

Response of soil microbial communities to agroecological versus conventional systems of extensive agriculture



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ARTICLE INFO

Keywords:

Agroecology
Sustainability
Microbial abundance
Metabolic efficiency

ABSTRACT

World population growth has led to a rise in resource demands imposed on agricultural systems, generating an increased global use of natural resources. However, agroecology proposes self-regulation in order to achieve a sustainable agricultural balance. Therefore, considering the rapid responses of microbial communities to small changes in soil use, the objective of this study was to assess the response of soil microbial communities to agroecological vs. conventional systems of extensive agriculture. Soil sampling was carried out in 2016 and 2017 with three different treatments using the sequence soybean/maize (*Glycine max* L./*Zea mays* L.) as the main crop: Agroecological (AE), conventional with cover crops (CC) and conventional without cover crops (control). Species used as cover crops were wheat (*Triticum aestivum*), vetch (*Vicia sativa* L.), oat (*Avena sativa* L.) and radish (*Raphanus sativus* L.). Agroecological treatment showed the lowest total nitrogen (0.18 mg N g^{-1}) and organic carbon (1.99 mg C g^{-1}) content of soil, and CC treatment showed the highest value of fluorescein diacetate hydrolysis, with values 63.2% and 12.1% higher than AE and the control, respectively. However, AE treatment also produced the highest F:B ratio (44.8) and the lowest metabolic quotient (1.14), which indicates an improvement in metabolic efficiency and soil quality. No significant differences were recorded in the abundance of fungal and bacterial communities between treatments. Our results suggest that agroecological management is characterised by fungal dominance in soil microbial communities and a higher microbial metabolic efficiency compared to conventional management. These results demonstrate more efficient use of carbon substrates in agroecological systems, which could counteract the negative effect of the lack of synthetic fertilisation and reduced-tillage in the long term. The findings demonstrate that sustainable agricultural tools with adequate management can be effectively used to preserve soil quality.

1. Introduction

In recent years, research studies of major agricultural regions of the world have focused on diversification and sustainable management of agricultural systems, in order to restore systems which have been misused and their resources over-exploited (Massawe et al., 2016; Kanter et al., 2016; Zeweld et al., 2017). In this sense, world population growth has led to a rise in resource demands imposed on agricultural systems, generating a greater global use of natural resources and a

significant decline in ecosystem services (Gianinazzi et al., 2010). High-intensity agriculture has mainly focused on productivity, instead of integrating natural resource management into food production security; monocultures and increased use of synthetic inputs, such as chemical fertilisers and pesticides, have reduced soil fertility (Foley et al., 2005). Therefore, there is no doubt that an alternative agricultural development paradigm is required that encourages more durable, greener, more resilient forms of agriculture that favour biodiversity and are socially just.

Abbreviations: $q\text{CO}_2$, metabolic quotient; TN, total nitrogen; eP, extractable phosphorus; SOC, soil organic carbon; AP, acid phosphatase; FDA, fluorescein diacetate; MBC, microbial biomass carbon; 18S, fungal rDNA copies; 16S, bacterial rDNA copies; F:B, fungal:bacterial ratio; C:N, carbon:nitrogen ratio

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<https://doi.org/10.1016/j.agee.2018.05.008>

Received 3 November 2017; Received in revised form 17 April 2018; Accepted 9 May 2018
0167-8809/ © 2018 Published by Elsevier B.V.

Given the high economic and environmental costs of the conventional agricultural system and future predictions of climate change, agroecology may represent the theoretic basis that could promote the equity and sustainability of agroecosystems. Conventional agriculture is based in transgenic crops developed for pest control using a single control mechanism (pesticide) which has frequently failed to control insect pests, pathogens and weeds (Altieri and Nicholls, 2000). Moreover, transgenic crops use tends to accelerate the evolution of resistant plagues (Tabashnik and Carrière, 2017), thus, agroecology involves biodiversification as a primary technique for inducing self-regulation in order to achieve a sustainable balance. Agroecology represents a scientific, methodological, and technological basis for a new “agrarian revolution” worldwide (Ferguson and Morales, 2010), since agroecology-based production systems are resilient, energetically efficient, biodiverse, socially just, and provide the basis for an energy, productive and food sovereignty strategy (Altieri et al., 2012). Hence, the study of the effect of agroecological management on natural resources in comparison with conventional management is extremely useful.

Since sustainability of agricultural systems is based on conservative practices which encompass the entire productive chain within an efficient resource use framework (Ferreira et al., 2011), microbiological processes occurring in the soil constitute the basis on which agroecological farming is sustained (Faria and Franco, 2002). This is because soil microbial communities develop a fundamental role in nutrient cycling and organic matter decomposition and, considering their interactions with crops, the study of microbiology provides an accurate means of analysing different agricultural management systems (Burton et al., 2010). Thus, the ability of microbial communities to respond rapidly to the changes in land use (Singh, 2015) can be employed to compare the effects of agroecological and conventional management. Since agroecological practices include an integration of several agricultural tools, such as cattle dung, reduced-tillage and crop diversification (Altieri et al., 2012), it would be expected that changes in microbial dynamics compared to conventional management would be observed. Therefore, considering that the higher diversity of microbes in ecosystems could establish a functional equilibrium which may enable sustainability to be preserved (Seneviratne, 2012), it is important to generate knowledge about the effect of agroecology on soil microbial communities.

Since agroecological systems use agricultural techniques to compensate for the lack of synthetic inputs, strategies such as integrated livestock-crop systems and reduced-tillage are commonly used by agroecological farmers (Toffolini et al., 2017). However, loss of soil quality caused by reduced-tillage compared to no-tillage and uncoupling of the nitrogen cycle from the carbon cycle caused by consumption of plant residues by cattle has been widely reported (Peigné et al., 2007; Favardin and Peyraud, 2010). Therefore, it is also necessary to take into account the negative aspects when studying the impact of agroecology on edaphic microbiology. Overall, the objectives of this study are: 1) to compare the response of soil microbial community structure and functionality to an agroecological system and a conventional agricultural system including a cover crop mixture; 2) to evaluate soil chemical parameters with both agricultural management systems evaluated; and 3) to study the relationships between the response of soil microbial functionality and chemical parameters with both agricultural management systems evaluated. We hypothesised that agroecological agriculture generates an increase in the structure and functionality of soil microbial communities, which is related to a greater microbial metabolic efficiency and macronutrient availability.

2. Material and methods

2.1. Field experiment

The study was carried out at the Pergamino Experimental Station of the National Institute of Agricultural Technology (INTA) (33°51'S,

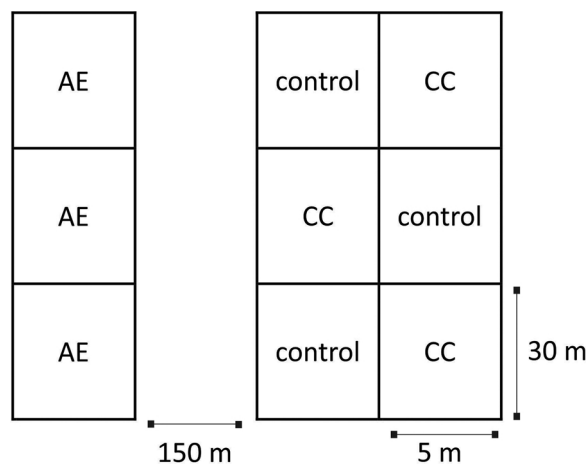


Fig. 1. Representative scheme of the field trial with the three treatments: agroecological management (AE), conventional management with cover crops (CC) and conventional management without cover crops (control).

60°40'W), Buenos Aires province, Argentina, in 2016 and 2017. Two long-term field trials set up in 2006 at the experimental station were evaluated, an agroecological trial and a conventional trial. Even though both trials were physically separated from each other (1500 m) to ensure that agroecological treatment was not exposed to agrochemicals, they were considered as a single trial for the purpose of statistical design and data analysis. The climate in this site is temperate humid, with mean annual temperature of 16.5 °C and rainfall occurring mostly in autumn and spring, with a mean annual rainfall of 971 mm for the 1910–2010 period (Agroclimatological Network Database, INTA; <http://climayagua.inta.gob.ar/>). The soil at the study site is predominantly Typic Argiudoll (USDA Soil Taxonomy) of the Pergamino series with a silt loam A horizon without eroded phase (< 0.3% slope). The experimental design was based on a one-way factorial design with three replications. The experiment consisted of three treatments with three replicates of each, totaling nine plots (Fig. 1). The treatments were: 1) Agroecological management (AE), 2) Conventional management with cover crops (CC), 3) Conventional management without cover crops (control). Agroecological plots were managed without the use of external synthetic inputs, such as herbicides, pesticides, mineral fertilisation or genetically modified crops. The plots were sown with soybean/maize (*Glycine max* L./*Zea mays* L.) sequence as main crops, in rotation with triticale (*Triticosecale*) and vetch (*Vicia sativa* L.) as cover crops, with crops sown by a reduced-tillage method. The plots included the presence of cattle in order to provide the addition of cattle dung to the soil. Weed control was performed using mechanical methods, such as chisel-ploughing and killing cover crops by disc harrowing which incorporated the vegetable residues into the soil. Conventional plots (including CC treatment and the control treatment) were managed with the application of herbicides, mineral fertilisers, and pesticides, and the use of genetically modified crops. The plots were also sown with soybean/maize sequence as main crops, with both crops being sown using a no-tillage method. Maize was fertilised at sowing with calcium superphosphate (150 kg ha⁻¹) and between V5-V6 with 32 kg N ha⁻¹. The species used as cover crops in CC treatment were: oat (*Avena sativa* L.), vetch (*Vicia sativa* L.) and radish (*Raphanus sativus* L.), which were sown as a mixture of species (oat-radish-vetch). In CC treatment, cover crops were killed using 3–4 l ha⁻¹ of glyphosate (48% active ingredient), and their residue left on the surface without tilling into the soil.

2.2. Soil sampling

Soil sampling was performed at soybean and maize harvest in March 2016 and 2017, according to a previous study (Restovich et al., 2012).

Six composite soil samples were collected 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 for molecular analysis.

2.3. Soil chemical properties

The soil pH was measured at a soil-to-water ratio of 1:2.5. Total carbon (SOC) was determined by wet oxidation following the Walkley and Black procedure (Black et al., 1965). As these soils are free of carbonates, the total carbon content is equivalent to the soil organic carbon (SOC) content. Total nitrogen and extractable phosphorus (eP) were determined by the micro-Kjeldhal method (Bremner, 1996) and Bray-Kurtz method (Bray and Kurtz, 1945), respectively.

2.4. Microbial biomass and respiration

Microbial biomass carbon was determined using the chloroform fumigation-inoculation technique according to Jenkinson and Powlson (1976). Soil microbial respiration was determined as potentially mineralizable carbon (CO₂-C respiration) according to Alef (1995). The amount of CO₂ released was measured from chloroform-treated and untreated soil samples (ca. 20 g). Treated samples were previously fumigated with chloroform, inoculated with fresh soil, and incubated with NaOH 0.2 M at room temperature in the dark for no longer than two weeks. Released CO₂ was estimated using HCl 0.2 N. For the quantification of microbial respiration, flasks containing no soil served as the control treatment.

2.5. Soil enzyme activities

Microbial activity was estimated by hydrolysis of fluorescein diacetate activity (FDA), according to Adam and Duncan (2001). 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. Substrate (FDA, 1000 mg 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. Once removed from the incubator, 15 ml of chloroform/methanol (2:1 v/v) was immediately added to terminate the reaction. The contents of the conical flasks were then centrifuged at 2000 rpm for 5 min. The supernatant was then filtered and analysed at 490 nm using a spectrophotometer.

Acid phosphatase (AP) was assayed using 1 g soil, 4 ml 0.1 M universal buffer (pH 6.5), and 1 ml 25 mM *p*-nitrophenyl phosphate (Tabatabai and Bremner, 1969). After incubation at 37 ± 1 °C for 1 h, the enzyme reaction was stopped by adding 4 ml 0.5 M NaOH and 1 ml 0.5 M CaCl₂ to prevent the dispersion of humic substances. Absorbance was measured in the supernatant at 400 nm.

2.6. Fungal and bacterial genes abundances

DNA was extracted from 0.5 g of soil. Extraction was performed with the soil NucleoSpin® Soil Kit for soil (Macherey-Nagel) using the manufacturer's protocol. DNA yield and purity were measured using a

microvolume fluorospectrometer (NanoDrop Technologies, Delaware).

The bacterial 16S rRNA gene copy numbers (primer set 338F/518R; Fierer et al., 2005) and fungal 18S rRNA gene copy numbers (primer set NS1-F/Fung R; May et al., 2001) of all samples were determined following the protocol of Liu et al. (2009). PCR amplification was quantified in a Line-Gene 9600 Plus by fluorometric monitoring with a Power SYBR Green PCR master mix (Applied Biosystems). The reaction was performed in a 25 µl volume containing 10 ng DNA, 0.2 mg ml⁻¹ BSA, 0.2 µM of each primer and 12.5 µl of SYBR premix EX Taq™ (Takara Shuzo, Shiga, Japan). The standard curves were separately constructed for bacteria and fungi using plasmids from cloned rRNA genes (Takara). Negative (ultrapure water) and positive DNA controls (*Pseudomonas aeruginosa*, 10-fold serial dilution) were also included. The number of copies of the standards was calculated from the concentration of extracted plasmid DNA. Standard curves were generated using triplicate 10-fold dilutions of plasmid DNA, ranging from 2.07 × 10² to 2.07 × 10⁸ copies for the bacterial 16S rRNA gene and 4.60 × 10² to 4.60 × 10⁸ copies of template for fungal 18S rRNA gene per assay. An amplification efficiency of quantification of 105% was obtained for the bacterial 16S rRNA gene and 98.2% for the fungal 18S rRNA gene, with a R² value and a slope being 0.998 and –3.197, and 0.993 and –3.365, respectively. Melting curve analysis was conducted after each assay to confirm specific amplification. A 2% agarose gel electrophoresis was further performed on the PCR amplification products and blanks to check whether an appropriate size was achieved. We used a negative control master mix to test possible inhibitory effects on quantitative PCR amplification caused by coextracted humic substances. The amplification efficiencies were calculated using the formula $Eff = [10(-1/slope)-1]$ (Hai et al., 2009). A relative fungal-to-bacterial ratio was directly calculated from the qPCR assays (Fierer et al., 2005).

2.7. Statistical analyses

Data calculation and statistical analyses were performed using Microsoft Office Excel and INFOSAT software (Di Rienzo et al., 2015) for Windows. Analysis of variance (ANOVA) was used with LSD (least significant difference) to test differences in the microbial parameters and soil chemical properties ($p \leq 0.05$). Data obtained from the two year evaluations were pooled in order to study the effect of the treatments on soil microbial and chemical variables. In all cases, residuals were tested for normality with the Shapiro-Wilks' test. A principal component analysis (PCA) was performed to determine separation among treatments, and to identify the microbial activities, fungal and bacterial gene abundances and chemical variables that best contributed to the separation of treatments. In addition, a correlation analysis was performed with the variables studied using Pearson's coefficient with $p \leq 0.05$ and $p \leq 0.001$ indicating statistical significance.

Table 1

Mean values ± standard errors for total nitrogen (TN), soil organic carbon (SOC), extractable P (eP), C:N ratio and pH, measured in 2016 and 2017 in three different management systems: Agroecological (AE), conventional with cover crops (CC), conventional without cover crops (control). Different letters indicate values that are significantly different ($p < 0.05$).

Treatments	TN (mg N g ⁻¹)	SOC (mg C g ⁻¹)	EP (mg P g ⁻¹)	C:N	pH
AE	1.8 ± 0.05 c	19.9 ± 0.33 b	3.28 ± 0.30 a	10.58 ± 0.32 a	5.76 ± 0.02 a
CC	2.2 ± 0.08 a	23.2 ± 0.52 a	2.62 ± 0.19 a	10.58 ± 0.20 a	5.86 ± 0.07 a
control	2.0 ± 0.06 b	22.0 ± 0.46 a	3.08 ± 0.34 a	10.81 ± 0.17 a	5.82 ± 0.04 a
<i>p</i> value	0.0046	0.0013	0.5879	0.4897	0.3630

Table 2

Mean values \pm standard errors for fluorescein diacetate (FDA) hydrolysis, acid phosphatase (AP), microbial respiration, microbial biomass carbon (MBC) and qCO_2 , measured in 2016 and 2017 with three different management systems: Agroecological (AE), conventional with cover crops (CC), conventional without cover crops (control). Different letters indicate values that are significantly different ($p < 0.05$).

Treatments	FDA (ug FDA g ⁻¹ h ⁻¹)	AP (ug p-nitrophenyl g ⁻¹ h ⁻¹)	Respiration (mg g ⁻¹)	MBC (mg g ⁻¹)	qCO_2
AE	74.13 \pm 2.80 c	649.81 \pm 22.22 a	0.43 \pm 0.02 a	0.47 \pm 0.02 a	1.14 \pm 0.24 b
CC	121.03 \pm 4.80 a	684.63 \pm 32.08 a	0.43 \pm 0.05 a	0.29 \pm 0.06 b	2.21 \pm 0.57 a
control	108 0.00 \pm 4.15 b	599.58 \pm 25.79 b	0.41 \pm 0.07 a	0.26 \pm 0.04 b	3.17 \pm 0.82 a
p value	< 0.0001	0.0230	0.9169	0.0073	< 0.0001

3. Results

3.1. Soil chemical properties

TN and SOC were clearly different in both systems studied (Table 1). TN was increased by CC treatment, which was 10.0% and 22.2% higher than control and AE, respectively; AE presented the lowest value for TN. The same trend was recorded for SOC, with AE being 16.6% and 10.5% lower than CC and control, respectively. The content of eP did not show significant difference between treatments, with mean values between 2.62 and 3.28 mg g⁻¹. Also, no significant differences were observed for C:N ratio and pH among treatments.

3.2. Soil enzyme activities

Interesting differences were observed in the soil enzyme activities between the different agricultural systems (Table 2). Concerning FDA hydrolysis, CC treatment showed the highest value, being 63.2% and 12.1% higher than AE and control, respectively; AE presented the lowest value for this variable. Regarding AP values, control treatment showed the lowest enzyme activity, being 8.3% and 14.2% lower than AE and CC, respectively.

3.3. Components of metabolic quotient

Microbial respiration values did not show significant differences between treatments (Table 2). On the contrary, MBC was clearly influenced by agroecological management, with AE treatment being 62.1% and 80.7% higher than CC and control, respectively. Metabolic quotient, calculated as the ratio between microbial respiration and MBC, showed the lowest value with AE, being 93.8% and 178.1% lower than CC and control, respectively.

3.4. Composition and abundance of fungal and bacterial communities

Quantitative PCR analysis performed in 2016 and 2017 did not show a significant variation between treatments in the abundance of fungal and bacterial communities (Fig. 2). The mean value of bacterial copy varied between 7.6 E + 26 (AE) and 9.9 E + 26 (control) 16S rDNA copy numbers g⁻¹. The mean value of fungal copy varied between 1.8 E + 27 (control) and 2.4 E + 28 (AE) 18S rDNA copy numbers g⁻¹.

The fungal:bacterial ratio (Fig. 2C) was lower in control samples in comparison with other treatments, with control values that were 24.3% and 56.7% lower than CC and AE, respectively.

3.5. Relationship among parameters

The information obtained from the study of microbial and chemical variables was analysed using a PCA (Fig. 3). PC1 and PC2 accounted for 62.5% and 18.2% of the variance, respectively. The AE treatment was clearly separated from the rest of the treatments along PC1, with control values being close to CC values. No significant separation between treatments was observed along PC2. The variables SOC, TN, FDA, qCO_2 and MBC were the most influential variables in the separation of

treatments. On the contrary, microbial structure variables (16S and 18S) did not have a great influence on the separation of treatments, as shown in Fig. 2. The PCA showed clear relationships between variables which were tested through a correlation analysis (Table 3). The correlation analysis recorded a significant and positive correlation between FDA and SOC and TN, with a negative correlation between FDA and F:B. Also, SOC was positively correlated with TN and eP. A significant and marked negative correlation was recorded between and F:B and qCO_2 , which was also positively correlated with 18S.

4. Discussion

4.1. Soil chemical properties response to agroecological management

The adoption of agroecological management systems in agricultural raises the need to understand the system as a whole, adequately managing the factors that influence crop production in order to make up for the lack of external chemical inputs. In this regard, the use of reduced-tillage methods that involves the incorporation of stubble into the soil represents a useful strategy for providing nutrients to the soil and reducing the inoculum of fungal diseases, also contributing to weed control (Peigné et al., 2007). However, it has been widely reported that the long-term practice of reduced-tillage can decrease the content of SOC in comparison with no-tillage methods (Angers et al., 1997; Wei et al., 2014). An increase in SOC with AE treatment was expected in our research, but the use of reduced-tillage could have negatively influenced this variable. In accordance with this, Chivenge et al. (2007) found that tillage disturbance was the dominant factor reducing carbon stabilisation in soil, probably by reducing carbon stabilisation within microaggregates. In this sense, alternative agricultural practices, such as the use of fabric or organic mulches, can also contribute to weed and pest control without the necessity for tillage (Feldman et al., 2000). Therefore, this kind of alternative practice could be introduced to prevent SOC loss in agroecological systems. Contrary to our results, Teasdale et al. (2007) found that organic farming including organic amendments produced higher carbon content than conventional farming with no-tillage, despite the use of tillage in organic management. Even though our trial included cattle dung in AE, which can increase the content of SOC, this was not enough to raise SOC levels to those achieved with conventional management. This was probably due to consumption of crop residues by cattle and the lack of organic amendments, which could have ensured an adequate content of organic matter.

Our results showed a clear diminution in the values of TN in the agroecological plots. This is probably due to the lack of synchronisation of crop nitrogen requirements and availability of soil nitrogen from organic materials, such as crop residues. In this regard, the use of synthetic fertiliser can help to achieve optimum production, though improper applications can cause environmental damage to air, water and soil quality (Wezel et al., 2014). Nonetheless, the C:N ratio in the agroecological plots was in an optimal balance, equaling that of conventional treatments. This suggests a balance between the immobilisation and the mineralization of nitrogen which is beneficial for crop production (Janssen, 1996), in spite of the lack of synthetic fertilisers in the AE plots. Although the exclusion of synthetic fertilisers in

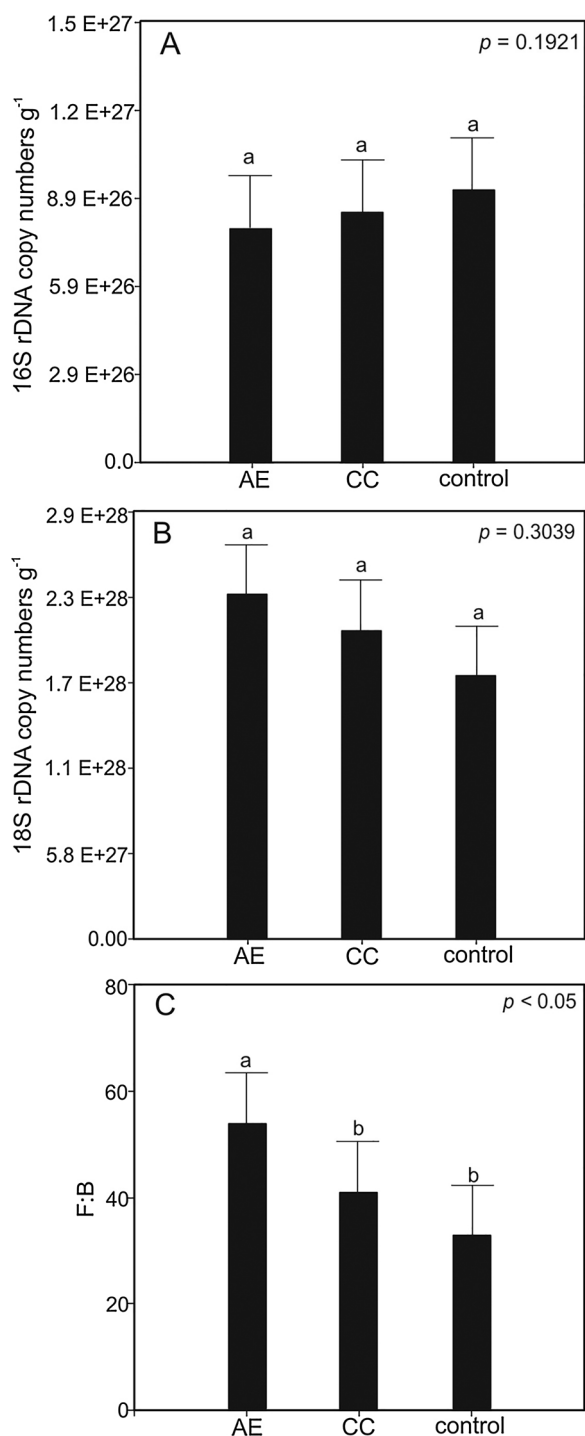


Fig. 2. Mean values of bacterial (16S) (A) and fungal (18S) (B) rDNA copy numbers g⁻¹ and fungal:bacterial ratio (F:B) (C), measured in 2016 and 2017 with three different management systems: Agroecological (AE), conventional with cover crops (CC), conventional without cover crops (control). Different letters indicate values that are significantly different ($p < 0.05$). Error bars indicate \pm one standard error.

agroecological systems has important environmental benefits, it represents a challenge for the production of extensive crops. In this regard, organic fertilisers provide a means of substituting inorganic fertilisers, but can also enhance soil biological activity and potentially increase soil mineralisation and nutrient loss (Birkhofer et al., 2008). Moreover, crop residue consumption by cattle and reduced-tillage could have benefited the lower content of TN observed in AE plots compared

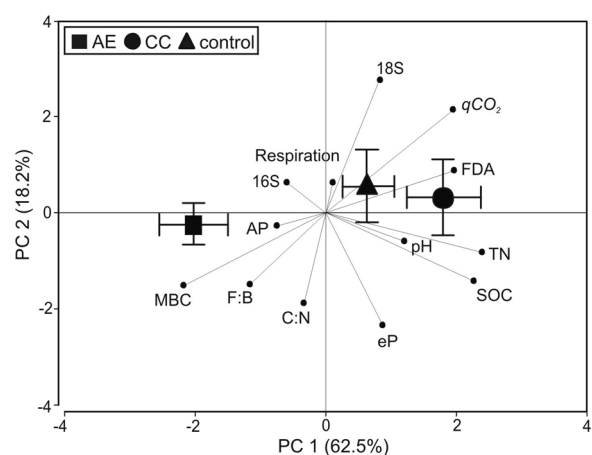


Fig. 3. Principal component (PC) analysis including microbial activity, diversity variables and chemical variables, measured in 2016 and 2017 with three different management systems: Agroecological (AE), conventional with cover crops (CC), conventional without cover crops (control).

with the other treatments. However, agroecological management did not affect the content of soil eP in our study, probably due to the fact that phosphate rapidly precipitates in soil which reduces leaching, which represents an important hazard with tillage management (Sims et al., 1998). This may be the reason why the inclusion of stubble into the soil resulted in a greater supply of eP with agroecological management, equaling that of conventional management. Overall, conventional treatment with the inclusion of a mixture of cover crops presented the best chemical conditions for crop production.

4.2. Agroecological management and soil microbial functioning

General soil microbial activity, determined by FDA hydrolysis, was significantly reduced by agroecological management in our study. This suggests that crop diversification and cattle dung did not provide enough energy sources in order to support microbial activity in AE that was comparable with conventional management. Since FDA was positively correlated with SOC and TN, it is possible that the higher content of those macronutrients in soil with conventional management, particularly with CC treatment, enhanced the general microbial activity. In accordance with this, Rakshit et al. (2016) reported that FDA hydrolysis and AP activity were positively correlated with the level of nitrogen fertilisation. However, Stark et al. (2007) demonstrated that FDA hydrolysis decreased with excessive fertiliser application, suggesting a negative impact of intensive fertilisation on soil microbiological activity. Moreover, reduced-tillage and its detrimental effect on SOC could have contributed to reduced energy sources in AE plots in our study, promoting a reduction in general microbial activity. Several recent studies (Glaser et al., 2015; Trupiano et al., 2017) proposed the use of biochar in combination with compost as a substitute for mineral fertilisers to close nutrient cycles in agroecological systems. Therefore, the use of organic compounds could represent an alternative means of enhancing general microbial activity in agroecological management systems.

Contrary to FDA results, AP activity was increased by agroecological management and conventional with cover crops in our study. In accordance with this, Marinari et al. (2006) studied the effect of seven years of organic management on soil fertility and found a higher AP activity and higher available phosphorus content in comparison with conventional management, despite the use of reduced-tillage. Since different plant species stimulate the growth of different microorganism species (el Zahar Haichar et al., 2014), the use of cover crops probably promoted a greater diversity of microbial functions, stimulating the AP activity in plots under cover crops. This effect was observed in

Table 3
Correlation analysis including microbial activity and diversity variables and chemical variables measured in 2016 and 2017.

	FDA	AP	16S	18S	F:B	qCO_2	pH	SOC	TN	eP
FDA	1	–	–	–	–	–	–	–	–	–
AP	–0.110	1	–	–	–	–	–	–	–	–
16S	–0.110	0.140	1	–	–	–	–	–	–	–
18S	–0.050	–0.060	–0.060	1	–	–	–	–	–	–
F:B	–0.380 ^a	–0.040	0.180	–0.070	1	–	–	–	–	–
qCO_2	0.240 ^a	–0.070	–0.170	0.320	–0.730 ^b	1	–	–	–	–
pH	0.210	–0.280 ^a	–0.210	0.180	–0.090	–0.090	1	–	–	–
SOC	0.550 ^b	–0.060	–0.180	0.490	–0.090	0.340	0.240	1	–	–
TN	0.470 ^a	–0.130	0.120	0.300	–0.010	0.490	0.130	0.800 ^a	1	–
eP	0.270	0.270	0.040	0.530	–0.001	–0.040	0.260	0.410 ^a	0.410 ^a	1

Abbreviations: FDA, fluorescein diacetate; AP, acid phosphatase; 16S, bacterial rDNA copies; 18S, fungal rDNA copies; F:B, fungal:bacterial ratio; qCO_2 , metabolic quotient; SOC, soil organic carbon; TN, total nitrogen; eP, extractable phosphorus.

^a $p \leq 0.05$.

^b $p \leq 0.001$.

agroecological plots despite the lack of synthetic fertilisers and the use of reduced-tillage methods.

Although the greater hydrolysis of FDA suggests a higher level of soil organic matter degradation in conventional plots, these plots also showed a high metabolic quotient (qCO_2), which suggests a low efficiency in the use of carbon sources. In this regard, Masciandaro et al. (1998) demonstrated that a lower qCO_2 reflects improved soil biophysical conditions, while a higher qCO_2 indicates soil degradation under intensive land use. Therefore, considering the high qCO_2 values reported in CC and control plots, our results indicates a lower metabolic efficiency of soil microbial communities with conventional management in comparison with agroecological management. In accordance with our results, Fließbach et al. (2007) compared organic and conventional management and found that microbial respiration did not vary between farming systems, but qCO_2 was significantly higher in conventional soils as compared to organic soils, suggesting a higher maintenance requirement of microbial biomass in soils from conventional systems. Probably, the increased nutrient supply from synthetic fertilisers in conventional plots in our study promoted greater microbial activity, but with a low use efficiency of carbon compounds which were poorly fixed by the microbial biomass. This effect was reflected in the high mean value of MBC in agroecological plots. Braman et al. (2016) also reported higher values of MBC and lower qCO_2 with organic management as compared to conventional management. Our results suggest that soil metabolic efficiency can be increased by agroecological management, which could lead to an improvement in soil biophysical conditions in the long term.

4.3. Effect of agroecological management on fungal and bacterial communities

No clear differences were recorded between treatments with regard to fungal and bacterial communities in our investigation. Nevertheless, it is notable that agroecological plots presented similar abundance of fungal and bacterial rDNA copies compared to conventional management despite consumption of crop residues by cattle and the lack of external synthetic fertilisers with AE treatment. In this regard, it has been reported that synthetic fertiliser stimulates fungal growth while inhibiting that of bacteria (Demoling et al., 2007), although bacterial biomass has also been shown to increase with fertilisation (Högberg et al., 2003). Moreover, fungal communities are known to be sensitive to soil disturbance caused by tillage (Helgason et al., 2009; 2010). However, our results suggests that energy sources provided in AE plots by crop diversification and cattle dung in conjunction with a high metabolic efficiency, were enough to maintain the same abundance of bacterial and fungal communities observed with conventional management using synthetic fertilisers and no-tillage. Probably, the use of reduced-tillage was not a sufficiently significant disturbance to reduce

the soil fungal communities with agroecological management. In accordance with this, Hydbom et al. (2017) found that fungal and bacterial growth rate were unaffected by conventional tillage treatment, and reduced-tillage had a stimulating effect on arbuscular mycorrhizal fungi and saprotrophic fungi compared to conventional tillage.

Agroecological plots also showed a significant increase in the F:B ratio compared to the other treatments. In this regard, higher F:B ratios are suggested to be indicative of a more sustainable agroecosystem, in which organic matter decomposition and nitrogen mineralisation dominate the provision of plant nutrients for crop growth (De Vries et al., 2006). It has been reported that mineral fertilisers reduce the F:B ratio, while organic manure with a high C:N ratio stimulates fungal growth and thus increases the F:B ratio (Buyer et al., 2010). Therefore, it is possible that the provision of easily assimilated mineral nutrients promoted the dominance of bacteria in soils under conventional management in our study, while cattle dung promoted the dominance of fungi in agroecological soils. Related to this, there is a general consensus in the literature that fungi are capable of degrading lignin and bacteria are not, and that fungi also dominate the decomposition of cellulose and hemicellulose, which are important components of organic matter (Strickland and Rousk, 2010). Hence, our results suggest that agroecological management may be related to a greater ability to degrade highly complex organic compounds by microbial soil communities in relation to conventional management. Malik et al. (2016) studied microbial dynamics and demonstrated the significant role of fungi in litter decomposition, showing that the increase in the F:B ratio was linked to higher carbon storage potential. This potential effect may represent an important way to increase carbon content in the long term in agroecological plots, which showed lower SOC values than conventional plots in our study, as reported above.

4.4. Relationships between parameters

It is known that abundant coverage of soils and use of nitrogen fertiliser promotes an increase in the synthesis of extracellular microbial enzymes in order to decompose the organic matter to obtain carbon (Hargreaves and Hofmöckel, 2014). This explains the positive correlation observed between FDA hydrolysis and TN and SOC. Thus, cover crop residues and synthetic fertilisation provided the necessary energy sources with CC treatment to sustain a high microbial enzyme production. In contrast, the lack of synthetic fertiliser in agroecological plots and consumption of crop residues by cattle may have been a determinant in reducing microbial enzymatic activity with AE treatment. Also, reduced-tillage may have contributed to reduced SOC content compared to no-tillage in agroecological soils, since no-tillage is an effective management practice for improving carbon sequestration (Liu et al., 2014). This greater soil disturbance and the consequent carbon reduction with AE treatment was related to lower FDA activity.

However, our results indicate that agroecological plots seem to make a more efficient use of carbon sources in comparison with conventional plots, as demonstrated by a low qCO_2 . This coefficient showed a negative and significant correlation to the F:B ratio, suggesting that an increase in metabolic efficiency may be related to the dominance of fungal communities in soils. Consistent with this, it has been demonstrated that fungi on average produce more biomass carbon per unit of carbon metabolised than bacteria, leading to a greater proportion of carbon stored in fungal-dominated systems when compared to bacterial-dominated systems (Strickland and Rousk, 2010). Hence, the correlations reported in our study could indicate that the high metabolic efficiency of the agroecological plots could lead to an increase in the soil storage of carbon in the long term, as a consequence of the fixation of carbon in the fungal biomass. In accordance with our results, De Vries et al. (2006) observed a decrease in the F:B ratio when synthetic fertilisation was employed, concluding that a higher fungal biomass indicates lower nitrogen leaching. It is possible that agroecological management, by promoting a higher metabolic efficiency and fungal dominance, could benefit nitrogen fixation in the microbial biomass, in this way preventing the leaching of nitrogen.

5. Conclusion

This study demonstrates the response of soil microbial communities to the adoption of agroecological management in extensive crop production. Our results suggest that agroecological management is characterised by fungal dominance in soil microbial communities and a higher microbial metabolic efficiency compared to a conventional management system. These characteristics demonstrate more efficient use of carbon substrates in agroecological systems, which could counteract the negative effect of lack of synthetic fertilisation and reduced-tillage in the long term. It is necessary to investigate the effect of alternative techniques to mineral fertilisation in order to complement cattle dung to ensure adequate provision of nutrients for crop production. However, in spite of the lack of synthetic fertilisation and the consequent reduction in general microbial enzymatic activity, agroecological management did not negatively affect soil fungal and bacterial abundance and increased the metabolic efficiency. This demonstrates that sustainable agricultural tools can be used effectively to preserve soil quality. Further research on agroecological practices is needed in order to generate information that allows farmers to make decisions on adopting this type of alternative management, which can provide food whilst reducing production costs and environmental risk.

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, CIAC 940140, and REDAE 1136021; and also by Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) through PIP N° 11220150100061CO and Secretaría de Ciencia y Tecnología, Universidad Nacional de Córdoba (SECyT-UNC).

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