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RESILIENCE OF NITRIFICATION PROCESS IN AGRICULTURAL AND RESTORED SOILS OF CENTRAL ARGENTINA

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Abstract

The aim of this study was to assess the resilience degree of soil nitrification process (by nitrate content, nitrification rate, and nitrifier abundance measurements), in soils of central Argentina. Soil nitrification process from cultivated farmlands (no-tillage soybean/sorghum and soybean monoculture and plow tillage peanut/sorghum) was compared to those from under restoration and control sites (native woodland). Nitrate content increased in peanut and soybean monoculture fields. Nitrification rate was higher in the control site, although nitrifier abundance did not differ between all the analyzed sites. All productive sites lead to the loss of the resilience capacity of global nitrification process in the semi-arid central area of Argentina. We conclude that no-tillage and crop rotations have a poor interactive effect on resilience of nitrification process in the short term of the transition from plow tillage. Moreover, closure is not a suitable practice for restoring nitrification process in the short-term.

Key words: Semiarid area, Nitrifiers, Peanut, Soybean, Closure.

1. Introduction

Soil is a dynamic and living entity used to produce goods and services of value to humans but not necessarily with perpetual ability to withstand the degradative processes (e.g nutrient depletion) unless appropriately managed (Tenywa *et al.*, 2001). Several management practices have been proposed to cope with soil degradation around the world, although information about novel management practices is necessary in order to decrease fertility losses. Since long-term changes can take place over time scales much greater than those at which management decisions are made, land users often do not perceive degradation as a concern (Vetter, 2009).

Although, soils are mainly composed by inorganic substances, the processes that involve fertility are strictly biological and performed by microorganisms (Nannipieri *et al.*, 2003; Wardle *et al.*, 2004). It is a well known that productive practices lead to a shift in the activity, abundance and structure of microbial communities compromising the conservation of soil fertility (Roper and Ophel-Keller, 1998; Walker and Desanker, 2004; Jinbo *et al.*, 2006).

Microbial nitrification process is considered of great importance in maintaining soil fertility (Arshad and Martin, 2002). Moreover, the understanding of nitrification in agricultural soils has practical importance for management of N fertilizers and animal waste (Norton, 2008). However, little is known about impact of agricultural practices on the degree of change and rate of restoration capacity (resilience) of nitrification process. Consequently, several authors have proposed the evaluation of the degree and threshold of soil resilience based on scales of variation magnitudes in key processes (such us nitrification) compared with an undisturbed site (Lal, 1997).

Since resilience capacity relies on geomorphological and climatic conditions, there is not a general pattern to predict this edaphic property (Beeby, 1995). However, soils can be grouped into different classes according to their degree of resilience. Highly resilient soils have high recovery rates and buffer capacity, while fragile soils exhibit an instability that prevents their return to their original state (Lal, 1997).

During the last 25 years there has been an expansion of no-till systems in Argentina altering the traditional livestock and agricultural production (Duval *et al.*, 2013). The semi-arid central area of Argentina is currently covered by flat fields under extensive agriculture with soybean (*Glicine max*) as the main crop. No-till systems (NT) have been widely adopted due to economic, productive and environmental aspects (Casado-Murillo and Abril, 2013) replacing the traditional local production under plow tillage systems (PT).

Agricultural research and development have generally remained focused on sustainable yields and reducing the effects of environmental variability (Vetter, 2009).Consequently, little information is known about soil resilience capacity to restore fertility in different agricultural practices.

The aim of this study was to assess the resilience degree of soil nitrification process (by nitrate content, nitrification rate, and nitrifier abundance measurements), in agricultural and restored soils from semi-arid central area of Argentina in order to establish sustainable management practices according to specific eco-regional conditions.

2. Materials and Methods

2.1 Study Area

The study area corresponds to the Espinal eco-region, which is mainly characterized by extensive areas of cultivated fields and scarce mosaics of native woodlands. Sorghum (*Sorghum spp*.), maize (*Zea mays*), soybean (*Glycine max*) and peanut (*Arachis hipogea*) are common agricultural crops in the area. Espinal woodlands are forests whose tree layer is dominated by *Prosopis alba*, *Celtis tala* and *Geoffroea decorticans*, with an abundant shrub layer of *Berberis rustifolia* and *Cestrum parqui*, and presence of grasses, mainly perennial Poaceae C4 species (genera *Trichloris*, *Chloris*). Soils of Espinal are alluvial forms related to sediment deposition, classified as Typic Haplustoll with a high content of sand. The climate of the region is semi-arid with a mean annual temperature of 17º C and a mean annual rainfall of 760 mm concentrated in summer (Jarsun *et al.*, 2003). The study area was located nearly Rio Tercero village (29º 54´ S, 63º 41´ W) at 341m asl, from 120 km south of Cordoba city, Argentina.

2.2 Experimental Design

In the study area, seven sites were selected, which present different crop systems managements: a) non-tillage soybean monoculture site (NTS); b) non-tillage sorghum/soybean rotation site (NTSgS); c) non-tillage soybean/sorghum rotation site (NTSSg); d) plow tillage sorghum-peanut rotation site (TSgP); e) plow tillage peanut-sorghum rotation site (TPSg); f) restoration site (R); and g) control site (C).

NTS site was intensively cultivated since five years and previously used to livestock production with alfalfa implantation. Non-tillage rotations sites consist in summer gramineous-leguminous crops with winter fallow. Annually, the sorghum residue in these sites is removed for forage use. The NT rotation sites were previously used for peanut production with rye and oats as winter crops under plow tillage. NTSSg site was fertilized with 80 kg ha^{-1} of P and S (20:12), and NTSgS with 80 kg ha^{-1} of N, P and S (20:20:12).

Plow tillage sites were cultivated since 40 years with summer gramineous-leguminous rotation and winter fallow. R site (since 4 years) was previously used for agricultural production and currently covered by natural vegetation and devoid of trees and woody species. Typical species include various annual herbaceous species such as *Cestrum parqui*, *Sorghum halepense*, *Silybum marianum*, *Chenopodium álbum*, *Ipomea purpurea*, *Tagete minuta* and *Chloris berroi*. C site is an area with Espinal woodlands.

2.3 Soil Sampling Design

In each site, three composite samples (10 subsamples) of soil (0-20 cm) were taken. The samples were collected in the period without crops to avoid the influence of living roots. The sampling date was on August 2012, in the middle of the winter bare fallow period (three months) in cropping sites.

2.4 Laboratory Analysis

Soil samples were air-dried for 24 hours and sieved through a 2 mm mesh. For each soil sample, we measured: a) nitrate content by colorimetric cadmium reduction method (Mulvaley, 1996), b) nitrifier bacteria abundance by the most probable number method with an specific culture media (Lorch *et al.*, 1995), and c) nitrification rate by 24 h incubation following Verchot (1999). Previously, soils were characterized for texture and pH (Klute, 1986) and total organic matter content (SOM) by the wet digestion method of Walkey and Black (Nelson and Sommers, 1982).

2. 5 Calculations and Statistical Analysis

The nitrification index was calculated as the ratio between nitrifier abundance and nitrification rate. The resilience degrees were established by means of the variations (%) of each nitrification parameters between productive sites and control site, following Lal's scale (1997): a) Class 0 (variation range: 0-25 %), low degradation and high resilience soils; b) Class 1 (26-50 %), moderate degradation and moderate resilience soils; c) Class 2 (51-75 %), strong degradation and low resilience soils; and d) Class 3 (76-100 %), total degradation and non-resilient soils. We assumed 75 % of variation as the resilience threshold.

Differences in the parameters among sites were analyzed by ANOVA and LSD Fisher test ($P \leq 0.05$). Principal component analysis (PCA) was performed to identify patterns of variations. Linear regressions between soil characteristic and nitrification parameters were carried out by Pearson test. All statistical calculations were carried out using the software program InfoStat (InfoStat, 2001).

3. Results

Soils were neutral and sandy, with low organic matter content. C site presented the highest content of SOM and the lowest pH value among sites. The lowest content of SOM was measured in NTSgS and the highest value of pH in TSgP site (Table 1).

Table 1. Soil characteristics (mean ± SD) of study sites. NTSSg: no tillage soybean/sorghum rotation, NTSgS: no tillage sorghum/soybean rotation; NTS: soybean monoculture; TPSg: plow tillage peanut/sorghum rotation; TSgP: plow tillage sorghum/peanut rotation; R: restoration site; C: control site; SOM: soil organic matter. Letters indicate significant differences among sites (LSD test, $p \leq 0.05$)

	NTSSg	NTSgS	NTS	TPSg	TSgP	R	C
Texture	loamy-sand	sandy-loam	loamy-sand	sandy-loam	loamy-sand	loamy-sand	loamy-sand
pH	6.76 bc (± 0.29)	6.48 cd (± 0.22)	6.50 c (± 0.12)	6.61 bc (± 0.07)	7.24 a (± 0.37)	6.96 ab (± 0.07)	6.30 d (± 0.08)

SOM ($g\ kg^{-1}$)	17.87 bc (± 1.19)	10.07 c (± 0.78)	13.20 bc (± 5.33)	14.03 bc (± 2.65)	20.27 b (± 7.55)	15.20 bc (± 2.74)	37.73 a (± 6.13)

Nitrate content and nitrification rate showed significant differences among sites. The nitrate content was higher in NTS and TPSg, not differing from C site; while NTSSg, NTSgS, TSgP and R sites presented lower contents of nitrate and with no significant differences between them (Table 2). Nitrification rate was highest in C site (Table 2) while nitrifier abundance (CV 79.11 %) and nitrification index showed no significant differences between sites (Table 2). Only a significant correlation was detected between nitrification rate and SOM (R 0.87, $p \leq 0.0001$) (Figure 1).

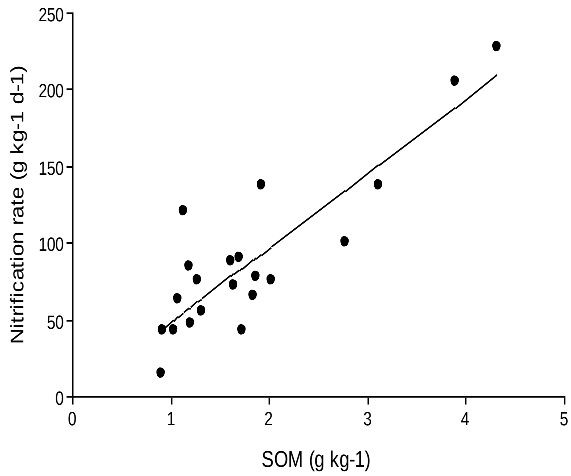
The variations between values of each productive site and C site were highly variable depending of each parameter measured. The highest percentage value was obtained in NTSgS site for nitrification rate (-74.12%) and the lowest in R site for nitrifier abundance (1.61 %). Accordingly, the Lal's scale application showed the following results: a) in Class 0 were included nitrifier abundance of TPSg and R site; b) in Class 1, the nitrate content (even positive values in NTS and TPSg) of all sites; and nitrifier abundance in NTSSg and TSgP; and in Class 2, nitrifier abundance of NTSgS and NTS and nitrification rate in all sites (Figure 2).

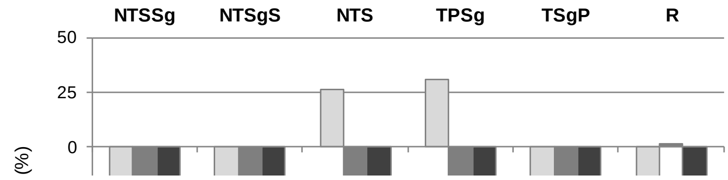
Table 2. Nitrification parameters (mean ± SD) in the analyzed sites. NTSSg: no tillage soybean/sorghum rotation; NTSgS: no tillage sorghum/soybean rotation; NTS: soybean monoculture; TPSg: plow tillage peanut/sorghum rotation; TSgP: plow tillage sorghum/peanut rotation; R: restoration site; C: control site. Letters indicate significant differences among sites (LSD test, $p \leq 0.05$).

	NTSSg	**NTSgS**	**NTS**	**TPSg**	**TSgP**	**R**	**C**
Nitrate content ($mg\ kg^{-1}$)	45.83 b (± 8.78)	44.17 b (± 5.77)	102.50 a (± 45.21)	106.67 a (± 44.81)	41.67 b (± 5.20)	41.67 b (± 13.77)	80.83 ab (± 1.44)
Nitrifier abundance ($\log_{10} g^{-1}$)	0.31 (± 0.32)	0.20 (± 0.34)	0.19 (± 0.22)	0.53 (± 0.49)	0.45 (± 0.16)	0.63 (± 0.00)	0.62 (± 0.22)
Nitrification rate ($mg\ kg^{-1}\ d^{-1}$)	71.67 b (± 6.29)	49.17 b (± 11.55)	90.83 b (± 6.25)	76.67 b (± 18.93)	83.33 b (± 14.43)	59.17 b (± 24.66)	190.00 a (± 46.84)
Nitrification index (%)	0.43 (± 0.30)	0.46 (± 0.62)	1.00 (± 1.26)	0.6 (±0.40)	0.56 (± 0.16)	1.17 (± 0.31)	0.37 (± 0.15)

Principal component analysis resulted in two components, which explained 68.17 % of the total variance (Figure 3). The first component (CP1) explaining 40.9 % of the data variance showed high loading for nitrification rate (0.73). The second component (CP2) accounting for 27.8 % of the total variation loaded heavily on nitrification index (0.85) and nitrifier abundance (0.38). This analysis clearly separated the C site from all the remaining sites, but not R site from productive sites.

Figure 1. Significant correlation (Pearson test, P<0.05) between soil organic matter (SOM) content and nitrification rate.





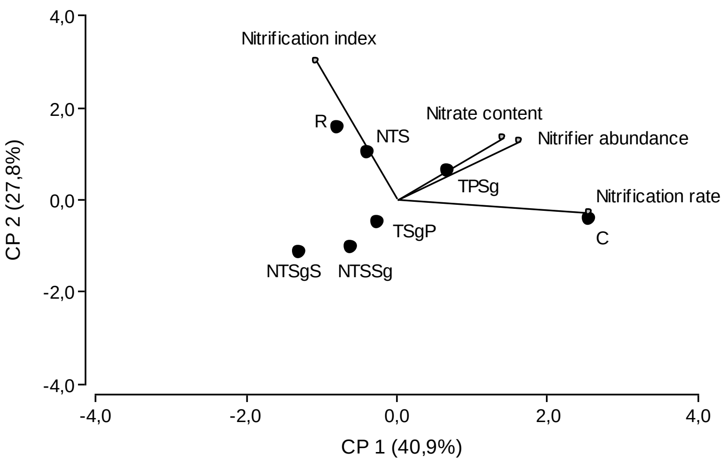


Figure 2. Variation (%) of nitrification parameters between productive sites and control site. NTSSg: no tillage soybean/sorghum rotation; NTSgS: no tillage sorghum/soybean rotation; NTS: soybean monoculture; TPSg: plow tillage peanut/sorghum rotation; TSgP: plow tillage sorghum/peanut rotation; R: restoration site.

Figure 3. Principal component analysis of nitrification parameters. NTSSg: no tillage soybean/sorghum rotation; NTSgS: no tillage sorghum/soybean rotation; NTS: soybean monoculture; TPSg: plow tillage peanut/sorghum rotation; TSgP: plow tillage sorghum/peanut rotation; R: restoration site; C: control site.

4. Discussion

4.1 Nitrate Content

Detected differences in nitrate content among sites could be explained by: a) the sampling date, b) the agricultural practice (tillage and rotations), and c) crop residues type. Regarding sampling date, our results agree with the widely known concept that the content of soil nitrate is highly affected by precipitations (Rimski-Korsakov *et al.*, 2004) due to the high nitrate solubility (Schmidt, 1982). The high nitrate values registered during the winter fallow (dry season) result from lack of rainfall to leach nitrate to deeper horizons and from lack of crops to uptake nitrate (Nielsen and Calderon, 2011).

Although several studies have evaluated the effect of tillage on soil nitrate content, they are not in agreement. For example, Bono *et al.* (2008) and Alvarez and Steinbach (2009) reported high nitrate values under plow tillage, while Casado-Murillo and Abril (2011) reported high nitrate under NT. In our results, sites under NT had slightly lower nitrate values than PT (64.16 vs 74.17 mg kg^{-1}), whereas significant differences were established between rotation systems.

Rotation with legumes clearly increase soil nitrate content, which is related to the ability of legumes to fix N_2, and its lower residue C:N than gramineous plants (Stevenson *et al.*, 1996; Blanco-Canqui and Lal, 2009). Deposition of soybean and peanut fresh leaves (low C:N) in periods of high temperatures and rainfall favor the mineralization process and the consequent nitrate release. In contrast, deposition of sorghum residue with high C:N, occurs under unfavorable climatic conditions (drought and low temperatures), which limit the nitrification (Huggins et al. 2007; Casado-Murillo and Abril 2011).

The fact that the C site recorded lower nitrate values than TSSgP and NTS is in agreement with information about soils covered with a diverse plant community. It is mentioned that the presence of arboreal and herbaceous plants with different growth patterns, make greater and more efficient the use of N throughout the year (Schmidt, 1982; Maly *et al.*, 2000). However, the lower nitrate content in R than C site, do not agree with reports from restored soils in semi-arid regions where soil N mineralization was higher (Raiesi, 2012). Probably, this is due to differences in the long of restoration periods. The available information reports nitrate values from closure of more than 18 years, while in our study it was of four years ago.

4.2 Nitrifier Abundance

Soil nitrifying microorganisms are less abundant than other functional groups (Sparling, 1998) due to the low energy obtained from the oxidation of ammonia (Schmidt, 1982; Norton, 2008), making them highly susceptible to environmental impacts (Abril 2003). In our results, nitrifier abundance show a trend to decreases in productive sites in relation to C site, which should indicate the negative impact of agricultural practices. Conversely, the similar abundance in R site respect to C site reflects a recovery trend because of closure.

The fact that no significant differences were recorded for nitrifier abundance among contrasting agricultural practice is in agreement with the findings of Noe and Abril (2013) in the arid Chaco region of Argentina, where nitrifier abundance was no influenced by contrasting livestock practices, which was explained by a possible replacement of species.

4.3 Nitrification Rate

The lower nitrification rate values in all agricultural sites than C site indicate the strong impact of agricultural practice on the nitrification process (Raiesi, 2012). Some authors have reported the highest nitrification rate in soils covered by low C:N crops residues (Zeller *et al.*, 2000; Breuer *et al.*, 2002; Vityakon, 2007), However, in this study the highest nitrification rate was observed in C site, characterized by woody residues with high C:N, in agreement with Noe and Abril (2013) who found the highest nitrification rates in soils of undisturbed woodlands.

The high SOM content in C site (37 mg kg^{-1}) and the positive correlation between SOM (Fortuna *et al.*, 2012) and nitrification rate, indicate that undisturbed soils provide optimal environmental conditions for the nitrification process (Breuer *et al.*, 2002; Noe and Abril, 2013). This is in agreement with Neill *et al.* (1999)'s report in relation to the drastic reduction of nitrification rate when woody vegetation is converted to pasture.

The fact that R site had recovered nitrifying microorganism abundance but the nitrification rates had remained low, indicate a scarce metabolic activity of nitrifier microorganisms community. This statement is consistent with Maly et al. (2000), who found no effect of short-term closure on N mineralization. The lower nitrification rate recorded in R site could be explained by the low availability of nitrifiable N (ammonium, urea, aminoacids, etc), due to lack of crop fertilization and change in the above plant community (Zeller *et al.*, 2000).

Sampling date on winter, could be affected the nitrification rate due to the direct relationship with climatic conditions (low temperature and dry period). It is know that during winter fallow, the scarce residues coverage (in tilled soils and soybean monoculture), favors fluctuations in temperature and low soil water retention, which would negatively affect the nitrification process (Nielsen and Calderon, 2011). However in our results this effect did not account since all agricultural sites present similar nitrification rate values.

4.4 Nitrification Index

Nitrification index relates abundance and activity of nitrifier communities being a useful indicator of the nitrifier microorganism's efficiency (Chu *et al.*, 2008). Our results do not show significant differences among analyzed sites, possibly due to mentioned high variability in nitrifier abundance, which resulted in a high CV for nitrification index (94.99%). However, a strong trend to negative impact on nitrification index in productive sites was detected (lowest values in C site).

4.5 Soil Resilience

The fact that nitrate resilience had been included in class 1 in all sites (even positive values) has relation with management practices. For example, the positive value of NTS (26 %) should account to N release from remaining alfalfa decomposition of the previous land use (livestock on alfalfa pasture) (Raiesi, 2012). Moreover, the positive values of TPSg could be addressed to recently peanut residue incorporation by tillage (Alvarez and Steinbach, 2009) at sampling date. Similarly, differences of nitrifier abundance resilience indicate the impact of agricultural practices (Abril 2003). Higher losses of resilience capacity of nitrifier abundances are registered in systems under NT (classes 1 and 2), while PT systems exhibit a resilience class 0 and 1. Tillage enhances soil aeration, favoring growth of strict aerobic microorganisms like nitrifiers. The result of this effect is visualized in R site, which recovers nitrifier abundance in the short term after closure, indicating that abundance has not exceeded the threshold of resilience.

As nitrification rate is the core of nitrification process, the inclusion of nitrification rate in class 2 for of all sites (R site, inclusive) indicates that nitrification process has a high risk to overcome the resilience capacity threshold (-75 %) in all productive and restoration practices. Our results show that an improvement of soil resilience capacity is not reached after 5 years of NT and rotations (with crop residue removal) in comparison with conventional tillage and rotations (plow tillage + peanut). This state is not in agreement with the differences recorded for soil resilience attributed to tillage and crop rotation by Tenywa *et al.* (2001). Restoration of productive areas is often slow, difficult and unpredictable (Vetter, 2009). We observed that nitrification rate has not recovered in R site despite its 4 years of closure. If we applied the concept stated by Seybold *et al.* (1999), respect two component of restoration processes: recovery rate (defined as the time required for return to the original state) and extend of recovery (degree of recovery reached), our result indicate that the nitrification process has a low recovery rate and a null extent of recovery after 4 years.

5. Conclusions

All productive sites lead to the loss of the resilience capacity of global nitrification process in the semi-arid central area of Argentina. No-tillage and crop rotations have a poor interactive effect on resilience of nitrification process in the short term of the transition from plow tillage. Closure is not a suitable practice for restoring nitrification process in the short-term. By these criteria, current agricultural development approaches in the semi-arid region of Argentina, are unlikely to enhance local soil´s nitrification resilience to intensive land use and other perturbations.

Accordingly, the main sustainable management practices to be suggested for environmental condition of this eco-region should be: a) perennial leguminous sowing in closured sites with periodic cuts to enhance N availability; b) incorporation of winter crops (oats, rye, wheat, etc.) into rotations to avoid bare fallow; and c) no removal of gramineous residue of summer crop.

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