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THE EVOLUTION OF MICROBIAL FUNCTIONAL PROFILE INVOLVED IN DECOMPOSITION PROCESSES IN LONG-TERM FERTILIZED EXPERIMENTS

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Abstract

The decomposition of organic matter plays an important role in the release and cycling of nutrients in agricultural soils. The use of 30-day decomposing straw technique, instead of the soil substrate itself, was chosen to highlight the microbial community involved in the decomposition of soil organic matter. EcoPlate (Biolog) readings were used to record daily changes in functional guilds. Dynamics of the Biolog EcoPlate showed the fluctuations within the functional microbial community active in the decomposition process. Both functional guilds presented a differentiated pattern over time. This functional analysis allows a better understanding microbial ability to break down and utilize the available compounds, underline their adaptability and metabolic diversity. The sum of activities recorded was more than 350% increase in NPK-amended variants. AWCD revealed a daily change in the performance of decomposing microbiomes with more than 525% increase in the same variants. Polymers, CH and AM guilds presented increases of over 400%. The interaction of microorganisms with organic matter is visible in the specific dynamics of functional microbiomes.

***Key words**: functional microbiome, long-term impact, microbial dynamics, input-based fluctuations.*

INTRODUCTION

It was shown that soil stores twice as much carbon than the atmosphere (Dasgupta et al., 2024), making it the largest carbon reservoir on Earth (Devi et al., 2024; Juhos et al., 2021). But huge amounts of carbon have been lost from agricultural soil due to the application of an intensive technology requires its return in sequestered forms (Singh & Gupta, 2018). The global annual carbon losses are estimated at between 50% and 70%. (Dasgupta et al., 2024).
The agricultural sector is experiencing significant pressure due to the continuous growth of the world's population (Kopittke et al., 2019), which has led to a constant increasing demand for food. This demographic expansion requires a corresponding increase in agricultural yield, thereby exacerbating existing challenges within the sector (Fróna & Harangi-Rákos, 2019). Consequently, it has become imperative for agricultural practices to evolve and adopt sustainable solutions to effectively address the rising food requirements. The urgency of this situation highlights the need for innovation and adaptability in agricultural strategies to ensure food security for the growing population (Wang et al., 2024).
In addition, chemical fertilizers are an important part of modern agriculture (Kumar et al., 2019) but, in high quantities, they have a negative impact on the soil microorganisms and affect soil health (Pahalvi et al., 2021). These microorganisms work toward breaking down organic matter such as straw and turn it into the nutrients that the plants need to grow. Each type of soil microorganism has its own role, such as breaking down organic matter and supporting plant uptake of nutrients (Hayat et al., 2010; Nabi et al., 2024; Mo et al., 2024).
Currently, agroecosystems show significant potential to increase their organic carbon amount, and the recovery of lost carbon is a key to maintaining ecosystem functions. In this process soil micro-organisms play an important role (Singh & Gupta, 2018). They can use various organic compounds, such as carbohydrates, proteins, lipids and lignin, either individually or synergistically through the production of coupled enzymes during the decomposition process (Mohammadi et al., 2011; Wallenstein et al., 2013; Sigoillot et al.,

2012; Nicolás et al., 2019). Micro-organisms facilitate biogeochemical cycles, converting organic matter into forms accessible to other organisms and contributing to carbon sequestration (Shilky et al., 2023). It was shown that soil organic carbon (SOC) has increased in most cases with the return of plant residues (Castellano et al., 2015; Guenet et al., 2021) such as straw, into the soil for a long time. This technique aims to improve soil health and maintain fertility by increasing organic matter levels through the decomposition of straw. The incorporation of straw into the soil allows to sequester organic carbon which is a necessary part of sustainable agriculture (Fan et al., 2024). Straw are plant residues and represent a valuable agricultural resource that plays an important role in improving soil quality (Jin et al., 2020). They are a rich source of carbon, (Liu et al., 2014) and contain large amounts of nitrogen, phosphorus, potassium and micronutrients, which are important for crop growth (Xin et al., 2024). Their use as soil amendment has a high efficiency in maintaining and increasing the carbon (C) stock in arable soils (Saharan et al., 2024; Ji et al., 2024).

Wheat straw is an abundant and globally valuable resource of biomass with a structure composed of a mixture of natural polymers (Wang et al., 2017). Mainly wheat stalks cell walls contain cellulose, which is characterized by a linear and crystalline structure, providing rigidity and strength. Apart from cellulose, these cell walls also include hemicellulose, a non-cellulosic but complex polymer consisting of ramified chains of various sugars as well as lignin, which is a branched amorphous polymeric compound that contributes to the rigidity and hardness of the wall cell structure, but without crystalline organization. These components give wheat straw valuable physical and chemical properties, making it a useful plant material for multiple applications (Shanmugam et al., 2024). Straw acts as a bulking agent that increasing soil porosity, improving aeration and thereby promoting the efficient decomposition of organic matter in the soil (Ren et al., 2023).

The aim of this study is to evaluate the kinetics of functional microbiomes involved in wheat straw decomposition. The main objective was to monitor microbial activity over 24 h time intervals in 5 days (96 h), based on the activity recorded in incubated Biolog EcoPlates. The secondary objectives were to assess the changes in microbial community diversity and its ability to metabolize the different substrates. There were formulated two additional research questions: i) which carbon sources have shown the greatest effectiveness in stimulating the growth of microorganisms in fertilized versus unfertilized soil? ii) what are the observed trends in the use of carbon sources in Biolog EcoPlate in relation to the type of fertilization applied?

MATERIALS AND METHODS

Experimental design

The study was carried out in Research and Development Station for Agriculture Livada (RDSA Livada) Satu Mare County, Romania.

An experiment in 11 variants with 4 replicates (Table 1) each one was organized on a Luvisol type soil. Brown Luvisol is a common soil type in temperate regions (Lavkulich & Arocena, 2011), having a well-stratified structure and a clay horizon. In this soil, acid reaction can affect fertility and the availability of necessary plant nutrients. To correct this acidity and improve the conditions for plants, amendments like calcium carbonate ($CaCO_3$) are applied, which increase the soil pH. In our study amendments were applied using two different doses, 2.5 tons per hectare and 5 tons per hectare. Each dose of amendment was designed to help neutralize acidity and improve soil structure and fertility.

Table 1. Experimental variants and inputs applied over a long time

Variant	Amendment (t/ha)	Fertilizer (kg/ha)
V1	0	N0P0K0
V2	2.5	N0P0K0
V3	2.5	N100P0K0
V4	2.5	N0P70K0
V5	2.5	N100P70K0
V6	2.5	N100P70K60
V7	5	N0P0K0
V8	5	N100P0K0
V9	5	N0P70K0
V10	5	N100P70K0
V11	5	N100P70K60

For a complete analysis of input effects, these two types of doses of amendments are tested on soils with different fertilization regimes. First, unamended and non-fertilized soils are compared as controls. Second, soil options treated with different types of chemical fertilizers are analyzed: nitrogen (N), phosphorus (P), an NP complex combining the advantages of nitrogen and phosphorus for optimal nutrient balance; and an NPK complex (nitrogen, phosphorus and potassium). Thus, by testing these amendment and fertilization combinations, it is possible to determine how each treatment contributes to improving the properties of brown luvisol, helping to increase agricultural productivity.

Decomposition protocol 30-day interval

The study of straw decomposition in soil involved the use of polypropylene bags (Ng et al., 2024), like tea bags. These bags are made from a fine, permeable material that allows microorganisms and air to penetrate, which are vital for decomposition process, but retains the plant material inside to facilitate observation.

Each bag contained 1 gram of wheat straw, dried at room temperature and ground to aid decomposition in experimental field. A total of 44 bags were placed in the soil, one in each of the 44 experimental plots at ARDS Livada, Satu Mare County.

Thus, 11 different variants were tested to provide a better understanding of the factors affecting the decomposition of wheat straw in the soil (Gheorghiţă et al., 2024).

The plant material decomposition experiment was conducted over a 30-day period, at a depth of 5-10 cm below the soil surface, near the root zone of the plants. This depth and positioning near the roots allowed direct contact with microorganisms around the roots, facilitating the observation of the decomposition process under natural conditions, directly influenced by the plant rhizosphere. The rhizosphere is known for the multitude of microorganisms that actively contribute to the decomposition of organic matter and to the nutrient cycle in the agricultural soil.

To maintain soil fertility and prevent nutrient depletion, experimental plots are cultivated using a crop rotation method. This involves changing the type of crop grown on the same plot each year. Through crop rotation, the soil maintains its nutritional diversity and long-term health, as each plant has different requirements and contributes its own organic substances to the soil. Thus, the experiment was conducted on plots with consistently fertilized soil, allowing for more precise monitoring of the decomposition process and the interaction between plant residues and the specific rhizosphere microbiome.

The protocol used for Biolog EcoPlate underwent slight modifications in terms of the material used. Instead of soil, which was traditionally used according to the method described by Garland & Mills (1991) and Garland (1997), decomposing wheat straw was used. This material originates from experiments carried out at ARDS Livada, Satu Mare County, where the bags were inoculated into the soil and left to decompose for 30 days. In the preparation stage, to realize the serial dilution, the decomposed plant material was processed until a dilution of 10^{-4} was obtained (Stoian et al., 2022). In addition, each well of the Biolog EcoPlate was inoculated with 120 µl of this dilution, thus adapting the method to the new experimental conditions. After inoculation, the plates were incubated at controlled temperature for the growth of microorganisms from the decomposed plant biomass. During incubation of these plates, microbial activity was monitored by measuring absorption, indicating utilization of carbon sources (Garland & Mills, 1991; Garland, 1997).

The substrates in Biolog EcoPlates represent different organic compounds - carbohydrates (CH), polymers (P), carboxylic acids (CX), amino acids (AA), and amines/amides (AM) that microorganisms metabolize for energy and growth, based on the method proposed by Stoian et al. (2022) and described as DEMSA concept. In this approach all chemical similar substrates are considered a guild, the term being used to describe an association of microbial groups with similar activities.

Analysis of microbial functional activity - in Biolog EcoPlate

The metabolic activity of soil microbial community was evaluated collectively using the Biolog EcoPlate, which measures their ability to utilize a variety of carbon sources.

The differences between organic and inorganic inputs (fertilized and unfertilized soils, as well as the effect of the amounts of amendments used), were analyzed by comparing the percentages of different substrates use (as functional guilds) in 11 variants. The microbial evolution was monitored over periods of 48, 72, and 96 hours.

Measurements were made every 24 hours, starting 48 hours after inoculation and continuing up to 96 hours. The measurements were carried out using a spectrophotometer. With this device, the intensity of light absorption at different wavelengths was analyzed, as it provides precise information about the microorganisms in the analyzed substrates. This tool allows monitoring of the dynamics of microorganisms and other relevant factors for soil quality assessment, and the results help to compare the influence of different types of inputs on soil microbiome growth.

Data analysis

All data were analyzed in RStudio (version 2024.12.0; R Core Team, 2024). The package “agricolae” (de Mendiburu, 2023) was used to explore the differences between treatments, based on ANOVA and Least Significant Difference (LSD) test. The Nonmetric Multidimensional Scaling (NMDS) Ordination metaMDS in “vegan” package, (Oksanen et al., 2022; Corcoz et al., 2022) was performed to assess the interaction between functional guilds and treatments, and to explore the stability of functional communities within and between treatments.

RESULTS AND DISCUSSIONS

Changing the Biolog EcoPlate relevance for decomposition purposes

The Biolog EcoPlates analysis provides important information about the ability of the functional diversity of soil microorganisms (Jezierska-Tys et al., 2020) to metabolize different substrates present in the soil. The technique can be applied in long-term fertilized soils to assess the ability of soil microbial communities to use a set of substrates as a complex of species with similar metabolic activities. This analysis is important to better understand how microorganisms in different soils break down and use available compounds, highlighting their adaptability and metabolic diversity.

Based on the values after 48 hours of incubation the control variant (V1), showed the lowest values for all the functional guilds in Biolog EcoPlate profile. In particular, the lowest value was recorded for carboxylic acids (215%), followed by the amino acid guild, which had an increase of only 6% (221%), compared to CX, contributing to the lowest total value of activities (SUM). After the incubation period AWCD (Average Well Color Development) reaches 283%, indicating a high level of microbial activity. This value was based on the color intensity changes in EcoPlates, which reflected the overall metabolic activity of the microbial community in response to the available substrates. The results showed that a natural, unfertilized and un-amended soil does not significantly improve its quality or fertility in time, highlighting the limitations in the metabolic potential of the microbiota where the inputs are absent (Table 2).

After 48 hours the amines microbiome had the highest value of all the biochemical categories analyzed, reaching 458% in V3. This variant was fertilized with N and moderately amended with 2.5 t/ha of calcium carbonate. The carbohydrate functional microbiome, showed a 453% increase in activity value in the V10 variant, based on both amendment and complex application of NP (the position occupied by the polymer guild is higher in V4, which showed a 14% percentage reduction compared to the values recorded by the previous association.

For the basal respiration (Wat), the lowest value of 119%, representing almost half of the lowest levels observed in the other studied microbiomes, was recorded in V11. This variant was fertilized with NPK and strongly amended. The deficiency of carbon sources, even in the presence of nutrient inputs, can generate significant differences in the metabolic activity of soil microorganisms.

For a hierarchical analysis of amendment and input influence on microbial functional guilds, the values observed were classified separately for each functional type of microbiome. The basal community (Wat) showed increases due to the application of the medium levels of amendment, while the sum of the entire activity and AWCD showed fluctuations and reached higher values in highly amended variants that lack of fertilizer application. Carboxylic acid guild presented the maximum values in highly amended variants.

Table 2. Evolution of the functional guilds at 48, 72 and 96 hours of incubation as shaped by long-term applied inputs

	V	Wat %	Sum %	AWCD %	P %	CH %	CX %	AA %	AM %
48 h	1	154.86±15.67^{b}	240.67±35.12^{b}	283.55±52.41^{d}	280.48±44.60^{b}	279.65±51.22^{c}	215.07±27.56^{b}	221.73±24.68^{c}	249.77±46.48^{b}
	2	130.91±12.40^{b}	294.65±15.30^{ab}	346.67±27.03^{bcd}	344.59±11.06^{ab}	322.37±25.01^{bc}	280.09±21.39^{ab}	261.11±16.51^{abc}	295.41±38.55^{ab}
	3	236.70±76.91^{ab}	321.91±28.99^{a}	352.78±27.54^{bcd}	355.50±31.77^{ab}	317.32±32.38^{bc}	319.22±40.21^{a}	320.55±31.96^{ab}	458.11±40.18^{a}
	4	322.36±68.85^{a}	305.36±24.77^{ab}	299.13±21.49^{cd}	439.21±44.60^{a}	330.07±36.56^{bc}	285.08±31.65^{ab}	240.04±10.52^{bc}	329.49±32.21^{ab}
	5	203.05±43.37^{ab}	327.87±16.29^{a}	392.10±45.41^{abcd}	409.50±45.70^{a}	355.45±4.515^{abc}	302.02±38.69^{ab}	291.73±9.816^{abc}	355.63±43.53^{ab}
	6	250.32±139.23^{ab}	331.41±33.29^{a}	355.33±34.32^{bcd}	430.62±25.12^{a}	324.16±36.31^{bc}	311.76±33.49^{a}	347.48±43.57^{a}	326.08±27.85^{ab}
	7	138.46±27.23^{b}	363.92±31.90^{a}	525.36±119.31^{a}	386.48±37.00^{a}	400.62±38.67^{ab}	371.99±66.16^{a}	321.88±38.30^{ab}	389.28±59.30^{ab}
	8	160.16±51.36^{b}	340.96±20.51^{a}	421.22±31.67^{abcd}	385.11±29.28^{a}	391.08±20.10^{ab}	311.50±18.82^{a}	281.19±19.76^{abc}	424.47±68.08^{a}
	9	141.81±31.89^{b}	339.44±39.00^{a}	426.62±58.96^{abc}	404.20±27.75^{a}	388.98±39.80^{ab}	317.67±36.42^{a}	282.48±55.16^{abc}	302.33±40.62^{ab}
	10	129.70±20.31^{b}	343.72±16.58^{a}	434.89±38.16^{abc}	324.98±38.55^{ab}	453.16±36.41^{a}	304.51±28.22^{ab}	330.76±16.52^{a}	310.32±57.62^{ab}
	11	119.59±18.24^{b}	357.20±6.842^{a}	476.15±24.68^{ab}	421.84±13.86^{a}	364.15±22.09^{abc}	356.43±3.402^{a}	310.65±15.04^{ab}	398.49±104.19^{a}
72 h	1	174.07±22.50^{a}	148.55±2.42^{a}	145.92±3.79^{a}	167.92±13.59^{ab}	145.38±4.27^{ab}	144.24±1.82^{a}	157.94±5.81^{abc}	134.46±6.19^{abc}
	2	170.33±21.37^{a}	131.43±1.08^{ab}	126.81±3.19^{a}	135.75±3.22^{bc}	135.97±2.47^{ab}	126.32±2.70^{ab}	137.38±2.22^{bc}	109.10±3.20^{c}
	3	216.70±53.80^{a}	147.56±6.28^{a}	143.13±18.64^{a}	176.78±17.27^{a}	148.58±9.29^{ab}	132.34±4.06^{ab}	164.75±9.23^{ab}	139.28±13.97^{abc}
	4	221.05±113.90^{a}	143.80±2.54^{a}	140.74±18.09^{a}	139.00±3.51^{bc}	148.04±6.04^{ab}	131.07±3.41^{ab}	165.86±5.88^{ab}	149.60±13.60^{a}
	5	214.56±63.15^{a}	141.77±5.44^{ab}	130.52±6.34^{a}	135.25±1.39^{c}	143.22±9.60^{ab}	130.56±3.68^{ab}	166.33±7.08^{ab}	129.20±7.76^{abc}
	6	110.69±14.65^{a}	122.25±4.82^{b}	131.22±8.11^{a}	137.96±6.13^{bc}	124.36±2.95^{b}	114.80±5.09^{b}	127.47±8.38^{c}	110.73±12.26^{bc}
	7	199.63±46.33^{a}	143.31±14.67^{a}	131.07±9.16^{a}	154.31±16.49^{abc}	140.46±11.72^{ab}	142.65±17.50^{a}	152.33±16.89^{abc}	116.96±9.23^{abc}
	8	213.99±95.76^{a}	151.17±15.46^{a}	144.58±9.95^{a}	167.52±15.99^{abc}	149.60±20.89^{ab}	140.31±13.40^{a}	174.50±23.90^{a}	148.78±29.93^{ab}
	9	128.09±4.76^{a}	148.86±3.60^{a}	151.86±4.41^{a}	157.61±12.07^{abc}	148.92±8.04^{ab}	144.11±2.75^{a}	160.38±13.12^{ab}	127.44±13.99^{abc}
	10	154.60±19.15^{a}	140.81±1.15^{ab}	138.28±3.31^{a}	162.18±13.93^{abc}	140.49±3.26^{ab}	133.73±2.13^{ab}	153.23±6.96^{abc}	126.42±9.64^{abc}
	11	187.97±50.31^{a}	146.29±1.72^{a}	139.69±6.01^{a}	150.68±2.42^{abc}	152.96±5.93^{a}	140.56±2.04^{a}	156.08±6.05^{abc}	114.49±5.53^{abc}
96 h	1	131.75±8.22^{ab}	123.13±1.08^{ab}	121.32±1.40^{abc}	127.56±2.29^{ab}	121.67±1.88^{ab}	125.12±1.11^{a}	124.75±3.46^{a}	108.63±2.87^{ab}
	2	100.47±6.81^{b}	113.67±1.85^{bc}	115.87±2.25^{bc}	121.53±1.99^{b}	109.12±3.66^{bc}	113.48±1.19^{bc}	118.um19±3.78^{ab}	111.01±2.61^{a}
	3	113.50±9.26^{ab}	116.50±1.11^{abc}	118.35±4.02^{bc}	125.63±2.99^{ab}	115.48±2.53^{abc}	114.18±0.59^{bc}	120.18±2.68^{ab}	109.22±9.53^{ab}
	4	86.30±2.93^{b}	118.09±1.40^{abc}	135.77±9.72^{a}	128.23±4.11^{ab}	119.53±3.21^{abc}	114.67±1.16^{bc}	115.42±3.03^{ab}	115.85±4.27^{a}
	5	101.83±7.20^{b}	119.41±0.38^{ab}	127.28±4.94^{ab}	126.08±1.45^{ab}	115.26±1.46^{abc}	118.95±0.78^{ab}	123.32±2.86^{ab}	120.13±2.14^{a}
	6	141.93±15.33^{ab}	119.15±1.90^{ab}	117.18±3.07^{bc}	126.92±1.55^{ab}	114.34±2.82^{abc}	119.94±1.13^{ab}	123.92±3.83^{a}	111.50±4.04^{a}
	7	169.49±43.44^{a}	118.31±2.34^{ab}	116.16±6.10^{bc}	128.98±7.42^{ab}	120.35±4.88^{ab}	112.97±0.93^{bc}	119.85±2.62^{ab}	118.12±8.72^{a}
	8	104.08±30.66^{b}	107.95±8.06^{c}	114.20±5.86^{bc}	131.75±11.49^{ab}	105.06±10.74^{c}	105.00±6.42^{c}	111.91±7.03^{b}	89.18±17.25^{b}
	9	169.28±24.44^{a}	113.93±6.92^{bc}	109.72±8.18^{c}	127.19±3.07^{ab}	107.19±9.23^{bc}	114.52±8.38^{bc}	120.73±4.46^{ab}	116.80±6.19^{a}
	10	137.60±14.30^{ab}	122.76±1.79^{ab}	121.50±1.83^{abc}	134.59±3.28^{ab}	120.55±1.28^{ab}	122.04±3.12^{ab}	123.07±4.19^{ab}	120.64±4.75^{a}
	11	126.35±11.95^{ab}	125.47±2.40^{a}	127.88±4.16^{ab}	136.87±4.27^{a}	126.64±3.42^{a}	120.27±2.71^{ab}	125.90±4.17^{a}	114.89±5.19^{a}

Note: Means±s.e. followed by different letters indicate significant differences at $p<0.05$ based on LSD post-hoc test. Legend: V1 - control, V2-V6 - Am1, V7-V11 - Am 2, V2/V7 - 0N, V3/V8 - N100, V4/V9 - P70, V5/V10 - N100P70, V6/V11 - N100P70K60; Am1 - 2.5 T/ calcium carbonate /ha, Am2 - 5 T/ calcium carbonate /ha. Wat – basal respiration; Sum – total metabolic activity; AWCD – average well color development; P – polymers; CH – carbohydrates; CX – carboxylic acids; AA – amino acids; AM – amines/amides.

After 72 hours of incubation, the dynamics in functional microbiomes change quickly, and the lowest values are mainly seen in three of the five guilds analyzed (Table 2). In V6, the carbohydrate association reached 124%, the carboxylic acid microbiome 114%, and the amino acid microbiome 127%, due to complex treatments.

Basal respiration also recorded the lowest value of all guilds at 110%. In this variant, the combined value exceeds the basal respiration value observed at 72 hours by 12 percent.

As for the highest values, they varied between the different guild variants studied, reaching a maximum in the case of basal respiration in variant 4 (V4), at 221%. This variant was medium-amended and fertilized with phosphorus. The polymer microbiome shows the highest metabolic activity in variant 3 (V3), which was fertilized with mineral nitrogen and amended to a medium level, like the treatment applied in the previous case. A special case is observed within the carboxylic acid microbial association, where the same maximum value of 144% was recorded in two of the 11 variants analyzed: in the control variant (V1), but also in variant 9, which was fertilized with phosphorus and intensively amended.

The 72-hour analysis shows fluctuations in the basal respiration values, with similar results for the two doses of amendments applied (2.5 t/ha and 5 t/ha), with no significant differences between them. The total activity of guilds fluctuated, reaching a minimum of 122% in V6 (NPK fertilized and moderately amended) and a maximum of 148% in native soil (V1) and in V9 (Am2+P70). Within the guilds analyzed, the highest percentage value was recorded in variant V3 (Am1+N100) with 176% in the polymer microbiome, followed by 174% in variant V8 (Am2+N100) in the amino acid. These findings highlight the predominant role of nitrogen to support an active microbiome, whether the amendment intensity varies from moderate to high.

When read after a 96-hour interval, the amine values in V5 and V10 are similar, representing 120% of the NP-fertilized variant in both cases (Table 1.). The difference between these variants is determined by the amount of amendment used, which goes from a moderate to an intense level. However, these variations don't generate significant differences between the observed values.

The highest value is found in the WAT group, associated with basal respiration. Unlike the other variants analyzed, the absence of carbon sources contributing to these higher values. In this context, the percentage recorded 169% in the V7 and V9 variants, which represents a notable difference of 49% compared to the amine microbiome.

Variant V8 shows the lowest values recorded for four of the five guilds analyzed. This variant, characterized by intensive amendment and supplementation with nitrogen fertilizer, shows values ranging from 89% for the amine's guild to 105% for carbohydrates and carboxylic acids. On the other hand, in three of the five guilds studied, the highest values were observed in V11, characterized by intensive amendment and fertilization with an NPK complex. The polymer microbiome recorded a maximum of 136%, followed by an easy decrease of 10 percent in the case of carbohydrates. The amino acid microbiome showed 11 percent lower values compared to polymers, thus showing a distinct distribution of values according to treatment.

Basal respiration measurements for 96 hours showed higher values for Am2 compared to Am1, although there were small fluctuations. Percentage values of SUM varied from a minimum in V8 of 107% (Am2+N100) to a maximum of 125% in V11 (Am2+NPK). Intensive amendment had mixed effects, both reducing and increasing values, while nitrogen applied alone produced minor decreases and NPK complex increased microbial metabolism. All the measured results in the guild, values lower than 100% were detected. In V8 (Am2+N100), 89% were associated with the amine microbiome, indicating a possible inhibitory effect of the treatment.

Non-metric multidimensional scaling (NMDS) analysis has shown that the functionality of microorganisms involved in soil decomposition varies with the type of fertilization applied whether organic or inorganic over a long period of time as well as with the natural soil condition and agricultural land use.

The microbial community at 48 h shows fluctuations between untreated samples (Figure 1), without requiring specific inputs for carbohydrate and carboxylic acid vectors. A precise transition to activities associated with basal respiration (WAT) is observed, as well as the functioning of the amine guild (AM). The guilds, including CH (carbohydrates), CX (carboxylic acids), AM (amines) and WAT (basal respiration), guilds demonstrate significant activity even in the absence of organic amendment or mineral fertilizer interventions. These findings suggest that undisturbed soil has the capacity to support a significant diversity of the microbial community.

The dominant microbiomes are associated with unfertilized soil, but the addition of organic inputs influences the specific accumulation of the microbial community. The intensity of these effects varies from moderate to pronounced and is particularly evident in soils under long-term fertilization. The intensive amendment with 5 t/ha of organic material, carried out without the application of mineral fertilizers induces a distinct change in the microbial community structure. This is visible in the position parallel to the 2-axis near carboxylic acid (CX) and carbohydrate (CH) vectors, without interacting directly with them.

In contrast, the NPK fertilizer complex, combined with an intensive amendment of 5 t/ha of carbonate, shows a marked effect on the microbial community, interacting directly with carbohydrates (CH), carboxylic acids (CX), amino acids (AA) and total microbial activities (SUM). This intersection indicates a significant influence of these factors on microbial functional guilds, suggesting that the simultaneous application of mineral fertilizers and organic amendments not only supports, but also actively shapes the structure and functioning of the soil microbial community. This highlights the importance of environmental conditions on microbial activity (Conant et al., 2011), and the continuous adaptation of the metabolic pathways used to decompose different organic materials. The relation with the environmental conditions is visible in the efficiency of the decomposition processes and in the composition and diversity of the microbial communities involved in this process. Therefore, studying these changes in microbial communities is a step forward to understanding the mechanism behind decomposition process (Chen et al., 2024). The combination of these inputs creates a favorable environment for enhancing microbial diversity and activity by stimulating metabolic processes associated with these vectors.

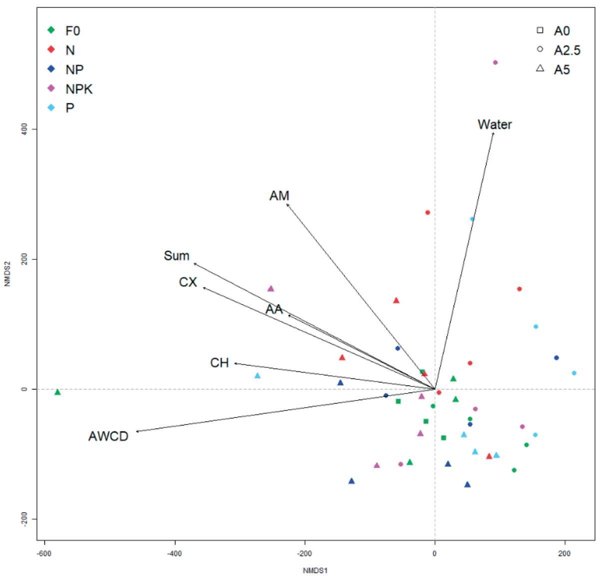

Figure 1. NMDS analysis of functional microbiomes after 48 h

In the 72-hour microbial diversity analysis (Figure 2), the distribution of the NP complex is characterized by a compact arrangement, indicating a uniform microbial diversity without major differences. In addition to the use of NP fertilizers, the presence of organic amendments is also observed, whose intensity varies, like the previous scenario, from moderate to intensive. In this context, two amendment points intersect the vectors associated with basal respiration (WAT) and carbohydrates (CH). The analysis reveals important differences between intensively and moderately amended points, suggesting that intensive amendment contributes significantly to sustaining higher microbial diversity in soils subjected to long-term fertilization. This phenomenon highlights the positive impact of intensive amendment practices on structure and functionality of microbial community. Extensive research has shown that the populations, structures and metabolic rates of these microorganisms react differently to climate and technology changes. The diversity of microbial communities (bacterial and fungal) is also modified during the decomposition process (Das et al., 2007; McGuire & Treseder, 2010).

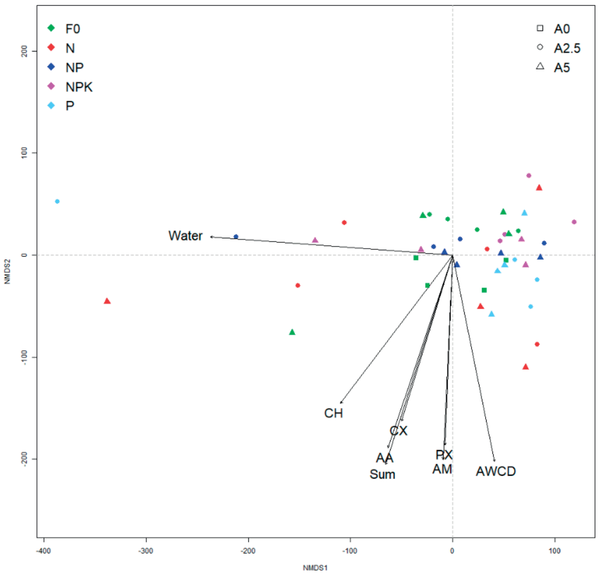

Figure 2. NMDS analysis of functional microbiomes after 72 h

At 72 hours, the lowest values are observed in variant 6, which is medium amended with 2.5 t/ha of calcium carbonate and fertilized with an NPK fertilizer complex. The values observed in this variant show a slight variation, ranging between 114, 124 and 127 percent, suggesting that the microbial diversity is relatively uniform, with no significant differences between the measured values.

The absence of mineral fertilizers results in a homogeneous microbiome with minimal functional differences. In this context, the absence of organic amendment correlates with the vectors of basal respiration (WAT), carbohydrates (CH), amines (AM), polymers (PX), and AWCD. These intersections suggest a less diversified microbial structure, character-rized by restricted metabolic activity in the absence of amendment or mineral fertilizer inputs.

At the second reading, there is a clear connection and a significant functional similarity between the amine and polymer guilds (it was completely absent in the first NMDS). These guilds appear to be closely related to each other or react directly in the presence of unfertilized and unamended soil when the soil is in its natural state.

On the other hand, when phosphorus is added and intensive amendments are applied, a synergistic effect is created, which means that the diversity of micro-organisms increases considerably. This shows how soil interventions can modify and influence the relationships between microorganisms, sometimes amplifying their diversity. This suggests that

fertilization and amendment interventions can positively influence microbial diversity, especially in interaction with these functional guilds.
Nitrogen, used as a mineral fertilizer, interacts with functional guilds of basal respiration (WAT), carbohydrates (CH), carboxylic acids (CX), amines (AM), and AWCD. The N-associated microbial community dominates in this situation, being supported by moderate amendment, both in terms of basal respiration and carbohydrates, and in the assessment of total microbial diversity (AWCD). In contrast, intensive amendment with 5 t/ha of calcium carbonate exerts a significant influence on the microbial community, particularly affecting the guilds associated with carboxylic acids and amines, suggesting a more pronounced functional diversification in the presence of this intense amendment.
Analysis of the three carbon sources evaluated - carbohydrates, carboxylic acids and amino acids - shows that they show roughly similar values. However, the highest percentage was recorded in variant 8, in the amino acids microbiome, with 174%. This result is associated with the application of a high level of amendment, consisting of 5 t/ha of calcium carbonate, together with mineral nitrogen fertilization.
A positive relation can be observed between the intense level of amendment and fertilization and the increase in the values recorded in variant 8, particularly in the amino acid guild. In fact, variant 6, with a lower level of amendment and fertilization, showed lower values and a relatively constant microbial diversity over time. This contrast underlines the influence of fertilization and amendment regime on the measured values and thus on microbial diversity.
The microbial diversity assessed at a 96-hour time interval (Figure 3) shows a relatively uniform distribution of the focus points studied under long-term fertilization, indicating stability and equilibrium of the microbial communities under most of the conditions analyzed. However, there is a significant exception in the use of nitrogen fertilizer in combination with amendment at an intensive level, where notable differences in function are observed. These differences are evident both in comparison to the phosphorus-treated samples and to the native sample, which received no mineral or organic input, highlighting the distinct effects of fertilization on soil microbiota diversity and function.

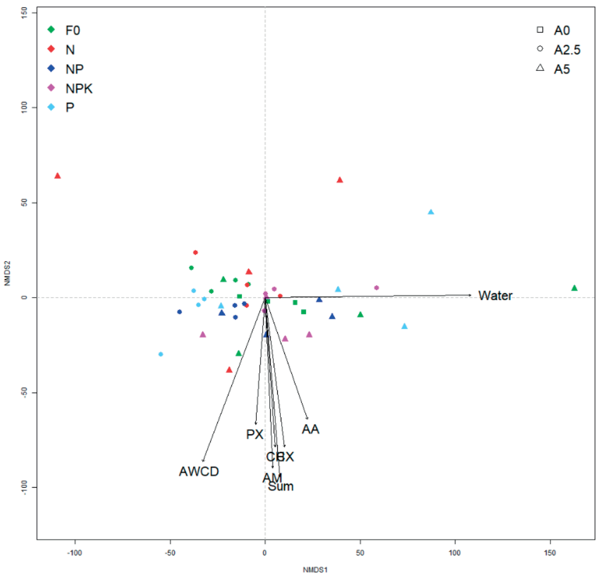

Figure 3. NMDS analysis of functional microbiomes after 96 h

Returning wheat straw back to agriculture can really help boost the soil micro-ecosystem and make this substrate more fertile (Li et al., 2024; Altieri, 2005). This method helps maintain the right moisture and temperature in the soil, which is great for the organisms that live there. As straw breaks down, it provides important nutrients for the soil (Jin et al., 2020), and by adding straw back into the ground, farmers can improve natural fertility of their fields and help keep the soil ecosystem healthy (ZHANG et al., 2024).

The unfertilized variant, without the presence of amendments or with the addition of organic inputs such as calcium carbonate, shows a relatively uniform distribution of the points of interest on the vectors analyzed. In this context, the microbial community associated with undisturbed soil overlaps with the various functional measures, including basal respiration, amino acids, carbohydrates, amines and AWCD (total microbial community activity). Instead, intensive amendment in the absence of mineral fertilizer addition intersects with the same functional measures, such as WAT, AM, CH and AWCD, indicating a similar influence on functional microbial diversity. It is important to mention that amendment at a medium level is not detected in the studied guilds, being outside of them, and that it brings just minor changes in the functional microbiome of the experimental soil, emphasizing a low influence on microbial activity and structure in this condition.

CONCLUSIONS

Measurements at regular intervals showed a progressive decrease in values, with significant differences between 48, 72 and 96h. The microbial community involved in the decomposition of wheat straw acted rapidly, reaching a peak at 48h (458% in V3 - amines), followed by a considerable reduction at 72h (174% in V8 - amino acids) and at 96h (136% in V11 - polymers).

This development suggests the continuous use of available resources, and their depletion over time, on a general trend of decreasing values over time.

The 48-hour analysis showed a major increase in microbial activity, in the presence of inputs, determining an increase in the following functional guilds: amines, carbohydrates and polymers, which recorded values of over 400%.

After 72 hours, a change in the microbial guild dynamics was observed for amines polymer and carbohydrate, along with carboxylic acids and amino acid, all of them showing lower values.

Basal respiration is at its maximum level after 96 hours, indicating that the soil microbiome is performing at its optimum level in its natural environment under long-term fertilization.

The microbial community responds rapidly to changes in the environment by utilizing available nutrients and energy resources. As these are consumed, the micro-organisms adjust their metabolic activity, which can lead to a gradual decrease in biological processes dependent on existing carbon sources.

Based on NMDS analysis, the absence of polymer guilds at 48 and 96 hours suggests that other metabolic guilds are dominant at these time intervals, suppressing polymer utilization.

The NMDS projection at 72 hours showed the polymer guild intersection with the amine guild, indicating a similar microbial activity between the two guilds or even an overlap of the microbial communities responsible for the degradation of these compounds.

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