Long-term fertilization does not affect soil C:N:S or the proportion between labile/non-labile fractions in Mollisols

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CORE IDEAS

- A 14-yr experiment involving 6 fertilization treatments and 5 farms was evaluated
- C sequestration efficiency of crop residues and soil N and S concentrations were quantified
- Soil C:N, C:S, and N:S ratios were not affected by long-term fertilization
- C, N and S distribution between organic fractions remained stable across treatments
- Labile N: total N ratio varied in a very short range

Abbreviations

SOM, soil organic matter; Nan, mineralizable N; POM- C, particulate organic carbon; POM- S, particulate organic sulfur; Δresidue C, accumulated difference in residue C input between control and fertilized treatments; Δtotal C, Δtotal N,Δtotal S, differences in total soil concentration of C, N and S between control and fertilized treatments.

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ABSTRACT

Agricultural management practices such as fertilization may affect the overall soil quality and nutrient supply capacity. Here, we hypothesized that long-term fertilization promotes: i) an increase in the proportion of easily degradable soil organic C, N and S fractions; and ii) changes in the soil C, N and S stoichiometric ratios in the direction of favoring the specific nutrient added to the soil. We included S in our study due to the increasing importance of S fertilization in many agricultural soils. A long-term experiment involving six fertilization treatments and maize, soybean and wheat as cash crops was conducted for 14 yr in an on-farm network located at the Pampean Region (Argentina). Long-term fertilization did not have a central role at defining the C, N and S distribution between soil organic fractions or their stoichiometric ratios since the particulate organic matter (POM)-C:total C, POM-S:total S; C:N, C:N, and N:S ratios were not affected by fertilization treatments and the mineralizable N:total N ratio varied in a very short range. Instead, long-term fertilization increased residue C inputs to the soil (8.7 - 19 Mg ha-1 over the non-fertilized control) and, in turn, increases in soil total C, N and S followed a linear relationship with residue inputs. This relationship was not affected by fertilization or site factors. Obtained data contributes to understand the effects of continued fertilization on soil properties such as the distribution of fast- and slow-cycling organic matter and nutrient concentration, which are essential for effectively managing soils for sustainable agriculture.

INTRODUCTION

Understanding soil carbon (C), nitrogen (N) and sulfur (S) dynamics in agroecosystems is relevant to maximize soil quality and nutrient use efficiency while minimizing environmental risks. Most C, N and S soil forms are associated to soil organic matter (SOM) and its continuum of labile (i.e. easily degradable) and less labile fractions, which vary in nutrient content, molecular structure and turnover time (Oades, 1988; Johnston et al., 2009; Scherer et al., 2009). The fast-cycling labile fractions have an active role in soil nutrient bioavailability and promoting microbial activity, whereas the slow-cycling less labile fractions contribute to long term storage of C and nutrients and water holding capacity (Six et al., 2002; Blanco-Moure et al., 2016).

Agricultural management practices affect the SOM labile fractions and the proportion labile / less-labile fractions (Haynes, 2000; Blanco-Moure et al., 2016; Chaudhary et al., 2017), which may result in changes in the overall soil nutrient cycling and storage capacity (Shahid et al., 2017). Among agricultural practices, fertilization would be a particular model to study this issue because it involves the direct addition of nutrients to the soil. Besides the classical N, P and K fertilization, over the past decades the need of S fertilization has become an increasingly important issue for crop management in many agricultural regions of the world, which suffered a reduction in their soil S availability (Salvagiotti et al., 2012; Aula et al., 2019). This has been attributed to the reduction of atmospheric deposition due to increased control of S emissions by industry, the limited use of S fertilizers, and the intensification of crop rotations (Scherer, 2001). Fertilization (mainly N, P and potassium, as mentioned) is a widespread practice performed by farmers to overcome yield nutritional constraints and, except in nutrient-rich soils, usually results in higher residue inputs (stover) (Sucunza et al., 2018). Functions relating how these additional residue inputs translate into soil C and SOM fractions would be useful to predict the trajectories of soil compounds and the stoichiometric ratios between them in response to fertilization (Campbell et al. 1991). This issue has been extensively studied for C in different agroecosystems (Tian et al., 2015; Chaudhary et al., 2017). In contrast, fertilization effects on soil nutrient concentrations and their partition between labile and less labile SOM fractions has received considerably less attention and studies reported so far has shown divergent results (Campbell et al., 1991). Among soil nutrients, N and S are suitable candidates to approach this issue as they are the plant nutrients with the higher proportion of organic forms. For example, continuous fertilization caused a decline in total soil N (Mulvaney et al., 2009), negligible effects on total or labile fractions of soil N (Divito et al., 2011), or increased total and labile N contents (Shahid et al., 2017). The partition of soil nutrients between SOM fractions would be determined by a trade-off between enhancing SOM mineralization and nutrient release, decomposing additional residue inputs and increasing storage concentrations. The extent of fertilization effects is expected to vary with the magnitude of the crop response to fertilization and the initial levels of SOM and soil nutrients (Campbell et al., 1991). If the effects of fertilization on the organic fractions of C, N and S are not parallel, the stoichiometric ratios between these elements would be modified.

Among the different SOM fractionation methods, the particle-size approach has been widely adopted to distinguish pools of different turnover rates (Poeplau et al., 2018). Since the pioneering work of Cambardella and Elliot (1992), particulate organic matter (POM) has become one of the reference methods for this purpose (Poeplau et al., 2018). Although originally conceived for C, it has been successfully adopted to characterize the labile fractions of organic P (e.g. Ciampitti et al., 2011). For N and S, the POM method has been relatively less adopted, although arises as a sound approach to distinguish its labile fractions (Amelung et al., 1998; Sindelar et al. 2015; Cates et al. 2016). A lack of reliable indicators of soil labile fractions of S has been claimed since some time ago (Blum et al., 2013) and continues today (Carciochi et al., 2016). However, a recent work showed that the S content in the POM was related (r=0.84) to the potentially mineralizable S estimated through soil aerobic incubation (24 weeks; Wyngaard and Cabrera, 2015). In contrast to S, there is a long list of methods to evaluate soil labile N (Schomberg et al., 2009; Martinez et al., 2018). One of the more adopted soil labile N tests is based on the N-NH_4 produced after short term anaerobic incubations (Nan) (Soon et al. 2007; Divito et al., 2011). In the Argentinean Pampas, Orcellet et al (2017) demonstrated that adding the Nan indicator to the traditional methods of diagnosing fertilizer N requirements improves the predictions of corn response to N fertilization.

As changes in SOM and soil nutrient concentration are relatively slow processes, long-term field studies are a valuable tool to determine the magnitude of the effects of crop management practices, such as fertilization, on the distribution of C and nutrients between SOM fractions. The objective of this report was to evaluate the effect of long-term N, P and S fertilization on: i) the relationship between residue inputs and changes in soil C, N and S; and ii) the distribution of C, N and S between the labile and non-labile soil organic fractions and the stoichiometric ratios between them. We hypothesized that long-term fertilization promotes; i) an increase in the proportion of soil labile fractions of C, N and S; and ii) changes in the soil C, N and S stoichiometric ratios in the direction of favoring the specific nutrient added to the soil. Our approach was to analyze the results from five field fertilization trials located on the northern Pampas of Argentina after 14 years of fertilizer additions.

MATERIALS AND METHODS

Long-term field experiment

A field experiment network was started in 2000 on private farms of the Regional Consortium for Agricultural Experimentation (CREA) to evaluate the long-term effects of different fertilization regimes on soil fertility and crop yields (Ciampitti et al., 2011; Sucunza et al., 2018). At present, the network comprises five sites located in Argentinean Pampas, four at

Santa Fe province (Balducci, San Alfredo, La Hansa and Lambare), and one at Cordoba (La Blanca). The five sites are managed following no-tillage practices and their soils belong to the Mollisol order and present some differences in their main characteristics (Table 1). Each site followed one of the following two crop rotations: 1) bi-annual rotation: maize and double cropping wheat/soybean (M-W/S); 2) tri-annual rotation: maize, full season soybean, and double cropping wheat/soybean (M-S-W/S). In this study, we evaluated the period between 2000/01 and 2013/14 growing seasons (Table 1).

Treatments

All sites followed a similar experimental protocol. In each site, the design was a randomized complete block with three replicates (except San Alfredo: two replicates). The replicates (plots) were 25-30 m wide and 65-70 m long. Six treatments were compared: a) control without

fertilization, b) NP fertilization, c) NS fertilization, d) PS fertilization, e) NPS fertilization; f) NPS+ (NPS + K, Mg and micronutrients fertilization). Nitrogen rates for wheat and maize were determined annually according to the models employed by local farmers to obtain maximum or near-maximum yields. These models are based on the N-nitrate soil content before sowing and the predicted N demand. Soybean crops were not fertilized with N. Along the experimental period, applied N (as urea) varied between 90 and 175 kg N ha^{-1} year^{-1}. Phosphorus and S rates were determined annually according to the maintenance/ build-up criterion and averaged 37 and 21 kg ha^{-1} year^{-1}, respectively. The P source was mono-ammonium phosphate and the S source was gypsum. Fertilizers were banded and incorporated at 5 cm depth at sowing. The rates of K, Mg, B, Cu and Zn averaged 12, 7, 1, 2 and 2 kg ha^{-1} year^{-1} respectively.

Analytical determinations

In order to minimize the effects of the most recent crop and concentrate on the effects of the long-term fertilization treatments, we selected the 2013-2014 season for soil sampling because all farms had the same crop (soybean). Soil samples (0-5, 5-10 and 10-20 cm) were collected after soybean harvest. For each soil layer, one composite sample, composed by 30 subsamples extracted with a 2 cm-diameter soil probe, was taken at each plot. The samples were air-dried and sieved with a 2 mm mesh. Besides C, in this work we focused in N and S, the two macronutrients with the higher proportion (>90%) of organic forms and whose availability depends on SOM mineralization. Regarding the other main macronutrients, in an on-going and parallel research, we are working on P (e.g. Sucunza et al., 2018), whereas potassium is not a limiting nutrient in Pampean soils. The following analytical determinations were made: organic C by the Walkley and Black method (Nelson and Sommers 1982.); total N by wet digestion followed by colorimetry (Baethgen and Alley, 1989); total S by wet digestion followed by turbidimetric measurements (Tabatabai, 1996). The POM fraction was separated following a modified version of Cambardella and Elliott (1992), in which the original Na hexametaphosphate was replaced by 0.05 M potassium chloride (Salas et al., 2003). Samples were stirred 15 hours at 150 rpm and the fraction >53 ɥm was collected after sieving, dried in an oven at 60°C until constant weight and weighed. The C and S POM concentration was measured following the same protocol described for total soil C and S. The mineralizable N (Nan) was measured after an anaerobic incubation in test tubes (150 x 16 mm) filled with 5 g of soil and 12.5 ml of distilled water (Keeney and Bremner, 1966). The tubes were hermetically sealed and incubated for 7 days at 40°C. At the end of the incubation period, the content was transferred to Erlenmeyer flasks with a

4M KCl solution that were stirred for 30 minutes and then filtered. Released N-NH_4 was measured by colorimetry (Baethgen and Alley, 1989).

Carbon inputs from crop residues (remaining aboveground biomass after harvest) were calculated annually from the yields of each crop, treatment and site, a uniform harvest index of 0.45, 0.53 and 0.46 for wheat, corn and soybean crops, respectively and a uniform C concentration of 0.4 kg kg^{-1} for all crop residues (Johnson et al., 2006). The accumulated differences in residue C input between control and fertilized treatments (Δ residue C) were calculated for each site by subtracting the accumulated residue C input of the control from the residue C of each other treatment. The differences in the soil concentration of total C, N and S between control treatment and fertilized treatments were calculated in each site at the end of the study period (Δ total C, Δ total N and Δ total S).

Statistical analysis

ANOVA's were performed with fertilization treatments, sites and interactions as fixed effects. Means of each level were compared using the least significant difference LSD test ($p < 0.05$). Stoichiometric relationships between the total and labile C, N and S contents were expressed in units of mass (kg kg^{-1}) and compared through regression analysis and F tests. When no significant differences were detected, the regression lines for those treatments were represented by a single function. The rotation variable was not included in the statistical comparisons, so the comparisons between both rotations are considered only descriptive. Statistical analyses were performed using INFOSTAT (Di Rienzo et al. 2018).

The relationships between the accumulated differences in residue C inputs (Δ residue C) and the changes in soil total C, N and S concentration (Δ total C, Δ total N and Δ total S) were evaluated by regression analysis. The effects of crop rotation, site or treatments on these relationships were evaluated by an F test. When there was no rotation, site or treatment effect, a single function was used to represent the relationship. When the intercept was not significantly different from zero, the function was forced through the origin.

RESULTS

Total and particulate organic carbon

At the end of the evaluated period, soil total C values closely followed the ranking of sites observed at the beginning of the experiment (2001; Tables 1; 2) with Lambare and San Alfredo as the highest and La Hansa, La Blanca and Balducchi as the lowest locations in terms of C concentration (Tables 2; 3). The joint application of N, P and S for fourteen consecutive years caused a significant increase (average across sites: +11%) on total C compared to the unfertilized control. Particulate organic C also varied significantly among sites (Tables 2; 4). The highest values were observed in San Alfredo and the lowest in La Blanca. The five treatments involving fertilizer additions showed slight differences between them in POM-C, but consistent higher values than the unfertilized control (average: + 13%, contrast between control and the five fertilized treatments resulted in $p<0.05$) (Table 4).

The POM-C: total C ratio varied between sites: the highest values were found in Balducchi and San Alfredo (0.32±0.005 and 0.31±0.006, mean and SE, respectively), while the other sites ranged between 0.26 and 0.27 (±0.005 in all cases). In terms of vertical stratification, as expected POM-C decrease with depth was more accentuated than total C (Fig. 1). At the 10-20 cm layer, POM-C decreased 69% from values found at 0-5 cm whereas the decrease in total C was around 40%. Interestingly, the POM-C:total C ratio remained unaffected by fertilization treatments in the five study sites (Table 2; Fig. 2).

Total and labile nitrogen

The interaction site x fertilization was not statistically significant for total N and Nan (Table

2), which allowed the analysis of the individual effects of both factors. Regarding the site effect, as expected total N resembled the pattern observed for total C: the highest values were found in

Lambare and San Alfredo and the lowest in Balducchi (Table 3). The same ranking of sites were observed for Nan (Table 4). In terms of fertilization, the unfertilized control had significantly lower total N than the five fertilized treatments (average difference over the control: 9.7%). As expected and similarly to what was observed for grain yields (Table 5), the two treatments involving the addition of three nutrients (NPS and NPS+) had 12% more total N than the non-fertilized control, whereas the treatment that did not receive N (PS) had lower (-6.2%) total N than the NPS+ treatment. Unexpectedly, Nan was not affected by fertilization treatments ($p = 0.09$) (Table 2). The Nan: total N ratio differed between sites and fertilization treatments (Table 2) and was higher in the control (0.034±0.001 SE) than in the fertilized treatments (0.029-0.03±0.001 SE in all cases).

As expected, Nan was more stratified in depth than the total N (Fig. 1). At the 10-20 cm layer, Nan decreased 78% compared to values found at 0-5 cm whereas the decrease in total N was around 42% (Fig. 1).

Total and particulate sulfur

The interaction site x fertilization was not statistically significant for total and POM- S (Table 2). As observed for total C and N, San Alfredo and Lambare had the highest and Balducchi the lowest

total S concentration (Table 3). Fertilization increased soil total S values by 4.8% (average for the five fertilization treatments, which did not show significant differences between them). The ranking of sites in terms of POM-S differed from what was observed for total S. In this case, La Hansa and Lambare had the lowest values while La Blanca the highest (Table

4). In contrast, responses to fertilization for POM-S were similar to total S: no differences within the five treatments involving nutrient additions but significant differences with the control (+12.5%, $p>0.05$,Table 4). The POM-S:total

S ratio varied between sites but remained unaffected by fertilization (Fig. 2). As observed for C and N labile fractions, the POM-S fraction was more concentrated in the surface layers than total S (Fig. 1). At the 10-20 cm layer, POM-S decreased 35% compared to values found at 0-5 cm whereas the decrease in total S was around 21% (Fig. 1). The vertical distribution of POM-S:total S ratio was not affected by site or fertilization.

C:N, C:S and N:S stoichiometric ratios

Variations in total C and total N were fairly proportional in all cases, resulting in no significant effects of fertilization nor soil sampling depth on the C:N ratio, which averaged 11.1 (Fig. 3). The C:S ratio was also not affected by fertilization treatments but was higher at the 0-5 than at the 5-20 cm soil layer (55±0.63 vs 43±0.64, mean ± SE, respectively) (Fig. 3). Similarly, the N:S ratio was unaffected by fertilization treatments and decreased with depth: at 0-5 cm averaged 4.9±0.06 whereas at 5-20 cm averaged 3.8±0.06 (mean ± SE) (Fig. 3).

Residue C inputs in relation to changes in soil C, N and S

All treatments involving nutrient additions increased the amount of residue inputs, as denoted by the positive values of the accumulated difference on residue C inputs between fertilized and non-fertilized treatments (Δ residue C). The treatment without N addition (PS) had the smallest Δ residue C) whereas the NS and NP treatments had intermediate values, and the two treatments that added the three nutrients (NPS and NPS+) had the largest values (Table 5). The Δ residue C of these three groups significantly differed from each other ($p < 0.05$). Fertilization increased aboveground residue C inputs up to 1.31 Mg ha^{-1} year $^{-1}$.

The two sites that included a more intensive crop rotation (Balducchi and San Alfredo, 1.5 crops per year) had a higher Δresidue C input than the three sites with a less intensive rotation sites (1.33 crops per year, La Hansa, La Blanca and Lambare) (averaged values of all fertilized treatments, $p < 0.05$). In Balducchi and San Alfredo the Δ residue C was 20 (± 3.4) and 16 (± 2.6) Mg ha^{-1}, respectively, while in La Hansa, La Blanca and Lambare the Δ residue C was 12 (± 1.6), 12 (± 1.2) and 8.6 (± 0.8) Mg ha^{-1}, respectively (means ± SE) .

A significant linear relationship was observed between the Δ residue C inputs and the change in the soil total C, N and S concentration (0-20 cm) of the fertilized treatments compared to the non-fertilized control at the end of the study period (Δ total C, N and S) (Fig. 4). The slopes of these relationships were not affected by site, rotation or treatment ($p > 0.05$), thus a common relationship was fitted to all the sites and treatments for each element (Fig. 4). The slopes of these relationships represent the change in the concentration of soil total C, N and S per Mg ha^{-1} of residue C input. Following this approach, for each Mg ha^{-1} of residue C input over the control treatment, an increase in soil total C, N and S concentration of 89.2±9.5, 8.89±0.54 and 1.06±0.11 mg kg^{-1} was observed, respectively (means ± SE) .

DISCUSSION

The continuous addition of fertilizers to the soil may affect several components of the agroecosystem such as soil quality and C and nutrient stocks (Russell et al., 2005; Shahid et al., 2017). Here, we explored the impacts of six fertilization treatments applied for 14 yr to a maize, wheat and soybean rotation on the soil C, N and S distribution in Pampean Mollisols. Changes in the total content and labile fractions of N and S are closely linked to C, since their inorganic forms do not usually accumulate in soils of humid areas. This is not the case for other nutrients such as P, whose labile fractions dynamics are determined by the soil P budget, estimated as the difference between P inputs (typically fertilizers) and P outputs (i.e. harvested grains) rather than by C or the residue dynamics, as observed in the study area by Sucunza et al. (2018). Fertilization increased aboveground residue C inputs up to 1.31 Mg ha^{-1} year^{-1} over the non-fertilized control which consistently promoted gains in total soil C that were not affected by either site or the type of fertilizer (Fig. 4). The increase in soil total N and S also followed a linear relationship with the residue inputs (Δ total N and S vs Δ residue C inputs; R^2= 0.74 and 0.43, respectively, Fig. 4), which, as observed for soil C (R^2= 0.53), was not affected by either fertilization or site factors. Fitted functions indicate that each additional Mg ha^{-1} of aboveground residue C input to the soil caused an increase of 8.89 (± 0.54 SE) and 1.06 mg kg^{-1} (±0.11 SE) in soil total N and S, respectively. Obtained values constitute a measure of the effects of fertilization on C sequestration efficiency and soil N and S concentrations. As expressed on both per area or per unit of residue basis, they would be useful for C- and nutrient-budgeting analyses since the amount of residues left by a fertilized crop is highly variable, from a null effect when fertilizing nutrient rich soils to a dramatic effect when applying fertilizers to very poor soils. If these effects depend on the amount of residues and less on the type of fertilizers, as shown by our data, estimated values could be applied to a wide array of fertilization treatments.

Regression lines shown in Fig. 4 allow further insights on soil C, N and S responses to the increased residue inputs of the fertilized treatments. The ratio between C and N slopes was 10.1 (i.e. 0.0892 / 0.0088 see Fig. 4), which indicates that each unit of additional residue translated into 10.1 times more C than N in the soil. This value is consistent with the average soil C:N ratio (11.1; Table 1), and is in line with the close relationship between soil C and N dynamics (e.g. Kumar and Goh, 1999), even in the presence of fertilization. In contrast, the ratio between the C and S slopes was 83.6 (0.0892 / 0.00106 –check that Fig. 4 indicates a slope of 1.06 but the unit is mg kg^{-1} and here is expressed in g kg^{-1} for comparison purposes-), a much higher value than the observed soil C:S ratio (~ 45). This result indicates that the S accumulation in the soil due to increased residue inputs was much slower than that of C (and also of N) which suggests a relatively slower conversion of residue S to soil organic S and predicts an increase of the soil total C: total S ratio in the medium/long term. The contrast between the stable nature of soil C:N ratio and the variable nature of soil C:S ratio has been reported previously. For example, Wang et al. (2006) observed that the C:N ratio of a native grassland grown on Mollisols of mesic regimen remained stable (~10) after being cropped for 60 to 83 yr (with diminished residue inputs, conversely to our approach with increased residue inputs promoted by fertilization), while the soil C:S ratio decreased from 61-72 to 35-55. Differences in the dynamics of the C:N and C:S ratios are generally attributed to the different decomposition/mineralization mechanisms of organic N and S and the way both nutrients are linked to C (Mc Gill and Cole, 1981; Blum et al., 2013). Whereas N is linked to C mostly through C-N unions, S is linked either through C-S or C-O-S bondages (Blum et al., 2013).

Taking the non-fertilized treatment as reference, the two treatments involving NPS additions showed an average annual gain in soil total C of 0.12 g kg soil $^{-1}$ yr^{-1} (0-20 cm). This value is within the range observed in long-term fertilization trials performed in the Southern Pampas for 11 years (Studdert and Echeverría, 2000), in the U.S. Great Plains for 11 years (Halvorson et al., 1999) and in Canada for 32 years (Gregorich et al., 1996). However, observed values are considerable lower than those reported by Tian et al. (2015), who observed that Chinese paddy soils fertilized with NPK for 14 years accumulated C at a mean rate of 0.21 g kg^{-1}yr^{-1} above the non-fertilized control. This contrast may be explained by the slow rate of SOM decomposition in frequently flooded soils (Tian et al., 2015). In our experiments, the positive responses to NPS fertilization on soil total N were in the same range of soil total C (+ 12% over the non-fertilized control), averaging 0.152 g N kg soil $^{-1}$. The annual accumulation rate found for N (11.2 mg kg^{-1} yr^{-1}), was in the same range than other long-term studies (e.g. Halvorson et al 1999, Yang et al 2012). The accumulation rate of soil total S in response to fertilization was relatively lower than rates observed for C and N (+6% between NPS treatment and the non-fertilized control), with final gains of the NPS over the control treatment of 19 mg S per kg of soil and a mean annual difference of 1.4 mg kg^{-1} yr^{-1}. Previous much longer studies (i.e. 35 and 82 years), showed greater final increases in soil S due to fertilization (30 - 90 mg kg^{-1}), and lower mean annual differences (0.85 and 1.09 mg kg^{-1} yr^{-1}) (Kirchmann et al., 1996; Yang et al., 2007).

In our experiments, NPS fertilization increased the absolute values of the labile and total contents for C and S in a rather similar magnitude, determining that the POM-C: total C and POM-S: total S ratios remained unaltered across fertilization treatments. For N, the labile fraction was not affected by fertilization treatments and the Nan:total N ratio varied in a very short range (0.029-0.034). Previous reports showed divergent results on the response of labile N fractions to fertilization treatments, from null effects (Fabrizzi et al., 2003), to positive (Yan et al 2006) or detrimental effects (Divito et al., 2011). In fertilized soils, it is known that the size of the labile N pool results from a tradeoff between the increases in the total N concentration and the acceleration of N turnover that reduce the mineralizable N pool (i.e. priming effect caused by N fertilization) (Kuzyakov et al., 2000). In our experiments none of these processes seems to have predominated over each other, with the consequence of a fairly constant Nan content across the fertilization treatments. Overall, the null or very small effect of fertilization treatments on the POM-C: total C, POM-

S: total S and Nan: total N ratios lead to reject the proposed first hypothesis for the increase in the proportion of labile fractions in response to fertilization. Instead, obtained results suggest that other stronger and more permanent factors regulate the relative proportion of labile fractions and the internal composition of SOM. The high resilience of the proportion of labile fractions is also reflected by the lack of correlation between the ratio POM-C: total C / Δresidue C which means that a larger amount of crop residues (generated by the fertilized crops) was not sufficient to affect the proportion of labile fractions. In a recent work, Cates et al. (2016) observed that POM may be more sensitive to belowground than to aboveground residue C inputs. In such sense, given that our findings on Δ residue C were based on aboveground biomass and no specific root to shoot changes across the different treatments were assumed, extrapolation of our results should be used with caution in those cases were the proportion of belowground residue inputs are expected to vary between compared treatments. On the other hand, the labile C, N and S fractions were more stratified along soil depth than the total contents of each element. This is reasonable in no tillage systems like the studied ones, in which the labile fractions concentrates the forms recently incorporated into the topsoil where crop residues and soil layers are not mixed as occurred in tilled soils (Dolan et al., 2006).

The proportion of fine soil fractions and associated small pores affects the dynamics of C and nutrients related to SOM through the protection against microbial attack (Van Veen and Kuikman, 1990; Amelung et al., 1998). Accordingly, the studied sites, which have a relatively wide range of soil textures (Table 1), differed in their stoichiometric soil C:N, C:S or N:S ratios. However, and in contrast to the proposed second hypothesis, these ratios were not affected by long-term fertilization treatments, including those involving N and S additions (Fig. 3, Table 2). Interestingly, the lack of fertilization effects on the C:N, C:S or N:S stoichiometric ratios was rather independent of other features proper of each site, as inferred by the non-significant interaction site x fertilization. This robustness implies that obtained results may be extrapolated to other Mollisols. On the other hand, the lower variability of the C:N compared to the C:S ratio (average R^2 0.84 vs 0.35) may be attributed to the closer relationship between the dynamics of C and N than between C and S in the soil (Maynard et al., 1983).

In conclusion, our results suggest that fertilization does not have a central role at defining the C, N and S distribution between SOM fractions or their stoichiometric ratios since the POM-C:total C, POM-S:total S; C:N, C:N, and N:S ratios were not affected by fertilization treatments and the Nan: total N ratio varied in a very short range. Instead, continuous fertilization increased residue inputs to the soil which boosted the provision of soil ecosystem services such as increased soil total C, N and S contents. Obtained data contributes to understand and quantify the effects of continued fertilization on soil properties such as nutrient storage and the distribution of fast- and slow-cycling SOM fractions, which are essential for effectively managing soils for sustainable agriculture.

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Legend of the figures

Figure 1: Topsoil vertical distribution of (a) Soil C, (b) Soil N, (c) Soil S. Vertical distribution was not affected by site or fertilization factors. Horizontal bar represents the standard error.

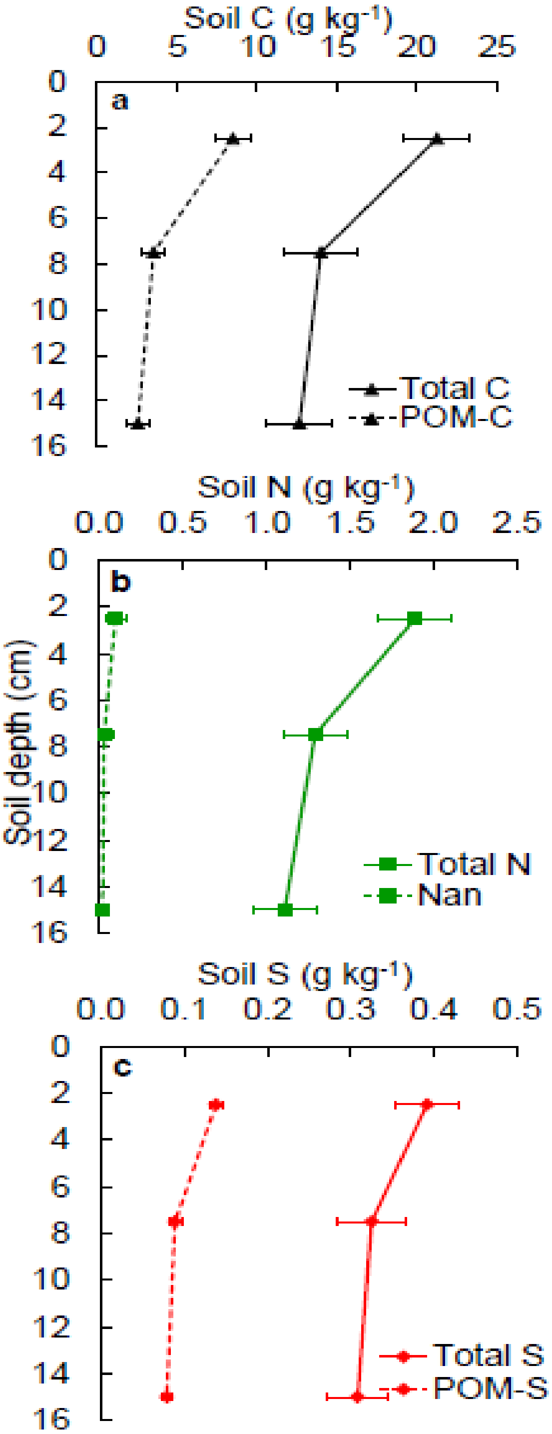

Figure 2: Bivariate plots illustrating the relationships between POM-C:Total C, Nan:Total N, POM-S:Total S at three soil depths (0-5, 5-10 y 10-20 cm). Data from five experimental sites and six fertilization treatments are included. Significant effects of soil depth were detected for the POM-C:Total C and Nan:Total N ratios and the individual functions are shown. Equations and coefficients of determination for the linear regressions are included.

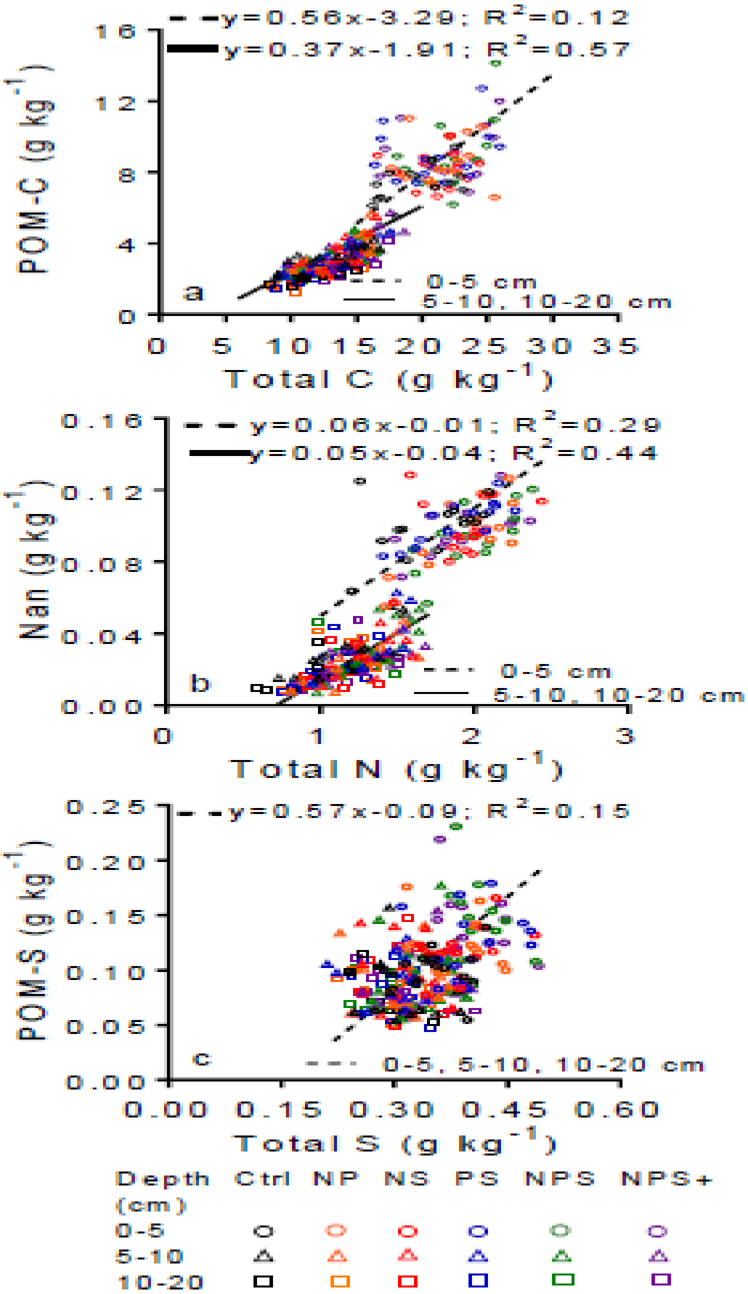

Figure 3: Bivariate plots illustrating the total soil C: N, C:S and N:S ratios at three soil depths (0-5, 5-10 and 10-20 cm). Data from the five experimental sites and six fertilization treatments are included. Significant effects of soil layer were detected for the C:N and C:S ratios and the individual functions are shown. Equations and coefficients of determination for the linear regressions are included.

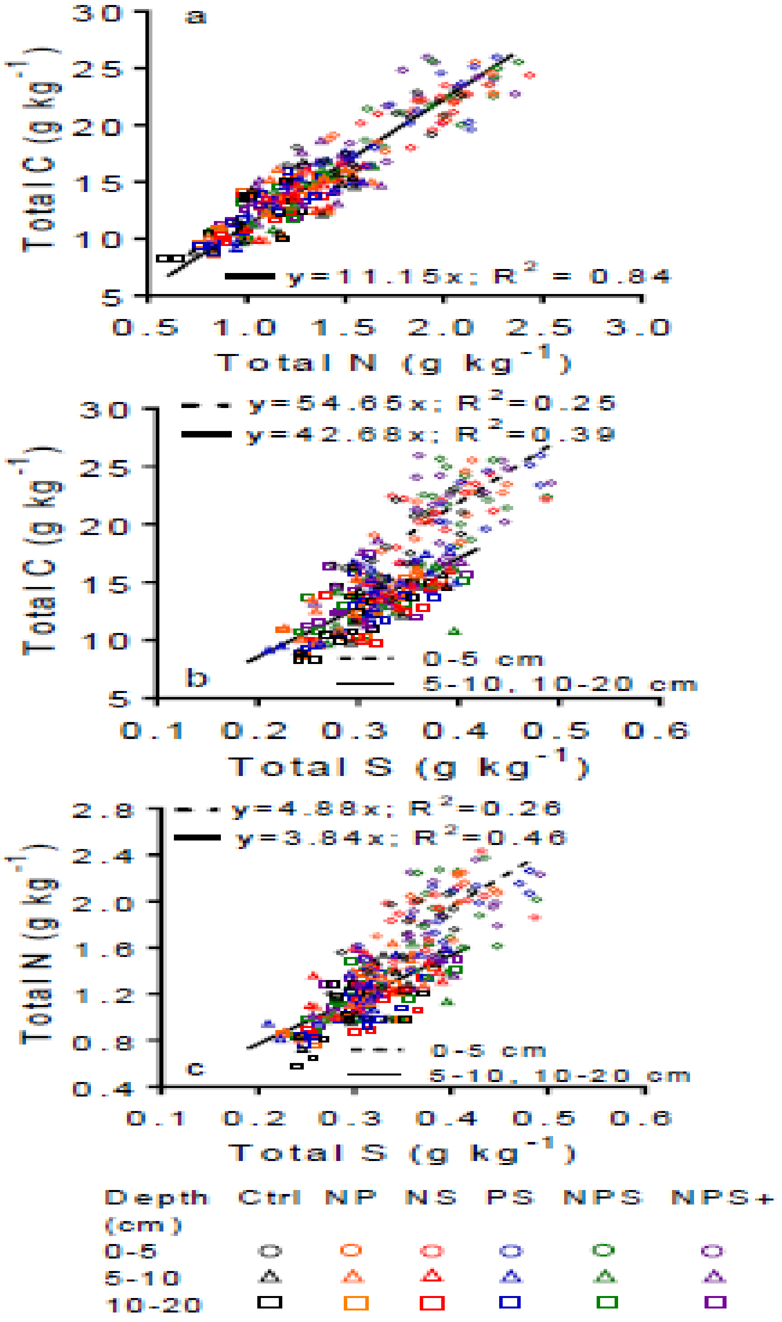

Figure 4: Bivariate plots illustrating the relationships between Δ Total C, Δ Total N, Δ Total S and Δ Residue C. Data from five experimental sites and six fertilization treatments are included. Individual functions and coefficients of determination for the linear regressions are included.

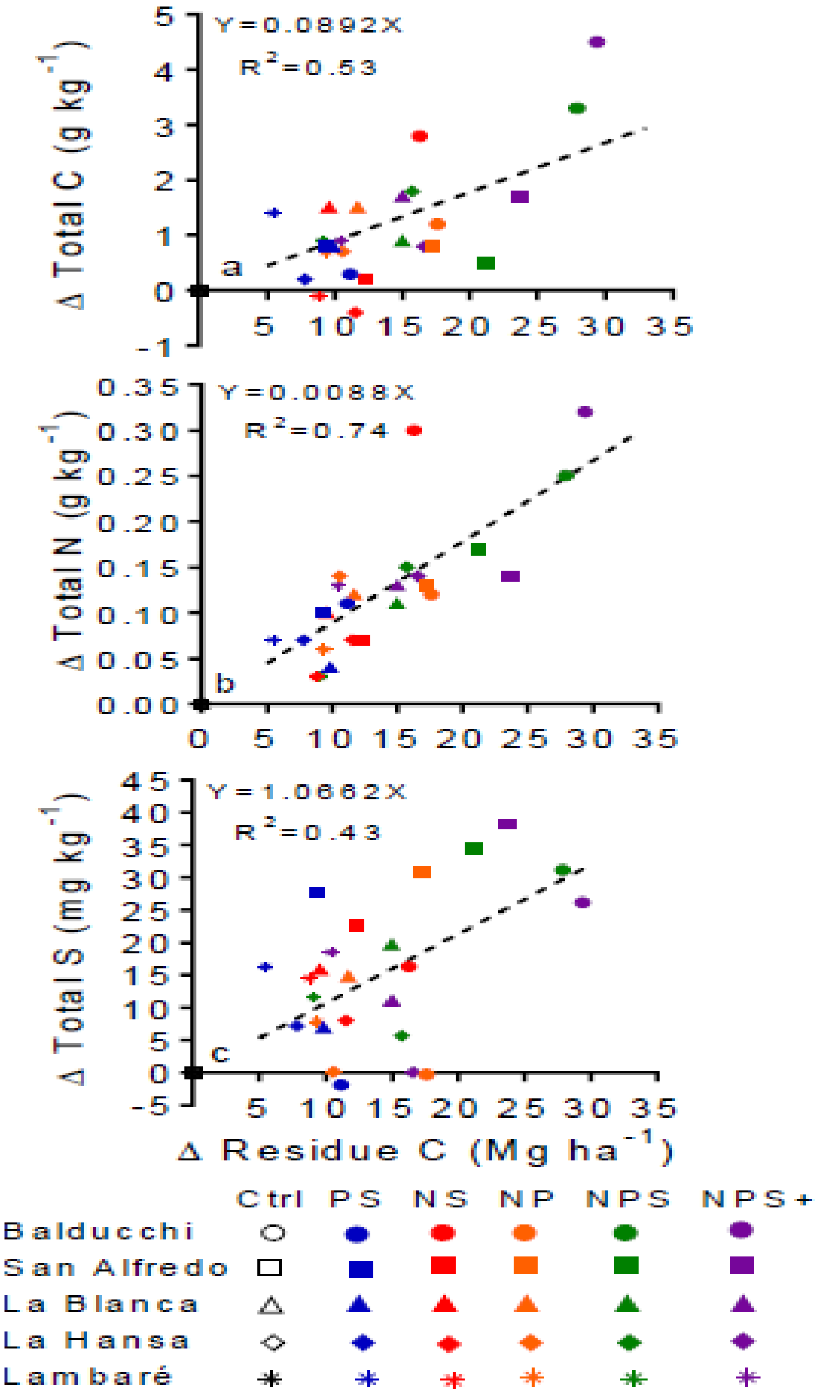

Table 1. Soil classification, location, and main soil properties (0- 20-cm depth) at the beginning of the experimental period (September 2000) for the five experimental sites.

Experimental site	**Balducchi**	**San Alfredo**	**La Blanca**	**La Hansa**	**Lambare**
Soil classification	Typic Hapludoll	Typic Argiudoll	Typic Hapludoll	Aquic Argiudoll	Typic Argiudoll
Location	34º09.461`S; 61º36.465`W	33°51'35.57"S; 61°28'7.84"W	33º29.923 S; 62º37.958`W	32º38.405`S; 61º19.967`W	32º 10.236`S; 61º 48.674`W
Soil series	Santa Isabel	Hughes	La Belgica	Bustinza	Los Cardos
Total organic C (g kg^{-1})	13.5	19.8	13.3	12.2	18.7
C: N ratio	11.6	11.1	10.3	11.6	10.9
pH	5.9	6.0	6.6	5.5	5.6
Ca (cmol kg^{-1})	8.1	11.0	7.2	7.6	9.9
Mg (cmol kg^{-1})	2.0	2.1	2.0	1.6	3.0
K (cmol kg^{-1})	1.4	1.7	1.9	1.7	2.6
Clay (g kg^{-1})	118	180	155	180	205
Silt (g kg^{-1})	531	620	564	789	765
Sand (g kg^{-1})	351	200	281	31	30
Rotation	Bi-annual: maize-wheat/soybean		Tri-annual: maize-soybean-wheat/soybean		

Table 2. ANOVA (F-Statistic and p values) results for the total and labile soil C, N and S fractions, the proportion of labile fractions and the stoichometric ratios. Main factors were: site (Balducchi, San Alfredo, La Blanca, La Hansa, Lambare) fertilization (control, PS, NS, NP, NPS and NPS+) and soil depth (0-5; 5-10; 10-20 cm).

		Total Carbon	Total Nitrogen	Total Sulfur	POM-C	Nan	POM-S	POM-C /total C	Nan/total N	POM-S /total S	C:N	C:S	N:S
Site	F	52.61	61.36	101.91	14.61	211.42	42.69	14.2	82.88	93.34	12.29	8.01	3.51
	p	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**0.02**
Fertilization	F	3.59	6.33	5.21	3.17	2.19	6.32	0.74	10.30	1.95	1.25	1.35	1.60
	p	**0.03**	**<0.01**	**<0.01**	**0.03**	0.09	**0.01**	0.60	**<0.01**	0.13	0.32	0.28	0.20
Soil depth	F	527.04	484.79	199.33	1330.2	2526.6	85.46	595.83	824.41	10.72	2.51	121.7	98.14
	p	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	0.08	**<0.01**	**<0.01**
site x fertilization	F	2.02	0.90	0.81	1.78	0.56	0.47	1.76	0.46	0.35	0.89	1.14	0.65
	p	**<0.01**	0.59	0.69	**0.03**	0.96	0.97	0.06	0.97	0.99	0.59	0.31	0.87
Site x soil depth	F	1.91	3.42	2.15	4.89	7.29	0.21	9.07	12.14	0.75	2.32	0.90	1.64
	p	0.11	**<0.01**	**0.03**	**<0.01**	**<0.01**	0.99	**<0.01**	**<0.01**	0.65	**0.02**	0.51	0.11
fertilization x soil depth	F	0.42	0.80	1.71	1.97	0.77	2.19	1.14	1.68	0.86	0.51	0.66	0.78
	p	0.79	0.63	0.08	**0.04**	0.66	**0.02**	0.33	0.09	0.57	0.88	0.75	0.64
Site x fertilization x soil depth	F	0.42	0.48	0.62	0.95	0.49	0.67	1.15	0.70	0.38	0.74	0.46	0.57
	p	0.99	0.99	0.96	0.57	0.99	0.93	0.26	0.90	0.99	0.87	0.99	0.97

Table 3. Organic carbon, total nitrogen and total sulfur contents at the 0-20 cm soil layer as affected by site and fertilization. Experimental sites

were located in the Northern Pampas (Balducchi, San Alfredo, La Blanca, La Hansa, Lambare). Fertilization treatments were: control, PS, NS, NP, NPS and NPS+.

	Fertilization treatments†												
	Control		PS		NS		NP		NPS		NPS+		Avg‡
Organic C (g kg^{-1} C)	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	
Balducchi	10.9b	0.31	11.2b	0.17	13.7a	1.20	12.1b	0.51	14.2a	1.32	15.4a	1.85	12.9D
San Alfredo	16.9a	0.02	17.7a	0.59	17.1a	0.28	17.7a	0.29	17.4a	0.58	18.6a	0.39	17.6A
La Blanca	13.2a	1.06	14.0a	0.57	14.7a	1.53	14.7a	1.57	14.1a	1.03	14.9a	1.17	14.3C
La Hansa	14.9a	0.72	15.1a	0.39	14.5a	0.44	15.6a	0.36	16.7a	0.53	15.7a	0.28	15.3B
Lambare	15.9a	0.26	17.3a	0.47	15.8a	0.09	16.6a	0.20	16.8a	0.09	16.8a	0.41	16.5A
All Sites §	14.4C		15.1BC		15.2BC		15.3ABC		15.7AB		16.3A		
Total N (g kg^{-1} N)													
Balducchi	0.90	0-05	1.01	0.02	1.20	0.07	1.02	0.05	1.15	0.05	1.22	0.11	1.08C
San Alfredo	1.41	0.04	1.51	0.06	1.48	0.02	1.54	0.01	1.58	0.07	1.55	0.01	1.51A
La Blanca	1.27	0.09	1.31	0.09	1.37	0.10	1.39	0.11	1.38	0.10	1.40	0.09	1.36B
La Hansa	1.26	0.12	1.33	0.09	1.33	0.11	1.40	0.05	1.41	0.09	1.40	0.10	1.35B
Lambare	1.45	0.03	1.52	0.06	1.48	0.04	1.51	0.03	1.48	0.03	1.58	0.02	1.50A
All Sites §	1.26C		1.34B		1.37AB		1.37AB		1.40AB		1.43A		
Total S (mg kg^{-1} S)													
Balducchi	265.9	3.89	264.0	8.38	282.2	1.18	265.6	9.22	297.1	7.75	292.1	6.79	277.8D
San Alfredo	336.9	38.84	364.7	34.91	359.5	45.53	367.8	28.87	371.4	33.03	375.2	41.31	362.6A
La Blanca	323.8	19.80	330.7	6.78	339.6	11.92	338.6	12.19	343.5	5.92	334.9	6.67	335.2B
La Hansa	320.7	5.20	327.9	4.93	328.7	1.97	320.8	4.84	326.4	1.99	320.7	4.96	324.2C
Lambaré	354.4	12.03	370.7	15.69	368.9	6.61	362.2	7.34	366.0	17.51	372.9	16.64	365.9A
All Sites §	320.3B		331.6A		335.8A		330.9A		340.9A		339.2A		

†Values in the same row followed by the same letter are not significantly different at the 5% level according to the LSD test.

‡Values in the column Average were obtained after pooling the fertilization treatments. Values in this column followed by the same letter are not significantly different at the 5% level according to the LSD test.

§ Values in the rows "All Sites" were obtained after pooling the five sites.

Table 4. Carbon and sulfur in particulate organic matter (POM-C and POM-S) and mineralized N under anaerobic incubation (Nan) at the 0-20 cm soil layer as affected by site and fertilization. Experimental sites were located in the Northern Pampas (Balducchi, San Alfredo, La Blanca, La Hansa, Lambare). Fertilization treatments were: control, PS, NS, NP, NPS and NPS+.

	Fertilization treatments†												
	Control		PS		NS		NP		NPS		NPS+		Avg‡
POM C (g kg^{-1} C)	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	
Balducchi	3.2c	0.02	4.0bc	0.29	4.2ab	0.40	4.0b	0.41	4.8ab	0.65	4.9a	0.57	4.2B
San	4.7b	0.16	5.4ab	0.54	5.3ab	0.15	5.9a	0.02	5.9a	0.21	5.6a	0.11	5.5A
Alfredo	3.7ab	0.23	3.9ab	0.25	3.8ab	0.16	3.4b	0.13	3.9ab	0.11	4.3a	0.29	3.8C
La Blanca	3.9a	0.39	4.3a	0.19	3.9a	0.16	4.1a	0.16	4.1a	0.19	4.2a	0.14	4.1BC
La Hansa	4.1a	0.18	4.6a	0.21	4.0a	0.21	4.5a	0.07	4.2a	0.20	4.3a	0.28	4.3B
Lambare													
All Sites§	3.9B		4.4A		4.2AB		4.3A		4.5A		4.6A		
Nan (mg kg^{-1} N)													
Balducchi	28.3	2.13	28.9	0.51	29.1	3.70	24.7	2.21	26.9	3.71	30.2	2.70	28.0D
San	43.4	2.51	45.1	0.26	41.9	1.24	42.8	1.53	43.8	3.20	42.6	0.46	43.3B
Alfredo	42.2	0.48	42.4	1.70	39.4	2.63	39.5	2.60	38.7	1.24	40.3	2.94	40.4C
La Blanca	43.0	1.11	42.8	0.83	41.0	2.30	42.4	1.93	40.3	1.07	40.9	1.68	41.7BC
La Hansa	55.5	3.13	60.2	5.22	57.4	2.64	57.8	3.35	56.6	4.70	58.9	4.85	57.7A
Lambare													
All Sites§	50.0		51.3		49.1		48.1		47.8		49.3		
POM S (mg kg^{-1} S)													
Balducchi	96.3	9.19	96.3	15.46	96.7	13.80	103.9	12.29	107.9	18.55	110.6	12.48	101.9B
San	81.3	2.16	107.3	4.49	110.9	14.49	102.0	2.75	106.1	0.75	93.6	4.57	100.2B
Alfredo	104.3	5.89	114.5	6.52	119.2	16.21	103.3	9.11	111.5	5.99	109.9	4.73	110.4A
La Blanca	69.9	0.73	76.7	3.67	78.8	5.57	72.2	2.48	82.8	7.66	82.8	3.42	77.2C
La Hansa	76.7	4.16	85.1	5.67	82.2	3.87	86.2	3.58	84.6	4.03	85.6	2.30	83.4C
Lambare													
All Sites§	85.7B		95.9A		97.6A		93.5A		98.6A		96.5A		

†Values in the same row followed by the same letter are not significantly different at the 5% level according to the LSD test.

‡ Values in the column Avg (Average) were obtained after pooling the fertilization treatments. Values in this column followed by the same letter are not significantly different at the 5% level according to the LSD test.

§ Values in the rows "All Sites" were obtained after pooling the five sites.

Table 5: Grain yields for each crop (average across sites) as affected by fertilization treatments, and accumulated difference on residue C inputs between fertilized treatments and the control treatment (mean and standard error).

	Fertilization treatments†											
	Control		PS		NS		NP		NPS		NPS+	
Grain yields (kg ha^{-1})	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE
Maize	6928 d	410	8724 c	426	10643 b	408	10689 b	451	11787 a	403	12035 a	400

Wheat	1954 c	154	3056 b	187	3033 b	176	3768 a	222	4016 a	241	4265 a	274
Soybean (full season)	3796 b	205	4386 a	190	4226 ab	196	4250 ab	197	4558 a	181	4572 a	178
Soybean (double crop)	2390 d	150	3017 ab	127	2787 bc	140	2631 cd	144	3140 ab	136	3169 a	137
Δ residue C (Mg ha^{-1})												
All sites	-----		8.7 c	0.9	11.6 b	1.3	13.3 b	1.7	17.7 a	3.1	19.0 a	3.3

† Means in the same row followed by the same letter are not significantly different at the 5% level according to the LSD test.