CROP YIELD, WEED INFESTATION AND SOIL FERTILITY RESPONSES TO CONTRASTED PLOUGHING INTENSITY AND MANURE ADDITIONS IN A MEDITERRANEAN ORGANIC CROP ROTATION

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Abstract

Conservation agriculture and organic farming are two alternative strategies that aim to improve soil quality and fertility in arable cropping systems through reducing tillage intensity, maintaining soil cover and increasing nutrient recycling, using farmyard and green manures. However, these practices can increase weed infestation or decrease nutrient availability. The objectives of this study were to evaluate the effects of tillage type (mouldboard vs. chisel ploughing), fertilization and green manure on soil parameters (SOC, N, bulk density, carbon stocks, and soil microbial biomass C_{mic} and N_{mic}), weed abundance and crop yields in a four-year rotation of spelt, chickpea, winter wheat and lentil in the Mediterranean region (Catalonia, Spain). Tillage and green manure did not affect crop yields or weed biomass, although during the last year of the experiment, plots with mouldboard ploughing had less weed biomass and higher lentil biomass. Fertilization was the most important factor, increasing the cereal yields, SOC, N and soil microbial biomass (C_{mic} and N_{mic}) content of the soil. However, fertilization did not favour chickpea and lentil crops because weed competition limited legume crop growth. Overall, there was a loss of SOC and a reduction of carbon stocks over the four years of the trial in the soil because of the deep soil tillage (25 cm) and low crop productivity irrespective of tillage type. In contrast, N content increased in all of the plots and was enhanced by fertilization. The use of chisel plough stratified the distribution of SOC and N in the surface layers (0-10 cm). Both C_{mic} and C_{mic}/SOC ratio increased in fertilized treatments, suggesting an increased lability of SOC. The application of more stabilized organic matter may be a better practice to build up soil organic matter and to maintain crop yields in organic farming systems.

Keywords: chisel plough; carbon stock; amendments; microbial biomass; cover crop

1. INTRODUCTION

Soils play a key role in agricultural systems because they represent the basis of food production (Fließbach et al., 2007). However, most arable soils are prone to degradation, mainly caused by intensive soil use (Gadermaier et al., 2012). Crop rotation, cover crops and reduced or no tillage practices aim to improve soil quality in arable cropping systems. Farmyard manure and green manure (organic fertilizers) can also contribute to soil fertility and quality. While most of these practices are used in organic farming cropping systems, the adoption of reduced tillage practices is not widespread in such systems (Gadermaier et al., 2012). The increase of weed infestation and the limited availability of N mainly at the beginning of the growing season are probably the main problems that reduced tillage pose to organic farmers (Gadermaier et al., 2012; Peigné et al., 2007; Sans et al., 2011). On the other hand, reduced tillage is highly suited to conserve soil fertility and prevent erosion (Berner et al., 2008; Gadermaier et al., 2012) by enhancing soil organic carbon (SOC) content, microbial activity and soil structure (Mäder and Berner, 2012; Peigné et al., 2013).

Cover crops can also contribute to the accumulation of organic matter in the upper soil layer and they can reduce weed infestation (Hobbs et al., 2008; Masilionyte et al., 2017). However, the use of cover crops must consider the possible consequences of competition for nutrients and water with cash crops (Plaza-Bonilla et al., 2017).

Crop production in organic farms is often limited by the lack of nitrogen. In such farms nitrogen inputs are needed to restore the amount of N depleted by crops (Fließbach et al., 2007). The use of organic fertilizers, in one hand, is an effective way to increase soil organic matter content (Alvarez, 2005) and N availability (Krauss et al., 2010; Lal, 2009; Maltas et al., 2013). On the other hand, suitable crop rotations containing legumes are fundamental to produce surpluses in N budgets (Gadermaier et al., 2012). However, the residue from a cover crop rich in legume species is often mineralised very fast, and nutrients can be released before the demands of the subsequent cash crop (Pang and Letey, 2000) and thus be lost or used by weeds. Therefore, the use of cover crops for supplying N to crops must be adapted to the reduced tillage systems (Peigné et al., 2007). In consequence, it is considered of great interest to gain knowledge on the N dynamics after the introduction of green manures and reduced tillage practices in organic arable cropping systems.

Links between C and N cycling are important to understand N supply in arable systems. The application of organic manures, and reducing tillage intensity can increase the SOC in topsoil, improve soil physical and biological properties and lead to reduced carbon losses or even to increased soil carbon storage in the soil (Cooper et al., 2016; Gattinger et al., 2012). In addition, soil microbiological activity is of primary importance in organic farming because N supply is mainly dependent on the degradation of soil organic matter by soil micro-organisms (Vian et al., 2009). In this case, and because of their high sensitivity, C and N in soil microbial biomass can be used as indicators of changes in soil owing to management in the short term (Fließbach et al., 2007).

Few experiments integrate reduced tillage into organic farming systems, and most of them are performed in temperate climates (Berner et al., 2008; Krauss et al., 2010; Peigné et al., 2007; Pekrun et al., 2003). So far, in Mediterranean climates reduced tillage practices have been studied only in conventional systems (Kassam et al., 2012; López-Garrido et al., 2014; Ward et al., 2012), and thus there is a lack of long term reduced tillage studies in organic systems. The low organic matter content with poor soil structure of the Mediterranean arable soils and the climatic constraints that limit plant growth during summer may constrain the chances to improve soil quality by means of reduced tillage and green manures (Kassam et al., 2012; Romanyà and Rovira, 2011; Hernanz et al, 2009).

Our aims were to study the effects of reduced tillage, farmyard manure and green manure (cover crop) on crop yields, weed abundance and soil organic C stocks and N availability. To address these aims we set in 2011 a mid-term experiment that was monitored during a four-year rotation of spelt (*Triticum spelta* L., 2011-12), chickpea (*Cicer arietinum* L., spring 2013), winter wheat (*Triticum aestivum* L., 2013-14) and lentil (*Lens culinaris* Medik., spring 2015).

We hypothesized that a) the lower disturbance of the soil profile by reduced tillage plus the addition of farmyard and green manures contribute to an increase, or at least maintain SOC and N stocks. These changes, combined with the increased stability of the soil system, b) will increase microbial biomass and N availability; and c) will allow a sustainable crop performance in reduced tillage organic crops.

2. MATERIALS AND METHODS

2.1. Site conditions

In November of 2011, a midterm field experiment was initiated in Gallecs (41°33'31.9"N 2°11'59.5"E), a peri-urban agricultural area of 753 ha situated 15 km north of Barcelona (Catalonia, Spain). Gallecs has a Mediterranean climate; the mean annual temperature and precipitation are 14.9 °C and 647 mm, respectively. At the beginning of the experiment, the soil properties of the field were evaluated. On average, the mineral fraction consisted of 43.3 ± 6.9 % sand, 26.9 ± 4.7 % loam and 29.7 ± 3.7 % clay; the texture was classified as loamy-clay (Soil Survey Staff, 1998); the soil type was Haplic Luvisol (IUSS Working Group WRB, 2015); the average soil organic matter was 1.5 ± 0.1 % (Walkley-Black); and the pH (H_2O) was 8.1 ± 0.1.

2.2. Field experiment

The trial consisted of a four-year crop rotation in a strip-split-block design of three factors (with two levels each): tillage system (mouldboard ploughing (P) vs. chisel (C)), fertilization (composted farmyard manure (+F) vs. no fertilizer (-F)) and green manure (with green manure

(+G) vs. no green manure (-G)). The factors were arranged with tillage treatments laid out in strips; fertilization was applied in perpendicular strips across the experiment, and the tillage strips were split into subplots for the green manure treatment. In total, 32 plots measuring 13 m × 12 m were established, comprising four replicates of each treatment (Figure 1). The field had been under organic management for five years prior to the trial establishment, with a typical dryland Mediterranean crop rotation that alternated winter cereals and legumes in spring for human consumption. The crop rotation of this trial consisted of spelt (2011–2012), chickpea (2013), winter wheat (2013-2014) and lentil (2015) (Figure 2).

Two tillage systems were used: a mouldboard plough (P) (soil inversion at 25 cm depth) plus a rotary harrow (5 cm depth), and a chisel plough (C) (no soil inversion at 25 cm depth) plus a rotary harrow (same as for the mouldboard plough). The fertilization treatment (+F) consisted of partially composted farmyard manure, composed of cattle manure and plant residues, obtained without managing and controlling the process, by gradually accumulating the material that was seasonally available, according to the normal practice used in the area. In consequence, the composted manure had a variable composition. The manure was applied every year before sowing the main crop. The total amount of manure applied each year differed in relation to the nutrient availability in the fertilizer and the nutritional demands of each crop (Table 1). The organic fertilizers were mixed in the soil by means of a chisel or mouldboard plough in accordance with the tillage treatment. In September 2012 and 2014, cover crops (+G) were sown in the corresponding 16 plots, consisting of a mixture of oat (*Avena sativa* L.), white mustard (*Sinapis alba* L.*)*, bitter vetch (*Vicia ervilia* (L.) Willd.) and common vetch (*Vicia sativa* L.) (Table 1). At the end of March of the following year, cover crops (as well as the weeds developing in –G treatment) were incorporated into the soil as green manure by disc harrowing.

Weeds were not controlled during the first year of the crop rotation due to an extremely prolonged rainy period that prevented the mechanical post-emergence weeding. In the second year of the rotation, weeds were controlled with an inter-row cultivator adapted to pass between the seeding rows of chickpea. The third year of the rotation, weeds were controlled with a flex-tine harrow during the wheat crop season. Finally, the last year of the rotation, lentil was established poorly because of drought and was outcompeted by weeds despite the manual removal of lamb's quarters individuals (*Chenopodium album* L.), which was the most important weed during the lentils' growth (Table 1).

2.3. Weed and crop assessment

Crop density was evaluated every year once the crop plants were well-established. The individuals were counted in a sample 0.5 m long, comprising two crop lines in four replicates in each plot.

Before crop harvest, four permanent square frames of 1 m^2 were randomly established, one in each quarter of the plot, to assess weed and crop aboveground biomass. The total aboveground biomass of weeds and crop was harvested in each frame and oven-dried at 60 °C for 48 h. The aboveground biomass of green manure and weeds was also evaluated during the green manure period. Grain crop yield was assessed in the inner 9 m × 8 m of each plot by a plot combine each year (except for lentils). The straw of the crops was not removed from the field and was incorporated with the stubble into the soil by disc harrowing at 10 cm deep. The spelt straw was chopped by a hammer straw chopper before being incorporated

2.4. Soil sampling and analyses of SOC, N, bulk density and carbon stocks

In November 2011 and 2015, the soil was studied at four depths: from 0 to 10 cm, from 10 to 20 cm, from 20 to 30 cm and from 30 to 40 cm. The first two depths were sampled in all of the plots, whereas the two deepest soil layers were sampled only in plots with farmyard manure and green manure with mouldboard ploughing and with chisel ploughing (P +F + GM and C + F + GM). To study soil bulk density, 3 soil cores of 6.2 cm diameter and 10 cm deep were extracted in each soil layer at each plot. Soil samples were oven-dried at 90-100 °C for 48 h. Soil bulk density was calculated according to the following formula: Bulk density (g cm^{-3}) = dry soil weight (g) / core volume (cm^3).

To study total soil organic carbon (SOC) and total nitrogen content (N), 20 soil cores of 2.5 cm of diameter were systematically extracted every 2 meters of distance in each plot. Each set of 20 cores extracted at each plot and depth constituted a sample. Soil samples were kept in plastic bags, properly labelled, in a fridge at 4 °C until analysis. Samples were air dried and sieved on a 2 mm mesh. A minimum amount of 50 g dried soil was prepared for SOC and N analysis, and the rest was separated for the soil microbial analyses (see below section 2.5). Total carbon and total nitrogen were analysed through dry combustion with a LECO© Truspec CHNS analyser (Bremner, 1996). The Walkley-Black procedure/ISO 14235 was finally chosen to indirectly estimate the soil organic carbon (SOC) due to the high proportion of carbonates.

Based on the soil bulk density and SOC, carbon stocks were calculated according to the following formula (Lee et al., 2009): Soil carbon stock (g m^{-2}) = soil carbon content (mg g^{-1}) × depth of soil layer (m) × area (m^2) × bulk density (g cm^{-3}) × 10^6.

2.5. Soil microbial biomass analyses

All of the soil microbial analyses were carried out on moist soil samples adjusted to a water content corresponding to 40–50 % of maximum water retention capacity. The soil microbial biomass (C_{mic} and N_{mic}) was estimated using chloroform fumigation extraction (CFE) following Vance et al. (1987). CFE was done in triplicate on 20 g (dry matter) subsamples that were extracted with 80 ml of a 0.5 M K_2SO_4 solution. Total organic carbon (SOC) in soil extracts was determined by infrared spectrometry after combustion at 850°C. Total nitrogen (N) was measured subsequently in the same sample by chemoluminescence. The soil microbial biomass was then calculated according to the formula: C_{mic} (µg g^{-1} oven dry soil) = EC/k_{EC}, where EC = (SOC in fumigated samples - SOC in control samples) and $k_{EC} = 0.45$ (Joergensen, 1996). N_{mic}

(μg g^{-1} oven dry soil) = EN/k_{EN}, where EN = (N extracted from fumigated samples – N extracted from control samples) and k_{EN} = 0.40 (Joergensen and Mueller, 1996).

2.6. Statistical analyses

The individual and combined effects of the type of tillage (P vs. C), fertilization (+F vs. -F) and green manure (+G vs. -G) on crop yields (spelt, chickpea, winter wheat), lentil aboveground biomass and weed aboveground biomass were evaluated using linear mixed effects models. For spelt crop, the factor of green manure was not analysed because the green manure crop was implemented after it. The weed biomass was introduced in the models as a covariate to evaluate the effect of weeds on grain yields (or crop biomass, when yield was not available). Tillage, fertilization and green manure were used as fixed factors, and the block was introduced as a random factor. The normality of residuals was verified using the Shapiro-Wilk test, and homoscedasticity was assessed using Bartlett's test. To meet the normality and homoscedasticity requirements, we used logarithmic or square root transformation on the data when necessary. The same statistical procedure was followed to analyse the effects of tillage, fertilization, green manure and depth of the soil layers and the interaction between the factors on the following soil parameters: SOC, N, soil bulk density, carbon stocks, and soil microbial biomass (C_{mic} and N_{mic}). The changes in soil quality indicators over the 4-year rotation were also studied, comparing soil samplings carried out twice during the experiment ($\Delta = t_f - t_i$). The first analysis was performed at the beginning of the trial, representing the initial status of the soil (t_i), and the second analysis was performed at the end of the experiment (t_f). All the analyses were performed in R version 3.2.2 (R Development Core Team, 2015) using the package lme4 (Bates et al., 2015) for linear mixed effects model fitting.

3. RESULTS

3.1. Crop yields and weed biomass

No differences in the density (individuals/m^2) of the established crops were found between treatments in the first two years (spelt and chickpea), although the establishment of winter wheat and lentil differed according to the type of tillage and the presence or not of green manure the previous year (wheat) or months (lentil). Wheat establishment was significantly higher in plots with mouldboard ploughing and no green manure compared to chisel (T (P vs. C) × G (+G vs. -G): $p = 0.009$). More plants of lentil emerged in plots with no green manure in general, and in plots with green manure, crop emergence was significantly higher in plots with mouldboard ploughing ((T (P vs. C) × G (+G vs. -G): $p = 0.04$).

The winter wheat crop had the highest yields (3200 ± 280.08 kg ha^{-1}), followed by spelt (2328 ± 100.51 kg ha^{-1}) and chickpea (384 ± 65.38 kg ha^{-1}). Lentil did not produce grain because extended drought dramatically affected both flowering and fruiting. Cereal yields were significantly higher in plots with fertilization; both the spelt and winter wheat yields were higher in plots with farmyard manure (Table 2 and Figure 3). Legumes did not follow the same trend; the chickpea yield and lentil biomass did not vary in relation to fertilization. Regarding the effects of the type of tillage and the incorporation of cover crops as green manure, crop yields did not vary significantly, with the exception of lentil biomass. The lentil biomass was significantly higher in plots that underwent mouldboard ploughing (Table 2 and Figure 3).

The effect of tillage on aboveground weed biomass varied over time. Although no significant differences were found in the first two crops in the rotation, the aboveground weed biomass was significantly lower in plots tilled with mouldboard ploughing than in plots tilled with chisel ploughing during wheat and lentil crop. The incorporation of the cover crop as green manure did not affect weed biomass during subsequent crops of chickpea (in the same year) and winter wheat (in the following year). However, in the fourth year (during the lentil crop), weed biomass was significantly higher in plots in which cover crops had been incorporated into the soil prior to lentil seeding. No statistically significant interaction between factors were found, with the exception of a significant lower weed biomass in plots with fertilization and mouldboard ploughing in the spelt crop (Table 2).

The results showed that the weed biomass did not affect spelt and winter wheat grain yield (slope for the effect of weed biomass on spelt yield: 1.60 ± 4.17, $p = 0.7$ and slope for the effect of weed biomass on winter wheat yield: -6.54 ± 26.99, $p = 0.8$). In contrast, chickpea yield and lentil biomass correlated negatively with weed biomass (slope for the effect of weed biomass on chickpea yield: $p < 0.001$ and slope for the effect of weed biomass on lentil biomass: $p = 0.003$).

Green manure biomass did not differ between treatments in 2013 or 2015. The analysis of the effect of the green manure on weed abundance and on the crop yield of the subsequent crop demonstrates that cover crop was effective in controlling weeds during its growing season but not the following year. The effect of green manure on the control of weed biomass was statistically significant (+G vs. -G: $p < 0.001$ in 2013 and 2015).

3.2. Changes in SOC and N during the four years of the experiment

Overall, SOC decreased significantly (t_f vs. t_i: $p < 0.001$) in all of the treatments over the 4-year rotation of the experiment, with the exception of the soil layer between 0 to 10 cm deep in plots with chisel plough and fertilization. In contrast, N content increased across all the treatments (t_f vs. t_i: $p < 0.001$) (Table 3 and Figure 4). The highest SOC losses occurred at superficial soil layers (0 to 10 cm) of plots without fertilization. SOC decreases were significantly higher at deeper soil layers (10 to 20 cm) of plots with chisel plough (C) than of plots with soil layers inversion using mouldboard ploughing (P) (Table 4). Although no significant interaction was found between the type of tillage and fertilization, our results showed that SOC content at 0 to 10 cm was maintained over the 4-year rotation in plots with chisel and fertilization (Table 3 and Figure 4).

Regarding the changes in N content, the highest increases occurred in plots with fertilization (Table 3). The type of tillage also affected ΔN; plots with chisel ploughing had higher increase than plots with mouldboard ploughing (Table 4 and Figure 4). However, this significant increase in N_t content occurred at the top soil layer of plots with chisel and fertilization, as indicated by the significant interaction between fertilization, tillage and soil layer (Table 4). Green manure did not show any effect. No significant differences were found in ΔSOC and ΔN_{tot} over the 4-year rotation of the trial according to the presence of green manure.

Overall, the C:N ratio of the soil decreased by 32 % after the four years (t_f vs. t_i: $p < 0.001$), and there was a significant interaction between tillage and fertilization, indicating a higher C:N ratio in plots with fertilization and reduced tillage compared to plots with mouldboard ploughing, irrespective of the soil layer (Table 4).

3.3. Bulk density and carbon stocks after four years of reduced tillage and organic inputs

After four years of the experiment, soil bulk density did not vary significantly in relation to the different experimental factors. Deeper soil layers had a higher bulk density than surface layers, but this pattern was not associated with the type of tillage or the organic fertilizer inputs, such as composted farmyard manure and green manure (Table 5).

Carbon stocks, assessed from the SOC content and the soil bulk density of soil samples in different soil layers, were significantly higher in plots fertilized with composted farmyard manure and were higher at deeper soil layers from 10 to 20 cm (Table 5), although this is mainly associated with higher bulk density. Furthermore, there was a significant interaction with the type of tillage and green manure; higher carbon stocks were detected in plots with chisel and green manure. The effect of the treatments at different soil layers showed some significant results as well. Carbon stocks were higher at deeper soil layers in plots with fertilization, and the plots with mouldboard ploughing presented lower carbon stocks at superficial soil layers (Table 5).

The diachronic analyses of carbon stocks over the 4-year rotation at four different soil layers (0 to 10 cm, 10 to 20 cm, 20 to 30 cm and 30 to 40 cm) in relation to the tillage (P +F + GM and C + F + GM) indicate that carbon stocks were significantly lower in deeper soil layers (soil layer 20 to 30 cm vs. superficial soil layers: $p < 0.001$; and 30 to 40 cm vs. superficial soil layers: $p < 0.001$). Overall, carbon stocks decreased after four years, irrespective of the soil layer ($p = 0.01$), and the negative effect of soil layer inversion using mouldboard ploughing was only statistically significant in the two upper soil layers (0 to 10 and 10 to 20 cm, $p < 0.001$).

3.4. Changes in soil microbial biomass

Soil microbial biomass (assessed as the C_{mic} and N_{mic}) was significantly higher in plots with farmyard manure (Table 6 and 7). Furthermore, soil microbial biomass was lower at deeper soil layers, and the significant interaction with fertilization reflects differences in C_{mic} and N_{mic} in fertilized and unfertilized plots (Table 7). Superficial soil layers showed greater differences between fertilized and plots without fertilization in C_{mic} and $N_{mic,}$ compared to soil layers at 10 to 20 cm. Plots with mouldboard ploughing showed similar C_{mic} at 0-10 cm depth and at 10 to

20 cm depth; conversely, plots with chisel ploughing showed significantly higher microbial biomass at superficial soil layers compared to the deeper soil layers (Table 6 and Figure 5). The highest C_{mic} was observed in superficial layers (0 to 10 cm) in plots with farmyard manure and chisel ploughing (Figure 5). N_{mic} did not vary significantly between soil layers in interaction with tillage, and C_{mic} and N_{mic} were not significantly affected by the presence or absence of green manure (Table 6 and 7).

The comparison of C_{mic} and N_{mic} between superficial and deeper soil layers in relation to the tillage (plots P +F + GM vs. plots C + F + GM) indicate that both C_{mic} and N_{mic} were decreased in deeper soil layers (20 to 30 cm C_{mic}: $p<0.001$, N_{mic}: $p<0.001$ and 30 to 40 cm C_{mic}: $p<0.001$, N_{mic}: $p<0.001$), although no significant differences were found in relation to tillage (data not shown).

The differences in C_{mic} and N_{mic} between the first and last year of the trial (ΔC_{mic} and ΔN_{mic}) did not vary in relation to the individual factors (tillage, fertilization and green manure). C_{mic} increased overall after the four years of the trial (t_f vs. t_i: $p<0.001$). Significant interactions were found between fertilization, tillage and soil depth, indicating higher increases of C_{mic} in plots with chisel plough (T: P vs. C: $p<0.001$) and fertilization (F: + vs. -: $p<0.001$) at 0 to 10 cm and decreases in the 10 to 20 cm layer (depth 10 to 20 vs. 0 to 10 cm: $p<0.001$). In contrast, plots with mouldboard ploughing did not show significant changes in C_{mic} at different soil depths. The N_{mic} decreased, in general, in all the plots after the four years of the trial (t_f vs. t_i: $p<0.001$), but the highest losses of N_{mic} were at superficial soil layers (depth 10 to 20 vs. 0 to 10 cm: $p<0.001$). Additionally, there was a significant interaction between the year and the type of tillage, indicating lower N_{mic} values in the last year in plots with chisel (T: P vs. C: $p = 0.02$); this was associated with the superficial layers, although no significant interactions were found (data not shown).

The C_{mic}/SOC ratio increased in all of the plots after the four years of the experiment (t_f vs. t_i: $p<0.001$). Furthermore, the C_{mic}/SOC after the four years of the experiment varied significantly with soil depth, and this factor also interacted significantly with the fertilization and the type of tillage (Table 7). We found the highest ratio at the superficial layers in plots with chisel plough and no fertilization compared to plots with chisel plough and no fertilization at deeper soil layers (Table 7 and Figure 5).

4. DISCUSSION

4.1. Crop yields and weed biomass

Our study reveals that fertilization is the most important factor affecting crop yields, particularly during the cereal cropping period. Organic systems rely upon the use of organic fertilizers and amendments that typically release nutrients (especially N) at a slower rate compared with mineral fertilizers. Nitrogen inputs are critical to the productivity of these systems, and the application of farmyard manure seems to be effective to maintain cereal yields (Fließbach et al., 2007; Maltas et al., 2013). Conversely, the grain yield of chickpea and aboveground biomass of lentil were not increased by fertilization. In general, legumes do not need supplemental N fertilization (Clayton et al., 2003) because they can obtain a significant proportion of its N by symbiotic nitrogen fixation (Walley et al., 2005).

The type of tillage had no significant effects on grain yields of cereals (spelt and winter wheat) and chickpea. Other studies under Mediterranean conditions obtained similar results (López-Garrido et al. 2014). However, many studies from temperate regions reveal lower crop yields in systems with no soil layer inversion by chiselling (Cooper et al., 2016) because of a combination of a shortage of nutrients and competition from weeds (Mäder and Berner, 2012; Peigné et al., 2013). Indeed, the lower biomass of lentil in plots with reduced tillage can be explained by the higher weed biomass under these conditions.

The positive effect of fertilization and mouldboard ploughing in controlling weeds in spelt and winter wheat highlights the importance of both factors in enhancing the competitive ability of crops. Weed abundance did not affect significantly spelt and winter wheat grain yield, indicating that the crop was able to suppress the growth of weeds to a point where their effect on crop growth was negligible. In contrast, the effect of weed biomass on chickpea yields and lentil crop biomass was statistically significant, indicating a strong negative correlation between weeds and legume crops. The growth of weeds was significantly enhanced by fertilization during legume crops and, consequently, they significantly reduced the growth of chickpea and lentil. Some studies indicated that lentil is very vulnerable to weed competition because of its short stature, slow establishment, and limited vegetative growth (Ahmadi et al., 2016). The high amount of weed biomass in chickpea and lentil irrespective of the treatment can be related to the inadequate post-emergence weed control. Our results indicated that mouldboard ploughing increased weed control and consequently lentil crop biomass. Therefore, improving weed management in legume crops is critical to their feasibility in organic farming because of the high susceptibility of such crops to weed competition.

Although we expected a negative or neutral effect of green manures on weed abundance, green manure increased weed abundance during the subsequent lentil crop. The extremely weak growth of lentil as a result of drought may have reduced the competitive ability of the crop and promoted weed growth. These results call for a careful evaluation of the insertion of cover crops in Mediterranean crop rotations (Plaza-Bonilla et al. 2017).

4.2. Changes in SOC, carbon stocks and N change during the experiment?

The amount of SOC stored in the soil is determined by the balance production of organic matter by plants and decomposition of organic matter by soil organisms. Each of these processes is controlled by physical, chemical, and biological factors (Guo and Gifford, 2002). In organic arable cropping systems, the intensity of soil disturbance, the farmyard manure and green manure fertilization are overriding factors that determine the amounts of SOC and N and their pattern of distribution in the soil profile (Gattinger et al., 2012). Some authors have indicated that SOC is enhanced by reduced tillage practices after several years (Mäder and Berner, 2012; Peigné et al., 2013). However, other studies were unable to demonstrate such a positive effect (Berner et al., 2008). Our study shows losses of SOC irrespective of the ploughing intensity, indicating that Mediterranean low input farming systems may reduce SOC content and consequently soil carbon stocks. It is interesting to note that such losses were lower in plots with chisel than in plots with mouldboard ploughing. Similar to other studies, reduced tillage stratified SOC and consequently soil carbon stocks and microbial biomass concentrated in the upper layers especially in fertilized plots (Berner et al., 2008, Cooper et al., 2016, Gadermaier et al., 2012). It is worthy to highlight that soil inversion by mouldboard ploughing reduces carbon stocks in the topsoil mainly in unfertilized soils while it increases SOC at deeper layers.

As the SOC losses also occurred in reduced tillage treatments, they must relate to other aspects of organic farming practices. Crop productivity is one of the main drivers of carbon stocks in arable systems. Both carbon stocks and crop productivity may be enhanced by crop fertilization practices (Johnston et al., 2009). The cereal grain yield even in our fertilized plots was less than half that of conventional systems in the region (Ministerio de Agricultura y Pesca, Alimentación y Medio Ambiente, 2009), indicating a low plant productivity in comparison to neighbouring cereal monocultures. This may explain SOC losses throughout the soil profile. Moreover, low productivity of legume crops as compared to cereals may further contribute to decrease soil organic matter in such crop rotations. These SOC loses may be partly compensated by the addition of farmyard manure. In these experimental systems, organic fertilization was crucial to maintain SOC level and to enhance cereal crop productivity, but it reduced productivity in legume crops. This suggests in one hand nutrient limitation for cereal productivity and on the other hand a negative effect of organic fertilization in rotations including legumes. As the use of fresh, unstabilized materials may induce the mineralization of native SOC stocks (Molina-Herrera and Romanyà, 2015; Romanyà et al., 2012), the addition of more stabilized composted organic materials may have contributed to building up SOC stocks.

There is a broad support in the literature for the positive effects of cover crops on SOC (Poeplau and Don, 2015). However, our results suggest that the incorporation of crop residues with low C:N ratio, such as those from legumes, into the soil can accelerate SOC decomposition, although other studies show positive responses on SOC levels in response to legumes (Beedy et al., 2010). The lack of response of green manure in our experiment may have been due to the general low plant productivity in the experimental area or lack of effect over short time-spans (Biederbeck et al., 1998).

N content was clearly enhanced after the 4-year rotation in all the plots. Our results show that N increase was significantly higher with the incorporation of farmyard manure, and the highest increase occurred in plots with reduced tillage. Increased N levels after adding manures have also been reported by other authors (Krauss et al., 2010; Maltas et al., 2013). In contrast, we did not find any significant effect of the incorporation of green manure on the increase of the N, indicating that the effect of applying farmyard manure was more important. Slight increases of N amount in unfertilized plots can be attributed to N fixation or to atmospheric deposition (Pang and Letey, 2000), which in the area may be as high a 15-22 kg ha^{-1} $year^{-1}$ (Vallejo et al., 2005). In our experimental site in Gallecs this value can be especially high because of its proximity to urban areas (highways, industry, etc.) (Ochoa-Hueso et al., 2011).

4.3. Changes in soil microbial biomass and N availability

Reduced tillage caused a stratification of soil microbial biomass in the soil profile, which parallels total SOC content. In agreement with previous studies (Vian et al., 2009), our results on microbial biomass (C_{mic} and N_{mic}) indicated that shifting from conventional to reduced tillage modifies crop residue distribution in the soil profile and environmental conditions for soil micro-organisms. Increased organic residues in top layers may go along with a decrease in the turnover rate of the SOC that may increase N immobilization and produce N shortages for crops (Pekrun et al., 2003; Vian et al., 2009).

In our experiment, the increase of C_{mic} after four years of trial can be mainly explained by the addition of manures. The addition of labile sources of SOC promotes soil microbial activity and consequently an increase of microbial biomass (Molina-Herrera and Romanyà, 2015; Fließbach and Mäder, 2000). Reduced tillage has also been found to increase microbial biomass in surface soils, although its effects have been found to be much stronger when combined with fertilization. These increases, however, have not been related to increases in N availability. Indeed, some studies in temperate climates reported a decrease of N availability for the crop under reduced tillage due to lower mineralization rates (Berner et al., 2008; Peigné et al., 2007).

The decrease of N_{mic} that ocurred in all treatments after four years of trial coincided with a 32 % decrease in the C:N ratio (see section 3.2). A low C:N ratio indicates an increased degree of humification (Bayer et al., 2002). Humified organic matter strongly holds N in highly recalcitrant forms and is thus unavailable to soil microbiota. In our experiment, low N availability was indicated by the decreased N_{mic}. N mobilization in such soils may involve destabilization of soil organic matter and its subsequent mineralization (Clarholm et al., 2015). This may coincide with increases in fungi and Gram (+) bacteria as has been reported by other authors studying organically managed minimum tillage farming systems (Sun et al., 2016).

The C_{mic}/SOC ratio can indicate the soil microbial efficiency of conversion of organic matter to microbial biomass and the stabilization of SOC by the soil mineral fractions (Sparling, 1992). In our mid-term trial, the loss of SOC in all the plots coincides with the increase of the C_{mic}/SOC ratio, which indicates that the microorganisms are integrating a greater proportion of soil organic matter.

5. CONCLUSIONS

Farmyard manure is the main factor affecting crop yields and weed biomass, as well as soil fertility and quality. Organic fertilization is crucial to sustain cereal yields, but can also exert a negative effect on legume crops by increasing the competitive effects of weeds. Although farmers are concerned that reduced tillage could reduce the already low crop yields under organic farming by increasing weed pressure and delaying nutrient mineralization, we have found that the concerns are unfounded. The tillage system does not have a consistent negative effect on yields, and the increased weed control of mouldboard plough only occurs in the mid- to long-term. The implementation of green manure in dryland areas requires a careful redesign of the cropping system. Although applying green manure could alleviate some fertility and weed control issues, we have not found positive effects on crop yields.

In the Mediterranean region of Spain, soils have low N availability and the organic fertilization might not be enough to maintain SOC content. Future research should explore the effects of applying more stabilized organic matter, which may be a better practice for the enhancement of soil quality and the build-up of soil organic matter in the soil.

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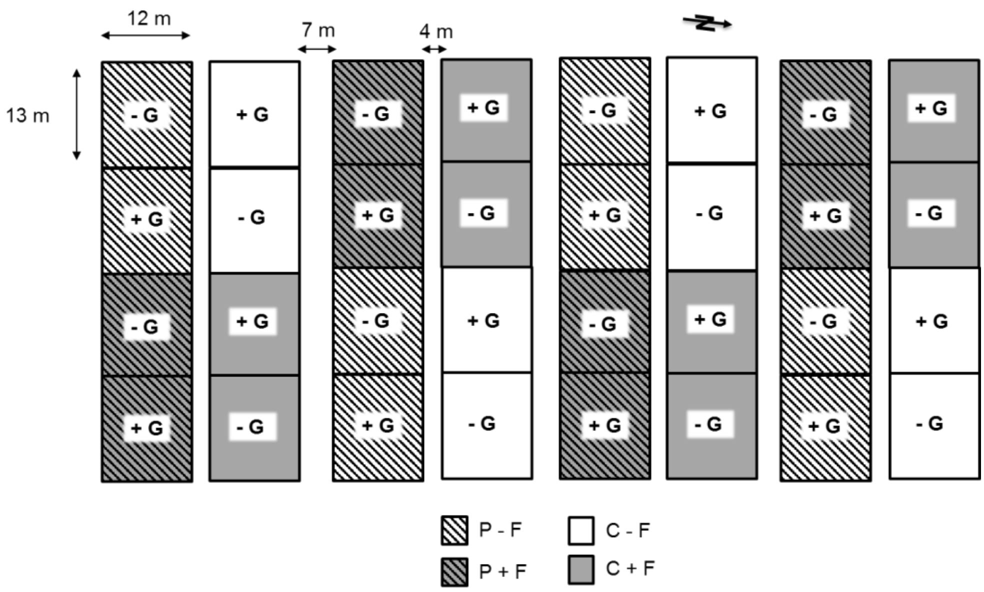

Figure 1. Experimental design in a strip-split-block with three factors of two levels each. P, mouldboard ploughing; C, chisel ploughing; + F, fertilization with farmyard manure, - F, not fertilized: + G, with green manure, - G, no green manure. Each treatment is replicated four times, totalling 32 plots.

Table 1. Date of field operations, sowing characteristics, and fertilization inputs for each crop of the rotation. The type and brand of agricultural equipment are also indicated.

Year of rotation	1st	2nd	3rd	4th
	Spelt	Chickpea	Winter wheat	Lentil
Tillage				
Conventional tillage				
Mouldboard ploughing, depth 25 cm EG 85-240-8, Kverneland	December 12th, 2011	March 28th, 2013	December 10th, 2013	March 20th, 2015
Rotative, depth 5 cm HR3003D, Kuhn	December 14th, 2011	April 13th, 2013	December 16th, 2013	March 30th, 2015
Reduced tillage				
Chisel, depth 25 cm KCCC 1187 - A00, Kverneland	December 14th, 2011	March 28th, 2013	November 12th, 2013	March 20th, 2015
Rotative, depth 5 cm HR3003D, Kuhn	December 14th, 2011	April 13th, 2013	December 16th, 2013	March 30th, 2015
Fertilization				
Composted cow farmyard manure	December 12th, 2011	March 28th, 2013	November 12th, 2013	March 19th, 2015
tn ha^{-1}	21.5	22	38	22
N_t kg ha^{-1}	134.60	40.04	138.28	62.36
Weed control	No control	May 30th, 2013	March 4th, 2014	June 2nd, 2015
Machinery for weed control		Inter-row cultivator	Flex-tine harrow Herse-6M, PICHON	Hand weeding
Sowing Amazone D09- 30	Spelt	Chickpea	Winter wheat	Lentil
Date of sowing	December 14th, 2011	April 13th, 2013	December 16th, 2013	March 31st, 2015
Sowing density	195 kg ha^{-1}	30 kg ha^{-1}	220 kg ha^{-1}	180 kg ha^{-1}
Spacing between rows	12 cm	75 cm	12 cm	12 cm
Harvest Plot combine Elite, Wintersteiger, Inc. Deuthz fhar	July, 12th 2012	July 31st, 2013	August 12th, 2014	-
Disc harrowing Norma RLBH 32, RAU	September 18th, 2012	October 26th, 2013	September 9th, 2014	September 20th, 2015
Green manure	October 17th, 2012	-	September 22th, 2014	-
Sowing density kg ha^{-1}	45.8 oat 1.5 mustard 61 bitter vetch 39.7 common vetch	-	45.8 oat 1.5 mustard 61 bitter vetch 39.7 common vetch	-

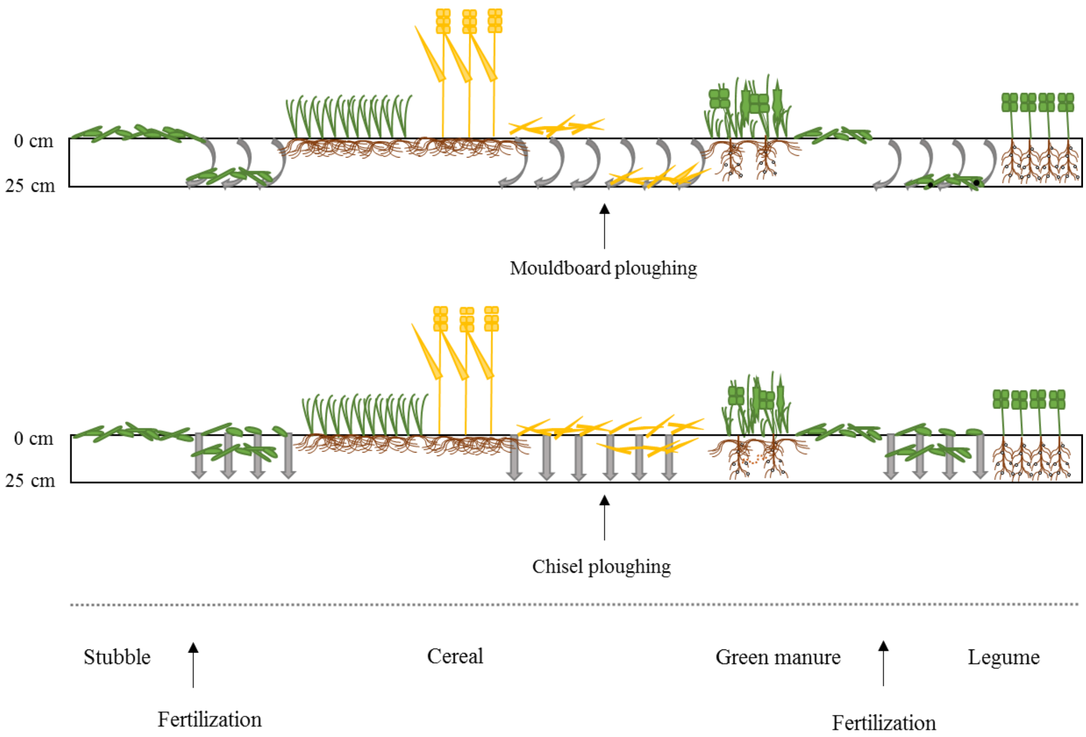

Figure 2. Cereal-legume crop rotation schemes of the experiment with two different tillage systems in two years. The first scheme (top) represents the rotation with mouldboard ploughing (inversion of soil layers) and the second scheme (bottom) represents the rotation with chisel ploughing (no soil inversion). The schemes start from the stubble incorporation of the previous crop, followed by the fertilization (in the corresponding plots) and the soil tillage for the cereal crop season. After cereal harvest and the stubble incorporation (disc harrowing), the cover crops are sown and later incorporated into the soil (in the corresponding plots), before the fertilization and tillage for the legume crop season.

Table 2. Results of the linear mixed effects models of the effect of fertilization (F +, fertilization with farmyard manure; F -,not fertilized), tillage system (T P: mouldboard ploughing; T C: chisel ploughing), green manure (G +, with green manure; G -, no green manure) and their interaction on the different variables measured (crop yields of spelt, chickpea and winter wheat, crop biomass of lentil, and weed biomass during each cropping season).

	F (+ vs -)	T (P vs C)	G (+ vs -)	F (+ vs -) × T (P vs C)	F (+ vs -) × G (+ vs -)	T(P vs C) × G(+ vs -)	F × T × G
Crop yields (kg ha^{-1})							
Spelt	163.06 ± 38.25*	6.72 ± 58.29^{NS}	23.28 ± 22.48^{NS}	-26.44 ± 22.48^{NS}	-2.25 ± 22.48^{NS}	13.95 ± 22.48^{NS}	16.92 ± 22.48^{NS}
Chickpea	-61.47 ± 29.10^{NS}	-0.12 ± 30.46^{NS}	5.35 ± 15.76^{NS}	9.34 ± 15.76^{NS}	-29.57 ± 15.76^{NS}	-9.06 ± 15.76^{NS}	-1.06 ± 15.76^{NS}
Wheat	484.96 ± 121.61*	36.82 ± 120.04^{NS}	28.92 ± 68.36^{NS}	-45.32 ± 68.36^{NS}	-8.45 ± 68.36^{NS}	-4.80 ± 68.36^{NS}	-1.42 ± 68.36^{NS}
Lentil (biomass g m^{-2})	-7.33 ± 5.13^{NS}	12.251 ± 4.70*	2.47 ± 3.87^{NS}	-2.16 ± 3.87^{NS}	-4.18 ± 3.87^{NS}	5.29 ± 3.87^{NS}	-4.91 ± 3.87^{NS}
Weed biomass (g m^{-2})							
Spelt	-0.16 ± 0.04**	-0.26 ± 0.12^{NS}	-0.07 ± 0.04^{NS}	-0.24 ± 0.04***	0.001 ± 0.04^{NS}	-0.01 ± 0.04^{NS}	-0.05 ± 0.04^{NS}
Chickpea	12.46 ± 5.47^{NS}	-2.46 ± 7.53^{NS}	1.92 ± 3.40^{NS}	-2.77 ± 3.40^{NS}	3.67 ± 3.40^{NS}	1.09 ± 3.40^{NS}	2.19 ± 3.40^{NS}
Wheat	-0.31 ± 0.08*	-0.28 ± 0.08*	-0.03 ± 0.07^{NS}	0.11 ± 0.07^{NS}	0.10 ± 0.07^{NS}	0.03 ± 0.07^{NS}	-0.04 ± 0.07^{NS}
Lentil	32.45 ± 7.75**	-12.79 ± 4.25**	11.55 ± 4.25*	-6.88 ± 4.25^{NS}	6.98 ± 4.25^{NS}	-3.95 ± 4.25^{NS}	0.81 ± 4.25^{NS}

The values are estimated differences, standard errors and their significance levels, which are indicated according to the following codes: *** $p \leq 0.001$, ** $p \leq 0.01$, * $p \leq 0.05$, ^{NS} not significant.

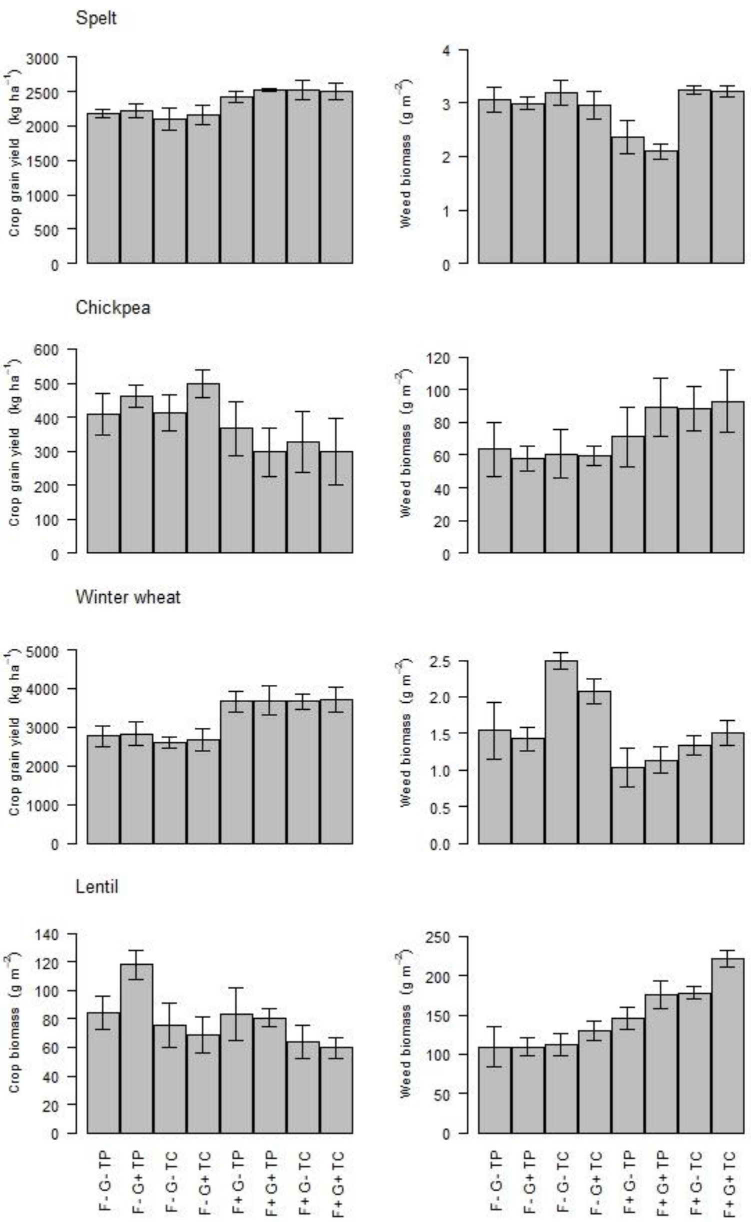

Figure 3. Mean (± SE standard error of differences) crop yields of spelt, chickpea, winter wheat and crop biomass of lentil (left) and the corresponding aboveground weed biomass (right) in each treatment: fertilization (F - , not fertilized; F +, fertilization with farmyard manure); green manure (G -, no green manure; G + sown with green manure); tillage systems (T P, mouldboard ploughing; T C, chisel ploughing). Because lentil crop was not harvested, aboveground lentil biomass was evaluated. Note the different vertical scale for each crop.

Table 3. Mean (± SE) of SOC and N_{tot} at two soil depths (0 – 10 cm and 10 – 20 cm) in each treatment: fertilization (F +, fertilization with farmyard manure; F -, not fertilized); tillage system (T P, mouldboard ploughing; T C, chisel ploughing); green manure (G +, sown with green manure; G -, no green manure) in the first and last year of the trial (t_i and t_f).

	0-10 cm		**10-20 cm**		**0-10cm**		**10-20 cm**	
	SOC t_i	**SOC** t_f	**SOC** t_i	**SOC** t_f	**N** t_i	**N** t_f	**N** t_i	**N** t_f
F +	1.170 ± 0.090	1.118 ±0.06 0	1.110 ±0.090	0.970 ± 0.060	0.124 ± 0.005	0.163 ±0.006	0.113 ± 0.006	0.148 ± 0.006
F -	1.150 ± 0.130	0.827 ± 0.060	1.090 ±0.120	0.800 ± 0.040	0.119 ± 0.007	0.127 ± 0.005	0.112 ± 0.005	0.123 ± 0.005
T P	1.140 ± 0.100	0.904 ±0.036	1.090 ±0.100	0.900 ± 0.030	0.120 ± 0.005	0.139 ± 0.003	0.110 ± 0.005	0.137 ± 0.005
T C	1.180 ± 0.120	1.042 ± 0.080	1.110 ±0.120	0.860 ± 0.070	0.121 ± 0.006	0.152 ± 0.008	0.114 ± 0.006	0.134 ± 0.006
G +	1.180 ± 0.120	0.97 ± 0.060	1.120 ±0.120	0.880 ± 0.050	0.121 ± 0.006	0.144 ± 0.005	0.114 ± 0.006	0.136 ± 0.006
G -	1.140 ± 0.100	0.975 ± 0.050	1.080 ±0.100	0.890 ± 0.040	0.122 ± 0.006	0.146 ± 0.006	0.110 ± 0.005	0.135 ± 0.005

Table 4. Results of the linear mixed effects models of the effect of the different experimental factors, plus depth and year and their interactions on the changes in soil organic carbon, in total nitrogen and in the ratio among these (ΔSOC, ΔN and ΔC:N). F +, fertilization with farmyard manure; F -, not fertilized; T P, mouldboard ploughing; T C, chisel ploughing; G +, sown with green manure; G -, no green manure.

	ΔSOC	**ΔN**	**ΔC:N**
F (+ vs -)	0.137± 0.075^{NS}	0.015± 0.002***	0.264 ± 0.675^{NS}
T (P vs C)	-0.049± 0.060^{NS}	-0.008±0.001***	0.085 ± 0.579^{NS}
G (+ vs -)	-0.021± 0.020^{NS}	-0.001±0.001^{NS}	-0.169 ± 0.188^{NS}
F (+ vs -) × T (P vs C)	0.009± 0.020^{NS}	-0.006±0.001**	0.452 ± 0.188*
F (+ vs -) × G (+ vs -)	-0.007± 0.020^{NS}	-0.002±0.001^{NS}	0.082 ± 0.188^{NS}
T (P vs C) × G (+ vs -)	-0.022± 0.020^{NS}	-0.002±0.001^{NS}	0.007 ± 0.189^{NS}
Depth (10-20 vs 0-10 cm)	-0.025±0.029^{NS}	-0.001±0.002^{NS}	-0.461 ± 0.267^{NS}
F (+ vs -) × depth (10-20 vs 0-10 cm)	-0.060±0.029*	-0.003±0.002^{NS}	-0.297 ± 0.267^{NS}
T (P vs C) × depth (10-20 vs 0-10 cm)	0.084±0.029**	0.012±0.002***	-0.021 ± 0.267^{NS}
G (+ vs -) × depth (10-20 vs 0-10 cm)	-0.002±0.029^{NS}	-0.002±0.002^{NS}	0.180 ± 0.267^{NS}
F (+ vs -) × T (P vs C) × depth (10-20 vs 0-10 cm)	0.0578±0.29^{NS}	0.006±0.002*	0.190 ± 0.267^{NS}

The values are estimated differences in marginal means, standard errors and their significance levels, which are indicated according to the following codes: *** p < 0.001, ** p < 0.01, * p < 0.05, NS not significant.

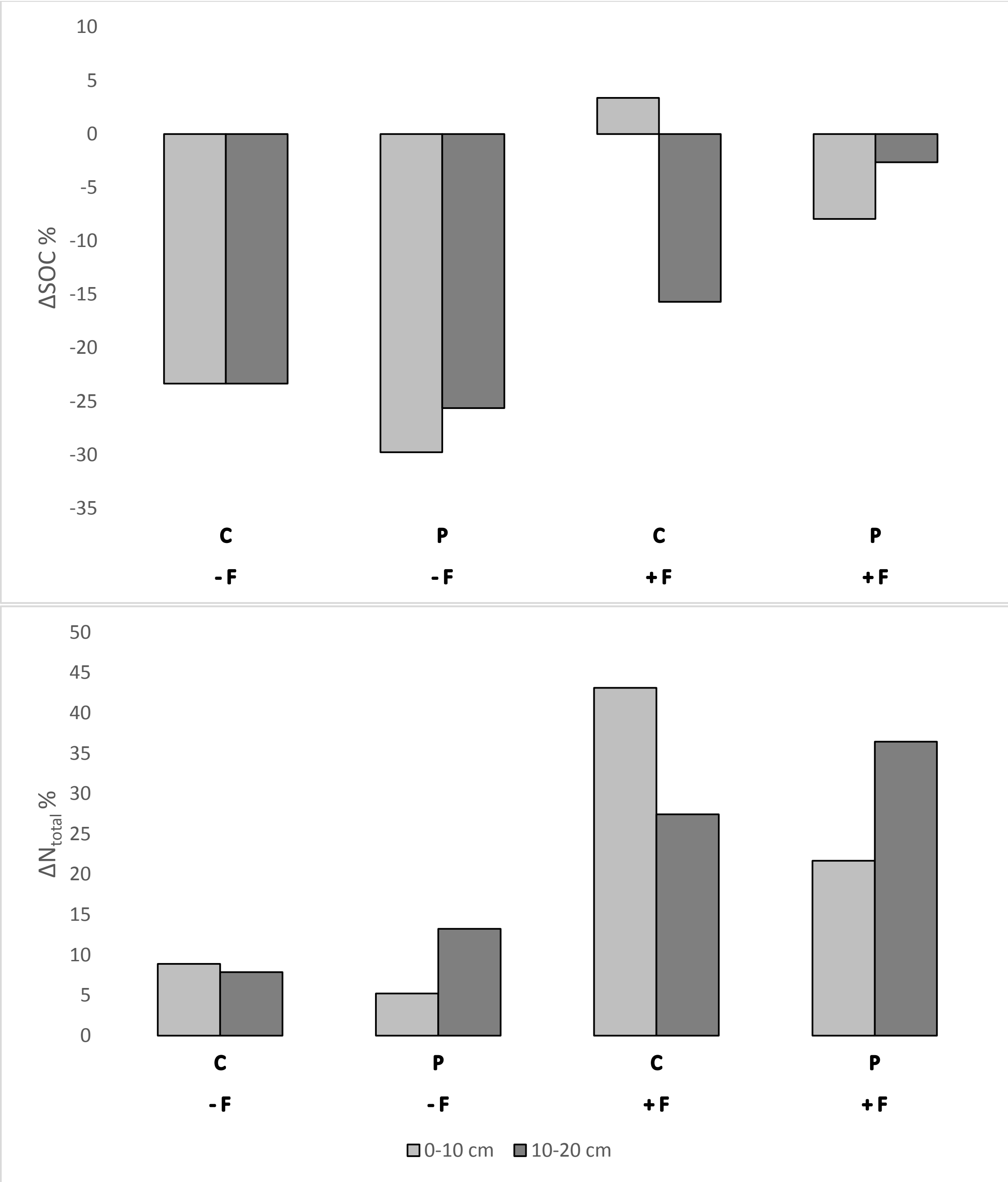

Figure 4. Changes in SOC and N contents, at two soil depths (0 to 10 cm and 10 to 20 cm), in plots under different treatments over the 4-year rotation of the experiment. Fertilization (F -, not fertilized; F +, fertilization with farmyard manure); tillage system (P, mouldboard ploughing; C chisel ploughing). ΔSOC and ΔN_{tot} represent the differences between the t_f and the t_i.

Table 5. Results of the linear mixed effects models of the effect of the different factors: fertilization (F + fertilization with farmyard, F - not fertilized; manure); tillage system (T P, mouldboard ploughing; T C, chisel ploughing); green manure (G -, no green manure; G +, sown with green manure); depth (1, 0 – 10 cm; 2, 10 – 20cm); and the interaction between them on soil bulk density and carbon stocks after the four years of trial (t_f).

	Bulk density (t_f)	**Carbon stocks (t_f)**
F (+ vs -)	-0.0038 ± 0.017^{NS}	1064.77 ± 295.35*
T (P vs C)	-0.007 ± 0.015^{NS}	415.15 ± 334.56^{NS}
G (+ vs -)	-0.003 ± 0.015^{NS}	-149.21 ± 203.76^{NS}
F (+ vs -) × T (P vs C)	0.012 ± 0.015^{NS}	18.22 ± 203.76^{NS}
F (+ vs -) × G (+ vs -)	0.008 ± 0.015^{NS}	130.66 ± 203.76^{NS}
T (P vs C) × G (+ vs -)	0.003 ± 0.015^{NS}	597.26 ± 203.76**
Depth (10-20 vs 0-10 cm)	0.259 ± 0.022***	1234.81 ± 269.12***
F (+ vs -) × depth (10-20 vs 0-10 cm)	-0.017 ± 0.022^{NS}	716.31 ± 269.12*
T (P vs C) × depth (10-20 vs 0-10 cm)	0.021 ± 0.022^{NS}	-1287.24 ± 269.12***
G (+ vs –) × depth (10-20 vs 0-10 cm)	-0.010 ± 0.022^{NS}	96.99 ± 269.12^{NS}

The values are estimated differences in marginal means, standard errors and their significance levels, which are indicated according to the following codes: *** p < 0.001, ** p < 0.01, * p < 0.05, NS not significant.

Table 6. Mean (± SE) of soil microbial biomass (C_{mic} and N_{mic}) and the ratio (C_{mic}/SOC) in the last year of the trial (t_f) at two soil depths (0 -10 cm and 10 – 20 cm) in each treatment: fertilization (F +, fertilization with farmyard manure; F -, not fertilized); tillage system (T P, mouldboard ploughing; T C, chisel ploughing); green manure (G +, sown with green manure; G - no green manure).

System	**C_{mic} (μ g^{-1}) (t_f)**		**N_{mic} (μ g^{-1}) (t_f)**		**C_{mic}/SOC (%)(t_f)**	
Depth	**0 -10 cm**	**10 – 20 cm**	**0 -10 cm**	**10 – 20 cm**	**0 -10 cm**	**10 – 20 cm**
F +	297.13 ± 15.66	250.48 ± 11.66	31.81 ± 7.00	25.27 ± 4.13	2.64 ± 0.31	2.57 ± 0.36
F -	234.73 ± 13.59	210.48 ± 12.65	19.70 ± 3.51	24.37 ± 4.75	2.88 ± 0.41	2.60 ± 0.24
T P	243.34 ±12.93	234.41 ± 11.00	26.37 ± 4.62	27.60 ± 4.86	2.72 ± 0.31	2.61 ± 0.26
T C	288.53 ± 16.31	226.56 ± 13.31	25.14 ± 5.89	22.04 ± 4.02	2.80 ± 0.41	2.56 ± 0.33
G +	269.04 ± 14.50	231.69 ± 11.85	26.33 ± 5.85	25.44 ± 5.66	2.80 ± 0.39	2.61 ± 0.32
G -	262.82 ± 14.74	229.27 ± 12.45	25.18 ± 4.65	24.19 ± 3.22	2.72 ± 0.34	2.55 ± 0.28

Table 7. Results of the linear mixed effects models of the effect of the different factors: fertilization (F +, fertilization with farmyard manure; F -, not fertilized); tillage system (T P, mouldboard ploughing; T C, chisel ploughing); green manure (G +, sown with green manure; G - no green manure; depth (0 – 10 cm and 10 – 20cm); and the interaction between them on C_{mic}, N_{mic} and C_{mic}/SOC in the last year of the trial (t_f).

System	**C_{mic} (t_f)**	**N_{mic} (t_f)**	**C_{mic}/SOC (t_f)**
F (+ vs -)	30.77 ± 3.56***	5.91 ± 2.71*	-0.10 ± 0.21^{NS}
T (P vs C)	-21.66 ± 14.93^{NS}	0.74 ± 2.25^{NS}	-0.07 ± 0.05^{NS}
G (+ vs -)	3.02 ± 4.02^{NS}	0.58 ± 2.25^{NS}	0.03 ± 0.03^{NS}
Depth (10-20 vs 0-10 cm)	-35.69 ± 3.77***	-1.06 ± 2.05^{NS}	-0.15 ± 0.07*
F (+ vs -) × depth (10-20 vs 0-10 cm)	-11.57 ± 3.78**	-5.04 ± 2.05*	0.11 ± 0.07^{NS}
T (P vs C) × depth (10-20 vs 0-10 cm)	26.38 ± 3.78***	1.40 ± 2.04^{NS}	0.07 ± 0.07^{NS}
F (+ vs -) × T (P vs C)	-3.13 ± 3.42^{NS}	-1.41 ± 2.25^{NS}	0.11 ± 0.05*
F (+ vs -) × T (P vs C) × depth (10-20 vs 0-10 cm)	-6.04 ± 6.23^{NS}	-0.25 ± 2.05^{NS}	-0.27 ± 0.07***

Significance levels are indicated according to the following codes: *** p < 0.001, ** p < 0.01, * p < 0.05, ^{NS} not significant. Interactions with the green manure were not significant.

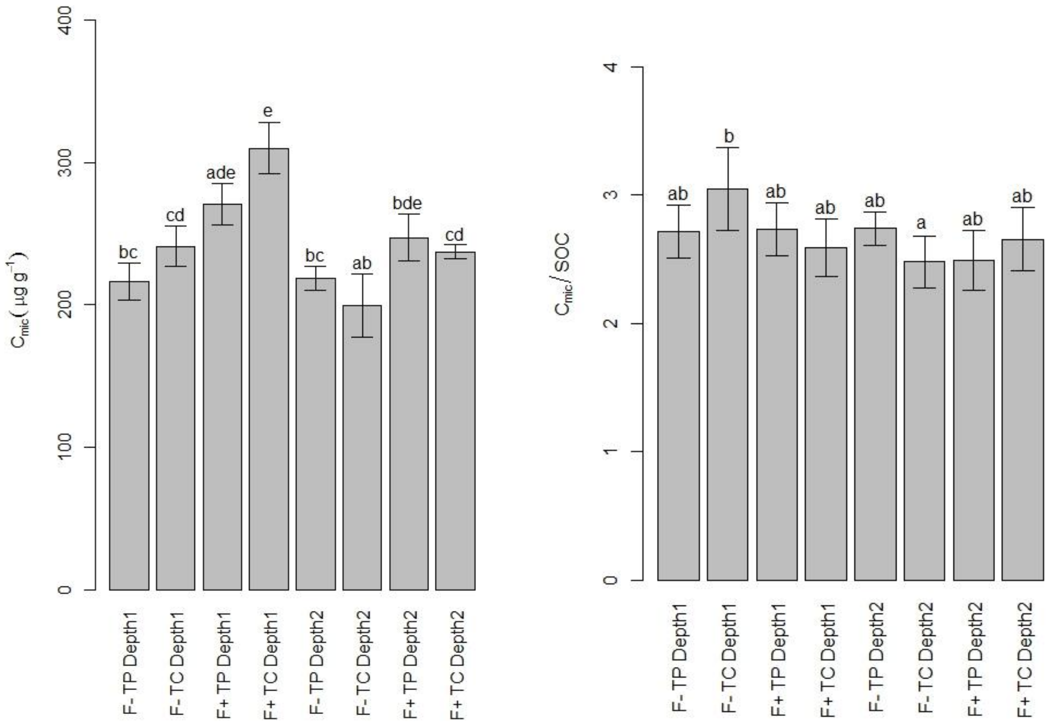

Figure 5. Soil microbial biomass C (left) and C_{mic}/SOC ratio (right, in percentage) after four years of the trial (t_f) in the different treatments: fertilization (F + fertilization with farmyard manure, F - not fertilized); tillage system (T P mouldboard ploughing, T C chisel ploughing); green manure (G + sown with green manure, G - no green manure); depth (1: 0 – 10 cm, 2: 10 – 20cm). Bars with no letters in common are significantly different (Tukey HSD test, $p < 0.05$).