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Article

Effects of doses of rice husk ash on microbial biomass and soil fauna in a natural grassland

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Daniel Hanke[1]
Julio Cordeiro do Nascimento[2]
Shirley Grazieli da Silva Nascimento[2]
Mariana Rockenbach de Ávila[3,§]
Clenio Nailto Pillon3

1 Universidad Federal de Paraná-Departamento de Suelos e Ingeniería Agrícola-Sector de Ciencias Agrarias. Curitiba, PR. Brasil (danielhanke@ufpr.br).

2 Universidad Federal de Pampa-Campus Don Pedrito, Don Pedrito, RS. Brasil. (nascimento.shy@gmail.com; juliocordeirodp@hotmail.com).

3 EMBRAPA. Clima Templado, Pelotas, RS, Brasil. (clenio.pillon@embrapa.br).

Autora para correspondencia: mariana.avila@colaborador.embrapa.br

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Abstract

Rice husks are the residue of rice processing in industries and are generally reused in thermoelectric reactors for power generation thanks to their calorific value. This process produces rice husk ash (RHA), which can be applied alone or in combination with other raw materials in agriculture. This work aimed to verify the relationship between microbial organisms and soil fauna with soil attributes using different doses of rice husk ash. The soil was classified as Palic Haplic Luvisol, maintained under natural grassland and grazing. The experimental design consisted of randomized blocks with four treatments (T= control; T1= 5 mg; T2= 10 mg; T3= 20 mg of RHA ha^{-1}) and four replications. Edaphic mesofauna was collected using the Provids method six months after the treatments were applied. Regarding the microbial biomass, the C and N contents of the soil were determined by dry combustion. The data were subjected to Anova (Tukey test, where $p< 0.05$). It was concluded that rice husk ash increases soil organic matter and microbial biomass. No differences were observed in the soil fauna with the application of ash; nonetheless, it was possible to identify trends of ecological changes with the application of these carbonized materials.

Keywords:

agro-industrial waste, natural field, soil fauna, soil quality.

Introduction

One of the main agricultural products grown in Brazil is rice as it is part of the daily diet of the population. According to data from the Brazilian Institute of Geography and Statistics (IBGE, for its acronym in Portuguese), 11 003 699 t of rice were produced in the Brazilian territory in 2020. Among the producing states, Rio Grande do Sul plays a prominent role, being the largest producer of the grain, contributing about 70% of the national value in the 2019-2020 harvest (IBGE, 2020).

Rice husks are generally used in thermoelectric reactors to generate energy, supplying the industries themselves. The rice husk ash (RHA) resulting from this process can be used in the manufacture of bricks (Mattey *et al*., 2014), ceramics, and cement for civil construction.

According to the study by Martins Filho *et al*. (2020), RHA can be used as a strategy to promote plant growth, acting as a soil conditioner, with the aim of reducing acidity, increasing nutrient availability, and improving structural conditions. Kath (2018) also analyzed a greater effectiveness of RHA compared to the use of liming products since these materials usually have a high composition of oxides and carbonates, which are capable of attenuating the activity of the main components of the active and potential acidity of the soil.

Results obtained by Góes *et al*. (2019) observed that the soil is inhabited by tens of thousands of organisms responsible for various ecosystem functions and processes. Microbial biomass is considered a living and most active fraction of soil organic matter (SOM) and plays a key role in nutrient availability and cycling (Agrawal and Gosha, 2016). The rate of nutrient flux in the soil depends mainly on the microbial biomass and is therefore used as an indicator of soil fertility.

The research carried out by Nguyen and Marschner (2017) indicates that microbial biomass is of great importance in several biological and biochemical processes in the soil since it is directly related to the SOM, the incorporation of residues, and the C and N cycles. The C and N of microbial biomass have been used as indicators of soil quality as microorganisms are extremely sensitive to changes that may occur (Kushwaha and Singh, 2005).

The role of fauna, mainly the edaphic mesofauna (organisms <2 mm), involves the fragmentation and redistribution of organic matter (Correia and Oliveira, 2000). This redistribution process stimulates microbial activity and can regulate the decomposition of materials carried out mainly by fungal populations. In addition to contributing to the decomposition of organic matter and consequently to soil fertility, the invertebrates that inhabit the ‘soil-leaf litter’ system process roots and make galleries and in this way, they also influence soil aggregation.

Soil organisms play an extremely important role in nutrient cycling (Paz-Ferreiro and Fu, 2016), incorporation and decomposition of SOM, carbon sequestration (Anjum and Khan, 2021), porosity, aeration, and water infiltration into the soil. In view of the above, the objective of this work was to verify the relationship between microbial organisms and soil fauna with soil attributes using different doses of RHA.

Materials and methods

The site of the experiment is located at the Pampa Technological Center-Fazenda Escola da Estância do Pampa, belonging to the Federal University of Pampa-UNIPAMPA-Dom Pedrito *Campus*. The experimental unit is located in the municipality of Dom Pedrito-RS (Physiographic Region of the Southern Gaucho Campaign) to 441.4 km from the capital of the state of Rio Grande do Sul (Porto Alegre), at the geographical coordinates 30° 59’ 58.4” South, 54° 36’ 56.4” West, with an approximate altitude of 150 m.

The climate of the region, according to the Köppen classification, is identified as Cfa, with an average temperature between 14° and 18° and annual rainfall ranging from 1 200 to 1 300 mm (Kuinchtner and Buriol, 2001). The soil in the area was classified as Abruptic Palic Haplic Luvisol (Haplic Luvisol) (Embrapa, 2018). The area has historically been occupied by natural pastures that contain the presence of the following botanical genera: Paspalum, Oxalis, Trifolium, Bromeliad, Prosopis, and Axonopus (Boldrini, 1997).

The experiment was implemented in May 2020. The experimental design was structured in randomized blocks (RBD) with four treatments (T= control; T1= 5 Mg of RHA ha^{-1}; T2= 10 mg of RHA ha^{-1}; T3= 20 Mg of RHA ha^{-1}) and four replications, totaling 16 experimental plots (25 m^2 each). In turn, each plot was divided into two subplots, one of which received fertilization with urea (20 kg ha^{-1}) and 5 NPK (4-14-8-5 kg ha^{-1}) to evaluate possible effects of interaction between RHA and mineral fertilizer in the soil.

Six months after implementing the experiment, soil samples (0-5 cm) were collected in each subplot by opening micro-trenches. The samples were properly packaged and subjected to characterization analysis.

Determination of C and N contents in soil and microbial biomass

Soil C and N contents were determined by dry combustion in a CHN elemental analyzer (Vario El III) in soil samples previously air-dried, ground, and passed through a 0.2 mm sieve. From the contents of these elements, the C/N ratio of the soil was calculated. The concentration of C and N in microbial biomass (MB) was determined by the procedures described in Embrapa (1997), with extraction performed by the microwave method and determination performed by dry combustion in a CHN elemental analyzer (Vario El III). After the determination of MB C (MBC) and MB N (MBN), the MB C/N ratio was also calculated.

Collection and identification of soil fauna

The collection of soil fauna was carried out under the pitfall trap methodology using Provid-type containers designed by Antoniolli *et al*. (2006) as traps. The trap containers were made from 600 ml PET bottles, making three openings of approximately 6 x 4 cm. The traps were arranged so that the openings were at the level of the ‘soil-leaf litter’ surface. Inside each trap, 70 ml of ethyl alcohol (70%) were added.

For each experimental replication, two traps were placed (plot with and without mineral fertilizer), totaling 32. The traps remained in the field for seven days and when they were removed from the experiment, another 70 ml of alcohol was added. Subsequently, the samples were sent to the Microscopy Laboratory of UNIPAMPA, where the organisms were classified and identified using optical microscopy technique.

Data processing

The data of C and N, C/N ratio, MBC, MBN, MB C/N ratio and identified edaphic fauna groups were subjected to an analysis of variance (Anova), followed by Tukey test ($p< 0.05$). This was done since any difference in these attributes and parameters, within the experimental design, was considered as an additive (development of environmental changes in the natural conditions of the ‘soil x vegetation’ system, caused by the doses of ash applied). Thus, the possibility of dividing the variance of the data between what can be verified by adding materials (gray) and what cannot be explained by this variation factor (residuals/errors) was assumed.

In addition to the data from the fauna study (number of individuals belonging to large groups of soils-taxonomic class/order), the following biodiversity indices were calculated: i) Shannon-Weaver diversity index (H= -#Pi log Pi. Where: Pi= the proportion of group i in the total sample e; and ii) Simpson’s index (form of dominance given by S= #(ni/N)2. Where: ni= the number of individuals in group i; and N= the sum of the density of all groups) (Odum, 1988). These procedures were performed using the statistical software of Sisvar (version 5.8) and Bioestat (version 5.0).

For exploratory purposes of the data distribution pattern, simple linear regression analyses (1st and 2nd order polynomials) and multivariate principal component analysis (PCA) were also applied, considering a linear correlation matrix as a measure of similarity and applying the bootstrap resampling test -with ten thousand interactions with replacement- to assess the importance of the stability of the data dispersion pattern (Paste software, version 4.03). As stated in the results and discussion section, the exploratory analyses were related to the results obtained in this research and the bromatological results of the vegetation described in Pinto *et al*. (2022).

Results and discussion

Microbiological attributes and soil organic matter (SOM)

The results for these attributes showed significant differences (Anova + Tukey test, considering: $p < 0.05$), which can be explained by the differences in the contribution of these elements through the doses of RHA (Table 1). The C/N ratio of the soil varied numerically from 10.8 to 14.2, being statistically higher in T2 (10 Mg ha^{-1}), followed by T3 (20 Mg ha^{-1}) and lower in T1 (5 Mg ha^{-1}) and the control (Table 1).

Table 1. Soil chemical and microbiological attributes, as well as abundance of soil fauna and biodiversity indices in each experimental treatment.

Soil attributes	C	N	C/N	MBC	MBN	MB C/N	Arachnida	Hymenoptera	Coleoptera	Collembola	Shannon-Weaver	Simpson	Dominance
	(g kg^{-1})			(mg kg^{-1})									
Cont	9.7cA	0.9bA	11.2aA	30.1cA	5.6cA	5.2cA	9 ±3	27 ±12	4 ±1	56 ±29	1.02	2.31	0.43
Cont NPK	9.9cA	0.9bA	10.8aA	23. 7cA	4.3cB	5.6cA	6 ±2	8 ±1	2 ±1	98 ±51	1.04	2.34	0.45
T1	9cA	0.8cA	11. 6aA	32.8cA	5.2cA	6.4bA	12 ±9	22 ±10	3 ±1	81 ±38	0.88	1. 91	0.52
T1 NPK	10.2cA	0.8cA	13aA	32. 3cA	4.4cB	7.3bA	12 ±8	14 ±7	5 ±4	62 ±19	0.98	2.06	0.49
T2	11.3bA	0.8bA	14. 2aA	53.2bA	6.3bB	8.5bA	13 ±9	25 ±17	6 ±2	76 ±60	1.01	2.18	0.46
T2 NPK	11.9bA	0.9bA	13aA	73.1bA	8.8bA	8.3bA	16 ±10	24 ±12	5 ±2	59 ±28	1.1	2.5	0.4
T3	14aA	1.2aA	11.5aA	84.3aA	8.3aB	10. 1aA	17 ±3	24 ±12	4 ±3	130 ±70	0.79	1.71	0.59
T3 NPK	13.4aA	1.1aA	12.7aA	93.6aA	9.4aA	9.9aA	14 ±13	36 ±20	4 ±3	212 ±89	0.66	1.51	0.66

There were no significant differences between the soil C/N ratio between the experimental treatments, that is, the addition of higher doses of ash (more recalcitrant material) was not sufficient to significantly change the C/N ratio of the soil samples, unlike what was observed for the MB C/N ratio (discussed below) (Table 1).

MBC and MBN values (in the 0-5 cm layer) ranged from 23.7 to 93.6 mg kg^{-1} and from 4.3 to 9.6 mg kg^{-1}, respectively, and increased with the magnification of the RHA dose (Table 1). This increase in soil microbial biomass indicates a significant change in biological activity (Liu *et al*., 2023), which is related to the specialization of the environment and the addition of pyrogenic materials that can affect nutrient cycling and, consequently, plant development.

The MBN showed significant differences between the subplot conditions, being higher in the plot that received mineral fertilizer (Table 1). This was to be expected since, with a greater contribution of N via fertilization, microorganisms can immobilize part of this element in the cell growth process (Grzyb *et al*., 2021) and metabolic processes. In turn, the MB C/N ratio ranged from 5.2 to 10.1, tending to increase along with increasing the dose of RHA in T2 and T3 treatments (Table 1).

MB C/N values above 8.0 with the highest doses of RHA suggest a predominance of fungi (Totola and Chaer, 2002), which make up an ecological group recognized for its importance in the decomposition of organic materials and nutritional cycle. In addition, fungi are preferred food sources for detritivorous-fungivorous soil fauna groups, such as springtails (Chauvat *et al*., 2014).

Soil fauna and ecological diversity

The main groups of edaphic fauna identified were: i) Arachnida; ii) Hymenoptera (83% of Formicidae, mainly 'cutters'); iii) Coleoptera (mainly Staphylinidae and Curculionidae); and iv)

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Collembola (65% Isotomidae) (Table 1). Among the experimental treatments, on average, the variation of these groups was: i) Arachnida (from 6 to 17 individuals); ii) Hymenoptera (8 to 36 individuals); iii) Coleoptera (2 to 6 individuals) and iv) Collembola (56 to 212 individuals).

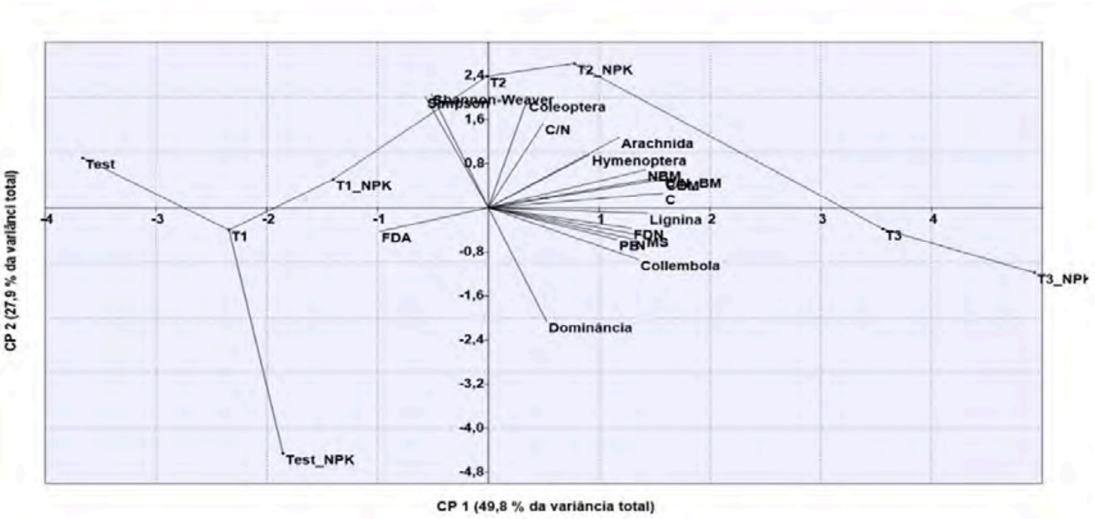
There was no significant difference between the experimental conditions and the subplots for any of the edaphic groups, which is possibly due to the high coefficients of variation verified (65.5%, 62.5%, 39.9%, and 51.9% for Arachnida, Hymenoptera, Coleoptera and Collembola, respectively).

The ecological diversity indices of Shannon-Weaver and Simpson ranged from 0.66 to 1.04 and from 1.51 to 2.34, respectively (Table 1); that is, as the dose of the added materials increases, which implies a higher C/N content, a decrease in the diversity of the edaphic fauna expressed by the Shannon-Weaver index and a greater ecological dominance of these communities were observed. Following the opposite trend, the ecological dominance index increased in T3 (20 Mg ha^{-1}), suggesting that the application of high amounts of these carbonized materials tends to reduce the diversity of soil fauna and increase the ecological dominance of some groups (Table 1).

Principal component analysis

A multivariate principal component analysis (PCA) was performed in order to relate the determined soil microbiological and fauna data or attributes with SOM data and vegetation bromatological data; the latter are described and discussed by Pinto *et al*. (2022) (Figure 1). PC 1 (x-axis), which explains most of the variance in the data, is a complex variable compiled from the following soil attributes/ parameters: i) C and N contents; ii) MBC; iii) MBN; iv) MB C/N; v) Arachnida; vi) Hymenoptera; vii) Collembola; viii) dry matter (DM) content of the grass; ix) crude protein (CP) content of the grass; x) neutral detergent fiber (NDF) content of the grass; xi) acid detergent fiber (ADF) content of the grass and xii) lignin content of grass (study results).

Figure 1. Diagram of ordering by principal components of variables and sampling units.



Thus, PC 1 contains the variation of SOM data, microbiological data, fauna data (predators, social insects, and cyclers), and vegetation data. The PCA described more than 80% of the total variance of the experimental data, with 77.7% of the variance contained in the first two ordering axes (principal components). PC 1 (x-axis) contained 49.8% of the data variation, while PC2 (y-axis) contained approximately 27.9% of the total variance (Figure 1). Both components 1 and 2, were considered stable by the probabilistic bootstrap resampling test, so the pattern of data dispersion was considered valid for discussion (Figure 1).

In other words, the position of the groups of organisms (soil fauna and flora) responds to variations in the content of SOM and constituents of plant tissues. PC 2 (y-axis) is a variable that contains

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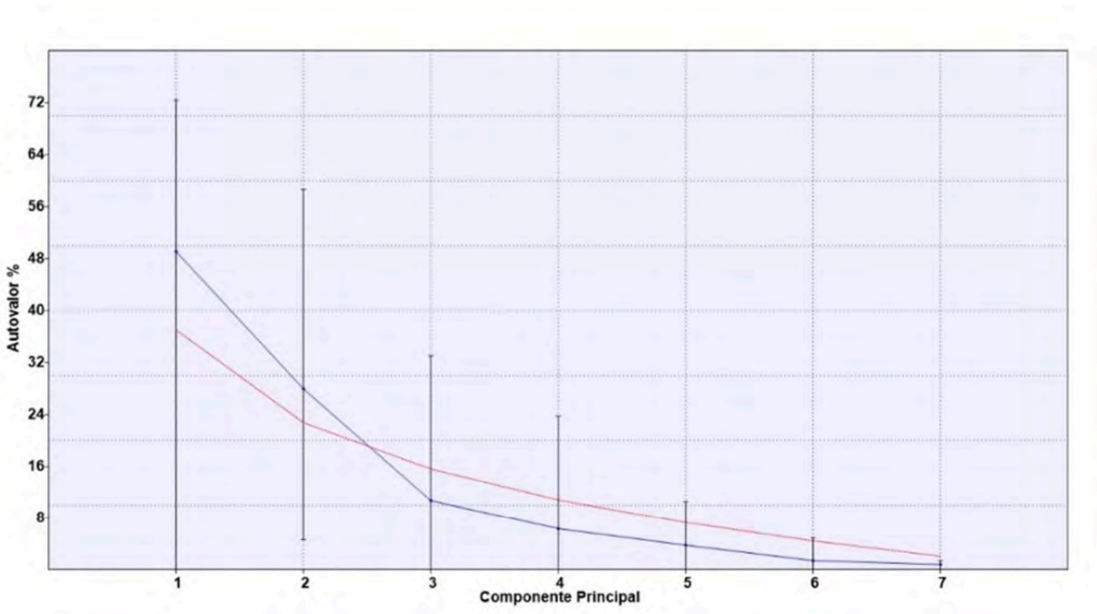
the variation of the parameter C/N ratio, Coleoptera group, and diversity indices (Shannon-Weaver, Simpson, and ecological dominance). Thus, PC 2 (y-axis) has as its main thematic meaning the ecological diversity of experimental conditions.

The results obtained suggest that the Coleoptera group is less sensitive to variations in soil and vegetation than the other groups of fauna and tends to concentrate more in experimental positions where there is a lower rate of SOM decomposition. The sampling units (average of the subplots of the experimental treatments) that appear to the right of the origin, in relation to PC 1, tend to present a greater number of Arachnida, Hymenoptera, Collembola, higher C and N contents, higher MBC, MBN and MB C/N ratio, as well as vegetation with higher phytomass and higher NDF, PC, and lignin composition. These sampling units correspond exactly to the highest doses of RHA (T2 and T3) (Figure 2).

Figure 2. Probability test of the stability of the scatter plot of the principal components using Bootstrap resampling. Note: Actual values represented by the blue line and bootstrap sample values represented by the red line.



Therefore, there is a proven trend that RHA has the ability to change not only the SOM and microbiological attributes of the soil, but also the bromatological composition of vegetation and certain groups of edaphic fauna. These taxonomic groups represent social insects (Hymenoptera), detritivorous-fungivorous cyclers (Collembola) and predators (Arachnida). As the RHA is composed of more recalcitrant (aromatic) carbonaceous structures, it is expected that, with the addition of these materials, there will be a progressive increase in the C/N ratio of the soil, which would favor greater fungal development.

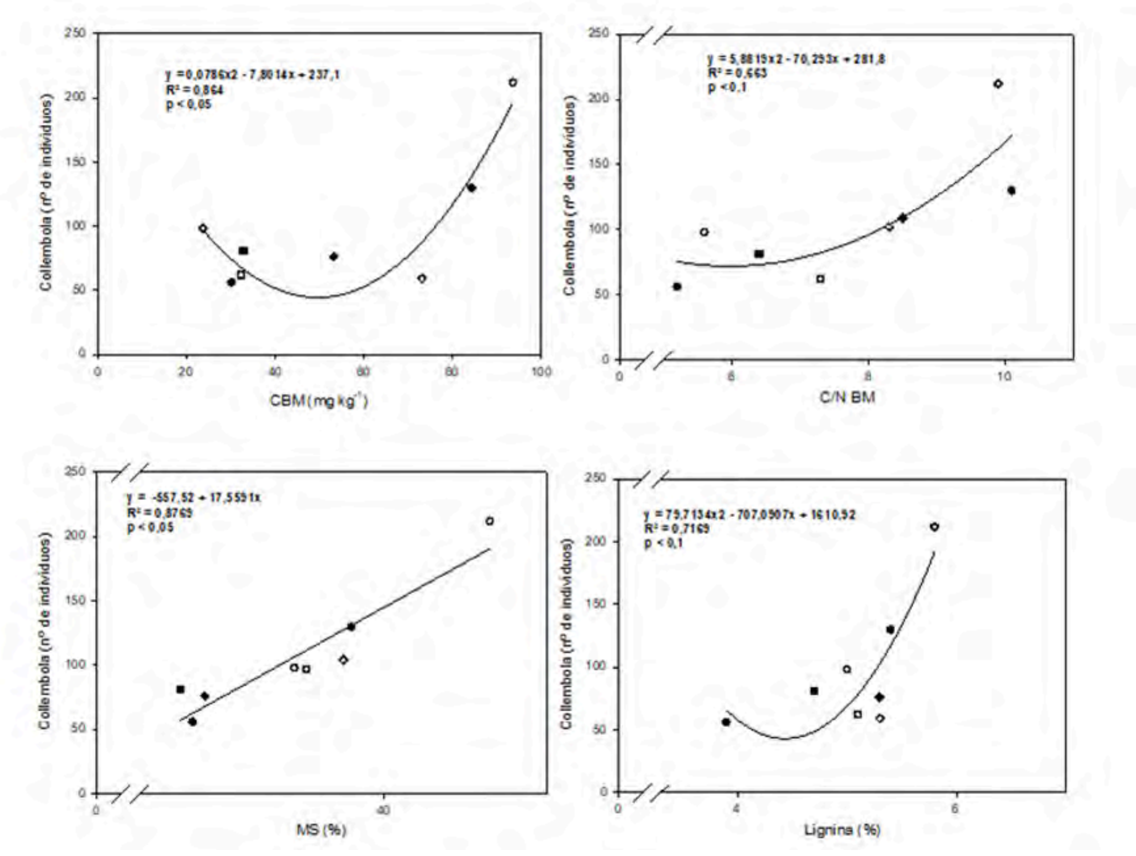
With the increase in the C/N ratio and biomass of the most recalcitrant forages, as well as the increase in fungal growth, there is likely to be ecological pressure for population growth of detritivorous and fungivorous organisms (Collembola). In this way, other organisms, predators of Collembola, such as arachnids, would also tend to increase.

On the other hand, the change in soil and vegetation characteristics brought about by the progressive addition of RHA would also create environmental conditions for an increase in the density of social insects (Hymenoptera), mainly ants (as observed in the selection process). Possibly, this trend would be related to the increase in the supply of biomass and the change in soil structure to a better drained environment. Regarding PC 2 (y-axis), it was observed that the application of RHA up to a dose of 10 Mg ha-1 has little influence on the ecological diversity of the system (Figures 1 and 2).

However, at the highest dose of RHA (T3= 20 Mg ha^{-1}), there was a substantial decrease in the diversity of organisms, concomitant with the increase in the dominance of certain soil groups. These data suggest that high doses of this material can have significant effects on the ecological dynamics of the edaphic fauna in grassland physiognomies, causing a decrease in diversity and an increase in the specialization of the environment in relation to certain groups.

The application of the spanning tree algorithm, represented through the edges that interconnect the sample units in the ordering diagram, highlights the pattern of similarity between the experimental conditions (Figure 3). This pattern shows that the control and T1 samples are more similar to each other, comparing the application of the intermediate dose of RHA (T2= 10 Mg ha^{-1}) and the highest dose (T3= 20 Mg ha^{-1}), with some variation between the subplots evaluated (with and without supplementary mineral fertilization).

Figure 3. Linear regression models between the Collembola population and MBC; MB C/N and lignin content in vegetation.



That is, although no significant differences were verified by the ANOVA -due to the high coefficient of variation of the data between the experimental replications, as already mentioned- there is, in fact, a tendency of alteration of soil attributes, in microbiology, in vegetation and fauna for the application of the RHA, which should not be ignored.

The relative temporal proximity between the fauna sampling and the time of installation of the experiment may have contributed to the results still being unstable from the point of view of the variation of the attributes/parameters evaluated. Nevertheless, a probabilistic sampling with a longer temporal distance from the installation period can elucidate how these ecological patterns will appear after further stabilization of environmental conditions by the application of RHA.

Conclusions

The application of rice husk ash resulted in a significant change in the amount of C and N in the soil and in the microbial biomass of the soil, conditioning a selectivity of fungal microorganisms (higher C/N ratio) with the increase in the dose used. The distribution pattern of certain detritivorous and fungivorous groups of the fauna (represented here by springtails) responds to the quantity and quality of the plant biomass added.

The increase in these organisms also results in a change in the population of predators (represented here by arachnids). In addition, the alteration of soil and vegetation characteristics, with the application of RHA, also resulted in the alteration of the community of social insects (represented here by Hymenoptera).

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