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RESEARCH ARTICLE

Cover crop mixtures increase ecosystem multifunctionality in summer crop rotations with low N fertilization

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Abstract

Cropping diversification with cover crop mixtures combined with low N fertilization represents an ecological alternative that may promote sustainability. Our objective was to evaluate changes on soil organic fractions and structure, cover crop biomass, and main crop yield 5 years after the introduction of two cover crop mixtures, oats+forage radish (CC1) and oats+forage radish+vetch (CC2), in a soybean-soybean and maize-soybean sequence with low N fertilization of maize. After 5 years, the soil from sequences with cover crops had higher concentrations of soil organic carbon (SOC) (23.3 vs 20.1 g kg^{-1}), soil organic nitrogen (SON) (2.4 vs 2.0 g kg^{-1}), and particulate organic carbon (POC) (4.4 vs 2.9 g kg^{-1}) at 0–5 cm depth than the controls without cover crops, in association with C input from cover crops aboveground biomass, which averaged 2.2 and 3.0 Mg ha^{-1} year^{-1} for CC1 and CC2, respectively. Soil aggregation at 0–5 cm depth was more stable with than without cover crops (33.4 vs 16.4%), and it was positively related to SOC ($R^2 = 0.44$, $p < 0.01$) and POC ($R^2 = 0.50$, $p < 0.01$) concentrations. Soil from CC2 had a higher proportion of macropores and mesopores over 300 μm than soil from CC1 and the controls without cover crops at 0–5 and 10–30 cm depth, respectively. Maize yield was affected by rainfall: it was similar among treatments in dry growing seasons (<5.0 Mg ha^{-1}) and higher in CC2 and the control without cover crops than in CC1 in more humid seasons (9.2 vs 7.9 Mg ha^{-1}). Soybean yield was similar among treatments except after dry cover crop growing seasons, when control treatments yielded more than cover crop treatments (3.4 vs 2.8 Mg ha^{-1}). This study demonstrates that summer crop sequences with cover crop mixtures increase ecosystem multifunctionality and that including vetch in the mixture increases its production potential and benefits, especially in the soybean-soybean sequence.

Keywords Maize · Soybean · Yield · Soil organic carbon · Soil organic nitrogen · Soil structure · Cover crop biomass · C/N ratio · Nitrate leaching · Available water

1 Introduction

Agricultural systems are under considerable pressure to increase productivity, decrease the pollution of waters and the atmosphere, and buffer against climate change (Schipanski et al. 2016). Over the past 50 years, global agricultural systems have evolved towards very simplified schemes based on sole crops in a year and long fallow periods. These systems are characterized by a high dependence on fossil energy (fertilizers, pesticides, and fuel), low efficiency of inputs, intensification of outputs (grain exports and nutrient losses), vulnerability to climate variability, and loss of soil quality and capacity to provide some ecosystem services (Tonitto et al. 2006; Viglizzo et al. 2011; Wingeyer et al. 2015). This type of intensification has improved yields: global agricultural production increased by 47%, supported by 5.6-fold and 2.5-fold increases in nitrogen (N) and phosphorus fertilizer inputs, but to the detriment of environmental quality and system resilience (Schipanski et al. 2016). Unfortunately, N fertilizer sources are not utilized efficiently in agricultural systems, and plant uptake seldom exceeds 50% of the N applied (Peoples et al. 2004). The volatile and mobile nature of some inorganic N forms as well as the asynchronies in N supply to and use by annual crops in most cropping systems results in losses to the environment. Growing concern about the environmental impacts of current agroecosystems boosted interest in alternative cropping schemes designed to increase

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taxonomic diversity, restore degraded soil functions, and provide ecosystem benefits beyond maximizing crop yield (Thorup-Kristensen et al. 2003, Schipanski et al. 2014). In this sense, previous studies have demonstrated that introducing cover crops in a rotation increases soil organic matter and aggregation (Poeplau and Don 2015, Restovich et al. 2019) and enhances N conservation and recycling within the soil-plant system (Portela et al. 2016; Restovich et al. 2012). Furthermore, some studies showed that cover crops supply nutrients to the succeeding crops through mineralization of residues, enabling the reduction of fertilization rates. Through the uptake and subsequent N release by residue decomposition in synchrony with the main crop demand, cover crops can maintain maize and soybean yields similar to or higher than those obtained without cover crops (Restovich et al. 2012, Schipanski et al. 2014). On the other hand, other studies have documented yield reductions with cover crops, and these differences seem to depend on cover crop management, local soil, and climate conditions (Abdalla et al. 2019). Tribouillois et al. (2018) reported that the consumption of soil water by cover crops and immobilization of soil N in cover crop biomass may reduce subsequent crop yield.

Each cover crop species is associated to specific functions related to different ecosystem services. Legumes fix N biologically through symbiosis with bacteria enabling the possibility of reducing N additions through inorganic fertilization (Kaye and Quemada 2017). Grasses generally produce abundant and slow decomposing biomass due to its relatively high C/N ratio, providing protection from erosion as well as regulating soil temperature and moisture content (Daryanto et al. 2018). Grasses also have fibrous roots with a large number of branches that act as a mesh, improving soil structure (Loades et al. 2013). On the other hand, crucifers produce tap-roots that penetrate deep soil layers reducing compaction (Chen et al. 2014). Other functions are related to traits rather than to a particular species or family. For example, weed suppression, N absorption, and soil C input are positively correlated with cover crop biomass production (Finney et al. 2016).

The incorporation of cover crop mixtures in rotation with cash crops is an innovative emerging strategy to enhance ecosystem multifunctionality (Finney and Kaye 2017). Mixtures of cover crops should be composed by species that provide complementary ecosystem services that increase the resilience and sustainability of agricultural systems (Schipanky et al. 2014). Restovich et al. (2012) found that maize yield after a mixture of oats (*Avena sativa* L.) and vetch (*Vicia sativa* L.) was similar than after vetch, but residual N at maize harvest was lower, demonstrating the potential of combining these two species for simultaneously supplying and retaining N within the soil-plant system. In the same trial, Restovich et al. (2011) found that forage radish (*Raphanus sativus* L.) increased macroporosity and that oats increased aggregation stability more than the other species evaluated as cover crops. The benefits reported for these species used as cover crops encouraged us to explore mixtures of species seeking for combinations of benefits and potential synergies that could increase productivity and long-term system sustainability. In this sense, combining vetch with forage radish and oats as cover crops could potentiate their capacity to supply N and enhance soil structure simultaneously.

In this paper, we evaluated how two cover crop mixtures that combine species with diverse functional traits impact on different ecosystem functions simultaneously when introduced in summer crop rotations with low N fertilization. We hypothesize that cover crop mixtures enhance soil organic matter accumulation and improve soil structure in association with an increase in carbon (C) input and N retention through cover crop biomass and that the extra N input when including vetch enhances productivity of the mixture and of the main crops. Our objective was to evaluate changes on soil properties (soil organic C and N and structure) and productivity (cover crop biomass and main crop yield) after 5 years of rotation with two cover crop mixtures introduced in two summer crop sequences. The mixtures evaluated combined a grass (oats) with a crucifer (forage radish) with or without a legume (vetch), and they were introduced in a soybean-soybean and a maize-soybean sequence combined with low N fertilization of maize (Fig. 1).

2 Materials and methods

2.1 Field experiment

A field experiment was set up in 2011 at the Pergamino Experimental Station of the Instituto Nacional de Tecnología Agropecuaria (INTA) (33° 51′ S, 60° 40′ W) introducing fall-winter cover crops in two rain-fed summer cash crop sequences (soybean-soybean and maize-soybean) sown under no tillage. The species used as cover crops were oats, vetch, and forage radish, combined in two mixtures: oats+forage radish (CC1) with densities of 80 and 20 kg seed ha^{-1}, respectively, and oats+forage radish+vetch (CC2) with densities of 20, 20, and 40 kg seed ha^{-1}, respectively. We also included a control without cover crops for each summer crop sequence which was maintained free of weeds chemically. The experiment resulted in six treatments: (a) soybean-CC1-soybean, (b) soybean-CC2-soybean, (c) soybean-fallow-soybean (control), (d) soybean-CC1-maize, (e) soybean-CC2-maize, and (f) soybean-fallow-maize (control). The experimental design consisted of a split plot in a randomized complete block arrangement with three blocks. The main plots (30 m long and 15 m wide) corresponded to the main crop sequence and were divided lengthwise into three subplots that corresponded to the cover crop treatments. In Pergamino, the climate is temperate humid, without a dry season, with a mean annual

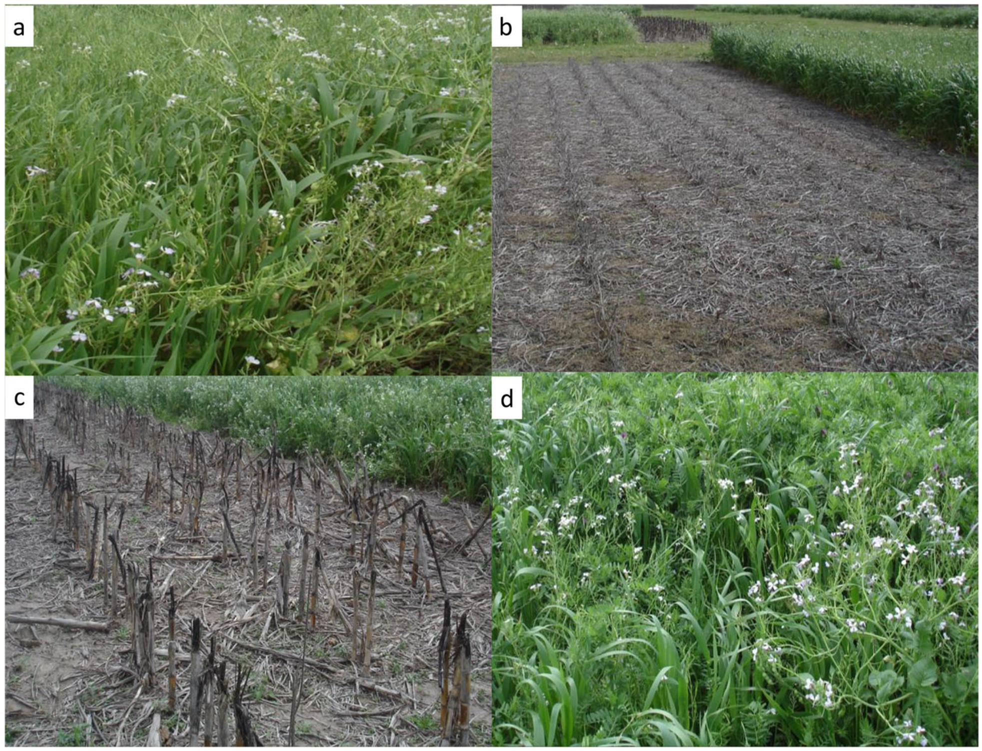

Figure 1. Two mixtures of fall-winter cover crops were introduced in two rain-fed summer cash crop sequences (soybean-soybean and maize-soybean) sown under no tillage. The species used as cover crops were oats, vetch, and forage radish, in two combinations: oats+forage radish (**a**) and oats+forage radish+vetch (**d**), and there were control treatments where the intercropping period remained fallow (**b** and **c**). Photographs by Silvina Beatriz Restovich.

temperature of 16.5 °C and mean annual rainfall of 988 mm for the 1910–2018 period (Agroclimatological Network Database, INTA). Average annual rainfall during this study (2011–2016) was 1230 mm. Rainfall occurs mainly during fall and spring, with the summer months being characterized by rainfall deficits of varying intensity (Hall et al. 1992) which may impact, particularly, on maize productivity.

Maize (*Zea mays* L.) hybrid DK 747 was sown in early October in rows spaced 0.70 m apart (75,000 plants ha^{-1}) in 2011, 2013, 2015, and 2017, and soybean (*Glycine max* L.) var. DM 5.1 was sown in November in rows spaced 0.52 m apart (500,000 plants ha^{-1}) in 2012, 2014, and 2016 in the maize-soybean sequence or every year in the soybean-soybean sequence (Table 1). Cover crop mixtures were sown in April or early May in rows spaced 0.17 m apart. Cover crop sowing dates were determined according to the previous main crop harvest date and to the distribution of rainfall during fall. Cover crops and maize were fertilized at sowing with 14.7 and 32 kg P_2O_5 ha^{-1}, respectively, and maize was fertilized at $V_{5\text{-}6}$ stage with 32 kg N ha^{-1}. Vetch and soybean were inoculated with *Rhizobium leguminosarum* biovar *Viceae* and *Bradyrhizobium* sp., respectively, immediately before sowing. The cover crop killing dates were determined according to soybean and maize sowing dates and to the distribution of rainfall, to ensure adequate soil moisture at main crop sowing. Cover crops were terminated in winter or early spring (August–September) before maize and in spring (October) when preceding soybean, rendering growing periods of 4 and 5–7 months, respectively. Cover crops were terminated with 3–4 L ha^{-1} of glyphosate (48% active ingredient). Weeds were controlled with pre-emergence application of atrazine (2 kg ha^{-1}) and post-emergence application of glyphosate (3–4 L ha^{-1}) for maize and soybean, respectively. Pest and disease controls were not necessary because they were below the economic damage threshold.

2.2 Plant and soil measurements

At the beginning of the experiment (April 2011) and after five cover crop-main crop cycles (April 2016), a disturbed and an undisturbed (cylinder) soil sample was extracted from 0 to 5, 5 to 10, 10 to 20, and 20 to 30 cm depths from each subplot to determine bulk density, pore size distribution, aggregate stability, soil organic carbon (SOC), soil organic nitrogen (SON), and particulate organic carbon (POC). Disturbed samples were extracted with a 5-cm-diameter auger and used to determine aggregate stability, SOC, SON, and POC. Undisturbed cylinders were used to determine bulk density and pore size distribution. Additionally, during the first 3 years of experiment (2011, 2012, and 2013), soil samples were extracted from each subplot from 0 to 20, 20 to 27, 27 to 52, 52 to 82, and 82 to 100 cm depths (corresponding to each soil horizon) at cover crop termination, to determine nitrate (NO_3) and available water content.

Bulk density was determined by the cylinder method (58.9 cm^3 volume) (Burke et al. 1986). Pore size distribution was calculated using the relationship between soil water content and matric potential (Hillel 1980). Soil pores were classified as micropores (<15 μm diameter), mesopores (15–60 μm),

Table 1. Cropping calendar, rainfall, temperature, and fertilization rates of the experimental plots. Harvest date refers to main crops, and killing date corresponds to cover crop termination. At termination of 2011, 2012, and 2013 cover crops (*), soil samples were extracted from 0 to 100 cm depth to determine nitrate (NO_3) and available water content. At harvest of 2015/2016 main crop (**), soil samples were extracted from 0 to 30 cm depth to determine pore size distribution, aggregate stability, soil organic carbon (SOC), soil organic nitrogen (SON), and particulate organic carbon (POC).

Crop	Sowing date	Harvest or killing date	Rainfall	Average temperature (°C)		Fertilization	
			mm	Max	Min	kg N ha^{-1}	kg P_2O_5 ha^{-1}
Maize-soybean							
CC 2011(*)	28 Apr 11	17 Aug 11	96	16.2	2.8	--	14.7
Maize 2011/12	3 Oct 11	19 Mar 12	592	28.2	13.0	32	31.5
CC 2012(*)	4 Apr 12	12 Oct 12	571	19.0	6.3	--	14.7
Soybean 2012/13	6 Nov 12	10 Apr 13	533	27.3	13.1	--	--
CC 2013(*)	16 Apr 13	16 Aug 13	145	18.6	5.2	--	14.7
Maize 2013/14	9 Oct 13	25 Mar 14	910	27.5	14.7	32	31.5
CC 2014	24 Apr 14	16 Oct 14	356	19.5	6.5	--	14.7
Soybean 2014/15	7 Nov 14	10 Apr 15	748	26.9	14.1	--	--
CC 2015	24 Apr 15	21 Aug 15	477	18.6	7.5	--	14.7
Maize 2015/16(**)	5 Oct 15	28 Mar 16	705	27.4	13.5	32	31.5
CC 2016	3 May 16	28 Oct 16	238	16.3	6.2	--	14.7
Soybean 2016/17	15 Nov 16	20 Apr 17	841	26.8	15.0	--	--
CC 2017	4 May 17	6 Sep 17	272	19.0	8.5	--	14.7
Maize 2017/18	4 Oct 17	20 Mar 18	317	28.1	12.5	32	31.5
Soybean-soybean							
CC 2011	28 Apr 11	17 Oct 11	185	18.1	4.2	--	14.7
Soybean 2011/12	8 Nov 11	12 Apr 12	564	28.4	13.0	--	--
CC 2012	20 Apr 12	12 Oct 12	557	18.3	5.9	--	14.7
Soybean 2012/13	6 Nov 12	10 Apr 13	533	27.3	13.1	--	--
CC 2013	16 Apr 13	10 Oct 13	196	19.6	6.1	--	14.7
Soybean 2013/14	21 Nov 13	21 Apr 14	866	27.3	15.1	--	--
CC 2014	24 Apr 14	16 Oct 14	356	19.5	7.7	--	14.7
Soybean 2014/15	7 Nov 14	10 Apr 15	748	26.9	14.1	--	--
CC 2015	24 Apr 15	17 Oct 15	584	19.1	7.8	--	14.7
Soybean 2015/16(**)	6 Nov 15	28 Apr 16	826	27.7	14.0	--	--
CC 2016	3 May 16	28 Oct 16	238	16.3	6.2	--	14.7
Soybean 2016/17	15 Nov 16	20 Apr 17	841	26.8	15.0	--	--
CC 2017	4 May 17	24 Oct 17	401	19.7	9.0	--	14.7
Soybean 2017/18	15 Nov 17	13 Apr 18	351	28.5	13.2	--	--

and macropores (60–300 and > 300 μm) (Hillel 1980). Cores in the sampling cylinders were saturated under vacuum for 24 h to minimize structural breakdown and subsequently taken to −1, −5, and −20 kPa matric potential using a tension table with a hanging water column (Bezerra de Oliveira 1968). Soil water retention at each potential was expressed in volumetric water content by using bulk densities for conversion from gravimetric to volumetric water contents. Aggregate stability was determined through water sieving using the Douglas and Goss (1982) method with slight modifications. Ten grams of air-dried 1–2-mm aggregates were placed on a 0.5-mm sieve and mechanically raised and lowered into water for 5 min. This size of aggregates was used because they are sensitive to short-term management changes (Rillig et al. 2002). The stability index was calculated as the ratio between the dry weight of over-0.5-mm aggregates and the dry weight of 1–2-mm aggregates and expressed as a percentage (Kemper 1965). Aggregate stability was classified as unstable (<20%), moderately stable (20–40%), and stable (>40%) (Irizar et al. 2015). Soil organic carbon was determined by wet digestion by Walkley-Black method (Nelson and Sommers 1982), SON was determined by Kjeldahl method (Mulvaney 1996), and POC by the method of Cambardella and Elliot (1992), replacing chemical dispersion in the original method by mechanical dispersion through water agitation of the soil sample with glass balls during 5 h at 40 rpm (Irizar et al. 2010). Soil organic carbon and SON were adjusted to an equivalent topsoil mass of 2300 Mg ha^{-1} to account for bulk density differences between 2011 and 2016 (Poulton et al. 2003). Soil nitrate concentration was determined by the phenol disulfonic method, and water content was measured gravimetrically. Nitrate-N content (kg ha^{-1}) at 0–100 cm depth was calculated as the sum of the products between nitrate concentration, layer thickness (m), and bulk density (kg dm^{-3}) across soil layers (0–20, 20–27, 27–52, 52–82, and 82–100 cm depths). Soil available water was determined as the sum of the differences between the volumetric water content at each sampling date and at permanent wilting point across soil layers.

At cover crop killing, aboveground biomass was harvested from two randomly selected 0.25-m^2 samples per subplot during 6 years. The harvested material was oven dried at 65 °C, and dry matter weights were recorded. Carbon concentration was assumed to be 40% of dry matter (Saffih-Hdadi and Mary 2008). Then, a subsample was ground, homogenized, and passed through a 0.25-mm diameter sieve for N determination by Kjeldahl method (Mulvaney 1996). Maize and soybean aboveground biomass production at harvest was obtained from two randomly selected 1-m^2 samples per subplot for 7 years. The harvested material was oven dried at 65 °C before separating grains to determine yield.

2.3 Data analyses

Soil and plant variables measured in each crop sequence with and without the cover crop mixtures were integrated using spider plots with a “more is better” normalized scale and a multifunctionality index (MF). To build the spider plots, variables were normalized to a scale from 0 to 1 using Equation 1 (Schipanski et al. 2014), for each main crop rotation separately.

$$Y_{norm} = \frac{Y_{treat}}{Y_{mean} \times 2} \quad (1)$$

where Y_{norm} is the normalized value of each variable; Y_{treat} is the value of the variable for CC1, CC2, or the control without cover crop; and Y_{mean} is the mean value of each variable for CC1, CC2, and control treatments.

The variables included in each spider plot comprised plant measurements (cover crop aboveground biomass and soybean and maize yield) and soil chemical (SOC, SON, and POC concentrations at 0–5, SOC and SON stocks at 0–20, and nitrate content at 0–100 cm depth) and physical measurements (aggregate stability and proportion of >300-µm macropores at 0–5, proportion of mesopores at 10–30, and soil available water content at 0–100 cm depth). Additionally, four biological variables (acid phosphatase, dehydrogenase and esterase activity, and total phospholipid fatty acids (PLFAs)) measured at 0–10 cm depth in the same field experiment in March 2013 and 2014 (main crop harvest) were included (Chavarría et al. 2016). In the case of soil nitrate content at cover crop killing, we redefined the variable as “N leaching control” by calculating the complementary value of Y_{norm} ($1-Y_{norm}$) to be consistent with the “more is better” criterion adopted for the other variables.

The MF was calculated as the average of the normalized difference between each cover crop treatment (CC1 and CC2) and the control without cover crops within each main crop rotation and across all the variables and depths included in the spider plots (Equation 2, adapted from Finney et al. 2016).

$$MF = \frac{\sum \frac{(Y_{CC} - Y_{control})}{Y_{mean} \times 2}}{N} \quad (2)$$

where Y_{CC} is the value of each variable for CC1 or CC2; $Y_{control}$ is the value of the variable for the control treatment; Y_{mean} is the mean value of each variable for CC1, CC2, and control treatments; and N is the number of variables used.

The effect of main crop and cover crop treatments and their interaction was evaluated for each variable using the mixed linear models approach. Plant variables (cover crop aboveground biomass production, N content, N concentration and C/N ratio, and main crop yield) were analyzed every year separately. Soil organic carbon, SON and POC concentrations, aggregate stability, and pore size distribution were analyzed at different depths after five cover crop-main crop cycles (April 2016). Soil nitrate and available water content at 0–100 cm were analyzed at cover crop termination during the first 3 years of the experiment. Homogeneity of variance was tested for each variable using the scatter plot of the residuals vs predicted values, and the normal distribution of the errors was tested using the Shapiro-Wilks test. Main crop and cover crop treatments were included as fixed effects, and blocks were included as a random effect, and analyses were performed with the MIXED procedure of SAS (SAS 2009). When differences between treatments were detected, mean values were compared using Fisher’s protected least significant difference (LSD) test ($p < 0.05$). A simple regression analysis (REG procedure of SAS) was used to determine the relationships between C input from cover crop aboveground biomass and SOC, SON, and POC and between soil carbon fractions and aggregate stability. To compare SOC and SON stock changes between the initial situation and after 5 years of rotation, we used an analysis of variance based on a split-split-plot design, where the main plots corresponded to the main crop sequence, the sub-plots corresponded to the cover crop treatment, and the sub-sub-plots to the time of evaluation (initial and after 5 years).

3 Results and discussion

3.1 Effect of mixtures of cover crops on soil organic fractions and structure

Soil organic C and N concentrations varied with cover crop treatment at 0–5 cm depths but not with main crop sequence (i.e., there was no significant interaction between main crop sequence and cover crop treatment at any depth). After 5 years of rotation, soil from maize-soybean and soybean-soybean sequences that included mixtures of cover crops had higher concentrations of SOC (23.3 vs 20.1 g kg^{-1}), SON (2.4 vs 2.0 g kg^{-1}), and POC (4.4 vs 2.9 g kg^{-1}) at 0–5 cm depth than the controls without cover crops (Table 2). Below 0–5 cm depth, all treatments had similar concentration of C and N organic fractions. Carbon input from aboveground biomass of cover crops was related to SOC (R^2 = 0.70, $p < 0.05$) and SON (R^2 = 0.95, $p < 0.05$) concentrations at 0–5 cm, and N input from aboveground biomass of cover crops was related to SON concentration at 0–5 cm (R^2 = 0.77, $p < 0.05$). These relationships reinforce the importance of C and N input from cover crops for organic matter increase in the first centimeters of soil in no-till systems (Poeplau and Don 2015, Restovich et al. 2019). In agreement with Duval et al. (2016), C input from cover crops aboveground biomass was also associated with POC (R^2 = 0.55, $p < 0.1$), although less strongly than with SOC and SON.

Initial SOC and SON stocks of the A horizon (0–20 cm) were 36.4 and 4.0 Mg ha^{-1}, respectively. After 5 years of rotation, SOC and SON stocks were similar with and without cover crops (mean stocks were 36.2 and 3.8 Mg ha^{-1} for SOC and SON, respectively). When compared with the initial situation, SOC stocks were maintained in the maize-soybean and soybean-soybean sequences with or without cover crops, while SON stocks were maintained in the maize-soybean sequence and decreased from 4.0 to 3.65 Mg ha^{-1} in the soybean-soybean sequence, regardless of the inclusion of cover crops (Fig. 2). Restovich et al. (2019) reported C sequestration at 0–20 cm depth 6 years after the introduction of cover crops in a maize-soybean rotation. The inclusion of maize in a rotation usually provides higher residue inputs, with higher C/N ratios, and a more favorable C balance compared to soybean monoculture (Mazzilli et al. 2014). However, in this study, SOC concentration increase at 0–5 cm did not result in a SOC stock increase at 0–20 cm, presumably due to the low C input of maize associated to its low productivity in two out of the three growing seasons that occurred before soil sampling (Fig. 4), and this was not offset by the use of cover crops. Additionally, periods of abundant rainfall during the study may have accelerated the rate of overall (cover crop + main crop) residue decomposition (Alvarez and Lavado 1998; Hutchinson et al. 2007). In the case of the soybean-soybean sequence, we also associate SOC stock conservation with the low C input of soybean residues compared to maize: maize C inputs usually exceed those of soybean by 1.4 to 1.8 fold (Mazzilli et al. 2014). On the other hand, SON stock decrease in this rotation is probably associated with the soil N enrichment and increased potential loss that characterize rotations with a high soybean cropping frequency (Plaza-Bonilla et al. 2015). Nitrogen enrichment can be related to the novel N input from biological fixation of legumes and to the high turnover of leguminous residues due to their low C/N ratio and higher soil/residue contact compared to maize (Chaves et al. 2021). Soil N enrichment after soybean harvest can be used by the succeeding cover crop or may be lost into the air or water (Della Chiesa et al. 2019; Nemecek et al. 2008). Plaza-Bonilla et al. (2016) reported that the use of cover crops mitigates the loss of SOC and SON in rotations with leguminous cash crops; however, in this study, cover crop mixtures did not revert SON stock decrease. To be sustainable, cropping systems based on grain legumes need to balance residual N and subsequent crop requirements, to avoid augmenting N losses through leaching and/or nitrous oxide emission (Nemecek et al. 2008).

Soil aggregate stability varied with cover crop treatment at 0–5 and 5–10 cm depths and with main crop sequence at 5–10 cm depth (i.e., there was no significant interaction between main crop sequence and cover crop treatment at any depth) (Table 2). After 5 years of rotation, soil aggregates from maize-soybean and soybean-soybean sequences that included cover crops were moderately stable, while those from sequences without cover crops were unstable (33.1% vs 16.4% at 0–5 cm depth). Aggregation stability at 0–5 cm depth was positively related with SOC (R^2 = 0.44, $p < 0.01$) and POC (R^2 = 0.50, $p < 0.01$) concentrations across both rotations, highlighting the importance of organic materials as binding agents (Six et al. 2004). At 5–10 cm depth, soil aggregation was slightly more stable with CC1 than with CC2 (17.7 vs 14.0% for CC1 and CC2, respectively), probably because CC1 had a higher proportion of oats, which has a fibrous root system that acts as a mesh, binding soil particles (Loades et al. 2013, Restovich et al. 2019). Also at 5–10 cm depth, soil aggregates from the soybean-soybean sequence were slightly more stable than those from the maize-soybean sequence (16.7 vs 12.0% for soybean-soybean and maize-soybean, respectively), presumably because cover crops have longer growing periods and biomass production when sown before soybean. These results suggest the importance of different soil binding agents in the formation and stabilization of soil aggregates. In addition to SOC and POC, roots and fungal hyphae (e.g., of arbuscular mycorrhizal fungi) release organic materials into the soil that also contribute to the bonding between particles (Goss and Kay 2005; Restovich et al. 2019; Rillig and Mummey 2006). Below the 5–10 cm depth, aggregation stability was similar between main crop sequences and cover crop treatments. This may be because no-till systems maintain

Table 2. Soil properties after 5 years of maize-soybean (M-S) and soybean-soybean (S-S) rotation, with (CC1 and CC2) or without (control) mixtures of cover crops at 0–5, 5–10, 10–20, and 20–30 cm depths. Different upper- and lower-case letters indicate significant differences ($p < 0.05$) between main crop sequences and cover crop treatments, respectively, within each soil depth (no significant interaction between main crop sequence and cover crop treatment). CC1, oats+forage radish; CC2, oats+forage radish+vetch; SOC soil organic carbon, SON soil organic nitrogen, POC particulate organic carbon.

	SOC (g kg^{-1})	SON (g kg^{-1})	POC (g kg^{-1})	Aggregate stability (%)	Macropores (>300 μm) (%)	Macropores (60–300 μm) (%)	Mesopores (15–60 μm) (%)	Micropores (< 15 μm) (%)
0–5 cm								
M-S	22.1 A	2.2 A	3.7 A	25.2 A	17.5 A	5.0 A	3.4 A	34.5 A
S-S	22.4 A	2.3 A	4.3 A	30.3 A	17.2 A	3.8 A	3.0 A	35.0 A
CC1	23.9 a	2.3 a	4.6 a	33.7 a	14.0 b	4.0 a	2.8 a	37.0 a
CC2	22.8 a	2.4 a	4.3 a	33.1 a	22.7 a	5.2 a	3.6 a	32.7 a
Control	20.1 b	2.0 b	2.9 b	16.4 b	16.3 b	4.1 a	3.2 a	35.3 a
5–10 cm								
M-S	15.6 A	1.6 A	0.9 A	12.0 B	14.0 A	4.0 A	2.8 A	31.3 A
S-S	14.4 A	1.5 A	0.8 A	16.7 A	12.8 A	3.2 A	3.5 A	31.2 A
CC1	15.5 a	1.6 a	0.9 a	17.7 a	13.3 a	3.5 a	2.9 a	31.5 a
CC2	14.7 a	1.6 a	0.9 a	14.0 ab	14.3 a	3.1 a	3.4 a	31.0 a
Control	14.9 a	1.5 a	0.7 a	11.3 b	12.7 a	4.0 a	3.3 a	31.2 a
10–20 cm								
M-S	13.4 A	1.4 A	0.6 A	6.2 A	13.6 A	2.6 A	3.1 A	31.0 A
S-S	12.9 A	1.3 A	0.5 A	6.4 A	12.6 A	2.7 A	3.0 A	32.0 A
CC1	12.9 a	1.3 a	0.6 a	4.8 a	13.6 a	3.1 a	3.0 b	31.0 a
CC2	13.5 a	1.4 a	0.5 a	7.6 a	12.2 a	2.6 a	3.8 a	31.5 a
Control	13.0 a	1.4 a	0.5 a	6.3 a	13.3 a	2.3 a	2.4 c	32.0 a
20–30 cm								
M-S	10.7 A	1.1 A	0.3 A	6.3 A	12.4 A	2.5 A	2.4 A	32.3 A
S-S	11.4 A	1.2 A	0.3 A	6.9 A	15.1 A	2.7 A	3.2 A	32.0 A
CC1	11.0 a	1.2 a	0.3 a	6.0 a	13.7 a	2.8 a	2.5 b	32.0 a
CC2	10.9 a	1,1 a	0.2 a	7.3 a	14.6 a	2.4 a	3.6 a	31.7 a
Control	11.3 a	1.1 a	0.3 a	6.5 a	12.8 a	2.6 a	2.4 b	33.3 a

high residue cover on the soil surface which promotes the increase of soil organic matter and aggregation stability in top soil (Chellappa et al. 2021).

Soil porosity varied with cover crop treatment at 0–5, 10–20, and 20–30 cm depths but not with main crop sequence (i.e., there was not a significant interaction between main crop sequence and cover crop treatment at any depth) (Table 2). Soil from plots with the three-species cover crop mixture (CC2) had a higher proportion of over-300-μm macropores at 0–5 cm depth than soil from plots with the two-species mixture (CC1) and the control without cover crops (Table 2). At 10–20 cm depth, soil from plots with both cover crop mixtures had a higher proportion of mesopores than soil from the controls without cover crops (3.8, 3.0, and 2.4% mesopores for CC2, CC1, and the control, respectively), and only in the case of CC2, this effect was maintained through the 20–30 cm depth. Similar results were previously reported in rotations that included grasses alone (Haruna et al. 2018) or combined with vetch as cover crop (Restovich et al. 2019). Cover crops can influence soil pore size distribution through direct and indirect mechanisms: they extend the time-frame with vegetation cover and living roots creating biopores (Haruna et al. 2020) and increase soil organic matter and aggregation stability which, in turn, create a soil environment more favorable for future root growth (Logsdon 2013). This is probably why, after 5 years of rotation, there was more porosity in soil from plots sown with cover crops than in the controls without cover crop. In this study, both cover crop mixtures increased the proportion of mesopores, but the inclusion of CC2 in rotations created more macro- and mesopores throughout the 0–30 cm depth. The presence of vetch in CC2 enabled biological fixation and transfer of N to non-legumes (Giacomini et al. 2003), probably enhancing below-ground biomass production and the formation of biopores.

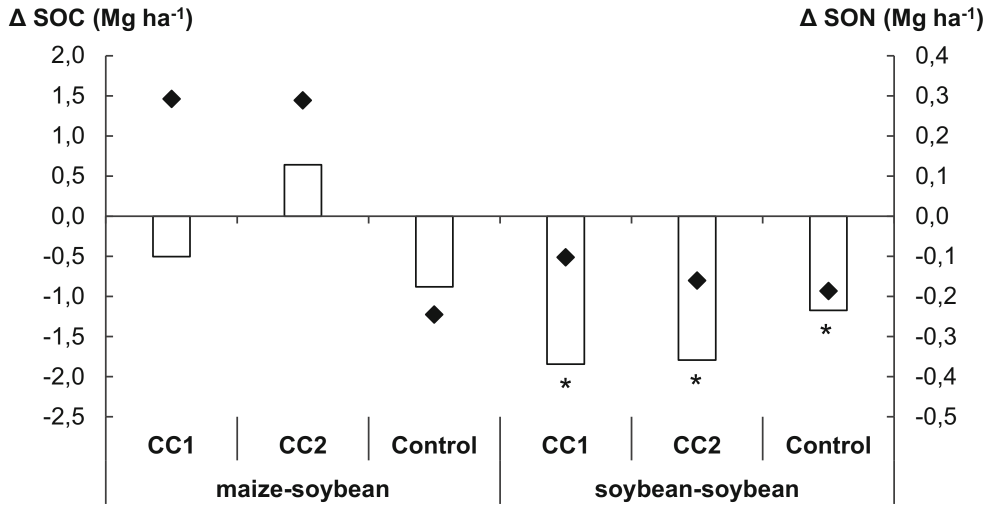

Figure 2. Change in soil organic carbon (SOC, black diamonds) and soil organic nitrogen (SON, bars) stocks in 2300 Mg soil ha^{-1} after 5 years of cultivation with or without (control) mixtures of cover crops (CC1 and CC2) in a maize-soybean and a soybean-soybean rotation. Asterisks indicate significant ($p < 0.01$) change in SON compared with the beginning of the experiment. CC1: oats + forage radish; CC2: oats + forage radish + vetch.

Furthermore, the combination of species with different root structure and distribution probably enhanced soil exploration and aggregation (Loades et al. 2013). Interestingly, the increase in macroporosity was registered at maize and soybean harvest, demonstrating that the effect of the cover crop's rooting system is persistent in time.

3.2 Effect of mixtures of cover crops on soil nitrate and available water content

Soil nitrate and available water content (0–100 cm depth) at cover crop termination varied with cover crop and main crop treatment, and there was no significant interaction between main crop sequence and cover crop treatment (Fig. 3). Soil NO_3-N content of the maize-soybean and soybean-soybean sequences with mixtures of cover crops was 52–82% lower than in the controls without cover crops (Fig. 3a). Cover crops also reduced soil available water content by ≈50% compared to the controls without cover crops in 2011 and 2013 growing seasons (Fig. 3b). However, in 2012, all cover crop treatments had similar available water content. In 2011 and 2013, soil NO_3-N content at cover crop termination was similar in both main crop sequences, but in 2012, the soybean-soybean sequence had more N-NO_3 than the maize-soybean sequence (63 vs 43 kg N-NO_3 ha^{-1}). Soil available water content at cover crop termination was always similar in the maize-soybean and soybean-soybean sequence.

These results were related to the amount of rainfall during the cover crop growing season: the differences in NO_3 and water content with and without cover crops became smaller in rainy growing seasons like 2012 (571 mm), when soil available water content was similar with and without cover crops (156 mm in 0–100 cm depth) and soil NO_3 content of the controls was less than twice that of the cover crop treatments (89 vs 54 kg N-NO_3 ha^{-1}). This was probably associated to the loss of N through leaching, which was ≈100 kg ha^{-1} during the fall-winter fallow of 2012 without cover crops (Restovich 2021). Soil nitrate reduction by cover crops has been reported for sole species cover crops (Constantin et al. 2010; Restovich et al. 2012). This reduction demonstrates the potential of cover crops to reduce N losses through leaching towards the end of the fallow period and during the early stages of summer crops when rainfall usually exceeds evapotranspiration (Portela et al. 2016; Rimski-Korsakov et al. 2015).

Cover crop water consumption from the upper soil horizons is generally recharged by spring rainfall. In this sense, cover crop killing dates are usually determined according to soybean and maize sowing dates, to ensure adequate soil moisture recharge before main crop sowing and high yield (Pinto et al. 2017). However, Restovich et al. (2012) found that water consumption from the deeper soil horizons may affect maize yield in dry years.

3.3 Biomass production and N use by mixtures of cover crops

Cover crop aboveground biomass accumulation ranged between 2.1 and 11.1 Mg ha^{-1} and differed between mixtures of cover crops, main crop sequences, and killing dates (Table 3). In 2011, 2014, and 2015, the biomass production of each mixture of cover crops varied with the main crop sequence (i.e., there was a significant interaction between main crop sequence and cover crop treatment). In the maize-soybean sequence, the biomass production of CC1 and CC2 was similar except in 2014, when CC2 almost doubled the production of CC1. In this sequence, cover crops have a shorter growing season before maize (2011, 2013, and 2015) than before soybean (2012, 2014, and 2016), because maize is sown in September–October and soybean is sown in November. In this sense, the longer growing season before soybean combined with a rainy growing season boosted biomass production of the triple mixture in 2014.

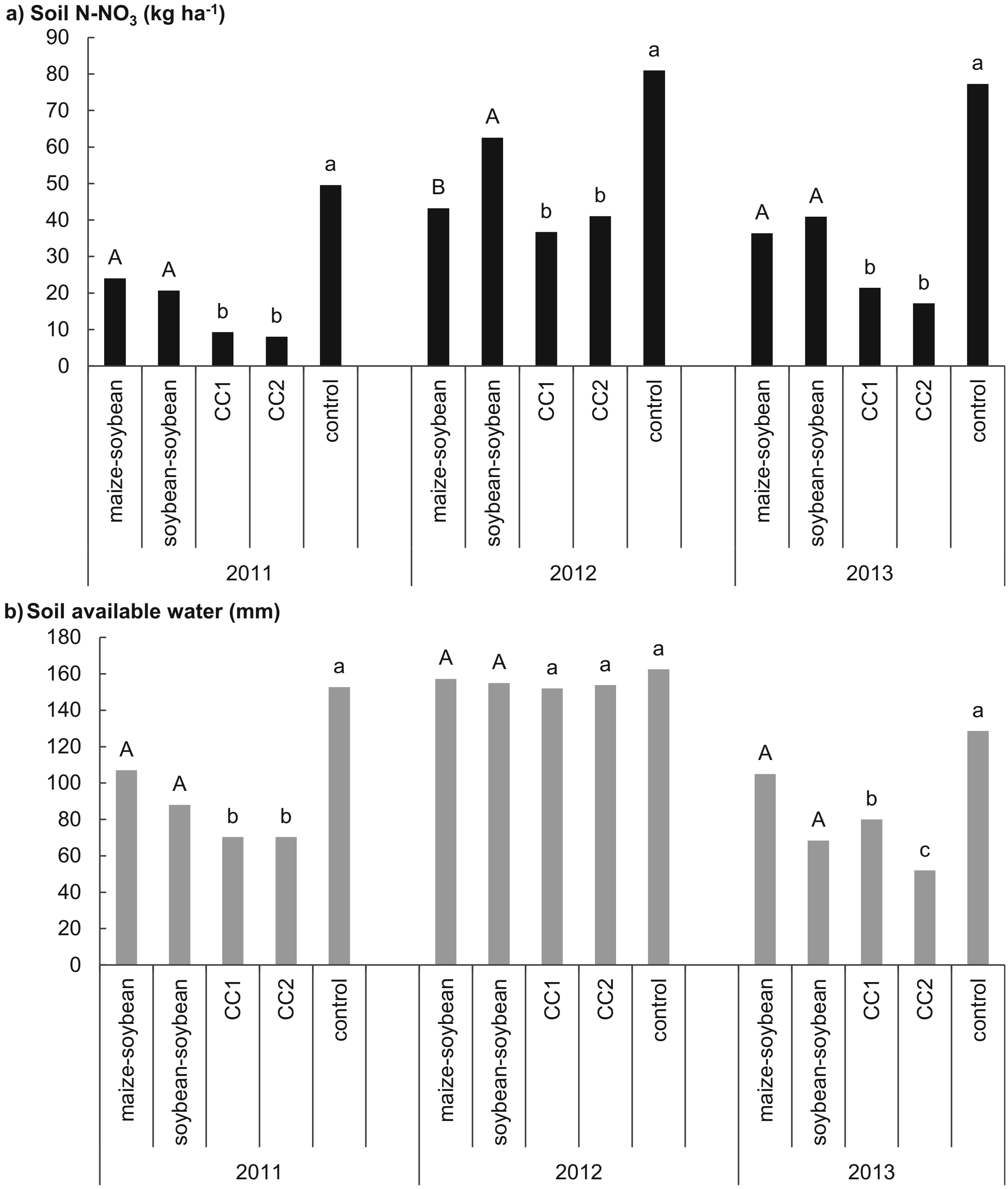

Figure 3. Soil NO_3-N **(a)** and available water content **(b)** at 0–100 cm depth at cover crop termination between 2011 and 2013. Different upper- and lower-case letters indicate significant differences ($p < 0.05$) between main crop sequences and cover crop treatments, respectively, within each year (no significant interaction between main crop sequence and cover crop treatment).

In the soybean-soybean sequence, CC2 produced more biomass than CC1 in years that presented a significant interaction between main crop sequence and cover crop treatment (2011 and 2015) and when cover crop treatment effect was independent from the main crop sequence (2016). This was related with the longer growing season of cover crops that preceded soybean, which extended towards spring, enabling the expression of the production potential of the triple mixture. In the case of wet growing seasons (e.g., 2012 with >550 mm), biomass production was high (≈10 Mg ha^{-1}) independently from the sequence and cover crop mixture. Wendling et al. (2019) observed that biomass production was more closely associated with the species that compose the mixture than with species richness. In Restovich et al. (2012), aboveground biomass production of sole species of grasses, crucifers, and legumes used as

Table 3. Aboveground biomass production, N content, N concentration, and C/N ratio of the mixtures of cover crops throughout the study period. Different lower-case letters indicate significant differences ($p < 0.05$) between cover crop mixtures within each main crop sequence (when there was a significant interaction between main crop sequence and cover crop treatment) or overall (when there was no significant interaction). Different upper-case letters indicate significant differences ($p < 0.05$) between main crop sequences when there was no significant interaction between main crop sequence and cover crop treatment. CC1, oats+forage radish; CC2, oats+forage radish+vetch; M maize, S soybean, CC cover crop.

	2011		2012		2013		2014		2015		2016	
Sequence/cover crops												
Maize-Soybean	CC	M	CC	S	CC	M	CC	S	CC	M	CC	S
Soybean-Soybean	CC	S	CC	S	CC	S	CC	S	CC	S	CC	S
Biomass (Mg ha-1)												
Maize-CC1-soybean	2.3a		-		-		4.7 b		3.3a		-	
Maize-CC2-soybean	2.1a		-		-		8.8 a		4.8a		-	
Soybean-CC1-soybean	5.9 b		-		-		7.2 a		5.7 b		-	
Soybean-CC2-soybean	7.6 a		-		-		6.8 a		11.1 a		-	
Maize/soybean	-		9.8 A		3.2 B		-		-		6.2 B	
Soybean-Soybean	-		10.0 A		7.5 A		-		-		7.8 A	
CC1	-		8.9 a		4.5 a		-		-		5.7 b	
CC2	-		10.8 a		6.3 a		-		-		8.2 a	
N (kg ha-1)												
Maize-CC1-soybean	38 a		-		-		-		-		-	
Maize-CC2-soybean	45 a		-		-		-		-		-	
Soybean-CC1-soybean	45 b		-		-		-		-		-	
Soybean-CC2-soybean	109 a		-		-		-		-		-	
Maize/soybean	-		109 A		53 B		91 A		77 A		75 A	
Soybean-Soybean	-		142 A		90 A		63 A		96 A		82 A	
CC1	-		70 b		43 b		37 b		45 b		36 b	
CC2	-		181 a		97 a		117 a		127 a		120 a	
N (g kg-1)												
Maize/soybean	18.8 A		10.8 A		16.1 A		11.4 A		18.7 A		10.9 A	
Soybean-Soybean	10.9 B		13.7 A		12.0 A		9.0 A		10.2 B		10.4 A	
CC1	12.1 b		8.1 b		11.3 b		6.1 b		11.2 b		6.2 b	
CC2	17.7 a		16.4 a		16.7 a		14.3 a		17.7 a		15.2 a	
C/N ratio												
Maize/soybean	22 B		44 A		25 B		45 B		23 B		44 A	
Soybean-Soybean	40 A		34 A		36 A		53 A		45 A		49 A	
CC1	38 a		51 a		37 a		66 a		43 a		65 a	
CC2	23 b		27 b		25 b		31 b		25 b		27 b	

cover crops varied between 1.1 and 8.1 Mg ha^{-1}, and this variability was associated to the length and rainfall accumulated during the growing season, cover crop family, and antecedent main crop.

Biomass N concentration was always higher (16.3 vs 9.2 g kg^{-1}), and C/N ratio was always lower (26 vs 50) in CC2 than in CC1 due to the inclusion of vetch in the triple mixture (Thorup-Kristensen et al. 2003) (Table 3). In relation to this and to the higher biomass production of CC2 in some growing seasons, N absorption by cover crops was also higher in CC2 than in CC1 (128 vs 46 kg N ha^{-1}). Cover crops that preceded maize in the maize-soybean sequence also had higher N concentrations (17.9 vs 11.0 g kg^{-1}) and lower C/N ratios (23 vs 40) than those preceding soybean in the soybean-soybean sequence, because the former were terminated during the vegetative stage and the latter were terminated during the reproductive stage (Thorup-Kristensen et al. 2003, Restovich et al. 2012).

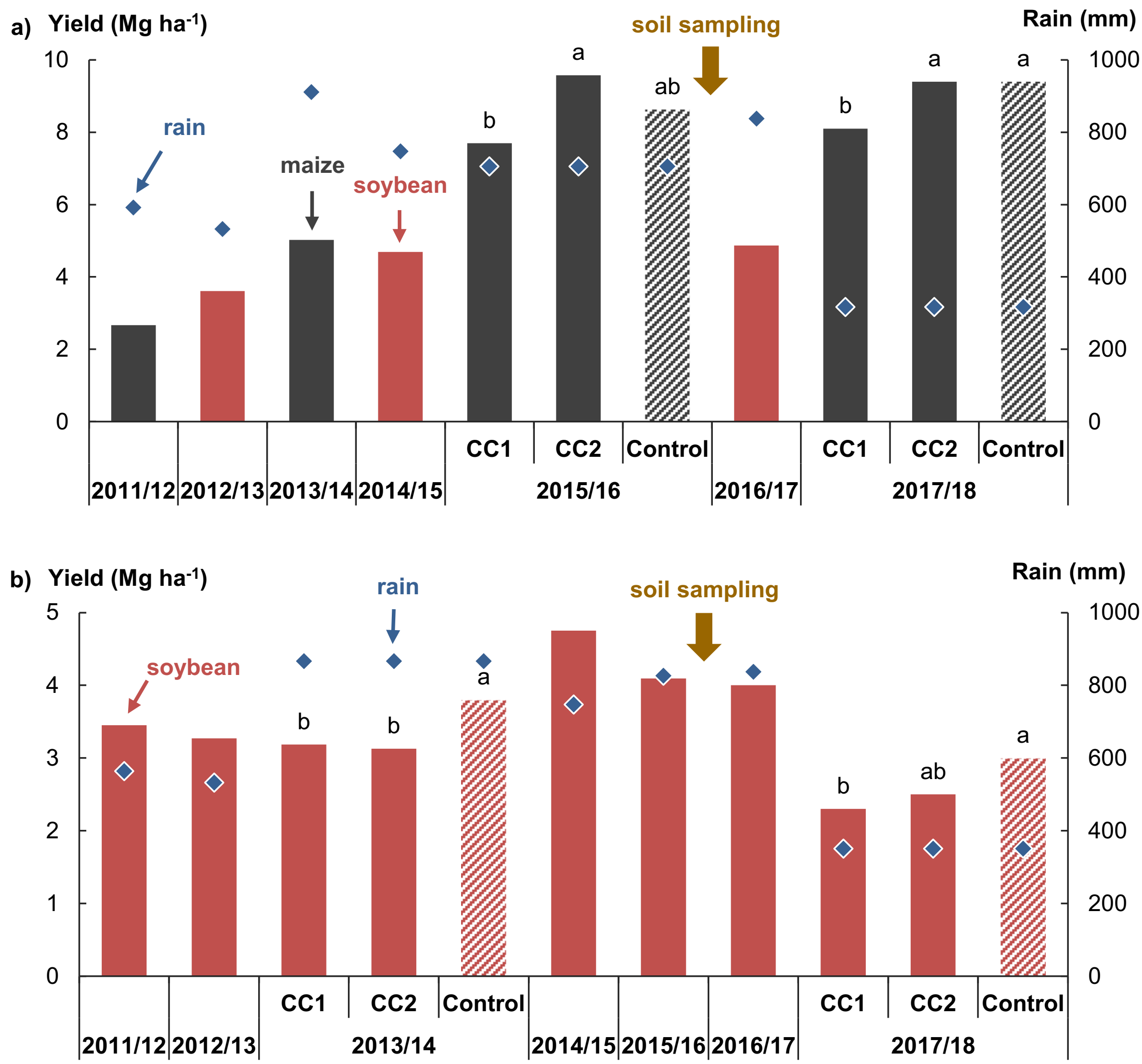

Figure 4. Harvest crop yield (bars) for the maize-soybean **(a)** and soybean-soybean **(b)** sequence and rainfall (symbols) between 2012 and 2018. Yields are expressed at 14.5% and 13.5% moisture, for maize and soybean, respectively. Different letters indicate significant differences ($p < 0.05$) between cover crop treatments within each main crop sequence and growing season. CC1, oats + forage radish; CC2, oats + forage radish + vetch; control, without cover crop.

3.4 Effect of mixtures of cover crops on maize and soybean yield

Maize yield was <5.0 Mg ha^{-1} and similar with or without cover crops in 2011/2012 and 2013/2014 growing seasons, because rainfall was scarce during the preceding cover crop growing seasons (<150 mm), between cover crop termination and maize sowing (<45 mm) and in December (<20 mm) when maize was at flowering which is a critical stage for yield determination (Fig. 3a and Table 1). In 2015/2016 and 2017/2018 growing seasons, average maize yield was 7.9 Mg ha^{-1} after CC1 and 9.5 Mg ha^{-1} after CC2. These growing seasons were preceded by rainy cover crop growing seasons (>270 mm) and a rainy period between cover crop termination and maize sowing (>85 mm) and presented average rain during December (74 and 128 mm in 2015 and 2017, respectively). The yield difference between CC1 and CC2 was probably related to more N availability after CC2, which had 50% vetch, than after CC1 (Tribouillois et al. 2016). Our results show that aboveground biomass of CC2 contains three times the N content of CC1 (Table 3), which may become available to maize after residue decomposition (Restovich et al. 2012). However, in 2017/2018 growing season, yield after CC2 was similar than that of the control without cover crops (9.4 Mg ha^{-1}), presumably because a very dry maize growing season (317 mm) restricted N provision through mineralization of cover crop residues, barely compensating soil nitrate content in control plots (Thorup-Kristensen et al. 2003). Additionally, cover crop residue decomposition may have resulted in a transient N limitation probably associated to microbial immobilization, further enhancing soil nitrate reduction (Redin et al. 2014). In 2015/2016,

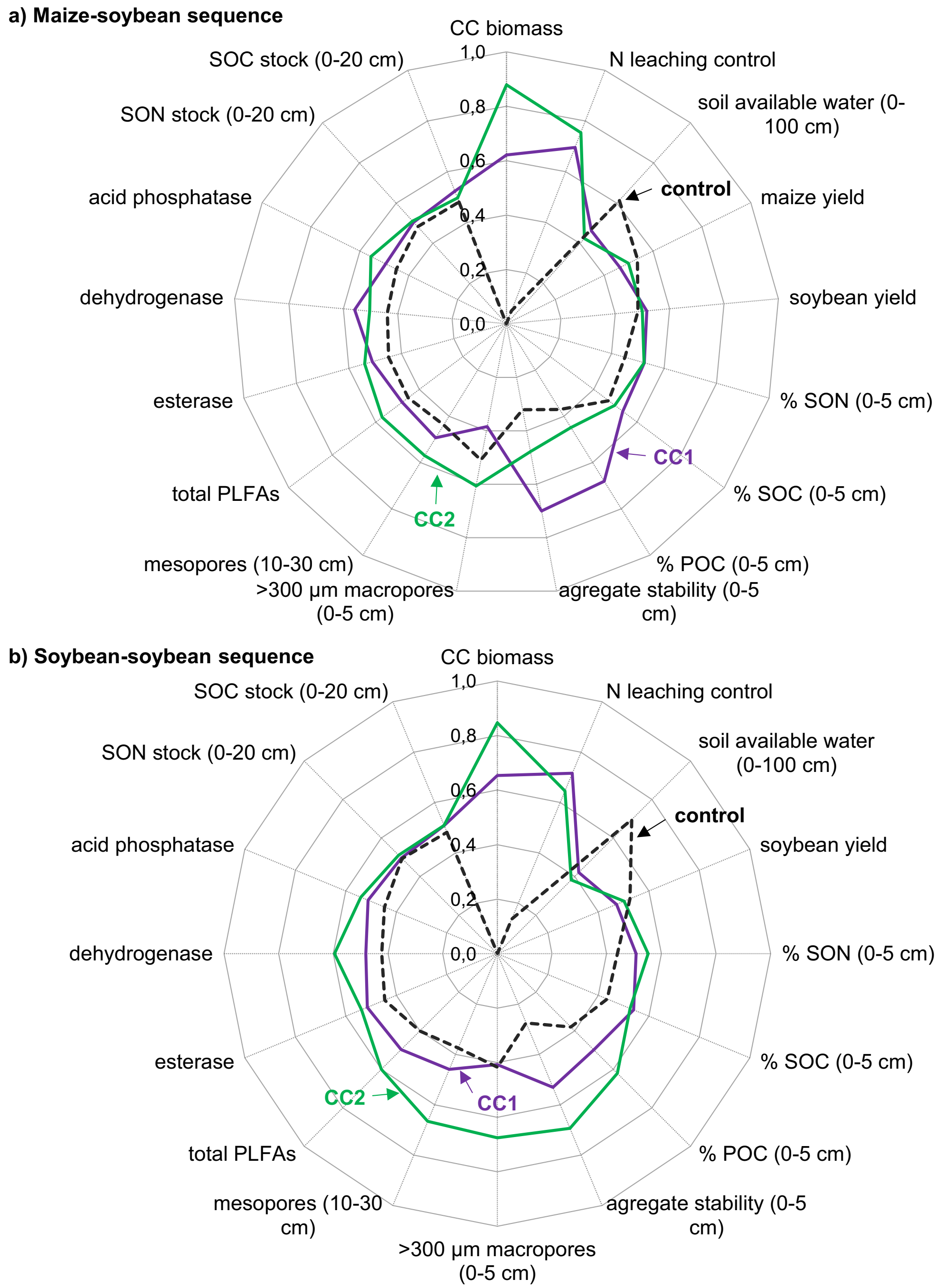

Figure 5. Integrated representation of soil and plant-related variables chosen as indicators of different ecosystem functions after 5 years of rotation with or without (control) two cover crop mixtures (CC1 and CC2) introduced in a maize-soybean (**a**) and a soybean-soybean (**b**) sequence. The variables included were those reported in this paper and four soil biological variables (acid phosphatase, dehydrogenase, esterase activity, and total phospholipid fatty acids (PLFAs)) reported by Chavarría et al. (2016). CC1 (purple contours), oats + forage radish; CC2 (green contours), oats + forage radish + vetch; control without cover crop (dashed black contour).

maize yield after CC2 was slightly higher than that of the control (9.6 vs 8.6 Mg ha^{-1}), presumably because a more humid growing season (705 mm) enabled more N availability from cover crop residue decomposition.

Soybean yield was similar with or without cover crops in 2012/2013, 2014/2015, and 2016/2017 in both main crop rotations (Fig. 4a and b), in agreement with previous results of Restovich et al. (2012). On the other hand, soybean yield of the control without cover crops was higher than that of the cover crop treatments (3.4 vs 2.8 kg ha^{-1}) in 2013/2014 and 2017/2018 growing seasons (Fig. 4b). At cover crop termination, soil nitrate and available water contents were higher in control plots without cover crops than in plots with cover crop mixtures, particularly after dry to normal cover crop growing seasons (Fig. 3). The higher nitrate and water availability of the control plots was probably associated to increased yield.

Yield reduction is often pointed out as a disadvantage of including cover crops before main crops (Abdalla et al. 2019).

In the case of rain-fed systems, yield reduction occurs some years and is directly or indirectly (through its interaction with N cycling) associated to the amount and distribution of rainfall. On the other hand, cover crops may enhance grain yield in rainy growing seasons through the uptake of potentially leachable nitrate and subsequent release, by residue decomposition, in synchrony with the main crop demand (Restovich et al. 2012).

3.5 Multifunctionality of agricultural systems with mixtures of cover crops

In the maize-soybean and soybean-soybean sequences, increasing the species richness using mixtures of cover crops had a positive impact on several ecosystem functions (Fig. 5). As previously demonstrated for sole species cover crops (Restovich et al. 2012, Tribouillois et al. 2016), biomass production by mixtures reduced soil nitrate content by 52–82% at the time of cover crop killing, reducing N leaching potential in humid growing seasons compared to the control. Soil nitrate and water absorption by cover crop mixtures, however, had a negative impact on soybean yield after dry to normal cover crop growing seasons. Although cover crops reduced soil water content by ≈50% at killing, soil water recharge before maize sowing was sufficient to maintain yields similar to those of the control without cover crops. However, maize could not benefit from CC2 N supply with reduced rainfall.

After 5 years of rotation, the inclusion of cover crop mixtures in the maize-soybean and soybean-soybean sequences increased SOC, SON, and POC concentrations by 16, 18, and 53%, respectively, and soil aggregation stability by 200% in the upper 0–5-cm layer, but did not modify soil C and N stocks of the A horizon in relation to the controls without cover crops. Both cover crop mixtures increased the proportion of soil mesopores at 10–20 cm by 42% with respect to the controls without cover crops, CC2 also increased the proportion of mesopores at 20–30 cm by 50%, and the proportion of macropores at 0–5 cm by 40% with respect to CC1 and the controls. Chavarría et al. (2016) evaluated the effect of including CC1 and CC2 mixtures on the microbial community structure analyzing PLFA biomarkers and soil enzyme activities after 3 years of rotation. They showed that the use of multispecies cover crops, especially the one with vetch, increased bacterial PLFA biomarkers in both main crop sequences, in association with an increased microbial biomass. Cover crop mixtures also increased the activity of microbial extracellular enzymes as dehydrogenase, acid phosphatase, and esterase, which reflect a greater metabolic capacity to process organic compounds derived from residues and root exudates and transform them into available nutrients.

Our results show the potential of two cover crop mixtures to enhance ecosystem multifunctionality with respect to the controls without cover crops. Interestingly, both cover crop mixtures showed similar multifunctionality indexes when introduced in the maize-soybean sequence (0.13 and 0.14 for CC1 and CC2, respectively), but CC2 outranged CC1 in the case of the soybean-soybean sequence (0.19 vs 0.11) and resulted in the highest index overall. Graphically, the contours corresponding to CC1 and CC2 are, on average, equidistant from the control in the spider plot that represents the maize-soybean sequence, but the contour corresponding to CC2 is further away from the control than the contour of CC1 in the case of the soybean-soybean sequence (Figure 5). The inclusion of maize in the rotation represents a benefit by itself, because it returns more residues with higher C/N ratios than soybean, stimulating the abundance of soil microorganisms (Vargas Gil et al. 2011) (Fig. 5). On the other hand, we have detected some trade-offs in the arena of N cycling. Nitrogen pre-emptive competition by cover crops resulted in an advantage in terms of main crop yield when the alternative was losing N through leaching; however, during dry years, it becomes a disadvantage because it reduces initial N availability for the subsequent main crop (Thorup-Kristensen et al. 2003, Tribouillois et al. 2016).

4 Conclusion

In this study, we evaluated two mixtures of cover crops designed on the basis of previous experiences and introduced in a soybean-soybean and maize-soybean sequence with low N fertilization of maize, using an original approach that integrates ecosystem functions and trade-offs. We demonstrate that replacing long bare fallow periods with mixtures of cover crops increases residue inputs, N absorption and recycling, and soil biodiversity, leading to increases in soil organic C and N concentrations in the top soil (0–5 cm). The mixtures evaluated also improved soil aggregation stability at 0–5 cm, and only the triple mixture CC2 increased the proportion of macro- and mesopores at 0–30 cm depth, presenting a biological option for the improvement of degraded soil structure in cropping systems. Longer and more humid growing seasons enhanced the biomass production potential of CC2 compared to CC1, and biomass from CC2 always had more N concentration and content and lower C/N ratio due to the inclusion of vetch. Nitrogen absorption by cover crop mixtures reduced soil nitrate and water content at cover crop termination with respect to the controls without cover crops, and the magnitude of these differences was inversely related to the amount of rainfall during the cover crop growing season. Maize and soybean yields after CC1 were similar or lower than those from the control plots without cover crops, and in the case of CC2, yields were usually similar to those of the controls. This was probably related to more N availability after CC2 than after CC1, although the amount and distribution of rainfall during some growing seasons limited N release from cover crop residues and absorption by main crops, reducing the expression of the mixture's potential on yield. Both combinations of species used as cover crops increased ecosystem multifunctionality compared to the controls without cover crops, and CC2 presented the highest index when introduced in the soybean-soybean sequence. In the maize-soybean sequence, CC2 enhanced porosity and CC1 increased aggregation stability and POC concentration. In the soybean-soybean sequence, CC2 improved soil structure (porosity and aggregation stability) and POC concentration. Our results show that cover crop mixtures can be an important component of sustainable agricultural systems, through their positive impacts on various soil physical, chemical, and biological properties. However, this practice poses potential trade-offs related to the effect of rainfall variability on soil water and N dynamics, and their impact on system productivity, which may in turn enhance or offset the effect of cover crops on C sequestration. The interaction of rainfall variability and cropping intensification with cover crop mixtures should be further studied in long-term experiments and/or through modeling.

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Authors' contributions Conceptualization: SBR, AEA, and SIP. Methodology: SBR and AEA. Data collection: SBR. Data handling and analysis: SBR, AEA, and SIP. Writing and visualization: SBR and SIP. Manuscript review and editing: SBR, AEA, and SIP. Funding acquisition: SBR, AEA, and SIP.

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Declarations

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

Conflict of interests The authors declare no competing interests.

Code availability Not applicable.

Data availability The data used in this study is not publicly available but could be provided by the corresponding author on reasonable request. In addition to the newly generated data, soil biological variables previously measured in the same experimental trial, and published by Chavarría et al. (2016), were incorporated to provide a comprehensive analysis of the ecosystem functions influenced by cover crop mixtures.

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