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INFLUENCE OF 12-YEARS OF NPS FERTILIZATION ON SOIL QUALITY, MICROBIAL COMMUNITY PROFILE AND ACTIVITY UNDER CONSERVATION AGRICULTURAL MANAGEMENT

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ABSTRACT

Microbial parameters are considered to be potential indicators of soil quality since soil microorganisms can respond rapidly to agricultural management systems. The effect of long-term fertilizer applications on soil, especially nitrogen (N), phosphorus (P), sulphur (S), and micronutrients (m) can alter soil chemistry, microbial community structure and function. The purposes of this study were to analyse the long-term effect of chemical fertilizers on soil properties, microbiological communities and function in relation to grain yields in two growing seasons (2012-2013 and 2013-2014). All fertilization treatments were applied to a maize-wheat/soybean rotation under no-till farming system. Six fertilization treatments were evaluated: PS, NS, NP, NPS, NPSm, and CK (unfertilized control). Application of N, P, and S, with several exceptions, tended to increase both total and available nutrient in comparison with nutrient-deficient treatments. In both growing seasons, CK treatment tended to show the lowest value of TOC (total organic carbon), whereas NPS and NPSm showed the highest values of grain yield. Except for dehydrogenase activity, soil basal respiration and enzyme activities tended to increase in fertilized treatments compared to CK. In 2012-2013, NPSm had the highest abundance of Gram-negative and Gram-positive bacteria. In 2013-2014, CK showed the highest abundance of actinomycetes and the lowest of Gram-negative bacteria. In addition, NPSm had the highest values of total PLFA (phospholipid fatty acid) biomass in both growing seasons. Soil microbial enzymes were mainly correlated with soil pH, indicating that soil alkalinity is a key factor governing soil enzyme functionality.

Key words: Soil microorganisms, PLFA, Enzyme activity, Microbial biomass

INFLUENCIA DE 12 AÑOS DE FERTILIZACIÓN NPS SOBRE LA CALIDAD DEL SUELO, PERFIL Y ACTIVIDAD DE LAS COMUNIDADES MICROBIANAS BAJO UN SISTEMA AGRÍCOLA CONSERVACIONISTA

RESUMEN

Los parámetros microbianos se consideran indicadores potenciales de la calidad del suelo, ya que los microorganismos del suelo pueden responder rápidamente a los sistemas de manejo agrícola. El efecto de las aplicaciones de fertilizantes a largo plazo en el suelo, especialmente el nitrógeno (N), el fósforo (P), el azufre (S) y los micronutrientes (m) puede alterar la química del suelo, la estructura y función de la comunidad microbiana. Los objetivos de este estudio fueron analizar el efecto a largo plazo de los fertilizantes químicos sobre las propiedades del suelo, las comunidades microbiológicas y la función en relación con los rendimientos de granos en dos campañas consecutivas (2012-2013 y 2013-2014). Todos los tratamientos de fertilización se aplicaron a una rotación de maíz-trigo/soja bajo siembra directa. Se evaluaron seis tratamientos de fertilización: PS, NS, NP, NPS, NPSm y CK (control sin fertilizantes). La aplicación de N, P y S, con algunas excepciones, tendió a aumentar los nutrientes totales y disponibles en comparación con los tratamientos con deficiencia de nutrientes. En ambas campañas, el tratamiento CK tendió a mostrar el valor más bajo de TOC. NPS y NPSm mostraron los valores más altos de rendimiento de granos. Excepto para la actividad deshidrogenasa, la respiración basal del suelo y las actividades enzimáticas tendieron a aumentar en tratamientos fertilizantes en comparación con CK. En 2012-2013, NPSm tuvo la mayor abundancia de bacterias Gram-negativas y Gram-positivas. En 2013-2014, CK mostró la mayor abundancia de actinomicetes y la más baja abundancia en bacterias Gram-negativas. Además, NPSm tuvo los valores más altos de la biomasa total (PLFA) en ambas campañas. Las enzimas microbianas del suelo se correlacionaron principalmente con el pH del suelo, lo que indica que la alcalinidad del suelo es un factor clave que rige la funcionalidad enzimática del suelo.

Palabras clave: Microorganismos de suelo, PLFA, Actividad enzimática, Biomasa Microbiana.

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INTRODUCTION

Conservation agriculture in its simplest form includes minimum soil disturbance, permanent soil cover, and crop rotation. The implementation of conservation practices has been identified as an effective tool for increasing yields through sustainable agriculture in various regions of the world (Pittelkow *et al.,* 2015). In contrast, the use of intensive crop production and conventional management practices may affect chemical, physical, and biological properties of the soil (Echeverría & García, 2005). These consequences are the incentive to assess the importance of planning and regulating to reduce future negative effects as soil desertification, loss of soil fertility and consequently loss of productivity. Conservation management systems can improve soil quality through increased infiltration, aggregate stability, and concentration of soil total organic C (Govaerts *et al.,* 2007), which can result in differences in soil organic matter quality and shift in soil microbial community profiles and functions. The conservation practices are usually complementary with the application of fertilizers according to specific crop species, edaphic, and climatic conditions (Bending *et al.*, 2002). Inorganic fertilizers, usually nitrogen (N), phosphorus (P), potassium (K), and sulphur (S) not only serve to maintain crop yields; but also to induce significant changes in soil properties and microbial communities. However, available information is conflicting and uncertainties still remain about the impact of inorganic fertilizers on soil microbial communities and its functions. Numerous studies have reported that the use of mineral fertilizers may have induced either direct or indirect changes in microbial biomass (Zhong & Cai, 2007), metabolic activities (Liu *et al.*, 2011), and may have affected the rate of decomposition and the structure of the soil microbial communities (Wyngaard *et al.*, 2012). Alvarez (2005) demonstrated that 50% of 20 medium-term experiments (10 years) involving fertilizer treatments in a Typic Argiudoll soil showed an increase in total organic carbon (TOC) in fertilized compared to unfertilized plots. Zhang *et al.* (2007) found that mineral fertilizer application stimulated Gram-positive bacteria population in paddy soil, as characterized by phospholipids fatty acids (PLFA) profiling. In contrast, excessive use of mineral fertilizers can produce serious soil degradation, soil compaction, reduction in soil organic matter, and decline of selected microbial taxa; consequently, the efficacy of mineral fertilizers on crop yields decreases over time (Bono & Romano, 2007). To our knowledge, few studies have been done in order to investigate the effect of mineral fertilizers on microbial communities under conservation agriculture systems.

The aims of this study were (i) to analyze the effect of different mineral fertilizer treatments on selected soil properties, (ii) to analyze the effect of different mineral fertilizer treatments on microbiological parameters that may serve as early indicators of changes in soil microbial communities in a conservation agriculture system and (iii) to evaluate the effect of mineral fertilizers on crop yields.

MATERIALS AND METHODS

The long-term field fertilizer experiment was carried out in Teodelina (34010´S, 61002´W), Santa Fe Province, Argentina. The trial was established in 2000 on a productive soil that had been cultivated under agricultural management for more of 60 years. The soil is a sandy loam (Typic Hapludol) with 35,1% sand, 53,2% silt, and 11,8% clay. The experiment was designed using a randomized complete block, with three replicates. All fertilization treatments were applied to a maize-wheat/soybean rotation with no-till farming. Fertilizer treatments were applied before maize and wheat sowing. No other fertilizers were applied before planting soybean. Maize was planted in October 2012, and harvested in March 2013. Soybean was planted in December 2013, and harvested in the third week of April 2014. Six different fertilizer treatments were evaluated: control (CK, without fertilization), PS (application of P and S), NS (application of N and S), NP (application of N and P), NPS (application of N, P, and S), and NPSm (application of N, P, S, and micronutrients) (**Table 1**). Twelve composite soil samples were randomly collected from each treatment at a depth of 0-10 cm during the physiological maturity stage of maize and soybean in early March 2013 and March 2014, respectively. Soil

Table 1. Detail of nutrients and fertilizers (kg ha^{-1}) applied in the mineral fertilization experiment.
Tabla 1. Detalle de nutrientes y fertilizantes (kg ha^{-1}) aplicados en el experimento de fertilización mineral.

	Maize 2012-2013						Wheat/Soybean *2013-2014*						Fertilizers
	CK	PS	NS	NP	NPS	NPSm	CK	PS	NS	NP	NPS	NPSm	
N		18	160	160	160	160		20	101	102	102	102	Urea
P		35		35	35	35		44		44	44	44	MAP ($NH_4H_2PO_4$)
K						12						25	K_2O
Mg						7						14	MgO
S		15	15		15	17		21	21		21	21	$CaSO_4$
B						1						1	B10
Zn						2						2	Zn 40
Cu						2						2	Cu25
Total Nutrients	0	68	175	195	210	234	0	85	122	146	167	234	

CK: unfertilized control; PS: application of P and S fertilizers; NS: application of N and S fertilizers; NP: application of N and P fertilizers; NPS: application of N, P, and S fertilizers; NPSM: application of N, P, S, and micronutrients fertilizers. B: boron; Zn: zinc; Cu: copper; N nitrogen; P: phosphorus; S: sulfur; Cl: chlorine, K: potassium; Mg: magnesium
CK: control sin fertilizar; PS: aplicación de fertilizantes P + S; NS: aplicación de fertilizantes N + S; NP: aplicación de fertilizantes N + P; NPS: aplicación de fertilizantes N, P y S; NPSM: aplicación de fertilizantes N, P, S más micronutrientes. B: boro; Zn: zinc; Cu: cobre; N nitrógeno; P: fósforo; S: azufre; Cl: cloro, K: potasio; Mg: magnesio.

samples were stored at -20 ºC for PLFA analysis or at -4 ºC for chemical and physiological analysis.

Soil samples were analyzed for pH, total organic carbon (TOC), total and available N, P, and S. Soil pH was measured in a 1:2.5 (soil to water ratio). Total OC and total S were quantified by an auto analyzer PE 2400 Series II C, H, N, S (Perkin Elmer) according to Jimenez and Ladha (1993). Total N and P and available N were determined by using an auto-analyzer nutrient SmartChem 200 (Westco, Scientific Instruments, Inc.), following the manufacturer's instructions (Goloran et al., 2014). Available P was extracted by using $NaHCO_3$ (pH 8.5) and determined with a spectrophotometer (882 nm), according to Olsen *et al.* (1954). Available S was extracted with Cl_2Ba and then quantified by a turbidmetric method (Kolmert *et al.,* 2000).

Basal respiration was determined by estimating the CO_2 released from approximately 10 g of soil, by titration with hydrochloric acid 0,2N (Alef, 1995). Soil samples were incubated in septa-stoppered acrylic tubes for 10 days at 25 ºC. Flasks that did not contain soil served as the control. Hydrolyzing activity of fluorescein diacetate (FDA) was estimated according to Adam and Duncan (2001) and was measured on a spectrophotometer UV-vis Perkin-Elmer Lamda 25 at 490 nm. The concentration of fluorescein was calculated by using a calibration curve constructed with patterns (0-5 μg/ml). Dehydrogenase activity (DHA) was determined according to García *et al.* (1997). Soil samples (1 g) were exposed to 0,2 ml of 0,4% INT (2-p iodophenyl-3-pnitrophenyl-5-phenyltetrazolium chloride) in distilled water at 22ºC in darkness for 20 h. The iodonitrotetrazolium formazan (INTF) was extracted with 10 ml of methanol and then measured spectrophotometrically (490 nm). Acid phosphatase activity (PHA) was determined as described by Tabatabai and Bremner (1969). Briefly, soil samples (100 mg) were placed in a centrifuged flask and then p-nitrophenyl phosphate in a buffered solution was added. After incubation, $CaCl_2$ (0,5 M) and NaOH (0,5 M) solutions were added followed by centrifugation and measured as para-nitrophenol on a spectrophotometer (400 nm).

The soil microbial community structure was determined by analyzing the phospholipid fatty acids (PLFA) composition (Bossio & Scow, 1998). A standard nomenclature was used. Branched fatty acids i15:0, a15:0, i16:0, i17:0 and a17:0 being chosen to represent Gram-positive bacteria, and the monoenoic and cyclopropane fatty acids 16:1ω9, 16:1ω11, cy17:0, 18:1ω9c, 18:1ω9t,

and cy19:0 selected to represent Gram-negative bacteria. The fatty acids 10 methyl 18:0, 16:1ω9c and the polyenoic 18:2ω6,9 were used as indicators of actinomycetes, arbuscular mycorrhizal fungi (VAM), and fungal biomass, respectively. Total microbial biomass (total PLFAs) was estimated as the sum of all the extracted PLFAs.

Data were analyzed using InfoStat Professional version 2017 (Di Rienzo *et al*., 2017). Differences among fertilizer treatments were evaluated by analysis of variance (ANOVA) under the linear, general, and mixed models (LGMM). Means were compared using the least significant difference (LSD) ($p \leq 0,05$). Shifts in community PLFA profiles were also analyzed by principal component analysis (PCA) to reduce the dimensionality. Stepwise multiple regression analysis ($p \leq 0,05$) was applied to detect the soil properties influencing microbial structure and function.

RESULTS AND DISCUSSION

General soil properties and crop yield

The impact of the application of mineral fertilizers on selected soil properties are shown in **Table 2**. Long-term fertilizer applications, especially treatments containing N, showed acidifying effects resulting in low soil pH values in comparison with CK treatment in both growing seasons. In comparison with CK, soil pH under NPSm decreased 0,40 and 0,55 units in 2012-2013 and 2013-2014, respectively. Several studies have shown that urea fertilization increased soil acidification in soils of Argentina (Sainz Rozas *et al.,* 2011) and other parts of the world (Graham *et al.,* 2002). Other authors also reported that the application of nitrogen fertilizers, under crop rotation, may increase soil acidification (Wyngaard *et al.,* 2012). In addition, soil organic matter offers many negatively charged sites to bind cations.

Table 2. Soil nutrient content under different mineral fertilizer treatments.
Tabla 2. Contenido de nutrientes del suelo bajo diferentes tratamientos fertilizantes.

Treatments	pH	Total content (g Kg^{-1})				Available nutrients (g Kg^{-1})			C/N
		TOC	N	P	S	N	P	S	
Maize 2012-2013									
NPSm	5,86 bc	37,80 ab	3,10 a	1,24 a	0,63 a	26,99 bc	57,29 a	15,0 b	12,84 a
NPS	5,86 bc	38,50 a	3,27 a	1,20 a	0,43 a	29,85 ab	55,59 a	15,0 b	12,11 ab
NP	5,85 c	25,80 c	2,33 b	1,23 a	0,37 a	33,88 a	55,47 a	15,0 b	11,29 ab
NS	5,75 c	27,40 bc	2,80 ab	0,81 b	0,50 a	29,50 abc	36,75 c	13,0 b	9,76 ab
PS	6,03 ab	21,63 c	2,20 b	1,37 a	0,40 a	26,80 bc	46,65 b	20,0 a	9,74 ab
CK	6,25 a	19,93 c	2,23 b	0,57 c	0,37 a	22,96 c	24,49 d	15,0 b	9,15 b
Soybean 2013-2014									
NPSm	5,57 c	29,43 a	4,20 a	1,49 a	0,50 ab	28,47 a	68,65 a	16,5 ab	7,34 a
NPS	5,70 bc	31,10 a	4,08 a	1,32 a	0,57 ab	28,47 a	60,94 ab	16,25 ab	8,03 a
NP	5,64 bc	23,83 b	3,51 a	1,32 a	0,33 b	25,47 ab	59,42 cd	12,0 b	7,45 a
NS	5,77 bc	30,93 a	3,59 a	1,19 a	0,63 a	25,25 ab	52,63 d	19,25 a	10,01 a
PS	5,81 b	27,23 ab	4,04 a	1,60 a	0,57 ab	19,99 b	71,98 a	17,92 a	6,74 a
CK	6,12 a	17,67 c	2,40 b	1,14 a	0,33 b	20,83 b	34,85 c	16,5 ab	8,90 a

Data with different letter indicates significant difference between treatments according to LSD test ($p \leq 0,05$). CK: unfertilized control; PS: application of P and S fertilizers; NS: application of N and S fertilizers; NP: application of N and P fertilizers; NPS: application of N, P and S fertilizers; NPSM: application of N, P, S and micronutrients fertilizers, TOC: total organic C; C/N: C/N ratio.
Letras diferentes indican diferencias significativas entre tratamientos según el test LSD ($p \leq 0,05$). CK: control no fertilizado; PS: aplicación de fertilizantes P + S; NS: aplicación de fertilizantes N + S; NP: aplicación de fertilizantes N + P; NPS: aplicación de fertilizantes N, P y S; NPSM: aplicación de N, P, S más micronutrientes, TOC: C orgánico total; C/N: relación C/N.

Thus, many authors have observed a decrease of soil pH with increasing TOC (Kanianska *et al*., 2014). However, the magnitude of the acidifying effect depends on several physic-chemical factors, such as soil type, soil pH, dose of N, and frequency of application.

In our study, CK treatment tended to show the lowest value of TOC in both growing seasons. In 2013-2014, TOC under NPSm and NPS increased by approximately 66,5% and 76,0% compared with CK, respectively. Although less evident, a similar trend was observed in the previous growing season 2012-2013. This change in TOC content may be associated with the variation of the amount and number of nutrients added to the soil. The increase in TOC with the application of mineral fertilizers may be due to greater input of root biomass due to improved crop growth. Thus, our results validated that mineral fertilization was essential for improving TOC, in particular under NPS and NPSm treatments. In support of this, other authors have reported that application of NPS increased TOC in the superficial layer of an argiudoll soil under no-tillage practice (Wyngaard *et al.,* 2012).

Application of N, P, and S, with several exceptions, tended to increase both total and available nutrients in comparison with the nutrient-deficient treatments. In 2012-2013, total N under NPSm and NPS increased by 39,0% and 46,5% compared with CK, respectively. In 2013-2014, the lowest value of total N was observed under the unfertilized treatment (CK). A similar trend was observed for available N in both growing seasons. In agreement with these results, other authors have observed an increase of total and available N in soils treated with urea (Abbasi & Khizar, 2012). In 2013-2014, PS treatment also increased total N, probably due to the presence of N in the chemical formulation of phosphorus fertilizer (Garcia *et al.*, 2010). Our results showed that total P was higher under soybean compared to maize. This result was probably due, at least in part, to a specific nutritional request of each crop, and should be considered in future studies. On the other hand, in 2012-2013, total and available P content was lowest under P-deficient treatments (NS and CK), and the maximum values of total P were observed under both NPS and NPSm. However, no significant differences among treatments were observed for total P and S in 2013-2014 and 2012/2013, respectively. This result may be explained by the fact that P and S contents were efficiently removed, and thus, soil P and S contents showed no differences in relation to CK. In our study, the soil C/N ratio was poorly affected by the fertilizer treatments.

Our results clearly showed that the different combinations of N, P, and S increased grain yield under a soybean-maize rotation (**Table 3**). These values of grain yield response to applied fertilizers agree with other studies carried out in the same area (Pampas region, Argentina) (Aramburu Merlos *et al.,* 2015). Both triple-fertilized treatments NPSm and NPS showed the highest values of grain yield in 2012-2013 and 2013-2014, respectively, while CK had the lowest values in both growing seasons. In 2012-2013 and 2013-2014, crop yield under NPS increased by 110% and 63% compared with CK, respectively. The influence of a NPS fertilization on grain yield has been previously reported by other authors (Wyngaard *et al.,* 2012: Verdenelli *et al.,* 2013).

Table 3. Crop yields (kg.ha^{-1}) under different mineral fertilizer treatments.
Tabla 3. Rendimientos de los cultivos (kg. ha^{-1}) bajo diferentes tratamientos fertilizantes.

Treatments	Maize 2012-2013	Soybean 2013-2014
NPSm	9653 a	3648 b
NPS	9357 b	4088 a
NP	8222 d	3268 c
NS	8829 c	3260 cd
PS	5962 e	3218 d
CK	4444 f	2497 e

Data with different letter indicates significant difference between treatments according to LSD test ($p \leq 0,05$). CK: unfertilized control; PS: application of P and S fertilizers; NS: application of N and S fertilizers; NP: application of N and P fertilizers; NPS: application of N, P, and S fertilizers; NPSM: application of N, P, S, and micronutrients fertilizers,
Letras diferentes indican diferencias significativas entre tratamientos según el test LSD ($p \leq 0,05$).CK: control no fertilizado; PS: aplicación de fertilizantes P + S; NS: aplicación de fertilizantes N + S; NP: aplicación de fertilizantes N + P; NPS: aplicación de fertilizantes N, P y S; NPSM: aplicación de N, P, S más micronutrientes.

Basal respiration and enzyme activities

The effect of agricultural practices on microbial activity is especially important because microbial functions play a central role in ecological and biological stability and are therefore closely related to soil quality. In 2012-2013 and 2013-2014, CK and NS showed the lowest soil basal respiration, respectively (**Table 4**). In 2012-2013, basal respiration under NPS and NPSm significantly increased by 47,8% and 34,7% compared with CK, respectively. The metabolic activity of microorganisms referred to as 'soil respiration' is a parameter which depends on the physiological condition of soil microorganisms and environmental factors. Several authors have observed that addition of organic or inorganic fertilizers may increase soil microbial activity and respiration (Hu *et al.,* 2011). This is due to the introduction of a source of easily accessible nutrient (such as mineral fertilizers) into the soil microorganism habitat. In this sense, the increase in nutrient contents would stimulate microbial activity and biomass cycling, thus leading to an increase in soil basal respiration. However, in our experiment, this effect was statistically significant only in the 2012-2013.

Several authors have stated that the application of mineral fertilizers had negligible effects on soil enzyme activities in contrast to organic amendments (Iovieno *et al.,* 2009). However, our study demonstrated that mineral fertilizer may alter soil enzyme activities (**Table 4**). In general, in both growing seasons, the unfertilized treatment showed the lowest values of FDA and PHA. However, statistical differences in PHA values were only observed in 2013-2014. In 2012-2013, NPS increased the FDA activity by 35.3% compared to CK. Similarly, in 2013-2014, FDA values also significantly differed between CK and the other fertilizer treatments. Furthermore, the higher TOC level under fertilized treatments in comparison with CK provides enough substrate to support higher microbial biomass and enzyme

Table 4. Basal respiration and enzyme activities under different mineral fertilizer treatments.
Tabla 4. Respiración basal y actividades enzimáticas bajo diferentes tratamientos fertilizantes minerales.

Treatments	RES ($g\ CO_2\ Kg^{-1}$)	FDA ($g\ fls\ Kg^{-1}$)	DHA ($mg\ INTF\ gr^{-1}$)	PHA ($\mu mol\ gr^{-1}\ h^{-1}$)
Maize 2012-2013				
NPSm	0,31 a	106,8 ab	3,59 bc	15,48 a
NPS	0,34 a	110,8 a	3,48 bc	15,74 a
NP	0,33 a	117,4 a	2,60 c	16,28 a
NS	0,29 ab	105,9 ab	3,75 bc	15,85 a
PS	0,27 ab	101,6 ab	3,80 b	15,08 a
CK	0,23 b	81,9 b	5,68 a	14,36 a
Soybean 2013-2014				
NPSm	0,37 a	155,6 a	5,74 a	14,70 ab
NPS	0,39 a	154,3 a	5,01 ab	13,71 bc
NP	0,34 a	168,8 a	3,92 bc	15,76 a
NS	0,32 a	160,65 a	1,52 d	13,55 bc
PS	0,36 a	156,1 a	3,21 c	13,34 cd
CK	0,34 a	127,8 b	5,20 a	12,31 d

Data with different letter indicates significant difference between treatments according to LSD test ($p \leq 0,05$). CK: unfertilized control; PS: application of P and S fertilizers; NS: application of N and S fertilizers; NP: application of N and P fertilizers; NPS: application of N, P, and S fertilizers; NPSM: application of N, P, S, and micronutrients fertilizers, RES: basal respiration, FDA: fluorescein diacetate, DHA: dehydrogenase activity, PHA: acid phosphatase activity.
Letras diferentes indican diferencias significativas entre tratamientos según el test LSD ($p \leq 0,05$). CK: control no fertilizado; PS: aplicación de fertilizantes P + S; NS: aplicación de fertilizantes N + S; NP: aplicación de fertilizantes N + P; NPS: aplicación de fertilizantes N, P y S; NPSM: aplicación de N, P, S más micronutrientes, RES: respiración basal, FDA: diacetato de fluoresceína, DHA: actividad deshidrogenasa, PHA: actividad fosfatasa ácida.

production (Yuan & Yue, 2012). The highest values of FDA under fertilized treatments indicated a positive effect of mineral fertilizers on soil biological quality, according to the findings of other authors (Cicatelli *et al.* 2014). In contrast to previously mentioned enzyme activities, mineral fertilizer application tended to decrease DHA values, the highest values of this enzyme activity being observed under CK. This apparent contradiction is resolved by the finding that DHA activity can be greatly inhibited in soils with lower pH values (Mukhopadhyay & Maiti, 2010). Other authors have also found that DHA activity was weekly influenced by mineral nitrogen fertilization (Hu *et al.,* 2014). As a useful tool of soil biochemical quality, soil enzyme activities have often been suggested as sensitive indicators of soil ecological quality. For example, several authors observed an increase of DHA activity in soils treated with organic fertilizers (Gaind & Singh, 2016). Our data support the idea that DHA activity should be carefully used as a soil quality indicator when the experimental design compared between soils under mineral fertilization.

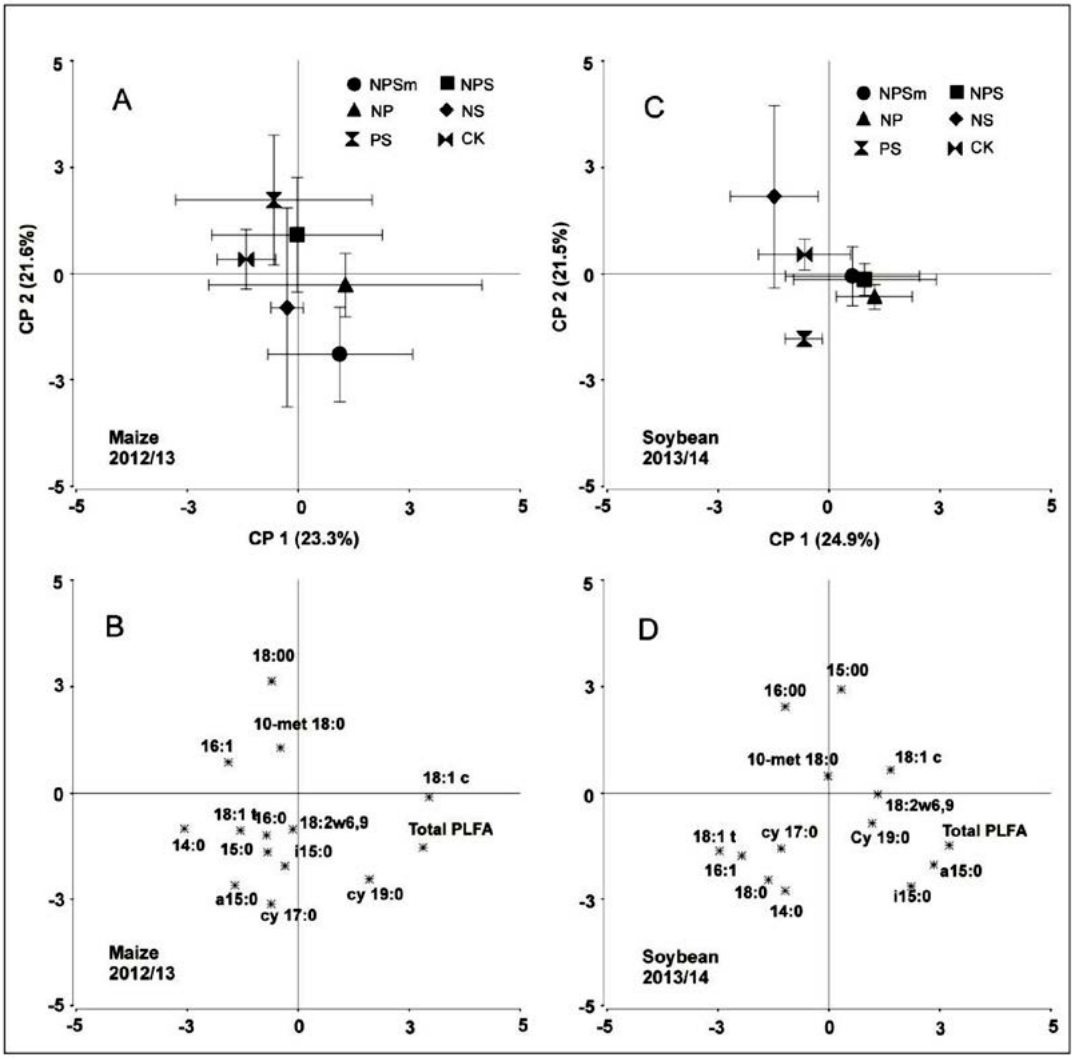

Figure 1. (A and C) Principal component (PC) analysis of phospholipids fatty acids (PLFA) profiles of soil under different mineral fertilizer treatments (2012-2013 and 2013-2014), (B and D) PCA showing loading values of selected PLFAs, CK: unfertilized control; PS: application of P and S fertilizers; NS: application of N and S fertilizers; NP: application of N and P fertilizers; NPS: application of N, P, and S fertilizers; NPSM: application of N, P, S, and micronutrients fertilizers.

Figura 1. (A y C) Análisis de los componentes principales (CP) de los perfiles de los ácidos grasos fosfolípidos (PLFA) de los diferentes tratamientos fertilizantes (2012-2013 y 2013-2014); (B y D) CP que muestran los valores de carga sobre las variables seleccionadas de PLFAs. CK: control no fertilizado; PS: aplicación de fertilizantes P + S; NS: aplicación de fertilizantes N + S; NP: aplicación de fertilizantes N + P; NPS: aplicación de fertilizantes N, P y S; NPSM: aplicación de N, P, S más micronutrientes.

Microbial community profiles

The response of the microbial community to mineral fertilization was determined from the mol percentage of all PLFAs identified in the extracts (**Figures 1 and 2**). Soil PLFA patterns contain a great deal of information about microbial communities, and total PLFAs per gram of soil has been widely recognized as an indicator of viable soil biomass (Hedrick *et al.*, 2000). According to our results, in both growing seasons, the PLFA profiles were dominated by the group of saturated fatty acids, principally 16:0 and 18:0. Levels of molar percentages were consistent with literature evidences for most PLFAs. The PC analysis explained approximately 45% and 46% of the total variance in 2012-2013 and 2013-2014, respectively. In 2012-2013, soil microbial communities from NPSm and NP clustered in the right-hand side of PC1, and were separated from the other fertilizer treatments. This discrimination was based on the higher relative abundance of several monounsaturated FAs (principally cy19:0, cy17:0 and 18:1c), a branched FA (a15:0) and the total PLFAs content (**Figure 1**). Thus, NPSm had the highest abundance of Gram-negative and Gram-positive bacteria (**Figure 2**). In 2013-2014,

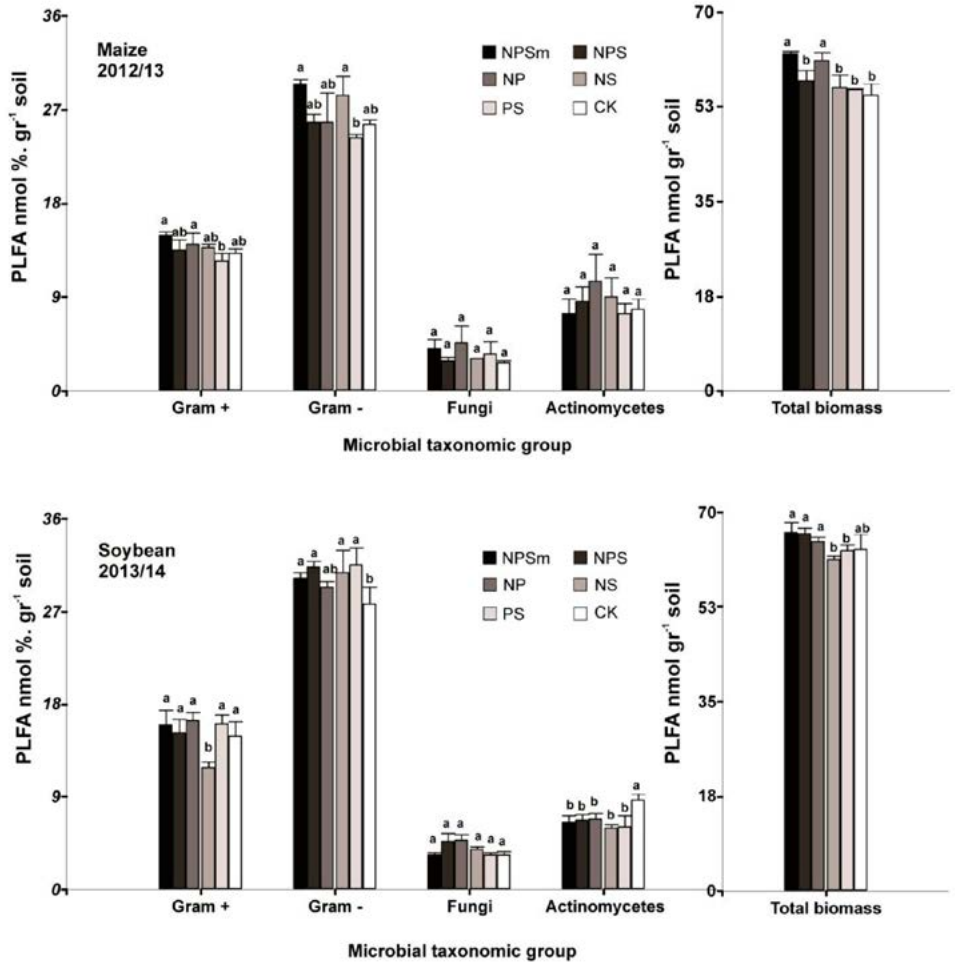

Figure 2. Microbial taxonomic groups (nmol% de PLFA g^{-1} soil) and total microbial biomass (nmol de PLFA g^{-1} soil) of soil under different mineral fertilizer treatments (2012-2013 and 2013-2014). Bars topped with the same letter are not significantly different according with LSD test ($p \leq 0,05$), CK: unfertilized control; PS: application of P and S fertilizers; NS: application of N and S fertilizers; NP: application of N and NPS: application of N, P and S fertilizers; NPSm: application of N, P, S and micronutrients fertilizers.

Figura 2. Grupos taxonómicos microbianos (nmol% de PLFA g^{-1} suelo) y biomasa microbiana total (nmol de PLFA g^{-1} suelo) del suelo bajo diferentes tratamientos fertilizantes (2012-2013 y 2013-2014). Misma letra no son significativamente diferentes de acuerdo con la prueba de LSD ($p \leq 0,05$). CK: control no fertilizado; PS: aplicación de fertilizantes P + S; NS: aplicación de fertilizantes N + S; NP: aplicación de fertilizantes N + P; NPS: aplicación de fertilizantes N, P y S; NPSM: aplicación de N, P, S más micronutrientes.

NPSm, NPS, and NP clustered in the right-hand side of PC1, while NS, PS, and CK were located in the other side. Moreover, in this growing season, CK showed the highest abundance of actinomycetes and the lowest of Gram-negative bacteria (**Figure 2**). However, differences in the abundance of fungi populations were not found in any of the growing seasons. The higher impact of mineral fertilization on bacterial communities in comparison with fungi may be due to the different ability to degrade simple substrates. In support of this, Li *et al.* (2015) demonstrated that fungi can degrade more recalcitrant organic compounds and those with high C:N compared to bacteria. Our results also showed that mineral fertilization tended to increase total microbial biomass. Particularly, the triple-fertilized treatment with micronutrients (NPSm) had the highest values of microbial biomass in both growing seasons. Since our experiment was performed under a conservation system, the increase in soil microbial biomass could be a result of increased crop growth under fertilized plots, and larger amounts of crop residue left in the soil, which in turn influence microbial processes and development (McDaniel *et al.,* 2014).

Table 5. Soil characteristic parameters which were found by stepwise regression analysis to be correlated with microbial properties and crop yield.
Tabla 5. Parámetros característicos del suelo que correlacionaron por análisis de regresión stepwise con las propiedades microbianas y el rendimiento de los cultivos.

Dependents	Related variables	R2
Maize 2012-2013		
Respiration	AP, TP	0,51
DHA	pH, TP	0,68
FDA	pH, AN	0,72
Gram-positive	AS	0,27
Gram-negative	TN	0,23
PLFA biomass	AP	0,33
Yield	AP, AS	0,76
Soybean 2013-2014		
PHA	pH	0,36
FDA	pH	0,71
Gram-negative	AP	0,56
Actinomycetes	TOC	0,74
Yield	AP	0,36

AN: available N; AP: available P; AS: available S; TN, total N; TP: total P; TS: total S, TOC: total organic carbon.
AN: N disponible; AP: P disponible; AS: S disponible; TN, N total; TP: P total; TS: S total, TOC: carbono orgánico total.

RESULTS INTEGRATION

Stepwise regression analysis showed that available P greatly affected crop yield in both growing seasons 2012-2013 and 2013-2014. The relevance of P fertilizer to increase soybean and maize grain yield has been extensively studied (Dodd & Mallarino 2005). In 2012/2013, available P was significantly correlated with basal respiration and PLFA biomass. In 2013-2014, available P significantly correlated with Gram-negative bacteria. In addition, stepwise analysis also revealed that soil pH significantly correlated with soil enzymatic activities (DHA and FDA in 2112/13, and PHA and FDA in 2013-2014). These results suggest that soil acidity is a key factor governing soil enzyme functionality. In particular, the relationship between soil pH and enzymatic activities has been reported by other authors (Grahams & Haynes, 2005).

CONCLUSIONS

Mineral fertilizers can alter soil properties, microbial structure and function. In particular, triple fertilizer treatments (NPS and NPSm) increased TOC and crop yields in both growing seasons. Mineral fertilizers also increased basal respiration and microbial activities (FDA and PHA), probably due to an increase in root biomass and a better crop growth. Our study showed that the triple fertilization (NPSm and NPS) can increase soil bacteria populations and total microbial biomass. In contrast, fungal populations were poorly affected. Further studies are needed to study the role of soil acidity to regulate the functioning of intracellular enzymes.

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