



Original article



Seasonal responses of soil microbial biomass C and enzymatic activity comparing no-tillage and integrated crop-livestock systems

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ABSTRACT

Sustainable agricultural systems, such as integrated crop-livestock (ICL) and no-tillage (NT), aim to sustainably produce crops and livestock while simultaneously conserving soil and its microbial properties, mainly in tropical regions. However, little is known about how microbial properties respond seasonally to management applied in NT and ICL. Thus, this study assessed the seasonal responses of soil microbial biomass C and enzymatic activity comparing both NT and ICL. The experimental area, under a block design with four replicates, with both NT and ICL management, was implemented in December 2022 on Yellow Argisol soil in Maranhao state, Brazil. Soil samples were collected (0–20 cm depth) in March, June, September, December, and March (2023). The results showed an effect size varying between 0.06 and 0.95 for agricultural systems, and 0.63 to 0.95 for sampling time. For the interaction between agricultural systems and sampling time, the effect size was superior to 0.86. NT showed initially higher microbial biomass C (~50%), leveling with ICL by the end of the sampling period. Phosphatase and dehydrogenase increased in ICL from March to June (~200% and ~700% for phosphatase and dehydrogenase, respectively), while fluorescein diacetate hydrolysis fluctuated in NT. Urease was higher (~100%) during all sampling times in NT. Linear discriminant analysis revealed distinct responses across sampling times, with a positive effect of pH on enzymatic activity in both systems and soil moisture and P impacting positively on microbial biomass in ICL. Our results revealed significant seasonal responses of soil microbial biomass and enzymatic activity comparing NT and ICL, but with distinct responses to agricultural systems. The study showed seasonal variation of soil microbial biomass and enzymatic activity dependent on the characteristics of NT and ICL. Therefore, understanding these differences helps farmers make better decisions for healthier soil and better crops.

1. Introduction

Sustainable agricultural systems are recognized as farming techniques that produce crops or livestock while conserving the environment, including the protection of soil and biodiversity, and being economically and socially viable [1]. Integrated crop-livestock (ICL) and no-tillage (NT) are particularly important agricultural systems used

worldwide in crop production. Despite both ICL and NT being considered sustainable systems, they differ in terms of soil and crop management. ICL focuses on integrating crop and livestock production within the same system, promoting the recycling of organic residues from crops and livestock [2]. In contrast, NT involves directly seeding crops under the straw of the previous crop without disturbing the soil through tillage [3]. This maintains soil structure, reduces erosion, and conserves soil

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moisture [4]. Both systems contribute similarly to cycling nutrients, increasing soil organic matter, improving soil health, and decreasing greenhouse gas emissions [5,6]. In Brazil, these systems are significantly important, occupying approximately 50 million hectares. Specifically, ICL is characterized by the annual rotation of pastures, used for meat or milk production [7], while NT involves maintaining permanent soil cover with straw and crop rotation, primarily using soybean [8].

An important aspect observed with the use of both ICL and NT is their potential benefits to soil biological properties [9–11], which are essential for plant productivity. Particularly, soil microbial biomass (SMB) is one of the most important attributes of soil biological properties [12], performing various functions in soils and showing high sensitivity to agricultural management [13]. Additionally, SMB produces and releases enzymes that enhance biological activity in soils [14], influencing the cycling of C, N, and P [15]. For instance, phosphatase and urease are the primary enzymes involved in the cycling of P and N, respectively, contributing to increased availability of these elements in soils [16,17]. Therefore, agricultural systems that conserve and increase SMB have shown higher soil enzymatic activity, leading to increased cycling of nutrients and crop yield. Indeed, previous studies have demonstrated increased SMB content and enzymatic activity with the adoption of both ICL and NT compared to conventional systems [18,19].

Although land use change, such as soil management, drives the responses of both SMB and enzymatic activity, some environmental factors, such as soil temperature and moisture, can also affect these biological properties [20]. This is particularly important in tropical regions with high seasonal variation in environmental conditions, where both SMB and enzymatic activity can exhibit significant annual variation [21]. Interestingly, previous studies have shown significant seasonal variation of SMB in NT [22] and ICL [23] compared to conventional systems. For instance, Lopes et al. [22] compared no-tillage and conventional systems and observed that the soil microbial properties, mainly microbial biomass C and N, changed significantly between seasons, being influenced by soil moisture and temperature. However, little is yet known about the seasonal variation in SMB and enzymatic activity comparing both NT and ICL, mainly in tropical regions, such as Brazil. Seasonal analyses of both SMB and enzymatic activity can provide key insights into the main drivers affecting these

biological properties in both NT and ICL, contributing to a better understanding of potential soil biological activity, particularly related to nutrient cycling. In this study, we hypothesize that there will be significant differences in the seasonal variations of SMB and enzymatic activity under NT and ICL across a year due to their specific management practices. To address this hypothesis, we assessed both SMB and enzymatic activity in soils under ICL and NT during five sampling times across a year.

2. Material and methods

2.1. Study area

The study was conducted at Farm Barbosa, Brejo, Maranhao state ($3^{\circ}42'66''S$; $42^{\circ}56'25''W$, 102 m). The soil is classified as Yellow Argisol containing 76 % sand, 8 % silt, and 16 % clay. The annual precipitation and temperature are shown in Fig. 1A. This farm has agricultural areas under NT and ICL systems that were implemented after the deforestation of native Cerrado in 2003. This was followed by applying 2.0 tons ha^{-1} of calcitic limestone +0.5 ton ha^{-1} of gypsum. In 2004, NT was implemented by applying reduced tillage. This area has been annually cultivated with soybean and millet, used as cover, under crop succession. Every year, soil fertilization consists of 100 kg ha^{-1} of ammonium sulfate, 150 kg ha^{-1} of monoammonium phosphate, and 170 kg ha^{-1} of potassium chloride. ICL was implemented in 2009 with soybean being cultivated followed by pasture (*Urochloa brizantha* cv. Marandu) and intercropped with maize. Annually at the sowing of soybean, the soil is fertilized with 340 kg ha^{-1} (formulation NPK 12-30-00). After 30 days, there is the application of 200 kg ha^{-1} (formulation NPK 10-00-30). After the crop harvest (soybean and maize), the pasture is grazed by cattle (three units of animals per hectare).

To evaluate the effect of these systems on soil properties, experimental plots (30 m \times 40 m) were established in December 2022 under a block design with four replicates. The agricultural practices of both NT and ICL were applied in these plots. In NT, soybean was sown in January and harvested in April, followed by millet sown in May and left as soil cover until September (Fig. 1B). In ICL, maize was sown in January and harvested in May, followed by pastures sown and cattle grazing from

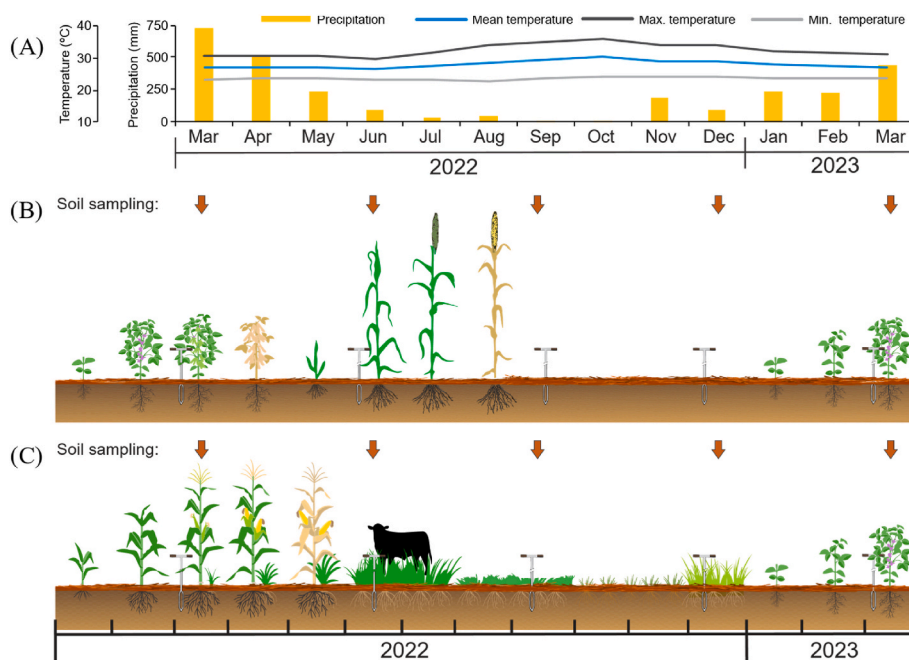


Fig. 1. Climatic characteristics during the 2022/2023 crop season (A) and the agricultural management applied in no-tillage (B) and integrated crop-livestock (C) systems. The arrows correspond to soil sampling periods (2022; March, June, September, December, and 2023; March).

June to December (Fig. 1C). In 2023, soybean was sown in both NT and ICL in January.

2.2. Soil sampling and analysis

To assess the seasonal variation of SMB and enzymatic activity, soil samples were collected at a depth of 0–20 cm in 2022 (March, June, September, and December) and 2023 (March) (Fig. 1B and C). In each period, five randomized points were sampled in each plot, and the samples were combined to form four composite samples in each system (NT and ICL), totaling eight composite soil samples per period. Since the experiment had five sampling times (March, June, September, December, and March 2023), a total of 40 soil composite samples were obtained (8 composite samples x 5 sampling times). All composite samples were placed in labeled plastic bags and stored in an icebox for transportation. In the laboratory, the samples were sieved (2 mm) and stored at 4 °C before soil biological analysis. Soil properties (Table S1) were analyzed according to Embrapa [24]. Soil pH was determined in a 1:2.5 soil/water extract. Available phosphorus (P) was estimated by photometry. Soil temperature was measured for 5 min at a depth of 10 cm using a probe thermometer during each soil sampling. Soil moisture content was estimated using the gravimetric method, expressed as the mass of water per mass of dry soil.

The microbial biomass carbon (MBC) was assessed according to Vance et al. [25], through the extraction of C from fumigated and unfumigated soils by potassium dichromate. An extraction efficiency coefficient of 0.41 was applied to convert the difference in C between fumigated and unfumigated soil into microbial biomass C. Total organic C (TOC) was determined by the wet combustion method using a mixture of potassium dichromate and sulfuric acid under heating [26]. The microbial quotient (qMic) was defined as the ratio between MBC and TOC [27]. The hydrolysis of fluorescein diacetate (FDA) and the dehydrogenase activity (DHA) were estimated according to Schnürer and Rosswall [28] and Casida et al. [29], respectively. The evaluation of FDA, which measures the activities of esterases, proteases, and lipases [30], was conducted by incubating soil samples with and without fluorescein diacetate, followed by the measurement of fluorescein content using spectrophotometry. The evaluation of DHA was based on the spectrophotometric determination of triphenyl tetrazolium formazan (TTF). The urease activity was determined according to Kandeler and Gerber [31], using urea as a substrate and the amount of ammonia produced being measured by spectrophotometry. The activity of acid phosphatase was evaluated through colorimetric estimation of *p*-nitrophenol [32].

2.3. Statistical analysis

The significance tests of the treatment effects (soil management [no-tillage (NT) and integrated crop-livestock (ICL)], and collection time (five sample times) and their interactions were performed using the analysis of variance with repeated measures (ANOVAR) in RStudio 2023.12.0–369 software. Shapiro-Wilk, Levene's and Mauchly's tests for normality, homogeneity of variances and sphericity, respectively, were used to ensure non-violation of the ANOVAR assumptions. Effect size was calculated as generalized eta squared (η^2_G) [33]. All data showed sphericity and Greenhouse-Geisser correction was not applied. The normality and homogeneity of variances were observed in our dataset. Bonferroni's test was used to access significant pair-wise differences. Linear discriminant analysis (LDA) was performed in Past 4.13 software. Permutational multivariate analysis of variance (PERMANOVA), based on Bray-Curtis's index, was used to confirm the groups formed in LDA. Redundancy analysis (RDA) was carried out to evaluate the relationships between soil properties (explanatory variables) and microbial properties (response variables) using the vegan package in RStudio software. The temporal stability of soil microbial biomass and enzymatic activity was assessed according to Tilman et al. [34]. Temporal stability was calculated as the ratio between the mean and its temporal standard

deviation for each variable evaluated in the experiment, subsequently tested using the non-parametric Wilcoxon test ($p < 0.05$).

3. Results

3.1. Soil microbial biomass C

Soil microbial biomass C, microbial quotient, phosphatase, and urease were significantly affected by agricultural system, sampling time, and their interaction (Table 1). The results showed an effect size varying between 0.06 and 0.95 for agricultural systems, and between 0.63 and 0.95 for sampling time. The effect size for the interaction between agricultural systems and sampling time was superior to 0.86. Soil microbial biomass C was significantly higher in NT (March 2022), with a temporary decrease observed in June and a consistent increase from September to March (2023) (Fig. 2A). In contrast, microbial biomass C was initially lower in ICL (March 2022), temporarily increased in June, and then stabilized from September to March (2023). By the end of the sampling period (March 2023), the levels of soil microbial biomass C were similar in both NT and ICL. The microbial quotient initially showed similar values in both NT and ICL, while it increased in ICL and decreased in NT by June (Fig. 2B). From September (2022) to March (2023), the microbial quotient increased in NT, while ICL showed a decrease in this soil parameter.

3.2. Soil enzymatic activity

From March to June (2022), both phosphatase and dehydrogenase increased in ICL, followed by a subsequent decrease from September (2022) to March (2023) (Fig. 2C and D). In NT, both phosphatase and dehydrogenase consistently increased over time. Urease activity was consistently higher in NT and increased by the end of the sampling period (March 2023) (Fig. 2E). Regarding fluorescein diacetate hydrolysis, NT showed a temporary increase in June followed by a decrease in September. Afterwards, fluorescein diacetate hydrolysis stabilized from December to March (2023). In ICL, fluorescein diacetate hydrolysis consistently decreased over time (Fig. 2F).

3.3. Multivariate responses and temporal stability of microbial biomass and enzymatic activity

The linear discriminant analysis showed distinct responses of soil microbial biomass and enzymatic activity at different sampling times (Fig. 3). In March 2022, we observed that both NT (with soybean) and ICL (with maize) exhibited a clear separation from other sampling times. From June (2022) through March (2023), the responses of each system differed. In NT, soil microbial properties clustered separately in June (with millet) compared to September and December (with straw), and March (2023; with soybean) which were closer. In ICL, soil microbial properties were closer in June and September (with pasture), while they showed separation in December (dried pasture) and March (2023; with soybean).

On the other hand, when comparing the main drivers of soil biological properties (Fig. 4), we observed soil pH positively influencing both dehydrogenase and phosphatase in NT (September and December) and ICL (September). Conversely, soil moisture and P positively drove the responses of microbial biomass C, microbial quotient, and TOC, mainly in ICL. The temporal stability of microbial biomass C and enzymatic activity did not differ when comparing both NT and ICL (Fig. 5). The exception was dehydrogenase activity, where temporal stability was higher in NT ($P = 0.029$; Fig. 5E).

4. Discussion

In this study, we compared two well-known sustainable agricultural systems: no-tillage versus integrated crop-livestock, to assess their

Table 1

ANOVA with repeated measures for the effects of soil management (SM), sampling time (ST) and their interaction (SM x ST) on all measured variables. Effect size was calculated as generalized eta squared (η_G^2).

| | SM | | ST | | SM x ST | | Sphericity ^a |
|-----------------------|--------------------|------------|---------|------------|---------|------------|-------------------------|
| | p-value | η_G^2 | p-value | η_G^2 | p-value | η_G^2 | |
| Total organic C | 0.41 ^{NS} | 0.06 | <0.05 | 0.63 | <0.05 | 0.90 | 0.15 |
| Microbial biomass C | <0.05 | 0.78 | <0.05 | 0.63 | <0.05 | 0.94 | 0.60 |
| Microbial quotient | <0.05 | 0.84 | <0.05 | 0.58 | <0.05 | 0.94 | 0.42 |
| Acid phosphatase | <0.05 | 0.90 | <0.05 | 0.94 | <0.05 | 0.93 | 0.92 |
| Urease | <0.05 | 0.95 | <0.05 | 0.95 | <0.05 | 0.86 | 0.26 |
| Dehydrogenase | 0.18 ^{NS} | 0.07 | <0.05 | 0.94 | <0.05 | 0.87 | 0.24 |
| Fluorescein diacetate | 0.31 ^{NS} | 0.07 | <0.05 | 0.97 | <0.05 | 0.91 | 0.50 |

^a Sphericity was examined with the Mauchly's Test. NS = non-significant.

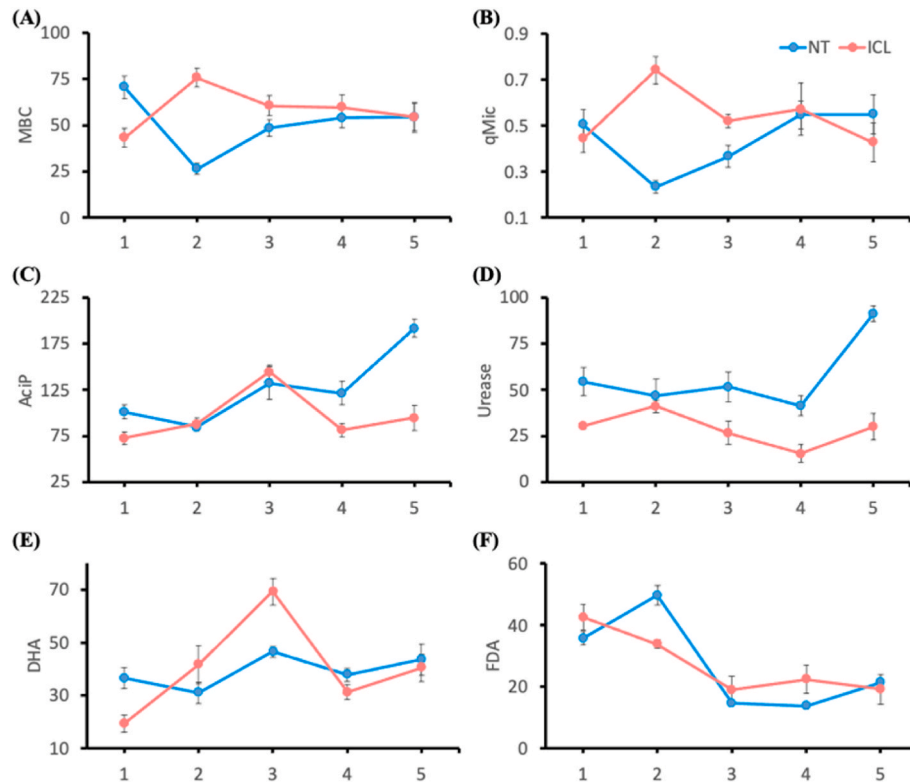


Fig. 2. (A) microbial biomass C (MBC; mg C kg⁻¹ soil), (B) microbial quotient (qMic; %), (C) acid phosphatase (AcIP; μ g *p*-nitrophenol g soil h⁻¹), (D) urease (μ g N-NH₄ g⁻¹ soil h⁻¹), (E) dehydrogenase (DHA; mg TPF kg⁻¹ soil d⁻¹), and (F) fluorescein diacetate hydrolysis (FDA; μ g FDA g⁻¹ soil) comparing no-tillage (NT) and integrated crop-livestock (ICL) systems and sampling times (1- March 2022; 2- June 2022; 3- September 2022; 4- December 2022; 5- March 2023).

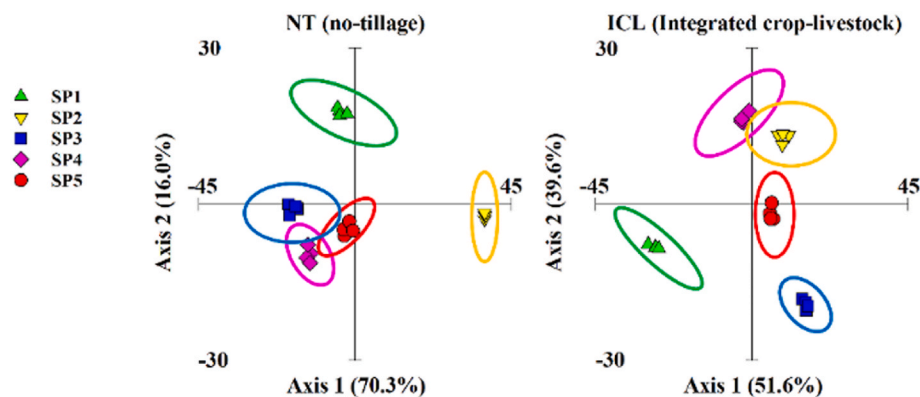


Fig. 3. Linear discriminant analysis (LDA) showing differences between the five collection times in soil under no-tillage (NT) or integrated crop-livestock (ICL). Sampling times: SP1 (March 2022); SP2 (June 2022); SP3 (September 2022); SP4 (December 2022); SP5 (March 2023).

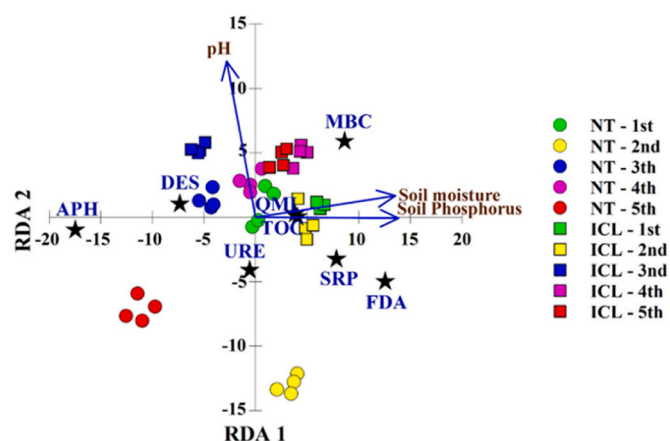


Fig. 4. Redundance analysis showing the relationships between chemical soil properties (pH, soil phosphorus and soil moistures) and biological soil parameters (SRP = soil respiration, TOC = total organic C, MBC = microbial biomass C, QMI = microbial quotient, APH = acid phosphatase, URE = urease, DES = dehydrogenase, FDA = fluorescein diacetate). RDA model was significant by ANOVA ($F = 3.18$; $p = 0.012$).

impact on soil microbial biomass and enzymatic activity across seasons. In tropical regions, soil moisture is the main factor influenced by seasonal variation. Typically, higher levels of soil moisture are observed during the rainy season than the dry season, and soil moisture is recognized as a main driver of soil microbial biomass and enzymatic activity [21,35]. Therefore, it is expected that the responses of soil microbial properties will be primarily influenced by soil moisture in tropical regions. However, our results showed that, regardless of typical seasonal variation, differences in soil management distinctly affected soil microbial biomass C and both urease and phosphatase activities. These results confirm our hypothesis, indicating varied responses of soil microbial biomass C and enzymatic activity to seasonal variation when comparing no-tillage and integrated crop-livestock. Our results also showed different effect sizes when comparing agricultural systems,

sampling time, and interactions, where the effect size was significant for all variables when analyzing the interaction between agricultural systems and sampling time [33]. Regardless of seasonal variation, the results showed higher values of microbial biomass C and microbial quotient under integrated crop-livestock, while urease was more prominent under no-tillage.

Specifically, higher microbial biomass C and microbial quotient were found from June to December in integrated crop-livestock. These results can be attributed to the permanent presence of pasture species, which provide abundant living roots [10], associated with the contribution of cattle excreta [9]. These characteristics support higher microbial biomass content and microbial quotient due to the input of more decomposable organic residues [36,37]. Indeed, a high microbial quotient may indicate the presence of less stable organic matter [38]. Previous studies in Brazilian cerrado observed high microbial biomass C and microbial quotient in soil under integrated crop-livestock due to the presence of pastures and the contribution of animal excreta [9,10]. On the other hand, no-tillage is influenced by the addition of more stabilized C from long-term straw cover, which contributes less available C to microbial biomass [39]. Thus, this factor promotes a lower microbial quotient [38].

At the beginning of the experiment, we observed crop-specific effects on soil microbial biomass C, with soybean in no-tillage increasing microbial biomass C compared to maize in integrated crop-livestock. This difference might be attributed to distinct root traits, such as root exudates, probably providing distinct C sources for microbial biomass [40]. For instance, soybean releases root exudates that provide more energy sources to soil microorganisms [41], leading to increased microbial biomass. Indeed, a previous study identified several C sources in the form of sugars released by soybean roots, such as glucose, arabinose, galactose, sucrose, kojibiose, and oligosaccharides [42]. Interestingly, both no-tillage and integrated crop-livestock under soybean in March 2023 showed similar microbial biomass C, confirming this positive effect of soybean on soil microbial biomass C.

Enzymatic activity is crucial for soil functioning, with various processes depending on soil enzymes, including residue decomposition and mineralization of N, P, and S [43]. At the beginning and end of the

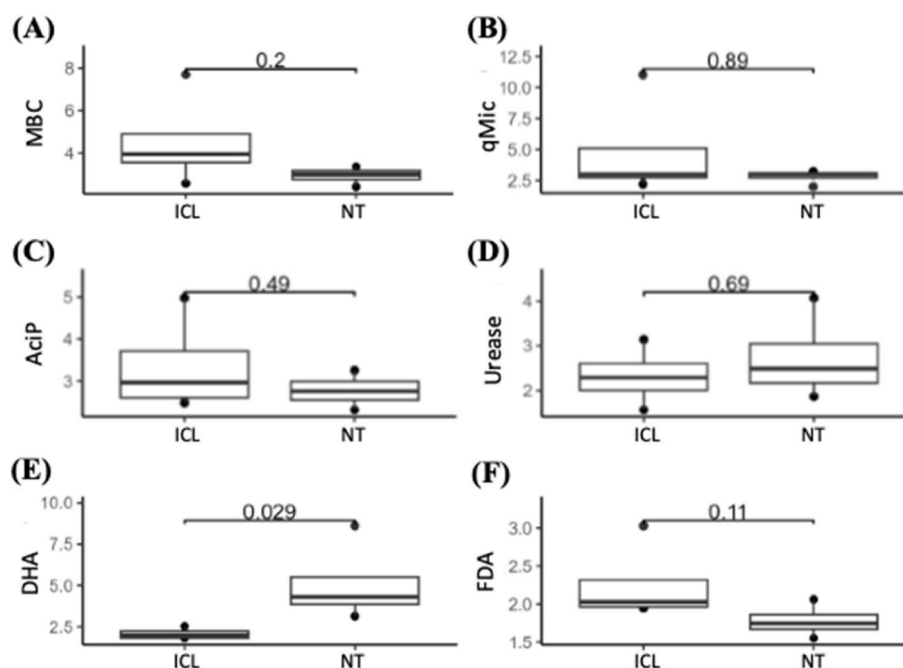


Fig. 5. Temporal stability comparing no-tillage (NT) and integrated crop-livestock (ICL) systems for (A) microbial biomass C (MBC), (B) microbial quotient (qMic), (C) acid phosphatase (AcIP), (D) urease, (E) dehydrogenase (DHA), and (F) fluorescein diacetate hydrolysis (FDA). Values on the top of each figure were tested using the non-parametric test of Wilcoxon ($p < 0.05$).

experiment, phosphatase activity increased in no-tillage, suggesting a potential contribution of soybean associated with straw cover [44]. For instance, Tyler [44] reported cover crops enhancing phosphatase activity under no-tillage, due to increased substrate availability. In integrated crop-livestock, lower phosphatase activity may be due to higher P-fertilizer addition in maize (2022) and soybean (2023) [45]. Regarding urease activity, this remained consistently higher in no-tillage and increased by the end of the sampling time (under soybean) (Fig. 2D). Similar to phosphatase activity, this higher urease activity can be attributed to straw presence [46]. In addition, high input of organic C [47] and the N-fixing ability by soybean contribute to increased urease activity [48]. Recently, Wen et al. [49] showed through a global meta-analysis that no-tillage associated with straw input effectively increases soil enzyme activity.

We found no significant effect of both no-tillage and integrated crop-livestock on dehydrogenase and fluorescein diacetate hydrolysis. Since dehydrogenase and fluorescein diacetate hydrolysis are indicators of microbial activity [50], these results suggest that the practices applied in both systems were not enough to distinctly influence soil microbial activity. Particularly, both no-tillage and integrated crop-livestock maintain permanent soil cover, by organic residues and plants, respectively, and presence of roots [51]. These factors contribute to the maintenance of soil microbial activity [52]. When comparing the effect of sampling time, the transition from soybean to pastures, in integrated crop-livestock, increased dehydrogenase activity, indicating a positive effect of pastures stimulating this enzyme due to high plant biomass [53]. In no-tillage, dehydrogenase showed lower variation, slightly increasing with straw presence, as also reported by Yan et al. [46] and Yang et al. [54]. In no-tillage, fluorescein diacetate hydrolysis temporarily increased in June (under millet) and decreased in September (under straw). In integrated crop-livestock, fluorescein diacetate hydrolysis consistently decreased over time. This result could be due to high substrate availability, potentially saturating esterase, which is an extracellular enzyme that degrades fluorescein, thus reducing fluorescein diacetate hydrolysis [55].

The linear discriminant analysis showed that soil microbial biomass and enzymatic activity responded to different plant species and soil management practices. When comparing soybean and maize grown under no-tillage and integrated crop-livestock, respectively, distinct responses in soil microbial properties were observed. For instance, soil microbial biomass C temporarily decreased in no-tillage when millet was cultivated and increased in integrated crop-livestock when pasture and cattle were present. This means that changing plant species within these systems (e.g., switching soybean to millet, and maize to pastures, in no-tillage and integrated crop-livestock, respectively) led to different microbial responses, particularly when straw cover was present on the soil surface in no-tillage. The presence of millet adds a more recalcitrant C in no-tillage, while pastures and cattle manure, in integrated livestock, contribute to a more labile C and N. This main difference can contribute to distinct responses of soil microbial biomass and enzymatic activity. Our results confirm that the distinct plant species [56], grown and the way the soil is managed significantly impact soil microbial biomass and activity [57,58]. In addition, the presence of organic residues, in no-tillage, modulates the response of microbial biomass and enzymatic activity, and this occurs due to the chemical composition of the organic residues returned to the soil, which influences microbial responses [59]. In our study, millet straw has a more recalcitrant C, which temporarily reduces soil microbial biomass in the no-tillage system when this residue is present.

Soil pH was the main driver of dehydrogenase in NT (September and December) and ICL (September). During these periods, there is the presence of straw cover in no-tillage (millet straw) and integrated crop-livestock (dried pastures), confirming the effect of straw associated with pH on soil dehydrogenase activity [60]. Soil moisture, as an important driver in tropical regions, was found to be related to microbial biomass and organic C in integrated crop-livestock, suggesting the effect of living

plants and roots [61]. Additionally, available P was shown to be an important driver of microbial biomass in integrated crop-livestock. This means that higher availability of P can increase soil microbial biomass C [62]. However, greater microbial biomass could increase the availability of P from organic matter in integrated crop-livestock through enzymatic processes [63].

Our results showed that the temporal stability of microbial biomass and enzymatic activity does not differ when comparing integrated crop-livestock and no-tillage. This may be due to the continuous input of organic residues associated with less disturbance by zero or reduced tillage in both systems [64]. Indeed, Kostin et al. [65] reported that agricultural systems with fewer perturbations have higher stabilization of microbial properties. Thus, the adoption of agricultural practices with lower soil disturbance associated with the addition of organic residues will contribute to maintaining more stable and resilient microbial properties over time [65], promoting long-term soil health and ecosystem sustainability.

Although different temporal dynamics were observed comparing both agricultural systems, the no-tillage promoted in general higher phosphatase and urease activities. These findings support the idea of continuous input of straw into the soil to increase enzymatic activity related to P and N cycling [66]. This increases the availability of plant nutrients and crop productivity [12]. These findings reinforce the importance of agricultural management practices, such as the no-tillage system, in stimulating enzymatic activity. These microbial indicators are critical for maintaining soil health and long-term agricultural productivity [67]. Future studies should elucidate the mechanisms driving these seasonal variations to direct agricultural management strategies for enhancing soil health and sustainability.

5. Conclusion

This study demonstrated that the responses of soil microbial biomass and enzymatic activity differ between no-tillage and integrated crop-livestock systems. Furthermore, the impact of seasonal variations in tropical soils on soil microbial biomass and enzymatic activity depends on the characteristics of each agricultural system. Our findings showed the effects of crop succession and millet straw cover in integrated crop-livestock and no-tillage, respectively, on microbial biomass C, urease, and phosphatase activity. Additionally, the distinct responses of soil microbial properties observed at different sampling times suggest a direct effect of agricultural systems, which can have implications for system-specific management practices.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

CRediT authorship contribution statement

Romario Martins Costa: Writing – original draft, Investigation. **Erica Maria Batista Araujo:** Writing – original draft, Investigation. **Davila Esmelinda Oliveira Silva:** Writing – original draft, Investigation. **Sandra Mara Barbosa Rocha:** Writing – original draft, Investigation. **Aurenivia Bonifacio:** Writing – review & editing, Writing – original draft, Formal analysis. **Ricardo Silva Sousa:** Writing – review & editing, Writing – original draft, Formal analysis. **Arthur Prudencio de Araujo Pereira:** Writing – review & editing, Writing – original draft. **Erika Valente de Medeiros:** Writing – review & editing, Writing – original draft. **Edvaldo Sagrilo:** Writing – review & editing, Writing – original draft, Visualization. **José Oscar Lustosa de Oliveira Junior:** Writing – review & editing, Writing – original draft. **Henrique Antunes de Souza:** Writing – review & editing, Writing – original draft. **Ademir Sergio Ferreira Araujo:** Writing – review & editing, Writing – original draft, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ejsobi.2024.103628>.

References

- [1] R. Çakmakçı, M.A. Salık, S. Çakmakçı, Assessment and principles of environmentally sustainable food and agriculture systems, *Agriculture* 13 (2023) 1073, <https://doi.org/10.3390/agriculture13051073>.
- [2] J.C. Reis, G.S. Rodrigues, I. Barros, R. de A.R. Rodrigues, R.D. Garrett, J. F. Valentim, M.Y.T. Kamoi, M. Michetti, F.J. Wruck, S. Rodrigues-Filho, P.E. O. Pimentel, S. Smukler, Integrated crop-livestock systems: a sustainable land-use alternative for food production in the Brazilian Cerrado and Amazon, *J. Clean. Prod.* 283 (2021) 124580, <https://doi.org/10.1016/j.jclepro.2020.124580>.
- [3] E.J. Possamai, P.C. Conceição, C. Amadori, M.L.C. Bartz, R. Ralisch, M. Vicensi, E. F. Marx, Adoption of the no-tillage system in Paraná State: a (re)view, *Rev Bras Cienc Solo* 46 (2022), <https://doi.org/10.36783/18069657rbcs20210104>.
- [4] B. Bekele, T. Habtemariam, Y. Gemi, Evaluation of conservation tillage methods for soil moisture conservation and maize grain yield in low moisture areas of SNNPR, Ethiopia, *Water Conservation Science and Engineering* 7 (2022) 119–130, <https://doi.org/10.1007/s41101-022-00129-0>.
- [5] W. Bieluczyk, M. de C. Piccolo, M.G. Pereira, M.T. Moraes, A. Soltangheisi, A.C. C. Bernardi, J.R.M. Pezzopane, P.P.A. Oliveira, M.Z. Moreira, P.B. Camargo, C.T. dos S. Dias, I. Batista, M.R. Cherubin, Integrated farming systems influence soil organic matter dynamics in southeastern Brazil, *Geoderma* 371 (2020) 114368, <https://doi.org/10.1016/j.geoderma.2020.114368>.
- [6] L.A. Alves, L.G.O. Denardin, G.D. Farias, J.P.M. Flores, D. Filippi, C. Bremm, P.C. F. Carvalho, A.P. Martins, L.C. Gatiboni, T. Tiecher, Fertilization strategies and liming in no-till integrated crop-livestock systems: effects on phosphorus and potassium use efficiency, *Rev Bras Cienc Solo* 46 (2022), <https://doi.org/10.36783/18069657rbcs20210125>.
- [7] A. Moraes, P.C.F. Carvalho, I. Anghinoni, S.B.C. Lustosa, S.E.V.G.A. Costa, T. R. Kunrath, Integrated crop-livestock systems in the Brazilian subtropics, *Eur. J. Agron.* 57 (2014) 4–9, <https://doi.org/10.1016/j.eja.2013.10.004>.
- [8] R. Fuentes-Llanillo, T.S. Telles, D. Soares Junior, T.R. Melo, T. Friedrich, A. Kassam, Expansion of no-tillage practice in conservation agriculture in Brazil, *Soil Tillage Res.* 208 (2021) 104877, <https://doi.org/10.1016/j.still.2020.104877>.
- [9] J.A. Bonetti, H.B. Paulino, E.D. Souza, M.A.C. Carneiro, J.O. Caetano, Soil physical and biological properties in an integrated crop-livestock system in the Brazilian Cerrado, *Pesqui. Agropecu. Bras.* 53 (2018) 1239–1247, <https://doi.org/10.1590/s0100-204x2018001100006>.
- [10] J.V. Laroça, J.M.A. Souza, G.C. Pires, G.J.C. Pires, L.P. Pacheco, F.D. Silva, F. J. Wruck, M.A.C. Carneiro, L.S. Silva, E.D. Souza, Soil quality and soybean productivity in crop-livestock integrated system in no-tillage, *Pesqui. Agropecu. Bras.* 53 (2018) 1248–1258, <https://doi.org/10.1590/s0100-204x2018001100007>.
- [11] S. Bansal, P. Chakraborty, S. Kumar, Crop-livestock integration enhanced soil aggregate-associated carbon and nitrogen, and phospholipid fatty acid, *Sci. Rep.* 12 (2022) 2781, <https://doi.org/10.1038/s41598-022-06560-6>.
- [12] Y. Li, S.X. Chang, L. Tian, Q. Zhang, Conservation agriculture practices increase soil microbial biomass carbon and nitrogen in agricultural soils: a global meta-analysis, *Soil Biol. Biochem.* 121 (2018) 50–58, <https://doi.org/10.1016/j.soilbio.2018.02.024>.
- [13] Y. Yan, C. Wang, J. Zhang, Y. Sun, X. Xu, N. Zhu, Y. Cai, D. Xu, X. Wang, X. Xin, J. Chen, Response of soil microbial biomass C, N, and P and microbial quotient to agriculture and agricultural abandonment in a meadow steppe of northeast China, *Soil Tillage Res.* 223 (2022) 105475, <https://doi.org/10.1016/j.still.2022.105475>.
- [14] C. Wang, H. Li, X. Sun, T. Cai, Responses of soil microbial biomass and enzyme activities to natural restoration of reclaimed temperate marshes after abandonment, *Front. Environ. Sci.* 9 (2021), <https://doi.org/10.3389/fenvs.2021.701610>.
- [15] M.C.H. Moraes, E.V. Medeiros, D.S. Andrade, L.D. Lima, I.C.S. Santos, A.P. Martins Filho, Microbial biomass and enzymatic activities in sandy soil cultivated with lettuce inoculated with plant growth promoters, *Revista Caatinga* 31 (2018) 860–870, <https://doi.org/10.1590/1983-21252018v31n408rc>.
- [16] O. Margalef, J. Sardans, J. Maspons, R. Molowny-Horas, M. Fernández-Martínez, I. A. Janssens, A. Richter, P. Ciais, M. Obersteiner, J. Peñuelas, The effect of global change on soil phosphatase activity, *Global Change Biol.* 27 (2021) 5989–6003, <https://doi.org/10.1111/gcb.15832>.
- [17] A. Piotrowska-Długosz, J. Długosz, A. Gryta, M. Fraç, Responses of N-cycling enzyme activities and functional diversity of soil microorganisms to soil depth, pedogenic processes and cultivated plants, *Agronomy* 12 (2022) 264, <https://doi.org/10.3390/agronomy12020264>.
- [18] A.J. Franco, A.P.V. Silva, A.B.S. Souza, R.L. Oliveira, É.R. Batista, E.D. Souza, A. O. Silva, M.A.C. Carneiro, Plant diversity in integrated crop-livestock systems increases the soil enzymatic activity in the short term, *Pesqui. Agropecuária Trop.* 50 (2020), <https://doi.org/10.1590/1983-40632020v50e04026>.
- [19] M.V.M. Sarto, W.L.B. Borges, J.R.W. Sarto, C.A.B. Pires, C.W. Rice, C.A. Rosolem, Soil microbial community and activity in a tropical integrated crop-livestock system, *Appl. Soil Ecol.* 145 (2020) 103350, <https://doi.org/10.1016/j.apsoil.2019.08.012>.
- [20] J. Deng, Y. Chong, D. Zhang, C. Ren, F. Zhao, X. Zhang, X. Han, G. Yang, Temporal variations in soil enzyme activities and responses to land-use change in the Loess plateau, China, *Appl. Sci.* 9 (2019) 3129, <https://doi.org/10.3390/app9153129>.
- [21] H.F. Amaral, K.R.F. Schwan-Estrada, J.O.A. de Sena, A. Colozzi-Filho, D. S. Andrade, Seasonal variations in soil chemical and microbial indicators under conventional and organic vineyards, *Acta Sci. Agron.* 45 (2023) e56158, <https://doi.org/10.4025/actasciagron.v45i1.56158>.
- [22] L.D. Lopes, R.C. Fontes Junior, E.P. Pacheco, M.F. Fernandes, Shifts in microbial and physicochemical parameters associated with increasing soil quality in a tropical Ultisol under high seasonal variation, *Soil Tillage Res.* 206 (2021) 104819, <https://doi.org/10.1016/j.still.2020.104819>.
- [23] N.J.C. Maia, M.C.P. da Cruz, J.C.B. Dubeux Junior, L.S. Menegatto, J.G. Augusto, G.G. Mendonça, M.C. Terçariol, J.G. Oliveira, F.F. Simili, Integrated crop-livestock versus conventional systems: use of soil indicators to detect short-term changes during seasonal variation, *Bragantia* 80 (2021), <https://doi.org/10.1590/1678-4499.20210127>.
- [24] EMBRAPA, *Manual de análises químicas de solos, plantas e fertilizantes, first ed., Brasília, 1999.*
- [25] E.D. Vance, P.C. Brookes, D.S. Jenkinson, An extraction method for measuring soil microbial biomass C, *Soil Biol. Biochem.* 19 (1987) 703–707, [https://doi.org/10.1016/0038-0717\(87\)90052-6](https://doi.org/10.1016/0038-0717(87)90052-6).
- [26] J.C. Yeomans, J.M. Bremner, A rapid and precise method for routine determination of organic carbon in soil, *Commun. Soil Sci. Plant Anal.* 19 (1988) 1467–1476, <https://doi.org/10.1080/00103628809368027>.
- [27] G. Kaschuk, O. Alberton, M. Hungria, Three decades of soil microbial biomass studies in Brazilian ecosystems: lessons learned about soil quality and indications for improving sustainability, *Soil Biol. Biochem.* 42 (2010) 1–13, <https://doi.org/10.1016/j.soilbio.2009.08.020>.
- [28] J. Schnürer, T. Rosswall, Fluorescein diacetate hydrolysis as a measure of total microbial activity in soil and litter, *Appl. Environ. Microbiol.* 43 (1982) 1256–1261, <https://doi.org/10.1128/aem.43.6.1256-1261.1982>.
- [29] L.E.J.R. Casida, D.A. Klein, T. Santoro, Soil dehydrogenase activity, *Soil Sci.* 98 (1964) 371–376.
- [30] G. Adam, H. Duncan, Development of a sensitive and rapid method for the measurement of total microbial activity using fluorescein diacetate (FDA) in a range of soils, *Soil Biol. Biochem.* 33 (2001) 943–951, [https://doi.org/10.1016/S0038-0717\(00\)00244-3](https://doi.org/10.1016/S0038-0717(00)00244-3).
- [31] E. Kandeler, H. Gerber, Short-term assay of soil urease activity using colorimetric determination of ammonium, *Biol. Fertil. Soils* 6 (1988), <https://doi.org/10.1007/BF00257924>.
- [32] K. Alef, P. Nannipieri, *Methods in Applied Soil Microbiology and Biochemistry*, Academic Press, New York, 1995, <https://doi.org/10.1016/B978-0-12-513840-6.X5014-9>.
- [33] J. Cohen, *Statistical Power Analysis for the Behavioral Sciences*, second ed., 1988.
- [34] D. Tilman, P.B. Reich, J.M.H. Knops, Biodiversity and ecosystem stability in a decade-long grassland experiment, *Nature* 441 (2006) 629–632, <https://doi.org/10.1038/nature04742>.
- [35] A.S.F. Araújo, S. Cesarz, L.F.C. Leite, C.D. Borges, S.M. Tsai, N. Eisenhauer, Soil microbial properties and temporal stability in degraded and restored lands of Northeast Brazil, *Soil Biol. Biochem.* 66 (2013) 175–181, <https://doi.org/10.1016/j.soilbio.2013.07.013>.
- [36] K.-H. Wang, R. McSorley, P. Bohlen, S.M. Gathumbi, Cattle grazing increases microbial biomass and alters soil nematode communities in subtropical pastures, *Soil Biol. Biochem.* 38 (2006) 1956–1965, <https://doi.org/10.1016/j.soilbio.2005.12.019>.
- [37] G. Egan, X. Zhou, D. Wang, Z. Jia, M.J. Crawley, D. Fornara, Long-term effects of grassland management on soil microbial abundance: implications for soil carbon and nitrogen storage, *Biogeochemistry* 141 (2018) 213–228, <https://doi.org/10.1007/s10533-018-0515-1>.
- [38] Y. Rui, D.V. Murphy, X. Wang, F.C. Hoyle, Microbial respiration, but not biomass, responded linearly to increasing light fraction organic matter input: consequences for carbon sequestration, *Sci. Rep.* 6 (2016) 35496, <https://doi.org/10.1038/srep35496>.
- [39] T. Zheng, A. Miltner, C. Liang, K.M. Nowak, M. Kästner, Turnover of bacterial biomass to soil organic matter via fungal biomass and its metabolic implications, *Soil Biol. Biochem.* 180 (2023) 108995, <https://doi.org/10.1016/j.soilbio.2023.108995>.

- [40] W. Ma, S. Tang, Z. Dengzeng, D. Zhang, T. Zhang, X. Ma, Root exudates contribute to belowground ecosystem hotspots: a review, *Front. Microbiol.* 13 (2022), <https://doi.org/10.3389/fmicb.2022.937940>.
- [41] A. Sugiyama, The soybean rhizosphere: metabolites, microbes, and beyond—a review, *J. Adv. Res.* 19 (2019) 67–73, <https://doi.org/10.1016/j.jare.2019.03.005>.
- [42] P.B. Timotiwiu, N. Sakurai, Identification of mono-, oligo-, and polysaccharides secreted from soybean roots, *J. Plant Res.* 115 (2002) 77–85, <https://doi.org/10.1007/s102650200012>.
- [43] L. Sobucki, R.F. Ramos, L.A. Meireles, Z.I. Antonioli, R.J.S. Jacques, Contribution of enzymes to soil quality and the evolution of research in Brazil, *Rev Bras Cienc Solo* 45 (2021), <https://doi.org/10.36783/18069657rbc20210109>.
- [44] H.L. Tyler, Winter cover crops and no till management enhance enzyme activities in soybean field soils, *Pedobiologia* 81–82 (2020) 150666, <https://doi.org/10.1016/j.pedobi.2020.150666>.
- [45] T. Mori, Greater impacts of phosphorus fertilization on soil phosphatase activity in tropical forests than in non-tropical natural terrestrial ecosystems: a meta-analysis, *Pedobiologia* 91–92 (2022) 150808, <https://doi.org/10.1016/j.pedobi.2022.150808>.
- [46] F. Yan, W. Zhou, Y. Sun, C. Guo, K. Xiang, N. Li, Z. Yang, Y. Wu, Q. Zhang, Y. Sun, X. Wang, J. Ma, No-tillage with straw mulching promotes the utilization of soil nitrogen by rice under wheat–rice and oilseed rape–rice cropping systems, *Front. Plant Sci.* 14 (2023), <https://doi.org/10.3389/fpls.2023.1170739>.
- [47] M.R.L. Garcia, A.A.M. Sampaio, E. Nahas, Impact of different grazing systems for bovine cattle on the soil microbiological and chemical characteristics, *Rev. Bras. Zootec.* 40 (2011) 1568–1575, <https://doi.org/10.1590/S1516-35982011000700024>.
- [48] M.D. Nakei, P.B. Venkataramana, P.A. Nkaidemi, Soybean-nodulating rhizobia: ecology, characterization, diversity, and growth promoting functions, *Front. Sustain. Food Syst.* 6 (2022), <https://doi.org/10.3389/fsufs.2022.824444>.
- [49] L. Wen, Y. Peng, Y. Zhou, G. Cai, Y. Lin, B. Li, Effects of conservation tillage on soil enzyme activities of global cultivated land: a meta-analysis, *J. Environ. Manag.* 345 (2023) 118904, <https://doi.org/10.1016/j.jenvman.2023.118904>.
- [50] W.J. Melo, G.M.P. Melo, V.P. Melo, A.S.F. Araujo, A.S. Ferraudo, L.M. A. Bertipaglia, Soil microbial biomass and enzyme activity in six Brazilian oxisols under cropland and native vegetation, *Bragantia* 79 (2020) 623–629, <https://doi.org/10.1590/1678-4499.20200242>.
- [51] R. Francaviglia, M. Almagro, J.L. Vicente-Vicente, Conservation agriculture and soil organic carbon: principles, processes, practices and policy options, *Soil Syst* 7 (2023) 17, <https://doi.org/10.3390/soilsystems7010017>.
- [52] N.L. Breil, T. Lamaze, V. Bustillo, C.-E. Marcato-Romain, B. Coudert, S. Queguiner, N. Jarosz-Pellé, Combined impact of no-tillage and cover crops on soil carbon stocks and fluxes in maize crops, *Soil Tillage Res.* 233 (2023) 105782, <https://doi.org/10.1016/j.still.2023.105782>.
- [53] C.A. Santos, C.C. Krawulski, D. Bini, T. Goulart Filho, A. Knob, C.C. Medina, G. Andrade Filho, M.A. Nogueira, Reclamation status of a degraded pasture based on soil health indicators, *Sci. Agric.* 72 (2015) 195–202, <https://doi.org/10.1590/0103-9016-2013-0274>.
- [54] Y. Yang, H. Liu, J. Wu, S. Zhang, C. Gao, S. Zhang, D.W.S. Tang, Soil enzyme activities, soil physical properties, photosynthetic physical characteristics and water use of winter wheat after long-term straw mulch and organic fertilizer application, *Front. Plant Sci.* 14 (2023), <https://doi.org/10.3389/fpls.2023.1186376>.
- [55] A. Dzionek, J. Dzik, D. Wojcieszynska, U. Guzik, Fluorescein diacetate hydrolysis using the whole biofilm as a sensitive tool to evaluate the physiological state of immobilized bacterial cells, *Catalysts* 8 (2018) 434, <https://doi.org/10.3390/catal8100434>.
- [56] L. Sun, S. Wang, M.P. Narsing Rao, Y. Shi, Z.-H. Lian, P.-J. Jin, W. Wang, Y.-M. Li, K.-K. Wang, A. Banerjee, X.-Y. Cui, D. Wei, The shift of soil microbial community induced by cropping sequence affect soil properties and crop yield, *Front. Microbiol.* 14 (2023), <https://doi.org/10.3389/fmicb.2023.1095688>.
- [57] K. Hartman, M.G.A. van der Heijden, R.A. Wittwer, S. Banerjee, J.-C. Walsler, K. Schlaeppli, Cropping practices manipulate abundance patterns of root and soil microbiome members paving the way to smart farming, *Microbiome* 6 (2018) 14, <https://doi.org/10.1186/s40168-017-0389-9>.
- [58] Y. Tian, D. Qiao, S. Xu, N. Wang, Effects of tree species and topography on soil and microbial biomass stoichiometry in Funiu Mountain, China, *BMC Ecol.* 20 (2020) 67, <https://doi.org/10.1186/s12898-020-00332-4>.
- [59] M. Almagro, A. Ruiz-Navarro, E. Diaz-Pereira, J. Albaladejo, M. Martínez-Mena, Plant residue chemical quality modulates the soil microbial response related to decomposition and soil organic carbon and nitrogen stabilization in a rainfed Mediterranean agroecosystem, *Soil Biol. Biochem.* 156 (2021) 108198, <https://doi.org/10.1016/j.soilbio.2021.108198>.
- [60] S. Sharma, N. Gupta, A.S. Chakkal, N. Sharma, S. Alamri, M.H. Siddiqui, F. U. Haider, Changes in enzyme activities in salt-affected soils during incubation study of diverse particle sizes of rice straw, *Agriculture* 13 (2023) 1694, <https://doi.org/10.3390/agriculture13091694>.
- [61] W. Cheng, D.C. Coleman, Effect of living roots on soil organic matter decomposition, *Soil Biol. Biochem.* 22 (1990) 781–787, [https://doi.org/10.1016/0038-0717\(90\)90157-U](https://doi.org/10.1016/0038-0717(90)90157-U).
- [62] T.A. de Camargo, L.A. Alves, I.C. Mendes, L.R. Gasques, L.G.S. de Oliveira, G. C. Pires, T.O. Almeida, P.C. de F. Carvalho, E.D. de Souza, Enhancing soil quality and grain yields through fertilization strategies in integrated crop-livestock system under no-till in Brazilian Cerrado, *Eur. J. Soil Biol.* 121 (2024) 103613, <https://doi.org/10.1016/j.ejsobi.2024.103613>.
- [63] L. Deiss, A. Moraes, J. Dieckow, A.J. Franzluebbers, L.C. Gatiboni, G. Ianzi Sasaki, P.C.F. Carvalho, Soil phosphorus compounds in integrated crop-livestock systems of subtropical Brazil, *Geoderma* 274 (2016) 88–96, <https://doi.org/10.1016/j.geoderma.2016.03.028>.
- [64] K.L. Page, Y.P. Dang, R.C. Dalal, The ability of conservation agriculture to conserve soil organic carbon and the subsequent impact on soil physical, chemical, and biological properties and yield, *Front. Sustain. Food Syst.* 4 (2020), <https://doi.org/10.3389/fsufs.2020.00031>.
- [65] J.E. Kostin, S. Cesarz, A. Lochner, M. Schädler, C.A. Macdonald, N. Eisenhauer, Land-use drives the temporal stability and magnitude of soil microbial functions and modulates climate effects, *Ecol. Appl.* 31 (2021), <https://doi.org/10.1002/eap.2325>.
- [66] W. Xie, Y. Zhang, J. Li, S. Wei, X. Li, H. Yu, B. Guan, Straw application coupled with N and P supply enhanced microbial biomass, enzymatic activity, and carbon use efficiency in saline soil, *Appl. Soil Ecol.* 168 (2021) 104128, <https://doi.org/10.1016/j.apsoil.2021.104128>.
- [67] J. Daunoras, A. Kačergius, R. Gudikiškaitė, Role of soil microbiota enzymes in soil health and activity changes depending on climate change and the type of soil ecosystem, *Biology* 13 (2024) 85, <https://doi.org/10.3390/biology13020085>.