Applied Soil Ecology 75 (2014) 172–180

Contents lists available at ScienceDirect

Applied Soil Ecology

journal homepage: www.elsevier.com/locate/apsoil

The influence of soil properties on denitrifying bacterial communities and denitrification potential in no-till production farms under contrasting management in the Argentinean Pampas

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ARTICLE INFO

Article history:
Received 20 August 2013
Received in revised form 1 November 2013
Accepted 18 November 2013

Keywords:
No tillage
Denitrification
Structural stability indices
Pampa region
Sustainable agriculture
Mollisols and Vertisols

ABSTRACT

The aim of this work was to investigate the response of the structure, abundance and potential activity of denitrifiers to contrasting agricultural management in no-till production fields, across a regional scale within the highly productive Argentine northern Pampas. Treatment categories were grouped according to the sustainability of the soil management, in terms of crop rotation, fertilization, agrochemicals use and pest control, as good no-till agricultural practices (GAP) and poor no-till agricultural practices (PAP). Non-cultivated soils in each geographic location were also evaluated as reference groups.

Mixed models, with sites treated as random factors, indicated that the potential denitrification activity and the size of the *nirS* community differed significantly between non-cultivated and PAP soils. Soil properties were separated into dynamic and inherent according to their variance components. The former had the largest part of their variances explained by agricultural management, while the latter were more affected by edaphic–climatic differences between sites. Both inherent and dynamic properties could explain the changes in potential denitrification activity, whereas changes in the abundance of denitrifiers were only related to inherent soil properties. Results from principal components analysis suggested site-specific response of most dynamic soil properties. Among the latter, only aggregate stability indices were strongly associated with potential denitrification activity after removing the geographical effect.

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1. Introduction

Reduction of tillage, which has been increasingly established in the last decades, aims to prevent soil erosion, reduce labour and machinery use and increase soil carbon sequestration (Pierce et al., 1994; Kirkegaard and Hunt, 2010). However, reduced soil disturbance does not entail sustainability if it is not accompanied by other measures. No-till needs to be coupled with crop rotation for effective control of residue-borne pathogens (Bockus and Shroyer, 1998), and permanent organic soil cover is required to offset some negative aspects of no-till farming on soil architecture (Vazquez et al., 1989; Bonel et al., 2005), which could have detrimental consequences on water infiltration (Pierce et al., 1994; Sasal et al., 2006).

Argentina is an early adopter of no-till management practices, first for soybean production and later for other crops, and their application has been growing steadily (Viglizzo et al., 2011). An estimated 80% of the soybean, corn, sunflower, wheat and sorghum are grown by this mode of agriculture, covering at present a total area of about 27 million hectares (source: Argentine No Till Farmers Association (AAPRESID); http://www.aapresid.org.ar/wp-content/uploads/2013/02/aapresid.evolucion_superficie_sd_argentina.1977_a_2011.pdf, last visited august 11, 2013). Regrettably, the widespread use of no-till in the Argentine Pampas, the central region of agricultural production, has not been always implemented in conjunction with the other recommendations included in the concept of conservation agriculture, especially regarding to crop rotation and fertilization (Austin et al., 2006).

Sustainability of soil resource management practices can be evaluated using the soil quality concept, defined as the capacity of a soil to function, i.e. to sustain plant and animal productivity,

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http://dx.doi.org/10.1016/j.apsoil.2013.11.012

promote plant and animal health and maintain environmental quality (Karlen et al., 1997). In this context, it is essential to recognize that soil quality depends on both inherent and dynamic properties and processes (Karlen et al., 2001). Inherent characteristics are determined by basic soil forming factors that show little change over time, such as mineralogy, texture and particle size distribution (Karlen et al., 2003). On the other hand, dynamic soil properties refer to soil attributes, such as organic matter, soil structure and macroporosity, which can change considerably over relatively short time periods in response to human use and are strongly affected by agronomic activities (Carter, 2002). As the influence of land use in soil properties is not independent of soil type, topography, vegetation, and climate, and soil attributes are strongly interrelated (Blanco and Lal, 2008), some soils properties may be both, dynamic and inherent. That is, for instance, the case of soil organic matter, which is not only related to continuous inputs of organic material to the soil but also to particle size distribution (Carter, 2002).

Denitrification is an alternative respiration pathway used by bacteria in the absence of oxygen. During denitrification, nitrate is reduced to dinitrogen gas by four reaction steps catalyzed by nitrate reductase, nitrite reductase, nitric oxide reductase and nitrous oxide reductase (Bothe et al., 2007). The reduction of nitrite to nitric oxide is the key reaction of the denitrifying pathway (Philippot, 2002). This reaction is catalyzed by two different types of nitrite reductases (Nir), a cytochrome cd1 enzyme, encoded by the *nirS* gene, or by a Cu-containing enzyme encoded by the *nirK* gene. These two genes, *nirK* and *nirS*, are commonly selected as functional markers to survey denitrifying community diversity and composition in environmental samples, including soils (Throback et al., 2004).

Potential denitrification activity has been widely used to examine denitrification in soils as a function of environmental conditions and management (Qin et al., 2012). Because the ability to denitrify is widespread among phylogenetically unrelated organisms, it is considered that the denitrifying community structure might serve as proxies of the soil microbial diversity (Throback et al., 2004; Philippot and Hallin, 2005; Falcão Salles et al., 2012).

Several studies have improved our understanding of the relationships between the ecology of denitrifiers and N loss from agroecosystems by analyzing the effect of agricultural practices on soil denitrifying communities (Philippot et al., 2007), and the spatial distribution of denitrifiers in relation to abiotic parameters and land management (Baudoin et al., 2009; Enwall et al., 2010; Attard et al., 2011). However, most studies are conducted under highly contrasting farming practices, such as conventional versus no-till farming, within a limited local scale (Baudoin et al., 2009; Attard et al., 2011). The objective of this study was to compare the community structure and the abundance of denitrifiers in production agricultural soils on a regional scale. We investigated soils with a documented history of different management practices under no-till, located along an edaphic-climatic gradient in northern Argentinean Pampas.

2. Materials and methods

2.1. Study sites and soil sampling

The sites and managements of soils for this study were selected between scientists and farmers participants of the BIOSPAS consortium (www.biospas.org), an interdisciplinary project, whose long term goal is to define ecological indicators of sustainability under no-till farming (Wall, 2011). Because of the difficulty in establishing strict replication of management practices in actual production fields, we have adopted the criteria described by the program of Certification in Good Agricultural Practices of AAPRESID (http://www.aapresid.org.ar/ac/buenas-practicas-agricolas/, last visited August 11, 2013) and the guidelines of Good Agricultural Practices developed by the Food and Agricultural Organization of the United Nations (www.fao.org/prods/GAP/index_en.htm, last visited August 11, 2013). Hence, soils from production fields were selected in accordance to the definition of the following three treatments (Figuerola et al., 2012): (1) "Good no-till Agricultural Practice" (GAP): Sustainable agricultural management under no-till, subjected to crop rotation, and minimum use of agrochemicals (herbicides, insecticides and fungicides); (2) "Poor no-till Agricultural Practice" (PAP): Non-sustainable agricultural management under no-till with crop monoculture, low nutrient addition and high agrochemical use; (3) "Natural Environment" (NE): grasslands or xerophytic forest situated in an area of approximately 1 ha close to the cultivated soils (less than 5 km). The type of agricultural management of the fields under production compared in this work had been managed under no-till for the previous 6 years before sampling, with the exception of a single year (2004/2005) in Bengolea, where the PAP site was chisel-plowed. Under this framework, the principal difference between both agricultural managements was the predominance of soybean in the crops succession in the PAP and a more balanced proportion of corn, wheat and soybean in the rotation in the GAP management, which resulted in higher crop yields. Other agronomic practices that distinguished GAP soils from PAP soils were the incorporation of cover crops and the lower amount of herbicides used. Details are given in Figuerola et al. (2012), including the farming practices, crop sequences and yields in each production site.

Production soils managed under the indicated contrasting practices were sampled in 4 locations across a west-east transect in the northern part of the Argentinean Pampas (Fig. 1): Bengolea (33°01′31″S; 63°37′53″W), Monte Buey (32°58′14″S; 62°27′06″1W), Pergamino (33°56′36″S; 60°33′57″W) and Viale (31°52′59.6″S; 59°40′07″W) (Fig. 1). The annual precipitation declines to the west (1023 mm to 795 mm; source: Servicio Meterológico Nacional (SMN), http://www.smn.gov.ar, retrieved November 2012) and the temperature falls with the same trend (mean annual temperature 18.0 °C to 16.3 °C; source: SMN, http://www.smn.gov.ar, retrieved November 2012). Precipitation varies with season; rainfall is concentrated during spring and summer periods.

The soil in Bengolea is a sandy loam Entic Haplustoll located in the Piedmont Pampa, where the moisture regime is mostly ustic and where fluviatile sands and silts are found together with the loessial sediments. The soil in Monte Buey is in a flat area in the limit between the Piedmont Pampa and the Rolling Pampa; it is a silty loam Typic Argiudoll with a moderately developed illuvial *Bt* horizon and an increased proportion of the silt fraction. The soil in Pergamino is a silty loam Typic Argiudoll with a well developed *Bt* horizon, representative of the Rolling Pampa; the precipitation is slightly higher than in the previous location, the drainage network is well defined and the relief is gently undulating (slopes of about 2% and up to 5%). The soil in Viale is a silty clay loam Hapludert; the precipitation is the highest in the transect, the landscape is also gently undulating and the drainage system is well developed. The clay mineralogy of the soil surface horizons of Bengolea, Monte Buey and Pergamino is rather similar, consisting of 2:1 clays, mainly illites with a small proportion of irregular interstratified illite-smectite minerals, and traces of kaolinite. Contrarily, the soil in Viale is characterized by a considerable proportion of smectite together with lower proportions of the previously mentioned clay minerals (Kraemer et al., 2012).

Field measurements and soil sampling were carried out in winter 2011. Samples were taken as triplicate for each treatment-site in three 5 m^2 sampling points separated at least 50 m from each other, taking care not to follow the sowing line in the field. Each sample of the top 10 cm of mineral soil was collected as a composite of

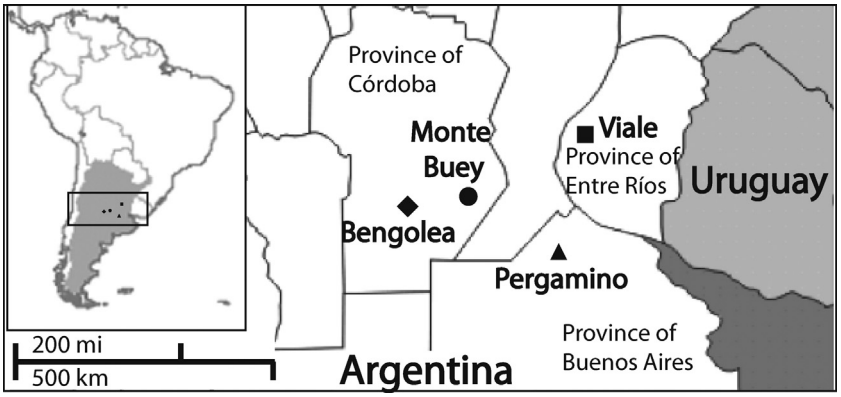

Fig. 1. Localization of sampling sites located across in the northern part of the Argentinean Pampas (see text for further details).

25–35 randomly selected subsamples. Soil samples were homogenized in the field and 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.

2.2. Soil physical and chemical characterization

Soil bulk density (BD) was determined by the core method (Blake and Hartge, 1986a) using 138.5 cm^3 volume cores ($n = 9$). Soil penetration resistance (PR) from 0.0 to 0.10 m depth was determined ($n = 10$) using a static digital penetrometer (Fieldscout SC-900®) with 30° tip angle. Total soil porosity (TP) was calculated according Danielson and Sutherland (1986) as follows:

$$TP(\%) = 100 \times (1 - BD/PD)$$

where PD means particle density (Mg m^{-3}) and BD is bulk density (Mg m^{-3}).

Particle density was measured in all combination (treatment/sites) according the picnometer method (Blake and Hartge, 1986b). Aggregate density (AggD) and Aggregate specific volume (AggV) were measured in 3–5 mm diameter aggregates with kerosene as non polar liquid with the methodology described by Stengel (1979). The soil water retention curve was measured in undisturbed soil samples (cores: at matric suctions of 10, 30, 60, 90, 330, 2.000 and 15,000 cm) in a pressure plate apparatus (Klute, 1986). Plant available water (PAW) was considered as the difference between field capacity (330 cm) and wilting point (15,000 cm), easily available water (EAW) was considered as the difference between field capacity and 2.000 cm. Air capacity (AC) was determined by the difference between water saturation and 60 cm suction. The van Genuchten–Mualem (VG–M) model was fitted with the soil water retention curve data using RETC program in order to calculate Dexter's index of soil physical quality (S) (Dexter, 2004). Three undisturbed samples were taken from the first 0.20 m to determine their aggregate stability index (AS) according to the three pretreaments proposed by Le Bissonnais method (Le Bissonnais, 1996) (AS1—fast wetting-; AS2—stir wetting-; AS3—slow wetting-). Soil temperature at 5 cm depth was measured with a pirometer (Rapitemp_Soft 4) and was later corrected by comparison with the air temperature along the measurement period (Soil-Air temperature). The following parameters were determined in the crushed and 2 mm sieved soil samples: pH (1:2.5 soil:water) by potentiometry, organic carbon (OC) by dry combustion method using a LECO CR12 19 equipment, and electric conductivity (EC) with a conductimeter. Exchangeable (Exch) ions and cation exchange capacity (CEC) were determined by the ammonium acetate 1 N method. Exch Na^+ and K^+ were measured by means of flame photometry, whereas Exch Mg^{2+} and Ca^{2+} were measured by atomic absorption spectrometry. Particle size distribution was measured by means of the Robinson's pipette method for the clay and silt fractions and by sieving for the sand fractions (Soil Conservation Service, 1972). Total Nitrogen (TN) was measured by the Kjeldahl method (Bremner, 1996). NO_3^- and NH_4^+ were extracted according Wheatley et al. (2001) , incubating 5 g of soil with 20 ml 1 M KCl in Erlenmeyer flasks at 250 rpm for 1 h. After centrifuging (4000 rpm for 30 min), supernatants were filtered. NH_4^+–N and NO_3^-–N were determined spectrophotometrically using the indophenol method and by reduction by vanadium(III) followed by Griess reaction (Miranda et al., 2001), respectively.

2.3. Potential denitrification rates

Denitrification enzyme activity, i.e., potential denitrification (PD), was measured in duplicate using the acetylene inhibition technique described by Patra et al. (2005). Shortly, fresh soil (10 g) was placed into 150 ml erlenmeyer flasks sealed with rubber stoppers, whose atmosphere was evacuated and replaced by a 90:10 He:C_2H_2 mixture. Substrate solution (10 ml) was added to reach a final concentration of 1 mM glucose and 1 mM KNO_3. The soil slurries were incubated on a rotary shaker at 25 °C and 120 rpm. Gas samples (0.5 ml) were taken at 1–6 h and analyzed immediately for N_2O using a gas chromatograph (Agilent Technologies 6890N) equipped with an HP-Plot Molesieve column and a ^{63}Ni electron capture detector (Agilent Network GC System (μECD)). Potential denitrification was expressed as μg N_2O–N h^{-1} g^{-1} dry soil.

2.4. Denitrifying community structure and size

Total DNA was extracted from a 0.5 g of each soil sample by use of Fast-DNA spin kit for soil extraction kit (MPbio), following the manufacturer's instructions. In order to reduce the presence of humic substances that inhibited the subsequent PCR reaction, an additional purification step was performed on the DNA sample using polyvinyl polypyrrolidone (PVPP) (Carter and Gregorich, 2008)

The size of denitrifying community was estimated by quantitative PCR (qPCR) of *nirK* and *nirS* using the primer combinations nirK876/nirK1040 and nirSCd3aFm/nirSR3cdm, and the thermal conditions described by Hallin et al. (2009). Quantification was based on the increasing fluorescence intensity of the SYBR Green dye during amplification. The qPCR assays were carried out in triplicate in 20 μl reaction volume, containing the SYBR green PCR Master Mix (Applied Biosystems), 1 μM of each primer, 0.4 μg μl^{-1}

of BSA and 10 ng of soil DNA. Standard curves were obtained using serial dilutions of purified PCR products amplified under the same conditions.

The structures of denitrifying communities were characterized by DGGE analysis of *nirK* and *nirS* as described by Throback et al. (2004), with modifications. Equal amounts of DNA extracts from the three subsamples of each site/treatment were pooled resulting in a total of 12 DNA samples. PCR amplification was performed in a volume of 50 μl containing 5 μl of 10X PCR buffer, 1.5 mM $MgCl_2$, 50 μM of each deoxynucleotide triphosphate, 1 μM of each primer, 0.4 μg μl^{-1} of BSA, 0.75 U of Platinum® Taq DNA Polymerase (Invitrogen) and 20 ng of soil DNA. The *nirK* fragments (ca. 500 bp) were amplified using nirK1F and GCR3Cu primers with an initial denaturation of the DNA at 94 °C for 5 min, followed by 35 cycles of 45 s at 94 °C, 45 s at 58 °C and 1 min at 72 °C, plus a final elongation step at 72 °C for 10 min. For *nirS*, a semi-nested PCR was carried out. In this case, the number of cycles and annealing temperature were adjusted to avoid unspecific amplification: 35 cycles and 57 °C in the first reaction, using primers nirSCd3aF/nirSR3cdm, and 20 cycles and 58 °C in the second reaction, using primers nirSCd3aF/GCR3Cd. As in some samples a non-specific amplicon persisted, the fragment of the expected size of 500 bp obtained in the first PCR was loaded into an agarose gel, eluted in 50 μl of distilled water, and used as substrate for the second PCR. DGGE was performed in 7% (wt/v) acrylamide gels containing a linear gradient ranging from 55% to 70% denaturant (100% denaturant was defined as 7 M urea and 40% (vol/vol) formamide). The gels were run in 1X TAE at 105 V and 60 °C for 17 h. Migration patterns were visualized by staining with 1: 10,000 SYBR Gold for 40 min followed by UV transillumination in a G:BOX Chemi HR16 (Syngene). These analyses were repeated in three independent experiments for each gene.

2.5. Data analysis and statistical tests

Mixed models were constructed to study the effect of treatments (sustainable or unsustainable agricultural practice, or undisturbed soil) on biological variables (Zuur et al., 2009). A reduction strategy was applied whenever possible to build compact models: we started with complete models and in a step-wise fashion non-informative factors and interactions were removed. All statistical analyses were performed using the R statistical software (R Development Core Team, 2011). The four sites were considered as a random sample of possible sites, and in some models the random structure included the treatments nested within sites. Linear mixed model were built and analyzed with the *nlme* library of R (Zuur et al., 2009). Biological data (PD, *nirS* and *nirK* copy number values) were log transformed to achieve normal distribution.

In order to analyze matrices obtained from DGGE fingerprints, cluster analysis of *nirS* and *nirK* banding profiles were performed with Ward' minimum variance algorithm. The bootstrap values (approximately unbiased p-values) were obtained from 1000 resamplings with the *pvclust* library of R (Suzuki and Shimodaira, 2006).

Relationships between PD, *nirS* and *nirK* copy number and physicochemical variables were analysed by both Pearson correlations and mixed models (using treatments as fixed effect and sites as random effect representing the nested structure of the experimental design, as explained above); the P-values were corrected for multiple inference using Holm's method. The degree of association between the genetic structure of the denitrifying community and potential denitrification was tested by quantifying the correlation between the rank similarity matrices obtained for genetic structure and activity, using Spearman correlation coefficient and associated significance level by a permutation test with 5000 permutations (Clarke and Ainsworth, 1993). A variance components analysis was used to determine the variance proportions attributable to treatments and sites in soil properties as a proxy to discriminate the dynamic and inherent behaviour of each soil feature.

Table 1
Regression coefficients, standard error (SE) and contrasts of the mixed linear models (MLM) for potential denitrification (PD), *nirK* and *nirS* gene copy number.

		Regression coefficient	SE	Contrasts
Log (PD)	NE	1.206	0.122	a
	GAP	1.019	0.122	a
	PAP	0.678	0.122	b
Log (*nirK*)	NE	7.459	0.086	a
	GAP	7.350	0.086	a
	PAP	7.394	0.086	a
Log (*nirS*)	NE	5.868	0.102	a
	GAP	5.947	0.102	a,b
	PAP	6.121	0.102	b

Identical letters indicates non-significant differences ($P < 0.05$). The models were built based on log-transformed values of PD, *nirK* and *nirS* gene copy number, using treatments as fixed effect (NE, Natural Environment; GAP, Good no-till Agricultural Practice; PAP, Poor no-till Agricultural Practice) and sites as random effect.

3. Results

3.1. Effect of agricultural management on the potential activity, the size and the structure of denitrifier communities

Potential denitrification (PD) rates exhibited large variations, and ranged from 0.14 to 113.36 μg N_2O–N h^{-1} g^{-1} dry soil, depending on the location and the agricultural management (Table S1). There was a strong site effect on PD, with differences spanning more than one order of magnitude between the values registered in Bengolea and the rest of the locations. Given that the influence of edaphic-climatic gradient on denitrifiers is not a central theme of this work, a detailed analysis of these responses is not reported. Yet different locations provide the context for analyzing the effects of agricultural practices at a regional scale, and thus the four sites were treated in mixed models as random factors (Table 1). Soils subjected to poor no-till agricultural practices (PAP) had significantly lower PD values than the same soils under GAP treatment ($P = 0.009$) and NE control ($P < 0.001$), whereas no significant differences were found between the two latter groups (Table 1).

Concerning the size of denitrifier communities, estimated by qPCR of *nirK* and *nirS* gene copies, copies of *nirK* per gram of soil (1.6×10^7 to 4.9×10^7) were at least one order of magnitude more abundant than *nirS* (1.2×10^5 to 6.2×10^6) (Table S1). For both genes, Viale and Pergamino had higher copy numbers than Bengolea and Monte Buey (Table S1). Agricultural management did not have a significant effect on the size of *nirK* community (Table 1). In contrast, the abundance of *nirS* in NE was significantly lower than the abundance of *nirS* in PAP ($P = 0.020$), but comparable to the abundance of *nirS* in GAP, whereas no statistical differences were detected between GAP and PAP (Table 1).

Differences in denitrifier community structure were analyzed by DGGE (Fig. S1). Clustering analysis indicated a clear separation based on geographic location for the *nirS* community structure. Interestingly *nirS* banding patterns of agricultural soils under good no-till practices were more closely related to natural environments than to agricultural soils under poor no-till practices in three of the four locations (Fig. 2), a grouping supported by high bootstrap values. In opposition, the highly variable fingerprints did not enable to find any association between *nirK* community structure and geographical position or agricultural managements (Fig. S2).

Potential denitrification activity was significantly correlated with both *nirS* abundance (Pearson coefficient = 0.764, $P < 0.001$), and *nirK* abundance (Pearson coefficient = 0.547, $P < 0.001$). However, a graphical inspection of the relationship between PD and

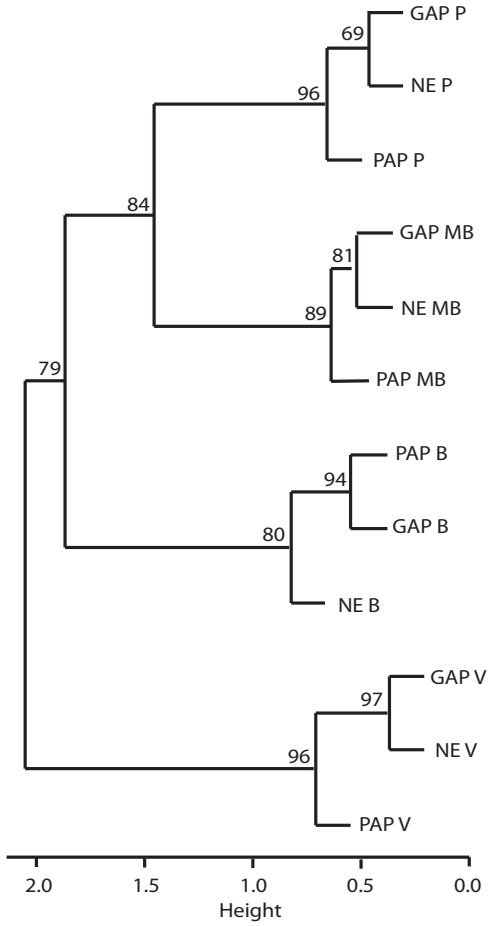

Fig. 2. Cluster analysis (Ward's minimum variance) of *nirS*-based DGGE fingerprints of soil bacterial communities subjected to contrasting agricultural managements under no-till in the four sites across Argentina's Pampas. Samples are indicated by treatment (GAP: Good no-till agricultural practices; PAP: Poor no-till agricultural practices; NE: natural environment), followed by location (B: Bengolea; MB: Monte Buey; P: Pergamino; V: Viale). Bootstrap values were obtained from 1000 resamplings.

nirS copy revealed that the correlation was forced by a site-specific effect (Fig. S3). Accordingly, the association between PD and *nirS* abundance (as well as *nirK* abundance) was no longer significant after it was analyzed using mixed models, which took into account the nested structure determined by sites ($P = 0.909$ and $P = 0.343$).

When evaluating the correlation between dissimilarity matrices from DGGE fingerprintings and denitrification potential by Mantel tests, only the *nirS* community structure exhibited an association, albeit weak, with potential denitrification activity ($P = 0.050$).

3.2. Relationship between denitrifier community changes and soil properties

The main physical and chemical properties of soils are summarized in Table S2. In order to determine which soil characteristics could explain the changes in denitrifier communities, an exploratory analysis was initially performed using all data sets. Next, soil parameters that did not vary significantly with management or site were discarded. Finally, in order to obtain a proxy for discriminating between the dynamic and inherent nature of each of the remaining 25 soil properties, we used a variance components analysis to determine the contribution of land management and site to the total variation. Table 2 shows that a higher percentage of the variance of seven soil properties was largely explained by agricultural practices, and to a lesser extent by soil geographical location. These properties, referred hereafter as dynamic, are mostly related to soil structure, including three measurements of soil stability (AS1, AS2 and AS3), total porosity (TP), bulk density (BD), and an index of soil physical quality (S). Exchangeable Potassium level (Exch K^+), which had similar values in all sites, was also included in this category due to the differences between non-cultivated and agricultural soils found in Monte Buey and Pergamino (Table S2). Soil organic carbon (OC) and total nitrogen (TN) were highly influenced by geographical location, and hence classified as inherent (Table 2). Nevertheless both properties were also influenced by treatments, showing higher values in GAP than PAP fields at the four sites (Table S2).

Potential denitrification activity was significantly correlated with several inherent soil properties (Table 2). PD also correlated with two dynamic properties, the aggregate stability indices AS1 and AS3. On the other hand, *nirS* and *nirK* abundance were almost exclusively related to those properties considered inherent (Table 2). Whereas *nirS* abundance was related with several inherent soil features (sand, clay, cation exchange capacity, aggregate density, aggregate specific volume and exchangeable Na^+ and Ca^{2+} contents), *nirK* only showed significant associations with exchangeable Ca^{2+} and sand content. Again, when the treatments in the mixed models were nested within sites to eliminate the associations forced by geographical locations, the correlations that remained significant were those between potential denitrification and soil stability indices, soil organic carbon and nitrogen, exchangeable potassium content, electrical conductivity and sand content (Table 2). Unexpectedly, the latter correlated significantly with *nirS* abundance as well (Table 2).

Principal components analysis of the dynamic soil parameters, organic carbon and potential denitrification showed that differences in agricultural management were captured largely by the first axis, which accounted for 41.2% of all variability between samples (Fig. 3). However, different variables influenced the separation of agricultural management within sites, suggesting a site-specific response of dynamic soil properties. For instance, separation of samples subjected to different management in Viale soils are mostly influenced by porosity, whereas in Pergamino different agricultural practices are separated along a gradient of aggregate stability indices (AS1 and AS3). Remarkably, a measurement of soil stability (AS2) was the soil feature most strongly associated with potential denitrification.

4. Discussion

Key factors controlling denitrification in soil, such as pH, organic carbon, nitrate, and nitrite availability, soil moisture, porosity, aeration, temperature and drying–wetting events, are influenced not only by soil inherent characteristics and climatic conditions, but also by land use (Philippot et al., 2007). The aim of this work was to investigate the response of denitrifying communities to the changes in soil properties exerted by no-till agricultural management, i.e. dynamic soil properties, across an ecological gradient. We were able to find strong relationships between potential denitrification activity and selected parameters of soil structure, particularly soil stability, in actual commercial farms located at distances exceeding 400 km. Understandably, because of the difference in soil types along the gradient, inherent properties had significant effects on the denitrification potential and on the abundance of denitrifier communities as well.

4.1. Influence of inherent soil characteristics on denitrifiers

Shifts in microbial community composition across a given landscape may be more influenced by soil inherent properties than

Table 2
Soil properties ordered by decreasing contribution of land management (treatment) to their variance (a), and their relationship with potential denitrification rates (PD), *nirK* and *nirS* gene copy number, evaluated by multiple correlations (b) or mixed linear models (c).

Soil property	(a) Variance Comp (%)			(b) Correlations coefficients			(c) MLM coefficients		
	Site	Treatment	Residual	*nirK*	*nirS*	PD	*nirK*	*nirS*	PD
AS1	15.7	65.7	18.6	0.33	−0.04	0.65***	0.07	−0.11	0.32**
BD	10.3	64.7	25.0	−0.24	−0.09	−0.33	−0.47	0.20	−0.42
AS3	24.4	56.3	19.3	0.29	−0.03	0.65***	0.07	−0.06	0.33***
Exch K^+	26.9	51.8	21.3	−0.36	−0.52*	−0.15	−0.01	−0.08	0.46**
AS2	41.1	42.0	17.0	0.23	−0.15	0.41	0.07	−0.08	0.43***
TP	28.7	35.3	35.9	0.16	−0.01	0.21	0.01	−0.01	0.03
S	14.5	28.3	57.3	−0.32	−0.43	−0.14	−1.88	−1.55	6.57
OC	63.2	15.7	21.2	0.48	0.37	0.81***	0.09	−0.09	0.35***
TN	56.3	14.6	29.2	0.47	0.26	0.74***	1.16	−1.35	3.78**
EC	65.6	14.4	20.0	0.22	0.51*	0.68***	0.06	0.08	1.94***
PAW	43.1	8.6	48.2	0.17	−0.14	−0.21	0.31	−1.88	0.33
PR	76.1	7.4	16.6	−0.56**	−0.51*	−0.60**	−0.01	0.01	−0.01
Exch Mg^{2+}	78.7	6.2	15.2	0.35	0.51*	0.66***	0.04	−0.07	0.10
Exch Ca^{2+}	64.3	6.0	29.8	0.51*	0.70***	0.51*	0.02	0.01	−0.01
CEC	91.6	5.0	3.4	0.38	0.69***	0.72***	0.01	0.01	0.05
AggV	85.3	2.2	12.6	−0.35	−0.74***	−0.52*	0.11	−3.20	0.88
AggD	85.3	1.8	12.9	0.35	0.75***	0.51*	0.02	1.40	−0.35
AC	42.8	0.8	56.5	−0.07	−0.26	0.12	0.01	−0.01	0.03
Clay (<2 μm)	95.6	0.6	3.8	0.48	0.79***	0.66***	0.01	0.03	0.03
Silt (2–50 μm)	96.5	0.6	3.0	0.37	0.34	0.48*	0.01	0.01	−0.01
Sand (>50 μm)	99.8	0.0	0.2	−0.50*	−0.66***	−0.68***	−0.01	0.02***	−0.04***
Exch Na^+	74.5	0.0	25.5	0.19	0.53*	0.40	−0.18	0.15	0.30
ESP	90.5	0.0	9.5	−0.15	−0.21	−0.36	−0.04	0.03	−0.06
EAW	66.7	0.0	33.4	0.27	−0.07	−0.03	−0.03	−0.42	−0.93
BS	33.3	0.0	66.7	0.05	−0.11	−0.51*	0.01	0.01	−0.02

Dashed line indicates limit between soil properties termed *dynamic* (above) and *inherent* (below), defined in base of which factor explained mainly their variances (higher variance percentage attributable to treatments for those classified as dynamic properties and higher variance percentage attributable to sites for those classified as inherent properties. Aggregate Stability 1—Fast wetting—(AS1), Bulk Density (BD), Aggregate Stability 3—Slow Wetting—(AS3), Exchangeable K^+ (Exch K^+), Aggregate Stability 2—Stir wetting—(AS2), Total Porosity (TP), S—Dexter (S), Organic Carbon (OC), Total Nitrogen (TN), Electrical conductivity (EC), Plant Available Water (330–15,000 cm) (PAW), Penetration Resistance (PR), Exchangeable Mg^{2+} (Exch Mg^{2+}), Exchangeable Ca^{2+}(Exch Ca^{2+}), Cation Exchangeable Capacity (CEC), Aggregate Specific Volume (AggV), Aggregate Density (AggD), Air Capacity (AC), Clay, Silt, Sand, Exchangeable Na^+ (Exch Na^+), Exchangeable Sodium Percentage (ESP)Easily Available Water (330–2000 cm) (EAW), Base Saturation (BS). Asterisks represent *P* values (*$P<0.05$, **$P<0.01$, ***$P<0.001$).

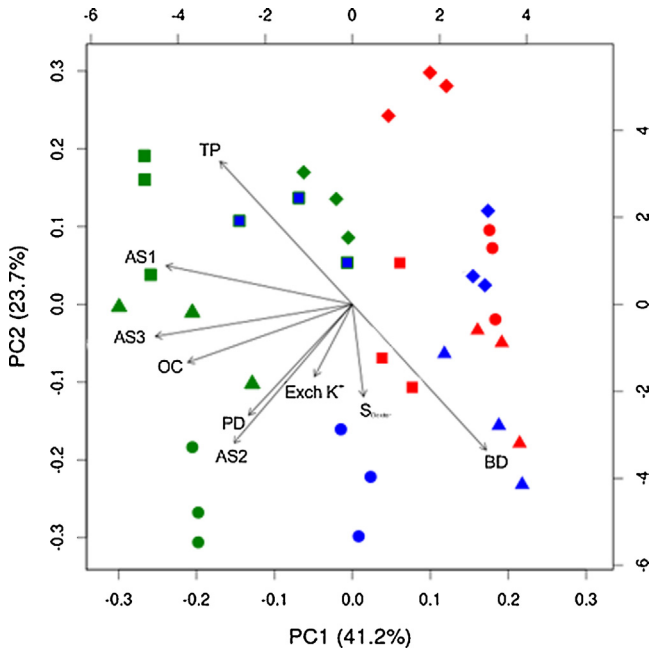

Fig. 3. Principal component analysis (PCA) of dynamic soil parameters. Organic carbon (OC), potential denitrification (PD), bulk density (BD), total porosity (TP), Dexter's index of soil physical quality (S_{Dexter}), exchangeable K^+ (Exch K^+), structural stability indices (AS1—fast wetting-; AS2—stir wetting-; AS3—slow wetting-). Amount of variability explained by each axis is presented in parenthesis. Geometric shapes indicate sites (Bengolea, diamond; Monte Buey, circle; Pergamino, triangle; Viale, square) and colours indicate agricultural treatments (NE, green; GAP, blue; PAP, red). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

by land-use (Lauber et al., 2008). Our results indicated that several inherent soil features that influence the development of soil aggregation, such as texture, cation exchange capacity and exchangeable Ca^{2+} (Bronick and Lal, 2005) exhibited a tight relationship with the variations of both denitrification potential and abundance of denitrifiers along the ecological gradient. This is a likely consequence of denitrification occurring within the anaerobic microsites (Parkin, 1987) at the interior of soil aggregates. Accordingly, the soil from Viale, which contained the highest values of clay, cation exchange capacity and exchangeable Ca^{2+} displayed higher potential denitrification rates, whereas the lowest values of PD were observed in Bengolea soil. Soils from Pergamino and Monte Buey, having a more balanced texture and intermediate exchangeable Ca^{2+} values, expressed intermediate denitrification potentials. The significant associations of sand content with potential denitrification, and also with *nirS* abundance, persisted even after modelling the nested structure determined by multiple sites. These results are consistent with a previous case, in which denitrification rates were higher in poorly-drained clay loam forest soils, than in well-drained sandy soils (Groffman and Tiedje, 1989).

4.2. Influence of dynamic soil properties on denitrification potential

Potential denitrification activity has been widely used to examine the effect of environmental conditions and management on soils (Qin et al., 2012). Our results agree with previous findings showing that PD is lower in crop soils than in grasslands (Bijay et al., 1989; Lensi et al., 1995), and also support the idea that conservative land-use practices tend to increase the potential denitrification activity (Baudoin et al., 2009; Attard et al., 2011).

It has recently been suggested that changes in potential denitrification activity are driven by soil environmental conditions rather than by denitrifier abundance or diversity (Attard et al., 2011). Among a wide variety of chemical and physical soil parameters, we have found that soil structural properties are the most affected by unsustainable agricultural management. In particular, the three aggregate stability indices evaluated here presented the most significant associations with PD. Aggregate stability is involved in maintaining important ecosystem functions in soil including organic carbon accumulation, infiltration capacity, movement and storage of water, and root and microbial community activity (Allen et al., 2011), and has been proposed as a main indicator of soil quality (Arshad and Coen, 1992) regarding different managements (Orellana and Pilatti, 1984; Lacerda et al., 2005). A more structured soil favours bacterial survival by offering them a higher diversity of physical habitats (Chenu and Cosentino, 2011).

Organic carbon and total nitrogen availability are critical attributes of soil health that drive the majority of the soil functions (Allen et al., 2011). Correspondingly, organic carbon has been highlighted as one of the most important factors affecting potential denitrification in soils (Beauchamp et al., 1989; Attard et al., 2011). The dependence of organic carbon and total nitrogen on both inherent and dynamic soil properties, and the significant correlation of these two parameters with the denitrification potential ratified here, exemplify the strong linkage among heterotrophic microorganisms activity, carbon input and soil structure, whose interactions are key to implement favourable management practices (Chenu and Cosentino, 2011).

Unexpectedly, agricultural management influenced potassium levels in two of the four sites examined. Although this property is not commonly used as a basic indicator for assessing soil quality, the rapid depletion of K^+ in soil is a well-known problem, which may become a constraint for crop production (Cakmak, 2010). Significant relationships between PD and electrical conductivity and potassium levels have not been reported previously, although Adviento-Borbe et al. (2006) did find direct effects of electrical conductivity on microbial respiration and N_2O emissions in soils under intensive cropping. Electrical conductivity is a measurement of salt concentration, which can reveal trends in salinity, crop performance, nutrient cycling and biological activity (Arnold et al., 2005). Understanding the influence of salt concentration on the activity of denitrifying needs more experimental evidence as soil properties are interdependent and do not respond independently to management changes (Blanco and Lal, 2008).

4.3. Relationship between the potential denitrification, abundance and structure of the denitrifying community

Denitrifying bacteria may serve as model systems for understanding the relationship between community structure and activity (Philippot and Hallin, 2005). Yet whereas some studies showed that in agroecosystems denitrification enzyme activity was correlated with the size (Hallin et al., 2009; Philippot et al., 2009; Attard et al., 2011) and the structure (Wertz et al., 2009) of denitrifying communities, in other cases potential denitrification activity was uncoupled from community composition (Dandie et al., 2008, 2011; Henderson et al., 2010). We have found that only the denitrifier communities harbouring the *nirS* gene, but not *nirK* gene, correlated with PD, a finding similar to the one observed in a previous study (Enwall et al., 2010).

Although not a universal occurrence (Hallin et al., 2009; Enwall et al., 2010; Attard et al., 2011), the dominance of *nirK* denitrifiers over *nirS* denitrifiers found in these soils appears to be a common feature of agroecosystems (Dandie et al., 2008, 2011; Yoshida et al., 2010; Baudoin et al., 2009; Pastorelli et al., 2011). Nonetheless, the response of denitrifier communities to agricultural management reported in the literature varied greatly, ranging from the absence of change (Hallin et al., 2009) to a differential change in the size of *nirK*, but not *nirS* communities (Yoshida et al., 2010; Attard et al., 2011). We do not think that the lack of association between *nirK* gene copy and most of the variables analyzed here, including soil properties, agricultural practices and potential denitrifying activity denotes a lack of response to perturbations. Rather, it seems to be related with the high variability of *nirK* denitrifiers between and within geographical locations. Since denitrifying bacteria possess either NirS or NirK, but not both enzymes (Glockner et al., 1993), the different distribution of the *nirS* and *nirK* gene abundance, suggests habitat selection on the *nirS* and *nirK* denitrifiers (Enwall et al., 2010). Interestingly, niche differentiation between denitrifiers can be inferred in this study from the fact that *nirS* community size and structure changed according to agricultural management but this effect was not apparent for *nirK* community. Based on the results presented here, it would be interesting to see whether changes in the smaller and less variable *nirS* community could be a more tractable marker at the regional scale.

4.4. Conclusions

The structure of denitrifying bacterial communities and the denitrification potential exhibited strong site effects along an edaphic-climatic gradient of the northern Argentinean Pampas. Mixed models were used to determine the effect of agricultural management on the potential of denitrification and on the abundance and structure of the denitrifier community structure across such gradient. In general, PD values of agricultural soils managed under good no-till practices were closer to those from natural environments than to agricultural soils under poor no-till practices, an observation that also applied to the structure and abundance of *nirS* communities. We found that both inherent and dynamic properties could explain the changes in potential denitrification activity, whereas changes in the abundance of denitrifiers were only related to inherent soil properties. After removing the geographical effect, the dynamic property most strongly associated with potential denitrification was an index of soil structural stability.

Acknowledgements

The authors are members of the BIOSPAS consortium (http://www.biospas.org/en), whose work is supported in part by the Argentine Ministry of Science, Technology and Innovation (Grant no. ANPCyT-PAE-36976) and by the National Institute for Agricultural Technology (INTA). ELMF, and LE are members of Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina. SR received fellowships from ANPCyT, Argentina. FBK and LDG were fellows of CONICET. We are grateful to Dr. J. Galantini and Lic. M. Duval (CERZOS-CONICET-UNS-Argentina) for helping us with OC and TN determinations.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.apsoil.2013.11.012.

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