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# Use of Soil Microorganisms to Improve Plant Growth and Ecosystem Sustainability

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## 1. Introduction

The productive capacity of soils is directly associated with their organic matter content which is the main reservoir of organic carbon and main source of nutrients for plants (Grandy et al., 2006; Studdert et al. 1997; Sisti et al., 2004; Urquiaga et al., 2004). A sustained increase in the concentration of organic carbon is needed to establish a land management system where the degradation of soil organic matter occurs slowly and consequently its quality increases. Despite being underestimated in traditional farming, the cycle of organic carbon decomposition, governs various agronomic processes that occur on the soil and appear to affect productivity (Scow, 1997).

Microorganisms are an essential and large component of the living biomass of the soil (Whitman et al., 1998). They play a key role in the biogeochemical cycles and have a great potential in both agriculture use and environmental protection (Doran & Zeiss, 2000; Grandy et al., 2006). For that reason, the operation of any terrestrial ecosystem depends largely on soil microbial activity (Barea, 2004). It is known that the systems of crop management can modify the structure and biodiversity of soil microbial communities.

## 2. Microorganisms and sustainable agriculture

Soil microorganisms control the mineralization of organic carbon and thus regulate nutrient recycling in it. Crops are the basis of the trophic pyramid because they represent the source of energy and available nutrients to the heterotrophic microorganisms which contribute largely to the decomposition of plant residues. Soil microorganisms can act not only as a destination of the available nutrients to produce their microbial biomass but also as a source of them for the plant (Andrén et al., 1993). It is known that the quality of plant residues, as defined by the C/N ratio and lignin content, determines their rate of decomposition and the mineralization-immobilization cycle. The understanding of the impact of interactions between degradation and mineralization of carbon, nitrogen and phosphorus occurring in the soil within any particular crop sequence is a relevant goal of the ecological and agronomic research (Rorig et al., 2004a; Triplett & Dick, 2008). Changes in the rate of carbon and mineral nutrient movement in the soil as a result of the interactions between plants and other organisms, apparently, would involve changes in the structure and physiology of soil biotic communities (Rorig et al., 2004b; Garcia de Salamone et al., 2006a; Grandy et al., 2006). The prompt response of the

microbial processes and community structure when physical, chemical and biological changes occur is necessary and beneficial to maintain soil quality. Changes in the structure of microbial communities in perturbed systems are generally associated with emissions of greenhouse gases such as, CO<sub>2</sub>, NO or N<sub>2</sub>O and nitrate leaching loss (Jackson et al., 2003).

The use of no-tillage in agricultural systems can contribute positively to the sustainability of agro-ecosystems (Doran & Zeiss, 2000). This is due to the occurrence of changes in the pore space structure and water dynamics, increasing infiltration (macropores) and storage of water and in consequence reducing soil loss by erosion. In addition, there is a marked stratification of organic matter in the top layer which corresponds to a better structure and greater mesoporosity (Michelena et al., (2001). However, it is necessary to study in depth the dynamics of microbial diversity and activity in order to obtain biotic indicators to diagnose soil health (Garcia de Salamone et al., 2004, 2005). Based on nitrogen balance studies in the soil-plant system, it is known that the soybean crops leave a negative nitrogen balance so the excessive number of cycles of this crop in the sequence may contribute to the impoverishment of the soil (Zotarelli et al., 2002, Cheng et al., 2003). By contrast, cereal crops provide the most important contribution of plant residues in continuous cropping systems (Grandy et al., 2006). Several experiments were performed with the aim to find variables related to the activity of soil microorganisms that may show the influence of crop sequences on the biological properties of no-tillage managed soils. García de Salamone et al., (2006b) have shown that different crops have great influence on the mycorrhiza occurrence and root length (Figure 1). The amount of root biomass indicates that the introduction of maize or soybean in the crop sequence could have a great influence on nutrient cycling and both physiological and structural microbial diversity.

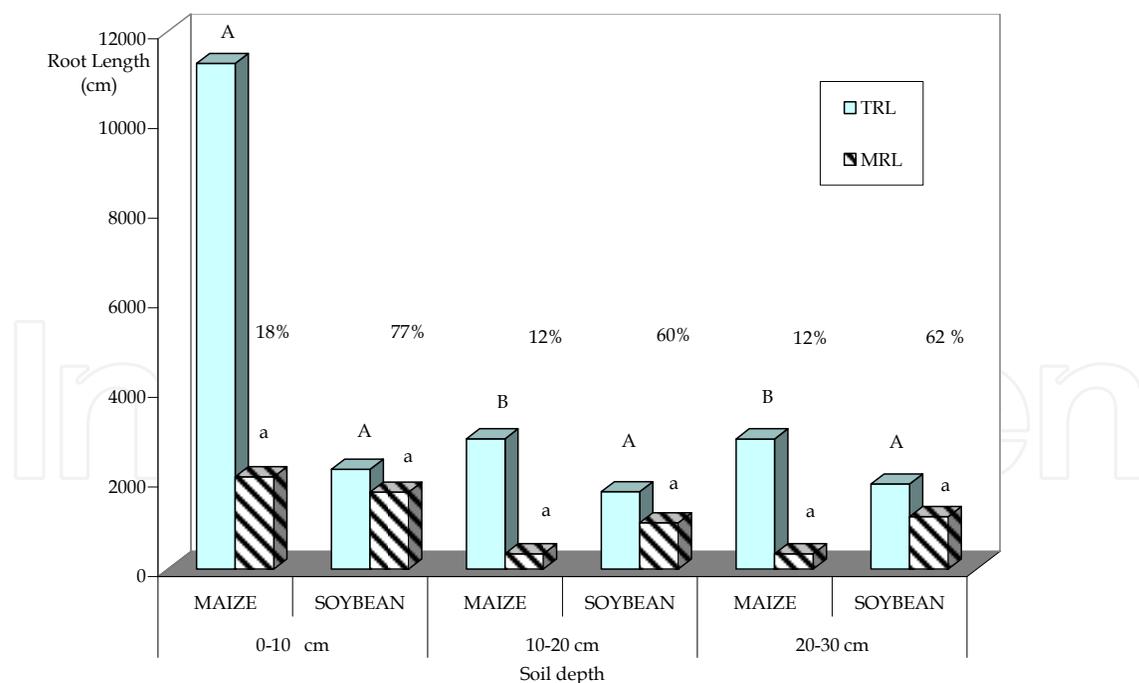


Fig. 1. Total root length (TRL) and Mycorrhiza root length (MRL) of maize and soybean in three soil depth layers at field conditions, (Adapted from García de Salamone et al., 2006b). Bars with the same letter are similar as indicated the Tukey's test ( $p < 0.05$ ). The comparisons between soil depth layers of TRL and MRL are in uppercase and lowercase, respectively. Numbers on each bar indicated percentages of the total root length with mycorrhiza.

### 3. Numerical dominance and diversity of soil microorganisms

Whitman et al., (1998) could show the numerical superiority of soil microorganisms in different ecosystems. Their estimation of the number of soil microbial cells in the Earth is approximately  $10^{30}$ . The estimated values are probably lower than the real numbers because in many cases the samples did not cover the entire depth of the soil profile. The samples were drawn on average from the first meter of depth and the authors applied methodologies to count cultivable microorganisms. It is known that on their best estimates, these methods cover less than 14% of the microbial community present in a soil sample. Despite these considerations, this figure is elevated when it is compared with the orders of magnitude of other living organisms such as humans ( $10^9$ ), termites ( $10^{17}$ ) or other magnitudes of the planet as ocean volume ( $10^{18} \text{ m}^3$ ) and Earth's mass ( $10^{27} \text{ g}$ ) or the mean distance from Sun to Earth ( $10^{11} \text{ m}$ ) and the radius of the universe ( $10^{26} \text{ m}$ ). Based on these data, soil microbial community can be named as "the invisible most." Its ecological significance is very strong as well in terms of macro reservoirs, because soil microorganisms contain 350-550 Pg of carbon, 85-130 Pg of nitrogen and 9-14 Pg of phosphorus; while plants only contain 560 Pg of carbon and 10 Pg, 1 Pg of nitrogen and phosphorus, respectively. The analysis of biogeochemical cycles (Schlesinger, 1991) indicates that the global carbon cycle is driven by  $\text{CO}_2$  fixation in plant photosynthesis but the soil compartment is more relevant than the atmosphere due to the intensity of flows, amounts and changes. The global nitrogen cycle is driven entirely by microbial reactions (Paul & Clark, 1996). The largest nitrogen compartment is inert and is represented by the atmospheric  $\text{N}_2$  ( $3.9 \times 10^{21} \text{ g}$  or 78% of gas composition). Microbial processes dominate the exchange between atmosphere and land-sea compartment of that element. Microbial diversity associated with each of the processes involved is clearly evident. The balance between nitrogen fixation and denitrification regulates inputs and outputs of soil nitrogen. Nitrification is the only process of conversion of ammonium to nitrate and is carried out mainly by autotrophic bacteria with fairly tight aeration and pH requirements. In summary, soil microorganisms are an enormous reservoir of genetic diversity, a significant reservoir of nutrients and a primary catalyst for the transformation of nutrients. A number of abiotic and biotic factors modify the genetic and physiological diversity of soil microbial communities (Maddoni et al., 2004).

Various estimates of the total number of bacterial species (Table 1) indicate that they are highly variable and the techniques used probably are the source of this variability. A simplistic analysis would seem to indicate that if there are between 1000 and 10000 species per gram of soil, it would not be necessary to worry about losing a certain amount of critical functions. But even if one accepts that a significant functional redundancy exists in most of the microbial communities (Naeem et al., 1995), we must take into account that: i. The occurrence of certain microbial processes is limited to very specific environments. For example, nitrogen-fixing trees in a rainforest and ii. There are interactions between diversity and invasion in a new habitat. For example, interactions between native microbes and introduced pathogens or biocontrol agents into a developing root. Anderson, (2003) established categories of organisms based on their sensitivity to changes in diversity. It can be found groups of microorganisms with different degrees of sensitivity for

Source	Methodology	Estimated Values species per gram of soil
Bergey's Manual of Systematic Bacteriology (Vol. 2)	Phenotypic Numerical Taxonomy	~3100 spp.
Vitek Database (Biomerieux)	Phenotypic Numerical Taxonomy	~1000 spp.
Torsvik et al. 1990 Torsvik et al. 2002	Hybridization DNA:DNA	~4000-10,000

Table 1. Various estimates of the total number of bacterial species

the same biogeochemical cycle. An example of this is related to the nitrifiers and N<sub>2</sub> fixing microorganisms which are located in the high and low sensitivity groups, respectively.

#### 4. Functionality and microbial diversity

Problems related to the study of microbial diversity and functionality are linked to two main aspects that are characteristic of soil microbial communities. The first is "hyperdensity" since it can be found between 10<sup>7</sup> and 10<sup>9</sup> cells per gram of soil. It can be accepted that it is impossible to obtain a complete catalog of all types present in any particular soil. The second drawback is the "hyperdiversity". As it is pointed out, it is possible that there are 10<sup>3</sup> - 10<sup>4</sup> types per gram of soil. These figures indicated that there is no method that can encompass all of them. On the other hand, the existence of low-density microbial types complicates the possibility of studying by traditional methods of cultivation. In this sense, the frequent problem is related to perform manipulative experiments to study microbial diversity when only a small percentage of soil microorganisms can be recovered by cultivation. This is because when we tried to cultivate microorganisms from a soil sample, even when various culture media and physicochemical conditions of incubation are used, the numbers of organisms that we can count is very low and because of that they are called "the cultivated minority." However, the evaluation of a microbial subgroup should be sufficient to detect changes in the dynamics of the community, if there are interactions between the measured subgroup and the other members of the community.

Techniques for studying microbial diversity can be classified into those that analyze the physiological diversity and those that address structural diversity. In the latter case, it is necessary to apply molecular techniques and the information obtained is based on comparisons with patterns of gene library. In the first case, most of the work, instead of analyzing communities and species abundance matrices as in ecology, physiological profiles are analyzed based on the use of substrates or abundance-based structural profiles of biomarkers such as, acids nucleic membrane lipids. The analysis of the physiological profiles of microbial communities can be achieved through a rapid assessment of multiple physiological properties such as, the use of carbon sources based on various tools such as: i. The reduction of redox dye and measuring the change in absorbance microplate wells after a short period of incubation (Garland, 1996, Di Salvo, García de Salamone 2008), ii. O<sub>2</sub> consumption on a sensitive fluorometer microplate cell (Garland et al., 2003) and iii. The production of carbon dioxide in the bottle headspace at the top of each cell (CS multi-

induced respiration). On the other hand, the analysis of fatty acid esters with phospholipids union (Haack et al., 1997) can be based on: i. The detection of the presence of lipids associated with viable cells (prokaryotes and eukaryotes), ii. Its rapid transformation from polar to neutral lipids (diglycerides) and iii. Cell viability assays based on membrane integrity. This information can be used to evaluate the total biomass of viable cells. Moreover certain lipids are specific and they can be used as biomarkers to detect both the physiological state and composition of the microbial community present in the sample. The counts of cultivable microorganisms belonging to different physiological and taxonomic groups can provide information about the dynamics of the microbial communities along a crop sequence and/or a crop cycle.

### 5. Crops, sequences and microbial diversity

Regarding the impact of cereal crops on the structure and physiology of the microbial communities, the author's research group has focused on finding variables related to the activity of soil microorganisms that indicate the influence of crop sequence in different soils of the Argentinean Pampas which are managed under no-tillage system. In this sense, it was found that the amount of total bacteria, fungi and actinomycetes in the soil could be used as biological indicators to detect seasonal variations in the wheat-soybean sequence at two Argentinean locations: Ramirez (Entre Rios) and Bengolea (Córdoba) (Figure 2). Both locations are sited at the north and northeast of Buenos Aires (Rorig et al., 2004b, Garcia de Salamone et al., 2006a). These authors also showed that count data for the bacterial genus *Pseudomonas* had a significant variation along the crop sequence which would be indicating versatility. The genus includes pathogenic and non-pathogenic strains. The latter ones have shown great diversity because some of them can detoxify the environment, others are plant growth promoters and/or biological control organisms (Garcia de Salamone et al., 2005).

On the other hand, the potentially mineralizable nitrogen (PMN) has also shown seasonal variations in the wheat-soybean sequence in Typical Argiudolls soils (Zubillaga et al., 2007). It could be also considered that these oscillations are related to changes in the food chains of microorganisms in the soil. The amount of nitrifying microorganisms and chemical availability of nitrate showed significant differences between sampling times in the crop cycle of wheat but not between production environments within a particular field plot (D'Auria et al., 2011). The potential nitrification did not vary between environments and sampling times, indicating that field conditions determine the activity of this group of soil microorganisms (Table 2). Regression analysis between these two variables indicated that the amount of ammonium oxidizing microorganisms increases in anthesis while soil nitrate concentration is reduced between the stages of stem elongation and anthesis of the wheat canopy (Figure 3). The chemical and biological variables analyze in a comprehensive manner the dynamic of nitrogen mineralization in field plots and the use of available nitrogen by the wheat crop.

With respect to carbon and nitrogen mineralization, the counts of cellulolytic and nitrifier microbial communities vary with the conditions imposed by the crop sequence (Rorig et al., 2004b). These authors found that in two different situations of soil and climate, the cultivation of wheat favors the activity of these two physiological groups of microorganisms in contrast to soybean which reduces their numbers. In addition, the

amounts of nitrifiers are significantly enhanced by the presence of plants and reduced in absence of them. The set of microbiological variables determined at different times of the wheat-soybean sequence showed that the conditions imposed cause measurable changes in soil microbiota. Although it is known that these techniques quantify a very small sample of the microbial community, detection of changes within culturable communities can be accepted if functional alterations of them can be estimated (Garcia de Salamone et al. 2006a). Previously, Garcia de Salamone et al., (2004) have observed differences in the physiological profiles of carbon use in the soil microbial communities when several levels of soil compaction were applied to simulate increasing intensities of machinery use (Figure 4). These different patterns of carbon use are indicating that the functional diversity of the microbial communities is changing because of the compaction treatments. The observed changes can be temporary or permanent. The management applied to the soil could finally define each situation. Using the same technique, as described below, it was possible to establish that inoculation with certain soil bacteria also alter temporarily microbial communities associated with wheat (Naiman et al., 2009) and rice (Garcia de Salamone et al., 2010) crops.

	Potential Mineralizable Nitrogen (mg kg <sup>-1</sup> )	Potential Nitrification (NO <sub>3</sub> g <sup>-1</sup> dry soil)	Most Probable Number of Nitrifiers Log g <sup>-1</sup> dry soil	Soil Nitrate concentration (mg kg <sup>-1</sup> )
Stem Elongation				
H	9.3a	170.7a	5.3a	22.0 bc
M	11.3a	153.1a	5.0a	28.1 c
Anthesis				
H	9.7a	744.3 b	7.7 b	10.0ab
M	16.4a	353.4ab	7.6 b	7.8a

Table 2. Biological and chemical variables of the nitrogen cycle estimated at two different stages of the wheat crop grown at field conditions on two historic levels of crop production. H: High, M: Medium, respectively. For each taxonomic group of microorganisms, means followed by the same letters are similar as indicated by the Tukey's test ( $p=0.05$ ).

A significant number of soil microorganisms are involved in mineralization of organic phosphorus. This must be converted to inorganic phosphorus to be used by plants. The mineralization of organic phosphorus is mostly carried out by phosphatase type enzymes, with significant levels of soil microbial phosphatases (García, 1999). Fixation and precipitation of phosphorus in soils is a phenomenon highly dependent on both pH and soil type. An important mechanism of solubilization of mineral phosphates is through the production and release of organic acids synthesized by soil microorganisms (Paul & Clark 1996). This causes an acidification of the medium and inorganic phosphorus release. There would be other ways to solubilize mineral phosphates, such as the production of inorganic acids and chelating substances. The ability of microorganisms to solubilize mineral phosphates depend on the nitrogen and/or carbon sources which are environmentally available. Related to the use of phosphorus by plants is of paramount importance to study arbuscular mycorrhiza. These symbiotic associations increase the area explored by the roots by promoting a better use of available resources (Chiocchio et al., 2002) and soil aggregation

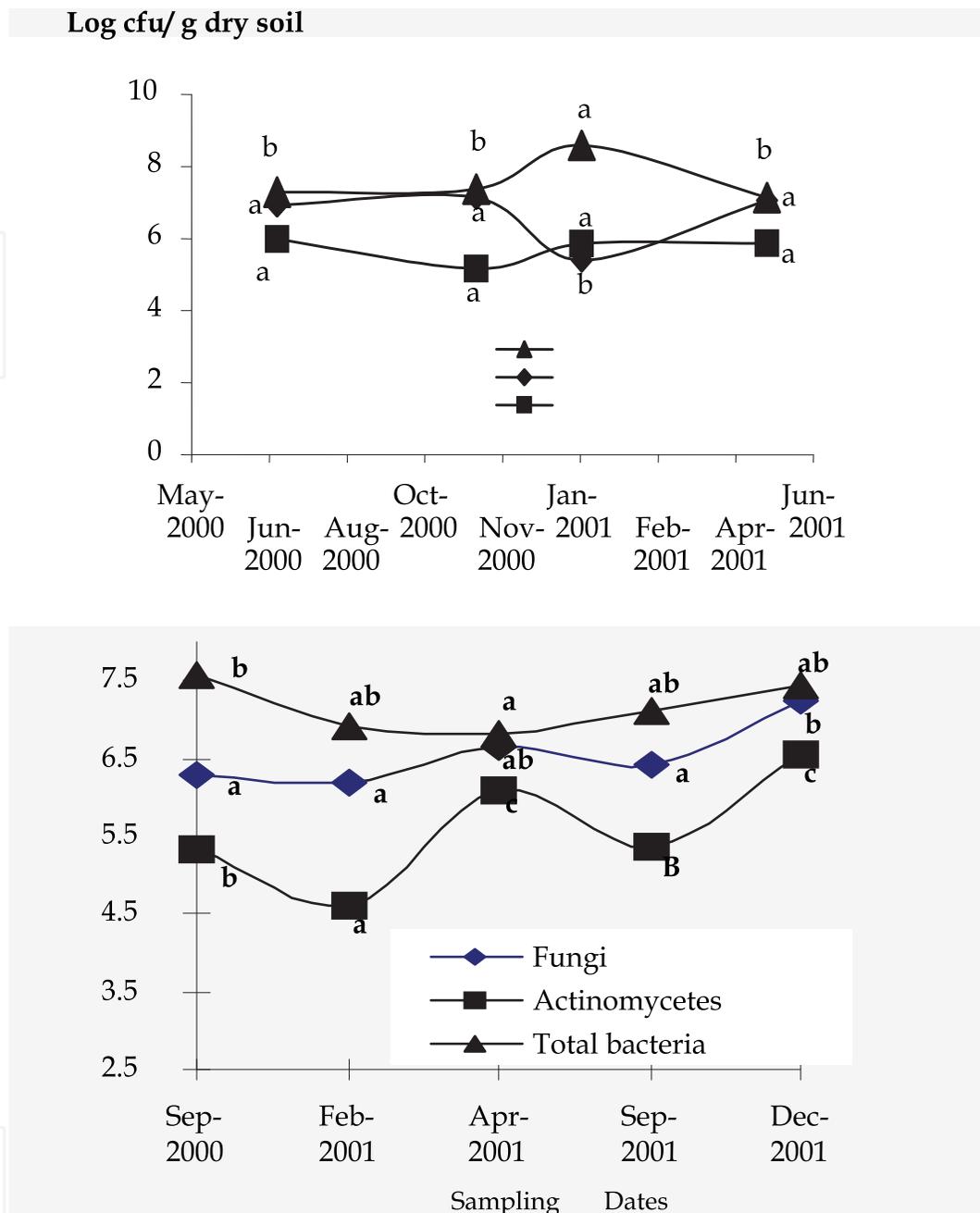


Fig. 2. Dynamics of fungi, actinomycetes and total bacteria during wheat/soybean sequence at two Argentinean locations. Top part: Typic Argiudoll soil in Ramírez (Entre Ríos). Bottom part: Entic Haplustoll soil in Bengolea (Córdoba). Means of each variable followed by the same letters are similar as indicated by the Tukey's test ( $p=0.05$ ).

(Rillig et al., 2002). The magnitude of natural mycorrhiza is considered a bioindicator of soil quality that responds to management practices (Garcia de Salamone et al., 2006a; Schalamuk et al., 2003) and can be promoted with the application of certain microorganisms (Naiman et al., 2008). The arbuscular mycorrhiza fungi are biotrophic symbionts and they associate with about 80% of the plant species. It has been observed in agroecosystems where the monocropping is applied, that the conventional tillage and the applications of both fertilizers and high soluble pesticides reduced more than 50% the

occurrence of natural mycorrhiza in comparison to the respective non-disturbed systems (Sieverding, 1991). Thus, it is also essential to consider that the maintenance of natural mycorrhiza for each crop in a given edaphoclimatic situation is highly relevant to maintain the sustainability (García de Salamone et al., 2006b; Schalamuk et al., 2006). In this sense,

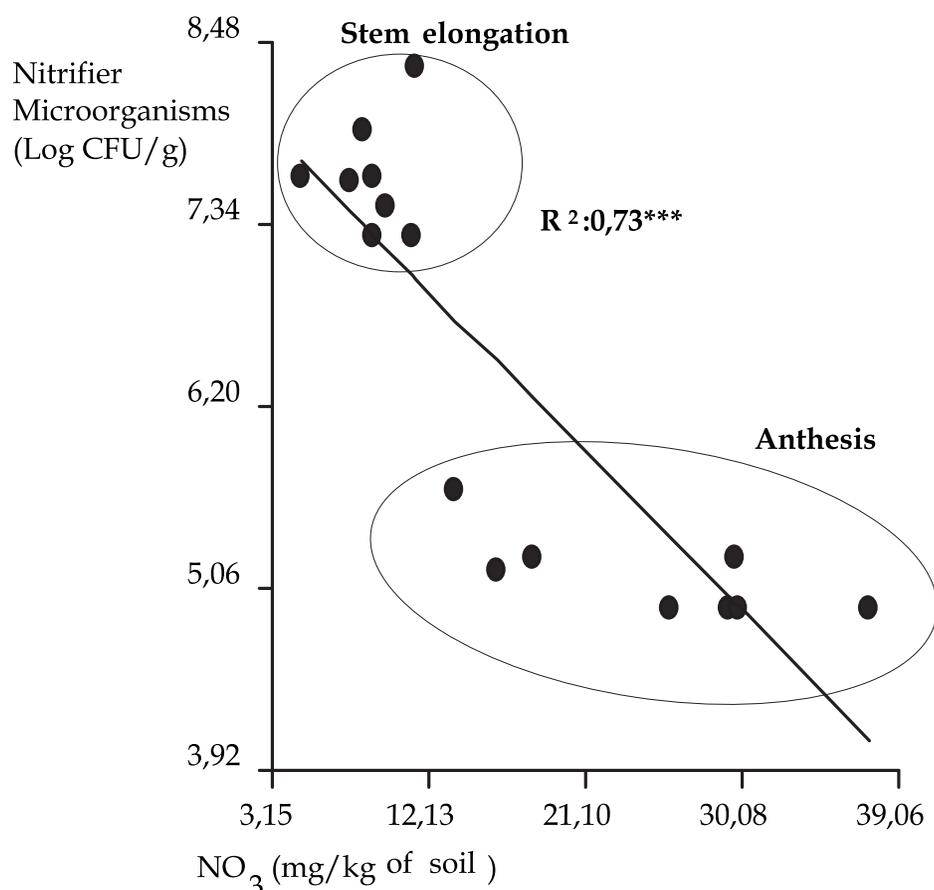


Fig. 3. Regression analysis of soil nitrate concentration and amount of cultivable nitrifiers at two stage of a wheat crop grown at field conditions on a Typic Argiudoll soil in Junin (Buenos Aires).

several studies were performed to evaluate soybean mycorrhiza associations under controlled conditions with different types and doses of pesticides (Martinez et al., 1998; Venedekian et al., 1999). Besides, trials have also been conducted with corn, wheat and soybeans (Garcia de Salamone et al., 2006b), which have shown that natural mycorrhiza is highly dependent on the historical and current management of the soil. Thus, both predecessor crop and phosphorus fertilizer addition exert significant effects on it. For these reasons, it is important to know the microbial dynamics of cereal crops included in the crop

sequence (Chiocchio et al., 2000; Menéndez et al., 1999; Studdert et al., 1997) and the effects of the introduction of microorganisms in the soil-plant system to provide a better understanding of these interactions which are known to help to agroecosystem sustainability (Naiman et al., 2009). It was observed that inoculation of wheat with a phosphorus solubilizer bacterium such as, *Pseudomonas fluorescens* can modify the mycorrhiza percentage and certain fungi structures during the growth cycle (Table 3). This PGPR is changing the population dynamic of the native mycorrhiza associated with the wheat crop and this could probably change the diversity of these microbial communities.

## 6. From soil microorganisms to microbial inoculants

Several soil microorganisms have natural properties which make them suitable to be used as biotechnological tools. However, any particular soil microorganism should show at least one beneficial characteristic in order to be considered for commercial propagation and consequent field application. There are different alternatives or markets for soil microbial products. In general, they can be included in some of the following types: nitrogen-fixing microorganisms, pest control agents, plant growth promoting rhizobacteria, usually named PGPR, and microorganisms for bioremediation. Any particular isolate should move through several steps before becoming a microbial inoculant. First of all, the development of a new soil microbial product for agriculture or bioremediation begins with the discovery of a useful naturally occurring organism. This can only be obtained establishing specific screening objectives which should be tested

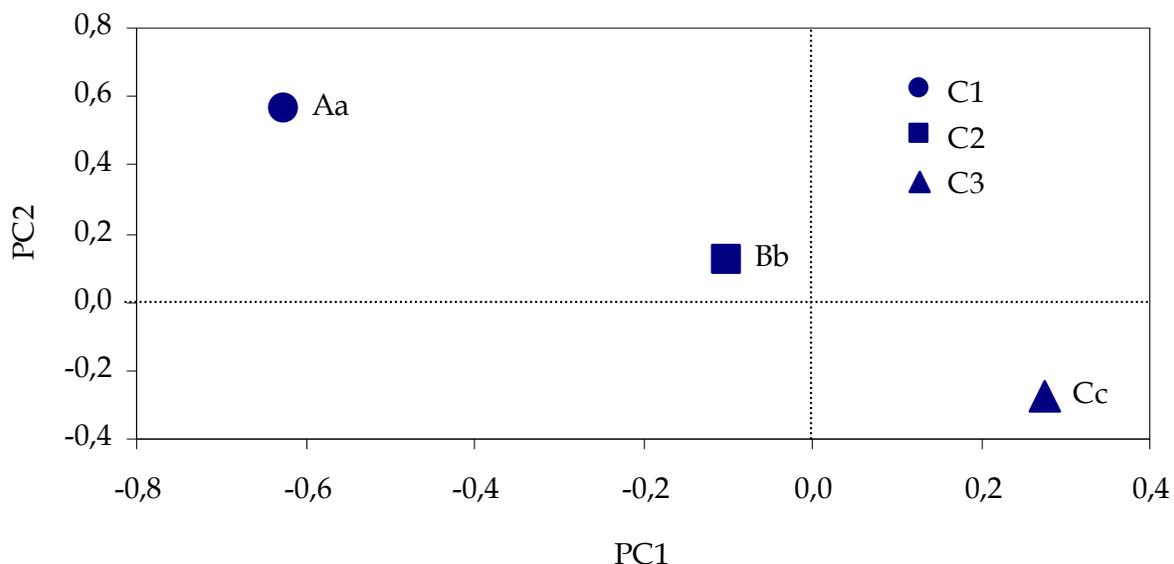


Fig. 4. Principal component analysis of physiological profiles of carbon use of the microbial communities of an Entic Haplustol soil, Bengolea (Córdoba) when three levels of compaction were applied to simulate increasing intensities of machinery use. Values are means of each compaction treatments, C1: control or field conditions, C2: 100 Kpa and C3: 400 Kpa. C2 and C3 were applied on the soil contained in cylinders with a diameter of 7.1 cm and a height of 6.1 cm. Data of absorbance of 72 h reading were used. PC1 and PC2 scores with the same uppercase and lowercases, respectively, indicate that they were not significantly different as determined by Tukey test at the rejection level of  $P < 0.05$ .

Inoculation treatments	Mycorrhiza percentage at two stages of growth	
	<i>Tillering</i>	<i>Grain filling</i>
Control	34 a	36 a
<b><i>Pseudomonas fluorescens</i></b>	48 b	51 b
	Arbuscules (%)	
Control	18 a	20 a
<b><i>Pseudomonas fluorescens</i></b>	25 b	24 b

Table 3. Impact of the inoculation with *Pseudomonas fluorescens* on mycorrhiza and arbuscules percentages found in wheat roots at two stages of growth. Means of each growth stage followed by the same letter are similar as indicated the Tukey's test ( $p < 0.05$ ), (Adapted from Naiman et al., 2008).

in every isolate. A sequence of laboratory, greenhouse and field experiments are usually performed in order to reduce the initial huge number of isolates to a small group of selected strains which have a combination of characteristics with commercial potential. Screening programs are usually carried out by companies or university laboratories. Most of the promising soil isolates have been obtained in university laboratories and then they were licensed to private companies to develop commercial formulations. Microbial inoculants for agriculture should be sold in a way that the farmers can easily apply. Liquid inoculants are the most accepted because they can be easily applied over the seeds before sowing. In this chapter, special emphasis is dedicated to a particular group of soil microorganisms. These are the PGPR. They are associated with plants of agricultural interest and in many cases direct beneficial effects on growth and both nitrogen and phosphorous nutrition are observed. Because of that, they constitute an ecological and economical alternative to increase food production (Bashan et al., 2004; Caballero Mellado, 2004; Díaz-Zorita & Canigia Fernandez, 2008; Ferraris & Courerot, 2004; Naiman et al., 2009; Reed & Glick, 2004). However, it should be noted that the impact of interactions between crops and soil microbes on the circulation of nutrients can be considerable. In this respect, plant biomass increases due to inoculation with PGPR can affect nutrient mineralization, solubilization of phosphorus, biological nitrogen fixation (BNF) and other biological processes associated with nutrient dynamics in the productive system. Therefore, it is required a detailed knowledge of the interrelationships between microorganisms added to the system (inoculants) and the native ones in both soil and associated plants. However, we know very little about the influence of management practices, such as PGPR inoculation and fertilization of cereal crops on the structure and functioning of the microbial communities. According to Wardle (2002) the generation of evidence-based information capable of connecting processes occurring in the air portion of the system with processes that take

place in the underground portion is one of the current challenges facing agroecological research.

There is abundant evidence in the literature indicating that the use of PGPR can have a significant role in agroecosystem sustainability (Antoun & Prévost 2006). PGPR microbial inoculants represent an emerging technology designed to improve the productivity of agricultural systems in the long run. However, the current use of microorganisms in agriculture remains at a low level despite the significant investment and scientific work (Catroux, 2007). They can be seen as a technology aligned with principles of sustainable agriculture, as opposed to the increasing use of pesticides and fertilizers. Several microorganisms are used in the normal agricultural practice, and others have potential for future use (Cassan & García de Salamone 2008; Maddonni et al., 2004b]. Most of them have the ability to colonize and establish an ongoing relationship with plants producing increases in biomass, root growth and commercial yield (Glick, 1995). At this regard, in the early '80s, the association between the diazotrophic bacteria *Azospirillum* and cereal crops was already considered a phenomenon of considerable scientific and economic value. Experimentation has also shown that a large number of factors influence the inoculation response in field conditions. Among PGPR, bacteria of the *Azospirillum* genus are the most used. The potential of this bacterium-plant association to increase crop production was first reported several years ago (Baldani et al., 1987). Positive impacts on plant growth through several mechanisms include enhancement of root development, production of growth regulators and nitrogen fixation (García de Salamone et al., 1996; Okon, 1994).

The content of nitrogen, phosphorus, potassium and various micronutrients is higher in plants inoculated with *Azospirillum* (Caballero Mellado et al., 1992; García de Salamone et al., 1996). Significant effects have been observed in wheat (Boddey et al., 1986; Caballero Mellado et al., 1992; Naiman et al., 2009), maize (Caballero Mellado, 2004; García de Salamone & Döbereiner, 1996), soybean (Bashan et al., 1990) and rice (Baldani & Baldani 2005; García de Salamone et al., 2010) among other species including more than a hundred crops and environmentally important plant species (Bashan et al., 2004). A recent publication compiled data from different countries showing the state-of-the-art in the *Azospirillum* inoculation responses (Cassan & García de Salamone, 2008).

The sustainability and profitability analysis requires a detailed knowledge of the interrelationships that exist between microorganisms commonly added as inoculants and those within the natural system (García de Salamone et al., 2006b; Naiman et al., 2009). Several authors have reported the impact of *Azospirillum* in root development with the consequent advantage on the absorption of water and nutrients (Bashan & Levanony 1990; García de Salamone et al., 1996; Okon, 1994). However, most of this information corresponds to experiments performed under controlled conditions. Thus, it is necessary to analyze the effects of commercial and new experimental inoculants in order to obtain better crops and a better use of the environmental resources. To date, several PGPR including *Pseudomonas* strains have been characterized as phosphorus solubilizers with the ability to produce organic acids (such as oxalic acid, fumaric acid and citric acid) and phosphatases that facilitate the solubilization of phosphorus and other nutrients (de Freitas et al., 1997; Rodriguez et al., 2006). In addition, it has been demonstrated that some strains can produce and supply several cytokinins in the rhizosphere of wheat and radish (García de Salamone et al., 2001, 2005). However, the largest amount of information about the activity of

*Pseudomonas* strains is associated with the indirect effects, through the control of pathogenic microorganisms. This can reduce the incidence of diseases through a number of mechanisms, including increased competitive ability by available nutrients, production of antibiotics, siderophores and induction of systemic resistance (Dowling & O'Gara, 1994; Kloepper, 1993).

The information analyzed in this chapter will show that the inoculation with PGPR such as *Azospirillum brasilense* and *Pseudomonas fluorescens* can modify cultivable microbial rhizosphere communities and plant growth at field conditions. The inoculation with these two rhizosphere microorganisms contributes to the emergence, development and grain production of crops such as wheat (Table 4), rice (Table 5), and maize (Table 6) (Baldani et al., 2008; Garcia de Salamone et al., 2010; Naiman et al., 2009). There is some evidence in controlled conditions for some crops that the application of certain PGPR does not change the structure of microbial communities (Herschkovitz et al., 2005). It was observed that there are some indications of the effect of PGPR inoculation have certain effects on the Shannon-Weaver diversity index of the microbial communities associated to the rhizosphere of wheat (Table 7). However the changes during the crop cycle are stronger than those produced by the PGPR inoculation. However, it is still necessary to understand better the microbial interactions that occur in soil-plant system under field conditions to assess the overall impact of this inoculation technology on the agroecosystem and to achieve the maximum efficiency (García de Salamone & Monzón de Asconegui 2008).

The average of two field experiments has showed that several commercial inoculants have different impact on grain yield of wheat crop in the west semiarid region of the Buenos Aires province (Figure 5). Although, all these commercial inoculants had the same strain of *Azospirillum brasilense*, these observations indicate that the interaction between the crop

Soil Type <sup>a</sup>	Number of plant cultivars	Inoculants		Range of Yield Increase due to Inoculation <sup>b</sup> (%)
		Number	Type	
Humic Hapludoll	2	3	Experimental	18-34
Humic Hapludoll	2	3	Experimental	0-2
Typic Arguidoll	2	3	Experimental	0-5
Typic Arguidoll	1	2	Experimental	7-9
Humic Hapludoll	1	3	Experimental	18-35
Entic Hapludoll	1	4	Commercial	2-32
Typic Arguidoll	1	2	Commercial	1-8
Typic Arguidoll	6	2	Experimental and commercial	9-30
Typic Arguidoll	1	2	Commercial	1-24
Aquatic Arguidoll	1	3	Experimental	0-10

Table 4. Characteristics of wheat field inoculation experiments and PGPR inoculation responses.

<sup>a</sup>All soil are located at the Province of Buenos Aires, Argentina.

<sup>b</sup>Yield increase with respect to the control plants without inoculation

and the microbial inoculant do not allow to confirm an average response with a particular PGPR strain when different formulations are used. Certain unknown ingredients other than the included PGPR strain can change the quality of the inoculants and consequently their efficiency. Standardization of quality control methodologies is still required.

Soil Type <sup>a</sup>	Number of plant genotypes	Inoculants		Range of Yield Increase due to Inoculation <sup>b</sup> (%)
		Number	Type	
Vertic Arguidoll	1	2	Experimental	4-8
Vertic Arguidoll	1	2	Experimental	17 - 20
Vertic Arguidoll	3	1	Commercial	- 7 - 20

Table 5. Characteristics of rice field inoculation experiments and PGPR inoculation response.

<sup>a</sup>All soil are located at the Province of Entre Rios, Argentina.

<sup>b</sup>Yield increase with respect to the control plants without inoculation

The combined analysis of the experiments described in Table 4 shows that inoculation with *Azospirillum* always increases the crop yield of wheat. In addition, the partition spikes and total biomass production can be increased through the practice of inoculation with this PGPR. An example of this kind of inoculation response is reported by Naiman et al (2009) who have observed that, *Azospirillum* inoculation increased aerial biomass by 12%, root biomass by 40% and grain yield by 16% (Table 8). Grain yield increases represent important earnings for the farmer and the increment of both aerial and root biomass may help to obtain a greater sustainability of the agroecosystems because the plant residues added to the system could help to maintain soil organic matter.

Rice is the third largest global food crop. Because of that its cultivation should be part of sustainable agriculture programs. Three experiments performed during the crop seasons 2006-2007, 2008-2009 and 2009-2010 in the rice belt in the province of Entre Rios, NE region of Argentina allowed the evaluation of the response of PGPR inoculation treatments (Table 5). In the first two cases, we studied the effect of *Azospirillum brasilense* on biomass production and grain yield (Garcia de Salamone et al., 2010, Gatica et al., 2009). The control biomass was 7256 and 15183 kg ha<sup>-1</sup> at tillering and grain filling, respectively. Treatment with *A. brasilense* significantly increased these values by 15 and 35% for tillering and 28 and 50% for grain filling. The control yielded 8370 kg ha<sup>-1</sup> and inoculation increased that value by 7.5%. In these studies we analyzed the rhizosphere microflora with potential to fix nitrogen using various methodologies. Also we observed increases in the most probable number (MPN) of cultivable rhizosphere diazotrophs when the seeds were inoculated with a mix of two *Azospirillum brasilense* strains (Table 9). However this MPN decreased between tillering and grain-filling stages. In the same experimental work it was observed that the ability to use four carbon sources by the microbial communities associated with rice rhizosphere was different between treatments at the grain-filling stage (Table 9). This approach utilizes the absorbance values on several carbon sources and the carbon level physiological profiles of the microbial communities are obtained through principal components analysis. In this case, malic acid, mannitol, ammonium oxalate and maltose

Soil Type <sup>a</sup>	Number of plant genotypes	Inoculants		Range of Yield Increase due to Inoculation <sup>b</sup> (%)
		Number	Type	
Typic Arguidoll	3	3	Experimental	0 - 4
Aeric Arguidoll	15	8	Experimental	-32 - 94
Aeric Arguidoll	7	8	Experimental	-39 - 42
Humic Hapludoll	4	6	Experimental	-6 - 77
Aeric Arguidoll	6	1	Experimental	2 - 5
Vertic Arguidoll	1	1	Experimental	0 - 5
Humic Arguidoll	1	1	Experimental	22 - 46
Entic Arguidoll	1	1	Experimental	3 - 13
Typic Arguidoll	3	2	Commercial	2 - 7

Table 6. Characteristics of maize field inoculation experiments and PGPR inoculation response

<sup>a</sup>All soil are located at the Province of Buenos Aires, Argentina.

<sup>b</sup>Yield increase with respect to the control plants without inoculation

Treatments	Shanon Weaver Diversity index <sup>(1)</sup>	
	Tillering	Grain filling
Without N-addition		
Control	3.0	2.9
CI 1	2.9	2.8
CI 2	3.0	2.9
CI 3	3.0	2.9
With N addition <sup>(2)</sup>		
Control	3.0	2.9
CI 1	2.9	2.9
CI 2	3.0	2.9
CI 3	3.0	2.9
<i>P</i>	0.56	0.29
Growth Stage Mean	2.9 a	2.8 b

Table 7. Effect of three commercial inoculants of PGPB and two levels of nitrogen (N) addition on the Shanon Weaver diversity index of the microbial communities during the wheat growth cycle.

Different letters indicate significant differences with Tukey's test ( $p < 0.05$ ). CI: Commercial Inoculant.

<sup>(1)</sup>Calculated from absorbance values obtained from a set of 23 carbon sources as described Naiman et al., (2009) using the methodology described by Gómez et al., (2004).

<sup>(2)</sup>45 kg ha<sup>-1</sup> of N applied as urea at sowing.

showed the highest Pearson correlation coefficients and their averages are in Table 9. Due to these carbon sources are usually frequent in the rhizosphere, the changes observed in their utilization can be related to changes in the physiological diversity of rice associated microbial communities after PGPR inoculation. In the case of the last crop season (Table 5), it was analyzed the effect of combined inoculation of two PGPR. These were *Azospirillum brasilense* and *Pseudomonas fluorescens*. In this experiment, it was analyzed the response to inoculation of three rice genotypes which were coming from different breeding programs. The response rates were significantly different among them (Table 5). This demonstrates the significant interaction between plant genotypes and applied soil bacteria. The analysis of both physiological and structural diversity of microbial communities indicated that they were also associated with the genotypes of the plant and the inoculation did not change those (Garcia de Salamone et al 2011 in press). The results of this work are indicating the high potential of the inoculation practice with PGPR for this crop and the possibility of increasing its efficiency through breeding programs that consider plant-bacteria association during the process. At this regard, Garcia de Salamone & Dobereiner (1996), proposed that the successful use of these plant-bacteria associations in agricultural scale can be achieved through plant breeding programs which consider “the ideotype approach” proposed by Donald (1968). Thus, it can help to obtain better combinations of both partners for any particular environment. Based on the results of several trials, it is possible to conclude about the relevance of the interaction between both maize genotypes and PGPR strains (Table 6). Garcia de Salamone et al., (1993) analyzed the available information about the response to *Azospirillum* inoculation in

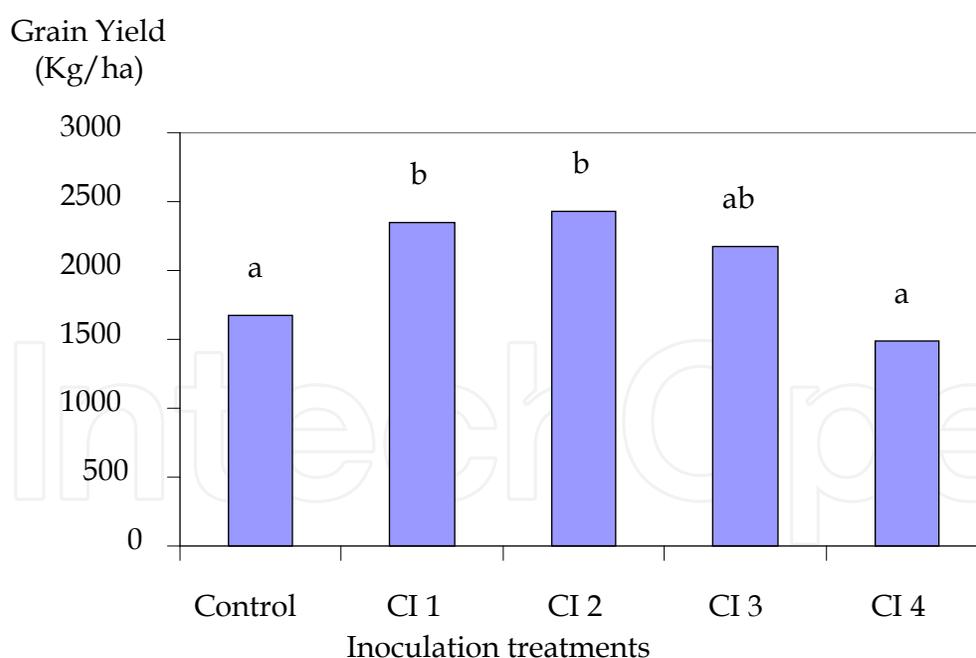


Fig. 5. Impact of *Azospirillum brasilense* inoculation treatments on grain yield of wheat grown at semiarid field conditions on an Entisol soil located in Saliquelo, West of the Buenos Aires, Argentina. CI: Commercial Inoculant.

Different letters indicate significant differences with Tukey's test ( $p < 0.05$ ).

association with maize and they observed a great consistency of the results in different crop seasons and environmental conditions. The analysis of consistency revealed significant grain yield increases ( $p < 0.05$ ) due to the inoculation with *Azospirillum* for all the evaluated experiments (Garcia de Salamone & Monzón de Asconegui 2008).

Inoculation Treatments	Grain yield		Biomass			
	PM	T <sup>(1)</sup>	Aerial		Roots	
			GF <sup>(2)</sup>	PM <sup>(3)</sup>	T	GF
(Kg ha <sup>-1</sup> )						
Control	2600a	3129a	4911a	8248a	12501a	22969a
CI-1	3400b	3130 <sup>a</sup>	5153a	8799a	23992b	25622a
CI-2	2900ab	3189a	5765a	9846a	16737ab	24624a
CI-3	3200b	3168a	5298a	8868a	18367ab	23467a
<i>p</i>	0.02	0,92	0,48	0,41	0,005	0,83

Table 8. Effect of several commercial inoculants of PGPB on grain yield, aerial and root biomass production during the wheat growth cycle.

Different letters indicate significant differences with Tukey's test ( $p < 0.05$ ). CI: Commercial Inoculant.

Tillering, GF: Grain-filling, PM: Physiological maturity.

Treatments	Diazotrophic microaerophilic bacteria (Log MPN g <sup>-1</sup> of root)		Average values of absorbance at 590 nm <sup>(1)</sup>
	Tillering <sup>(2)</sup>	Grain filling <sup>(3)</sup>	
Control	8.5 a	6.2 a	0.184 b
Experimental Inoculants (EI) <sup>(4)</sup>			
EI 1	9.3 b	6.4 ab	0.137 a
EI 2	8.9 ab	6.7 b	0.128 a

Table 9. Most Probable Number (MPN) of microaerophilic nitrogen-fixing bacteria and average of absorbance values shown by cultivable microorganisms in the rhizosphere of rice plants inoculated at sowing with *Azospirillum brasilense* (Compiled data from García de Salamone et al., 2010).

<sup>(1)</sup> Absorbance values for four carbon sources showing the highest Pearson correlation coefficients in the principal components analysis of carbon level physiological profiles.

<sup>(2)</sup> 35 and <sup>(3)</sup> 117 days after sowing. <sup>(4)</sup> Experimental inoculants 1 and 2 contained the same two *Azospirillum brasilense* strains but they were differently applied on the rice seeds.

## 7. Biological nitrogen fixation (BNF)

Biological nitrogen fixation becomes relevant and it can be incorporated by certain cereal-PGPB associations as a relevant source of nitrogen for the agroecosystem (Garcia de Salamone et al., 1996; Urquiaga et al., 2004). It has been shown that inoculation with certain bacteria-plant combinations, such as *Azospirillum*-maize can provide nitrogen from BNF at levels equivalent to 100 kg ha<sup>-1</sup> of this element (Garcia de Salamone et al., 1996). The contribution of nitrogen could help to the sustainability of the agroecosystem because it can

improve the quality of the plant residues and enable a reduction in the use of nitrogen fertilizers (Figure 6). Moreover, it was observed that inoculation with *A. brasilense* modify the  $^{15}\text{N}/^{14}\text{N}$  ratio of both rice and wheat plants in the grain filling stage (Garcia de Salamone et al., 2009). In the case of rice, it could be concluded that the amount of nitrogen derived from BNF increased when inoculation with certain *Azospirillum* strains was applied (Table 10). Three weeds plants growing contemporarily with the wheat crop were used as non-fixing controls to estimate the occurrence of nitrogen fixation through the  $^{15}\text{N}$  dilution technique (Boddey, 1987). Wheat plants showed significant differences among genotypes in the values of  $^{15}\text{N}/^{14}\text{N}$  ratios (Table 11). The great variability is evident in the  $^{15}\text{N}/^{14}\text{N}$  relationships of uninoculated wheat plants and it would be indicating the possibility of using these values to calculate the amount of nitrogen derived from BNF in inoculated wheat plants. The mean  $^{15}\text{N}/^{14}\text{N}$  ratio of the three weed plants in the experiment of wheat was 8.64 and it was used to estimate the BNF rates. They showed variability between the genotypes of the plant as reported earlier with maize genotypes (Garcia de Salamone et al., 1996) in association with PGPR. The relationship between the  $^{15}\text{N}/^{14}\text{N}$  ratios in both control and inoculated wheat plants has shown that, the average percentage range of nitrogen derived from BNF was 13-55%.

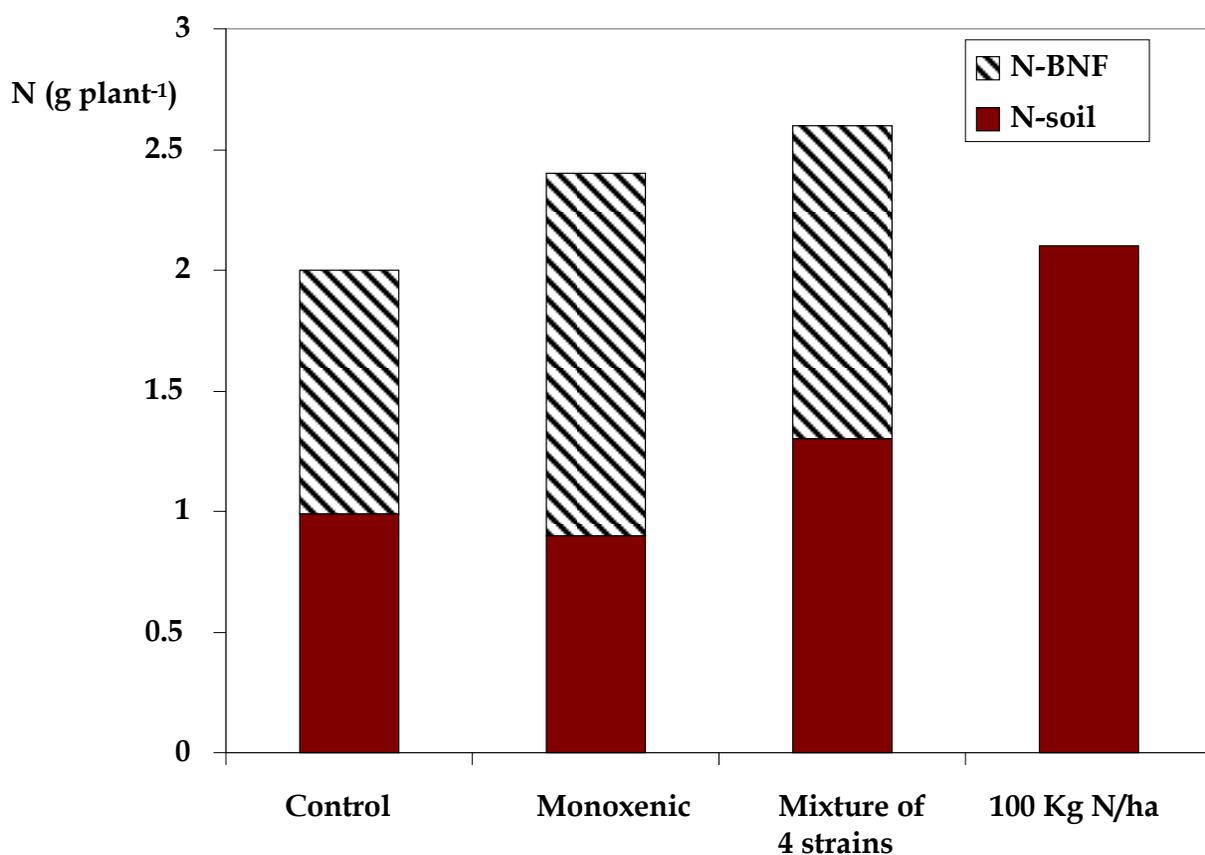


Fig. 6. Nitrogen content derived from soil and biological fixation in aerial plant tissues of maize plants fertilized with nitrogen and inoculated with *Azospirillum* strains. Data compiled from García de Salamone, et al., (1996).

These data indicate that inoculation with *Azospirillum* can produce changes in plant physiology which can be related to the occurrence of BNF. To increase accuracy, it is

necessary to obtain better estimates of soil  $^{15}\text{N}/^{14}\text{N}$  values (Alves et al., 2008). The most probable number counts of ammonium oxidizing microorganisms increased when the seeds of rice and wheat crops were inoculated with *Azospirillum brasilense*. However, potentially mineralizable nitrogen (PMN) showed different responses to inoculation for these two crops (D'Auria et al., 2011). PMN values for the rice plants were significantly lower in the soil associated with the inoculated plants. However, the soil PMN associated with uninoculated wheat plants was  $5.3 \text{ mg N-NH}_4 \text{ kg}^{-1}$  soil, which was significantly ( $p < 0.05$ ) lower to  $10.3 \text{ mg N-NH}_4 \text{ kg}^{-1}$  soil for the soil observed associated with the inoculated plants. From these data, we can infer that changes of plant physiology of rice and wheat plants produced by *Azospirillum* inoculation could favor BNF of these cereal crops and changed activity and numbers of the microorganisms associated to N-cycling.

Inoculation Treatments	Delta $^{15}\text{N}/^{14}\text{N}$				% N-BNF	PMN N-NH <sub>4</sub> (mg Kg <sup>-1</sup> dry soil)	Log MPN Nitrifiers	
	(1)	Rice plants	Weed Plants (2)					(3)
			<i>E.p</i>	<i>Cyp</i>				
C	16.31	16.9	15.48	18.52	3.9	8.4	5,9	
ExIn 1	11.68	13.16	1052	16.25	12.2	3.1	6,0	
ExIn 2	11.31	12.22	10.61	16.64	14.1	Wd	6,3	

Table 10. Values of Delta  $^{15}\text{N}/^{14}\text{N}$  ratios for rice and associated weed plants, percentages of nitrogen derived from biological fixation (N-BNF), potential mineralizable nitrogen (PMN) and most probable number (MPN) of nitrifiers in the rhizosphere of field-grown rice at grain-filling stage.

$^{15}\text{N}/^{14}\text{N}$  ratio of weed plants in the experiment of wheat was 8.64. Estimates of BNF rates showed variability between (1) C: control without inoculation; ExIn 1 and 2: Inoculation treatments including the same mixture of two *Azospirillum brasilense* strains but using two different application techniques. (2) Weed plants sampled at the same moment of the crop: *E.p*: *E. polystachia*, *Cyp*: *Cyperus sp.*, *P.v*: *P. viscose*. (3) % N-FBN: Percentage of N derived from BNF calculated using for calculations the average  $^{15}\text{N}/^{14}\text{N}$  ratio of control plants for each treatment as the value of control. wd: without data.

## 8. Summary and outlook

The results included in this review show the ability of certain PGPR to modify the ecophysiology of crops such as wheat, corn and rice in field conditions. However, the information available would indicate that the plant-strain-environment interaction is relevant to the results of inoculation that can be obtained (Garcia de Salamone, Monzón de Asconegui 2008). It was observed that the inoculation response is variable and the introduced bacteria can colonize and remain in the rhizosphere. Yield increases and biomass production have ecological relevance and they should be studied from the point of view of microbial ecology. Besides, these aspects should be studied using isolates with ability to fix nitrogen in association with the plant. This could increase the response level and improve the efficiency of use of the available resources. The introduction of PGPR could cause changes in the microbial activity in the rhizosphere and they have to be studied in detail

Genotype of wheat	Inoculation Treatments (1)	Delta $^{15}\text{N}/^{14}\text{N}$ (2)	% N-FBN (3)	% N-FBN (4)
Buck 75	C	10.08	-16.67	-
	ExIn	8.70	-0.69	13.66
Buck Chacarero	C	7.05	18.34	29.41
	ExIn	8.18	5.38	19.05
Buck Sureño	C	7.72	10.65	22.98
	ExIn	6.06	29.86	39.58
Klein Zorro	C	4.16	51.85	58.57
	ExIn	5.44	37.03	45.99
Klein Castor	C	4.48	48.21	55.27
	ExIn	5.74	33.56	42.83
Austaliano	C	4.97	42.53	50.55
	ExIn	5.89	31.89	41.35

Table 11. Impact of *Azospirillum brasilense* inoculation on values of Delta  $^{15}\text{N}/^{14}\text{N}$  ratios and percentages of nitrogen derived from biological fixation for six genotypes of wheat at grain-filling stage grown at field conditions.

(1) C: control without inoculation, ExIn: Experimental Inoculant formulation of *Azospirillum brasilense*. (2) Delta  $^{15}\text{N}/^{14}\text{N}$  of test plants, average of four replicates. %N-FBN: Percentage of N derived from BNF calculated using as the value of control plants in [F1]; (3) average  $^{15}\text{N}/^{14}\text{N}$  ratio of weeds; (4):  $^{15}\text{N}/^{14}\text{N}$  ratio of Buck 75.

(Garcia de Salamone & Cassan 2010). It is known that microbial diversity can be used as an index of soil quality and the management conditions can modify it (Garcia de Salamone et al., 2006, 2010). In connection with this and because the practice of PGPR inoculation is being used by a growing number of farmers in various agricultural areas of the world, it is necessary to provide knowledge about the microbial ecology of the rhizosphere of crops under field conditions when PGPR are applied repeatedly crop after crop.

The results compiled in this review are also consistent and they can be extended with those reviewed by Reed & Glick (2004), Bashan et al., (2004), Cassan & Garcia de Salamone, (2008), Garcia de Salamone & Cassan (2010). All available information indicates that inoculation with PGPR should be favored. However, the variability in both PGPR and plant capabilities should be adjusted and enhanced in order to include alternative mechanisms such as BNF, production of plant growth regulators, biological control of pathogens, among others in order to improve response rates to inoculation in the field.

In summary, the direct effects of the use of PGPR in cereal crops exert a significant role in fundamental processes of the ecosystem such as carbon and nitrogen recycling. The approach to these problems with field experiments provides a set of evidence that has involved the author's interaction in several research teams. It is expected that this review results in a contribution to connect processes occurring in both aerial and soil portions of the ecosystem.

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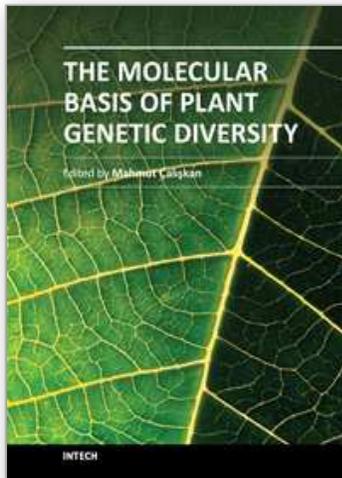
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## **The Molecular Basis of Plant Genetic Diversity**

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The Molecular Basis of Plant Genetic Diversity presents chapters revealing the magnitude of genetic variations existing in plant populations. Natural populations contain a considerable genetic variability which provides a genomic flexibility that can be used as a raw material for adaptation to changing environmental conditions. The analysis of genetic diversity provides information about allelic variation at a given locus. The increasing availability of PCR-based molecular markers allows the detailed analyses and evaluation of genetic diversity in plants and also, the detection of genes influencing economically important traits. The purpose of the book is to provide a glimpse into the dynamic process of genetic variation by presenting the thoughts of scientists who are engaged in the generation of new ideas and techniques employed for the assessment of genetic diversity, often from very different perspectives. The book should prove useful to students, researchers, and experts in the area of conservation biology, genetic diversity, and molecular biology.

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