European Journal of Soil Biology 76 (2016) 74–82

Contents lists available at ScienceDirect

European Journal of Soil Biology

journal homepage: http://www.elsevier.com/locate/ejsobi

Original article

Effect of cover crops on microbial community structure and related enzyme activities and macronutrient availability

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ARTICLE INFO

Article history:
Received 23 November 2015
Received in revised form
28 June 2016
Accepted 14 July 2016

Handling Editor: C.C. Tebbe

Keywords:
Microbiota
Diversification
Sustainability
Microbial interactions
Soil microbiology

ABSTRACT

Continuous monoculture in major agricultural regions has been characterized by the loss of fertility, increased soil erosion and surface runoff. Thus, the inclusion of cover crops (CC) is a promising option aimed at better preserving the sustainable production of agricultural systems. The objective of this study was to assess the short-term effect of CC on soil microbial community structure and related enzyme activities and macronutrient availability. Species tested as CC were: oat (*Avena sativa* L.), vetch (*Vicia sativa* L.) and radish (*Raphanus sativus* L.), which were sown in two species mixtures, oat/radish (CC1) and oat/radish/vetch (CC2), with soybean monoculture and soybean/corn being the cash crops. The field trial was performed under no-tillage, and soil sampling was carried out in 2013 and 2014. The analysis of soil phospholipid fatty acid (PLFA) showed an increase of total bacterial PLFA and Gram-positive PLFA under CC treatments, being on average 6.8% significantly higher in CC2 and CC1 than in the control treatment. In addition, soil enzyme activities (esterase activity, dehydrogenase activity and acid phosphatase activity) were on average 20% higher in plots under CC in comparison to control treatments. The total N was significantly higher after CC2 treatment (3.13 mg g^{-1}) than in either the CC1 (2.00 mg g^{-1}) or control (2.19 mg g^{-1}). This research revealed that the inclusion of CC mixtures in crop rotation produced an increase in bacterial PLFA, in particular Gram-positive bacteria, in the short term. These changes were related to soil enzyme activities and to the availability of the main macronutrients N and P, with a CC mixture including oat/radish/vetch being highly recommended not only to improve soil biological processes but also to provide additional N to the cash crop.

1. Introduction

Currently, there is a growing concern about the large areas of monoculture in major agricultural regions, since these systems are characterized by loss of fertility, increased soil erosion and surface runoff [1]. Furthermore, monoculture-based agro-ecosystems show little soil coverage, low aggregate stability and subsequent compaction and poor water infiltration [2]. Soybean crop, which is one of the main crops in the humid pampas of Argentina due to its high profitability, is often repeatedly grown in monoculture rather than being rotated with other crops. Continuous soybean monoculture results in yield decline and quality deterioration [3], as well as degradation of soil quality, through loss of soil nutrients, autotoxicity of root exudates and changes in microbial community [4,5]. Therefore, in order to maintain high soybean productivity levels, it is necessary to include agricultural practices that preserve the richness of soil microbial communities, with the final aim of improving crop yield and conserving the sustainability of the system [6].

Abbreviations: CC, cover crop; CC1, oat/radish; CC2, oat/radish/vetch; PLFA, phospholipid fatty acid; FDA, fluorescein diacetate; DHA, dehydrogenase activity; MBC, microbial biomass C; TN, total N.

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http://dx.doi.org/10.1016/j.ejsobi.2016.07.002
1164-5563/

The inclusion of cover crops (CC) is a possible alternative cropping tool in sustainable agricultural production. In contrast to crop monoculture, the use of CC can result in an important supply of plant nutrients, and therefore stimulate soil microbiological activity [7]. In fact, microorganisms are key players in important ecological processes, with soil microorganisms being responsible for regulating the main processes occurring in the soil, such as nutrient transformation, plant growth promotion, decomposition of organic material and various interactions with physical soil processes [8]. Accordingly, microbial parameters have been used as indicators of changes in soil quality under different soil management practices, as in the inclusion of CC [9]. Furthermore, compared with soil physicochemical properties, microbial properties appear to be more sensitive, especially in the early evaluation of management and land use change [10]. However, the specific changes in soil community structure produced by CC mixture inclusion in extensive agricultural systems and how those changes are associated with soil enzymatic activity are two aspects that still remain unclear.

Implementing alternative management practices stimulates the development of diverse microbial community, which can support suitable soil enzyme activities [11–13]. Thus, the study of the relationships between microbial community composition and microbial activity may be fundamental to be able to explain the soil biological processes that regulate plant growth. Accordingly, the ratio between enzyme activity and microbial biomarkers has been used to indicate the relative contribution of stabilized enzyme activity and enzyme activity associated with microorganisms [14,15]. In fact, this ratio represents a combination of two different measurements in a single criterion, which can give some indications of any changes occurring in microbiological activity. Since soil functions are the result of microbial communities that closely interact with one another [16], it is necessary to understand how the composition of microbial communities varies with the inclusion of different species of CC in agricultural rotation systems.

Agronomic and environmental consequences associated with monoculture also influence soil macronutrients processes, such as N losses [17]. In contrast, CC are known to improve the agro-system services by increasing soil organic matter and improving nutrient retention capacity of agricultural soils [18]. The use of CC plays an important role in maintaining cash crop productivity by recycling N from legumes and increasing the amount of C returned to the soil [19]. Since there is recent evidence that diversifying monoculture systems impact positively on the provision of main macronutrients [20,21], it is important to analyze the relationships between the effects of agricultural diversification on soil microbial communities and macronutrient supply.

We hypothesize that CC inclusion generates changes in specific groups of soil microorganisms and leads to an increase in the microbial biomass. Subsequently, these effects generate an increase in soil enzyme activity, mainly due to a rise in the enzyme activities associated with active microbial cells, which as a consequence, stimulates N and P availability. The aims of this research were to 1) assess the short-term effect of the inclusion of CC mixtures on microbial community structure in agro-systems under soybean monoculture and soybean/corn rotation, 2) evaluate the ratio between fungal and bacterial PLFA biomarkers and soil enzyme activity; and 3) assess the impact of the inclusion of CC mixtures on the two principal soil macronutrients: N and P.

2. Materials and methodology

2.1. Field experiment characteristics

This study was conducted at the Pergamino Experimental Station of the Instituto Nacional de Tecnología Agropecuaria (INTA) (33°51′S, 60°40′W), Buenos Aires province, Argentina. The predominant soil in the area is Luvic Phaeozem (World Reference Base – FAO classification system). The climate is temperate humid, without a dry season, with a mean annual temperature of 16.5 °C [22] and mean annual rainfall of 971 mm for the 1910–2010 period (Agroclimatological network database, INTA). Rainfall and drainage occur mainly in fall and spring, with the summer months being characterized by rainfall deficits of varying intensity [22]. The field trial was performed under no-tillage and started in March 2011, and soil sampling was carried out in 2013 and 2014. The experimental design consisted of a split plot design in a randomized complete block arrangement. The main plot (30 m long × 15 m wide) corresponded to the cash crop sequence (soybean monoculture and soybean/corn rotation) and was divided into three subplots (30 m long × 5 m wide), corresponding to the CC treatments (covering) and the control. The species used as CC were: oat (*Avena sativa* L.), vetch (*Vicia sativa* L.) and radish (*Raphanus sativus* L.), which were sown in two mixtures of species: oat/radish (CC1) and oat/radish/vetch (CC2), with a control treatment without CC. The experiment consisted of six treatments with three replicates each, in three blocks, totaling 18 subplots and 6 main plots. The treatments were: a) soybean/soybean CC1; b) soybean/soybean CC2; c) soybean/soybean control; d) soybean/corn CC1; e) soybean/corn CC2; and f) soybean/corn control.

Soybean (*Glycine max* L.) hybrid DM5.1 (Don Mario) was sown at a 0.52 m-row spacing and a density of 500,000 plants ha^{-1}; and corn (*Zea mays* L.) hybrid DK 747 (Monsanto) was sown in rows spaced 0.70 m apart (75,000 plants ha^{-1}), with both crops being sown by direct drilling. Corn was fertilized at seeding with calcium superphosphate (150 kg ha^{-1}) and between V5-6 with 32 kg N ha^{-1}. CC were sown under no-tillage immediately after harvest of the cash crop (soybean or corn) and fertilized at seeding with 14.7 kg of P_2O_5 ha^{-1}, with vetch being inoculated with *Rhizobium leguminosarum* biovar *Viceae*, immediately before sowing. The soybean and corn sowing dates and the distribution of rainfall determined the end of the CC growing periods. Before planting corn, CC were dried in winter or early spring (August–September) during their vegetative stage, and when soybean preceded, they were dried in spring (October) at the reproductive stage. CC were killed with 3–4 l ha^{-1} of glyphosate (48% active ingredient).

2.2. Soil sampling

Soil sampling was performed at cash crop harvest in March for two crop cycles in 2013 and 2014, and the sampling methodology was carried out according to Restovich et al. [23]. Six composite soil samples were taken per plot from horizon A, at a depth of 10 cm, from six sampling stations. Samples were passed through a 2-mm sieve and stored at 4 °C until analysis. A subsample of 20 g from each sample was stored at −20 °C until PLFA analysis.

2.3. Phospholipid fatty acid analysis

PLFA analysis was conducted according to the process described by Meriles et al. [24], based on Zelles [16]. Soil samples (8 g soil) were extracted overnight with 40 ml of a one-phase buffer containing chloroform, methanol, and phosphate buffer at a ratio of 1:2:0.8 (8.7 g K_2HPO_4 l^{-1}, pH 7.4). The total lipid extract was fractionated into neutral lipids, glycolipids, and polar lipids in a silicic acid column. The polar lipid fraction containing the phospholipids was isolated and transesterified into fatty acid methyl esters (FAMEs) using a mild acid methanolysis reaction. FAMEs were analyzed by capillary gas chromatography with flame ionization

detection on a PerkinElmer (Clarus 500 GC) using a 30-m nonpolar column (Col-Elite-5), with both the injector and detector being maintained at 290 °C. The column temperature was programmed with an initial temperature of 180 °C for 4 min and then ramped up at a rate of 4 °C a minute to a final temperature of 280 °C. The separated FAMEs were identified by chromatography retention time, using a standard bacterial acid methyl ester mix (Supelco, Supelco UK, Poole, Dorset, UK). Methyl-nonadecanoate (C19:0) was used as an internal standard for quantification of the PLFA. For each sample, the abundance of individual PLFA was expressed as nmol%, and the PLFA peak areas were combined into biomarker groups (Table 1).

2.4. Enzyme activities

Total microbial activity was estimated by analyzing the esterase activity measured by fluorescein diacetate (FDA) hydrolysis, according to Adam and Duncan [25]. Briefly, 2 g of soil and 15 ml of 60 mM potassium phosphate buffer pH 7.6 were placed in a 50-ml conical flask and substrate (FDA, 1.000 μg ml^{-1}) was added to start the reaction. The flasks were placed in an orbital incubator at 30 °C and 100 rpm for 20 min. The reaction was terminated by adding 15 ml of chloroform/methanol (2:1 v/v) to the flask immediately after removal from the incubator. The contents of the conical flasks were then centrifuged at 2.000 rpm for 5 min and the supernatant was measured at 490 nm on a spectrophotometer.

The procedure for measuring dehydrogenase activity (DHA) followed García et al. [26]. Soil (1 g) was exposed to 0.2 ml of 0.4% INT (2-p iodophenyl-3-pnitrophenyl-5-phenyltetrazolium chloride) in distilled water at 22 °C in darkness for 24 h. The INTF (iodonitrotetrazolium formazan) formed was extracted with 10 ml of methanol by shaking vigorously for 1 min, and the INTF was measured spectrophotometrically at 490 nm.

Acid phosphatase activity was determined according to Tabatabai and Bremner [27]. Soil (1 g) and a buffered solution of *p*-nitrophenyl phosphate were placed in a 50-ml conical flask and incubated at 37 °C for 1 h. Before filtration of the solution, 1 ml of $CaCl_2$ and 4 ml of NaOH (both 0.5 M) were added, and the content of *p*-nitrophenyl was measured by a spectrophotometer at 490 nm.

2.5. Microbial biomass C (MBC)

An aliquot of moistened soil, previously incubated for 15 h at 30 °C, was fumigated with chloroform as a biocide and then extracted with 0.5 M K_2SO_4 [28]. The solution was centrifuged, filtered and an extract of 4 ml was added to 1 ml 0.06 M of $K_2Cr_2O_7$ and 4 ml H_2SO_4. This solution was digested using a digester plate at 140 °C for 30 min, and absorbance was measured at 590 nm on a spectrophotometer. MBC was estimated by calculating the difference between fumigated and non-fumigated samples, using a correction factor (kc: 0.35).

2.6. Total N and extractable P

The content of total N (TN) and extractable P (TP) of soil samples was determined by an auto-analyzer nutrient SmartChem 200 (Westco, Scientific Instruments, Inc.). For TN, the method is based on the conversion of organic N compounds of biological origin, such as amino acids, proteins, peptides and ammonium through an acid digestion process. The application range for this technique is 0.10–5.00 mg/l TN, and the method is based on USEPA 351.2, .Rev. 2.0 [29]. The procedure for TP consists of the conversion of P compounds into inorganic P by an acid digestion process. The application range for this technique is 0.01–20.00 mg P/l, and the method is based on USEPA 365.4 [30]. For both acid digestions, 134 g of potassium sulfate (K_2SO_4) and 7.3 g of copper sulfate pentahydrate III ($CuSO_4 \cdot 5H2O$) were dissolved in 800 ml of water and 134 ml of concentrated sulfuric acid (H_2SO_4). An aliquot of this solution (10 ml) was added per g of soil sample.

2.7. Statistical analyses

The studied parameters were analyzed using linear mixed models of InfoStat-Professional [31]. Data obtained from both years were pooled in order to study the effect of the treatments on the soil microbial variables. The fixed effects were covering (CC1, CC2 and control), sequence (soybean/soybean and soybean/corn) and their interaction (covering × sequence), with year, block and sequence being used as random effects. All assumptions required for the analysis of variance (ANOVA) were verified. In all cases, residuals were tested for normality using the Shapiro-Wilks' test. When confirming a statistically significant P value, the Fisher test ($P < 0.05$) was used for comparison. For the PLFA analysis, a principal component analysis (PCA) and a cluster analysis based on Euclidean distance were performed to characterize the composition of the microbial communities. In addition, correlations between enzyme activities and MBC, TN and extractable P were estimated using Pearson's coefficient with $p \leq 0.05$ and $p \leq 0.01$.

3. Results

3.1. Microbial community structure

The microbial community structure estimated by PLFA profiles is shown in Table 2, where it can be observed that the total bacterial and Gram-positive bacterial PLFA were significantly higher with the inclusion of CC than in control treatments. A PCA was performed using the PLFA data (Fig. 1, A) and the first two principal components, PC1 and PC2, accounted for 55.2% and 23.7% of the total variation, respectively. In the soybean monoculture plots, a clear separation between control treatment and CC treatments was observed along PC1. For the soybean/corn rotation plots, the control treatment was separated from the CC1 and CC2 treatments along PC1. The greatest variability in this analysis was explained by the bacterial PLFA biomarkers (Total bacteria, Gram-positive, Gram-negative), with these results being confirmed by the cluster analysis (Fig. 1, B). The dendrogram of the PLFA profiles revealed that control treatments of both soybean monoculture and soybean/corn rotation plots were linked together, forming a cluster separated from the remaining treatments. The CC treatments corresponding to soybean/corn rotation were separated from soybean monoculture CC treatments, with both clusters being linked together at a

Table 1
Fatty acid biomarker groups.

Biomarker	Fatty acids
Fungi	C18:2ω6,9
Actinomycetes	10-methyl 16:0; 10-methyl 18:0
Gram-positive bacteria	Iso- and ante-iso branched fatty acids: i15:0, a15:0, i16:0, i17:0, a17:0
Gram-negative bacteria	Monoenoic and cyclopropane fatty acids 16:1ω9, 16:1ω11, cy17:0, 18:1ω9c, 18:1ω9t, cy19:0

high Euclidean distance.

3.2. Enzyme activities

A significant effect of CC inclusion was recorded for enzyme activities (Table 3). The FDA hydrolysis was significantly higher under the inclusion of CC, with CC2 and CC1 treatments being 20.4 and 14.6% higher than the control, respectively. The same trend was observed for DHA, with both CC2 and CC1 showing increases of 27.2 and 21%, respectively, in comparison with the control treatment. Acid phosphatase values revealed the same behaviour, with CC2 and CC1 treatments being 21.9 and 12.1%, respectively, higher than the control.

3.3. Ratios of enzyme activity to bacterial and fungal PLFA

The ratio of enzyme activity to bacteria PLFA (Fig. 2) was significantly increased by the CC2 treatment in particular, which was 14.2, 19.2 and 15.3% higher than control treatment for FDA hydrolysis, DHA and acid phosphatase, respectively. The ratio of enzyme activity to fungal PLFA was higher for both CC treatments, being on average 47.5, 59.0 and 50.0% higher than that of the control treatment for FDA hydrolysis, DHA and acid phosphatase, respectively.

3.4. Microbial biomass C

The results revealed an evident rise in MBC content after the inclusion of the CC treatments (Fig. 3), which was 35.0 and 19.3% significantly higher than the control for CC1 and CC2, respectively. The results of the correlation analysis between MBC and enzyme activities are shown in Table 5. According to the Pearson coefficients, FDA hydrolysis (0.29), DHA (0.16) and acid phosphatase (0.16) had a significant ($p < 0.01$) and positive correlation with MBC.

3.5. Total N and extractable P

A significant effect of CC inclusion was recorded for TN (Table 4), with the TN value increasing in response to the inclusion of CC2, which was 56.5% and 42.9% higher than CC1 and the control, respectively. The correlation analysis (Table 5) showed a significant ($p < 0.05$) and positive correlation between TN and FDA hydrolysis (0.29) and DHA (0.31). A significant ($p < 0.01$) and positive correlation was also found between acid phosphatase (0.57) and extractable P, which was negatively correlated ($p < 0.01$) with FDA hydrolysis (−0.46).

Soil organic C and pH showed higher values in response to the inclusion of CC (Table 4). Soil organic C was 8.8% and 7.9% higher in CC2 and CC1 than in control treatments, respectively. Values of pH followed the same trend, with Soy/Soy-CC2 treatment showing the highest value and both Soy/Soy-control and Soy/Corn-control treatments showing the lowest pH.

4. Discussion

4.1. Changes in microbial community composition

Different plant species included in agricultural rotation can release specific compounds in the rhizosphere [32], which can lead to changes in the composition of microbial communities associated with the type of crop employed. Root exudates include low-molecular weight compounds such as amino acids, organic acids, sugars and other secondary metabolites and high-molecular weight compounds, such as mucilage (polysaccharides) and proteins [33]. Therefore, the contribution of nutrients to the soil generated by CC inclusion and the continuous presence of live root systems in the soil may have led to an overall increase in microbial PLFA biomarkers, which impacted positively on the soil biological processes. In particular, soil bacterial PLFA were the most increased in response to CC inclusion, with the CC mixture including vetch (CC2) showing the highest concentration of PLFA. Moreover, independently of the CC species employed, the use of CC was demonstrated to be a useful tool for increasing microbial PLFA biomarkers. This finding was reflected by the multivariate analysis (Fig. 1), which separated controls from the rest of the treatments and revealed a differentiation in PLFA composition when CC were included in the rotation.

In our investigation, bacterial PLFA biomarkers were the ones with highest increases generated by the inclusion of CC mixtures. Thus, these findings suggest that the diversification of agricultural

Table 2
Content of various biomarkers of phospholipid fatty acids (nmol PLFA g^{-1}) measured at cash crop harvest in 2013 and 2014 in soybean monoculture plots and soybean/corn rotation plots under the different cover crop (CC) treatments CC2 (oat/radish/vetch), CC1 (oat/radish) and Control; small letters within a column reflect significant differences between treatments ($P < 0.05$).

	Total PLFAs	Total bacteria	Total fungi	Gram+	Gram−	Actino*
Sequence						
Soy/Corn	49.49 a	25.72 a	4.18 a	16.36 a	9.36 a	5.3 a
Soy/soy	52.2 a	27.28 a	4.01 a	17.17 a	10.11 a	5.25 a
Covering						
CC2	57.38 a	27.22 a	3.87 a	16.96 a	9.97 a	5.22 a
CC1	50.82 a	26.94 a	3.78 a	17.27 a	9.95 a	4.63 a
Control	44.49 a	25.34 b	4.75 a	16.03 b	9.29 a	5.9 a
Covering*Sequence						
Soy/corn-CC2	52.09 ab	25.92 ab	4.08 a	16.21 a	9.71 a	5.29 a
Soy/CornCC1	49.61 ab	26.29 ab	3.88 a	16.88 a	9.41 a	4.76 a
Soy/Corn-Control	46.77 ab	24.95 b	4.57 a	16.01 a	8.97 a	5.83 a
Soy/Soy-CC2	62.66 a	27.95 ab	3.66 a	17.71 a	10.24 a	5.15 a
Soy/Soy-CC1	52.03 ab	28.16 a	3.68 a	17.67 a	10.48 a	4.49 a
Soy/Soy-Control	41.91 b	25.73 b	4.7 a	16.14 a	9.59 a	6.09 a
P						
Covering	0.0852	0.0434	0.1349	0.038	0.3456	0.1858
Sequence	0.5274	0.0965	0.6487	0.163	0.0928	0.9305
Covering*sequence	0.4105	0.7511	0.8296	0.5827	0.8541	0.9266

Actino, actinomycetes.
*($P < 0.05$).

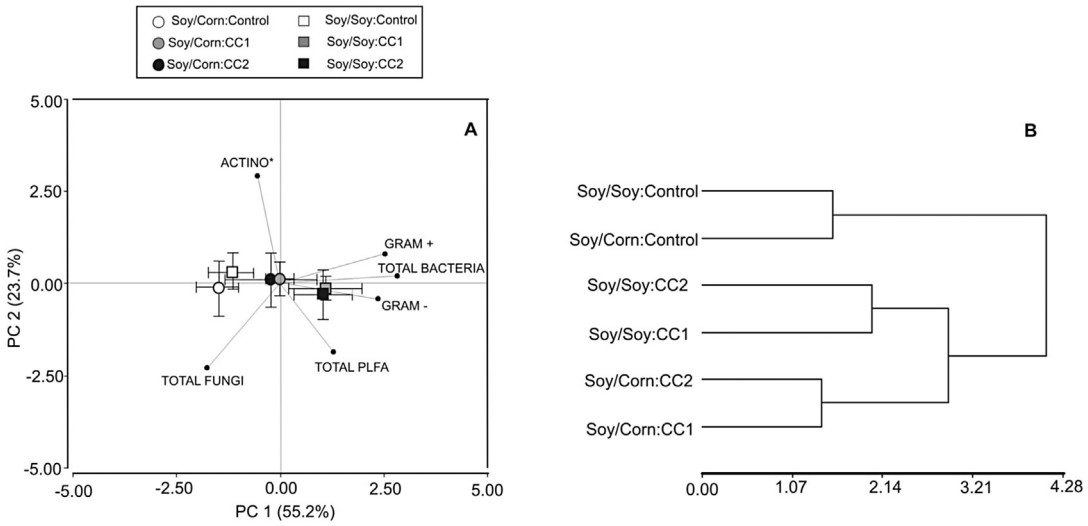

Fig. 1. Principal component analysis (PCA) (A) and cluster analysis based on Euclidean distance (B) of soil phospholipid fatty acids (PLFA), measured at cash crop harvest in the agricultural cycles 2013 and 2014 in soybean monoculture plots and soybean/corn rotation plots under the different cover crop treatments CC2 (oat/radish/vetch), CC1 (oat/radish) and Control.; error bars indicate standard deviation. Actino, actinomycetes.

Table 3
Esterase activity, dehydrogenase activity (DHA) and acid phosphatase activity measured at cash crop harvest in 2013 and 2014 in soybean monoculture and soybean/corn rotation plots under the different cover crop treatments CC2 (oat/radish/vetch), CC1 (oat/radish) and Control; small letters within a column reflect significant differences between treatments ($P \leq 0.05$).

	Esterase activity (μg FDA g^{-1} h^{-1})	DHA (μg INTF g^{-1} h^{-1})	Acid phosphatase (μg *p*-nitrophenyl g^{-1} h^{-1})
Sequence			
Soy/Corn	115.11 a	66.73 a	1205.3 b
Soy/soy	114.52 a	62.78 a	1285.92 a
Covering			
CC2	123.77 a	70.98 a	1363.87 a
CC1	117.85 a	67.50 a	1254.49 a
Control	102.83 b	55.78 b	1118.48 b
Covering*Sequence			
Soy/corn-CC2	124.27 a	67.15 ab	1336.27 a
Soy/CornCC1	117.63 a	74.57 a	1192.3 bc
Soy/Corn-Control	103.43 b	58.46 bc	1087.34 c
Soy/Soy-CC2	123.27 a	74.81 a	1391.47 a
Soy/Soy-CC1	118.07 a	60.43 bc	1316.68 ab
Soy/Soy-Control	102.22 b	53.1 c	1149.62 c
P			
Covering	<0.0001	0.0013	0.0407
Sequence	0.8844	0.547	0.4119
Covering*sequence	0.9795	0.088	0.9245

systems using CC can produce a general rise in soil bacterial PLFA, with the Gram-positive bacteria being more efficient than the remaining microorganisms in the use of the nutrient supply generated by CC plant litter and root exudates. Our results are consistent with those of Bossio et al. [34] and Vargas Gil et al. [6], who reported an increase in populations of Gram-positive bacteria in agricultural soils subjected to conservation agricultural practices. Related to this, it was previously reported that growth patterns of fungi, together with their ability to degrade recalcitrant compounds such as lignin and cellulose, demonstrate the lower nutrient requirements of fungi compared with bacteria [35,36]. Therefore, these physiological differences between fungi and bacteria and their resulting biological interactions may be the reason for the dominance of bacterial PLFA in the soil in CC treatments, at least in the short term, as observed in our research.

Our findings suggest that the effect of including corn in the rotation did not generate major changes in the composition of soil microbial communities in the short term. In contrast, Zhang et al. [12] reported significant effects of agricultural management practices on PLFA profiles in the short term, with increases in total biomass and changes in the structure of microbial communities through the employment of zero tillage and crop rotation. However, this expected effect was not evident in our research during the studied period.

4.2. Microbial biomass and its relationship with soil enzyme activities

In our study, there was an evident increase in MBC generated by CC inclusion in comparison with control treatment, which supports the results observed by PLFA analysis. These findings are probably related to the amount and diversity of crop residues and the proportion of easily decomposable organic products returned to the soil in response to CC inclusion. Our results are in agreement with Zhu et al. [37], who reported that MBC was significantly increased by CC inclusion compared to fallow in extensive agricultural

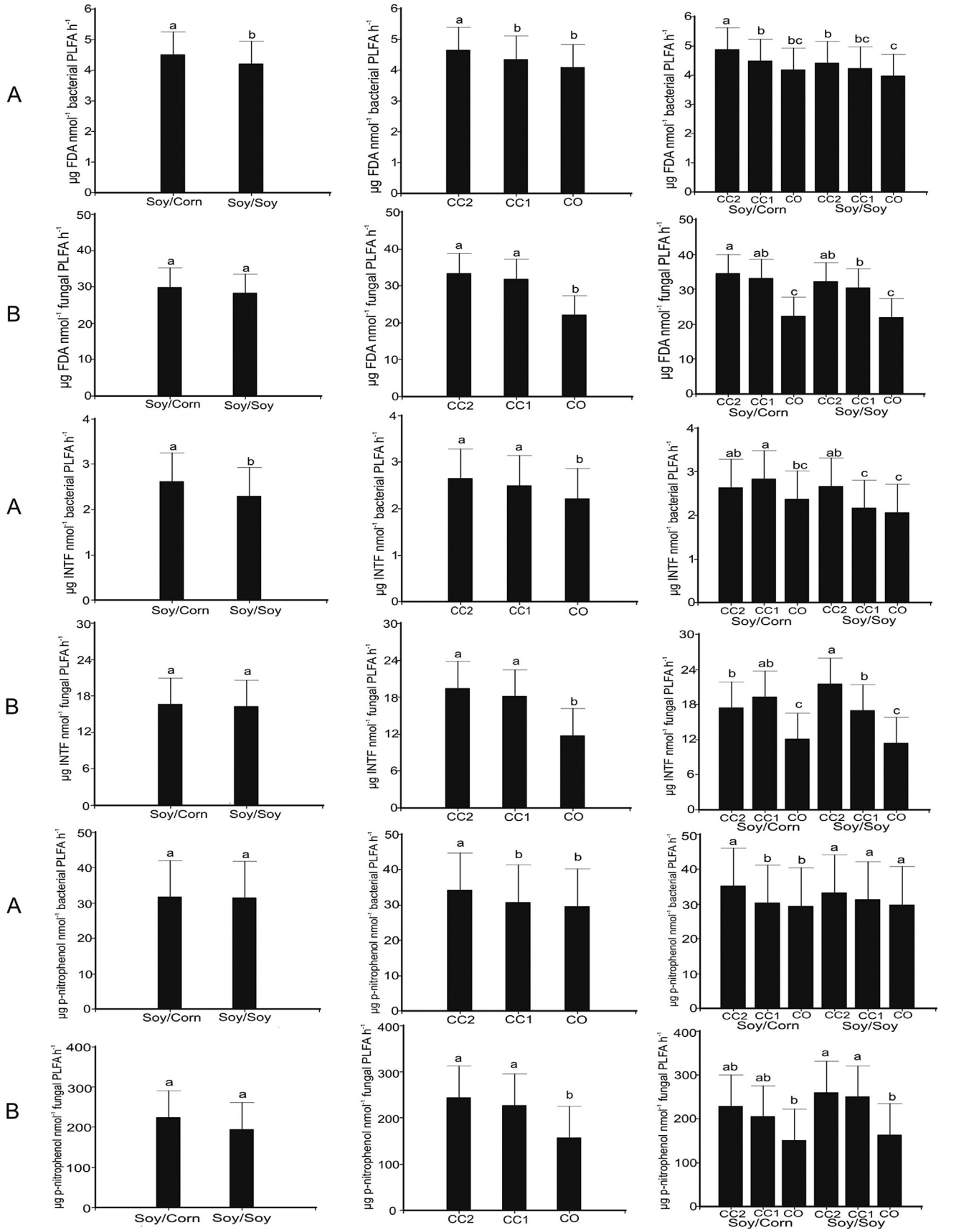

Fig. 2. Ratios of enzyme activity to bacterial (A) and fungal (B) phospholipid fatty acids (PLFA) measured at cash crop harvest in 2013 and 2014 in soybean monoculture plots and soybean/corn rotation plots under the different cover crop treatments CC2 (oat/radish/vetch), CC1 (oat/radish) and Control (CO); small letters above the columns reflect significant differences between treatments ($p < 0.05$).

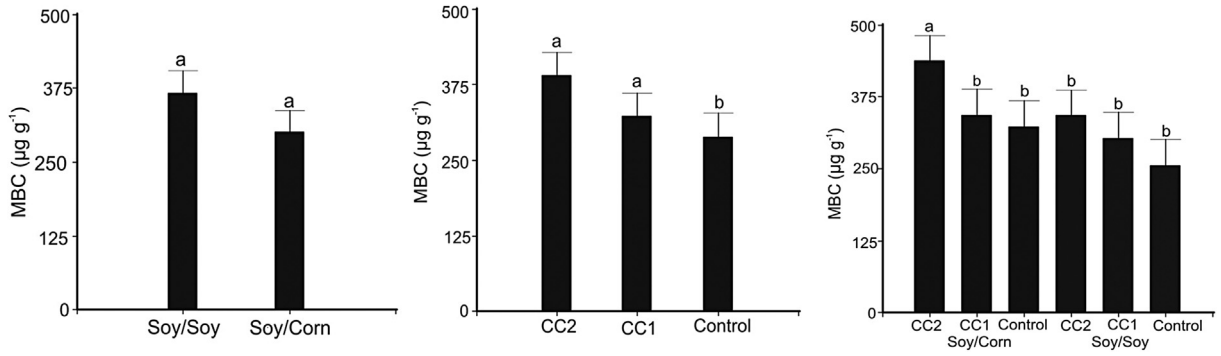

Fig. 3. Microbial biomass C (MBC) measured at cash crop harvest in the agricultural cycles 2013 and 2014 in soybean monoculture plots and soybean/corn rotation plots under the different cover crop treatments CC2 (oat/radish/vetch), CC1 (oat/radish) and Control; bars with different letters are significantly different according to the LSD test ($P \leq 0.05$).

Table 4
Total N (TN), extractable P (EP), soil organic C and pH measured at cash crop harvest in 2013 and 2014 in soybean monoculture and soybean/corn rotation plots under the different cover crop treatments CC2 (oat/radish/vetch), CC1 (oat/radish) and Control; small letters within a column reflect significant differences between treatments ($p < 0.05$).

	TN (mg N g^{-1})	EP (mg P g^{-1})	Soil organic C (mg C g^{-1})	pH
Sequence				
Soy/Corn	2.81 a	1.89 a	22.14 a	5.57 b
Soy/soy	2.07 a	1.67 a	22.52 a	5.79 a
Covering				
CC2	3.13 a	1.88 a	23.02 a	5.78 a
CC1	2.00 b	1.90 a	22.83 a	5.75 a
Control	2.19 b	1.56 a	21.15 b	5.52 b
Covering*Sequence				
Soy/corn-CC2	3.79 a	2.16 a	22.57 a	5.56 c
Soy/Corn-CC1	1.85 b	2.04 a	22.67 a	5.64 bc
Soy/Corn-Control	2.79 ab	1.47 a	21.18 b	5.52 c
Soy/Soy-CC2	2.47 ab	1.60 a	23.08 a	6.00 a
Soy/Soy-CC1	2.15 b	1.75 a	23.37 a	5.85 ab
Soy/Soy-Control	1.59 b	1.65 a	21.12 b	5.52 c
P				
Covering	0.0498	0.474	0.0018	0.012
Sequence	0.0737	0.5575	0.4883	0.0046
Covering*sequence	0.1958	0.4074	0.7771	0.0417

Table 5
Correlation analysis between enzyme activities and microbial biomass C (MBC), total N (TN) and extractable P (EP) measured at cash crop harvest in the agricultural cycles 2013 and 2014.

	Pearson coefficients		
	MBC	TN	EP
FDA hydrolysis (µg FDA g^{-1} h^{-1})	0.29**	0.29*	−0.46**
DHA (µg INTF g^{-1} h^{-1})	0.16**	0.31*	−0.24
Acid phosphatase (µg *p*-nitrophenyl g^{-1} h^{-1})	0.16**	−0.31	0.57**

*Significant at $P < 0.05$.
**Significant at $P < 0.01$.

systems. In the present investigation, we observed that drying the CC mixtures without tilling them into the soil resulted in an increased soil microbial biomass C. Thus, CC inclusion had a positive effect on soil microbial biomass C, which persisted until cash crop harvest.

MBC was found to be weakly positively correlated with the three enzyme activities analyzed in our study, which were significantly higher under the inclusion of CC mixtures. FDA hydrolysis is widely accepted as an accurate and simple method to measure total microbial activity in soils [25] whereas DHA in soil depends on the content of soluble organic C [38]. The higher values of esterase activity and DHA found in plots under CC inclusion than in controls reflect a great metabolic capacity to process organic compounds and transform them into available nutrients. In addition, the increase in acid phosphatase activity under CC inclusion suggests a greater decomposition of organic P and more P being available for cash crops than in control treatments. Since phosphatase activity plays a significant role in P bioavailability to plants from native organic P compounds, it is frequently used as an indicator of P availability in soils [39]. The increase in enzyme activities found under CC treatments is as important as the increase of microbial biomass, since the capacity of soil microbial communities to maintain their functional diversity may be more important for agro-system productivity and stability than microbial structural diversity [40]. The activity of soil enzymes of the microbial community can be used as an indicator of soil organic matter decomposition potential and nutrient availability [41]. Hence, the high content of soil enzyme activities found in this research revealed an enhanced capacity of soils under CC inclusion to process soil organic matter, which may improve nutrient availability in the long term. In agreement, Xu et al. [13] observed a strong positive relationship between soil organic matter decomposition rate and soil enzyme activities.

Higher values of the ratios between enzyme activities and bacterial and fungal PLFA, the main organic matter decomposers, were observed in CC treatments than in the control treatment. The increase in these ratios when PLFA increase or remain stable

indicates a desynchronized response of enzyme activities and bacterial and fungal PLFA [15,12]. Therefore, our finding suggests that CC inclusion promoted a higher activity of both intra- or pericellular enzymes associated with the microorganisms and also activities of extracellular enzymes stabilized by surface reactive particles or entrapped by humic substances [21]. The CC2 treatment revealed higher ratios between enzyme activities and bacterial PLFA than CC1, indicating an increase in the activities of stabilized extracellular enzymes in the case of the more diverse CC2 mixture than in the other treatments. Moreover, the presence of a leguminous crop included in the CC mixture may have enhanced the quality and quantity of root exudates released to the soil, compared with a non-leguminous crop. Likewise, Shahzad et al. [42] stated that plants strongly influence soil organic matter mineralization through their exudates, as the result of years of plant-microbe coevolution. Consequently, the use of a diverse CC mixture including a legume may have also enhanced microbial activity in our study, with beneficial effects on soil enzyme activity and nutrient cycling. These results demonstrate the control exerted by different CC mixtures on soil microbial communities and the close relationship between the structure of these communities and enzyme production.

4.3. Macronutrient responses to CC inclusion

TN was found to be higher under the CC mixture including a leguminous crop. Related to this, Samarappuli et al. [43] compared different species of CC from different families and reported that leguminous CC were the most suitable for providing additional N for subsequent cash crops. In our study, a more diverse and active microbial community generated by the diversification of the agrosystem may increase the availability of macronutrients such as N, which is fundamental for cash crop development. In particular, our investigation indicated that a CC mixture such as oat/radish/vetch not only enhanced microbial PLFA biomarkers and microbial activity, but also provided additional N, which contributes to plant growth.

Although extractable P was positively correlated with acid phosphatase activity, no differences were found after the inclusion of CC in the short term. Our results are consistent with findings of Varela et al. [44], who reported that the presence of CC did not affect P content in a soybean monoculture system in Argentina. However, the above-mentioned positive correlation between acid phosphatase and extractable P observed in our study suggests that extractable P content may be increased by CC treatment in the long term.

5. Conclusions

Through the assessment of soil PLFA we were able to detect and characterize changes in the structure of soil microbial communities generated by the inclusion of CC mixtures in the short term. These alterations were characterized by a notable increase in the bacterial PLFA biomarkers, particularly in Gram-positive bacteria, with the greater amount of MBC observed in plots under CC inclusion being complemented with increased soil enzyme activities. The higher ratios between soil enzyme activity and bacterial and fungal PLFA observed in CC treatments demonstrate that the increase in soil enzyme activity is a response not only to the increase of microbial biomarkers, but also to activities of extracellular enzymes stabilized by soil components.

The rise in microbial biomarkers and microbial activity reported in this study reflects a positive impact on main macronutrient availability as a consequence of the diversification of agricultural systems with a predominance of soybean. A CC mixture including oat/radish/vetch is highly recommended not only to improve soil biological processes, as described above, but also to provide additional N to the cash crop. The positive effects of CC inclusion on soil microbial structure and related enzyme activity and macronutrient availability described in this study were observed at the cash crop harvest. This demonstrates that the use of CC mixtures is a valuable agricultural tool to improve soil fertility and contributes to the sustainability of the agricultural system.

Acknowledgments

The authors thank the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina, for the fellowship granted to D. Chavarria. This work was financially supported by Instituto Nacional de Tecnología Agropecuaria (INTA) through the research projects PNSUELO 1134043 and CIAC 940140; and also by Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) and Secretaría de Ciencia y Tecnología, Universidad Nacional de Córdoba (SECyT-UNC). We thank Dr. Paul David Hobson, native speaker, for the revision of the manuscript.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejsobi.2016.07.002.

References

[1] G. Triplett, W. Dick, No-tillage crop production: a revolution in agriculture!, Agron. J. 100 (2008) 153–165.
[2] M.C. Sasal, A. Andriulo, M. Taboada, Soil porosity characteristics and water movement under zero tillage in silty soils in Argentinian pampas, Soil Till. Res. 87 (2006) 9–18.
[3] C. Li, X. Li, W. Kong, Y. Wu, J. Wang, Effect of monoculture soybean on soil microbial community in the Northeast China, Plant Soil 330 (1–2) (2010) 423–433.
[4] L. Wu, H. Wang, Z. Zhang, R. Lin, Z. Zhong, W. Lin, Comparative metaproteomic analysis on consecutively Rehmannia glutinosa–monocultured rhizosphere soil, PLoS One 6 (5) (2011) 20611.
[5] L. Wu, Z. Li, J. Li, M.A. Khan, W. Huang, Z. Zhang, W. Lin, Assessment of shifts in microbial community structure and catabolic diversity in response to Rehmannia glutinosa monoculture, Appl. Soil Ecol. 67 (2013) 1–9.
[6] S. Vargas Gil, J.M. Meriles, C. Conforto, M. Basanta, V. Radl, A. Hagn, M. Schloter, G. March, Response of soil microbial communities to different management practices in surface soils of a soybean agroecosystem in Argentina, Eur. J. Soil Biol. 47 (2011) 55–60.
[7] M.A. Bustamante, D. Said-Pullicino, E. Agulló, J. Andreu, C. Paredes, R. Moral, Application of winery and distillery waste composts to a Jumilla (SE Spain) vineyard: effects on the characteristics of a calcareous sandy-loam soil, Agric. Ecosyst. Environ. 140 (2011) 80–87.
[8] J. Burton, C. Chen, Z. Xu, H. Ghadiri, Soil microbial biomass, activity and community composition in adjacent native and plantation forests of subtropical Australia, J. Soil Sediments 10 (2010) 1267–1277.
[9] E.L. Balota, P.A. Auler, Soil microbial biomass under different tillage systems and permanent groundcover cultivation between orange trees, Rev. Bras. Ciênc. Solo. 35 (6) (2011) 1873–1883.
[10] E. Zagal, C. Muñoz, M. Quiroz, C. Córdova, Sensitivity of early indicators for evaluating quality changes in soil organic matter, Geoderma 151 (3) (2009) 191–198.
[11] E.L. Balota, A. Calegari, A.S. Nakatani, M.S. Coyne, Benefits of winter cover crops and no-tillage for microbial parameters in a Brazilian Oxisol: a long-term study, Agric. Ecosyst. Environ. 197 (2014) 31–40.
[12] B. Zhang, Y. Li, T. Ren, Z. Tian, G. Wang, X. He, C. Tian, Short-term effect of tillage and crop rotation on microbial community structure and enzyme activities of a clay loam soil, Biol. Fertil. Soils 50 (2014) 1077–1085.
[13] Z. Xu, G. Yu, X. Zhang, J. Ge, N. He, Q. Wang, D. Wang, The variations in soil microbial communities, enzyme activities and their relationships with soil organic matter decomposition along the northern slope of Changbai Mountain, Appl. Soil Ecol. 86 (2015) 19–29.
[14] L. Landi, G. Renella, J.L. Moreno, L. Falchini, P. Nannipieri, Influence of cadmium on the metabolic quotient, L-:D-glutamic acid respiration ratio and enzyme activity:microbial biomass ratio under laboratory conditions, Biol. Fertil. Soils 32 (2000) 8–16.
[15] P. Nannipieri, L. Giagnoni, G. Renella, E. Puglisi, B. Ceccanti, G. Masciandaro, F. Fornasier, Soil enzymology: classical and molecular approaches, Biol. Fertil. Soils 48 (2012) 743–762.

[16] N. Li, S.H. Yao, M.Y. You, Y.L. Zhang, Y.F. Qiao, W.X. Zou, X. Han, B. Zhang, Contrasting development of soil microbial community structure under no-tilled perennial and tilled cropping during early pedogenesis of a Mollisol, Soil Biol. Biochem. 77 (2014) 221–232.
[17] S.W. Culman, S.S. Snapp, J.M. Green, L.E. Gentry, Short- and long-term labile soil carbon and nitrogen dynamics reflect management and predict corn agronomic performance, Agron. J. 105 (2013) 493–502.
[18] K. Steenwerth, K.M. Belina, Cover crops enhance soil organic matter, carbon dynamics and microbiological function in a vineyard agroecosystem, Appl. Soil Ecol. 40 (2008) 359–369.
[19] D. Plaza-Bonilla, J.M. Nolot, S. Passot, D. Raffaillac, E. Justes, Grain legume-based rotations managed under conventional tillage need cover crops to mitigate soil organic matter losses, Soil Till. Res. 156 (2016) 33–43.
[20] A.C. Gaudin, K. Janovicek, B. Deen, D.C. Hooker, Wheat improves nitrogen use efficiency of maize and soybean-based cropping systems, Agric. Ecosyst. Environ. 210 (2015) 1–10.
[21] M.D. McDaniel, L. Tiemann, A. Grandy, Does agricultural crop diversity enhance soil microbial biomass and organic matter dynamics? a meta-analysis, Ecol. Appl. 24 (2014a) 560–570.
[22] R. Hall, C. Rebella, J. Ghersa, Culot Field-crop systems of the pampas, in: C.J. Pearson (Ed.), Field Crops Ecosystems. Ecosystems of the World, Elsevier, Amsterdam, 1992, pp. 413–450.
[23] S.B. Restovich, A. Andriulo, S. Portela, Introduction of cover crops in a maize–soybean rotation of the Humid Pampas: effect on nitrogen and water dynamics, Field Crops Res. 128 (2012) 62–70.
[24] J.M. Meriles, S. Vargas Gil, C. Conforto, G. Figoni, E. Lovera, G.J. March, C. Guzmán, Soil microbial communities under different soybean cropping systems: characterization of microbial population dynamics, soil microbial activity, microbial biomass, and fatty acid profiles, Soil Till. Res. 103 (2009) 271–281.
[25] G. Adam, H. Duncan, Development of a sensitive and rapid method for the measurement of total microbial activity using fluorescein diacetate (FDA) in a range of soils, Soil Biol. Biochem. 33 (2001) 943–951.
[26] C. García, M.T. Hernández, F. Costa, Potential use of dehydrogenase activity as an index of microbial activity in degraded soils, Commun. Soil Sci. Plant Anal. 28 (1997) 123–134.
[27] M. Tabatabai, J. Bremner, Use of p-nitrophenyl phosphate for assay of soil phosphatase activity, Soil Biol. Biochem. 1 (4) (1969) 301–307.
[28] E. Vance, P. Brookes, D. Jenkinson, An extraction method for measuring soil microbial biomass C, Soil Biol. Biochem. 19 (1987) 703–707.
[29] USEPA U. Environmental Protection Agency, Methods for Chemical Analysis of Water and Wastes, US Environmental Protection Agency, Environmental Monitoring Support Laboratory, Cincinnati, OH, USA, 1993.
[30] USEPA U. Environmental Protection Agency, Methods for Chemical Analysis of Water and Wastes, US Environmental Protection Agency, Environmental Monitoring Support Laboratory, Cincinnati, OH, USA, 1974.
[31] J.A. Di Rienzo, F. Casanoves, M.G. Balzarini, L. Gonzalez, M. Tablada, C.W. Robledo, InfoStat Versión, Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina, 2013. http://www.infostat.com.ar.
[32] S. Vargas Gil, J.M. Meriles, C. Conforto, G. Figoni, M. Basanta, E. Lovera, G.J. March, Field assessment of soil biological and chemical quality in response to crop management practices, World J. Microbiol. Biotechnol. 25 (2009) 439–448.
[33] F. el Zahar Haichar, C. Santaella, T. Heulin, W. Achouak, Root exudates mediated interactions below ground, Soil Biol. Biochem. 77 (2014) 69–80.
[34] D. Bossio, M. Girvan, L. Verchot, J. Bullimore, T. Borelli, A. Albrecht, K. Scow, A. Ball, J. Pretty, A. Osborn, Soil microbial community response to land use change in an agricultural landscape of western Kenya, Microb. Ecol. 49 (2005) 50–62.
[35] S. Güsewell, M.O. Gessner, N: P ratios influence litter decomposition and colonization by fungi and bacteria in microcosms, Funct. Ecol. 23 (2009) 211–219.
[36] M.S. Strickland, J. Rousk, Considering fungal:bacterial dominance in soils-methods, controls, and ecosystem implications, Soil Biol. Biochem. 42 (2010) 1385–1395.
[37] B. Zhu, L. Yi, L. Guo, G. Chen, Y. Hu, H. Tang, C. Xiao, X. Xiao, G. Yang, S.N. Acharya, Z. Zeng, Performance of two winter cover crops and their impacts on soil properties and two subsequent rice crops in Dongting Lake Plain, Hunan, China, Soil Till. Res. 124 (2012) 95–101.
[38] J. Furczak, J. Joniec, Changes in biochemical activity of podzolic soil under willow culture in the second year of treatment with municipal–industrial sewage sludge, Int. Agrophys. 21 (2007) 145–152.
[39] J. Spears, K. Lajtha, B. Caldwell, S. Pennington, K. Vanderbilt, Species effects of Ceanothus velutinus versus Pseudotsuga menziesii, Douglas-fir, on soil phosphorus and nitrogen properties in the Oregon cascades, For. Ecol. Manag. 149 (2001) 205–216.
[40] B. Caldwell, Enzyme activities as a component of soil biodiversity: a review, Pedobiologia 49 (2005) 637–644.
[41] M.P. Waldrop, J.W. Harden, M.R. Turetsky, D.G. Petersen, A.D. McGuire, M.J. Briones, A.C. Churchill, D.H. Doctor, L.E. Pruett, Bacterial and enchytraeid abundance accelerate soil carbon turnover along a lowland vegetation gradient in interior Alaska, Soil Biol. Biochem. 50 (2012) 188–198.
[42] T. Shahzad, C. Chenu, P. Genet, S. Barot, N. Perveen, C. Mougin, S. Fontaine, Contribution of exudates, arbuscular mycorrhizal fungi and litter depositions to the rhizosphere priming effect induced by grassland species, Soil Biol. Biochem. 80 (2015) 146–155.
[43] D. Samarappuli, B. Johnson, H. Kandel, M. Berti, Biomass yield and nitrogen content of annual energy/forage crops preceded by cover crops, Field Crops Res. 167 (2014) 31–39.
[44] M. Varela, C. Scianca, M. Taboada, G. Rubio, Cover crop effects on soybean residue decomposition and P release in no-tillage systems of Argentina, Soil Till. Res. 143 (2014) 59–66.