Crop monoculture rather than agriculture reduces the spatial turnover of soil bacterial communities at a regional scale

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Summary

The goal of this study was to investigate the spatial turnover of soil bacterial communities in response to environmental changes introduced by the practices of soybean monoculture or crop rotations, relative to grassland soils. Amplicon sequencing of the 16S rRNA gene was used to analyse bacterial diversity in producer fields through three successive cropping cycles within one and a half years, across a regional scale of the Argentinean Pampas. Unlike local diversity, which was not significantly affected by land use type, agricultural management had a strong influence on β-diversity patterns. Distributions of pairwise distances between all soils samples under soybean monoculture had significantly lower β-diversity and narrower breadth compared with distributions of pairwise distances between soils managed with crop rotation. Interestingly, good agricultural practices had similar degree of β-diversity as natural grasslands. The higher phylogenetic relatedness of bacterial communities in soils under monoculture across the region was likely determined by the observed loss of endemic species, and affected mostly to phyla with low regional diversity, such as *Acidobacteria*, *Verrucomicrobia* and the candidates phyla *SPAM* and *WS3*. These results suggest that the implementation of good agricultural practices, including crop rotation, may be critical for the long-term conservation of soil biodiversity.

Introduction

No-till (also known as direct drilling and zero tillage) is an agricultural practice in which crop residues from previous harvesting are left on the soil surface, and the soil is not disturbed other than by the passage of the drill coulters. By reducing tillage, soil erosion is prevented, carbon storage is increased and available moisture is used more efficiently, making the soil management more sustainable (Díaz-Zorita *et al.*, 2002; Derpsch *et al.*, 2010). In Argentina, no-till presently dominates cropping practices, covering almost 26 million hectare, i.e. 75% of the total cultivated area (source: AAPRESID; www.aapresid.org.ar). On the basis of the associated gain in productivity, this conservation agricultural practice has been steadily gaining acceptance by farmers.

Regrettably, market forces have encouraged farmers to grow soybean in monoculture. This is often combined with low nutrient restoration, which eventually may offset the advantages of no-tillage, a practice that should be accompanied by additional actions to ensure sustainability. These include permanent organic soil cover, required to improve water storage and to avoid negative effects of no-till on soil architecture (Shaver *et al.*, 2002), and crop rotation, which is needed to reduce pathogen carryover on crop residues from previous harvesting (Bockus and Shroyer, 1998).

Loss of biodiversity caused by intensive agriculture is a major worldwide concern. Declining of species related to intensive agriculture has been documented for several organisms, such as birds (Bockus and Shroyer, 1998), insects (Tscharntke *et al.*, 2008), stream invertebrates (Beketov *et al.*, 2013), butterflies (Ekroos *et al.*, 2010) and soil macrofauna (Domínguez *et al.*, 2010). However, despite the crucial role of bacteria in the cycling of nutrients, carbon storage and plant growth, the impact of no-till agriculture with either crop rotation or monoculture on bacterial diversity in soil is not well understood.

Many previous studies investigating the impact of agricultural practices on microbial diversity focused on the

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richness and total abundance of bacteria at a small geographical scale (α-diversity), which did not appear to be the most suitable way to detect clear differences, even across highly contrasting land uses (Domínguez *et al.*, 2010). On the other hand, valuable insights into the mechanisms of community assembly can be gained by examining between-habitat differences in species composition along ecological gradients (Chase and Myers, 2011). Spatial turnover is a measure of β-diversity that determines how species composition changes across two or more local assemblages or across local and regional assemblages (Koleff *et al.*, 2003). Because local and regional diversity are related to each other, comparing processes of community assembly along biogeographic gradients also requires the testing of the size of the regional species pool (γ-diversity) (Chase and Myers, 2011). Patterns of spatial diversity have led to a greater understanding of the effect of the transition to agriculture of forest soils (Rodrigues *et al.*, 2013) and scrublands (Ding *et al.*, 2013). Similarly, studies of temporal variability in bacterial community have been useful in elucidating the dependence of bacterial dynamics on land use type (Lauber *et al.*, 2013). These previous studies have convincingly demonstrated the influence of human activities on the spatial and temporal turnover of microbial communities. Yet to our knowledge, these approaches have not been used to address less contrasting, but ecologically relevant scenarios, such as the impact on microbial biodiversity associated with monoculture farming.

The goal of this study was to investigate the spatial turnover of soil bacterial communities at the regional level, in response to environmental changes introduced by the practices of soybean monoculture and crop rotations, including soybean, in comparison to natural grassland. Patterns of β-diversity of bacterial communities were examined in no-till production fields subjected to contrasting crop rotation regimes under realistic conditions, replicated in four different locations across a scale of 400 km, during three successive cropping seasons. We hypothesized that β-diversity across a relatively large spatial scale would likely be sensitive to agricultural management even if local diversity was not affected. The results show evidence that crop monoculture drives the homogenization of bulk soil bacterial communities by leading to the loss of endemic species.

Results and Discussion

Bacterial community structure of soil in the Pampa region

The agricultural sites were selected in according to the operational description of good no-till agricultural practices (GAP) and poor no-till agricultural practices (PAP), defined in terms of crop rotation, fertilization, agrochemicals use and pest control (Wall, 2011; Figuerola *et al.*, 2012). The bacterial communities exhibited an overall phylum-level distribution pattern characteristic for agricultural soils (Fig. 1). Taken together, the most abun-
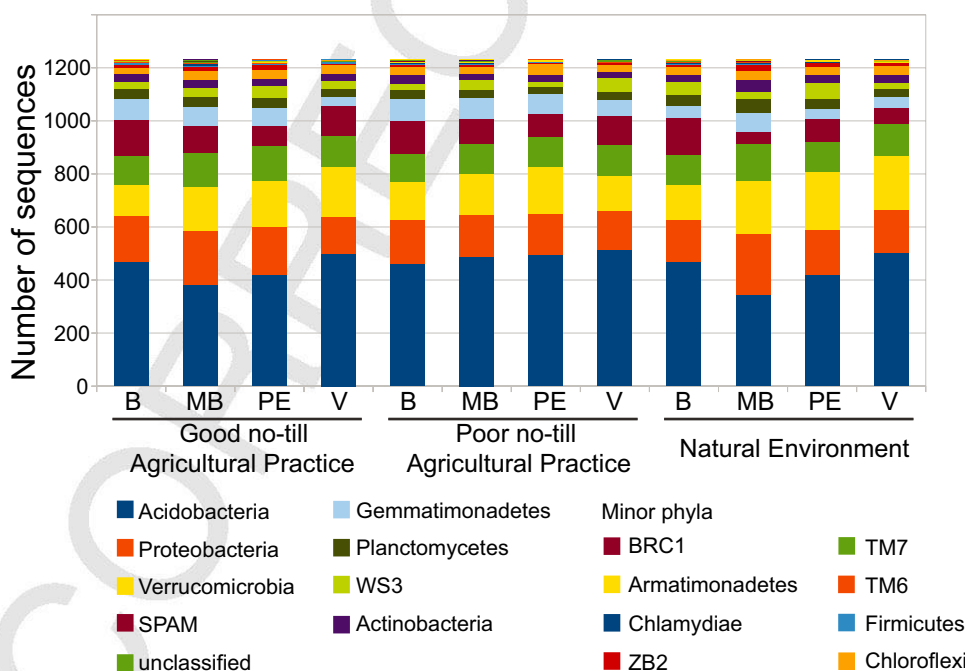
Fig. 1. Average distribution of 16S rRNA sequences classified at phylum level in the 12 studied sites. All samples were rarefied to 1230 sequences. B: BENGOLEA, MB: Monte Buey, P: Pergamino, V: Viale.

dant bacterial phyla across all samples were *Acidobacteria*, *Proteobacteria* and *Verrucomicrobia*. The sequence data set also revealed a relatively high abundance of the candidate phylum *SPAM* (Lipson and Schmidt, 2004). Other phyla commonly encountered in soils, such as *Gemmatimonadetes*, *Actinobacteria*, *WS3*, *Planctomycetes* and *Chloroflexi*, were also well represented in the pyrosequencing data set (Fig. 1).

The compositional profiles based on the V4 region observed in this study are not entirely consistent with our previous V1-V3 amplicon study performed on samples from the same sites, obtained during a previous sampling date in winter 2009 (Figuerola *et al.*, 2012). Although it has been shown that the use of different primers that target different variable regions of the 16S rRNA imposes biases that affect the output of community composition surveys (Schmalenberger *et al.*, 2001; Claesson *et al.*, 2010; Pinto and Raskin, 2012), it was particularly striking that the representation of *Acidobacteria* was considerably larger, and the one of *Actinobacteria* was much smaller, than those determined from pyrosequencing the V1-V3 region. Yet the results of ordination based on the V4 region are similar to those of the ordination based on the V1-V3 region, in that there is a good separation of samples according to the geographical region (Figuerola *et al.*, 2012; Supporting information Fig. S1A). Results based on the V4 region could additionally discriminate between PAP soils and the other soil samples (Supporting information Fig. S1B), a finding that could likely be due to the gain in statistical power obtained by the much larger sample size in the present study.

The average overlap of operational taxonomic units (OTUs) between the three pseudoreplicates was for natural environments (NE) 16.5 ± 2.5, for GAP 18.3 ± 1.3 and for PAP 20.3 ± 2.0, similar to the overlap obtained for technical replicates in previous microbiome surveys (Zhou *et al.*, 2011). Therefore, this low level of reproducibility was likely due to undersampling and could be attributed to the random sampling bias (Zhou *et al.*, 2013).

α-Diversity

The patterns of the rarefaction curves of samples from all sites, types of management and times were roughly similar, suggesting comparable unsampled diversity for all samples, regardless of soil location and agricultural management (Supporting information Fig. S2). Differences in diversity indices due to soil management were not significant when analysed by mixed models, with site-treatment interaction and sampling as random factors (Supporting information Table S1, see also Figuerola *et al.*, 2012). The fact that local bacterial diversity is not markedly altered as a consequence of farming activities is not unexpected. In fact, climax communities in NE may

be limited by nutrient availability and therefore fertilizer addition may allow colonization by new species from the regional pool. This could explain the observation that soil bacterial diversity even increased upon conversion of rainforest and semi-arid soils to agriculture (Jesus *et al.*, 2009; Ding *et al.*, 2013).

β-Diversity

The values of β-diversity and the distribution breadth increased in the order PAP < GAP < NE (Fig. 2). These results were also true for taxonomic measures of dissimilarity (Bray–Curtis) (Fig. 2). Distributions of pairwise distances (weighted normalized UniFrac) between samples within the poor agricultural practices were significantly lower from both good agricultural practices (KS test for PAP vs GAP, *P* = 0.024) and grassland samples (KS test for PAP vs NE, *P* < 0.0001). According to the Kolmogorov–Smirnov test, the differences in the distributions of pairwise distances between well-managed soils (GAP) and NE were not significant. At the phylum level, the magnitude of this effect depended on the richness of each taxon at the regional scale (γ-diversity). For phyla with low regional diversity, i.e. *Acidobacteria*, SPAM, WS3 and *Verrucomicrobia* (Table 1, Supporting information Fig. S3), distributions of pairwise distances between all soils samples indicated lower β-diversity and narrower breadth in soils under soybean monoculture compared with soils managed with crop rotation (Table 1 and Supporting information Fig. S4). Kolmogorov–Smirnov test confirmed that the distributions were significantly different (Table 1). In contrast, distributions corresponding to phyla with higher γ-diversity (*Actinobacteria*, *Planctomycetes* and *Proteobacteria*) were highly similar, regardless of the soil management (Table 1 and Supporting information Fig. S4). For all phyla, differences in the distribution of pairwise distances between well-managed soils (GAP) and NE were not significant (Table 1).

Earlier applications of culture-independent, molecular-based approaches have described changes in the overall bacterial community structure (Girvan *et al.*, 2003) or in particular microbial populations (Smalla *et al.*, 2001) in response to soil type and agricultural practices. While those and other pioneer studies have contributed to our understanding of the impact of agriculture on microbial diversity (Van Elsas and Rutger, 2006), the available techniques at the time were limited to the detection of a few taxa. More recent data, from deep sequencing studies, have suggested differential responses of selected taxa to land use (Ding *et al.*, 2013; Lauber *et al.*, 2013). Using a slightly different approach, we have previously detected taxa, whose abundances were significantly correlated to each soil management, which prompted us to propose a potential management indicator to discriminate between

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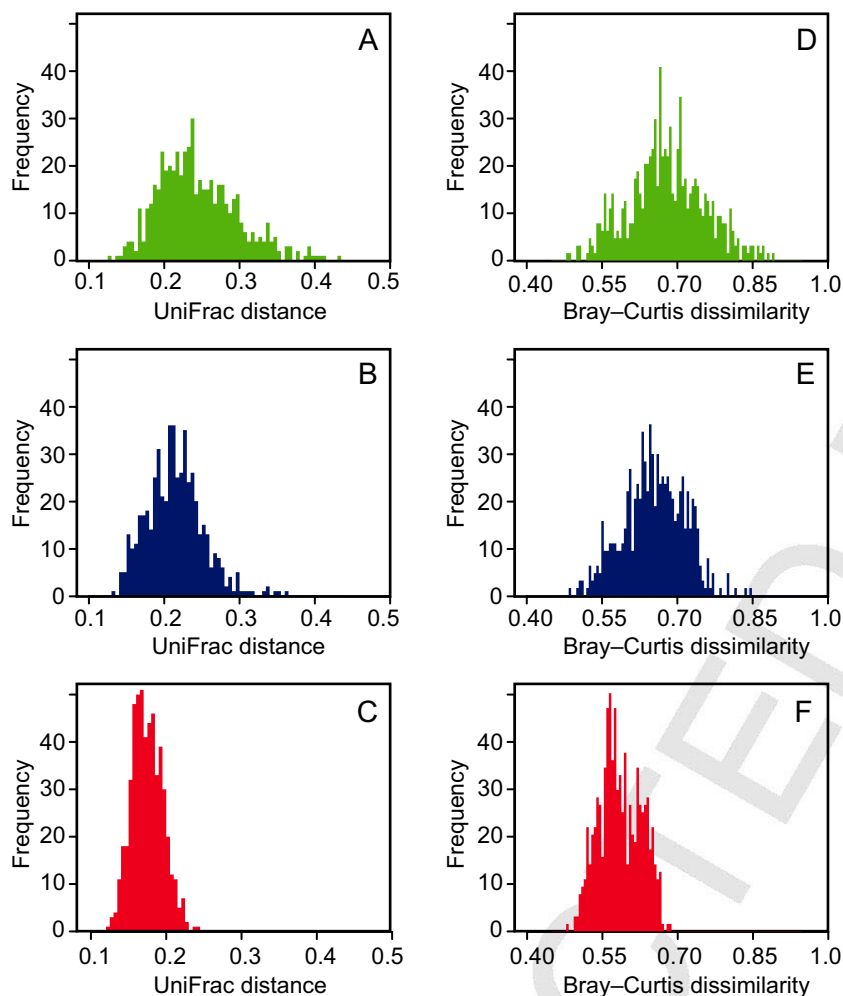
Fig. 2. Distributions of pairwise phylogenetic distances (A–C) and Bray–Curtis dissimilarities (D–F) between soils samples from within each land use type. (A, D) Natural environments; (B, E) good no-till agricultural practices; (C, F) poor no-till agricultural practices.

sustainable vs non-sustainable agricultural practices in the Pampa region (Figuerola *et al.*, 2012). While our results confirm that some phyla with low regional diversity are clearly affected by soil management, phyla with high regional diversity seemed to be unaffected. These observations do not necessarily imply that phyla not showing differences in β-diversity were not influenced by land use. It may instead indicate that species pool of phyla with high

Table 1. Comparison between the histograms of pairwise phylogenetic distance for major phyla detected in samples from different soil use types.

| Phylum	Richness[a]	NE vs GAP		GAP vs PAP		NE vs PAP	
		P value	D[c]	P value	D[c]	P value	D[c]
Planctomycetes	1149 ± 11	0.847	0.125	1	0.0341	1	0.0341
Proteobacteria	968 ± 19	0.847	0.125	0.851	0.1061	0.571	0.1364
Actinobacteria	638 ± 3	0.745	0.0392	0.956	0.0745	0.990	0.0638
Acidobacteria	541 ± 14	0.289	0.2143	0.784	0.1429	**0.009**[d]	0.3571
Gemmatimonadetes	449 ± 10	0.847	0.125	1	0.0455	1	0.0455
Verrucomicrobia	419 ± 12	0.847	0.125	0.998	0.0568	0.2154	0.1591
WS3	188 ± 4	0.2154	0.1591	0.620	0.1136	**0.004**[d]	0.2614
SPAM	180 ± 8	0.782	0.0957	0.782	0.0957	**0.063**[d]	0.1915

The Kolmogorov–Smirnov two-sample test was used to test the null hypothesis that the distribution of phylogenetic distances between samples, considering only the indicated phylum, did not depend on the soil management.
a. Rarefied to 2900 sequences.
b. NE: natural environment, GAP: good no-till agricultural practice, PAP: poor no-till agricultural practice.
c. The Kolmogorov–Smirnov D-statistic is the largest absolute value of the difference between the two relative cumulative frequencies.
d. Significant comparisons are indicated in bold.
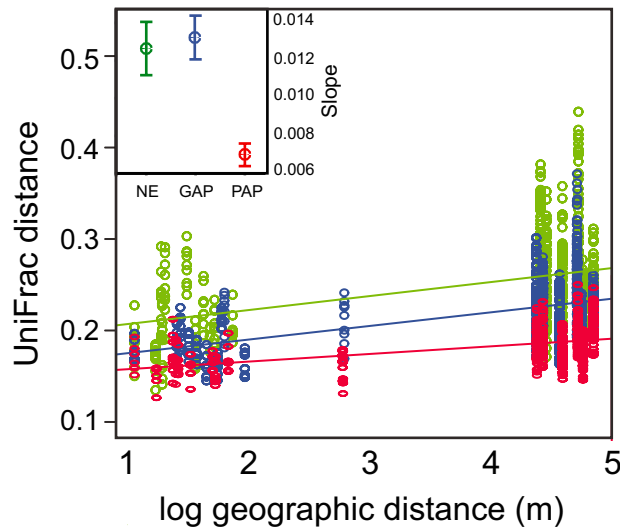
Fig. 3. Decay of phylogenetic similarity (UniFrac) with geographic distance in natural environments (green), good no-till agricultural practices (blue) and poor no-till agricultural practices (red). The inset shows the slope of the relationship between geographic distance and community dissimilarity for each land use type. The mean values and standard errors were estimated from the linear models. All slopes were significantly different from zero ($P < 0.001$).

γ-diversity are large in comparison to the number of species in the community, and since only a smaller fraction of the species pool can co-occur in any given local community, β-diversity is therefore overestimated (Chase, 2003; Fukami, 2004).

The turnover of species for each type of management was also examined along the geographical and environmental gradient (Fig. 3). Sampling locations were distributed across a geographical scale of 400 km, with major differences in physicochemical characteristics of the soils, ranging from sandy loam in Bengolea to silty loam in Monte Buey and Pergamino and silty clay loam in Viale (Castiglioni *et al.*, 2013; Rosa *et al.*, 2014). Bacterial communities from well-managed soils and grassland soils displayed similar turnover across the spatial scale [analysis of covariance (ANCOVA), $P = 0.719$], whereas soils under soybean monoculture had a lower spatial turnover than both GAP and NE (ANCOVA, $P < 0.001$).

The increase of β-diversity with geographic distance (Soininen *et al.*, 2007) can result from dispersal limitation (Chase and Myers, 2011; Hanson *et al.*, 2012) or as a consequence of the habitat heterogeneity that leads to species sorting by environmental selection (Kallimanis *et al.*, 2008; Hanson *et al.*, 2012). The co-occurrence in NE and GAP of taxa that were not detected in PAP samples taken at the same locality, observed in this study and in Figuerola and colleagues (2012), permitted us to rule out any significant role for dispersal limitation to account for the observed decrease in β-diversity. This

leaves habitat conditions as the most likely explanation of the differences in community composition among management types. Thus, the decrease in the spatial turnover observed in soils managed with unsustainable practices is consistent with a higher contribution of deterministic forces (Hanson *et al.*, 2012). Accordingly, we tested the proposition that because closely related taxa have common niche preferences (Philippot *et al.*, 2010; Barberán *et al.*, 2011), reduction of ecological niches would have led to more homogeneous bacterial distribution at the phylogenetic level. As expected, significantly higher phylogenetic relatedness was observed in soils managed under soybean monoculture compared with soils managed by crop rotation and grasslands (Table 2).

Niche breadth

It has been demonstrated that habitat disturbance, as well as habitat loss or habitat fragmentation favour generalists over specialist species (Marvier *et al.*, 2004). Considering that habitat specialist would be more sensitive to species sorting processes (Lindström and Langenheder, 2012), we asked whether the mechanisms that lead to more homogeneous communities involved the loss of habitat specialists (Olden *et al.*, 2004; Doxa *et al.*, 2012). We defined '*endemic taxa*' as OTUs that are unique to only one location, i.e. with niche breadth B equal to one (Bengolea, Monte Buey, Pergamino or Viale), and '*widely distributed taxa*' as OTUs common to three or four sampling location (niche width B = 3–4).

Poorly managed agricultural soils (PAP) had significantly higher number of individuals belonging to taxa occurring in all types of soils than GAP ($P = 0.004$) and NE ($P = 0.002$) (Fig. 4A) and significantly lower number of individual belonging to endemic taxa than natural environment ($P < 0.001$) and GAP ($P = 0.008$) (Fig. 4B). Overall, these results suggest that poor agricultural practices negatively affect endemic OTUs, with a concomitant increment in widely distributed taxa.

Table 2. Average phylogenetic relatedness between communities belonging to each land type use.

	Least squares means ± SE	Contrasts[a]
Grassland (NE)	0.0469 ± 0.0011	a
Good no-till agricultural practices (GAP)	0.0467 ± 0.0011	a
Poor no-till agricultural practices (PAP)	0.0443 ± 0.0011	b

The mean values and standard error (SE) were estimated from the linear mixed model, where land use type was treated as a fixed effect with site and land use-sample interaction as random effects.
a. Identical letter indicates non-significant differences.

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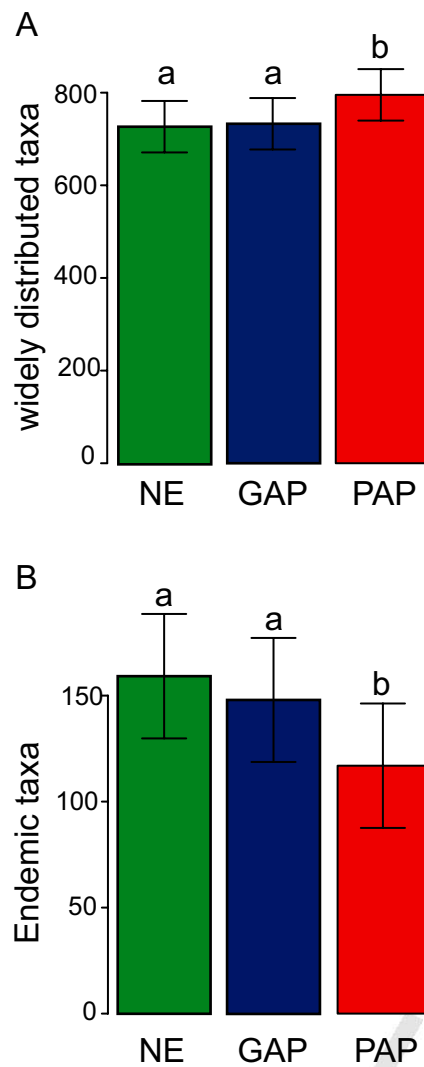
Fig. 4. A. Distribution of OTUs that are common to three or four sampling location (niche breadth B = 3–4).
B. Distribution of OTUs that are unique to only one location (niche breadth B = 1). The mean values represent the estimates from the linear mixed models, where land use type was treated as a fixed effect, and site (for widely distributed), or sampling and site-land use interaction (endemic) as random factors. Error bars are confidence intervals.

The loss of endemic species reduced significantly β-diversity, whereas α-diversity, which measures largely cosmopolitan species, was not significantly affected. This is consistent with the previous observation that copiotrophic taxa are enhanced in agricultural soils (Fierer *et al.*, 2011; Ding *et al.*, 2013).

Similar diversity decline at the regional scale has been reported for macroscopic organisms, where extinction of local specialists or poor dispersers results in the dominance of generalist species (Ekroos *et al.*, 2010; Doxa *et al.*, 2012). Birds and insects assemblages with specific requirements in tropical forest ecosystems are replaced in agricultural habitats by species with greater habitat and diet breath (Tscharntke *et al.*, 2008). To the best of our knowledge, such changes also occurred in prokaryotes with the rather more dramatic conversion of the Amazon rainforest to agriculture (Rodrigues *et al.*, 2013).

Canonical analysis of principal coordinates (CAP)

CAP based on UniFrac distances was performed to study the relationship between phylogenetic composition and environmental gradients (Fig. 5). The two first constrained axes explained 43% of variance. The first axis CAP1, which was characterized by lower values of total organic carbon (TOC), total nitrogen (Nt), extractable phosphorus (Pe) and pH, separated the samples of soils with unsustainable practices (PAP) from well-managed soils (GAP) and grassland (NE). Correlations with the mentioned environmental variables were significant in all cases ($P = 0.01$).

The observed site effects respond to the well-known fact that pools of carbon, nitrogen and phosphorus in soils may vary greatly with location (Syers, 1997; Ross *et al.*, 1999). CAP shows in addition the effect of agricultural management. Previous surveys have determined that soil C and N declined after land use changes from pasture to crop (Guo and Gifford, 2002; Steenwerth *et al.*, 2002).
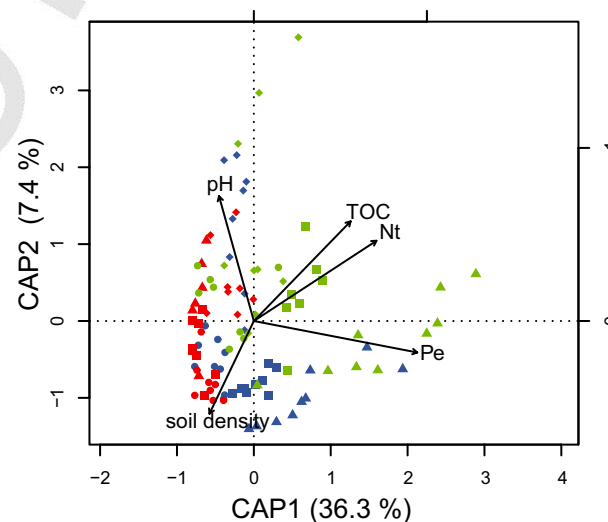
Fig. 5. Constrained analysis of principal coordinates (CAP) using bacterial OTUs relative abundances and measured environmental soil parameters that had significant correlations with bacterial community structure: total organic carbon (TOC), total nitrogen (Nt), extractable phosphorus (Pe), ~~and~~ pH, and soil density. Arrows indicate the direction and magnitude of the environmental parameter. Samples are colour-coded to indicate the different managements: Green corresponds to natural environments (NE), blue to good no-till agricultural practices (GAP); red are poor no-till agricultural practices (PAP). Localities are indicated by symbols: squares (Bengolea), circles (Monte Buey), diamonds (Pergamino) and triangles (Viale).

The association of low C and bacterial community structure was more pronounced in PAP soils, in agreement with previous long-term experiments, which showed that crop rotations sustained higher TOC and soil Nt than crop monoculture (Heenan *et al.*, 2004; González-Chávez *et al.*, 2010). On the other hand, it is difficult to disentangle the influence of crop rotation and nutrient amendment on the observed correlations with P and Nt because in the selected PAP soils crop monoculture was accompanied by low nutrient restoration, and fertilization alters the composition of the soil nutrient pools (Stevenson and Cole, 1999; Beauregard *et al.*, 2010).

Conclusions

The results of the analyses using different measures of β-diversity in this study were consistent, and suggest that soybean monoculture drives the homogenization of bacterial communities at a regional scale in no-till fields of Argentinean Pampa. Although environmental impact of agricultural practices is inevitable, sustainable agricultural management should be essential to minimize the damage to the environment. Results described in this work show that good agricultural practices, essentially defined by crop rotation, sustained bacterial β-diversity almost as well as natural grasslands and did not cause a significant loss of endemic species. According to the insurance hypothesis, biodiversity is considered essential for ecosystem functioning (Loreau *et al.*, 2001), since the occurrence of functionally redundant species helps to maintain ecosystem services (Allison and Martiny, 2008). Previous results from a long-term trial recommended the combination of no-tillage together with a correct stubble management and crop sequence to preserve and improve the diversity of soil bacterial communities (Ceja-Navarro *et al.*, 2010). One of the strengths of this work is that the analyses were performed on productive fields belonging to different farmers, rather than on highly controlled research plots. We show here that under realistic field conditions the homogenization of bacterial communities caused by soybean monoculture was primarily determined by the loss of endemic species and affected mostly phyla with low regional diversity. Since both local and regional diversity contribute to the ecosystem multifunctionality (Pasari *et al.*, 2013), our results show, at the level of soil bacterial diversity, that the implementation of good agricultural practices including crop rotation may be critical for the long-term conservation of the soil function.

Experimental procedures

Experimental design and sites description

Blocks of treatments were sampled at four different locations across a scale of 400 km through three successive cropping cycles. Sampling points were situated on a west-east transect within the Argentinean Pampa region. The annual precipitation and temperatures decline from east to west. The ~~1000–800 mm average~~ annual rainfall is concentrated in spring and summer. The sampled region comprised the provinces of Córdoba, Buenos Aires and Entre Ríos: Bengolea (33° 01′ 31″ S; 63° 37′ 53″ W), a sandy loam Entic Haplustoll soil; Monte Buey (32° 58′ 14″ S; 62° 27′ 06″ W), a silty loam Typic Argiudoll soil with a moderately developed illuvial Bt horizon and an increased proportion of the silt fraction; Pergamino (33° 56′ 36″ S; 60° 33′ 57″ W), a silty loam Typic Argiudoll soil with a well developed Bt horizon, and Viale (31° 52′ 59,6″ S; 59° 40′ 07″ W), a silty clay loam Hapludert soil. The clay mineralogy of the soil surface horizons of Bengolea, Monte Buey and Pergamino consist of 2:1 clays, mainly illites with a small proportion of irregular interstratified illite-smectite minerals and traces of kaolinite. The soil in Viale is characterized by a considerable proportion of smectite with lower proportions of clay minerals (Castiglioni *et al.*, 2013; Rosa *et al.*, 2014).

In each location, we sampled two production fields with different crop rotation regimes, and a grassland soil in close proximity to the agricultural sites, as a reference for NE. The agricultural soils were selected from farms that were managed for no less than 5 years under no-till, according to the following standards: (i) crop rotations, including soybean, and appropriate nutrient amendment, i.e. managed according to criteria considered as GAP (Figuerola *et al.*, 2012), www.ac.org.ar/~~descargas/PyC_eng.pdf~~; www.fao.org/prods/GAP/index_en .htm); (ii) mostly soybean monoculture with minimal nutrient inputs, a regime of management that fits an operational definition of PAP (Figuerola *et al.*, 2012). Crop sequences over 5–10 years previous to the initial sampling are given in Supporting information Table S2. Note that crop sequences were not necessarily replicated within each management type, since this was not a randomized controlled trial but an observational study of soils managed under realistic conditions.

Sampling protocol and storage

Three samples from the top 10 cm of bulk soil were collected, as a composite of 16–20 randomly selected cores from the top 10 cm of mineral bulk soil within an area of 5 m². Composite soil samples were homogenized in the field. Each sample was separated at least 50 m from each other, without following the sowing line in the field. Soil samples were transported to the laboratory at 4°C. Within 3 days after collection, samples were sieved through 2 mm mesh to remove roots and plant detritus. Soils were stored at −20°C until DNA extraction.

Samplings were performed in February 2010 (summer), September 2010 (late winter) and February 2011 (summer). Thus, we analysed a total of 108 soil samples (4 geographical locations × 3 land-use types × 3 samples per land use × 3 time points per site). A previous deep sequencing analysis of the same 12 soils sites, sampled in the previous winter season (June 2009), was already reported (Figuerola *et al.*, 2012).

Nucleic acid isolation and PCR amplification

DNA was extracted from 0.5 g of soil samples using FastDNA spin for soil extraction kit (MP Biomedicals Inc), following the

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manufacturer's instructions. We introduced a further purification step, using polyvinylpolypyrrolidone spin columns (Berthelet *et al.*, 1996), in order to eliminate residual humic substances that could inhibit the subsequent PCR reaction. Eluted DNA was stored at −20°C.

A fragment of approximately 250 bp spanning the V4 region of 16S rRNA was amplified by PCR using primers F563-R802. This is a different target region from that used in our previous study of the same study sites (Figuerola *et al.*, 2012), and was selected on the basis on the recommendation of the Ribosomal Database Project (RDP) for titanium pyrosequencing (Sul *et al.*, 2011).

Library preparation and sequencing

Sequencing 454 adapters and MIDs (Supporting information Table S1) were added by a second amplification round. Libraries were pooled in equimolar concentration and sequenced in two half plates on a 454/Roche GS FLX Titanium platform at INDEAR, Rosario, Argentina.

Pyrosequencing raw reads were deposited in the NCBI Short-Read Archive under accession number SRP035435.

Read quality assessment

A total of 718 340 sequences were retrieved with an average length of 269 bp. Initially each plate region was processed separately according Mothur SOP (Schloss *et al.*, 2011). Sequence errors were reduced by denoising with the implemented version of Pyronoise in Mothur. Sequences shorter than 200 bp or with more than 1 mismatch to the barcode and/or 2 mismatches to the primers were discarded. After filtering, 206 394 quality sequences remained from plate-region 1 (average length 255 bp), and 149 809 quality sequences from plate-region 2 (average length 254 bp). Next, both sets of sequences were combined to continue with the alignment using Silva database v. 111 (http://www.arb-silva.de/no_cache/download/archive/release_111/Exports). Those sequences that aligned out of the range of interest were removed. Thereafter, chimeras were detected using the chimera.uchime command in Mothur. Sequences were classified against the RDP training set 6. All chloroplast, mitochondria and reads unclassified at the kingdom level were discarded. Finally, OTUs were established at a genetic distance of 0.03 using the furthest neighbour algorithm in Mothur. In order to avoid the bias caused by differences in sequencing depth in the estimation of α- and β-diversity (Lundin *et al.*, 2012), a subset of 1230 sequences were randomly subsampled from each group using Mothur's sub.sample function. Three samples from NE and three from PAP had low number of sequences; therefore, three samples of GAP were removed randomly to achieve a balanced design of 33 samples per treatment, yielding a total of 99 samples for downstream analysis. Rarefaction curves were calculated in the PAST package, version 2.05 (Hammer *et al.*, 2001).

Diversity analysis

α-Diversity indices were calculated using PAST (Hammer *et al.*, 2001). Different measures of β-diversity were employed. Bray–Curtis measure of dissimilarity (Bray and Curtis, 1957), which consider OTUs abundance, was used to calculate the pairwise compositional dissimilarity between communities within each land-use type, and also to describe the increase in pairwise dissimilarity with increasing geographic distance. The Bray–Curtis dissimilarity matrix was computed in R with 'vegan' package. The weighted UniFrac distance (Lozupone *et al.*, 2010) was used to measure the phylogenetic dissimilarity between pairs of communities subjected to the same land use and the increase in phylogenetic distance with geographic distance. The UniFrac distance metric between each pair of samples was calculated online (http://unifrac.colorado.edu/).

Histograms for comparing β-diversity were made with *make_distance_histograms.py* script in QIIME (Caporaso *et al.*, 2010) or with the function *hist* in R. The Kolmogorov–Smirnov test in R package 'stats' was used to assess the significance of the difference between pairs of distributions.

Spatial turnover was calculated by linear regression of phylogenetic distances vs geographic distances, utilizing *lm* function from library 'stats' in R. Geographic distances between sampling sites were calculated from GPS data with the aid of packages 'gmt' and 'Fields' in R. Turnover rates were compared by ANCOVA using function *aov* in R.

Gamma diversity for the eight dominant phyla was estimated using species (OTUs) accumulation curves from the total observed number of OTUs belonging to each phylum in the complete sequence dataset.

Phylogenetic relatedness was calculated using the function *ses.mntd* of R library 'Picante'. Niche width was calculated by the Levins' method using *niche.width* in R library '*sppa*'.

Multivariate analysis

Relationship between environmental variables and community composition was assessed using CAP, a method of constrained ordination that can be applied to non-Euclidean dissimilarity measures (Anderson and Willis, 2003). The analysis was performed with function *capscale* in R 'vegan' package, on the UniFrac weighted and normalized distance matrix. Physicochemical data comprised: pH, TOC, Nt, Pe, soil humidity and soil density. Significance of each term was determined by permutation test with *anova* in 'vegan' R. Non-significant parameters were removed from the analysis.

Statistical analysis

Soil management effect was examined using linear mixed models adjusted in R 3.0.2 with *lmer* function from package '*lme4*'. The use of mixed linear models allows handling of correlated data such as repeated measures and/or pseudoreplicates. Considering these factors as random avoids the violation of the assumption of independence resulting from sets of measurements that are spatially or temporally linked or correlated in any other way (Chaves, 2010). Rather than seeking for temporal patterns, the reason for including three sample dates was to increase the sample size and the power of statistical tests, with the added advantage of not relying on differences based on single time points. To avoid implicit nesting (Bates, 2010), a new factor named 'Sample' was created by numbering all pseudoreplicates from

1 to 99. In the full models, agricultural management was treated as the only fixed effect. Random terms included site, sampling, sample and all possible interactions. Non-significant random effects were removed after an automatic backward elimination procedure with *step* function (lmerTest), leading to the final models described in Supporting information Appendix S1. Reduced model contrasts were calculated by *difflsmeans* from R package 'lmerTest'.

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Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Fig. S1. Principal coordinates analysis (PCoA) of weighted UniFrac distances between soil samples. Samples are colour-coded to indicate the different managements: Green corresponds to natural environments (NE), blue to good no-till agricultural practices (GAP); red are poor no-till agricultural practices (PAP). Localities are indicated by symbols: squares (Bengolea), circles (Monte Buey), diamonds (Pergamino) and triangles (Viale). (A) Ellipses are 99% confidence intervals of group centroids for each geographical location [ordiellipses function in R vegan package (Oksanen *et al.*, 2012)]; (B) Ellipses are 99% confidence intervals of group centroids for each management type (Oksanen *et al.*, 2012).

Fig. S2. Rarefaction curves indicating the observed number of OTUs within the 16S rRNA gene sequences in each of the 12 sites, considering the three pseudo-replicates per sampling and the three sampling dates. OTUs are shown at the 3% genetic distance level. Samples are colour-coded to indicate the different managements: Green corresponds to natural environments (NE), blue to good no-till agricultural practices (GAP); red are poor no-till agricultural practices (PAP). Localities are indicated by symbols: squares (Bengolea), circles (Monte Buey), diamonds (Pergamino) and triangles (Viale).

Fig. S3. Species (OTUs) rarefaction curves of 16S sequences classified at the phylum level for all the soil samples. Purple: Planctomycetes; green: Proteobacteria; black: Actinobacteria; blue: Acidobacteria; grey: Gemmatimonadetes; light blue: Verrucomicrobia; yellow: WS3; Red: SPAM.

Fig. S4. Histograms of pairwise phylogenetic distances for major phyla, between soils samples from within each soil management type. Distributions are colour-coded to indicate the different managements: Green corresponds to natural environments (NE), blue to good no-till agricultural practices (GAP); red are poor no-till agricultural practices (PAP).

Table S1. Measures of α-diversity and pairwise statistics between different soil managements.

Table S2. Sequence of crops practiced from 2000 to 2011 in the eight agricultural sites examined in this study. PAP refers to the operational definition of poor no-till agricultural practices; GAP refers to good no-till agricultural practices.

Appendix S1. Mixed effect linear models. Proposed full models containing agricultural practice as fixed factor and site, sampling, sample and all possible interactions as random effects. Reduced models derived from automatic backward elimination of non-significant effects.