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KINETICS OF THE MICROBIAL FUNCTIONAL PROFILE INVOLVED IN DECOMPOSITION SHAPPED BY LONG-TERM APPLICATION OF INPUTES

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Abstract

Fertilization methods have the capacity to modify both soil fertility and biological characteristics, consequently affecting the ecological functions of the soil. Straws are a hard crop residue to decompose, and stimulate the appearance of a specific functional microbiome. The microbial functional profile is correlated with the application of calcium carbonate (CaCO3) and fertilizers containing nitrogen, phosphorus, potassium (NPK). The present study aims to analyze the effect of long-term application of inorganic fertilizers and liming on the functional soil microbial communities. A modified EcoPlate method was used to incorporates the utilization of straw subjected to a 30-day incubation period in the soil at the Livada Agricultural Research and Development Station. In the aftermath of the Ecoplate experiment, discernible alterations in substrate solubilization rates have been noted across diverse soil compositions, spanning from untreated soil to those enriched with nitrogen, phosphorus, and an NPK complex. The results provide information on important functional soil microbial assemblages influenced by fertilizer application and the detection of the most active functional gropus associated with straw decomposition process.

Key words*: EcoPlate, microbial communities, fertilization, long-term field experiment.*

INTRODUCTION

Soil microbial communities are essential components of agricultural ecosystems (Singh et al., 2011), having a significant impact not only on fundamental soil processes, but also actively contributing to improving soil fertility and increasing agricultural productivity (Itelima et al., 2018). Their ecological function can be evidenced by their diversity, carbon utilization and community composition. (Wu et al., 2021).

Agriculture has always been an effective solution to meet the food needs of the global population. (Ortiz and Sansinenea, 2022). With today's rapidly growing population, it is crucial that agriculture is able to meet this increased demand. It is therefore also vital to increase both crop productivity and quality.

Agricultural soil is hosts for a variety of microbial species (Herrera and Lebeis, 2016) sensitive to environmental changes. Changes in nutrients and pH after fertilisation significantly affect soil microorganisms (Geisseler and Scow, 2014). For example, organic fertilisers encourage the growth of microorganisms (Marinari et al., 2000), while excess phosphorus reduces their diversity (Wang et al., 2018; Ma et al., 2022). Soil pH influences the composition of bacteria, as the proportion of bacteria varies with the acidity level.

Soil microorganisms are essential to nutrient cycling and ecological processes (Hopkins, and Dungait, 2010), influencing soil health, plant nutrient availability and ecosystem stability. During their interactions, these micro-organisms can influence their community composition, organic matter decomposition rates (Eskelinen et al., 2009), nitrogen fixation (Li et al., 2015) and other key processes affecting the overall functioning of the soil. Thus, understanding these interactions and their long-term effects is essential for the sustainable management of agricultural land and natural ecosystems.

Soil microorganisms have a significant contribution in the ecosystem, playing a key role in biogeochemical processes (Smith et al., 2015; Corcoz et al., 2022a; Oneț et al., 2024) that influence soil health and fertility. These organisms are involved in the transformation and recycling of nutrients in a complex and

dynamic way. They break down organic matter, facilitating the release of nutrients such as nitrogen, phosphorus, and potassium from organic material into the soil (Li et al., 2022).

Chemical fertilizers are synthetic compounds with a high concentration of essential nutrients crucial for plant growth (Kumar et al., 2019). In simpler terms, these are human-made substances that provide plants with the necessary nutrients. While the use of pesticides and fertilizers carries inherent risks in agriculture, they remain indispensable for global food security (Nieder et al., 2018).

Primary nutrients are essential elements required by plants in sufficient quantities for their growth and development, including nitrogen (N), phosphorus (P), and potassium (K) (Shrivastav et al., 2020).

Nitrogen (N) is essential for chlorophyll production, promoting plant growth (Fageria, 2001).

Phosphorus (P) is crucial for energy transfer, root growth, and fruiting in plants (Malhotra et al. 2018; Vance et al., 2003).

Potassium (K) activates enzymes, supports respiration and photosynthesis, and aids water movement, flowering, and fruiting (Pathak et al., 2020).

When inputs such as nitrogen (N), phosphorus (P), combined (NP) or complete (NPK) fertilisation, along with different types of amendment, from moderate to intense, influence the microbial community (Li et al., 2018), these microorganisms are involved in complex interactions, both long and short term.

The incorporation of inputs, such as fertilizers and organic or mineral amendments (Francioli et al., 2016), plays an essential role in the assembly and evolution of this microbial community. Nitrogen fertilisation can stimulate the growth of nitrogen-fixing bacteria, which convert atmospheric nitrogen into plant-usable forms, helping to improve soil fertility (Soumare et al., 2020; Saha et al., 2017). On the other hand, the addition of large amounts of inputs can lead to imbalances in the microbial community, favoring some species over others and potentially having negative consequences for soil health and crop production.

In general, the ability of the microbial community to break down organic matter is essential for nutrient cycling and maintaining soil fertility (Kong et al., 2011). Soil microorganisms are involved in processes such as degradation of organic matter, nutrient mineralization and nitrogen fixation (Powlson et al., 2001), thus contributing to the availability of essential plant nutrients. Therefore, it is crucial to understand how inputs influence the structure and functioning of the microbial community (Zhang et al., 2012) in order to develop sustainable agricultural practices and promote efficient soil and resource management.

The microbial community is a complex system of interactions that can be characterized by both positive and negative influences, depending on the nature of the inputs and their ability to break down organic matter. This community is a dynamic entity, and its structure and functioning are significantly shaped by inputs to the agricultural soil.

The aim of this research is to investigate the complex interactions that occur between plant biomass, represented by wheat straw, the microorganisms involved in its decomposition process and the buffer material, in this case the inoculated litterbag. Here, instead of focusing directly on the soil, we focus on the fine soil particles persisting on the walls of the litterbag, which have been in direct contact with the plant biomass in the decomposition process over a period of 30 days. The aim is to gain a detailed understanding of how these elements interact and to highlight their impact on microbial community dynamics and structure, thus providing a deeper insight into the decomposition cycle of plant biomass.

The specific objectives of the research are:

1. Evaluating the diversity and activity of microorganisms present in the buffer material (inoculated litterbags) using Biolog EcoPlates to analyze changes in the functional microbial community during the decomposition process of plant biomass.
2. Assessing the impact of different types of agricultural inputs (fertilizers and amendments) on the diversity and composition of the soil functional microbial community.
3. Analyzing the interactions between agricultural inputs and the functional microbial community.

MATERIALS AND METHODS

The experiments using the litterbag method were conducted in long-term experiments, on a brown luvic soil, from Livada Agricultural Research and Development Station, in Satu Mare county, Romania (Kurtinecz et al., 2023). These experiments focused primarily on the 30-day decomposition process of wheat straw, to investigate the dynamics of functional microbiomes involved in the breakdown of organic matter under different applied treatments. The chopped straw was placed in litterbags and inserted into plots at a depth of 5-10 cm. After 30 days in soil conditions, the litterbags (without the organic matter that reside in interior) were supposed to a serial dilution in distilled water. The 10^{-4} dilution was used to inoculate the Biolog EcoPlates (Stoian et al., 2022). The procedure allows the analysis of microbiomes that is present on the litterbag walls, and offer information on the functional profile of microorganism that are at the border between the soil and biomass contained by litterbags. EcoPlates were incubated at 20°C for 96 hours. Readings were performed with a Spectrophotometer at 590 nm, every 24 h from the total of 96 hours, when a plateau in absorbance was observed.

Microplates developed in the late 1980s consisted of plates equipped with 96 individual wells. Each well contained various carbon sources and a redox dye called tetrazolium violet. This dye underwent a colour change to purple when microorganisms from the soil, inoculated onto the plate, utilized these carbon sources (Garland, 1996). A subsequent innovation was the EcoPlate, an improved version of the plate designed for the analysis of microbial communities and ecological research (Feigl et al., 2017). The EcoPlate comprises three replicates for each of the 31 carbon sources, thus providing the ability to monitor metabolic activity and study the development of microorganisms under plate conditions.

For this experiment, a specific buffer medium was used, namely a litterbag, which was inoculated into the agricultural soil together with the plant biomass, in this case, straw, for a period of 30 days. Our aim was to reinterpret and adapt the Biolog EcoPlate method, a recognized technique in the field of microbial ecology, by conducting an experiment where conventional soil is replaced with this buffer material, specifically the inoculated litterbag together with the plant biomass, so that it is in direct contact with both soil particles and plant biomass. Through the Biolog EcoPlate method, we aimed to assess the diversity and activity of microorganisms present in soil subjected to long-term fertilization, in the presence of different types of carbon supplied by litterbag in the EcoPlate. This approach allows us to better understand how microbial communities react to changes brought about by long-term fertilization and to analyse the impact of their diversity on different types of carbon, thus contributing to the understanding of microbial ecology in the context of sustainable agricultural practices.

Litterbags were placed in 11 variants of the ARDS Livada long-term experiment, each numbered from V1 to V11 (Table 1). Variant V1 served as the control sample, and variants V2-V6 involved moderate amendments (Am1) - 2.5 t ha^{-1} every 5 years. The second amendment (Am2) comprised 5 t ha^{-1} variants V7-V11, which are characterized by higher dose of amendments. In both cases, amendment consisted of the use of calcium carbonate to adjust soil acidity, together with mineral fertilizers such as N, P, NP and NPK associated with each of the V2-V6 and V7-V11 variants.

The data used in the assessment of litterbag associated functional microbiomes consists of the percentages recorded between 24-48 h (R3), 48-72 h (R4) and 72-96 h (R5), which represent the increases in the dynamics of functional microbiomes as a reaction to the substrates in EcoPlates.

Data analysis was performed with RStudio (RStudio Team, 2019.), version 2022.02.3. Basic statistics were extracted with formulas in the “psych” package (Revelle, 2019) with for the comparison of differences caused by both amendments and fertilizers, LSD test was used (at $p<0.05$), with formulas from “agricolae” package (de Mendiburu F., 2020; Corcoz et al., 2022b).

Table 1. Experimental design and factors used in experimentation

		Am 1 - 2.5 t ha^{-1}					Am 2 - 5 t ha^{-1}				
Fertilizers (kg ha^{-1})	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11
N			100		100	100		100		100	100
P				70	70	70			70	70	70
K						60					60

RESULTS AND DISCUSSIONS

Biolog EcoPlate associated with the decomposition of organic matter in agricultural soils are a valuable tool in the study of nutrient cycling and soil microbial processes (Feigl et al., 2017; Huang et al., 2024). Instead of analysing the soil directly, we can use the buffer material with fine soil particles on its walls to investigate the activity of the soil microbiome. The use of Biolog EcoPlate in combination with litterbags as a buffer instead of soil is an interesting and useful approach in the investigation of ecological and agronomic processes.

The results from Biolog EcoPlate analysis will provide valuable information on how soil microorganisms use soil carbon and help to understand the dynamics of decomposition processes and carbon cycling in terrestrial ecosystems (Fang et al., 2014).

The AWCD reading shows the highest value for V7 at 525%, heavily amended with 5t ha^{-1} calcium carbonate (Tables 2, 3, 4). This value of 525 indicates a high metabolic activity, suggesting that the soil microflora has the ability to metabolize the carbon sources available on the Biolog plates, showing a more intense colour in wells that present higher rates of metabolic activity. Compared to V7, the AWCD for the intensive amended and phosphorus fertilized treatment (V9), drops from 525% to 151 % in the fourth reading. At the fifth reading, there is a slight but not so pronounced decrease of 26% compared to the previous reading, which reaches 125% for the V11. This soil was heavily amended and fertilized with a complex of NPK fertilizer. In general, the activity of microorganisms in agricultural soils can be positively influenced by this fertilizer complex (Martyna, 2019; Mohammadi et al., 2011; Vidican & Sandor, 2015). The NPK complex has the potential to stimulate the activity of microorganisms, leading to more efficient decomposition of soil organic matter and better nutrient recycling (Yang et al., 2019). It also supports soil symbioses such as nitrogen-fixing bacteria and mycorrhizae, thereby improving soil fertility through more efficient nutrient uptake (Kafle et al., 2019; Stoian et al., 2019; Pop-Moldovan et al., 2022). The use of complex NPK formulas in the context of long-term fertilization therefore plays an important role in stimulating soil microflora activity, contributing to the health and fertility of agricultural soil (Kracmarova et al., 2020).

The distilled water (WAT) group analysis, in the third reading, reveal V4 that showed a significant increase in metabolic activity, reaching 322% under moderate and phosphorus-fertilized amendment (Tables 2, 3, 4). These high levels of metabolic activity were followed by a slight decrease in the fourth reading, where the V4 variant maintained its dominant position, but with a reduced percentage at 221, indicating a difference of 101% from the third reading. From another perspective, in the fifth reading, an interesting situation is observed. Or this time, the V4 stands out as having the smallest percentage value, with only 86%. In contrast, variants V7 and V9, in the context of intensive amendment, show 169%, a decrease from the previous readings. The 169 percent recorded for V7 and V9 reflects a similar trend, with V7 benefiting from intensive amendment only, while V9 also benefits from phosphorus fertilization. Phosphorus, as a mineral element applied in the right doses, has the ability to support the metabolic activity of soil microorganisms (Sindhu et al., 2014; Richardson and Simpson, 2011).

Table 2. Dynamics of functional guilds after 48 hours due to long-term applied treatments

V	Wat	Sum	AWCD	P	CH	CX	AA	AM
1	135.65±19.87b	332.79±9.78ab	421.18±13.4abc	393.04±25.6ab	413.93±14.44ab	285.90±14.07ab	285.51±9.91abc	365.07±34.36ab
2	130.91±12.41b	294.65±15.3b	346.67±27.03bc	344.59±11.07ab	322.37±25.02bc	280.09±21.4b	261.11±16.51bc	295.41±38.56b
3	236.70±76.92ab	321.91±28.99ab	352.78±27.55bc	355.50±31.77ab	317.32±32.39c	319.22±40.22ab	320.55±31.96abc	458.11±40.19a
4	322.36±68.86a	305.36±24.78ab	299.13±21.5c	439.21±44.6a	330.07±36.57bc	285.08±31.65ab	240.04±10.53c	329.49±32.22ab
5	203.05±43.38ab	327.87±16.3ab	392.10±45.41abc	409.50±45.71ab	355.45±4.52bc	302.02±38.7ab	291.73±9.82abc	355.63±43.53ab
6	250.32±139.23ab	331.41±33.3ab	355.33±34.33bc	430.62±25.13 a	324.16±36.31bc	311.76±33.49ab	347.48±43.57a	326.08±27.85ab
7	138.46±27.24b	363.92±31.91a	525.36±119.31a	386.48±37ab	400.62±38.68abc	371.99±66.16a	321.88±38.31abc	389.28±59.31ab
8	160.17±51.36ab	340.96±20.51ab	421.22±31.67abc	385.11±29.29ab	391.08±20.11abc	311.50±18.82ab	281.19±19.76abc	424.47±68.09ab
9	141.81±31.90b	339.44±39.01ab	426.62±58.97abc	404.20±27.76ab	388.98±39.8abc	317.67±36.42ab	282.48±55.16abc	302.33±40.63b
10	129.7±20.32b	343.72±16.59ab	434.89±38.17abc	324.98±38.55b	453.16±36.42a	304.51±28.22ab	330.76±16.52ab	310.32±57.63ab
11	119.59±18.24b	357.20±6.84ab	476.15±24.68ab	421.84±13.87a	364.15±22.09abc	356.43±3.4ab	310.65±15.05abc	398.49±104.2ab

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

Table 3. Dynamics of functional guilds after 72 hours due to long-term applied treatments

V	Wat	Sum	AWCD	P	CH	CX	AA	AM
1	174.07±22.50a	100.62±10.11b	145.92±3.79a	167.92±13.59ab	145.38±4.27ab	144.24±1.82a	157.94±5.81abc	134.46±6.19abc
2	170.33±21.37a	146.29±1.72a	126.81±3.19a	135.75±3.22b	135.97±2.47ab	126.32±2.70ab	137.38±2.22bc	109.10±3.20c
3	216.70±53.81a	131.43±1.08ab	143.13±18.64a	176.78±17.27a	148.58±9.29ab	132.34±4.06ab	164.75±9.23ab	139.28±13.97abc
4	221.05±113.90a	147.56±6.28a	140.74±18.09a	139.00±3.51b	148.04±6.04ab	131.07±3.41ab	165.86±5.88ab	149.60±13.60a
5	214.56±63.14a	143.80±2.54ab	130.52±6.34a	135.25±1.39b	143.22±9.60ab	130.56±3.68ab	166.33±7.08ab	129.20±7.76abc
6	110.69±14.65a	141.77±5.44ab	131.22±8.11a	137.96±6.13b	124.36±2.95b	114.80±5.09b	127.47±8.38c	110.73±12.26bc
7	199.64±46.33a	122.25±4.82b	131.07±9.16a	154.31±16.49ab	140.46±11.72ab	142.65±17.50a	152.33±16.89abc	116.96±9.23abc
8	213.99±95.76a	143.31±14.67ab	144.58±9.95a	167.52±15.99ab	149.60±20.89ab	140.31±13.40a	174.50±23.90a	148.78±29.93ab
9	128.09±4.76a	151.17±15.46a	151.86±4.41a	157.61±12.07ab	148.92±8.04ab	144.11±2.75a	160.38±13.12abc	127.44±13.99abc
10	154.61±19.15a	148.55±2.42a	138.28±3.31a	162.18±13.93ab	140.49±3.26ab	133.73±2.13ab	153.23±6.96abc	126.42±9.64abc
11	187.97±50.31a	140.81±1.15ab	139.69±6.01a	150.68±2.42ab	152.96±5.93a	140.56±2.04a	156.08±6.05abc	114.49±5.53abc

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

Table 4. Dynamics of functional guilds after 96 hours due to long-term applied treatments

V	Wat	Sum	AWCD	P	CH	CX	AA	AM
1	131.75±8.22ab	123.13±1.08ab	121.32±1.40abc	127.56±2.29ab	121.67±1.88ab	125.12±1.11a	124.75±3.46a	108.63±2.87ab
2	100.47±6.81b	113.67±1.85bc	115.87±2.25bc	121.53±1.99b	109.12±3.66bc	113.48±1.19abc	118.19±3.78ab	111.01±2.61ab
3	113.50±9.26ab	116.50±1.11abc	118.35±4.02bc	125.63±2.99ab	115.48±2.53abc	114.18±0.59abc	120.18±2.68ab	109.22±9.53ab
4	86.30±2.93b	118.09±1.40abc	135.77±9.72a	128.23±4.11ab	119.53±3.21abc	114.67±1.16abc	115.42±3.03ab	115.85±4.27a
5	101.83±7.20b	119.41±0.38ab	127.28±4.94ab	126.08±1.45ab	115.26±1.46abc	118.95±0.78ab	123.32±2.86ab	120.13±2.14a
6	141.93±15.33ab	119.15±1.90abc	117.18±3.07bc	126.92±1.55ab	114.34±2.82abc	119.94±1.13ab	123.92±3.83a	111.50±4.04ab
7	169.49±43.44a	118.31±2.34abc	116.16±6.10bc	128.98±7.42ab	120.35±4.88abc	112.97±0.93bc	119.85±2.62ab	118.12±8.72a
8	104.08±30.66b	107.95±8.06c	114.20±5.86bc	131.75±11.4ab	105.06±10.74c	105.00±6.42c	111.91±7.03b	89.18±17.25b
9	169.28±24.44a	113.93±6.92bc	109.72±8.18c	127.19±3.07ab	107.19±9.23bc	114.52±8.38abc	120.73±4.46ab	116.80±6.19a
10	137.6±14.30ab	122.76±1.79ab	121.50±1.83abc	134.59±3.28a	120.55±1.28abc	122.04±3.12ab	123.07±4.19ab	120.64±4.75a
11	126.35±11.95ab	125.47±2.40a	127.88±4.16ab	136.87±4.27a	126.64±3.42a	120.27±2.71ab	125.90±4.17a	114.89±5.19a

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.

A similar situation is observed for the amines group, where reading 3 shows the highest value in V3, from Am1 and fertilized with mineral nitrogen, reaching 458% (Tables 2, 3, 4). This is succeeded by V4, also in Am1, but fertilized with phosphorus, which shows a significant decrease to 149% in the fourth reading and 120% in the fifth reading in variants V5 and V10. V5 comes from moderate amendment, while V10 is associated with intensive

amendment, both benefiting from fertilization with an NP complex. This complex contributes to maintaining a high diversity of microorganisms in the agricultural soil suggesting its potential to support long-term soil fertility under constant and optimal fertilization (Ge et al., 2008).

On the other hand, the carbohydrate group shows quite high percentage values compared to amines (Tables 2, 3, 4). A maximum is observed in reading 3, with a value of 453% in the V10 variant, which was intensively amended and fertilized with an NP complex. This is followed by variant V11 in reading 4, also under intensive Am2 amendment, but this time fertilized with an NPK complex, where there is visible also a decrease (152%), representing a reduction to 75% compared to reading 3. Reading 5 under variant V11 then shows a value of 126 %.

In reading 3, polymers reach a maximum value in V4, following an average amendment of 2.5 t ha^{-1}, reaching 439% (Tables 2, 3, 4). In measurement 4, the polymer group reaches a maximum in variant V9, but shows a decrease of 176% compared to the value present in variant V4, which was fertilized with phosphorus in an average amendment scheme. This type and dose of fertilizer has a positive effect on the microflora of the agricultural soil. At opposite, in reading 5, variant V11 shows a maximum of 136% under intensive fertilization and fertilized with NPK complex, indicating that this complex contributes to the increase of functional activity in agricultural soil. Therefore, this fertilizer complex has the ability to stimulate the development of a rich and active variety of microorganisms in the soil, which contribute to the processes of decomposition of organic matter (Kaur et al., 2008; Dincă et al., 2022).

From the functional guilds analysed (Tables 2, 3 and 4), there are two possible directions for the amines, carbohydrate and polymer guilds: the first one - is that are these substrates are more easily metabolized or, the second one is - that the diversity of microorganisms capable of metabolizing these substrates is greater. In the case of amines, the V3 variant, treated with an average dose of 2.5 t ha^{-1} of calcium carbonate, predominates, and in the case of polymers, the V4 variant.

In the V3 of amines, in addition to the amount of amendment, nitrogen is included, which can favor microorganisms capable of converting this nitrogen into more accessible forms. This indicates that the presence of nitrogen can stimulate microbial activity and thus the mineralization and nutrient transfer (Zhang et al., 2019; Chen et al., 2003).

For the V4 of the polymer guild, in addition to the amount of amendment present, the presence of phosphorus as a mineral fertilizer is also observed. Phosphorus, a crucial element for the activity of micro-organisms in agricultural soil with long-term fertilization (Billah et al., 2019), can facilitate the metabolism of carbon sources, such as polymers, and supports the decomposition process by activating specific enzymes (Zhu et al., 2018).

As regards intensive amendment with 5t ha^{-1} of calcium carbonate from the carbohydrate guild, the predominance of the V10 variant, which, in addition to intensive amendment, also includes the NP fertilizer complex, is recorded (Tables 2, 3 and 4). The NP fertilizer complex plays an essential role in soil microflora activity, supporting the growth of microorganisms (Zhang et al., 2015; Achari and Kowshik, 2018), for a more efficient metabolism of the carbon source, in this case carbohydrates, and thus promoting organic matter degradation and nutrient recycling in agricultural soil with long term fertilization (Zechmeister-Boltenstern et al., 2015).

The three periods analyzed showed a different pattern of low and high activity in functional guilds, due to the long-term applied treatments (Figure 1). The differences between the dynamics of activity (Tables 2, 3, 4) enable the detection of different functional guild trends associated with a specific treatment (Figure 1). The highest basal functional activity (Wat) is associated with P application at low levels of amendment (V4) for readings 3 and 4 but decrease drastically at the end of incubation period. This phenomenon indicates a high generalist microbial community that have a high activity rate, but only for a short period of time in the absence of resources. The dynamic of lowest basal activity is changing in a decrease trend, from V11 (highest doses of treatment) to V6 (highest dose of fertilizers + medium amendment) and V4 in 5th reading.

This trend indicates a low functional microbiome in V11, that slowly increases its activity, followed by a microbiome that have a fluctuating activity (V6).

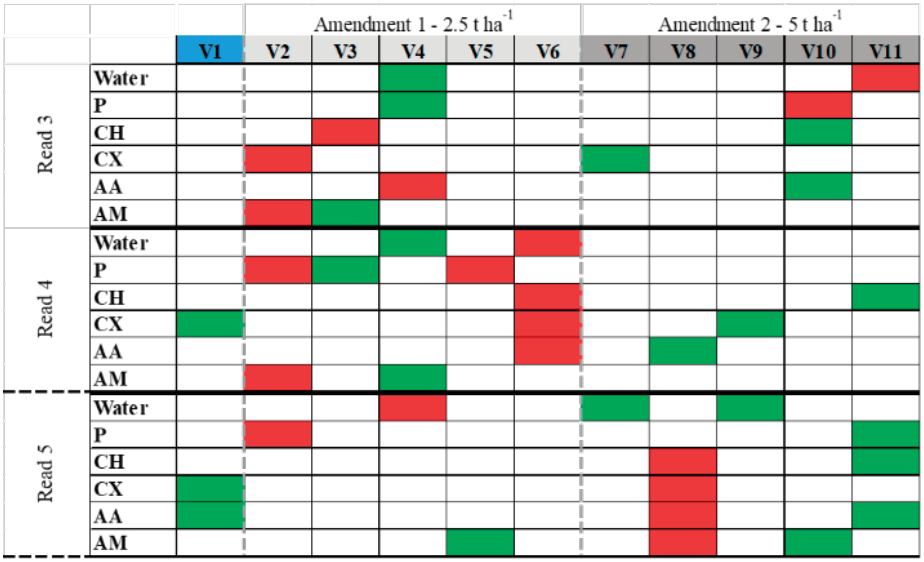

Figure 1. Trends and dynamics of functional guilds due to long-term applied treatments

The Polymers guild show an interesting dynamic trend, with the highest activity associated with V4 (phosphorus only) in the 3rd reading, followed by an increase of a nitrogen microbiome (V3 - 4th reading) and the activation of a dual microbiome in V11 (5th reading, NPK + amendment). The decrease trend of daily activity shows a pattern associated with NP fertilizers (V10, V5) up to the absence of these minerals (V2) and is coupled with the decrease in amendment from 5 to 2.5 t ha^{-1}. The presence of amendments at different levels sustains this lowering trend as the separation of microbiome for two functions - the use of fertilizers (different levels) and the use of amendments. In this context, the share of each microbiome is different in their communities which imply a lack of synergy toward the efficient use of polymers.

Carbohydrates highest activity is associated with highest doses of fertilizers and their complexity (V10, V11) in the presence of amendments. The change produced in the pH of these variants enabled the emergence of functional communities that use efficiently the carbohydrates. The trend of decreases between readings is very interesting for this functional guild microbiome. In the 3rd day high doses of N and medium amendment on soil (V3) decrease the potential activity, followed after one day by the V6 functional microbiome and the lowest one in V8 (5th reading). In this context, both the microbiome in medium amended soils and NPK fertilized (V6) and high amended soils and P fertilized (V8) presents similar characteristics of carbohydrates decomposition, but with a 24 h gap between them.

Carboxylic and acetic acids microbiome is very sensitive to medium amendment doses (V2, V6) in the early and middle stages of activity and to high amendment completed by P (V8) in the late stages. For this lowest activity trend, the use of NPK (V6) sustain another 24 h of supplementary activity until the decrease, while the use of only P (but on high amendments - V8) extend this decrease process up to 48 h. An interesting case its represented by the maximum recorded activity for this functional guild, the microbiome having the highest activity in 5 t ha^{-1} amended soils in the 3rd reading (V7), but until the end of incubation period is maintained at maximum in the control variant. The mechanism is based on the activity performed by specialized microorganism for

mineral fertilizers that cannot decompose efficiently these substrates.

Amino acids show an identical pattern of decrease as for carbohydrates, and a fluctuating high activity trend associated with the application of amendments. An interesting case is that at the 5th reading, a high activity of this functional guild is visible also in the control variant, which indicates the presence of same similar functional microbiomes in both variants, but at a lower share in the control one., which cause this gap in the activity. For the control variant it indicates a shift of functional microbiome from a generalist activity to an amino acid specialized one.

Amides/Amines functional microbiomes are more associated with medium amended soils, with an interesting increase from 3rd to 5th reading. In the 3rd reading the maximum is associated with N application (V3), followed after 24 h by V4 (P based variant) and V5 (NP based variant). Both type of fertilizers activates the microbiome of this guild, faster for N, and obtain the synergy of elements after 48 h. The decrease trend is identical with the one recorded for Carboxylic and acetic acids, which indicates the low presence of these functional microbiomes in these variants.

CONCLUSIONS

The long-term application of inputs have a direct influence on the activity dynamics of decomposing microorganisms.

Analysis of basal soil respiration highlights the importance of phosphorus application at low amendment levels (V4), which initially generated the higher functional activity, indicating a generalist microbial community.

High treatment doses initially showed a low basal activity, which gradually increased over time, while phosphorus produce a fluctuating activity.

The dynamic activity of the Polymers guild is significantly influenced especially by the phosphorus application, which alters this specific microbiome.

Maximum activity of carbohydrate and amino acid functional microbiomes is observed in treatments with the highest fertilizer rates and complexity, when amendments are present.

The microbiome's sensitivity to carboxylic and acetic acids shows variations due the synergy of amendment and fertilization, with NPK prolonging activity by 24 hours, while the exclusive application of phosphorus delaying it.

Amide/amine-associated microbiomes are more commonly found in medium amendment soils, indicating a significant increase in activity over time.

The Biolog EcoPlate method is an efficient technique for monitoring the functional activity of soil microbial communities involved in decomposing processes.

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