

Improving the quality of the production environment

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ABSTRACT: This study aimed to investigate different production environments, through diagnosis of the chemical, physical attributes and enzymatic activity of the soil, in addition to evaluating strategies to improve the quality of these environments and their impact on the productivity of commercial crops. Low productivity environments were characterized by lower dry matter production of cover crops with a reduction of up to 46.2%, greater resistance to soil penetration at a depth of 40-60 cm, lower levels of soil organic matter in the uppermost layer superficial and also in depth, combined with lower enzyme activity. It was possible to restore productivity in the low corn crop environment, by up to 15.34% in the vegetative treatment + organic conditioner + biological input and 5.11% for vegetative + biological input. There was a positive synergism as a residual effect for both environments in the off-season soybean crop in the vegetative treatment + organic conditioner + biological input, up to 39.53% in the low environment and 25% for the high environment. For wheat cultivation, it was possible to increase productivity in the low environment by up to 1.92% in the vegetative method, 9.09% vegetative + organic conditioner and 24.4% vegetative + biological input in relation to the high environment.

Key words: biological soil activation; enzyme activity; grain productivity; organic matter

Melhoria da qualidade do ambiente de produção

RESUMO: Neste estudo objetivou-se investigar diferentes ambientes de produção, através de diagnóstico dos atributos químicos, físicos e atividade enzimática do solo, além de avaliar estratégias para melhorar a qualidade destes ambientes e seu reflexo na produtividade de culturas comerciais. Os ambientes de baixa produtividade foram caracterizados pela menor produção de matéria seca das plantas de cobertura com redução de até 46,2%, maior resistência a penetração do solo na profundidade de 40-60 cm, menores teores de matéria orgânica do solo na camada mais superficial e também em profundidade, aliado a menor atividade das enzimática. Foi possível restaurar a produtividade no ambiente de baixa na cultura do milho, em até 15,34% no tratamento vegetativo + condicionador orgânico + insumo biológico e 5,11% para vegetativo + insumo biológico. Houve um sinergismo positivo como efeito residual para os dois ambientes na cultura da soja safrinha no tratamento vegetativo + condicionador orgânico + insumo biológico em até 39,53% no ambiente de baixa e 25% para o ambiente de alta. Para a cultura do trigo, foi possível aumentar a produtividade no ambiente de baixa em até 1,92% no método vegetativo, 9,09% vegetativo + condicionador orgânico e 24,4% vegetativo + insumo biológico em relação ao ambiente de alta.

Palavras-chave: ativação biológica do solo; atividade enzimática; produtividade de grãos; matéria orgânica



Introduction

The global demand for food is showing a linear upward trend, coupled with the demand for quality. Global population growth by 2050 is projected at up to 2 billion people, rising from 7.7 billion individuals to 9.7 billion by 2050 (Salgado et al., 2024). Brazil is considered one of the main options for increasing food production in the coming years, mainly due to its natural resources, especially agricultural soil with the potential to intensify cultivation systems associated with the humid tropical and subtropical climate that predominates in different agricultural regions of the country. In addition to this challenge, there is also the challenge of guaranteeing grain and fiber production in the face of the climate change scenario that is intensifying around the world (Foyer et al., 2016; Gupta et al., 2020) with prolonged periods of drought during the crop cycle, high temperatures and high volumes of precipitation in a short period of time (Amado, 2023; Wuebbles et al., 2017), putting the agricultural production base at risk, mainly due to the occurrence of erosion, decline in organic matter and loss of soil nutrients.

In this context, it is necessary to provide quality and longevity to production systems, and regenerative soil management can be an efficient alternative. In this sense, it is necessary to redesign production systems in order to build a production environment that can provide productivity increases and at the same time temporal stability. Furthermore, considering the spatial variability of soil attributes and crop performance, it is necessary to use site-specific management tools. The identification of production environments with high and low production potential can be carried out using various tools such as harvest maps, vigor maps and the farmers own experience (Santi et al., 2013; Amado et al., 2016; Basso & Brinton, 2021). However, the causes that characterize such environments require a careful diagnosis of the soils chemical, physical and biological attributes, with a systemic view of agricultural systems. High productivity environments generally correlate with soil water storage, organic matter content, biological diversity and attributes that favor the deepening of the plant root system, which can be expressed as soil health (Mendes et al., 2020; Bonine Pires et al., 2021; Müller et al., 2021; Passinato et al., 2021).

Resilience is a characteristic of production environments, which are built on diversified production system models. It is the guiding principle of a quality no-till system, with roots active for as long as possible in the system, high plant residue input and minimal mechanical tillage. These principles enable synergism between soil-plant-atmosphere, bringing a constant flow of energy to the system, mainly through the cycling of organic macronutrients, carbon, hydrogen and oxygen from photosynthesis (gas cycles) (Primavesi, 2002). In addition, the construction of the production environment is mainly aimed at reducing plant stress during its cycle, which is fundamental for the maximum efficiency of photosynthesis by plants, resulting from the maximization

of stomatal functioning (opening of stomata), assimilation of carbon dioxide CO₂ and photosynthetic rate by plants (Zhang et al., 2016).

This study sought to advance the management of different production environments within the same area, starting with an initial diagnosis through cover crops, soil analysis stratified in depth, characterizing the chemical and physical aspects of the soil, as well as enzymatic activity (β -glucosidase and arylsulfatase). With this information, chemical and biological interventions in isolation and in combination were evaluated in terms of crop productivity and the improvement of productive environments. The main objective of this study was to advance the short-term and residual effects of interventions that promote soil health in each production environment.

Materials and Methods

Study site, environmental conditions and experimental design

The research began at the end of the winter season, in the 2022 agricultural year, in a rural producers area in the municipality of Frederico Westphalen, located in the northwest of Rio Grande do Sul, Brazil (27° 23' 51" S and 53° 35' 19" W), at an altitude of 490 m, with an average annual rainfall of 1,881 mm, an average temperature of 19.1 °C and a humid subtropical climate (Alvares et al., 2013).

The production system established during the research was winter cover crops (black oats + forage turnip)/summer corn (AG 1666)/safrinha soybeans (TMG 7062)/fall cover crops (millet + forage turnip + buckwheat)/wheat (TBIO ponteiro). As for phytosanitary management, base and top dressing fertilization was the same as that used throughout the area, the producers standard management.

The experiment was a factorial design with three replications. The two production environments (main plots) were established through the vigor of the cover crops, containing the following dimensions (10 × 30 m), followed by three treatments (sub-plots) containing dimensions of (10 × 10 m).

Biomass production of cover crops

The dry matter production of the cover crops was estimated in a useful area of 0.250 m² (three repetitions), the samples were kept in a forced-air oven at a temperature of 60 °C until the weight of the samples stabilized.

Chemical soil analysis

As for the chemical attributes of the soil, stratified samples were collected using an auger in the following layers (0-10, 10-20, 20-40, 40-60 and 60-80 cm), totaling five samples per experimental plot. The samples were sent to the Laboratório de Solos e Tecidos Vegetais of the Universidade Regional Integrada de Frederico Westphalen, Rio Grande do Sul, Brazil, for determination of soil organic matter (OM), soil pH in water (1:1), clay, phosphorus (P), potassium (K⁺),

calcium (Ca^{2+}), magnesium (Mg^{2+}), sulphur (S), aluminum (Al^{3+}) and CEC base saturation (BS). For analysis of soil pH (in H_2O), clay, OM (sulphuric solution with external heat and spectrophotometric determination of Cr^{3+}), exchangeable Ca^{2+} , Mg^{2+} and Al^{3+} (extracted by 1 mol L^{-1} KCl solution), SO_4^{2-} (extracted by calcium phosphate, barium chloride gelatine and determined by turbidimetry), available P and K^+ content (extracted by Mehlich-1) and soil BS (%) which was calculated as: $(100((\text{Ca}^{2+} + \text{Mg}^{2+} + \text{K}^+)/\text{CEC at pH 7.0}))$, where CEC is the exchange capacity of the soil (Tedesco et al., 1995).

Physical soil analysis

Soil penetration resistance (PR) was measured at moisture levels close to field capacity, using a digital penetrometer (PenetroLOG, Falker®, model PLG1020). The readings were taken after the cover crops had been rolled (three repetitions/plot), with readings every cm up to 60 cm deep, using a load cell and inserting the rod at a speed of 0.018 m s^{-1} . A type 2 cone (diameter 12.83 mm) with a 30° angle was used (Asabe, 2009).

Soil enzyme activity

For soil enzymatic analysis, three soil samples were taken from each experimental plot in the 0-10 cm layer, forming a composite sample. The β -glucosidase and arylsulfatase enzymes were analyzed according to the methodology proposed by Tabatabai (1994).

Interventions with vegetative methods, conditioner/organic and biological inputs applied prior to the pre-planting of corn, off-season soybean and wheat crops

The interventions were established in the two production environments, with the following treatments: T1 (vegetative method), T2 (vegetative method + conditioner/organic + biological inputs) and T3 (vegetative method + biological inputs). For the plant adjustment, the cover crops that preceded the main crop were kept, with their respective biomass production. As for the organic conditioner, 3.5 t ha^{-1} of poultry litter was used for the corn crop and 2.5 t ha^{-1} prior to the wheat crop, containing the following chemical compositions N (3.24%), P_2O_5 (3.62%), K_2O (3.65%), Ca (9.19%) and Mg (3.48%). In addition, 2.6 t ha^{-1} of dolomitic limestone with a concentration of CaO (26%), MgO (13%) and PRNT (60.7%); 2 t ha^{-1} of gypsum with a concentration of Ca (18.98%), CaO (26.57%) and S (13.87%) were added before the wheat crop. In the biological inputs intervention, the biological actives *Azospirillum brasilense* strains AbV5 and AbV6, concentration of $2 \times 10^8 \text{ CFU mL}^{-1}$, dose (4 L ha^{-1}), *Trichoderma asperellum* strain CCT 2165, concentration of 1×10^{10} , dose (2 L ha^{-1}) and *Flex roots*, dose (1 L ha^{-1}), were applied immediately after sowing the corn via aerial spraying, with a spray volume of 80 L ha^{-1} . For the wheat crop, the application was only carried out in environments with vegetative method treatment + biological input at full bloom, *Azospirillum brasilense* strains AbV5 and AbV6 concentration of $2 \times 10^8 \text{ CFU mL}^{-1}$, dose (2 L ha^{-1}), *Trichoderma*

asperellum strain CCT 2165 concentration of 1×10^{10} , dose (1 L ha^{-1}) and *Flex roots*, dose (2.5 L ha^{-1}), in a spray volume of 80 L ha^{-1} . In the safrinha soybean crop after the corn crop, these adjustments added up to a residual effect.

Crop productivity

Corn yields were assessed on a useful area of 1.8 m^2 , safrinha soybeans 0.9 m^2 and wheat 1.36 m^2 for each treatment in the production environments, with grain moisture corrected to 13%.

Statistical analysis

The data on dry matter production of cover crops, soil penetration resistance (PR), final grain yield of corn, soybeans and wheat were subjected to analysis of variance (ANOVA) and compared using the Tukey test ($p < 0.05$). All statistical analyses were carried out using the statistical program Sisvar, version 5.3 (Ferreira, 2014).

Results and Discussion

The dry matter production of the ground cover plants in the fall/winter (black oats + forage turnip) differed between the production environments (Figure 1). Thus, the low productivity environment had an average production of $3,600 \text{ kg ha}^{-1}$ while the high productivity environment produced $5,266 \text{ kg ha}^{-1}$, representing a superiority of up to 46.2% in the dry matter production of the cover crops.

This difference in the dry matter production of the cover crops confirms that the production environments were well designed and, above all, explains the high spatial variability of productivity in the plot under investigation. The greater dry matter production of cover crops is reflected in greater

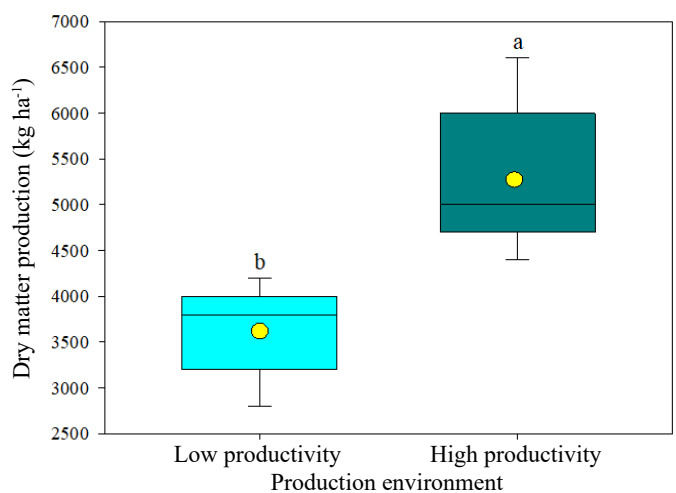


Figure 1. Dry matter production of cover crops in two production environments (low and high productivity). The bars indicate the maximum and minimum values, the median and the yellow dot represents the average. The letters refer to the statistical difference between yield environments in the Tukey test ($p < 0.05$). Frederico Westphalen - RS, Brazil, 2022 harvest.

nutrient cycling in environments with greater production potential (Santi et al., 2016), greater root production, greater addition of carbon to the soil and root exudates.

With regard to physical attributes, soil penetration resistance (PR) (Figure 2) in both production environments showed values lower than those limiting the development of crop roots (< 2.5 MPa), according to studies carried out in different regions of Brazil involving management zones and production environments (Dantas, 2018; Passinato et al., 2021). The environments only differed at depths > 40 cm, where the high productivity environment had a lower PR than the low productivity environment, probably due to deep rooting in this subsoil layer in the first environment.

As for the soil chemical attributes (Table 1), phosphorus (P) was generally low ($P \leq 6.0 \text{ mg dm}^{-3}$) at all depths, regardless of the production environment (CQFS, 2016). Phosphorus is an important macronutrient as a source of energy and promoter of plant root growth during the early stages

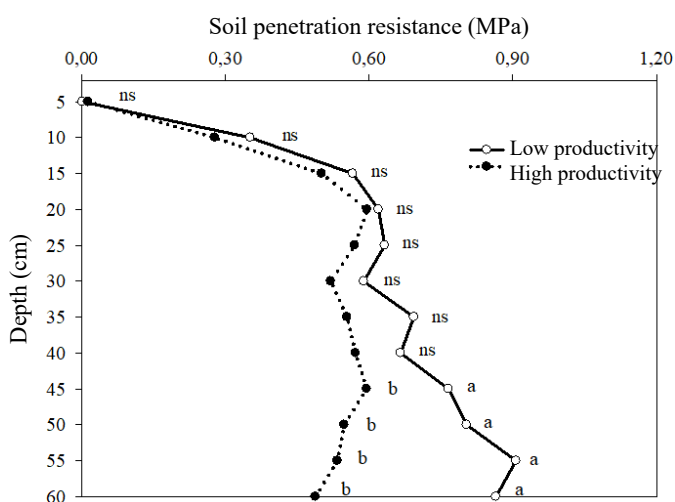


Figure 2. Soil penetration resistance in two production environments (low and high productivity). The letters refer to the statistical difference between yield environments Tukey test ($p < 0.05$). Frederico Westphalen - RS, Brazil, 2022 harvest.

Table 1. Soil attributes: clay, soil organic matter (OM), pH in water, phosphorus (P), potassium (K^+), calcium (Ca^{+2}), magnesium (Mg^{+2}), sulphur (S), aluminum (Al^{+3}) and CEC base saturation (BS), in two production environments, Frederico Westphalen - RS, Brazil, harvest 2022.

Depth (cm)	Clay (%)	OM (%)	pH	P			K^+ (mg dm^{-3})			S		Mg^{+2}		Ca^{+2} ($\text{cmol}_c \text{ dm}^{-3}$)		Al^{+3}		BS (%)	
Low productivity																			
0-10	79	2.8	5.4	3.7	229.5	39.9	3.2	8.0	0.0	63.1									
10-20	69	1.7	5.1	1.0	103.2	46.3	2.0	6.2	0.2	55.1									
20-40	79	1.3	5.3	1.0	51.0	50.6	1.6	5.7	0.1	57.5									
40-60	79	1.2	5.4	1.0	44.5	57.5	2.1	6.7	0.0	67.0									
60-80	79	1.0	5.5	1.0	37.5	41.5	2.7	6.8	0.0	71.1									
High productivity																			
0-10	59	3.6	5.2	1.4	270.5	23.9	2.6	6.2	0.1	57.9									
10-20	79	2.4	5.0	1.0	190.0	32.2	1.6	4.6	0.3	49.2									
20-40	79	1.9	5.1	1.0	111.0	38.0	1.4	5.2	0.2	52.6									
40-60	79	1.4	5.2	1.0	54.5	38.6	1.1	4.9	0.3	49.8									
60-80	79	1.3	5.2	1.0	40.0	44.0	1.4	5.2	0.3	57.8									

(Dantas, 2018; Müller et al. 2021). Potassium (K^+), which plays an important role in enzyme activation and stomatal regulation, showed adequate levels ($K \geq 91.0 \text{ mg dm}^{-3}$) regardless of the production environment. However, in the high productivity environment, the levels of these nutrients were sufficient up to the 20-40 cm layer, while in the low productivity environment they were sufficient up to the 10-20 cm layer (CQFS, 2016).

As for the macronutrients calcium (Ca^{+2}), magnesium (Mg^{+2}) and sulphur (S), these are in sufficient levels at all the depths and production environments investigated. The critical levels are Ca^{+2} ($\geq 4.0 \text{ mg dm}^{-3}$), Mg^{+2} ($\geq 1.0 \text{ mg dm}^{-3}$) and S ($\geq 5.0 \text{ mg dm}^{-3}$) (CQFS, 2016). Table 1 shows that the low productivity environment has higher concentrations of these nutrients: 27.9% (Ca^{+2}), 43.2% (Mg^{+2}) and 33.4% (S) compared to the high productivity environment in the general average of depths (CQFS, 2016). These lower levels of various nutrients can be explained by the greater export of nutrients that occurs in the high-yield environment, which maintains higher grain yields over several harvests.

For the aluminum content (Al^{+3}) and base saturation (BS), it should be noted that at the depths of 40-60 and 60-80 cm in the high productivity environment, slightly higher values were observed for Al ($\cong 0.3\%$) and lower for BS ($\cong 28.2\%$) compared to the low productivity environment (CQFS, 2016). This result may be associated with the lower soil pH value of < 3.8% and the Ca^{+2} content of < 33.6% in the high environment compared to the low environment at these depths. In relation to the greater presence of Al^{+3} in the soil, this is mainly dependent on management and the pH value and Ca and BS contents, which can affect productivity by affecting plant root growth, nutrient absorption, energy expenditure to perform their physiological functions such as photosynthetic activity, enzyme activation, CO_2 fixation and photoassimilates (Bossolani et al., 2021).

The soil organic matter (OM) content was 28.6, 41.2 and 46.1% higher in the 0-10, 10-20 and 20-40 cm layers in the high productivity environment than in the low productivity environment. The greater decrease in OM content at depth

between the productive environments was previously reported by [Santi et al. \(2013\)](#) and is probably associated with the lower volume of roots in this layer. The lower OM levels are in line with the lower biomass production of the cover crops (46.2%) in the low productivity environment ([Figure 1](#)). Previously, [Bayer et al. \(2011\)](#) reported a linear relationship between the biomass input and the OM content in the surface layer, suggesting an input of 8-10 Mg ha⁻¹ year⁻¹ to maintain the OM stock under no-till ([Calegari et al., 2020](#)). It is known that OM interacts with the chemical, physical and biological characteristics of the soil, accounting for 70-80% of the soil CEC ([Bayer & Bertol, 1999](#)). In addition, this OM can block the toxicity of Al⁺³ ([Salet, 1998](#)), act on the structure of the soil and the architecture of micro and macro-pores ([Sá, 2001](#)), which govern the storage and availability of water to plants, as well as being a source of energy through carbon and nitrogen for the soil biota ([Corassa, 2018](#); [Pires et al., 2020](#); [Passinato et al., 2021](#)). [Conceição et al. \(2005\)](#) argued that since OM is an attribute that integrates the functioning of chemical, physical and biological properties, it could be used to characterize the quality of productive environments.

The activity of the enzymes β -glucosidase and arylsulfatase also proved to be a sensitive indicator of production environments ([Figure 3](#)). Thus, the activity of these enzymes was higher in the high productivity environment by 17 and 41% for β -glucosidase and arylsulfatase, respectively. The higher enzyme activity is associated with the greater biomass input from the cover crops ([Figure 1](#)) and the higher OM content ([Table 1](#)). Similar results were reported by [Mendes et al. \(2020\)](#), [Pires et al. \(2020\)](#), and [Passinato et al. \(2021\)](#). In a study by [Rodrigues et al. \(2022\)](#), increases in β -glucosidase and arylsulfatase of 33 and 46% were found in treatments with brachiaria compared to treatments without cover crops between the rows for coffee. These enzymes have been studied globally and are part of the BioAS technology

recently proposed by Embrapa as sensitive indicators of soil quality ([Mendes et al., 2020](#)).

In a long-term study, [Pires et al. \(2020\)](#) confirmed a higher β -glucosidase activity of up to 69% in the no-till system (compared to the conventional tillage system). The higher enzyme activity was mainly associated with an increase in OM in the topsoil due to management practices such as crop rotation, maintaining permanent soil cover and introducing legumes into the system. The activities of these soil enzymes are often closely related to OM ([Lopes et al., 2013](#)) and exhibit strong links with microbial communities and functional gene abundance ([Trivedi et al., 2016](#); [Peixoto et al., 2010](#)), and have shown positive correlations with soybean and corn yields ([Lopes et al., 2013](#); [Mendes et al., 2021](#)).

As for the final grain yield for the maize crop ([Figure 4A](#)), there was no statistical difference in the interaction between environment and treatments, but it is important to note that for the different treatments, the high-yield vegetative method environment had a higher average yield than the low productivity environment by up to 31.3% in the vegetative method intervention, 15.3% in the vegetative method + conditioner/organic + biological inputs intervention and 14.05% in the vegetative method + biological inputs intervention. The adoption of synergistic strategies in environments with different production potential are opportunities to exploit the potential of the high-productivity environment and, on the other hand, to reduce the yield gap in the low productivity environment.

[Figure 4A](#) shows that in the low productivity environment there was a 15.4% increase in corn yields when the vegetative method + conditioner/organic + biological inputs were used and 5.11% for the vegetative method + biological inputs compared to the treatment with the vegetative method alone.

As for the safrinha soybean crop established after corn, a similar response was observed in the low productivity

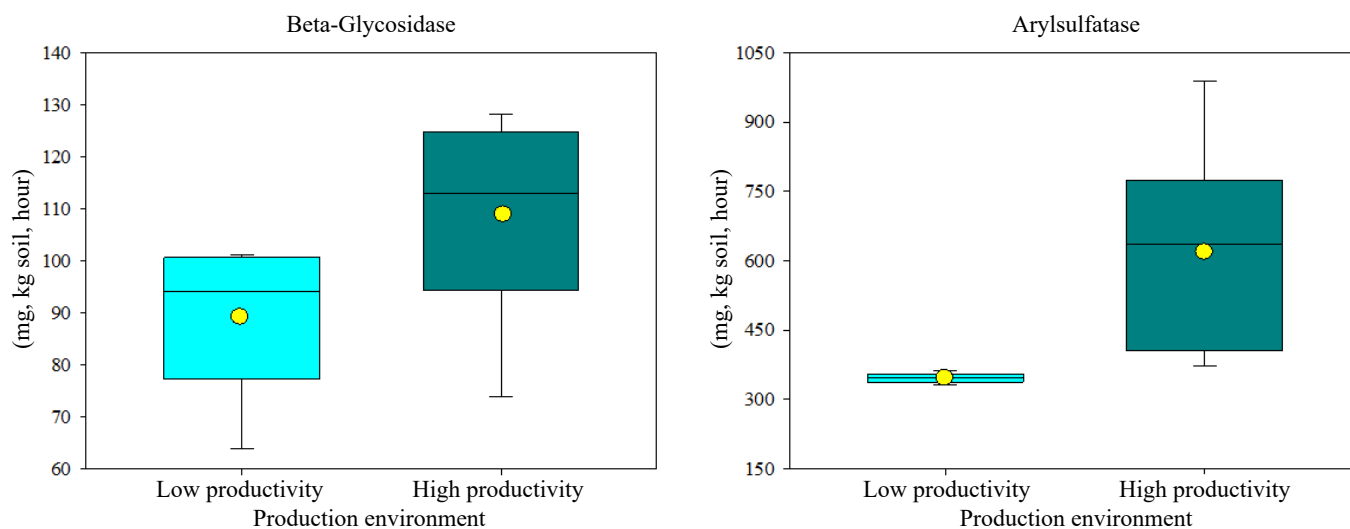


Figure 3. Soil enzyme activity, Beta-Glycosidase and Arylsulfatase in two production environments (high and low productivity). The bars indicate the maximum and minimum values, the median and the yellow dot represents the average. Frederico Westphalen - RS, Brazil, 2022 harvest.

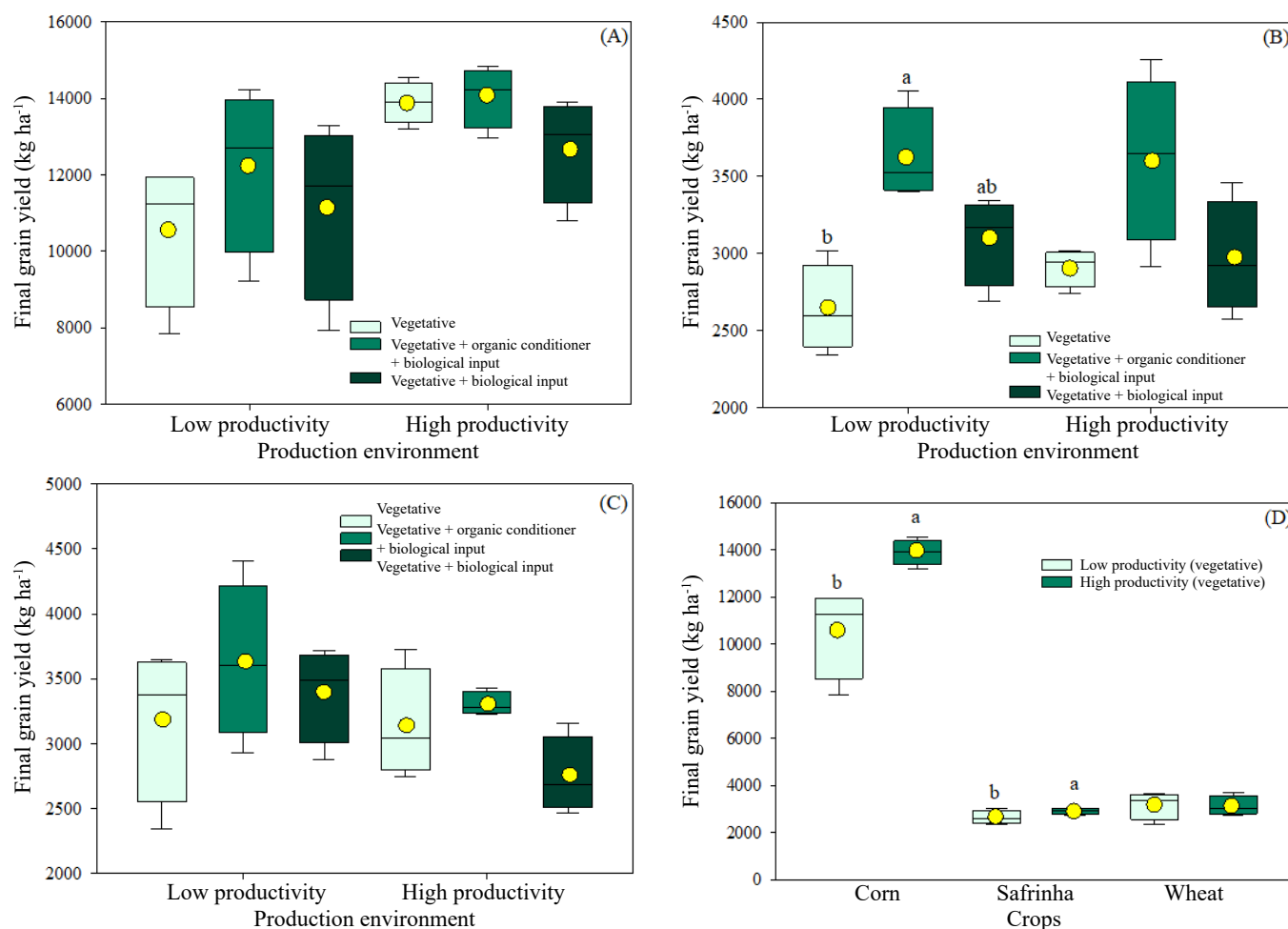


Figure 4. Grain yield for corn (A), 'safrinha' soybeans (B) and wheat (C) in two production environments (low and high productivity) subjected to interventions with vegetative methods, conditioner/organic and biological inputs, (D) average yield of the three crops for the vegetative method for the low and high productivity environment. The bars indicate the maximum and minimum values, the median and the yellow dot represents the average. The letters refer to the statistical difference between yield environments Tukey test ($p < 0.05$). Frederico Westphalen - RS, Brazil, 2022/2023 harvest.

environment (Figure 4B), with a yield increase of up to 39.5% in the intervention with vegetative method + organic conditioner/fertilizer + biological inputs and 18.6% vegetative method + biological inputs compared to the adjustment with vegetative methods alone. In the high productivity environment, there was a 25% increase in productivity for the joint intervention of the vegetative method + organic conditioner/fertilizer + biological inputs compared to the intervention with the vegetative method applied alone. An analysis of the harvest and the safrinha shows the potential of strategies applied together to increase crop yields in both production environments, reducing the difference between them.

Figure 4C shows the yield results for the wheat crop, which show no statistical difference between the environments and different treatments, but it can be seen that after introducing autumn cover crops, system fertilization and the application of biological inputs, giving up the safrinha soybean crop after the corn crop, it was possible to increase the yield of the low productivity environment in the vegetative method by 1.92%, vegetative method + organic conditioner by 9.09%

and for the vegetative method + biological input by 24.4% compared to the high productivity environment.

Figure 4D shows that there is a difference of 3,300 kg ha⁻¹ in the corn crop and 300 kg ha⁻¹ in the safrinha soybean crop from the high to the low productivity environment. In the wheat crop, the low productivity environment had a higher yield of 60 kg ha⁻¹ after the use of autumn cover crops and system fertilization.

These results indicate that it is possible to raise the productivity of low to high productivity environments by improving soil quality, especially with the use of cover crops, combined with organic conditioners and biological inputs, with the interaction that takes place in the rhizosphere of the plants involving the soil, the roots, microorganisms, the rapid cycling of nutrients, the diversity of elements and organic strata are the main factors responsible for causing this rapid recovery of the production environment and increasing crop productivity, where the new labile carbon added to the system orchestrates these changes and provides an increase in soil life (Lange et al., 2015).

Finally, these findings indicate that, especially in less productive environments, it is important to restore microorganisms such as *Azospirillum*, *Trichoderma* and a set of bacteria that will perform functions in the soil and plant, such as stimulating rooting, fixing N in the soil, promoting defense metabolites and suppressing pathogenic microorganisms, associated with mineral diversity, which in this case is the conditioner/organic that also activates the soil biota through the contribution of C and N to the system, enriching it with other important nutrients for the plant such as phosphorus, potassium, calcium, magnesium, sulphur, boron, copper, zinc and manganese.

This positive signaling on productivity, both immediate and as a residual effect for the next crop, proposed by the synergism between cover crops, biological assets and poultry litter is fundamental for restoring life in the soil, the diversity of microorganisms, the increase in C and N content in the system which, in general terms, build quality OM throughout the harvests.

Conclusions

The OM content at the surface and depth, the activity of the enzymes β -glucosidase and arylsulfatase, soil penetration resistance and the dry matter production potential of the cover crops are important diagnostic factors for defining production environments.

The vegetative + conditioner/organic + biological inputs method was responsible for restoring yields in the low productivity environment, 1,620 kg ha⁻¹ for corn crop, 1,020 kg ha⁻¹ for safrinha soybeans and 420 kg ha⁻¹ for wheat. The biological inputs method increased corn yields by 540 kg ha⁻¹, safrinha soybeans by 480 kg ha⁻¹ and wheat by 180 kg ha⁻¹ in the low productivity environment.

The high productivity environment in the vegetative method had higher yields, 3,300 kg ha⁻¹ for the corn crop and 300 kg ha⁻¹ for safrinha soybeans compared to the low productivity environment. Finally, it was possible to equalize wheat yields between the low productivity environment (3,180 kg ha⁻¹) and the high productivity environment (3,120 kg ha⁻¹), through synergism between cover crops and system fertilization, without growing safrinha soybeans after the corn harvest.

Compliance with Ethical Standards

Author contributions: Conceptualization: MJRS, CJB; Data curation: MJRS, CJB; Investigation: MJRS, CJB, TJCA, DCF, ALS, LPA; Methodology: MJRS, CJB, TJCA, DCF, ALS, LPA; Software: MJRS, CJB, TJCA, DCF, ALS, LPA; Supervision: MJRS, CJB, TJCA, DCF, ALS, LPA; Validation: MJRS, CJB, TJCA, DCF, ALS, LPA; Visualization: MJRS, CJB, TJCA, DCF, ALS, LPA; Writing – original draft: MJRS, CJB, TJCA, DCF, ALS, LPA; Writing – review & editing: MJRS, CJB, TJCA, DCF, ALS, LPA.

Conflict of interest: There is no conflict of interest on the part of any of the authors.

Funding source: The Universidade Federal de Santa Maria (UFSM).

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