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Soil organic carbon, macro- and micronutrient changes in soil fractions with different lability in response to crop intensification

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ABSTRACT

Soils under no tillage have experienced unfavorable changes, mainly due to current agricultural practices that consist in monocultures that leave little residue cover. The inclusion of grass as cover crops during the winter season could be a sustainable strategy to increase crop intensification in sequences where soybean predominates, helping to maintain soil fertility, organic matter levels and enhance soil physical properties. The aim of this research was to evaluate the effects of 8 years of sustainable crop intensification (by increasing the proportion of cereals in crop rotations) on soil organic carbon, macro- and micronutrients associated with granulometric fractions of different lability in a Typic Argiudoll of the Rolling Pampa, Argentina. The experiment included two crop sequences commonly used in this area: soybean-soybean (S-S) and maize-soybean-wheat/soybean (M-S-W/S) combined with the inclusion of wheat (*Triticum aestivum* L.) as cover crop (CC) in winter. The intensification sequence indices (ISI) were 0.39, 0.69, 0.55 and 0.64 for S-S, S-CC-S, M-S-W/S and M-CC-S-W/S, respectively. The carbon measured in the coarse particulate fraction (Pcf) in the 0–5 cm soil depth was 3 times larger in S-CC-S than in S-S. Cropping intensity also modified N, S, P, Ca and Mn in the Pcf with no changes in Mg, K, Zn, Fe and Cu contents. Among the carbon fractions studied, only the carbon measured in the Pcf and the easy mineralizable carbon estimated by the soil respiration in the first soil layer (0–5 cm), were positively correlated with the ISI. In the present study, 8 years under sustainable crop intensification were sufficient to show changes in the mineral associated fraction (Maf). Increases in the C in the Maf in maize legume-based rotation, suggest SOC accumulation in more stable carbon pools.

1. Introduction

The humid Argentine Pampa is one of the largest areas dedicated to cereal and oil crops in the southern hemisphere, due to a temperate climate, adequate rainfall and a large proportion of soils belonging to the great group Argiudolls, with high productivity (Rimski-Korsakov et al., 2015). One of the most successful soil management practices for recovering degraded agricultural soils has been no-tillage (NT) cultivation (Lal et al., 2007). In the last 20 years, NT has been increasingly adopted in this area, reaching almost 80% of cultivated lands. However, areas under NT have experienced unfavorable changes over the last 15 years, mainly due to soybean monoculture that leave little residue cover (Novelli et al., 2011). This production system dominates field crop acreage averaging around 60% (MAGyP 2014). When not planted in monoculture, soybean is included in sequences with maize or as a double-crop succeeding wheat. On the other hand, when soybean is not planted as a double-crop, the soil is under long periods of fall-winter fallow, with low annual carbon input to the system that threaten the physical and chemical fertility of the soil (Novelli et al., 2017).

Sustainable intensification of agriculture addresses a simultaneous enhancement of agricultural productivity, while conserving and protecting the environment. There are several approaches for minimizing environmental externalities (Wezel et al., 2015). Among them, reducing soil disturbance (e.g. reduced tillage practices for avoiding soil erosion), maintaining permanent soil cover (e.g. cover crops), and implementing crop rotations including nitrogen fixing crops, are key components of conservation agriculture (Petersen and Snapp, 2015). In silty soils of the Pampas Region, soil physical conditions are usually negatively affected by soybean monoculture (Sasal et al., 2010; Novelli et al., 2017). Then, the inclusion of grass species either as crops or as

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cover crops during the winter season could be one strategy to increase soil cover in sequences where soybean monoculture predominates (Restovich et al., 2011). Cover crops may favor soil organic matter stocks (Duval et al., 2016), soil biodiversity (Frasier et al., 2016b) and soil fertility by restoring considerable amounts of nutrients (Martinez et al., 2013; Varela et al., 2014) among others ecosystem services. Duval et al. (2016) reported that more than 3.0 Mg ha^{-1} $year^{-1}$ of aboveground C were supplied by grass cover crops within soybean monoculture, with a concomitant increase in soil organic carbon (SOC) of 7% for each Mg of C supplied by the cover crops at 0–5 cm soil depth as compared with soybean monoculture.

The evaluation of total organic carbon (TOC) and total nitrogen (TN) has been traditionally used for assessing changes in soil chemical properties because of the inclusion of cover crops (Mazzoncini et al., 2011). However, evaluating labile soil organic carbon fractions associated with residue decomposition in the short term (3–4 years) (Plaza-Bonilla et al., 2014), may be more accurate for evaluating soil changes in response to cover crop inclusion (Ensinas et al., 2016). Likewise, microbial biomass measurements may reveal potential changes in soil organic matter, long before changes in total soil organic C (Powlson et al., 1987).

Cover crops can take nutrients from deeper soil layers and after chemical desiccation, release these nutrients on the soil surface during straw degradation (Pacheco et al., 2011) and therefore changing the chemical properties of uppermost layers of the soil (Nascente et al., 2015). Several authors studied the effects of cover crops in different granulometric fractions of SOM (Santos et al., 2011; Rosolem et al., 2016) and some evaluated soil N associated with these fractions (Sainju et al., 2003). Other studies showed the stratification of macro and micronutrients in soil (Crusciol et al., 2008; Beltran et al., 2016). However, little is known about the impact of different crop sequences on macro- and micronutrients content and availability in different fractions.

The purpose of this research was to evaluate changes in organic carbon, macronutrients and micronutrients content in different soil granulometric fractions as affected by crop sequences with different participation of grass crops. We hypothesized that increasing grass participation in crop sequences will increase biomass inputs and thus increase carbon, macronutrients and micronutrients content in the particulate coarse (105–2000 μm) and fine fractions (53–105 μm).

2. Materials and methods

2.1. Study site and experimental design

The experiment was located at INTA Oliveros Research Station (32° 32' S; 60° 51' W) Argentina, on a Typic Argiudoll (USDA Soil Taxonomy), deep, well-drained, carbonates free until the 240 cm depth, with a superficial horizon characterized by a silt loam texture (clay 209 g kg^{-1}, silt 708 g kg^{-1}, sand 83 g kg^{-1}) with a predominance of illite in the clay fraction, pH 6 (1/2.5 soil/water), C.E.C. 19.4 $cmol_c$ kg^{-1}. The climate is humid temperate with mean annual temperature of 17.6 °C and mean annual rainfall of 1042 mm. Rainfall and drainage occur mainly in fall and spring, while in the summer months usually present deficits of varying intensity in the agro-climatic balance.

A long-term experiment designed to evaluate crop sequences with different intensity of grass and soybean participation was set up in 2006 under no-tillage system. The area has been under agriculture for the last 50 years, and under no-tillage from the last 8 years previous to the beginning of the trial. The experiment included two crop sequences commonly used in this area: soybean–soybean (S-S) and maize–soybean–wheat/soybean (M-S-W/S) combined with the inclusion of wheat (*Triticum aestivum* L.) as cover crop in winter. All treatments were arranged in a randomized complete block design with three replicates. Each experimental unit was 13 m wide × 50 m long. At sowing, soybean was inoculated with *Bradyrhizobium* sp. and fertilized with 70 kg ha^{-1} of triple super phosphate. Maize and wheat were fertilized at sowing with 80 kg ha^{-1} of mono ammonium phosphate. Wheat was fertilized again one month later with an average rate of ca. 100 kg N ha^{-1} as urea, while maize was fertilized in order to reach ca. 165 kg N ha^{-1} at planting (summing up nitrates in soil at planting + N fertilizer). Cover crops were killed at mid-booting stage corresponding to Z45 in the Zadoks growth scale, with 3–4 L ha^{-1} of glyphosate (48% active principle).

The intensification sequence index (ISI) that expresses the relative number of days of the year occupied by crops in a given crop sequence, was calculated as the ratio between the number of days with crops in each crop sequence and the length of the sequence (Caviglia and Andrade, 2010). For the sequences analyzed, the ISI was 0.39, 0.69, 0.55 and 0.64 for S-S, S-CC-S, M-S-W/S and M-CC-S-W/S, respectively.

In each experimental unit grain yields were determined with a small plot combine at harvest maturity from a 10 m^2 area and grain moisture was also determined. At physiological maturity of each crop in the sequence, dry matter in plant tissue (grain and vegetative biomass of each crop) were determined in a 1 m^2 area by hand harvesting. Samples were dried at 70 °C and harvest index was calculated. These harvest indexes were averaged for each treatment and used in combination with the small plot combine grain yield to calculate whole-plot aboveground biomass. Carbon content (Table 1) was calculated by multiplying biomass by a factor of 0.45 (Williams et al., 1987).

2.2. Soil sampling and measurements

In April 2014 (after the 2013–14 cropping season ended), soil samples were taken at 0–5 and 5–20 cm depth, replicated three times in each experimental unit. Subsamples were mixed and homogenized in the field, air-dried, and sieved to pass a 2 mm screen. A subset of soil samples were stored at field moisture in a refrigerator at 4 °C until analysis for microbial measurements.

Total N (TN) was determined as proposed by Bremner and Mulvaney (1982) and total soil organic carbon (TOC) content was quantified by using the wet oxidation method (Nelson and Sommers, 1982). Total macro (phosphorus (P), calcium (Ca), potassium (K), magnesium (Mg) and sulfur (S)) and micronutrients (zinc (Zn), iron (Fe), manganese (Mn) and cupper (Cu)) were extracted by a digestion of 1 g of soil sample using 2:1 nitric acid/hydrochloric acid (adapted from Ref. Malavolta et al., 1997). Total Ca, K, Mg, Zn, Fe, Mn and Cu were determined using a VARIAN 2005 atomic absorption spectrophotometer, total P was determined by molybdenum blue colorimetric method and total S by the barium chloride turbidimetric method (Olson and Engelstad, 1972).

Soil particle size fractionation was performed by wet sieving (Cambardella and Elliott, 1992; Galantini, 2005). Briefly, 50 g of previously air-dried and sieved (2 mm) soil was dispersed and mixed with 100 mL of distilled water. Then samples were subjected to mechanical dispersion through a rotary shaker for 16 h at 40 rpm to disintegrate the

Table 1
Cumulative values of annual inputs of carbon in vegetative biomass, and nitrogen (N), phosphorus (S) and sulfur (S) applied as fertilizer after 8 years in each sequence: soybean monoculture (S-S) and maize, soybean, wheat/soybean (M-S-W/S) combined with the inclusion of cover crops (CC): (M-S-CC-W/S) (S-CC-S).

	S-S	M-S-W/S	M-CC-S-W/S	S-CC-S
ISI	0.39	0.55	0.64	0.69
C in vegetative biomass (Mg ha^{-1})	14.80	22.28	23.83	24.15
N applied by fertilization (kg ha^{-1})	0	843	843	400
P applied by fertilization (kg ha^{-1})	126	140	140	126
S applied by fertilization (kg ha^{-1})	22	22	22	22

ISI: intensification sequence index for each crop rotation.

aggregates. Sieving was done with 53 μm and 105 μm diameter mesh, until the water coming out through the sieve was clear to the naked eye. Three fractions were obtained: (i) coarse fraction (105–2000 μm) containing coarse particulate organic fraction and fine to coarse sands (Pcf); (ii) medium fraction (53–105 μm) which included fine particulate organic fraction and very fine sand (Pff); and (iii) fine fraction (< 53 μm) containing mineral associated organic fraction (Maf), silt and clay minerals and dissolved organic carbon. The material retained in each sieve was transferred to aluminum pots, and oven-dried at 105 °C for 24 h for later weighing. Particulate total fraction (Pf) was then calculated as the sum of Pcf and Pff.

Total C and macro- and micronutrients concentration of the different size fractions were analyzed following the procedures described above for total soil nutrient content.

Nutrient content in the fraction < 53 μm (Maf) was calculated as the difference between total nutrient content in the bulk soil sample (Ts) and nutrient content in the Pcf + Pff fractions.

Soil basal respiration (Resp) was measured in the Ts according to Jenkinson and Powlson (1976), by incubating soil samples (20 g at 55% WHC) at 25 °C for 7 days. Soil microbial biomass C (MBC) was performed by the chloroform fumigation–extraction method (Vance et al., 1987) using a conversion factor of 0.33. Both the respiration and microbial biomass were used to calculate the metabolic quotient (qCO_2), which expresses the quantity of CO_2 emitted per microbial biomass unit and time (Anderson and Domsh, 1990).

Carbon in the different size fractions and microbiological measurements were analyzed for both soil layers (0–5 and 5–20 cm), while the macro and micronutrients were measured only for the 0–5 cm soil depth.

2.3. *Statistical analyses*

Data were processed using the InfoStat statistics program (Di Rienzo et al., 2013). Homogeneity of variance and normality tests were performed for each variable. Data was analyzed with ANOVA. Mean separation was accomplished using a least squares difference (LSD) comparison test with a significance level of 0.05.

3. Results and discussion

3.1. *Organic carbon in the different soil size fractions*

Considering the C measured in the three size fractions (Pcf, Pff and Maf) and referring them to the C measured in the Ts, there was an average loss of 15.8% due to the wet sieving. For the Pcf, Pff and Maf the losses were calculated in the order 2.28%, 2.25% and 10.9%, respectively.

Carbon in the coarse fraction (Pcf) in 0–5 cm soil depth was ca. 3 times larger in S-CC-S than in S-S ($p < 0.05$) (Fig. 1a). These results are in agreement with previous results in the same experiment after the second cycle of rotation (Beltran et al., 2016). When soybean is cropped as monoculture, even under no tillage, the low C input and high decomposition rates of residues increased erosion risk and C loses. Using the AMG model, Milesi Delaye et al. (2014) calculated that including cover crops during autumn-winter between soybean crops may maintain SOC as compared with soybean monoculture. These authors estimated that oat or oat/vetch as CC would incorporate between 2.3 and 3.1 Mg C ha^{-1} y^{-1}, respectively. In the present study the contribution of wheat as CC was 1.4 Mg C ha^{-1} y^{-1} and that from the S-CC-S rotation was 3 Mg C ha^{-1} y^{-1}, while for the M-S-W/S it was 2.8 t Mg C ha^{-1} y^{-1}. Thus, the C input of a CC/soybean sequence would not be different than the C input of a maize monoculture (Rimski-Korsakov et al., 2015) or maize –wheat/soybean sequence.

The inclusion of cover crops in rotations that already include grass (maize and wheat) did not produce increases in the particulate fraction of carbon (Fig. 1a) and only differentiated from the S-S rotation.

Carbon in the fine fraction (Pff) and in the total particulate fraction (Pf) presented no significant differences among crop rotations (Fig. 1a). However, a trend of increasing carbon in these two fractions when cover crops were incorporated within soybean monoculture was detected ($p < 0.10$). The highest ISI (0.69) of this sequence among the rotations may have impacted in this increase since it has the largest C inputs to the soil (Table 1). Duval et al. (2013) found that Pff was the most sensitive C fraction to show differences due to changes in soil management. However, in the present research carbon in the Pcf, and not in the Pff, was the most sensitive among crop sequences.

Carbon in the mineral associated fraction (Maf) in the 0–5 cm soil depth was 41% and 43% higher ($p < 0.05$) in the M-CC-S-W/S as compared with S-S and S-CC-S, respectively. The C:N ratio of residues is a major quality factor which modifies the efficiency in transforming residues to stable soil carbon. Higher C-input by grasses, and the high concentration of soluble compounds and a low C:N provided by the soybean tissues (Duval et al., 2016) could enhance microbial activity and stubble decomposition promoting soil stable C accumulation (Frasier et al., 2016b). Lehmann and Kleber (2015) proposed a new conceptual model for organic matter formation, where the decomposer community breakdown the large molecules decreasing its size and increasing their solubility, and thus promote the interaction with mineral surface increasing its protection and facilitating its incorporation into soil aggregates. Recently, it was suggested that the main source of C binding to the mineral fraction comes from plant-derived labile compounds (Cotrufo et al., 2015).

The use of both grasses and legumes has been proposed as a sustainable strategy to increase stable soil carbon accumulation (Li et al., 2016). However, our results showed that inclusion of wheat as CC in the soybean monoculture did not increase the C in the Maf, but an increase was observed with the inclusion of maize and wheat in the crop rotation (Fig. 1a). Spiesman et al. (2018) found that in potentially productive soils, C storage can be enhanced by favoring C4 over C3 grasses through increased above- and belowground net primary productivity and reduced heterotrophic respiration. Fornara and Tilman (2008) conclude that crop rotations that include C4 grasses and leguminous species may improve stable carbon accumulation in soils, since legumes provides an extra N supply that can be used by C4 grasses to increase below and above-ground biomass, and by microbes promoting stubble decomposition and SOC formation. Puget et al. (1995) found that the larger

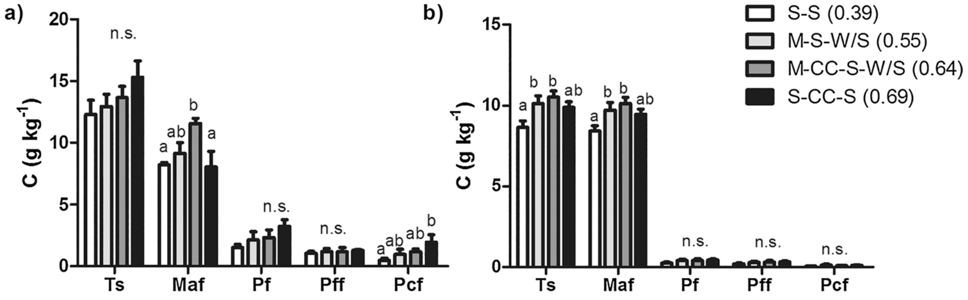

Fig. 1. Mean values and standard error for the carbon content (C) in the bulk sample (Ts), the mineral associated fraction (Maf), total particulate fraction (Pf), particulate fine fraction (Pff) and particulate coarse fraction (Pcf) in two sequences: soybean monoculture (S-S) and maize, soybean, wheat/soybean (M-S-W/S) combined with the inclusion of cover crops (CC): (M-S-CC-W/S) (S-CC-S) for the a) 0–5 cm soil depth, and 2) 5–20 cm soil depth. Numbers in the legend between parentheses indicates the intensification sequence index for each crop rotation. Means with different letters indicates significant differences ($p < 0.05$).

carbon content of water-stable macro-aggregates was due to young carbon from the maize.

Total organic C measured in the bulk soil sample (C in the Ts) in the 0–5 cm depth did not present significant differences among crop intensification sequences (Fig. 1a). Changes in SOC generally occur only after long periods (Sá and Lal, 2009), and short-term changes in total SOC in response to soil management practices are often small and difficult to assess (Zotarelli et al., 2007). In the present study, after 8 years of different crop sequences under NT, changes in SOC were found both in the coarse fraction and in the mineral associated fraction. However, it has to be considered that the Pcf only represents a minor contribution to the total C pool. This fraction consists mostly of OM coatings on the sand particles, making this C not physically protected and thus available for microbial decomposition. On the other hand, most of the soil C is stored in the mineral associated fraction (Maf). The C measured in the Maf mainly represents the more stable carbon since it is part of water-stable physically protected microaggregates (Denef et al., 2004).

There were not significant differences among crop rotations for the soil organic labile fractions (Pff and Pcf) in the 5–20 cm depth (Fig. 1b). Differences found in the 0–5 cm depth were only statistically significant for carbon in coarse fraction, mainly related to inputs from crop residues. On the other hand, total carbon (C in the Ts) and C in the mineral associated fraction were 20% higher in the crop rotations that include maize (M-S-W/S and M-CC-S-W/S) in comparison to the soybean monoculture (S-S) ($p < 0.05$). Boddey et al. (2010) suggest that legumes play an important role in promoting soil C accumulation in soils under no-tillage, perhaps due to a slow-release of N from decaying surface residues/roots which favored maize root growth. Santos et al. (2011) conclude that most of the cover crops contribution to C sequestration in no-till soils is from roots and is stored in the mineral-associated fraction. The results found in our research suggested the grass-legume-based rotation, especially when maize was included, was more efficient in converting biomass C into soil organic C in this deeper 5–20 cm layer than legume monoculture, possibly related to C roots contribution. Gale and Cambardella (2000) primarily attributed the soil organic C accumulation in no-till soils because of an increased retention of root-derived C.

Sequences with maize and legume participation were more efficient to sequestrate stable C at both soil depths (0–5 and 5–20 cm). Thus, combine C rich sources (grasses) with legume species that increase the N inputs, has to be considered as a major strategy to increase SOM levels and improve soil quality.

3.2. Microbial measurements

Soil respiration under incubation (Resp) and MBC are considered as indicators of easily mineralizable carbon (Franzluebbers et al., 1995). Resp at 0–5 cm depth (Fig. 2a) was 64% higher in the soil with the highest crop intensity (S-CC-S) in comparison with S-S and M-S-W/S, which could be explained not only due to the mix of grasses and legumes in the rotation as compared with soybean monoculture, but also by a greater quantity of organic material in the soil (Fig. 1a). The easy mineralizable carbon measured as soil respiration was more sensitive to detect differences among crop sequences than total particulate organic carbon (Fig. 1a).

Soil respiration was positively correlated with carbon measured in the Pf and Pcf ($r = 0.81$, $p < 0.05$; $r = 0.82$, $p < 0.05$, respectively), and no correlation were found between Resp and carbon in the Pff and Maf. The lack of relation among the C measured in the Pff and Maf with soil respiration, support the assertion that SOC is moving into the physically-protected microaggregates.

The microbial biomass carbon (MBC) (Fig. 2b) followed a similar trend than the observed for soil respiration, where the soybean monoculture (S-S) with the lowest ISI was 52% lower than S-CC-S. However, MBC was not sensitive enough to detect changes due to including CC in rotations with maize and wheat. The present study shows that after 8 years, differences in MBC between treatments could be detected, as was previously observed in similar experiments in the Pampas region (Costantini et al., 1995, 1996).

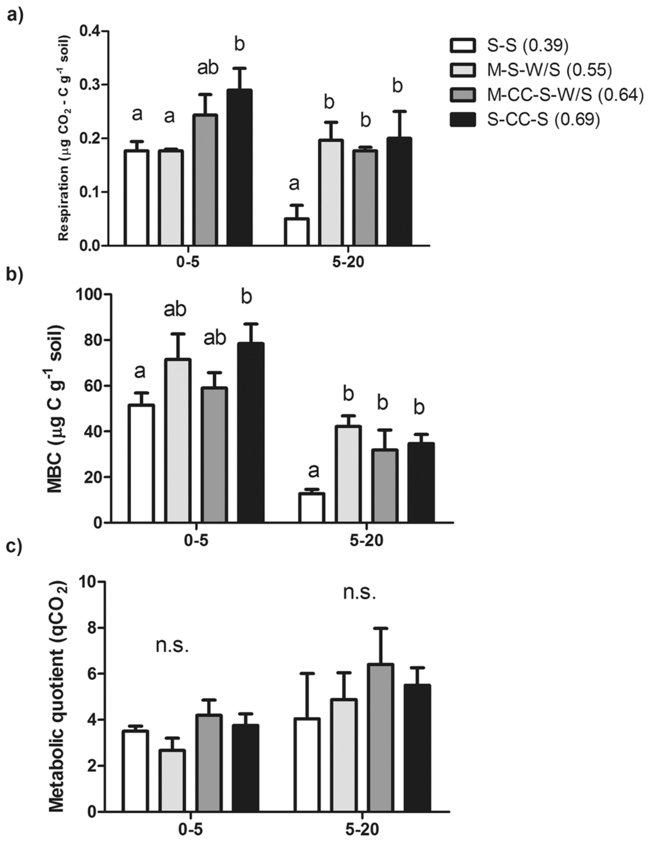

Fig. 2. Mean values and standard error for: a) Soil respiration b) Microbial biomass carbon (MBC) c) Metabolic quotient in two sequences: soybean monoculture (S-S) and maize, soybean, wheat/soybean (M-S-W/S) combined with the inclusion of cover crops (CC): (M-S-CC-W/S) (S-CC-S) for the 0–5 cm soil depth. Numbers between parentheses are the intensification sequence index for each crop rotation. Means with different letters indicates significant differences ($p < 0.05$).

There is increased evidence that transformation of residue C into microbial biomass could determine increases in soil C (Cotrufo et al., 2013; Kallenbach et al., 2015; Frasier et al., 2016a,b). Our results showed that increases in MBC in S-CC-S at 0–5 cm depth (Fig. 2a) agreed with the higher increase in the labile C fractions at the same soil depth (Fig. 1a).

For the 5–20 cm soil depth the differences in Resp and MBC among the soybean monoculture and the other crop rotations were relatively greater than in the upper layer (0–5 cm). Resp and MBC for S-S were 282% and 183%, respectively, lower than any of the other crop sequences. Also, as observed in the 0–5 cm layer, there was a positive correlation between Resp and carbon in the total particulate carbon fraction (Pf) (R^2 0.75, $p < 0.05$). Thus, the increment in the labile fraction may favor soil microbial growth and activity. For this soil depth, the labile carbon measured by Resp and MBC were both more sensitive than the carbon in the particulate fractions (Fig. 1b). Metabolic quotient (qCO_2) did not present significant difference between treatments (Fig. 2c). High values of this quotient are usually associated with nutritional stress conditions. However, the lack of difference may occur under similar environmental conditions (Anderson and Domsh, 1990).

Among the soil variables studied, those associated with more lability, i.e. carbon in the particulate coarse fraction (Pcf) and Resp in the first soil layer (0–5 cm), were positively correlated with the ISI (Fig. 3). These results suggest that these variables are closely related to the

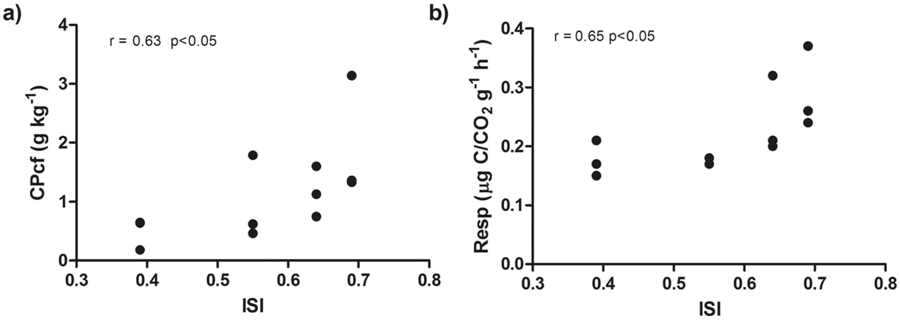

Fig. 3. Correlation between the intensification sequence index (ISI) and a) the soil organic carbon in the particulate coarse fraction (CPcf) and b) soil respiration (Resp) for the 0–5 cm soil layer.

increase in intensification index.

According to our findings, the response of the microbial community at 0–5 cm depth seemed to be mainly influenced by the crop intensification and the subsequent increase in the stubble, since the highest increases in Resp and MBC were found in the crop rotation with the highest ISI (S-CC-S). However, for the 5–20 cm soil depth, increases in both biological indicators were found when grasses were included in the sequence, independently of the crop intensification. These results suggest that microbial behavior in the 5–20 cm soil depth could be mainly related to root decomposition dynamics, and not with the stubble increases. Frasier et al. (2016a) reported that root biomass was better than aboveground biomass or litter to explain the response of MBC in a no-tilled system in the Argentinean pampas.

3.3. Macronutrients and micronutrients in different soil size fractions

Cropping intensity modified N, S, P and Ca content in the Pcf with no changes in Mg and K content. Sulfur content was the only nutrient that was modified in both Pcf and Pff. Variations in nutrient content in different fractions of particulate organic matter depend on several factors that affect nutrient return to soil. One of them is the nutrient harvest index, that determines the magnitude of nutrient export with grains from the system (and inversely nutrient returns via residues) and the C:nutrient relationship of the residues. Other, is the amount of fertilizer added to the crops in the sequence. In the present experiment N, P and S were applied in maize and wheat, P and S in soybean and N in the cover crop. Nitrogen content in Pcf increased ca. 4 times in S-CC-S as compared with S-S. However, no significant increases were detected among S-S and all sequences that included maize or wheat, even when C content increased in these sequences (Fig. 1). This increase in N content in the Pcf fraction only in S-CC-S may be likely due to two processes: (i) the C:N ratio in cover crops is lower than in maize or wheat, since they are killed before anthesis, and thus, a higher mineralization rate of the residues is expected and (ii) soybean is present each year in this sequence, with also a low C:N ratio. As opposed to this, in the other sequences soybean is present in 2 out of 4 years (when completing one cycle). Therefore, in both cases, there is a higher supply of residues with low C:N ratios that may have contributed to the Pcf fraction. The Phosphorus in Pcf increased ca. 3 times in S-CC-S respect to S-S, and the trend between sequences was similar to that observed with N, even when the cover crop was not fertilized with this nutrient (Fig. 4b). However, P was applied in all crops in the sequence. The sequences that included maize or wheat received from the beginning of the experiment 11% more P as fertilizer than S-S or S-CC-S. The positive effect observed in the present experiment in S-CC-S may be due to the effect of CC taking up residual P applied in soybean, in addition to the low C:P relationship (as compared with the other sequences) that may have increased P availability in S-CC-S. Varela et al. (2014) showed that soybean residues released ca. 20–30% of P independent of the succeeding cover crop. Also, it is important to highlight that in M-S-W/S and M-CC-S-W/S, P exportation with grains is more intense, and thus, residues may have low amount of P to be recycled. The S content in the Pcf was duplicated when a cover crop was included within a soybean monoculture when compared with S-S. Sulfur content in Pcf also increased by 67–100% respect to S-S, when sequences that included maize or wheat were analyzed (Fig. 4c). It is important to highlight that S addition as fertilizer was similar among sequences (Table 1), and thus this additional S should have come from recycling. As opposed to P and N, S harvest index is low in all species (Ciampitti et al., 2013) included in the sequence, and thus, a larger amount of S may have returned to the soil and contributed to increase S content in particulate fractions. In contrast to what was observed for N and P, all treatments that included CC or maize and wheat as grain crops increased S in the Pff fraction.

Sequences that have higher proportion of soybean (i.e. S-S and S-CC-S) showed an increase in Ca content in the particulate fractions, and no changes among sequences when analyzing the content of Mg and K. All these nutrients were not added with fertilizers, and thus any changes in organic matter content in surface are expected to happen from nutrient recycling from deeper depths. Soybean requires larger quantities of Ca than maize or wheat, but also Ca harvest index is low. Therefore, a large demand for calcium coupled with low export with seeds may have contributed to residues with higher Ca content that was reflected in the medium term in this increase in Ca content in particulate organic matter.

When analyzing nutrient ratios in the different compartments (Table 2), N:P, N:S or P:S ratios were similar among different fractions, except for Pcf. This fraction showed ratios 46, 96 and 55% higher than the other fractions for N:P, N:S and P:S, respectively. These increases suggest active biological processes in Pcf that cannot stabilize stoichiometry among nutrients and thus producing imbalances (Sun et al., 2018). In this analysis, it seems that N and P mineralization in the Pcf fraction surpassed S mineralization. Also, S addition to the system was relatively low (ca. 22 kg per ha), and for instance, P addition was 6 times larger than S addition (Table 1), then a progressive imbalance between P and S may have occurred, reflected in the relative content of both nutrients in Pcf, a very labile fraction that may early indicate imbalances of both nutrients. On the other hand, when analyzing the effects of crop sequences, the S-CC-S showed the higher imbalances for the N:S and P:S ratios, being 76 and 110 larger under than the other sequences, suggesting also a larger mineralization of N and P respect to S. Similar to this, Yang et al. (2007) found significant increases in N:S ratio in organic matter associated with aggregates larger than 2 mm, in response to long term application of manure in agricultural soils.

Zn, Fe and Cu content in particulate organic matter in the 0–5 cm soil depth (Fig. 5) showed no changes when sequences varied, however Mn in the coarse particulate fraction (Pcf) showed changes in the M-CC-S-W/S sequence, that increased 43% over the others (Fig. 5b). Since all these nutrients were not added as fertilizers, it seems that Mn, as described with Ca above, may have been recycled from deeper areas of soil. Wei et al. (2006) found that long-term use of leguminous crops led to a large increase in available Mn. Guimaraes Moreira et al. (2016) observed a positive relationship between Mn availability and SOM

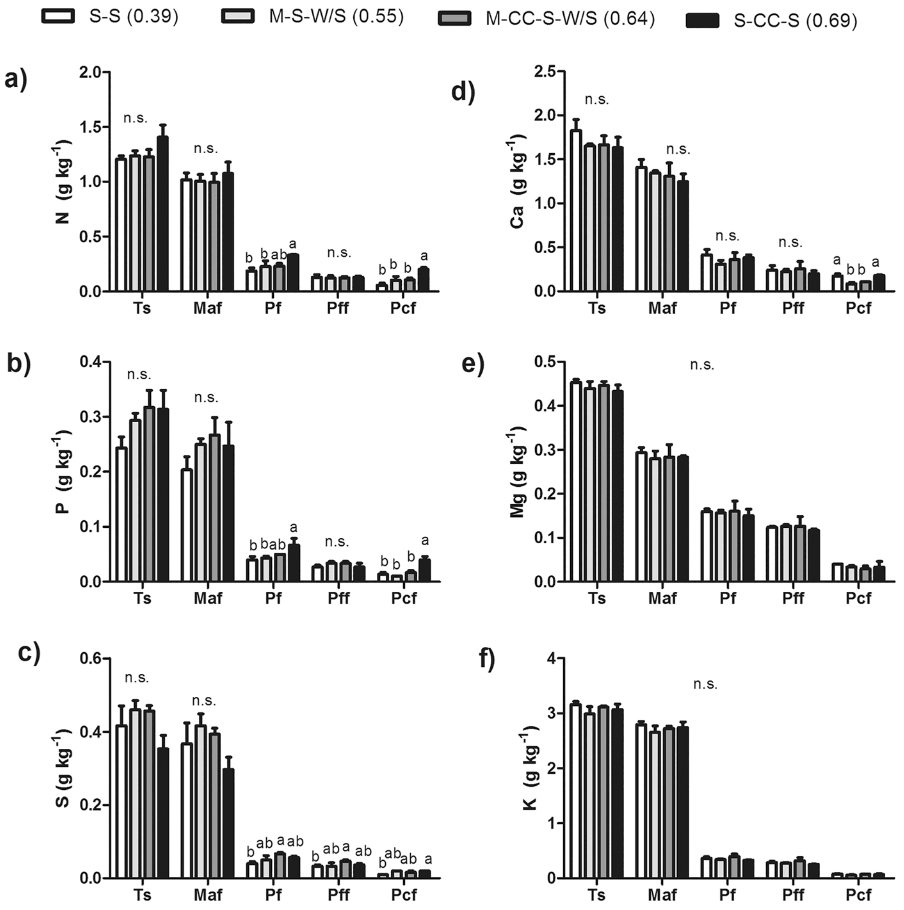

Fig. 4. Mean values and standard error for the total macronutrients a) N, b) P, c) S, d) Ca, e) Mg, f) K measured in the total soil sample (Ts), mineral associated fraction (Maf), total particulate fraction (Pf), particulate fine fraction (Pff) and particulate coarse fraction (Pcf) in two sequences: soybean monoculture (S-S) and maize, soybean, wheat/soybean (M-S-W/S) combined with the inclusion of cover crops (CC): (M-S-CC-W/S) (S-CC-S) for the 0–5 cm soil depth. Numbers between parentheses are the intensification sequence index for each crop rotation. Means with different letters indicates significant differences ($p < 0.05$).

concentration. According to Havlin (2005), increases in soil organic soluble complex may improve Zn availability in soil. Based in those previous results, we expected increases in micronutrient concentration bound to organic complex. However, our results did not show any relation among the total micronutrients and the carbon measured in the labile particulate fractions (data not shown). Previous results from this same experiment showed an increase in available Mn and Zn in M-S-W/S over the others treatments, with the lowest values in the soybean monoculture (Beltran et al., 2016).

4. Conclusions

Crop sequences with increasing participation of grasses under NT increased the C, N, S, P, Ca and Mn contents in the 0–5 cm soil depth in the more labile fraction (coarse particulate fraction – Pcf). Changes in the P and S content in Pcf, suggested recycling from fertilizer applied in previous crops in the rotation and/or previous legume crop in case of soybean. On the other hand, Ca and Mn seem to be recycled from deeper layers since these nutrients were not added as fertilizer.

Table 2

Mean values ± standard error for the total nitrogen/phosphorus ratio (N/P), total nitrogen/Sulfur ratio (N/S), total phosphorus/sulfur ratio (P/S) measured in the total soil sample (Ts), mineral associated fraction (Maf), total particulate fraction (Pf), particulate fine fraction (Pff) and particulate coarse fraction (Pcf) in two sequences: soybean monoculture (S-S) and maize, soybean, wheat/soybean (M-S-W/S) combined with the inclusion of cover crops (CC): (M-S-CC-W/S) (S-CC-S) for 0-5 cm soil depth.

		S-S (0.39)	M-S-W/S (0.55)	M-CC-S-W/S (0.64)	S-CC-S (0.69)
N/P	Ts	5.05 ± 5.03 a	4.19 ± 0.23 a	3.91 ± 0.23 a	4.52 ± 0.35 a
	Maf	5.23 ± 0.82 a	4.03 ± 0.36 a	3.78 ± 0.35 a	4.52 ± 0.73 a
	Pf	4.92 ± 1.31 a	5.04 ± 0.67 a	4.73 ± 0.67 a	5.27 ± 0.79 a
	Pff	5.15 ± 1.34 a	3.68 ± 0.09 a	3.64 ± 0.42 a	4.78 ± 0.44 a
	Pcf	4.70 ± 1.78 a	9.52 ± 2.87 a	7.43 ± 1.86 a	5.61 ± 1.06 a
N/S	Ts	3.02 ± 0.44 a	2.70 ± 0.18 a	2.68 ± 0.20 a	4.19 ± 0.85 a
	Maf	2.92 ± 0.55 a	2.49 ± 0.30 a	2.54 ± 0.31 a	3.82 ± 0.92 a
	Pf	4.45 ± 1.17 a	5.73 ± 2.51 a	3.65 ± 0.53 a	6.34 ± 0.35 a
	Pff	4.40 ± 1.25 a	5.20 ± 2.32 a	2.61 ± 0.39 a	4.00 ± 0.39 a
	Pcf	5.08 ± 1.82 a	6.52 ± 2.60 a	7.31 ± 1.88 a	10.2 ± 0.77 a
P/S	Ts	0.59 ± 0.05 a	0.64 ± 0.01 a	0.69 ± 0.07 a	0.95 ± 0.23 a
	Maf	0.56 ± 0.05 a	0.61 ± 0.03 a	0.68 ± 0.08 a	0.90 ± 0.28 a
	Pf	0.91 ± 0.06 a	1.05 ± 0.33 a	0.78 ± 0.05 a	1.26 ± 0.18 a
	Pff	0.84 ± 0.06 a	1.39 ± 0.58 a	0.71 ± 0.05 a	0.86 ± 0.15 a
	Pcf	1.11 ± 0.04 a	0.66 ± 0.08 a	1.10 ± 1.38 a	1.91 ± 0.26 b

Numbers between parentheses are the intensification sequence index for each crop rotation.
Means with different letters within each row indicates significant differences ($p < 0.05$).

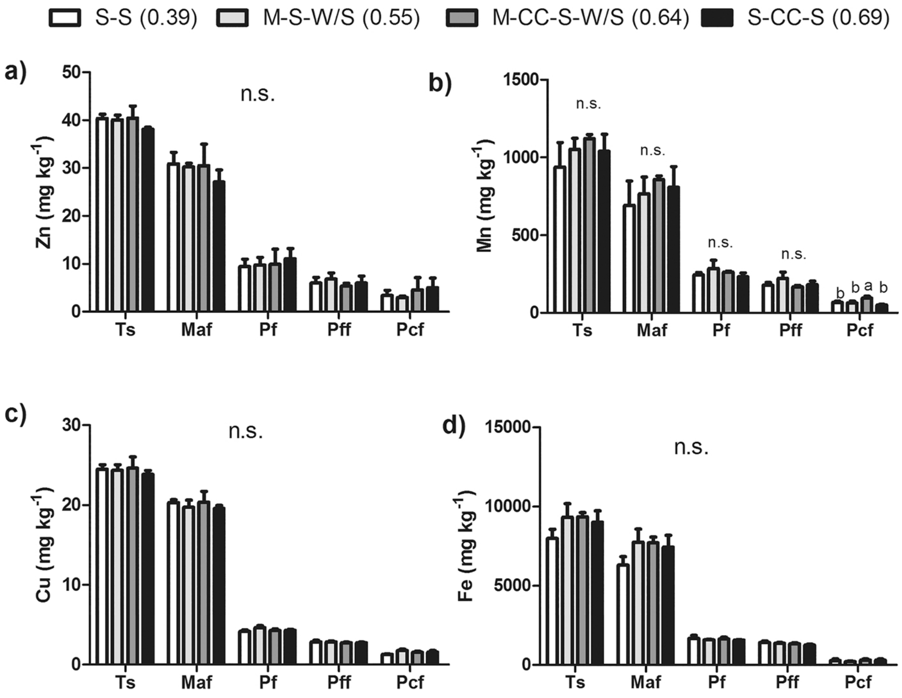

Fig. 5. Mean values and standard error for the total micronutrients a) Zn, b) Mn, c) Cu, d) Fe measured in the total soil sample (Ts), mineral associated fraction (Maf), total particulate fraction (Pf), particulate fine fraction (Pff) and particulate coarse fraction (Pcf) in two sequences: soybean monoculture (S-S) and maize, soybean, wheat/soybean (M-S-W/S) combined with the inclusion of cover crops (CC): (M-S-CC-W/S) (S-CC-S) for the 0–5 cm soil depth. Numbers between parentheses are the intensification sequence index for each crop rotation. Means with different letters indicates significant differences ($p < 0.05$).

Indicators of labile C like Pcf and soil respiration were positively correlated with the time occupied by crops (ISI). Thus, suggesting that the ISI could be a good predictor of the increases in soil labile C for the sequences studied.

In the present study, 8 years under different crop intensity produced changes in SOC in the coarse fraction, and also in the mineral associated fraction (Maf), indicating SOC accumulation into more stable carbon pools in the maize-legume-based rotation.

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