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The Diversification and Intensification of Crop Rotations under No-Till Promote Earthworm Abundance and Biomass

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Abstract: The diversification and intensification of crop rotations (DICR) in no-till systems is a novel approach that aims to increase crop production, together with decreasing environmental impact. Our objective was to analyze the effect of different levels of DICR on the abundance, biomass, and species composition of earthworm communities in Argentinean Pampas. We studied three levels of DICR—typical rotation (TY), high intensification with grass (HG), and with legume (HL); along with three references—natural grassland (NG), pasture (PA), and an agricultural external reference (ER). The NG had the highest earthworm abundance. Among the DICR treatments, abundance and biomass were higher in HL than in HG and, in both, these were higher than in TY. The NG and PA had a distinctive taxonomic composition and higher species richness. Instead, the DICR treatments had a similar richness and species composition. Earthworm abundance and biomass were positively related to rotation intensity and legume proportion indices, carbon input, and particulate organic matter content. The application of DICR for four years, mainly with legumes, favors the development of earthworm populations. This means that a subtle change in management, as DICR, can have a positive impact on earthworms, and thus on earthworm-mediated ecosystem services, which are important for crop production.

Keywords: soil; soil properties; macrofauna; earthworms; biodiversity; sustainability; soil invertebrates; farming systems

1. Introduction

In the early 1990s in Argentina, genetically modified soybean cropping was approved. After this, soybean monocropping, a wide adoption of no-till and an expansion of the agricultural area in detriment of natural ecosystems occurred, and is still being carried out in the Pampas region. The current agricultural system that prevails in our country is based on simplified practices, with very low crop diversity, and it generates soils impoverished in structure and nutrients. Furthermore, it is a system that is highly dependent on chemical inputs and GMOs [1–3]. However, in the last years, no-till farmers attempted to improve the simplified, low rotation, or monocropping systems, with the inclusion of "good agricultural practices" (GAP), as an integral part of no-till, i.e., mixed crop rotation, cover crops, integrated pest-weed and disease management, nutrient recycling, and a rational use of agrochemicals [4–6]. The GAP promoted a higher particulate organic carbon (POC) content [7] and induced favorable structural features [8] in the crop soils of the study region. Recently, some producers began to explore a new no-till alternative, which implies the diversification and intensification of crop rotations (DICR). Through means of intensifying the rotation sequences by including a greater number of crops per unit of time, a more efficient and intensive use of environmental resources, such as water and solar radiation is achieved. This higher efficiency allows us to maintain or increase crop production per unit of time and area, in a less harmful way, and contributes to a higher C return to the soil [9–11]. Moreover, well-balanced sequences between grasses and legumes provide stubbles that increase the contribution of C and N to the soil and consequently the productivity of the next crops [11].

Soil fauna perform important functions, including soil structure improvement, nutrient cycling, and organic matter decomposition. These processes might become much more important in no-till systems where there is no mechanical loosening of soil or mixing of soil and residues [12]. Among soil fauna, earthworms are a fundamental component. They are considered "ecosystem engineers" for their ability to directly or indirectly transform the availability of resources for their own benefit and for other species [13]. Earthworms improve soil structure, renew the organic matter, participate in the cycling of nutrients, and modify the bacterial community [14–17], directly and indirectly favoring plant productivity [18]. In a recent meta-analysis, Van Groenigen et al. [19] estimated that the presence of earthworms in agroecosystems produced an average increase of 25% in crop yields and a 23% increase in the aerial plant biomass. In a previous study in the Pampas region, we demonstrated that in no-till systems with GAP, earthworms significantly contributed to C incorporation, via differential consumption of soils enriched in organic matter and the consequent enrichment of earthworm aggregates (in no-till, 100% more POC was found in earthworm aggregates than in the surrounding soil). Furthermore, we also demonstrated that they contributed to soil structure through the production of macroaggregates that are more stable to water disruption than those physically generated [20]. Therefore, the conservation of the earthworm community is a key aspect to develop strategies that aim to increase agricultural productivity in a more sustainable way.

Soil management practices affect earthworm populations by affecting the food supply, mulch protection, and the chemical and physical environment [12]. The beneficial effects of no-till on earthworm populations compared to conventional tillage is widely demonstrated [21–23]. Some authors also highlight the importance of incorporating cover crops and of diversifying rotations to increase earthworm biomass and abundance in agricultural systems (e.g., [18,24]). Although several studies were carried out on earthworm communities in the agricultural soils of the Pampas region [4,25–27], and the positive effect of the inclusion of GAP was demonstrated [6], there are no studies that evaluated the effect of the different levels of diversification and intensification of crop rotation in no-till, on earthworm communities. The DICR is a relatively recent management approach through which economic sustainability is being tested by some producers, and whose impact on environmental sustainability indicators is extremely necessary to be evaluated. Therefore, the objective of the present study was to analyze the effect of different levels of diversification and intensification of crop rotations on the abundance, biomass, and species composition of the earthworm community. For this, we studied three levels of DICR in no-till systems—(1) typical rotation, (2) high intensification with grass, and (3) high intensification with legume. In addition, two internal references systems, a natural grassland and a long-term pasture, and an external reference of no-till with low rotation were studied. We hypothesized that—(1) earthworm abundance and biomass will be higher in the natural grassland than in the agricultural sites, and among them, on the higher rotations with higher DICR levels, with the highest positive effect on legume than grass rotation; and (2) the natural grassland will have a higher species richness and different community composition, compared to the agricultural treatments, because they can harbor species that are highly sensitive to the disturbances produced by agriculture. Among agricultural treatments, the most intensified and diversified rotation will have a community composition more similar to that of the natural grassland than the lower rotation treatments, because they contributed to a more diverse and better quality food for earthworms.

2. Material and Methods

2.1. Study Area

A field experiment with different levels of DICR was carried out in four localities in the most productive area of the Pampas region of Argentina. Two localities were localized in the Buenos Aires province, close to the Ines Indart (34°23′48′′ S, 60°32′29′′ W; "La Matilde" farm) and Baradero (33°48′37′′ S, 59°30′17′′ W; "Las Matreras" farm) cities. The two others were localized in the Santa Fe province, near the Venado Tuerto (33°44′40′′ S, 61°58′09′′ W; "Carmen" farm) and Uranga (33°15′44′′ S, 60°42′28′′ W; "San Nicolas" farm) cities (Figure 1). Soils in the area were Mollisols (USDA Soil Taxonomy) or Phaeozem (World Reference Base for Soil Resources); in La Matilde, Las Matreras, and San Nicolas, the soils were Typic Argiudolls (silty clay loam), according to the USDA classification, with a well-developed illuvial horizon (Bt), while the Hapludolls soils dominate in Carmen, with a higher sand proportion [28,29]. Climate in the region was temperate sub-humid with a dry season in winter, and with a mean annual temperature at about 16–18 °C. The mean annual precipitation in the La Matilde, Las Matreras, and San Nicolas was about 950–1100 mm, while in Carmen, it was about 850–950 mm. The relief in the region was flat with a gentle slope, which was, in all sites, lower than 0.5%.

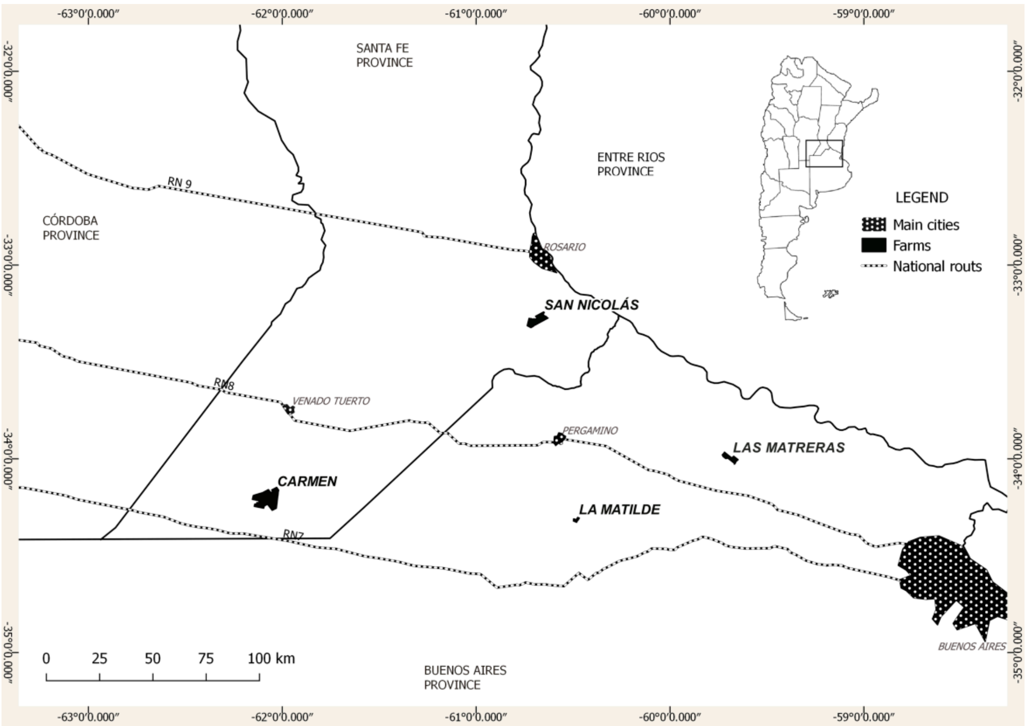
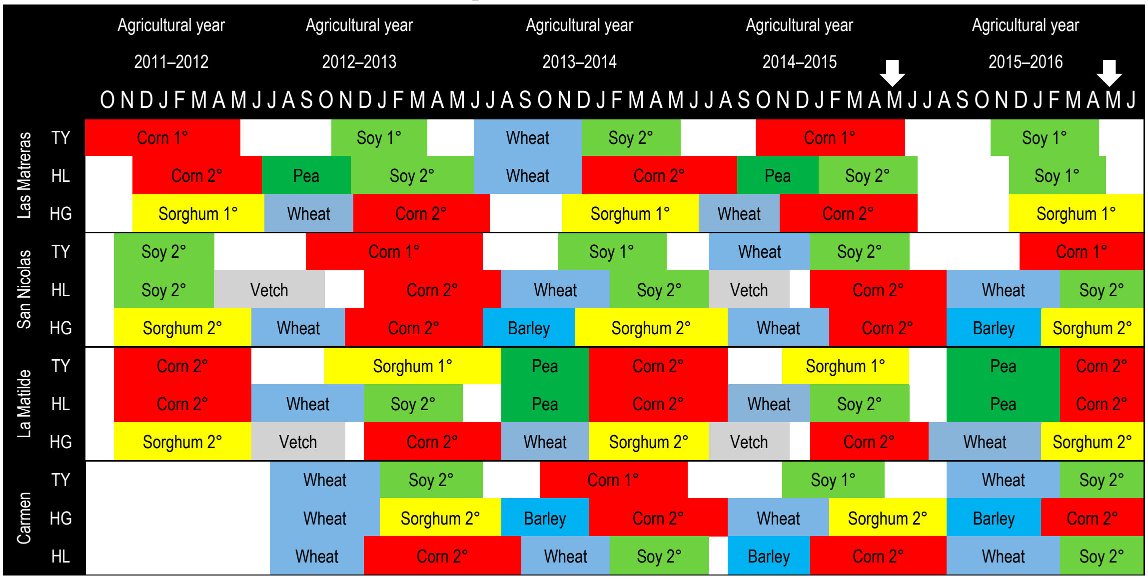


Figure 1. Study area in the Pampas region of Argentina. The farms are represented by black-filled figures, the main cities are represented by the dotted figures and the routes are shown by the dotted lines.

2.2. Experimental Design and Sampling

In 2011, farmers started the DICR field experiment in 4 localities. In each one, a large plot with a single and homogeneous land-use history was selected for performing the DIRC experiment. In the 10 years prior to the beginning of the experiment, every plot was managed under no-till, with soybean in the low-rotation scheme, and corn as the unique summer crop. Wheat was the only winter crop, although bare soil during winter was a frequent situation. In each locality, these plots were subdivided into four smaller plots, about 10 to 25 hectares for each one. In three of these plots, a crop rotation scheme of 3-year cycles with variations in crop intensity and diversity was established. The rotation scheme involved three DICR levels—TY (typical rotation), HG (high intensification with grass), and HL (high intensification with legumes) (Table 1). The fourth plot was cropped with a consociated pasture legume/grass (PA), which is considered to be an internal reference system. Additionally, two other references were selected—(1) natural grassland (NG), a site located in each farm with more than 30 years without agricultural intervention, with a mix of native and exotic grasses but without trees; and (2) an agricultural external reference (ER), an agricultural plot located near each farm, selected as representing the usual agricultural management of the region (no-till with low-crop rotation or soybean monocropping).

Table 1. Rotation scheme applied in each farm from the beginning of the essay. The sampling months are highlighted with an arrow. 1°: First sowing date (between October–December), 2°: second sowing date (from December) of the summer crops.



TY—typical rotation, HG—high intensification with grass, HL—high intensification with legume, PA—pasture, NG—natural grassland, and ER—external reference. Soy —Soybean.

Once complete rotation cycle was attempted, we conducted our first sampling in May 2015 and a second (monitoring) sampling in May 2016. In 2015, 24 plots were sampled, consisting of 6 treatments (TY, HG, HL, NG, PA, and ER) in 4 localities (6 treatments × 4 localities), while in 2016, 12 plots were sampled since (i) the trial in Carmen farm was discontinued by the farmers and (ii) only three DICR levels, plus the PA as a reference were monitored (4 treatments × 3 localities). NG and ER were not sampled because both are expected to be more stable over time, given the absence of environmental or management changes; although this is also true for the PA, it was kept in the 2016 sampling, as an important reference for being part of the same initial large plot than DICR treatments.

Two rotation indices were used to characterize the DICR levels, this is, the occupation time by crops in rotations, expressed as crops per year of rotation [29,30]:

$$IRI\ (Intensification\ rotation\ index) = EPM/TDR,$$

where "*EPM*" are the days since crop emergence until physiological maturity, and "*TDR*" are the total days of rotation; and

$$ILI\ (Intensification\ legume\ index) = LEPM/TDR,$$

where "*LEPM*" are the days since emergence until physiological maturity of the legume crop.

Additionally, the average carbon input (CIn) by year for each rotation was calculated as:

$$CIn = \sum CIn_c\ (Bio \times 0.4 \times HC)/YR$$

where "CIn_c" is the humified carbon input by each crop in rotation, "*Bio*" is the total biomass contributed by the crop (aerial and root biomass), "0.4" is the C content of dry matter, "*HC*" is the humification coefficient of the crop, and "*YR*" refers to the years of the rotation [29,31].

2.3. Earthworms

Earthworms were sampled by the standardized sampling method in ISO [32]. In each sampling plot, five random sampling points were selected while avoiding the plot edges. In each point, a soil monolith of 25 × 25 × 20 cm was extracted and split into layers—0 to 10 cm and 10 to 20 cm in depth. The earthworms were obtained carefully by hand-sorting the soil sample and then fixed in 96% alcohol. Once in the laboratory, earthworms were counted and weighed, and the adults were identified to the species level, using the taxonomic keys of Righi [33], Mischis and Moreno [34], Blakemore [35], and Momo and Falco [36]. Numbers and biomass of earthworms obtained from each monolith (0.0125 m^2) were expressed to 1 m^2. Biomass was not obtained in 2016 because juveniles were bred to maturity for taxonomic identification.

2.4. Soil Parameters

At each sampling point, soil samples were collected to determinate the following soil parameters:

Bulk density (BD) was determined by the cylinder method [37]. Soil samples were taken from 0–10 cm and 10–20 cm soil depth as duplicate undisturbed samples, using 100 cm^3 cylinders. Each sample was wet-weighted, dried in the oven for 24 h at 105 °C, and weighted again, to perform the calculation.

Particulate organic carbon (POC) after earthworm hand-sorting was carried out by collecting 100 g of soil from each monolith. The physical soil fractionation by particle size was conducted by the wet sieving method described in [38], obtaining two fractions: <53 μ and >53 μ. The determination of the CO content of each fraction was quantified by the Walkley & Black method [39].

Stubble biomass (Bio), i.e., the vegetable cover of each monolith was collected, dried in the oven at 40 °C, and weighted.

2.5. Statistical Analyses

The effect of the treatments on earthworm abundance was analyzed by generalized mixed linear models (GMLM). The models selected were those that presented the lowest AIC value [40] and a ratio (deviance)/(degrees of freedom) lower than 2.5 [41]. The model selected for the 2015 data was as follows—the treatment (TY, HG, HL, PA, NG, and ER) was considered as fixed factor; the locality (4), the depth (0–10 and 10–20 cm), and the sample (nested into treatment and locality) were the random factors. To analyze the effect of DICR treatments plus PA (reference) in both sampling years, a second model was performed by considering the abundance data of 2015 and 2016, where only the three DICR levels and the pastures were monitored. The selected model had the treatment (TY, HG, HL and PA) as a fixed factor, and the locality (except CA), the year, and the sample (nested into treatment and locality) as random factors. Due to data overdispersion, the abundance values were adjusted with a negative binomial distribution. The Di Rienzo, Guzman, and Casanoves (DGC) a posteriori test [42] was used to evaluate the significance of differences between treatments, when the p values were significant ($p < 0.01$). For earthworm biomass, the effect of the treatments was analyzed by the general mixed linear model and the model with the lowest AIC value was selected [43]. Prior to the analysis, data were transformed with base 10 logarithm (Log 10 (x + 1)) to fit the normal distribution. The *VarIdent* function was used to decrease the heterocedasticity of the data. In the selected model, the treatment (TY, HG, HL, PA, NG, and ER) was considered to be a fixed factor and the locality and the depth (0–10 and 10–20 cm) were the random factors. To assess the significant differences between management ($p < 0.01$), DGC was used as a posteriori test [42]. The species richness was analyzed by the general mixed linear model [43]. The treatment (TY, HG, HL, PA, NG, and ER) was considered as a fixed factor, while the locality was considered as a random factor. To assess the significant differences between management ($p < 0.01$), DGC was used as a posteriori test [42]. In both, biomass and richness models, the assumptions of variance homogeneity and normality were analyzed graphically, and in addition, normality was corroborated with the Shapiro-Wilks test.

To evaluate changes in the species composition of earthworm communities between the different treatments, principal component analysis (PCA) was performed. Prior to the analysis the abundance data were transformed according to Hellinger [44].

The relationships between earthworm abundance and biomass in 2015, with the soil and the rotation parameters, were analyzed by mixed models. Each soil (Bio, BD, and POC) and rotation (IRI, ILI, and CIn) parameter was considered as a fixed factor, and the locality was considered to be a random factor. The earthworm abundance data were analyzed by generalized mixed linear models with negative binomial distribution, while the log-transformed biomass data were analyzed by general mixed linear models. For each model, conditional R^2 was calculated using the "*r.squaredGLMM*" function (which describes the proportion of variance explained by both the fixed and random factors). The regressions of mixed models were plotted using the *VISREG* function [45].

All analyses were performed in R [46] and the Infostat [47] software.

3. Results

3.1. Earthworm Communities

The earthworm abundance in 2015 was affected by treatments ($p < 0.0001$) (Figure 2). The highest abundance was observed in NG, which presented more than twice the observed abundance in HL and ER. Among the DICR treatments, earthworm abundance was about twice in HL than in the HG and TY rotations, which had the lowest earthworm abundances. As well as earthworm abundance, the biomass in 2015 was also different between treatments ($p = 0.0034$) (Figure 3). The PA, NG, HL, and ER had the highest biomass values. Among the DICR treatments, the HL had the highest biomass, almost twice than in HG and TY, which had the lowest biomass of all treatments.

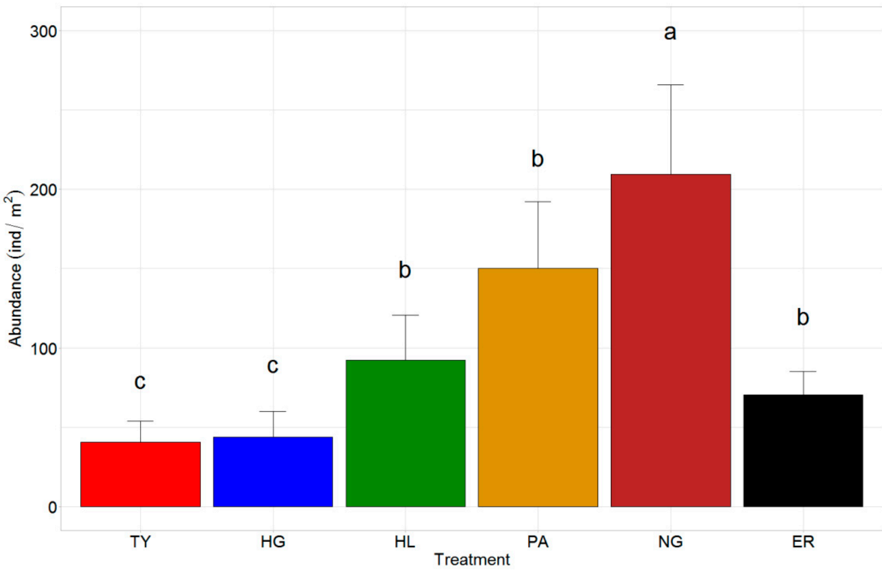


Figure 2. Earthworm abundance in the different treatments in 2015. Different letters indicate significant differences between treatments (DGC, $p < 0.05$). TY—typical rotation, HG—high intensification with grass, HL—high intensification with legume, PA—pasture, NG—natural grassland, ER—external reference, and AIC—1140.72.

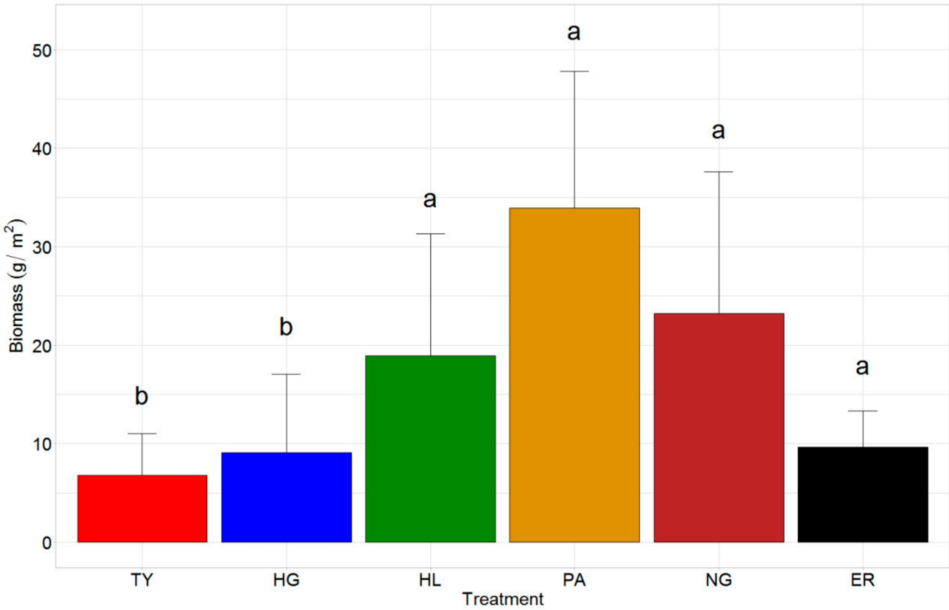


Figure 3. Earthworm biomass in the different treatments in 2015. Different letters indicate significant differences between treatments (DGC, $p < 0.05$). TY—typical rotation, HG—high intensification with grass, HL—high intensification with legume, PA—pasture, NG—natural grassland, ER—external reference, and AIC—11.72.

In the second abundance model, where the 2015 data of the DICR levels and the PA were analyzed together with the data from 2016 monitoring, the effect of treatments on earthworm abundance was significant ($p = 0.0102$); variance explained by year as a random factor was very low (0.11) suggesting DICR treatment as the main explaining factor. The PA, HL, and HG had greater abundances with respect to TY, confirming the observed differences in the first sampling year.

The species richness was significantly higher in NG (10 spp.) and in PA (7 spp.), than in ER (4 spp.), HL (2 spp.), and in both HG and TY (3 spp.).

Regarding species composition of earthworm communities in the NG, a distinctive species composition from the rest of the treatments was observed (PC1 50.4%), given by the presence of some exclusive species like *Metaphire californica*, *Amynthas gracilis*, *Glossodrilus parecis*, *Kenleenus armadas* and *Aporrectodea rosea* (Figure 4). The PA also presented a different species composition with respect to most of the treatments, which is mainly evident through axis 2 (PC2 23.9%). An association of HL, HG, and TY was observed, mainly due to the high abundance of *Aporrectodea caliginosa* and *Octolasion cyaneum* in the three treatments.

3.2. Earthworm Relationships with Soil Properties and Management Parameters

Earthworm abundance and biomass were positively related to intensification rotation index (IRI) (vs. abundance = R^2 0.7769, $p = 0.0012$; vs. biomass = R^2 0.7455, $p = 0.0059$) and ILI (vs. abundance = R^2 0.9329, $p = 3.95 \times 10^{-12}$; vs. biomass = R^2 0.7284, $p = 0.0109$) indices and to CIn (vs. abundance = R^2 0.7076, $p = 0.00562$; vs. biomass = R^2 0.7132, $p = 0.00728$) (Figure 5). In the case of IRI, the observed pattern was also associated with treatments (Figure 5a,b); the PA was associated to the highest index values and the highest values of abundance or biomass, the TY to the lowest and the HG and HL to the intermediate values of both index and earthworm abundance or biomass. Regarding ILI, a pattern of treatments association similar to that of IRI was observed, but the lowest values were observed in the HG and not in TY treatment (Figure 5c,d). In the case of CIn, there was only an association of the PA with the highest index values and highest earthworm abundance or biomass, but no pattern of treatment association was clear among the other treatments (Figure 5e,f).

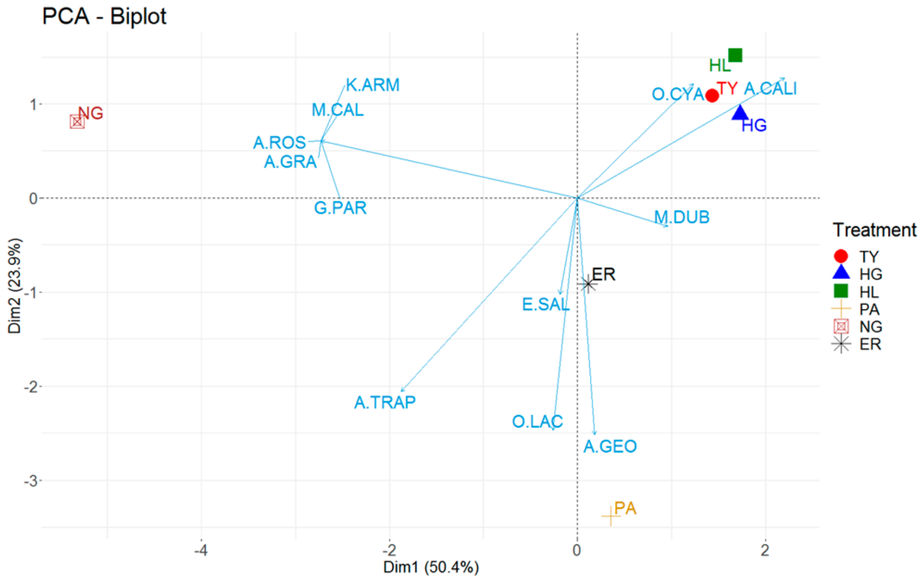


Figure 4. Principal component ordination diagram of Hellinger transformed earthworm community data showing the species vectors projected in the space formed by PCA axes 1 (50.4% of the variation) and 2 (23.9%); for all treatments in the four localities in 2015. Multiple arrows at the same point indicate an overlap of species. O.CYA (*Octolasion cyaneum*), A.CALI (*Aporrectodea caliginosa*), K.ARM (*Kenleenus armadas*), G.PAR (*Glossodrilus parecis*), O.LAC (*Octolasion lacteum*), M.DUB (*Microscolex dubius*), A.TRAP (*Aporrectodea trapezoides*), E.SAL (*Eukerria saltensis*), M.CAL (*Metaphire californica*), A.ROS (*Aporrectodea rosea*), A.GRA (*Amynthas gracilis*), and A.GEO (*Allolobophora georgii*). TY—typical rotation, HG—high intensification with grass, HL—high intensification with legume, PA—pasture, NG—natural grassland, and ER—external reference.

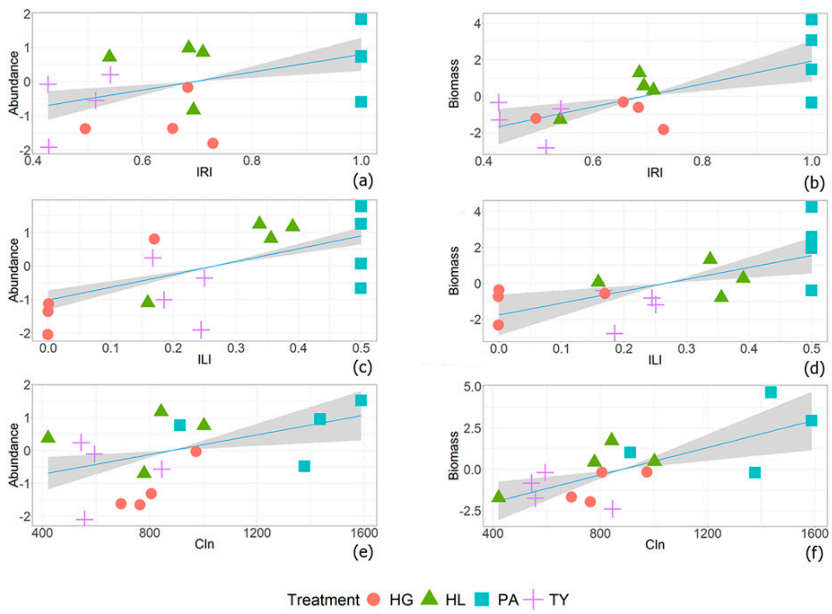


Figure 5. Relationship between intensification rotation index (IRI), intensification legume index (ILI), and Carbon input (CIn) with earthworm abundance and biomass, in the diversified and intensified rotations and pasture in 2015. (**a**) IRI vs. abundance, (**b**) IRI vs. biomass, (**c**) ILI vs. abundance, (**d**) ILI vs. biomass, (**e**) CIn vs. abundance, (**f**) CIn vs. biomass. Shading areas are the confidence intervals 95%. TY—typical rotation, HG—high intensification with grass, HL—high intensification with legume, and PA—pasture.

Regarding the relationships with soil parameters, earthworm abundance and biomass showed a positive marginally significant regression with POC content (vs. abundance, $R^2 = 0.6704$, $p = 0.0584$; vs. biomass $R^2 = 0.6902$, $p = 0.0481$) (Figure 6). The PA was associated with a higher POC and abundance and biomass values, while among DICR treatments, no clear pattern of treatment association was detected. There were no significant relationships between BD and Bio with earthworm abundance and biomass (BD vs. abundance $p = 0.165$; vs. biomass, $p = 0.654$), (Bio vs. abundance $p = 0.2614$; vs. biomass, $p = 0.3392$).

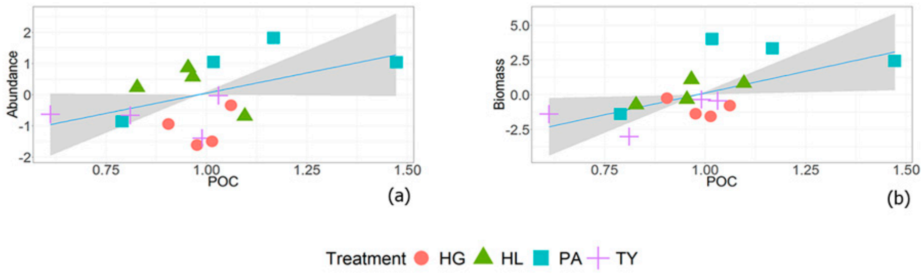


Figure 6. Relationship between earthworm abundance and biomass with soil particulate organic carbon content (POC) in 2015, in the DICR treatments, and PA. (**a**) POC vs. abundance, (**b**) POC vs. biomass. Shading areas are the confidence intervals of 95%. TY—typical rotation, HG—high intensification with grass, HL—high intensification with legume, and PA—pasture.

4. Discussion

The inclusion of good agricultural practices (GAP) in no-till systems, was shown to be positive on litter and soil fauna, however it was suggested that GAP as a management strategy, might be improved by increasing and diversifying crop-rotation intensity [6]. Therefore, in this contribution we studied the effect of three different levels of diversification and intensification of crop rotations under NT. The typical rotation, that could be considered as a similar intensification level as GAP, and two more intensified and diversified systems—high intensification with legumes and high intensification with grass.

4.1. DICR Effects on Earthworm Communities

As expected, the unmanaged grasslands supported the most abundant earthworm community. The abundance decline in the agricultural treatments was consistent with our hypothesis and with previous studies, either in other parts of the world [48] or in the study region [20,25,27,49,50]. The conversion of natural soils to agricultural ones implies deep, unfavorable changes in environmental conditions for earthworm communities [48,51]. In the Pampas region, the simplification of plant diversity, the soil compaction, and the strong dependence on herbicides, insecticides, and fungicides, are considered to be the main reasons of soil fauna reduction in agroecosystems [4].

As expected, the abundance of earthworms in the PA was significantly lower than in NG but higher than in the agricultural treatments. Moreover, both, the PA and NG showed the highest earthworm biomass. The positive effect of pastures on earthworms is likely related to the production of stubble of high nutritive quality, dead roots, and the presence of a permanent vegetation layer that protects earthworms from predation and extreme temperature fluctuations, which favors the development of earthworm populations, in a similar way to natural systems, reaching similar or even higher earthworm biomass [48,52,53]. Grass and legume consociate pastures (as implemented here) improve soil properties through nitrogen fixation by legumes and soil aggregation by grass root systems [54]. Furthermore, they offer a stubble that keeps quantity, quality, and continuity of food supply for earthworms through time [55,56].

Regarding the main objective of our study, our results showed that a four-year period (2011–2015) of intensification and diversification of crop rotations produced a clear positive effect on earthworm abundance and biomass, especially when the high DICR level included legumes. Moreover, this positive effect of DICR was sustained over time, according to the results obtained in the second sampling (2016). In the 2015 sampling, the rotation intensified with legumes (HL) showed a higher earthworm abundance and biomass than the typical rotation (TY), in agreement with our expectations of a higher positive effect of legume than grass rotation. A positive effect of legumes was previously observed on earthworm abundance and biomass [24,57]. Earthworm number and biomass were negatively correlated with the C/N ratio of the roots [57]; and, in short essays, larger weight gains in earthworms fed with residues with low C/N ratios was observed compared to high C/N diet [58]. Legume cover crops used in HL showed markedly lower C/N ratios (12 for pea [59] and 10 for vetch [60]) than the grass species used in HG (barley and wheat with C/N ratios of 109 and 102, respectively [61]). However, including data from the second sampling, the rotation with grasses promoted an earthworm abundance similar to the HL, both being higher than in TY. The difference in the response timing between HL and HG could be explained by the quality of the stubble provided, according to the time necessary for its decomposition and for its nutrients to be available. Crop species of low C/N ratio as legumes provide soil with residues of high nutritional quality and fast decomposition rate, being a fast-food source for earthworms in the short-term, while the opposite was observed for high C/N ratio residues, as grasses [62]. Thus, legume residues in HL provided a fast and better quality food resource for earthworms, promoting a greater abundance and biomass in the first year of sampling, while in HG due to the lower quality and slower decomposition rate of grass residues, the benefits on earthworm populations were observed when the data from the second year of sampling were included. At the same time, the TY rotation presented the lowest abundance and biomass values, demonstrating that the absence of winter cover crops and a high input of chemical herbicides, are unfavorable conditions for earthworm populations.

Regarding the external agricultural reference, in the 2015 sampling, it did not show statistical differences with HL, neither in abundance nor in biomass, although both parameters showed a trend to be higher in HL than in ER. The ER were fields external to the farm where the DICR treatments were performed, therefore, other unknown factors, mainly a different land use and management history, with respect to the field where the DICR assay was stablished, might have influenced this result. Species composition was influenced by biogeographical and historical factors, and thus different species react differently to management practices. Otherwise, this result highlights the importance of the positive effect of HL with respect to TY rotation, since the performance of the different treatments of the assay in the same field, guarantees that the observed differences were caused by the studied crop rotation changes.

In accordance with our hypothesis and with previous findings [63,64], the NG was characterized by an earthworm community different to the agricultural treatments, and also had the highest species richness. Habitat disturbance and physical and chemical alterations due to the change in land use differentially affect earthworm species, reducing the diversity of communities. It was suggested that this occurs because disturbances mainly affect the native species that are more susceptible to environmental change [63,65]. However, in our study the distinctive species composition in NG was given mainly by exotic but not by native earthworm species. This could be because the natural sites we sampled were small relicts that only partially conserve the characteristics from the original landscape and are often exposed to some degree of anthropogenic impact.

The PA had a community different to the DICR treatments and a higher richness with regards to them, similar to the NG. This was likely due to the favorable conditions that are generated in the pasture, where a permanent cover layer offered food and protection to earthworms [48,52,53], which favored a reconstitution of species number over time [48]. Although the species richness in the pasture was high (r = 7), close to that of the NG (r = 10), the composition of the community was different. As Decaens and Jimenez argued [48], when conditions provided by pastures were highly different from those of the initial natural vegetation, the recovery of the original community would be difficult to achieve. In this sense, the characteristics of consociate grass–legume pastures of 3 years sampled in this study were quite far from those of natural sites. However, its benefits in preserving a relatively diverse community are remarkable, considering that the original plot was the same than that for other DICR treatments.

Unlike what we hypothesized, there was no change in community composition between high intensified DICR treatments and TY. The richness in the three DICR systems was low (2 or 3 species), with a dominance of two exotic species—*Aporrectodea caliginosa* and *Octolasion cyaneum*. After four years of starting the DICR experiment, the intensification and diversification of crop rotations promoted earthworm abundance and biomass, but it did not change species composition. It is possible that, due to the low movement rate of earthworms, in general less than 10 m per year, and the limited availability of nearby natural patches that could act as species sources [64,66,67], four years are not enough to increase the richness and to cause changes in species composition. This is especially true when considering that all DICR treatments started from a single field with a homogeneous community. On the other hand, the successful adaptation of the species from the exotic family Lumbricidae, as *Aporrectodea caliginosa* and *Octolasion cyaneum*, in agricultural and cattle-raising fields in the region [68], might make it more difficult for the recolonization of native species in the short-term, due to competitive exclusion effects [64,69].

The ER had a community structure different to all other treatments, mainly characterized by higher *E. saltensis* abundances than the other systems. As we have said, ER system had a different location to the other treatments, and therefore its species composition result from different historical and geographical processes. The difference in earthworm communities among ER and the other systems, might also be related to the relative high abundances that we found, since different species might have different susceptibility to specific management practices.

4.2. Earthworm Relationships with Soil Properties and Management Parameters

The DICR implies an increase in the number of crops in the rotation, in order to increase the level of C input to the system and to improve the balance of C [29]. As expected, the regression models showed a positive relationship between earthworm abundance and biomass with both, IRI and ILI indices, and also with the CIn, showing that, for all analyzed samples, the DICR favored both earthworm abundance and biomass. The PA was generally associated with the highest values of intensification indices and CIn, and the highest values of abundance and biomass of earthworms. The already discussed benefits of the pastures for earthworms are highlighted by the regression analysis. Even more, because in this assay the PA was consociated with legumes and grasses, so in addition to providing a large biomass of litter as food for earthworms, it was of high quality. As the intensification indices decreased from the PA values, the abundance and biomass of earthworms also decreased. Regarding the three intensification treatments, both highly intensified rotations were different from the typical rotation in terms of IRI and both, earthworm abundance and biomass, followed that trend. In case of ILI, the clear response of earthworm abundance and biomass to the index increase confirmed the importance of legumes for earthworms, at least in the short-to-medium term, as in our study. Although the degree of intensification and the theoretical contribution of carbon were similar between the two highest intensified treatments, the rotation with legumes favored earthworms more than the rotation with grasses. As we have pointed out, legumes provide nutritious and high-quality stubble, being a fast food source for earthworms [55,62,70].

Among the soil parameters, the particulate organic carbon (POC) showed a positive relationship with earthworm abundance and biomass. This result agreed with previous studies, which recognize soil organic matter as a key factor for earthworm community development [25,71]. Moreover, the SOM is especially important for endogeic species, which live and feed within the soil [19] and that are the dominating species in the study region. There was no clear pattern relating the treatments with the results of the regression, which indicates that the relationship of POC with earthworms is independent of the treatments. There is a general link between the theoretical calculated C input with the POC levels measured in the soil, except for the typical rotation.

While the C input was higher in both highly intensified treatments than in the TY, this difference was not reflected in the soil POC content. It was likely that this four-year period of DICR was not enough to produce changes in SOM, even in a relatively rapid response parameter as POC.

5. Conclusions

In the present study, the diversification and intensification of crop rotations had a positive effect on both the abundance and the biomass of earthworms, mainly in the rotation that was highly intensified with legumes. We consider the magnitude of the effect to be compelling because of the short-term of the DICR experiment (4 years), and fundamentally because the differences among DICR treatments were relatively minor, compared to what is usually studied in agricultural systems (for example, monoculture vs. rotation, no-tillage vs. plow tillage). The greater input of high-quality trophic resources and the all-year growing roots, promotes the reproduction (more abundance) and growth (biomass) of the earthworms. However, the community structure did not change. For such a change, more time is needed, mainly because the earthworms have a limited migration capacity, moving slowly from one plot to another. In addition, a change in species composition depends on the landscape characteristics, such as the proximity of the cultivated plot to areas with greater species richness.

Overall, our results highlight the earthworm sensitivity to subtle changes in agricultural management and their importance as indicators. Moreover, in this region, earthworms are key drivers of C incorporation and the soil-structure maintenance processes [20]. This means that farmers' decisions (in this case applying DICR) are able to favor earthworm populations, and therefore to improve ecosystem services that are important for crop production. Then, we suggest that the earthworms should be considered when making decisions about agricultural managements.

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