2).

Leaf N concentrations were significantly higher for nodulating legumes (mean across soil types, 22.3 mg g^{-1}) compared with non-nodulating legumes (15.8 mg g^{-1}) and nonleguminous species (16.3 mg g^{-1}) (Table 2). Concentrations of N were lowest for trees on nutrient-poor white sands (mean for all species, 14.9 mg g^{-1}), particularly for nodulating legumes. The upper 12 cm of this soil type also had the lowest soil N concentration (i.e. 0.59 mg g^{-1}), but, overall, leaf N was not clearly related to total soil N (Tables 2 and 3).

Soil was enriched with ^{15}N, except for white sand soil that had a $\delta^{15}N$ value of 0.6‰ (Fig. 1). Leaves of trees were depleted in ^{15}N relative to the soil on which they were growing. For nonleguminous species, the depletion was systematically *c.* 2.8‰, resulting in a close relationship between soil $\delta^{15}N$ and leaf $\delta^{15}N$ of non-leguminous species (Fig. 1). A similar relationship was observed for non-nodulating legumes (Table 2) with an average depletion of 3.4‰. Soil $\delta^{15}N$ was thus

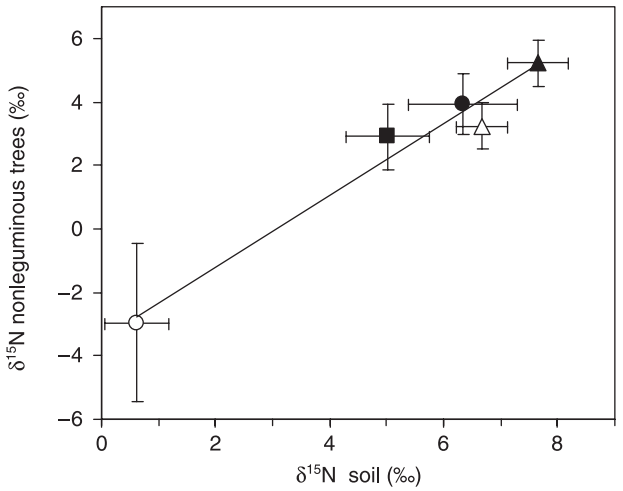

Fig. 1 Relationship between $\delta^{15}N$ values of leaf nitrogen (N) of nonleguminous trees and soil N collected near the sampled trees (upper 12 cm). Means (± SD) are shown per soil type: white sand, open circle; brown sand, square; alluvial creek deposits, closed circle; laterite F, open triangle; laterite L, closed triangle. Sample frequencies, species names and soil classification names are given in Table 1. Linear regression, $r^2 = 0.97$.

reflected in N isotopic composition of non-nodulating species, legumes and nonlegumes. Although variability was observed for leaf $\delta^{15}N$ sampled on white sand, that variability was not associated with the three functional groups identified (Table 2, Fig. 2). As the forest on brown sand occupied the largest segment of the study area, it was sampled the most frequently with respect to species and individual trees (Table 1). Trees on this soil type showed the lowest $\delta^{15}N$ values for nodulating species, intermediate $\delta^{15}N$ values for non-nodulating legumes and the highest $\delta^{15}N$ values for nonlegumes (Table 2, Fig. 2). Leaf $\delta^{15}N$ of nodulating legumes on brown sand ranged from −1.6‰ in *Ormosia coutinhoi* to +2.9‰ in an unidentified *Inga* species. The three highest values were not significantly different from the nonleguminous species, but all other nodulating legumes showed significantly lower $\delta^{15}N$ values (Table 4, Fig. 2). Significant differences in $\delta^{15}N$ with non-nodulating legumes were found in nine of the 15 nodulating species (Table 4, Fig. 2). Similarly, on alluvial soil along creeks and on the two lateritic soils, the lowest $\delta^{15}N$ values were found for nodulating legumes with substantial variation between species and higher $\delta^{15}N$ values for non-nodulating species (Table 4, Fig. 2). Such differences in ^{15}N abundance between functional groups in four of the five soil types can potentially be used to infer the source of N nutrition of trees.

Estimates of N_2 fixation in forest trees

To be able to estimate N_2 fixation by the ^{15}N natural abundance method, it is required that the non-nodulating reference species has a $\delta^{15}N$ value ($\delta^{15}N_{ref}$) which is significantly different from zero, that variation in $\delta^{15}N$ between reference species is relatively small, and that $\delta^{15}N$ values of nodulating legumes ($\delta^{15}N_{nod}$) are between $\delta^{15}N_{fix}$ and $\delta^{15}N_{ref}$ (Shearer & Kohl, 1986; Högberg, 1997; Boddey *et al.*, 2000). Non-nodulating legumes and nonlegumes are potential reference plants for the estimation of %Ndfa from $\delta^{15}N$ values and can thus be used to derive values for $\delta^{15}N_{ref}$. Two previous studies with Neotropical forest trees used a value for $\delta^{15}N_{fix}$ of −1.7‰ (Yoneyama *et al.*, 1993; Sprent *et al.*, 1996). Excluding forest on white sand, most $\delta^{15}N$ values for nodulating legumes on the other four soil types ranged between that for the non-nodulating reference species and this value of −1.7‰ (Fig. 2). The conditions mentioned in the Introduction for successful application of the ^{15}N natural abundance method for estimating N_2 fixation were thus reasonably well fulfilled for trees on brown sand, alluvial soil and the two lateritic soils, but not for trees growing on white sand (Table 2, Fig. 2). The isotope data for trees on white sand could thus not be used for estimating N_2 fixation. Estimates for N_2 fixation were only performed for the nodulating legumes on the four remaining soils. Since $\delta^{15}N$ of non-nodulating legumes was significantly lower than that of nonlegumes on two soils (Table 2), we made separate %Ndfa estimates with the two non-nodulating groups as reference.

As the $\delta^{15}N$ values of the nodulating species covered the entire range between the $\delta^{15}N$ value of non-nodulating species ($\delta^{15}N_{ref}$) on each soil and the $\delta^{15}N_{fix}$ value of −1.7‰ (Fig. 2), the estimated values for %Ndfa varied between zero and 100% depending on species and soil type (Table 4). The higher $\delta^{15}N$ of some nodulating species compared with the mean of the non-nodulating legumes (Table 2, Fig. 2) resulted in negative estimates of %Ndfa. These were recorded as zero (Table 4). With the exception of the dystric Leptosol soil, the %Ndfa was not very different between the different soil types (Table 4). Low %Ndfa values were found for the three nodulating caesalpinioids: 9–24% as a mean across soil types and calculation scenarios. The estimates for %Ndfa for the nodulating mimosoids ranged from 0% for *Inga* spp. to 49% for *Pentaclethra macroloba*. Some of the 10 papilionoids, such as *Ormosia coutinhoi* and *Swartzia oblanceolata*, showed high %Ndfa estimates of almost 100%, whereas other species, such as *Swartzia leiocalycina* (16%) and *Clathropis macrocarpa* (22%), showed lower values for %Ndfa. The liana *Dioclea eliptica* also showed high N_2-fixing activity (Table 4). These results provide evidence that high rates of N_2 fixation (%Ndfa > 33) can be achieved by nine of the 17 investigated nodulating legumes in undisturbed rainforest of central Guyana.

Nitrogen fixation in pot experiments

Expt 1 was carried out to estimate the potential of N_2 fixation under favourable growing conditions of high availability of all nutrients except N and to establish a possible reduction of N_2 fixation activity at high availability of soil N. In order

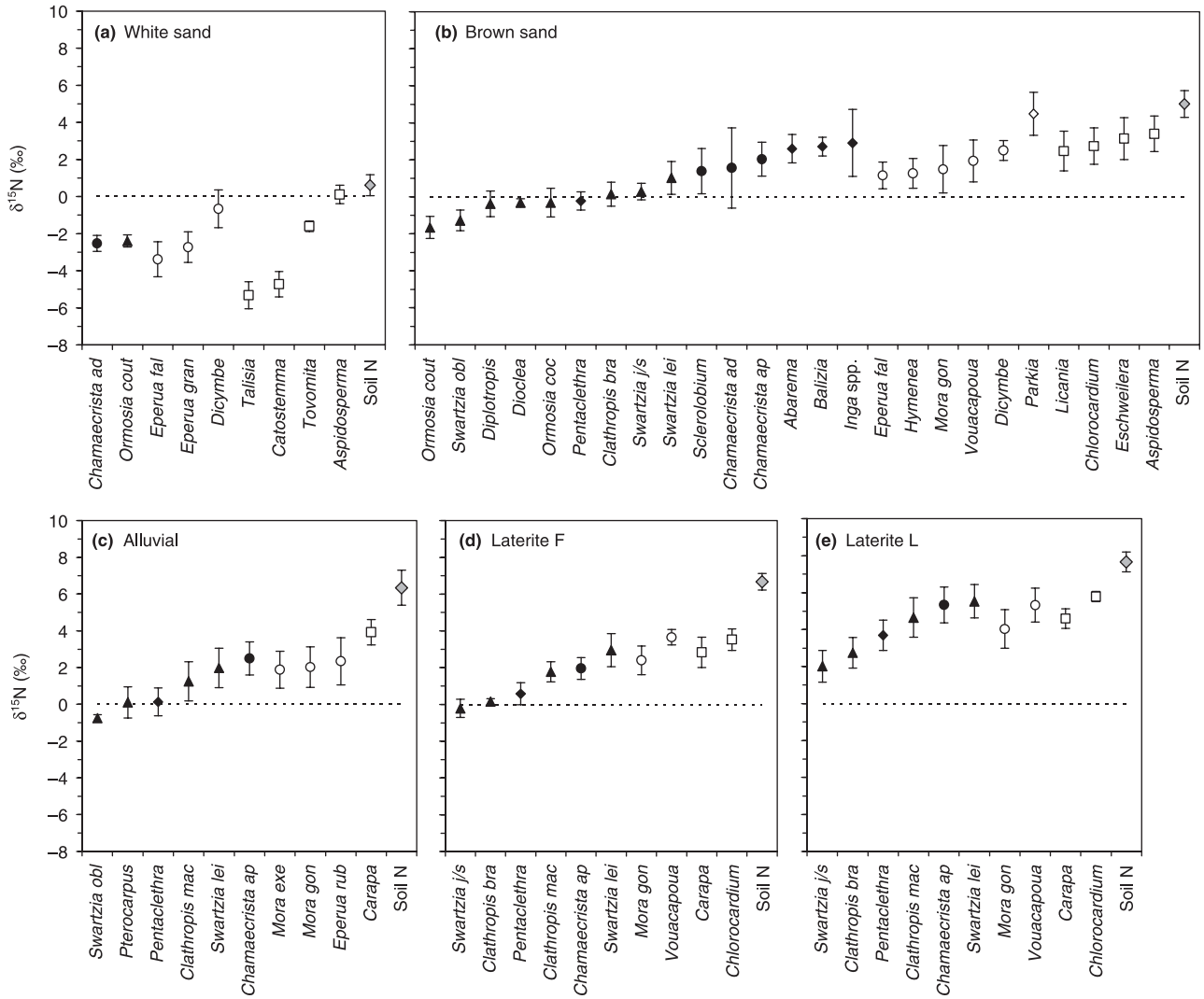

Fig. 2 The $\delta^{15}N$ values of leaves of 34 woody species sampled from trees growing on the five soil types. Species are arranged in order of ascending $\delta^{15}N$ value per soil type within each functional group of nodulating legumes (●, ◆, ▲), non-nodulating legumes (○, ◇) and nonleguminous species (□). Legumes are classified as Caesalpinioideae (●, ○), Mimosoideae (◆, ◇) and Papilinoidae (▲). Each panel represents one soil type: (a) white sand, (b) brown sand, (c) alluvial creek deposits, (d) laterite F, and (e) laterite L. Means (± SD) are shown per species and soil type. Sample frequencies, full species names and soil classification names are given in Table 1.

to investigate possible species-specific differences in these parameters as well, the nodulating species *Pentaclethra macroloba*, *Sclerolobium guianense* and *Chamaecrista apoucouita* were selected for their different estimates of N_2 fixation (Table 4). The two reference species used in this experiment had similar ^{15}N atomic excess percentages and N uptake rates in the two treatments and thus gave similar estimates for %Ndfa. The average of the two estimates is presented in Fig. 3. The results show clearly that *P. macroloba* and *S. guianense* derived almost all their N from atmospheric N_2 at low soil N availability, whereas %Ndfa was reduced to *c.* 40% at high N (Fig. 3). Both species had similar increases in biomass under low and high N availability during the experimental period (Table 5). Nevertheless, *S. guianense* had a lower N uptake in the low-N treatment, as is evident from its lower N concentration (Table 5). The increased N_2 fixation at low N thus compensated fully for the reduced availability of soil N in the case of *P. macroloba* but not for *S. guianense. C. apoucouita* derived about half of its N from N_2 at low N, but that was reduced to nondetectable rates at high soil N availability (Fig. 3). Nodulation was substantial at low N but negligible at high N in this species (Fig. 3), consistent with the effect on N_2 fixation. Contrary to the other two species, biomass increment and N acquisition were strongly reduced in *C. apoucouita* at low compared with high soil N availability, as was the case with the two reference species (Table 5). In this species, N_2

Table 4 Estimates of the percentage leaf nitrogen (N) derived from atmospheric N_2 fixation (%Ndfa) for the nodulating species sampled on four soil types

	Brown sand		Alluvial clay		Laterite F		Laterite L		
	Non-nodulating legumes	Nonlegumes	Non-nodulating leg	Nonlegumes	Non-nodulating legumes	Nonlegumes	Non-nodulating legumes	Nonlegumes	Mean of all estimates
Caesalpinioideae									
Chamaecrista adiantifolia	9 ns	29							19
Chamaecrista apoucouita	0 ns	19*	0 ns	25*	0 ns	26	0 ns	0 ns	11
Sclerolobium guianense	14 ns	33							23
Mimosoideae									
Abarema jupunba	0 ns	7 ns							3
Balizia pedicilaris	0 ns	4 ns							2
Inga spp.	0 ns	0 ns							0
Pentclethra macroloba	59	68	51	67	50	54	17	22	48
Papilionoidae									
Clathropis brachypetala	49	60			59	62	31	36	44
Clathropis macrocarpa			21 ns	47	24	30	2 ns	8 ns	22
Dioclea elliptica	61	70							66
Diplotropis purpurea	63	71							67
Ormosia coccinea	61	70							66
Ormosia coutinhoi	99	99							99
Pterocarpus officinalis			51	68					60
Swartzia jenmanii/ schomburgkii	44	57*			68	70	42	46	55
Swartzia leiocalycina	24	41*	1 ns	35	20 ns	6 ns	0 ns	0 ns	13
Swartzia oblanceolata	88	91	75	83*					84

The mean $\delta^{15}N$ per group of reference species and per soil type (Table 2) was used for the calculations. These groups were non-nodulating legumes and nonleguminous species. Calculations were further based on the $\delta^{15}N$ values of the nodulating species (Fig. 2) and a ^{15}N fractionation of –1.7‰ during the fixation process. Negative values were set at 0. For number of samples and abbreviation of soil types, see Table 1.

ns, $\delta^{15}N$ not significantly different from reference species at $P < 0.05$.

*, the %Ndfa estimate with nonleguminous species as a reference was significantly higher than that with non-nodulating legumes as a reference.

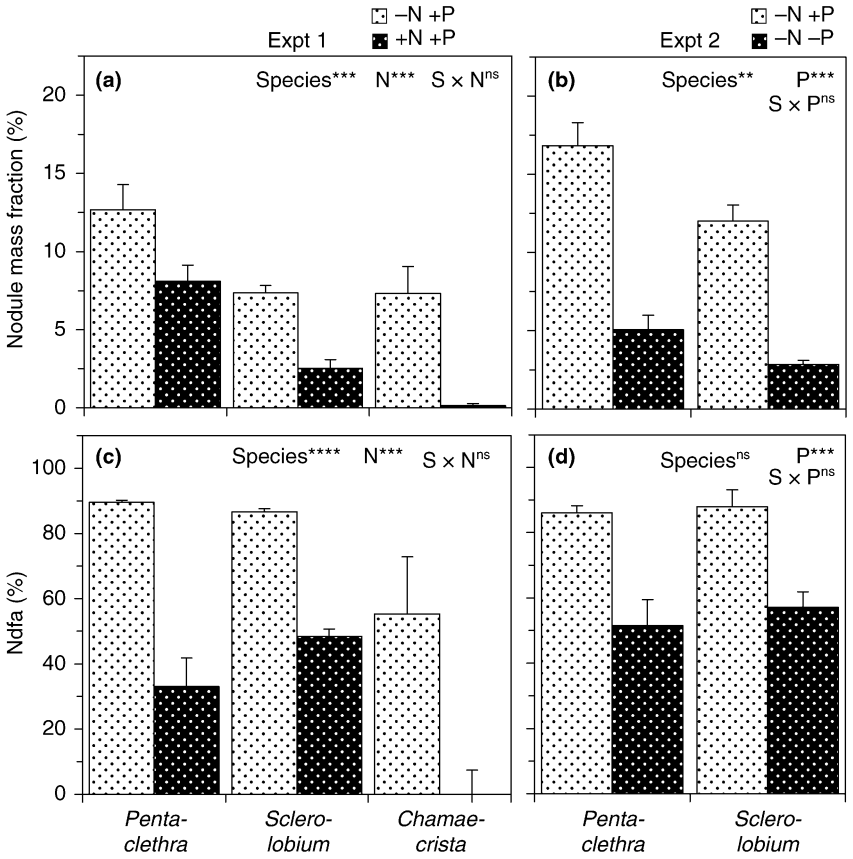

Fig. 3 Nodule dry mass as a percentage of total root mass (nodule mass fraction) and the percentage of plant nitrogen (N) derived from N_2 fixation (%Ndfa) as affected by N and phosphorus (P) availability on *Pentaclethra macroloba*, *Sclerolobium guianense* and *Chamaecrista apoucouita*. (a, c) Results of Expt 1 on N availability; (b, d) results of Expt 2 on P availability. Results of a two-way ANOVA for species and nutrient availability effects and their interaction (S × N; S × P) performed on arcsin-transformed data are shown in the panels: ns, not significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

fixation could thus only compensate to a limited extent for reduced soil N availability.

In Expt 2, no N fertilizer was applied and P fertilizer was given in one treatment to establish a possible limitation of N_2 fixation under the low soil P availability. The two species which had shown high N_2 fixation capacity in Expt 1, *P. macroloba* and *S. guianense*, were used. Similarly to Expt 1, N_2 fixation was high when P was applied without N (Fig. 3). No addition of P effectively resulted in a low soil available P, as evident from reduced growth (Table 6) and low leaf P concentrations (Table 7). Ndfa was similarly reduced in the two species from 87 to 54% (Fig. 3) and nodule biomass as a fraction of root biomass decreased from 17 to 5% in *P. macroloba* and from 13 to 3% in *S. guianense* (Fig. 3), which is consistent with the reduced N_2 fixation rates.

Discussion

Estimates of N_2 fixation by the ^{15}N natural abundance method

The ^{15}N natural abundance method is the only method available for quantitative estimations of symbiotic N_2 fixation in natural forest ecosystems (Högberg, 1997; Boddey *et al.*, 2000; Sprent & Parson, 2000). Several studies have shown that, in natural forest, the $\delta^{15}N$ values of many nodulating trees were lower compared with non-nodulating trees and that $\delta^{15}N$ values of the latter ones were sufficiently different from atmospheric N_2 to make an estimate of N_2 fixation possible (Yoneyama *et al.*, 1993; Högberg & Alexander, 1995; Guehl *et al.*, 1998; Roggy *et al.*, 1999a,b; Koponen *et al.*, 2003; Kreibich *et al.*, 2006). However, these conditions were absent in other studies (Högberg, 1990; Pate *et al.*, 1993; Handley *et al.*, 1994; Handley & Scrimgeour, 1997; Gehring & Vlek, 2004), which precluded these estimates. In this study, a sufficiently high $\delta^{15}N$ of non-nodulating trees and a mostly lower $\delta^{15}N$ of nodulating trees were found on brown sands, alluvial creek deposits and the lateritic soils, but not on white sand (Fig. 2). Hence, with the exception of trees growing on white sand, the ^{15}N natural abundance method could be used to estimate N_2 fixation rates by mature trees in the forest. The references noted in this paragraph and the contrast between white sand and the other soils in our study illustrate that the possibility of using the natural ^{15}N abundance method is dependent on the local conditions.

Reference trees should ideally utilize the same soil available N in the same space and time compared with the potentially N_2-fixing nodulating species. However, insufficient information is available on the N uptake patterns of the mature forest trees for pairing nodulating with non-nodulating species if

Table 5 Plant parameters for nodulating and non-nodulating potted tree saplings as affected by nitrogen (N) availability in Expt 1

	Pentaclethra macroloba		*Sclerolobium guianense*		*Chamaecrista apoucouita*		*Eperua falcata*		*Dicymbe altsonii*		ANOVA		
	–N	+N	–N	+N	–N	+N	–N	+N	–N	+N	Species	N	S × N
Plant dry mass start (g)	5.26 ± 0.83		5.64 ± 0.53		1.29 ± 0.08		2.68 ± 0.21		2.98 ± 0.18		***	–	–
Plant dry mass end (g)	248.4 ± 21.6	308.2 ± 30.9	232.8 ± 27.5	268.9 ± 9.5	73.2 ± 14.9	171.2 ± 20.6	44.2 ± 5.0	80.7 ± 8.9	45.2 ± 4.3	137.8 ± 9.6	***	***	***
Plant [N] (mg g^{-1})	14.6 ± 0.5	14.1 ± 0.4	12.3 ± 0.5	16.6 ± 1.0	9.9 ± 0.7	13.4 ± 0.9	7.2 ± 0.4	16.8 ± 1.3	6.7 ± 0.3	10.7 ± 0.4	***	***	***
Net N uptake (g per plant)	3.54 ± 0.31	4.28 ± 0.48	2.81 ± 0.39	4.41 ± 0.35	0.73 ± 0.19	2.26 ± 0.28	0.28 ± 0.02	1.32 ± 0.16	0.25 ± 0.03	1.43 ± 0.11	***	***	***
^{15}N atom excess (%)	0.140 ± 0.008	0.167 ± 0.021	0.178 ± 0.013	0.129 ± 0.006	0.586 ± 0.022	0.259 ± 0.024	1.129 ± 0.035	0.218 ± 0.026	1.240 ± 0.111	0.286 ± 0.021	***	***	***

The nodulating species are *P. macroloba*, *S. guianense* and *C. apoucouita*, and the nonnodulating species are *E. falcata* and *D. altsonii*. Presented are plant dry mass at the beginning and end of the experimental treatment, whole-plant N concentration, net N uptake and the percentage ^{15}N atomic excess of whole-plant N (means ± SE; $n = 5$). Results of a two-way ANOVA are shown: ***, $P < 0.001$; S × P, interaction. Data on percentages atomic excess of N taken up by the plants during the experimental treatment are presented as supplementary material (Table S1).

Table 6 Plant parameters for nodulating and non-nodulating potted tree saplings as affected by phosphorus (P) availability in Expt 2

	Pentaclethra macroloba		*Sclerolobium guianense*		*Carapa guianensis*		ANOVA		
	–P	+P	–P	+P	–P	+P	Species	P	S × P
Plant dry mass start (g)	17.8 ± 1.2		32.4 ± 4.2		5.9 ± 0.5		***	–	–
Plant dry mass end (g)	44.3 ± 4.0	89.5 ± 10.6	75.0 ± 10.8	104.5 ± 10.0	31.1 ± 2.7	32.3 ± 2.0	***	***	*
Plant [N] (mg g^{-1})	9.2 ± 0.3	13.8 ± 0.6	7.7 ± 0.2	11.4 ± 0.7	4.6 ± 0.2	4.9 ± 0.2	***	***	***
Net N uptake (g per plant)	0.162 ± 0.036	1.010 ± 0.182	0.244 ± 0.74	0.881 ± 0.154	0.080 ± 0.009	0.095 ± 0.006	***	***	***
^{15}N atom excess (%)	0.355 ± 0.016	0.179 ± 0.023	0.300 ± 0.045	0.151 ± 0.023	1.111 ± 0.059	1.138 ± 0.045	***	**	*

The nodulating species are *P. macroloba* and *S. guianense*, and the nonnodulating species is *C. guianensis*. Presented are plant dry mass at the beginning and end of the experimental treatment, whole-plant nitrogen (N) concentration, net N uptake and the percentage ^{15}N atomic excess of whole-plant N (means ± SE; $n = 8$). Results of a two-way ANOVA are shown: *, $P < 0.05$; **, $P < 0.001$***, $P < 0.001$; S × P, interaction. Data on percentages atomic excess of N taken up by the plants during the experimental treatment are presented as supplementary material (Table S2).

Table 7 Concentrations of nitrogen (N) and phosphorus (P) in leaf dry matter of the species used for the two pot experiments

	Expt 1, N availability			
Leaf [N] (mg g^{-1})	–N +P	+N +P	Small saplings in forest	Mature forest trees
Pentaclethra macroloba	29.3 ± 0.8	26.9 ± 0.6	28.9 ± 0.9	26.4 ± 0.5
Sclerolobium guianense	16.8 ± 1.3	20.5 ± 1.3	17.4 ± 0.7	16.1 ± 0.7
Chamaecrista apoucouita	15.2 ± 0.9	23.5 ± 1.2	21.1 ± 0.7	22.1 ± 0.5
Dicymbe altsonii	10.7 ± 0.6	17.3 ± 0.5	23.1 ± 0.7	16.9 ± 1.9
Eperua falcata	13.7 ± 0.7	30.5 ± 2.7	22.1 ± 0.8	15.9 ± 0.4
	Expt 2, P availability			
Leaf [P] (mg g^{-1})	–N –P	–N +P	Small saplings in forest	Mature forest trees
Pentaclethra macroloba	0.49 ± 0.02	1.16 ± 0.04	0.53 ± 0.08	0.77 ± 0.03
Sclerolobium guianense	0.22 ± 0.01	0.77 ± 0.19	0.40 ± 0.05	0.52 ± 0.03
Carapa guianensis	0.35 ± 0.04	1.11 ± 0.19	0.66 ± 0.03	0.63 ± 0.19

Data are for saplings after the experimental treatments, for saplings collected in the forest on brown sand soil (plants sampled at the start of the experiments), and for mature forest trees (means ± SE of all samples across soil types).

that would be possible at all. A possible complication with the estimations of %Ndfa could be the type of mycorrhizal association (Högberg & Alexander, 1995), but these are almost exclusively of the arbuscular type (Béreau *et al.*, 1997; Perreijn, 2002), which reduces the likelihood of interference. We have used a broad range of reference species that were divided into two groups, non-nodulating legumes and nonlegumes. This averages over possible difference in N uptake patterns, but cannot exclude that variation in $\delta^{15}N$ is caused by other reasons than N_2 fixation only. The difference in the %Ndfa estimates using the two reference groups (Table 4) illustrates their semiquantitative nature. Species with the highest %Ndfa estimates in one soil also generally had the highest values when growing in another soil (Table 4). This similar hierarchy of %Ndfa per soil type for species growing in more than one soil adds to the confidence in the method, at least in a relative sense.

A soil $\delta^{15}N$ value close to unity and an absence of a relationship of $\delta^{15}N$ of the tree leaves with nodulation observed on white sands in our study (Table 2, Fig. 1) was also reported for similar forests in French Guiana (Roggy *et al.*, 1999a) and Brazil (Martinelli *et al.*, 1999). Roggy *et al.* (1999a) also concluded that reliable estimates of N_2 fixation were not possible for trees grown on white sand. However, the reason for the contrasting $\delta^{15}N$ of the white sand system compared with the other soils is unknown. Nodulating legumes on white sand are different from nodulating legumes on the other soils because they do not show a higher leaf N concentration compared with non-nodulating trees. Nevertheless, it is likely that, for instance, *Ormosia coutinhoi* fixes atmospheric N_2 because it nodulates profusely on white sand where it is most common, as it did on brown sand where a high %Ndfa value was found (Table 4).

The forests in central Guyana have some species and several genera in common with other Neotropical forests. The %Ndfa estimates can thus be compared with the studies where such estimates are also reported. Higher %Ndfa values for *Chamaecrista* species were found in the Brazilian cerrado (Sprent *et al.*, 1996) than for *Chamaecrista* species in the central Guyana rainforest, but the species were not identical. Our %Ndfa estimates were on average somewhat lower (40%) than those for a forest in French Guiana that has similarity in species composition and soil (52%) (Roggy *et al.*, 1999a). In particular, the $\delta^{15}N$ value of the nodulating *Inga* species in Guyana showed no evidence of N_2 fixation activity, whereas %Ndfa estimates were, on average, 52% in French Guiana (Roggy *et al.*, 1999a). However, in both studies, *Sclerolobium*, *Swartzia* and *Ormosia* species were identified as active N_2 fixers. The same is true for *Pterocarpus officinalis*, which was identified as an active N_2-fixing tree on alluvial soil along creeks in central Guyana (Table 4) and in a swamp forest in French Guiana (Koponen *et al.*, 2003).

N and P availability

In the three species that were investigated, nodulation and %Ndfa estimates were significantly reduced when soil available N was experimentally increased (Fig. 3). High soil N availability leading to reduced N_2 fixation is a general phenomenon not only limited to herbaceous crops but also found for trees (van Kessel & Roskoski, 1983; Goi *et al.*, 1993; Hartwig, 1998). Application of P increased nodulation and N_2 fixation significantly (Fig. 3), a response which is well known for leguminous crops (Israel, 1987; Sprent, 1999) and from a limited number of studies with trees (Ribet & Drevon, 1996). Nitrogen is considered not to be a growth-limiting nutrient element in primary tropical rainforest, whereas P availability is seen as a principal limiting factor on heavily weathered

phosphate-binding soils such as at our site (Raaimakers, 1995; Brouwer, 1996; van Kekem *et al.*, 1996; Pons *et al.*, 2005). This evidence suggests that the putative high N and low P availability can reduce N_2 fixation in rainforest trees below their capacity. What further evidence do we have?

Available P in the shallow topsoil layer where the organic matter is concentrated is generally low (Table 3) and reduces to very low quantities at greater depths (van Kekem *et al.*, 1996). Total soil N concentrations (Table 3) are not conclusive with respect to N availability. However, comparison of nutrient element concentrations in leaves allows a better interpretation of their availability in the system. Mature forest trees and small saplings harvested in the forest had intermediate concentrations of P compared with those of high- and low-P grown plants in Expt 2 (Table 7). This would indicate that the availability of P for these trees is indeed low, although probably not as low as at the end of Expt 2 in the low-P treatment. Some reduction in N_2 fixation as a result of low P availability can thus be expected in the forest. High leaf N concentrations were found in the high-N treatment of Expt 1 for the non-nodulating reference species *Dicymbe altsonii* and *Eperua falcata*, and for *Chamaecrista apoucouita* that showed negligible nodulation in that treatment. Leaf N was also high for these species in the forest, except for mature *Eperua falcata* trees (Table 7). This is evidence for a generally high N availability in the undisturbed forest at our site. It is thus likely that, in addition to the limiting effect of a low P availability, N_2 fixation by forest trees can also be reduced as a result of the prevailing high N availability.

P. macroloba had a high capacity to fix N_2, as was evident from the high %Ndfa in the low-N and high-P availability treatment in the experiments and from the fact that the reduced soil N availability was fully compensated by N_2 fixation (Fig. 3, Table 5). N_2 fixation estimates were also high for *S. guianense* but reduced soil available N was not fully compensated by N_2 fixation (Table 5), which is evidence for a somewhat lower N_2 fixation capability of this species. *C. apoucouita* had a clearly lower capacity of N_2 fixation and was highly sensitive to the reducing effect of additional N (Fig. 3, Table 5). Estimates of %Ndfa for forest trees were highest for *P. macroloba*, intermediate for *S. guianense* and lowest for *C. apoucouita* (Table 4), the same as the hierarchy found for N_2-fixing capability in the pot experiments. The consistency between the field data and the results of the pot experiments, which are based on the ^{15}N enrichment method, supports the confidence in the %Ndfa estimates based on the ^{15}N natural abundance method used for mature trees in the forest. The %Ndfa estimates for the mature forest trees are lower compared with saplings grown in pots in the more optimal treatment (high P and low N). This could be because mature trees in general have lower N_2 fixation rates. It is also possible, as argued earlier, that N_2 fixation was reduced below their capacity at the low P and high N availability in the forest.

The concentrations of P tend to be higher in leaves of nodulating legumes compared with non-nodulating species (Sprent, 1999). Although a limitation of N_2 fixation at low P availability was experimentally established for Guyanese forest tree species (Fig. 3), higher P concentrations were not found in nodulating legumes (Table 2). Lack of evidence for a higher P requirement of nodulating tree legumes has been reported (Tuohy *et al.*, 1991). A lower P requirement, and consequently a higher P utilization efficiency, could be an adaptation to the prevailing low P availability in the forest. Another observed trend in legumes is a high N concentration in leaf tissue (McKey, 1994). Such higher N concentrations were indeed observed but only for nodulating legumes (Table 2). This is thus not an intrinsic trait of all legumes, as is sometimes suggested (McKey, 1994), but only for nodulating legumes. The higher N concentrations are likely to be the result of the additional N source that is available for the N_2-fixing trees. Since a large fraction of leaf N is involved in the photosynthetic apparatus, higher leaf N concentrations can result in higher photosynthetic capacities, as was also observed for trees in central Guyana (Raaimakers *et al.*, 1995). The two late successional nodulating legumes, for which photosynthesis was measured (*Pentaclethra macroloba* and *Ormosia coccinea*), had higher photosynthetic capacities compared with non-nodulating late successionals legumes (N. C. Houter, unpublished). The higher leaf N concentrations can thus be utilized for a higher photosynthetic activity in nodulating trees.

Nitrogen fixation at the ecosystem level

Although leguminous trees were the dominant species in the forest we investigated (ter Steege *et al.*, 1993, 2000b), most are members of the non-nodulating caesalpinioids. Nodulating legumes are less abundant. Although N_2 fixation can be an important source of N for nodulating trees (Table 4), the question remains as to what the quantitative significance is of the N_2-fixing process at the ecosystem level. Using available data on basal area and leaf turnover times, an estimate of N_2 fixation at the ecosystem level was constructed. The estimates for N_2 fixation ranged between 4 and 7 kg ha^{-1} yr^{-1} (Table 8), which amounts to between 5 and 9% of net leaf N uptake by the tree community. Although the N uptake data account for leaf turnover, root turnover and fruiting can also require a substantial percentage of the N budget of a tree. However, if the same %Ndfa value found for leaves also applies to the N taken up for growth of other parts of the trees, then these percentages of leaf N uptake also apply to whole tree N-uptake. Comparable to brown sands in Guyana, Roggy *et al.* (1999a) estimated a similar 5.5% N_2 fixation of total N uptake for a forest on oxysols where nodulating legumes had a similar percentage of the total tree biomass. Our estimates of annually fixed N_2 input are also in the same range as reported for other Neotropical forests (Sylvester-Bradley *et al.*, 1980; Jordan *et al.*, 1982; Kreibich *et al.*, 2006). However,

Table 8 Estimates of N_2 fixation at the forest canopy level and related parameters

	Brown sand	Alluvial clay	Laterite F	Laterite L
Leaf N (kg ha^{-1})	162	136	122	128
Leaf N (%)				
Nodulating legumes	14	13	28	31
Non-nod legumes	47	64	34	22
Non-legumes	39	23	38	47
N_2 fixation (kg ha^{-1} yr^{-1})	6.3	5.5	7.0	3.9
N_2 fixation (%)	5.8	6.0	8.6	4.6

Calculations were performed for the four soil types where %Ndfa estimates at the tree level were available (Table 4). Leaf N and its distribution over the three functional groups was calculated from leaf biomass estimates based on stem diameter measurements (ter Steege *et al*., 2000b) and allometric relationships (Lescure *et al*., 1983). Leaf N derived from N_2 fixation in the forests as absolute value and as a percentage of total leaf N uptake by all trees was calculated using a leaf turnover time of 1.5 yr, which was based on leaf litterfall data for the same forests (Brouwer, 1996; Thomas, 2001). When no data on %Ndfa for less abundant species were available, the means per functional group and soil type were used (Perreijn, 2002).

higher estimates of fixed N_2 input were reported for a swamp forest in French Guiana (8–28%; Koponen *et al*., 2003). We conclude that symbiotic N_2 fixation constitutes a large source of N input in tropical rainforest ecosystems and is important for maintaining relatively high amounts of plant N availability. Substantial losses of N can occur after disturbance (ter Steege *et al*., 1995) and symbiotic N_2 fixation is potentially an important mechanism for restoring N pools, provided that sufficient N_2-fixing legumes remain after the disturbance event.

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Supplementary Material

The following supplementary material is available for the article online:

Table S1 Supplemental data for pot Expt 1: the ^{15}N atomic excess percentages of N taken up by the plant during the experimental period (means ± SE; $n = 5$)

Table S2 Supplemental data for pot Expt 2: ^{15}N atomic excess percentages of N taken up by the plant during the experimental period (means ± SE; $n = 8$)

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