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Uncultivated margins are source of soil microbial diversity in an agricultural landscape

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ABSTRACT

Agricultural intensification simplified environments, reduced their diversity, and hindered their ecosystem processes. Permanently vegetated areas (uncultivated margins) embedded in the cultivated matrix play a critical role in maintaining diversity and soil properties, and so mitigate the negative impact of intensification. We performed two studies aimed at evaluating the role of uncultivated margins on soil heterotrophic bacteria. In the first study, we sampled soybean fields and herbaceous and woody margins in three locations along a 100-kilometer transect. In a second study, in one location we sampled uncultivated margins and perpendicular 50-meter transects from each margin towards the centre of its adjacent soybean field. As control, we sampled similar transects in soybean fields that had cropped fields as margins. In both studies, we characterized the catabolic profiles and diversity of the heterotrophic bacterial community and soil properties. Soil microbial communities of uncultivated margins differed in composition and were more diverse than the cropped matrix. In turn, these differences positively correlated with soil pH. Woody margins also influenced the soil microbial composition, diversity and soil pH of neighbouring cultivated fields. In contrast, herbaceous margins did not influence their cultivated neighbours. These results broaden our understanding of soil heterotrophic bacterial community in agro-ecosystems and its implications for ecosystem functioning.

1. Introduction

Agricultural intensification fragmented, simplified and homogenized the landscape of the most productive lands of the world. This process involved an increasing use of inputs (e.g. fertilizers, pesticides) and the specialization in a few annual crops. As a result, agroecosystems produced more food, but lost some of their capacity to provide other services (Foley et al., 2005). In the Rolling Pampa region, mixed farming systems combining extensive animal husbandry with annual crops were largely replaced by continuous cropping (Baldi et al., 2006). As a consequence, food provision increased, but biodiversity (de la Fuente et al., 2006; Bilenca et al., 2007) and carbon sequestration decreased (Caride et al., 2012; Viglizzo et al., 2011).

Uncultivated elements (margins) embedded in the agricultural landscape increase spatial heterogeneity, provide habitat for many species (Burel et al., 1998; Tscharntke et al., 2005), and preserve ecosystem functions (Klein et al., 2003; Follain et al., 2007). In the Rolling Pampa, uncultivated margins are either herbaceous or woody and sustain diverse communities of plants, small mammal and arthropods that spill over adjacent cropped areas (Bilenca et al., 2007; de la Fuente et al., 2010; Poggio et al., 2010, 2013; Molina et al., 2014). Margins dominated by woody species are particularly complex and diverse, harbour a diverse herbaceous understory, and are more the result of an invasion process than a planned design (Ghersa et al., 2002). Their greater plant diversity and temporal cover seemed to boost larger soil carbon contents and litter (D'Acunto et al., 2014), which could also influence soil microbial diversity.

Landscape context (i.e. agricultural mosaic) and crop management critically affect soil properties and the composition and diversity of soil biota. At the landscape scale, the heterogeneity of land use (e.g. crops, pastures, woodlands, grasslands) alter carbon cycling and the organisms involved in this process (Liao and Boutton, 2008; Castro et al., 2010). Moreover, greater litter production and plant diversity are associated with more diverse soil microbial communities (Zak et al., 2003). Because plant species differ in histochemical composition, changes in plant diversity and composition might indirectly alter the composition and function of heterotrophic microbial communities (Zak et al., 2003). However, the impact of uncultivated margins in the Rolling Pampa types on soil microbial communities has not been evaluated yet. At the field

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scale, management decisions such as tillage system, crop sequence, fertilization and pest management, largely control both primary production and eventually the litter incorporated into soil (Follett, 2001). Ultimately, these practices alter soil biota by changing plant litter amount and quality, soil pH and nutrient availability (Zak et al., 2003; Fierer and Jackson, 2006; Lauber et al., 2008, 2009; Gomez and Garland, 2012).

Here we investigated to what extent the changes previously observed in soil properties of uncultivated margins and cultivated fields (D'Acunto et al., 2014) correspond to changes in heterotrophic soil microorganisms. First, we sampled the composition and functional diversity of the heterotrophic soil bacterial community and other soil properties in herbaceous and woody margins and in soybean fields, the most frequent land use. We sampled them in three locations along a northeast-southwest 100-km transect in the Rolling Pampa. Second, in one of the three locations, we identified uncultivated margins adjacent to fields cropped with soybean. In each of this 'margin-soybean' pairs, we sampled the margin and the first 50 m-interface with the soybean fields. We also sampled a third 'margin-soybean' pair as control, consisting of cropped fields (cultivated with maize or soybean) and the interface with the corresponding adjacent soybean field. Based on the properties of woody margins described above, we expected them to have the largest bacterial diversity and soil carbon stocks. We also expected woody margins to have the greatest effect on composition and functional diversity of soil bacterial community of neighbouring soybean fields as a consequence of the litter spill over previously detected (D'Acunto et al., 2014).

2. Materials and methods

2.1. Description of the study area and landscape elements

The study was carried out between 2011 and 2012 in the central Rolling Pampa which extends from 32°S to 34°S and 60°W to 61°W (Argentina). Climate is temperate sub-humid, without a marked dry season. Mean annual rainfall is ~1000 mm and mean annual temperature is 17 °C. Soils are Argiudolls, characterized by a subsurface horizon with clay accumulation (Soriano et al., 1991). Since the 1990s, reduced tillage, genetically modified crops, and higher soybean prices led to a strong agricultural intensification process (Viglizzo et al., 2011). Sown perennial pastures and natural grasslands were extensively cultivated and nowadays continuous cropping dominates the landscape. Most fencerows have been removed to enlarge and simplify the cropped area. Therefore, in the current landscape, spontaneous vegetation occur only as small, scattered fragments of semi-natural vegetation in grazing paddocks, wire-fencerows and roadside verges (Viglizzo et al., 2011).

Current landscape is characterized by three main elements: cultivated fields with soybean, representing the most frequent situation, and two uncultivated margin types dominated by spontaneous herbaceous vegetation (hereafter herbaceous margins) or woody vegetation (hereafter woody margins). These uncultivated margins represent between 1 and 2% of the landscape (Ghersa et al., 2002). Herbaceous margins are linear environments (5–10 m wide), year-round vegetated by annual and perennial species that account for 80% of landscape plant diversity (de la Fuente et al., 2010; Poggio et al., 2010). The most abundant species are grasses (*Cynodon dactylon*, *Digitaria sanguinalis*, *Lolium multiflorum*, *Poa annua* and *Paspalum dilatatum*) and forbs (*Apium leptophyllum*, *Artemisia annua*, *Anthemis cotula*, *Bidens subalternans*, *Capsella bursa-pastoris*, *Chenopodium album*, *Hypochaeris radicata*, *Matricaria chamomilla*, *Portulaca oleracea*, *Silene gallica*, *Tagetes minuta* and *Trifolium repens*). Woody margins have an average area of 1 ha, are covered by tree species and also have an herbaceous understory. The most abundant tree species are *Broussonetia papyrifera*, *Fraxinus* spp., *Gleditsia triacanthos*, *Ligustrum* sp., *Melia azedarach* and *Morus alba*, and the most abundant species of the understory are *Ammi majus*, *Bromus catharticus*, *Chenopodium album* and *Tagetes minuta*. Woody margins are not directly sprayed with herbicides, but they may receive drift from neighbouring crops. In contrast, herbaceous margins are usually sprayed with herbicides to reduce weeding. The cropped matrix encompasses fields of approximately 50 ha, most of them cultivated with soybean as single year crop or as a wheat–soybean double crop (Andrade et al., 2015). Crops are sprayed with systemic and contact pesticides during spring and summer, until harvest, in early autumn (Ferraro et al., 2003).

2.2. Sampling design and analysis

Firstly, we characterized the composition and functional diversity of the heterotrophic bacterial community and soil properties in herbaceous and woody uncultivated margins and in soybean fields. They were located in three areas along a northeast-southwest 100-km transect: San Pedro (33°47′S; 60°00′W), Pergamino (33°55′S; 60°23′W) and Junín (34°23′S; 60°48′W). Secondly, in a separate experiment only performed in Pergamino, we evaluated the influence of uncultivated margins on the composition and functional diversity of the heterotrophic bacterial community of adjacent field cropped with soybean.

2.2.1. Uncultivated margins and cropped matrix

In each location, we randomly selected 5 replicates for each uncultivated margin type (herbaceous and woody) and 2 replicates for the cropped matrix, represented by soybeans fields. In the spring (November, 2011), we sampled soil at each replicate and determined total and labile carbon, total nitrogen, pH, respiration rates, and the composition and functional diversity of the heterotrophic bacterial community.

In order to determine the functional composition and diversity (richness and evenness) of the heterotrophic bacterial community, in each replicate we sampled 5 soil cores of the top 10 cm and mixed them into a single composite sample. Soil samples were kept in the refrigerator (4 °C) until laboratory analyses. We used sterile micro plates that contained 96 wells with one of 17 carbon sources: amino acids (alanine, arginine, histidine, and proline), organic acids (benzoic acid, salicylic acid and pyruvic acid), a carboxylic acid (itaconic acid), carbohydrates (cellobiose, fructose, dextrose, lactose, mannitol, rhamnose and xylose), a fatty acid (Tween 80) and an alcohol (glycerol), and a blank with sterile distilled water (Garland and Mills, 1991 adapted by Di Salvo and García de Salamone, 2012). Each well received 50 μl of a standard basal media, 50 μl of tetrazolium violet, which develops colour under CO_2 production, and a soil aliquot of 50 μl from 10^{-4} soil suspensions. Incubations were 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 average well colour development of the 17 carbon sources of each sample was calculated. Then it was used to transform individual well values to eliminate variation in colour development caused by different cell densities (Garland, 1997). Functional diversity was calculated using the Shannon–Weaver index (H) which combines richness and evenness in the distribution of metabolic activity. Briefly, this index is calculated as the sum of the product of the optical density recorded at each carbon source and its own log for the 17 carbon sources used. We used an optical density of 0.25 as a threshold of a positive response (Garland, 1997). Catabolic richness is the number of different carbon sources that were used by the bacterial community (i.e. equivalent to species richness in the soil), and was calculated by counting all the positive optical density readings.

Catabolic evenness is a measure of the equitability of activities across all carbon sources, equivalent to species relative abundance in the soil (Lupwayi et al., 1998).

Soil respiration was quantified under controlled greenhouse conditions with a portable, closed dynamic chamber (PPSystems, SRC-1, Soil CO2 Flux System, UK) in microcosms containing soil from each situation (herbaceous-woody margins and soybean). 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). For this purpose, we used the same samples collected for heterotrophic bacterial community determination. Five hundred grams of soil were placed in individual trays of 20 cm × 15 cm and 5 cm in height. In each tray we placed a litterbag with 1 g of soybean litter (standard substrate), previously collected from the study site. Litterbags were covered with 1 cm of soil and were softly pressed to enhance soil–litter contact. The experimental units were randomly assigned to a site in the greenhouse and incubated at 25 °C for a maximum period of 90 days. Gravimetric water content of soil was maintained constant by adding distilled water by daily evaluation. After litterbags were harvested (at 30, 60, and 90 days of incubation), remaining soil was incubated for 48 h in darkness and then soil respiration rate was estimated.

Soil properties were estimated by coring the top 15 cm of the soil profile (each replicate was a composite sample of 5 subsamples). Samples were kept in the refrigerator (4 °C) until determination. Total organic carbon and nitrogen were determined by Walkley Black and Kjeldahl methods respectively and labile carbon was determined by the density fractionation method (Richter et al., 1975). Soil pH was measured with an electronic pH meter in a water solution: soil of 2.5:1.

2.2.2. Influence of margins on neighbouring croplands

To investigate the effect of each margin type on the composition and functional diversity of the interface with adjacent soybean fields, in Pergamino (November, 2012), we selected soybean fields of approximately 50 ha, adjacent to (a) an herbaceous margin, (b) a woody margin, or (c) another soybean or maize crop (cropped margin) ($n = 5$). In each replicate (margin-soybean field interface), we sampled points along a perpendicular transect from the margin toward the soybean field. Transects were randomly initiated along the field margin, avoiding corners, gates, troughs, ditches and any other margin discontinuity (Poggio et al., 2010). Points inside the soybean fields were sampled at increasing distances from the margin: 0 m (fencerow), 2 m, 4 m, and 50 m. Distances were established based on the assumption that differences in soil microorganisms would be more probable in micro sites closer to the margin. At each point of the interface we collected a composite sample from five soil cores from the top 10 cm of the soil profile and estimated the composition and functional diversity of the heterotrophic bacterial community and soil pH following the same procedures described above.

2.3. Statistical analysis

For the first study, soil carbon (total and labile), total nitrogen, soil pH, soil respiration rate, microbial functional diversity (Shannon–Weaver index), catabolic richness and evenness were analyzed in a nested design, with landscape elements nested within the location factor (San Pedro, Pergamino and Junín). Because location had no significant effect on any of the analyzed variables, we calculated an average for each landscape element (uncultivated margins and soybean) per location and data was analyzed using a one-way ANOVA ($n = 3$). When statistical effects were detected ($p \leq 0.05$) means were compared by Tukey tests. Catabolic profiles of the heterotrophic bacterial community were analyzed using a PCA and the position of treatments along the first axis was compared through an ANOVA (Semmartin et al., 2010). In addition, functional diversity, soil carbon (total and labile), soil total nitrogen and soil pH were related by regression analyses. Soil respiration was analyzed in a factorial design considering the landscape element, the incubation period (30, 60 and 90 days) and their interaction as sources of variation. Because the interaction between landscape element and incubation period had no significant effect, we calculated an average for each landscape element per location and data were analyzed using a one-way ANOVA ($n = 3$).

For the second study, at each point along the margin-crop interfaces, catabolic profiles of the heterotrophic bacterial community of margin types were analyzed as described above for landscape element, using a PCA and the position of treatments along the first axis was compared through an ANOVA (Semmartin et al., 2010). Functional diversity and soil pH were evaluated by linear and non linear regressions against the distance from margin. Then, we compared each point of the margin-crop interfaces (2 m, 4 m and 50 m) using a one-way ANOVA. When statistical effects were detected ($p \leq 0.05$) means were compared by Tukey tests. Finally, functional diversity and soil pH were related by regression analysis.

3. Results

3.1. Uncultivated margins and cropped matrix

Landscape elements (herbaceous and woody margins, and soybean fields) significantly differed in most of the studied variables, whereas locations were similar (Table 1).

Landscape elements differed in the functional composition of their soil heterotrophic bacterial community (Fig. 1). The first axis explained 69.5% of the total variance and the second explained 12% (Fig. 1, left panel). The three landscape elements occupied different positions on the first axis ($F_{2,33} = 24.75$, $p = 0.0001$, data not shown). Four carbon sources accounted for the greatest variation of the catabolic profiles. Woody margins showed larger carbon dioxide production on two carbohydrates, rhamnose and xylose, and one amino acid, alanine, and lower on benzoic acid (Fig. 1, left panel). In addition, woody margins displayed a greater heterogeneity among sites (San Pedro, Pergamino and Junín) than the others, whose

Table 1

ANOVA (F values) results of three landscape elements (herbaceous and woody margins and soybean fields) in three locations (San Pedro, Pergamino and Junín) along a northeast-southwest 100-km transect in the Rolling Pampa compared by a nested design where landscape element is nested within location (location > landscape element).

Soil and microbial biota variables	Variation sources	
	Location	Location > element
Total carbon	0.08	3.81^{**}
Labile carbon	0.32	3.47^{**}
Total nitrogen	0.56	3.04^{*}
pH	1.92	6.72^{***}
Soil respiration rate under controlled conditions		
After 30 days	0.10	3.11^{*}
After 60 days	1.69	1.11
After 90 days	1.11	0.64
Microbial diversity (Shannon–Weaver index)	0.57	5.44^{**}

* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

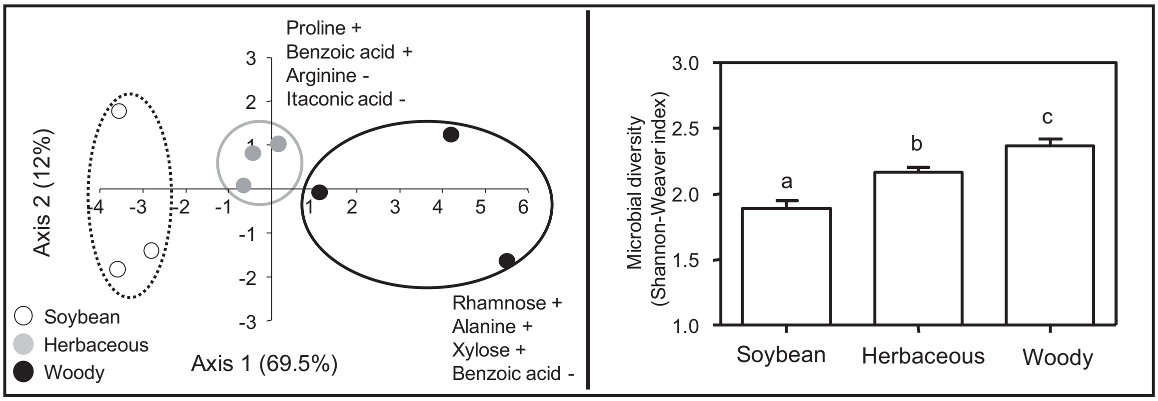

Fig. 1. Principal components analysis of catabolic soil profiles (left) and microbial diversity (right) of three landscape elements: cropped (soybean) and two uncultivated margin types (permanent herbaceous and woody vegetation) in three locations (San Pedro, Pergamino and Junín) along a northeast-southwest100-kilometer transect in the Rolling Pampa. On the left, each point represents the mean of each landscape element at each location. Carbon sources on both axes are those that showed greater, positive or negative, variation in the bacterial activity pattern (larger eigenvectors). On the right, each bar represents the mean of the three locations, error bars indicate +1 standard error and different letters indicate significant differences, $p \leq 0.05$).

points were more closely grouped on the first axis (Fig. 1, left panel).

The microbial functional diversity index also differed among elements (Fig. 1, right panel). It was highest in the woody margins, intermediate in the herbaceous margins and lowest in the soybean fields ($F_{2,33} = 18.4$, $p = 0.0001$). Catabolic richness paralleled the response of the diversity index ($F_{2,33} = 15.8$, $p = 0.0001$, data not shown). Catabolic evenness was also highest in the woody margins, but herbaceous margins did not differ from soybean fields ($F_{2,33} = 6.9$, $p = 0.003$, data not shown).

Soil respiration rates of woody margins under controlled incubations were significantly greater than the rest after 30 days of incubation ($F_{2,6} = 24.2$, $p = 0.001$, Table 2), and greater than soybean fields after 60 days of incubation ($F_{2,6} = 3.9$, $p = 0.05$, Table 2). These differences were no longer detected after 90 days of incubation ($p = 0.2$, Table 2).

Landscape elements diverged in their soil properties. Soil total carbon of woody margins was 40% greater than the rest, whereas the labile fraction showed larger, though more variable, contents. Soil total nitrogen of woody margins was 50 and 30% greater than soybean and herbaceous margins, respectively (Table 2). Soil pH also differed between uncultivated margins and soybean (Table 2). Woody margins showed more neutral values, soybean fields were slightly acidic and herbaceous margins had intermediate values.

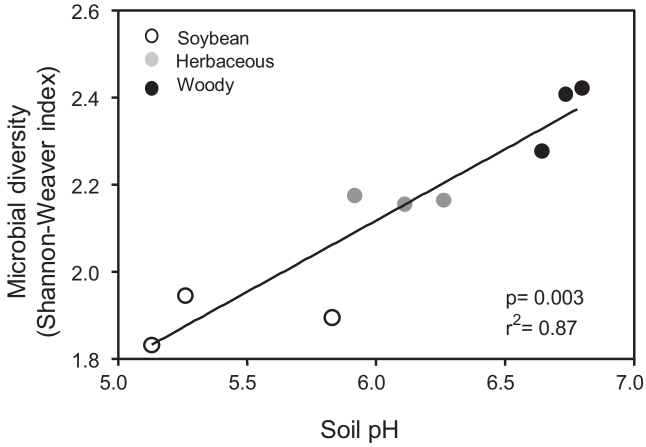

Fig. 2. Relationship between microbial diversity and soil pH in three landscape elements: soybean fields and uncultivated margins with permanent herbaceous or woody vegetation. Samples were obtained in three locations (San Pedro, Pergamino and Junín) along a northeast-southwest 100-km transect in the Rolling Pampa (Argentina). Each point represents the mean of each landscape element at each site. Best-fit line is shown.

Table 2
Soil properties and respiration rates of three landscape elements (herbaceous and woody margins and soybean fields) along a 100-km transect in the Rolling Pampa. Data show means of each margin type, with ±1 standard error in parentheses.

Soil and microbial biota variables	Landscape element		
	Soybean	Herbaceous margin	Woody margin
Soil total carbon (%)**	2.35^{a} (0.35)	2.54^{a} (0.34)	4.33^{b} (0.71)
Soil labile carbon (%)*	0.40^{a} (0.08)	0.69^{a} (0.73)	1.34^{b} (0.79)
Soil total nitrogen (%)**	0.21^{a} (0.03)	0.23^{a} (0.11)	0.36^{b} (0.09)
Soil pH***	5.41^{a} (0.46)	6.16^{b} (0.53)	6.72^{c} (0.44)
Soil respiration rate under controlled conditions (mg CO_2/m^2 h)			
After 30 days***	0.04^{a} (0.01)	0.07^{a} (0.01)	0.11^{b} (0.01)
After 60 days*	0.02^{a} (0.01)	0.04^{ab} (0.01)	0.05^{b} (0.01)
After 90 days	0.01^{a} (0.01)	0.02^{a} (0.01)	0.03^{a} (0.01)

Different letters indicate significant differences among margin types from an ANOVA test.
[*] $p < 0.05$.
[**] $p < 0.01$.
[***] $p < 0.001$.

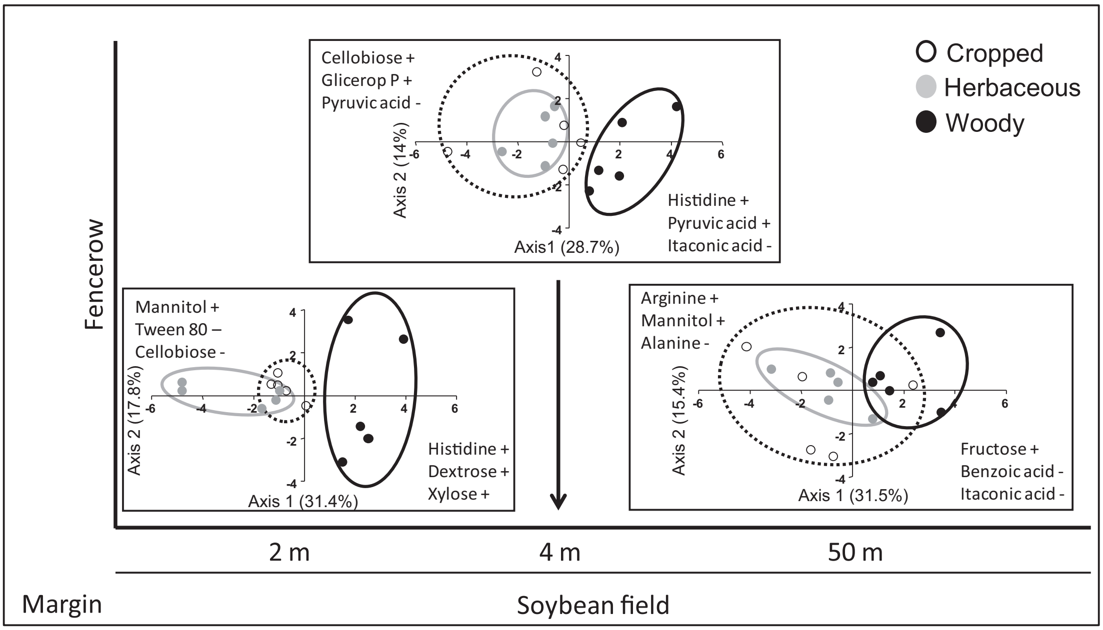

Fig. 3. Principal component analysis of catabolic soil profiles across margin-soybean field interface for three margins types in Pergamino: cropped, herbaceous and woody margins. Values on the soybean field line indicate the distance from the fencerow (in meters). Carbon sources indicated on both axes are those that showed greater, positive or negative, variation in the bacterial activity pattern (larger eigenvectors).

Soil functional diversity was positively and highly correlated with soil pH (Fig. 2).

3.2. Influence of margins on their neighbouring croplands

Woody margins affected the functional composition of the heterotrophic soil bacterial community of their neighbouring soybean fields. The bacterial composition of the 50 m interface associated to woody margins significantly differed from the interfaces associated to herbaceous and crop margins (Fig. 3). The position of the woody-margin interface on the first axis of the principal component analysis was consistently different from the rest (2 m: $F_{2,11} = 17.03$, $p = 0.0004$; 4 m: $F_{2,11} = 5.76$, $p = 0.01$; 50 m: $F_{2,11} = 4.84$, $p = 0.03$) (Fig. 3). The identity of the most indicative carbon sources varied among distances from the margin, although histidine appeared more related to higher values of the first ordination axis for samples close to the margin (2 and 4 m) whereas it was not important in the centre of the field (50 m) (Fig. 3). Overall, interfaces corresponding to herbaceous and cropped margins showed a relatively similar heterotrophic soil bacterial composition along the 50 m of margin-field interface (Fig. 3).

Woody margins also influenced the microbial functional diversity of their neighbouring soybean fields. Microbial diversity of woody-margin interfaces was greater than the interfaces associated to other margin types, and these differences decreased as the distance from the margin increased (Fig. 4, upper panel; 2 m: $F_{2,11} = 7.19$, $p = 0.008$, 4 m: $F_{2,11} = 2.92$, $p = 0.01$, 50 m: $F_{2,11} = 2.04$, $p = 0.17$).

Woody margins increased soil pH of their neighbouring soybean fields only at 2 m ($F_{2,11} = 4.04$, $p = 0.02$) (Fig. 4, intermediate panel). As with microbial functional diversity, soil pH differences vanished at increasing distances from the margin (Fig. 4, intermediate panel). In turn, microbial functional diversity was positively correlated with soil pH at 2 and 4 m, but the pattern depended on margin type (Fig. 4, lower panel). At 50 m we did not detect any correlation between these variables ($p > 0.05$).

4. Discussion

Uncultivated margins had a different and more diverse soil microbial biota than the cropped matrix and a moderate capacity to influence their neighbouring croplands. Soil microbial communities of uncultivated woody and herbaceous margins had a different catabolic profile and were more diverse than those of soybean fields. In turn, woody margins had higher soil pH. Woody margins also influenced soil microbial composition, diversity and soil pH of their neighbouring cultivated fields while herbaceous margins did not. Therefore, even representing an extremely reduced fraction of the total area, uncultivated margins seem to be effective for conserving landscape soil microbial diversity and sequestering carbon, without soil acidification. Furthermore, these findings highlight the need to broaden our understanding of the factors governing the final effect of trees on these ecosystems.

Uncultivated woody margins had a different, more diverse and active soil microbial community than herbaceous margins and crops. This greater diversity coincided with greater soil total carbon, total nitrogen and pH, and with larger amounts of a less decomposable litter (D'Acunto et al., 2014). The positive relationship between microbial diversity and soil pH validates the patterns recently documented for regional and local scales (Fierer and Jackson, 2006; Lauber et al., 2009; Rousk et al., 2010). Nevertheless, the higher pH and microbial diversity of woody margins with respect to the cropped matrix contradicts the generalized soil acidification and loss of biological fertility documented for afforestation and spontaneous encroachment (Chen et al., 2008; Berthrong et al., 2009a,b; Eldridge et al., 2011; Berhongaray et al., 2013). We need to disentangle the conditions that, as in our case, determine greater soil pH and a more diverse and active microbial community under tree cover (Zavaleta and Kettley, 2006; Tighe et al., 2009; Alberio and Comparatore, 2014; Fu et al., 2015).

The dominating patterns in the literature on afforestation may not properly reflect other situations that, as our woody margins, combine a diverse community of tree species with eventually different effects. Since the most widespread species used in

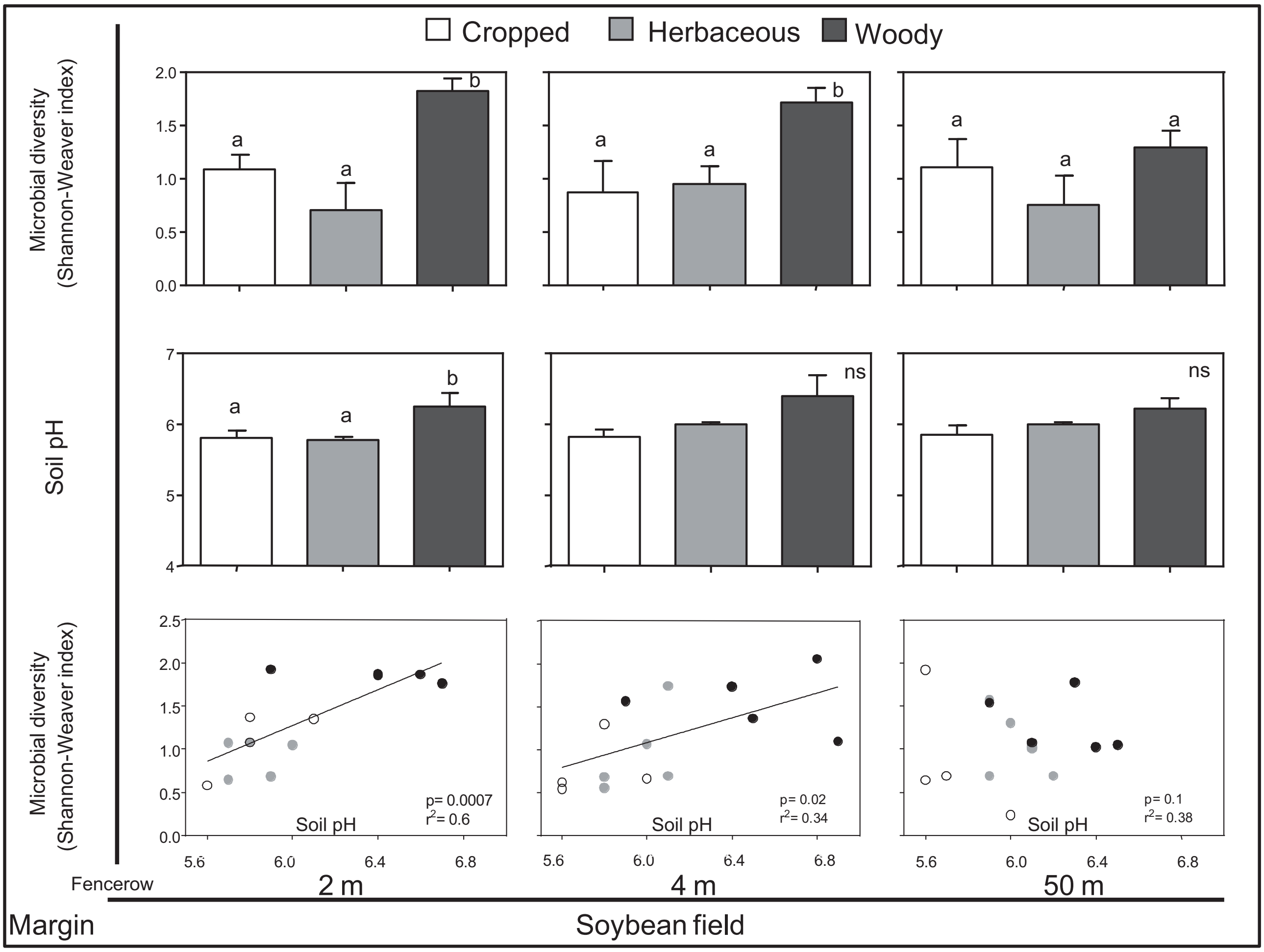

Fig. 4. Microbial diversity (upper panel), soil pH (intermediate panel) and relationship between microbial diversity and soil pH (lower panel) at 2, 4 and 50 m point across the margin-soybean field interface for three margins types in Pergamino: cropped, herbaceous and woody margins. Values on the soybean field line indicate the distance from the fencerow (in meters). In upper and intermediate panels, data are means ($n = 5$), error bars indicate +1 standard error and different letters indicate significant differences, $p \leq 0.05$). In lower panel, each point represents the average of each sampling point (2, 4 and 50 m) in each margin-soybean field interface and the lines represent the best-fit line.

afforestation are rapid-growth coniferous and myrtaceous (mainly *Pinus* and *Eucalyptus* genera), they strongly influence the vertical distribution of soil exchangeable cations and soil acidity (Jobbágy and Jackson, 2003). In the woody margins of the Rolling Pampa, trees were not planted with a productive purpose, but resulted from an invasion process promoted by the introduction of zero tillage (Ghersa et al., 2002). Unlike monospecific tree plantations, woody margins are vegetated by more than one tree species and also have an herbaceous understory. Therefore, the greater plant diversity and cover throughout the year, combined with a lower canopy density (Ghersa et al., 2002; Zalba and Villamil, 2002), seemed to boost soil microbial biota diversity and potential respiration rate.

Woody margins moderately influenced soil of their neighbouring croplands. This margin-crop relationship has received little attention. Here we showed that the proven role of margins as refuge for plants and animals (Poggio et al., 2010; Weyland et al., 2012; Molina et al., 2014) is also found for microbes, presumably by offering a greater variety resources and micro-sites. While margin influence on neighbouring crop might vary from 100 meters for arthropods to a several kilometres for birds (Weyland et al., 2012; Molina et al., 2014), we found a woody footprint on soil bacterial composition even at 50 meters from margin. The reduction of litter mass and soil carbon as the distance from margin increased (D'Acunto et al., 2014) suggests the existence of a positive feedback among litter fall, organic matter accumulation, and soil pH. In spite of their high functional microbial diversity, herbaceous margins did not affect neighbouring soybean fields. The frequent herbicide applications they receive affect their species composition (Poggio et al., 2010) and presumably explain their reduced plant litter and soil carbon accumulation and, ultimately, the lack of influence on their neighbouring fields (D'Acunto et al., 2014).

In conclusion, soil microbial composition and functional diversity distribution in the Rolling Pampa is spatially structured according to landscape patterns of vegetation margins and soil properties. Uncultivated margins acted as source of microbial diversity and those with trees spilled over into the neighbouring cultivated fields. Their greater soil microbial diversity occurred along with changes in other ecosystem properties which may positively impact on regulation and support ecosystem services. These results broaden our understanding of soil microbial community in agro-ecosystems, and its implications for ecosystem functioning.

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