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Microbiological process in agroforestry systems. A review

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Abstract Soils around the world are degraded due to inappropriate management practices. There is thus the necessity to find more conservationist agricultural systems. Agroforestry system is an alternative system that helps prevent land degradation while allowing continuing use of land to produce crops and livestock on a sustainable basis. Agroforestry system is a form of sustainable land use that combines trees and shrubs with crops and livestock in ways that increase and diversify farm and forest production while also conserving natural resources. This system enhances organic carbon accumulation in soils by the inclusion of cover crops and permanent vegetation, which is expected to increase the soil microbial biomass. The use of microorgan-

isms aims at improving nutrient availability for plants. Currently, there is an emerging demand to decrease the dependence on chemical fertilizers and achieve sustainable agriculture and agroforestry. Arbuscular mycorrhizal fungi, plant growth-promoting rhizobacteria, and the association of rhizobia with leguminous plants are mutualistic symbioses of high economic importance for increasing agricultural production. The biological nitrogen fixation (BNF) process is an economically attractive and ecologically sound method to reduce external nitrogen input and improve the quality and quantity of internal resources. BNF by associative diazotrophic bacteria is a spontaneous process where soil nitrogen is limited and adequate carbon sources are available. However, the ability of these bacteria to contribute to increased crop yields is only partly a result of BNF. The successful use of legumes is dependent upon appropriate attention to the formation of effective symbioses with root nodule bacteria. An essential component for increasing the use of legumes is the integration of plant breeding and cultivar development, with appropriate research leading to the selection of elite strains of root nodule bacteria. An expansion of the utility of inoculants is also necessary to develop a broad conceptual framework and methodology that is supported by scientific arguments; it is destined to impact assessment of the use of new biological products in agriculture.

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1 Introduction

Soil degradation can be attributed to unsuitable land use and inappropriate land management practices. Deforestation of fragile land, overcutting of vegetation, shifting cultivation, overgrazing, repetitive tillage, and unbalanced fertilizer use have resulted in progressive loss of soil quality (Miralles et al. 2009). Therefore, there is a need to develop sustainable agricultural systems that maintain soil biological processes and are less dependent on external inputs (i.e., fertilizers and herbicides) and mechanical cultivation to reduce impacts on the environment and conserve soils (Moonen and Bàrberi 2008). Agroforestry system (AFS) can be considered an alternative system that help prevent land degradation while allowing continuing use of land to produce crops and livestock on a sustainable basis (Cacho 2001; FAO 2005).

AFS is a form of multi-cropping which involves combining at least one woody-perennial species with a crop which results in ecological and economic interactions between the two components (Palma et al. 2007). Such systems are effective at improving and conserving soil quality by continuous deposition of plant biomass and turnover of leaf litter. This provides a continuous stream of organic material to the soil (Nair et al. 2008), especially because of the long roots of the forest component that go deep into the soil (Albrecht and Kandji 2003; Barreto et al. 2010), increasing soil organic matter stocks (Manley et al. 2007; Fontes et al. 2010) and the carbon sequestration potential (Roshetko et al. 2007; Sharrow and Ismail 2004; Kirby and Potvin 2007; Nair et al. 2009).

Biological properties can be also optimized in the soil under AFS (Yan et al., 2000; Udawatta et al. 2008; Yadav, et al. 2010). Several authors have reported that soil microbial biomass and microbial diversity are greater in the AFS due to the ameliorative effects of trees and organic matter inputs and the differences in litter quality and quantity and root exudates (Gomez et al. 2000; Myers et al. 2001; Mungai et al. 2005; Sørensen and Sessitsch 2007). The presence of a large and diverse soil microbial community is crucial to the productivity of any agroecosystem. Moreover, mixtures of

plant species in AFS usually allow a larger diversity and/or abundance of mycorrhizal fungi than monocultures (Cardoso and Kuyper 2006) and becomes more efficient the nitrogen biological fixation, especially in tropical soils (Serraj 2004; Freitas et al. 2010).

Although several benefits of agroforestry practices to soil microorganisms are reported in the literature, more research is needed to fill key knowledge gaps for a comprehensive understanding of buffer effects on overall environmental quality (Lowrance et al. 2002; Loveall and Sullivan 2006).

2 Soil microbial biomass

The soil microbial biomass comprises all soil organisms with a volume less than approximately $5 \times 10^3 \mu\text{m}^3$ (other than plant tissue) and can be considered as the living part of soil organic matter (Brookes 2001). The proportion present as living microbial cells (microbial biomass carbon in milligrams per kilogram of soil) typically comprises 1% to 5% (w/w) of the total organic carbon, and microbial nitrogen accounts for 1% to 6% (w/w) of the total organic nitrogen (Jenkinson and Ladd 1981).

The soil microbial biomass has important functions in the soil, including nutrient cycling and the degradation of pollutants (e.g., pesticides, urban and industrial waste) (Dick 1997; Haney et al. 2003; Watanabe and Hamamura 2003; Araujo et al. 2003; Araujo and Monteiro 2006). According to Powlson et al. (1987), the main function of microorganisms is to mediate soil processes and high rates of turnover, which is a sensitive indicator of changes in the soil organic matter.

The adoption of different management systems can have negative or positive effects on the soil properties. AFS are being increasingly recommended as a sustainable form of land use because they are believed to provide the optimum level of food production, a supply of firewood, and cash benefits, while maintaining soil fertility (Heuvelop et al. 1988). AFS generally enhance organic matter accumulation in soils through the inclusion of different crops and permanent vegetation cover, which would be expected to increase the soil microbial biomass. Additionally, AFS have been recognized as an alternative for the rehabilitation of degraded areas through the use of different tree species with crops. This management strategy improves the biological properties of degraded soils due the high quantity of sources of organic residues (Mendonça et al. 2001). These systems may restore soil biodiversity and other important functions of the soil community (Macdicken and Vergara 1990). Fisher (1995) suggested that trees might improve soil quality in several ways. Many tropical tree species can fix atmospheric nitrogen and may therefore increase the soil nitrogen content. The large root system of trees potentially accumulates nutrients from a large volume of soil, whereas fallen litter

concentrates nutrients near the soil surface. Fallen litter and fine-root turnover may increase the soil organic matter concentration. Trees may also enhance the above- and belowground microclimate around plant roots and may alter the soil biological properties. In Kenya, Belsky et al. (1989) found 35% to 60% greater soil microbial biomass under *Adansonia digitata* and *Acacia tortilis* crowns than in the open grassland areas, due to a better microclimate for the soil microorganisms. Tangiang et al. (2009) noticed that plant residues, added organic matter, vegetation, plant species composition, and soil mineral nutrients altered the microbial population and their species composition under traditional AFS in Northeast, India. Instead of the effects of growing trees in combination with field crops on soil microbial biomass was evaluated by Chander et al. (1998) in soils under a 12-year-old AFS, and the soil microbial biomass was significantly affected by the AFS. Both the amount of microbial biomass carbon and the enzymatic activities were greater in soils under AFS than in conventional systems. According to the same authors, the greater microbial biomass reflected the response of the increased input of organic matter to the soil under the AFS. Previously, Tornquist et al. (1999) found that the soil microbial biomass carbon and nitrogen were not significantly different in pastures and AFS in Costa Rica. According to the authors, the soils evaluated had a high soil microbial biomass.

Kaur et al. (2000) investigated the effects of the monocropping of rice, forestry, and agroforestry on the soil microbial biomass in India. The authors observed that the soil microbial biomass was increased by 42% (microbial carbon) and 13% (microbial nitrogen) in AFS compared to monocropping. They attributed the higher soil microbial biomass to the high quantity of carbon released by the AFS. Their findings supported a study by Rao and Pathak (1996) that demonstrated an increase in the microbial carbon and nitrogen (10–60% and 17–43%, respectively) in AFS with a large carbon source than in conventional soil with low carbon.

It seems likely that litter quality regulated the level of soil microbial biomass in tree-based systems. The availability of carbonaceous materials and substrates such as sugars, amino acids, and organic acids to the soil from the roots is important for supplying energy for the microbial populations (Bowen and Rovira 1991). The importance of root exudates in maintaining a larger microbial biomass closer to the trees has also been reported by Browaldh (1997).

In a Brazilian ecosystem, Almeida et al. (1997) compared the influence of an AFS with coffee, native vegetation, or a conventional system on the soil microbial biomass. The author observed a decrease in the soil microbial biomass under the conventional system; however, the AFS was similar to the native vegetation. Furthermore, the AFS showed greater potential to cycle nutrients than the conventional system.

3 Biological nitrogen fixation

Biological nitrogen fixation (BNF) is known to occur to various degrees and in different environments, including soils, fresh and salt waters, and sediments; on or within the roots, stems, and leaves of certain higher plants; and within the digestive tracts of some animals. The potential for nitrogen fixation exists for any environment capable of supporting the growth of microorganisms. Biological systems that are capable of fixing nitrogen are historically classified as non-symbiotic or symbiotic, depending on the involvement of one or more organisms, respectively (Hubbell and Kidder 2003).

Over the last few years, studies examining the nodulation of legume tree species and the selection of highly efficient rhizobial strains for legume trees have received more attention. Species with potential use in different agrosystems, efficient nitrogen-fixing rhizobia, have been selected and are available for inoculant production (Faria 1995; Faria and Lima 1998; Chen et al. 2005; Balachandar et al. 2007). New species of rhizobia or bradyrhizobia have been described, and large collections of isolates are being developed. These rhizobia are of economic importance in low-input sustainable agriculture, agroforestry, and land reclamation (Balachandar et al. 2007).

Until recently, it has been generally accepted that legumes (and the non-legume genus *Parasponia*) are nodulated exclusively by members of the family *Rhizobiaceae* in the α -proteobacteria, which includes the genera *Azorhizobium*, *Bradyrhizobium*, *Mesorhizobium*, *Rhizobium*, and *Sinorhizobium* (Sprent 2001). Over the last few years, however, several other species of α -proteobacteria have been shown to nodulate legumes (Moulin et al. 2002). These include strains of *Methylobacterium* that nodulate *Crotalaria* and *Lotononis* (Jaftha et al. 2002; Sy et al. 2001a and b); *Blastobacter denitrificans*, which nodulates *Aeschynomene indica* (van Berkum and Eardly 2002); and *Devosia* strains that nodulate *Neptunia natans* (Rivas et al. 2002). More controversially, a few members of the β -proteobacteria, such as *Burkholderia* spp. (originally isolated from *Aspalathus carnosa* and *Machaerium lunatum*, Moulin et al. 2001) and *Ralstonia taiwanensis* (isolated from *Mimosa pudica*; Chen et al. 2001) have been discovered in nodules of tropical legumes. The terms α - and β -rhizobia have been proposed to distinguish the rhizobial α - and β -proteobacteria (Moulin et al. 2001). Phylogenetic analysis of the available *nodA* and *nifH* genes from α - and β -proteobacteria suggests that β -rhizobia evolved from diazotrophs through multiple lateral gene transfers (Chen et al. 2003).

All α -rhizobial genera belong to the Rhizobiales order, whereas β -rhizobial genera belong to the Burkholderiales order. A same genus or even species often contains both rhizobial and nonrhizobial strains, each *Rhizobium* has a defined host spectrum, but there is no strict correlation between legume and bacterium taxonomy, although some

associations are favored (e.g., *Azorhizobium*—*Sesbania* and *Burkholderia*—*Mimosa*) (Masson-Boivin et al. 2009).

The taxonomic classification of rhizobia strains has undergone a transformation since 2001, with the reclassification of some species to the β -proteobacteria suborder, especially *Burkholderia* species (Moulin et al. 2001). *Burkholderia* species are effective symbionts of several plants species (Barrett and Parke 2005, 2006; Chen et al. 2005; Chen et al. 2003). Although some are important pathogens for humans and animals (e.g., *Burkholderia cepacia*, *Burkholderia pseudomallei*, and *Burkholderia mallei*), studies show that the symbiont species are phylogenetically distant of these (Bontemps et al. 2009).

According to Moulin et al. (2001), the characterization of the symbionts of the yet unexplored legumes may reveal the rhizobial nature of additional members of the beta-proteobacteria and possibly other taxonomic classes. Such a study may contribute significantly to the understanding of the origin, and the evolution of, the legume–rhizobia symbioses, and may open new perspectives for engineering beneficial associations.

Besides, a number of isolates have been reported from legume nodules, capable of nitrogen fixation but phylogenetically located outside the traditional groups of rhizobia in the alpha-proteobacteria. New lines that contain nitrogen-fixing legume symbionts include *Methylobacterium*, *Devosia*, *Ochrobactrum*, and *Phyllobacterium* in the alpha-proteobacteria and *Burkholderia*, *Ralstonia*, and *Cupriavidus* in the beta-proteobacteria. All these new nodulating bacteria are phylogenetically (16S rDNA) distinct from the rhizobia, but do carry nod genes similar to those of rhizobia. These genes encode for Nod factors, signal molecules in the bacterium–legume communication that accompanies nodulation (Willems 2006).

Recently, the presence of γ -proteobacteria of the genera *Pseudomonas*, *Pantoea*, *Enterobacter*, and *Escherichia* was described in the nodules of *Hedysarum* spp. (Benhizia et al. 2004; Muresu et al. 2008), suggesting potential synergistic interactions among various species and the existence of other unidentified mechanisms. Another species belonging to the *Cohnella phaseoli* suborder of γ -proteobacteria was recently isolated from the nodules of *Phaseolus* in Spain. However, this species was not detected in nodules from three species of this genus, and no reported *Nod* genes have been identified in this bacteria (García-Fraile et al. 2008).

Among the new genera capable of nodulating leguminous species, the association between plants of the genus *Mimosa* and bacteria should be emphasized. *Burkholderia* spp. is more competitive when co-inoculated with strains of *Rhizobium tropici* and *Rhizobium etli* (Elliott et al. 2009). Another interesting point is the inability of *Mimosa* spp. to form nodules when inoculated with *Rhizobium* sp. strain NGR234, which has a broad spectrum of hosts (over 250

plants including *Parasponia* sp.) (Pueppke and Broughton 1999).

Studies using native plant species in tropical regions have been conducted, and new rhizobia species have been described (Martinez-Romero et al. 1991; Vandamme et al. 2002; Chen et al. 2008), suggesting wide diversity for this bacteria group. The selection of partners (macro and microsymbionts) based on symbiotic specificity and the identification of those with greater efficiency is part of the strategy to optimize the BNF, especially given that nodulation in legumes does not occur equally among the three subfamilies. Hirsch et al. (2001) estimated that less than 25% of Caesalpinoideae are able to nodulate, whereas over 90% of the Mimosoideae and Papilionoideae subfamilies are capable of nodulation.

3.1 Improving the nodulation and nitrogen fixation ability

Microbial interactions, which are regulated by specific molecules/signals, are responsible for key environmental processes, such as the biogeochemical cycling of nutrients and matter and the maintenance of plant health and soil quality (Barea et al. 2004). Plant growth-promoting rhizobacteria (PGPR), in combination with efficient rhizobia, could improve growth and nitrogen fixation by inducing the occupancy of the introduced rhizobia in the nodules of the legume (Tilak et al. 2006). Some PGPR can improve nodulation and nitrogen fixation in legume plants (Zhang et al. 1996; Andrade et al. 1998; Lucas-Garcia et al. 2004). Studies carried out under field conditions (Bai et al. 2003), particularly those using ^{15}N -based techniques (Dashti et al. 1998), have highlighted these beneficial cooperative effects between microbes. Research on the mechanisms by which PGPR enhance nodule formation has implicated their production of plant hormones in the co-inoculation benefits. For example, Chebotar et al. (2001) demonstrated that some *Pseudomonas* strains, but not all, increased the number of nodules and lowered the acetylene activity in soybean plants inoculated with *Bradyrhizobium japonicum*. Silva et al. (2006) verified that some *Bacillus* strains with effective Rhizobia resulted in enhanced nodulation in cowpeas (*Vigna unguiculata* L.), soybeans (*Glycine max* L.) (Araújo and Hungria 1999), and beans (*Phaseolus vulgaris* L.) (Figueiredo et al. 2008). Furthermore, Vessey and Buss (2002) demonstrated that the application of *Bacillus* species to seeds or roots caused variation in the composition of the rhizosphere, which resulted in increased growth and yield of different crops.

According to Saravana-Kumar and Samiyappan (2007), *Bradyrhizobium* prompted the nodulation and growth of legumes in combination with active 1-aminocyclopropane-1-carboxylate (ACC) deaminase-expressing PGPR. Moreover, certain rhizobacteria also possess the enzyme ACC deaminase, which hydrolyses ACC into ammonia and α -

ketobutyrate (Mayak et al. 1999). ACC deaminase activity in PGPR plays an important role in the host nodulation response (Remans et al. 2007). PGPR expressing ACC deaminase could suppress accelerated endogenous ethylene synthesis and, therefore, may facilitate root elongation and nodulation, which in turn could improve the growth and yield of the plants (Zafar-ul-Hye 2008). In addition, the gene for ACC deaminase has also been found in some rhizobia, including *Mesorhizobium loti*, *B. japonicum*, and *Rhizobium* sp. ACC deaminase also facilitates symbiosis by decreasing the ethylene levels in the roots of the host (Okasaki et al. 2007).

Endophytes can also produce ACC deaminase. Whereas this enzyme has no function in bacteria, it cleaves ACC, the precursor of ethylene in plants, and therefore modulates the ethylene levels that promote plant growth (Sziderics et al. 2007; Sun et al. 2009). Plant growth is also promoted by the production of phytohormones, such as auxins, cytokinins, and gibberellins (Steenhoudt and Vanderleyden 2000). The most related phytohormone produced by endophytic bacteria is the auxin indole-3-acetic acid, which is produced by *Gluconoacetobacter*, *Azospirillum*, *Herbaspirillum*, *Methylobacterium*, *Erwinia*, *Pantoea*, and *Pseudomonas* (Kuklinsky-Sobral et al. 2004).

3.2 The role of arbuscular mycorrhiza fungi in improving nodulation and rhizobial activity

The widespread presence of symbiotic arbuscular mycorrhiza (AM) fungi in nodulated legumes and the role of AM fungi in improving nodulation and rhizobial activity within the nodules are both universally recognized processes (Barea et al. 2005). AM fungi and rhizobia are two of the most important plant symbionts; they play a key role in natural ecosystems and influence plant productivity, plant nutrition, and plant disease resistance (Demir and Akkopru 2007). Mycorrhizas benefit the host through the mobilization of phosphorus from non-labile sources, whereas *Rhizobium* fixes nitrogen (Scheublin and Van der Heijden 2006). The interactions between AM fungi and bacteria suggest a beneficial effect of the fungi on bacterial development and vice versa (De Boer et al. 2005). AM colonization has been shown to improve nodulation and nitrogen fixation, and the use of the isotope ^{15}N has made it possible to ascertain and quantify both the amount of nitrogen that is fixed in a particular situation and the contribution of the AM symbiosis to nitrogen fixation (Barea et al. 2002). Studies examining the biochemical and physiological basis of the interactions between AM fungi and rhizobia that improve legume productivity have suggested that the main effect of AM fungi in enhancing rhizobia activity is through the generalized stimulation of host nutrition. However, it should be noted that some localized effects may also occur at the root or nodule level (Barea et al. 1992). Additional experiments

have corroborated the positive effect of the interactions between AM fungi and rhizobia under drought conditions (Ruiz-Lozano et al. 2001). For example, inoculation with AM fungi protected soybean plants against the detrimental effects of drought and helped the plants tolerate the premature nodule senescence induced by drought stress (Porcel et al. 2003). Suitable combinations of AM fungi and rhizobia bacteria may increase plant growth and resistance to pathogens (Aysan and Demir 2009) and improve nodulation and nitrogen fixation (Barea et al. 2002).

The bacteria involved in the establishment of mycorrhiza and/or mycorrhiza function were therefore designated mycorrhiza helper bacteria (MHB) by Garbaye (1994) and are currently the most investigated group of bacteria that interact with mycorrhizas (Frey-Klett et al. 2007). The MHB strains that have been identified to date belong to many bacterial groups and genera, such as Gram-negative proteobacteria (*Agrobacterium*, *Azospirillum*, *Azotobacter*, *Burkholderia*, *Bradyrhizobium*, *Enterobacter*, *Pseudomonas*, *Klebsiella*, and *Rhizobium*), Gram-positive firmicutes (*Bacillus*, *Brevibacillus*, and *Paenibacillus*) and Gram-positive actinomycetes (*Rhodococcus*, *Streptomyces*, and *Arthrobacter*) (Frey-Klett et al. 2007). Some species are responsible for multiple helper effects because they influence both plants and the associated mycorrhizal fungi. The stimulating effect of MHB has been evaluated mostly when symbiotic associations are exposed to stresses ranging from drought to contamination with heavy metals (Vivas et al. 2003a, b). A classic example illustrating the helper effect is the rhizobia-producing ACC deaminase, which modulates plant ethylene levels, thereby increasing plant tolerance to environmental stress and stimulating nodulation (Ma et al. 2002). Changes in auxin and cytokinin levels have been implicated in the normal infection and nodulation processes of various species of legumes. However, rhizobial inoculation has been reported to both increase and decrease auxin levels in the roots (Ferguson and Mathesius 2003).

3.3 Inoculation technology: a strategy for sustainable development

The success of inoculation technology is a result of the knowledge accumulated over a century of research. The first inoculants containing *Rhizobium* were marketed in the United States. Today, the inoculants are marketed in most of the world and are mainly destined for the agribusiness cultures, and it has been estimated that some 2,000 t of rhizobial inoculants are produced worldwide every year (Ben et al. 2007). Nevertheless, its use is still restricted for other species of legumes, such as those of interest in AFS. In Brazil, this scenario does not reflect the potential use of biological resources in a country that has an estimated 15–20% of the planet's biological diversity (Salati et al. 2006).

However, in addition to the selection of the strain, the inoculation vehicle is also part of the technology. Peat has been used for about a century as the principal support vehicle for inoculation of legumes. A number of practical issues, such as unevenness in the composition and difficulties in obtaining, handling, and the sterilization of peat, suggest new formulations need to be tested, developed, and adequately assessed in the field. Liquid formulations also have limitations, including difficulties in transportation and storage (because they often require refrigeration) and the difficulties in coating and homogeneous mixtures in the seeds. The proper formulation should be biodegradable and easy to handle and is even more critical when mixed inoculants are developed or when the inoculants contain additional plant growth-promoting bacteria (Dobbelaere et al. 2003; Deaker et al. 2004). In order to increase the inoculant quality and efficiency and to reduce costs and environmental impacts, alternative carrier materials have been studied (Ben et al. 2007; Albareda et al. 2008).

In an attempt to improve the inoculant quality in Brazil, different carrier blends, together with diazotrophs, were evaluated at room temperature, and their performance as an inoculant was compared to a peat-based inoculant carrier. Polymer blends functioned as an efficient carrier to rhizobial inoculants and show competitive advantages including biodegradability and being non-toxic and water soluble. These blends enabled the pre-inoculation of the seeds and the maintenance of the rhizobia numbers at room temperature that were comparable to that of traditional peat inoculants (Xavier et al. 2010). This product has been tested in association with *Bradyrhizobium* and cowpeas (Fernandes Júnior et al. 2009) and the inoculant for cane sugar (*Gluconacetobacter diazotrophicus*, *Herbaspirillum seropedicae*, *Herbaspirillum rubrisubalbicans*, *Azospirillum amazonense*, and *Burkholderia tropica*) (Silva et al. 2009).

The use of inoculant strains differs from traditional associations with rhizobia and legumes and is a promising alternative to agriculture. Over the next few years, there will be greater environmental awareness and sustainable use of natural resources, leading to a policy of increased demand for alternative sources of farm inputs and more efficient use of plants. With these conditions, there will be opportunities arising from the increased demand for more efficient plant uptake of nitrogen from the air.

However, quality control standards must be established, and few countries have identity standards and well-defined parameters for the inoculants. Since 2004, Brazil, through the Ministry of Agriculture and Livestock, has had legislation dealing with registration and the patterns and a catalog of authorized inoculants strains.

Expansion of the inoculants' utility is also necessary to develop a broad conceptual framework and methodology. With regard to scientific arguments, it is destined to impact the assessment of new biological products in agriculture.

Extensive discussions, with the participation of competent institutions responsible for the standardization of biosafety issues in the country, should be promoted, especially for the recommendation of new microbial species for use as inoculants.

To increase the efficiency of the product, the application of a mixture of strains with different characteristics to stimulate the synergy between the strains and adaptation to the environment should also be considered. However, these mixtures should be recommended cautiously because of the difficulties involved in the registration and quality control of these products.

Currently, BFN has also taken on a new meaning by the Brazilian government as it is among the four measures (in addition to the recovery of pasture, crop-livestock integration, and no-tillage on straw) to be adopted extensively in agriculture in order to voluntarily reduce greenhouse gas production. Therefore, BFN can also be viewed as a central part of the strategy for the integration of the other proposed actions.

3.4 Rhizobial biodiversity and its importance for the inoculant industry

Bacterial symbionts are extraordinarily diverse for some extremely beneficial traits, such as biological nitrogen fixation efficiency and rate, soil survival, and competitiveness for nodule formation (Brockman and Bezdicek 1989; Anyango et al. 1995; Doyle and Doyle 1998; Handley et al. 1998; Andronov et al. 1999; Caballero-Mellado and Martinez-Romero 1999; Collins et al. 2002; Galli-Terasawa et al. 2003; Fagerli and Svenning 2005; Grange and Hungria 2004; Jesus et al. 2005; Alexandre et al. 2006; Bala and Giller 2006; Duodu et al. 2006; Duodu et al. 2007; Langer et al. 2008; Alexandre et al. 2009).

This diversity is considered one of the most basic foundations of the inoculant industry, as it allows soil microbiology researchers to find, assess, and identify strains with higher nitrogen fixation potential, as well as other useful traits, that would allow a commercial rhizobial inoculant to increase the yield of legumes (Date 2000; Hungria and Vargas 2000; Aguilar et al. 2001; Lesueur et al. 2001; Faria 2002; Faria and Franco 2002; Mostasso et al. 2002; Martins et al. 2003; Howieson and Ballard 2004; Yates et al. 2005a; Yates et al. 2005b; Alberton et al. 2006; Silva et al. 2007).

On the other hand, this potential benefit can only be achieved by continuing research and evaluation of different bacterial strains (Daba and Haile 2000; Catroux et al. 2001), resulting in similarities between the inoculant and plant breeding industries. Both industries may be considered, from a pragmatic point of view, as essentially consisting of finding new genetic resources to achieve

higher plant yields than currently possible with the use of the available genetic material.

This constant renewal leads to the development and evaluation of a continually increasing amount of new genetic material to maximize the probability of finding new genetic resources to achieve higher plant yields, be it via greater nutrient use efficiency, water use efficiency, drought tolerance etc., than currently available. It would seem to be a “Red Queen” phenomenon, in which the industry has to run harder to be able to outpace its own past successes. For example, the São Paulo state sugarcane breeding program evaluated 300,000 new progenies in 2001, of which only 17 were deemed desirable for performance testing (Bressiani 2001). On the other hand, most scientific papers detailing strain evaluation cite much lower evaluated strains numbers. Some examples are the selection of strains for red clover (*Trifolium pratense*) inoculation in northern Scandinavia based on 431 strains (Duodu et al. 2007), high temperature-resistant strains for soybean (*G. max*) in Iran based on 56 strains (Rhamani et al. 2009), salt-resistant common bean (*P. vulgaris*) in Tunisia based on 19 strains (Mnasri et al. 2007), and several Mediterranean legume shrubs and trees in Central Spain with an exceedingly narrow basis of only nine strains (Ruiz-Díez et al. 2009).

An approach more similar to that of the plant breeding can be seen on a 1997 paper describing selection of strains for a forage legume under evaluation in Australia, which evaluated 1,200 rhizobial isolates for *Stylosanthes* sp. Aff. *Stylosanthes scabra* (*Stylosanthes seabrana*). Of those strains, 18 were selected for field experimentation in four different areas, resulting in a 10% to 20% yield increase in areas without established compatible populations in the first year, followed by a 400% gain in the second year compared to the un-inoculated control (Date 1997).

Evaluated strain numbers may be underestimated in the scientific literature in comparison to works of a more technical nature, such as research reports or pamphlets such as those from EMBRAPA describing strain selection for Brazilian legume tree species (Faria et al. 1984; Faria 1997; 2000; 2002). Unfortunately, this kind of information is usually not available to research papers, basically for space limitations and the inability to include the entire bacteria collection with all of the relevant details.

While inoculant production, as well as crop breeding, centers on economically important legumes, biodiversity research efforts have focused on several legume species with limited economical importance today, but which show potentially important uses for reforestation, improved fallows, or AFS (Chikowo et al. 2006; Makatiani and Odee 2007; Manassila et al. 2007; Ceccon 2008; Chesney 2008; Daudin and Sierra 2008; Bashan et al. 2009; Jalonen et al. 2009; Nygren and Leblanc 2009; Freitas et al. 2010). For

example, work has been conducted for several *Mimosa* species in Brazil (Bontemps et al. 2009), for *Prosopis* species in Morocco (Benata et al. 2008), and for several genera in Africa (Doignon-Bourcier et al. 1999; Bala et al. 2002; Odee et al. 2002; Bala et al. 2003a; Bala et al. 2003b; Wolde-meskel et al. 2004; Diabate et al. 2005; Diouf et al. 2007; Law et al. 2007).

4 Conclusion

AFS promote the permanent input of litter to increase the organic matter content of the soil and positively influence the soil microbial community by providing a large source of carbon and energy. The improvements in the soil microorganism status are important because microorganisms provide many functions in a soil ecosystem, including organic matter decomposition, nitrogen fixation, uptake of phosphorus by mycorrhiza, and the promotion of plant growth. The soil microbial populations are immersed in a framework of interactions known to affect plant fitness and soil quality. They are involved in fundamental activities that ensure the stability and productivity of both agricultural systems and natural ecosystems. Strategic and applied research has demonstrated that certain co-operative microbial activities can be exploited, as a low-input biotechnology, to help sustainable agriculture and agroforestry.

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