



A win-win situation – Increasing protein production and reducing synthetic N fertilizer use by integrating soybean into irrigated Mediterranean cropping systems

Genís Simon-Miquel^{a,*}, Moritz Reckling^{b,c}, Jorge Lampurlanés^d, Daniel Plaza-Bonilla^a

^a Department of Crop and Forest Sciences - Agrotecnio-CERCA Center, Universitat de Lleida, Av. Rovira Roure 191, 25198 Lleida, Spain

^b Leibniz Centre for Agricultural Landscape Research (ZALF), 15374 Müncheberg, Germany

^c Department of Crop Production Ecology, Swedish University of Agricultural Sciences (SLU), Sweden

^d Department of Agricultural and Forest Engineering - Agrotecnio-CERCA Center, Universitat de Lleida, Av. Rovira Roure 191, 25198 Lleida, Spain

ARTICLE INFO

Keywords:

Diversification
Energy
Protein
Single cropping systems (SCS)
Double cropping systems (DCS)

ABSTRACT

Over the last decades, non-cereal crops have been displaced in European cropping systems leading to a significant dependency on imported soybean. Continuous maize cropping under Mediterranean irrigated conditions can lead to agronomic and environmental problems. The objective of this work was to assess diversified Mediterranean irrigated cropping systems to maximize protein production while reducing synthetic N fertilizer use. A field experiment was carried out from 2019 to 2021 in an irrigated area in NE Spain. Four cropping systems, (i) continuous maize (MM), (ii) soybean in a rotation one out of three years (MSrt), (iii) barley-maize double cropping system (BM), and (iv) barley-soybean double cropping system (BS) were assessed at the crop, pre-crop and cropping system level. Productivity in terms of grain, energy and protein yield was measured at the crop and calculated for the cropping system level. As well, synthetic N fertilizer use efficiency was calculated for each cropping system. At the pre-crop level, soybean introduction led to a 28% yield increase in the following cereal (maize or barley) mainly due to the residual N effect. At the cropping system level, soybean in rotation (MSrt) did not lead to a significant increase in total protein production compared to MM (from 895 to 947 kg ha⁻¹ yr⁻¹), but it mildly increased synthetic N fertilizer use efficiency. Protein production in the BS system (1778 kg protein ha⁻¹ yr⁻¹) was significantly higher than in all other cropping systems (990 kg protein ha⁻¹ yr⁻¹ on average). As well, BS was the cropping system with the highest synthetic N fertilizer use efficiency compared to the other cropping systems (251 and 88 kg grain kg synthetic N fertilizer⁻¹). Our results demonstrate that introducing soybean as a double crop following barley is a successful strategy to reduce environmental impacts resulting from N fertilizer use and increase protein production, contributing to plant protein self-sufficiency and cropping systems diversification.

1. Introduction

European farmers have specialised in cereal production over the last decades (Zander et al., 2016). This specialisation was facilitated by the availability of synthetic N fertilizers at relatively low prices and EU Common Agricultural Policies (CAP) during the last 60 years (Magrini et al., 2016). This situation has been aggravated by the changes in diets during this same period, where meat consumption increased across Europe, thus maximizing cereal demand for feed production (Lassaletta et al., 2014). Consequently, non-cereal crops have been displaced in the European cropping systems. For instance, the current surface devoted to

legume crops in the EU is significantly lower than the worldwide average (1.5% compared to 14% of the arable land) (Watson et al., 2017). The EU Green Deal aims to trigger a transition towards more diversified cropping systems with lower synthetic pesticide and fertilizer use, and a greater share of organic farming, all supported by the inclusion of legumes into cropping systems among other measures (European Commission, 2019). Nearly 80% of the EU's deficit in high protein crop produce is covered by soybean imports (58 Mt yr⁻¹) from overseas. This number represents 90% of EU soybean consumption (Guilpart et al., 2022). Along with the CAP efforts to promote grain legumes (especially those with high protein content), new trends in human diets indicate

* Corresponding author.

E-mail address: genis.simon@udl.cat (G. Simon-Miquel).

<https://doi.org/10.1016/j.eja.2023.126817>

Received 31 January 2023; Received in revised form 10 March 2023; Accepted 10 March 2023

Available online 13 March 2023

1161-0301/© 2023 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

that grain legume consumption in the EU is increasing (Moller et al., 2019). This situation is opening a market for EU farmers to grow local, GM-free food-grade grain legumes, especially soybean, with a higher profitability level than feed-grade products (Karges et al., 2022).

In semiarid Mediterranean areas such as NE Spain, irrigation availability allows high-yielding maize (*Zea mays* L.) production, with average yields between 15 and 17 t ha⁻¹ when grown as a single crop in one year (E. Martínez et al., 2017). Given the high maize productivity achieved, continuous maize monoculture is common in the area with its production mainly devoted to feed production. Traditionally, this type of cropping system entails high N fertilization rates of 300–400 kg N ha⁻¹ and winter bare fallow managed with intensive soil tillage practices e.g. one or two subsoiler passes followed by a pass of rototiller (Sisquella et al., 2004). However, its continuous cropping can lead to agronomic and environmental problems such as N leaching due to N fertilisation not well synchronized with crop needs (Cavero et al., 2003), weeds proliferation (Saulic et al., 2022), soil degradation (Paraja-Sánchez et al., 2017), increased greenhouse gas emissions and loss of biodiversity (Reckling et al., 2016a). Therefore, there is a need to investigate competitive and sustainable alternatives to continuous maize cropping. Soybean introduction can help alleviate these problems. As a legume crop, soybean contributes to cropping system diversity, increased N flow coming from biological N fixation (Peoples et al., 2009), and thus reduced synthetic N use at the cropping system level (Plaza-Bonilla et al., 2017). In addition, soybean can have a positive effect on the following crops through several mechanisms (N credit, pest control, soil structure, etc.) (Angus et al., 2015). Although soybean pre-crop effects have been documented extensively in other areas (Karlen et al., 2013; Munkholm et al., 2013; Rathke et al., 2007), scarce information exists on European cropping systems. For example, Preissel et al. (2015) reported 179 site-year comparisons of break crop and reference pre-crop across Europe, with none of them including soybean.

Sequential double cropping systems i.e. two sole crops per year, intensify the described single cropping systems. A winter cereal (often barley (*Hordeum vulgare* L.)) is grown from autumn to early summer and is followed by a late sown and early-maturing maize, established under no-till, allowing to harvest two grain crops in one year (Maresma et al., 2019). With double cropping systems, soil cover over time increases significantly as the winter bare fallow is replaced by a winter cereal (Hisse et al., 2022). However, winter cereal and maize double cropping systems are still high N-demanding to maintain productivity. Introducing soybean as a double crop, instead of maize, can have benefits at the cropping system level. Similarly to the SCS, double-cropped soybean reduces synthetic N demand due to biological N fixation and can positively impact the following crop performance (e.g. barley) (Franchini et al., 2012). In addition, climate change is likely to expand the area suitable for double cropping in Europe (Nendel et al., 2022). In that regard, information on soybean performance as a double crop in European cropping systems is lacking.

Favourable policies for legume production, new diet trends with higher plant protein intake, and the need for an increased N use efficiency open the path to design and assess innovative cropping systems with grain legumes. The performance of a novel cropping system can be assessed at different levels (Preissel et al., 2017). At the crop level, specific management practices (tillage, N fertilisation, sowing dates, etc.) are the research target, generally aimed at maximizing the productivity of the targeted crop. At the pre-crop level, part of the crop sequence is studied, as the aim is to disentangle the effects of different preceding crops on a following crop. However, the effects are still reported at the crop level (Preissel et al., 2015). Studies at a cropping system level allow a comparison between cropping systems performance as a whole, independently of the species or the number of crops in a rotation and not focusing just on one of the crop species (Reckling et al., 2016b). Therefore, more than one factor (i.e. tillage, fertilisation, cultivars, etc.) will differ in the cropping systems approach (Drinkwater et al., 2000), as the aims are to investigate the performance of different

cropping systems when managed accordingly to the best management practices available in each case (Debaeke et al., 2009). Within this framework, the use of different indicators is suggested to avoid biases (Costa et al., 2021). For example, when introducing legumes in a rotation, it can be expected that the total protein productivity of the cropping system will increase. However, that is the result of the removal or reduction in presence of another crop. This crop is often a cereal, mainly grown as a source of energy. Thus, to achieve a fairer comparison, the energy output of the different cropping systems should also be computed (Costa et al., 2021). Resource needs and use can also be impacted by the cropping system (e.g. inclusion of legumes reduces the need for synthetic N fertilizers). In consequence, resource use efficiency is another example of a systems performance indicator (López-Bellido and López-Bellido, 2001). Under Mediterranean irrigated conditions, research has focused on the crop and pre-crop level for specific crops, wheat (Abad et al., 2005) and maize (Berenguer et al., 2009; Salama et al., 2021), especially. However, little research has addressed diversification and management impacts using a multi-scale framework, crop, pre-crop and cropping system levels, and none of them including soybean.

The objective of this work was to redesign and assess Mediterranean irrigated cropping systems through crop diversification to maximize protein production while reducing synthetic N use. The performance was assessed at the cropping system level, as the cropping systems involved different crops with different uses, as well as the crop and pre-crop level, to disentangle the mechanisms behind the cropping systems' performance.

2. Materials and methods

2.1. Experimental site and design

An on-farm field experiment was carried out in NE Spain (Sucs, Lleida, 41°41'51.16" N, 0°25'57.08" E, 287 masl) from 2019 to 2021. This area has a semi-arid Mediterranean climate with a continental influence. The average annual precipitation and potential evapotranspiration for the last 30 years were 352 mm and 1073 mm, respectively. The annual mean air temperature was 14.4 °C for the same period. Summer months concentrate the lowest precipitation (15–20% of the annual amount) and the highest potential evapotranspiration (44% of the total). Climate data were retrieved from the Catalan Agriculture Department, specifically from the Raimat weather station (2 km SE from the field experiment). At the beginning of the experiment, some key soil characteristics were measured (0–30 cm depth): soil particle size was 29% clay, 37% silt and 34% sand (pipette method). Organic C was 17.3 g kg⁻¹ (Walkley-Black with external heating), organic N (Kjeldahl) was 2.3 g kg⁻¹, available P (Olsen) was 44.1 mg kg⁻¹, available K (ammonium acetate) was 434 mg kg⁻¹, pH (ext. 1:2.5 H₂O) was 8.1, bulk density (cylinder method) was 1.4 g cm⁻³ and stoniness (excavation method) was 0.3 cm³ cm⁻³. The soil of the experiment was classified as a Typic Calcixerept (Soil Survey Staff, 2014).

Four cropping systems (Fig. 1) were assessed over three years in a randomized block design with four replications. The four cropping systems were divided into two groups: single cropping systems (SCS, one cash crop and one cover crop per year) and sequential double cropping systems (DCS, two cash crops per year). In the SCS, continuous maize (MM), as the reference treatment for the area, and the introduction of soybean one out of three years in the crop rotation (MSrt) were tested. In both cases (MM and MSrt), the cash crop was preceded by winter rye (*Secale cereale* L.) cover crop (Fig. 1) terminated with roller crimper simultaneously to soybean and maize planting. For the MSrt, the three phases of the sequence were present each year to account for the interannual climate variability. In the case of DCS, barley-maize (BM) and barley-soybean (BS) were tested. In these cases, the same sequence was repeated over the experimental period (Fig. 1). Irrigation was provided to overcome the water deficit (especially in summer) by a solid set of sprinklers arranged in an 18 × 16 m framework. The irrigation rate

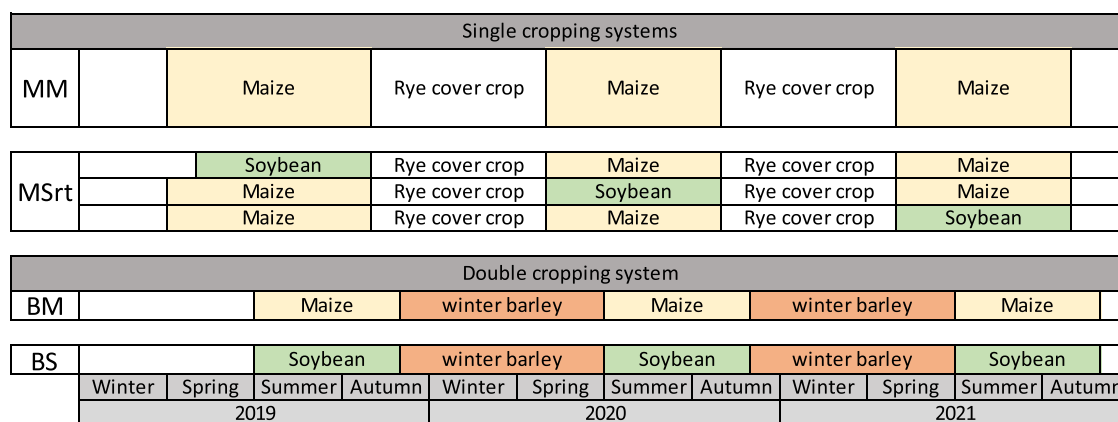


Fig. 1. Conceptual diagram of the four cropping systems assessed (MM: continuous maize, MSrt: soybean in a three-year rotation with maize; BM: barley-maize double cropping system; BS: barley-soybean double cropping system).

was applied according to crop needs and local management practices. The plot size was 75×18 m and the full experiment (plots, corridors and margins) covered a surface of 7 ha.

2.2. Cropping system management

In the SCS, in 2019 maize and soybean were planted in early April and May (Table 1), respectively, on bare soil. After their harvest, sub-soiler and rototiller passes were performed before rye sowing (cv. Amber) at 80 kg ha^{-1} (Table 1) with a 6 m drill machine (row width of

12.5 cm). In 2020, the rye cover crop was broadcasted on summer crop residues at a rate of 120 kg ha^{-1} in early December (Table 1). In 2020 and 2021, maize and soybean were planted both in early May (Table 1), coinciding with the rye cover crop anthesis. Cover crop termination and maize and soybean planting were performed simultaneously, green planting, with individual roller crimpers attached to each unit of a no-till planting machine (ZRX Plus – With Integral row cleaner from Dawn® coupled with a John Deere 1705 MaxEmerge®). Late-maturity maize cultivar was planted at 8.8 seeds m^{-2} . For soybean, cv. ES Isidor MG I was used the three years, given its acceptance by the local food industry, and was planted at 50 seeds m^{-2} .

In the DCS, hybrid barley (Cv. Hyvido Zoo) was sown at 90 kg ha^{-1} after summer crops harvest with a 6 m drill machine in November 2019 (Table 1). In December 2020, barley was drilled with a no-till planter. Maize and soybean in the DCS were planted with the same no-till planting machine as in the SCS a few days after the barley harvest (Table 1). Maize cultivar P0312 was used and planted at 8.8 seeds m^{-1} . For soybean, ES Isidor MG I was used (except in 2019 where ES Mentor MG 00 was used), planted at 50 seeds m^{-2} . In 2019, only the summer crops (maize and soybean) were present, as the experiment started in April 2019.

In both systems, DCS and SCS, swine manure and slurry were applied just before the experimental period started, by March-April 2019 (Table 1). After the 2019 summer crops, $134 \text{ kg NH}_4\text{N ha}^{-1}$ were applied as swine manure to all plots. Top-dressing N fertilization was applied to maize at the V2-V4 stage (Table 1) and the rates and sources (Table 2) were decided according to residual soil nitrate contents, and expected crop needs, following the method used by Plaza-Bonilla et al. (2017). The soybean did not receive top-dressing N fertilization, except in the SCS in 2021 where 50 kg N ha^{-1} were applied as a starter fertilizer to overcome a possible competition with the cover crop decomposition. Winter rye cover crops did not receive mineral N fertilisation. Barley did not receive synthetic N fertilizer in 2020 (given the manure application in November 2019), while 75 kg N ha^{-1} were applied in 2021 (Table 2). All cash crops (maize, soybean and barley) were harvested with a commercial combine harvester (CLAAS Lexion 8800) (Table 1). Grain yield was measured by individually harvesting and weighing the grain in each plot. Grain moisture and specific grain weight were measured using a grain analysis computer (DICKEY-John Gac 2100®). Grain yield was standardized to dry matter content before proceeding with further calculations. Herbicide applications were performed either pre or post-emergence depending on the weed species present and according to the local advisors. When no-till planting the summer crops on the rolled rye cover crop (2020 and 2021), metaldehyde (40 g Kg^{-1} at 10 kg ha^{-1}) was applied to control slug attacks.

Table 1

Management practices dates in the three experimental years on each cropping system (MM: Continuous maize, MSrt: soybean rotation one out of three years; BM: barley-maize double cropping system; BS: barley-soybean double cropping system). Rows with only one date indicate that the operation was carried out the same for all the treatments.

Year	Management practice	Single cropping system		Double cropping system	
		MM	MSrt*	BM	BS
2019	Organic fertilization		27 Mar		
	Tillage		4 Apr		
	Maize and soybean planting	12 Apr	13 May	20 Jun	
	Synthetic N fertilization maize	3 Jun	-	2 Aug	-
	Maize and soybean harvest	18 Oct	7 Nov	18 Nov	18 Nov
	Organic fertilization		25 Nov		
2020	Tillage		26 Nov		
	Winter crop sowing	29 Nov (rye)		29 Nov (barley)	
	Cover crop roller-crimping	3 May			
	Winter barley harvest			16 Jun	
	Maize and soybean planting	3 May	3 May	22 Jun	22 Jun
	Synthetic N fertilization maize	7 May	7 May	26 Jun	26 Jun
2021	Maize and soybean harvest	10 Oct	10 Oct	2 Dec	2 Dec
	Winter crop sowing	14 Dec (rye)		5 Dec (barley)	
	Synthetic N fertilization barley	-	-	3 Feb	
	Cover crop roller-crimping	6 May	7 May		
	Winter barley harvest			26 Jun	
	Maize and soybean planting	6 May	7 May	28 Jun	28 Jun
	N fertilization maize	12 May + 2 Jul	12 May	2 Jul	2 Jul
	Maize and soybean harvest	22 Oct	22 Oct	22 Nov	22 Oct

*The dates stated in this table refer to the soybean phase in the MSrt system. The maize phase was managed as in the MM system.

Table 2

Rates of synthetic N fertilizer applied to each crop and cropping system over the experimental period. + sign indicates that the application was composed of more than one type of fertilizer. In 2019 the experiment began with the summer crops phase. Each value between brackets corresponds to one fertilization event. The absence of brackets indicates that fertilization was carried out in a single event. The use of **bold** refers to the mean applied to each cropping system, while non-bold refers to each crop in the rotation.

Cropping system	Crop	Year						Mean (kg N ha ⁻¹ yr ⁻¹)	Total N applied (kg N ha ⁻¹ 3 yr ⁻¹)
		2019		2020		2021			
		Rate (kg N ha ⁻¹)	Source [§]	Rate (kg N ha ⁻¹)	Source	Rate (kg N ha ⁻¹)	Source		
MM	Maize	150 + 50	U+AS	80 + 8	U+MAP	(50 + 8)+ (75 + 75)	(U+MAP)+ (U+AS)	165	495
MSrt*		133		61		158		117	352
	Maize	150 + 50	U+AS	80 + 8	U+MAP	(50 + 8)+ (75 + 75)	(U+MAP)+ (U+AS)	165	495
BM	Soybean	0		8	MAP	50 + 8	U+MAP	22	65
		100		88		233		140	420
	Barley	-		0		75		38	75
BS	Maize	100	AN	80 + 8	U+MAP	75 + 75 + 8	U+AS+MAP	100	345
		0		8		83		30	90
	Barley	-		0		75	AN	38	75
	Soybean	0		8	MAP	8	MAP	5	15

*These values are calculated as the mean of the N rates applied to each phase of the rotation: 2 phases of maize and one of soybean.

[§]N: Ammonium nitrate; AS: Ammonium sulphate; MAP: Monoammonium phosphate; U: Urea.

2.3. Data acquisition and calculation of cropping systems performance indexes

Soil sampling was performed twice per year, once in May (right before summer crops' planting for the SCS and during barley grain filling for the DCS) and the second one in November (after summer crops harvest and right before winter crops sowing). Two observations were taken in each plot at two depth intervals covering the rooting depth (0–30 and 30–60 cm). Soil water content was determined gravimetrically and soil nitrate (NO₃) was extracted with deionized pure water at a soil:water ratio of 1:5. The extracts were analysed with a continuous flow autoanalyzer (Multi-element analyser, Smartchem 200). Both water and soil nitrate contents were corrected by the soil stoniness. Crop biomass was sampled at physiological maturity for cash crops (barley, soybean and maize) to quantify the yield components (pods or ears m⁻², grains pod⁻¹ or ear⁻¹, and thousand-grain weight (TGW)) for each of them. Rye cover crop biomass was sampled at its termination moment. For winter crops (barley and rye cover crop) the sampling area was 0.1 m², while for maize and soybean it was 1.5 and 0.75 m², respectively, in three observations per plot. As the different cropping systems involved different crop species, protein and energy yields were calculated so that the different crop yields can be compared among them (Costa et al., 2021; Plaza-Bonilla et al., 2017). Grain N concentration was determined by dry combustion (model Truspec CN, LECO, St Joseph, MI, USA) and then was multiplied by 5.5 (soybean), 5.6 (maize) or 5.4 (barley) to obtain the grain protein concentration (Mariotti et al., 2008). As well, grain energy content was calculated based on the gross energy content for the different crops. These values were extracted from www.feedipedia.com and they were 23.6, 18.7 and 17.4 MJ kg⁻¹ for soybean, maize, and barley, respectively, computed on a dry matter basis. The grain yield for the whole experimental period and the annual yield was calculated for each replication of each cropping system and it is presented in kg ha⁻¹ year⁻¹ on a 14% moisture level. In the SCS, the yield of three experimental years (2019, 2020 and 2021) was averaged for each replication in the MM system. In the MSrt system, the yield of the three phases of the rotation was averaged every year before the computation of the three-year average. For the DCS, the annual yield was computed by summing the yield of the two crops harvested each year (winter barley and maize or winter barley and soybean for BM and BS, respectively). Then, the mean annual yield for the DCS is the average of the total annual yield obtained in 2020 and 2021. The data from 2019 in the DCS was excluded since that year only the summer crops were

present in the experiment.

Synthetic nitrogen use efficiency (NUE_{synt}) was computed for each system and replication over the whole experimental period (summer 2019 to autumn 2021). This indicator was calculated using equations [1], [2] and [3] for the grain, energy and protein yields, respectively, to standardize the different cropping systems to a comparable indicator.

$$NUE_{synt-g} = \frac{\sum \text{grain yield (kg ha}^{-1}\text{)}}{\sum \text{Synthetic N fertilizer applied (kg ha}^{-1}\text{)}} \quad (1)$$

$$NUE_{synt-e} = \frac{\sum \text{energy yield (GJ ha}^{-1}\text{)}}{\sum \text{Synthetic N fertilizer applied (kg ha}^{-1}\text{)}} \quad (2)$$

$$NUE_{synt-p} = \frac{\sum \text{protein yield (kg ha}^{-1}\text{)}}{\sum \text{Synthetic N fertilizer applied (kg ha}^{-1}\text{)}} \quad (3)$$

Where NUE_{synt-g}, NUE_{synt-e} and NUE_{synt-p} refer to nitrogen use efficiency of the synthetic fertilizer for grain, energy and protein yields, respectively. The Synthetic N fertilizer applied refers to the total amount of N applied as mineral fertilizer through the experimental period in each cropping system. Single-year NUE_{synt} values were not calculated as in some year-treatments no synthetic N fertilizer was applied.

2.4. Statistical analyses

All statistical analyses were carried out with JMP Pro 16 (SAS Institute Inc, 2019). Statistical analyses were performed independently at the (i) crop, (ii) pre-crop and (iii) cropping system levels. At the crop level, grain, energy, and protein yields of the summer cash crops (maize and soybean) were subjected to an analysis of variance (ANOVA). The model used included the type of cropping system (SCS or DCS), the year effect, their interaction and the block as fixed effects. For the case of barley (winter cash crop), the year and the block were the only factors included in the model, as barley was not included in any of the single cropping systems. At the pre-crop level, the preceding crop effects of soybean and maize were analysed on the maize in the SCS and on the barley in the DCS. Separate ANOVAs for maize and barley were performed. Pre-crop, year, their interaction, and block were included as fixed effects in the models. The variables analysed with these models were yield, total grain N, yield components, aboveground biomass, and N uptake. In addition, soybean and maize preceding crop effects were analysed (in the SCS) on the rye cover crop biomass, biomass N content

and total N uptake using the same statistical model as the maize and the barley.

At the cropping system level, annual productivity and NUE_{synt} (of grain, energy and protein yields) were subjected to an ANOVA where the cropping system and block were kept as fixed effects. The cropping system effect included four levels, with the soybean rotation system having the three phases averaged per year. Year factor was not necessary since the productivities were standardized to $\text{kg grain ha}^{-1} \text{ year}^{-1}$, $\text{MJ ha}^{-1} \text{ year}^{-1}$, and $\text{kg protein ha}^{-1} \text{ year}^{-1}$, and the NUE indicators were calculated for the whole experimental period. Soil nitrate contents were analysed independently after winter crops (May) and after summer crops harvesting (November). This separation allows the comparison between years reflecting similar conditions: soil nitrate contents in May are a direct consequence of the crop and crop management of the winter crops, while the contents in November depend mostly on the summer crop. In both cases, the ANOVA included the cropping system (four levels), year (two and three levels for the May and November soil samplings, respectively), their interaction and block as fixed effects of the model. For the MSrt system, the average of the three phases was computed for each sampling date. The first sampling date (21/03/2019) was not included in the analyses since it took place right before the experiment began. Soil nitrate content data were log-transformed to meet the normal residue distribution. Back-transformed data is presented. In all levels, Student's *t* or Tukey HSD were used to perform a means separation test when a fixed effect was significant ($p\text{-value} < 0.05$).

3. Results

Annual precipitation during the experimental period was 32 mm below and 138 mm above the average for the area in 2019 and 2020, respectively. In 2021 it coincided with the 30-year average for the area (363 mm). Mean yearly air temperatures did not differ remarkably from the average (14.9°C). Maximum daylight temperatures over 35°C were registered 15, 12 and 7 days in 2019, 2020 and 2021, respectively, during the June-August period. No extreme cold events were registered during the experimental period. Irrigation was applied to overcome the water stress caused by the high temperatures and scarce precipitations, especially in summer crops, as it is required in the area. On average,

700 mm yr^{-1} were applied to each cropping system. In the SCS, this rate was split into 50 and 650 mm applied in the rye cover crop and the summer crops (maize and soybean), respectively. In the DCS, the split was 150 and 550 mm for the winter barley and the summer crops (maize and soybean), respectively.

3.1. Crop level performance

Maize grain yield was significantly affected by the cropping systems x year interaction (Table 3). In 2019, maize yields were the highest and did not differ significantly between cropping systems (18.8 t ha^{-1} on average). In 2020 and 2021, maize grain yield was higher in the SCS than in the DCS with 13.2 and 9.2 t ha^{-1} , respectively, on average for 2020 and 2021 (Table 3). Energy yields followed the same trend as maize grain yields, as they were obtained by multiplying dry matter grain yields by 18.7 MJ kg^{-1} . For the maize protein yield only the single effects, year and cropping system, were significant. Following the grain yield trend, in 2019 higher maize protein yields were observed ($1201 \text{ kg protein ha}^{-1}$) than in the other years (622 and $668 \text{ kg protein ha}^{-1}$ on average in 2020 and 2021). Maize in the SCS showed higher protein yields compared to the DCS, with 941 and $720 \text{ kg protein ha}^{-1}$, respectively, on average for the three years.

Soybean grain yield was affected by the cropping system x year interaction (Table 3). Soybean yield in DCS was 29% lower in 2019 and 59% higher in 2021 compared to SCS (4.1 and 2.2 t ha^{-1} in 2019 and 2021, respectively) (Table 3), while no significant differences were found in 2020, with a yield of 3.4 t ha^{-1} on average. As in maize, soybean energy yields presented the same trend as grain yield. Soybean protein yield was not significantly different between SCS and DCS in 2019 and 2020, with values ranging from 949 to $1141 \text{ kg protein ha}^{-1}$ (Table 3). In 2021, soybean protein yield in DCS was significantly higher than in SCS (1010 and $508 \text{ kg protein ha}^{-1}$, respectively) (Table 3).

Barley yields were only tested for the year effect, as this crop was only grown in DCS. No year effect was found on any of the variables analysed with an average grain, energy and protein yield of 7.7 t ha^{-1} , 122 GJ ha^{-1} and $703 \text{ kg protein ha}^{-1}$, respectively (Table 3).

Table 3

Grain (14% moisture), energy and protein yield (on a dry matter basis) at the crop level affected by cropping systems (SCS: single cropping system; DCS: double cropping system), year (2019, 2020 and 2021) and their interaction. For each crop, P-values are shown below the mean values for each variable. Values in bold indicate $p\text{-value} < 0.05$. For each variable and crop, levels not connected by the same letter are significantly different at $\alpha = 0.05$.

Crop	Cropping system	Year	Grain yield (t ha^{-1})		Energy yield* (GJ ha^{-1})		Protein yield (kg ha^{-1})	
Maize	SCS	2019	19.7	a	316	a	1273	
		2020	14.0	b	225	b	740	
		2021	12.5	b	201	b	809	
	DCS	2019	17.8	a	286	a	1129	
		2020	8.8	c	142	c	504	
		2021	9.3	c	150	c	526	
	Cropping system (CS)		0.0001		0.0001		0.0001 [§]	
	Year (Y)		0.0001		0.0001		0.0001	
	CS x Y		0.0258		0.0258		0.321	
Soybean	SCS	2019	4.1	a	84	a	1141	a
		2020	3.7	ab	75	ab	1100	a
		2021	2.2	c	45	c	508	b
	DCS	2019	2.9	bc	59	bc	949	a
		2020	3.1	abc	62	abc	964	a
		2021	3.5	ab	71	ab	1010	a
	Cropping system (CS)		0.370		0.370		0.404	
	Year (Y)		0.050		0.050		0.005	
	CS x Y		0.001		0.001		0.001	
Barley	DCS	2020	7.9		125		703	
		2021	7.5		119		703	
	Cropping system (CS)		NA* *		NA		NA	
	Year (Y)		0.635		0.635		0.998	

*Grain yield x gross energy content. * *This factor was not included in the ANOVA as barley was only grown in the DCS. §As the interaction was not significant, the letters of significance are not presented in this table. Significant single effects are described in the text.

3.2. Preceding effects of soybean on maize and barley

Soybean positively affected the following crop yields and grain N, maize in the SCS and barley in the DCS. In maize, grain yield increased from 11.2 to 14.4 t ha⁻¹ when preceded by soybean compared to maize as the preceding crop (Fig. 2a). Maize grain N followed the trend of grain yield with 110 and 151 kg ha⁻¹ for the pre-crop maize and soybean, respectively (Fig. 2c). Above-ground biomass production of maize was only affected by the pre-crop, with 23.4 and 27.3 t ha⁻¹ for maize and soybean preceding crops, respectively, as an average of 2020 and 2021 (Table 4). Maize N uptake in the above-ground biomass followed a similar trend (although not significant) with 204 and 246 kg N ha⁻¹ for the maize and soybean preceding crops, respectively (values averaged across years). Maize ears m⁻² and grains ear⁻¹ were not significantly affected by the pre-crop or the year effect (Table 4). Maize TGW was affected by the pre-crop and year single effects, with higher TGW in 2020 than in 2021 (237 and 280 g, respectively). As well, soybean pre-crop showed higher TGW compared to maize pre-crop (272 and 246 g, respectively, averaged across years) (Table 4).

In barley, the pre-crop x year interaction significantly affected grain yield and grain N (Figs. 2b, 2d). In season 2019–20, the two pre-crops, soybean and maize, led to a similar barley grain yield and grain N uptake. In contrast, soybean compared to maize as a pre-crop led to significantly higher barley grain yields (9.6 vs 6.5 t ha⁻¹) and N uptake (174 vs 108 kg N ha⁻¹) in 2021 (Figs. 2b and 2d). Barley aboveground biomass was not significantly affected by any factor, with an average of

17.8 t ha⁻¹ across pre-crops and years (Table 4). Pre-crop x year interaction affected barley N uptake. In 2020, the differences between maize and soybean pre-crops were not statistically significant (204 and 230 kg N ha⁻¹, respectively), while in 2021, barley after soybean had a higher N uptake compared to maize as the pre-crop (252 and 169 kg ha⁻¹, respectively) (Table 4). Barley ears m⁻² were affected by the year, with 599 and 399 ears m⁻² in 2020 and 2021, respectively (Table 4). Differently, the number of grains ear⁻¹ was significantly lower in 2020 than in 2021 (43 and 54 grains ear⁻¹, respectively) (Table 4). The TGW was not significantly affected by the pre-crop or the year and presented an average value of 36 g. Preceding crop effects were also tested on rye cover crop biomass at termination. No effect of the pre-crop was found (Table 4). However, a strong effect of the year was observed, with 10.8 and 4.9 t ha⁻¹ produced in 2020 and 2021, respectively (Table 4). As well, cover crop N uptake at termination was significantly higher in 2020 than in 2021 (162 and 87 kg N ha⁻¹, respectively) (Table 4).

3.3. Cropping system level performance

Barley-soybean cropping system achieved the highest protein productivity (1779 kg protein ha⁻¹ yr⁻¹) with 99%, 88% and 58% higher protein production than the MM, MSrt and BM systems, respectively (Fig. 3c). Except in the MM system (895 kg protein ha⁻¹ yr⁻¹), the protein productivity of each cropping system is split into different crops. For the MSrt (948 kg protein ha⁻¹ yr⁻¹) and the BM (1129 kg protein

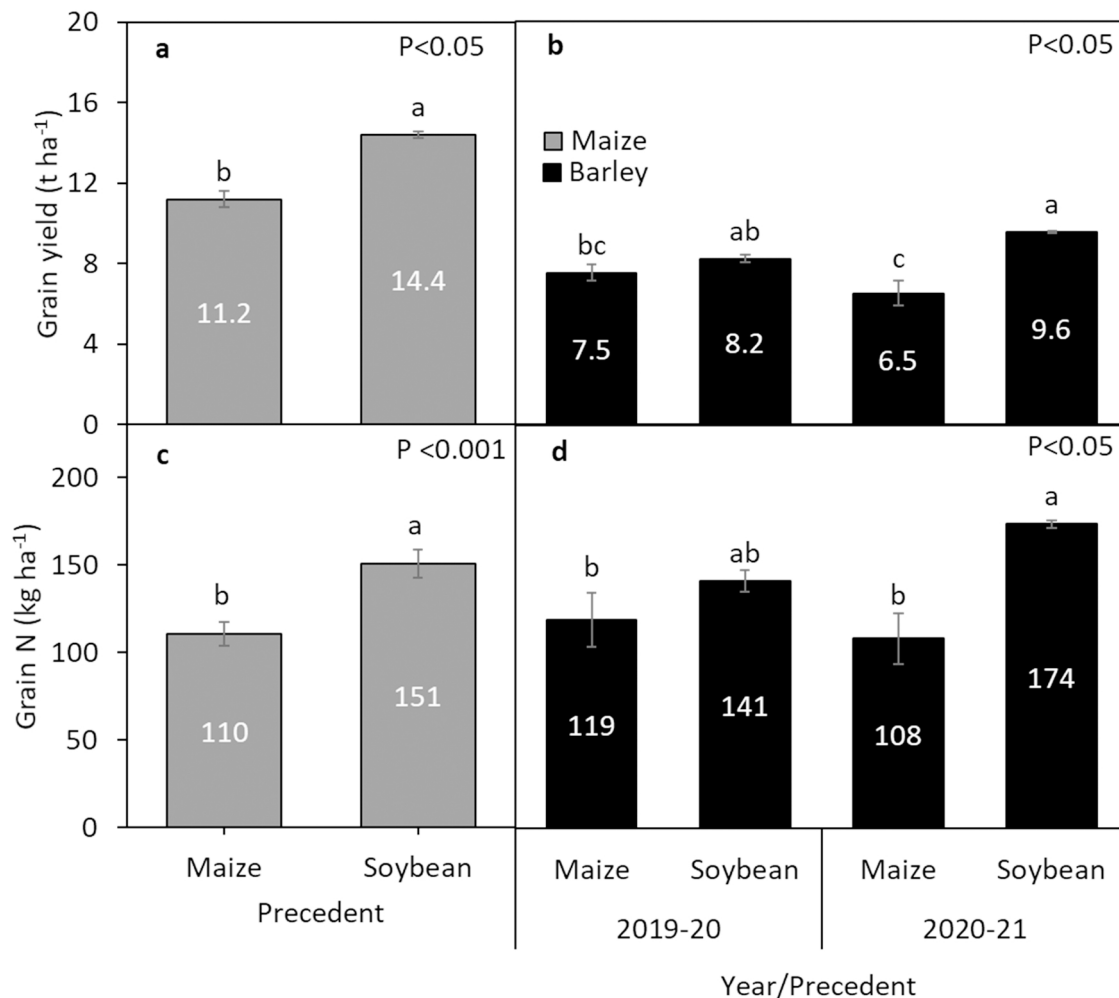


Fig. 2. Grain yield (14% moisture) and grain N for maize (a and c) and barley (b and d) depending on the pre-crop in maize, and the pre-crop x year interaction in barley. Within each sub-figure, levels not connected by the same letter are significantly different at $\alpha = 0.05$. Error bars refer to standard error.

Table 4

Above-ground biomass and N uptake (Nupt) of maize, barley and rye and yield components for maize and barley as affected by pre-crop (maize or soybean), year (2020 and 2021) and their interaction. For each crop, P-values are shown below the mean values for each variable. Values in bold indicate **p-value** < 0.05. For each variable and crop, levels not connected by the same letter are significantly different at $\alpha = 0.05$.

Crop	Year	Pre-crop		Biomass (t ha ⁻¹)		Nupt (kg ha ⁻¹)		ears m ⁻²		grains ear ⁻¹		TGW (g)			
Maize	2020	Maize		21.3	23.9	163	188	B	7.67	7.74	661	725	221	237	b
		Soybean		26.6		213			7.78		757		253		
	2021	Maize		25.5	26.7	244	261	A	7.28	7.33	679	670	271	280	a
		Soybean		27.9		278			7.39		661		290		
	Pre-crop			0.0257		0.081			0.4420		0.1780		0.0359		
	Year			0.0629		0.012			0.5360		0.2130		0.0049		
	Pre-crop x year			0.2047		0.602			0.6400		0.0661		0.3870		
Barley	2020	Maize	19.3	19.2	204	bc	217	649	599	A	42	43	B	33	34
		Soybean	19.1		230	ab		548			43			36	
	2021	Maize	16.9	17.6	169	c	211	361	399	B	54	54	A	39	38
		Soybean	18.2		252	a		436			53			38	
	Pre-crop			0.0825		0.0018			0.9318		0.7883		0.4015		
	Year			0.2267		0.5285			0.0029		0.0052		0.0690		
	Pre-crop x year			0.0644		0.0177			0.0557		0.9240		0.3509		
Rye	2020	Maize	10.2	10.9	A	175		162	A						
		Soybean	11.5			150									
	2021	Maize	5.1	4.9	B	73	87	B							
		Soybean	4.8			101									
	Pre-crop			0.5068		0.9418									
	Year			0.0001		0.0013									
	Pre-crop x year			0.3264		0.1440									

ha⁻¹ yr⁻¹) systems, maize contributed 68% and 46% of the protein yield, respectively. The remaining protein production in these systems was attributed either to soybean (305 kg protein ha⁻¹ yr⁻¹ in the MSrt) or barley (615 kg protein ha⁻¹ yr⁻¹ in the BM). In the case of BS DCS, 55% of the protein yield was produced by soybean (987 kg protein ha⁻¹ yr⁻¹), while the rest was produced by barley.

Grain yield and energy productivity at the cropping system level were highest for continuous maize and barley-maize (Figs. 3a, 3b). The MM system yielded 12.7 t ha⁻¹ yr⁻¹ and 238 GJ ha⁻¹ yr⁻¹ of maize. The BM system yielded a total of 13.8 t ha⁻¹ yr⁻¹ and 256 GJ ha⁻¹ yr⁻¹, where maize and barley accounted for 56% and 44% of the total grain and energy yield, respectively. In the SCS with soybean, MSrt, total grain and energy yield was 9.9 t ha⁻¹ yr⁻¹ and 190 GJ ha⁻¹ yr⁻¹, respectively. Ninety per cent and 88% of the grain and energy yield was attributed to the maize proportion (with two out of the three years) in the cropping system. In the DCS, BS grain and energy yield was 10.1 t ha⁻¹ yr⁻¹ and 200 GJ ha⁻¹ yr⁻¹, respectively. Seventy-two per cent of the grain yield and 67% of the energy yield were attributed to the barley phase of the system (Figs. 3a, 3b).

Within the SCS, no differences were found between MM and MSrt for the N_{UE_{synt-g}} and N_{UE_{synt-e}}, with an average value of 81 kg grain kg synthetic N⁻¹ and 1.5 GJ kg synthetic N⁻¹, respectively (Figs. 4a, 4b). In DCS, N_{UE_{synt-g}} and N_{UE_{synt-e}} were higher than in SCS. Within DCS, BS presented significantly higher N_{UE_{synt-g}} and N_{UE_{synt-e}} compared to the BM system (102 and 251 kg grain kg synthetic N⁻¹ and 5.1 and 1.9 GJ kg synthetic N⁻¹, respectively, Figs. 4a, 4b). For the N_{UE_{synt-p}} indicator, BS presented the highest value, 50.1 kg protein kg synthetic N fertilizer⁻¹, compared to the other three cropping systems (ranging between 5.4 and 8.2 kg protein kg synthetic N fertilizer⁻¹) (Fig. 4c).

Soil nitrate contents in November (at 0–60 cm depth) were affected by the year but not by the cropping systems. Across the three experimental years, residual soil nitrate content after summer crops decreased from 137 in 2019–50 kg NO₃-N ha⁻¹ in 2021 (Fig. 5a). Soil nitrate content in May (after winter crops) was affected by the cropping system x year interaction. Single cropping systems (MM and MSrt) presented contrasting values in the two seasons, with values around 30 and 90 kg NO₃-N ha⁻¹ (0–60 cm depth) in 2020 and 2021, respectively, without differences between MM and MSrt (Fig. 5b). On the other hand, the double cropping systems (BM and BS) had a similar soil nitrate content in May in both years of around 30 kg NO₃-N ha⁻¹ (Figure 50b).

4. Discussion

4.1. Productivity and synthetic N use efficiency at the cropping system level

Protein yield in the SCS increased by 5% (without being significant) when including soybean. In this regard, Costa et al. (2021) simulated the productivity of different crop rotations across three contrasting areas in Europe. In their scenario of Romania, they found a 50% increase in protein yield (from 312 to 458 kg ha⁻¹ yr⁻¹) when soybean was added in a four-year rotation. Compared to our study, soybean introduction led to a higher relative increase in protein yield due to lower maize productivity, likely caused by the colder climate and rainfed conditions in Romania. From these results, it can be drawn that a larger inclusion of soybean in Mediterranean irrigated SCS would not increase protein output. Nonetheless, the values reported in our work refer to crude protein content derived from grain N content, while protein quality was out of the scope of this study. In that regard, soybean protein amino acid distribution is essential for feed formulas (Gatel, 1994) and soybean-based food, thus stressing the importance of soybean in European cropping systems beyond the agronomic benefits.

Grain and energy yield at the cropping system level was reduced by 20% when soybean was introduced in the rotation. Soybean contribution to energy yield was 11% (in a three-year rotation, MSrt), demonstrating that energy yield was largely driven by maize. These results are in agreement with Hisse et al. (2022), who reported a soybean contribution to energy yield of 30% in a two-year rotation with soybean and maize in Argentina. In both cases, soybean contribution to energy yield was 20% lower than its proportion in the rotation, indicating that its inclusion leads to a decrease in energy yield. These results also agree with Notz et al. (2023), who reported lower energy yields in 16 out of 19 crop rotations comparisons without and with legumes across Europe using regional statistics and expert-based data.

Grain DCS are not common in European cropping systems due to limited thermal time in most of the continent. To the extent of our knowledge, double cropping systems with soybean under European conditions have not been addressed further than the crop level and only in Turkey (e.g. Arslan et al., 2006; Çalişkan et al., 2007; Gulluoglu et al., 2018). Compared to the continuous maize system (MM), energy yield increased by 7.5% in BM (256 GJ ha⁻¹ yr⁻¹) and decreased by 16% in BS (200 GJ ha⁻¹ yr⁻¹). In the case of BM, these results agree with the ones

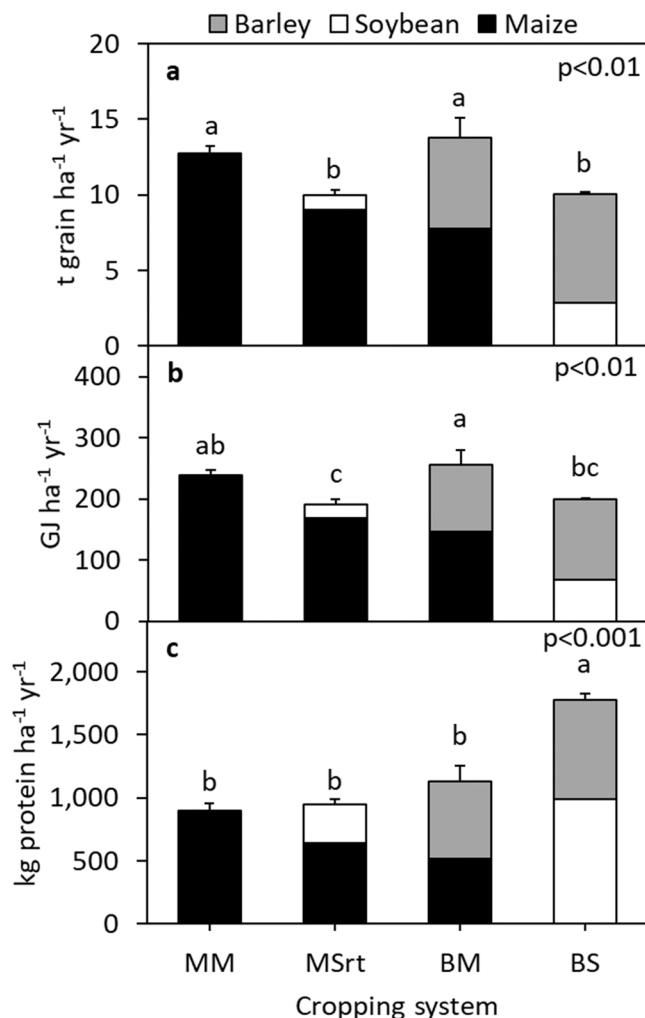


Fig. 3. Grain (a), energy (b) and protein (c) annual yield of the four cropping systems assessed. MM: Continuous maize; MSrt: soybean in a three-year rotation with maize; BM: barley-maize double cropping system; BS: barley-soybean double cropping system. Within each sub-figure, levels not connected by the same letter are significantly different at $\alpha = 0.05$. Error bars refer to standard error for the whole cropping system.

reported by Liang et al. (2011) after surveying 362 farms in China, where they found an average energy yield of 260 GJ ha⁻¹ yr⁻¹ (values calculated from the yields reported). Protein yield in the BS system was significantly higher than the rest of the cropping systems assessed, given the larger share of soybean in the cropping system and the pre-crop effect on the winter barley. In this regard, BS double cropping system is a promising strategy to increase protein output, contributing to plant protein self-sufficiency and cropping systems diversification in Mediterranean irrigated areas. Although a wider adoption of this cropping system across northern Europe is currently limited by the temperatures largely, climate change can expand the areas suitable for sequential double cropping, an underexplored management option in Northern areas (Nendel et al., 2022). Similarly, Seifert and Lobell (2015) simulated a likely increase in double cropping surface ranging from 126% to 239% under RCP4.5 and RCP8.5 scenarios, respectively.

Legume introduction in cropping systems decreases synthetic N dependence (Peoples et al., 2009). In our case, the N fertilizer rate in the MSrt was reduced by 30% compared to the MM system. However, soybean introduction in the SCS (MSrt) had little effect on N_{syn}-g and N_{syn}-e compared to MM, as the reduction in grain and energy production was proportional to the reduction in the synthetic N fertilizer applied. Regarding protein production efficiency, the increase in

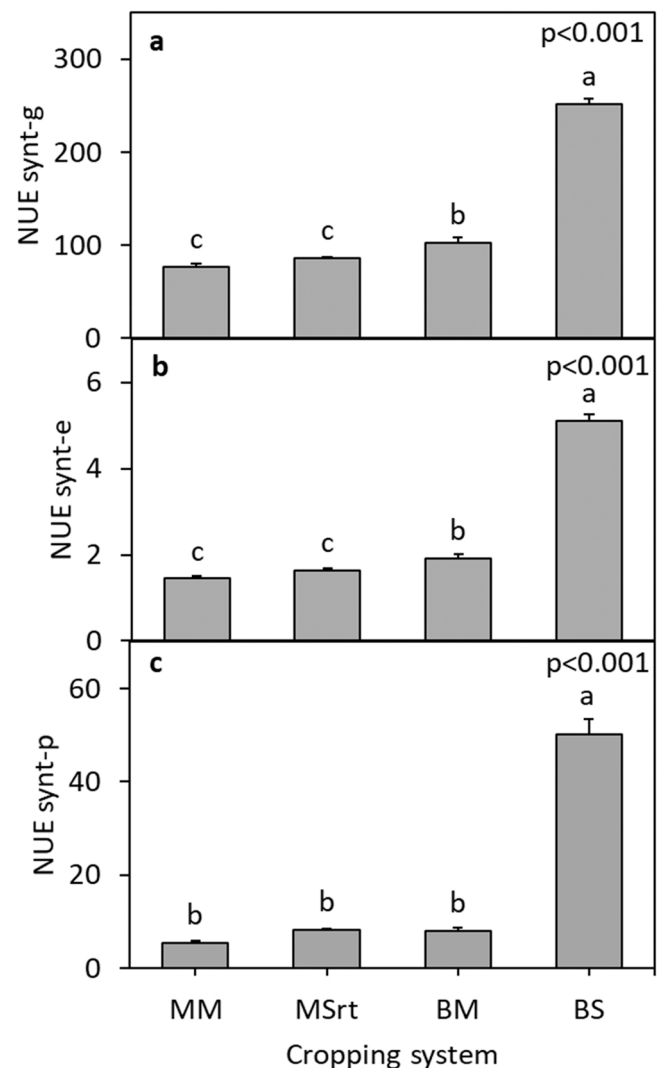


Fig. 4. Synthetic N use efficiency for grain (a), energy (b) and protein (c) production of the four cropping systems assessed. MM: Continuous maize; MSrt: soybean in a three-year rotation with maize; BM: barley-maize double cropping system; BS: barley-soybean double cropping system. NUE values are expressed as kg grain kg synthetic N⁻¹ (a), GJ kg synthetic N⁻¹ (b) and kg protein kg synthetic N⁻¹ (c). Within each sub-figure, levels not connected by the same letter are significantly different at $\alpha = 0.05$. Error bars refer to standard error.

NUE_{syn}-p (although not statistically significant) was 49%, as the protein production of soybean was similar to that of maize, but without N fertilizer requirements. The magnitude of N_{syn}-g observed (82 kg grain kg synthetic N fertilizer⁻¹, on average) was higher than average values for previous studies in the Ebro valley. For instance, Villar-Mir et al. (2002) tracked the maize yield and synthetic N rates applied to ten commercial farms in NE Spain for two years and reported an average N_{syn}-g of 40 kg grain kg synthetic N fertilizer⁻¹ (calculated from their data). The efficiencies reported in the present study indicate that redesigned cropping systems through diversification, use of a cover crop and the adaptation of N fertilizer rates contribute to the reduction in the use of N fertilizers while maintaining the system's productivity.

In the case of DCS, N_{syn}-g and N_{syn}-e increased by 30% and 253% in the BM and BS compared to MM, respectively. The increase in the BM system was due to the low N fertilizer rates applied to barley in relation to its productivity. The N_{syn}-e of the system was 1.9 GJ kg synthetic N fertilizer⁻¹, which is close to the values calculated from Maresma et al. (2019) of 1.8 GJ kg synthetic N fertilizer⁻¹. Barley-soybean DCS presented the highest level of independence from

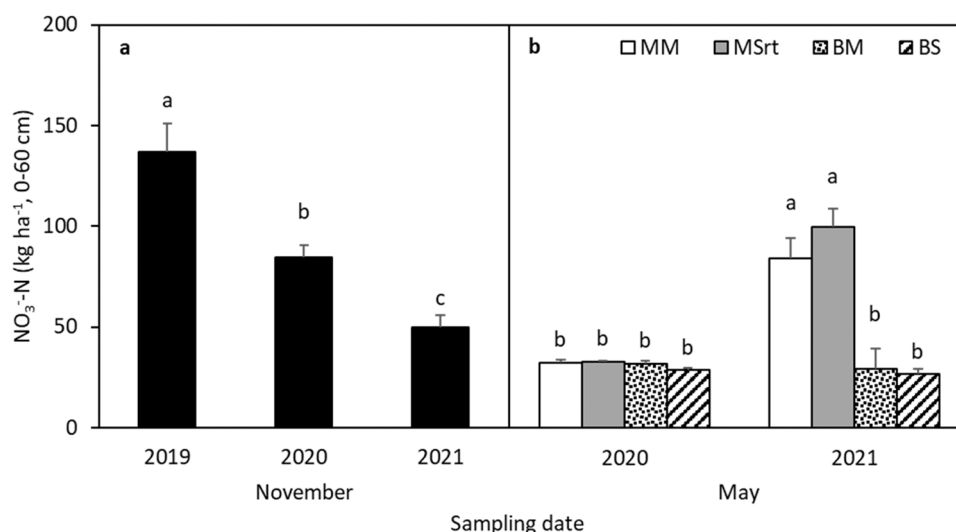


Fig. 5. Soil nitrate contents in November for the year effect (a) and in May for the cropping systems (MM: Continuous maize, MSrt: soybean in a three-year rotation with maize; BM: barley-maize double cropping system; BS: barley-soybean double cropping system) by year interaction (b). Within each sub-figure, levels not connected by the same letter are significantly different at $\alpha = 0.05$. Error bars refer to standard error.

synthetic N inputs compared to the other cropping systems (Fig. 4). The main drivers of increased $\text{NUE}_{\text{synth-p}}$ in the BS cropping system were the larger share of soybean, the low N rates applied to barley and the effect of soybean as the preceding crop on barley. Therefore, diversification with BS double cropping systems led to a win-win situation for protein production and reducing synthetic N fertilizer. In addition, these diversified systems can contribute to a potential reduction in environmental impacts through lower N_2O emission directly linked to the grain legume independence from nitrogen synthetic fertilizers (Peyrard et al., 2016) and the provision of several ecosystem services (Kremen and Miles, 2012).

Such cropping systems under Mediterranean conditions are dependent on substantial irrigation water amounts. The four cropping systems assessed present a similar pattern and rate of irrigation, around 700 mm distributed between April and September. In the SCS, all irrigation water is devoted to the summer crop (maize or soybean), while in the DCS, irrigation is split into barley (to achieve higher yields) and the summer crops. Therefore, the alternatives proposed do not imply an increase in irrigation water use compared to traditional cropping systems (i.e. MM). Nevertheless, future research could tackle adaptation strategies to potential irrigation water deficits for these cropping systems such as more efficient irrigation systems, less water-demanding crops, etc. (Harro-Monteagudo et al., 2022).

After maize and soybean harvest, no differences were found in residual soil nitrate content between cropping systems. However, a decreasing trend through the years of the experiment indicates that the cropping system redesign, especially the reduction of synthetic N rates applied and the use of a winter cover crop, led to a lower amount of soil nitrate susceptible of being lost through leaching during the periods of low N intake (i.e. autumn months). Ensuring a living crop cover throughout the year through the use of cover crops has been widely reported as a beneficial practice to reduce N leaching, as well as increasing soil organic carbon stocks (Poelau and Don, 2015). In cold areas (e.g. Blombäck et al., 2003) or temperate climates under rainfed cropping systems (e.g. Plaza-Bonilla et al., 2016), the use of cover crops plays a key role in maintaining crop cover throughout the year, as the resources (either water or radiation) might not be sufficient to grow two cash crops. In contrast, in our case study, winter crop development is rarely limited by low temperatures and summer drought can be offset with irrigation. In that sense, including a winter cash crop with adequate N fertilizer rates can bring similar benefits to the use of the cover crop, thus contributing to the system's productivity and the farm's

profitability.

The larger amount of rye biomass, and thus a larger N uptake, accumulated in 2020 than in 2021 explain the contrasting soil mineral N contents at rye termination in 2020 and 2021 (Fig. 5b). In that regard, a simulation study demonstrated that the use of cover crops, as well as the adaptation of N fertilisation to the cash crops' needs, reduced N leaching in the long term in three locations in N France (Constantin et al., 2012). Soil nitrate contents during the barley phase in the DCS decreased from the sowing to grain filling period (Fig. 5a & 5b). These results confirm that the barley phase in the DCS can lead to similar (2020) or lower (2021) soil nitrate contents than the use of cover crops, even when N fertilizer is applied to it. In that regard, Heggenstaller et al. (2008) reported a reduced risk of N leaching in a forage-rye and grain-maize double cropping system in Iowa (US), even with the N fertilizer demand being higher than in their sole-crop reference (maize).

4.2. Mechanisms behind the system's performance explained at the crop and pre-crop level

Maize grain yield was reduced by 24% when grown as a double crop compared to single cropping. Fewer grains per ear (714 and 556 grains ear^{-1} in the SCS and DCS, respectively, data not shown) could be the main factor explaining the grain yield differences between single and double-cropped maize. Similar yield reductions in double-cropped maize in Argentina have been found, with grain number and TGW as the factors responsible for the yield decrease (Andrade et al., 2000; Crespo et al., 2022; R.D. Martínez et al., 2017).

Single-cropped soybean presented a high interannual yield variability. The low yield recorded in 2021 compared to 2019 and 2020 was due to an exceptionally low TGW (128 g) compared to the average of 2019 and 2020 (193 g). A heat wave during the early phases of grain filling (R4-5 stages) could have been the reason for a such phenomenon (Dornbos and Mullen, 1991). Double-cropped soybean achieved similar grain yields compared to single cropping. These results contrast with the ones reported by Shrestha et al. (2021) in Ohio, in a colder area than our study, where a reduction of 65% in soybean yield was found in DCS compared to SCS. As well, reductions of 35% in yield were reported by Calviño and Monzon (2009) in Argentina (temperate to subtropical climates). In the cited studies, the authors identify the dryer conditions and the shorter grain filling period as the main drivers for the yield reductions observed in double-cropped soybean. In our case, irrigation offsets any water shortage for soybean production. However, the fact

that no reduction was observed when shortening the growing period by two months (i.e. soybean planting is delayed from early May to late June in DCS compared to SCS) suggests that the potential soybean yield in a SCS might be higher than the one reported in the present study. Local food industry constraints and seed availability limit the use of later maturity groups than I or II in the SCS. Therefore, we hypothesize that the use of later maturity groups in single-cropped soybean would allow to better exploit the potential of soybean under Mediterranean irrigated conditions. Nendel et al. (2022) simulated (mapped) current and climate change scenarios for soybean suitability across Europe, pointing out the suitability of maturity group II (under current conditions) and III (under climate change conditions, RCP 4.5) for Mediterranean areas.

Soybean introduction led to an average yield increase of 28% in the following cereal. As well, an increase in total N uptake was observed in maize and barley preceded by soybean. Increased N uptake after a break crop has been identified as one of the main drivers for yield increase in maize production in the Corn Belt in the US (Gentry et al., 2013; Pikul et al., 2005) and wheat in several temperate areas across the globe (Kirkegaard et al., 2008) and even rice in South Brazil (Ribas et al., 2021). In our case, the larger N uptake found in soybean-preceded cereals suggests that soybean crop residues left a larger amount of mineralizable N for the following crop compared to maize crop residues. This hypothesis was confirmed by Omay et al. (1998), who demonstrated that soybean crop residues do not necessarily leave a larger amount of N in the soil, but the N content in soybean crop residues is rapidly available for the next crop uptake in comparison to the N contained in maize crop residues. In our case, the higher TGW in 2020 and 2021 with soybean as pre-crop compared to maize would indicate that N release from soybean crop residues was likely absorbed by the crop at the latter stages of maize development.

5. Conclusions

The multi-scale methodology used in this study (crop, pre-crop and cropping system level) allowed a fair comparison among different cropping systems. It shows that the individual crop performance is closely linked to the cropping system where it is grown i.e. the grain yield of maize after a soybean pre-crop can only be accounted for in a cropping system assessment. Including the crop and pre-crop level analyses in this study brought insights on specific mechanisms explaining the cropping system performance e.g. soybean yield analysis at the crop level allowed to formulate the hypothesis that longer maturity groups might be needed for single cropping systems. Through an on-farm experimental approach, we assessed the performance and sustainability of four cropping systems under Mediterranean irrigated conditions. While soybean introduction in single cropping systems showed little increase in protein yield, barley-soybean double cropping system led to the highest protein yields (1778 kg protein ha⁻¹ yr⁻¹) compared to traditional continuous maize single cropping system (895 kg protein ha⁻¹ yr⁻¹). Sustainable cropping system intensification through double cropping also led to a reduction in synthetic N fertilizer use and an increased synthetic N use efficiency compared to maize-based systems (77 and 251 kg grain kg synthetic N fertilizer⁻¹, respectively). These novel findings on double cropping systems with soybean in Europe need to be explored further as climate change expands the current area suitable to double cropping. We conclude that soybean introduction in maize-based cropping systems is an essential pillar towards increasing plant protein production in Europe, as well as a path to more sustainable cropping systems.

CRedit authorship contribution statement

Genís Simon-Miquel: Conceptualization, Methodology, Data analysis, Visualization, Writing – original draft. **Moritz Reckling:** Data analysis, Supervision, Writing – review & editing. **Jorge Lampurlanés:** Experimental design, Writing – review & editing. **Daniel Plaza-Bonilla:**

Conceptualization, Experimental design, Methodology, Supervision, Project administration, Funding acquisition, Writing – review & 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

This research was developed in the framework of the SusCrop-ERA-NET LegumeGap project, PCI2019-103597 funded by MCIN/AEI/10.13039/501100011033 and co-funded by the European Union, and the ECO-TRACE Research project, TED2021-131895A-I00 funded by MCIN/AEI/10.13039/501100011033 and the European Union Next-GenerationEU/PRTR. Daniel Plaza-Bonilla is Ramón y Cajal fellow (RYC-2018-024536-I) co-funded by MICIN/AEI/10.13039/501100011033 and European Social Fund. Genís Simon-Miquel thanks Universitat de Lleida for the funding granted to do an international research stay at ZALF where this work was conceptualized. Moritz Reckling was funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) – 420661662. The authors of this manuscript kindly thank Ramon Pujol Chambert and Ramon Pujol Figueras from Jolbertal SL for their goodwill to carry out this research in their fields and with their machinery and for providing precious feedback. This acknowledgement also extends to Ariadna Marquilles, Josep Maria Perera, Gerard Piñol, Pablo Redondo, Louise Blanc, Andreu Dago, and several bachelor students from Universitat de Lleida for their field, lab and technical support.

References

- Abad, A., Michelena, A., Lloveras, J., 2005. Effects of nitrogen supply on wheat and on soil nitrate. *Agron. Sustain. Dev.* 25, 439–446. <https://doi.org/10.1051/AGRO:2005042>.
- Andrade, F.H., Otegui, M.E., Vega, C., 2000. Intercepted radiation at flowering and kernel number in maize. *Agron. J.* 92, 92–97. <https://doi.org/10.2134/AGRONJ2000.92192X>.
- Angus, J.F., Kirkegaard, J.A., Hunt, J.R., Ryan, M.H., Ohlander, L., Peoples, M.B., 2015. Break crops and rotations for wheat. *Crop Pasture Sci.* 66, 523–552. <https://doi.org/10.1071/CP14252>.
- Arslan, M., Uremis, I., Uludag, A., 2006. The critical period of weed control in double-cropped soybean. *Phytoparasitica* 34, 159–166. <https://doi.org/10.1007/BF02981316>.
- Berenguer, P., Santiveri, F., Boixadera, J., Lloveras, J., 2009. Nitrogen fertilisation of irrigated maize under Mediterranean conditions. *Eur. J. Agron.* 30, 163–171. <https://doi.org/10.1016/j.eja.2008.09.005>.
- Blombäck, K., Eckersten, H., Lewan, E., Aronsson, H., 2003. Simulations of soil carbon and nitrogen dynamics during seven years in a catch crop experiment. *Agric. Syst.* 76, 95–114. [https://doi.org/10.1016/S0308-521X\(02\)00030-6](https://doi.org/10.1016/S0308-521X(02)00030-6).
- Çalışkan, S., Arslan, M., Üremiş, I., Çalışkan, M.E., 2007. The effects of row spacing on yield and yield components of full season and double-cropped soybean. *Turk. J. Agric.* 31, 147–154. <https://doi.org/10.3906/tar-0703-10>.
- Calviño, P., Monzon, J.P., 2009. Farming systems of argentina: yield constraints and risk management. *Crop Physiol. Appl. Genet. Improv. Agron* 55–70. <https://doi.org/10.1016/B978-0-12-374431-9.00003-7>.
- Cavero, J., Beltrán, A., Aragüés, R., 2003. Nitrate exported in drainage waters of two sprinkler-irrigated watersheds. *J. Environ. Qual.* 32, 916–926. <https://doi.org/10.2134/jeq2003.9160>.
- Constantin, J., Beaudoin, N., Launay, M., Duval, J., Mary, B., 2012. Long-term nitrogen dynamics in various catch crop scenarios: test and simulations with STICS model in a temperate climate. *Agric. Ecosyst. Environ.* 147, 36–46. <https://doi.org/10.1016/j.agee.2011.06.006>.
- Costa, M.P., Reckling, M., Chadwick, D., Rees, R.M., Saget, S., Williams, M., Styles, D., 2021. Legume-modified rotations deliver nutrition with lower environmental impact. *Front. Sustain. Food Syst.* 5, 113. <https://doi.org/10.3389/FSUFS.2021.656005/BIBTEX>.
- Crespo, C., Martínez, R.D., Wyngaard, N., Divito, G., Martínez Cuesta, N., Barbieri, P., 2022. Nitrogen diagnosis for double-cropped maize. *Eur. J. Agron.* 140. <https://doi.org/10.1016/j.eja.2022.126600>.

- Debaeke, P., Munier-Jolain, N., Bertrand, M., Guichard, L., Nolot, J.-M., Faloya, V., Saulas, P., 2009. Iterative design and evaluation of rule-based cropping systems: methodology and case studies. A review. *Agron. Sustain. Dev.* 29, 73–86. <https://doi.org/10.1051/agro:2008050>.
- Dornbos Jr., D.L., Mullen, R.E., 1991. Influence of stress during soybean seed fill on seed weight, germination, and seedling growth rate. *Can. J. Plant Sci.* 71, 373–383. <https://doi.org/10.4141/cjps91-052>.
- Drinkwater, L.E., Janke, R.R., Rossoni-Longnecker, L., 2000. Effects of tillage intensity on nitrogen dynamics and productivity in legume-based grain systems. *Plant Soil* 227, 99–113. <https://doi.org/10.1023/A:1026569715168>.
- European Commission, 2019. The European green deal. *Introd. Knowl. Manag. Commun.* 106–117. <https://doi.org/10.4324/9780080495781-12>.
- Franchini, J.C., Debiassi, H., Balbinot Junior, A.A., Tonon, B.C., Farias, J.R.B., Oliveira, M.C.N. de, Torres, E., 2012. Evolution of crop yields in different tillage and cropping systems over two decades in southern Brazil. *F. Crop. Res.* 137, 178–185. <https://doi.org/10.1016/j.fcr.2012.09.003>.
- Gatel, F., 1994. Protein quality of legume seeds for non-ruminant animals: a literature review. *Anim. Feed Sci. Technol.* 45, 317–348. [https://doi.org/10.1016/0377-8401\(94\)90036-1](https://doi.org/10.1016/0377-8401(94)90036-1).
- Gentry, L.F., Ruffo, M.L., Below, F.E., 2013. Identifying factors controlling the continuous corn yield penalty. *Agron. J.* 105, 295–303. <https://doi.org/10.2134/agronj2012.0246>.
- Guilpart, N., Iizumi, T., Makowski, D., 2022. Data-driven projections suggest large opportunities to improve Europe's soybean self-sufficiency under climate change. *Nat. Food*. <https://doi.org/10.1038/s43016-022-00481-3>.
- Gulluoglu, L., Bakal, H., Arioglu, H., 2018. Oil content and composition of soybean genotypes grown in different growing seasons under Mediterranean conditions. *J. Environ. Biol.* 39, 211–215. <https://doi.org/10.22438/jeb/39/2/MRN-318>.
- Haro-Montegudo, D., Palazón, L., Zoumides, C., Beguería, S., 2022. Optimal implementation of climate change adaptation measures to ensure long-term sustainability on large irrigation systems. *Water Resour. Manag.* <https://doi.org/10.1007/s11269-022-03225-x>.
- Heggenstaller, A.H., Anex, R.P., Liebman, M., Sundberg, D.N., Gibson, L.R., 2008. Productivity and nutrient dynamics in bioenergy double-cropping systems. *Agron. J.* 100, 1740–1748. <https://doi.org/10.2134/agronj2008.0087>.
- Hisse, I.R., Biganzoli, F., Peper, A.M., Poggio, S.L., 2022. Annual productivity of cropping sequences: responses to increased intensification levels. *Eur. J. Agron.*
- Karges, K., Bellingrath-Kimura, S.D., Watson, C.A., Stoddard, F.L., Halwani, M., Reckling, M., 2022. Agro-economic prospects for expanding soybean production beyond its current northerly limit in Europe. *Eur. J. Agron.* 133, 126415 <https://doi.org/10.1016/j.eja.2021.126415>.
- Karlen, D.L., Kovar, J.L., Cambardella, C.A., Colvin, T.S., 2013. Thirty-year tillage effects on crop yield and soil fertility indicators. *Soil Tillage Res.* 130, 24–41. <https://doi.org/10.1016/j.still.2013.02.003>.
- Kirkegaard, J., Christen, O., Krupinsky, J., Layzell, D., 2008. Break crop benefits in temperate wheat production. *F. Crop. Res.* 107, 185–195. <https://doi.org/10.1016/j.fcr.2008.02.010>.
- Kremen, C., Miles, A., 2012. Ecosystem services in biologically diversified versus conventional farming systems: benefits, externalities, and trade-offs. *Ecol. Soc.* 17. <https://doi.org/10.5751/ES-05035-170440>.
- Lassale, L., Billen, G., Romero, E., Garnier, J., Aguilera, E., 2014. How changes in diet and trade patterns have shaped the N cycle at the national scale: Spain (1961–2009). *Reg. Environ. Chang.* 14, 785–797. <https://doi.org/10.1007/S10113-013-0536-1/FIGURES/7>.
- Liang, W., Li, Carberry, P., Wang, G., Yan, L., R. Hai, L., H., Zhan, Xia, Ping, A., 2011. Quantifying the yield gap in wheat-maize cropping systems of the Hebei Plain, China. *F. Crop. Res.* 124, 180–185. <https://doi.org/10.1016/j.fcr.2011.07.010>.
- López-Bellido, R.J., López-Bellido, L., 2001. Efficiency of nitrogen in wheat under Mediterranean conditions: effect of tillage, crop rotation and N fertilization. *F. Crop. Res.* 71, 31–46. [https://doi.org/10.1016/S0378-4290\(01\)00146-0](https://doi.org/10.1016/S0378-4290(01)00146-0).
- Magrini, M.B., Anton, M., Chole, C., Corre-Hellou, G., Duc, G., Jeuffroy, M.H., Meynard, J.M., Pelzer, E., Voisin, A.S., Walrand, S., 2016. Why are grain-legumes rarely present in cropping systems despite their environmental and nutritional benefits? Analyzing lock-in in the French agrifood system. *Ecol. Econ.* 126, 152–162. <https://doi.org/10.1016/j.ecolecon.2016.03.024>.
- Maresma, Á., Martínez-Casasnovas, J.A., Santiveri, F., Lloveras, J., 2019. Nitrogen management in double-annual cropping system (barley-maize) under irrigated Mediterranean environments. *Eur. J. Agron.* 103, 98–107. <https://doi.org/10.1016/j.eja.2018.12.002>.
- Mariotti, F., Tomé, D., Mirand, P.P., 2008. Converting nitrogen into protein - Beyond 6.25 and Jones' factors. *Crit. Rev. Food Sci. Nutr.* 48, 177–184. <https://doi.org/10.1080/10408390701279749>.
- Martínez, E., Maresma, A., Biau, A., Cela, S., Berenguer, P., Santiveri, F., Michelena, A., Lloveras, J., 2017. Long-term effects of mineral nitrogen fertilizer on irrigated maize and soil properties. *Agron. J.* 109, 1880–1890. <https://doi.org/10.2134/AGRONJ2017.01.0020>.
- Martínez, R.D., Cirilo, A.G., Cerrudo, A., Andrade, F.H., Reinoso, L., Valentinuz, O.R., Balbi, C.N., Izquierdo, N.G., 2017. Changes of starch composition by postflowering environmental conditions in kernels of maize hybrids with different endosperm hardness. *Eur. J. Agron.* 86, 71–77. <https://doi.org/10.1016/j.eja.2017.04.001>.
- Möller, B., Voglhuber-Slavinsky, A., Donitz, E., Rosa, A., 2019. 50 trends influencing Europe's food sector by 2035 - The FOX project. Fraunhofer Inst.
- Munkholm, L.J., Heck, R.J., Deen, B., 2013. Long-term rotation and tillage effects on soil structure and crop yield. *Soil Tillage Res.* 127, 85–91. <https://doi.org/10.1016/j.still.2012.02.007>.
- Nendel, C., Reckling, M., Debaeke, P., Schulz, S., Mohnicke, M.B., Constant, J., Fronzek, S., Hoffmann, M., Jakšić, S., Kersebaum, C., Kopyra, A.K., Raynal, H., Schoving, C., Stella, T., Battisti, R., 2022. Future area expansion outweighs increasing drought risk for soybean in Europe. *Glob. Chang. Biol.* 00, 1–19. <https://doi.org/10.1111/gcb.16562>.
- Notz, I., Topp, C.F.E., Schuler, J., Alves, S., Gallardo, L.A., Dauber, J., Haase, T., Hargreaves, P.R., Hennessy, M., Iantcheva, A., Jeanneret, P., Kay, S., Recknagel, J., Rittler, L., Vasiljević, M., Watson, C.A., Reckling, M., 2023. Transition to legume-supported farming in Europe through redesigning cropping systems. *Agron. Sustain. Dev.* 43. <https://doi.org/10.1007/s13593-022-00861-w>.
- Omay, A.B., Rice, C.W., Maddux, L.D., Gordon, W.B., 1998. Corn Yield and Nitrogen Uptake in Monoculture and in Rotation with Soybean. *Soil Sci. Soc. Am. J.* 62, 1596–1603. <https://doi.org/10.2136/sssaj1998.03615995006200060017x>.
- Pareja-Sánchez, E., Plaza-Bonilla, D., Ramos, M.C., Lampurlanés, J., Álvaro-Fuentes, J., Cantero-Martínez, C., 2017. Long-term no-till as a means to maintain soil surface structure in an agroecosystem transformed into irrigation. *Soil Tillage Res.* 174, 221–230. <https://doi.org/10.1016/j.still.2017.07.012>.
- Peoples, M.B., Brockwell, J., Herridge, D.F., Rochester, I.J., Alves, B.J.R., Urquiaga, S., Boddey, R.M., Dakra, F.D., Bhattarai, S., Maskey, S.L., Sampet, C., Rerkasem, B., Khan, D.F., Hauggaard-Nielsen, H., Jensen, E.S., 2009. The contributions of nitrogen-fixing crop legumes to the productivity of agricultural systems. *Symbiosis* 48, 1–17. <https://doi.org/10.1007/BF03179980>.
- Peyrard, C., Mary, B., Perrin, P., Véricel, G., Gréhan, E., Justes, E., Léonard, J., 2016. N2O emissions of low input cropping systems as affected by legume and cover crops use. *Agric. Ecosyst. Environ.* 224, 145–156. <https://doi.org/10.1016/j.agee.2016.03.028>.
- Pikul, J.L., Hammack, L., Riedell, W.E., 2005. Corn yield, nitrogen use, and corn rootworm infestation of rotations in the northern corn belt. *Agron. J.* 97, 854–863. <https://doi.org/10.2134/agronj2004.0263>.
- Plaza-Bonilla, D., Nolot, J.M., Passot, S., Raffailac, D., Justes, E., 2016. Grain legume-based rotations managed under conventional tillage need cover crops to mitigate soil organic matter losses. *Soil Tillage Res.* 156, 33–43. <https://doi.org/10.1016/j.still.2015.09.021>.
- Plaza-Bonilla, D., Nolot, J.M., Raffailac, D., Justes, E., 2017. Innovative cropping systems to reduce N inputs and maintain wheat yields by inserting grain legumes and cover crops in southwestern France. *Eur. J. Agron.* 82, 331–341. <https://doi.org/10.1016/j.eja.2016.05.010>.
- Poeplau, C., Don, A., 2015. Carbon sequestration in agricultural soils via cultivation of cover crops - a meta-analysis. *Agric. Ecosyst. Environ.* 200, 33–41. <https://doi.org/10.1016/j.agee.2014.10.024>.
- Preissel, S., Reckling, M., Schläpke, N., Zander, P., 2015. Magnitude and farm-economic value of grain legume pre-crop benefits in Europe: a review. *F. Crop. Res.* 175, 64–79. <https://doi.org/10.1016/j.fcr.2015.01.012>.
- Preissel, S., Reckling, M., Bachinger, J., Zander, P., 2017. Introducing legumes into European cropping systems: farm-level economic effects. *Legumes Crop. Syst.* 280.
- Rathke, G.W., Wienhold, B.J., Wilhelm, W.W., Diepenbrock, W., 2007. Tillage and rotation effect on corn-soybean energy balances in eastern Nebraska. *Soil Tillage Res.* 97, 60–70. <https://doi.org/10.1016/j.still.2007.08.008>.
- Reckling, M., Bergkvist, G., Watson, C.A., Stoddard, F.L., Zander, P.M., Walker, R.L., Pristeri, A., Toncea, I., Bachinger, J., 2016a. Trade-offs between economic and environmental impacts of introducing legumes into cropping systems. *Front. Plant Sci.* 7, 1–15. <https://doi.org/10.3389/fpls.2016.00669>.
- Reckling, M., Hecker, J.M., Bergkvist, G., Watson, C.A., Zander, P., Schläpke, N., Stoddard, F.L., Eory, V., Topp, C.F.E., Maire, J., Bachinger, J., 2016b. A cropping system assessment framework—Evaluating effects of introducing legumes into crop rotations. *Eur. J. Agron.* 76, 186–197. <https://doi.org/10.1016/j.eja.2015.11.005>.
- Ribas, G.G., Zanon, A.J., Streck, N.A., Pilecco, I.B., de Souza, P.M., Heinemann, A.B., Grassini, P., 2021. Assessing yield and economic impact of introducing soybean to the lowland rice system in southern Brazil. *Agric. Syst.* 188, 103036 <https://doi.org/10.1016/j.agsy.2020.103036>.
- Salama, H.S.A., Nawar, A.I., Khalil, H.E., Shaalan, A.M., 2021. Improvement of maize productivity and N use efficiency in a No-tillage irrigated farming system: effect of cropping sequence and fertilization management. *Plants* 2021 Vol. 10, 1459. <https://doi.org/10.3390/PLANTS10071459>.
- SAS Institute Inc., 2019. JMP®, Version 15 Pro, SAS Institute Inc, 2019., Cary, NC, 1989–2021.
- Saulic, M., Oveisi, M., Djalovic, I., Bozic, D., Pishyar, A., Savić, A., Prasad, P.V., Vrbničanin, S., 2022. How Do Long Term Crop Rotations Influence Weed Populations: Exploring the Impacts of More than 50 Years of Crop Management in Serbia. *Agron* 2022 Vol. 12, 1772. <https://doi.org/10.3390/AGRONOMY12081772>.
- Seifert, C.A., Lobell, D.B., 2015. Response of double cropping suitability to climate change in the United States. *Environ. Res. Lett.* 10. <https://doi.org/10.1088/1748-9326/10/2/024002>.
- Shrestha, R.K., Richer, E., Clevenger, W.B., Davis, M., Lindsey, L.E., 2021. Effect of mono-, relay-, and double-crop systems on yield and profitability. *Agron. J.* 113, 1747–1757. <https://doi.org/10.1002/agj2.20598>.
- Soil Survey Staff, 2014. Keys to soil taxonomy. *Soil Conserv. Serv.* 12, 410. <https://doi.org/10.1109/TIP.2005.854494>.
- Sisquella, M., Lloveras, J., Álvaro-Fuentes, J., Santiveri, F., Cantero-Martínez, C., 2004. Técnicas de cultivo para la producción de maíz, trigo y alfalfa en regadíos del valle del Ebro.

- Villar-Mir, J.M., Villar-Mir, P., Stockle, C.O., Ferrer, F., Aran, M., 2002. On-farm monitoring of soil nitrate-nitrogen in irrigated cornfields in the Ebro Valley (northeast Spain). *Agron. J.* 94, 373–380. <https://doi.org/10.2134/agronj2002.0373>.
- Watson, C.A., Reckling, M., Preissel, S., Bachinger, J., Bergkvist, G., Kuhlman, T., Lindstrom, K., Nemecek, T., Topf, C.F.E., Vanhatalo, A., Zander, P., Murphy-Bokern, D., Stoddard, F.L., 2017. In: Sparks, D. (Ed.), *Grain Legume Production and Use in European Agricultural Systems*. *Advances in Agronomy*, *Advances in Agronomy*, pp. 235–303. <https://doi.org/10.1016/bs.agron.2017.03.003>.
- Zander, P., Amjath-Babu, T.S., Preissel, S., Reckling, M., Bues, A., Schläpke, N., Kuhlman, T., Bachinger, J., Uthes, S., Stoddard, F., Murphy-Bokern, D., Watson, C., 2016. Grain legume decline and potential recovery in European agriculture: a review. *Agron. Sustain. Dev.* 36. <https://doi.org/10.1007/s13593-016-0365-y>.