Chapter 6
Biological Nitrogen Fixation (BNF) in Mixed-Forest Plantations

Sergio Miana de Faria, Fabiano de Carvalho Balieiro, Ranieri Ribeiro Paula, Felipe Martini Santos, and Jerri Edson Zilli

6.1 Introduction

The emergence of symbiosis between leguminous plants and bacteria of the Rhizobia group (bacteria able to induce nodules and fix nitrogen) remains clouded in mystery and speculation (Sprent 1994; Brockwell et al. 2005; Doyle 2016; Parniske 2008). However, because the legume plants also possess the ability to take up N from soil, it is assumed that they were associated with diazotrophic bacteria (nitrogen-fixing bacteria) primitively in a parasitic mode, later evolving to infection, nodulation, and fixation patterns (Faria et al. 1987; Sprent 1994, 2007). It is important to realize that symbiosis is not obligatory for the plant or bacteria, but when associated both symbionts have ecological advantages of survival and competition (Sprent 2007).

The reason why some legume species do not nodulate, even though different leguminous species are capable of similarly accumulating N in the tissue, is not fully understood. For example, the genus *Cassia* comprises about 30 tree species, in which nodulation and nitrogen fixation remain unconfirmed. On the other hand, the genus *Chamaecrista* (closely related to *Cassia* genus) with more than 250 species that are herbs, shrubs, and arboreal types is an exclusive nodulant (de Faria et al.

S. M. de Faria · J. E. Zilli (✉)
EMBRAPA Agrobiology, Brazilian Agricultural Research Corporation, Seropédica, RJ, Brazil
e-mail: jerri.zilli@embrapa.br

F. de Carvalho Balieiro
EMBRAPA Soils, Brazilian Agricultural Research Corporation, Rio de Janeiro, RJ, Brazil

R. R. Paula
Center for Agricultural Sciences and Engineering, Federal University of Espirito Santo, Alegre, ES, Brazil

F. M. Santos
Federal Rural University of Rio de Janeiro, Seropédica, RJ, Brazil

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2010; Sprent 2007, 2009; de Faria et al. 1999; Moreira et al. 1992). It is intriguing, however, that these two leguminous genera with similar capability to accumulate N in their tissues vary in their nodulation features.

Is it possible that the ability of some legumes to accumulate N may have exerted a selection pressure driving towards symbioses, being a way of legumes adapting to the increasing demand for N in a limiting environment? If so, and given that the biological nitrogen fixation (BNF) event in prokaryotes is so old, it is questionable why plants would not have acquired the ability to form a structure ("rhizoplast"), resembling an organelle. Moreover, why most plants do not nodulate, including some legumes, is unclear. It is probable that the selection pressure was not strong enough for the plants to acquire *nif* genes (genes found in all diazotrophs required for structure, biosynthesis, and regulation of nitrogenase, the enzyme responsible for fixing N) and the plant-bacteria relationship is still evolving (Postgate 1992; Coba de la Peña et al. 2018). Probably, some groups of plants (especially the Papilionoideae subfamily and Mimosoideae clade) shared a close relationship with bacteria that had this enzymatic apparatus and took advantage of this association (Polhill et al. 1981; Sprent 2007).

BNF is the primary N intake form in agroecosystems, promoting equilibrium between atmospheric N_2, being the reactive forms incorporated in soil and organisms. For a number of reasons (such as the cost of production and environmental impact of synthetic fertilizers), BNF has become indispensable for sustainable agriculture on the planet (Crews and Peoples 2004), with Brazil being an excellent example of leveraging this process in annual crops, such as soybeans, beans, and others (Hungria and Mendes 2015).

Besides, BNF has also been useful in the restoration of degraded areas, part of the recovery technologies, which are based on the introduction of pioneering, fast-growing N_2 -fixing legumes (Franco et al. 1995; Chaer et al. 2011; Balieiro et al. 2018). In this case, the inoculation of legume seeds with rhizobia supplies plants with nitrogen often scarce on severely degraded lands, improves soil quality, and supports plant growth and ecological succession (Parrota et al. 1997; Franco and Faria 1997; Batterman et al. 2013).

Tropical soils, like most in Brazil, are highly weathered and poor in nutrients and organic matter, which require the supply of nutrients for adequate plant growth. Forest plantations, whether pure or mixed, depend on external inputs for adequate growth, although water availability exerts substantial control over growth and biomass accumulation (Stape et al. 2010; Moraes Gonçalves et al. 2013). This is especially important when it comes to fast-growing species, such as eucalyptus, which can accumulate about 155 kg ha^{-1} of N in biomass during the first year after planting (Laclau et al. 2010). It has been shown, however, that the introduction of N_2-fixing legumes in a consortium with fast-growing non-N_2-fixing species may be a strategy to promote gains in biomass production (wood), decrease the dependence on chemical nitrogen fertilizer by companion species (Chap. 1), and contribute to several soil processes, especially those dependent on the soil organic matter (see Chaps. 4 and 10).

This chapter addresses the biological fixation of N_2 as a critical ecological facilitation strategy in mixed-forest plantations, allowing N fixation to act positively on the development of non-N_2 -fixing species. The mixture of fast-growing N_2-fixing species is capable of improving the quality of soil organic matter, improving N status in the system and crop productivity (Forrester et al. 2005; Voigtlaender et al. 2012; Rachid et al. 2013; Santos et al. 2018). A compilation of information, without the commitment to exhaust the literature, was made by trying to give numbers to the benefits of nodulating bacteria. The potential use of the plant microbiome and growth-promoting microorganisms in forestry is still incipient, but very promising. This subject is discussed in Chap. 5.

6.2 Nodulating Bacteria and Symbiosis Establishment

Into the plant kingdom, members of the family Leguminosae have the ability to interact with diazotrophic bacteria and form nodules (Fig. 6.1). The nodules can be located in the roots of major legume species and in the stem of a few, as seen in the genera *Aeschynomene*, *Sesbania*, and *Neptunia* that grow in the flooded regions (Fig. 6.1).

The term “rhizobium” has always represented a group of gram-negative, obligate aerobic, non-endospore-forming alpha-proteobacteria that induce plants to form nodules through highly complex molecular signaling (Parniske 2008; Doyle 2011, 2016; Clúa et al. 2018). The taxonomy of nitrogen-fixing bacteria that associate with legumes has been frequently reviewed, especially as they have been identified as nodulating (Chen et al. 2006; Peix et al. 2015; Andrews and Andrews 2017).

Nowadays, rhizobia represent several lineages within the alpha-proteobacteria, and hence the term “rhizobia” does not represent a single taxon but refers to a polyphyletic cluster of bacterial lineages having similar functions. Most known rhizobia

Fig. 6.1 Spherical stem nodules of *Aeschynomene* sp. (approx. 3 mm in diameter); spherical radicular nodule of *Dalbergia nigra* (approx. 2 mm in diameter) and branched root nodules of *Andira nitida* (approx. 5–10 mm in length)

still belong to the family Rhizobiaceae [*Rhizobium*, *Ensifer* (*Sinorhizobium*), *Allorhizobium*, *Parahizobium*, *Neorhizobium*, *Shinella*], Phyllobacteriaceae, *Mesorhizobium*, *Aminobacter*, *Phyllobacterium*, Brucellaceae (*Ochrobactrum*, *Methylobacterium*, *Microvirga*), Bradyrhizobiaceae (*Bradyrhizobium*), Xanthobacteraceae (*Azorhizobium*), and Hyphomicrobiaceae (*Devosia*), but some others belong to beta-proteobacterial genera in the family Burkholderiaceae (*Paraburkholderia*, *Cupravidus*, and *Trinickia*) (Andrews and Andrews 2017; Peix et al. 2015; Sprent et al. 2017).

For the establishment of a mutual symbiosis, as is usual in the symbiosis between rhizobia and nodulating legumes, it is necessary that a series of physical, biochemical, physiological, and environmental factors complement each other. Several authors (Moreira and Siqueira 2006; Parniske 2008; Doyle 2011, 2016) describe that the fundamental stages for the establishment of symbioses are (1) preinfection, in which symbionts are recognized and interactions occur between surface bacteria and plant; (2) plant infection by the bacteria and formation of nodules; and (3) functioning of nodules, i.e., nitrogen fixation. According to the authors, several dozen genes are involved in the process of N_2 fixation in nodule-fixing bacteria, which influence everything from the recognition of the host plant by the bacteria to the transport of carbon from the plant to the bacteroid (the active form of nitrogen-fixing bacteria).

The pink color inside the nodule indicates the effectiveness of nodulation and the efficiency of nitrogen fixation. It shows the presence of active leghemoglobin, which is needed to supply oxygen at low tension for the nodules to function. The oxygen tension inside the nodules, which is usually low, is necessary because nitrogenase (the enzyme responsible for nitrogen fixation) is irreversibly inhibited in the presence of high O_2 concentration (Raymond et al. 2004). The efficiency of nodulation can be measured by the benefits of symbiosis to the host and the system as a whole, such as higher production of plant biomass, significant accumulation of nutrients (including N), and even transfer, directly or indirectly, of N to non-N_2- fixing plants.

Among the principal genera of nodulating bacteria of native forest legumes studied from the Amazon, Cerrado, Caatinga, and Brazilian Atlantic Forest *Bradyrhizobium*, *Rhizobium*, *Ensifer*, *Mesorhizobium*, and *Paraburkholderia* are the most common (Moreira and Siqueira 2006; Bournaud et al. 2013; da Silva et al. 2014; Zilli et al. 2014; Reis Jr et al. 2010).

The induction of nodules of forest legumes occurs through several bacterial genera; however, there is a certain specificity of response in terms of efficiency in nitrogen fixation (de Faria et al. 1999). This specificity appears to increase as symbionts coevolve in the same geographic region. For example, the species *Mimosa pudica* and even other members of Mimosa tribes are efficiently and almost exclusively nodulated by bacteria of the genus *Rhizobium* in Central America, whereas, in the Brazilian Cerrado, nodulation is almost exclusively by *Paraburkholderia* (Bontemps et al. 2016). Local climate conditions and specifically edaphic (pH) factors contribute to the emergence of this specificity (Bontemps et al. 2016; Pires et al. 2018; Reis Jr et al. 2010). Patterns relating to strains of *Rhizobium* nodulating species originated in Central America and *Paraburkholderia* in South America have also been observed in the *Calliandra* genus (tribe Ingae) (Silva et al. 2018).

Species of the *Acacia* genus, commonly used in mixed plantations, are nodulated by both *Rhizobium* and *Bradyrhizobium* and less frequently by *Ensifer*, *Mesorhizobium*, and *Paraburkholderia* (Lawrie 1981; Barberi et al. 1998, Sakrouhi et al., 2016). However, there are important differences in the nodulation efficiency and N_2 fixation even within a genus and species to which the strains belong (Galiana et al. 2002).

6.3 The Ability of the Forest Legumes to Nodulate and Fix Nitrogen

The family Leguminosae was recently reviewed and classified into six subfamilies: Duparquetioideae, Cercidoideae, Dialioideae, Detarioideae, Papilionoideae, and Caesalpinioideae, with the latter also encompassing the traditional subfamily Mimosoideae, which became a clade of Caesalpinioideae (LPWG 2017). The nodulation is mostly concentrated in the subfamily Papilionoideae, in which about 97% of the investigated species can nodulate. In this subfamily, only some tribes and genera do not nodulate, such as Dipteryxeae, part of the Dalbergieae (*Vaitarea* and *Vataereopsis*), Swartzieae (only some species of the genus *Swartzia* nodulate), and some genera of Sophoreae. Similarly, in the subfamily Papilionoideae, the Mimosoid clade within the subfamily Caesalpinioideae, 95% of the species fix nitrogen associated with rhizobia. For the other members of the old subfamily Caesalpinioideae, until recently, only 25% were associated with rhizobia-producing nodules and these are concentrated in some genera of the Cassieae (*Chamaecrista*), Caesalpinieae (*Melanoxylon*, *Moldenhawrea*, *Tachigali*, *Dimorphandra*, and other genera in this tribe) (Allen and Allen 1981, de Faria et al. 1989).

Nodulation and consequently the BNF benefits usually occur when nitrogen is scarce in the environment, and therefore in mature forests it is rare to find nodules in the species capable of associating with rhizobia (de Faria et al. 1984; Winbourne et al. 2018; Piotto et al. 2009), with reduced BNF contributions (Nardoto et al. 2014). Likewise, nitrogen fixation will occur at a higher intensity when the species requires more nitrogen, that is, during its exponential growth phase. Although the majority of soils have native bacteria capable of nodulating tree species typically used in mixed plantations, significant gains in establishment, growth, and productivity can be obtained when seedlings are inoculated with selected strains (Franco and de Faria 1999; Galiana et al. 2002).

6.4 Brazilian Rhizobia Selection Program for Leguminous Trees

Embrapa Agrobiologia is a pioneer and internationally recognized for its work in the selection and maintenance of diazotrophic bacterial germplasm associated with native and introduced leguminous trees. Since 1960, this research center isolated over 5000 strains of rhizobia from different regions of Brazil. More than 2600 botanical specimens were investigated for nodulation capacity, which includes more than 80 genera and 400 forest species reported for the first time as nodulants or non-nodulants. It is worth remembering that the pioneering work developed with tree species was performed by Dr. Döbereiner and her group in the 1960s, including the preliminary studies on host specificity of the sabiá (*Mimosa caesalpiniifolia*) (Campelo and Döbereiner 1969). Native of the Caatinga biome, this legume originated from Caatinga (Brazilian Northeast), and is widely distributed throughout the country as it has several uses such as live fences, charcoal, firewood, erosion control, forage, honey flowers, and others.

A program to obtain and select strains of rhizobia for legumes initially requires the confirmation of the ability of the isolate to induce nodulation in a host, but in Brazil, given the large diversity of legume species and the limited knowledge of the flora it is often necessary first to evaluate nodulation capacity.

Roots of individual plants can be examined directly in the field for the presence of nodules. If present, the nodules can be collected for bacteria in the laboratory. Subsequent purification and selection of the most efficient isolates for nitrogen fixation in the target plant species can be performed. The presence of nodulation can also be confirmed in the greenhouse by inoculating seed collected in the field where a target legume grows with a set of several bacteria from different groups. The inoculation with a mixture of rhizobia strains of different origin along with the soil from the native location where the legume grows is another strategy.

After confirmation of nodulation in specific species, the selection of most efficient strains for biological N_2 fixation is the next step. It is important to note that some specific responses exist in terms of nitrogen fixation efficiency by a particular group and/or several bacterial strains, and that due to such preferences it may be necessary to select the most efficient strain for each forest species.

Embrapa develops trials that are divided into three phases and follow the official rules of the Ministry of Agriculture, Livestock and Food Supply—MAPA (Brazil 2011). In the first phase, each legume species is tested aseptically in “Leonard jars” containing a mixture of sand and vermiculite (Vincent 1970), with strains of several different origins. In this phase, the nature of the isolates (if they are rhizobia) is confirmed, besides the N_2 fixation potential. The best strains are tested in soil, and unsterilized conditions (second-phase test). This phase evaluates the competitiveness and efficiency of bacteria in comparison to those in the native soil. Nursery and field conditions are part of the third phase.

Studies developed over the last decades by Embrapa Agrobiologia have led to the selection of several rhizobia strains for different legume species. These strains were

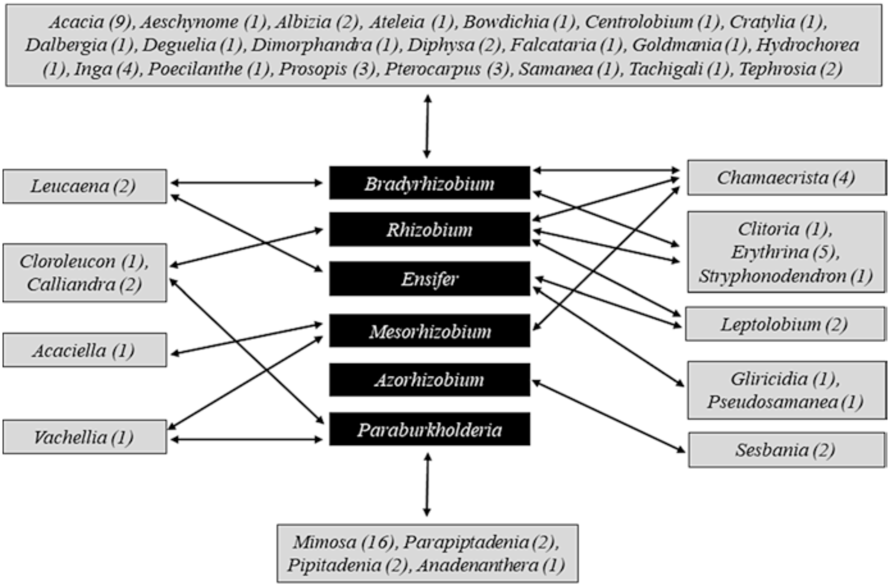

Fig. 6.2 Legume tree genera and respective rhizobia genera with strain efficient in the BNF symbiosis. The numbers in parentheses indicate the number of legume species within each genus for which strains of rhizobium have already been selected

selected for approximately 90 forest species belonging to 38 genera (Fig. 6.2). For most legume genera studied (60%), among 57 species, strains of the genus *Bradyrhizobium* were selected (Fig. 6.2), followed by the genera *Rhizobium* and *Paraburkholderia*. It is clear, therefore, that the *Bradyrhizobium* genus not only is the most common symbiont of native and introduced legumes growing in Brazil, but also tends to be the most efficient for N_2 fixation among most genera (Fig. 6.2). However, this cannot be generalized as plant-microbe specificity involving another bacteria genus that can be important. For example, within *Mimosa*, 16 species that were identified and selected strains all are members of the genus *Paraburkholderia* (Fig. 6.2). The same has also been observed for the plant genera *Piptadenia*, *Parapiptadenia*, and *Anadenathera* that probably only are nodulated by *Paraburkholderia* strains (Fig. 6.2).

For 24 of these legumes, there is at least one strain authorized by MAPA for the production of inoculants (Brazil 2011). Other forest legumes, for which although currently there is at least one strain already selected, are not yet included in the official MAPA list, which still requires significant efforts, including support from the industry to validate the efficiency for registration (Fig. 6.2). This is the case of the introduced forest legume such as some *Acacia* species, and most of the native species already tested or with potential for use in mixed planting, such as the species within the genera *Enterolobium*, *Inga*, *Erythrina*, *Mimosa*, *Dalbergia*, *Tachigali*, and others.

6.5 Dependence of Biological N_2 Fixation on Mycorrhization

Several microorganisms colonize the rhizosphere, which include bacteria, actinomycetes, and fungi. These microbes perform activities that are related to the physiology and nutrition of plants. In this sense, the decomposition of soil organic compounds, their mineralization, BNF, release of substances that stimulate growth or antagonism to pathogens, as well as availability of nutrients are important for plant growth (Grayston et al. 1997; Andrade et al. 2000; Kuiper et al. 2004; Moreira and Siqueira 2006).

As N and P are usually the most limiting nutrients for plant growth in the tropics, more attention has been paid to research on these elements and to alternatives for the use of biological inputs, such as inoculation with rhizobia and mycorrhizal fungi. Inoculation of tree legumes with rhizobial strains and mycorrhizal fungi can meet all the N and P requirements for plant growth, taking into account the other factors that are not limiting (Oliveira Júnior et al. 2016; Patreze et al. 2004; Moreira and Siqueira 2006).

Several tree species, including *A. mangium*, have the ability to associate with arbuscular mycorrhizal fungi as well as ectomycorrhizal fungi, in addition to establishing efficient nodulation with rhizobia. Mycorrhizal fungi can help increase the biological nitrogen fixation, by enhancing P availability that is in demand for the BNF process, as well as the nitrogen-fixing bacteria tend to influence mycorrhizal colonization. This pattern of synergistic response is commonly observed in the Mimosoid clade that responds to both types of symbioses (Oliveira Júnior et al. 2016; Bournaud et al. 2017).

In fact, there are complex interactions between legumes with their symbiotic partners, which is the result of an old coevolution leading plants and microsymbionts to respond more or less effectively to this interaction (Parniske 2008). Variations in responses by both mycorrhizal fungi and rhizobial inoculation are typical, because they are also associated with plant genetics and microsymbiotic performance (Monteiro 1990a; Patreze and Cordeiro 2004). Monteiro (1990a) studied the interaction between *Mimosa caesalpiniifolia* and *M. scabrella* with rhizobia and arbuscular mycorrhizal fungi and concluded that the microsymbionts acted synergistically for the production of biomass and nutrient accumulation in plants, with biomass production exceeding 400% compared to the treatment without the microorganisms even with nutrient addition. In the same way Founoune et al. (2002) evaluated the influence of two isolates of the ectomycorrhizal fungi, *Pisolithus* sp. (COI 007, COI 024), and one isolate of *Scleroderma dictyosporum* (Sd 109) on the growth of *A. mangium* and the synergy with rhizobium inoculation. Compared to the control treatment that lacked inoculation with both the symbionts, *A. mangium* plants treated with COI 007 and Sd 109, respectively, had significantly higher biomass of roots and leaves. In addition, treatment with COI 007 resulted in a higher number of nodules per plant. In the case of *Acacia holosericea*, however, the highest number of nodules was present when inoculated with the COI 024 isolate.

These results show that, although symbiosis with mycorrhizal fungi is a rule among higher plants, there may be additional benefits from certain isolates (Moreira and Siqueira 2006; Shiavo and Martins 2002; Diagne et al. 2013).

The vast majority of the tree legumes can benefit from the association with mycorrhizal fungi and through this association the BNF is improved as well. However, certain groups of legumes not only benefit from this tripartite association, but are also highly dependent on the mycorrhization to establish efficient nodulation, even when supplied with phosphorus (Jesus et al. 2005). For example, recent studies have shown that *Piptadenia gonoacantha* is only capable of inducing the formation of inefficient nodules in the absence of mycorrhiza, and in this case, the color and shape of the nodules formed are different (Bournaud et al. 2017; Oliveira Júnior et al. 2016).

The importance of field-level and nursery studies with mycorrhizal fungi (mycorrhizal fungi and diazotrophic bacteria) is essential to evaluate their efficiency. Laboratory and greenhouse conditions often do not represent the tougher field conditions, even though nursery conditions for seedling production can be similar to those in the greenhouse. For practical purposes inoculation with rhizosphere soil of plants growing in the field will provide well-adapted AMF.

6.6 The Contribution of BNF in Mixed-Forest Plantations

N_2-fixing trees, mainly species from the Leguminosae family, have been widely used to improve N status of non-N_2-fixing species in agroforestry systems (Mafongoya et al. 1998) and mixed-forest planting for timber production (Binkley and Giardina 1997; Richards et al. 2010) and for recovery of degraded lands (Franco and Faria 1997; Chaer et al. 2011). However, the contribution of BNF (percentage of N derived from the atmospheric fixation—% Ndfa) to tree and shrub species under field conditions is not easy to evaluate, mainly due to the difficulties in estimating the amount of N accumulated in the above- and belowground plant components (Khanna 1998; Boddey et al. 2000). Interactions with the abiotic (climate and soil in particular) and biotic factors (inter- and intraspecific interaction of mixed plantations) also complicate these estimations, since they affect competition and facilitation between plants and species, especially in mixed plantations (see Chap. 2).

6.6.1 Measuring the Biological Nitrogen Fixation (BNF) in Woody Perennial Species

Determining the BNF contribution in trees and shrubs, both in planted forests and agroecosystems or in the native forests, has been the subject of several studies and reviews (Boddey et al. 2000; Galiana et al. 2004; Gehring and Vlek 2004; Gehring et al. 2005; Bouillet et al. 2008; Chalk 2016; Paula et al. 2018). Among the methodologies developed for the quantification of BNF and its applicability to woody perennial species either under greenhouse (pots) or field conditions, Peoples et al. (1989) cited acetylene reduction analysis (ARA), determination of relative abundance of ureides in plant sap, and use of ^{15}N (isotopic enrichment and natural abundance) isotope dilution (ID) techniques. The N balance and nitrogen accretion method can also be used to estimate the total N input (kg ha^{-1}) via BNF (Peoples et al. 1989; Forrester et al. 2007; Voigtlaender et al. 2018) in different ecosystems. It should be noted, however, that each of these methodologies mentioned has specifications and limitations (see Boddey et al. 2000; Unkovich et al. 2008; Chalk 2016).

The ARA method uses the activity of the nitrogenase enzyme because under high acetylene concentration, it can be used as a substrate to be reduced to ethylene. This analysis represents a qualitative evaluation of BNF as a point analysis of the nitrogenase activity in the nodules from the plant. The evaluation of the abundance of ureides (allantoins and allantoic acid) in the xylem relies on the ability of the species to transport these compounds preferentially, to the detriment of nitrate and other amino compounds, such as asparagine and glutamine (Peoples et al. 1996). For example, plants from the genus *Acacia*, the most transported BNF products, are asparagine and glutamine (Brockwell et al. 2005).

Isotopic dilution (ID) using the natural abundance of ^{15}N is currently a good option to determine the proportion of N derived from the BNF from the air (% Ndfa) under field conditions (Boddey et al. 2000). This method relies on the fact that under the same natural condition plants that fix some or all of their nitrogen will have lower ^{15}N signal than plants that obtain their entire N from the soils, which are ^{15}N enriched.

For the method based on ^{15}N enrichment (E), the soil is enriched with a labeled fertilizer and paired plots—one containing the legume and the other an N_2-fixing reference plot—are used for the application. However, the ID (NA or E) technique presents some limitations as plant selection, tissue sampling, unpredictability in the levels of ^{15}N, N available to plants from organic matter decomposition, quality and quantity of organic matter, and selective absorption of N sources by ecto- and endomycorrhizal fungi (Högberg 1997; Natelhoffer and Fry 1988; Boddey et al. 2000; Gehring and Vlek 2004).

For the N balance and N accretion method, the BNF rate is estimated as the difference in accumulated N in the plant biomass (aerial and root), in the litter deposited in the soil, or in the soil between plots of the non-N_2-fixing species and plots containing the N_2-fixing species (monocultures or mixed species). Thus, it is assumed that the differences (in kg ha^{-1}) in N in the treatment are mainly due to the biological process. The amounts of N_2 fixation could be underestimated if the N in the belowground area is not considered, especially in planted forests (Forrester et al. 2007).

In addition, Chalk (2016) suggested that the uncertainty in BNF rate could be attributed to the B-value (the relative isotopic abundance of legumes growing in N-free medium). The reasons are as follows: (1) it is not usually determined similarly in the field or pot experiments; (2) it is dependent on the rhizobial strain used as the inoculant; and (3) it depends on the part of the plant tissue sampled. Differences in the root density among the plants in each treatment (monocultures and mixed plantation) of the topsoil, and variations in nitrate and ammonium availability, were reported by Bouillet et al. (2008) as factors contributing to uncertainty in N_2 fixation estimates. These can lead to differences in ™^{15}N of mineral N uptake (NH_4^+ is less depleted in ^{15}N than NO_3^-) by both plant species from soil or from fertilizer application. The costs of the enriched fertilizer and the isotope analyses also restrict the use of these two techniques (NA or E).

6.6.2 Higher Nitrogen Fixation in Mixed Plantations

The rate of the BNF estimates (% Ndfa) in tree species under mixed planting conditions is scarce in Brazil, especially when dealing with native flora. Increased attention was paid to *Acacia mangium* because of the increased growth seen in degraded lands and low-fertility soils in the 1980–1990s of the last century (Franco and Faria 1999; Coelho et al. 2007), additionally to the interest of forestry companies in studies of silvicultural performance and interaction of the species with eucalyptus at the end of the twentieth century (Harwood and Nambiar 2014; Parrota and Knowles 1999), and the growing demands in Southeast Asia (Harwood and Nambiar 2014; Balieiro et al. 2018).

A few reports estimating the %Ndfa in pure and mixed plantations with legumes, using the NA or E techniques, across the Brazilian states of São Paulo and Rio de Janeiro, showed a significant contribution of BNF to mixed plantations (Balieiro et al. 2004, Paula et al. 2018), thus corroborating the work performed in other locations, such as Puerto Rico (Parrotta et al. 1996) and Ivory Coast (Tchichele et al. 2016). However, Forrester et al. (2007) studied mixed plantations of *Acacia mearnsii* and *E. globulus*, but observed opposite results. The higher BNF in mixed plantations was attributed to the elevated N requirement of eucalyptus under mixed plantation regimes. This requirement led to a strong competition for soil N by the plants and a consequent elevation of the N demand in the system (Balieiro et al. 2004; Paula et al. 2018), by the high litter decomposition rate (Santos et al. 2016) and soil N mineralization (Voigtlaender et al. 2019). Table 6.1 contains the %Ndfa estimates for tree legumes in mixed and pure plantations in Brazil and other countries, using natural abundance (NA) and enrichment of ^{15}N (E) techniques.

The results in Table 6.1 corroborate the work of Brockwell et al. (2005), who reported that the biological nitrogen fixation rates observed under the field conditions for *Acacia*, shrubs, and tree species occur in the 2–90% range. The authors further describe that this range denotes the genetic variability within the genus *Acacia*, the efficiency of strains and different species of rhizobia, and the different estimation techniques of BNF.

In general, BNF contributions are higher when the planting is younger (Parrotta et al. 1996; Paula et al. 2018; Balieiro et al. unpublished date; Balieiro et al. 2002—Tables 6.1 and 6.2) and with local infertile soil (Bernhard- Reversat et al. 1996;

Table 6.1 Percentage of percentage of nitrogen derived from atmosphere (Ndfa, %) for native and exotic woody periannias l species estimated by natural abundance (NA) and enrichment ^{15}N (E) techniques, under pure and mixed plantation

N_2-fixing species	Reference species[(a)]	Country	Aboveground component or litter sampled	Age months	Ndfa %	Method	Reference
Peltophorum dubium (mixed, 50:50)	*Eucalyptus grandis*	Brazil	Le	24		NA	Coelho et al. (2007)
Inga sp. (mixed, 50:50)	*E. grandis*	Brazil	Le	24	74	NA	Coelho et al. (2007)
Mimosa scabrella (mixed, 50:50)	*E. grandis*	Brazil	Le	24	92	NA	Coelho et al. (2007)
Mimosa caesalpiniaefolia (mixed, 50:50)	*E. grandis*	Brazil	Le	24	74	NA	Coelho et al. (2007)
Pseudosamanea guachapele (G100)	*E. grandis* (E100)	Brazil	Le+Ba+Br+st[#]	84	17–36	NA	Balieiro et al. (2004)
P. guachapele (E50:G50)	*E. grandis* (E100)	Brazil	Le+Ba+Br+st[#]	84	34–84	NA	Balieiro et al. (2004)
Acacia mangium (A100)	*E. grandis* (E100)	Brazil	Le	39	0–14	E	Paula et al. (2018)
A. mangium (E50:A50)	*E. grandis* (E100)	Brazil	Le	60	30–52	E	Paula et al. (2018)
A. mangium (A100)	*E. urograndis* (E100)		Le+Ba+Br+St+Cr+Mr+Fr+Li[#]	24	59	E	Tchichelle et al. (2017)
A. mangium (E50:A50)	*E. urograndis* (E100)		Le+Ba+Br+St+Cr+Mr+Fr+Li[#]	24	64	E	Tchichelle et al. (2017)
A. mangium (A100)	*E. grandis* (E100)	Brazil	Le+Ba+Br+St+Cr+Mr+Fr+Li[#]	30	20	NA	Bouillet et al. (2008)
A. mangium (E50:A50)	*E. grandis* (E100)	Brazil	Le+Ba+Br+St+Cr+Mr+Fr+Li[#]	30	10	NA	Bouillet et al. (2008)
A. mangium (E50:A50)	*E. grandis* (E100)	Brazil	Le+Ba+Br+St+Cr+Mr+Fr+Li[#]	30	59	E	Bouillet et al. (2008)
A. mangium (A100) (plots low fertility)	*E. urograndis* (E100)	Ivory Coast	Le	19	27	NA	Galiana et al. (2002)
A. mangium (A100) (plots medium fertility)	*E. urograndis* (E100)	Ivory Coast	Le	19	64	NA	Galiana et al. (2002)
A. mangium (A100) (plots medium fertility)	*E. urograndis* (E100)	Ivory Coast	Le	19	67	NA	Galiana et al. (2002)

Leucaena leucocephala (E50:L50)	*E. robusta*	Puerto Rico	Le	12	98	E	Parrota et al. (1996)
Leucaena leucocephala (E50:L50)	*E. robusta*	Puerto Rico	Le	42	98	E	Parrota et al. (1996)
A. mearnsii (A100)	*E. globulus* (E100)	Australia	Le	120	52–107	NA	Forrester et al. (2007)
A. mearnsii (E50:A:50)	*E. globulus* (E100)	Australia	Le	120	8–17	NA	Forrester et al. (2007)
A. mangium (A100)	*E. urograndis* (E100)	Brazil	Le	12	65	NA	Balieiro et al. (data not published)
A. mangium (A100)	*E. urograndis* (E100)	Brazil	Le	24	26	NA	Balieiro et al. (data not published)
A. mangium (A100)	*E. urograndis* (E100)	Brazil	Le	60	0	NA	Balieiro et al. (data not published)
A. mangium (E50:A50)	*E. grandis* (E100)	Brazil	Le	12	70	NA	Balieiro et al. (data not published)
A. mangium (E50:A50)	*E. grandis* (E100)	Brazil	Le	24	50	NA	Balieiro et al. (data not published)
A. mangium (E50:A50)	*E. grandis* (E100)	Brazil	Le	60	0	NA	Balieiro et al. (data not published)

(a) non-N_2-fixing species used to calculate the %Ndfa, (b) componet used by authors to estimate the %Nfda, *Le* leaves, *Ba* barck, *Br* branches, *St* stem; *Cr* coarse roots, *Mr* medium roots, *Fr* fine roots, *Li* litter

Table 6.2 N_2 fixed (kg ha^{-1}) by woody perennial legumes in pure and mixed plantations based on difference in aboveground N accumulation using the accretion method

N_2-fixing species (A)	Reference species (B)	Country	Age (months)	N acumulated (kg ha^{-1})	N accretion (A−B or (A+B)-B	Reference
Acacia mangium (A100)	*Eucalyptus grandis* (E100)	Brazil	39	204.6	−19.6	Paula et al. (2018)
A. mangium (E50:A50)	*E. grandis* (E100)	Brazil	39	444.7	+220.5	Paula et al. (2018)
	E. grandis (E100)	Brazil		224.2		Paula et al. (2018)
Acacia mangium (A100)	*E. urograndis* (E100)	Brazil	60	186.3	+17.6	Santos et al. (2018)
A. mangium (E50:A50)	*E. urograndis* (E100)	Brazil	60	231.8	+63.1	Santos et al. (2018)
A. mangium (E100:A100)	*E. urograndis* (E100)	Brazil	60	285.5	+116.8	Santos et al. (2018)
	E. urograndis (E100)	Brazil	60	168.7		Santos et al. (2018)
Mimosa scabrella (mixed, 50:50)	*E. grandis*	Brazil	24	317.7	+170.9	Coelho et al. (2007)
Mimosa caesalpiniaefolia (mixed, 50:50)	*E. grandis*	Brazil	24	149.6	+2.8	Coelho et al. (2007)
A. mangium	*E. grandis*	Brazil	24	188.4	+41.6	Coelho et al. (2007)
	E. grandis	Brazil	24	146.8		Coelho et al. (2007)
A. mangium (A100)		Brazil				Balieiro et al. (2004)
	E. grandis (E100)	Brazil	60	410.5		Balieiro et al. (2002)
Pseudosamanea guachapele (G100)	*E. grandis* (E100)	Brazil	60	756.8	−57.0	Balieiro et al. (2002)
P. guachapele (E50:G50)	*E. grandis* (E50:G50)	Brazil	60	467.5	+289.3	Balieiro et al. (2002)
	E. grandis (E100)	Brazil	60	410.5		Balieiro et al. (2002)
Acacia mangium (A100)	*Eucalyptus grandis* (E100)	Ivory Coast	24	30.1	+23.8	Tchichele et al. (2017)
A. mangium (E50:A50)	*E. grandis* (E100)	Ivory Coast	24	23.5	+17.2	Tchichele et al. (2017)
	E. grandis (E100)	Ivory Coast	24	6.3		Tchichele et al. (2017)

(continued)

Table 6.2 (continued)

N_2-fixing species (A)	Reference species (B)	Country	Age (months)	N acumulated (kg ha^{-1})	N accretion (A−B or (A+B)-B	Reference
A. mearnsii (A100)	*E. globulus* (E100)	Brazil	120	496	+358	Forrester et al. (2007)
A. mearnsii (A50:E50)	*E. globulus* (E100)	Brazil	120	480	+342	Forrester et al. (2007)
	E. globulus (E100)	Brazil	120	138		Forrester et al. (2007)
A. mangium (A100)—Itatinga	*E. grandis* (E100)	Brazil	72	2050	+413	Voigtlander et al. (2018)
A. mangium (E50:A50)—Itatinga	*E. grandis* (E100)	Brazil	72	1836	+199	Voigtlander et al. (2018)
	E. grandis (E100)	Brazil	72	1637		Voigtlander et al. (2018)
A. mangium (A100)—Bofete	*E. grandis* (E100)	Brazil	72	2242	+348	Voigtlander et al. (2018)
A. mangium (E50:A50)—Bofete	*E. grandis* (E100)	Brazil	72	2111	+217	Voigtlander et al. (2018)
	E. grandis (E100)	Brazil	72	1895		Voigtlander et al. (2018)
A. mangium (A100)—Luiz Antônio	*E. urograndis* (E100)	Brazil	72	1622	–	Voigtlander et al. (2018)
A. mangium (E50:A50)—Luiz Antônio	*E. urograndis* (E100)	Brazil	72	1848	+84	Voigtlander et al. (2018)
	E. urograndis (E100)	Brazil	72	1764		Voigtlander et al. (2018)
A. mangium (A100)—Santana do Paraíso	*E. urograndis* (E100)	Brazil	72	3258	+367	Voigtlander et al. (2018)
A. mangium (E50:A50)—Santana do Paraíso	*E. urograndis* (E100)	Brazil	72	3168	+277	Voigtlander et al. (2018)
	E. urograndis (E100)	Brazil	72	2891		Voigtlander et al. (2018)

Galiana et al. 2002, Balieiro et al. 2004). Both factors are related to the upregulation of N_2 fixation depending on the soil N status (Vitousek et al. 2002; Galiana et al. 2002). Galiana et al. (2002) observed the spatial variability in %Ndfa for *A. mangium* as a result of soil fertility variation between plots. The %Ndfa reached 64 and 67% in blocks II and III, respectively, versus 27% in block I, following a parallel increase in N and P soil content.

The total N accumulated in the biomass, litterfall, and soil-derived BNF is calculated as the difference in total N found in N_2-fixing species and reference species (non-fixing) (Parrotta et al. 1996; Forrester et al. 2007). From some previous work, the additional amount of N introduced by tree legumes in mixed and pure planting conditions is estimated, in order to measure the benefits of introducing legumes in these systems. As shown in Table 6.2, the total N accumulated in the aerial parts of the plants at a given stage is underestimated, as much of the N_2 fixed may be related to the roots (coarse and fine) and the litter (deposited and on the ground). Although the N_2-fixed N by the legume in the plantation is proportional to its capacity to compete in a specific local, the BNF contribution is always higher in the mixed plantation than in the eucalyptus monocultures. It is expected to contribute up to 60 kg ha^{-1} $year^{-1}$ under mixed-forest plantations. These values corroborate with previous reports on plantations under field conditions, with *Acacia* spp. in Africa and Australia, with up to 50 kg ha^{-1} $year^{-1}$ of fixed N (Sprent 1993; Sutherland and Sprent 1993). As much as BNF contribution of leguminous species depends on its adaptability and growth in local edaphoclimatic conditions, it is imperative that breeding and selection of these species be carried out for different Brazilian conditions, as it has been done in Southeast Asia (Griffin et al. 2015).

Likewise, the new mixed planting arrangement using Brazilian native species may offer some benefits, especially for the biological conservation and the associated ecosystem services (see Chaps. 10 and 12). Silvicultural management of these plantations needs to be better understood as the pruning, thinning, or proper cleaning and maintenance of the plants are activities that could disturb the system and affect BNF in the legumes.

6.6.3 Nitrogen Transfer between Plants in Mixed Plantations

Although N transfer between plants occurs in both directions, i.e., from N_2-fixing tree to non-N_2-fixing and vice versa, the magnitude of the transfer is greater from N_2-fixing tree to non-N_2-fixing species (see review by Chalk et al. 2014). Several studies show that 0–50% of N contained in plants associated with N_2-fixing trees could be derived from such transfers. Due to the transfer, non-N_2-fixing trees growing in a consortium of N_2-fixing trees sometimes accumulate more N in their biomass than the individually growing trees. This additional N is assumed to derive from the transfer (Chalk et al. 2014).

The N present in the N_2-fixing trees can be transferred directly or indirectly to non-N_2-fixing species growing within a consortium (Munroe and Isaac 2014) (Fig. 6.3). Low-molecular-weight nitrogen compounds, such as nitrate, ammonium, and amino acids, are transferred directly between the plants without transforming, from the root exudates or by the action of mycorrhiza. The decomposition of the vegetative tissue, above- and belowground, and its mineralization by soil microorganisms lead to the indirect transfer of N between plants. These N transfer rates vary in speed and significance, and are poorly understood, as well as the limiting or

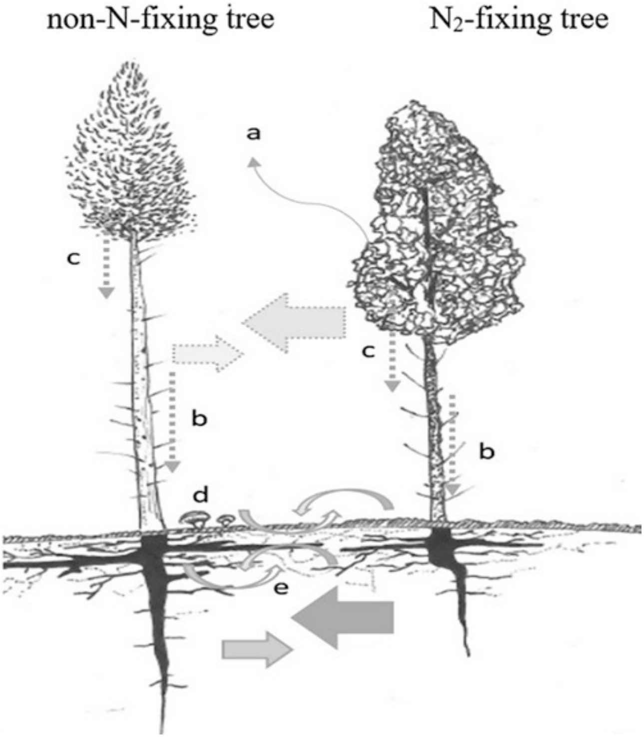

Fig. 6.3 Schematic showing pathways of N transfer between trees in mixed-species plantation with non-N_2-fixing and N_2-fixing trees. Direct transfer of N begins with an N compound with low molecular weight without undergoing transformation by (**a**) ammonium volatilization, (**b**) stemflow, (**c**) throughfall, (**d**) soluble N in litterfall and pruning residues, and (**e**) root exudates, soluble N in nodule and roots, and mycorrhiza network. Indirect transfer of N occurs after transformation of N substance by (**d**) decomposition and mineralization of litterfall and pruning residues, (**e**) root and nodule decomposition and mineralization, and *by mycorrhizal network (both ectomycorrhizal and arbuscular fungi)*

facilitating factors of this transfer (Mafongoya et al. 1998; Munroe and Isaac 2014; Chalk et al. 2014; Peoples et al. 2015).

Among the main source of N potentially transferable in the mixed plantation, the branches and green leaves of litterfall and the fine roots and nodules represent the major sources of N in quantitative terms. It is important to notice however that the amount of N accumulated in the fine roots is still poorly understood (Mafongoya et al. 1998; Munroe and Isaac 2014; Peoples et al. 2015). The biomass of these roots (diameter <2 mm) at a depth of 17 m was estimated for pure and mixed plantations of acacia and eucalyptus at 4 years of age in Itatinga, Brazil (Germon et al. 2018). Acacia trees produced approximately 4.2 tons ha^{-1} of fine roots in the mixed plantation up to a depth of 12 m. Using an average of 2.3% N content in fine acacia roots (Paula 2015), the N content in the fine roots was 98.3 kg ha^{-1}.

Living tissue derived from pruning or harvesting is an important source of N since the total and soluble N content is higher than that in the senescent tissues. Paula (2015) used crop residues (i.e., leaves, branches, and bark) of ^{15}N-enriched *A. mangium* and *E. grandis* in eucalyptus seedlings to trace the path of the residue-derived N in the soil-plant system. Three months after the application of these residues, young leaves of eucalyptus seedlings were significantly enriched with ^{15}N when they received *A. mangium* residues, which was not the case when the plants received *E. grandis* residues or unlabeled residues. This result shows rapid transferability of a large fraction of soluble N from the legume to other plants.

Direct N transfer from legumes through root exudation and mycorrhizal represents a substantial source of N during tree growth (Munroe and Isaac 2014). This transfer is important because it occurs in the short term (e.g., hours, days), and even trees distant from the source can benefit from the transferred N (Paula et al. 2015). Paula et al. (2015) applied potassium nitrate enriched with ^{15}N to the stem of *A. mangium* trees and observed values of ^{15}N above natural abundance in eucalyptus tissues located within a radius of up to 6.2 m around the acacia plants marked for 60 days after the application. N transfer between plants is facilitated by the presence of both arbuscular mycorrhizae and ectomycorrhizas associated with the vast majority of plant species and can be modulated by source and drain relationship (He et al. 2003). These organisms are able to absorb mineral and organic forms of N derived from N_2-fixing species and assimilate N as needed before transferring to plants growing in a consortium (He et al. 2003; Munroe and Isaac 2014). Ectomycorrhizae can also act on the transfer of N between plants, as they can break down complex organic compounds present in the soil and transform them into forms that are assimilated by plants (He et al. 2003). Estimates of N transfer through mycorrhizae from legumes to non-legumes vary between 20 and 50% of accumulated N (He et al. 2003).

Direct approaches to estimate N transfer involve the application of a nitrogen source enriched with ^{15}N to the nitrogen-fixing tree, and subsequent isotope tracing in the tissues of the reference species (Chalk et al. 2014). The N_2-fixing tree can be labled with ^{15}N via foliar absorption, via injection in the branches and stem, and by root absorption, each one with its particularities (Yasmin et al. 2006; Chalk et al. 2014). Paula et al. (2015) used the ^{15}N values observed in the fine roots of *E. grandis* and *A. mangium* to calculate the N ratio of *E. grandis* derived from *A. mangium* and concluded that the average N transfer reached values of approximately 43%. Based on mass balance, the authors calculated the proportion of ^{15}N injected into the stem of *A. mangium* that was transferred to *E. grandis* trees within a radius of 6.2 m around the acacia, which reached an estimate of N transfer of approximately 3%.

Other potential high-throughput N transfer routes involve leaf leaching, foliar ammonia gas release, root leaching, and herbivory of nodules (Peoples et al. 2015). The first two routes were studied by Paula (2015). At different dates after ^{15}N application in *A. mangium* trees, ^{15}N was determined in the stemflow and throughfall samples collected below the labled acacia, as well as samples derived from collectors installed above the acacia to capture ammonia. The researcher observed that there was no enrichment of these sources of N with ^{15}N above the natural one, and that both sources of N had negative values of $\delta\ ^{15}$N.

Although many advances are being made to understand the facilitation and the ecological relationships involving the transfer of N between plants (mainly N_2-fixing to non-N_2-fixing species), it is also urgent that studies with key nutrients for the process of decomposition, growth, and biological stabilization of N_2 and stabilization of soil organic matter, such as P, are initiated.

6.7 Brazilian Native Legume Tree Species with Potential for Mixed Plantations

In this section, we present some Brazilian legume tree species with potential for mixed planting with *Eucalyptus* spp. The N_2-fixing legume tree species were categorized into two groups: "fertilizer" and "timber" species. The former one included fast-growing species that have high rates of N_2 fixation, which can be used to increase the N and other nutrient levels through aboveground biomass pruning, litter deposition, and/or root exudates and decomposition. In addition, they are species that generally produce light to moderately heavy wood with lower commercial-value timber. The second group included the species which present longer rotation than *Eucalyptus* spp. and produce wood for multiple uses with high commercial value.

6.7.1 Fertilizing Legume Trees

The genera *Enterolobium*, *Erythrina*, *Inga*, and *Mimosa* include tree species with high levels of nodulation in natural environments or under controlled conditions (de Faria et al. 2006; Canosa et al. 2012; Lorenzi 1992). They can be suggested as species of the Brazilian flora with the potential to be introduced in mixed plantations with *Eucalyptus* spp. Table 6.3 lists some of these species.

In the genus *Enterolobium*, popularly known as "tamboril," it is possible to distinguish *E. maximum* and *E. contortisiliquum* as potential species to be introduced in the mixed plantations with *Eucalyptus* spp. *E. maximum*, an Amazonian species, has wood with easy workability and good finishing, for use in boats, toys, household utensils, and plates (Souza et al. 2002). *E. contortisiliquum* is seen along the Brazilian east-coast, including the Atlantic rainforest and Caatinga biomes. It has lightwood (density of 0.54 g cm^{-3} at 12% of moisture content), which can be used in the manufacture of boats and crates. The flowers are mellifluous, and the fruits contain saponin, a substance used in the manufacture of soap.

Inga and *Erythrina* are commonly used as arboreal components of the agroforestry systems with banana, cocoa, and rubber trees in the state of Bahia and the Amazon. Frequently, both genera have also been planted under different arrangements of agroforestry systems in Latin America (Bolivia, Peru, and Colombia).

Table 6.3 Species of "fertilizer" tree legumes of Brazilian flora suggested for mixed plantations with *Eucalyptus* spp.

Genus	Species	Geographic distribution (Brazilian states[a])	Phytogeographical domains (Brazilian biomes)
Erythrina	*E. verna* Vell.	North (AC); Southeast (ES, MG, RJ, SP)	Amazon, Atlantic rainforest
	E. poeppigiana (Walp.) O. F. Cook	North (AC, AM, PA, RO)	Amazon
	E. fusca Lour.	North (AC, AM, AP, PA, RO) Midwest (MT)	Amazon, Cerrado
Inga	*I. edulis* Mart.	North (AC, AM, AP, PA, RO, RR); Northeast (BA, PB, PE); Midwest (MT); Southeast (ES, MG, RJ, SP) South (PR, SC)	Amazon, Caatinga, Cerrado, Atlantic rainforest
	I. laurina (Sw.) Willd.	North (AC, AM, PA); Northeast (BA, CE, MA, PB, PE); Midwest (DF, GO, MS, MT); Southeast (ES, MG, RJ, SP) South (PR)	Amazon, Caatinga, Cerrado, Atlantic rainforest
	I. cinnamomea Spruce ex Benth.	North (AC, AM, AP, PA, RO)	Amazon
Enterolobium	*E. maximum* Ducke	North (AC, AM, PA, RO, RR); Midwest (MT)	Amazon
	E. contortisiliquum (Vell.) Morong	Northeast (BA, CE, PB, PE, PI, RN); Midwest (DF, GO, MS, MT); Southeast (MG, RJ, SP); South (PR, RS, SC)	Caatinga, Cerrado, Atlantic rainforest
Anadenanthera	*A. colubrina* var. *cebil* (Griseb.) Altschul	Northeast (BA, CE, PB, PE, PI, RN, SE); Midwest (DF, GO, MS, MT); Southeast (MG)	Caatinga, Cerrado, Atlantic rainforest
	A. colubrina (Vell.) Brenan var. *colubrina*	Northeast (BA); Southeast (MG, RJ, SP); South (PR)	Caatinga, Cerrado, Atlantic rainforest
	A. peregrina (L.) Speg. var. *peregrina*	North (AM, PA, RR); Midwest (DF, GO, MS); Southeast (MG)	Amazon, Cerrado
	A. peregrina var. *falcata* (Benth.) Altschul	Northeast (BA, PB); Midwest (MS, MT); Southeast (MG, RJ, SP); South (PR)	Caatinga, Cerrado, Atlantic rainforest
Mimosa	*M. scabrella* Benth.	Southeast (MG, RJ, SP); South (PR, RS, SC)	Atlantic rainforest
	M. caesalpiniaefolia Benth.	North (AM, PA, RO); Northeast (AL, BA, CE, MA, PB, PE, PI, RN); Midwest (DF, GO, MS); Southeast (ES, MG, RJ, SP); South (PR, SC)	Amazon, Caatinga, Cerrado, Atlantic rainforest

[a] Brazilian states abbreviations: *AC* Acre, *AL* Alagoas, *AM* Amazonas, *AP* Amapá, *BA* Bahia, *CE* Ceará, *DF* Distrito Federal, *ES* Espírito Santo, *GO* Goiás, *MA* Maranhão, *MG* Minas Gerais, *MS* Mato Grosso do Sul, *MT* Mato Grosso, *PA* Pará, *PB* Paraíba, *PE* Pernambuco, *PI* Piauí, *PR* Paraná, *RJ* Rio de Janeiro, *RN* Rio Grande do Norte, *RO* Rondônia, *RR* Roraima, *RS* Rio Grande do Sul, *SC* Santa Catarina, *SP* São Paulo, *SE* Sergipe

There are several species of *Inga* in the Brazilian flora that are generally adapted to hot and humid climates. Many of them are responsive to pruning in the aerial part, where all green biomass generated can be enclosed along the planting lines of *Eucalyptus* spp. The trees of *Inga* spp. produce lightwood that can be used for coinage and energy. The fruits are edible and can be commercially exploited for the regional markets. Similarly, the genus *Erythrina* also has trees with light- and softwood, used mainly for crates, furniture linings, shoes, and toys.

Mimosa has smaller trees (varying from 5 to 15 m) distributed in several biomes. *M. scabrella*, for example, is a species found in cold places or altitudes in the south and southeast of Brazil. The wood from this genus has an average density of 0.67 g cm^{-3}, and is widely used in interior finishes, in the manufacture of plywood and packaging and for energy. *M. caesalpiniaefolia* however adapts well to warm, dry, or humid climates. In the Brazilian Northeast, it is commonly cultivated. It is a spiny tree, even though with non-spiny variants, that produces multiple stems, requiring significant maintenance. Some of the tree characteristics are helpful, as living fences. The wood is suitable for firewood, charcoal, cable tools, and external uses such as wood posts. Its wood is long lasting under external conditions, even without chemical treatment.

6.7.2 Timber Species

Among the trees of this group, we highlight the following genera: *Anadenanthera, Bowdichia*, *Centrolobium*, *Dalbergia*, *Hymenolobium*, *Plathymenia*, and *Tachigali* (Table 6.4).

Some species of *Anadenanthera* are known as "angicos" in Brazil. These are fast-growing plants and can be found in several Brazilian regions. *A. colubrina* has heavy woods (density ranging from 0.80 to 1.00 g cm^{-3}) suitable for building (indoor), planks, packaging, firewood, and charcoal. *A. peregrina* has a dense wood (0.70 to 0.97 g cm^{-3}) and is suitable for the manufacture of pieces of rafts, frames, roof slats, rural constructions, and outdoor construction materials such as sleepers, stakes, fence posts, and posts. In addition to the wood uses, the mixed plantations of *Anadenanthera* with *Eucalyptus* offer honey from the flowers.

Species of the genus *Bowdichia* are popularly known as "sucupira" in Brazil. *B. nitida* is an Amazonian species with the potential to reach heights of up to 35 m in natural conditions. The most common uses include the timber for making furniture, decorative laminates, bridges, and civil and naval constructions (Souza et al. 1997). The wood is very dense (exceeding 0.96 g cm^{-3}) and dark brown in color. *B. virgilioides* is distributed in the different Brazilian biomes and generally reaches heights of about 20 m. The wood is of high density (0.91 g cm^{-3}), is long-lasting, and is used in construction (outdoor areas) and furniture.

The genus *Centrolobium* has some of the important timber species such as *C. tomentosum*, *C. robustum*, and *C. paraense*, known in Brazil as "putumuju" or "araribá." The woods of these species are heavy, dense (over 0.75 g cm^{-3}), and easy

Table 6.4 Timber species legumes with late rotations of Brazilian flora suggested for mixed plantations with *Eucalyptus* spp.

Genus	Species	Geographic distribution (Brazilian states[a])	Phytogeographical domains (Brazilian biomes)
Plathymenia	*P. reticulata* Benth.	North (PA); Northeast (BA, CE, MA, PI); Midwest (DF, GO, MS, MT); Southeast (ES, MG, RJ, SP); South (PR)	Amazon, Caatinga, Cerrado, Atlantic rainforest
Dalbergia	*D. nigra* (Vell.) Allemãoex Benth.	Northeast (AL, BA, PB, PE, SE); Southeast (ES, MG, RJ, SP); South (PR)	Atlantic rainforest
Tachigali	*T. vulgaris* L. G. Silva & H. C. Lima	North (AM, PA, TO); Northeast (BA, CE, MA, PI); Midwest (DF, GO, MS, MT); Southeast (MG, SP)	Amazon, Caatinga, Cerrado
Centrolobium	*C. robustum* (Vell.) Mart. ex Benth.	Northeast (BA); Southeast (ES, MG, RJ, SP)	Atlantic rainforest
	C. tomentosum Guillem. ex Benth.	Northeast (BA); Midwest (DF, GO); Southeast (ES, MG, RJ, SP); South (PR)	Caatinga, Cerrado, Atlantic rainforest
	C. paraense Tul.	North (RR)	Amazon
Hymenolobium	*H. modestum* Ducke	North (AM, PA)	Amazon
	H. petraeum Ducke	North (AM, AP, PA); Northeast (MA)	Amazon
	H. excelsum Ducke	North (AM, PA)	Amazon
Bowdichia	*B. virgilioides* Kunth	North (AM, AP, PA, RO, RR, TO); Northeast (AL, BA, CE, MA, PB, PE, PI, RN, SE); Midwest (DF, GO, MS, MT); Southeast (ES, MG, SP)South (PR)	Amazon, Caatinga, Cerrado, Atlantic rainforest, Pantanal
	B. nitida Spruce ex Benth.	North (AC, AM, AP, PA, RO, RR)	Amazon

[a]Brazilian states abbreviations: *AC* Acre, *AL* Alagoas, *AM* Amazonas, *AP* Amapá, *BA* Bahia, *CE* Ceará, *DF* Distrito Federal, *ES* Espírito Santo, *GO* Goiás, *MA* Maranhão, *MG* Minas Gerais, *MS* Mato Grosso do Sul, *MT* Mato Grosso, *PA* Pará, *PB* Paraíba, *PE* Pernambuco, *PI* Piauí, *PR* Paraná, *RJ* Rio de Janeiro, *RN* Rio Grande do Norte, *RO* Rondônia, *RR* Roraima, *RS* Rio Grande do Sul, *SC* Santa Catarina, *SP* São Paulo, *SE* Sergipe, *TO* Tocantins

to work. The wood colors range from brown to yellow, with veins or orange spots. The wood is employed typically in luxury carpentry and fine furniture, but it is also used in civil and naval constructions, and in hydraulic work.

Dalbergia nigra, known as "jacarandá-da-Bahia," produces one of the most beautiful and premium woods of the Atlantic rainforest. Consequently, it happens to be one of the threatened species facing extinction in the Brazilian forests. In Brazil, there are other timber species of the same genus, for example, *D. spruceana*, which is found in the Amazon. These species produce heavy (ranging from 0.80 to 1.00 g cm^{-3}), smooth, fine-textured, natural-toned, dark-colored (sometimes blasted) wood that offers an excellent finish to luxury furniture and interior decoration. It is one of the well-known Brazilian woods and used in the manufacture of musical instruments (piano, violin, and others).

Species of the genus *Hymenolobium* are known commercially as "angelim", although this vernacular name has also been attributed to other Amazonian legume tree species, such as *Dinizia excelsa* Ducke ("angelim-vermelho, angelim pedra"), *Vatairea paraensis* Ducke, *Vatairea sericea* (Ducke) Ducke, *Vataireopsis speciosa* Ducke ("angelim-amargoso"), and *Pithecellobium racemosum* (Ducke) Killip (angelim-rajado). However, only *Hymenolobium* and *Pithecellobium* species are N_2 fixing. Some *Hymenolobium* species reach heights up to 40 to 50 m and 80 to 100 cm of diameter at breast height (DBH) in their natural habitats, with rectilinear and cylindrical shafts up to 25 m in length. The wood has a reddish-brown core, with darker brown spots due to oil-resin exudation and pale-brown sapwood. The wood is of medium to high density (0.71 g cm^{-3}), with easy workability, and offers a good finish. It is currently one of the largest woods used in the Brazilian domestic market, and commonly used in the manufacture of furniture and civil construction (beams, rafters, frames, linings, and others).

Plathymenia foliolosa, popularly known as "vinhático," is widely distributed in Brazil. The vinhático trees reach a height between 15–30 m and 40–70 cm of DBH under natural conditions. A striking feature of the adult trees is that the bark emerges from the trunk as large plaques. The wood is light (density of 0.50 g cm^{-3}) and has easy workability and longer durability. The color of the wood ranges from yellow-gold to yellow-brown, and is therefore commonly used in luxury articles, furniture and civil construction (decorative interior panels), and internal ship finishing.

Tachigali vulgaris, known as "tachi-branco or tachi-dos-campos," has been widely cultivated for over two decades in monoculture stands in northern Brazil and has shown good silvicultural potential. Castro et al. (1990) found annual mean increments (AMI) in height, DBH, and volume of 2.2 m year $^{-1}$, 2.9 cm $year^{-1}$, and 9.2 m^3 $year^{-1}$, respectively, when evaluating 3.5-year-old monocultures, established with a spacing of 3 m × 3 m. Narducci (2014) found AMI of 2.53 m $year^{-1}$ and height of 2.05 cm $year^{-1}$ at DBH in 7.5-year-old monocultures planted with a spacing of 4 m × 4 m. *T. vulgaris* presents a medium to high wood density (0.60 g cm^{-3} to 0.74 g cm^{-3}) which can be suitable for the production of sawwood and roundwood, especially posts, beams, and civil construction, and for energy purposes. It is considered to be moderately dense wood (0.65 g cm^{-3} to 0.81 g cm^{-3}) (Carvalho 2005).

6.7.3 *Other Introduced-Potential Species (Trees and Shrubs)*

In Brazil, some legume trees were introduced and have been cultivated/domesticated by farmers and foresters. Such species could also be tested in different arrangements of mixed-species plantations with *Eucalyptus* and/or other native species, including the cultivation of shrubby legumes for green manure production. All these species are listed in Table 6.5.

6.8 Final Considerations

In recent years, studies on nitrogen-fixing tree species have been intensified, including in mixed-forest plantations. The ability to fix nitrogen and accumulate large amounts of N as part of their biomass confers adaptive characteristics to the legumes that excel over other species. There is a large diversity of legume trees, such as the early and fast growers, and some which are slow growers but produce better quality timber.

Most studies on mixed-species plantations seem to be focused on *Acacia* and *Eucalyptus*. From these studies, many technical recommendations are readily available for the productive sector, although its large-scale use is challenging. *A. mangium* and *A. mearnsii* seem to be the main N_2-fixing species studied (Forrester et al. 2005; Bouillet et al. 2013). These species have shown great adaptation to South American edaphoclimatic conditions and other tropical and subtropical countries. Furthermore, both species have been widely cultivated in their native regions (i.e., Southeast Asia and Oceania). This is due to the multiple wood uses, which can be applied to the production of cellulosic pulp, firewood, and charcoal. In Indonesia and Vietnam, the branches and dead leaves are used as fuel and the leaves as fodder for cattle due to their high protein content (Krisnawati et al. 2011). Some non-timber uses still include the production of honey (due to apiculture flowers and the presence of extrafloral nectaries) (Tonini et al. 2010), glue, and tannin extraction from the bark (mainly for *A. mearnsii*). The barks of *A. mangium* additionally provide a good substrate for edible mushrooms (Lim et al. 2011).

However, in Brazil, *A. mangium* behaves as a aggressive and invasive species, which in part seems to be due to its broad N_2-fixing capability even in marginal soils with low nutrients (Souza et al. 2018; Le Maitre et al. (2011) Delnatte and Meyer 2012, Aguiar et al. 2014, Morais and Montagner 2015, see Chap. 11). Its capacity to absorb P and the highly efficient nutrient recycling within the plants make it a strong competitor for this element. Therefore, silvicultural programs must consider the phosphate fertilization management in successive rotations that include these acacia species.

In this context, we stimulate the test with native legumes from Brazil and in neotropical region, in general, in order to generate information on optimal growth conditions of these legumes. In Brazil, despite the high diversity of woody species,

Table 6.5 Other introduced-potential species (trees and shrubs) with potential to use in forest planting

Genera	Species	Origin	Habit	Potential invasion risk reported in Brazil[a]	Uses
Acacia	*A. auriculiformis* Ex Benth.	Southeastern Asia, Australia, Papua New Guinea	Woody	YES+	Fertilizer/ timber
	A. mangium Willd.	Indonesia, Australia, Papua New Guinea	Woody	Yes+	Fertilizer/ timber
	A. mearnsii Willd.	Australia, Papua New Guinea, Tasmania	Woody	Yes	Fertilizer/ timber
Acaciella	*A. angustissima* (Mill.) Kuntze	Central America, Colombia	Woody	Yes	Fertilizer
Albizia	*A. lebbeck* (L.) Benth.	Southern Asia, South Africa, Australia	Woody	Yes+	Fertilizer/ timber
Cajanus	*C. cajan* (L.) Millsp.	Probably from India	Shrubby	No	Fertilizer
Crotalaria	*C. grahamiana* Wight & Arn.	Probably from India	Shrubby	No	Fertilizer
	C. juncea L.	Asia	Shrubby	No	Fertilizer
	C. spectabilis Roth	Asia	Shrubby	No	Fertilizer
Falcataria	*F. moluccana* (Miq.) Barneby and Grimes	Southeastern Asia, Papua New Guinea	Woody	No	Fertilizer
Gliricidia	*G. sepium* (Jacq.) Steud.	Central America	Shrubby/ woody	No	Fertilizer
Leucaena	*L. leucocephala* (Roxb.) Benth.	Central America	Woody	Yes+	Fertilizer
Pithecellobium	*P. dulce* (Roxb.) Benth.	Mexico, Central America and northern of South America	Woody	Yes	Fertilizer
Pseudosamanea	*P. guachapele* (Kunth) Harms	Central America, Colombia, Ecuador, Peru, Venezuela	Woody	Yes+	Fertilizer

(continued)

Table 6.5 (continued)

Genera	Species	Origin	Habit	Potential invasion risk reported in Brazil[a]	Uses
Sesbania	*S. grandiflora* (L.) Pers.	From southeastern Asia to northern Australia	Woody	No	Fertilizer
	S. sesban (L.) Merr.	Africa, Asia	Woody	No	Fertilizer
Tephrosia	*T. vogelii* Hook. f.	Tropical Africa	Shrubby	No	Fertilizer

[a]"+" means high

there are few long-term experiments on silviculture and management of native species under mixed plantations. One of the pioneering works was conducted by Dr. Renato de Jesus and his collaborators at the Reserva Natural Vale (Linhares, ES, Brazil) since the 1970s (Rolim and Piotto 2018). In general, results have shown that many native species (including N_2-fixing trees) present great potential for reforestation and agroforestry systems, increasing the supply of high-quality timber and reducing the pressure on remnants of the Atlantic Forest. Clearly, several barriers must be overcome because many of the native species mentioned above require further characterization and knowledge for their domestication, especially for people relying on timber for their livelihood. Other barriers include the identification of species adapted to the different Brazilian edaphoclimatic conditions, responses to silvicultural treatments (i.e., thinning or pruning regimes), and need to ensure adequate seed availability.

As for the benefits of BNF, it can be a major factor to enhance the productivity and sustainability of a forest plantation. What we still need is the increased adoption of inoculation of legume seeds during the seedling production and transplanting stages. Selecting the appropriate strain and inoculating during these two stages have been improving the symbiosis.

It is recommended that the species discussed in this chapter be prioritized in future studies of *Eucalyptus* plantations mixed with N_2-fixing legumes. At the time of species selection, the farmer should opt for the availability of seeds and seedlings in the region, as well as check for compatibility to the local climate.

This is an activity involving multilocation field trials and selection of genetically superior material with the desired phenotype suitable for forest stands and for timber yield; particularly, the shape and size of the trunk may be key considerations. Besides, additional studies are needed to understand the behavior and interaction of these species in mixed plantations with *Eucalyptus* spp. and others non-N_2-fixing species in diverse Brazilian soil and climate conditions. A series of experiments, collaborations by different institutions, and participation of forestry experts from different regions would be immensely valuable.

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