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RESEARCH ARTICLE

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Effect of replacing conventional Italian ryegrass by organic nitrogen source systems on chemical soil properties

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Abstract

Aim of study: To evaluate agronomic performance and changes on soil chemical properties in two types of managements: conventional or sustainable.

Area of study: Principality of Asturias, Spain.

Material and methods: On a sandy-clay-loam texture soil, three winter forage legumes (faba bean, red clover and white lupin), in monoculture or mixed with Italian ryegrass and with organic fertilization (sustainable management) *versus* Italian ryegrass in monoculture and inorganic fertilization (conventional management) were evaluated during three consecutive years. After the harvest in spring, the rotations were completed with maize crop with the purpose to evaluate the effect of the sustainable management on forage yield and soil chemical parameters.

Main results: The results showed that faba bean and red clover in monoculture and mixed with Italian ryegrass had better edaphic quality than Italian ryegrass in monoculture, and white lupin in monoculture or mixed with Italian ryegrass. Faba bean in monoculture and mixed with Italian ryegrass, both with organic fertilization, could be competitive crops since both had yields comparable to Italian ryegrass in monoculture with inorganic fertilization.

Research highlights: Current agricultural practice could be changed for a more sustainable management system, including organic fertilization and legume crops.

Additional key words: legumes; sustainable farming; biological nitrogen fixation; manure; yield

Abbreviations used: 0C (test without crop); 0L (test without legume); 0L0C (spontaneous vegetation); BNF (biological nitrogen fixation); CAP (Common Agricultural Policy); C/N (carbon-nitrogen ratio); DM (dry matter); ECEC (effective cation exchange capacity); ET0 (reference evapotranspiration); FB (faba bean); FBIR (faba bean-Italian ryegrass intercrop); FC (field capacity); GHG (greenhouse gases); IR (Italian ryegrass); NUE (nitrogen use efficiency); OM (organic matter); RC (red clover); RCIR (red clover-Italian ryegrass intercrop); T0 (soil chemical characteristics before starting the experiment); WL (white lupin); WLIR (white lupin-Italian ryegrass intercrop).

Authors' contributions: Conceived and designed the experiments: AMF. Performed the experiments: SB and AMF. Soil analysis: SB, JAO and EAK. Analyzed the data: SB, FV and AMF. Wrote the paper: SB, FV and AMF. Critical revision of the manuscript: FV and AMF. Supervising the work: AMF. All authors read and approved the final manuscript.

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Introduction

Nitrogen is one of the most important nutrients that plants need to grow, limiting crop production of many agricultural soils, and therefore N addition is needed to increase yields and sustain production (IPCC, 2013). However, the excessive use of inorganic N fertilizers has posed serious threats to environment and human health (Ahmed *et al.*, 2017). The excessive use of N fertilizer results in low N use efficiency (NUE) and has no yield benefits. NUE for most of the plant species ranges from 30 to 50%, while the remaining 50-70% N is either utilized by soil microorganisms (Wuebbles, 2009; Ng *et al.*, 2016), lost through leaching mainly of nitrate (NO_3^-)

and/or by the emission of gaseous forms of N, of which nitrous oxide (N_2O) and ammonia (NH_3) are the main environmental concern (Sutton *et al.*, 2011). This affects natural ecosystems by N enrichment, nitrate contamination in surface and groundwater, thereby changing the biodiversity and causing the greenhouse gas emission (Ward, 2009). In addition, unsuitable land management can lead to a loss of soil fertility and a reduction in the abundance and diversity of soil microorganisms. For example, intensive arable farming causes a progressive decline of soil organic matter (OM) affecting physical, chemical, biochemical and microbiological properties (Caravaca *et al.*, 2002). For these reasons, there is an urgent need to change paradigms towards sustainable agricultural practices that aim to use applied resources as efficiently as possible to ensure sufficient yields and reduce environmental impacts (Schlesinger, 2009).

Environmental care and climate-change mitigation are specific priorities of the Common Agricultural Policy (CAP). In this situation, farmers, which are heavily dependent on aid from Europe to maintain profitability on their farms, are forced to implement new systems that meet these environmental requirements. The "green direct payment" ("greening"), which account for 30% of direct payments, require farmers to diversify crops, maintain permanent grassland and to dedicate 5% of arable land to ecological focus areas. These requirements involve the use of few inputs such as nitrogenous fertilizers. However, the forage rotation common in many dairy farms in Northwest of Spain (Italian ryegrass as winter crop and maize as summer crop), has high demand for N. Therefore, more environment-friendly alternatives must be considered (Jiménez-Calderón *et al.*, 2020). The maize is the main option as summer crop due to its high yield, high energy contribution and good ensilability (Martínez-Fernández *et al.*, 2013). Thus, alternative winter crops to Italian ryegrass must be found.

Legume crops could play an important role in this context by delivering multiple services in line with sustainability principles (Stagnari *et al.*, 2017). One of the most important characteristics of legumes is their ability to fix atmospheric nitrogen (N_2) through the Legume-*Rhizobium* symbiosis (Ramírez-Bahena *et al.*, 2016). The amount of symbiotic N_2 fixation by legumes can range from 100 to 380 kg N ha^{-1} $year^{-1}$ in northern temperate-boreal regions and more than 500 kg N ha^{-1} $year^{-1}$ in the tropics (Ledgard & Steele, 1992; Gibson *et al.*, 1982). This amount will depend on several factors such as plant-bacterial efficiency (Clemente, 2016), the plant species, soil properties or environmental conditions (N'Dayegamiye *et al.*, 2015). Thanks to this particularity, legumes can improve edaphic fertility (Rubiales, 2016) by incorporating that N into the soil and, therefore, the use of fertilizers is reduced, the economic cost of excessive use of external inputs and the negative environmental impact caused by excessive use of inorganic fertilizers (Lüscher *et al.*, 2014; Crème *et al.*, 2016; Clemente, 2016; Stagnari *et al.*, 2017).

The N fixed by the legumes can also be used by intercropping (Ledgard & Steele, 1992). For example, in grass-legume mixture, N transfer from legumes to grasses allows the capture of more energy by natural pigments to support photosynthesis. Therefore, the photosynthetic capacity and productivity of the grasses is improved in the mixtures (Liu *et al.*, 2016). The provision of biological nitrogen fixation (BNF) has also positive effects on yield and quality characteristics of subsequent crops (Rochon *et al.*, 2004; Jensen *et al.*, 2011; Preissel *et al.*, 2015; N'Dayegamiye *et al.*, 2015; Clemente, 2016). These agronomic pre-crop benefits are known as the so-called "nitrogen effect" (Peoples *et al.*, 2009).

In addition to the BNF and sparing processes, legumes provide other benefits like the so-called "break crop effect". These effects include benefits to soil OM and structure (Köpke & Nemecek, 2010; Clemente, 2016). For example, species such as white lupin have deep roots that facilitate the absorption/recycling of water from the deeper layers of the soil. Moreover, roots of this species produce exudates that improve the nutrient solubilization (Stagnari *et al.*, 2017). In addition, the root exudates exert phytotoxic and allopathic effects that can be used for weed control in the subsequent crops (Baldock *et al.*, 1981; Hesterman, 1988). Legumes are considered ideal for crop diversification (Kumar *et al.*, 2018). For example, including legumes in rotations, especially with cereals, can break the biological cycles of insects avoiding diseases and pests reducing the use of pesticides (Köpke & Nemecek, 2010; Preissel *et al.*, 2015; Clemente, 2016) while contributing to the biodiversity of the ecosystems (Clemente, 2016). Leguminous crops, such as faba bean, attract pollinating insects during the flowering season (Miguelañez, 2017), so they provide an indirect ecological service by allowing cross-pollination of nearby fruit trees such as apple trees (Miñarro, 2014).

The introduction of legumes into agricultural rotations helps in reducing the use of fertilizers in arable systems contributing to climate change mitigation. This fact reduces greenhouse gases (GHG) emissions, such as carbon dioxide (CO_2) and nitrous oxide (N_2O), mainly related to energy consuming during fertilizer manufacture and transport (Lemke *et al.*, 2007; Lüscher *et al.*, 2014). It has been reported that N input savings are approximately 277 kg ha^{-1} of CO_2 per year and that legume crops emit 5 to 7 times less GHG per unit area compared with other crops. Therefore, carbon sequestration in the soil is greater (Stagnari *et al.*, 2017). A meta-analysis carried out by Kumar *et al.* (2018) suggest that legumes have 30% higher capacity to store soil organic carbon than other species.

On the other hand, manure is a valuable by-product for the livestock industry and a sustainable and economic opportunity in agricultural systems (Perramon *et al.*, 2016). Animal manure may constitute wastes if not managed appropriately; however, when sustainable manure management technologies are adopted it can provide health, environmental, economic and social benefits. Malomo *et al.* (2018) mentions, among others, the following associated benefits to sound manure management: prevention environmental impacts on air, water, soil, wildlife and marine; reduces GHG emissions from waste; provides savings to farms by waste prevention actions recovery and/or recycling activities leading to lower dependence on chemical fertilizers; delivers more attractive and pleasant human settlements and better social amenity, encourages changes in community attitudes and behaviours. However, is necessary to enact relevant guidelines to promote its sustainable management so that the basic functions of production, collection, storage, treatment, transfer and utilization manure management systems be managed holistically to minimize nutrient losses, prevent pollution and other potential risks.

The use of manure and slurry as fertilizers is an alternative to reduce inorganic fertilizer inputs without reducing yield agricultural (Jiménez-Calderón *et al.*, 2018). These resources provide nutrients to plants, OM to soil and allow complete the nutrient cycle, making that part of the N fixed by legumes and harvested as forage can return to the soil, where it will be available again for subsequent crops (Ren *et al.*, 2014). Their application improves the biological and physicochemical soil properties and also they are a source of energy and nutrients for the edaphic ecosystem (Butler & Muir, 2006).

The aim of this study was to evaluate agronomic performance and changes on soil chemical properties during three years with two types of management, conventional management constituted by Italian ryegrass in monoculture and inorganic fertilization *vs* sustainable management constituted by three legumes in monoculture or in mixed with Italian ryegrass and organic fertilization.

Materials and methods

Experimental area and crops

The study was undertaken at the SERIDA experimental farm (Grado, Spain), located at 43°22’35” N, 6°03’45” W and 65 m above sea level. This region's climate is classified as Mediterranean template according to Papadakis climate classification (Papadakis, 1966), with an average annual temperature of 13.4ºC, average annual rainfall of 980 mm (SIGA, 2019) and average annual reference evapotranspiration (ET0), estimated by Hargreaves equation, of 2.7 mm day^{-1} (Allen *et al.*, 1998). Complementary, the weather data were recorded by meteorological station of the experimental farm. Surface formations of the zone comprise alluvial and fluvial-glacial deposits. Lithological presents mixed units (conglomerates, sands, marls and limestone levels) from the Upper Eocene - Lower Oligocene (Cenozoic). Potential natural vegetation units are the mature riparian forests (alder grove) and the meadows and grasslands. The land capability class is II and it defines the soils with some limitations that reduce the choice of plants or require moderate conservation practices (SITPA, 2019). The experiment was established on Inceptisol Order, Udepts Suborder, Dystrudepts Group and Fluventic Humic Subgroup (Soil Survey Staff, 2014) with a sandy-clay-loam texture (20% clay, 17% silt and 63% sand), adequate bulk density for root growth (1.46 g cm^{-3}) and admissible field capacity (FC = 18.24%). These parameters were defined at 20 cm of depth before the beginning of experiment. Soil was prepared for sowing by a cross-field pass subsoiler, disc harrow, basal fertilization and cross-field pass milling machine. A split-plot design in a randomized complete block design, with three repetitions was used during three consecutive agronomic years (2012/13, 2013/14 and 2014/15). Three arable plots with a surface of 11 m × 22 m each one under fallow conditions at the beginning of the experiment were used. Main plots were constituted by a test without legume (0L) and three legumes: faba bean (*Vicia faba* L. -FB-), red clover (*Trifolium pratense* L. -RC-) and white lupin (*Lupinus albu*s L. -WL-). Sub-plots were constituted by a test without crop (0C) and Italian ryegrass (*Lolium multiflorum* Lam. -IR-). 0L0C combination corresponds to spontaneous vegetation. The allocation of the crops in each main plot and sub-plot combination was maintained throughout the three years of the study. Monoculture seed rates were 40, 150, 20 and 100 kg ha^{-1} for IR (var. ‘Barextra’), FB (var. ‘Prothabon 101’), RC (var. ‘Quiñequeli’) and WL spp. respectively. The seed mixtures were made reducing the amount of seed contributed from each species until obtaining a 1:1 ratio. All seeds were sown broadcast. To complete the crop rotations, a short-cycle variety (FAO 200) of maize (*Zea mays* L. var. ‘SY Kairo’) was used as summer crop in all experimental allotments with a seed rate of 90,000 plants per hectare. Showing dates for all crops are shown in Table 1.

Soil analysis was made at the beginning of the experiment, according to the methodology described below, and the fertilization requirements, based on P content, were calculated according its baseline chemical characteristics before starting the experiment (T0).

Composted manure from the SERIDA beef cattle herd was applied on the legumes in monoculture and in intercrop, and in 0L0C allotments as described in Table 2. The monoculture IR allotments were fertilized with the same N-P-K amounts but using inorganic fertilizers in order to maintain a conventional management, and in addition, in these subplots, 60 kg ha^{-1} of N as calcium ammonium nitrate (27%) was applied after the first cut of IR.

Table 1. Sown and harvest dates for spontaneous vegetation (0L0C), Italian ryegrass (IR), faba bean (FB), red clover (RC) and white lupin (WL) monocultures and their intercrops with IR (FBIR, RCIR and WLIR) in three agronomic years (2012-2013, 2013-2014 and 2014-2015).

Year	Dates	0L0C	IR	FB	RC	WL	FBIR	RCIR	WLIR	Maize
2012/13	Sown	16/11/12	16/11/12	16/11/12	16/11/12	16/11/12	16/11/12	16/11/12	16/11/12	26/06/13
	Harvest	22/05/13	1st cut: 24/04/13 2nd cut: 11/06/13	22/05/13	11/06/13	n.d.	22/05/13	11/06/13	n.d.	09/10/13
2013/14	Sown	31/10/13	31/10/13	31/10/13	31/10/13	31/10/13	31/10/13	31/10/13	31/10/13	04/06/14
	Harvest	14/04/14	1st cut: 17/03/14 2nd cut: 28/04/14	14/04/14	28/04/14	23/04/14	14/04/14	28/04/14	23/04/14	29/09/14
2014/15	Sown	27/10/14	27/10/14	27/10/14	27/10/14	27/10/14	27/10/14	27/10/14	27/10/14	23/06/15
	Harvest	21/05/15	1st cut: 23/02/15 2nd cut: 26/05/15	28/04/15	26/05/15	21/05/15	28/04/15	26/05/15	21/05/15	15/10/15

n.d: no data because these crops did not complete their development.

Before sowing maize as summer crop, the allotment corresponding with IR was fertilized with 125 kg N ha^{-1}, 150 kg P_2O_5 ha^{-1} and 250 kg K_2O ha^{-1} of a chemical fertilizer using a 9-18-27 complex combined with calcium ammonium nitrate (27%). The rest of the allotments were fertilized with organic fertilizer using mature cattle manure with the same amount of nutrients that the chemical one.

All winter crops were harvested in spring (Table 1). IR in two cuts and the rest of them in a single cut. IR was harvested in phenological stage 51 (beginning of heading), RC in phenological stage 61 (beginning of flowering: 10 % of flowers open) and FB and WL in phenological stage 70 (bean-grain) according to Hack *et al.* (1992). The intercrops were harvested at the optimum stage for the most developed crop. Winter crops were harvested and sampled with a motor mower and weighing the forage in two areas of 4.5 m^2 in each allotment. Maize was harvested for silage when plants were on grain maturity stage between quarter milkline and two-thirds milkline. The sampling was carried out taking the plants on two parallel rows with 0.70 m distance between them along three meters, constituting a sampling area of 4.2 m^2. Dry matter yields were determined according to de la Roza *et al.* (2002) in all crops.

Table 2. Composition and dosage of manure used in winter crops and spontaneous vegetation (0L0C) allotments during the three years of the experiment.

Fertilization date	Contribution kg N-P_2O_5-K_2O t^{-1}	Manure dose (t ha^{-1})
31/10/2012	9 - 8 - 9	6[1]
21/10/2013	4 - 4 -12	12
20/10/2014	8 -5 -10	8

[1]: The deficit of K in soil at the beginning of the experiment was solved by applying 115 kg ha^{-1} of K_2O as potassium chloride (60%) to prevent any limitation in the crops growth.

Soil samples were collected after each winter crop harvest to track the evolution of soil characteristics. For this purpose, ten sub-samples were taken from each allotment at 20 cm depths using a Dutch auger. All sub-samples were then pooled to make one composite soil sample for further analyses.

Soil chemical analysis

Soil parameters were determined at the Forest Engineering Area of the University of Oviedo. Samples were air-dried at room temperature, crumbled, finely crushed and sieved through a 2-mm screen, before proceeding to analysis. Particle-size distribution was determined by the pipette method using sodium hexametaphosphate and Na_2CO_3 to disperse the samples after destruction of soil OM with H_2O_2 at 6% (Gee & Bauder, 1996). Soil pH was measured with a glass electrode in a suspension of soil and water 1:2.5. OM was determined by weight loss-on-ignition. C was estimated according to the relation C (%) = OM (%) / 1.724 (Oliveira *et al.*, 2006). Total N was determined by a Kjeldahl digest method (Klute, 1996). Exchangeable cations (Ca, Mg, K and Na) were extracted with 1 M NH_4Cl, and exchangeable Al (Al_{ECEC}) was extracted with 1 M KCl and later were determined by atomic absorption/emission spectrophotometry (Pansu & Gautheyrou, 2006). Effective cation exchange capacity (ECEC) was calculated as the sum of the values of exchangeable cations and exchangeable Al. Available P was determined colorimetrically with Mehlich 3 reagent (Mehlich, 1984).

Statistical analysis

The statistical analysis was carried out in the R environment for statistical computing (R Core Team, 2017).

Two-way ANOVA was used to compare the effect of management (crop and type of fertilization) as main factor on soil properties. Year was considered as random effect. Means were compared using Duncan's Multiple Range Test. Significance was set at $p<0.05$.

Results

Weather conditions of the study area during the crop developing periods are shown in the Figure 1. Average annual temperature was similar for all periods and comparable with the historical data; however, average annual precipitation and its distribution were different among years and with the historical data. The average temperature recorded during the three years for the months in which the winter crops were developed (November - May) was 10.4°C, with an average of minimum temperatures of 5.3°C and an average of maximum temperatures of 15.5°C. For the months in which the summer crop was developed (June - October), the average annual temperature was 18.2°C, with an average of the minimum of 12.5°C and an average of the maximum of 23.8°C. The first year (2012-2013) was the rainiest one, with an annual rainfall of 1253 mm accumulated in 159 rainy days mainly distributed during the months of January, February and March. In the second year (2013-2014), the annual rainfall decreased with respect to the previous year, recording 995 mm in 124 rainy days with a homogeneous distribution during the winter months. In the last year of this study (2014-2015) there was an annual rainfall of 1123 mm in a total of 133 rainy days, highlighting the month of February with 252 mm collected in 16 days of rain. In these weather conditions, all crops had a regular germination except WL, which neither produced forage in the first year of study in monoculture nor intercrop with IR.

Chemical properties in soil are shown in Table 3. The pH value did not show significant differences ($p>0.05$) with respect to initial situation (T0). OM content increased in all treatments compared with the initial situation ($p<0.001$). Allotments under WL and WLIR had higher OM content and higher C/N ratio than the other legumes in monoculture or intercropped with ryegrass ($p<0.001$). An increase in Ca concentration was observed in all treatments with respect to initial situation ($p<0.001$), especially in WL and WLIR. Soil had low K content (0.31 cmol$^{(+)}$ kg^{-1}) at the beginning of experiment. Fertilizers application, both organic and inorganic, increased ($p<0.05$) soil K content to high values (0.84 - 1.02 cmol$^{(+)}$ kg^{-1}) for 0L0C, IR, FB, WL, FBIR and WLIR, and very high values (K>1.02 cmol$^{(+)}$ kg^{-1}) for RC and RCIR, although there were no significant differences between treatments. Na content was elevated at the beginning of study (Na>1.5 cmol$^{(+)}$ kg^{-1}) and decreased significantly ($p<0.05$) in all treatments except RC. ECEC was increased with the treatments although it remained within the range considered as normal (10-20 cmol$^{(+)}$ kg^{-1}). There was no significant difference for P content with respect to initial situation.

Average winter forages yields are shown in the Figure 2 (A). In monocultures, the highest yields were obtained with IR (7237 kg DM ha^{-1}) and FB (6496 kg DM ha^{-1}), with no difference between them. The yields of the other legumes in monoculture were lower ($p<0.05$) than IR and FB, with 2699 kg DM ha^{-1} and 2414 kg DM ha^{-1} for RC and WL respectively. The production of RC was 3.8 t ha^{-1} in the first year, but declined in the second and third year of experiment with 1.8 and 1.9 t ha^{-1} respectively. Likewise, white lupin did not develop during the first year neither in monoculture nor in mixture. Although Italian ryegrass emerged, the biomass obtained in the WLIR plots was not considered. Likewise, maize was not sown in these plots the first year. The most productive intercrop was FBIR (7367 kg DM ha^{-1}), showing differences with the other intercrops ($p<0.05$) but without differences with IR and FB. Average maize yields are showed in the Figure 2 (B). There were no significant differences as a result of the previous crop and fertilization type.

Discussion

In this study, soil texture was classified, based on the granulometric analysis, as sandy-clay-loam. Loam soils are balanced, suitable from the agricultural point of view since their properties are compensated (Porta-Casanellas *et al.*, 2003). However, the clay component of the texture makes the soil susceptible to waterlogging during prolonged periods of rain, such as February 2013 and 2015, losing permeability and aeration capacity. Under these circumstances, IR, FB, RC, FBIR and RCIR were able to complete their development the three years of study regardless of the meteorological and edaphic conditions. However, WL and WLIR failed in its establishment the first year of study. The lower rainfall regime during the second year allowed its development without problem. It is known that the root system of this crop has the ability to improve soil texture and drainage (Fumagalli *et al.*, 2014; Reddy, 2016). Therefore, the fact that in the second year the white lupin completed its development, could have improved some edaphic conditions such as soil structure, porosity or amount of OM among others, preventing the waterlogging caused the root asphyxiation and ensuring its implementation the following year despite having been as rainy as the first.

The application of fertilizers such as elemental sulphur, urea or ammonium salts and the growth of legumes such as clover can drive soil acidification (Goulding, 2016); however, in this study soil acidification was not observed.

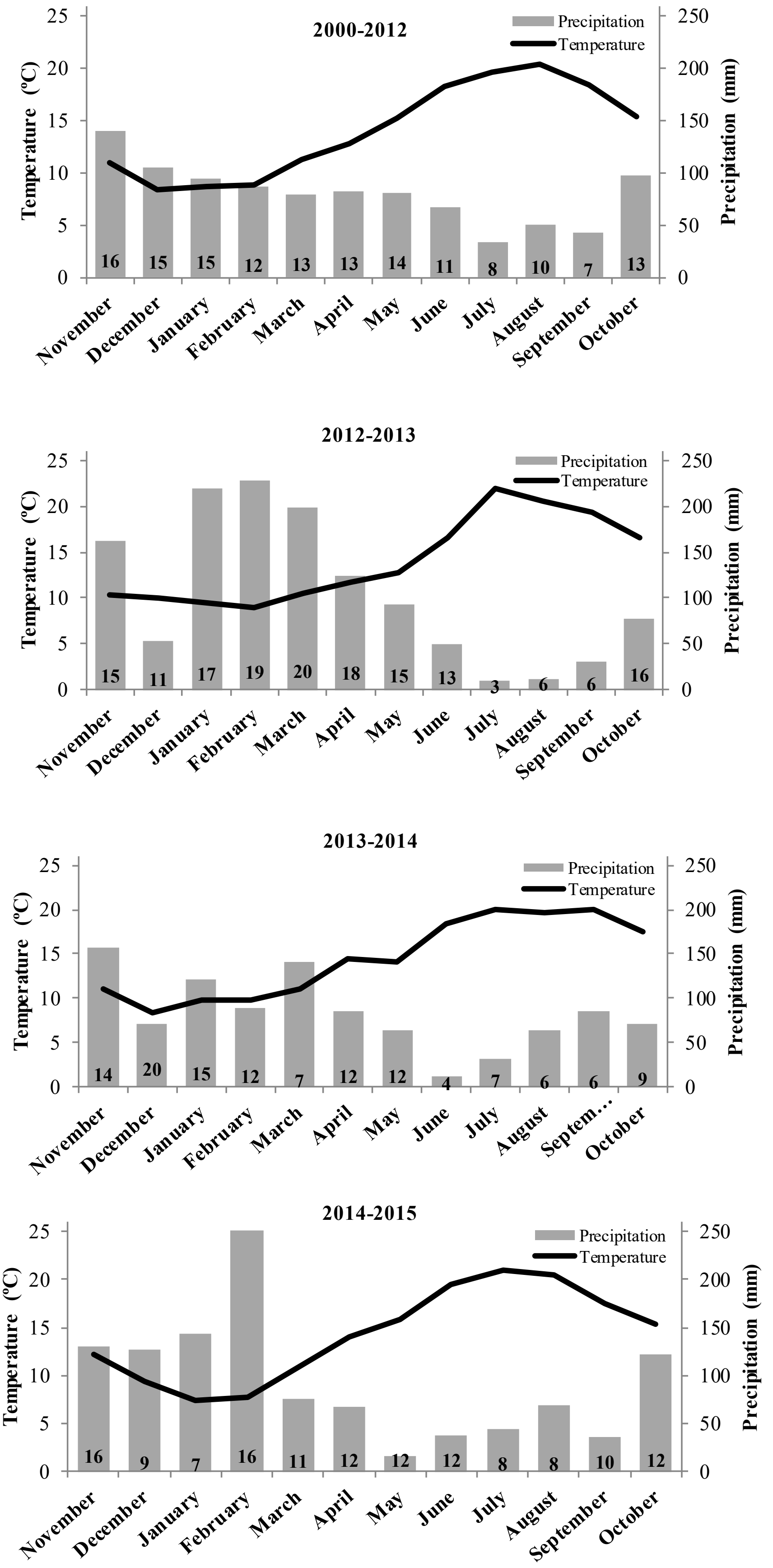

Figure 1. Climographs of the experimental area over the historic period (2000-2012) and the three agronomic years (2012-2013, 2013-2014 and 2014-2015). Number of rainy days by month are indicated in the bottom of each histogram bar.

Table 3. Chemical properties in soil under spontaneous vegetation (0L0C), Italian ryegrass (IR), faba bean (FB), red clover (RC) and white lupin (WL) monocultures and their intercrops with IR (FBIR, RCIR and WLIR).

	T0	**0L0C**	**IR**	**FB**	**RC**	**WL**	**FBIR**	**RCIR**	**WLIR**	**S.E.**	***p***
pH	6.46	6.76	6.51	6.66	6.65	6.73	6.73	6.65	6.70	0.180	0.082
OM (%)	2.09^{c}	4.27^{b}	4.17^{b}	4.32^{b}	4.07^{b}	5.82^{a}	4.08^{b}	4.44^{b}	5.53^{a}	0.615	0.001
C (%)	1.21^{c}	2.48^{b}	2.42^{b}	2.51^{b}	2.36^{b}	3.38^{a}	2.37^{b}	2.58^{b}	3.21^{a}	0.357	0.001
N (%)	0.32^{ab}	0.30^{ab}	0.33^{ab}	0.32^{ab}	0.34^{ab}	0.25^{b}	0.32^{ab}	0.42^{a}	0.21^{b}	0.098	0.030
C/N	3.76^{d}	9.50^{cd}	11.23^{abc}	8.73^{cd}	9.05^{cd}	16.12^{ab}	9.78^{bcd}	7.76^{cd}	17.53^{a}	5.575	0.008
Ca (cmol$^{(+)}$ kg^{-1})	6.50^{d}	8.92^{c}	8.83^{c}	9.33^{bc}	9.32^{bc}	10.50^{a}	9.32^{bc}	9.13^{bc}	10.10^{ab}	0.767	0.001
Mg (cmol$^{(+)}$ kg^{-1})	2.66	1.97	1.81	1.96	2.00	1.63	2.13	2.06	1.81	0.654	0.583
K (cmol$^{(+)}$ kg^{-1})	0.31^{b}	1.02^{a}	0.97^{a}	0.84^{a}	1.06^{a}	0.85^{a}	0.93^{a}	1.10^{a}	0.86^{a}	0.277	0.011
Na (cmol$^{(+)}$ kg^{-1})	1.75^{a}	1.26^{bc}	1.28^{bc}	1.24^{bc}	1.50^{ab}	1.19^{c}	1.38^{bc}	1.31^{bc}	1.28^{bc}	0.235	0.026
Al (cmol$^{(+)}$ kg^{-1})	0.17	0.12	0.15	0.12	0.12	0.11	0.13	0.14	0.13	0.034	0.156
ECEC (cmol$^{(+)}$ kg^{-1})	11.40^{b}	13.30^{a}	13.04^{a}	13.49^{a}	14.00^{a}	14.29^{a}	13.89^{a}	13.74^{a}	14.18^{a}	1.023	0.013
Al$_{ECEC}$ (%)	1.53^{a}	0.90^{b}	1.17^{b}	0.89^{b}	0.90^{b}	0.79^{c}	0.93^{b}	0.99^{b}	0.90^{b}	0.259	0.013
P (mg kg^{-1})	26.34	21.91	22.18	23.91	20.91	23.60	23.57	23.98	23.88	3.988	0.521

T0 corresponds to soil properties before the beginning of the experiment in 2012. OM: organic matter; C/N: carbon/nitrogen ratio; ECEC: effective cation exchange capacity; Al$_{ECEC}$: Al exchangeable. $^{a, b, c:}$ Different letters indicate significant differences between treatments. Data are presented as mean of three agronomic years (2012-2013, 2013-2014 and 2014-2015).

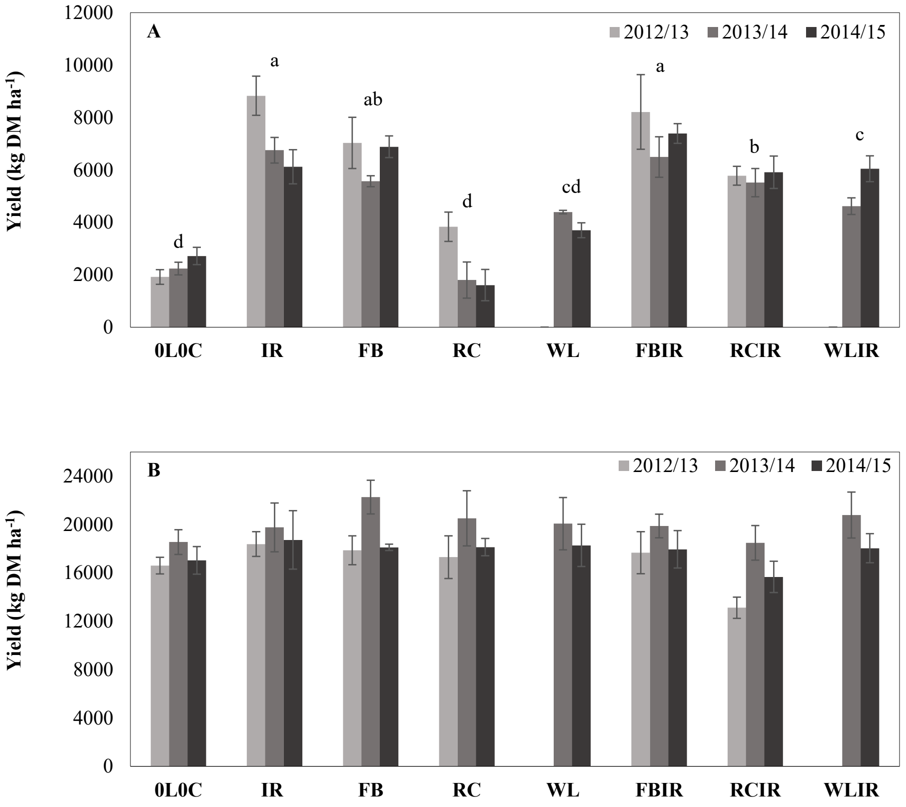

Figure 2. Dry matter yield per hectare (kg DM ha^{-1}) of winter forages (A) and of maize cultivated after the different winter crops (B) studied during agronomic years 2012-2013, 2013-2014 and 2014-2015. 0L0C: spontaneous vegetation; IR: Italian ryegrass; FB: faba bean; RC: red clover and WL: white lupin monocultures and their intercrops with IR (FBIR, RCIR and WLIR). $^{a, b, c:}$ Different letters indicate significant differences between treatments.

Organic matter is a fundamental indicator of soil quality and it constitutes one of the largest reservoirs of carbon (Ciais *et al.*, 2014). In uncultivated soils, OM content is depending of kind of vegetation that support, soil properties as texture, pH, temperature, moisture, aeration, clay mineralogy and soil biological activities (Bot & Benites, 2005). In agricultural soils, the application of fertilizers increases the crops yields and therefore has effects on the OM content (Bauer & Black, 1994). In this study, fertilizer application, both organic and inorganic, increased soil OM content with respect to its content at the beginning of experiment. Organic matter contents were similar for all treatments except for WL and WLIR. This fact may be due to WL deep taproot that improves soil structure and drainage (Fumagalli *et al.*, 2014). These results are in accordance with a study carried out by Crème *et al.* (2016), which concludes that different plant species and mixtures may have specific effects on soil parameters related to OM most probably due to plant specific rhizosphere effects.

The C/N ratio reports soil health and determines the degree of mineralization of the OM that exists in the soil as well as the type of humus, therefore has an important impact on plant N availability. A small value of this ratio, indicates a great degree of OM mineralization and, therefore, a high soil quality. According to Junta de Extremadura (1992), a C/N ratio between 8 to 10 represents a good edaphic quality, *i.e.* balanced soil with a controlled release of mineral N and carbon content, and with OM well balanced between mineralization and humification, and high fertility. In this study, FB, RC and FBIR were within the optimum range. RCIR showed a C/N ratio <8, which indicates a rapid mineralization or excess of N. WL and WLIR showed very high C/N ratio (C/N>15). This higher C/N ratio is probably due to an excess carbon and energy and indicate a slow decomposition kinetics of this crop therefore, practically all the released N is taken by the microorganisms of the soil, leaving very little free to be used by the plants.

According to Junta de Extremadura (1992) the Ca and Mg concentrations of this study were appropriate since in all cases were higher than 4 cmol$^{(+)}$ kg^{-1} for Ca and higher than 0.7 cmol$^{(+)}$ kg^{-1} for Mg. Beside this it is necessary to take into account antagonistic effects between cations. Too much Ca and/or K can interfere in Mg assimilation. Ca/Mg ratio is optimum around 5 cmol$^{(+)}$ kg^{-1}, but higher than 10 indicates Mg deficit. K/Mg ratio must be between 0.2 and 0.3. When this ratio is higher than 0.5, it indicates Mg deficit while if it is lower than 0.1, it it indicates K deficit (Junta de Extremadura, 1992). In the initial situation of this study, the Ca/Mg ratio was 2 and the K/Mg ratio was 0.1. After three years, allotments with monoculture crops (IR, FB and RC) showed a Ca/Mg ratio of 5 while that intercrops (FBIR and RCIR) showed a Ca/Mg ratio of 4. WL and WLIR showed a Ca/Mg ratio of 6. K/Mg ratio was 0.4 for FB and FBIR and 0.5 for the rest of the alternatives.

Legumes generally have higher P demands than grasses and P availability may limit their growth (Roscher *et al.*, 2011). In this study, only RC presented, although not significantly, lower P content after three years compared with the initial situation. Despite significant difference between treatments were not found, red clover tended to be higher in P content when it was associate with Italian ryegrass. Similar results were observed by Crème *et al.* (2016) who reported that negative effects on legumes on soil P may be attenuated when they grow in mixture with grasses.

Italian ryegrass showed high yield as a result of two accumulated spring cuts. This amount of production requires considerable mechanization and excessive use of external inputs, such as extra doses of inorganic fertilizer. FBIR showed a competitive yield using only organic fertilization (manure) and reduced mechanization as a result of a single cut harvest system. Several authors (Tosti & Guiducci, 2010; Monti *et al.*, 2016) have studied this competitive yield with reduced inputs, better pest control (Lopes *et al.*, 2016), pollution mitigation (Luo *et al.*, 2016) and more stable aggregate food or forage yields per unit area (Smith *et al.*, 2013).

Red clover offers high forage yield potential and fast establishing (Eriksen *et al.*, 2014); however, it uses may be limited by its lack of persistence (Marshall *et al.*, 2017). Thus, in this study, the RC yield was lower in the second and third years than the first year. However, when RC was intercropped with IR, better yields were obtained and they were similar during the three years of study. Hoekstra *et al.* (2016) also reported that, compared to monocultures, simple grassland mixtures can result in increased yields, greater stability in response to disturbance (*i.e.* drought), reduced invasion by weeds and improved nutrient retention. WL trended to be less productive that WLIR. This fact could be caused because *Lupinus* spp. are poor weed competitors during early establishment since canopy development is slow, facilitating light penetration and subsequent weed seed germination and yield loss due to competition (Putnam *et al.*, 1989).

In summary, traditional winter crop of Italian ryegrass chemically fertilized can be replaced by some legumes organically fertilized. Faba bean in monoculture and in intercrop with Italian ryegrass, both with organic fertilization, could be competitive crops since both had yields comparable to Italian ryegrass with chemical fertilization. Faba bean and red clover and their intercrops with Italian ryegrass had better edaphic quality than Italian ryegrass and white lupin and their intercrop with Italian ryegrass.

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