Dynamics of Rhizosphere Microbial Communities of Cover Crops Dried with Glyphosate

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Abstract

The use of cover crops (CC) may be associated with other management practices recommended to achieve high yields and collaborate to use available resources more efficiently. Glyphosate is a nonselective systemic herbicide, which is commonly used for drying CC. Here we included a review of the related topics and showed the effects of drying oats and rye with glyphosate, inoculation with two plant growth-promoting rhizobacteria, and nitrogen fertilization on rhizosphere microbial communities at field conditions in the western Pampas of Argentina. Rhizosphere samples were obtained at three times: before drying the CC, a month after this, and at harvest time of soybean which was grown after each CC. Counts of viable cells and physiology of rhizosphere microbial communities were analyzed. The inclusion of CC dried with glyphosate modifies their associated rhizosphere microbial communities. Their numbers significantly decreased or increased. For some microorganisms, these changes were temporary because their amounts at soybean harvest time did not differ from those obtained when the sampling was done before drying CC with glyphosate application. Besides, our results indicate that the drying time must be chosen taking into account CC types and their phenology. This scientific information is evidence of changes on rhizosphere microbial communities due to the management of CC with glyphosate in combination with or without both inoculation and fertilization of CC. These data are agronomic and environmentally relevant because they have shown that the type of management would impact on the quality and health of the soil and therefore in agroecosystem sustainability.

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D.P. Singh et al. (eds.), *Plant-Microbe Interactions in Agro-Ecological Perspectives*, DOI 10.1007/978-981-10-5813-4_2

Keywords
Rhizosphere microorganisms • Soybean • Rye • Oats • *Pseudomonas fluorescens* • *Azospirillum*

2.1 Introduction

In recent years, agriculture has evolved to long agricultural cycles and in some cases to continuous agriculture (Ruffo and Parsons 2004) combined with non-tillage and the use of agrochemicals (Pound 1998; García 1999). This situation led to intensive land use, driven by the expansion of the agricultural frontier over areas not suitable for agriculture, where soils are much more fragile and more susceptible to water and wind erosion (Pound 1998; Pengue 2009; Sasal et al. 2006; Salsal 2012, 2013). The most relevant characteristics of this phenomenon called "agriculturization" are, on the one hand, the increase of the annual summer crops at the expense of the stagnation of the winter crops and, on the other hand, the exponential growth of the soybean (*Glycine max* L. Merrill) in comparison with the rest of the species (Carreño and Viglizzo 2011; ACSOJA 2015). In each region, other crops and livestock were replaced by this legume (Ruffo and Parsons 2004). Currently, soybean monoculture, the intensive use of agrochemicals, and the low replenishment of carbon and nutrients are common (Scianca et al. 2009). Due to their high profitability, the extensive crop farmers focused exclusively on cultivating transgenic soybeans with resistance to the herbicide glyphosate. Thus, this crop went from occupying almost 5 million hectares in 1990 to 20.6 million hectares for the summer season 2015–2016, only in Argentina. Grain production, for its part, increased from 10 to 57.6 million tons for the same period of time (MinAgri 2016). In this way, Argentina became the first country to export soybean oils and flour and the third exporter of soybean grains (ACSOJA 2015). Similar situation was developed in Brazil.

On the other hand, the limited contributions of soybean stubble, even under non-tillage, have shown to affect the contents of organic matter (Andriulo et al. 1999; Satorre 2003) with a consequent negative carbon balance in the soil favoring degradation processes (Alvarez et al. 2006). This is worrying, because approximately 0.1% of organic matter is lost per centimeter of degraded soil (Casas 2013). In addition, Salsal (2013) indicates that the monoculture does not provide ecological and agronomic benefits, as it leads to a decrease in the biodiversity of both the quality and the amount of organic matter available in the soil. In this environment, microorganisms degrade organic matter and directly impact soil properties (Ferreras et al. 2009).

Thus, due to the great economic importance of soybean cultivation worldwide and the low input of residues with a low C/N ratio (Studdert and Echeverría 2000), a negative N balance is also established, which contributes to soil impoverishment (Zotarelli et al. 2002; Cheng et al. 2003). In addition, since its residue decomposes rapidly and leaves the soil exposed to erosive action (Salsal 2013), the need arises to incorporate tools to favor the sustainability of the system.

2.2 Use of Cover Crops as a Management Alternative

One of the alternatives is to include cover crops (CC) in the crop sequence in order to increase the soil carbon input through their residues. Thus, the quality of the soil is improved (Alvarez et al. 2006; Martinez et al. 2013), and in the medium term, the negative carbon balance suffered by the extensive agricultural systems is mitigated (Scianca et al. 2011). The productive capacity of soils is directly associated with their organic matter content, which is the main reserve of organic carbon and the main source of nutrients for the crops (Urquiaga et al. 2004). For this reason, CC represents a technological alternative that balances the carbon of the soil, contributing a significant improvement for the physical and chemical properties of it (Cordone and Hansen 1986; Altieri 1994; Ruffo and Parsons 2004; Carfagno et al. 2008). However, very little is known about the dynamics of the microbiological properties associated with this system.

CC can be defined as those crops that grow specifically to keep the soil covered, protecting it from erosion, avoiding the loss of nutrients by washing and runoff. In addition, they are used to reduce compaction, minimize residual nitrate leaching, increase carbon content, improve plant nutrition, lower soil temperature, increase water use efficiency, contribute to water table depression in very humid periods, as well as reduce the level of weeds and the use of agrochemicals (Ruffo and Parsons 2003, 2004, Scianca et al. 2011; Fernández et al. 2012). Therefore, the use of CC and zero tillage is an effective measurement to conserve and maintain productive potential of the soil (Altieri 1994).

CC are species with desirable characteristics to include in the crop sequence with the commercial crops (Espindola et al. 2005) such as soybean. CC are different from pasture because they do not produce a direct rent due to the reason that they are not harvested. They grow out between two commercial crops and are not incorporated into the soil and are not grazed unlike the green manures (Ruffo and Parsons 2003, 2004; Restovich et al. 2012). The residues of CC remain on the surface, releasing nutrients contained in the aerial and radical biomass of the plants, providing energy to the microbial and mesofauna communities, and thus improving soil fertility (Ruffo and Parsons 2004; Álvarez et al. 2008). In some cases, CC are legume species that can receive N inputs through biological fixation, while other CC act by limiting the leaching of nutrients, especially N, to the underground aquifer (Parkin et al. 2006). On the other hand, CC residues inhibit weed growth, creating conditions similar to those that can be found at greater depth, i.e., less light and low daily thermal amplitude (Pérez and Scianca 2009). In addition, these residues sometimes release phytotoxic substances resulting from their degradation processes (Teasdale 1996; Teasdale et al. 2007). These characteristics of CC residues would be related to the amount of biomass they produce (Liebman and Davis 2000). The biomass produced can vary according to the species, so it is very important to consider the rate of decomposition of the residues, water contribution to the soil profile, types of crops of the sequence and the nutritional requirements of the next crop in the sequence (Carfagno et al. 2007).

The most used grasses are oats (*Avena sativa* L.), black oats (*Avena strigosa* L.), yellow oats (*Avena byzantine* L.), rye (*Secale cereale* L.), and Italian ryegrass (*Lolium multiflorum* L.), which are used as winter-rainfed crops to suppress weeds and reduce erosion in the season prior to the sowing of maize or soybean (FAO 1994; Amigone and Tomaso 2006; Restovich et al. 2012). In the Pampa region of Argentina, the grasses most commonly used are rye and oats (Pérez and Scianca 2009). Rye is widely used because it contributes large volumes of plant residues that decompose slowly, compared to other winter grasses. Once their decomposition begins, they release harmful substances such as phenols, terpenes or alkaloids, which affect the germination of weed seeds (Ruffo and Parsons 2004; Pérez and Scianca 2009; Carfagno et al. 2013). In addition, rye is considered one of the most tolerant crops to cold and water stress. Oats are used as CC for the wide availability of varieties which are adapted to different areas of the Pampa region (Ruffo and Parsons 2004) and for the production of high volumes of vegetal biomass added to the soil (Cordone and Hansen 1986). However, oat cultivars are generally not resistant to very low temperatures. For that reason, this CC is used in temperate zones (Ruffo and Parsons 2004). Although oats can grow in any types of soil, it is important that they do not have moisture retention problems, because this CC has high water consumption due to its high transpiratory coefficient (Ruffo and Parsons 2004; Infoagro 2015). Some authors consider that, in addition to rye and oats, the most commonly used species in the Pampas region are raigrass and triticale (Melo et al. 1993; Garza et al. 2007; Carfagno et al. 2013). In the case of legumes, the most adapted to this region are the *Vicia sativa*, *Vicia villosa*, and the clovers. In the north of Argentina, species of *Crotalaria*, *Vigna*, lupines, and soft clover are also used, with very promising results. These CC are sown without soil remotion, generally once the soybean has been harvested (Melo et al. 1993; Carfagno et al. 2007; Garza et al. 2007).

2.3 Use of Plant Growth-Promoting Rhizobacteria (PGPR) for Cover Crops

Furthermore, the inclusion of CC can be combined with other technological alternatives, such as the use of plant growth-promoting rhizobacteria or PGPR. These have a significant effect on agroecosystem sustainability (Antoun and Prevost 2006), since PGPR inoculation contributes to the implantation, development, biomass, and grain production of crops, such as rice, wheat, and maize (Lucy et al. 2004; Siddiqui 2006; García de Salamone 2012). However, very little information is available about its effects on forage plants that are used as CC. It is necessary to know the microbial interactions that can occur in the CC's rhizosphere under field conditions, in order to evaluate the overall impact of CC inoculation technology on this type of agroecosystem for achieving maximum efficiency. PGPR are particularly important in the soil-plant relationship and are responsible for the increase of nutrient supply as well as for the production of growth factors or phytohormones. Bacteria belonging to the genera *Azospirillum*, *Pseudomonas*, *Azotobacter*, and *Arthrobacter* and

Bacillus subtilis stand out because of their potential as PGPR biofertilizers and they have a significant impact on crop yield and quality (Glick 1995; Bashan and Holguin 1997; Dobbelaere et al. 2003; García de Salamone 2012). Studies with microorganisms of the genus *Azospirillum* and *Azotobacter* have demonstrated that these bacteria besides fixing nitrogen in nonsymbiotic associations with plants also segregate growth-promoting substances such as auxins, gibberellins, and cytokinins, which directly benefit the plant (Bashan et al. 2004; Halda-Alija 2003; Pedraza et al. 2010). The genus *Azospirillum* stands out because, besides being a supplier of phytohormones, it can fix nitrogen under microaerobiosis conditions (García de Salamone et al. 1996; Okon 1994). Higher levels of nitrogen, phosphorus, potassium, and various micronutrients in plants inoculated with *Azospirillum* has been reported (Caballero-Mellado 2004; García de Salamone et al. 1996; Pedraza et al. 2010). In addition, significant effects have been observed on the development and production of wheat (Caballero-Mellado 2004; Naiman et al. 2009; Bashan et al. 1990), maize (García de Salamone 2012) and rice (Baldani and Baldani 2005; Garcia de Salamone et al. 2010, 2012). On the other hand, there are reports which showed experiments carried out including PGPR of the genus *Pseudomonas*, which can solubilize phosphorus (P) and thus supply the soluble P to plants through several mechanisms (De Freitas et al. 1997; Rodriguez et al. 2006). In addition, some strains of *P. fluorescens* are capable of producing cytokinins (García de Salamone et al. 2001, 2006). However, the greater amount of information on the activity of *Pseudomonas* strains is associated with the indirect effects that they produce, through the control of pathogenic microorganisms (Siddiqui 2006). This can reduce the incidence of diseases through a number of mechanisms, including increases in available nutrients, production of antibiotics, and induction of siderophores as a mechanism of control of phytopathogenic agents (Dowling and O'Gara 1994). In addition, PGPR can increase crop performance and shorten their cycles, as well as reduce both the use of chemical fertilizers and in consequence the environmental pollution (Park et al. 2005).

Thus, the inoculation with PGPR, based on two microorganisms such as *Azospirillum brasilense*, which can provide nitrogen via biological fixation and promotes a greater root and vegetative development, and *Pseudomonas fluorescens* which stimulates growth because it can facilitate phosphorus solubilization and provide phytosanitary protection and cytokinin supply, could be associated with other recommended management practices to achieve high yields or collaborate to use the available resources more efficiently (García de Salamone et al. 2001, 2012; García de Salamone and Monzón de Asconegui 2008). In this sense, the biological fixation of N_2 by *A. brasilense* acquires relevance and can be incorporated through the plant-PGPR association to contribute N to the agroecosystem (García de Salamone et al. 1996; Urquiaga et al. 2004), where the soybean crop leaves a negative balance of N. This constitutes an economic and ecological alternative to increase plant production (Cassan and García de Salamone 2008). It is recognized that the use of these PGPR would bring about an improvement of sustainability, contributing to the recovery of soil fertility while preserving the environment (García de Salamone et al. 2012; Lara Mantilla et al. 2011).

On the other hand, it should be taken into account that CC should not compete with profitable crops or affect their yield. Because of that, suppression of their growth is necessary to avoid excessive consumption of water. The date of planting and the type of CC should be taken into account to manage the time of growth interruption. That moment should be prior to the maximum demand of the plants, which is flowering for both legumes and grasses (Casas 2007). The achievement of the greatest coverage and the contribution of carbon to the soil will depend on the number of days of CC growth, and this in turn is strongly determined by the environmental conditions of the site under study (Álvarez et al. 2005; Caviglia et al. 2013). Therefore, the available water and the carbon input that CC leaves in the soil for the next summer crop can be modified by managing the time of their growth interruption (Alvarez et al. 2005; Carfagno et al. 2013).

2.4 Impact of the Use of Glyphosate to Stop the Growth of CC

The time of interruption of CC growth should be adjusted to the conditions of each region to ensure the recharge of the soil profile with spring precipitation (Carfagno et al. 2008). The herbicide glyphosate (N phosphonomethylglycine, C3H8NO5P) is usually used to stop the growth or drying of CC. The molecule belongs to the class of organophosphates. It is a nonselective, broad-spectrum, postemergent herbicide that is mainly used for the removal of undesirable grasses and shrubs, in agricultural areas (Gómez et al. 2008), forests, and landscape environments (Busse et al. 2001; Nivia 2001). This herbicide exerts its action through inhibition of the 5-enolpyruvy l-shikimate-3-phosphate synthetase (EPSPS) enzyme, thus preventing plants from making three essential aromatic amino acids, namely, tryptophan, phenylalanine, and tyrosine, which are important for growth and survival of plants (Jaworski 1972; Steinrücken and Amrhein 1980; Duke et al. 2003; Gómez et al. 2008). This herbicide is absorbed by the leaves and stems and then translocated to the roots and vegetative underground organs causing the death of nonresistant plants (Villalba 2009).

Because the metabolic pathway of shikimic acid does not exist in animals, the acute toxicity of glyphosate is considered low (Levesque and Rahe 1992). However, this herbicide may interfere with some enzymatic functions in animals, but the symptoms of poisoning only occur at very high doses. Commercial products of this herbicide contain other compounds which may be highly toxic (Nivia 2001), such as different surfactants or adjuvants that serve to achieve herbicide penetration into plant tissues. Therefore, the toxicological characteristics of the market products are different from those of glyphosate (Cox 2004). Many authors emphasize the need to study the toxic effects of the glyphosate blend plus the surfactant or adjuvant used in the field rather than studying only the individual components (Monosson 2005; Cox and Surgan 2006; Mesnage et al. 2010). Several studies have reported the emergence of resistant weeds (Mueller et al. 2003; Papa 2009; Villalba 2009; Papa et al. 2012; Papa and Tuesca 2014) and a higher incidence of diseases (Levesque et al.

1993; Johal and Huber 2009). Despite the information on glyphosate and its commercial formulations, their use has been intensified due to the good results that have been obtained after application. However, the available information on the long-term effect of continuous herbicide use is scarce (Gómez et al. 2008).

On the other hand, while it is stated that glyphosate has a very short half-life, it can be maintained in the environment for long periods, mainly because it adheres to soil minerals and sediments (Andréa et al. 2003). Some authors have pointed out that it has a moderate persistence in the soil, approximately 47 days (Tejada 2009). However, this cannot be generalized since other authors have pointed out that glyphosate can be very mobile in soil and slowly degraded (Piccolo et al. 1994). In this regard, it has been noted that when this herbicide is bound to other compounds, it cannot be degraded. Moreover, when it binds to soil minerals, it can be released and dispersed again after long periods of time after application (Pessagno and dos Santos Afonso 2006). Thus, the availability of glyphosate depends mainly on two factors: the rate of degradation by soil microorganisms and the degree of adsorption to soil particles that immobilize and temporarily inactivate it (Zabaloy and Gómez 2005; Zabaloy et al. 2008). Once the glyphosate begins to be degraded by the microorganisms, carbonated and phosphatized components are released to the soil, which can also be used by soil microorganisms (Shushkova et al. 2009). Thus, glyphosate can affect the functioning of the terrestrial ecosystem, which depends heavily on soil microbial activity (Paul and Clark 1996; Doran and Zeiss 2000). This is because microorganisms actively participate in the degradation of organic matter and consequently in all biogeochemical cycles (Schlesinger 1997; León et al. 2008).

Glyphosate can affect microbial activity (Tejada 2009) by the mentioned intervention in the metabolic cycle of shikimic acid that is present in the majority of the microorganisms (Jaworski 1972; Bode et al. 1986; Bentley 1990). This herbicide may be considered to interfere with the decomposition of organic matter (Abdel-Maller et al. 1994; Alef and Nannipieri 1995), and thus the nutrients would be retained and their availability to plants would be reduced (De Baets et al. 2011). In addition, the soil physical characteristics would be affected, as it would reduce the release of microbial products, which participate in particle aggregation and in consequence in soil structure (Paul and Clark 1996). Therefore, the potential degradation of glyphosate depends on the ability of the microorganisms to adapt to the new environmental conditions, and this needs to be analyzed in detail for each system under study (Zucchi et al. 2003). However, most of the trials to evaluate the effects of glyphosate on soil microbial communities have been carried out under controlled conditions and not over the rhizosphere under field conditions. Therefore, in the case of CC in succession with soybean, it was necessary to carry out field studies to know the possible effects that would be producing on the native microbial communities, growth interruption or drying of the CC with glyphosate at doses used year after year by farmers. This is because no studies were found in relations with the influence of this herbicide on the microbial communities associated to CC. For this purpose, we performed a series of field experiments in the west of Buenos Aires Province, Argentina, to study the effect of CC dried with glyphosate in sequence in soybean crops. Thus, we could observe that the amount of the glyphosate degrader

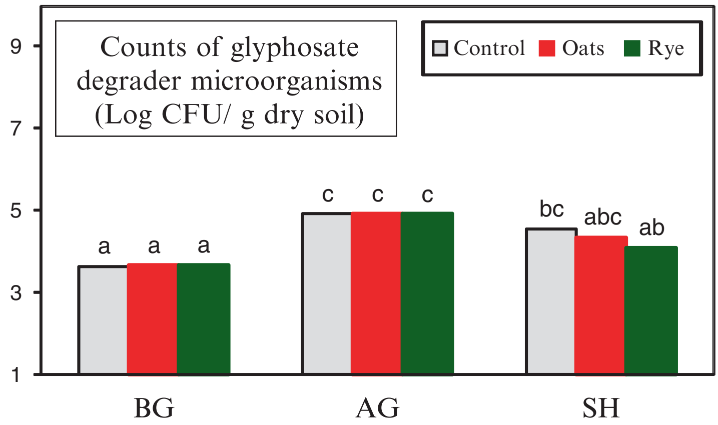

Fig. 2.1 Counts of glyphosate degrader microorganisms in the rhizosphere for the interaction between three coverage treatments and three sampling times in average of two drying times. *BG* Before glyphosate application, *AG* a month later glyphosate application, *SH* Soybean harvest, *Log CFU* Logarithm of colony forming units. *Bars* with different letters indicated differences between coverage treatments by Tukey's test ($p \leq 0{,}05$)

microorganisms increased a month after glyphosate application and their numbers stayed higher at the end of the agricultural cycle at soybean harvest (Fig. 2.1). We also observed that the number of fungi in the rhizosphere of three coverage treatments did not change due to glyphosate application but at soybean harvest time the amounts of fungi decreased significantly but the rhizosphere of oats showed the highest numbers of this type of soil microorganisms (Fig. 2.2). It was depicted that this herbicide increased the presence of certain species of fungi and decreased others. In addition, some authors observed that this herbicide decreased respiration and decomposition rates of organic matter (Abdel-Maller et al. 1994). The influence of glyphosate on the arbuscular mycorrhizal fungus *Glomus intraradices* in carrot roots (Wan et al. 1998) and transgenic soybean (Powell et al. 2009) was also identified under and showed to be contradictory in controlled condition experiments. This effect should be analyzed under conditions that allow evaluating its ecological relevance, since most plants grow poorly without this symbiotic relationship and there is evidence that could be affected by fumigations with glyphosate, but there is no information about what happens at field conditions. At this regard, we did not observe glyphosate effects on native *Mycorrhiza* However, we did observe that oats had higher percentages of root fungal colonization, arbúsculos and vesicles than rye, but there were no effects of fertilization and inoculation on native *Mycorrhiza* of these crops (Table 2.1). This demonstrates that certain management decisions imposed to the systems provoked significant changes in certain microbial communities with their particularities.

On the other hand, in plants of transgenic soybean with resistance to glyphosate, it was found that the bacterium *Bradyrhizobium japonicum*, which fixes nitrogen in the roots of this plant, possesses a glyphosate sensitive enzyme and that when it is

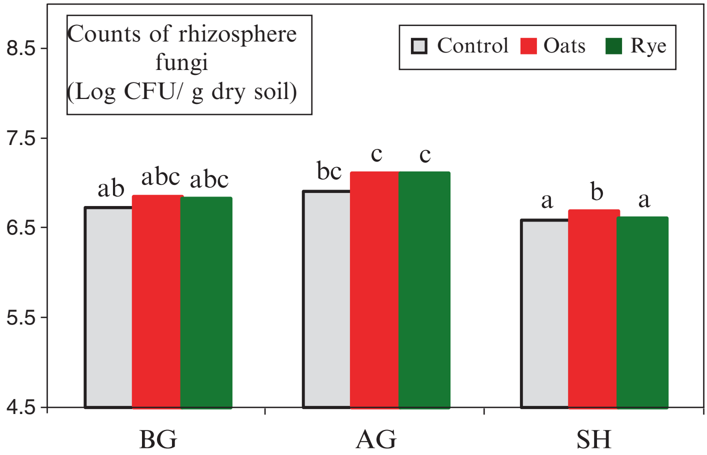

Fig. 2.2 Counts of rhizosphere fungi for the interaction between sampling times after glyphosate application and coverage treatments *BG* before glyphosate application, *AG* a month later glyphosate application, *SH* Soybean harvest, *Log CFU* Logarithm of colony forming units. *Bars* with different letters indicated differences between coverage treatments by Tukey's test ($p \leq 0{,}05$)

Table 2.1 Fungal colonization and structures of native arbuscular mycorhiza of oats and rye at tillering stage growing at field conditions

Cover crops	Root fungal colonization (%)	Arbuscules (%)	Vesícles (%)	Spores (%)
Oats	50.9 b	34.4 b	14.8 B	8.7 A
Rye	33.2 a	19.3 a	6.8 A	5.3 A

Different lower and capital letters indicate significant differences by Tukey's test ($P \leq 0.05$) and Kruskal Wallis's test ($P \leq 0.05$), respectively, for each variable

exposed to this herbicide accumulates shikimic acid and hydroxybenzoic acids, which cause inhibition of growth and even death of the bacteria when high concentrations of the acids mentioned are present. It was also found that glyphosate accumulates in nodules of soybean roots (Zablotowicz and Reddy 2004). In this regard, in the sequence of field experiments that we performed to study the effect of cover crops dried with glyphosate, it could be detected that the amount of rhizosphere native nitrogen fixers associated with both oats and rye were decreased after glyphosate application (Fig. 2.3). It can be assumed that glyphosate can affect the growth of all leguminous plants and overall soil health, as this herbicide would be affecting the nitrogen cycle in the agroecosystem. It was also possible to determine, in contrast to some short-term research reports, effects on soil microorganisms that depended on the concentration of glyphosate used. Roslycky (1982) conducted an experiment under controlled conditions where the soil was mixed with different concentrations of glyphosate and sampled during the 214 days later. Thus, concentrations of 1, 10, 50, and 100 μg g^{-1} of glyphosate soil had no effect on bacterial, fungal, and actinomycete populations, whereas concentrations of 500 and 1000 μg g^{-1} of soil of this herbicide increased initially the number of bacteria, fungi, and

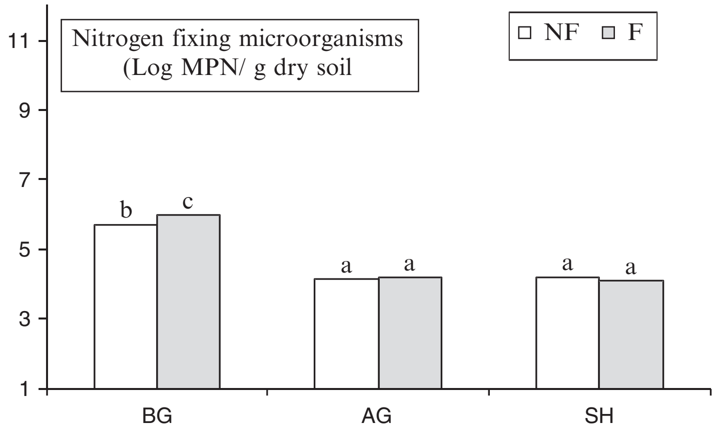

Fig. 2.3 Counts of Most Probable Number (MPN) of nitrogen fixers microorganisms associated with oats and rye for the interaction between sampling times with respect to the glyphosate application and urea application *BG* Before glyphosate application, *AG* a month later glyphosate application, *SH* Soybean harvest, *NF* Non Fertilized, *F* Fertilized. *Bars* with different letters indicated differences between fertilization treatments by Tukey's test ($p \leq 0{,}05$)

actinomycetes, followed by a decrease and then an increase but not as marked as initially observed. Other authors also observed under controlled conditions increases in respiration and enzymatic activity of the soil as a consequence of the application of different concentrations of glyphosate (Gianfreda et al. 1995; Haney et al. 2000). In the field experiments performed by our group, interesting information was obtained in relation with the rhizosphere community of actinomycetes because this taxonomic group has shown to have high sensitivity to glyphosate application (Fig. 2.4). The interaction between the phenological stage of CC when glyphosate was applied to drying them, sampling time and CC displayed differences between both tillering and jointing stages. In the latter, no effects due to the coverage treatments were observed, but in the former, it was possible to detect significant differences among treatments and sampling time. This microbial community is highly sensitive in the case of the control without CC, and it could be differentiated to oat and rye rhizospheres. Thus, actinomycetes associated with rye were less affected than those in oats' rhizosphere by glyphosate application.

On the other hand, there is very little information about the influence of management practices on the structure and functioning of the microorganisms, due to the inoculation of CC with PGPR such as *A. brasilense* and *P. fluorescens*. Therefore, in attending this need, we have studied the effects of the CC oats and rye and their inoculation on rhizosphere microbial communities at field conditions (Fig. 2.5). We observed that there were not effect of the CC but the application of glyphosate produced a permanent impact on the community level physiological profiles analysis using the technique described by Di Salvo and García de Salamone (2012) because there were significant differences between the PC of microbial communities of the sampling time before glyphosate application at jointing phenological stage of the

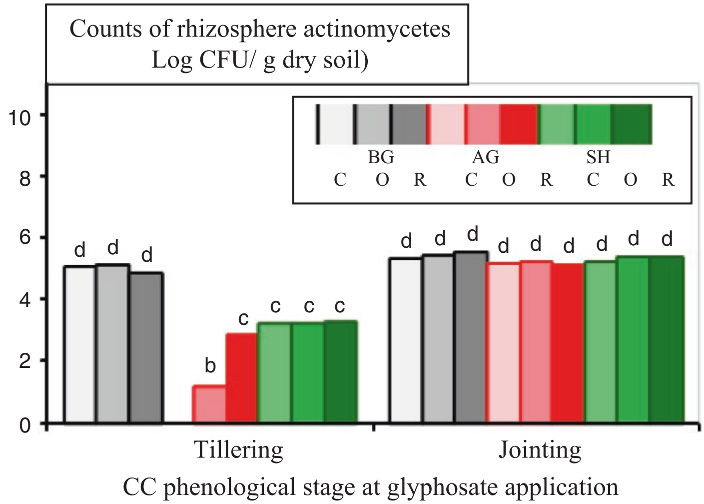

Fig. 2.4 Counts of rhizosphere actinomycetes for the interaction between phenological stages of glyphosate application at Tillering and Jointing, sampling times *BG* before glyphosate application, *AG* a month later glyphosate application, *SH* Soybean harvest and coverage treatments C control without CC, *O* oats, *R* rye, *Log CFU* Logarithm of colony forming units. *Bars* with different letters indicated differences among means performed with Tukey's test ($p \leq 0.05$)

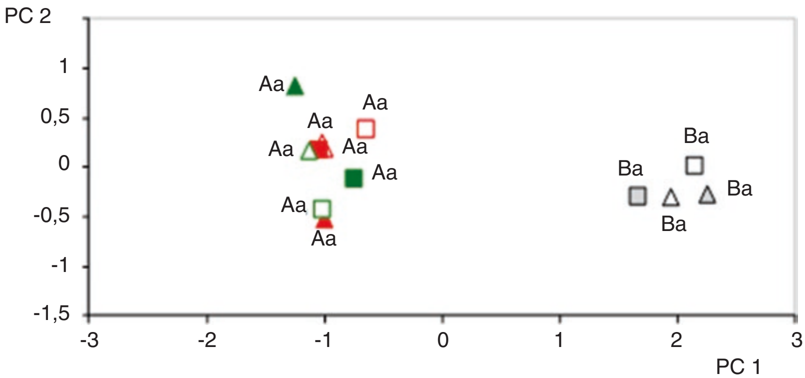

Fig. 2.5 Principal components (PC) multivariate analysis of the rhizosphere microbial communities for the interaction between sampling time, inoculation and two cover crops, rye (*triángules*) and oat (*squares*) grown at field conditions. Sampling times are before glyphosate application (*grey*) at jointing phenological stage, *red*: a month later glyphosate application and *green*: at soybean harvest grown after cover crops. Empty and full symbols are without and with PGPR inoculation on the seeds. *Capital letters* indicate significant differences for PC 1 and lower letters indicate significant differences for PC 2 obtained through mean comparison with Tukey's test ($p \leq 0{,}05$). *Numbers in parenthesis* indicate the percentages of the explained variance by each PC

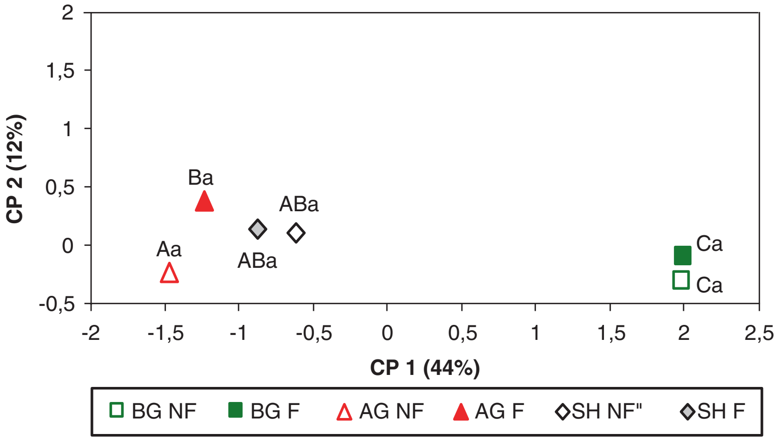

Fig. 2.6 Principal components (PC) multivariate analysis of the rhizosphere microbial communities for the interaction between sampling time and fertilization. *BG* Before application of Glyphosate, *AG* After Application of Glyphosate, *SH* At Soybean Harvest, *NF* Non Fertilized, *F* Fertilized. *Capital letters* indicate significant differences for PC 1 and lower letters indicate significant differences for PC 2 obtained through mean comparison with Tukey's test ($p \leq 0{,}05$). *Numbers in parenthesis* indicate the percentages of the explained variance by each PC

CC with respect to the other two sampling time whose physiological profiles were not different. It was also observed that fertilizer addition at sowing of CC had impact of the physiological profiles of the microbial communities a month later of the application of glyphosate (Fig. 2.6).

2.5 Conclusion

In relation to what has been expressed so far, we observed significant changes produced by the management of the CC oats and rye on the dynamics of their rhizosphere microbial communities. It has generated information capable of connecting processes that occur in the aerial portion of the system with processes taking place in the underground portion. This has been described as one of the challenges of agroecological research (Wardle 2002).

Acknowledgments The works reported in this chapter had been partially supported by the following grants coordinated by IEGS, PICT1864-FONCYT 2008 from the MINCyT, UBACyT projects 20020090100255 and 20020130100716 of the Universidad de Buenos Aires (UBA) in Argentina. JSEO had a scholarship of the National Council of Scientific and Technical Research.

We would like to dedicate this work in memory of Dr. Katia RS Teixeira, Brazilian researcher of the EMBRAPA, Rio de Janeiro, Brazil, who always will be in our hearts.

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