

1 INTRODUCTION

Current forest cover estimates for Indonesia range from 0.9 to 1.2 million km², or 48 to 69% of Indonesia's total land area of 1.9 million km². This represents 10% of all tropical forests in the world and nearly half of tropical Asia's remaining forest (FAO 2001a). However, the annual deforestation rate has increased from 1.2 million ha at the beginning of the 1990's (FAO 1993) to 1.3 million ha, or 1.2% of the country's forest cover at the end of the 1990's (FAO 2001a). This increase is driven by industrial and logging operations, population pressure, transmigration programs, and expanding agriculture. Furthermore, the road network built for commercial logging, which provides access to forest areas, increases the fragmentation of the forest (Sunderlin 1999) and leads to an expansion of margin areas, consequently increasing the vulnerability of the forest (Skole and Tucker 1993). The stability of rainforest margins is a critical factor in the preservation of tropical forests. Thus, one of the most important challenges is to find solutions for stabilizing these margin areas. Agroforestry may offer economically viable windows of opportunity for the sustainable use of tropical forests (Wassmann and Vlek 2003).

Nitrogen (N) is one of the most important nutrients affecting crop yields. However, low levels of N are often a limiting factor especially in developing countries (Crews 1999). Natural or anthropogenic disturbances are associated with drastic reductions in biomass and loss of N from the system. In shifting land-use systems, for instance, N rapidly becomes one of the growth-limiting nutrients causing the abandonment of the land (Franco and de Faria 1997). Slashing and burning 3.5- and 7-year-old secondary forest in the Eastern Amazon resulted in a negative N balance of about 291 and 403 kg N ha⁻¹, respectively (Sommer et al. 2004). Furthermore, in more open bush vegetation and grassland, repeated burning volatilized between 56 and 90 % of the aboveground N pool (Kauffman et al. 1994). Sustained land productivity and high crop yields can only be maintained by replenishing the soil nutrients removed by harvested products or lost via erosion, leaching, runoff, or volatilization; the replenishment should provide at least an equivalent amount of inputs, either from fertilizer and manure, or from natural processes. Therefore, the strategy of closing the nutrient cycle at the farm level through integrated soil fertility management by returning

residue, fallowing fields and enhancing biological nitrogen fixation (BNF) in farming practices is essential for sustainability (Vlek et al. 1997).

In the tropics, cultivation of cacao (*Theobroma cacao* L.) with N₂ fixing trees (NFTs) such as Gliricidia (*Gliricidia sepium* (Jacq.) Walp) as shade trees has been practiced for generations. In this agroforestry system, NFTs are mainly used to reduce the heat stress of the cacao through the amelioration of the micro-climatic conditions (Beer et al. 1998). In addition, NFTs provide a possible solution for the restoration and maintenance of soil fertility, the reduction of soil erosion, and the maintenance of productivity for a longer period of time (Khanna 1998; Jose et al. 2000). Studies carried out in coffee and cacao plantations in Latin America showed that the aboveground N from NFTs varied from 60-340 kg N ha⁻¹ yr⁻¹ (Beer 1988). Roskoski and van Kessel (1985) reported N₂ fixation between 35-60 kg N ha⁻¹ yr⁻¹ by different NFTs in an unfertilized coffee and cacao plantation. Meanwhile, BNF by Gliricidia was estimated to range between 100 and 300 kg N ha⁻¹ yr⁻¹ (Khanna 1998; Franco and de Faria 1997; Sanginga et al. 1995). Thus, N₂ fixation by Gliricidia can play a major role for the input of N into the soil. However, shade trees also have physiological drawbacks, such as competition that reduces the production of the main crops. As a consequence, farmers often alter their cacao agroforestry system to “full-sun” cacao monoculture without NFTs. This practice leads to a short-term increase in cacao production, but in the long run it increases stress and the need for nutrients, especially N, and pesticides, and reduces the period of productivity (Beer et al. 1998; Siebert 2002). The farmers’ lack of information regarding the amount of N₂ fixed by the NFTs and their contribution to the N economy of the cacao agroforestry systems is the main reason for this practice. This lack of information is not only due to the low economic value of shrub and leguminous trees compared to food grain legumes (Moufhe and Dakora 1999), but also to the fact that N₂ fixation by NFTs is difficult to measure (Boddey et al. 2000).

It is clear that BNF can play a major role in the restoration of soil fertility in agroecosystems. However, its role in a specific cacao agroforestry system where the NFTs such as Gliricidia are mainly used as shade trees remains unclear. Field research on this question is very limited and mainly based on the assumption that NFTs will fix N₂ from the atmosphere, thus increasing the availability of N to the system. However, there are still some open questions such as: Does Gliricidia play a major role in the

restoration of soil fertility in cacao agroforestry systems? How much N_2 is fixed by the *Gliricidia* annually? And what are the consequent implications for management practices (e.g. pruning and litterfall input)? In order to answer these questions, quantifying the proportion of N derived from atmospheric N_2 (%Ndfa) in the N balance of the cacao agroforestry system is crucial.

There are several methods for estimating N_2 fixation in NFTs such as the total N difference method (Gauthier et al. 1985; Ndoye and Dreyfus 1988), the acetylene reduction assay method (Roskoski 1981; Roskoski et al. 1982), or the ureide assay method (Herridge et al. 1994; Peoples et al. 1996). These methods, except the N difference method that is direct criteria, are based on indirect, qualitative, yield-dependent criteria, and furthermore, not all NFTs carry ureides in their xylem sap. Therefore, the ^{15}N isotope method has become a widely used technique for estimating N_2 fixation in legumes, because it provides yield-independent and time-integrated estimates of %Ndfa (Chalk 1985; Shearer and Kohl 1986; Peoples et al. 1995; Boddey et al. 2000). The ^{15}N isotope method depends upon differences in isotopic composition of the sources of N available for plant growth, i.e., soil N, fertilizer N and atmospheric N_2 (Bergersen and Turner 1983). There are two main variations of the technique: One involves enrichment of soil N by addition of ^{15}N -enriched fertilizers (^{15}N enrichment method, ^{15}NEM), and the other makes use of the natural ^{15}N enrichment of available soil N (^{15}N natural abundance method, ^{15}N NAM).

The ^{15}NEM is widely used and has found widespread acceptability for annual crops and herbaceous forage legumes. It has also been reviewed by Chalk (1985), Danso (1988), Witty et al. (1988) and Giller and Wilson (1991). The underlying assumption of the technique is that fixing and reference plants absorb the same relative amount of nitrogen of ^{15}N -enriched fertilizer from the soil; in theory the added enriched fertilizer must be homogeneously distributed vertically and horizontally over the rooting zone of the plants. Some reviewers point out that in a number of cases this assumption is violated, producing considerable errors in the estimation of BNF. This is especially true for natural conditions encountered in the field, when woody, deep-rooting perennials are involved extracting different (possibly non-labeled) pools of N (Danso et al. 1992; Parotta et al. 1994). Although some “ideal” reference plants have been defined in the literature (Witty 1983; Danso et al. 1992), it is difficult to choose an appropriate

reference plant to satisfy the requirements in the case of long-term studies with NFTs, especially in a permanent system such as cacao agroforestry, where only two or three plant species are available. This may also not be easily or consistently met when faced with a rapid change of ^{15}N enrichment in the soil both with time and depth (Chalk 1985; Giller and Wilson 1991; Danso et al. 1992).

The ^{15}N NNAM is, on the other hand, seen as the most promising methodology for quantifying the contribution of N_2 fixation in natural systems (Boddey et al. 2000). It is based on the difference in $\delta^{15}\text{N}$ values (‰, between the two sources of N nutrition, soil-mineral N and atmospheric N_2 calculated as $1000 \times (\text{atom}\% \text{ }^{15}\text{N} \text{ sample} - \text{atom}\% \text{ }^{15}\text{N} \text{ reference}) / \text{atom}\% \text{ }^{15}\text{N} \text{ reference}$, with $\text{atom}\% \text{ }^{15}\text{N} \text{ reference}$ at 0.3663 ‰. The accuracy of the estimates of N_2 fixation using this technique is influenced by the degree and uniformity of the $\delta^{15}\text{N}$ values in the plant-available soil N (Shearer and Kohl 1986; Gathumbi et al. 2002). In many cases, the variation of $\delta^{15}\text{N}$ values of total N in the soil is small and reasonably uniform and stable with time (Högberg 1997); therefore, the choice of a reference plant appears less critical. However, the $\delta^{15}\text{N}$ value of the plant-available soil N may vary spatially and temporally (Ledgard et al. 1984), which complicates the assessment of N_2 fixation by NFTs. Though the ^{15}N pool is not enriched artificially, isotopic discrimination is the main bottleneck in this method (Sutherland et al. 1993; Androssoff et al. 1995). A minimum of 2 ‰ $\delta^{15}\text{N}$ unit differences between the plant-available soil N (detected in reference plant) and atmospheric N_2 (detected in fixing plant) is recommended (Unkovich et al. 1994). Shearer and Kohl (1986) recommend a minimum 5-7 ‰ $\delta^{15}\text{N}$ value for plant-available soil N, which appears to be more adequate given the potential problem with spatial variability and isotopic discrimination. Gathumbi et al. (2002) suggest a >5 ‰ $\delta^{15}\text{N}$ value of plant-available soil N for tree-based fallow systems. Additionally, variation in the $\delta^{15}\text{N}$ value of the legumes under study and seasonal changes as well as differences between plant compartments might cause uncertainties, a fact widely recognized in the literature (Shearer et al. 1983; Bremer and van Kessel 1990; Sutherland et al. 1991; Unkovich et al. 1994; Pate et al. 1994; Sanginga et al. 1996).

A number of field and greenhouse studies have compared the ^{15}N NEM and the ^{15}N NNAM for estimating %Ndfa in different legume species under a variety of growing conditions. Both methods provide similar estimates of N_2 fixation (Bremer and van

Kessel 1990; Peoples et al. 1996; Cadisch et al. 2000). However, a good agreement does not always imply that both methods provide a correct estimate (Hamilton et al. 1993; Cadisch et al. 2000), as correlations between the two methods in individual estimates are poor (Androssoft et al. 1995; Stevenson et al. 1995). This is probably caused by a high spatial variability in controlling environmental variables (Boddey et al. 2000; Walley et al. 2001). In addition, a small standard error of the estimate does not always indicate that the estimate is accurate (Witty and Ritz 1984). Furthermore, most experiments on different methods report the overall average of the estimates and it remains unclear whether individual values are correlated or not.

Based on the problems discussed above, this study was conducted to test the hypotheses that

- (1) Gliricidia can play a major role in cacao agroforestry systems not only as a shade tree but also for the restoration of soil N fertility;
- (2) The $^{15}\text{NNAM}$ can be used successfully to estimate %Ndfa of Gliricidia in cacao agroforestry systems as an alternative to the ^{15}NEM , as long as appropriate precautions are taken.

Therefore, the objectives of this study are:

- (I) to quantify the %Ndfa of Gliricidia in a cacao agroforestry system, and
- (II) to compare ^{15}NEM and $^{15}\text{NNAM}$ in estimating the %Ndfa of Gliricidia in a cacao agroforestry system.

2 LITERATURE REVIEW

2.1 Biological nitrogen fixation

Frequently, subsistence farmers face the problem that the capacity of their soil to supply N declines rapidly once agriculture intensifies (Herridge et al. 1994). This condition is more often encountered in the tropics, where many soils are extremely fragile and give very poor yields after only a few years of cultivation without expensive fertilizer inputs. Soil erosion and further decline in soil nutrient status is often a consequence. Therefore, to conserve productivity and to achieve sustainable management, it is necessary to replenish nutrients that have been removed or lost from the soil. In the case of N, this can be achieved either by applying nitrogenous fertilizers or through BNF (Peoples et al. 1995). However, the working concept of sustainable agriculture for tropical developing countries aims to avoid the excessive use of mineral fertilizers, energy and pesticides. For that reason, and since atmospheric N₂ is a virtually inexhaustible source, and the energy used for BNF is 'free', the use of BNF is the most 'environmentally friendly' approach to supply N and organic matter to an agroecosystem (Bohloul et al. 1992; Danso et al. 1992). It also plays a role in reducing the production risk and in the management of the agriculture resource base (Giller and Cadisch 1995). In contrast to the optimistic view that soil-improving legumes may play a significant role in agricultural systems, there are also growing concerns that the use of soil-improving legumes is declining worldwide (Becker et al. 1995)

Galloway et al. (1995) estimated the total annual global bio-fixation in agroecosystems at around 40-48 million Mg N yr⁻¹, which represents almost half of the 90 million Mg N yr⁻¹ from industrial fertilizer production (FAO 2001b), and approximately 20 % of all N available to the world's crops (Smil 2002). This means that a great effort would be necessary if BNF were to replace industrial fertilizers (Bumb 1995). On the other hand, with recent international emphasis on environmentally sustainable development and the use of renewable resources, greater attention will be given to the role of BNF in supplying N for agriculture (Peoples et al. 1995).

Biological nitrogen fixation, without doubt, improves the N status of soil. But this does not mean that legumes always contribute large amounts of N to the soils in which they grow. Perennial agroforestry systems are especially predestinated to provide

a more positive N balance and to minimize N losses to the environment (Peoples et al. 1995). Biological nitrogen fixation can also be controlled by manipulating various physical, environmental, nutritional, and biological factors (Hansen 1994) and may be more open to management (Khanna 1998). The use of inorganic N-fertilizer is on the other hand regulated by economic considerations, the fertilizer itself is generally utilized inefficiently by crops, and there is an increasing awareness of the environmental costs involved (Craswell and Godwin 1984; Peoples et al. 1995).

The fixation of the unavailable gaseous form of N₂ from the atmosphere into forms that higher plants can use (either NH₄⁺ or NO₃⁻) is mediated by: (1) bacteria in symbiotic relationship with vascular plants, (2) symbiosis between cyanobacteria and fungi (lichen) or plants, (3) free-living heterotrophic or autotrophic bacteria that are typically associated with soil or detritus, and (4) abiotic reactions in the atmosphere associated with lightning (Sprent and Sprent 1990). Biological nitrogen fixation by symbiosis of legumes with the bacteria *Rhizobium/Bradyrhizobium*, by rhizosphere associations (e.g., with the *Gramineae* or the *Ulmaceae*) and by free-living microorganisms in the phyllosphere and in the soil varies widely regarding their quantitative importance. Of those inputs, symbiotic N₂ fixation by legume-rhizobia association provides the largest inputs of N for agriculture (Peoples et al. 2002) and is an important component of the N cycle in agroecosystems.

2.1.1 Biological nitrogen fixation in agroforestry systems

In the conceptual development of ecologically sustainable production, systems that require minimal industrial inputs, such as agroforestry systems with legumes and non-legume crops, have raised much hope in recent years. The energy crisis will continue to increase the cost of chemically fixed nitrogen (Burrish 1999). Hardy (1980) estimated that 1-2 % of the world's fossil energy is used for fertilizer. Therefore, integrating trees, especially NFTs, into agroforestry systems can make a major contribution to sustainable agriculture (Giller and Wilson 1991; Cinnamani 1993; Peoples et al. 1995; Palm 1995). Nitrogen fixing trees are especially valued for their ability to grow in soils poor in N (Boddey et al. 1997; Bhatia et al. 2001).

The use of legumes as shade trees is one of the earliest examples of the use of NFTs in agroforestry (Giller 2001) despite the fact that in this system, NFTs are mainly

used to protect the main crops, e.g., cacao or coffee, from the ‘full’ sun, rather than as a source of N to improve soil fertility. Two factors regulate the amount of N₂ fixed by legumes: the amount of N accumulated during growth, and the proportion of N that is derived from symbiotic N₂ fixation (Peoples et al. 1997). Transfer of N from N₂-fixing plants to non-N₂-fixing plants in mixed stands can occur in various ways, i.e., aboveground processes influenced by the production of litter fall (or pruning), its nutrient content, and the rate of decomposition, and belowground processes influenced by the turnover of fine roots and nodules (Khanna 1998). On the other hand, competition between plants for light, space, nutrients, and water in many cases reduces the productivity of the main crops.

Little information is available on NTFs regarding their lower economic value compared to food grain legumes (Muofhe and Dakora 2000). The problems associated with BNF measurement are much more complex with NTFs than with annual crops, largely due to the size of trees and their perennial nature (Danso et al. 1992), which make an assessment of total biomass and N content difficult. Moreover, high variability may exist among isolines and provenances (Sanginga et al. 1992). Hence only few studies have been conducted on BNF in trees.

2.1.2 Gliricidia (*Gliricidia sepium* [Jacq.] Walp.)

Gliricidia, also named “madre de cacao” (Spanish for “mother of cacao”) to describe its use as a cacao shade tree, is one of the most common multipurpose woody legumes throughout the tropics. It is a member of the sub-family *Papilionoideae* and of the tribe *Robinieae* (Lavin 1987). It is closely related to but not synonymous with a less common white flowered taxon, *Gliricidia maculata*. It is native to the Pacific coast of Central America and Mexico (Simons and Stewart 1998; Nygren et al. 2000). In Indonesia, especially in Sulawesi, most cacao agroforestry systems use Gliricidia as a shade tree due to its resistance to the defoliating psyllid (*Heterosphylla cubana*), which has devastated the NTF *Leucaena leucocephala* in cacao and coffee agroforestry systems in Sumatra (Swaminathan 1987).

Gliricidia residue is rich in N. With low concentrations of lignin and active polyphenol, it decomposes rapidly (Handayanto et al. 1994; Vanlauwe 1996); therefore, when used as a green manure or organic fertilizer, in a short time it contributes a large

amount of N available to the other plants. It has also been reported that decomposed litter of *Gliricidia* increases nutritional status, water holding capacity and bulk density of the soil (Rosecrance et al. 1992; Arachchi and Liyanage 1998). Nitrogen release from litterfall of *Gliricidia* trees may reach 20 kg N ha⁻¹ yr⁻¹ (Nygren et al. 2000). The nodule turnover may also reach 20 kg N ha⁻¹ yr⁻¹ (Nygren and Cruz 1998). *Gliricidia*, with a deep-rooting system, has shown the ability to loosen hard soils (Toky and Bisht 1992).

Table 2.1 shows the %Ndfa of *Gliricidia* estimated with different methods. The %Ndfa estimate of *Gliricidia* ranges from 37-55 % in hedgerow trees to 41-43 % in alley cropping and 49-87 % in monoculture. The input of atmospheric N₂ by *Gliricidia* varies considerably depending on the method used to estimate BNF, the age and the population of the trees and the management practices. Studies carried out in coffee and cacao plantations in Latin America with 120-560 leguminous trees ha⁻¹ show that the aboveground N transfer input varied from 3-14 Mg ha⁻¹ yr⁻¹ of dry matter containing 60-340 kg N ha⁻¹ yr⁻¹ (Beer, 1988). Roskoski and van Kessel (1985), applying the acetylene reduction assay to determine the input of atmospheric N₂ in an unfertilized coffee and cacao plantation by the shade trees *Inga junicuil*, *Gliricidia sepium*, or *Erythrina poeppigiana*, report N₂ fixation between 35 and 60 kg N ha⁻¹ yr⁻¹. Use of the same technique in field monoculture resulted in estimates of only 13 kg N ha⁻¹ yr⁻¹ (Roskoski et al. 1982); estimates using isotope dilution methods showed that *Gliricidia* contributed 86-309 kg N ha⁻¹ yr⁻¹ in alley cropping in the Philippines (Ladha et al. 1993) and 70-274 kg N ha⁻¹ yr⁻¹ in field monoculture in Australia (Peoples et al. 1996). Though BNF by *Gliricidia* varied depending on the management and method used, the results show that BNF by *Gliricidia* can play a significant role in agricultural systems.