Research Article

Anne Karine Boulet*, Carlos Alarcão, Carla Ferreira, Zahra Kalantari, Adelcia Veiga, Lara Campos, António Ferreira, Rudi Hessel

Agro-ecological services delivered by legume cover crops grown in succession with grain corn crops in the Mediterranean region

<https://doi.org/10.1515/opag-2021-0041>

received October 29, 2020; accepted July 21, 2021

Abstract: Grain corn is the main cereal produced in Portugal. It is grown in intensive monoculture cropping systems that may have negative effects on soil quality, affecting long-term fertility and productivity, and, therefore, the sustainability of the production. A promising management practice to mitigate soil degradation is to grow winter cover crops used as green manure. This study examined the effectiveness of six legume cover crops (LCCs) species in providing agro-ecological services for grain corn systems in the Mediterranean region, specifically in terms of nutrient leaching, nutrient recycling, weed control, and soil fertility. The study was performed in Central Portugal during 2 years, and it assessed legumes/weeds dry biomass yield, their nutrients content, and soil organic matter evolution. Results show that, in general, LCC are well adapted to Mediterranean conditions,

yielding large amounts of biomass (up to 8 ton/ha for some clover species). In terms of nutrient leaching mitigation, the overall N–P–K nutrient uptake was 176–20–172 kg/ha. Green manure services enabled a reduction of 35% of N, 50% of P, and 100% of mineral fertilizers for a grain corn production of 12 ton/ha. Weed control by LCC was effective only in the second year of the study and for three clover species (crimson, balansa, and arrowleaf) due to their early establishment and/or high biomass production competing with weeds. Soil fertility was not improved in the short term, LCC incorporation into the soil to a slight depletion of the soil organic matter content.

Keywords: cover crop, grain corn, nutrient supply, leaching, weed control, Mediterranean region

1 Introduction

In recent decades, grain corn (*Zea mays*) has become the main agricultural crop cultivated globally, with more than 1 billion tons produced annually [1]. In Portugal, grain corn is the major cereal grown, representing 56% of total cereal yield, followed by wheat (19%) and rice (16%) [2]. Portugal has extremely favorable edapho-climatic conditions for corn cropping, with its irrigated corn fields being among the most productive in the world [3]. However, intensive cropping systems, in particular corn monoculture, can have negative effects on soil quality, affecting long-term fertility and productivity, and, therefore, the sustainability of agriculture. In order to improve the sustainability of cropping systems, different management practices have been implemented and investigated. One of the most promising practices suggested to prevent soil degradation is growing cover crops [4–6]. Cover crops are a specific form of mixed cropping in which a secondary crop is planted and grown after the main crop is harvested [5].

Cover crops provide a large number of benefits and vital agro-ecological services [6]. They can protect the

* **Corresponding author: Anne Karine Boulet**, CERNAS, Coimbra Agriculture School, Polytechnic Institute of Coimbra, Bencanta, 3045-601 Coimbra, Portugal, e-mail: anne.karine@esac.pt

Carlos Alarcão: Regional Directorate of Agriculture and Fisheries of the Central Region (DRAPC) – Baixo Mondego Experimental Center, Quinta de Na Sra do Loreto, 3020-201 Coimbra, Portugal

Carla Ferreira: CERNAS, Coimbra Agriculture School, Polytechnic Institute of Coimbra, Bencanta, 3045-601 Coimbra, Portugal; Department of Physical Geography, Stockholm University, Bolin Centre for Climate Research, SE-10691 Stockholm, Sweden;

Navarino Environmental Observatory, 24001 Messinia, Greece

Zahra Kalantari: Department of Physical Geography, Stockholm University, Bolin Centre for Climate Research, SE-10691 Stockholm, Sweden; Navarino Environmental Observatory, 24001 Messinia, Greece; School of Architecture and the Built Environment, KTH Royal Institute of Technology, Stockholm, Sweden

Adelcia Veiga, Lara Campos, António Ferreira: CERNAS, Coimbra Agriculture School, Polytechnic Institute of Coimbra, Bencanta, 3045-601 Coimbra, Portugal

Rudi Hessel: Wageningen Environmental Research, Subdivision Soil, Water and Land Use, PO Box 47, 6700AA Wageningen, The Netherlands

soil in periods between crop seasons or the soil surface in perennial crops, reducing runoff and soil erosion [5]. They also reduce the risk of nutrient leaching during the rainy period and avoid groundwater pollution, by taking up and immobilizing water-soluble nutrients in their roots and aerial parts [7]. Cover crops can be used as green manure, recycling nutrients, providing a gradual release of nutrients to the subsequent main crop, and reducing the need for mineral fertilizers [8,9]. Cover crops can produce large amounts of biomass, thus improving carbon sequestration and soil organic matter (OM) content [10]. They can also compete directly with weeds and limit their growth during the fallow period and, as a consequence, reduce seedbank deposits [11–14]. When flowering cover crops are included in the cropping system, they can also attract more beneficial insects and reduce plant diseases [5]. In addition, cover crops can improve water retention [15] and have a positive impact on the microbial community relevant for nutrient cycling [16].

The success of cover crops in providing agro-ecological services depends on their development and plant density [5]. Adaptation of cover crop species to local conditions is a critical and indispensable aspect in effectively providing agro-ecological services and enabling sustainable intensification of cropping systems.

Growing corn (*Zea mays* L.) after a legume cover crop (LCC) is a common practice in many countries, including Portugal, due to the new orientations of the common agricultural policy (CAP) relative to the greening subsidies attribution conditions. The benefits and vital agro-ecological services provided by cover crops have mainly been studied and quantified in the United States, Canada, North Europe or Asia as established in the meta-analysis of Miguez and Bollero [17] and Tonitto *et al.* [18] and more recently Abdalla *et al.* [19]. Nevertheless, few publications are available for Mediterranean countries as a reduced number of scientific teams are investigating LCCs under Mediterranean climate as Gabriel and Quemada in Spain [20], Ballesta Remy and Lloveras Vilamanyà in Italy [21], or Perdigão *et al.* in Portugal [22].

In the Low Mondego Region, farmers sow winter cover crops and most commonly yellow lupin with the single purpose to be eligible for CAP subsidies and generally do not take in account the potential benefits of cover crop managed as green manure. This fact leads, in many cases, to a lack of valorization of the LCC potential, an over fertilization of the main crop, and a systematic use of pesticides as precaution, reducing the profit of the farmer and leading to important nutrients lost and potential ground water pollution. Therefore, it is needed to test, describe, and quantify the agronomic performances of promising

legumes cover crops species in order to identify the most adapted to our regional edaphoclimatic conditions. It is important to determine their effectiveness in terms of biomass production as well as their nutrient content allowing to predict their potential to mitigate nutrient leaching or improving nutrient recycling, weed control, or soil fertility (SF). It is an essential issue to provide farmers with precise tools and information allowing them to produce in a more efficient and sustainable way.

This study investigated the capacity of six species of LCCs to provide agro-ecological services in grain corn systems, to identify the most suitable species for the Mediterranean region. Specific objectives were to:

1. determine the effectiveness of the six legumes species in producing high dry biomass yields;
2. discuss the impact of different LCC on management of the crop system, including mitigation of nutrient leaching, nutrient recycling (and fertilization gains associated with nutrient recycling provided by different LCC), and weed control; and
3. identify if there are short-term impacts on SF (i.e., soil OM content) driven by different LCC.

2 Materials and methods

2.1 Study area

The study was conducted in the Lower Mondego Valley (Figure 1), an alluvial plain in Central Portugal traditionally used for maize and rice cultivation [23]. The climate at the study site is Mediterranean, characterized by rainy winters and dry summers. The mean annual temperature is 15.5°C, with smooth variations, and the mean annual precipitation is 1,016 mm, occurring essentially between October and March [24]. The soils are modern alluvial soils, with texture ranging from silt-loam to sandy-clay-loam.

In the Mondego Valley, grain corn is usually sown in spring and harvested in autumn. It is typically cultivated in intensive irrigated monoculture systems, using water from the Mondego River through the irrigation infrastructure of the Lower Mondego Valley, extending over 12,300 ha. In Portugal, farms specializing in corn cultivation receive direct payments from the European Union (EU) CAP. To qualify for EU “greening” payments, they also need to meet the mandatory requirement of “crop diversification,” in this case less means <75% of area cropped with corn. Highly specialized farms that do not comply with this requirement have the option of adopting the equivalent practice of “soil cover during winter.” Use of legume cover species is one of ways

Figure 1: Maps showing the location of (left) the Mondego catchment in Central Portugal and (right) the Mondego Valley and the corn fields used in the study. Source: Atlas de Portugal, IPG.

in which farmers can qualify for CAP subsidies. Cover crops are usually sown in autumn after harvest of the corn, are cut and milled mechanically, and are buried in the soil during the spring of the following year.

This study was performed at the Baixo Mondego Experimental Center, an agricultural station managed by the Regional Directorate of Agriculture and Fisheries of the Central Region of Portugal. The experimental site includes 10 ha devoted to arable research since 1981. Various crop varieties and farm management practices have been investigated at the station over the years.

Soil presented a loamy sand texture (79% of sand, 14% of silt, and 7% of clay), the medium values of fertility classes obtained in spring 2020 are presented in Table 1.

Figure 2 presents the monthly total rainfall amount (mm) and monthly average temperature ($^{\circ}\text{C}$) for the two study periods (2018/2019 and 2019/2020) compared with a reference period of 1961–1990.

The average temperatures recorded during the two growing periods (from December to May) were slightly higher than the normal average temperature distribution for the reference 30 year period (1961–1990), about $+0.4^{\circ}\text{C}$ for 2018/2019 and $+1.2^{\circ}\text{C}$ for 2019/2020.

More specifically, the first growing period (2018–2019) presented a colder January about -1.2°C , lower than the

reference average and a warmer May $+1.6^{\circ}\text{C}$. The second growing period (2019/2020) presented February and May temperature much higher than the reference, that is, 2.1 and 2.5°C , respectively.

In terms of precipitation, both growing periods showed lower rainfall amounts than the reference, with an important decrease in rainfall amount over the period from December to May of 40% for 2018/2019 and 17% for 2019/2020 compared to the reference period 1961–1990.

Despite the overall dry periods, the second year (2019/2020) of study suffered 2 months (November and December) of intense rainfall events with monthly rainfall amount two times superior to the reference (240 mm instead of 120 mm), leading to a punctual pounding of the study area. In particular, the 19–20 of December 2019, a rainfall event totalizing 110 mm of precipitation, led to the immersion of a large part the trial field during 36 h.

2.2 Experimental design

In autumn 2018, a study was initiated to investigate the behavior of six species of LCCs: forage pea (*Pisum sativum* L.), yellow lupin (*Lupinus luteus*), crimson clover (*Trifolium*

Table 1: Average SF parameters at the study site

	pH	Total	Available		Exchangeable cations			
		N (mg/kg)	P ₂ O ₅ (mg/kg)	K ₂ O (mg/kg)	K ⁺ (cmol/100 g)	Na ⁺ (cmol/100 g)	Ca ²⁺ (cmol/100 g)	Mg ²⁺ (cmol/100 g)
Value	6	1,100	210	170	0.40	0.05	6.00	0.60
Fertility classes	Few acid	High	Very high	High	Medium	Very low	Medium	Low

Figure 2: Monthly total rainfall amount (mm) and monthly average temperature (°C) for the two study periods (2018/2019 and 2019/2020) and the reference period (1961–1990).

incarnatum), balansa clover (*Trifolium michelianum*), Persian clover (*Trifolium suaveolens*), and arrowleaf clover (*Trifolium vesiculosum*). These were selected because they are among the species qualifying for greening through CAP. The experimental design comprised seven plots (Figure 3), one per legume species, and one control plot left in fallow with natural vegetation that included hop clover (*Medicago lupulina*), crown daisy (*Chrysanthemum coronarium*), Roman chamomile (*Chamaemelum mixtum*), corn spurry (*Spergula arvensis*), wild radish (*Raphanus raphanistrum*), sorrel (*Rumex induratus*), and opium poppy (*Papaver somniferum*).

Each plot occupied an area of 750 m² (10 m × 75 m) and was divided into three subplots of 25 m × 25 m (Z1, Z2, and Z3), in order to provide three replicates for each experiment (Section 2.3). The cover crops were sown as winter crops and used as green manure for grain corn production in the following spring. In autumn 2019, the cover crops were sown again in the same plots. Both the 2018/2019 and 2019/2020 seasons are analyzed in this study (Figure 3).

2.3 Management of the cover crops during the study period

The legume species were sown as winter cover crops in autumn following the harvest of the main crop, grain corn (cv. Food and Agriculture Organization of the United Nations

[FAO] 300) sown in spring. All six LCC were cut at full flowering stage, as typically done by farmers due to optimal C/N ratio in biomass at that time and avoiding seeds production. During the first year of the study, due to technical limitations the cut was performed on the same date for all LCC species. In the second year, the cut was performed on different dates according to stage of maturity of the species.

Soil preparation for LCC seeding included two passes with a disk harrow to break up and incorporate corn

Figure 3: Experimental design of the field trial, including seven plots and three subplots per plot.

residues into the soil, followed by seedbed preparation with a rotary hoe. Legume seeds were manually broadcast. Seed density and other information on agronomics parameters are summarized in Table 2. No fertilizer or pesticide was applied during the LCC growing season.

Corn seeding in spring was performed mechanically after two passes with a disk harrow and soil surface leveling with a rotary hoe. Mineral fertilizer was applied during corn seeding and after 4 weeks. The fertilizer applied comprised N–P–K at 110–24–24 in the first year and 110–0–0 in the second year. Fertilization differences were based on local soil properties assessed prior to each corn cropping season.

2.4 Data collection

2.4.1 Vegetation assessment

Vegetation samples were taken immediately before cutting the LCC, to quantify the ability of the cover crops to produce biomass and their effectiveness in weed control. Sampling was performed using the quadrat method. In each plot, 12 random quadrats of vegetation were collected per plot (0.5 m² per quadrat, four quadrats in each of the three subplots Z1, Z2, and Z3, corresponding to 6 m² per plot). Vegetation samples were taken by first cutting all the aboveground biomass. For each sample, LCC and weed plants were then sorted and weighed separately in order to determine green biomass of LCC and weeds. Dry biomass of LCC and weeds was quantified separately after drying the samples at 65°C for 72 h in an oven with forced air circulation.

The production yield of the main crop was calculated in the base of 20 linear meter of corn cob manual sampling, for each subplot Z1, Z2, and Z3 at each plot. Corn was threshed and weighted, grain moisture was determined, and corn yield results were converted to final dry yield for a standard of 14% moist.

Compound subsamples of about 20 g of each legume species and weeds were homogenized by grinding in a stainless steel mill and sieving through a 0.5 mm mesh and used for chemical quantification of nutrient concentrations, including total N, P, and K. N content was quantified after extraction by digestion with sulfuric acid and subsequent distillation and titration according to the Kjeldahl method. Samples for P and K determination were prepared by incineration at 550°C overnight, extraction, and redissolution with hydrochloric acid. P concentration was determined by molecular absorption

Table 2: Agronomic management practices applied within the seven plots with six different cover crop species and the control

Code	Common name	Latin name	Seeding rate (kg/ha)	Years 1–2	Sowing date Years 1–2	Cutting date Years 1–2	Growing days Years 1–2
FP	Forage pea	<i>Pisum sativum</i>	60–60		11/12/2018–03/12/2019	18/04/2019–02/04/2020	128–121
CC	Crimson clover	<i>Trifolium incarnatum</i>	30–30		11/12/2018–03/12/2019	18/04/2019–23/04/2020	128–142
YL	Yellow lupin	<i>Lupinus luteus</i>	60–60		11/12/2018–03/12/2019	18/04/2019–02/04/2020	128–121
BC	Balansa clover	<i>Trifolium michelianum</i>	20–30		11/12/2018–03/12/2019	18/04/2019–23/04/2020	128–142
PC	Persian clover	<i>Trifolium suaveolens</i>	**–25		**/**/****–03/12/2019	**/**/****–07/05/2020	***–156
AC	Arrowleaf clover	<i>Trifolium vesiculosum</i>	20–35		11/12/2018–03/12/2019	18/04/2019–07/05/2020	128–156
NV	Natural vegetation	***	**–**		**/**/****–**/**/****	18/04/2019–02/04/2020	128–121

*, No data available.

spectrophotometry, after reaction with vanadate–molybdate reagent, and K concentration determination by flame atomic absorption spectrophotometry.

2.4.2 Soil properties

Soil samples were collected at 0–30 cm depth on two occasions in both the 2018/2019 and 2019/2020 campaigns: before seeding the LCC (Table 2) and after cutting the LCC but before incorporating the cut biomass into the soil. Within each plot, three soil samples were taken in each of subplots Z1, Z2, and Z3 to assess overall SF. All soil samples were prepared by drying at 65°C and sieving through a 2 mm mesh and then analyzed for chemical properties. Soil carbon content was determined by oxidation at 600°C and quantified using an infrared analyzer (SC-144DR-Sulfur/Carbon Analyzer, LECO Europe B.V., Geleen, The Netherlands) and converted to soil OM content using a conversion factor of 1.72. Total nitrogen (TN) was determined using the Kjeldahl method, which quantifies the organic fraction and some inorganic (NH_3 and NH_4^+) nitrogenous compounds. Plant-available soil P (P_2O_5) and soil K (K_2O) were quantified after extraction, using the Egnér–Riehm method. Quantification of P_2O_5 and K_2O was performed by molecular spectrophotometry and atomic absorption spectrometry (PerkinElmer, Inc., Waltham, MA, USA), respectively.

2.5 Data analyses

2.5.1 Soil fertility

Overall fertility coefficient was calculated for each subplot, considering the spatial variability in SF. The coefficient was computed by attributing to each fertility parameter (OM, TN, P_2O_5 , and K_2O) determined for each subplot a value of 1, 2, or 3, corresponding to low, medium, and high

fertility level, respectively (Table 3). The values for the four parameters were combined to give an overall fertility coefficient for each subplot (min–max range 4–12), which was used for statistical data analyses. Overall fertility coefficient was also calculated for soil samples collected during both spring seasons.

To consider the spatial variability of the SF in the statistical treatment of the soil OM content temporal pattern evolution for each sampling date (five campaigns), the data set has been separated into two series. The first of the two series included the values of soil OM from the two subplots Z1 and Z2 from the six LCC plots ($n = 12$) and the second of the two series included the values of the subplot Z3 of the six LCC ($n = 6$). Control plot's data were also separated in two series Z1–Z2 ($n = 2$) and Z3 ($n = 1$), but due to the reduced number of samples, no statistical treatment has been performed.

2.5.2 Nutrient uptake

Dry biomass per hectare of LCC and of weeds was calculated for each plot (legume species), and for each subplot (Z1, Z2, and Z3), based on average values of the vegetation, samples were taken within the four quadrats per subplot.

The ability of each LCC and of weeds to take up nutrients from the soil was estimated by multiplying the average dry biomass (kg/ha) obtained for each plot by the associated nutrient composition (NPK) determined in chemical analyses in the laboratory.

2.5.3 Nutrient release

The FAO 300 grain corn cultivar grown in the study site has a short cycle, with vegetative development from 96 to 105 days. Assuming expected yield of the corn crop of about 12 ton/ha, based on typical local yields, and considering the nutrient composition of this corn variety, expected nutrient uptake by the corn during the growing period was 280–50–245 kg NPK per hectare [25]. However, in the fertilization guidelines [26], the recommended NPK dose for this corn cultivar, based on local SF status assessed through analyses and expected yield of 12 ton/ha, is 240–50–50 kg NPK per hectare.

The fertilizer effect provided by the green manure (LCC + weeds) to the corn crop was calculated by applying a coefficient of degradation to each macroelement (N, P, K) present in the green manure to give the quantity of nutrients effectively available for corn uptake and not only present in the soil. The coefficients used for N, P, and K

Table 3: SF classes used to calculate overall SF coefficient for each subplot prior to corn seeding

	1 – Low	2 – Medium	3 – High
OM (%)	OM < 1.5	1.5 < OM < 1.8	OM > 1.8
Total N (mg/kg)	N < 900	900 < N < 1,050	N > 1,050
Available P_2O_5 (mg/kg)	P_2O_5 < 150	150 < P_2O_5 < 200	P_2O_5 > 200
Available K_2O (mg/kg)	K_2O < 150	150 < K_2O < 200	K_2O > 200

Table 4: SF coefficient within each plot and subplot for each soil chemical parameter analyzed

	OM			Total N			Available K ₂ O			Available P ₂ O ₅			Fertility Coeff.		
	Z1	Z2	Z3	Z1	Z2	Z3	Z1	Z2	Z3	Z1	Z2	Z3	Z1	Z2	Z3
Forage pea	3	3	2	3	3	2	3	3	1	2	2	1	11	11	6
Crimson clover	3	3	2	3	3	3	3	3	1	2	2	2	11	11	8
Yellow lupin	3	3	2	3	3	2	3	3	1	3	3	3	12	12	8
Balansa clover	3	3	1	3	3	2	3	2	1	2	2	1	11	10	5
Persian clover	3	3	1	3	3	1	3	2	1	2	2	1	11	10	4
Arrowleaf clover	3	3	1	3	3	1	2	1	1	2	2	1	10	9	4
Natural vegetation	3	3	1	3	3	2	2	1	2	2	2	1	10	9	6

1, Low; 2, medium; 3, high fertility and overall SF coefficient (sum of individual coefficients).

were 0.5, 0.6, and 1.0, respectively [27–29]. In order to allow the estimation of the potential reduction of mineral fertilizer represented by the incorporation the LCC, the P and K total were converted in P₂O₅ and K₂O using factors of 2.29 and 1.20, respectively.

2.5.4 Weed control

Pearson's correlation coefficient was used to investigate the statistical relationship between cover crop biomass, weed biomass (WB), weed cover percentage, and SF. It was only applied to the 2019/2020 dataset as during the 2018/2019 crop season, the weed control capacity of the LCC was not observable.

3 Results

3.1 Assessment of SF

The SF coefficient values used to assess SF within each subplot and plot are presented in Table 4. SF was not homogenous across the plots and subplots. The forage pea, crimson clover, and yellow lupin plots showed higher fertility than the other plots, with overall fertility coefficient of 11–12 for subplots Z1 and Z2, and 6–8 for subplot Z3. The other plots had lower values, ranging from 9 to 11 for Z1 and Z2, and from 4 to 5 for Z3. SF coefficient differed between subplots within the same plot, with Z3 showing systematically lower fertility (4–8) than Z1 and Z2 (9–12).

The high spatial variability of the SF in the trial field can be explained, on one hand, by heterogeneous cultural precedents leading to different nutrients quantities

remaining in the soil, and, on the other hand, by a slightly sandier soil texture for the subplots Z3 increasing the nutrient leaching susceptibility, as a closer position relatively to the water line increasing the flooding risk and nutrient losses and also the presence of high trees near the water line originated some shadow in the Z3 area at the end of the day.

3.2 Production of cover crop biomass and main crop yield

Overall LCC biomass production varied widely in function of the legume species, SF (Z1, Z2, Z3), and year (Figure 4; Table 5). In terms of overall biomass production, clover species produced the higher yields of biomass attaining maximum production for one-third of the subplots superior to 8 ton/ha for arrowleaf and balansa clover, with a maximum biomass of almost 10 ton/ha for balansa clover. Crimson and Persian clovers presented biomass production slightly lower varying between 4 and 6 ton/ha. Yellow lupin and forage pea showed lower biomass production between 2–5 and 2–4 ton/ha, respectively.

Biomass production varied also in function of the year. The second year revealed a clear increase in biomass production for three species of clover but particularly for crimson clover that passed from 3 ton/ha the first year to almost 6 ton/ha the second year. This clover species showed lower overall biomass production than the other clover species at the first year. In contrast, yellow lupin and forage pea showed a smooth decrease in biomass production from the first to the second year.

Biomass production varied in function of SF. For the subplots, Z3 presented fertility coefficient equal or inferior to 5, the biomass production decrease widely. Balansa and arrowleaf clovers showed around 50% lower productivity in

Figure 4: Dry biomass production for the six LCCs plots in the first year (2018/2019) and the second year (2019/2020).

areas with low SF (subplot Z3). Persian clover productivity was less affected by SF decrease. Forage pea, yellow lupin, and crimson clover grown in subplots with only medium and high fertility (fertility coefficient superior or equal to 6) did not show relevant biomass differences across the subplots.

Main crop yields are presented in Figure 5. Grain corn yields are comprised between 10 and 11 ton/ha for the two campaigns, with larger variations in corn yield between plots the second year. In the second year of the study, a significant decrease in the corn yield for the plot in fallow that only attained 6 ton/ha was noted.

3.3 Nutrient uptake and immobilization by LCC

The first year of study showed globally a more homogeneous nutrient uptake (legumes + weeds) between species

than the second year, with minimum and maximum N uptake of 140 and 210 kg/ha in the first year and 66 and 239 kg/ha in the second year; P uptake of 19 and 25 kg/ha in the first year and 7 and 29 kg/ha in the second year; and K uptake of 147 and 241 kg/ha in the first year and 47 and 208 kg/ha in the second year (Table 6).

The overall median N–P–K nutrient uptake over the two study years considering all the species was 176–20–172 kg/ha with an interquartile range of 58–5–56 kg/ha. Compared with the N–P–K uptake by weeds in plots in fallow with natural vegetation (45–8–60 kg/ha), the nutrient uptake was around threefold higher in the LCC plots, corresponding to additional NPK nutrient uptake by plant biomass (legume crops and weeds) of 118–14–127 kg NPK/ha.

Plots with clover species presented globally the higher nutrient uptake (legumes + weeds) at the second year of the study with maximum NPK uptake values for balansa clover of about 240–30–210, followed by Persian clover 220–25–200, and arrowleaf clover 200–20–195.

Even if the legume biomass (LB) of Persian clover plots was lower than that of arrowleaf clover plots, a higher biomass of weeds in the plots with Persian clover leading to these plots globally presented a higher uptake of nutrients.

Forage pea and yellow lupin global NPK uptake (legumes + weeds), at the first year of the study, was quite similar and presented good performance about 180–20–150, the weaker production of forage pea (legumes) relatively to yellow lupin being compensated by a higher amount of weeds. These performances reduced during the second year of the study with NPK uptake almost two times lower.

Globally the nutrient uptake (legumes) by LCC alone without weeds showed higher variation than uptake by

Table 5: Dry biomass production in different subplots (Z1, Z2, and Z3) by the six LCCs in year 1 (2018/2019) and year 2 (2019/2020)

	LCC dry biomass (ton/ha)					
	Year 1			Year 2		
	Z1	Z2	Z3	Z1	Z2	Z3
Forage pea	4.2	3.3	4.5	3.3	2.0	1.5
Crimson clover	4.2	2.9	3.5	6.3	7.1	6.4
Yellow lupin	4.9	4.2	2.9	3.9	2.3	3.3
Balansa clover	8.4	3.6	1.7	7.5	9.8	3.9
Persian clover		6.4		6.4		4.5
Arrowleaf clover	7.5	6.2	2.7	8.9	9.3	3.1

Figure 5: Average corn yield for each plot for the two study years.

the overall biomass production (legumes + weeds). The median NPK content was lower (131–11–104 kg/ha), whereas the interquartile range was higher (78–7–99 kg/ha).

These results show that in general, lower nutrient uptake of legumes was compensated for by higher nutrient uptake by weeds (Figure 6).

3.4 Nutrients available for the main crop (grain corn)

Figure 7 presents the amount of nutrients provided by (1) the legume incorporation in the soil, (2) the weed incorporation, and (3) the complementary mineral fertilizer amount necessary to cover the NPK needs of the grain corn for each legume species. Considering an expected

corn grain yield of 12 ton/ha cultivated with good fertility conditions, the NPK amendment need of the corn was estimated as 240–50–50 kg/ha.

In average for all the species and the years, the legume green manure contribution (legumes + weeds) after the application of mineralization rates, equaled to about 85–25–180 kg of N/P₂O₅/K₂O mineral fertilizer, with clovers species presented the highest values.

The LCC (legumes + weeds) were able to supply 35% of N, 50% of P₂O₅, and 100% of K₂O of the required amendment. In the fallow (control) plot, the natural vegetation provided much less nutrients to the corn, about 10% of N, 20% of P₂O₅, and 100% of K₂O.

Larger variations in nutrient supply also exist between species at the second year of the study. The nutrient supply between the first and the second year varied from 27 to 40% the first year and from 13 to 46% the second year

Table 6: Median uptake of N, P, and K in the six LCC plots and in the control plot (fallow with natural vegetation) during year 1 (2018/2019) and year 2 (2019/2020)

	Total N (kg/ha)						Total P (kg/ha)						Total K (kg/ha)					
	Year 1			Year 2			Year 1			Year 2			Year 1			Year 2		
	L	W	L + W	L	W	L + W	L	W	L + W	L	W	L + W	L	W	L + W	L	W	L + W
Forage pea	96	80	176	44	21	66	9	13	22	3	4	7	45	111	156	18	30	47
Red clover	82	59	140	145	5	151	10	10	20	16	1	17	91	81	172	151	7	159
Yellow lupin	131	52	183	87	32	119	11	9	19	7	6	13	75	72	147	52	45	96
Balansa clover	91	66	157	228	11	239	10	11	21	27	2	29	104	91	195	194	15	208
Persian clover				172	50	222				17	9	26				130	69	199
Arrowleaf clover	161	49	210	196	0	196	17	8	25	20	0	20	173	68	241	194	0	194
Natural vegetation		52	52		39	39		9	9		8	8		72	72		46	46

L, Legumes; W, weeds; L + W, legumes and weeds.

Figure 6: Statistical parameters (minimum, quartile 1, quartile 2 [median], quartile 3, maximum, and interquartile range [IQR]) of the biomass results for the six LCC over the 2 years of the study.

for N, and from 25 to 46% the first year and from 20 to 79% the second year for P_2O_5 , around 100% for K_2O .

During the first year, a relevant proportion of the nutrients incorporated into the soil through green manuring was provided by weeds. During the second year, the LB yield was higher, especially for the clover species, which led to an increase in the relative contribution of nutrients by the legumes and a decrease in the contribution from the weeds in absolute and relative terms.

3.5 Weed control capacity

In terms of weed control performance, the results in the first year did not show any greater efficiency of LCC in decreasing the WB compared with the control biomass (natural vegetation; Table 7; Figure 8). The LCC species did not seem to influence weed emergence and

development, with WB in LCC plots being similar to those in the control lot (1–3 ton/ha of dry biomass).

However, in the second year of the study, there was a clear reduction in WB in the LCC plots for three of the four clover species (crimson, balansa, and arrowleaf). In these plots, the WB decreased from 2–3 ton/ha in the first year to <1 ton/ha in the second year (Figure 8). In general, the clover species (except Persian clover) showed higher efficiency in controlling weed emergence during the winter than forage pea and yellow lupin, particularly in the second year of the study. The crimson, balansa, and arrowleaf clovers kept WB below 0.5 ton/ha in six of the nine subplots, whereas WB reached 3–4 ton/ha in the control plot. This indicates that overall weed infestation in the crimson, balansa, and arrowleaf clover subplots was less than 10%. Forage pea, yellow lupin, and Persian clover plots had similar WB to the control plot, indicating weaker capacity for weed control by these LCC species.

Figure 7: Amount of macronutrients (N/ P_2O_5 / K_2O) provided to the main crop (grain corn) by the LCC (legumes and weeds) used as green manure and by complementary mineral fertilizer on average for the two study years.

Table 7: Dry biomass production of weeds by weight in all plots and subplots (Z1, Z2, and Z3) in year 1 (2018/2019) and year 2 (2019/2020)

	Weeds dry biomass (ton/ha)					
	Year 1			Year 2		
	Z1	Z2	Z3	Z1	Z2	Z3
Forage pea	3.4	3.7	1.8	3.6	1.2	1.0
Crimson clover	3.3	3.5	2.5	0.8	0.3	0.3
Yellow lupin	2.2	2.0	2.8	1.4	1.8	1.8
Balansa clover	0.6	2.8	2.6	0.6	0.5	1.3
Persian clover				0.7	2.6	2.9
Arrowleaf clover	0.9	2.1	2.1	0.0	0.5	1.9
Natural vegetation	2.2	3.9	0.9	3.3	2.6	1.1

Considering the overall biomass production (Table 8), there was a weak negative correlation between LB and WB (-0.63 ; $p < 0.05$) but a stronger negative correlation between LB and weed percentage (WP) (-0.91 ; $p < 0.05$). This discrepancy disappeared when the correlation was evaluated by species, which highlights the importance of legume species in controlling weeds. Arrowleaf and balansa clovers showed the highest negative correlation between LB and WB production ($>(-0.95)$; $p < 0.05$), showing that weed control is strongly related to LB production. The crimson and Persian clovers and yellow lupin showed weaker correlation values between weed and LCC biomass (-0.62 to -0.81 ; $p < 0.05$).

SF played an important role in terms of WP for the balansa, Persian, and arrowleaf clovers, as indicated by high negative correlation coefficients (-0.96 , -0.87 , -1.00 , respectively; $p < 0.05$), indicating that the efficiency of

Table 8: Pearson's correlation coefficient between LB, WB, WP, and SF (based on overall fertility coefficient)

	LB/WB	LB/WP	LB/SF	WB/SF	WP/SF
Forage pea	0.98	0.88	0.73	0.56	0.31
Crimson clover	-0.68	-0.71	0.34	0.45	0.42
Yellow lupin	-0.81	-0.99	-0.14	-0.47	0.00
Balansa clover	-0.96	-0.96	0.85	-0.97	-0.96
Persian clover	-0.62	-0.80	0.99	-0.71	-0.87
Arrowleaf clover	-0.95	-0.99	0.98	-1.00	-1.00
Total	-0.63	-0.91	0.28	0.05	-0.16

these clover species in controlling weeds decreases with decreasing SF. The other legume species did not display clear relationships between SF and LB or WP.

3.6 Spatiotemporal changes in soil OM

Over the two study years were realized five soil sampling campaigns: three campaigns in autumn (after harvesting of corn and before seeding the LCC) and two campaigns in spring before incorporation of LCC in the soil. Overall OM contents in soil measured in the study area were low and showed large temporal and spatial variations within plots ranging from 1.53 to 1.98% and with minimum and maximum in the subplots, ranging from 1.23 to 2.13% (Figure 9), with lower values consistently registered at for the subplots Z3.

The analysis of OM evolution (Figure 10) highlighted for the LCC plots a seasonal behavior of the OM content in soil for the most fertile areas (Z1–Z2), which presented

Figure 8: Dry biomass production of weeds in kg/ha per plot and per year.

higher soil OM content in spring (in median from 1.93 to 1.96%) and lower OM content in autumn (in median from 1.71 to 1.89%). The increase of the OM during the winter was between 0.07 and 0.15% and the decrease during the summer was between -0.18 and -0.22% , which leads to a general progressive decrease in the soil OM content that lost 0.18% passing from 1.89% at the beginning of the study to 1.71% after 2 years. Nevertheless, the decrease concerned more specifically the autumn measurements, the spring measurements presenting more stable OM content values (1.96 and 1.93%). At the control plot (with winter fallow) for the most fertile areas (Z1–Z2) the seasonal variability amplitude of the OM content was reduced and did not exceed $\pm 0.6\%$, with a general diminution over time also much lower about 0.09% decreasing in median from 2.03 to 1.92% after 2 years.

In relation with the LCC plots situated in the less fertile areas Z3, any seasonal behavior of the OM content could be demonstrated. In fact a regular general decrease in the OM through time existed, with a loss of 0.38% of OM that passed in median from 1.82 to 1.44% after 2 years. The control plot (with winter fallow) also presented a large decrease in the OM content about 0.40% passing from 1.73 to 1.33% but also a clear seasonality with an increase in the OM during the winter between 0.06 and 0.08% and decrease during the summer from -0.19 to -0.35 . In general, the less fertile zones Z3 showed a more important decrease in the OM content than the most fertile areas.

4 Discussion

4.1 Biomass production

The six cover crop species produced large amounts of biomass, especially the clover species (6–10 ton/ha). All the species of legumes tested were able to adapt to local conditions, leading to biomass production higher than the 4–6 ton/ha reported elsewhere [11,13,14].

All clover species produced higher dry biomass yields during the second year of the study, whereas yellow lupin and forage pea showed decreasing yield production from the first to the second year. This can be partly explained by earlier flowering of these two species during the second year, with 1 week less of growth before cutting than in the first year, whereas the clover species were cut 2–4 weeks later than in the first year, which led to considerably higher dry biomass production. Also during the second year of the project, field observations identified after the period of ponding at the end of December, the existence of seeds entrainment by the water very clear for the forage pea plot.

4.2 Impact of LCC on nutrient leaching

The results obtained in this study showed that it is possible to double or triple the nutrient uptake by vegetation

Figure 9: Differences in soil OM content for the six species of LCCs and the fallow (natural vegetation) plots during the five soil sampling campaigns conducted over the study period.

Figure 10: (top left) Statistical treatment (minimum, Q1, Q3, maximum) of OM content evolution of LCC plots for the Z1/Z2 data series ($n = 14$ for each date); (top right) median of OM content evolution of LCC plots vs control plots for Z1/Z2 data series; (bottom left) statistical treatment (minimum, Q1, Q3, maximum and median) of OM content evolution for the Z3 data series ($n = 17$ for each date); (bottom right) median of OM content evolution of LCC plots vs control plots for Z3 data series.

during the winter by sowing a LCC instead of letting the soil in fallow. Nutrient uptake by cover crops could thus mitigate possible leaching losses of nutrients such as nitrates, which are highly mobile in the soil [30].

One limitation of this study was that it did not consider the capacity of the legumes for biological N fixation. However, Voisin et al. [31] demonstrated that biological fixation of N by legumes decreases with the mineral N content of the soil and is completely inhibited at values above 380 kg N/ha. Thus, N uptake from the soil, which is less energy demanding for legume plants than biological N fixation, will be greater on soils with a high or medium mineral N content and that was the case of the trial fields.

Immobilization of nutrients by LCC is particularly relevant in the Mediterranean region, where the soils are typically exposed to intense rainfall events, creating a high risk of nutrient leaching during the winter. Soil with poor capacity to retain nutrients during intense rainfall in autumn and winter will suffer a greater leaching of nutrients, leading to loss of SF and risk of groundwater pollution. Although legumes are not as efficient catch crops as crucifers, which can capture around 60% of soil mineral N during the fallow period, they can still prevent around 35% of N losses [18,32].

In the first two years of the study, the seeding date of the LCC was delayed, and as a result of technical problems, seeding was performed in December instead of October. Thus while final LCC biomass obtained in spring was

high, these crops could not have been fully efficient relatively to nutrient leaching mitigation due to the late start of growth under low temperatures during the autumn/winter. The main LCC growing period was thus the spring and not the autumn, the critical period of intensive rainfall. Future studies are needed to test earlier seeding dates (October instead of December) and investigate the growth behavior of the six species for different seeding dates.

4.3 The role of LCC as green manure

This study demonstrated that LCC can supply an important proportion of the macronutrients required by the main crop, thus acting as a green manure and providing an agro-ecological service. Trials with clover species presented the best performances and were able to provide the first year about 40% of N amendment requirements to the subsequent grain corn crop, 60% of P_2O_5 and 100% of K_2O . The other legume species investigated (yellow lupin and forage pea) can supply 28% of N, 43% of P_2O_5 , and 100% of K_2O , whereas natural vegetation (fallow plot) provided only about 9% of the N, 24% of P_2O_5 , and 100% of the K_2O required for corn amendment.

It is important to note that at the second year of green manure incorporation, this nutrient availability will increase as one part of the nutrients that was not available in the first year after burring can be degraded in the second year and

then increase the quantity of nutrients in the soil available for the main culture the second year.

In fact, the estimation of nutrient release by LCC for the next crop is particularly complex, because it depends on many factors. The characteristics of the species used as green manure and its cutting phase influence the C/N ratio of plant biomass, which is a major driver of soil OM mineralization [33]. Climate is another important factor in mineralization of nutrients as the Mediterranean warm climate accelerates the OM decomposition rate [34]. The type and intensity of soil mobilization also influence the presence of oxygen in the soil that intensifies mineralization processes, still favor the increase of the surface contact of green manure with soil, and represent important drivers in terms of OM decomposition rates and nutrients release [35].

It is important to note that the LCCs in this study were cut at the full flowering stage, and the biomass obtained had $C/N < 20$. These low C/N ratios are known to lead to a fast decomposition of the OM when incorporated into the soil and provide a nutrient source for the main crop growing from spring to autumn. Shi [36] demonstrated that decomposition and nutrient release from LCC occur during 0–3 months after incorporation into the soil, which is in synchrony with the growing period of the grain corn crop. The synchronization of nutrient release with crop requirement is a very important issue that allows to optimize the nutrient uptake of the main crop and avoids the nutrient leaching [37].

Although the mineral NPK fertilizer amount applied to the grain corn crop (110–24–24 kg/ha in the first year, 110–0–0 kg/ha in the second year) was lower than the recommended level based on soil analysis (240–50–50 kg/ha) after deduction of the green manure contribution (85–25–180 kg/ha), the corn crop almost achieved the expected yields indicating that it happened a higher nutrient release by the LCC in the soil than theoretically estimated. The fall in corn production for the fallow plot the second year of the study compared to the LCC plots illustrated the capacity of the LCC in providing nutrients to the next crop allowing to reduce substantially the amount of mineral fertilizers.

4.4 Weed control by LCC

LCCs reduced WB only in the second year of the study. The arrowleaf, balansa, and crimson clovers showed the highest efficiency in controlling weeds in the field. Considering the overall biomass production of legumes

and weeds, arrowleaf and balansa clovers showed the highest negative correlation between LB and WB production ($>(-0.95)$; $p < 0.05$). In the case of crimson clover, Persian clover, and yellow lupin, the correlation was weaker but still significant, so weed control capacity was not as strongly related to LB production in those cases.

During the second year of the study, three clover species (arrowleaf, balansa, and crimson clovers) kept weed production under 0.5 ton/ha in the majority of the subplots, corresponding to less than 10% of the overall biomass produced within the plots, compared with 4 ton/ha in the control. The performance of legume species in controlling weeds is influenced by several parameters; however, based on field observations, crimson clover showed the most uniform emergence among the LCC species tested and established most rapidly in the field. This hampered weed emergence, despite limited production of biomass. In the case of arrowleaf and balansa clovers, plant establishment was slower and allowed some weed emergence at an initial stage. However, the high biomass production of these species (mainly >8 ton/ha) led to recumbent stems that formed a dense and thick soil cover, preventing weed emergence in later stages. These observations confirm findings by Dorn *et al.* [12] and Melander *et al.* [38], and stress the overwhelming importance of successful early stage establishment of cover crops and soil surface cover. This implies that for species with rapid initial growth, biomass production is not as important as for species with later emergence. The latter depend on the amount of biomass developed in a later phase to prevent weed emergence. Other studies have highlighted the importance of high biomass production to efficiently compete for resources with weeds [39].

SF played an important role in LCC biomass production, with lower SF resulting in lower biomass production and higher WB. This was clearly apparent in the plots sown with arrowleaf and balansa clovers. The field experiments indicated that some LCC species are more adaptable to poor soil conditions. This is extremely relevant for choice of cover crop species by farmers, depending on the SF level of their fields.

4.5 Improvement of soil OM content by LCC

The implementation of corn intensive monoculture cropping systems in the study region led in the long term to depletion of soil OM content that decreased commonly below the threshold of 2%. Soil OM content is the result

of the balance between the carbon input and the output caused by decomposition, leaching, and erosion. The increase of carbon input through the introduction of winter cover crops used as green manure to substitute the fallow should lead to improve soil OM content. Cover crops are also known to reduce the output, mobilizing the excess of N remaining in the soil at the end of the main crop cultivation and then mitigating the N leaching [40].

Nevertheless, our study highlighted a slight tendency of the soil OM content decrease after the 2 years of winter cover crop managing, which is in contradiction with the consensual meaning reported in the literature. The recent metadata analysis performed by Poeplau and Don [41] inventoried 139 observations, and only 10% of the plots presented a soil organic carbon stock depletion. The principal reason evoked to explain this soil organic carbon depletion was the addition of rapidly decomposable plant material (low C/N ratio) that leads to microbial community growth, and the availability of enough energy to break up more stable compounds of old soil organic carbon as compared to the no cover crop treatment. In our study, the legumes cover crops tested in fact presented very low C/N ratios (in general <20) leading to fast decomposition of OM in the soil that occurred principally during the summer. The warm median temperatures (above 20°C) during the summer and high soil moisture content maintained by irrigation practice still promoted faster soil OM decomposition of the biomass. At the control plot (fallow with natural vegetation) for the most fertile areas Z1–Z2, the decline of soil OM during the summer was lower than that for LCC plots. In fact, for the control plot, the amount of biomass incorporated in the soil was two to three times inferior than that for LCC plots and presented a higher C/N ration for weeds superior to 20, leading to a slower decomposition of the fresh OM.

The subplots situated in the less fertile area Z3 presented a slightly larger decrease of the soil OM. These subplots presented a sandier texture and lower clay content that could influence the OM storage as described by Hassink and Whitmore [42], soil OM accumulation is influenced by soil textural class and the ability of the soil to fix soil OM is positively correlated with the clay content of the soil.

The fast decomposition characteristic of the LCC is an advantage in terms of green manure management, and nutrient recycling militates against the SF improvement in terms of OM content. The use of legumes cover crop in the conditions of the study highlighted that it is not suitable to improve SF at least in the first two years, and led to the opposite, a decrease of soil OM after 2 years of winter cover cropping. Then if the purpose of the

farmer is to increase soil OM, the use of a mix of legumes and nonlegumes cover crop with higher C/N would be recommended.

The level of soil mobilization can also influence the evolution of soil organic content. Olson et al. [43] described that cover crops managed under different tillage systems increased soil OM content in the order: no-till > chisel plow > moldboard plow. The introduction of LCC in the cropping system led to the multiplication of soil mobilization operations. It was necessary for two more passes of chisel and a pass of rototiller before sowing the LCC in autumn and two more passes of chisel to uniformly incorporate the LCC in the soil. These extra tillage operations potentially also originated an acceleration of the OM decomposition in the soil. The practice of direct sowing would be an interesting alternative that would limit soil oxygenation and then the OM decomposition rate in the soil and also save expensive agricultural operations. Nevertheless, the installation of the culture could be difficult by the high quantity of corn straw remaining at the soil surface that will avoid one part of the seeds to be in contact with the soil. In the case of clover species, the seeds presented very small diameters that normally require a fine preparation of the seeds bed, and this specificity could be an important handicap for the adoption of direct seeding technique. Forage pea and yellow lupin presented larger seeds size and may be more adapted to that practice, even if identified as less productive species. This aspect needs to be investigated and validated.

5 Conclusion

This study demonstrated that the six legume species used as cover crops were adapted to the regional conditions and provided interesting agro-ecological services.

The six LCC species produced high amounts of biomass far above the quantities registered for most of the studies developed in colder climate as they survived to the winter and presented an important growing phase in spring before being cut.

Dry biomass production reached yields of up to 8 ton/ha for good SF conditions. Nevertheless, the variability of the results inter- and intraspecies was very high due to the influence of many parameters such as the precipitation amount and intensity (leaving potentially to soil pounding and lethality of the plants), the spatial variability of SF, or the sowing date or the cutting date.

Clover species, even if the reduced size of their seeds that turn the installation more delicate (obliging to a finer

preparation of the seeds bed) and a very slow start-up of the growing phase, presented a final biomass production much higher than forage pea or yellow lupin even if the initial growing phase of these two species was earlier and quicker. This fact led to potential best performance of the forage pea and yellow lupin (and also crimson clover that is the most precocious of the clovers) in terms of nutrient leaching mitigation during the autumn season. Nevertheless, it is important to notice that no pesticides have been used for legumes cultivation, and then at the initial growing phase, the percentage of weed infection was high, and a large part of the initial mitigation of the nutrient leaching was provided by the weed and not by the legumes. Considering the entire vegetative period, legumes and weeds allowed an important uptake of nutrients from the soil, contributing to mitigate the leaching of nutrients, but majority during spring period, and not during winter the most critical period in terms of nutrient leaching. It would be recommending then precocious sowing date in order to avail the last weeks of soft temperatures allowing a rapid installation of the legumes and an optimization of the nutrient immobilization by the legumes during the autumn and winter.

In terms of green manure services, it is important to divulgate these results and deliver simple tools to the farmers, allowing them to estimate the amount of nutrients that various species of legumes are able to provide for diverse conditions and the corresponding amount of mineral fertilizer that they could save. This study highlights for an expected grain corn yield of 12 ton/ha, grown in good SF conditions, that it is possible theoretically to reduce the amount of NPK mineral fertilizer (35, 50, and 100%) corresponding to saving 85, 25, and 180 kg/ha of N, P_2O_5 , and K_2O , respectively, on account of the nutrient recycling provided by the green manure incorporation.

During the second year of the project, a maize yield of 11 ton/ha, with a mineral fertilization NPK rate extremely low (100–0–0 kg/ha) indicated that the quantity of nutrients effectively available for the corn growth was higher than the expected following our calculations and estimations (the OM degradation velocity and rate being extremely difficult to estimate). This led to the next step of the study that should be to test various quantity of mineral fertilization in order to determine empirically the optimal rate of fertilization to maintain the level of production and limit the loss of nutrients.

The study of the effect of some environmental conditions (like effect of ponding or cold weather) cannot be planned, just be observed when happened and needs various consecutive years of study to cover a vast set of

conditions. For example, it was possible to determine during the second year of the study that presented a very wet autumn, that some species were more resistant to ponding than others, like yellow lupin or crimson clover, what is an important factor in a region where terrain is frequently immersed. The effect of the freeze should be possible to evaluate for the third year of the project in course that presented 2 weeks of negative temperatures in January.

In terms of weed control, LCC efficiency is variable in function of the study year and was only highlighted at the second year for the clover species. Three clover species (crimson, balansa, and arrowleaf clovers) performed best in terms of weed control due to early establishment and/or high biomass production in later growth stages, ensuring strong competition with weed species. In a general way, weed control capacity is strongly related to LB production. The success in weed control also depends on the early stage establishment of cover crops and soil surface cover than can compensate positively the lower biomass from some species. It is important to observe the agronomic behavior of the LCC in the region in the long term to identify distinct climatic conditions, the species with most regular potential in weed control. SF played also an important role in terms of biomass production, as in this study, lower SF resulted in lower LCC biomass production and relatively higher WB. It is important to identify the adaption of the species at the soil conditions to orient the choice of the farmers in function of the SF status of their fields.

In terms of soil organic content improvement capacity, the LCC failed completely at least during the first year of the study. That could be explained by the fast decomposition characteristics of the LCC due to their low C/N, combined with optimal weather conditions (warm and humid through the irrigation system) leading to an extremely fast mineralization of the biomass in the soil and then an important decline of OM content during the warm period. The intensification of the soil mobilizations due to the LCC cultivation could also have increased the oxygenation of the soil, leading to the acceleration of the OM decomposition in the soil, and, finally, the massive addition of biomass to the soil could have led to microbial community growth providing enough energy to mineralize more stable OM and leading to a global decrease of the soil OM content. It would be then important in the future to plan trial fields with management practices able to mitigate this negative effect on soil OM content, such as the use of mix seeds (legumes, crucifers, gramineous) in order to increase the C/N and slow the OM decomposition; direct sowing of the LCC seeds; and also the no burring of the

LCC biomass in the soil in order to limit the tillage operations and avoid the massive input of biomass in the soil.

Acknowledgments: We would like to thank the Regional Directorate of Agriculture and Fisheries of the Central Region (DRAPC) – Baixo Mondego Experimental Center, who provided trial fields and workers to carry out the study field work, as well as Vasco Abreu from the Nutriprado company who graciously provided the legume seeds.

Funding information: This research was supported by the European Union Horizon 2020 Programme for research and innovation, through the Soil Care project “Soil Care for profitable and sustainable crop production in Europe,” Grant agreement no. 677407.

Conflict of interest: The authors state no conflict of interest.

Data availability statement: The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

References

- [1] United States Department of Agriculture (USDA), Foreign Agricultural Service (FAS) grain: World markets and trade, 2020, marketing year: October 1, 2019–September 30, 2020.
- [2] Estratégia Nacional para a Promoção da Produção de Cereais (ENPPC). Grupo de Trabalho de Cereais, Gabinete de Planeamento, Políticas e Administração Geral (GPP); 2018. p. 95.
- [3] Vasconcellos-Souza L. A produção de milho em Portugal. Agronegócios Notícias Cerealicultura. Porto, Portugal: Agropress, Publindústria eds; 2015. Available from: <http://www.agronegocios.eu/noticias/a-producao-de-milho-em-portugal/>
- [4] De Baets S, Poesen J, Meersmans J, Serlet L. Cover crops and their erosion-crimsonucing effects during concentrated flow erosion. *Catena*. 2011;85(3):237–44. doi: 10.1016/j.catena.2011.01.009.
- [5] Barão L, Alaoui A, Ferreira C, Basch G, Schwilch G, Geissen V, et al. Assessment of promising agricultural management practices. *Sci Total Environ*. 2019;649:610–9. doi: 10.1016/j.scitotenv.2018.08.257.
- [6] Alaoui A, Barão L, Ferreira C, Schwilch G, Basch G, Garcia-Orenes F, et al. Visual assessment of the impact of agricultural management practices on soil quality. *Agron Soils Environ Qual*. 2020;112(4):2608–23. doi: 10.1002/agj2.20216.
- [7] Gabriel JL, Muñoz-Carpena R, Quemada M. The role of cover crops in irrigated systems: water balance, nitrate leaching and soil mineral nitrogen accumulation. *Agric Ecosyst Environ*. 2012;155:50–61. doi: 10.1016/j.agee.2012.03.021.
- [8] Thorup-Kristensen K, Dresbøll DB, Kristensen HL. Crop yield, root growth, and nutrient dynamics in a conventional and three organic cropping systems with different levels of external inputs and N re-cycling through fertility building crops. *Eur J Agron*. 2012;37(1):66–82. doi: 10.1016/j.eja.2011.11.004.
- [9] Plaza-Bonilla D, Nolot JM, Passot S, Raffaillac D, Justes E. Grain legume-based rotations managed under conventional tillage need cover crops to mitigate soil organic matter losses. *Soil Tillage Res*. 2016;156:33–43. doi: 10.1016/j.still.2015.09.021.
- [10] Raphael JPA, Calonego JC, Marcondes D, Milori BP, Rosolem CA. Soil organic matter in crop rotations under no-till. *Soil Tillage Res*. 2016;155:45–53. doi: 10.1016/j.still.2015.07.020.
- [11] Brust J, Claupein W, Gerhards R. Growth and weed suppression ability of common and new cover crops in Germany. *Crop Prot*. 2014;63:1–8. doi: 10.1016/j.cropro.2014.04.022.
- [12] Dorn B, Joss W, van der Heijden MGA. Weed suppression by cover crops: comparative on-farm experiments under integrated and organic conservation tillage. *Weed Res*. 2015;55(6):586–97. doi: 10.1111/wre.12175.
- [13] Alonso-Ayuso M, Gabriel JL, García-González I, Del Monte JP, Quemada M. Weed density and diversity in a long-term cover crop experiment background. *Crop Prot*. 2018;112:103–11. doi: 10.1016/j.cropro.2018.04.012.
- [14] Büchi L, Wendling M, Amossé C, Jeangros B, Charles R. Cover crops to secure weed control strategies in a maize crop with reduced tillage. *Field Crop Res*. 2020;247:107583. doi: 10.1016/j.fcr.2019.107583.
- [15] Hubbard RK, Strickland TC, Phatak S. Effects of cover crop systems on soil physical properties and carbon/nitrogen relationships in the coastal plain of southeastern USA. *Soil Tillage Res*. 2013;126:276–83. doi: 10.1016/j.still.2012.07.009.
- [16] Brennan EB, Acosta-Martinez V. Cover cropping frequency is the main driver of soil microbial changes during six years of organic vegetable production. *Soil Biol Biochem*. 2017;109:188–204. doi: 10.1016/j.soilbio.2017.01.014.
- [17] Miguez FE, Bollero GA. Review of corn yield response under winter cover cropping systems using meta-analytic methods. *Crop Sci*. 2005;45:2318–29.
- [18] Tonitto C, David MB, Drinkwater LE. Replacing bare fallows with cover crops in fertilizer-intensive cropping systems: a meta-analysis of crop yield and N dynamics. *Agric Ecosyst Env*. 2006;112:58–72. doi: 10.1016/j.agee.2005.07.003.
- [19] Abdalla M, Hastings A, Cheng K, Yue Q, Chadwick D, Espenberg M, et al. A critical review of the impacts of cover crops on nitrogen leaching, net greenhouse gas balance and crop productivity. *Glob Chang Biol*. 2019;25(8):2530–43. doi: 10.1111/gcb.14644.
- [20] Gabriel JL, Quemada M. Replacing bare fallow with cover crops in a maize cropping system: yield, N uptake and fertiliser fate. *Eur J Agron*. 2011;34(3):133–43. doi: 10.1016/j.eja.2010.11.006.
- [21] Ballesta Remy A, Lloveras Vilamanyà J. Nitrogen replacement value of alfalfa to corn and wheat under irrigated Mediterranean conditions. *Span J Agric Res*. 2010;8(1):159–69. doi: 10.5424/sjar/2010081-1155.
- [22] Perdigão A, Coutinho J, Moreira N. Cover crops as nitrogen source for organic farming in southwest Europe.

- Acta Hortic. 2012;933:355–61. doi: 10.17660/ActaHortic.2012.933.46.
- [23] Soares NO. Desenvolvimento da Região do Baixo Mondego a partir dos anos 60. A Cultura do Arroz. Dissertations Theses in Sociology, Coimbra, Portugal: Faculdade de Economia, Universidade de Coimbra (FEUC); 2011. p. 25.
- [24] Instituto Nacional de Meteorologia e Geofísica (INMG), 1941–2000. Anuário climatológico de Portugal. I Parte, Continente, Açores e Madeira – Observações de superfície. Lisboa.
- [25] Coelho AM, França GE. Seja o doutor do seu milho: nutrição e adubação. 2nd edn. Piracicaba: Potafos; 1995. p. 9. Available from: <http://ainfo.cnptia.embrapa.br/digital/bitstream/item/77363/1/Seja-doutor.pdf>
- [26] Instituto Nacional de Investigação Agrária e Veterinária (INIAV). Manual de Fertilização das Culturas. Lisboa: Laboratório Químico Agrícola Rebelo da Silva; 2000. p. 282.
- [27] Jobin P, Douville Y. Engrais verts et cultures intercalaires. Ste-Élizabéth-de-Warwick: Centre de développement d'agrobio-logie; 1996. p. 20. Available from: https://www.agrireseau.net/agriculturebiologique/documents/Engvert_couleur_basse.pdf
- [28] Leplatôis Vedie H. Les engrais verts en maraîchage biologique. ITAB-GRAB Fiches-techniques_maraichage; 2005. Available from: http://itab.asso.fr/downloads/Fiches-techniques_maraichage/ENGRAIS%20VERTS.pdf
- [29] Sullivan DM, Andrews ND. Estimating plant-available nitrogen release from cover crops. Oregon State University Extension Service, PNW 236; 2012. Available from: <https://catalog.extension.oregonstate.edu/sites/catalog/files/project/pdf/pnw636.pdf>
- [30] Oelmann Y, Kreutziger Y, Bol R, Wilcke W. Nitrate leaching in soil: tracing the NO₃– sources with the help of stable N and O isotopes. Soil Biol Biochem. 2007;39(12):3024–33. doi: 10.1016/j.soilbio.2007.05.036.
- [31] Voisin AS, Salon C, Munier-Jolain NG, Ney B. Effect of mineral nitrogen on nitrogen nutrition and biomass partitioning between the shoot and roots of pea (*Pisum sativum* L.). Plant Soil. 2002;242:251–62. doi: 10.1023/A:1016214223900.
- [32] Couëdel A, Alletto L, Tribouillois H, Justes E. Cover crop crucifer-legume mixtures provide effective nitrate catch crop and nitrogen green manure ecosystem services. Agric Ecosyst Environ. 2018;254:50–9. doi: 10.1016/j.agee.2017.11.017.
- [33] Janssen BH. Nitrogen mineralization in relation to C:N ratio and decomposability of organic materials. Plant Soil. 1996;181:39–45. doi: 10.1007/BF00011290.
- [34] Gutiérrez-Girón A, Díaz-Pinés E, Rubio A, Gavilán RG. Both altitude and vegetation affect temperature sensitivity of soil organic matter decomposition in Mediterranean high mountain soils. Geoderma. 2015;237–238:1–8. doi: 10.1016/j.geoderma.2014.08.005.
- [35] Liu X, Herbert S, Hashemi M, Zhang X, Ding G. Effects of agricultural management on soil organic matter and carbon transformation – a review. Plant Soil Environ. 2006;52(12):531–43. doi: 10.17221/3544-PSE.
- [36] Shi J. Decomposition and nutrient release of different cover crops in organic farm systems. Dissertations & Theses in Natural Resources. 75. Lincoln: University of Nebraska; 2013. Available from: <https://digitalcommons.unl.edu/natresdiss/75>
- [37] Cook JC, Gallagher RS, Kaye JP, Lynch J, Bradley B. Optimizing vetch nitrogen production and corn nitrogen accumulation under no-till management. Agron J. 2010;102:1491–9.
- [38] Melander B, Munier-Jolain N, Charles R. European perspectives on the adoption of nonchemical weed management in reduced-tillage systems for arable crops. Weed Technol. 2013;27:231–40. doi: 10.1614/WT-D-12-00066.1.
- [39] Reberg-Horton SC, Grossman JM, Kornecki TS. Utilizing cover crop mulches to reduce tillage in organic systems in the southeastern USA. Renew Agriculture Food Syst. 2012;27:41–8. doi: 10.1017/S1742170511000469.
- [40] Premrov A, Coxon CE, Hackett R, Kirwan L, Richards KG. Effects of over-winter green cover on soil solution nitrate concentrations beneath tillage land. Sci Total Env. 2014;470–471:967–74. doi: 10.1016/j.scitotenv.2013.10.057.
- [41] Poeplau C, Don A. Carbon sequestration in agricultural soils via cultivation of cover crops – a meta-analysis. Agricult Ecosyst Environ. 2015;200:33–41. doi: 10.1016/j.agee.2014.10.024.
- [42] Hassink J, Whitmore AP. A model of the physical protection of organic matter in soils. Soil Sci Soc Am J. 1997;61:131–9. doi: 10.2136/sssaj1997.0361599.
- [43] Olson K, Ebelhar SA, Lang JM. Long-term effects of cover crops on crop yields, soil organic carbon stocks and sequestration. Open J Soil Sci. 2014;4:284–92. doi: 10.4236/ojss.2014.48030.