

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 $\frac{1}{3}$ (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 N₂-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 (Burriss 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 $\delta^{15}\text{N}$ -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 N₂-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 ¹⁵N natural abundance method'

2.5.1 General considerations

This study uses the '¹⁵N 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 ¹⁵N-enrichment ('dilution') method is hampered by the need of homogenous ¹⁵N-enrichment, both throughout the soil profile and over the major N-pools available to vegetation (Fried et al., 1983; Boddey et al., 1995).