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Inclusion of Cover Crops in Cropping Sequences with Soybean Predominance in the Southeast of the Humid Argentine Pampa

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Abstract: The incorporation of a cover crop (CC) may be an alternative to increase soil carbon (C) and nitrogen (N) supply in crop sequences with a high frequency of soybean. The aims of this study were (i) to determine shoot biomass production, C and N contents in the CC under two N additions, (ii) to evaluate the effect on soybean grain yield of including a CC, and (iii) to evaluate the water productivity of the crop sequences. Crops were evaluated during three growing seasons of a long-term field trial under no tillage on Typic Argiudoll in Balcarce, Buenos Aires, Argentina. Mean CC shoot biomass was 6.6 Mg ha⁻¹ yr⁻¹ for N-fertilized and 5.2 Mg ha⁻¹ yr⁻¹ for non-fertilized treatments. Mean C and N accumulation in the CC shoot biomass were 2.8 Mg C ha⁻¹ yr⁻¹ and 131 kg N ha⁻¹ yr⁻¹ in N-fertilized treatments, and 2.3 Mg C ha⁻¹ yr⁻¹ and 67 kg N ha⁻¹ yr⁻¹ in nonfertilized treatments. Soybean grain yield ranged from 2.4 to 4.3 Mg ha^{-I}. In two out of the three growing seasons, soybean grain yield was greater ($p<0.05$) for crop sequences that included a CC. Water productivity was higher ($p<0.05$) in crop sequences with a CC and even higher when they were N-fertilized. The inclusion of a CC preceding soybean in crop sequences is an alternative to improve soil C and N budgets and would be a promising strategy to increase productivity and cropping system sustainability in this region.

Keywords: Yield, sustainability, carbon, nitrogen.

INTRODUCTION

The southeast of the humid Argentine Pampa is one of the regions in the world with superior conditions for grain crop production [1], due to the temperate climate, adequate rainfall and a large proportion of soils belonging to the great group Argiudolls, with high productivity. Because of this, the region has a high potential for winter and summer crop production. In recent years, soybean (*Glycine max* (L.) Merr.) production has increased significantly, covering over 50% of the total cultivated area of the country and recording a mean annual production of 45.3 million Mg over the past five years [2]. The increase in soybean production is due mainly to an increase in soybean frequency in the crop rotation. However, soybean monoculture, in particular, may have negative effects on soil quality, affecting productivity and therefore the sustainability of the system.

Soybean has adapted to different conditions, which is why it is able to keep relatively high grain yields in low fertility soils, although it has nutrient requirements similar to or higher than other crops [3]. Soybean even has the ability to take up atmospheric nitrogen (N) through biological N fixation (BNF), although this process does not usually fulfill the crop N requirements, resulting in a negative balance of this nutrient in the soil [4]. A review by Salvagiotti *et al*. [5] reported that BNF only contributes between 50% and 60% of the soybean crop demand. However, if N contribution of the roots is considered, soil N balance could become neutral or just slightly negative [5]. According to estimates by Collino *et al*. [6] made in the southeast of Buenos Aires Province, BNF contributed about 44% of the total N demand for the soybean crop. Despite this, generally soybean did not have greater yield due to N fertilization [7], and this is related to decreases in the contribution of N by BNF [5]. Therefore, N fertilization of soybean would not be the best alternative to improve soil N balance.

Another negative feature of the increase of soybean frequency in the rotation is the low quantity of crop residues returned to the soil. In addition, the soybean residues have a low carbon:nitrogen ratio (C:N) resulting in rapid decomposition rates. These crop characteristics can lead to decreases in the soil organic carbon (SOC) [8]. SOC is considered a key component of the soil, directly or indirectly affecting many of the parameters that define its quality [9, 10]. Sainz Rozas *et al*. [11] identified significant reductions in SOC due to agricultural intensification, particularly in the north and west of the Argentine Pampa. In the southeast of Buenos Aires Province, the decrease in SOC was lower but also relevant, showing SOC content 36% lower than pristine soil conditions [11]. Therefore, it is important to identify management practices that tend to improve the C and N balance in crop sequences with a predominance of soybean, in order

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to maintain soil quality and the sustainability of the system. The integration of practices that promotes a positive C balance, such as no-tillage (NT), crop rotation, fertilization, inclusion of cover crops, and proper management of available water would maintain or increase the SOC level [10].

An alternative to increase the amount of biomass returned to the soil is through the sustainable intensification of agriculture. Indeed, the intensification sequence index (ISI) is an intuitive indicator that expresses the number of crops per year in a given crop sequence, which is associated with a more efficient capture of resources [12, 13]. In a study carried out by Caviglia *et al*. [14] in southern Buenos Aires Province, it was determined that wheat (*Triticum aestivum* L.)/soybean sequential double crops increased water and radiation productivity compared to sole crops due to an improvement in the capture of these resources. The inclusion of cover crops (CCs) during the winter season could be one strategy to increase the ISI in crop sequences where soybean predominates. The CCs provide additional biomass, increasing the amount of C that is put into the soil, which can improve its quality due to the positive effect on its physical, chemical and biological properties [15, 16]. Accordingly, Villamil *et al*. [17] and Blanco-Canqui *et al*. [18] reported that the inclusion of a CC increased SOC and this was related to improvements in the soil's physical properties.

Grass CCs generally increase C and N accumulation in response to N fertilization [19]. In addition, N uptake by grass CCs decreases the risk of $NO₃$ -leaching in fallow periods [20, 21]. As a consequence, CCs could increase N capture with no direct effects on the BNF [22]. Sainju *et al*. [23] reported that the inclusion of CCs increased SOC and soil organic N (SON), improving the productivity of the soil.

Although it is accepted that CCs have the potential to improve soil quality, contradictory information exists about the effect on the yield of subsequent crops. Several authors reported that the inclusion of CCs in a cropping sequence did not reduce soybean grain yield when water did not limit crop production [19, 20]. However, Singer and Kohler [24], and Westgate *et al*. [25], reported reductions in soybean yield and shoot biomass accumulation, utilizing rye (*Secale cereale* L.) as the CC in their studies in Iowa. On the other hand, Nielsen *et al*., [26] in a semi-arid condition, determined reduction in wheat (*Triticum aestivum* L.) yield seeded following a legumes CC, due to a decrease in water availability.

In general, there is little information in the current research literature on the introduction of a CC with or without N fertilization in systems with a predominance of soybean as an alternative to increase the balance of C and N, and of the effect of this management practice on the productivity of the system. In southern Buenos Aires Province, due to the increase in the cultivation of soybean in crop rotation and the current state of degradation of the soil, this information would be extremely useful. This study evaluates the importance of CC on biomass production, C and N accumulation, and soybean grain yield. This information is useful in order to define strategies to improve or at least attenuate soil degradation, in cropping sequences with high soybean frequency, and in this way increasing the sustainability of the production system.

The aims of this study after three years of implementating different cropping sequences with a predominance of soybean in a Typic Argiudoll in Balcarce were (i) to determine shoot biomass production, C and N contents in the CC under two N rates, (ii) to evaluate the effect of including a CC on soybean grain yield and (iii) to evaluate the water productivity of the crop sequences.

MATERIALS AND METHODOLOGY

A long-term field trial under no-tillage was started in 2006 at the *Unidad Integrada Balcarce*, in Balcarce, Buenos Aires Province, Argentina (37º45' S and 58º18' W; 870 mm mean annual rainfall; 13.8 °C mean temperature; 138 m above sea level). The soil found at the experimental site is a Typic Argiudoll with less than a 2% slope. The soil has a loam texture at the surface layer (0-20 cm depth), with an average particlesize distribution of 23% clay, 36% silt and 41% sand. The subsurface layer (25-110 cm depth) has clay-loam texture. Prior to the establishment of the experiment, the site had been under conventional tillage for more than 25 years. Tillage comprised moldboard plowing (from 10 cm to 15 cm deep), disking and field cultivation with the least tillage operations necessary to get an appropriate seedbed. In 2006, at the beginning of the study, soil pH in the 0-20 cm depth was 5.4, and SOC and P-Bray were 26.7 g C kg⁻¹ and 14 mg P kg⁻¹, respectively [Barbieri, personal communication].

The experimental design consisted of a randomized completely blocks design (RCBD) with three replications per treatment. Three treatments were evaluated: soybean monoculture (Sb); cover crop/soybean (CC/Sb) and Nfertilized cover crop/soybean (CC_F/Sb). The experimental units were 15 m x 5 m plots. The CC was oats (*Avena sativa* L.), which was chosen because it is a grass with a high growth rate spring−winter. It was sown with seed density of 120 kg ha⁻¹ and 0.175 m row spacing. In the N-fertilized CC treatment, the dose was 90 kg N ha^{-1} broadcast at tillering, corresponding to decimal state Z23 [27]. Cover crops were killed at flag leaf just visible, corresponding to state Z37 [27], with $3-4$ L ha⁻¹ of glyphosate (48% active principle). Treatment Sb was maintained without weeds during the fallow period using an application of glyphosate at the beginning of spring. Soybean was sown in rows spaced 0.35 m apart and with a density of 450,000 seeds ha⁻¹ inoculated with *Bradyrhizobium japonicum*. At sowing, it was fertilized with 20 kg P ha⁻¹ as triple superphosphate $(0-46-0)$ and 15 kg S ha⁻¹ as gypsum (SO₄Ca 2H₂O, 16% S, 20% Ca). Pests, weeds and diseases were controlled with chemical methods. Crop sequences were assessed three years after beginning the trial, in growing seasons 2009/10, 2010/11 and 2011/12. More information about crop management and total rainfall during crop growing seasons are shown in Table **1**. Decadic rainfall and temperate data were obtained from INTA's weather station, situated 500 m from the experimental site (Fig. **1** and **2**).

Immediately before applying glyphosate to CCs, above-ground biomass was harvested from three 0.35 m2 sub-samples per plot. For this, shoot biomass was clipped just above the surface of the soil. The samples were dried at 60°C in an oven until a constant weight was achieved (this took approximately a week). Similar methodology was utilized in 2010/11 and 2011/12 at soybean final grain filling,

Table 1. Management data for crops and total rainfall during the growing period of cover crops and soybean in the growing seasons 2009/10, 2010/11 and 2011/12 in Balcarce, Argentina

	2009/10	2010/11	2011/12
	Cover crop (oats)		
Sowing date	22/05/2009	14/05/2010	01/06/2011
Killing date	21/10/2009	18/10/2010	17/10/2011
Total rainfall (mm)	347	334	276
	Soybean		
Sowing date	13/11/2009	20/11/2010	24/11/2011
Variety	Nidera 4209	DM 4970	DM 3810
Harvest date	10/4/2010	27/4/2011	13/4/2012
Total rainfall (mm)	570	433	457

Fig. (1). Average monthly temperature in 2009/10, 2010/11 and 2011/12 growing seasons and historical average monthly temperature 1971- 2012 in Balcarce, Argentina.

Fig. (2). Decadic rainfall in 2009/10, 2010/11 and 2011/12 soybean growing seasons in Balcarce, Argentina.

corresponding to R6 stage [28], to determine soybean shoot biomass. Dry samples of CC shoot biomass were ground (1 mm) to determine C and N concentration using the Dumas

method of dry combustion at 950°C and CN thermoconductivity detection using a TruSpec CN analyzer [29]. At soybean maturity, defined as R8 [28], harvest was carried Table 2. Nitrogen (N) and Carbon (C) concentration, C:N ratio, Shoot Biomass Production and N and C content of the cover crops in the growing seasons 2009/10, 2010/11 and 2011/12 in the cropping sequences: cover crop/soybean (CC/Sb); N-fertilized cover crop/soybean (CC_F/Sb) in Balcarce, Argentina. When interaction cropping sequence (CS) x growing season (GS) was significant (p< 0.05) simple effects were evaluated. The main effect was evaluated when interaction GS x CS was not significant. Means were compared using the LSD test. different lower case letters indicate significant (p<0.05) differences between CS. Different capital letters indicate significant (p<0.05) differences between GS. Values in parenthesis indicate standard **error of the Mean**

out by plot combine and grain yield was determined by harvesting a surface of 1.05 m wide and 10 m long (10.5 m^2) per plot. The moisture of grain samples was measured and soybean yield was corrected to grain moisture 13.5%.

Water productivity (WP) was calculated as the ratio between total accumulated shoot biomass for each sequence $(Sb, CC/Sb \text{ and } CC_F/Sb)$ and the total accumulated rainfall in the three growing seasons. In growing season 2009/10, soybean shoot biomass was not determined, so this was estimated as the ratio between soybean grain yield and of harvest index. For this, a value of harvest index of 0.4 was used [8]. ISI was calculated as the ratio between the number of crops in each crop sequence and the length of the sequence [13].

Homogeneity of variance and normality tests were performed for each analyzed variable. C and N concentrations, C:N ratio, shoot biomass, C and N content of the CC and soybean grain yield were analyzed through a repeated measure model. This model was used to incorporate the correlations for the errors arising from measurements on the same experimental unit through the years. These analyses were done using the MIXED procedure and REPEATED option of the Statistical Analysis System (SAS) [30]. When the interaction cropping sequences x growing season was significant (p<0.05), simple effects were evaluated. The main effect was evaluated when interaction cropping sequence x growing season was not significant. Total accumulated shoot biomass and WP was analyzed using the MIXED procedure of SAS, which treated cropping sequence as fixed effect and block as random effect. When F statistic was significant, Least Significant Difference (LSD) at the 0.05 level was used to separate the means among treatments.

RESULTS AND DISCUSSION

Shoot Biomass, C and N Contents in CC

In the growing season 2009/10 and 2010/11, the total rainfall during the CC's growing period was close to the historical median of 337 mm (Table **1**), while in 2011/12 it was 61 mm lower. Shoot biomass production, C concentration and C content of CC were not affected by a significant (p<0.05) interaction year and cropping sequences, and ranged between 4 Mg ha⁻¹ and 8.4 Mg ha⁻¹ (Table 2). This is in line with the report by Restovich *et al*. [20], who used oats as the CC in the northern region of the Argentine Pampa

Fig. (3). Soybean grain yield (Mg ha⁻¹) in the growing season 2009/10, 2010/11 and 2011/12 in the cropping sequences: soybean monoculture (Sb); cover crop/soybean (CC/Sb); N-fertilized cover crop/soybean (CC_F/Sb) in Balcarce, Argentina. Vertical bars for each column indicate standard error of the mean. Means were compared using the LSD test. Different lower case letters indicate significant (p<0.05) differences between cropping sequences. Different capital letters indicate significant (p<0.05) differences between growing seasons.

without N fertilization. In addition, these values were in the range reported by other studies using grasses as the CC [31, 19]. In 2010/11, the CC shoot biomass was higher $(p<0.05)$ than in the other growing seasons (Table **2**). This could be attributed to different causes, such as the earlier sowing date in 2010/11, which gave a longer CC growing period compared to other years, the lowest accumulated rainfall in the cycle 2011/12, and the lower temperatures in late winter and early spring (September) in 2009/10 (Table **1** and Fig. **1**). The average shoot biomass production in the three growing seasons was 6.6 Mg ha⁻¹ yr ⁻¹ in CC_F/Sb and 5.2 Mg ha⁻¹ yr ⁻¹ in CC/Sb. This represents 2.8 Mg C ha^{-1} yr⁻¹ and 2.3 Mg C ha⁻¹ yr⁻¹, respectively. Shoot biomass was greater (p <0.05) in CCF/Sb (Table **2**). Similar results were reported by Ruffo *et al*. [19] in Illinois. They determined a higher shoot biomass production of rye CC when N availability increased due to N fertilizers applied to the previous corn (*Zea mays* L.) crop.

Beyond the differences among treatments with or without N fertilization, CC produced high amounts of biomass in the winter period. This may increase the amount of residues in the soil compared to soybean monoculture. It could, therefore, have a beneficial effect on the soil, diminishing the risk of erosion and soil surface crusting [32], during the CC and soybean growing season. Furthermore, a greater amount of residues on the surface may improve water conservation due to an increase in infiltration and reduced evaporation, mainly in the spring and summer seasons [15, 33]. The additional C input to the soil by CC improves the C balance in cropping sequences with soybean predominance. Therefore, it could be beneficial for productivity and sustainability of the cropping system [10].

Shoot biomass N concentration, C:N ratio and N content of the CC were affected by significant $(p<0.05)$ interaction growing season x cropping sequence (Table **2**). In 2010/11, N concentration was lower (p <0.05) than in other growing seasons. An explanation for this would be an N dilution effect due to a great shoot biomass accumulation in this grow-

ing season. This dilution effect was more evident in CC/Sb due to no N fertilization. Therefore, CC/Sb registered the higher (p<0.05) C:N ratio in 2010/11, while in CC_F/Sb it was not significantly different $(p<0.05)$ across years. In the three growing seasons, N concentration and N content in CC shoot biomass was higher $(p<0.05)$ in the N fertilized treatment. As a consequence, the C:N ratio was lower ($p<0.05$) compared to treatment without N fertilization. This is in line with the report by Ruffo *et al*. [19], which determined higher N concentration and a lower C:N ratio in rye when there was an increase in N availability. The low C:N ratio is associated with an increase of N release via mineralization [34, 35], which may be available in the soil for subsequent crops. On average, for the three growing seasons, N-fertilized CC accumulated 131 kg N ha^{-1} yr h^{-1} in shoot biomass, while nonfertilized CC accumulated 67 kg N ha⁻¹. The literature shows similar CC N content in grasses, and this depends on N availability in the soil and shoot biomass production [19, 21, 31]. Although the sequence CC/Sb showed the lowest ($p<0.05$) N accumulation in every cycle (Table **2**), due to the lowest shoot biomass production and the lowest N concentration, the amount of N accumulated can be regarded as important because it is derived from the soil and not from the fertilizer. Therefore, N content in CC/Sb indicates a beneficial effect of CC due to the reduced risk of N leaching [19-21]. **Solution**
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In addition, the N immobilized by the CC may later be mineralized in the soil and absorbed by subsequent crops in the rotation [33]. Is important to consider that the reported values of C and N accumulation in CC were determined only in shoot biomass, so if the root biomass and root exudates are included [36], these values would be increased and the beneficial effect of the CC on C and N accumulation would be higher.

Soybean Grain Yield

Soybean grain yield ranged from 2.4 to 4.3 Mg ha^{-1} (Fig. **3**), similar to what was reported in other regional studies

Table 3. Total Accumulated shoot biomass (soybean + cover crop) in the growing season 2009/10, 2010/11 and 2011/12, and water **productivity (WP) in the cropping sequences: soybean monoculture (Sb); cover crop/soybean (CC/Sb); N-fertilized cover** crop/soybean (CC_F/Sb) in balcarce, argentina. WP was calculated as a ratio between total accumulated shoot biomass and total accumulated rainfall in the Three Growing Seasons (2,503 mm). different letters indicate significant (p<0.05) differences between cropping sequences using the LSD test. Values in parenthesis indicate standard error of the mean

ity is the major determinant [37]. For the three growing seasons, the average grain yield was 3.0 Mg ha⁻¹ in the monoculture, and 3.3 Mg ha⁻¹ and 3.2 Mg ha⁻¹ when it was sown after a CC with and without N addition, respectively.

There was a significant $(p<0.05)$ interaction growing season x cropping sequence for grain yield. In 2009/10, every cropping sequences registered higher grain yield $(p< 0.05)$, which ranged between 3.7 Mg ha⁻¹ and 4.3 Mg ha⁻¹ (Fig. **3**). One explanation for this would be the adequate rainfall during the soybean's growing period, which accumulated between the third decade of October (2009) and the second decade of March (2010) (covering the period between CC killing and soybean maturity). It was 71 mm above the historical median for this period (499 mm) (Table **1**). In addition, there was abundant rainfall between the second decade of February and the first decade of March (Fig. **2**), coinciding with the period between R4 (fully developed pod) to R6.5 (fully developed seed) growth stages [28]. This period is proposed as critical for kernel set [38]. In this growing season, grain yield was greater ($p<0.05$) in CC_F/Sb but lower in CC/Sb and Sb (Fig. **2**). On the other hand, in the 2010/11 growing season, rainfall was less favorable for soybean growth. Accumulated rainfall was 66 mm below the historical median (Table **1**). Low rainfall was also recorded during the critical period for kernel set, and, as a consequence, soybean grain yield was affected (Fig. **2**). Grain yields did not differ $(p \le 0.05)$ between cropping sequences and were in the range of 2.4 \overline{Mg} ha⁻¹ to 2.7 \overline{Mg} ha⁻¹ (Fig. 3). This is in line with what was reported by Restovich *et al*. [20] who determined, in a long-term study, that soybean grain yield was no different with oats as the CC under different rainfall conditions, from normal to high or exceptionally dry, during soybean growing seasons. During the 2011/12 growing season, accumulated rainfall was 41.2 mm below the historical median although the rainfall during the critical period for kernel set (which coincided with previous years) was higher than in 2010/2011 (Fig. 2). Soybean yield ranged from 2.7 Mg ha⁻¹ to 3.1 Mg ha⁻¹, and was higher ($p \le 0.05$) in CC/Sb and CC_F/Sb , than Sb (Fig. 3).

Under temperate weather conditions in Illinois, with mean soybean grain yield of 2.8 Mg ha⁻¹, Ruffo *et al.* [19] reported no yield differences with or without a CC. However, in studies performed in Iowa with a range of soybean grain yield similar to those found in our experiment, decreases were reported in shoot biomass and soybean grain yield using rye as the CC [24, 25]. Despite these results, Pantoja *et al*. [39], working in four locations in Iowa, reported no significant differences in soybean grain yield following rye, although in their studies the rye shoot biomass production was low $\left($ <2500 Mg ha).

Results of our study indicate that soybean grain yield did not decrease after the inclusion of a CC in three growing seasons beyond differences in rainfall. In addition, in 2009/10 when environmental conditions were favorable to achieve high yields and in 2011/12 when, although rainfall was lower, it did not affect the yield much, the sequences with CC had higher yields than monoculture soybean (except for CC/Sb in 2009 that did not differ from Sb). This indicates a possible beneficial effect of these management practices on soil productivity, beyond the short period after the implementation. Villamil *et al*. [17] and Blanco-Canqui *et al*., [18] reported that the inclusion of CCs in different cropping sequences increased the organic C content and improved physical properties that affect soil productivity.

Water Productivity

Analyzing the three growing seasons, the total rainfall was 2,503 mm (from April 2009 to March 2012), total accumulated shoot biomass by the cropping sequences in this period was higher (p<0.05) in CC_F/Sb followed by CC/Sb , while Sb was the lowest (Table **3**). Therefore, WP was higher ($p<0.05$) for sequences with a CC and even higher when they were N-fertilized, showing WP values that were twice as high as soybean monoculture (Table **3**). This is consistent with the reports of Caviglia *et al*. [14] and Caviglia and Andrade [13], who determined a more efficient use of the resources due to the intensification of the production system by increasing the number of crops per year. For the sequences analyzed, the ISI was 2 in CC_F/Sb and CC/Sb (two crops in one year) while in Sb it was 1 (one crop in one year). This indicates that increases in the ISI were associated with improvements in WP [13].

CONCLUSIONS

The results obtained in this experiment showed that the inclusion of a CC in cropping sequences with soybean predominance improves water productivity and these effects were greater in CCs fertilized with N. On the other hand, CCs increased the amount of C provided to the soil through residues, which can improve C balance. The use of a CC also takes up substantial amounts of N, which would prevent N-

leaching mainly in regions with higher rainfall during the winter period, as is the case in southeast of the Humid Argentine Pampa. This N immobilized in residues would be recycling back into the soil, and be utilized by following crops in the rotation. Inclusion of a CC did not restrict soybean grain yield in the three growing seasons when different crop water conditions occurred. In addition, when water availability favored high grain yield, sequences with CCs had higher yields than soybean monoculture. This supports the incorporation of a CC in crop sequences with a high frequency of soybean in southeast of the Humid Argentine Pampa as a feasible alternative to increase the contribution of C and N, and to improve the balance of both elements in the soil. It would therefore be useful to evaluate the effects of grass CCs with or without N fertilization following soybean over the long term, in different environments and production scenarios, on processes that intervene in the dynamic of C and N and other parameters that define soil quality. This would allow us a greater understanding around the impact of this management practice on soil productivity and sustainability of the production system.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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