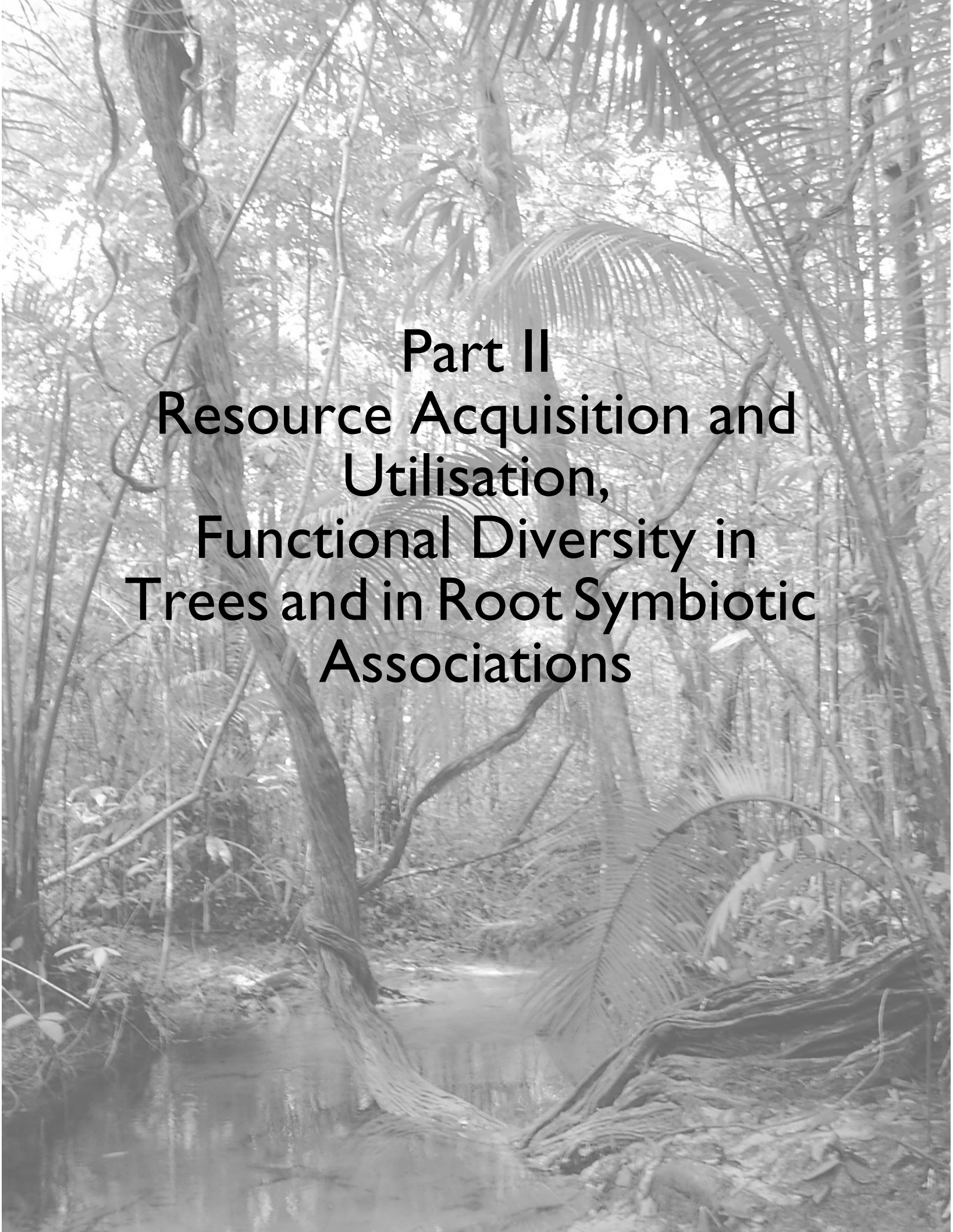

Part II
Resource Acquisition and Utilisation, Functional Diversity in Trees and in Root Symbiotic Associations

Ecology and Management of a Neotropical Rainforest

Resource Acquisition and Utilisation, Functional Diversity in Trees and in Root Symbiotic Associations

Jean-Marc Guehl

The five chapters of this part address different aspects of functional diversity – i.e. the identification of functions, and the range and spatial assembling of functional traits, exhibited by organisms or species of the different ecosystem components – in tropical rainforests. These aspects are relevant for two main questions in the field of tropical rainforest ecology:

– The understanding of the mechanisms underlying species coexistence, and the maintenance of species diversity, in hyper-diverse systems such as tropical rainforests. Functional – and in particular ecophysiological – traits have hardly been considered so far, with the exception of the light adaptation and growth dynamics features of trees. Traits related to resource acquisition and utilisation are of particular relevance here, e.g. to address aspects of niche complementarity, mutualism or competition in the relationships among organisms and species.
– The assessment of the role of biodiversity in ecosystem function. For instance, is there a relationship between the high tree species richness and the high primary productivity, or the high evapotranspiration, which characterise the tropical rainforest? There is still a considerable lag between our recognition of the importance of tropical rainforests in global hydrological and carbon cycles and our knowledge of the biological determinants of ecosystem function.

The functional diversity of tree species is addressed in three chapters.

(1) Light adaptation features studied in shade tunnels provide original information on differential plastic responses to light in major species (Barigah et al., Chapter 1) allowing to better understand the behaviour of species in forest conditions.

(2) Using the stable carbon isotope discrimination approach, which allows broad screenings of tree species, Guehl et al. (Chapter 2) show the existence of considerable differences in water-use efficiency, as well as in sensitivity to drought and the potential depth of soil water extraction among species.

(3) The successful use of the nitrogen isotopic screening approach allowed Domenach et al. (chapter 5) to depict the different N acquisition groups of trees and spot N_2-fixing species existing at the community level.

Root symbionts constitute an essential biological component of forest ecosystems. Béreau et al. (Chapter 4) provide a first screening of the types of mycorrhizal associations encountered in French Guiana, clearly showing the prominence of endomycorrhizae, as well as the dependence of seedling growth on mycorrhizal infection. Domenach et al. (Chapter 5) provide preliminary insights into the diversity of rhizobia associated with N_2-fixing trees, pointing to the predominance of the ubiquitous and promiscuous *Bradyrhizobia*.

Ecosystem integration is achieved by two different approaches.

(1) Scaling up from tree to ecosystem is carried out for N fixation (Chapter 5), providing a first picture of the different terms of the ecosystem-level N balance and its modulation by soil properties. A similar exercise is performed for canopy carbon isotope discrimination (Buchmann et al., Chapter 3), a parameter used in global carbon cycle modelling.

(2) Direct ecosystem-level assessment of C dynamics – involving both CO_2 exchange of different ecosystem components and transformations, characterised by isotopic fractionations, along the leaf-litter-soil continuum – is performed by Buchmann et al. (Chapter 3).

Ecology and Management of a Neotropical Rainforest

Chapter 1

Contrasting responses of growth traits to light regime in seedlings of three tropical rainforest species

Têtè Sévérien Barigah, Pascal Imbert, Moïse Béreau, Roland Huc

Abstract – The light regime under forest canopies varies considerably in space and time, ranging from full sunlight (in very large gaps) to total shade, and affecting the establishment and early growth of tree seedlings. We studied the effects of light regime on growth, biomass accumulation and biomass distribution among plant components in potted forest tree seedlings. Shade tunnels were used to provide partial shade (10%, 30%, 45%) and deep shade (2%) light conditions. Seedlings of three tree species were grown from locally field-gathered seeds. Seedlings of the studied species usually grow in contrasting forest environments: openings (*Carapa guianensis*), partial shade (*Dicorynia guianensis*) and deep shade (*Vouacapoua americana*), and differ in their ecological strategies of regeneration. Growth analysis was performed in order to separate ontogenic drifts and phenotypic plasticity of growth parameters in their responses to light regime. In comparison with the two other species, *V. americana* clearly exhibited lower growth rates in a high light regime and other typical features of shade tolerance: high values for root mass ratio (RMR) (RMR = root mass/plant mass) and root/shoot ratio (R/S) (R/S = root system mass/above-ground plant mass), low plasticity of growth parameters in response to light regime. Surprisingly, *D. guianensis* clearly displayed features of a light-demanding species (high growth rates under high light and high plasticity of growth parameters), whereas it is considered as a species with intermediate shade tolerance, frequently occurring under dense canopies at the seedling stage. From the stand dynamics point of view, this result points to a high ability in *D. guianensis* to exploit locally favourable or improving light conditions (sunflecks, gap formation).

Keywords: Shade tunnels, Juvenile growth, Dry matter production, Biomass allocation, Seedling establishment

1. Introduction

The light environment in tropical rainforests is highly variable spatially and in time (Hartshorn, 1990; Clark and Clark, 1992; Bongers, 1998). Irradiance at ground level in tropical forests varies between less than 2% of full sunlight in the understorey to practically 100% in very large forest gaps. Seedlings of rainforest tree species must cope with low light availability until a gap is formed in the forest canopy (Clark and Clark, 1992)

and many species grow faster and exhibit lower mortality rates in canopy openings than under dense forest canopy (Augspurger, 1984a, 1984b, Clark and Clark, 1987, Denslow et al., 1990).

Tree species are known to differ in light requirements for their establishment, which in turn has been associated with differences in their ability to maintain a favourable carbon balance of seedlings and small saplings in the different light environments (Fetcher et al., 1983). Plants are capable of adjusting the relative size of their components (e.g. shoots and root systems) in response to changes in the availability of light (Robinson, 1986; Johnson and Thornley, 1987; McConnaughay and Coleman, 1999) and these adjustments may ultimately determine plant growth rate (Poorter, 1989). Shade-tolerant species can germinate and establish in the shade of the forest understorey, whereas light-demanding species can germinate in the shade, but need gaps for establishment, (Swaine and Whitmore, 1988; Favrichon, 1994; Whitmore, 1996). In fact, adaptation to light does not merely reflect such a simple dual system; rather a range of intermediate behaviours can be defined along a gradient of shade tolerance.

Plant traits related to carbon acquisition and its partitioning among plant components are the main determinants of the different light adaptation strategies (McGraw and Wulff, 1983). Plasticity in resource allocation patterns of plants under different growing conditions is thought to be of adaptive nature, and to reflect a tendency towards optimisation of growth with respect to environmental conditions. However, resource partitioning also changes significantly during plant development (Evans, 1972; Coleman et al., 1994), and the effects of growing conditions on resource partitioning will interact with those of ontogeny. Consequently, differences in resource partitioning cannot be ascribed clearly either to growing conditions or to ontogenetic state, unless plants are compared at the same size or developmental stage rather than at the same age (Walters et al., 1993a,b). Little is yet known about the specific requirements for establishment, growth and development of most tropical tree species (Bazzaz and Pickett, 1980; Mooney et al., 1980; Fetcher et al., 1994).

This study was focused on the dependence of seedling's growth traits – and their biomass allocation to shoots and roots – on the light regime in three tropical rainforest tree species differing in ecological requirements for their natural regeneration. An experimental approach was carried out on potted seedlings under five contrasting light regimes in shaded tunnels. The first objective was to assess how light influences seedlings growth and biomass production, the ultimate aim being to confirm experimentally the differential behaviours of the species observed in the forest. The second objective was to check whether under the assigned light conditions species do adjust their allocation of biomass to different plant components because of ontogenetic drifts and/or because of phenotypic plasticity in their morphological traits. An approach based on analysis of allometric relationships between growth traits was used to disentangle these two effects.

2. Materials and methods

2.1. Plant material and growth conditions

This study was conducted in French Guiana near the Paracou experimental plots of CIRAD-Forêt (see Part I) from July 1994 to January 1996.

Seedlings of abundant tree species which differed in light adaptation were used. Light adaptation features were taken from Favrichon (1994) (see also Picard et al., Chapter 6, Part IV). Favrichon's classification is based on growth dynamics observations made on trees according to classes of diameter at breast height (DBH), either in the pristine cover or in response to microclimatic changes induced by thinnings of various intensities. *Carapa guianensis* (Meliaceae) is a light-demanding species often found in recently disturbed or secondary forests; *Dicorynia guianensis* (Caesalpiniaceae) has intermediate light requirements; and *Vouacapoua americana* (Caesalpiniaceae) is a shade-tolerant species. Seeds of these species were collected in their natural habitats. *D. guianensis* seeds required a chemical seed coat abrasion treatment for dormancy breaking (Béreau et al., 2000).

Forty potted seedlings per species were assigned to five light regimes (light treatments). Five plants per species and per treatment were randomly selected and harvested three and a half months after sowing, then every other month from October 1994 until mid-January 1996.

The light regimes included full sunlight (100%), partial shade (45%, 30%, 10% of full sunlight) and full shade (2%). These treatments mimicked variations in irradiance from large gaps to dense forest canopy. Seeds

were germinated in 22-litre plastic bags filled with a mix (1/3, v/v) of sand and the upper 30 cm layer of a forest soil (sandy loam) collected near Paracou. Soil moisture was kept close to field capacity by providing automated drip irrigation together with natural rainfall. Plants were grown under full sunlight conditions and in open-ended tunnels at Paracou where the mean annual air temperature is 26 °C. Air temperature in the tunnels occasionally rose to 35 °C (windless days during the dry season) and relative humidity ranged from 80% (dry season) to 100% (rainy season). The various light regimes most likely created air temperature and humidity differences that may be a component of treatment effects.

2.2. Measurements and analyses

The following variables were measured on all sampled seedlings: height of the main stem, biomass of the different plant components (leaf lamina, branches including rachis and petioles, stem, taproot and fine roots with diameter < 2 mm). The three species have compound leaves and in the following the term "leaf lamina" in fact denotes "leaflet lamina". The leaf lamina area of harvested seedlings was measured using an automated leaf area meter (Li-Cor model 3000-A equipped with Li-Cor Belt Conveyor 3050-A, Li-Cor Instruments, Lincoln, Nebraska). Afterwards, the leaf lamina, shoot and root fractions were dried to constant mass in a gravity convection oven at 70 °C before weighing. The following variables were calculated for growth and biomass allocation analyses:

- leaf mass per unit leaf area (LMA) (g m^{-2});
- leaf area ratio (LAR) (leaf area/plant mass, m^2 kg^{-1});
- leaf mass ratio (LMR) (leaf mass/plant mass, g g^{-1});
- root mass ratio (RMR) (root mass/plant mass, g g^{-1});
- stem mass ratio (SMR) (stem mass/plant mass, g g^{-1});
- root/shoot ratio (R/S) (root system mass/above-ground plant mass, g g^{-1}).

Data were analysed using the general linear model (GLM) program of the statistical analysis system (SAS Institute, Cary, NC). The means of biomass variables and that of whole-plant leaf area were transformed in their natural logarithms before analysis to meet the assumptions of normality and equality of variances associated with GLM procedures. Computed ANOVA outputs were then used to compare species for the different growth variables. Natural logarithmic transformed growth variables were also used for assessing allometric relationships.

3. Results

3.1. Leaf area, biomass and height growth

Seedlings of the three species grown under high light had higher total leaf area, total biomass and main stem height than did those grown under a low light regime (Fig. 1). Seedlings of *C. guianensis* and *D. guianensis* displayed the lowest leaf area under full shade (2%) and full sunlight (100%), and the leaf development was highest for the three intermediate light regimes (10%, 30% and 45%). The highest leaf area development in *V. americana* was observed at 30% of full sunlight. During the first three months of the growing period (before day 100), both *C. guianensis* and *V. americana* showed a higher biomass production than *D. guianensis* under the five light regimes, but the latter species displayed very fast growth rates from day 100 of the study period. Initial height growth (before day 100) was highest in *C. guianensis* and *V. americana*, the two species also being characterized by large seed size. *D. guianensis* displayed fast height growth from day 100 onwards. In *V. americana* seedlings grown under more than 30% of full sunlight, no height growth was observed during the measurement period.

D. guianensis clearly showed the highest LAR values (Fig. 2). A gradual decreasing effect, very closely related to the increasing light level (Fig. 2A), was observed for LAR in the three species. LAR was high in the small plants (Fig. 2B). Clear decreasing trends in LAR were observed with increasing plant size in *C. guianensis* and *D. guianensis*. In *V. americana*, a decreasing trend in LAR with total biomass was observed in plants grown at 30%, 45% and 100% of full sunlight regimes. In the three species, the LAR vs. total biomass allometric relationships were clearly affected by the light regime (Fig. 2B). *V. americana* seedlings were less responsive to light than the other species.

3.2. Allometry of root vs. shoot mass

The seedlings of the three species responded qualitatively in a similar way to the light regimes, increasing RMR being induced by increasing light levels (Fig. 3). *D. guianensis* displayed the lowest RMR values at low light intensity, but the sensitivity to increasing light was higher than in the other two species. The highest RMR values were recorded in

V. americana seedlings. Altering the light regime led to shifts in the RMR vs. total biomass relationships (Fig. 3B).

Allometric plots of root vs. shoot mass of the seedlings (Fig. 4) indicated distinct biomass allocation patterns in the three species. *C. guianensis* and *D. guianensis*

Table 1
Growth and morphological variables for seedlings of the three species at the final harvest, 20 months after sowing. For a given variable and light treatment, species mean values not sharing the same symbol are significantly different. For a given variable and species, mean values for the different light treatments not sharing common letters are significantly different ($n = 5$, ANOVA, $P < 0.05$). Cg: *Carapa guianensis;* Dg: *Dicorynia guianensis;* Va: *Vouacapoua americana.*

Morphological variables	*Species*	*Light environment (% of full sunlight)*				
		2%	*10%*	*30%*	*45%*	*100%*
Leaf area (m^2)	Cg	$0.148^{\S b}$	$0.402^{\S a}$	$0.315^{\S ab}$	$0.292^{\S ab}$	$0.147^{\S b}$
	Dg	0.075^{+c}	$0.321^{\S a}$	$0.259^{\S ab}$	$0.220^{\S ab}$	$0.126^{\S bc}$
	Va	$0.154^{\S ab}$	$0.260^{\S a}$	$0.238^{\S ab}$	$0.191^{\S ab}$	$0.079^{\S b}$
Total biomass (g)	Cg	$18.65^{\S b}$	$88.54^{\S ab}$	$104.74^{\S a}$	$128.39^{\S a}$	$92.32^{\S ab}$
	Dg	3.66^{+b}	34.88^{+a}	$45.28^{\S a}$	48.28^{+a}	29.74^{+ab}
	Va	$19.02^{\S b}$	38.76^{+ab}	$67.77^{\S a}$	57.61^{+a}	33.23^{+ab}
Leaf mass per unit area ($g\ m^{-2}$)	Cg	40.60^{+d}	$54.15^{\S +cd}$	$68.70^{\S bc}$	$83.32^{\S b}$	$103.51^{\S a}$
	Dg	$26.94^{\#d}$	46.48^{+c}	$58.90^{\S b}$	66.75^{+ab}	75.48^{+a}
	Va	$50.20^{\S b}$	$55.06^{\S b}$	$73.28^{\S a}$	$74.00^{\S +a}$	80.56^{+a}
Height (m)	Cg	$0.63^{\S a}$	$0.99^{\S a}$	$0.86^{\S a}$	$0.93^{\S a}$	$0.72^{\S a}$
	Dg	0.23^{+b}	0.44^{+ab}	$0.55^{\S +a}$	0.44^{+ab}	0.37^{+ab}
	Va	0.31^{+ab}	0.39^{+ab}	0.44^{+a}	0.37^{+ab}	0.28^{+b}
Root/shoot ratio ($g\ g^{-1}$)	Cg	0.32^{+b}	$0.37^{\S +b}$	$0.54^{\S +ab}$	0.46^{+b}	$0.73^{\S +a}$
	Dg	$0.15^{\#b}$	0.19^{+b}	0.47^{+a}	0.53^{+a}	0.52^{+a}
	Va	$0.48^{\S b}$	$0.52^{\S b}$	$0.75^{\S ab}$	$0.91^{\S ab}$	$1.32^{\S a}$
Leaf area ratio ($m^2\ kg^{-1}$)	Cg	8.1^{+a}	4.6^{+b}	3.3^{+bc}	2.4^{+cd}	1.6^{+d}
	Dg	$21.1^{\S a}$	$10.0^{\S b}$	$6.0^{\S c}$	$4.7^{\S c}$	$4.2^{\S c}$
	Va	8.2^{+a}	6.5^{+b}	3.5^{+c}	3.2^{+c}	2.3^{+c}
Leaf mass ratio ($g\ g^{-1}$)	Cg	$0.32^{\#a}$	$0.25^{\#b}$	0.22^{+cb}	0.19^{+cd}	0.16^{+d}
	Dg	$0.56^{\S a}$	$0.45^{\S b}$	$0.34^{\S c}$	$0.31^{\S c}$	$0.31^{\S c}$
	Va	0.41^{+a}	0.36^{+a}	$0.25^{\S b}$	0.24^{+b}	0.18^{+b}
Stem mass ratio ($g\ g^{-1}$)	Cg	$0.43^{\S a}$	$0.48^{\S a}$	$0.43^{\S a}$	$0.49^{\S a}$	$0.42^{\S a}$
	Dg	0.31^{+a}	$0.39^{\S +a}$	$0.34^{\S +a}$	0.34^{+a}	$0.35^{\S +a}$
	Va	0.27^{+a}	0.31^{+a}	0.32^{+a}	0.30^{+a}	0.28^{+a}
Root mass ratio ($g\ g^{-1}$)	Cg	0.24^{+c}	$0.27^{\S bc}$	$0.34^{\S +ab}$	0.31^{+bc}	$0.42^{\S +a}$
	Dg	$0.13^{\#b}$	0.16^{+b}	0.32^{+a}	0.35^{+a}	0.34^{+a}
	Va	$0.32^{\S b}$	$0.33^{\S b}$	$0.43^{\S ab}$	$0.46^{\S ab}$	$0.54^{\S a}$

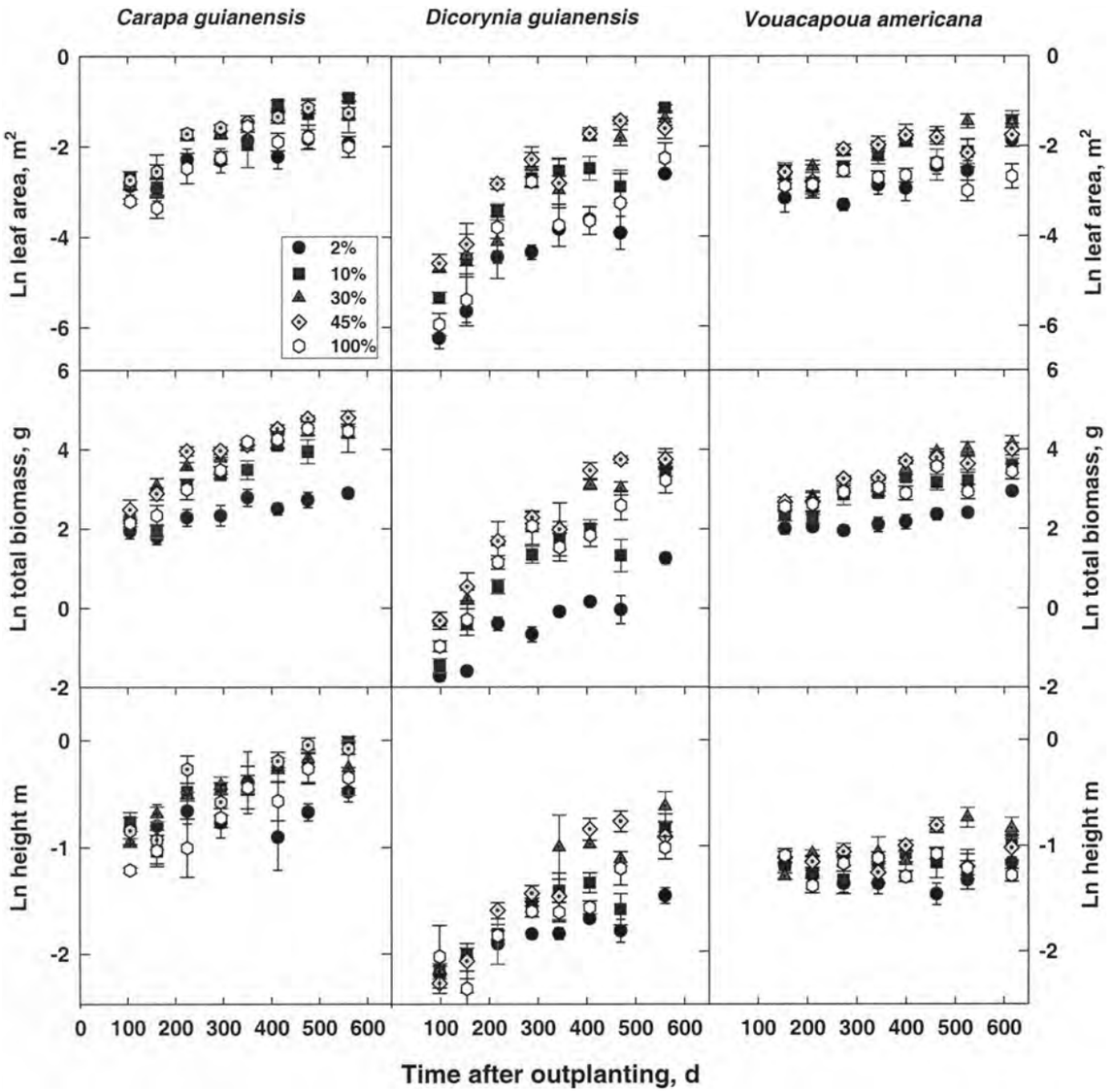

Fig. 1. Time course of leaf area, total biomass and height in seedlings of the three species over a period covering 616 days after sowing for the five different light regimes (2%, 10%, 30%, 45% and 100% of full sunlight). The *Y*-axis is represented in natural logarithmic scales so that the local slopes of the relationships correspond to relative growth rates.

seedlings tended to favour biomass investment in their above-ground structures, while *V. americana* seedlings favoured below-ground biomass.

3.3. Variations in biomass allocation and morphological traits at the final harvest

Seedlings grown under 30% and 45% of full sunlight yielded the highest biomass accumulation (Table 1) in the three species. The total biomass of *C. guianensis* seedlings receiving 45% of full sunlight was more than 1.4 times higher than that of seedlings which received 100%, and 18.6 times greater than that of plants grown in 2% of full sunlight, while the difference in magnitude of total biomass between plants grown in the most favourable light-regime and plants grown in 2% of full sunlight was less in *D. guianensis* and *V. americana.*

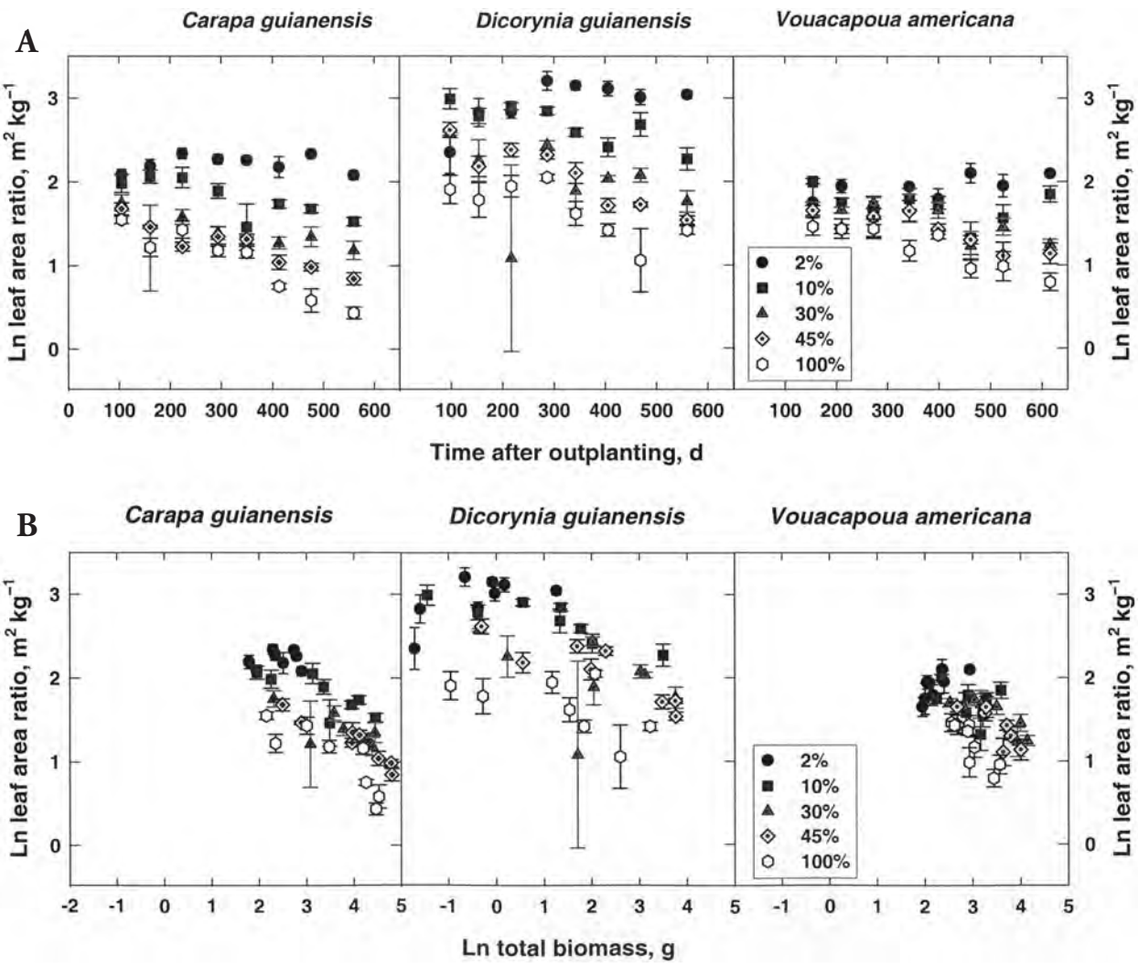

Fig. 2A. Time course of leaf area ratio (LAR) in seedlings of the three species over a period covering 616 days after sowing for the five different light regimes (2%, 10%, 30%, 45% and 100% of full sunlight); **B.** Allometric relationships between LAR and total biomass.

The leaf area ratio in the three species decreased in response to increasing light (Table 1), whereas RMR increased (Table 1).

Despite the fact that shading decreased biomass partitioning to fine roots, biomass allocation did not show the same pattern in the three species (Fig. 5). Under low light regimes, in all three species the investment in leaves was mainly made at the expense of taproots. Biomass allocation to branches was favoured for plants grown under light regime higher than 10% at the expense of leaf and main stem for *C. guianensis* and *D. guianensis* (Fig. 5). For *V. americana,* allocation to taproots was favoured at the expense of leaves and main stem compartment when seedlings grew under high light conditions.

4. Discussion

The large initial (day 100) differences observed among species for growth variables can be ascribed to differences in seed size (not measured in this work, unfortunately) rather than to specific light effects, because light availability does not limit seedling growth until cotyledon reserves are depleted (Kitajima, 1992; Osunkoya et al., 1994; Baraloto, 2001). *D. guianensis* is a small-seeded species, while

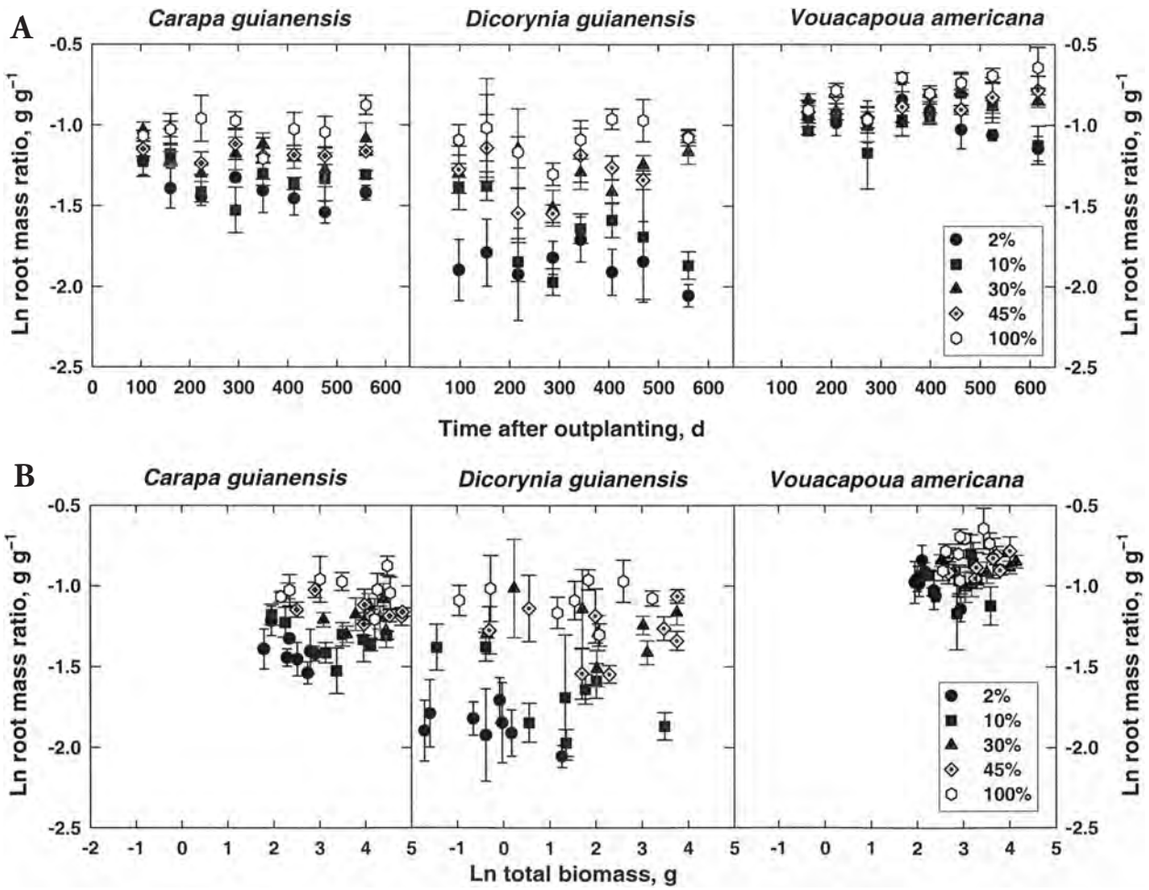

Fig. 3A. Time course of RMR in seedlings of the three species over a period covering 616 days after sowing for the five different light regimes (2%, 10%, 30%, 45% and 100% of full sunlight); **B.** Allometric relationships between RMR and total biomass.

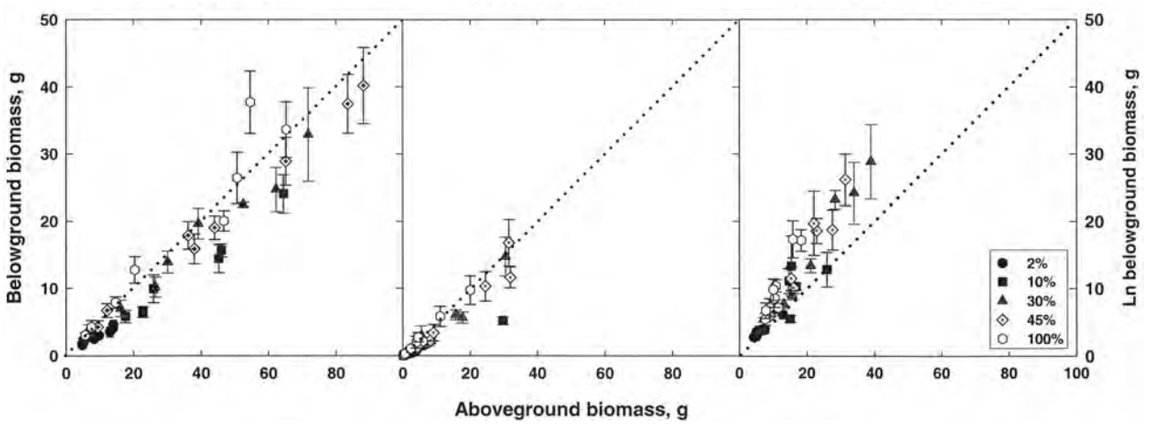

Fig. 4. Allometric plots of root biomass vs. shoot biomass in the three species for the five different light regimes (2%, 10%, 30%, 45% and 100% of full sunlight). The dotted line ($Y = 0.5\times$) is represented for sake of clarity.

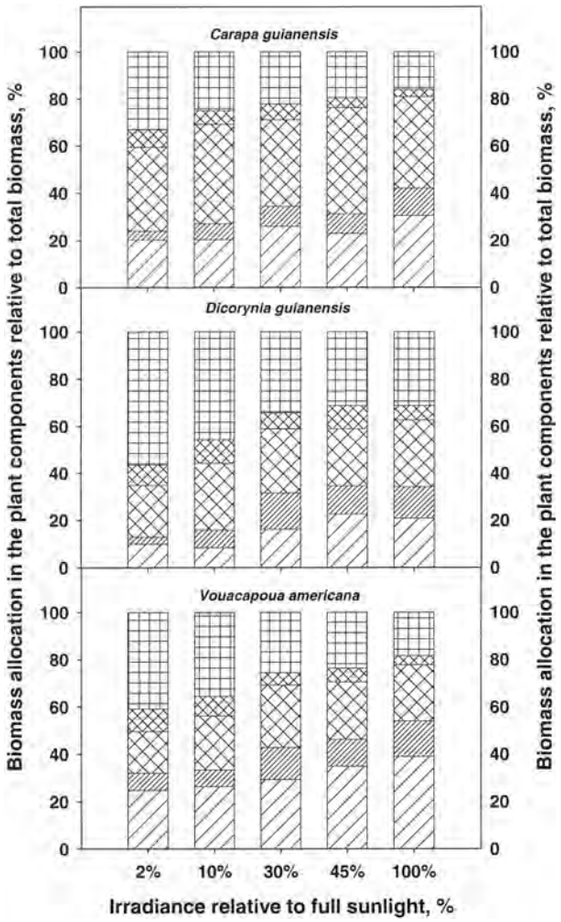

Fig. 5. Allocation of biomass to the different plant components at the final harvest day for the different light regimes. Error bars were omitted for sake of clarity. From bottom to top: taproot, fine roots, branches, stem and leaf lamina.

C. guianensis and particularly *V. americana* are large-seeded species (Baraloto and Forget, Chapter 2, Part IV). Relative and absolute growth rates became faster in *D. guianensis* than in the two other species from day 100 after sowing. Seedlings of *C. guianensis* and *V. americana* have hypogeal storage cotyledons, while *D. guianensis* has thin, epigeal leaf-like cotyledons. This characteristic is likely to have allowed *D. guianensis* seedlings to start using light as the main autotrophic source of energy earlier than did the seedlings of the two other species.

The different allometric relationships between growth variables, derived from the data after day 100, were clearly modulated by light regime (Fig. 2B, Fig. 3B, Fig. 4), which denotes the existence of phenotypic plasticity in the growth responses to light independently of ontogenic drift. The three species displayed different degrees of plasticity in morphological responses to light regime from deep shade to full sunlight, the responses in *C. guianensis* and *D. guianensis* being higher than in *V. americana*.

Seedlings grown under extreme light regimes (2% and 100%) achieved a considerably lower leaf area, as well as lower biomass and height growth, than those grown in the intermediate conditions. Optimal light conditions for biomass growth were 45% of the full sunlight for *C. guianensis* and *D. guianensis* and 30% for *V. americana* (Table 1).

In *V. americana*, leaf area decreased with time (Fig. 1) under full sunlight, pointing to the absence of tolerance to full sunlight conditions. *C. guianensis* seedlings produced higher total biomass and grew better than *V. americana* and *D. guianensis* individuals, even under the 2% light regime. Under these low light conditions, growth advantages were expected to be in favour of the latter two species – and especially *V. americana* – because of their habitat preferences. Findings similar to ours have been observed in previous reports (Barigah et al., 1998; Veneklaas and Poorter, 1998). Physiological and morphological adaptation to light may not be the only determinants of survival and performance in dense canopy understorey conditions; other factors such as nutritional, water or herbivory constraints, also including competition or mutualism (mycorrhizae, etc.) interspecific relationships, may play key roles (Baraloto and Forget, Chapter 2, Part IV; Béreau et al., Chapter 4, Part II). Studies in real field conditions are needed to substantiate, or complement, conclusions drawn from approaches carried out in simplified experimental systems.

In order to complete its life cycle, a plant must function as a balanced system in terms of resource uptake and use, and its different functional components must remain in balanced relative proportions (Mooney, 1972). A main equilibrium to be achieved is between the amount of shoot biomass and that of root biomass. It was remarkable that seedlings of *V. americana*, the most shade-tolerant species, had the highest RMRs by investing up to 50% of their biomass in their root system (Table 1). In contrast, *C. guianensis* and *D. guianensis* allocated less biomass towards their below-ground compartment.

Low light levels clearly induced a preferential biomass allocation to shoots, leading to improved light harvesting (Fig. 5). Holmgren et al. (1997) suggested that shade from canopy trees may alternatively facilitate or inhibit success of woody seedlings, depending on soil water conditions. However, these authors did not point out the consequence of disturbances such as gap formation on seedling mortality, as seedlings with a weak root system would withstand a long period of water shortage less well than those which allocated more biomass for root growth. As a result, in water shortage conditions, some seedlings which would have developed a large below-ground biomass would respond better to improved light conditions resulting from canopy gaps than those having a weak root mass (Shumway et al., 1993; Gottschalk, 1994).

In conclusion, we provided relevant data for the understanding of the differential adaptations in rainforest tree seedlings to contrasting light regimes. In comparison with the two other species, *V. americana* clearly exhibited low growth rates in a high light regime, and other typical features of shade tolerance: high RMR and R/S values (Table 1), and low plasticity of growth parameters in response to light regime. For LAR and LMA, often reported to be higher in shade-tolerance than in light-demanding species (Bazzaz and Pickett, 1980), we did not find values that consistently substantiated such a ranking. Surprisingly, *D. guianensis* clearly displayed features of a light-demanding species (high growth rates under high light, high plasticity of growth parameters), whereas it is considered to be a species with intermediate shade tolerance (Favrichon, 1994), frequently occurring under dense canopies at the seedling stage. From the stand dynamics point of view, these results point to a high ability to exploit locally favourable or improving light conditions (sunflecks, gap formation).

Acknowledgements

We thank Eliane Louisanna, Audin Patient, Saintano Dufort, Dumaine Duchant for technical assistance and Erwin Dreyer and Jean-Marc Guehl for the critical reading and comments on an earlier draft of this chapter.

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Ecology and Management of a Neotropical Rainforest

Chapter 2

Community-level diversity of carbon-water relations in rainforest trees

Jean-Marc Guehl, Damien Bonal, André Ferhi, Têtè Sévérien Barigah, Graham Farquhar, André Granier

Abstract – We analysed the functional diversity at tree community level by screening 65 species for leaf carbon isotope composition ($\delta^{13}C$) – an indicator of time-integrated leaf-level water-use efficiency (WUE, the ratio CO_2 assimilation rate (*A*)/stomatal conductance for water vapour (*g*)). We also assessed leaf water potential, as well as stomatal sensitivity to drought and soil water extraction features for selected species. We analysed possible trade-offs among these ecophysiological traits, as well as associations with taxonomic position and with ecological features such as leaf phenology or shade tolerance. We found an extremely high variability in sunlit leaf $\delta^{13}C$ among canopy tree species (range of 6‰), corresponding to WUE values varying over a threefold range. An original and environmentally robust result of our studies consisted in the association found between species $\delta^{13}C$ and species shade tolerance type. Heliophilic species exhibited more negative $\delta^{13}C$ values (i.e. lower WUE) than hemitolerant species. However, tolerant species displayed $\delta^{13}C$ values even more negative than the heliophilic species, thus pointing to a non-linear pattern for the association between $\delta^{13}C$ and the gradient of shade tolerance. Differences in $\delta^{13}C$ among species appeared to be primarily driven by differences in *g*. We also observed contrasting patterns of stomatal sensitivity to drought. Species midday leaf water potential spanned a wide range of values, from –0.2 MPa to ca –3.5 MPa, possibly reflecting a decreasing gradient of stomatal sensitivity to drought. No tight relationship between leaf $\delta^{13}C$ and midday water potential arose among species. Our results are not fully in agreement with the concept of functional types of species (Grime 1977, Tilman 1988) stating a unique grouping of species with respect to various traits. "Cross" diversity among main functions or traits may reflect an important aspect of functional diversity in tropical rainforests.

Key words: Functional diversity, Interspecific variability, Water-use efficiency, Stable isotopes, Shade tolerance types, Stomatal function, Drought responses.

1. Introduction

There is growing evidence that terrestrial ecosystem function is determined by functional diversity – i.e. the value, range and spatial assembling of species traits – rather than species numbers per se (Diaz and Cabido, 2001). Experimental manipulation of the species composition of herbaceous plant communities has proved a useful means of demonstrating the role of biodiversity for essential ecosystem functions such as net primary productivity, nutrient cycling, trophic transfer (Loreau, 2000) or water-relations (Caldeira et al., 2001). In the case of forests, the interest of manipulating species composition is limited, due to the high lifespan of trees. In hyper-diverse communities such as tropical rainforests, a further constraint in imposed by the huge number of species (ca. 200 species per ha, Part I), and in particular the large proportion of rare species. A relevant approach to the role of biodiversity in ecosystem function is then the assessment of ecophysiological functions and traits in natural communities, for which the necessary steps are (1) the grouping of species based on functional traits (Diaz and Cabido, 2001; Part II, chapter 5) and (2) an understanding of the mechanisms underlying the coexistence and spatial assembling of species or functional types of species (Wright, 2002).

Tropical rainforests play an important role in the global carbon cycle and climate regulation through their CO_2 and H_2O exchange with the atmosphere (Part II, Chapter 3). It is therefore essential to assess the role of tree functional diversity in the carbon and hydrological cycles in such systems (Nepstad et al., 1994). A main component in modelling the CO_2 and H_2O fluxes of the different terrestrial vegetation types, and in particular forests, is consideration of the functions related to the coupling of these two fluxes (Lloyd and Farquhar, 1994).

Carbon isotope discrimination (Δ) – roughly the difference in carbon isotope composition ($\delta^{13}C$) between the carbon source for photosynthesis (i.e. atmospheric CO_2) and the photosynthetic products (i.e. plant material) – is a convenient measure of long-term intercellular CO_2 concentration (Farquhar et al., 1982) and is an indicator of the set point for regulation of gas exchange, reflecting leaf-level water-use efficiency and overall trade-offs between carbon gain and transpirational water loss (Ehleringer, 1993). Hence, Δ measurements are suited to assess the biological determinants of coupling between CO_2 and H_2O fluxes at canopy level, and are particularly relevant to address the role of functional diversity among species in this coupling.

Trade-offs among species between Δ and plant water relations, or drought tolerance features, have been found in different biomes (Ehleringer, 1993; Sobrado and Ehleringer, 1997). Despite high rainfall, tropical rainforests can experience seasonal soil drought (Guehl, 1984; Huc et al., 1994) and drought is known to have been a main determinant of post-glacial species migration (Prance, 1982), as well as a factor shaping the present species distribution and biodiversity in neotropical forests.

We assessed the diversity of $\delta^{13}C$ among numerous tree species, mainly in the Paracou forest and in a nearby plantation. We also assessed leaf water potential as a parameter of tree water relations, as well as stomatal sensitivity to drought and soil water extraction features for selected species. We analysed possible trade-offs between these ecophysiological traits as well as associations with taxonomic position and with ecological features such as leaf phenology or shade tolerance, thereby addressing Grime's (1977) and Tilman's (1988) hypotheses stating the possibility of a unique grouping of species with respect to various functions and traits within communities.

2. Methodology and study sites

2.1. Methodological approach

• The first step of our approach consisted in a screening of 65 species for $\delta^{13}C$ in a 1-ha homogenous stand in Paracou forest (see Part I) in which all trees having a diameter at breast height (DBH) higher than 10 cm were inventoried, identified, and named according to the checklist of the plants of the Guianas (Boggan et al., 1997). Only dominant and co-dominant trees that had access to full sun were screened, so as to avoid confounding effects due to vertical within-canopy gradients in leaf $\delta^{13}C$ induced by light attenuation and gradients in canopy air carbon isotope composition ($\delta^{13}C_a$) (Buchmann et al., 1997; Guehl et al., 1998; Part II, Chapter 3). The light adaptation features of species were taken from Favrichon (1994) (Part IV, Chapter 5), who used the following characteristics of trees: species mean DBH, absolute radial growth rate in the different diameter classes, mortality, and recruitment (i.e. accession rate

to the 10 cm DBH class). Three shade tolerance types are distinguished for tall trees:

– Heliophilic species, needing openings in the forest to establish and reproduce, having high growth rates and displaying higher growth rates in lower diameter classes than in higher ones.
– Hemitolerant species, able to tolerate low light levels for seedling establishment but needing high light levels to reproduce once in the canopy. Many species within this group are potentially emergent (i.e. with tree crowns above the canopy).
– Shade tolerant species, reaching the upper canopy, but able to establish and reproduce under shade, with low growth rates in all DBH classes.

Short-lived heliophilics as well as lower canopy and understorey shade tolerant species were not included. Species were also characterized by their pattern of leaf phenology (deciduous-leaved/evergreen-leaved) (Loubry, 1994; Sabatier, unpublished data) or by their range of distribution in tropical South America (1, Guianas; 2, Guianas + North-Eastern Amazonia; 3, Guianas + Amazon; 4, Amazon to Panama; 5, Tropical South America) (Flora Neotropica).

• The second step involved less extensive screenings aimed at assessing both $\delta^{13}C$ and leaf water potential as well as their relationship in (1) monospecific plots of a 15 year-old plantation near Paracou forest, i.e. in more homogenous conditions (even-aged stands, no interspecific competition) than the pristine forest and (2) in a second forest site (piste de St-Elie, 30 km at the NW of Paracou forest) having similar soil and climate conditions (Sarrailh, 1984). Predawn (Ψ_{wp}) and midday (Ψ_{wm}) leaf water potentials were measured (Scholander et al., 1965) on the same trees on sunny days during both the wet and the dry seasons.

• In a third step, a few species were chosen on the basis of contrasting $\delta^{13}C$ values (Bonal et al., 2000b,c) and ecological characteristics, for a thorough ecophysiological characterisation, with complementary analyses of (1) field water relations in contrasting wet season and severe dry season conditions (Paracou plantation) and (2) drought responses in controlled conditions (containerised seedlings in climate chambers at INRA Kourou). *Eperua falcata* Aub., Caesalpiniaceae, is a hemitolerant species occurring in various soil conditions, but is more common on thinned out soils (Barthes, 1991; Sabatier et al., 1997). *Virola surinamensis* Warb., and *Virola michelii*, Myristicaceae, are heliophilic species usually associated with very moist soils in bottom flats (Bena, 1960). *Diplotropis purpurea* (Rich.) Amsh., Caesalpiniaceae, is also a heliophilic species which is common on deep well-drained soils (Bena, 1960).

2.2. Carbon isotope composition and relationship with leaf gas exchange and tree hydraulic functioning

Ten to fifteen fully expanded sunlit leaves per tree were shot down using a rifle. A sub-sample of 1 mg of dry powdered material was combusted and analysed for carbon isotope composition using an isotope ratio mass spectrometer at the stable isotope facility of INRA at Nancy. The abundance of stable isotopes was expressed according to the conventional delta notation as the molar $^{13}C/^{12}C$ ratio of the sample (R_{sample}) relative to that of an international standard ($R_{standard}$):

$$\delta^{13}C(‰) = \frac{R_{sample} - R_{standard}}{R_{standard}}\ 1000 \qquad (1)$$

Since only sunlit leaves were sampled, the characteristics of atmospheric CO_2, the photosynthetic C source, could be considered as constant: atmospheric CO_2 concentration (C_a) = 358 µmol mol^{-1}, carbon isotope composition of atmospheric CO_2 ($\delta^{13}C_a$) = – 7.85‰. (Buchmann et al., 1997). Carbon isotope discrimination was thus calculated as :

$$\Delta‰ = \frac{\delta^{13}C_a - \delta^{13}C}{1000 + \delta^{13}C}\ 1000 \qquad (2)$$

According to Farquhar et al. (1982), Δ is related to the time-integrated ratio of intercellular to ambient CO_2 concentration (C_i/C_a), which is also related to time-integrated leaf intrinsic water-use efficiency (WUE = leaf area based CO_2 assimilation rate (A)/leaf conductance for water vapour (g)):

$$\Delta‰ = a + (b - a)\ \frac{C_i}{C_a} = a + (b - a)\left(1 - \frac{1.6WUE}{C_a}\right),$$

$$WUE = \frac{A}{g} \qquad (3)$$

where a (4.4‰) and b (27‰) are the isotopic fractionation constants during CO_2 diffusion through

the stomatal pores and during carboxylation, respectively.

The second term in the denominator of equation 2 is quite small and can be neglected. Thus, leaf $\delta^{13}C$ can be considered as negatively and linearly related to Δ and positively related to WUE.

A theoretical background to analyse combined interspecific differences in $\delta^{13}C$ and in Ψ_{wm} is provided by considering a simple steady-state model of water flow through the soil-tree-atmosphere continuum (Panek, 1996). Equating the expressions of transpirational water flow per unit tree leaf area, in the liquid and vapour phases, and assuming Ψ_{wp} to be an estimation of the average water potential of the soil surrounding the root system (Ritchie and Hinckley, 1975), yields:

$$g\nu = K_L(\Psi_{wm} - \Psi_{wp}) \quad (4)$$

where ν (mol mol^{-1}) denotes the leaf-to-air vapour molar fraction difference and K_L (mmol m^{-2} s^{-1} MPa^{-1}) denotes the whole tree leaf specific hydraulic conductance (i.e. the whole tree hydraulic conductance divided by the tree leaf area).

Combining equations 3 and 4, one obtains, after some transformations, an expression of Δ, or leaf $\delta^{13}C$, as a function of the gradient of water potential:

$$\Delta = b - \frac{(b-a)1.6\nu}{C_a}\,\frac{A}{K_L}\,\frac{1}{\Psi_{wp}-\Psi_{wm}} \text{ or,}$$

$$\delta^{13}C \approx \delta^{13}C_a - b + (b-a)\,\frac{1.6\nu}{C_a}\,\frac{A}{K_L}\,\frac{1}{\Psi_{wp}-\Psi_{wm}} \quad (5)$$

2.3. Field water relations

The following components of field water relations were assessed in trees mainly of the Paracou plantation (see Bonal et al., 2000b for details):

- Diurnal and seasonal changes in leaf gas exchange and water potential. Daily time-courses of leaf water potential were established simultaneously to the gas exchange (A and g) measurements, starting with a predawn measurement (Ψ_{wp}).
- Sap flow, whole-tree hydraulic conductance and canopy conductance for water vapour. Constant heating radial flowmeters (Granier, 1985) were used. At tree level, they allowed estimation of sap flow density (the transpirational water flow per unit sapwood cross-sectional area Ju, kg dm^{-2} h^{-1}). Whole-tree hydraulic conductance was estimated either on a leaf area basis (K_L, leaf area specific hydraulic conductance, mmol m^{-2} s^{-1} MPa^{-1}) or on a sapwood area basis (K_S, sapwood area specific hydraulic conductance, mmol m^{-2} s^{-1} MPa^{-1}). At canopy level, sap flow measurements were used (1) to assess the variability of tree transpiration, and (2) to estimate whole stand transpiration and canopy conductance to water vapour by considering the big leaf model, which states an analogy between leaf and canopy H_2O exchange regulation (Granier et al., 1992; Granier et al., 1996)
- Soil water content and $H_2{}^{18}O$ extraction profiles. Vertical profiles of soil water content (SWC, % on a dry soil gravimetric basis) were established in the three plots for both seasons. In order to assess soil water extraction features in the plots corresponding to the different species, we used an approach based on the assessment of the natural composition of stable oxygen isotopes ($\delta^{18}O$) in soil and sapwood xylem water. This methodology is based on the fact that (1) soil water extraction by roots and xylem transfer does not induce isotopic fractionation of oxygen isotopes of water (Allison et al., 1983), and (2) gradients in $\delta^{18}O$ of soil water with soil depth may arise from seasonal variations in rainfall isotope signature (Craig, 1961; Dansgaard, 1964) and from the isotope fractionation that occurs during surface soil water evaporation (Ehleringer and Dawson, 1992). Therefore, by comparing instantaneous $\delta^{18}O$ of xylem sap water with that of soil water, it is possible to interpolate a mean soil depth where roots extract water (Jackson et al., 1995; Bonal et al., 2000a).

3. Interspecific variability of leaf $\delta^{13}C$ and gas exchange traits

3.1. Range of variations and environmental stability

We found a high variability of sunlit leaf $\delta^{13}C$ among species. Mean species $\delta^{13}C$ values varied over a ca. 6‰ range (Fig. 1, upper panels). Differences among species were highly significant ($P < 0.001$) and were

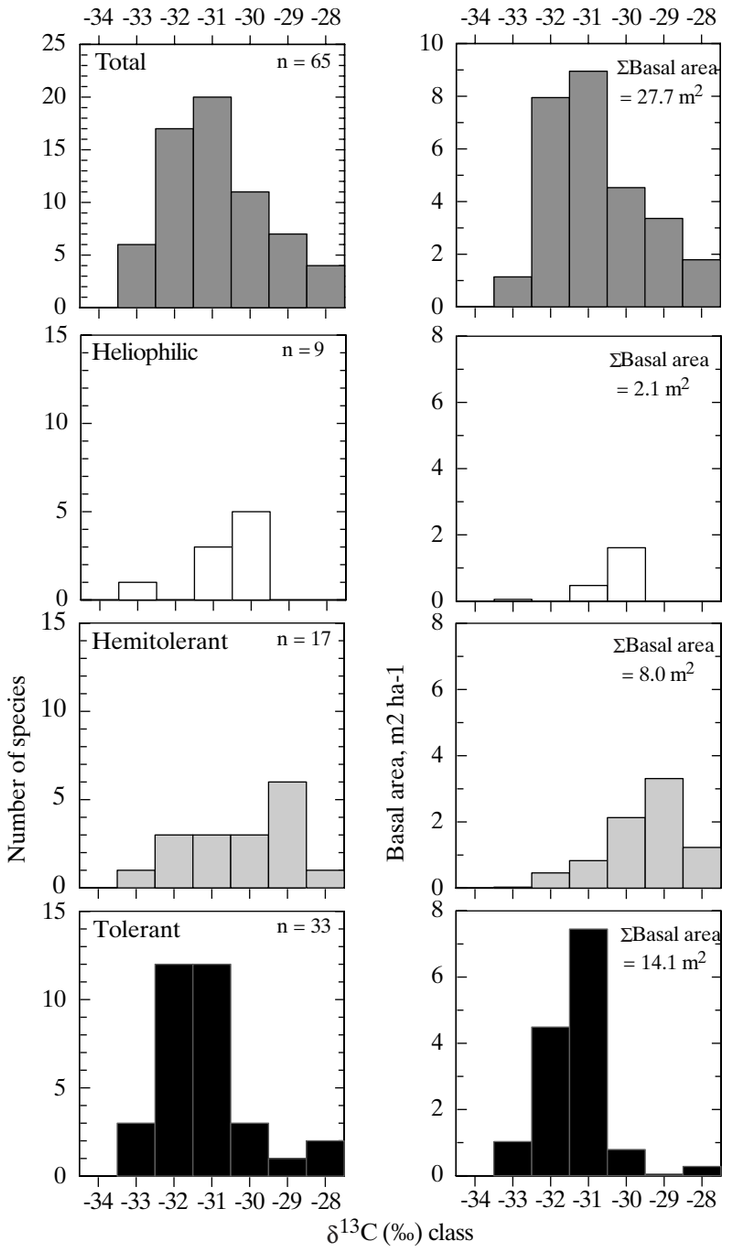

Fig. 1. Frequency distributions of sampled species (left) and the corresponding basal area values represented by these species (right) by $\delta^{13}C$ classes for the three different shade tolerance types (heliophilic, hemitolerant, tolerant). Six species could not be attributed to shade tolerance types. The total basal area of the stand was 34.1 m^2 ha^{-1}. Species for which $\delta^{13}C$ was not assessed represented a basal area of 6.4 m^2 ha^{-1}. Adapted from Bonal et al. (2000c).

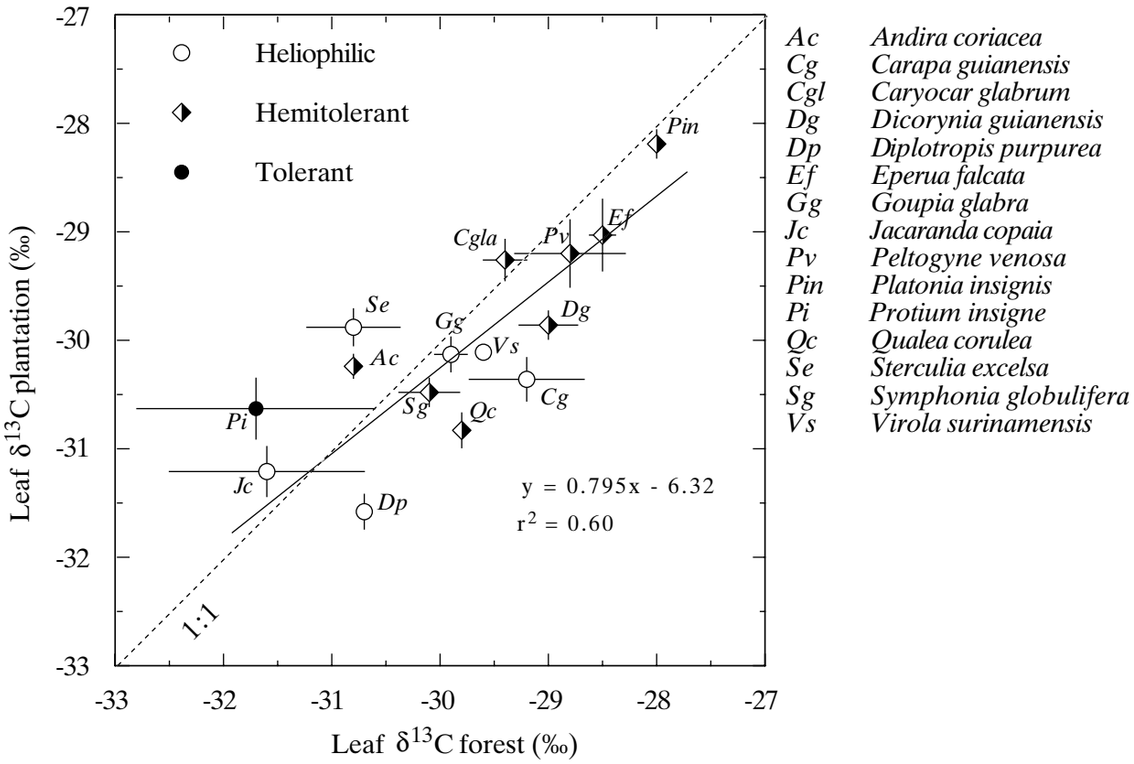

Fig. 2. Relationship between sunlit leaf carbon isotope composition ($\delta^{13}C$, mean values ±SE) in trees of different species growing either in the forest or in the monospecific plots of the Paracou plantation. Different shade tolerance types were distinguished.

consistent with the results found for a nearby stand of Paracou forest for a lower number of species (Guehl et al., 1998). Martinelli et al. (1998) assessed the interspecific variability of tree leaves $\delta^{13}C$ in an Amazonian rainforest in Rondônia (Brazil), considering both upper and lower canopy trees. They found an overall range of variability of 7.1‰ for the average species $\delta^{13}C$ values. Considering only upper canopy trees (total height > 25 m), this range was 5.5‰ (from –34.3 to –28.8‰), which is remarkably consistent with our results.

Despite the differences in (1) soil characteristics between Paracou forest and plantation, due to the severe perturbation of the upper soil layers in the plantation, and (2) the type of competition between trees (interspecific vs. intraspecific), mean species $\delta^{13}C$ was tightly correlated between the two situations, the slope of regression line not being significantly different from unity (Fig. 2). This stability suggests a predominant genetic intrinsic control of species $\delta^{13}C$ under conditions prevailing in the two situations.

According to the classical two-step model of carbon isotope discrimination (equation 3) during photosynthesis (Farquhar et al., 1982), the range of about 6.0‰ we observed between species in the Paracou forest (Fig. 1) would correspond to a difference of 85 µmol mol^{-1} in average C_i and to WUE = A/g ranging from 28 to 82 µmol mol^{-1}, i.e. WUE values varying over a threefold range.

Gas exchange measurements made on several species in the Paracou plantation allowed to assess the consistency of gas exchange with $\delta^{13}C$ and Δ differences among species and to analyse the physiological components underlying these differences according to equation 3. We obtained a negative relationship between Δ and A/g which did not significantly (P = 0.05) differ from the theoretical relationship expected from equation 3 (Fig. 3). Clearly, for the set of species considered, the differences in Δ

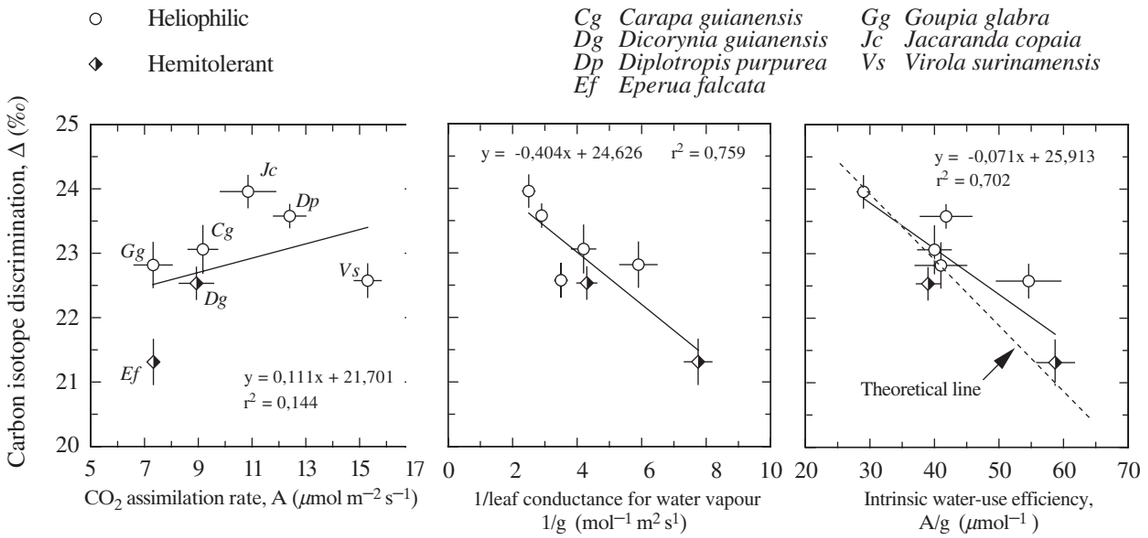

Fig. 3. Relationships between carbon isotope discrimination (Δ) and leaf gas exchange in trees of different species, representing two distinct shade tolerance groups (no shade tolerant species were available), growing in the monospecific plots of the Paracou plantation. The dashed line in the right hand side panel represents the theoretical Δ vs. A/g relationship according to equation 3; the coefficients of the observed relationship did not significantly differ ($p = 0.05$) from the theoretical values. Data from Huc et al. (1994) and Bonal et al. (2000b).

and $\delta^{13}C$ among species were driven by differences in g, since a negative relationship was observed between Δ and $1/g$. Species also displayed differences in A, which were however positively related to Δ, thereby tending to offset the influence of differences in g on Δ (equation 3).

3.2. Association with leaf phenology patterns and shade tolerance types

Evergreen-leaved species displayed more negative $\delta^{13}C$ values than deciduous-leaved ones (Table 1). Sobrado and Ehleringer (1997) found similar results in a tropical dry forest. Because, in wet tropical conditions, the drought-deciduous type is only one among different deciduous patterns (Loubry, 1994), differences in phenology cannot yet be clearly interpreted from an ecological or ecophysiological point of view. The association found here between $\delta^{13}C$ and phenology patterns remains to be elucidated (Terwilliger et al., 2001).

Our results also clearly point to the absence of an association between the gradients in $\delta^{13}C$ values and the area of distribution of the species (Table 1); namely, there was no peculiar $\delta^{13}C$ characteristic for those species extending towards the dry tropics.

Marked differences in species $\delta^{13}C$ were found with respect to the different types of shade tolerance (Table 1). This is also reflected in distinct species frequency and basal area distributions with respect to $\delta^{13}C$ classes for the different types of shade tolerance (Fig. 1).

A main environmentally robust result of our studies consisted in the non linear association between $\delta^{13}C$ and the gradient of shade tolerance. Heliophilic species indeed exhibited more negative $\delta^{13}C$ (i.e. lower WUE) than did hemitolerant species. However, tolerant species displayed even more negative $\delta^{13}C$ values than did heliophilic species (Fig. 1, Table 1).

3.3. Association with taxonomic position

Interestingly, differences in $\delta^{13}C$ among species were associated with their taxonomic situation at family level. Differences between families were highly significant ($P < 0.001$) and corresponded to a range of 3.5‰. (Fig. 4), i.e. WUE varying over almost a twofold range. It is worth noting that families considered as archaic from an evolutionary point of view (Myristicaceae, Lauraceae, Chrysobalanaceae) (Judd et al., 2002) displayed extremely low $\delta^{13}C$ and thus low WUE. However, due to the low number of

Table 1
Mean leaf $\delta^{13}C$ values (±1 S.E.) of canopy tree species growing in Paracou forest. Species were grouped according to their tolerance to shade (Tolerant; Hemitolerant; Heliophilic), or to their range of distribution in South America (1, Guianas; 2, Guianas + North-eastern Amazon; 3, Guianas + Amazon; 4, Amazon to Panama; 5, Tropical South America), or to their pattern of leaf phenology (deciduous-leaved; evergreen-leaved). Mean values not sharing common letters are significantly different (Duncan's multiple range test, $P < 0.05$).

	$\delta^{13}C$ (‰)
Shade tolerance	$P < 0.001$
Tolerant	-31.6 ± 0.1^{c}
Hemitolerant	-29.7 ± 0.2^{a}
Heliophilic	-30.6 ± 0.3^{b}
Area of distribution	$P = 0.12$
1	-31.1 ± 0.2
2	-31.0 ± 0.2
3	-31.4 ± 0.4
4	-30.8 ± 0.4
5	-30.2 ± 0.3
Leaf phenology	$P < 0.001$
Deciduous	-30.3 ± 0.2^{a}
Evergreen	-31.2 ± 0.1^{b}

trees considered in some families, it is not possible here to draw clear conclusions for the overall association between systematic position and $\delta^{13}C$.

Furthermore, considering the within-family variations of $\delta^{13}C$, the non linear association between $\delta^{13}C$ and shade tolerance types described above still held (Table 2). Clearly, when considering the most abundant families, within-family $\delta^{13}C$ differences among species occurred for those families (Caesalpiniaceae, Clusiaceae) encompassing both hemitolerant (high $\delta^{13}C$) and heliophilic or tolerant (low $\delta^{13}C$) species, whereas in families not encompassing hemitolerant species, but only heliophilic and/or tolerant species (Chrysobalanaceae, Lecythidaceae, Sapotaceae), no significant species effects were noticed. This similarity in patterns points to the existence of convergent evolution in the different families. Analysing species $\delta^{13}C$ values with a systematic and phylogenetic perspective may be a worthy approach for the future.

4. Ecophysiological interpretation of the interspecific variability in $\delta^{13}C$ and water potential

4.1. Range of variations of water potential and environmental stability

Predawn leaf water potential (Ψ_{wp}) remained higher than –0.2 MPa in all species in the different situations (data not shown), pointing to the absence of important tree water deficit, even in the pronounced dry season situation. This is consistent with the fact that there was no substantial difference in midday leaf water potential (Ψ_{wm}) between wet season and dry season values both in the two Paracou sites (forest and plantation) and in piste de St-Elie forest (Fig. 5). Mean species Ψ_{wm} values spanned a wide range of values from –0.2 MPa to ca –3.5 MPa. For those species occurring in different sites, the Ψ_{wm} values were stable with respect to the different environments (Fig. 5). This suggests, as for the $\delta^{13}C$ values, a predominant intrinsic genetic control of Ψ_{wm} at species level.

4.2. Ecophysiological basis of ecological strategies

The pattern of association between $\delta^{13}C$ and the three shade tolerance types in piste de St-Elie forest (Y-axis of Fig. 6) was similar to that found in Paracou (Fig. 1), heliophilic species being intermediate between hemitolerant and tolerant species ($P < 0.001$). $\delta^{13}C$-derived average WUE = A/g values (equation 3) were: 44 (heliophilic), 60 (hemitolerant) and 36 (tolerant) mmol mol^{-1}, respectively.

Contrastingly, there was no significant difference ($P = 0.05$) in Ψ_{wm} among the shade tolerance types (X-axis of Fig. 6). A loose, but significant, negative relationship was found between the average species values of $\delta^{13}C$ and Ψ_{wm} (Fig. 6). Rather than representing a tight trade-off between traits, this relationship is merely to be ascribed to the absence of data points in two distinct areas of the graph (see dotted areas in Fig. 6), possibly reflecting functional domains not allowed by natural selection:

– (α), low $\delta^{13}C$ together with low Ψ_{wm}. Typically, this would be achieved by trees combining high g and low K_l (equation 4), which would be prone to drought induced

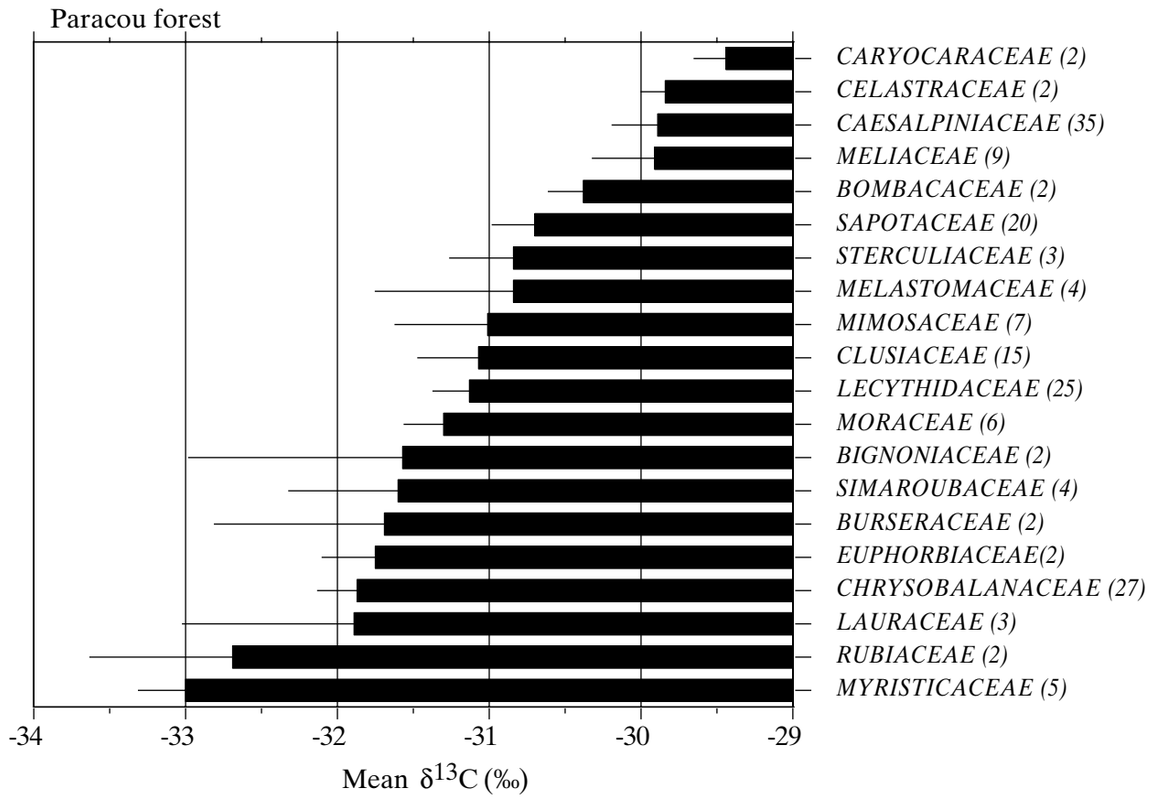

Fig. 4. Sunlit leaf carbon isotope composition ($\delta^{13}C$, mean values ±SE) in the different families occurring in the studied plot of Paracou forest. The number of sampled trees is given for each family. Data from Bonal et al. (2000c).

irreversible xylem embolism (Tyree and Sperry, 1988; Tyree, 2002; see also paragraph 5.2 below)

– (β), low $\delta^{13}C$ together with low Ψ_{wm}. We cannot provide a straightforward interpretation for this exclusion zone so far. Diffusional limitations in CO_2 assimilation due to low *g*, precluding sufficient C supply to trees, can be evoked.

The main result in Fig. 6 consists in the fact that species average data points were ordered along the direction of maximum variation of A/K_L (i.e. normal to iso-values). Calculated A/K_l values differed among the three shade tolerance types (4.9, 6.7 and 3.5 10^{-3} MPa in the heliophic, hemitolerant and tolerant groups, respectively). Considering the average A values found (Bonal, personal communication) in an experiment with potted young trees for these three groups (10.2, 8.3 and 7.15 µmol m^{-2} s^{-1}), the following average values can be calculated: $K_l = 2.1$ (heliophilic), 1.3 (hemitolerant) and 2.0 (tolerant) mmol m^{-2} s^{-1} MPa^{-1}. Similarly, average g values can be calculated from $\delta^{13}C$ by using equation 3: $g = 233$ (heliophilic), 138 (hemitolerant) and 200 (tolerant) mmol m^{-2} s^{-1}. For the two former groups, these indirectly assessed g values were quite consistent with those directly measured in the Paracou plantation (Fig. 3). The K_L values calculated for the heliophilic and hemitolerant groups were also in accord with those measured for *Eperua falcata* and *Diplotropis purpurea* in Paracou plantation (Table 3).

The low $\delta^{13}C$ and WUE found for heliophilic species is in agreement with the 'gambler' (i.e. resource waster) ecological strategy proposed by Oldeman and van Dijk (1991), leading to the ability of heliophilics to rapidly dominate neighbours. A main result of our investigations consists in the distinction of two groups among the late stage species (heliophilic species

Table 2
Sunlit average species, or shade tolerance type, leaf $\delta^{13}C$ (±SD) and ANOVA results at family level for species and shade tolerance type effects. Only the most represented botanical families of the Paracou forest (n° of sampled tree ≥ 15) were considered.

FAMILY – *Shade tolerance* *Species*	*Nb. of trees*	*Leaf $\delta^{13}C$* *ANOVA at family level (p > F)* *Mean values (‰)*	
		Shade tolerance	*Species*
CAESALPINIACEAE	(35)	***	***
– *Hemitolerant*		–28.6 ± 0.7	
Recordoxylon speciosum	(5)		–28.0 ± 0.8
Eperua falcata Aubl.	(5)		–28.5 ± 0.3
Eperua grandiflora (Aubl.) Benth.	(4)		–28.9 ± 0.3
Dicorynia guianensis Amsh.	(5)		–29.0 ± 0.6
Swartzia polyphylla D.C.	(1)		–29.2
– *Heliophilic*		–30.2 ± 0.5	
Sclerolobium melinonii	(3)		–30.0 ± 0.5
Diplotropis purpurea	(1)		–30.7
– *Tolerant*		–32.1 ± 1.2	
Bocoa prouacensis Aubl.	(5)		–31.8 ± 1.4
Vouacapoua americana	(6)		–32.3 ± 1.0
CHRYSOBALANACEAE	(27)	ns[1]	ns
– *Heliophilic*		–30.9 ± 1.2	
Parinari montana	(1)		–29.8
Parinari sp.	(2)		–31.5 ± 1.0
– *Tolerant*		–32.0 ± 1.3	
Licania majuscula	(1)		–30.6
Licania ovalifolia	(2)		–31.7 ± 0.4
Licania robusta	(5)		–31.8 ± 1.6
Licania membranacea Sagot	(5)		–32.0 ± 1.7
Licania alba (Bernoulli) Cuatr.	(6)		–32.1 ± 1.4
Licania minutiflora	(5)		–32,4 ± 1.0
CLUSIACEAE	(15)	**	*
– *Hemitolerant*		–30,3 ± 0.8	
Symphonia globulifera L.f.	(6)		–30.1 ± 0.7
Monorobea coccinea	(4)		–30.7 ± 0.9
– *Tolerant*		–32.5 ± 1.7	
Tovomita sp.	(5)		–32.5 ± 1.7

Table 2 *(continued)*

FAMILY – <u>Shade tolerance</u> *Species*	*Nb. of trees*	*Leaf $\delta^{13}C$* *ANOVA at family level ($p > F$)* *Mean values (‰)*	
		Shade tolerance	*Species*
LECYTHIDACEAE	(24)	nr[2]	ns
– <u>Tolerant</u>		–31.2 ± 1.2	
Lecythis chartacea	(3)		–30.8 ± 1.1
Couratari pulchra	(4)		–30.8 ± 0.8
Eschweilera sp1.	(6)		–31.0 ± 0.9
Lecythis idatimon Aubl.	(6)		–31,4 ± 1.1
Gustavia hexapetala	(5)		–32.0 ± 0.7
SAPOTACEAE	(20)	nr	ns
– <u>Tolerant</u>		–30.7 ± 1.3	
Chrysophyllum prieurii	(2)		–29.7 ± 0.1
Chrysophyllum sanguinolentum	(4)		–30.3 ± 0.6
Pradosia cochleria	(4)		–30.5 ± 1.4
Pouteria melanopoda	(5)		–30.8 ± 1.3
Micropholis guianensis	(5)		–31.4 ± 1.7

[1]ns: not significant ($P < 0.05$); [2]nr: not relevant.
*: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$.

excluded) based on the $\delta^{13}C$ values, in correspondence with Favrichon's hemitolerant and tolerant groups. Tolerant species are able to grow in understorey conditions where light is the main limiting factor. The extremely negative $\delta^{13}C$ found in this group appears to be associated with high g, possibly allowing to maximise carbon assimilation under low light (Givnish, 1988). It is noteworthy that low $\delta^{13}C$ was maintained for trees reaching the upper canopy. The hemitolerant group encompasses most emergent species, whereas emergent species are not included in the tolerant group (Favrichon, 1994). High WUE in the former group may be considered as an adaptive trait due to the high evaporative demand prevailing in the emerging tree crowns. The low K_L values found for the hemitolerant group, together with the tendency for low Ψ_{wm}, suggests that transpiration is constrained by hydraulic architecture in this group (Tyree, 2002).

5. Contrasting patterns of water relations and leaf gas exchange responses to drought

5.1. Patterns of drought responses

The responses of leaf gas exchange to soil drought assessed in controlled climate chamber conditions with potted plants differed among the three species (Fig. 7). Anisohydric species are characterized by leaf water potential (Ψ_w) which decreases markedly with increasing atmospheric evaporative demand during the day and with increasing soil drought. In contrast, isohydric species maintain Ψ_w constant during the day at a value which does not depend on soil water status until plants are close to death (Stocker, 1956; Tardieu and Simonneau, 1998). While in *Eperua falcata* stomatal closure occurred from soil water content (Θ) of ca. 0.15 $m^3\ m^{-3}$, *Diplotropis purpurea* and

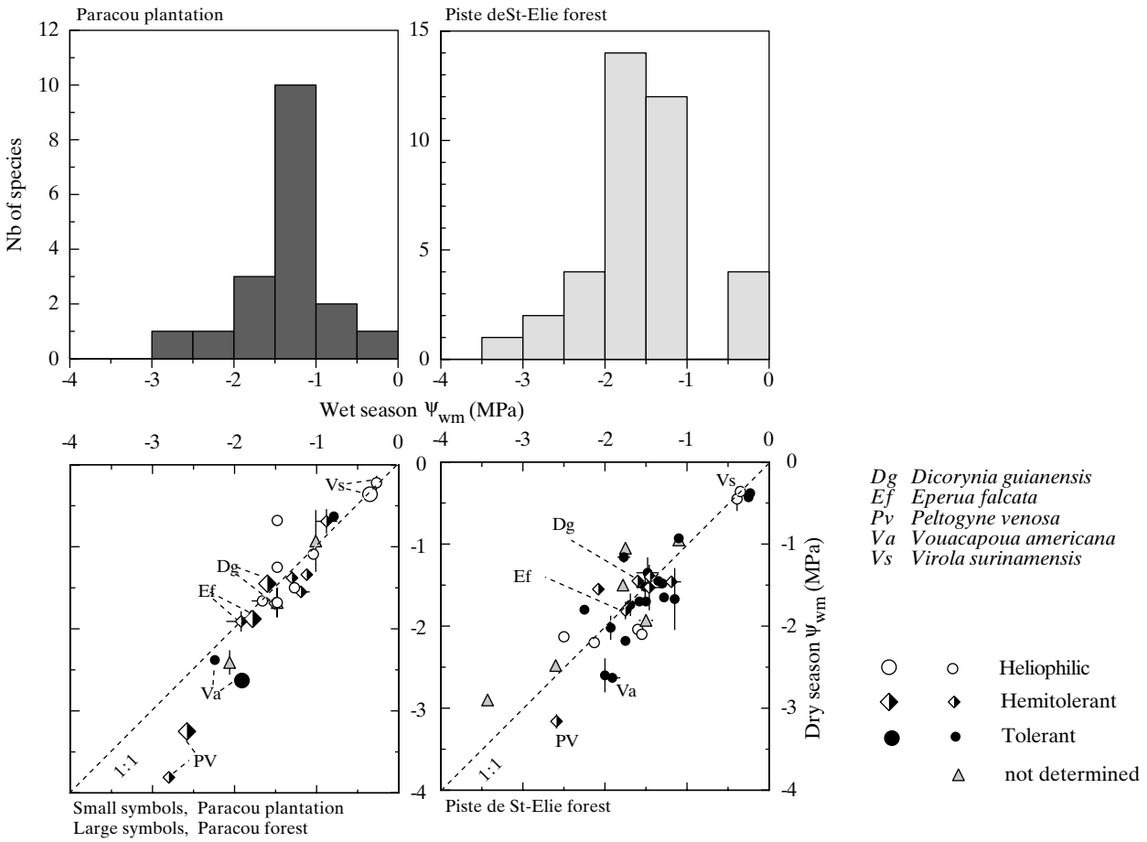

Fig. 5. (Bottom) Relationships between dry season (October 1998) and wet season (May 1999) midday water potential (Ψ_{wm}) in a range of species (average species values ±SEM) in Paracou (forest and plantation) and in the piste de St-Elie forest. Different shade tolerant types were distinguished. Only species occurring both in Paracou and in the St-Elie forest were identified (abbreviations). (Top) Frequency distributions of sampled species by wet season Ψ_{wm} classes in the Paracou plantation and in the piste de St-Elie forest.

particularly *Virola michelii* were characterised by higher thresholds for the onset of stomatal closure (Fig. 7). *Eperua falcata* had characteristics of stomatal control consistent with the anisohydry. *Diplotropis purpurea* had an isohydric behaviour at least at the beginning of the drying cycle, since Ψ_{wm} did not decrease – and $\Psi_{wp} - \Psi_{wm}$ did not increase – over a large range of Θ (down to $< 0.1 m^3 m^{-3}$). Such a stomatal control involves an interaction between hydraulic (i.e. leaf water status) and root-to-shoot chemical signals (Tardieu and Simonneau, 1998). In drying soil, chemical signals induce stomatal closure in isohydric species (Davies and Zhang, 1991; Tardieu, Zhang and Gowing, 1993) and stomata are more sensitive to these signals when the transpiration rate – which depends on leaf-to-air vapour pressure difference – is high, or when Ψ_w is low (Tardieu and Simonneau 1998). The sensitivity of g_s to Θ in *Diplotropis purpurea* was even accompanied by a slight increase in Ψ_{wm} with Θ decreasing down to $0.1 m^3 m^{-3}$. More pronounced features of isohydry were found in *Virola michelii*, combining (1) Ψ_{wm} which remained remarkably stable over the drying cycle, at least as long as leaves did not dehydrate, so that Ψ_{wm} did not depend on soil water content, and (2) extremely high stomatal sensitivity to soil drought (Fig. 7). Similar results were found by Reekie and Bazzaz (1989) for three tropical gap-tree species (*Cecropia obtusifolia*, *Myriocarpa longipes*, *Piper auritum*).

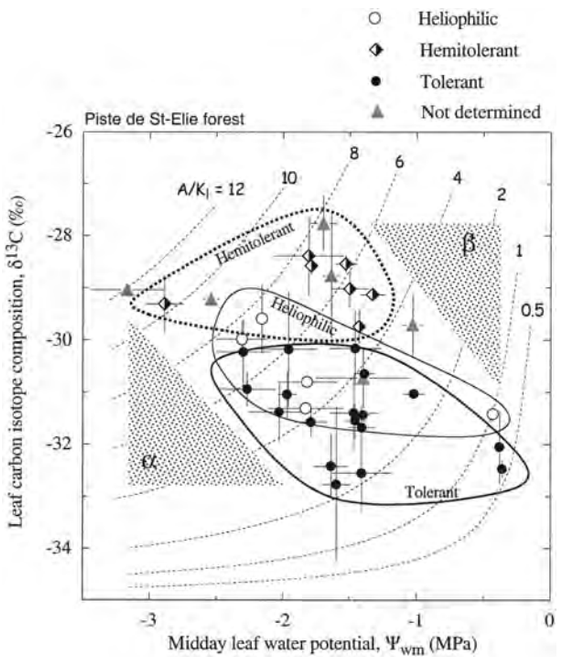

Fig. 6. Relationship between midday leaf water potential (Ψ_{wm}) and sunlit leaf ($\delta^{13}C$). Average species values (±SE) are reported. Different shade tolerant types were distinguished (outlines drawn by hand). A significant correlation was observed between the two variables ($y = -2.09\times -33.7$, $r^2 = 0.17$, $P < 0.05$). Dotted lines represent iso-values for A/K_L and were drawn based on equation 5 considering typical daily mean values for $\delta^{13}C_a$ (–7.85‰), ν (15 mmol mol^{-1}) and C_a (358 µmol mol^{-1}) (Buchmann et al., 1997 ; Bonal et al., 2000b) and assuming $\Psi_{wp} = 0$. Dotted areas α and β represent functional domains without species datapoints (see text).

Table 3
Predawn (Ψ_{wp}) and midday (Ψ_{wm}) water potential, calculated leaf area specific hydraulic conductance (K_L, mmol m^{-2} s^{-1} MPa^{-1}) and sapwood area specific hydraulic conductance (K_S, mmol m^{-2} s^{-1} MPa^{-1}) of three rainforest species in Paracou plantation in October 1997 (dry season) and July 1998 (wet season). Mean values ±S.E. For a given line, different letters indicate statistical differences ($P < 0.05$).

	Units	*Species*		
		Eperua falcata	*Virola surinamensis*	*Diplotropis purpurea*
Ψ_{wp}	MPa			
October 1997		–0.14 ± 0.01^a	–0.14 ± 0.01^a	–0.13 ± 0.02^a
July 1998		–0.16 ± 0.01^a	–0.15 ± 0.01^a	–0.20 ± 0.01^b
Ψ_{wm}	MPa			
October 1997		–1.82 ± 0.04^c	–0.14 ± 0.01^a	–0.62 ± 0.07^b
July 1998		–1.90 ± 0.18^c	–0.27 ± 0.04^a	–1.52 ± 0.05^b
K_L	mmol m^{-2} s^{-1} MPa^{-1}			
October 1997		1.5 ± 0.1^c	29.3 ± 22.3^a	7.7 ± 1.5^b
July 1998		1.4 ± 0.3^c	99.6 ± 65.8^a	2.8 ± 0.1^b
K_S	mmol m^{-2} s^{-1} MPa^{-1}			
October 1997		7.8 ± 1.0^c	54.4 ± 6.7^a	31.2 ± 4.5^b
July 1998		19.0 ± 3.8^c	296.5 ± 48.5^a	27.5 ± 3.8^b

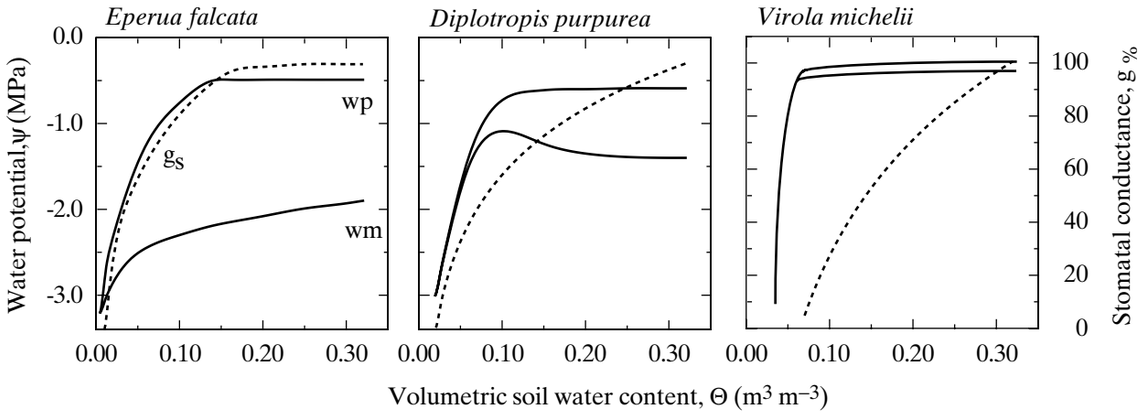

Fig. 7. Contrasting responses of predawn (Ψ_{wp}) and midday (Ψ_{wm}) leaf water potential and of stomatal conductance (*g*, dotted line) to decreasing soil water content in seedlings of three species. Data from a soil drying cycle experiment with containerised seedlings grown in a climate chamber. Adapted from Bonal and Guehl (2001).

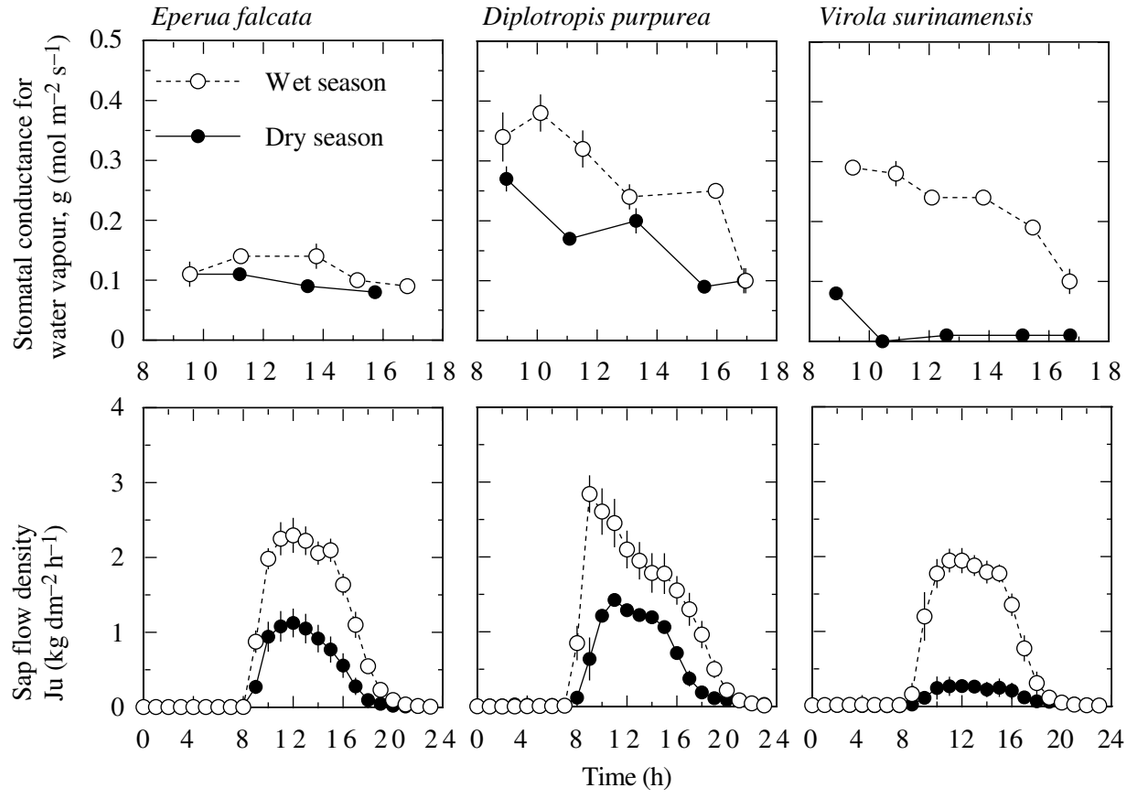

Fig. 8. Daily time-course of sunlit leaf stomatal conductance (*g*) and of stem sap flow density (Ju) for three rainforest tree species in the monospecific plots of the Paracou plantation in wet (July 1998) and dry (October 1997) seasons. Mean values (±SE) for three or four trees per species. Adapted from Bonal et al. (2000b).

5.2. Field water relations

These contrasting patterns of drought responses were clearly validated by the results from the in situ assessment (field experiment in Paracou plantation) of water relations (*Virola michelii* was replaced by *Virola surinamensis*). In none of the species was predawn water potential markedly reduced in the dry season as compared to the wet season (Table 3), pointing to the absence of significant tree water stress. In this situation, *Virola surinamensis* displayed an almost complete stomatal closure as of 10h00, confirming the extremely high stomatal sensitivity to soil drought in this species (Fig. 8). Contrastingly, *g* as well as sap flow density (Ju) was less affected in the dry season in *Diplotropis purpurea* and particularly in *Eperua falcata*.

The small differences between Ψ_{wm} and Ψ_{wp} in *Virola surinamensis* were associated with a large whole-tree hydraulic conductance for both leaf gas exchange derived K_l values (leaf area specific hydraulic conductance) and sapflow derived K_s values (sapwood area specific hydraulic conductance). Large hydraulic conductance combined with high stomatal sensitivity to soil drought may allow *Virola* to avoid xylem embolism under pronounced soil drought (Tyree and Sperry, 1988; Tyree and Ewers, 1996).

Combining the information from the vertical soil water content and $H_2{}^{18}O$ extraction profiles, we could also assess mean water extraction depths in the three monospecific plots. In the *Virola surinamensis* plot, no seasonal change in soil water content was observed under 250 cm depth (Fig. 9, right side panels), pointing to the absence of water extraction. Thus, the only likely mean extraction depth in this species that arose from the isotopic data (Fig. 9, main panel) for the harsh dry season conditions corresponded to the upper 20 cm soil layer. In *Diplotropis purpurea*, and particularly *Eperua falcata*, seasonal changes in soil water content and isotopic data pointed to deeper water extraction zones in the dry season. Deep water extraction, associated with a deep rooting pattern, was also found for *Eperua falcata* in a pristine forest situation (Bonal et al., 2000a) and has been shown to play a prominent role in the high annual evapotranspiration values characterising rainforests (Nepstad et al., 1994).

For the species selected for the assessment of drought responses and field water relations, a clear correspondence was found between (1) increasing difference ($\Psi_{wp} - \Psi_{wm}$) in the absence of drought (Fig. 7), which relates to the degree of anysohydry and possibly of drought tolerance, (2) decreasing stomatal sensitivity to drought, and (3) increasing potential soil water extraction depth. Whether this association can be generalised is still an open, but worthy, question.

5.3. Scaling up from tree to canopy transpiration

When considering sap flow density of the trees in Paracou forest, large differences were found among species (Fig. 10), both in maximum values and in the daily patterns. High Ju was recorded in *Eperua falcata* and *Dicorynia guianensis*, while lower values were observed in *Vouacapoua americana* and in *Carapa procera*. *Dicorynia guianensis* showed a stomatal closure around 10h00, when vapour pressure deficit (VPD) increased above ca. 10 hPa, indicating a high sensitivity of this species to atmospheric drought. Combining individual tree sap flow measurements and sapwood area distribution within the stand (Granier et al., 1996), we were able to scale up tree transpiration to the stand level. Canopy-level transpiration amounted to ca. 75% of Penman potential evapotranspiration, i.e. close to that often observed in high leaf area index temperate forests during the growing season. Canopy conductance to water vapour (g_c) was derived from climate measurements and from estimated stand transpiration. A negative effect of VPD on g_c was found, as previously observed in temperate forests (Fig. 11). Nevertheless, this negative effect appeared to be stronger in the Paracou forest than in a beech forest (Granier et al., 2000), indicating a higher stomatal sensitivity to VPD for the tropical than for the temperate broadleaved tree species, which is somewhat in contradiction with Shuttleworth's (1989) conclusion that the response of the whole canopy to climate is similar when comparing tropical to temperate forests.

6. Conclusions

Leaf $\delta^{13}C$ and water potential proved useful to assess community-level functional diversity in rainforests. We found an extremely high interspecific variability in sunlit $\delta^{13}C$ among canopy trees, the range of variations being similar to that found over broad climatic gradients (Schulze et al., 1998; Leffler and Enquist, 2002) or among distinct life forms within

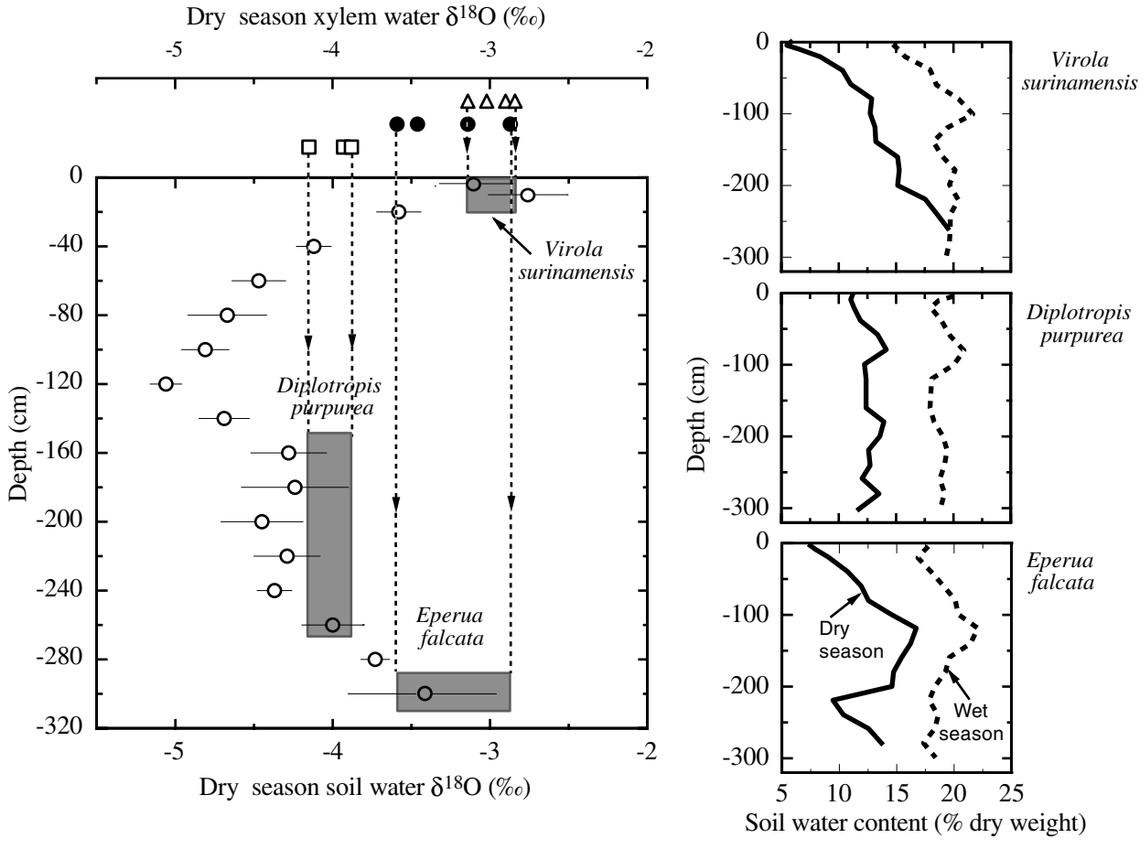

Fig. 9. (Left) Vertical profile of soil water oxygen isotope composition ($\delta^{18}O$) in a severe dry season situation (October 1997) in the Paracou plantation (average values ±SEM, four holes x three species) and corresponding xylem $\delta^{18}O$ in trees of three species ($n = 3$ or 4). Vertical soil water $\delta^{18}O$ profiles were identical for the three monospecific plots and data were pooled between the plots. Shaded areas correspond to the estimated mean depths of soil water extraction for each species inferred from water $\delta^{18}O$ measurements (projection of xylem values on the soil profiles) and by considering soil water content data for the three species (see text). (Right) Vertical profiles of soil water content in the three monospecific plots ($n = 4$ for each plot) in wet (July 1998) and dry (October 1997) seasons.

communities (Brooks et al. 1997) in different types of forests. This variability can clearly be ascribed to intrinsic species differences and appears to be at least partly driven by differences in stomatal conductance and also in hydraulic features among species, as it arises from the indirect approach we used by combining species $\delta^{13}C$ values and leaf water potential values.

A main and original result of our studies is the association established between species $\delta^{13}C$, a trait related to leaf gas exchange regulation, on the one hand, and species shade tolerance types, on the other hand. This result points to the existence of potential deterministic links between ecosystem function (gas exchange regulation) and tree community dynamic features (e.g. perturbations that may affect the relative proportions of the different shade tolerance types).

We could provide here a clear confirmation, based on a leaf functional trait ($\delta^{13}C$), of the existence of two distinct groups within the non heliophilic late stage species (Favrichon, 1994) as well as elements for the functional characterisation of these groups. This distinction does not conform to the simple paradigm of heliophilic/shade-tolerant dichotomy. The non monotony in the relationship between species $\delta^{13}C$ and the gradient of species shade tolerance (heliophilic – hemitolerant – tolerant) proved robust over the

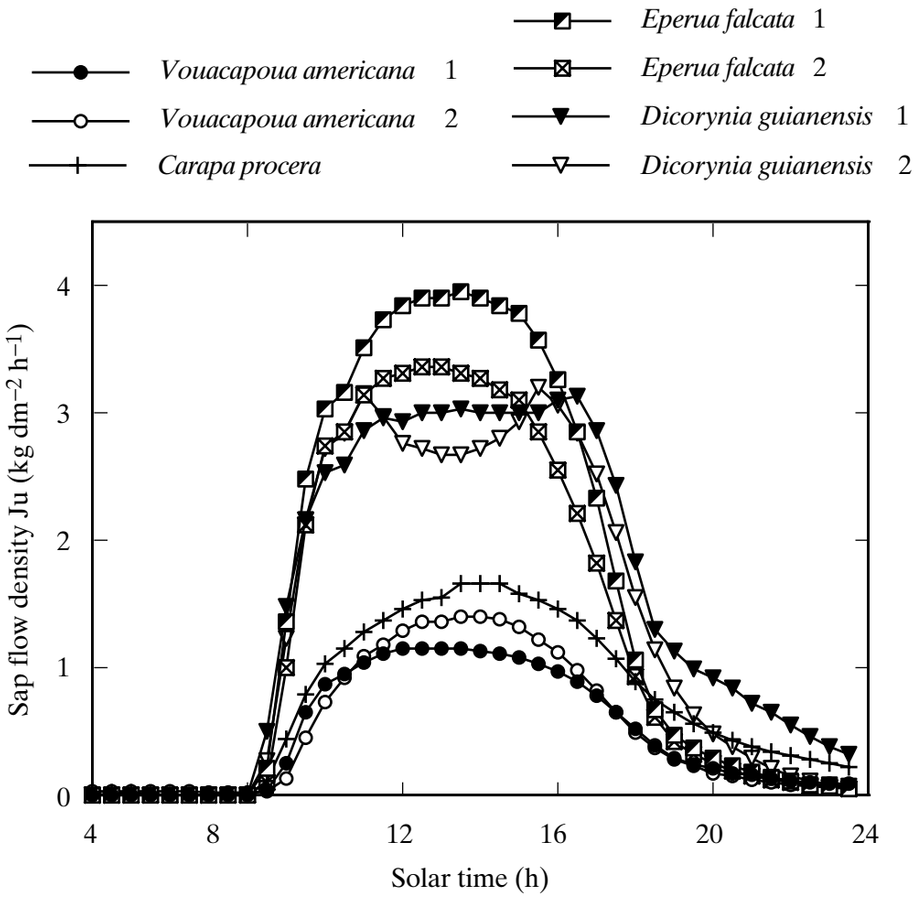

Fig. 10. Daily time course of sap flow density (Ju) in trees of different species in Paracou forest. Adapted from Granier et al. (1996).

different sites and environmental conditions. This result deserves further attention and should now be considered within the framework of assessment of trade-offs between life-span and structural and functional leaf traits over different life-forms and functional types within plant communities (Reich et al 1997). Carbon isotope composition, or any other trait related to leaf gas exchange regulation, has not so far been considered in such approaches.

Our results clearly point to the fact that in the tropical rainforest co-existing species display contrasting patterns of stomatal regulation and avoidance of plant water deficit, in response to drought. These results allow to infer some conclusions from an ecological point of view. Among the species selected for thorough ecophysiological analysis, *Eperua falcata* was the least sensitive to drought and appeared to be tolerant to drought. This species will present a positive CO_2 assimilation balance under moderate to severe drought conditions and will remain competitive in a large range of environmental conditions. In contrast, *Diplotropis purpurea* seedlings will dramatically close stomata under drought. Although such a response will allow to maintain a favourable tree water status, it will induce severe restriction in the CO_2 assimilation balance when drought conditions become durable. This might result in the reduction of this species' competitivity and its ability to establish under unfavourable environmental conditions. This effect will be even more pronounced for *Virola*. Regarding the dynamics of species distributions, it might be expected that species such as

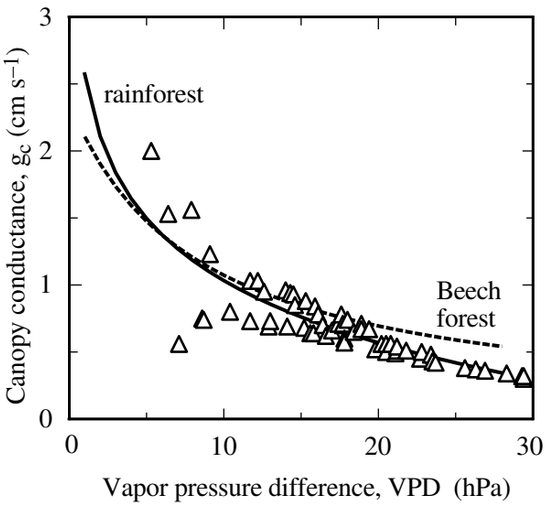

Fig. 11. Relationship between canopy conductance for water vapour diffusion (g_c) and vapour pressure deficit (VPD) for global irradiance values above 400 µmol m^{-2} s^{-1} in the Paracou forest (symbols and bold line) and in a temperate beech forest (dotted line). Canopy conductance values were scalled up from tree-level transpiration measurements. Data from Granier et al. (1996, 2000).

Diplotropis purpurea and *Virola* would be very sensitive to long-lasting drought periods such as occurred over the Guiana plateau during the Quaternary (Prance 1982). In contrast, the distribution of species like *Eperua* might be less affected by these changes.

We have provided evidence for the existence of considerable community-level diversity in functional traits among tree species related to $\delta^{13}C$, on the one hand, and water potential, stomatal sensitivity to drought and potential soil water extraction depths, on the other hand. However, no clear relationship arose between the two types of traits that could point to the existence of trade-offs between WUE and tolerance to drought, as has been suggested for biomes with dryer environments (Ehleringer, 1993). Thus, even though we found an association between $\delta^{13}C$ and shade tolerance types, our results are not fully in agreement with the concept of functional types of species (Grime 1977, Tilman 1988) stating a unique grouping of species with respect to various traits. "Cross" diversity among main functions or traits may reflect an important aspect of functional diversity in tropical rainforests.

The implications of the diversity of $\delta^{13}C$ should now also be considered within the framework of 'bottom-up' approaches which try to relate community level tree functional diversity to canopy or ecosystem carbon isotope discrimination and C and H_2O exchange, as illustrated by the scaling-up attempt made in Fig. 11 (see also Part II, Chapter 3).

Acknowledgements

We would like to thank Jean-Yves Goret, Damien Trémeaux and Pascal Imbert for their participation in the field work, as well as Claude Brechet for the isotopic analyses. This work was supported by a grant from the 'Ministère de l'Environnement' (SOFT Programme) and by an INRA PhD grant for Damien Bonal.

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Ecology and Management of a Neotropical Rainforest

Chapter 3

Insights into the carbon dynamics of tropical primary rainforests using stable carbon isotope analyses

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Abstract – Analyzing the carbon isotopic composition of ecosystem carbon pools and fluxes provides valuable information about the origin of carbon compounds, the partitioning of composite fluxes into individual flux components, as well as their major environmental and biotic controls. We synthesize the stable isotope data available for undisturbed lowland tropical rainforests, assess reasons for their variability, and address the representativeness of the French Guiana site Paracou. At Paracou, $\delta^{13}C_{leaf}$ values decreased from about –29‰ at the top of the canopy to about –34‰ in the understory. These intra-canopy changes of $\delta^{13}C_{leaf}$ values were mainly due to changes in leaf function and, to a minor extent, to changes in $\delta^{13}C_{air}$. Leaf carbon isotope discrimination (Δ_{leaf}) was lowest for leaves growing at the top of the canopy (approximately 22‰) and highest for shade leaves in the understory (approximately 26‰), reflecting increasing foliar C_i/C_a ratios from about 0.7 at the top of the canopy to 0.90 in the understory. Very similar patterns were found for other tropical lowland forests. A progressive enrichment in ^{13}C was found along the leaf–litter–soil continuum at Paracou, as observed in many tropical forest soils. Surprisingly, constant $\delta^{13}C$ values for soil organic matter were found for all tropical lowland forests, ranging from –28.3‰ to –28.0‰. Canopy air CO_2 concentrations [CO_2] throughout the Paracou canopy showed a pronounced daily pattern: [CO_2] profiles were well stratified, showing decreasing [CO_2] with increasing height above the ground. Clear seasonal patterns (wet and dry seasons) were observed only for [CO_2] close to the ground, indicating lower soil respiration fluxes in the wet season. In general, soil respiration rates measured at Paracou averaged 4 µmol CO_2 m^{-2} s^{-1} and fit well within the range reported for other lowland tropical forests, most of them studied in the neotropics. Temporal and spatial variations in [CO_2] in the Paracou canopy as well as in tropical forests of other regions were reflected in changes of $\delta^{13}C_{air}$. Isotopic mixing equations provided estimates of (1) the isotopic composition of CO_2 originating from ecosystem respiration, $\delta^{13}C_{ER}$, and (2) the integrated ecosystem carbon isotope discrimination, Δ_e. No intra-annual variability was detectable for the two parameters: $\delta^{13}C_{ER}$ was –27.5‰ in the dry season and

–27.9‰ in the wet season; (Δ_e) was estimated as 20.3‰ and 20.5‰, respectively. Both integrating isotopic measures, $\delta^{13}C_{ER}$ and Δ_e, for the Paracou site were in the median range reported for other primary rainforests. Thus, for all ecosystem compartments and at all levels examined, carbon isotopic signatures demonstrated the representativeness of the French Guiana site Paracou for lowland pristine forests globally.

Keywords: CO_2, $\delta^{13}C$, Carbon isotope discrimination, Canopy profiles, Soil respiration, Tropical ecosystems

1. Introduction

Detailed information about carbon pools and fluxes, and their controlling factors, is needed to elucidate the role of tropical forests for the global carbon budget. Tropical forests cover 17% of the earth's continents (Olson et al., 1983). More than half of the carbon in the terrestrial biomass (above and below ground) and about one third of the world's soil carbon are found here (Solomon et al., 1993). However, ecosystem studies in the tropics, which use a combination of classical and modern techniques (from biomass estimates and nutrient analyses to measurements of net ecosystem exchange and stable isotope analyses), are still limited. This lack of information considerably increases the uncertainty for global carbon models.

Internal carbon fluxes within tropical forest canopies and their interactions with soil and atmospheric exchange processes can be addressed by various means. Canopy air CO_2 concentrations ($[CO_2]$) can be used as an indicator of differences in gas exchange activities among different forest components and of interactions between the soil and the atmosphere. In response to turbulent mixing as well as to photosynthesis and respiration by vegetation and soil, $[CO_2]$ vary daily, from very high nocturnal $[CO_2]$ to low concentrations during the day (Wofsy et al., 1988). Stable isotopes (e.g. ^{13}C, ^{18}O) can be used to separate the different flux components of the biospheric–atmospheric net CO_2 exchange, because atmospheric–biospheric CO_2 sources have typically different isotopic signatures. Turbulent mixing between these two sources within the canopy and discrimination against $^{13}CO_2$ during photosynthesis results in $\delta^{13}C$ ratios of canopy air that are more depleted near the soil surface than at the top of the canopy (Buchmann et al., 1997; Lloyd et al., 1996). This effect is even more pronounced, the denser the canopy or the more active the soils or vegetation (Buchmann et al., 1996). At the leaf level, carbon isotope ratios, $^{13}C_{leaf}$, and leaf carbon isotope discrimination (Δ_{leaf}) provide useful information about long-term gas exchange, particularly in combination with classical, short-term measurements of plant water potentials or leaf assimilation rates (Bonal et al., 2000a). Analyzing the change of carbon isotopic composition along the leaf–litter–soil continuum and its spatial variability provides useful information about the carbon cycling within ecosystems (Buchmann et al., 1997, 1998a; Guehl et al., 1998). Thus, the knowledge of the isotopic signature of carbon pools and fluxes provides additional information that could not be obtained otherwise.

Tropical forests are very diverse, not only in terms of species composition and their ecophysiology or biogeochemistry, but also in their services to human society. Thus, current tropical ecology needs to answer the questions of how representative any given field site is for a certain forest type, e.g. for lowland primary tropical forests, by which factors ecological processes are controlled under current conditions, and how these processes might behave under changing climate and land use regimes. In this chapter, we will synthesize our results on isotopic signatures of carbon pools and fluxes at Paracou, a lowland primary forest site in French Guiana. We will compile a database on carbon isotope signatures for undisturbed lowland tropical forests, assess their spatial and temporal variability, and address potential reasons for those patterns. Finally, we will ask whether or not our results from Paracou are representative for primary lowland forests in the humid tropics.

2. Experimental site and methodology

In the following, we compare several datasets collected at Paracou to datasets from other lowland primary

tropical forests. However, we consider only data for undisturbed forests in the humid tropics; data for dry tropical forests or for forests subjected to anthropogenic land use change were omitted.

The most detailed data sets described here were collected at the Paracou site, close to the CIRAD-Forêt experimental plots (see Part I of this book for a description of the site and its environment). This lowland tropical forest with a leaf area index of about eight (Granier, pers. comm.) consists of three canopy layers, with the top canopy reaching 30–35 m height, the middle layer ranging from about 15 to 20 m and an understory layer of less than 2 m height.

$[CO_2]$ were measured continuously during 5 days in the dry season in 1994 and the wet season in 1995. Air was sampled from six different heights in the canopy (between 0.02 and 37 m) and $[CO_2]$ were analyzed using an infra-red gas analyzer (LI-6200, LiCor, Lincoln, Nebraska, USA). Flasks, filled with canopy air, were sampled during day- and nighttime for isotopic analyses ($n = 62$ in 1994 and 36 in 1995; Buchmann et al., 1997). The CO_2 was extracted cryogenically using a three-trap vacuum line in the laboratory within 12 h after collection. Soil respiration rates were measured regularly using a soil respiration chamber connected to a portable photosynthesis system (LI-6200). PVC tubes were installed at the site 24 h prior to measurements. This set-up was changed to collect soil- respired CO_2 for isotopic analyses by introducing a flask (filled with nitrogen) and a water trap into the closed circuit between the chamber and the analyzer ($n = 4$ in each year). Gravimetric soil water contents and soil temperatures were measured each time the flux rate was determined. Foliage samples were collected from all three canopy layers using a shot-gun in 1994 and 1995 ($n = 5$ per species and canopy layer). During the 1998 short dry season (February/March), sunlit, mature foliage ($n = 10$–15 per tree) was sampled for analyses of carbon isotope ratios and nitrogen concentrations from 187 individual trees representing 64 species (Bonal et al., 2000b). All samples were dried for 48 h at 70 °C and then ground to a fine powder. A 1-mg subsample was used for isotopic analyses (Delta S, Finnigan MAT, Bremen, Germany). For more details on the methods, see Buchmann et al. (1997).

The carbon isotope ratio ($\delta^{13}C$) was calculated as:

$$\delta^{13}C = \left(\frac{R_{sample}}{R_{standard}} - 1 \right) \times 1000‰ \quad (1)$$

where R_{sample} and $R_{standard}$ are the molar $^{13}C/^{12}C$ ratios of the sample and the standard (PDB), respectively (Farquhar et al., 1989). The overall precision for the carbon isotope measurements was 0.03‰ for gas samples and 0.11‰ for foliage samples. Leaf carbon discrimination was calculated as:

$$\Delta_{leaf} = \frac{\delta^{13}C_{air} - \delta^{13}C_{leaf}}{1 + \delta^{13}C_{leaf}} \quad (2)$$

where $\delta^{13}C_{air}$ and $\delta^{13}C_{leaf}$ represent the carbon isotope ratios of canopy CO_2 and foliage, respectively. For more details, see Buchmann et al. (1997). The ratio of internal mesophyll $[CO_2]$ to ambient concentrations (C_i/C_a) was calculated as:

$$\Delta_{leaf} = a + (b - a) \times \frac{C_i}{C_a} \quad (3)$$

where a and b are fractionation factors associated with fractionation against ^{13}C during diffusion ($a = 4.4‰$) and photosynthetic carboxylation ($b = 29‰$; O'Leary et al., 1992).

3. Leaf and soil isotopic signatures

3.1. Inter-specific variability of $\delta^{13}C_{leaf}$

Foliar $\delta^{13}C$ ratios ($\delta^{13}C_{leaf}$) and Δ_{leaf} varied considerably among different tree species within the Paracou site (Table 1). At the top canopy, inter-specific variability was higher (approximately 4‰ at 28–32 m height) than in the mid-canopy (approximately 1.5–2.5‰ at 13–18 m height) or in the understory (approximately 1.5–2‰ at 2 m height). No significant differences in $\delta^{13}C_{leaf}$ or Δ_{leaf} were found between the dry and the wet seasons. A larger survey in the same area with 102 species (406 individuals) supported these results (Bonal et al., 2000b). Mean species $\delta^{13}C$ values for sunlit, mature leaves varied by over 6‰, reflecting differences in leaf phenology (deciduous vs. evergreen) and shade tolerance (see Chapter 2, Part II, in this volume). Leaf stomatal regulation was considered to be dominating this inter-specific variation while nitrogen concentrations, varying widely, had no effect on $\delta^{13}C_{leaf}$ (Fig. 1). Sun foliage $\delta^{13}C_{leaf}$ values averaged $-31.2 \pm 1.0‰$ for evergreen species and $-30.4 \pm 1.6‰$ for deciduous tree species (±S.D.; Bonal et al., 2000b). Generally, standard deviations of sunlit $\delta^{13}C_{leaf}$ are reported as 1–2‰ for tropical forests (Broadmeadow et al., 1992; Ducatti et al., 1991; Kapos et al., 1993;

Table 1
Inter-specific variability of leaf carbon isotopic signatures ($\delta^{13}C_{leaf}$ and Δ_{leaf} in ‰) and C_i/C_a estimates (µmol mol^{-1}) within a tropical lowland forest in French Guiana (modified after Buchmann et al., 1997)

	$\delta^{13}C_{air}$	$\delta^{13}C_{leaf}$	Δ_{leaf}	C_i/C_a
Dry season (September 1994)				
Top canopy, 28 m	–7.49			
Eschweilera odora		–30.3	23.52	0.78
Eperua grandiflora		–28.4	21.52	0.70
Minquartia guianensis		–27.7	20.79	0.67
Recordoxylon speciosum		–26.6	19.63	0.62
Vouacapoua americana		–29.7	22.89	0.75
Mid-canopy, 13 m	–7.55			
Eschweilera amara		–33.4	26.74	0.91
Eschweilera odora		–32.8	26.11	0.88
Licania alba		–33.5	26.85	0.91
Ocotea glomerata		–34.4	27.81	0.95
Oxandra asbeckii		–31.9	25.15	0.84
Understory trees, 2 m	–8.37			
Eschweilera amara		–33.1	25.58	0.86
Eschweilera odora		–34.0	26.53	0.90
Licania alba		–34.4	26.96	0.92
Nectandra grandis		–33.6	26.11	0.88
Oxandra asbeckii		–33.2	25.68	0.87
Wet season (July 1995)				
Top canopy, 32 m	–7.67			
Eschweilera odora		–31.7	24.82	0.83
Eperua grandiflora		–31.9	25.03	0.84
Minquartia guianensis		–27.6	20.50	0.65
Recordoxylon speciosum		–27.6	20.50	0.65
Vouacapoua americana		–30.3	23.34	0.77
Mid-canopy, 18 m	–7.84			
Eschweilera amara		–34.0	27.08	0.92
Eschweilera odora		–32.8	25.81	0.87
Licania alba		–33.5	26.55	0.90
Ocotea glomerata		–33.3	26.34	0.89
Oxandra asbeckii		–32.6	25.59	0.86
Understory trees, 2 m	–8.54			
Eschweilera amara		–34.0	26.36	0.89
Eschweilera odora		–34.4	26.78	0.91
Licania alba		–34.2	26.57	0.90
Nectandra grandis		–35.1	27.53	0.94
Oxandra asbeckii		–33.2	25.51	0.86

All values, including the carbon isotope ratios of canopy air ($\delta^{13}C_{air}$ in ‰), are given for the dry and the wet season.

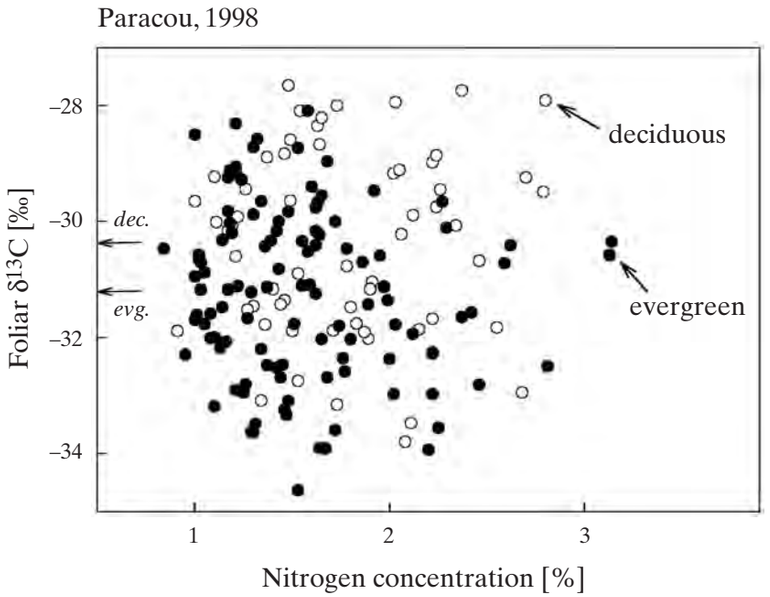

Fig. 1. Relationship between foliar $\delta^{13}C$ values and nitrogen concentrations for deciduous and evergreen tree species ($n = 171$). Each data point represents a bulk sample of 10 to 15 sunlit, mature leaves per tree individual. Arrows indicate mean values for deciduous and evergreen foliage (data from Bonal et al., 2000b).

Martinelli et al., 1998; Medina and Minchin 1980; van der Merwe and Medina 1989). Thus, the French Guiana site falls well within this range of variability for other tropical lowland forests in Brazil, Venezuela or Trinidad. The similarity among these tropical South American forests was further supported by the fact that different geographical distributions could not explain any variation in the $\delta^{13}C_{leaf}$ values of tree species growing at Paracou (Bonal et al., 2000b).

3.2. Spatial variations in $^{13}C_{leaf}$

Foliar $\delta^{13}C$ values were always higher for leaves growing in the sunny, top canopy than for leaves growing in the shade, closer to the ground (Table 1). While $\delta^{13}C_{leaf}$ values averaged -28.6 ± 0.7‰ (dry season) and -29.9 ± 0.9‰ (wet season) in the top canopy, mean foliar $\delta^{13}C$ values ranged from –33.6‰ to –33.2‰ (dry season) and from –34.2‰ to –33.3‰ (wet season) in the two lower canopy strata. For the Paracou forest, the most pronounced change in $\delta^{13}C_{leaf}$ occurred between the top and the mid-canopy, while other forests, e.g. in Brazil or Trinidad showed a more gradual change with decreasing height above the ground (Fig. 2). At Paracou, foliage in the mid- and understory canopy strata was about 4–5‰ depleted relative to the top, sunlit foliage, while in other lowland tropical forests, this depletion of shade foliage was smaller (about 3‰). However, whether these differences are consistent across all lowland rainforests cannot be decided, because the dataset on intra-canopy variation of foliar carbon isotope ratios from moist tropical lowland forests is still very limited (Table 2). In general, the isotopic signatures of sunlit, mature leaves at the top of the canopy stayed surprisingly constant, around –30‰. The depletion in $\delta^{13}C_{leaf}$ with decreasing height above the ground varied between 0.06‰ and 0.37‰ m^{-1} for all the sites, averaging 0.17‰ m^{-1} height. The French Guiana site, with 0.19‰ m^{-1} for the dry season and 0.14‰ m^{-1} for the wet season, was well within the typical range, similar to the south Venezuelan sites (between 0.13‰ and 0.37‰ m^{-1}) and the Brazilian sites (between 0.06‰ and 0.26 ‰ m^{-1}).

Source air effects could not explain the vertical gradients in $\delta^{13}C_{leaf}$ values at Paracou: $\delta^{13}C_{air}$ differed by only 1‰ within the canopy while $\delta^{13}C_{leaf}$ differed by 4‰–5‰ (Table 1). Thus, ecophysiological responses to changing environmental factors, e.g.

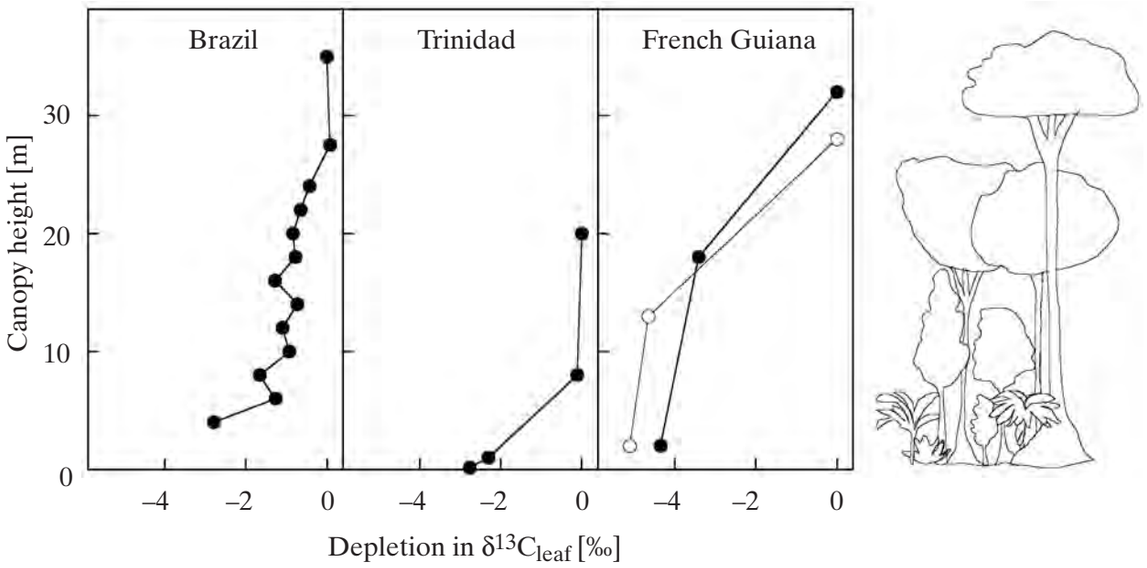

Fig. 2. Typical spatial pattern of leaf carbon isotope ratios within tropical canopies ($\delta^{13}C_{leaf}$ in ‰). The $\delta^{13}C_{leaf}$ of foliage at the top of the canopy was used as a reference to calculate the intra-canopy depletion in $\delta^{13}C_{leaf}$ with decreasing height above the ground. Examples are taken from three lowland tropical forests in Brazil (Martinelli et al., 1998), Trinidad (Broadmeadow et al., 1992) and French Guiana (Buchmann et al., 1997). Data for French Guiana are given for the 1994 dry season (open symbols) and the 1995 wet season (closed symbols).

Table 2
Comparison of carbon isotope ratios of foliage ($\delta^{13}C_{leaf}$), litter ($\delta^{13}C_{litter}$), soil organic matter at 10 cm depth ($\delta^{13}C_{SOM}$) and soil respired CO_2 ($\delta^{13}C_{SR}$) in undisturbed tropical forests

Site	*Latitude/ longitude*	*Time*	*Height (m)*	$\delta^{13}C_{leaf}$ *(‰)*	$\delta^{13}C_{litter}$ *(‰)*	$\delta^{13}C_{SOM}$ *(‰)*	$\delta^{13}C_{SR}$ *(‰)*	*Reference*
Paracou, French Guiana	5° 2′ N 53° 0′ W	September 1994	28	–28.6 ± 0.7	–30.5 ± 0.5	–28.3 ± 0.3	–26.5 ± 0.1	Buchmann et al. (1997)
			13	–33.2 ± 0.4				
			2	–33.6 ± 0.2				
		July 1995	32	–29.9 ± 0.9	–28.9 ± 0.2	–28.0 ± 0.1	–26.8 ± 0.3	Buchmann et al. (1997)
			18	–33.3 ± 0.3				
			2	–34.2 ± 0.3				
Paracou, French Guiana	5° 2′ N 53° 0′ W	March 1993	30	–29.5 ± 0.1	–29.5 ± 0.2	–28.0 ± 0.2		Guehl et al. (1998)
Paracou, French Guiana	5° 2′ N 53° 0′ W	February 1998	30	–31.0 ± 0.1				Bonal et al. (2000b)

Table 2 *(continued)*
Comparison of carbon isotope ratios of foliage ($\delta^{13}C_{leaf}$), litter ($\delta^{13}C_{litter}$), soil organic matter at 10 cm depth ($\delta^{13}C_{SOM}$) and soil respired CO_2 ($\delta^{13}C_{SR}$) in undisturbed tropical forests

Site	*Latitude/ longitude*	*Time*	*Height (m)*	$\delta^{13}C_{leaf}$ *(‰)*	$\delta^{13}C_{litter}$ *(‰)*	$\delta^{13}C_{SOM}$ *(‰)*	$\delta^{13}C_{SR}$ *(‰)*	*Reference*
Simla, Trinidad	10° N 61° W	n.a.	20	–24.7 ± 0.1				Broadmeadow et al. (1992)
			8	–28.6± 0.7				
			1	–29.0 ± 0.5				
Aripo, Trinidad	10° N 61° W	n.a.	20	–29.7 ± 1.4				Broadmeadow et al. (1992)
			8	–29.9 ± 0.8				
			1	–32.0 ± 0.8				
South Venezuela	2° N 67° W	n.a.	20	–30.4	–30.9			van der Merwe and Medina (1989)
			8	–33.4				
			1	–32.9				
South Venezuela	2° N 67° W	n.a.	>20	–28.7 ± 0.8				Medina and Minchin (1980)
			<5	–34.3 ± 0.7				
South Venezuela	2° N 67° W	n.a.	>20	–30.5 ± 0.7	–29.4			Medina and Minchin (1980)
			2–10	–33.4 ± 0.5				
			>2	–35.2 ± 0.4				
Dimona Ranch, Brazil	2° S 60° W	September/ October 89	>20	–30.7 ± 0.3				Kapos et al. (1993)
		January/ July 90	1.3	–35.5				
Reserva Ducke, Brazil	2° 6′ S 59° 6′ W	July 1985					–24.8/–27.4	Quay et al. (1989)
Rondônia, Brazil	3° 8′ S 60° 1′ W	1974–1976	17.5	–30.5 ± 0.4	–30.5 ± 0.4			Ducatti et al. (1991)
			9	–31.1 ± 0.6				
			3.5	–31.6 ± 1.1				

Table 2 *(continued)*
Comparison of carbon isotope ratios of foliage ($\delta^{13}C_{leaf}$), litter ($\delta^{13}C_{litter}$), soil organic matter at 10 cm depth ($\delta^{13}C_{SOM}$) and soil respired CO_2 ($\delta^{13}C_{SR}$) in undisturbed tropical forests

Site	*Latitude/ longitude*	*Time*	*Height (m)*	$\delta^{13}C_{leaf}$ *(‰)*	$\delta^{13}C_{litter}$ *(‰)*	$\delta^{13}C_{SOM}$ *(‰)*	$\delta^{13}C_{SR}$ *(‰)*	*Reference*
Rondônia, Brazil	3° 8′ S 60° 1′ W	1974–1976	17.5	–30.2 ± 0.6	–29.9 ± 0.4			Ducatti et al. (1991)
			9	–30.1 ± 0.5				
			3.5	–31.1 ± 0.4				
Rondônia, Brazil	8° 45′ S 63° 23′ W	n.a.	35	–31.2	–28.7 ± 0.5			Martinelli et al. (1998)
			20–32.1					
			4	–34.0				
Rondônia, Brazil	10° 1′ S 62° 49′ W	n.a.				–28.3		Pessenda et al. (1998)
Bahia, Brazil	n.a.	October 1993			–29.4 ± 0.1	–28.0 ± 0.1		Cadisch et al. (1996)

n.a.: not available.

$[CO_2]$, light or relative air humidity, contributed more to those vertical patterns in $\delta^{13}C_{leaf}$ than did $\delta^{13}C_{air}$. An important parameter for plant gas exchange, the C_i/C_a ratio, indicated changing gas exchange patterns within the canopy. Leaves at the top canopy had lower C_i/C_a (between 0.62 and 0.78) than foliage in the mid- and the understory canopy strata (between 0.84 and 0.95). Obviously, photosynthetic rates within this tropical forest decreased faster than stomatal conductance rates with increasing $[CO_2]$ and decreasing light intensity (i.e. with decreasing height above the ground). Similar patterns were also found in other forest canopies (e.g. Brooks et al., 1997; Ehleringer et al., 1986).

3.3. $\delta^{13}C$ along the leaf–litter–soil continuum and its spatial variability

The $\delta^{13}C$ values along the leaf–litter–soil continuum tended to increase steadily at the French Guiana site (Table 2), with the effect being most obvious from litter to soil. The total enrichment in ^{13}C from leaf to soil organic carbon varied between 1‰ and 2‰ (relative to sun foliage) or 2–4‰ (relative to mid canopy foliage). In other lowland tropical forests, very similar patterns were observed. The $\delta^{13}C_{litter}$ values were either close to the corresponding $\delta^{13}C_{leaf}$ values of sun foliage (Ducatti et al., 1991; van der Merwe and Medina, 1989) or increased by 1–2‰ (Cadisch et al., 1996; Martinelli et al., 1998; Medina and Minchin, 1980). In general, $\delta^{13}C_{litter}$ showed a smaller within-site variation than did $\delta^{13}C_{leaf}$ for all sites where data are available (S.D. of 0.3‰ vs. 0.5‰, respectively), indicating spatial and temporal integration of plant organic matter of different origins. Further ^{13}C enrichment could be observed for soil organic matter (at 10 cm depth) and soil respired CO_2 at the French Guiana site. Surprisingly constant $\delta^{13}C$ values for soil organic matter ($\delta^{13}C_{SOM}$) were found for all tropical lowland forests, ranging from –28.3‰ to –28.0‰. The same trend of progressive enrichment was observed in temperate and boreal forest ecosystems (Buchmann et al., 1997, 1998a; Flanagan et al., 1996) and is probably due mainly to a gradual shift in the relative contributions of microbial vs. plant components in the residual soil organic matter (Ehleringer et al., 2000).

Unfortunately, the isotopic data for soil respired CO_2 ($\delta^{13}C_{SR}$) at Paracou cannot be compared to any other tropical forest site since no other datasets are known to the authors. No significant differences were found between $\delta^{13}C_{SR}$ of the wet and the dry seasons (–26.8‰ vs. –26.5‰). Neill et al. (1996) determined the $\delta^{13}C$ of soil respired CO_2 using lab incubations

(sieved soil, no roots) with soil organic matter from a forest in Rondônia, Brazil and found a very similar $\delta^{13}C_{SR}$ value of –26.5‰.

Guehl et al. (1998) assessed the spatial variability of $\delta^{13}C$ in litter and soil organic matter under dominant trees in the Paracou forest, as they relate to the variability of sunlit leaf $\delta^{13}C$ values. In contrast to findings in a mixed temperate forest (Balesdent et al., 1993), Guehl and co-workers did not observe significant correlations between litter $\delta^{13}C$ values or leaf and soil $\delta^{13}C$ values (Fig. 3). Due to the non-synchronous leaf fall of the Paracou tree species (Loubry, 1994) and the high turnover rate of litter, both characteristic for tropical rainforests, litter $\delta^{13}C$ values are likely to display seasonal changes at a given site, whereas the isotopic signatures of soil organic matter are expected to be more stable over time, integrating time periods of about 5 years (Bird et al., 1996). It is also noteworthy that the spatial variability of soil $\delta^{13}C$ was low in this highly heterogeneous pristine forest, with a maximum range of 1.5‰ among different sampling locations. Using a similar sampling design, Balesdent et al. (1993) found a range of approximately 4‰ for soil $\delta^{13}C$ values for mixed broad-leaved and coniferous temperate forests.

4. Variation in $[CO_2]$ and $^{13}C_{air}$

The source air for foliage photosynthesis is highly variable temporally and spatially in both its $[CO_2]$ and $\delta^{13}C$ ratios (Fig. 4). For the French Guiana site, daily variations were larger than seasonal variations. Changes in turbulent mixing of the atmosphere, varying soil respiration rates as well as changing contributions of canopy assimilation or respiration do affect these canopy profiles. Generally, $[CO_2]$ are the

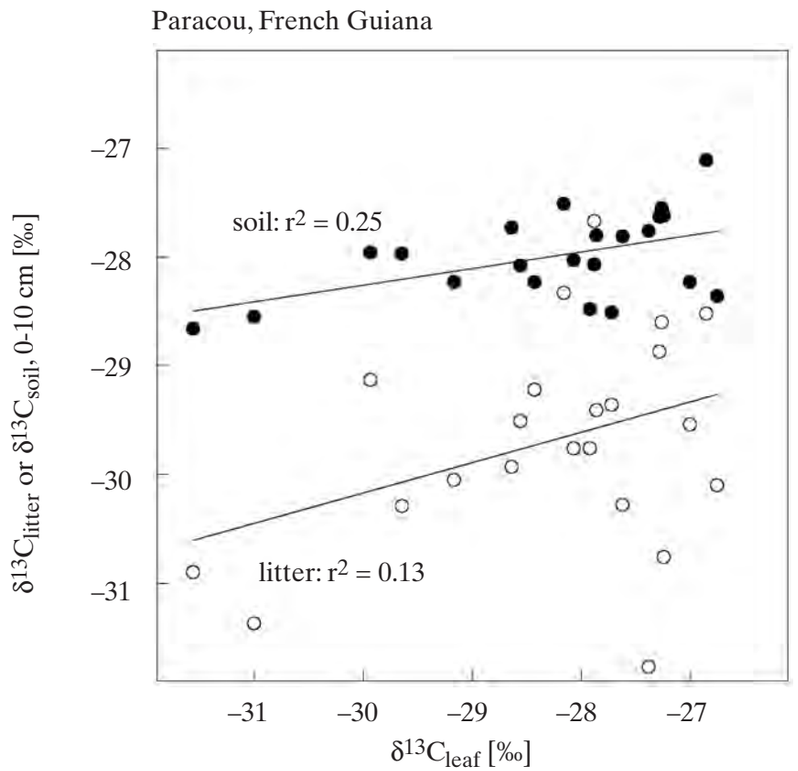

Fig. 3. Relationships between foliar $\delta^{13}C$ (sun leaves) and litter $\delta^{13}C$ (open circles) or soil $\delta^{13}C$ (0–10 cm depth; closed circles). Litter and soil samples were taken under those trees from which leaf samples were collected (data from Guehl et al., 1998). None of the regressions were significant at the 0.05 level.

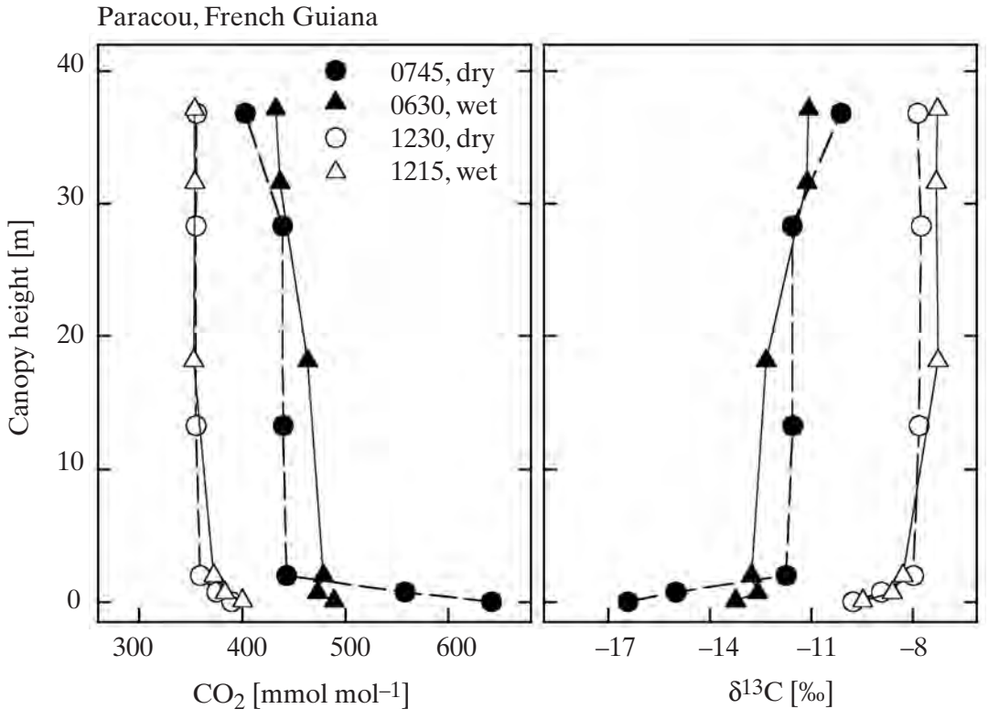

Fig. 4. Spatial and temporal variation in canopy CO_2 concentrations and canopy $\delta^{13}C_{air}$ values during the dry and the wet season in 1994 and 1995, respectively. Original flask data are given (from Buchmann et al., 1997).

highest and $\delta^{13}C_{air}$ values are the lowest at times when turbulence is low and respiration dominates ecosystem CO_2 gas exchange (i.e. at night and during the morning). At these times, high $[CO_2]$ can built up from respiration in the canopy and the CO_2 is not "flushed" out of the ecosystem until the next morning, when turbulence sets in due to heating of the atmosphere. In contrast, during the day, turbulence is high, ensuring a well-mixed main canopy (i.e. top and mid-canopy strata) with quite constant $[CO_2]$ and $\delta^{13}C_{air}$ values, close to the tropospheric background. Since photosynthesis discriminates against the heavier $^{13}CO_2$ (Farquhar et al., 1989), this heavier $^{13}CO_2$ (higher $\delta^{13}C_{air}$ values) remains in the canopy. Thus, daytime $\delta^{13}C_{air}$ values up to –7.75‰ were measured for the top Paracou canopy, while at night the $\delta^{13}C_{air}$ values were as low as –11.6‰ for the same height. Closer to the ground, the effect of soil respiration became apparent. $[CO_2]$ were always higher (up to 400 ppm during the day) compared to those in the main canopy due to the constant soil CO_2 efflux. The corresponding $\delta^{13}C_{air}$ values were always lower (reaching minimum values of –16.4‰ during the night) due to respiration and decomposition of plant material depleted in ^{13}C (see Tables 1 and 2).

These patterns found at the Paracou site in French Guiana were very similar to those reported from other tropical lowland forests (Table 3). Night- and daytime differences of $[CO_2]$ and $\delta^{13}C_{air}$ between the top height and close to the ground were well within the range for comparable intra-canopy profiles. Nighttime intra-canopy differences for $[CO_2]$ and $\delta^{13}C_{air}$ were 90–130 ppm and between 3‰ and 3.7‰ at Paracou, while other forest canopies showed a range of 10–110 ppm and 1–4‰, respectively. During the day, we found intra-canopy profiles of 60–100 ppm and 3–4.7‰ at Paracou, while profiles in other tropical canopies were between 10 and 130 ppm and between 1‰ and 3‰. Differences in stand structure (and therefore turbulence regime), as well as species composition and water availability (and therefore ecosystem gas exchange), might be

Table 3
Intra-canopy differences for CO_2 concentrations (ΔC in ppm) and carbon isotope ratios of canopy air ($\Delta\delta_{air}$ in ‰) along height profiles (in m) in low land tropical forests for two different times (nighttime/early morning vs. noon)

Site	*Latitude/ longitude*	*Season*	*Month*	*Profile (m)*	*Night/morning*		*Noon*		*Reference*
					ΔC *(ppm)*	$\Delta\delta_{air}$ *(‰)*	ΔC *(ppm)*	$\Delta\delta_{air}$ *(‰)*	
Paracou, French Guiana	5° 2′ N 53° 0′ W	D	September 1994	0.02–28	130	3.7	100	4.7	Buchmann et al. (1997)
		W	July 1995	0.10–32	90	3.0	60	3.0	
Simla, Trinidad	10° N 61° W	W + D	n.a.	0.15–20	40	1	12	1	Broadmeadow et al. (1992)
Aripo, Trinidad	10° N 61° W	W + D	n.a.	0.15–20	40	1	30	1	Broadmeadow et al. (1992)
BCI, Panama	9° 10′ N 79° 51′ W	W + D	1985–1987	0.5–25	n.a.	n.a.	40	2.5	Sternberg et al. (1989)
Turrialba, Costa Rica	9° 6′ N 83° 4′ W	n.a.	November 1967	1–40	40	n.a.	15	n.a.	Lemon et al. (1970)
Reserva Ducke, Brazil	2° 6′ S 59° 6′ W	D	July 1985	0–28	70	4	70	2	Quay et al. (1989)
		D	August 1985	0–27	110	3	120	1.5	Wofsy et al. (1988)
		W	April/May 1987	0.02–37	60	n.a.	100	n.a.	Fan et al. (1990); Trumbore et al. (1990)
Para, Brazil	2° 6′ S 47° 31′ W	D	June 1995	0.5–18	50	2	120	4	Sternberg et al. (1997)
		D	October 1995	0.5–18	40	2	30	2	
		D	December 1995	0.5–18	40	2	40	3	
Rondônia, Brazil	3° 8′ S 60° 1′ W	D	May 1995	0.5–45	60	3	40	1	Sternberg et al. (1997)
		D	October 1995	0.5–45	20	1	50	1	
		D	December 1995	0.5–45	30	2	10	2	
Rondônia, Brazil	10° 5′ S 61° 55′ W	W	April/June ‘93	1–31	40	1	70	2	Grace et al. (1996)
Rondônia, Brazil	10° 5′ S 61° 55′ W	W	May 1993	1–26	10	n.a.	15	3	Kruijt et al. (1996)
		D	September 1992	1–26	10	n.a.	130	2.8	
West Malaysia	2° 59′ N 102° 18′ E	n.a.	November 1973	0.05–30	50	n.a.	115	n.a.	Aoki et al. (1975)

D = dry season, W = wet season, n.a. = not available.

responsible for the regional differences among various tropical forests for $[CO_2]$ and $\delta^{13}C_{air}$. Although the dataset is small, intra-canopy differences during the dry season seem to be often larger than those during the wet season (Table 3). Effects of cloudiness and soil water logging, reducing both canopy assimilation and soil respiration rates during the wet season, could be responsible for this observation.

Net ecosystem CO_2 exchange (NEE) measurements in the Amazon (using eddy covariance techniques) might provide further insight (Malhi et al., 1998, 1999). However, the annual NEE between an Amazon tropical forest and the atmosphere was relatively constant during the year. Although canopy photosynthetic capacity declined during the dry season, this decrease was compensated by increased hours of sunshine. Nevertheless, highest daytime CO_2 uptake rates were measured during the wet season. In addition, ecosystem respiration showed no pronounced seasonal fluctuation. Although temperatures were typically higher during the dry season, soil moisture was lower compared to the wet season. The authors concluded that NEE peaked in the early wet season and then declined progressively to minimum numbers in the late dry season (Malhi et al., 1999). Thus, the interplay between phenology (including litterfall), cloudiness and water availability determined NEE, which in turn will affect canopy $[CO_2]$ and $\delta^{13}C_{air}$ profiles.

5. Soil CO_2 efflux

Reduced soil respiration rates during the wet season were indeed found at Paracou (Table 4). Whereas soil CO_2 efflux from this well-drained site averaged 4.8 µmol CO_2 m^{-2} s^{-1} during the dry season, it fluctuated between 2.4 and 6.3 µmol CO_2 m^{-2} s^{-1} during the wet season. Just after heavy rain events (about 20 mm), soil respiration rates dropped by 30–50% relative to the rates prior to rain. Similar results were reported for a tropical forest on Barro Colorado Island (BCI), Panama (Kursar, 1989). There, soil respiration rates dropped by 29% 1.5 h after a 17 mm rain event. Five hours later, the rates were back to those prior to the rain. However, despite these short-term reductions immediately following rain events, it cannot be concluded that soil respiratory losses during the entire wet season are significantly smaller than those during the dry season. To the contrary, maximum rates have also been measured during the wet season, including at the Paracou site (Table 5; see also Kursar, 1989; Maggs and Hewett, 1990). In general, soil respiration rates measured in French Guiana (Buchmann et al., 1997; Janssens et al., 1998) fit well within the range reported for other lowland tropical forests, most of them studied in the neotropics. While the rates can vary between 1 and 16 µmol CO_2 m^{-2} s^{-1}, the average value was closer to 4 µmol CO_2 m^{-2} s^{-1}.

Similar to temperate and boreal forest soils, temporal and spatial variations in soil CO_2 efflux are typically large (S.D. between 0.3 and 1 µmol CO_2 m^{-2} s^{-1}). At the French Guiana site, soil temperature could not explain this variation since it stayed rather constant. Soil moisture (see Table 4), but also biological parameters such as root density were found to be important factors (Janssens et al., 1998). This is in contrast to the situation in most temperate and boreal

Table 4
The effect of precipitation events on soil respiration rates at the primary lowland forest site in Paracou (after Buchmann et al., 1997)

Time	*Soil* CO_2 *efflux* (µmol m^{-2} s^{-1})		
	Before rain	*After rain*	*Reduction by*
Wet season 1995	5.00 ± 0.26	2.41 ± 0.05	52%
	4.75 ± 0.44	3.34 ± 0.08	30%
	6.27 ± 0.19	3.50 ± 0.02	44%
Dry season 1994	4.77 ± 0.12		

For more details see text.

Table 5
Soil respiration rates in moist tropical forests

Site	*Latitude/longitude*	*Season*	*Time*	*Soil respiration rate* (μmol CO_2 m^{-2} s^{-1})		*Reference*
				Range	*Average*	
Paracou, French Guiana	5° 2′ N 53° 0′ W	D	September 1994		4.8 ± 0.1	Buchmann et al. (1997)
		W	July 1995		2.4–6.3	Buchmann et al. (1997)
Paracou, French Guiana	5° 2′ N 53° 0′ W	W + D	July–September 1994	2.0–2.7	2.3	Janssens et al. (1998)
Hawaii, high elevation	19° 45′ N 155° 15′ W	W + D	1991–1992	1.0–2.3		Raich (1998)
BCI, Panama	9° 10′ N 79° 51′ W	W + D	1984–1986		3.8	Kursar (1989)
South Venezuela, podsol	2° N 67° W		n.a.		3.8	Medina et al. (1980)
South Venezuela, laterite	2° N 67° W		n.a.		2.7	Medina et al. (1980)
Reserva Ducke, Brazil	2° 6′ S 59° 6′ W	W	April/May 1987		5.3	Fan et al. (1990)
		D	August 1985		4.1	Wofsy et al. (1988)
Para, Brazil	2° 6′ S 47° 31′ W	W	May 1992		6.7 ± 0.5	Davidson and Trumbore (1995)
		D	November 1992		5.6 ± 0.5	
Rondônia, Brazil	10° 5′ S 61° 57′ W	W + D	1992 and 1993		5.5 ± 1.6	Meir et al. (1996)
Orissa, India	21.5° N 83.5° E	D	September 1988		8.1 ± 0.6	Behera et al. (1990)
Sarawak, Malaysia	2° N 11° E	n.a.	June 1970	4.1–6.2		Wanner et al. (1973)
NE Queensland, Australia	17° 17′ S 145° 37′ E	W + D	July 1986–October 1987	7–14		Maggs and Hewett (1990)
Multiple sites	1–18° N or S		n.a.	1.0–16.1		Schlesinger (1977)
Multiple sites	1–18° N or S		n.a.		3.3 ± 0.2	Raich and Schlesinger (1992)

D = dry season, W = wet season, n.a. = not available.

forests, where variability of soil CO_2 efflux is mainly explained by those two factors, temperature and moisture (typically between 60% and 80%). Only limited knowledge is available about the contribution of root/rhizosphere vs. microbial respiration. Medina et al. (1980) conclude that root respiration is responsible for 67–82% of bulk soil respiration. No information about the partitioning between different sources is available for the Paracou site.

6. Integrating ecosystem $^{13}CO_2$ exchange

Integrating information from all ecosystem compartments is of major importance in order to understand ecosystem fluxes, particularly within a global context. Using canopy air and its isotopic signatures can help in both spatial and temporal integration since the major ecosystem processes, assimilation and respiration, leave their "fingerprint" on $\delta^{13}C_{air}$. Two parameters are available to characterize the $^{13}CO_2$ exchange of ecosystems: the isotopic signature of ecosystem respiration ($\delta^{13}C_{ER}$) and ecosystem discrimination (Δ_e).

6.1. Ecosystem respiration

The $\delta^{13}C_{ER}$ was estimated from a so-called "Keeling plot" (Buchmann et al., 1997; Keeling 1958). If one plots the inverse [CO_2] vs. the corresponding $\delta^{13}C_{air}$ value, a linear relationship evolves that often holds for day- and nighttime data (Fig. 5). This relationship depicts a two-ended mixing model: two CO_2 sources with different $\delta^{13}C$ values mix within the canopy. Atmospheric CO_2 with its $\delta^{13}C$ of about –7.8‰ mixes with respired CO_2 that typically has a much lower $\delta^{13}C$ value (about –26‰ for C_3 stands or –13‰ for C_4 stands). The intercept from a geometric mean regression analysis represents the isotopic signature of total ecosystem respiration, the second source of CO_2 within any given canopy. Typically, the error associated with this approach is about 1‰ for $\delta^{13}C_{ER}$ estimates (Buchmann et al., 1998b).

For the primary forest at Paracou, the $\delta^{13}C_{ER}$ values were –27.5‰ during the dry season and –27.9‰ during the wet season (Table 6). Thus, intra-annual variability was negligible. The $\delta^{13}C_{ER}$ estimates for this lowland tropical forest were well within estimates for other tropical forests, ranging from –28.3‰ to 22.8‰. Most of the $\delta^{13}C_{ER}$ estimates were higher than the corresponding $\delta^{13}C_{leaf}$ values (compare Table 2), indicating the influence of soil respiratory fluxes to ecosystem respiration. Based on chamber measurements for soil, stem and foliage respiration, Meir et al. (1996) scaled these flux rates to the ecosystem level and compared this estimate to total ecosystem respiration measured by eddy covariance techniques. Both approaches gave very similar results for ecosystem respiration: 7.2 µmol CO_2 m^{-2} s^{-1} based on scaling and 6.6 µmol CO_2 m^{-2} s^{-1} based on eddy covariance techniques. Soil respiration accounted for 76% of the total ecosystem flux, compared to 10% for stem respiration and 14% for foliage respiration. Thus, stable isotope analyses can provide important information about the dominant flux component, although only the combination of isotope and flux measurements allows the quantitative partitioning of net ecosystem exchange (e.g. Bowling et al., 1999, 2001).

6.2. Ecosystem discrimination

Both $\delta^{13}C_{ER}$ as well as ecosystem discrimination (Δ_e) integrate over all the fluxes from all compartments within a given ecosystem (Buchmann et al., 1998b; Flanagan and Ehleringer, 1998). While $\delta^{13}C_{ER}$ gives direct information about the isotopic signature of the respiratory CO_2 leaving the ecosystem, ecosystem discrimination provides information about the CO_2 assimilated by the canopy and potentially about the ratio between CO_2 assimilation and canopy conductance (Buchmann and Kaplan, 2001). The well-known relationship between Δ_{leaf} and $\delta^{13}C_{leaf}$ (Farquhar et al., 1989) is transferred to the next higher organizational level, by replacing the $\delta^{13}C_{leaf}$ with $\delta^{13}C_{ER}$ and $\delta^{13}C_{air}$ with $\delta^{13}C_{trop}$. Δ_e was calculated as:

$$\Delta_e = \frac{\delta^{13}C_{trop} - \delta^{13}C_{ER}}{1 + \delta^{13}C_{ER}} \quad (4)$$

where $\delta^{13}C_{ER}$ represents the intercept of the corresponding Keeling plot (see Fig. 5) and $\delta^{13}C_{trop}$ represents the tropospheric background value.

Ecosystem discrimination of the Paracou site was estimated as 20.3‰ during the dry season and 20.5‰ during the wet season, reflecting the small intra-annual variability already seen in the $\delta^{13}C_{ER}$ values (Table 6). Both Δ_e estimates were well within the range reported in the literature, from 17.3‰ to 21.1‰. Thus, the similarity among different lowland tropical forests growing within a 20° latitudinal band that we found for all individual compartments of these

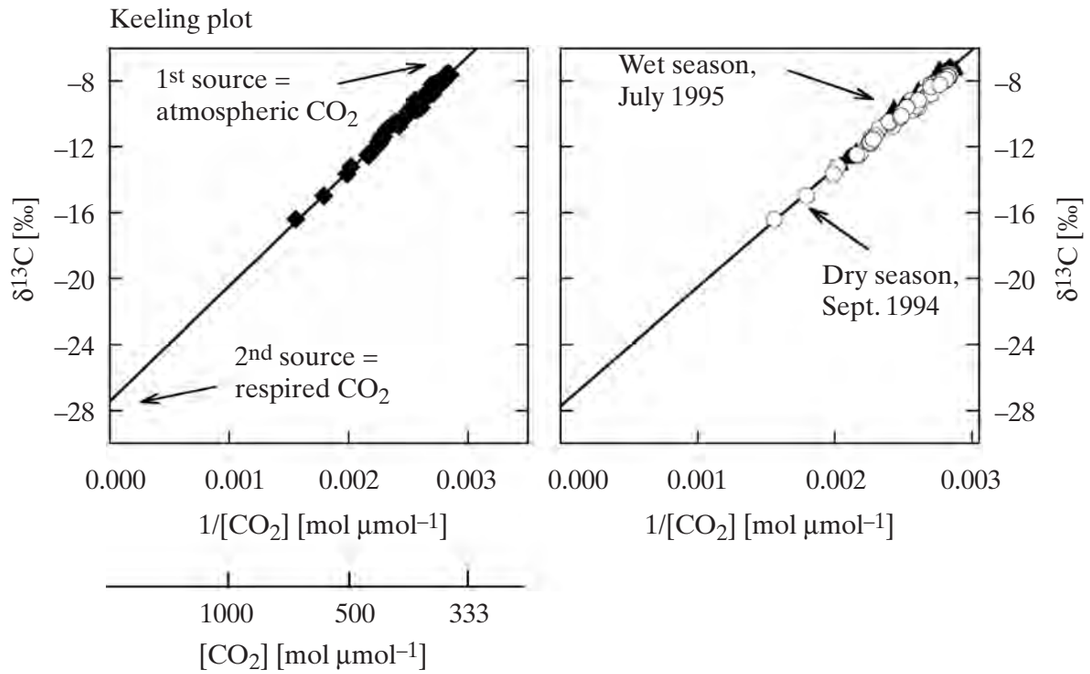

Fig. 5. Relationship between the inverse canopy CO_2 concentrations and the respective $\delta^{13}C_{air}$ values. **A.** Conceptual Keeling plot. **B.** Keeling plots for the dry and the wet season in 1994 and 1995, respectively. Original flask data are presented (from Buchmann et al., 1997).

Table 6
Estimates for carbon isotopic signatures of the CO_2 exchange between tropical forests and the atmosphere

Site	*Latitude/longitude*	*Season*	*Time*	*Ecosystem carbon isotopic signature*		*Reference*
				$\delta^{13}C_{ER}$ (‰)	Δ_e (‰)	
Paracou, French Guiana	5° 2′ N 53° 0′ W	D	September 1994	–27.5 ± 0.2	20.3 ± 0.2	Buchmann et al. (1997)
		W	July 1995	–27.9 ± 0.4	20.5 ± 0.4	Buchmann et al. (1997)
Chamela, Mexico	20° N 105° W	n.a.	October 88	–27.3	20.2	Lancaster (1990)
BCI, Panama	9° 10′ N 79° 51′ W	W + D	1985 and 1987	–28.3	21.1	Sternberg et al. (1989)
BCI, Panama	9° 10′ N 79° 51′ W	n.a.	March 88	–27.8	20.7	Lancaster (1990)
Trinidad	10° N 61° W	W + D	n.a.	–22.8	17.3	Broadmeadow et al. (1992)
Rondônia, Brazil	2° S 60° W	W	1990	–25.8	18.6	Kapos et al. (1993)

Table 6 *(continued)*
Estimates for carbon isotopic signatures of the CO_2 exchange between tropical forests and the atmosphere

Site	*Latitude/longitude*	*Season*	*Time*	*Ecosystem carbon isotopic signature*		*Reference*
				$\delta^{13}C_{ER}$ (‰)	Δ_e (‰)	
Reserva Ducke, Brazil	2° 6′ S 59° 6′ W	D	July 1985	–27.6	20.3	Quay et al. (1989)
Rondônia, Brazil	10° 5′ S 61° 55′ W	W + D	1992 and 1993	–27.1	19.5	Lloyd et al. (1996)

Carbon isotope ratios of ecosystem respiration ($\delta^{13}C_{ER}$) were determined as the intercepts of Keeling plots (see text). Ecosystem discrimination (Δ_e) was calculated according to Buchmann et al. (1997). D = dry season, W = wet season. n.a. = not available.

forests (see above) is also valid at the higher ecosystem level. Most of these Δ_e estimates were well above the modeled canopy discrimination Δ_A of 18.4‰ given by Lloyd and Farquhar (1994), but well below the up-scaled canopy discrimination for Paracou and adjacent stands (Δ_A = 23.1‰) estimated by Bonal et al. (2000b), both of which do not include the soil compartment. The estimate by Lloyd and Farquhar (1994) is based on a limited dataset for $\delta^{13}C_{leaf}$, with variations around this value of about 2–3‰ (personal communication J. Lloyd). Bonal et al. (2000b) scaled up $\delta^{13}C_{leaf}$ to the canopy level, weighing $\delta^{13}C_{leaf}$ of all species present by their basal stem area. Thus, identifying the reasons for this 3.8‰ spread in Δ_e and for the discrepancy between Δ_e and Δ_A will be the next major task for biogeochemical ecosystem research.

Global carbon models increasingly use stable carbon and oxygen isotopes to identify the distribution and the magnitude of global carbon sinks and sources (e.g. Ciais et al., 1995; Fung et al., 1997; Tans et al., 1993). Thus, detailed isotopic information is needed to describe the terrestrial biospheric CO_2 exchange adequately. However, all current models use only one value for Δ_e across the globe, neglecting spatial and temporal variations, although a 3‰ difference between modeled and real ecosystem discrimination will result in a 20% change of the global carbon sink (Fung et al., 1997). Kaplan et al. (2002) modeled ecosystem discrimination for 26 different biomes, including three tropical forest types. Based on their equilibrium ecosystem model (BIOME4), ecosystem discrimination for evergreen broadleaf and semi-evergreen tropical forests averaged 22‰, about 2‰ larger than the mean Δ_e observed with field measurements (around 20‰; Table 6).

7. Conclusions

Our knowledge about the internal carbon dynamics of tropical forests and their contribution to the global carbon budget is still rather poor. Often, only a handful of datasets exists for any given research question, resulting in high uncertainties of whether or not an observed phenomenon reflects a general behavior of lowland tropical forests or rather a site-specific pattern caused by the interplay of the many environmental factors characteristic of a particular site. However, we found an outstanding similarity in the isotopic signatures of carbon pools and fluxes among a wide range of tropical forests in Central and South America, Asia and Australia. Despite temporal and spatial variations within any given site, carbon isotopic signatures showed similar trends worldwide. Thus, the French Guiana site, Paracou can well be considered a model ecosystem, representative for many lowland pristine forests globally.

Stable isotopes proved useful for many aspects that are relevant for the internal carbon dynamics of tropical ecosystems, ranging from the identification of major CO_2 sources within a canopy, to understanding the dominant controls of foliage and ecosystem gas exchange, to the partitioning of net ecosystem fluxes. However, the number of studies using stable isotopes is still rather small, and site selection for ecosystem studies has often been biased towards “ideal”, undisturbed forests sites (Buchmann and Kaplan, 2001), leading to neglect of the effects of land use

change on these pristine forest sites. Filling this gap by studying these disturbed ecosystems will also benefit from using stable isotopes, not only in classical applications to determine the photosynthetic type of the dominant vegetation, but also to quantify the then changing contributions of different carbon fluxes to the overall ecosystem flux. Combining eddy covariance measurements of net ecosystem CO_2 exchange with ecophysiological measurements (Buchmann, 2002) and stable isotope analyses (Bowling et al., 1999a,b; Knohl et al., 2002) will probably be the most promising avenue in future ecosystem research. We strongly believe that determining the consequences of human impacts on these pristine forests will be the next challenging step in tropical ecology.

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Ecology and Management of a Neotropical Rainforest

Chapter 4

Mycorrhizal symbiosis in the tropical rainforest of French Guiana and its potential contribution to tree regeneration and growth

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Abstract – The tropical rainforest of French Guiana is characterized by a high diversity of tree species and generally poor soils. There is little knowledge about the mycorrhizal status of these trees. The mycorrhizal status of 75 species belonging to 28 families was first surveyed. Fine roots of seedlings and adult trees were collected in the upper 5 cm of the soil. This study showed that endomycorrhizas with vesicles and hyphal coils prevail in this forest. These results are consistent with observations in tropical rainforests in other parts of the world, where arbuscular mycorrhizas (AM) and ectomycorrhizas are frequently associated with Caesalpiniaceae. In our sample, ectomycorrhizas occur in two families only, Nyctaginaceae and Polygonaceae. Seedlings of *Dicorynia guianensis* Amshoff, an economically important tree species in French Guiana, were then studied in greenhouse conditions. Different microbial treatments affecting the soil, such as disinfection and inoculation with humus or root pieces, were compared; seedling growth proved to be very mycorrhiza dependent. These results emphasize that the endomycorrhizal status is an important factor capable of controlling the natural regeneration of tree species and contributing to the stability of forest ecosystems on poor soils, given that under natural conditions early seedling mortality is common, with a survival rate of only about 7% due to microbial pathogens, insects, drought, etc. Investigations on the soil AM community in diverse forest are now being carried out, considering the AM inoculum potential of the rhizosphere soil of two tree species: *D. guianensis* and *Eperua falcata* Aublet.

Keywords: Tropical rainforest, Mycorrhizal symbiosis, French Guiana, *Dicorynia guianensis*, Seedlings, Experimental approach

1. Introduction

In most natural ecosystems, plants with mycorrhizal associations are predominant and endomycorrhizas, obligately symbiotic fungi in the order of Glomales, are the most common underground symbiosis (Smith and Read, 1997). Compared to temperate regions, relatively little mycorrhizal research has been carried out in neotropical forests. Investigations on vesicular arbuscular mycorrhizas (AM) in Central and South America began around 1972 (Janos, 1975; Mosse, 1972; Thomazini, 1974).

In the Amazonian rainforest, Janos (1975), St John (1980), and Moyersöen (1993) found a dominance of host trees forming associations with endomycorrhizas without specific relationships between plant and fungi. Nevertheless, substantial evidence indicates that AM provides benefits to host seedlings by transferring mineral nutrients and water (Smith and Read, 1997).

No data about the symbiotic status of trees in the French Guiana rainforest existed before 1992 (Béreau and Garbaye, 1994). A survey of the coastal zone of French Guiana and an experimental assay conducted in greenhouse on *Dicorynia guianensis* Amshoff (an economically important timber species) have confirmed the dominance of vesicular AM (Béreau, Gazel and Garbaye, 1997), the apparent weak role of soil inoculum density on root colonization (Martin et al., 2001), and the potential role of this symbiosis in forest regeneration and growth (Alexander et al., 1992; Béreau, Louisanna and Garbaye, 1997; Ingleby et al., 1990).

2. Mycorrhizal root survey in the natural rainforest of French Guiana

French Guiana in South America (2–6 °N) has an annual rainfall of 2–4 m with a dry season from August to October which presents many days without rain. Soils are acidic with a high potential aluminium toxicity and a low available phosphorus rapidly converted to insoluble forms. The natural rainforest is characterized by a high species richness and a wide botanical diversity (Riera et al., 1990; Sabatier and Prevost, 1990) (see Part I for more detailed information).

The mycorrhizal infection types of different tree species (seedling and adult) belonging to 28 families, classified according to the nomenclature of Boggan et al. (1992) and represented by 75 species, have been surveyed on two of the soil types common in the region: podzols and ferralitic soils.

The species were chosen according to their ecology and because they belong to families (Caesalpiniaceae, Euphorbiaceae, Lauraceae, Myrtaceae, Nyctaginaceae, Tiliaceae) known to be ectomycorrhizal in Africa and other parts of South America (Alexander and Högberg, 1986). Most species (85%) were also present and sampled in the experimental plots of Paracou where the soils are ferralitic, developped on migmatite and shales (see Part I for details).

Superficial fine roots from seedlings and mature trees previously identified at the experiment sites were harvested. The roots were placed in plastic bags and analyzed in the laboratory after a careful washing. Ectomycorrhizas (ECM) with sheath and Hartig net were examined following the methods described by Ingleby et al. (1990) under the stereomicroscope and light microscope, after sectioning and staining.

The presence of intracellular, non-septate mycelium was used as a diagnosis of vesicular asbuscular mycorrhiza. The structural diversity of endomycorrhizas was determined after staining according to the Kormanic and Mc Graw (1982) technique, referring to the two major structural classes described by Gallaud (1905): *Arum*-type (with intercellular hyphae and intracellular arbuscules) and *Paris*-type (with only intracellular, coiled hyphae and rare arbuscules). These classes corresponded with morphological differences in host plant roots.

Our results (Béreau, Gazel and Garbaye, 1997) revealed that ECM could not be detected in species proposed as potentially ectomycorrhizal in the literature (Caesalpiniaceae, Euphorbiaceae, Lauraceae, Myrtaceae, Tiliaceae; Alexander and Högberg, 1986). Typical fungal sheaths and Hartig nets were present in only two families, Nyctaginaceae (*Neea* sp. R. et Pav.) and Polygonaceae (*Coccoloba* sp. L.), confirming the observations of Janos (1980b) in Amazonia and Moyersöen (1993) in Venezuela. It was verified that the lack of ECM in putatively receptive species was not due to local soil conditions: these species lacked ECM even when standing adjacent to ectomycorrhizal *Neea* or *Coccoloba.* In contrast, all the species studied on the different sites showed endomycorrhizas characterized by mycelium, vesicules and hyphal coils corresponding to the *Paris*-type of Gallaud (1905). Typical permanent arbuscules have not been found; as observed by Janos (1984), they are absent or rare and ephemeral in tropical trees.

Our results also contradict the hypothesis that ectomycorrhizal species are dominant in tropical podzols (Kubitzky, 1989): in the western part of French Guiana, on podzolic soils, we could not find this type of symbiosis on *D. guianensis* Amshoff and *Dimorphandra polyandra* Benoist (Caesalpiniaceae).

Table 1 lists the 16 tree species of Legumes (Caesalpiniaceae, Papilionaceae, Mimosaceae) and their endomycorrhizal structures observed in Paracou. *D. guianensis*, *Hymenolobium flavum* and *Pterocarpus rohrii* were more than 95% mycorrhizal with the three structure types, regardless of the presence or not of bacterial nodules.

Table 1
Adult trees and seedlings of the legumes species studied in Paracou (nomenclature of Boggan et al., 1992): percentage of microscopic fields showing endomycorrhizal structures (mycelium, coils and/or vesicles)

Tree species	*% of mycorrhization*	*structures*		
		mycelium	*coils*	*vesicles*
CAESALPINIACEAE				
Tribe Caesalpinieae				
Dimorphandra hohenkerkii (= *D. polyandra* Benoist)	42	6	32	4
Recordoxylon speciosum (Benoist) Norm. et Mar.	86	82	0	4
Sclerolobium melinonii Harms	40	22	16	2
Vouacapoua americana Aublet	30	5	22	3
Tribe Cassieae				
Dicorynia guianensis Amshoff	100	35	35	30
Tribe Detarieae				
Eperua falcata Aublet	87	40	22	25
Eperua grandiflora (Aublet) Bentham	78	24	33	21
Peltogyne venosa (Vahl) Bentham	30	29	22	9
FABACEAE				
Tribe Swartzieae				
Bocoa prouacencis Aublet	70	50	16	12
Swartzia remiger Amshoff (**a)**	60			
Tribe Sophoreae				
Diplotropis purpurea (Richard) Amshoff	81	27	31	23
Tribe Dalbergieae				
Andira coriacea Pulle	20	13	5	2
Hymenolobium flavum Kleinhoonte	98	30	33	35
Pterocarpus rohrii Vahl	95	29	38	28
MIMOSACEAE				
Tribe Parkieae				
Parkia nitida Miquel	12	8	0	4
Tribe Ingeae				
Inga sp P. Müller	68	57	0	11

a: adult trees only.

3. Mycorrhizal dependency

Arbuscular mycorrhizal fungi colonize the roots of most plant species; however, they differ in their growth response to mycorrhizal inoculation. This is often refered to as mycorrhizal dependency (Janos, 1980c; Plenchette et al., 1983). Alexander et al. (1992) suggest that mycorrhizas are obligate for the establishment of tree seedlings in Malaysian forests, and Janos (1980a and 1984) that they might be of great importance for plant growth in mineral-poor soils of neotropical rainforests. The prevalence of endomycorrhizas in the

rainforest of French Guiana led us to address experimentally the contribution of this symbiosis to the growth of *D. guianensis* seedlings. This species was chosen because it is of major commercial importance in the region and because of its high mortality rate in regeneration (Bariteau and Geoffroy, 1989; Forget, 1998).

In a greenhouse with a shade screen intercepting 85% of the incident light, a ferralitic forest soil of Paracou, steam-disinfected at 90 °C, was inoculated with fresh forest soil (30% V/V), mycorrhizal root fragments of adult trees (10% W/W) or left un-inoculated, and planted with aseptically pre-germinated seeds of *D. guianensis*. The seeds were extracted from pods collected at the experimental site of Paracou and their dormancy broken by chemical treatment: pure sulfuric acid for 10 mm and washing five times with sterile distilled water. Twenty-nine weeks later, growth parameters (height, leaf area, leaf and stem weights, root density) were determined and mycorrhizal colonization according to Kormanic and Mc Graw (1982) was quantified (percent of fields with colonized roots). The analysis of variance of the data was performed to detect significant differences between individual treatments.

Fig. 1 shows that the seedlings in both inoculated treatments had a higher shoot dry weight than those in the control treatment (Béreau, Louisanna and Garbaye, 1997). Similar results were obtained for total leaf area, total leaf number and height. The root system was smaller in the control than in the inoculated treatments. Mycorrhizal colonization was equally high in both inoculated treatments (over 80%) and absent in the non-inoculated control. As in the forest, *D. guianensis* seedlings had no bacterial nodules in the inoculated treatment of the experiment. Growth differences with the control, where soil bacteria were not introduced, can therefore be attributed to mycorrhizas. The same endomycorrhizal structures, found in the roots of forest seedlings and of potted ones, suggest that endomycorrhizal symbiosis counts among the factors controlling the regeneration of *D. guianensis* in the primary rainforest.

4. Soil inoculum of AM fungi

It has been shown that there are few viable AM fungal spores in the soil of lowland tropical rainforests and that colonization is assumed to pass from living roots to living roots (Janos, 1983).

The most probable number of arbuscular mycorrhizal propagules and the spore density in relation with mycorrhizal colonization of two Caesalpiniaceae species *(D guianensis* and *Eperua falcata*) have been studied in three sites of the primary forest of French Guiana (Martin et al., 2001). A very high number of viable spores (50–150/g soil) were found compared with soils in the Catinga Amazonica of Venezuela (1.1–1.8/g soil) (Moyersoen, 1993) or with the results of other works in lowland tropical rainforests. In two of the three sites studied, more spores were found in *D. guianensi*s than in *E. falcata* rhizosphere. Spores belonging to the Glomaceae and Acaulosporaceae families were dominant.

As in other tropical forest soils (Fisher et al., 1994), a significant correlation between numbers of spores and propagules was found but not with the AM colonization level of the roots.

Fig. 1. Shoot growth and mycorrhizal colonization of *D. guianensis* seedlings 29 weeks after sowing and inoculation. Values with the same letter are not significantly different (Fisher pooled least significant difference, $P \leq 0{,}05$, one factor ANOVA).

5. Conclusion and perspectives

Whatever the soil type, the trees in the rainforest of French Guiana have *Paris*-type endomycorrhizas, dominated by intracellular hyphal coils and very short-lived arbuscules. In contrast with rainforests in Africa, South-East Asia and even other regions of South America, ECM are extremely rare.

Our experimental results on the mycorrhizal dependency of *D. guianensis* seedlings confirm those of Janos (1980a) who found that endomycorrhizas increased seedling growth. They also support the hypothesis that the endomycorrhizal status of seedlings may be one of the determinant factors (Béreau, Louisanna and Garbaye, 1997) controlling the regeneration of *D. guianensis*. In forest, despite the early colonization of young seedlings (13% after 4 weeks of growth), their establishment is impeded by many biotic and abiotic factors (high rate of insect phyllophagy, attacks by fungal pathogens, drought stress, etc.) which result in a high mortality rate for this timber species (80–90%) (Forget, 1998). However, we have little information about the role of fungal communities in colonization (specificity, rapidity and efficacy). The latter observation underlines the need of more work on spore types to describe the diversity of the resident inoculum and identify possible specificity patterns. The use of molecular taxonomy should help to progress in our understanding of the community structure of the inoculum.

In addition, in situ experiments in the natural ecosystem have been initiated to provide more information about the role of the endomycorrhizal symbiosis in the dynamics of the rainforest ecosystem: the survival, growth and endomycorrhizal colonization structures of *D. guianensis* seedlings. Pre-germinated seeds were planted along a natural gradient of environmental resources in primary rainforest.

Finally, interrogations persist concerning the determinism of the dominant mycorrhizal status of a forest ecosystem. For instance, it has been suggested that the extensive network of the external mycelium favours ectomycorrhizal tree species in poor soils. In the case of endomycorrhizas, it is possible that the carbon cost on the host for the installation of the fungus is lower, and that the lack of specificity may play a role in the preservation of biodiversity and in the stability of the ecosystem, as suggested by Janos (1985). According to Sélosse and Le Tacon (1995), the appearance of endomycorrhizas in plants preceded that of ECM; we may therefore suggest that the occurrence of refuge areas in the Guianas, linked to climatic variation during the Quaternary (Grandville, 1991), could be involved in the extreme rarity of ECM in this region.

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Ecology and Management of a Neotropical Rainforest

Chapter 5

Diversity of the leguminous tree *Rhizobium* associations and role of the nitrogen fixation on the stability of the rainforest in French Guiana

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Abstract – In the rainforest ecosystem of French Guiana, known for its low nutrient availability, more than 1200 tree species belonging to 68 families have been identified so far. The contribution of N_2-fixing trees to the nitrogen input could be one of the possible explanations for the paradoxical occurrence of luxuriant and highly species-rich forest growing on nutrient-poor soils. Consequently, the representation of N_2-fixing species (number of taxa, density, biomass, N_2-fixation) and the diversity and specificity of their associated rhizobia have been studied. The three families of legumes (Caesalpiniaceae, Mimosaceae, Papilionaceae) are among the six most important families in terms of species richness with more than 180 species, but their capacity to fix nitrogen in situ is difficult to estimate by conventional means. We recommend the use of ^{15}N natural abundance which combined with the total N content of leaves allows to screen for actively performing N_2-fixing species. This method is based on the natural differences in ^{15}N composition between N soil and N atmosphere and allows to identify the relative proportion of these two sources in the plant N content. By this method, we showed that 43% of Leguminosae were fixing species and that the contribution of the fixation to their nitrogen nutrition averaged 54%. The N_2-fixing species play a part in the functioning of the ecosystem by increasing the N-biomass by 10%. The high species richness of fixing trees did not appear to be related to a high diversity of associated rhizobia. This diversity was studied by PCR-RFLP using universal primers targeting the 16S-rDNA genes. Results showed the predominance of ubiquitous and promiscuous *Bradyrhizobia*.

Keywords: texte à venir sur épreuves prévu sur 2 lignes

1. Introduction

One thousand and two hundred tree species belonging to 68 families have been recorded in the rainforest ecosystem of French Guiana (Sabatier, 1994). Nevertheless, this rainforest on crystalline rocks is characterized by a limited stock of mineral nutrients (Turenne, 1979) related to acid unsaturated soils, intense drainage and rapid decomposition of soil organic matter. Several researchers have attempted to

explain the paradoxical occurrence of luxuriant and highly species-rich rainforests on nutrient-poor soils (Medina and Cuevas, 1989, 1994; Vitousek and Stanford, 1986). Reviewing nutrient relations, they showed that large amounts of organic matter are fixed per unit of nutrient absorbed and concluded that most rainforests are more nutrient efficient (i.e. conservative) than temperate forests. This concept supports the possibility of low nutrient losses from the system as well as nutrient limitation to primary production (Vitousek, 1984). Even though there is evidence that P is the limiting element in most rainforests (Vitousek, 1984), many reports indicate substantial losses of N in highly leached ultisols and oxisols (Arora and Juo, 1982; Pleysier and Juo, 1981). The role of N is essential to the biomass accumulation and the fixation of atmospheric N_2 by nodulated-legume species possibly counterbalances for N losses at the ecosystem level (Pate et al., 1993).

The relative importance of N_2-fixing species in rainforests is poorly documented in terms of biomass, number of taxa and number of individuals. Only twenty percent of over 19,000 indexed species of tropical Leguminosae have been examined for bacterial nodulation so far (Allen and Allen, 1981; De Faria and Sprent, 1994; De Faria et al., 1989; Moreira et al., 1992). The level of host specificity, the diversity of the associated microorganisms and their efficiency in fixing N_2 are also unknown.

Prospecting at random is difficult in unexplored rainforests. So, as suggested by Delwiche et al. (1979) and Virginia and Delwiche (1982), the natural N isotopic composition of plant tissues may constitute a presumptive indicator of N_2-fixation and a means of screening for N_2-fixing plants in natural ecosystems. Moreover, N_2-fixation is difficult to estimate by conventional means in such systems, and for this type of survey the natural ^{15}N abundance method is valuable (Domenach et al., 1989; Shearer and Kohl, 1986). Observation of the nodulation can be additional to this method and the molecular diversity of the bacterial strains can be analyzed from DNA extracted and purified directly from nodules, avoiding the arduous strain isolating step method (Rouvier et al., 1996; Simonet et al., 1994).

This chapter presents a data synthesis on the N_2-fixing rainforest tree species of northwestern French Guiana (Guehl et al., 1998; Roggy, 1998; Roggy et al., 1999a,b) including the Paracou site. In this work, the significance of the N_2-fixing species in the plant community was estimated in terms of density, biomass and mass of fixed leaf N. Then, the diversity of the species was investigated at the plant and bacteria levels.

2. Experimental procedure

2.1. The process relies on existing botanical surveys in the study areas

Field work took place in French Guiana, South America (2–6°N; 51°50′–54°50′ W). Annual rainfall ranges from 2 to 4 m and monthly average temperatures vary slightly around 26 °C (Boye et al., 1979). The climate is of the seasonal equatorial type.

Studies were conducted on two natural forest sites on oxisols (Van Wambeke, 1975) developed on the weathered mantle of a Precambrian metamorphic rock (schists of the Armina series, see Part I, Chapter 1, Section 3.1) (i) at IRD's Piste de St Elie research station (PSE, formerly ECEREX (Sarrailh, 1984), 5°18′ N, 53°30′ W); the climate is equatorial with an annual rainfall of 3,250 mm (Sabatier, 1994) and slight variations of the monthly temperatures around 26 °C; and (ii) more recently, in the experimental zone of Paracou (CIRAD-Forêt, see Part I, Chapter 1) focused on in this book. In the first area, Sabatier et al. (1997) inventoried all woody plants with a DBH of 10 cm or more in a 10-ha plot. As for trees only, 6,118 individuals were censused, labelled and mapped. Based on the herbarium vouchers collection, the botanical inventory led to the identification of 59 families (according to Cronquist's taxonomy, 1981), 192 genera and 448 species or morphospecies. Three families represented more than 50% of trees: Lecythidaceae 26.2%, Leguminosae (*sensu lato*, with Caesalpiniaceae being the most common) 18.4% and Chrysobalanaceae 7.2%. In the second area, Molino and Sabatier (unpublished data) inventoried all woody plants with a DBH of 2 cm or more in ten 0.5-ha transects, settled in the Paracou plots (see Part I, Chapter 1, Sections 2.2. and 3.2, and Annex 1, Table 2). A total of 17,016 trees were censused using the same methodology as at PSE. Considering only the trees with a DBH of 10 cm or more (2,863 trees distributed in 54 families, 162 genera and 318 species or morphospecies), four families represented more than 50% of individuals: Lecythidaceae 18.8%, Leguminosae 17.1%, Chrysobalanaceae 10.3% and Clusiaceae 6.2%. In this chapter, only the trees with a DBH ≥10 cm were considered for comparing the two sites, St Elie (PSE) and Paracou.

2.2. The putative N_2-fixing status of a tree species was detected using the $\delta^{15}N$ approach

The ^{15}N abundance of atmospheric dinitrogen is invariant (Mariotti, 1983) and is used as the standard reference value ($\delta^{15}N = 0‰$). The $\delta^{15}N$ of biologically fixed-N ranges between –2‰ and 0‰ (Kurdali et al., 1993; Yoneyama, 1987), while that of the soil ranges between –10‰ and +20‰ depending on the type of ecosystem (Shearer et al., 1974). Non-fixing plants, using only soil N, reflect the $\delta^{15}N$ of the soil N sources; fixing plants, using both atmospheric and soil N, present intermediate values. Whenever the $\delta^{15}N$ value of a tree is close to that of fixed-N, but different from that of neighboring trees known as non-fixing, this suggests that the former tree is highly dependent on fixation for its N supply (Kurdali et al., 1993).

Sampling was essentially focused in the first area (PSE) on two groups of tree species, 57 legume species (169 individuals) and 20 pioneer species (55 individuals). Leaves were collected from trees neighboring the non-N_2-fixing reference species *Dicorynia guianensis* (Caesalpiniaceae, 45 individuals). This species was taken as the non-fixing reference that is essentially ubiquitous and abundant in the forest cover (Guehl et al., 1998). Samples were analyzed for $\delta^{15}N$ and total N concentration as described by Casabianca (1993), using an elementar analyzer (SCA, CNRS, Solaize, France) coupled to a mass spectrometer (Delta S Finningan Mat, Bremen, Germany). Isotopic composition was expressed according to the classical delta notation as the molar $^{15}N/^{14}N$ ratio of the sample (R_{sample}) relative to that of an international standard ($R_{standard}$):

$$\delta^{15}N(‰) = \frac{R_{sample} - R_{standard}}{R_{standard}} \times 1000$$

Only a few data for $\delta^{15}N$ were available at Paracou because the identification of trees species was still in progress at the time of sampling: 10 individuals of *Dicorynia* and a few identified *Leguminosae* and pioneer species were sampled. These data permitted to extrapolate the results of PSE to Paracou in terms of classification of the species according to their putative N_2-fixing ability or inability. They also allowed to verify whether the $\delta^{15}N$ values were homogenous at a larger scale.

2.3. The N_2-fixing status of the species was confirmed by looking for root nodules

When nodules were found, the roots which carried them were traced up to the base of the parent tree and its identity recorded. Acetylene reduction assays were then used as an indicator of efficient N_2-fixation (Hardy et al., 1968).

2.4. The significance of fixing species and their contribution to the N input in the forest system

This was evaluated on the basis of:

(1) The distribution of trees into diameter classes and estimation of plant biomass

Leaf and total aboveground (leaves and trunks) plant biomass (P) were assessed from DBH according to the allometric equation of Lescure et al. (1983) and Puig et al. (1990) where $P = k.DBH^a$ with $k = 0.00873$ and $a = 2.1360$ for leaf biomass and $k = 0.05635$ and $a = 2.7248$ for total biomass.

These estimates are semi-quantitative but the model proposed by Lescure et al. (1983) and Puig et al. (1990) has been developed from French Guiana data and is more specific to this area.

(2) The estimates of N_2-fixation

Estimates of the fractional contribution of fixed-N_2 (% N derived from atmosphere, % Ndfa) to the total leaf N concentration of legumes were calculated as follows (Amarger et al., 1977; Bardin et al., 1977; Shearer and Kohl, 1986):

$$\%Ndfa = \left(\frac{\delta^{15}N_o - \delta^{15}N_t}{\delta^{15}N_o - \delta^{15}N_a}\right) \times 100$$

where $\delta^{15}N_o$ is the $\delta^{15}N$ value of leaves of non-N_2-fixing plants, $\delta^{15}N_t$ is the $\delta^{15}N$ value of leaves of N_2-fixing plants and $\delta^{15}N_a$ is the ^{15}N value of fixed-N in N_2-fixing plants when their only source of N is atmospheric N_2 (i.e. when grown hydroponically with N-free nutrient solution). In our study, $\delta^{15}N_a$ was not available and we assumed that it ranged between 0‰ and –2‰ according to Shearer and Kohl (1986) and Yoneyama et al. (1991). Therefore, two estimates of the fractional contribution of fixed-N_2 to the N nutrition of the N_2-fixing species were done: we used 0‰ and –2‰ as the $\delta^{15}N_a$ value (assumptions I and II, respectively), and two-tailed unpaired *t*-tests were made at the species level (for $n = 3$ individuals) and at the

family level (for $n < 3$) to determine statistical differences between estimates ($P = 0.01$, Statview 4.02).

The standard error of the estimate (SE_{est}) of Ndfa was calculated as follows (Schearer and Kohl, 1986):

$$SE_{est} = \sqrt{\frac{(y-c)^2 SE_x^2}{(x-c)^4} + \frac{SE_y^2}{(x-c)^2}}$$

where y refers to $\delta^{15}N_t$, c to $\delta^{15}N_a$, x to $\delta^{15}N_o$, and SE_x and SE_y to the SE of x and y. The calculation was made by comparing separately the $\delta^{15}N$ value of each N_2-fixing species with the average $\delta^{15}N$ value of all non-N_2-fixing trees collected at the same site. This allowed the integration of the heterogeneity of the soil ^{15}N abundance into the SE of $\delta^{15}N_o$.

2.5. The diversity of the associated bacteria was investigated from divergent legume tree species

Laguerre et al. (1996) showed that the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) allowed to differentiate 16s DNAr genes of *Rhizobium* reference strains at species and higher levels. They proposed a combination of restriction enzymes to resolve these strains into species in agreement with a classification based on DNA–DNA homology. On the basis of these results, our aim was to compare type and (or) reference strains of *Bradyrhizobium*, *Rhizobium*, *Mesorhizobium*, *Sinorhyzobium*, *Azorhizobium* and of two genera close to *Rhizobium*, *Agrobacterium Rhizogenes* and *Blastobacter aggregatus* (Willems and Collins, 1993; Young and Haukka, 1996) with unknown unisolated strains amplified directly from root nodules of different divergent rainforest legume trees. Extraction and amplification of DNA directly from nodules have successfully been used for identifying *Frankia* strains of different actinorhizal species (Bosco et al., 1994; Mirza et al., 1994; Normand et al., 1996; Rouvier et al., 1996). This approach avoids biases introduced by isolation procedures (i.e. selection of culture-adapted strains) and allows a rapid examination of numerous strains.

Nodules of 17 rain forest legume tree species were harvested from one tree per species. These tree species had been previously identified as N_2-fixing species and belong to the three taxonomic families of Cronquist (1981). We concentrated on the *Inga* genus, which was the most diversified legume genus in the stand in terms of species richness. The whole 16s DNAr genes of the reference strains and of the unisolated strains in the nodules were amplified by using universal bacterial primers FGPS5–281 bis (5′-ATG GAR AGY TTG ATC CTG GCT CA-3′) where R is G or A and Y is T or C in the ratio of 50% and FGPS1509′-153 (5′-AAG GAG GGG ATC CAG CCG CA-3′). These primers are derived from conserved region of 16s DNAr gene and are designed to amplify nearly the full length of this gene in most bacteria (Normand et al., 1996).

A total of 137 nodules were collected and their restriction patterns of amplified 16s DNAr gene (obtained with five enzymes: NdeII, CfoI, HinfI, HaeII and MspI) were compared with the RFLP profiles of the 19 reference strains.

3. Results and discussion

3.1. Distribution of the Leguminosae in the two sites

The importance of the legumes in the two sites is reported in Table 1. They are represented by 76 species distributed in 31 genera in the first site and

Table 1
Importance of legume woody species in two rainforests of French Guiana

Family	*Number of genera*		*Number of species*		*Number of individuals*	
	St Elie	*Paracou*	*St Elie*	*Paracou*	*St Elie*	*Paracou*
Caesalpiniaceae	12	8	24	14	940	409
Mimosaceae	8	6	37	22	140	98
Papilionaceae	11	4	17	6	58	17

St Elie: inventory plot of 10 contiguous ha; Paracou: 10 inventories, plot of 0.5 ha.

by 44 species distributed in 20 genera in the second site. Botanical inventories revealed, on both sites, that there was no correlation between the importance of a family in terms of its species diversity and the rank it occupied within the stand. On both sites, the Caesalpiniaceae were the most represented in terms of density of individuals and the Mimosaceae in terms of specific diversity. The Papilionaceae were very poorly represented in terms of density of individuals but presented an important generic diversity. Eighty-six percent of the Mimosaceae and all the Caesalpiniaceae and Papilionaceae inventoried in Paracou were found in PSE. One may conclude that the floristic composition and the density of legumes are similar in the two sites provided that similar areas are compared.

3.2. General distribution of leaf $\delta^{15}N$ and N concentration

The species sampled in the first site gave a bimodal distribution for mean $\delta^{15}N$ (Fig. 1a). Eighty-two percent of the species ranged from –1 to 3‰ and 18% from 4 to 7‰, revealing two statistically separate groups of plant species. The individuals in the reference species gave an unimodal $\delta^{15}N$ distribution, with values of 4–6‰ (Fig. 1c). This latter range of $\delta^{15}N$ values was superimposed on that of one group of sampled species, suggesting identical $\delta^{15}N$ signatures of plant foliage.

The distributions of the mean leaf N concentration in sampled species and the leaf N concentration in the reference individuals are given in Fig. 1b,d. The observed variability between species was larger than that observed for individuals in the reference species: 22 species were below 2%. The mean leaf N concentration of the reference species was quite high (2.17 ± 0.05%) and was used as threshold value to divide species with low $\delta^{15}N$ values into statistically separate clusters of plants (Fig. 2a). The "supposed non-N_2-fixers" group of species (mean $\delta^{15}N = 5.14 \pm 0.30$‰ and mean leaf N = 2.61 ± 0.09%) had $\delta^{15}N$ values statistically similar to the reference species ($\delta^{15}N = 4.93 \pm 0.10$‰). The "supposed N_2-fixers" group (mean $\delta^{15}N = 2.09 \pm 0.18$‰ and mean leaf N = 2.48 ± 0.05%) included species with $\delta^{15}N$ values significantly lower than the reference species and no distinct or significantly higher leaf N concentrations. The "uncertain other plants" group (mean $\delta^{15}N = 1.65 \pm 0.17$‰ and mean leaf N = 1.69 ± 0.04%) contained species with $\delta^{15}N$ and leaf N concentrations significantly lower than the reference. There was no statistical difference between this group and the "supposed N_2-fixers" for $\delta^{15}N$ but there was a significant difference in leaf N concentration. This group is mainly made up of pioneer species, except for one legume genus (*Eperua*). The results showed that similar patterns of $\delta^{15}N$ and leaf N concentration values are observed in Paracou (Fig. 2b). The species with low $\delta^{15}N$ values divide into statistically separate clusters on the basis of the leaf N concentration. The distribution of the species within these two groups corresponded to the one observed in PSE. A difference of about 1.5 δ between the two sites is observed for the reference species ($\delta^{15}N = 3.5 \pm 0.13$ vs. $\delta^{15}N = 4.93 \pm 0.10$). On both sites, the $\delta^{15}N$ value of the reference species is high compared to those for fixed-N_2. This allowed the detection of trees with a small contribution of fixed-N_2 to their N supply. The low variability in these values (0.10 in PSE and 0.13 in Paracou) also indicated a low spatial heterogeneity of the $\delta^{15}N$ for this soil N source. Both supposed non-N_2-fixing species and reference species exhibited similar $\delta^{15}N$ values. This suggested similar pathways for the acquisition of N and thus, that the $\delta^{15}N$ of *Dicorynia guianensis* was representative of that of the primary non-N_2-fixing species. These results confirmed that *Dicorynia guianensis* was a good reference species, as suggested by Guehl et al. (1998) on the Paracou site.

Nodulation occurred on most of the "supposed N_2-fixers" species, except for the pioneer species (Table 2). All Mimosaceae and Papilionaceae had nodules, while the Caesalpiniaceae, *Bocoa prouacensis* and *Vouacapoua americana* had none. A recent survey, however, found typical bacterial nodules on *Bocoa prouacensis* and "unidentified black glossy nodules" on *Vouacapoua americana* (Béreau and Garbaye, 1994). *Vouacapoua pallidior* was also found to be nodulated in the Amazon region of Brazil (Moreira et al., 1992). The acetylene reduction assays revealed efficient N_2-fixation for all the nodulated-species tested (Table 2). No nodules were found in the "supposed non-N_2-fixers" group of species except for the *Inga* genus, in spite of the high $\delta^{15}N$ values. No nodules were found in the "uncertain other plants" group, which confirmed the putative non-N_2-fixing status of these species.

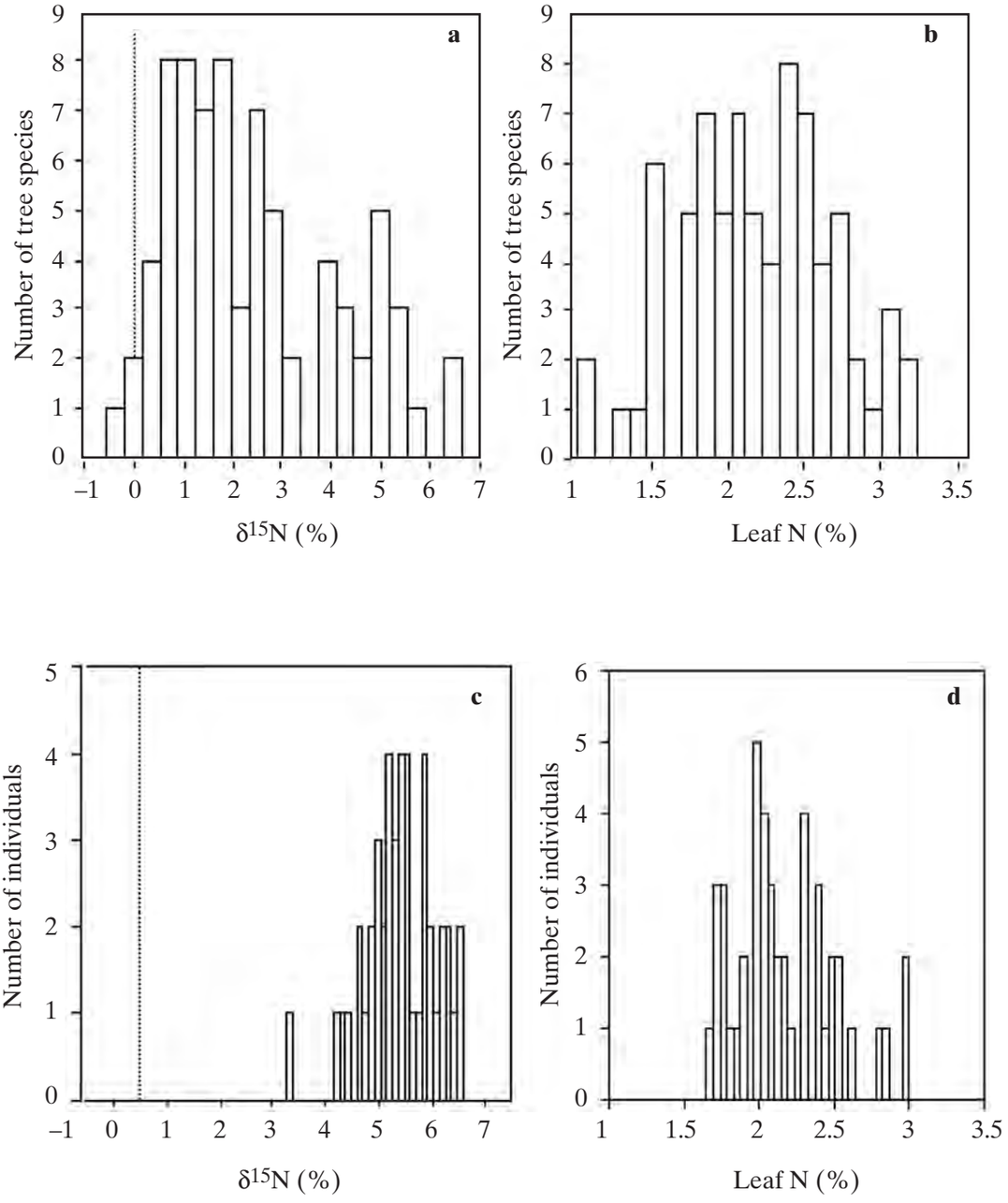

Fig. 1. Taken from Roggy et al. (1999). Frequency distribution of leaf $\delta^{15}N$ and total leaf N concentration in tree species (a, b: mean values) and in individuals of the non-N_2-fixing reference tree species *Dicorynia guianensis* (c, d) sampled in a rainforest of French Guiana (Piste de St Elie).

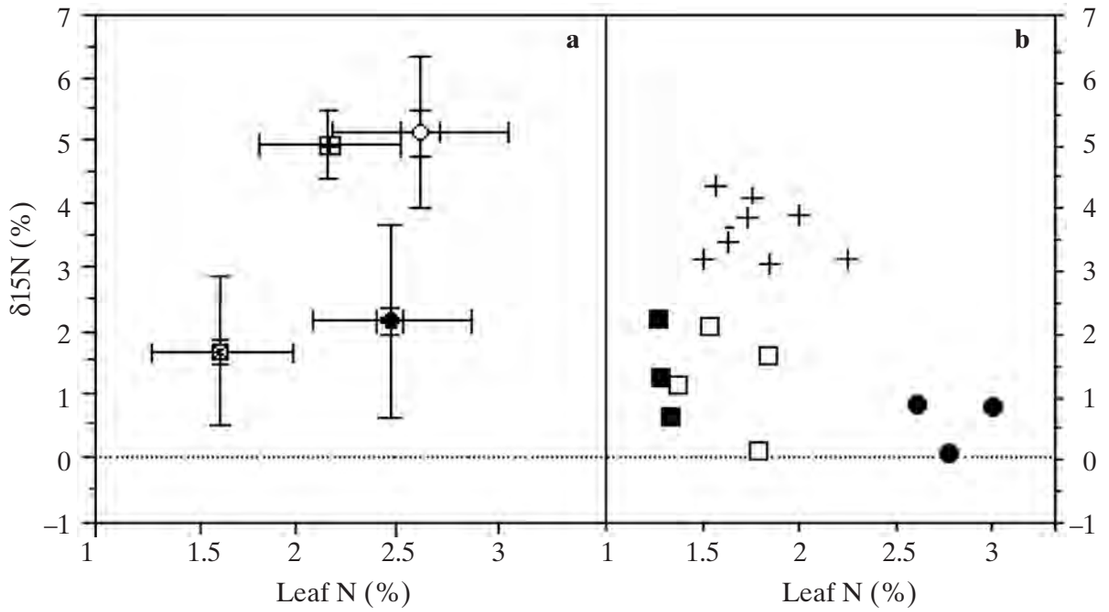

Fig. 2. Relationship between leaf ^{15}N abundance ($\delta^{15}N$ ‰) and total leaf N concentration (leaf N %) in legume trees in two rainforests on oxisol, compared to the non-N_2-fixing reference species *Dicorynia guianensis* (crosses). (a) Piste de St Elie, vertical and horizontal bars indicate SE (short bars) and SD (long bars). (b) Paracou, each data point represents a separate individual tree. Crosses: *Dicorynia guianensis* a ($n = 45$), b ($n = 10$). Open circles: supposed non-N_2-fixers: a ($n = 19$); closed circles: supposed N_2-fixers: a ($n = 70$), b (*Sclerolobium melinonii*); squares: uncertain other plants: a ($n = 57$), b *Eperua falcata* (open), *Carapa procera* (closed).

Table 2
N_2-fixing ability of some legumes and pioneer tree species collected in a rainforest of French Guiana (Piste de St Elie): evidence from leaf $\delta^{15}N$ (‰) and total leaf N concentration (leaf N %)

Plant type[1]	*n*	*δ15N (‰)*	*leaf N (%)*	*Nodulation occurs*[2]	*ARA*[3]
Reference species					
Caesalpiniaceae					
Dicorynia guianensis	45	4.93 ± 0.10	2.17 ± 0.05	–	
Supposed N_2-fixers					
Legumes					
Caesalpiniaceae					
Bocoa prouacensis	15	1.92 ± 0.34	2.21 ± 0.06	–	
Crudia bracteata	13	3.83 ± 0.27	2.82 ± 0.08	+	**nco**
Peltogyne venosa	1	1.08	1.52	**ne**	
Sclerolobium spp.	5	1.32 ± 0.73	3.01 ± 0.10		
S. albiflorum	1	3.00	2.80	**ne**	
S. melinonii	4	0.90 ± 0.77	3.06 ± 0.11	+	+

Table 2 (*continued*)
N_2-fixing ability of some legumes and pioneer tree species collected in a rainforest of French Guiana (Piste de St Elie): evidence from leaf $\delta^{15}N$ (‰) and total leaf N concentration (leaf N %)

Plant type[1]	*n*	*$\delta^{15}N$ (‰)*	*leaf N (%)*	*Nodulation occurs*[2]	*ARA*[3]
*Senna quiquangulata**	1	0.55	1.55	+	**nco**
Swartzia spp.	11	0.64 ± 0.25	2.50 ± 0.08		
S. arborescens	1	1.56	2.71	+	**nco**
S. guianensis	2	0.58 ± 0.85	2.55 ± 0.19	+	**nco**
S. panacoco	2	1.19 ± 0.93	2.37 ± 0.06	+	+
S. polyphylla	6	0.32 ± 0.23	2.49 ± 0.13	+	+
Tachigalia paniculata	1	3.20	1.90	+	+
Vouacapoua americana	11	2.64 ± 0.20	2.36 ± 0.08	–	
Mimosaceae					
Abarema spp.	4	2.07 ± 0.24	2.21 ± 0.17		
A. jupunba	2	1.78 ± 0.41	2.11 ± 0.08	+	+
A. mataybifolia	2	2.35 ± 0.15	2.30 ± 0.40	+	+
Balizia pedicellaris	1	0.87	1.72	+	+
Inga spp.	26	1.60 ± 0.26	2.47 ± 0.08		
I. alba	1	3.29	2.58	**ne**	
I. cayennensis	1	0.82	2.12	+	**nco**
I. cinnamomea	1	2.82	2.65	+	**nco**
I. fanchoniana	4	1.19 ± 0.88	3.04 ± 0.12	+	**nco**
I. gracilifolia	1	2.14	2.36	+	+
I. leiocalycina	1	0.24	1.72	+	+
I. paraensis	1	1.71	2.44	**ne**	
I. pezizifera	2	2.36 ± 0.49	3.01 ± 0.03	+	+
I. rubiginosa	1	0.76	2.68	+	+
I. sarmentosa	1	1.6	2.59	**ne**	
I. semialata	1	1.07	1.85	+	+
I. splendens	2	1.36 ± 0.74	2.36 ± 0.07	+	**nco**
I. spp.	3	1.84 ± 0.7	2.31 ± 0.19	+	+
I. stipularis	4	2.11 ± 1.2	2.09 ± 0.12	+	+
I. thibaudiana	1	1.03	3.17	+	+
I. tubaeformis	1	0.04	1.78	**ne**	
Stryphnodendron polystachyum	1	1.02	2.2	+	+

Table 2 (*continued*)
N_2-fixing ability of some legumes and pioneer tree species collected in a rainforest of French Guiana (Piste de St Elie): evidence from leaf $\delta^{15}N$ (‰) and total leaf N concentration (leaf N %)

Plant type[1]	*n*	*δ15N (‰)*	*leaf N (%)*	*Nodulation occurs*[2]	*ARA*[3]
Papilionaceae					
Alexa wachenheimii	6	2.66 ± 0.35	2.72 ± 0.16	+	+
Andira coriacea	2	0.62 ± 0.40	1.97 ± 0.03	+	+
Hymenolobium cf. *flavum*	1	–0.56	2.02	+	+
Ormosia spp.	4	1.08 ± 0.33	2.00 ± 0.07		
O. melanocarpa	2	1.65 ± 0.15	1.90 ± 0.10	+	+
O. sp.	2	0.51 ± 0.08	2.10 ± 0.00	+	+
Paramachaerium ormosioides	4	0.73 ± 0.11	2.74 ±0.05	+	+
Poecilanthe hostmanii	2	2.50 ± 0.22	2.15 ± 0.08	+	+
Vatairea erythrocarpa	1	2.88	1.80	**ne**	
Pioneer species					
Bignoniaceae					
Jacaranda copaia	2	1.47 ± 0.16	2.10 ± 0.14	–	
Cecropiaceae					
Cecropia spp.	2	1.74 ± 0.13	2.49 ± 0.21		
C. obtusa	1	1.87	2.71	–	
C. sciadophylla	1	1.61	2.28	–	
Celastraceae					
Goupia glabra	7	1.40 ± 0.7	1.95 ± 0.13	–	
Flacourtiaceae					
Banara guianensis	1	0.81	2.24	–	
Melastomataceae					
Loreya arborescens	1	2.44	2	–	
Rubiaceae					
Palicourea guianensis	2	2.12 ± 0.28	1.99 ± 0.32	–	
Supposed non-N_2-fixers					
Legumes					
Caesalpiniaceae					
Cassia spruceana	1	4.90	1.70	**ne**	
Crudia aromatica	2	5.21 ± 0.49	2.76 ± 0.16	–	
Dialium guianensis	1	4.36	2.51	–	
Sclerolobium guianensis	1	4.74	2.56	**Â**	

Table 2 (*continued*)
N_2-fixing ability of some legumes and pioneer tree species collected in a rainforest of French Guiana (Piste de St Elie): evidence from leaf $\delta^{15}N$ (‰) and total leaf N concentration (leaf N %)

Plant type[1]	*n*	*δ15N (‰)*	*leaf N (%)*	*Nodulation occurs*[2]	*ARA*[3]
Mimosaceae					
Inga spp.	16	4.93 ± 0.35	2.46 ± 0.08		
I. acrocephala	2	3.84 ± 1.15	2.59 ± 0.30	+	+
I. edulis	1	4.91	3.23	+	+
I. huberi	1	4.43	2.27	+	+
I. lomatophylla	2	5.40 ± 1.20	2.10 ± 0.10	–	
I. sp4	8	5.33 ± 0.54	2.43 ± 0.05	–	
I. jenmanii	2	4.19 ± 1.19	2.5 ± 0.4	–	
Parkia spp.	2	6.63 ± 2.41	2.55 ± 0.26	–	
Pseudopiptadenia suaveolens	2	4.62 ± 0.32	1.81 ± 0.01	–	
Zygia racemosa	7	5.67 ± 0.15	2.97 ± 0.14	–	
Papilionaceae					
Dipteryx odorata	1	5.04	1.9	–	
Pioneer species					
Melastomataceae					
Loreya mespiloides	4	3.87 ± 0.56	2.35 ± 0.11	–	
Miconia spp.	3	4.41 ± 0.56	1.73 ± 0.24		
M. fragilis Z	1	3.82	2.21	–	
M. tschudyoides	2	4.91 ± 0.45	1.49 ± 0.06	–	
Bellucia grosssularioides	1	6.62	2.41	–	
Uncertain other plants					
Legumes					
Caesalpiniaceae					
Eperua spp.	26	1.48 ± 0.17	1.69 ± 0.05		
E. falcata	18	1.52 ± 0.19	1.83 ± 0.04	–	
E. grandiflora	8	1.39 ± 0.40	1.37 ± 0.06	–	
Pioneer species					
Annonaceae					
Xylopia nitida	8	0.96 ± 0.25	1.75 ± 0.07	–	

Table 2 (*continued*)
N_2-fixing ability of some legumes and pioneer tree species collected in a rainforest of French Guiana (Piste de St Elie): evidence from leaf $\delta^{15}N$ (‰) and total leaf N concentration (leaf N %)

Plant type[1]	*n*	*δ15N (‰)*	*leaf N (%)*	*Nodulation occurs*[2]	*ARA*[3]
Clusiaceae					
Vismia spp.	4	0.96 ± 0.56	1.41 ± 0.11		
V. latifolia	2	0.01 ± 0.07	1.26 ± 0.17	–	
V. sessilifolia	2	1.90 ± 0.34	1.46 ± 0.01	–	
Flacourtiaceae					
Laetia procera	7	2.50 ± 0.47	1.77 ± 0.11	–	
Meliaceae					
Carapa procera	9	1.34 ± 0.29	1.56 ± 0.07	–	
Rubiaceae					
Isertia spiciformis	1	0.18	1.03	–	
Rutaceae					
Zanthoxylum sp.1	1	2.90	1.07	–	
Tiliaceae					
Apeiba glabra	1	2.95	1.48	–	
Ulmaceae					
Trema guianensis	1	2.99	1.43	–	

Values are mean ± SE (*n*: number of samples). For species with at least three replicates, the non-parametric Mann-Whitney *U*-test was used to test significant differences with the reference species, for both variables ($P < 0.05$); otherwise trends were determined according to whether individuals were within or outside the confidence interval of individuals in the reference species at 98% for the $\delta^{15}N$ and 95% for the leaf N.
[1]Classification according to the taxonomy of Cronquist (1981).
[2]Under nodulation: ne: not examined; –: plants without nodules; +: plants with nodules (for positive reports, roots were always traced up to the base of the tree).
[3]Acetylene reduction assay used as an indicator of efficient N_2-fixation. +: positive reports; nco: not carried out; *: sarmentos shrub.

As suggested by Virginia and Delwiche (1982), our results showed that measurements of natural ^{15}N abundance were relevant indicators of symbiotic diazotrophy in plants when combined with measurements of total leaf N. A significant correlation between the two variables is not necessary, as indicated by our results.

The presence or absence of nodules on the species has confirmed the indications given by the $\delta^{15}N$ results in about 80% of cases for the N_2-fixing species and in 90% of cases for the non-N_2-fixing species. A few *Inga* spp. were found nodulated in spite of their high $\delta^{15}N$ values. However, the $\delta^{15}N$ signature in young leaves reflects the form of the N supply during growth. Thus, we assumed that assimilation rather than fixation contributed to the N supply of these species at the time of sampling.

The $\delta^{15}N$ varied greatly among non-N_2-fixing plants (Table 3) suggesting that plant-available soil N cannot be a single source. The $\delta^{15}N$ data also indicated that non-fixing plants were clearly organized into two statistically distinct groups: one group of legumes species (78% of the species in PSE) and one group of pioneer species (82% of the species in PSE). Such organization undoubtedly provides much information about the functioning of the non-N_2^- fixing species. The use of NO_3^- or NH_4^+ by tree species is related to their ecological behavior. Freeden

Table 3
Summary of isotopic patterns $\delta^{15}N$ (‰) and total leaf N concentration (leaf N (%)) in tree species collected in two rainforests in French Guiana (Piste de St Elie and Paracou)

Plant type	*N*		*$\delta^{15}N$ (‰)*		*Leaf N (%)*	
Reference species						
St Elie	45		4.93 ± 0.10^{a}		2.17 ± 0.05^{a}	
Paracou	10		3.5 ± 0.12^{f}		1.75 ± 0.06^{f}	
	legumes	*pioneers*	*legumes*	*pioneers*	*legumes*	*pioneers*
Supposed N_2 fixers						
St Elie	110	15	1.90 ± 0.13^{b}	1.58 ± 0.35^{b}	2.43 ± 0.04^{c}	2.07 ± 0.09^{d}
Paracou	3		0.48 ± 0.24^{g}	–	2.77 ± 0.11	–
Supposed non-N_2 fixers						
St Elie	33	8	5.17 ± 0.22^{a}	4.42 ± 0.46^{a}	2.51 ± 0.07^{c}	$2.12 \pm 0.14^{a,d}$
Paracou	–	–	–	–	–	–
Uncertain other species						
St Elie	26	32	1.48 ± 0.17^{b}	1.78 ± 0.25^{b}	1.69 ± 0.05^{e}	1.59 ± 0.05^{e}
Paracou	4	3	1.14 ± 0.42^{g}	1.27 ± 0.44^{g}	1.62 ± 0.11^{fg}	1.29 ± 0.02^{g}

Values are mean ± SE (*n*: number of individuals). The non-parametric Mann–Whitney *U*-test was used to test significant differences for both variables with the reference species specific to each site. Values with the same superscript are not statistically different ($P < 0.01$).

et al. (1991) and Stewart et al. (1992) showed, in different rainforests, that pioneer species had high nitrate reductase activities in both leaves and roots while canopy and emergent species did not. They concluded a preferential use of NO_3^- by the gap species and of NH_4^+ by the closed forest species. Our results are consistent with the NO_3^- /NH_4^+ hypothesis and have been confirmed by measuring low NO_3^- $\delta^{15}N$ and high NH_4^+ $\delta^{15}N$ values in soil (data not shown).

This also allows the $\delta^{15}N$ method to be successfully used to screen for N_2-fixing species, in contrast to Handley et al. (1994) who found that statistically different groups of non-fixing plants made it impossible to distinguish them from the fixing ones. They concluded that, in such cases, Shearer and Kohl's (1986) linear two-source model could not be used for estimating N_2-fixation in situ. Our results are also consistent with those of Handley et al. (1994) on the irrelevance of the linear two-source model when there are more than two soil N_2 sources. However, it is possible to estimate N_2-fixation in our system. The system is slightly more complex and, despite the variability in the $\delta^{15}N$ of the non-N_2-fixing species, N_2-fixing groups could still be identified, provided that plants are simultaneously classified taxonomically, by the presence or absence of nodules, by their leaf $\delta^{15}N$ and total N concentration.

3.3. Distribution of the legumes in diameter classes

The distributions of the legume trees in the different diameter classes are reported in Figs. 3a,b. The patterns observed in the two sites are similar and are characteristic of the structure of the rainforest stands in French Guiana (the "open forests" Favrichon, 1995; Puig et al., 1990; Sabatier and Prevost, 1989;

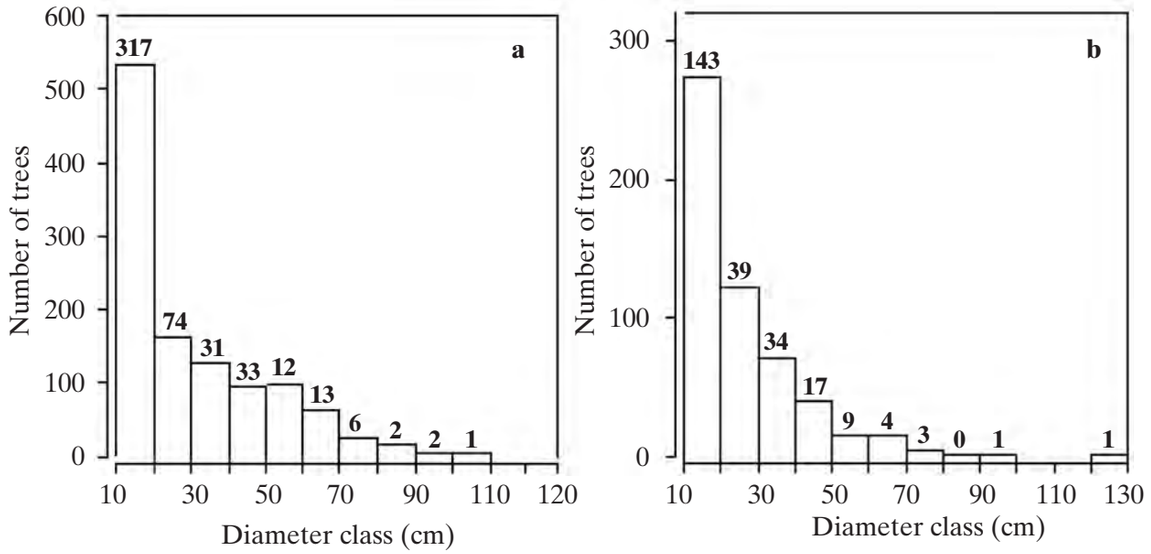

Fig. 3. Diameter class distribution of legume trees in two rainforests on oxisol, French Guiana. (a) Piste de St Elie (1 plot of 10 ha), (b) Paracou (10 plots of 0.5 ha). The number of N_2-fixing trees is indicated in each diameter class.

see Part IV, Chapter 5, Section 2.2 and Fig. 5): about 70% of the legume trees have a DBH ranging from 10 to 30 cm. N_2-fixing trees show a similar distribution: 85% of them ranged from 10 to 30 cm DBH on the first site (Fig. 3a) and 72% on the second site (Fig. 3b). The relative abundance of N_2-fixing trees in the low diameter classes does not necessarily mean a preferential N_2-fixation process for trees with low DBH. The comparison of the two distributions (Fig. 3a,b) indicates on both sites that 50% of the trees in the 10–30 cm DBH classes are fixing trees. This ratio is nearly similar for trees in the 80–110 cm DBH classes in the first site and in the 60–130 DBH classes in the second site. These ratio decreases in the intermediate classes. Therefore, the observed pattern for fixing trees reflects more the structure of the two stands than an N_2-fixation inability for trees with high diameters. Thus, the N_2-fixing ability occurred in all diameter classes, indicating the important role played by these species in the silvigenesis process.

3.4. Phytomass estimates

Estimates of leaf and total aboveground phytomass were calculated in the two sites from DBH data (Table 4). The results showed that the biomasses varied widely between the two sites whereas densities of trees were nearly similar (about 600 individuals per ha). The lowest values of biomass were found in Paracou with a difference of 53 t ha^{-1} for the total aboveground phytomass of the non-N_2-fixing legumes species and of 85 t $ha^{-1}$for the non-legumes species. The N_2-fixing species had similar values of leaf and total aboveground phytomass on the two sites, respectively around 0.6 and 40 t ha^{-1}. These results showed that the stands in PSE and Paracou had different structures, although they had similar densities of trees: 6.5% of the individuals indeed ranged in the 40–130 cm DBH classes in Paracou whereas they were 11.1% in PSE. However, those differences must be considered cautiously as, considering the whole plots of Paracou and not only the transects studied here, the same proportion of trees (between 9.6% and 10.4% according to the Paracou plots) appears above 40 cm DBH on both sites.

Our estimates of phytomass are consistent with those of Lescure et al. (1983) and Puig et al. (1990) in different nearby stands. Their results yield total aboveground and leaf phytomass, respectively ranging from 245 to 440 t $ha^{-1}$and from 3.7 to 6.4 t ha^{-1}. They are also consistent with phytomass data from six steepland rainforests, with mean values, respectively, of 7.2 and 282 t ha^{-1} for the total leaf and the total aboveground biomass (Scatena et al., 1993). Brown and Iverson (1992) also estimated that the total

Table 4
Biomass (*t* dry matter ha^{-1}) and density of trees in two rainforests on oxisol in French Guiana (Piste de St Elie, Paracou)

Plant type	*Number of trees*		*Leaf biomass[1] (t ha^{-1})*		*Total aboveground plant biomass[1] (t ha^{-1})*	
	*St Elie**	*Paracou***	*St Elie*	*Paracou*	*St Elie*	*Paracou*
Legumes species	1139	532	2.0	1.4	135.6	83.0
Non-N_2-fixing species	677	263	1.4	0.8	97.2	42.3
N_2-fixing species	462	251	0.6	0.6	38.4	40.7
Non-legume species	5017	2575	4.4	3.3	250.5	165.3
All species	6156	3107	6.4	4.7	384.1	248.3

Assessed from DBH data (diameter at breast height) according to the allometric equation of Lescure et al. (1983) and Puig et al. (1990) where: $P = k\,DBH^a$ with $k = 0.00873$ and $a = 2.1360$ for leaf biomass and $k = 0.05635$ and $a = 2.7248$ for total aboveground plant biomass. *: inventory plot of 10 ha. **: inventory plot of 0.5 ha.

aboveground biomass ranges from 175 to 400 t ha^{-1} in 28 rainforest stands of the Brazilian Amazon. These estimates, however, are semi-quantitative because specific factors such as architectural models of development (Hallé et al., 1978), wood density and canopy position are not considered. Several linear and non-linear models have been tested to correct for these biases, and the relevant choice of the model depends on the independant variables that are used (Brown et al., 1989). The non-linear models are more reliable for biomass estimates from DBH data. Brown et al. (1989) proposed a general model for tropical forests by live zone. This is based on data gathered from many statistically selected plots over a large area of tropical rainforests. However, Brown and Iverson (1992) showed that the total aboveground biomass varies widely in these areas, which is why we preferred to use that proposed by Lescure et al. (1983) and Puig et al. (1990), which is more specific to the rainforest of French Guiana. We estimated the total aboveground phytomass at 384 t ha^{-1} in St Elie and at 248.3 t ha^{-1} in Paracou. Brown and Iverson (1992) showed that the absolute range of total aboveground phytomass for moist forests in tropical America was 120–410 t ha^{-1}, with a mean value of 221 ± 10.3 t ha^{-1}. These results showed that the total aboveground phytomasses estimated in the two sites are the highest among the moist forests in tropical America.

The results in Fig. 4 reveal the significance of the N_2-fixing legumes in the two stands in terms of density and biomass. They represented 7.5% of the individuals in PSE (40% of the legume trees) and 8%

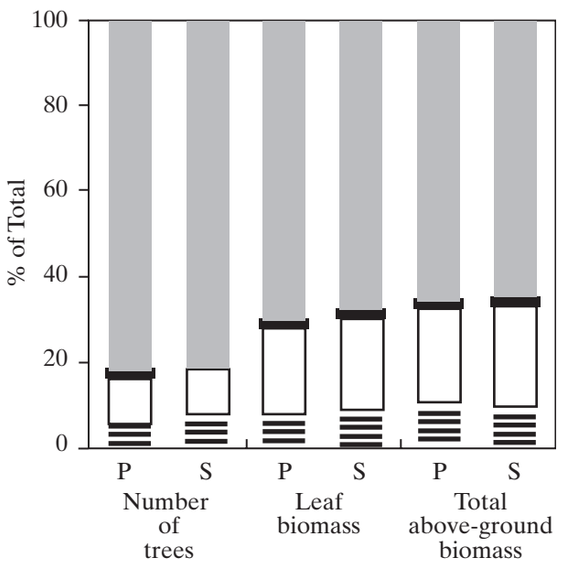

Fig. 4. Density and biomass of trees in two rainforests on oxisol. P: Paracou station; S: Piste de St Elie, French Guiana. ▫: non-legumes; ■: legumes not studied for their N_2-fixing status; □: non-N_2-fixing legumes; ◨: N_2-fixing legumes.

of the individuals in Paracou (47% of the legume trees). The results also indicated that, on both sites, about 1/3 of the aboveground phytomass came from the legumes with 10% from N_2-fixing species. We also observed that the percentage of N_2-fixing species was nearly similar in the two sites, with 7% of the species (60% of the legumes species) and 8% of the species (50% of the legumes species) in Paracou and PSE, respectively.

3.5. Estimates of N_2-fixation and N input in the system

The estimates were based on the data obtained in the site of PSE (Table 5). The conditions for a reliable estimate of N_2-fixation were met, as proposed by Shearer and Kohl (1986): a significant difference in $\delta^{15}N$ between non-fixing species on the one hand, and both fixed-N and N_2-fixing species (5.01‰ vs. –2‰ = $\delta^{15}N$ fixed-N = 0‰ (Yoneyama et al., 1991) and 5.01‰ vs. 2.19‰, 1.91‰ and 1.52‰ for Caesalpiniaceae, Mimosaceae and Papilionaceae, respectively) on the other hand. A low $\delta^{15}N$ variability (SE) also occurred for both N_2-fixing and non-N_2-fixing species with 0.20, 0.25 and 0.27 for the three legume families and 0.11 for the non-N_2-fixing species; this generated a low SE of the estimates. In our study we did not have the $\delta^{15}N_a$ of fixed-N for these species and we assumed that it lies between 0‰ and –2‰ as described by Shearer and Kohl (1986) and Yoneyama et al. (1991). Two estimates of the fractional contribution of fixed-N to the N nutrition of N_2-fixing species were made. We used 0‰ and –2‰ as $\delta^{15}N_a$ values (assumptions I and II) and two-tailed unpaired *t*-tests were done at species level (for $n = 3$ individuals) and at family level to seek statistical differences between estimates ($P = 0.01$, Statview 4.02 Software).

Statistical differences were found between assumptions I and II in each family, which means that estimates nevertheless remained sensitive to a 2‰ variation in the $\delta^{15}N$ of fixed-N. It is finally important to note that reliable estimates of N_2-fixation cannot be based on single measurements. In this survey, the small number of replicates for most species (due to the high species diversity and the low individual density per species) led us to analyze the results at the family level.

Based on the two assumptions, the SE of the Ndfa ranged from 3% to 5% for the N_2-fixing families (Table 5). These values were low compared to the SE described in favorable estimate cases (5–10%, Shearer and Kohl, 1986). Caesalpiniaceae displayed mean contributions of fixed-N ranging from 40% to 56% with SE from 3% to 4%. Well-represented N_2-fixing Caesalpiniaceae species ($n = 4$) displayed a Ndfa ranging from 24% to 94% and 17% to 67% under assumptions I and II, respectively. The highest values were found for the genus *Swartzia*. The Mimosaceae displayed mean values of Ndfa ranging from 44% to 62% with also slight SE (4–5%). In this family the *Inga* genus is the most diversified legume genus in the stand. The Papilionaceae finally displayed the highest mean values of Ndfa, which ranged from 50% to 70% and a low SE (4–5%). The results indicated a substantial contribution of fixed-N to the N nutrition of the legume species in this rainforest, the Papilionaceae and Caesalpiniaceae having the highest and lowest contributions, respectively, and the Mimosaceae showing *an* intermediate value.

3.6. Estimate of the leaf N mineralomass

The leaf N mineralomass was estimated from the leaf N concentration of the legumes and from a mean value of 2% N in the leaf dry matter for the other species (mean value of all non-N_2-fixing species sampled in St Elie: 55 legume trees and 55 pioneer trees). The estimate yielded a mineralomass of 129.88 kg of leaf N ha^{-1} for the whole stand with 42.68 kg from all legumes and 13.68 kg from the N_2-fixing ones (Table 6). Sixty-one percent of the leaf N of the fixing legumes came from the Caesalpiniaceae, the most represented legume family with 8.29 kg leaf N ha^{-1}. The Papilionaceae showed the lowest contribution with 2.32 kg leaf N ha^{-1} (17%) and the Mimosaceae an intermediate value with 3.07 kg leaf N ha^{-1} (22%). Based on the two assumptions, these results led to a contribution of fixed-N to the total leaf N ranging from 6 to 8 kg ha^{-1} for all N_2-fixing legumes in the stand. These data, compared to the total leaf N mineralomass, showed that the contribution of fixed-N could be estimated at 5.5%.

In order to calculate the net annual N_2-fixation in the stand, we needed to know the annual accumulation of dry matter in the leaf phytomass (annual leaf production). Parker (1994) showed, from data of leaf phytomass and annual leaf litter production, that the leaf turnover time ranged from 0.9 to 2.2 years in five tropical rainforests. Puig

Table 5
Estimation of the contribution of fixed-N (%Ndfa) in N_2-fixing tree species collected in a rainforest on oxisol in French Guiana (Piste de St Elie)

		Ndfa (%)[1]				*Ndfa (%)*[1]	
N_2-fixing tree species	*n*	*Assumption I*	*Assumption II*	*N_2-fixing tree species*	*n*	*Assumption I*	*Assumption II*
Mimosaceae				***Caesalpiniaceae***			
Abarema jupunba	2	64 ± 8	46 ± 6	*Bocoa prouacensis*	15	62 ± 7	44 ± 5
Abarema mataybifolia	2	53 ± 3	38 ± 2	*Crudia bracteata*	13	24 ± 6	17 ± 4
Balizia pedicellaris	1	83	59	*Sclero. albiflorum*	1	40	29
Inga acrocephala	2	23 ± 23	17 ± 16	*Sclerolobium melinonii*	4	82 ± 15	59 ± 11
Inga alba	1	34	25	*Senna quiquangulata**	1	89	64
Inga cayennensis	1	84	60	*Swartzia arborescens*	1	69	49
Inga cinnamomea	1	44	31	*Swartzia guianensis*	2	88 ± 17	63 ± 12
Inga edulis	1	2	1	*Swartzia panacoco*	2	76 ± 19	54 ± 13
Inga fanchoniana	4	76 ± 18	54 ± 13	*Swartzia polyphylla*	6	94 ± 5	67 ± 3
Inga gracifolia	1	57	41	*Tachigali paniculata*	1	36	26
Inga huberi	1	12	8	*Vouacap. americana*	11	47 ± 4	34 ± 3
Inga leiocalycina	1	95	68	*All Caesalpiniaceae*	57	56 ± 4	40 ± 3
Inga cf. *semialata*	1	79	56				
Inga pezizifera	2	53 ± 10	38 ± 7	***Papilionaceae***			
Inga rubiginosa	1	85	61	*Alexa wachenheimii*	6	47 ± 7	34 ± 5
Inga sarmentosa	1	68	48	*Andira coriacea*	2	88 ± 8	63 ± 6
Inga splendens	2	73 ± 15	52 ± 11	*Hymenolobium* cf. *flavum*	1	111	79
Inga spp.	3	63 ± 14	45 ± 10	*Ormosia melanocarpa*	2	67 ± 3	48 ± 2
Inga paraensis	1	66	47	*Ormosia* sp.	2	90 ± 2	64 ± 1
Inga stipularis	4	58 ± 24	41 ± 17	*Paramachaeri. Ormosioides*	4	85 ± 2	61 ± 2
Inga thibaudiana	1	79	57	*Poecilanthe hostmanii*	2	50 ± 4	36 ± 3
Inga tubaeformis	1	99	71	All *Papilionaceae*[2]	19	70 ± 5	50 ± 4
Stryphnodend. Polystachyum	1	80	57				
All Mimosaceae[2]	36	62 ± 5	44 ± 4				

Values are mean ± SE (*n*: number of trees sampled).
[1]Estimates are based on the assumption that the $\delta^{15}N$ of fixed-N_2 in legumes leaves is 0‰ (assumption I) or –2‰ (assumption II). The non-fixed-N_2 $\delta^{15}N$ (5.01 ± 0.11) used in the calculation is the mean of 82 trees (including 45 *Dicorynia*). Error terms only include the variation of N_2-fixing and control plants SE_y and SE_x as described by Shearer and Kohl (1986).
[2]Differences between estimates are significant at the 99% confidence level (based on two-tailed unpaired *t*-test). *: sarmentous shrub.

Table 6
Leaf N mineralomass (kg ha^{-1}) and contribution of fixed-N_2 to the N mineralomass of tree species in a rainforest on oxisols, "Piste de St Elie", French Guiana

Plant families	*Leaf N mass kg Ha^{-1}*	*Leaf fixed-N_2[2] assumption I (kg ha^{-1})*	*Leaf fixed-N_2[2] assumption II (kg ha^{-1})*	*Contribution of fixed-N_2 to N mineralomass[3] (%)*
Fixing legumes				
Caesalpiniace	8.3	4.7	3.3	48
Mimosaceae	3.1	1.9	1.3	53
Papilionaceae	2.3	1.6	1.2	60
All fixing legumes	13.7	8.2	5.8	54
All legumes	42.7	8.2	5.8	16.4
Whole stand	**129.9**	**8.2**	**5.83**	**5.5**

[1]Estimated from the leaf N content of the legumes and from a mean value of 2% N in the leaf dry matter for the other species (mean value of all non-N_2-fixing species sampled: 55 legume trees and 55 pioneer trees).
[2]Estimated from leaf N mineralomass and Ndfa values under the assumption that $\delta^{15}N$ of fixed-N was 0‰ (assumption I) and –2‰ (assumption II).
[3]Mean % between the two assumptions.

and Delobelle (1988) found similar results in French Guiana with a leaf turnover time of 1 year in a stand next to ours. This estimate is consistent with the results of Puig and Delobelle (1988) and Puig et al. (1990), which gave 5.64 t ha^{-1} year^{-1} for the leaf production (leaf litterfall) and 4.45 t ha^{-1} for the leaf phytomass of the trees in the same stand. The difference between these two values results from the leaf litterfalls of epiphytes and lianas. On the basis of these results (turnover time of 1 year for the leaves) and from our data of leaf phytomass, leaf N mineralomass and Ndfa (5.5%), we estimated that N-fixation contributed to about 7 kg N ha^{-1} year^{-1} to the leaf N mineralomass in PSE. In both mixed and pure stands of alder grown in Canada, Coté and Camire (1984) estimated fixed-N at 53 kg N ha^{-1} year^{-1}. *Prosopis glandulosa,* representing about 33% of the cover, would have fixed 40 kg N ha^{-1} year^{-1} in a desert ecosystem (Shearer et al., 1983). In field conditions, Sougoufara et al. (1990) estimated fixed-N at 40–60 kg ha^{-1} year^{-1} for *Casuarina equisetifolia.* Most of the values obtained were high compared to our results and the density of fixing trees in stands (7.5% of the cover). Such values were not available in other surveys and comparison of data from field or plantation experiments with data from natural ecosystems would not be relevant.

On one hand, Sylvester-Bradley et al. (1980) estimated at 5 kg N ha^{-1} year^{-1} the N_2-fixation associated with termites and showed that the occurrence *of Azospirillum* spp. was very low in Amazonian primary forests on latosol and, on the other hand, in Costa Rican rain forests, phyllospheric N_2-fixation ranged from 2 to 7 kg N ha^{-1} year^{-1} (Freiberg, 1994). Using these estimates and the value of 7 kg N ha^{-1} year^{-1} coming from N_2-fixing legumes in the stand, we estimated the N input through biological N_2-fixation at about 14–19 kg N ha^{-1} year^{-1}. In addition, 6–7.5 kg inorganic N ha^{-1} year^{-1} would be deposed from the atmosphere in lowland rainforests of Malaysia and Panama (Cavelier et al., 1997; Manokaran, 1980). Medina and Cuevas (1994) gave a loss of about 13 kg N ha^{-1} year^{-1} due to denitrification in a range of tropical rainforests. These data combined with our estimates suggested that N output could be balanced by N input in climax tropical rainforests. This also suggested that the small contributions of fixed-N by the different

systems are, all together, significant in maintaining the N biogeochimal cycle at equilibrium.

A significant difference in isotope identity between assimilated and fixed-N was the basic requirement for a successful use of the $\delta^{15}N$ method to evaluate biological N_2-fixation. Such conditions were found and $\delta^{15}N$ analysis, combined with N mineralomass data, provided the first estimate of N input through N_2-fixing legumes in a natural rainforest. Although both the $\delta^{15}N$ method and phytomass assessments yielded semi-quantitative data of N_2-fixation and N productivity, these results allowed a better understanding of how N balances proceed in the rainforest of French Guiana.

3.7. Identification of rhizobia strains by PCR-RFLP of 16S DNAr genes

The restriction patterns obtained for the unisolated strains with the five endonucleases revealed four to six 16s DNAr profiles depending on the enzyme used (Table 7).

The restriction patterns of the reference strains were also used to validate the theoretical profiles obtained with the RFLP ANALYSE software. Such softwares are very useful for comparing unknown strains to numerous references, while avoiding the time-consuming DNA amplifications and digestions. The number of full-length 16s DNAr sequences available in Genbank was, however, limiting because most of the sequences recorded had less than 800 pb. We used the 19 rhizobia strains for which a complete sequence was available but many more sequences are needed for an optimal use of such softwares.

The RFLP analysis of the 137 non-isolated strains revealed that they were organized in 11 different combinaisons of the 16s DNAr profiles we obtained with the five endonucleases (the group S5 with chloroplast-like sequences was not considered).

Considering the care taken to minimize the topological errors, the neighbor-joining method revealed two distinct clusters of RFLP groups which were closely related to *Bradyrhizobium* (seven RFLP groups on nine: S1, S2, S7, S9, S10, S11 and S12) and to *Azorhizobium caulinodans* (two RFLP groups on nine: S3 and S4) (Table 7). Most strains were close to

Table 7
Restriction patterns of 16s DNAr genes directly amplified from 137 root nodules of tropical legume trees in French Guiana (Piste de St Elie)

Host of origin[a]	*n*[b]	*16s DNAr type*	*Related reference strains*[c]	*Restriction patterns*				
				NdeII	*CfoI*	*HinfI*	*HaeIII*	*MspI*
Caesalpinaceae								
Crudia brachteata	2	S8	Not distinguished	N3	C4	Hi5	Ha3	M3
Mimosaceae								
Abarema jupunba	3	S7	*B. elkanii*	N1	C3	Hi4	Ha1	M5
Balizia pedicellaris	5	S10 + S5	*B. elkanii*	N1 + N3	C3 + C3	Hi3 + Hi2	Ha1 + Ha3	M2 + M3
Inga acrocephala	2	S2 + ?	*B. japonicum*	N1 + N3	C1 + C3	Hi3 + ?	Ha2 + Ha3	M2 + ?
Inga leiocalycina	10	S11 + S5	*B. elkanii*	N1 + N3	C2 + C3	Hi1 + Hi2	Ha2 + Ha3	M1 + M3
Inga huberi	1	S6		N4	C5	Hi3	Ha4	M6
Inga jenmanii	1	S1 + S5		N1 + N3	C1 + C3	Hi1 + Hi2	Ha1 + Ha3	M1 + M3
	3	S1	*B. elkanii*	N1	C1	Hi1	Ha1	M1
Inga rubiginosa	5	S12 + S5	*B. japonicum*	N1 + N3	C1 + C3	Hi1 + Hi2	Ha5 + Ha3	M1 + M3
Inga semialata	2	S11	*B. elkanii*	N1	C2	Hi1 + Hi2	Ha2 + Ha3	M1 + M3
	4	S11 + S5		N1 + N3	C2 + C3	Hi3	Ha4	M1
	1	S6		N4	C5	Hi3	Ha4	M6
Inga cf. *semialata.*	5	S2 + S5	*B. japonicum*	N1 + N3	C1 + C3	Hi3 + Hi2	Ha2 + Ha3	M2 + M3
Inga stipularis[d]	9[d]	S2 + S5	*B. japonicum*	N1 + N3	C1 + C3	Hi3 + Hi2	Ha2 + Ha3	M2 + M3

Table 7 (*continued*)
Restriction patterns of 16s DNAr genes directly amplified from 137 root nodules of tropical legume trees in French Guiana (Piste de St Elie)

Host of origin[a]		*n*[b]	*16s DNAr type*	*Related reference strains*[c]	*Restriction patterns*				
					NdeII	*CfoI*	*HinfI*	*HaeIII*	*MspI*
Inga thibaudiana	Oxisols	12	S1 + S5		N1 + N3	C1 + C3	Hi1 + Hi2	Ha1 + Ha3	M1 + M3
		2	S1	*B. elkanii*	N1	C1	Hi1	Ha1	M1
		7	S5		N3	C3	Hi2	Ha3	M3
		1	S9	*B. elkanii*	N2	C2	Hi1	Ha2	M1
		5	S9 + S5		N2 + N3	C2 + C3	Hi1 + Hi2	Ha2 + Ha3	M1 + M3
	Spodosols	10	S1 + S5		N1 + N3	C1 + C3	Hi1 + Hi2	Ha1 + Ha3	M1 + M3
		3	S1	*B. elkanii*	N1	C1	Hi1	Ha1	M1
		4	S5		N3	C3	Hi1	Ha1	M1
		2	S9	*B. elkanii*	N2	C2	Hi1	Ha2	M1
		7	S9 + S5		N2 + N3	C2 + C3	Hi1 + Hi2	Ha2 + Ha3	M1 + M3
Papilionacee									
Dioclea sp. (liana)		3	S7	*B. elkanii*	N1	C3	Hi4	Ha1	M5
Hymenolobium cf *flavum*		1	S8	not distinguished	N3	C4	Hi5	Ha3	M3
		2	S3	*A. caulinodans*	N1	C3	Hi3	Ha1	M4
Ormosia coutinoii		2	S10	*B. elkanii*	N1	C3	Hi3	Ha1	M2
Paramachaerium ormosioides		11	S4	*A. caulinodans*	N1	C3	Hi3	Ha5	M2
Poecilanthe hostmanii		7[d]	S3	*A. caulinodans*	N1	C3	Hi3	Ha1	M4
		5	S3	*A. caulinodans*	N1	C3	Hi3	Ha1	M4

Each restriction pattern was designated by a letter representing the enzyme and a number representing the profile type. The number of profiles obtained with the five enzymes: NdeII, CfoI, HinfI, HaeIII and MspI was 4, 5, 5, 5 and 6, respectively. The number of bands larger than 75 pb obtained per profile with the same five enzymes was 3–5, 3–7, 3–5, 7–10 and 4–7, respectively.
[a]According to the taxonomy of Cronquist (1981).
[b]Number of nodules.
[c]The closest reference strains for the RFLP profiles were infered by the neighbour-joining method of Saitou and Nei (1987). The profile S8 was not distinguished. The groups S5 and S6 were not taken into consideration since identified as chloroplast-like sequences.
[d]Aerial nodules on adventive roots on trunk at height ranging from 1.5 to 3 m.

Bradyrhizobium elkanii in this cluster, except the strains from the S2 and S12 groups that were found close to *B. japonicum* by using the neighbor-joining algorithm of Saitou and Nei (1987). By using the UPGMA method (Sneath and Sokal, 1973) for tree reconstruction, the groups S2 and S12 were found close to *B. elkanii*. The S8 profile, which shared no or few restriction patterns with references, could not be analyzed. This showed that information provided by the restriction with five enzymes was not always sufficient for identifying the known relatives of unknown strains at species level, even when the strains were separately analyzed. The distance matrix showed that the groups S6 and S8 were close to *B. japonicum* and *A. caulinodans*, respectively. However, these groups showed 99% of divergence with all the other reference strains. This did not allow us to reliably conclude about their taxonomic situation. The taxonomic question concerning these strains can only be resolved by using molecular sequence data. We noticed, however, that the group S8 clustered strains derived from nodules of two divergent legume species. The group S6 also had clustered strains deriving from two *Inga* spp. This suggested that the host specificity of these strains was quite slight. Our results agreed with those suggesting the predominance of slow-growing *Bradyrhizobia* in tropical legumes (Dupuy et al., 1992; Moreira et al., 1993; Stackebrandt et al.,

1993; Wong et al., 1994). We did not observe strains related to fast-growing *Rhizobium* spp., whatever the host plant of origin and the number of nodules treated. Some rhizobia nodulating tropical legume trees were, however, found as fast-growing (De Lajudie et al., 1992, 1993, 1994; Dreyfus and Dommergues, 1981). The occurrence of slow- and fast-growing strains in the same genus or plant species or even the same nodule has been also frequently observed (Dreyfus and Dommergues, 1981; Gao et al., 1994; Moreira et al., 1993; Zhang et al., 1991). Considering the confused state of the taxonomy of *Bradyrhizobia* and the methods of analysis used in these different works, most strains were identified as *Bradyrhizobium* sp. However, Chakrabarti and Chakrabarti (1989) found that strains isolated from Indian plant species were related to the species *B. elkanii*, formerly referred to *B. japonicum* group II (Kuykendall et al., 1992). Rumjanek et al. (1993) also showed that strains of *B. elkanii* were predominant in field soils of Brazil and were common inoculum strains for soybeans. Our results showed that the predominance of strains related to *B. elkanii* may be extended to natural soils as well as to legume tree species in rainforests of French Guiana. We noticed that these strains were derived from nodules of Mimosaceae as well as from nodules of Papilionaceae. These results indicated that clustering of strains was related neither to the species nor to the family of their host plants. This agreed with the promiscuous status of the Bradhyrhizobia already proposed by Norris (1956), and also suggested that there was no link between the evolution of the strains and the evolution of the plants they nodulated, as was suggested by Moreira et al. (1993) in Brazil.

Strains closely related to *A. caulinodans* were only found in three species of the Papilionaceae. This suggested a narrow host specifity for these strains compared to the *Bradyrhizobium* spp. Molecular sequence data would be interesting since *A. caulinodans* of *Sesbania rostrata* is still the only named species in the genus (Dreyfus et al., 1988). *Poecilante hostmanii* and *Inga stipularis* species constituted two new records of tropical tree species with aerial nodules. Based on the structure of the nodulation sites of Alazard and Duhoux (1988), these legumes belong to the group of *Pentaclethra macroloba*, with evolved predetermined sites from which a profuse nodulation occurs on well-developed adventitious roots. The strains from the aerial nodules of *Inga stipularis* were found close to *Bradyrhizobium*. This was also suggested by Young et al. (1991) and Wong et al. (1994) for strains from stem nodules of different *Aeschynomene* spp. We also noted that *A. caulinodans* and *B. elkanii* were found in the distance matrix as close neighbors of the strains derived from aerial nodules of both *Poecilante hostmanii* and *Inga stipularis*. These results agreed with the assumption that *Bradyrhizobium* and *A. caulinodans* are both members of one DNAr branch (Dreyfus et al., 1988; Willems and Collins, 1993).

The results in greenhouse with *Inga thibaudiana* revealed that the diversity of rhizobia was very slight and was also similar at individual and plant species level. It seems that these results might be extrapolated to the other plant species we studied because, in most cases, one RFLP group was found for each tree species whatever the number of nodules treated. Moreover the soil type did not act on the diversity of the nodulating strains, suggesting that *Bradyrhizobia* were ubiquitous in rainforests of French Guiana.

From the data described above, little diversity is observed among strains and the predominance of ubiquitous and promiscuous *Bradyrhizobia* may therefore be suggested. However, it has to be noted that the 16s DNAr genes may be too conserved to have the power to sort out the relationships between close species as well as to be used to show differences in host specificity (Martinez-Romero, 1994; Young and Haukka, 1996). Less conserved sequences such as intergenic spacer between 16s and 23s have been successfully used to compare organisms at the species and infra species level (Hönerlage et al., 1994; Jensen et al., 1993; Laguerre et al., 1996; Martinez-Romero, 1994; Navarro et al., 1992; Nour et al., 1994a,b; Nour et al., 1995; Rouvier et al., 1992; Rouvier et al., 1996; Woese, 1987). Moreover, it has also been shown that the phylogeny of *nod* genes was better correlated with the host taxonomy than that of 16s genes (Ueda et al., 1995; Young and Johnston, 1989).

Despite its limits, the PCR-RFLP method remains useful for rapid examination of numerous strains as well as to detect potential new taxa in natural ecosystems. This method did not allow to precisely identify the strains but it did, at least, determine their known relatives. The final resolution of the taxonomy of these rhizobia strains can only be resolved by 16s DNAr sequences and DNA–DNA hybridation (Graham et al., 1991).

4. Conclusion

These results showed that the $\delta^{15}N$ signature of plant foliage may be a relevant indicator for functional groups in the community of non-fixing plants, as well as a good diagnosis tool for diazotrophy when combined with total leaf N analysis. We also concluded that measurements of natural ^{15}N abundance made it possible to prospect in new environments and could be successfully extended to larger tree screenings in tropical rainforests.

The highest total leaf N concentrations were found in legumes with no significant differences between fixing and non-fixing plants within this family. This indicated that forest legumes had a great ability to extract large amounts of soil N, and that N_2-fixation for rainforest legumes should not be considered as a competitive advantage for N nutrition, but rather as a means among others of N acquisition.

Legume trees represented 17% and 18.5% of the inventoried woody plants with a DBH ≥10 cm in St Elie and Paracou, respectively. Forty-seven percent of these trees in Paracou (29 species) and 40% in PSE (40 species) had an N_2-fixing ability. Among the nodules tested, only four 16S DNAr types were listed, which were found close to *Bradyrhizobium* (*elkanii* and *japonicum*) and to *A. caulinodans*. The last had an unknown profile. Therefore, it seemed that the high species richness of the legumes in the rainforest of French Guiana did not necessarily correspond to a high diversity of the associated rhizobia.

Non-nodulated-legume trees were good non-fixing reference trees for using the ^{15}N natural abundance method to estimate N_2-fixation. The contribution of fixed-N to the N nutrition of the legume species was important and ranged from 50% to 70% for the Papilionaceae, from 44% to 62% for the Mimosaceae, and from 40% to 56% for the Caesalpiniaceae. The contribution of the N_2-fixation to the leaf N mineralomass of the stand was estimated at about 7 kg N ha^{-1} $year^{-1}$ from these data combined with those on leaf phytomass and leaf N concentration. This value constitutes a slight N input in the system and is undoubtedly underestimated: only leaves were considered and not the root trees or the possible root-to-root N_2 transfer occurring between plants. The N_2-fixed by other plants (jungle vine), the N_2-fixation associated with termites, the phyllospheric and rhizospheric N_2-fixation, as well as the N deposed from atmosphere by rains, are additional means of N input in these ecosystems. The sum of all these small contributions contribute to the stability of the rainforests by maintaining the N biogeochimal cycle at equilibrium. These processes have to be taken into consideration for sustainable management of these ecosystems.

Acknowledgements

This work was supported in part by a grant (J.C. Roggy's PhD) from INRA/IRD within the SILVOLAB group in French Guiana, SOFT programmes of the French Ministry of Environment and by the Laboratory of Ecologie Microbienne, Université Lyon I.

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