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RESEARCH ARTICLE

Is the nitrification a redundant process in arid regions?: activity, abundance and diversity of nitrifier microorganisms

Es la nitrificación un proceso redundante en regiones áridas?: actividad, abundancia y diversidad de microorganismos nitrificadores

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ABSTRACT

We tested if the microbial functional redundancy concept (theory relating changes in ecosystem functioning by species loss) apply for changes in the ammonium-oxidizing community (nitrifier bacteria) due to: a) grazing disturbance, b) seasonal variations (dry season vs. wet season), c) habitat type (soil vs. organic residues), and d) ecological conditions (two eco-regions), in an arid region of central-western of Argentina. We determined: a) abundance of culturable nitrifier bacteria, b) nitrification activity and efficiency, and c) nitrifier community genetic structure by PCR-DGGE (richness and similarity index). Grazing did not change the nitrifier abundance, activity and richness, while seasonally and eco-region effects were scarce. Contrarily, the habitat type affected nitrifier activity, which was higher in organic residues than in soil. All similarity indexes were low (average 0.50, range 0.76-0.18) which suggest high species diversity in these arid eco-regions. Our results indicate that the nitrification process in arid region of Argentina is redundant but it applies only for each habitat. To our knowledge, this is the first functional redundancy study on microbial ecological process in disturbed arid zones, in which we report an important species replacement but without modifications in microorganism abundance and activity.

Key words: arid Chaco, Monte desert, PCR-DGGE, similarity index, species replacement.

RESUMEN

Nosotros testeamos si el concepto de redundancia funcional microbiana (teoría que relaciona los cambios en el funcionamiento del ecosistema y la pérdida de especies) se aplica para la comunidad de microorganismos oxidadores de amonio (bacterias nitrificadoras) afectada por el pastoreo, la estacionalidad (estación seca y húmeda), el tipo de hábitat (suelo y restos orgánicos), y las condiciones ecológicas (eco-región del Chaco Árido y el Monte), en la región árida del centro-oeste de Argentina. Se determinó: a) la abundancia de bacterias nitrificadoras cultivables, b) la actividad y eficiencia de la nitrificación, y c) la estructura genética de la comunidad de nitrificadores mediante PCR-DGGE (riqueza e índice de similitud). El pastoreo no modificó la abundancia de nitrificadores, la actividad y la riqueza, mientras que el efecto de la estacionalidad y la eco-región fue escaso. Contrariamente, el tipo de hábitat afectó la actividad nitrificadora, la cual fue más alta en los restos orgánicos que en el suelo. Todos los índices de similitud fueron bajos (promedio = 0.5; rango entre 0.76-0.18), lo cual sugiere una alta diversidad de especies en estas regiones áridas. Nuestros resultados indican que el proceso de nitrificación en la región árida central de Argentina es redundante, pero que se puede aplicar solo para cada tipo de hábitat. En nuestro conocimiento, este es el primer estudio de redundancia funcional sobre procesos ecológicos microbianos en zonas áridas disturbadas, en el cual se detectó un importante remplazo de especies, sin modificaciones en la abundancia y actividad de los microorganismos.

Palabras clave: Chaco Árido, índice de similitud, desierto el Monte, PCR-DGGE, reemplazo de especies.

INTRODUCTION

The potential effects of biodiversity loss on ecosystem functioning and services have been a primary concern of ecologists during the last decade (Hooper et al. 2005). The concept of functional redundancy is important to theories relating changes in ecosystem processes due to species loss (Sasaki et al. 2009). There is a basic assumption that some species perform similar roles in communities and ecosystem, and redundant species can therefore be lost

with minimal impacts on ecosystem processes (Diaz & Cabido 2001, Sasaki et al. 2009). In other words, redundant species are necessary to ensure ecosystem resilience to disturbance or invasion and regulation in the organic matter flux (Villéger et al. 2008, Sasaki et al. 2009). The effects of species loss or changes in composition, and the mechanisms by which effects manifest themselves, can differ among ecosystem properties, ecosystem type, environmental conditions, organism's type (animals, plants and microorganisms) and pathways of potential community change (Hooper et al. 2005, Chaer et al. 2009).

Due to microorganisms are not a single homogeneously functioning entities, the ecosystemic significance of microorganism's functional diversity is a challenge (Strickland et al. 2009). It has frequently been assumed that there is a high degree of functional redundancy for broad-scale process, such as organic matter decomposition, however, a specific redundancy concept for microbial process have been scarcely analyzed (Nannipieri et al. 2003, Chaer et al. 2009).

Among the specialized microbial processes, the nitrification is a primary concern of ecologists because it is the source of N availability for plants in terrestrial ecosystems (Vitousek et al. 1991, Paul 2007, Zhou et al. 2009). Nitrification is the ammonia oxidation (and the consequently nitrate production) during organic residues and/or soil organic matter decomposition carried out by nitrifier bacteria, as well as by the recently discovered nitrifier archaea (Bollmann et al. 2011, Fortuna et al. 2012). This process consists in a lito-autotrophic metabolism, which includes two biochemical steps: the ammonium oxidation that produces nitrite and the following nitrite oxidation for nitrate production (Paul 2007, Fortuna et al. 2012). These metabolisms generate a small amount of energy resulting in low growth rates and yields and diversity of nitrifier microorganisms in relation to other microorganism groups (Adair & Schwartz 2008, Bollmann et al. 2011). In consequence, nitrifiers should be highly sensitive to environmental conditions, and they should change in abundance and activity under anthropic disturbs (Sparling 1998, Abril et al. 2001, Paul 2007).

Arid zones are interesting ecosystems for testing the nitrifier functional redundancy due to the frequency in environmental and land use disturbances. It is known that microbial community respond to disturbances with changes in: a) the microbial abundance, b) the activity, and/or c) the community structure (Patra et al. 2005, Dell et al. 2008, Chaer et al. 2009).

Therefore, we tested the functional redundancy concept of the nitrifier community in the western-central arid region of Argentina, determining: a) the abundance of culturable nitrifier bacteria; b) the nitrification activity, and c) the nitrifier community genetic structure. We aim to establish if the functional redundancy concept apply for grazing disturbances, climatic seasons, habitat type (soil vs. organic residues), and eco-regions.

METHODS

Study area

The western-central arid region of Argentina is an extensive area with considerable climatic variations (daily, annual and inter-annual). The precipitations quantity and frequency limit primary productivity and decrease soil microbial activity (Austin et al. 2004, Bell et al. 2008). Besides, the region undergoes strong grazing pressure, because livestock is the unique possible land use (Huxman et al. 2004, Guevara et al. 2009).

The study was conducted in two arid eco-regions of western-central Argentina: Arid Chaco and Monte (Fig. 1). The Arid Chaco eco-region is a wood-land in which tree layer is dominated by *Aspidosperma quebracho-blanco* Schlecht and, to a lesser degree, by *Prosopis* spp., with an abundant shrub layer of *Larrea divaricata* Cav, *Mimozyganthus carinatus* (Griseb.) Burkart and *Acacia furcatispina* Burkart. Grasses (genera: *Trichloris*, *Gouinia*, *Setaria* and *Pappophorum*) are present in sites with low woody cover. Mean annual rainfall range 350-500 mm, concentrated during the summer. In the dry winter season, water balance is negative resulting in a soil moisture deficit. Mean annual temperature is 20 ºC (Cabrera 1976, Morello 1977). The soil is of alluvial origin, classified as Molic Ultifluvent, with neutral pH, 25 mg g^{-1} of organic matter and 2.0 mg g^{-1} of total N (Abril et al. 2005).

The Monte eco-region is an extensive shrubland dominated by *Larrea* spp., interspersed with open forest of *Prosopis flexuosa* DC. The herb layer is composed of grasses, mainly perennial *Poaceae* C4 species (genera *Pappophorus*, *Digitaria*, *Trichloris*, *Aristida* and *Sporobolus*). Mean annual rainfall range 80-350 mm, with a strong water deficit along year, and mean annual temperature range 13-15 ºC (Cabrera 1976, Claver & Roig-Juñent 2001). The soils are typic Torrifluvent, sandy-loam of aeolian and alluvial origin, neutral to slightly alkaline pH, 13 mg g^{-1} of organic matter and 2.4 mg g^{-1} total N (Abril et al. 2009).

In each eco-region, two 1ha-sites (grazed and ungrazed) were selected. In Arid Chaco eco-region,

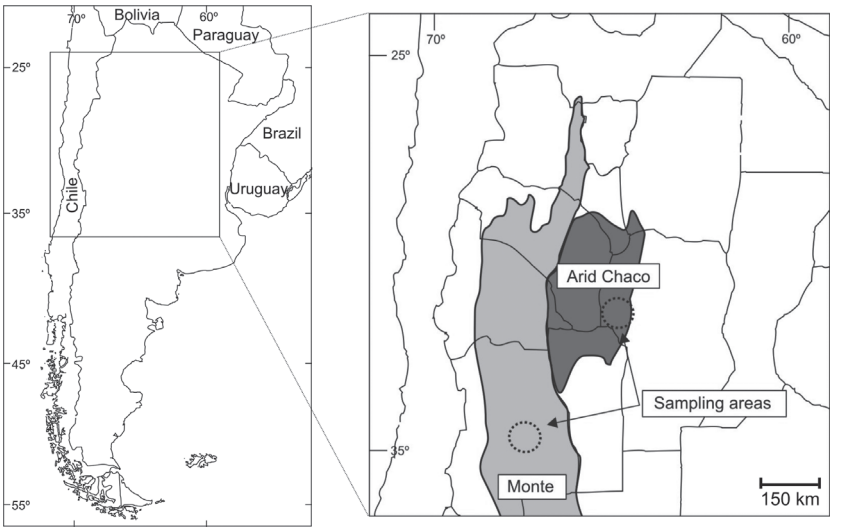

Fig. 1: Arid Chaco and Monte eco-regions sampling areas in western-central region of Argentina.

Áreas de muestreo en las eco-regiones del Chaco Árido y el Monte en el centro-oeste de Argentina.

ungrazed site (ACh-U) was Chancaní Reserve (31° 22' S and 65° 26' W) and grazed site (ACh-G) was San Miguel ranch (31° 45' S and 65° 24' W) located 40 km from Chancaní Reserve, which undergo overgrazing (2.8-3.8 ha AU^{-1}, including cattle, sheeps and goats) since more than 60 years (W. Rodriguez, personal communication, 2009). In Monte eco-region, ungrazed site (M-U) was Ñacuñán Reserve (34° 03' S and 67° 58' W) and grazed site (M-G) was La Pampa ranch (34° 03'S and 67° 54' W) located 5 km from Ñacuñan Reserve, which is a highly degraded site with cattle (8.3-34 ha AU^{-1}) since more than 50 years (P. Blanco, personal communication, 2009).

Sampling design

In each study site, three sampling 100 m-transects were randomly selected. In each transect, one composite sample (15 subsamples at random) of approximately 250 g of soil (0-20 cm) and one composite sample (15 subsamples at random) of surface organic residues in 0.16 m^2 (including litterfall, wood material and cattle manure) were collected. The samplings were taken in two periods: dry season and wet season, within the same week for all study sites.

The soil and organic residues samples of each site (n = 3) were stored refrigerated (4 °C) during transport (10 h), to avoid alterations in sample compositions. Samples (soil and organic residues) were air dried for 24 h (25 °C), sieved through a 2 mm mesh (soil) and milled (organic residues) in laboratory steel mill. Samples were stored at: a) room temperature for chemical analysis, b) 4 °C for biological analysis and c) -20 °C for molecular analysis. Previously, an aliquot of each sample was utilized for water content determination by gravimetric method. Samples for molecular analysis were pooled (45 sub-samples).

Chemical characteristics

Soil and organic residues samples from dry season were characterized according to the following parameters: total organic C by the wet-digestion method of Walkley and Black (Nelson & Sommer 1996), and total N content by micro Kjeldahl (Bremner 1996). Moreover, in soil samples, texture and pH were measured using the standard methods recommended by Klute (1986), and in organic residues, total masses were determined by gravimetric method. Precipitations were recorded in meteorological stations at Ñacuñan Reserve and San Miguel ranch, during the study year.

Nitrifier abundance and activity

In soil and organic residue samples, the nitrifier abundance was determined by the Most Probable Number (MPN) method in specific liquid media (mineral base plus 0.5 g L^{-1} $SO_4(NH_4)_2$) (Lorch 1995). Nitrification activity was determined by aerobic incubation (24 h on 1.5 mM NH_4^+ and 1 mM PO_4^{3-} buffer), and nitrate quantification (Abril et al. 2001, Verchot et al. 2003, Ceccherini et al. 2008). Briefly, for each situation, two 2g-samples soil/organic residues were suspended in 50 ml of buffer: in one 2g-sample, the liquid phase was extracted and the nitrate was quantified immediately by spectrometry (initial values), while in the second one, previous nitrate quantification, the samples were incubated in a shaking incubator (100 rpm), at room temperate, and 16/8 h light/dark (incubated values). The nitrification activity was determined by difference between incubated and initial nitrate concentration values.

Nitrifier community genetic structure

Community total DNA was extracted in duplicates from 0.5 g of soil and 0.3 g of organic residues (Fast DNA Kit for Soil BIO 101, Qbiogen). DNA purity was checked spectrophotometrically (260/280 nm ratios) (Picodrop), while DNA extracts were also checked for shearing and degradation by agarose gel electrophoresis (Ascher et al. 2010). Nitrifiers were assessed by bacterial amoA gene fragment-PCR-DGGE of 40 ng target DNA with the

primer set GC-amoA 1f (5'-CGC CCG GGG CGC GCC CCG GGC GGG GCG GGG GCA CGG GGG TTT CTA CTG GTG GT-3')/ amoA 2r (5'-CCC CTC KGS AAA GCC TTC TTC-3'; K = G/T y S = G/C), generating 506 bp amplicons (Rotthauwe et al. 1997, Ceccherini et al. 2007). PCR products were checked for correct size and quantity by gel-electrophoresis in comparison to standardized DNA marker (Mass Ladder DNA Mix, Fermentas).

Genetic fingerprinting (DGGE) of nitrifiers were performed with 100 ng amplicons on 6 % polyacrylamide at 60 °C for 16 h, at 100 V, and 45-65 % denaturing gradient (100 % denaturant contains 7 M urea and 40 % formamide). The samples were run in duplicates, stained by SybrGreen, and the total number of different bands (band richness) was determined for the compared samples (Ceccherini et al. 2008). A standardized DNA was used as intra-gel DGGE marker. PCR and DGGE were performed using Thermocycler (My-Cycler, Biorad) and Ingeny PhorU system (Ingeny, Leiden, NL), respectively.

Calculations and statistical analysis

The nitrification efficiency index (NEI % = nitrification activity/nitrifier abundance), (Chu et al. 2008), and band richness (band quantity for each situation) were calculated. The effects of grazing, seasonally, habitat type and ecological conditions and their interactions were tested using ANOVA (four factors) and comparison of means by LSD test ($P \leq 0.05$). Data analyses were performed using the InfoStat (2001) software.

The bands relative abundances were expressed as band frequencies: ratio between quantity of situations that present x-band and all situations. The similarity of the PCR-DGGE profiles was assessed by similarity index (Dice Coefficient) according to the following relationship $Cd = 2a/(b + c)$ where a is the number of bands shared by two samples, b is the number of bands in sample 1, and c is the number of bands in sample 2 (Clegg et al. 2003). An index value ≥ 0.80 was considered that correspond to a similar community (Clegg et al. 2003).

RESULTS

Soil and organic residues characteristics

The soils of all situations were characterized as neutral (in ACh-U) to alkaline pH, in the remaining sites (Table 1). In Arid Chaco eco-region, total organic C was higher in ACh-U than grazed site, while in Monte eco-region the organic C did not differ between sites. Contrarily, the total N was higher in both ungrazed sites (ACh-U and M-U) (Table 1). Soil water content was low in all situations (range: 1.28 - 6.14 %), and values were higher in Arid Chaco than Monte (4.26 vs. 3.10 %; $P = 0.041$).

The organic residue masses were different among sites. The lowest values were detected in ACh-G and the highest in ACh-U. In each eco-region, the grazed sites presented lower values than ungrazed sites. Total C and N contents in organic residues did not differ among situations (Table 1). Organic residue water content did not differ among sites; however, significant

TABLE 1

Soil and organic residues characteristics in the study sites. ACh-U: Arid Chaco-ungrazed site, ACh-G: Arid Chaco-grazed site, M-U: Monte-ungrazed site, M-G: Monte-grazed site. Letters indicate significant differences among situations (LSD test, $P \leq 0.05$).

Características del suelo y los restos orgánicos en los sitios de estudio. ACh-U: Chaco Árido-sitio no pastoreado, ACh-G: Chaco Árido-sitio pastoreado, M-U: Monte- sitio no pastoreado, M-G: Monte- sitio pastoreado. Las letras indican diferencias significativas entre las situaciones (test LSD, $P \leq 0.05$).

	ACh-U	ACh-G	M-U	M-G
Soil				
Texture	sandy-clay loam	clay loam	sandy-clay	sandy-clay loam
pH	7.30 b	8.80 a	8.59 a	9.08 a
Total organic C (g kg^{-1})	30.2 a	20.0 b	8.9 c	10.5 c
Total N (g kg^{-1})	5.9 a	3.3 b	2.1 c	1.7 d
Organic residues				
Total organic C (g kg^{-1})	360.3	401.3	322.5	399.7
Total N (g kg^{-1})	14.7	14.1	15.6	12.9
Mass (kg ha^{-1})	7338.89 a	762.38 d	6518.08 b	2306.32 c

differences between seasons were detected (wet season 13.16 % vs. dry season 6.90 %).

Nitrifier abundance and activity

The nitrifier abundances among the analyzed situations were similar in both soil and organic residues in dry and wet season, while the nitrification activity significantly varied in soil only in both seasons (dry and wet). In soil, the highest activity values were detected in ACh-G in dry season and in ACh-U in wet season, and the lowest in M-U in wet season. Accordingly, soil NEI was highest in ACh-G (in dry season) and ACh-U (in wet season) (Table 2).

The effects of grazing, season, habitat type and eco-region were scarce. The seasonal effect was only significant for the nitrifier abundance (wet season 3.12 vs. dry season 2.67 log g^{-1}; P = 0.0005), whereas the habitat type effect was significant for nitrification activity (organic residues: 1.01 vs. soil: 0.19 mg nitrate g^{-1}; P =

TABLE 2

Nitrifier abundance (log g^{-1}), nitrification activity (mg nitrate g^{-1}), and nitrification efficiency index (%), from soil and organic residues in the analyzed sites (dry and wet season) (mean ± SD). ACh-U: Arid Chaco-ungrazed site, ACh-G: Arid Chaco-grazed site, M-U: Monte-ungrazed site, M-G: Monte-grazed site. For each season, letters indicate significant differences among situations (LSD test, P ≤ 0.05). NEI: Nitrification efficiency index.

Abundancia de bacterias nitrificadoras (log g^{-1}), actividad nitrificadora (mg nitrato g^{-1}), e índice de eficiencia de nitrificación (%), en el suelo y los restos orgánicos de los sitios de estudio (estación seca y húmeda) (media ± DE). ACh-U: Chaco Árido-sitio no pastoreado, ACh-G: Chaco Árido-sitio pastoreado, M-U: Monte- sitio no pastoreado, M-G: Monte- sitio pastoreado. Las letras indican diferencias significativas entre situaciones para cada estación (test LSD, P ≤ 0.05). NEI: índice de eficiencia de nitrificación.

	Nitrifier abundance (log g^{-1})		Nitrification activity (mg nitrate g^{-1})		NEI (%)	
	Dry season	Wet season	Dry season	Wet season	Dry season	Wet season
Soil						
ACh-U	2.71 (± 0.45)	3.38 (± 1.01)	0.22 ab (± 0.21)	0.34 a (± 0.14)	8.39 ab (± 7.91)	11.06 a (± 5.99)
ACh-G	2.38 (± 0.36)	3.27 (± 0.64)	0.35 a (± 0.31)	0.19 b (± 0.10)	15.04 a (± 14.72)	5.80 b (± 3.00)
M-U	2.39 (± 0.47)	2.45 (± 0.24)	0.10 b (± 0.06)	0.10 c (± 0.04)	4.32 b (± 2.71)	3.90 b (± 1.69)
M-G	2.66 (± 0.61)	3.21 (± 1.32)	0.09 b (± 0.07)	0.13 bc (± 0.06)	3.49 b (± 2.43)	4.39 b (± 2.17)
P	0.3243	0.1351	0.0222	< 0.0001	0.0288	0.0007
Organic residues						
ACh-U	2.79 (± 0.58)	3.56 (± 0.93)	1.15 (± 0.68)	0.82 (± 0.30)	41.23 (± 23.73)	24.74 (± 11.70)
ACh-G	2.63 (± 0.39)	3.30 (± 0.62)	0.93 (± 0.55)	0.93 (± 0.55)	37.46 (± 24.82)	29.11 (± 17.33)
M-U	2.58 (± 0.86)	2.86 (± 0.43)	1.20 (± 0.34)	1.26 (± 0.55)	51.02 (± 17.99)	45.41 (± 22.16)
M-G	2.98 (± 1.12)	2.91 (± 0.84)	0.75 (± 0.18)	1.00 (± 0.26)	28.71 (± 12.80)	37.14 (± 16.00)
P	0.7112	0.1602	0.1830	0.1967	0.1587	0.0717

TABLE 3

Presence (+)/absence (-) of grazing, season, habitat type and eco-region effects on nitrifier abundance, nitrification activity, nitrification efficiency index (NEI), richness and similarity index.

Presencia (+)/ausencia (-) del efecto del pastoreo, la estacionalidad, el tipo de hábitat y la eco-región sobre la abundancia de nitrificadores, la actividad nitrificadora, el índice de eficiencia de nitrificación (NEI), la riqueza y el índice de similitud genética.

	Grazed	Seasonally	Habitat type	Eco-region
Abundance	-	+	-	-
Activity	-	-	+	-
NEI	-	-	+	-
Richness	-	-	-	-
Similarity	+	+	+	+

0.0001) and NEI (organic residues 36.85 vs. soil 7.05 %; P = 0.0025). Grazing did not affect nitrifiers abundance, activity and NEI (Table 3).

We detected the following interactions: a) grazing x eco-region for nitrifier abundance (ACh-U = M-G > ACh-G > M-U; P = 0.0196); b) eco-region x seasonally for nitrifier abundance (ACh-wet > M-wet = M-dry = ACh-dry; P = 0.0304), c) eco-region x habitat type for nitrification activity (M-organic residues > ACh-organic residues > ACh-soil = M-soil; P = 0.0192), and d) eco-region x habitat type for NEI (M-organic residues > ACh-organic residues > ACh-soil = M-soil; P = 0.0048) (Fig. 2).

Nitrifier genetic structure

Forty-one bands were identified in DGGE profiles from 16 samples: 35 in soil and 28 in organic residues. Total average and range of band richness were 13.31 (9-17). In soil, the band average was 14 (range 10-17) and in organic residues 12.6 (range 9-17). Grazing, season, habitat type and eco-region effect and its interactions were not detected in band richness.

No band was represented in all the samples. Thirteen bands were exclusive of soil and six bands were exclusive of organic residues. In soil, 12 bands were presented in one sample only, whereas in organic residues two bands were detected in one sample only. The number-33 band was detected in all soil samples and number-14 band in all organic residue samples (Fig. 3).

None of the possible pairwise comparisons (120) exceed 0.80 of similarity index. The highest similarity index was presented in organic residues of M-G between dry/wet seasons (0.76) and the lowest in soil M-U in wet

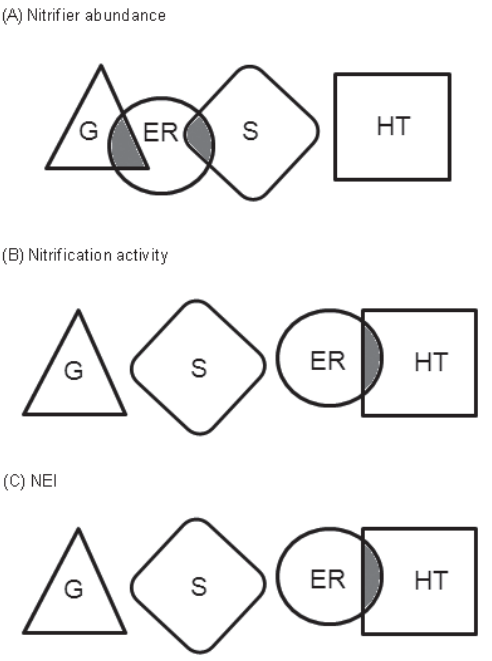

Fig. 2: Significant interactions (grey zones) among grazing, season, habit type and eco-region effect on nitrifier abundance (a), nitrification activity (b) and nitrification efficiency index (c; NEI) (LSD test, P ≤ 0.05). G: grazing, S: season, HT: habitat type, ER: eco-region.

Interacciones significativas (zonas grises) entre el efecto del pastoreo, la estacionalidad, el tipo de hábitat y la eco-región sobre la abundancia de nitrificadores (a), la actividad nitrificadora (b) y el índice de eficiencia de nitrificación (c; NEI) (LSD test, P ≤ 0.05). G: pastoreo, S: estación, HT: tipo de hábitat, ER: eco-región.

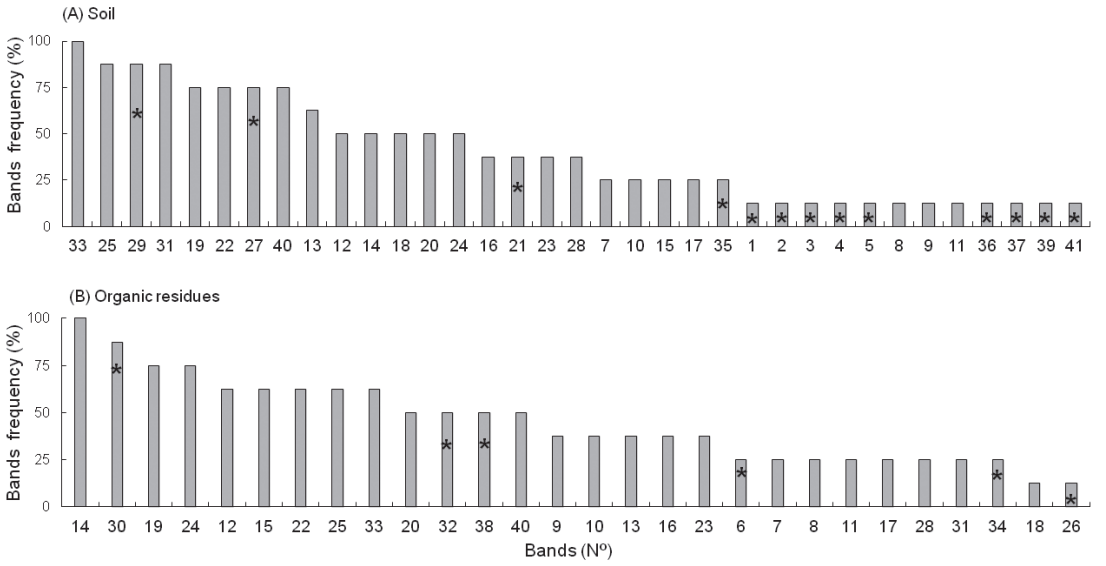

Fig. 3: Relative abundance of bands (%) detected using PCR-DGGE analysis. (a) bands detected in soils, (b) bands detected in organic residues. * indicate bands exclusive for each habitat.

Abundancia relativa de bandas (%) detectadas mediante el análisis de PCR-DGGE. (a) bandas detectadas en el suelo, (b) bandas detectadas en los restos orgánicos. * indica bandas exclusivas para cada hábitat.

season/ organic residues M-G in wet season (0.18) (Fig. 4). The effects of disturbs (grazing, seasonally, habitat type and eco-region) were detected in nitrifier similarity index (Table 3).

DISCUSSION

In our results, the few differences detected in nitrifier abundance and nitrification activity, suggest high functional stability in nitrification process, whereas the high diversity would be the result of a species replacement. These observations are in agreement with Bell et al. (2008), who suggested that when biotic or abiotic factors limited microbial individual function, other microorganisms provided to ecosystem process by replacing repressed microbial components.

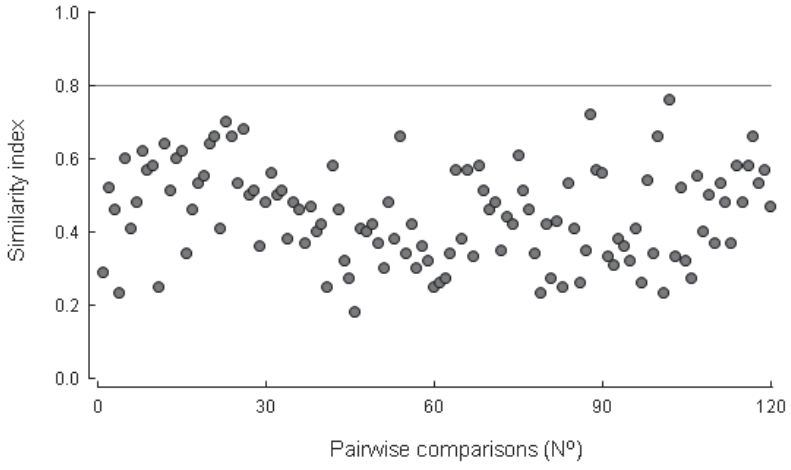

Fig. 4: Genetic similarity indexes (pairwise comparison using Dice coefficient) between the analyzed situations.

Índice de similitud genética (pares de comparación según Coeficiente Dice) entre las situaciones analizadas.

Effect of grazing

Contrarily to Patra et al. (2005) and Zhang et al. (2008), we detected few changes due to grazing (decrease in nitrification activity in the grazed site of Monte eco-region only). Although land use is one of the main factors that strongly impact N cycle (because it modifies biotic and abiotic soil characteristics), the effects of land use on soil net N mineralization still remain controversial (Wang et al. 2006, Bisigato et al. 2008).

Despite the fact that in our study grazed sites showed few changes, it is important to note that both eco-regions grazed sites have lower soil total N content than ungrazed sites. It is accepted that soil total N decrease is due to a less N release from plant litter (Wang et al. 2006), and our results are consistent with this statement. When the nitrate production (24 h) is calculated in relation to the organic residues mass per ha, grazed sites have approximately -75 % of organic residues, which result in -85 % nitrate production per ha (Fig. 5).

Although the nitrifier band richness was similar among grazed and ungrazed sites, the fact that the sites only share a 55 % of bands indicating that the grazing induce strong population changes. These results disagree with Chear et al. (2009) who proposed that the effect of stress or disturbance on specialized functions such as nitrification decrease as biodiversity decreases. However, our results clearly show high nitrifier functional redundancy for grazing disturbances because the species replacement does not involve the abundance and activity modifications.

Effect of seasonality

Our results show that season climatic changes affect the nitrifier abundance without modifying the nitrification activity. However, these abundance changes did not reach to equalize the magnitude mentioned by other authors (Meier et al. 2008). The high nitrifier abundance in Arid Chaco during wet season has a direct relation to the precipitations recorded during the period, which was twelve fold higher than the accumulated in dry season (469 vs. 39 mm). Contrarily, in Monte eco-region the variation of precipitations between seasons was four fold only (167 vs. 36.4 mm), and this difference did not produce changes in nitrifier abundance.

It is well know the influence of temperature and humidity over the microorganisms, but

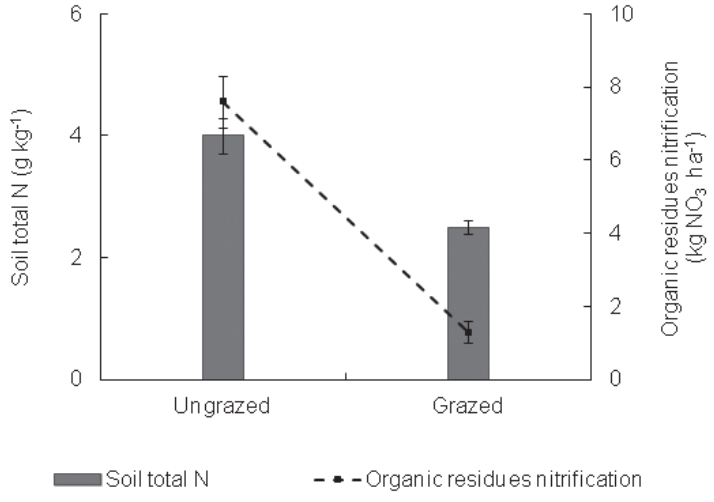

Fig.5: Relationships between organic residues nitrification and soil total N in ungrazed and grazed sites. Bars indicate standard error.

Relación entre el proceso de nitrificación en los restos orgánicos y el N total del suelo en los sitios no pastoreados y pastoreados. Las barras indican el error estándar.

the size and activity of functional group are not necessarily tightly coupled (Mazzarino et al. 1991, Patra et al. 2005). This aspect is clearly reflected in our results: abundance increase (dry season = 2.67 vs. wet season = 3.12 log g^{-1}) but nitrification activity is similar, even with high precipitations. This observation also is in agreement with Yadkjian et al. (2006) who did not find a significant relationship between annual net N mineralization and annual water input.

According to Wang et al. (2006) the 15 % of soil moisture would be the threshold for the nitrification activity in soils. However, we detected nitrification activity (0.10 mg nitrate g^{-1}) with soil moisture as low as 1.28 %. This disagreement could be due to that Wang et al. (2006) measured the nitrification activity for longer incubated periods than our study (35 d vs. 24 h). It has been shown that long incubated period underestimated the nitrification activity because the O_2 deficiency diffusion (Abril et al. 2001).

Our results show that temperature range in Monte eco-region (-7 to 42 ºC) (Claver & Roig-Juñent 2001) does not affect the nitrification activity, which suggests the high functional bacterial adaptation to arid region conditions. However, our result do not agree with Wang et al. (2006) and Zhang et al. (2008), who reported that the temperature has a strong effect on nitrification activity.

It is important to note that the analyzed situation show more bands shared between seasons (mean 62 %) that between habitat type, eco-region and grazing disturbance. These results agree with several authors who stated that few microbial population changes along the year (Laverman et al. 2001, Meier et al. 2008).

Effect of habitat type

The habitat was an important factor to the diversity and activity variation of nitrifier community, corroborating the importance of nitrification in organic residues (Chu et al. 2008, Casado-Murillo & Abril 2011). The strong differences in diversity and activity between soil and organic residues would be related to factors that define nitrification process. Ross et al. (2009) consider that nitrification is related with a high availability of low C/N ratio compounds, and state that 23-25 is the threshold above which nitrification is minimal. However, we found higher nitrification in organic residues than soil (C/N 25.7 vs. 5.2), indicating that the C/N ratio would not be the most important factor in nitrification process.

Our results of elevated NEI in organic residues clearly indicate that nitrifier cells are more metabolically active in organic residues than in soil, which agree with the high dissimilarity in the community genetic structure (44 % of similarity) between soil and organic residues. Meier et al. (2009) found different microbial populations in habitats with different physicochemical characteristic.

Effect of eco-region

The fact that the eco-region did not influence the nitrifier abundance and activity, but it did influence on the interaction with site, season and habitat, suggest a relationship between environmental factors (Ross et al. 2009, Rooney et al. 2010). For example, nitrifier abundance gradation in eco-region x season interaction (ACh-wet > M-wet = M-dry = ACh-dry) is probably due to the highest precipitations received by Arid Chaco during wet season.

Contrarily to abundance and activity, genetic structure of nitrifier community was different between eco-regions, and it presented the lower similarity of the pairwise comparisons (40 %). It is expected that two eco-regions with great ecological differences (in precipitations magnitude and frequency, temperature, soil characteristics, and vegetation structure) have different nitrifier community structures (Avrahami & Conrad 2003, Chaer et al. 2009, Fierer et al. 2009).

Conclusions

Our results indicate that the nitrification process in the studied arid region of Argentina is redundant but it applies only for each habitat (soil and organic residues), due to nitrifier microorganisms are highly affected by resource characteristics. To our knowledge, this is the first functional redundancy study on microbial ecological process in disturbed arid zones, in which we report an important species replacement but no modifications in nitrifier abundance and activity.

From an ecological perspective, this nitrifier redundancy indicates high ecosystem stability (resilience and resistance) (Chaer et al. 2009). Due to the high resistance of the archaea, it would be possible to think that this high stability in nitrification process results from nitrifier-archaea (Bollmann et al. 2011). However, little is known about the archaea diversity in arid regions, particularly in central-western of Argentina. This area is in great need of further investigation. Indeed, the fact that nitrification process was only affected by organic residue quantity deposited in soil has a special relevance to strengthen criteria for cattle management practices in arid zones, which must tend to improve the organic residues accumulation.

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