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Effect of different cover crops on C and N cycling in sorghum NT systems

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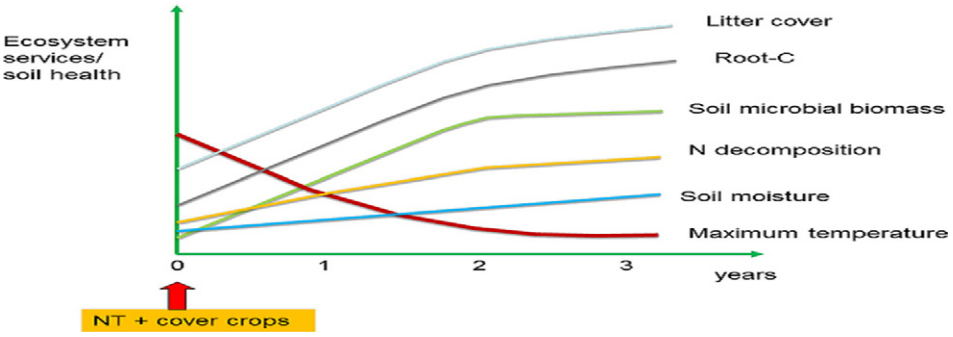
HIGHLIGHTS

- Cover crops increased root biomass, total carbon, microbial biomass, and nitrogen.
- Cover crops improved soil moisture and soil temperatures.
- Soil litter disappearance was a good indicator of mineral N availability.
- Microbial biomass reached carrying capacity of 20.1 g C m^{-2} and 1.9 g N m^{-2}.

GRAPHICAL ABSTRACT



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ABSTRACT

In many no-till (NT) systems, residue input is low and fallow periods excessive, for which reasons soil degradation occurs. Cover crops could improve organic matter, biological activity, and soil structure. In order to study changes in soil carbon, nitrogen and microbial biomass a field experiment (2010−2012) was set up with sorghum (*Sorghum bicolor* Moench.) monoculture and with cover crops. Treatments were control (NT with bare fallow), rye (*Secale cereale* L.) (R), rye with nitrogen fertilization (R + N), vetch (*Vicia villosa* Roth.) (V), and rye-vetch mixture (VR) cover crops. A completely randomized block design with 4 replicates was used. Soil was sampled once a year at 0.06 and 0.12 m depth for total C, microbial biomass carbon (MBC) and-nitrogen (MBN) determinations. Shoot and root biomass of sorghum and cover crops, litter biomass, and their respective carbon and nitrogen contents were determined. Soil temperatures at 0.06 and 0.12 m depth, volumetric water contents and nitrate concentrations were determined at sowing, and harvest of each crop, and during sorghum's vegetative phase. NT led to a small increase in MBC and MBN, despite low litter and root biomass residue. Cover crops increased litter, root biomass, total C, MBC, and MBN. Relationships between MBC, MBN, and root-C and –N adjusted to logistic models ($R^2 = 0.61$ and 0.43 for C and N respectively). Litter cover improved soil moisture to 45–50% water filled pore space and soil temperatures not exceeding 25 °C during the warmest month. Microbial biomass stabilized at 20.1 g C m^{-2} and 1.9 g N m^{-2} in the upper 0.06 m. Soil litter disappearance was a good indicator of mineral N availability. These findings support the view that cover crops, specifically legumes in NT systems can increase soil ecosystem services related to water and carbon storage, habitat for biodiversity, and nutrient availability.

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1. Introduction

As the agricultural frontier advances, driven by the growing global food demand (Huber et al., 2014; Sakschewski et al., 2014), important land use changes occur especially in marginal and semiarid regions (Coutinho et al., 2014; Guida Johnson and Zuleta, 2013). The soils of these areas are not resilient to the effects of inappropriate use and management which rapidly leads to their permanent degradation and associated loss of productivity (Rojas et al., 2016; Zach et al., 2006). Degradation and organic matter loss also bring about a decline in biological activity (Noellemeyer et al., 2008) and biodiversity (Jangid et al., 2011; Thiele-Bruhn et al., 2012). In order to restore soil productivity and attain sustainable production systems it is crucial to improve and maintain soil quality specifically in terms of ecosystem functions sustained by microbial activity (Bastida et al., 2008). One of the most successful and common practices to recover degraded agricultural soils has been no-tillage (NT) cultivation (Lal et al., 2007). Adoption of NT has been shown to improve soil carbon contents and structural stability when compared to conventional tillage systems (Blanco-Canqui et al., 2011; Fernández et al., 2010; Varvel and Wilhelm, 2011; Zotarelli et al., 2007). However, there persist many doubts about whether NT on its own can effectively increase or even stabilize soil C pools (Baker et al., 2007; Doran et al., 1998). The plant residue mulch cover on the soil's surface associated to NT systems also has a strong influence on environmental conditions for biological processes, protecting the soil from drying or flooding, and from abrupt temperature changes (Doran et al., 1998; Fernandez et al., 2008).

Current agricultural practices in many areas under no-till in the Argentinean Pampas as well as in many parts of the world consist in monocultures that leave very little residue cover (e.g. soybean, bioenergy or silage crops) and that jeopardize the possible benefits of NT cultivation (Denef et al., 2007; Fengrui et al., 2000; Kou et al., 2012; Lal, 2009; Powlson et al., 2014). The detrimental effect of the lack of diversity (i.e. monoculture versus crop rotation) on soil biological activity was already mentioned by Dick (1992). On the other hand, the fallow periods in soybean or corn monocultures commonly used in the Argentinean Pampas or elsewhere are very long and the soil remains without vegetation during five months or more (Andrade and Satorre, 2015; Milne et al., 2007; Powlson et al., 2011; Restovich et al., 2012; Tanaka et al., 2005). These long bare fallow periods also have detrimental effects on hydrological processes (Nosetto et al., 2011), promote nutrient leaching, and they might reduce the biological activity of soils (Doran et al., 1998; Ferrari et al., 2015; Schutter and Dick, 2002; Tonitto et al., 2006). These problems were sought to be overcome by the introduction of cover or catch crops, which increase plant residue on the soil's surface and were shown to be effective in preventing excessive rise of water tables and nitrogen contamination of ground water (Dabney et al., 2001; Dinesh et al., 2004; Poeplau and Don, 2015; Tonitto et al., 2006). Cover crops also have the additional benefit of providing an active rhizosphere during the entire growing season, providing better conditions for microbial activity than bare fallows (Galvez and Douds, 1995; Kong and Six, 2012; Lehman et al., 2012; Mendes et al., 1999; Schutter and Dick, 2002).

Annual grasses such as rye, oats and ryegrass have been much used as cover crops but generally inclusion of an annual legume is preferred since these mixtures apparently promote soil organic matter formation (Ding et al., 2006; Kong and Six, 2012; Mazzoncini et al., 2011) and nitrogen availability for subsequent cash crops (Schröder et al., 2013; Venkateswarlu et al., 2007). For pure grass cover crops in NT systems especially in cooler environments an excessive accumulation of aboveground residue may occur, presumably related to their high C:N that might limit decomposition. Although it is well known that the relation between C and N regulates microbial decomposition of plant residues (Dijkstra et al., 2008), there is little information about the range of C:N that would favor C sequestration and whether a synthetic fertilizer N has the same impact as organic N sources.

We hypothesized that the inclusion of cover crops under NT would produce higher residue cover on the soil, which in turn would lead to attenuated moisture and temperature conditions resulting in a more favorable environment for microbial activity and growth. The extra supply of high C:N residues from a cereal cover crop could represent N limitation for soil biota and consequently carbon would accumulate under NT management, while the utilization of an N source, organic by including vetch or inorganic via fertilizer, would compensate this demand accelerating carbon and nitrogen cycling. Therefore, the objective was to elucidate the changes in soil environmental conditions and soil microbial biomass with the goal to assess the restoration of soil biological activity and C and N cycling brought about by no-till and cover crops on a degraded agricultural soil.

This work is part of a more comprehensive study on the effect of cover crops on ecosystem services, C and N budgets with special emphasis on the changes induced in microbial biomass, its composition and the effect on C and N sequestration and stabilization.

2. Materials and methods

2.1. Site description

The study was carried out on a *petrocalcic Paleustoll* (USDA, NRCS, 2010) with a calcium carbonate hardpan at depths between 0.6 and 1.5 m, located at the National Institute of Agricultural Technology (INTA) Experimental Station at Anguil, La Pampa, Argentina (S 36° 36′ 37.95″; W 63° 58′ 48.22″). The area is within the arid and semiarid Pampa with an annual rainfall of 700 mm and mean temperature of 13 °C. Cropping history at the experimental site was annual cropping for livestock fodder under conventional tillage (disk and harrow) for more than 70 years. The most relevant soil properties are summarized in Table 1.

2.2. Experimental design

The experiment comprised three growing seasons (2010 − 2013) of the rotation of sorghum (*Sorghum bicolor* Moench.) with and without cover crops (*Secale cereale* L. and *Vicia villosa* sp. *dasycarpa*) under no-till. Sorghum growing season was between mid-November to April when it was cut for silage or hay. This practice is common in the semiarid Pampas where livestock production has undergone intensification (feedlot and dairy farming) in recent times. Treatments with cover crops (CC) were rye + vetch (RV), rye (R), vetch (V), rye fertilized with 40 kg ha^{-1} of urea-nitrogen (R + N) growing between April and October. A control without cover crop (C) was also established to represent a typical situation of monoculture with long bare fallow between each summer crop. These fallows were maintained weed-free by one or two applications of glyphosate (2 L ha^{-1}). The experiment was performed in a completely randomized block design with 4 replicates. Plot size was 10 m wide by 50 m long. Cover crops were drilled at a density of 200 seeds m^{-2} for both R and V, and 80 and 120 seeds m^{-2} of rye and vetch, respectively for the RV treatment with a 0.17 m distance between lines. All treatments were fertilized with 20 kg ha^{-1} of phosphorus as triple superphosphate broadcast before seeding, simultaneous with broadcast urea in the R + N treatment. Cover crops were terminated by application of a mixture of glyphosate (3 L ha^{-1}) and 2.4-dichlorophenoxiacetic acid (0.4 kg ha^{-1}). Sorghum was planted in all plots at a density of 15 seeds m^{-2} and with a row spacing of 0.52 m.

2.3. Sampling procedure and analytic determinations

The sampling was timed as to coincide with the early vegetative phase of the sorghum crop (4–6 leaves with visible ligule) taking into account the time required for cover crop residues to undergo decomposition, the development of rhizosphere activity and adequate

Table 1
Soil properties at the beginning of the experiment (2010). Composite samples of at least 32 subsamples were taken at each depth in the area where the experimental plots were to be established. Total organic carbon (SOC), total nitrogen (N), available phosphorus (P Bray), cation exchange capacity (CEC), pH and bulk density (BD).

Soil depth	Texture	Sand	Silt	Clay	SOC	Total N	Available P	CEC	pH	BD
m				g kg^{-1}			g kg^{-1}	cmol kg^{-1}		kg m^{-3}
0.00–0.06	Sandy loam	561	270	149	9.7	0.92	0.0092	8.65	5.9	1.238
0.06–0.12	Sandy loam	567	280	153	9.1	0.89	0.007	8.16	5.7	1.243

temperatures for microbial activity (Poll et al., 2008). Soil samples were taken during the 2010, 2011 and 2012 cropping seasons in a 0.25 m^2 area between sorghum lines, replicated three times in each plot, and 7 subsamples were collected with a tubular auger of 0.032 m diameter at 0–0.06 and 0.06–0.12 m depth within this area. Subsamples were mixed and homogenized in the field, and subsamples for microbial biomass were stored at field moisture in a refrigerator at 2 °C until analysis (in any case less than 2 months) in order to prevent mineralization (Wu and Brookes, 2005). A diagram of the crop sequence and the sampling dates is shown in Fig. 1.

2.3.1. Aerial and root biomass of sorghum and cover crops

In October, immediately after terminating cover crops with herbicides, aboveground biomass was determined by cutting the plants at ground level in an area of 0.25 m^2, with three replicates per plot. Samples were oven-dried at 60 °C until constant weight, dry weight was recorded, and then they were milled for total C and N determination (LECO — TrueSpec® CN).

For root sampling, soil samples were taken with a tubular auger (0.032 m diameter) at 4 points between two crop rows (0.17 m distance) three times in each plot, coinciding the first and last point with a crop row. Soil volume extracted at each point was 0.006 m^3. Between row samples were pooled into a single sample per depth for each replicate. Roots were separated from soil by wet-sieving through a submerged 500 μm sieve with tap water (Barley, 1970), the roots retained and floating on the sieve were collected with tweezers, oven-dried to constant weight at 60 °C, weighed, and milled for C and N determinations (LECO — TrueSpec® CN).

To avoid overestimating total root biomass (RB), the influence-percentage of roots on crop row (CR) and between rows (BR) was calculated (I %). It was determined that 71% of the transect was occupied by BR (0.12 m/0.17 m × 100) and 29% by CR (0.05 m/0.17 m × 100) for CC, while for sorghum these values were 90% (0.47/0.52 m) and 10% (0.05/0.52 m) respectively. Total root biomass (RB) was calculated as a sum of root biomass in BR and CR:

$$\mathrm{RB}(\mathrm{g\ m^{-2}}) = \mathrm{BR} + \mathrm{CR} \quad (1)$$

$$\mathrm{BR} = [(\textstyle\sum \text{dry weight BR})/(\pi \times r^2 \times \text{number of points in BR}]/0.0001 \times I\ (\%) \quad (2)$$

$$\mathrm{CR} = \left[(\textstyle\sum \text{dry weight CR})/(\pi \times r^2 \times \text{number of points in CR})\right]/0.0001 \times I\ (\%) \quad (3)$$

0.0001 is a unit conversion factor (g cm^{-2} to g m^{-2}).

2.3.2. Litter biomass on the soil surface

The litter on the soil surface was collected in 0.25 m^2 areas with three replicates for each experimental plot at the beginning of the experiment and during the early vegetative phase of sorghum crop (4–6 leaves with visible ligule) each year. The material was sieved through 2 mm mesh to remove soil particles, and was dried at 60 °C until constant weight. Dry weight was registered for each sample and then the replicates of each plot were mixed for total C and N determinations (TrueSpec® CN auto analyzer).

Litter carbon and nitrogen disappearance were calculated according to the balance method proposed by Singh and Yadava (1974). This method seems to be more appropriate than Olson's model (Olson, 1963) when initial litter values are variable between periods of analysis (Singh and Gupta, 1977). The model equation is:

$$\text{Litter disappearance}\ (\mathrm{g\ m^{-2}}) = \text{initial litter} + \text{litter production} - \text{final litter}. \quad (4)$$

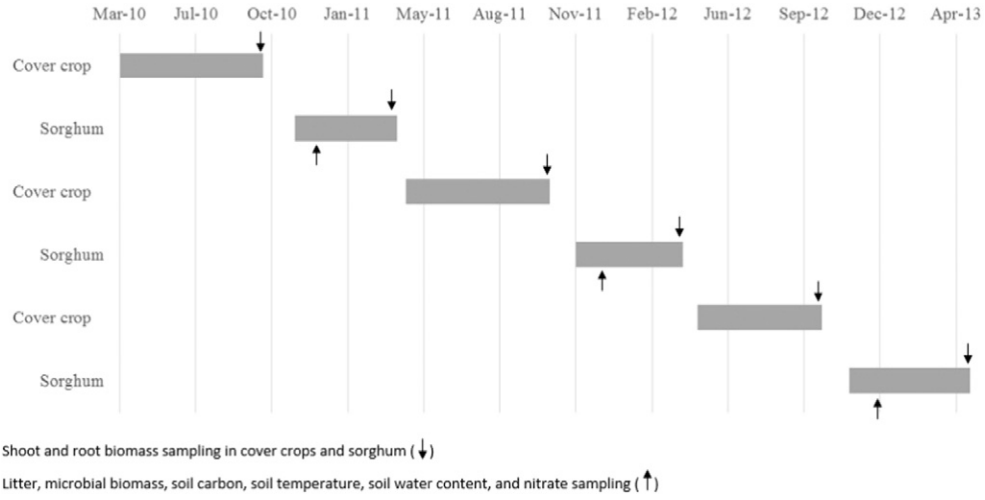

Fig. 1. Sequence of cover crops and sorghum, and sampling dates for the different determinations. Soil temperature, nitrate and soil water content were also measured at planting and harvesting of each crop.

2.3.3. Soil microbial biomass carbon and nitrogen

Soil subsamples were extracted using the fumigation-extraction method (Voroney, 2006), and before extraction all visible roots were removed from the samples with metallic tweezers (Mueller et al., 1992). For both fumigated and non-fumigated samples, 30 g of soil and 60 mL of solution (K_2SO_4, 0.5 M) was used, and these were shaken during 1 h and then filtered through Whatman GF 934-AH filter paper using a vacuum pump. Gravimetric moisture contents were determined simultaneously and used to correct data to dry soil weight. Microbial biomass carbon (MBC) was determined according to Vance et al. (1987) and was calculated by the following formula:

$$\mathrm{MBC}\left(\mu g\ C\ g\ soil^{-1}\right) = (C_f - C_{nf})\ k_{ec}{}^{-1} \quad (5)$$

where C_f and C_{nf} are the carbon concentrations of fumigated and non-fumigated samples, respectively, and $k_{ec} = 0.45$ (Wu et al., 1990).

Microbial biomass nitrogen was determined by Kjeldahl (ISO Norm 11261) and was calculated according to:

$$\mathrm{MBN}\left(\mu g\ N\ g\ soil^{-1}\right) = (N_f - N_{nf})\ k_{en}{}^{-1} \quad (6)$$

where N_f and N_{nf} are the nitrogen concentrations of fumigated and non-fumigated samples, respectively, and $k_{en} = 0.54$ (Pruden et al., 1985).

The obtained values were transformed into MB mass (g m^{-2}) for each soil depth layer using the average bulk density (BD) for each depth (Table 1).

2.3.4. Soil environment

Soil temperature (digital thermometer −50 to 150 °C, Luft Germany, at 0.06 and 0.12 m), gravimetric soil moisture content (at 0.20 m intervals to 1 m depth), and N-nitrate contents (at 0.20 and 0.60 m; Cataldo et al., 1975) were determined in the vegetative phase of the sorghum crop, and at planting and harvest of cover crops and sorghum (Fig. 1). Soil moisture content at 0.20 m depth was used to calculate water filled pore space (Robertson and Groffman, 2015):

$$\mathrm{WFPS}\ (\%) = \mathrm{WS} * \mathrm{BD} * 100/1 - (\mathrm{BD}/2.65) \quad (7)$$

$$\mathrm{WS} = (\text{soil wet weight} - \text{soil dry weight})/\text{soil dry weight} \quad (8)$$

where WS is soil water content at 0–0.20 m (g g^{-1}), BD is bulk density (g cm^{-3}), and 2.65 is soil particle density (g cm^{-3}).

2.3.5. Soil carbon content

Soil total carbon was determined the first (2010) and third year (2012) of the experiment in samples taken from two layer depths (0–0.06 and 0.06–0.12 m) by dry combustion with a CN auto analyzer (LECO-TrueSpec®).

2.4. Statistical analyses

Analyses of variance (ANOVA) were carried out using the mixed linear model procedure for time-repeated measurements of longitudinal data. Means were compared with Fisher's test at a significance level of 0.05 (α). The logistic equation model was used to describe the relationship between root biomass (RB) and soil microbial biomass considering that this model is generally used to describe population growth (Tsoularis and Wallace, 2002). The model equation was:

$$\mathrm{MB} = \alpha/\left[1 + \beta\, e^{-\gamma\, \mathrm{RB}}\right]. \quad (9)$$

Threshold levels of root biomass at which MBC and MBN stabilization occurred in each depth were estimated according to analysis of semivariograms with 5% error (Cressie, 1993). All statistical analyses were carried out using InfoStat software (Di Rienzo et al., 2009).

3. Results

3.1. Cover crop and sorghum aerial biomass

Sorghum shoot residues had a C:N of 77 and the total carbon they contributed amounted to 609 to 856 g C m^{-2} yr^{-1}. Sorghum crops were used for hay baling in the first and last year of the experiment with almost no remnant left on the field, whereas in the 2010/2011 season it was cut for silage, which left a remnant of approximately 25% of total aboveground biomass. All cover crops that included rye had similar aerial biomass, and on average contributed between 251 and 184 g C m^{-2} yr^{-1} while vetch produced significantly less

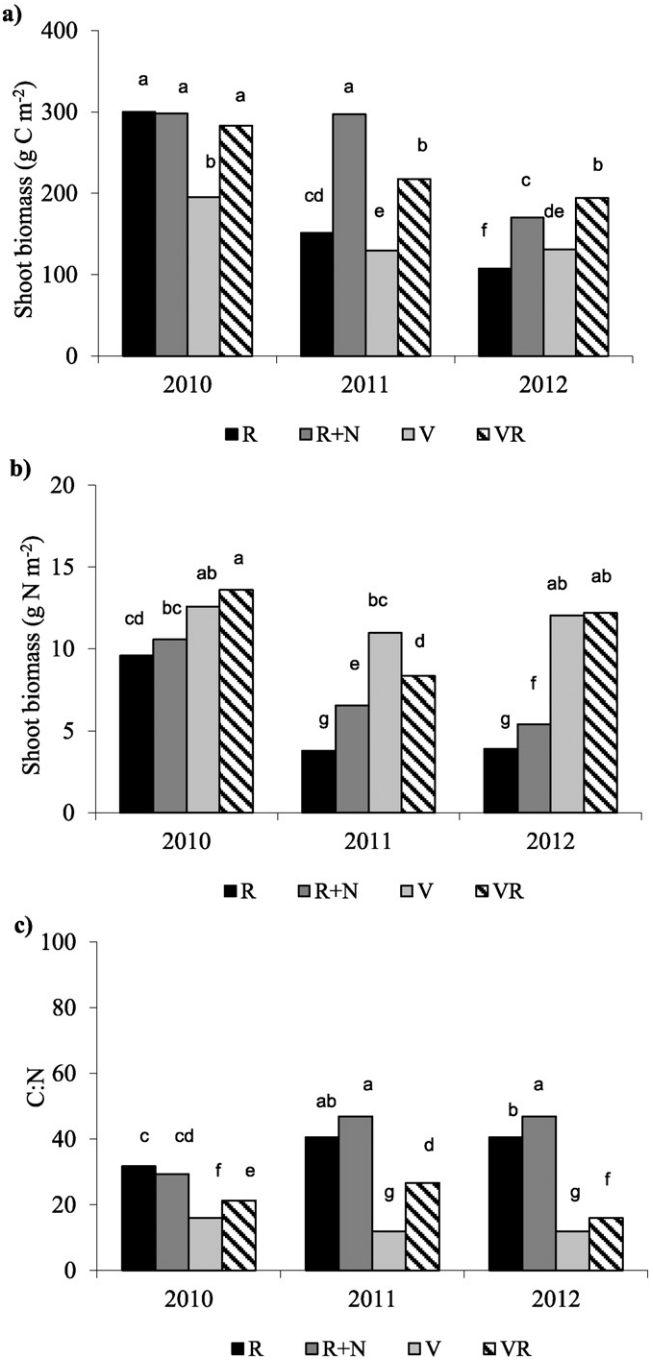

Fig. 2. Cover crop shoot biomass produced during the three year experiment in terms of carbon (a) and nitrogen contents (b) (g m^{-2}) and its quality (C:N) (c). Means with different letters are not significantly different ($p < 0.05$). Treatments were rye (R), rye plus nitrogen (R + N), vetch (V), vetch plus rye (VR), and control without cover crop (C).

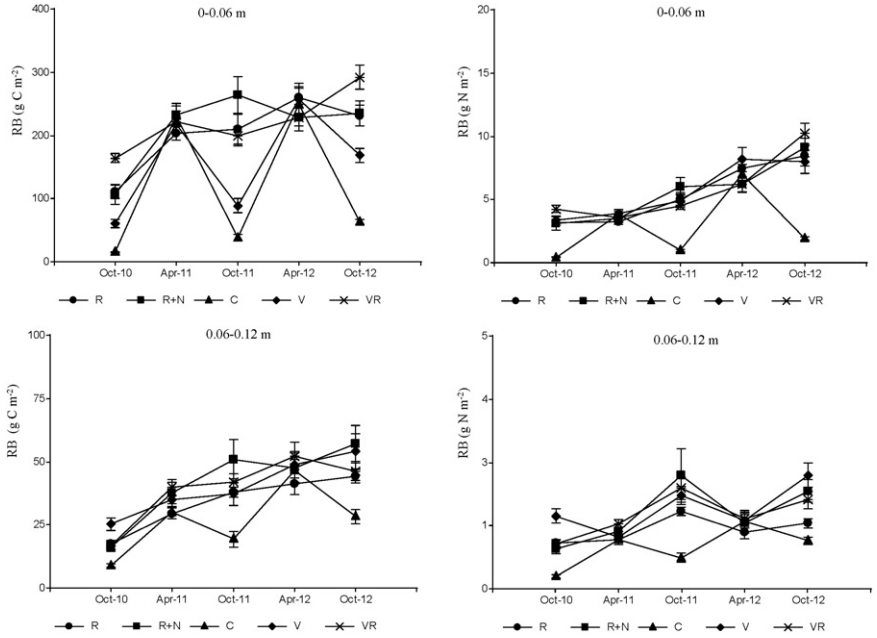

Fig. 3. Evolution of root biomass carbon and nitrogen of cover crop treatments (rye = R, rye plus nitrogen = R + N, control without cover crop = C, vetch = V, vetch plus rye = VR) through the experimental period in the two soil depths (0–0.06 and 0.06–0.12 m). October sampling represented cover crop roots and April sampling sorghum roots. Error bars denote standard error.

(148 g C m^{-2} yr^{-1}) during the 3- year experiment. However, treatments with vetch (V and VR) had higher aerial biomass N contents and contributed with a better quality material (Fig. 2).

Cover crop biomass production varied among years and treatments with a tendency to decline in successive years. During the first growing season, all rye treatments generated the highest biomass and there were no differences according to nitrogen availability, as supplied via fertilizer (R + N) or through biological fixation (VR). In the second year, rye showed a high response to N fertilization, R + N produced 146 g C m^{-2} more than R. This response declined in the 3rd year to 63.2 g C m^{-2}. During the 2nd year, VR produced 66.4 g C m^{-2} more compared to R, and in the 3rd year, the response was 87.1 g C m^{-2} more. In general, vetch alone or combined with rye produced higher N-shoot biomass (Fig. 2b) that represented an input of better quality material to the litter pool. Shoot biomass quality changed according to sampling time ($p < 0.05$), however, V and VR presented the lowest C:N (12–16 and 16–27) compared to R and R + N treatments (30–47) (Fig. 2c).

3.2. Cover crop and sorghum root biomass

The October sampling represented the cover crop roots while the April sampling collected sorghum roots (Fig. 3). Root biomass carbon differed among treatments in the October sampling dates and no

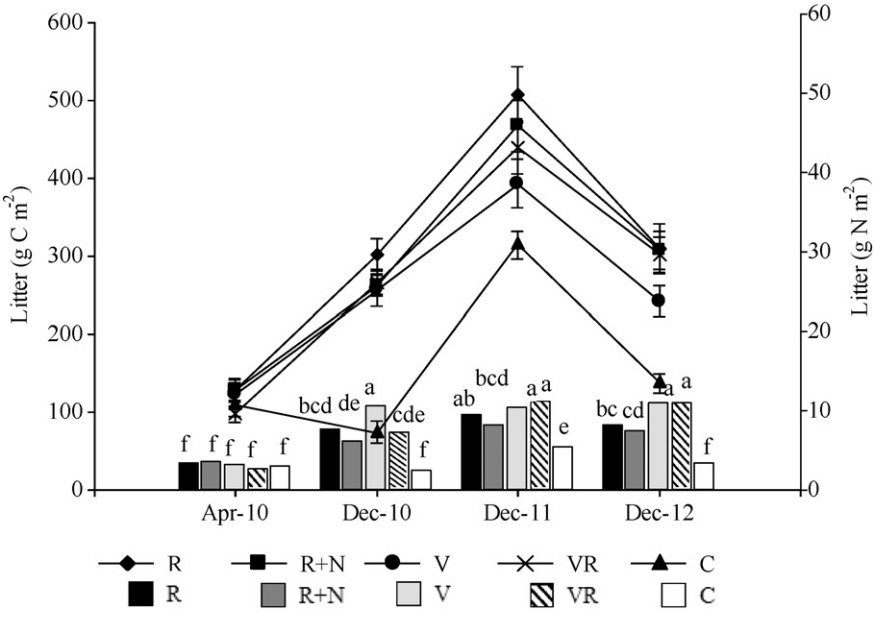

Fig. 4. Litter carbon (lines) and nitrogen (bars) contents (g m^{-2}) in cover crop treatments rye (R), rye plus nitrogen (R + N), vetch (V), vetch plus rye (VR), and control without cover crop (C) at the beginning of the experiment (April 2010) and at the beginning of each sorghum growing season. Means with different letters are not significantly different ($p < 0.05$). Error bars denoted standard error.

differences among treatments were seen in the April samplings, indicating that the effect of cover crop root contributions were diluted by sorghum root mass.

The lowest values of root-C in October sampling corresponded to control treatments (15–63 g C-BRT m^{-2} in 0–0.06 m and 9–28 g C-BRT m^{-2} in 0.06–0.12 m) (Fig. 3). Treatments with rye produced between 107 and 292 g C-BRT m^{-2} at 0–0.06 m depth, while in the deeper stratum there was less root biomass (16 to 52 g C-BRT m^{-2}). An accumulative effect was observed for R treatments in the superficial soil stratum ($p = 0.0083$), and for all cover crop treatments except V and for sorghum at the deeper stratum (p values between 0.0002 and 0.0295).

October sampling N-BRT contents (cover crop roots) were higher at 0–0.06 m compared to 0.06–0.12 m soil depth and varied between 0.4 to 10 g N-BRT m^{-2} and 0.2 to 2.1 g N-BRT m^{-2} respectively. All treatments showed significant increases in their root biomass N contents towards the end of the experiment ($p < 0.0001$) in the superficial soil. Sorghum contributed with 3.6 to 6.9 g N-BRT m^{-2} in the surface soil in each season (April sampling), while in the deeper stratum these values were considerably lower (1.1 to 1.3 g N-BRT m^{-2}).

3.3. Litter accumulation and dynamics

At the beginning of the experiment litter mass on the soil surface was between 97 and 129 g C m^{-2}. Cover crops increased litter C by 50 to 60% compared to the control treatment (Fig. 4), although no differences among CC treatments were detected.

Differences in litter C between cover crop treatments became apparent in the 2nd year, and the highest values corresponded to R, R + N and VR (Fig. 4). Litter C was lowest in the 1st year with 185 g C m^{-2} average across CC treatments, increasing in the 2nd year to 452 g C m^{-2} (mostly attributable to the relatively high sorghum stover remnant when cut for silage) and then declining to 290 g C m^{-2} in the last year. Treatments with vetch (V and VR) contributed with a material rich in N and were efficient in reducing litter C:N in the crop sequence (22–28) in contrast to R and R + N treatments (41–60) (data not shown). Litter carbon and nitrogen disappearance showed a linear relationship in all treatments (Fig. 5a). The slopes of these regressions indicate N mineralization per unit of C-loss from litter in an annual period with significant differences between V and R ($p = 0.0005$), R + N ($p < 0.0001$), VR ($p < 0.0001$) and C ($p = 0.0703$). Moreover, N-litter disappearance from the second year on explained changes in mineral N contents of soil (Fig. 5b).

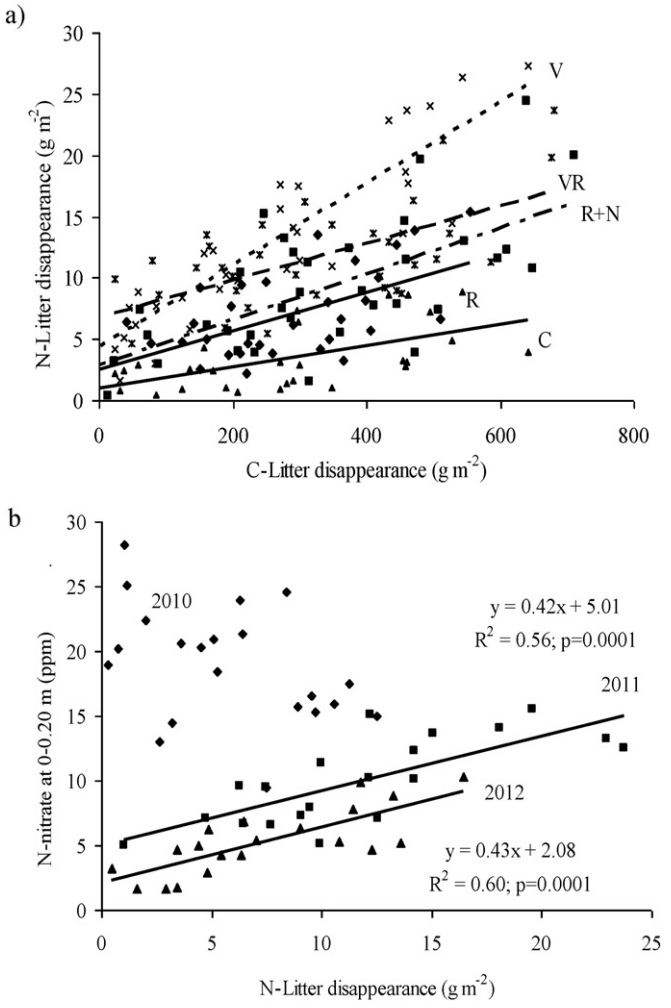

Fig. 5. a) Litter carbon and nitrogen disappearance in cover crop treatments rye (R), rye plus nitrogen (R + N), vetch (V), vetch plus rye (VR), and control without cover crop (C) during the three-year experiment; and b) the relationship between N-nitrate concentration (ppm) and N-litter disappearance (g m^{-2}) for each year (2010, 2011 and 2012).

3.4. Soil microbial biomass carbon and nitrogen

From the first year, there were significant differences between the control and CC treatments (Fig. 6). On average, CC treatments had 30% more C and 60% more N in the microbial biomass compared to NT without CC. In the first 0.06 m of soil the highest values for microbial biomass C and N were found in VR (24 g MBC m^{-2} and 2.3 g MBN m^{-2}), while in the deeper soil stratum V had more MBC and MBN (21 and 2 g m^{-2}, respectively). The control was the only treatment that showed a consistent trend to increase over the 3-year period, from an initial MBC of 13.3 g m^{-2} to 18.1 g m^{-2} at the end of the experiment. In terms of annual variations, in the surface layer a very rapid response in MBC was observed after the first growing season of cover crops (2010) in all treatments except for R + N, attributable to cover crop root input. The following years R + N evidenced changes in time with an increase in 2011 and a decrease in 2012 ($p = 0.008$) but the rest of cover crop treatments showed similar average values each year. In the deeper soil layer, a very sharp increase occurred in all treatments from year 1 to year 2, which then maintained this level in year 3. Only R + N showed a decreased the last year at this depth ($p = 0.09$). All treatments with cover crops presented higher values of MBN than control throughout the three year period at 0–0.06 m depth ($p < 0.0001$). In general, variations in time resulted significant between the first and second year ($p = 0.0004$). At 0.06–0.12 m, MBN showed differences between treatments and time ($p = 0.0002$). Treatments with cover crop increased MBN in 2011 ($p = 0.0002$) except for R. Control MBN increased in time with significant differences between 2010 and 2012.

3.5. Soil environment and its relationship with soil microbial biomass

Litter cover had an attenuating effect on soil temperature and moisture. The accumulation of residues on soil surface explained 66 and 71% of soil temperature variability at 0.06 and 0.12 m depth in the warmest month of the year (December) coinciding with early sorghum vegetative phase (Fig. 7).

Soil temperatures ranged between 41–23 °C (0–0.06 m) and 35–22 °C (0.06–0.12 m) during the experiment. Soil microbial biomass and soil temperature showed negative relationships only at 0–0.06 m the first and second year of the experiment when temperatures were higher than 25 °C (Fig. 8). No apparent effects were observed for the 3rd year.

Soil moisture contents in December (0–0.20 m) increased with time in a range between 23 and 61 mm (data not shown). Soil moisture content poorly explained the variability observed in microbial biomass carbon ($R^2 = 0.04$; $y = 0.27\times + 197.1$; $p = 0.0057$). Water filled pore space showed a better relationship with soil biota, indicating a

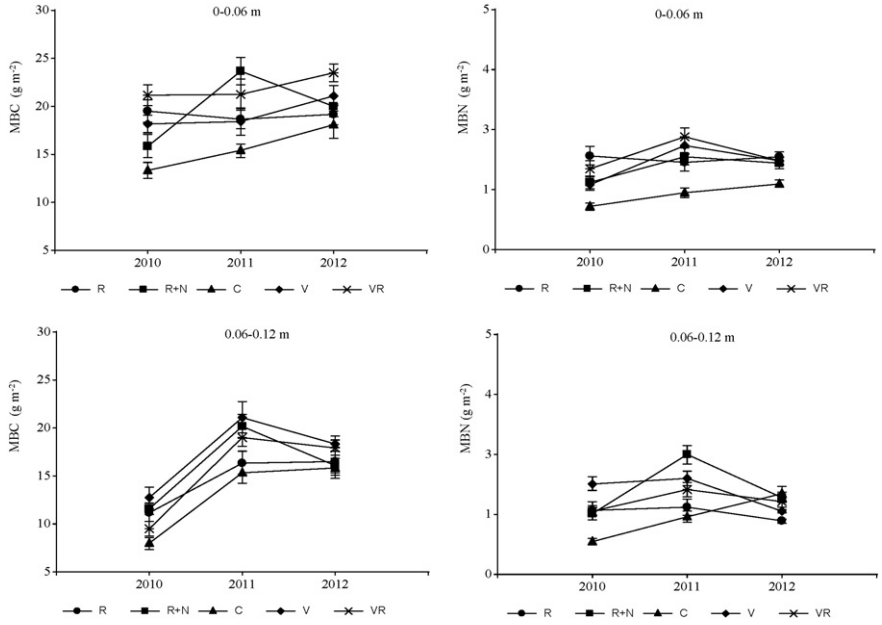

Fig. 6. Evolution of microbial biomass carbon and nitrogen (MBC; MBN) in cover crop and control treatments (rye = R, rye plus nitrogen = R + N, control without cover crop = C, vetch = V, vetch plus rye = VR) at 0.06 m and 0.12 m soil depth. Sampling was carried out in early vegetative stages of sorghum (4–6 leaves) each year. Error bars represent standard error.

threshold value of 50% above and below which the availability of oxygen or moisture respectively limited the size of soil microbial biomass (Fig. 9).

3.6. Relationships between above- and belowground plant biomass and microbial biomass

Regression analyses between cover crop shoot biomass and MBC did not result in significant relationships ($p = 0.9376$ for 0–0.06 m depth). Litter carbon did not explain much of the variability of MBC although p values were significant ($R^2 = 0.18$, $p < 0.0001$ for 0–0.12 m and $R^2 = 0.16$, $p < 0.0020$ for 0–0.06 m depth).

The relationship between root carbon and MBC adjusted to the logistic growth curve (Fig. 10).

This curve showed an exponential growth up to a value of 160 and 48 g root C m^{-2} at 0–0.06 and 0.06–0.12 m and stabilized growth at a value of 20.9 and 18.8 g MBC m^{-2} for each depth respectively, corresponding to the horizontal asymptote. The regression equations were:

$$\text{MBC (0–0.06 m)} = 20.9/\left[1 + 0.64\, e^{-0.02\ RB}\right]$$

$$\text{MBC (0.06–0.12 m)} = 18.8/\left[1 + 3.27\, e^{-0.10\ RB}\right].$$

Similar results were observed for MBN and the N contents of root biomass for 0–0.06 m depth (Fig. 10). The exponential growth was up to a value of 5.2 g root N m^{-2} and stabilized at a value of 1.91 g MBN m^{-2}. At 0.06–0.12 m, the relationship was linear ($R^2 = 0.39$; $p < 0.0001$). The regression equations were:

$$\text{MBN (0–0.06 m)} = 1.91/\left[1 + 1.55\, e^{-0.89\ RB}\right]$$

$$\text{MBN (0.06–0.12 m)} = 0.48\ RB + 0.83.$$

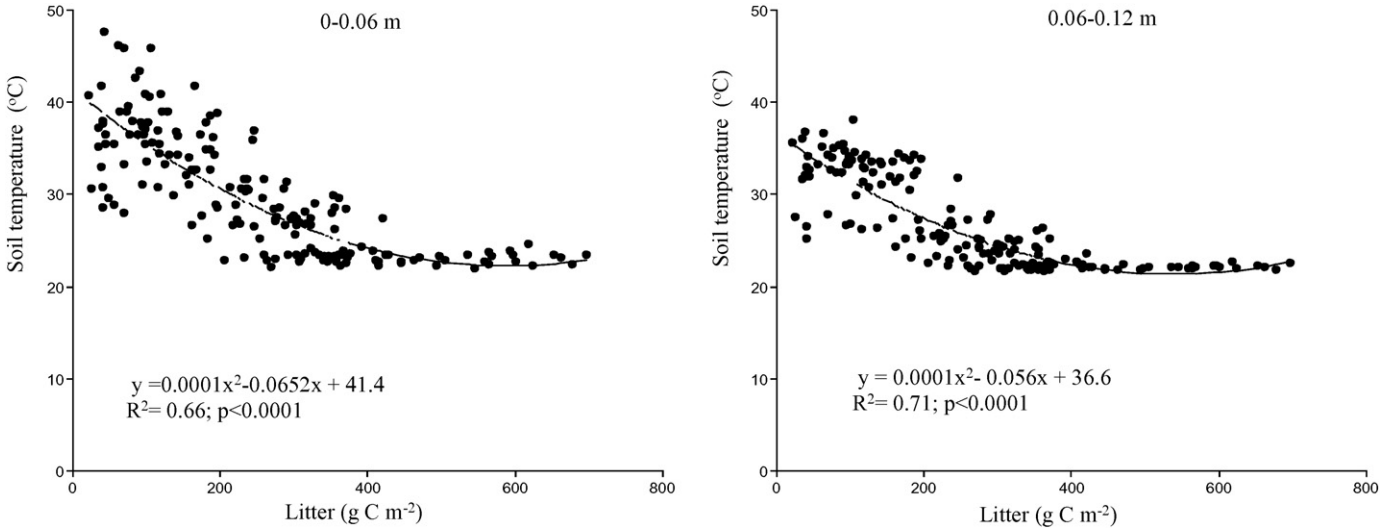

Fig. 7. Relationship between litter carbon (g m^{-2}) and soil temperature (°C) at 0–0.06 m and 0.06–0.12 m depth ($n = 240$).

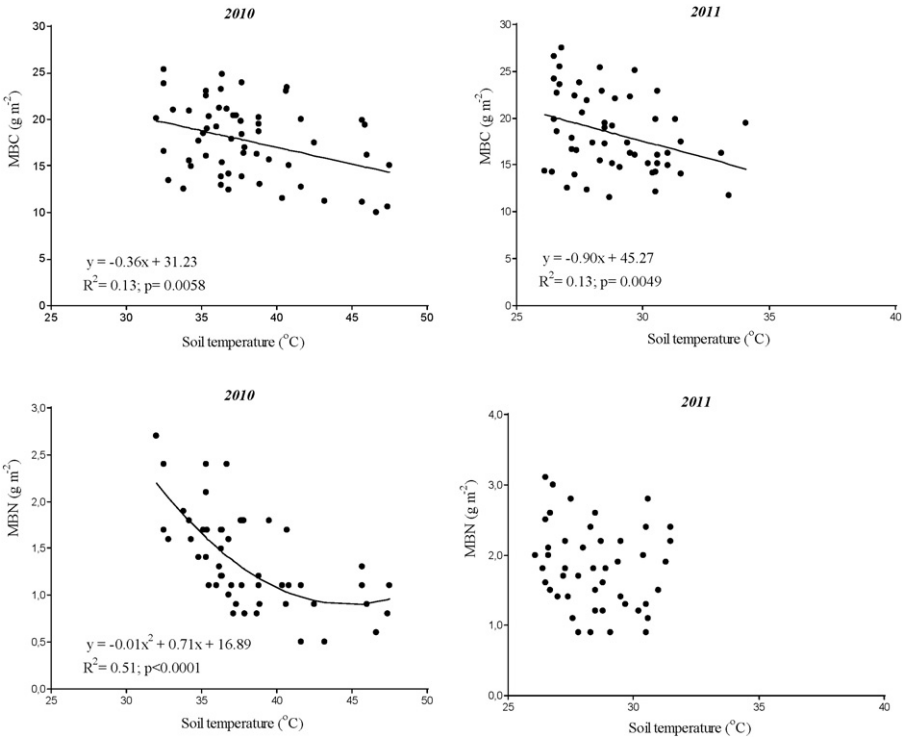

Fig. 8. Relationship between soil temperature at 0–0.06 m and soil microbial biomass carbon and nitrogen (MBC, MBN) the first (2010) and second (2011) year of the experiment.

3.7. Soil carbon contents

The results indicated differences between treatments at the end of the experiment in the 0–0.06 m soil layer. Three years of vetch inclusion as cover crop (V and VR) resulted in higher levels of total carbon in soil ($p < 0.006$) (Fig. 11). No differences between treatments in the deeper layer (0.06–0.12 m) were found, but in general all treatments showed higher carbon contents the third year ($p < 0.0003$).

4. Discussion

The change from conventional cultivation to no-till had little effect on litter cover on the soil surface (Fig. 2), except in 2011 when more sorghum stover remained in the field. Sorghum monoculture under NT contributed with very little root biomass (Fig. 4) and had an insignificant effect on microbial biomass (Fig. 5). Nevertheless, NT led to a small but steady increase in microbial biomass carbon and nitrogen,

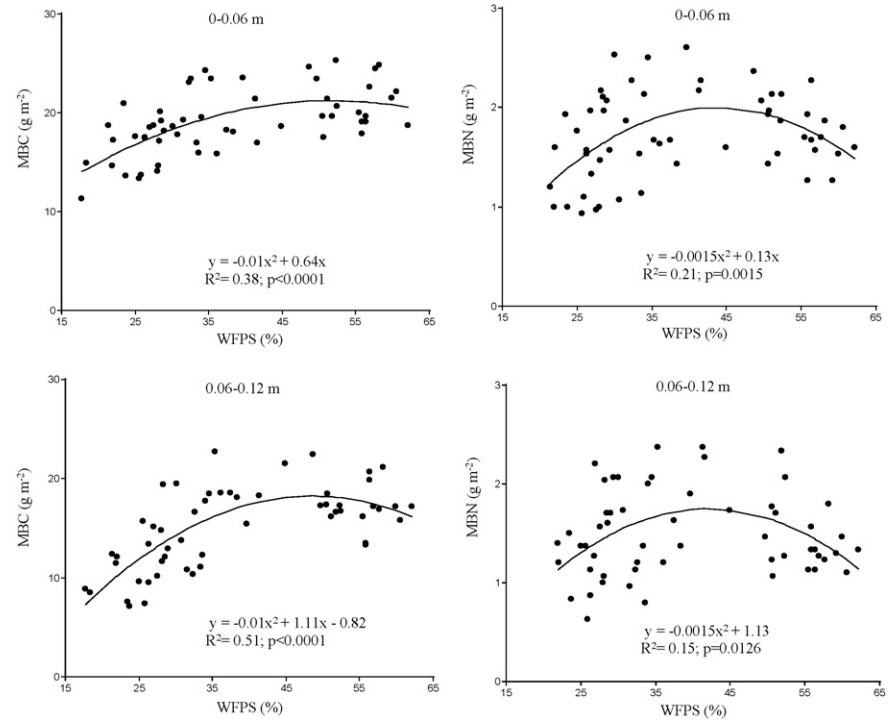

Fig. 9. Relationships between water filled pore space (WFPS) and soil microbial biomass carbon and nitrogen (MBC, MBN) for 0–0.06 and 0.06–0.12 m depth layers.

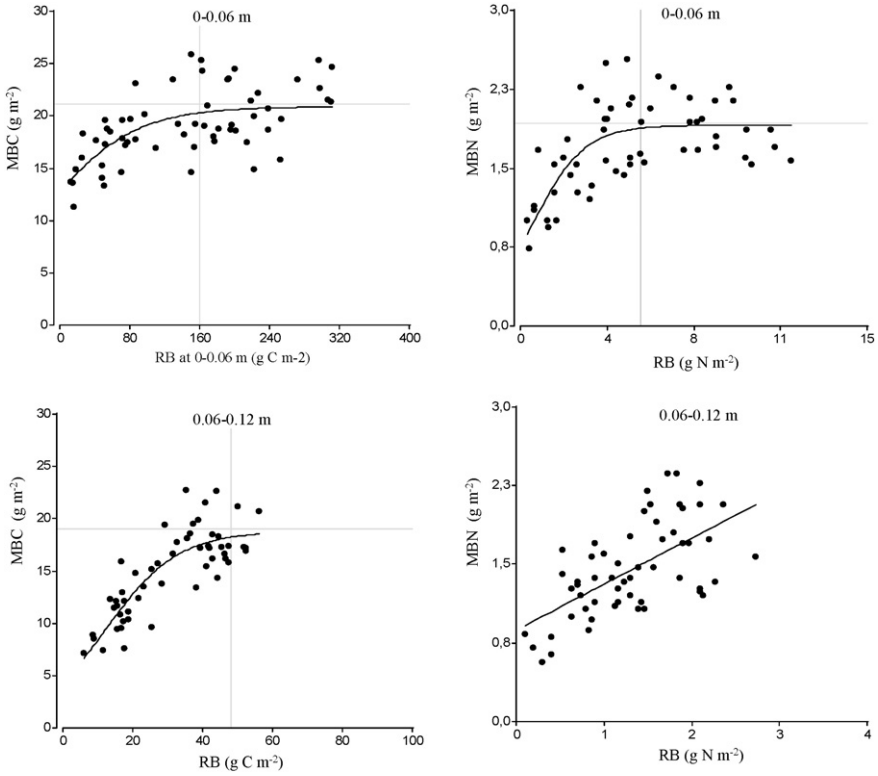

Fig. 10. Relationships between root biomass (RB) carbon (C) and nitrogen (N), and microbial biomass carbon (MBC) and nitrogen (MBN) (g m^{-2}) for the two soil depth layers (0–0.06 m and 0.06–0.12 m).

especially in the deeper soil stratum. This might be attributed to the positive effect of NT on the soil's structure and environmental conditions (Doran et al., 1998), thus improving conditions for microbial growth and reducing stress factors (Helgason et al., 2010). Yet it has been shown that microbial response is mostly to residue management and to a lesser degree to tillage itself (Spedding et al., 2004), which is what was observed when comparing sorghum monoculture with cover crop treatments. High cover crop biomass additions occurred in the experiment, especially in the fertilized and legume associated treatments, and these resulted in increased litter mass on the soil surface. However, neither aboveground biomass nor litter explained the response of soil microbial biomass satisfactorily. The R^2 values obtained in these regressions with litter were all very low (0.16 to 0.18). One possible explanation of the lack of relationship between litter and MBC is the spatial separation of these substrates in no-till systems (Garnier et al., 2008). Some authors reported a delay in microbial biomass response to litter accumulation on soil surface under no-till systems and attributed this to the spatial separation between litter and soil microorganisms (Beare et al., 1992; Poll et al., 2008). This could well be one of the reasons why Baker et al. (2007) in a recent review could not find sufficient evidence for enhanced C sequestration in NT systems. Yet these authors stipulate that the short cropping seasons and long fallow periods of modern agricultural systems that deplete the soil of living roots during long spans would be more responsible for the high C loss of agricultural soils when compared to perennial systems. Cover crop inclusion might constitute an avenue for recreating a root input similar to perennial plant cover in soils. Rhizodeposition according to Rees et al. (2005) would be rapidly respired and therefore not contribute to C sequestration, but the authors consider root biomass as an important aspect of plants when considering their potential to enhance C accrual.

Cover crop root biomass better explained the variations in microbial biomass for both soil depth stratums than aboveground biomass (R^2 = 0.46 and 0.61 for MBC, and R^2 = 0.43 and 0.39 for MBN at 0.06 and 0.12 m respectively). The strong relationships between root and soil microbial biomass can be explained by the intimate contact between roots and the soil's microbiota (Dos Santos et al., 2011). Root exudates and the turnover of fine roots provide abundant and readily accessible substrates for microbial growth (Haichar et al., 2014; Puget and Drinkwater, 2001). The response of MBC to the presence of cover crops was very rapid — after only 6 months, the difference in MBC compared to sorghum without cover crop resulted significant.

Sorghum contributed with the lowest values of shoot quantity and quality (C:N 77). Long winter fallow periods resulted in decreases in root-C biomass in the soil suggesting its decomposition, but a considerable proportion remained in soil (30–40%), which could be associated with its low quality (C:N 55). Cover crop inclusion in the crop sequence represented an extra input of shoots- and roots- C and N. In response to our hypothesis, the rye-sorghum sequence evidenced greater litter accumulation on the soil surface throughout the years, indicating that the contribution of low quality residues of sorghum and rye (C:N 40) represented a limitation for its decomposition. The inclusion of a legume was effective to improve overall litter quality in the rotation, and when vetch was combined with rye, it had the highest aerial biomass carbon contribution. Low root residue quality of both grasses (rye and sorghum) could represent a limitation, since the C:N is one of the factors that influence root decomposition rates (Redin et al., 2014; Li et al., 2015). When the crop rotation included either vetch or fertilized rye there was no accumulation of root residues. This might be due to the enhanced decomposition of low quality sorghum roots by the release of N from the legume or the higher N availability in the fertilized treatment. Some recent studies have suggested this synergistic effect of N-rich sources for decomposition of high C:N residues (Handa et al., 2014; Pérez Harguindeguy et al., 2008; Vos et al., 2013). The higher microbial biomass, lower residue accumulation and enhanced decomposition under rotation with cover crops led to higher carbon stocks compared to the NT sorghum monoculture (Fig. 11). These findings confirm the speculations of Baker and Griffis (2009) and Baker et al. (2007) that NT systems by themselves will not substantially improve

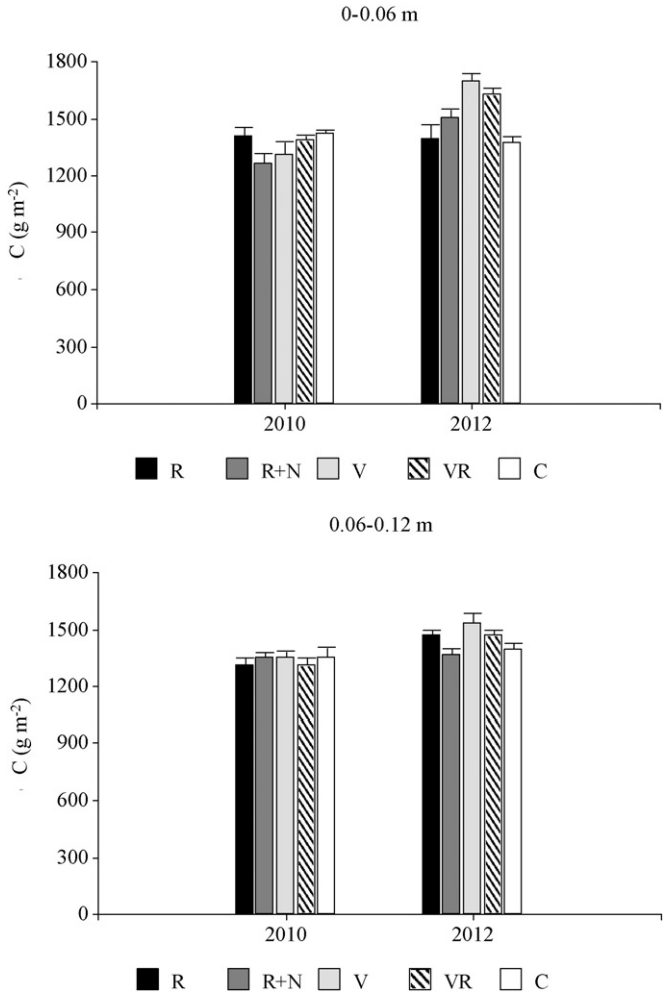

Fig. 11. Soil total carbon (g m^{-2}) at the beginning (2010) and end (2012) of the experiment with cover crop inclusion (rye = R, rye plus nitrogen = R + N, vetch = V, vetch plus rye = VR) and without cover crop (C) at two soil depth layers (0–0.06 and 0.06–0.12 m). Error bars denote standard error.

C sequestration, and that cover crop inclusion could represent an alternative for more sustainable agricultural production systems.

Cover crops' effects on soil cover also resulted in better environmental conditions for microbial activity. The resultant improved moisture conditions at 45 to 50% water filled pore space and temperatures below 25 °C during the warmest month (December) contributed to microbial biomass reaching the apparent carrying capacity for this soil (Smith and Paul, 1990).

There are several reports comparing values of microbial biomass between tillage systems, crop rotations, and land-use (Bradford et al., 2013; Ekenler and Tabatabai, 2003; Pereira da Silva et al., 2014, 2010; Wagai et al., 1998), and these values range from 250 up to 600 μg MBC g $soil^{-1}$. The results showed MBC values in the lower end of this range (143 and 295 μg g^{-1}), possibly attributable to the climate of the semiarid Pampas. There are to date no reported field studies that have monitored MBC in particular soil management systems during a longer period, therefore we expected to find a cumulative effect of continuous input of shoot and root biomass to the soil. This was the case of the sorghum monoculture showing a linear response of MBC in time. However, all cover crop treatments adjusted to logistic growth models, reaching a plateau during the second year of the experiment. According to the logistic equation any initial population will grow until reaching the maximum sustainable level, i.e. the carrying capacity. The results suggested that the soil microbial biomass was in a steady state at 20.9 g MBC and 1.9 g MBN m^{-2} (equivalent to 276 μg MBC g $soil^{-1}$, or 1.3% of SOC) for the superficial soil stratum. Although there are no previous reports from field studies, some model predictions propose steady state values for MBC. Li et al. (2014) obtained a similar value (260 μg g $soil^{-1}$) by using three different models (Allison et al., 2010; German et al., 2012; Wang and Post, 2012).

Apparently, the carrying capacity of the soil with regards to the microbial population it can sustain is not only regulated by substrate but also by environmental and intrinsic conditions that form the habitat, such as aggregation (Smith and Paul, 1990). Changing environment could affect microbial biomass by varying the carrying capacity and possibly varying the intrinsic growth rate. In fact, when applying the logistic model for each depth, both these parameters were different. Carrying capacity values were higher in surface soil (20.9 and 18.8 MBC g m^{-2} for 0.06 and 0.12 m depth respectively) indicating a larger size of microbial biomass. Annual intrinsic growth rate was lower in the surface stratum (0.02 and 0.10 for each depth respectively) indicating that microbial biomass exponential growths rates are lower in this depth, suggesting that the environmental conditions would be less favorable, i.e. higher fluctuation in moisture and temperature than in the deeper soil stratum. The fact that MBC and MBN reached carrying capacity indicated that the change in soil management simulated in the experiment led to an improved microbiological habitat.

Anderson and Domsch (2010) asked a key question: "How much substrate carbon is needed to maintain constant the soil microbial biomass?" For the *petrocalcic Paleustolls* of the semiarid Pampa which have a SOC content of ~1.5% (11.2 Mg C ha^{-1}), least an annual input of 102 g root C m^{-2} and 5.2 g root N m^{-2} is required to maintain a stable soil microbial biomass level of 20.1 g C m^{-2} and 1.9 g N m^{-2} in the topsoil.

5. Conclusions

Our findings support the hypothesis that extra residue input by cover crops increased soil microbial biomass and led to enhanced biological activity and C sequestration, thus overcoming some limitations of monoculture NT systems. Within the 3-year time-span of the experiment, microbial biomass reached the soil's carrying capacity, attributable to greater root biomass inputs and favorable moisture and temperature conditions, brought about by higher residue cover. Soil litter disappearance and its relation to mineral N availability was a good indicator of the enhanced microbial biomass activity induced by cover crops. These findings support the view that diversified systems including cover crops and specifically legumes in NT can increase soil health and ecosystem services related to water storage, element cycling, habitat for biodiversity, carbon sequestration, and plant nutrient provision.

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