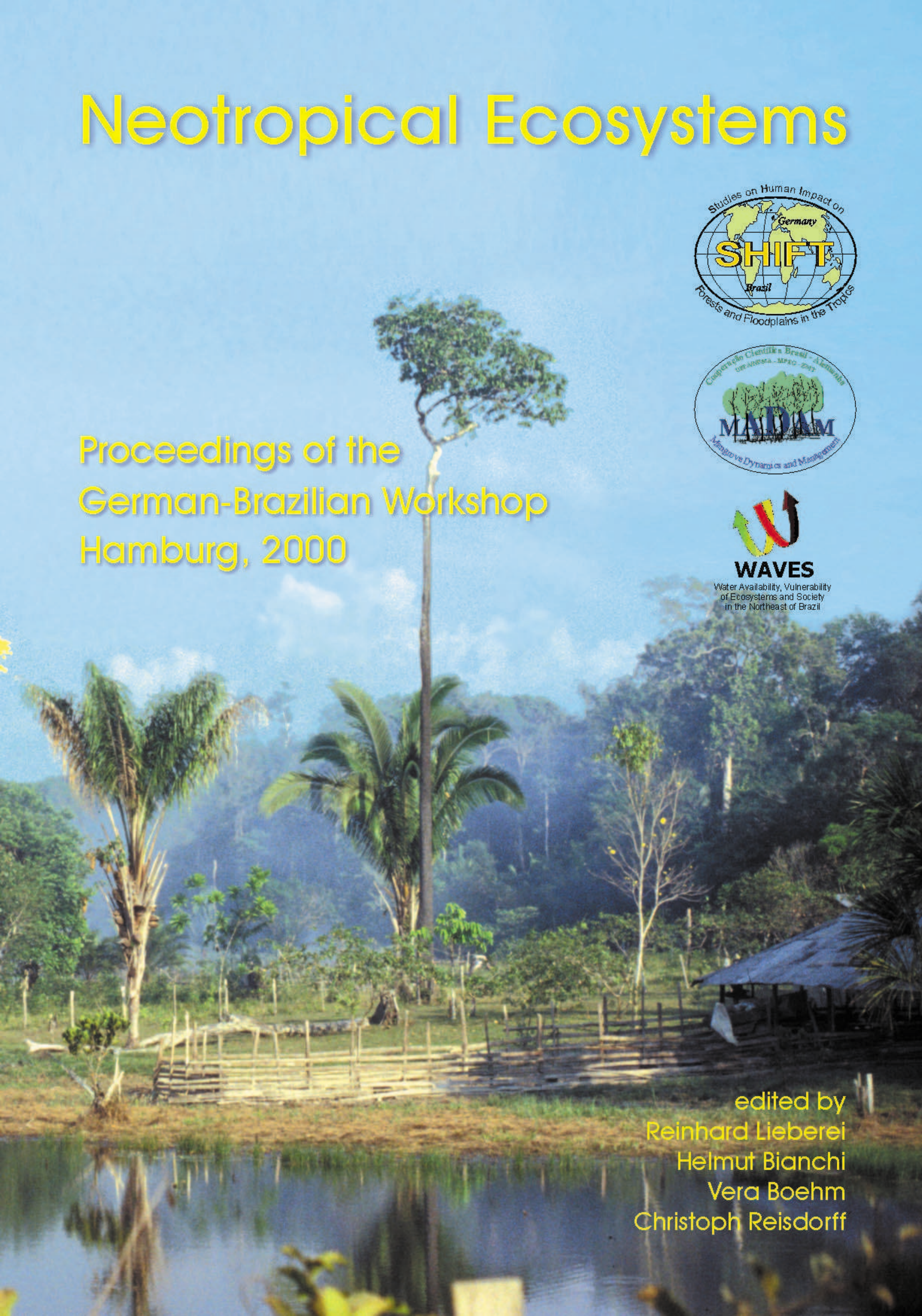

Neotropical Ecosystems

Proceedings of the German-Brazilian Workshop Hamburg, 2000

Studies on Human Impact on Forests and Floodplains in the Tropics
SHIFT
Germany
Brazil

MADAM
Mangrove Dynamics and Management

WAVES
Water Availability, Vulnerability of Ecosystems and Society in the Northeast of Brazil

edited by
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Layout Helmut K. Bianchi, GKSS, Karsten Bittner, Documedia, Geesthacht, Germany

Printing GKSS-Forschungszentrum Geesthacht GmbH, Geesthacht, Germany

ISBN 3-00-010691-X

Lieberei, R., Bianchi, H-K., Boehm, V., Reisdorff, C., (eds.) 2002:
Neotropical Ecosystems, Proceedings of the German-Brazilian Workshop,
Hamburg 2000. GKSS-Geesthacht .

The research cooperation has been carried out under the auspices of the German - Brazilian Govemmental Agreement on the Cooperation in Scientific Research and Technological Development.

The issuance of the Proceedings and the production of the CD-ROM was sponsored (Code 0339991) by the

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Bundesministerium für
Bildung, Wissenschaft,
Forschung und Technologie

Federal Ministry of
Education and Research

Field Estimation of Biological N_2-Fixation by Five Tropical Tree Species Using Two ^{15}N Isotope Dilution Methods

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Abstract

One aim of the SHIFT project "Secondary Forests and Fallow Vegetation in the Agricultural Landscape of the Eastern Amazon Region (ENV 25/2)" is to shorten the fallow period by planting fast-growing leguminous trees. Therefore, the screening and identification of suitable leguminous trees for improved fallows in term of biological N_2-fixation (BNF) was an important objective of the present work. Previous studies in the main project area showed that an improved fallow system with *Acacia mangium* planted at a spacing of 1 m x 1 m accumulated about 56 t ha^{-1} above-ground biomass within two years, which is about twice as much as the above-ground biomass production by Clitorea racemosa (27 t ha^{-1}) at a spacing of 2 m x 2 m or by the spontaneous natural fallow (24 t ha^{-1}).

In order to determine the contribution of biological N_2 fixation to total N uptake of five tropical tree legumes in the field, two ^{15}N isotope dilution methods were used. In February 1997, ^{15}N-labelled ammonium sulphate was applied to an area of 768 m^2 at a rate of 9 kg N ha^{-1} while a second experimental area remained unlabelled. One month later improved fallow was established on both areas. The following nursery-grown seedlings of N_2-fixing tree species (NFTs) were planted at a spacing of 1 m x 1 m (density of 10000 plants per hectare): *A. mangium, A. angustissima, Inga edulis, C. racemosa* and *Sclerolobium paniculatum.* Three non-N_2-fixing tree (NNFT) species *Eucalypthus urophylla, Jacaranda copaia* and *Schyzolobium amazonicum* were used as reference plants for the isotope dilution calculations. Leaf samples were taken after 19 months of growth from N_2-fixing and non-N_2-fixing trees and analyzed for their % N-content and $^{15}N/^{14}N$ ratio by ANCA mass spectrometry. On the basis of the obtained results for ^{15}N content in NFTs and NNFTs, the percentage of nitrogen derived from the atmosphere (%Ndfa) of the five NFTs was calculated.

We determined that significant amounts of N (~90%), accumulated during the early growth stage of *A. mangium, I. edulis* and *S. paniculatum*, were derived from biological nitrogen fixation. However, we observed significant and systematic differences between ^{15}N enrichment and ^{15}N natural abundance methods. Based on the results of these three tree species, ^{15}N natural abundance method appears to underestimate BNF by a facto 1.7 as compared with the ^{15}N enrichment method. This factor may be applied in studies of natural ecosystems of the region.

Keywords

BNF, ^{15}N, Isotope dilution methods, Natural abundance method, ^{15}N enrichment method, Improved fallow, Legumes, Trees, Agroforestry, Amazon

1 Introduction

Slash-and-burn agriculture is one of the most important causes for rain forest conversion. Natural fallow vegetation, called "capoeira" in Brazil, plays a key role in the success and sustainability of this land-use-system. The main functions of this vegetation are the recuperation of the large amounts of biomass and nutrients lost during cultivation. High demographic pressure and expansion of cash crops are causing an ongoing reduction of fallow periods. Short periods of spontaneous fallow do not provide enough time for complete recuperation, calling for an improvement of the fallow.

SANCHEZ (1999) distinguishes the following three types of tropical fallows: natural, enriched and improved. Natural fallows are early successional stages of spontaneous secondary regrowth – capoeira. The term "enriched fallow" refers to a low density enrichment of the spontaneous fallow vegetation with species of economic value (such as fruits, medicines etc.). In contrast, "improved fallows" aim at increasing soil fertility, usually by incorporating rapidly growing legume species into the crop-fallow rotation, with the primary purpose of nitrogen fixation. However, reliable quantification of biological nitrogen fixation (BNF) under field-conditions is lacking, due to the substantial methodological difficulties involved.

The most promising methods for determining BNF in trees are the ^{15}N isotope dilution (ID) methods. According to

Danso (1995) these can be distinguished into: a) the natural abundance method (^{15}N NAM), first applied by Shearer and Kohl (1986) – which is based on the naturally higher $^{15}N/^{14}N$ ratio of soil N than of atmospheric N_2 and b) the enrichment method (^{15}N EM) – where a ^{15}N enriched inorganic (labelled fertilizer) or organic material has been purposely added to the soil to artificially increase the ^{15}N content of soil N over that of atmospheric N_2 (McAuliffe et al., 1958; Fried and Middelboe, 1977). Both the ^{15}N natural abundance method and ^{15}N enrichment method involve growing N_2-fixing plants (NFT) in soil with $^{15}N/^{14}N$ ratios measurably different from the almost worldwide constant $^{15}N/^{14}N$ ratio of 0.3663 atom-% present in the atmosphere. If the ^{15}N-content in legume plants is significantly different from the soil ^{15}N-pool, the %Ndfa (percentage of N derived from atmosphere) can be calculated. The ^{15}N enrichment method is a robust and accurate method of BNF- determination. However, costs involved for highly-enriched ^{15}N-fertilizer are prohibitive for large-scale studies. In contrast, the ^{15}N natural abundance method is a simpler and common method for research in undisturbed natural ecosystems.

The identification of fast-growing leguminous trees suitable for improved fallows through their large biomass and nutrient accumulation was one of the important objectives of the SHIFT Project "Secondary Forests and Fallow Vegetation in the Agricultural Landscape of the Eastern Amazon Region (ENV25). Previous studies in the main project area showed that an improved fallow system formed by planting *A. mangium* accumulated about 56 t ha^{-1} above-ground biomass within two years, which is about twice the above-ground biomass production by fallow improved with *C. racemosa* (27 t ha^{-1}) or by the spontaneous natural fallow (24 t ha^{-1}).

2 Material and Methods

Two field experiments were conducted to compare the two ^{15}N isotope dilution methods and to determine the contribution of five tropical tree legumes in terms of BNF. The study was conducted in the oldest agrarian frontier of the Amazon, the micro-region Bragantina, in the municipality of Igarapé-Açu, northeastern Pará, Brazil. The region has a gentle relief, but is well drained by many rivers that flow to the Atlantic Ocean. The Bragantina is dominated by nutrient-poor Oxisols and Ultisols. The climate is humid, characterized by an average annual temperature of 26 °C and average annual rainfall in the order of 2500 mm. The dry season, with a monthly precipitation of < 60 mm, lasts from September to November.

In order to determine the contribution of BNF to the total N uptake of five tropical tree legumes in the field by the two ^{15}N ID methods, ^{15}N-labelled ammonium sulphate was applied to an area of 768 m^2 at a rate of 9 kg N ha^{-1} (^{15}N EM) while a second experimental area remained unlabelled (^{15}N NAM). One month later improved fallows were established on both areas. The following nursery-grown N_2-fixing tree species (NFTs) were planted at a spacing of 1 m x 1 m: on the ^{15}N-labelled experiment (^{15}N-enriched): *A. mangium, A. angustissima, Inga edulis, Sclerolobium paniculatum.* Additionally, *C. racemosa* was planted on the ^{15}N natural abundance experiment (NAM). Three non-N_2-fixing tree (NNFT) species, *Eucalyptus urophylla, Jacaranda copaia* (^{15}N EM) and *Schyzolobium amazonicum* (NAM) were used as reference plants for BNF-calculations. Leaf samples were taken after 19 months of growth from NFTs and NNFTs and analyzed for their % N-contents and $^{15}N/^{14}N$ ratio by ANCA mass spectrometry.

3 Results

Fig. 1 illustrates the aboveground biomass production of NFTs 19 months after planting for both areas. The above-ground biomass production 19 months after planting varied widely between the tested species (1.3 to 30.6 t ha^{-1}). *A. mangium* produced in average 30.4 (±0.3) t ha^{-1}, which is about 10 times the biomass produced by *A. angustissima* and *C. racemosa* with 3.6 (±2.3) t ha^{-1} and 3.0 (±0.6) t ha^{-1}, respectively. There were no systematic differences in biomass values between the two experiments, ensuring comparability of the experiments.

Nineteen months after planting, *A. magium* and *I. edulis*

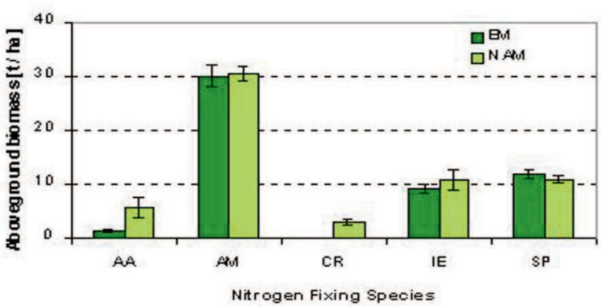

Fig. 1: Average with standard error of aboveground biomass accumulation [t ha^{-1}] of AA = *Acacia angustissima*, AM = *A. mangium*, CR = *Clitorea racemosa*, IE = *Inga edulis* and SP = *Sclerolobium paniculatum* on both areas 19 months after planting (MAP).

produced between 70 % and *S. paniculatum* 90 % of the above-ground biomass of a previously established improved fallow experiment in the same study region after 30 months (BRIENZA, 1999; Fig. 2). In contrast, *A. angustissima* and *C. racemosa* performed poorly in this improved fallow experiment. Both produced only approximately 25 % of that in the earlier experiment. These low biomass values were possibly caused by the El Niño anomaly, affecting these two species more severely than the other three.

Tab. 1 shows the mean values for percentage N in leaves of

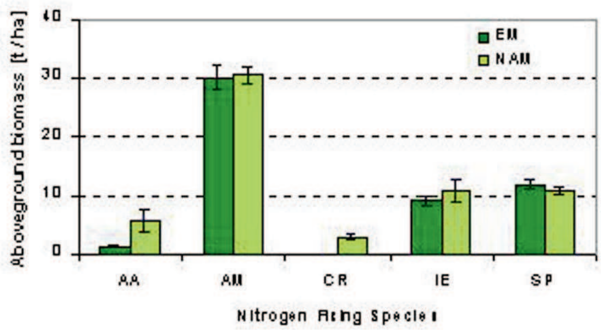

Fig. 2: Comparison of above-ground biomass production [t ha^{-1}] of nitrogen fixing trees (AA = *Acacia angustissima*, AM = *A. mangium,* CR = *Clitorea racemosa*, IE = *Inga edulis* and SP = *Sclerolobium paniculatum*) after 19 and 30 months of growth.

all tested species after 19 months of growth. Nitrogen contents are remarkably similar between both experiment, ensuring the comparability of the methods tested. Significant differences were observed in N contents between N_2-fixing and non-N_2-fixing tree species indicating the importance of BNF in N-accumulation.

The ^{15}N-abundances in the leaves, as obtained with the ^{15}N-

Tree species	-----------% N----------	
	^{15}N enriched	NAM
A. mangium	2.4 (0.1)	2.7 (0.1)
I. edulis	2.4 (0.1)	2.6 (0.1)
S. paniculatum	2.1 (0.1)	2.2 (0.1)
A. angustissima	3.2 (0.1)	3.4 (0.2)
C. racemosa	-	3.1 (.1)
Mean of NNFTs	**1.5 (0.3)**	**1.6 (0.2)**
E. urophylla	1.2 (0.1)	1.3 (0.1)
J. copaia	1.8 (0.4)	1.9 (0.1)
S. amazonicum	-	1.6 (.1)

Tab. 1: Average and standard error of Nitrogen content [% N] in leaves of planted tree species after 19 months growth

enrichment and with the ^{15}N natural abundance method are shown in Table 2. The ^{15}N excess values of the N_2-fixing trees ranged from 0.007 to 0.021 atom-%. In contrast, the ^{15}N excess values of non-N_2-fixing trees were approximately 10 times higher, with a mean-value of 0.081 atom-%. The same tendency could also be observed with the ^{15}N values. Whereas the ^{15}N values of the N_2-fixing trees ranged from –0.14 ‰ to –1.28 ‰, the mean non-N_2-fixing trees averaged a value of +1.22 ‰. Nitrogen fixing trees and non-fixing trees thus differ clearly, both in the ^{15}N natural abundance method and the ^{15}N enrichment method. The use of the natural abundance method (NAM) resulted in

Tree species	atom-% ^{15}N excess	^{15}N ‰
A. mangium	0.007 (0.001)	-0.14 (0.1)
I. edulis	0.009 (0.003)	-0.21 (0.1)
S. paniculatum	0.008 (0.003)	-0.52 (0.2)
A. angustissima	0.021 (0.006)	-1.28 (0.2)
C. racemosa	-	-1.02 (0.2)
Mean of NNFTs	**0.081 (0.025)**	**1.22 (0.1)**
E. urophylla	0.088 (0.016)	1.14 (0.1)
J. copaia	0.074 (0.028)	1.30 (0.2)
S. amazonicum	-	1.23 (0.2)

Tab. 2: Average and standard error of four samples of ^{15}N content (atom-% ^{15}N excess and ^{15}N ‰) in sampled leaves of N_2-fixing and non-N_2-fixing tree species after 19 months growth.

lower %Ndfa-estimations, than the enrichment method (Tab. 3). As the %Ndfa-values estimated by the enrichment method likely represent actual BNF-activities, we assume a systematic under-estimation of BNF by the natural abundance method – with the exception of the deviating values of *A. angustissima*. The latter presumably was caused by the exceptionally low biomass-values of this specie (as demonstrated in Fig. 2). The %Ndfa-values of the other 3 tree species attained Ndfa rates of 89% (by ^{15}N enriched) and 52% (by NAM). Based on the results of these three tree species, ^{15}N natural abundance method appears to underestimate BNF by a facto 1.7 as compared with the ^{15}N enrichment method.

4 Conclusions

Tree species	------%Ndfa------	
	^{15}N enriched	NAM
A. mangium	91 (1)	47 (4)
I. edulis	87 (3)	49 (3)
S. paniculatum	90 (1)	59 (7)
A. angustissima	72 (7)	86 (7)
C. racemosa	-	77 (6)

Tab. 3: Average and standard error of calculation of proportion of nitrogen derived from the atmosphere [% Ndfa]

From our results we determined that significant amounts of N (~90%), accumulated during the early growth stage of *A. mangium, I. edulis* and *S. paniculatum*, were derived from biological nitrogen fixation. However, we observed significant and systematic differences between ^{15}N

enrichment and ^{15}N natural abundance methods. The estimation of tree BNF using the natural abundance method appears feasible if the estimated %-Ndfa is multiplied by the factor 1.7. This factor may be applied in studies of natural ecosystems of the region.

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6 Acknowledgement

This study was partly financed by BMBF (FKZ: 01LT0012) and CNPq (Process No. 680099/95-4). The authors wish to express their gratitude to EMBRAPA – Amazônia Oriental, Belém and the Faculdade das Ciências Agrárias do Pará (FCAP) for enabling and supporting this research work as well as Mr. U. M. F. Marques for his great help and patience.