Grain legume-based rotations managed under conventional tillage need cover crops to mitigate soil organic matter losses

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Grain legume-based rotations managed under conventional tillage need cover crops to mitigate soil organic matter losses

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Abstract

Inserting legumes in low-input innovative cropping systems can represent a good strategy to reduce current N fertilizer dependency while enhancing ecosystem services. However, although the impact of the use of legumes as cover crops has been broadly studied, very little is known about the effects of grain legume-based rotations on soil organic carbon (SOC) and nitrogen (SON). A cropping system experiment with three 3-year rotations with different levels of inclusion of grain legumes: GL0, GL1 and GL2 (none, one, and two grain legumes, respectively), with (CC) or without (BF, bare fallow) cover crops was established in SW France (Auzeville) under temperate climate. Durum wheat was present in all the rotations to act as an indicator of their performance. Soil organic C and SON were quantified before the beginning of the experiment and after 3 and 6 years (i.e. after one and two complete 3-yr rotations). Aboveground C and N inputs to the soil, and C and N harvest indexes and grain yield of the cash crops were also measured. Inserting grain legumes in the rotations significantly affected the amount of C and N inputs and consequently SOC and SON. After two cycles of the 3-yr rotation, the GL1 and GL2 treatments showed a greater decrease in SOC and SON when compared to GL0. However, the inclusion of cover crops in the rotations led to mitigate this loss. Durum wheat produced significantly greater grain yields in GL1 when compared to GL0, while GL2 presented intermediate values. In turn, the incorporation of cover crops did not reduce C and N harvest indexes or the grain yield of the different cash crops. We concluded that, in such conventionally-tilled grain legume-based rotations, the use of cover crops was efficient to mitigate SOC and SON losses and then increase N use efficiency at the cropping system level without reducing productivity.

Keywords

Grain legumes; Cover crop; Rotation; Soil organic carbon; Soil organic nitrogen.

1. Introduction

Agricultural activity faces the challenge of maintaining current productivity while minimizing environmental risks in order to attain its sustainability. Nitrogen nutrition is a key element to reach this objective due to its implications in crop productivity. Since the discovery of the Haber-Bosch process, the anthropogenic fixation of reactive nitrogen has doubled the natural terrestrial sources of this element (Fowler et al., 2013). In arable cropping systems an overuse of this nutrient has led to major losses such as nitrate leaching to groundwater or gaseous emissions to the atmosphere as nitrous oxide (N_2O) (Bouwman et al., 2013). Moreover, substantial quantities of carbon dioxide (CO_2) are produced during the synthetic fixation of N_2 to NH_3, given the large energetic requirements of this process (Jenkinson, 2001). As a consequence, research efforts must be placed on the design and optimization of arable cropping systems in order to better couple crop N needs and nutrient availability while reducing current dependence on synthetic N fertilizers.

The design of low-input innovative cropping systems with the inclusion of legumes in the rotations as cash or cover crops is a major tool for reducing N losses and N fertilizer-dependence. Crop diversification with legumes not only has advantages in terms of plant N nutrition but also contributes to a breakage effect on pest and disease cycles and to the development of populations of beneficials for crop defense (Voisin et al., 2014). Moreover, the establishment of those cropping systems can improve the current budget of N in areas of the world such as Europe, where nitrogen efficiency (being the quotient between the product output and the total N inputs in the system) is calculated to be around 36% compared to a global average of 50% (Erisman et al., 2011). However, a proper cropping systems design must also take into account the effects on soil quality and fertility, natural resource conservation and agricultural productivity.

Soil organic matter represents one of the main indicators of soil quality and fertility (Franzluebbers, 2002). As a consequence, it has a direct impact on plant productivity. Carbon sequestration in soils is assumed to be a useful strategy to reduce the concentration of CO_2 in the atmosphere (Lal, 2004). Recent meta-analyses have summarized the impact of cropping intensification and different management practices such as tillage and fertilization on soil organic matter (e.g. Luo et al., 2010; Maillard and Angers, 2014; McDaniel et al., 2014). Cropping intensification, i.e. the reduction of the bare fallow periods between cash crops, usually results in SOC and SON sequestration due to the increase in the amount of C and N returned to soil as a result of a longer photosynthesis and improved N cycling by legumes (Franzluebbers, 2005; Sainju et al., 2003). In this line, the use of autumn cover crops with the ability to scavenge nutrients can lead to the reduction of nitrate leaching to groundwater (Brennan and Boyd, 2012; Tosti et al., 2014) and other ecosystem services such as an increase in biodiversity (Lal, 2004) and the abatement of soil erosion (Dabney et al., 2001).

In addition to C and N inputs, one determinant aspect of the cropping system capacity to increase SOC and SON is the C:N ratio of crop residues. This ratio is affected by the nutrition status of the plants and their phenological stage. The ratio is greater in mature plants as a consequence of a reduction in N concentration followed by a diminution of water soluble compounds and an increase of more lignified constituents. All the last biochemical attributes are directly related to the decomposition of biomass (Justes et al., 2009; Thorup-Kristensen et al., 2003) which is faster for crop residues with a low C:N ratio such as the leguminous (Sanchez et al., 2004). The addition of easily decomposable plant material to the soil can also lead to a priming effect which entails a burst of microbial activity leading to enhanced SOC decomposition (Kuzyakov, 2010). Regarding to this, Jensen et al., (2012) pointed out the key role of the net N-balance of the soil-plant system as a driver of soil C changes. They also suggested that legumes with a high N harvest index such as soybean do not maintain SOC levels due to the large amount of N that is exported. Moreover, other aspects can also regulate the stocks of SOC and SON when legumes are incorporated in the rotations: (i) the inherent characteristics of legumes in below-ground biomass production, (ii) the amount and quality of root exudates, (iii) the impacts on the microbial community and (iv) the effects on soil structure and C and N protection within soil aggregates (Drinkwater et al., 1998).

In contrast to cereal-based cropping systems, less attention has been paid to the effect of cover crop use on SOC and SON in grain legume-based rotations. Therefore, our objective was to quantify the impact of the incorporation of grain legumes and cover crops on SOC and SON in rotations with an increasing number of grain legumes managed under conventional tillage. We hypothesized that the inclusion of cover crops in grain legume-based rotations would mitigate the loss of SOC caused by the lower C inputs and C:N ratio of leguminous crop residues.

2. Materials and methods

2.1 Experimental site and treatments

The study was carried out in one experimental field of the Institut National de la Recherche Agronomique (INRA) station in Auzeville (SW France) established in 2003. Site characteristics and soil properties at the beginning of the experiment are detailed in Table 1. The location represents a temperate climate. Three 3-year rotations with different number of grain legumes (GL0, GL1 and GL2; no, one and two grain legumes included in the rotation, respectively) with (CC) and without (BF, bare fallow) cover crops were compared, resulting in six different cropping systems. The GL0 treatment consisted of a Sorghum (*Sorghum bicolor L.*) – sunflower (*Helianthus annuus L.*) – durum wheat (*Triticum turgidum L.*) rotation. The GL1 treatment was based on a sunflower – winter pea (*Pisum sativum L.*) – durum wheat rotation while the GL2 treatment consisted of a soybean (*Glycine max L.*) – spring pea – durum wheat rotation. Mustard (*Sinapis alba L.*), vetch (*Vicia sativa L.*) and a vetch – oat (*Avena sativa L.*) mixture were used as cover crops. Durum wheat was established in all rotations as an indicator of the performance of the different cropping systems studied. A conceptual diagram of the six cropping systems studied in the experiment is given in Fig. 1. The different cultivars and seeding rates of the cash and cover crops used in each rotation are shown in Table 2. Depending on the rotation, the use of cover crops aimed to (i) reduce nitrate leaching and (ii) reduce mineral fertilization needs with the use of a legume as a cover crop. The present work covers two rotation cycles (i.e. 2003-2006 for the first cycle and 2006-2009 for the second one). The experiment was laid out with a split-plot design with three blocks with the amount of grain legumes in the rotation as the main plot and the cover crop treatments as the sub-plot. Sub-plot size was 200 x 15 m. Within each rotation, each crop was grown each year in order to take into account the interannual weather variability; hence each rotation was replicated three times but started with a different crop.

Prior to the establishment of the experiment the field had been devoted to low-input field crops production under conventional tillage. Further information regarding the set-up of the experiment can be found at Plaza-Bonilla et al., (2015).

2.2 Crop management

Soil tillage was performed with one pass of rotary harrow to prepare the soil for seeding, followed, when needed, by a pass of cultipacker. A disk plow was used to incorporate the cover crops into the soil. Moreover, when needed, a pass of moldboard plough was performed to 30 cm depth to mechanically control weeds reducing the use of herbicides. Durum wheat and winter and spring peas were seeded with a commercial combined seeding machine while summer crops (soybean, sunflower and sorghum) were seeded with a precision air seeder. Durum wheat was sown in November and harvested within the first fortnight of July. Winter pea was sown in December and harvested during June. Spring pea was sown during February and harvested late June. Sorghum cycle covered the period from late April-beginning of May to late September. Sunflower was sown during the last fortnight of April and harvested during September. Finally, soybean was sown the first week of May and harvested between late September and early October. Crops were sown at a depth of 2-4 cm. The distance between rows was 17 and 50 cm for winter and summer crops, respectively. Mechanical hoeing was performed one or two times on sorghum and sunflower depending on the presence of weeds. The amount of N fertilizer applied in each crop was calculated by taking into account the mineral N in the soil before seeding, the requirements of the crop and an estimation of mineralization. Ammonium nitrate was used as fertilizer for wheat, while urea was used for sorghum and sunflower. No N fertilization was applied to legume crops. Nitrogen fertilization was performed with a pneumatic precision applicator being split two or three times in durum wheat according to the preceding crop: tillering (only if preceded by sunflower), beginning of stem elongation and booting; corresponding to the 21, 31 and 41 stages of the Zadoks scale (Zadoks et al., 1974). Contrarily, a single application of fertilizer was performed on sunflower and sorghum right after sowing. The use of crop protection products was decided according to plant sanitary status being minimized as much as possible following the principles of integrated pest management. The combination of high temperatures and low amounts of rainfall during the summer led to the need of irrigating soybean and sorghum crops with an annual average of 171 and 102 mm, respectively, in order to reach a target of ca. 80% of the maximum evapotranspiration of the crop. Irrigation was applied with the use of a large-volume sprinkler. Grain harvest was performed with a commercial harvesting machine. All crop residues were chopped and incorporated into the soil. Air temperature and rainfall values were recorded daily using an automated weather station located 200 m far from the experiment.

2.3 Soil and biomass samplings and analysis

In order to quantify the effects of the different treatments on SOC and SON, three soil samplings were performed in 2003, 2006 and 2009 in autumn, right after the harvest of summer crops. For each sub-plot (i.e. combination of the amount of legumes in the rotation and cover crop treatment), two sampling areas of 18 x 15 m were identified and 10 soil samples of 0-30 cm depth were obtained using a hydraulic coring device with a 15-mm diameter auger (MCL3, Geonor, Oslo, Norway). In turn, in 2006 and 2009 soil samples of the 30-60 cm soil depth were also analyzed in order to check if the different cropping systems affected SOC and SON below the upper 30 cm. Since no differences between treatments were observed in this depth (30-60 cm), the present study is focused on the plough layer (0-30 cm).

In order to minimize the effect of soil heterogeneity on SOC and SON and ease subsequent samplings, the coordinates of each sampling point were recorded with a GPS device. Soil bulk density (0-30 cm depth) was quantified with the cylinder method (Grossman and Reinsch, 2002) at the end of the experiment. Once in the laboratory, soil samples were air dried at room temperature and sieved to 2 mm. Soil C and N concentration were determined with the Dumas combustion method with a Leco-2000 analyzer (LECO, St. Joseph, MI, US).

Cash crop aboveground biomass was measured right before their harvest and before the termination of cover crops by cutting 6 m^2 of plants at soil surface level. Once in the laboratory, the samples were dried at 80 ºC for 48 h and weighed. Afterwards, the grain was threshed (in the case of cash crops) and the rest of biomass was finely grinded and analyzed for C and N concentration using the same method as for soil samples. The aboveground C and N inputs to the soil were calculated by multiplying the weight of biomass excluding the grain (in the case of cash crops) by its C and N concentration, respectively, and dividing by the area sampled. In turn, grain yield was calculated dividing the weight of grain by the area sampled and adjusted to 100 g kg^{-1} moisture content. The C:N ratio of the residues of the different crops was also calculated by dividing the C concentration of the biomass by the N concentration. Finally, C and N harvest indexes of the different cash crops were calculated by dividing the amount of C and N in the grain by the amount of C and N in the whole aboveground biomass (i.e. including grain).

2.4 Data analysis

A linear relationship between SOC and SON and the year of sampling was performed using a mixed linear model with random effect in order to take into account that the intercept could differ between replications (i.e. random effect). This procedure aimed to take into account differences in initial soil organic C and N in the experimental plots as a result of their previous history and soil spatial variability. In turn, the slope (i.e. rate of SOC and SON change over time) was considered equal for the different replications of a given treatment (i.e. fixed effect). The regressions were carried out taking into account SOC and SON concentration values since no differences between treatments were found on soil bulk density.

An analysis of variance (ANOVA) was performed to analyze differences between cropping systems (i.e. combinations of number of grain legumes in the rotation and cover crop treatments) on soil aboveground C and N inputs. Moreover, for each cash crop, differences between cover crop treatments on grain yield and C and N harvest indexes were also analyzed. Grain yield and C and N harvest indexes of durum wheat were used as indicators of the performance of the different cropping systems studied, since durum wheat was used in all the rotations. When significant, differences between treatments were identified at 0.05 probability level of significance using a LSD test. Finally, the impact of C and N inputs and their C:N ratio on the changes of SOC and SON stocks was tested using the standard least square personality of the JMP 11 Pro statistical package (SAS Institute Inc., 2014).

3. Results

3.1 Environmental conditions during the experiment

Rainfall and air temperature during the experimental period are shown in Fig. 2. Annual rainfall ranged from 501 to 744 mm, values that represent only 55% and 82% of the mean potential evapotranspiration of the area (i.e. 905 mm). Rainfall was quite lower than the 30-year average (685 mm) in four of the six cropping seasons studied. February, June and July were the months with the lowest amount of rainfall with 25, 33 and 26 mm, respectively, as an average of the six cropping seasons. Mean annual temperature varied from 13.3 to 14.2 ºC, being lower than the 30-yr average (13.7 ºC) in four of the six cropping seasons.

3.2 Grain yields and C and N harvest indexes

The results of the analysis of variance of the effects of the different treatments on grain yields and C and N harvest indexes are shown in Table 3. Climatic variability led to differences between years in grain production of the different cash crops (Tables 3 and 4). For instance, over the 6 years of the experiment, durum wheat grain yield ranged between 4143 and 7414 kg ha^{-1} and sunflower grain yield between 1881 and 3982 kg ha^{-1}. The use of cover crops did not lead to significant changes in grain production of the different cash crops as it was planned with the adaptation of fertilizer-N to the preceding crop (Tables 3 and 4). Contrarily, significant differences between cropping systems were found on durum wheat grain yield (Table 3), with significantly greater values under the GL1-BF, GL1-CC and GL2-BF treatments when compared to GL0-BF and GL0-CC, as an average of the 2003-2009 period (Table 4). Cover crop treatments did not significantly affect C and N harvest indexes of the different cash crops studied, except for sorghum N harvest index (Tables 3 and 5). The different cropping systems significantly affected the C harvest index of durum wheat, with slightly greater values in the GL0 and GL2 treatments, when compared to GL1 (Table 5).

Contrarily, N harvest index was not significantly affected by the cropping systems compared (Table 3).

3.3 Cash crop and cover crop residues C and N concentration and C and N inputs to the soil

Average aboveground crop residue C inputs ranged between 2192 and 3118 kg ha^{-1} yr^{-1} for the different treatments studied (Fig. 3A). Aboveground biomass of cover crops represented 12, 23 and 25% of the whole amount of aboveground C residue inputs incorporated to the soil in the GL0-CC, GL1-CC and GL2-CC treatments, respectively. Cover crop treatments significantly affected C inputs with greater values when using cover crops (2978 kg C ha^{-1} yr^{-1}), compared to the bare fallow treatment (2385 kg C ha^{-1} yr^{-1}) as an average of the three GL rotations (Fig. 3A). Contrarily, no significant effects of the number of grain legumes and the interaction between cover crops and grain legumes were found on this variable. Similarly, the use of cover crops significantly affected the inputs of N with greater values in the CC treatment compared to BF (Fig. 3B). A greater amount of N inputs as crop residues was found in the GL1-CC treatment when compared to GL0-CC, with intermediate values in GL2-CC (Fig. 3B).

The aboveground biomass C concentration ranged between 414 and 472 kg C t^{-1} dry matter, being similar for all crops of the experiment (data not shown). Contrarily, and as expected, the N concentration presented great variation among crops, ranging from 6.6 (wheat and sorghum) to 18.5 kg N t^{-1} dry matter (winter pea) for cash crops and from 20.8 (mustard) to 40.9 kg N t^{-1} dry matter (vetch) for cover crops (Table 6). In turn, the C:N ratio of crop residues presented a great variation between the different crops of the experiment being 66, 74 and 37 for durum wheat, sorghum and sunflower, respectively (data not shown). In the case of the grain legumes, the C:N ratios were 27, 32 and 37 for winter pea, spring pea and soybean, respectively. Cover crops presented the lowest C:N ratio: 12 for vetch, 16 for mustard and 17 for the vetch-oat mixture (data not shown). Once weighted by the amount of residues incorporated to the soil by each crop, mean C:N ratios were 59, 39 and 41 for the GL0-CC, GL1-CC and GL2-CC treatments, respectively. In the case of the treatments without cover crops the incorporation of grain legumes in the rotations also reduced the weighted C:N ratios of aboveground crop residues being 62, 47 and 50 for the GL0-BF, GL1-BF and GL2-BF treatments, respectively (data not shown). As an average of BF and CC treatments, the amounts of C from aboveground crop residues returned to the soil were 3122, 1905 and 2718 kg C ha^{-1} yr^{-1} for the sorghum, sunflower and durum wheat crops in GL0, 1946, 2004 and 3171 kg C ha^{-1} yr^{-1} for the winter pea, sunflower and durum wheat crops of GL1 and 2090, 1647 and 2829 kg C ha^{-1} yr^{-1} for the spring pea, soybean and durum wheat crops of GL2 (Fig. 4). However, no differences between BF and CC treatments were found on crop residue C inputs for any of the cash crops of the experiment (Fig. 4). In the case of the cover crops the amount of C returned to the soil by the cover crops ranged between 349 and 1287 kg C ha^{-1} yr^{-1} for the vetch and mustard cover crops of the GL0-CC and GL2-CC treatments, respectively (Fig. 4).

3.4 SOC and SON change versus time

The mixed effects linear regressions between the number of years since the beginning of the experiment and SOC and SON concentrations are shown in Figs. 5 and 6, respectively. Soil organic carbon and SON concentration values were used since no differences between treatments were found on soil bulk density. A significant reduction in SOC was observed in the GL1-BF, GL2-BF and GL1-CC treatments (Fig 5). Taking into account the average bulk density measured in the experiment (1.55±0.10 Mg m^{-3}) and the slope of the regressions, the GL0-BF, GL1-BF and GL2-BF treatments led to a change in SOC stocks in the plough layer (0-30 cm) of -233, -595 and -735 kg C ha^{-1} yr^{-1}, respectively. In turn, the estimated change in

SOC stocks was +284, -493 and -246 kg C ha^{-1} yr^{-1}, for the GL0-CC, GL1-CC and GL2-CC treatments, respectively.

A significant reduction of SON concentration was observed in the GL1-BF and GL2-BF treatments (Fig. 6). In this case, the change in SON stocks in the plough layer (0-30 cm soil depth) was -33, -47 and -74 kg N ha^{-1} yr^{-1} in the GL0-BF, GL1-BF and GL2-BF treatments, respectively. However, when cover crops were used, the change in SON stocks was not significant with +9, -19 and -9 kg N ha^{-1} yr^{-1} for the GL0-CC, GL1-CC and GL2-CC treatments, respectively. No significant relationships were found between the amount and quality of the aboveground crop residues and SOC and SON changes.

4. Discussion

4.1 Cash crop performance under the cropping systems studied

The use of cover crops did not significantly have a negative impact on the yield and C and N mobilization into the grain of the subsequent cash crops. Then, the similar global performance of the cash crops in the BF and CC treatments could be the result of three causes: (i) the correct degree of synchronization attained between cash crop N uptake and cover crops N release (Dabney et al., 2001), (ii) the early finalization of cover crops in autumn that was carried out to avoid competition for water and nutrients in particular for spring and summer cash crops, appearing then as a relevant management in such pedoclimatic conditions (Plaza-Bonilla et al., 2015) and (iii) the slight increase in durum wheat N fertilization that was carried out in the GL1-CC and GL2-CC when compared to GL1-BF and GL2-BF (Plaza-Bonilla et al., 2015). In turn, the inclusion of one grain legume every three years (i.e. GL1 treatment) improved the performance of durum wheat (i.e. the common crop of all the cropping systems studied used as a revelator of GL effect) when compared to the rotation without grain legumes (GL0). This finding would be explained by the positive effect of the presence of winter pea before durum wheat in GL1 instead of sunflower in GL0 due to the greater quality of pea crop residues in terms of N concentration (i.e. 12 and 19% in sunflower and winter pea, respectively). As a summer crop, sunflower is harvested later than pea and usually depletes soil water and mineral N at depth fact that could have led to pre-emptive competition for resources with the succeeding durum wheat (Kirkegaard et al., 2008; Rachidi et al., 1993). Moreover, the time of crop residue incorporation, later for sunflower when compared to pea (i.e. three months in our conditions), could have increased N immobilization after the emergence of the crop and during autumn, reducing tillering due to temporary N deficiency which in turn could reduce the potential performance of the succeeding durum wheat crop.

4.2 SOC and SON as affected by the incorporation of grain legumes in the rotations

The loss of SOC was aggravated in the bare fallow treatment, which reached an estimated rate of -595 and -735 kg C ha^{-1} yr^{-1} when one (GL1-BF) and two (GL2-BF) grain legumes were incorporated as cash crops, respectively. In contrast, the rotation without grain legumes (GL0-BF) lost SOC at a lower rate of 233 kg C ha^{-1} yr^{-1}. Similar to our results, in a 10-yr study carried out in southwestern Saskatchewan (Canada), Campbell et al., (2000) found a loss of SOC of 100 kg C ha^{-1} yr^{-1} when incorporating a lentil (*Lens culinaris* Medik.*)* every three years in a continuous wheat monocropping. Different findings were reported by Rochester (2011) when comparing different cotton-based cropping systems. After 10 years of experiment he observed an increase in SOC when cropping systems produced crop residues of greater N concentration, this is those that included legumes. However, as a difference with our study, the cropping systems studied by Rochester (2011) were based on minimum tillage. Regarding to this, Jensen et al., (2012) pointed out that N_2-fixing grain legumes have potential for SOC sequestration provided that no-tillage is used. This requirement is related to the deep soil C sequestration that occurs when no-till is performed. Thus, the conventional tillage management of our experiment could explain the high loss of C in the GL1 and GL2 treatments. Moreover, the time of tillage operations could have also influenced the dynamics of SOC in our experiment. Studdert and Echeverría (2000) observed greater SOC loss in summer crops-based sequences involving more tillage operations during spring. They hypothesized that those results could be the consequence of faster SOC decomposition when tillage is performed at greater temperatures.

Since C inputs were similar among grain legume treatments in BF and CC, we hypothesize that other variables could have played a major role on SOC dynamics in our experiment. It has to be taken into account that sorghum residues of high C:N ratio and lignin content were incorporated into the soil in the GL0 treatment. It is known that greater residue lignin and C:N ratios impact positively on SOC accumulation (Paustian et al., 1992). Contrarily, legume crop residues tend to decompose faster (Schomberg et al., 1994). Thus, the lower C:N ratio of crop residues returned to the soil in the GL1 and GL2 treatments would have hastened the decomposition of SOC. Interestingly, it must be noted that the weighed (taking into account all crop sequences) crop residue C:N ratios in the GL1 and GL2 treatments were 43 and 44, as an average of the BF and CC treatments, respectively. Those values are close to the threshold established by Vigil and Kissel (1991) below which net mineralization occurs. Low C:N ratio crop residues not only decompose faster but can also lead to a priming effect by altering soil microbial community due to a greater C use efficiency which accelerates the mineralization of native organic matter (Derrien et al., 2014; Kuzyakov, 2010; Manzoni et al., 2008). Thus, given the short-term nature of our experiment (i.e. 6 years), we could hypothesize that this effect could have played a major role in the turnover of the initial SOC in the GL1 and GL2 treatments. In addition, it has also been suggested that the incorporation of highly-decomposable plant material usually leads to a lower protection of C and N within aggregates. For instance, Martens (2000a) observed a decrease in soil aggregate stability when adding soybean (C3 plant) crop residues to the soil in comparison to maize (C4 plant) residues. This hypothesis is supported by the findings of Huggins et al., (1998), who observed a greater degree of humification under maize than under soybean due to the greater content of phenolic acids in the first. Similarly, Martens (2000b) observed a significant relationship between crop residue phenolic acid content and aggregate stability. Thus, a lower stability of soil aggregates could have led to a greater decomposition of soil organic matter in the GL1 and GL2 rotations. Contrarily, the presence of a C4 plant with a high phenolic acid content in the GL0 treatment (i.e. sorghum) would have led to a greater degree of soil organic matter humification reducing SOC losses.

4.3 SOC and SON as affected by the use of cover crops in grain legume-based rotations

The use of cover crops mitigated the loss of SOC and SON in the rotations studied. The rate of SOC loss was reduced by 13 and 67% when incorporating cover crops in the GL1 and GL2 treatments, respectively, while the loss of SON was reduced by a 59 and a 88% in the same treatments. Those findings are in line to those reported by Poeplau and Don (2015) in their 139-plots meta-analysis, who found an annual increase of SOC of 320 kg C ha^{-1} when including cover crops. The low C:N ratio of cover crop residues (i.e. lower than the one of grain legumes) would indicate that other factors different than the greater amount of aboveground C inputs would have counterbalanced the above-supposed priming effect. Different aspects could explain the mitigation of SOC and SON loss when using cover crops in arable cropping systems. First, cover crops usually grow during a short period of time in our conditions (2-4 months after emergence) and periods of autumn where the temperatures decrease, fact that leads to both reduced shoot to root ratios (Marcelis et al., 1998) and increasing soil C and N sequestration. As it was concluded by Rasse et al., (2005) and Kätterer et al., (2011), root-derived C usually contributes more significantly to the stable soil C pool than aboveground biomass-C. Secondly, the growth of cover crops implies a consumption of soil water and mineral N could result in less advantageous conditions for soil organic matter decomposition compared to a long bare fallow, due to N limiting conditions for microbial biomass.

In the literature different studies reported an increase of SOC with cover crops. For instance, Franzluebbers (2005) reported a sequestration rate of 250 kg C ha^{-1} yr^{-1} when using cover crops compared to bare fallow in southeastern United States. In turn, Follett (2001) reported an accumulation of SOC between 100 and 600 kg C ha^{-1} yr^{-1} when eliminating bare fallow periods. In the same line, in a factorial tillage, nitrogen and cover crop long-term experiment carried out in Italy, Mazzoncini et al., (2011) observed an increase of 80, 330 kg C ha^{-1} yr^{-1} and -10 and 35 kg N ha^{-1} yr^{-1} when non-legume, and legume cover crops were used, respectively, compared to bare fallow. Similarly, in a simulation study with the STICS soil-crop model, Constantin et al., (2012) reported a long-term maintenance of soil organic matter when cover crops were used in using the data of one rainfed arable cropping system of the center of France, under the same type of climate than our experiment. .

One explanation to the lack of SOC and SON sequestration in our experiment could be related by the low amount of biomass produced by cover crops due to the short growing time that was managed in order to avoid (i) delays in the sowing time of the succeeding cash crops and (ii) an excessive competition for water and nutrients. In turn, in our experiment the use of conventional tillage –with ploughing two times within three years and incorporation of residues by disking each year after harvest– could have maximized the decomposition of soil organic matter. When green manures are added to conventionally-tilled rotations, the increase in tillage frequency could mask their effect on organic matter accretion (Campbell et al., 1992; Drinkwater et al., 2000). Tillage accelerates SOC decomposition due to the breakage of soil macroaggregates reducing the formation of microaggregates with long-lasting C and N protective capacity (e.g. Plaza-Bonilla et al., 2013). Moreover, tillage buries crop residues deep in the soil where better conditions for decomposition occur (i.e. greater moisture and mineral nutrient contact) (Paustian et al., 1997). Regarding to this, Franzluebbers (2005) showed that SOC sequestration potential at the 0-20 cm soil depth increased around 20% when using cover crops under conservation tillage systems. In turn, Mazzoncini et al., (2011) concluded that cover crops can help to compensate the high mineralization rate found under intensively-tilled cropping systems. Those findings are in line with the results of Boddey et al., (2010) who studied the impact of cover crop legumes on SOC stocks in subtropical Oxisols concluding that C sequestration strongly depended on the type of tillage performed. The last authors found an increase of SOC from 5000 to 8000 kg ha^{-1} when using no-tillage in three long-term (15-26 years) rotations containing intercropped or cover-crop legumes, in comparison with the use of conventional tillage.

5. Conclusions

The results of our experiment show a significant decrease in SOC when introducing grain legumes in conventionally-tilled rotations cropped in European temperate climatic conditions. This finding suggests that the incorporation of crop residues with a low C:N ratio into the soil enhanced SOC decomposition that is aggravated due to tillage during bare fallow periods. However, the use of cover crops in these arable rotations mitigated the loss of SOC and SON without affecting the transfer of C and N to the grain and the final production of the cash crops. Given the low C:N ratio of cover crop residues, other factors supplemental to the amount of C incorporated to the soil would have contributed to the mitigation of soil organic matter losses. Thus, in grain legume-based cropping systems managed under conventional tillage, cover crops play an important role to maintain soil organic matter and crop productivity by recycling N from legumes and increasing the amount of C returned to the soil. Consequently, in these temperate climatic conditions, inserting grain legumes in cropping systems must be accompanied to the concomitant inclusion of cover crops in order to obtain a benefit from grain legumes.

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Figure captions

Fig. 1 Conceptual diagram of the six 3-year cropping systems tested in the experiment. GL0, GL1 and GL2 refer to the number of grain legumes included in each rotation (0, 1 and 2, respectively), CC and BF refer to cover crop and bare fallow, respectively. Main crops are shown in continuous line rectangles. Cover crops and bare fallow periods (in italics) are shown in dashed line rectangles.

Fig. 2 Monthly rainfall (columns) and air temperature (line) in the experimental area starting in September 2003 until end of August 2010. Rainfall and mean temperature for each growing season are shown at the top of the figure.

Fig. 3 Aboveground C (A) and N (B) inputs as affected by the number of grain legumes in a 3-year rotation (GL0, GL1 and GL2, 0, 1 and 2 grain legumes in the rotation) and cover crop treatments (CC, cover crop; BF, bare fallow). Values correspond to the annual average of two 3-year rotations (2003-2009). For total inputs, different lower-case letters indicate significant differences between rotations and cover crops at $P < 0.05$. * Indicates significant differences between cover crop treatments on the amount of inputs at $P < 0.05$. Vertical bars indicate standard deviation.

Fig. 4 Crop residue-C inputs of the different cash (A) and cover crops (B) (Mu, mustard; Sf, sunflower; Sg, sorghum; sP, spring pea; Sy, soybean; V, vetch; VO, vetch-oat mixture; W, durum wheat; wP, winter pea) as affected by the number of legumes in a 3-year rotation (GL0, GL1 and GL2, 0, 1 and 2 grain legumes in the rotation). The values are the mean of two 3-year rotations (2003-2009). For the cover crops, the preceding cash crop is shown between brackets. Vertical bars indicate standard deviation.

Fig. 5 Linear relationship (mixed linear model) between soil organic carbon concentration (0-30 cm soil depth) and the number of years since the beginning of the experiment as affected by the number of grain legumes in a 3-year rotation (GL0, GL1 and GL2, 0, 1 and 2 grain legumes in the rotation) and cover crop treatment (cover crop, CC; bare fallow, BF). Vertical bars indicate standard deviation.

Fig. 6 Linear relationship (mixed linear model) between soil organic nitrogen concentration (0-30 cm soil depth and the number of years since the beginning of the experiment as affected by the number of grain legumes in a 3-year rotation (GL0, GL1 and GL2, 0, 1 and 2 grain legumes in the rotation) and cover crop treatment (cover crop, CC; bare fallow, BF). Vertical bars indicate standard deviation.

Table 1. General site and soil characteristics in the 0- to 30-cm and 30- to 60-cm soil depths at the beginning of the experiment in 2003. Values between brackets correspond to the standard deviation.

Site and soil characteristics		
Latitude	43º 31' N	
Longitude	1º 30' E	
Elevation, m	150	
Soil depth (cm)	0 - 30	30 - 60
pH (H_2O, 1:2.5)	7.0 (0.5)	7.3 (0.7)
CEC, cmol+ kg^{-1}	18.1 (3.6)	
Organic C, g kg^{-1}	8.7 (1.0)	6.6 (0.8)
Organic N, g kg^{-1}	1.1 (0.1)	0.9 (0.1)
Particle size distribution, %		
Sand (2000-50 μm)	37.6 (6.4)	29.9 (5.1)
Silt (50-2 μm)	36.8 (2.9)	37.9 (2.1)
Clay (<2 μm)	25.6 (3.7)	30.0 (2.8)

Table 2. Cultivars of the different cash and cover crops (in italics) established in the cropping systems studied. GL0, GL1 and GL2 stand for 0, 1 and 2 grain legumes in the rotation. Values between brackets correspond to the seeding rate (seeds m^{-2}).

Rotation	Crop *Cover crop*	Year						
		First cycle of the three-year rotation				Second cycle of the three-year rotation		
		2003	2004	2005	2006	2007	2008	2009
GL0	Durum wheat	Provenzal (317)	Biensur (337)	Biensur (301)	Biensur (278)	Biensur (300)	Biensur (413)	Biensur (322)
	Vetch-Oat	-	*Topaze+Chantilly (73+170)*	*Topaze+Chantilly (37+170)*	*Topaze+Chantilly (50+43)*	*Topaze+Chantilly (31+140)*	*Topaze+Ranch (83+93)*	*Topaze+Ranch (44+100)*
	Sorghum	-	Solarius (31)	Solarius (29)	Solarius (29)	Fulgus (30)	Fulgus (30)	Fulgus (30)
	Sunflower	-	Melody (7)	Melody (7)	Melody (7)	Melody (7)	Melody (7)	Melody (7)
	Vetch	-	*Alfalfa undersown (550)*	*Topaze (73)*	*Topaze (50)*	*Topaze (50)*	*Topaze (73)*	*Topaze (76)*
GL1	Durum wheat	Provenzal (317)	Biensur (337)	Biensur (301)	Biensur (278)	Biensur (300)	Biensur (413)	Biensur (322)
	Vetch-Oat	-	*Topaze+Chantilly (73+170)*	*Topaze+Chantilly (37+170)*	*Topaze+Chantilly (50+43)*	*Topaze+Chantilly (31+140)*	*Topaze+Ranch (83+93)*	*Topaze+Ranch (44+100)*
	Sunflower	-	Melody (7)	Melody (7)	Melody (7)	Melody (7)	Melody (7)	Melody (7)
	Mustard	-	*Twist+Concerta (160)*	*Twist+Concerta (172)*	*Twist+Concerta (172)*	*Twist+Concerta (160)*	*Albatros (171)*	*Albatros (135)*
	Winter pea	Ideal (72)	Ideal (71)	Ideal (79)	Cartouche (70)	Cartouche (79)	Hardy* (100)	Izar (83)
	Mustard	-	*Twist+Concerta (160)*	*Twist+Concerta (172)*	*Twist+Concerta (172)*	*Twist+Concerta (160)*	*Albatros (171)*	*Albatros (135)*
GL2	Durum wheat	Provenzal (317)	Biensur (337)	Biensur (301)	Biensur (278)	Biensur (300)	Biensur (413)	Biensur (322)
	Mustard	-	*Twist+Concerta (160)*	*Twist+Concerta (172)*	*Twist+Concerta (172)*	*Twist+Concerta (160)*	*Albatros (171)*	*Albatros (135)*
	Soybean	-	Dekabig (39)	Dekabig (38)	Dekabig (39)	Isidor (39)	Isidor (36)	Isidor (36)
	Spring pea	-	Hardy (110)	Hardy (109)	Hardy (100)	Hardy (101)	Hardy (113)	Hardy (100)
	Mustard	-	*Twist+Concerta (160)*	*Twist+Concerta (172)*	*Twist+Concerta (172)*	*Twist+Concerta (160)*	*Albatros (171)*	*Albatros (135)*

* In 2008 a spring pea cultivar was sown instead the winter one due to excessively humid soil conditions at the end of autumn.

Table 3. Results of the analysis of variance (probability level) of the effects of the six cropping systems studied (combination of number of grain legumes in a 3-year rotation and cover crop treatments), the year of sampling and their interaction on durum wheat grain yield, and durum wheat C and N harvest indexes. For the rest of crops (i.e. sorghum, sunflower, winter pea, soybean and spring pea) the results of the analysis of variance of the effects of the use of cover crops, year of sampling and their interaction on grain yield and C and N harvest indexes are shown.

ANOVA	Durum wheat	Sorghum	Sunflower	Winter pea	Soybean	Spring pea
			Grain yield			
Cover crop		0.531	0.657	0.388	0.874	0.482
Year	<0.001	<0.001	0.012	<0.001	0.583	<0.001
Cover crop x Year		0.076	0.966	0.454	0.944	0.577
Cropping system	0.047					
Cropping system x Year	0.045					
			C harvest index			
Cover crop		0.820	0.958	0.592	0.250	0.661
Year	<0.001	<0.001	<0.001	0.026	0.002	0.002
Cover crop x Year		0.993	0.518	0.933	0.190	0.567
Cropping system	0.013					
Cropping system x Year	0.005					
			N harvest index			
Cover crop		0.747	0.628	0.247	0.800	0.936
Year	<0.001	0.102	<0.001	<0.001	0.566	<0.001
Cover crop x Year		0.013	0.897	0.853	0.622	0.454
Cropping system	0.230					
Cropping system x Year	0.373					

Table 4. Grain yield of the different cash crops used in each treatment (GL0, GL1 and GL2, 3-year rotation with 0, 1 and 2 grain legumes, respectively; BF and CC, bare fallow and cover crops, respectively) at 100 g kg^{-1} grain moisture content. Values between brackets correspond to the standard deviation. For durum wheat, different lower-case letters indicate differences between cropping systems (i.e. combination of GL rotations and cover crop treatments) at *P*<0.05.

Rotation	Crop	Year												Average	
		First cycle of the three-year rotation						Second cycle of the three-year rotation							
		2004		2005		2006		2007		2008		2009			
		BF	CC	BF	CC	BF	CC	BF	CC	BF	CC	BF	CC	BF	CC
GL0	Sorghum	9410	8443	7008	7017	7673	6813	7444	7522	8182	8477	7501	8348	7870 (840)	7709 (786)
	Sunflower	3140	2196	3191	3164	2346	2148	3322	3261	3312	3163	2717	2460	3004 (653)	2692 (765)
	Durum wheat	6208 c	6309 bc	5617	6181	5085 b	4637 b	6519	6331	5015 b	5859 ab	4840 a	4309 ab	5547 (897) c	5604 (918) c
GL1	Sunflower	3982	3976	3139	3112	1881	2256	3298	3261	2836	3031	2452	2769	2931 (852)	3067 (674)
	Winter pea	3854	4394	5252	4834	2947	2806	2111	2117	2725	2931	869	1638	2960 (1464)	3120 (1240)
	Durum wheat	7077 abc	7200 abc	6019	5615	6859 a	5604 b	6387	6977	5965 ab	5918 ab	4143 ab	4842 a	6075 (1026) a	6026 (893) ab
GL2	Soybean	2989	3894	4025	3715	3803	3737	3371	3534	3708	3842	2888	2459	3464 (787)	3530 (915)
	Spring pea	3699	3893	3735	4560	3797	3711	4738	4743	5032	4755	1541	1639	3757 (1236)	3883 (1179)
	Durum wheat	7414 a	7272 ab	5573	5657	5423 b	5294 b	6207	6285	6167 a	6073 a	4865 a	3329 b	5941 (919) abc	5652 (1365) bc

Table 5. Carbon and nitrogen harvest indexes of the different crops used in each rotation (GL0, GL1 and GL2, 3-year rotation with 0, 1 and 2 grain legumes, respectively). Values are the mean of two 3-year rotations (from 2003 to 2009). Values between brackets correspond to the standard deviation.

Rotation	Crop	C harvest index		N harvest index	
		Bare fallow	Cover crop	Bare fallow	Cover crop
GL0	Sorghum	0.50 (0.04)	0.50 (0.04)	0.67 (0.04)	0.68 (0.04)
	Sunflower	0.37 (0.03)	0.36 (0.05)	0.52 (0.06)	0.50 (0.07)
	Durum wheat	0.46 (0.04) a	0.46 (0.05) a	0.74 (0.07)	0.75 (0.06)
GL1	Sunflower	0.36 (0.03)	0.37 (0.03)	0.53 (0.08)	0.53 (0.07)
	Winter pea	0.40 (0.06)	0.40 (0.05)	0.53 (0.11)	0.56 (0.08)
	Durum wheat	0.43 (0.04) b	0.43 (0.04) b	0.72 (0.06)	0.72 (0.05)
GL2	Soybean	0.48 (0.03)	0.47 (0.05)	0.81 (0.03)	0.80 (0.04)
	Spring pea	0.44 (0.06)	0.43 (0.07)	0.59 (0.09)	0.60 (0.11)
	Durum wheat	0.45 (0.04) a	0.46 (0.04) a	0.74 (0.08)	0.74 (0.07)

Table 6. Nitrogen concentration of the different crops used in each rotation (GL0, GL1 and GL2, 3-year rotation with 0, 1 and 2 grain legumes, respectively) (kg N t^{-1} dry matter). Values are the mean of two 3-year rotations (from 2003 to 2009). In the vetch-oat mixture, the values correspond to the crop between brackets. Values between brackets correspond to the standard deviation.

Rotation	Crop	Cover crop treatments	
		Bare fallow	Cover crop
GL0	Sorghum	6.6 (1.7)	6.6 (1.6)
	Sunflower	11.9 (2.3)	12.7 (3.6)
	Vetch	-	*40.9 (4.4)*
	Durum wheat	7.2 (1.9)	6.6 (1.0)
	(Vetch)-Oat	-	*38.2 (5.3)*
	Vetch-(Oat)	-	*26.8 (8.3)*
GL1	Sunflower	12.1 (2.0)	12.0 (1.9)
	Mustard	-	*28.6 (8.2)*
	Winter pea	18.5 (4.3)	17.7 (3.2)
	Mustard	-	*29.1 (5.5)*
	Durum wheat	7.3 (2.3)	7.3 (1.6)
	(Vetch)-Oat	-	*39.9 (5.1)*
	Vetch-(Oat)	-	*26.9 (10.0)*
GL2	Soybean	14.4 (3.2)	14.1 (3.0)
	Spring pea	15.6 (4.4)	15.4 (1.0)
	Mustard	-	*26.3 (5.6)*
	Durum wheat	6.8 (1.8)	7.2 (2.3)
	Mustard	-	*20.8 (7.0)*

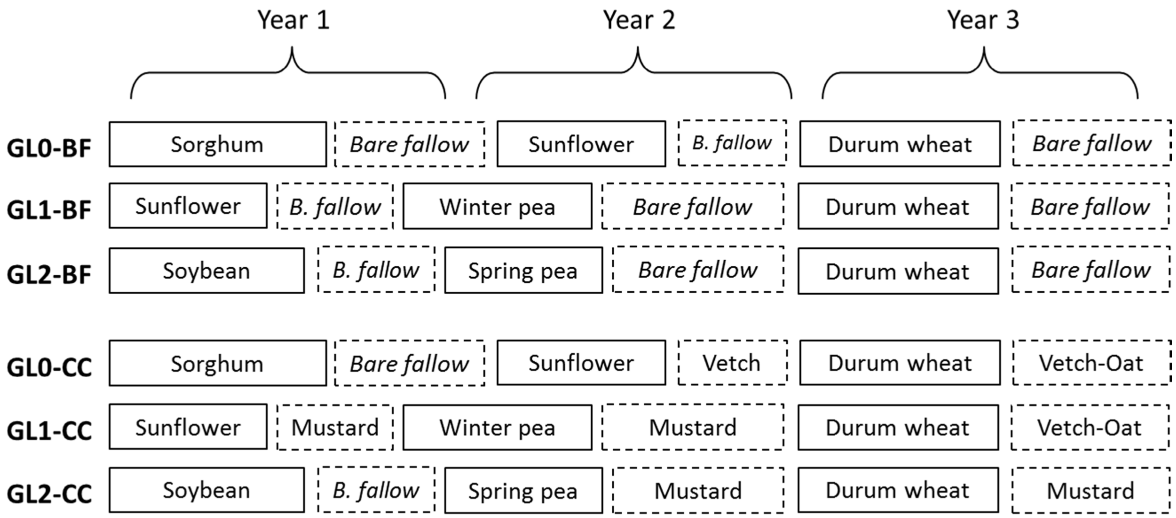

Year 1
Year 2
Year 3
GL0-BF
Sorghum
Bare fallow
Sunflower
B. fallow
Durum wheat
Bare fallow
GL1-BF
Sunflower
B. fallow
Winter pea
Bare fallow
Durum wheat
Bare fallow
GL2-BF
Soybean
B. fallow
Spring pea
Bare fallow
Durum wheat
Bare fallow
GL0-CC
Sorghum
Bare fallow
Sunflower
Vetch
Durum wheat
Vetch-Oat
GL1-CC
Sunflower
Mustard
Winter pea
Mustard
Durum wheat
Vetch-Oat
GL2-CC
Soybean
B. fallow
Spring pea
Mustard
Durum wheat
Mustard

Fig. 1

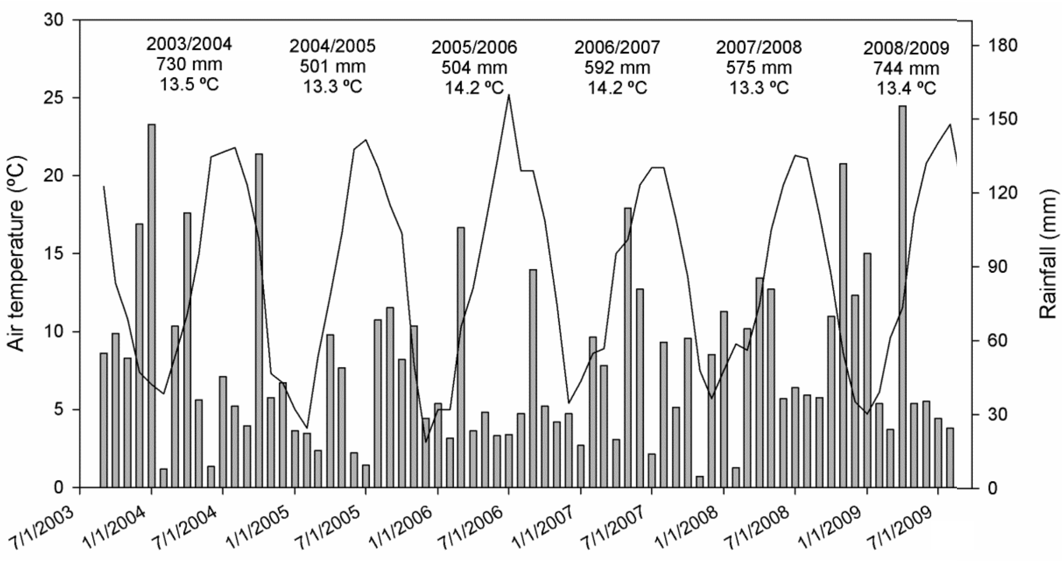
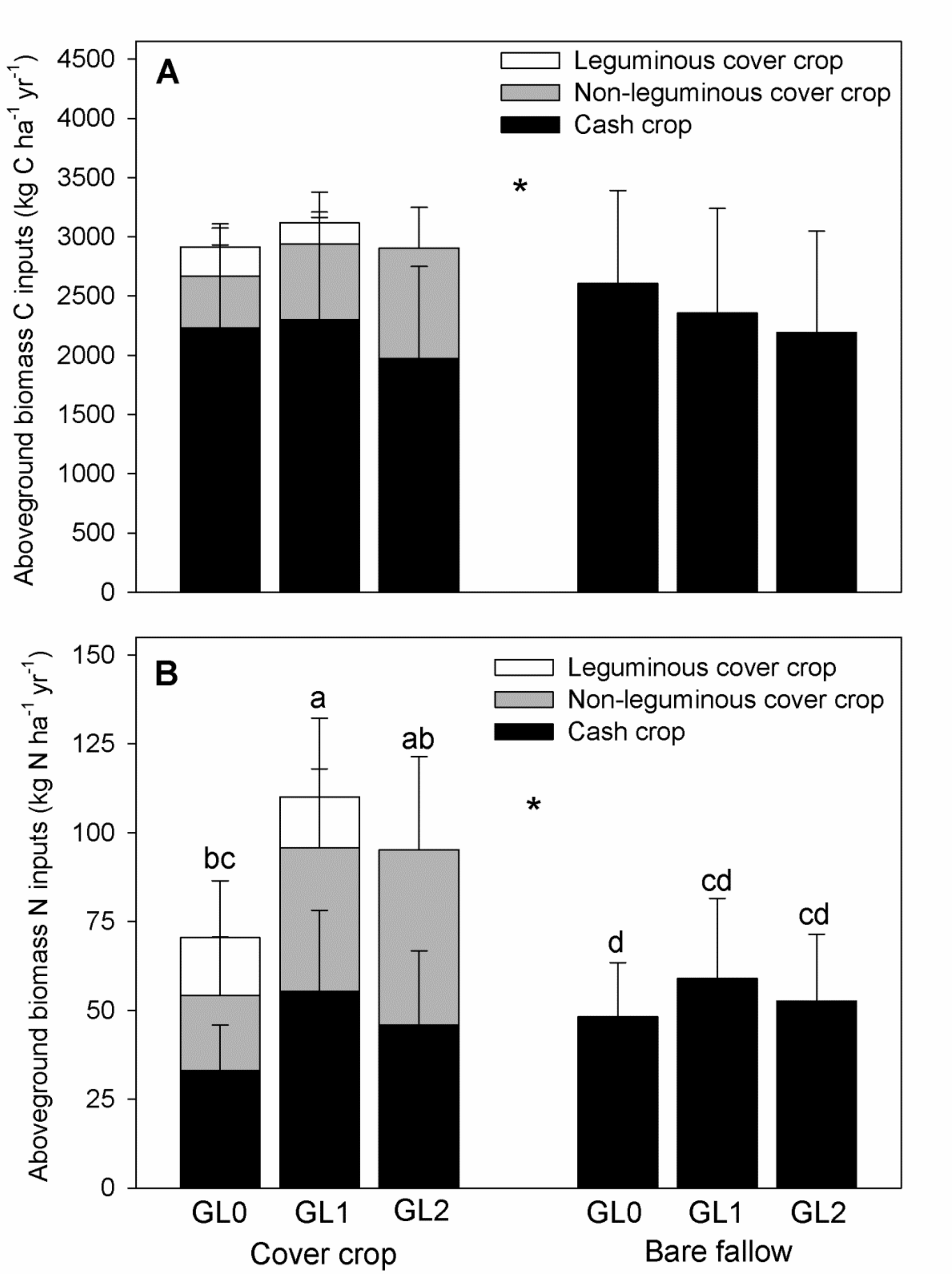

2003/2004
730 mm
13.5 ºC
2004/2005
501 mm
13.3 ºC
2005/2006
504 mm
14.2 ºC
2006/2007
592 mm
14.2 ºC
2007/2008
575 mm
13.3 ºC
2008/2009
744 mm
13.4 ºC
Air temperature (ºC)
Rainfall (mm)
0
5
10
15
20
25
30
0
30
60
90
120
150
180
7/1/2003
1/1/2004
7/1/2004
1/1/2005
7/1/2005
1/1/2006
7/1/2006
1/1/2007
7/1/2007
1/1/2008
7/1/2008
1/1/2009
7/1/2009

Fig. 2



A
Aboveground biomass C inputs (kg C ha^{-1} yr^{-1})
4500
4000
3500
3000
2500
2000
1500
1000
500
0
Leguminous cover crop
Non-leguminous cover crop
Cash crop
*
B
Aboveground biomass N inputs (kg N ha^{-1} yr^{-1})
150
125
100
75
50
25
0
Leguminous cover crop
Non-leguminous cover crop
Cash crop
a
ab
bc
*
cd
d
cd
GL0
GL1
GL2
GL0
GL1
GL2
Cover crop
Bare fallow

Fig. 3

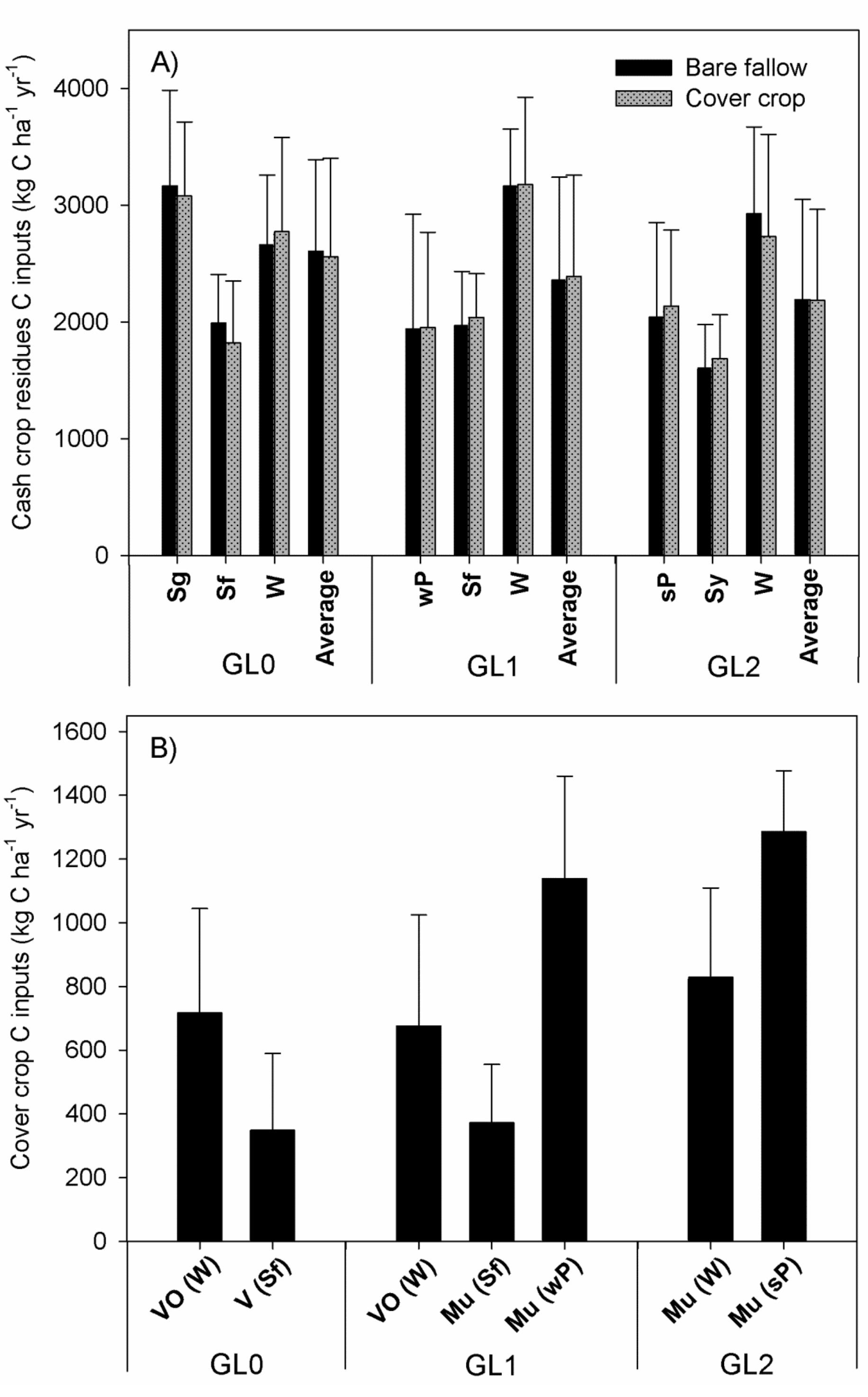

A)
Bare fallow
Cover crop
Cash crop residues C inputs (kg C ha-1 yr-1)
0
1000
2000
3000
4000
Sg
Sf
W
Average
wP
Sf
W
Average
sP
Sy
W
Average
GL0
GL1
GL2
B)
Cover crop C inputs (kg C ha-1 yr-1)
0
200
400
600
800
1000
1200
1400
1600
VO (W)
V (Sf)
VO (W)
Mu (Sf)
Mu (wP)
Mu (W)
Mu (sP)
GL0
GL1
GL2

Fig. 4

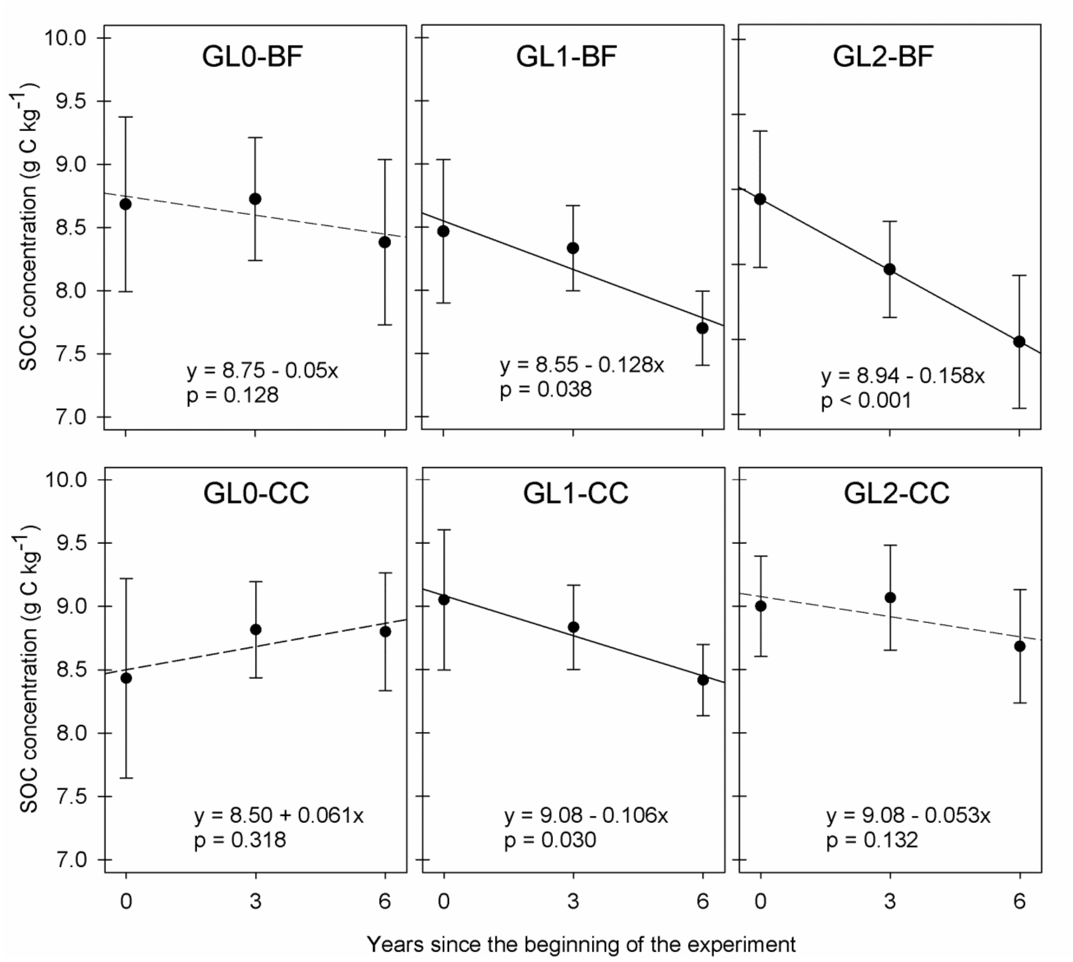

GL0-BF
GL1-BF
GL2-BF
GL0-CC
GL1-CC
GL2-CC
SOC concentration (g C kg^{-1})
10.0
9.5
9.0
8.5
8.0
7.5
7.0
y = 8.75 - 0.05x
p = 0.128
y = 8.55 - 0.128x
p = 0.038
y = 8.94 - 0.158x
p < 0.001
y = 8.50 + 0.061x
p = 0.318
y = 9.08 - 0.106x
p = 0.030
y = 9.08 - 0.053x
p = 0.132
0
3
6
Years since the beginning of the experiment

Fig. 5

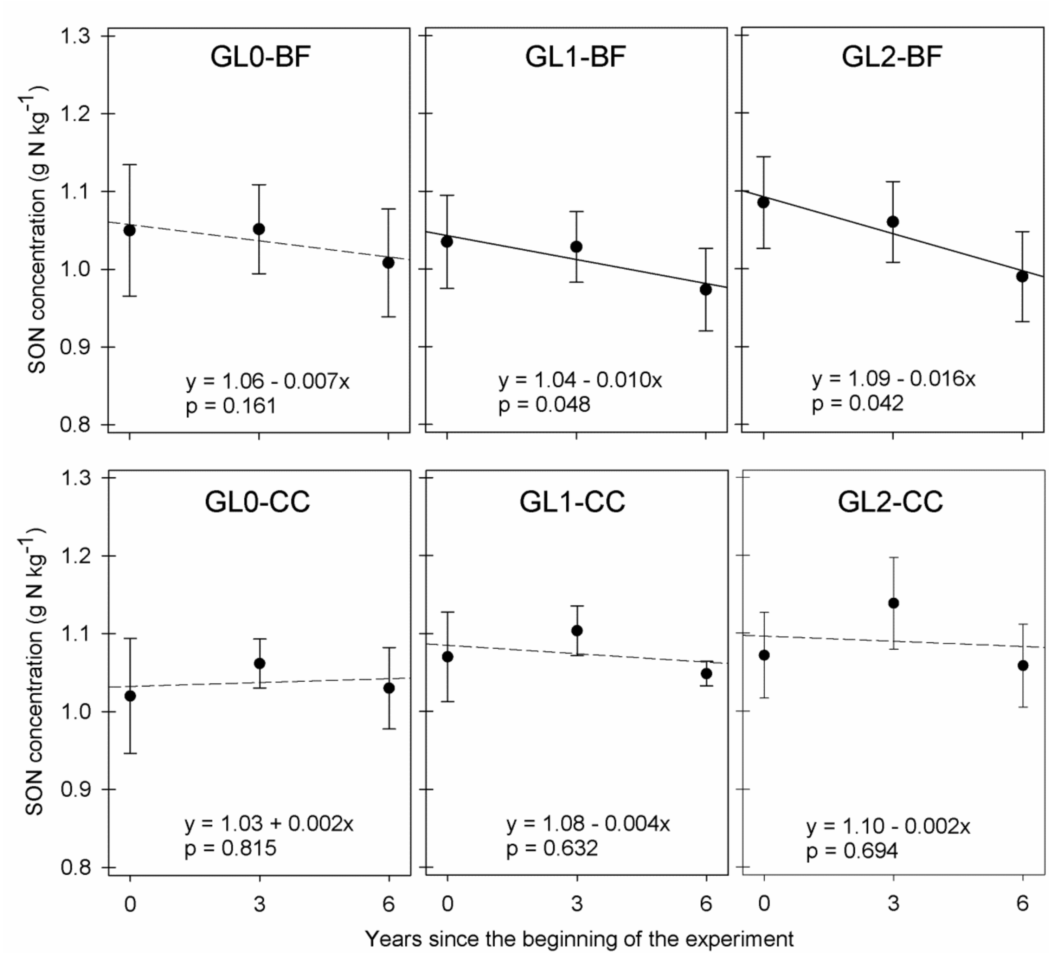

GL0-BF
GL1-BF
GL2-BF
GL0-CC
GL1-CC
GL2-CC
SON concentration (g N kg^{-1})
1.3
1.2
1.1
1.0
0.9
0.8
y = 1.06 - 0.007x
p = 0.161
y = 1.04 - 0.010x
p = 0.048
y = 1.09 - 0.016x
p = 0.042
y = 1.03 + 0.002x
p = 0.815
y = 1.08 - 0.004x
p = 0.632
y = 1.10 - 0.002x
p = 0.694
0
3
6
Years since the beginning of the experiment

Fig. 6