
Chapter 5

POTENTIAL IMPACT OF BIOLOGICAL NITROGEN FIXATION AND ORGANIC FERTILIZATION ON CORN GROWTH AND YIELD IN LOW EXTERNAL INPUT SYSTEMS

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ABSTRACT

Maize productivity in tropical low external input systems is usually limited by low soil fertility because crop uptake leads to a gradual depletion of soil nutrient stocks. Since the use of chemical fertilizers is unfeasible or undesired, the management of the fertility of these soils depends primarily on low-cost processes based on nutrient recycling. The main processes that may contribute to this are: 1) Biological Nitrogen Fixation (BNF); and 2) nutrient recycling through organic fertilization using plant residues or animal manures; 3) where feasible, use of industrial and/or urban waste. BNF may contribute to maize growth and yield by direct fixation in corn, or through the use of legume plants either as green manure or as crops in rotation or intercropped with corn. Either way, BNF can usually be considered as long term

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sustainable, and usually would be one of the preferred nitrogen sources for low external input corn production systems. Since almost all soil nitrogen is derived from the atmosphere, in the absence of substantial use of nitrogen fertilizer most of the remaining nitrogen pool is a product of BNF, either recent or past. The main difference between on-field BNF and use of plant residues and animal manures is that nitrogen was fixed or obtained from the soil pool on another field and later taken to the corn field. At the same time, nutrient recycling through organic fertilization is usually limited due to the low amounts of organic matter available for this use, especially considering the concurrent demands for this material. Therefore, the efficient use of the different types of organic matter used as fertilizer requires knowledge about its quality and patterns of decomposition in order to guarantee synchronization between nutrient supply and crop demand. Finally the third approach in these systems centers on the use of urban waste, most usually compost or sewage sludge, or industrial by-products. Some of these may be quite rich in several nutrients at the same time, but usually require careful investigation in possible negative effects of items such as heavy metals and pathogens. So, we review information regarding BNF directly on corn, in green manure or crop rotations involving this culture, strategies to improve the amount and quality of organic fertilizers produced in low input systems, and some possible alternatives of urban or industrial by-products, describing the current rationale to supply nutrients to maize crops at a low cost using the resources available within the agroecosystems.

Keywords: BNF, legume, green manure, sludge, compost

1- INTRODUCTION

Corn (*Zea mays*) is a major user of synthetic nitrogen fertilizer, therefore if Biological Nitrogen Fixation (BNF) in corn is successful; there could be far-reaching economic

consequences (Halbrendt & Blase, 1989). With the current cost of fertilizer approaching half the total variable cost of producing corn, the potential savings could be substantial if BNF is developed and adopted (Mendonça et al., 2006). By definition, BNF is synonymous with sustainability. This process offers an economically attractive and ecologically sound means of reducing external nitrogen input and improving the quality and quantity of internal resources (Saikia & Jain, 2007). Clearly, it is not realistic to consider sustainable agriculture on a broad scale in the absence of BNF.

Some cereal crops of commercial importance like corn, rice, wheat, and millets are found to have association with microorganisms that are capable of assimilating atmospheric nitrogen (Döbereiner & Boddey, 1981; Okon & Kapulnik, 1986; Baldani et al., 1986; Urquiaga et al., 1992; Chelius & Triplett, 2001; Riggs et al., 2001; Boddey et al., 2003; Tejera et al., 2006; Barassi et al., 2007; Herridge et al., 2008). The yields of corn have also risen steadily, largely because of use of hybrids and increased inputs of fertilizer nitrogen. To accommodate the world's expanding population, which is projected to double by 2050, an ever-increasing production of food crops will be necessary. This must be achieved primarily by increasing productivity of currently farmed areas, since suitable new land is very limited. An obvious goal of BNF research is to find ways to enable the major cereal crops to utilize BNF directly as a partial or major source of their nitrogen needs (Raymond et al., 2004).

2- BIOLOGICAL NITROGEN FIXATION (BNF)

The BNF is the process by which the bulk of the atmospheric nitrogen was incorporated into the living matter, throughout the evolution of our planet. Even today, this process is the main pathway of nitrogen incorporation to the ecosystem, which is constantly recycled into the atmosphere primarily by the action of organisms decomposers of soil

organic matter. Therefore, the action of microorganisms nitrogen fixers and denitrification warrants an inexhaustible reservoir of nitrogen in the atmosphere. In addition to ensuring an ecosystem in balance, a reduction in the application of excessive doses of nitrogen compounds, such as nitrate, which contaminates water and plants consumed by humans, enables the development of less aggressive agriculture to the environment. The estimate is that the contribution of biologically fixed nitrogen ranges from 139 to 170.10⁶ tons of nitrogen a year, at least the double of chemical fixation (Peoples & Craswell, 1992).

The microorganisms that promote the Biological Nitrogen Fixation (BNF) have great importance, since this element is essential component of proteins, nucleic acids and other nitrogen compounds, and therefore of life for all living beings (Döbereiner, 1997). Ecosystems in climax, the microbiote is in balance in the soil, maintaining its biodiversity and sustainability, but this balance can easily be broken by man or by natural phenomena (Döbereiner, 1992). Even though the greatest contributions of the BNF has been detected in oceans and leguminous, some plants of the family Gramineae have shown a very significant potential in obtaining nitrogen, by the action of nitrogen fixing bacteria (Baldani et al., 2002; Alves et al., 2006). These plants have a fascicule root system, taking advantage on the leguminous' pivoting system to extract water and soil nutrients, and because they are widely used as food by humans. Therefore, even if only part of N could be provided by the association with fixing bacteria, the economy in nitrogen fertilizers would be equal to or higher than that observed with leguminous that can be self-sufficient in nitrogen (Döbereiner, 1992; Boddey et al., 2003).

Undoubtedly, after carbon, oxygen and hydrogen, nitrogen is quantitatively the most important element required by plants and animals for growth both in water and on land reaching about 1.5% of the dry weight of innumerable agricultural crops (Van Loon & Duffy, 2001). So, this was one of nutrients that most contributed to the so-called Green Revolution.

Due to its indiscriminate use led to environmental problems (Bouchard et al., 1992). Some of the adverse environmental effects of excessive use of nitrogenous fertilizers are: (i) methemoglobinemia in infants due to NO_3 and NO_2 in waters and food, (ii) cancer due to secondary amines, (iii) respiratory illness due to NO_3 , aerosols, NO_2 and HNO_3 (iv) eutrophication due to N in surface water, (v) material and ecosystem damage due to HNO_3 in rainwater, (vi) plant toxicity due to high levels of NO_2 and NH_4 in soils, and (vii) excessive plant growth due to more available N, depletion of stratospheric ozone due to NO and N_2O (Saikia & Jain, 2007).

In tropical countries, 40% of the costs of maize cultivation, for instance, are committed to the purchase of mineral nitrogen (Majerowicz et al., 2002). Overall, cereals cultivation consumes 60% of the total nitrogen fertilizer used in the world. However, on average, only 33% of all N applied are recovered in the grains promoting the loss of 15.9 billion dollars in 1999 (Raun et al., 2002). Questions like these encourage the creation of technologies that reduce the excessive amount of fertilizer applied. The study of efficiency in the use of N allows multiple ways, from the simplest, which are based on the mere reduction of doses of fertilizers to productive levels, even those based on genetic improvement able to set up productive plants in poor N soils. Both in physiological level, as molecular, the study of the acquisition and use of N should be linked to the understanding of absorption, assimilation and redistribution of this nutrient in cell and its balance between storage and use in cellular and whole plant (Majerowicz et al., 2002).

Even now, new methods for its use are intensely studied. The quest for greater efficiency in its use, through recognition of biochemical and molecular pathways of absorption and assimilation in plants, as well as the agroecological methods, such as the BNF, are proposals to allow the sustainable use of this material without production loss (Traore & Maranville, 1999; Pradella et al., 2001).

A number of reviews of plant-associated N₂ fixation have clearly highlighted the many methodological problems and inconsistencies in the published studies (Boddey, 1987; Chalk, 1991; Giller, 2001; Giller & Merckx, 2003). One of the key problems is distinguishing between inputs of N by free-living and associative agents and other external sources of N contributing to agricultural soils, e.g. N in rainfall and dry deposition (Herridge et al., 2008). Such inputs can represent 3–50 kg N/ha/year (McNeill & Unkovich 2007). Roper & Ladha (1995) concluded that the free-living, heterotrophic bacteria may fix significant amounts of N in agricultural systems, using crop residues as an energy source.

2.1. DIAZOTROPHIC BACTERIA

The diazotrophic bacteria occupy separate niches, and may be free-living, symbiotic or associative. The BNF was first described in diazotrophic bacteria from the rhizosphere and rizoplane of a wide variety of non-leguminous plants (Döbereiner, 1992; Boddey et al., 2003).

Common diazotrophs found in the rhizosphere of maize are *Enterobacter spp.*, *Rahnella aquatilis*, *Paenibacillus azotofixans*, *Azospirillum spp.*, *Herbaspirillum seropedicae*, *Bacillus circulans* and *Klebsiella sp.* (Chelius & Triplett, 2001).

The positive effects of *Azospirillum* on maize growth are mainly derived from physiological changes of the inoculated plant roots, which enhance water and mineral nutrient uptake (Okon & Kapulnik, 1986; Barassi et al., 2007). Both *A. brasiliense* and *A. irakense* are used as inoculant biofertilizers for maize. Other species of *Azospirillum* capable of increasing the yield of maize are *A. lipoferum* and *A. indigens*, and *Azorhizobium caulinodans* was also capable of giving such beneficial effects (Riggs et al., 2001). The magnitude of this increase varies with the *Azospirillum* strain and maize cultivar and depending on soil conditions.

Cereals of economic importance, such as corn, sugar cane, rice, wheat, sorghum, and some fodder were identified with hosts of different species of endophytic diazotrophic bacteria. Among endophytic diazotrophic are: *Gluconacetobacter diazotrophicus* (Cavalcante & Döbereiner, 1988; Boddey et al., 2003), *Azoarcus spp.* (Reinhold-Hurek et al., 1993), *Herbaspirillum seropedicae* (Baldani et al., 1986; James, 2000), *Herbaspirillum rubrisubalbicans* (Baldani, 1996; Gillis et al., 1991), *Burkholderia spp.* (Baldani et al., 1997) and *H. lusitanum* (Valverde et al., 2003).

Initially the endophytic microorganisms were considered harmless to plants, but from the '70s its importance to the plants started to be observed (Azevedo et al., 2002). There are several positive effects attributed to endophytic bacteria, such as the promotion of plant growth (Okon & Labandera- Gonzalez, 1994; Okon & Itzlgsohn, 1995; Raja et al., 2006), biological control of pests and diseases in plants (Mariano et al., 2004), biological nitrogen fixation (Döbereiner & Boddey, 1981; Downing et al., 2000; Verma et al., 2004; Reis Junior et al., 2008), induction of systemic resistance (Hallmann et al., 1997), production of siderophores (Burd et al., 1998; Wenbo et al., 2001) and production of antibiotics (Strobel & Daisy, 2003).

The promotion of plant growth occurs mainly by the production of phytohormones as auxins, cytokinins, gibberellins, abscise acid and ethylene by the endophytic bacteria. The production of these phytohormones has been reported in bacteria as *Gluconoacetobacter*, *Azospirillum*, *Herbaspirillum*, *Erwinia*, *Pseudomonas* and *Pantoea* (Kuklinsky-Sobral et al., 2004). The indoleacetic acid is a naturally occurring important auxin that causes physiological effects on the plant, such as increased growth (Lambrecht et al., 2000; Nefedieve, 2003; Figueiredo et al., 2008).

The association of cereals and grasses with endophytic diazotrophic bacteria may represent one of the most promising alternatives for the promotion of plant growth, soil

management and environmental quality since bacteria are able to promote growth, increase disease resistance, through biological fixation of nitrogen or the phytohormones production (Thuler et al., 2003; Bashan et al., 2004). In addition, diazotrophic endophytic may present advantages in relation to diazotrophic associated with roots once they are better located to explore the carbon sources released by plants and these bacteria have been isolated from several grasses species (Riggs et al., 2001; Tejera et al., 2006).

There is a great interest in characterizing the diversity of these microorganisms in order to use their potential in different cultures, especially the corn, where a wide diversity of diazotrophic has been found colonizing this plant (Baldani et al., 1997; Chelius & Triplett, 2001; Pitnner et al., 2007). In general, studies on diversity are based on cultivation techniques and subsequent characterization of isolates. However, the cultivation of microorganisms provides limited information about diversity since most existing organisms is not easily isolated by conventional cultivation techniques. Techniques of Random Amplified Polimorphic RAPD-DNA, Polymerase chain reaction-BOX-PCR, Amplified Fragment Length Polymorphism-AFLP and Amplified ribosomal DNA Restriction Analysis-ARDRA are applied in assessing the diversity of microbial community cultured. The application of independent cultivation techniques such as Denaturing Gradient Gel Electrophoresis-DGGE, the construction and analysis of clone libraries and qPCR real time quantitative are applied to the study of microbial communities (Andreote et al., 2008). For evaluation of BFN diversity in different ecosystems, universal primers have been used to amplify the gene *nifH* through techniques of independent cultivation (Bashan et al., 2004). Such techniques make possible to obtain a more complete characterization of the diazotrophic community than dependent techniques of cultivation (Roesch et al., 2007).

2.2- PLANT GROWTH-PROMOTING RHIZOBACTERIA (PGPR) INCREASE CROP PERFORMANCE

The mechanisms by which PGPR increase crop performance is not well understood. There are several inoculants currently commercialized that seem to promote growth through at least one mechanism; suppression of plant disease (termed Bioprotectants), improved nutrient acquisition (termed Biofertilizers), or phytohormone production (termed Biostimulants). Inoculant development has been most successful to deliver biological control agents of plant disease, that is organisms capable of killing other organisms pathogenic or disease causing to crops (Tenuta, 2003).

BNF by associative diazotrophic bacteria is a spontaneous process where soil N is limited and adequate C sources are available. Yet the ability of these bacteria to contribute to yields in crops is only partly a result of BNF. A range of diazotrophic plant growth-promoting rhizobacteria participate in interactions with C₃ and C₄ crop plants (e.g. rice, wheat, maize, sugarcane and cotton), significantly increasing their vegetative growth and grain yield. The mechanisms involved have a significant plant growth-promoting potential, retaining more soil organic-N and other nutrients in the plant–soil system, thus reducing the need for fertilizer N and P. Table 1 suggests that the diversity of habitat and effectiveness might logically require more than one bacterial strain to obtain the maximum biological effects on plant growth are summarized indicating the proposed mechanisms of PGP (plant growth promoting) effects (Kennedy, et al., 2004).

Table 1. Biology, and potential role of some diazotrophs promoting crop production (adapted by Kennedy et al., 2004).

Diazotrophs	Condition for BNF	Habitat	Energy source	Mechanism of effect	References
<i>Azotobacter chroococcum</i>	Aerobic	Rhizosphere	Organics in soil	BNF	Kennedy & Tchan (1992)
<i>Clostridium spp.</i>	Anaerobic	Soil saprophyte	Organics in soil	BNF	Kennedy & Tchan (1992)
<i>Azospirillum spp.</i>	Microaerobic	Rhizosphere, mildly endophytic in roots, stems and leaves	Organics in soil, root exudates and plant tissue	BNF, PGP	Reinhold & Hurek (1998) Mirza et al., (2000) Okon & Kapulnik (1986)
<i>Herbaspirillum seropedicae</i>	Microaerobic	Endophytic, Rhizosphere	Root exudates	BNF, PGP	Baldani et al., (1986, 2000) Hurek et al., (1994)
<i>Azoarcus sp.</i>	Microaerobic	Endophytic	Root exudates	BNF	Reinhold-Hurek et al., (1993)
<i>Burkholderia vietnamiensis</i>	-	Rhizosphere, Endophytic,	Organics in soil and root exudates	BNF, PGP	Baldani et al., (1997, 2000)
<i>Rhizobium leguminosarum</i> bv <i>phaseoli</i>	-	Endophytic in roots	Root exudates	PGP	Yanni et al., (1997) Biswas et al., (2000)
<i>Rhizobium etli</i> bv <i>phaseoli</i>	-	Endophytic in roots	Root exudates	PGP	Gutiérrez-Zamora (2001) Martínez-Romero (2001)
<i>A. caulinodans</i>	Microaerobic	Endophytic in roots	Root exudates	PGP	Matthews et al., (2001)
<i>Glucanoacetobacter diazotrophicus</i>	Microaerobic	Endophytic in roots, stems and leaves	Root exudates and plant tissue	BNF	Baldani et al., (1997) Boddey et al., (1991)

* BFN, Biological nitrogen fixation; PGP, plant growth promoting.

According to Kennedy et al. (2004) this diversity will need to be carefully considered in the future design of the most efficient inoculant biofertilisers. For example, an important question is whether inoculants should be restricted to a single strain of bacterium, such as *Azospirillum*, or not. If all of the PGP mechanisms can be well expressed in a single strain of bacterium this would simplify the design of inoculant products. However, it would be unlikely that a single strain of bacterium would be capable of optimal activity.

3- LEGUME NITROGEN FIXATION

Another approach to biological nitrogen fixation used with corn is the inclusion of legumes as part of the cropping system, most commonly as grain crops or green manures. The widespread knowledge, and even to a large degree “faith”, that legumes always improve soil nutritional status has historically led most farmers to include legume in the cropping systems, either directly or indirectly through fallow periods (Van Kessel & Roskoski, 1988; Gathumbi et al., 2002; P hoomthaisong et al., 2003; Sanginga, 2003; Sanginga et al., 2003; Okito et al., 2004).

3.1- CROP ROTATIONS

Legume crops are a key component of most traditional tropical cropping systems (Van Kessel & Roskoski, 1988; Peoples et al., 1995; Sanginga, 2003), either cultivated at the same time as the main commercial crop, which would most frequently be corn, sorghum or millet (Anthofer, 2005; Fan et al., 2006; Okogun et al., 2007), depending on the region, or in sequential cropping on the same field, which is usually the preferred solution for current commercial cropping due to easier management (Goss et al., 2002; Smith et al., 2008). Some of the results achieved with the use of a legume crop in the production system can be shown as examples. For instance Fan et al. (2006) have found that when corn and faba bean

(*Vicia faba*) were intercropped in Northern China, corn yield was not significantly different from the achieved by corn single-cropped which did not receive nitrogen fertilizer, and slightly lower than what corn would yield if nitrogen fertilizer was used (12.04 t.ha⁻¹ for the intercrop, and 13.31 t.ha⁻¹ for single corn), but this difference was not significant. In another paper, yields in plots previously sown to soybean were significantly larger than yields in the fallow plots, with averages of about 3 t.ha⁻¹ and 0.5 t.ha⁻¹, respectively (Osunde et al., 2003). Under either of the approaches, although common sense indicates that there is always a gain in soil fertility due to the inclusion of the legume crop, this frequently is not the case with modern cultivars (Singh et al., 2003). This happens because these cultivars may achieve higher nitrogen harvest indexes than the total nitrogen fixation. This possible negative nitrogen balance may be the main responsible for the huge variation in results from the several papers dealing with the effect of inclusion of legume crops in the cropping system (Gentry et al., 2001; Tilman et al., 2002; Fortuna et al., 2003; Rosolem et al., 2004; Robertson et al., 2005; Smith et al., 2008).

Besides leaving crop residues on the field, which would usually be the recommended practice for reasons besides nitrogen balance, such as weed control, reduction of soil loss through erosion and of superficial soil temperature (Oliveira et al., 2002; Cabezas, 2004; Balkcom & Reeves, 2005; Silva, 2006), other manageable aspects of the legume crop that allow a positive nitrogen yield for the following crop would be selection of legume cultivars with longer growth period, lower nitrogen harvest indexes or higher nitrogen fixation potential.

Increasing growing period for the legume in the system will usually allow larger biomass accumulation with all of the above-mentioned advantages, and may be applied both for cover and grain legume crops (Anthofer, 2005; Fan et al., 2006; Chikoye et al., 2008). On the other hand, it may be interesting when the pulse crop is grown on alternate years on the

same plot with corn, as is usually the case on the corn-soybean rotation, very common on some of the main soybean growing regions of the world, such as Brazil and the United States. The corn-soybean rotation is probably the most important cereal-legume rotation in large scale intensive agriculture, since both crops are major commodities. Since both are also important potential sources for biofuels, respectively ethanol under current American practice, and biodiesel an increase in area under cultivation is predicted for both (Salvagiotti et al., 2008).

The importance of the possibly negative nitrogen balance in legume crops such as soybean may be observed in Salvagiotti et al. (2008). These authors have examined 637 data sets (site-year-treatment combinations) from field studies that had examined nitrogen balance data and had been published in refereed journals from 1966 to 2006. In most situations they found that the amount of N fixed was not sufficient to replace N export from the field in harvested seed. The partial N balance (fixed N in aboveground biomass - N in seeds) was negative in 80% of all data sets, with a mean net soil N mining of $-40 \text{ kg N}\cdot\text{ha}^{-1}$. However, when an average estimated belowground N contribution of 24% of total plant N was included, the average N balance was close to neutral ($-4 \text{ kg N}\cdot\text{ha}^{-1}$). This gap between crop N uptake and N supplied by BNF tended to increase at higher seed yields for which the associated crop N demand is higher

On the other hand, as long as NHI is lower than the nitrogen obtained from biological nitrogen fixation, nitrogen export through seeds would be lower than nitrogen fixation, and there would be a net increase of available soil nitrogen (Phoomthaisong et al., 2003; Singh et al., 2003; Alves et al., 2006). This increase in nitrogen fixation is one of the main aims of most soil microbiologists currently working hand in hand with the legume inoculants' industry (Date, 2000; Graham & Vance, 2000; Catroux et al., 2001; Hardarson & Atkins, 2003; Deaker et al., 2005; McInnes et al., 2005). There are strong indications for some of the

crops commonly used in rotation with corn that another feasible approach is cultivar selection for higher nitrogen fixation potential (Tsai et al., 1998; Hardarson & Atkins, 2003; Bouton, 2007).

While the results for inclusion of legume green manures on the nitrogen balance are more positively consistent than those for legume crops, this practice has not achieved the same degree of grower adoption as the former (Chikowo et al., 2004; Anthofer, 2005; Crews and Peoples, 2005; Musiyiwa et al., 2005; Shelton et al., 2005; Rufino et al., 2006; Ojiem et al., 2007).

A recent paper (Tonitto et al., 2006) discusses 36 papers on the effect of the inclusion of legumes as green manure in North American cropping systems, of which 28 had corn as the main crop. The authors conclude that in half of the 228 individual experiments the legume green manure resulted in a net input of 50 to 150 kg of N.ha⁻¹. They also conclude that with that level of biological nitrogen input there was usually no significant difference on yield between conventionally managed systems and legume based ones. They also conclude that if fixed nitrogen was higher than 180 kg of N.ha⁻¹ there was usually a 5% gain on crop yield, while if input was below 110 kg of N.ha⁻¹ the expected result would be loss of yield. These results were achieved with loss of a growing season, since all experiments included only short season green manure crops.

In another paper, a field experiment was conducted in Michigan, USA, studying how corn was affected by inclusion of crop rotation with soybean and/or short season legume green manures (Smith et al., 2008). The experimental design was unique in that no fertilizer or pesticides were used, and the only management variable manipulated was number of species in the rotation, thus providing a strong comparison to grassland diversity-ecosystem function experiments. Corn grain yield increased linearly in response to the number of crops in the rotation, with yields in the treatment with corn, soybean and winter wheat as crops and

red or crimson clovers and rye as short season cover crops were over 100% higher than in continuous monoculture. Most importantly, the yields were not significantly different from the county average for each of the 3 years despite the absence of chemical inputs.

An important point concerning use of green manures is that it is highly knowledge dependent, since it must be highly environmentally adapted, or it won't achieve the expected result. Although low formal education levels are widespread among developing world farmers, usually they have high level knowledge of their farming environment. Both of these aspects indicate that green manure research should ideally be conducted under field conditions, preferably with farmer management at least on final research stages (Jensen & Hauggaard-Nielsen, 2003; Chikowo et al., 2004; Crews & Peoples, 2005; Mapfumo et al., 2005).

4- USE OF SEWAGE SLUDGE ON MAIZE CULTIVATION

The sewage sludge (SS) or pie is waste of urban and / or industrial origin which results from the treatment of effluents, presenting highly variable composition. The differences vary with the type of process employed (primary, raw sludge produced in primary decanters; activated sludge, produced in biological reactors and, digested sludge, process of biological stabilization), with the physiographic location of Wastewater Treatment of Sewage - WTSs (which reflects the dietary habits of the population), with the balance of nutrients from food consumed, with a time of year and with the waste discharge (Saito, 2007; Tsutiya, 2000; PROSAB, 1999; Vidor, 1999). According to Bettioli & Camargo (2000) depending on the origin and the process of obtainment used, the sewage sludge presents quite variable composition, being rich in organic matter (40 to 60 %), nitrogen and some micronutrients

such as iron, copper, zinc and manganese. Typical sewage sludge contains 40 % organic matter, 4 % of nitrogen, 2 % phosphorus and 0.4 % potassium.

When there is possibility of sterilization with sulfate / calcium carbonate in the process of WTS, the resulting product becomes the biosolids. Therefore, biosolids is the name given to the sludge resulting from the sewage treatment, with features that allow recycling in rational and environmentally safe way. The term biosolids was created and disseminated throughout the world to encourage the use of sewage as fertilizer and soil-conditioners (Oliveira et al., 2005; Smith, 2005; USEPA, 1999). The urban sludge, when treated, may eliminate the pathogenicity of viruses, bacteria, fungi, protozoa and helminthes (Barbosa et al., 2007). However, the possible presence of pollutants such as pathogens agents and potentially toxic elements - heavy metals are factors that may cause negative impacts, so its application requires special care to be avoided damage to the population and the environment.

The application to soil is one of the oldest practices of final destination for sanitary sewer. The "sewage farms", became known as the first experiments in England at the beginning of the nineteenth century, quickly spread through Europe and the United States (Bastos, 2003). The best known information is those from China. In the West, particularly in Prussia, irrigation with sewage effluent was practiced since 1560. In England, around 1800, many projects were developed for agricultural use of sewage effluent, especially due to combat cholera's epidemics. The adoption of the practice of using soil as means of sewage or sludge disposal has been frequent in many countries (Nascimento et al., 2004).

The use of sewage sludge as organic fertilizer has been mentioned as an alternative to the final destination of the waste, mainly by the predominant concentration of organic matter and source of nutrients (Messias, 1993; Messias & Moraes, 1992; Pires, 2005; Andreoli et al., 2004; Gadioli & Fortes Neto, 2004; Faria, 2007).

Besides the environmental and economical point of view, the use of sludge in agriculture is advantageous once it promotes greater soil's water holding capacity, porosity (aeration of the roots) and aggregate stability. Also, greater resistance to erosion, residual effect usable for subsequent crops, and possibly induce the suppression of soil to phytopathogens (Bettioli & Fernandes, 2004; Silva et al., 2002; Berton et al., 1997; Melo et al. 1994). The criteria for application of SS should be based as well in soil attributes and not only in their total levels of metals. The knowledge of how these attributes influence metals behavior is then able to show the amount of waste that can receive the soil (Borges & Coutinho, 2004). Some inventories were created for monitoring and managing the sludge disposal in space, including surveys of environmental data (soil, water, geology, geomorphology and vegetation), current use of rural and urban soil and institutional context. Gomes et al. (2001) proposed an inventory, followed by the location of areas potentially suitable for the sludge recycling, by eliminating areas incompatible with the necessary environmental attributes and the legislation requirements. Therefore, it was considered the distance of water resources, urban spot, flooded areas and land slope, among other factors. Soils' agricultural ability was assessed from the current levels of fertility, its ability to recover physically and chemically by organic addition, beyond the risk of erosion of them.

Most studies have aimed to verify the effect of organic fertilizers on the yield, compared to, in general, with complete (NPK) or incomplete mineral fertilization including corrective soil. With rare exceptions, most studies shows effects of fertilization, both organic and mineral, in relation to the control, but the differences between organic and mineral fertilizers are variable, depending on the soil characteristics, doses of fertilizers, crop and study area. According to studies performed, results expected with organic and mineral fertilization, isolate, may be represented by a curve of quadratic type, in which production rises relative to the dose (Malavolta, 1981). The application of organic compounds in a

continuous manner raises the nitrogen level to the point of making dispensable its application in the form of chemical fertilizer. In poor soils, the amount of organic matter may increase considerably its potential productivity. The efficiency of organic fertilizers to improve soil productivity depends on several factors that should be considered: (a) quality and quantity of application, (b) ages and conditions of use; (c) methods of implementation; (d) the adequacy to farming systems prevailing in the region, (e) and, especially, the relative cost of their use.

Daros et al. (1993) verified residual effect of N and P in soil sludge-fertilized in the production of millet with subsequent associated cultivation of oats and vetch. Mazzarino et al. (1998) state that the release of P by sludge depends on the soil type and origin of the residue. Silva et al. (2002) corroborated that the sludge used presented 25 % more efficiency than the triple superphosphate as phosphorus source for corn. The application of increasing doses of sewage sludge promoted decrease in pH and increase in levels of organic matter, total N, P, K, Na, Ca and Mg in soils with crops of maize and beans, however dry material of both crops was lower than that obtained by complete mineral fertilization (Nascimento et al., 2004). Galdo et al. (2004) and Tsadilas et al. (1995) observed higher grains yield in maize cultivation, with application of sewage sludge, as well as Cripps et al. (1992) found grains yield 47 % higher with application of sewage sludge in comparison with conventional fertilization. Silva et al. (2002) and Biscay & Miranda (1996) reported higher grain yield in relation to the control and the NPK fertilizer for three years, after sludge application, demonstrating its residual effect. Some authors showed an increase in levels of Cd and Cu (Logan et al., 1997; Favaretto, 1997; Pierrisnard, 1996; Al-Jaloud et al., 1995; Reddy et al., 1989, Ritter & Eastburn, 1978), Cr, Ni and Zn in corn, beans and sorghum, with increase in the doses of sewage sludge application from 40.5 t ha⁻¹ (Boaretto et al., 1992; Oliveira, 1995; Angels & Mattiazzo, 2000).

The absorption of large quantities of Zn by plants in treatments with sewage sludge considered above the adequate range for the cultivation of corn, according to Malavolta et al. (1989), may have caused lower productivity of this treatment. The application of increasing doses (10, 20, 30, 40 and 60 t.ha⁻¹) of sewage sludge promoted decrease in pH and increase in levels of organic matter, N, P, K, Na, Ca and Mg in crops of maize and beans, however dry matter of both cultures was lower than that obtained by mineral fertilization (Nascimento et al., 2004). Maize's dry matter production increased with the dose of sludge in the presence or absence of potassium (Simonete et al., 2003; Simonete & Kiehl, 2002; Simonete, 2001; Berton et al., 1989). Gomes et al., (2007), to evaluate the chemical changes in soil caused by the addition of sewage sludge, installed an experiment with corn in the field conditions, in Yellow Argisol, which consisted of six treatments (0; 7.7; 15.4; 29.7; 45.1 and 60.5 t. ha⁻¹). The production of grains increased depending on the doses of sewage sludge up to the application of 26 t. ha⁻¹ which provided the maximum agronomic efficiency for the corn production. The sludge dose of maximum agronomic efficiency was effective at raising the levels of Ca, Mg, Cu, Mn and Zn in the leaves of maize to nutritionally adequate concentrations. Although the levels of N and Fe in the leaf have increased depending on the doses of sewage sludge, they were insufficient to supply the nutritional demands. The levels of P and K in the leaves have not changed by the doses of sewage sludge. Martins et al., (2003) in the field experiment found that the production of grains and dry weight from the shoots of maize increased linearly with the addition of SS (0, 20, 40, 60 and 80 t/ ha) in the period from 1983 to 1987. The concentrations of metals Cu, Fe, Mn and Zn in grains were not influenced in any significant way by the addition of SS, and even in larger doses, within the acceptable levels, without causing restriction for human consumption.

The field experiment, aiming to evaluate the agricultural use of biosolids in corn was installed by Melo et al., (1994) in two soil types, Latosol Purple - LR and Red-Dark - LE,

medium texture, whose treatments were: control (without application of fertilizer in the first agricultural season and with application of mineral fertilizers in the second year), 2.5 (L1), 5.0 (L2) and 10.0 (L3) t.ha⁻¹ of biosolids (base dried in stove). Phosphorus required by the crop was supplemented with mineral fertilizer, considering that the whole of the P from biosolid would be available for culture. The potassium required by plants has been applied in the form of KCl, completing the content in the biosolids in the first year and without considering the content of biosolids in the second agricultural season. In the first season, in which the control received no mineral fertilizer, the highest dose of biosolids affected the production of straw in the soil LE, not being enough to increase grain yield; in the LR soil the highest dose of biosolids affected the production of stem + leaf and grains production in the second season, in which the control received mineral fertilization. In the soil LE none of the variables was affected, meaning that the dose 2.5 t.ha⁻¹ biosolids provided to the culture performance comparable to that of mineral fertilizer, in the soil LR the highest dose of biosolids affected the production of stem + leaves and the grains production, which were higher than in the control treatment. Similar experiments were conducted by Rodrigues et al., (2006) and Barbosa et al., (2007).

5- THE USE OF PLANT RESIDUES AND ANIMAL MANURE AS FERTILIZERS

The use of manure and plant residues as organic fertilizers may lead to beneficial effects besides the addition of nutrients. First of all, the incorporation of organic residues increases the levels of soil organic matter (SOM), which is vital for having a good growth media as it will improve soil structure, water-holding capacity, increase microbial biomass and soil fauna, and is essential to the cycling of nutrients. Also, a high content of organic material in the soil will enhance soil aggregation and help decrease runoff and erosion.

(Goulding et al, 2001; Weil & Magdoff, 2004). However, in many areas of the world the use of organic fertilizers is often limited both by the relatively low amounts of these materials normally available within farms and also by the cost of purchasing these fertilizers from off-farm sources (Sanchez, 1995). For these reasons, managing the fertility of soils in low external input systems requires detailed knowledge about SOM and nutrient cycling in order to improve the use efficiency of organic fertilizers and, in its turn, improve income generation while preserving the quality of the soils and other natural resources.

However, there is limited information regarding the use of organic residues to ameliorate soil fertility in low external input systems. For example, very little is known about the relationships between the quality of residues and their effects on soil nutrient availability (Palm et al., 2001a, 2001b; Vanlauwe, 2005). As mentioned previously in this chapter, these relationships require site-specific information that may be very important to the success of the fertilization strategy. For example, animal manure is one of the most common organic fertilizers used in many areas of the world. However, the manure available is often of very low quality and, therefore, may mineralize low amounts, or even immobilize, nitrogen (Silva & Menezes, 2007; Menezes & Salcedo, 2007). In order to increase N supply to crops, many studies have proposed the use of other organic amendments, such as green manures or cover crops using legume species (Palm et al., 1997; Marin et al., 2007; Silva et al., 2007; Holmes, 1998). Other studies have investigated management options to optimize the use of organic residues, such as mixing residues of different qualities, applying residues in the surface or incorporated, or applying residues at different moments of the cropping cycling, among others. In this section, we present some of these studies and discuss the strategies to optimize the use of animal and green manures to improve maize crops within low external input systems.

5.1- SYNCHRONIZATION BETWEEN SOIL NUTRIENT SUPPLY AND PLANT DEMAND

Overall, most fertilization strategies have a common goal, which is to achieve the best possible synchronization between soil nutrient supply and plant demand, as explained by the 'synchrony' concept (Myers et al., 1994), with the goal of minimizing losses and increasing the use efficiency of nutrient within agroecosystems (Mundus et al., 2008). Many experiments have demonstrated that N use efficiency when applying fertilizers or crop residues may vary greatly, ranging from 5 to 74%, depending on the material and the location (Ibewiro et al., 2000; Mubarak et al., 2003; Myers et al., 1997; Palm, 1995; Sisworo, 1990).

These questions are very relevant for the production of maize, since nutrient requirements for optimal maize growth can be demanding, given that this crop has potentially high yields and that nutrient uptake is directly related to dry matter production (Fageria et al., 1991). The actual amounts of maize nutrient uptake will vary among locations depending on productivity. In tropical countries, for instance, national averages of maize yield may range from just above 1 t ha⁻¹ until up to 5-10 t ha⁻¹, while in many sub-tropical or temperate regions this may be over 20 t ha⁻¹ (Fisher & Palmer, 1983; Norman et al., 1995). However, nutrient demand is not uniform throughout the whole life cycle of the maize plant. The actual amount of nutrients taken up is generally largest in the period near tasseling and silking and then again later during the grain filling period, peaking from 6 to 9 weeks after germination (Karlen et al., 1988). Similarly, Hagggar et al., (1993) found maize N uptake to be highest in the period from 30 to 60 days after planting.

There are several ways of improving synchrony between maize demand and soil nitrogen supply, such as controlling the date of planting, the length of crop growth, or using different crops in a form of multiple cropping systems. Alternatively it is also possible to

affect the release and availability of nutrients to the plants by managing the time of application and the quality of organic inputs, such as manure and crop residues (Mundus et al., 2008). However, as mentioned previously, the adoption of these management options requires detailed knowledge of the relationships between organic fertilizer quality and decomposition.

5.2- RELATIONSHIPS BETWEEN ORGANIC FERTILIZER QUALITY AND N MINERALIZATION

The quality of organic residues available for use as fertilizers may vary greatly and this may affect significantly the patterns of N release to the soil. Basically, materials with a slow decomposition rate and limited release of nutrients are considered as low quality, while high quality materials are more labile, i.e., release nutrients quickly. The addition of materials of different qualities have significant impacts on the build-up of SOM, since more recalcitrant material will be more likely to form stable complexes and increase SOM, while fast decomposing material will do little for the maintenance and build-up of SOM (Giller, 2001; Nyberg et al., 2002; Palm et al., 2001).

Traditionally, factors such as the C:N ratio (Mafongoya et al., 2000) as well as the initial N content (Constantinides & Fownes, 1994) of organic fertilizers were regarded as good indicators of fertilizer quality. However, there is consistent evidence that the contents of lignin and polyphenols in relation to N are better indicators of decomposition rate for many organic residues, particularly plant biomass used as green manure. The (lignin+polyphenol):N ratio have repeatedly been found to correlate well with the rate of decomposition (Constantinides & Fownes, 1994; Handayanto et al., 1994; Hartemink & O'Sullivan, 2001; Mafongoya et al., 1998; Mafongoya et al. 2000; Palm 1995). On the other hand, Lehmann et

al. (1995), Oglesby & Fownes (1992) and Palm & Sanchez (1991) found that the polyphenol:N ratio gave the best correlation. The reason for this is related to the fact that polyphenols can form complexes with proteins (which contain N, P and S) that are resistant to degradation from most decomposing organisms. In a similar way lignin is also capable of complexing with carbohydrates and N and thereby slowing down degradation (Berg & McClaugherty, 2003; Handayanto et al., 1994).

In practice, the discussion about fertilizer quality and nitrogen availability is of great relevance. For example, even though animal manure is widely used as organic fertilizer, its effects on soil may be highly variable but, in general, will lead to an immediate immobilization of N (Calderón et al., 2005; Lupwayi & Haque, 1999; Kirchmann, 1991; Nyamangara et al., 1999). This immobilization is mainly due to a much higher C:N ratio in the manure as compared to that found in most leguminous green manures (Lupwayi & Haque, 1999). Many studies have reported depletion of soil N after manure application (Menezes & Salcedo, 2007; Silva et al., 2007; Mafongoya et al., 2000). However, it is important to remember that even though application of manure may lead to immobilization there will still be some benefits (Coulter, 1998; Kapkiyai et al., 1999), such as the addition of other nutrients like P, K and trace elements that may limit plant productivity in some systems. Besides, since manure is less prone to degradation, it will stay longer in the soil and experiments have shown that applications of manure will lead to a build up of SOM (Kapkiyai et al., 1999; Nyamangara et al., 2001).

In contrast, the use of legume prunings as green manure may lead to fast decomposition and N release, which may bring benefits to maize when well managed. For example, Mundus et al., (2008) found that *Gliricidia sepium* prunings decomposed twice as fast as cattle manure after incorporated to the soil. Additionally, Cadish et al., (1998) found that treatments with 11 materials that led to a large immediate N release continued to provide

greater benefits to the maize plants in later growth stages, compared to materials that released very little N immediately after application. The immediate N mineralization was thus more important than the possible release later in the cropping cycle (Cadish et al., 1998).

However, it is important to mention that, if slow decomposition of organic fertilizers may cause nutrient limitations to crops, there are also some problems when decomposition happens too fast. For example, many studies have reported that fertilization with fast decomposition legumes used as green manures can cause leaching losses when applied at planting, when the crop is unable to take up all the released N (Chirwa et al., 2006; Hagedorn et al., 1997; Lehmann et al., 1995; Zaharah & Bah, 1999). Peinetti et al., (2008) found that the excess of water in years with high precipitation events may reduce maize productivity due to nitrogen leaching to deeper soil layers after the fertilization with gliricidia prunings. Hasegawa & Denison (2005) also observed significant increases in N leaching when increasing the rate of legume cover crop incorporated to the soil.

Other reported strategies to improve the synchronization between nutrient availability and plant demand are the mixture of high and low-quality material (Chirwa et al., 2006; Handayanto et al., 1997); the splitting of the application of materials into several minor applications (Hagedorn et al., 1997; Schroth et al., 1992); or the application of the materials on the soil surface, instead of incorporating it. The strategy of mixing low and high-quality materials might to some degree help solve the N immobilization or leaching problems as sometimes seen early in the maize growing period. However, this may not be enough to solve the problem of the extra N requirement later in the growth period (Palm et al., 2001). Mafongoya et al., (1997) found that mixtures of high and low quality prunings did not behave as simple additions of the two materials. This finding was also supported by Zingore et al., (2003), who suggested that some of these unpredicted release patterns showed potential for improving N synchrony.

Zaharah & Bah (1999) found that the incorporation of the fast decomposing *Gliricidia* could be problematic, since much of the nutrients could be lost by leaching. These authors suggested that split applications of the prunings would be a way of controlling the release and thereby limit N losses. In a related experiment Zaharah et al., (1999) found that split applications of *Gliricidia*, 21 and 45 days after planting was more beneficial to maize crops than applications earlier in the cropping cycle. Similarly, Mundus et al., (2008) concluded that split applications of a mixture of low-quality animal manure with prunings of the highly labile *Gliricidia sepium* led to a more synchronized N release to maize when compared to the single application of these materials.

It is important to emphasize the usual limited availability of organic fertilizers in low external input systems. For this reason, the fertilizers available must be efficiently used to minimize losses (such as N leaching, denitrification or volatilization) while maximizing the benefits to farmers and the environment. In this respect, using animal manure and the biomass of cultivated or spontaneous plants available within farms is the main process to increase the amount of nutrients to be used for maize fertilization. However, the management of organic residues as fertilizers present several challenges, such as the high variability of the quality of the materials available which, in its turn, will result in different patterns of decomposition and nutrient release, particularly nitrogen. Since maize demand is greater during the period of approximately 4 to 9 weeks after planting, there is need to synchronize this demand to high soil nutrient availability. The literature during the last decade has reported several strategies that may improve this synchrony, such as the mixture of organic materials of different qualities, splitting the application of the fertilizers, or even adjusting the time between fertilizer incorporation and crop planting. However, the most relevant underlying message from the information available regarding this theme is the importance of site-specific information, such as the quality of the materials available, the patterns of decomposition of

these materials and the nutrient demand of the maize varieties adopted. The integration of this information will allow the most efficient use of the available fertilizers to supply N to maize crops.

6- CONCLUSION

Agricultural studies of soil systems have historically been directed toward the physical and chemical aspects of crop production, with lower importance given to the ecological dimension. Currently, there is a need to develop greater knowledge of soil ecosystems, and their biological diversity and ecological functions, to build a broad basis for sustainable agricultural development, which should rely as much as feasible on renewable resources such as solar energy, rain water, atmospheric nitrogen, soil organic matter. Crop rotation, based on the inclusion of legumes, is considered one of the most powerful management practices for pursuing such aims because of its implications for maintaining soil fertility, saving energy and avoiding pollution. Some other options would include use of organic residues or sewage sludge. One of the main characteristics observed on most of the available literature for any of the above mentioned approaches is the high variability observed from experiment to experiment, indicating that studies should be conducted as much on-site as possible, including whenever feasible, farmer participation.

The adaptability of maize genotypes to environments where the nutrients are not readily available can be related, among other factors, to the association with benefic microorganisms as diazotrophic bacteria and plant growth promoters. These microorganisms are potential tools for sustainable agriculture and the trend for future and the process BNF offers an economically attractive and ecologically sound means of reducing external nitrogen input and improving the quality and quantity of internal resources.

REFERENCES

Alves, B. J. R., Zotarelli, L., Fernandes, F. M., Heckler, J. C., Macedo, R. A. T., Boddey, R. M., Jantalia, C. P., & Urquiaga, S. (2006) Fixação biológica de nitrogênio e fertilizantes nitrogenados no balanço de nitrogênio em soja, milho e algodão. *Pesquisa Agropecuária Brasileira* 41, 449-456.

Alves, B. J. R. et al. (2006) Fixação biológica de nitrogênio e fertilizantes nitrogenados no balanço de nitrogênio em soja, milho e algodão. *Pesquisa Agropecuária Brasileira*, Brasília, v.41, n.3, p.449-456,

Al-Jaloud, A. A.; Hussain, G.; Al-Saati, A. J. & Karimulla, S.(1995) Effect of wastewater irrigation on mineral composition of corn and sorghum plants in a pot experiment. *Journal of Plant Nutrition*, v.18, p.1677-1692.

Andreote, F.; Lacava, P.T. & Azevedo, J. L. (2008) Diversidade molecular de microrganismos endofíticos, p.233-257, In: Figueiredo, M. V. B.; Burity, H.A.; Stamford, N.P.; Santos, C.E.R.S. *Microrganismos e Agrobiodiversidade: o novo desafio para a agricultura*. Guaíba-RS: Agrolivros, v.1, 565 p.

Andreoli, C. V.; Sperling, M. V. & Fernandes, F. (2004) Princípios do tratamento biológico de águas residuais. Curitiba: FCO, v.6.

Anjos, A.R.M.dos & Mattiazzo, M. E. (2000) Metais pesados em plantas de milho cultivadas em Latossolos repetidamente tratados com biossólidos. *Scientia Agrícola*, Piracicaba, v.57, n.4, p. 769-776.

Anthofer, J. (2005) Above-ground biomass, nutrients, and persistence of an early and a late maturing *Mucuna* variety in the Forest-Savannah Transitional Zone of Ghana. *Agriculture,- Ecosystems-and-Environment* 110, 59-77.

- Azevedo, J.L.; Maccheroni Junior, W.; Araújo, W. L. & Pereira, J. O. (2002) Microrganismos endofíticos e seu papel em plantas tropicais, p.233-268. In: Biotecnologia: avanços na agricultura e na agroindústria p.433
- Baldani, J. I; Baldani, V.L.D.; Seldin, L. & Döbereiner, J. (1986) Characterization of *Herbaspirillum seropedicae* gen. nov., sp. nov., a root , Internation Journal Systemic Bacteriology, v. 36, p. 86-93.
- Baldani, J.I.; Caruso, L.; Baldani, V.L.D.; Goi, S.R. & Döbereiner, J. (1997) Recent advances in BNF with non-legume plants. Soil Biology and Biochemistry, v. 29, p.911-922.
- Baldani, V.L.D. Efeito da inoculação de *Herbaspirillum spp.* no processo de colonização e infecção de plantas de arroz e, ocorrência e caracterização parcial de uma nova bactéria diazotrófica. Tese de Doutorado, UFRRJ, Itaguaí, 1996.
- Baldani, V.L.D.; Oliveira, E.; Balota, E.; Baldani, J.I.; Kirchof, G. & Döbereiner, J. (1997) *Burkholderia brasilensis* sp. nov. Uma nova espécie de bactéria diazotrófica endofítica. Anais da Academia Brasileira de Ciências, Rio de Janeiro, v.69, p.116.
- Baldani, J.I.; Reis, V.M.; Teixeira, K.R.S. & Baldani, V.L.D. (2002) Biotecnologia: avanços na agricultura e na agroindústria, cap. potencial biotecnológica de bactérias diazotróficas associativas e endofíticas. Caxias do Sul – RS: Educs, 433 p.
- Balkcom, K.S. & Reeves, D.W. (2005) Sunn-hemp utilized as a legume cover crop for corn production. Agronomy Journal 97, 26-31.
- Barassi et al. (2007) *Azospirillum spp.*, a Dynamic Soil Bacterium Favourable to Vegetable Crop Production. Dynamic Soil, Dynamic Plant , v. 1(2) 68-82.
- Barbosa, G.M.C.; Tavares Filho, J.; Brito, O.R. & Fonseca, I.C.B. (2007) Efeito residual do lodo de esgoto na produtividade do milho safrinha. Revista Brasileira de Ciência do Solo, n. 31, p. 601- 605.

Bastos, R.K.X. (2003) Utilização de esgotos tratados em fertirrigação, hidroponia e piscicultura. Programa de Pesquisa em Saneamento Básico – PROSAB. Rio de Janeiro: ABES.

Bashan, Y. et al. (2004) *Azospirillum*-plant relationships: physiological, molecular, agricultural, and environmental advances (1997-2003). Canadian Journal of Microbiology v.50, p.521-577.

Berg B. & McClaugherty C. (2003) Plant Litter, Decomposition, Humus Formation, Carbon Sequestration. 1st edition. Springer-Verlag, Berlin, Germany.

Berton, R.S.; Camargo, O.A. & Valadares, J.M.A.S. (1989) Absorção de nutrientes pelo milho em resposta à adição de lodo de esgoto a cinco solos paulistas. Revista Brasileira de Ciência do Solo, Campinas, v.13, n.1, p. 187-192.

Berton, R.S.; Valadares, J.M.A.S.; Camargo, O.A. & Bataglia, O.C. (1997) Peletização de lodo de esgoto e adição de CaCO₃ na produção de matéria seca e absorção de Zn, Cu e Ni pelo milho em três Latossolos. Revista Brasileira de Ciência do Solo, Viçosa, v.21, p. 685-691.

Bettiol, W. & Camargo, O.A. (2000) Impacto ambiental do uso agrícola do lodo de esgoto. Jaguariúna, SP: EMBRAPA. 312p.

Bettiol, W. & Fernandes, S. A. P. (2004) Efeito do lodo de esgoto na comunidade microbiana e atributos químicos do solo. Embrapa, Jaguariúna, SP.

Biscaia, R.C.M. & Miranda, G.M. (1996) Uso do lodo de esgoto calado na produção de milho. Sanare, Curitiba, v.5, n.1, p.86-89.

Boddey, R.M. (1987) Methods for quantification of nitrogen fixation associated with gramineae. Crit. Ver. Plant Sci., v.6, p.209–266.

- Boddey, L.H. (2003) Ocorrência e diversidade de bactérias diazotróficas do gênero *Burkholderia*, isoladas de cana-de-açúcar (*Saccharum sp.*) cultivadas na Austrália e no Brasil. Tese Doutorado. PPG Ciência do Solo, IA, UFRRJ, 109 p.
- Boddey, R. M.; Urquiaga, S.; Alves, B. J. R. & Reis, V. (2003) Endophytic nitrogen fixation in sugarcane: present knowledge and future applications, *Plant and Soil* 252: 139–149.
- Borges, M. R. & Coutinho, E. L. M. (2004) Metais pesados do solo após aplicação de biossólido. I – Fracionamento. *Revista Brasileira de Ciência do Solo*, v.28: p.543-555.
- Bouchard, D.C.; Willians, M.K. & Surampalli, R.Y. (1992) Nitrate contamination of ground water: sources and potential health effects. *Journal of the American Water Works Association*, v. 84, p. 85-90.
- Bouton, J. (2007) The economic benefits of forage improvement in the United States. *Euphytica* 154, 263-270.
- Burd, G.I.; Dixon, D.G. & Glick, B.R. (1998) A plant growth-promoting bacterium that decreases nickel toxicity in Seedlings. *Applied and Environmental Microbiology*, v. 64, p.3663-3668.
- Cabezas,W.-A.R.L., (2004) Influência da cultura antecessora e da adubação nitrogenada na produtividade de milho em sistema plantio direto e solo preparado. *Ciência Rural* 34, 1005-1013.
- Cadisch G., Handayanto E., Malama C., Seyni F. & Giller K.E. (1998) N recovery from legume prunings and priming effects are governed by the residue quality. *Plant and Soil* 205: 125-134.
- Calderón F.J., McCarty G.W. & Reeves J.B. III. (2005) Analysis of manure and soil nitrogen mineralization during incubation. *Biology and Fertility of Soils* 41: 328-336.

- Catroux, G., Hartmann, A., & Revellin, C. (2001) Trends in rhizobial inoculant production and use. *Plant and Soil* 230, 21-30.
- Cavalcante, V.A. & Döbereiner, J.A. (1988) New acid-tolerant nitrogen-fixing bacterium associated with sugar-cane. *Plant and Soil*, v.108, p. 23-31.
- Chalk, P.M. (1991) The contribution of associative and symbiotic nitrogen fixation to the nitrogen nutrition of non-legumes. *Plant and Soil*, v.132, p.29–39.
- Chelius, M.K. & Triplett, E.W. (2001) The diversity of archaea and bacteria in association with the roots of *Zea mays* L. *Microbiology Ecology*, v.41, p.252-263.
- Chikowo, R., Mapfumo, P., Nyamugafata, P. & Giller, K.E. (2004) Maize productivity and mineral N dynamics following different soil fertility management practices on a depleted sandy soil in Zimbabwe. *Agriculture, Ecosystems and Environment* 102, 119-131.
- Chikoye, D., Ekeleme, F., Lum, A.F. & Schulz, S. (2008) Legume-maize rotation and nitrogen effects on weed performance in the humid and subhumid tropics of West Africa. *Crop Protection* 27, 638-647.
- Chirwa P.W., Black C.R., Ong C.K. & Maghembe J. (2006) Nitrogen dynamics in cropping systems in southern Malawi containing *Cliricidia sepium*, pigeonpea and maize. *Agroforestry Systems* 67: 93-106.
- Constantinides M. & Fownes J.H. (1994) Nitrogen Mineralization from Leaves and Litter of Tropical Plants: Relationship to Nitrogen, Lignin and Soluble Polyphenol Concentrations. *Soil Biology and Biochemistry* 26 (1): 49-55.
- Coulter J.K. (1998) Rain-fed Arable Farming Systems and their Improvement. In: Webster C.C. and Wilson P.N. (eds.): *Agriculture in the Tropics*. 3rd edition. Blackwell Science Ltd, Cambridge, England. pp. 144-178.

Crews, T. E. & Peoples, M. B. (2005) Can the Synchrony of Nitrogen Supply and Crop Demand be Improved in Legume and Fertilizer-based Agroecosystems? A Review. *Nutrient Cycling in Agroecosystems* 72, 101-120.

Cripps, R. W.; Winfree, S. K. & Reagan, J. L. (1992) Effects of swage sludge applications method on corn production. *Community Soil Science and Plant Analitical*, v.23, p.1705-1715.

Daros, C. O.; Aita, C.; Cerita, C. A. & Fries, M. R. (1993) Lodo de esgoto: Efeito imediato no milho e residual na associação aveia e ervilhaca. *Revista Brasileira de Ciência do Solo*, Campinas, v.17, n.2, p.257-261.

Date, R. A. (2000) Inoculated legumes in cropping systems of the tropics. *Field Crops Research* 65, 123-136.

Deaker, R., Roughley, R. J., & Kennedy, I. R. (2005) Legume seed inoculation technology—a review. *Soil Biology and Biochemistry*.

Döbereiner, J. & Boddey, R.M. (1981) Nitrogen fixation in association with gramineae. *Current Perspectives in Nitrogen Fixation*. Australia Academic Science, p.305-312.

Döbereiner, J. (1992) Recent changes in concepts of plant bacteria interactions, endophytic N_2 fixing bacteria. *Ciência e cultura*, v. 44 p.310-313.

Döbereiner, J. Fixação de nitrogênio em associação com gramíneas (1992) In: Cardoso, E.J.B. N. *Microbiologia do Solo*, Sociedade Brasileira de Ciências do Solo, Campinas, p.173-180.

Döbereiner, J. (1997) A importância da fixação biológica de nitrogênio para a agricultura sustentável. *Biotecnologia Ciência e Desenvolvimento*, v. 1, n. 1, p. 02-03 (Encarte Especial).

Downing, K.J et al. (2000) Biocontrol of the sugarcane borer *Eldana saccharina* by expression of the *Bacillus thuringiensis cryIAc7* and *Serratia marcescens chiA* genes in

sugarcane-associated bacteria. *Applied and Environmental Microbiology*, v. 66, p. 2804-2810.

Fageria N.K., Baligar V.C. and Jones C.A. (1991) *Growth and Mineral Nutrition of Field Crops*. Marcel Dekker, Inc. New York.

Fan,F., Zhang,F., Song,Y., Sun,J., Bao,X., Guo,T. & Li,L. (2006) Nitrogen fixation of faba bean (*Vicia faba* L.) interacting with a non-legume in two contrasting intercropping systems. *Plant and Soil* 283, 275-286.

Faria, L. C. de. (2007) *Uso do lodo de esgoto (biossólido) como fertilizante em eucaliptos: demanda, potencial e crescimento das árvores e viabilidade econômica*. Universidade de São Paulo: Tese de Doutorado (Escola Superior de Agricultura Luiz de Queiroz). Piracicaba.

Favaretto, N. (1997) Efeito do lodo de esgoto na fertilidade do solo e no crescimento e produtividade do milho (*Zea mays* L.). *Brazilian Archives of Biology and Technology*, Curitiba, v.40, n.4, p.836-847.

Figueiredo, M. V. B.; Burity, H.A.; Martinez, C.R. & Chanway, C.P. (2008) Alleviation of water stress effects in common bean (*Phaseolus vulgaris* L. by co-inoculation *Paenibacillus x Rhizobium tropici*. *Applied Soil Ecology*, v. 40, p. 182-188.

Fisher K.S. & Palmer A.F.E. (1983) Maize. In: *Symposium on Potential Productivity of Field Crops Under Different Environments*. International Rice Research Institute, Los Baños, Laguna, Philippines, pp. 155-180.

Fortuna, A., Harwood, R., Kizilkaya, K., & Paul, E. A. (2003) Optimizing nutrient availability and potential carbon sequestration in an agroecosystem. *Soil Biology and Biochemistry* 35, 1005-1013.

Gadioli, J.L. & Fortes Neto, P. (2004) Rendimento de milho e de feijão preto cultivado em solo acrescido de lodo de esgoto. *Sanare*, v.21, p.53-58.

Galdos, M. V.; De Maria, I. C. & Camargo, O. A. (2004) Atributos químicos e produção de milho em um latossolo vermelho eutroférico tratado com lodo de esgoto. *Revista Brasileira de Ciência do Solo*, v.28: p.569-577.

Gathumbi, S.M., Cadisch, G. & Giller, K.E. (2002) ^{15}N natural abundance as a tool for assessing N_2 -fixation of herbaceous, shrub and tree legumes in improved fallows. *Soil Biology and Biochemistry* 34, 1059-1071.

Gentry, L.E., Below, F.E., David, M.B. & Bergerou, J.A. (2001) Source of the soybean N credit in maize production. *Plant and Soil* 236, 175-184.

Gillis, M.; Döbereiner, J.; Pot, P.; Goor, M.; Falsen, E.; Hoste, B.; Reinhold, B. & Kersters, K. (1991) Taxonomic relationship between (*Pseudomonas*) *Rubrisubalbicans*, some clinical isolates (EF group 1), *Herbaspirillum seropedicae* and (*Aquaspirillum*) *Autotrophicum*. In: Polsinelli, M.; Materassi, R.; Vicenzini, M. eds. *Nitrogen Fixation*. Kluwer Academic Publishers. p. 293-294.

Giller K.E. (2001) *Nitrogen fixation in tropical cropping systems*, 2nd edn. CABI, Wallingford, p 423

Giller, K.E. & Merckx, R. (2003) Exploring the boundaries of N_2 - fixation in cereals and grasses: an hypothetical and experimental framework. *Symbiosis*, v.35, p.3–17.

Gomes, L.P.; Coelho, O.W.; Costa, A.N. & Marques, M.O. (2001) Resíduos sólidos do saneamento: processamento, reciclagem e disposição final. *RiMa- ABES*. Rio de Janeiro, cap.7: p. 165-187.

Gomes, S. B. V.; Nascimento, C. W. A. & Biondi, C. M. (2007) Produtividade e composição mineral de plantas de milho em solo adubado com lodo de esgoto. *Revista Brasileira de Engenharia Agrícola e Ambiental*, v.11, n.5, p.459–465.

Goss, M.J., de Varennes, A., Smith, P.S. & Ferguson, J.A. (2002) N₂ fixation by soybeans grown with different levels of mineral nitrogen, and the fertilizer replacement value for a following crop. *Canadian Journal of Soil Science* 82, 139-145.

Goulding K.W.T., Murphy D.V., Macdonald A., Stockdale E.A., Gaunt J.L., Blake L., Ayaga G. & Brookes P. (2001) The Role of Soil Organic Matter and Manures in Sustainable Nutrient Cycling. In: Rees R.M., Ball B.C., Campbell C.D. and Watson C.A. (eds): *Sustainable Management of Soil Organic Matter*. 1st edition. CAB International, Wallingford, UK. pp. 221-232.

Graham, P. H. & Vance, C. P. (2000) Nitrogen fixation in perspective: an overview of research and extension needs. *Field Crops Research* 65, 93-106.

Hagedorn F., Steiner K.G., Sekayange L. & Zech W. (1997) Effects of rainfall pattern on nitrogen mineralization and leaching in a green manure experiment in South Rwanda. *Plant and Soil* 195: 365-375.

Haggar J. P., Tanner E. V. J., Beer J. W. & Kass D. C. L. (1993) Nitrogen dynamics of tropical agroforestry and annual cropping systems. *Soil Biology and Biochemistry* 25 (10): 1363-1378.

Halbrendt, C. & Blase, M. (1989) Potential impact of biological nitrogen fixation: the case of corn. *North Central Journal of Agricultural Economics*, v.11, p.145-156.

Halmann et al. (1997) Bacterial endophytes in agricultural crops. *Canadian Journal Microbiology*, v.43, p. 895-914.

Halbrendt, C. & Blase, M. (1989) Potential impact of biological nitrogen fixation: the case of corn. *North central. North Central Journal of agricultural Economics*, vol 11, 145-156.

Handayanto E., Cadish G. & Giller K. E. (1994) Nitrogen release from prunings of legume trees in relation to quality of the prunings and incubation method. *Plant and Soil* 160: 237-248.

Handayanto E., Cadish G. & Giller K.E. (1997) Regulating N Mineralization from Plant Residues by Manipulation of Quality. In: Cadish G. and Giller K.E. (eds.). *Driven by Nature – Plant Litter Quality and Decomposition*. 1st edition. CAB International, Wallingford, UK. pp. 175-185.

Hardarson, G. & Atkins, C. (2003) Optimising biological N₂ fixation by legumes in farming systems. *Plant and Soil* 252, 41-54.

Hartemink A. E. & O’Sullivan J. N. (2001) Leaf litter decomposition of *Piper aduncum*, *Gliricidia sepium* and *Imperata cylindrical* in the humid lowlands of Papua New Guinea. *Plant and Soil* 230: 115-124.

Hasegawa, H. & Denison, R.F. (2005) Model predictions of winter rainfall effects on N dynamics of winter wheat rotation following legume cover crop or fallow. *Field Crops Res.* 91, 251–261.

Herridge, D.F.; Peoples, M.B. & Boddey, R.M. (2008) Global inputs of biological nitrogen fixation in agricultural systems. *Plant and Soil*, v.311, p.1–18.

Holmes J.C. (1998) Socio-economic Background: The World Food Problem. In: Webster C.C. and Wilson P.N. (eds.). *Agriculture in the Tropics*. 3rd edition. Blackwell Science Ltd, Cambridge, England. pp. 1-19.

Ibewiro B., Sanginga N., Vanlauwe B.& Merckx R. (2000) Nitrogen contributions from decomposing cover crop residues to maize in tropical derived savanna. *Nutrient Cycling in Agroecosystems* 57: 131-140.

James, E.K. Nitrogen fixation in endophytic and associative symbiosis (2000) *Field Crops Research*, v. 65, p. 197-209.

Jensen, E. S. & Hauggaard-Nielsen, H. (2003) How can increased use of biological N₂ fixation in agriculture benefit the environment? *Plant and Soil* 252, 177-186.

Kapkiyai J.J., Karanja N.K., Qureshi J.N., Smithson P.C. & Woomer P.L. (1999) Soil organic matter and nutrient dynamics in a Kenyan nitisol under long-term fertilizer and organic input management. *Soil Biology and Biochemistry* 31: 1773-1782.

Karlen D.L., Flannery R.L. & Sadler E.J. (1988) Aerial Accumulation and Partitioning of Nutrients by Corn. *Agronomy Journal* 80: 232-242.

Kennedy, I.R.; Choudhury, A.T.M.A. & Kecske's, M. L. (2004) Non-symbiotic bacterial diazotrophs in crop-farming systems: can their potential for plant growth promotion be better exploited? *Soil Biology & Biochemistry* v. 36: 1229–1244.

Kirchmann H. (1991) Carbon and nitrogen mineralization of fresh, aerobic and anaerobic animal manures during incubation with soil. *Swedish Journal of Agricultural Research* 21: 165-173.

Kuklinsky-Sobral, J.; Araujo, W.L.; Mendes, R.; Geraldi, I.O.; Pizzirani-Kleiner, A.A.& Azevedo, J.L. (2004) Isolation and characterization of soybean-associated bacteria and their potential for plant growth promotion. *Environmental Microbiology*, v.6, p.1244-1251.

Lambrecht, M.; Okon, Y.; Vande Broek, A. & Vanderleyden, J. (2000) Indole-3-acetic acid: a reciprocal signaling molecule in bacteria-plant interactions. *TIM*, v.8, 298-300.

Lehmann J., Schroth G. & Zech W. (1995) Decomposition and nutrient release from leaves, twigs and roots of three alley-cropping tree legumes in central Togo. *Agroforestry Systems* 29: 21-36.

- Logan, T. J.; Lindsay, B. J.; Goins, L. E. & Ryan, J. A. (1997) Field assessment of sludge metal bioavailability to crops: sludge rate response. *Journal of Environmental Quality*, v.26, p.534-550.
- Lupwayi N.Z. & Haque I. (1999) *Leucaena* hedgerow intercropping and cattle manure application in the Ethiopian highlands. I. Decomposition and nutrient release. *Biology and Fertility of Soils* 28: 182-195.
- Mafongoya P.L., Barak P. & Reed J.D. (2000) Carbon, nitrogen and phosphorus mineralization of tree leaves and manure. *Biology and Fertility of Soils* 30: 298-305.
- Mafongoya P.L., Nair P.K.R., & Dzwela B.H. (1997) Multipurpose tree prunings as a source of nitrogen to maize under semiarid conditions in Zimbabwe. 3. Interactions of pruning quality and time and method of application on nitrogen recovery by maize in two soil types. *Agroforestry Systems* 35: 57-70.
- Mafongoya P.L., Nair P.K.R., & Dzwela B.H. (1998) Mineralization of nitrogen from decomposing leaves of multipurpose trees as affected by their chemical composition. *Biology and Fertility of Soils* 27: 143-148.
- Malavolta, E. (1981) Adubos minerais e orgânicos. In: ENCONTRO DE TÉCNICOS EM SOLO, Rio de Janeiro, 25p.
- Malavolta, E.; Vitti, G. G. & Oliveira, S. A. (1989) Avaliação do estado nutricional das plantas: princípios e aplicações. Piracicaba, Associação Brasileira para Pesquisas de Potássio e Fosfato, 201p.
- Mapfumo, P., Mtambanengwe, F., Giller, K. E., & Mpeperekwi, S. (2005) Tapping indigenous herbaceous legumes for soil fertility management by resource-poor farmers in Zimbabwe. *Agriculture, Ecosystems and Environment* 109, 221-233.

Mariano, R. L. R. et al. (2004) Importância de bactérias promotoras de crescimento e de biocontrole de doenças de plantas para uma agricultura sustentável Anais da Academia Pernambucana de Ciência Agronômica, Recife, vol. 1, p.89-111.

Martins, A.L.C.; Bataglia, O.C.; Camargo, O.A. & Cantarella, H. (2003) Produção de grão e absorção de Cu, Fe, Mn e Zn pelo milho em solo adubado com lodo de esgoto, com e sem calcário. Revista Brasileira de Ciência do Solo, Viçosa, v. 27 n.3, p. 563-574.

Majerowicz, N.; Pereira, J. M. S.; Médici, L.O.; Bizon, O.; Pereira, M. B. & Santos Júnior, U. M. (2002) Estudo da eficiência de uso do nitrogênio em variedades locais e melhoradas de milho. Revista Brasileira de Botânica, v. 25, p. 129-136.

Marin, A.M.P., Menezes, R. S. C., Salcedo, I. H. (2007) Produtividade de milho solteiro ou em aléias de gliricídia adubado com duas fontes orgânicas. Pesquisa Agropecuária Brasileira, v.42, p.669 – 677.

Mazzarino, M. J.; Laos, F.; Satti, P. & Moyano, S. (1998) Agronomic and environmental aspects of utilization of organic residues in soils of the Andean-atagonian region. Soil Science and Plant Nutrition, v.44, p.105-113.

McInnes, A., Thies, J. E., Abbott, L. K., & Howieson, J. G. (2005) Structure and diversity among rhizobial strains, populations and communities—a review. Soil Biology and Biochemistry.

McNeill, A.M. & Unkovich, M.J. (2007) The nitrogen cycle in terrestrial ecosystems. In: Marschner, P.; Rengel, Z. (eds) Nutrient cycling in terrestrial ecosystems. Soil Biology, v.10, p.37–64.

Mendonça, M.M. & Urquiaga, S. S.; Reis, V. M. (2006) Variabilidade genotípica de milho para acumulação de nitrogênio e contribuição da fixação biológica de nitrogênio. Pesquisa Agropecuária Brasileira, v.41, p.1681-1685.

Menezes, R. S. C. & Salcedo, I. H. (2007) Mineralização de N após incorporação de adubos orgânicos em um Neossolo Regolítico cultivado com milho. *Revista Brasileira de Engenharia Agrícola e Ambiental.* , v.11, p.361 – 367.

Melo, W.J.; Marques, M.O.; Santiago, G. & Chelli, R.A. (1994). Efeito de doses crescentes de lodo de esgoto sobre frações da matéria orgânica e CTC de um Latossolo cultivado com cana-de-açúcar. *Revista Brasileira de Ciência do Solo*, v. 18, p. 449-455.

Messias, A. S. (1993) Fertilizantes orgânicos (lixo urbano). Recife, Universidade Católica de Pernambuco. 48 p.

Messias, A.S. & Moraes, F.A. (1992) Emprego do lixo urbano na adubação do milheto. In: REUNIÃO BRASILEIRA DE FERTILIDADE DO SOLO E NUTRIÇÃO DE PLANTAS, 20., 1992, Piracicaba / SP. Anais... Piracicaba, p.384.

Mubarak A.R., Rosenani A.B., Anuar A.R. & Zauyah D.S. (2003) Recovery of Nitrogen from Maize Residue and Inorganic Fertilizer in a Maize-Groundnut Rotation System in Humid Tropics of Malaysia. *Communications in Soil Science and Plant Analysis* 34 (17- 18): 2375-2394.

Mundus, S., Menezes, R. S. C., Neergaard, A. & Garrido, M. S.(2008) Maize growth and soil nitrogen availability after fertilization with cattle manure and/or gliricidia in semi-arid NE Brazil. *Nutrient Cycling in Agroecosystems.* , v.82, p.61 – 73.

Musiyiwa, K., Mpepereki, S., & Giller, K. E. (2005) Symbiotic effectiveness and host ranges of indigenous rhizobia nodulating promiscuous soyabean varieties in Zimbabwean soils. *Soil Biology and Biochemistry* 37, 1169-1176.

Myers R.J.K., Palm C.A., Cuevas E. Gunatilleke I.U.N. & Brossard M. (1994) The synchronisation of nutrient mineralisation and plant nutrient demand. In: Woomer P.L. and

Swift M.J. (eds.). The Biological Management of Tropical Soil Fertility. Edition. Wiley-Sayce Publishing, Exeter, UK, pp. 81-116.

Myers R.J.K., Van Noordwijk M. & Vityakon P. (1997) Synchrony of Nutrient Release and Plant Demand: Plant Litter Quality, Soil Environment and Farmer Management Options. In: Cadisch G. and Giller K.E. (eds.). Driven by Nature – Plant Litter Quality and Decomposition. 1st edition. CAB International, Wallingford, UK. pp. 215-229.

Nascimento, C. W. A.; Barros, D. A. S.; Melo, E. E. C. & Oliveira, A. B. (2004) Alterações químicas em solos e crescimento de milho e feijoeiro após aplicação de lodo de esgoto. Revista Brasileira de Ciência do Solo. Viçosa. v.28, n.2, p.385-392.

Nefedieva, E.E. (2003) The influence of impulse pressure on the phytohormone content, growth and crop productivity of buckwheat plants (*Fagopyrum esculentum* Moench. cv. Aromat). Pacific Journal Science Technology, v.3, p.123–135.

Norman M.J.T., Pearson C.J. & Searle P.G.E. (1995) The Ecology of Tropical Food Crops. 2nd edition, Cambridge University Press.

Nyamangara J., Gotosa J. & Mpfu S.E. (2001) Cattle manure effects on structural stability and water retention capacity of a granitic sandy soil in Zimbabwe. Soil & Tillage Research 62: 157-162.

Nyamangara J., Piha M.I. & Kirchmann H. (1999) Interactions of aerobically decomposed cattle manure and nitrogen fertilizer applied to soil. Nutrient Cycling in Agroecosystems 54: 183-188.

Nyberg G., Ekblad A., Buresh R. & Högberg P. (2002) Short-term patterns of carbon and nitrogen mineralization in a fallow field amended with green manures from agroforestry trees. Biology and Fertility of Soils 36: 18-25.

Oglesby K.A. & Fownes J.H. (1992) Effects of chemical composition on nitrogen mineralization from green manures of seven tropical leguminous trees. *Plant and Soil* 143: 127-132.

Ojiem, J. O., Vanlauwe, B., de Ridder, N., & Giller, K. E. (2007) Niche-based assessment of contributions of legumes to the nitrogen economy of Western Kenya smallholder farms. *Plant and Soil* 292, 119-135.

Okito, A., Alves, B.J.R., Urquiaga, S. & Boddey, R.M. (2004) Nitrogen fixation by groundnut and velvet bean and residual benefit to a subsequent maize crop. *Pesquisa Agropecuaria Brasileira* 39, 1183-1190.

Okogun, J.A., Sangina, N. & Abaidoo, R.C. (2007) Evaluation of maize yield in an on-farm maize-soybean and maize-Lablab crop rotation systems in the Northern Guinea savanna of Nigeria. *Pakistan Journal of Biological Sciences* 10, 3905-3909.

Okon, Y. & Kapulnik, Y. (1986) Development and function of *Azospirillum*-inoculated roots. *Plant and Soil*, v.90, p.3-16.

Okon, Y. & Labandera-Gonzalez, C. (1994) Agronomic application of *Azospirillum*: An evaluation of 20 years worldwide field incubation. *Soil Biology and Biochemistry*, Oxford, v.26, n.12, p.1591-1601.

Okon, Y. & Itzgsohn, R. (1995) The development of *Azospirillum* as a commercial inoculant for improving crop yields. *BioUschnology Advances*, Vol. 13, No. 3, p. 415-424.

Oliveira, F. C. (1995) Metais pesados e formas nitrogenadas em solos tratados com lodo de esgoto. Piracicaba, ESALQ/USP, 90p. (Tese de Doutorado).

Oliveira, F.C.; Melo, W. J.; Pereira, G.T.; Melo, V.P. & Melo, G. M. P.H H. (2005) Heavy metals in oxisols amended with biosolids and cropped with maize in a long-term experiment. *Scientia Agricola*. (Piracicaba, Brazil), v.62, n.4, p.381-388.

Oliveira, T.K., Carvalho, G.J. & Moraes, R.N.S. (2002) Plantas de cobertura e seus efeitos sobre o feijoeiro em plantio direto. *Pesquisa Agropecuaria Brasileira* 37, 1079-1087.

Olivares, F.L.; Baldani, V.L.D.; Reis, V.M.; Baldani, J.I. & Döbereiner, J. (1996) Occurrence of the endophytic diazotrophs *Herbaspirillum* spp. In roots, stems and leaves, predominantly of Gramineae. *Biology Fertility Soils*, v.21, p.197-200.

Osunde, A. O., Bala, A., Gwam, M. S., Tsado, P. A., Sanginga, N. & Okogun, A. (2003) Residual benefits of promiscuous soybean to maize (*Zea mays* L.) grown on farmers' fields around Minna in the southern Guinea savanna zone of Nigeria. *Agriculture, Ecosystems & Environments* 100, 209-220.

Palm C.A. (1995) Contribution of agro forestry trees to nutrient requirements of intercropped plants. *Agro forestry Systems* 30: 105-124.

Palm C.A. & Sanchez P.A. (1991) Nitrogen release from the leaves of some tropical legumes as affected by their lignin and polyphenolic contents. *Soil Biology and Biochemistry* 23: 83-88.

Palm, C.A & Rowland, A.P. A minimum dataset for characterization of plant quality for decomposition. In: Cadisch G and Giller KE (eds) *Driven by nature: Plant litter quality and decomposition*, CAB International, Wallingford, 1997. 379–392p.

Palm, C.A; Catherine, N; Gachengo, Delve, R.J; Cadisch, G & Giller, K.E. (2001b) Organic inputs for soil fertility management in tropical agroecosystems: application of an organic resource database. *Agriculture, Ecosystems and Environment*. 83:27-42.

Palm, C.A; Giller, K. E; Mafongoya P. L & Swift M.J. (2001) Management of organic in the tropics: translating theory into practice. *Nutrient Cycling in Agroecosystem*. 61:63-75.

Peinetti, H. R.; Menezes, R. S. C.; Tiessen, H. & Marin, A.M.P (2008) Simulating plant productivity under different organic fertilization practices in a maize/native pasture rotation system in semi-arid NE Brazil. *Computers and Electronics in Agriculture*. , v.62, p.204 – 222.

Peoples, M.B. & Craswell, E.T. (1992) Biological nitrogen fixation: investments, expectations and actual contributions to agriculture. *Plant and Soil*, v.141, p.13-39.

Peoples, M. B., Herridge, D. F. & Ladha, J. K.(1995) Biological nitrogen fixation: An efficient source of nitrogen for sustainable agricultural production? *Plant and Soil* 174, 3-28.

Phoomthaisong, J., Toomsan, B., Limpinuntana,V., Cadisch, G. & Patanothai, A. (2003) Attributes affecting residual benefits of N₂-fixing mungbean and groundnut cultivars. *Biology and Fertility of Soils* 39, 16-24.

Pierrisnard, F. (1996) Impact de l'amendement des boues residuaires de la ville de Marseille sur des sols a vocation agricole: comportement du Cd, Cr, Cu, Ni, Pb, Zn, des hydrocarbures et des composes polaires. Marseille, 1996. 408p. These (Docteur) - Facolte des Sciences et Techniques de Saint-Jerome, Universite de Droit d'Economie et des Sciences d'AIX-MARSEILLE.

Pires, A.M.M. (2005) Lodo de esgoto. *AMBIENTE BRASIL*. EMBRAPA Meio Ambiente, p.1-2.

Pittner, E. et al. (2007) Flutuação populacional de bactérias do gênero *Azospirillum* em solo cultivado com milho e em campo nativo. *Ambiência Guarapuava*, v.3 n.2 p. 243-252.

Pradella, J.G.C.; Oliveira, M.S. & Urenha, L.C. (2001) Produção de inoculantes Agrícolas. In: Lima, U.A.; Aquarone, E.; Borzani, W.; Schmidell, W. *Biotecnologia industrial*. São Paulo: Edgard Blücher, v.3, p.279-305.

PROSAB - Programa de Pesquisa em Saneamento Básico. (1999) Uso e manejo do lodo de esgoto na agricultura. Rio de Janeiro: PROSAB, 97p.

Raja, P.; Uma, S.; Gopal, H. & Govindarajan, K. (2006) Impacto of Bio inoculants consortium on rice root exudates, biological nitrogen fixation and plant growth. *Journal of Biological Sciences* v. 6(5): 815-823.

Raun, W.R. et al. (2002) Improving nitrogen use efficiency in cereal grain production with optical sensing and variable rate application. *Agronomy Journal*, v.94, p.815-820.

Raymond, J.; Siefert, J. L.; Christopher R. Staples, C. R. & Blankenship, R. E. (2004) The natural history of nitrogen fixation. *Molecular Biology Evolution* 21(3):541–554.

Reddy, M. R.; Lameck, D. & Rezanian, M.E. (1989) Uptake and distribution of copper and zinc by soybean and corn from soil treated with sewage sludge. *Plant and Soil*, v.113, p.271-274.

Reinhold-Hurek, B.; Hurek, T.; Gillis, M.; Hoste, B.; Vancanneyt, M.; Kersters, K. & De Ley, J. (1993) Nitrogen-fixing proteobacteria associated with roots of kallar grass (*Leptochloa fusca* (L.) Kunth), and description of two species, *Azoarcus indigenus* sp. nov. and *Azoarcus communis* sp. nov. *International Journal of Systematic Bacteriology*, v.43, p.574-584.

Reis Junior, F.B.; Toledo, C.T.; Machado, A. T. & Sodek, L. (2008) Inoculação de *Azospirillum amazonense* em dois genótipos de milho sob diferentes regimes de nitrogênio. *Revista Brasileira de Ciências do Solo*, 32:1139-1146.

Riggs, P.J.; Chelius, M.K.; Iniguez, A.L.; Kaeppler, S.M. & Triplett, E.W. (2001) Enhanced maize productivity by inoculation with diazotrophic bacteria. *Australian Journal of Plant Physiology*, v.28, p.829-836.

Ritter, W.F. & Eastburn, R.P. (1978) Leaching of heavy metals from sewage sludge through coastal plain soils. *Communications in Soil Science and Plant Analysis*, v.9, p.785-798.

- Robertson, M. J., Sakala, W., Benson, T. & Shamudzarira, Z. (2005) Simulating response of maize to previous velvet bean (*Mucuna pruriens*) crop and nitrogen fertiliser in Malawi. *Field Crops Research* 91, 91-105.
- Roesch, L.F.W. et al. (2007) Diversidade de bactérias diazotróficas endofíticas associadas a plantas de milho. *Revista Brasileira de Ciências do Solo*, v.31, p.1367-1380.
- Rodríguez, H.; Fraga, R.; Gonzalez, T. & Y. Bashan, Y. (2006) Genetics of phosphate solubilization and its potential applications for improving plant growth-promoting bacteria. *Plant and Soil* 287:15–21.
- Rodrigues, T.A.N.; Arruda, R.S. & Soares, C. F. (2006) Produtividade de milho e de feijão consorciados adubados com diferentes formas de lodo de esgoto. *Suelo Nutrição Vegetal*, v.6, n.1, p.52-63. ISSN 0718-2791.
- Roper, M.M. & Ladha, J.K. (1995) Biological N₂ fixation by heterotrophic and phototrophic bacteria in association with straw. *Plant and Soil*, v.174, p.211–224.
- Rosolem, C.A., Pace, L. & Crusciol, C.A.C. (2004) Nitrogen management in maize cover crop rotations. *Plant and Soil* 264, 261-271.
- Rufino, M. C., Rowe, E. C., Delveb, R. J. & Giller, K. E. (2006) Nitrogen cycling efficiencies through resource-poor African crop-livestock systems. *Agriculture, Ecosystems & Environment* 112, 261-282.
- Saikia, S.P. & Jain, V. (2007) Biological nitrogen fixation with non-legumes: An achievable target or a dogma? *Current Science*, v.92.(n 3) p.317-322.
- Saito, M. L. (2007) O uso do lodo de esgoto na agricultura: precauções com os contaminantes orgânicos. Embrapa Meio Ambiente, Jaguariúna, SP, p.28.

Salvagiotti, F., Cassman, K.G., Specht, J. E., Walters, D.T., Weiss, A. & Dobermann, A. (2008) Nitrogen uptake, fixation and response to fertilizer N in soybeans: A review. *Field Crops Research* 108, 1-13.

Sanchez, P. A. Science in Agroforestry. *Agrof. Systems*, 30:5-55, 1995.

Sanginga, N. (2003) Role of biological nitrogen fixation in legume based cropping systems; a case study of West Africa farming systems. *Plant and Soil* 252, 25-39.

Sanginga, N., Dashiell, K.E., Diels, J., Vanlauwe, B., Lyasse, O., Carsky, R.J., Tarawali, S., Safo-Adjei, B., Menkir, A., Schulz, S., Singh, B.B., Chikoye, D., Keatinge, D. & Ortiz, R. (2003) Sustainable resource management coupled to resilient germplasm to provide new intensive cereal-grain-legume-livestock systems in the dry savanna. *Agriculture, Ecosystems and Environment* 100, 305-314.

Schroth G., Zech W. & Heimann G. (1992) Mulch decomposition under agroforestry conditions in a sub-humid tropical savannah processes and influence of perennial plants. *Plant and Soil* 147: 1-11.

Shelton, H.M., Franzel, S. & Peters, M. (2005) Adoption of tropical legume technology around the world: Analysis of success. *Tropical Grasslands* 39, 198-209.

Silva, J. E.; Resck, D.V.S. & Sharma, R.D. (2002) Alternativa agronômica para o bio-sólido produzido no Distrito Federal. I — Efeito na produção de milho e na adição de metais pesados em Latossolo no cerrado. *Revista Brasileira de Ciência do Solo*, Viçosa, v. 26, n.2, p. 487-495.

Silva, E.-C.d. (2006) Aproveitamento do nitrogênio (^{15}N) da crotalaria e do milheto pelo milho sob plantio direto em Latossolo Vermelho de Cerrado. *Ciência Rural* 36, 739-746.

Silva, T. O.; Menezes, R. S. C.; Tiessen, H.; Sampaio, E. V. S. B. & Salcedo, I. H. (2007) Adubação orgânica da Batata com esterco e, ou *Crotalaria juncea*. I-Produtividade vegetal e estoque de nutrientes no solo em longo prazo. Revista Brasileira de Ciência do Solo. , v.31, p.39 - 49.

Silva, T.O. & Menezes, R. S. C. (2007) Adubação orgânica da Batata com esterco e, ou, *Crotalaria juncea*. II- Disponibilidade de N, P e K no solo ao longo do ciclo de cultivo. Revista Brasileira de Ciência do Solo. , v.31, p.51 - 61.

Singh,A., Carsky,R.J., Lucas,E.O. & Dashiell,K. (2003) Soil N balance as affected by soybean maturity class in the Guinea savanna of Nigeria. Agriculture, Ecosystems and Environment 100, 231-240.

Simonete, M. A. (2001) Alterações nas propriedades químicas de um argissolo adubado com lodo de esgoto e desenvolvimento e acúmulo de nutrientes em plantas de milho. 2001. 89p. Tese (Doutorado) - Escola Superior de Agricultura Luiz de Queiroz, Piracicaba, São Paulo.

Simonete, M. A. & Kiehl, J. C. (2002) Extração e fitodisponibilidade de metais em resposta à adição de lodo de esgoto no solo. Scientia Agrícola, v.59, p. 555-563.

Simonete, M. A.; Kiehl, J. C.; Andrade, C.A. & Teixeira, C.F.A. (2003) Efeito do lodo de esgoto em um Argissolo e no crescimento e nutrição de milho. Pesquisa Agropecuária Brasileira, Brasília, n.10, v. 38, p. 1187-1195.

Smith,R.G., Gross,K.L.& Robertson,G.P. (2008) Effects of crop diversity on agroecosystem function: Crop yield response. Ecosystems 11, 355-366.

Sisworo W.H., Mitrosuhardjo M.M., Rasjid H. & Myers R.J.K. (1990) The relative roles of N, fertilizer, crop residues and soil in supplying N in multiple cropping systems in a humid, tropical upland cropping systems. Plant and Soil 121: 73-82.

Strobel, G.; Daisy, B. (2003) Bioprospecting for microbial endophytes and their natural products. *Microbiology and Molecular Biology Reviews*, v.67, p.491-502.

Tejera, N; Ortega, E. & Rodes, R. Lluch, C. (2006) Nitrogen compounds in the apoplastic sap of sugarcane stem: Some implications in the association with endophytes. *Journal of Plant Physiology* v. 163 80: 85.

Tenuta,2003.www.umanitoba.ca/afs/agronomists_conf/2003/pdf/tenuta_rhizobacteria.pdf

Thuler, D.S. et al. (2003) Plant growth regulators and amino acids released by *Azospirillum* sp. in chemically defined media. *Letter Applied Microbiology*, v.37, p.174-178.

Tilman, D., Cassman, K. G., Matson, P. A., Naylor, R. & Polasky, S. (2002) Agricultural sustainability and intensive production practices. *Nature* 418, 671-677.

Tonitto,C., David,M.B. & Drinkwater, L.E. (2006) Replacing bare fallows with cover crops in fertilizer-intensive cropping systems: A meta-analysis of crop yield and N dynamics. *Agriculture, Ecosystems and Environment* 112, 58-72.

Traore, A.; Maranville, J. W. (1999) Nitrate reductase activity of diverse grain sorghum genotype and its relationship to nitrogen use efficiency. *Agronomy Journal, Madison*, v. 91, p. 863-869.

Tsai, S. M., Nodari, R. O., Moon, D. H., Camargo, L. E. A., Vencovsky, R. & Gepts, P. (1998) QTL mapping for nodule number and common bacterial blight in *Phaseolus vulgaris* L. *Plant and Soil* 204, 135-145.

Tsadilas, C. D.; Matsi, T.; Barbayiannis, N. & Dimoyiannis, D. (1995) Influence of sewage sludge application on soil properties on the distribution and availability of heavy metals fractions. *Community Soil Science and Plant Analytical*, v.26, p.2603-2619.

Tsutiya, M. T. (2000) Alternativas de disposição final de biossólidos gerados em estações de tratamento de esgoto. In: Bettioli, W. & Camargo, O. A. (Ed) Impacto ambiental do uso agrícola do lodo de esgoto. Jaguariúna: EMBRAPA Meio Ambiente, cap. 4, p. 69-106.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY-USEPA. EPA 625/10-84-003. (1999). Environmental regulations and technology: use and disposal of municipal wastewater sludge. Federal Register, p. 15-16.

Urquiaga S., Cruz K. H. S. & Boddey R. M. (1992) Contribution of nitrogen fixation to sugar cane: Nitrogen-15 and nitrogen balance estimates. Soil Science Societies American Journal 56, 105–114.

Valverde, A. et al. (2003) *Herbaspirillum lusitanum* sp. nov., a novel nitrogen-fixing bacterium associated with root nodules of *Phaseolus vulgaris*. International Journal of Systematic and Evolutionary Microbiology, v.53, p.1979-1983.

Van Kessel, C. & Roskoski, J.P. (1988) Row spacing effects on N₂-fixation, N-yield and soil N uptake of intercropped cowpea and maize. Plant and Soil 111, 17-23.

Van Loon, G. W. & Duffy, S. J. (2001) Microbiological processes. In: Environmental Chemistry. New York: Oxford University, Cap.15, p. 492.

Vanlauwe, B; Gachengo, K; Shepherd, E; Barrios, G; Cadisch, G & Palm, C.A. (2005) Laboratory validation of a resource quality-based conceptual framework for organic matter management. Soil Science Society American Journal. 69:1135-1145.

Verma, C.S.; Singh, A.; Chowdhury, S.P. & Tripathi, A.K. (2004) Endophytic ability of two deep-water rice endophytes, *Pantoea* sp. and *Ochrobactrum* sp. using green fluorescent protein reporter. Biotechnology Letters, v. 26, p. 425-429.

Vidor, C. (1999) Descarte de lodo de estações de tratamento de efluentes domésticos no solo. In: Tedesco, M. J. & Gianello, C. (ed.) Manejo racional de resíduos no solo. Porto Alegre: DS/UFRGS, p. 128-150.

Weil R.R. & Magdoff F. (2004) Significance of Soil Organic Matter to Soil Quality and Health. In: Magdoff F. and Weil R.R. (eds): Soil Organic Matter in Sustainable Agriculture. 1st edition. CRC Press LLC, Florida, USA.

Wenbo, M.; Zalec, K. & Glick, B. R. (2001) Biological activity and colonization pattern of the bioluminescence-labeled plant growth-promoting bacterium *Kluyvera ascorbata* SUD165/26. FEMS Microbiology Ecology, v. 35, p. 137-144.

Zaharah A.R. & Bah A.R. (1999) Patterns of decomposition and nutrient release by fresh *Gliricidia (Gliricidia sepium)* leaves in an ultisol. Nutrient Cycling in Agroecosystems 55: 269-277.

Zaharah A.R., Bah A.R., Mwangi N.K., Kathuli P. & Juma P. (1999) Management of *Gliricidia (Gliricidia sepium)* residues for improved sweet corn yield in an ultisol. Nutrient Cycling in Agroecosystems 54: 31-39.

Zingore S., Mafongoya P., Nyamugafata P. & Giller K.E. (2003) Nitrogen mineralization and maize yields following application of tree prunings to a sandy soil in Zimbabwe. Agroforestry Systems 57: 199-211.