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# Management Influence on the Quality of an Agricultural Soil Destined for Forage Production and Evaluated by Physico-Chemical and Biological Indicators

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**Abstract:** The European Common Agricultural Policy promotes the sustainable use of soils through the principle of cross-compliance that links direct payments to good farming practices. Thus, it is necessary to find sustainable alternatives to the conventional management for forage production in the Atlantic Arc dairy farms. Two alternative managements (faba bean in monoculture—FB—and faba bean–Italian ryegrass intercrop—FBIR) were cultivated with organic fertilization during two consecutive years, and compared to the conventional management (Italian ryegrass in monoculture—IR—under chemical fertilization) as winter crops. Maize was used as the summer crop to complete the rotations using organic and chemical fertilization, respectively. The forage yield of winter fodder was quantified. Soil samples and summer harvests were performed to analyse the physico-chemical and biological parameters. The best forage yield corresponded to FBIR with 9.2 t dry matter (DM) $ha^{-1}$ vs. 7.2 and 5.7 t DM $ha^{-1}$ for FB and IR, respectively. The soil organic matter did not show significant differences among treatments, suggesting that it may be a poor indicator of the management influence on edaphic quality in the short term. Biological indicators were more sensitive and faster to differentiate among managements than chemical indicators. Earthworm abundance was higher in FB and FBIR than IR ($p < 0.001$), and consequently, soil infiltration was improved with the alternative management (13.90 vs. 2.08 and 0.90 min for IR, FB and FBIR, respectively, $p < 0.01$). As a result, the soil health diagnosis of the agroecosystem was better for alternative management.

**Keywords:** sustainable farming; faba bean; organic fertilization; slurry; yield

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## 1. Introduction

Environmental care and climate change mitigation are specific priorities of the European Common Agricultural Policy (CAP). The CAP implementation for the period 2014–2020 has guided the agricultural holdings towards a more sustainable model to be able to meet the environment and climate change challenges. Apart from crop yield and nutritional quality, the sustainability of the farming practices that help meet environmental and climate goals must also be considered in the agricultural policy. Under this scenario, the farmers, who are heavily dependent on European subsidies to maintain the profitability of their farms, are forced to implement new management systems to achieve the environmental requirements. The CAP (2014–2020) promoted environmental conservation through three routes: cross compliance, greening payments, and agri-environmental schemes [1]. The "green direct payment" (or "greening") accounts for up to 30% of direct payments and it requires crop diversification to maintain the permanent grassland and to dedicate at least 5% of arable land to "ecological focus areas" [2]. Therefore, the selection of alternative and competitive crops would be the first step. In June 2018, the European Commission presented proposals for a future CAP in which the term of "conditionality" replaced the terms of "greening" and "cross-compliance" of the current CAP. The future CAP will include more ambitious and sustainable agricultural commitments through the adoption

of better farming practices and standards by farmers. The conditionality links income support (and other area- and animal-based payments) to environment- and climate-friendly farming practices and its standards are known as "Good Agricultural and Environmental Conditions" (GAECs) and "Statutory Management Requirements" (SMRs). These practices and standards aim at delivering a higher level of environmental and climate actions. The GAECs set standards for mitigating and adapting to climate change, which include addressing water challenges, soil protection and quality, land management, and protection and quality of biodiversity [3]. For the specific case of soil protection and quality, farmers should meet the following requirements: minimum land management under tillage to reduce the risk of soil degradation including on slopes, no bare soil in the most sensitive period and crop rotation. These practices will replace the previous condition of crop diversification.

The common forage rotation in many dairy farms from the Spanish Atlantic area is Italian ryegrass (*Lolium multiflorum* Lam.) as the winter crop and maize (*Zea mays* L.) as the summer crop. This crop rotation is very productive, but highly demanding of nitrogen that could have negative effects on soil fertility [4,5]. Therefore, more environmentally friendly alternatives must be considered. Maize is the main option as the summer crop due to its high yield, high energy contribution and good ensilability [6]. Therefore, winter crops are a key focus to achieve these environmental requirements. Previous studies carried out in the experimental plots located in the SERIDA farm showed that the traditional winter and chemically fertilized Italian ryegrass (IR) crop can be replaced by certain organically fertilized legumes [7,8]. The authors concluded that faba bean (FB) in monoculture or intercropped with IR, both with organic fertilization, could be competitive crops since they obtained comparable yields to IR with chemical fertilization and less mechanization as a result of a single cut harvest system. However, further trials in real plots must be carried out to evaluate those factors that cannot be considered in the experimental plots, such as the effect on the field of heavy machinery.

The preservation of the agricultural land in good agricultural and environmental condition plays an important role in soil protection. Simple, fast, and reliable soil quality indicators are necessary to evaluate the short-term agro-ecosystem sustainability and to predict soil alterations which will help farmers on decision making. Several soil parameters (physical, chemical, and biological ones) could be good candidates to verify the soil use and the management strategy within a desired timescale. Among the physical indicators, soil texture, aggregation, moisture, porosity, and bulk density have already been used, while among chemical indicators, total C and N, mineral nutrients, organic matter, cation exchange capacity, among others, are also well established. However, most of them have generally shown a slow response compared to the biological ones, such as microbial biomass C and N, soil biodiversity, soil enzymes, soil respiration, macro and mesofauna, etc. [9].

The aims of this trial were: (1) to study and compare the agronomic behaviour of two alternative management strategies and a conventional one in real scale plots; and (2) to evaluate the impact of strategies on the soil health of the agroecosystem.

## 2. Materials and Methods

### *2.1. Experimental Area and Crops*

The study was undertaken at the SERIDA experimental farm (Villaviciosa, Asturias, Spain), located at 43°28′20″ N, 5°25′10″ W and at 10 m above the sea level. The region's climate is classified as Warm Temperate Oceanic Climate according to Papadakis climate classification [10], with an average annual temperature of 12.8 °C, average annual rainfall of 1180 mm [11] and average annual reference evapotranspiration (ET0), estimated by Hargreaves equation, of 2.1 mm day$^{-1}$ [12]. Additional weather data collection was performed during the experimental period from the nearest official meteorological station located at 43°29′7″ N, 5°16′13″ W (https://datosclima.es/, accessed on 6 July 2019). The experimental farm is located on superficial clastic formations with abundant matrix

and mixed lithological units from the Triassic, Jurassic Dogger and Jurassic Lias periods. Land capability can be assigned to class II, subclass s [13]. The trial was established on Mollisol Order, Udoll Suborder, Hapludoll Group and Fluventic Subgroup [14] with a sandy-loam texture (11% clay, 13% silt and 76% sand) and under fallow conditions at the beginning of the experiment. The soil physico-chemical and biological parameters were defined before the start the experiment. Soil was prepared for sowing with a cross-field pass subsoiler, a harrow disc, applying a basal fertilization and using a cross-field pass milling machine.

Three adjacent plots with a surface of one hectare were used to test the different managements: (1) chemical fertilization and Italian ryegrass (*Lolium multiflorum* Lam.) crop (IR); (2) organic fertilization and faba bean (*Vicia faba* L.) crop (FB); and (3) organic fertilization and faba bean–Italian ryegrass intercrop (FBIR). The crop assignment in each plot was maintained throughout the two consecutive agronomic years (2014–2015 and 2015–2016). Seed rates were 50 kg $ha^{-1}$ for IR, 150 kg $ha^{-1}$ for FB and 75 + 25 kg $ha^{-1}$ of FB and IR, respectively, for FBIR. Broadcast seed sowing was applied for all the seeds. To complete the crop rotations, 75,000 plants per hectare of short-cycle variety (FAO 200) of maize (*Zea mays* L.) were used as summer crop in all the experimental allotments. Maize was chemically fertilized in the IR rotation and organically fertilized in the rotations with FB and FBIR. The sowing and harvest dates for all crops are shown in Table 1.

**Table 1.** Sown and harvest dates for all crops in two agronomic years (2014–2015 and 2015–2016).

| Agronomic Year | | IR | FB | FBIR | Maize |
|---|---|---|---|---|---|
| | | | Dates | | |
| 2014/15 | Sown | 20/10/2014 | 20/10/2014 | 20/10/2014 | 11/06/2015 |
| | Harvest | 1st cut: 20/04/2015<br>2nd cut: 25/05/2015 | 05/05/2015 | 05/05/2015 | 30/09/2015 |
| 2015/16 | Sown | 30/10/2015 | 30/10/2015 | 30/10/2015 | 11/06/2016 |
| | Harvest | 1st cut: 13/04/2016<br>2nd cut: 31/05/2016 | 22/04/2016 | 13/04/2016 | 03/10/2016 |

In the maize crops, a selective pre-emergence herbicide (Primextra Gold, Syngenta AG, Basel, Switzerland) was applied for weed control, and an organophosphate insecticide (Dursban 48, Syngenta AG, Basel, Switzerland) for pest control. Both treatments followed the doses recommended by the manufacturer.

The annual fertilization was adjusted according to the results of initial soil analysis. The IR plot was fertilized with an inorganic fertilizer to maintain a conventional management. A basal dressing fertilization of 60 kg N $ha^{-1}$, 60 kg $P_2O_5$ $ha^{-1}$ and 130 kg $K_2O$ $ha^{-1}$ was applied before sowing according to requirements at the beginning of the experiment. In addition, 60 kg $ha^{-1}$ of N was applied as topdressing after the first IR cut for silage. Furthermore, 125 kg N $ha^{-1}$, 150 kg $P_2O_5$ $ha^{-1}$ and 220 kg $K_2O$ $ha^{-1}$ were added after the second silage cut and before sowing the maize as the summer crop. Finally, when maize plants were 20 cm height, 75 kg N $ha^{-1}$ was applied as topdressing. The fertilization of the FB and FBIR plots consisted of manure and slurry from the SERIDA dairy herd which contained the same amount of nutrients as the chemical fertilization. Topdressing fertilization was not added in these plots to compensate the amount of atmospheric N fixed by the legumes.

All winter crops were harvested in spring (Table 1). IR was harvested in two cuts after a cleaning cut at the end of winter. FB and FBIR were harvested in a single cut. An additional cut of the Italian ryegrass regrowth was possible in the second agronomic year in the FBIR plot. The first cut of IR was made when it was at 51 phenological stage (beginning of heading) according to Hack et al. [15], and the second cut occurred around 5–7 weeks later (stage 55, middle of heading). The FB and FBIR crops were both harvested when

FB was in phenological stage 70 (bean-grain). The forage yield controls were performed at harvest from three sampling areas in each plot which were as separated as possible among them. The IR yield was measured by tracing a diagonal transect across each sampling area and after cutting 1 $m^2$ of forage in five subsamples of 2 × 0.1 $m^2$ at ground level with a hand mower. The FB and FBIR plots were sampled by randomly delimiting 1 $m^2$ in each sampling area and cutting the forage inside it with a hand mower at ground level. In all cases, the forage yield measurements followed Martínez-Fernández et al. [16].

### 2.2. Soil Analysis

Soil samples for the evaluation of the physico-chemical parameters were obtained at the beginning of the experiment (autumn of 2014) and after each winter and summer crop harvest in four occasions (spring and autumn of 2015 and 2016). The soil samples from the upper layer soil (20 cm) were collected in three random points per plot with a Dutch auger. Once in the laboratory, the samples were air-dried at room temperature, crumbled, finely crushed and sieved through a 2 mm screen. The particle-size distribution was determined by the pipette method using sodium hexametaphosphate and $Na_2CO_3$ to disperse the samples after the destruction of soil organic matter (OM) with $H_2O_2$ at 6% [17]. 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 [18]. Total N was assessed with the Kjeldahl method [19]. Exchangeable cations (Ca, Mg, K and Na) were extracted with 1 M $NH_4Cl$, exchangeable Al ($Al_{ECEC}$) with 1 M KCl, and they were all subsequently evaluated by atomic absorption/emission spectrophotometry [20]. Effective cation exchange capacity (ECEC) was calculated as the sum of the values of exchangeable cations and the exchangeable Al. Available P was determined colourimetrically with Mehlich 3 reagent [21].

To evaluate the impact of the agricultural practices on the agroecosystem health, soil samples were collected in three points/plot at the beginning (autumn of 2014) and at the end (autumn of 2016) of the experiment. The sampling points during autumn of 2014 were georeferenced to repeat the final sampling in the same locations. The soil health diagnosis was carried out with the Agroecosystem Health Cards (AHC) system [22]. AHC consist of handbooks that provide straightforward and practical explanations to assess agroecosystem health through indicators classified into two categories, "basic" and "advanced". Those indicators are grouped into three ecosystem services: "biodiversity conservation", "soil conservation" and "global change mitigation". The basic indicators are macrofauna diversity, number of earthworms, infiltration time and soil colour. The advanced indicators are mesofauna diversity, bacteria, basal respiration, induced respiration, respiratory quotient, compaction, and $CO_2$ emissions from soil. The protocol to obtain the value of each indicator is briefly described below:

Macrofauna diversity (n°): four cubic blocks of soil 25 cm per side and 30 cm deep were extracted and the number of the different species of macrofauna was counted.

Number of earthworms (n° $m^{-2}$): four soil blocks (25 × 25 × 30 $cm^3$) were extracted and the number of individuals was counted and multiplied by four to obtain the number of earthworms per $m^2$.

Infiltration time (min): it is defined as the time necessary for the amount of fallen water to percolate during one hour of heavy–very heavy rain (30 L $m^{-2}$ according to the State Meteorological Agency of Spain, AEMET). A metal cylinder of 10 cm in length and 10 cm in internal diameter was inserted into the soil, and 235 mL of water was gently poured into it. The minutes taken for the water to disappear were timed.

Soil colour: the colour of the extracted soil fraction was evaluated with a scale from 1 to 9, which ranges from light to dark according to the reference values on AHC included in the user guide [22].

Mesofauna diversity (biological quality index—BQ): it was evaluated with the Berlese–Tullgren extraction method [23]. A cylindrical soil sample of 10 cm in diameter and 5 cm deep was placed on a 2 mm metal mesh and over a funnel. A 50 W bulb was applied at

20 cm distance. A container with alcohol was placed at the end of the funnel to collect the organisms after seven days. The species of mesofauna were then counted with a magnifying glass.

Bacteria (H'): the ability of soil bacteria to degrade the 31 substrates present in the Biolog ™ ECO microplates (Biolog Inc, Hayward, CA, USA) at 595 nm was measured. The Shannon functional diversity index (H') was calculated according to the procedure described by Mijangos et al. [24].

Basal respiration (mg C-$CO_2$ $kg^{-1}$ $h^{-1}$): the $CO_2$ emission rate from the soil during the first three days of incubation was measured in hermetic flasks at 30 °C, with a NaOH solution and without addition of external nutrients, according to the ISO 16072:2002 method.

Induced respiration (mg C-$CO_2$ $kg^{-1}$ $h^{-1}$): the $CO_2$ emission rate was determined over the soil incubated at 30 °C for the basal respiration measurement and during the six hours of incubation at 30 °C. A NaOH solution was present after the addition of a nutrient solution of a mixture of glucose, $KH_2PO_4$ and $(NH_4)_2SO_4$, according to the ISO 17155:2002 method.

Respiratory quotient ($qCO_2$): it was calculated as the quotient of basal respiration between induced respiration.

Compaction (MPa): it is defined as the resistance offered by the soil to be penetrated by the roots. It was measured with a CP4011 cone penetrometer (Rimik Pty Ltd., Toowoomba, Australia) along a profile from 0 to 75 cm deep.

$CO_2$ emission: soil respiration was determined in situ using a camera linked to an infrared gas analyser (SCR-1 and IRGA EGM-4, PP Systems, Amesbury, MA, USA) that records the $CO_2$ concentration.

The value of each indicator is classified on a scale from 1 to 9 according to the reference values from the AHC user guide. The status of each of the services is calculated as an average of their indicators, and the global health diagnosis of the agroecosystem, "basic" and "advanced" categories taken separately, as the average of the three services. The scores between 7 and 9 are considered "good", between 4 and 6 "average", and between 1 and 3 "bad".

### *2.3. Statistical Analysis*

The statistical analysis was performed using the R statistical package [25]. The homogeneity of variances was checked a priori using Levene's test. The forage production, soil physico-chemical properties and soil health services values were contrasted by two-way ANOVA considering management (crop and type of fertilization) as the main factor and year as the random effect. Each plot was considered as an experimental unit. Means were compared using Duncan's Multiple Range Test. Significance was set at $p < 0.05$.

## 3. Results

### *3.1. Weather Conditions*

The weather conditions during the winter and summer forage cropping are shown in Table 2. Thermal regime was similar between years. The average temperature of the winter growing season (October–May) was 12.0 °C, with minimum and maximum temperatures of 8.5 °C and 15.6 °C, respectively. For the summer forage growing season (June–September), the average temperature was 18.4 °C, with an average minimum of 15.1 °C and an average maximum of 21.8 °C. Rainfall showed slight variations between years. The second agronomic year was lightly rainier than the first one, with 1192 mm and 1115 mm, respectively, but with fewer rainy days (137 vs. 165). Average rainfall during the winter months was similar between years for the total rainfall, but the average rainfall in the summer months of the second agronomic year was 101 mm higher, with three less rainy days.

**Table 2.** Weather conditions during growth periods of the winter and summer forage crops throughout the agronomic years 2014–2015 and 2015–2016.

| Agronomic Years | 2014–2015 | | 2015–2016 | |
|---|---|---|---|---|
| **Growing Season** | **Winter** | **Summer** | **Winter** | **Summer** |
| Period considered | 20/10/14–25/05/15 | 11/06/15–30/09/15 | 30/10/15–30/05/16 | 11/06/16–03/10/16 |
| Days of crop | 218 | 112 | 214 | 115 |
| Minimum temperature (°C) | 8.4 | 14.9 | 8.6 | 15.3 |
| Maximum temperature (°C) | 15.1 | 21.8 | 16.1 | 21.7 |
| Average temperature (°C) | 11.7 | 18.3 | 12.3 | 18.5 |
| Rainy days | 129 | 36 | 104 | 33 |
| Rainfall accumulated (mm) | 945 | 170 | 921 | 271 |

### 3.2. Forage Yields

The average winter forage yields are shown in Figure 1. The most productive crop was FBIR with 9206 kg DM ha$^{-1}$ compared to 7241 and 5730 kg DM ha$^{-1}$ for FB and IR, respectively.

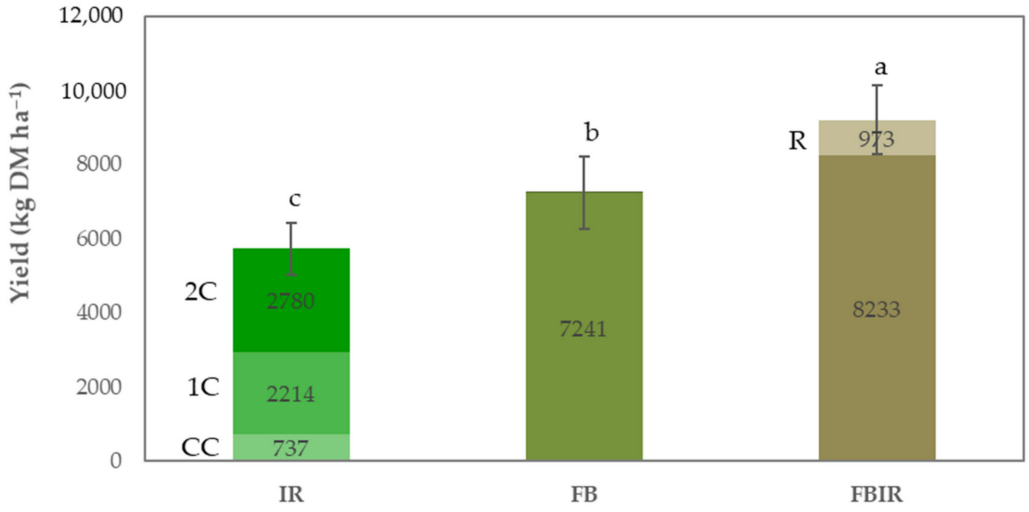



**Figure 1.** Average yields (kg DM ha$^{-1}$) of Italian ryegrass (IR), faba bean (FB) and FBIR during agronomic years 2014–2015 and 2015–2016. CC: cleaning cut; 1C: first cut; 2C: second cut and R: regrowth. a, b, c: Different letters indicate significant differences among treatments.

### 3.3. Soil Physico-Chemical Properties

Chemical properties in the soil are shown in Table 3. pH values were higher ($p < 0.001$) in the alternative managements (FB and FBIR) than in the conventional management. Electrical conductivity (EC), OM, C, Na, and $Al_{ECEC}$ did not differ ($p > 0.05$) between treatments. FBIR had a higher ($p < 0.05$) C/N ratio, ECEC, and Ca, Mg, K, and P contents than IR. These parameters were similar between alternative treatments, except K content, which was higher in FB than FBIR ($p < 0.001$). N content in the IR plot was higher ($p < 0.05$) than in the plots with legumes. All chemical parameters, except pH, N and Na, differed between years ($p < 0.001$). EC, OM, C and C/N increased over time, while Ca, Mg, K, and ECEC declined. Interaction effects between treatments and years were observed for N, Ca, Mg, K and ECEC parameters. Soil N content and ECEC in conventional management were constant over time, but they declined in the last year in alternative management. The concentration of Mg and K declined over time, especially in the FBIR treatment. Soil Ca increased in the second agronomic year in conventional management, while it declined in both alternative managements.

**Table 3.** Chemical properties in the soil under conventional management (Italian ryegrass and chemical fertilization—IR) and alternative managements (faba bean monoculture and organic fertilization—FB—and faba bean–Italian ryegrass intercrop and organic fertilization—FBIR) in two consecutive agronomic years. Results of ANOVA analyses (significance) to test effect of the management strategies (M), the agronomic years (Y) and the interactions between management (M) and year (Y). Values are means for n = 15 per treatment.

| Chemical Properties | Management Strategies (M) | | | | Significance | | |
|---|---|---|---|---|---|---|---|
| | IR | FB | FBIR | rse | *p* (M) | *p* (Y) | *p* (MxY) |
| pH | 6.18 $^{b}$ | 7.16 $^{a}$ | 7.07 $^{a}$ | 0.562 | <0.001 | 0.574 | 0.267 |
| Electrical conductivity (dS $m^{-1}$) | 0.06 | 0.09 | 0.07 | 0.034 | 0.130 | <0.001 | 0.050 |
| Organic matter (g $kg^{-1}$) | 5.17 | 5.25 | 5.38 | 0.567 | 0.599 | <0.001 | 0.158 |
| C (g $kg^{-1}$) | 3.00 | 3.04 | 3.12 | 0.328 | 0.597 | <0.001 | 0.155 |
| N (g $kg^{-1}$) | 0.21 $^{a}$ | 0.18 $^{b}$ | 0.18 $^{b}$ | 0.033 | 0.031 | 0.294 | 0.044 |
| C/N ratio | 14.89 $^{b}$ | 17.48 $^{ab}$ | 18.29 $^{a}$ | 3.566 | 0.036 | <0.001 | 0.131 |
| Ca ($cmol^{(+)}$ $kg^{-1}$) | 11.42 $^{b}$ | 12.04 $^{ab}$ | 12.74 $^{a}$ | 1.111 | 0.011 | <0.001 | 0.024 |
| Mg ($cmol^{(+)}$ $kg^{-1}$) | 0.61 $^{b}$ | 0.69 $^{ab}$ | 0.76 $^{a}$ | 0.132 | 0.015 | <0.001 | 0.004 |
| K ($cmol^{(+)}$ $kg^{-1}$) | 0.24 $^{c}$ | 0.42 $^{a}$ | 0.30 $^{b}$ | 0.060 | <0.001 | <0.001 | <0.001 |
| Na ($cmol^{(+)}$ $kg^{-1}$) | 0.91 | 0.94 | 0.89 | 0.252 | 0.890 | 0.094 | 0.981 |
| Al ($cmol^{(+)}$ $kg^{-1}$) | 0.01 | 0.01 | 0.02 | 0.022 | 0.246 | <0.001 | 0.210 |
| Cation exchange capacity ($cmol^{(+)}$ $kg^{-1}$) | 13.19 $^{b}$ | 14.10 $^{ab}$ | 14.72 $^{a}$ | 1.213 | 0.006 | <0.001 | 0.010 |
| Al exchangeable (%) | 0.06 | 0.04 | 0.10 | 0.115 | 0.336 | <0.001 | 0.372 |
| P (mg $kg^{-1}$) | 17.86 $^{b}$ | 20.94 $^{ab}$ | 23.84 $^{a}$ | 4.084 | 0.002 | <0.001 | 0.147 |

rse: residual standard error; M: management (crop and fertilization); Y: year (2014–2015 and 2015–2016). $^{a, b, c}$: Different letters indicate significant differences among managements ($p < 0.05$).

### *3.4. Soil Health Diagnosis*

The results of soil health diagnosis are shown in Table 4. In the basic diagnosis, macrofauna and soil colour indicators did not differ ($p > 0.05$) among managements. However, earthworm abundance and infiltration time were more favourable in allotments with legumes and organic fertilization (FB and FBIR) than IR and chemical fertilization. Although the abundance of earthworms was notably higher under alternative management (FB, FBIR, $p < 0.001$), the scores are considered "average" according to the AHC reference values. Infiltration time was significantly lower in plots under alternative management (2.08 and 0.90 vs. 13.90 min for FB, FBIR and IR, respectively, $p < 0.05$). Its score is considered "good" opposite to the "average" score obtained by the conventional management. The final scores were 5.72 and 5.85 vs. 4.50 for FB, FBIR and IR, respectively. Therefore, the basic diagnosis indicated that soil health under alternative management was better ($p < 0.001$) than under conventional management. The agronomic year had a significant effect on the records for the macrofauna, the earthworms, and the infiltration time.

In the advanced diagnosis, the mesofauna differed ($p < 0.001$) among the three managements, with the highest diversity values in the FBIR plot. Bacteria diversity was different between conventional and alternative managements, but it was similar between FB and FBIR. In any case, the scores for both indicators were relatively low according to the AHC reference values. Basal and induced respiration results were higher ($p < 0.001$) under alternative management (FB and FBIR), and the scores were considered as "good". Regardless, the induced respiration was significantly higher in the alternative management, and the respiratory quotient was similar between treatments. Compaction results were in accordance with the infiltration results obtained in the basic diagnosis, so alternative management tended ($p = 0.053$) to be more favourable compared to the other managements. Contrary to the rest of the parameters, $CO_2$ emissions were lower with conventional management ($p < 0.001$) and obtained a better score in the global change mitigation service. Advanced diagnosis of agroecosystem health was also better with alternative management, although only FBIR presented significant differences ($p < 0.05$) compared to the conventional management. All indicators of soil health diagnosis, except colour, $qCO_2$ and $CO_2$ emissions, showed significant differences between years. Mesofauna and bacteria increased over time,

while basal and induced respirations decreased. Interaction effects between treatments and years were observed for infiltration, mesofauna and compaction.

Chemical and biological indicators of soil health are compared in Figure 2. Regarding the chemical indicators, K and N had the lowest balance, although a light separation between managements was observed with K (Figure 2A). The most unbalanced biological indicator was the respiratory quotient, while the abundance of earthworms and the mesofauna showed a clear separation among managements (Figure 2B).

**Table 4.** Soil health diagnosis results under conventional management (Italian ryegrass and chemical fertilization—IR) and alternative managements (faba bean monoculture and organic fertilization—FB—and faba bean–Italian ryegrass intercrop and organic fertilization—FBIR) in two consecutive agronomic years and their interactions between management (M) and year (Y). Values and scores are means of n = 6 per treatment. Results of ANOVA analyses (significance) testing the differences between management strategies (M), years (Y) and the interaction between both factors.

| | Management Strategies (M) | | | | | | | Significance | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | IR | | FB | | FBIR | | rse | *p* (M) | *p* (Y) | *p* (MxY) |
| **Health Services:** | **Value** | **Score** | **Value** | **Score** | **Value** | **Score** | | | | |
| Biodiversity conservation | | | | | | | | | | |
| Macrofauna (n°) | 2.33 | 3.28 | 2.83 | 3.72 | 3.17 | 4.02 | 0.682 | 0.213 | <0.001 | 0.467 |
| Soil conservation | | | | | | | | | | |
| Earthworms (n° $m^{-2}$) | 2.67 | 1.47 $^{b}$ | 50.67 | 5.03 $^{a}$ | 48.00 | 5.25 $^{a}$ | 1.068 | <0.001 | <0.001 | 0.099 |
| Infiltration (min) | 13.90 | 6.52 $^{b}$ | 2.08 | 8.40 $^{a}$ | 0.90 | 8.73 $^{a}$ | 0.887 | 0.002 | 0.001 | 0.003 |
| Global change mitigation | | | | | | | | | | |
| Soil colour (score) | 6.17 | 6.17 | 6.67 | 6.67 | 6.58 | 6.58 | 0.425 | 0.134 | 0.422 | 0.797 |
| Basic diagnosis score | | 4.50 $^{b}$ | | 5.72 $^{a}$ | | 5.85 $^{a}$ | 0.420 | <0.001 | 0.387 | 0.942 |
| Biodiversity conservation | | | | | | | | | | |
| Mesofauna (BQ) | 38.48 | 3.87 $^{c}$ | 48.07 | 4.52 $^{b}$ | 58.57 | 5.22 $^{a}$ | 0.361 | <0.001 | 0.001 | 0.019 |
| Bacteria (H′) | 2.95 | 3.93 $^{b}$ | 3.52 | 5.05 $^{a}$ | 3.78 | 5.78 $^{a}$ | 0.660 | 0.001 | <0.001 | 0.104 |
| Soil conservation | | | | | | | | | | |
| Basal respiration (mg C-$CO_2$ $kg^{-1}h^{-1}$) | 1.17 | 6.82 $^{b}$ | 1.92 | 8.62 $^{a}$ | 2.00 | 8.78 $^{a}$ | 0.514 | <0.001 | 0.002 | 0.128 |
| Induced respiration (mg C-$CO_2$ $kg^{-1}h^{-1}$) | 5.25 | 2.58 $^{b}$ | 8.02 | 3.43 $^{a}$ | 3.37 $^{a}$ | 7.90 | 0.258 | <0.001 | <0.001 | 0.158 |
| Respiratory quotient ($qCO_2$) | 0.23 | 1.00 | 0.25 | 1.00 | 0.27 | 1.00 | <0.001 | 0.397 | 0.337 | 0.397 |
| Compaction (MPa) | 2131.68 | 5.75 $^{b}$ | 1625.08 | 6.68 $^{a}$ | 1658.72 | 6.73 $^{a}$ | 0.698 | 0.053 | 0.058 | 0.004 |
| Global change mitigation | | | | | | | | | | |
| $CO_2$ emissions (g $CO_2$ $m^{-2}$ $h^{-1}$) | 0.52 | 7.98 $^{a}$ | 0.94 | 7.13 $^{b}$ | 1.28 | 6.52 $^{b}$ | 0.493 | <0.001 | 0.358 | 0.130 |
| Advanced diagnosis score | | 5.18 $^{b}$ | | 5.53 $^{ab}$ | | 5.70 $^{a}$ | 0.282 | 0.023 | <0.001 | 0.680 |

BQ: biological quality index; H′: Shannon Index; $qCO_2$: microbial metabolic quotient; rse: residual standard error; M: management (crop and fertilization); Y: year (2014–2015 and 2015–2016). Scores were obtained by comparing the values observed with the reference values (scale from 1 to 9 for each parameter) in the AHC guide [22]. $^{a, b, c}$: Different letters indicate significant differences among managements ($p < 0.05$).

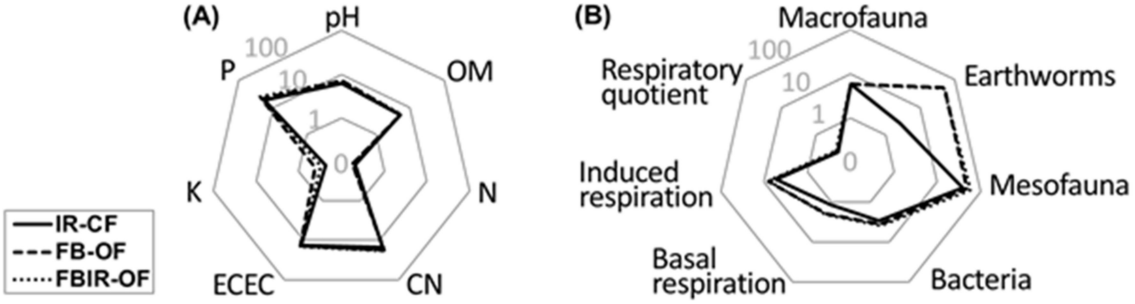



**Figure 2.** Comparison of the soil chemical (**A**) and biological indicators (**B**) between treatments. IR: Italian ryegrass crop and chemical fertilization; FB: faba bean and organic fertilization; FBIR: faba bean–Italian ryegrass intercrop and organic fertilization; OM: organic matter; CN: carbon/nitrogen ratio; ECEC: effective cation exchange capacity. Indicators' units were normalized using a logarithmic scale.

## 4. Discussion

Italian ryegrass (*Lolium multiflorum* Lam.) is widely used in the dairy farms of the Principality of Asturias (North of Spain) due to its easy and quickly establishment, high forage yield and high quality silage [26]. Studies carried out by Martínez-Fernández et al. [27] in the same area reported productions for IR up to 8700 kg DM $ha^{-1}$. This high production is achieved by applying high rates of N fertilizers. This intensively managed system requires many farming tasks and it could result in a continuous environmental degradation, particularly of the soil [28]. Furthermore, mowing during the winter months is often difficult due to the adverse environmental conditions, including an excess of soil moisture, which makes the machinery work difficult. Additional disadvantages include the increased cost of several cutting systems, losses of protein content and the risk of lodging if a single cut is made [29]. The alternatives tested in this experiment (FB and FBIR, both under organic fertilization) use less agricultural machinery because all the production would be obtained in a single cut. FBIR was the most productive alternative, even without including the additional cut of the Italian ryegrass regrowth in the second year of the experiment. Numerous studies verified that the management systems based on grass-legume mixtures would improve forage productivity and they contribute substantially to a more sustainable and environmentally friendly agriculture [30,31]. Additional studies have shown that the intercropping of FB with other species significantly increases the production and it provides very profitable yields [32–34], mainly due to the increase in soil fertility [35]. Other studies indicate that the increased biodiversity of crop associations may improve ecosystem services, including forage yield [36]. According to Cardinale et al. [37], such diversity effects can be explained by an improved acquisition of resources in time and space, called niche complementarity, the generation of positive interspecific interactions (facilitation) and the selection of the most productive species (sampling or selection effect).

The soil chemical parameters were not able to establish clear differences between conventional and alternative management after two years of study. For example, the OM is considered a fundamental indicator of soil quality [38], but it did not show differences among managements. However, the OM content was higher at the end of the assay due to the fertilization effect. A similar effect was observed for the C concentration and the C/N ratio. Therefore, in this study, soil OM may be a poor indicator for agricultural land when comparing different managements, since its content is influenced by the fertilizers' application [39]. The C/N ratio is a good index of soil OM mineralization and, in warm climates, its value must be between 10 and 12 [40]. In this study, the C/N ratios were equal or higher than 15, which indicates a slow mineralization and humification of the OM in all managements. The EC records differed significantly between years, with higher values during the second agronomic year. However, its values remain within the range of soils classified as non-saline (<0.35 dS $m^{-1}$) [14]. Therefore, this parameter does not seem to be a good indicator in areas with abundant rainfall. Similarly, the records of ECEC decreased between years, especially with both alternative managements, but they remained within the range considered normal (10–20 $cmol^{(+)}$ $kg^{-1}$) [14]. The pH under conventional management (IR) was slightly more acidic than under the alternative managements (FB and FBIR), although it remained within the pH range, which is considered suitable for most crops. A pH between 5.6 and 7.3 is generally most favourable for plant growth because most of the nutrients are readily available in this range [14]. The K content was the only chemical parameter that showed significant differences among managements, between years and their interactions. The high variability of K in cattle slurry could explain these differences and interactions. FB and FBIR had higher K concentrations than IR. However, these contents are low considering that these plots were fertilized with cattle manure. Differences between years and interaction effects between management and year were observed in Ca and Mg. The fertilization with K could lead to a decrease in the availability of Mg, and an excess of Ca inhibits the absorption of Mg. The Mg deficiency in the soil was observed in all subplots regardless of type of management (crop and type of fertilization) due to the antagonistic effect when the Ca/Mg ratio is above 10 and the K/Mg ratio is

above 0.3. A phosphoric fertilization was carried out in both managements to balance the extractions due to the harvest. This could explain the small losses by erosion that are reflected in significant differences between years while medium fertility values were maintained (16–25 mg $kg^{-1}$) [40].

The biological and biochemical properties of the soil can respond more quickly to management activities and disturbances than the physical and/or chemical ones [41,42], and thus they can be more suitable for short-term assessments of soil quality and degradation. Indeed, biological indicators provide early warnings of system collapse and allow us to react before irreversible damage to the integrity and functioning of the soil ecosystem occurs [43]. The results of the soil health study show that the biological indicators, such as soil biodiversity and its activity, were affected by the conventional management (Italian ryegrass crop and chemical fertilization), with significant decreases compared to the alternative management (faba bean crop and bean–Italian ryegrass intercrop and organic fertilization with cattle slurry).

The macrofauna (>1 mm) is the first link of the soil trophic chain and it is responsible for starting the decomposition of the organic remains to make them available to the lower trophic levels. In the three plots, the soils showed low values of this indicator and no differences among managements and years, and these results may correlate with the slow organic matter mineralization observed through the C/N relationship in all of them. Within soil macrofauna, the most studied group to assess soil structure are earthworms [44]. The abundance of this indicator was notably higher in the alternative management and it concurred with higher values of the physical parameters, such as infiltration and compaction, with the alternative managements and along time. In the study, both alternative managements decreased the soil compaction and consequently improved the infiltration capacity over time. The movement of earthworms through the soil profile creates a network of interconnected channels that favour infiltration and allow the passage of roots without the need to break the aggregates [45], and this is vital for the agricultural soils to mitigate soil compaction. In this study, the infiltration time was very low in the plots with alternative management against the plot with conventional management, which required about a quarter of an hour to infiltrate the same amount of water. Under these circumstances, a moderate risk of flooding and/or surface runoff problems in the higher slope areas during rainy episodes could occur. The greatest earthworm abundance and the consequent improvement in the water and air circulation provided a greater availability of labile substrates, which could explain the higher microbial activity estimated through basal respiration. A high basal respiration indicates that soil is active and therefore functioning, but it also means its $CO_2$ emissions are higher. Soil mesofauna (0.1–1 mm) is very sensitive to edaphic environmental changes, so they are considered good indicators of soil health [46]. HBRI showed the best values for this indicator, especially during the last agronomic year, which suggests that crop associations promote biodiversity in the soil–plant system. According to Hervé and Vidal [47], the edaphic invertebrate communities are favoured by environments with high floristic diversity.

Considering the soil biological parameters, the greatest bacteria diversity in the soil was detected under alternative management and it reveals the potential of the bacterial communities of these plots to catabolize several C substrates [48]. On the other hand, the microbial abundance (biomass), estimated indirectly from substrate-induced respiration under non-limiting food conditions, was significantly higher under the alternative management. The microbial biomass refers to the living part of soil OM and its value is well established as a bioindicator of soil fertility [49]. It is a more sensitive measure of the changes in soil health than the OM content, since it reveals trends in time periods of 1–5 years as in this study. The reductions in the microbial biomass are usually related to a decrease in the soil carbon input or, for example, to intensive tillage and/or the use of toxic substances [50]. Although the values of this indicator increased under the alternative management, they were low, so the respiratory quotient (basal/induced respiration) is also low, even under the alternative management. This result is likely related to an inefficient use of

the available carbon by the microbial populations, which may maintain high respiration rates, but they cannot increase their biomass. This situation is typical of young ecosystems under evolution (as they mature, they gain biomass and slow their energy flows) or could even suggest the presence of microbial stress conditions as a consequence of the tillage system, if we rule out the presence of toxic substances. On the other hand, a high respiratory quotient could favour the release of carbon from the soil into the atmosphere as $CO_2$. For this reason, $CO_2$ emissions were higher under alternative management because the increase in microbial activity (basal respiration) was not accompanied by a simultaneous increase in its biomass (induced respiration), leading to negative results for the edaphic service of "Fight against climate change" (carbon sequestration).

In addition to yield and soil protection, one of the fundamental criteria to be considered before replacing Italian ryegrass must be the protein self-sufficiency. The new policies increasingly support alternative strategies based on local and sustainable protein sources to reduce the dependence on soybean for ruminants. Thus, the legume winter forages proposed in the present study could play an important role in animal nutrition and farm self-sufficiency, since they show higher levels of protein than Italian ryegrass.

## 5. Conclusions

Chemically fertilized Italian ryegrass can be replaced by faba bean in monoculture or intercropped with Italian ryegrass with organic fertilization since these crops present similar or higher yields than IR and they concentrate all the production in a single cut.

The inclusion of legumes and organic fertilization in the alternative managements improves key soil parameters linked to important ecosystem services such as the abundance of earthworms, soil infiltration, soil biodiversity and soil compaction. This strategy improves the edaphic quality of an agricultural soil aimed at forage production.

The biological indicators were more sensitive and differentiated the managements faster than the chemical indicators.

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