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Liming and Nitrogen Fertilization Effects on Soil Microbial Community in Long Term No-till

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HIGHLIGHTS

- Soil respiration and metabolic quotient showed to be good biological indicators of soil quality.
- Soil pH was a driven factor for soil microbial community.
- Nitrogen fertilization negatively affected diazotrophic microbial community.

Abstract: Soil management influences organic matter decomposition rates as well soil microbial community functional behavior. No-till (NT) is the most used management system by farmers due to its conservation practices and high productivity. The main objective of this study was to evaluate the impact of surface-applied lime, nitrogen (N) application, and black oat residues on soil microbial community of a Typic Hapludox under continuous NT. Therefore, soil chemical attributes, microbial biomass carbon, basal respiration, metabolic quotient, most probable number of diazotrophs, as well as bacterial functional analysis were performed. The effect of liming and N fertilization amendments inputs were saw in soil respiration and metabolic quotient measurements, showing them to be good indicators of soil quality. Further studies should be carried out in order to molecularly identify microbial communities present in soils with different liming and N fertilization management to evaluate the behavior of specific bacterial taxa under such conditions.

Keywords: soil microbial biomass; diazotroph microorganisms; soil management; Biolog EcoPlates.

INTRODUCTION

Soils are complex environments, which are subject to variation according to factors such as climate, management, and geography, exhibiting spatial and temporal heterogeneity [1], being one of the main components of terrestrial ecosystems. Soil equilibrium has been constantly changed through anthropic

actions, which have been causing its degradation and quality reduction [2]. Its quality can be evaluated mainly for agroecosystems where the objective is to achieve high productivity [3]. Soil chemical indicators such as available carbon (C) content, pH, phosphorus (P) and potassium (K), total nitrogen (N), electrical conductivity, cation-exchange capacity (CEC), and mineral N as well as biological attributes such as soil respiration and biomass are among the most frequently used indicators of soil quality [3].

Microbial biomass is the portion of soil organic matter fully determined by the biotic and abiotic factors of the environment, therefore it is quickly changed due to soil use and management [4]. The metabolic potential of the microbial community is strongly associated with C levels, which by itself is related to the decomposition processes and nutrient release from organic residues [5], affected by crop rotation.

Crop rotation in a continuous no-till (NT) system is one of the main factors that determine the increase in the superficial amount of organic matter and promote the recycling of some essential nutrients in depth [6]. Black oat (Avena strigosa Schreb) is commonly used as a cover crop for being a rustic species, undemanding in terms of soil fertility, and has been well adopted in the following states of Brazil: Paraná, Santa Catarina, Rio Grande do Sul, São Paulo, and Mato Grosso do Sul [7]. Besides, it has a high biomass production as well as presents a fast early growth, and produces seeds that generate a low production cost [6].

On the other hand, soil fertility limitations require constant amelioration for hold crops productivity. Low soil N availability is the main limiting factor on black oat growth and biomass production [8]. Therefore, when black oat is used as winter cover, N fertilization should stimulate growth and root activity as well as biomass production and uptake of other nutrients [8]. More than 95% of the total N in soil is in organic form, and only 2-5% is inorganic ammonium (NH_4^+), and/or nitrate (NO_3^-) forms [9]. The inorganic N comes from the mineralization processes, carried out by the soil microbiota [10] and/or from the N fertilizer application. Nitrogen application with ammoniacal fertilizers decreases soil pH [8], which has a strong influence on the decomposition of organic matter and, consequently, on the soil microbial community.

Surface liming ameliorates topsoil acidity and minimizes the effect on soil acidification caused by the addition of ammoniacal fertilizers, stimulating nitrification in acidic soils [11]. As a result, the surface application of lime can increase the possibility of downward movement of exchangeable Ca^{2+} and Mg^{2+}, which accompanies NO_3^- anions [8,12]. The uptake of NO_3^- by plants can increase the rhizosphere pH in subsoil rovided that roots are established in deeper layers [13].

As alternatives for evaluate soil fertility, biological indicators have been reported as highly promising and among the factors that influence microbial composition in soils, soil pH and N availability has been reported as important ones. Soil microbial community can vary constantly due to the different forms of soil management, therefore some of the soil microbiological properties are more sensitive and can be considered as best indicators of soil quality [14]. Changes in C microbial biomass (SMB-C), basal respiration (CO_2-C), metabolic quotient (qCO_2), and microbial quotient (qMIC) are information that provide a better prediction of ecological processes occurring in the soil [15].

Several studies and techniques have been developed to evaluate the behavior of microbial communities in the face of changes in the most diverse terrestrial ecosystems, among these the BiologMicroPlates™. This technique has been widely used to evaluate the physiological profile of soil bacteria community in microbial ecological studies [16,17]. Assessing soil microbial community behavior allows better evaluation of soil use and management techniques and practices that can maintain or improve soil fertility and quality over the years [15].

In this way, this study reports a field trial that examined the effects of black oat residues associated with surface liming and N application over soil microbial community under a continuous NT system.

MATERIAL AND METHODS

Site location and historical crop rotation

The experiment was performed in Ponta Grossa, PR, Brazil (25º 10’ S, 50º 05’ W), on an Oxisol (loamy, kaolinitic, thermic Typic Hapludox). The climate at the site is categorized as a Cfb (mesothermal, humid subtropical) with mild summer and frequent frosts during the winter. The average altitude is 970 m with average maximum and minimum temperatures of 22 and 13 ºC, respectively. The annual precipitation is about 1550 mm. A continuous NT system was established in 1978, and during the period from 1978 to 2004 were cultivated wheat, triticale or black oat in the winter and soybean or corn in the summer. No-till involved no disturbance to the soil other than the sowing operation. The historical use of the experimental area from 2004 is detailed in figure 1. Dolomitic lime was applied to a soil surface at a rate of 12 Mg ha^{-1} in May 2004.

Nitrogen fertilization was performed annually through the application of 180 kg ha^{-1} of N as ammonium nitrate (NH_4NO_3), during the tillering of winter crops (black oat or wheat).

Treatments were randomly distributed in triplicates in the experimental area: without winter cover crop (Fallow), with black oat as cover crop (Oat), with black oat and N fertilization (Oat-N), with black oat and lime application (Oat-L), and with black oat and N fertilization and lime addition (Oat-NL). The area of each plot collected was 41.6 m^2. In plots without cover crop, black oat was burnt down with glyphosate soon after emergence.

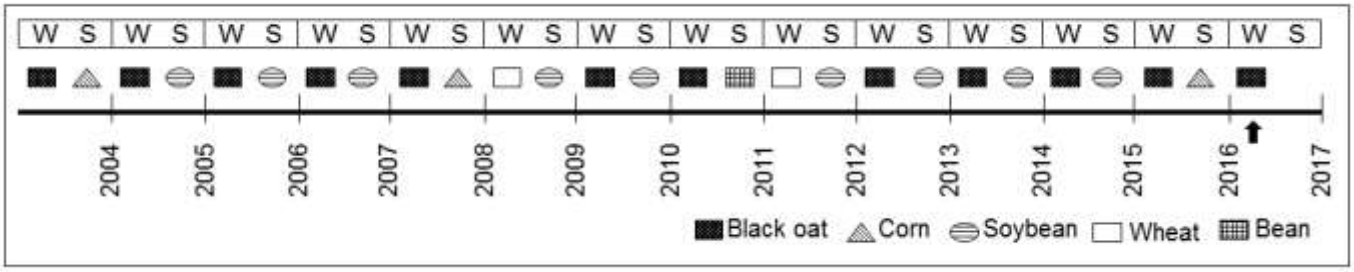

Figure 1. Historical crop rotation in the experimental area from 2004 to 2017. W: winter. S: summer. Arrow: current experiment.

Soil sampling and chemical analyses

Soil samples were collected at 0-10 cm depth in the flowering of black oats in 2017, stage where N is more required [10]. The sampling took place 30 days after N fertilization. Composite samples of 12 subsamples were collected within a circle of 6 m in diameter. Soil samples were dried at 30 °C or 40 °C, for microbiological and chemical analysis, respectively. It was determined the soil pH in a 0.01 M $CaCl_2$ suspension, potential acidity (H + Al), and contents of exchangeable Al^{+3}, Ca^{+2}, Mg^{+2}, and K^{+} [18].

Functional analysis of soil microbial communities

Community level substrate utilization (CLSU) was determined using Biolog™ EcoPlate. Three replicates of 10 g (fresh weight) were suspended during 30 min at 300 rpm, in 90 mL of sterile saline solution (0.85% NaCl). The suspensions were sedimented for 10 min, and then 125 µl of a 10-fold dilution of supernatant was applied in each well of plate. Finally, plates were incubated at 25 °C and optical density at 590 nm and 750 nm (OD_{590} and OD_{750}) was read at 24 h intervals for 5 days. Each well was analyzed, and values of individual absorbance for 31 single substrates were corrected subtracting the blank control value (raw difference; RD). Negative RD values were set to zero. The different inoculum densities were normalized using RD values and dividing by their respective average well colour development (AWCD) values [19].

Most probable number (MPN) of diazotrophic bacteria

Soil samples in triplicate (10 g), were incubated at 28 ºC during 48 h after addition of 2 mL de H_2O, and then suspended at 180 rpm during 20 min in 90 mL of sterile saline solution (0.85% NaCl). A 10-fold dilution of suspension until 10^{-6} was made, and 0,1 ml of each dilution was inoculated in tubes (10 mL) content 3 mL of semi-solid medium [20,21].

The presence of N-fixing organisms has been verified by pellicle formation in each tube. To calculate the most probable number of diazotrophs bacteria in the original sample was used the Mc Crady's table [20].

Microbial biomass carbon, basal respiration and metabolic quotient

Soil microbial biomass C (SMB-C) was estimated by fumigation-extraction method using fresh samples [22], in snap-caps flasks of 300 ml containing 20 g of soil. The samples were fumigated or not with alcohol-free chloroform overnight. The fumigation-extraction method is based on the fact that the C of microorganisms killed by fumigation is made available in the soil, where it is subsequently extracted and quantified. For determination of soil basal respiration (CO_2-C), 20 g of soil was placed in a flask sealed and incubated during 72 h. The CO_2-C emitted was captured in a NaOH solution 0.5 M, titrated with HCl 0.5 M [23]. The metabolic quotient (qCO_2) was determined by ratio CO_2-C/SMB-C [24]. The obtained values were adjusted for humidity that was determined drying 20 g of soil at 105 C.

Statistical analysis

The means of variables that had a significant difference by the F test ($p < 0.05$) were compared by the Duncan test ($p < 0.05$). The data were also submitted to multivariate analysis by principal component analysis (PCA) using the RStudio program [25], with the Vegan statistical package [26].

RESULTS

Soil chemical attributes

Table 1 shows soil chemical attributes in the 0–10 cm layer for the different treatments.

Table 1. Soil chemical attributes in the surface layer (0-10 cm) of soil samples collected at the flowering of black oat in winter 2017.

Treatments	pH	Al^{3+}	Ca^{2+}	Mg^{2+}	K^{+}	C	M	V
	($CaCl_2$)	(mmolc dm^{-3})				(g dm^{-3})	(%)	
Fallow	4.7 b	8.1 a	29.0 bc	5.7 b	1.5 ab	20 a	27.4 a	33.6 bc
Oat	4.9 b	6.7 b	35.3 b	6.5 b	1.8 a	21 a	22.1ab	41.3 b
Oat-N	4.4 b	9.5 a	22.7 c	4.8 b	1.1 b	22 a	32.8 a	26.0 c
Oat-L	6.1 a	0.0 c	52.0 a	17.0 a	1.9 a	25 a	0.0 d	68.1 a
Oat-NL	5.5 ab	3.3 bc	43.7 ab	11.8 ab	1.8 a	22 a	11.0 c	54.7 ab

M %= aluminum saturation; V%= base saturation; Fallow, without winter cover crop; Oat, with black oat as cover crop; Oat-N, with black oat and N fertilization; Oat-L, with black oat and lime application, and Oat-NL, with black oat, N fertilization and liming. Values followed by the same letter were not statistically different (Duncan, $p \leq 0.05$).

Fallow, Oat, and Oat-N presented lower pH values (4.7, 4.9 and 4.4, respectively). Oat-L presented the highest pH value (6.1), followed by Oat-NL treatment, 5.5.

Exchangeable Al^{3+} contents were high in Fallow and Oat-N, while exchangeable Ca^{2+} contents were higher in Oat-L and Oat-NL. It's important to mention that although soil pH is high for exchangeable aluminum in the form of Al^{3+} the observed values are common in the studied region. Also, some exchangeable Al^{3+} may appear at pH values up to 5,5 [10]. The highest contents of exchangeable Mg^{2+} and K^{+} were registered in Oat-L treatment, associated with Ca^{2+}.

Biological soil indicators

The Fallow, Oat and Oat-L treatments showed the highest values of bacterial density per gram of soil (6.66, 6.49, and 5.87 Log MPN g^{-1}, respectively). The lowest values were observed in Oat-N and Oat-NL (Figure 2).

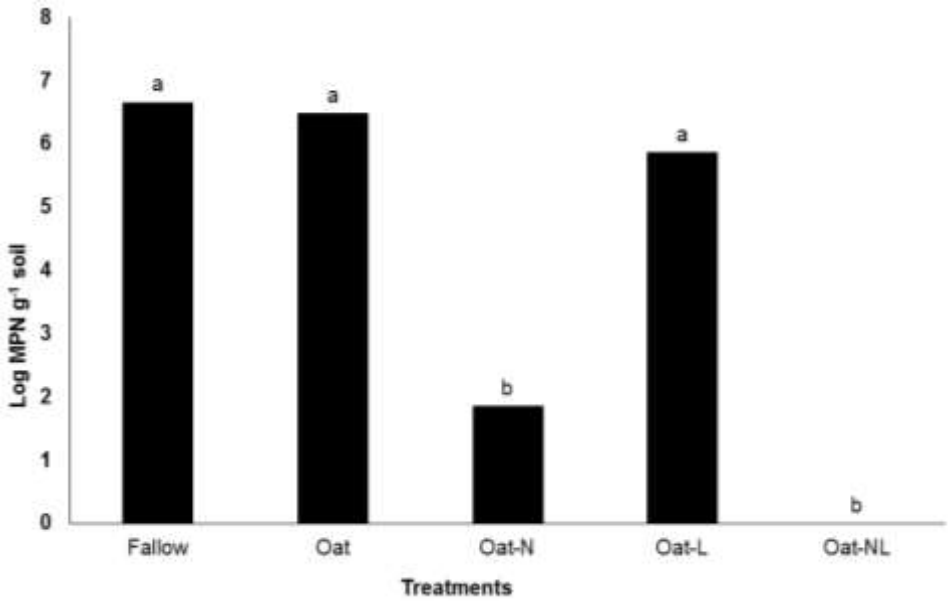

Figure 2. MPN values of diazotrophic bacteria for each treatment during black oat cultivation in winter 2017. Fallow, without winter cover crop; Oat , with black oat as cover crop; Oat-N, with black oat and N fertilization; Oat-L, with black oat and lime application, and Oat-NL, with black oat, N fertilization and liming. Values followed by the same letter were not statistically different (Duncan, $p \leq 0.05$).

Table 2. Changes in soil microbial biomass carbon, basal respiration and metabolic quotient during black oat cultivation.

Treatments	**SMB-C** $\mu g\ g^{-1}$	**CO_2-C** $\mu g\ g^{-1}\ day^{-1}$	**qCO_2** $\mu g\ \mu g^{-1}$ C microbial h^{-1}
Fallow	91.37 b	0.47 b	5.19 b
Oat	124.51 a	0.54 ab	4.36 b
Oat-N	76.46 b	0.74 a	9.77 a
Oat-L	84.29 b	0.33 b	3.99 b
Oat-NL	87.11 b	0.47 b	5.43 b

SMB-C, soil microbial biomass carbon; CO_2-C, basal respiration; qCO_2, metabolic quotient; Fallow, without winter cover crop; Oat , with black oat as cover crop; Oat-N, with black oat and N fertilization; Oat-L, with black oat and lime application, and Oat-NL, with black oat, N fertilization and liming. Values followed by the same letter were not statistically different (Duncan, $p \leq 0.05$).

The {SMB-C}, as well as respiration and metabolic quotient values showed remarkable variations among treatments (Table 2). The average values of SMB-C ranged from 76.46 to 124.51 $\mu g\ g^{-1}$ soil. The highest value was observed in Oat treatment (124.51 $\mu g\ g^{-1}$ soil), while in Fallow, Oat-N, Oat-L, and Oat-NL the values showed no significant differences. Oat-N was the treatment that stood out in basal respiration analyses, with an average value of 0.74 µg of CO_2-C $g^{-1}\ day^{-1}$, followed by Oat treatment (0.54 µg of CO_2-C $g^{-1}\ day^{-1}$). The lowest values of basal respiration were observed in Fallow, Oat-L and Oat-NL treatments.

The metabolic quotient value results from the relationship between basal respiration and microbial biomass, which presented a high rate in the Oat-N treatment (9.77 µg of CO_2 μg^{-1} C microbial h^{-1}), demonstrating that the application of N at high doses can interfere in the microbial community.

The C sources presented in Ecoplate system were grouped according to their chemical functions: Carboxylic Acids, Carbohydrates, Amino Acids, Polymers, Phenolic Compounds and Amines, and tabulated according to consumption in each treatment. Carboxylic acids and polymers were the most consumed C sources by soil bacterial community, followed by carbohydrates, phenolic compounds, amino acids and amines. For carbohydrates C source, there is no significant difference among treatments. For the other C

sources, only Oat-L treatment was statistically different from the other treatments and presented the lowest consumption values (Figure 3).

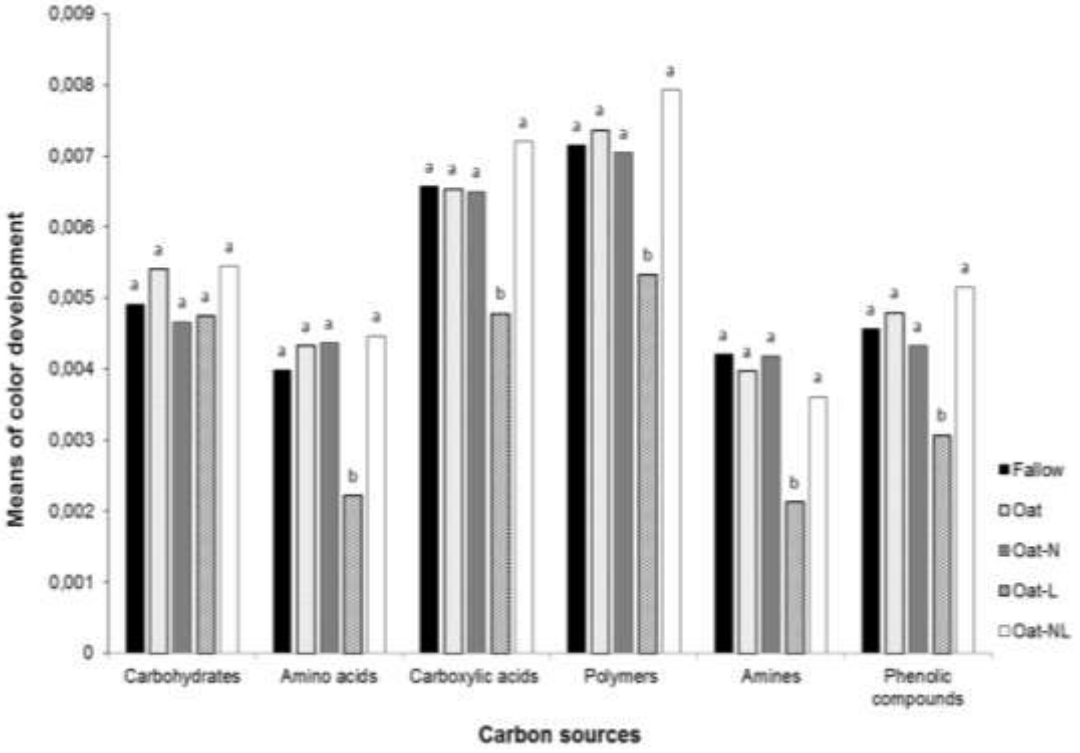

Figure 3. Functional analysis of soil microbial communities based on carbon source consumption. Fallow, without winter cover crop; Oat, with black oat as cover crop; Oat-N, with black oat and N fertilization; Oat-L, with black oat and lime application, and Oat-NL, with black oat, N fertilization and liming. Values followed by the same letter were not statistically different (Duncan, $p \leq 0.05$).

The C sources with highest consumption were γ-Hidróxibutírico acid, D-Malic acid, L-Phenylalanine and Tween 40 in Fallow treatment; L-Arginine and D,L-α-Glycerol phosphate in Oat treatment; α-D-Lactose and Tween 80 in Oat-N treatment; D-Galactonic acid, γ-Lactone and L-Threonine in Oat-L treatment; and Pyruvic Acid Methyl Ester, L- Asparagine and L-Serine in Oat-NL treatment.

Principal component analysis (PCA) organized 54% of total variance in the first component and 18% in the second component, completing 72% of total variance. It was generated by correlating galacturonic acid from Biolog matrix, which presented significant correlation with microbial biomass measurements, with microbial biomass C, basal respiration, metabolic quotient, most probably number of diazotrophs and all soil chemical attributes (Figure 4). Oat-N treatment was positively correlated with metabolic quotient and microbial respiration parameters. Fallow treatment presented high exchangeable Al^{3+} content. Oat-L and Oat-N-L treatments had a positive correlation with soil pH and exchangeable Ca^{2+} and Mg^{2+} contents. Finally, the Oat treatment was positively correlated with MPN, galacturonic acid, and microbial biomass C.

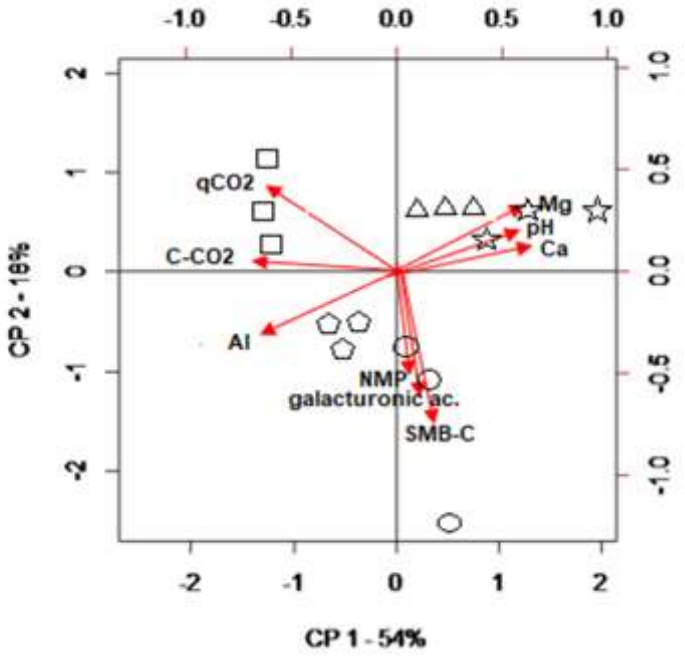

Figure 4. Principal component analysis (PCA) generated from biological parameter data and all chemical analysis data correlated with galacturonic acid. qCO_2: metabolic quotient, CO_2-C: soil basal respiration, SMB-C: soil microbial biomass carbon, NMP: most probable number of diazotrophs, Al: aluminum, Mg: magnesium, Ca: calcium, galacturonic ac: galacturonic acid. The treatments are: square (Oat + N); star (Oat + L), triangle (Oat + N + L); pentagon (Fallow) and circle (Oat).

DISCUSSION

Soil chemical indicators

Soil pH values were higher in oat – limed treatment. The Fallow, Oat, and Oat-N treatments presented lower soil pH values. A possible explanation lies on NH_4NO_3-N applications. The increase in soil acidity with the use of ammoniacal fertilizers is caused by the nitrification process, the oxidation of NH_4^+ to NO_3^- releases H^+ protons, which occupy cation exchange sites leached with NO_3^-, reducing the soil pH [27,28]. The high values of Al^{3+} observed in N fertilized treatments also match with lower pH values.

Soil acidification under NT systems due to fertilization with ammoniacal fertilizers caused a decrease in exchangeable Ca^{2+} and Mg^{2+} contents in a superficial layer (0 – 10 cm), and an increase in exchangeable Al^{3+} content to a depth of 10–20 cm [8]. Liming has long been reported as an efficient way to reduce soil acidity in both conventional and NT systems [8, 9]; this fact was observed in the treatments with liming as Oat-L and Oat-NL in which the soil pH increased and, consequently, the Al^{3+} contents were lower. Increasing soil pH with liming favors a reductions in Al^{3+} content due to its precipitation as $Al(OH)_3$ [28].

The Fallow treatment presented a high exchangeable Al^{3+} content (Figure 4). This correlation may be justified since this treatment did not receive lime to correct soil acidity, and under these conditions, there was an increase in exchangeable Al^{3+} content.

Reduced levels of exchangeable Ca^{2+} in soil surface layer were founded in treatments without lime, i.e. Fallow, Oat, and Oat-N (22.7; 29.0 and 35.3 $mmol_c\ dm^{-3}$, respectively) [28]. Exchangeable Mg^{2+} content was higher in Oat-L treatment (17 $mmol_c\ dm^{-3}$) due to surface liming [8], while a lower exchangeable Mg^{2+} content in Oat-N (4.8 $mmol_c\ dm^{-3}$) occurred by soil acidification causing movement of Mg^{2+} due to the formation of ionic pair with NO_3^-. Liming effects are positive due to increasing the possibility of downward movement of Ca^{2+} and Mg^{2+} accompanying NO_3^- [12].

The exchangeable K^+ contents were higher in Oat-L (1.9 $mmol_c\ dm^{-3}$), Oat and Oat-NL (1.8 $mmol_c\ dm^{-3}$) treatments compared to Oat-N treatment (1.1 mmolc dm^{-3}). Cover crop recycles large amounts of K^+ and liming increases soil K^+ retention in soil CEC [8]. On the other hand, soil acidification caused by N fertilization with NH_4NO_3 decreases the exchangeable K^+ content in the soil surface layer.

Biological soil indicators

Most probable number of diazotrophic bacteria values showed a positive correlation with Oat treatment (Figure 4). Its values were lower in treatments that received N fertilization, i.e. Oat-N and Oat-NL, evidencing that the applied N via NH_4NO_3 had a negative effect on the community of diazotrophic bacteria in soil. Another study also found a low or almost non-existent density of diazotrophic bacteria in soils with high N doses applications [30]. The reasons why this phenomenal is observed are not very clear, but it may include: i) bacterial competition; and ii) plant physiological state alteration by N input affecting N-fixing organisms.

Oat treatment stood out from the other treatments in (SMB-C) analysis (Figure 4 and Table 2). Depending on soil management and crop, C levels of microbial biomass varied. Because soil microbial biomass is higher in undisturbed soils [31], SMB-C is higher in NT systems than in conventional tillage [32].

In general, information on changes in (SMB-C), basal respiration (CO_2-C), metabolic quotient (qCO_2), and microbial quotient (qMIC) tends to provide a better prediction of ecological processes occurring in soil [33]. High basal respiration rates may indicate environmental stress, which justifies the fact that Oat-N treatment had a high basal respiration rate (0.74 µg CO_2-C g^{-1} day^{-1}), which contributes to low C (7.46 µg g^{-1}) (Table 2). These results agree with the report that the C losses are reduced as microbial biomass becomes more stable and efficient [34]. Therefore, less C will be lost as CO_2 by respiration and a good fraction of C will be incorporated into the microbial biomass. As well as basal respiration, metabolic quotient was positive correlated with Oat- N (Figure 4). Metabolic quotient may vary depending on the amount of C present in soil microbiota versus basal respiration [35]. High values of the metabolic quotient reveal that the microbial population is oxidizing C from its cells for maintenance and adaptation to the soil, demonstrating stressful situations [24, 36].

Biolog Ecoplate analysis indicated that amino acids, carboxylic acids, amines, polymers, phenolic compounds, and carbohydrates are potential C sources consumed by the microbiota in soil covered with black oat residues (Figure 4). However, black oat management showed to affect their consumption by the native microbiome: at the Oat-L treatment, there was a higher consumption of all C sources except carbohydrates (Figure 3). Soil management and tillage are decisive factors and have a great influence on the decomposition rates of organic matter provided by microbial activity [37].

Galacturonic acid was shown to have a positive correlation with Oat treatment. This treatment presented the largest microbial mass, which is associated with high C availability. This treatment, showed to be less impacted by imputed amendments inputs and it was not changed by the lime and N applications. The ability to use galacturonic acid as C source might be related to the fact that it constitutes the pectic substances, which form the lamella of upper plant cells [38].

CONCLUSION

Soil microbial community composition was influenced by N fertilization, surface liming and soil cover management. The soil pH influenced the growth and development of microbial community.

The N fertilization under NT system decreased the population of N-fixing bacteria and both liming and N fertilization effects were captured in soil respiration and metabolic quotient measurements, showing them to be good indicators of soil quality.

The effect of adding N on the diversity of diazotrophs community is still not very well understood. Thus, further studies should be carried out in order to identify microbial communities and evaluate the behavior of specific bacterial taxa under such conditions.

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Conflicts of Interest: The authors declare no conflict of interest.

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