N_2 FIXATION AND DENITRIFICATION IN A FLOODPLAIN FOREST IN CENTRAL AMAZONIA, BRAZIL

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List of abbreviations

β	isotopic fractionation coefficients: $\beta = (^{15}N/(^{14}N+^{15}N))_{substrate} / (^{15}N/(^{14}N+^{15}N))_{product}$ or approximate: $\beta = 1- (\delta^{15}N_{substarte} - \delta^{15}N_{product})/1000$ (KOHL and SHEARER, 1980)
$\delta^{15}N_A$	isotopic ratio of biologically fixed nitrogen from atmosphere
$\delta^{15}N_R$	reference value (isotopic ratio of non-N_2 fixing reference plants)
%Ndfa	percentage of N derived from atmosphere
Abs_{260}	absorption $_{wavelength}$
ANOVA	analyses of variance
ARA	acetylene reduction assay
ARDRA	amplified ribosomal DNA restriction analysis
a.s.l.	above sea level
ATP	Adenosin-triphosphat
bp	base pair(s)
CCD	charge coupled device
CENA	Centro de Energia Nuclear na Agricultura
cfu	colony forming unit(s)
CTAB	cetyltrimethylammonium bromide
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
dw	dry weight
EDTA	ethylenediamine tetraacetic acid
ECD	electron capture detector
EtBr	ethidium bromide
FID	flame ionisation detector
FIV	family important value
FZJ	Forschungszentrum Jülich
fw	fresh weight
GC	gas chromatograph(y)
INPA	Instituto Nacional de Pesquisas da Amazônia
IVI	important value index
kb	kilo base pair(s)
LSD	least significant difference

M	molar
MPN	Most Probable Number
n	number of samples
ND	not determined
p	probability value
PCR	polymerase chain reaction
r	Spearman correlation coefficient
R^2	degree of explanation
rep-PCR	repetitive extragenic palindromic sequence-based PCR
RFLP	restriction fragment length polymorphism
SDS	sodium dodecil sulphate
SHIFT	Studies of Human Impact on Forests and Floodplains in the Tropics
sp(p).	species
TAE	tris-acetate-EDTA buffer
TBE	tris-borate-EDTA buffer
TE	tris-EDTA
tiff	tagged image file format
UGPMA	unpaired group method using arithmetic averages
UV	ultra violet radiation
v/v	volume by volume
w/v	weight by volume

used chemical formulas, symbols and unit abbreviations are conform with international standards

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1. Introduction

South America is the most densely forested continent in the world, with an average of 51% of the land area forested and 2.6 ha of forest per capita (FAO, 2001). The Amazon region holds the world's largest area of tropical rainforest, which is divided into different forest types (PIRES and PRANCE, 1985; FERREIRA and PRANCE, 1999). Large areas are covered by "terra firme" forest, growing on high topographic elevations, which are not subject to flooding. In contrast, there are the inundation forests which periodically oscillate between terrestrial and aquatic periods. The Amazon forests are essential for ecological equilibrium on a local, regional and global scale, so that all possible effort has to be taken in order to protect and preserve these ecosystems.

The inundation areas of the Rio Amazonas and its large tributaries cover an area of approximately 30 million hectares, of which about 30% are forested (KLINGE et al., 1990; JUNK, 1993). Due to the pronounced seasonality of rainfall there are big fluctuations in the water level of the rivers, resulting in a predictable mono-modal flood pulse which is the main factor controlling ecological processes in the inundation areas (JUNK et al., 1989). This also influences the interesting adaptations of animals and plants present (ADIS, 1997; PAROLIN, 2001). Microbiological community composition and activities could also be strongly influenced by the drastic changes in the soil physical and chemical properties during the hydrological cycle (DENDOOVEN et al., 1996; BOSSIO and SCOWN, 1998).

Due to the geological structure of their catchments, there are large chemical differences between Amazonian rivers (SIOLI, 1950; FURCH, 1987), which strongly influences their floodplains (FURCH et al., 1983; KLINGE and FURCH, 1991). Clear- and black-water rivers have only very small quantities of suspended inorganic sediments and solutes, whereas the white-water rivers are rich in both (FURCH and KLINGE, 1989). Accordingly 2 types of inundation areas are distinguishable. The ones flooded by clear- or black-water are named "igapó", the areas flooded by nutrient rich white-water are called "várzea" (IRMLER, 1977; PRANCE, 1979). Due to the annual water and nutrient input, várzea soils are the most fertile ones in Amazonia, supporting the most productive plant communities known (PIEDADE et al., 1991; 1994). Despite the problems associated with annual flooding, small-scale agriculture and shifting cultivation has been practised for centuries (FEARNSIDE, 1984; COOMES and BURT, 1997). However, during recent decades land pressure has increased and the detrimental

consequences for the forest cover have become obvious (FEARNSIDE, 1997a; KOLK, 1998). Concerns about this development, and the consequent need to establish sustainable management for existing forests in order to preserve their protective function in conserving soil and productivity of the ecosystem, make knowledge about biomass production and nutrient cycling important. Therefore, besides this study about the gaseous N turnover, several other aspects, for example respiration, photosynthesis, increment and growth dynamic etc. were studied in the same study area (KREIBICH and KERN, 2000; HORNA et al., 2000; WALDHOFF et al., 2000; LUDWIGSHAUSEN and KAPPEN, 2000; SCHÖNGART and WORBES, 2000).

Since sustainable management is only possible within the limits of ecosystem nutrient cycles (WHITMORE, 1998), this study focussed on this essential aspect and investigated the gaseous N turnover in the várzea forest. Enclosure experiments and field studies indicate that N can be a limiting factor for biomass production because the white-water is rich in alkali and alkaline-earth cations but not in N compounds (ZARET et al., 1981; SETARO and MELACK, 1984; FORSBERG, 1984; FURCH and JUNK, 1993). This is also indicated by the fact that in várzea soil there are enough nutrients available for the annual requirements of the forest except for N (FURCH, 1997). Additionally, as mycorrhizal infection is common, P limitation is reduced (MEYER, 1991; BROUWER, 1996). N input and output pathways of the forest are the water exchange with river and groundwater, dry and wet deposition and the gaseous N fluxes. N_2 fixation and denitrification may play important roles for this ecosystem. Both processes have already been reported for some ecotopes within the Amazon floodplain (SALATI et al., 1982; MELACK and FISHER, 1988; MARTINELLI et al., 1992; DOYLE and FISHER, 1994; KERN et al., 1996). With about 17% mainly abundantly nodulated legume trees in the várzea forest (SYLVESTER-BRADLEY et al., 1980; WORBES, 1986), there could be a high potential of N_2 fixation, but so far, actual N gains via symbiotic N_2 fixation are unknown. A previous study on Ilha de Marchantaria identified denitrification as the major pathway for N loss from a várzea lake (KERN et al., 1998), but reports about gaseous N turnover in várzea forest soil are scarce (KOSCHORRECK and DARWICH, submitted).

The main questions addressed here are whether N_2 fixation and denitrification are significant processes for the várzea forest, to what extent the flood pulse directly or indirectly regulates the gaseous N turnover, and finally, if sustainable management of the várzea forest is possible. Therefore, for the first time in a várzea forest, symbiotic and non-symbiotic

N_2 fixation and denitrification were monitored simultaneously over nearly 2 complete hydrological cycles (20 months). Physical, chemical and microbiological soil properties were determined to identify and investigate factors influencing and regulating the gaseous N turnover, with particular focus on the impact of the flood pulse. Characterisation of rhizobia and legumes was undertaken to provide background information about their diversity and possible symbioses within the study area. The forest stand structure was analysed to evaluate the importance of the legume species and therefore the impact of their N_2 fixation potential for the entire várzea forest. A pot experiment utilising the isotope dilution method was designed to compare different methods. An attempt to balance the gaseous N turnover and put it into context with the N cycle of the studied várzea forest, was undertaken.

The objective of this study was, to measure the gaseous N turnover, identify the regulating factors and to assess the extent of N_2 fixation and denitrification as a part of the entire N cycle of the várzea forest.

1.1 N cycle

N is one of the most important macro-nutrients and it is essential to all organisms. It plays a crucial role in the organisation and functioning of the world's ecosystems (VITOUSEK et al., 1997) and is found in all spheres. The major amount is bound in the lithosphere ($16.4 * 10^{19}$ kg N; STEPHENSON, 1986), and the atmosphere contains about $4 * 10^{18}$ kg N in form of N_2 (CAMPBELL, 1986). As organic matter and in mineral form $24 * 10^{13}$ kg N are available (STEPHENSON, 1986). The gaseous N being fixed from or released to the atmosphere represents only small fluxes by comparison with the previously fixed N that cycles among the components of the ecosystems. Global data about the 2 main processes of the gaseous N turnover, N_2 fixation and denitrification, are listed in Table 1.1.

Pre-industrial N deposition was greatest for tropical ecosystems, related to soil emissions, biomass burning and lightning emissions. In contrast, contemporary N deposition onto the northern hemisphere far exceeds contemporary tropical N deposition (HOLLAND et al., 1999). In the Amazon basin in total, regional gains and losses of N appear to be balanced. Inputs through bulk deposition and output via the Rio Amazonas are each $36 * 10^8$ kg N y^{-1} and the gaseous input and output are each $120 * 10^8$ kg N y^{-1} (SALATI et al., 1982). Comparing these

values with the global amounts shown in Table 1.1, about 3-10% of the terrestrial gaseous N turnover occurs in the Amazon basin.

Table 1.1 Literature data about global N_2 fixation and denitrification given in annual N fluxes of 10^9 kg N y^{-1}

global N_2 fixation	**N flux [10^9 kg N y^{-1}]**	**references**
natural N_2 fixation on the continents	90-195	GALLOWAY, 1998; CLEVELAND et al., 1999
agricultural N_2 fixation by crops	32-53	VITOUSEK et al., 1997; JENKINSON, 2001
marine N_2 fixation	30-300	VITOUSEK et al., 1997
industrial N_2 fixation	80-98	GALLOWAY et al., 1995; JENKINSON, 2001
global denitrification	**N flux [10^9 kg N y^{-1}]**	**references**
denitrification on the continents	125	HAHN and JUNGE, 1977
marine denitrification	450	CODISPOTI et al., 2001

Since N is a key nutrient for the production of crops, it is added as fertiliser to agricultural land in high quantities to meet the crop demands. With the increasing world population, the pressure on the production systems and agricultural research and policy will continue to increase. Nitrogenous fertiliser input is high in Western Europe and in East Asia, where China consumes the greatest amount (KAWASHIMA et al., 2000). In comparison, in Brazil N fertiliser use per hectare is rather low which might also be due to the importance of associative and symbiotic N_2 fixation in tropical agriculture (DÖBEREINER, 1977; GILLER and WILSON, 1991). However, human activities during the past century have approximately doubled the rate of N input into the terrestrial N cycle, leading to serious environmental consequences which are already apparent (VITOUSEK et al., 1997; GALLOWAY, 1998). Due to its mobility, N is easily lost to the environment and has become a major pollutant in the atmosphere, water and soil. Therefore, sound management of N resources is a prerequisite for sustainable agriculture and environmental protection (VEEN, 2000). In this context, wetlands are particularly important. Periodic wetting and drying cycles and the associated changes between aerobic and anaerobic conditions enable biochemical transformations, for example increased denitrification, which are highly significant for nutrient cycling and for maintenance and enhancement of water quality (MALTBY et al., 2000).

Various chemical, physical but mainly microbiological processes, strongly influenced by redox potentials, lead to transformation, mobilisation and immobilisation as well as to exchange fluxes between the various N pools (Figure 1.1). The N components included in the residues of organisms and organic matter are transformed by extra-cellular enzymes of decomposers into peptides and amino acids. These products are either incorporated into the cell structures or desaminated to NH_4^+ which is the first product of mineralisation. In aerobic soils nitrification, an oxidation process, takes place: *Nitrosomonas* and other genera convert NH_4^+ to NO_2^- and *Nitrobacter* and other genera transform NO_2^- to NO_3^-. Nitrification is regulated by soil NH_4^+ and O_2 concentrations and the pH optimum is between 7 and 8 (SCHLEGEL, 1992). Both, NH_4^+ and NO_3^- can be assimilated and used for biosynthesis of amino acids, proteins and nucleic acids. Plant tissue is the main N source for animals. Denitrification is a chemical as well as a biological process, leading to N loss mainly in form of N_2, but also NO and N_2O are released. Under anaerobic conditions, NO_3^- is denitrified by heterotrophic bacteria which use NO_3^- as an electron acceptor. N_2 in the atmosphere can be transformed by volcanic action and lightning, or fixed by industrial or biological processes. Non-symbiotic and symbiotic microorganisms like actinomycetes, cyanobacteria or rhizobia are able to fix N_2 and convert it to NH_3. Denitrification and biological N_2 fixation are described in more detail later on. NH_4^+, NO_2^- and NO_3^- are sensitive to leaching and runoff. Volatilisation of NH_3 from soil is another possible N output pathway, mainly important in soils with high N contents and high pH.

Generally, N is thought to be more limiting in temperate than tropical forests (MARTINELLI et al., 1999), however, even within the tropics, there are many regions where N is limiting, including flooded forests (HECKMAN, 1998; SPRENT and PARSONS, 2000), especially on young soils. In the short term, N availability is mainly dependent on the rate of internal cycling (PROCTOR, 1987) but in the long term the balance between inputs and outputs determines the ecosystem nutrient status (VITOUSEK and SANFORD, 1986).

N enters a forest ecosystem with rain, deposition of dust and aerosols, and by N_2 fixation, whereas N losses occur through leaching from the root zone, erosion and denitrification (Figure 1.2). For floodplains the exchange with the river is fundamental. For example, the N gain via the river was, at over 70%, the main N source for a várzea lake. And the N loss via the river extended to nearly 44% of the total N loss, only exceeded by denitrification (KERN et al., 1998).

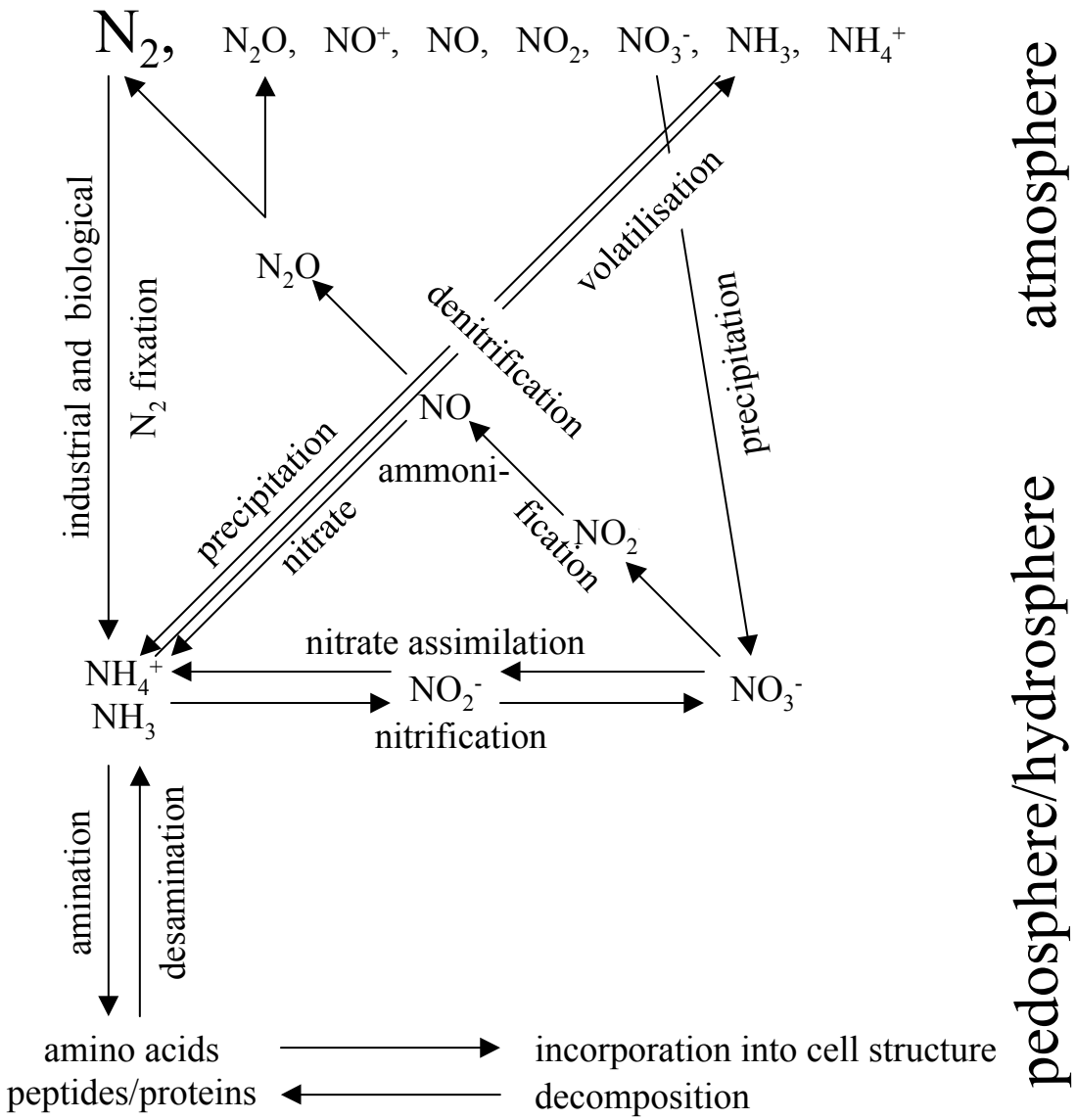


Figure 1.1 Scheme of the various processes involved in the complex biogeochemical N cycle (modified from Werner D., University of Marburg)

In terra firme climax forests internal N cycling is often very tight. In the absence of harvesting, input and output fluxes are small (SPRENT, 1987) and N turnover through microbial processes is rapid, maintaining the ecosystems productivity (KLINGE et al., 1975). The major above ground pool of N is the plant community from which N is transferred to the forest floor in small and large litter fall and in throughfall and stemflow. Assimilation of N by plants can be a large sink, leading to an efficient long-term retention of N in the forest (PERAKIS and HEDIN, 2001). This is additionally supported by the very common mycorrhizal infection in tropical forests, which leads to enhanced mineral uptake (BROUWER, 1996). A proportion of the above ground N is in dead organic matter such as small and large litter. N is

gradually released by decomposition mediated by soil animals and microorganisms. Roots take up N from soil and water solution or perhaps directly from the litter layer (HERRERA et al., 1978; CUEVAS and MEDINA, 1988) and transfer the N to the canopy. Roots are a considerable below-ground N pool, releasing N to the soil by secretion as well as by death and decomposition of their parts.

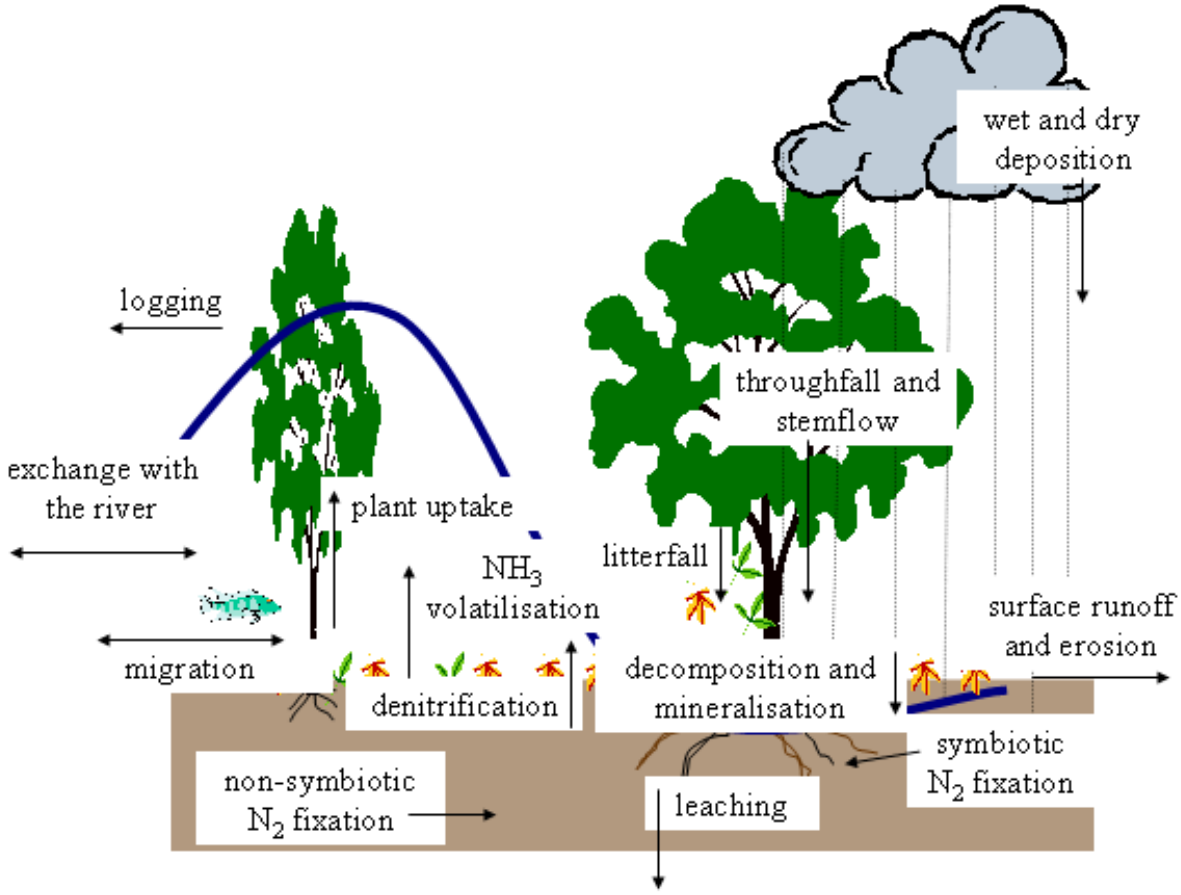


Figure 1.2 Scheme of the input and output N fluxes as well as internal N cycling in the várzea forest. The blue line symbolises the flood pulse.

1.2 N_2 fixation

Since prokaryotes containing nitrogenase, are the only organisms able to access the large N pool of the atmosphere, they have a vital role in the global N cycle. Diazotrophs cover a wide temperature range, from arctic conditions to hot springs (KIMBLE et al., 1995; LIENGEN, 1999) and are present in basically all ecosystems, with representatives in environments as different as aerobic soils (*Azotobacter*), the ocean surface layer (*Trichodesmium*) and specialised nodules in legume roots (*Rhizobium*) (HALBLEIB and LUDDEN, 2000). Correspondingly, they

either live freely, are associated to other organisms or form real symbioses and result in considerably different N gains via N_2 fixation in various habitats (Table 1.2).

Biological N_2 fixation, where N_2 reduction leads to $2NH_3$ and H_2, is catalysed by the nitrogenase enzyme complex (CHRISTIANSEN et al., 2001). The dinitrogenase (Mo-Fe-protein) binds and reduces N_2, whereas the dinitrogenase-reductase (Fe-protein) regenerates the nitrogenase (KIM and REES, 1994). The enzyme complex is highly O_2 sensitive (PAERL and CARLTON, 1988), so that various protection mechanisms, e.g. diffusion barriers or respiratory protection, were developed. Theoretically 16 ATP-molecules are needed for the N_2 reduction (including the reduced protons), but at about 20-30 ATP molecules the actual need is much higher (BURRIS and ROBERTS, 1993). The nitrogenase complex requires specific conditions, particularly related to the redox conditions of its environment. Other favourable factors are low mineral N contents (HANSEN et al., 1992; COLNAGHI et al., 1997), but good availability of other nutrients especially P, Fe and Mo (GILLER and WILSON, 1991). Temperature changes may also influence N_2 fixation (TROLLDENIER, 1982).

Table 1.2 Examples of N_2 fixation rates of different phytocoenosis in various habitats

	N_2 fixation [kg N ha^{-1} y^{-1}]	**references**
coastal marine sediments	< 1	SEITZINGER and GARBER, 1987
intertidal microbial mats	153	JOYE and PAERL, 1993
unvegetated semi-aquatic várzea sediments	12	KERN, 1995
mangrove litter and sediment	30	PELEGRÍ et al., 1997
grasses *Brachiaria* spp., *Paspalum notatum*	21-45	BODDEY et al., 1983; BODDEY and VICTORIA, 1986
associative N_2 fixation in Amazonian terra firme forest	16	JORDAN et al., 1982
associative N_2 fixation in Amazonian caatinga vegetation	35-200	HERRERA and JORDAN, 1981
epiphytes on Amazonian vegetation	0-20	SALATI et al., 1982
root mats in the várzea	243	SYLVESTER-BRADLEY et al., 1980
actinorhizal tree *Alnus*	266	BORMANN et al., 1993
tree legume *Chamaecytisus proliferus*	8-82	OVALLE et al., 1996
Chilean tree legumes (*Acacia caven, Prosopis alba, Prosopis chilensis*)	0-9	OVALLE et al., 1996
herbal legume *Vigna* sp.	9-201	PEOPLES et al., 1995
terra firme secondary vegetation	0-5	THIELEN-KLINGE, 1997

Non-symbiotic N_2 fixation

Free-living diazotrophs are heterotrophic (e.g. *Clostridium, Klebsiella, Azotobacter*), chemoautotrophic (e.g. *Thiobacillus, Methylosinus*) or phototrophic (e.g. *Rhodospirillum*, *Rhodopseudomonas*). The ability to fix N_2 is widespread within the soil- and water-bacteria (SCHLEGEL, 1992), which are preferably abundant in waters or soils with reduced O_2 concentrations (PAERL and CARLTON, 1988). Soil temperature, pH value and water content influence the free-living diazotrophs (STEWART, 1975). Non-symbiotic N_2 fixation is positively correlated with soil organic matter (JAEGER and WERNER, 1981). Mineral nutrition also influences N_2 fixation by free-living prokaryotes, where high concentrations of combined N inhibit N_2 fixation, whereas a good supply of other nutrients, mainly K, Ca, Mg, Mo, Ni, favours N_2 fixation (CHRISTIANSEN et al., 2001; O'HARA, 2001). Associative N_2 fixation in the rhizosphere is also influenced by environmental factors, such as temperature, O_2 concentration, pH values and the presence of aromatic compounds. Also different plant characteristics, for example composition, production and release of root exudates, or plant age, have an influencing potential (KROTZKY et al., 1988; WERNER et al., 1989). While in temperate climates non-symbiotic N_2 fixation is relatively unimportant (ECKARDT and BIESBOER, 1988), the associated N_2 fixing bacteria in the rhizosphere of tropical grasses are important N sources for agriculture and natural ecosystems (DÖBEREINER, 1977; TROLLDENIER, 1984).

Symbiotic N_2 fixation

Symbiotic N_2 fixation is generally able to fix between 10 and 300 kg N ha^{-1} y^{-1} and provides 80% of the biologically fixed N on land (PEOPLES and CRASWELL, 1992). 11 plant families are characterised by N_2 fixation, namely members of the legume family and actinorhizal plants which form nodule symbioses with rhizobia and actinomycetes (*Frankia*), respectively. An exception is *Parasponia*, an Ulmaceae, which forms symbioses with rhizobia (GRESSHOFF et al., 1986). N_2 fixing trees are mainly tropical and subtropical, whereas herbaceous legumes are also abundant in temperate regions (BREWBAKER, 1990). The bacteria receive energy, carbohydrates and live within special structures (nodules) where they can exist in a favourable environment. For the plants, N_2 fixing symbioses alleviate any soil N limitation to growth since they receive NH_4^+ from the bacteria, but N_2 fixation requires more energy and a better supply of nutrients other than just the assimilation of NO_3^- and NH_4^+ (SPRENT, 1995). Therefore, the occurrence of mycorrhiza is widespread in nodulated legumes and actinorhizal

plants, which provide for efficient uptake of nutrients, but also require an increased supply of C (SPRENT and PARSONS, 2000).

Legumes form the 3rd largest flowering plant family, with around 18000 species divided into the 3 subfamilies Caesalpiniodeae, Mimosoideae and Papilionoideae (SPRENT and PARSONS, 2000). They are highly diverse and adaptable to ecological niches. Nodulation is least common in Caesalpinoideae (23% nodulated), most common in Papilionoideae (97% nodulated), with the Mimosoideae (90% nodulated) being intermediate (FARIA et al., 1989; SPRENT, 1995). It was believed, that rhizobia belong exclusively to the alpha-subclass of Proteobacteria, where they are distributed in 4 distinct phylogenetic branches (*Rhizobium*, *Bradyrhizobium*, *Azorhizobium*, *Sinorhizobium*) (JONES, 1998). But recently, the identification of Proteobacteria from the beta-subclass nodulating legumes was reported (MOULIN et al., 2001). Which suggests, that the ability to establish a symbiosis with legumes is more widespread in bacteria than previously anticipated.

To form an effective symbiosis, rhizobia require several specific genes. These include *nod* genes, which encode the production of *nod*-factors, which stimulate the plants to produce symbiotic nodules, *nif* genes, which produce the N_2 fixing nitrogenase enzyme (HAUKKA et al., 1998) and others (*rhi*, *hem, lps* etc.) involved in nodule formation, N_2 fixation or the production of surface components required for bacterial invasion (BECKER et al., 1997; KISS et al., 1997).

Associations between the symbiotic partners are quite specific, so when legume species are introduced into new regions it is often necessary to inoculate them with the appropriate rhizobia in order to establish an effective symbiosis (HAUKKA et al., 1998). For woody legumes, effective rhizobia seem to be widely distributed and some species can even be nodulated by a wide range of strains from both fast- and slow-growing rhizobia (BARNET et al., 1985). The specificity of the legume-rhizobia interaction is determined by distinct patterns of signal release and response. Flavonoids and *nod*-factors are involved as components of the signal exchange chain between micro-symbionts and legume host plants (PUEPPKE et al., 1998). Flavonoids excreted by plant roots or seeds interact with the *nod*D gene product of the rhizobia and this induces the transcription of bacterial *nod* genes (SCHMIDT et al., 1994; STEELE et al., 1999). Then, the bacteria produce lipo-oligosaccharide signals to induce the

nodule meristem. The development that results in a N_2 fixing nodule can be characterised by 4 major stages (HIRSCH, 1992; NINER and HIRSCH, 1998):

1. the infection of the plant by the bacteria
2. the organisation of the newly divided plant cells into a nodule
3. the maintenance of N_2 fixation
4. the senescence of the nodule tissue

Generally, 2 morphologically different types of nodules are distinguished, the determinate and indeterminate forms. Determinate ones reach their final size by cell expansion after an initial period of meristem growth, indeterminate ones recapitulate in space the temporal phases of nodule development so that there is a gradient of differentiation from the distal to the proximal end of the nodule (NINER and HIRSCH, 1998). Determinate nodules have a continuous vascular system surrounding the nodule, where lenticels facilitating gas exchange at the surface are common. Woody legumes are not restricted, they bear nodules of all known morphological and anatomical types (SPRENT and SPRENT, 1990).

Carbohydrates produced by photosynthesis in host plants are used in the nodule to produce ATP, reductants and α-ketoacias for the initial assimilation of the NH_3. Depending on the legume species, either amino acids or ureides are produced as the main export compound to rapidly remove NH_3 from its site of formation and transport it to the xylem. The requirement of O_2 for oxidative ATP formation by the bacteroids is balanced against the sensitivity to O_2 of nitrogenase. Therefore, O_2 diffusion into the nodule is controlled by a gaseous diffusion barrier, which balances the inward O_2 flux with respiratory O_2 consumption (WITTY et al., 1986). The major resistance to the diffusion of O_2 is provided by a layer of cells in the inner cortex, so that adaptations to different O_2 pressures involve a regulation of cortical intercellular air-spaces (PARSONS and DAY, 1990).

Environmental factors can significantly influence nodulation by either inhibiting or stimulating nitrogenase activity or nodule formation. Outstanding N_2 fixing plants, growing in harsh environments are for example *Acacia* or *Inga* growing on acid soils and *Aeschynomene* or *Sesbania* growing in waterlogged soils (BREWBAKER, 1990). It has been suggested, that in tropical primary forest, nodulation might be repressed due to substances from the vegetation e.g. tannins (MOREIRA et al., 1992), but abundant nodulation of seedlings and young trees in these forests, might rather indicate a strong influence of the plant age (NORRIS, 1969).

Nodulation varies with the nutrient availability in soil (SYLVESTER-BRADLEY et al., 1980). High mineral N concentrations generally reduce or even inhibit root nodule formation (TSAI et al., 1998), while in some cases NH_4^+ may even have a stimulating effect (SPRENT, 1995). Good supply with other nutrients, especially K has a positive effect on symbiotic N_2 fixation and may improve the plants resistance against environmental stress (SANGAKKARA et al., 1996).

Generally, water stress significantly reduces N_2 fixation and nodulation (CASTELLANOS et al., 1996). Flooding and waterlogging is a drastic environmental change, where many plants react with a reduction of plant growth, mainly of roots, because it leads to a reduction in O_2 supply and thus to accumulation of toxic substances. Although nitrogenase is strongly O_2 sensitive, O_2 is required for bacteroid respiration to meet the ATP requirements of N_2 reduction. Therefore, for some nodulated legumes, already after a few days of waterlogging, nodule number and weight are reduced and nitrogenase activity suppressed (BUTTERY, 1987; SUNG, 1993). But sensitivity to waterlogging is quite variable between legume species, various tropical and temperate legumes can grow, nodulate, and fix N_2 while flooded (PUGH et al., 1995; JAMES et al., 2001). Flood tolerant species show adaptations such as the production of adventitious roots, increased aerenchyma or lenticel production, larger vacuoles or nodule cells, thickening of submerged stems etc. (SHIFERAW et al., 1992). The continuation of nitrogenase activity under waterlogging for soil grown plants implies the development of an internal route for gaseous exchange between submerged nodules and the atmosphere, namely a downward movement of O_2 through air spaces within the plant tissue (PUGH et al., 1995), but also N_2 exchange through aerenchyma exists (ARTH et al., 1998). Also, aquatic legumes grow aerenchyma and hollow stems to ensure a sufficient O_2 supply. In addition, they may set the diffusion resistance to a minimum level and take up O_2 directly from the water (JAMES et al., 1992).

Apparently there is a difference between sudden changes in water conditions or O_2 concentrations and long-term environmental conditions. For example, white clover is able to adapt to long-term waterlogging, resulting in even increased dry weight and N_2 fixation, but is sensitive to sudden changes in moisture conditions (PUGH et al., 1995). And, the nitrogenase activity of aquatically grown *Neptunia plena* was irreversibly inhibited by rapid exposure of nodules to ambient air, whereas this species is also able to grow and fix N_2 when grown in well aerated vermiculite cultures (JAMES et al., 1992). Plants in the floodplain forest are well

adapted to the annual flooding which lasts several months, showing morphological and physiological adaptations, such as pressurised gas transport (GRAFFMANN, 2000; PAROLIN, 2001).

Legume-rhizobia symbioses are most efficient at fixing N_2, making them the main contributors of fixed N in all pristine ecosystems (CLEVELAND et al., 1999). They also contribute significantly to the N-balance of tropical floodplain forests (MARTINELLI et al., 1992; BARRIOS and HERRERA, 1994; SPRENT, 1999).

1.3 Denitrification

Denitrification leads to N loss in the form of gaseous N_2, NO and N_2O from the hydrosphere and pedosphere. It is the only biological process transforming N compounds to N_2, thus it has a vital role for the global N cycle. Chemical denitrification, which is a disproportionation of HNO_2, leading to NO, NO_2 and H_2O, occurs mainly at pH values below 5 and at low soil moisture contents directly after wetting (DAVIDSON, 1992; DAVIDSON et al., 1993). More important is biological denitrification, where denitrifying bacteria reduce NO_3^- or NO_2^- using these substances as electron acceptor instead of O_2. This microbiological process is nearly exclusively a facultative trait, triggered in the cell by low O_2 tension and availability of a N oxide (ZUMFT, 1997).

A diversity of bacteria from various subclasses of the Proteobacteria and even Archaea are able to denitrify when environmental conditions are favourable, examples are *Pseudomonas denitrificans*, *Thiobacillus denitrificans* or *Paracoccus denitrificans* (SCHLEGEL, 1992). Denitrification is a modular process, proceeding in 4 stages: NO_3^- respiration, NO_2^- respiration, NO respiration and N_2O respiration, with the intermediates NO_2^-, NO and N_2O. Each of these reductive steps is catalysed by different reductase enzymes, which are regulated by distinct gene sets (BURNS et al., 1996). The total number of genes necessary for denitrification may be about 50 for a single organism, where in many bacteria the genes *nar* (NO_3^- respiration), *nir* (NO_2^- respiration), *nor* (NO respiration), and *nos* (N_2O respiration), are assembled in clusters (ZUMFT, 1997). O_2 is recognised as a major regulator of denitrification as it affects both, the synthesis and activity of the 4 denitrifying enzymes (DENDOOVEN and ANDERSON, 1995). The 4 reductases differ with respect to optimum conditions, so that

environmental factors can play a vital role in determining whether denitrification is complete or if intermediates of this process accumulate (BURNS et al., 1996).

Denitrifiers are ubiquitous in sub-soils and groundwater, but their activity often is limited by available C, leading to a decrease of denitrification with soil depth (LUO et al., 1998; PARAMASIVAM et al., 1999). Denitrification shows significant seasonal variation in temperate and tropical habitats (PINAY et al., 1993, KERN, 1995). Under field conditions, the regulating factor, O_2 availability, is influenced by physical factors such as soil water content and soil compaction, thus also soil texture and drainage have significant influences on denitrification (GROFFMAN and TIEDJE, 1991). Denitrification rates may be positively correlated with soil water content, NO_3^--N content, NH_4^+-N content and C availability and negatively correlated with soil temperature and pH (PINAY et al., 1993). Whereas the importance of the different regulators depends on the different habitats, e.g. O_2 should be most important in soils, while NO_3^- should be most important in sediments. In a swamp forest NO_3^- availability had a high impact, whereas in upland forests the water content was most important (MERRILL and ZAK, 1992). In a temperate agricultural soil, soil water content and soil NO_3^- status together explained 67% of the variation in denitrification (KILIAN and WERNER, 1996). Due to diminutive spatial differences in soil properties creating “hot spots” (CHRISTENSEN et al., 1990; PARKIN, 1987), denitrification rates are highly variable within small areas of forest ground, with coefficients of variance higher than 500% (FOLORUNSO and ROLSTON, 1984).

Due to its sensitivity to environmental factors, denitrification rates vary greatly between different habitats (Table 1.3). The fate and transport of N in wetlands was modelled by MARTIN and REDDY (1997). A seasonally flooded regime has a great impact on microbiological N cycling in the soil. Flooding and drying out lead to changes in the soil physical and chemical environment (PONNAMPERUMA, 1984) which influence microbial community composition (BOSSIO and SCOW, 1998) and activities (NEILL, 1995; DENDOOVEN et al., 1996). The highest nutrient dynamic, especially denitrification, is expected during the transition between aquatic and terrestrial period, due to small localised changes in aerobic and anaerobic conditions. Denitrification is dependent on NO_3^- and favoured by anoxic conditions (TIEDJE, 1988), but nitrification, necessary for the replenishment of NO_3^-, requires the presence of O_2. Water filled pore space around 60%, provides the best conditions for nitrification, since neither the diffusion of substrates, nor the diffusion of O_2 are restricted (LINN and DORAN, 1984). At higher soil water contents and O_2 partial pressures below 0.5%,

denitrification is favoured (BOLLMANN and CONRAD, 1998). Exposure of soil to air with flood recession is favourable for the oxidation of NH_4^+, and with NO_3^- production, denitrification rates increase. With increasing soil water content during rising water, O_2 availability decreases, supporting the reduction of NO_3^-. Denitrification may be even more stimulated by flooding than by glucose addition (CHRISTENSEN et al., 1990).

Table 1.3 Examples of denitrification rates in different habitats

	denitrification [kg N ha^{-1} y^{-1}]	**references**
intertidal microbial mats	0-38	JOYE and PAERL, 1993
unvegetated semi-aquatic várzea sediments	32	KERN, 1995
different cropping systems	3-17	SVENSSON et al., 1991
temperate riparian forest	55-115	PINAY et al., 1993
temperate well-drained forest	< 1	MERRILL and ZAK, 1992
tropical terra firme forest	3	JORDAN et al., 1982

In rivers, highest denitrification is in the surface sediment layer, where continuous replenishment of NO_3^- occurs (KESSEL, 1977). When O_2 is available, and NO_3^- concentration is low, sediment internal nitrification is important. But coupled nitrification-denitrification within lake and estuarine sediments may contribute only up to 10% of the total denitrification (RYSGAARD et al., 1993). In contrast, in the rhizosphere of flooded rice paddies, the regeneration of NO_3^- and NH_4^+ leads to a tight coupling of nitrification and denitrification, where the aerenchymateous rice plants are important for the transport of O_2 and N_2 into and from the rhizosphere (ARTH et al., 1998; LIESACK et al., 2000).

Exceptionally, denitrification may occur in apparently aerobic environments due to facultative anaerobic denitrifiers (LLOYD, 1993) and nitrification may proceed at relatively low O_2 concentrations (GUNDERSEN, 1966). In waterlogged soils, NO_3^- reductase activity may remain high also under aerobic conditions (DENDOOVEN and ANDERSON, 1994), when high quantities of readily decomposable C substrate are available, and a high concentration of NO_3^- reductase will be an advantage for the microorganisms when anaerobiosis is induced (DENDOOVEN and ANDERSON, 1995). Therefore, with sufficient NO_3^- and C availability high denitrification in flooded or waterlogged soils is expected (MERRILL and ZAK, 1992), resulting in a high CO_2 production in soil (GROFFMAN and TIEDJE, 1991). Denitrification is stimulated by bioturbation through the activity of soil fauna (LENSI et al., 1992; SVENSSON, 1998). Also, denitrification is lower at unvegetated areas in contrast to vegetated areas, when plants do not use up water and available N but reduce the O_2 concentration due to root respiration

(GRIFFITH et al., 1993). Direct competition for NO_3^- between plants and denitrifiers seem to be relatively unimportant, due to the different O_2 requirements and seasonal variations of these processes, with high plant N uptake during the terrestrial period and high denitrification during the aquatic period (CHRISTENSEN and TIEDJE, 1988; PINAY et al., 1995). Significant influence of plant species on denitrification rates was also reported (KILIAN and WERNER, 1996; KAISER et al., 1998). Wetland species with greater evapotranspiration rates will promote greater N loss by denitrification due to stimulated transport of soluble N by vegetation induced water movement (MARTIN and REDDY, 1997). Additionally, high denitrification rates occur when the plant cover decays (CHRISTENSEN et al., 1990). Therefore, denitrification leads to high N losses from floodplains, so that in eutrophic regions, rehabilitation of riparian zones with riparian vegetation appears to be a good start to restoring the buffering capacities of river ecosystems against N loads (PINAY et al., 1993).

2. Study area

2.1 Geography

The studied forest is next to Lago Camaleão on Ilha de Marchantaria (3°15'S and 59°58'W), an island in the Rio Solimões in Central Amazonia. In Brazil, the Amazon river between the Peruvian border and the confluence with the Rio Negro is called Rio Solimões. Ilha de Marchantaria is located 15 km upstream from this confluence, close to the city of Manaus (Figure 2.1). The study area (várzea) is only influenced by suspended matter and nutrient rich white-water. At the edges of the inhabited island, at high elevations, farmers grow vegetables, and fishing activities were observed mainly during low water. Due to the relatively high population density in the várzea (JUNK and FURCH, 1985), and the proximity to Manaus, human influence on the study area cannot be excluded. The studied forest is located inland on the island on a ridge between 2 lakes, at an elevational range of 22 m - 25 m a.s.l.. The south-east edge is relatively steep, rising from the water line of Lago Camaleão to the highest elevations of the forest within a couple of meters. On the other side, the forest floor smoothly descends to the level of the lake. The study area is 1.5 km long and between 80 and 120 m wide, therefore it covers an area of about 15 ha.

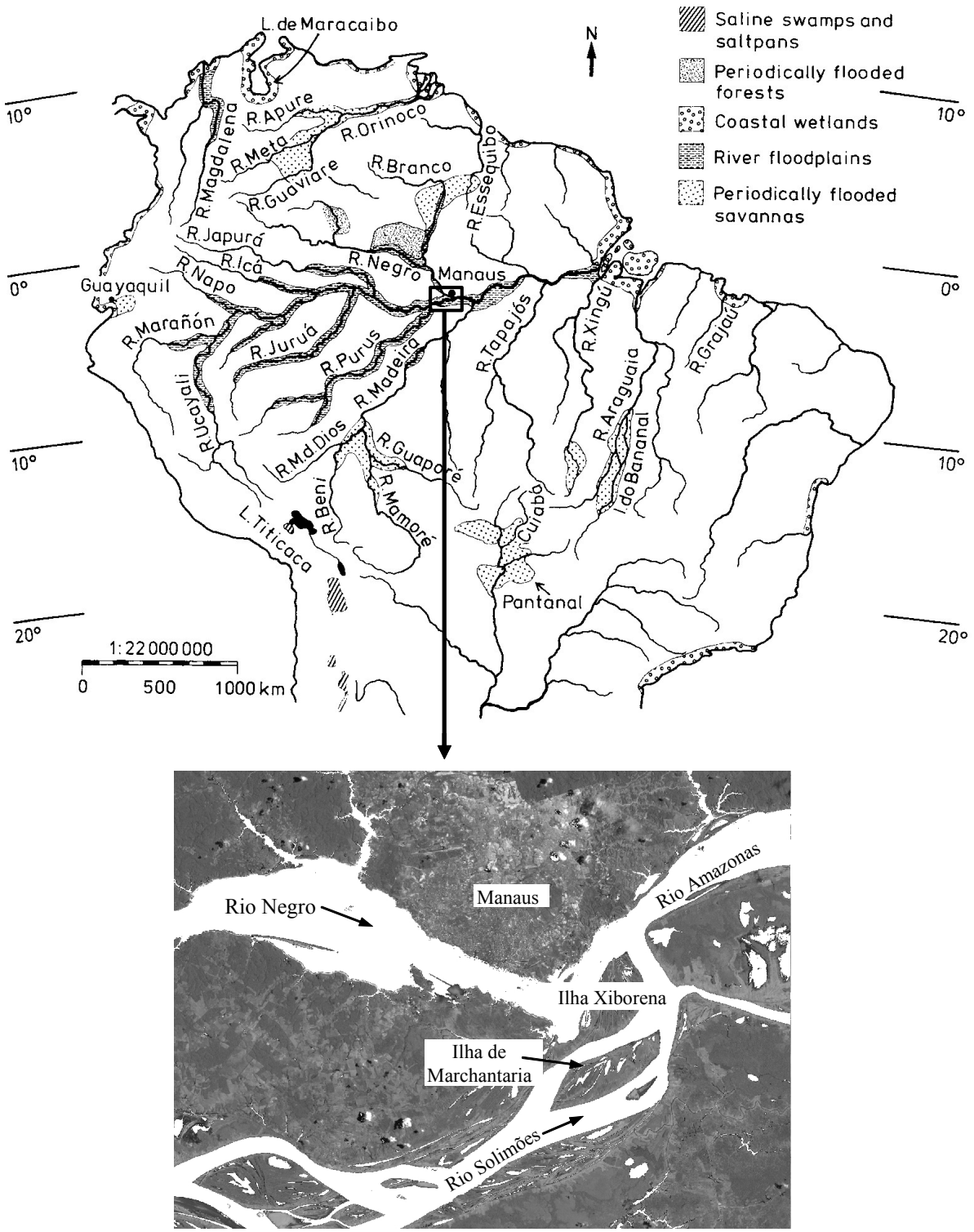


Figure 2.1 Location of the study area in Central Amazonia, Brazil. Distribution of different neotropical wetlands (top) including the Amazon floodplain (JUNK, 1993). Modified satellite image (bottom) of the Ilha de Marchantaria and the confluence of the Rio Negro and the Rio Solimões near Manaus (Instituto Nacional de Pesquisas Espaciais (INPE) 18/09/98).

2.2 Climate

The climate is permanently hot and humid. The mean annual temperature at Manaus is 26.6° C, with an average temperature of 27.9° C during the dry season, and 25.8° C during the rainy season (JUNK and FURCH, 1985). The warmest months are August to November, the coolest are January to April. But daily fluctuations are greater than seasonal variations and can exceed 10° C (IRION et al., 1997).

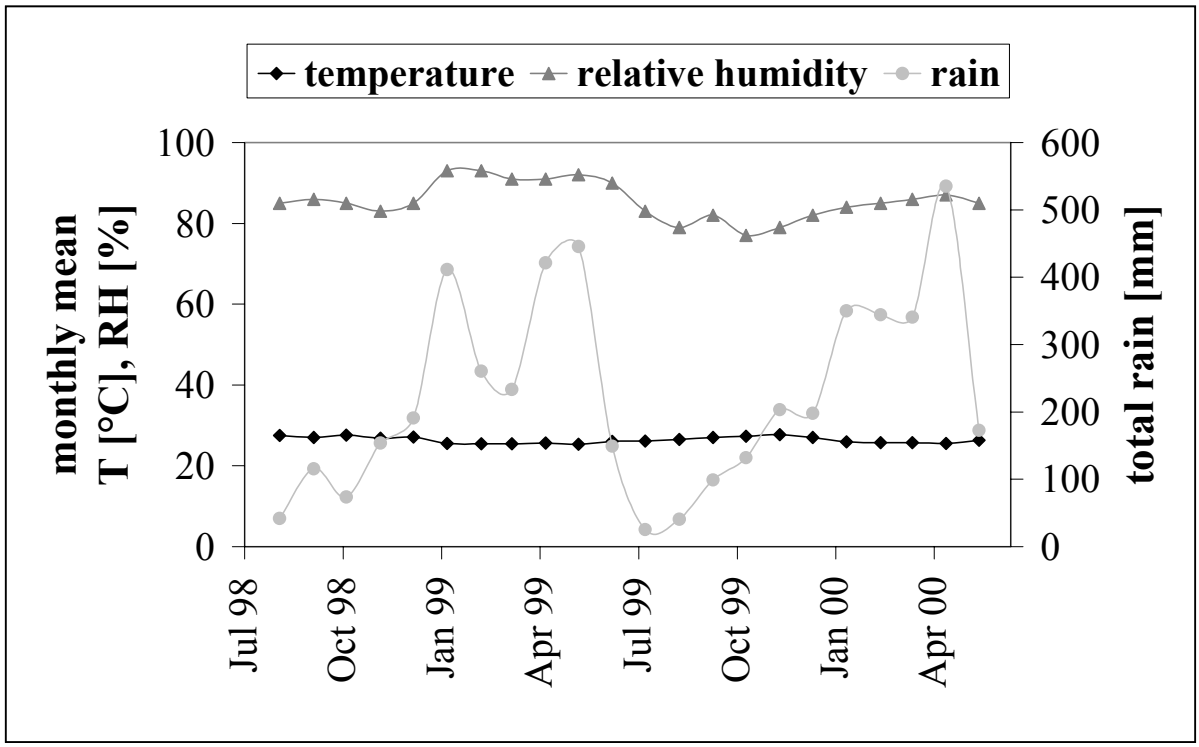


Figure 2.2 Monthly means of temperature and relative humidity as well as total rain during the study period measured by the meteorological station in Manaus (Instituto Nacional de Meteorologia, Estação 82331)

In Central Amazonia, air humidity is permanently high, varying between an average of 75.6% in September and 86.7% in April (SALATI and MARQUES, 1984; RIBEIRO and ADIS, 1984). During the study period it was on average 86% in Manaus (Figure 2.2). Long term mean total rainfall is 2100 mm $year^{-1}$, 1999 total rainfall was 2620 mm (Figure 2.2). More or less developed seasons exist, with the rainy season from January to May and the dry season from July to November.

The rainwater has a low pH value of 4.3-5.3 (FURCH and JUNK, 1997). N concentration in rain is about 0.41 mg l^{-1} including 0.169 mg l^{-1} NH_4^+-N, 0.110 mg l^{-1} NO_3^--N and 0.002 mg l^{-1}

NO_2^--N (JUNK and FURCH, 1985). When passing through the canopy of the forest, concentrations of dissolved mineral nutrients in the water increase significantly, N compounds are on average 15 times higher in stem flow than in rain water. Therefore, precipitation and canopy leaching add about 8.2 kg N, 0.22 kg P, 2.0 kg K and 1.4 kg Ca per hectare and year to the ecosystem (JUNK and FURCH, 1985).

2.3 Geology and pedology

The Amazon basin, which lies less than 100 m a.s.l. is bordered in the west by relatively young mountains, the Andes (tertiary). In the north and the south are the Guyanan and Brazilian Shields. Cretaceous, tertiary and pleistocene sediments characterise the Amazon basin (IRION et al., 1983). Some active floodplains are only a few thousand years old and therefore the most recent structural elements of the Amazonian lowlands (IRION et al., 1997). The different Amazonian water types are related to the specific conditions of their catchment areas (SIOLI, 1950). White-water arises from the Andean and Pre-Andean region, areas with intensive erosion processes. In the archaic shields of Central Brazil and Guyana where erosion is small, clear- and black-water rivers originate.

Sedimentation and erosion processes are obvious effects of the Rio Amazonas on its floodplain, which is strongly influenced by the high sediment load, the strong current and the large annual water level fluctuations (IRION et al., 1983; 1984). On Ilha de Marchantaria, until 30 cm depth the soil consists of silty clay, below there is sand with small proportions of silt and clay (IRION et al., 1983). The fine sand and coarse silt fraction consists mainly of quartz with minor amounts of feldspar and clay minerals. The clay and fine silt fractions contain predominantly smectite and illite followed by chlorite and kaolinite (IRION et al., 1997).

The Ilha de Marchantaria is most likely a very young island which was formed only several centuries ago. It arose from a sand bank in the middle of Rio Solimões. The island is characterised by narrow ridges which concentrically cover the whole island, and with permanent lakes in between (IRION et al., 1984). Ilha de Marchantaria is typical for the várzea, which is a small scale mosaic of different structural elements of fluvial origin (IRION et al., 1997).

The alluvial loamy várzea soils are relatively rich in nutrients sustaining a high natural productivity (SOMBROEK, 1984, MEGGERS, 1985). Additionally, they have a relatively high cation exchange capacity (IRION et al., 1997). The contents of Na, K, and Ca are in the upper range of soils, the content of Mg is even higher. C, N, S and to some extend P are concentrated in the humic soil fraction and show a strong depletion with soil depth (FURCH, 1997). Generally a low proportion of N and a high proportion of Ca are characteristic for the várzea (FURCH and KLINGE, 1989). NH_4^+-N occupies a major portion of the inorganic N, which shows a high mobility and an easy transfer to deeper soil layers (FURCH, 1997).

2.4 Hydrology

The seasonal distribution of the rainfall produces large water level fluctuations of the rivers. The discharge of Rio Solimões represents the rainfall of its large catchment area with local rains being of little importance (JUNK and FURCH, 1985). Its water level changes follow a sinoidal curve with an average flood amplitude of 10 m at Manaus (1903-2000). Low water is usually in October/November, high water in June/July, several weeks after the end of the rainy season. But the water level fluctuations show also marked variations between years (IRMLER, 1977), for instance low water levels varied between 13.64 m a.s.l. in the year 1963 and 21.84 m a.s.l. 1974 (1903-2000).

The studied forest area is influenced by the water level of Lago Camaleão, and is located on an elevational range of 22 m - 25 m a.s.l. (Figure 2.3). Therefore, on average it is inundated between 4.7 and 7.6 month per year (1903-2000). The maximum water level during the study period was 29.5 m, the lowest spot of the study area was then flooded by 7.5 m. In 1998 the lowest sampling points fell dry mid-September and the highest points were flooded again mid-February 1999 (Figure 2.3). During the next hydrological cycle the study sites fell dry at the beginning of October 1999 and were flooded again at the end of March 2000. Therefore, October to February was considered as the terrestrial period and March to September as the aquatic period, transition periods were in September/October and February/March.

The water of Rio Solimões is relatively rich chemically, particularly with high concentrations of P, Mg and Ca (FURCH et al., 1982). Mean total N concentration is 603 μg N l^{-1} (FURCH and KLINGE, 1989). But a clear relationship exists between the total element content and the proportional contributions of Ca and N: the lower the total elements, the lower the Ca and the

higher the N concentrations (FURCH and KLINGE, 1989). For várzea lakes the greatest pool of total N input is provided by river water flowing into the floodplain. From Rio Solimões into Lago Camaleão an input of 10.5 kg N ha^{-1} was calculated for a period of 9.5 months (KERN et al., 1998).

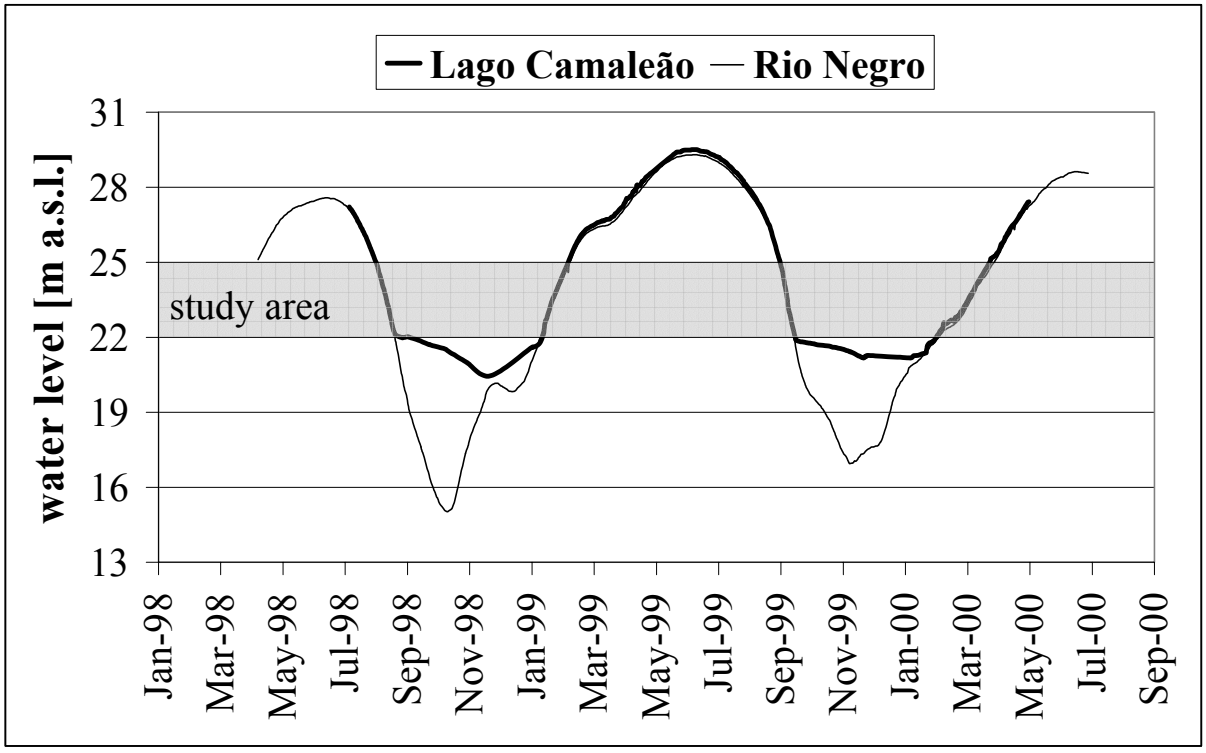


Figure 2.3 Fluctuations in water level of Rio Negro (provided by J.A. Nunes de Mello in co-operation with the Administração do Porto de Manaus) and Lago Camaleão during the study period. The elevational range of the study area is shaded in grey.

2.5 Vegetation

Conditions are favourable for plant growth in the várzea with respect to all aspects of soil chemical characterisation (FURCH, 1997). But an important stress factor is the limitation of O_2 in water and rhizosphere of flooded trees. Aquatic periods can be up to 10 month, where trees might stand in up to 15 m deep water (KUBITZKI, 1989).

The várzea forest has a high nutrient requirement, which is reflected by a high nutrient content in the biomass and by a high productivity. The annual biomass production is 17.4 t ha^{-1} (Furch, 1997). In tropical South America only mountain rain forests have similarly high values. The species *Pseudobombax munguba*, *Crateva benthamii*, *Vitex cymosa,*

Pterocarpus amazonicus, *Cecropia* spp. and *Piranhea trifoliata* are characteristic for white-water areas (PRANCE, 1979). Frequently present are also the shrubs *Anacampta* sp. and *Elaeoluma glabrescens,* and the liana *Dalbergia riparia* (WORBES, 1983). 13.3% of the species belong to the family Leguminosae (WORBES, 1986). Many trees in the várzea are deciduous, and species of the understorey are mainly evergreen shrubs. Roots form a very dense layer on the soil surface of Amazonian rainforests, and about half of the fine roots were found just beneath the soil surface (Meyer, 1991). Inundation forests have smaller species diversity than terra firme forests (BRÜNIG, 1973) and a gradient in the duration of flooding is reflected by the species composition. As well, basal area and wood-volume decrease with increasing time of flooding (WORBES, 1983).

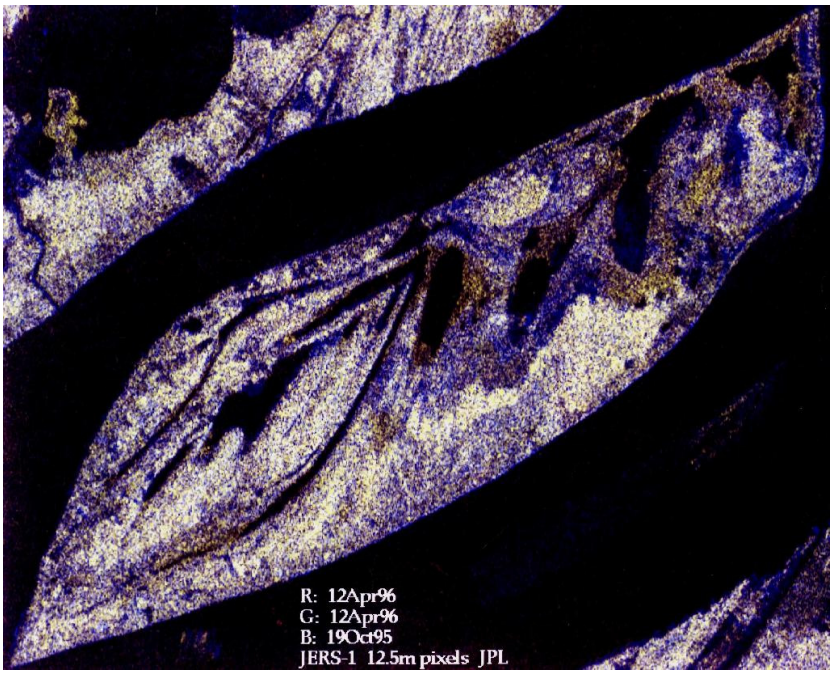
The deciduous várzea forest on Ilha de Marchantaria is a secondary forest with an age of 60 to 100 years (FURCH and KLINGE, 1989; WORBES et al., 1992; KLINGE et al., 1995). The canopy is divided into 4 layers and has a height of 25-30 m (WORBES, 1983). At maximum water level a majority of the trees loose their leaves, and during the terrestrial period in December all trees have recovered their leaves again. WORBES (1983) counted 167 tree individuals (dbh > 5 cm) on 2100 m², which belonged to 30 species. Additionally, there were 2 species with shrub habit and 1 liana species. There were less than 5 tree seedlings per m² and no herbaceous plants. *Pseudobombax munguba* dominated the forest stand and *Crateva benthamii* and *Vitex cymosa* were very frequent as well. 17.4% of the tree individuals belong to the Leguminosae (WORBES, 1986). The lack of typical pioneer species which are frequent at the lower elevational areas as well as the lack of big tree species which characterise the higher elevational areas, suggests that the forest on Ilha de Marchantaria with regard to elevational level and development status is between these 2 extremes (KLINGE et al., 1983; WORBES, 1986).

3. Material and methods

3.1 Forest structure analysis

Water level, time of flooding and the elevation of the study area were recorded since these are important determinants for biological processes (JUNK et al., 1989), as well as for type and structure of vegetation in the floodplain (PRANCE, 1979). Long-term records of the water level at Manaus are maintained by the Manaus Harbour Authority and were provided by J.A. Nunes de Mello. Due to the small downstream gradient of approximately 2 cm km^{-1}, and the determining influence of the high water discharge from the Rio Solimões on the water level at Manaus, these records, within a range of approximately ± 30 cm, provide information on the hydrological cycle at Ilha de Marchantaria (KERN, 1995). During the sampling period, the current water level of Lago Camaleão, which directly influences the study area, was measured with a stadia rod at 1 gage point. Close to many trees, and at other sampling points, the water depth was measured during high water and the elevation calculated using water level data from Lago Camaleão. Therefore, it was always possible to calculate the difference between soil surface and water level, as well as the time of flooding. The average annual time of flooding during the last century (1903–2000) was calculated for the study area using the long-term records of the water level at Manaus.

Identification of plants in the field and of dried samples (branches, leaves, blossoms or fruits) was done by Michael Hopkins and his research group of botanists in co-operation with the INPA herbarium. Additional help, especially with the legume species, was provided by Luiz Augusto Gomes de Souza and Marlene Feitas da Silva. However, as botanical identification of tree species is difficult in Amazonia (FEARNSIDE, 1997b), the problem of misidentification cannot be totally excluded. The most common system of classification, which is also adopted here, divides the Leguminosae into the 3 subfamilies: Caesalpiniodeae, Mimosoideae and Papilionoideae (SPRENT and PARSONS, 2000). During 3 field excursions with the botanists, and from many fruit, blossoms and branch samples, a list of all legume species identified in the study area irrespective of size and frequency was completed. During the terrestrial period, plants were spot checked for nodulation.



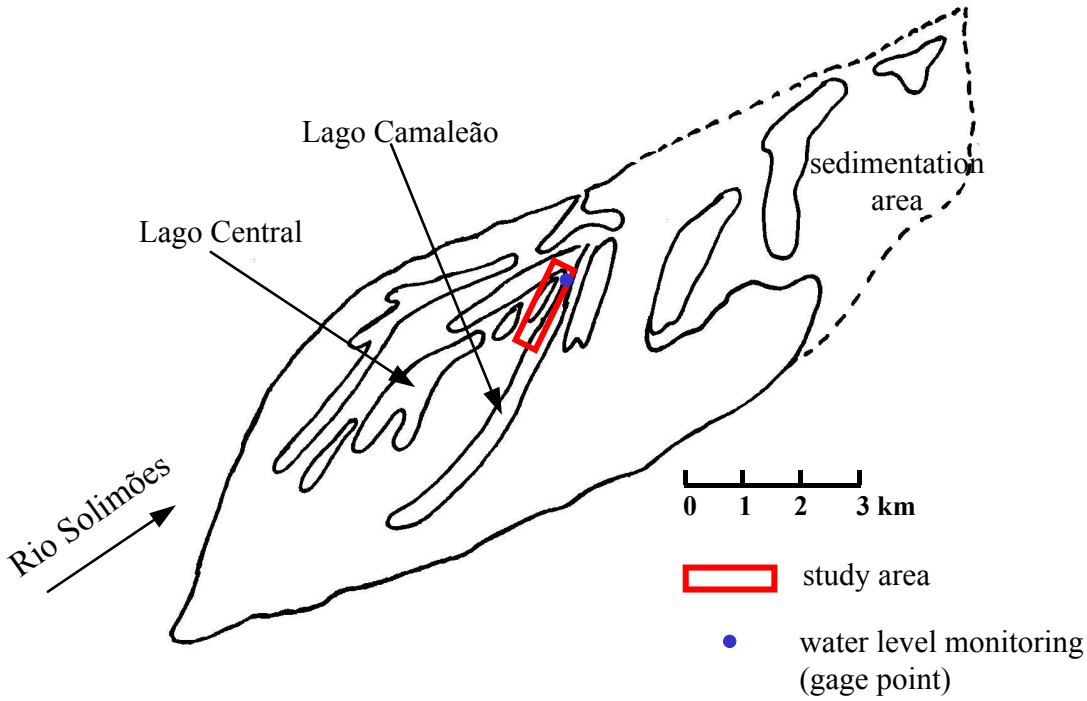


Figure 3.1 Study area on Ilha de Marchantaria. Multi-temporal satellite image (top) of the Marchantaria island composed from October 1995 (terrestrial period) and April 1996 (aquatic period) images. Bright colours represent high elevations with seasonally non-flooded areas, dark colours represent low areas subject to extended annual flooding (Image provided by J. Holt, NASA-Jet Propulsion Laboratory Pasadena, California). Location of the study area (bottom) and the gage point for water level monitoring on Ilha de Marchantaria (modified from HORNA-DE-ZIMMERMANN, 2001)

A stand structure analysis was undertaken during the terrestrial period 1999/2000. The 4 plots for tree identification were equally distributed along the study area (Figure 3.1), each covering the whole width of the forest in exact north-south direction from one lake to the other, and with a length of 50 m. This plot shape was used due to the exceptional local situation on the island with the forests growing on narrow ridges. Thus, differences in stand structure and composition on the forest edges and at different elevations were taken into account. In total an area of 2.1 ha was covered, which for frequency determination of species was divided into 24 subplots. This plot size was appropriate for the relatively homogenous várzea forest (WORBES, 1997). Plots should be large enough for representative sampling and small enough to cover only the forest type being studied (KLINGE and RODRIGUES, 1973), but no general rule for plot size in tropical forests can be given (UNESCO et al., 1978). Areas between 0.1 and 4 ha were suggested for terra firme and floodplain forests (CURTIS and COTTAM, 1964; LAMPRECHT, 1977; UNESCO et al., 1978; WORBES, 1986).

In these 4 plots, all trees with a stem diameter of 3.5 cm or more at 1.3 m height (or above the highest buttress), and all woody climbers with a base diameter of 3.5 cm or more were measured with a tape at the target height, mapped, and if possible identified to species level (CURTIS and COTTAM, 1964). Lianas were included, since they are an integral and substantial part of tropical forest ecosystems and their consideration important for ecological studies (GENTRY, 1983).

Conventional methods were used to describe the forest stand (CURTIS and COTTAM, 1964; MUELLER-DOMBOIS and ELLENBERG, 1974). Relative richness is the percentage of species which belong to a specific family. Relative frequency is a measure of the distribution of a species within the stand, and it is calculated as the number of subplots in which the species occurs divided by the total number of subplots (here: 24). Relative density is the number of individuals of one family (or species) as a percentage of the total number of individuals of all species, and the absolute density is the average number of individuals of a family (or species) per area. Relative dominance represents the importance of a family or species in relation to their area covered, calculated as the total basal area of one family (or species) as a percentage of the total basal area of all species. Therefore, absolute dominance is the average basal area of each family (or species) per hectare. The family important value (FIV) is calculated as the sum of relative richness, relative density and relative dominance at the family level (MORI et al., 1983) whereas the important value index (IVI) is the sum of relative frequency, relative

density and relative dominance at the species level. These indexes are particularly valuable for the tree members of forest stands (CURTIS and COTTAM, 1964).

Stand structure data and the equations shown in Table 3.1 were used to estimate the above ground dry biomass of the forest stand in the study area. Unfortunately, no equations for volumetric based biomass estimations are available for the várzea. Therefore, different equations deriving from secondary and primary terra firme forests were used to give an idea about the possible range of biomass in the study area. Only equations using the parameters diameter and wood density were applied, since the height of trees was not measured. The application of models using only diameter as independent variable was acceptable, since HIGUCHI et al. (1998) found for an Amazonian forest the results of these models are as consistent and precise as those with height as additional variable.

Table 3.1 Volume based estimates for above ground dry biomass deriving from secondary and primary terra firme forest stands

references		**equations**
BROWN et al., 1989	for trees with D > 10 cm:	$H=4.722*\ln(D^2)-13.323$ $B=(\exp(-2.409+0.9522*\ln(D^2HWD)))*1.03$
	for trees with D < 10 cm:	$B=(\exp(4.9375+1.0583*\ln(D^2)))*1.14/10^3$
	for palm trees:	$B=(\exp(3.6272+0.5768*\ln(D^2H)))*1.02/10^3$
	for lianas:	$B=10^{(0.12+0.91*\log10(BA))}$
	for *Cecropia* sp. with D > 10 cm:	$B(wood)=\exp(-3.78+0.95*\ln(D^2)+\ln(H)$ $B(leaves)=-0.56+0.02(D^2)+0.04(H)$
NELSON et al., 1999	general:	$\ln(B)=-1.4278+2.3836*\ln(D)+0.7655*\ln(aWD)$
	for *Cecropia* sp.:	$\ln(B)=-2.5118+2.4257*\ln(D)$
LAURANCE et al., 1997	general:	$B=(\exp(3.232+(2.546*\ln(D/100))))*600$
HIGUCHI et al., 1998	general:	$B=0.6*Bf$
	for trees with 5 < D < 20 cm:	$\ln Bf=-1.754+2.665*\ln(D)$
	for trees with D > 20 cm:	$\ln Bf=-0.151+2.170*\ln(D)$

B: above ground biomass (dry weight) [kg]
Bf: above ground biomass (fresh weight) [kg]
D: diameter at 1.3 m height for trees or base diameters for lianas [cm]
WD: wood density [g cm^{-3}], aWD: average wood density, for this study it was 0.57 g cm^{-3} (weighted average wood density according to FEARNSIDE (1997b))
H: height of tree [m]
BA: basal area [cm^2]

The phenology of 10 tree species (*Albizia multiflora, Macrolobium acaciifolium, Pterocarpus amazonum, Zygia inaequalis, Cecropia latiloba, Crateva benthamii, Nectandra amazonum, Pseudobombax munguba, Tabebuia barbata* and *Vitex cymosa*) legumes and non-legumes was monitored once a month. For some species, leaf area was measured with a leaf area meter (ΔT Area Meter, Delta-T Devices). Leaf dry weights were determined gravimetrically after drying until weight-consistency at 60° C. The specific leaf weights were then calculated from dry weight per leaf area (MEDINA, 1983). Wood samples were taken with an increment borer (Suunto 200 mm, Grube KG) and oven dried at 60° C. The wood cores had a diameter of 0.5 cm, their length was measured with a caliper and from this data their volume calculated. Basic density was then calculated from the ratio of dry weight to fresh volume, which is the most appropriate measure for biomass (FEARNSIDE, 1997b).

3.2 Physical and chemical soil analyses

Soil sampling

Before soil sampling, the litter layer was removed and also sampled. During the terrestrial period, soil samples down to 100 cm were taken with a Pürkhauer soil sampler, for 100 to 450 cm depth a liner-sampler (04.15, Eijkelkamp) was used. During the aquatic period sampling was done with a gravity corer (UWITEC-CORER). The soil column was divided in the depths 0-5 cm, 5-20 cm, 20-40 cm, 40-60 cm, 60-100 cm and below in 50 cm segments, and the fractions packed in plastic bags with as little as possible air contact. Always 3 replicate soil samples were mixed together. They were stored on ice until extraction the same day. For nutrient analyses 10 g of fresh soil were extracted with 90 ml 1M KCl for 1 h on a shaker, filtered and stored frozen until analysed.

Monthly samples down to a depth of 40 cm were taken at 10 locations during the terrestrial period and down to a depth of 20 cm at 3 locations during the aquatic period. Locations were selected, so that the range of elevations was covered and areas close to legumes, close to non-legumes and without vegetation were included. Once at the beginning of the terrestrial period, mid-October 1999 soil was sampled down to a depth of 450 cm at 3 locations. All 3 sampling points were on an elevation of 24 m a.s.l., the water level was at 2.3 m underground. At the end of the terrestrial period (February 2000) soil sampling of the soil profile was undertaken again at 3 locations but only down to 100 cm.

Physical and chemical analyses

Most physical and chemical properties were determined by standard methods (GRASSHOFF, 1976; DIN, 2000), details are listed in Table 3.2.

Table 3.2 List of methods, on site measurements and laboratory analyses, for the determination of the physical and chemical soil properties

physical properties	**on site measurements**
O_2 partial pressure	measurement with a soil O_2 analyses system (14.35, Eijkelkamp) (during the aquatic period, O_2 concentration and O_2 saturation in the flooding water was measured with a WTW Oxi 91 with profundity-probe (EOT 190; BR 160))
temperature	measurement with a soil-thermometer (J. Bibby Science Products) (air temperature was measured with a normal air-thermometer, during the aquatic period, temperature of the flooding water was determined with a WTW Oxi 91 with profundity-probe (EOT 190; BR 160))
	laboratory analyses
bulk density	gravimetric determination, using exact volumetric cylinders (95 cm³) and drying at 105° C
density of soil solids	pycnometer method
porosity	calculation, using bulk density and density of soil solids data
particle size	pipette analyses and sieving after dispersion in ammonia solution
pH	measurement with WTW pH 91 with glass electrode in KCl extract (10 g-fw soil in 90 ml 1M KCl)
organic matter	gravimetric determination after combustion at 500°C for 4 h (residue of ignition)
water content	gravimetric determination after oven drying at 105° C, conversion to water filled pore space using soil-solid density data

chemical properties	**laboratory analyses**
total N	determination with an elemental analyser in combination with a mass spectrometer at CENA Piracicaba/Brazil and FZJ Jülich/Germany
total C	determination with an elemental analyser in combination with a mass spectrometer at CENA Piracicaba/Brazil and FZJ Jülich/Germany
total P	photometric analyses of phosphate after alkaline persulfate-pulping
NO_2^-	photometric analyses using the sulfanil-amide method
NO_3^-	photometric analyses using the sulfanil-amide method after reduction to NO_2^- with a cadmium-amalgam column
NH_4^+	photometric analyses using the indophenol-blue method

3.3 Microbiological methods

Once during the terrestrial period and once during the aquatic period, at 5 locations soil samples were taken from 3 layers (litter, 0-5 cm and 5-20 cm). Locations included different elevations and areas close to legumes, close to non-legumes and without vegetation. Sampling

was under sterile conditions and the soil was immediately packed in aluminium boxes and stored cool until analysis. Bacterial biomass determination of different physiological groups were undertaken by standard microbiological methods (ALEF, 1991; SCHINNER et al., 1996).

Table 3.3 Specifications of the MPN methods used for determining the numbers of N_2 fixers, ammonifiers, nitrifiers and denitrifiers in soil (ALEF, 1991; SCHINNER et al., 1996)

physiological groups	nutrient media	growth detection
N_2 fixers	*solution 1:* 5 g sodium malate, 0.1 g yeast extract, 139 mg K_2HPO_4, 27 mg KH_2PO_4, 1 ml iron chelate solution, 1 ml micronutrient solution were diluted to 900 ml with distilled water; *solution 2:* 5 g glucose, 10 ml magnesium sulfate solution, 10 ml calcium chloride solution were diluted to 100 ml with distilled water; *complete media:* after sterilisation and cooling, both solutions were mixed and 1 ml sterile filtered biotin solution (2 mg D(+)biotin in 400 ml dest. water) added	acetylene reduction assay (Chapter 3.5)
ammonifiers	10 g peptone, 5 g NaCl were dissolved in 1000 ml distilled water	colour-reaction (brown, red) with Nesslers-reagent
nitrifiers	1 g K_2HPO_4, 0.5 g $MgSO_4$, 1 g NaCl, 5 g $CaCO_3$ were dissolved in 1000 ml distilled water and sterilised; then 50 ml of sterile $(NH_4)_2SO_4$ solution (2%) was added	NO_2^- detection with colour-reaction (red) (sulfanilic acid and naphthylamin reagents) and NO_3^- detection (after NO_2^- destruction with sulfuric acid and urea) with colour-reaction (blue) (diphenylamin reagent)
denitrifiers	1 g glucose, 1 g yeast extract, 0.2 g $MgSO_4$, 0.1 g NaCl, 0.2 g KNO_3, 10 ml 0.1% $FeSO_4$/EDTA solution, 10 ml 0.5 M KH_2PO_4 buffer were diluted to 1000 ml with distilled water	observation of gas formation (bubbles in Durham-tubes; and NO_2^- detection (positive), NO_3^- detection (negative))

To estimate the number of culturable proteolytic bacteria, decimal diluted soil suspensions were primed on calcium-caseinate/milk powder agar which were then incubated at 30° C for 2 to 3 days. Colonies with transparent coronas, indicating extracellular protein digestion, were counted to specify the number of colony forming units (cfu) of the proteolytic bacteria. Most probable number methods (MPN) were used for the other physiological groups (Table 3.3). Triplicate decimal soil dilution series in liquid nutrient media were prepared in culture tubes. After incubation at 30° C, for 1 to 4 weeks depending on physiological group, number of tubes per dilution showing growth characteristics of the physiological group being investigated were recorded. The most probable number of microorganisms in the sample was calculated using the McCrady probability table (MAN, 1975).

3.4 Genotypic characterisation of rhizobia

Isolation of rhizobia from legume nodules

Bacteroids were isolated from dried nodules of *Albizia multiflora* and *Pterocarpus amazonum,* collected in the study area, according to the following protocol:

1. rehydrate nodules by soaking them in water over night
2. rinse nodules in ethanol (95%) for 1 min
3. rinse nodules in H_2O_2 (5%) for 1 min
4. wash the nodules 6 times thoroughly in sterile tap water
5. place nodules individually in microtiter plate wells, and squash them in a drop of yeast mannitol broth with a sterile glass rod (Table A.3.1)
6. streak one loopful of the resulting suspension onto a yeast-mannitol-agar-plate (primary isolation plates; Table A.3.1)
7. incubate the plates at 30° C for up to 10 days, with daily growth control
8. check well-isolated colonies for typical rhizobial colony and cell morphology characteristics (Figure 3.2), perform a Gram stain

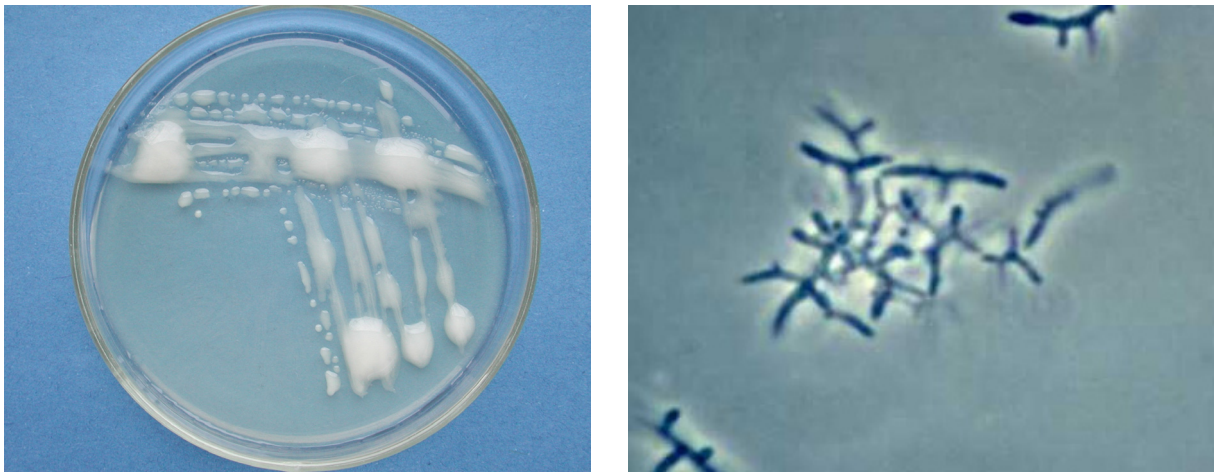


Figure 3.2 Examples of colonies (left) and cells (right; magnified 1000 times) of an isolate from nodules of *Albizia multiflora*.

Isolation of DNA from rhizobia

The DNA was prepared from 1.5-2 ml liquid cultures grown to late exponential phase, according to the protocol shown in Table A.3.2 (AUSUBEL et al., 1990). Additional useful information about DNA extraction methods is presented by JOHNSON (1991). To determine the concentration of the DNA preparations, the DNA sample solutions were diluted 1:50 (2 µl sample in 98 µl TE buffer) and the concentration measured with a GeneQuantII photometer

(Pharmacia Biotech). Only reasonably pure DNA preparations with an absorption ratio Abs_{260}:Abs_{280} between 1.8 and 2.0 were used. The DNA samples were diluted with TE buffer to a final concentration of 50 ng μl^{-1} in a 50 µl volume.

Amplification of partial nodC and nifDK gene fragments

The strains were tested for the presence of *nod* and *nif* genes. For the *nod* gene region, the *nod*C primer set (UEDA et al., 1995) was used. *Nif* gene region was amplified using the *nif*D and *nif*K primers (LAGUERRE et al., 1996). DNA sample solution (1 µl) was added to the standard PCR mix shown in Table A.3.3. The Perkin Elmer Gen Amp PCR System 2400 was used for amplification (Table A.3.4).

Amplification of the 16S rDNA region

Almost complete 16S rDNA sequences can be amplified from nearly any prokaryote using universal primers (WEISBURG et al., 1991). The primer pair fD1 and rD1 were used to amplify the 16S rDNA gene region of the isolated strains. DNA sample solution (1 µl) was added to the standard PCR mix shown in Table A.3.3. The used PCR cycling parameters are shown in Table A.3.4 (Biometra TGradient Thermocycler).

Restriction fragment length polymorphism analysis of the 16S rDNA PCR products

RFLP analysis is able to group strains at the genus to species level (FOX et al., 1992). The 16S rDNA amplification products were digested for 2 h at 37° C with the enzymes: *Hha*I, *Dde*I, *Sau*3AI, *Msp*I (New England BioLabs Inc.) (VINUESA-FLEISCHMANN, 1998). The choice of enzymes was based on the studies of LAGUERRE et al. (1994) and MOYER et al. (1996). The restriction buffers supplied by the manufacturer for each enzyme were used. The digestion mixture was: 10 µl sample (PCR product)

2 µl reaction buffer

1 µl enzyme (8-10 units)

7 µl H_2O

Rep-PCR genomic fingerprinting

The repetitive extragenic palindromic PCR permits differentiation of closely related strains within species and is therefore well suited for biodiversity studies. The fingerprint patterns can be compared to estimate relative degrees of similarity between isolates (VERSALOVIC et al., 1994). The method is based on primers complementary to naturally occurring highly

conserved, repetitive DNA sequences, present in multiple copies in the genomes of most gram negative bacteria (LUPSKI and WEINSTOCK, 1992). With the use of the GTG_5 primer and PCR selective amplification of distinct genomic regions was achieved. 1 µl of DNA sample (50 ng) was used for the 25 µl rep-PCR mix which is shown in Table A.3.5. The following cycling parameters were used for 30 cycles and with a final extension at 65° C for 8 min (Perkin Elmer Gen Amp PCR System 2400):

95° C for 2 min
93° C for 45 sec
50° C for 1 min
65° C for 8 min

Gel image documentation

The efficiency of the *nod*C, *nif*DK and 16S rDNA amplification was checked on 0.8% TAE agarose gels as described in SAMBROOK et al. (1989) (Table A.3.6). The sample (5 µl) was loaded on the gels by adding about 1 µl of glycerol-loading buffer including bromphenol-blue.

The RFLP products were resolved on 7 cm long 2% metaphor agarose gels in TBE (Table A.3.6). Electrophoresis was done at 55 V for 2.8 h. 100 bp ladder (Gibco-BRL) as molecular size markers were included on the gels for gel-to-gel normalisation and estimation of restriction fragment sizes. The sample (5 µl) was loaded on the gels by adding about 1 µl of glycerol-loading buffer including bromphenol-blue. The DNA was stained after electrophoresis in an EtBr solution for 15-20 min, and visualised under short wavelength (254 nm) UV light. The images were digitised using a CCD camera (INTAS, Göttingen) and saved as tiff files for further image processing and analysis.

The rep-PCR products were resolved on 18 cm long 1.5% agarose gels in TAE buffer. 6 µl of reaction mixture were loaded onto the gel. Electrophoresis was performed at room temperature at 4 V/cm for 4.2 h. 1 kb ladders (Gibco-BRL) were included for normalisation purposes. The strain specific banding profile is named the rep-PCR genomic fingerprint.

The digitised electrophoretic patterns were analysed with the GelCompar software package (Version 4.0, Applied Maths, Kortrijk, Belgium). Cluster analyses of similarity matrices were performed by the unweighted pair group method using arithmetic averages (UGPMA).

The sizes of restriction fragments from 16s rDNA - RFLPs of reference strains (Table A.3.7) were used for comparison with the strains isolated from *Albizia multiflora* and *Pterocarpus amazonum*. Hierarchical cluster analyses was undertaken with SPSS (SPSS Inc., Chicago, USA, Version 9.01, 1999). Fusion method was "linkage between groups" with quadratic Euclidean distance.

3.5 Acetylene reduction assay

With the combination of the acetylene reduction assay and the acetylene block method it is possible to simultaneously measure N_2 fixation and denitrification in one unique sample (HARDY et al., 1968; YOSHINARI et al., 1977). A prerequisite is, that the C_2H_2 (Ethin) concentration used for incubation is suitable for both methods, and that both processes show a similar kinetic.

The acetylene reduction assay (ARA), developed by HARDY et al. (1968), is commonly used for N_2 fixation measurements (BERGMAN et al., 1992; HARDARSON and DANSO, 1993; VESSEY, 1994; BARKMANN and SCHWINTZER, 1998; PELEGRÍ and TWILLEY, 1998; VITOUSEK and HOBBIE, 2000), although it is an indirect method. The reduction of C_2H_2 to C_2H_4 is very specific for all N_2 fixing organisms and the method is convenient and sensitive. Measurement is based on the ability of the nitrogenase enzyme to reduce other substances besides N_2 (Figure 3.3). The reduction of C_2H_2 to C_2H_4 requires only 2 electrons by comparison with the reduction of N_2 to $2NH_3$ which requires 6 electrons (LUDEN, 1991). For quantitative measurements it is important to use a suitable C_2H_2 concentration (usually 10%-30%) during sample incubation (KNOWLES, 1980; JOYE and PAERL, 1993). The amount of C_2H_4 produced by incubating soil samples, litter, nodules or whole plants can be analysed by gas chromatography (GC).

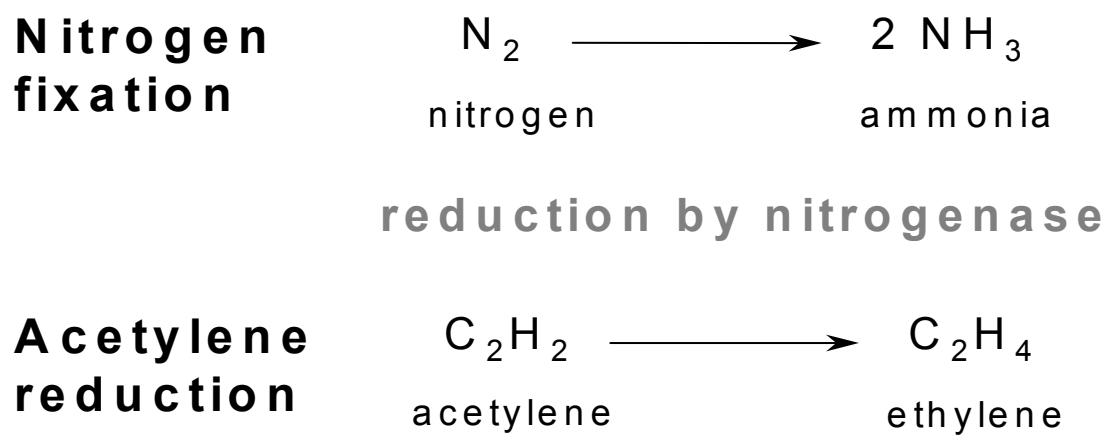


Figure 3.3 Principle of the acetylene reduction assay, used for determination of N_2 fixation, above is the natural pathway, below the process occurring in presence of C_2H_2

From August 1998 until September 1999, monthly measurements with a minimum of n = 9 during the aquatic period and up to n = 30 during the terrestrial period in 0-5 cm soil depth at locations with different elevations and vegetation were undertaken. Once at the beginning of the terrestrial period (October 1999) microbiological activities in soil were measured down to 450 cm at 3 locations (Chapter 3.2), and down to 100 cm at the end of the terrestrial period (February 2000). Each measurement included 2 to 3 replicates and for comparison 1 sample which was incubated without C_2H_2. To investigate the influence of vegetation and soil properties, 10 plots each with a legume (*Zygia inaequalis*), a non-legume (*Crateva benthamii*) and a location without trees were marked. Measurements in 0-5 cm soil depth (each location: 5 replicates, 2 references without C_2H_2) were undertaken at the beginning (October 1999), the middle (December 1999) and the end (February 2000) of the terrestrial period. Simultaneously, elevation and soil properties were investigated (NH_4^+, NO_3^-, pH, temperature, gravimetric water content). During terrestrial periods, at 9 random locations litter samples were taken and incubated during the rainy season (January 1999) and during the dry season (December 1999). Additionally, from 4 legume species (*Albizia multiflora*, *Campsiandra comosa*, *Pterocarpus amazonum* and *Zygia inaequalis*) root samples with nodules (n = 10 root samples per species) were incubated during the terrestrial period to investigate symbiotic N_2 fixation of nodules.

Different methods, modifications of those described by KERN (1995), were used to take into account the different conditions during the terrestrial and aquatic period. To correct for natural nitrous-oxide evolution, measurements included 1 to 2 samples which were incubated without C_2H_2. Soil equivalent to 20-40 g dry weight or litter equivalent to 3-7 g dry weight

was placed in pre-weighed, sterile, gas-tight, glass bottles (118 ml) immediately after sampling. During the terrestrial period, the bottles were closed and 15 ml C_2H_2 (99.5%, Linde) was added (approximately 15%). After mixing, 7 ml gas sample was taken with a vacutainer (7 ml Becton Dickinson 367625) and 8 ml were released to avoid over-pressure. While the ground was flooded, the incubation bottles were completely filled with floodplain water. Bottles were closed immediately with rubber stoppers, which allowed a gas-bubble-free and air-tight containment. To create 50 ml head-space, water was replaced by N_2 (Linde). Directly after 8 ml C_2H_2 was added and mixed, 7 ml gas sample was taken for the start value. Nodulated roots were excavated in a way that the connection with the stem remained intact allowing species classification. Soil was carefully shaken off and roots immediately placed in pre-weighed, sterile, gas-tight, glass bottles (21 ml). Associative N_2 fixation in the rhizosphere of aquatic and terrestrial plants was not measured. Bottles were closed, 3 ml of air removed and 3 ml of C_2H_2 injected. Measurements were normally carried out in the morning, most bottles were incubated for 4 h, root/nodule incubation was for 1 h only. Subsurface soil and roots were incubated under dark conditions, in an insulated box to avoid artificial heating of the samples. Litter was incubated under daylight. After incubation a gas sample was taken with a vacutainer. The weight of all samples was determined, from the roots only the fresh weight of the nodules was recorded. The headspaces of all incubations were quantified, and also the water volumes from the anaerobic incubations. Conversion of mass-values to area-values was done with bulk density data (Table 4.5). Annual N_2 fixation in the soil layer 0-5 cm was calculated as the sum of the average monthly values from the measurements from September 1998 to August 1999.

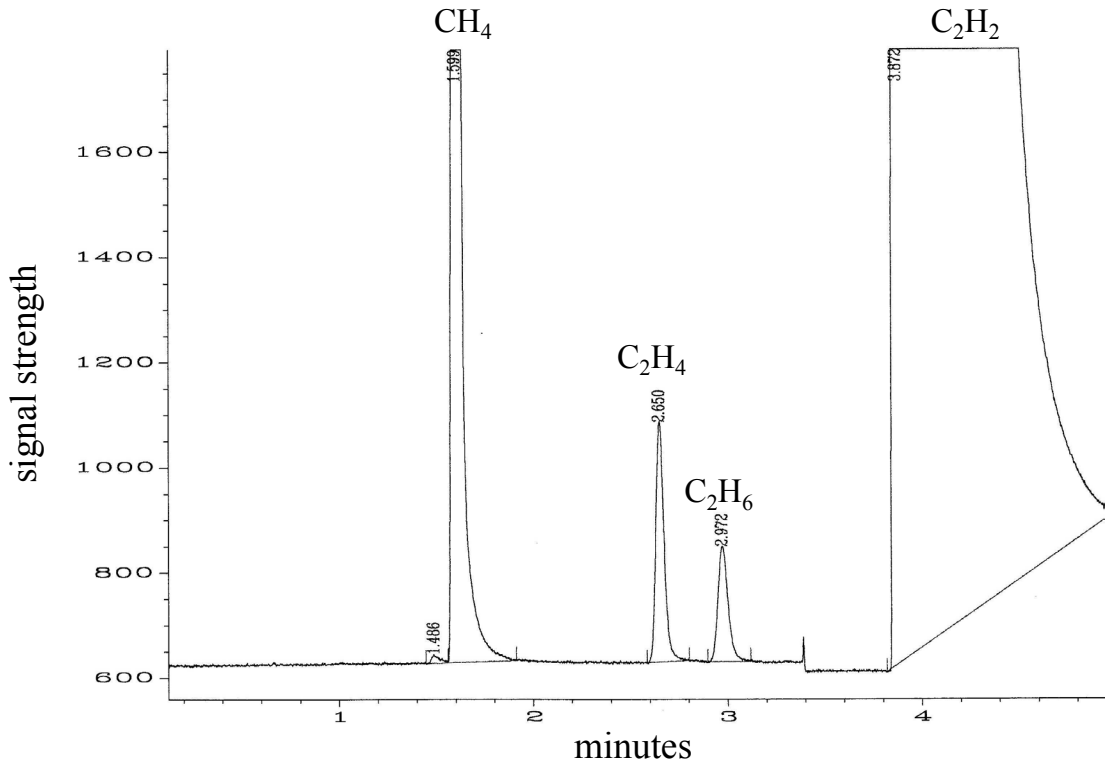


Figure 3.4 GC chromatogram of a gas sample detected by FID

The C_2H_4 was analysed by GC (Hewlett Packard 5890 Series II), detected by Flame Ionisation Detector (FID) (Figure 3.4). As occasionally C_2H_2 reduction can lead to C_2H_6 formation, production of C_2H_6 was also controlled and measured by GC. A gas sample of 250 µl was injected with a gas tight syringe (Pressure lock). With the used configuration, previously described by KERN (1995), it was possible to analyse gas samples for N_2O, O_2, CH_4, C_2H_2, C_2H_4 and C_2H_6. N_2 (ECD-quality, Linde) was used as carrier gas at a flow rate of 15 ml min^{-1}. FID gases were hydrogen (carbon-hydrate free, Linde) and synthetic air (carbon-hydrate free, Linde). The gases passed a cleaning system (Chrompack) with O_2, moisture and activated C filter before they reached the GC. The gas compounds of the sample were separated in a 25 m capillary column with an inner diameter of 530 µm (Pora-Plot U) and then split between the 2 detectors (Electron Capture Detector (ECD) and FID). Column head pressure was 100 kPA. The GC parameters (Table 3.4) and the detector signals were controlled by the computer program HP-Chem, which automatically integrates the resulting peaks. Calibration of the system was done at the beginning of each series of measurements, and at least once a day; check standards were run after every 20 samples. C_2H_4 standards of 14.6 ppm (gas mix CO, CO_2, CH_4, C_2H_2, C_2H_4, C_2H_6 at 15-20 ppm in N_2, Scotty speciality gases, Alltech) and 100 ppm (in He Scotty 2, Alltech) were used. The mean of 16 blanks,

which were air samples after incubations with C_2H_2 but without sample material, was 0.3 ppm C_2H_4. The detection limit of the used method was 1.9 ppm C_2H_4.

Table 3.4 GC and detector parameters for the analysis mainly of C_2H_4 and N_2O

injector temperature	100° C
column temperature (oven)	40° C
temperature program:	40° C for 4 min (analyses)
	40° C - 107° C (temperature increase with 70° C min^{-1})
	107° C for 0.5 min
	107° C - 40° C (temperature decrease with 70° C min^{-1})
FID-temperature	200° C
ECD-temperature	300° C

Concentrations in the incubated water were calculated from the C_2H_4 measurements in the gas phase and the use of solubility tables (WILHELM et al., 1977; WEISS and PRICE, 1980). C_2H_4 production was calculated by subtracting the final sample value from the start concentration.

To calculate the amount of fixed N from the C_2H_4 measurements, it is advisable to determine the correct conversion factor for the specific system using ^{15}N gas (POTTS, 1984; BODDEY and VICTORIA, 1986; STAL, 1988). Unfortunately, during this study it was not possible to use this method for calibration. Therefore, conversion into total N_2 fixed was done by the theoretical conversion factor 3, which is based on the electron ratio of N_2:C_2H_2 reduction.

Further information and descriptions of the ARA were published by HARDY et al. (1973), BODDEY (1987), SEITZINGER and GARBER (1987) and MCNEILL et al. (1996).

3.6 Acetylene block method

The acetylene block method is widely used for measuring denitrification because of its high sensitivity and its practicability in the field with relatively short incubation times (VINER, 1982; JOYE and PAERL, 1993; HATCH et al., 1998). It is based on the inhibition of bacterial nitrous-oxide reduction to N_2 in the presence of C_2H_2 (YOSHINARI and KNOWLES, 1976) and assesses the denitrification of all NO_3^--N irrespective of its source (Figure 3.5). A minimum C_2H_2 concentration of 15% is needed for blockage of the N_2O reductase also at low NO_3^- concentrations. The amount of produced N_2O by incubating soil or litter samples in a C_2H_2-atmosphere can be analysed by GC. This N_2O concentration needs correction with natural N_2O production data to calculate the denitrification rate.

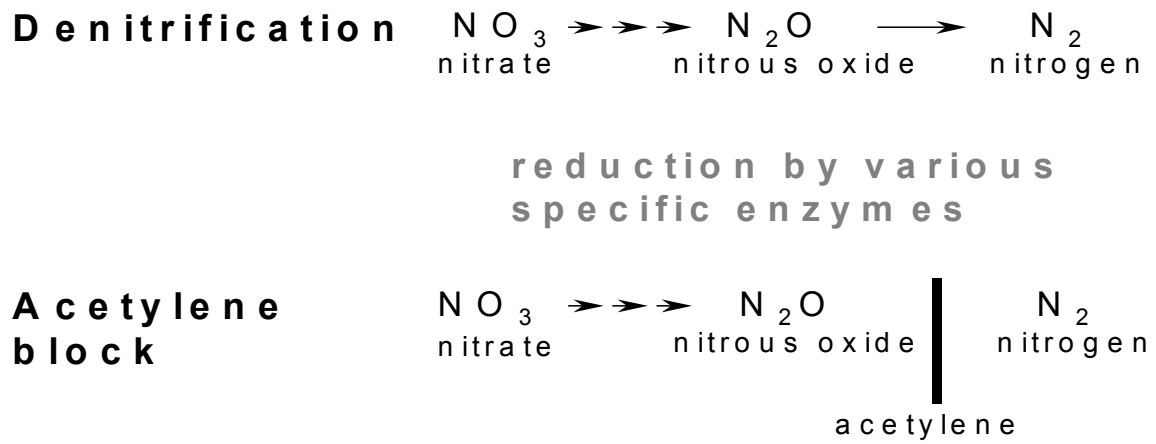


Figure 3.5 Principle of the acetylene block method, used for determination of denitrification, above is the natural pathway, below the blocked process in presence of C_2H_2

The same sampling procedures as described in Chapter 3.5 were used, since denitrification and N_2 fixation were measured simultaneously in the same sample (HARDY et al., 1968; YOSHINARI et al., 1977). Incubations without C_2H_2 were undertaken to measure the natural N_2O production in the soil.

The nitrous oxide was analysed by GC (Hewlett Packard 5890 Series II), detected by ^{63}Ni-ECD (Figure 3.6). GC-analysis were undertaken as described in Chapter 3.5. N_2O standards of 1 ppm (in N_2 Scotty speciality gases 2, Alltech), 25 ppm and 100 ppm (in N_2 Scotty speciality gases 1, Alltech) were used. The mean of 16 blanks was 0.5 ppm N_2O. The detection limit of the used method was 3.3 ppm N_2O. N_2O concentrations in the incubated water were calculated from the measurements in the gas phase and the use of solubility tables (WILHELM et al., 1977; WEISS and PRICE, 1980). Natural N_2O release was calculated by subtracting the final gas sample value from the start concentration of the incubations without C_2H_2. Denitrification rates were calculated from the incubations with C_2H_2 by subtracting the final gas sample value from the start concentration and additional correction for the natural N_2O release.

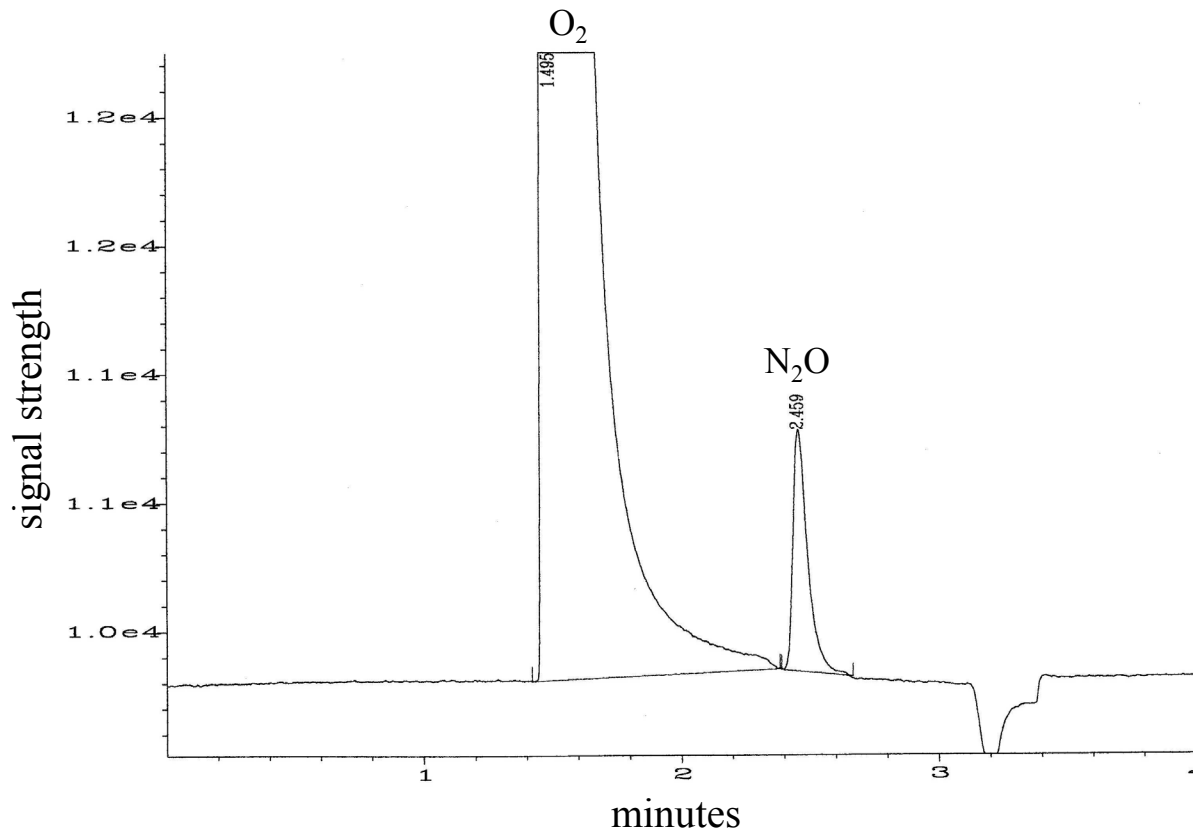


Figure 3.6 GC chromatogram of a gas sample detected by ^{63}Ni-ECD

Further information and descriptions of the acetylene block method were published by KNOWLES (1990), RUDOLPH et al. (1991), MALONE et al. (1998) and WATTS and SEITZINGER (2000).

3.7 ^{15}N natural abundance method

The ^{15}N natural abundance method for estimating the fractional contribution of biologically fixed N to N_2 fixing systems is a valuable method mainly to assess the N_2 fixation by trees in natural ecosystems (SHEARER and KOHL, 1986; VIRGINIA et al., 1988, HÖGBERG, 1990; YONEYAMA et al., 1993). It provides a time-integrated estimate of the percentage of N derived from atmosphere (%Ndfa), without disturbing the system. The method is based on small, but measurable differences in ^{15}N abundance between the N sources for plants, namely atmospheric N_2 and other N sources, mainly soil. The ^{15}N fraction in atmospheric N_2 (0.3663%) is globally constant (MARIOTTI, 1983), in comparison, soil N is usually more enriched in ^{15}N (SHEARER and KOHL, 1988b). The isotopic ratios of these different sources of N influencing nodulated legumes are estimated on the one side by N_2 fixing legumes grown

without soil N and on the other side by non-N_2 fixing reference plants. This is necessary because direct measurement of $\delta^{15}N$ of atmospheric N_2 and soil N is inadequate due to isotopic fractionation which occurs during incorporation of N from these sources into plant tissue (SHEARER and KOHL, 1988b; YONEYAMA et al., 1991a).

Sampling in the forest

Plant internal transport of isotopically altered metabolites causes variation in isotopic abundance among plant parts. This also raises the question about which plant part is appropriate to estimate %Ndfa. To eliminate any problems connected with isotopic fractionation, the entire plant has to be analysed. But obviously this is only possible with herbaceous plants and not with trees. It is therefore necessary to select a part of the plant for analyses which has a ^{15}N abundance similar to the entire plant, or which has a ^{15}N abundance which deviates from the entire plant in the same direction and by the same magnitude in both N_2 fixing and reference plants (SHEARER and KOHL, 1988b). Generally, different above ground plant parts have ^{15}N abundances similar to each other and to the entire plant (SHEARER and KOHL, 1988b), which makes these suitable for the %Ndfa calculation. Leaves seem to be the most representative plant parts (THIELEN-KLINGE, 1997), and normally they are used. Due to N translocations (MEDINA, 1981) and their relative short life span (KLINGE et al., 1975; PAROLIN, 1997) they are best suited for the monitoring of seasonal variation. Additionally, leaves have the highest N concentrations of all plant parts (FURCH and KLINGE, 1989). To see if leaves from the species in the study area are really suitable plant parts to characterise the isotopic ratio of the whole plant, or at least to calculate the %Ndfa (SHEARER and KOHL, 1988b), besides leaves, also roots, wood from stems, branches and twigs, blossoms, fruits and nodules were sampled.

Generally, 3 or more young but fully developed leaves from the canopy were sampled from the target plants, termed as “medium sun-leaves” in this study. For all legume species, identified and located in the study area, samples were taken from at least 3 individual plants during the terrestrial period. Additionally, a selection of non-leguminous species were sampled in the same way as potential reference plants. Since young leaves are not always available and specific sampling of leaves is difficult, also sun- and shade-leaves, young not yet fully developed, old and submerged leaves were collected, to assess the danger of artefacts due to sample composition. Additionally, leaves from tree seedlings were sampled in the forest, to facilitate the interpretation of experimental results obtained with seedlings.

Since leaf sampling for isotopic characterisation and N_2 fixation calculations should be done in the period of highest biomass production (PATE et al., 1994) most samples were taken during the terrestrial period, which is regarded as the main growth period of the várzea forest (WORBES, 1986). But to investigate possible differences in isotopic ratios between hydrological periods, leaf samples of 16 species were taken from the same individual plants once in the terrestrial period and once in the aquatic period. Additionally, once a month, leaves of 10 tree species (*Albizia multiflora, Macrolobium acaciifolium, Pterocarpus amazonum, Zygia inaequalis, Cecropia latiloba, Crateva benthamii, Pseudobombax munguba, Nectandra amazonum, Tabebuia barbata* and *Vitex cymosa*), each with 3 parallels, were collected to monitor their $\delta^{15}N$ values during the hydrological cycle. For statistical analyses of possible seasonal trends, the hydrological cycle was divided into 4 time periods: Beginning of the terrestrial period (October + November), end of the terrestrial period (December - February), beginning of the aquatic period (March - July) and end of the aquatic period (August + September).

Rain, river and lake water was sampled randomly and also soil, sampled as described in Chapter 3.2, was used for isotopic analysis.

Pot-experiment

A pot-experiment was undertaken to determine the isotopic ratio of biologically fixed N from atmosphere ($\delta^{15}N_A$) for the nodulated legumes (SHEARER and KOHL, 1986; 1988b). Strictly, the $\delta^{15}N_A$ value needs to be determined for each nodulated legume under study. Since this was impossible due to a lack of seedlings, a variety of 5 nodulated legume species from the study area (*Albizia multiflora*, *Campsiandra comosa*, *Mimosa pigra*, *Pterocarpus amazonum* and *Zygia inaequalis*) were grown on N free substrate. Additionally, growth conditions in the experiment were artificial and transfer of the results obtained with seedlings to trees grown in the forest had its shortcomings, but this experiment seemed to be the only possibility to determine the $\delta^{15}N_A$ value for the nodulated legumes in the study area. Unfortunately, when sterilised seeds were planted and inoculated in leonard-jars, containing N free nutrient solution for legumes, very few seedlings germinated and many plants died, so that no useful results could be obtained. Therefore, an alternative experiment was undertaken. In the forest, small but already nodulated seedlings of *Albizia multiflora (*12 plants*), Campsiandra comosa* (13 plants), *Mimosa pigra* (11 plants), *Pterocarpus amazonum* (11 plants) and *Zygia inaequalis* (17 plants) were collected in October and November 1999. All soil was removed

and their roots carefully washed. 1 seedling per pot was planted in a mixture of Vermiculite/Sand (50:50), which was intensively washed before with de-ionised water. Since a greenhouse was not available, the plants were kept outside at the INPA premises in Manaus under a transparent roof to protect them from rain and other deposits. The plants were supplied with a N free nutrient solution for legumes (Table A 3.8). Every month 1 leaf per plant was sampled and all leaves of 1 species pooled as 1 sample. On the sampling days also the height and number of leaves of each plant were recorded. On 7th May 2000 all plants were harvested and the roots thoroughly washed. Each plant was divided into the 3 samples: leaves, stem and roots. After drying of the samples at 60°C until weight consistency, the dry weight of each sample was determined. As contamination control, substrate of each pot was sampled at the end of the experiment and analysed for N compounds (NH_4^+, NO_3^-, total N). For the growth curve, only the surviving plants were included. The N accumulated per plant was calculated as shown in Equation 3.1.

Equation 3.1:

$$N_{accp}\,[mg] = \left\{ \underbrace{\left(\frac{bm_l[g\text{-}dw]}{100} * N_l\,[\%] \right)}_{N_{accl}} + \underbrace{\left(\frac{bm_r[g\text{-}dw]}{100} * N_r\,[\%] \right)}_{N_{accr}} + \underbrace{\left(\frac{bm_s[g\text{-}dw]}{100} * N_s\,[\%] \right)}_{N_{accs}} \right\} * 1000$$

N_{accp}, N_{accl}, N_{accr}, N_{accs}	N accumulated of plant, leaves, roots and stem;
bm_l, bm_r, bm_s	biomass of leaves, roots and stem
N_l, N_r, N_s	N content of leaves, roots and stem

The $\delta^{15}N$ value of the whole plant was calculated according to Equation 3.2.

Equation 3.2:

$$\delta^{15}N_{plant} = \left(\frac{N_{accl}}{N_{accp}} * \delta^{15}N_{leave} \right) + \left(\frac{N_{accr}}{N_{accp}} * \delta^{15}N_{root} \right) + \left(\frac{N_{accs}}{N_{accp}} * \delta^{15}N_{stem} \right)$$

Sample preparation and analyses

The plant material was dried at 60° C for 1 week and ground to a fine powder in a mill or mortar. The homogenised material was weighed into tin-capsules (5*9 mm, IVA Meerbusch) using a µg-precise balance. Leaf, nodule, fruit and blossom samples of 1.5 to 2 mg were analysed, of wood samples 2-3 mg were weighed in. 100 ml of rain, river and flooding water

was lyophilised and also transferred into tin-capsules. Additionally, to determine the isotopic ratio of the total N in the ground, soil samples were dried at 60° C. The dry soil was finely ground and about 15-20 mg was weighed into tin-capsules. Analyses were undertaken in the same way as the analyses of the plant material, only that Leco was used as analytical standard. To determine the isotopic ratio of the plant available N in soil, 10 g of fresh soil was extracted with 100 ml 1M KCl at the same day of sampling. The extract-solutions were stored frozen. To separate the dissolved N from the large amount of salt, a steam distillation was performed. For this process 0.2 g activated MgO and 0.4 g Devarda-reagent were added. Thus, NO_3^- was reduced and all dissolved N gassed out as NH_3, which was after condensation captured in 20 ml 0.01 M H_2SO_4. This acid solution was lyophilised to reduce the sample volume ready for isotopic analysis.

Analyses of most samples were undertaken in co-operation with Reynaldo L. Victoria, Plínio B. de Camargo and Marcelo Moreiro at the Centro de Energia Nuclear na Agricultura (CENA) in Piracicaba, about 20% to 25% of the samples were analysed in co-operation with Hilmar Förstel and Marcus Boner at the Forschungszentrum Jülich (FZJ, IsoLab) in Jülich. An elemental analyser in combination with mass spectrometer was used (EA 1110 CHN Finnigan Delta plus). Antropin was used as standard. At the beginning of each sample run, first 2 blanks, then 2-3 antropin standards were analysed, every 5th sample was analysed in duplicate, in the sequence with 5 samples distance, to check the reproducibility. After every 12th sample a check antropin standard was run. The results of ^{15}N abundance are expressed as $\delta^{15}N$ (Equation 3.3), low values indicating N_2 fixation. Analyses resulted in the $\delta^{15}N$ values and N content of each sample (as well $\delta^{13}C$ and C content were analysed).

Equation 3.3:

$$\delta^{15}\mathrm{N} = \frac{(\text{atom\% }^{15}\mathrm{N})_{\text{sample}} - (\text{atom\% }^{15}\mathrm{N})_{\text{standard}}}{(\text{atom\% }^{15}\mathrm{N})_{\text{standard}}} * 1000$$

The standard is atmospheric N_2 with an atome% ^{15}N value of 0.3663.

The percentage of N derived from atmosphere (%Ndfa) was calculated according to Equation 3.4 (SHEARER and KOHL, 1986; YONEYAMA et al., 1993).

Equation 3.4:

$$\% \text{ Ndfa} = \frac{\delta^{15}N_R - \delta^{15}N_F}{\delta^{15}N_R - \delta^{15}N_A}$$

$\delta^{15}N_R$: reference value (determined with non-N_2 fixing reference plants)

$\delta^{15}N_A$: value of fixed N (determined with legumes grown without soil N)

$\delta^{15}N_F$: value of potentially N_2 fixing target plant

Since results can vary significantly dependent on the reference used, it is quite crucial to determine the "right" reference value ($\delta^{15}N_R$) (HAMILTON et al., 1992; AWONAIKE et al., 1993). The larger and the more constant the difference between the $\delta^{15}N$ values of the reference and the legumes is, the more precise are the calculations. Different possibilities for the selection and calculation of the reference value were used and compared in this study (Chapter 4.5.3), as suggested by CHALK et al. (1996). As described before, the $\delta^{15}N_A$ value was determined in a pot-experiment.

The ^{15}N natural abundance method has been discusses in detail in the following papers: KOHL et al., 1980; RENNIE and RENNIE, 1983; SHEARER and KOHL, 1986; 1988a.

3.8 Isotope dilution method

A pot experiment using the isotope dilution method including 2 treatments with and without ^{15}N labelling was undertaken. The nodulated legumes *Albizia multiflora* and *Campsiandra comosa* were used as well as *Cecropia latiloba* and *Macrolobium acaciifolium* as reference species. Clearly, the transfer of results obtained with seedlings, to trees in the forest is problematic, nevertheless, the experimental design provides a suitable approach for method comparison.

Várzea soil was used, but unfortunately, due to logistic problems not from the study area. It was collected from the other side of the Rio Solimões at the waterfront of Ilha Xiborena. During the terrestrial period 1998/99, seeds and seedlings of the 4 species (*Albizia multiflora*, *Campsiandra comosa*, *Cecropia latiloba* and *Macrolobium acaciifolium*) were collected in the study area. Seedlings were immediately planted in growing-bags, the seeds were sown in March 1999. The seedlings were cultivated outside at the INPA premises in Manaus, where also later the experimental-pots were kept. In August 1999, 156 seedlings (56 *Albizia*

multiflora, 45 *Campsiandra comosa*, 14 *Cecropia latiloba* and 41 *Macrolobium acaciifolium*) were available. The plants of each species were divided into 7 similar groups. Therefore, of *Albizia multiflora*, *Campsiandra comosa* and *Macrolobium acaciifolium* 5-8 seedlings formed an experimental group. The seeds of *Cecropia latiloba* did not germinate, only few plants of this species were available, so that there were only 2 seedlings per group. 28 plastic-pots with a diameter of 54 cm and a height of 37 cm were used. Each pot was filled with approximately 100 kg soil. For each pot, 0.15 g $(NH_4)_2SO_4$ was dissolved in 3 litres water, which was then well mixed with the soil for uniform distribution. The different treatments are described in Table 3.5. The next day, on the 28th of August, the groups of seedlings were randomly planted in the pots.

Table 3.5 Description of the 2 treatments used in the isotope dilution pot experiment

Treatments	No. of pots	Labelling	Specification
unlabelled	16 (4/species)	single application of $(NH_4)_2SO_4$[a] at a rate of 0.3 mg N kg^{-1} soil	"^{15}N natural abundance method" Isotopic ratio in the soil was not meant to be influenced, labelling was undertaken to avoid systematic errors due to different N status of the soil.
^{15}N labelled	12 (3/species)	single application of $(*NH_4)_2SO_4$ (10% $^{15}N_2$)[b] at a rate of 0.3 mg N kg^{-1} soil	The amount of ^{15}N in the soil was increased at the beginning of the experiment and naturally decreased with time.

a (1012171; Merck & Co. Inc.)
b (85-702-31-4; Isotec Inc.)

Every 1 to 2 months, the height and number of leaves of each plant was recorded. From each plant the second youngest leaf was sampled, material from each pot was pooled as 1 sample. From each pot 3 soil samples (whole profile) were taken and all samples from pots with the same plant species and treatment were mixed together. Soil preparation and analyses was done in the same way as described for the ^{15}N natural abundance method. On the 8th of May 2000 the plants were harvested and the roots thoroughly washed. The plants were divided into the 3 samples: leaves, stem and roots. Again, material from each pot was pooled together. After drying of the samples at 60°C until weight consistency the dry weight of each sample was determined. Sample preparation and analyses was done in the same way as described for the ^{15}N natural abundance method. For the growth curves, only the surviving plants were included. N accumulated and the δ^{15}N value of the whole plants were calculated according to Equations 3.1 and 3.2. %Ndfa was calculated for *Albizia multiflora* and *Campsiandra comosa*

separately for each treatment and both reference plants (Equation 3.4). Therefore, 4 different calculation models were used and compared. The amount of fixed N per plant was calculated according to Equation 3.5.

Equation 3.5

$$N_{fixed}\,[mg\,plant^{-1}] = \frac{Naccp\,[mg]}{100} * \%Ndfa\,[\%]$$

N_{fixed}	amount of fixed N per plant
N_{accp}	accumulated N of the plant
%Ndfa	percentage of N derived from atmosphere

3.9 Statistics

Statistics were calculated with SPSS (SPSS Inc., Chicago, USA, Version 9.01, 1999) and Excel 97 (Microsoft Corporation, 1997) according to standard methods (BÜHL and ZÖFEL, 1998; KÖHLER et al., 1996). As a measure of the variation of data around the arithmetic mean, generally the standard deviation is shown. Normal distribution of data sets was checked by the Kolmogorov-Smirnov-Test, homogeneity of variances was checked with the Levene-Test. The level of significance was set at $p = 0.05$.

Analyses of variance (ANOVA) were carried out. The significant difference between 2 groups of data was determined by the t-test. Significant differences between 3 or more groups of data were determined with the one-factorial ANOVA with post hoc LSD test. Seasonal differences or differences with soil depth of physical and chemical soil properties were investigated with one-factorial ANOVA for replication samplings (dependent data). Differences between terrestrial and aquatic periods, as well as differences between leaf types or plant parts were, investigated by the t-test for paired samples.

If data was not normally distributed, non-parametric tests were used. The Kruskal-Wallis-H-Test and the Mann-Whitney-U-Test were applied for 3 or more independent groups of data and for 2 independent groups of data, respectively. For dependent data the Friedman and the Wilcoxon-Test respectively were applied. This was the case for microbiological soil property data.

To investigate the correlation of N_2 fixation and denitrification rates with soil parameters, Spearman correlation coefficients (r) were determined. Additionally, multivariate linear regressions were calculated to provide a degree of explanation (R^2).

4. Results

4.1 Forest

The stand structure was analysed to evaluate the importance of the legume species and the impact of their N_2 fixation potential for the entire várzea forest in the study area. Additionally, the legume species were investigated and characterised to evaluate their biodiversity and potential to form symbioses with rhizobia.

4.1.1 Stand structure

1441 woody plants were inventoried in the 4 plots (total area: 2.1 ha), of which 22 belonged to the Caesalpiniodeae, 74 to the Papilionoideae and 47 to the Mimosoideae. Therefore, legumes had an average absolute density of 68 individuals per hectare. In total, 44 woody species from 25 families were determined, whereas 3 species could not be identified and 8 were only identified to genus level.

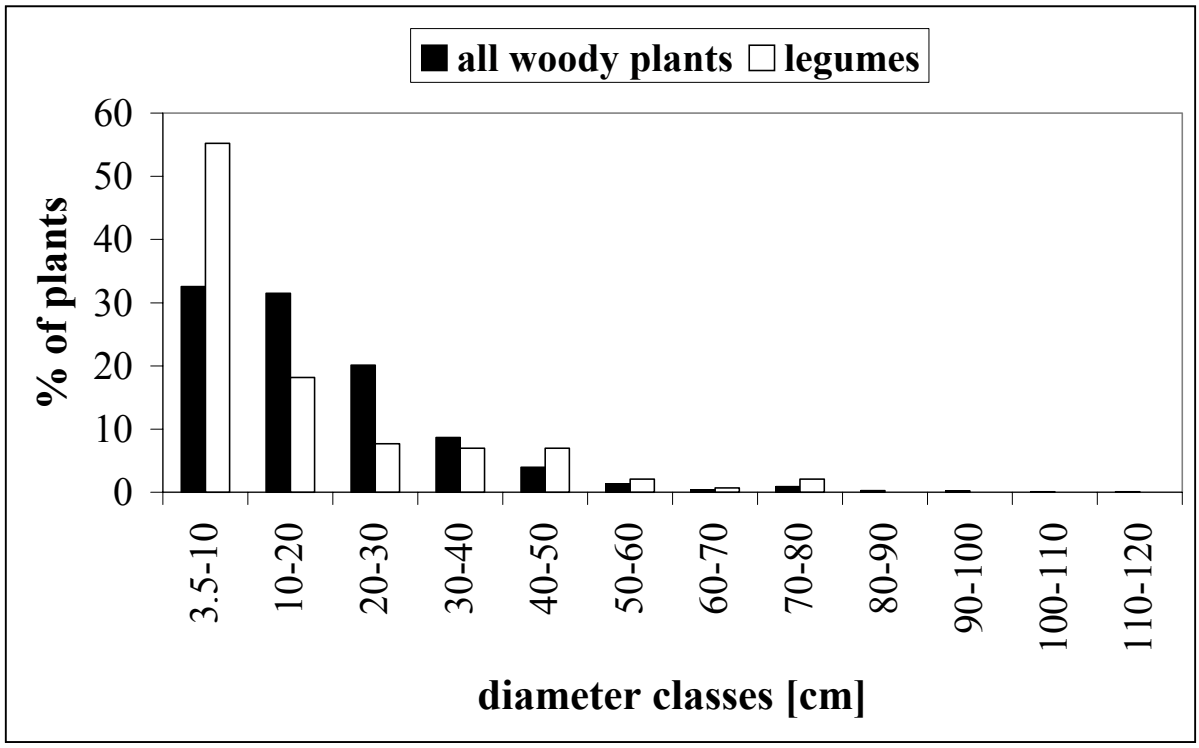


Figure 4.1 Distribution of diameter classes for all woody plants (including legumes; n=1441) and for legumes separately (n=143) which were found in the 2.1 ha area (4 plots) where the stand structure analysis was undertaken.

Diameter classes for all woody plants (including legumes), and for legumes separately, showed typical distributions, with over 80% of the plants with diameters between 3.5 and 30 cm (Figure 4.1). Diameters ranged between 3.5 and 114 cm (max.: *Pseudobombax munguba*) with a mean diameter of 18 cm. Due to the high proportion of leguminous shrubs and lianas, which have small diameters between 3.5 and 10 cm, the Leguminosae had a lower average diameter of 16 cm. Legume diameters ranged from 3.5–73 cm (max.: *Macrolobium acaciifolium*). The following 5 species attained diameters over 70 cm, in descending order of importance (basal area and number of trees with diameter over 70): *Pseudobombax munguba* (Bombacaceae), *Luehea cymulosa* (Tiliaceae), *Triplaris* sp. (Polygonaceae) and the legume species *Macrolobium acaciifolium* (subfamily: Caesalpiniodeae) and *Campsiandra comosa* (subfamily: Caesalpiniodeae). Total basal area of all woody species in the 2.1 ha area was 62 m², of which legumes covered 6 m².

In terms of FIV Capparaceae and Verbenaceae were the most important families, the legumes represented the 3rd most important family, specially with a high relative richness of 27% (Table 4.1). Looking separately at the 3 legume subfamilies, Papilionoideae was the 3rd, Caesalpiniodeae the 5th and Mimosoideae the 8th most important group.

Table 4.1 Family importance value, relative richness, relative density and relative dominance of the 8 most important families. Additionally, the values of the 3 legume subfamilies are shown. Families are listed in order of their importance.

Plant families (subfamilies)	**family importance value**	**relative richness [%]**	**relative density [%]**	**relative dominance [%]**
Capparaceae	59	2	37	20
Verbenaceae	54	2	23	29
Leguminosae	47	27	10	10
Papilionoideae	18	11	5	2
Caesalpiniodeae	17	9	2	6
Mimosoideae	12	7	3	2
Flacourtiaceae	18	5	7	6
Tiliaceae	16	2	2	12
Bombacaceae	14	2	2	10
Myrtaceae	11	2	7	2
Euphorbiaceae	11	7	3	1
remaining 17 Families	69	50	9	10

Crateva benthamii (Capparaceae) and *Vitex cymosa* (Verbenaceae) prevailed with a relative dominance of 20% and 29%, respectively, and a relative density of 37% and 23%,

respectively. Despite its low dominance (< 1%; 0.12 m² ha^{-1}) the liana *Dalbergia riparia* (Papilionoideae) was the most important legume species with an IVI of 10 (Table 4.2). *Macrolobium acaciifolium* (Caesalpiniodeae), *Albizia multiflora* (Mimosoideae) and *Pterocarpus amazonum* (Papilionoideae) were the most important legume trees with IVIs of 9, 8 and 5, respectively. *Macrolobium acaciifolium* trees had a high mean diameter of 46 cm and a relative dominance of 5% (1.51 m² ha^{-1}). Also *Albizia multiflora* and *Pterocarpus amazonum* had, with 19 cm and 26 cm, respectively, mean diameters above average, but their relative dominances were evidently lower at 2% (0.45 m² ha^{-1}) and 1% (0.42 m² ha^{-1}), respectively.

Table 4.2 Relative and absolute stand structure characteristics (frequency, density, dominance) and important value index (IVI) of the legume species. Species are listed in order of their importance.

legume species	**sub-family**	**IVI**	**relative frequ. [%]**	**relative density [%]**	**relative domin. [%]**	**absol. density [ind. ha^{-1}]**	**absol. domin. [m² ha^{-1}]**
Dalbergia riparia	Papil.	10	6	3	0	23	0.12
Macrolobium acaciifolium	Caes.	9	2	1	5	9	1.51
Albizia multiflora	Mimos.	8	5	2	2	15	0.45
Pterocarpus amazonum	Papil.	5	2	1	1	8	0.42
Mimosa pigra	Mimos.	2	2	1	0	6	0.01
Machaerium aristulatum	Papil.	2	1	0	0	2	0.02
Lonchocarpus sp.	Papil.	1	1	0	0	1	0.02
Campsiandra comosa	Caes.	1	0	0	1	0	0.19
Zygia inaequalis	Mimos.	1	1	0	0	1	0.03
Dalbergia inundata	Papil.	1	1	0	0	1	0.01
Acosmium nitens	Caes.	1	0	0	0	0	0.03
Crudia amazonica	Caes.	1	0	0	0	0	0.03

All species identified in the study area, irrespective of their size or abundance, are listed in Table A.4.1. Characterisation of the species was done by observations and measurements as well as comparison with data from the literature about their growth habit, basal area, absolute density, wood density, fruiting season, leaf characteristics (growth, periodicity, size, N content, weight) and mycorrhiza status.

According to different volume based estimation models, the above ground forest biomass of the study area is between 227 and 343 t ha^{-1} (Table 4.3). Legumes represent a fraction of 10-12%, of which Caesalpiniodeae account for 7-8%, and Papilionoideae and Mimosoideae each for about 2%.

Table 4.3 Above ground dry biomass estimates for the study area according to the models from 4 different studies undertaken in terra firme forests. The estimated biomass of the 3 legume subfamilies and of the remaining families together is also shown.

	biomass estimate [t ha^{-1}] according to			
	BROWN et al., 1989	**NELSON et al., 1999**	**LAURANCE et al., 1997**	**HIGUCHI et al., 1998**
Caesalpiniodeae	18	16	24	23
Papilionoideae	4	4	5	6
Mimosoideae	4	4	5	5
non-legumes	201	211	307	309
sum	227	235	342	343

4.1.2 Legume species

In the study area 24 legume species were found, of which 12 belonged to the Papilionoideae, 6 to the Caesalpiniodeae and 6 to the Mimosoideae. 10 species were trees, 5 shrubs, 6 woody climbers, 2 herbs and 1 aquatic plant (Table 4.4).

Of the legume species 21 were nodulated and only 3 were non-nodulated (Table 4.4). For 11 species the nodulation status was actually verified in the study area, for the others reports from the literature were taken. This is the first report of the observation of nodules on a *Pterocarpus* sp. in Brazil. There is no literature report about the nodulation status of the genus *Cymbosema*, but since this genus belongs to the tribe Phaseoleae which contains predominantly nodulated genus/species (FARIA et al., 1989), *Cymbosema roseum* was treated as a nodulated species. A further indication of its nodulation is its low $\delta^{15}N$ value (0.2‰) (Figure 4.18).

Table 4.4 Characterisation of the identified legume species in the study area. Empirical and literature data is included, the grey shading indicates data which were determined in this study.

species	**sub-family**	**growth habit**	**[a]wood-density [g cm^{-3}]**	**[b]leaf period-icity**	**leaf N content [%]**	**nodul-ation status**	**reference for nodulation status**
Acosmium nitens	Caes.	**tree**	0.74	deciduous	ND	+	FARIA and LIMA, 1998
Aeschynomene sp.	Papil.	**shrub**	ND	ND	**4.7**	+	FARIA et al., 1989; FARIA and LIMA, 1998
Albizia multiflora	Mimos.	**tree**	**0.62**	**deciduous**	**3.9**	**+**	FARIA et al., 1984; 1987; FARIA and LIMA, 1998
Bauhinia sp.	Caes.	**shrub**	ND	ND	**4.4**	-	FARIA et al., 1987; 1989; FARIA and LIMA, 1998
Campsiandra comosa	Caes.	**tree**	0.81; **0.86**	deciduous	**2.2**	**+**	FARIA et al., 1989; FARIA and LIMA, 1998

species	sub-family	growth habit	[a]wood-density [g cm^{-3}]	[b]leaf period-icity	leaf N content [%]	nodul-ation status	reference for nodulation status
Chamaecrista sp.	Caes.	**tree**	ND	ND	**2.7**	+	FARIA et al., 1984; 1987; FARIA and LIMA, 1998
Crudia amazonica	Caes.	**tree**	0.87	deciduous	**2.8**	-	FARIA et al., 1989; FARIA and LIMA, 1998
Cymbosema roseum	Papil.	**woody climb.**	ND	ND	**3.6**	(+)	FARIA et al., 1989
Dalbergia inundata	Papil.	**woody climb.**	0.75-1.00	ND	**2.7**	+	FARIA et al., 1989; FARIA and LIMA, 1998
Dalbergia riparia	Papil.	**woody climb.**	0.75-1.00	ND	**3.0**	+	FARIA et al., 1987; 1989
Entada polyphylla	Mimos.	**shrub**	ND	ND	**4.2**	+	FARIA et al., 1989; FARIA and LIMA, 1998
Inga splendens	Mimos.	**tree**	ND	ND	**3.8**	+	FARIA et al., 1984; 1987; 1989; FARIA and LIMA, 1998
Lonchocarpus sp.	Papil.	**woody climb.**	0.70-0.95	ND	**2.4**	+	FARIA et al., 1984; FARIA and LIMA, 1998
Machaerium aristulatum	Papil.	**woody climb.**	0.7	ND	**2.3**	+	FARIA et al., 1984; 1987; 1989; FARIA and LIMA, 1998
Machaerium ferox	Papil.	**woody climb.**	0.7	ND	ND	+	FARIA et al., 1984; 1987; 1989; FARIA and LIMA, 1998
Macrolobium acaciifolium	Caesalp.	**tree**	0.43; 0.49; **0.56**	**semi-deciduous**	**2.6**	-	FARIA et al., 1987; 1989; FARIA and LIMA, 1998
Mimosa pigra	Mimos.	**shrub**	ND	deciduous	**3.5**	+	FARIA et al., 1987; 1989; FARIA and LIMA, 1998
Neptunia oleracea	Mimos.	**aquat. plant**	ND	ND	**3.8**	+	ALLEN and ALLEN, 1981; FARIA et al., 1989
Pterocarpus amazonum	Papil.	**tree**	0.33; **0.40**	**deciduous**	**2.8**	+	FARIA et al., 1989; FARIA and LIMA, 1998
Sesbania exasperata	Papil.	**shrub**	ND	ND	**5.3**	+	FARIA et al., 1989; SPRENT, 1995
Swartzia sp.	Papil.	**tree**	0.63; 0.86	evergreen	**2.4**	+	FARIA et al., 1984; 1989; FARIA and LIMA, 1998
Teramnus volubilis	Papil.	**herb**	ND	ND	**3.8**	+	GALLI, 1958 cited in ALLEN and ALLEN, 1981; FARIA et al., 1989
Vigna sp.	Papil.	**herb**	ND	ND	**3.9**	+	FARIA et al., 1989; FARIA and LIMA, 1998
Zygia inaequalis	Mimos.	**tree**	**0.72**	**evergreen**	**2.6**	+	FARIA et al., 1989; FARIA and LIMA, 1998

a) wood-density data is published by ALLEN and ALLEN (1981), WORBES et al. (1992), FEARNSIDE (1997b) and PAROLIN et al. (1998)
b) leaf periodicity data is published by KLINGE et al. (1983) and SCHÖNGART et al. (2002)
ND: not determined and no literature data available

Summing-up, legumes were the 3rd most important plant family with an exceptionally high species richness of 27%. On average, 68 individual legumes per hectare accounted for 10-12% of the total above ground biomass. The liana *Dalbergia riparia* (Papilionoideae) and the trees *Macrolobium acaciifolium* (Caesalpiniodeae), *Albizia multiflora* (Mimosoideae) and *Pterocarpus amazonum* (Papilionoideae) were the most important legume species in the study area. Of the 24 legume species found in the study area, 87.5% were nodulated.

4.2 Soil

Physical, chemical and microbiological soil properties were determined to identify and investigate factors influencing and regulating the gaseous N turnover. Particular focus was on the impact of the flood pulse, since many soil properties are directly controlled by it.

4.2.1 Physical properties

In the terrestrial period, average bulk density varied between 1.0 and 1.4 Mg m^{-3} according to soil depth (Table 4.5). Density of soil solids was 2.26 ± 0.1 Mg m^{-3}, thus porosity was between 40 and 56% (Table 4.5). The silt fraction was dominant, and increased with depth. During the terrestrial period, soil had a low pH of 4.3 in the surface soil layer, but it increased with soil depth reaching a value of 6.2 at 350 cm depth. In comparison, during the aquatic period pH was higher, ranging between 4.9 at 5-20 cm depth and 5.2 in litter. Organic matter varied considerably between soil layers, it was over 13% at 0–5 cm depth and below 4% in the 60–100 cm layer. During the entire hydrological cycle, soil temperature varied between 27° C and 30° C depending on depth and time.

Table 4.5 Physical soil properties during the terrestrial period (mean ± standard deviation).

Depth	Bulk density	Porosity	Particle size			pH	Organic matter	O_2 par. press.
			clay	silt	sand	(in		
[cm]	[Mg m^{-3}]	[%]	[%]	[%]	[%]	KCl)	[%]	[%]
litter	ND	ND	ND	ND	ND	5.1 ± 0.6	30.1 ± 12.1	ND
0-5	1.0 ± 0.1	56 ± 6	11.4 ± 2.6	78.5 ± 6.7	1.4 ± 1.5	4.3 ± 0.2	13.4 ± 5.0	20 ± 1
5-20	1.2 ± 0.1	48 ± 5	14.1 ± 3.0	80.4 ± 6.3	0.4 ± 0.6	4.1 ± 0.2	7.3 ± 1.6	19 ± 2
20-40	ND	ND	10.6 ± 3.6	81.0 ± 4.6	0.9 ± 0.5	4.5 ± 0.3	5.9 ± 1.3	18 ± 2
40-60	1.3 ± 0.0	42 ± 2	ND	ND	ND	4.9 ± 0.3	4.7 ± 0.3	17 ± 2
60-100	1.4 ± 0.0	40 ± 1	9.7 ± 0.2	89.4 ± 1.0	0.0 ± 0.0	5.3 ± 0.2	3.9 ± 0.0	17 ± 2

Oxygen

During the terrestrial period, O_2 partial pressure in soil-air declined with depth on average from 20% at 0-5 cm to 17% at 60-100 cm depth (Table 4.5). Additionally, there were seasonal changes in the surface soil layer (0-5 cm), during the first months of the terrestrial period the O_2 partial pressure increased from 19% in September to 21% in December, then it slightly decreased again to 20% in February. Also, the gradient in the soil profile changed significantly with time.

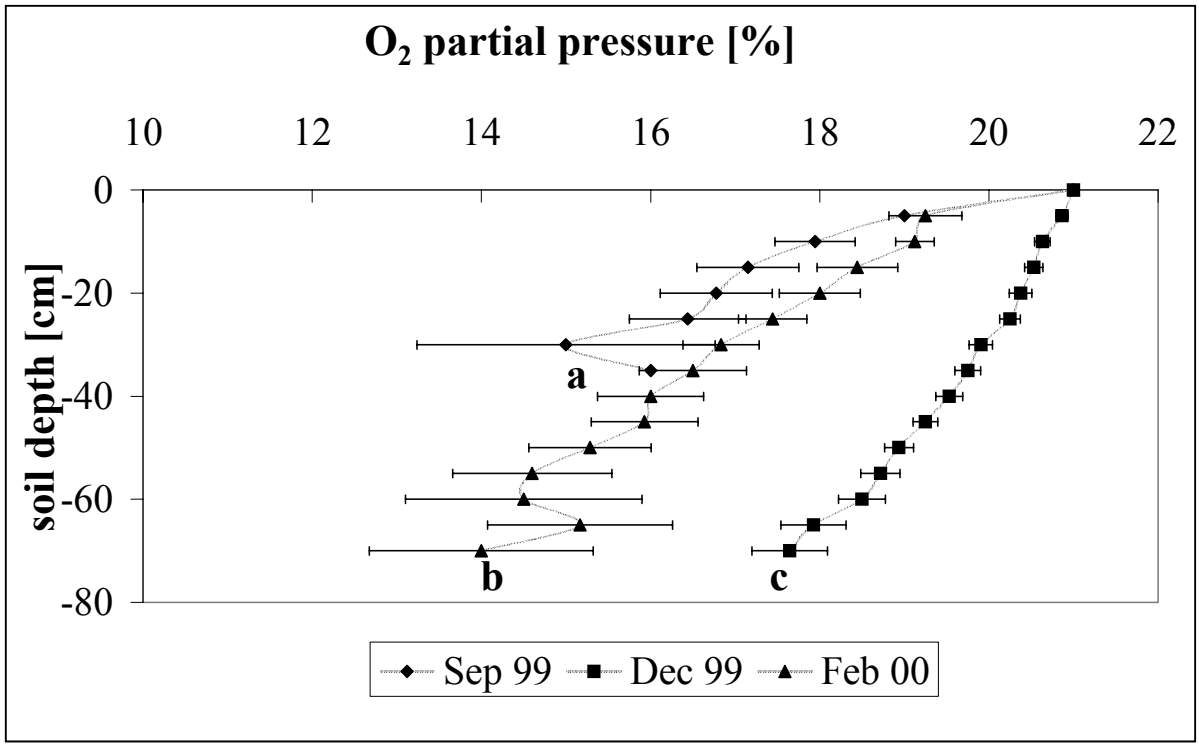


Figure 4.2 O_2 partial pressure in soil-air at different depths and during 3 different measurement periods (n=14; means and standard deviation). Graphs marked with different letters are significantly different by $p<0.05$

Lowest O_2 levels in the soil profile were determined right at the beginning of the terrestrial period in September 1999 (Figure 4.2). Measurements were taken shortly after drying of the soil and then only down to a depth of 35 cm as below this point extraction of soil-air was impossible due to the main pore space filled with water (water level: 22.4 m a.s.l.). Already at 30 cm depth O_2 partial pressure was only 15%. Also, at the end of the terrestrial period in February 2000 O_2 partial pressure was relatively low, continually decreasing to 17% at 30 cm and 14% at 70 cm soil depth. In the middle of the terrestrial period in December 1999 O_2 levels were relatively high, only slightly decreasing to 18% at 70 cm depth.

During the aquatic period, concentration of dissolved O_2 varied between 1.2 and 3.8 mg l^{-1} in surface water, whereas in deep water, just above the sediment, concentrations were between 0.9 and 3.5 mg O_2 l^{-1} (Table 4.6). That means for this temperature regime that the O_2 saturation level of the water was between 12% and 50%. O_2 concentration in the flooding water varied strongly with time and flooding level. In March O_2 concentrations were low, probably due to intensive biodegradation of organic material. Due to in-flowing river water, O_2 concentration increased and reached its maximum in August 1999.

Table 4.6 O_2 concentration and saturation level in the flooding water within the studied forest area (n=3; mean ± standard deviation).

month 1999	surface water O_2 concentration [mg/l]	deep water O_2 concentration [mg/l]	surface water O_2 saturation level [%]	deep water O_2 saturation level [%]
March	1.2 ± 0.2	0.9 ± 0.2	15 ± 2	12 ± 3
May	2.0 ± 0.5	1.2 ± 0.1	26 ± 6	16 ± 1
June	1.5 ± 0.0	1.5 ± 0.0	20 ± 0	20 ± 0
July	2.7 ± 0.1	2.7 ± 0.1	35 ± 1	35 ± 1
August	3.8 ± 0.2	3.5 ± 0.1	50 ± 2	45 ± 1
September	2.8 ± 0.2	1.4 ± 0.1	36 ± 2	18 ± 1

Water content

Water content in soil varied with depth and time, mainly influenced by precipitation and the flood pulse. During receding water, at a water level of 21.7 m a.s.l. (October 99), mean water content in the soil profile varied between 22.7% and 32.7% (Figure 4.3). Differences between soil layers were significant, with the driest parts at 20-60 cm depth, and the wettest layers below the water table at 300-450 cm depth.

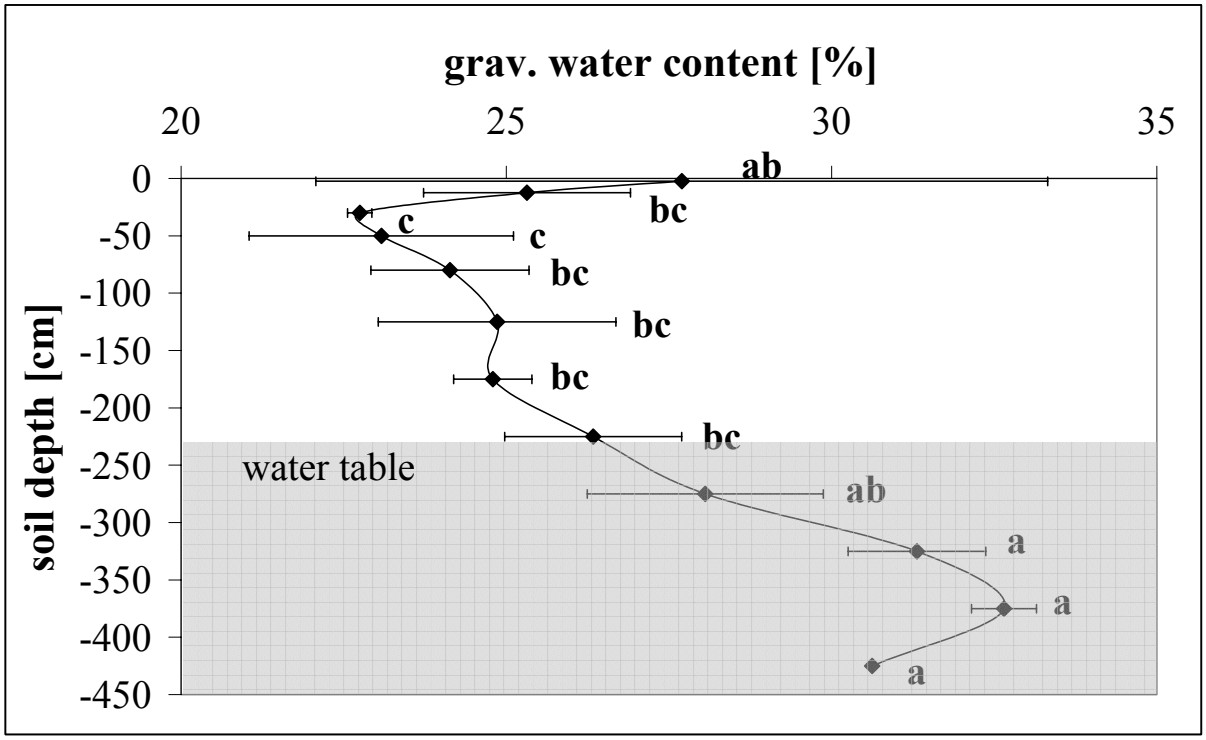


Figure 4.3 Gravimetric water content in the soil profile, measured in October 1999 (n=3; mean and standard deviation). Soil below the water table (230 cm depth) is shaded grey, points followed by the same letter are not significantly different by $p<0.05$.

Significant seasonal variation in water content existed in litter and the surface soil layer ($p<0.05$) (Figure 4.4). Litter had the highest gravimetric water contents with an average of 42% during the terrestrial period and 66% during the aquatic period. In the surface soil layer, the mean water content was on average 33% during the terrestrial period and 42% during the aquatic period. In deeper soil layers, no significant seasonal variation existed. The soil layer 5-20 cm had an average water content of 30%, at 20-40 cm depth the average water content was 27%. The soil was driest in November/December, during the terrestrial period in the dry season. Actual and short term variations should be much larger than the differences between these average values. Gravimetric water contents of 33% and 42% in the soil layer 0-5 cm is equivalent to 66% and 84% water filled pore space, respectively. A water content of 30% at 5-20 cm depth and 27% at 20-40 cm depth is equal to 90% and 89% water filled pore space, respectively. The water holding capacity was not determined.

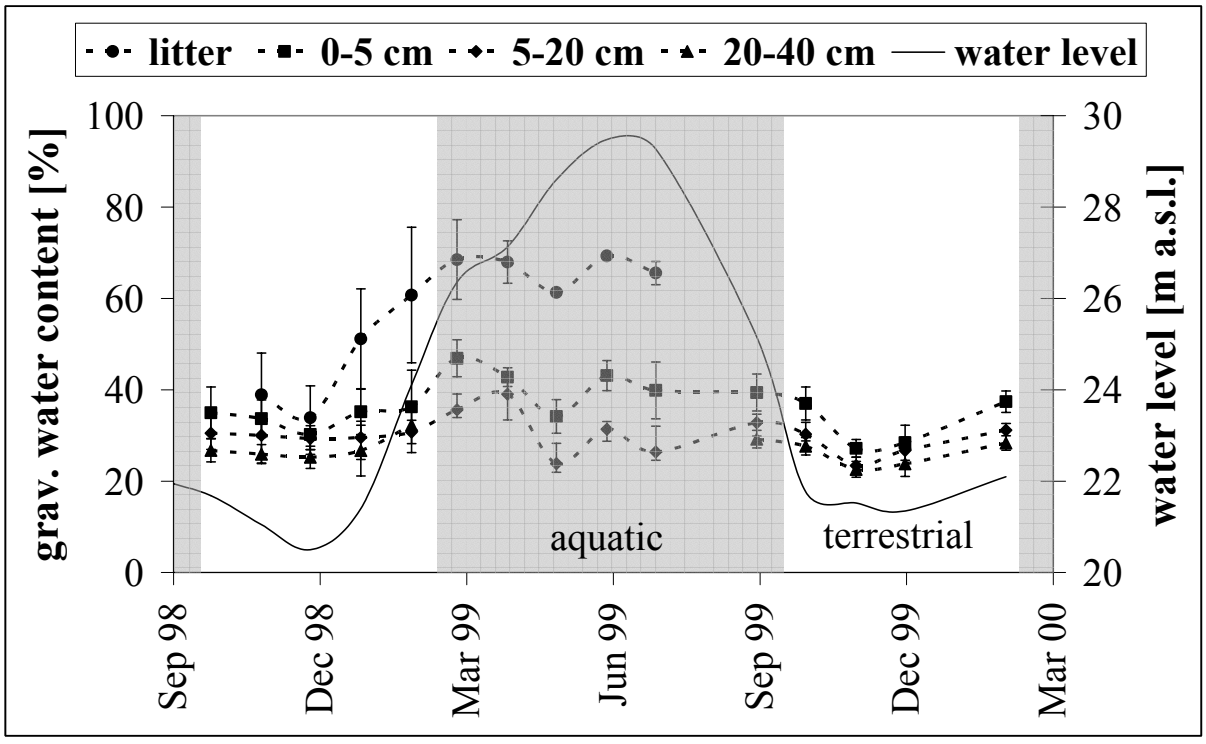


Figure 4.4 Gravimetric water content in 4 different soil layers during the time course from October 1998 until February 2000 (n=30 during the terrestrial period, n=9 during the aquatic period; means and standard deviations). The aquatic periods are shaded in grey.

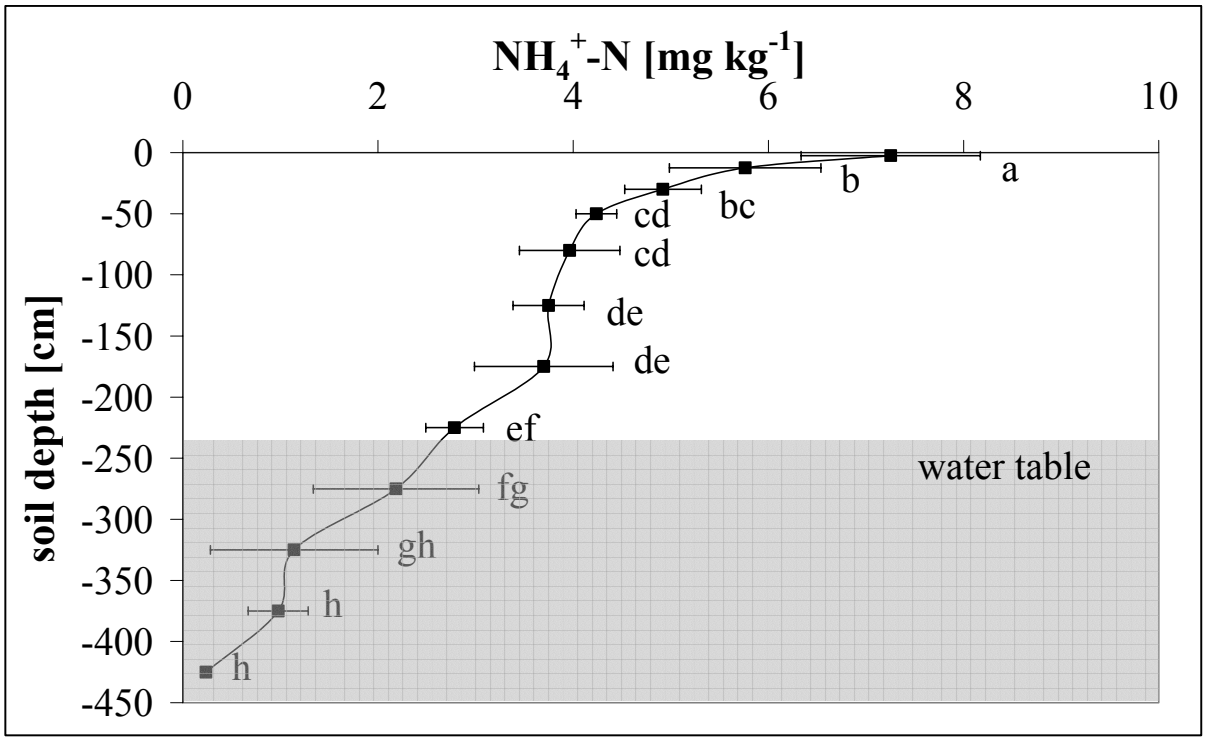
4.2.2 Chemical properties

In the terrestrial period, average total N decreased with soil depth from 9.8 g kg^{-1} in litter to 0.7 g kg^{-1} at 60-100 cm depth (Table 4.7). Average C content had a similar trend with a high value of 196.7 g kg^{-1} in litter and a reduction to 5.8 g kg^{-1} at 60-100 cm depth. Also the C/N ratio decreased with soil depth, in litter it was highest with 19.9 and significantly different from the mineral soil. Mean total P varied between 0.5 g kg^{-1} in litter and 0.7 g kg^{-1} in soil, but no significant differences between litter or soil layers existed.

Table 4.7 Chemical soil properties during the terrestrial period (mean ± standard deviation)

Depth [cm]	NO_3^--N [mg kg^{-1}]	NO_2^--N [mg kg^{-1}]	NH_4^+-N [mg kg^{-1}]	total N [g kg^{-1}]	total C [g kg^{-1}]	C/N-ratio	total P [g kg^{-1}]
litter	ND	0.02 ± 0.02	ND	9.8 ± 3.1	196.7 ± 76.2	19.9 ± 3.9	0.5 ± 0.1
0–5	0.7 ± 0.1	0.02 ± 0.02	7.3 ± 0.9	2.5 ± 0.9	32.5 ± 14.3	14.0 ± 10.7	0.7 ± 0.3
5–20	0.6 ± 0.1	0.02 ± 0.02	5.8 ± 0.7	1.3 ± 0.3	13.0 ± 4.2	10.1 ± 2.0	0.7 ± 0.4
20–40	0.4 ± 0.1	0.03 ± 0.03	4.9 ± 0.4	0.8 ± 0.2	8.4 ± 3.1	10.8 ± 4.1	0.7 ± 0.4
40–60	0.5 ± 0.1	ND	4.2 ± 0.2	0.8 ± 0.2	5.3 ± 1.4	7.2 ± 0.7	ND
60-100	0.4 ± 0.2	ND	4.0 ± 0.5	0.7 ± 0.4	5.8 ± 4.7	7.7 ± 2.0	ND

Mean NH_4^+-N content decreased significantly with soil depth, but it was always the dominant form at about one order of magnitude higher than NO_3^--N (Figure 4.5).



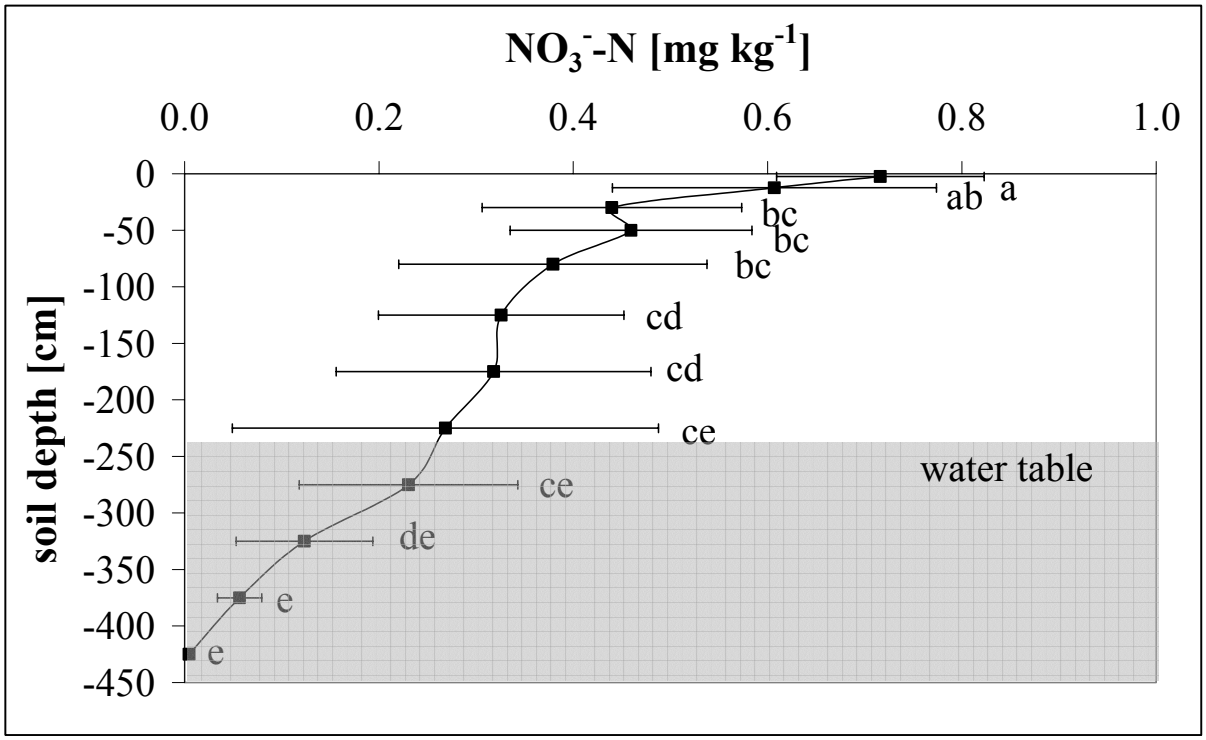


Figure 4.5 NH_4^+-N (page 58) and NO_3^--N (page 59) content in the soil profile, measured in October 1999 (n=3; mean and standard deviation). Soil below the water table (230 cm depth) is shaded grey, points followed by the same letter are not significantly different by $p<0.05$.

NO_3^--N content was at a very low level even in the top soil. NO_3^--N decreased from 0.7 mg kg^{-1} at 0-5 cm to 0.4 mg kg^{-1} at 60-100 cm and to <0.1 mg kg^{-1} at 400-450 cm depth (Figure 4.5). NH_4^+-N content decreased from 7.3 mg kg^{-1} at 0-5 cm to 4.0 mg kg^{-1} at 60-100 cm depth. At 400-450 cm depth only 0.2 mg kg^{-1} NH_4^+-N was measured. On average 0.6% of total N was mineral N.

In the soil layers which were continuously sampled during the hydrological cycle (0-5 and 5-20 cm) NH_4^+-N and NO_3^--N content showed significant seasonal differences (Figure 4.6). NH_4^+-N content showed an increase during the transition from terrestrial to aquatic period, and then concentrations stayed relatively high during the aquatic period. NH_4^+-N content at 0-5 cm soil depth were, with an average of 7.7 ± 2.6 mg kg^{-1} during the aquatic period, significantly higher than during the terrestrial period (3.3 ± 2.6 mg kg^{-1}). In contrast, NO_3^--N was significantly higher during the terrestrial period (0.3 ± 0.2 mg kg^{-1}) compared to the aquatic period (0.1 ± 0.1 mg kg^{-1}).

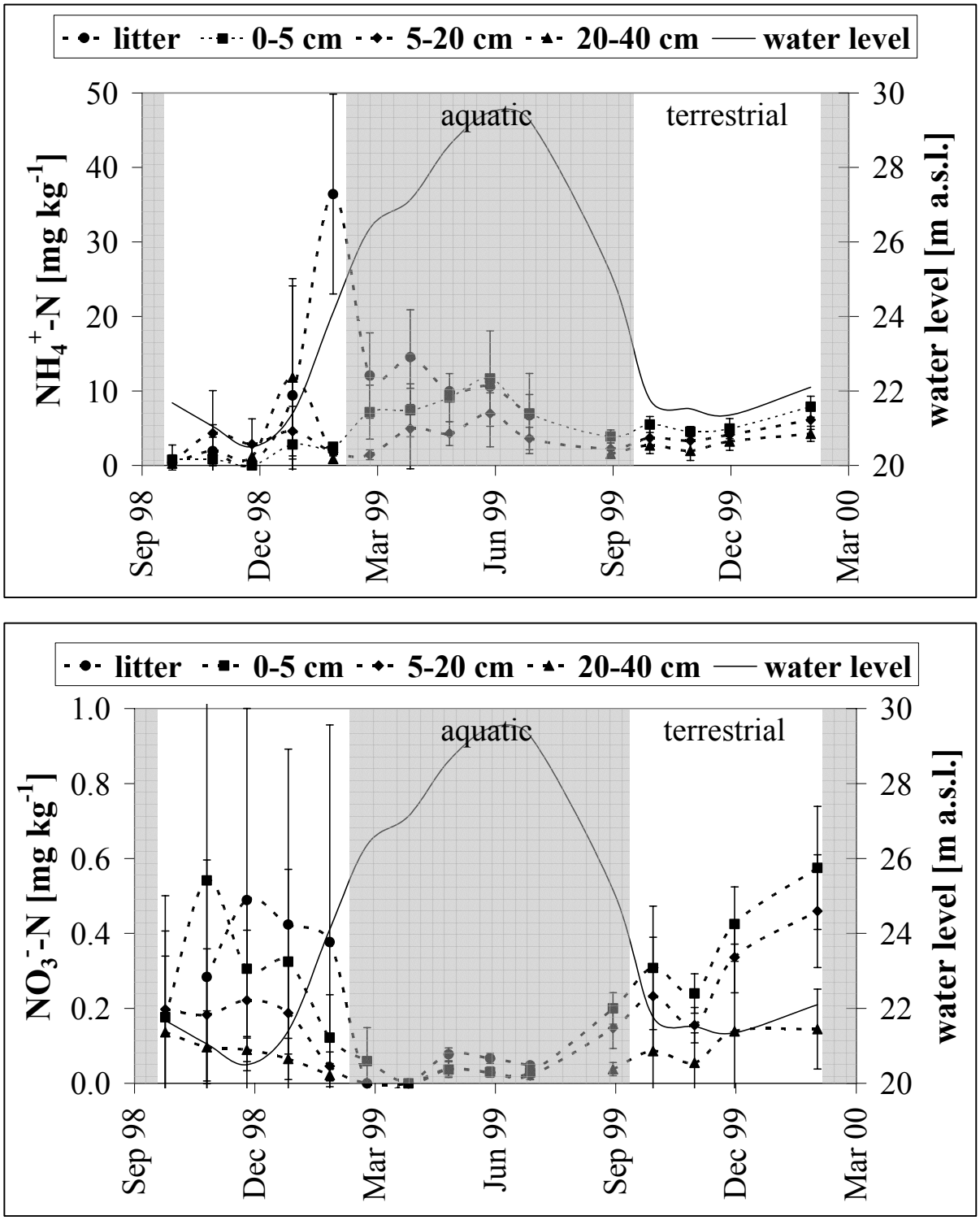


Figure 4.6 NH_4^+-N (top) and NO_3^--N (bottom) content in 4 different soil layers during the time course from October 1998 until February 2000 (n=30 during the terrestrial period, n=9 during the aquatic period; means and standard deviations). The aquatic periods are shaded in grey.

4.2.3 Microbiological properties

Analysis of the samples revealed a high heterogeneity in bacterial distribution within the soil. This strongly affected the average values obtained, and means that the results are not necessarily representative for the study area during the hydrological cycle. No significant differences in bacterial numbers were observed between the terrestrial and the aquatic period, and between litter and soil depths (Table 4.8). The results do provide insight into the microbiological status of the soil.

Free-living N_2 fixing bacteria were present in numbers between 10^1 and 10^7 g-dw^{-1}. Average values showed the tendency of high numbers in litter and the surface soil layer (10^5-10^6 g-dw^{-1}) and a lower density at 5-20 cm depth. Colony forming units of proteolytic bacteria (10^4 to 10^6 cfu g-dw^{-1}) and numbers of ammonifiers (10^6 and 10^8 g-dw^{-1}) were high, even down to a soil depth of 20 cm. Low numbers of nitrifying bacteria, mainly below 10^3 g-dw^{-1}, were observed. In 19% of the samples no nitrifying bacteria were present. Maximum numbers of 10^6 g-dw^{-1} were found at one location at 0-5 cm soil depth and at 5-20 cm soil depth. Due to better O_2 supply, and therefore more favourable conditions for nitrification, higher numbers of nitrifiers were expected during the terrestrial period, but no such trend was observed. Denitrifiers were present in the soil in relatively high numbers between 10^2 and 10^8 g-dw^{-1}. Although conditions for denitrification were more favourable during the aquatic period, numbers tended to be higher during the terrestrial period.

Table 4.8 Most probable numbers or colony forming units of diazotrophs, proteolytic bacteria, nitrifiers, ammonifiers and denitrifiers in litter and soil (0-5 cm and 5-20 cm depth). Samples were taken during the terrestrial (December 1998) and the aquatic period (March 1999). Values in a row, followed by the same letter are not significantly different by $p<0.05$ (n=5; means).

		litter		soil (0-5 cm)		soil (5-20 cm)	
		terrest.	**aquatic**	**terrest.**	**aquatic**	**terrest.**	**aquatic**
diazotrophs	MPN [g-dw^{-1}]	ND	$10^5{}_a$	ND	$10^6{}_a$	ND	$10^3{}_a$
proteolytic bacteria	cfu [g-dw^{-1}]	$10^5{}_a$	$10^6{}_a$	$10^5{}_{ab}$	$10^5{}_{ab}$	$10^4{}_b$	$10^4{}_b$
nitrifiers	MPN [g-dw^{-1}]	$10^1{}_a$	$10^2{}_b$	6_a	$10^5{}_{ab}$	7_a	$10^5{}_{ab}$
ammonifiers	MPN [g-dw^{-1}]	$10^8{}_a$	$10^7{}_a$	$10^8{}_a$	$10^8{}_a$	$10^8{}_a$	$10^6{}_a$
denitrifiers	MPN [g-dw^{-1}]	$10^7{}_a$	$10^6{}_a$	$10^6{}_a$	$10^5{}_a$	$10^7{}_a$	$10^3{}_a$

In summary, the acidic soil in the study area was rich in organic matter, specially in the surface soil layer, where also N concentrations were highest. Significant seasonal variations

due to the flood pulse were apparent for many soil properties (pH, O_2 availability, soil water content, NH_4^+-N and NO_3^--N contents) but not for bacterial numbers. During the terrestrial period aeration was good, while hypoxic or even anoxic conditions were characteristic for the aquatic period. NH_4^+-N content was higher during the aquatic period by comparison with the terrestrial period, whereas for NO_3^--N content it was the other way round. NH_4^+-N content was always about one order of magnitude higher than NO_3^--N.

4.3 Rhizobia

Characterisation of rhizobia was undertaken to provide information about their biodiversity and possible symbioses with legume species.

A total of 18 isolates were obtained, which exhibited typical rhizobial colony and cell morphology. All isolates grew at a medium rate, forming well defined colonies (2-4 mm) after 4-6 days of incubation at 30° C. The 14 isolates from *Albizia multiflora* and 4 isolates from *Pterocarpus amazonum* (Table A.4.2) all had *nod* as well as *nif* gene regions which were amplified with the *nod*C and *nif*DK primers respectively (Figures 4.7 and 4.8), which strongly suggests that they are rhizobia.

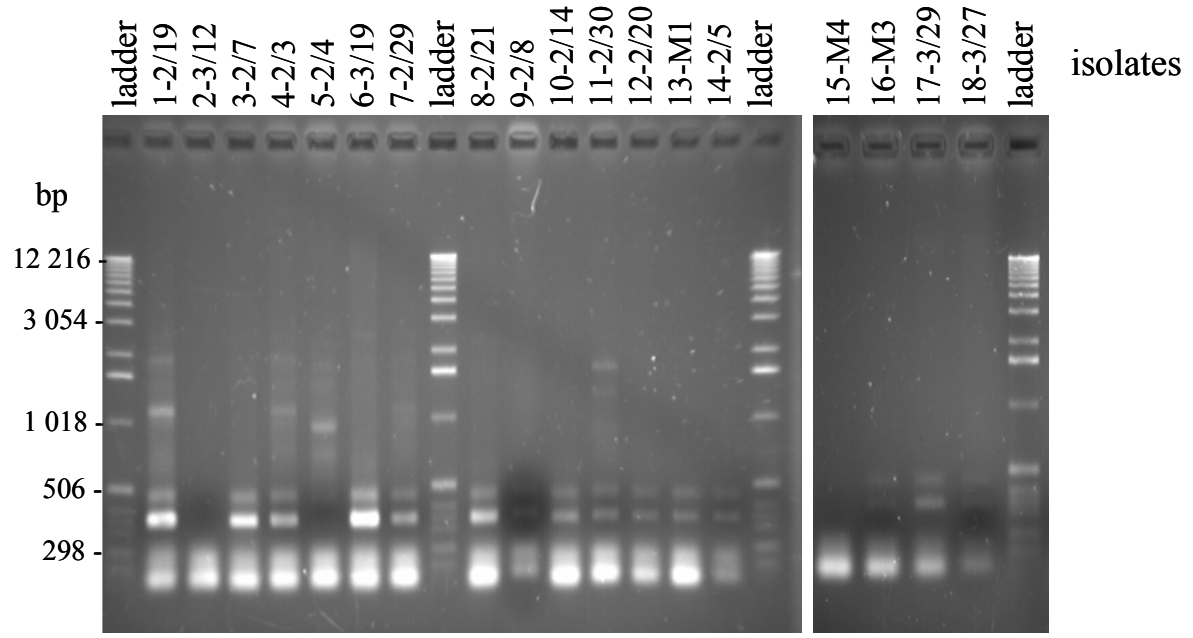


Figure 4.7 Gel images of the amplified *nod* gene region (274 bp) of all isolates from *Albizia multiflora* and *Pterocarpus amazonum*, including 1kb DNA ladders

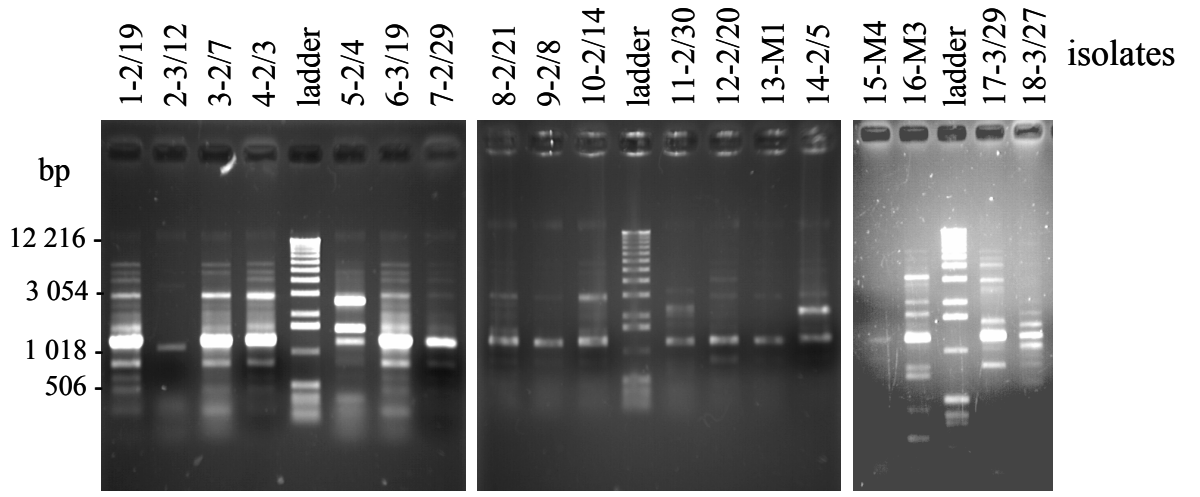


Figure 4.8 Gel images of the amplified *nif* gene region (1250 bp) of all isolates from *Albizia multiflora* and *Pterocarpus amazonum*, including 1kb DNA ladders

Nearly full length 16S rDNA fragments (1.5 kb) could be amplified from all isolates. The individual RFLP patterns resulting from the restriction with enzymes *Dde*I, *Hha*I, *Msp*I, and *Sau*3AI are shown in Figure 4.9. The sizes of the restriction fragments are shown in Table A.4.2. The *Dde*I restriction grouped the isolates in 3 genotypes, with 4 or 5 bands. The *Hha*I digestion formed 4 different groups with 3, 4 or 5 bands. All isolates had a restriction fragment of 454 bp size after *Hha*I digestion. With *Msp*I restriction 4 or 5 fragments emerged, dividing the isolates into 5 genotypes. Analysis of the *Sau*3AI restriction pattern was more difficult due to aberrantly running gels, but the same genotypes as revealed by the *Hha*I enzyme were confirmed. With *Sau*3AI enzyme 3 or 4 fragments were detected, all isolates sharing a fragment of 218 bp size. Although only 2 host species in 1 forest area were sampled, the isolates investigated were heterogeneous in their RFLP profiles, indicating the existence of an appreciable genotypic diversity within the small isolate collection analysed. Cluster analysis of the combined RFLP patterns obtained with the 4 enzymes resolved 5 16S rDNA alleles (I-V) grouped in 2 major clusters (A with alleles I and II, and B comprising alleles III-V) with a mean linkage level of 50% and 65% similarity, respectively. These 2 major branches most likely constituted 2 different genera, with at least 2 species (Figure 4.9). These major clusters did not correlate with the 2 host species. The 4 isolates from *Pterocarpus amazonum* were clustered together with isolates from *Albizia multiflora* in 3 similarity groups.

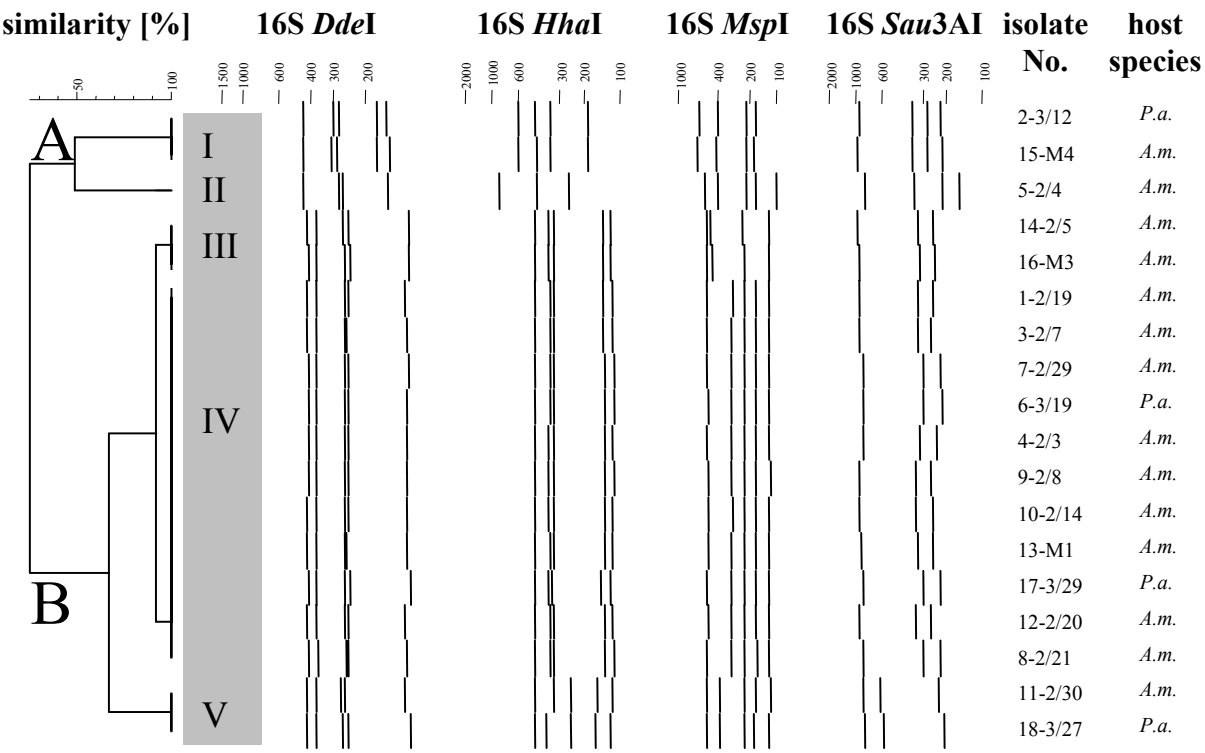


Figure 4.9 Cluster analyses of combined *Dde*I, *Hha*I, *Msp*I and *Sau*3AI restriction patterns of amplified 16S rDNA of all isolates from *Albizia multiflora* (*A.m.*) and *Pterocarpus amazonum* (*P.a.*).

The rep-PCR fingerprinting with the GTG_5 primer revealed a high genotypic diversity. 1 isolate (No. 15-M4) was excluded from this analysis since it did not show a clearly visible fingerprint. All other isolates displayed an individual genomic fingerprint, indicating that each isolate also represents a different strain. The 17 isolates included in the rep-PCR analysis were subdivided into 5 groups at a similarity level ≥ 60% (Figure 4.10). These groupings were highly consistent with those obtained by 16S ARDRA. Isolates with 16S ARDRA genotypes III and IV were all found within the major rep-PCR cluster, except for 16-M3. Isolates 2-3/12 and 5-2/4 had individual rep-PCR patterns that were more related to each other than to those of all the other isolates, which was consistent with their grouping in the 16S ARDRA cluster A.

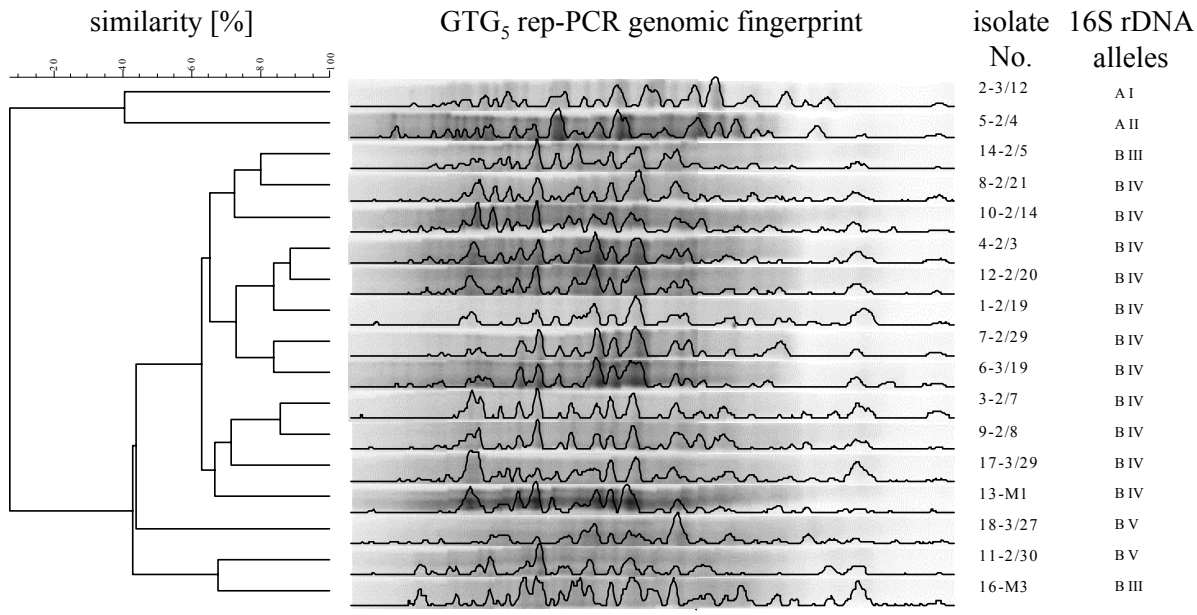


Figure 4.10 Cluster analyses of the GTG_5 rep-PCR fingerprints of all isolates except for 15-M4

In an attempt to classify the environmental isolates at the genus level based on their 16S rDNA PCR-RFLPs, the theoretical restriction patterns of 13 reference strains were calculated for the 4 enzymes used for ARDRA based on their full-length 16S rDNA nucleotide sequence (Table A.3.7). Cluster analyses of these patterns revealed that none of the isolates matched 100% the RFLPs of the reference strains (Figure 4.11). However, the *Albizia multiflora* and *Pterocarpus amazonum* isolates with 16S ARDRA genotypes III, IV and V cluster with the *Mesorhizobium* spp. reference strains. Isolate 5-2/4 clustered with the 2 *Bradyrhizobium japonicum* reference strains. Finally, the environmental isolates 2-3/12 and 15-M4 did not fit within any of the clusters of the reference strains used, and consequently cannot be classified at the genus level.

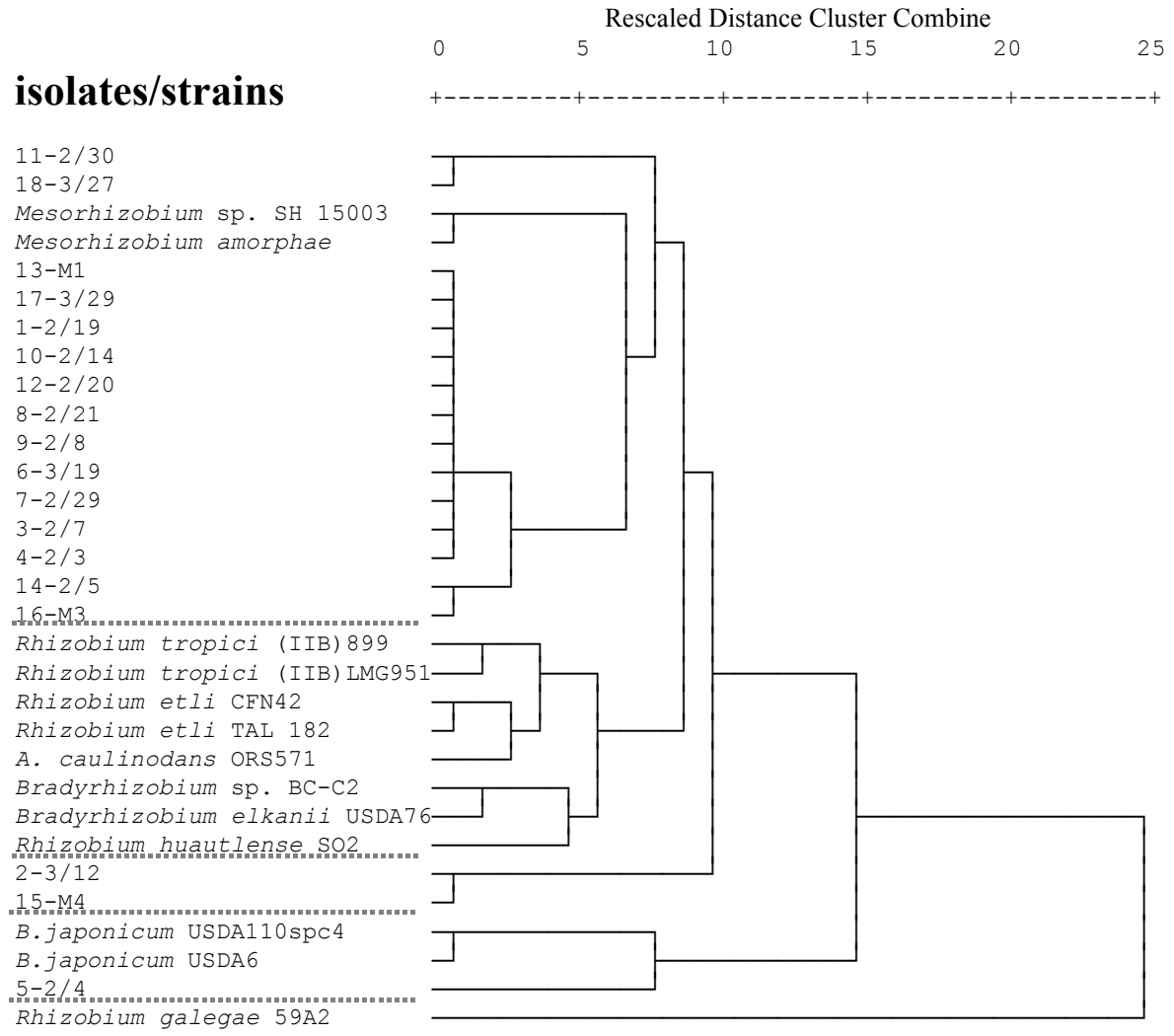


Figure 4.11 Cluster analyses of combined *Dde*I, *Hha*I, *Msp*I and *Sau*3AI restriction patterns of amplified 16S rDNA. Empirical RFLP patterns of all isolates and theoretical RFLP patterns of 13 reference strains were included.

In summary, the genotypic diversity was remarkable, considering the small isolate collection analysed. 5 16S rDNA similarity groups, divided in 2 major clusters, were revealed by analysis of combined RFLP patterns, and a similar diversity was confirmed by rep-PCR fingerprinting. Most isolates were grouped with *Mesorhizobium* spp., 1 with *Bradyrhizobium japonicum*. Genotypic grouping was not correlated with the 2 host species, suggesting a relatively high diversity of possible legume-rhizobia symbioses.

4.4 Non-symbiotic N_2 fixation

Non-symbiotic N_2 fixation was monitored using the acetylene reduction assay (ARA), simultaneously with assessment of soil properties during the hydrological cycle, to identify

the regulating factors with particular focus on the flood pulse. The N gain via non-symbiotic N_2 fixation was estimated to assess its importance for the várzea forest.

4.4.1 N_2 fixation rates and influencing factors

Spatial variation

N_2 fixation measurements at 0-5 cm soil depth during the transition period of receding water (October 1999) and during the terrestrial period (December 1999 and February 2000) were used to investigate correlations with soil factors measured simultaneously: NH_4^+-N and NO_3^--N content, pH, temperature, water content and elevation. Unfortunately, other potential influencing factors such as O_2 concentration, organic matter and total C in soil (GRANHALL, 1981; JAEGER and WERNER, 1981) were not measured simultaneously with N_2 fixation and were therefore not included in this statistical analysis. Significant correlations existed between non-symbiotic N_2 fixation rates and NO_3^--N content ($r = 0.56$; $p<0.05$) as well as with water content ($r = -0.29$; $p<0.05$) and elevation ($r = 0.25$; $p<0.05$). The combination of all measured influencing factors explained 22% of the variability of N_2 fixation, whereas, the degree of explanation was 13% for NO_3^--N content, 5% for water content and 2% for elevation (Figure 4.12).

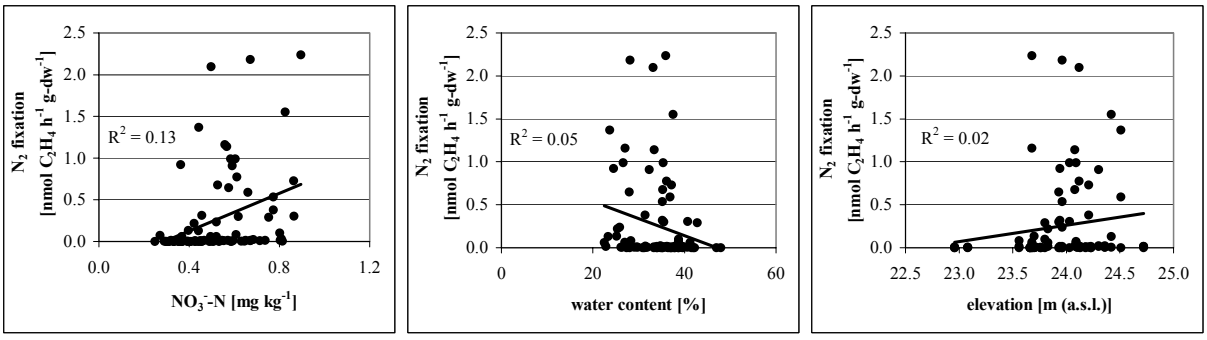


Figure 4.12 Regressions between non-symbiotic N_2 fixation and soil NO_3^--N content (left), soil water content (middle) and elevation (right). The coefficients of explanation (R^2) are given (n=90).

The regression of increasing N_2 fixation with increasing soil NO_3^--N content is remarkable, and might not be based on a causal connection, but rather be due to coincidental similar seasonal trends of both factors. N_2 fixation was significantly higher during the terrestrial period (December 1999 and February 2000) compared to the transition period of receding water in October 1999 (Figure 4.13). The NO_3^--N content was also significantly higher during the terrestrial period (Figure 4.6), when sufficient O_2 availability should have supported

nitrification. Soil water content influences transportation and exchange processes in the soil and thus affects non-symbiotic N_2 fixation. In the study area N_2 fixation increased with decreasing water content. Water content in the soil itself, was mainly influenced by the water level and precipitation (Chapter 4.2.1). Elevation in combination with the water level changes defines time and period of flooding. Higher elevations, and therefore shorter periods of flooding, seem to favour nitrogenase activity of free-living diazotrophs.

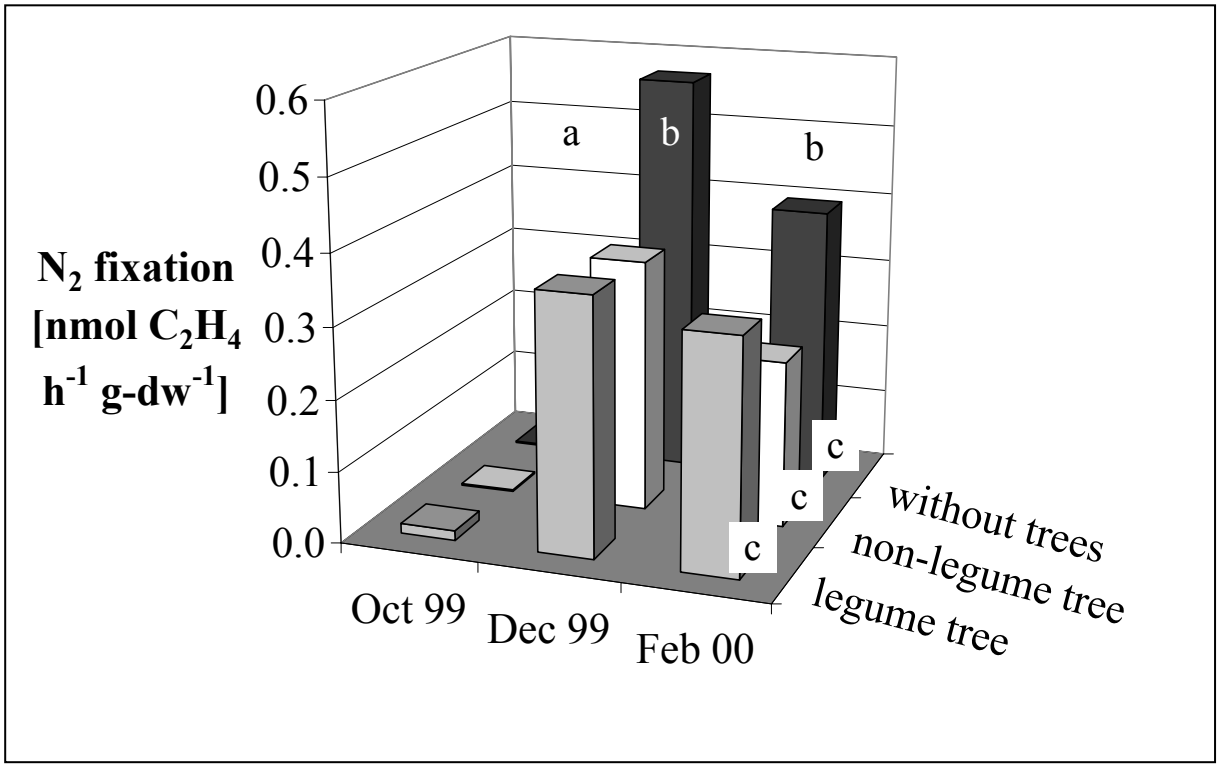


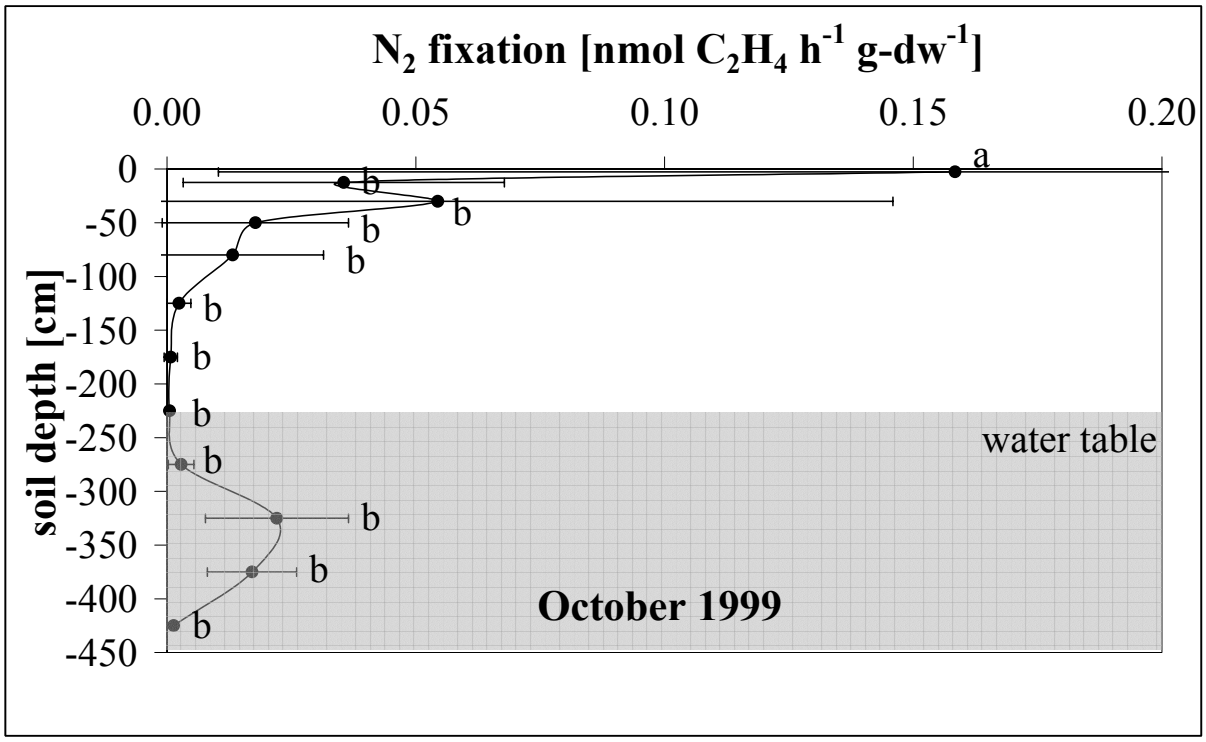
Figure 4.13 N_2 fixation rates during 3 sampling periods at different locations in the surface soil layer (n=10). Monthly rows marked with the same letter are not significantly different at $p<0.05$. Differences between sites, rows indicated with the same letter, are not significant at $p<0.05$.

Whether there is an influence of the vegetation on non-symbiotic N_2 fixation was investigated. Measurements at 3 sampling periods (October 1999, December 1999 and February 2000) showed no significant differences in N_2 fixation in the surface soil layer (0-5 cm) between areas next to legumes, next to non-legumes and locations without trees (Figure 4.13). Therefore, in the floodplain forest there was no effect of the different plant species on relevant soil properties, which would have led to significant differences in N_2 fixation. Apparently, spatial differences in soil properties, for example uneven distribution of organic C exceeded vegetation effects and caused a high variability of N_2 fixation within small areas.

The coefficients of variance for replicate soil samples taken from one site < 4 m² were 3–224%.

Variations in soil profile and litter

Incubations of soil from different depths (0-450 cm) at the beginning of the terrestrial period (October 1999) resulted in average N_2 fixation rates between 0.00 and 0.16 nmol C_2H_4 h^{-1} g-dw^{-1} (Figure 4.14). The maximum rate, which is significantly different from all other values measured in the soil profile ($p<0.05$), occurred in the surface soil layer (0–5 cm). Medium values were measured between 5 and 60 cm, and at 300–400 cm soil depth. Measurements down to 100 cm soil depth at the end of the terrestrial period (February 2000) revealed N_2 fixation rates between 0.00 and 0.06 nmol C_2H_4 h^{-1} g-dw^{-1}, whereas no significant differences occurred between soil layers (Figure 4.14).



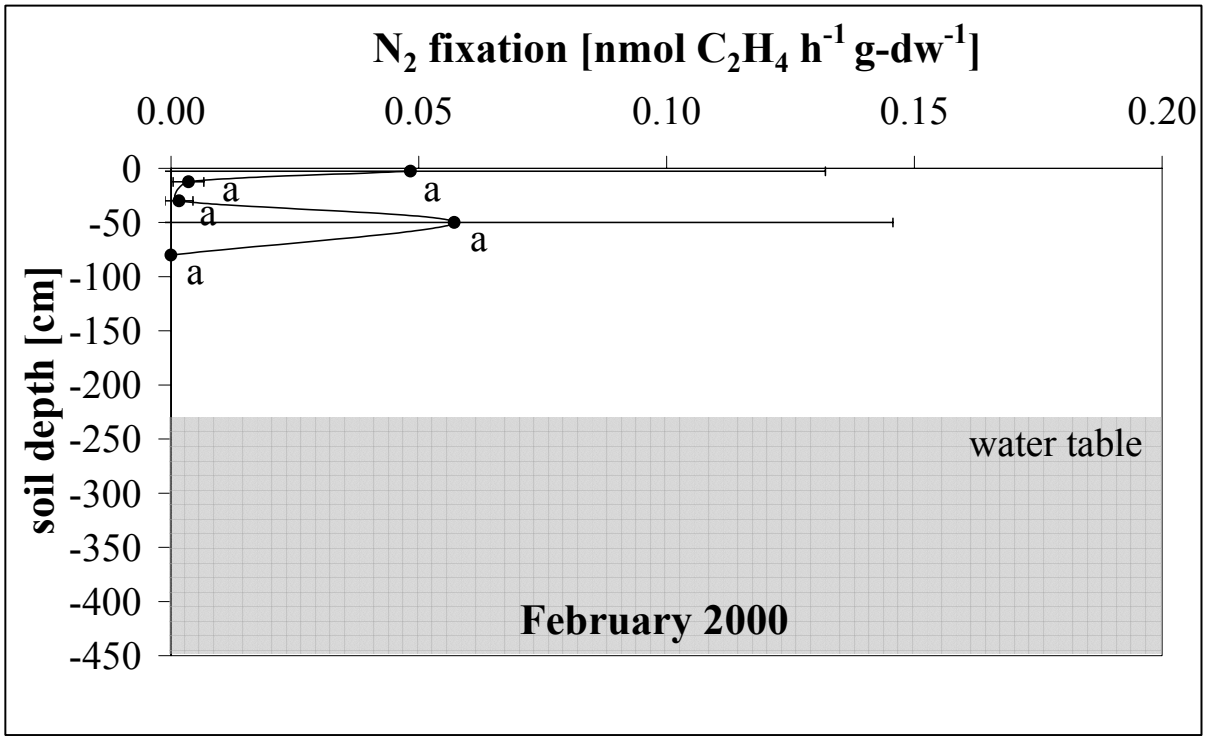


Figure 4.14 N_2 fixation rates measured in the soil profile at the beginning (October 1999) (page 69), and at the end of the terrestrial period (February 2000) (page 70) (n=3; means and standard deviations). The soil below the water table is marked in grey, points followed by the same letter are not significantly different by $p<0.05$.

Incubations of litter during the terrestrial period revealed significantly higher rates of N_2 fixation than incubations of soil ($p<0.05$). Mean N_2 fixation was 1.34 ± 2.50 nmol C_2H_4 h^{-1} g-dw^{-1} in January 1999 (n=9) and 0.24 ± 0.43 nmol C_2H_4 h^{-1} g-dw^{-1} in December 1999 (n=9), with an individual sample maximum of 7.50 nmol C_2H_4 h^{-1} g-dw^{-1} (January 1999). Results differed between the 2 sampling periods with $p=0.1$.

Seasonal variation

The microbiological activity varied seasonally in the floodplain forest soil. Mean N_2 fixation in the surface soil layer (0–5 cm) varied between 0.00 and 0.40 nmol C_2H_4 h^{-1} g-dw^{-1} with an individual sample maximum rate of 5.86 nmol C_2H_4 h^{-1} g-dw^{-1} in October 1998. During the aquatic period N_2 fixation was negligible (Figure 4.15). The same pattern in N_2 fixation was repeated during the next hydrological cycle, when measurements were undertaken during 3 sampling periods. During the transition period of receding water, mean N_2 fixation was negligible and significantly different ($p < 0.05$) from the N_2 fixation during the terrestrial

period in December 1999 (0.43 ± 0.63 nmol C_2H_4 h^{-1} g-dw^{-1}) and February 2000 (0.32 ± 0.52 nmol C_2H_4 h^{-1} g-dw^{-1}) (Figure 4.13).

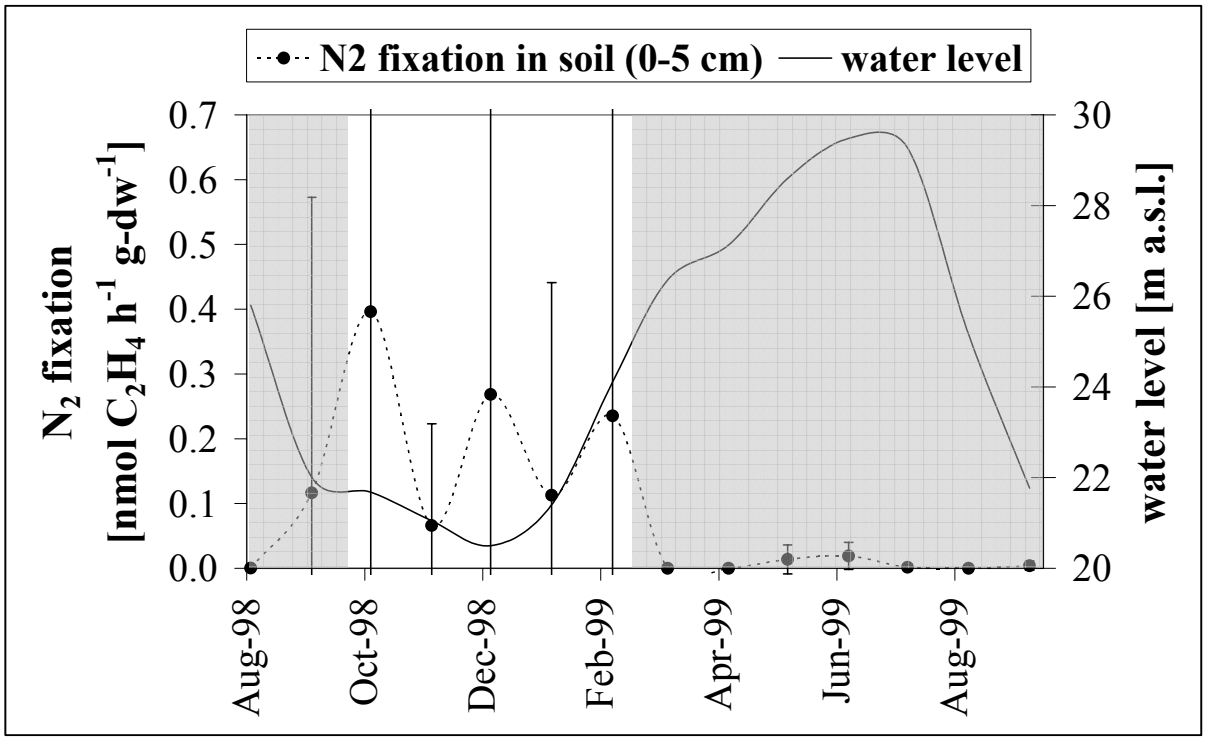


Figure 4.15 N_2 fixation rates in the surface soil layer (0-5 cm) during the hydrological cycle. Monthly measurements were undertaken at different locations in the study area from August 1998 to September 1999, with n=9 when the study area was inundated and n=30 during the terrestrial period (mean and standard deviations).

4.4.2 N gain via non-symbiotic N_2 fixation

N gain via non-symbiotic N_2 fixation in the surface soil layer (0-5 cm) was significantly different between the terrestrial and the aquatic period ($p<0.05$; Figure 4.16). Sum of the mean monthly N gains during the 5 months of terrestrial period was 3.6 kg N ha^{-1}, and the amount of N fixed during the 7 months of aquatic period was only 0.5 kg N ha^{-1}. This is interesting, since also the main biomass production of the várzea forest takes place during the terrestrial period (WORBES, 1985). So, non-symbiotic N_2 fixation could stimulate vegetation growth during this period. The annual N gain in the surface soil layer was 4.1 kg N ha^{-1}.

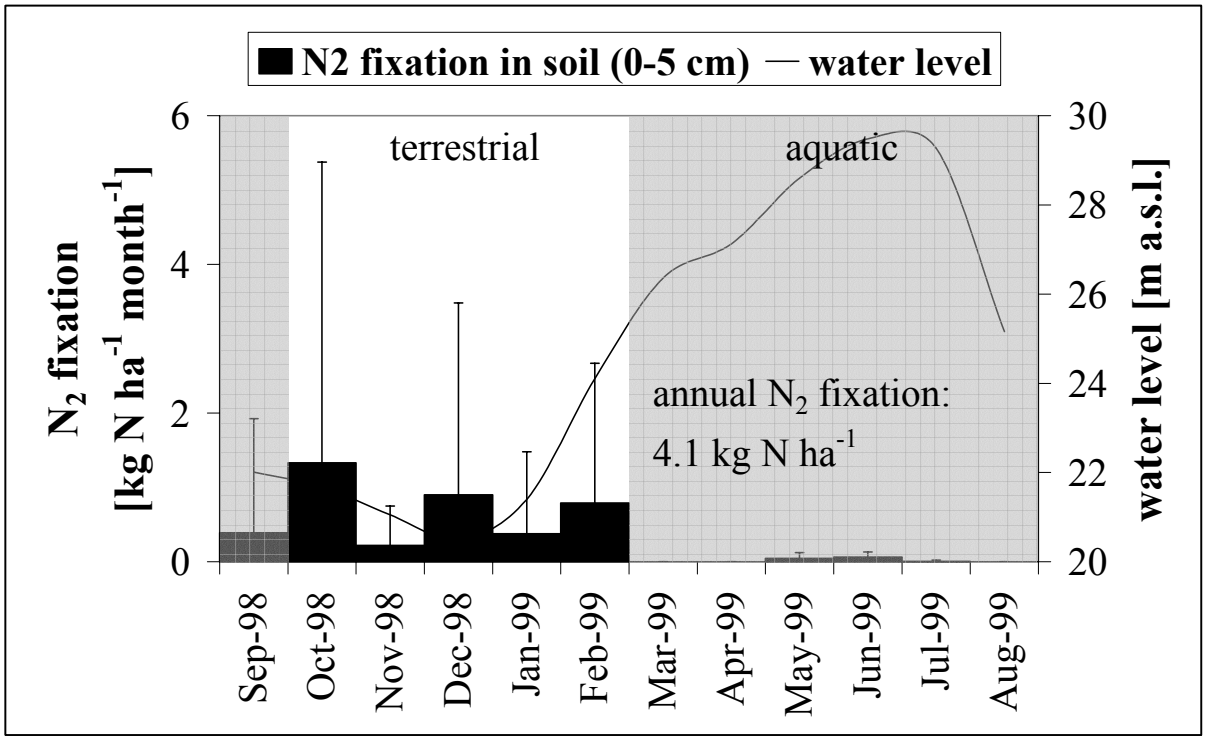


Figure 4.16 Monthly N gain via non-symbiotic N_2 fixation in the surface soil layer (0-5 cm) during 1 year (mean and standard deviation). The aquatic periods are shaded in grey.

Non-symbiotic N_2 fixation was measured at 0-5 cm soil depth throughout the whole hydrological cycle. Therefore, annual N gain can only be calculated for the surface soil layer. This horizon is especially relevant, since the N dynamic is most important in the surface soil layer, where most fine roots exist and the main biological activity occurs (MEYER, 1991). Although maximum N_2 fixation rates were measured in 0-5 cm soil depth, incubations in the soil profile resulted in remarkable N_2 fixation rates also in deeper soil layers (Figure 4.14), so that annual N gain via non-symbiotic N_2 fixation is higher when the whole soil profile is considered. However, seasonal variation of N_2 fixation (Figure 4.15) and environmental factors (Chapter 4.2) during the hydrological cycle make it impossible to extrapolate N_2 fixation in soil below 5 cm depth from 2 measurements during the terrestrial period (Figure 4.14) to 1 year.

Summarising, non-symbiotic N_2 fixation in the soil was influenced by various factors, which itself were strongly influenced by the flood pulse, restricting N_2 fixation in the surface soil layer to the terrestrial period. In the soil profile, maximum N_2 fixation rates occurred in litter, followed by rates at 0-5 cm soil depth. N_2 fixation was also detected in deeper soil layers. Annual N gain in the surface soil layer was 4.1 kg N ha^{-1}.

4.5 Symbiotic N_2 fixation in the forest

The ^{15}N natural abundance method was used to calculate the percentage of N derived from atmosphere (%Ndfa) for the nodulated species in the study area. $\delta^{15}N$ values of plants were investigated simultaneously with potential influencing factors during the hydrological cycle, to identify the regulating factors with particular focus on the flood pulse. The N gain via symbiotic N_2 fixation was estimated to assess its importance for the várzea forest.

4.5.1 $\delta^{15}N$ of water and soil

The mean $\delta^{15}N$ value of rain in the study area was 3.1‰ ± 1.4‰ (n=6), of Rio Solimões it was 3.9‰ ± 0.9‰ (n=5) and of Lago Camaleão it was 3.7‰ ± 0.8‰ (n=8). The $\delta^{15}N$ values of the soil total N pool were all positive, ranging from 3.4‰ to 11.2‰, and the values of the soil extractable N (NH_4^+, NO_3^-) ranged from 1.8‰ to 7.1‰ (Table 4.9).

Table 4.9 $\delta^{15}N$ values and coefficients of variance of the soil total N pool and of the extractable N from soil (NH_4^+, NO_3^-) sampled at different locations in the study area during the terrestrial period (mean ± standard deviations).

	soil total N pool			soil extractable N (NH_4^+, NO_3^-)		
soil depth [cm]	**$\delta^{15}N$ [‰]**	**coef. of variance [%]**	**number of samples**	**$\delta^{15}N$ [‰]**	**coef. of variance [%]**	**number of samples**
litter	4.2 ± 1.0	23	11	3.7 ± 0.2	6	2
0-5	5.4 ± 2.1	40	30	3.5 ± 2.3	66	3
5-20	4.8 ± 2.8	58	31	3.7 ± 1.5	40	3
20-40	5.6 ± 3.6	64	23	2.5 ± 0.8	30	3
40-60	4.6 ± 4.3	94	6	3.5 ± 1.4	40	3
60-100	6.6 ± 6.2	95	10	4.4 ± 1.4	31	3
100-150	6.1 ± 5.9	96	3	3.5	ND	1
150-200	7.7 ± 6.7	87	3	2.4	ND	1
200-250	3.9 ± 0.4	11	3	7.1	ND	1
250-300	3.4 ± 1.3	39	3	1.8	ND	1
300-350	6.6 ± 5.5	82	3	3.5	ND	1
350-400	11.2 ± 12.9	115	2	3.1	ND	1

Variability was high with coefficients of variance between 11% and 115% for the soil total N pool and lower with coefficients of variance between 6% and 66% for the soil extractable N. Although it seemed that the $\delta^{15}N$ values of the soil extractable N were lower compared with the values of the soil total N, no significant differences existed ($p>0.05$). In accordance with previous findings in the Amazon basin (PICCOLO et al., 1994; 1996), $\delta^{15}N$ values seemed to

increase with soil depth, at least for the soil total N pool, differences between soil layers of both measurements were not significant ($p>0.05$). $\delta^{15}N$ values of soil total N showed no significant differences between areas next to legumes, next to non-legumes and locations without trees, and also no significant difference between the terrestrial and the aquatic period. $\delta^{15}N$ values were not significantly correlated with any of the simultaneously determined soil properties (total N, total C, C/N ratio, water content).

4.5.2 $\delta^{15}N$ of the plants

$\delta^{15}N$ values in different plant parts

Due to fractionation processes during N uptake, isotopic ratios differ within one plant. $\delta^{15}N$ values of different plant parts from the same individual trees pair-wise compared revealed significant differences between the $\delta^{15}N$ values of leaves and of woody parts for nearly all investigated legume and non-legume species (Table A.4.3). Also, differences in $\delta^{15}N$ values between twigs and stems, and between roots and nodules were predominantly significant. Stems, roots, branches and twigs show the lowest $\delta^{15}N$ values, followed by leaves (Figure 4.17). $\delta^{15}N$ values of fruits and blossoms were in the same range as the values of leaves, and no significant differences existed. Highest $\delta^{15}N$ values were measured in nodules. The general pattern of $\delta^{15}N$ values in leaves and woody parts are the same in nodulated and non-nodulated plants (Figure 4.17). Therefore, it is adequate to use the isotopic ratios of the leaves for %Ndfa calculations (SHEARER and KOHL, 1988b).

Additionally, $\delta^{15}N$ in roots had the highest intraspecific coefficients of variance, the lowest were detected in fruits, blossoms and nodules, followed by leaves, which supports the selection of leaves for %Ndfa calculations. There was no difference in the pattern of N content between nodulated and non-nodulated plants. Leaves (blossoms and nodules) had always the highest and stems the lowest N content.

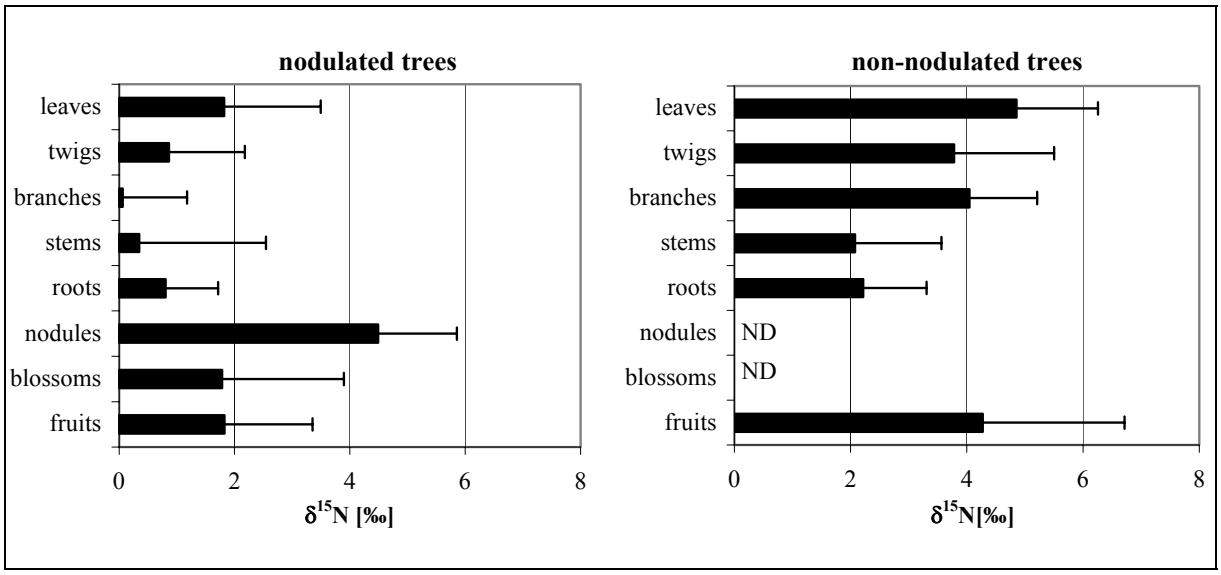


Figure 4.17 Mean $\delta^{15}N$ values in different plant parts of nodulated (4 species) and non-nodulated (7 species) trees (mean and standard deviation). Values of the individual species are given in Table A.4.3

$\delta^{15}N$ values in different leaf types

$\delta^{15}N$ values of different leaf types from the same individual trees pair-wise compared revealed only few significant differences, which were mainly due to the leaf age (Table A.4.4). For *Pterocarpus amazonum* and *Nectandra amazonum* the medium sun leaves differed significantly from the young, not yet completely developed, sun leaves, and for *Campsiandra comosa* and *Albizia multiflora* the age induced difference was significant within the shade leaves. Generally, the young leaves of nodulated and non-nodulated trees had higher $\delta^{15}N$ values by comparison with the medium ones (Table A.4.4). This is consistent with results from the secondary vegetation in the eastern Amazon region, where new leaves had about 0.5‰ units higher $\delta^{15}N$ values than old leaves (THIELEN-KLINGE, 1997). Correspondingly, also the N contents of the leaf types showed significant differences with the leaf ages. Young leaves had the highest N contents, followed by medium sun, medium shade and submerged leaves. The old sun leaves had the lowest N contents.

The danger of invalid determination of the $\delta^{15}N$ value of the species due to slight differences in sample composition was small because there were mainly insignificant differences between the leaf types. The only exception might have been during the flushing of new leaves, when only young leaves were available or were predominant.

Overview of $\delta^{15}N$ values

The mean $\delta^{15}N$ values of leaves of 22 leguminous species and 11 non-leguminous species, including plants of all growth habits from aquatic plants to trees, varied over a range of 8 $\delta^{15}N$ units from -1.4‰ to 6.8‰ (Figure 4.18 and Table A.4.5). More than half of the species showed clear positive $\delta^{15}N$ values, only 2 species had clear negative values, and the others had values about ±1‰ around 0. Growth habit and leaf periodicity of the legumes and non-legumes had no significant influence on their isotopic ratios, which is in contrast to findings of YONEYAMA et al. (1990b). The $\delta^{15}N$ values of the legumes ranged from -1.4‰ to 5.1‰ and the values of the non-legumes from 3.2‰ to 6.8‰, means were 1.4‰ and 4.6‰, respectively. The range of $\delta^{15}N$ values of the non-nodulated species was about the same as the mean difference between the non-nodulated and the nodulated species. Although $\delta^{15}N$ values of both groups were overlapping, their $\delta^{15}N$ values and N contents were significantly different by $p<0.05$. Mean N content in leaves of the legume plants was 3.3%, and of the non-legumes it was 2.5%. There was a slight negative correlation between $\delta^{15}N$ values and N content ($r=-0.21$; $p=0.002$).

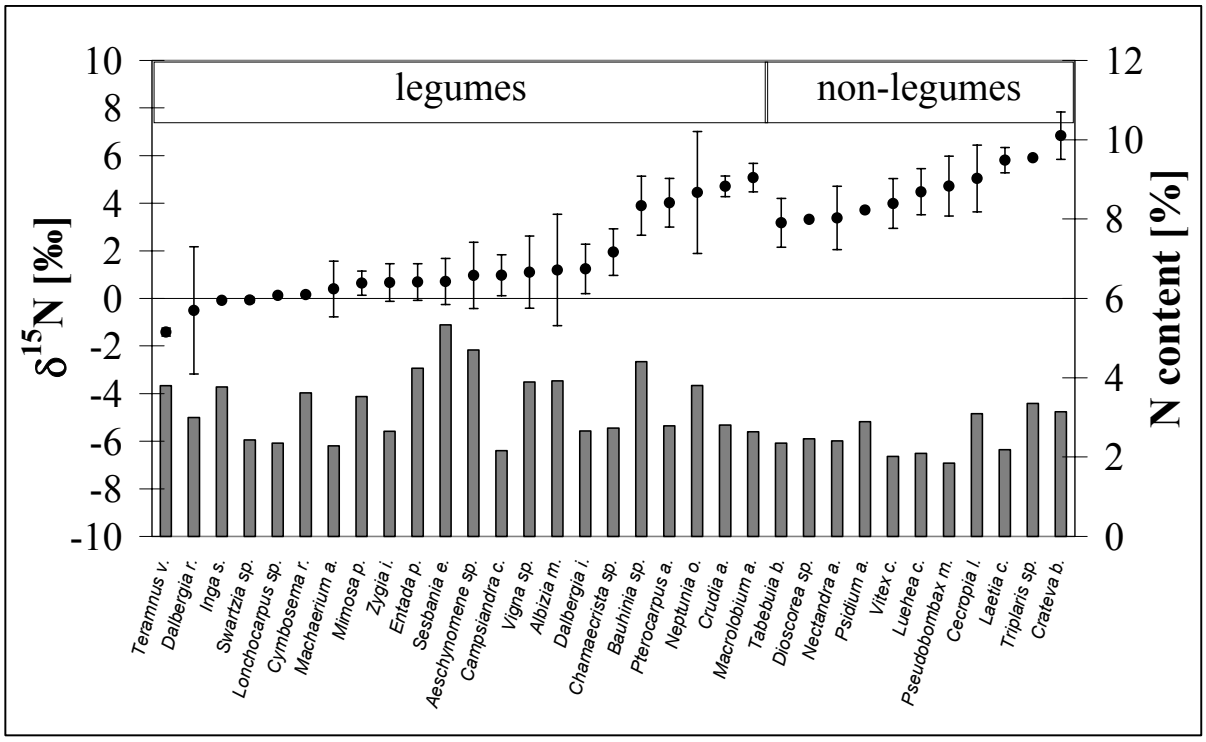


Figure 4.18 Overview of the $\delta^{15}N$ values (points) and the mean N contents (columns) of all investigated woody and non-woody species, organised according to legumes/non-legumes and increasing $\delta^{15}N$ values. Means and standard deviations of the $\delta^{15}N$ values are shown.

13 species had intermediate $\delta^{15}N$ values in the intersection of legumes and non-legumes. All non-legumes showed significant differences in their $\delta^{15}N$ values by comparison with the nodulated legumes. As expected, the $\delta^{15}N$ values of the 3 non-nodulated legumes *Bauhinia* sp., *Crudia amazonica* and *Macrolobium acaciifolium* were in the same range as the values of the non-legumes. Most nodulated legume species had average $\delta^{15}N$ values below 2‰, which were significantly lower than the values of the non-legumes. However, the $\delta^{15}N$ values of the nodulated legumes *Neptunia oleracea* and *Pterocarpus amazonum* were not significantly different from the values of the non-nodulated plants. On *Pterocarpus amazonum* and *Neptunia oleracea* nodules were actually observed in the study area, and at least for the nodules of *Pterocarpus amazonum* it was confirmed that they did actually fix N_2 (Table 4.10). This indicates, that despite nodulation, the percentage of their plant N derived from atmosphere via symbiotic N_2 fixation was negligible.

N_2 fixation of nodules from *Zygia inaequalis*, *Campsiandra comosa*, *Albizia multiflora* and *Pterocarpus amazonum* was measured on 10 root samples per species. Mean N_2 fixation was between 5 and 15 nmol C_2H_4 h^{-1} mg^{-1} nodule fresh weight (Table 4.10). Intraspecific variation in N_2 fixation was larger than the variation between the 4 species (interspecific coefficient of variance: 49%). Therefore, it was not surprising, that the nodule N_2 fixation rates were not significantly correlated with the $\delta^{15}N$ values of the species.

Table 4.10 N_2 fixation of nodules collected from 4 legume species in the study area was measured with the ARA (n=10; mean ± standard deviation)

	N_2 fixation [nmol C_2H_4 h^{-1} mg^{-1}]	**coefficient of variance [%]**	**number of nodules**
Campsiandra comosa	14.8 ± 12.9	87	168
Pterocarpus amazonum	13.7 ± 9.5	69	52
Albizia multiflora	6.9 ± 6.3	92	622
Zygia inaequalis	5.0 ± 2.8	57	588

Intraspecific variation

$\delta^{15}N$ values of different individuals of one species varied considerably, with intraspecific coefficients of variance between 9% and 531%. The coefficients of variance were negatively correlated with the mean $\delta^{15}N$ values of the species (r=-0.38; p=0.041). Thus, non-nodulated plants had relatively small coefficients of variance, all below 40% (Table A.4.5). The nodulated woody climbers *Dalbergia riparia* and *Machaerium aristulatum* had the highest variance with coefficients over 200%. But, despite the significant trend that the higher the

$\delta^{15}N$ values the lower the coefficients of variance, there was no significant difference in intraspecific variation between nodulated and non-nodulated species. The variability between individuals of one species may be due to site effects, plant conditions and differences in N_2 fixation. But correlations between the $\delta^{15}N$ values and elevation of growing site, leaf N content and basal area were weak. For legume species, $\delta^{15}N$ values were mainly negatively correlated with leaf N contents, and $\delta^{15}N$ values seemed to increase with increasing basal area. For *Pterocarpus amazonum,* $\delta^{15}N$ values increased significantly with increasing elevation, but this trend was not found for the other investigated species.

Half of the investigated species showed significant differences in $\delta^{15}N$ values and N contents between seedlings and trees (Figure 4.19). No consistent trend in $\delta^{15}N$ values existed between seedlings and trees. Most species had lower $\delta^{15}N$ values in seedlings by comparison with trees, but 1 nodulated and 1 non-nodulated species showed the opposite trend. For all investigated species, the leaf N content was larger for seedlings by comparison with trees.

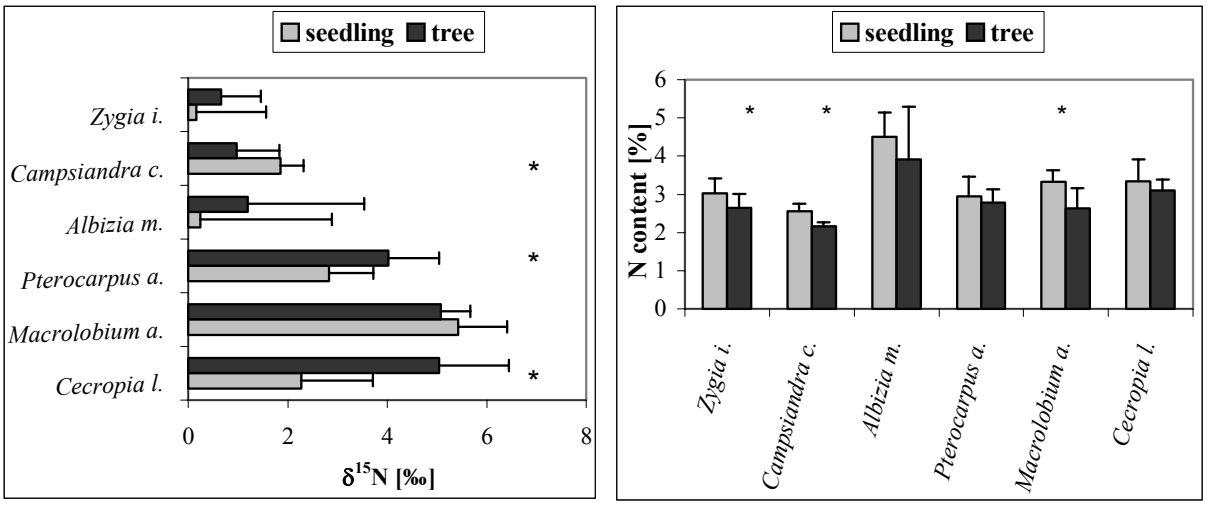


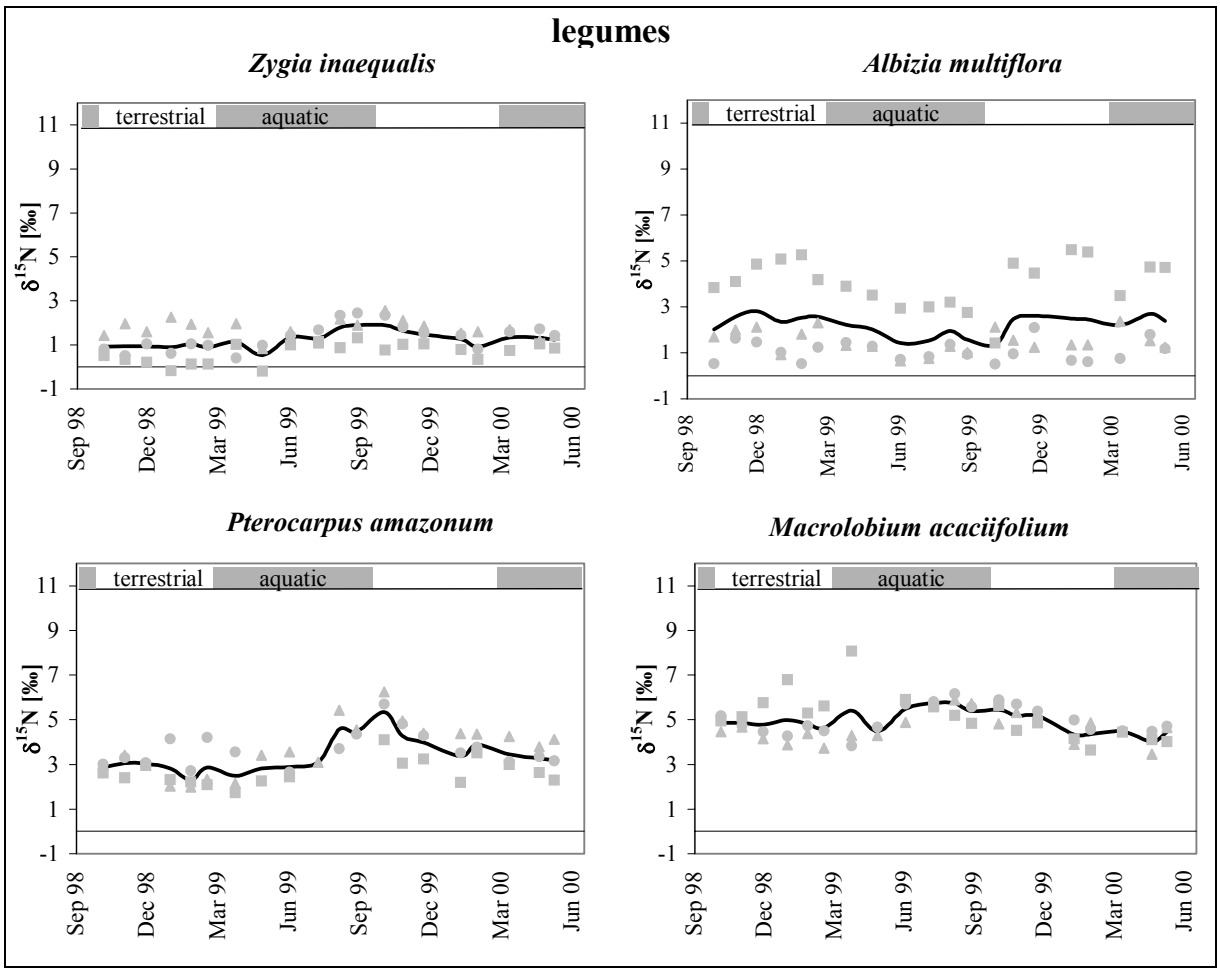
Figure 4.19 $\delta^{15}N$ values and N contents of seedlings and trees of 6 investigated species (n=10; mean and standard deviation). Significant differences at $p<0.05$ are marked with a star

Seasonal variation

Despite drastic environmental changes between the terrestrial and aquatic periods, significant differences in the $\delta^{15}N$ values or N contents of 16 investigated legume and non-legume species were scarce (Table A.4.6). Most species had slightly higher $\delta^{15}N$ values during the aquatic period by comparison with the terrestrial period, but only for 2 nodulated (*Zygia inaequalis, Machaerium aristulatum*) and 1 non-nodulated legume (*Macrolobium*

acaciifolium) was this difference significant. 10 species showed higher leaf N contents during the aquatic period in comparison with the terrestrial period (Table A.4.6). For most legume and non-legume species, the intraspecific variation of their $\delta^{15}N$ values in leaves was smaller during the aquatic period than during the terrestrial period (Table A.4.6), indicating a reduced variability of influencing factors between sites under waterlogged conditions. Concerning the seasonal pattern in $\delta^{15}N$ values and N contents, there was no significant difference between nodulated and non-nodulated plants.

More detailed investigations with monthly samplings during nearly 2 hydrological cycles confirmed that most species showed no significant seasonal variation in their $\delta^{15}N$ values (Figure 4.20).



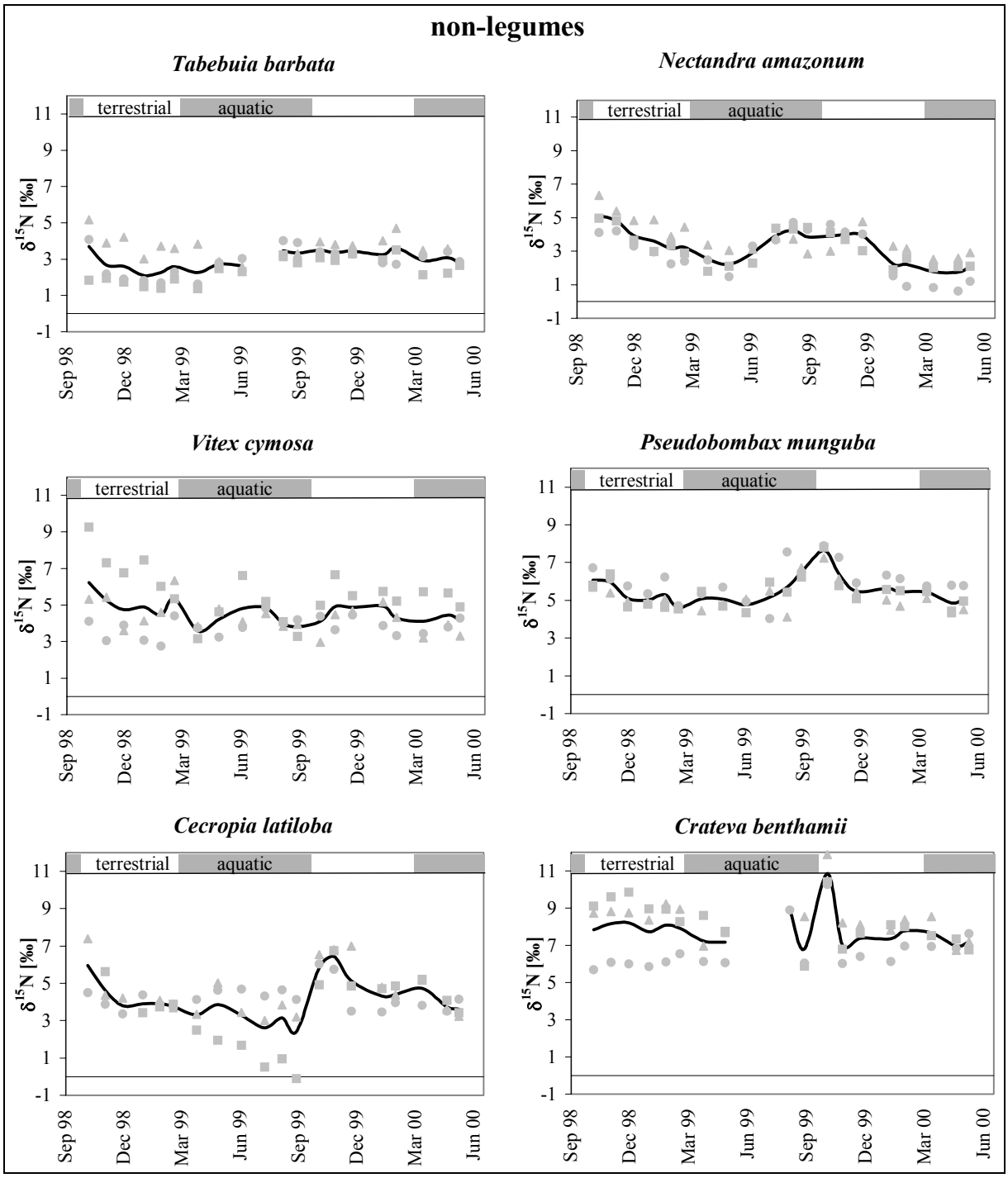


Figure 4.20 Seasonal variation of the $\delta^{15}N$ values of legume and non-legume trees over the period of 20 months. The black line represents the average value of 3 individual trees whose individual results are shown as grey dots. The line is interrupted and no values are shown during the time when the trees had no leaves

From the 10 investigated species, only 1 legume (*Pterocarpus amazonum*) and 3 non-legumes (*Nectandra amazonum, Pseudobombax munguba, Cecropia latiloba*) showed clear seasonal trends. The $\delta^{15}N$ values of *Pterocarpus amazonum, Nectandra amazonum* and *Pseudobombax*

munguba at the end of the aquatic period/beginning of the terrestrial period were significantly higher than the values during the rest of the hydrological cycle. The $\delta^{15}N$ values of *Cecropia latiloba* at the beginning of the terrestrial period were significantly higher than the values in all other periods. Correspondingly, significantly higher N contents in leaves at the end of the aquatic period/beginning of the terrestrial period in comparison with the rest of the hydrological cycle existed for the majority of the species. Since mainly non-nodulated plants showed significant seasonal differences it is proposed that these changes in the isotopic ratios were not induced by changes in symbiotic N_2 fixation.

4.5.3 Reference plants

To determine the $\delta^{15}N$ value of the N taken up from the soil by the nodulated legumes in the study area, reference plants are used. Ideally the non-N_2 fixing species would obtain N from the same N source in the same proportion with the same temporal and spatial pattern as the N_2 fixing plants (HÖGBERG, 1997), and have the same physiology and metabolism as the nodulated legumes. Since this is impossible to predict, suitable and highly similar reference species were selected, and a mixture of reference species which grow in proximity to the target legumes were used as well.

Selection of reference species

The following criteria for the selection of the most suitable reference plant were used: small intraspecific variation in $\delta^{15}N$ values of the reference species, high dissimilarity in $\delta^{15}N$ with those of the legumes, high similarity in seasonal $\delta^{15}N$ pattern with the legumes, high similarity in $\delta^{15}N$ pattern between the parts of one plant, high physiological similarity to the legumes (Table 4.11). All non-nodulated species of which the $\delta^{15}N$ values were determined, were taken into consideration, although it was impossible to apply all selection criteria to all species.

Table 4.11 Suitability as reference species according to the selection criteria (** = very suitable; * = suitable; ND = not determined, criteria was not applied to the species)

non-nodulated species	small intraspecific variation in $\delta^{15}N$ values[1)]	high $\delta^{15}N$ difference to nodulated plants[2)]	similarity in seasonal $\delta^{15}N$ pattern[3)]	similarity in intraplant isotopic fractio-nation[4)]	physiologic similarity[5)]
Bauhinia sp.			ND	ND	ND
Crudia amazonica	**	*	ND	ND	**
Macrolobium acaciifolium	**	*	*	*	**
Tabebuia barbata			**		*
Dioscorea sp.	ND		ND	ND	ND
Nectandra amazonum					*
Psidium acutangulum	ND		ND	ND	
Vitex cymosa			**	*	*
Luehea cymulosa	*		ND		ND
Pseudobombax munguba		*	*	*	
Cecropia latiloba		*		*	*
Laetia corombulosa	**	**	ND	ND	*
Triplaris sp.	ND	**	ND	ND	
Crateva benthamii	**	**	*		**

1) The species was considered as very suitable with regard to a small intraspecific variation if the $\delta^{15}N$ coefficient of variance is ≤10% and as suitable if it is ≤20% (Table A.4.5).
2) The higher the $\delta^{15}N$ value of the non-nodulated species, the higher the difference to the $\delta^{15}N$ values of the nodulated species. Therefore, the species was considered as very suitable if its $\delta^{15}N$ value was 6-7‰, and as suitable if it was 5‰ (Table A.4.5).
3) The similarity of the 10 species which were investigated for their seasonal variation in $\delta^{15}N$ values (Figure 4.20) was determined by hierarchical cluster analysis. The species which were grouped directly next to nodulated legumes were considered as very suitable, the species which were in one cluster with nodulated legumes were considered as suitable (Figure A.4.1).
4) The similarity of the 11 species which were investigated for their plant internal $\delta^{15}N$ pattern (Table A.4.3) was determined by hierarchical cluster analysis with the ranking of $\delta^{15}N$ values in roots, stems, branches and leaves as variables. The species which were in one cluster with nodulated legumes, were considered as suitable (Figure A.4.2).
5) The similarity of 15 species (for which sufficient data was available) was determined by hierarchical cluster analysis with standardised values (z-values) of family (legume or non-legume), growing habit, basal area, wood density, fruiting season, leaf periodicity and N content in leaves. The species which were grouped directly next to nodulated legumes were considered as very suitable, the species which were in one cluster with nodulated legumes were considered as suitable (Figure A.4.3).

According to these selection criteria, the non-legume *Crateva benthamii* and the non-nodulated legume *Macrolobium acaciifolium* were the most suitable reference species. Therefore, their mean $\delta^{15}N$ values of 6.8‰ and 5.1‰, respectively, were used as reference values.

Mixture of reference species

Since all plants from which samples were taken are growing relatively close together in a forest area of 15 ha, simply all non-nodulated species can be included as reference species. The mean $\delta^{15}N$ value of all non-nodulated species investigated was 4.6‰, which was also used as reference value.

Since it is possible to compensate for regular local variability within the study area in selecting reference plants in proximity to the N_2 fixing legumes (SHEARER and KOHL, 1988b), another approach was to select single trees of nodulated species and collect samples of non-nodulated species growing around it to a maximum of 10 m away (Table 4.12).

Table 4.12 Reference groups of 3 to 5 non-nodulated trees referring to one nodulated legume. The $\delta^{15}N$ values of the legume and the non-nodulated trees as well as the average $\delta^{15}N$ value of the single groups (reference value) are given.

nodulated legume and its $\delta^{15}N$ value	**species and $\delta^{15}N$ value of the nearest non-nodulated trees**	**ref. value**
Zygia inaequalis 1 1.9‰	*Crateva benthamii* 7.1‰, *Crateva benthamii* 6.8‰, *Crateva benthamii* 7.2‰, *Tabebuia barbata* 1.9‰, *Vitex cymosa* 3.8‰	5.4‰
Zygia inaequalis 2 1.5‰	*Crateva benthamii* 7.1‰, *Crateva benthamii* 6.3‰, *Macrolobium acaciifolium* 4.7‰, *Vitex cymosa* 3.2‰, *Vitex cymosa* 4.5‰	5.2‰
Zygia inaequalis 3 1.1‰	*Crateva benthamii* 5.2‰, *Crateva benthamii* 6.6‰, *Pseudobombax munguba* 2.6‰, *Tabebuia barbata* 2.2‰, *Vitex cymosa* 3.3‰	4.0‰
Zygia inaequalis 4 0.7‰	*Crateva benthamii* 5.7‰, *Crateva benthamii* 7.4‰, *Macrolobium acaciifolium* 5.1‰, *Nectandra amazonum* 3.8‰, *Vitex cymosa* 3.3‰	5.1‰
Zygia inaequalis 5 0.0‰	*Crateva benthamii* 6.7‰, *Crateva benthamii* 5.9‰, *Crateva benthamii* 4.7‰, *Macrolobium acaciifolium* 4.9‰, *Vitex cymosa* 2.6‰	5.0‰
Zygia inaequalis 6 -0.8‰	*Crateva benthamii* 7.2‰, *Crateva benthamii* 7.9‰, *Macrolobium acaciifolium* 4.5‰, *Tabebuia barbata* 2.3‰, *Vitex cymosa* 3.5‰	5.1‰
Zygia inaequalis 7 0.1‰	*Crateva benthamii* 5.2‰, *Crateva benthamii* 6.6‰, *Pseudobombax munguba* 2.6‰, *Tabebuia barbata* 2.2‰, *Vitex cymosa* 3.3‰	4.0‰
Zygia inaequalis 8 1.3‰	*Crateva benthamii* 5.2‰, *Crateva benthamii* 6.6‰, *Pseudobombax munguba* 2.6‰, *Tabebuia barbata* 2.2‰, *Vitex cymosa* 3.3‰	4.0‰
Zygia inaequalis 9 0.6‰	*Crateva benthamii* 5.2‰, *Crateva benthamii* 6.6‰, *Pseudobombax munguba* 2.6‰, *Tabebuia barbata* 2.2‰, *Vitex cymosa* 3.3‰	4.0‰
Zygia inaequalis 10 0.3‰	*Crateva benthamii* 5.7‰, *Crateva benthamii* 7.3‰, *Crateva benthamii* 5.3‰, *Tabebuia barbata* 5.2‰, *Vitex cymosa* 6.6‰	6.0‰
Albizia multiflora 1 1.2‰	*Crateva benthamii* 8.5‰, *Crateva benthamii* 7.1‰, *Nectandra amazonum* 1.3‰, *Vitex cymosa* 4.5‰, *Vitex cymosa* 4.1‰	5.1‰

nodulated legume and its $\delta^{15}N$ value	species and $\delta^{15}N$ value of the nearest non-nodulated trees	ref. value
Albizia multiflora 2 2.4‰	*Crateva benthamii* 6.2‰, *Crateva benthamii* 5.3‰, *Crateva benthamii* 4.6‰, *Vitex cymosa* 4.5‰, *Vitex cymosa* 4.4‰	5.0‰
Albizia multiflora 3 4.5‰	*Crateva benthamii* 7.0‰, *Crateva benthamii* 7.2‰, *Crateva benthamii* 6.8‰, *Vitex cymosa* 4.3‰, *Vitex cymosa* 3.4‰	5.7‰
Albizia multiflora 4 6.6‰	*Crateva benthamii* 7.5‰, *Crateva benthamii* 6.9‰, *Pseudobombax munguba* 6.0‰, *Vitex cymosa* 6.0‰	6.6‰
Albizia multiflora 5 2.8‰	*Crateva benthamii* 5.4‰, *Crateva benthamii* 6.5‰, *Crateva benthamii* 6.9‰, *Vitex cymosa* 3.5‰, *Vitex cymosa* 2.9‰	5.0‰
Albizia multiflora 6 -0.4‰	*Crateva benthamii* 7.1‰, *Crateva benthamii* 6.8‰, *Crateva benthamii* 7.2‰, *Tabebuia barbata* 1.9‰, *Vitex cymosa* 3.8‰	5.4‰
Albizia multiflora 7 0.1‰	*Crateva benthamii* 7.1‰, *Crateva benthamii* 6.3‰, *Macrolobium acaciifolium* 4.7‰, *Vitex cymosa* 3.2‰, *Vitex cymosa* 4.5‰	5.2‰
Albizia multiflora 8 2.5‰	*Crateva benthamii* 6.7‰, *Crateva benthamii* 7.2‰, *Macrolobium acaciifolium* 4.7‰, *Tabebuia barbata* 2.8‰, *Vitex cymosa* 5.0‰	5.3‰
Albizia multiflora 9 3.8‰	*Crateva benthamii* 6.6‰, *Crateva benthamii* 8.0‰, *Pseudobombax munguba* 6.4‰	7.0‰
Albizia multiflora 10 -0.5‰	*Crateva benthamii* 5.2‰, *Crateva benthamii* 6.6‰, *Pseudobombax munguba* 2.6‰, *Tabebuia barbata* 2.2‰, *Vitex cymosa* 3.3‰	4.0‰
Pterocarpus amazonum 1 4.4	*Crateva benthamii* 6.7‰, *Crateva benthamii* 6.3‰, *Vitex cymosa* 3.7‰, *Vitex cymosa* 3.1‰	5.0‰
Pterocarpus amazonum 2 4.3	*Crateva benthamii* 7.8‰, *Macrolobium acaciifolium* 4.7‰, *Pseudobombax munguba* 4.2‰, *Vitex cymosa* 3.6‰, *Vitex cymosa* 4.1‰	4.9‰
Pterocarpus amazonum 3 3.3	*Crateva benthamii* 7.1‰, *Crateva benthamii* 6.3‰, *Macrolobium acaciifolium* 4.7‰, *Vitex cymosa* 3.2‰, *Vitex cymosa* 4.5‰	5.2‰
Pterocarpus amazonum 4 4.3	*Crateva benthamii* 5.7‰, *Crateva benthamii* 7.4‰, *Macrolobium acaciifolium* 5.1‰, *Nectandra amazonum* 3.8‰, *Vitex cymosa* 3.3‰	5.1‰
Pterocarpus amazonum 5 3.0	*Crateva benthamii* 7.1‰, *Crateva benthamii* 6.8‰, *Crateva benthamii* 7.2‰, *Tabebuia barbata* 1.9‰, *Vitex cymosa* 3.8‰	5.4‰
Pterocarpus amazonum 6 2.7	*Crateva benthamii* 7.1‰, *Crateva benthamii* 6.3‰, *Macrolobium acaciifolium* 4.7‰, *Vitex cymosa* 3.2‰, *Vitex cymosa* 4.5‰	5.2‰
Pterocarpus amazonum 7 3.9	*Crateva benthamii* 7.1‰, *Crateva benthamii* 10.4‰, *Pseudobombax munguba* 3.7‰	7.1‰
Pterocarpus amazonum 8 4.9	*Crateva benthamii* 5.3‰, *Crateva benthamii* 6.3‰, *Macrolobium acaciifolium* 6.6‰, *Tabebuia barbata* 3.8‰, *Vitex cymosa* 3.7‰	5.1‰
Pterocarpus amazonum 9 4.9	*Crateva benthamii* 5.7‰, *Crateva benthamii* 7.3‰, *Crateva benthamii* 5.3‰, *Tabebuia barbata* 5.2‰, *Vitex cymosa* 6.6‰	6.0‰
Pterocarpus amazonum 10 5.9	*Crateva benthamii* 5.7‰, *Crateva benthamii* 7.3‰, *Crateva benthamii* 5.3‰, *Tabebuia barbata* 5.2‰, *Vitex cymosa* 6.6‰	6.0‰

Since these tree groups were growing close together all should be influenced by the same site effects. 10 individual trees of each *Zygia inaequalis*, *Albizia multiflora* and *Pterocarpus amazonum* were marked and small tree reference groups formed. Since *Crateva benthamii* and *Vitex cymosa* are the most abundant species in the study area, they were also most often found

next to the target legume trees. The resulting single reference values for each target legume are listed in Table 4.12.

4.5.4. $\delta^{15}N$ of N_2 fixing plants grown without soil N

Growth of plants

The legume seedlings of 5 species grown in N free substrate for 6 months had a relatively high mortality of 47%. From the surviving plants, average amount of leaves per plant decreased from 3-5 leaves at the beginning to 2-3 leaves at the end of the experiment. However, this means that at least 2-3 new leaves per plant have been grown, and increase in height was observed as well (Figure 4.21), thus most seedlings accumulated N during the experiment. At the end of the experiment, the seedlings had a total biomass between 0.23 and 1.26 g-dw, of which 49-73% was stem, 19-42% were roots and 8-22% leaves (Table A.4.7). The leaves contained on average 3.4% N, the roots and stems 1.7% and 1.8% N, respectively. Therefore, the plants contained on average between 3.8 mg and 19.3 mg N in total. N compounds (total N, NH_4^+, NO_3^-) in the substrate sampled at the end of the experiment were below the limits of detection. Apparently, despite nodulation, the plants suffered due to the lack of N, because all other nutrients were supplied sufficiently (Table A.3.8).

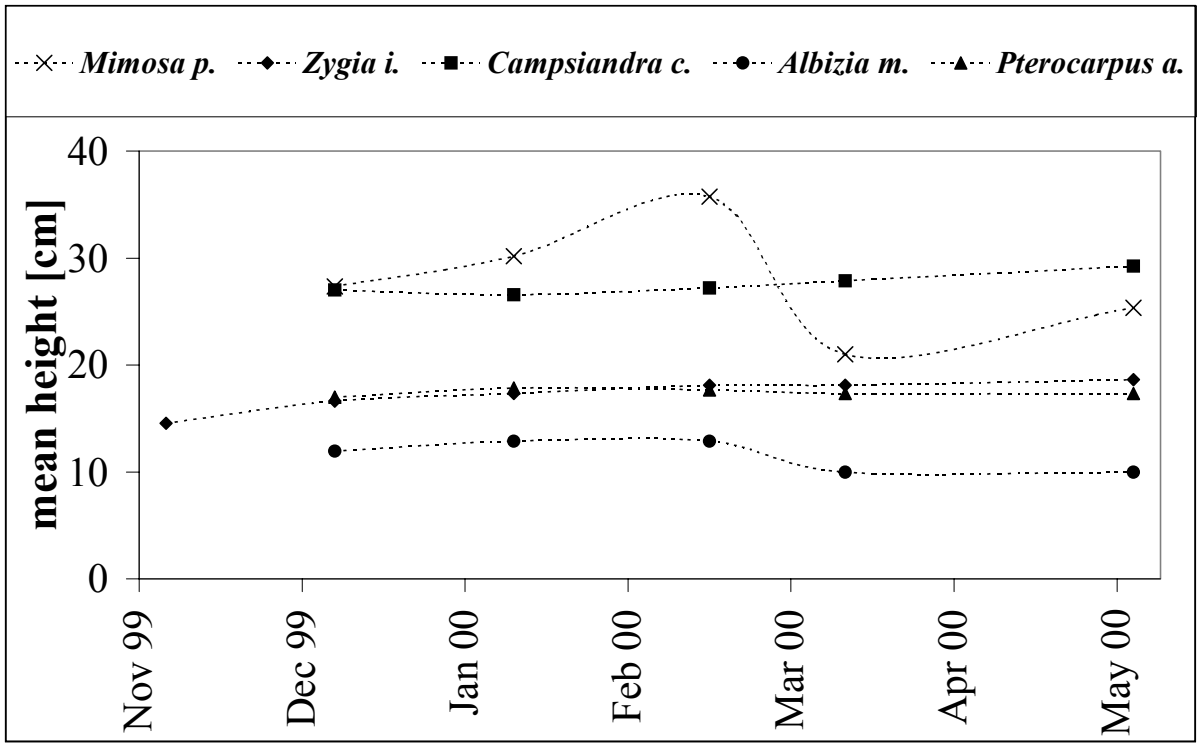


Figure 4.21 Growth pattern of the nodulated legumes grown without soil N over the whole run of the experiment. Mean heights of the species are shown, numbers of plants were between 3 and 11 (Table A.4.7).

$\delta^{15}N$ values of plants

The $\delta^{15}N$ values of the leaves decreased from a mean value of -0.7‰ in December 1999 to -4.9‰ at the end of the experiment in May 2000 (Figure 4.22). All species showed the similar trend of decreasing $\delta^{15}N$ units at the beginning of the experiment, and a more or less levelling out of the $\delta^{15}N$ value from January 2000 onwards when most likely the leaf N content was derived entirely from N_2 fixation. Differences in absolute $\delta^{15}N$ values between the species were high, and at the end of the experiment the mean values ranged over nearly 9‰ units. *Pterocarpus amazonum* and *Campsiandra comosa* had the highest $\delta^{15}N$ values in leaves of -0.1‰ and -1.0‰, respectively. *Albizia multiflora* and *Mimosa pigra* had the lowest values of -8.7‰ and -8.6‰, respectively (Figure 4.22). Most plants did not show significant differences between the plant parts, the others (*Campsiandra comosa, Pterocarpus amazonum)* showed a similar pattern as the one found for the trees in the forest, with lowest $\delta^{15}N$ values in stems, followed by roots, and highest $\delta^{15}N$ values in leaves (Table A.4.7.). Therefore, the result of this experiment is a mean leaf $\delta^{15}N$ value of -4.9‰ for plants grown without soil N, characterising the isotopic ratio of biologically fixed N from atmosphere.

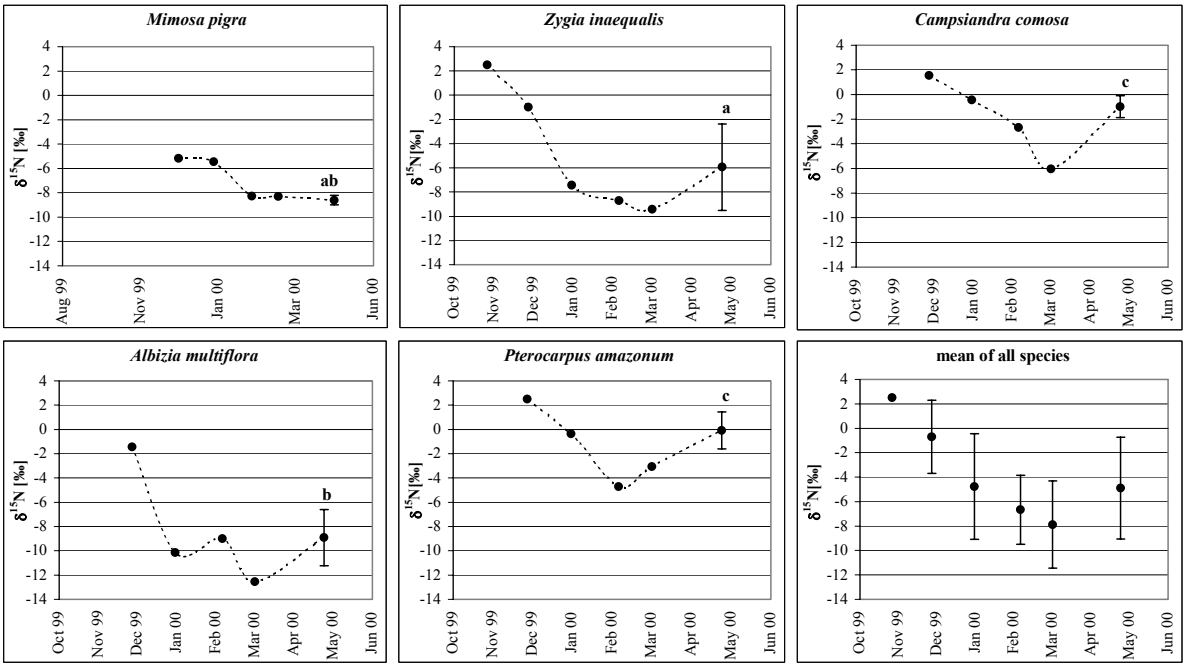


Figure 4.22 Mean leaf $\delta^{15}N$ values of nodulated species grown without soil N over the run of the experiment. During the experiment, all leaves of 1 species were mixed as 1 sample. Only at the end, the single plant values were determined, their standard deviation is shown. Points marked with the same letter are not significantly different by $p<0.05$. The mean $\delta^{15}N$ value and standard deviation of all species grown without soil N during the run of the experiment is shown as well.

4.5.5 %Ndfa and N gain of N_2 fixing plants

Calculations of the percentage of N derived from atmosphere for all woody and non-woody nodulated legumes applying the different reference values resulted in %Ndfa values between 2% and 70% (Table 4.13). The different reference values lead to high variations in %Ndfa between 7% and 19% units per species. Naturally, the species with $\delta^{15}N$ values close to the values of the non-nodulated legumes were most influenced by the calculation method.

Table 4.13 %Ndfa of nodulated species in the study area, including plants of all growth habits from aquatic plant to trees, calculated according to different approaches and with 4 reference values in order to show minimum and maximum scenarios. The mean $\delta^{15}N$ value of *Macrolobium acaciifolium* was 5.1‰ and of *Cecropia latiloba* it was 6.8‰. All non-nodulated species had a mean $\delta^{15}N$ value of 4.6‰ and the $\delta^{15}N$ value of the reference groups are listed in Table 4.12.

		calculation approaches with			
		single reference species		**mixture of ref. species**	
		legume (***Macrolobium acaciifolium***)	**non-legume** (***Crateva benthamii***)	**all non-nod. species**	**reference groups***
nodulated legume species	**$\delta^{15}N$ [‰]**	**%Ndfa [%]**	**%Ndfa [%]**	**%Ndfa [%]**	**%Ndfa [%]**
Teramnus volubilis	-1.4 ± 0.2	65	70	63	ND
Dalbergia riparia	-0.5 ± 2.7	56	62	54	ND
Inga splendens	-0.1	52	59	49	ND
Swartzia sp.	-0.1 ± 0.1	52	59	49	ND
Lonchocarpus sp.	0.1 ± 0.1	50	57	47	ND
Cymbosema roseum	0.2	49	57	47	ND
Machaerium aristulatum	0.4 ± 1.2	47	55	44	ND
Mimosa pigra	0.6 ± 0.5	45	53	42	ND
Zygia inaequalis	0.7 ± 0.8	44	52	41	42
Entada polyphylla	0.7 ± 0.8	44	52	41	ND
Sesbania exasperata	0.7 ± 1.0	44	52	41	ND
Aeschynomene sp.	1.0 ± 1.4	41	50	38	ND
Campsiandra comosa	1.0 ± 0.9	41	50	38	ND
Vigna sp.	1.1 ± 1.5	40	49	37	ND
Albizia multiflora	1.2 ± 2.3	39	48	36	31
Dalbergia inundata	1.2 ± 1.0	39	48	35	ND
Chamaecrista sp.	1.9 ± 1.0	32	42	28	ND
Pterocarpus amazonum	4.0 ± 1.0	11	24	6	13
Neptunia oleracea	4.5 ± 2.6	6	20	2	ND

* %Ndfa of the single target legumes were calculated with their specific reference values as shown in Table 4.12, then the mean for each species was calculated.

Combining the %Ndfa results (Table 4.13) with the results from the stand structure analyses (Table 4.2), the average percentage of plant N derived from atmosphere for the várzea vegetation was calculated. But, since legume species with low frequency were not found in the "stand structure analyses plots", and since herbs and other small plants were *per se* not included in the stand structure analyses, it was only possible to consider about half of the legume species for this calculation. This limitation is unfortunate, but acceptable, since the excluded species do not contribute a substantial amount to the biomass production of the forest due to their low number and small size. No samples for isotopic analyses were taken

from the nodulated legume tree *Acosmium nitens*, so it could not be included in the calculation. For the non-nodulated species the %Ndfa was set zero, and for the nodulated legumes minimum and maximum %Ndfa values were taken to calculate the 2 possible extremes. Depending on the important value index of the species, the weighted average %Ndfa for the forest was calculated (Table 4.14). This weighting appeared to be the most suitable, since it considers frequency, density and dominance of the species. Relative density alone would probably overestimate the productivity of small plants, whereas relative dominance, would probably far underestimate the relatively high productivity of lianas (GENTRY, 1983; PUTZ, 1983). Since biomass is generally not a reliable indicator of productivity (UNESCO et al., 1978) the estimated species fraction of total biomass was also rejected as a measure.

Table 4.14 Important value index (IVI) and %Ndfa (minimum and maximum value) for legume and non-legume species. From this data the weighted average %Ndfa of the várzea forest was calculated.

woody plants	**IVI**	**%Ndfa (min) [%]**	**%Ndfa (max) [%]**
Dalbergia riparia	10	54	62
Lonchocarpus sp.	1	47	57
Machaerium aristulatum	2	44	55
Mimosa pigra	2	42	53
Zygia inaequalis	1	41	52
Campsiandra comosa	1	38	50
Albizia multiflora	8	31	48
Dalbergia inundata	1	35	48
Pterocarpus amazonum	5	6	24
Crudia amazonica	1	0	0
Macrolobium acaciifolium	9	0	0
non-legumes	259	0	0
weighted average*		4	5

$$* \text{ weighted average} = \frac{\sum_{i=1}^{12} \%\text{Ndfa}_i * \text{IVI}_i}{\sum_{i=1}^{12} \text{IVI}_i}$$

The minimum and maximum weighted averages of the várzea forest were 4% and 5% Ndfa respectively. This means that between 4% and 5% of plant N of the forest in the study area derived from symbiotic N_2 fixation.

For the net biomass production of the várzea forest (19.6 t ha^{-1} y^{-1} of leaves, wood and roots) on average 322.7 kg N ha^{-1} y^{-1} are used according to FURCH (1997, 1999) who considered for this estimation the results of several studies undertaken on Ilha de Marchantaria (ADIS et al., 1979; KLINGE et al., 1983; FURCH and KLINGE, 1989; MEYER, 1991). Although these productivity measurements were not undertaken in exactly the same study area, it is plausible to take these numbers as a basis for the N gain estimation, since studies were undertaken in similar várzea forest areas on the same island. Therefore, since 4% to 5% of the total plant N of the forest derived from symbiotic N_2 fixation, the N gain via nodulated legumes was between 12.9 and 16.1 kg N ha^{-1} y^{-1}. Clearly, this estimation has its shortcomings due to average productivity and %Ndfa values, but since productivity measurements of the plants under study were not undertaken, it seems to be the most accurate possibility to estimate the N gain via symbiotic N_2 fixation for the study area.

Summarising, the flood pulse seemed to have no influence on symbiotic N_2 fixation, since no seasonal variation in $\delta^{15}N$ values, which would suggest differences in N_2 fixation rates between terrestrial and aquatic period, was found. The nodulated legumes with *Neptinia oleracea* on the one side and *Teramnus volubilis* on the other, fixed between 2% and 70% of their plant N from atmosphere, respectively. Altogether, between 4% and 5% of plant N of the várzea forest derived from symbiotic N_2 fixation, which resulted in an N gain between 12.9 and 16.1 kg N ha^{-1} y^{-1}.

4.6 Symbiotic N_2 fixation under experimental conditions

A selection of 2 nodulated and 2 non-nodulated species from the study area were treated with ^{15}N labelling in a pot experiment, to compare the results obtained with the isotope dilution method with results of the ^{15}N natural abundance method.

4.6.1 $\delta^{15}N$ of the soil

$\delta^{15}N$ values of the soil total N pool of the ^{15}N labelled treatment decreased significantly during the experiment from 22.3‰ in November 1999 to 5.2‰ and 6.2‰ in March and May 2000, respectively (Figure 4.23). This trend was due to a constant loss of ^{15}N after the labelling with $(^{15}NH_4)_2SO_4$ at the start of the experiment in August 1999. For the unlabelled treatment $\delta^{15}N$ values of the soil total N pool varied between 3.2‰ and 4.8‰. Despite the

strong decrease of $\delta^{15}N$ values of the ^{15}N labelled treatment, $\delta^{15}N$ values of the ^{15}N labelled treatment were significantly higher than the $\delta^{15}N$ values of the unlabelled treatment at all sampling times. Soil N content was 0.8 ± 0.4 g kg^{-1}.

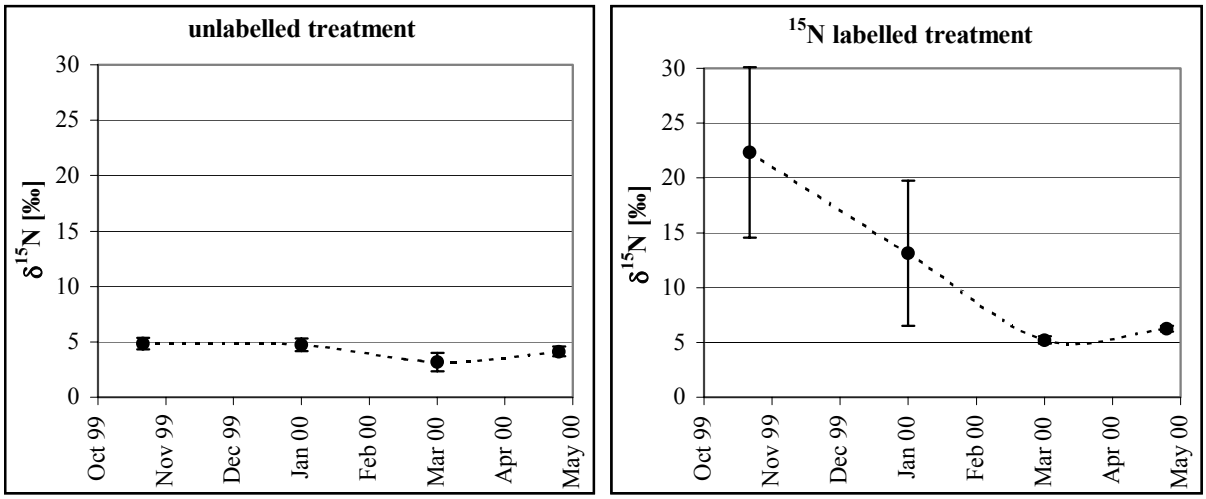


Figure 4.23 $\delta^{15}N$ values of the soil total N pool of the unlabelled (left) and the ^{15}N labelled (right) treatment (n=4; mean and standard deviation).

4.6.2 $\delta^{15}N$ values of the plants grown under experimental conditions

Growth of plants

The seedlings had a mortality of 26.5%, so that from the 5 to 8 plants per pot (2 of *Cecropia latiloba*), only 3 to 7 per pot (0-2 of *Cecropia latiloba*) were left for harvest at the end of the experiment. The plants grew on average between 5 and 18 cm during the 8 months of the isotope dilution experiment. *Cecropia latiloba* plants showed the largest increase and *Campsiandra comosa* plants the smallest (Figure 4.24). The main growth period was between October 1999 and December 1999, when also most leaves were grown, on average between 2 and 3 new ones per plant. Afterwards, average leaf growth per plant was reduced to 0-1 leaf per month which led to a reduction in leaf number per plant from 4-8 in October 1999 to 2-6 in May 2000. For *Macrolobium acaciifolium* and *Cecropia latiloba* this growth pattern and ageing of leaves was reflected by a significant decrease of leaf N content during the last months of the experiment. At the end of the experiment, on all *Albizia multiflora* and most *Campsiandra comosa* plants nodules were found.

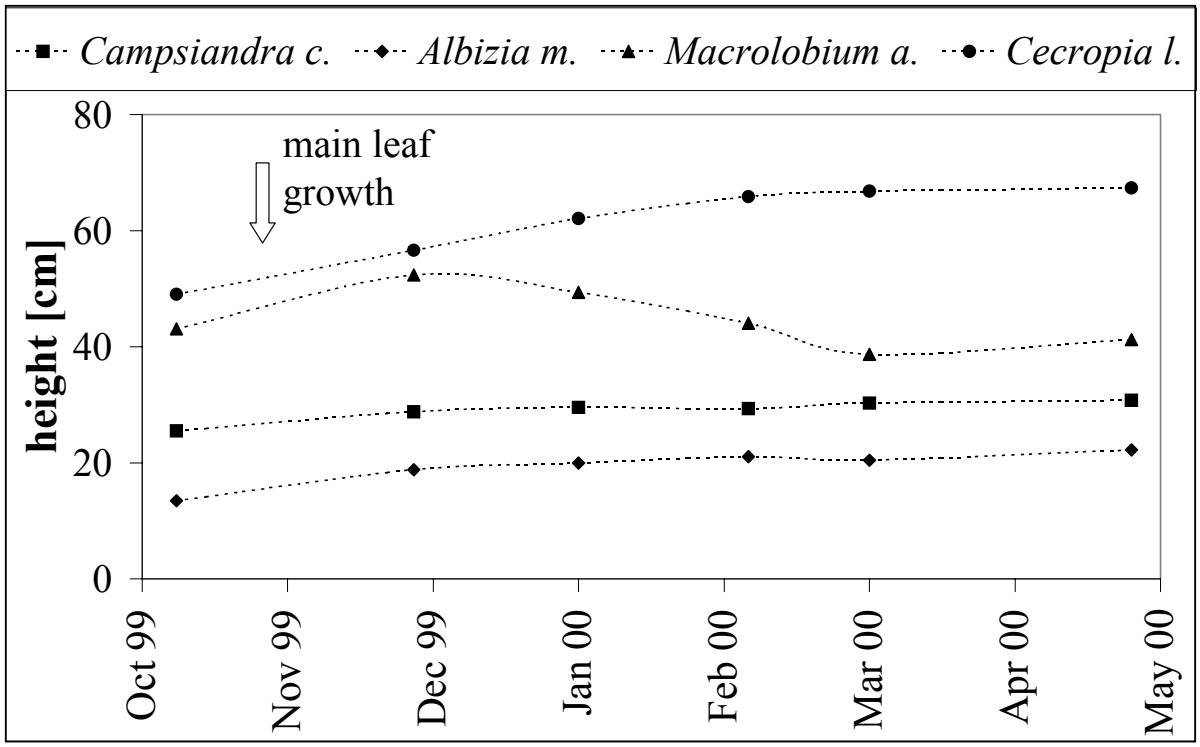


Figure 4.24 Growth pattern of the plants during the isotope dilution experiment. The average height per species irrespective of the treatment is shown.

The plants did not show significant differences between treatments in height, number or N content of leaves at any time, except for *Macrolobium acaciifolium*. From February 2000 onwards, plants of *Macrolobium acaciifolium* in the unlabelled pots were significantly smaller and in December 1999 and January 2000 their leaf N contents were significantly lower in comparison with the plants in the ^{15}N labelled pots.

At the end of the experiment, the plants had a total biomass between 1.56 and 20.23 g-dw, of which 58-76% was stem, 15-34% roots and 5-21% leaves (Table A.4.8). *Cecropia latiloba* were the largest plants and *Albizia multiflora* the smallest. *Campsiandra comosa* and *Cecropia latiloba* had relatively high fractions of leaf biomass in comparison with *Albizia multiflora* and *Macrolobium acaciifolium* whose leaf biomass was below 10% of their total biomass. The leaves of *Albizia multiflora* had the highest N contents, *Campsiandra comosa* the lowest. For all species the N contents in roots and stems were 46% to 69% lower in comparison with the leaf N contents. Therefore, the plants had accumulated during their lifetime, on average between 26.1 mg and 228.2 mg N in total, strongly dependent on their size (Table A.4.8).

$\delta^{15}N$ values of plants

Significant temporal variation or differences between species in leaf $\delta^{15}N$ values were rare also due to high variation especially between the plants in the ^{15}N labelled pots, but trends were visible (Figure 4.25). In the unlabelled treatment, *Campsiandra comosa* and *Albizia multiflora* showed a significant increase in their leaf $\delta^{15}N$ values after December 1999. Also, whereas $\delta^{15}N$ values of the nodulated legumes were significantly lower than the values of the non-nodulated species in the first months of the experiment, by March and May 2000 no significant differences between species existed any more. Indicating N_2 fixation by the nodulated legumes, especially at the beginning of the experiment, which decreased with time. The $\delta^{15}N$ values of *Macrolobium acaciifolium* and *Cecropia latiloba* in the unlabelled treatment were on a constant level during the whole run of the experiment. Although a slight decrease of leaf $\delta^{15}N$ values was visible for most species (except *Albizia multiflora*) in the ^{15}N labelled pots, no significant temporal variation existed (Figure 4.25), which is astonishing considering the strong decrease of soil $\delta^{15}N$ values in this treatment. No significant differences between species existed, although the low $\delta^{15}N$ values of *Albizia multiflora* may suggest N_2 fixation.

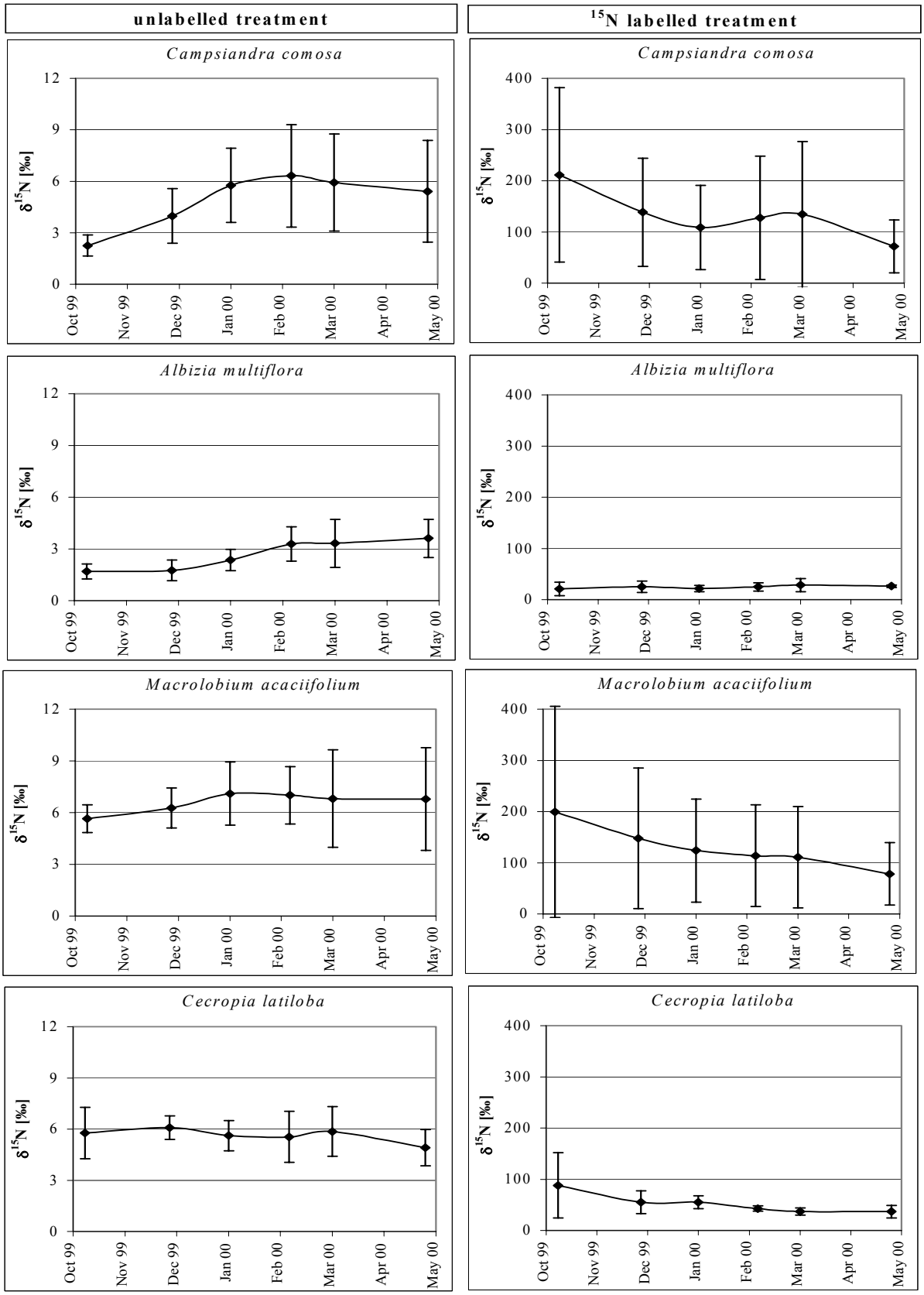


Figure 4.25 Temporal variation of the leaf $\delta^{15}N$ values of all species and treatments of the isotope dilution experiment (n=4 for the unlabelled treatment, n=3 for the ^{15}N labelled treatment; mean and standard deviation).

The isotopic ratios in the different plant parts were in the same range for most species and treatments (Table A.4.8) and did not show any consistent pattern. Significant differences in $\delta^{15}N$ values between leaves and roots/stems existed only for ^{15}N labelled *Campsiandra comosa* and unlabelled *Albizia multiflora*. Therefore, differences between the leaf $\delta^{15}N$ values and the calculated values for the whole plants were negligible.

$\delta^{15}N$ values of all plant parts of *Campsiandra comosa* were not significantly lower than the values of the non-nodulated species, which indicates negligible N_2 fixation of *Campsiandra comosa* at least at the end of the experiment. $\delta^{15}N$ values of *Albizia multiflora* indicated N_2 fixation during the whole run of the experiment. At the end of the experiment, in the unlabelled treatment, *Albizia multiflora* had the lowest $\delta^{15}N$ values in all plant parts, differences were significant for stem and root $\delta^{15}N$ values (Table A.4.8). In the ^{15}N labelled treatment no significant differences between nodulated and non-nodulated species existed, and again, *Albizia multiflora* had the lowest $\delta^{15}N$ values in leaves and stems (Table A.4.8).

4.6.3 %Ndfa and the amount of fixed N per nodulated legume

Percentage of N derived from atmosphere calculated for *Campsiandra comosa* resulted in decreasing %Ndfa values in the unlabelled treatment; but in the ^{15}N labelled treatment values stayed more or less on the same low level (Figure 4.26). In October 1999 %Ndfa of *Campsiandra comosa* was between 0% and 33%, in May 2000 it was between 0% and 12% depending on the calculation approach. In contrast to *Albizia multiflora*, the unlabelled treatment resulted in higher %Ndfa values of *Campsiandra comosa* by comparison with the ^{15}N labelled treatment. But generally, all calculation approaches resulted in low %Ndfa values of *Campsiandra comosa* with temporal means between 0% and 15% (Table A.4.8). It was estimated, that about 1 year old *Campsiandra comosa* plants fixed between 0 and 10.2 mg N during their lifetime, depending on their biomass and the calculation approach for %Ndfa.

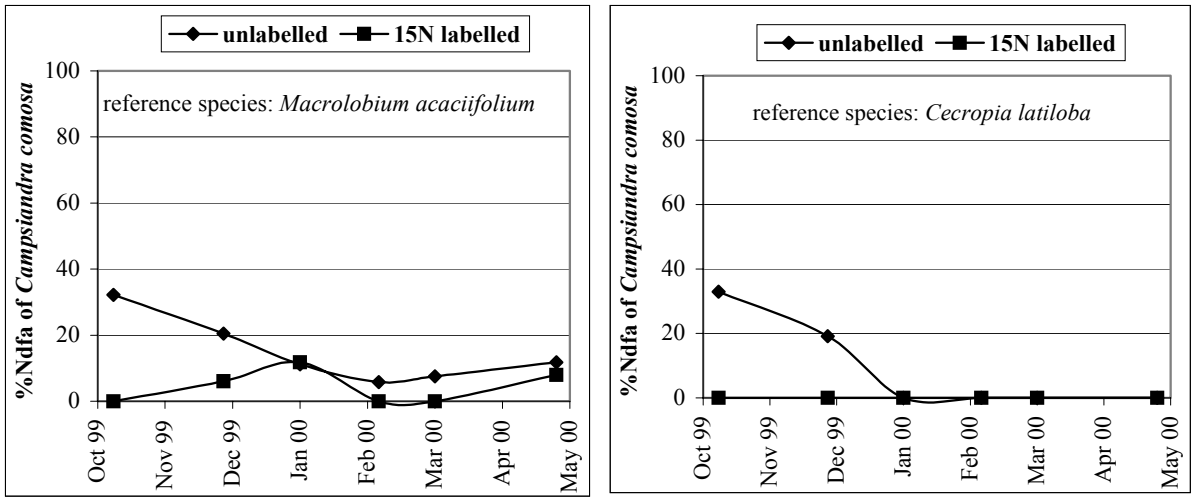


Figure 4.26 %Ndfa of *Campsiandra comosa* over the run of the experiment, calculated for both treatments and with *Macrolobium acaciifolium* (left) and *Cecropia latiloba* (right) as reference species.

Calculations of the percentage of N derived from atmosphere resulted in continuously decreasing %Ndfa values of *Albizia multiflora* in both treatments, and with *Macrolobium acaciifolium* and *Cecropia latiloba* as reference plants (Figure 4.27). In October 1999 %Ndfa of *Albizia multiflora* was between 38% and 87%, and in May 2000 it was only between 13% and 63% depending on the calculation approach. The ^{15}N labelled treatment and *Macrolobium acaciifolium* as reference species resulted in higher %Ndfa values than the unlabelled treatment and *Cecropia latiloba* as reference species. Temporal means of %Ndfa resulted in values between 28% and 76% for *Albizia multiflora* (Table A.4.8). It was estimated that about 1 year old *Albizia multiflora* plants fixed between 8.1 and 19.8 mg N during their lifetime, depending on their biomass and the calculation approach for %Ndfa.

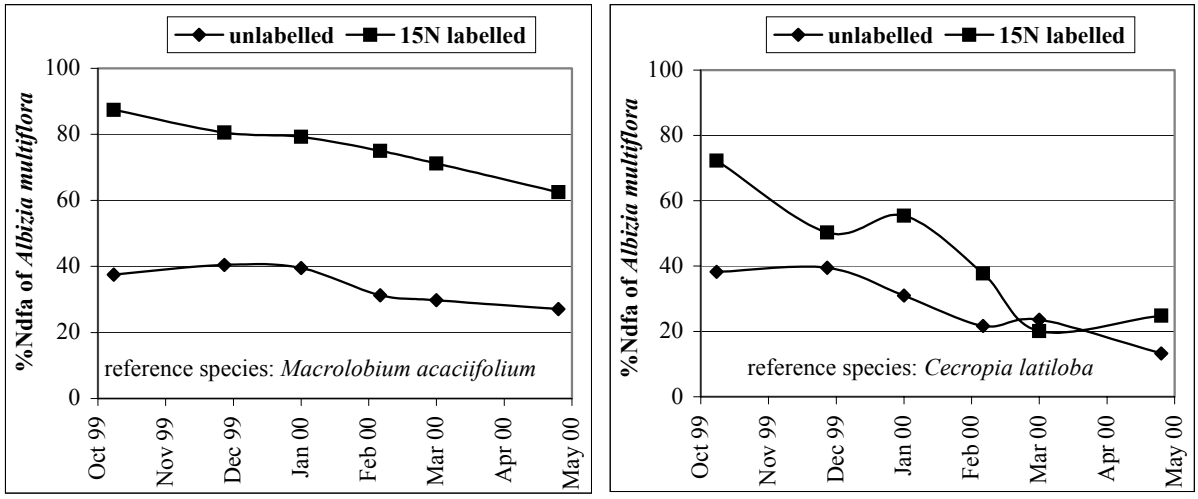


Figure 4.27 %Ndfa of *Albizia multiflora* over the run of the experiment, calculated for both treatments and with *Macrolobium acaciifolium* (left) and *Cecropia latiloba* (right) as reference species.

4.6.4 Comparison of experimental with forest results

$\delta^{15}N$ values of the unlabelled seedlings and forest trees showed large differences of 0‰ to 5.5‰ units (Table 4.15), with generally higher $\delta^{15}N$ values for the experimental seedlings by comparison with the forest trees. These differences were mostly higher within roots and stems by comparison with leaves, which was probably due to the fact that the experimental seedlings did not show a clear inner-plant isotopic fractionation pattern (Table A.4.8) like the trees (Figure 4.17). Since the differences between $\delta^{15}N$ values in leaves were not generally smaller when forest seedlings were compared with the experimental seedlings (Table 4.15), the differences between leaf $\delta^{15}N$ values of the experiment and the forest might be rather due to differences in soil parameters than due to physiological differences between seedlings and trees.

Table 4.15 $\delta^{15}N$ values of plants under experimental conditions (unlabelled treatment) and of seedlings and trees from the forest (mean ± standard deviation).

	$\delta^{15}N$ values [‰]						
	leaves			roots		stems	
	experim. seedlings	forest seedlings	forest trees	experim. seedlings	forest trees	experim. seedlings	forest trees
Campsiandra comosa	5.4 ± 3.0	1.9 ± 0.5	1.0 ± 0.9	4.7 ± 1.2	0.8 ± 0.3	4.4 ± 2.3	-1.1 ± 0.5
Albizia multiflora	3.6 ± 1.1	0.2 ± 2.7	1.2 ± 2.3	2.3 ± 0.9	0.3 ± 1.2	0.8 ± 0.8	0.5 ± 1.6
Macrolobium acaciifolium	6.8 ± 3.0	5.4 ± 1.0	5.1 ± 0.6	8.8 ± 1.7	3.8 ± 2.1	7.0 ± 1.9	3.1 ± 1.2
Cecropia latiloba	4.9 ± 1.2	2.3 ± 1.4	5.0 ± 1.4	4.7 ± 1.0	3.8 ± 0.7	5.4 ± 3.0	2.6 ± 1.4

For *Campsiandra comosa*, the ^{15}N natural abundance method in the forest revealed a 10 times higher %Ndfa value (41%) compared to the isotope dilution method (4%) when both results were calculated with *Macrolobium acaciifolium* as reference species. The difference between the 2 experimental treatments was not as dramatic, but still results of the unlabelled treatment were remarkably higher by comparison with the ^{15}N labelled treatment (Table 4.16). In contrast, for *Albizia multiflora*, the isotope dilution method resulted in a %Ndfa value (76%) which was nearly double the value obtained using the ^{15}N natural abundance method in the forest (39%) when both were calculated with *Macrolobium acaciifolium* as reference species. The difference between the results of the 2 methods was even larger, when the 2 experimental treatments were compared (Table 4.16).

Table 4.16 %Ndfa values of *Campsiandra comosa* and *Albizia multiflora* obtained with the isotope dilution method and the ^{15}N natural abundance method in the experiment (Table A.4.8) and the forest (Table 4.13) calculated with different reference species.

	%Ndfa		
	isotope dilution method experiment: ^{15}N labelled treatment	^{15}N natural abundance method experiment: unlabelled treatm.	forest
Campsiandra comosa	0 + 4	9 + 15	38-50
Albizia multiflora	43 + 76	28 + 34	31-48

Summarising, the %Ndfa values determined with the isotope dilution method varied strongly from the results obtained with the ^{15}N natural abundance method in the experiment and in the forest. The 2 nodulated legume species showed diametrical deviations comparing the 2 methods. Whereas for *Campsiandra comosa* the isotope dilution method resulted in much

lower %Ndfa values than the ^{15}N natural abundance method, it was the opposite for *Albizia multiflora*.

4.7 Denitrification

Denitrification was monitored using the acetylene block method, simultaneously with assessment of soil properties during the hydrological cycle, to identify the regulating factors with particular focus on the flood pulse. The N loss via denitrification was estimated to assess its effect on the várzea forest.

4.7.1 Denitrification rates and influencing factors

Spatial variation

Denitrification measurements at 0-5 cm soil depth during the transition period of receding water (October 1999) and during the terrestrial period (December 1999 and February 2000) were used to investigate correlations with soil factors measured simultaneously: NH_4^+-N and NO_3^--N content, pH, temperature, water content and elevation. Unfortunately, other potential influencing factors such as O_2 concentration, organic matter and total C in soil (PARKIN and TIEDJE, 1984; MERRILL and ZAK, 1992) were not measured simultaneously with denitrification and were therefore not included in these statistical analyses. Significant correlations existed between denitrification rates and water content ($r = 0.63$; $p < 0.05$) as well as between denitrification and soil pH ($r = 0.35$; $p < 0.05$). The combination of all measured influencing factors explained 29% of the variability of denitrification, whereas, the degree of explanation was 15% for water content and 3% for pH values (Figure 4.28).

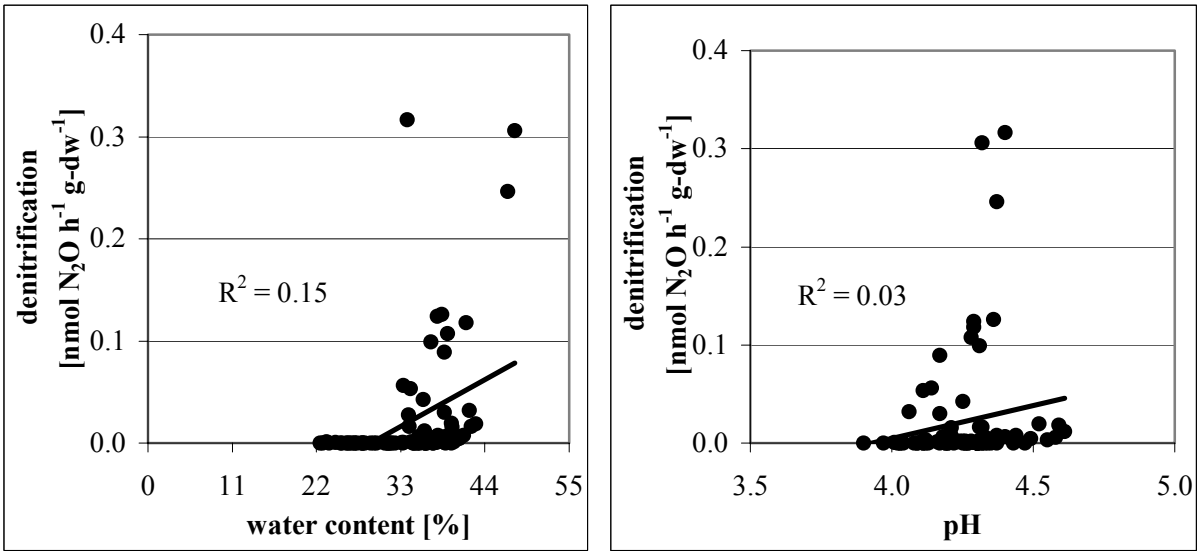


Figure 4.28 Regressions between denitrification and gravimetric water content (left) and between denitrification and soil pH values (right). The coefficients of explanation (R^2) are given (n=90).

Denitrification increased with increasing soil water content. When the gravimetric water content was below 33% denitrification rates were negligible (Figure 4.28), very likely due to the absence of anoxic conditions (SEXSTONE et al., 1988). Denitrification increased as well with increasing soil pH and therefore more neutral conditions (Figure 4.28). Remarkable is the lack of significant correlation between denitrification and NO_3^--N content, since NO_3^--N availability was identified as the most important factor controlling denitrification in a swamp ecosystem by MERRILL and ZAK (1992).

Another influencing factor might be the vegetation (KILIAN and WERNER, 1996). But measurements at 3 sampling periods (October 1999, December 1999 and February 2000) showed no significant differences in denitrification in the surface soil layer between areas next to legumes, next to non-legumes and locations without trees (Figure 4.29). Therefore, in the floodplain forest there was no effect of different plant species which would have led to significant differences in denitrification.

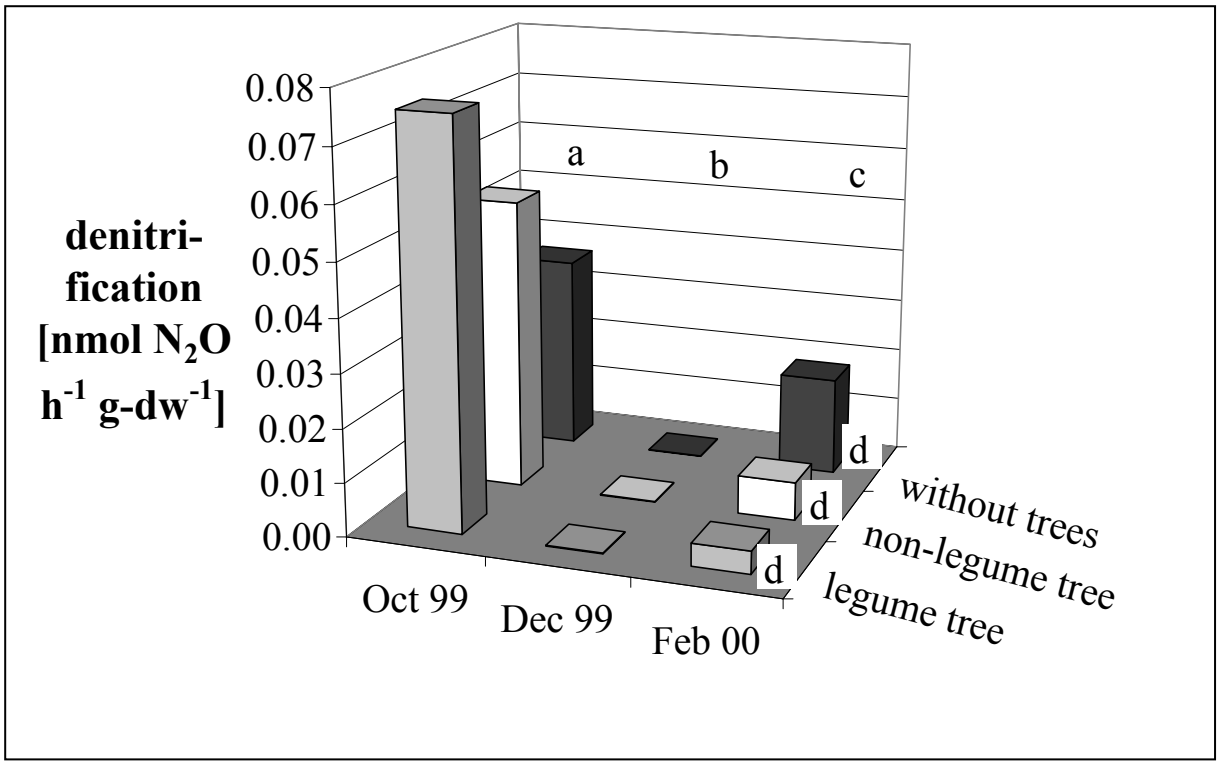


Figure 4.29 Denitrification rates during 3 sampling periods at different locations in the surface soil layer (n=10). Monthly rows marked with the same letter are not significantly different at $p<0.05$. Differences between sites, rows indicated with the same letter, are not significant at $p<0.05$.

The coefficients of variance for replicate soil samples taken from sites < 4 m² were 9–224% for denitrification. This relatively high variability within small areas is consistent with previous soil studies (FOLORUNSO and ROLSTON, 1984; MERILL and ZAK, 1992; WATTS and SEITZINGER, 2000).

Variation in soil profile and litter

Incubations of soil from different depths (0-450 cm) at the beginning of the terrestrial period (October 1999) resulted in average denitrification rates between 0.00 and 0.03 nmol N_2O h^{-1} $g\text{-}dw^{-1}$ (Figure 4.30). The maximum rate, which was significantly higher than all other values measured in the soil profile ($p<0.05$), occurred at 250 to 300 cm depth, just below the water table. Medium values were measured at 5-20 cm, 40-60 cm and 100-200 cm soil depth. Measurements down to 100 cm soil depth at the end of the terrestrial period (February 2000) revealed significantly lower denitrification rates between 0.00 and 0.01 nmol N_2O h^{-1} $g\text{-}dw^{-1}$.

In February 2000, no significant differences in denitrification rates between soil layers occurred (Figure 4.30).

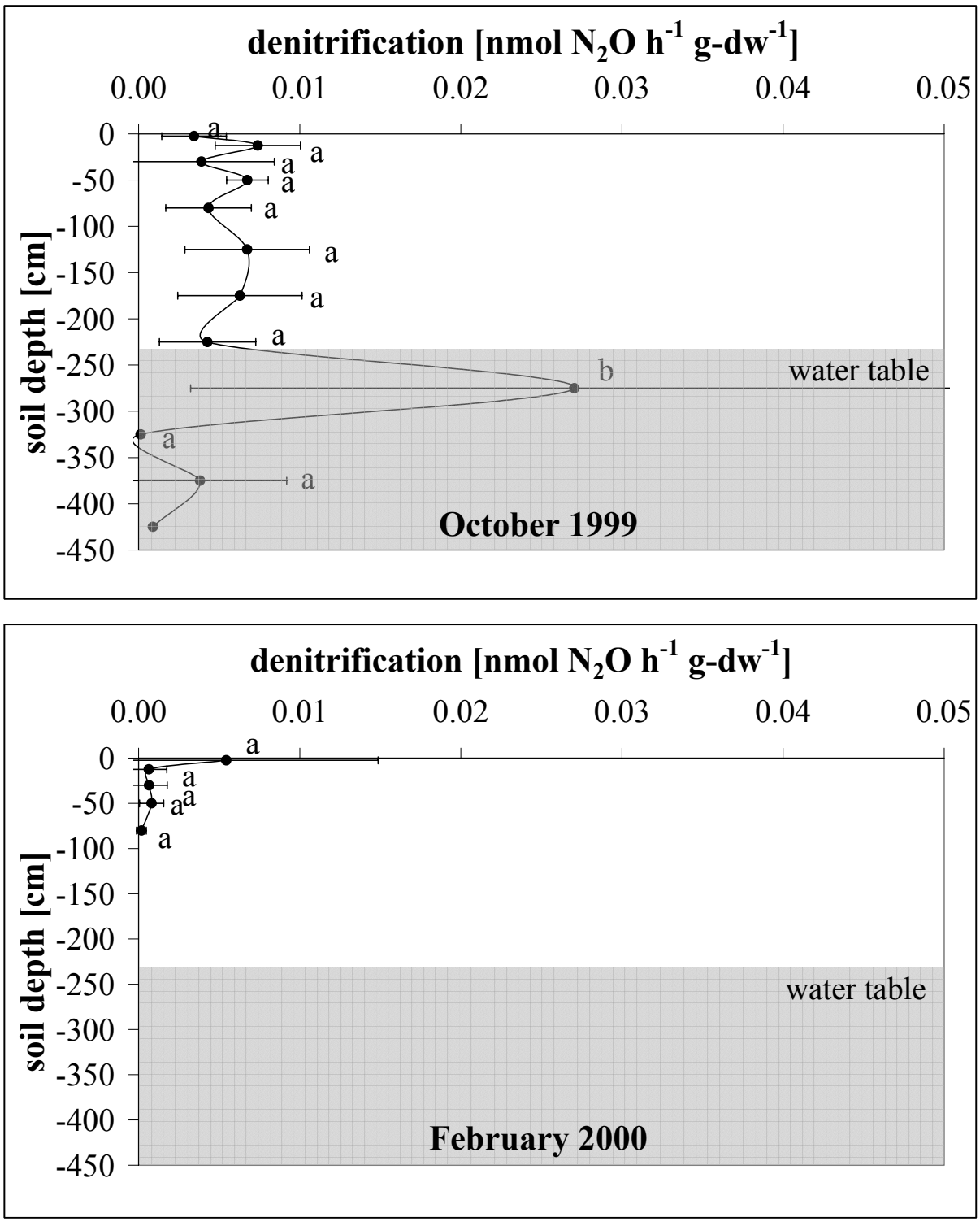


Figure 4.30 Denitrification rates measured in the soil profile at the beginning (October 1999) (top), and at the end of the terrestrial period (February 2000) (bottom) (n=3; means and standard deviations). The soil below the water table is marked in grey, points followed by the same letter are not significantly different by $p<0.05$.

Incubations of litter during the terrestrial period revealed significantly higher rates of denitrification than incubations of soil ($p<0.05$). Mean denitrification was 0.05 ± 0.08 nmol N_2O h^{-1} g-dw^{-1} in January 1999 (n=9) and 0.04 ± 0.09 nmol N_2O h^{-1} g-dw^{-1} in December 1999 (n=9), with an individual sample maximum of 0.53 nmol N_2O h^{-1} g-dw^{-1} (December 1999). There was no significant difference between denitrification rates in litter at the 2 sampling periods.

Seasonal variation

From August 1998 to September 1999 mean denitrification in the soil layer 0–5 cm varied between 0 and 0.70 nmol N_2O h^{-1} g-dw^{-1}; the individual sample maximum was 1.76 nmol N_2O h^{-1} g-dw^{-1} in May 1999. Denitrification varied seasonally in the floodplain forest soil, and the highest rates occurred during flooding and the transition periods (Figure 4.31). The same pattern in denitrification was repeated during the next hydrological cycle. Denitrification rates differed significantly between the 3 sampling periods in October 1999, December 1999 and February 2000 ($p < 0.05$). During the transition period of receding water in October 1999, mean denitrification was highest with 0.06 ± 0.09 nmol N_2O h^{-1} g-dw^{-1}. In the middle of the terrestrial period, in December 1999, denitrification was not detectable and at the end of the terrestrial period during the rainy season (February 2000) it was 0.01 ± 0.03 nmol N_2O h^{-1} g-dw^{-1} (Figure 4.29).

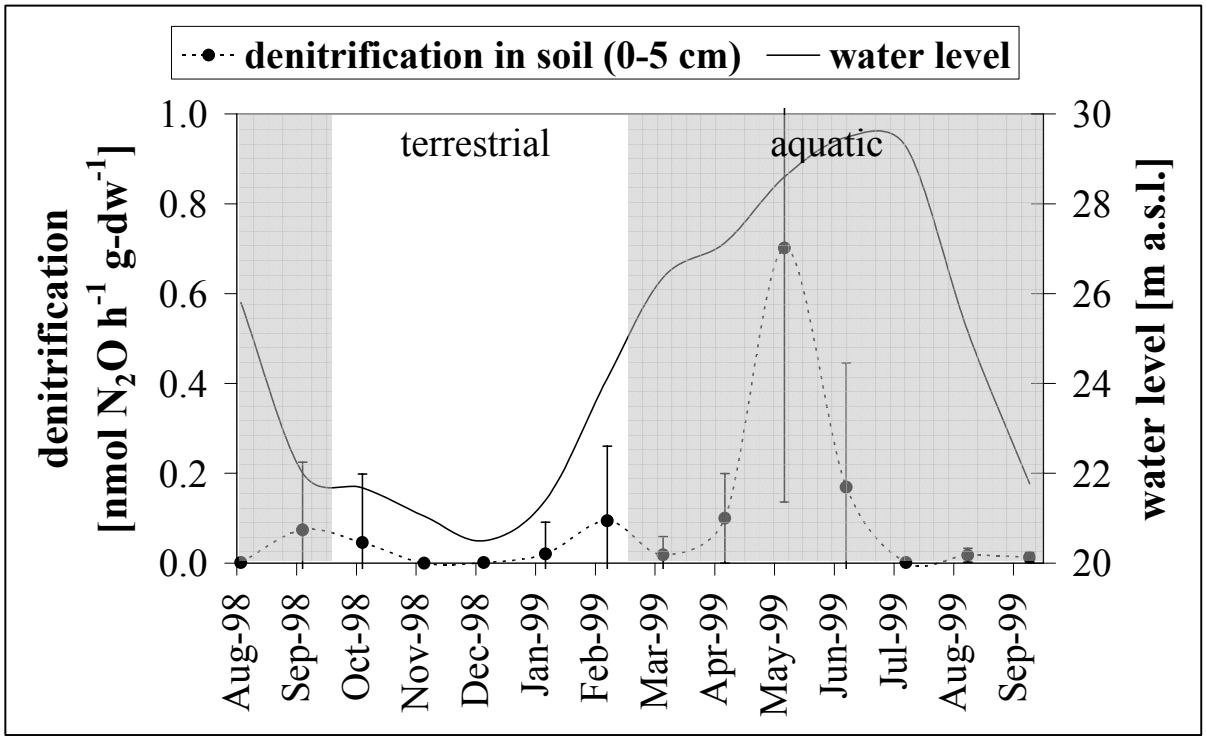


Figure 4.31 Denitrification rates in the surface soil layer (0-5 cm) during the hydrological cycle. Monthly measurements were undertaken at different locations in the study area from August 1998 to September 1999, with n=9 when the study area was inundated and n=30 during the terrestrial period (mean and standard deviations).

4.7.2 N loss via denitrification

N loss via denitrification in the surface soil layer (0-5 cm) was significantly different between the terrestrial and the aquatic period ($p<0.05$; Figure 4.32). Sum of the mean monthly N losses during the 7 months of the aquatic period was 10.9 kg N ha^{-1}, and the amount denitrified in the 5 months of the terrestrial period was 1.6 kg N ha^{-1}. Therefore, the annual N loss via denitrification in the surface soil layer was 12.5 kg N ha^{-1}.

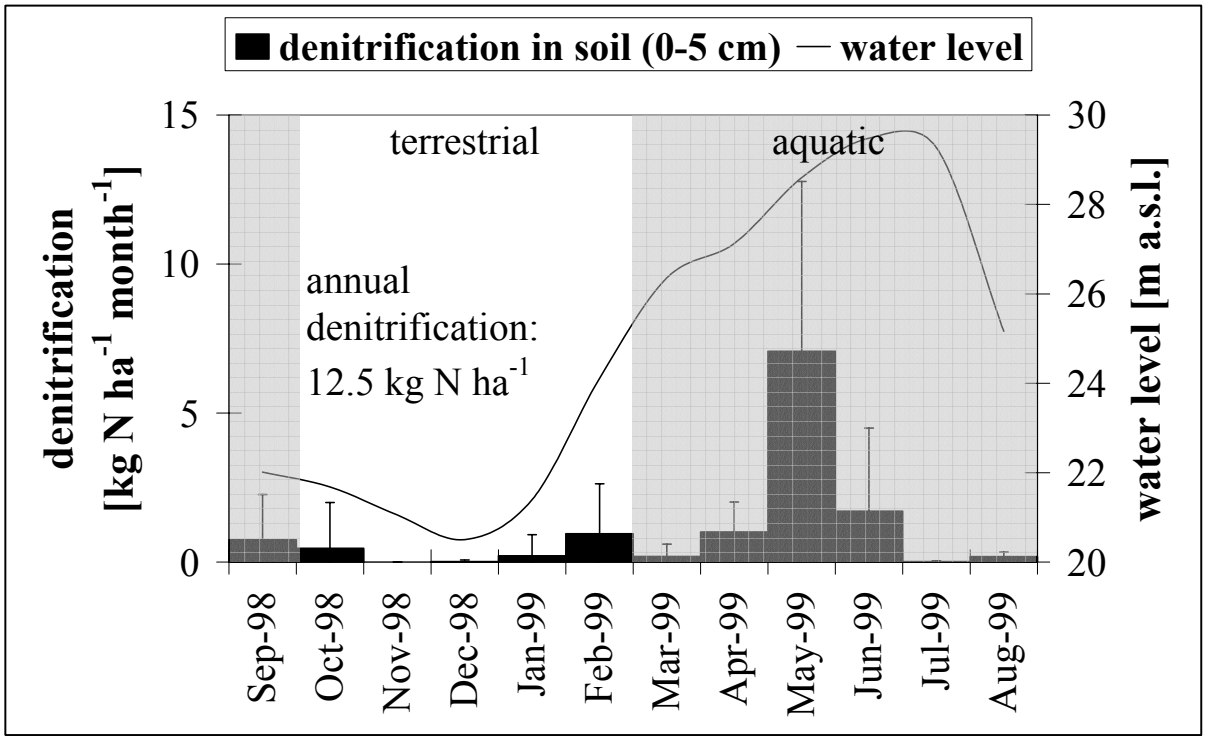


Figure 4.32 Mean monthly N loss via denitrification in the surface soil layer (0-5 cm) during 1 year (mean and standard deviation). The aquatic periods are shaded in grey.

Only at 0-5 cm soil depth, denitrification was constantly measured during the whole hydrological cycle. Therefore, annual N loss can only be calculated for the surface soil layer. This horizon is especially relevant, since the N dynamic is most important in the surface soil layer, where the main biological activity occurs (MEYER, 1991). Although, incubations in the soil profile undertaken in October 1999 revealed maximum denitrification rates below the water table at 250-300 cm depth (Figure 4.30). Therefore, annual N loss via denitrification is much higher when the whole soil profile is considered. But seasonal variation of denitrification rates (Figure 4.31) and of environmental factors (Chapter 4.2) during the hydrological cycle make it impossible to extrapolate denitrification in soil below 5 cm depth from 2 measurements during the terrestrial period (Figure 4.30) to 1 year.

Summarising, highest denitrification rates in the surface soil layer were measured during flooding and the transition periods, revealing the strong influence of the flood pulse. In the soil profile, maximum rates occurred at 250 to 300 cm soil depth, just below the water table, only in litter denitrification was even higher. Annual N loss via denitrification in the surface soil layer (0-5 cm) was 12.5 kg N ha^{-1}.

5. Discussion

Results obtained within 2 years are limited, and how far conclusions for long term ecological processes can be drawn must be discussed not only in such a dynamic system as the várzea, where height and time of flooding, which differ greatly from year to year, have regulating effects on physical, chemical and biological processes. Interannual climate variability strongly affects element cycles also in non-flooded areas (HESSEN et al., 1997; TIAN et al., 1998), for example yearly differences of over 60% were reported for litter production (UNESCO et al., 1978), or 30% differences in soil N_2O-N emissions (KAISER et al., 1998). Influences of El Nino-Southern Oscillation weather phenomenon on vegetation and animal life (ADIS and LATIF, 1996; WORBES and SCHÖNGART, 2002) in the Amazon floodplain are apparent, so that effects of the strong El Nino (1997/1998) and La Nina (1998/1999) events also on this study are possible. Therefore, the results strictly apply for the study period, and for generalisations precaution needs to be taken.

5.1 Forest, soil and rhizobia

Forest

The forest type on Ilha de Marchantaria, generally, represents a mid-stage tree community according to the successional classification of WORBES (1997), but due to the observed moderate human disturbance, the studied forest may not completely represent a pristine várzea succession. 1441 woody plants of 44 species were counted on 2.1 ha. This relatively low species diversity is on the one hand typical for inundation forests, due to selection and adaptation to the periodic flooding (WORBES, 1986), but on the other hand, it is even smaller than in other várzea forests (WORBES, 1983; KLINGE et al., 1995), which is probably due to the relatively young age and low elevation of the studied forest. The most abundant species *Crateva benthamii* and *Vitex cymosa* are characteristic for the várzea (PRANCE, 1979), and they are specially important in early successional stages (KLINGE et al., 1995). But their high dominance in the study area was remarkable and lead to the predominance of the plant families Capparaceae and Verbenaceae of which they were the only representatives. Due to this also anthropogenically influenced situation, Leguminosae were only the 3rd important family, with the highest relative richness (27%) and a relative density of 10% (Table 4.1). In contrast, previous studies in the várzea revealed the legumes as the most important family not only due to their species richness, but also with relative densities of 12-17% (WORBES, 1986;

KLINGE et al., 1995). Therefore, the high importance of the legumes in the study area is characteristic for the várzea and might generally be even higher than in the forest under study. Moreover, legumes are even more abundant in igapó and terra firme forests (KLINGE et al., 1975; WORBES, 1986), so that their N_2 fixation potential due to legume-rhizobia symbioses might be of high ecological relevance in the whole Amazon basin. It is not surprising, that the liana *Dalbergia riparia* is the most important legume in the study area, because this species is highly abundant in the várzea (WORBES, 1983; 1986; KLINGE et al., 1995) and lianas are generally an important ecological component in tropical forests (GENTRY, 1983; PUTZ, 1983). That *Macrolobium acaciifolium* and *Pterocarpus amazonum* belonged to the most important legume trees is in accordance with the findings of KLINGE et al. (1995). The surprising fact, that none of the previous studies mentioned *Albizia multiflora*, which was the 3rd important legume species in the study area, might be due to misidentification or the use of its synonym *Pithecellobium paucipinnatum*. On Ilha de Marchantaria, previously *Pithecellobium* spp. were found with relative densities of 1% and 6% by KLINGE et al. (1995) and WORBES (1983), respectively.

Estimates of the forest biomass showed a quite broad range between 227 and 343 t ha^{-1} (Table 4.3) where the model of BROWN et al. (1989) is the most complex and detailed one, it differentiates between trees of different sizes and types and most importantly between trees and lianas. This should lead to a more precise estimate, since relationships between diameter and leaf biomass for trees and lianas are very different (OGAWA et al., 1965; PUTZ, 1983). Another considerable factor for volume based biomass, the wood density (FEARNSIDE, 1997b), is only included in the equations of BROWN et al. (1989) and NELSON et al. (1999). Therefore, and considering the fact that forest biomass estimates for tropical America range from 190 to 300 t ha^{-1} (BROWN and LUGO, 1984), and for the várzea from 97 to 265 t ha^{-1} (KLINGE et al., 1995; FURCH, 1997), the estimates by BROWN et al. (1989) and NELSON et al. (1999) seem more realistic, so that an above ground biomass of ca. 230 t ha^{-1} was estimated for the study area.

In the study area 24 woody and non-woody legumes were identified (Table 4.4). The nodulation status was checked randomly for several species, although nodulation appears to be a generic character and in most cases is consistent within continents (FARIA et al., 1989). However, a nodulated genus may only be nodulated under particular conditions (SPRENT, 2000). Considering this it is interesting that on some *Pterocarpus amazonum* trees nodules

were found, since previously nodules have been found on all species of *Pterocarpus* examined, for example *Pterocarpus podocarpus* in Venezuela (BARRIOS and GONZALES, 1971 cited in ALLEN and ALLEN, 1981), but not on those from Brazil (ALLEN and ALLEN, 1981; FARIA et al., 1984; 1989; FARIA and LIMA, 1998; SPRENT and PARSONS, 2000). Despite nodulation, its $\delta^{15}N$ values were relatively high, indicating a low %Ndfa of *Pterocarpus amazonum*. However, 87.5% of the identified legume species were nodulated, which may, as well as the high importance of the legumes, indicate an ecological advantage of the N_2 fixing plants in the study area. The approximate age of the forest is 60 to 100 years (FURCH and KLINGE 1989; WORBES et al., 1992; KLINGE et al., 1995), so that the forest has not reached a climax stage yet and hence is probably not in equilibrium of growth dynamic and nutrient cycling. Thus, N input might still have had stimulating and supporting effects for biomass production by comparison with climax forests (SPRENT, 1987), where absence of nodules in potentially nodulated species is common (NORRIS, 1969; FARIA et al., 1984). It was also expected, that the fertile várzea soils provide favourable conditions for nodulation, since a good nutrient availability especially of P, K, S and micro-nutrients stimulates nodulation (TSAI et al., 1993; OLIVEIRA et al., 1998). Furthermore, nodulated legumes contribute significantly to the N-balance of Amazonian wetlands (SALATI et al., 1982; MARTINELLI et al., 1992), where nodulation of legumes is much more abundant than in the drier terra firme regions (MOREIRA et al., 1992). Due to adaptations to permanent or seasonal flooding, nodules appear to be active during the whole hydrological cycle, especially for herbaceous legumes like *Aeschynomene* sp., *Mimosa* sp., *Neptunia* sp., *Sesbania* sp., *Vigna* sp. etc. where function of nodules during flooding has been proven (JAMES et al., 2001).

Nodulated legumes might not only be important for the native floodplain forest, but might also support the protection of the area due to their value for sustainable management (PASTOR and BINKLEY, 1998). In agroforestry, herbal- and woody-climbers are used as soil cover and mainly tree-legumes are used in alley-cropping and mixed-cropping systems (PEOPLES et al., 1995). Hardwood timber production represents the major economic use of leguminous trees, although other roles in providing shelter, land conservation, land reclamation and as medicinal plants are important as well (LADHA et al., 1993; JOHNSTON, 1998). For example, wood of *Campsiandra* sp. and *Swartzia* sp. is highly durable, and therefore preferably used for heavy construction purposes (RECORD and HESS, 1943 cited in ALLEN and ALLEN, 1981; SPRENT and PARSONS, 2000). *Vigna* sp., *Aeschynomene* sp., *Sesbania* sp., *Teramnus* sp. etc. are used as good forage, hay and green-manure and due to their ability to maintain soil

fertility and prevent erosion they are used as soil cover (ALLEN and ALLEN, 1981). Since nodulated legumes generally improve the soil N status and transfer fixed N also to adjacent or following plants, the development of small scale agroforestry systems which support the cultivation of legumes may open new perspectives for the várzea.

Soil

The silty soil of the study area was typical for the várzea (IRION et al., 1997), and also the very low pH values during the terrestrial period were characteristic for soils in Latin America (ANYANGO et al., 1998). The increase of the pH during the transition period to a higher level and more neutral conditions in the aquatic period, was due to the flooding with river and lake water which have a pH of 6-8 (KERN, 1995). Still, mainly legume species and rhizobia strains which tolerate the acid conditions during the terrestrial period (pH 4-5) were expected in the study area, since soil pH has a high impact on nodule bacteria and the process of nodulation (STREIT et al., 1992; ANYANGO et al., 1998; GRAHAM and VANCE, 2000). Generally, denitrifiers are more pH tolerant than nitrifiers (PARKIN, 1987; TIETEMA et al., 1992), still acidity may limit denitrification (HALL et al., 1998; STEVENS et al., 1998). The richness in organic matter particularly in the surface soil layers was favourable for microbiological processes, especially denitrification (PU et al., 1999). Since soil temperature variation was small, no effect on the enzyme activities involved in mineralisation and denitrification was expected, because in experiments the response to temperature changes was small (TSCHERKO et al., 2001).

Generally, O_2 availability in soil is influenced by the O_2 partial pressure in the gas phase, the moisture content, the soil texture and respiration activity. In the study area, the flood pulse mainly controlled both the soil O_2 and water status. During the terrestrial period, especially in the surface soil layer, aeration was good with a mean air filled pore space of 34% and an average O_2 partial pressure of 20%. Anaerobic conditions which stimulate denitrification were probably restricted to soil micro-pores, because denitrification is mainly favoured at O_2 partial pressures lower then 0.5% and at 65% water filled pore space about 10% of the soil volume are anoxic (BOLLMANN and CONRAD, 1998). O_2 availability decreased with soil depth, for example, mean air filled pore space was only 11% and O_2 partial pressure ranged from 15% to 20% at 30 cm soil depth (Figure 4.2). High water contents mainly corresponded to low O_2 partial pressure, but a clear relation as shown by SMITH and DOWDELL (1974) could not be calculated. Also the gravimetric water content in soil varied with depth and time, additionally

influenced by precipitation. At the beginning of the terrestrial period, the driest soil layers were in the 20-60 cm depth, and naturally the wettest layers below the water table. In the surface soil layer, the mean water content was on average 42% during the aquatic period and 33% during the terrestrial period. During the aquatic period, O_2 concentrations in the flooding water directly over the sediment were low, mainly due to intensive biodegradation of organic material (MELACK and FISHER, 1983). Additionally, the thermal stratification favours hypoxic/anoxic conditions in deep water (JUNK and FURCH, 1985). With increasing water level above approximately 27 m a.s.l. the Rio Solimões starts to flow from 2 sides into the inundation area until it overflows the whole island. Then O_2 concentrations increase due to the O_2 rich river water and the turbulence (JUNK, 1990; KERN, 1995). In 1999 a water level of 27 m a.s.l. was reached mid April, and the maximum level with 29.5 m a.s.l. was reached in June. An increase of O_2 concentrations was measured in deep water, but surprisingly, the maximum was reached not until August, during receding water (Table 4.6).

Total C and N contents were highest in the surface soil layers and also NH_4^+-N and NO_3^--N contents decreased significantly with soil depth. NH_4^+-N was always the dominant form over NO_3^--N, which might indicate reduced conditions in the soil even during the terrestrial period. This dominance of NH_4^+-N in wetland soils (BRUNE et al., 2000) is in contrast to results from terra firme forests on Amazonian upland soils where NO_3^--N is dominant (NEILL et al., 1999). This prevalence of NH_4^+ might favour symbiotic N_2 fixation in the study area, since for some legume species even enhanced nodulation and N_2 fixation occurs in the presence of NH_4^+, but not of NO_3^- (SPRENT, 1995). However, when mineral N concentrations are too high, root nodule formation and also non-symbiotic N_2 fixation is reduced or even inhibited (STEWART, 1975; TSAI et al., 1998). This might be the case during the aquatic period, when significantly higher NH_4^+-N contents in the 0-5 cm soil depth by comparison with the terrestrial period, correspond with negligible non-symbiotic N_2 fixation rates. In contrast, favoured by good O_2 availability, NO_3^--N concentrations were higher during the terrestrial period by comparison with the aquatic period.

Except for nitrifiers, bacterial numbers were relatively high (Table 4.8), indicating adapted bacterial populations, not affected by the water stress, which generally may suppress microbial population growth and activity rates (TSCHERKO et al., 2001). NH_4^+-N and NO_3^--N concentrations showed clear changes during the hydrological cycle, which was not reflected by numbers of bacteria involved in soil N dynamics. The lack of variation in bacterial

numbers during the hydrological cycle was probably due to the complex regulation mechanisms in the rapidly changing environment of the várzea. Where activity changes without population changes should be much more efficient than a regulation of the population size depending on soil conditions. These results were in accordance with findings by CHAO and CHAO (1997), who theorised, that differences in NO_3^--N concentrations resulted from differences in the activities and composition of the nitrifiers. The small numbers of nitrifiers were probably due to the low soil pH (TIETEMA et al., 1992) and reduced or even anoxic conditions in the soil. In the study area free-living N_2 fixing bacteria were present in high numbers of 10^5-10^6 g^{-1} in litter and the surface soil layer, which is in the range of diazotroph numbers in N-poor soils with considerable amounts of available C (ABD-EL-MALEK, 1971). Diazotrophs grew also in deeper soil layers, which might be correlated to sufficient C availability and suitable water contents as reported by GADKARI and MEYER (1992). Denitrifiers were also present in high numbers between 10^2 and 10^8 g^{-1}, which was in the range of denitrifier numbers in aquatic sediment of Lago Camaleão (KERN, 1995), indicating favourable conditions for denitrifier populations in various ecotopes of the várzea and therefore, a high N loss via denitrification in the floodplain was possible.

Rhizobia

The biodiversity of rhizobia nodulating tropical legumes is large, including fast- and slow-growing strains (HAUKKA et al., 1996; 1998; LAFAY and BURDON, 1998; DOIGNON-BOURCIER et al., 1999). Isolates from legume plants from the Amazon region were found to belong to the genera *Azorhizobium*, *Bradyrhizobium*, *Mesorhizobium*, *Rhizobium* and *Sinorhizobium* (MOREIRA et al., 1993; MOREIRA et al., 1998). Growth rates can already be used as a rough indication to what genus a nodule isolate might belong (JORDAN, 1984). In this study, growth rate of all isolates was intermediate, which is consistent with the clustering of most of them with *Mesorhizobium* spp.

New rhizobial isolates are generally confirmed to be such by inoculation experiments. This authentication was not possible in this work due to the lack of seeds of the corresponding primary host plants. A molecular genetic approach was chosen instead for this purpose. For the formation of an effective symbiosis, rhizobia require several classes of symbiosis-relevant genes (NINER and HIRSCH, 1998). These include *nod* genes, which encode for enzymes involved in *nod*-factor biosynthesis (BROUGHTON et al., 2000), which stimulate the plants to produce symbiotic nodules, and the nitrogenase structural and regulatory *nif* genes (HAUKKA

et al., 1998). The *nod* genes are unique to rhizobia, whereas *nif* genes are found in all N_2 fixing bacteria (YOUNG and HAUKKA, 1996). Therefore, the presence of both, *nod* and *nif* genes, in the isolates from *Pterocarpus amazonum* and *Albizia multiflora* is a strong indication, that these isolates are rhizobia.

16S rDNA is considered one of the minimal standards for genetic characterisation of bacteria (GRAHAM et al., 1991) and sequence polymorphisms of individual rhizobial 16S rDNAs can be detected by the means of restriction fragment length polymorphism (RFLP) analyses (LAGUERRE et al., 1994). Almost the entire *rrs* gene can be amplified with the fD1 and rD1 PCR primers (WEISBURG et al., 1991). Sequence and restriction analyses of amplified 16S rDNA has been successfully used for studying rhizobia (WILLEMS and COLLINS, 1993; LAGUERRE et al., 1994). The RFLP analyses based on the combination of 3 to 5 restriction enzymes is reliable for phylogenetic and taxonomic studies of large sets of strains (HEYNDRICKX et al., 1996; MOYER et al., 1996). According to their 16S rDNA-RFLP patterns, 5 *rrs* alleles were found among the 18 isolates from nodules of *Albizia multiflora* and *Pterocarpus amazonum* (Figure 4.9). These were grouped in 2 major clusters that most likely correspond to at least 2 different genera and species, based on their linkage level. This is a relatively large genotypic diversity, considering that only 2 host species in 1 forest area were sampled. For comparison, 745 rhizobia isolates from 32 legume host species at 12 sites in south-east Australia were divided into 21 genomic species by small-subunit rDNA PCR-RFLP and phylogenetic analyses (LAFAY and BURDON, 1998). 15 isolates from *Acacia mangium* sampled at various locations in Indonesia were classified into 9 groups with RFLP-16S rDNA analyses (NUSWANTARA et al., 1997) and 171 isolates from tropical legumes in the Amazon region and south-east of Brazil were grouped into 18 clusters by comparative polyacrylamide gel electrophoresis (MOREIRA et al., 1993). Host specificity of rhizobia is a well known phenomenon, but it does not necessarily lead to a correlation between genotypic clusters obtained and the divergence groups of legumes from which the strains were isolated (MOREIRA et al., 1993; HAUKKA et al., 1998). This was also the case in this study.

To analyse the diversity of the isolates at stricter taxonomic resolution, rep-PCR genomic fingerprinting with the GTG_5 primer was undertaken. With the GTG_5-PCR, all isolates yielded distinct patterns (Figure 4.10), which may suggest that all isolates correspond to distinct bacterial strains (VERSALOVIC et al., 1994). But since the same sample 2-3 times analysed in the same PCR experiment and electrophoreses revealed patterns with a similarity

of only 90-95% and only 85-90% when analysed independently (VINUESA et al., 1998), it is suspected that isolates with high similarities are not really different. Most of the sample isolates were grouped in a cluster with ≥ 60% similarity, which is consistent with their close relatedness as revealed by 16S ARDRA (genotypes III and IV).

Many taxonomic studies using rhizobia of tree or shrubby legumes found, that the vast majority of sampled isolates did not correspond to reference strains (MOREIRA et al., 1993; HAUKKA et al., 1998; LAFAY and BURDON, 1998). This may be due to the focus of research on agriculturally relevant legumes, which keeps the taxonomic position of most of the tree-associated rhizobia uncertain (VINUESA et al., 1998). Therefore, it is not surprising that none of the sampled isolates had ARDRA patterns identical to that of any of the reference strains (Figure 4.11). It can only be concluded that isolates with the ARDRA genotype III and IV (and probably also V) were *Mesorhizobium* spp., and isolate 5-2/4 (ARDRA genotype II) most likely belonged to the genus *Bradyrhizobium*. The generic classification of the 2 isolates with the ARDRA genotype I could not be achieved. Interestingly, most isolates were grouped with *Mesorhizobium* spp. which was quite atypical as mainly *Bradyrhizobium* strains nodulate tropical legumes (NORRIS, 1965; JORDAN, 1984). This had been confirmed by many independent studies, which found that the vast majority of isolates from tropical legumes belong to the *Bradyrhizobium* group (MOREIRA et al., 1993; LAFAY and BURDON, 1998; VINUESA et al., 1998; DOIGNON-BOURCIER et al., 1999). However, at least partial 16S rDNA sequencing of representatives of each of the 5 ARDRA genotypes detected in this study would be required to classify the corresponding isolates at the genus to species levels of taxonomic resolution.

In summary, nodulated legume species might be of high ecological relevance in the várzea forest. Most soil properties, except for bacterial numbers showed significant variations regulated by the flood pulse, so that highly adapted plant and bacteria species were believed to be present in the study area. Mainly *Mesorhizobium* but also *Bradyrhizobium* species were nodulating *Albizia multiflora* and *Pterocarpus amazonum* on Ilha de Marchantaria.

5.2 Non-symbiotic N_2 fixation

Non-symbiotic N_2 fixation showed high spatial variability, as well as significant variation with time and soil depth. The soil was incubated under dark conditions, which selected for

heterotrophic diazotrophs. But since natural light within the forest under the litter layer is low (GRAFFMANN, 2000), no significant artificial suppression of nitrogenase activity was expected. In contrast is the N_2 fixation on semi-aquatic sediments in the várzea, where N_2 fixation was mainly due to photo-autotrophic organisms (KERN, 1995).

Many environmental factors and soil properties like O_2 supply, soil water content, mineral nutrients, temperature, pH and availability of energy have an influence on N_2 fixation (STEWART, 1975; DÖBEREINER, 1977; GRANHALL, 1981). Measurements during the terrestrial period showed, that N_2 fixation in soil seemed to be mainly influenced by the soil water content and the elevation (Figure 4.12). The regression of increasing N_2 fixation with increasing soil NO_3^--N contents was most likely not a causal relation, but rather due to coincidentally similar seasonal trends, as N_2 fixation in self-regulation naturally declines with increasing contents of mineral N (GRANHALL, 1981). The soil water content influences O_2 and nutrient supply and general metabolic activities (STARK and FIRESTONE, 1995) and thus also affects non-symbiotic N_2 fixation. In the study area, N_2 fixation declined with increasing soil water content, probably due to the resulting reduction in O_2 and increase in NH_4^+-N availability. This corresponds to the inhibition of non-symbiotic N_2 fixation during the aquatic period. Higher elevations, and therefore shorter periods of flooding, additionally favoured N_2 fixation, suggesting an aerobic diazotroph population. N_2 fixation was not significantly correlated with soil pH or temperature, probably due to acid tolerant bacteria (BECKING, 1961) and small temperature changes in the study area. These simultaneously measured soil properties could only explain 22% of the variation in N_2 fixation during the terrestrial period. Thus, other factors, for example O_2 and organic matter/total C availability must have had significant influence as well.

Corresponding results of high O_2 partial pressure in soil air and high N_2 fixation rates existed, but since O_2 concentrations were not measured simultaneously with N_2 fixation, they could not be statistically proven. At the beginning of the terrestrial period in September/October 1999, the minimum of the O_2 partial pressure in soil air was measured (Figure 4.2), as well as minimum N_2 fixation rates (Figure 4.13), and high O_2 concentrations in December 1999 corresponded with high N_2 fixation rates. Apparently, the aerobic N_2 fixing bacteria population was well adapted to air (STEWART, 1975; SCHLEGEL, 1992), because also in the soil profile, N_2 fixation was highest in litter and the surface soil layer (Figure 4.14). Furthermore, N_2 fixation seemed to be favoured by high organic matter and total C contents,

since these were highest in litter and the surface soil layer as was N_2 fixation. Other influencing factors like K, Ca, Mg, Mo, Ni availabilities (STEWART, 1975; CHRISTIANSEN et al., 2001; O'HARA, 2001) were not measured. In the mixed forest stand of the várzea, no significant effect of different plant species on non-symbiotic N_2 fixation existed. The high variability in N_2 fixation rates within small areas of forest ground was probably due to diminutive spatial differences in soil properties.

Incubations of soil from different depths resulted in maximum N_2 fixation rates in the surface soil layer (0–5 cm), followed by 20–60 cm and 300–400 cm (Figure 4.14). The maximum N_2 fixation in 0–5 cm soil depth was favoured by high C concentrations, relatively low bulk density and good aeration. The relatively high N_2 fixation rates below the water table were surprising, especially by comparison with negligible N_2 fixation during the aquatic period. It might be explained through the activity of anaerobic diazotrophs forming stable and active populations in this soil layer. Some diazotroph populations prefer deeper soil layers, where the O_2 supply is lower and the water content suitable (STEWART, 1975; GADKARI and MEYER, 1992).

During the terrestrial period N_2 fixation was higher in the litter than in the mineral soil, which is in accordance with literature results (PELEGRÍ et al., 1997; PELEGRÍ and TWILLEY, 1998). But, mean N_2 fixation was low by comparison with values of mangrove litter reported from 7.3 to 1350 nmol C_2H_4 h^{-1} g-dw^{-1} (ZUBERER and SILVER, 1978; PELEGRÍ et al., 1997). The litter was incubated only under natural daylight and therefore it is not possible to distinguish between autotrophic and heterotrophic N_2 fixation. High N_2 fixation due to cyanobacteria was measured in unvegetated semi-aquatic sediment close to the water line of Lago Camaleão (KERN, 1995). However, in the forest on average only 10% of light radiation reaches the ground (GRAFFMANN, 2000), and drier conditions at the higher elevations do not favour cyanobacterial growth. Therefore, the high N_2 fixation was hypothesised to be mainly due to heterotrophic bacteria which grow on the leaves that act as a C source. A previous study in mangrove forest found that N_2 fixation in litter is associated with C loss (PELEGRÍ et al., 1997), and ZUBERER and SILVER (1978) found no light-induced stimulation of nitrogenase activity in litter. Additionally, a temporal trend in N_2 fixation on leaf litter associated with the availability of organic substrates leached from the litter mainly with the intermediate stage of litter decomposition was reported (PELEGRÍ et al., 1997; PELEGRÍ and TWILLEY, 1998). These mechanisms might explain the difference in N_2 fixation at the 2 sampling times.

Considerable seasonal differences in N_2 fixation rates occurred in the floodplain forest soil, due to the flood pulse. N_2 fixation was restricted to the terrestrial period (Figure 4.15). Shortly after drying of the soil, nitrogenase activity of free-living N_2 fixing bacteria increased to a level, which was in the lower range of N_2 fixation rates measured in mangrove forest sediments (ZUBERER and SILVER, 1978; PELEGRÍ and TWILLEY, 1998) and high by comparison with N_2 fixation rates on the shore of a temperate lake (JAEGER and WERNER, 1981). Sufficient organic matter in the surface soil layer supported nitrogenase activity, which was apparently not eliminated by NH_4^+, and relatively low pH. Negligible N_2 fixation during the aquatic period is in accordance with previous studies on Ilha de Marchantaria (KERN and DARWICH, 1997). KERN (1995) reported that high NH_4^+-N concentrations in aquatic sediment do not support N_2 fixation. ESTEVES and ENRICH-PRAST (1998) found N_2 fixation in Amazonian aquatic sediment repressed by low organic C concentrations, but this effect is unlikely in the study area, since organic matter contents were high at least in the surface soil layer. More likely is an inhibition of N_2 fixation due to anoxic conditions during the aquatic period.

Annual N input in the surface soil layer was calculated to be 4.1 kg N ha^{-1}, which was lower than the N gain of 12 kg N ha^{-1} y^{-1} measured for unvegetated semi-aquatic sediments on Ilha de Marchantaria (KERN, 1995), corresponding to generally relatively small N gains via non-symbiotic N_2 fixation, whereas free-living diazotrophs are only important in special environments, for example 30-50 kg N ha^{-1} y^{-1} are gained by cyanobacteria in rice fields (SCHLEGEL, 1992). Associative N_2 fixation in the rhizosphere of terrestrial and aquatic plants was not measured. However, since the main biomass production of the várzea forest takes place during the terrestrial period, when also non-symbiotic N_2 fixation occurred in the soil, it might have some stimulating effect for the várzea forest.

The acetylene reduction assay (ARA) is commonly used to measure non-symbiotic N_2 fixation, although it is an indirect method. The reduction of C_2H_2 to C_2H_4 is very specific for all N_2 fixing organisms and the method is convenient and sensitive. However, the N_2 fixation rates in this study might be overestimated, since the theoretical ratio of 3:1 between C_2H_2 reduction and N_2 fixation was assumed, while higher measured ratios have been reported (HARDY et al., 1973; SEITZINGER and GARBER, 1987; DOYLE and FISHER, 1994; O'DONOHUE et al. 1991). On the other side, a flow through system of the ARA would have produced

higher figures. Nevertheless, the seasonal and spatial (depth) trends of N_2 fixation should be valid.

In summary, non-symbiotic N_2 fixation in soil was controlled by the flood pulse. Due to the restriction to the terrestrial period, the N gain by free-living diazotrophs, although it was small, could have positive effects on the biomass production.

5.3 Symbiotic N_2 fixation

5.3.1 Symbiotic N_2 fixation in the forest

The ^{15}N natural abundance method is a temporally integrating method, but due to relatively short leaf ages and the ability of plants to relocate N between plant parts (MEDINA, 1981), differences in the $\delta^{15}N$ value of the N source of the plants within months are able to detect (SHEARER et al., 1983). In the várzea most leaves become between 6 and 12 months old, whereas leaf ages vary according to leaf periodicity (PAROLIN, 1997).

$\delta^{15}N$ values of soil

Isotopic fractionation leads to different ^{15}N abundance in product and substrate of incomplete processes. Generally, due to reaction kinetic reasons, lighter molecules with the ^{14}N are favoured. This isotopic discrimination is naturally also associated with various processes in the soil e.g. NH_3 volatilisation or denitrification (SHEARER and KOHL, 1991), so that soil N is usually more enriched in ^{15}N than atmospheric N_2. This is not universally the case (BINKLEY et al., 1985) and seems to be positively correlated with soil age (SHEARER and KOHL, 1991). However, although the várzea is geologically relatively young (IRION et al., 1997), all soil and water $\delta^{15}N$ values were clearly above zero in the study area (Table 4.9). $\delta^{15}N$ values of the soil total N ranged from 3.4‰ to 11.2‰ with a mean of 5.5 ± 3.9‰, which is in the range of soil $\delta^{15}N$ values of the várzea (MARTINELLI et al., 1992) and of whole Brazil (YONEYAMA et al., 1993). Generally, $\delta^{15}N$ of soil N declines with depth in the profile, because small highly degraded particles are translocated to deeper soil layers in contrast to larger, less degraded ones (LEDGARD et al., 1984). This trend was also seen in the study area, but no significant differences between the soil layers existed. This might be due to the relatively small number of samples and the high variability. Normally, $\delta^{15}N$ values of soil total N are significantly higher than the values of reference plants (YONEYAMA et al., 1993), which is due to

fractionation processes during N uptake (YONEYAMA et al., 1991b) and also to lower $\delta^{15}N$ values of the plant available N (BINKLEY et al., 1985). In the study area, the values of the reference plants, ranging from 3.2‰ to 6.8‰ were in the range of the $\delta^{15}N$ values of the plant available N pool, but also not significantly different from the values of the soil total N. Although the $\delta^{15}N$ value of the soil extractable N ranging from 1.8‰ to 7.1‰ were lower than the values of the soil total N, no significant differences existed. Normally, isotopic fractionation during ammonification and nitrification leads to lower ^{15}N abundance in NH_4^+ and NO_3^- in comparison to total N (MARIOTTI et al., 1982).

$\delta^{15}N$ of plants

Application of the ^{15}N natural abundance method in the study area was possible. The prerequisite of significant differences in $\delta^{15}N$ between N derived from atmosphere ($\delta^{15}N$ value of the N_2 fixing plants grown without soil N ranging from -8.7‰ to -0.1‰) and N derived from soil ($\delta^{15}N$ value of the reference plants ranging from 3.2‰ to 6.8‰) was given, and the observed variability between the non-N_2 fixing reference plants (difference between the maximum and the minimum $\delta^{15}N$ value of the reference plants was 3.6‰ units) was small compared to the difference between the mean $\delta^{15}N$ value of reference plants (reference values ranging from 4.0‰ to 7.1‰) and N_2 fixing legumes grown without soil N (-4.9‰; difference was 8.9‰ to 12‰ units) (SHEARER and KOHL, 1988b; 1991).

Plant internal differences in $\delta^{15}N$ values existed for several legume and non-legume trees in the study area, woody tissue had the lowest $\delta^{15}N$ values, leaves, fruits and blossoms had medium values, nodules had the highest $\delta^{15}N$ values (Figure 4.17). This pattern is in accordance with studies on several species (SHEARER et al., 1982; 1983; THIELEN-KLINGE, 1997). In contrast to the woody parts, the nodules with their strongly elevated $\delta^{15}N$ value in comparison with the rest of the plant had little effect on the $\delta^{15}N$ value of the plant, since only a small fraction of the plant N was in the nodules (SHEARER et al., 1983). The reasons behind these differences in $\delta^{15}N$ value between plant parts are isotopic fractionation during metabolic processes and N uptake and transport of isotopic altered metabolites. Internal cycling of N within the tree results in a depletion of ^{15}N in N sinks, e.g. the translocation of N to stem-wood causing the low $\delta^{15}N$ value of stems (SHEARER et al., 1983). Since in the study area N_2 fixing and reference trees showed basically the same plant internal $\delta^{15}N$ pattern, it is

acceptable that the $\delta^{15}N$ values of leaves are used for calculating the percentage of N derived from atmosphere (SHEARER and KOHL, 1988b).

High $\delta^{15}N$ values of young leaves by comparison with medium or old leaves were in accordance with findings of PEOPLES et al. (1991). The difference between the $\delta^{15}N$ values between young and medium leaves can be explained by fractionation processes during the development of the leaves. In young leaves mainly anabolic processes with isotopic fractionation coefficients (β) of 1.0091 to 1.0184 take place, while in older leaves catabolic processes with lower β values of 1.0017 to 1.0170 are taking over (YONEYAMA et al., 1991a; 1991b).

The significantly lower $\delta^{15}N$ values of the legumes by comparison with the non-legumes indicated a remarkable contribution of fixed N for these plants and for the ecosystem (Figure 4.18). The $\delta^{15}N$ values of the non-legumes were basically in the same range as other non-N_2 fixing trees in Brazil (YONEYAMA et al., 1993). Significant N_2 fixation of the legumes was probably due to favourable conditions for legume-rhizobia symbioses in the várzea (OLIVEIRA et al., 1998; MOREIRA et al., 1992), and the relatively young age of the studied forest (SPRENT, 1987). But it is in contrast to other findings in Brazil (SALATI et al., 1982; YONEYAMA et al., 1993) and Costa Rica (SHEARER and KOHL, 1986), where N_2 fixation of woody legumes was negligible. That woody legumes nodulate abundantly and fix significant amounts of N has been shown before (SANGINGA et al., 1990; KADIATA et al., 1996). The nodule nitrogenase activities of the 4 investigated legume trees were remarkable, values were in the lower range of legume crop activities (WERNER, 1992; VÁSQUEZ-ARROYO et al., 1998). The slightly higher N contents in legumes by comparison with non-legumes, due to N_2 fixation (KADIATA et al., 1996) were in accordance with findings of YONEYAMA et al. (1993).

For most species isotope data correlated well with nodulation, which was in accordance with previous studies (NDIAYE and GANRY, 1997; PATE et al., 1998). In contrast, the nodulated legumes *Pterocarpus amazonum* and *Neptunia oleraceae* had high $\delta^{15}N$ values not significantly different from the ones of the non-nodulated plants. For *Pterocarpus amazonum* N_2 fixation per nodule dry weight was relatively high, so that the high $\delta^{15}N$ value should be due to scarce nodulation, and correlation of N_2 fixation with nodule numbers (VÁSQUEZ-ARROYO et al., 1998). Low nodule numbers were suspected due to observations in the forest, where it was difficult to find nodules on *Pterocarpus amazonum*, from 20 trees searched,

nodules could only be found on 4. *Neptunia oleraceae* was abundantly nodulated in the study area, and N_2 fixation by it was reported before (SUBBA-RAO et al., 1995), so that its high $\delta^{15}N$ value was surprising. It might be due to low nitrogenase activity of the nodules or different fractionation during N uptake or metabolism, since the $\delta^{15}N$ value of the water was not different from the value of the soil extractable N. On the other hand, some non-legumes had relatively low $\delta^{15}N$ values similar to the ones of the legumes. One explanation might be the effects of different rooting depth and mycorrhizal infection on the $\delta^{15}N$ values of the plants (HÖGBERG, 1990; FREY and SCHÜEPP, 1992; RAO and GILLER, 1993). These are due to the utilisation of different N sources and improved soil N uptake (REDECKER et al., 1997). 81% of the tree species in the várzea have vesicular-arbuscular mycorrhizal symbioses. For instance, the roots of *Tabebuia barbata*, the non-legume with the lowest $\delta^{15}N$ value, show a high density of intercellular hyphae and vesicles in contrast to *Crateva benthamii* with no mycorrhiza and the highest $\delta^{15}N$ value (MEYER, 1991). But since the root system was unknown and the mycorrhiza status was only know for less than 40% of the plants under study, a systematic or statistical analysis of the variability of leaf $\delta^{15}N$ values in correlation to this effect was impossible in this study.

The relatively large intraspecific variation of $\delta^{15}N$ values found in the study area and the trend of high coefficients of variance for species with low $\delta^{15}N$ values was in accordance with results of YONEYAMA et al. (1993), who had coefficients of variance up to 200%. The large intraspecific variation of the 3 monthly sampled *Albizia multiflora* trees was due to 1 selected tree, which apparently did not fix N_2. This tree appeared to be old or unhealthy with dead branches and it lost the leaves earlier than the majority of the *Albizia multiflora* trees. Generally, the variability in $\delta^{15}N$ values between individual trees of one species was probably due to site effects, plant conditions and differences in N_2 fixation, but only few factors have been investigated. The negative correlation between leaf N content and $\delta^{15}N$ values was probably due to isotopic fractionation accompanying mobilisation of N from leaves, which results in ^{15}N enrichment of the leaf progressively as more leaf protein is degraded and translocated to other tissue (SHEARER et al., 1983). The positive correlation of $\delta^{15}N$ values and basal area might be due to decreasing nodulation with tree growth and age (INGRAM, 1990), or metabolic changes with tree ageing. The increase of $\delta^{15}N$ values with increasing elevation for *Pterocarpus amazonum* should be due to soil conditions, like different biological activity, N availability or denitrification resulting in different soil isotopic fractionation with elevational

changes (GARTEN, 1993). Generally, nodulation is greatly influenced by the environment, and varies enormously from plant to plant (ODEE et al., 1995), and factors influencing N_2 fixation are micro-symbionts efficiency, plant condition and soil properties like pH, moisture content, temperature, and nutrient availability (WILLIAMS et al., 1988; OLIVEIRA et al., 1998; MARTÍNEZ-ROMERO et al., 1998). The largest intraspecific differences were expected between seedlings and trees, but only half of the investigated species showed significant differences in $\delta^{15}N$ values and N contents of leaves. Differences in $\delta^{15}N$ values were probably due to different metabolic activities of trees and seedlings, and to differences in nodulation. Time and efficiency of seedling nodulation is dependent on the plant species and on the rhizobial strains (OLIVEIRA et al., 1998). A special case are ant-plants, like *Cecropia latiloba* where basically all trees were occupied and seedlings were unoccupied by ants. Plants occupied by ants use different N sources than plants unoccupied by ants, and therefore show significantly different $\delta^{15}N$ values (SAGERS et al., 2000). This may explain the significant difference between $\delta^{15}N$ values of *Cecropia latiloba* seedlings and trees (Figure 4.19).

Most N_2 fixing and non-N_2 fixing species did not show significant seasonal variation in their $\delta^{15}N$ values, only *Pterocarpus amazonum, Nectandra amazonum, Pseudobombax munguba* and *Cecropia latiloba* had significantly higher $\delta^{15}N$ values at the end of the aquatic period and/or at the beginning of the terrestrial period compared to the rest of the hydrological cycle (Figure 4.20). Since these differences were mainly apparent for non-legumes, they could not be due to changes in N_2 fixation. It is believed, that also during the aquatic period leaf $\delta^{15}N$ values reflect the isotopic ratio of the N sources, although due to a reduction in biomass production (WORBES, 1986), N uptake was probably reduced as well. At least during leaf flushing, which was for most species at the end of the aquatic period, N uptake of plants should be high. Transfer of stored N from woody tissue or roots to leaves during the first flush did not happen, because the effect of isotopic fractionation accompanying such translocation would result in new developing leaves being depleted in ^{15}N corresponding to the woody plant parts (SHEARER et al., 1983) whereas in fact leaf tissue was enriched in ^{15}N. The increased $\delta^{15}N$ values correspond to increased leaf N contents, and to the time of the flushing of new leaves for all species, except for *Cecropia latiloba* which grows new leaves during the whole year. Since the high $\delta^{15}N$ values of young leaves were evident in the study area, it was believed that the seasonal variability in leaf $\delta^{15}N$ values was due to leaf flushing and thus plant phenology. Apparently, the nodulated legumes were well adapted to the periodical flooding, so that they were able to fix N_2 during the whole hydrological cycle. Wetland

legumes are able to grow active nodules and fix N_2 under flooded conditions (BECKER and GEORGE, 1995; JAMES et al., 2001), in contrast to other legumes for which water-logging reduces nodulation and N_2 fixation (MINCHIN and SUMMERFIELD, 1976; SUNG, 1993). Normally, low O_2 concentrations, which occur during flooding, reduce root growth and alter root N metabolism significantly (WERNER et al., 1980). However, sensitivity to low O_2 concentrations is quite variable between legume species (THYNN and WERNER, 1996) and the várzea plants need to be well adapted to periodical flooding, for example with pressurised gas transport, to support a sufficient O_2 supply for the root system (GRAFFMANN, 2000), and probably also to maintain symbiotic N_2 fixation during flooding. Nodule adaptations to low O_2 partial pressures are possible (DAKORA and ATKINS, 1991) and may be necessary to avoid a significant decrease of N_2 fixation due to a lack of O_2 (ARRESE-IGOR et al., 1993).

The reference plant concept is quite critical, since results of %Ndfa calculations vary significantly dependent on the reference used (HAMILTON et al., 1992; AWONAIKE et al., 1993). The reference value obtained with non-N_2 fixing plants differs depending on soil type (YONEYAMA et al., 1993), the soil layers where roots are developing (LEDGARD et al., 1984), the form of N available for plants (YONEYAMA et al., 1990a) etc. Therefore, ideally the non-N_2 fixing species should obtain N in exactly the same way and have the same physiology and metabolism as the N_2 fixing plant. For some crop legumes, this is well achieved with a non-nodulated cultivar of the same species (KILIAN et al., 2001), but this is not an option in natural ecosystems. Therefore, as suggested by CHALK et al. (1996), different approaches were used for the selection of appropriate reference values. One was to select species which were suitable and most similar to the nodulated legumes (THIELEN-KLINGE, 1997), the other was to evaluate a diverse range of non-N_2 fixing plants and use their average as reference value (AWONAIKE et al., 1993; CHALK et al., 1996; BODDEY et al., 2000). Accordingly, different models for the determination of the reference value were used, so that the results, ranging from 4.0‰ to 7.1‰, provide a basis for the calculation of minimum and maximum scenarios of the percentage of N derived from atmosphere for the nodulated species. However, this broad range of possible reference values reveals, that only a semi-quantitative estimate of the N gain via symbiotic N_2 fixation can be achieved (UNKOVICH et al., 1994).

The $\delta^{15}N$ values of the nodulated legumes grown without soil N differed greatly between species (Figure 4.22), probably due to different rhizobia (YONEYAMA et al., 1986) and N transport systems of the plants (YONEYAMA et al., 1991a). The average value of -4.9‰ was

low, compared to literature data. Most reported $\delta^{15}N$ values for biologically fixed N are in the range from -4‰ to 2‰ (DOMENACH and CORMAN, 1984; SHEARER and KOHL, 1986; 1988b). LEDGARD (1989) reported more negative $\delta^{15}N$ values in plants under nutrient deficiency by comparison with plants under optimal nutrient supply, but this explanation seemed unlikely, due to a good supply with N free nutrient solution during this experiment. As far as it is possible to judge, no experimental errors occurred, the seedlings grew (Figure 4.21) and thus, needed to take up N from air during the experiment and expectedly, the $\delta^{15}N$ values decreased with time along with the change from the N sources air and soil while they were growing in the forest, to exclusively air while they were growing in N free substrate during the experiment. However, due to the remarkable low $\delta^{15}N$ values of *Mimosa pigra* and *Albizia multiflora* grown without soil N, the average $\delta^{15}N$ value of biologically fixed N of the legumes under study was -4.9‰.

The %Ndfa values of the woody and non-woody nodulated legumes were between 2% and 70% (Table 4.13), similar to the results of YONEYAMA et al. (1993) who reported values from negligible to 80% Ndfa for tropical plants in Thailand. But their finding of high %Ndfa of small plants and negligible %Ndfa for legume trees was not confirmed, probably due to favourable conditions for nodulated legumes in the study area as already mentioned. More than half of the species had %Ndfa values over 40%, similar to values of legume crops. For example, lentils have %Ndfa values of 52% to 56%, clover have values between 43% and 67%, common bean have values of 0% to 58% (CASTELLANOS et al., 1996; VÁSQUEZ-ARROYO et al., 1998; MOAWAD et al., 1998). Depending on the reference value, the %Ndfa values of the whole várzea forest (4-5%) varied by 22%, which is a similar level of precision as reported by SANGINGA et al. (1996). The estimated N gain via symbiotic N_2 fixation of 12.9 to 16.1 kg N ha^{-1} y^{-1} was relatively high by comparison with tropical secondary vegetation which was found to fix between 0.1 and 4.7 kg N ha^{-1} y^{-1} (THIELEN-KLINGE, 1997). This might be due to more abundant nodulation in floodplain forests by comparison with terra firme forests (SYLVESTER-BRADLEY et al., 1980; MOREIRA et al., 1992), counteracting the fact that generally N_2 fixation of nodulated legumes is believed to be more important in disturbed areas by comparison with natural forests (FARIA et al., 1984; SPRENT, 1987). But of course, the estimated N gain was small by comparison with mono-specific agricultural stands of legume crops. Lentils, for example, may fix 127-139 kg N ha^{-1} y^{-1}, clover plants fix 27-205 kg N ha^{-1} y^{-1} and faba bean fix between 200 and 360 kg N ha^{-1} y^{-1} (MOAWAD et al., 1998; KILIAN et al., 2001).

The advantage of the isotopic methods is that they provide a yield-independent estimate of the proportional dependence of legumes on atmospheric N_2 (CHALK 1985; CHALK et al., 1996). The ^{15}N natural abundance method has certain advantages, particularly in natural ecosystems, since disturbance of the system is not required and comparisons of estimates of N_2 fixation based on the ^{15}N natural abundance method with estimates based on other methods revealed good accordance (SHEARER and KOHL, 1988b). Difficulties with the method are mainly due to the reference-plant concept, which is subject to remarkable error (LEDGARD et al., 1984; YONEYAMA et al., 1990a; HÖGBERG, 1990), so that estimates of %Ndfa based on these values may become semi-quantitative. Therefore, investigations of $\delta^{15}N$ values of plant-available soil N (PEOPLES et al., 1991), of nodulation, mycorrhizal infection and plant growth patterns (YONEYAMA et al., 1993) are additionally required. In this study, these problems were taken into consideration during the selection of the reference plants and most importantly in using different approaches for the calculation of the reference value. However, in spite of its many limitations, the ^{15}N natural abundance method remains the best option to estimate N_2 fixation in natural ecosystems (HÖGBERG, 1997).

5.3.2 Symbiotic N_2 fixation under experimental conditions

As expected, in the ^{15}N labelled treatment, due to a constant loss of ^{15}N after the labelling, $\delta^{15}N$ values of the soil total N pool decreased significantly during the experiment (Figure 4.23). A problem was the high variability between pots, which was due to inhomogeneous distribution of ^{15}N. Apparently, despite the high precaution which was taken with adding of $(^{15}NH_4)_2SO_4$ and mixing of the soils, homogeneous labelling in all pots could not be achieved. In the unlabelled treatment mean $\delta^{15}N$ value of the soil total N pool was 4.2‰, corresponding to the litter values in the study area, but slightly lower than the values of the mineral soil. The mean soil N content of 0.8 g $kg^{-1}$of the experiment, corresponded to the soil N contents at 20-60 cm depth in the study area.

Growth of the seedlings occurred, but inexplicably biomass production decreased during the last months of the experiment (Figure 4.24). For *Campsiandra comosa* and *Albizia multiflora* the decrease in growth corresponded with an increase of $\delta^{15}N$ value, due to N_2 fixation being directly related to plant growth (CECCATTO et al., 1988). Critical for the evaluation of the experiment was the high variability of leaf $\delta^{15}N$ values of plants in different pots, which prevented significant differences between nodulated and non-nodulated species, especially in

the ^{15}N labelled treatment (Figure 4.25). $\delta^{15}N$ values of *Campsiandra comosa* in both treatments indicated negligible N_2 fixation at least at the end of the experiment, in contrast to *Albizia multiflora* which $\delta^{15}N$ values indicated remarkable N_2 fixation during the whole experiment. Consequently, temporal means of %Ndfa values of *Campsiandra comosa* were only between 0% and 15%, whereas for *Albizia multiflora* temporal means were between 28% and 76% Ndfa.

The %Ndfa results of the isotope dilution method varied strongly from the results obtained with the ^{15}N natural abundance method. Differences may be due to methodological differences and differences between the environmental conditions in the experiment and in the forest. Additionally, differences between seedlings and trees might be important, since generally nodulation and N_2 fixation potential of legumes vary with plant age (SANGINGA et al., 1990; KADIATA et al., 1996). But in this case it seemed to be insignificant, since generally significant differences between seedlings and trees in the forest were scarce and for all experimentally investigated species, except for *Cecropia latiloba*, differences between leaf $\delta^{15}N$ values between seedlings and trees in the forest were small by comparison with the difference between $\delta^{15}N$ values between seedlings in the experiment and seedlings in the forest (Table 4.15). Particularly, for *Campsiandra comosa*, the ^{15}N natural abundance method in the forest revealed significantly higher %Ndfa values than the isotope dilution method but they were also higher than the unlabelled treatment of the experiment, although this difference was smaller (Table 4.16). In contrast, for *Albizia multiflora*, the isotope dilution method revealed significantly higher %Ndfa values than the ^{15}N natural abundance method in the forest, even with a higher difference than the unlabelled treatment of the experiment. Therefore, the 2 nodulated legume species showed diametrical deviations comparing the 2 methods. For *Campsiandra comosa*, the differences between the results seemed to be due to methodological differences and due to the difference between environmental conditions in the experiment and in the forest. For *Albizia multiflora* the results seemed to differ especially due to the difference between the isotope dilution method and the ^{15}N natural abundance method.

The isotope dilution method had been used successfully, especially for small trees and in pot experiments (DANSO et al., 1992; 1995), but it may cause problems and is not feasible for large specimens, mainly because of the difficulties associated with incorporation of the isotopes in the total soil root volume. There was also the additional difficulty for the isotope labelling in the várzea forest due to the periodic flooding, thus a pot experiment was

undertaken. The disadvantage of the isotope dilution method is clear, that the isotopes are normally not distributed uniformly in either time or space (WITTY, 1983; BODDEY and VICTORIA, 1986). Consequently, there is no guarantee that the isotopic composition of the soil N taken up by different plant species will be the same, so that even negative values of %Ndfa may be obtained (CHALK, 1985), due to the estimates depending on reference plants (BODDEY et al., 1990; BREMER et al., 1993). It may be possible to overcome the problem connected with reference plants, when the ^{15}N enrichment of mineral N extracted from soil is used as the reference criterion to estimate the percentage of N derived from atmosphere of legumes (CHALK et al., 1996). Unfortunately, due to analytical problems, this was impossible in this study. With the limitations of the isotope dilution method, the data obtained by the ^{15}N natural abundance method are considered to be more reliable.

In summary, symbiotic N_2 fixation by legume-rhizobia symbioses was independent from the flood pulse. Due to the high N_2 fixation potential of the legume species and their importance in the studied forest area, their N gain might have been important for the várzea forest during the whole hydrological cycle.

5.4 Denitrification

Denitrification showed high spatial variability, as well as significant variation with time and soil depth. Control of denitrification is complex, since it is dependent on NO_3^- and favoured by anoxic conditions (TIEDJE, 1988). On the other hand nitrification, necessary for replenishment of NO_3^- in the soil, depends on oxic conditions and NH_4^+. Measurements during the terrestrial period showed that denitrification seemed to be mainly influenced by the gravimetric water content and the soil pH. The soil water content influences O_2 supply, transportation of mineral nutrients and general metabolic activities and thus affects also denitrification (STARK and FIRESTONE, 1995). In the study area, denitrification increased with increasing soil water content, and when gravimetric water content in the surface soil layer was below 33% no considerable denitrification occurred (Figure 4.28). This is mainly due to increasing O_2 availability with low soil water contents and therefore, unfavourable conditions for denitrification (TIEDJE, 1988). This correlation between water content and denitrification was according to results from experiments and field measurements (KILIAN and WERNER, 1996; BRETTAR et al., 1998; BOLLMANN and CONRAD, 1998). These findings during the terrestrial period corresponded to maximum denitrification rates during the aquatic period.

Generally, denitrification is not strongly acid sensitive (PARKIN, 1987), but still more neutral conditions are favourable for denitrification (Figure 4.28). Denitrification slightly increased with increasing pH values, confirming results of HALL et al. (1998) and STEVENS et al. (1998) who found denitrification limited by acidity. Denitrification was not significantly correlated with NH_4^+-N and NO_3^--N contents, (temperature and elevation), which might be due to small spatial differences in soil properties, which were not measured in sufficient resolution. Still, the lack of correlation between denitrification and mineral N contents is surprising, especially in the case of NO_3^--N, since denitrification depends on this substrate (TIEDJE, 1988). These simultaneously measured soil properties could only explain 29% of the variation in denitrification during the terrestrial period. Thus, other factors, for example O_2 and organic matter/total C availability must have had a significant influence as well.

Corresponding results of low O_2 partial pressure in soil air and high denitrification rates existed, but since O_2 concentrations were not measured simultaneously with denitrification, they could not be statistically proven. At the beginning of the terrestrial period in September/October 1999, the minimum O_2 partial pressure in soil air was measured (Figure 4.2), as well as high denitrification rates (Figure 4.29), and high O_2 concentrations in December 1999 were accompanied by low denitrification rates. Furthermore, denitrification seemed to be favoured by high organic matter and total C content in litter, since despite good aeration, denitrification was highest in litter in comparison to the mineral soil. No significant effect of different plant species on denitrification existed, which was in contrast to studies in agricultural systems. For example, KILIAN and WERNER (1996) found a fourfold increase in denitrification in plots of N_2-fixing plants compared with non-N_2 fixing plants, and CHAO and CHAO (1997) found increased nitrification in soils under legumes by comparison with other soils. But conditions in an agricultural system are relatively homogeneous by comparison with forest environments. In the mixed forest stand of the várzea, roots are intertwined of many different species, so that soil below non-legumes might be influenced by legumes as well, and vice versa. The high variability in denitrification rates within small areas of forest ground was consistent with previous soil studies (GROFFMAN et al., 1992; MERILL and ZAK, 1992; WATTS and SEITZINGER, 2000), where coefficients of variance higher than 500% were measured (FOLORUNSO and ROLSTON, 1984). The reason were probably diminutive spatial differences in soil properties, like uneven dispersion of particulate organic C, creating “hot spots”, small areas with extremely high denitrification rates (CHRISTENSEN et al., 1990; PARKIN, 1987).

Incubations of soil from different depths during receding water resulted in the maximum denitrification rate at 250 to 300 cm soil depth, just below the water table (Figure 4.30). Apparently, available C was sufficient to support denitrification in the sub-soil (RICHARDS and WEBSTER, 1999). Anaerobic conditions at 250 to 300 cm depth favoured denitrification, but sufficient NO_3^- must be available to enable a high denitrification rate. The most important source of NO_3^- apparently resulted from leaching from the surface soil layers during flood recession and precipitation (LUO et al., 1998). The lower denitrification rates in the soil profile at the end of the terrestrial period in comparison with those in October 1999 were most likely due to a loss of NO_3^- via leaching and plant uptake during the terrestrial period. Denitrification was higher in litter than in mineral soil, which might be due to stimulation of denitrifying bacteria as the C source of the leaf litter becomes available. In comparison, denitrification rates in litter were slightly lower than rates measured in a Mediterranean *Pinus* forest (MUSACCHIO et al., 1996).

Considerable seasonal differences in denitrification rates occurred in the floodplain forest soil, due to the flood pulse (Figure 4.31). High rates occurred during flooding and the transition periods. The occurrence of the maximum denitrification during flooding was surprising, since KERN et al. (1996) reported much higher denitrification rates in exposed sediments of Lago Camaleão than in flooded sediments. In the forest soil however, O_2 release from the roots may support nitrification within the otherwise anoxic soil and therefore also denitrification (ARTH and FRENZEL, 2000). And possibly increasing O_2 and NO_3^- concentrations in the water column due to the change of water flow and through-flow of river water within the floodplain, starting mid April 1999, created this denitrification maximum, although surprisingly maximum O_2 concentrations in the flooding water were not measured until August 1999. The relatively high denitrification rates during the transition periods are due to small localised changes in aerobic and anaerobic conditions. Due to its low solubility in water, oxic-anoxic interfaces develop, which separate aerobic from anaerobic processes (BRUNE et al., 2000). Exposure of soil to air with receding water is favourable for oxidation of NH_4^+, and with possible NO_3^- production denitrification rates could have increased. With increasing soil water content during rising water, O_2 availability decreases, supporting the reduction of NO_3^-. Annual N loss in the surface soil layer was calculated to be 12.5 kg N ha^{-1}, which was lower than the N loss of nearly 32 kg N ha^{-1} y^{-1} measured for unvegetated semi-aquatic sediments on Ilha de Marchantaria (KERN, 1995). However, N loss via denitrification was still remarkable and might play an important role for the várzea forest.

The acetylene block method is widely used to measure denitrification because of its high sensitivity and its practicability in the field. However, it has also been criticised because of problems associated, with uneven penetration of C_2H_2 into soil microsites, and falsifications due to the C_2H_2 gas (RUDOLPH et al., 1991; GROSS and BREMNER, 1992; BOLLMANN and CONRAD, 1997; WATTS and SEITZINGER, 2000). Main problems in the várzea may be the inhibition of nitrification (SCHUSTER and CONRAD, 1992) and incomplete blockage of the N_2O reductase mainly at low NO_3^- concentrations (CHRISTENSEN et al., 1989). Thus denitrification may have been underestimated, so that for interpretation and comparison of absolute denitrification rates, results should be treated as minimum values. Nevertheless, the seasonal and spatial (depth) trends of denitrification should be valid.

In summary, denitrification was strongly influenced by the flood pulse, showing highest rates during the aquatic period. Due to the relatively high N losses, denitrification could have an important influence on the N cycle of the várzea forest.

6. N_2 fixation and denitrification in the context of the N cycle

The N cycle of the várzea forest and the importance of the gaseous N turnover were assessed. An attempt, to balance the input and output N fluxes from and to the forest ecosystem, was undertaken. Values of N_2 fixation and denitrification determined in this study were used and as far as possible the picture completed with data from the literature (Figure 6.1).

N input into the forest ecosystem was possible via symbiotic and non-symbiotic N_2 fixation and via dry and wet deposition. Anthropogenic additions are negligible in most parts of the várzea (KERN and DARWICH, 1997). Output pathways might be denitrification, leaching and erosion. Fires or extractions in the form of logging are possible in the várzea, but they did not occur in the investigated forest during the time of study. N loss via NH_3 volatilisation can be excluded due to the low soil pH. Migration and the exchange with the river due to the periodic flooding were input as well as output pathways for N.

Symbiotic N_2 fixation via nodulated legumes was estimated to be between 12.9 and 16.1 kg N ha^{-1} y^{-1}. Non-symbiotic N_2 fixation in the surface soil layer was 4.1 kg N ha^{-1} y^{-1}. Since not all nodulated legume species could be included in the estimate, and non-symbiotic N_2 fixation in deeper soil layers was neglected as well as associative N_2 fixation, these are minimum values and actual N gain via N_2 fixation should be higher in the study area.

Due to wet, and less importantly dry, deposition N is transferred from the atmosphere to Amazonian forest and soil (LESACK and MELACK, 1991). KERN and DARWICH (1997) reported a deposition of 2.6 kg N ha^{-1} y^{-1} at Lago Camaleão on Ilha de Marchantaria, which is an appropriate value for this balance since it was measured right next to the study area, and spatial variability in quality and quantity of the rain is considerable in Central Amazonia (RIBEIRO and ADIS, 1984; ANDREAE et al., 1990; LESACK and MELACK, 1991). Due to high N concentrations in the rain water during the dry season, over 80% of the N deposition occurs between June and November (KERN, 1995).

Rio Solimões floods the forest on average between 4.7 and 7.6 months per year depending on the elevational range. For the N exchange with the river, the interactions between the forest and the flooding water during that time are crucial. Important processes are sedimentation,

diffusion, re-suspension, fish migration and the exchange of coarse organic detritus and macrophytes.

Sedimentation of particulate N was estimated using a sedimentation rate of 3.14 mg N m^{-2} d^{-1} (KERN, 1995). Therefore, during a flooding period of 4.7 to 7.6 months per year sedimentation would introduce 4.4 to 7.2 kg N ha^{-1} y^{-1}. Uncertainties with this estimation are due to different sedimentation rates within the forest by comparison with the lake and during the period of overbank flow at high water. KERN (1995) calculated a total N input via the river of 10.5 kg N ha^{-1} y^{-1} including dissolved N. Since the other N input pathways via the river were not measured and no appropriate estimation models are known, they could not be included in the balance, and a N input via the river of 4.4 to 10.5 kg N ha^{-1} y^{-1} was assumed.

N output from the várzea to the river due to re-suspension of sediments and dissolved N is 6.0 kg N ha^{-1} y^{-1} (KERN, 1995). Some N loss is assumed by the feeding of fish, which are migrating into the forest with rising water feeding on fruits, and therefore extracting N when they return to lakes and rivers (GOTTSBERGER, 1978). With receding water on the one hand organic detritus from the trees is carried away with the water, on the other hand dead wood and macrophytes are deposited on the forest floor (MARTIUS, 1997). But these exchange processes could not be included in the balance, due to a lack of suitable estimation models.

Denitrification in the surface soil layer was 12.5 kg N ha^{-1} y^{-1}. Since denitrification in deeper soil layers was neglected, this is a minimum value and actual N loss via denitrification should be higher in the study area. N leached from the soil at 0-20 cm depth was measured to be 1.2-3.8 kg N ha^{-1} y^{-1} in the study area (KREIBICH et al., in preparation). Erosion is probably not very high, due to the protective vegetation cover with dense root-systems in litter and the surface soil layers (MEYER, 1991), and probably high infiltration due to silty soil, but it was not measured and no suitable estimation models are known.

Table 6.1 Overview of N input and output fluxes to and from the studied várzea forest, measured or estimated as described in this chapter. Percentages of the input and output fractions are calculated for the single pathways.

	N input to the forest	
	[kg N ha^{-1} y^{-1}]	**[%]**
symbiotic N_2 fixation	12.9 - 16.1	48 - 54
non-symbiotic N_2 fixation	4.1	12 - 17
dry and wet deposition	2.6	8 - 11
exchange with the river	4.4 - 10.5	18 - 32
total N input	24.0 - 33.3	
	N output from the forest	
	[kg N ha^{-1} y^{-1}]	**[%]**
denitrification	12.5	56 - 63
leaching	1.2 - 3.8	6 - 17
exchange with the river	6.0	27 - 30
total N output	19.7 - 22.3	

The attempted balance (Table 6.1) has to be seen as a preliminary result. Nevertheless, the following conclusions can be drawn. The gaseous N turnover plays an important role in the studied forest area, covering 60% - 71% of the N input and 56% - 63% of the N output. The high abundance of N_2 fixing legumes provides the main source for N, even surpassing the N gain via the river. Denitrification is the main pathway for N loss from the várzea soil, confirming results of KERN and DARWICH (1997). Since total N input was assumed to be 24.0 - 33.3 kg N ha^{-1} y^{-1}, and total N output only 19.7 - 22.3 kg N ha^{-1} y^{-1}, there was a surplus of N in the study area. Suggesting a N retention in the studied forest and therefore increasing growth and succession of the mid-stage forest.

The nutrient balance of a tropical forest determines whether it can be utilised on a sustainable basis or not (COLE, 1995; WHITMORE, 1998). Due to the considerable N input via N_2 fixation and the flooding water, an open N cycle of the forest is suggested according to the definition of BAILLIE (1989), who defined closed nutrient cycles as such, when the ecosystem is sustained entirely by atmospheric inputs and internal cycling of nutrients whereas in open nutrient cycles also other sources exist. In the várzea forest, the amount of N stored in the biomass (1479 kg N ha^{-1}; FURCH, 1997) is about 53 times the annual N input (24.0 - 33.3 kg N ha^{-1} y^{-1}), in terra firme forests this ratio is about 500 (BROUWER, 1996). Therefore, and since the N gain clearly surpasses the N loss of the studied forest, a sustainable utilisation of the várzea forest rather than of terra firme forests seems possible as far as the N budget is concerned. Sustainability means that the nutrient loss needs to be balanced with the N gain over time, so that the N loss of a harvest needs to be replenished before a next harvest.

Therefore, due to the high amounts of N stored in the vegetation, clearing the várzea forest is not an option. Extensive agroforestry systems for continuous production of timber and associated crops seem to be a suitable method to use the floodplain forest. Since the main N gain was due to symbiotic N_2 fixation, and growing perennial N_2 fixing species improves the soil N status (PASTOR and BINKLEY, 1998), sustainable management systems necessarily rely on nodulated legumes (JONES, 1998). Whether additional inoculation with effective rhizobia strains may lead to a significant plant dry weight and N content increase may be tested (KIPE-NOLT et al., 1992; PEREIRA et al., 1993; MOAWAD et al., 1998). Generally, how nodulated legumes may be used in sustainable management, to support the local population and protect the várzea forest ecosystem should be investigated by future research.

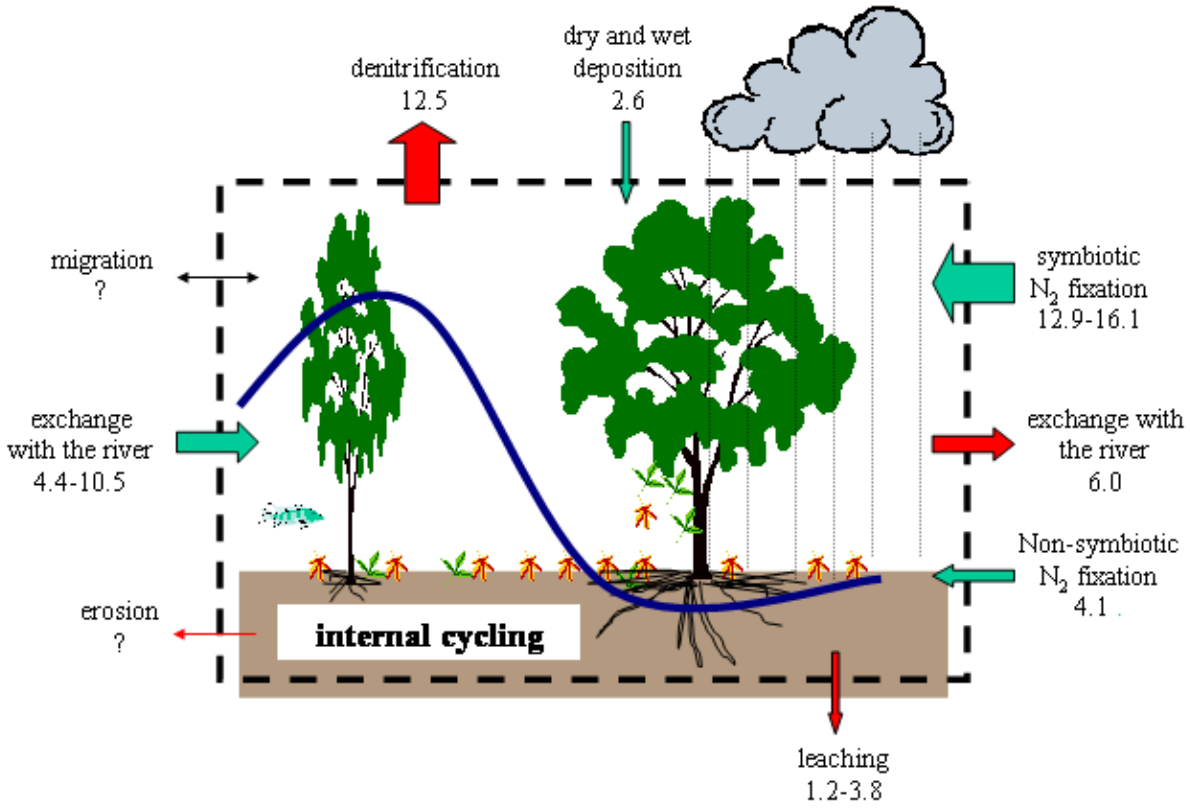


Figure 6.1 Average N fluxes from and to the studied várzea forest (in kg N ha^{-1} y^{-1}) measured or estimated as described in this chapter. The width of the arrows reflect the amount of the input (green) and output (red) N fluxes. When an estimation seemed impossible, N fluxes are indicated by a line.

7. Summary

7.1 Summary

In Amazonia, the most fertile soils are found in the várzea with possibly N as a limiting factor. Small-scale agriculture and shifting cultivation has been practised for centuries, but due to increasing land pressure, sustainable management concepts which support the local population and protect the forest ecosystem are needed. This study focused on the N cycle of a várzea forest, since sustainable agroforestry is only possible within the limits of ecosystem nutrient cycles.

The objective was, to measure the gaseous N turnover, identify the regulating factors and to assess the extent of N_2 fixation and denitrification as a part of the entire N cycle of the várzea forest. Therefore, for the first time in a várzea forest symbiotic and non-symbiotic N_2 fixation and denitrification were monitored simultaneously over 20 months, and their variability related to vegetation and hydrological factors. With an elevational range of 22 to 25 m a.s.l. the studied forest was inundated on average between 4.7 and 7.6 months per year. The forest stand structure was analysed in order to evaluate the importance, diversity and nodulation potential of legume species. Physical, chemical and microbiological soil properties were determined using standard methods, to identify and investigate factors influencing and regulating the gaseous N turnover. For more information about possible symbioses and typical populations of symbiotic N_2 fixing bacteria in the várzea, a cluster analysis of 16S rDNA-PCR restriction fragment length polymorphism and rep-PCR fingerprinting was performed. Analysis of ^{15}N in plants according to the ^{15}N natural abundance method allowed the calculation of the percentage of plant N derived from atmosphere via symbiotic N_2 fixation. A selection of 2 nodulated and 2 non-nodulated species from the study area were treated with ^{15}N labelling in a pot experiment, to compare the results obtained with the ^{15}N natural abundance method with results of the isotope dilution method. Non-symbiotic N_2 fixation and denitrification in forest soil were monitored simultaneously using the acetylene reduction assay and the acetylene block method, respectively. The attempt to balance the gaseous N turnover and put it into context with the N cycle of the studied várzea forest, was undertaken.

Leguminosae were the 3rd important plant family with an exceptionally high species richness of 27%. On average, 68 individual legumes per hectare accounted for 10-12% of the total above ground biomass. The liana *Dalbergia riparia* and the trees *Macrolobium acaciifolium*, *Albizia multiflora* and *Pterocarpus amazonum* were the most important legume species in the study area. Of the 24 legume species found in the study area, 87.5% were nodulated. Therefore, the formation of symbioses between legume species and rhizobia seemed to be highly abundant.

The acidic soil in the study area was rich in organic matter, specially in the surface soil layer, where also N contents were highest. NH_4^+-N contents were constantly about 1 order of magnitude higher than NO_3^--N. During the terrestrial period aeration was good, while hypoxic or even anoxic conditions were characteristic for the aquatic period. Significant seasonal variations due to the flood pulse were apparent for many soil properties but not for bacterial numbers, so that highly flood adapted bacteria species were believed to be present in the study area.

Genotypic diversity of rhizobia was remarkable, considering that only 14 isolates from *Albizia multiflora* and 4 isolates from *Pterocarpus amazonum* were analysed. 5 16S rDNA similarity groups, divided in 2 major clusters, were revealed by analysis of combined RFLP patterns, and a similar diversity was confirmed by rep-PCR fingerprinting. Genotypic grouping was not correlated with the 2 host species. Most isolates were grouped with *Mesorhizobium* spp., 1 with *Bradyrhizobium japonicum*, and 2 isolates could not be classified in the present taxonomic units. Mainly *Mesorhizobium* but also *Bradyrhizobium* species were nodulating *Albizia multiflora* and *Pterocarpus amazonum* on Ilha de Marchantaria.

Non-symbiotic N_2 fixation in the soil was influenced by various factors, which themselves were strongly influenced by the flood pulse, preventing N_2 fixation in the surface soil layer during the aquatic period. In the soil profile, maximum N_2 fixation rates occurred in litter, followed by rates at 0-5 cm soil depth, but also in deeper soil layers N_2 fixation occurred. Annual N gain in the surface soil layer was 4.1 kg N ha^{-1}. Due to the restriction to the terrestrial period, the N gain by free-living diazotrophs, although it was small, might have had positive effects on the biomass production.

The flood pulse seemed to have no influence on symbiotic N_2 fixation, since no seasonal variation in $\delta^{15}N$ values which would suggest differences in N_2 fixation rates between terrestrial and aquatic period was found. The nodulated legumes with *Neptinia oleracea* on the one side and *Teramnus volubilis* on the other, fixed between 2% and 70% of their plant N from atmosphere, respectively. Altogether, between 4% and 5% of plant N of the várzea forest derived from symbiotic N_2 fixation, which resulted in a N gain between 12.9 and 16.1 kg N ha^{-1} y^{-1}. The relatively high N gains via symbiotic N_2 fixation may be important for the várzea forest during the whole hydrological cycle.

Highest denitrification rates in the surface soil layer were measured during flooding and the transition periods, revealing the strong influence of the flood pulse. In the soil profile, maximum rates occurred at 250 to 300 cm soil depth just below the water table, and only in litter was denitrification even higher. Annual N loss via denitrification in the surface soil layer was 12.5 kg N ha^{-1}. Due to the relatively high N losses, denitrification can have an important influence on the N cycle of the várzea forest.

The complete N balance has to be seen as a preliminary result. Nevertheless, conclusions can be drawn. The gaseous N turnover plays an important role in the studied forest area, covering 60% - 71% of the N input and 56% - 63% of the N output. The high abundance of N_2 fixing legumes provided the main N source, even surpassing the N input by the river. Denitrification was the main reason for N loss from the várzea soil. Since the N input clearly surpassed the N output of the studied forest, a sustainable utilisation of the várzea forest seems possible as far as the N budget is concerned. Besides other reasons, due to the high amounts of N stored in the vegetation, clearing the várzea forest is not an option. Extensive agroforestry systems, including nodulated legumes seem to be a suitable method to use the floodplain forest. How nodulated legumes may be used in sustainable management, to support the local population and protect the ecosystem should be investigated by future research.

7.2 Zusammenfassung

Die Várzea beherbergt die fruchtbarsten Böden Amazoniens; wahrscheinlich ist N hier der limitierende Faktor. Seit Jahrhunderten wird kleinflächig Landwirtschaft und Wanderfeldbau betrieben, aber durch den steigenden Bevölkerungsdruck werden nachhaltige Bewirtschaftungskonzepte, die die Ernährung der lokalen Bevölkerung und den Schutz des

Waldökosystems sicherstellen, dringend erforderlich. Die vorliegende Arbeit konzentrierte sich auf den N-Kreislauf des Várzea-Waldes, da nachhaltige Agrarforstwirtschaft nur innerhalb der Grenzen der Nährstoffkreisläufe des Ökosystems möglich ist.

Ziel war es, den gasförmigen N-Umsatz zu messen, die regulierenden Faktoren zu identifizieren und den Anteil der N_2-Fixierung sowie der Denitrifikation am gesamten N-Kreislauf des Várzea-Waldes abzuschätzen. Erstmals in einem Várzea-Wald wurden daher simultan symbiontische und asymbiontische N_2-Fixierung und Denitrifikation über einen Zeitraum von 20 Monaten gemessen und ihre Variabilität im Bezug zu Vegetation und hydrologischen Faktoren untersucht. Durch seine Höhenlage von 22 bis 25 m ü. NN war das untersuchte Waldgebiet im Durchschnitt zwischen 4,7 und 7,6 Monate im Jahr überschwemmt. Die Bestandsstruktur des Waldes wurde untersucht, um Bedeutung, Diversität und Nodulation der Leguminosen festzustellen. Physikalische, chemische und mikrobiologische Bodenbedingungen wurden mit Standardmethoden gemessen, um die beeinflussenden und regulierenden Faktoren des gasförmigen N-Umsatzes zu identifizieren und zu untersuchen. Um mehr Informationen über mögliche Symbiosen und typische Populationen symbiontischer N_2-fixierender Bakterien in der Várzea zu gewinnen, wurden Clusteranalysen der „16S rDNA-PCR restriction fragment length polymorphism“ und „rep-PCR fingerprinting“ durchgeführt. Die Analyse des ^{15}N in den Pflanzen entsprechend der „^{15}N natural abundance“-Methode ermöglichte die Berechnung des Prozentanteils des pflanzlichen N, der mittels symbiontischer N_2-Fixierung aus der Atmosphäre bezogen wurde. In einem Topfpflanzenexperiment wurden zwei nodulierende und zwei nicht nodulierende Baumarten aus dem Untersuchungsgebiet mit ^{15}N markiert, um die Ergebnisse der „^{15}N natural abundance“-Methode mit den Ergebnissen der „Isotope dilution“-Methode zu vergleichen. Asymbiontische N_2-Fixierung und Denitrifikation im Boden wurden simultan mit der Acetylen-Reduktionsmethode und mit der Acetylen-Blockmethode gemessen. Zusammenfassend wurde der Versuch unternommen, den gasförmigen N-Umsatz zu bilanzieren und ihn im Kontext des N-Kreislaufes des untersuchten Waldgebietes zu betrachten.

Leguminosae waren die drittwichtigste Pflanzenfamilie mit einem außergewöhnlich hohen relativen Artenreichtum von 27 %. Im Durchschnitt bildeten 68 Leguminosen-Pflanzen pro Hektar 10–12 % der gesamten oberirdischen Biomasse. Die Liane *Dalbergia riparia* und die Bäume *Macrolobium acacciifolium*, *Albizia multiflora* und *Pterocarpus amazonum* waren die

wichtigsten Leguminosenarten im Untersuchungsgebiet. Von den 24 identifizierten Leguminosenarten waren 87,5 % nodulierend, so dass die Ausbildung von Symbiosen zwischen Leguminosen und Rhizobien sehr häufig und divers erschien.

Der Boden im Untersuchungsgebiet war sauer und reich an organischem Material, vor allem in der obersten Bodenschicht, wo auch die N-Konzentrationen am höchsten waren. Die Konzentration von NH_4^+-N lag immer ungefähr eine Größenordnung über der von NO_3^--N. Während der terrestrischen Periode war die Bodendurchlüftung gut, hingegen waren hypoxische oder sogar anoxische Bedingungen für die aquatische Periode charakteristisch. Signifikante saisonale Veränderungen, hervorgerufen durch den Flutpuls, waren für viele Bodeneigenschaften gegeben, nicht aber für die Bakterienzahlen, so dass sehr flutungstolerante Bakterien im Untersuchungsgebiet vermutet werden.

Die genotypische Diversität der Rhizobien war bemerkenswert, vor allem, da nur 14 Isolate von *Albizia multiflora* und 4 Isolate von *Pterocarpus amazonum* untersucht wurden. Fünf 16S rDNA-Ähnlichkeitsgruppen wurden durch die Analyse kombinierter RFLP-Muster identifiziert, die sich in zwei Obergruppen aufteilten. Ein ähnliche Diversität wurde durch „rep-PCR fingerprinting“ bestätigt. Die genotypischen Gruppen korrelierten nicht mit den zwei Wirtspflanzenarten. Die meisten Isolate waren zusammen mit *Mesorhizobium* spp. eingruppiert, ein Isolat mit *Bradyrhizobium japonicum*, zwei Isolate konnten nicht klassifiziert werden. Auf der Ilha de Marchantaria nodulieren hauptsächlich *Mesorhizobium*-, aber auch *Bradyrhizobium*-Arten die Leguminosen *Albizia multiflora* und *Pterocarpus amazonum*.

Die asymbiontische N_2-Fixierung im Boden wurde von unterschiedlichen Faktoren beeinflusst, die selbst stark vom Flutpuls beeinflusst waren. Dies führte zur Unterdrückung der N_2-Fixierung während der aquatischen Periode in der obersten Bodenschicht. Im Bodenprofil wurden maximale N_2-Fixierungsraten in der Streuschicht gemessen, gefolgt von den Fixierungsraten in 0–5 cm Tiefe. Aber auch in tieferen Bodenschichten trat N_2-Fixierung auf. Der N-Gewinn in der obersten Bodenschicht betrug 4,1 kg N ha^{-1} a^{-1}. Obwohl er gering ist, könnte der N-Gewinn durch frei lebende diazotrophe Bakterien durch die Beschränkung auf die terrestrische Periode doch positive Effekte auf die Biomasseproduktion haben.

Der Flutpuls scheint keinen Einfluss auf die symbiontische N_2-Fixierung zu haben, denn es wurden keine saisonalen Veränderungen der $\delta^{15}N$-Werte gefunden, die auf einen Unterschied in der N_2-Fixierung zwischen der terrestrischen und der aquatischen Periode hindeuteten. Die nodulierten Leguminosen mit *Neptunia oleraceae* auf der einen Seite und *Teramnus volubilis* auf der anderen fixierten zwischen 2 % und 70 % ihres pflanzlichen N aus der Atmosphäre. Insgesamt wurden zwischen 4 % und 5 % des pflanzlichen N des Várzea-Waldes durch symbiontische N_2-Fixierung gewonnen, was in einen N-Gewinn zwischen 12,9 und 16,1 kg N ha^{-1} a^{-1} resultierte. Die relativ hohen N-Gewinne durch die symbiontische N_2-Fixierung waren für den Várzea-Wald wahrscheinlich während des gesamten hydrologischen Zyklus von Wichtigkeit.

In der obersten Bodenschicht wurden höchste Denitrifikationsraten während der Überschwemmung und der Übergangsperioden gemessen, was den starken Einfluss des Flutpulses verdeutlicht. Im Bodenprofil wurden maximale Denitrifikationsraten in 250 bis 300 cm Bodentiefe, genau unterhalb des Wasserspiegels, gemessen, nur in der Streuschicht war die Denitrifikation noch höher. Der Stickstoffverlust in der obersten Bodenschicht betrug 12,5 kg N ha^{-1} a^{-1}. Durch die relativ hohen N-Verluste hat die Denitrifikation einen wichtigen Einfluss auf den N-Kreislauf des Várzea-Waldes.

Die aufgestellte N-Bilanz muss als vorläufiges Ergebnis angesehen werden, trotzdem können folgende Schlüsse gezogen werden: Der gasförmige N-Umsatz spielt eine wichtige Rolle im untersuchten Waldgebiet. Die N_2-Fixierung macht 60 % bis 71 % der N-Einträge aus, während die Denitrifikation für 56 % bis 63 % der N-Austräge verantwortlich ist. Die sehr häufigen N_2-fixierenden Leguminosen stellen die hauptsächliche N-Quelle dar, die an Bedeutung sogar die N-Einträge durch den Fluss übertrifft. Hauptsächlich die Denitrifikation führte zu den hohen N-Verlusten aus dem Várzea-Boden. Da der N-Eintrag den N-Austrag deutlich überstieg, erscheint – zumindest aus Sicht des N-Haushalts – eine nachhaltige Nutzung des Várzea-Waldes möglich. Da große N-Mengen in der Vegetation gebunden sind, stellt die Abholzung des Waldes auch aus diesem Grunde keine gangbare Alternative dar. Extensive Agrarforstwirtschaftssysteme, die nodulierende Leguminosen einschließen, scheinen die passendste Methode zur Nutzung des Überschwemmungswaldes zu sein. Wie im Detail nodulierende Leguminosen in nachhaltigen Managementsystemen eingesetzt werden könnten, um die lokale Bevölkerung zu ernähren und das Ökosystem zu schützen, sollte in zukünftigen Forschungsprojekten untersucht werden.

7.3 Resumo

A várzea da Amazônia Central apresenta os solos mais férteis da região e N provavelmente é o fator limitante local. Há séculos pratica-se na região uma agricultura restringida a pequenas áreas e um cultivo a curto prazo. Devido à uma crescente pressão demográfica surge a nesessidade de divulgar os conceitos para o manejo sustentável da várzea, que suporta a população local e protege o ecossistema das florestas. Este estudo focalizou o ciclo de N de uma floresta de várzea, pois o manejo agro-florestal sustentável apenas é viável dentro dos limites dos ciclos de nutrientes do ecossistema.

Os objetivos foram medir a transformação do N gasoso, identificar os fatores reguladores e avaliar a extensão da fixação e da denitrificação do N_2 como uma parte do ciclo completo de N da floresta de várzea. Pela primeira vez em uma floresta de várzea foram monitorados a fixação simbiótica e assimbiótica e a denitrificação durante um periodo de 20 meses. Sua variabilidade foi relacionada à vegetação e a fatores hidrológicos. A floresta estudada encontra-se a uma altitude de 22 - 25 m a.n.m. e é inunda em média entre 4.7 e 7.6 meses por ano. A estrutura da floresta foi analisada para avaliar a importância, a diversidade e o potencial do nodulação das espécies leguminosas. Para identificar e investigar os fatores que influenciam e que regulam o ciclo do N gasoso foram determinadas as propriedades físicas, químicas e micro-biológicas do solo usando-se métodos padrões. Para obter mais informações sobre possíveis simbioses e populações típicas de bactérias que fixam N_2 em simbioses na várzea, uma análise do conjunto de "16S rDNA-PCR restriction fragment length polymorphism" e do "rep-PCR fingerprinting" foram executados. A análise de ^{15}N das plantas, que foi feita de acordo com o método "^{15}N natural abundance", permitiu o cálculo da porcentagem de N da planta derivada da atmosfera através da fixação simbiótica de N_2. Para uma comparação entre os resultados obtidos com o método "^{15}N natural abundance" e os do "^{15}N isotope dilution method" foram selecionadas duas espécies na área de estudo com nodulação e 2 espécies sem nodulação e marcadas com ^{15}N em uma experiência com plantulas em vasos. A fixação assimbiótica e a denitrificação no solo da floresta foram monitoradas simultaneamente usando o método da redução do acetileno e o método do bloco de acetileno, respectivamente. Resumidamente pode-se dizer que foi feita a tentativa de bilanciar a transformação do N gasoso e analisá-la dentro do contexto do ciclo de N da floresta de várzea em questão.

As leguminosas são a terceira família mais importante de plantas encontradas, apresentando uma riqueza de espécies excepcionalmente alta de 27%. Em média, 68 legumes individuais por hectare implicaram em 10-12% do total da biomassa acima da terra. A liana *Dalbergia riparia* e as árvores *Macrolobium acaciifolium*, *Albizia multiflora* e *Pterocarpus amazonum* foram as espécies leguminosas mais importantes na área de estudo. Das 24 espécies de leguminosas encontradas na área de estudo, 87.5% são noduladas, assim que a formação de simbioses entre espécies leguminosas e rhizobia é abundante.

O solo acídico na área do estudo é rico em matéria orgânica, especialmente na camada na superfície do solo, aonde também as concentrações de N são mais elevadas. Os conteúdos de NH_4^+-N encontrados foram constantemente por volta de uma ordem de valor mais altos do que os de NO_3^--N. Durante o período terrestre a aeração é boa, enquanto que no período aquático condições hypóxicas ou anóxicas são características. Existem muitas variações sasonais significativas nas propriedades do solo devido ao pulso da inundação mas para o número de bactérias não, assim que suspeitamos existirem muitas espécies de bactérias extremamente tolerantes à inundação na área do estudo.

Considerando-se que apenas 14 isolados de *Albizia multiflora* e 4 isolados de *Pterocarpus amazonum* foram analisados, a diversidade genotípica de rhizobia foi notável. A análise de combinados de RFLP identificou 5 grupos de similaridade de 16S rDNA, divididos em 2 conjuntos principais. Uma diversidade similar foi confirmada pelo rep-PCR fingerprinting. Os grupos genotípicos não estão correlacionados às 2 espécies hospedeiras. A maioria de isolados foi agrupada com *Mesorhizobium* spp., uma com *Bradyrhizobium japonicum* e 2 isolados não puderam ser classificados. Na Ilha de Marchantaria principalmente *Mesorhizobium,* mas também espécie de *Bradyrhizobium,* formam simbioses com *Albizia multiflora* e *Pterocarpus amazonum*.

A fixação assimbiótica de N_2 no solo foi influenciada por vários fatores, que por sua vez foram fortemente influenciados pelo pulso da inundação, impedindo o fixação de N_2 na camada de superfície do solo durante o período aquático. No perfil do solo a atividade máxima de fixação de N_2 ocorreu na camada de palha, seguida pela atividade a de 0-5 cm de profundidade. Porém também em camadas mais profundas ocorreu a fixação de N_2. O ganho anual de N na camada de superfície do solo foi de 4.1 kg N ha^{-1}. Devido à limitação ao

período terrestre, o ganho de N por bactérias diazotrophs vivendo livremente, embora pequeno, pode ter tido efeitos positivos na produção da biomassa.

O pulso de inundação não pareceu ter influência na fixação simbiótica de N_2, pois não foram encontradas variações sasonais nos valores de $\delta^{15}N$ que indicariam diferenças nas taxas de fixação de N_2 no período terrestre e aquático. Leguminosas noduladas com *Neptunia oleracea* por um lado e *Teramnus volubilis* por outro, fixaram entre 2% e 70% do seu N vegetal da atmosfera, respectivamente. Em soma entre 4% e 5% do N vegetal da floresta de várzea vem da fixação simbiótica de N_2, o que resulta em um ganho de N entre 12.9 e 16.1 kg N ha^{-1} y^{-1}. Os ganhos relativamente altos de N através da fixação simbiótica de N_2 provavelmente são importantes para a floresta do várzea durante todo o ciclo hidrológico.

As mais altas taxas de denitrificação foram medidas na camada de superfície do solo durante a inundação e os períodos de transição, indicando uma forte influência do pulso de inundação. No perfil do solo as taxas máximas de denitrificação ocorreram a uma profundidade de 250 a 300 cm, justo abaixo do espelho da água. Apenas na camada de palha a denitrificação foi mais alta. A perda anual de N através da denitrificação na camada de superfície do solo foi de 12.5 kg N ha^{-1}. Devido às perdas relativamente altas de N, a denitrificação pôde ter tido uma influência importante no ciclo de N da floresta de várzea.

O balanço de N aqui apresentado deve ser visto como um resultado preliminar. Mesmo assim podemos tirar algumas conclusões. O N gasoso é importante na área de estudo, cobrindo 60% - 71% do input de N e 56% - 63% do output de N. A abundância de leguminosas que fixam N_2 é a principal fonte de N, seu input é até mais alto do que o input de N pelo rio. A denitrificação é o principal motivo para a perda de N do solo da várzea. Como o input de N é significativamente mais alto do que o output de N na floresta estudada, o manejo sustentável da floresta de várzea parece possível ao menos em relação ao balanço de N. Devido às grandes quantidades de N armazenadas na vegetação, o desmatamento da floresta da várzea não é uma opção. Os sistemas agro-florestais extensivos, que incluem leguminosas noduladas, parecem ser um método apropriado para usar a floresta da várzea. Em projetos futuros de pesquisa deveriam ser estudadas as possibilidades de aproveitar as leguminosas noduladas no manejo sustentável, a fins de nutrir a população local e proteger o ecossistema.

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Appendix

Table A.3.1 Yeast-mannitol-broth and -agar medium for rhizobia isolation (DSM catalogue, 1993)

yeast extract	1.0 g
mannitol	10.0 g
agar+	15.0 g
soil extract*	200 ml
distilled water	800 ml

+ include the agar only for yeast-mannitol-agar (solid medium)

*soil extract: Add 80 g air-dried garden soil and 0.2 g $NaCO_3$ to 200 ml distilled water and autoclave the suspension for 1 hour at 121° C. To obtain a clear supernatant allow to settle and centrifuge. Adjust pH to 7.2.

Adjust pH to 7.0

Table A.3.2 Protocol for the isolation of DNA and the necessary solutions (AUSUBEL et al., 1990)

1.	spin the liquid culture in a centrifuge for 7 min with 14 000 rpm, discard the supernatant; after a short-spin, completely remove the remaining supernatant
2.	re-suspend the cell pellet totally in 756 µl TE buffer, which removes magnesium ions from the cell walls and represses DNA degrading enzymes
3.	add 40 µl 10% SDS and 4 µl of proteinase K and incubate for 1 hour at 37° C to lyse the cell walls
4.	add 133.3 µl 5 M NaCl and mix thoroughly, the high salt concentration shall prevent the formation of CTAB-nucleic acid complexes
5.	add 106.7 µl CTAB solution, mix thoroughly and incubate 10 min at 65° C to precipitate denatured protein and polysaccharides as CTAB complexes while the nucleic acids stay dissolved
6.	add 850 µl chloroform/isoamyl alcohol (24:1, v/v) and centrifuge (7 min at 14 000 rpm) to extract the CTAB-protein/polysaccharide complexes
7.	Remove the aqueous top phase to a fresh eppendorf-cup, leaving the interface behind
8.	re-extract with 850 µl chloroform/phenol solution (1:1, v/v), shake carefully and centrifuge again
9.	transfer the top aqueous phase to a fresh eppendorf-cup and add 470 µl isopropanol to precipitate the DNA, shake carefully and centrifuge again for 2 min at room temperature, decant the isopropanol
10.	wash the DNA with 470 µl 70% ethanol, centrifuge again at room temperature, decant the supernatant and let it dry for 2 hours
11.	re-dissolve the DNA pellet in 50 µl TE buffer at 4° C over night

solution	contents
TE- buffer	1 mM EDTA, 10 mM Tris-HCl, pH 8.0
SDS	sodium dodecyl sulfate 10% (w/v)
proteinase K solution	proteinase K 20 mg/ml H_2O (stocks, keep frozen)
CTAB solution	10% Cetyl trimethyl ammonium bromide (CTAB) in 0.7 M NaCl solution: dissolve 4.09 g NaCl in 80 ml H_2O, slowly add 10 g CTAB while stirring, warm up to 60° C and complete to 100 ml with H_2O

Table A.3.3 Standard 50 µl PCR mix

amount	solution
0.3 µl	*Taq* polymerase (10 U/µl)
1.5 µl	sense primer (10 pmol/µl)
1.5 µl	antisense primer (10 pmol/µl)
1.0 µl	dNTPs mix (10 mM each)
2.5 µl	DMSO
5.0 µl	PCR buffer (with 15 mM $MgCl_2$)
37.2 µl	H_2O bidist.

Table A.3.4 PCR cycling parameters

Amplification of the *nod*C gene region

temperature [° C]	time [sec]	
37	30	
60	120	* 5
94	30	
50	30	
72	30	* 27
94	30	
4	∞	

Amplification of the *nif*D and *nif*K gene regions

temperature [° C]	time [sec]	
95	180	
94	60	
63	60	* 35
72	120	
72	180	
4	∞	

Amplification of the 16S rDNA region

temperature [° C]	time [sec]	
95	120	
94	15	
93	45	
55	45	* 10
72	120	
94	15	
93	45	
55	45	* 20
72	120 + 1 sec/cycle	
72	300	
4	∞	

Table A.3.5 Standard rep-PCR mix

amount [µl]	solution
10.4	H_2O (bid.)
2.5	PCR buffer
5.2	$MgCl_2$ solution (25 mM)
2.5	DMSO (dimethylsulfoxid, 99.9%)
2.5	dNTP's (12.5 mM each)
0.5	GTG_5 primer
0.4	*Taq* polymerase (10 U/µl)

Table A.3.6 Gels and solutions used for the gel image documentation

gels and solutions	contents
agarose gel	1.2 g agarose, 150 ml TAE buffer
metaphor agarose gel	2% metaphor agarose in TBE buffer
loading buffer	125 mM EDTA; 45% (v/v) glycerol; 0.125% (w/v) bromphenol blue
TAE buffer	2 M Tris-HCl (pH 8); 1 M acetic acid; 50 mM EDTA
TBE buffer	90 mM Tris-borate; 2 mM EDTA
TE buffer	10 mM Tris-HCl (pH 7.4); 1 M EDTA (pH 8)
molecular size marker	10 µl marker, 20 µl loading buffer, 170 µl TE buffer
EtBr staining solution	Ethidium bromide 1 µg/ml H_2O

Table A.3.7 Theoretical restriction patterns of reference strains calculated for 4 enzymes based on their full-length 16S rDNA nucleotide sequence (calculated sizes of restriction fragments [bp]).

reference strains	*Dde*I	*Hha*I	*Msp*I	*Sau*3AI
Rhizobium galegae 59A2	193 179 147 147 135 133 115 113 101 58	334 280 276 172 135 115 113 79	496 273 222 169 146 123	363 312 223 173 119 65 59
Rhizobium huautlense SO2	363 312 261 138 147 97	334 280 278 172 135 115 114 79	496 273 222 170 146 123	845 232 174 87 83
Mesorhizobium sp. SH 15003	409 367 261 238 147	450 329 280 139 119 115 79	309 254 222 170 146 122 109 86	945 232 174 83
Mesorhizobium amorphae	409 367 260 238 147	450 329 280 139 118 115 79	309 254 222 169 146 122 109 86	945 232 173 83
Rhizobium etli CFN42T	409 364 261 238 147	456 334 280 135 116 114 79	497 419 222 170 123	707 178 175 174 83 56 54
Rhizobium etli TAL 182	408 363 261 238 147	456 334 279 135 114 114 79	495 419 222 170 123	705 178 175 174 83 56 54
Rhizobium tropici (IIB) 899T	410 364 261 239 147	333 280 280 172 117 114 79 69 65	656 421 222 170	706 233 175 174 84 56
Rhizobium tropici (IIB) LMG951	409 362 261 205 147 105	334 294 280 172 114 114 79 70 65 56	655 491 222 170	705 205 175 174 99 83 56
Azorhizobium caulinodans ORS 571T	539 312 261 238 97	334 280 278 172 137 114 114 79	506 419 222 170 151	707 178 175 174 83 60 54
Bradyrhizobium japonicum USDA110spc4	409 365 263 242 111	813 334 137 116 115	502 289 222 170 156 91 81	708 235 182 176 83 54
Bradyrhizobium sp. BC-C2	365 296 263 242 113 111	452 361 334 137 116 115	502 289 222 170 156 91 81	708 236 235 176 83
Bradyrhizobium japonicum USDA 6T	365 296 263 242 113 111	813 334 137 116 115	502 289 222 170 156 91 81	708 235 182 176 83 54
Bradyrhizobium elkanii USDA 76T	409 367 261 128 114 111	452 361 334 139 115 114	495 289 222 170 122 91 79	710 235 174 133 83 54

Table A.3.8 N free nutrient solution for legumes

1000 ml nutrient solution contain:	
[mg]	compounds
279	K_2SO_4
493	$MgSO_4 * 7 H_2O$
23	KH_2PO_4
145	K_2HPO_4
371	$CaCl_2 * 2 H_2O$
1.43	H_3BO_3
1.02	$MnSO_4 * 4 H_2O$
0.22	$ZnSO_4 * 7 H_2O$
0.08	$CuSO_4 * 5 H_2O$
0.05	$Na_2MoO_4 * 4 H_2O$
0.10	$CoCl_2 * 4 H_2O$
16.70	Fe-solution

Table A.4.1 Overview and characterisation of all identified plant species found in the research area in alphabetical order, empirical and literature data (mean values) is included, the grey shading indicates data which was determined in this study. (Leguminosae (C.): Caesalpiniodeae; Leguminosae (P.): Papilionoideae; Leguminosae (M.): Mimosoideae)

species	family	habit	basal area [cm²]	individ. ha^{-1}	wood-density[a] [g cm³]	fruiting season[b]	flushing of new leaves[b]	leaf periodic.[b]	leaf size[b] [cm²]	spec. leaf weight[b] [g m^{-2}]	nodul. status[c]	mycorrh. status[d]	leaf N content [%]
Acosmium nitens	Leguminosae (C.)	tree	602	0.5	0.74	Jun - Aug	ND	deciduous	ND	ND	+	ND	ND
Aeschynomene sp.	Leguminosae (P.)	shrub	ND	ND	ND	ND	ND	ND	ND	ND	+	ND	4.7
Albizia multiflora	Leguminosae (M.)	tree	297	15	0.62	Dec - Mar	Jul - Dec	deciduous	42.8	195	+	ND	3.9
Alchornea sp.	Euphorbiaceae	shrub	67	14	0.34	ND	ND	semi-deciduous	ND	ND	ND	++	ND
Annona montana	Annonaceae	tree	284	4	0.35	Apr - Aug	ND	semi-deciduous	ND	ND	ND	ND	ND
Astrocaryum jauari	Arecaceae	palm tree	ND	ND	ND	Apr - Jun	ND	evergreen	ND	ND	ND	++	ND
Bactris sp.	Arecaceae	palm tree	94	7	ND	ND	ND	ND	ND	ND	ND	ND	ND
Bauhinia sp.	Leguminosae (C.)	shrub	ND	ND	ND	ND	ND	ND	ND	ND	-	ND	4.4
Buchenavia oxycarpa	Combretaceae	tree	13	0.5	ND	Mar - Jun	ND	deciduous	17.1	117.2	ND	ND	ND
Campsiandra comosa	Leguminosae (C.)	tree	3978	0.5	0.81; 0.86	Jan - Jun	ND	deciduous	464.5	84.5	+	ND	2.2
Casearia aculeata	Flacourtiaceae	tree	66	5	0.56; 0.66	ND	ND	ND	20.0; 24.3	47.2; 56.5	ND	+	ND
Cecropia latiloba	Cecropiaceae	tree	262	9	0.32; 0.33; 0.48	Mar - Jun	all year	evergreen	816.9; 819.0	132.4; 210.6	ND	ND	3.1
Chamaecrista sp.	Leguminosae (C.)	tree	ND	ND	ND	ND	ND	ND	ND	ND	+	ND	2.7
Crateva benthamii	Capparaceae	tree	236	249	0.42; 0.49; 0.50	Mar - Jun	Sep - Dec	deciduous	45.9; 58.4	133.5; 133.6	ND	-	3.1
Crescentia sp.	Bignoniaceae	tree	121	0.5	0.5	All year	ND	deciduous	72.7	50.2	ND	++	ND
Crudia amazonica	Leguminosae (C.)	tree	548	0.5	0.87	Feb - Apr	ND	deciduous	ND	ND	-	ND	2.8
Cymbosema roseum	Leguminosae (P.)	w. climb.	ND	ND	ND	ND	ND	ND	ND	ND	(+)	ND	3.6
Dalbergia inundata	Leguminosae (P.)	w. climb.	78	1	0.75-1.00	ND	ND	ND	ND	ND	+	++	2.7
Dalbergia riparia	Leguminosae (P.)	w. climb.	52	23	ND	ND	ND	ND	80.5	62.5	+	ND	3
Dioscorea sp.	Dioscoreaceae	her. climb	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	2.5
Entada polyphylla	Leguminosae (M.)	shrub	ND	ND	ND	ND	ND	ND	ND	ND	+	ND	4.2
Eschweilera ovalifolia	Lecythidaceae	tree	608	1	0.70; 0.75	Apr - Jun	ND	evergreen	ND	ND	ND	+	ND
Gustavia augusta	Lecythiadaceae	tree	373	1	0.55; 0.67	ND	ND	evergreen	104.3	80	ND	+	ND
Ilex inundata	Aquifoliaceae	tree	1494	0.5	0.43	ND	Dec	deciduous	ND	ND	ND	++	ND
Inga splendens	Leguminosae (M.)	tree	ND	ND	ND	ND	ND	ND	ND	ND	+	++	3.8
Laetia corymbulosa	Flacourtiaceae	tree	435	42	0.61; 0.66	Feb - Jun	ND	evergreen	42.8	78.9	ND	ND	2.2
Lonchocarpus sp.	Leguminosae (P.)	w. climb.	120	1	0.70-0.95	ND	ND	ND	ND	ND	+	ND	2.4
Luehea cymulosa	Tiliaceae	tree	2417	14	0.39; 0.48	ND	ND	semi-deciduous	ND	ND	ND	ND	2.1
Mabea nitida	Euphorbiaceae	tree	718	0.5	ND	ND	ND	ND	ND	ND	ND	ND	ND

species	family	habit	basal area [cm²]	individ. ha^{-1}	wood-density[a] [g cm³]	fruiting season[b]	flushing of new leaves[b]	leaf periodic.[b]	leaf size[b] [cm²]	spec. leaf weight[b] [g m^{-2}]	nodul. status[c]	mycorrh. status[d]	leaf N content [%]
Machaerium aristulatum	Leguminosae (P.)	w. climb.	121	2	ND	ND	ND	ND	ND	ND	+	ND	2.3
Machaerium ferox	Leguminosae (P.)	w. climb.	ND	ND	ND	ND	ND	ND	ND	ND	+	ND	ND
Macrolobium acaciifolium	Leguminosae (C.)	tree	1678	9	0.43; 0.49; 0.56	Feb - May	Aug - Dec	semi-deciduous	49.8	192.1	-	++	2.6
Mimosa pigra	Leguminosae (M.)	shrub	11	6	ND	ND	ND	deciduous	5.3	48.4	+	ND	3.5
Nectandra amazonum	Lauraceae	tree	1045	5	0.44; 0.47; 0.52	Apr - Aug	May - Nov	evergreen	29.4; 50.7	91.4; 150.4	ND	ND	2.4
Neptunia oleracea	Leguminosae (M.)	aqua. pl.	ND	ND	ND	ND	ND	ND	11.3	182.3	+	ND	3.8
Oryza perennis	Poaceae	herb	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Piranhea trifoliata	Euphorbiaceae	tree	265	6	0.77; 0.91	ND	ND	semi-deciduous	ND	ND	ND	ND	ND
Pouteria glomerata	Sapotaceae	tree	147	11	0.68	ND	ND	evergreen	ND	ND	ND	ND	ND
Pseudobombax munguba	Bombacaceae	tree	1775	17	0.20; 0.22; 0.25	Jun - Oct	Sep - Jan Feb - Mar	deciduous	74.8; 188.8	112.4; 194.1	ND	++	1.8
Pseudoxandra polyphleba	Annonaceae	tree	25	1	0.51	ND	ND	ND	ND	ND	ND	ND	ND
Psidium acutangulum	Myrtaceae	shrub	146	46	0.74; 0.82	Apr - Jul	ND	deciduous	19.2	104.3		++	2.9
Pterocarpus amazonum	Leguminosae (P.)	tree	525	8	0.33; 0.40	May - Jun	Aug - Nov	deciduous	302.2	138	+	+++	2.8
Salix humboldtiana	Salicaceae	tree	987	2	0.39; 0.42	ND	ND	evergreen	ND	ND	ND	ND	ND
Sesbania exasperata	Leguminosae (P.)	shrub	ND	ND	ND	ND	ND	ND	ND	ND	+	ND	5.3
Simaba sp.	Simaroubaceae	tree	382	3	0.42	Feb - May	ND	evergreen	ND	ND	ND	+++	ND
Sloanea sp.	Elaeocarpaceae	tree	99	0.5	ND	ND	ND	ND	ND	ND	ND	ND	ND
Sorocea duckei	Moraceae	tree	23	2	0.58	May - Aug	ND	ND	ND	ND	ND	+++	ND
Swartzia sp.	Leguminosae (P.)	tree	ND	ND	0.63; 0.86	Apr - Jun	ND	evergreen	ND	ND	+	++	2.4
Tabebuia barbata	Bignoniaceae	tree	383	4	0.65; 0.71; 0.77	Apr - May	Aug - Oct	deciduous	61.5; 168.0	88.3; 153.6	ND	+++	2.3
Tabernaemontana juruana	Apocynaceae	shrub	22	3	ND	ND	ND	evergreen	ND	ND	ND	-	ND
Teramnus volubilis	Leguminosae (P.)	herb	ND	ND	ND	ND	ND	ND	ND	ND	+	ND	3.8
Trichilia sp.	Meliaceae	tree	26	0.5	0.51	ND	ND	ND	100.9	135.3	ND	ND	ND
Triplaris sp.	Polygonaceae	tree	2888	3	0.59; 0.63	Sep - Nov	ND	semi-deciduous	ND	ND	ND	++	3.4
Vigna sp.	Leguminosae (P.)	herb	ND	ND	ND	ND	ND	ND	21.3	23.4	+	ND	3.9
Vitex cymosa	Verbenaceae	tree	533	157	0.56; 0.59; 0.64	Jun - Sep	Jul - Dec	deciduous	98.8; 100.2	51.3; 84.2	ND	+++	2
Zygia inaequalis	Leguminosae (M.)	tree	291	1	0.72	May - Jun	May - Nov	evergreen	153.8	107.9	+	ND	2.6

a) wood-density data is published by ALLEN and ALLEN (1981), WORBES et al. (1992); FEARNSIDE (1997); PAROLIN et al. (1998)

b) periodicity data and leave characterisation is published by KLINGE et al. (1983); ZIBURSKI (1990); AYRES (1995); SCHÖNGART et al. (2002)

c) nodulation data is published by ALLEN and ALLEN (1981); FARIA et al. (1984; 1987; 1989); FARIA and LIMA (1998)

d) mycorrhiza data is published by MEYER (1991), mycorrhiza infection: + low; ++ medium, +++ high

ND not determined and no literature data available

Table A.4.2 Results of the RFLP digestion: sizes of the resulted restriction fragments [bp] after digestion with the enzymes *Dde*I, *Hha*I, *Msp*I and *Sau*3AI

isolate no.	**host species**	***Dde*I**					***Hha*I**					***Msp*I**					***Sau*3AI**			
2-3/12	*Pterocarpus amazonum*	437	297	280	173	148	594	454	361	188		624	397	223	170		941	356	281	218
15-M4	*Albizia multiflora*	437	297	280	173	148	594	454	361	188		624	397	223	170		941	356	281	218
5-2/4	*Albizia multiflora*	437	280	260	148		868	454	260			539	397	213	170	100	823	356	218	157
14-2/5	*Albizia multiflora*	414	370	260	248	113	454	361	336	137	116	500	463	397	223	123	863	300	218	
16-M3	*Albizia multiflora*	414	370	260	248	113	454	361	336	137	116	500	463	397	223	123	863	300	218	
1-2/19	*Albizia multiflora*	414	370	260	248	113	454	361	336	137	116	500	293	223	170	123	863	300	218	
3-2/7	*Albizia multiflora*	414	370	260	248	113	454	361	336	137	116	500	293	223	170	123	863	300	218	
4-2/3	*Albizia multiflora*	414	370	260	248	113	454	361	336	137	116	500	293	223	170	123	863	300	218	
7-2/29	*Albizia multiflora*	414	370	260	248	113	454	361	336	137	116	500	293	223	170	123	863	300	218	
8-2/21	*Albizia multiflora*	414	370	260	248	113	454	361	336	137	116	500	293	223	170	123	863	300	218	
9-2/8	*Albizia. multiflora*	414	370	260	248	113	454	361	336	137	116	500	293	223	170	123	863	300	218	
10-2/14	*Albizia multiflora*	414	370	260	248	113	454	361	336	137	116	500	293	223	170	123	863	300	218	
12-2/20	*Albizia multiflora*	414	370	260	248	113	454	361	336	137	116	500	293	223	170	123	863	300	218	
13-M1	*Albizia. multiflora*	414	370	260	248	113	454	361	336	137	116	500	293	223	170	123	863	300	218	
6-3/19	*Pterocarpus amazonum*	414	370	260	248	113	454	361	336	137	116	500	293	223	170	123	863	300	218	
17-3/29	*Pterocarpus amazonum*	414	370	260	248	113	454	361	336	137	116	500	293	223	170	123	863	300	218	
11-2/30	*Albizia multiflora*	414	370	260	248	113	454	361	260	149	116	500	454	223	170	123	863	612	218	
18-3/27	*Pterocarpus amazonum*	414	370	260	248	113	454	361	260	149	116	500	454	223	170	123	863	612	218	

Table A.4.3 $\delta^{15}N$ values of different plant parts from the same individual trees (mean ± standard deviation; leaves marked in grey) and the p-values of the pair-wise comparisons (significant differences with $p < 0.05$ are printed in bolt)

	$\delta^{15}N$ values [‰]										
			legumes			**non-legumes**					
plant parts:	*Zygia i.*	*Campsia. c.*	*Albizia m.*	*Pterocar. a.*	*Macrolob. a*	*Cecropia l.*	*Crateva b.*	*Tabebuia b.*	*Nectand. a.*	*Vitex c.*	*Pseudob. m.*
leaves	1.2 ± 0.9	0.6 ± 0.7	1.3 ± 1.7	4.3 ± 1.0	5.5 ± 0.4	4.7 ± 1.5	7.5 ± 1.3	3.2 ± 0.9	4.2 ± 0.7	3.8 ± 0.9	5.1 ± 1.1
twigs	-0.4 ± 1.7	0.4 ± 0.3	0.8 ± 2.0	2.7 ± 1.0	4.7	ND	4.8	1.8 ± 1.1	ND	ND	ND
branches	-0.4 ± 1.3	-0.8 ± 0.5	-0.3 ± 2.2	1.7 ± 0.9	4.5 ± 0.6	4.6 ± 1.2	5.5 ± 1.4	2.0 ± 0.4	4.5 ± 0.6	3.1 ± 1.1	4.0 ± 0.8
stems	-1.4 ± 0.8	-1.1 ± 0.5	0.5 ± 1.6	3.4 ± 1.0	3.1 ± 1.2	2.6 ± 1.4	2.7 ± 1.4	1.5 ± 0.9	3.9 ± 1.5	1.2 ± 1.9	-0.6 ± 2.8
roots	0.0 ± 1.1	0.8 ± 0.3	0.3 ± 1.1	2.1 ± 0.7	3.8 ± 2.1	3.8 ± 0.7	1.3 ± 1.2	1.5 ± 0.4	2.1 ± 1.7	1.4 ± 1.8	1.6 ± 1.1
nodules	5.3 ± 1.0	5.5 ± 0.2	2.5 ± 1.4	4.6	ND	ND	ND	ND	ND	ND	ND
blossoms	0.5	0.1	1.7	4.8 ± 0.0	ND	ND	ND	ND	ND	ND	ND
fruits	0.3	0.8 ± 0.4	2.8 ± 1.2	3.5 ± 0.8	6.0	ND	ND	ND	ND	2.6	ND

	p-values										
			legumes			**non-legumes**					
comparisons:	*Zygia i.*	*Campsia. c.*	*Albizia m.*	*Pterocar. a.*	*Macrolob. a*	*Cecropia l.*	*Crateva b.*	*Tabebuia b.*	*Nectand. a.*	*Vitex c.*	*Pseudob. m.*
leaves - twigs	**0.000**	0.564	**0.002**	**0.000**	N.D.	N.D.	N.D.	0.137	N.D.	N.D.	N.D.
leaves - branches	**0.000**	0.120	**0.000**	**0.000**	**0.007**	0.473	**0.000**	**0.002**	0.548	**0.000**	**0.000**
leaves - stems	**0.000**	**0.007**	**0.001**	**0.002**	0.084	**0.000**	**0.026**	0.282	0.622	0.166	**0.041**
leaves - roots	0.103	0.977	**0.036**	**0.000**	0.105	0.056	**0.000**	**0.017**	**0.043**	**0.009**	**0.000**
leaves - nodules	**0.008**	0.124	0.681	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
leaves - fruits	N.D.	0.944	0.284	0.724	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
leaves - blossoms	N.D.	N.D.	N.D.	0.054	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
twigs - branches	0.511	**0.021**	0.286	**0.007**	N.D.	N.D.	N.D.	0.891	N.D.	N.D.	N.D.
twigs - stems	**0.038**	**0.012**	0.432	**0.046**	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
twigs - roots	0.210	0.632	0.719	0.985	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
branches - stems	**0.002**	0.455	0.142	**0.000**	0.134	0.130	N.D.	0.560	0.677	0.259	N.D.
branches - roots	0.643	N.D.	0.412	0.058	0.178	0.771	**0.015**	0.055	0.109	0.715	**0.002**
stems - roots	**0.006**	0.159	0.370	**0.003**	0.893	**0.013**	0.261	0.988	0.325	0.525	0.479
roots - nodules	**0.050**	**0.009**	0.269	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.

Table A.4.4 $\delta^{15}N$ values of different leave types (the standard leaf type is marked in grey) from the same individual trees (mean ± standard deviation) and the p-values of the pair-wise comparisons (significant differences with $p < 0.05$ are printed in bolt)

	$\delta^{15}N$ values [‰]							
	legumes				non-legumes			
leave types:	*Zygia i.*	*Campsian. c.*	*Albizia m.*	*Pterocarp. a.*	*Tabebuia b.*	*Nectandra a.*	*Vitex c.*	*Pseudob. m*
medium sun leaves	1.2 ± 0.9	0.6 ± 0.7	1.7 ± 2.4	4.5 ± 0.9	3.6 ± 0.4	4.3 ± 0.5	4.0 ± 0.1	6.0 ± 1.1
young sun leaves	1.6 ± 0.8	ND	2.4 ± 1.5	5.1 ± 0.8	3.7 ± 0.5	5.8 ± 0.2	3.7 ± 0.4	ND
old sun leaves	0.6 ± 0.6	ND	2.0 ± 2.1	4.5 ± 1.1	ND	3.5 ± 1.5	6.1	ND
young shade leaves	1.2 ± 1.0	1.4 ± 0.4	2.3 ± 1.3	5.0 ± 1.3	2.8	ND	5.7	7.7
medium shade leaves	0.2 ± 1.3	0.9 ± 0.5	1.4 ± 2.2	4.5 ± 0.5	2.9 ± 0.0	ND	5.4	6.4 ± 1.1
submerged leaves	0.7 ± 0.7	1.4 ± 0.1	ND	ND	ND	3.3	ND	ND

	p-values							
	legumes				non-legumes			
comparisons:	*Zygia i.*	*Campsian. c.*	*Albizia m.*	*Pterocarp. a.*	*Tabebuia b.*	*Nectandra a.*	*Vitex c.*	*Pseudob. m*
medium sun l. - young sun l.	0.602	ND	0.396	**0.026**	0.362	**0.030**	0.440	ND
medium sun l. - old sun l.	0.879	ND	0.325	0.066	ND	0.470	ND	ND
medium sun l. - submerged l.	**0.006**	0.052	ND	ND	ND	ND	ND	ND
old sun l. - young sun l.	0.503	ND	0.894	0.985	ND	0.257	ND	ND
medium sun l. - medium shade l.	**0.001**	0.191	0.640	0.851	0.316	ND	ND	0.134
medium shade l. - young shade l.	0.089	**0.031**	**0.040**	0.290	ND	ND	ND	ND

Table A.4.5 Overview of the $\delta^{15}N$ values and N contents of all investigated woody and non-woody species, organised according to legumes/non-legumes and increasing $\delta^{15}N$ values (mean ±standard deviation).

	number of plants	$\delta^{15}N$ value [‰]	coefficient of variance [%]	N content [%]
legumes				
Teramnus volubilis	3	**-1.4** ± 0.2	12	**3.8** ± 0.3
Dalbergia riparia	5	**-0.5** ± 2.7	531	**3.0** ± 0.1
Inga splendens	1	**-0.1**		**3.8**
Swartzia sp.	2	**-0.1** ± 0.1	163	**2.4** ± 0.2
Lonchocarpus sp.	2	**0.1** ± 0.1	62	**2.4** ± 0.1
Cymbosema roseum	1	**0.2**		**3.6**
Machaerium aristulatum	3	**0.4** ± 1.2	295	**2.3** ± 0.1
Mimosa pigra	7	**0.6** ± 0.5	79	**3.5** ± 0.6
Zygia inaequalis	10	**0.7** ± 0.8	119	**2.6** ± 0.4
Entada polyphylla	3	**0.7** ± 0.8	112	**4.2** ± 0.3
Sesbania exasperata	3	**0.7** ± 1.0	137	**5.3** ± 0.1
Aeschynomene sp.	2	**1.0** ± 1.4	144	**4.7** ± 1.4
Campsiandra comosa	2	**1.0** ± 0.9	89	**2.2** ± 0.1
Vigna sp.	4	**1.1** ± 1.5	138	**3.9** ± 0.6
Albizia multiflora	16	**1.2** ± 2.3	196	**3.9** ± 1.4
Dalbergia inundata	5	**1.2** ± 1.0	84	**2.7** ± 0.5
Chamaecrista sp.	4	**1.9** ± 1.0	50	**2.7** ± 0.6
Bauhinia sp.	3	**3.9** ± 1.2	32	**4.4** ± 0.2
Pterocarpus amazonum	11	**4.0** ± 1.0	25	**2.8** ± 0.3
Neptunia oleracea	1	**4.5** ± 2.6	58	**3.8** ± 0.3
Crudia amazonica	5	**4.7** ± 0.4	9	**2.8** ± 0.3
Macrolobium acaciifolium	10	**5.1** ± 0.6	12	**2.6** ± 0.5
non-legumes				
Tabebuia barbata	9	**3.2** ± 1.0	32	**2.3** ± 0.4
Dioscorea sp.	1	**3.3**		**2.5**
Nectandra amazonum	5	**3.4** ± 1.3	39	**2.4** ± 0.4
Psidium acutangulum	1	**3.7**		**2.9**
Vitex cymosa	26	**4.0** ± 1.0	26	**2.0** ± 0.4
Luehea cymulosa	3	**4.5** ± 1.0	22	**2.1** ± 0.3
Pseudobombax munguba	7	**4.7** ± 1.3	27	**1.8** ± 0.3
Cecropia latiloba	14	**5.0** ± 1.4	28	**3.1** ± 0.3
Laetia corombulosa	3	**5.8** ± 0.5	9	**2.2** ± 0.1
Triplaris sp.	1	**5.9**		**3.4**
Crateva benthamii	42	**6.8** ± 1.0	15	**3.1** ± 0.6

Table A.4.6 $\delta^{15}N$ values (top) and N contents (bottom) of 16 investigated legume and non-legume species, from which samples were taken from the same individual plants during the terrestrial and the aquatic period (mean ± standard deviation; coefficient of variance). Species which had significantly different $\delta^{15}N$ values or N contents during the aquatic period than during the terrestrial period are marked with a star.

	terrestrial period		**aquatic period**		
	$\delta^{15}N$ values [‰]	coef. of. variance [%]	$\delta^{15}N$ values [‰]	coef. of variance [%]	number of plants
legumes					
Dalbergia riparia	0.7 ± 3.0	417	-0.7 ± 1.6	251	3
*Machaerium aristulatum**	0.4 ± 1.2	295	2.6 ± 0.9	33	3
Mimosa pigra	0.8 ± 0.4	51	0.9 ± 1.9	220	6
*Zygia inaequalis**	0.7 ±0.8	119	1.7 ± 0.8	45	10
Campsiandra comosa	0.7 ±0.8	115	0.4 ± 0.3	72	2
Albizia multiflora	1.7 ± 1.7	102	1.4 ± 1.1	80	10
Pterocarpus amazonum	4.1 ± 1.0	24	4.6 ± 1.0	22	10
Crudia amazonica	4.8 ± 0.5	11	5.1 ±0.5	10	3
*Macrolobium acaciifolium**	4.9 ±0.3	6	5.5 ±0.6	10	9
non-legumes					
Tabebuia barbata	2.9 ± 0.7	25	3.2 ± 0.7	22	8
Nectandra amazonum	3.4 ± 1.3	39	4.2 ± 0.6	13	5
Vitex cymosa	4.0 ± 1.1	26	3.7 ± 0.8	22	25
Pseudobombax munguba	4.7 ± 1.3	27	5.6 ± 1.2	22	7
Cecropia latiloba	4.9 ± 1.3	27	4.0 ± 1.2	29	13
Laetia corombulosa	5.8 ± 0.5	9	5.8 ± 1.5	26	3
Crateva benthamii	6.9 ± 1.0	14	7.3 ± 1.3	18	40

	terrestrial period		**aquatic period**		
	N content [%]	coef. of. variance [%]	N content [%]	coef. of variance [%]	number of plants
legumes					
Dalbergia riparia	3.0 ± 0.2	6	2.8 ± 0.2	7	3
*Machaerium aristulatum**	2.3 ± 0.1	5	2.8 ± 0.2	8	3
Mimosa pigra	3.6 ± 0.5	15	4.1 ± 1.3	32	6
Zygia inaequalis	2.6 ± 0.4	13	2.5 ± 0.2	8	10
Campsiandra comosa	2.3 ± 0.3	13	2.0 ± 0.1	4	2
Albizia multiflora	3.4 ± 0.7	19	3.4 ± 0.6	19	10
Pterocarpus amazonum	2.8 ± 0.3	11	3.4 ± 0.7	21	10
Crudia amazonica	2.8 ± 0.3	12	3.2 ± 0.2	5	3
Macrolobium acaciifolium	2.7 ± 0.5	21	2.7 ± 0.4	14	9
non-legumes					
*Tabebuia barbata**	2.4 ± 0.4	18	3.3 ± 0.4	13	8
Nectandra amazonum	2.4 ± 0.4	17	2.4 ± 0.5	20	5
*Vitex cymosa**	2.0 ± 0.4	21	2.3 ± 0.4	18	25
Pseudobombax munguba	1.8 ± 0.3	16	1.9 ± 0.4	23	7
Cecropia latiloba	3.1 ± 0.3	9	3.0 ± 0.3	10	13
Laetia corombulosa	2.2 ± 0.1	4	1.9 ± 0.2	10	3
*Crateva benthami**	3.2 ± 0.5	17	4.0 ± 0.8	21	40

Table A.4.7 Biomass, accumulated N and $\delta^{15}N$ values of the nodulated legumes grown without soil N at the end of the experiment (mean ± standard deviation)

		biomass [g-dw]			
	number of plants	stem	roots	leaves	whole plant
Mimosa pigra	3	**0.21** ± 0.07	**0.05** ± 0.02	**0.02** ± 0.02	**0.28** ± 0.08
Zygia inaequalis	11	**0.23** ± 0.18	**0.13** ± 0.11	**0.10** ± 0.18	**0.46** ± 0.47
Campsiandra comosa	8	**0.71** ± 0.32	**0.35** ± 0.18	**0.20** ± 0.14	**1.26** ± 0.60
Albizia multiflora	9	**0.11** ± 0.06	**0.10** ± 0.08	**0.02** ± 0.02	**0.23** ± 0.13
Pterocarpus amazonum	3	**0.27** ± 0.16	**0.11** ± 0.07	**0.10** ± 0.06	**0.48** ± 0.28

		accumulated N [mg]			
	number of plants	stem	roots	leaves	whole plant
Mimosa pigra	3	**2.6** ± 0.7	**0.6** ± 0.2	**0.9** ± 1.0	**3.8** ± 1.6
Zygia inaequalis	11	**4.0** ± 3.7	**2.6** ± 2.0	**3.3** ± 6.2	**10.0** ± 11.4
Campsiandra comosa	8	**10.0** ± 3.1	**4.8** ± 2.2	**4.5** ± 2.8	**19.3** ± 7.6
Albizia multiflora	9	**2.8** ± 1.8	**2.0** ± 1.6	**1.0** ± 0.8	**5.7** ± 3.3
Pterocarpus amazonum	3	**5.3** ± 2.1	**1.6** ± 0.8	**2.8** ± 1.6	**9.7** ± 4.5

		$\delta^{15}N$ values [‰]			
	number of plants	stem	roots	leaves	whole plant
Mimosa pigra	3	**-7.6** ± 0.9	**-7.4** ± 1.5	**-8.6** ± 0.4	**-7.9** ± 0.6
Zygia inaequalis	11	**-5.4** ± 3.3	**-5.6** ± 3.2	**-5.9** ± 3.6	**-5.6** ± 3.2
Campsiandra comosa	8	**-6.0** ± 1.7	**-4.2** ± 1.5	**-1.0** ± 0.9	**-4.4** ± 1.2
Albizia multiflora	9	**-8.3** ± 1.9	**-7.1** ± 2.2	**-8.7** ± 2.4	**-7.9** ± 1.7
Pterocarpus amazonum	3	**-6.5** ± 6.3	**-4.9** ± 6.1	**-0.1** ± 1.5	**-4.3** ± 4.8

Table A.4.8 Results of the unlabelled and ^{15}N labelled treatments of the pot experiment: measured and calculated biomass, N contents, accumulated plant N and $\delta^{15}N$ values of the plants at the end of the experiment (mean ± standard deviation). Additionally temporal mean %Ndfa values and the amount of fixed N per plant for the two nodulated legume species calculated with both reference species are shown.

	biomass				N contents				$\delta^{15}N$ values				reference species: *Macrolobium acaciifol.*		reference species: *Cecropia latiloba*	
treatments	stems [g-dw]	roots [g-dw]	leaves [g-dw]	plants [g-dw]	stems [% N]	roots [% N]	leaves [% N]	plant N [mg]	stems [‰]	roots [‰]	leaves [‰]	plants [‰]	%Ndfa [%]	fixed N [mg]	%Ndfa [%]	fixed N [mg]
Campsiandra comosa																
unlabelled	2.44 ± 0.89	0.57 ± 0.21	0.82 ± 0.46	3.83 ± 1.53	0.7 ± 0.1	1.0 ± 0.1	2.1 ± 0.5	41.0 ± 21.9	4.4 ± 2.3	4.7 ± 1.2	5.4 ± 3.0	5.0 ± 2.4	15	6.1	9	3.6
^{15}N labelled	2.90 ± 1.65	0.73 ± 0.43	0.77 ± 0.43	4.40 ± 2.49	0.7 ± 0.2	1.0 ± 0.1	1.9 ± 0.4	40.9 ± 21.2	77.4 ± 66.9	105.7 ± 55.9	71.7 ± 51.5	83.9 ± 54.5	4	1.8	0	0.0
Albizia multiflora																
unlabelled	1.10 ± 0.80	0.62 ± 0.57	0.16 ± 0.17	1.87 ± 1.49	1.2 ± 0.2	1.4 ± 0.3	3.7 ± 1.0	29.1 ± 28.8	0.8 ± 0.8	2.3 ± 0.9	3.6 ± 1.1	2.0 ± 0.7	34	10.0	28	8.1
^{15}N labelled	0.95 ± 0.67	0.53 ± 0.29	0.07 ± 0.06	1.56 ± 1.00	1.7 ± 0.1	1.4 ± 0.5	4.2 ± 0.8	26.1 ± 16.2	21.6 ± 4.6	124.7 ± 176.5	26.3 ± 2.5	38.5 ± 25.0	76	19.8	43	11.3
Macrolobium acaciifolium																
unlabelled	1.30 ± 0.97	0.74 ± 0.21	0.19 ± 0.15	2.22 ± 1.27	0.9 ± 0.1	0.9 ± 0.1	2.8 ± 0.2	22.5 ± 11.9	7.0 ± 1.9	8.8 ± 1.7	6.8 ± 3.0	7.6 ± 1.9				
^{15}N labelled	5.24 ± 0.53	1.27 ± 0.97	0.39 ± 0.23	6.90 ± 1.68	1.0 ± 0.1	1.0 ± 0.2	2.8 ± 0.3	75.0 ± 13.1	35.0 ± 26.1	112.0 ± 73.6	78.3 ± 60.7	44.3 ± 20.2				
Cecropia latiloba																
unlabelled	14.18 ± 11.90	3.05 ± 2.21	3.00 ± 3.24	20.23 ± 17.33	0.9 ± 0.5	1.2 ± 0.1	3.0 ± 0.6	228.2 ± 157.8	5.4 ± 3.0	4.7 ± 1.0	4.9 ± 1.2	5.5 ± 1.6				
^{15}N labelled	6.87 ± 7.45	1.46 ± 1.28	1.66 ± 2.35	10.00 ± 11.03	1.0 ± 0.1	0.9 ± 0.0	2.6 ± 0.6	132.6 ± 163.1	40.1 ± 16.6	37.2 ± 3.8	36.6 ± 12.0	38.1 ± 13.0				

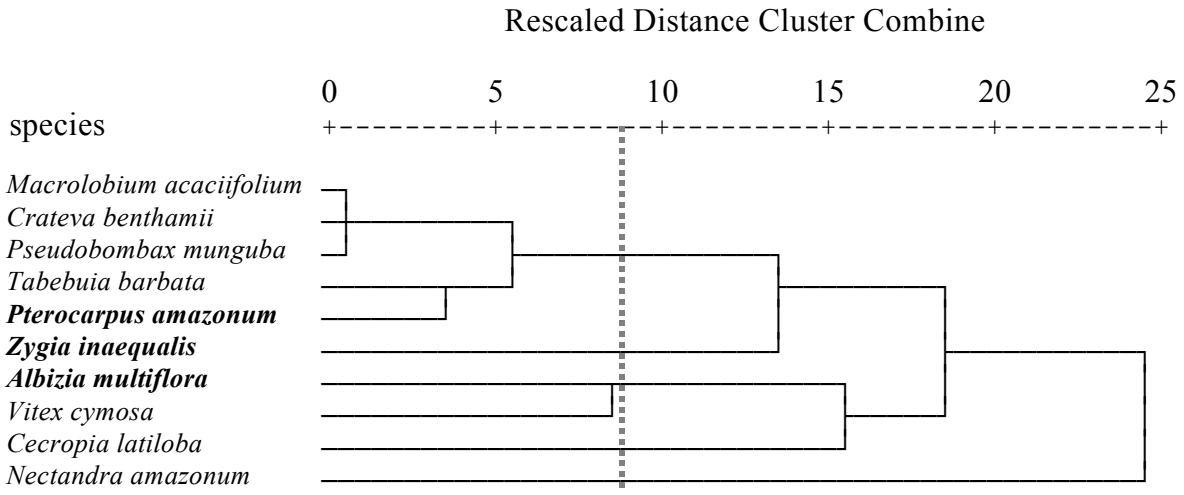

Figure A.4.1 Dendrogram using average linkage between groups showing the similarity of 10 species in respect to their seasonal variation in $\delta^{15}N$ values (Figure 4.20) determined by hierarchical cluster analysis. The 3 nodulated legumes are printed in bolt.

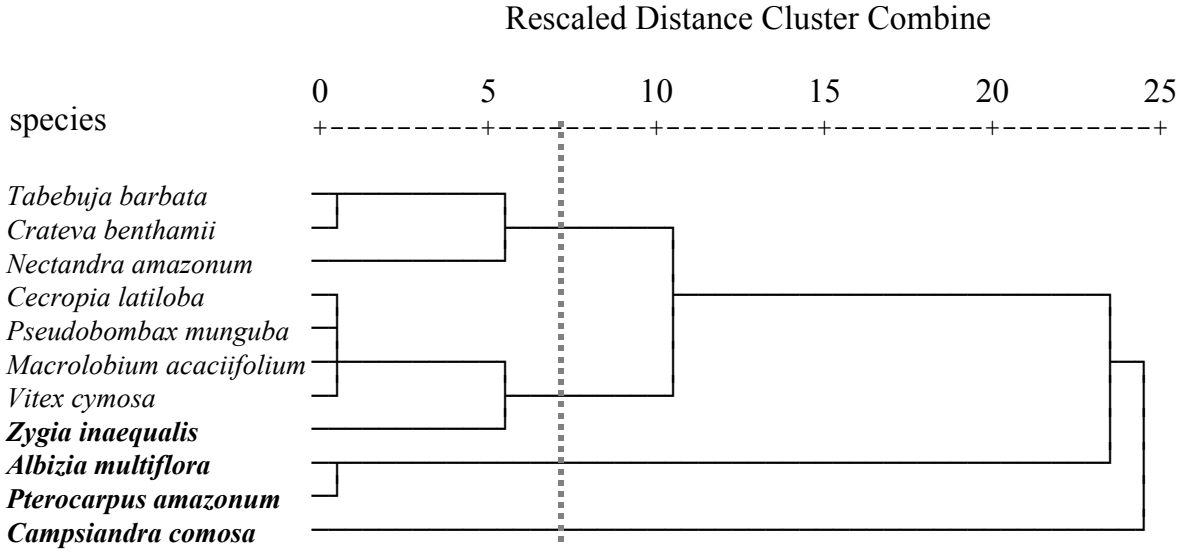

Figure A.4.2 Dendrogram using average linkage between groups showing the similarity of 11 species in respect to their plant internal $\delta^{15}N$ pattern (Table A.4.3) determined by hierarchical cluster analysis with the ranking of $\delta^{15}N$ values in roots, stems, branches and leaves as variables. The 4 nodulated legumes are printed in bolt.

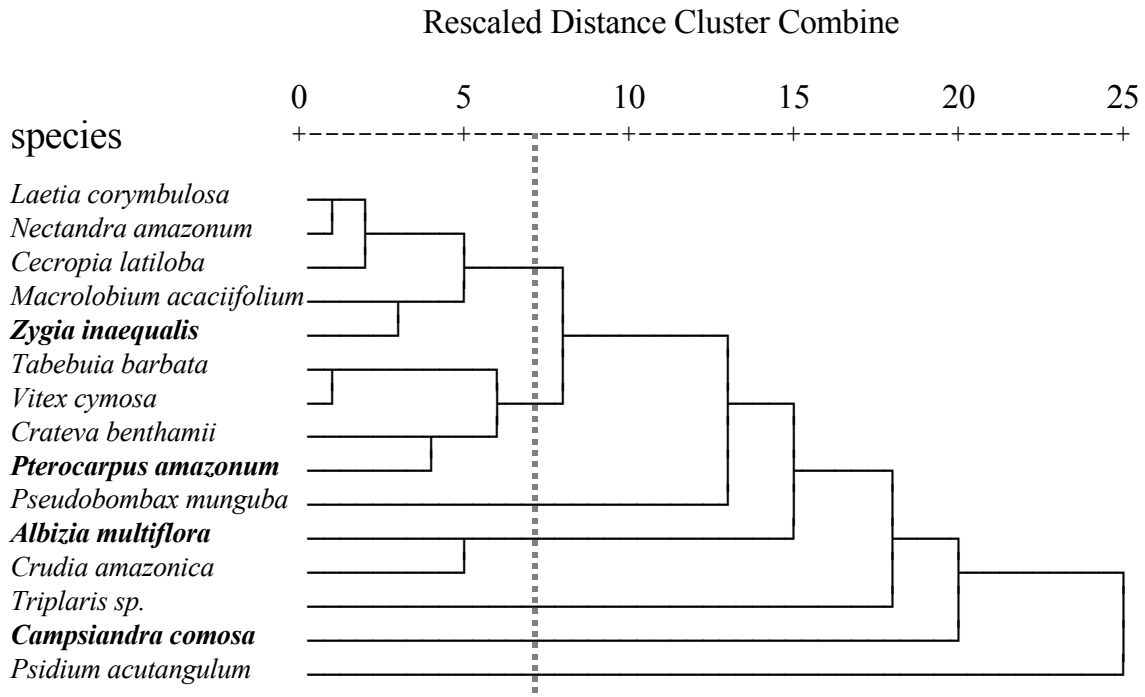

Figure A.4.3 Dendrogram using average linkage between groups showing the physiological similarity of 15 species from the study area, determined by hirarchical cluster analysis with standardised values (z-values) of family (legume or non-legume), growing habit, basal area, wood density, fruiting season, leaf periodicity and leaf N content. The 4 nodulated legumes are printed in bolt.

Summary

In Amazonia, the most fertile soils are found in the várzea with possibly N as a limiting factor. Small-scale agriculture and shifting cultivation has been practised for centuries, but due to increasing land pressure, sustainable management concepts which support the local population and protect the forest ecosystem are needed. This study focused on the N cycle of a várzea forest, since sustainable agroforestry is only possible within the limits of ecosystem nutrient cycles.

The objective was, to measure the gaseous N turnover, identify the regulating factors and to assess the extent of N_2 fixation and denitrification as a part of the entire N cycle of the várzea forest. Therefore, for the first time in a várzea forest symbiotic and non-symbiotic N_2 fixation and denitrification were monitored simultaneously over 20 months, and their variability related to vegetation and hydrological factors. With an elevational range of 22 to 25 m a.s.l. the studied forest was inundated on average between 4.7 and 7.6 months per year. The forest stand structure was analysed in order to evaluate the importance, diversity and nodulation potential of legume species. Physical, chemical and microbiological soil properties were determined using standard methods, to identify and investigate factors influencing and regulating the gaseous N turnover. For more information about possible symbioses and typical populations of symbiotic N_2 fixing bacteria in the várzea, a cluster analysis of 16S rDNA-PCR restriction fragment length polymorphism and rep-PCR fingerprinting was performed. Analysis of ^{15}N in plants according to the ^{15}N natural abundance method allowed the calculation of the percentage of plant N derived from atmosphere via symbiotic N_2 fixation. A selection of 2 nodulated and 2 non-nodulated species from the study area were treated with ^{15}N labelling in a pot experiment, to compare the results obtained with the ^{15}N natural abundance method with results of the isotope dilution method. Non-symbiotic N_2 fixation and denitrification in forest soil were monitored simultaneously using the acetylene reduction assay and the acetylene block method, respectively. The attempt to balance the gaseous N turnover and put it into context with the N cycle of the studied várzea forest, was undertaken.

Leguminosae were the 3rd important plant family with an exceptionally high species richness of 27%. On average, 68 individual legumes per hectare accounted for 10-12% of the total above ground biomass. The liana *Dalbergia riparia* and the trees *Macrolobium acaciifolium*,

Albizia multiflora and *Pterocarpus amazonum* were the most important legume species in the study area. Of the 24 legume species found in the study area, 87.5% were nodulated. Therefore, the formation of symbioses between legume species and rhizobia seemed to be highly abundant.

The acidic soil in the study area was rich in organic matter, specially in the surface soil layer, where also N contents were highest. NH_4^+-N contents were constantly about 1 order of magnitude higher than NO_3^--N. During the terrestrial period aeration was good, while hypoxic or even anoxic conditions were characteristic for the aquatic period. Significant seasonal variations due to the flood pulse were apparent for many soil properties but not for bacterial numbers, so that highly flood adapted bacteria species were believed to be present in the study area.

Genotypic diversity of rhizobia was remarkable, considering that only 14 isolates from *Albizia multiflora* and 4 isolates from *Pterocarpus amazonum* were analysed. 5 16S rDNA similarity groups, divided in 2 major clusters, were revealed by analysis of combined RFLP patterns, and a similar diversity was confirmed by rep-PCR fingerprinting. Genotypic grouping was not correlated with the 2 host species. Most isolates were grouped with *Mesorhizobium* spp., 1 with *Bradyrhizobium japonicum*, and 2 isolates could not be classified in the present taxonomic units. Mainly *Mesorhizobium* but also *Bradyrhizobium* species were nodulating *Albizia multiflora* and *Pterocarpus amazonum* on Ilha de Marchantaria.

Non-symbiotic N_2 fixation in the soil was influenced by various factors, which themselves were strongly influenced by the flood pulse, preventing N_2 fixation in the surface soil layer during the aquatic period. In the soil profile, maximum N_2 fixation rates occurred in litter, followed by rates at 0-5 cm soil depth, but also in deeper soil layers N_2 fixation occurred. Annual N gain in the surface soil layer was 4.1 kg N ha^{-1}. Due to the restriction to the terrestrial period, the N gain by free-living diazotrophs, although it was small, might have had positive effects on the biomass production.

The flood pulse seemed to have no influence on symbiotic N_2 fixation, since no seasonal variation in $\delta^{15}N$ values which would suggest differences in N_2 fixation rates between terrestrial and aquatic period was found. The nodulated legumes with *Neptinia oleracea* on the one side and *Teramnus volubilis* on the other, fixed between 2% and 70% of their plant N

from atmosphere, respectively. Altogether, between 4% and 5% of plant N of the várzea forest derived from symbiotic N_2 fixation, which resulted in a N gain between 12.9 and 16.1 kg N ha^{-1} y^{-1}. The relatively high N gains via symbiotic N_2 fixation may be important for the várzea forest during the whole hydrological cycle.

Highest denitrification rates in the surface soil layer were measured during flooding and the transition periods, revealing the strong influence of the flood pulse. In the soil profile, maximum rates occurred at 250 to 300 cm soil depth just below the water table, and only in litter was denitrification even higher. Annual N loss via denitrification in the surface soil layer was 12.5 kg N ha^{-1}. Due to the relatively high N losses, denitrification can have an important influence on the N cycle of the várzea forest.

The complete N balance has to be seen as a preliminary result. Nevertheless, conclusions can be drawn. The gaseous N turnover plays an important role in the studied forest area, covering 60% - 71% of the N input and 56% - 63% of the N output. The high abundance of N_2 fixing legumes provided the main N source, even surpassing the N input by the river. Denitrification was the main reason for N loss from the várzea soil. Since the N input clearly surpassed the N output of the studied forest, a sustainable utilisation of the várzea forest seems possible as far as the N budget is concerned. Besides other reasons, due to the high amounts of N stored in the vegetation, clearing the várzea forest is not an option. Extensive agroforestry systems, including nodulated legumes seem to be a suitable method to use the floodplain forest. How nodulated legumes may be used in sustainable management, to support the local population and protect the ecosystem should be investigated by future research.