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Research Article

Soil enzymatic activity and wheat grain yield under cover crop systems[1]

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ABSTRACT

For years, the impacts of using cover crops in productive systems have been measured by their relation with soil chemical and physical characteristics. Consequently, the effects on the soil microbiological characteristics have been little explored. This research aimed to measure the short-term effects of cover crop systems on the enzymatic activity of arylsulfatase and beta-glycosidase, as well as the wheat grain yield. Thirty-five cover crop systems (18 single and 17 intercropped) were implemented, with 3 replications of the following variables for each treatment: enzymatic activity of arylsulfatase and beta-glycosidase, soil organic matter and sulfur contents, and wheat grain yield. The data were submitted to descriptive analysis, multivariate cluster analysis by dendrograms for the single and intercropped plant systems, and t-test for independent samples between the average scores of each group in the dendrograms. Independently of the crop system, there were short-term effects on the enzymatic activity and grain yield. Plants from the same botanic family presented different effects among them. Therefore, in the short-term, cover crops affect the enzymatic activity, and plants that present a higher enzymatic activity do not necessarily result in higher grain yields.

KEYWORDS: Bioindicators, soil enzymes, arylsulfatase, beta-glycosidase.

RESUMO

Atividade enzimática do solo e rendimento de grãos de trigo sob sistemas de plantas de cobertura

Por anos, os impactos da utilização de plantas de cobertura em sistemas produtivos foram avaliados por meio de sua relação com propriedades químicas e físicas do solo. Consequentemente, os efeitos nas características microbiológicas do solo foram pouco explorados. Objetivou-se avaliar o efeito a curto prazo de sistemas de plantas de cobertura na atividade enzimática de arilsulfatase e betaglicosidase, bem como o rendimento de grãos de trigo. Foram implantados 35 sistemas de plantas de cobertura (18 solteiros e 17 consorciados), com 3 repetições para cada tratamento das seguintes variáveis: atividade enzimática de arilsulfatase e betaglicosidase, teores de matéria orgânica e enxofre no solo e rendimento de grãos de trigo. Os dados foram submetidos a análise descritiva, análise multivariada de agrupamento por dendrogramas para os sistemas solteiro e consorciado e teste de t para amostras independentes entre as médias de cada grupo dentro dos dendrogramas. Independentemente do sistema de cultivo, houve efeitos a curto prazo na atividade enzimática e no rendimento de grãos. Plantas da mesma família botânica apresentaram efeitos distintos entre si. Portanto, a curto prazo, as plantas de cobertura afetam a atividade enzimática, e plantas que apresentam maior atividade enzimática não necessariamente resultam em maiores rendimentos de grãos.

PALAVRAS-CHAVE: Bioindicadores, enzimas de solo, arilsulfatase, betaglicosidase.

INTRODUCTION

The growing demand for food, allied to concerns regarding environment, shows that the improvement in cultivation techniques and the better use of resources like soil, water and fertilizers are fundamental to promote a sustainable production. In the productive system, one of the main conservationist techniques used to promote sustainability is the no-tillage system, which benefits the soil restoring and restructuring (Salomão et. al 2020).

For Vezzani & Mielniczul (2011) and Salomão et. al (2020), the no-tillage system intends to create a continuous soil coverage through crop rotation and direct sowing on straw, whose main objective is to create the least soil tillage possible, being really

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efficient in minimizing the impacts of agricultural production over soil, and keeping its physical, chemical and biological properties conserved.

In the no-tillage system, one of the main practices is crop rotation with cover crops, which allows an increase in organic matter, improvements in physical properties, decrease in erosion, suppression of weeds and enhancement in soil microbiological characteristics, besides promoting the nutrients cycling, consequently increasing the soil quality in general (Nunes et al. 2011, Skora Neto & Campos 2017).

Many studies on the benefits of cover crops are directed to soil chemical and physical properties (Bressan et al. 2013, Salomão et al. 2020, Bertolino et al. 2021), being necessary more researches on the dynamics between cover crops and soil biological quality. The main indicators used worldwide analyze the activities of microorganisms in the soil through basal respiration or result from the activities of certain enzymes, with this last method presenting a high sensitivity to detect earlier alterations, in addition to present a higher correlation to nutrients cycling (Silveira 2007).

Enzymes are biomolecules that have an important participation in the cycles of elements in the soil, having a great contribution for the stability of ecosystems (Martens et al. 1992). Such is the case of the arylsulfatase enzyme, which acts directly in providing sulfur for plants, and the beta-glycosidase enzyme, which acts in the final stage of cellulose decomposition, being really important for the carbon cycle (Tabatabai 1994). Since it is related to microorganisms, flora and fauna, the enzymatic activity has a great potential to be used as a soil quality indicator, once it is sensitive to variations induced by management (Revoredo 2005).

Therefore, the hypothesis of this research is that the use of cover crop systems may influence directly on the enzymatic activity of arylsulfatase and beta-glycosidase in the soil and wheat grain yield. Thus, this study aimed to evaluate the short-term effects of cover crop systems on the enzymatic activity of arylsulfatase and beta-glycosidase, as well as on the wheat grain yield.

MATERIAL AND METHODS

The study took place in a commercial area in Cruz Alta, Rio Grande do Sul state, Brazil (28º45'S, 53º35'W and altitude of approximately 410 m), in the 2021 harvest. The soil of the region is classified as Latossolo Vermelho Distrófico (Embrapa 2013) or Ferralsol (FAO 2015), with a slightly wavy relief and more than 20 years under no-tillage system, being managed for some years with precision farming tools. The experimental area presents a high fertility and uniformity, with the following soil physical-chemical features: clay: 44.24 %; pH (H_2O): 6.12; P: 19.2 mg dm^{-3}; K^+: 160.3 mg dm^{-3}; Ca^{2+}: 5.7 cmol$_c$ dm^{-3}; Mg^{2+}: 2.4 cmol$_c$ dm^{-3}; organic matter: 3.0 %; base saturation: 75.1 %; Al^{3+}: 0.00 cmol$_c$ dm^{-3}; cation exchange capacity at pH 7.0: 11.1 cmol$_c$ dm^{-3}.

According to the Köppen classification, the region is Cfa, with humid subtropical climate, presenting an average rainfall rate of 1,881 mm and average air temperature of 19.1 ºC (Alvares et al. 2014), with well-defined summer and winter seasons and without a defined dry season (Silva Filho et al. 2021). Figure 1 presents the meteorological data during the experiment.

Thirty-five cover crop systems were analyzed in the fall season, planted after corn harvest and with development before the wheat crop, consisting of 18 single systems [T1: IAV Veloz bean; T2: DKB240 corn; T3: *Cajanus cajan* (20 kg ha^{-1}); T4: *Lupinus albus* (60 kg ha^{-1}); T7: *Pennisetum glaucum* (30 kg ha^{-1}); T12: *Crotalaria juncea* (30 kg ha^{-1}); T14: *Crotalaria spectabilis* (20 kg ha^{-1}); T15: *Coracana eleusine* (10 kg ha^{-1}); T17: *Sorghum bicolor* (20 kg ha^{-1}); T18: *Vicia sativa* L. (40 kg ha^{-1}); T19: *Vicia villosa* (40 kg ha^{-1}); T21: *Raphanus sativus* L. (20 kg ha^{-1}); T22: *Fagopyrum esculentum* (60 kg ha^{-1}); T23: *Avena sativa* (100 kg ha^{-1}); T26: *Avena strigosa* cv. Ucraniana (65 kg ha^{-1}); T29: *Avena strigosa* (80 kg ha^{-1}); T34: *Mucuna pruriens* (80 kg ha^{-1}); T35: *Canavalia ensiformis* (100 kg ha^{-1})] and 17 intercropped systems [T5: RX 520 (*A. sativa* + *Secale cereale* + field pea + pivoting turnip (50 kg ha^{-1}); T6: RX 410 (IPR Afrodite oat + *A. strigosa* + *R. sativus* L.) (40 kg ha^{-1}); T8: *F. esculentum* (30 kg ha^{-1}) + *P. glaucum* (10 kg ha^{-1}); T9: *P. glaucum* (15 kg ha^{-1}) + *R. sativus* L. (10 kg ha^{-1}) + *F. esculentum* (30 kg ha^{-1}); T10: *P. glaucum* (20 kg ha^{-1}) + *R. sativus* L. (10 kg ha^{-1}); T11: *C. juncea* (20 kg ha^{-1}) + *P. glaucum* (15 kg ha^{-1}); T13: *C. juncea* (20 kg ha^{-1}) + *R. sativus* L. (10 kg ha^{-1}); T16: *C. eleusine* (5 kg ha^{-1}) + *P. glaucum* (20 kg ha^{-1}); T20: *R. sativus* L. (10 kg ha^{-1}) + *V. villosa* (15 kg ha^{-1}); T24: *A. sativa* (40 kg ha^{-1}) + *V. villosa* (20 kg ha^{-1}) + *R. sativus* L. (10 kg ha^{-1}); T25: *A. sativa* (30 kg ha^{-1}) +

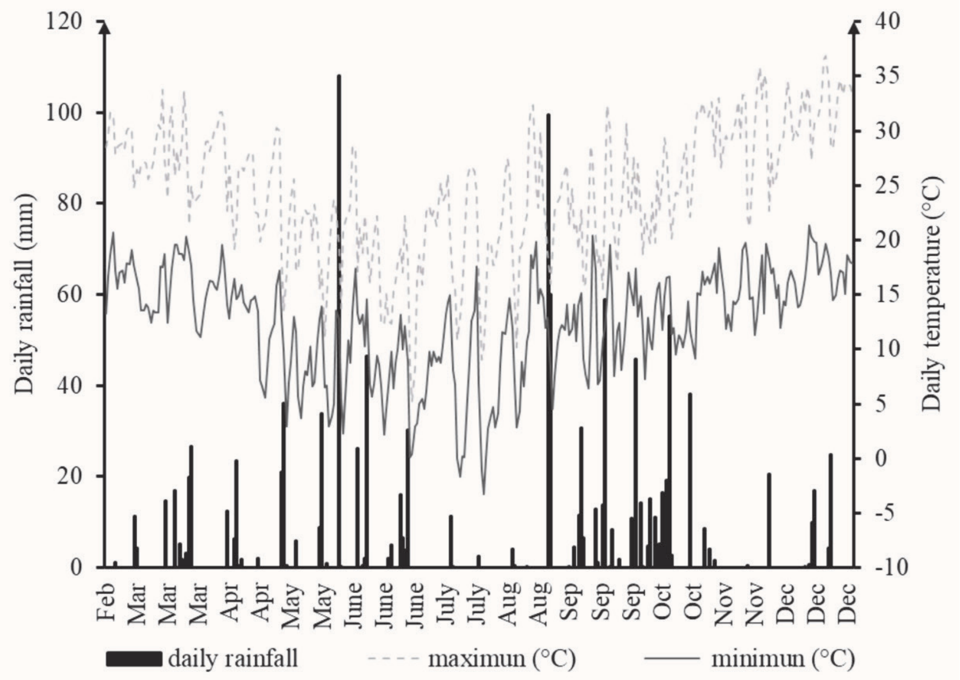

Figure 1. Rainfall, minimum and maximum daily temperatures, from February to December 2021, in Cruz Alta, Rio Grande do Sul state, Brazil. Source: (Brasil 2022).

V. villosa (15 kg ha^{-1}) + *R. sativus* L. (5 kg ha^{-1}) + *P. glaucum* (15 kg ha^{-1}); T27: *A. strigosa* (30 kg ha^{-1}) + *P. glaucum* (20 kg ha^{-1}); T28: *A. strigosa* (30 kg ha^{-1}) + *P. glaucum* (15 kg ha^{-1}) + *R. sativus* L. (10 kg ha^{-1}); T30: *A. strigosa* (40 kg ha^{-1}) + *P. glaucum* (20 kg ha^{-1}); T31: *A. strigosa* (40 kg ha^{-1}) + *P. glaucum* (30 kg ha^{-1}) + *R. sati*vus L. (10 kg ha^{-1}); T32: *A. strigosa* (30 kg ha^{-1}) + *P. glaucum* (15 kg ha^{-1}) + *R. sativus* L. (10 kg ha^{-1}); T33: *A. strigosa* (30 kg ha^{-1}) + *P. glaucum* (15 kg ha^{-1}) + *R. sativus* L. (5 kg ha^{-1}) + *V. villosa* (15 kg ha^{-1})]. The systems were sown by using a mechanical seeder between February 22 and 26, in plots measuring approximately 20 m wide and 200 m long each. The cover crop systems were dried and managed with a knife roller after the soil collection for enzymatic evaluation, around 10 days before sowing the wheat.

The soil collection was carried out at the depth of 0-0.15 m, on May 27, with three replications for each treatment, totaling 105 samples. To make each sample, eight subsamples were prepared, in which the soil was catalogued and sent to the laboratory for chemical determination of sulfur and organic matter contents, as well as to measure the enzymatic activities of arylsulfatase and beta-glycosidase (adapted from Tabatabai 1994), determined in µg p-nitrophenol g^{-1} soil h^{-1}.

On June 28, the sowing of the wheat TBIO Audaz cultivar was performed with a mechanical seeder, in an average density of 330 seeds m^{-2} and spacing of 0.17 m. The base fertilization used 200 kg ha^{-1} of the commercial formula 07-40-00 (N-P_2O_5-K_2O). Cover fertilizations were carried out with 150 kg ha^{-1} of KCl and 100 kg ha^{-1} of urea, when the wheat was in the tillering phenological stage. On the stretch phenological stage, 100 kg ha^{-1} of YaraBella, commercial formula 27-00-00 (N-P_2O_5-K_2O), were applied (CQFS-RS/SC 2016).

The wheat crop procedures were the same in all the treatments. The wheat was mechanically harvested on Nov. 11, and data obtained with sensors that measured the grain yield (Shiratsuchi 2004). At the harvest final stage, a file with georeferenced grain yield was generated, processed by the SMS Advance® Ag Leader Tecnology software, and exported to the CR - Campeiro 7 software (Giotto & Robaina 2007). Then, spatialized wheat grain yield data for each cover crop system were obtained, with values adjusted for humidity of 13 %, and three wheat grain yield values for each treatment.

The data were submitted to an exploratory analysis (descriptive statistics), intending to verify their position and dispersion. Arylsulfatase, beta-glycosidase, organic matter, sulfur in the soil and wheat grain yield values were used in a grouping multivariate statistical analysis by dendrograms, with a complete linkage, and the Euclidean distance was used as a measure of dissimilarity (Callegaro & Longhi 2013).

In order to find treatments with similar answers in the evaluated variables, the cover crop systems were previously subdivided into two: single and intercropped. Based on this, the isolated grouping analysis for each system was performed. After that, the average scores were calculated for each variable, in each group, and compared two by two with the Student t-test ($p < 0.05$) for independent samples. The statistical analysis was carried out by the R software version 3.2.2 (R Core Team 2018) and the Bioestat 5.0 software (Ayres et al. 2007).

RESULTS AND DISCUSSION

The results regarding the descriptive analysis of soil features are presented in Table 1. In relation to the coefficient of variation (CV), for Warrick & Nielsen (1980), the classification of the soil features is divided into low (CV < 12 %), moderate (12 % < CV < 60 %) and high (CV > 60 %) variability. Therefore, the data presented a low variability for organic matter, moderate variability for both enzymes and a high variability for sulfur contents in the soil. The coefficient of variation for the wheat grain yield was only 13.7 %, being considered a moderate variation.

For the multivariate dendrograms analysis performed only with single systems, the cover crops were subdivided into four groups (Figure 2), in which the group S1 is formed by different crop species, like legumes (bean, white lupin, *Crotalaria spectabilis* and common vetch) and grasses (corn and *Coracana eleusine*); group S2 by grasses (white forage sorghum, white oat and black oat), legumes (pingeon pea, *Crotalaria juncea* and hairy vetch), a cruciferous one (fodder radish) and a polygonaceae (buckwheat); group S3 only by grasses (millet and common oat); and group S4 only by legumes (gray velvet bean and jack bean).

The groups presented medium values for the variables (Table 2). It is possible to observe that the S1 and S2 groups differed from each other only due to the enzymatic activities, presenting organic matter and sulfur contents in the soil and wheat grain yield without a significative difference. So, despite this variation in the enzymatic activity, there was no relation to wheat grain yield, when comparing the groups S1 and S2.

Table 1. Descriptive analysis of soil features and wheat grain yield.

Parameter	Organic matter	Sulfur	Beta-glycosidase	Arylsulfatase	Wheat grain yield
	%	mg dm^{-3}	µg p-nitrophenol g^{-1} soil h^{-1}		kg ha^{-1}
Average	2.92	20.13	109.48	127.72	3,646.23
Maximum	3.60	140.00	407.17	378.10	5,932.00
Minimum	2.40	7.30	30.47	42.15	3,097.00
Median	2.90	17.20	104.75	119.63	3,567.00
Variance	0.06	220.32	3,639.90	3,939.77	250,324.70
Standard deviation	0.24	14.84	60.33	62.76	500.32
CV (%)	8.44	73.72	55.10	49.14	13.72

Table 2. Average levels for each group of single systems.

Group	Nº of treatments	Beta-glycosidase	Arylsulfatase	Organic matter	Sulfur	Wheat grain yield
		µg p-nitrophenol g^{-1} soil h^{-1}		%	mg dm^{-3}	kg ha^{-1}
S1	6	170.5 A*	176.8 A	2.9 A	21.1 AB	3,713.0 B
S2	8	86.2 B	93.8 B	2.9 A	22.7 A	3,768.2 B
S3	2	147.1 A	158.8 ABC	3.0 A	14.2 AB	3,246.4 C
S4	2	61.4 B	71.1 C	2.9 A	14.3 B	4,672.0 A

* For each variable, average scores are followed by different letters in the column and differ from each other by the t test for independent samples at $p < 0.05$. S1: bean, white lupin, *Crotalaria spectabilis*, common vetch, corn and *Coracana eleusine*; S2: white forage sorghum, white oat, black oat, pigeon pea, *Crotalaria juncea*, hairy vetch, fodder radish and buckwheat; S3: millet and common oat; S4: gray velvet bean and jack bean.

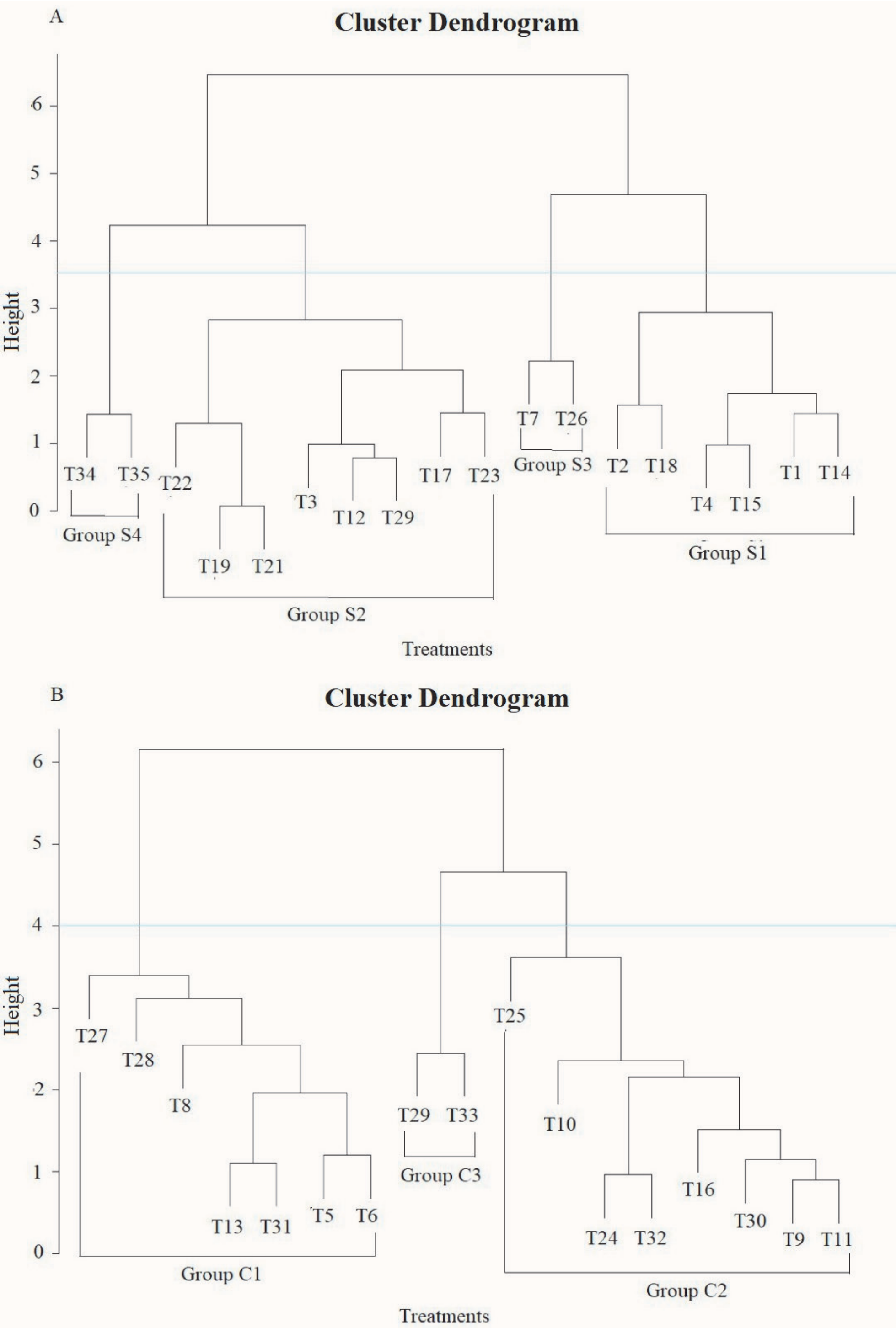

Figure 2. Dendrogram for single (A) and intercropped (B) cover crop systems. S1: T1 (bean), T2 (corn), T4 (white lupin), T14 (*Crotalaria spectabilis*), T15 (*Coracana eleusine*) and T18 (common vetch); S2: T3 (pigeon pea), T12 (*Crotalaria juncea*), T17 (forage white sorghum), T19 (hairy vetch), T21 (fodder radish), T22 (buckwheat), T23 (white oat) and T29 (black oat); S3: T7 (millet) and T26 (common oat); S4: T34 (gray velvet bean) and T35 (jack bean); C1: T5 [RX 520 (white oat + rye + field pea + pivoting turnip)], T6 [RX 410 (oat IPR Afrodite + black oat + fodder radish)], T8 (buckwheat + millet), T13 (*C. juncea* + fodder radish), T27 (common oat + millet), T28 (common oat + millet + fodder radish) and T31 (black oat + millet + fodder radish); C2: T9 (millet + fodder radish + buckwheat), T10 (millet + fodder radish), T11 (*C. juncea* + millet), T16 (*C. eleusine* + millet), T24 (white oat + hairy vetch + fodder radish), T25 (white oat + hairy vetch + fodder radish + millet), T30 (black oat + millet) and T32 (black oat + millet + fodder radish); C3: T20 (fodder radish + hairy vetch) and T33 (black oat + millet + fodder radish + hairy vetch).

Simon et. al (2017), evaluating cover crops in succession to the corn crop, observed that, for the enzymatic activity of arylsulfatase, the crops of the Fabaceae family (legumes) showed lower activity values than for the cover crops of the Poaceae family (grasses), except for the millet crop, which did not differ from the legumes in the study, and, in turn, does not corroborate the data found for the groups S3 (158.8 µg p-nitrophenol g^{-1} soil h^{-1}) and S4 (71.1 µg p-nitrophenol g^{-1} soil h^{-1}) from single crops, where the arylsulfatase activity in the only legume group did not differ from the only grass group. On the other hand, in a study developed by Balota et al. (2004), in a no-tillage system, the enzymatic activity of arylsulfatase presented higher levels in the grass rotation (corn/wheat) at the layers of 0-0.05 m and 0.10-0.20 m than in the ones with legume and grass rotation (soy/wheat) at the same layers.

The wheat grain yield in the groups showed a better result (4,672.0 kg ha^{-1}) for single legumes (S4) and the lowest one (3,246.4 kg ha^{-1}) for single grasses (S3) (Table 2). The lower grain yield in groups with grasses is explained by the high C/N ratio of grasses in general, where, due to the succession of crops (corn/grass/wheat), the immobilization of mineral N by organisms in the soil is higher for straw decomposition (Aita & Giacomini 2006).

In the dendrograms with only intercropped systems (Figure 2), the cover crops were subdivided into three groups with similar data behavior: group C1 with the treatments T5 [RX 520 (white oat + rye + field pea + pivoting turnip)], T6 [RX 410 (oat IPR Afrodite + black oat + fodder radish)], T8 (buckwheat + millet), T13 (*Crotalaria juncea* + fodder radish), T27 (common oat + millet), T28 (common oat + millet + fodder radish) and T31 (black oat + millet + fodder radish); group C2 with T9 (millet + fodder radish + buckwheat), T10 (millet + fodder radish), T11 (*C. juncea* + millet), T16 (*Coracana eleusine* + millet), T24 (white oat + hairy vetch + fodder radish), T25 (white oat + hairy vetch + fodder radish + millet), T30 (black oat + millet) and T32 (black oat + millet + fodder radish); group C3 with T20 (fodder radish + hairy vetch) and T33 (black oat + millet + fodder radish + hairy vetch). It is possible to observe some tendencies in the crops of each group, being the group C1 the one that presents only 2 treatments among the 7 composed by legumes (T5 with field pea and T13 with *Crotalaria Juncea*), and all the groups, except for the T13, present grasses as one of the species. Meanwhile, the group C2 has grasses in all the treatments, and the main one is the millet crop, which is not only present in the T24 treatment, composed by white oat, pea and fodder radish. Finally, the group C3 presents hairy vetch and fodder radish in all the treatments.

In table 3 are presented the average scores of variables for each intercropped group, in which, similarly to the single crop systems, no significant difference was observed between organic matter and sulfur levels in the soil, showing that they do not have a direct influence on the variables.

Regarding the wheat grain yield reached in the intercropped systems, the group C3 was the one that presented the best result (4,156.6 kg ha^{-1}), when compared to the others, pointing out the presence of radish + vetch in both the treatments that compose the group. Zanata et al. (2015) also have reached a better grain yield when using vetch as a cover crop, but, differently, the study analyzed corn yield in succession. Deuschle et al. (2015), even using cover crop species like *Crotalaria juncea*, *Mucuna aterrima*, *Cajanus cajan*, *Pennisetum glaucum* and *Phaseolus vulgaris*, did not find any difference for wheat grain yield in the treatments. Moreover, Skora Neto & Campos (2017),

Table 3. Average levels for each group of intercropped systems.

Group	Nº of treatments	Beta-glycosidase	Arylsulfatase	Organic matter	Sulfur	Wheat grain yield
		µg p-nitrophenol g^{-1} soil h^{-1}		%	mg dm^{-3}	kg ha^{-1}
C1	7	122.4 A*	160.0 A	2.9 A	16.4 A	3,414.1 B
C2	8	87.2 B	112.7 B	2.9 A	23.4 A	3,393.3 B
C3	2	74.1 B	89.0 B	3.1 A	18.8 A	4,156.6 A

* For each variable, average levels followed by different letters in the column differ from each other by the t test in independent samples at $p < 0.05$. C1: T5 [RX 520 (white oat + rye + field pea + pivoting turnip)], T6 [RX 410 (oat IPR Afrodite + black oat + fodder radish)], T8 (buckwheat + millet), T13(*Crotalaria juncea* + fodder radish), T27 (common oat + millet), T28 (common oat + millet + fodder radish) and T31 (black oat + millet + fodder radish); C2: T9 (millet + fodder radish + buckwheat), T10 (millet + fodder radish), T11 (*Crotalaria juncea* + millet), T16 (*Coracana eleusine* + millet), T24 (white oat + *Vicia villosa* + fodder radish), T25 (white oat + *Vicia villosa* + fodder radish + millet), T30 (black oat + millet) and T32 (black oat + millet + fodder radish); C3: T20 (fodder radish + *Vicia villosa*) and T33 (black oat + millet + fodder radish + *Vicia villosa*).

when studying *Helianthus annuus* L., *Crotalaria juncea* L., *Raphanus sativus* L., *Pisum sativum* L. and *Fagopyrum esculentum* Moench, noticed that sunflower as cover crop resulted in higher levels for wheat grain yield, while the other cover crops showed no difference, when compared to the control treatment. Despite that, some researches show differences for wheat grain yield when using different cover crops, even in fertilization associated to N. For example, in a study analyzing crotalaria and hairy vetch under different N doses, Nunes et al. (2011) verified that the use of *Crotalaria juncea* resulted in the best results, in relation to hairy vetch and fallow, not corroborating the present study, since the crotalaria and vetch crops are in the same group (S2).

Enzyme synthesis is carried out mainly by soil microorganisms, so the enzymatic activity is a consequence of the relationship between microorganisms and several factors, is favored by vegetation (rhizosphere) (Carvalho 2005), and is connected to organic matter and biomass (Ajwa et al. 1999), organic carbon levels and soil cation exchange capacity (Tabatabai & Bremner 1972), besides being influenced by different substracts (plants) present in the area (Tabatabai 1994).

In the same way, wheat grain yield is influenced by several factors in the production system, some of them related to previous cover crops. The biomass production in different crop systems may be directly related to wheat plantability. Chen et al. (2007) noticed a reduction in the number of emerged plants after systems with more mass production.

In the present research, as observed by Sá (1993), it was identified that, in successive grass crops, it is necessary more N supplementation, due to the high C/N ratio that causes nitrogen immobilization by microorganisms for decomposition of plant material (Calonego et al. 2012). Because legumes have the ability to fix atmospheric N through symbiosis, in addition to their low C/N ratio, decomposition becomes faster, thus releasing nutrients to the successor crop (Ceretta et al. 1994). Therefore, the wheat grain yield in single systems with legumes was 1,425.6 kg ha^{-1} higher than in single systems with grasses.

CONCLUSIONS

1. In the short term, cover crops affect the enzymatic activity of arylsulfatase and beta-glycosidase;
2. Cover crops have a significant effect on wheat grain yield;
3. Plants from the same botanical family impact differently the enzymatic activity and wheat grain yield;
4. It was possible to group cropping systems with similar response in the enzymatic activity of the different enzymes and in the wheat grain yield;
5. The groups of plants that show a higher enzymatic activity do not necessarily result in higher wheat grain yield.

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