

# **Intensive short-duration rotational grazing is associated with improved soil quality within one year after establishment in Colombia**

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## **Abstract**

Large-scale conversion of natural ecosystems to grazed grasslands and subsequent soil degradation due to overgrazing and inadequate pasture management in tropical agroecosystems urgently call for sustainable intensification of grazing systems, *i.e.* increasing animal productivity while maintaining or improving soil quality and ecosystem services. We investigated the impact of intensive short-duration rotational grazing (IRG) management on soil properties in two study sites in Colombian Eastern Plains. In each site, one farm with stocking rates as high as 4.2 livestock units (LU) ha<sup>-1</sup> managed by IRG was compared with an adjacent traditionally managed (reference) farm with low animal stocking rate (1 LU ha<sup>-1</sup>), where cattle grazing was either continuous (Morichal site), or

rotational with long grazing period and short periods of pasture recovery (Villasol site). As early as nine months after the adoption of IRG management, both farms managed by IRG had lower bulk density and higher water retention capacity than their respective reference farms, despite the more than four-times higher stocking rates. The animal feed supplement at the IRG farm at Morichal likely contributed to higher soil organic carbon stocks and improved soil aggregation when compared to the reference farm and to the Villasol site, where no supplement was applied. The improvement of soil properties found in IRG farms, compared to reference farms, was associated with a higher macrofauna abundance, particularly that of earthworms and beetles, which play a crucial role in soil structure improvement through bioturbation. Our results demonstrate the capacity of IRG management to intensify cattle production per unit area, while simultaneously improving soil properties and increasing soil macrofauna biodiversity as early as nine months since the implementation of IRG management.

## **Keywords**

soil macrofauna; soil aggregation; tropical forage; grazing management; Colombian Eastern Plains.

## **1 Introduction**

Over 70% of agricultural land in the tropics is used for livestock grazing (Rao et al., 2011). In Latin America, cattle ranching has traditionally been characterized by extensive pasture management with low stocking rates, driven by low costs of deforestation compared to the cost of production intensification (Murgueitio et al., 2011; White et al., 2001). As a consequence of growing demand for animal products in recent years,

particularly in developing countries, large areas have been deforested and converted to pastures to increase production (Lerner et al., 2017). For example, in Colombia more than 30% of the country's land area is covered by grazed grasslands as is typical for many Latin American countries. Furthermore, the average stocking rate in Colombia remains as low as 0.6 animals ha<sup>-1</sup> with 81% of the farms possessing less than 50 livestock units (LU) (Lerner et al., 2017; Murgueitio et al., 2011). Nevertheless, in recent years the negative environmental and social impacts of deforestation have become more apparent, which, together with increasing land scarcity and higher land prices has resulted in a push for sustainable intensification (Murgueitio et al., 2011; White et al., 2001). Sustainable intensification seeks increasing outputs per unit land area, while simultaneously reverting soil degradation and enhancing ecosystem services (Lerner et al., 2017). To that end, practices such as forage diversification, silvopastoral systems establishment or multi-paddock rotational grazing, including intensive short-rotational grazing (IRG) across multiple paddocks, have been recommended (Latawiec et al., 2014; Lerner et al., 2017; White et al., 2001).

In large parts of the tropics, the most common management of rangelands is continuous grazing which doesn't allow for grasslands recovery. Because cattle movement is driven by the localized presence of palatable forages (Teague et al., 2013), continuous grazing management leads to an excessive defoliation of preferred forage species and to a reduction of their abundance in the pasture over time. This selective grazing can lead to localized soil degradation and erosion in small patches around palatable species or in rest areas (McDonald et al., 2018; Teague et al., 2011; Waters et al., 2017). Alternatively, short-duration intensive rotational grazing (IRG) across multiple paddocks is based on high stocking rates, short periods of grazing and long periods of pasture recovery in order to intensify the production and reduce soil and pasture degradation (Teague et al., 2013).

Among the most commonly discussed environmental benefits of IRG are increased soil organic carbon (SOC) content, reduced soil compaction, higher soil water retention and improved soil aggregation (McDonald et al., 2018; Park et al., 2017; Teague et al., 2013; Waters et al., 2017).

However, the number of published studies assessing the effects of IRG systems on (soil-based) ecosystem services in replicated experiments is limited and results are inconsistent (Conant et al., 2017), differing greatly from on-farm observations. Such discrepancy is likely caused by the use of (i) small experimental plots, and (ii) following a strict pre-defined rotational plan without adapting to current conditions on the pasture, as well as the general high costs of the establishment and maintenance of grazing management experiments. To overcome these limitations, the paired comparison of farms managed by IRG with traditionally managed farms in their vicinity has been proposed (Alfaro-Arguello et al., 2010; Ferguson et al., 2013). Such comparisons, reflecting the management practiced by the farmers, who adapt the duration of the grazing periods and the selection of the paddocks to observed pasture conditions in order to optimize forage and animal production (Teague et al., 2011), are therefore a feasible alternative to overcoming such limitations and provide reliable results if potential differences among farms are carefully accounted for.

As in other tropical areas (Alfaro-Arguello et al., 2010; Ferguson et al., 2013), some farmers in the Eastern Plains (“Los Llanos Orientales”) of Colombia have started to manage their farms based on a more holistic approach using IRG management to claim that forage and animal productivity will increase. Nevertheless, robust scientific evidence is needed to inform practices for sustainable intensification as well as the policies that help overcome the economical and knowledge barrier for their adoption and scaling (Tapasco et al., 2019).

In the current study, we aimed to compare the effects of contrasting grazing management on soil properties while focusing on farm-level comparisons. Therefore, soil chemical, physical and biological parameters were assessed in two farms managed by IRG, and two traditionally-managed farms, with particular focus on soil macrofauna, because of its rapid response and high sensitivity to changes in agro-ecosystem management (Lavelle et al., 2006; Rousseau et al., 2013). Soil macrofauna plays a key role in organic matter decomposition and nutrient cycling, while improving soil physical properties and soil structure (Lavelle et al., 2006). However, despite its usefulness as an early indicator of management impact on soil quality, soil macrofauna on farms managed by IRG management has been largely under-investigated. We hypothesized that (i) IRG improves soil physico-chemical properties and soil macrofauna parameters due to an expected increase in above and belowground biomass production and reduced soil compaction; and that (ii) soil macrofauna responds clearly to changes in grazing management while being positively correlated with the changes in soil physical and chemical properties.

## **2 Materials and Methods**

### **2.1 Study sites**

The study was performed in Colombian Eastern Plains, where decades of continuous grazing have caused soil fertility decline, reflected in low cattle stocking rates and meat production. Two sites were selected (Fig. S1), each with paired adjacent farms for comparison of IRG management with high stocking rate ( $4.2 \text{ LU ha}^{-1}$ ), with traditionally managed reference farm with low cattle density ( $<1 \text{ LU ha}^{-1}$ ). While the two IRG-managed farms may represent somewhat different soil, landscape and climate conditions, the IRG- and traditionally-managed reference farm pairs within each study site (Morichal and Villasol) were selected in the near proximity (directly adjoining farms, the maximum

distance between transects 500m) to reflect the same climate, terrain and landscape setting. Thus, it can be assumed that the dramatic difference in cattle stocking rates between IRG and reference farms are the result of different grazing management, rather than landscape variability. This is also confirmed by comparable stocking rates between IRG and reference farms before the IRG management adoption (personal communication, farm managers Nelson Velasquez and Disney Baquero). Prior the IRG adoption, both IRG farms were managed with low cattle density management ( $<1 \text{ LU ha}^{-1}$ ) with occasional seeding of pasture grasses of higher nutritional value and productivity. Nevertheless, these grasses did not appear until the adoption of IRG management. In both IRG farms, areas with different dominant forage species were identified and sampled separately. No such areas with different forage species were identified in the two traditionally managed reference farms. Moreover, the current weight gain of grazing cattle at IRG farms (without receiving fertilization) of  $570 \pm 157 \text{ g animal}^{-1} \text{ day}^{-1}$  (unpublished data) exceeds the average animal weight gain in pastures of *B. humidicola* cv. Llanero without fertilization ( $484 \pm 148 \text{ g animal}^{-1} \text{ day}^{-1}$ ) and the mean animal weight gain of the area ( $325 \pm 106 \text{ g animal}^{-1} \text{ g}^{-1}$ ) (Rincón et al., 2018).

### 2.1.1 Morichal

The first study site, comprising one IRG farm and one reference farm with continuous grazing, was located in Vereda La Llanerita ( $4^{\circ}6'N$ ,  $73^{\circ}26'W$ , 590 m.a.s.l.) near Villavicencio (Meta department, Colombia). The mean annual precipitation of the area is 3856 mm and the mean temperature is  $25.5^{\circ}C$ . The farm managed by IRG covers 43.6 ha and accommodates around 180 LU. Prior the adoption of the IRG management at this farm, the cattle rotated among eight paddocks with long periods of grazing at low stocking rates ( $1 \text{ LU ha}^{-1}$ ). The IRG management at Morichal site was implemented in September 2017 and consists of high cattle stocking rates ( $4.2 \text{ LU ha}^{-1}$ ) and rapid rotation across

multiple paddocks (cattle density 188 LU ha<sup>-1</sup> within a paddock) (Fig. 1a). Animals stayed in the same paddock (approximately 800 m<sup>2</sup>) for a maximum of 24 h before being moved to a next paddock (Table 1). Each paddock was left to recover for at least 60 days before the next grazing event. At the time of sampling (1<sup>st</sup> of June 2018, nine months after IRG implementation), patches of different improved forage species had appeared, and the farm had developed into pasture areas covered by *Brachiaria humidicola* (Rendle) Schweick cv. Tully (IRG-Tully) and pastures of *Brachiaria ruziziensis* R. Germ. and C.M. Evrard) (IRG-Ruz) (Fig. S2). In addition, one kg animal<sup>-1</sup> day<sup>-1</sup> of a local product called *Palmix* is spread over the pastures. *Palmix* is a mixture of waste materials from palm oil production (palm kernel cake, and palm fiber), glycerine and urea (N content 1.28 %), which is used by farmers as a supplementary feed to improve animal nutrition. Adjacent farm was selected as an example of traditional grazing management where cattle is continuously grazed (TM-cont) on large areas with low stocking rates (<1 LU ha<sup>-1</sup>) due to low soil fertility and productivity of pastures formed dominantly by *Brachiaria humidicola* cv. Llanero (Fig. 1b, Fig. S2).

### 2.1.2 Villasol

The second study site is located in Vereda Sardinata (4° 1'N, 73°46'W, 550 m.a.s.l), near Acacias (Meta department, Colombia). The mean annual precipitation of the area is 3250 mm and the mean temperature is 25°C. The farm managed by IRG in the Villasol area occupies 70 ha and accommodates around 300 LU. Before to IRG adoption, the area was managed rotationally with long grazing period (eight days) and short pasture recuperation time (15 days), similar to the current management of the directly adjacent reference farm (traditional management-rotations; TM-rot). The reference farm was covered with a mixture of *Brachiaria humidicola* cv. Llanero and Marandú grass (*Brachiaria brizantha* (Hochst ex A. Rich) Stapf cv. Marandú). The pastures were

frequently flooded due to soil compaction, low soil coverage by plants and poor soil properties. In December 2017, the IRG grazing system was implemented by dividing the area into paddocks (Table 1). After IRG establishment, patches of different forage species became apparent and the farm evolved into areas covered by *Brachiaria humidicola* (Rendle) Schweick cv. Tully (IRG-Tully), and with Marandú grass (IRG-Mar). During the period between the IRG establishment (Dec 2017) and sampling (August 2018) an average of 300 animals (4.3 LU ha<sup>-1</sup>) rotated in small paddocks (1.5 - 1.8 ha) with high stocking rates (180 LU ha<sup>-1</sup>) within the paddocks (Table 1) with a minimum period of 60 days between grazing events to ensure pasture regeneration. Cattle feed in Villasol is not supplemented and their nutrition relies exclusively on grazing.

## **2.2 Sampling design**

One transect was laid out in each of the reference farms where pasture composition was relatively homogeneous, and four samples were collected at least 100 m apart. The four samples within each farm were considered replications despite the lack of spatially independent due to the large of the area and long distances between sampling points. Similar design in has been used in previous studies focusing on the effect of land use management on soil properties in large single monitoring areas when no spatially separated areas were available (Francaviglia et al., 2017; Lagomarsino et al., 2011; Moghimian et al., 2017; Soleimani et al., 2019). At both IRG farms (one in each study site), two transects were laid out to account for the observed variability in dominant forage species: IRG-Tully (pastures dominated by *B. humidicola* cv. Tully) and IRG-Ruz (pastures dominated by *B. ruziziensis*) in Morichal site; and IRG-Tully (*B. humidicola* cv. Tully) and IRG-Mar (*B. brizantha* cv. Marandú) in Villasol.

## **2.3 Soil macrofauna**

Soil invertebrates were extracted in June and August 2018 (middle of rainy season) using the standard Tropical Soil Biology and Fertility Institute (TSBF) method (Anderson and Ingram, 1993). At each sampling point, one soil monolith (25 x 25 cm) was collected for each sampling depth (0–10 cm and 10–20 cm). Soil invertebrates (larger than 2 mm) were hand-sorted and preserved in ethanol (70%) until the identification in the laboratory of the International Centre for Tropical Agriculture (CIAT) in Palmira, Colombia. All individuals were classified into broad taxonomic groups (*Formicidae*, *Oligochaeta*, *Chilopoda*, *Diplopoda*, *Coleoptera*, *Hemiptera*, *Araneae*, *Dermaptera*, *Gastropoda*, *Isopoda*, *Blattodea*, and others), and the abundance of each group was noted. Taxa were further allocated into functional groups, according to Cherubin et al. (2016), with the exception of *Coleoptera*, which were classified to family level, which was consequently attributed a functional group (predators and detritivores). Due to the high abundance and functional diversity of *Formicidae* we did not include them in any of the functional groups and treated them separately.

#### **2.4 Root biomass, root length and average diameter**

Roots from tropical grasses were extracted from soil (0–20 cm) using a metal auger (5 cm diameter). The extracted soil core was then split into two samples representing 0–10 cm and 10–20 cm depths. All roots were washed on a sieve and then manually picked with tweezers, thoroughly washed with tap water and scanned (Epson 10000xl). Images were then processed using WinRHIZO software (Regent Instrument Inc, Sainte-Foy, Canada) to calculate the mean root length and diameter. After scanning, roots were dried at 60°C until constant weight to determine root biomass. Root volume and biomass were then calculated based on the total volume of extracted soil.

#### **2.5 Soil properties**

Undisturbed soil samples were collected from both soil layers (0-10 cm and 10-20 cm) using a metal ring (5 cm height, 5 cm diameter). Soil cores were saturated with water by capillarity during 48 h and weighed. Afterwards, the same soil cores were used to determine sequentially the water retention at matric potentials of -33 kPa (in a sand box Eijkelkamp, The Netherlands) and of -1500 kPa (15 bar pressure plate extractor Eijkelkamp, The Netherlands). Finally, the cores were dried at 105°C until constant weight and the bulk density was calculated as a ratio of dry soil weight and volume. The total soil porosity was determined according to:

$$\text{Soil porosity} = 1 - (\text{soil bulk density}/\text{particle density}),$$

where soil particle density of 2.65 g cm<sup>-3</sup> was assumed (Danielson and Sutherland, 1986).

For the assessment of soil aggregate stability, large blocks of soil were carefully collected from the same soil layers (0-10 and 10-20 cm) with a spade next to each soil monolith and transported in a box to laboratory. Large aggregates and soil clogs were broken down along the natural planes of fracture, air-dried and the soil was carefully passed through a 1.2 cm sieve. Then, wet sieving was performed as follows: A subsample (60 g) of dry soil with aggregates was placed on the largest sieve of a stack of 5 sieves (6.3, 4.75, 2, 0.25 and 0.125 mm). After the re-wetting of the soil by capillarity (20 min), the set of sieves was moving up and down (3 cm) for 20 minutes using a Yoder apparatus. The material that remained on each sieve was carefully washed off and weighed after oven drying. The mean weight diameter (MWD) was calculated using the proportion by weight and the mean diameter of each size fraction, using the mean of the size 1.2 cm (the initial sieve used to pass the soil) and 6.3 mm (the size of the largest sieve). Soil texture was determined using the hydrometer method after soil dispersion with sodium hexametaphosphate.

Air-dried and sieved (<2 mm) soil collected together with soil aggregate samples (0-10 and 10-20 cm) was used for chemical soil analyses. The soil organic C (SOC) content was quantified according to Walkley and Black (1934) and the total N (TN) content with the Kjeldahl digestion and steam distillation method (Bremner and Mulvaney, 1982), respectively. The soil C stocks were calculated on an equivalent soil mass (ESM) basis according to Wendt and Hauser (2013) to consider the impact on SOC stocks of the changes of BD caused by soil compaction. The selected ESM layer was 2000 Mg soil ha<sup>-1</sup> which corresponded with an average depth of the reference mass being 15.8 and 17.3 cm in Morichal and Villasol, respectively.

Plant available phosphorus and other macro- and micronutrients were extracted by Mehlich-3 and P was determined colorimetrically (Murphy and Riley, 1962). The contents of Ca, Mg, K and Na were quantified by atomic absorption spectrophotometry (AAAnalyst 400, PerkinElmer, Wellesley, Massachusetts, USA).

The potential activities of soil enzymes were determined in sieved (<2 mm) fresh soil samples conserved at 4°C until processing (within 14 days since samples collection). The activity of protease and urease was quantified colorimetrically using the method proposed by Nannipieri et al. (1980) and Kandeler and Gerber (1988), respectively, by measuring the content of NH<sub>4</sub><sup>+</sup>-N after soil sample incubation with substrate (N-benzoyl-L-arginine amide hydrochloride monohydrate and urea for soil protease and urease, respectively). Soil acid phosphomonoesterase activity was measured according to (Tabatabai and Bremner, 1969) using p-nitrophenol as a substrate. β-glucosidase activity was determined using salicin as substrate using the methodology of Hoffmann and Dedeken (1965).

## **2.6 Statistical analysis**

Data were analyzed using a generalized linear mixed model (GLMM) with the grazing management (IRG and TM), study site (Morichal and Villasol) and the soil depth (0-10

cm and 10-20 cm) as fixed factors. Because two transects were laid in each IRG farm corresponding to different forage species, the forage type was considered a random factor nested within the grazing management (Forage type (Grazing Management)). The forage type was not considered as a fixed factor because the forages differed in Morichal and Villasol study sites and in different grazing management. When applicable, the means of the different grazing management practices were separated using LSD test ( $p < 0.05$ ). In the case of SOC stocks, only grazing management and study site were considered as fixed factors. The GLMM was used because macrofauna abundances are often not normally distributed. Furthermore, due to the high abundance of zero values in macrofauna abundance, a negative binomial distribution with a log-link function as an extension of Poisson distribution was used (Kamau et al., 2017). The studied chemical and physical soil properties and enzymatic activities were compared selecting a normal distribution after the evaluation of data normality and transformation ( $\log_{10}(X + 1)$ ) when necessary. In all the cases, the used distribution was selected according to the (lowest) Akaike Information Criterion (AIC)

The principal components analysis (PCA) was performed to analyze the relations among studied variables combining the results from both study sites. In order to reduce the number of variables included in the PCA, we calculated the geometric mean of the assayed enzymatic activities (Gmean) according to Hinojosa et al. (2004) and the available water content (AWC) as the difference of the soil water content at FC and at WP (Supplementary material Table S1). Furthermore, we included the most representative soil parameters (Gmean, AWC, BD, MWD and SOC), plant variables (root length), and the three macrofauna functional groups (predators, herbivores and detritivores) and the richness of observed taxa. Additionally, the sampling points were

plotted in the space defined by the first two PCA axes. All the analyses were performed using SPSS 22 software (IBM SPSS Inc., Chicago, USA).

### **3 Results**

#### **3.1 Soil properties and enzymatic activity**

The IRG-managed farm in Morichal had a considerably higher SOC content and C/N ratio compared to low-density adjacent TM-cont reference (Table 2). The TN content was higher in both farms managed by IRG than in their respective reference farms (Table 2). Furthermore, soil BD was lower under IRG management when compared to their respective reference farms in both study sites (Table 3), which was reflected in higher soil porosity at IRG-managed farms. Furthermore, soil at both IRG-managed farms had higher available water content (Supplementary material Table S1). In general, the differences of soil physical properties were most evident in the top layer. Differences in SOC contents were reflected in SOC stocks calculated in an ESM layer of 2000 Mg soil ha<sup>-1</sup>, where higher SOC stocks were observed under IRG when compared to the TM-cont reference farms (Fig. 2). Nevertheless, such differences were only visible in Morichal (IRG farm with SOC stocks 29% higher than the TM-cont reference farm), while SOC stocks were comparable at both farms in Villasol (Fig. 2).

The MWD of soil water-stable aggregates was higher under IRG when compared to the adjacent reference farm in Morichal (0-10 cm soil layer), but no differences were observed in Villasol (Table 3). While soil at the IRG-farm at Morichal had higher potential activity of soil  $\beta$ -glucosidase, protease and phosphomonoesterase in comparison to the TM-cont, only protease activity was higher in IRG than in reference farm at Villasol site (Table 2). Higher activity of urease was observed in the topsoil layer of IRG farms than in their corresponding reference farms.

### **3.2 Soil macrofauna**

In total, 4576 and 3654 soil macrofauna individuals were collected at Morichal and Villasol site, respectively, and the majority of macrofauna individuals was found in the upper soil layer (Table 4, Table 5). At both study sites, the most abundant macrofauna taxon was ants (*Formicidae*, 4307 and 1556 individuals m<sup>-2</sup> at Morichal and Villasol sites, respectively), and termites (*Isoptera*, 747 and 1785 individuals m<sup>-2</sup> at Morichal and Villasol sites, respectively).

The total abundance of macrofauna and ants was higher in the topsoil of IRG than in their respective reference farms (Table 4, Table 5). The abundances of earthworms (*Oligochaeta*), spiders (*Araneae*), beetles (*Coleoptera*, adults and larvae), woodlice (*Isopoda*) and earwigs (*Dermaptera*), as well as the total richness of macrofauna taxa (determined for each monolith) and the abundance of predators were higher in the IRG management than in the respective reference farms in both Morichal and Villasol (Table 4, Table 5).

### **3.3 Root density**

Root biomass and root diameter were both higher in Villasol site than in Morichal (Table 6) and in the topsoil (0-10 cm) when compared to 10-20 cm soil layer. Root diameter was higher in the 10-20 cm soil layer (Table 6). The root length was higher in the IRG than in the reference farms but only in the topsoil layer. Root biomass was also higher at IRG farm when compared to the references farm in Morichal site.

### **3.4 Correlation between soil macrofauna and selected soil properties**

In total, 74% of total variance in the data was explained by the first two principal components (Fig. 3, Table S2). Along the PC1 axis (accounting for 54% of data variance), the SOC was positively related with MWD, AWC, root length, geometric mean of soil enzymes (Gmean) and negatively with BD. The abundance of predators and the richness

of macrofauna taxa were loaded with PC1 variables. Along the PC2 axis, herbivores, detritivores and AWC together contributed with 21% to data variance. The PC1 axis clearly distinguished between both soil depths (0-10 cm depth sling points loaded on the right side of the axis and 10-20 cm samples loaded on the left side). Furthermore, all IRG-Tully points (in both IRG farms) were located further to the right along the PC1 respect to other management samples, while all IRG-Mar and IRG-Ruz were clearly separated by PC2 from other sampling points.

## **4 Discussion**

### **4.1 Effect of grazing management on soil properties**

Our study showed that higher stocking rates under IRG were not associated with soil compaction and instead, correspond with lower soil bulk density in the upper 10 cm of the soil, despite the four times higher stocking rates under IRG grazing management (4.2 LU ha<sup>-1</sup>) compared to <1 LU ha<sup>-1</sup> under traditional grazing (continuous grazing or rotational grazing with long grazing period and insufficient time for pasture recovery). This further coincided with a higher water retention capacity (Table 3) under IRG, similarly to findings of Teague et al. (2011). Reduced soil compaction and available water content are of particular interest in this region with bimodal climate where higher soil water storage capacity can reduce the waterlogging during the wet season while increasing water availability during the dry periods (Amezquita et al., 2004). The more favorable soil physical properties correlated with the higher SOC and higher soil coverage by plant biomass which reduces the trampling impact on soil surface (Beukes and Cowling, 2003; Teague et al., 2011). Similarly, the lower bulk density and higher water storage under IRG is likely caused by higher root biomass and root length of the improved plant genotypes. In fact, we observed a higher water retention capacity in the IRG-Mar and IRG-Ruz farm sections when compared to the IRG-Tully, which indicates either

positive impact of these forages on soil, or the possibility of Marandú and *B. ruziziensis* establishment only after the improvement of soil properties due to their higher sensitivity to water-logging when compared to *B. humidicola* cv. Tully.

The impact of root growth and turnover, and the architecture of the root systems of different forage species on the accumulation of SOC, soil physical properties and biological activity is well known (Amezquita et al., 2004). The IRG-managed pastures were dominated with *Brachiaria* cultivars/species such as Marandú or *B. ruziziensis*, which are known to develop denser and deeper root systems compared to *B. humidicola* cv. Llanero, similarly to the observations of Guenni et al. (2002). While the effect of other exogenous factors on the pasture composition cannot be ruled out, it is plausible that the change of management led to the establishment of improved forages due to more evenly distributed grazing pressure (Teague et al., 2013). A clear link between root length and SOC stocks has been observed in the present study, which confirmed the crucial role of plants in SOC accumulation in soil. Nevertheless, differences between IRG and traditional farm in soil properties were much less conspicuous in Villasol, where no differences in SOC stocks were detected. Hence, it could be speculated that continuous grazing may be more detrimental to soil properties when compared to rotational grazing regardless the intensity of rotations, which warrant future studies.

Soil functioning is largely determined by soil structure. The formation of soil aggregates, a common indicator of soil structure (Six et al., 2000), is mediated by SOC content and the soil biological activity, which in turn determines the SOC sequestration potential. The more productive IRG farm at Morichal is therefore likely associated with higher SOC stocks and to the higher root biomass, which are often well correlated with MWD of soil aggregates (Rillig et al., 2002), as was also observed in this study in the form of a strong positive correlation between SOC, MWD of soil aggregates and soil enzymatic activity.

Dense root systems generally supports higher abundance of soil microbiota, including arbuscular mycorrhizal fungi contributing to the stabilization of soil aggregates (Caravaca et al., 2002; Deneff et al., 2001). Higher soil quality under IRG at Morichal seems also related to soil microbial activity assessed as higher potential activity of soil enzymes, which resulted to be useful indicators of nutrient transformations suggesting higher mineralization of C, N and P under IRG management.

The climate change mitigation potential of regenerative agriculture has been the subject of discussions recently. Despite the short period since the IRG implementation, we observed higher SOC and TN content at IRG farm than at the reference farm in Morichal site, which confirms the results of Teague et al. (2011), Sanjari et al. (2008) and Waters et al. (2017). The higher SOC under IRG could be related with the factors above mentioned, however, the short period since the IRG establishment suggests the involvement of other factors. The increasing SOC stocks at Morichal and the lack of effect at Villasol could be related with the use of Palmix supplementation, which contributes approximately 20 kg of N ha<sup>-1</sup> year<sup>-1</sup>. If we consider that approximately 50% of the ingested N (corresponding to 10 kg of N ha<sup>-1</sup> year<sup>-1</sup>) is deposited as dung and urine (Haynes and Williams, 1993) the use of Palmix supplementation could explain the higher TN at Morichal compared to the adjacent farm managed by continuous grazing without any supplementation. Such a higher carbon and nutrient input could also contribute to the higher activity of soil microbes and soil macrofauna and thus, to the improvement of soil structure, particularly in the upper (20 cm) soil layer. The role of animal supplementation with organic residues in combination with different grazing strategies would be required to confirm such a hypothesis of its impact on soil quality. In order to assess co-benefits or trade-offs in terms of environmental impact of IRG, in particular the effects on animal-borne greenhouse gases emissions (methane) with increasing herd numbers, or soil-borne

emissions (nitrous oxide) from increased concentration of dung and urine in a smaller area compared to dung and urine patches sparse in a bigger area, either per unit of area or per unit of product, requires further study. Nevertheless, the strong positive impact on pasture productivity (hence potentially reduced need of further deforestation) indicate an overall positive impact of such practices not only on soil health but also on carbon sequestration, as suggested globally for regenerative agriculture practices (Paustian et al., 2020), contributing to the GHG emissions reduction in the livestock sector in Latin America (Arango et al., 2020)

#### **4.2 Soil macrofauna as an early indicator of grazing management**

We observed strong differences in soil macrofauna abundance and richness between the different grazing management systems. The abundance of earthworms, spiders, beetles, woodlice and earwigs was higher in the IRG-managed farms than in the reference farms, which may partly explain the positive impact of IRG on soil physical and chemical soil properties and enzyme activities or vice versa, *i.e.* reduced soil compaction under IRG management may stimulate soil fauna activity, or both (Lavelle et al 2006). Previous studies have demonstrated the positive effect of grazing intensification on soil macrofauna in tropical savannas and natural pastures (Decaëns et al., 2004; Webster et al., 2019). Such effect have been related with the increase of dung and urine inputs to soil or the root decay after grazing events which supply a huge amount of organic matter which stimulates the soil macrofauna development (Decaëns et al., 2004). Other factors affecting soil macrofauna abundance and diversity is the plant biomass growth and nutrient quality of the forage (Flegel and Schrader, 2000; Laossi et al., 2008). In our study, we observed a clear differentiation in PCA plot along the PC2 axis between IRG-Ruz and IRG-Mar from IRG-Tully which indicates the crucial impact of forage identity on the soil macrofauna. Nevertheless, the possible effect of soil properties cannot be ruled out

due the lack of baseline data. The observed better soil physical and chemical properties under *B. humidicola* cv. Tully when compared to other forages (either caused by the establishment of different forage species or permitting for *B. humidicola* cv. Tully establishment), were characterized by higher abundance of predators, which seemed to benefit from higher root length and/or improved soil condition. On the other hand, both IRG-Ruz and IRG-Mar pastures had higher abundance of herbivores and detritivores, which indicates the positive impact of higher nutritional value of these forages on litter-decomposing invertebrates.

Furthermore, the higher MWD observed in IRG in Morichal site could be related with the higher abundance of earthworms which play a key role in soil aggregation and SOC stabilization within aggregates (Lavelle, 1997). In addition, the observed higher enzymatic activities could be related with soil macrofauna because the biogenic structures could act as an incubator of microbial activity leading to a higher soil organic matter decomposition (Lavelle et al., 2006). Finally, the higher abundance of predators in IRG could be interpreted as a sign of greater ecosystem complexity and stability (Ruiz-Cobo et al. 2010; Vasconcellos et al. 2013), which confirms the high potential of IRG management for sustainable intensification. Our study confirmed that soil macrofauna serve as an early and easy-to-measure indicator of land-use change due to their rapid response to changing conditions including grazing management (Webster et al., 2019).

## **5. Conclusions**

Soil properties differed between IRG and traditionally managed reference farms, possibly as a result of direct (*i.e.* long-term pasture recovery, higher and more uniform distribution of animal dung and urine) and indirect (changes of pasture composition, stimulation of soil macrofauna) impacts of grazing management. Soil macrofauna resulted to be a useful

early indicator of land-use change and clearly responded to differences in pasture species and to changing soil environmental conditions. Especially earthworms and beetles responded positively to IRG in both study sites, mainly in *B.ruzizienses* and Marandú patches. Predators, considered indicators of ecosystems' stability, were the most abundant in *B. humidicola* cv. Tully patches under IRG.

Given the higher pasture and animal productivity and the positive impacts on soil quality, IRG management can be considered a suitable sustainable intensification approach, increasing resource use efficiency and output per unit of land area, without additional inputs. To provide more robust results, more independent replicates in the form of paired farms and long-term evaluations are recommended, without losing focus on the representability of the farming systems and the importance of scale. Lastly, the environmental impacts of IRG systems integrating impacts on deforestation, animal- and soil-borne GHG emissions and SOC sequestration potential require more attention in the future studies.

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**Table 1.** Overview of the properties and management (rotations details, total area etc.) of the selected farms

	Morichal		Villasol	
	IRG	TM-cont	IRG	TM-rot
Total area (ha)	44	60	70	10
Mean number of animals	180	70	300	12
Animal density (LU ha <sup>-1</sup> )	4.2	≤1	4.3	≤1
Animal density within paddock (LU ha <sup>-1</sup> )	188	n.a.	180	n.a
Grazing frequency	<1 day grazing, 60 days rest	Continuous	<1 day grazing, 60 days rest	8 days grazing, 15 days rest
Time since management adoption	9 months	>10 years	9 months	>10 years
Animal supplementation	Palmix	none	none	none

TM-cont, traditional management consisting of continuous grazing; TM-rot, traditional management based on rotational grazing with long grazing and short pasture recovery periods

**Table 2.** The effects of management (Mn), study site (Site) and soil depth (Depth) on soil chemical properties and enzymatic activity. Mean (S.E.) (n=4). The description of the IRG management and the reference farms is provided in Table 1.

	SOC (%)	TN (%)	C/N	P <sub>av.</sub> (mg kg <sup>-1</sup> )	pH	EC (μS cm <sup>-1</sup> )	β-gluc <sup>1)</sup>	Ure <sup>2)</sup>	Prot <sup>3)</sup>	Phos <sup>4)</sup>
<i>Morichal</i>										
0-10 cm										
IRG-Tully	2.21 (0.09)	0.27 (0.01)	8.09 (0.15)	6.44 (1.06)	5.10 (0.12)	92.2 (21.6)	0.50 (0.07)	91.7 (8.93)	64.8 (2.26)	6.36 (0.48)
IRG-Ruz	2.17 (0.06)	0.23 (0.02)	9.61 (0.84)	11.63 (0.58)	5.19 (0.02)	75.2 (2.26)	0.61 (0.07)	56.5 (8.53)	63.3 (0.69)	5.61 (0.32)
TM-cont	1.51 (0.05)	0.21 (0.01)	7.27 (0.29)	3.72 (0.70)	5.04 (0.05)	59.5 (4.36)	0.25 (0.05)	112 (7.34)	45.9 (2.95)	4.69 (0.24)
10-20 cm										
IRG-Tully	1.16 (0.05)	0.14 (0.01)	8.23 (0.22)	2.32 (0.27)	4.86 (0.07)	36.5 (2.38)	0.32 (0.07)	30.9 (3.92)	48.4 (4.71)	3.22 (0.15)
IRG-Ruz	1.03 (0.03)	0.13 (0.00)	7.67 (0.03)	3.36 (0.54)	4.93 (0.07)	36.1 (2.33)	0.20 (0.04)	31.3 (6.18)	47.8 (4.02)	2.69 (0.09)
TM-rot	0.95 (0.01)	0.13 (0.01)	7.26 (0.69)	1.87 (0.87)	4.79 (0.07)	28.6 (2.52)	0.09 (0.01)	31.7 (4.73)	40.8 (2.95)	2.85 (0.20)
<i>Villasol</i>										
0-10 cm										
IRG-Tully	2.44 (0.12)	0.34 (0.02)	7.24 (0.25)	6.85 (1.27)	5.07 (0.14)	89.3 (25.7)	0.51 (0.02)	47.7 (0.78)	112 (4.36)	4.93 (0.19)
IRG-Mar	2.67 (0.16)	0.33 (0.02)	8.08 (0.62)	20.01 (4.58)	5.46 (0.09)	93.6 (7.68)	0.77 (0.15)	59.3 (3.21)	115 (9.38)	4.77 (0.21)
TM-rot	2.28 (0.08)	0.30 (0.02)	7.72 (0.27)	7.28 (0.24)	5.47 (0.04)	102 (15.4)	0.63 (0.02)	49.1 (2.70)	103 (6.41)	5.57 (0.23)
10-20 cm										
IRG-Tully	0.99 (0.01)	0.18 (0.00)	5.57 (0.21)	0.83 (0.19)	4.94 (0.09)	22.1 (3.15)	0.20 (0.05)	13.9 (0.87)	67.1 (5.77)	2.82 (0.19)
IRG-Mar	1.07 (0.18)	0.18 (0.02)	5.97 (0.30)	4.61 (0.54)	4.99 (0.15)	37.7 (3.85)	0.16 (0.08)	20.9 (5.30)	72.4 (10.7)	2.56 (0.40)
TM-rot	1.14 (0.11)	0.16 (0.02)	7.12 (0.15)	2.39 (0.72)	5.13 (0.15)	36.8 (7.23)	0.21 (0.03)	16.0 (2.47)	71.9 (10.0)	2.70 (0.31)
<i>Effects</i>										
Mn.	***	*	n.s.	n.s.	n.s.	n.s.	*	n.s.	*	n.s.
Site	***	***	**	n.s.	***	n.s.	**	***	***	n.s.
Depth	***	***	**	***	***	***	***	***	***	***
Mn. x Site	**	n.s.	**	n.s.	n.s.	n.s.	**	n.s.	n.s.	**
Mn. x Depth	***	n.s.	*	*	n.s.	n.s.	n.s.	*	n.s.	n.s.
Site x Depth	***	**	n.s.	n.s.	n.s.	n.s.	*	***	***	n.s.
Mn x Site x Depth	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	**	n.s.	**

SOC, soil organic carbon; TN, total nitrogen; P<sub>av.</sub>, available phosphorus

IRG-Tully, intensive rotational grazing management with prevailing *B. humidicola* cv. Tully; IRG-Ruz, intensive rotational grazing with prevailing *B. ruziziensis*; TM-cont, traditional management consisting of continuous grazing; IRG-Mar, intensive rotational grazing with prevailing *B. brizantha* cv. Marandu; TM-rot, traditional management consisting of low-intensity rotational grazing.

1) Activity of soil β-glucosidase (μmoles saligenin g<sup>-1</sup> h<sup>-1</sup>)

2) Activity of soil urease (mg NH<sub>4</sub><sup>+</sup>-N g<sup>-1</sup> soil h<sup>-1</sup>)

3) Activity of soil protease ( $\text{mg NH}_4^+\text{-N g}^{-1} \text{ soil h}^{-1}$ )

4) Activity of soil acid phosphomonoesterase ( $\mu\text{moles p-nitrophenol g}^{-1} \text{ h}^{-1}$ )

\*, \*\*, \*\*\* indicate significant differences (Generalized linear mixed model) at  $p < 0.05$ ,  $p < 0.01$ ,  $p < 0.001$  level, respectively; n.s. not significant

**Table 3.** The effects of management (Mn), study site (Site) and soil depth (Depth) on soil moisture content at saturation (SAT), field capacity (-33 kPa, FC) and at wilting point (-1500 kPa, WP) and on soil bulk density (BD), soil porosity (SP), mean weight diameter (MWD) of water stable aggregates and the soil textural class. Mean (S.E.) (n=4). The description of the IRG management and the reference farms is provided in

Table 1.

	SAT	FC (%)	WP	Bulk density (g cm <sup>-3</sup> )	Soil porosity (%)	MWD (mm)	Clay	Silt	Sand	Soil textural class
<i>Morichal</i>										
0-10 cm										
IRG-Tully	50.03 (4.18)	38.97 (2.41)	30.29 (1.62)	1.12 (0.04)	57.92 (1.69)	6.18 (0.24)	19	39	42	Loam
IRG-Ruz	53.03 (1.71)	42.29 (0.78)	36.59 (0.99)	1.07 (0.03)	59.65 (1.03)	5.67 (0.36)	22	31	47	Loam
TM-cont	34.81 (0.75)	31.27 (0.75)	26.32 (1.38)	1.36 (0.01)	48.79 (0.46)	4.74 (0.22)	25	36	40	Loam
10-20 cm										
IRG-Tully	31.71 (0.49)	25.48 (0.44)	21.41 (0.34)	1.38 (0.02)	47.97 (0.73)	3.82 (0.29)	22	43	35	Loam
IRG-Ruz	28.29 (0.34)	23.77 (0.48)	19.42 (0.64)	1.45 (0.01)	45.17 (0.46)	3.55 (0.52)	24	29	47	Loam
TM-cont	28.48 (0.56)	24.08 (0.09)	19.57 (0.37)	1.47 (0.01)	44.61 (0.55)	3.08 (0.10)	28	44	28	Clay loam
<i>Villasol</i>										
0-10 cm										
IRG-Tully	60.16 (3.91)	53.93 (3.81)	46.64 (3.21)	1.02 (0.05)	61.41 (1.73)	5.47 (0.25)	22	37	41	Loam
IRG-Mar	75.52 (10.7)	63.42 (9.36)	54.41 (9.57)	0.89 (0.08)	66.57 (2.88)	5.78 (0.22)	16	49	35	Loam
TM-rot	47.33 (1.85)	44.45 (1.60)	39.63 (1.91)	1.14 (0.02)	57.01 (0.82)	5.14 (0.45)	13	46	41	Loam
10-20 cm										
IRG-Tully	34.80 (1.72)	30.81 (1.42)	26.47 (1.24)	1.41 (0.10)	46.97 (3.67)	3.01 (0.23)	22	22	56	Sandy clay loam
IRG-Mar	37.25 (5.54)	32.45 (4.14)	27.40 (3.03)	1.35 (0.10)	49.01 (3.88)	3.82 (0.40)	20	43	37	Loam
TM-rot	29.13 (1.64)	25.37 (1.67)	21.23 (1.07)	1.47 (0.04)	44.66 (1.64)	4.55 (0.20)	25	25	50	Sandy clay loam
<i>Effects</i>										
Mn.	*	**	*	**	**	n.s.				
Site	**	***	***	**	**	n.s.				
Depth	***	***	***	***	***	***				
Mn x Site	n.s.	n.s.	n.s.	n.s.	n.s.	***				
Mn x Depth	**	*	n.s.	*	*	**				
Site x Depth	*	**	**	*	*	n.s.				
Mn x Site x Depth	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.				

IRG-Tully, intensive rotational grazing with prevailing *B. humidicola* cv. Tully; IRG-Ruz, intensive rotational grazing with prevailing *B. ruziziensis*; TM-cont, traditional management consisting of continuous grazing; IRG-Mar, intensive rotational grazing with prevailing *B. brizantha* cv. Marandu; TM-rot, traditional management consisting of rotational grazing.

\*, \*\*, \*\*\* indicate significant differences (Generalized linear mixed model) at  $p < 0.05$ ,  $p < 0.01$ ,  $p < 0.001$  level, respectively

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2 **Table 4.** The abundance of soil macrofauna groups. Mean (S.E.) (n=4). The description of the  
 3 IRG management and the reference farms is provided in Table 1.

	Morichal			Villasol		
	IRG-Tully	IRG-Ruz	TM-cont	IRG-Tully	IRG-Mar	TM-rot
Macrofauna group						
Individuals m <sup>-2</sup>						
0-10 cm						
Formicidae (ants)	7544 (4236)	1916 (742)	3460 (1380)	604 (247)	3996 (1938)	68 (26)
Isoptera (termites)	72 (72)	1156 (748)	1012 (361)	432 (288)	4876 (3316)	48 (48)
Oligochaeta adults (earthworms)	332 (180)	648 (99)	56 (51)	688 (227)	1112 (408)	108 (23)
Oligochaeta eggs (earthworms)	0 (0)	0 (0)	0 (0)	4 (4)	0 (0)	4 (4)
Coleoptera adults (beetles)	544 (119)	204 (67)	16 (7)	884 (165)	104 (45)	60 (22)
Coleoptera larvae (beetles)	316 (72)	104 (34)	40 (21)	56 (23)	80 (43)	60 (27)
Diplopoda (millipedes)	0 (0)	60 (15)	0 (0)	4 (4)	24 (19)	0 (0)
Chilopoda (centipedes)	0 (0)	0 (0)	0 (0)	0 (0)	40 (17)	0 (0)
Araneae (spiders)	4 (4)	16 (7)	0 (0)	20 (10)	36 (10)	4 (4)
Hemiptera (true bugs)	24 (5)	92 (87)	12 (12)	20 (20)	4 (4)	0 (0)
Blattodea (cockroaches)	0 (0)	12 (8)	0 (0)	24 (14)	4 (4)	0 (0)
Dermaptera (earwigs)	0 (0)	0 (0)	0 (0)	16 (11)	64 (44)	0 (0)
Isopoda (woodlice)	4 (4)	8 (5)	0 (0)	8 (5)	48 (22)	0 (0)
Total abundance	8852 (4219)	4216 (860)	4612 (1081)	2768 (736)	10420 (5117)	368 (51)
Richness	11.00 (0.82)	11.50 (0.96)	8.00 (0.91)	13.00 (1.68)	14.50 (1.19)	9.50 (0.5)
Detritivors	804 (204)	2008 (669)	1124 (401)	1332 (437)	6168 (3336)	232 (69)
Herbivors	24 (5)	92 (87)	12 (12)	36 (31)	92 (34)	12(12)
Predators	480 (113)	200 (59)	8 (5)	792 (168)	160 (54)	56 (10)
10-20 cm						
Formicidae (ants)	20 (20)	40 (14)	40 (35)	20 (12)	76 (34)	416 (416)
Isoptera (termites)	0 (0)	100 (100)	0 (0)	0 (0)	228 (0)	0 (0)
Oligochaeta adults(earthworms)	4 (4)	108 (63)	4 (4)	0 (0)	192 (130)	0 (0)
Oligochaeta eggs (earthworms)	60 (39)	120 (19)	0 (0)	0 (0)	24 (14)	84 (73)
Coleoptera adults (beetles)	20 (20)	12 (8)	0 (0)	48 (38)	16 (16)	0 (0)
Coleoptera larvae (beetles)	24 (5)	84 (48)	24 (14)	12 (4)	24 (19)	16 (9)

Diplopoda (millipedes)	0 (0)	8 (8)	0 (0)	0 (0)	0 (0)	0 (0)
Chilopoda (centipedes)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Araneae (spiders)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Hemiptera (true bugs)	8 (8)	92 (44)	28 (18)	0 (0)	0 (0)	0 (0)
Blattodea (cockroaches)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Dermaptera (earwigs)	0 (0)	0 (0)	0 (0)	8 (5)	0 (0)	0 (0)
Isopoda (woodlice)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Total abundance	80 (49)	444 (175)	100 (29)	92 (50)	536 (369)	432 (411)
Richness	5.75 (1.18)	7.75 (1.31)	3.25 (1.65)	4.00 (1.41)	4.50 (0.87)	2.75 (0.75)
Detritivors	88 (35)	420 (105)	28 (16)	12 (4)	472 (335)	100 (79)
Herbivors	8 (8)	92 (44)	28 (18)	12 (4)	0 (0)	0 (0)
Predators	20 (20)	12 (8)	0 (0)	48 (38)	12 (12)	0 (0)

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4 IRG-Tully, intensive rotational grazing with prevailing *B. humidicola* cv. Tully; IRG-Ruz, intensive rotational  
5 grazing with prevailing *B. ruziensis*; TM-cont, traditional management consisting of continuous grazing; IRG-  
6 Mar, intensive rotational grazing with prevailing *B. brizantha* cv. Marandú; TM-rot, traditional management  
7 consisting of rotational grazing. Richness was determined in each monolith separately.

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17 **Table 5.** The effects of management (Mn), study site (Site) and soil depth (Depth) on the  
 18 abundance of macrofauna groups, total abundance and richness and functional groups

	Mn.	Site	Depth	Mn x Site	Mn. x Depth	Site x Depth	Mn x Site x Depth
	<i>Effects</i>						
Formicidae (ants)	n.s.	n.s.	***	n.s.	**	***	*
Isoptera (termites)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Oligochaeta adults (earthworms)	*	n.s.	***	n.s.	n.s.	n.s.	n.s.
Oligochaeta eggs (earthworms)	n.s.	**	**	***	n.s.	n.s.	***
Coleoptera ad. (beetles)	**	n.s.	***	n.s.	n.s.	n.s.	n.s.
Coleoptera lv. (beetles)	*	n.s.	**	n.s.	n.s.	n.s.	n.s.
Diplopoda (millipedes)	n.s.	n.s.	*	n.s.	*	n.s.	n.s.
Chilopoda (centipedes)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Aranea (spiders)	*	n.s.	***	n.s.	**	n.s.	n.s.
Hemiptera (true bugs)	n.s.	***	n.s.	n.s.	*	n.s.	n.s.
Blattodea (cockroaches)	n.s.	n.s.	*	n.s.	*	n.s.	n.s.
Dermaptera (earwigs)	***	***	n.s.	***	n.s.	n.s.	n.s.
Isopoda (woodlice)	*	n.s.	***	n.s.	**	n.s.	n.s.
Total macrofauna	n.s.	n.s.	***	n.s.	*	***	**
Richness	***	n.s.	***	n.s.	n.s.	n.s.	**
Detritivores	n.s.	n.s.	***	n.s.	n.s.	n.s.	**
Herbivores	n.s.	n.s.	*	n.s.	n.s.	**	n.s.
Predators	**	n.s.	***	n.s.	n.s.	n.s.	n.s.

19 \*, \*\*, \*\*\* indicate significant differences at  $p < 0.05$ ,  $p < 0.01$ ,  $p < 0.001$  level, respectively; n.s. not significant  
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31 **Table 6.** The effects of management (Man) and soil depth on the root density, length and  
 32 average root diameter. Mean (SEM) (n=4).

	Root density (kg m <sup>-3</sup> )	Root length (cm cm <sup>-3</sup> )	Average root diameter (mm)
<i>Morichal</i>			
0-10 cm			
IRG-Tully	2.16 (0.47)	7.33 (0.76)	0.69 (0.02)
IRG-Ruz	2.34 (0.36)	6.36 (0.91)	0.70 (0.01)
TM-cont	1.08 (0.31)	3.83 (0.69)	0.72 (0.02)
10-20 cm			
IRG-Tully	0.53 (0.05)	1.55 (0.25)	0.84 (0.03)
IRG-Ruz	0.24 (0.04)	1.09 (0.16)	0.75 (0.01)
TM-cont	0.17 (0.07)	0.60 (0.07)	0.84 (0.04)
<i>Villasol</i>			
0-10 cm			
IRG-Tully	2.00 (0.38)	7.32 (0.94)	0.69 (0.02)
IRG-Mar	2.76 (0.75)	3.98 (0.65)	0.81 (0.03)
TM-rot	3.49 (1.12)	4.06 (0.53)	0.86 (0.04)
10-20 cm			
IRG-Tully	0.73 (0.19)	1.50 (0.22)	0.91 (0.04)
IRG-Mar	0.47 (0.10)	0.95 (0.23)	0.93 (0.06)
TM-rot	0.66 (0.26)	0.83 (0.20)	0.97 (0.02)
<i>Effects</i>			
Mn.	n.s.	n.s.	n.s.
Site	**	n.s.	***
Depth	***	***	***
Mn x Site	*	n.s.	n.s.
Mn x Depth	n.s.	*	n.s.
Site x Depth	n.s.	n.s.	n.s.
Mn x Site x Depth	n.s.	n.s.	n.s.

33 AMP-Tully, adaptive multi-paddock grazing with prevailing *B. humidicola* cv. Tully; AMP-Ruz, adaptive multi-  
 34 paddock grazing with prevailing *B. ruziensis*; TM-cont, traditional management consisting of continuous  
 35 grazing; AMP-Mar, adaptive, multi-paddock grazing with prevailing *B. brizantha* cv. Marandu; TM-rot, traditional  
 36 management consisting of rotational grazing.

37 \*, \*\*, \*\*\* indicate significant differences at p<0.05, p<0.01, p<0.001 level, respectively; n.s. not significant

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45 **Fig. 1.** High stocking rate within a *B. humidicola* cv. Tully paddock managed by IRG in  
46 Morichal site (a); adjacent farm managed by continuous grazing at low (<1 LU ha<sup>-1</sup>) stocking  
47 rate (b)

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49 **Fig. 2.** Mean ( $\pm$ S.E.) of soil organic carbon stocks (Mg C ha<sup>-1</sup>) of the different paddocks  
50 (calculated on an equivalent soil mass (ESM) of 2000 Mg soil ha<sup>-1</sup>) in Morichal and Villasol  
51 sites as affected by grazing management (Mn), study site (Site) and their interaction. (n = 4)  
52 \*, \*\*, \*\*\* indicate significant differences (Generalized linear mixed model) at p<0.05, p<0.01,  
53 p<0.001 level, respectively; n.s. not significant

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55 **Fig. 3.** (a) Ordination of the samples from Morichal (represented by black circles (● and ○ for  
56 0-10 cm and 10-20 cm depth, respectively)) and Villasol (represented by red squares (■ and □  
57 for 0-10 cm and 10-20 cm depth, respectively) in the orthogonal space defined by PC1 and PC2  
58 axis of the PCA. IRG-Tully, paddock covered predominantly with *B. humidicola* cv. Tully  
59 managed by intensive rotational grazing; IRG-Mar, paddock covered predominantly with *B.*  
60 *brizantha* cv. Marandú managed by intensive rotational grazing; IRG-Ruz, paddock covered  
61 predominantly by *B. ruziziensis* managed by intensive rotational grazing; TM-cont, traditional  
62 management consisting of continuous grazing (Morichal area); TM-rot, traditional management  
63 consisting of rotations with long grazing period and short pasture recovery (Villasol area). (b)  
64 Loading plots for measured soil parameters in Principal Components Analysis (PCA). SOC,  
65 soil organic carbon; Gmean, geometric mean of enzymatic activities; MWD, mean weight  
66 diameter of soil aggregates; BD, bulk density; AWC, available water content.

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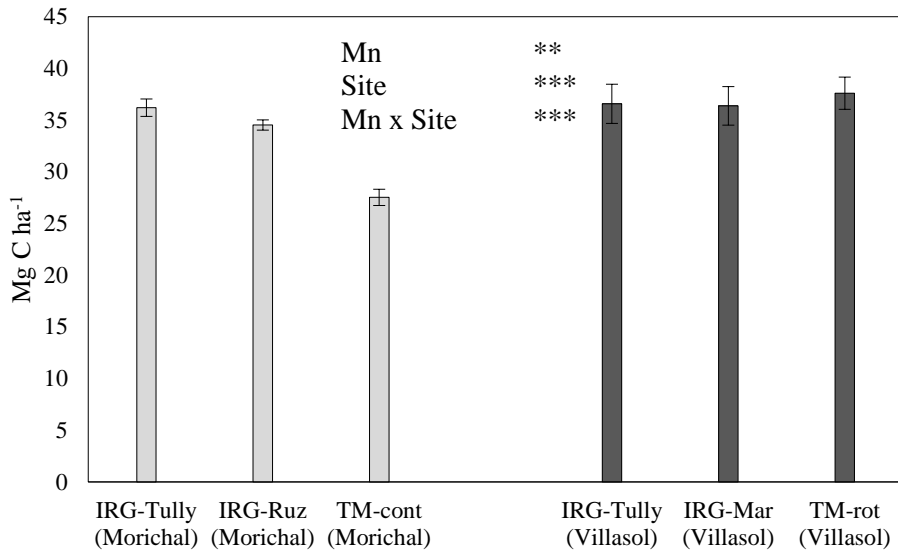


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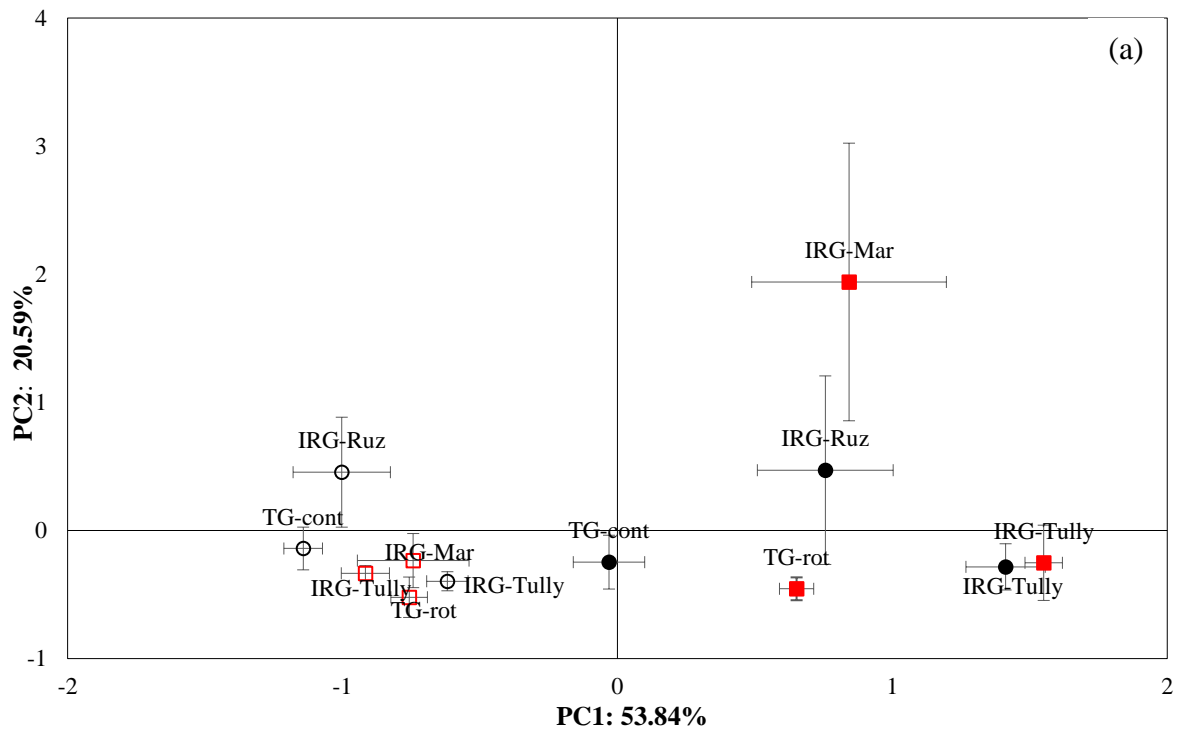
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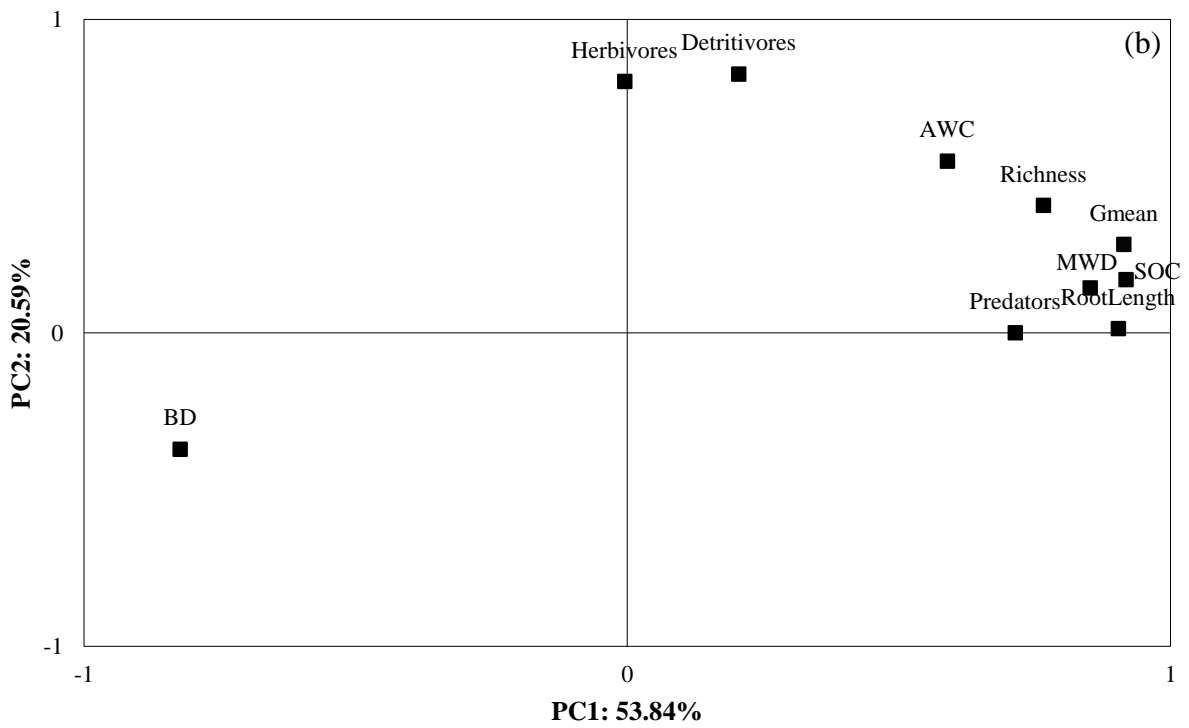
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