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The Role of Biological Nitrogen Fixation in Secondary and Primary Forests of Central Amazonia

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ABSTRACT

Slash-and-burn agriculture causes heavy nitrogen losses both to the atmosphere and via nitrate leaching. Biological Nitrogen Fixation (BNF) is believed to be the main mechanism through which secondary forest regrowth makes up for these losses, thus ensuring the sustainability of slash-and-burn land use. BNF is regulated by a wide range of factors, mainly the degree of N-limitation, availability of P and other nutrients, and access to light, all of which change along succession.

Our knowledge on the role of BNF in tropical secondary and primary forests is uncertain, as research is scarce so far, and involves serious methodological problems in estimating BNF. The present study was conducted in a central Amazonian slash-and-burn system at the frontier of agricultural colonization. First-cycle secondary succession is investigated with the false-time-series approach (13 sites) and takes primary forests as controls (6 sites). Another 6 sites address the effects of higher preceding land use intensity and of soil fertility.

Indirect evidence on the role of BNF along succession is obtained by investigating legume species composition and the impact of N_2-fixing legumes on vegetation and topsoil. In a second step, BNF is estimated quantitatively with the '^{15}N natural abundance method'. The are findings are as follows:

High primary forest phytomass stocks (460t ha^{-1}) are paired with record initial rates of phytomass recovery (170t in 10 yrs.), pointing to an extremely dynamic agroecosystem. However, structural characteristics of the vegetation suggest that resilience of regrowth is lower and susceptible to degradation on three sites with moderately increased land use intensity (two burns or extended cultivation period).

Legume species differ in their capability to nodulate and fix N_2. Of the total of 157 legume species identified in this study, 78% were classified as potentially capable of BNF. Legume species composition changes along succession and gives strong evidence on the role of BNF in these forests: species capable of BNF dominate the legume community in all stages of secondary succession (86-91% of total legume phytomass), indicating that this plant characteristic confers competitive advantages in fallow regrowth. In contrast, the phytomass share of potential N_2-fixers is markedly lower (57%) in primary forests.

Evidence on the role of BNF is further obtained by investigating possible effects of potentially N_2-fixing legumes on their surroundings, quantified in 278 plots ranging from 25-225m^2 in size and covering an average 18% of the study sites. The vegetation shares of potential N_2-fixers are significantly positively correlated with total vegetation phytomass in secondary regrowth, whereas no such relationships are apparent in primary forests.

The vegetation shares of potential N_2-fixers are, furthermore, significantly negatively correlated with the δ^{15}N-signals in the leaf litter of secondary regrowth. Again, no relationships were observed in the primary forests.

Lower vegetation shares of potentially N_2-fixing legumes, and the lacking impact of these on total phytomass and on the leaf litter δ^{15}N-signals in their surroundings all point to lower BNF in the three 'degraded' sites. In contrast, BNF is high in a 4 yr.-old regrowth growing on more fertile 'terra morena do índio', which is dominated by *Inga edulis* and *I. thibaudiana*.

All above-outlined evidence on the role of BNF proves significant BNF in secondary regrowth as opposed to low or negligible BNF in primary forests. However, results do not support the idea of any BNF peak at some stage of succession and rather suggest a constant role of BNF throughout the first 25 years of first-cycle secondary regrowth.

N_2-fixing lianas play a yet insufficiently recognized key functional role as agents of BNF, notably in early stages of succession. Potentially N_2-fixing lianas combine 42% of all legume phytomass in young first-cycle regrowth; shares decline along succession and are only 4% in primary forests. The foliar δ^{15}N-signals of potentially N_2-fixing lianas are on average 0.5‰ lower than for trees, suggesting that lianas may also be the more efficient N_2-fixers.

Foliar δ^{15}N-signals are negatively correlated with the individual legume phytomass mainly in primary forests. This gives room to the hypothesis that BNF may be physiologically limited in the tree giants, which dominate the primary forest legume vegetation.

Point pattern analysis reveals a clustered distribution of potential N_2-fixers, and interpolation of georeferenced isotopic data suggests that BNF is concentrated in 'hotspots'. Spatial distribution of BNF is positively or negatively associated with the occurrence of legume groups, genera or single species. However, relationships are complex and so far unpredictable.

The ^{15}N natural abundance method fails as a quantitative method of estimating BNF in these tropical forests, since with all BNF-estimation methods large portions of estimates are invalid (mainly negative). Nevertheless, differences in the plausibility of methods become apparent, mainly concerning differences in the type of reference species representing the 'soil-derived δ^{15}N'. On average 1.1‰ higher foliar δ^{15}N-signals of non N_2-fixing legumes result in the lowest portion of invalid cases (19% of plants). Foliar N-concentrations suggest that non N_2-fixing legumes are more similar to potentially N_2-fixing legumes than non-legume species. Non N_2-fixing legume species are, therefore, recommended as reference species. This study shows the N_2-fixing vegetation to reduce the δ^{15}N-signals in their surroundings. Future research must therefore envisage this effect as a systematic source of error for BNF estimation.

Die Rolle der biologischen Stickstoffixierung in Sekundär- und Primärwäldern Zentralamazoniens

KURZFASSUNG

Brandrodungsfeldbau verursacht große Stickstoffverluste in die Atmosphäre und durch Nitratauswaschung. Die Biologische Stickstoffixierung (BSF) ist vermutlich der wichtigste Mechanismus, um diese Verluste im Sekundärwald wieder auszugleichen und ist somit entscheidend für die Nachhaltigkeit des Brandrodungsfeldbaus. Die BSF wird durch ein breites Spektrum von Faktoren geregelt die sich im Sukzessionsverlauf ändern, v.a. von der Verfügbarkeit an N, P und anderen Nährstoffen und dem Zugang zum Licht.

Unser Wissen über die Rolle der BSF im Sukzessionsverlauf und in Primärwäldern bleibt ungewiß, da Feldforschung bislang selten und mit schwerwiegenden Problemen der BSF-Messung behaftet ist. Diese Arbeit wurde in einem zentralamazonischen Brandrodungsgebiet durchgeführt. Sekundäre Sukzession im ersten Rodungszyklus wird in falscher Zeitreihe untersucht (13 Flächen) und mit Primärwald-Kontrollen (6 Flächen) verglichen. Weitere 6 Flächen untersuchen den Einfluß einer intensiveren vorangegangenen Landnutzung sowie der Bodenfruchtbarkeit.

Indirekte Erkenntnisse über die Rolle der BSF im Sukzessionsverlauf werden durch die Untersuchung der Leguminosen-Artenzusammensetzung, sowie des Einflusses von N_2-fixierenden Leguminosen auf die umliegende Vegetation und dem Boden gewonnen. In einem zweiten Schritt wird die BSF quantitativ mit der „^{15}N natürliche Abundanz Methode“ geschätzt. Die wichtigsten Ergebnisse lassen sich wie folgt zusammenfassen:

Eine hohe Primärwald-Phytomasse (460 t ha^{-1}) ist gepaart mit einer sehr schnellen anfänglichen Phytomassenregeneration (170t in 10 Jahren), was auf ein sehr dynamisches Agroökosystem weist. Allerdings deuten strukturelle Vegetationscharakteristika auf eine leichte Degradierbarkeit bereits nach nur leicht gesteigerter Landnutzungsintensität (zwei Brände oder ausgedehnte Kulturphase) hin.

Die Leguminosenarten unterscheiden sich in ihrer Fähigkeit zu nodulieren, von allen 157 in dieser Arbeit identifizierten Leguminosenarten wurden 78% als potentielle N_2-Fixierer klassifiziert. Die Leguminosen-Artenzusammensetzung ändert sich im Sukzessionsverlauf und liefert dabei deutliche Hinweise auf die Bedeutung der BSF in diesen Wäldern: potentiell N_2-fixierende Arten dominieren die Leguminosengemeinschaft zu allen Stadien der sekundären Sukzession (86-91% der Leguminosen-Phytomasse), deutlicher Hinweis, daß diese Pflanzeneigenschaft im Sekundärwald einen Konkurrenzvorteil darstellt. Im Gegensatz hierzu ist dieser Anteil deutlich niedriger (57%) im Primärwald.

Hinweise auf die Rolle der BSF finden sich auch durch die Untersuchung der Einflüsse, die potrentielle N_2-Fixierer auf ihre Umgebung ausüben. Diese wurden untersucht in 278 Parzellen, die 25 bis 225m^2 groß sind und durchschnittlich 18% der Flächen erfassen. Die Vegetationsanteile potentieller N_2-Fixierer sind im Sekundärwald signifikant positiv mit der Gesamtphytomasse korreliert, dagegen sind keine Zusammenhänge im Primärwald erkennbar. Sie sind außerdem im Sekundärwald signifikant negativ mit den $\delta^{15}N$-Signalen in der Blattstreu korreliert, auch hier sind keine Zusammenhänge im Primärwald erkennbar.

Die geringeren Vegetationsanteile potentieller N_2-Fixierer, sowie der fehlende Einfluß von diesen auf die Gesamtphytomasse und auf die $\delta^{15}N$-Signale in der Blattstreu weisen auf eine geringe BSF in den drei „degradierten“ Flächen. Dagegen ist die BSF hoch in einem von *Inga edulis* und *I. thibaudiana* dominierten Wiederaufwuchs auf „terra morena do índio“.

Alle diese Indikatoren beweisen eine signifikante BSF im Sekundärwald, im Gegensatz zu geringer oder unbedeutender BSF im Primärwald. Die Ergebnisse dieser Arbeit stützen aber nicht die These von einem BSF-Maximum zu einer bestimmten Sukzessionsphase, sondern weisen vielmehr auf eine recht konstante Rolle der BSF in den ersten 25 Jahren Regeneration.

N_2-fixierende Lianen spielen eine bislang nur unzureichend gewürdigte funktionelle Schlüsselrolle v.a. in frühen Stadien der Sekundärsukzession: potentiell N_2-fixierende Lianen machen 42% der Phytomasse aller Leguminosen in jungem Wiederaufwuchs aus, dieser Anteil sinkt im Sukzessionsverlauf und beträgt nur noch 4% in Primärwäldern. Die δ^{15}N-Signale in den Blättern potentiell N_2-fixierender Lianen sind durchschnittlich 0.5‰ niedriger als diejenigen der Bäume, Lianen könnten demnach auch die effizienteren N_2-Fixierer sein.

Die Blatt δ^{15}N-Signale sind v.a. in den Primärwäldern negativ mit der individuellen Leguminosen-Phytomasse korreliert. Dies erlaubt die Hypothese, daß die BSF in den die Primärwald-Leguminosen dominierenden Baumriesen physiologisch limitiert ist.

Punktmuster-Analysen zeigen eine geklumpte Verteilung der potentiellen N_2-Fixierer, und Interpolation der δ^{15}N-Signale deutet auf eine BSF-Konzentration in eng begrenzten „hotspots“. Die räumliche Verteilung der BSF ist positiv oder negativ assoziiert mit den Leguminosen, einzelnen Gattungen oder Arten, diese Beziehungen sind aber sehr komplex.

Die ^{15}N natürliche Abundanz Methode scheitert als Methode zur quantitativen BSF-Schätzung, da viele Schätzungen ungültig (v.a. negativ) sind. Dennoch werden Unterschiede in der Plausibilität der Schätzmethoden deutlich, v.a. bezüglich der verschiedenen Referenzpflanzenarten. Die um 1.1‰ höheren δ^{15}N-Signale in den Blättern nicht N_2-fixierender Legumionosen erbringen die geringste Häufigkeit an ungültigen Schätzungen (19% der Pflanzen). Die Blatt N-Konzentrationen weisen auf ihre größere Ähnlichkeit mit den potentiellen N_2-Fixierern hin als nicht-Leguminosen, nicht N_2-fixierende Leguminosen werden daher als Referenz empfohlen. N_2-fixierende Leguminosen reduzieren das δ^{15}N-Signal in ihrer Umgebung, was als systematische Fehlerquelle berücksichtigt werden muß.

O papel da fixação biológica de nitrogênio em floresta secundária e primária da Amazônia Central

SUMÁRIO

A agricultura de derruba e queima causa grandes perdas de nitrogênio tanto para a atmosfera quanto para a lixiviação de nitrato. Acredita-se que a Fixação Biológica de Nitrogênio (FBN) é o mecanismo mais importante da vegetação secundária para recuperar essas perdas, mantendo, assim, a sustentabilidade dessa forma tradicional do uso da terra. A FBN está regulada por diversos fatores que variam no decorrer da sucessão, especialmente a limitação de nitrogênio, a disponibilidade de fósforo e outros nutrientes, e o acesso à luz.

Nosso nível de conhecimento sobre o papel da FBN em mata tropical ainda é precário, devido, principalmente, à escassez de pesquisas e à sérios problemas metodológicos na quantificação da FBN. O presente trabalho foi desenvolvido na Amazônia Central, numa área de colonização agrícola recente. A sucessão secundária após a primeira roça é investigada em cronoseqüências (13 áreas) e toma a mata primária como controle (6 áreas). Em outras 6 áreas pesquisa-se os efeitos de um uso de terra intensificado e da fertilidade do solo.

Obteu-se provas indiretas sobre o papel da FBN no decorrer da sucessão ao se investigar a composição taxonômica das leguminosas e a influência destas na vegetação e no solo aos arredores. Num segundo passo, quantificou-se a FBN pelo ‘método da abundância natural de ^{15}N’, adquirindo-se os seguintes resultados:

A fitomassa da mata primária (460t ha^{-1}) convive com uma reacumulação de fitomassa inicialmente muita rápida (170t em 10 anos), indicando um agroecosistema extremamente dinâmico. Porém, características estruturais da vegetação sugerem que a regeneração é propícia à degradação em 3 áreas com uso de terra ligeiramente intensificado (2 queimadas ou cultivo mais longo).

As espécies de leguminosas variam em sua capacidade de nodulação. Do total de 157 espécies identificadas, 78% foi classificada como potencialmente capaz da FBN. A composição taxonômica das leguminosas muda no decorrer da sucessão, evidenciando, assim, o papel da FBN nessas florestas: as espécies capazes de FBN dominam as leguminosas em todas as fases da sucessão secundária (86-91% da fitomassa das leguminosas) e indicam que essa capacidade confere vantagens competitivas na floresta secundária. Por outro lado, a contribuição dos potenciais fixadores é bem mais baixa (57%) na mata primária.

Obteu-se, também, evidência do papel da FBN ao investigar seus efeitos nos arredores, quantificados em 278 parcelas de 25 à 225m^2 de tamanho e cobrindo, em média, 18% das áreas. A contribuição da fitomassa dos potenciais fixadores está positivamente correlacionada com a fitomassa total da vegetação em mata secundária. Em contraste, não aparenta nenhuma relação na mata primária. A contribuição da fitomassa dos potenciais fixadores está negativamente correlacionada com os sinais de δ^{15}N na liteira foliar em mata secundária, mas não em mata primária.

A menor ocorrência das leguminosas potencialmente fixadoras e a ausência de impacto delas tanto na fitomassa total quanto no sinal de δ^{15}N da liteira indicam para uma FBN baixa nas três áreas ‘degradadas’. Em contraste, a FBN é alta numa floresta secundária em ‘terra morena do índio’, dominada por *Inga edulis* e *I. thibaudiana*.

Todos os indicadores acima listados comprovam uma FBN substancial na floresta secundária, ao contrário da FBN baixa ou insignificante na mata primária. Os resultados não confirmam, porém, a idéia de um pico da FBN em um determinado estágio da sucessão. Ao invés disso, eles sugerem um papel constante da FBN nos primeiros 25 anos de sucessão após a primeira roça.

Cipós fixadores de nitrogênio assumem uma função-chave como agentes da FBN nas fases iniciais da sucessão. Em vegetação secundária nova os cipós potencialmente fixadores constituem 42% da fitomassa das leguminosas. Essa quota diminui no decorrer da sucessão e atinge, na mata primária, somente 4%. Como os sinais de $\delta^{15}N$ nas folhas dos cipós potencialmente fixadores são em média 0.5‰ mais baixos do que nas árvores, os cipós possivelmente também são mais eficientes na FBN.

Os sinais de $\delta^{15}N$ nas folhas são negativamente correlacionados com a fitomassa individual das leguminosas. Isso leva à hipótese de que a FBN seria fisiologicamente limitada nas árvores gigantes que dominam as leguminosas na mata primária.

A análise do padrão espacial revela uma distribuição agregada dos potenciais fixadores e a interpolação dos dados isotópicos sugere que a FBN concentra-se em 'hotspots'. A distribuição espacial da FBN está positiva ou negativamente associada à ocorrência de grupos, gêneros ou espécies de leguminosas. Essas relações são complexas e até agora imprevisíveis.

O método da abundância natural de ^{15}N demonstra não ser adequado como método quantitativo para estimar a FBN nessas florestas, dado que uma grande parte das estimativas é inválida (na maioria negativa). No entanto, surgem diferenças na plausibilidade dos métodos para estimar a FBN, especialmente com respeito às diversas espécies de referência. Por causa dos sinais em promédio 1.1‰ mais altos, o uso de leguminosas incapazes de FBN resulta na mais baixa ocorrência de estimativas inválidas (19% das plantas). Como as concentrações folhares de nitrogênio são mais similares do que nas não-leguminosas, recomenda-se as leguminosas não-fixadoras como plantas de referência. Mostra-se, finalmente, que a FBN reduz o sinal de $\delta^{15}N$ nos seus arredores. Futuras pesquisas devem levar isso em conta como fonte sistemática de erro.

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LIST OF ABBREVIATIONS

BNF	Biological Nitrogen Fixation
BSF	Biologische Stickstiffixierung
FBN	Fixação Biológica de Nitrogênio
CSR	complete spatial randomness
EMBRAPA/CPAA	Empresa Brasileira de Pesquisa Agropecuaria – Centro de Pesquisa Agroflorestal da Amazônia Ocidental
first-cycle slash-and-burn	manual slash-and-burn of primary forest, followed by one year cassava cultivation
IDW	Inverse Distance Weighing interpolation method
INPA	Instituto Nacional de Pesquisas da Amazônia
%Ndfa	% Nitrogen derived from the atmosphere (i.e. via BNF)
n.d.	not determined or no data
n.s.	not significant (i.e. $p > 0.05$ if not specified otherwise)
PF	primary forest: old growth forest without obvious signs of human disturbance
TAGP	total aboveground phytomass (in t ha^{-1}): including litter, but excluding dead logs and roots on the soil surface

1 INTRODUCTION

In Brazilian Amazonia alone, small-scale slash-and-burn agriculture affects an area of more than 10 million ha, sustains half a million people and provides about 80% of the region's food production (Serrão, 1995). Regeneration dynamics of naturally regrowing fallow vegetation is decisive for the productivity and sustainability of this traditional land use system, as it recuperates the productive potential lost during the burn and brief cultivation-phase (Denich, 1989; Brown and Lugo, 1990; Palm et al., 1996; Szott et al., 1999). Management recommendations designed to improve the land use system (e.g., optimized fallow periods, fallow enrichment, etc.) require an understanding of the mechanisms, processes and interactions that drive fertility buildup along succession.

In terms of quantity, nitrogen is the most important plant nutrient which is lost during the slash-and-burn land use and which reaccumulates in the fallow period. Based on the absence of other major pathways of N-input, Biological Nitrogen Fixation (BNF) by legume-rhizobia symbiosis is believed to be the main mechanism for recuperating N-stocks. In contrast, BNF is believed to be low in primary forests with its tight nitrogen cycling within the system (Sprent, 1987; Jordan, 1989; Thielen-Klinge, 1997; Roggy et al., 1999). However, our knowledge on the role of BNF along succession and in primary forests is uncertain. This is due both to the scarcity of research to date and to the serious methodological problems involved in estimating BNF.

The present study pursues the following two basic questions:

(1) What is the role of legume-BNF along secondary succession and in primary forests?

(2) Is the ^{15}N natural abundance method successful in quantifying BNF in tropical forest vegetation?

Research was conducted in a central Amazonian slash-and-burn system at the frontier of agricultural colonization. Secondary succession is investigated with the false-time-series approach and takes primary forests as controls. Indirect evidence on the role of BNF along succession is obtained by investigating legume species composition and the impact of N_2-fixing legumes on vegetation and topsoil. In a second step, BNF is estimated quantitatively with the '^{15}N natural abundance method'.

2 STATE OF KNOWLEDGE

2.1 Nitrogen along succession and in primary forests

Slash-and-burn agriculture causes heavy losses of nitrogen both to the atmosphere (Mackensen et al., 1996) and via nitrate leaching (Mello Ivo et al., 1996; Williams and Melack, 1997). Hölscher et al. (1997) estimate that 97% of aboveground N-stocks are lost during the burn, brief cultivation phase, and early stage of successional regeneration. Nevertheless, N-availability is relatively high during the cultivation phase and initial regrowth. This is due to elevated rates of N-mineralization caused by the effects of ash fertilization (increased pH and P-availability), root decomposition and altered microclimate (Vitousek and Walker, 1987).

Nitrogen uptake increases rapidly with the buildup of phytomass and N-stocks in secondary regrowth, thereby depleting readily available soil nitrogen. Nitrogen accumulation slows down in late stages of succession, as the phytomass accumulation of regrowth saturates and internal N-cycling becomes increasingly tighter. Primary forests are believed to be in a 'state of equilibrium' with both small losses and small inputs (via BNF) of nitrogen leaving or entering the system (Odum, 1969; Vitousek and Reiners, 1975; Snedaker, 1980; Sprent, 1987; Jordan, 1989). Vegetation is rather limited by resources other than nitrogen, mainly by P-availability.

Lehmann et al. (in preparation) found nitrate peaks at 2-8m depth under 9 of this study's sites and prove that secondary regrowth partially recuperates previously leached nitrate by deepsoil N-pumping. Wet N deposition in central Amazonia is low and estimated at only 3-4 kg N ha^{-1} yr^{-1} (Kern, 1995; Williams et al. 1997), since about half of total rainfall has been recycled at least once (Lesak and Melack, 1991).

2.2 Biological nitrogen fixation (BNF)

2.2.1 Legume BNF

Leguminosae consistently rank among the most important plant families in Amazonia throughout all stages of secondary regrowth and in primary forests, in terms of their phytomass, abundance and diversity (Klinge et al., 1975; Gentry, 1982; Denich, 1989; Terborg and Andresen, 1998). Based on the typically above-average nitrogen tissue-contents of legumes, McKey (1994) postulated an 'N-demanding lifestyle' of legumes. However, this view may be too simplified (Bryan et al., 1996). The ability to nodulate (i.e., form a symbiosis with nodule bacteria) has been investigated so far for approximately $^1/_3$ (430 species) of Amazonian Leguminosae: 90% of Papilionoideae, 76% of Mimosoideae and 30% of Caesalpinoideae were found capable of nodulation (de Souza et al., 1994; de Souza and da Silva, 1997). Differences both in the occurrence of nodulation and in BNF-rates are related to taxonomic evolution (Sprent, 1995; de Souza et al., 1997). Native legume species will fix far less nitrogen than agroforestry species such as *Leucaena leucocephala* or *Gliricidia sepium*, with maximum annual fixation rates of 600kg N ha^{-1} (Giller and Wilson, 1991).

2.2.2 Non-legume BNF

By far the largest N-input by BNF is to be expected via legume-rhizobia symbiosis in secondary regrowth, though possibly less so in primary forests. Non-legume BNF (not investigated in this study) occurs via various pathways, which differ in their importance:

BNF by blue-green algae has been shown to occur on leaf surfaces of the primary forest understory and mid-canopy (Goosem and Lamb, 1986; Carpenter, 1992; Freiberg, 1998), with part of this nitrogen being taken up directly by the leaves (Bentley and Carpenter, 1984). Blue-green algae may also occur in old secondary regrowth but not in low vegetation, due to temporarily insufficient humidity.

Palms may fix nitrogen either directly via associative BNF (Magalhães, 1986; Baldani et al., 1997), or indirectly via large moss loads on their stems. Palms thus possibly form an important pathway of BNF in the palm-rich primary forests of central and western Amazonia.

Associative N_2-fixation by C4-grasses (Christiansen-Weniger, 1991; Baldani et al., 1997) and by ferns (Virginia and Delwiche, 1982) may be relevant in areas with more extended land use history (e.g., southern and eastern Amazonia) in which degraded regrowth is dominated by a dense grass or fern cover. Though actinorhizal plants occur in Amazonia, no symbiosis with *Frankia* has been detected (Magalhães et al., 1984). However, the research basis of this aspect is insufficient for the region (Ishizuka, 1992). Some wood-feeding termite species, ants and earthworms have also been reported to fix nitrogen via bacteria in their digestive tracts (Sylvester-Bradley et al., 1978; Tayasu, 1997).

Traditionally, non-legume BNF has been viewed as being only of minor importance in tropical forests, i.e., not exceeding 1-2kg N ha*yr^{-1} (Bothe et al., 1983;

Goosem and Lamb, 1986). However, knowledge remains insufficient and N-input via non-legume BNF may be higher notably in primary forests (see chap. 6.8).

2.3 Factors regulating legume BNF

Aside from the taxonomic differences within the Leguminosae (chap. 2.2.1), a wide range of factors regulates BNF:

BNF is a highly energy-consuming mechanism (Burris and Roberts, 1993); Chapin and Bloom (1987) give an overview over the energy costs of the main pathways of N-nutrition. According to Lynch and Whipps (1990), 30-50% of the net fixed C of the plant needs to be invested for the nodule bacteria. Consequently, a strict feedback mechanism suppresses BNF as soon as sufficient mineral nitrogen is available (Sanginga et al., 1989; Chalk, 2000). The large energy requirements of BNF are responsible for reduced BNF in shaded legumes as compared to legumes in full sunlight (van Kessel and Roskoski, 1983; Bryan et al., 1996).

Low light availability is expected to limit BNF especially in primary forest undergrowth, and legume nodulation sometimes observed in primary forests is mainly limited to treefall gaps (Vitousek and Denslow, 1986). Shade-induced BNF reductions vary according to the species-specific light demands of the host plants (Izaguirre-Mayoral et al., 1995).

BNF requires a wide range of nutrients. Due to the high energetic costs, P usually is most important. Cole and Heil (1981) estimate an average 7kg N-gain via increased BNF per kg fertilizer-P. This is caused by both a better nutritional status of the host plant and direct P-requirements of rhizobia (Beck and Munns, 1984; Smart et al., 1984; Israel, 1987; Giller and Wilson, 1991). Numerous fertilizing experiments have also demonstrated direct or indirect BNF limitations through other nutrients such as K, Ca, Mg, S and Mo (Giller and Wilson, 1991; Sangakkara, 1991; Chalk, 2000).

Some allelopathic substances, e.g., tannins and polyphenols, are known to reduce or inhibit BNF (Rice, 1964; Halsall et al., 1995). It is unknown if such effects are due to direct inhibition of nodulation or to reduced root hair development (Halsall et al., 1995). The influence of soil pH on BNF appears to be related to pH-optima of the host plants rather than to direct effects on the rhizobial populations (de Souza et al., 1997).

Woomer et al. (1988) describe the relationships between legume occurrence, cover and shoot phytomass on the infection potentials (determined by ‘trap plants’, MPN-method) in soils under different vegetation on Hawaii. Magalhães and da Silva (1986) report on differences in both the observation of root nodules and in acetylene reduction rates between sand and clay soils in Rondônia. A low rhizobia infection potential may be both a cause and a consequence of low BNF, and it remains unknown if infection potentials become a limiting factor for BNF (i.e., below minimum thresholds) in primary forests.

BNF may be subject to physiological limitations in large or very large primary forest legumes. Field observations by Norris (1969) indicate abundant nodulation in small juvenile legume trees throughout Amazonian primary forests, as opposed to scarce or absent nodulation in tree ‘giants’. Kreiblich (2002) report on a significant positive relationship between basal area and foliar δ^{15}N-signals in legume trees of a 100-yr.-old regrowth in central Amazonian varzea. Physiological limitations may offer an alternative explanation to the ‘theory of equilibrium’ for low or absent legume-BNF in primary forests. Since knowledge on this important aspect is so far lacking, the possibility of physiological limitation of BNF in large-sized plants is a special focus of the present study (chap. 5.6.3).

2.4 BNF along succession and in primary forests

The role of BNF may be expected to vary along secondary fallow regrowth and differ from primary forests as a result of successional shifts in N-availability and N-requirements and of constraints to BNF:

N-availability is initially relatively high (increased N-mineralization paired with low N-uptake of small plants), and N-requirements decline in late succession (saturated phytomass accumulation, efficient N-cycling). Whereas P-availability is likely to be limiting throughout all stages of succession and in primary forests (Vitousek, 1984; Raaimakers et al., 1995; Gehring et al., 1999), access to light will turn a limiting factor mainly in the undergrowth of tall forests. As a result of maximum N-limitation combined with the still relatively low BNF-constraints, BNF has been hypothesized to be maximum in initial succession, or to peak somewhere in ‘mid’ succession (Gorham et al., 1979; Thielen-Klinge, 1997).

Primary forests are supposedly in equilibrium with no significant BNF inputs (chap. 2.1). All ^{15}N natural abundance-based studies conducted so far indicate low or absent BNF in native legume trees of primary forests. Roggy et al. (1999a) estimate

BNF-derived nitrogen stocks to total 6-8kg N ha^{-1} or 5% of aboveground N-stocks in a primary forest in French Guyana. Högberg and Alexander (1995) and Thielen-Klinge (1997) likewise detected only insignificant or no BNF at all in primary forests of Cameroon and eastern Amazonia, respectively. Scarce nodulation and low rates of acetylene reduction are further indications of the insignificance of BNF inputs in Amazonian primary forests (Sylvester-Bradley et al., 1980; Moreira and Franco, 1994).

Next to the successional status, BNF is driven by soil-edaphic factors: The major Amazonian soil types differ widely both in the occurrence of nodulation and in N_2-fixation, as measured by acetylene reduction rates. These are consistently lowest in kaolinitic Oxisol, as compared to more sandy ('campina' and 'campinarana') or organic-rich ('terra preta') soils (Sylvester-Bradley et al., 1980; Moreira et al., 1992; Moreira et al., 1993). De Oliveira and Sylvester-Bradley (1982) describe corresponding variations in rhizobia infection potentials of such soils, assessed by trap plants on soils at <80km distance from the present study. Such differences are due to both chemical (P-fertility, availability of mineral nitrogen, nitrate-leaching losses) and physical (soil aeration, root penetration) effects. The high BNF rates in semiaquatic varzea ecosystems are caused by the combination of high fertility and seasonal (gaseous) N-losses (Martinelli et al., 1992; Kern, 1995; Kreiblich, 2002).

2.5 The '^{15}N natural abundance method'

2.5.1 General considerations

This study uses the '^{15}N natural abundance' method for the estimation of BNF. Handley and Scrimgeour (1997), Högberg (1997), and Boddey et al. (2000) provide thorough reviews on the method. This method was preferred in view of overriding problems with all other methods of BNF estimation, notably in complex spontaneous forest vegetation:

- The main disadvantages of acetylene reduction assays are the disturbances involved (excised root fragments or nodules), and the lack of temporal integration of measurements (Rennie and Rennie, 1983; Wani et al., 1983; Danso et al., 1992).
- The xylem sap method is not applicable to most woody legume species because of the heterogeneity of xylem sap compounds other than ureides (Hansen and Pate, 1987; Herridge et al., 1996).
- The ^{15}N-enrichment ('dilution') method is hampered by the need of homogenous ^{15}N-enrichment, both throughout the soil profile and over the major N-pools available to vegetation (Fried et al., 1983; Boddey et al., 1995).

Basic assumption of the ^{15}N natural abundance method is the ‘two-source N-model’: Nitrogen originates from the air ($\delta^{15}N$=0‰), or from ^{15}N-enriched soil and litter N-pools. The contribution of atmospherically derived N (‘% Ndfa’) can be calculated when the $\delta^{15}N$-signals in the leaves of putative N_2-fixers are lower than those in paired non N_2-fixers (‘reference plants’). The assumption of a uniform $\delta^{15}N$ signature of ‘soil-derived N’, common for N_2-fixers and reference plants, is the main uncertainty of the method.

The ^{15}N natural abundance method has been applied for investigating BNF in tropical forest trees by Högberg and Alexander (1995) in African primary forests, by Roggy et al. (1999a+b) in primary forests of French Guiana, and by Thielen-Klinge (1997) and Paparčikova (in preparation) in primary and secondary forests of an intensely exploited slash-and-burn area in eastern Amazonia. However, evidence remains uncertain and partially contradictory.

Two key methodological questions remain unresolved and are, therefore, given special attention in this study: the ‘correct’ $\delta^{15}N$-signal of soil-derived nitrogen, and the extent to which N_2-fixing legumes reduce the $\delta^{15}N$-signal in their surroundings (the ‘$\delta^{15}N$ dilution problem’).

2.5.2 Factors affecting the $\delta^{15}N$-signal

Nitrogen is subject to a wide range of transformation processes, all of which discriminate to differing degrees against the heavier nitrogen isotope. Discrimination against ^{15}N is a function of the completeness of N-transformation relative to the source N-pool. The extent of isotopic fractionation is expressed in ‘α-factors’. Högberg (1997) and Boddey et al. (2000) provide overviews of α-factors for the major pathways of N-transformation. Discrimination is especially high for nitrification (α = 1.015-1.035), microbial immobilization of ammonium causes fractionation of α = 1.012-1.020, whereas ammonification of organic substance is isotopically close to neutral. The process of BNF causes slight ^{15}N-dilution relative to the air standard, expressed as ‘B-value’. This alteration mostly ranges between -2‰ and 0‰ and is influenced by bacterial strains, host legume species, and nutrient availability (Peoples et al., 1990; Unkowich et al., 1994).

Ammonium and nitrate isotopic signatures differ, and both are typically ^{15}N-depleted in comparison to soil $\delta^{15}N_{tot}$ (Binkley et al., 1985; Pate et al., 1993). Nitrate nutrition is associated with low isotopic discrimination during plant uptake but with higher ^{15}N-fractionation within the plant as compared to ammonium nutrition (Handley and Scrimgeour, 1997). Foliar δ^{15}N-signals of preferentially nitrate-feeding pioneers tend to be lower than those of preferentially ammonium-feeding late successional or primary forest species (Roggy, 1999b; Boddey et al., 2000). Both mycorrhizal and nonmycorrhizal plants possibly have access to organic nitrogen compounds (Chapin et al., 1993; Näsholm et al., 1998). However, the importance and isotopic effects of this form of N-nutrition remain unknown.

Vegetation and topsoil are connected to one another by the litter cycle. Litter δ^{15}N-signals are lower than topsoil $\delta^{15}N_{tot}$ and both have been shown to correlate with each other (Piccolo et al., 1996; Thielen-Klinge, 1997). Interruption of the litter cycle (litter removal) causes ^{15}N-enrichment, and increased litter-input causes ^{15}N-dilution of topsoil-N (Nadelhoffer and Fry, 1988). $\delta^{15}N_{tot}$ usually increases with soil depth (People et al., 1996; Piccolo et al., 1996; Thielen-Klinge, 1997). Tiessen et al. (1984) observed 7‰ δ^{15}N-differences between sand- and clay-bound nitrogen, associated with differing N-turnover (radiocarbon) times. Ants and termites are further reasons both for localized hotspots of ^{15}N-enrichment, and for species-specific deviations of δ^{15}N (Högberg and Alexander, 1995; Tayasu, 1998).

Isotopic differences within individual plants range around 2-4‰ and are described a.o. by Handley and Raven (1992) and Yoneyama (1995). Foliar δ^{15}N-signals are affected by leaf expansion, subsequent NH_3-losses, and N-translocation during senescence.

Infection with vesicular arbuscular mycorrhiza (VAM) is widespread or general in secondary and primary forests of Amazonia (Janos, 1980; St John and Uhl, 1983; Allen and Allen, 1990). The beneficial effects of mycorrhizal infection on BNF are well documented (e.g., dela Cruz et al., 1988; Barea et al., 1991). Mycorhiza also directly access ammonium (Attiwill and Leeper, 1987) and possibly organic nitrogen (see above). Handley et al. (1993) describe the impact of inoculation with two different VAM species on the host plant δ^{15}N-signal. The isotopic effect is similar in dimension to the variability caused by different rhizobia strains on the B-values. Low host specificity of VAM facilitates both intra- and interspecific N-transfer between plants (Francis et al., 1986; Ozinga et al., 1997). The extent and isotopic effects of mycelian N-transfer are unknown and pose a potentially serious methodological problem for the ^{15}N natural abundance method.

The soil δ^{15}N-signature generally increases with time, as the result of repeated N-cycling and N-transformations discriminating against ^{15}N. Vitousek et al. (1989) describe such an increase on differently-aged lava flows in Hawaii. The soil-derived δ^{15}N-signature is thus expected to increase along secondary regrowth, and δ^{15}N-signals of both soil and vegetation are expected to be maximum in primary forests (Thielen-Klinge, 1997).

N_2-fixing plants possibly reduce the δ^{15}N-signature of topsoil and litter N-pools in their surroundings via litterfall and root sloughing. Such a 'δ^{15}N dilution-problem' would cause an underestimation of %Ndfa, and the question whether to select reference plants nearby or far away from paired potential N_2-fixers continues to be debated controversially (Binkley et al., 1985; Pate et al., 1994; van Kessel et al., 1994; Sanginga et al., 1995; Handley and Scrimgeour, 1997; Thielen-Klinge, 1997; Boddey et al., 2000). This question is, therefore, further pursued in the present study.

2.6 Summary of knowledge

The current state and lack of knowledge is summarized as follows:

1) Biological N_2-fixation (BNF) in secondary fallow regrowth is the main pathway for recuperating the heavy N-losses caused by slash-and-burn land use. The role of BNF possibly varies systematically along succession and is expected to be low in primary forests, resulting out of shifts in N-requirements and in the availability of other limiting resources (mainly P- and light-availability).
2) The ^{15}N natural abundance method is successful for BNF estimations in more homogenous settings such as grasslands or plowed fields. Evidence in tropical forests continues to be questionable in the face of the heterogeneity of such ecosystems and the multitude of processes affecting the δ^{15}N-signatures in vegetation and topsoil N-pools. Methodological uncertainties are related especially to the 'correct' measure of the soil-derived δ^{15}N-signal and to the 'δ^{15}N dilution problem'. The adequacy and functioning of the ^{15}N natural abundance method in such complex forest communities has so far not been conclusively proven.

3 RESEARCH STRATEGY

Both the complex nature of the slash-and-burn agroecosystem and the multitude of factors influencing the nitrogen isotopic signal call for a holistic research approach. The aim of this study is to gain information on (1) the role of BNF along succession, and (2) the functioning of the ^{15}N natural abundance method for estimating BNF. Research is based on the following three methodological features:

1) Inventory of the entirety of legume vegetation and of a wide array of other vegetation components,
2) Georeferenced data and a spatially systematic sampling scheme, and
3) Coverage of a variety of N-pools ranging from the leaves of different plant categories to N-pools in litter and topsoil.

Evidence on the role of BNF along succession is extracted from the taxonomic composition of the legume vegetation (chap. 5.2.2), based on the hypothesis that high phytomass shares of potentially N_2-fixing species are the outcome of competitive advantages inferred by this plant characteristic in N-limited and N_2-fixing vegetation. Further indirect evidence on the role of BNF is obtained by investigating the impact of the phytomass shares of potential N_2-fixers on total phytomass stocks (chap. 5.4) and on leaf litter nitrogen (chap. 5.5.2).

This research project gives a special focus on small-scale (within-site) spatial patterns along succession. The spatial organization of potentially N_2-fixing vegetation is investigated in Chapter 5.3. Chapter 5.8.1 interpolates the foliar $\delta^{15}N$-signals and %Ndfa-estimates, and Chapter 5.8.2 overlays these interpolations with the legume vegetation maps. Such spatial perspective is designed to yield insights into the causes and the methodological and ecological consequences of irregular legume- and BNF-distribution. The $\delta^{15}N$ dilution problem and the possibility of physiological limits to BNF in large plants are further factors included in investigations.

Chapter 5.7 evaluates the functioning of the ^{15}N natural abundance method for BNF estimation in tropical forests. For this purpose, %Ndfa is estimated in a variety of scenarios. These cover a range of definitions of the ‘soil derived’ $\delta^{15}N$-signal, based on different categories of reference species or groups of species, and on the manner of individual pairings of putative N_2-fixers and reference plants. The validity of estimation scenarios is assessed by taking the portion of valid estimates as plausibility criteria.

4 MATERIALS AND METHODS

4.1 Study area

This study was conducted in central Amazonia, in the municipalities of Presidente Figueiredo (110km N of Manaus) and Rio Preto da Eva (70km E of Manaus, Brazil) at 3°S60°W and 120-150m a.s.l. Figure 4.1 shows the location of the five study areas.

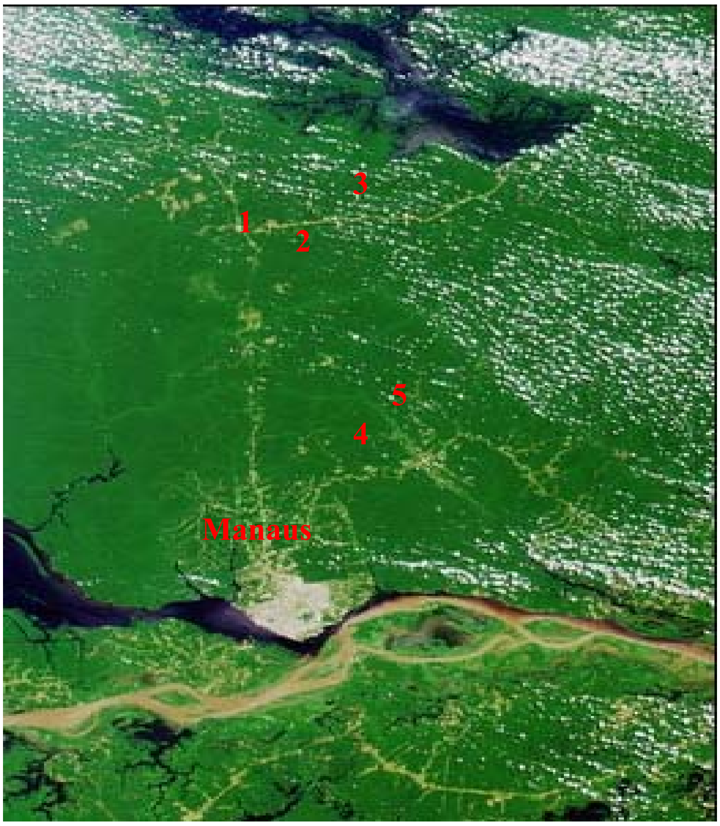


Figure 4.1. Satellite image of the study area (image source: NASA)

Land use in the region commenced in the mid 1980s, and primary forests are still omnipresent. Primary forests are old-growth dense tropical evergreen forest. Nearly 2000 species of vascular plants have been identified in a forest reserve at approximately 100km distance from the study region (Ribeiro et al., 1999). The high species diversity in central Amazonia is believed to be a consequence of the geographic location in the transition zone between the floristic provinces of western Amazonia, eastern Amazonia and the Guyanas (de Oliveira and Mori, 1999). Leguminosae consistently rank among the most important plant families concerning the number of recorded species, species diversity and vegetation shares. Palms are a further conspicuous component in these forests (Guillaumet, 1987; Terborg and Andresen, 1998).

As a consequence of the short land use history and of the relative scarcity of current or abandoned pastures, aggressive ruderal grasses (e.g., Imperata brasiliensis, Paspalum melanospermum) or ferns (e.g., Pteridium aquilinum) are entirely or nearly absent in the region. Such species are conspicuous components of regrowth in other areas of Amazonia with more intense land use, often retarding or even stalling secondary succession (Szott et al., 1999).

The climate is humid tropical and is classified as Am in the Köppen-system (Ribeiro and Adis, 1984). Average temperature is 26.7°C and annual rainfall is 2180mm (seven-year average). A dry season of approximately four months causes seasonal leaf shedding of some species but is altogether low in intensity (Sombroek, 2001). Figure 4.2 shows daily rainfall at the meteorological station of Presidente Figueiredo (20-40km distance from the main study area).

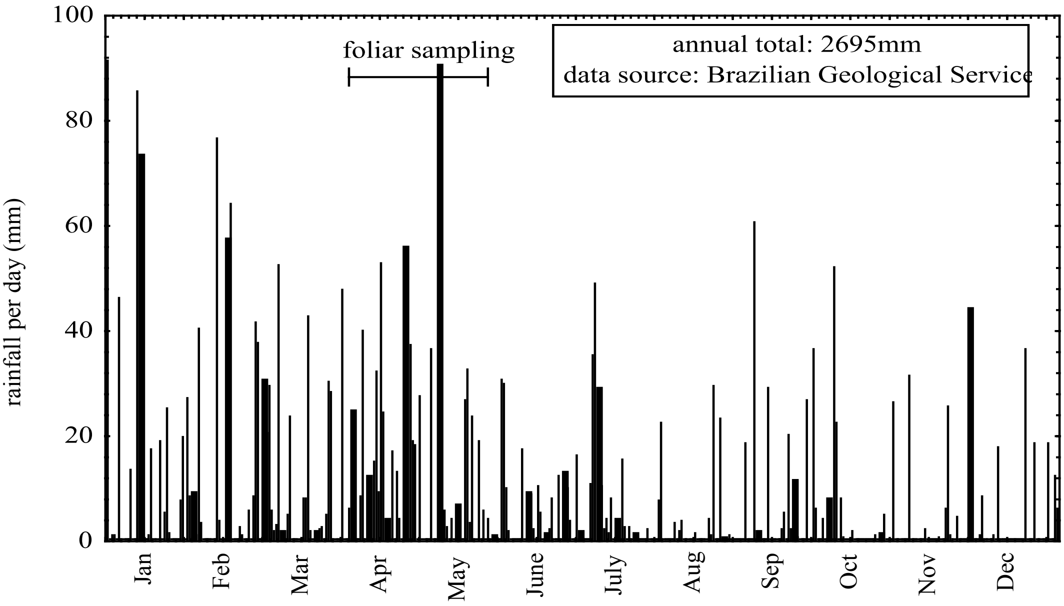


Figure 4.2. Daily rainfall at the Presidente Figueiredo meteorological station for the year 2000 (bar indicates foliar sampling period for %N/δ^{15}N-analyses, see chap. 4.6.1)

All sites are located on terra firme (not seasonally inundated). Soils of most sites are kaolinitic Oxisol (Aplic Acrorthox or 'Latossolo amarelo' according to the Brazilian classification). These soils are acidic, high in exchangeable Al, and very poor in nutrients such as N, P, Ca, and K (Chauvel et al., 1987). They are derived from the tertiary 'Barreiras' sediments and are remarkably homogenous throughout Amazonia with little variations in their fertility status (Cerri et al., 1991; Morais et al., 1995).

4.2 Study sites

A total of 25 study sites were investigated (Table 4.1). Thirteen secondary regrowth sites constitute the ‘normal’ succession in a false time-series, which isolates fallow age as single factor. A high level of standardization excludes land use related sources of variability: regrowth developed uniformly after first-cycle manual slash-and-burn (slash-and-burn of primary forest), followed by a one-year cassava cultivation phase. Sites range in fallow age from 2-25 years and are grouped into site classes of ‘young’ (2- to 3-yr.-old, 4 sites), ‘mid-aged’ (5- to 10-yr.-old, 5 sites) and ‘old’ (12- to 25-yr.-old, 4 sites) regrowth. Secondary regrowth is compared with 6 primary forest control sites, representing the theoretical endpoint of succession.

Three ‘degraded’ sites (4-, 5- and 11-yr.-old) are included for comparisons. Degradation is caused by more intense land use (two burns or extended cultivation phase). Land use intensity is nevertheless far lighter than, e.g., in abandoned pastures or mechanized agriculture. All above-mentioned sites are situated on kaolinitic Oxisol. Possible soil-edaphic effects are investigated in two ‘campinarana’ forests situated on more infertile sandy Ultisol (Spodic Paleudult: Bravard and Righi, 1989), and in one 4-yr.-old secondary forest site on more fertile ‘terra morena do índio’ (Humic Paleudult):

Campinarana forests are primary forests growing in the transition zone of the soil catena ranging from tall forests on kaolinitic Oxisol to low and sclerophyllous ‘campina’ forests growing on oligotrophic white sands. Their reduced soil fertility status is due to lower nutrient availability and lower water-holding capacity (Anderson, 1981; Coomes and Grubb, 1996). Terra morena do índio is rich in organic matter (minimum 50cm depth of the A-horizon), and soil formation was influenced by Amerindian land use (as indicated by pottery debris). This soil occurs in the surroundings of the more renowned high-fertility ‘terra preta do índio’ (which is typically under permanent agriculture).

Table 4.1. Site overview by site class and chronosequence (site sizes in ha; the location of the 5 chronosequences is shown in Figure 4.1; the 25 study sites are further detailed in Annex 1)

					Pres. Figueiredo series						Rio Preto series			
	fallow age	total			chronoseq. 1		chronoseq. 2		chronoseq. 3		chronoseq. 4		chronoseq. 5	
site class	yrs.	sites	size	plot-n*	sites	size	sites	size	sites	size	sites	size	sites	size
young degraded	4-5	2	0.86	26			1	0.38	1	0.48				
old degraded	11	1	0.90	12			1	0.90						
terra morena	4	1	0.37	12									1	0.37
young sec.veg.	2-3	4	1.73	48			2	0.51	2	1.22				
mid-aged sec.veg.	5-10	5	3.57	71	1	0.52	1	0.63	1	1.15	2	1.27		
old sec.veg.	12-25	4	2.47	52	2	1.83	1	0.32			1	0.32		
PF (clay)	PF	6	4.63	43	1	0.66	2	1.53	2	2.22	1	0.22		
PF (sand)	PF	2	1.14	14									2	1.14
	total:	25	15.67	278										

* see chap. 4.4.2

The 25 study sites are dispersed over an area of approximately 300km^2, thereby reducing the potential of regional singularities. Sites are clustered (blocked) into five chronosequences, each consisting of differently aged secondary regrowth and 1-2 primary forest control sites (Table 4.1).

Sites range in size from 0.2-1.2ha and total 15.7ha. Sites were selected to be homogenous, avoiding obvious gradients in soil appearance, history of land use, etc. All sites are level or slightly convex. Site delimitation excluded any depressions, which could be associated with waterlogging, causing denitrification losses after heavy rainfall events. Generously dimensioned border zones surround site boundaries, separating them from neighboring forests or clearings (see chap. 4.9.3).

Figure 4.1 illustrates the approximate locations of the chronosequences, maximum between-site distances within a chronosequence are <4km, minimum distance between neighboring chronosequences is about 15km. The validity of the false-time-series approach and the extent of regional variability are evaluated in chap. 4.9.1.

4.3 Species inventory

4.3.1 Plant mapping

Plant mapping covers all legume tree and liana species and additionally selected non-legume species. Results are based on a complete coverage of all plants >50cm height on the entirety of the 25 study sites. Plant position within the sites was mapped with measuring tapes. Mapping was conducted from a system of parallel access paths constructed with minimum disturbance at the beginning of this study (i.e., one year before leaf and soil sampling). The distance between access paths was adapted to the density of undergrowth vegetation. Mapping precision is estimated to be approximately one meter (i.e., ±50cm) at small- to mid-scale (up to 10m distance). Larger-scale mapping precision (between site extremities) is lower in some cases, due to error potentiation caused by not entirely parallel access paths.

Plant position is defined as the central position of all shoot bases belonging to the same plant individual. No attempt was made to distinguish between vegetative offshoots (ramets) and genetically distinct plants (genets). However, regeneration is thought to be almost exclusively by seeds both in primary forests and in the secondary forests derived from first-cycle slash-and-burn.

4.3.2 Leguminosae and ‘legume functional groups’

A total of 9383 legume plants were recorded on the 25 study sites, more than 98% of them were identified to the species level. Species were identified on-location or, in doubtful cases, at the INPA herbarium. Legume species are classified according to two criteria, resulting in four ‘legume functional groups’:

- growth form: trees vs. lianas
- ability to fix N_2 via BNF: potentially nodulating vs. non-nodulating species

The ability of legume species to nodulate is related to the evolution of both legumes and rhizobia. Species are classified as follows:

- According to literature data, where available (Allen and Allen, 1981; Magalhães et al., 1982; Faria et al., 1989; Moreira et al., 1993a+b; Moreira et al., 1994; de Souza et al., 1994 and 1997; Brito and de Souza, 1997; Faria and Lima, 1998). Whereas a single observation of nodulation is sufficient to prove a species’ nodulation capacity, it is almost impossible to prove a species’ incapacity to nodulate.
- Exploratory root excavations, establishing nodulation capacity for three important liana species previously not investigated (*Machaerium multifoliolatum* and *Dalbergia multiflora)*, or reported as non-nodulating (*M. hoehneanum*: Moreira et al., 1992). Rhizobia cultivars were subsequently isolated and stored in the rhizobia collection of INPA Soil Sciences Dept.
- The remaining 102 species are classified according to evolutionary considerations, evaluating the taxonomic position of the respective tribes and genera (Doyle, 1994; Sprent, 1995; Bryan et al., 1996; de Souza et al., 1997).

Annex 2 lists all legume species encountered in this study, their growth form and the classification of their nodulation capacity. Of the total of 157 legume species (42 genera), 123 are classified as certainly or probably capable of nodulation (52% of Caesalpinoideae, 82% of Mimosoideae, and 85% of Papilionoideae). Nodulation capacity is remarkably similar among growth forms, with 79% of the tree and 78% of the liana species classified as potential N_2-fixers.

The binary classification system of ‘potential N_2-fixers’ and ‘non N_2-fixers’ is obviously crude, as both the susceptibility to nodulate and the quantity of biologically fixed nitrogen vary widely among the group of potentially N_2-fixing species. Actual nodulation or BNF are regulated by many different edaphic factors (chap. 2.3).

4.3.3 Selected non-legume species

Evaluation of BNF with the ^{15}N natural abundance method requires the pairing of putative N_2-fixers with non N_2-fixing reference plants. Due to the prevalence of legume species potentially capable of nodulation, pairing with securely non N_2-fixing legume species alone does not yield sufficient replicates. The following seven non-legume tree and nine non-legume lianas (total of 8545 individuals) are, therefore, included in the botanical mapping (with species included in $\delta^{15}N$-sampling marked with *, see chap. 4.4.1):

Non-legume pioneer trees:

* *Jacaranda copaia* (AUBL.) D.DON (Bignoniaceae)

Cecropia sciadophylla MART., *C. distachya* HUBER and
C. purpurascens C.C.BERG (Cecropiaceae)

Goupia glabra AUBL. (Celastraceae)

* *Vismia guianensis* (AUBL.) CHOISY (Clusiaceae)

* *Laetia procera* (POEPP.) EICHLER (Flacourtiaceae)

Non-legume lianas:

* *Memora adenophera* SANDWITH,
M. moringifolia SANDWITH, and
Leucocalantha aromatica BARB. RODR. (Bignoniaceae)

* *Rourea cuspidata* BENTH. and
Pseudoconnarus rhynchosioides (STANDL.) PRANCE (Connaraceae)

* *Davilla rugosa* POIR. (Dilleniaceae)

Strychnos subcordata SPRUCE and *S. cogens* BENTH (Loganiaceae)

* *Securidaca rivinaefolia* ST.HILL (Polygalaceae)

The selection of these species aims at achieving as wide as possible coverage of sites and site classes, in adequate distribution throughout the area of the respective sites, and yielding sufficient replications. Chapter 5.1.3 reports on the most important of these species in terms of their vegetation shares and compares selected structural characteristics of these species, while chapter 5.6.1 provides the results of foliar N-analyses.

4.4 Sampling scheme

4.4.1 Leaf sampling

A total of 19 sites and 32 species were selected for analysis of foliar N-concentrations and δ^{15}N-signals. Table 4.2 gives an overview of the dataset of this study (comprising 2247 leaf samples after the exclusion of outliers and extremes, and of insufficiently replicated species).

The group of potential N_2-fixers covers 13 tree and 7 liana species, the group of non N_2-fixing legume reference species consists of 4 tree species (*Zygia racemosa, Macrolobium microcalyx, Parkia nitida* and *P. multijuga*) and 1 liana species (*Bauhinia alata*). Three pioneer tree species and 4 liana species were selected as non-legume reference plants. Species selected for δ^{15}N-analyses are marked with * in Annex 2 (legumes) and in chap. 4.3.3 (non-legumes).

Table 4.2. Foliar sampling scheme for %N- and δ^{15}N-analysis: sample replications of five species categories by site class (55 outliers and extremes previously excluded)

	pot. N_2-fixers		non N_2-fixing legumes	non-legumes		
	trees 13 species	lianas 7 species	5 species	trees 3 species	lianas 4 species	sub-total
4 yrs. (degraded) (1 site)	11	41	2	20	27	101
11 yrs. (degraded) (1 site)	27	78	22	61	46	234
4 yrs. (terra morena)* (1 site)	47	9	2	8	3	69
25 yrs. (‘outlier’)* (1 site)	28	30	5	14	21	98
3- yr.-old (2 sites)	39	69	22	38	49	217
5- to10-yr.-old (5 sites)	159	195	60	127	131	672
12- to 20-yr.-old (3 sites)	45	102	17	50	54	268
PF (clay) (5 sites)	201	158	80	14	135	588
sub-total (19 sites)	557	682	210	332	466	**2247**

* exceptional sites (see chapters 4.9.1 and 5.2.4)

The main criteria of species selection for foliar nitrogen analysis are the occurrence in as many as possible site classes and sites, and a regular within-site distribution. Many species were not considered because of insufficient replications. The three *Cecropia*-species were not included in sampling, because of probable isotopic effects caused by associations with *Azteca* ants (Sagers et al., 2000). *Goupia glabra* was excluded because of the prevalence of negative δ^{15}N-values reported in other studies, possibly caused by ‘some other pathway of N-nutrition’ (Guehl et al., 1998; Roggy et al., 1999b).

The selection of individual plants for foliar sampling is based on the results of botanical mapping and is designed to optimize information yield per sample. The sampling strategy follows three objectives: (1) to attain dense and regular within-site distributions, (2) to adequately represent small and large plant individuals, and (3) to cover locations possibly affected by BNF as well as areas not affected by N_2-fixing legumes:

(1) Sampling density averages $1.3/100m^2$ for potential N_2-fixers and $0.9/100m^2$ for non-legume references (mean of 18 sites) and does not differ systematically between site classes. Sampling density is lower for non N_2-fixing legume references ($0.5/100m^2$, mean of 6 sites), due to scarcer occurrence of these species. Sampling density within each site is approximately constant, allowing for interpolation of unsampled areas and spatial analysis of BNF (chap. 5.8).

(2) The phytomass of sampled plants is ln-normally distributed for (most) species and sites. This indicates that plant selection was successful in reflecting the skewed distributions of plant sizes along succession (chap. 5.1.5) and ensures the validity of investigations on possible plant size effects for BNF (chap. 5.6.3).

(3) Sampled plants were classified according to their proximity to potentially N_2-fixing legumes in their surroundings: 34.2% of all sampled plants were classified as ‘in direct neighborhood of large individuals or within clusters of potentially N_2-fixing plants’, and 25.1% as ‘not affected by neighboring potentially N_2-fixing plants’.

Maps 1-3 (in Annex 3) illustrate the foliar sampling strategy for the two main legume genera (7 *Inga* species and 5 *Machaerium* species covered by this investigation).

4.4.2 Plot-based sampling

Total aboveground phytomass, plant abundance and structural composition, as well as soil and litter chemistry were determined in 278 plots on the 25 study sites. The plots are distributed more or less regularly throughout the sites. They constitute independent samples, as they are not adjacent to another. Plot positioning in the primary forest sites avoided recent treefall gaps, all other characteristics of vegetation were explicitly ignored in order to avoid investigator bias. Maps 4-11 (in chap. 5.3.1 and Annex 6) illustrate the positioning and delimitation of plots, indicated as black squares.

Within the framework of a given total sampling area, a compromise needed to be found between adequate numbers of plot replications and reasonable plot sizes. Plot sizes were chosen to reflect the 'grain' of vegetation heterogeneity, which is related to the predominating size of 'plant influence zones' (Zinke, 1962; Rhoades, 1997). Plot size was, therefore, scaled from 5m x 5m (2- to 4 yr.-old regrowth), 7.50m x 7.50m (5 to 10-yr.-old regrowth), 10m x 10m (11- to 25-yr.-old regrowth) to 15m x 15m (primary forest). In compensation, the number of plots per area (plot density) is highest in young vegetation. Average area coverage over all sites is 18% (see Annex 1).

Litter mass was quantified in five subplots within each plot, in the plot center and at ¾-distance from the center to plot corners. The size of the subplots varies according to plot size: 50cm x 50cm (5m x 5m plots), 75cm x 75cm (7.50m x 7.50m and 10m x 10m plots), and 1m x 1m (15m x 15m plots). Fresh litter mass is calculated as the median of the five subplots. Litter dry weight and litter chemistry are based on composite subsamples taken from the five subplots.

Soil sampling is designed to account for small-scale variability, avoid investigator bias, and compensate for additional within-plot heterogeneity with increasing plot sizes (Palmer and Dixon, 1990). Topsoil (0-10cm) was sampled with an auger in a regular sampling grid, increasing with plot size from 50cm x 50cm to 1m x 1m and resulting in composite samples of 36-49 corings per plot.

The adequacy of the plot-based sampling scheme is discussed in chap. 4.9.2.

4.5 Quantification of phytomass

4.5.1 Allometric phytomass estimation

Aboveground phytomass of trees, lianas and arborescent palms was estimated allometrically, based on the stem diameters of all (up to 30) shoots per plant. Estimations used diameter-based mixed-species or species-specific log-linear allometric equations, established for primary forest trees in Colombian Amazonia (Overmann et al., 1994), secondary forest trees of central Amazonia (Nelson et al., 1999), and lianas of this study area (Gehring et al., submitted a). As there seem to be no diameter-based allometric equations for arborescent palms, tree allometric equations had to be used, in analogy to Laurance et al. (1999) and Clark and Clark (2000). The phytomass of arborescent palms is, therefore, probably overestimated.

Diameters were measured at breast height (dbh, i.e. at 1.30m shoot extension) or at 30cm shoot extension, individually decided on for optimal representation of the respective stem form and plant dimensions. Free choice between both diameter standards is regarded as advantageous compared to rigid definitions, as it allows flexibility in the transition-zone between 'small and thin' and 'large and thick' shoots. The compatibility between both diameter-standards was achieved with logistic regressions developed by Gehring et al. (submitted b), based on a subset of n=8916 tree, liana and palm shoots in which both diameters were recorded. Phytomass was subsequently estimated using the dbh for trees and arborescent palms, and using the diameter at 30cm extension for lianas.

Shoot length was not considered for allometric phytomass estimations, since the error caused by length estimation of large shoots is expected to be greater than the increase of precision achieved in allometric equations. This question is further discussed in Araújo et al. (1999) and Ketterings et al. (2001).

4.5.2 Destructive phytomass measurement

For estimation of total aboveground phytomass (TAGP), the phytomass of all plants smaller than approximately 1.30m height, and of all leaf palms within the 278 plots was determined by destructive harvesting and separation in wood and leaves for dry weight calculations. Dry weight was determined by oven-drying of representative subsamples for 1-2 weeks at 65°C. The phytomass of 'non-woody herbaceous' plants (forbs, Bromeliaceae, ferns) and of 'grasses and sedges' was likewise determined, but is negligible on all sites (data therefore not shown).

Litter was sampled in 5 subplots per plot, delimited by metal frames. The entire litter layer within these subplots was carefully collected and manually separated from fine roots. Litter was distinguished in mixed leaf litter, *Cecropia spp*. leaf litter, and wood/twigs. Composite samples for each litter compartment were taken for dry-weight calculations, and for P-, N- and δ^{15}N-analyses. The mass of large dead logs, mainly the remains of primary forest slash-and-burn, was not considered. The litter subplot position was, therefore, shifted when occupied by large logs.

4.6 Sample processing and analyses

4.6.1 Sample processing

Foliar samples are fully expanded mid-aged leaves collected as a mixture of different canopy positions, and of shade and sun-exposed leaves of the selected plants (Grubb, 1977; Weetman and Wells, 1990). Leaf petioles and rachis were removed, leaves with apparent symptoms of herbivory, fungus attack or other plant disease were discarded in order to avoid variability caused by such sources (Gebauer and Dietrich, 1993; Unkovich et al., 1993). Samples were immediately (within hours) transferred to a field oven maintaining constant 65°C, thereby reducing potential N-volatilization problems during drying (Thielen-Klinge, 1997). Samples were pre-ground in a conventional plant mill and subsequently pulverized in a ball mill to ensure homogenous samples for isotopic analysis.

Repeated sampling for quantification of seasonal δ^{15}N-variations was not feasible within the framework of this study. Foliar sampling was, therefore, restricted to two months (April - May 2000), at mid to end of the rainy season (see Figure 4.2). This sampling period is thought to provide the most stable δ^{15}N-signals (Högberg, 1986; Pate et al., 1994; Thielen-Klinge, 1997). It attempts to avoid sources of δ^{15}N-variation associated with the onset of the rainy season such as the nitrate pulse caused by soil rewetting after the dry spell and isotopic effects of leaf expansion (Abbadie et al., 1992; Lodge et al., 1994). It likewise avoids δ^{15}N-instabilities at the end of the rainy season or during the dry season, which are caused by N-retranslocation during leaf senescence (Virginia and Delwiche, 1982; Gebauer, 1991; Killingbeck, 1996).

Soil samples were processed in field fresh conditions. Samples were thoroughly homogenized, sieved to 2mm mesh-size and roots and charcoal fragments manually removed. Processed soil was air-dried and stored in an air-conditioned room until subsequent analyses.

4.6.2 Chemical analyses

Leaf and leaf litter N-concentrations were measured in an autoanalyser, and δ^{15}N-signal with a continuous flow mass spectrometer ANCA-SL 20-20 (Europa Scientific). Each sample was measured twice; every ten reading was calibrated with two wheat flower standards. Total P-content in litter was determined by HCl-extraction of ashed samples and subsequent analysis with the Mo-blue method.

Analysis of topsoil chemistry covers the pH (H_2O) and 'plant-available' P determined by resin extraction. Dry soil (250mg) was extracted with synthetic anion-exchange resin (BDH, N$^{o.}$ 55164) in 10ml of H_2O for 16hrs. Resin-P was thereafter completely extracted with HCl and determined colorimetrically by the Mo-blue method. This procedure is conform the first step of sequential P-extraction (Tiessen and Moir, 1993).

Table 4.3 shows the results of the litter and topsoil chemical analyses of the 25 study sites.

Table 4.3. Topsoil and leaf litter chemistry by site class: resin-extractable topsoil-P and pH, and mixed leaf litter P- and N-concentrations and δ^{15}N-signals (P-concentrations in ppm, P and pH as medians, nitrogen as means)

	topsoil (0-10cm)			mixed leaf litter			
		pH (H_2O)	resin-P		P	%N	δ^{15}N
4-5 yrs. (degraded)	(n=26)	4.53	0.75	(n=25)	0.26	1.91	3.46
11 yrs. (degraded)	(n=12)	4.05	1.09	(n=10)	0.16	1.97	2.67
4 yrs. (terra morena)	(n=12)	4.88	0.83	(n=11)	0.75	2.57	2.10
2- to 3-yr.-old	(n=56)	4.25	0.40	(n=54)	0.27	2.07	3.15
5- to10-yr.-old	(n=63)	4.31	0.51	(n=61)	0.25	1.98	2.56
12- to 25-yr.-old	(n=52)	4.41	0.91	(n=51)	0.26	1.59	3.36
primary forest (clay)	(n=42)	3.83	0.58	(n=41)	0.25	1.85	3.71
primary forest (sand)	(n=14)	3.77	0.63	(n=14)	0.33	2.42	3.10

4.6.3 'Plant-available' topsoil δ^{15}N-signal

The isotopic signature of plant-available ('mineral') nitrogen was investigated in a greenhouse experiment, using rice as bioextractor. The bioextraction approach avoids analytical problems encountered both with the KCl-extraction and solidification of the extractants (Lindau and Spalding, 1984; Sørensen and Jensen, 1991; Paparčikova, 1996).

Two composite soil samples each (0-10cm, 15-20 corings per sample) were taken from 17 sites. One sample represents locations with phytomass contributions of potential N_2-fixers far above the site average ('N_2-fixer clusters'), and the other represents locations with potential N_2-fixers absent in the surroundings (i.e., 5-8m radius), see chap. 4.3.2 for definitions. Comparison of δ^{15}N-signatures from the different sampling locations provides information on the degree of δ^{15}N-interactions between vegetation and topsoil and allows evaluation of the extent of possible 'δ^{15}N dilution' of the topsoil mineral N-pool by N_2-fixing vegetation.

Three rice seeds (*Oryza sativa* var. 'primavera') per sample were grown in ceramic pots containing 400g (dry weight) of 2mm-sieved soil. Rice was raised at 65-75% water-holding capacity and completely harvested after 6 weeks (i.e., before onset of flowering and leaf senescence), and analyzed for N-concentrations and δ^{15}N-signals. The experimental procedure is largely in analogy with that described by Ladha et al. (1993).

Rice required weekly amendments with (minus-N) nutrient solution, as stated by a parallel trial without fertilization, which failed because of overriding P-deficiency problems. Nutrient additions may have increased N-mineralization, the extent and isotopic effects are unknown.

Though rice extraction of '$\delta^{15}N_{min}$' avoids several analytical problems, this approach likewise suffers serious methodological drawbacks. Isotopic distortions may be caused a.o. by (1) disturbance of the soil sample (sieved to 2mm), (2) nutrient amendments required for raising the rice, or (3) the ^{15}N-discrimmination by rice N-uptake ('α-value', see chap. 2.5.2) differing from forest trees and lianas.

4.7 Estimation of biologically fixed nitrogen

The main features of the ^{15}N natural abundance method have been outlined in chapter 2.5. The method is based on the concept of two isotopically distinct sources of (foliar) nitrogen in potentially N_2-fixing legumes and paired non-fixing reference plants: air-derived and soil-derived N.

Atmospheric N_2 has a constant ^{15}N-content of 0.3663% and ‰-deviations of ^{15}N-contents from this air standard are expressed as '$\delta^{15}N$'. Positive $\delta^{15}N$ indicates ^{15}N-enrichment; negative $\delta^{15}N$ indicates ^{15}N-dilution relative to the air. The isotopic signal of soil-derived nitrogen is measured in the leaves of non N_2-fixing 'reference plants'. This study investigates differing categories of such reference plant species and takes mixed leaf litter as an additional reference.

The $\delta^{15}N$-signal of 'air-derived nitrogen' deviates from the air standard because of slight isotopic dilution in the process of N_2-fixation. A greenhouse experiment that was designed to measure these 'B-values' for the main legume species of this study failed. BNF is therefore estimated parallel in two scenarios, assuming B-values of -2‰ and 0‰. This is in analogy to the procedure applied by Roggy et al. (1999a) and represents the probable range of B-values occurring in vegetation.

The ^{15}N natural abundance method estimates the contribution of nitrogen in putatively N_2-fixing legumes which originates from BNF (%Ndfa). The calculation uses the following equation:

$$\%\text{Ndfa} = ((\delta^{15}N_{ref} - \delta^{15}N_{leg}) \div (\delta^{15}N_{ref} - \text{B-value})) * 100$$

where

$\delta^{15}N_{leg}$ = foliar isotopic signal of potentially N_2-fixing legumes, and
$\delta^{15}N_{ref}$ = foliar isotopic signal of soil-derived nitrogen in paired reference plants.

This study calculates %Ndfa in a range of scenarios covering differing reference plant categories (chap. 5.7.1) and differing pairing methods of legumes with reference plants (chap. 5.7.2).

4.8 Statistics

The assumption of normal distribution had to be refuted for many variables (Kolmogorov Smirnov and Shapiro-Wilks ‘W’ tests). Data were therefore either normalized, or nonparametric statistical procedures (medians, Kruskal-Wallis ANOVA, Spearman correlation) applied. Homogeneity of variance was routinely checked with partial regression plots.

Outliers or extremes (defined as >1.5-fold deviation beyond the 25-75% percentile range) were detected for the δ^{15}N-signals of 55 foliar and 1 leaf litter sample and were excluded from further data processing.

Histograms of plant phytomass are strongly positively skewed. This is true for all growth forms and for most species and genera (chap. 5.1.5). Natural logarithm (ln) data-transformations were most successful to achieve normality, though square root transformation was preferred for some species. Graphic scaling of individual plant phytomass in all maps presented in this thesis is done in seven steps, which are based on the 25%, 50%, 90%, 95%, and 99% percentiles of phytomass for the respective group of species and site.

Plot-based estimates of total aboveground phytomass (TAGP) and total abundance are normally distributed for all site classes; estimates of the structural composition of vegetation are given as medians. Both the δ^{15}N-signals and the calculated %Ndfa-estimates of all plant categories and of most species are normally distributed, but the exclusion of negative %Ndfa-estimates causes a negative skew.

Vegetation and taxonomic data are standardized to one hectare and no attempt is made to investigate the effect of site size on species composition (e.g., species:area-curves). Further standardizations of data aim at making TAGP comparable between sites (‘relative phytomass deviation’: %-difference of plot TAGP from the respective site means), and at eliminating species-specific differences of the reference δ^{15}N-signals (‘species-averaged references’: addition or subtraction of the difference of the species’ foliar δ^{15}N-signal from the median of the respective reference category and site).

The spatial distribution of legume plants is investigated with Ripley's point pattern statistics (Ripley, 1977; Diggle, 1983). Ripley's K(*d*) is a cumulative function expressing the expected number of further plants with increasing distance (*d*) from arbitrary plants, divided by the overall plant density of the site. This study uses L(*d*), the linearized estimator of Ripley's K(*d*) (Diggle, 1983). Distance is increased in 1m-steps, in accordance with mapping precision. Observed point pattern distribution is compared with mean complete spatial randomness (CSR), generated by 100 MonteCarlo simulations. Positive deviations from CSR indicate clustering, negative deviations indicate uniform plant distribution. Point pattern analysis is based on plant individuals only; the inclusion of plant phytomass in spatial analysis (via 'marked point processes') was not feasible within the framework of this thesis. Minimum plant numbers always exceed 100 per site.

Maximum distances for point pattern analysis (28-55m) were chosen conservatively, based on the minimum side lengths of the sites. Sites with directional anisotropy (identified by semivariogram modeling) were excluded. As border-effect correction algorithms require rectangular areas (Haase, 1995), irregular site edges were cut off prior to geostatistical analyses (causing an average area loss of 2.9%); the rectangularized borders are indicated on all maps. Point pattern analysis is stochastic, the results are, therefore, merely interpreted graphically and no attempts are made for further statistical analysis of graphed data.

The spatial distribution of %Ndfa-estimates within the study sites is investigated by interpolating the 'species-averaged' (see above) δ^{15}N-signals of potential N_2-fixers and of non-legume and legume reference plants. Interpolations were conducted parallel using two different interpolation algorithms (Watson and Philip, 1985; Mitas and Mitasova, 1988): Inverse Distance Weighed (IDW, based on the 12 nearest neighbors), and minimum curvature fitting (Spline, calculating regularized surfaces). Insufficient replications of δ^{15}N-samples impeded the modeling of semivariograms. Minimum-n was set at 30 δ^{15}N-samples each for potential N_2-fixers and reference plants for most sites (see chap. 4.4.1 for sampling densities, sampled plants are indicated as black circles and purple squares in Maps 22-31). Interpolated %Ndfa-estimates assume a B-value of 0‰, and are based on non-legumes and (six sites with sufficient sampling density) non N_2-fixing legumes as references. Interpolations were conducted in the same rectangularized site delimitation used for point pattern analyses (see above), spatial resolution was set to a 1m x 1m grid.

Significance values are given as $p<0.05$ (*), $p<0.01$ (**) and $p<0.001$ (***) if not specified otherwise. Statistical analyses were conducted with STATISTICA 5.1 (1998), and the S-Plus extensions 'EnvironmentalStats' (1998) and 'SpatialStats' (1995). Maps were produced with ArcView 3.1 (1998).

4.9 Sources of error

4.9.1 False-time-series approach and the constancy assumption

This study investigates secondary succession using the 'false-time-series' approach. Fallow age is isolated as a single factor, by maintaining all other factors constant. Such constancy assumption is the main drawback of any false-time-series approach, as this is obviously impossible to achieve in the field.

The present study achieves a high level of standardization of preceding land use history (uniformly first-cycle manual slash-and-burn and 1 year cassava cultivation). Though the fallow ages of some older sites represent best estimates, time precision is believed to be high due to repeated farmer questionings and pairings with historical events. In contrast, differences in soil texture and chemical composition are unavoidable sources of variability between sites, in spite of the high degree of regional homogeneity within the main soil order and careful field inspections prior to site selection.

Blocking of sites into chronosequences is designed to evaluate the extent of regional variability (i.e., at 15-100km distance). Table 4.4 shows the variation between chronosequences for the main parameters quantified by this study at the two endpoints of the time-series: 2- to 3-yr.-old regrowth and primary forest controls.

Table 4.4. Regional variability: comparison of key variables between chronosequences at the two endpoints of succession

	a) 2- to 3 yr.-old regrowth		b) primary forest (clay)			
chronosequence N$^{o.}$	2 (2 sites)	3 (2 sites)	1 (1 site)	2 (2 sites)	3 (2 sites)	4 (1 site)
total abundance (n ha^{-1})	14320	9564	4689	5484	4215	5541
TAGP (t ha^{-1})	87.4	128.8	418.1	394.6	545.3	452.8
structural composition of vegetation (in % of TAGP)						
lianas	5.0	3.9	3.0	1.3	0.9	0.2
palms	0.3	0.3	0.3	0.4	6.1	0.5
single-shoot plants	82.1	76.3	98.8	95.1	96.0	97.8
largest 5% of plants	21.7	36.7	84.6	85.8	87.9	85.8
Leguminosae	6.3	4.1	14.4	20.6	14.2	18.8
legume functional composition (in % of legume phytomass)						
potentially N_2-fixing trees	50.4	38.6	43.4	56.6	50.2	62.7
potentially N_2-fixing lianas	36.5	47.2	7.2	3.0	1.8	7.3
non N_2-fixing trees	7.7	6.7	46.4	40.3	48.0	28.0
non N_2-fixing lianas	5.4	7.6	3.1	0.2	0.1	2.0
important species and genera (in t ha^{-1})						
Goupia glabra	0.6	2.5	0	1.2	0.0	0.3
Laetia procera	0.6	1.8	0	2.1	0.3	0
Vismia guianensis	2.4	3.5	0	0.1	0.1	0
Cecropia spp.	32.3	4.6	0	0.2	0	0
Inga spp.	0.6	1.3	2.7	7.8	5.4	3.6
Machaerium spp.	1.7	2.2	4.3	2.5	0.6	6.2
soil chemistry						
pH (H_2O)	4.4	4.4	3.9	3.9	3.9	3.7
resin-P (ppm)	0.37	0.44	0.51	0.58	0.80	0.33
leaf litter						
mass (kg ha^{-1})	529	324	3400	5124	2031	2172
N-concentration	1.74	2.22	1.64	1.57	2.04	2.36
δ^{15}N-signal	+3.92	+2.80	+3.29	+3.93	+3.72	+3.69
P-concentration (ppm)	0.26	0.28	0.24	0.27	0.27	0.22
foliar δ^{15}N-signal						
Inga paraensis	n.d.	+7.30	+3.79	+4.05	+4.19	+4.41
Inga stipularis	+6.12	+5.77	+3.95	+4.03	+4.57	+4.99
Machaerium hoehneanum	+5.14	+4.49	+3.28	+3.66	+2.82	+4.12
Machaerium madeirensis	+4.18	+4.04	+2.19	+3.11	+3.11	n.d.
Zygia racemosa	+5.47	+6.15	+4.56	+4.87	+4.52	+5.47
Bauhinia alata	+5.13	n.d.	n.d.	+4.89	+4.72	+6.57
Laetia procera	+4.10	+3.84	n.d.	+3.30	n.d.	n.d.
Vismia guianensis	+4.43	+3.54	+3.86	+3.95	n.d.	n.d.
Davilla rugosa	+4.22	+3.46	+2.64	+2.72	+4.59	+4.90
Memora adenophera	+4.78	+4.71	+3.10	+3.11	+2.76	n.d.

Variation differs widely among the parameters. Both the structural vegetation characteristics and the functional composition of legumes are remarkably constant. In contrast, botanical composition at the genus and species level varies widely, with each site constituting ‘a case of its own’.

N-concentrations and δ^{15}N-signals of leaf litter and of most of the investigated species also differ significantly between some sites. However, no systematic variations between chronosequences are apparent, pointing to high between-site variability rather than to regional gradients.

Within the first-cycle successional series on clayey Oxisol, this study isolates a 25-yr.-old ‘outlier-site’ with consistently 2‰ lower foliar δ^{15}N-signals and highly variable leaf litter δ^{15}N-signals. This site is, therefore, excluded from further analysis. The underlying causes for such differences are unknown, as the site otherwise appears quite normal.

Site selection was not successful in maintaining P-levels constant, as both litter-P and resin-extractable P in the topsoil are significantly lower in chronosequence N$^{o.}$ 4 (Rio Preto asphalt road) than in the three Pres. Figueiredo chronosequences (N$^{o.}$ 1-3). Reversely, two (10- and 20-yr.-old regrowth) sites differ markedly having 2-3 times higher levels of resin-extractable P. Again, the underlying causes are unknown; topsoil pH was elevated in one of these sites, whereas litter-P did not differ. The false-time-series approach is thus unsuitable for the investigation of possible P- and pH-effects. Site-by-site correlations are too low in their statistical power due to insufficient plot replications per site and are, therefore, not shown.

4.9.2 Adequacy of the plot-based sampling scheme

Forest phytomass is highly skewed and skewedness increases with successional age (chap. 5.1.5). Especially in primary forests, a large portion of phytomass is concentrated in merely a few irregularly distributed big trees. Phytomass estimates will, therefore, become erratic if the sampling area is too small.

Site sizes (Table 4.1 and Annex 1) are deemed sufficient for an adequate representation of most of the common species and genera investigated by botanical mapping of the entire sites. In contrast, plot-based assessment of total vegetation is necessarily more limited in regard to the feasible total sampling area. The plot-based sampling scheme is designed to scale with the 'grain' of heterogeneity along succession (see chap. 4.4.2), i.e., with the predominant size of 'plant influence zones' (Zinke, 1962; Rhoades, 1997). The disadvantages of overdimensioned plot sizes are discussed in Palmer and Dixon (1990). In summary, a compromise between adequate repetitions and plot sizes is needed.

The coefficient of variation (CV in %) is taken as an indicator of the adequacy of sampling design. Average within-site variability of the 25 sites is 33% (for TAGP, 4 sites >50%). Variability within the site classes is fairly constant (Table 4.5), suggesting that a systematic sampling bias along succession was avoided.

Variation is consistently higher for lianas and palms (with low phytomass shares) as compared to total or tree phytomass. CVs of the litter horizon are intermediate, with variation presumably reduced by the sampling scheme involving 5 subplots per plot.

Table 4.5. Evaluation of the plot-based sampling scheme: plot replications, plot sampling area and CV (in %) per site class and phytomass compartment

site class	No. of plots	sampling area (m^2)	total phytomass	trees	lianas	palms	litter*
4 yrs. (terra morena) (1 site)	12	616	50.9	60.3	106.3	194.9	72.5
4-5 yrs. (degraded) (2 sites)	26	650	38.5	43.8	158.2	-	22.5
11 yrs. (degraded) (1 site)	12	1200	21.3	21.8	79.1	-	36.0
2- to 3-yr.-old (4 sites)	48	1294	41.6	44.6	94.0	-	44.7
5- to 10-yr.-old (5 sites)	71	4825	35.1	36.2	72.1	-	67.6
12- to 25-yr.-old (4 sites)	52	5200	30.6	32.2	177.4	137.4	47.0
PF (clay) (6 sites)	43	9675	37.1	38.4	128.0	119.8	52.1
PF (sand) (2 sites)	14	3150	30.4	31.6	84.5	73.6	35.1

*: calculated over median values of 5 subplots per plot (see chap. 4.4.2)

4.9.3 Vegetation components not covered

Vegetation may be inadequately quantified by (1) direct omission of certain compartments, or (2) types of vegetation not represented in the sampling areas.

(1) The present study constitutes an important improvement over many other studies, as small plants were not excluded. This provides data over the entire (aboveground) vegetation. Data presented in Table 4.6 are likely to be useful for other studies, for assessing the degree of phytomass underestimation caused by common minimum diameter thresholds, and comparing data sets published in the literature.

Table 4.6. Percentage of total tree and liana abundance (%n) and phytomass (%t) covered by commonly applied minimum diameter thresholds

	4-5 yrs. (degraded)		2- to 3-yr.-old		12- to 25-yr.-old		primary forest (clay)	
	% n	% t	% n	% t	% n	% t	% n	% t
>10cm dbh	4.6	27.9	3.4	24.1	12.2	59.5	11.4	89.9
5-10cm dbh	21.2	44.6	22.3	51.1	20.5	28.5	11.1	6.6
2.5 - 5cm dbh	23.9	19.3	24.8	16.0	18.2	7.6	19.1	2.5
<2.5cm dbh	50.4	7.9	49.5	8.5	49.1	4.2	58.4	0.8
<1.30m *	x	0.3	x	0.3	x	0.2	x	0.2

*: destructively determined trees and lianas <1.30m height (see chap. 4.5.2)

A tradeoff exists between area coverage and feasible minimum diameters. For large-scale inventories in primary forests, a minimum dbh of 10cm (Newbery et al., 1992; Laurance et al., 1999) is justified, as this will cover 90% of total phytomass. A minimum dbh of 2.5cm for primary forest lianas (Laurance et al., 2001) would result in an 82% coverage of liana phytomass, likewise acceptable in the face of low shares of liana phytomass in primary forests. In contrast, studies in young or degraded vegetation require far lower diameter thresholds or need to quantify small plants destructively.

Aside from the desired level of precision for phytomass estimations, the large pool of small plants merits consideration, as these plants are vital for regeneration dynamics and form an understory of particularly high diversity notably in primary forests (Gentry and Dodson, 1987).

Total aboveground phytomass (TAGP) includes the litter layer, but excludes large logs. Chambers et al. (2000) estimated the mass of dead coarse wood (>10cm diameter) at 21t ha-1 in a primary forest at approximately 50km distance from this study area.

Log remnants of the preceding primary forest, which were only partially consumed by the burn and subsequent decomposition, are a conspicuous feature of several young and mid-aged sites derived from first-cycle slash-and-burn. According to Kauffman et al. (1995), the burn consumes only 50% of the large (>20cm diameter) woody debris. Such coarse-wood mass influences soil formation and nutrient dynamics, and may create microsites favorable for the establishment of pioneer species (Lack, 1991).

(2) For methodological reasons (striving for within-site homogeneity), site and plot selection avoided the following vegetation components:

The present study is in line with the large majority of other studies in discarding 20- to 50m-wide ‘border zones’. These separate sites from neighboring primary forest margins, thus excluding border-related edaphic gradients (Galo et al., 1992). Border zones are very common in the study region, due to the prevailing small field sizes (typically less than one hectare) and the abundance of primary forests in their surroundings. Since border zones are higher in stature and basal area (Mesquita et al., 2001), their exclusion causes an underestimation of area-based phytomass accumulation, which is, therefore, expected to be even more rapid than indicated in Figure 5.1.

Primary forest margins also differ markedly in their species composition; seed dispersal gradients are one underlying reason. Willson and Crome (1989) found wind-dispersed seeds to move farther from the forest margin into neighboring fields than vertebrate-dispersed seeds, and Gorchov et al. (1993) found bird-dispersed seeds to be more distance-limited than bat-dispersed seeds. Botanical mapping of this study accidentally included one primary forest margin. The almost complete absence of otherwise dominant pioneer trees and the elevated occurrence of legume species incapable of N_2-fixation are characteristic features of this border zone (Table 4.7).

Table 4.7. Differing botanical composition in a primary forest border zone: case study in a 20-yr.-old regrowth site

		non-border ('core') area (0.80ha)		PF border zone (0.21ha)	
		n ha^{-1}	t ha^{-1}	n ha^{-1}	t ha^{-1}
trees	*Cecropia* spp.	209	21.8	0	0
	Goupia glabra	56	2.6	15	0.2
	Laetia procera	91	5.8	5	0.3
	Vismia guianensis	209	12.7	15	0.4
	Inga spp.	50	8.3	73	5.4
	Zygia racemosa	4	0.2	49	1.1
	Parkia spp.	4	0.2	15	0.1
lianas	*Machaerium* spp.	275	1.2	49	0.04
	3 *Memora* species*	35	0.02	24	0.01
	Davilla rugosa	26	0.2	5	0.01

* *Memora adenophera, M. moringifolia, M. flaviflora*

This study likewise excluded areas containing remnant primary forest trees. Such remnant trees are common in the secondary regrowth of the region. They favorably influence microclimate and accelerate the establishment of ornitochorous woody vegetation, thereby acting as nuclei of accelerated regeneration (Guevara et al., 1986; Otero-Arnaiz et al., 1999; Carrière et al., 2002).

Avoidance of recent treefall gaps in plot positioning causes an overestimation of area-based primary forest phytomass. In level primary forests, 0.5-6.3% of the area is affected by treefall gaps (Sanford, 1986; van der Meer and Bongers, 1996). Structural and floristic composition of primary forests is likewise affected by the differing vegetation in treefall gaps, and gap size has been shown to be a decisive factor for gap succession dynamics (Brokaw, 1985; Alvarez-Buylla and Martinez-Ramos, 1992; Cintra and Horna, 1997). The occurrence of nodulation in primary forest treefall gaps suggests that BNF may be elevated in such areas (Vitousek and Denslow, 1986).

5 RESULTS

5.1 General vegetation characteristics

This section gives a general description of the forest vegetation on the 25 study sites, showing the phytomass accumulation, growth form composition and a variety of structural vegetation characteristics along the successional time series. Information is required to understand the regrowth dynamics in this agroecosystem and to appraise the successional stage of each study site.

Results commence with the total aboveground phytomass (chap. 5.1.1), and subsequently detail the liana, palm and litter vegetation components (chap. 5.1.2) and the most characteristic pioneer tree genera (chap. 5.1.3). The number of shoots per plant (chap. 5.1.4) and the skewedness of plant-size distribution (chap. 5.1.5) are further 'structural' characteristics under investigation. Chapter 5.1.6 synthesizes these data and takes the degree of successional reapproximation to primary forest levels as a measure of resilience of this slash-and-burn agroecosystem.

5.1.1 Total aboveground phytomass

Total aboveground phytomass (TAGP, including the litter layer but excluding dead logs) is estimated at 460t ha^{-1} in primary forests on clayey Oxisol (mean of 6 sites); the TAGP of primary forests on the more infertile sandy Ultisol ('campinarana') is 25% lower (346t ha^{-1}: mean of 2 sites).

Figure 5.1 plots the phytomass accumulation with successional regrowth, taking the form of an asymptotic saturation-curve ($R^2=0.86$, $p<0.001$) and indicating a very rapid initial phytomass accumulation and a slowdown later in the succession:

$$\text{TAGP} = 44.4 + 131.94 * \log10 \text{ (yrs. after abandonment)}$$

Calculation is based on the 13 secondary regrowth sites constituting 'first-cycle succession' (circles); the degraded sites (open squares) and the terra morena site (open triangle) are plotted for comparison. Phytomass would reaccumulate to 75% of the primary forest level after an estimated 175 years of regeneration.

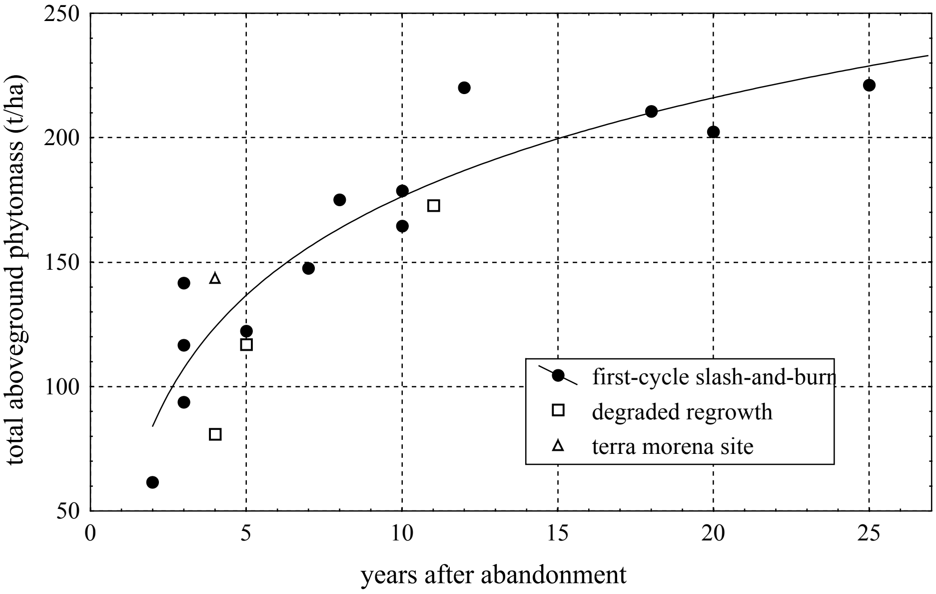


Figure 5.1. Phytomass accumulation along succession

Phytomass on the three degraded sites is below average, though the difference appears to narrow down with time. Phytomass on the more fertile terra morena site is 13% above average. In ‘young’ (2- to 5 yr.-old) regrowth, LSD-test indicates significantly higher TAGP for the terra morena site as compared to the degraded sites, whereas first-cycle regrowth is intermediate. The 11-yr.-old degraded site is significantly lower in TAGP than the 12-yr.-old first-cycle regrowth, but does not differ statistically from two 10-yr.-old first-cycle regrowth sites.

Total aboveground phytomass and total abundance of the 25 study sites are detailed in Annex 1.

5.1.2 Non-tree phytomass components along succession

Trees dominate both TAGP (t ha^{-1}) and total abundance (n ha^{-1}) in all types of vegetation. Shares range from 90-96% in TAGP and from 67-82% in total abundance. However, non-tree compartments vary systematically among site classes differing in their successional stage (see chap. 4.2 for site classification). This section presents data on (1) lianas, (2) palms, and (3) the litter layer along succession. Liana and palm phytomass contributions in the 25 study sites are detailed in Annex 1.

(1) Lianas

Table 5.1 shows liana phytomass and abundance and their percentage of total vegetation. These are highest in young and degraded regrowth and decrease with successional age. Lianas contribute 20-30% to total abundance in secondary regrowth with abundance shares being consistently one order of magnitude higher than phytomass shares. Liana phytomass contribution varies locally, attaining a maximum of up to 50% of TAGP in thickets (last column in Table 5.1).

Lianas appear to be an almost irrelevant component of primary forests, contributing only 0.8-1.8% of the total phytomass. They do, however, constitute a significant component of primary forest undergrowth, as indicated by abundance shares of 10-14%.

Table 5.1. Lianas along succession: phytomass, abundance, and percentage of total vegetation

		liana abundance		liana phytomass		
	site-n	plants ha^{-1}	% of total	tons ha^{-1}	% of total	plot max.%
4-5 yrs. (degraded)	2	2302	19.2	7.1	8.4	53.7
11 yrs. (degraded)	1	1883	23.7	3.4	2.1	6.9
4 yrs. (terra morena)	1	1758	26.4	1.8	1.6	7.2
2- to 3-yr.-old	4	3060	30.2	6.3	6.5	22.0
5- to 10-yr.-old	5	1583	26.6	4.8	3.5	16.3
12- to 25-yr.-old	4	1182	22.6	5.0	2.5	36.6
PF (clay)	6	724	14.2	8.2	1.8	9.1
PF (sand)	2	681	10.7	2.6	0.8	2.3

(2) Palms

Palms form a conspicuous element especially in central and western Amazonian primary forests (Terborgh and Andresen, 1998). Species diversity is high, Ribeiro et al. (1999) listing 45 species in 15 genera for a primary forest reserve at approximately 100km from this study area. Leaf palms (‘stemless’; anatomically the stems are often below the soil surface) dominate the undergrowth and mid-canopy levels (Kahn and de Castro, 1985).

Table 5.2 presents palm phytomass, growth form composition, and palm contribution to TAGP with successional age.

Table 5.2. Leaf palms and tree palms: phytomass and percentage of TAGP along succession

		phytomass (t ha^{-1})*		% of TAGP	
	site-n	tree palms	leaf palms	mean	plot max.
4-5 yrs. degraded	2	0.01	0.2	0.2	1.1
11 yrs. degraded	1	2.0	0.3	1.2	7.2
4 yrs. (terra morena)	1	4.3	4.0	9.2	54.8
2- to 3-yr.-old	4	1.0	0.3	1.8	26.9
5- to 10-yr.-old	5	0.2	0.4	0.4	11.0
12- to 25-yr.-old	4	3.7	0.4	2.1	7.6
PF (clay)	6	10.4	1.0	2.7	22.3
PF (sand)	2	8.9	0.1	2.7	5.2

* tree palms were determined allometrically, leaf palms were determined destructively and also include juvenile palms (see chap. 4.5)

With a phytomass share of 2.7%, palms surpass lianas in primary forests. The phytomass share of palms in secondary regrowth is about half of that of primary forests. The palm contribution is exceptionally high in the terra morena site and is locally considered as a soil-quality indicator.

(3) Litter layer

Total litter mass increases log-linearly with successional age ($R^2=0.29$, $p=0.01$, $n=17$ sites). Litter mass is six-fold higher in primary forests than in secondary regrowth, whereas the leaf litter contribution remains fairly constant among most site classes (Table 5.3). Within first-cycle secondary regrowth, phytomass of standing vegetation and litter mass are significantly correlated to one another (Pearson R = +0.32, n=169 plots, $p<0.001$).

Table 5.3. Litter horizon along succession: litter mass and leaf litter percentage

	site-n	total litter (kg ha^{-1})	% leaf litter
4-5 yrs. (degraded)	2	465	85.3
11 yrs. (degraded)	1	1258	67.1
4 yrs. (terra morena)	1	137	70.0
2- to 3-yr.-old	4	367	79.5
5- to 10-yr.-old	5	584	74.1
12- to 25-yr.-old	4	747	77.0
PF (clay)	6	3326	72.1
PF (sand)	2	3221	69.2

Since the litter layer was quantified during the rainy season, the low values are attributed to the combination of low litterfall and rapid decomposition (Luizão and Schubart, 1987). Values are, therefore, not directly comparable to the 5-12t ha^{-1} reported for Amazonian primary forests by Brown et al. (1995), Höfer et al. (1996), and Delaney et al. (1997).

5.1.3 Important pioneer tree species and genera

Four non-legume pioneer tree species and genera covered by this study are characteristic not only of the secondary vegetation of the study area, but are also very common in many other regions of Amazonia: *Goupia glabra, Laetia procera, Vismia guianensis* and *Cecropia* spp. Table 5.4 shows successional and land use related changes in the vegetation shares of these pioneers and additionally of the most important legume tree genus *Inga*. Species are listed in chap. 4.3.2 and Annex 2.

Table 5.4. Important pioneer tree species and genera:
contribution to total abundance (%n) and to TAGP (%t) by site class

	Goupia glabra		*Laetia procera*		*Vismia guianensis*		*Cecropia spp.**		*Inga spp.***	
	%n	%t	%n	%t	%n	%t	%n	%t	%n	%t
4-5 yrs. (degraded)	0.8	2.7	1.0	3.5	1.2	4.2	2.1	5.6	0.5	0.5
11 yrs. (degraded)	2.5	1.6	5.6	11.8	5.2	6.5	4.3	20.2	1.7	1.6
4 yrs. (terra morena)	0.1	0.3	0	0	0.1	0	0.3	1.0	10.4	15.4
2- to 3-yr.-old	1.1	1.3	1.5	1.1	1.7	3.1	6.6	19.2	1.2	0.8
5- to 10-yr.-old	2.0	6.5	2.4	4.2	4.2	3.9	2.2	15.5	1.1	2.1
12- to 25-yr.-old	2.2	4.3	1.7	3.5	3.5	2.0	2.9	8.8	2.2	2.5
PF (clay)	0.2	0.1	0.1	0.2	0.2	0.2	0.1	0	4.7	1.2
PF (sand)	0.1	0.7	0	0	0	0	0	0	3.5	0

* *C. sciadophylla, C. distachya, C. purpurascens*
** more details in chap. 5.2.3

The share of phytomass of these four non-legume pioneer tree species and genera in TAGP is maximum (30%) in mid (5- to 10-yr.-old) succession. Lower phytomass shares in late (12- to 25-yr.-old) succession are partly caused by declining abundance.

Successional optima differ among species: Phytomass shares of *Vismia guianensis* are high in the degraded sites, locally gaining dominance in almost monospecific clusters. This appears to be a general feature of this species (Uhl et al., 1988; Mesquita et al., 2001). *Cecropia* spp. dominates the top canopy in early and mid-succession, but rapidly declines in old regrowth. The genus *Cecropia* consists of three species in the study area (*C. purpurascens, C. distachya, C. sciadophylla*), which differ mainly in their degree of mutualism with *Azteca* ants (obligatory, facultative and no mutualism, respectively; Berg, 1978). No systematic differences between site classes are apparent (data not shown). The minor occurrence of *Cecropia spp.* and of *V. guianensis* in two primary forest sites is spatially aggregated and probably caused by old treefall gaps. In contrast, both *Goupia glabra* and *Laetia procera* occur as single tree giants in primary forests.

Differences in the structural composition (chapters 5.1.4 and 5.1.5) of the four pioneer tree species and genera are shown in Annex 4, and their combined contributions to TAGP on the 25 sites listed in Annex 1.

The spatial distribution of *L. procera, V. guianensis* and of the *Cecropia* species is illustrated in Maps 15-17 (Annex 7) for three exemplary regrowth sites.

5.1.4 Number of shoots per plant

The number of shoots per individual plant varies considerably (up to a maximum of 30 shoots). Figure 5.2 shows successional changes in phytomass share of four classes of shoot number per plant for all plants investigated in this study.

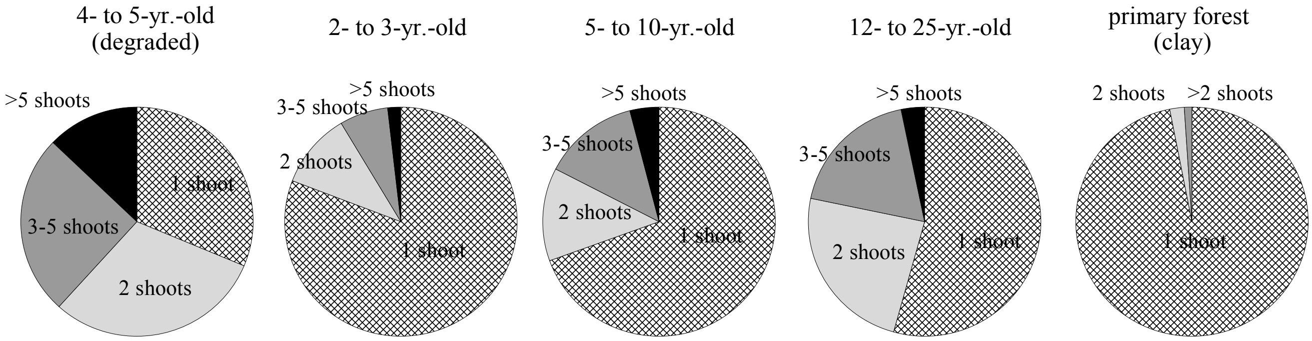


Figure 5.2. Successional changes in the phytomass share of plants with different shoot numbers

Single-shoot plants make up $^2/_3$ – $^3/_4$ of the total phytomass in secondary vegetation. This is in marked contrast to primary forests, where phytomass is entirely dominated by single-shoot plants (97% of TAGP). Young degraded regrowth is substantially different from first-cycle secondary succession, as single-shoot plants constitute only 31%, and plants with five or more shoots make up 13% of total phytomass (11-yr.-old degraded site: 19% and 6%, respectively). This may have been caused by an increase in vegetative resprouting.

Mean shoot quantity per plant is higher for lianas (1.50) than for trees (1.43) and palms (1.36, for first-cycle regrowth). This difference between lianas and trees is significant in first-cycle regrowth (t-test $p<0.001$), but not in primary forests. Annex 4 (1st column) compares the phytomass contributions of multiple-shoot plants to total phytomass of the four non-legume pioneer tree species and genera listed in Table 5.4.

5.1.5 Plant size distribution along succession

Total abundance decreases along succession from 11050 plants ha^{-1} in young regrowth to 4800 plants ha^{-1} in primary forest. Phytomass accumulation is, therefore, due to increased individual plant phytomass; however, this increase does not occur evenly for all plants. Figures 5.3 and 5.4 show histograms of phytomass and abundance shares of differently sized trees and lianas along succession, with plant size (allometrically estimated aboveground phytomass) given on a log-decimal scale. Plant phytomass distribution is positively skewed in all but the youngest sites, and skewedness increases continuously with succession. Skewedness is greatest in primary forests, differing substantially from any stage of secondary regrowth. Both the maximum individual phytomass and the degree of skewedness are lower for lianas than for trees, especially in primary forests. Nevertheless, the successional increase of skewedness is similar in pattern for the tree and liana compartments.

In general, the plant phytomass distribution of legume trees and lianas does not differ from that of non-legume trees and lianas. However, in primary forests the potentially N_2-fixing lianas appear less skewed than the non N_2-fixing lianas.

An increase of skewedness along succession is evident for all plant species, but species-specific differences are apparent. Annex 4 (2nd column) shows the phytomass contributions of the largest 5% of individuals of selected non-legume pioneer trees. Plant size of obligate pioneers (*Cecropia* spp. and *Vismia guianensis*) is more evenly distributed than that of pioneers, which also occur in primary forests (*Goupia glabra* and *Laetia procera*).

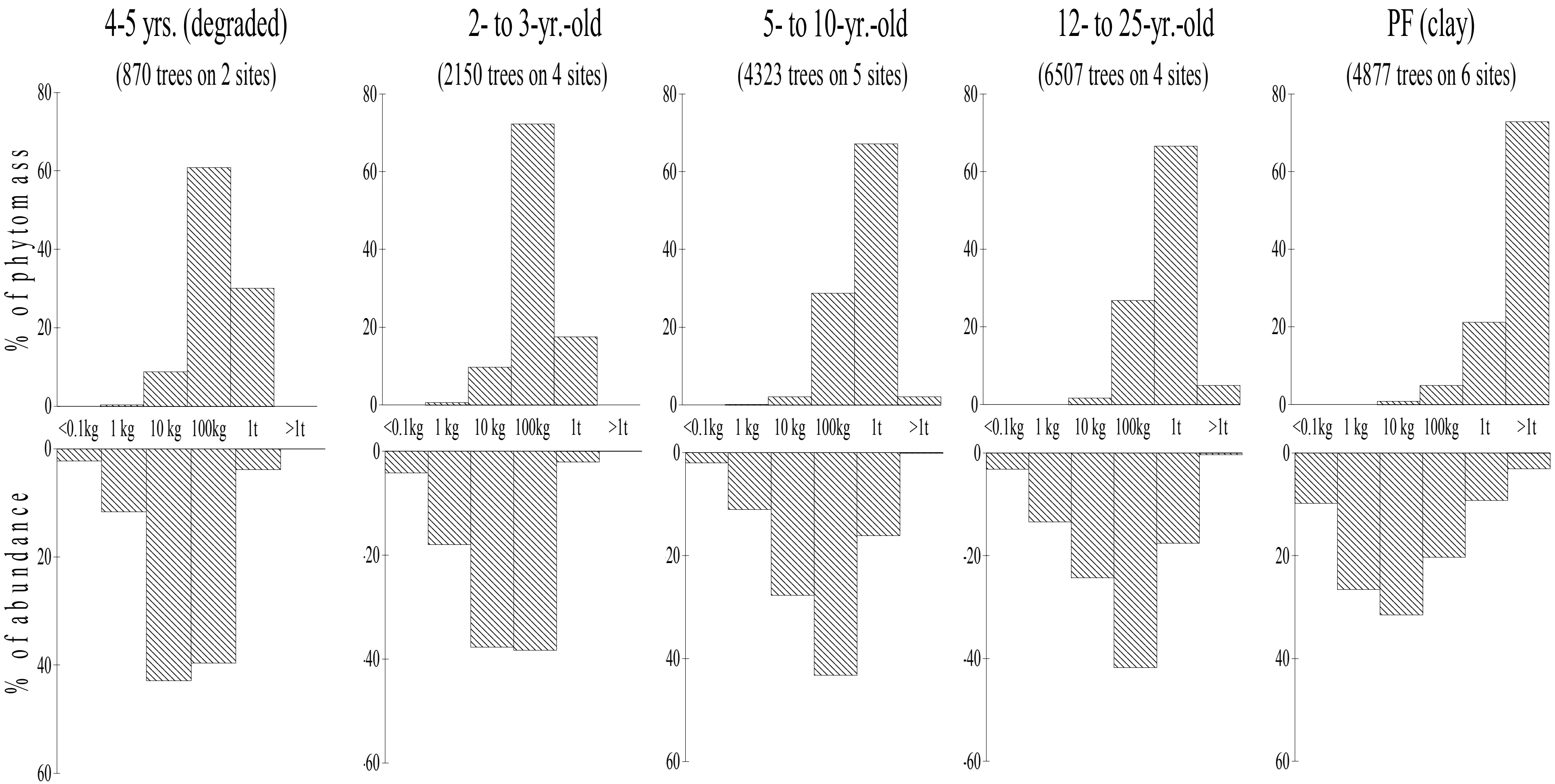


Figure 5.3. Successional changes in tree size distribution: histograms of relative composition of phytomass (top row) and of abundance (bottom row), size-classes are upper limits of estimated individual plant phytomass on a log(10)-scale

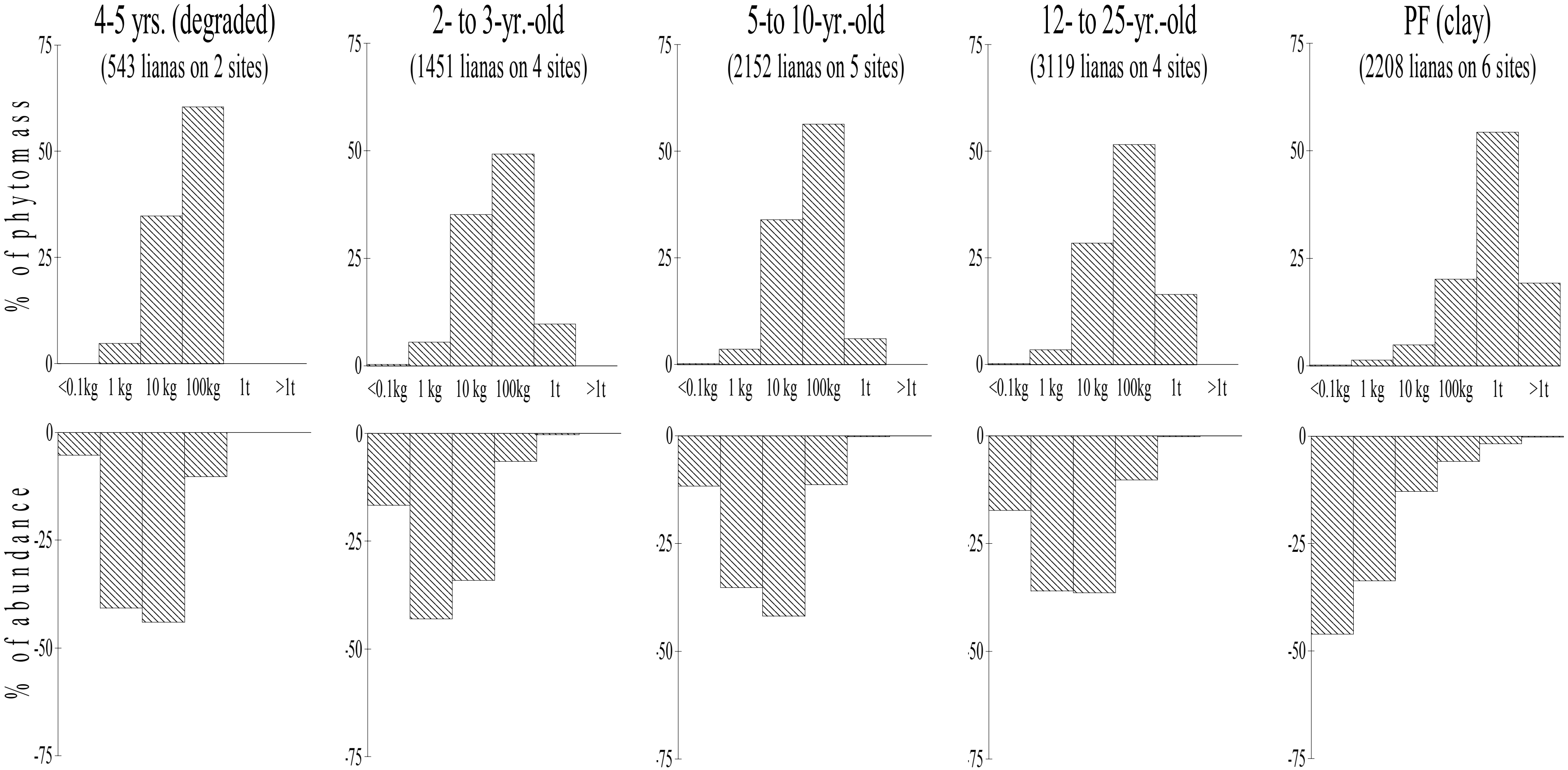


Figure 5.4. Successional changes in liana size distribution: histograms of relative composition of phytomass (top row) and of abundance (bottom row), size-classes are upper limits of estimated individual plant phytomass on a log(10)-scale

Table 5.5 shows the increasing concentration of total phytomass in the largest 5% of tree and liana individuals. Primary forest phytomass is dominated by a few 'giant' plants. Conversely, the half of the smallest vegetation ('undergrowth') contributes little to the phytomass of primary forests.

Table 5.5. Phytomass percentage of relative plant size classes along succession

trees

	2- to 3-yr.-old	5- to 10-yr.-old	12- to 25-yr.-old	primary forest
smallest 50%	4.7	4.8	2.6	0.3
50-95%	67.5	55.9	56.7	17.1
largest 5%	27.8	39.3	40.7	82.6

lianas

	2- to 3-yr.-old	5- to 10-yr.-old	12- to 25-yr.-old	primary forest
smallest 50%	3.3	2.5	1.5	0.2
50-95%	48.4	49.4	32.0	9.5
largest 5%	48.3	48.1	66.5	90.3

Inequality in phytomass shares not only increases with succession, but also differs as a result of differences in 'vigor' of vegetation (Table 5.6). The degraded sites exhibit the most evenly distributed phytomass. In contrast, the terra morena site is structurally similar to old secondary regrowth in terms of tree phytomass distribution. Liana phytomass distribution is less skewed because of the dominance of small-sized *Derris* spp.

Phytomass contribution of the largest 5% of all plants to the TAGP of the 25 study sites is listed in Annex 1.

Table 5.6. Phytomass percentage of relative plant size classes in young regrowth: impact of degradation and of soil fertility

trees

	4-5 yrs. (degraded)	2-3yrs. (first-cycle)	4 yrs. (terra morena)
smallest 50%	8.3	4.7	2.4
50-95%	74.6	67.5	55.0
largest 5%	17.1	27.8	42.6

lianas

	4-5 yrs. (degraded)	2-3yrs. (first-cycle)	4 yrs. (terra morena)
smallest 50%	5.5	3.3	5.6
50-95%	49.7	48.4	46.3
largest 5%	44.8	48.3	48.1

(=effect of *Derris* spp.)

5.1.6 Summary and conclusions

Table 5.7 summarizes the vegetation data presented in this section. It is designed as a means to evaluate the degree of successional approximation of vegetation characteristics to primary forest status. Though the biological significance of these parameters is unknown, their successional reapproximation to the original levels encountered in the primary forest controls is taken as a measure of ecosystem ‘resilience’, which in turn is an important measure of the sustainability of the slash-and-burn land use investigated by this study.

Table 5.7. Vegetation total and the structural composition of vegetation along succession: reapproximation to the levels of primary forest controls (total n=278 plots)

		mean vegetation total		median vegetation composition (in % of TAGP)				
	plot-n	n ha^{-1}	t ha^{-1}	lianas	palms	four pioneer trees	single-shoot plants	biggest 5% of plants
4-5 yrs. (degraded)	26	9246	100.3	7.9	0.2	32.9	36.3	34.7
11 yrs. (degraded)	12	7567	172.9	2.1	1.2	71.0	19.1	37.2
4 yrs. (terra morena)	12	6548	143.7	1.6	9.2	0	39.3	55.8
2- to 3-yr.-old	48	11050	115.8	6.2	1.6	31.3	83.5	32.0
5- to 10-yr.-old	71	6306	157.9	3.6	0.5	38.5	67.2	33.2
12- to 25-yr.-old	52	5025	212.2	2.5	1.8	27.8	57.1	53.1
PF (clay)	43	4779	469.6	1.9	3.4	0	98.0	86.5
PF (sand)	14	6257	342.4	0.8	2.8	0	94.5	86.8
		(chap. 5.1.1)		(chap. 5.1.2)		(chap. 5.1.3)	(chap. 5.1.4)	(chap. 5.1.5)

Whereas total abundance and the phytomass share of lianas decline rapidly, the increase of the degree of skewedness towards the conditions in primary forest is slower. The contribution of single-shoot plants declines along succession before attaining maximum levels in primary forests, suggesting that the approximation of vegetation characteristics is not necessarily unidirectional. Primary forests differ markedly from all stages of secondary regrowth even after 25 years of regeneration. The three degraded sites are even more behind in their structural composition than in their merely moderately lower total phytomass stocks.

The structural parameters investigated in this study also appear useful as a means of characterizing abundant species or genera; notably *Cecropia* spp. and *Vismia guianensis* differ systematically from another in all parameters throughout succession (Annex 4).

5.2 Legume vegetation

This section gives a description of the legume vegetation component along succession. Investigations are based on the complete inventory and phytomass estimation of all legume plants occurring on the 25 study sites, allowing for a quantitative analysis of the legume community.

Chapter 5.2.1 shows the legume share of the total vegetation; this information is necessary for evaluating the role legume-BNF may play in these forests. Chapter 5.2.2 describes changes of legume 'functional' composition along succession, classifying legumes by their ability to fix N_2 and by their growth form. The share of phytomass of the potentially N_2-fixing species will be taken as an indirect indicator of the role of BNF along succession. Chapter 5.2.3 describes the taxonomic composition of the legume community and identifies important tree and liana genera and species, and chapter 5.2.4 details the exceptional 4-yr.-old regrowth site on terra morena do índio dominated by N_2-fixing *Inga edulis*.

5.2.1 Legume vegetation share along succession

Legume contribution to the total vegetation varies from 5-8% (TAGP) and 9-12% (total abundance) in secondary regrowth (Table 5.8). With a phytomass of 40-80t ha^{-1}, the legume contribution to TAGP is two to three times higher in primary forests than in secondary regrowth (17% vs. 5-8%).

The legume shares in tree vegetation are similar to that in total vegetation. In contrast, legume share of the liana component is three to ten times higher, both in terms of phytomass and abundance. No systematic shifts in legume share are apparent with succession, but degradation appears to reduce the legume tree and increase the legume liana components.

Table 5.8. Leguminosae along succession: abundance (n ha^{-1}), phytomass (t ha^{-1}), and legume vegetation share (%n and %t)

	n ha^{-1}	t ha^{-1}	% of total vegetation %n	% of total vegetation %t	% of trees %n	% of trees %t	% of lianas %n	% of lianas %t
4-5 yrs. (degraded)	687	4.5	8.6	4.6	3.0	2.4	41.9	59.5
11 yrs. (degraded)	959	6.2	13.4	3.6	4.8	3.2	36.4	27.1
4 yrs (terra morena)	1139	23.2	18.6	16.2	14.0	17.1	26.7	23.2
2- to 3-yr.-old	1181	5.1	11.6	5.2	4.2	3.0	26.6	40.0
5- to 10-yr.-old	544	11.5	9.2	8.2	4.1	7.6	20.0	29.2
12- to 25-yr.-old	577	10.5	12.0	4.9	5.0	4.5	33.2	24.9
PF (clay)	706	78.4	15.5	17.1	9.9	17.1	42.6	80.1
PF (sand)	795	41.2	14.2	12.4	8.5	12.8	50.2	30.6

5.2.2 Legume nodulation capacity and growth forms

Figure 5.5 gives successional changes in the composition of legume phytomass and abundance, based on their capability to nodulate (i.e. to fix N_2) and on their growth form (‘legume functional groups’, see chap. 4.3.1).

Potentially N_2-fixing species account for 86-91% of both legume phytomass and abundance in first-cycle secondary regrowth. No shifts in nodulation capacity are apparent during the first 25 years of succession covered by this study. In contrast, the vegetation share of legume species incapable of nodulation is much higher in the primary forests growing on clayey Oxisol (42.7% of phytomass and 15.2% of abundance). This portion is fairly constant over all six sites (see Table 4.4).

Potentially N_2-fixing species dominate legume vegetation on the young degraded sites as well as on the terra morena site (not shown), with shares >95% in total legume phytomass and abundance.

The contribution of potentially N_2-fixing legumes to the TAGP of the 25 study sites is given in Annex 1.

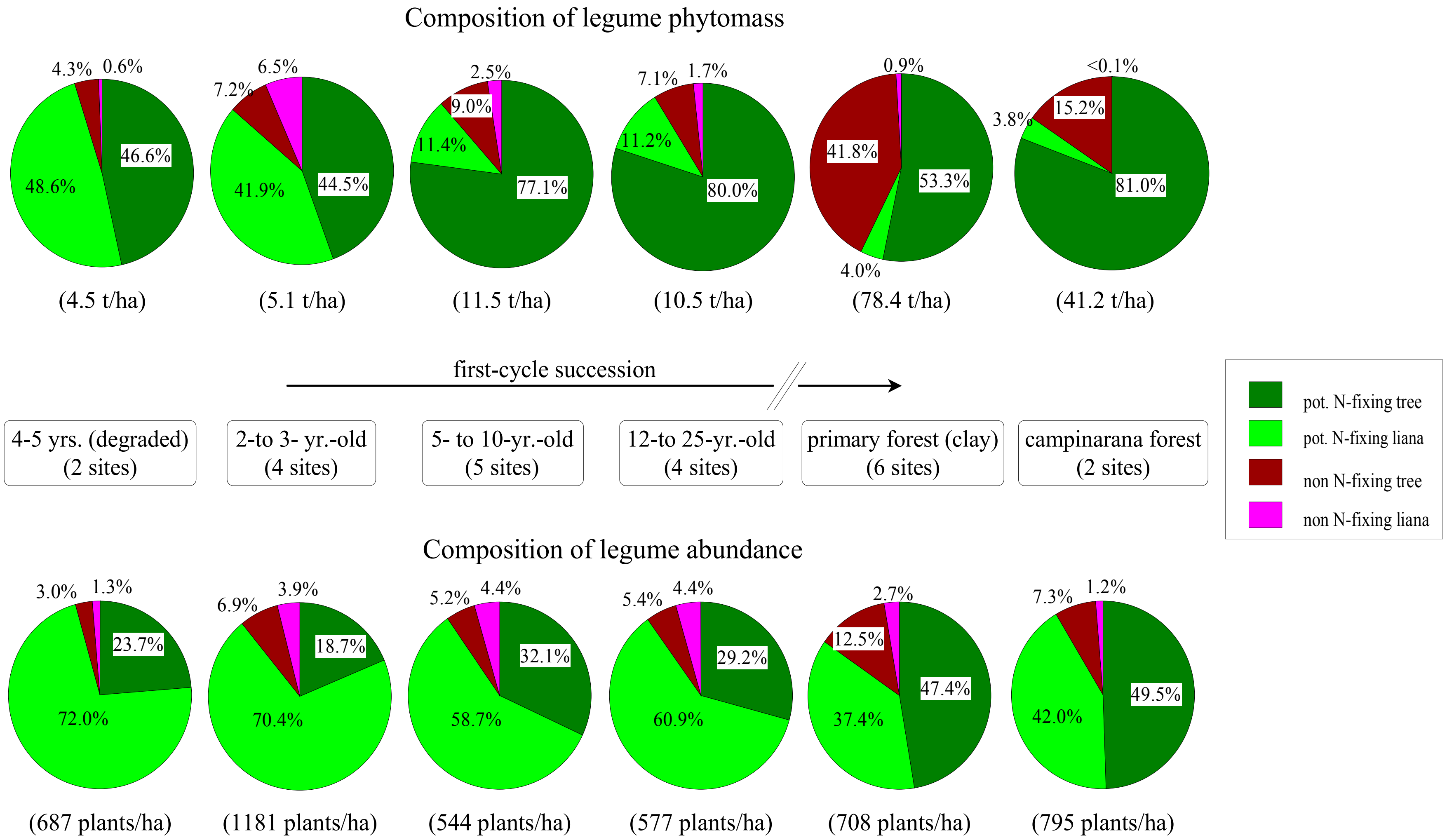


Figure 5.5. Legume functional composition along succession: share of potentially N_2-fixing and of non N_2-fixing trees and lianas in total legume phytomass (top row) and abundance (bottom row); values in brackets give total legume phytomass and abundance

Legume vegetation also differs in respect to growth form. The contribution of lianas to the legume phytomass is highest in young degraded sites (53%), declines with succession from 40% to 13%, and is low in the primary forests (4.9%). Due to the predominantly small size of lianas as a whole (Figure 5.4), the liana contribution to legume abundance is especially high (73%, 65% and 40% in young degraded, old secondary and primary forests, respectively).

Table 5.9 gives the share of potential N_2-fixers in the tree and liana vegetation components of total vegetation. The combination of high legume share in the liana-component (Table 5.5) and the higher portion of potential N_2-fixers within the legume lianas (Figure 5.5) results in a five- to tenfold higher overall share of potential N_2-fixers in lianas than in trees. Successional changes of shares of phytomass and abundance are similar in pattern.

Table 5.9. Potentially N_2-fixing trees and lianas along succession: abundance (n ha^{-1}), phytomass (t ha^{-1}), and shares in the tree and liana vegetation components (%n and %t)

	potentially N_2-fixing trees				potentially N_2-fixing lianas			
	abundance, phytomass and contribution to all trees				abundance, phytomass and contribution to all lianas			
	n ha^{-1}	%n	t ha^{-1}	%t	n ha^{-1}	%n	t ha^{-1}	%t
4-5 yrs. (degraded)	166	2.3	2.2	2.4	490	21.3	2.1	29.0
11 yrs. (degraded)	184	3.3	4.7	2.8	663	35.2	0.8	24.1
4 yrs. (terra morena)	667	14.0	22.8	17.1	467	26.5	0.3	16.5
2- to 3-yr.-old	264	3.7	2.3	2.4	785	25.6	2.0	32.6
5- to 10-yr.-old	196	3.8	9.9	6.5	297	18.7	0.9	17.9
12- to 25-yr.-old	165	4.2	8.4	4.1	360	30.5	1.2	23.4
PF (clay)	334	8.3	41.6	9.5	273	37.6	3.1	37.2
PF (sand)	402	7.4	36.2	10.9	327	48.0	0.8	29.6

5.2.3 Legume genera and species

The complete list of legume species and tribes identified in this study is given in Annex 2. Seven of the 19 legume tribes contribute more than 5% of total legume phytomass or abundance at some stage of succession. Species richness is much higher for trees (total of 121 species) than for lianas (total of 36 species). With 35 species, *Inga* is the most diverse legume genus recorded in this study, followed by *Swartzia* (15 species) and *Machaerium* (12 species).

Tables 5.10 and 5.11 give the phytomass and abundance percentage of the most important legume tree and liana genera. The taxonomic composition differs substantially between primary forests and secondary regrowth. The genera *Swartzia*, *Sclerolobium* and *Parkia* are characteristic for primary forests and to some extent for old secondary regrowth. The contribution of rare species (last columns in Tables 5.10 and 5.11) is high in primary forests.

The genus *Inga* dominates legume phytomass and abundance in all stages of secondary regrowth. The phytomass contribution of *Inga* is substantially lower in primary forests, as this genus is mainly limited to the understory and mid-canopy level. *Inga* spp. are generally classified as capable of nodulation, but 'N_2-fixation capacity' differs widely among species, ranging from the strongly N_2-fixing *I. edulis* to *I. panurensis*, which is probably incapable of nodulation (de Souza et al., 1994).

The genus *Machaerium* dominates legume lianas throughout succession, with shares of 72-90% of legume liana phytomass and abundance. This study expands knowledge on the nodulation status of *Machaerium* by establishing nodulation capacity in two further species (chap. 4.3.1). The entire genus *Machaerium* can, therefore, be expected to be capable of nodulation and N_2-fixation.

Table 5.12 compares the shares in TAGP and total abundance of the two main legume liana genera *Machaerium* and *Derris*. In contrast to the 22 study sites located on clayey Oxisol, the three edaphically differing sites in chronosequence N$^{o.}$ 5 (campinarana forests on sandy Ultisol and the terra morena site) are low in *Machaerium* and high in *Derris*. Thus, the genus *Derris* (three species likewise capable of N_2-fixation) partly assumes the role of *Machaerium* at these sites. The phytomass shares of *Derris* are lower, however, due to the smaller stature and lower maximum phytomass of the three *Derris* species.

Table 5.10. Legume tree genera along succession: contribution to total legume tree abundance (%n) and tree phytomass (%t)

	total legume trees		potentially N_2-fixing genera										non N_2-fixing genera					
			Inga		*Swartzia*		*Stryphnodend.*		*Dimorphandra*		*Sclerolobium*		*Parkia*		*Zygia*		others*	
	n ha^{-1}	t ha^{-1}	%n	%t	%n	%t	%n	%t	%n	%t	%n	%t	%n	%t	%n	%t	%n	%t
4-5 yrs. (degraded)	187	2.4	23.2	23.6	8.9	0.7	0.4	0.4	48.3	60.7	0.6	-	0.7	0.7	6.4	3.0	11.6	10.8
11 yrs. (degraded)	273	5.3	44.5	51.4	7.9	0.4	3.1	30.5	1.6	1.1	0.2	0	4.2	4.3	10.5	0.9	28.1	11.3
4 yrs. (terra morena)	669	22.8	94.6	96.8	0.8	0.1	0	0	0.8	1.1	0	0	0	0	0.4	0	3.3	2.1
2- to 3-yr.-old	264	2.3	35.9	37.2	12.4	4.9	6.0	22.5	2.4	5.1	3.8	1.4	1.4	1.3	13.4	3.9	16.6	13.3
5- to 10-yr.-old	196	9.9	33.5	39.8	8.6	2.5	16.4	28.7	10.3	6.6	1.3	1.8	4.4	6.9	11.0	4.4	9.8	5.1
12- to 25-yr.-old	194	9.1	53.5	54.3	10.8	10.0	2.3	5.4	7.5	2.3	4.8	11.9	6.5	2.5	6.6	3.2	8.0	10.3
PF (clay)	408	74.7	50.0	7.4	10.0	10.5	0.6	2.3	2.4	0.5	2.6	11.9	3.6	13.8	11.3	3.3	19.5	50.4
PF (sand)	458	40.4	39.7	0.8	30.0	27.4	0.3	5.9	1.3	0.02	8.2	4.2	5.8	12.0	2.6	1.2	12.0	48.6

* 22 genera, 44 species

Table 5.11. Legume liana genera along succession: contribution to total legume liana abundance (%n) and liana phytomass (%t)

	total legume lianas		potentially N_2-fixing genera						non N_2-fixing			
			Machaerium		*Derris*		*Clitoria*		*Bauhinia*		others*	
	n ha^{-1}	t ha^{-1}	%n	%t	%n	%t	%n	%t	%n	%t	%n	%t
4-5 yrs. (degraded)	450	2.1	88.5	89.5	3.5	1.4	3.5	3.5	0.3	0.1	4.2	5.5
11 yrs. (degraded)	686	0.9	89.4	83.9	1.0	0.4	4.0	2.4	3.1	10.9	2.5	2.5
4 yrs. (terra morena)	469	0.4	8.3	14.3	83.4	53.2	0	0	0.6	28.9	7.7	3.7
2- to 3-yr.-old	823	2.4	82.2	80.6	8.6	2.6	2.5	1.1	1.3	7.1	5.3	8.6
5- to 10-yr.-old	321	1.2	76.3	63.6	4.2	0.4	5.8	2.7	2.7	17.9	11.1	15.3
12- to 25-yr.-old	383	1.4	79.7	79.4	7.1	1.0	2.6	1.6	1.3	8.8	9.3	9.1
PF (clay)	298	3.7	75.2	72.7	8.0	0.4	2.1	0.2	6.1	11.9	8.6	14.8
PF (sand)	337	0.8	42.3	90.5	53.4	9.0	1.1	0.2	1.6	0.2	1.6	0.03

* 8 genera, 16 species

Table 5.12. Contribution to total abundance (%n) and TAGP (%t) by the two main legume liana genera *Machaerium* and *Derris* by site class

	Machaerium spp. (12 species)		*Derris* spp. (3 species)	
	%n	%t	%n	%t
4-5 yrs. (degraded)	5.1	2.1	3.2	1.0
11 yrs. (degraded)	8.6	0.4	0.7	0.1
4 yrs. (terra morena)	0.6	0.1	34.4	1.0
2- to 3-yr.-old	7.1	2.0	6.7	0.2
5- to 10-yr.-old	5.0	0.5	1.5	0.1
12- to 25-yr.-old	6.2	0.5	5.3	0.2
PF (clay)	4.7	0.6	3.4	0
PF (sand)	2.6	0.2	23.2	0.1

The species composition of *Inga* as the most important tree genus and of *Machaerium* as the most important liana genus is detailed in Annex 5. *Inga paraensis* is the most important *Inga* species in 10 out of the 25 study sites. *Machaerium hoehneanum* dominates in all stages of secondary regrowth, contributing more than half of the *Machaerium* phytomass and abundance. This species alone combines 12-14% of total liana abundance, and 4% (primary forest) to 20% (young regrowth) of total liana phytomass. The phytomass share of *M. madeirensis* is high in primary forests due to the occurrence of large plants (i.e., >100kg individual phytomass).

Though the common *Inga* and *Machaerium* species listed in Annex 5 occur on most sites and in all stages of succession, their species composition differs between secondary and primary forests. *Inga umbratica* and *Machaerium multifoliolatum* are characteristic primary forest species. In terms of phytomass share, *Machaerium hoehneanum* represents a dominant secondary regrowth species.

5.2.4 Exceptional terra morena site

This chapter describes the 4-yr.-old regrowth site on terra morena do índio. This site is exceptional in its botanical composition, which differs markedly from all other study sites. Table 5.13 compares key botanic characteristics of this site with ‘young’ regrowth on clayey Oxisol, and additionally shows some nitrogen parameters compiled later in this study.

Table 5.13. Comparison of the exceptional terra morena site with similarly aged regrowth on clayey Oxisol: characteristic vegetation components (in t ha^{-1}) and additional nitrogen isotopic data

	terra morena (4-yr.-old)	first-cycle 2- to 3-yr.-old	'degraded' (4- and 5-yr.-old)
Inga edulis	15.7	0	0
Inga thibaudiana	4.6	0	0
other *Inga* species	1.8	1.0	0.6
Derris spp. (3 species)	0.23	0.07	0.04
Machaerium spp. (12 species)	0.06	1.9	1.9
4 pioneer trees*	1.8	24.6	15.5
palms	7.7	1.2	0.2
litter δ^{15}N-signal**	+2.10	+3.15	+3.46
foliar δ^{15}N-signal of pot. N_2-fixer***	+1.93	+4.90	+5.19

* see chap. 5.1.3, ** see chap. 5.5.1, *** see chap. 5.6.1

The most striking feature of the terra morena site is the extraordinary dominance of *Inga edulis* and (to a lesser extent) of *I. thibaudiana*. Together, these two species account for a record 14.1% of TAGP. *Inga edulis* usually is absent in secondary or primary forests of central Amazonia. In all other 24 study sites it merely occurs in small numbers in the 11-yr.-old degraded site (located close to former habitation, probably planted).

Introduction of *Inga edulis* by former Indian land use is a plausible hypothesis. *Inga*-enrichment is common in the surroundings of Indian settlements, as their pods constitute a popular food source. Indian anthropogenic influence of this site was proven by pottery debris found at about 20cm soil depth, but the time since abandonment by Indians, and possible mechanisms of *Inga*-persistence remain unknown.

Inga edulis is a species renowned for its high rates of N_2-fixation. Indeed, abundant nodulation occurred in the topsoil and litter horizon over the entire site. The low δ^{15}N-signals both in the leaf litter and in the leaves of potential N_2-fixers (bottom rows in Table 5.13) point to high rates of BNF occurring at this site.

The terra morena site also is exceptional with regard to other botanical characteristics, notably the almost complete absence of pioneer trees such as *Cecropia* spp. or *Vismia guianensis,* the elevated contribution of palms, and the substitution of *Machaerium* by *Derris* as the main potentially N_2-fixing liana genus. The terra morena site also differs strongly from the other sites in all other parameters investigated by this study, suggesting both a faster development of vegetation and a stronger impact of BNF on this site.

5.2.5 Summary and conclusions

The share of legume phytomass in total vegetation ranges from 5-8% in secondary regrowth and 17% in primary forest controls. As to be expected, legume species composition differs substantially between site classes, with many species, genera or even tribes limited to primary forests or old regrowth. This may be caused by low competitive strength, unfavorable edaphic conditions, or limited seed input in early succession.

Based on the hypothesis that the species-specific capability to nodulate and fix N_2 will be competitively advantageous in N_2-fixing stages of succession only, the species composition of the legume community constitutes an indirect indicator of the role of BNF along succession. Legume composition in secondary and primary forests is shown to differ substantially in this respect, with potential N_2-fixers dominating the legume community throughout first-cycle regrowth (86-91% of legume phytomass), whereas their phytomass share is only 58% in primary forest controls. However, legume composition does not support the hypothesis of a BNF peak in early or mid-succession (chap. 2.1), but rather points to a constant role of BNF throughout the first 25 years of regrowth covered by this study. The taxonomic evidence on the role of BNF along succession and in primary forests is further discussed in chap. 6.2.

Legume growth form composition changes systematically along succession. The share of lianas is high in early regrowth (48.4% of legume phytomass), declines along succession, and is low in primary forests (4.9% of legume phytomass). Such successional shifts appear parallel in direction with the successional changes in growth form composition of the total vegetation (Table 5.1 in chap. 5.1.2), but they are far more expressed in their scale. The combination of a high legume share in all lianas and the higher portion of potential N_2-fixers within the legume lianas makes the potentially N_2-fixing lianas a ‘key functional group’ of species especially in early regrowth. This so far insufficiently recognized key role of potentially N_2-fixing lianas is further discussed in chap. 6.3.

Legume trees are taxonomically very diverse, the main tree genus *Inga* alone combining almost as many species (35 species) as all legume lianas together (36 species). The legume liana community is dominated by the potentially N_2-fixing genus *Machaerium* throughout all stages of succession, and *M. hoehneanum* as the single most important species may be classified as a ‘keystone species’. Legume species composition is affected by soil fertility, with low shares of *Machaerium* spp. partially compensated by high shares of *Derris* spp. on the three sites with different soil.

The terra morena site constitutes a ‘high-BNF’ case study, which is useful for comparison with the successional time series on clayey Oxisol for all parameters investigated by this study. The agronomic potential of terra morena do índio has so far been insufficiently investigated.

5.3 Spatial organization of legume vegetation

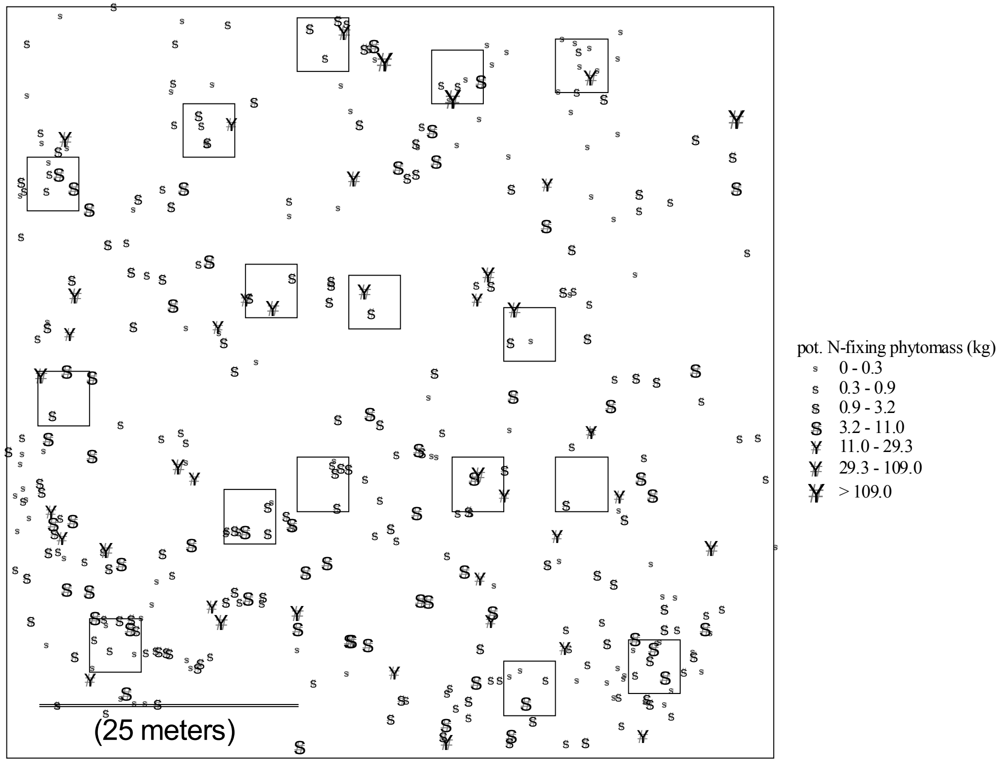
This section analyses (1) the within-site distribution of the potentially N_2-fixing legumes and (2) possible differences of spatial organization between species and along succession. Investigations are based on the complete mapping of all individuals growing on the study sites, and the data are interpreted optically (by maps) and geostatistically (by point pattern analysis).

Results are relevant for judging the degree of legume:vegetation interactions (chap. 5.4) and of legume:soil interactions (chap. 5.5), and are used for the interpretation of spatial patterns of $\delta^{15}N$-signals and of BNF-estimates (chap. 5.8.2).

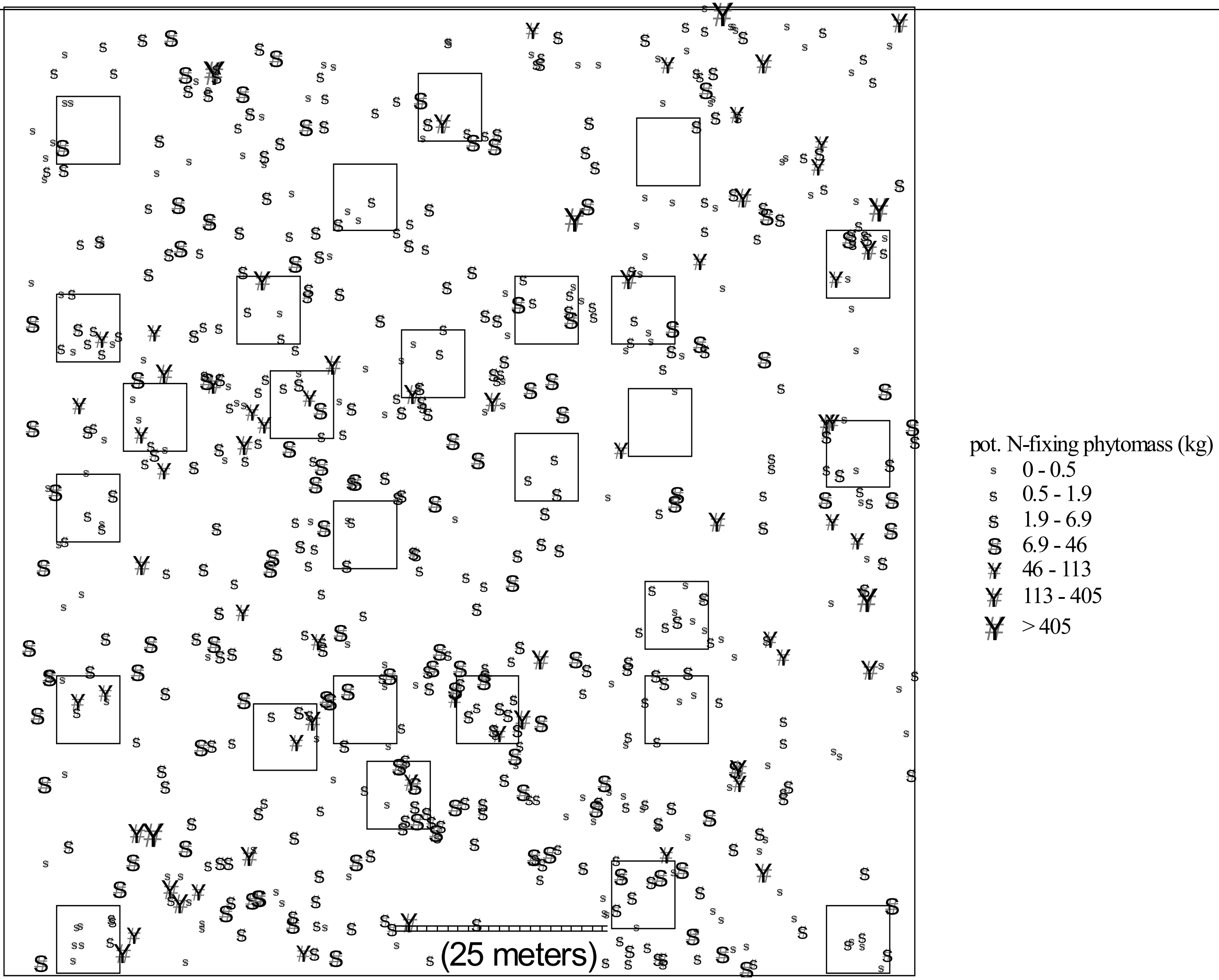
5.3.1 Distribution patterns of potentially N_2-fixing legumes

Maps 4-6 show the spatial organization of potentially N_2-fixing legumes for three exemplary sites located at <1km distance from one another, but differing in their successional stage (3- and 7-yr.-old first-cycle regrowth and primary forest control). The scaling of plant phytomass on these maps is designed to reflect the skewed distribution of plant sizes (chap. 5.1.5). Plants are, therefore, classified into the following seven percentile levels of all potential N_2-fixers occurring on the respective site: <25%, 25-50%, 50-75%, 75-90%, 90-95%, 95-99%, and >99%. Rectangular frames delimit the completely mapped areas, which will be used for subsequent point pattern analysis.

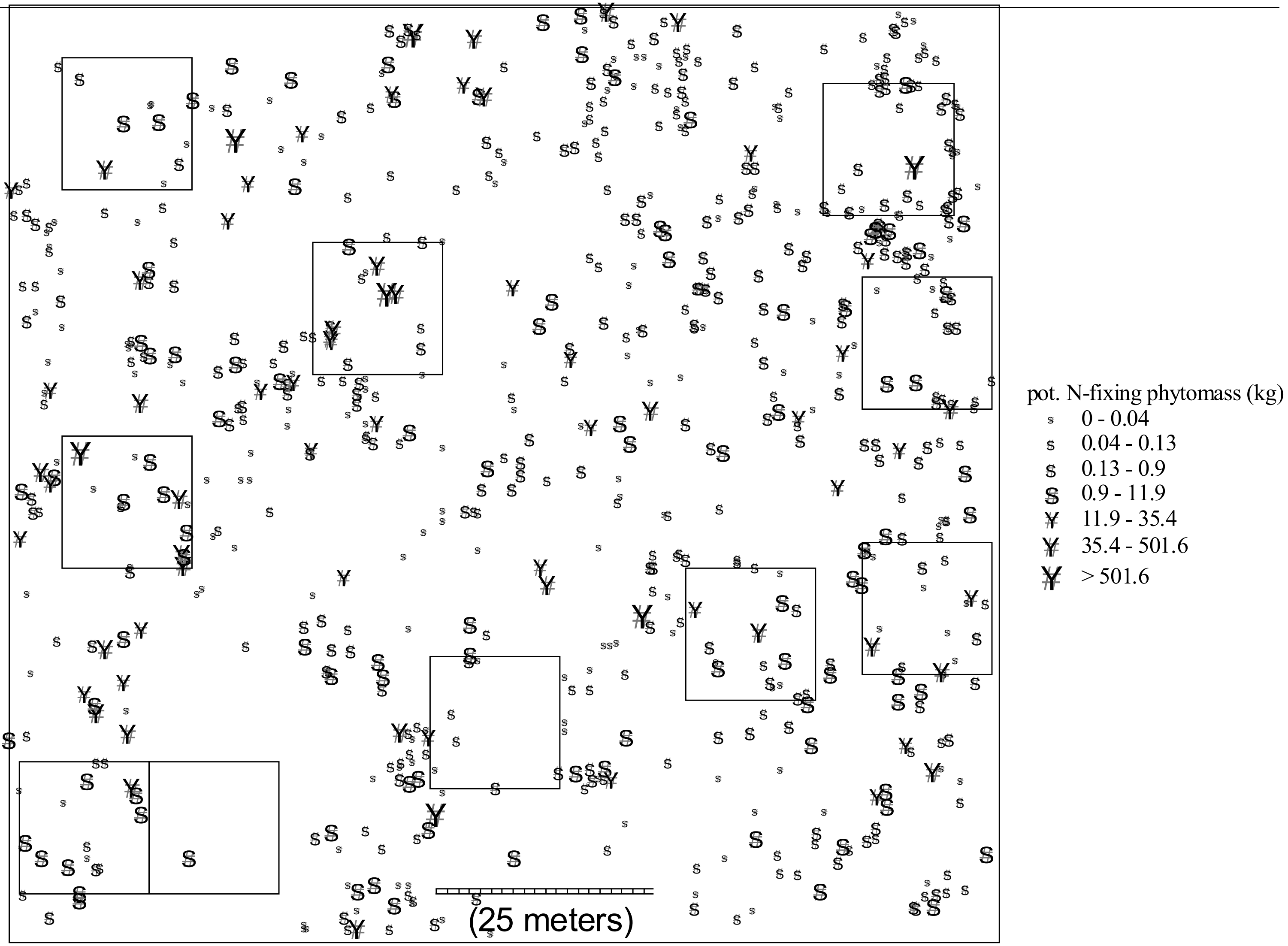
The spatial organization of potential N_2-fixers is shown for additional exemplary secondary and primary forest sites on Maps 7-11 in Annex 6.



Map 4. Potentially N_2-fixing legumes in a 3-yr.-old regrowth site ('Geraldo young')
Phytomass is scaled in seven percentile levels; squares show the spatial arrangement of 5 x 5m-sized sampling-plots (see chap. 4.4.2); frame delimits the completely mapped area used for geostatistical analyses.



Map 5. Potentially N_2-fixing legumes in a 7-yr.-old regrowth site ('Geraldo old')
Squares show the spatial arrangement of 7.50 x 7.50m-sized sampling-plots; for further explanations see Map 4



Map 6. Potentially N_2-fixing legumes in a primary forest site on clayey Oxisol ('Geraldo PF')
Squares show the spatial arrangement of 15 x 15m-sized sampling plots; for further explanations see Map 4

Visual assessment of all maps allows the following two conclusions:

(1) Clustered distribution of potential N_2-fixers on a small scale (i.e., several meters), and

(2) Random or uniform distribution of potential N_2-fixers on a larger scale (i.e., over the entire site).

As the human eye tends to identify patterns, which may not hold true in reality, visual assessment of maps may be biased. In a further step, spatial distribution is, therefore, investigated geostatistically. This study uses the Ripley's point pattern statistics, which has revolutionized spatial ecology by introducing scale (distance) in the analysis. This constitutes a significant improvement over the traditional indices of point pattern distribution (e.g., Morisita indices, variance-to-mean ratio etc.; Forman and Hahn, 1980; Hamill and Wright, 1986; Rossi and Higuchi, 1998).

Figure 5.6 shows the point pattern distribution of all potentially N_2-fixing plants on 15 secondary regrowth sites and on primary forest controls. Ripley's L(*d*) is plotted against simulated complete spatial randomness (CSR). Positive deviations indicate clustered, and negative deviations uniform (regular) distribution of potentially N_2-fixing plants. Distance is scaled in 1m steps.

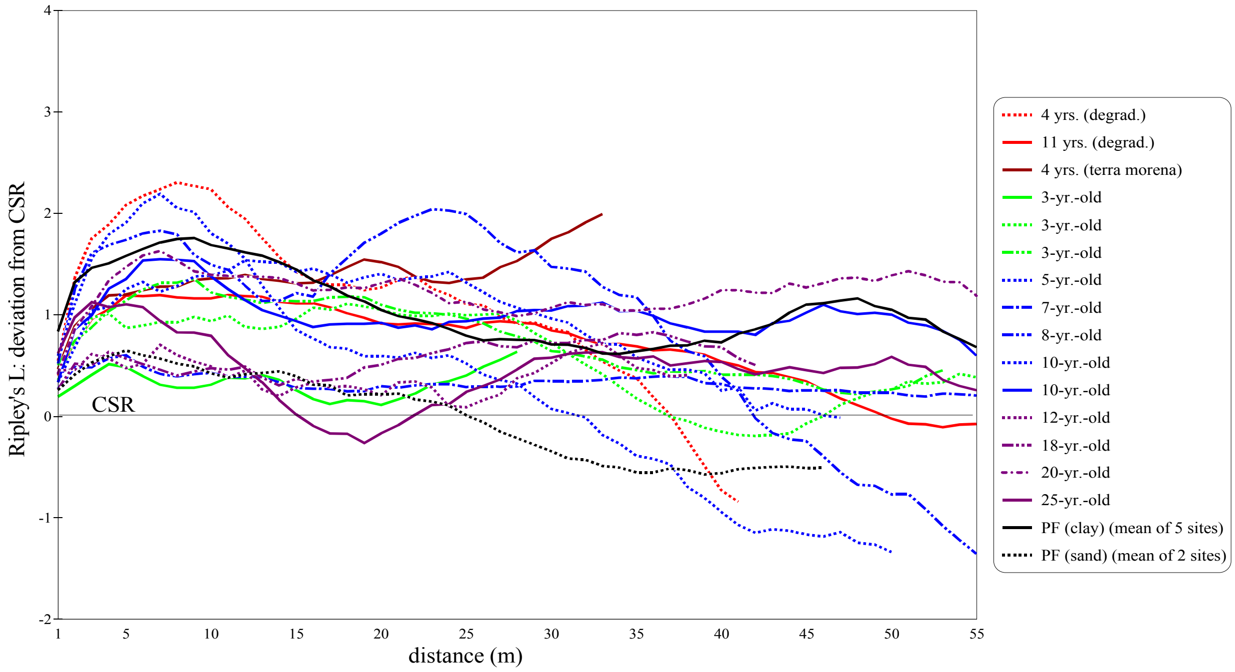


Figure 5.6. Spatial distribution of potentially N_2-fixing legume plants along succession: deviations of Ripley's L(d) from complete spatial randomness (CSR) (sequence of sites as in Annex 1)

Potentially N_2-fixing legumes are distributed in clusters at short to mid distances, with maximum clustering at 5-10m distance on all sites. No successional trends of legume distribution are apparent.

With increasing distance, statistical power degrades and values become erratic in absolute terms, but approach zero in relative terms (percentage deviations of Ripley's L(*d*) from CSR, data not shown). Ripley's statistics are based on plant individuals only. Visual interpretation of Maps 4-6 and of those of the other sites, as well as the skewed phytomass distributions (chap. 5.1.5) suggest that the clustering of potentially N_2-fixing phytomass is even greater than the clustering of plant individuals shown in Figure 5.6.

5.3.2 Distribution patterns at different levels of species aggregation

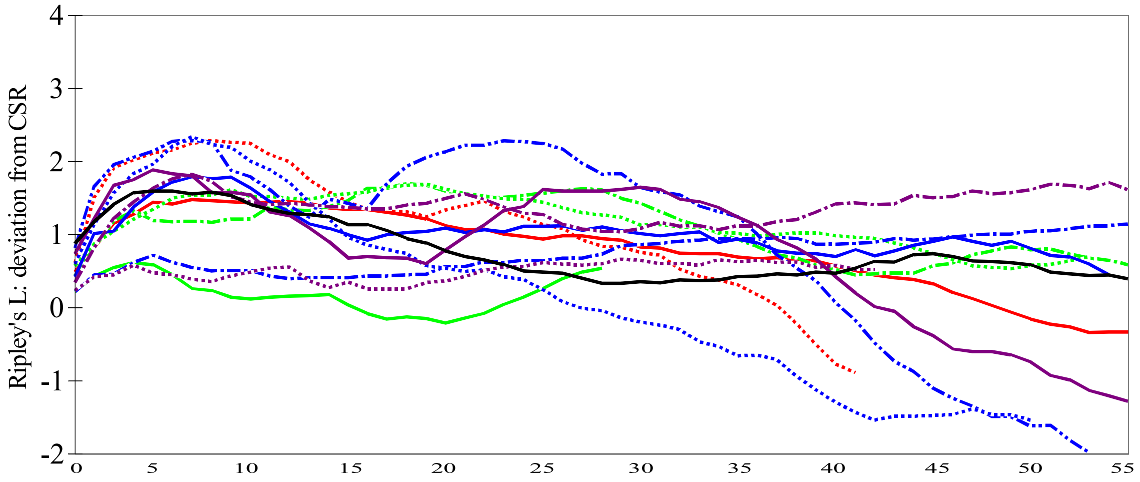
'Potentially N_2-fixing legumes' comprise a heterogeneous total of 123 tree and liana species (28 genera) as listed in Annex 2. This section investigates the spatial organization of legume vegetation at different levels of species aggregation, commencing with the entirety of potentially N_2-fixing species shown in Figure 5.6. Figure 5.7 presents continuous subsets, from growth form over genus to *Machaerium hoehneanum* as single species.

A serious drawback of Ripley's statistics is the high number of replications needed obtain meaningful patterns (Getis and Franklin, 1987). This study uses a minimum threshold of 100 plants per site. Analysis, therefore, had to be limited to *M. hoehneanum* as the single most abundant legume species.

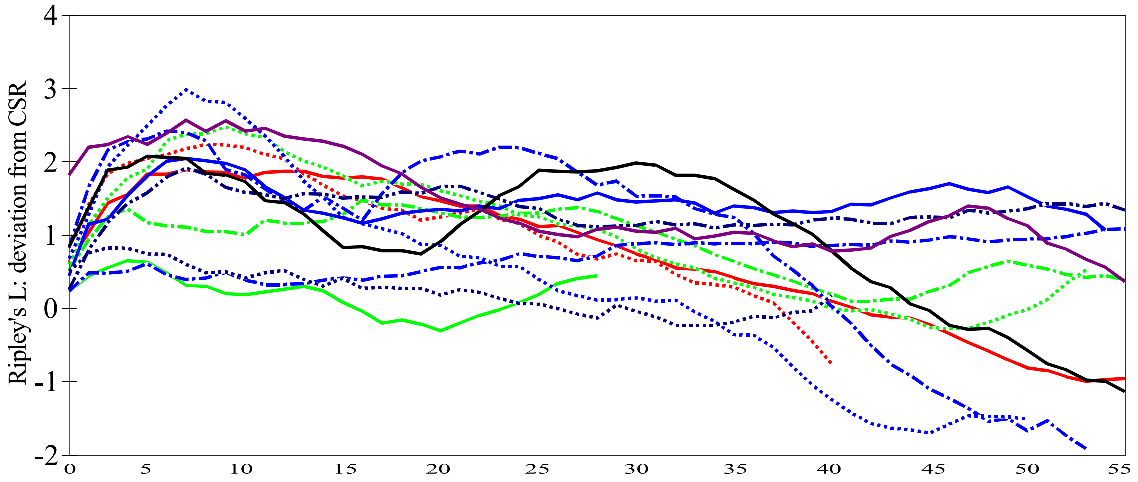
The degree of clustering increases with the narrowing of the group of plants, approximately doubling between 'all potential N_2-fixers' and *M. hoehneanum* as single species. The marked differences in the distribution pattern of *M. hoehneanum* indicate that vegetation is spatially organized at the species level. Distribution patterns become smoother and less pronounced when the number of species is increased. Moreover, ranking between sites changes during the process. The spatial scale remains similar with maximum clustering at about 5-10m distance.

Whereas no successional trends of spatial organization are apparent at higher levels of species grouping, *M. hoehneanum* shows a systematic change from strongly clustered in young regrowth to spatially random in old regrowth. The distribution in primary forests is again strongly clustered.

potentially N-fixing lianas



genus *Machaerium*



Machaerium hoehneanum

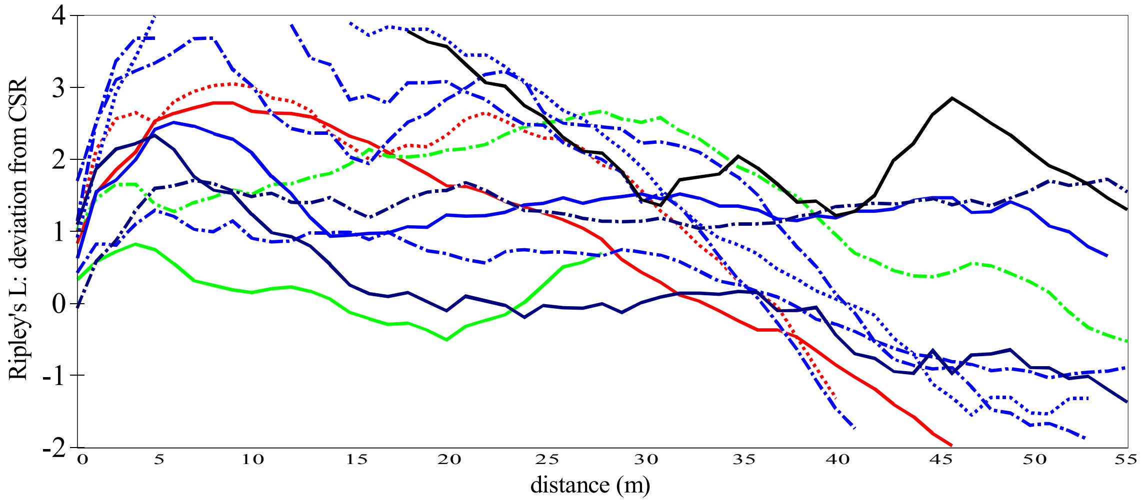
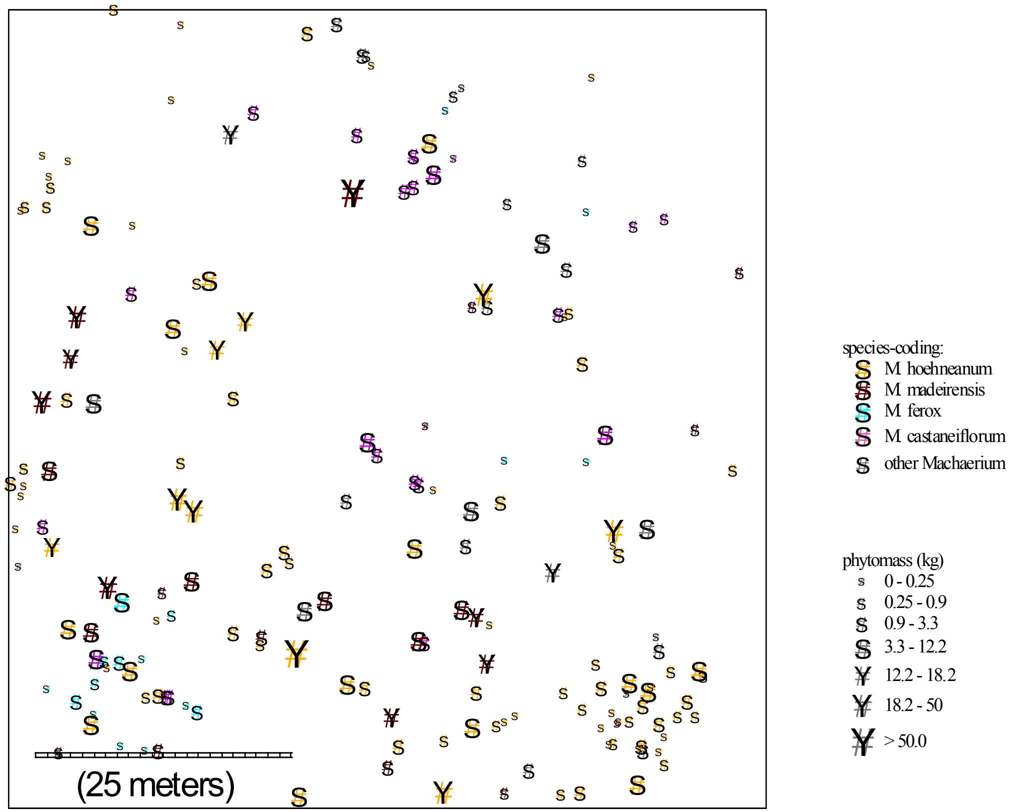


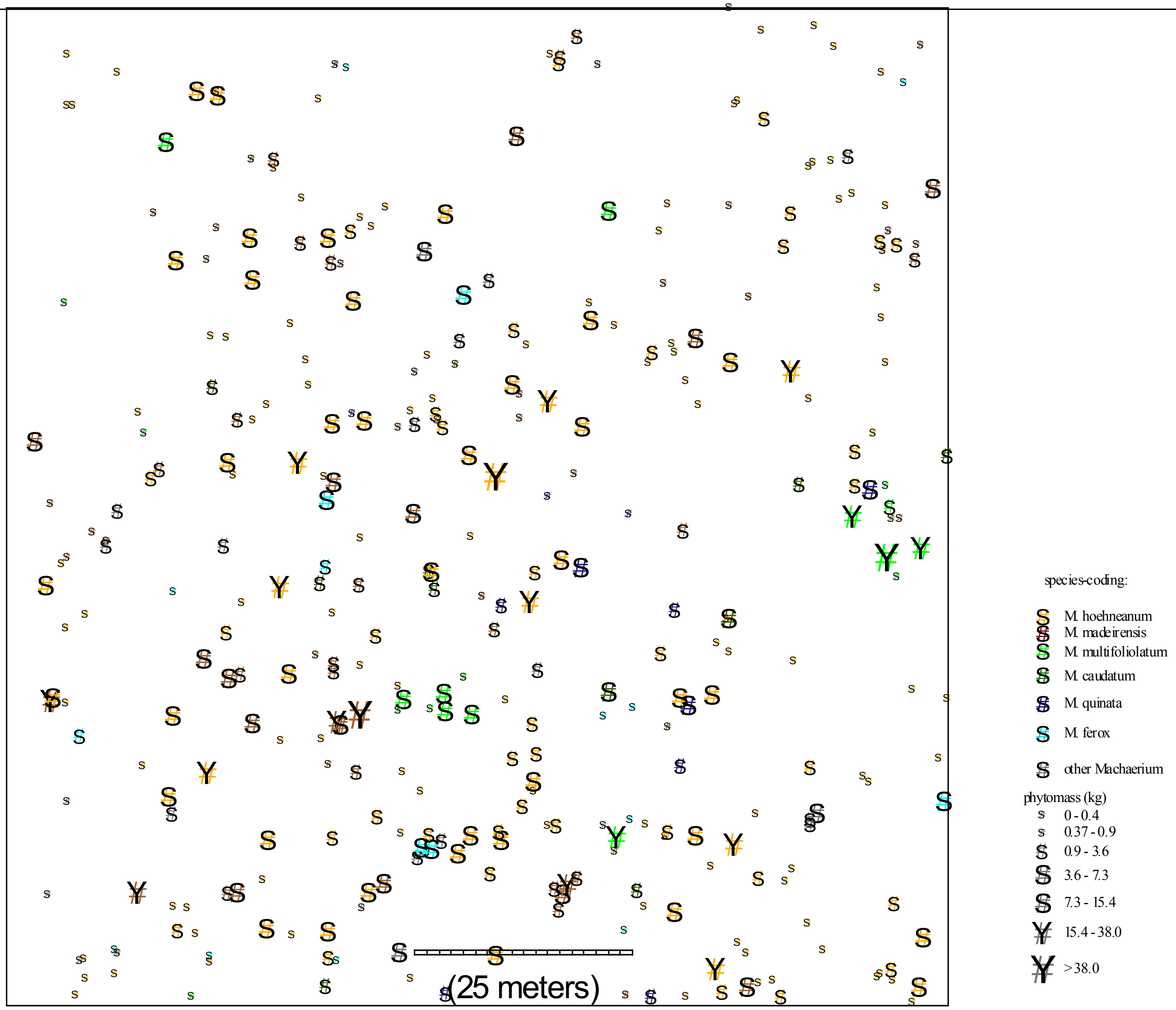
Figure 5.7. Plant pattern distributions at three levels of species grouping: legume functional group, genus, and single species (coding and scales as in Figure 5.6)

Maps 12-14 show the distribution patterns of the main *Machaerium* species on the same three sites shown in Maps 4-6. Visual assessment of these maps confirms that spatial organization of this genus is on the species level and large species-specific differences are apparent.

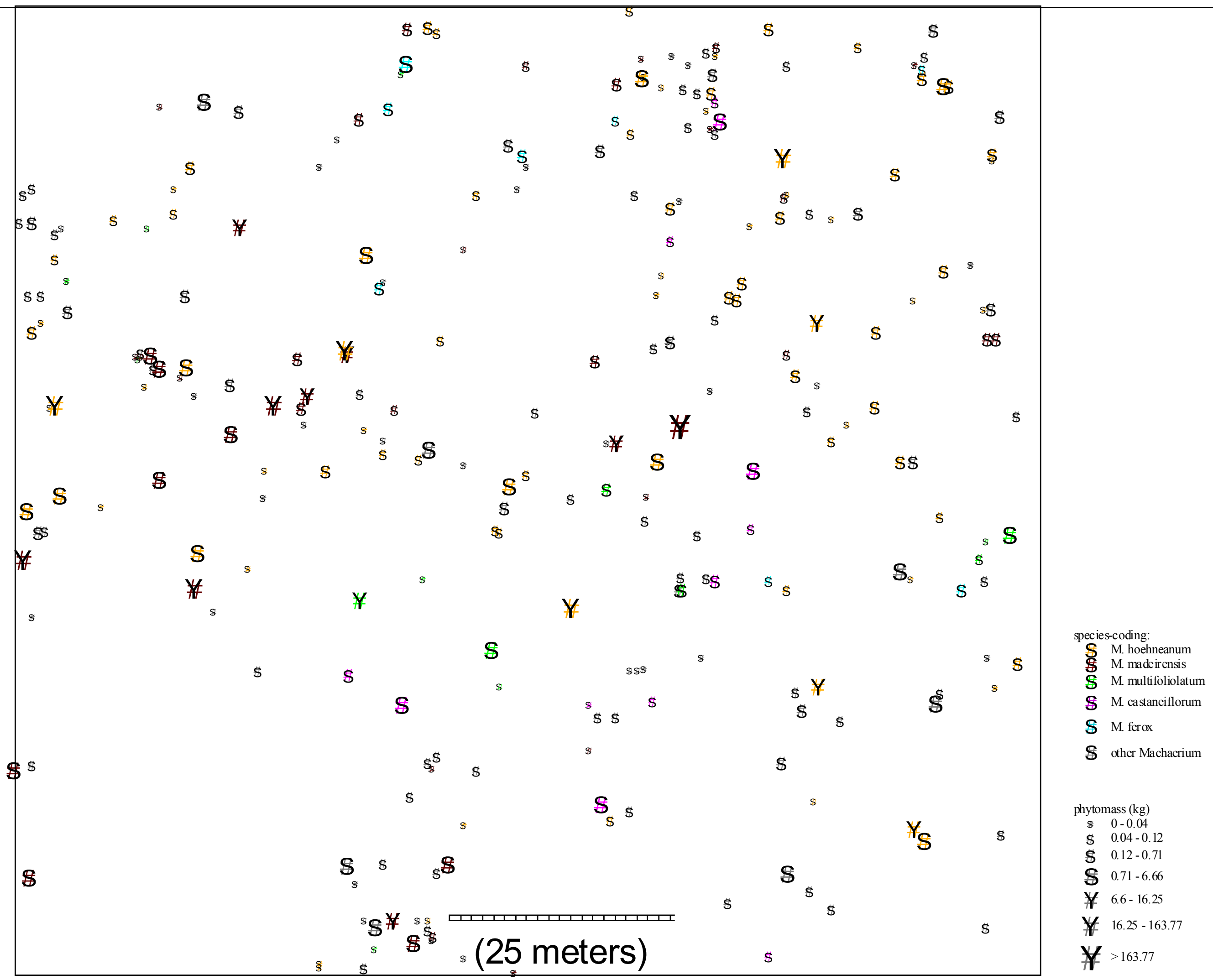
Species-specific differences in plant distribution patterns are likewise evident within the group of non-legume pioneer trees investigated in this study (chap. 5.1.3). Maps 15-17 (in Annex 7) illustrate such differences on three 10- to 20-yr.-old regrowth sites. Whereas the distribution of *Laetia procera* appears fairly homogenous, *Vismia guianensis* is spatially aggregated. Maps 15-16 indicate one possible mechanism, which drives plant distribution patterns: mutual (competitive) exclusion at high densities. Map 17 compares the distribution patterns of two *Cecropia* species, which differ mainly in the degree of their mutualism with *Azteca* ants (Berg, 1978). Neither attraction nor repulsion is apparent between these two species.



Map 12. *Machaerium* species distribution in a 3-yr.-old regrowth site ('Geraldo young') Phytomass is scaled in seven percentile levels of *Machaerium* spp. on the site.



Map 13. *Machaerium* species distribution in a 7-yr.-old regrowth site (‘Geraldo old’)
Phytomass is scaled in seven percentile levels of *Machaerium* spp. on the site.



Map 14. *Machaerium* species distribution in a primary forest site on clayey Oxisol (‘Geraldo PF’)
Phytomass is scaled in seven percentile levels of *Machaerium* spp. on the site.

5.3.3 Summary and conclusions

This is most probably the first study investigating spatial vegetation patterns along tropical forest succession. The results are summarized as follows:

- Potentially N_2-fixing legume vegetation is spatially clustered throughout all stages of succession, clustering is maximum at 5 to 10m distance.
- Spatial organization of vegetation is at the species level and is obscured by any level of species grouping.
- *Machaerium hoehneanum* as single species shows a systematic change from strongly clustered distribution in young regrowth to random distribution in old regrowth, whereas distribution in primary forests is again strongly clustered.

The finding that potentially N_2-fixing legumes are aggregated in clusters gives room to the hypothesis that N-input via BNF is likewise irregularly distributed within the sites. This could lead to the formation of microsites with elevated total phytomass (chap. 5.4) and $\delta^{15}N$-dilution of leaf litter nitrogen (chap. 5.5.2) and of foliar nitrogen (chap. 5.8.2) in areas affected by clusters of N_2-fixing legumes. The ecological and methodological consequences of the irregular distribution of potentially N_2-fixing vegetation are further discussed in chap. 6.4.

5.4 Legume - vegetation interactions

This section provides indirect evidence on the role of BNF along succession, based on the hypothesis that locally significant BNF can increase the phytomass stocks of vegetation by reducing N-availability constraints via BNF. The combination of high potential N_2-fixer phytomass shares and elevated total phytomass is interpreted as the consequence of significant BNF inputs. Such impacts are expected to differ widely within the heterogeneous group of potentially N_2-fixing species. Growth form is, therefore, in a second step taken as a broad means of species classification.

Phytomass differs between sites and increases asymptotically with fallow age, calling for phytomass standardization in order to group sites into site classes. For this purpose, the relative within-site deviation of TAGP from the respective site means is calculated. Only plots with more than a 2% phytomass share of potential N_2-fixers are considered, since below this level no sizeable BNF impact is expected. The skewed plant size distribution (chap. 5.1.5) and the clustered spatial distribution of potential N_2-

fixers (chap. 5.3.1) are reflected in the plot-based sampling scheme, with phytomass shares of potential N_2-fixers below 5% in 59% of the plots, above 15% in 26% of the plots, and attaining a maximum share of 80%.

5.4.1 Impact of potential N_2-fixers on vegetation phytomass

Figure 5.8 shows the relationship between the ln-normalized phytomass shares of all potentially N_2-fixing legumes and the within-site variation of TAGP for first-cycle secondary regrowth. The relationship is linear and significant (Pearson R = +0.30, $p<0.001$). Consequently, TAGP of plots with less than 5% of the phytomass contributed by potential N_2-fixers falls 6% below the site average, and plots with >15% phytomass contribution by potential N_2-fixers have 22% above-average TAGP-stocks (for first-cycle succession, difference significant at $p<0.001$).

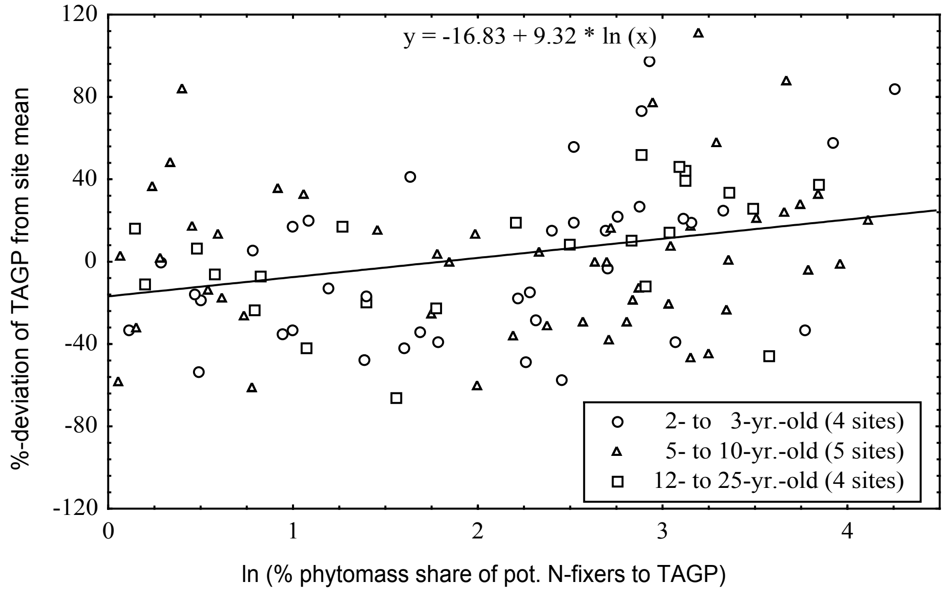


Figure 5.8. Impact of the phytomass shares of potential N_2-fixers on the within-site variation of TAGP (first-cycle regrowth, n=93 plots > 2% phytomass share)

The impact of potential N_2-fixers on the variation of TAGP differs among site classes (Table 5.14). The relationship is significantly positive for all stages of first-cycle secondary regrowth and for the terra morena site. The relationship is non-significant in degraded regrowth and in the campinarana forests. In contrast, no relationship whatsoever is apparent in the primary forest controls on clayey Oxisol.

Table 5.14. Spearman correlations between the phytomass share of potential N_2-fixers and the within-site variation of TAGP (using non-transformed data, minimum 2% phytomass contribution by pot. N_2-fixers)

	plot-n	phytomass share of all pot. N_2-fixers vs. TAGP-variation	
4-11 yrs. (degraded)	n=24	n.s.	
4 yrs. (terra morena)	n=12	+0.64	($p<0.03$)
2- to 3-yr.-old	n=39	+0.51	($p<0.001$)
5- to 10-yr.-old	n=39	+0.34	($p=0.03$)
12- to 25-yr.-old	n=24	+0.45	($p=0.03$)
PF (clay)	n=22	n.s.	
PF (sand)	n=8	n.s.	

This study also investigated the impact of potential N_2-fixer phytomass on the remaining phytomass in their surroundings (both ln-normalized, in t ha^{-1}). Contrary to TAGP variation, relationships are non-significant in all cases (data not shown).

5.4.2 Influence of growth form

The impact of potential N_2-fixers on TAGP is the outcome of both positive effects (i.e., reduced N-availability constraints via BNF) and negative effects (i.e., inhibition of surrounding vegetation via occupation of growth space, competition for light, nutrients etc.). Both may be expected to vary widely among the heterogeneous total of 123 potentially N_2-fixing species recorded in this study. Table 5.15 investigates such differential effects, taking growth form as a very broad means of species grouping.

Table 5.15. Impact of the phytomass share of potentially N_2-fixing trees and lianas on the within-site variation of TAGP (Spearman correlations of non-transformed data, minimum 2% phytomass share)

	phytomass share of pot. N_2-fixing trees vs. TAGP-variation			phytomass share of pot. N_2-fixing lianas vs. TAGP-variation	
4- and 11 yrs. (degrad.)	n.s.		n=10	n.s.	n=18
4 yrs. (terra morena)	+0.63	(p=0.03)	n=12	-	(n=2)
2- to 3-yr.-old	+0.57	(p=0.01)	n=19	n.s.	n=6
5- to 10-yr.-old	+0.41	(p=0.02)	n=34	n.s.	n=13
12- to 25-yr.-old	+0.46	(p=0.05)	n=18	n.s.	n=8
PF (clay)	n.s.		n=21	n.s.	(n=4)
PF (sand)	n.s.		n=8	-	(n=0)

The group of potentially N_2-fixing trees exerts a positive impact on TAGP-variation, similar in strength to the entirety of potential N_2-fixers (see Table 5.14). In contrast, the impact of the group of potentially N_2-fixing lianas is non-significant or absent in all stages of succession.

A likely explanation for lacking effects of potentially N_2-fixing lianas on TAGP-variation is the prevailing negative impact of lianas as a whole on phytomass stocks, which is in contrast to the prevailing positive relationships with trees (Table 5.16). The negative impact of lianas on TAGP is presumably due to their high competitive strength in accessing light, growth space and nutrients.

Table 5.16. Spearman correlations between the tree and liana phytomass shares and the within-site variation of TAGP (over all plots, using non-transformed data)

	plot-n	phytomass share of all trees vs. TAGP-variation	phytomass share of all lianas vs. TAGP-variation
4-5 yrs. (degraded)	n=26	n.s	n.s.
11 yrs. (degraded)	n=12	n.s.	-0.59 (p=0.04)
4 yrs. (terra morena)	n=12	+0.78 (p=0.003)	n.s.
2- to 3-yr.-old	n=48	+0.27 (p=0.05)	n.s.
5- to 10-yr.-old	n=71	+0.41 (p=0.003)	-0.34 (p=0.004)
12- to 25-yr.-old	n=52	+0.34 (p=0.01)	-0.24 (p=0.09)
PF (clay)	n=43	+0.27 (p=0.08)	n.s.
PF (sand)	n=14	n.s.	n.s.

5.4.3 Summary and conclusions

Significant BNF is proven for all stages of secondary succession, based on the observation of a positive association between the phytomass share of potential N_2-fixers and TAGP. In marked contrast, no BNF is apparent in primary forests. This pattern is in accordance with the expectations outlined in chap. 2.4.

The impact of potential N_2-fixers on TAGP is mainly caused by the direct phytomass contribution of this vegetation component, as the relationships with the phytomass of the surrounding vegetation are non-significant in all cases. Thus, the facilitation effect of potential N_2-fixers on surrounding vegetation (‘leakage’ of fixed N_2) is either low or offset by negative effects (competition for light and nutrients).

Facilitation and inhibition vary amongst the potentially N_2-fixing legume species. In contrast to potentially N_2-fixing trees, the effects of potentially N_2-fixing lianas on TAGP are non-significant in all cases. This is attributed to the negative effects of lianas on TAGP in general (suppression of surrounding vegetation due to their competitive strength), which offset the positive effects of liana-BNF. The arbitrary role of potentially N_2-fixing lianas is further discussed in chap. 6.3.

5.5 Soil nitrogen pools

This section investigates the nitrogen pools in the mixed leaf litter and in the ‘plant-available’ topsoil, which are believed to constitute the main sources of the ‘soil-derived nitrogen’ in vegetation. Focus is on (1) changes of N-concentrations and δ^{15}N-signals along succession and differences between secondary and primary forests, and (2) the possible impact of N_2-fixing vegetation on the N-concentrations and δ^{15}N-signals of the surrounding soil nitrogen pools. In analogy to chap. 5.4.1, high phytomass shares of potential N_2-fixers associated with low soil δ^{15}N-signals will be interpreted as causal relationships and thus as an indirect evidence of BNF. Furthermore, the possible reduction of soil δ^{15}N-signals in the surroundings of N_2-fixing vegetation is important from a methodological viewpoint, as this ‘δ^{15}N dilution problem’ potentially constitutes a systematic source of error for the ^{15}N natural abundance method (see chap. 2.5.2).

5.5.1 Leaf litter nitrogen along succession

Figure 5.9 shows the leaf litter δ^{15}N-signals and N-concentrations for the 25 study sites.

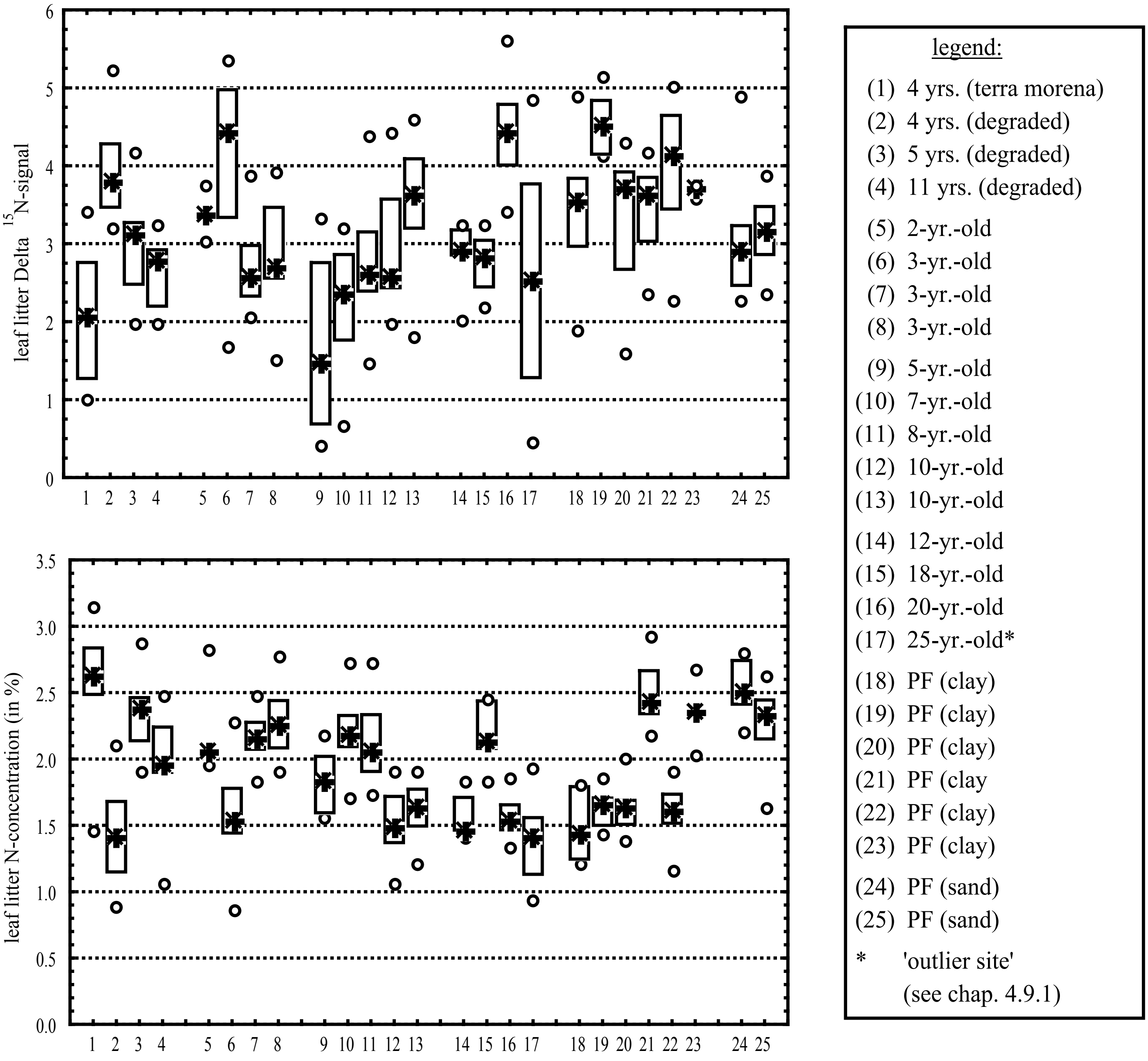


Figure 5.9. Leaf litter δ^{15}N-signals and N-concentrations on the 25 study sites: medians, 25-75 percentiles and minimum/maximum; sequence of sites as in Annex 1

Leaf litter δ^{15}N-signals vary largely between +2‰ and +5‰ and N-concentrations between 1% and 2.5%. The within-site variation of the litter δ^{15}N-signals tends to be lower in primary forests than in regrowth and is highest in the 25-yr.-old 'outlier' site (site N$^{o.}$ 17 marked with *, see chap. 4.9.1).

Table 5.17 gives the mean leaf litter N-concentrations and δ^{15}N-signals and the median N-stocks by site class. N-stocks are calculated by multiplying the N-concentrations of mixed leaf litter and of *Cecropia* leaf litter with their respective mass estimates.

Table 5.17. Mean leaf litter N-concentrations and δ^{15}N-signals, and median leaf litter N-stocks along succession

		mean		median
	plot-n	%N	δ^{15}N	kg N ha^{-1}
4-11 yrs. (degraded)	25	1.89	3.47	7.0
11 yrs. (degraded)	10	1.97	2.67	16.6
4 yrs. (terra morena)	11	2.57	2.10	3.2
2- to 3-yr.-old	47	2.07	3.15	5.9
5- to 10-yr.-old	70	1.98	2.56	6.9
12- to 25-yr.-old	51	1.59	3.36	10.0
primary forest (clay)	41	1.85	3.71	42.8
primary forest (sand)	14	2.42	3.10	46.0

Litter δ^{15}N-signals increase along succession and are significantly higher ($p<0.01$) in old than in young and mid-aged first-cycle regrowth (excluding 25-yr.-old outlier-site). Whereas the δ^{15}N-signals are significantly higher in primary forests than in secondary regrowth as a whole (t-test $p<0.001$), they do not differ statistically from 12- to 20-yr.-old regrowth. Litter δ^{15}N-signals are lowest in the *Inga*-dominated exceptional terra morena site, suggesting a high rate of BNF (chap. 5.2.4).

Leaf litter N-concentrations decline along succession when excluding a 4-yr.-old degraded site. N-concentrations are significantly higher in young and mid-aged regrowth as compared to old regrowth and primary forests (ANOVA $p<0.001$).

N-concentrations and δ^{15}N-signals are close together in the two campinarana sites (N$^{os.}$ 24 and 25 in Figure 5.9). Their δ^{15}N-signals are significantly lower and their N-concentrations are significantly higher than in primary forest controls on clayey Oxisol (t-test $p=0.02$ and $p<0.001$, respectively).

Leaf litter N-stocks are four to eight times as high in primary forests as in secondary forests. This is due to differences of similar magnitude in their litter mass (chap. 5.1.2).

In spite of higher N-concentrations, leaf litter N-stocks are low in the terra morena site, suggesting a rapid litter decomposition and fast circulation of fixed nitrogen. In contrast, leaf litter decomposition and N-cycling appear slow in the 11-yr.-old degraded site. This could be the consequence of a substantially higher share of woody litter (32.9% of total litter mass vs. 24.1% overall average, see Table 5.3).

5.5.2 Factors influencing leaf litter nitrogen

In first-cycle secondary regrowth, the phytomass shares of potentially N_2-fixing legumes are significantly negatively correlated with the δ^{15}N-signals of mixed leaf litter (Figure 5.10). In analogy to chap. 5.4.1, only plots with more than a 2% phytomass share of potential N_2-fixers are considered, as no sizeable impact of N_2-fixing vegetation is expected below this threshold.

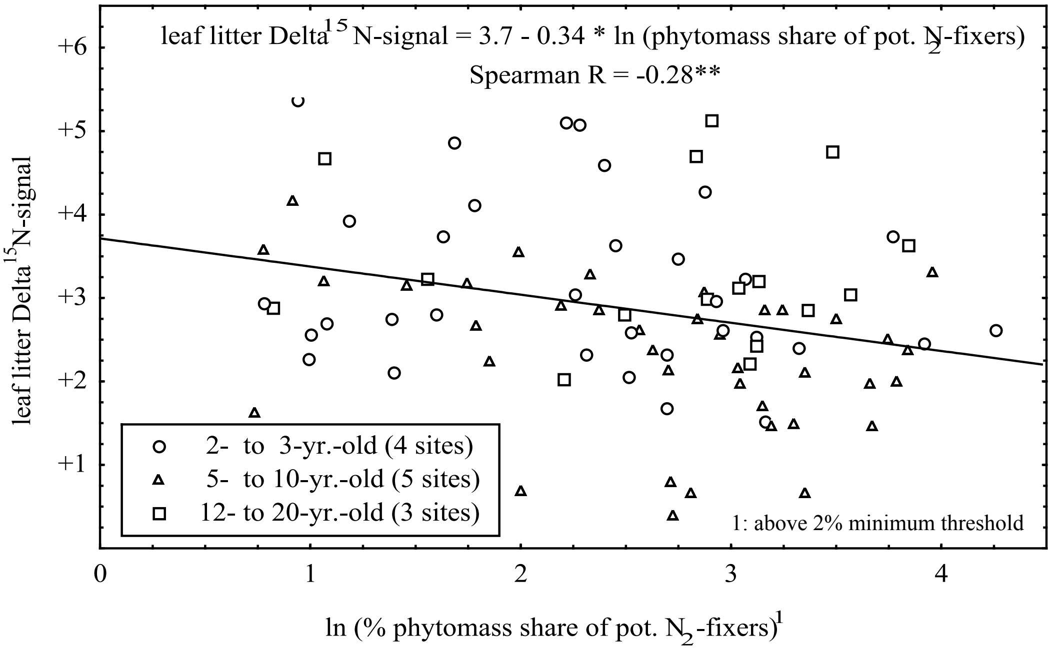


Figure 5.10. Influence of the phytomass share of potentially N_2-fixing legumes on the δ^{15}N-signal of mixed leaf litter

The mean leaf litter δ^{15}N-signal decreases from +3.15 (<5%) → +2.79 (5-15%) → +2.59 (>15%) phytomass share of potential N_2-fixers, these differences are significant (Kruskal-Wallis ANOVA p=0.04). The relationships between the phytomass shares of potential N_2-fixers and the litter δ^{15}N-signals are also significant in the terra morena regrowth site (Spearman R = -0.56*), and non-significant in the campinarana forests. In contrast, no relationships at all are apparent between the phytomass shares of N_2-fixers and the litter δ^{15}N-signals in the primary forests on clayey Oxisol, nor in the degraded regrowth. The phytomass shares of potential N_2-fixers do not affect the leaf litter N-concentrations in any stage of succession (first rows in Table 5.18).

Table 5.18. Factors affecting leaf litter nitrogen (Spearman correlations)

model:	first-cycle regrowth	PF (clay)	degraded regrowth
ln (pot. N_2-fixer phytomass share) vs. leaf litter N-concentration[1)]	n.s. (n=88)	n.s. (n=17)	n.s. (n=13)
ln (pot. N_2-fixer phytomass share) vs. leaf litter δ^{15}N-signal[1)]	-0.28**	n.s.	n.s.
foliar N-concentration vs. leaf litter N-concentration	n.s. (n=111)	n.s. (n=29)	n.s. (n=9)
foliar δ^{15}N-signal vs. leaf litter δ^{15}N-signal	+0.31***	n.s.	n.s.
ln (leaf litter mass) vs. leaf litter N-concentration	-0.60*** (n=151)	-0.29° (n=40)	-0.41° (n=23)
ln (leaf litter mass) vs. leaf litter δ^{15}N-signal	+0.41***	n.s.	n.s.

1) above minimum threshold of 2% phytomass share *** p<0.001, ** p<0.01, ° p<0.07

Leaf litter nitrogen is expected to be related with foliar nitrogen, 86% of all plots contain one or more plant included in the foliar sampling scheme. Plot-based correlations of mean foliar δ^{15}N-signals and leaf litter δ^{15}N-signals are significant in first-cycle secondary regrowth. In contrast, no correlations between leaf and leaf litter N-concentrations are significant at any stage of succession (mid rows in Table 5.18).

Leaf litter nitrogen is strongly affected by leaf litter mass, with litter N-concentrations negatively correlated with litter mass throughout succession and litter δ^{15}N-signals positively correlated with litter mass in secondary regrowth (bottom rows in Table 5.18). Such dependencies are seen as the outcome of leaf litter decomposition processes.

5.5.3 Topsoil 'plant-available' nitrogen

Figure 5.11 shows the 'plant-available' nitrogen in the topsoil, assessed by bioextraction with rice. Tissue N-concentrations and δ^{15}N-signals are positively correlated to one another (Pearson R = 0.59***, n=34), apparently a general observation in literature (Kitayama and Iwamoto, 2001).

Neither the N-concentrations nor the δ^{15}N-signals of the topsoil vary systematically along succession, and secondary and primary forests on kaolinitic Oxisol do not differ from another. In contrast, the δ^{15}N-signals are lower in the terra morena and campinarana forest sites.

No effect of potentially N_2-fixing legumes on plant-available topsoil-N is apparent, as values do not differ between sampling positions within clusters of potential N_2-fixers (full symbols) vs. outside of areas possibly affected by N_2-fixation (open symbols in Figure 5.11).

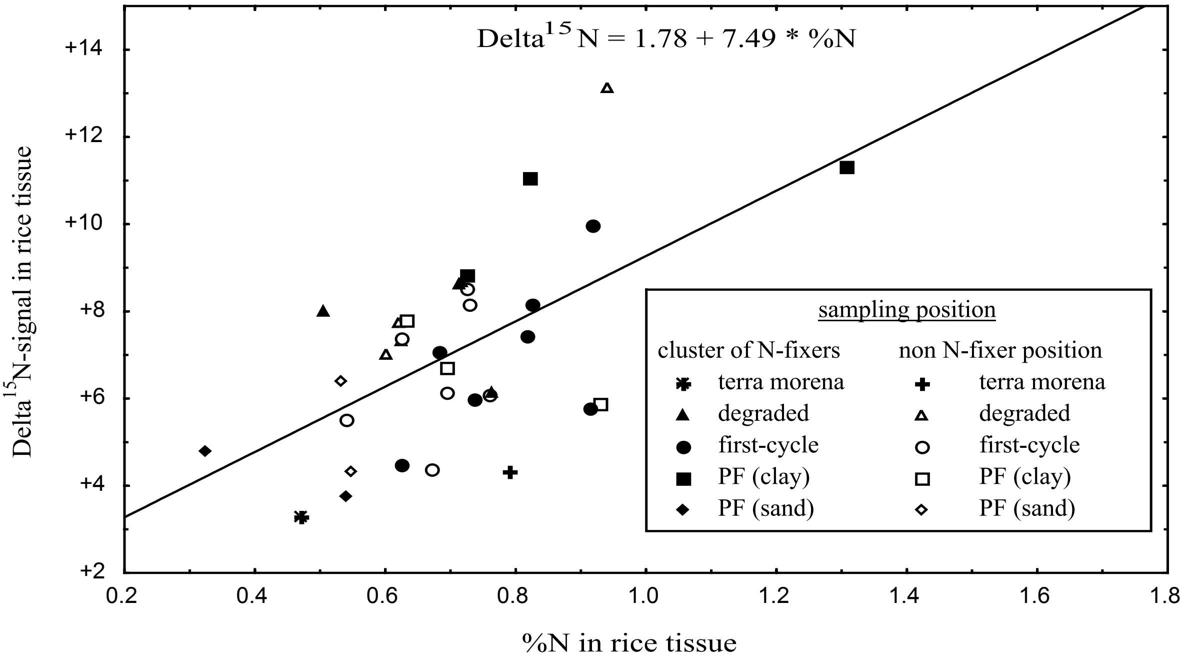


Figure 5.11. Rice-extracted ('plant-available') topsoil N-concentrations and δ^{15}N-signals: influence of site age, soil type and the vegetation share of potential N_2-fixers

5.5.4 Summary and conclusions

The leaf litter δ^{15}N-signals increase (moderately) along succession, and they are significantly higher in old secondary and in primary forests than in young and mid-aged regrowth. Conversely, the N-concentrations tend to sink along succession and are lower in old secondary and in primary forests than in young and mid-aged regrowth. Such low litter N-concentrations may result out of low tissue N-concentrations or out of high rates of N-retranslocation before leaf senescence, suggesting an elevated ‘N-use efficiency’ (Vitousek, 1982). This again points to low N-availability in these old growth forests (further discussed in chap. 6.8).

Leaf litter δ^{15}N-signals are significantly lower and N-concentrations are significantly higher both in the terra morena site as compared to similarly-aged regrowth on clayey Oxisol, and in the campinarana forests as compared to primary forest controls. This points to an elevated role of BNF on these edaphically differing sites. Topsoil ‘plant-available’ δ^{15}N-signals are likewise markedly lower in these sites, but contrary to leaf litter this is accompanied by low topsoil N-concentrations.

Both the leaf litter N-concentrations and δ^{15}N-signals are related to leaf litter mass, suggesting a strong impact of litter decomposition on the leaf litter nitrogen. Samples comprise the entire O-horizon and are thus composed of material from varying stages of decomposition, the sampling-period at mid rainy season implies high decomposition rates which result in the altogether low litter mass recorded in this study (chap. 5.1.2).

The lower leaf litter δ^{15}N-signals in plots with high phytomass shares of potential N_2-fixers allow the following two conclusions:

(1) Significant relationships are taken as indirect evidence of significant BNF in first-cycle secondary regrowth, whereas no BNF is detectable in primary forests.

(2) N_2-fixing vegetation reduces the δ^{15}N-signals in its surroundings via the litter-cycle. Thus, the ‘δ^{15}N dilution problem’ outlined in chap. 2.5.2 exists and constitutes a serious methodological problem for the ^{15}N natural abundance method. This problem is further discussed in chap. 6.6.

5.6 Nitrogen in the leaves

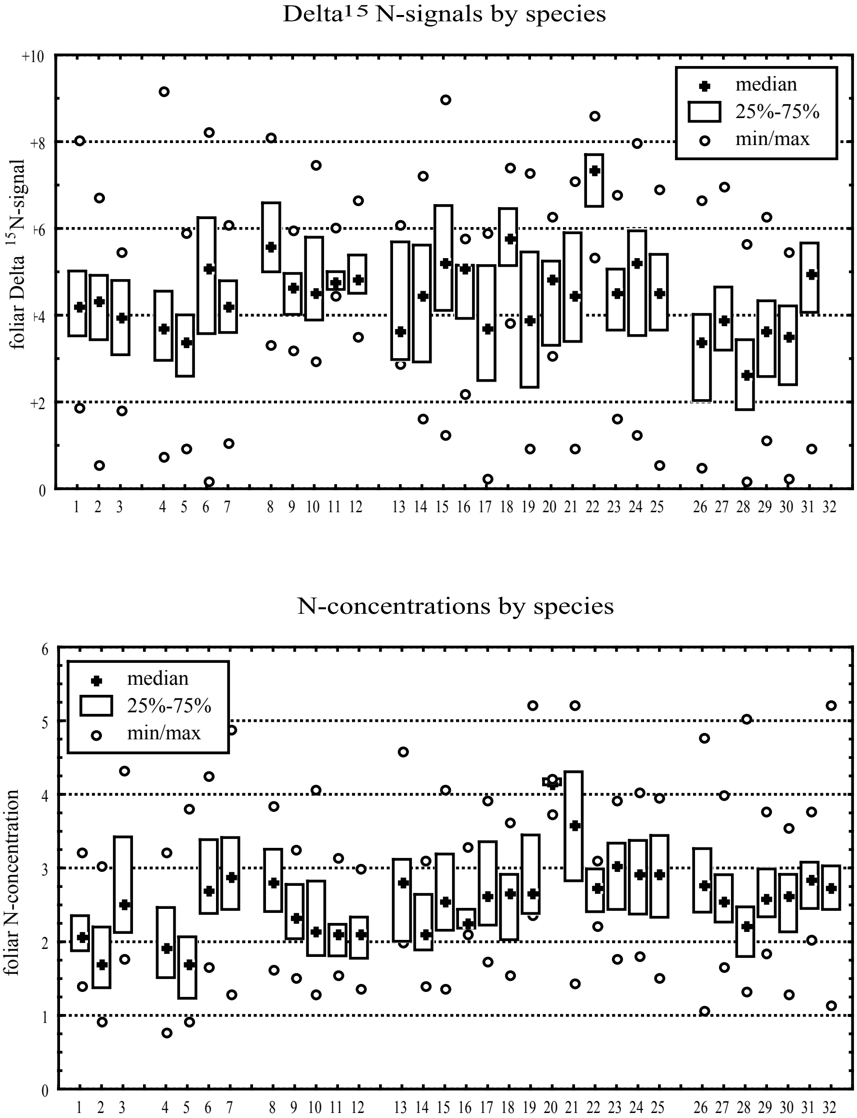
This section evaluates the results of the %N- and δ^{15}N-analysis of the 2247 leaf samples from the 19 analyzed sites; the sampling scheme is detailed in chap. 4.4.1.
Chap. 5.6.1 presents the differences in foliar nitrogen between the 32 selected species. In a second step, the species are grouped into five 'functional groups': potentially N_2-fixing trees and lianas, non N_2-fixing legumes, and non-legume trees and lianas. Systematic differences between these plant categories will be shown, both with respect to their δ^{15}N-signals and their N-concentrations.

Chap. 5.6.2 assesses the degree to which foliar N-concentrations and δ^{15}N-signals vary between sites and looks for possible shifts along succession or between secondary and primary forests. Such shifts may be caused by systematic changes in 'background' nitrogen along succession, or they may result from BNF at some stage of secondary regrowth.

Chap. 5.6.3 investigates the individual plant size as a factor, which could affect the isotopic composition of foliar nitrogen. This addresses the possibility that BNF may suffer physiological limitations in large and old legume plants, especially in old-growth forests (chap. 2.3). For this purpose, correlations between the (allometrically estimated and ln-transformed) individual plant phytomass and the foliar δ^{15}N-signals are investigated.

5.6.1 Differences between species and functional groups of species

Figure 5.12 shows the foliar δ^{15}N-signals (top) and N-concentrations (bottom) of the 32 investigated species. Species are grouped into five 'functional groups' of species; foliar N-concentrations and δ^{15}N-signals of all species are detailed in Annex 10.



non-legume trees:
(1) *Laetia procera*
(2) *Vismia guianensis*
(3) *Jacaranda copaia*

non-legume lianas:
(4) *Davilla rugosa*
(5) *Rourea cuspidata*
(6) *Memora adenophera*
(7) *Securidaca rivinaefolia*

non N-fixing legumes:
(8) *Zygia racemosa*
(9) *Macrolobium microcalyx*
(10) *Parkia multijuga*
(11) *Parkia nitida*
(12) *Bauhinia alata*

pot. N-fixing trees:
(13) *Inga edulis*
(14) *Inga stipularis*
(15) *Inga paraensis*
(16) *Inga umbellifera*
(17) *Inga cayennensis*
(18) *Inga umbratica*
(19) *Stryphnodendron pulcherrimum*
(20) *Stryphnodendron guianensis*
(21) *Dimorphandra parviflora*
(22) *Dimorphandra coccinea*
(23) *Swartzia ingifolia*
(24) *Swartzia cuspidata*
(25) *Enterolobium schomburgkii*

pot. N-fixing lianas:
(26) *Machaerium hoehneanum*
(27) *Machaerium multifoliolatum*
(28) *Machaerium madeirensis*
(29) *Machaerium quinata*
(30) *Machaerium ferox*
(31) *Dalbergia multiflora*
(32) *Derris negrensis*

Figure 5.12. Foliar δ^{15}N-signals and N-concentrations of the 32 selected species (over all sites, excluding exceptional sites and species <5 replicates; for taxonomy refer to chap. 4.3.3 and Annex 2)

The species' median δ^{15}N-signals range from +2.8 to +5.3‰, and median N-concentrations from 1.7 to 4.5 %N; the total range of values (minimum to maximum) is approximately twice (N-concentrations) to 4 times (δ^{15}N-signals) as high (0.9 to 5.2 %N and +0.1 to +9.0‰ δ^{15}N). No negative δ^{15}N-signals were recorded following the exclusion of 55 outliers and extremes. Data of most species and sites are normally distributed, allowing for parametric ANOVA and post-hoc comparisons with the Spjøtfoll-Stoline test (Spjøtfoll and Stoline, 1973) when $p<0.05$ and $n\geq3$.

The δ^{15}N-signals are record-high for *Dimorphandra coccinea* (+7.4‰), indicating that this species - though previously classified as a potential N_2-fixer - seems not to fix N_2 in the studied sites. *D. coccinea* was excluded from further analysis because of this uncertain N_2-fixation status.

The overall mean δ^{15}N-signal of potentially N_2-fixing lianas is 0.5‰ lower than that of potentially N_2-fixing trees, whereas the N-concentration does not differ between growth forms. Neither the δ^{15}N-signals, nor the N-concentrations are systematically related with taxonomy (genus, tribe or family).

Table 5.19 shows the N-concentrations and δ^{15}N-signals of the five functional groups of plants, with letters indicating statistical groupings of post-hoc comparisons.

Table 5.19. Foliar N-concentrations and δ^{15}N-signals of five functional groups of species: means ±SE and statistical groupings of post-hoc comparisons (all sites and species, excluding exceptional sites and *D. coccinea*)

			N-concentration (%)			δ^{15}N-signal		
			mean	±SE	group	mean	±SE	group
pot. N_2-fixing trees	(12 species)	(n=476)	2.86	0.04	c	4.28	0.07	b
pot. N_2-fixing lianas	(7 species)	(n=643)	2.67	0.02	c	3.73	0.06	a
non N_2-fixing legumes	(5 species)	(n=203)	2.52	0.04	b	5.05	0.08	c
non-legume trees	(3 species)	(n=310)	2.11	0.04	a	4.10	0.06	b
non-legume lianas	(4 species)	(n=442)	2.27	0.04	a	3.80	0.07	ab

The δ^{15}N-signals of potentially N_2-fixing trees and lianas do not differ significantly from the δ^{15}N-signals of non-legume trees and lianas. The main difference in the isotopic composition lies in the consistently higher δ^{15}N-signals of non N_2-fixing legume species as compared to both the potentially N_2-fixing legumes and non-legume species (average difference of 1.1‰, ANOVA significant at $p<0.001$).

The N-concentrations are significantly higher in legumes than in non-legumes, both in secondary and primary forests. They are also significantly higher in potential N_2-fixers than in non N_2-fixing legumes in secondary regrowth (excluding non-legumes from analysis, t-test $p<0.001$). In primary forests, N-concentrations are significantly ($p<0.01$) higher in potentially N_2-fixing lianas than in non N_2-fixing legume lianas, but do not differ for legume trees. All above-outlined differences are not caused by a single species, as the exclusion of any one single species from analysis does not alter the significance levels of the above-outlined differences.

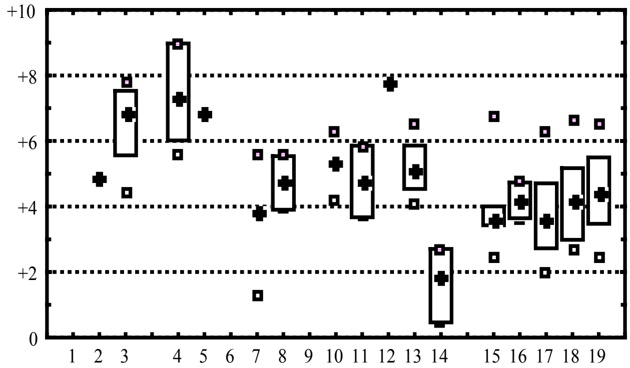
Foliar N-concentrations and δ^{15}N-signals tend to be positively correlated, which appears to be a general phenomenon encountered in literature (compiled by Kitayama and Iwamoto, 2001). Correlations are independent of successional age (data not shown), but they differ between species. In secondary regrowth, correlations are significant ($p<0.05$) for 4 out of the 7 investigated non-legume species. In contrast, correlations are significant in only 2 out of 20 potentially N_2-fixing species, and in 1 out of 5 non N_2-fixing legume species (all Spearman R ranging from +0.19 to +0.38).

Annex 8 shows the foliar δ^{15}N-signals of the 5 functional groups of species on the 19 investigated sites. ANOVA detected significant differences between groups in 15 out of the 19 sites; letters indicates the results of post-hoc comparisons. However, this study does not support the expectation of systematically lower δ^{15}N-signals in the leaves of potential N_2-fixers than those in non-legume reference plants at some stage of succession, since both higher and lower δ^{15}N-signals are encountered with similar frequency in all site classes. In contrast, the δ^{15}N-signals of the non N_2-fixing legumes are significantly higher than those of both the potentially N_2-fixing legumes and the non-legume reference species in most sites and throughout succession. Within-site δ^{15}N-differences between the 5 functional groups are about 2-3‰ and are thus higher than between-site δ^{15}N-variation.

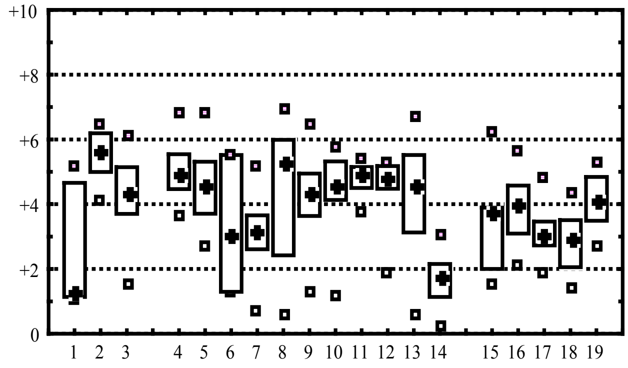
5.6.2 Foliar nitrogen along succession

Figures 5.13 and 5.14 show the foliar δ^{15}N-signals and N-concentrations of one exemplary species for each functional group of plants over the investigated 19 sites.

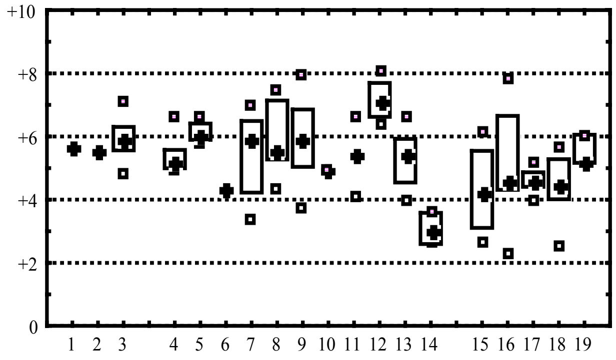
Inga paraensis (total n=85)



Machaerium hoehneanum(total n=299)



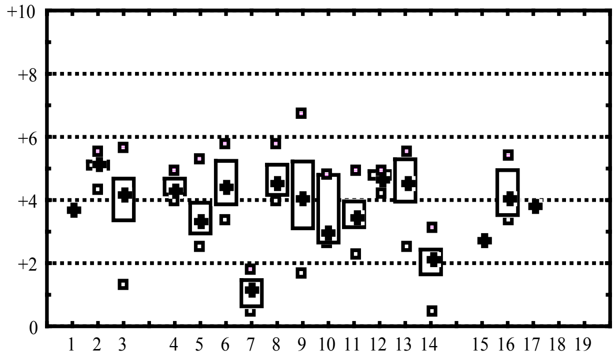
Zygia racemosa(total n=99)



species coding:

(1) terra morena
(2) 4 yrs. (degrad.)
(3) 11 yrs. (degrad.)
(4) 3-yr.-old
(5) 3-yr.-old
(6) 5-yr.-old
(7) 7-yr.-old
(8) 8-yr.-old
(9) 10-yr.-old
(10) 10-yr.-old
(11) 12-yr.-old
(12) 18-yr.-old
(13) 20-yr.-old
(14) 25-yr.-old*
(15) primary forest
(16) primary forest
(17) primary forest
(18) primary forest
(19) primary forest
* 'outlier site'

Vismia guianensis (total n=155)



Rourea cuspidata (total n=153)

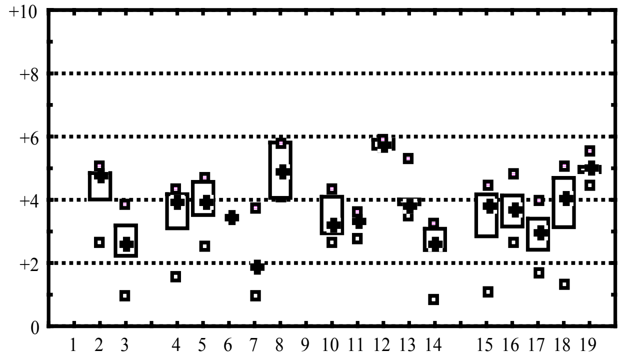
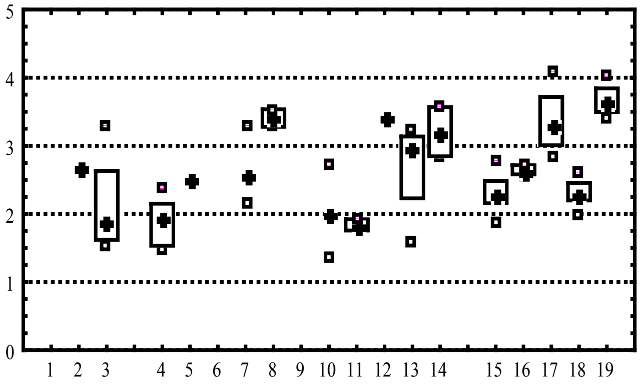


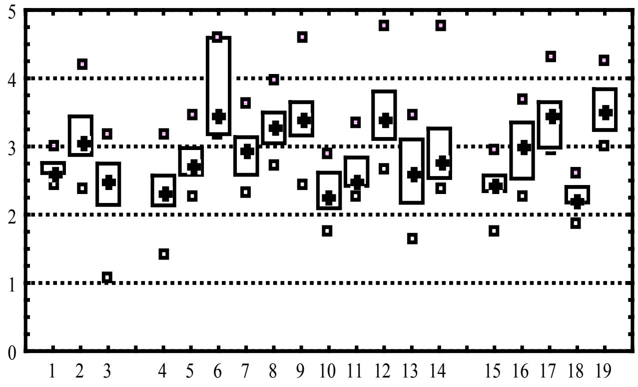
Figure 5.13. Foliar δ^{15}N-signals of exemplary species along succession (medians, 25-75 percentile range, and minimum/maximum; sequence of sites as in Annex 9)

Figure 5.14. Foliar N-concentrations of exemplary species along succession (medians, 25-75 percentile range, and minimum/maximum; sequence of sites as in Annex 9)

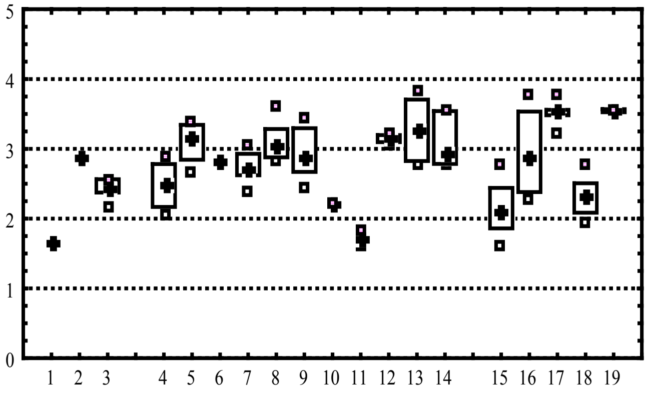
Inga paraensis (total n=85)



Machaerium hoehneanum(total n=299)



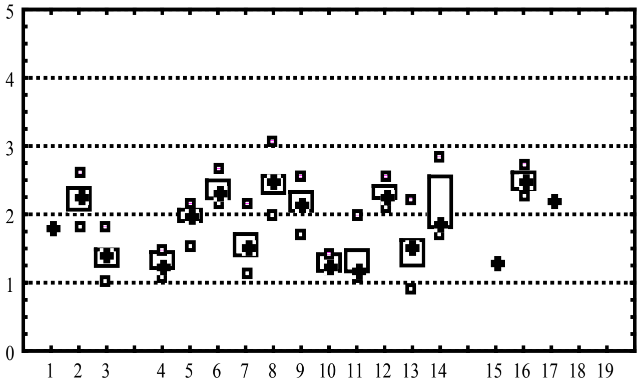
Zygia racemosa(total n=99)



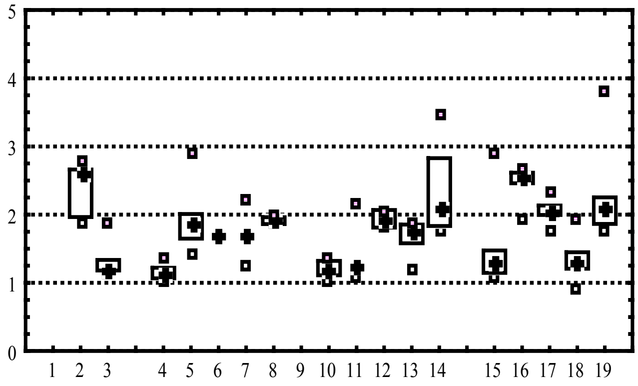
species coding:

(1) terra morena
(2) 4 yrs. (degrad.)
(3) 11 yrs. (degrad.)
(4) 3-yr.-old
(5) 3-yr.-old
(6) 5-yr.-old
(7) 7-yr.-old
(8) 8-yr.-old
(9) 10-yr.-old
(10) 10-yr.-old
(11) 12-yr.-old
(12) 18-yr.-old
(13) 20-yr.-old
(14) 25-yr.-old*
(15) primary forest
(16) primary forest
(17) primary forest
(18) primary forest
(19) primary forest
* 'outlier site'

Vismia guianensis (total n=155)



Rourea cuspidata (total n=153)



Neither the δ^{15}N-signals nor the N-concentrations show any clear trend along succession, nor do they differ systematically between secondary and primary forests. Thus, foliar nitrogen does not meet the expectations of a successional increase in δ^{15}N-signals (chap. 2.5.2), and does not follow the (weak) successional trends observed for leaf litter nitrogen (chap. 5.5.1).

The 25-yr.-old ‘outlier’ site (site N$^{o.}$ 14) is extraordinary in its consistently 2-3‰ lower δ^{15}N-signals for most species and for all functional groups of species. Foliar N-concentrations and δ^{15}N-signals are detailed by sites in Annex 9 and by potentially N_2-fixing species in Annex 10.

5.6.3 Impact of the individual plant size on foliar δ^{15}N-signals

The impact of the individual plant size on the foliar δ^{15}N-signals differs between potentially N_2-fixing and non N_2-fixing species:

No correlations between individual phytomass and foliar δ^{15}N-signals were detected either for the non N_2-fixing legumes, or for the non-legume trees or lianas. This holds true for all 12 investigated non N_2-fixing species and over all sites and site classes.

In marked contrast, individual phytomass is moderately but frequently significantly positively ln-linearly correlated with the foliar δ^{15}N-signals in potentially N_2-fixing legumes. Correlations differ in slope, significance and precision along succession and between growth forms and species (right columns in Annex 9 and 10).

Correlations are significant for either potentially N_2-fixing trees or lianas in 4 out of the 14 regrowth sites and in all 5 primary forest sites (Spearman R ranging from +0.27 to +0.79, see Annex 9). When combining sites into site classes, correlations are significant in primary forest only.

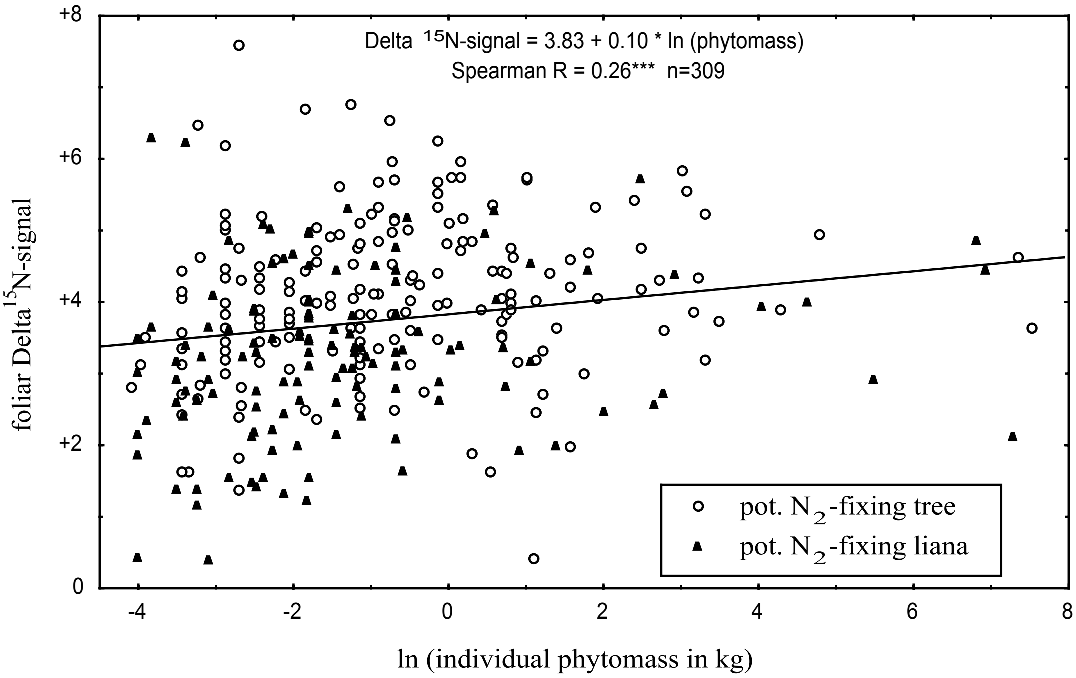


Figure 5.15. Impact of the individual plant size on the foliar δ^{15}N-signals of potentially N_2-fixing trees and lianas in primary forests

Figure 5.15 shows the relationship between the individual phytomass and the δ^{15}N-signals of potential N_2-fixers in primary forests; phytomass ranges from 15 grams to 10 tons. For the entirety of potential N_2-fixers, the slope of this relationship is moderate only, but slope increases at the growth form, genus and species levels. Correlations are significant for both growth forms (trees: R = +0.25***, lianas: R= +0.30***) and for the main genera (5 *Inga* species: R = +0.24**, 5 *Machaerium* species: R = +0.30***).

Correlations between individual phytomass and δ^{15}N-signals differ among species (Annex 10, secondary and primary forests combined for analysis). They are significant for 3 out of the 12 potentially N_2-fixing tree species and for 5 out of the 7 potentially N_2-fixing liana species investigated in this study.

Correlations between individual phytomass and foliar N-concentrations are not as precise as correlations with the δ^{15}N-signals, and they differ in their (mainly positive) signal between some species.

5.6.4 Summary and conclusions

The foliar δ^{15}N-signals of potentially N_2-fixing legumes are not systematically lower than those of the non-legume reference species at any stage of succession, violating the requirement of lower δ^{15}N-signals in putative N_2-fixers than of 'soil-derived' (reference) nitrogen. In contrast, the δ^{15}N-signals of the non N_2-fixing legume species are consistently (on average 1.1‰) higher than both the potentially N_2-fixing legumes and the non-legume references. The similar δ^{15}N-signals for both the potentially N_2-fixing legumes and the non-legume references, and the systematically higher δ^{15}N-signals in non N_2-fixing legumes will cause marked differences in the plausibility of the BNF estimations based on these reference categories (see following chap. 5.7.1).

Foliar N-concentrations are significantly higher in legumes than in non-legumes, both in secondary and in primary forests. This is in accordance with the expectations outlined in chap. 2.2.1 and appears to be a general feature of the Leguminosae. The group of non N_2-fixing legume species takes an intermediate position, with N-concentrations on average 0.2% lower than the potentially N_2-fixing legumes, and 0.3% higher than the non-legume species.

Legumes also differ from non-legumes in a further plant characteristic: Whereas N-concentrations and δ^{15}N-signals are significantly positively correlated in the leaves of most non-legume species (in analogy to rice tissue, see Figure 5.11), no relationships are apparent either for potentially N_2-fixing nor for non N_2-fixing legumes.

These results point to the closer proximity of non N_2-fixing legumes to putative N_2-fixers, as compared to the non-legume species. Such proximity could make them preferential candidates for the use as reference species in future studies (further discussed in chap. 6.5).

Chap. 5.6.3 describes the positive correlations between the individual plant size and the foliar δ^{15}N-signals of potentially N_2-fixing trees and lianas, especially in primary forests. No relationships are apparent for any of the 12 non N_2-fixing species or functional groups. This allows the conclusion that plant-size effects on foliar δ^{15}N-signals are BNF-related, and not caused by other factors (e.g., size effects on the within-plant δ^{15}N-distribution). Thus, BNF appears to be physiologically limited in large primary forest trees and lianas. Further implications of this finding are discussed in chap. 6.7.

5.7 Evaluation of the ^{15}N natural abundance method

This section evaluates the adequacy of the ^{15}N natural abundance method for estimation of BNF in tropical forest vegetation. For this purpose, %Ndfa-estimates are obtained parallel by a variety of estimations calculated assuming a range of reference plant categories (chap. 5.7.1), and using various modes of individual N_2-fixer and reference plant pairing (chap. 5.7.2). The portion of valid cases (estimates within the theoretical range of 0 to 100% Ndfa) is taken as a measure of plausibility of the different manners of BNF estimation.

5.7.1 Differences between reference categories

The correct quantification of the 'soil-derived' δ^{15}N-signature constitutes a critical methodological challenge for the ^{15}N natural abundance method. The assumption of a common N-pool demands that putative N_2-fixers and paired reference plants are similar in all isotopically relevant aspects of N-nutrition other than BNF, notably the main rooting depth, the portions of differing soil N-pools accessed by the plants (NO_3^-, NH_4^+, N_{org}), and the distribution of nitrogen within the plants (isotopic signature of mid-aged leaves relative to other plant organs). Since knowledge on these plant characteristics is obviously insufficient or absent in all studies dealing with spontaneous forests, there is general consensus on the need to measure several or many reference species (Handley and Scrimgeour, 1997; Högberg, 1997; Boddey et al., 2000). This approach, also pursued in the present study, avoids erroneous %Ndfa-estimates caused by a single 'atypical' reference species.

The non N_2-fixing reference species investigated in this study are grouped into non-legume trees, non-legume lianas, and non N_2-fixing legumes (see Table 5.19). In order to avoid distortions caused by differing species replications per site, this study calculates 'species-averaged' δ^{15}N-signals as the average of each of the 3-5 species per site and reference category (chap. 4.8). Table 5.20 shows the average δ^{15}N-signals of the three reference plant categories and additionally of mixed leaf litter along succession.

Table 5.20. Species-averaged δ^{15}N-signals of three categories of reference plants, and leaf litter δ^{15}N-signals along succession (sites with n ≥ 3 plants per species and site)

	non-legume tree	non-legume liana	non N_2-fixing legume	mixed leaf litter
4 and 11 yrs. (degrad.) (2 sites)	+4.49	+4.24	+5.21	+3.32
3-yr.-old (2 sites)	+4.06	+4.16	+4.81	+3.50
5- to 10-yr.-old (5 sites)	+3.94	+4.01	+5.03	+2.59
12- to 20-yr.-old (3 sites)	+4.09	+4.06	+5.93	+3.37
PF (clay) (5 sites)	+4.00	+4.37	+5.00	+3.73

The species-averaged δ^{15}N-signals of non N_2-fixing legumes are on average 1.5‰ higher than those of non-legume trees and lianas; the influence of growth form is only moderate. Lower δ^{15}N-signals of the leaf litter are to be seen as the outcome of various sources of isotopic discrimination occurring during litter decomposition, plant N-uptake and transport to the leaves.

Both, the mean site N-concentrations and δ^{15}N-signals of the differing categories of 'soil-derived nitrogen' are correlated to one another (Table 5.21). Relationships are approximately linear and significant in most cases, indicating that all measures of the 'soil-derived δ^{15}N-signal' are sensitive at least on a relative (site-by-site) basis.

Table 5.21. Spearman correlations of the site means of N-concentrations (top right) and of δ^{15}N-signals (bottom left) between the differing reference categories (n ≥ 3 plants per species and site)

	non-legume trees	non-legume lianas	non N_2-fixing legumes	mixed leaf litter
non-legume trees	-	0.62^{**}	0.53^{*}	0.49^{*}
non-legume lianas	0.69^{**}	-	0.85^{***}	n.s.
non N_2-fixing legumes	n.s.	0.85^{***}	-	n.s.
mixed leaf litter	0.91^{***}	0.91^{***}	0.54^{*}	-

N-concentrations

δ^{15}N-signals

In the following, %Ndfa-estimates will be calculated with different species-averaged references, with mixed leaf litter, and with the single most abundant tree and liana species of each reference category (*Vismia guianensis* and *Davilla rugosa* as the most abundant non-legume species, and *Zygia racemosa* and *Bauhinia alata* as the most abundant non N_2-fixing legume species). This single-species approach is designed to evaluate the validity of reference species grouping.

The use of *Zygia racemosa* and *Bauhinia alata* as single-species references encounters the problem of insufficient replications of *B. alata* on several sites. *Vismia guianensis* as a non-legume reference tree may be questionable in primary forests, as the occurrence of this species is limited to treefall gaps (chap. 5.1.3).

In analogy to Roggy et al. (1999a), %Ndfa is estimated assuming two different levels of ^{15}N-dilution in the process of BNF: B-values of -2‰ and 0‰, which represent the expected range for most N_2-fixing species and rhizobial strains. Calculations are done individually for each sampled potential N_2-fixer and assume the site means of reference δ^{15}N-signals ($n \geq 3$ per site and species for statistical reasons). The effect of other modes of reference plant pairings will be investigated in chap. 5.7.2.

Figure 5.16 shows the data distribution of all %Ndfa estimates obtained by the different definitions of the reference δ^{15}N-signal and assuming B-values of –2‰ (black) and 0‰ (gray). Data are classified in steps of 10% Ndfa, figure headings give additional information.

Due to the normal distribution of δ^{15}N-signals, %Ndfa-estimates are normally distributed for all sites, species, and modes of %Ndfa-estimation. Whereas a B-value of -2‰ reduces kurtosis and increases the range of %Ndfa-estimates as compared to B = 0‰, the B-value does not affect the quantity of negative %Ndfa-estimates, and the overall means are similar for both B-values. The assumption of isotopic neutrality of BNF causes %Ndfa-estimates above 100% in 1 - 9 cases (0.2 – 0.9% of cases), as opposed to zero cases assuming a B-value of –2‰.

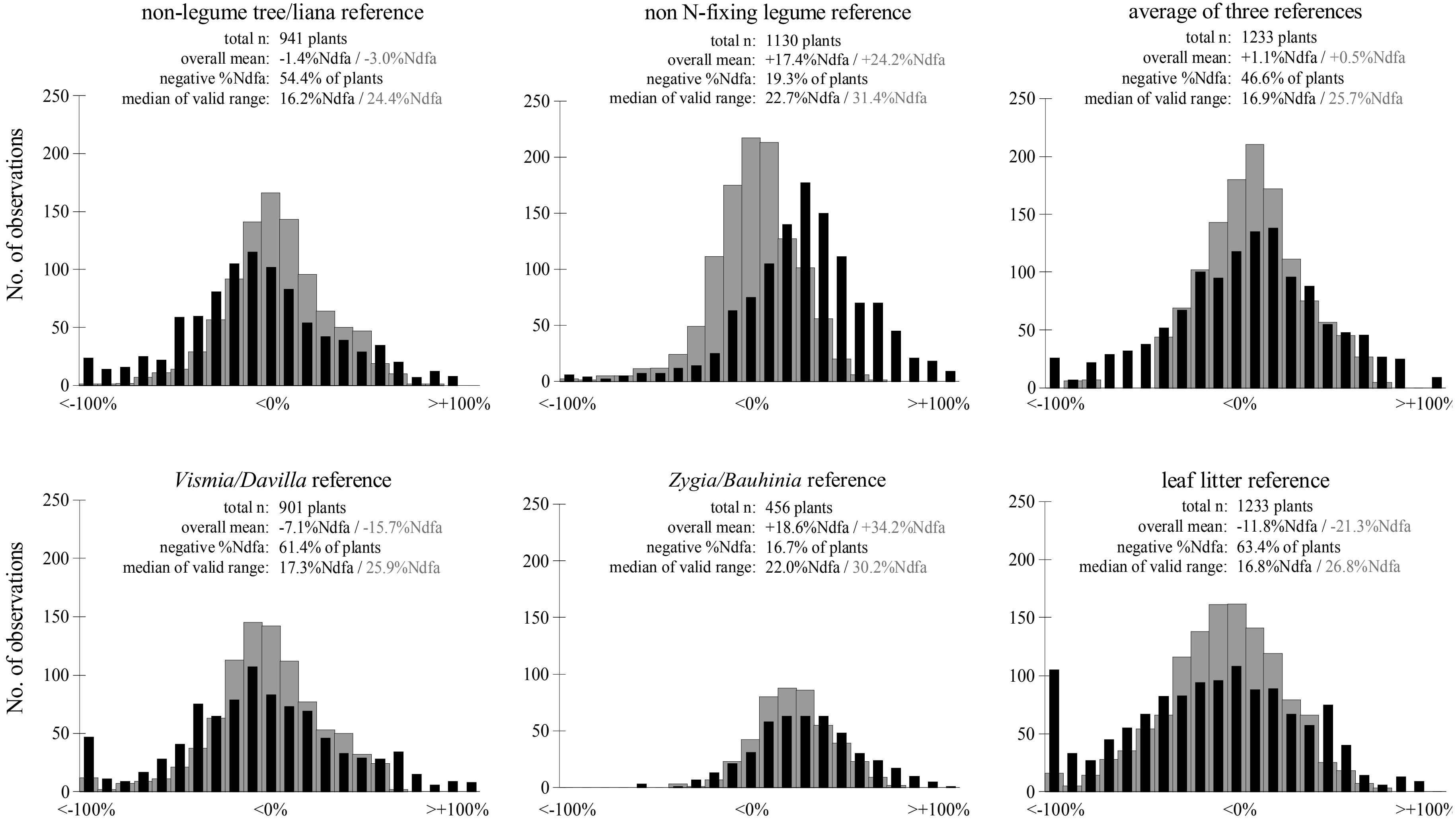


Figure 5.16. Histograms of %Ndfa estimates obtained by different definitions of the 'soil-derived δ^{15}N-signal' (over all sites with sufficient replicates, assuming B-values of -2‰ (black) or 0‰ (gray), bars represent 10%-classes of %Ndfa-estimates, ranging from <-100% to >+100%Ndfa)

The different categories of soil-derived $\delta^{15}N$ result in large and systematic differences in the portions of negative %Ndfa-estimates. Whereas more than half of all %Ndfa-estimates are negative when using non-legume species as references, the use of non N_2-fixing legumes as reference results in the lowest portion of negative %Ndfa-estimates (19% of plants). This is the consequence of the significantly higher $\delta^{15}N$-signals of the non N_2-fixing legume species (Table 5.19 and Annex 8). Due to their low $\delta^{15}N$-signals, mixed leaf litter as reference results in the highest portion of invalid estimates (63% of all potentially N_2-fixing plants). The variable impact of litter decomposition on leaf litter (chap. 5.5.2) is a further argument against the use of leaf litter as a reference.

Estimates based on the single-species approach (bottom row in Figure 5.16) and estimates based on the respective aggregated reference $\delta^{15}N$-signals (top row in Figure 5.16) result in very similar data distributions and portions of valid cases.

The portion of negative %Ndfa-estimates is not related to the successional stage of vegetation (Table 5.22), but it differs widely among the potentially N_2-fixing species (Annex 11).

Table 5.22. Percentage of potentially N_2-fixing plants with negative %Ndfa-estimates along succession: comparisons of different reference categories, single-species approach and leaf litter reference (based on sites with $n \geq 3$ plants per reference species)

	reference categories		single-species approach		
	non-legumes	non N_2-fixing legumes	*Vismia*/ *Davilla*	*Zygia*/ *Bauhinia*	mixed leaf litter
degraded regrowth	66.5	22.6	78.5	n.d.	88.6
3-yr.-old	72.5	48.6	79.8	57.1	88.1
5- to 10-yr.-old	57.3	21.9	68.0	7.1	77.2
12- to 20-yr.-old	45.3	7.4	41.2	13.5	56.8
PF (clay)	46.3	14.5	38.6	14.9	46.9

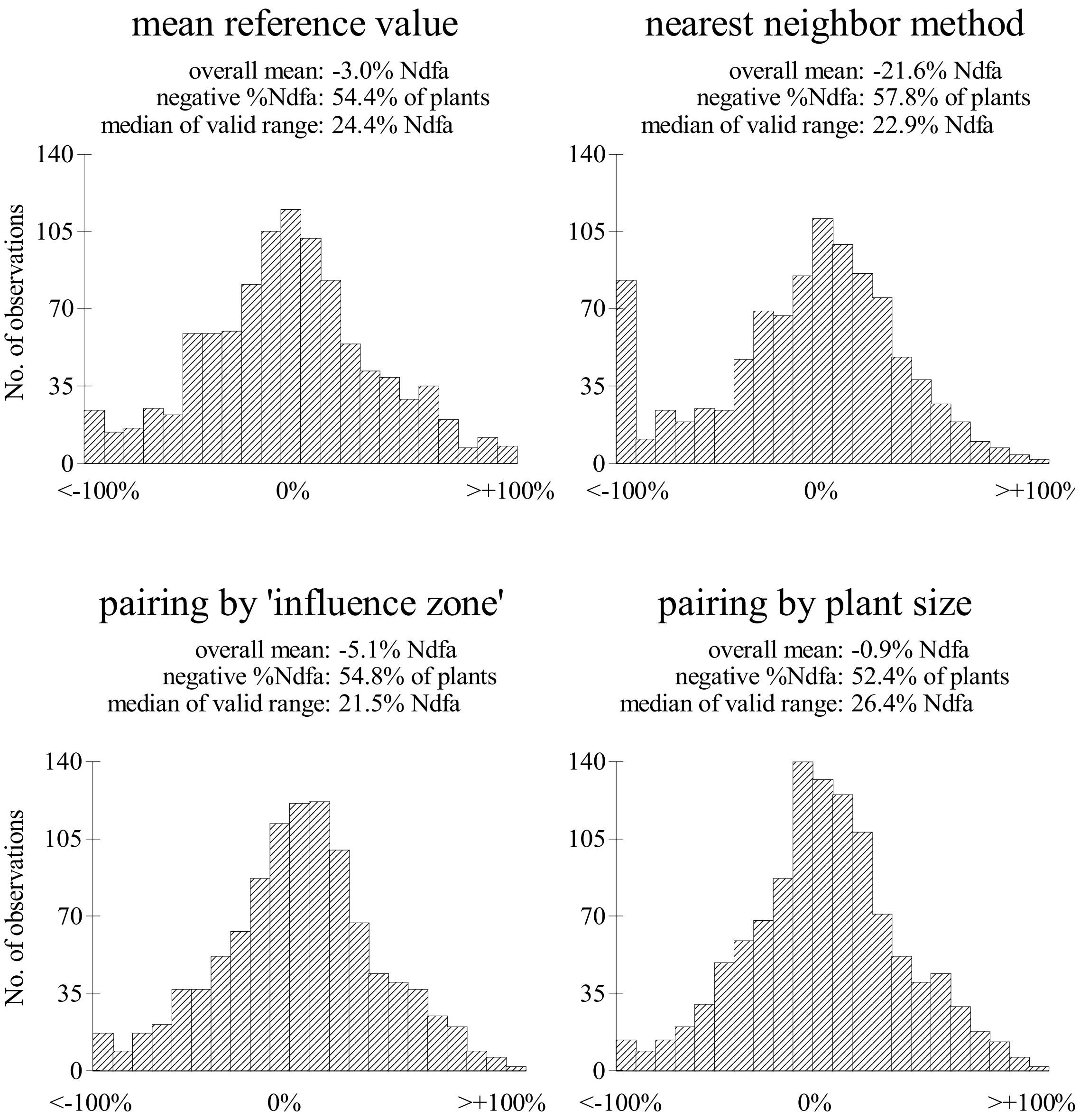
The medians of valid BNF estimates (estimates between 0% and 100%Ndfa) mainly range between 10% and 30%Ndfa in all stages of secondary succession and in primary forests, no successional trends are apparent (data not shown). The %Ndfa-estimates are 5-15% higher when using non N_2-fixing legumes as reference as compared to non-legume references. They are likewise 5-15% higher when assuming isotopic neutrality of BNF than when assuming a B-value of –2‰ (data not shown).

5.7.2 Effect of reference plant pairing method

In the following, different strategies of individual reference plant selection are evaluated by comparing a variety of plant-pairing routines. The %Ndfa estimations are developed under the assumption of (1) a single overall reference δ^{15}N-signal (site means, as presented in chap. 5.7.1), (2) by pairing putative N_2-fixers and reference plants with the nearest neighbor method, or by utilizing pairing routines which distinguish in (3) 'N_2-fixer influence-zones' or in (4) individual plant size classes. Calculations are conducted for non-legume reference trees and lianas and uniformly assume a B-value of 0‰.

Plant pairing by 'N_2-fixer influence zones' distinguishes plants growing in the direct vicinity of potential N_2-fixers, in the transition zone, or outside of areas possibly affected by BNF (chap. 4.4.1). Foliar N-concentrations of reference plants are 0.2% higher in the direct vicinity of potentially N_2-fixing legumes than of plants outside of areas possibly affected by BNF (significant at $p=0.01$ over all non-legumes in first-cycle regrowth). This suggests that BNF may indeed affect the soil-derived nitrogen. On the other hand, the δ^{15}N-signals are not significantly affected by the proximity to potentially N_2-fixing vegetation in any site or site class (data not shown).

Chapter 5.6.3 has shown significant plant-size effects on the δ^{15}N-signals of potentially N_2-fixing legume trees and lianas, indicating that plant pairing into size classes may make sense. For this purpose, potential N_2-fixers and reference plants are categorized into the following three plant-size classes: 'small' (i.e., smaller than the 25-percentile), 'mid-sized' (i.e., 25- to 75-percentiles) and 'large' (i.e., greater than the 75-percentile) of the legume tree and liana phytomass of the respective site.



mean reference value

overall mean: -3.0% Ndfa
negative %Ndfa: 54.4% of plants
median of valid range: 24.4% Ndfa

nearest neighbor method

overall mean: -21.6% Ndfa
negative %Ndfa: 57.8% of plants
median of valid range: 22.9% Ndfa

pairing by 'influence zone'

overall mean: -5.1% Ndfa
negative %Ndfa: 54.8% of plants
median of valid range: 21.5% Ndfa

pairing by plant size

overall mean: -0.9% Ndfa
negative %Ndfa: 52.4% of plants
median of valid range: 26.4% Ndfa

Figure 5.17. Histograms of %Ndfa estimates obtained by different manners of reference plant pairing; bars represent 10%-classes of %Ndfa-estimates ranging from <-100% to >+100%Ndfa

Figure 5.17 shows the data distribution of %Ndfa-estimates obtained with the above-outlined four plant pairing methods. As in Figure 5.16, all data are normally distributed. The ‘nearest neighbor method’ results in altogether lower %Ndfa-estimates, a larger portion of invalid cases and the occurrence of negative extremes (i.e., <-500%Ndfa). Plant pairing by ‘influence zones’ results in a data distribution almost identical with the assumption of the overall reference site means. Pairing by plant sizes slightly increases the %Ndfa-estimates and marginally reduces the portion of invalid cases.

Table 5.23 compares the performance of the four plant pairing methods for different stages of succession; no successional trends are apparent for any one of these methods.

Table 5.23. Percentage of invalid %Ndfa-estimates obtained by different manners of reference plant grouping and site class (see text for further explanations)

	overall reference mean	nearest neighbor method	grouped by 'influence zone'	grouped by plant-size class
degraded regrowth	66.46 (n=158)	64.23 (n=79)	50.34 (n=147)	56.33 (n=158)
3-yr.-old	72.48 (n=109)	79.80 (n=79)	74.75 (n=99)	76.15 (n=109)
5- to 10-yr.-old	57.30 (n=356)	50.16 (n=160)	56.79 (n=324)	51.97 (n=356)
12- to 20-yr.-old	45.27 (n=148)	47.33 (n=62)	45.45 (n=132)	47.30 (n=148)
PF (clay)	46.34 (n=359)	60.46 (n=185)	52.77 (n=343)	54.32 (n=359)

5.7.3 Summary and conclusions

All investigated calculation methods show a normal distribution of %Ndfa-estimates. However, many of the %Ndfa-estimates are invalid. The ^{15}N natural abundance method is thus unsuitable as a quantitative method for BNF-estimation in these forests. Nevertheless, the plausibility of estimates differs between the investigated estimation methods:

Chapter 5.7.1 illustrates considerable differences between different reference plant categories both in their foliar δ^{15}N-signals and in the resulting %Ndfa-estimates. Non N_2-fixing legume species perform far better as reference as compared to non-legume species. The overall mean %Ndfa-estimate is significantly in the positive and the portion of invalid estimates is much lower (19.3% of all estimates) than when using non-legume references. This is caused by the higher foliar δ^{15}N-signals of non N_2-fixing legumes (on average 1.1‰ higher than in non-legume reference plants).

The significance of correlations of both N-concentrations and δ^{15}N-signals between all types of references suggests that all reference categories reflect similar patterns between sites and are thus sensitive at least on a relative basis. Grouping of reference species into categories does not alter the portion of valid cases nor the resulting %Ndfa-estimates as compared to the single-species approach, and is thus a successful strategy to increase replications and reduce the risk of atypical references.

The successional stage does not affect the plausibility of %Ndfa-estimates in any method of %Ndfa-estimation. The B-value only moderately affects the %Ndfa-estimates and does not affect the portion of valid cases.

Since litter $\delta^{15}N$-signals are systematically 1-2‰ lower than foliar samples, %Ndfa-estimates based on leaf litter as reference show the highest portion of invalid cases. The strong impact of the decomposition stage on leaf litter nitrogen (litter mass positively correlated with the $\delta^{15}N$-signals and negatively with the N-concentrations; chap. 5.5.2) is a further argument against leaf litter, which is, therefore, considered an inadequate measure of the soil-derived $\delta^{15}N$-signal.

The manner of pairing putative N_2-fixers and reference plants affects the %Ndfa-estimates to a far lower extent than do the different categories of reference plants. Altogether, the assumption of an overall (site means) reference $\delta^{15}N$-signal is justified. Nevertheless, some differences are apparent:

The %Ndfa-estimates obtained by the nearest neighbor method are significantly more in the negative and result in the highest portion of invalid cases. ^{15}N dilution by N_2-fixing legumes may be partially responsible for this finding. On the other hand, %Ndfa-estimates obtained with plant pairings by N_2-fixer influence zones do not differ systematically from those based on the assumption of an overall mean site reference value. Finally, the %Ndfa-estimates improve marginally when distinguishing in 3 percentile classes of individual plant size.

The failure of the ^{15}N natural abundance method in estimating BNF in tropical forests, possible reasons for this, and recommended strategies for future research are further discussed in chap. 6.5.

5.8 Spatial pattern of BNF

Chapter 5.3 has shown potentially N_2-fixing legumes to be spatially aggregated, giving room to the hypothesis that BNF is likewise spatially irregularly distributed within the study sites. This section explores the spatial patterns of interpolated foliar δ^{15}N-signals and %Ndfa-estimates. In a second step, underlying causes are explored by comparing such isotopic patterns with the distribution patterns of potentially N_2-fixing species or species groups. Spatial analysis of BNF-patterns is undertaken with the full understanding that the absolute values of %Ndfa-estimates are erroneous and therefore irrelevant (chap. 5.7).

5.8.1 Spatial distribution of δ^{15}N-signals and of %Ndfa-estimates

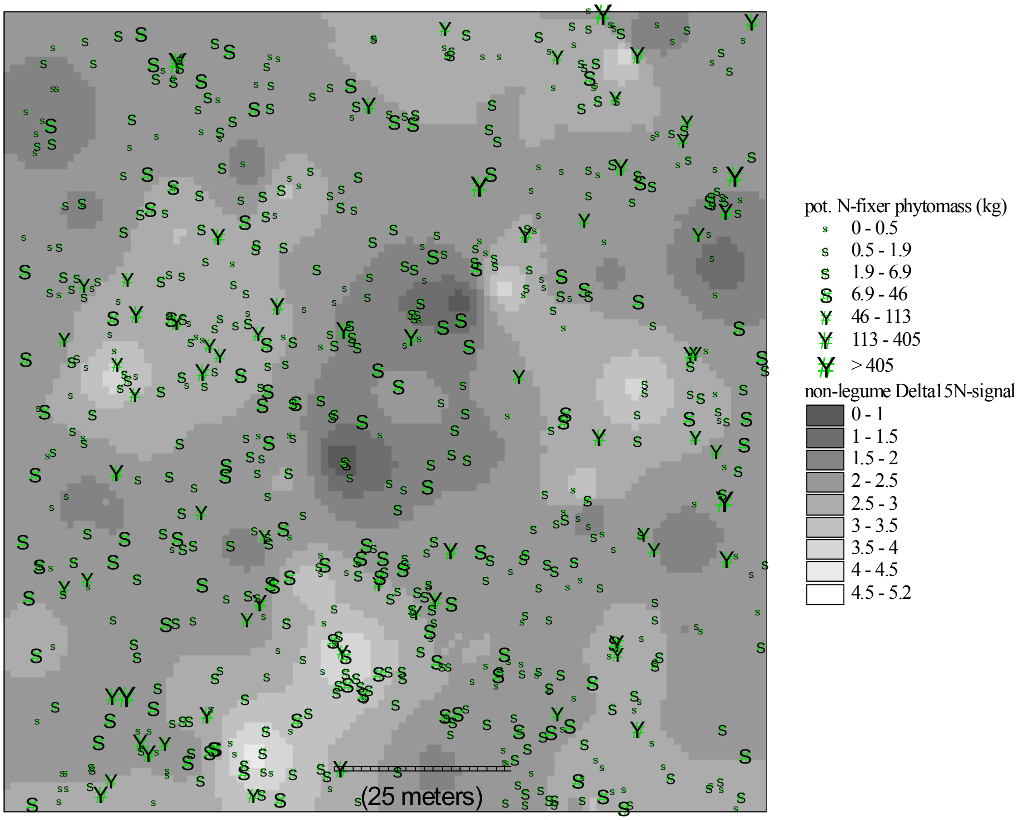
Foliar δ^{15}N was obtained in a spatially systematic sampling scheme, achieving approximately regular sampling densities throughout the sites (chap. 4.4.1) and thus allowing for interpolation of isotopic data. Both inverse distance weighing ('IDW'-method) and minimum curvature fitting ('Spline'-method) were tested as interpolation methods. Results of the IDW method are deemed more realistic, since histograms of %Ndfa-estimates for all 1m^2-cells per site are similar to the histograms presented in Figures 18 and 19 (data not shown). The Spline method was considered inadequate for this study because it 'overshoots' with both extremely positive (i.e., > +500%) and negative (i.e., < -500%) Ndfa-estimates.

The %Ndfa is calculated by overlaying interpolated δ^{15}N-signals of potential N_2-fixers and reference plants. Species-specific δ^{15}N-differences within the groups of potential N_2-fixers and of (non-legume/legume) reference species were previously eliminated by data standardization ('species-averaged references', see chap. 4.8). Since the ^{15}N natural abundance method has been shown to fail as a quantitative method of BNF estimation (chap. 5.7), the absolute values of spatial %Ndfa-estimates become irrelevant, especially when using non-legume species as reference; interpretations are valid on a relative basis only. The B-value affects the absolute scale of estimated %Ndfa, but only marginally influences its spatial distribution within the sites. For simplicity reasons, the %Ndfa-interpolations, therefore, uniformly assume isotopic neutrality of BNF (B=0‰).

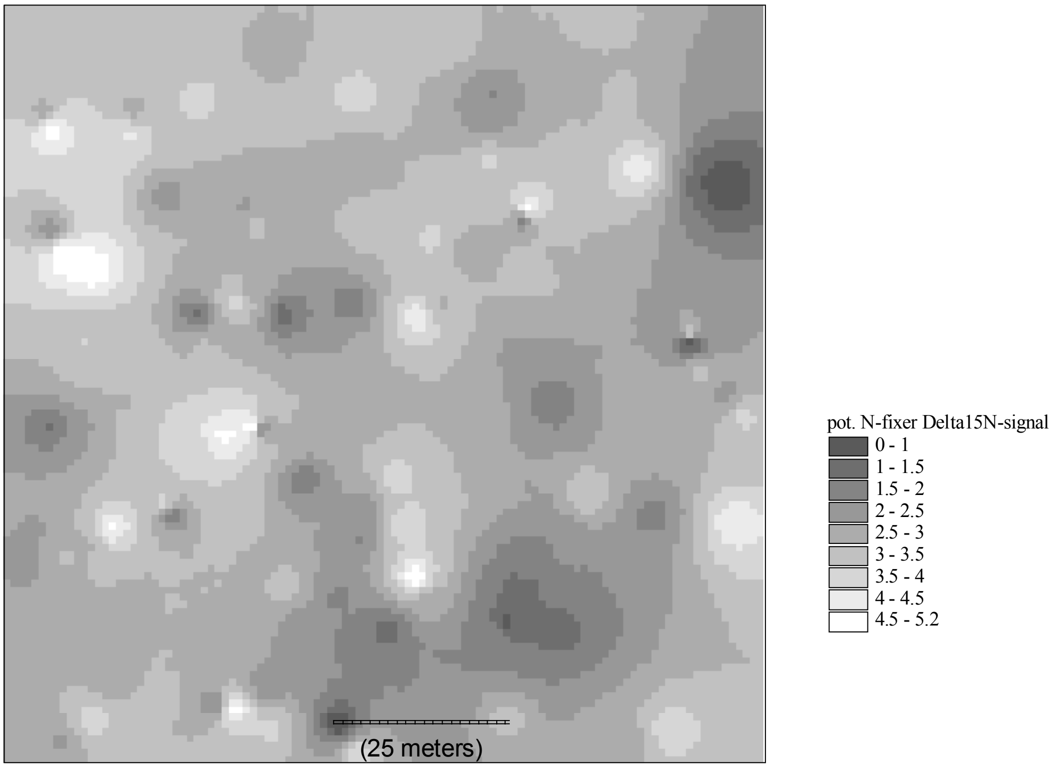
Maps 18-21 show the δ^{15}N-interpolations of potential N_2-fixers and of non-legume reference plants on two (7- and 20-yr.-old) regrowth sites, and Maps 22-23 show the resulting estimates of %Ndfa for these sites. Further maps showing the within-site distributions of %Ndfa as a result of IDW interpolations of potential N_2-fixers (purple squares) and of non-legume references (black circles) are presented in Maps 24-31 in Annex 12.

For comparison, %Ndfa calculated with non N_2-fixing legume references are shown for three of these sites (Maps 27, 29 and 31 in Annex 12). As to be expected (chap. 5.7.1), the portion of negative %Ndfa-estimates (blue areas) is much lower and the %Ndfa-estimates are higher when using non N_2-fixing legumes as reference, as compared to non-legume reference species. Spatial patterns, however, remain fairly similar for both methods.

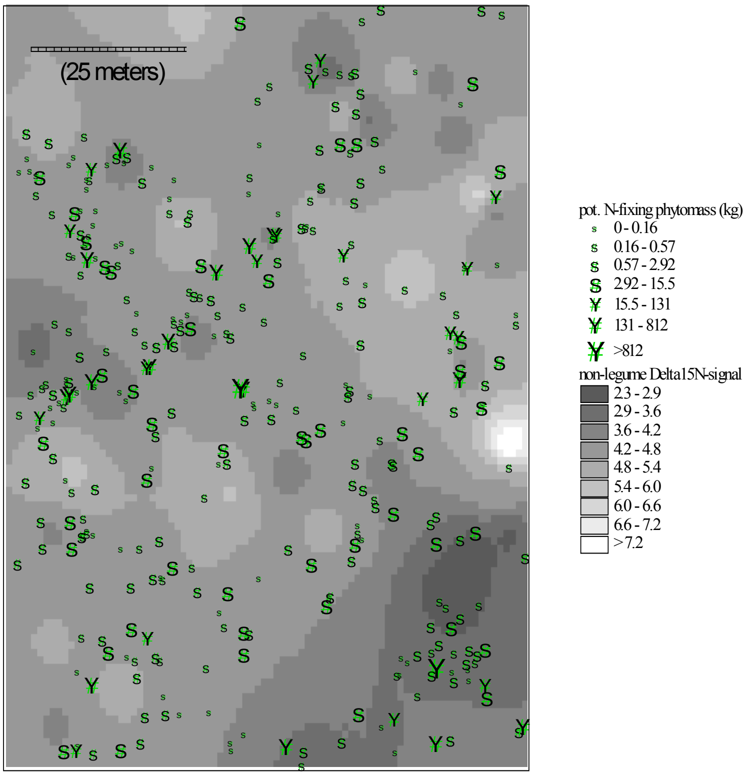
The spatial pattern of %Ndfa is irregular and patchy in all sites. In some cases, patches of high or low %Ndfa-estimates are caused by single N_2-fixer or reference plant individuals, and may, therefore, be considered methodological artifacts. Nevertheless, patchiness remains after discounting such single-plant effects. This suggests that BNF is locally concentrated in distinct hotspots, rather than occurring widespread over the entirety of the vegetation.



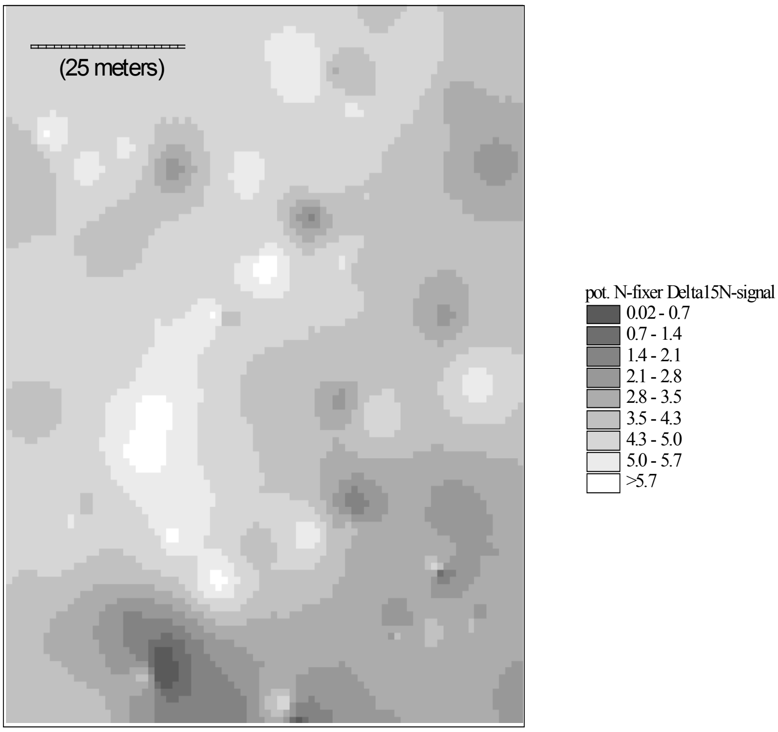
Map 18. IDW-interpolated non-legume reference δ^{15}N-signal of a 7-yr.-old first-cycle regrowth site, and the distribution of potentially N_2-fixing legumes (scaled in 7 percentile levels of estimated phytomass in kg)



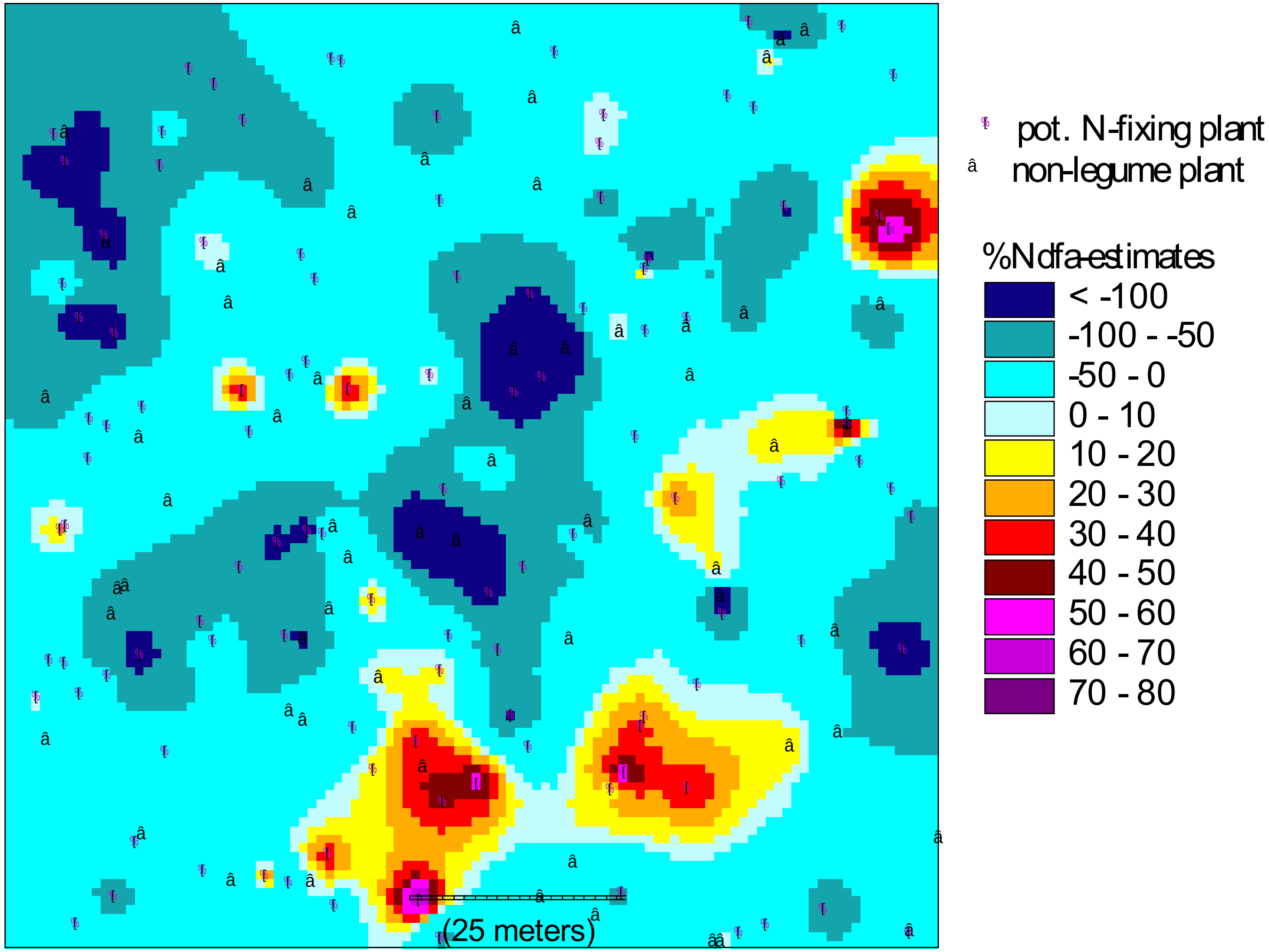
Map 19. IDW-interpolated potential N_2-fixer δ^{15}N-signal of the above-shown 7-yr.-old first-cycle regrowth site



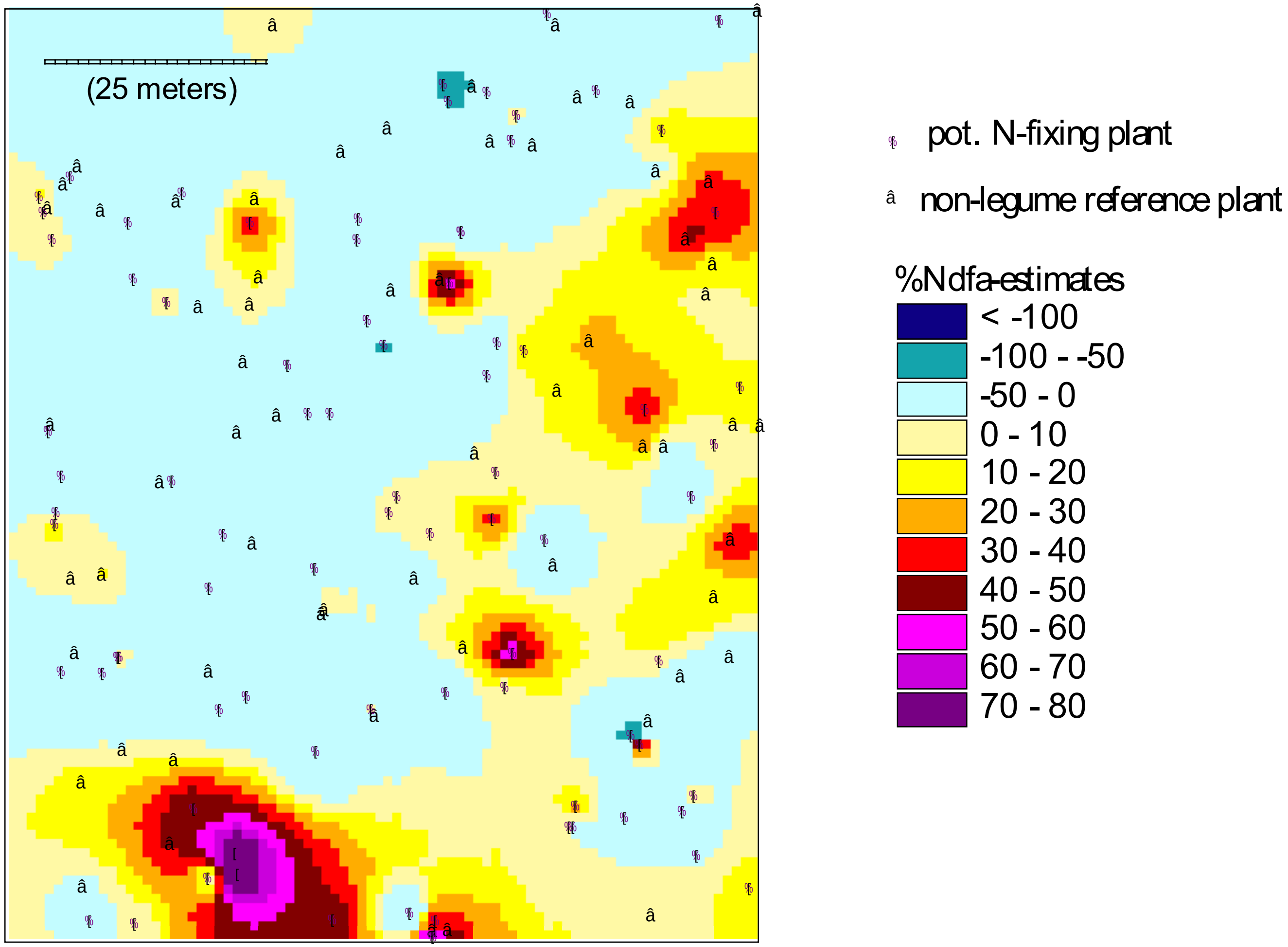
Map 20. IDW-interpolated non-legume reference δ^{15}N-signal of a 20-yr.-old first-cycle regrowth site, and the distribution of potentially N_2-fixing legumes (scaled in 7 percentile levels of estimated phytomass in kg)



Map 21. IDW-interpolated potential N_2-fixer δ^{15}N-signal of the above-shown 20-yr.-old first-cycle regrowth site



Map 22. Spatial distribution of %Ndfa-estimates on the 7-yr.-old first-cycle regrowth site (IDW interpolation, assuming a B-value of 0‰)



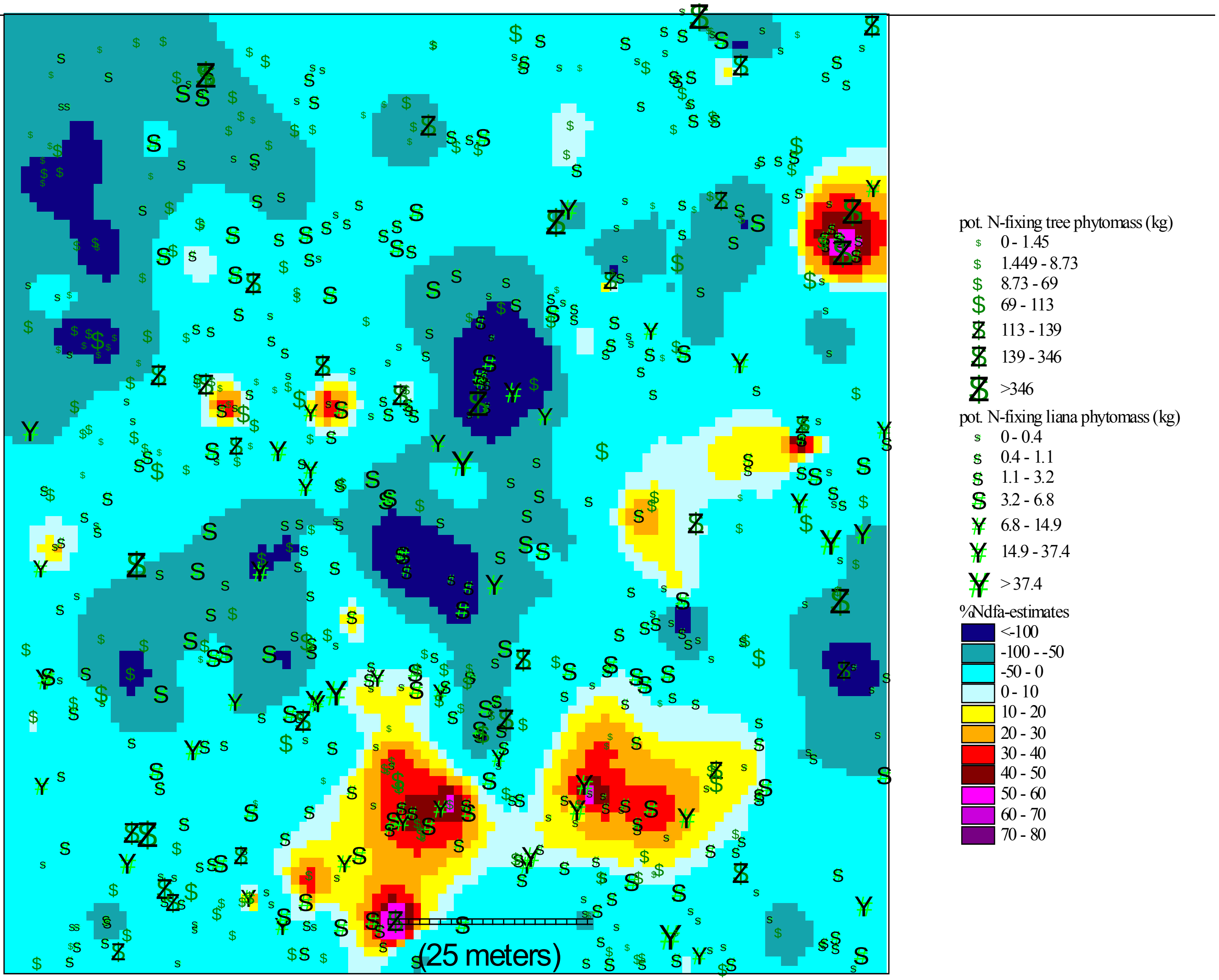
Map 23. Spatial distribution of %Ndfa-estimates on the 20-yr.-old first-cycle regrowth site (IDW interpolation, assuming a B-value of 0‰)

5.8.2 Causes and consequences of irregular BNF distribution

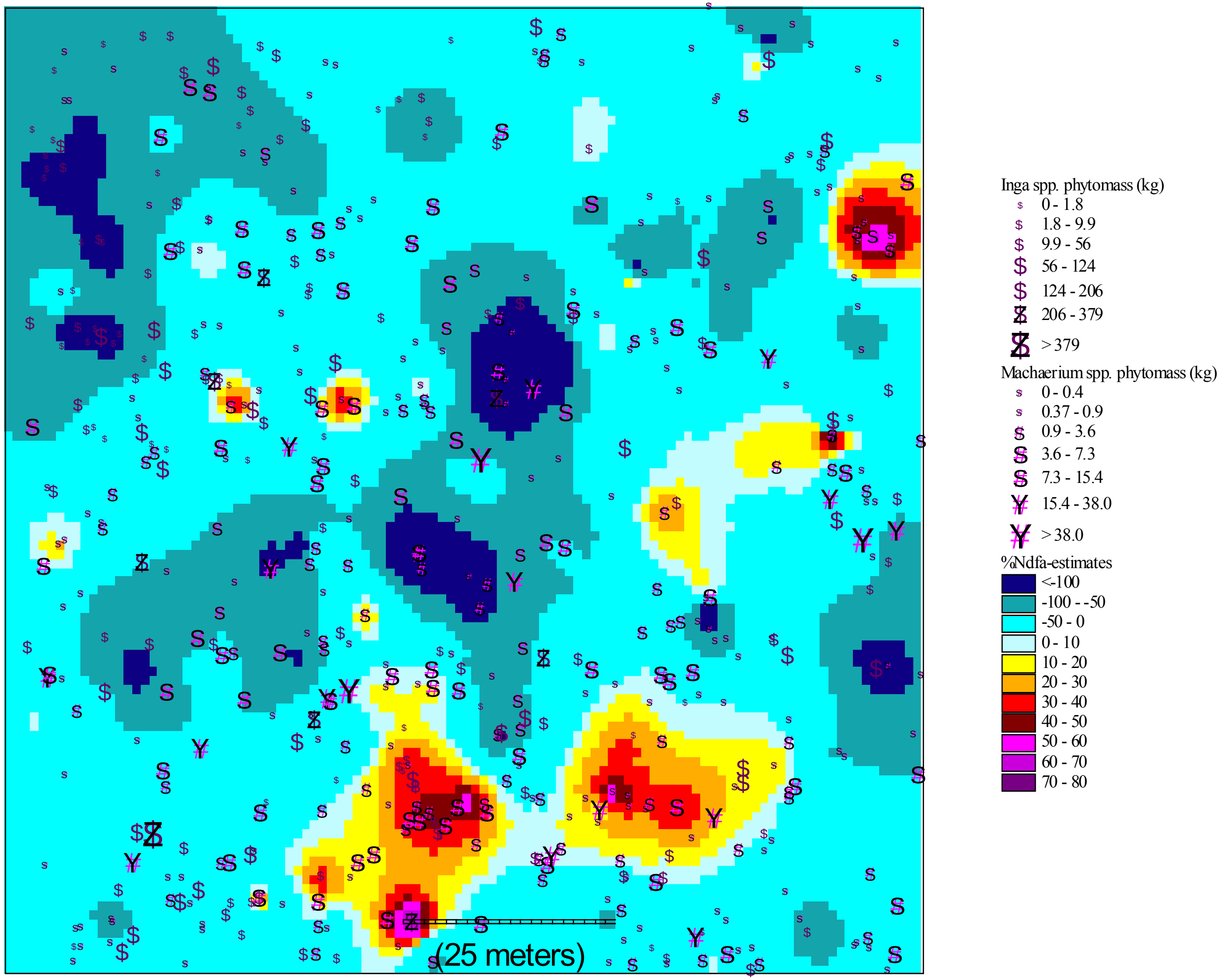
This chapter investigates the potentially N_2-fixing vegetation as a possible factor affecting the spatially irregular patterns of foliar $\delta^{15}N$-signals and %Ndfa-estimates. Investigations are based on visual comparisons of interpolated %Ndfa-estimates and of the ‘soil-derived’ (non-legume reference) $\delta^{15}N$-signals with overlaid point pattern distributions of potentially N_2-fixing species or species-groups.

In Maps 32-37 the %Ndfa-estimates of the previously detailed 7- and 20-yr.-old regrowth sites are compared with the plant distribution patterns formed by continuous subsets of potentially N_2-fixing species, distinguishing potentially N_2-fixing trees and lianas, the main tree and liana genera (*Inga* and *Machaerium*), and the two most abundant species of each of these genera (*I. paraensis* and *I. alba* / *I. stipularis*; *M. hoehneanum* and *M. multifoliolatum*). Plant phytomass is scaled in 7 percentile classes of the respective functional group or genus and site.

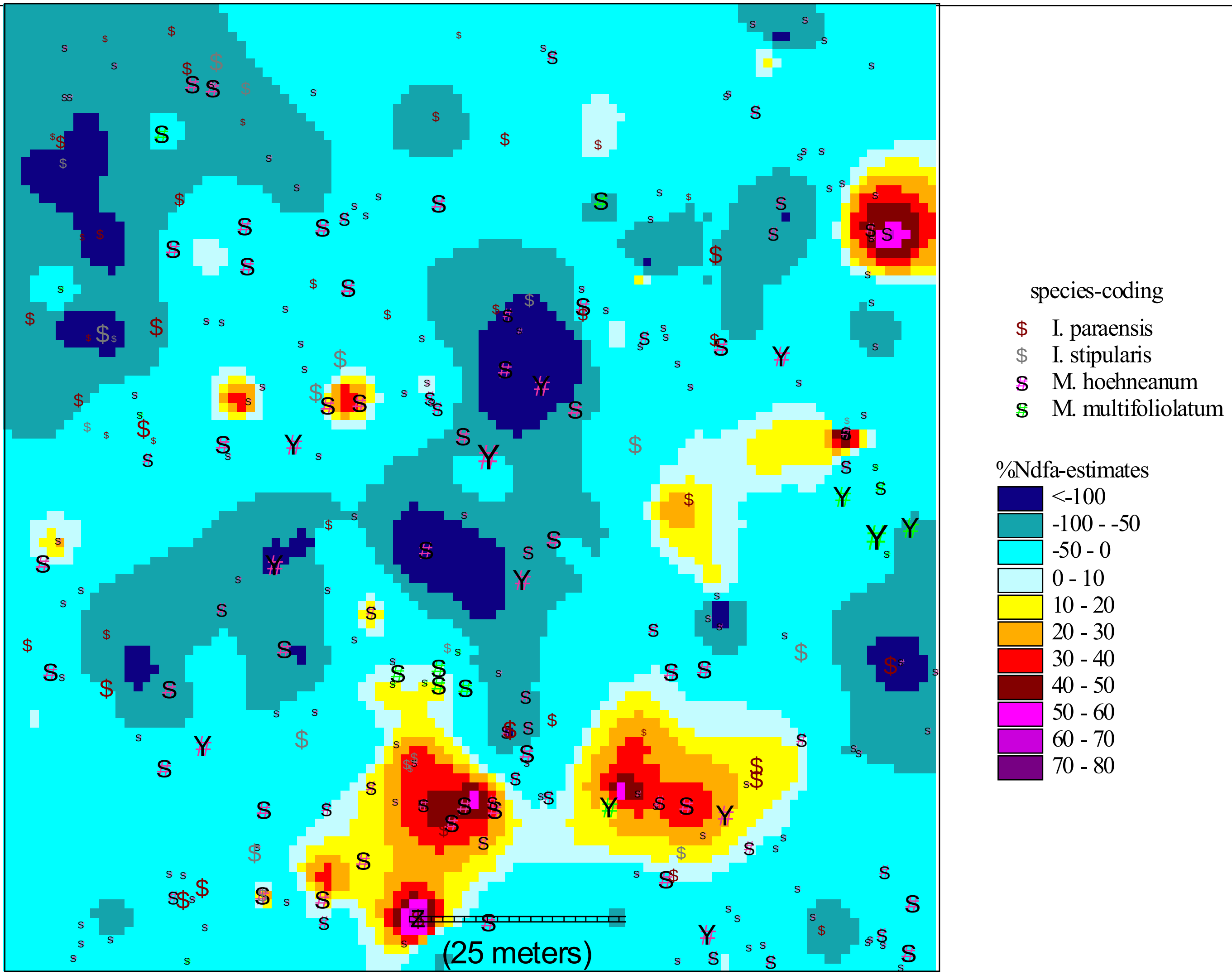
Clusters of the entirety of potential N_2-fixers are similarly often associated with positive and negative %Ndfa-deviations. Narrowing down by growth form and genus indicates a trend for potentially N_2-fixing trees and the tree genus *Inga* to be associated with low or negative %Ndfa-estimates, whereas potentially N_2-fixing lianas and the liana genus *Machaerium* appear to be associated with high %Ndfa-estimates. Further narrowing down to the species level increases the complexity of such patterns, since species-specific differences within these genera then become apparent. Whereas *I. stipularis* and *I. alba* appear to be associated with low or negative %Ndfa-estimates, both negative and positive associations exist for *I. paraensis*. Within the genus *Machaerium*, *M. hoehneanum* appears mainly associated with high %Ndfa-estimates, whereas *M. multifoliolatum* coincides with negative %Ndfa-estimates.



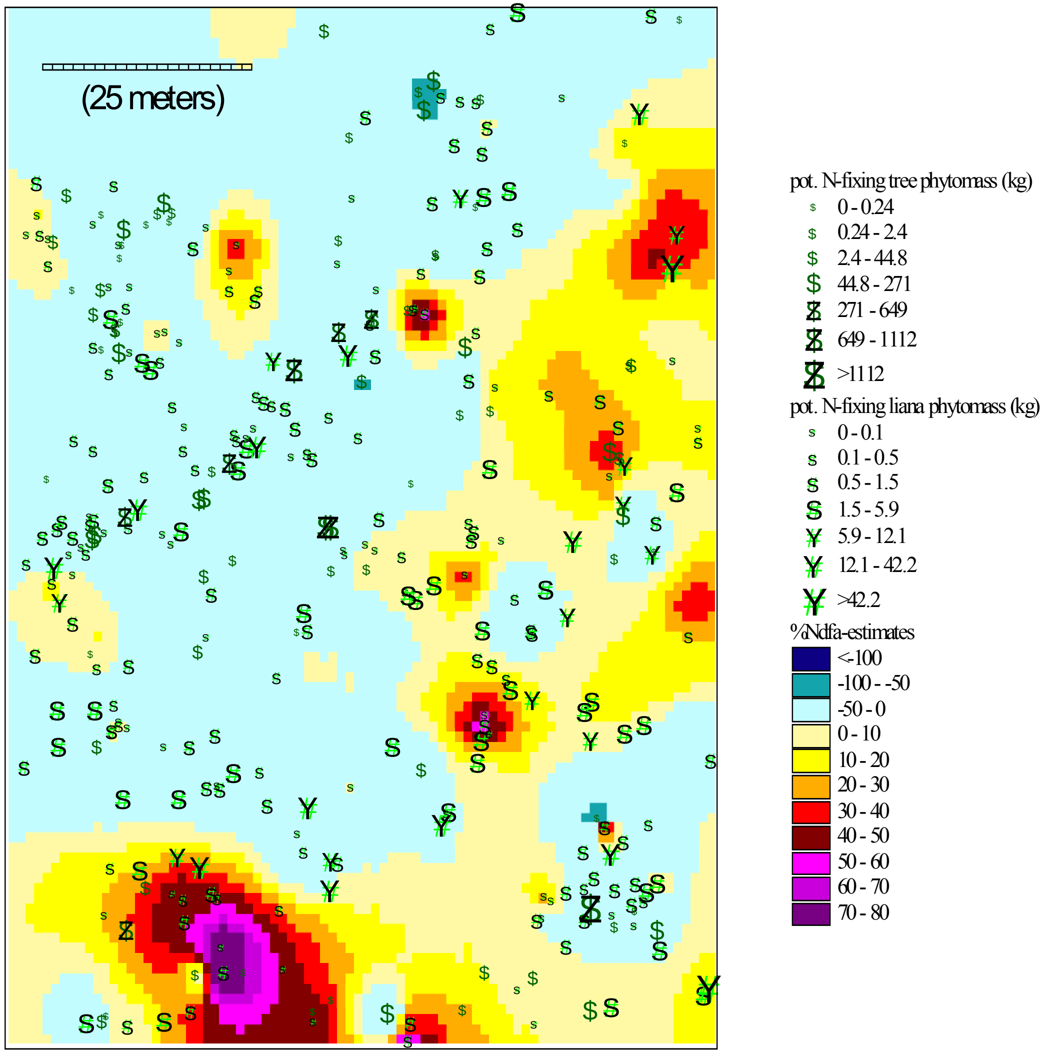
Map 32. 7-yr.-old regrowth: interpolated %Ndfa-estimates and the distribution of potentially N_2-fixing trees (green triangles) and lianas (green circles); phytomass is scaled in seven percentile levels.



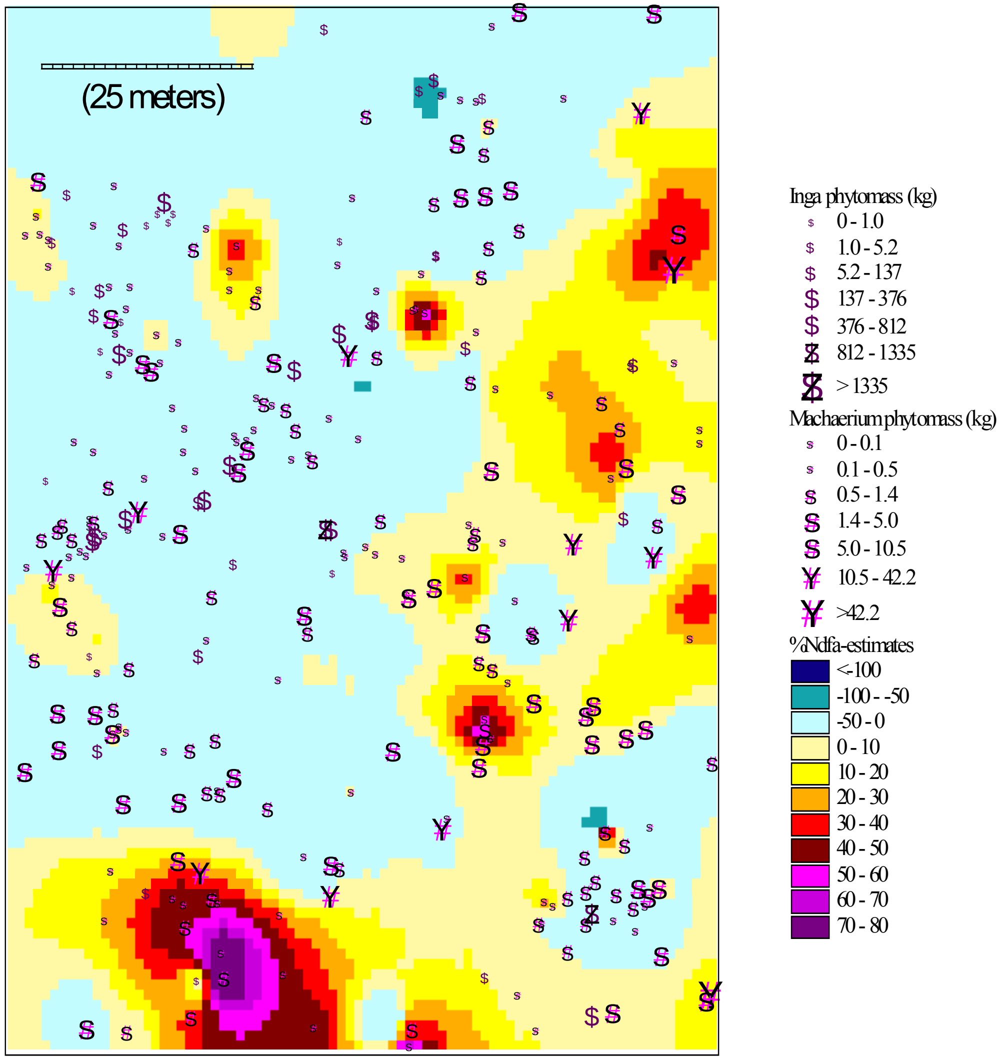
Map 33. 7-yr.-old regrowth: interpolated %Ndfa-estimates and the distribution of *Inga* spp. (purple triangles) and *Machaerium* spp. (pink circles); phytomass is scaled in seven percentile levels.



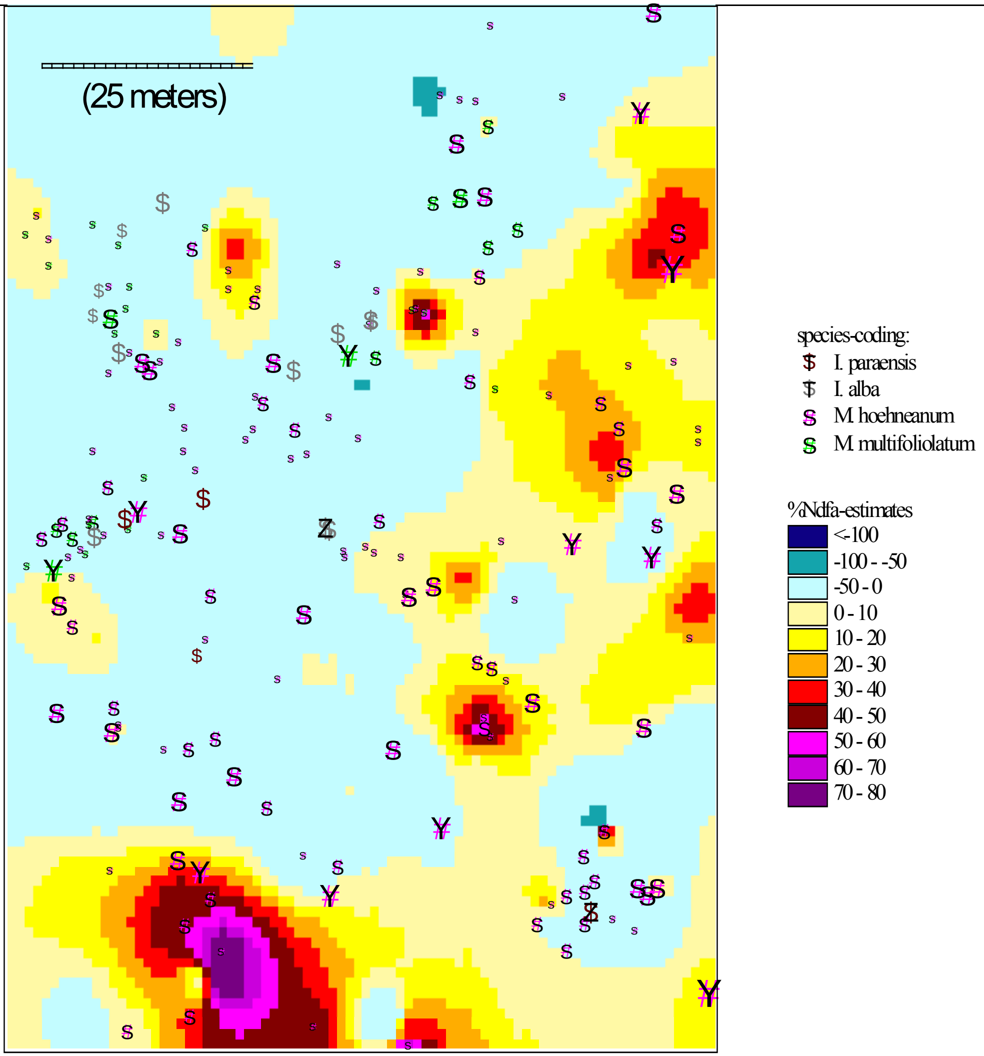
Map 34. 7-yr.-old regrowth: interpolated %Ndfa-estimates and the distribution of the two main *Inga*- and *Machaerium*-species (see legend for species-coding; phytomass is scaled in seven percentile levels of *Inga* spp. and *Machaerium* spp. as in Map 33).



Map 35. 20-yr.-old regrowth: interpolated %Ndfa-estimates and the distribution of potentially N_2-fixing trees (green triangles) and lianas (green circles); phytomass is scaled in seven percentile levels.



Map 36. 20-yr.-old regrowth: interpolated %Ndfa-estimates and the distribution of *Inga* spp. (purple triangles) and *Machaerium* spp. (pink circles); phytomass is scaled in seven percentile levels.



Map 37. 20-yr.-old regrowth: interpolated %Ndfa-estimates and the distribution of the two main *Inga*- and *Machaerium*-species (see legend for species-coding; phytomass is scaled in seven percentile levels of *Inga* spp. and *Machaerium* spp. as in Map 36).

Maps 18 and 20 assess the possible impact of potentially N_2-fixing legumes on the δ^{15}N-signal of soil-derived nitrogen (the 'δ^{15}N dilution problem') by comparing the distribution patterns of potential N_2-fixers with the interpolated non-legume reference δ^{15}N-signals. Whereas clusters of potentially N_2-fixing legumes appear to be associated with low reference δ^{15}N-signals in the 20-yr.-old site, no relationships are apparent in the 7-yr.-old site. Site-by-site comparisons between interpolated reference δ^{15}N-signals and plant distribution patterns reveal complex relationships, with both positive and negative associations apparent for legume and non-legume species groups, genera, and single species (maps not shown). Conclusions concerning single-species effects both on the interpolated δ^{15}N-signals and %Ndfa-estimates are limited, since these effects may be caused by a wide range of unknown factors, or just be chance-relationships.

5.8.3 Summary and conclusions

As outlined in chap. 5.7, the ^{15}N natural abundance method fails in providing secure %Ndfa-estimates in absolute terms. Spatial analysis of the isotopic data does not reduce this problem; in large parts of the investigated sites the estimates are, therefore, invalid (blue = negative). If the estimates do provide a relative assessment of BNF, the spatial perspective provided in this section allows the following two conclusions:

(1) BNF is irregularly distributed and high %Ndfa-estimates are concentrated in locally well-defined 'hotspots', which cover relatively small areas of the sites. Such hotspots could create microsites with elevated N-inputs and N-turnover. This hypothesis is supported by the evidence derived from plot-based correlations, in which N-inputs in plots with high phytomass shares of potential N_2-fixers appear high enough to affect both the leaf litter δ^{15}N-signals (chap. 5.5.2) and the TAGP (chap. 5.4).

(2) Putatively N_2-fixing plants affect both the isotopic composition of the 'soil-derived' δ^{15}N-signal and the resulting %Ndfa-estimates in their surroundings. However, effects are very complex and appear unpredictable, since the signal varies between growth forms, genera and single species. The 'δ^{15}N dilution problem' occurs on some locations, but relationships likewise remain unpredictable.

The spatial patterns of δ^{15}N-signals and %Ndfa-estimates and the impact of legume plant distribution are further discussed in chap.6.4.

6 DISCUSSION

6.1 Rapid regrowth and the sustainability of slash-and-burn

This study investigates the fallow component of the slash-and-burn agroecosystem at a newly developing frontier of agricultural colonization. Fallow age is captured with the false-time-series approach (chapters 4.2 and 4.9.1). Primary forest controls represent both the situation preceding human disturbance, and the theoretical endpoint of succession.

The present study is the first to systematically investigate secondary forest succession in central Amazonia, since research is mainly concentrated in the 'arc of deforestation' in eastern and southwestern Amazonia, in which deforestation problems are (so far) most severe. In contrast, research on primary forests is well represented in the Manaus region (i.e., at <100 km distance of this study).

Total aboveground phytomass in the primary forest sites used as controls gives an indication of the vegetation potential. The TAGP recorded in this study (460t ha^{-1}) and in all other studies in the region (Klinge et al., 1975; Laurance et al., 1999; Fearnside et al., 2001) are very high and well above phytomass records for most Amazonian primary forests (in the range of 250-350t ha^{-1}). Both climatic and soil-edaphic causes are responsible:

The climate of central and western Amazonia differs mainly in its weak expression of dry season (Figure 4.2). Raich et al. (1991) discuss the impacts of the duration and severity of the dry season on TAGP. This is probably the explanation for the lower TAGP recorded in semidecidous forests of peripheral Amazonia (e.g., Fölster et al., 1976; Uhl et al., 1988; Alves et al., 1997; Salomão et al., 1998; Gerwing and Farias, 2000). Brown and Lugo (1992) and Fearnside (1992) classify those forests as 'not dense'. Low phytomass in southern Venezuelan primary forests (Saldarriaga et al., 1988; Jordan, 1989) is due to the oligotrophic nature of that region, which is located on the precambrian Guiana shield. The present study confirms the impact of soil fertility on primary forest phytomass, recording 25% lower TAGP in the two 'campinarana' forests on sandy Ultisol.

Phytomass accumulation during secondary regrowth is in the form of a saturation curve with extremely rapid initial increments. For young and mid-aged (i.e., up to

10 yr.-old) regrowth, accumulation is substantially above the linear rates reported by Uhl (1987), Fearnside and Guimarães (1996), Alves et al. (1997), Hughes et al. (1999), and Steininger (2000). Next to methodological issues (chap. 4.9.3) and the elevated vegetation potential of central and possibly western Amazonia (outlined above), this is also due to the nature of first-cycle slash-and-burn as the lightest conceivable form of preceding land use. More research is required to distinguish between regional and land use related factors.

In their carbon balancing for Amazonia, Houghton et al. (2000) assume three levels of phytomass recovery, with 'high' rates of 5.5t carbon (or 11t phytomass) increment per hectare and year. The present study suggests substantially faster initial recovery in central Amazonia, and calls for an adaptation of future carbon models.

The curvilinear form of phytomass accumulation implies a sinking cost:benefit relationship for slash-and-burn agriculture (i.e., accumulation of phytomass and restoration of site fertility with fallow time). Conversely, the rapid initial recuperation points to the benefits of maintaining minimum fallow periods. In terms of phytomass stocks, this agroecosystem recovers remarkably quickly from disturbance, and may, therefore, be judged as ecologically resilient.

Next to total phytomass stocks, this study provides data on a wide range of structural parameters of vegetation, summarized in chap. 5.1.6. Reapproximation to primary forest levels is interpreted as resilience, even though the biological significance, causes and consequences of the various parameters are unknown or speculative. The strong impact of a very moderate increase in land use intensity (two burns or extended cultivation phase) observed in the three 'degraded' sites on all structural parameters suggests that this agroecosystem may be more vulnerable to degradation than indicated by the fast phytomass recovery. Any management recommendation developed for slash-and-burn land use (e.g., evaluation of optimum fallow periods), which is merely based on the phytomass of secondary regrowth, may therefore be misleading.

Williams et al. (1998) position the pioneer tree genera *Cecropia* and *Vismia* at the two endpoints of 'colonization strategies'. The present study confirms this view, with large differences in structural parameters between these genera at all stages of secondary regrowth (Annex 4).

Both the decreasing rates of phytomass accumulation in late succession and the marked differences of all structural parameters in primary forest indicate that long-term recovery of regrowth is a process requiring centuries rather than decades.

Phytomass is a poor measure for the exceptionality of the 4-yr.-old regrowth on ‘terra morena do índio’ (TAGP merely 16% above average). The outstanding position of this site holds true for virtually all investigated parameters, notably the botanical composition, the vegetation structure, and the δ^{15}N-signals in litter and leaves (chap. 5.2.4). Whereas ‘terra preta do índio’ has become into a focus of research (Smith, 1980; Costa and Kern, 1999), investigations so far are lacking on the soil dynamics and the agronomic potential of these adjacent terra morena soils.

6.2 Legume composition as an indicator of BNF

The taxonomically defined capability to nodulate and biologically fix nitrogen is a key functional characteristic of legumes, which may provide indications on the role of BNF along succession. This appears to be the first systematic investigation on successional changes in the legume community of tropical forest regrowth. Chapter 5.2.2 shows large differences in legume species composition between all stages of secondary regrowth and primary forests: With shares of 86-91% in legume phytomass and abundance, species capable of N_2-fixation entirely dominate the legume community in all stages of secondary succession. In marked contrast, the vegetation share of potentially N_2-fixing species is substantially lower in primary forests (85% in abundance and only 57% in phytomass). Since of all 157 legume species identified in this study 78% were classified as potentially capable of BNF (chap. 4.3.1), the share of potential N_2-fixers in the primary forest legume community appears close to chance.

According to theory, primary forests are supposedly in a ‘state of equilibrium’ with tight N-cycling and low gains and losses of nitrogen (chap. 2.1). Consequently, the capability to nodulate and fix N_2 is not expected to confer sizeable competitive advantages in this ecosystem. In contrast, the dominance of potentially N_2-fixing legumes in secondary regrowth indicates a distinct competitive advantage for this plant characteristic. Taking legume species composition as an indirect evidence of the role of BNF, this infers that legume-BNF occurs in significant quantities in secondary regrowth and is low or absent in primary forests.

The botanical data presented here do not confirm the hypothesis of an elevated BNF in early or mid-succession (see chap. 2.4), they rather point to a fairly constant role of BNF throughout the 2-25 years successional time span investigated.

6.3 Potentially N_2-fixing lianas: a key functional group?

A relationship between high liana dominance and low TAGP appears to be general knowledge in the literature and is confirmed by this study (chap. 5.4.2). Lianas have been frequently reported to suppress tree growth or fecundity, and to increase tree mortality (Putz, 1983; Clark and Clark, 1990). This is attributed to their competitive strength mainly for light, growth space, and water. According to Schnitzer et al. (2000) and Laurance et al. (2001), liana dominance in initial regrowth can retard or even indefinitely arrest further forest succession.

The results of this study emphasize a second, so far insufficiently recognized aspect of lianas: their key role in BNF, notably during early succession. This view is based on both (1) legume vegetation data and (2) the results of foliar $\delta^{15}N$-analyses:

(1) Legume contributions to total phytomass and abundance are one order of magnitude higher for lianas than for trees throughout all stages of succession and in primary forests (chap. 5.2.1). The data on legume functional composition demonstrate a dominance of potentially N_2-fixing lianas, mainly in early stages of succession. In 2- to 3-yr.-old regrowth, they comprise more than 40% of total legume phytomass and more than 70% of legume abundance (chap. 5.2.2). Phytomass contributions of potentially N_2-fixing lianas decline along succession and they are low in primary forest controls (4% of legume phytomass and only 0.7% of TAGP).

(2) Lianas appear to be more efficient N_2-fixers than trees. Foliar $\delta^{15}N$-signals of potentially N_2-fixing lianas are about 0.5‰ lower than those of trees (chap. 5.6.1) and visual comparisons between legume plant distributions and overlaid %Ndfa-interpolations indicate a predominantly positive impact of potentially N_2-fixing lianas on BNF, whereas potentially N_2-fixing trees show the opposite trend (chap. 5.8.2). The role of potentially N_2-fixing lianas on phytomass accumulation appears ambivalent, with positive effects (facilitation via BNF) offset by negative effects (competitive inhibition of the surrounding vegetation), see chap. 5.4.2.

Liana participation in BNF of primary forests is probably more prominent than suggested by their low phytomass shares. In contrast to potentially N_2-fixing trees, the $\delta^{15}N$-signals of potentially N_2-fixing lianas are even lower in primary forests than in secondary regrowth (chap. 5.6.1). Foliar N-concentrations give a further indication of the prominent role of lianas for primary forest BNF: whereas potentially N_2-fixing lianas are significantly higher in their N-concentrations than non N_2-fixing legume lianas, no differences are apparent for legume trees. Above-average liana-BNF probably relates to their efficiency in accessing light, which is presumably the main limiting resource for energy-intensive BNF in mature forests. Furthermore, physiological limitations of BNF in large-sized plants (chap. 5.6.3) are likely to have less impact on the BNF of lianas than on that of trees in primary forests, since plant size distribution is less skewed for lianas than for trees (chap. 5.1.5).

The participation of lianas in N-cycling is expected to be higher than their respective shares in legume phytomass and in BNF. This is because of their typically low wood:leaf-ratios (Putz, 1984; Gehring et al., submitted a), especially in the small lianas that dominate regrowth (chap. 5.1.5). Liana leaves apparently also have shorter life-spans (Hegarty, 1990) and decompose faster (Cornelissen, 1996) than tree leaves.

This study postulates that potentially N_2-fixing lianas constitute a ‘key functional group’ of species (sensu Körner, 1993) in tropical forest vegetation, notably in initial stages of successional regrowth. The genus *Machaerium* dominates this group in all stages of succession, and *M. hoehneanum* may be classified as a ‘keystone species’. Low shares of *Machaerium* spp. paired with high shares of *Derris* spp. on the three sites with different soils suggest that other species or genera can assume such a prominent position, depending on edaphic or regional variations. Liana-BNF may thus be a general phenomenon in tropical forest ecosystems, rather than be some local singularity of the study region.

The importance of potentially N_2-fixing lianas for re-establishing the N-cycle early along succession was first suggested by de Souza et al. (1994) and Reiners et al. (1994); the present study is the first to provide quantitative data. It recommends the inclusion of legume lianas in future BNF-research for a better evaluation of the role of this frequently overlooked vegetation component.

6.4 Spatial patterns of legumes and of BNF

Spatial distribution of tropical forest vegetation has so far been investigated in primary forest only. *Machaerium hoehneanum* shows a clear successional trend, beginning with strongly clustered distribution in initial regrowth and becoming randomly distributed in further succession (chap. 5.3.2). More research on other species appears warranted, in order to evaluate whether systematic changes in spatial organization are a general phenomenon of successional regrowth. This could open up fascinating perspectives for spatial and successional ecology; spatial organization may also prove to be a sensitive indicator of the successional status of vegetation (chap. 5.1.6).

Several studies report on the spatial distribution of selected primary forest tree species in Amazonia, conducted either for abundant species (Sterner et al., 1986) or on very large study sites (Condit et al., 1994; Forget et al., 1999). Condit et al. (2000) compile data on the distribution patterns of tropical primary forest trees in large-scale inventories worldwide (six sites ranging from 25-52ha). Strong clustering of *M. hoehneanum* in primary forests is in accordance with the results of the above-mentioned studies, in which almost all species were found to be clustered to some extent.

Research on successional changes of distribution patterns is scarce, though some research has been done on successional changes in the spatial patterns of other plant communities, e.g., in semiarid bushland in Spain (Carreira et al., 1992) or mine spoils in Germany (Felinks, 2000). However, spatial and temporal scales and statistical methods differ widely from this study, thus not allowing comparisons.

Whereas spatial organization of vegetation occurs on the species level, this study demonstrates that 'potentially N_2-fixing legumes' in their entirety are likewise aggregated in clusters in all stages of succession as well as in primary forests. This gives room to the hypothesis that BNF does not occur uniformly in vegetation. Interpolation of %Ndfa-estimates confirm such non-uniform distribution patterns and BNF indeed appears to be locally concentrated in 'hotspots' (chap. 5.8.1). The results of plot-based correlations indicate that BNF-input in such hotspots is high enough to affect the $\delta^{15}N$-signals of leaf litter (chap. 5.5.2) and ultimately to increase TAGP (chap. 5.4). Thus, clusters of N_2-fixing vegetation form important microsites and increase the environmental heterogeneity in secondary regrowth.

6.5 Validity of the ^{15}N natural abundance method for BNF-estimation

According to Högberg (1997), the ^{15}N natural abundance method requires a minimum 5‰ difference between the mean δ^{15}N-signals of putative N_2-fixers and paired reference plants for secure BNF-estimates. Such difference is not attained in any site or site class for any mode of %Ndfa-estimation. Consequently, all estimations encounter the overriding problem of large portions of invalid %Ndfa-estimates. Notably, the cutoff point at 0% Ndfa is problematic, as it is located close to the peak of data distribution and thus constitutes an artificial data boundary (Figures 5.16 and 5.17). The valid median %Ndfa-estimates do not differ systematically between secondary and primary forests and thus do not accompany the qualitative evidence gained in this study on a sizeable role of BNF in secondary regrowth as opposed to low or absent BNF in primary forests (see chapters 5.2.2, 5.4 and 5.5.3). The ^{15}N natural abundance method, therefore, fails as a quantitative method of estimating BNF in this study.

However, the investigated %Ndfa estimation methods differ in their degree of plausibility. The main difference is in the type of reference species representing the soil-derived δ^{15}N-signal, whereas the influence of the manner of individual plant pairing on the %Ndfa-estimates is much lower:

The mean %Ndfa-estimate obtained with non-legume reference species is close to zero and the portion of negative estimates is close to 50%. The estimates thus appear randomly distributed and, therefore, meaningless. In marked contrast, the use of non N_2-fixing legumes as reference results in a positive mean (+17% Ndfa) and a significantly lower portion of negative estimates (19% of cases). Systematically higher foliar δ^{15}N-signals and resulting higher %Ndfa-estimates of non N_2-fixing legumes as compared to non-legume references have also been reported by Sprent et al. (1996) in Brazilian cerrado vegetation, suggesting that this may be a general phenomenon of non N_2-fixing legume species. An argument in favor of non N_2-fixing legumes over other plant families as reference species is their taxonomic proximity:

Foliar N-concentrations are systematically higher in legumes than in non-legumes, an observation almost general in the literature and a characteristic of this plant family (chap. 2.2.1). Only part of this difference may be explained by BNF, since non N_2-fixing legumes (though lower in %N than potential N_2-fixers) are likewise higher in %N than non-legume species (chap. 5.6.1). A further distinction between legumes and non-legumes is the absence of correlations between foliar %N and $\delta^{15}N$ for potentially N_2-fixing as well as non N_2-fixing legume species, which is in contrast to most non-legume reference species with significant positive correlations. Such differences suggest that legumes differ from other plant families in some unknown, but isotopically relevant physiological aspect. Though almost all non-nodulating legumes have been shown to likewise fix N_2 (i.e., to reduce acetylene), N_2-fixation rates are very low and thus irrelevant as a source of error (Bryan et al., 1996). Non N_2-fixing legume species may thus be preferential candidates as reference species for future ^{15}N natural abundance studies. More research on this topic appears warranted.

The effect of the plant pairing method on the %Ndfa-estimates is only moderate, and the assumption of an overall mean site reference $\delta^{15}N$-signal appears sufficient (chap. 5.7.2). The nearest neighbor method resulted in more invalid %Ndfa-estimates and in the occurrence of negative extremes, $\delta^{15}N$ dilution (chap. 6.6) may be partly responsible for this. Pairing putative N_2-fixers and reference plants by classes of 'N_2-fixer influence zones' or by plant size-classes does not significantly improve the %Ndfa-estimates.

6.6 The '$\delta^{15}N$ dilution problem'

The '$\delta^{15}N$ dilution problem' expresses the possibility that N_2-fixing vegetation reduces the isotopic signal of the soil N-pools in its surroundings (chap. 2.5.2). This requires significant nitrogen inputs via leaf and root litter with low $\delta^{15}N$-signals, resulting out of the combination of significant BNF and 'leakage' of fixed N_2 (i.e., incomplete N-recovery before senescence). If not accounted for, the $\delta^{15}N$-dilution problem forms a systematic source of error for the ^{15}N natural abundance method. From a practical viewpoint, the question arises whether it is better to select the reference plants in direct vicinity of the putative N_2-fixer (reducing spatial variability of soil-N) or rather well beyond the reach of the supposed N_2-fixer (thus avoiding such $\delta^{15}N$ dilution).

This study shows the phytomass of potentially N_2-fixing legumes to be aggregated in clusters (chap. 5.3.1), and %Ndfa-estimates to be concentrated in hotspots (chap. 5.8.1) throughout all stages of succession. Consequently, $\delta^{15}N$ dilution of soil-derived nitrogen in the surroundings of N_2-fixing legumes may indeed become a locally significant problem for estimating BNF with the ^{15}N natural abundance method.

In first-cycle regrowth, high phytomass contributions of potentially N_2-fixing legumes significantly reduce the $\delta^{15}N$-signals of leaf litter in their surroundings (chap. 5.5.2), thus providing evidence on the existence of the $\delta^{15}N$ dilution problem. In contrast, potentially N_2-fixing legumes apparently do not affect the isotopic signature of 'plant-available' (rice-extracted) topsoil-N (chap. 5.5.3). Next to the methodological uncertainties of rice extraction, it is also conceivable that the 'leakage' of fixed N_2 into an N-limited system primarily affects less labile N-pools. In a ^{15}N-marker experiment, Kaye et al. (2002) did not detect any impacts of N_2-fixers (*Leucaena* and *Casuarina*) on 'labile N-pools' in their surroundings, but rather on the 'non-labile' soil-N fraction.

Whereas the foliar N-concentrations of reference plants were moderately (about 0.2%) higher in plants growing in the direct vicinity of potentially N_2-fixing legume clusters than those in plants growing outside areas possibly influenced by N_2-fixation, the $\delta^{15}N$-signals did not differ systematically between these locations. Consequently, the %Ndfa-estimates derived from plant pairings by 'N_2-fixer influence zones' differ only marginally from estimates based on the overall reference $\delta^{15}N$ site means (chap. 5.7.2). On the other hand, the $\delta^{15}N$ dilution problem may have contributed to the altogether lower %Ndfa-estimates obtained by the nearest neighbor method. Visual assessment of the impact of potential N_2-fixers on interpolated reference $\delta^{15}N$-signals does not allow definite conclusions, since the patterns encountered in different sites and species are very complex and remain unpredictable (chap. 5.8.2).

6.7 BNF along succession

The present study compiles different lines of evidence on the role of BNF in secondary forest succession as compared to primary forest controls. Evidence ranges from taxonomic and vegetation data to the results obtained by $\delta^{15}N$-analysis:

Legume species composition provides strong evidence on BNF in secondary regrowth, as species capable of N_2-fixation combine 86-96% of both legume phytomass and abundance throughout succession (chapters 5.2.2 and 6.2). Furthermore, plot-based correlations demonstrate that high phytomass contributions of potential N_2-fixers are associated both with elevated TAGP (chap. 5.4.1) and with reduced $\delta^{15}N$-signals of the leaf litter (chap. 5.5.2) in their surroundings.

The results of the present study do not support the hypothesis of a BNF maximum in early or mid-succession (chap. 2.4). No successional trend of BNF is apparent for any of the above-outlined BNF indicators along first-cycle succession. BNF rather appears to occur at fairly constant rates throughout secondary regrowth. Thus, BNF rates in secondary regrowth do not provide arguments for recommending minimum or optimum fallow periods in this slash-and-burn agroecosystem.

Lower contributions of legumes to TAGP, lower percentages of potential N_2-fixers in legume vegetation, and the missing impact of these on both TAGP and the litter $\delta^{15}N$-signals in their surroundings all point to lower BNF in the ‘degraded’ sites. From an agronomic point of view, it seems important to investigate the underlying reasons for such reduction in BNF. In contrast, BNF is high in the 4 yr.-old exceptional regrowth growing on ‘terra morena do índio’, with elevated shares of potential N_2-fixers both in legume and in total vegetation, a strong impact of these on TAGP and on the litter $\delta^{15}N$-signals, high foliar N-concentrations, and low foliar $\delta^{15}N$-signals.

6.8 Do primary forests fix nitrogen?

The general consensus in the literature is that primary forests of the lowland humid tropics are not primarily N-limited and, therefore, do not fix N_2 in significant quantities (chap. 2.4). These forests supposedly maintain sufficiently high N-availability by avoiding major N-losses via tight N-cycling within the system. Vegetation potential (total phytomass) is rather limited by overriding P-availability constraints (Vitousek, 1984; Raaimakers et al., 1995), and ultimately also by photosynthetic energy (light) and by the atmospheric CO_2-pressure (Grace et al., 1996; Chambers et al., 2001).

However, Laurance et al. (1999) found a significant relationship between soil nitrogen and TAGP in primary forests at approximately 80km from the present study area. Topsoil total N-content was the single most important soil variable, explaining 38% of TAGP-variation (the absent P-effects were probably due to the method of P-extraction employed by that study). The significantly lower leaf litter N-concentrations in old regrowth and primary forests than in young and mid-aged regrowth observed in the present study (chap. 5.5.1) likewise point to a high N-use efficiency and thus to a low N-availability in old-growth forests.

In primary forests, N-cycling is probably not free of losses out of the system. Due to excess humidity, N-loss via denitrification increases in late secondary succession and is high in primary forests (Groffman, 1995: 13kg N ha^{-1} yr^{-1} in a Costa Rican primary forest). Nitrate peaks in the subsoil under central Amazonian primary forests also indicate N-losses via leaching, though part of this nitrogen may subsequently be recovered by nitrate pumping (Schroth et al., 1999; Lehmann et al., in preparation).

All BNF indicators investigated in the present study appear to confirm low or negligible BNF in primary forests, as opposed to the constant BNF rates occurring throughout secondary regrowth. Primary forest legumes have low phytomass shares of potential N_2-fixers, which is interpreted as an absence of competitive advantages conferred by the ability to fix N_2 (chap. 6.2). Contrary to secondary regrowth, potential N_2-fixers do not affect TAGP (chap. 5.4.1), nor do they influence the $\delta^{15}N$-signals of leaf litter in their surroundings (chap. 5.5.2).

In contrast to primary forests on clayey Oxisol, several studies report on frequent nodulation in campinarana forests (Magalhães et al., 1982; Moreira and Franco, 1994). The present study confirms such observations, showing low $\delta^{15}N$-signals of plant-available (rice-extracted) nitrogen in the topsoil (chap. 5.5.3), lower leaf litter δ^{15}N-signals and higher litter N-concentrations (chap. 5.5.1), and higher phytomass shares of legume species capable of N_2-fixation (chap. 5.2.2) than in primary forests on clayey Oxisol. Furthermore, the phytomass shares of potential N_2-fixers (non-significant) tend to be positively associated with TAGP (chap. 5.4.1), and negatively with the litter δ^{15}N-signals (chap. 5.5.2) in campinarana forests as opposed to the primary forests on clayey Oxisol.

Individual plant size is positively correlated with the foliar δ^{15}N-signals of potential N_2-fixers (chap. 5.6.3). This effect is mainly limited to primary forests, where tree ‘giants’ dominate legume phytomass (chapters 5.1.5. and 5.2.2). In these forests, trees with more than one ton of individual phytomass comprise 84% of all potentially N_2-fixing phytomass, and trees with more than 10 tons 40%. This supports the hypothesis that BNF may be physiologically limited in mature forests. Field observations by Norris (1969) reforce this hypothesis, reporting on frequent nodulation in juvenile trees as opposed to low or absent nodulation in large primary forest trees. Low BNF could thus be caused by the inability of large trees to fix N_2, rather than by an absence of N-limitation. The extent of such physiological limits of primary forest BNF warrants future research, since the consequences will be far-reaching for our ecological understanding.

So far, research on legume BNF in tropical forests has been limited to trees (Högberg and Alexander, 1995; Thielen-Klinge, 1997; Högberg and Wester, 1998; Roggy et al., 1999a+b). However, such limitation may be misleading because primary forest BNF possibly occurs to a large extent in non-tree vegetation compartments. The possible role of lianas as agents of BNF in primary forests was discussed in chap. 6.3. Next to lianas, N-inputs by non-legume BNF may also be high. Especially palms could be important agents of BNF in these palm-rich forests, either directly (via associative BNF) or indirectly (stems heavily laden with mosses).

7 CONCLUSIONS

The following conclusions are drawn from this research-project:

1) Biological Nitrogen Fixation occurs in significant amounts at all stages of secondary forest regrowth following first-cycle slash-and-burn agriculture in central Amazonia. The importance of BNF appears constant throughout the 2 to 25 years time span covered by this study. In contrast, BNF is low or absent in primary forests. Evidence is based mainly on the legume species composition, and on the impact of legumes on total phytomass stocks and on the leaf litter δ^{15}N-signals.

2) N_2-fixing lianas play a key role in restoring N-stocks especially in young secondary regrowth. Physiological limitations are partly responsible for the low or absent BNF in large primary forest tree legumes, BNF possibly occurs mainly in other vegetation-compartments.

3) Potentially N_2-fixing vegetation is spatially aggregated in clusters at all stages of succession. Plant distribution affects the spatial patterns of δ^{15}N-signals and %Ndfa-estimates, but relationships are complex and unpredictable. BNF appears to be concentrated in relatively small ‘hotspots’, thereby creating microsites with elevated N-availability.

4) The ^{15}N natural abundance method fails as a quantitative method of BNF-estimation in these forests, since large portions of estimates are invalid in all estimation-scenarios. Our knowledge on the multitude of mechanisms and interaction-patterns remains insufficient. Non N_2-fixing legume species are to be preferred as reference in future research, and the problem of δ^{15}N-dilution in the surroundings of N_2-fixing legumes needs to be envisaged as a systematic source of error.

8 REFERENCES

Abbadie L, Mariotti A, and Menaut JC (1992) Independence of savanna grasses from soil organic matter for their nitrogen supply. Ecology 73(2): 608-613

Allen ON and Allen EK (1981) The Leguminosae: a source book of characteristics, uses and nodulation. University Wisconsin Press, Madison, WI

Allen EB and Allen MF (1990) The mediation of competition by mycorrhizae in successional and patchy environments. In: Grace JB and Tilman D (eds) Perspectives on competition. pp 367-368 Academic Press, San Diego

Alvarez-Buylla ER and Martinez-Ramos M (1992) Demography and allometry of *Cecropia obtusifolia*, a neotropical pioneer tree - an evaluation of the climax - pioneer paradigm for tropical rain forests. J Ecol 80: 275-290

Alves DS, Soares JV, Amaral S, Mello EMK, Almeida SAS, da Silva OF and Silveira AM (1997) Biomass of primary and secondary vegetation in Rondônia, Western Brazilian Amazon. Glob Change Biol 3: 451-461

Anderson AB (1981) White sand vegetation of Brazilian Amazonia. Biotropica 13(3): 199-210

Araújo TM, Higuchi N and Andrade JC (1999) Comparison of formulae for biomass content determination in a tropical rain forest site in the state of Pará, Brazil. Forest Ecol Manage 117: 43-52

Attiwill PM and Leeper GW (1987) Forest soils and nutrient cycles. Melbourne University Press, Melbourne

Baldani JI, Caruso L, Baldani VLD, Goi SR and Döbereiner J (1997) Recent advances in BNF with non-legume plants. Soil Biol Biochem 29(5/6): 911-922

Barea JM, Azcon-Aguilar C and Azcon R (1991) The role of vesicular-arbuscular mycorrhizae in improving plant N-acquisition from soil as assessed with ^{15}N. In: Stable isotopes in plant nutrition, soil fertility and environmental studies. Roc. Int. Symp. Vienna, Oct. 1-5, 1990 pp 209-216 IAEA and FAO, Vienna

Beck DP and Munns DN (1984) Phosphate nutrition of *Rhizobium spp*. Appl Env Microb 47(2): 278-282

Bentley BL and Carpenter EJ (1984) Direct transfer of newly-fixed nitrogen from free-living epiphyllous microorganisms to their host plant. Oecologia 63: 52-56

Berg CC (1978) Espécies de *Cecropia* da Amazônia Brasileira. Acta Amazonica 8(2): 149-182

Binkley D, Sollins P and McGill WB (1985) Natural abundance of nitrogen-15 as a tool for tracing alder-fixed nitrogen. Soil Sci Soc Am J 49: 444-447

Boddey RM, de Oliveira OC, Alves BJR and Urquiaga S (1995) Field application of the ^{15}N isotope dilution technique for the reliable quantification of plant-associated biological nitrogen fixation. Fert Res 42: 77-87

Boddey RM, Peoples MB, Palmer B and Dart P (2000) Use of the ^{15}N natural abundance technique to quantify biological Nitrogen fixtion by woody perennials. Nutr Cycl Agroecosyst 57: 235-270

Bothe H, Yates MG and Cannon FC (1983) The Nitrogen fixing organism and the nitrogenase reaction. In: Lauchli A and Bieleski RL (eds) Encyclopedia of plant physiology. pp 241-285 New series Vol. 15A. Springer, Berlin.

Burris RH and Roberts GP (1993) Biological nitrogen fixation. Ann Rev Nutrition 13: 317-335.

Bravard S and Righi D (1989) Geochemical differences in an Oxisol - Spodosol toposequence of Amazonia, Brazil. Geoderma 44: 29-42

Brito VM and de Souza LAG (1997) Isolamento e caracterização morfológica de rizóbios obtidos de leguminosas nativas da Amazônia. In: Jornada de iniciação científica do INPA pp 227-230. INPA, Manaus, Brazil.

Brokaw NVL (1985) Gap-phase regeneration in a tropical forest. Ecology 66(3): 682-687

Brown IF, Martinelli LA, Thomas WW, Moreira MZ, Cid Fereira CA and Victoria RA (1995) Uncertainty in the biomass of Amazonian forests: an example from Rondônia, Brazil. Forest Ecol Manage 75: 175-189

Brown S and Lugo AE (1990) Tropical secondary forests. J Trop Ecol 6: 1-32

Brown S and Lugo AE (1992) Aboveground biomass estimates for tropical moist forests of the Brazilian Amazon. Interciencia 17: 8-18

Bryan JA, Berlyn GP and Gordon JC (1996) Toward a new concept of the evolution of symbiotic nitrogen fixation in the Leguminosae. Plant Soil 186: 151-159

Carpenter EJ (1992) Nitrogen fixation in the epiphyllae and root nodules of trees in the lowland tropical rainforest of Costa Rica. Acta Oecologica 13(2): 153-160

Carreira JA, Sanchez-Vazquez F and Niell FX (1992) Short-term and small-scale patterns of post-fire regeneration in a semi-arid dolomitic basin of southern Spain. Acta Oecologica 13(3): 241-253

Carrière SM, André M, Letourmy P, Olivier I and McKey DB (2002) Seed rain beneeth remnant trees in a slash-and-burn agricultural system in southern Cameroon. J Trop Ecol 18: 353-374

Cerri CC, Volkoff B and Andreaux F (1991) Nature and behaviour of organic matter in soils under natural forest, and after deforestation, burning and cultivation, near Manaus. Forest Ecol Manage 38: 247-257

Chalk PM (2000) Integrated effects of mineral nutrition on legume performance. Soil Biol Biochem 32: 577-579

Chambers JQ, Higuchi N, Schimel JP, Ferreira LV and Melack JM. (2000) Decomposition and carbon cycling of dead trees in tropical forests of the central Amazon. Oecologia 122: 380-388

Chapin FS and Bloom AJ (1987) Plant response to multiple environmental factors. BioScience 37(1): 49-57

Chapin FS, Moilanen L and Kielland K. (1993) Preferential use of organic nitrogen for growth by a non-mycorrhizal arctic sedge. Nature 361: 150-153

Chauvel A, Lucas Y, and Boulet R (1987) On the genesis of the soil mantle of the region of Manaus, Central Amazonia, Brazil. Experientia 43: 234-241

Christiansen-Weniger C (1991) Influence of *Azospirillum spp.* on the nitrogen supply of a gramineous host. PhD thesis, Landbouwuniversiteit Wageningen, Netherlands

Cintra R and Horna V (1997) Seed and seedling survival of the palm *Astrocaryum murumuru* and the legume tree *Dipteryx micrantha* in gaps in Amazonian forest. J Trop Ecol 13: 257-277

Clark DB and Clark DA (1990) Distribution and effects on tree growth of lianas and woody hemiepiphytes in a Costa Rican wet forest. J Trop Ecol 6: 321-331

Clark DB and Clark DA (2000) Landscape-scale variation in forest structure and biomass in a tropical rain forest. Forest Ecol Manage 137: 185-198

Cole CV and Heil RD (1981) Phosphorus effects on terrestrial nitrogen cycling. Ecol Bulletins (Stockholm) 33: 363-374

Condit R, Hubbell SP and Foster RB (1994) Density dependence in two understory tree species in a neotropical forest. Ecology 75(3): 671-680

Condit R, Ashton PS, Baker P, Bunyavejchewin S, Gunatilleke S, Gunatilleke N, Hubbell SP, Foster RB, Itoh A, la Frankie JV, Lee HS, Losos E, Manokaran N, Sukumar R and Yamakura T (2000) Spatial patterns in the distribution of tropical tree species. Science 288: 1414-1418

Coomes DA and Grubb PJ (1996) Amazonian caatinga and related communities at La Esmeralda, Venezuela: forest structure, physiognomy and floristics, and control by soil factors. Vegetatio 122: 167-191

Cornelissen JH (1996) An experimental comparison of leaf decomposition rates in a wide range of temperate plant species and types. J Ecol 84: 573-582

Costa ML and Kern DC (1999) Geochemical signatures of archeological sites with black earth soils in the Amazon region. J Geochem Explor 66:369-385

Danso SKA, Bowen GD and Sanginga N (1992) Biological nitrogen fixation in trees in agro-ecosystems. Plant Soil 141: 177-196

de Oliveira AA and Mori SA (1999) A central Amazonian terra firme forest. I. High tree species richness on poor soils. Biodiv Conserv 8: 1219-1244

de Oliveira LA and Sylvester-Bradley R (1982) Effect of different Central Amazonian soils on growth, nodulation, and occurrence of N_2-fixing *Azospirillum* spp. in roots of some crop plants. Turrialba 32(4): 463-469

de Souza LAG and da Silva MF (1997) Estimativa atual da avaliação da habilidade nodulífera das Leguminosas da Amazônia associadas a rizóbios. Congresso Brasileiro de Ciência do Solo, Resumos Expandidos 26, Rio de Janeiro, Brazil

de Souza LAG, Silva MF and Moreira FW (1994) Capacidade de nodulação de cem leguminosas da Amazônia. Acta Amazonica 24(1-2): 9-18 de Souza LAG, da Silva MF and Moreira FMS (1997) Associações rizóbios-arbóreas na Amazônia. In: Noda H, de Souza LAG and Fonseca OJM (eds) Duas décadas de contribuições do INPA à pesquisa agronômica no trópico úmido. pp 193-219. INPA, Manaus, Brazil

dela Cruz RE, Manalo MQ, Aggangan NS and Tambalo JD (1988) Growth of three legume trees inoculated with VA mycorrhizal fungi and *Rhizobium*. Plant Soil 108: 111-115

Delaney M, Brown S, Lugo AE, Torres-Lezama A and Quintero NB (1997) The distribution of organic carbon in major components of forests located in five life zones of Venezuela. J Trop Ecol 13: 697-708

Denich M (1989) Untersuchungen zur Bedeutung junger Sekundärvegetation für die Nutzungssystemproduktivität im östlichen Amazonasgebiet, Brasilien. Göttinger Beiträge zur Land- und Forstwirtschaft in den Tropen und Subtropen 46

Diggle PJ (1983) Statistical analysis of spatial point patterns. Academic Press, London

Doyle JJ (1994) Phylogeny of the legume family: an approach to understanding the origins of nodulation. Annu Rev Ecol Syst 25: 325-349

Faria SM, Lewis GP, Sprent JI and Sutherland JM (1989) Occurrence of nodulation in Leguminosae. New Phytologist 111: 607-619

Faria SM and Lima HC (1998) Additional studies of the nodulation status of legume species in Brazil. Plant Soil 200: 185-192

Fearnside PM (1992) Forest biomass in Brazilian Amazônia: coments on the estimate by Brown and Lugo. Interciencia 17(1): 19-27

Fearnside PM and Guimaraes WM (1996) Carbon uptake by secondary forests in Brazilian Amazonia. Forest Ecol Manage 80: 35-46

Fearnside PM, Alencastro Graça PML and Alves Rodrigues FJ (2001) Burning of Amazonian rainforests: burning efficiency and charcoal formation in forest cleared for cattle pasture near Manaus, Brazil. Forest Ecol Manage 146: 115-128

Felinks B (2000) Primärsukzession von Phytozönosen in der Niederlausitzer Bergbaufolgelandschaft. Diploma thesis, Fakultät für Umweltwissenschaften und Verfahrenstechnik, TU Cottbus, Germany

Forget P-M, Mercier F and Collinet F (1999) Spatial patterns of two rodent-dispersed rain forest trees *Carapa procera* (Meliaceae) and *Vouacapoua americana* (Caesalpiniaceae) at Paracou, French Guiana. J Trop Ecol 15: 301-313

Forman RTT and Hahn DC (1980) Spatial patterns of trees in a Carribean semievergreen forest. Ecology 61(6): 1267-1274

Fölster H, de las Salas G and Khanna PK (1976) A tropical evergreen forest site with perched water table, Magdalena valley, Columbia: Biomass and bioelement inventory of primary and secondary vegetation. Oecol Plant 11(4): 297-320

Francis R, Finlay RD and Read DJ (1986) Vesicular-arbuscular mycorrhiza in natural vegetation systems IV.: Transfer of nutrients in inter- and intra-specific combinations of host plants. New Phytologist 102: 103-111

Freiberg E (1998) Microclimatic parameters influencing nitrogen fixation in the phyllosphere in a Costa Rican premontane rain forest. Oecologia 17: 9-18

Fried M, Danso SKA and Zapata F (1983) The methodology of measurement of N_2 fixation of nonlegumes as inferred from field experiments with legumes. Can J Microb 29: 1053-1062

Galo AT, Rich PM and Ewel JJ (1992) Effects of forest edges on the solar radiation regime in a series of reconstructed tropical ecosystems. American Society for Photogrammetry and Remote Sensing, Annual Meeting. pp 98-108 Albuquerque, NM

Gebauer G (1991) Natural nitrogen isotope ratios in different compartments of Norway spruce from a healthy and a declining stand. In: Stable isotopes in plant nutrition, soil fertility and environmental studies. pp 131-138. IAEA and FAO, Vienna

Gebauer G and Dietrich P (1993) Nitrogen isotope ratios in different compartments of a mixed stand of spruce, larch and beech trees and of understory vegetation including fungi. Isotopenpraxis/Env Health Stud 29: 35-44.

Gehring C, Denich M, Kanashiro M and Vlek PLG (1999) Response of secondary vegetation in Eastern Amazonia to relaxed nutrient availability constraints. Biogeochemistry 45: 223-241

Gehring C, Park SJ and Denich M (submitted a) Liana allometric biomass equations for Amazonian primary and secondary forest. Forest Ecol Manage

Gehring C, Park SJ and Denich M (submitted b) Close relationship between diameters at 30cm and at breast height (dbh). Forest Ecol Manage

Gentry AH (1982) Patterns of neotropical plant species diversity. Evolut Biol 15: 1-84

Gentry AH and Dodson C (1987) Contribution of nontrees to species richness of a tropical rain forest. Biotropica 19(2): 149-156

Gerwing JJ and Farias DL (2000) Integrating liana abundance and forest stature into an estimate of total aboveground biomass for an eastern Amazonian forest. J Trop Ecol 16: 327-335

Getis A and Franklin J (1987) Second-order neighborhood analysis of mapped point patterns. Ecology 68(3): 473-477

Giller KE and Wilson KJ (1991) Nitrogen fixation in tropical cropping systems. CAB International, Wallingford, UK

Goosem S and Lamb D (1986) Measurements of phyllosphere nitrogen fixation in a tropical and two subtropical rain forests. J Trop Ecol 2: 373-376

Gorchov DL, Cornejo F, Ascorra C and Jaramillo M (1993) The role of seed dispersal in the natural regeneration of rain forest after strip-cutting in the Peruvian Amazon. Vegetatio 107/108: 339-349

Gorham E, Vitousek PM and Reiners WA (1979) The regulation of chemical budgets over the course of terrestrial ecosystem succession. Annu Rev Ecol Syst 10: 53-84

Grace J, Lloyd L, McIntyre L, Miranda AC, Meir P and Miranda HC (1996) Carbon dioxide flux over Amazonian rainforest in Rondônia. In: Gash HJC et al. (eds): Amazonian deforestation and climate. pp 307-318 John Wiley & Sons, Chichester.

Groffman PM (1995) A coneptual assessment of the importance of denitrification as a source of soil nitrogen loss in tropical agro-ecosystems. Fertilizer Research 42(1-3): 39-148

Grubb PJ (1977) Control of forest growth and distribution on wet tropical mountains with special reference to mineral nutrition. Ann Rev Ecol Syst 8: 83-107

Guehl JM, Domenach AM, Barigah TS, Casabianca H, Ferhi A and Garbaye J (1998) Functional diversity in an Amazonian rainforest of French Guyana: a dual isotope approach (δ^{15}N and δ^{13}C). Oecologia 116: 316-330

Guevara S, Purata SE and van der Maarel E (1986) The role of remnant forest trees in tropical secondary succession. Vegetatio 66: 77-84

Guillaumet J-L (1987) Some structural and floristic aspects of the forest. Experientia 43: 241-251

Haase P (1995): Spatial pattern analysis in ecology based on Ripley's K-function. J Veget Sci 6: 575-582

Halsall DM, Leigh JH, Gollasch SE and Holgate M (1995) The role of allelopathy in legume decline in pastures. II.: Comparative effects of pasture, crop and weed residues on germination, nodulation and root growth. Aust J Agric Res 46: 189-207

Hamill DN and Wright SJ (1986) Testing the dispersion of juveniles relative to adults: a new analytical method. Ecology 67(4): 952-957

Handley LL, Daft MJ, Wilson JB, Scrimgeour CM, Ingleby K and Sattar MA (1993) Effects of the ecto- and VA-mycorrhizal fungi *Hydnagium carneum* and *Glomus clarum* on the Delta^{15}N and Delta^{13}C values of *Eucalyptus globulus* and *Ricinus communis*. Plant Cell Environ 16: 375-382

Handley LL and Raven JA (1992) The use of natural abundance of nitrogen isotopes in plant physiology and ecology. Plant Cell Environ 15: 965-985

Handley LL and Scrimgeour CM (1997) Terrestrial plant ecology and ^{15}N natural abundance: the present limits to interpretation for uncultivated systems with original data from a Scottish old field. Adv Ecol Res 27: 133-212

Hansen AP and Pate JS (1987) Evaluation of the ^{15}N natural abundance method and xylem sap analysis for assessing N_2 fixation of understory legumes in Jarrah (*Eucalyptus marginata* DONN ex SM.) forest in S.W. Australia. J Exp Bot 38(194): 1446-1458

Hegarty EE (1990) Leaf life-span and leafing phenology of lianes and associated trees during a rainforest succession. J Ecol 78: 300-312

Herridge DF, Palmer B, Nurhayati DP and Peoples MB (1996) Evaluation of the xylem ureide method for measuring N_2 fixation in six tree legume species. Soil Biol Biochem 28(3): 281-289

Houghton RA, Skole DL, Nobre CA, Hackler JL, Lawrence KT and Chometowski WH (2000) Annual fluxes of carbon from deforestation and regrowth in the Brazilian Amazon. Nature 403: 301-304

Höfer H, Martius C and Beck L (1996) Decomposition in an Amazonian rain forest after experimental litter addition in small plots. Pedobiologia 40: 570-576

Högberg P (1986) Nitrogen-fixation and nutrient relations in savanna woodland trees (Tanzania). J Appl Ecol 23: 675-688

Högberg P and Alexander IJ (1995) Roles of root symbioses in African woodland and forest: evidence from ^{15}N abundance and foliar analysis. J Ecol 83: 217-224

Högberg P (1997) Tansley review N$^{o.}$ 95: ^{15}N natural abundance in soil-plant systems. New Phytologist 137: 179-203

Högberg P and Wester J (1998) Root biomass and symbioses in *Acacia mangium* replacing tropical forest after logging. Forest Ecol Manage 102: 333-338

Hölscher D, Moeller RF, Denich M and Fölster H (1997) Nutrient input-output budget of shifting agriculture in eastern Amazonia. Nutr Cycl Agroecosyst 47: 49-57

Hughes RF, Kauffman JB, and Jaramillo VJ (1999) Biomass, carbon, and nutrient dynamics of secondary forests in a humid tropical region of Mexico. Ecology 80(6): 1892-1907

Ishizuka J (1992) Trends in biological nitrogen fixation research and application. Plant Soil 141: 197-209

Israel DW (1987) Investigation of the role of phosphorus in symbiotic dinitrogen fixation. Plant Physiology 84: 835-840

Izaguirre-Mayoral ML, Vivas AI and Oropeza T (1995) New insights into the symbiotic performance of native tropical legumes: I.: Analysis of the response of thirtyseven native legume species to artificial shade in a neotropical savanna. Symbiosis 19: 111-129

Janos DP (1980) Mycorrhizae influence tropical succession. Biotropica 12: 54-64

Jordan CF (1989) An Amazonian rain forest - the structure and function of a nutrient stressed ecosystem and the impact of slash-and-burn agriculture. UNESCO & The Parthenon Publishing Group, MAB Series, Park Ridge, NJ

Kahn F and de Castro A (1985) The palm community in a forest of central Amazonia, Brazil. Biotropica 17(3): 210-216

Kauffman JB, Cummings DL, Ward DE and Babbitt R (1995) Fire in the Brazilian Amazon. I.: Biomass, nutrient pools, and losses in slashed primary forest. Oecologia 104: 397-408

Kaye JP, Binkley D, Zou X and Parrotta JA (2002) Non-labile soil ^{15}Nitrogen retention beneath three tree species in a tropical plantation. Soil Sci Soc Am J 66: 612-619

Kern J (1995) Die Bedeutung der N_2-Fixierung und der Denitrifikation für den Stickstoffhaushalt des amazonischen Überschwemmungssees Lago Camaleão. PhD thesis, Institut für Biologie, University of Hamburg, Germany

Ketterings QM, Coe R, van Noordwijk M, Ambagau Y and Palm CA (2001) Reducing uncertainty in the use of allometric biomass equations for predicting above-ground tree biomass in mixed secondary forests. Forest Ecol Manage 146: 199-209

Killingbeck KT (1996) Nutrients in senesced leaves: keys to the search for potential resorption and resorption proficiency. Ecology 77(6): 1716-1727

Kitayama K and Iwamoto K (2001) Patterns of natural ^{15}N abundance in the leaf-to-soil continuum of tropical rain forests differing in N availability on Mount Kinabalu, Borneo. Plant Soil 229: 203-212.

Klinge H, Rodrigues WA, Brunig E and Fittkau EJ (1975) Biomass and structure in a central Amazonian rain forest. In: Golley FB and Medina E (eds) Tropical ecological systems - trends in terrestrial and aquatic research. Springer, Berlin

Körner C (1993) Scaling from species to vegetation: the usefulness of functional groups. In: Schulze E-D and Mooney HA (eds) Biodiversity and ecosystem function. Springer, Berlin

Kreiblich H (2002) N_2 fixation and denitrification in a floodplain forest in Central Amazonia, Brazil. PhD thesis. Institut für Agrartchnik Bornim, University of Pottsdam, Germany

Lack AJ (1991) Dead logs as a substrate for rain forest trees in Dominica. J Trop Ecol 7: 401-405

Ladha JK, Peoples MB, Garrity DP, Capuno VT and Dart PJ (1993) Estimating dinitrogen fixation of hedgerow vegetation using the Nitrogen-15 natural abundance method. Soil Sci Soc Am J 57: 732-737

Laurance WF, Fearnside PM, Laurance SG, Delamonica P, Lovejoy TE, Rankin-de Merona JM, Chambers JQ and Gascon C. Relationship between soils and Amazon forest biomass: a landscape scale study (1999). Forest Ecol Manage 118: 127-138

Laurance WF, Pérez-Salicrup D, Delamonica P, Fearnside PM, d'Angelo S, Jerozolinski A, Pohl L and Lovejoy TE (2001) Rain forest fragmentation and the structure of Amazonian liana communities. Ecology 82(1): 105-116

Lehmann J, Trujillo L, da Silva LF, Gehring C, da Silva JP and Zech W (in preparation) Deep subsoil nitrogen dynamics in secondary forest successions of the central Amazon.

Lesak LFW and Melack JM (1991) The deposition, composition, and potential sources of major ionic solutes in rain of the central Amazon basin. Water Ressources Research 27(11): 2953-2977

Lindau CW and Spalding R F (1984) Major procedural discrepancies in soil extracted nitrate levels and nitrogen isotopic values. Ground Water 22(3): 273-278

Lodge DJ, McDowell WH and McSwiney CP (1994) The importance of nutrient pulses in tropical forests. TREE 9(10): 384-387

Luizão FJ and Schubart HOR (1987) Litter production and decomposition in a terra-firme forest of Central Amazonia. Experientia 43: 259-265

Lynch JM and Whipps JM (1990) Substrate flow in the rhizosphere. Plant Soil 129: 1-10

Mackensen J, Hölscher D, Klinge R and Fölster H (1996) Nutrient transfer to the atmosphere by burning of debris in eastern Amazonia. Forest Ecol Manage 86: 121-128

Magalhães FMM, Magalhães LMS, de Oliveira LA and Döbereiner J (1982) Ocorrência de nodulação em leguminosas florestais de terra firme nativas da região de Manaus - AM. Acta Amazonica 12(3): 509-514

Magalhães FMM (1986) O estado atual do conhecimento sobre fixação biológica de nitrogênio na Amazônia. In: 1. Simpósio do Trópico Úmido. pp 499-512 EMBRAPA-CPATU, Belém, Brasil

Magalhães FMM and da Silva MF (1986) Associações Rhizobium-leguminosas no estado de Rondônia. Acta Amazônica 16-17: 7-17

Martinelli LA, Victoria RL, Trivelin PCO, Devol AH and Richey JE (1992) ^{15}N natural abundance in plants of the Amazon river floodplain and potential atmospheric N_2 fixation. Oecologia 90: 591-596

McKey D (1994) Legumes and Nitrogen: the evolutionary ecology of a Nitrogen-demanding lifestile. In: Sprent JI, McKey D (eds) Advances in legume systematics 5: the Nitrogen factor. pp 211-228 Royal Botanic Gardens, Kew, UK.

Medina E and Cuevas E (1994) Mineral nutrition: humid tropical forests. Progress in Botany 55: 115-129

Mello Ivo W, Ferreira S, Biot Y and Ross S (1996) Nutrients in soil solution following selective logging of a humid tropical "*terra firme*" forest north of Manaus. Env Geochem Health 18: 69-75

Mesquita R, Ickes K, Ganade G and Williamson GB (2001) Alternative successional pathways in the Amazon basin. J Ecol 89: 528-537

Mitas L and Mitasova H (1988) General variational approach to the interpolation problem. Comput Math Applic 16(12): 983-992

Morais JL, Cerri CC, Melillo JM, Kicklighter D, Neill C, Skole DL and Steudler PA (1995) Soil carbon stocks of the Brazilian Amazon basin. Soil Sci Soc Am J 59: 244-247

Moreira FMS, Silva MF and Faria SM (1992) Occurrence of nodulation in legume species in the Amazon region of Brazil. New Phytologist 121: 563-570

Moreira FMS and da Silva MF (1993) Associação Rizóbio-Leguminosas na Amazonia - I. Boletín Museo Paraense Emílio Goeldi, sér. Botánica 9(2): 129-141

Moreira FMS, Magalhães LMS, da Silva MF, Moreira FW and Scarcello JA (1993) Associação Rizóbio-Leguminosas na Amazonia - II. Boletín Museo Paraense Emílio Goeldi, sér. Botánica 9(2): 267-277

Moreira FMS, da Silva MF, Moreira FW and de Souza LAG (1994) Associação rizóbio-leguminosas na Amazônia - III. Boletín Museo Paraense Emílio Goeldi, sér. Botánica 10(2): 295-302

Moreira FMS and Franco AA (1994) Rhizobia-host interactions in tropical ecosystems in Brazil. In: Sprent JI and McKey D (eds) Advances in legume systematics. Part 5. The Nitrogen factor. pp 63-74 Royal Botanic Gardens, Kew, UK.

Nadelhoffer KJ and Fry B (1988) Controls on natural nitrogen-15 and carbon-13 abundances in forest soil organic matter. Soil Sci Soc Am J 52: 1633-1640

Näsholm T, Nordin A, Giesler R, Högberg M and Högberg P (1998) Boreal forest plants take up organic nitrogen. Nature 392: 914-916

Nelson BW, Mesquita R, Pererira JLG, de Souza SGA, Batista GT, and Couto LB (1999) Allometric regressions for improved estimate of secondary forest biomass in the central Amazon. Forest Ecol Manage 117: 149-167

Newbery DC, Campbell EJF, Lee YF, Ridsdale CE and Still MJ (1992) Primary lowland dipterocarp forest at Danum Valley, Sabah, Malaysia: structure, relative abundance and family composition. Phil Trans Royal Soc, London, Sect.B 335: 341-356

Norris DO (1969) Observations on the nodulation status of rainforest leguminous species in Amazonia and Guyana. Tropical Agriculture (Trinidade) 46:145-151

Odum EP (1969) The strategy of ecosystem development. Science 164: 262-270

Otero-Arnaiz A, Castillo S, Meave J and Ibarra-Manríquez G (1999) Isolated pasture trees and the vegetation under their canopies in the Chiapas coastal plain, Mexico. Biotropica 31(2): 243-254

Overman JPM, Witte HJL and Saldarriaga JG (1994) Evaluation of regression modes for above-ground biomass determination in Amazon rainforest. J Trop Ecol 10: 207-218

Ozinga WA, van Andel J and McDonell-Alexander MP (1997) Nutritional soil heterogeneity and mycorrhiza as determinants of plant species diversity. Acta Botanica Neerlandica 46(3): 237-254

Palm CA, Swift MJ and Woomer PL (1996) Soil biological dynamics in slash-and-burn agriculture. Agric Ecosys Environ 58: 61-74

Palmer MW and Dixon PM (1990) Small-scale environmental heterogeneity and the analysis of species distributions along gradients. J Veget Sci 1: 57-65

Paparčikova L (1996) Bestimmung von ^{15}N in der anorganischen N-Fraktion von Böden aus dem östlichen Amazonasgebiet Brasiliens. Diploma thesis. Institute of Agriculture in the Tropics, University of Göttingen, Germany

Pate JS, Stewart GR and Unkovich MJ (1993) ^{15}N natural abundance of plant and soil components of *Banksia* woodland ecosystem in relation to nitrate utilization, life form, mycorrhizal status and N_2-fixing abilities of component species. Plant Cell Environ 16: 365-373

Pate JS, Unkovich M J, Armstrong EL and Sanford P (1994) Selection of reference plants for ^{15}N natural abundance assessment of N_2 fixation by crop and pasture legumes in South-west Australia. Aust J Agric Res 45: 133-147

Peoples MB, Bergersen FJ, Turner GL, Sampet C, Rerkasem B, Bhromsiri A, Nurhayati DP, Faizah AW, Sudin MN, Morhayati M and Herridge DF (1991) Use of the natural enrichment of ^{15}N in plant available soil N for the measurement of symbiotic N_2 fixation. In: Stable isotopes in plant nutrition, soil fertility and environmental studies. pp 117-129 IAEA and FAO, Vienna

Piccolo MC, Neill C, Melillo JM, Cerri CC and Steudler PA (1996) ^{15}N natural abundance in forest and pasture soils of the Brazilian Amazon basin. Plant Soil 182: 249-258

Putz FE (1983) Liana biomass and leaf area of a 'tierra firme' forest in the Rio Negro basin, Venezuela. Biotropica 15(3): 185-189

Putz FE (1984) The natural history of lianas on Barro Colorado Island, Panama. Biotropica 22: 103-105

Raaimakers D, Boot RGA, Dijkstra P, Pot S and Pons T (1995) Photosynthetic rates in relation to leaf phosphorus content in pioneer versus climax tropical rainforest trees. Oecologia 192: 120-125

Raich JW, Rastetter EB, Melillo JM, Kicklighter DW, Steudler PA, Peterson BJ, Grace AL, Moore B and Vörösmarty CJ (1991) Potential net prmary productivity in South America: Application of a global model. Ecol Applic 1(4): 399-429

Reiners WA, Bouwman AF, Parsons WFJ and Keller M (1994) Tropical rain forest conversion to pasture: changes in vegetation and soil properties. Ecol Applic 4: 363-377.

Rennie RJ and Rennie DA (1983) Techniques for quantifying N_2 fixation in association with nonlegumes under field and greenhouse conditions. Can J Microb 29: 1022-1035

Rhoades C (1997) Single-tree influences on soil properties in agroforestry: lessons from natural forest and savanna ecosystems. Agrof Syst 34: 7-94

Ribeiro JEL, Hopkins MJG, Vicentini A, Sothers CA, Costa MAS, de Brito JM, de Souza MAD, Martins LHP, Lohmann LG, Assunção PACL, Pereira EC, da Silva CF, Mesquita MR, Procópio LC (1999) Flora da reserva Ducke – Guia de identificação das plantas vasculares de uma floresta de terra firme na Amazônia Central. INPA/DFID, Manaus, Brazil

Ribeiro MNG and Adis J (1984) Local rainfall variability - a potential bias for bioecological studies in the central Amazon. Acta Amazonica 14(1-2): 159-174

Rice EL (1964) Inhibition of nitrogen-fixing and nitrifying bacteria by seed plants. Ecology 45: 824-837

Ripley BD (1977) Modelling spatial patterns. J. Royal Stat Soc., Ser. B 39:172-212

Roggy JC, Prévost ME, Garbaye J and Domenach AM (1999a) Nitrogen cycling in the tropical rain forest of French Guiana: comparison of two sites with contrasting soil types using $\delta^{15}N$. J Trop Ecol 15: 1-22

Roggy JC, Prévost MF, Gourbiere F, Casabianca H, Garbaye J and Domenach AM (1999b) Leaf natural ^{15}N abundance and total N concentration as potential indicators of plant N nutrition in legumes and pioneer species in a rain forest of French Guiana. Oecologia 120: 171-182

Rossi LMB and Higuchi N (1998) Aplicação de métodos de análise do padrão espacial em oito espécies arbóreas da floresta tropical úmida. In: Gascon C and Moutinho P (eds) Floresta amazônica: dinâmica, regeneração e manejo. pp 41-60 INPA, Manaus, Brazil

Sagers CL, Ginger SM and Evans RD (2000) Carbon and nitrogen isotopes trace nutrient exchange in an ant-plant mutualism. Oecologia 123: 582-586

Saldarriaga JG, West DC, Tharp ML and Uhl C (1988) Long-term chronosequence of forest succession in the upper Rio Negro of Colombia and Venezuela. J Ecol 76: 938-958

Salomão RP, Nepstad DC and Vieira ICG (1998) Biomasa e estoque de carbono de florestas tropicais primária e secundária. In: Gascon C and Moutinho P (eds) Floresta Amazônica: dinâmica, regeeração e manejo. pp 99-119 INPA, Manaus, Brazil

Sanford RL (1986) Canopy openings in a primary neotropical lowland forest. J Trop Ecol 2: 277-282

Sangakkara UR (1991) Analysis of the effect of potassium fertilizer on nitrogen fixation in bushbeans using ^{15}N. Poster presentation in: Stable isotopes in plant nutrition, soil fertility and environmental studies. pp 226-228 IAEA and FAO, Vienna

Sanginga N, Danso SKA and Bowen GD (1989) Nodulation and growth response of *Allocasuarina* and *Casuarina* species to phosphorus fertilization. Plant Soil 118: 125-132

Sanginga N, Vanlauwe B and Danso SKA (1995) Management of biological N_2 fixation in alley cropping systems: estimation and contribution to N balance. Plant Soil 174: 119-141

Schnitzer S, Dalling J, Carson W (2000) The impact of lianas on tree regeneration in tropical forest canopy gaps: evidence for an alternative pathway of gap-phase regeneration. J Ecol 88: 655-666

Schroth G, da Silva LF, Seixas R, Teixeira WG, Macêdo JLV and Zech W Subsoil accumulation of mineral nitrogen under polyculture and monoculture plantations, fallow and primary forest in a ferralitic Amazonian upland soil. Agric Ecosyst Environ 75: 109-120

Serrão EAS (1995) Possibilities for sustainable agricultural developement in the Brazilian Amazon: an EMBRAPA proposal. In: Cluesener-Godt M and Sachs I (eds) Brazilian perspectives on sustainable developement of the Amazon region. pp 250-273 UNESCO, MAB Series, Vol. 15, London

Smart JB, Dilworth MJ and Robson AD (1984) Effect of phosphorus supply on phosphate uptake and alkaline phosphatase activity in Rhizobia. Archives Microbiol 140: 281-286

Smith NJH (1980) Anthrosols and human carrying capacity in Amazonia. Annals Assoc Am Geogr 70(4): 553-566

Snedaker SC (1980) Successional immobilization of nutrients and biologically mediated recycling in tropical forests. Biotropica 12: 16-22

Sombroek W (2000) Spatial and temporal patterns of Amazon rainfall. Ambio 30(7): 388-396

Sørensen P and Jensen ES (1991) Sequential diffusion of ammonium and nitrate from soil extracts to a polytetrafluoroetylene trap for ^{15}N determination. Analytica Chimica Acta 252: 201-203

Spjøtvoll E and Stoline MR (1973) An extension of the t-method of multiple comparison to include the cases with unequal sample sizes. J Am Stat Ass 68(344): 975-978

Sprent JI (1987) The ecology of the nitrogen cycle. Cambridge University Press, Cambridge.

Sprent JI (1995) Legume trees and shrubs in the tropics: N_2 fixation in perspective. Soil Biol Biochem 27(4/5): 401-407

Sprent JI, Geoghegan IE, Whitty PW and James EK (1996) Natural abundance of ^{15}N and ^{13}C in nodulated legumes and other plantsin the cerrado and neighbouring regions of Brazil. Oecologia 195: 440-446.

StJohn TV and Uhl C (1983) Mycorrhizae in the rain forest at San Carlos de Rio Negro, Venezuela. Acta Cientifica Venezolana 34: 233-237

Steininger MK (2000) Secondary forest structure and biomass following short and extended land-use in central and southern Amazonia. J Trop Ecol 16: 689-708

Sterner RW, Ribic CA and Schatz GE (1986) Testing for life historical changes in spatial patterns of four tropical tree species. J Ecol 74: 621-633

Sylvester-Bradley R, Bandeira AG and de Oliveira LA (1978) Fixação de nitrogênio (redução de acetileno) em cupins (Insecta: *Isoptera*) da Amazônia Central. Acta Amazonica 8(4): 621-627

Sylvester-Bradley R, de Oliveira LA, de Podesta Filho JA and StJohn TV (1980) Nodulation of legumes, nitrogenase activity of roots and occurence of nitrogen-fixing *Azospirillum spp*. in representative soils of central Amazonia. Agro-Ecosystems 6: 249-266

Szott LT, Palm CA and Buresh RJ (1999) Ecosystem fertility and fallow function in the humid and subhumid tropics. Agrof Syst 47: 163-196

Tayasu I (1997) Nutritional ecology of termite-symbiont systems using stable isotope techniques. PhD thesis, Center for Ecological Research, Kyoto University, Japan

Tayasu I (1998) The use of carbon and nitrogen isotope ratios in termite research. Ecol Res 13(3): 1-12

Terborgh J and Andresen E (1998) The composition of Amazonian forests: patterns at local and regional scales. J Trop Ecol 14: 645-664

Thielen-Klinge A (1997) Rolle der biologischen N_2-Fixierung von Baumleguminosen im östlichen Amazonasgebiet, Brasilien. PhD thesis. Institute of Agriculture in the Tropics, University of Göttingen, Germany

Tiessen H, Karamanos RE, Stewart WB and Selles F (1984) Natural Nitrogen-15 abundance as an indicator of soil organic matter transformations in native and cultivated soils. Soil Sci Soc Am J 48: 312-315

Tiessen H and Moir JO (1993) Characterisation of available P by sequential extraction. In: Carter MR (ed) Soil sampling and methods of soil analysis. pp 75-86 CRC Press, Boca Raton, FL

Uhl C (1987) Factors controlling succession following slash-and-burn agriculture in Amazonia. J Ecol 75: 377-407

Uhl C, Buschbacher R and Serrão EAS (1988) Abandoned pastures in eastern Amazonia. I. Patterns of plant succession. J Ecol 76: 663-681

Unkovich M, Pate JS and Sanford P (1993) Preparation of plant samples for high precision Nitrogen isotope ratio analysis. Comm Soil Sci Plant Analysis 24(15&16):2093-2106

Unkovich MJ, Pate JS, Sanford P and Armstrong EL (1994) Potential precision of the Delta ^{15}N natural abundance method in field estimates of nitrogen fixation by crop and pasture legumes in South-west Australia. Aust J Agric Res 45: 119-132

van der Meer PJ and Bongers F (1996) Patterns of tree-fall and branch-fall in a tropical rain forest in French Guiana. J Ecol 84: 19-29

van Kessel C and Roskoski JP (1983) Nodulation and N_2 fixation by *Inga jinicuil*, a woody legume in coffee plantations. III.: Effect of fertilizers and soil shading on nodulation and nitrogen fixation (acetylene reduction) of *I.jinicuil* seedlings. Plant Soil 72: 95-105

van Kessel C, Farrell RE, Roskoski JP and Keane KM (1994) Recycling of the naturally-occurring ^{15} N in an established stand of *Leucena leucocephala*. Soil Biol Biochem 26(6): 757-762

Virginia RA and Delwiche CC (1982) Natural ^{15}N abundance of presumed N_2-fixing and non-N_2-fixing plants from selected ecosystems. Oecologia 54: 317-325

Vitousek PM and Reiners WA (1975) Ecosystem succession and nutrient retention: a hypothesis. BioScience 25(6): 376-381

Vitousek PM (1982) Nutrient cycling and nutrient use efficiency. American Naturalist 119(4): 553-572

Vitousek PM (1984) Litterfall, nutrient cycling and nutrient limitation in tropical forests. Ecology 65(1): 285-298

Vitousek PM and Denslow JS (1986) Nitrogen and phosphorus availability in treefall gaps of a lowland tropical rainforest. J Ecol 74: 1167-1178

Vitousek PM and Walker LR (1987) Colonization, succession and resource availability: ecosystem-level interactions. In: Gray A J, Crawley MJ and Edwards PJ (eds) Colonization, succession and stability. pp 207-223 Blackwell Scientific Publications, Oxford

Vitousek PM, Shearer G and Kohl DH (1989) Foliar ^{15}N natural abundance in Hawaiian rainforest: patterns and possible mechanisms. Oecologia 78: 383-388

Wani ASP, Dart PJ and Upadhyaya MN (1983) Factors affecting nitrogenase activity (C_2H_2 reduction) associated with sorghum and millet estimated using the soil core assay. Can J Microb 29: 1063-1069

Watson DF and Philip GM (1985) A refinement of Inverse Distance Weighted interpolation. Geo-Processing 2:315-327

Weeetman GF and Wells CG (1990) Plant analysis as an aid in fertilizing forests. In: Westerman RL (ed) Soil testing and plant analysis. Soil Science Society of America Inc., Madison,WI

Williams MR, Fisher TR and Melack JM (1997) The composition and deposition of rain in the central Amazon, Brazil. Atmospheric Environment 31: 207-217

Williams MR and Melack JM (1997) Solute export from forested and partially deforested catchments in the central Amazon. Biogeochemistry 38: 67-102

Williamson GB, Mesquita R, Ickes K and Ganade G (1998) Estratégias de colonização de árvores pioneiras nos Neotrópicos. In: Gascon C and Moutinho P (eds) Floresta amazônica: dinâmica, regeneração e manejo. pp 131-143 INPA, Manaus

Willson MF and Crome FJH (1989) Patterns of seed rain at the edge of a tropical Queensland rain forest. J Trop Ecol 5: 301-308

Woomer PL, Singleton P and Bohlool BB (1988) Ecological indicators of native Rhizobia in tropical soils. Appl Env Microb 54(5): 1112-1116

Yoneyama T (1995) Nitrogen metabolism and fractionation of nitrogen isotopes in plants. In: Wada E, Yoneyama T, Minagawa M, Ando T and Fry BT (eds) Stable isotopes in the biosphere. pp 92-102 Kyoto University Press, Kyoto, Japan

Zinke PJ (1962) The pattern of influence of individual forest trees on soil properties. Ecology 43(1): 130-133

Appendix 1. The 25 study sites

					plot-based scheme		mean vegetation total		median % contributions to TAGP					
site N$^{o.}$	site name	age	CS(1)	size (ha)	plot-n	% area-coverage	abund. (n ha^{-1})	TAGP (t ha^{-1})	lianas	palms	pioneer trees(2)	single-shoot plants(3)	largest 5% plants(4)	pot. N$_2$-fixers(5)
	young first-cycle secondary vegetation													
1	Joaquim	2	2	0.23	3	7.3	5867	61.7	10.2	2.0	11.7	35.9	62.8	9.6
2	Francisco	3	2	0.16	12	18.8	16433	93.8	4.6	0.2	63.0	94.8	0	5.7
3	Gustavo (young)	3	3	0.60	17	7.1	8588	116.8	3.5	0.4	25.6	84.9	15.5	1.3
4	Geraldo (young)	3	3	0.47	16	8.5	10600	141.5	4.8	0.3	12.6	81.5	52.4	14.1
	mid-aged first-cycle secondary vegetation													
5	Adventista (young)	5	4	0.33	8	13.6	10044	122.4	2.7	0	13.2	56.5	46.2	19.9
6	Geraldo (old)	7	3	1.10	27	13.8	5505	147.7	2.8	0.4	8.3	73.5	35.4	9.1
7	Ilson	8	4	0.89	17	10.7	5773	175.1	4.3	0	26.6	56.8	44.2	1.9
8	Edson (young)	10	1	0.48	9	18.8	4011	164.6	4.2	0.1	11.7	45.0	38.9	1.4
9	Guillerme	10	2	0.63	10	15.9	8450	178.7	1.4	0.4	58.6	94.3	17.3	1.9
	old first-cycle secondary vegetation													
10	Anna (old)	12	2	0.28	10	35.7	5580	220.1	1.0	0.3	37.4	66.2	42.0	5.7
11	Adventista (old)	18	4	0.30	6	20.0	5533	210.6	3.4	1.5	0.8	66.5	57.9	20.0
12	Jasiel	20	1	0.98	21	21.4	4624	202.4	1.2	0.1	15.1	48.6	44.0	0.5
13	Edson (old)	25	1	0.63	15	23.8	5013	221.2	2.1	2.3	0	58.5	68.4	0.5
	primary forest controls (clayey Oxisol)													
14	Anna	PF	2	0.70	8	22.4	5422	353.1	1.3	0.4	0	99.1	84.2	2.0
15	Francisco	PF	2	0.63	6	21.4	5556	443.0	0.7	0	0	97.5	90.1	8.2
16	Edson	PF	1	0.66	8	27.3	4689	418.1	3.5	0.3	0	99.0	83.7	0.2
17	Gustavo	PF	3	1.09	9	18.6	2257	550.6	1.1	5.1	0	92.4	87.2	1.0
18	Geraldo	PF	3	1.16	10	19.4	5978	540.6	1.0	4.6	0	99.2	90.0	1.2
19	Adventista	PF	4	0.10	2	61.4	5541	452.8	1.5	0.5	0	99.2	84.1	0.2
	‘degraded’ secondary vegetation													
20	Anna (2nd cycle)	4	2	0.33	12	9.1	12433	81.0	10.2	0.2	14.7	29.5	46.2	5.2
21	Gustavo (2 burns)	5	3	0.45	14	7.8	6514	116.9	1.3	0	37.2	46.7	90.0	0.8
22	Bispo (extend. cultiv.)	11	2	0.70	12	17.1	7567	172.9	1.7	0.4	55.1	19.3	38.9	1.9
	sites on different soil													
23	Aurelio (terra morena)	4	5	0.36	12	17.1	6548	143.7	1.6	0.8	0	44.7	63.5	45.3
24	Maria (sandy)	PF	5	0.65	8	27.7	6106	320.8	0.5	4.0	0	96.3	88.3	6.7
25	Novo Jerusalém (sandy)	PF	5	0.48	6	28.2	6459	371.4	0.7	2.0	0	94.0	86.6	3.4

(1) chronosequence, see Table 1; (2) *G.glabra, L.procera, V.guianensis, Cecropia* spp., see Table 8; (3) see Figure 4; (4) see Tables 9 and 10; (5) see chap. 5.2.2

Appendix 2. List of all legume species of this study:

taxonomic order, growth form, and classification of nodulation capacity
(* indicates species selected for δ^{15}N-analysis)

Caesalpinioideae

		growth form	nodulation capacity
	Amherstieae		
	Elizabetha speciosa DUCKE	T	(+)
	Eperua glabriflora (DUCKE) R.S.COWAN	T	(-)
	Hymenaea parviflora HUBER	T	(--)
	Hymenaea reticulata DUCKE	T	(--)
	Peltogyne excelsa DUCKE	T	(-)
	Peltogyne paniculata BENTH.	T	(-)
	Tachigali polyphylla POEPPIG	T	(+)
	Tachigali myrmecophila DUCKE	T	(++)
	Tachigali venusta DWYER	T	(+)
	Cassieae		
	Cassia rubrifolia DUCKE	T	(-)
	Dialium guianense (AUBLET) SANDW.	T	(--)
	Senna tapajozensis H.S.IRWIN&BARNEBY	L	(--)
	Senna quinquangulata (L.C.RICH.) H.S.IRWIN&BARNEBY	L	(-)
	Cyonometreae		
	Copaifera multijuga HAYNE	T	(-)
	Detarieae		
	Macrolobium guianense (AUBLET) PULLE	T	(-)
	Macrolobium limbatum SPRUCE EX BENTH.	T	(--)
*	*Macrolobium microcalyx* DUCKE	T	(--)
	Macrolobium prancei R.S.COWAN	T	(--)
	Dimorphandreae		
	Dimorphandra coccinea DUCKE	T	(++)
*	*Dimorphandra parviflora* SPRUCE EX BENTH.	T	(+)
	Dimorphandra pennigera TUL.	T	(+)

---

growth form: T=tree L=liana
nodulation capacity: (++) proven capacity, (+) probable capacity, (-) probable incapacity, (--) ‘proven’ incapacity
note that a conclusive proof of the incapacity to nodulate is impossible

Sclerolobieae

	Sclerolobium chrysophyllum POEPP.&ENDL.	T	(++)
	Sclerolobium guianense BENTH.	T	(+)
	Sclerolobium melanocarpum DUCKE	T	(+)
	Sclerolobium melinonii HARMS	T	(+)
	Sclerolobium micropetalum DUCKE	T	(+)
	Sclerolobium paraense HUBER	T	(++)
	Sclerolobium setiferum DUCKE	T	(+)
	Vouacapoua pallidior DUCKE	T	(++)

Papilionoideae

Cercideae

*	*Bauhinia alata* DUCKE	L	(--)
	Bauhinia coronata BENTH.	L	(--)
	Bauhinia cupreonitens DUCKE	L	(-)
	Bauhinia guianensis AUBL.	L	(--)
	Bauhinia guianensis AUBL.var *splendens* (KUNTH) AMSHOFF	L	(--)

Dalbergieae

	Andira micrantha DUCKE	T	(++)
	Andira parviflora DUCKE	T	(++)
	Andira unifoliolata DUCKE	T	(+)
*	*Dalbergia multiflora* HEYNE EX WALL.	L	(++)
	Derris amazonica KILLIP	L	(++)
	Derris floribunda (BENTH.) DUCKE	L	(+)
*	*Derris negrensis* BENTH.	L	(++)
	Dipteryx magnifica (DUCKE) DUCKE	T	(--)
	Dipteryx odorata (AUBL.) WILLD.	T	(--)
	Dipteryx polyphylla HUBER	T	(-)
	Hymenolobium excelsum DUCKE	T	(++)
	Hymenolobium heterocarpum DUCKE	T	(++)
	Hymenolobium modestum DUCKE	T	(++)
	Hymenolobium pulcherrimum DUCKE	T	(+)
	Hymenolobium sericeum DUCKE	T	(+)
	Machaerium amplum BENTH.	L	(+)
	Machaerium aureiflorum DUCKE	L	(+)
	Machaerium castaneiflorum DUCKE	L	(+)
	Machaerium caudatum DUCKE	L	(+)
*	*Machaerium ferox* GLAZIOU	L	(++)
	Machaerium froesii RUDD	L	(++)
*	*Machaerium hoehneanum* DUCKE	L	(++)
	Machaerium humboltianum VOGEL	L	(+)
*	*Machaerium madeirensis* PITTIER	L	(++)
*	*Machaerium multifoliolatum* DUCKE	L	(++)
	Machaerium piresii RUDD	L	(+)
*	*Machaerium quinata* (AUBL.) SANDWITH	L	(++)

	Pterocarpus amazonum (BENTH.) AMSHOFF	T	(+)
	Pterocarpus officinalis JACQ	T	(++)
	Vatairea paraensis DUCKE	T	(-)
Hedysareae			
	Desmodium adscendens (SW.) DC.	L	(+)
Phaseoleae			
	Centrosema brasilianum (L.) BENTH.	L	(++)
	Clitoria amazonum BENTH	L	(+)
	Clitoria javitensis (H.B.K.) BENTH.	L	(++)
	Clitoria leptostachya BENTH.	L	(+)
	Dioclea megacarpa HUBER	L	(+)
Sophoreae			
	Ormosia grossa RUDD	T	(+)
	Ormosia paraensis DUCKE	T	(+)
Swartzieae			
	Bocoa racemulosa (HUBER) COWAN	T	(+)
	Bocoa viridiflora (DUCKE) R.S.COWAN	T	(+)
	Swartzia arborescens (AUBL.) PITTIER	T	(+)
	Swartzia brachyrachis HARMS	T	(+)
	Swartzia corrugata BENTH.	T	(++)
*	*Swartzia cuspidata* SPRUCE EX BENTH.	T	(+)
	Swartzia discocarpa DUCKE	T	(+)
*	*Swartzia ingifolia* DUCKE	T	(+)
	Swartzia lamellata DUCKE	T	(+)
	Swartzia oblanceolata SANDWITH	T	(+)
	Swartzia polyphylla DC.	T	(++)
	Swartzia recurva POEPP. IN POEPP. & ENDL.	T	(+)
	Swartzia reticulata DUCKE	T	(+)
	Swartzia robiniifolia VOGEL	T	(+)
	Swartzia schomburgkii BENTH.	T	(+)
	Swartzia tessmannii HARMS	T	(+)
	Swartzia ulei HARMS	T	(++)

Mimosoideae

Acacieae

	Acacia amazonica Benth.	T	(+)
	Acacia altiscandens Ducke	T	(+)
	Acacia multipinnata Ducke	L	(++)
	Acacia polyphylla DC.	L	(+)

Adenanthereae

	Dinizia excelsa Ducke	T	(--)
	Stryphnodendron duckeanum Occh.	T	(+)
*	*Stryphnodendron guianense* (Aubl.) Benth.	T	(++)
	Stryphnodendron paniculatum Poepp. & Endl.	T	(+)
*	*Stryphnodendron pulcherrimum* (Willd.) Hochr.	T	(++)
	Stryphnodendron racemiferum (Ducke) W.Rodrigues	T	(+)

Eumimoseae

	Mimosa guilandinae (DC.) Barneby	L	(++)
	Mimosa pudica L. var. *unijuga* (Duchass. & Walp.)	L	(+)
	Mimosa spruceana (Benth.) Barneby	L	(--)

Hedysareae

	Balizia elegans (Ducke) Barneby & J.W.Grimes	T	(+)

Ingeae

	Abarema adenophera (Ducke) Barneby & J.W.Grimes	T	(+)
	Abarema floribunda (Benth.) Barneby & J.W.Grimes	T	(+)
	Abarema jupunba (Willd.) Britton & Killip	T	(++)
	Abarema laeta (Poepp. & Endl.) Barneby	T	(+)
	Abarema mataybifolia (Sandwith) Barneby & J.W.Grimes	T	(+)
	Abarema piresii Barneby & J.W.Grimes	T	(+)
	Calliandra tenuiflora Benth.	T	(++)
*	*Enterolobium schomburgkii* Benth.	T	(++)
	Inga alata Benoist	T	(+)
	Inga alba (Sw.) Willd.	T	(++)
	Inga bicoloriflora Ducke	T	(+)
	Inga capitata Desv.	T	(++)
*	*Inga cayennensis* Sagot ex Benth.	T	(++)
	Inga chrysantha Bernardi & Spichiger	T	(+)
	Inga cordatoalata Ducke	T	(+)
*	*Inga edulis* Mart.	T	(++)
	Inga glomeriflora Ducke	T	(+)
	Inga gracilifolia Ducke	T	(+)
	Inga grandiflora Ducke	T	(+)

	Inga huberi DUCKE	T	(+)
	Inga lateriflora MIQ.	T	(++)
	Inga laurina WILLD.	T	(+)
	Inga leiocalycina BENTH.	T	(+)
	Inga longiflora BENTH.	T	(+)
	Inga macrophylla HUMB. & BONPL. EX WILLD.	T	(++)
	Inga marginata WILLD.	T	(++)
	Inga melinonis SAGOT	T	(+)
	Inga obidensis DUCKE	T	(+)
	Inga panurensis SPRUCE EX BENTH.	T	(-)
*	*Inga paraensis* DUCKE	T	(+)
	Inga pendula (WILLD.) WALP.	T	(+)
	Inga pezizifera BENTH.	T	(++)
	Inga rhynchocalyx SANDWITH	T	(+)
	Inga rubiginosa (RICH.) DC.	T	(++)
	Inga splendens WILLD.	T	(++)
	Inga stenoptera BENTH.	T	(+)
*	*Inga stipularis* DC.	T	(++)
	Inga suberosa T.D.PENN.	T	(+)
*	*Inga thibaudiana* DC.	T	(++)
*	*Inga umbellifera* (VAHL) STEUD. EX DC.	T	(+)
*	*Inga umbratica* POEPP. & ENDL.	T	(+)
	Inga velutina WILLD.	T	(+)
	Inga vera KUNTH	T	(+)
*	*Zygia racemosa* (DUCKE) BARNEBY & J.W.GRIMES	T	(--)
	Zygia ramiflora (DUCKE) BARNEBY & J.W.GRIMES	T	(--)

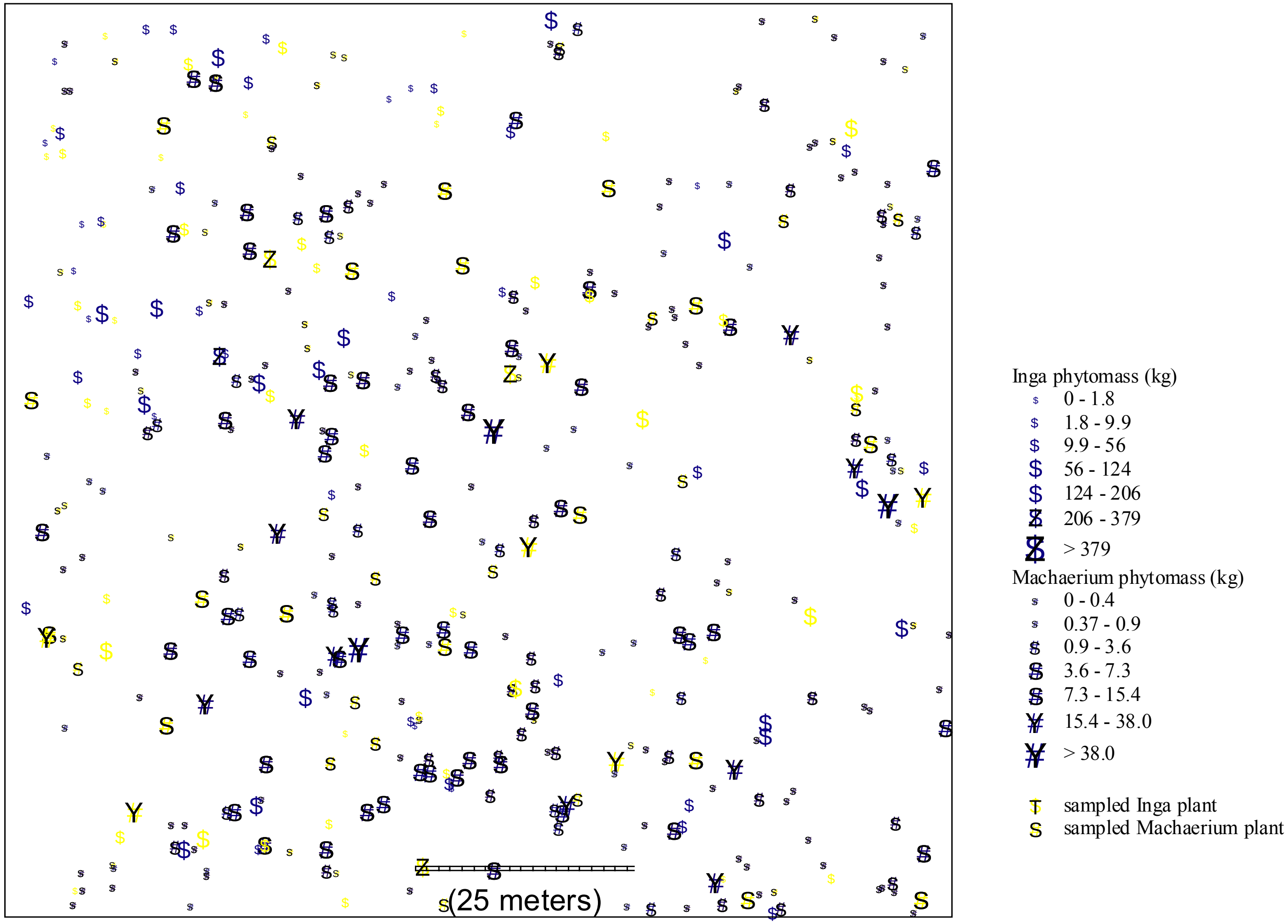
Parkieae

	Parkia decussata DUCKE	T	(-)
	Parkia igneiflora DUCKE	T	(-)
*	*Parkia multijuga* BENTH.	T	(--)
*	*Parkia nitida* MIQ.	T	(--)
	Parkia panurensis SPRUCE EX H.C.HOPKINS	T	(--)
	Parkia pendula (WILLD.) WALP.	T	(--)

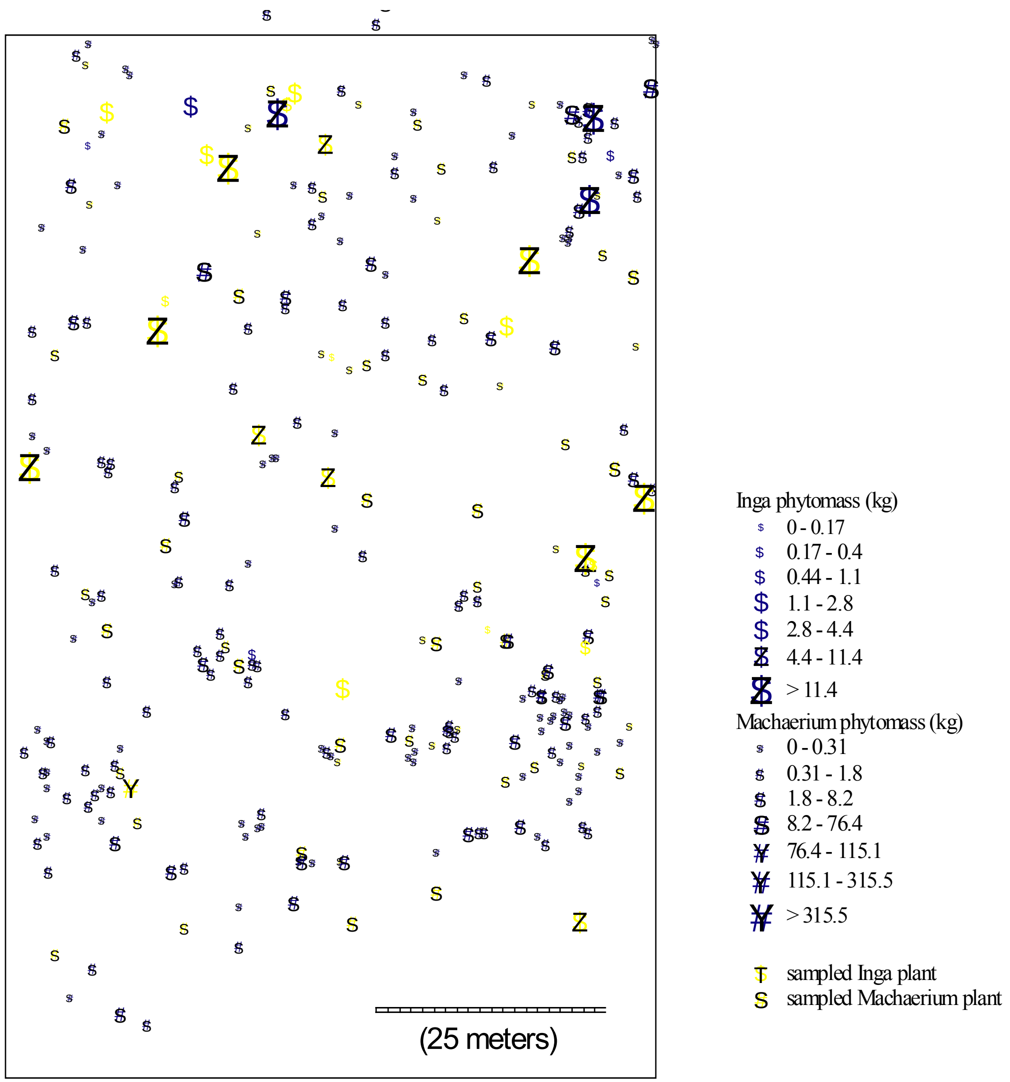
Piptadenieae

	Piptadenia minutiflora DUCKE	L	(+)
	Piptadenia suaveolens GRISEB.	L	(++)
	Pseudopiptadenia psilostachya (BENTH.)G.P.LEWIS & L.RICO	T	(+)

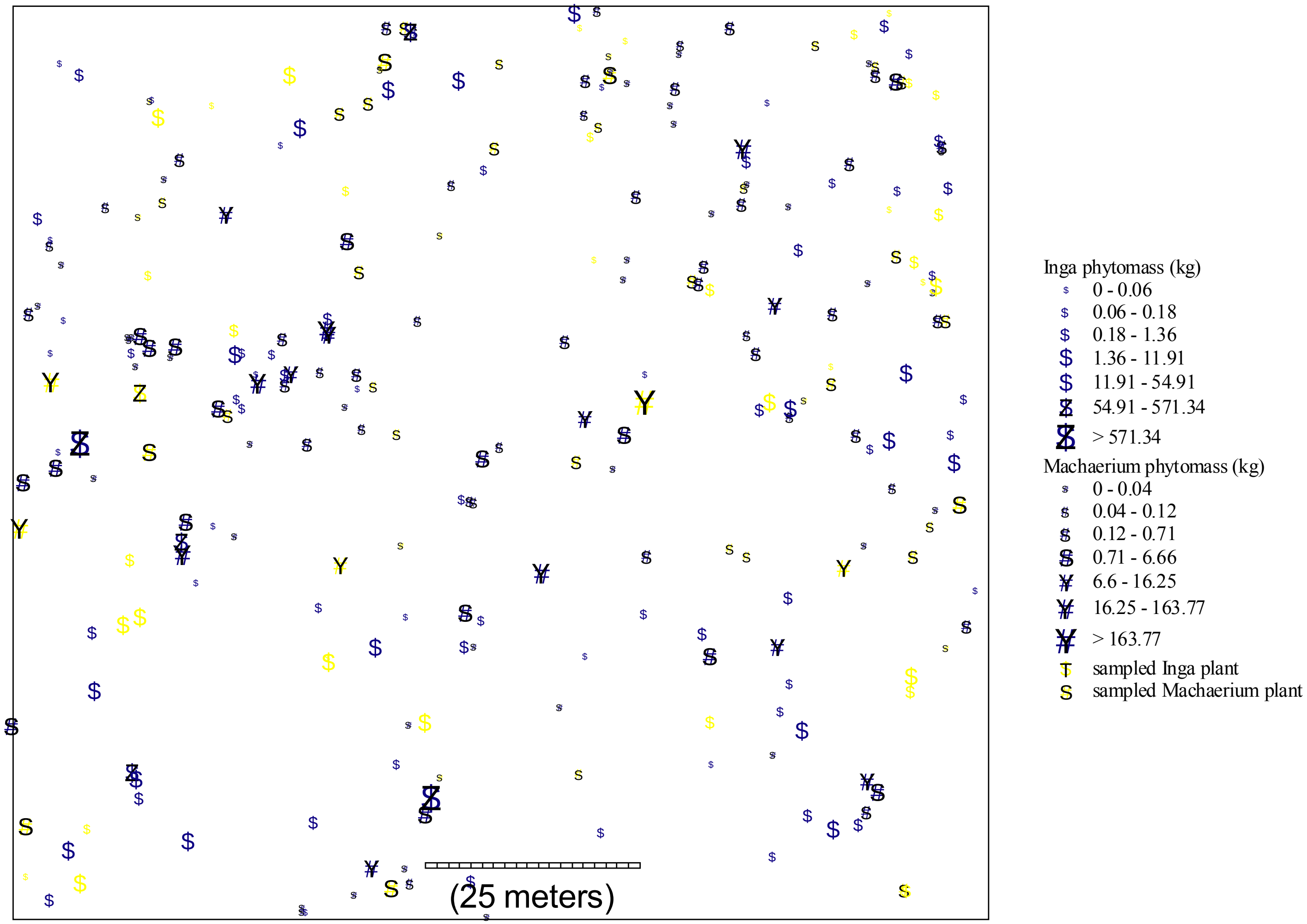
Appendix 3: Systematic foliar sampling scheme attaining a regular within-site distribution and an adequate plant size representation (see chap. 4.4.1), exemplified for seven *Inga*-species (triangles) and five *Machaerium*-species (circles)
(phytomass is scaled in seven percentile levels of these genera, frame delimits the completely mapped area)



Map 1. *Inga*- and *Machaerium*-sampling in a 7-yr.-old first-cycle regrowth ('Geraldo old')



Map 2. *Inga*- and *Machaerium*-sampling in an 11-yr.-old degraded regrowth (‘Bispo’)



Map 3. *Inga*- and *Machaerium*-sampling in a primary forest control site ('Geraldo PF')

Appendix 4. Structural characteristics of four non-legume pioneer trees along succession (phytomass contributions in % of total)

4- to 5-yr.-old degraded (2 sites)

	plant n	multiple-shoot plants (in %)	largest 5% of plants (in%)	max. indiv. phytomass (kg)
Goupia glabra	28	93.5	29.3	218.6
Laetia procera	31	52.9	16.5	117.2
Vismia guianensis	77	79.0	20.4	150.6
*Cecropia spp.**	147	69.2	15.4	456.4

2- to 3-yr.-old regrowth (4 sites)

	plant n	multiple-shoot plants (in %)	largest 5% of plants (in%)	max. indiv. phytomass (kg)
Goupia glabra	75	70.5	48.6	180.8
Laetia procera	72	99.2	52.5	179.6
Vismia guianensis	89	86.0	29.4	179.6
*Cecropia spp.**	699	88.8	19.9	420.2

5- to 10-yr.-old regrowth (5 sites)

	plant n	multiple-shoot plants (in %)	largest 5% of plants (in%)	max. indiv. phytomass (kg)
Goupia glabra	338	46.2	28.1	529.3
Laetia procera	569	10.7	19.5	328.1
Vismia guianensis	605	38.4	30.4	698.6
*Cecropia spp.**	466	13.4	20.3	1509.8

12- to 25-yr.-old regrowth (4 sites)

	plant n	multiple-shoot plants (in %)	largest 5% of plants (in%)	max. indiv. phytomass (kg)
Goupia glabra	126	64.9	36.1	1117.1
Laetia procera	126	36.4	20.0	971.5
Vismia guianensis	201	50.2	23.4	465.6
*Cecropia spp.**	293	44.8	21.6	1052.7

* *C.sciadophylla, C.distachya, C.purpurascens*

Appendix 5. Species composition of the two main legume genera:
species' shares of abundance (%n) and phytomass (%t) within the tree genus *Inga* and the liana genus *Machaerium*

Species composition of the tree genus *Inga*

	total *Inga*		%-contribution to legume trees		*I.alba*		*I. paraensis*		*I. cayennensis*		*I. stipularis*		*I. umbratica*		other *Inga*	
	n ha^{-1}	t ha^{-1}	% n	% t	% n	% t	% n	% t	% n	% t	% n	% t	% n	% t	% n	% t
4-5 yrs. (degraded)	35	0.6	23.2	23.6	-	-	32.2	41.4	33.3	21.6	6.5	1.5	2.2	0.2	25.7	35.5
11 yrs. (degraded)	121	2.7	44.5	51.4	8.2	25.3	14.1	20.4	4.7	0.3	2.4	0.4	1.2	0.7	69.4	53.0
4 yrs. (terra morena)	633	22.1	94.6	96.8	-	-	0.4	0.3	-	-	-	-	0.4	0.4	99.1	99.3
2- to 3-yr.-old	158	0.9	35.9	37.2	6.9	10.9	25.3	38.0	3.5	1.6	6.8	10.5	4.9	4.3	52.6	34.7
5- to 10-yr.-old	68	3.2	33.5	39.8	11.7	30.4	19.9	26.7	18.5	5.9	7.4	3.1	5.0	2.6	37.5	31.3
12- to 25-yr.-old	105	5.2	53.5	54.3	10.6	38.3	13.2	9.5	16.7	7.4	16.7	2.1	9.6	3.1	33.2	39.6
PF (clay)	220	5.5	50.0	7.4	7.8	25.0	20.2	23.7	8.7	2.9	9.4	0.8	22.2	19.4	31.8	28.2
PF (sand)	196	0.2	39.7	0.8	-	-	18.9	22.8	12.8	1.2	11.5	4.4	16.9	40.4	39.9	31.2

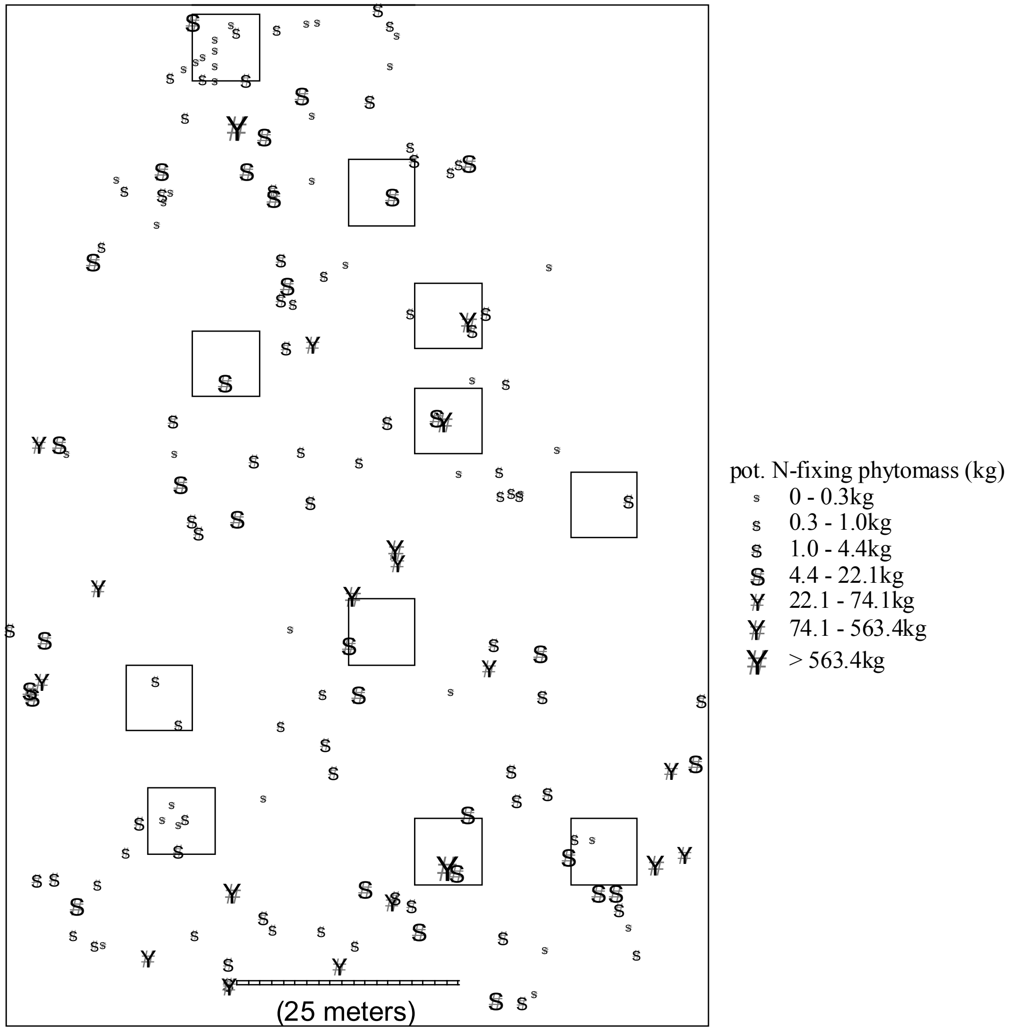
(28 species)

Species composition of the liana genus *Machaerium*

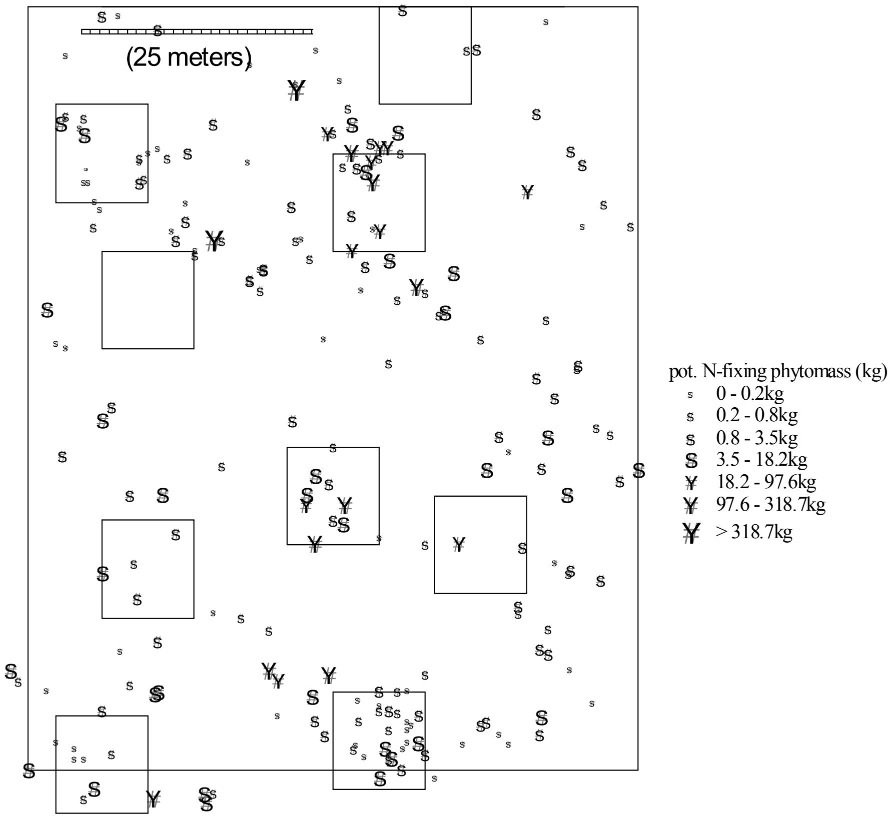
	total *Machaerium*		%-contribution to legume lianas		*M.hoehneanum*		*M.madeirensis*		*M.multifoliolatum*		*M.quinata*		*M.ferox*		other *Machaerium*	
	n ha^{-1}	t ha^{-1}	% n	% t	% n	% t	% n	% t	% n	% t	% n	% t	% n	% t	% n	% t
4-5 yrs. (degraded)	444	1.9	88.5	89.5	60.7	59.4	21.9	29.8	0.3	0.01	1.6	1.1	3.1	1.1	12.4	8.6
11 yrs. (degraded)	613	0.8	89.4	83.9	52.0	46.5	7.7	13.7	4.4	17.9	3.3	3.8	11.0	7.1	21.7	11.0
4 yrs. (terra morena)	39	0.1	8.3	14.3	71.4	85.4	-	-	-	-	-	-	-	-	28.6	14.6
2- to3-yr.-old	683	1.9	82.2	80.6	61.0	67.7	16.5	20.8	2.4	2.8	3.1	1.4	4.1	1.2	12.9	6.3
5- to 10-yr.-old	260	0.7	76.3	63.7	55.9	49.3	10.9	14.6	7.5	16.7	2.4	1.8	7.8	6.0	15.5	11.6
12- to 25-yr.-old	295	1.1	79.7	79.4	49.7	64.1	15.7	21.4	6.8	3.9	6.6	2.8	3.7	0.3	17.4	7.4
PF (clay)	218	2.8	75.2	72.7	50.2	27.6	16.5	44.5	12.4	24.8	2.1	0.2	5.3	0.2	13.6	2.7
PF (sand)	145	0.8	42.3	90.5	44.9	16.0	10.9	49.0	5.0	0.03	1.1	0.3	5.7	0.4	32.3	34.1

(6 species)

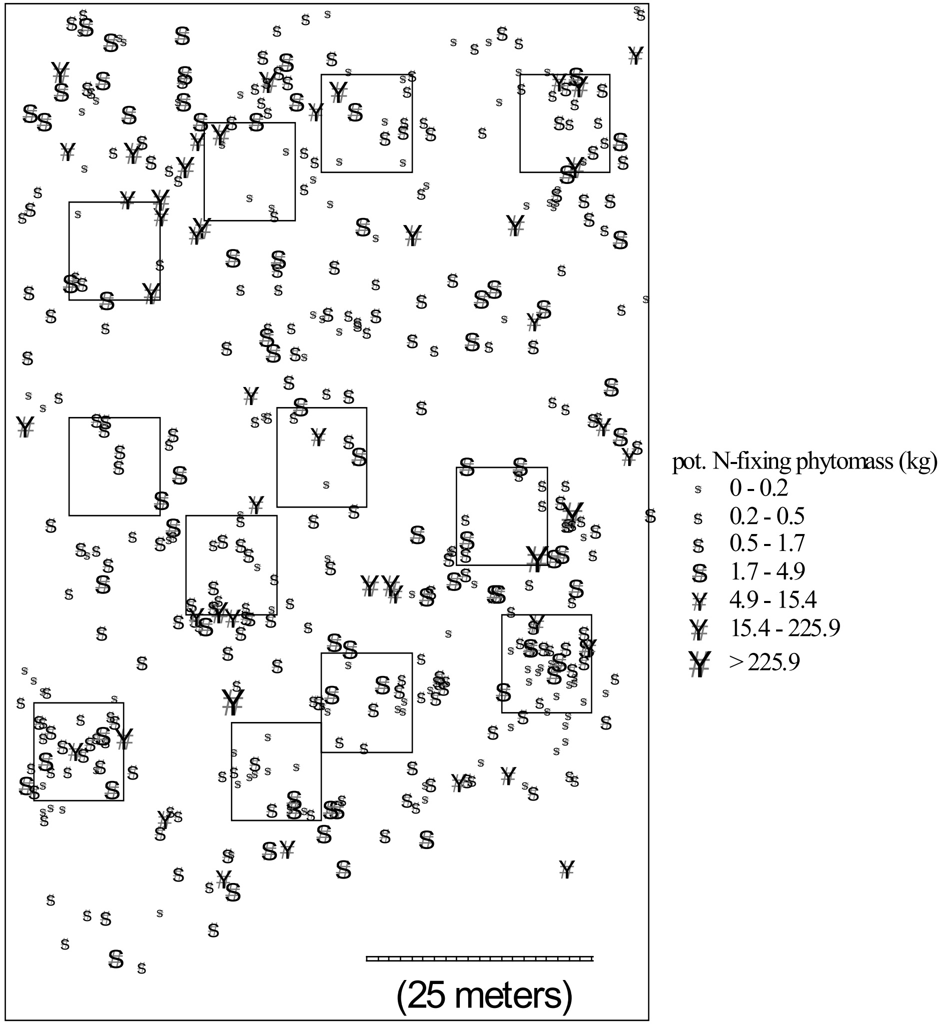
Appendix 6. Spatial distribution of potentially N_2-fixing vegetation (see chap. 5.3.1)
Plant phytomass is scaled in seven percentile levels, frames delimit the completely mapped area, squares show the plot-based sampling-scheme (see chap. 4.4.2)



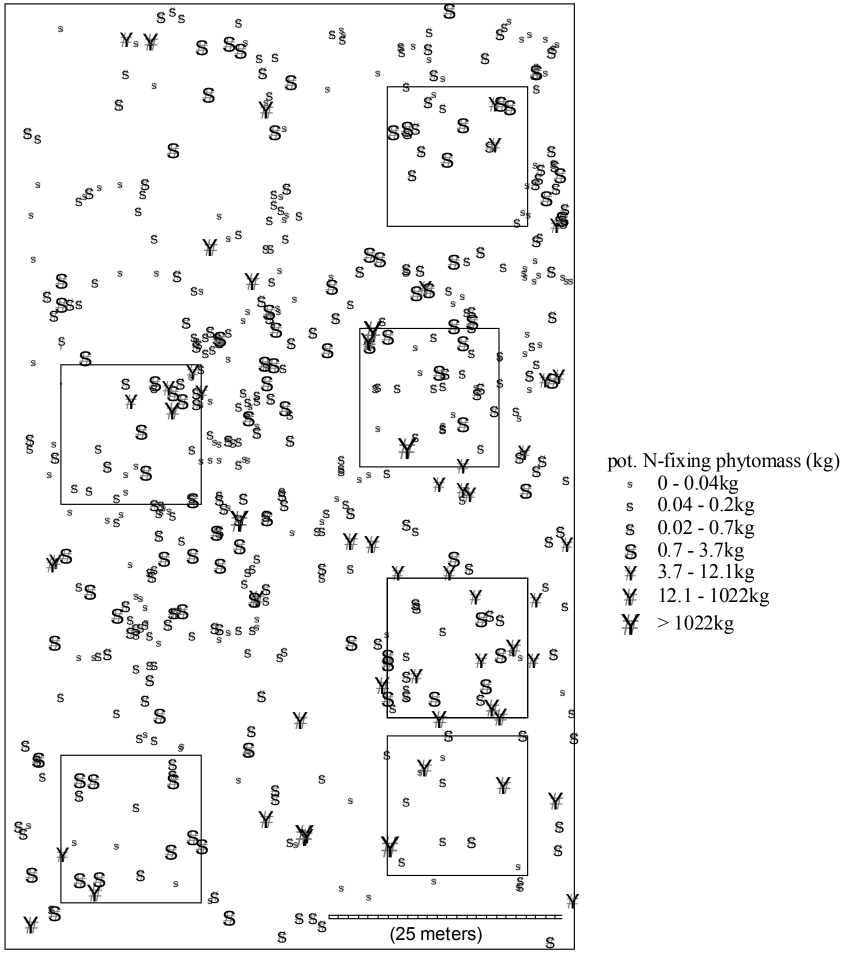
Map 7. Potentially N_2-fixing legumes in an 8-yr.-old first-cycle regrowth (‘Ilson’)



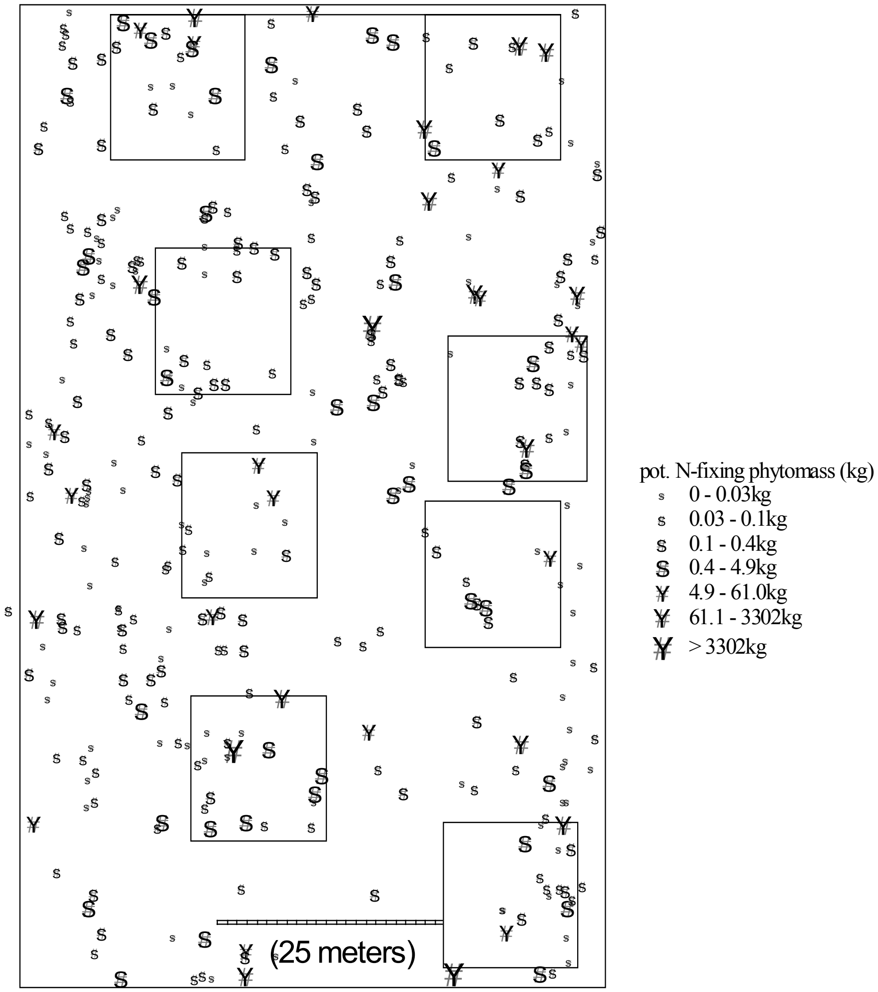
Map 8. Potentially N_2-fixing legumes in a 10-yr.-old first-cycle regrowth (‘Edson young’)



Map 9. Potentially N_2-fixing legumes in an 11-yr.-old degraded regrowth ('Bispo')

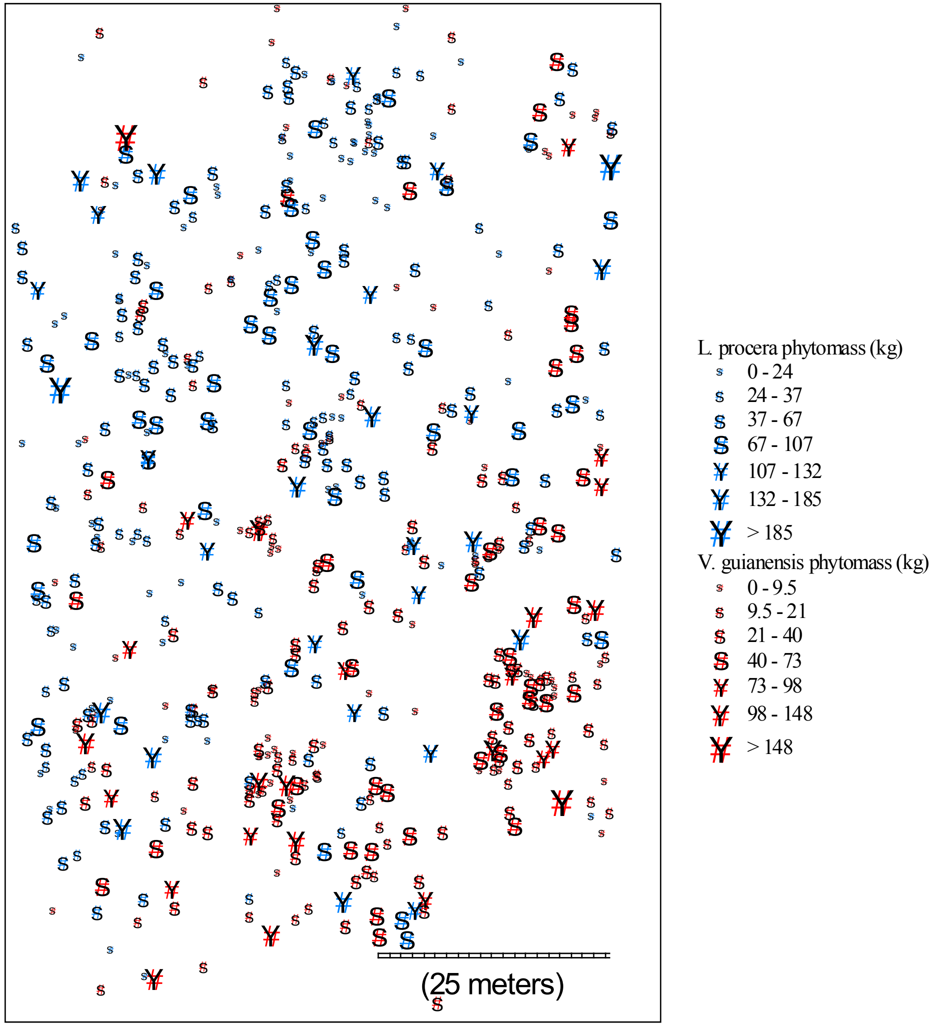


Map 10. Potentially N_2-fixing legumes in a primary forest (clay) (‘Francisco-PF’)

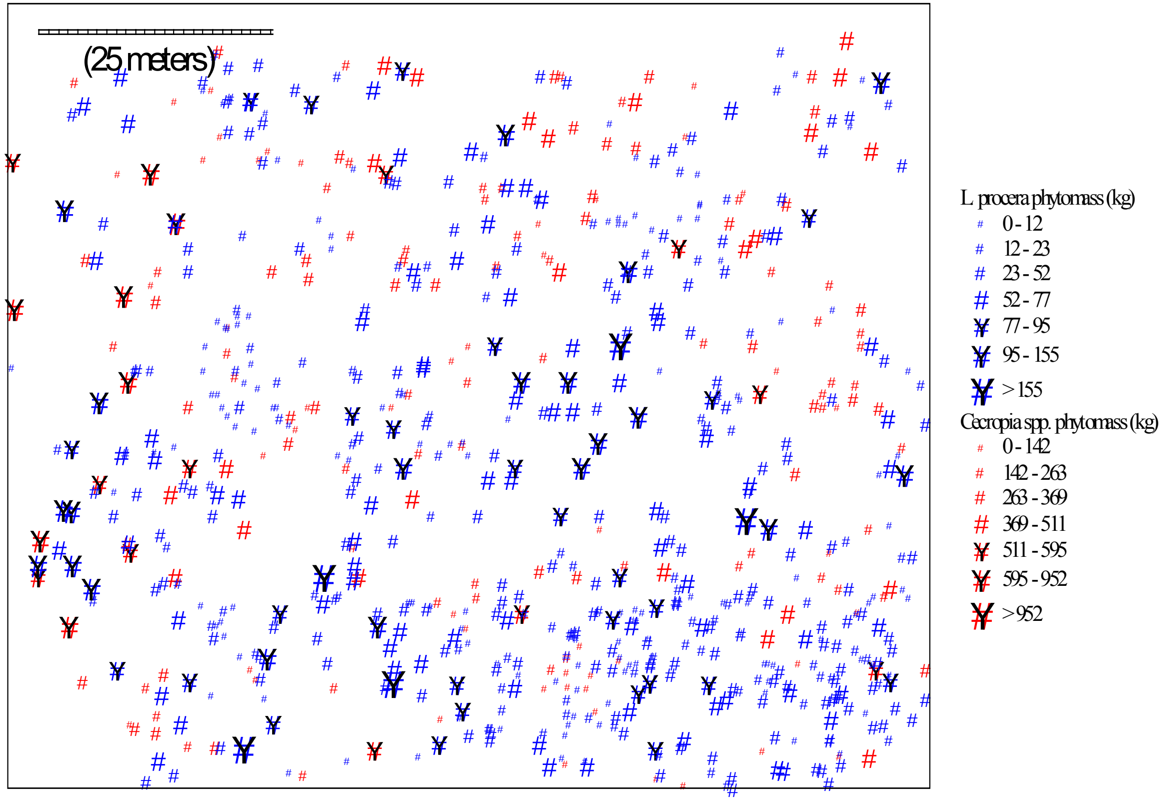


Map 11. Potentially N_2-fixing legumes in a campinarana forest (‘Maria’)

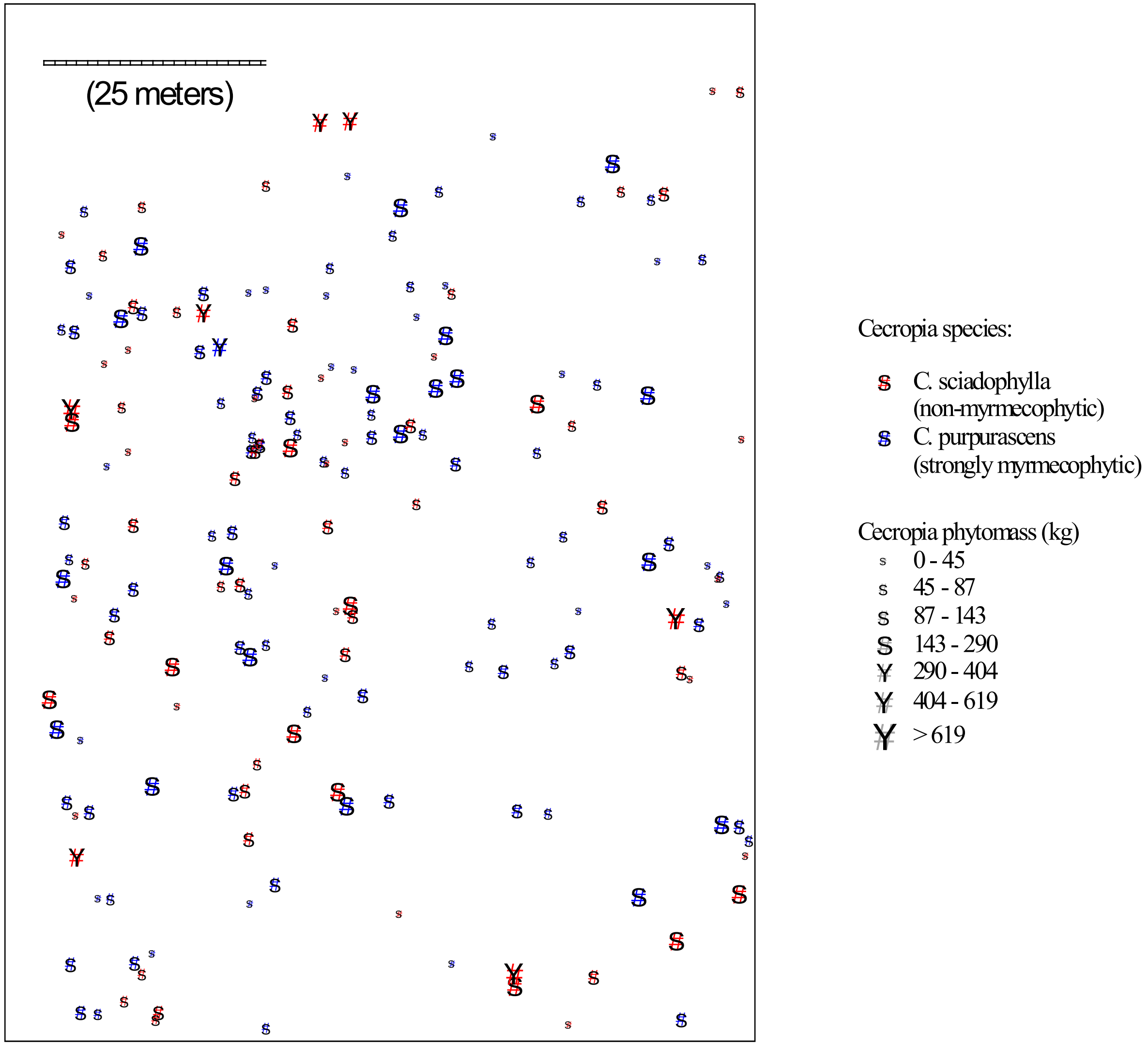
Appendix 7. Plant distribution patterns of selected non-legume pioneer trees
(plant biomass is scaled in seven percentile-levels of the respective species and site, see chap. 4.8)



Map 15. Distribution of *Laetia procera* and *Vismia guianensis* on an 11-yr.-old (degraded) regrowth ('Bispo')



Map 16. Distribution of *Laetia procera* and *Cecropia* spp. on a 10-yr.-old first-cycle regrowth ('Guillerme')



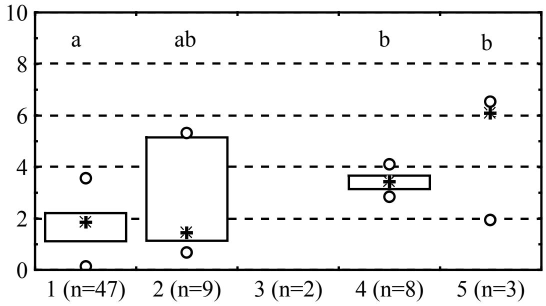
Map 17. Distribution of *Cecropia sciadophylla* (non-myrmecophytic) and *C. purpurascens* (strongly myrmecophytic) on a 20-yr.-old first-cycle regrowth (‘Jasiel’)

Appendix 8. Foliar δ^{15}N-signals of five 'functional groups' of plants in 19 sites: potentially N_2-fixing trees and lianas, non N_2-fixing legumes, non-legume trees and lianas (medians, 25-75 percentile range and minimum/maximum;
letters give statistical groupings of post-hoc comparisons when ANOVA $p<0.05$)

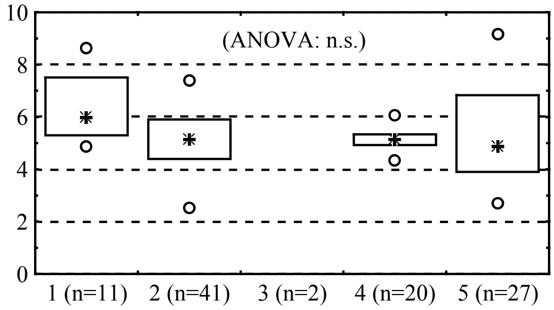
functional groups:
1: pot. N-fixing trees
2: pot. N-fixing lianas

3: non N-fixing legumes
4: non-legume trees
5: non-legume lianas

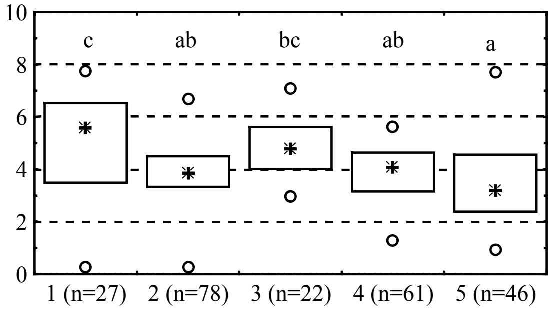
4 yrs. terra morena ('Aurelio')



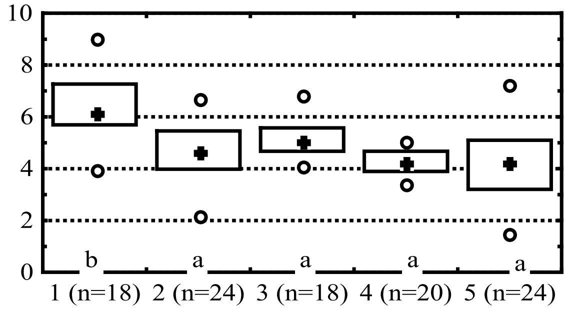
3 yrs. degraded ('Anna')



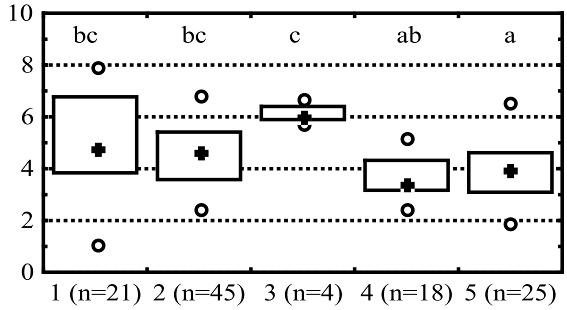
11 yrs. degraded ('Bispo')



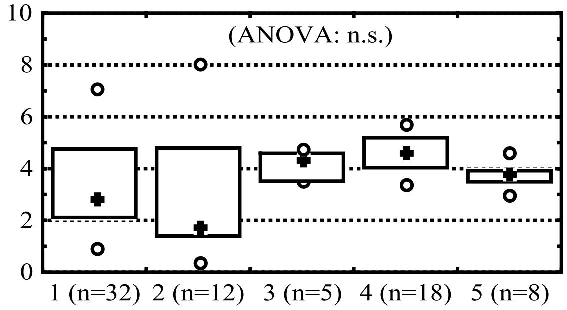
3-yr.-old ('Francisco')



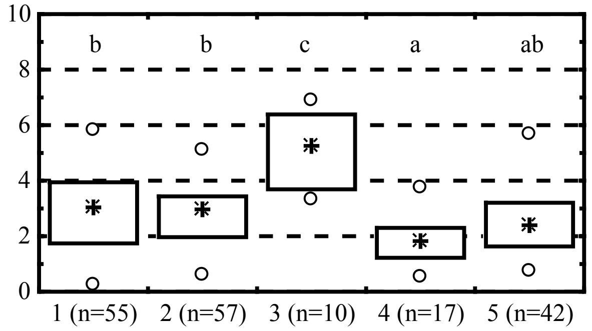
3-yr.-old ('Geraldo')



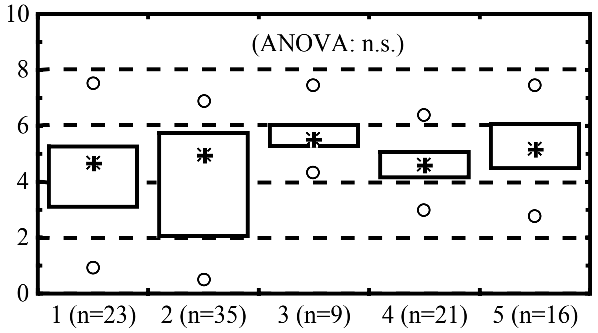
5-yr.-old ('Adventista')



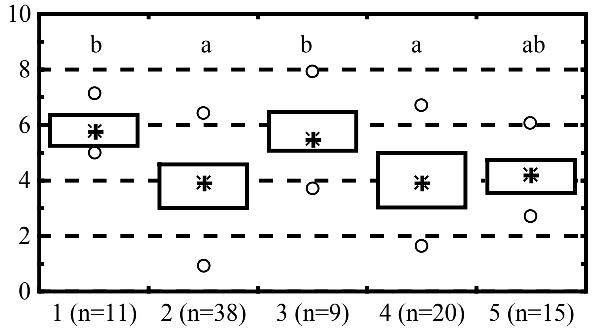
7 yrs. old ('Geraldo')



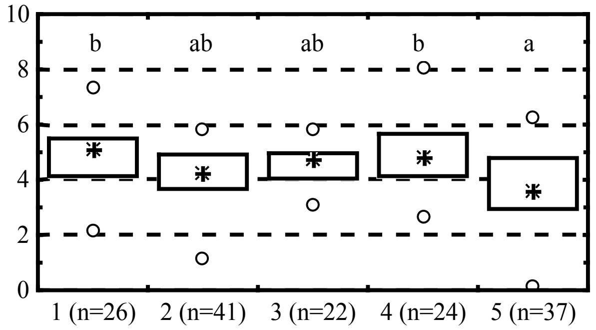
8 yrs. old ('Ilson')



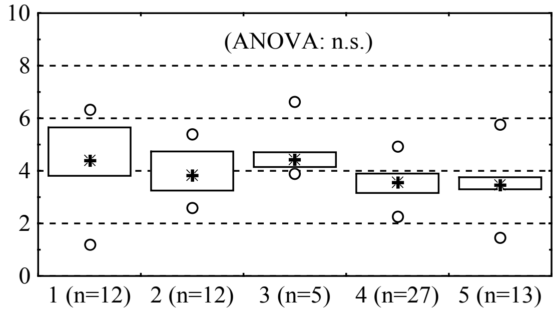
10 yrs. old ('Edson')



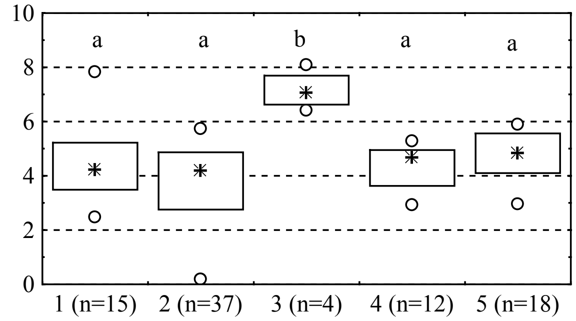
10 yrs. old ('Guillerme')



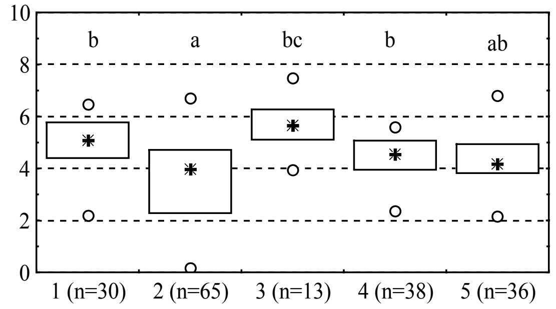
12 yrs. old ('Anna')



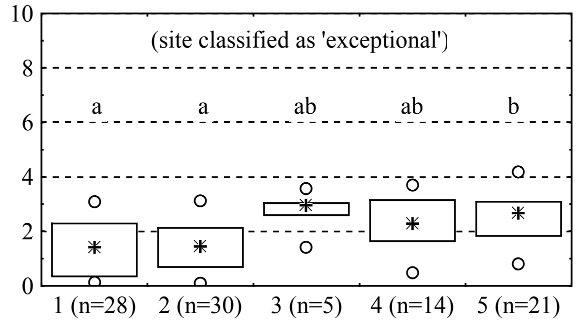
18 yrs. old ('Adventista')

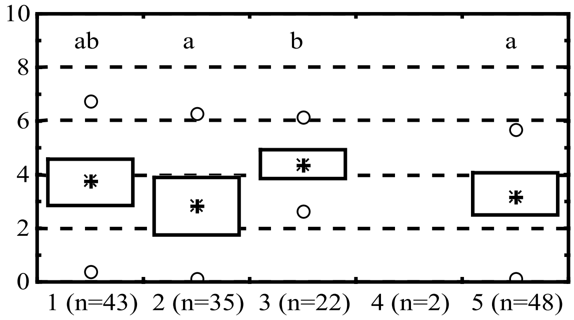


20 yrs. old ('Jasiel')

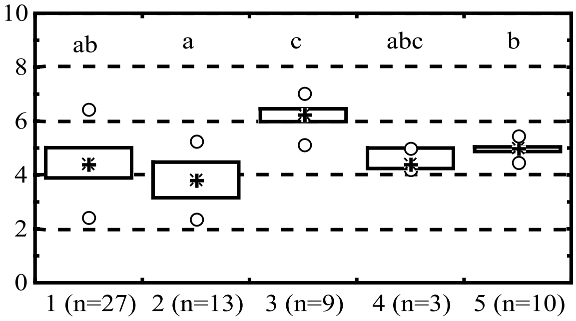


25 yrs. old ('Edson')

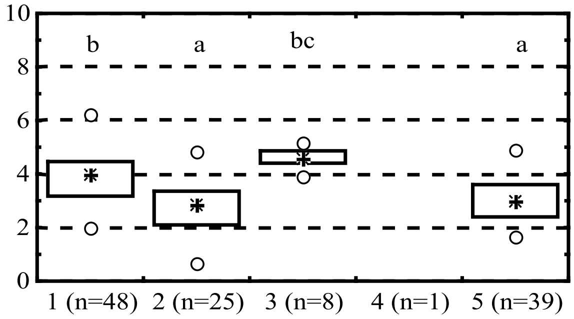




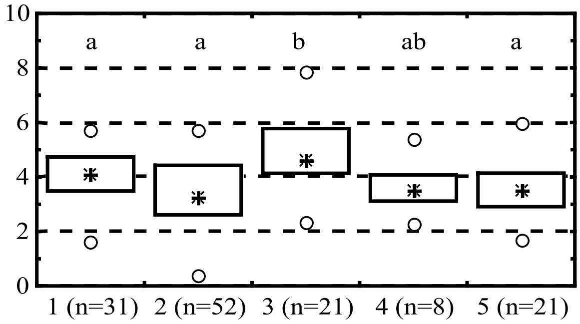
'Anna' primary forest



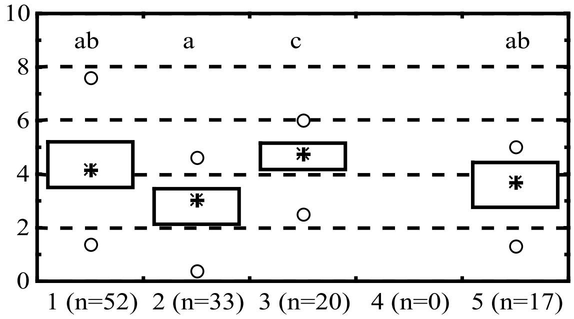
'Adventista' primary forest



'Edson' primary forest



'Francisco' primary forest



'Geraldo' primary forest

Appendix 9. Foliar nitrogen of potential N_2-fixers on 19 sites, and impact of individual plant size

	mean foliar nitrogen						impact of individual phytomass			
	pot. N_2-fixing trees			pot. N_2-fixing lianas			model: ln (phytomass) // $\delta^{15}N^{**}$			
	%N	$\delta^{15}N$	n	%N	$\delta^{15}N$	n	trees	n	lianas	n
4 yrs terra morena*	2.6	1.75	47	2.6	2.87	9	n.s.	47	-	9
5 yrs. (degraded)	3.2	5.46	6	3.1	5.15	41	-	6	n.s.	41
11 yrs. (degraded)	2.5	5.04	27	2.4	3.83	78	+0.71***	27	n.s.	78
3-yr.-old	2.3	6.37	18	2.3	4.69	24	+0.79***	18	n.s.	24
3-yr.-old	3.0	5.10	21	2.7	4.60	45	n.s.	21	n.s.	45
5-yr.-old	4.0	3.50	32	3.5	2.97	12	+0.44*	32	n.s.	12
7-yr.-old	2.8	2.99	55	2.6	2.74	57	n.s.	55	n.s.	57
8-yr.-old	3.7	4.38	23	3.1	4.22	35	n.s.	23	n.s.	35
10-yr.-old	3.5	5.86	11	3.2	3.81	38	n.s.	11	n.s.	38
10-yr.-old	2.2	4.86	26	2.1	4.10	41	n.s.	26	+0.29°	41
12-yr.-old	2.0	4.49	12	2.2	3.92	12	n.s.	12	n.s.	12
18-yr.-old	3.5	4.47	15	3.1	3.78	37	+0.50°	15	n.s.	37
20-yr.-old	2.3	4.92	29	2.4	3.65	65	n.s.	29	n.s.	65
25-yr.-old*	3.0	1.36	28	3.0	1.31	30	n.s.	28	n.s.	30
PF (clay)	2.3	3.72	43	2.2	2.99	35	n.s.	43	+0.31°	35
PF (clay)	2.9	3.96	31	2.9	3.36	52	+0.36*	31	+0.36*	52
PF (clay)	3.3	3.91	48	3.1	2.77	25	+0.27°	48	n.s.	25
PF (clay)	2.3	4.28	52	2.3	2.81	33	n.s.	52	+0.33°	33
PF (clay)	3.4	4.46	27	3.5	3.79	13	n.s.	27	+0.58*	13
all sites**	2.9	3.59	332	2.6	3.34	524	+0.20***	476	+0.22***	643
all regrowth**	3.0	3.52	175	2.6	3.49	374	+0.14*	275	+0.11*	485
all primary forest	2.8	3.66	157	2.7	2.99	150	+0.22**	201	+0.30***	158

* 'exceptional sites', see chapters 4.9.1 and 5.2.4

** Spearman correlations over all non-exceptional sites
significance-levels: ° ($p<0.07$) * ($p<0.05$) ** ($p<0.01$) *** ($p<0.001$) -: n<10 replicates

Appendix 10. Foliar nitrogen of potentially N$_2$-fixing species, and impact of individual plant size

	foliar nitrogen (mean over 17 sites)[1]			Impact of plant size[2] model: ln (phytomass) // foliar nitrogen	
	n	%N	$\delta^{15}N$	%N	$\delta^{15}N$
Inga edulis	6	2.9	4.16	-	-
Inga stipularis	57	2.3	4.25	n.s.	n.s.
Inga paraensis	82	2.6	4.79	n.s.	n.s.
Inga thibaudiana	4	2.4	4.50	-	-
Inga umbellifera	6	2.4	4.48	-	-
Inga cayennensis	66	2.8	3.56	-0.26*	+0.32**
Inga umbratica	60	2.5	4.46	n.s.	n.s.
Stryphnodendron pulcherrimum	26	4.2	3.99	n.s.	+0.42*
Stryphnodendron guianensis	5	4.1	4.55	-	-
Dimorphandra parviflora	45	3.5	4.65	-0.26°	n.s.
Swartzia ingifolia	39	2.9	3.57	n.s.	n.s.
Swartzia cuspidata	40	2.9	4.29	+0.41**	n.s.
Enterolobium schomburgkii	44	2.9	4.54	n.s.	+0.28°
Machaerium hoehneanum	279	2.8	4.16	+0.16*	+0.17**
Machaerium multifoliolatum	72	2.7	2.83	n.s.	n.s.
Machaerium madeirensis	108	2.2	3.62	+0.20**	+0.32***
Machaerium quinata	35	2.7	2.66	n.s.	n.s.
Machaerium ferox	74	2.5	3.31	n.s.	+0.34**
Dalbergia multiflora	28	2.8	3.28	n.s.	+0.39*
Derris negrensis	47	2.8	4.47	n.s.	+0.33**
all pot. N$_2$-fixers	1119	2.7	3.96	+0.11***	+0.22***
pot. N$_2$-fixing trees	476	2.9	4.28	+0.10**	+0.20***
pot. N$_2$-fixing lianas	643	2.7	3.73	+0.08*	+0.22***

1) excluding two 'exceptional' sites

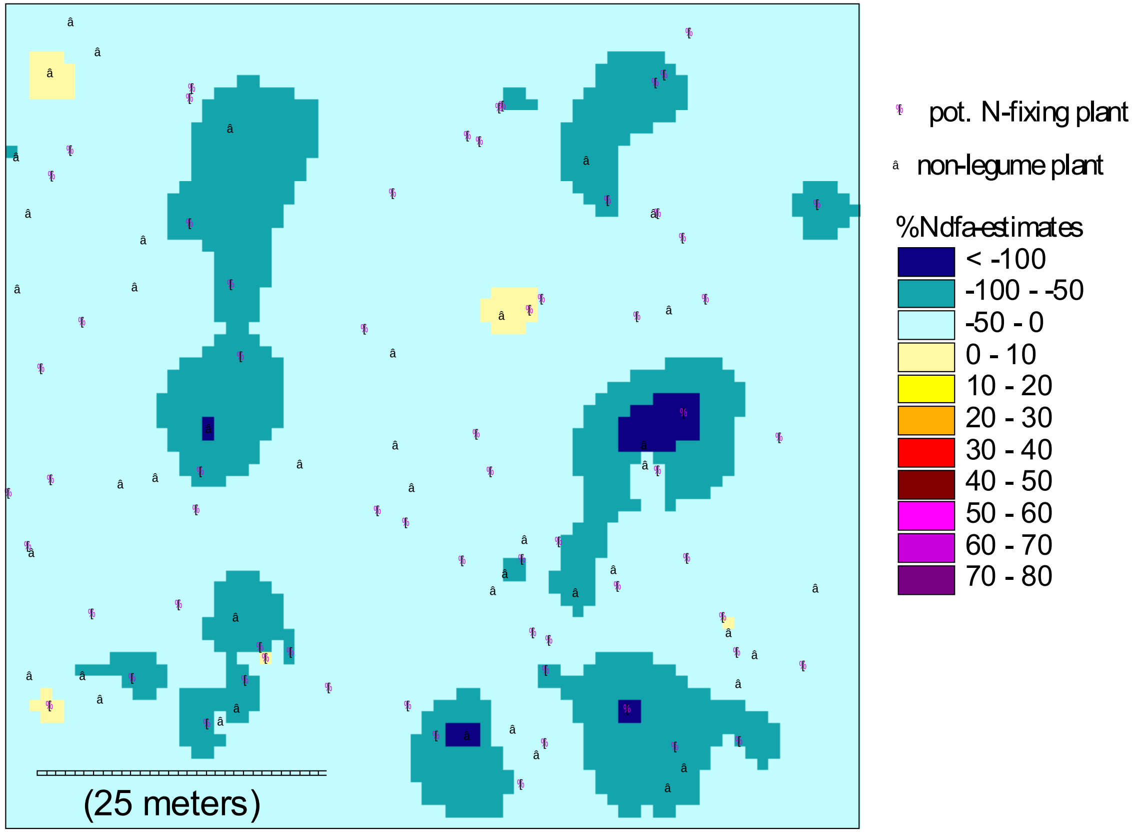
2) Spearman correlations
significance-levels: ° ($p<0.07$) * ($p<0.05$) ** ($p<0.01$) *** ($p<0.001$) -: n<10 replicates

Appendix 11. Percentage of pot. N_2-fixing legumes with negative %Ndfa-estimates:
differences between species and reference categories (in percent of all analyzed plants, minimum-n = 3 per species)

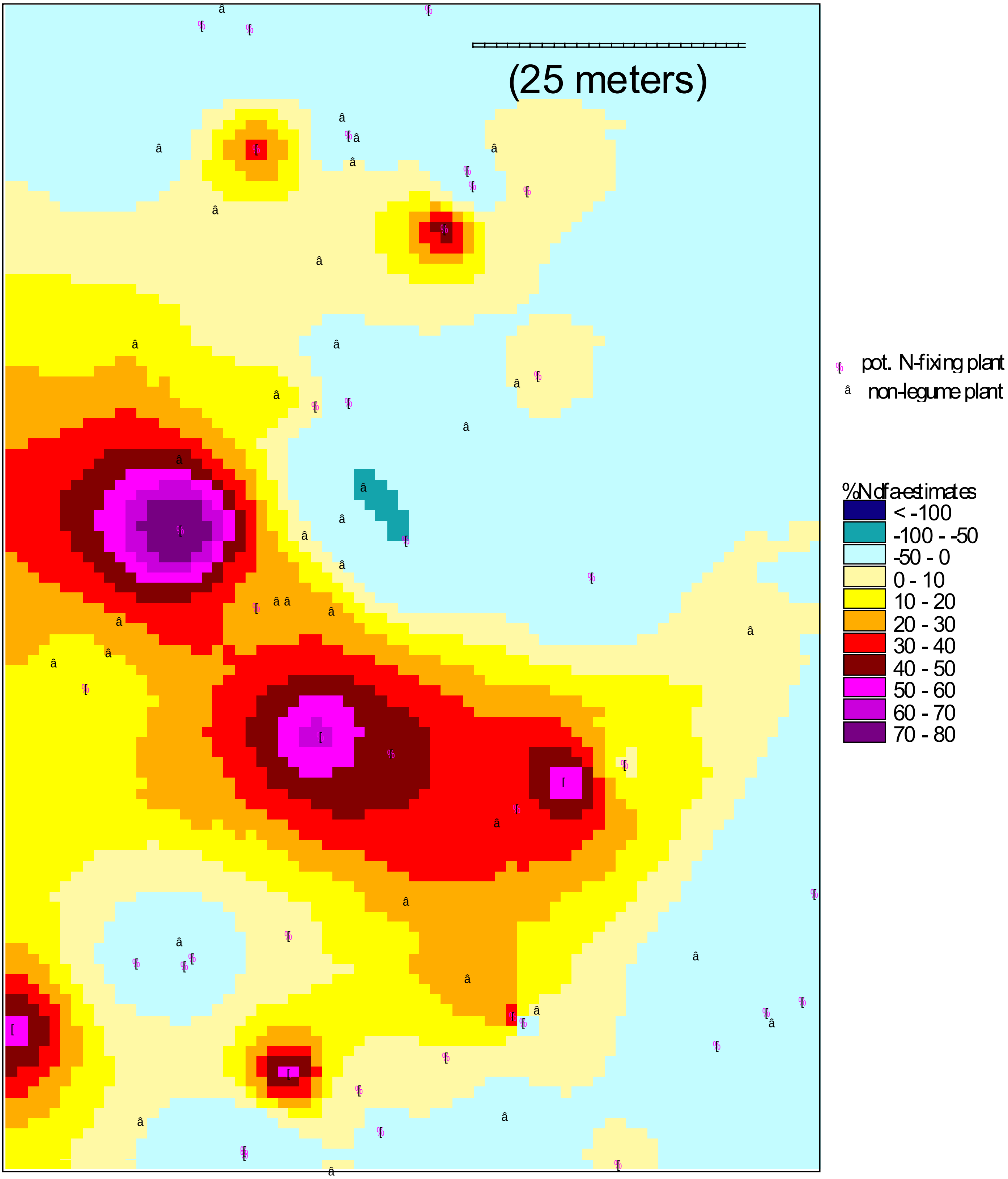
	3- to 20-yr.-old first-cycle regrowth (10 sites)					primary forest (clay) (5 sites)				
	non-legumes	legumes	*Vismia / Davilla*	*Zygia / Bauhinia*	leaf litter	non-legumes	legumes	*Vismia / Davilla*	*Zygia / Bauhinia*	leaf litter
Inga edulis	100	33	-	-	-	-	-	-	-	-
Inga stipularis	80	38	92	29	80	57	22	50	46	74
Inga paraensis	90	52	95	36	95	50	23	33	24	60
Inga thibaudiana	67	50	67	-	67	-	-	-	-	-
Inga umbellifera	60	20		33	60	-	-	-	-	-
Inga cayennensis	50	24	50	0	73	38	11	0	11	42
Inga umbratica	87	50	93	75	100	69	9	43	22	64
Stryphnodendron pulcherrimum	40	20	40	-	60	-	-	-	-	-
Stryphnodendron guianensis	60	20	60	-	100	-	-	-	-	-
Dimorphandra parviflora	68	23	68	25	76	100	61	50	67	89
Swartzia ingifolia	56	19	75	8	69	-	20	-	25	60
Swartzia cuspidata	77	19	77	10	81	33	27	-	0	82
Enterolobium schomburgkii	69	48	81	31	85	86	25	67	25	42
Machaerium hoehneanum	67	27	75	21	80	50	7	59	5	40
Machaerium multifoliolatum	24	3	24	0	38	8	0	20	0	13
Machaerium madeirensis	48	10	51	6	62	50	0	50	0	24
Machaerium quinata	22	0	26	0	39		0	-	0	0
Machaerium ferox	32	6	54	14	68	0	6	0	0	6
Dalbergia multiflora	21	0	21	0	36	-	-	-	-	-
Derris negrensis	74	17	79	-	89	25	15	-	0	38
all pot. N_2-fixers	60	22	66	19	74	46	14	39	15	47
pot. N_2-fixing trees	72	32	78	27	82	62	22	38	27	62
pot. N_2-fixing lianas	53	17	60	13	69	32	5	39	2	27

Appendix 12. Interpolated %Ndfa-estimates of exemplary sites

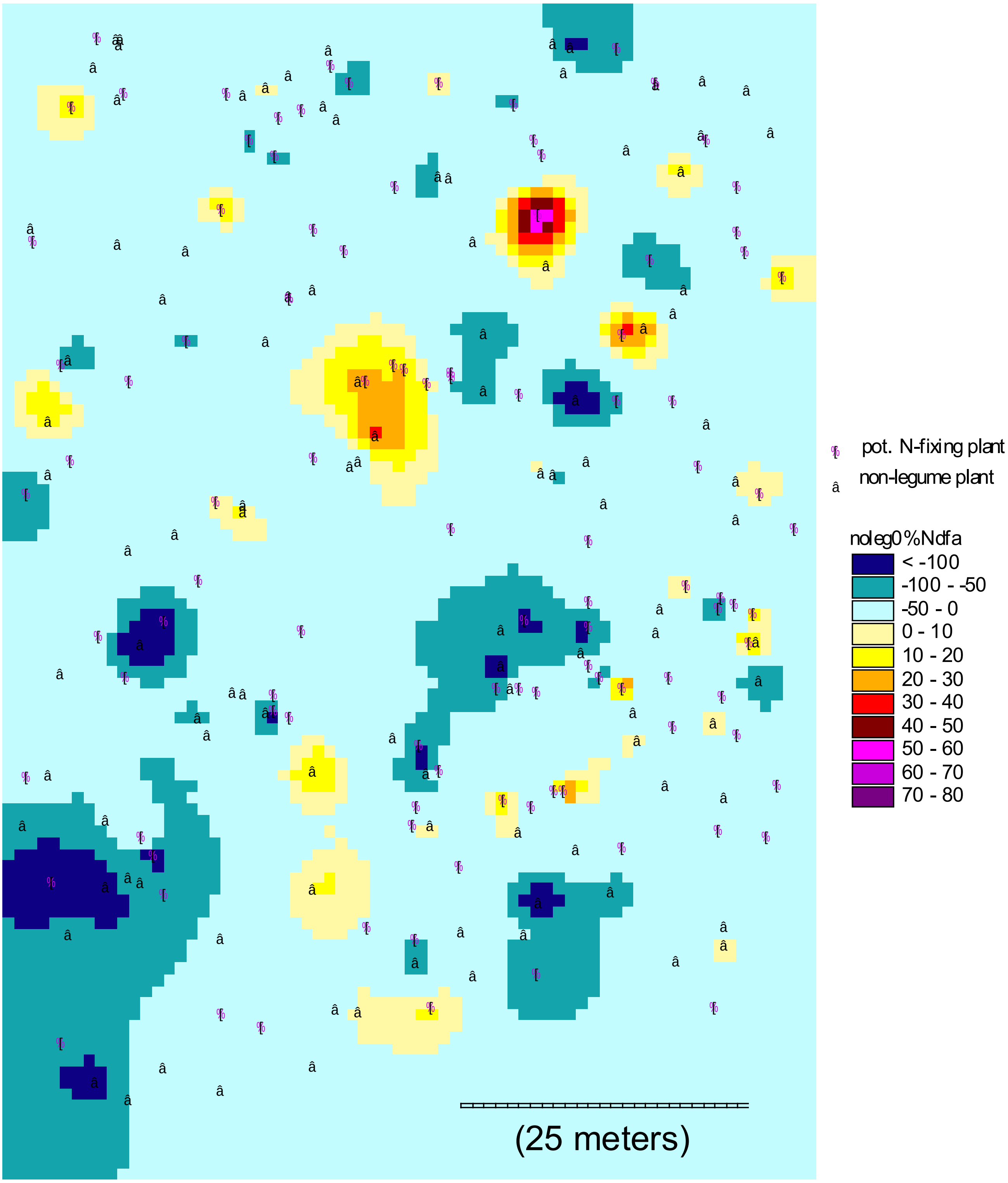
(assuming B=0‰, interpolation by IDW, purple squares and black circles indicate sampled plants, see chap. 5.8.1)



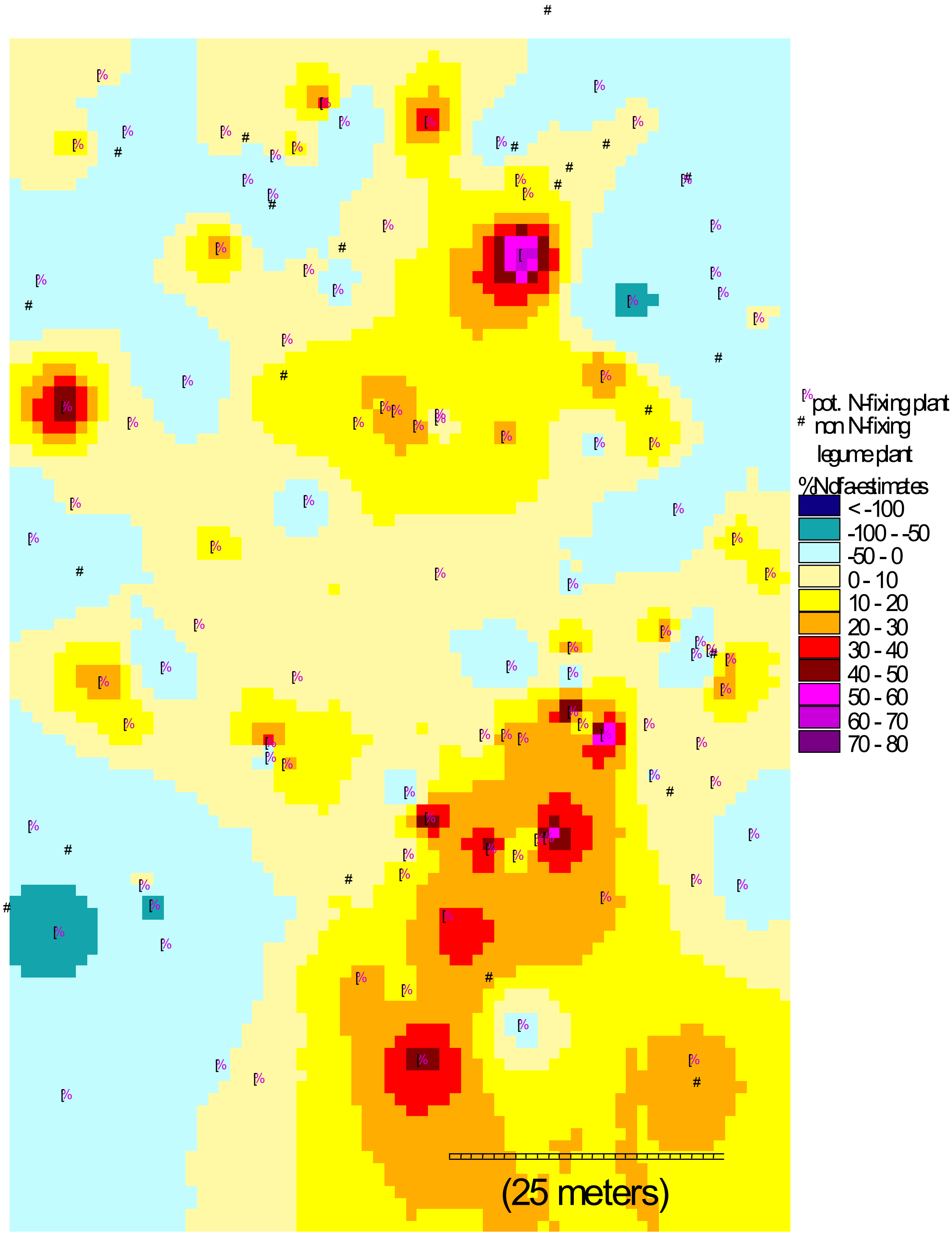
Map 24. %Ndfa-estimates for a 3-yr.-old first-cycle regrowth (‘Geraldo young’), based on non-legume reference



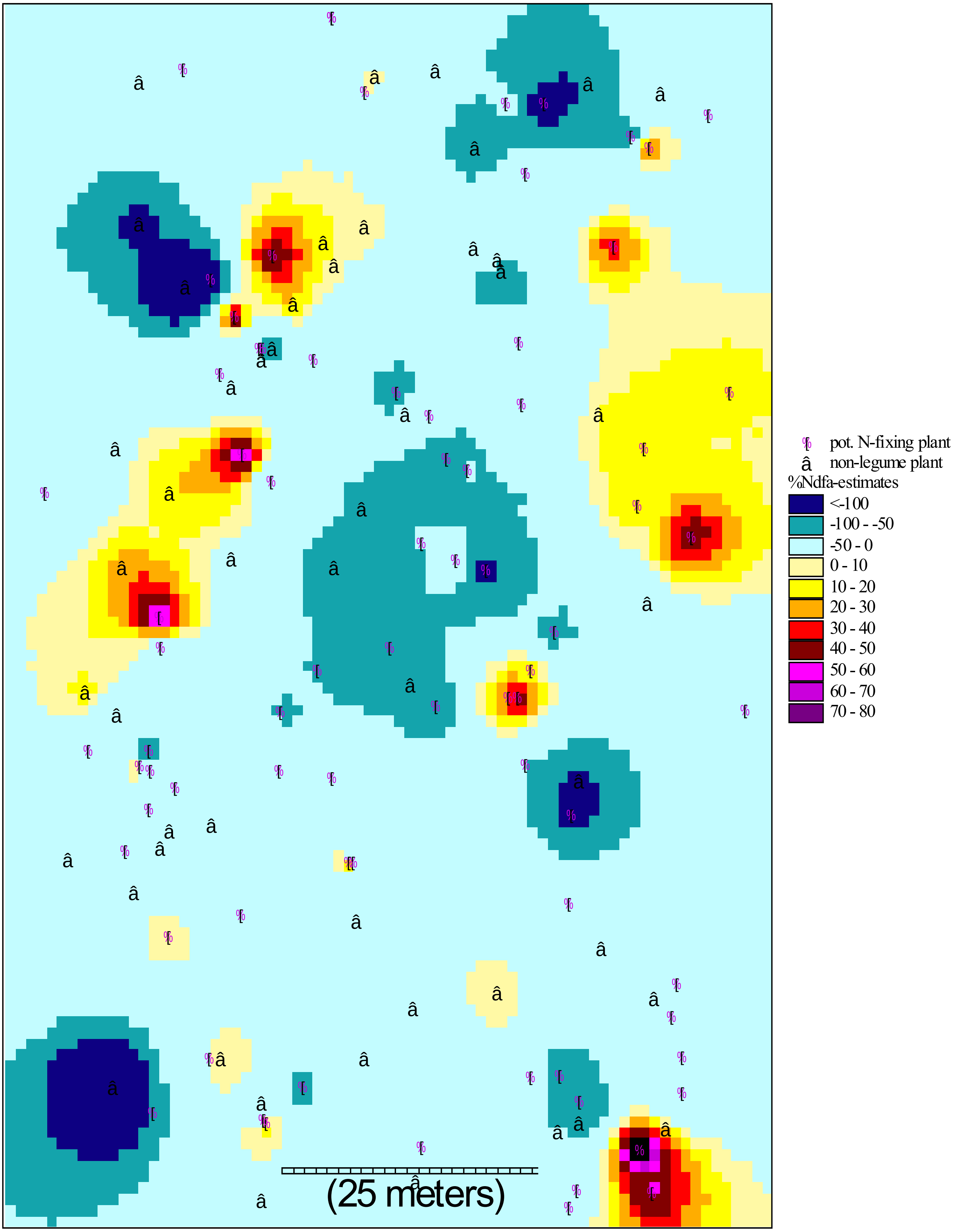
Map 25. %Ndfa-estimates for an 8-yr.-old first-cycle regrowth (‘Ilson’), based on non-legume reference



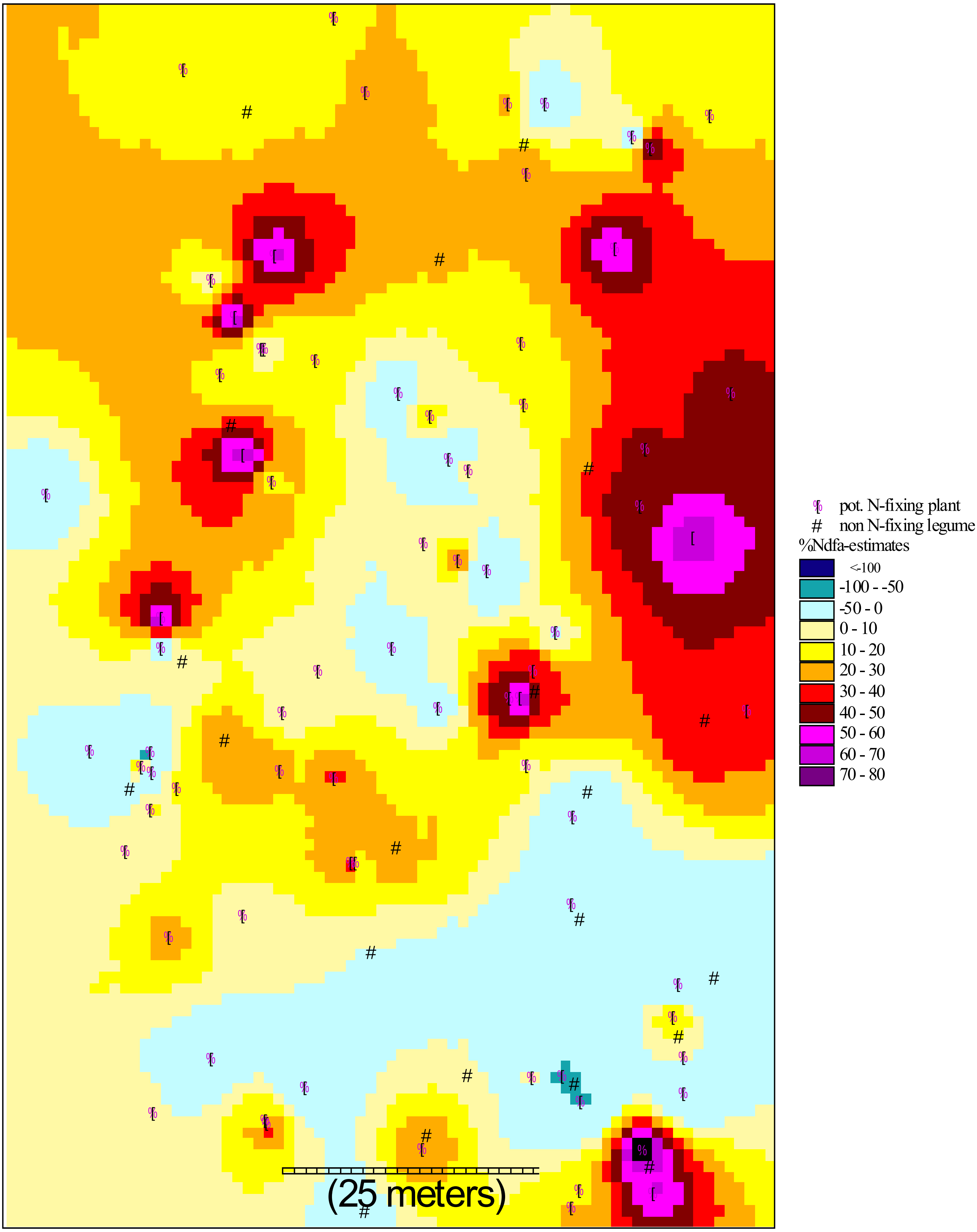
Map 26. %Ndfa-estimates for an 11-yr.-old degraded regrowth (‘Bispo’), based on non-legume reference



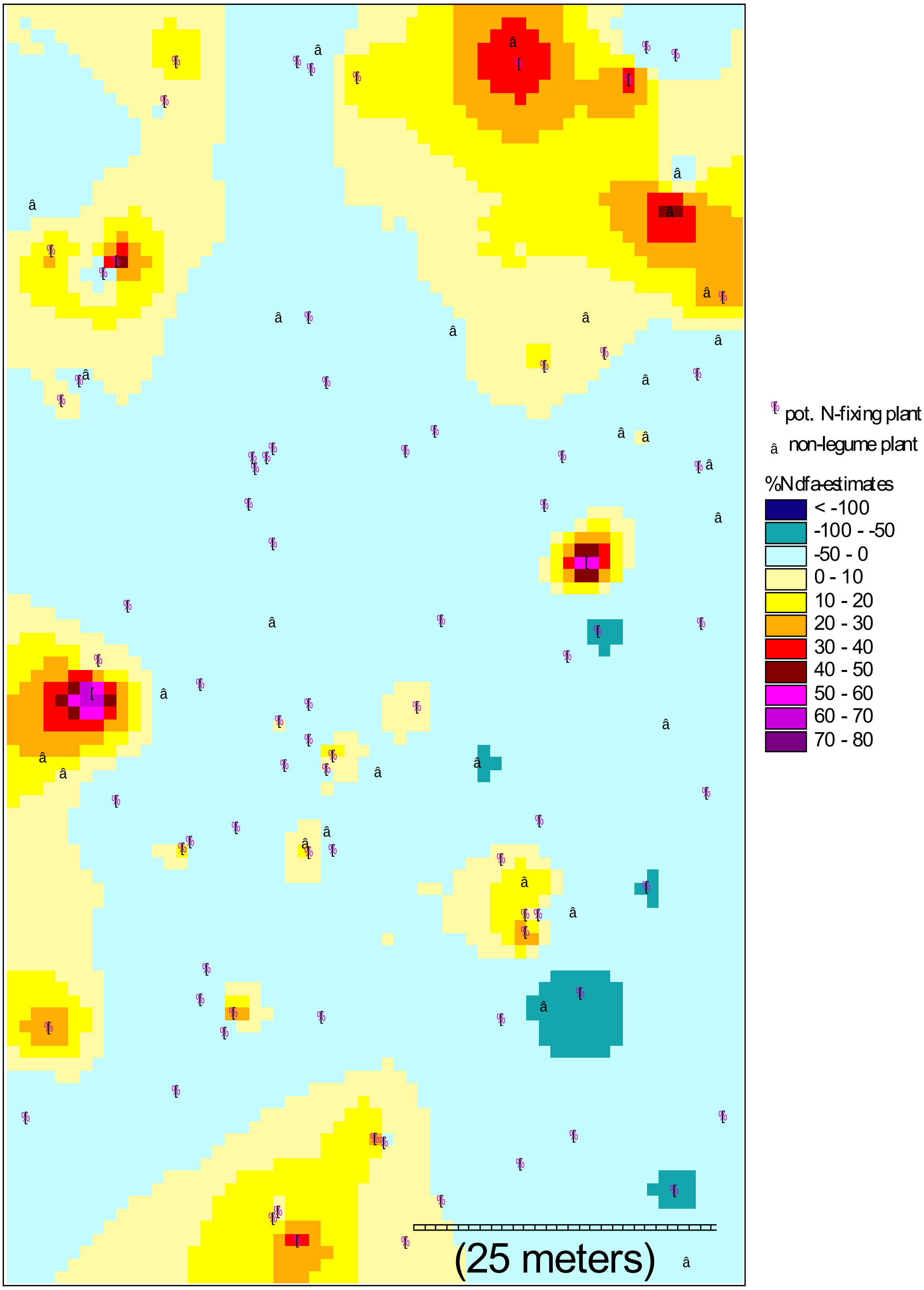
Map 27. %Ndfa-estimates for an 11-yr.-old degraded regrowth (‘Bispo’), based on non N_2-fixing legume reference



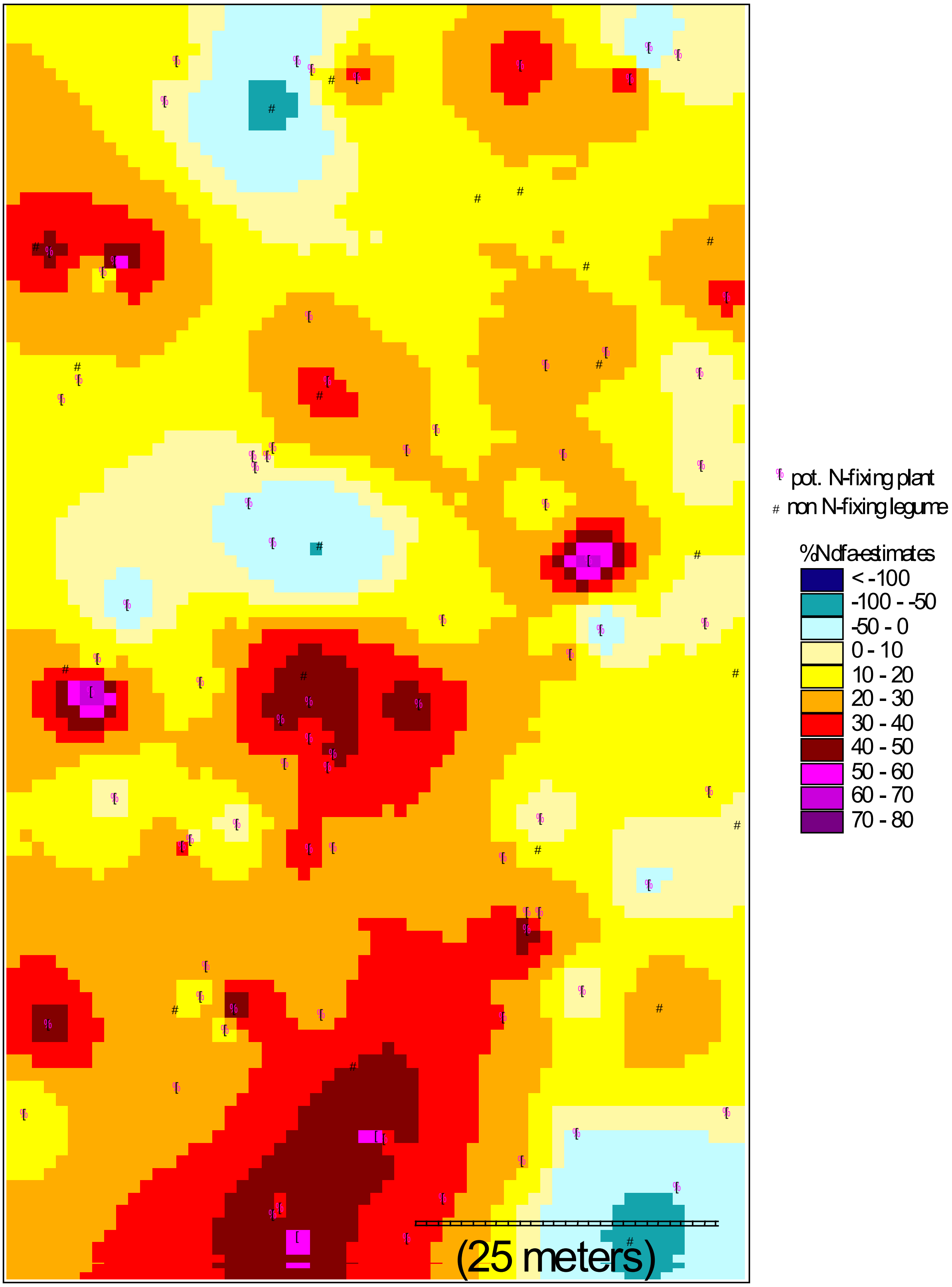
Map 28. %Ndfa-estimates for a primary forest (clay) ('Anna PF'), based on non-legume reference



Map 29. %Ndfa-estimates for a primary forest (clay) ('Anna PF'), based on non N_2-fixing legume reference



Map 30. %Ndfa-estimates for a primary forest (clay)
('Francisco PF'), based on non-legume reference



Map 31. %Ndfa-estimates for a primary forest (clay) ('Francisco PF'), based on non N_2-fixing legume reference