European Journal of Agronomy 149 (2023) 126915

Contents lists available at ScienceDirect

European Journal of Agronomy

journal homepage: www.elsevier.com/locate/eja

Soybean seed yield and protein response to crop rotation and fertilization strategies in previous seasons

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ARTICLE INFO

Keywords:
Crop intensity
Cropping systems
Diversification
Maize
Field pea
Soybean
Wheat

ABSTRACT

Annual crops dominate Argentinean landscapes with a majority of fields dedicated to soybean production. Consecutive years with full-season soybean crops are frequent and this practice is associated with organic carbon losses affecting soil chemical and physical properties. It is proposed that more intensive and diverse cropping sequences coupled with targeted fertilization may allow keeping near-neutral nutrient and carbon balances while improving successive crops performance. We conducted four on–farm experiments along six seasons in the Argentine Pampas where three crop rotations and two fertilization strategies (high and regular rates of N, P, and S) were evaluated. Crop rotations involved were i) soybean monoculture, ii) a typical wheat-soybean/maize/soybean rotation, and iii) a more intensified rotation consisting of wheat-soybean/field pea-maize/soybean. After five years, soil organic carbon was consistently lower under soybean monoculture compared to that under more intensive and diverse rotations. During the sixth and last season, identically managed soybean crops were grown in all treatments to determine the influence of previous management history on soil, plant nutrients, and on crop performance. Results indicate that crop rotation largely influenced soybean seed yield (range from 120 to 690 kg ha^{-1} yield increase) mainly through changes in water use affecting solar radiation interception and, by a lesser extent, the conversion efficiency of radiation into biomass and partition to seeds. Comparatively, increased fertilization rates in preceding seasons resulted in a lower seed yield response (range from nil to 180 kg ha^{-1}), which indicates that fertilization strategies alone are ineffective to restore productivity levels after years of soybean monoculture. However, in most cases, the high fertilization strategy in previous seasons resulted in greater soybean protein concentration in seeds and in overall protein yield.

1. Introduction

Natural ecosystems in the Argentine Pampas consisted of vast grasslands that transitioned into mixed farming systems alternating annual crops and pastures during the 20th century. In the last three decades, annual crops increased their dominance mostly driven by the expansion of soybean that now accounts for more than half of the cultivated land in the region (de Abelleyra and Verón, 2020; Viglizzo et al., 2011; MAGYP, 2021). This surge in soybean production was initially supported by the crop's suitability for the wet-temperate conditions of the Pampas and then promoted by the development of effective farming technologies in combination with a high relative price and low production costs compared to other crops in the area (Satorre and Andrade, 2021).

In Argentina, soybean is typically grown during the warm season in a 3-year wheat-soybean/maize/soybean rotation. Sometimes other crops such as sunflower, grain sorghum, barley, rapeseed, or field pea are included. However, consecutive years of full-season soybean are also frequent especially in rented fields where farmers aim to reduce production costs. At issue is that cropping systems dominated by low

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https://doi.org/10.1016/j.eja.2023.126915
Received 22 March 2023; Received in revised form 29 June 2023; Accepted 15 July 2023

biomass producing crops like soybean often result in organic carbon losses, which negatively affects soil chemical and physical properties that influence nutrient cycling, water dynamic, and plant root growth (Studdert, Echeverria, 2000; Novelli et al., 2011; Nosetto et al., 2012, 2015; Crespo et al., 2021). Furthermore, this type of cropping system has a substantial gap between nutrient exportation and reposition that results in nutrient mining and fertility losses (Andrade et al., 2022; Wyngaard et al., 2022). The inclusion of high biomass-producing crops in the sequence (Studdert, Echeverria, 2000) and/or increasing the crop intensity (*i.e.* with more crops per year) (Caviglia et al., 2004; Andrade et al., 2015; Novelli et al., 2017) can help mitigating losses in soil organic carbon and fertility. Effectiveness of the crop rotation in preventing soil deterioration could be assessed directly through determination of changes in soil properties over time. In addition, since such changes affects plant performance, soil deterioration may be also evaluated *via* a detailed ecophysiological analysis by assessing resource capture by crops and their efficiency to convert captured resources into biomass and seed yield when growing under the different soil conditions.

After decades of continue cropping in the region, little attention has been paid to holistic approaches that evaluate the long-term impact of management decisions over the cropping system and ways to alleviate soil degradation. Besides crop rotation diversification and intensification, management practices such as fertilization strategies may help attenuating soil deterioration processes. However, its capacity to successfully mitigating this phenomenon has not yet been explored. Given its importance as both a grain and industrial crop, our aim was to evaluate the residual response of soybean crops to changes in both crop rotation and fertilization strategies in preceding seasons. For this purpose, we conducted four on-farm experiments at various locations in the rolling and inland Pampas of Argentina.

2. Materials and methods

2.1. Experimental sites

Over a span of six seasons, from April 2014 to March 2020, we conducted four experiments in the Pampas region of Argentina. Our experiments involved three different crop rotations and two fertilization strategies, all under a no-till system and rainfed conditions, which is the most frequent scenario for grain production in Argentina. Experiments were situated in the north area of Buenos Aires province, close to Pergamino (1), San Pedro (2), and Bragado (3 and 4) cities (Fig. 1). Soil texture differed among sites, being silt-clay-loam in San Pedro, silt-loam in Pergamino, and sandy-loam in Bragado. Actual clay content in the sub-soil is high in San Pedro (48 % clay in Typic Argiudoll, Arrecifes series, 1–3 % slope) and Pergamino (43 % clay in Typic Argiudoll, Urquiza series, <1 % slope), and low in Bragado (25 % clay in Typic Hapludoll, Bragado series (I) and 16 % clay in Entic Hapludoll, Norumbega series (II), both <1 % slope; INTA, 1989). Additionally, experiments in Bragado and Pergamino presented a water-table oscillating between 1.2 and 1.8 m depth (Supplementary Table S1).

2.2. Experimental design and crop management

Initially, a randomized complete block design in which three crop rotations with two replicates were implemented in all four on-farm experiments. Thus, each experiment consisted of 6 large plots, each measuring 22 m wide and at least 200 m long (>0.44 ha per plot, >2.6 ha per trial). The reduced number of replicates within each site could be seen as one limitation of this study together with the lack of contemporary presence of all phases of each crop rotation in the same year, which is useful to determine the effect of crop by weather interaction (Cochran, 1939). Here, we explored the influence of spatial variation in weather and soil by setting experiments in four key sites to be representative of most usual environments in the studied area. The inclusion of all phases of each rotation every year, and one more replicate per site, would have implied to increase 3.5-fold the number of plots at the cost of having to reduce the number of experiments and/or plot size to keep trials manageable and within budget, losing both representativeness of commercial fields and regional coverage.

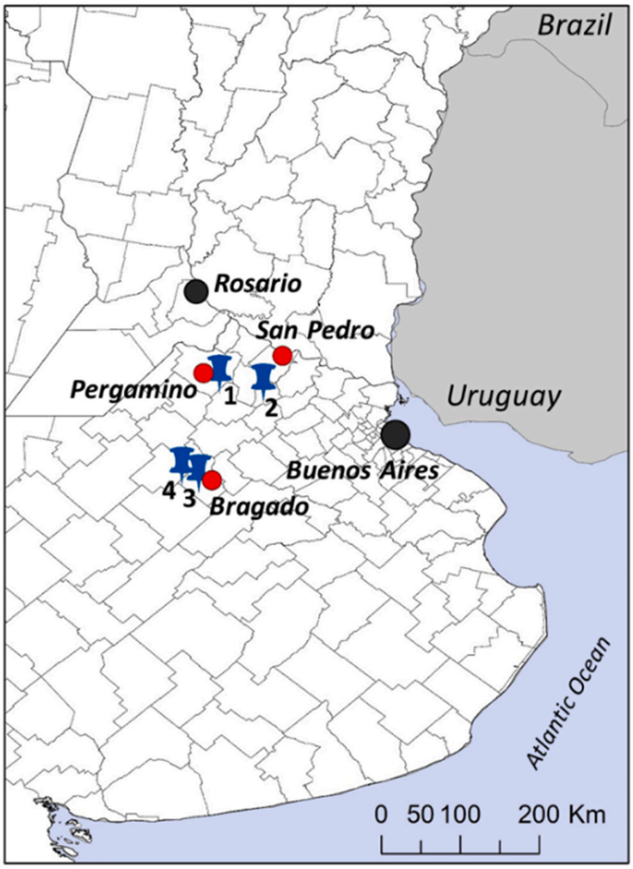

Fig. 1. Experimental sites (blue pins) and proximate main cities (red dots). Sites were named based on the proximate cities as follows: Pergamino (1; 33° 55′ S; 60° 23′ W), San Pedro (2; 34° 01′ S; 59° 54′ W), Bragado I and II (3 and 4; 35° 03′ S; 60° 38′ W).

Crop rotations evaluated were i) soybean monoculture (SOY), ii) a typical wheat-soybean/maize/soybean rotation (TYP), and iii) a more intensified rotation consisting of wheat-soybean/field pea-maize/soybean (INT) (Table 1). Two 3-yr cycles of each crop rotation were completed. We followed farmer regular fertilization strategy in the area, in which maize and wheat crops are fertilized at sowing with nitrogen (N) to reach 180–200 and 150–160 kg ha^{-1} of total soil plus fertilizer available N, respectively, after N-NO$_3$ soil analysis (0–60 cm). On these crops, 27–34 kg ha^{-1} of phosphorus (P) were also applied at sowing. Legume seeds were inoculated with nitrogen-fixing bacteria at sowing (*Bradiryzhobium japonicum* for soybean and *Rhizobium leguminosarum*

Table 1
Crop rotations evaluated.

Season	Soybean monoculture [SOY]	Typical rotation [TYP]	Intensified rotation [INT]
2014/15	Soybean	Wheat-Soybean	Wheat-Soybean
2015/16	Soybean	Maize	Field pea-Maize
2016/17	Soybean	Soybean	Soybean
2017/18*	Soybean	Wheat-Soybean	Wheat-Soybean
2018/19*	Soybean	Maize	Field pea-Maize
2019/20	***Soybean***	***Soybean***	***Soybean***

* plots were split by half in 2017/18 and set following i) high or ii) regular fertilization rate strategies.

biovar viceae for field pea) and 10–14 kg P ha^{-1} were applied according to expected yields except for double cropped soybeans (following wheat) that were not fertilized at all. Once the first three-year cycle was completed (2017), plots were split by half to set two different fertilization strategies (high and regular fertilization rates) in 2017/18 and 2018/19 seasons. The high fertilization scheme implemented consisted of additional 25–35 kg N ha^{-1} (in wheat and maize), 8–15 kg P ha^{-1}, and 10–30 kg S ha^{-1} (in all crops but double cropped soybeans) across sites. After this subdivision, a combination of three crop rotations and two fertilization managements was set, resulting in six treatments. In the last season (2019/2020), all treatments converged in full-season soybean crops following the exact same management (farmer's average), within each location, in order to assess the impact of previous seasons management on this crop. Management practices followed on each crop for the entire rotation (2014–2020) are available as Supplementary Material (Supplementary Table S2). Here, we detail the soybean crop management during the 2019/2020 season exclusively.

Soybean management practices in 2019/2020 were conducted following regular commercial field operations with typical machinery used by farmers in the region. This means soybean was sown with a no-till system and top yielding, maturity group IV, cultivars (DM40R16STS in Pergamino; DM4612 in Bragado I, Bragado II, and San Pedro; see DON MARIO Semillas – http://www.donmario.com for details on varieties). Sowing date (late October in Bragado I, Bragado II, and Pergamino; late November in San Pedro), seeding rate (37–40 seeds m^{-2}), and row spacing (20–35 cm) used in the experiments were also based on regional recommendations to achieve high yields. Weeds, insects, and diseases were maintained below damage thresholds by spraying agrochemicals when necessary.

2.3. Environmental and soil conditions

Daily incident PAR and temperature records during the 2019/2020 season were obtained from meteorological stations nearby experimental sites (INTA, 2022), except for incident PAR data at Pergamino that were retrieved from NASA-POWER (2022) (Fig. 2). Monthly mean temperatures during soybean growing season (November-March) were between 21 and 24°C, with the highest values in San Pedro. Daily incident PAR ranged between 10 and 12 MJ m^{-2} from November to February, showing an important drop at the end of the growing season (March). Rainfall data was measured at the experimental fields. Cumulated rainfall during the growing season was similar to the historical average for the region in Bragado but lower in Pergamino and San Pedro (Fig. 2). However, until February, when soybean yield is usually determined in the Pampas, all sites presented less cumulated rainfall than the historical records, with a large deficit in San Pedro, intermediate in Pergamino, and lowest in Bragado.

Several variables were measured in the main plot (crop rotation) to determine soil condition across treatments prior to soybean crop establishment in the 2019/20 season. Crop residues on the soil surface were obtained from three 1 m^2 samples per plot, and then dried and weighted to estimate stubble biomass. In addition, soil water content was calculated gravimetrically, at sowing and maturity, as the weight difference between soil samples before and after drying at 110°C for 72 h. This estimation was performed in layers of 20 cm up to a depth of 180 cm. Moisture content was calculated and expressed as volumetric water (%) considering soil apparent bulk density reported at each location (INTA, 1989; Saxton et al., 1986). Soil pH was determined through potentiometry (SAMLA, 2004). To evaluate soil physical condition in the various treatments, apparent bulk density in the topsoil (0–10 cm) was calculated from undisturbed samples as the relation between dry soil mass and its volume following the cylinder method (Blake and Hartge, 1986). The proportion of soil volume not occupied by solid particles (porosity) was estimated based on the measured apparent density (AD) and real density (RD) of solid particles (2.65 g cm^{-3}) as *1-(AD/RD)*. Soil aggregate stability (0–10 cm) was assessed based on average particle size after applying a mechanical stress on moisturized samples (Le Bissonnais, 1996). Water infiltration rate was also measured as the water lamina that the soil infiltrates per hour using an infiltrometer disc device after removing topsoil crop residues (Gil, 2006). Finally, both soil total organic carbon (SOC) and organic carbon in the soil light fraction (Galantini, 2008) were estimated based on Walkely and Black (1934). The organic carbon in the soil light fraction was isolated using bromoform (density = 2 g cm $^{-3}$) followed by agitation, centrifugation, and filtration of soil samples. This fraction of soil organic carbon corresponds to less degraded particles.

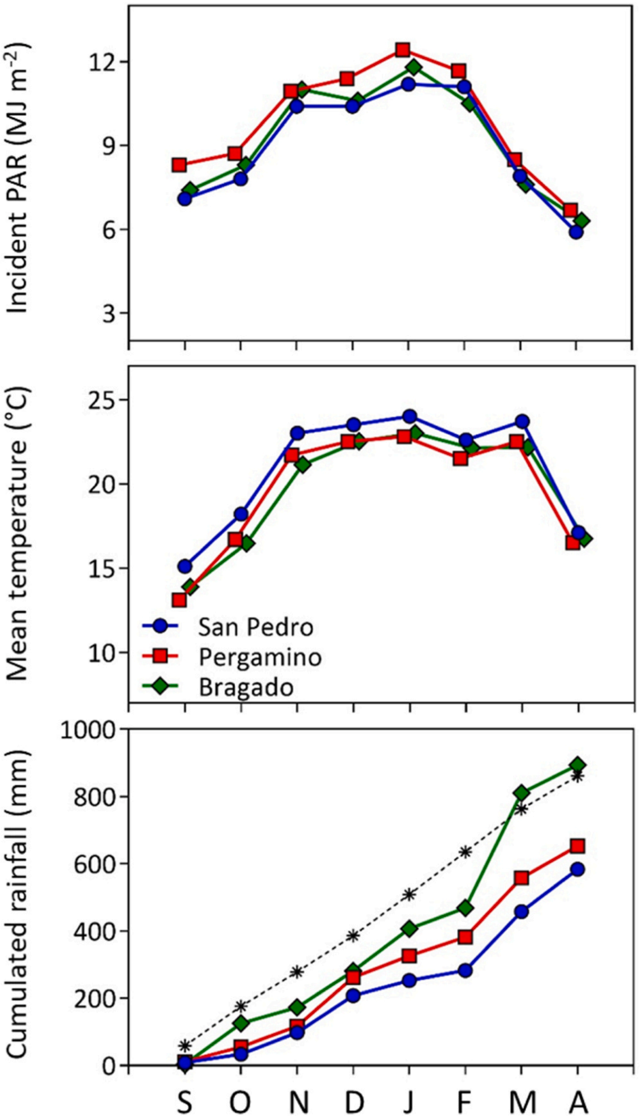

Fig. 2. Monthly average photosynthetically active solar radiation (PAR; MJ m^{-2}; top panel) and temperature (°C; middle panel) and monthly cumulated rainfall (mm; bottom panel) in all experimental sites during 2019–2020 cropping season. Historical average rainfall (1970–2020) for the area is shown in bottom panels with a discontinuous line.

2.4. Measurements

Incident photosynthetically active radiation (PAR) during the crop cycle, interception efficiency (ei), radiation use efficiency to produce aboveground biomass (RUEb), and harvest index (HI) were considered as the eco-physiological determinants of grain yield. During the 2019/2020 season, fraction of incoming radiation reaching the ground (PAR non-intercepted/incident PAR) was measured with a photosynthetic photon flux sensor bar (Cavadevices Argentina; http://www.cavadevices.com) every 10–15 days to estimate the interception efficiency and calculate overall PAR interception during soybean crop cycle. Moreover, a partial water balance (water lamina at sowing - water lamina at maturity + effective rainfall) was calculated to estimate crop evapotranspiration (ET) during the growing season. Effective rainfall (rainfall - runoff) was determined based on actual rainfall and runoff curve number method (Hjelmfelt, 1991). For this calculation, no changes in runoff were considered across treatments despite differences in stubble amounts. In San Pedro, water content in soil at maturity was assumed to remain constant beyond 120 cm depth. Crop ET was not determined at Bragado I given the unknown magnitude of the influence of a water table that prevented accurate estimations (Supplementary Table S1).

Aboveground biomass of every crop was estimated from three 1 m^2 samples in each plot at physiological maturity. Plants were cut at ground level, dried and weighted. For seed yield determination (kg ha^{-1}), plots were harvested with a combine harvest machine, transferred to a hopper with scale and weighed. Yields are expressed at 0 % moisture content in seeds. Crop residues were left on the soil after harvest. Nitrogen content (%N) in seeds samples were determined by Kjeldahl. Protein content (%) was estimated after multiplying %N by 6.25 (Krul, 2019), while protein yield (kg ha^{-1}) was estimated as the resultant of seed yield by protein content. Statistical analyses were performed with Infostat software (Di Rienzo et al., 2020). Treatments effects were evaluated using analysis of variance (ANOVA) and comparison of means (LSD). Associations between variables were evaluated with regression analyses.

3. Results

3.1. Soil conditions during the 2019/20 season

The amount of stubble on the soil surface was more than double in the typical (TYP) and intensified (INT) rotations (range 8.3–12.5 Mg ha^{-1}) than under continuous soybean (SOY) (3.1–5.3 Mg ha^{-1}) across sites ($p < 0.05$; Table 2). Similarly, at all sites, either average organic carbon (SOC) and/or the light fraction of organic carbon concentration in the topsoil (0–10 cm) was larger in TYP and INT than under SOY ($p < 0.10$; Table 2). However, differences in absolute SOC and its light fraction (0–10 cm) were comparably lower, given the higher soil bulk density found in SOY plots (1.27 g cm^{-3}; range 1.18–1.38) compared with TYP and INT (1.20 g cm^{-3}; range 1.08–1.29). Overall, the light fraction of SOC in the topsoil (0–10 cm) varied from 3.8 to 4.6 Mg ha^{-1} in SOY to 4.7–5.4 Mg ha^{-1} in TYP (average 25 % increase) and 4.9–6.3 Mg ha^{-1} in INT sequence (average 40 % increase; Table 2). Compared to the initial soil conditions (2014/15 season; Supplementary Table S1), SOY reduced absolute SOC and its light fraction while TYP and INT crop rotations resulted in no significant changes in three out of four sites (CI 90 %). Additionally, crop intensification resulted in larger water infiltration rates in Pergamino ($p < 0.05$), where a difference of 9 mm h^{-1} was found between TYP and INT *versus* SOY, while similar trends were found in Bragado I and II (Table 2). Finally, given the 6-month fallow period prior to full season soybean crops, soil initial water content was similar among treatments at all sites except Bragado II, where INT presented a higher water lamina above wilting point (389 mm) than TYP (280 mm) and SOY (226 mm) in the 1.8 m depth soil profile (Fig. 3).

3.2. Soybean crops performance

The differences in soil conditions mentioned above affected the performance of identically managed soybean crops in each experimental site during the 2019/2020 season. Water use varied across treatments and sites (Fig. 3). Specifically, water evapotranspiration (ET) ranged between 342 and 408 mm in San Pedro, 458–504 mm in Pergamino, and 520–594 mm in Bragado II. In fine-textured soils (Pergamino and San Pedro), SOY exhibited the smallest water depletion in the sub-soil indicating a restricted water use from deep soil layers when compared to other crop rotations. In the coarse-textured soil of Bragado II, water availability in SOY remained consistently as the lowest throughout the crop cycle. However, water depletion from sowing to maturity was smaller in SOY than in other treatments and, consequently, crop ET

Table 2
Average conditions during 2019/2020 soybean season at each experimental site and overall. All variables, except stubble amount, were measured prior to soybean sowing. Stubble was measured after soybean harvest. SOC: Soil organic carbon; Light C: carbon in the soil light fraction; EC: electric conductivity. Results for soybean monoculture (SOY), typical rotation (TYP), and intensified rotation (INT) are presented. Rotation x site interaction was not significant for all soil variables evaluated. Thus, overall results across sites are also shown. Significance levels: *$p < 0.1$; **$p < 0.05$; ***$P < 0.01$.

	SOC (0–10 cm, %)	**SOC** (0–10 cm, t ha^{-1})	**Light C** (0–10 cm, %)	**Light C** (0–10 cm, t ha^{-1})	**Stubble** (Mg ha^{-1})	**pH**	**EC** (ds m^{-1})	**Bulk density** (g cm^{-3})	**Infiltration** (mm h^{-1})	**Stability** (mm)	**Porosity** (%)
Pergamino											
SOY	2.3 (b)	26.9	0.40	4.6	3.8 (b)	5.5	0.69 (a)	1.18	30.3 (b)	1.3	57
TYP	2.3 (ab)	26.6	0.41	4.7	9.9 (a)	5.5	0.36 (b)	1.15	39.4 (a)	1.4	57
INT	2.5 (a)	27.2	0.50	5.3	12.3 (a)	5.5	0.32 (b)	1.08	39.3 (a)	1.4	59
	*				**		*		**		
San Pedro											
SOY	1.9	23.0	0.31 (b)	3.8	3.1 (b)	5.9	0.32	1.23	39.6	1.2	54
TYP	2.0	23.6	0.44 (ab)	5.1	8.3 (a)	5.8	0.18	1.17	35.0	1.2	56
INT	2.0	23.8	0.54 (a)	6.3	9.2 (a)	5.8	0.16	1.17	38.7	1.2	56
			*		***						
Bragado I											
SOY	1.7	22.4	0.30 (b)	3.9	5.3 (b)	5.6	0.38	1.30	33.5	1.8	51
TYP	1.9	23.1	0.43 (b)	5.4	11.7 (a)	5.7	0.27	1.25	37.9	1.8	53
INT	1.9	24.2	0.39 (ab)	4.9	12.5 (a)	5.7	0.27	1.25	39.6	1.7	53
			*		***						
Bragado II											
SOY	1.3 (b)	17.2 (b)	0.28 (c)	3.8	3.6 (b)	5.7	0.35	1.38 (a)	37.2	0.7 (b)	48 (b)
TYP	1.5 (a)	19.6 (a)	0.39 (b)	5.0	9.5 (a)	5.6	0.29	1.29 (ab)	42.8	1.2 (a)	51 (ab)
INT	1.7 (a)	20.7 (a)	0.48 (a)	5.9	10.9 (a)	5.7	0.30	1.23 (b)	41.6	1.1 (ab)	54 (a)
	***	**	***		***			**		*	*
Overall											
SOY	1.8 (c)	22.4	0.32 (b)	4.0 (b)	3.9 (c)	5.7	0.43 (a)	1.27 (a)	35.2	1.24	53 (b)
TYP	1.9 (b)	23.2	0.42 (a)	5.0 (a)	9.9 (b)	5.7	0.27 (b)	1.21 (b)	38.8	1.36	54 (a)
INT	2.0 (a)	24.0	0.48 (a)	5.6 (a)	11.2 (a)	5.7	0.26 (b)	1.18 (b)	39.8	1.38	55 (a)
	***		***	***	***		***	***			***

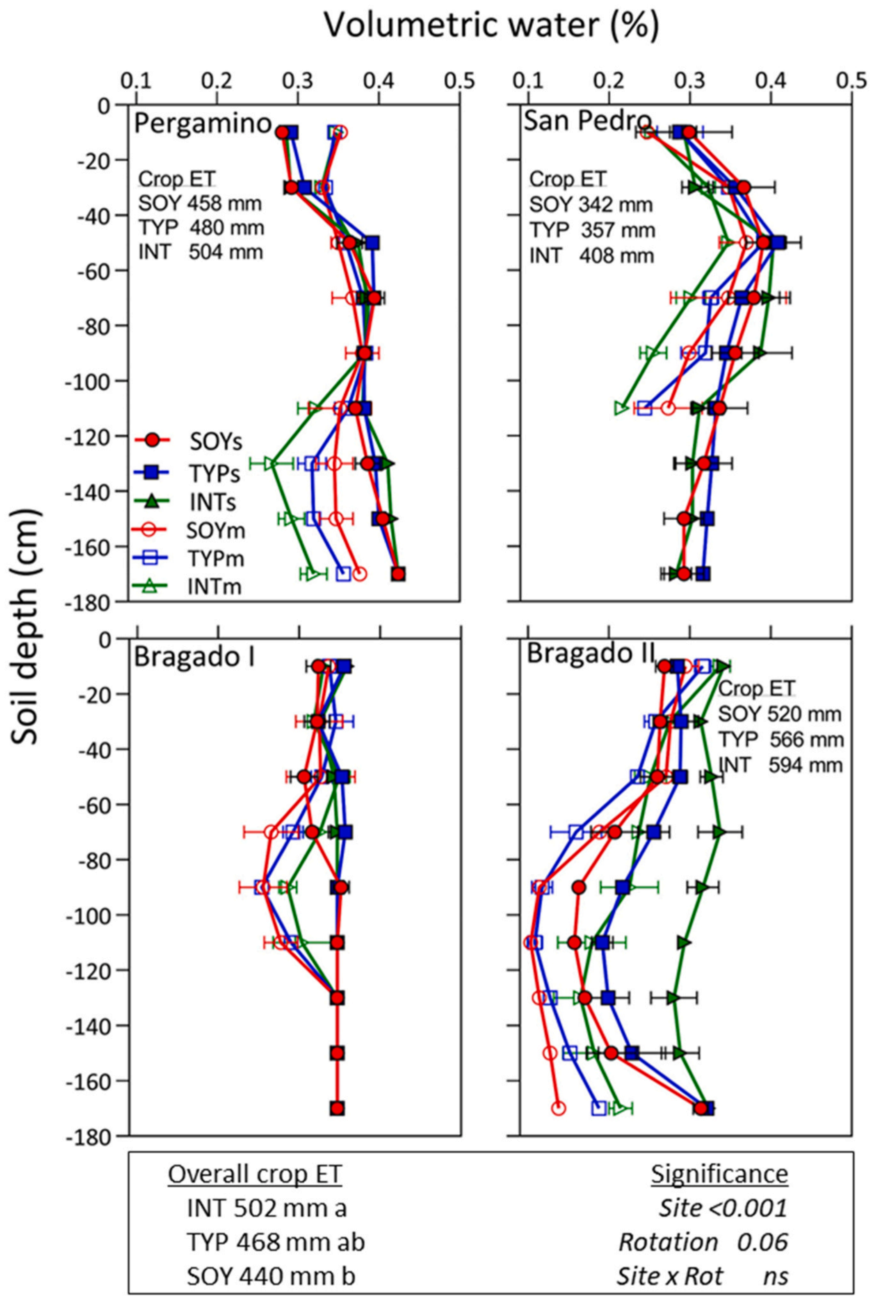

Fig. 3. Soil water content at soybean sowing (s) and maturity (m) from 0 to 180 cm depth during the 2019/2020 season. Soybean monoculture (SOY), typical (TYP), and intensified rotation (INT) are indicated for each experimental site. Water content in Bragado I (100–180 cm) was adjusted to saturation level given the presence of a water table. Estimated crop evapotranspiration (ET) is indicated inside the panels. Effective rainfall between sowing and maturity was 438 mm (Pergamino), 318 mm (San Pedro) and 444 mm (Bragado). Crop ET was not calculated at Bragado I due to unknown magnitude of water table influence. Different letters indicate significant differences among treatments ($p < 0.05$).

tended to be the smallest in SOY as well (Fig. 3). Therefore, soybean ET was the lowest in SOY in these three sites overall ($p = 0.06$).

Estimated soybean ET was positively associated with intercepted PAR across sites and treatments ($r^2 = 0.67$, $p < 0.01$). A step-by-step ecophysiological assessment revealed that crop rotation affected soybean interception of PAR (Table 3). Cumulated PAR interception was lower in SOY than in TYP and INT in Pergamino and San Pedro ($p < 0.01$), with a similar trend in Bragado II, and no significant differences in Bragado I ($p > 0.10$; Table 3). Differences in PAR interception dynamic among treatments were evident during January and February, when soybean seed yield is mostly determined (Fig. 4). In addition, INT crop rotation also increased radiation use efficiency to produce biomass (RUEb) compared to SOY in Bragado I and San Pedro ($p < 0.05$), while in Pergamino and Bragado II there where similar but not significant trends. Similarly, harvest index was increased at TYP and INT rotations compared to SOY in San Pedro ($p < 0.05$) while other sites showed no differences across treatments (Table 3). As a consequence of the cumulative responses, intensified crop rotations (INT) effect on soybean seed yield was 120–690 kg ha^{-1}, which is equivalent to a yield increment that ranged from 3 % (Bragado I) to 51 % (San Pedro) *versus* SOY (Fig. 5). Fertilization strategies alone (high [+] *versus* regular fertilizer rate for each given rotation) in previous seasons influence on soybean PAR interception, RUEb, and harvest index was comparatively smaller (Table 3). However, the relatively small but consistent positive effect of high-fertilization treatment across the ecophysiological components of seed yield resulted in a response that ranged from nil to 180 kg ha^{-1}, being equivalent to less than 4 % in any site by crop rotation combination (Table 3; Fig. 5). Overall, changes in soybean seed yield across treatments and locations were associated with variations in PAR interception (Fig. 6; $p < 0.001$).

3.3. N concentration in seeds and protein yield

Despite fertilization strategy employed in previous seasons resulted

Table 3
Solar PAR interception (PARint), RUE to produce biomass (RUEb), biomass, harvest index (HI), and seed yield for soybean crops in 2019/20 season. Different letters indicate significant differences among treatments within sites. Treatments include a combination of crop rotation (soybean monoculture [SOY], typical [TYP], and intensified rotation [INT]) and fertilization strategy (high [+] and regular). Changes in fertilization strategies were imposed only during the previous two seasons (2017/18 and 2018/19).

Site	Treatment	PARint (MJ m^{-2})		RUEb (g MJ^{-1})		Biomass (g m^{-2})		HI		Seed yield (g m^{-2})	
Pergamino											
	SOY	830	d	1.34		1113	b	0.36	b	398	f
	SOY+	844	cd	1.35		1139	b	0.36	b	408	e
	TYP	857	bc	1.33		1142	ab	0.37	ab	429	d
	TYP+	867	b	1.40		1214	ab	0.36	b	444	c
	INT	871	ab	1.41		1245	a	0.38	ab	456	b
	INT+	886	a	1.39		1235	a	0.40	a	473	a
	rotation	*0.01*		*ns*		*0.1*		*0.02*		*< 0.001*	
	fertilization	*0.03*		*ns*		*ns*		*ns*		*< 0.001*	
	rot x fert	*ns*		*ns*		*ns*		*ns*		*0.08*	
San Pedro											
	SOY	472	e	1.07	b	503	d	0.25	c	127	c
	SOY+	480	d	1.07	b	512	d	0.25	c	128	c
	TYP	531	c	1.06	b	562	c	0.31	b	174	b
	TYP+	538	b	1.08	b	580	bc	0.31	b	181	b
	INT	542	ab	1.13	a	610	a	0.32	a	196	a
	INT+	546	a	1.10	ab	601	ab	0.32	a	192	a
	rotation	*< 0.01*		*0.02*		*< 0.01*		*< 0.001*		*< 0.001*	
	fertilization	*< 0.01*		*ns*		*ns*		*ns*		*ns*	
	rot x fert	*ns*		*ns*		*ns*		*ns*		*ns*	
Bragado I											
	SOY	907	a	1.09	c	991	b	0.43	a	426	e
	SOY+	913	a	1.11	bc	1009	b	0.43	a	434	cd
	TYP	910	a	1.11	bc	1007	b	0.43	a	431	de
	TYP+	913	a	1.15	ab	1043	ab	0.42	a	441	b
	INT	893	b	1.16	a	1035	ab	0.42	a	438	bc
	INT+	896	b	1.19	a	1069	a	0.43	a	456	a
	rotation	*ns*		*0.03*		*0.1*		*0.02*		*ns*	
	fertilization	*0.1*		*0.05*		*0.06*		*ns*		*< 0.01*	
	rot x fert	*ns*		*ns*		*ns*		*ns*		*0.1*	
Bragado II											
	SOY	758		1.15	b	875	d	0.39	a	340	f
	SOY+	765		1.17	ab	895	cd	0.39	a	350	e
	TYP	794		1.16	ab	921	bc	0.39	a	363	d
	TYP+	792		1.19	a	942	ab	0.40	a	373	c
	INT	808		1.18	ab	956	a	0.39	a	376	b
	INT+	807		1.20	a	969	a	0.40	a	387	a
	rotation	*ns*		*ns*		*0.1*		*0.05*		*0.06*	
	fertilization	*ns*		*0.09*		*0.05*		*ns*		*0.001*	
	rot x fert	*ns*		*ns*		*ns*		*ns*		*ns*	
Overall											
	site	*< 0.001*		*< 0.001*		*< 0.001*		*< 0.001*		*< 0.001*	
	rotation	*0.09*		*< 0.001*		*< 0.001*		*ns*		*< 0.01*	
	fertilization	*0.03*		*0.1*		*< 0.01*		*ns*		*< 0.001*	
	rot x fert	*ns*		*0.1*		*ns*		*0.02*		*ns*	
	site x rot	*< 0.01*		*ns*		*0.1*		*< 0.001*		*< 0.001*	
	site x fert	*ns*		*ns*		*ns*		*ns*		*0.05*	

in low seed yield differences among soybean crops, changes in N concentration (and consequently protein) in seeds were detected across fertilization schemes overall ($p < 0.05$; Table 4). In contrast, crop rotation did not significantly affect N concentration in seeds at any site ($p > 0.1$). However, the response to fertilization seemed to be lower in the SOY+ (*versus* SOY) treatment compared to TYP+ or INT+ (*versus* TYP or INT). In terms of protein concentration, we found very little variation across sites and rotations at the average farmer fertilization rate (average 32.1 %, range 31.2–33.4 %). At high fertilization treatments, protein content increased by nearly 3 % points (average 35.3 %, range 33.7–37.6 %) (Table 4).

Protein yield, as the resultant of protein concentration in seeds and seed yield, increased with crop intensification ($p < 0.05$). Considering the three sites where protein content in seeds was assessed in all treatments (this is without San Pedro), an average of 1247, 1310, and 1350 kg ha^{-1} of protein were produced in SOY, TYP, and INT, respectively. In addition, increased fertilization rate in preceding seasons significantly ($p < 0.05$) increased protein yield by 103 kg ha^{-1} in SOY+ (*versus* SOY), by 195 kg ha^{-1} in TYP+ (*versus* TYP), and by 224 kg ha^{-1} in INT+ (*versus* INT) as average across sites. The combined effect of intensified crop rotation and high fertilization strategy produced the largest increase in protein yield (+327 kg ha^{-1} in INT+ *versus* SOY, as the average across the three sites) (Table 4).

4. Discussion

After the course of five years, our study found that soybean monoculture plots (SOY) had lower concentration of C in the topsoil compared to more diversified and intensified crop rotations (TYP and INT) overall across sites, which was occasionally accompanied by declines in total C stocks, stability of soil aggregates, porosity, and infiltration. These findings align with recent research in the region reporting crop sequence intensification as a way to keep higher carbon stocks in soils compared to soybean monoculture (Crespo et al., 2021; Semmartin et al., 2023). However, here, we did not find a trajectory of soil carbon accumulation at soybean monoculture plots as reported by Semmartin et al. (2023) nor

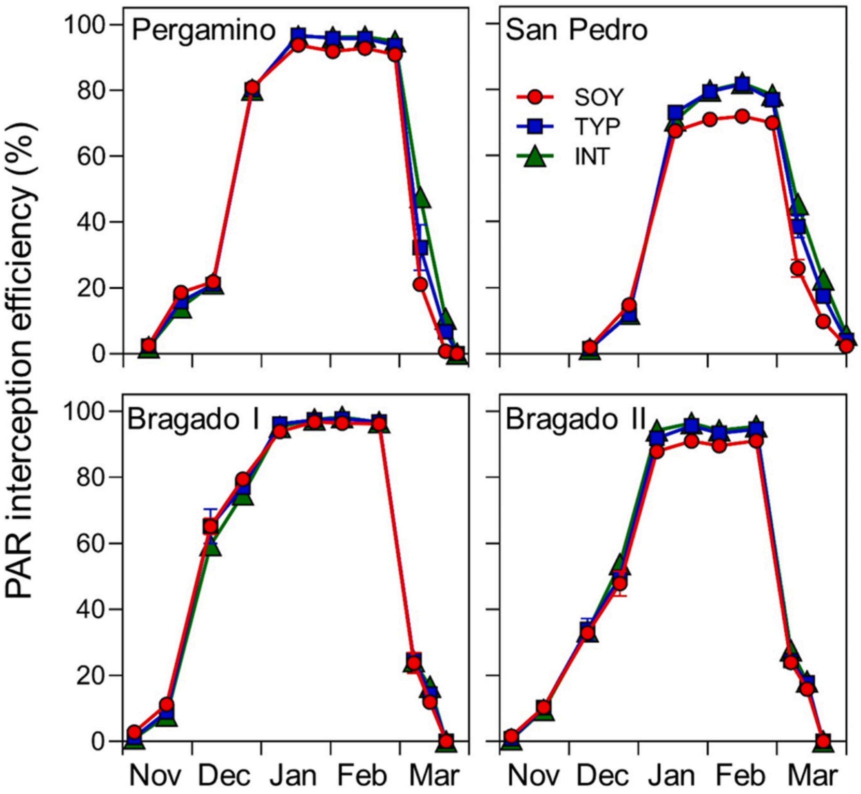

Fig. 4. Soybean photosynthetically active radiation (PAR) interception efficiency during the 2019/20 season. Soybean monoculture (SOY), typical rotation (TYP), and intensified rotation (INT) are displayed separately for each experiment. +Fertilized treatments are not displayed since differences compared to regular fertilization were negligible.

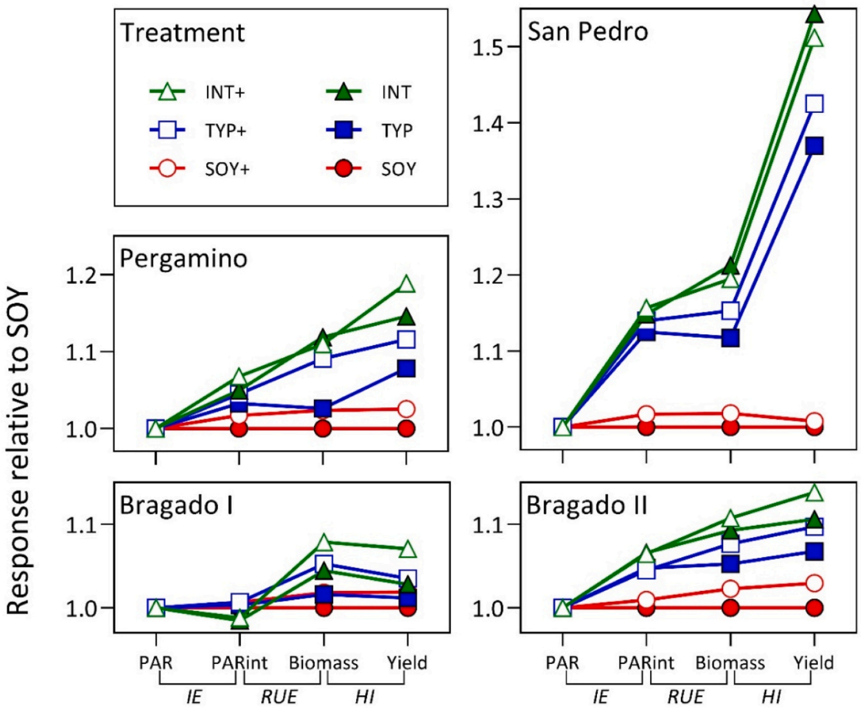

Fig. 5. Ecophysiological seed yield components (photosinthetically active radiation [PAR], solar radiation interception efficiency [IE], radiation use efficiency [RUE], and harvest index [HI]) influencing the cumulative response of PAR interception, biomass production and seed yield of different treatments compared to soybean in monoculture. Treatments include a combination of crop rotation (soybean monoculture [SOY], typical [TYP], and intensified rotation [INT]) and fertilization strategy (high [+] and regular). Statistical significances are shown in Table 2. /.

did we find consistent evidence of soil carbon losses over time in intensified crop sequences as indicated by Crespo et al. (2021) (Supplementary Table S1). One possible explanation for this divergence is that carbon stock trajectory is strongly influenced by initial soil conditions, such as carbon saturation deficit at the beginning of the experiments (Berhongaray et al., 2013; Alvarez and Berhongaray, 2021).

Additionally, we found evidence that evapotranspiration (ET) of a

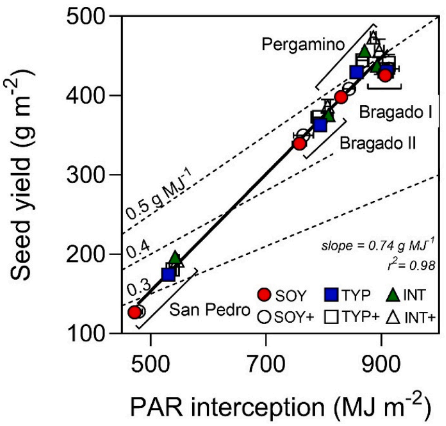

Fig. 6. - Soybean seed yield as a function of PAR interception. RUE to produce seed yield reference lines are displayed. Each dot corresponds to the average for each treatment and site. Treatments include a combination of crop rotation (soybean monoculture [SOY], typical [TYP], and intensified rotation [INT]) and fertilization strategy (high [+] and regular).

soybean crop can be significantly affected by the crop rotation (Fig. 3). Our results revealed that soybean ET can be lower in SOY than in more intensified crop rotations, either because of a reduction in soil available water (Bragado II) or because soybean was limited to extract water from deep soil layers (Pergamino and San Pedro). On one hand, a reduction in water availability driven by poor infiltration rates can be expected in soils with low stability, porosity, and topsoil cover like in SOY treatment at Bragado II (Dardanelli et al., 1994, 1997; Franzluebbers, 2002; Ranaivoson et al., 2017). On the other hand, a strong clayey subsoil in San Pedro and Pergamino represents a physical limitation for soybean root growth that may have been partially alleviated in INT and TYP treatments due to a higher and more diverse root activity in previous seasons, which creates root channels and facilitates soil exploration by successive soybean crops (Wang et al., 1986; Nickel et al., 1995).

The crop rotation effects on water dynamic discussed above are key determinants of crop yield under rainfed production systems. In fact, our assessment was mainly focused on quantifying the influence of these changes on a living sensor, a soybean crop, reflecting differences through solar radiation utilization and productivity. Despite this assessment was performed during a single season (2019/2020), we were able to explore a relatively large range of conditions as determined by different rainfall patterns and soils across experimental sites (Fig. 2; Supplementary Table S1). Overall, soybean seed yield was significantly lower after years of soybean monoculture (SOY) when compared with other crop rotations (TYP and INT) (Table 3). Water stress affects seed yield by reducing crop capacity to intercept solar radiation, its efficiency to convert intercepted PAR into biomass, or even the partition of biomass to harvestable organs depending on the magnitude and timing of the stress (Sadras and Connor, 1991; Cox and Jolliff, 1987; Dardanelli et al., 1991). Typically, sub-optimal water supply initially affects crop leaf expansion, reducing the interception efficiency of solar radiation (Sinclair and Horie, 1989). Our experiments evidenced larger reductions in PAR interception efficiency at SOY as water limitation increased, being largest in San Pedro (Fig. 4). In Bragado I, a water table alleviated differences among treatments and, thus, PAR interception resulted unaltered. As water stress intensifies, radiation use efficiency decreases given that CO_2 influx is limited by stomata closure (Andriani et al., 1991; Dardanelli et al., 1991) and HI could be reduced when water transpiration is restricted during late reproductive (Sadras and Connor, 1991). This was also the case of San Pedro, in which RUE and HI were largely reduced in SOY as compared with other crop rotations (TYP and INT).

Table 4

Nitrogen (N) and protein concentration in soybean seeds and total protein yield for all treatments and sites during the 2019/20 season. Different letters indicate significant difference among treatments within sites. Treatments include a combination of crop rotation (soybean monoculture [SOY], typical [TYP], and intensified rotation [INT]) and fertilization strategy (high [+] and regular). SOY+, TYP+, and INT+ treatments in San Pedro are missing values. Changes in fertilization strategies were imposed only during the previous two seasons (2017/18 and 2018/19).

Site	Treatment	N (%)	Protein (%)		Protein yield (kg ha^{-1})	
Pergamino						
	SOY	5.34	33.4		1328	c
	SOY+	5.45	34.0		1390	bc
	TYP	5.24	32.7		1405	abc
	TYP+	5.73	35.8		1591	ab
	INT	5.18	32.4		1476	abc
	INT+	5.41	33.8		1601	a
	rotation	*ns*	*ns*		*< 0.01*	
	fertilization	*ns*	*ns*		*0.05*	
	rot x fert	*ns*	*ns*		*ns*	
San Pedro						
	SOY	5.15	32.2		408	b
	SOY+	-	-			
	TYP	5.21	32.6		568	a
	TYP+	-	-			
	INT	5.16	32.3		633	a
	INT+	-	-			
	rotation	*ns*	*ns*		*0.03*	
	fertilization	-	-		-	
	rot x fert	-	-		-	
Bragado I						
	SOY	5.04	31.5	b	1339	c
	SOY+	5.40	33.7	ab	1463	bc
	TYP	5.12	32.0	b	1375	c
	TYP+	5.72	35.8	ab	1574	ab
	INT	4.99	31.2	b	1365	c
	INT+	6.01	37.6	a	1712	a
	rotation	*ns*	*ns*		*ns*	
	fertilization	*0.02*	*0.02*		*< 0.01*	
	rot x fert	*ns*	*ns*		*ns*	
Bragado II						
	SOY	5.07	31.6	b	1075	c
	SOY+	5.49	34.2	ab	1199	abc
	TYP	5.07	31.7	b	1150	bc
	TYP+	5.80	36.2	a	1350	ab
	INT	5.15	32.2	b	1210	abc
	INT+	5.84	36.5	a	1410	a
	rotation	*ns*	*ns*		*0.03*	
	fertilization	*0.05*	*0.05*		*0.03*	
	rot x fert	*ns*	*ns*		*ns*	
Overall (without San Pedro)						
	SOY	5.15	32.2	c	1247	d
	SOY+	5.44	34.0	b	1350	c
	TYP	5.14	32.1	c	1310	d
	TYP+	5.75	35.9	a	1505	b
	INT	5.10	31.9	c	1350	cd
	INT+	5.75	35.9	a	1574	a
	site	*ns*	*ns*		*< 0.001*	
	rotation	*ns*	*ns*		*< 0.001*	
	fertilization	*< 0.001*	*< 0.001*		*< 0.001*	
	rot x fert	*ns*	*ns*		*0.08*	
	site x rot	*ns*	*ns*		*ns*	
	site x fert	*ns*	*ns*		*ns*	

Higher N, P, and S fertilization rates in previous crops had a relatively lower influence (<4 % on soybean seed yield response) than crop rotation. This means that fertilization alone cannot restore productivity levels after years of soybean monoculture. Anyway, high fertilization schemes in previous crops combined with intensive and diverse crop rotations can help sustain higher protein concentration in soybean seeds (Table 4). This is highly relevant and could partially mitigate a current major concern in Argentina where, while seed yields have been increasing at *ca.* 30 kg ha^{-1} y^{-1}, national average protein content has decreased from nearly 40 % to 35 % along the last two decades

(Cuniberti et al., 2018).

Benefits derived from crop rotation and land use intensification on overall economic and productive terms have been previously assessed and contrasted against soybean monoculture across a range of conditions for the Pampas (Caviglia et al., 2019; Andrade et al., 2022). In this study, we provide evidence that soil conditions under soybean monoculture also potentially aggravate water and/or nutrient deficits. However, soybean monoculture still occurs when management decisions are based considering its lower production cost or higher relative prices compared to other crops. Moreover, once soil is deteriorated, soybean is the one crop that makes farmers avoid big economic losses given its low input costs, mainly associated to low seeding costs and reduced response to N and P fertilizer applications, and the extended critical period for yield determination that limits the influence of occasional water stresses under rainfed production systems. The necessary change to a more productive and sustainable crop production system depends on the tradeoff between short term productive and economic results and long-term benefits provided from diversified and intensive crop rotations. Previous results, and those reported here showed changes of magnitude with economic impact not only in soybean but in the system as a whole under diversified and properly run rotations (Andrade et al., 2022). Moreover, new alternatives, such as various harvestable winter and also cover crops need to be explored as tools to improve soil conditions and, thus, crop yield under long term continuous agriculture in the region.

CRediT authorship contribution statement

José F. Andrade: Conceptualization, Investigation, Data curation, Funding acquisition, Project administration, Formal analysis, Writing-original draft, Writing-review and editing. **Matías Ermácora:** Conceptualization; Investigation; Methodology; Resources; Writing-Review and editing. **Javier De Grazia:** Investigation; Methodology; Writing-Review and editing. **Hernan Rodriguez:** Investigation; Methodology; Writing-Review and editing. **Enrique Mc Grech:** Investigation, Data curation, Formal analysis, Writing-Review and editing. **Emilio H. Satorre:** Conceptualization and Visualization, Funding acquisition, Project administration, Supervision, Writing-Review and editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

The authors thank the advisors from 'Grupo CREA Norte de Buenos Aires' for their collaboration in experimental designing and results interpretation. This research was funded by CREA-Norte de Buenos Aires, Argentina and National Agency for Supporting Science and Technology of Argentina (Agencia, MINCyT, PICT 2018-02164 and PICT 2019-03768).

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.eja.2023.126915.

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