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# Diversifying crop rotation increased metabolic soil diversity and activity of the microbial community

Luciana D'Acunto[a,b], José F. Andrade[c], Santiago L. Poggio[a,d], María Semmartin[a,b,*]

[a] IFEVA, Universidad de Buenos Aires, CONICET, Buenos Aires, Argentina
[b] Universidad de Buenos Aires, Facultad de Agronomía, Cátedra de Ecología, Av. San Martín 4453 (C1417DSE), Buenos Aires, Argentina
[c] Universidad de Buenos Aires, Facultad de Agronomía, Cátedra de Cerealicultura, Argentina
[d] Universidad de Buenos Aires, Facultad de Agronomía, Cátedra de Producción Vegetal, Argentina

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ABSTRACT

Agricultural intensification has increased food production by reducing crop diversity and increasing fertilization and crop protection. Unfortunately, intensification has also reduced soil ecosystem services. Diversifying crop rotations could be a feasible alternative to promote positive feedbacks between soil biota and soil properties. Here, we investigated the impact of diversifying crop rotations on functional composition and diversity of the heterotrophic soil bacterial communities. We studied three frequent rotations with a total number of crops ranging from two to four. Before the experiment, all plots were cultivated with soybean. In the first experimental year, the crop sequences were (1) fallow/soybean, (2) barley/soybean, and (3) field pea/maize. In the second year, all plots were subjected to a wheat/soybean double crop. The experiment was replicated in three locations of the Rolling Pampa (Argentina). Soil and plant sampling took place immediately after the soybean harvest, in the second year. The most diverse rotation (field pea/maize, wheat/soybean) showed the highest standing biomass and litter and the most metabolically diverse and active soil microbial community ($P \leq 0.05$). In turn, metabolic diversity was positively associated with plant and litter biomass ($r^2 = 0.7$) and with soil pH ($r^2 = 0.72$). Our results revealed that crop rotation affects soil metabolic bacterial diversity and activity ($P \leq 0.05$). The most diverse rotation (four different crops) had also the most diverse and active soil microbial biota, concomitantly with a higher plant biomass production and soil pH. Because soil microbial activity and metabolic diversity detected in specific rotations potentially contribute to soil aggregate formation and other soil properties intimately related with nutrient cycling and plant production, the negative effect of agricultural intensification could be attenuated by designing specific and more diverse crop rotations.

## 1. Introduction

Sustainably increasing crop production is critical for modern agriculture. Agricultural intensification fragments farmed landscape by enlarging field size and decreasing crop diversity to a handful of species. Because the expansion of cropland area is unlikely, increasing crop yields and using double crops are essential parts of agricultural intensification (Andrade et al., 2015). Therefore, modern agroecosystems provide more food, but depend more on external inputs and lose self-regulation capacity (Foley et al., 2005). In particular, intensification may dramatically affect soil properties responsible for residue decomposition, nutrient re-cycling, and soil formation (Zak et al., 2003; McDaniel et al., 2014a; Lange et al., 2015).

Feedbacks between plants and soil microbes represent an important dimension of ecosystem regulation, which has been addressed in different contexts and scales (Zak et al., 2003; Lange et al., 2015). In agricultural landscapes, land use (e.g. crops, pastures, woodlands, grasslands) alters carbon cycling and soil organisms (Guo and Gifford, 2002). Woody patches show lower litter decomposition than the cultivated matrix in which they are embedded because of their production of large amounts of recalcitrant tissue. Therefore, they accumulate more soil carbon and sustain more diverse soil microbial communities than surrounding cropped areas (D'Acunto et al., 2014, 2016). Within cropped areas, long-term crop rotations also accumulate more soil carbon and microbial biomass than monocultures, particularly when rotations include cover crops (McDaniel et al., 2014a; Tiemann et al., 2015; Venter et al., 2016). Mechanisms for this influence involve variation in litter chemistry, soil pH and nutrient contents (Zak et al., 2003; Fierer and Jackson, 2006; Lauber et al., 2008, 2009; Wickings et al., 2012; Venter et al., 2016).

* Corresponding author at: IFEVA, Universidad de Buenos Aires, CONICET, Buenos Aires, Argentina.
*E-mail address:* semmartin@agro.uba.ar (M. Semmartin).

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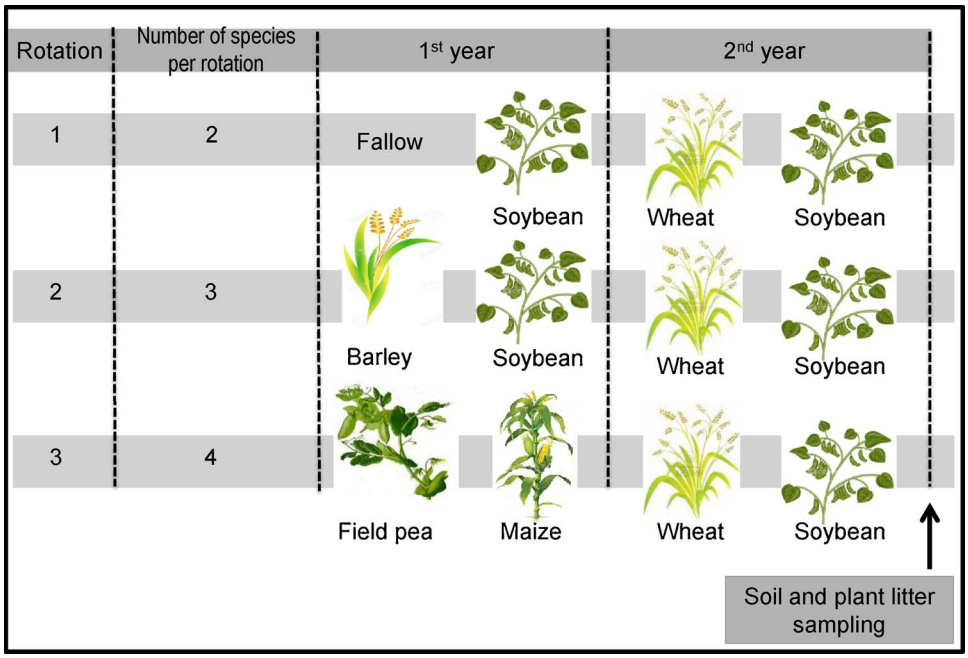


**Fig. 1.** Experimental design and sampling. Crop rotations differed in the composition and total number of different crop species (fallow/soybean,wheat/soybean, 2 species; barley/soybean, wheat/soybean, 3 species; and field pea/maize, wheat/soybean, 4 species). The same experimental design was replicated in three different locations (Junín, Pergamino and San Pedro) along a SW-NE 100-km transect in the Rolling Pampa (Argentina).

While the benefits of particular crop rotations in terms of yields, resource use, and pest control have been well established, those related to soil microbial organisms are less understood (Altieri, 1999; Liebman and Dyck, 1993; Govaerts et al., 2007; Andrade et al., 2015; Venter et al., 2016). Crop rotation involves changes in the amount, quality and timing of root metabolites, above-ground residue deposition, fertilization and pest control (Follett, 2001; Roger-Estrade et al., 2010). Therefore, the final impact of particular rotations on soil biota structure will depend on the net balance among such factors. Empirical evidence is scarce: even though rotations seem to increase microbial diversity compared to monocultures (Lupwayi et al., 1998; González-Chávez et al., 2010; Postma-Blaauw et al., 2010, Venter et al., 2016; but also see Jiang et al., 2016), the effects of different rotation schemes are not known. In schemes promoting sustainability, where monoculture is not conceived as a regular practice, the consequences of alternative rotations should be critically evaluated to identify the best combinations of crops. A complete knowledge would also require disentangling both the underlying mechanisms and the relationships with crop production.

The Rolling Pampa, the corn-belt of Argentina, provides a useful context to study crop rotations because agricultural intensification has been dramatic during the last decades (Baldi et al., 2006). In this region, intensively managed, continuous croplands replaced mixed systems that combined perennial pastures and annual crops. The widespread adoption of new technologies, such as no-tillage, fertilization, and genetically modified crops, as well as the increase of soybean international prices, led to a rapid removal of fencerows to enlarge fields. Rotations of maize, soybean and wheat/soybean double crops are dominant, even though double cropping of field pea/maize, barley/soybean, and rapeseed/soybean are also frequent to increase total biomass production and resource use efficiency (Andrade et al., 2015). In these cropping systems, relationships between crop yields and rotations have been well established (Caviglia et al., 2004; Andrade et al., 2015). Nevertheless, the effects of crop rotations on soil microbial communities remain contentious.

Here, we investigated the impact of diversifying crop rotations on the functional diversity of heterotrophic soil bacterial communities. We hypothesize that increasing the number of different crops, particularly by including double-cropping, increases the total inputs of root exudates and plant residues into soil in terms of both mass and substrate diversity. In turn, crop diversity might alter soil properties relevant to soil microbial structure and function such as soil pH. Therefore, a greater amount and diversity of resources will promote the growth and activity of different groups of the heterotrophic soil bacterial community.

## 2. Materials and methods

### 2.1. Study system

The study was carried out from 2010 to 2012 in three locations along a 100-km transect in the Rolling Pampa, northern of Buenos Aires province (Argentina): Junín (34°23′S; 60°48′W), Pergamino (33°55′S; 60°23′W), and San Pedro (33°47′S; 60°00′W). Climate is temperate subhumid, without a marked dry season but with frequent water deficit during summer. Mean annual rainfall is approximately 1000 mm and mean annual temperature is 17 °C (Hall et al., 1992). The frost period extends from mid-April to late-September. Soils are mostly Argiudolls, which are characterised by a topsoil horizon rich in organic matter and a clay accumulation subsurface horizon (Soriano et al., 1991). The original grassland vegetation was extensively ploughed, and nowadays continuous cropping dominates the landscape. Annual crops exceed 90% of the land surface, 8% is for feed cattle, and 2% correspond to uncultivated areas. Soybean occupies 60% of the area as a single crop, and 16% as a second crop right after wheat. Maize occupies 15% of the area and wheat is the main winter crop, with 13% of the sown area. Other winter crops are barley, rapeseed, and peas, with less than 5% of total cropped area (Satorre, 2011).

### 2.2. Experimental design and analyses

In each of the three locations (Junín, Pergamino and San Pedro), three crop rotations were implemented, based on their relevance in the region: in the first year, crops were (1) fallow/soybean, (2) barley/soybean and (3) field pea/maize, and in the second year there was a common double cropping of wheat/soybean (Fig. 1). In summary, rotations ranged from 2 to 4 different crops. Genotypes were those recommended as most productive in the region. Sowing dates, plant densities and row spacing were adjusted to the selected genotypes and the typical recommendations (Table 1). Each rotation had two replicates per location, each consisting of a 22 × 200 m plot. Because a

**Table 1**
Genotype, sowing date, plant density and nitrogen and phosphorus fertilization used in the three crop rotations with increasing number of species (fallow/soybean, wheat/soybean, 2 species; barley/soybean, wheat/soybean, 3 species; and field pea/maize, wheat/soybean, 4 species) in the three locations along a SW-NE 100 km transect in the Rolling Pampa (Argentina) (Andrade et al., 2015).

| Crop | Genotype | Sowing date | Plant density (plants/m2) | N-Fertilization (kg N/ha) | P-Fertilization (kg $P_2O_5$/ha) |
|---|---|---|---|---|---|
| **First year** | | | | | |
| Soybean | Don Mario DM (3810[1], 4670[2,3]) | October | 30–40 | 5 | 12–25 |
| Barley | Scarlett | June | 250 | 100–120 | 50–60 |
| Field pea | Vipper | July | 80–100 | 0 | 20 |
| Maize (second crop) | Dekalb DK747 | December | 6–7 | 60–80 | 50 |
| Soybean (second crop) | Don Mario (3700[1], 4670[2]), Nidera 4990[3] | Nov-Dec | 30–45 | 0 | 0 |
| **Second year** | | | | | |
| Wheat | Nidera – Baguette 11 | June | 280 | 160 | 60–80 |
| Soybean | Don Mario (4210[1], 3810[2], 4250[3]) | Dec–Jan | 30–40 | 0 | 0 |

[1] Junín.
[2] Pergamino.
[3] San Pedro.

hierarchical analysis with crop rotation nested in location did not reveal significant effects of location for any of the studied variables, we considered the locations as replicates (N = 9, n = 3) and the two plots per location as sub-replicates.

Experimental plots were located on commercial paddocks which had been previously cultivated with soybean. Crops were managed as most frequent practices to emulate regular commercial fields in the region. Therefore, no-till sowing system and fertilization at sowing were implemented. Soybean and field pea were inoculated with *Bradyrhizobium japonicum* and *Rhizobium leguminosarum* var. pisi, respectively. Crops were fertilized with nitrogen/phosphorus as urea, monoammonium phosphate or single superphosphate (Table 1). Weeds, insects and diseases were controlled with pesticides commonly used in the region. Briefly, for fallow, soybean and maize, total herbicides (glyphosate, paraquat) were applied. In barley and wheat dicamba was used for control of broadleaf weeds. Insects were controlled with chlorpyrifos, and fungal diseases were treated with products based on cyproconazole, tebuconazole, trifloxystrobin and azoxystrobin.

Soil and litter samples were collected immediately after soybean harvest in the second year (Fig. 1). In each sub-replicate, five soil samples (0–10 cm) were taken with a 2 cm diameter core and mixed in a composite sample. Approximately a third of each sample was kept at 4 °C until analyses of bacterial community and soil pH. Soil pH was estimated with an electronic pH-meter in a water solution:soil of 2.5:1. The remaining sample was sieved (2 mm mesh size) and conditioned for the estimation of potential soil respiration rate in laboratory. Plant litter was determined by collecting litter from soil surface, in six randomly located frames (0.4 × 0.4 m) in each replicate sample. Standing biomass (not including grain) of each crop was measured from three samples of 1 $m^2$ in each plot by cutting plants at ground level, then dried at 60 °C, and weighted (for more details see Andrade et al., 2015).

We characterised the composition and functional diversity of the heterotrophic bacterial community through community level physiological profile method (Garland and Mills, 1991 adapted by Di Salvo and García de Salamone, 2012) of each soil crop rotation. In sterile and single 200 µl microplates, we separately offered 15 different carbon sources to soil inocula from crop rotations. Carbon sources consisted of different compounds usually present in the rhizosphere. They included aminoacids (alanine, arginine, histidine, and proline), amine (putrescine), carboxylic acids (pyruvic and itaconic), carbohydrates (cellobiose, dextrose, mannitol, glycerol, rhamnose and xylose), a phenolic compound (benzoic acid), a polymer (tween 80), and a control with distilled water. Each well received 50 µl of a standard basal media, 50 µl of tetrazolium violet, which develops colour under $CO_2$ production. Finally, each well was inoculated with 50 µl from $10^{-4}$ soil suspensions corresponding to each rotation. Incubations were carried out at 25 °C for a maximum of 96 h. Well colour development was measured at 24, 48 and 72 h (only 48 h measurements are shown), as absorbance at 590 nm (Multiskan EX Spectrophotometer ®).

The optical density for each well was calculated by subtracting the control well values from each plate to the optical density value of the well (Garland and Mills, 1991). Microbial activity in each microplate was expressed using average well colour development (AWCD) and calculated following the method of Garland and Mills (1991). For richness estimation, we used an optical density of 0.25 as a threshold of a positive response (Garland, 1997). Therefore, our estimation of richness (S) of a given sample was the number of carbon sources with and optical density ≥ 0.25. Functional bacterial diversity was estimated using the Shannon-Wiener index (H'), which combines richness and evenness, as follows H' = Σ pi * (ln pi), where pi is the ratio between the optical density developed in each carbon source and the sum of all activities on the 15 substrates.

Potential soil respiration was quantified under controlled laboratory conditions along 3 months in soils coming from each of three sites (Junín, Pergamino and San Pedro) and the three crop rotations. We filled rectangular microcosms of 20 × 15 cm and 5 cm in height with 500 g of soil. The microcosms were incubated in darkness, at 25 °C for a maximum period of 90 days, without lid to avoid inhibitory effects due to carbon dioxide accumulation. Respiration was registered at 30, 60 and 90 days of incubation. Gravimetric water content of soil was maintained constant by adding distilled water by daily evaluation. Soil respiration rate was estimated with a portable, closed dynamic chamber (PPSystems, SRC-1, Soil $CO_2$ Flux System, UK). Briefly, this closed system estimates soil respiration by quantifying the variation in $CO_2$ concentration of the chamber during a limited lapse (up to 2 min). The soil chamber was provided with an external PVC collar that ensured a tight seal between the chamber and the PVC collars inserted into the soil (Le Dantec et al., 1999).

### 2.3. Statistical analyses

We first compared crop rotations by using a nested (hierarchical) design, with rotations nested within the location factor. Because location had no significant effect on any response variable, we averaged the two plots of each crop rotation per location and analysed the data by one-way ANOVAs with rotation as factor (n = 3). When statistical effects were detected, means were compared by Tukey tests. Catabolic profiles of the heterotrophic bacterial community of rotations were analysed using a PCA and the position on the first axis was compared through an ANOVA (Semmartin et al., 2010). In addition, bacterial use of individual substrates was analysed by independent ANOVA tests, with crop rotation as factor; when significant differences were detected, means were compared by Tukey tests. Soil respiration was analysed considering the incubation period (30, 60 and 90 days) as repeated

**Table 2**
Plant, soil and microbial traits of three crop rotations with increasing total number of species (fallow/soybean, wheat/soybean, 2 species; barley/soybean, wheat/soybean, 3 species; and field pea/maize, wheat/soybean, 4 species) in the three locations along a SW-NE 100 km transect in the Rolling Pampa (Argentina). Data show means with standard error within parentheses (n = 3). Different letters indicate significant differences among rotations.

| | Crop rotation | | |
|---|---|---|---|
| | Fallow/ Soybean | Barley/ Soybean | Field pea/ maize |
| Plant standing biomass ($g/m^2$)* | 891.4 (9.84) a | 1831.8 (67) b | 2361.1 (50.7) c |
| Plant litter ($g/m^2$)** | 506.3 (14) a | 748.2 (131.4) ab | 1067 (19.7) b |
| Soil pH*** | 5.32 (0.13) a | 5.25 (0.12) a | 5.82 (0.04) b |
| Soil potential respiration rate (μg $C\text{-}CO_2$ $m^{-2}$ $h^{-1}$) | | | |
| Day 30 ns | 43.3 (9.2) a | 36.6 (6.7) a | 46.6 (11.5) a |
| Day 60 ns | 20 (2.8) a | 30 (9.3) a | 35 (12.3) a |
| Day 90 ns | 13.3 (2.3) a | 20 (5.6) a | 23.3 (10.4) a |
| Community level physiological profiles | | | |
| Average well colour development (AWCD)*** | 0.26 (0.01) a | 0.28 (0.01) ab | 0.32 (0.02) b |
| Position on principal component (axis 1)*** | −1.63 (0.66) a | −0.85 (0.39) a | 2.48 (1.48) b |
| Metabolic richness (S) ns | 7.33 (0.17) a | 7 (0.33) a | 8 (0.5) a |
| Metabolic diversity (H') *** | 2.29 (0.03) a | 2.3 (0.03) a | 2.39 (0.01) b |

ns $P > 0.05$.
* $P < 0.001$.
** $P < 0.01$.
*** $P < 0.05$.

measures. Finally, metabolic diversity (Shannon index H') was related with standing biomass + litter and with soil pH by linear and non-linear regression analyses.

## 3. Results

Crop rotations had a consistent effect on plant and soil variables (Table 2). Plant standing biomass, litter, and soil pH varied among crop rotations. Standing biomass and litter were significantly greater in field pea/maize, in coincidence with a higher soil pH. Conversely, potential respiration rate did not differ among rotations (Table 2).

The community level physiological profiles also differed among rotations (Table 2, Figs. 2 and 3). The average well colour development, a measure of the overall metabolic activity, was greater in the most diverse rotation (field pea/maize, wheat/soybean) and lower in the least diverse one (fallow/soybean, wheat/soybean), while the barley/soybean, wheat/soybean rotation showed intermediate values

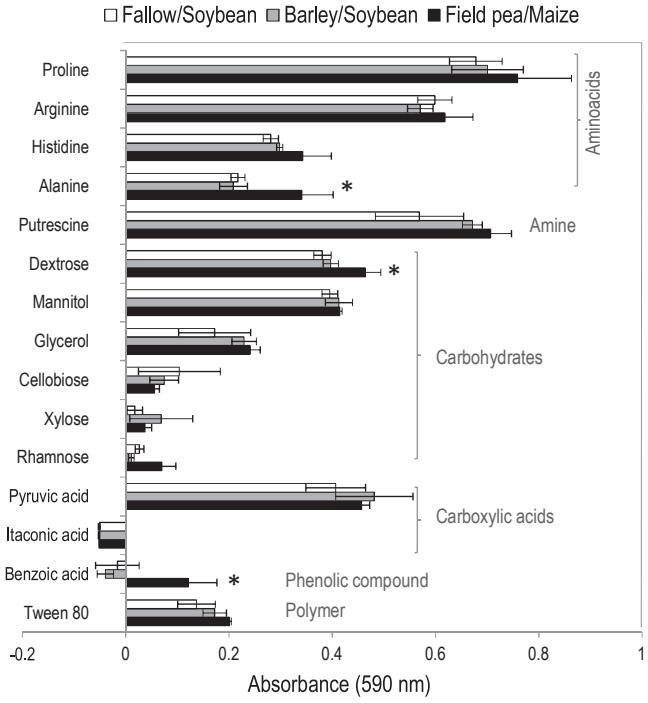


**Fig. 3.** Soil bacterial utilization of individual carbon sources, measured as absorbance at 590 nanometres. Soil inocula correspond to three crop rotations with increasing number of species: fallow/soybean, wheat/soybean (2 species); barley/soybean, wheat/soybean (3 species); and field pea/maize, wheat/soybean (4 species). Soil samples were collected in the second year, after soybean harvest (see Fig. 1 for more details). The experiment was replicated in three locations along a SW-NE 100-km transect in the Rolling Pampa (Argentina). Asterisks denote significant differences among rotations ($P \leq 0.05$).

(Table 2). Consistent with this result, the first axis of the principal component analysis (accounting for 37.7% of total variation), separated the field pea/maize rotation from the others (fallow/soybean and barley/soybean) (Fig. 2, Table 2). Overall, aminoacids/amine and carbohydrates were more used by the bacterial community than carboxylic acids, polymers and phenolic compounds (Fig. 3). Four carbon sources accounted for most of the variation of the catabolic profiles: two amino-acids, alanine and proline, one carbohydrate, dextrose, and benzoic acid revealed a greater bacterial utilization in the field pea/maize, wheat/soybean soil incubations (Figs. 2 and 3). In turn, within the carboxylic acids, pyruvic acid was more metabolized than itaconic acid (Fig. 3). Catabolic diversity (Shannon-Wiener index H') of field pea/maize, wheat/soybean rotation was greater than the rest, although the number of utilized carbon sources (richness S) did not significantly

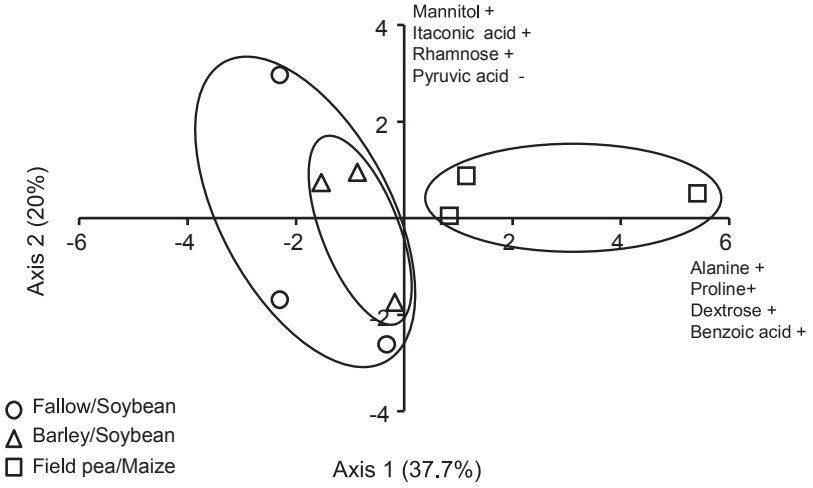


**Fig. 2.** Principal components analysis of community level physiological profiles of three crop rotations with different total crop diversity (2, 3, and 4 species): fallow/soybean, wheat/soybean; barley/soybean, wheat/soybean; and field pea/maize, wheat/soybean. Samples were obtained in three locations along a SW-NE 100-km transect in the Rolling Pampa (Argentina). Carbon sources on both axes are those with greater, positive (+) or negative (−), variation in the bacterial activity pattern (larger eigenvectors).

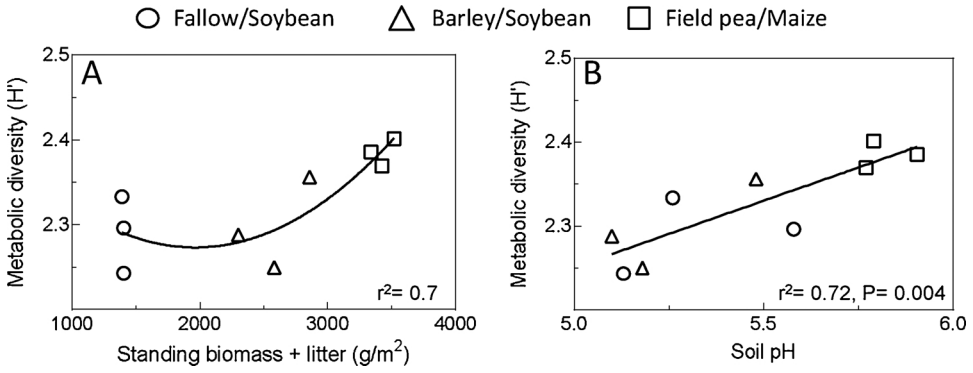


**Fig. 4.** Metabolic soil bacterial diversity (Shannon-Wiener index H') as a function of (A) plant standing biomass + litter, and (B) soil pH, from three crop rotations with increasing total number of species: fallow/soybean, wheat/soybean (2 species); barley/soybean, wheat/soybean (3 species); and field pea/maize, wheat/soybean (4 species). The experiment was replicated in three locations along a SW-NE 100-km transect in the Rolling Pampa. Points represent the average of each crop rotation in each location. Lines represent the least square fit of polynomic quadratic and linear functions respectively.

differ among rotations (Table 2). In turn, metabolic diversity positively correlated with standing biomass + litter and with soil pH (Fig. 4A and B).

## 4. Discussion

Here we showed that different rotations including two to four different crops modified soil and microbial properties, even when soil sampling took place right after the harvest of a common final crop sequence (wheat/soybean). The most diverse rotation (field pea/maize, wheat/soybean) showed the highest crop standing biomass, litter, soil pH, microbial metabolic diversity and activity. Moreover, metabolic diversity positively correlated with plant biomass, litter, and soil pH. The functional composition of soil heterotrophic bacterial community of this rotation also differed from the rest. Rotations did not differ in the number of carbon sources utilized or in potential soil respiration. In summary, the most diverse rotation had greater functional diversity of heterotrophic soil bacterial communities. This difference cannot be attributed to a short-term effect of different crop species because treatments were assessed at the end of a common crop sequence.

Our results showed that rotations differing in crop species number and composition impacted on the functional metabolic profile and diversity of heterotrophic soil microbial communities. The consumption of carbon substrates showed that the field pea/maize, wheat/soybean rotation, the one with greater number of crops species (four), metabolized more benzoic acid (phenolic compound), and specific aminoacids and carbohydrates (alanine and dextrose respectively) than the other rotations. This rotation had also a more intense substrate use (AWCD) and a greater metabolic diversity. A recent meta-analysis documented that crop rotation increases soil microbial diversity with respect to monocultures by an average of 3.5% (Venter et al., 2016), in coincidence with the pattern documented for grasslands (Zak et al., 2003; Lange et al., 2015). The magnitude and direction of the effect varied with crop species and the years of monoculture. About two thirds of the studies, including a large variety of crops and agronomic scenarios (e.g. tomatoes, potatoes, cucumber, wheat, maize, pastures, etc.), showed no differences of microbial diversity between monocultures and rotations, one third found positive rotation effects (Venter et al., 2016). In this context our study shows that even in sustainable agroecosystems, where monoculture is not conceived as a recommended practice, a rotation including four crops may increase soil microbial metabolic diversity by about 4% with respect to a two-species rotation.

There is an increasing understanding of the role of plant diversity on belowground ecosystem function through the effects on soil biota in both semi natural (Zak et al., 2003; Lange et al., 2015) and agricultural systems (Tiemann et al., 2015; Venter et al., 2016). More diverse plant communities are associated with greater primary production due to a more efficient and complementary spatial and temporal use of resources (Tilman et al., 2001). Accordingly, they produce greater quantity and diversity of litter and root exudates. Soil microbial communities respond with greater biomass, diversity and activity, which positively feedback on plant nutrition and productivity (Zak et al., 2003; Lange et al., 2015). Ultimately, these positive feedbacks impact on stable aggregate soil formation, which can sustain a greater plant productivity through an increased provision of water and nutrients (Lange et al., 2015). In agricultural systems, adding one or more crops in rotation to a monoculture increased soil aggregation, organic carbon, total nitrogen, microbial activity and microbial biomass (McDaniel et al., 2014a, 2014b; Tiemann et al., 2015). Our findings on functional diversity and composition of soil biota under different rotations suggest that these critical feedbacks can be strengthened by designing particular and more diverse crop rotations. Our results are consistent with the fact that the more diverse field pea/maize-wheat/soybean rotation had a greater and more efficient resource use by intercepting more total photosynthetically active radiation and having a greater yield and biomass production than less diverse rotations (Andrade et al., 2015). In turn, the lower metabolic diversity and colour intensity developed by the least diverse rotation, where soybean dominated (fallow/soybean-wheat soybean), coincided with the negative effects on metabolic diversity previously detected in legumes (Lupwayi et al., 2012b; McDaniel et al., 2014b; Lange et al., 2015).

Although this experiment did not allow us to separate the effects of crop diversity, biomass, identity, and management, the influence of each factor may be hypothesized based on previous evidence. While in the present study crop rotation effects on plant biomass were evident, other studies have documented species effects on rhizospheric and bulk soil microbial communities independently from plant biomass. In coincidence with our results, a recent study showed that maize exudates form more diverse rhizospheric microbial communities than soybean exudates (Wang et al., 2017). The dominance of Rhizobiales in soybean rhizosphere suggests that this group might outcompete other groups, reducing the diversity of soybean rhizosphere. In turn, empirical evidence showed that barley exudates promote soil dispersion around roots, whereas maize exudates promote soil aggregation, positively impacting on soil structure (Naveed et al., 2017). Mineral nutrient contents might also explain part of the variation observed in the functional diversity and composition of soil bacteria. Crops may dramatically alter their root exudates patterns under nutrient deficiency (Carvalhais et al., 2011). Nevertheless, as in our study nitrogen and phosphorus fertilization was relatively similar for different rotations, we do not consider this factor as a very relevant source of variation for the observed bacterial metabolic responses. Finally, the interaction of crop identity and/or biomass with other micro environmental properties such as soil pH may have also contributed to the observed patterns. The positive relationship between microbial metabolic diversity and soil pH documented in this study is consistent with the positive relationship largely documented at different spatial scales (Fierer and Jackson, 2006). Moreover, artificially increased soil pH in croplands showed stimulating effects on microbial activity (Kemmitt et al., 2006) which would positively interact with nutrient availability, soil aggregate formation and potential plant production.

The consumption of carbon substrates is a sensitive indicator of

variability in metabolic potential of the culturable portion of bacterial communities. Although the family of techniques that work with the culturable components of soil microbiota tend to better characterize the fast-growing populations (Smalla et al., 1998; Blagodatskaya and Kuzyakov, 2013), the community level physiological profiles have successfully detected functional microbial differences due to agricultural practices and changes in land use (Bending et al., 2004; Chaer et al., 2009; Lupwayi et al., 2012a; Brackin et al., 2013). Although we used fewer substrates than other studies, our substrates had representative members of the compound groups most frequently used. Consequently, our technique showed that the three rotations metabolized more aminoacids, amines and carbohydrates than carboxylic acids and polymers, in coincidence with findings from studies using more substrates (Brackin et al., 2013).

## 5. Conclusions

Rotations with different crop species affected the amount of litter, soil pH, and soil microbial composition and functional diversity. These impacts were detected even when sampling took place after a common final crop sequence (wheat/soybean), which reduced potential short-term effects given by different crops. Because higher soil microbial activity and metabolic diversity contribute to soil aggregate formation, and changes of soil pH are intimately related with nutrient cycling and plant production, the negative effect of agricultural intensification on soil ecosystem services could be attenuated by using diversified and particular crop rotations. These results broaden our understanding of soil microbial community in agro-ecosystems and its implications for ecosystem functioning.

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