

FUNCTIONAL ALTERATION AND DOMINANCE-CODOMINANCE PATTERNS IN DECOMPOSITION FUNCTIONAL MICROBIOMES FROM LONG-TERM FERTILIZED AND AMENDED EXPERIMENTS

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Abstract

The application of fertilizers and amendments in agroecosystems is a necessary management strategy to sustain crop productivity, but in the long term it causes changes in soil microbiomes. The aim of the research was to analyze in detail the changes on decomposition functional microbiome induced by both types of inputs in a long-term experiment. The functional microbial associations were analyzed using the DEMSA tool, for each of the experimental variants being extracted the segments of the ecological niche: intensification, expansion, narrowing and contraction. The functional alteration varied greatly ranging between 0.4% and 26.7%, with most of the communities showing a dominance of carbohydrates guilds (intensification and narrowing niche) and carboxylic acids (expansion and contraction niche). Within the communities, the dominant groups were associated mostly with amendments for the activities that exceeded the control. In contrast, the reduction of activities within the microbiomes was correlated with the applied fertilizers. Each of the analyzed microbiome segments presented a specific pattern of dominance-codominance of functional guilds and groups that were associated with the synergic impact of long-term applied amendments and fertilizers.

Key words: functional ecological niches, long-term experiment, microbial assemblage changes, straw decomposition, substrate microbial reaction.

INTRODUCTION

Soil microorganisms are important in maintaining ecosystem health and respond to long-term fertilization by changing the structure of their communities. The microorganisms create complex communities which support soil and plant health by regulating the nutrient cycle and protecting the ecosystem from biotic and abiotic stresses (Bhattacharjee et al., 2022).

The high diversity of microorganisms in soil and limited understanding of how they function often lead to their omission in carbon cycle models. However, microbial decomposers are responsible for the breakdown of most organic matter in the biosphere and play an important role in the carbon and nutrient cycles (Soares & Rousk, 2019).

Microbial coexistence means that multiple species can live together, even if they compete for the same resources, through mechanisms that reduce direct competition or balance the advantages of each species. Understanding these interactions, based on modern coexistence

theory, shows how niche differences allow microbial communities to maintain to maintain stability and functionality. Applying this concept in agriculture can help build and manage microbial communities to improve soil health and agroecosystem productivity (Zhang et al., 2021; Chang et al., 2023).

According to Vidican et al., 2017 on the assemblage of community, significant attention is given to dominant and co-dominant groups and provides an insight on the internal dynamics of ecosystems. Soil microbiome is structured around these dominant groups, which influence both the composition and functioning of the microbial community. The dominance of certain groups is closely linked to their ability to adapt to environmental conditions, allowing rapid responses to changes in resources or management activities. In contrast, co-dominant groups are more unstable, susceptible to pronounced variations due to environmental factors (Stoian et al., 2022).

Soil microbial communities possess functional redundancy, even though species have different

metabolic niches. All microorganisms perform basic functions, but only a few participate in rare processes. The conservation of phylogenetic niches explains this redundancy, as essential genes have been preserved over billions of years and transmitted between microorganisms. Soil provides many different microhabitats, and species from the same evolutionary branch may have overlapping niches, making them functionally redundant in certain processes.

In this context, the Detailed Exploration of Microbial Sociological Assemblages (DEMSA) method was created to improve the limitations of standard CLPP analyses by using Biolog EcoPlate substrates as chemically defined groups, facilitating the detailed study of microbial niche evolution in soil (Stoian et al., 2022). The soil microbiome is a dynamic micro-ecosystem that regulates biological processes vital to global ecosystems. Preserving it as "next-generation biological" could support future applications, but the challenge lies in finding storage methods that maintain its functionality. Current research is analyzing the integrity of the preserved microbiome by assessing its structural diversity and functional potential.

The aim of this research was to analyze in-depth the impact of long-term fertilizer and liming application on the functional microbiome assemblage associated with straw decomposition. The hypotheses proposed and tested were formulated as research questions: i) treatment combination show a visible pattern on the functional microbiome activity; ii) each treatment induce a specific increase or decrease in metabolic activities recorded in microbiome; iii) functional group analysis reveal specific patterns of ecological niche as defined by the activity level; iv) the use of DEMSA model is suitable for the extraction of relevant indicators of long-term treatments.

MATERIALS AND METHODS

The study was conducted in an experimental field with long-term fertilization (over 60 years), located at ARDS Livada, Satu Mare county, and aimed to investigate the decomposition process of wheat straw using the litterbag method. The experimental protocol was adapted from Gheorghită et al. (2024), with minor

modifications to the DEMSA model applied to the dataset.

Based on the analysis of activity trends in functional microbial profiles involved in organic matter decomposition across 10 variants (V2-V11) in experimental treatments after 60 years of long-term soil fertility management, this study aims to investigate the interactions between functional microbial communities and their diversity in long-term fertile soils and to determine if decomposition rates are associated with overall microbial diversity or with specific key species linked to the applied inputs. The DEMSA model by Stoian et al. 2022, evaluates the response of microbial communities involved in organic matter decomposition to long-term fertilization by comparing carbon substrate utilization against a control interval. It classifies functional shifts into four ecological niche segments-intensification, expansion, narrowing, and contraction-thereby revealing changes in microbial functional diversity and decomposition stability in soil.

The experiment included ten experimental variants (V2-V11), corresponding to different levels of liming (amendment): medium amendment (2.5 t ha^{-1}) for variants V2-V6 and intensive amendment (5 t ha^{-1}) for variants V7-V11. Variants V2 and V7 included only the application of the amendment (from moderate to intensive), without mineral fertilization. Variants V3 and V8 were fertilized with nitrogen ($\text{N} - 100 \text{ kg ha}^{-1}$), followed by V4 and V9 fertilized with phosphorus ($\text{P} - 70 \text{ kg ha}^{-1}$), V5 and V10 with nitrogen and phosphorus ($\text{NP} - 100+70 \text{ kg ha}^{-1}$), and V6 and V11 with nitrogen, phosphorus, and potassium ($\text{NPK} - 100+70+60 \text{ kg ha}^{-1}$). A major difference in the use of the DEMSA model is the comparison of all experimental variants with the control V1, represented by unfertilized soil (native soil). The Biolog EcoPlate protocol involved the use of plant material harvested after a 30-day decomposition period, followed by sample processing and serial dilutions up to 10^{-4} . The resulting suspensions were then inoculated into microplates, $120 \mu\text{L}$ per well. The microplates were incubated at room temperature in the dark, and readings were taken at 24, 48, 72, and 96 hours using a spectrophotometer (Gheorghită et al., 2024).

Within the methodology used, the substrates were classified as functional groups, each being assigned to a functional guild based on chemical indicators (Stoian et al., 2022). The impact of organic matter decomposition on the microbiome in soils with long-term fertilization was assessed using the functional groups: carbohydrates (CH), polymers (P), carboxylic acids (CX), amino acids (AA), and amines/amides (AM).

The entire database was explored using RStudio (R Core Team, 2024). Basic statistics were obtained using the 'psych' package (Revelle, 2024), while differences between variants (V2-V11) were evaluated using ANOVA and the LSD test from the 'agricolae' package (de Mendiburu, 2023). The stability of functional microbiomes was evaluated using diversity indices and NMDS ordination, calculated with the vegan package (Oksanen et al., 2022), and correlations vectors and communities were explored at niche level.

RESULTS AND DISCUSSIONS

The functional traits of microorganisms are measurable characteristics that show the performance of an organism under specific environmental conditions. These traits can be studied in microbial ecology to improve biogeochemical models and predict how ecosystems respond to global changes.

Patterns of functional proliferation in microbial communities under long-term application of treatments

In soils, the intensification process in microbial community implies an increase in activity that is maintained in the average-to-maximum range of the native soil. These values can be used as an indicator of natural activity associated with the decomposition process, which overscores the native potential but do not affect the natural balance of community. Expansion can be defined as a significant or considerable increase in the activity of decomposing microorganisms, that perform an accelerated process due to the significant increase in the abundance and variety of several functional groups.

Carbohydrates (CH) show a relatively homogeneous distribution of the intensity of microbial metabolism involved in the decomposition of organic matter. Microbial

activity associated with the use of carbohydrates, as well as other functional groups (P, CX, AA), remains relatively stable between different inputs applied over the long term. Of all the guilds analysed, carbohydrates show the most pronounced increase in activity in microbial communities, suggesting that they are preferred and more easily accessible substrates for soil microorganisms.

In the amine guild (AM1 and AM2), variants V5 and V6 - treated with organic amendment and 2.5 t ha⁻¹ CaCO₃ - recorded zero values, which indicates the absence of stimulation of the metabolic activity of microorganisms involved in the decomposition of organic matter (Table 1). This suggests that, under long-term fertilization conditions, microbial communities belonging to this functional guild respond by reducing their metabolic processes. Consequently, the additional application of mineral fertilizers (NP or NPK) does not result in higher values. Also, in the intensively amended variant (V8), no increase in activity is observed, even though nitrogen is present. This may indicate either a lower functional activity of the microorganisms in this guild or the fact that amine-specific substrates are more difficult to degrade, requiring metabolic conditions or a longer adaptation of the microbial community.

Table 1. Microbial functional guilds and diversity in Intensification and Expansion range of activities

	Guild performance in Intensification niche				
	Px	CH	CX	AA	AM
V2	19.8±3.9ab	41.4±3.4ab	17.5±1.5ab	18.4±2.9bcd	2.7±1.6bc
V3	14.4±3.4ab	38.5±3.5ab	26.5±7.3a	18.1±3.4bcd	2.2±2.2bc
V4	20.0±1.8ab	38.4±8.7ab	25.6±4.7a	14.7±4.1d	1.1±1.1c
V5	13.2±2.9b	39.3±7.7ab	17.9±2.3ab	29.4±5.6ab	
V6	15.1±1.3ab	42.2±2.5ab	16.1±1.0ab	26.4±3.0abc	
V7	13.3±5.3b	26.3±2.7b	24.9±5.4a	30.8±2.4a	4.4±2.2bc
V8	29.7±13.6a	42.8±7.8ab	8.5±2.0b	18.7±5.6bcd	
V9	17.1±2.8ab	52.3±5.0a	11.1±3.2b	17.8±3.6cd	1.5±1.5bc
V10	10.5±3.4b	31.8±7.9b	25.5±4.2a	21.0±2.9abcd	11.0±0.9a
V11	18.8±4.9ab	35.0±10.1ab	17.9±6.5ab	21.3±4.1abcd	6.7±4.5ab
	Guild performance in Expansion niche				
	Px	CH	CX	AA	AM
V2	21.1±5.7ab	43.8±9.1a	15.2±1.7ab	14.7±2.9b	4.9±3.2b
V3	7.6±2.1b	61.7±5.1a	16.7±6.0ab	13.4±5.1b	0.4±0.4b
V4	20.8±6.3ab	49.9±14.3a	18.1±6.4ab	10.4±7.6b	0.6±0.6b
V5	8.8±1.4b	63.1±2.5a	6.2±2.7b	21.7±5.3ab	
V6	10.9±1.5b	46.9±10.0a	9.0±4.5ab	33.0±11.0a	
V7	6.4±4.3b	42.9±12.5a	25.1±9.0a	22.2±5.9ab	3.1±2.1b
V8	30.3±13.3a	51.9±11.3a	12.5±1.7ab	5.1±2.0b	
V9	11.2±3.9b	67.6±11.6a	12.2±6.2ab	8.5±3.1b	0.3±0.3b
V10	9.1±2.4b	39.5±14.0a	23.8±6.7ab	12.3±3.3b	15.1±4.7a
V11	15.4±3.7ab	48.7±11.8a	15.2±9.7ab	14.9±7.7b	5.6±3.6b

Note: V2-V6 - liming L1 (2.5 t ha⁻¹ calcium carbonate), V7-V11 - liming L2 (5 t ha⁻¹ calcium carbonate), V2-V7 - liming without mineral fertilizer, V3-V8 - liming with N100, V4-V9 - liming with P70, V5-V10 - liming with N100P70, V6-V11 - liming with N100P70K60. Legend: Px - polymers; CH - carbohydrates; CX - carboxylic acids; AA - amino acids; AM - amines/amides. Values ± s.e. followed by different letters present significant differences according to LSD (p<0.05).

The microbial diversity of the soil responsible for decomposing organic matter can expand, leading to a richer microbiome in terms of groups involved in decomposition.

The V5 and V10 variant treated with NP, but different liming intensities show that an increase in the level of amendment does not lead to a significant increase in the microbiome diversity. The carbohydrate guild generates the highest values, as these substances are more easily solubilised and used as an energy source by decomposing microorganisms in the soil. Due to their simpler chemical structure and rapid degradation, microbial diversity expands within a short period of time. As a result, there is a significant increase in both the number of microorganisms and their diversity, a phenomenon that indicates a marked stimulation of biological processes in the soil.

The general trend of expansion indicates a shift towards L1, and in the case of polymers the amendment applied at the average level results in overall slightly higher values. A similar pattern can be observed in carboxylic acids, where there is a moderate expansion in values, also in L1. It appears that applying only 2.5 t ha⁻¹ of amendment is sufficient to stimulate higher microbial diversity.

Narrowing niche of the soil microbiome refers to the decrease or reduction in the microbiome in the average-minim range compared to the native community. Overall, the dominant microbial groups involved in decomposition processes become less abundant. In terms of contraction niche the microbiome suffers a compaction process and its diversity is reduced. The affected populations decrease considerably under the minimum native potential and the groups involved in the decomposition decrease or even disappear due to the application of different inputs.

Growth strategies in Intensification and Expansion niches

The microbial diversity involved in the decomposition process extends to the soil microbiome under long-term fertilization conditions. In the L1 variants, characterized by a moderate level of amendment, the CH guild shows an increase in microbial activity, as this level of amendment supports a highly active microbiome. Under these conditions, intensive amendment (L2) does not generally lead to

higher diversity values, except in variants V7 and V8.

A similar behaviour is observed for the P, CX, and AA guilds, where microbial diversity remains higher under L1 conditions. The carbon sources associated with these guilds seem to be preferred by microbial communities involved in decomposition when there is a buffering effect generated by soil pH correction. A particular case is represented by the AM guild (amines), which are more difficult to metabolize by the decomposing microbiome, which explains the lack of diversity expansion under conditions of intensive amendment (L2). In most situations, intensive amendment applied alone generates higher microbial indicator values compared to variants combined with chemical fertilizers (Liu et al., 2009). Thus, soil pH adjustment becomes a main factor for the structure and functioning of the microbiome (Hartmann & Six, 2023).

The intensification in the activity of microbial communities indicates a relative homogeneity of the soil microbiome, with no major functional differences between the guilds analysed under conditions offered by L1 and L2 (Ortega et al., 2024). Microorganisms generally respond similarly to the decomposition process, suggesting a relatively uniform microbial structure between guilds. The exception is the AM guild, which, in certain situations, does not show increases in microbial activity intensity.

Functional diversity supports stable metabolic activity at an optimal level of amendment (L1), correlated with expansion and intensity indicators. Amendment (L2), the increase in intensity is not accompanied by an expansion of the diversity of the agricultural soil microbiome, which indicates a selection effect on the dominant microbial communities. This is assured by a shift from a generalist to a specialized community, depending both on the inputs applied over a long period of time and on the availability of carbon sources in Biolog EcoPlate (Gheorghiu et al., 2026).

A significant positive influence is exerted by the application of calcium carbonate, which results in high values in the treated variants, with no major differences in microbial metabolism at the intensive dose of 5 t ha⁻¹. An average dose of 2.5 t ha⁻¹ CaCO₃ is sufficient to maintain the balance of the agricultural soil microbiome.

The expansion of microorganisms responsible for decomposition becomes more efficient in the presence of a moderate amount of fertilizer, which facilitates and stimulates a visible proliferation of microorganisms. This manifests itself both in the increase in the diversity of communities across a wide range and in the intensification of their metabolic functions, their activity becoming more pronounced (Maron et al., 2018).

Patterns of functional decline in microbial communities under long-term application of treatments

In conditions of long-term fertilization (Table 2), the narrowing manifests as a reduction in functional microbiome involved in the decomposition of organic matter, as a result of the rich supply of organic and inorganic nutrients in the soil. This phenomenon generates a limited or restricted number of groups adapted to nutrient-rich conditions, to the detriment of a diverse microbial community. The dominance of decomposing microorganism species is reduced, and mineralization processes become more limited in terms of functional diversity (Gessner et al., 2010).

Table 2. Microbial functional guilds and diversity in Narrowing and Contraction range of activities

	Guild performance in Narrowing niche				
	Px	CH	CX	AA	AM
V2	6.3±4.6b	18.1±6.6ab	41.4±4.9ab	25.8±6.6ab	8.1±0.8abc
V3	12.9±4.5ab	17.4±6.1ab	37.6±5.0ab	20.5±2.6abc	11.3±2.9a
V4	8.1±2.9b	17.0±4.2ab	36.6±2.5ab	27.9±4.6a	10.1±1.3a
V5	10.8±2.1ab	23.8±3.9ab	38.5±1.0ab	16.8±2.5abc	9.9±0.3ab
V6	9.3±0.6ab	21.2±2.2ab	45.3±2.5a	13.0±4.6c	10.9±0.7a
V7	13.4±2.2ab	33.2±3.6a	31.4±4.7b	16.4±1.9abc	5.4±0.1bc
V8	9.1±2.0ab	20.3±6.7ab	43.8±5.7ab	16.5±1.9abc	10.0±1.3a
V9	7.8±1.8b	11.1±3.1b	48.8±1.9a	22.3±2.7abc	9.7±1.5ab
V10	17.5±3.9a	26.1±9.1ab	40.7±7.2ab	15.6±2.1bc	
V11	11.5±2.0ab	18.9±7.8ab	42.6±3.4ab	21.7±6.2abc	5.1±2.9c
	Guild performance in Contraction niche				
	Px	CH	CX	AA	AM
V2	6.2±6.1b	10.1±3.6b	42.7±10.2ab	26.7±8.5a	14.0±2.8ab
V3	17.1±7.0b	11.2±8.2b	39.7±6.3ab	15.3±4.9ab	16.5±2.1a
V4	9.6±3.2b	16.4±6.5ab	41.3±6.2ab	22.8±4.8ab	9.7±3.2abc
V5	14.2±7.1b	13.7±3.3ab	44.6±4.0ab	13.9±2.2ab	13.4±1.6ab
V6	4.6±1.6b	21.9±5.8ab	42.7±5.6ab	17.1±7.8ab	13.4±3.1ab
V7	11.7±3.2b	31.9±4.3a	32.6±4.4ab	18.9±3.2ab	4.7±1.5bc
V8	10.8±4.2b	21.6±8.0ab	37.4±7.2ab	15.9±5.8ab	14.0±2.4ab
V9	12.8±2.0b	6.6±3.6b	50.5±5.0a	18.6±0.4ab	11.1±2.2ab
V10	34.0±9.7a	25.5±9.2ab	30.6±7.0b	9.8±3.7b	
V11	14.9±5.8b	22.5±12.0ab	38.3±8.4ab	9.3±5.2b	14.7±8.5ab

Note: V2-V6 - liming L1 (2.5 t ha⁻¹ calcium carbonate), V7-V11 - liming L2 (5 t ha⁻¹ calcium carbonate), V2-V7 - liming without mineral fertilizer, V3-V8 - liming with N100, V4-V9 - liming with P70, V5-V10 - liming with N100P70, V6-V11 - liming with N100P70K60. Legend: Px - polymers; CH - carbohydrates; CX - carboxylic acids; AA - amino acids; AM - amines/amides. Values ± s.e. followed by different letters present significant differences according to LSD (p<0.05).

The most pronounced reduction in microbial metabolism is observed in the case of carboxylic acids, with values ranging from 40.7% in variant V10 (treated with NP and intensively amended) to 48.8% in variant V9 (intensively treated and fertilized with P). These results indicate that the types of fertilizers applied lead to a decrease in microbial metabolic activity (Ge et al., 2010). Under these conditions, carboxylic acids are carbon sources that are more difficult to solubilize in the presence of added inputs.

A relatively uniform distribution of the communities involved in the decomposition of organic matter is particularly noticeable in the polymer guild, where narrowing in microbial metabolism are lower compared to the CX guild. The assessment of contraction phenomenon can be used to describe a sharp decrease in microbial activity, manifested by a reduction in metabolism below the minimum observed in native community (Table 2). This phenomenon can occur during the decomposition process, when the microorganisms involved are strongly influenced by environmental factors, which can lead to the inhibition of microbiome development and the cessation of its proliferation (Islam et al., 2020).

When contraction occurs, a relatively heterogeneous loss in microbiome can be observed in all treatments. Thus, microbial communities maintain their structure in a similar range of losses. Overall, they do not suffer an abrupt change into their metabolic activity, proliferation rate, or biological functions, even in the presence of different variants or specific types of substrates (Poyraz et al., 2021). This stability leads to a uniform loss of microbial groups, with no visible differences in the entire vital process of decomposition (Khatoun et al., 2017).

Carboxylic acids show the highest values in the contraction phenomenon analysed. As a result of prolonged exposure to the different types of inputs, the diversity of microbial communities tends to decrease significantly, and the metabolic activity of microorganisms is affected (Macias-Benitez et al., 2020). Over time, part of biological functions is inhibited or even completely inactivated, as the conditions created by the constant presence of treatments limit the development and adaptation of microbial populations (Gonzalez & Aranda, 2023).

Reduction strategies in Narrowing and contraction niches

The functional diversity involved in the decomposition process, in the context of the contraction phenomenon, is strongly influenced by the application of N, P, and NP fertilizer complexes (Luo et al., 2018). These treatments cause significant reductions in microbial diversity, especially in guilds associated with the use of carboxylic acids, which implicitly leads to a decrease in the microbial metabolism of various C sources.

Microbial diversity losses double in volume during the contraction phenomenon at the total CX guild level, reaching its maximum value in variant V9, characterized by the application of an intensive amendment treatment and phosphorus in the form of mineral fertilizer. In this variant, the soil microbiome is significantly reduced, followed by variant V5 fertilized with NP and variant V6 fertilized with NPK complex. In the case of carboxylic acids, the functional diversity of microorganisms involved in decomposition processes plays a significant role in the structure of this guild. The ability to use the 9 carboxylic acids may be limited by fertilization conditions and the type of amendment applied, as well as soil pH and nutrient availability (Zhang et al., 2023). Under these conditions, decomposing microorganisms will metabolize these compounds less efficiently, leading to a reduction in the metabolic activity of microorganisms (Phale et al., 2007).

On the other hand, the CX guild, both in the narrowing and contraction niches, causes significant decreases in both functional diversity and the structure of the communities of microorganisms responsible for decomposition, under conditions of fertilization with N, P, NP, and NPK.

At the same time, the NP and NPK fertilizer complex causes functional decreases in the Px, CH, and CX guilds. An exception is the AA guild, where the contraction phenomenon is reduced by almost half compared to Px. In the case of the AM guild, the absence of the contraction phenomenon in variant V10 is notable, a trend that is also maintained in the narrowing phenomenon.

In the CH and AA guilds, there is a relatively uniform decreasing distribution of microbial

diversity, with N and P treatments causing similar effects of microbial diversity reduction (Wang et al., 2023).

These two phenomena are closely related in terms of reducing both microbial diversity and the structure of communities, as the reduction in the availability of certain metabolic resources and the modification of soil fertilization conditions favour the selection of dominant microbial groups, while other groups become less active or are even eliminated, thus leading to a more selective microbiome (Islam et al., 2020).

Niche-functional alteration produced by long-term application of treatments

Within the positive phenomena (Table 3), the highest index of expansion and intensification is recorded, indicating a significant increase in the diversity of microbial communities, as well as an amplification of their metabolic activity when applying only moderate fertilization (L1), without the addition of mineral fertilizers. In this context, soil pH is a key factor in the decomposition process of straw (Yang et al., 2023).

Table 3. Microbial functional activity and changes in the range of the functional niches activities

	Sum Int	Index of Int	Sum Exp	Index of Exp
V2	0.6±0.1ab	1.6±0.1ab	5.8±2.1a	13.8±4.0a
V3	0.5±0.1ab	1.5±0.1ab	4.9±1.8ab	12.2±3.4ab
V4	0.6±0.1ab	1.5±0.3ab	4.2±1.1ab	11.0±2.7ab
V5	0.5±0.0ab	1.5±0.0ab	2.9±0.3ab	8.4±1.0ab
V6	0.6±0.1ab	1.8±0.1ab	3.9±0.9ab	10.1±2.0ab
V7	0.5±0.0ab	1.4±0.1ab	1.8±0.3b	5.1±0.9b
V8	0.4±0.1b	1.2±0.2b	3.1±1.5ab	8.5±3.8ab
V9	0.6±0.1ab	1.6±0.2ab	3.3±1.1ab	8.7±2.7ab
V10	0.7±0.0a	1.9±0.1a	3.9±0.8ab	10.0±1.9ab
V11	0.6±0.1ab	1.6±0.3ab	3.4±0.9ab	8.9±1.9ab
	Sum Narr	Index of Narr	Sum Contract	Index of Contract
V2	1.2±0.1ab	3.2±0.4ab	2.8±0.4b	7.3±1.3b
V3	1.4±0.2ab	4.2±1.1ab	4.2±1.3ab	12.5±4.9ab
V4	1.5±0.2ab	4.1±0.9ab	3.3±0.7b	9.2±2.4b
V5	1.6±0.0a	4.8±0.2ab	4.5±0.2ab	13.±2±0.8ab
V6	1.4±0.2ab	3.8±0.7ab	3.2±0.7b	9.0±2.5b
V7	1.6±0.1ab	4.7±0.3ab	3.7±0.4ab	10.9±1.6ab
V8	1.7±0.2a	5.5±1.2a	5.7±1.1a	18.0±4.8a
V9	1.5±0.1ab	4.3±0.7ab	3.6±0.8ab	10.4±3.1ab
V10	1.0±0.0b	2.7±0.2b	2.3±0.3b	6.2±0.9b
V11	1.4±0.2ab	3.9±0.9ab	2.7±0.7b	7.7±2.2b

Note: values ± s.e. followed by different letters present significant differences according to LSD ($p < 0.05$). Legend: Int - Intensification niche, Exp - Expansion niche, Narr - Narrowing niche, Contract - Contraction niche; Sum - the total recorded sum of activities in each functional niche, Index of - The index of general changes in community assemblage pattern recorded in each functional niche.

In contrast, variants V3, V4, and V6 show relatively similar values, but slightly lower than V2. The application of mineral fertilizers (N, P, and NPK) contributes to maintaining an active, more uniform, and balanced microbiome in soils treated in the long term. Although microbial

diversity reacts quickly to environmental changes, a functional balance and long-term adaptability are maintained within microbial communities. At the same time, the NP fertilizer complex in variant V10 (L2+NP) produces values like those observed previously, so this combination of inputs has a synergistic downgrade effect comparable to that observed in variant V6.

Significant decreases are registered in the V8 variant, fertilized with N, associated with an intensively applied amendment (L2). Under these conditions, the total microbial diversity is reduced, and within the microbial communities, there is a decrease in the microbiome involved in decomposition processes.

In the case of variants V5 (L1+NP) and V3 (L1+N), there is also a slight reduction in the microbiome associated with the phenomenon of narrowing and contraction, but the effect is less pronounced compared to V8.

At the same time, variants V7 and V9 (P as mineral fertilizer), characterized by the application of an intensive amendment (L2), show a relatively homogeneous dispersion of the contraction phenomenon. This uniform distribution suggests that the application of L2, both individually and in combination with P, contributes to the similar manifestation of this pattern in the analysed variants.

The intensification segment in microbial communities is highlighted by a star-like distribution in the NMDS projection (Figure 1). The carbohydrate vector shows an increase, based on the amplified microbial activity in the presence of nitrogen and organic amendments, regardless of their intensity. The intersection of this vector with phosphorus and the NP combination suggests an intensification in carbohydrate guild when additional mineral inputs are available.

Nitrogen treated communities are distributed pointwise, with a stellar structure characterized by unequal arms, ranging from medium to intensive organic amendments, with obvious differences depending on the microbial guild. Thus, microbial communities intensify their carbohydrates and polymers metabolism, while amine-associated activity is absent. In contrast, fertilization with NP and NPK has a significant effect on soil microorganisms, visibly stimulating the rate of amine guild metabolism.

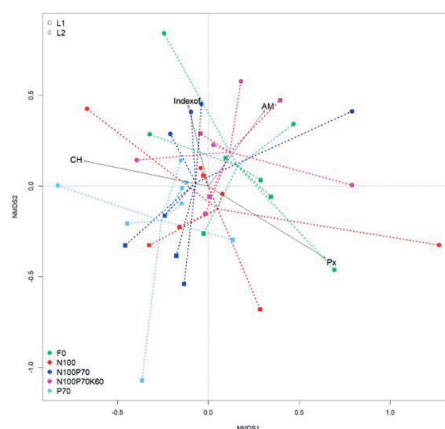


Figure 1. Functional microbiome dispersion in Intensification niche

Microbial communities associated with the guild of amines and polymers show a pronounced overlap and intensified metabolic activity in the absence of mineral fertilizers, but under conditions of organic amendments at both medium and intensive levels. In soils fertilized with NPK, microbial communities generate multiple intersection points across the entire NMDS projection, crossed by both polymers and amines vectors. This dynamic indicates an acceleration in the metabolism of these two guilds, associated with subtle but relevant functional changes in the structure of microbial communities.

The vector associated with the carbohydrate guild (CH) is the only one that significantly exhibits the phenomenon of expansion (Figure 2). This is an important image provided by the NMDS analysis, highlighting the dominant guild that expands along the microbial diversity gradient, consisting of a microbiome capable of metabolizing carbohydrates and amplifying its functional activity.

The absence of mineral fertilizers, in the context of the sole application of amendments L1 and L2, has a significant effect on soil microbiome diversity, as microorganisms are strongly and positively influenced by the CH vector. This process leads to a marked increase in microbiome diversity. The CH vector overlaps three distinct branches, corresponding to a gradient of amendment intensity, ranging from medium to intensive.

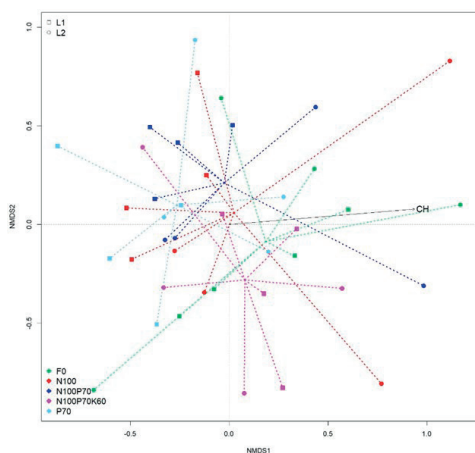


Figure 2. Functional microbiome dispersion in Expansion niche

Within this unique vector, it is also observed that the application of N and NP treatments leads to the formation of an extremely active microbiome, and the expansion of microorganisms involved in the decomposition of organic matter has a synergistic effect on the functionality of the soil ecosystem.

In the context of narrowing, microorganisms from the carbohydrate and amine guild vectors overlap directly with L1 and NP complexes (Figure 3). The carbohydrate-associated vector (CH) also interacts with the structure of microbial communities shaped by N, P, and NPK treatments. Given that the centroid of the NP complex also overlaps with that of NPK, suggest that the microorganisms responsible for the degradation of organic matter have a uniform and comparable effect in both types of fertilization systems.

In contrast, the application of amendments at an intense level significantly interferes with the amine vector, suggesting that high amendment input favours the formation of a microbial community like that developed under the influence of NP and NPK mineral input complexes. This indicates a convergence of microbial structure in response to intensified amendments.

The cumulative activity of microorganisms is modified by the long-term application of mineral fertilizers, which is reflected in a gradual decrease in metabolic activity associated with adaptation processes to a constant nutrient supply.

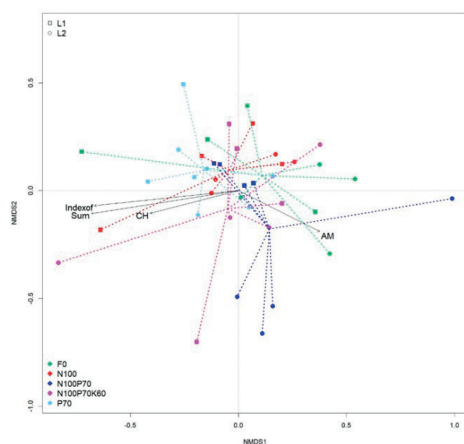


Figure 3. Functional microbiome dispersion in Narrowing niche

The NMDS of the contraction phenomenon show a more dispersed microbiome at a similar pattern with the narrowing (Figure 4). The CH vector from overlaps with the microbiome of unfertilized soils, but amended both moderately and intensively, through four points that intersect this vector. Under these conditions, the diversity of dominant microorganisms involved in the decomposition of organic matter is significantly reduced. An additional synergistic effect is exerted by N and NP fertilization, which leads to a decrease in diversity and, implicitly, in the activity of microorganisms in agricultural soils subjected to long-term fertilization.

Also, the base of the CH vector is positioned at a very short distance from the NPK complex, which indicates a close association between these factors. The proximity suggests that the presence of complete NPK fertilization further contributes to the inhibition of microbial community activity, through nutritional imbalances and changes in the physical-chemical conditions of the soil.

NMDS analysis reveals a star-like distribution of points, indicating the existence of relatively high structural microbial diversity. However, the reduced dispersion and partial overlap of the groups suggest a decrease in the abundance of microorganisms, as well a direction through a functional homogenization in communities, especially in soils affected by intense and long-term fertilization.

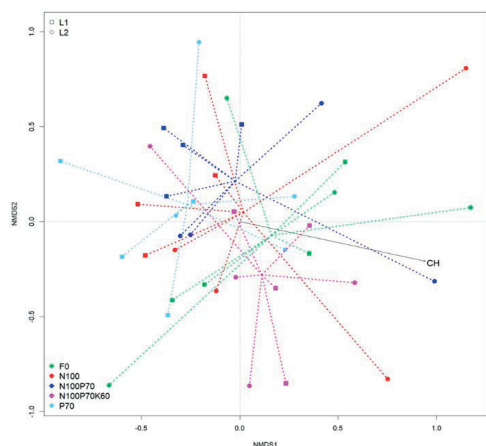


Figure 4. Functional microbiome dispersion in Contraction niche

Long-term fertilization, within the proliferation/decline phenomena, allows data to be divided into 4 quartiles (Q1-Q4) based on the general functional alteration, allowing the

evaluation of the microbial response to applied treatments (Figure 5). This phenomenon scores the functionality of the microbiome from low to high values.

Q1 and Q2 represent the quartiles with higher values, both in terms of positive and negative values recorded in microbial metabolism. On the other hand, Q3 and Q4 are represented by the quartiles with the lowest values, both positive and negative.

In the variants subjected to moderate amendment treatments, it can be observed that, in the upper quartile (Q1), variants V2 and V3(+N) reach a maximum of metabolic activity and diversity. Variant V2, which is subjected only to limiting inputs of 2.5 t ha⁻¹, records a maximum of 26% in Q1.

Q4 records, in 5 variants, represent functionally reduced values, with positive values between 0.43 and 5.81, both recorded in L2, while the maximum value was recorded in V8 and the minimum value in V9.

		L1					L2					
		V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	
+	Q1	26.69 + 20.03	26.69	22.21								
	Q2	20.02 + 13.35									15.81	
	Q3	13.34 + 6.68		10.30 7.73		13.20			7.65	7.79 9.82		
	Q4	6.67 + 0.00	5.38	0.54		3.48		5.81	0.43			
-	Q4	-0.00 - -6.68	-1.87 -4.27	-3.44	-2.24	-4.06	-5.06	-2.86 -5.88	-2.64	-2.00	-1.66 -2.41	-1.33
	Q3	-6.69 - -13.36			-10.11 -8.08 -7.71	-12.11	-11.36 -12.58				-7.09 -8.08	
	Q2	-13.37 - -20.05		-15.11				-17.66	-18.73			
	Q1	-20.06 - -26.73	-21.19					-26.73				

Figure 5. Microbial response as total functional alteration compared to native community.

Legend: Table representation by quartiles: Q1 - high values; Q2 - medium-high values; Q3 - medium-low values; Q4 - low values

Q3 predominates in variants V4, V6, V9, and V10, with semi-reduced values. Two approximately equal values are observed between V4, treated with medium-level amendment and fertilized with P, and V10, amended at an intensive level and treated with NP, which does not generate significant

differences between treatments in terms of the decomposer microbiome.

Regarding the lower negative quartiles, there is a general downward trend in microbial activity associated with specific treatments. Thus, in V5, fertilized with NP, activity decreases especially in Q3 and Q4. In contrast, in V6, amended at a

medium level and fertilized with NPK, there is a maximum decrease in activity in Q3. For V7, amended at an intensive level, there are significant decreases in activity in Q3, with values remaining low in Q4.

In the negative Q4 there is homogeneity between values, ranging from -1.33 in V11, treated intensively with CaCO_3 + NPK, to -5.88 in V7, treated only with intensive amendment. Metabolic activity shows a slight decrease in all variants analyzed. An interesting aspect is that this trend is present in all variants, the functional microbiome is influenced by long-term inputs. This distribution of the functional microbiome responds differently depending on the type and intensity of the treatments applied, and the effects are maintained in the long term, demonstrating that proper management of fertilization and amendments is vital for maintaining the balance and functionality of the soil microbiome.

Variant V8, intensively treated with CaCO_3 and fertilized with N, showed the highest decrease in Q1, reaching 26%, in the metabolic activity of the functional microbiome responsible for decomposition. A second case is represented by V3, amended at a medium level and fertilized with N, which showed a slight decrease of 5% from the initial value (-21%). The application of these inputs leads to some of the lowest values of metabolic activity, generating significant changes in the structure of the microbiome, from generalist species to more specialized species (Ren et al., 2023). As a general image on how soil microbiome change its functionality due to long-term treatment application, the intensification and expansion represent the processes by which microorganisms grow in number and activity, and their diversity expands, stimulated by favorable conditions, such as the application of a moderate amount of fertilizer, resulting in more intense metabolic activity and more varied communities (Dincă et al., 2022). On the other hand, contraction and narrowing represent the opposite process whereby the diversity of microbial communities shrinks and becomes more selective: certain dominant groups continue to function, while others decline in activity or disappear, reducing the diversity and functionality of the agricultural soil microbiome (Coban et al., 2022).

Metabolic plasticity provides a new way to differentiate generalist microorganisms from specialist ones (Chen et al., 2021). In microbial biology, generalists and specialists are often defined by their frequency in different habitats: microbes that show up in lots of environments have a broad metabolic niche and are considered generalists, while those with limited distribution have a narrower metabolic niche and are considered specialists (Liao et al., 2016). Generalist microorganisms have a wide distribution and a large metabolic niche, and can adapt their metabolism to various environmental conditions, while specialists, with a restricted distribution and a smaller metabolic niche, have fewer biological mechanisms for adaptation (Malard & Guisan, 2023; Vidican et al., 2025). Using the DEMSA model, it was beneficial to refine the biological framework related to organic matter decomposition for the specific objectives of our study. Since the model describes general functional microbial responses, we introduced more specific links between microbial indices and the stages of the decomposition process of wheat straw. Thus, microbial indices were correlated with the complex dynamics of organic matter decomposition to improve the interpretation and visualization of the dataset and to better highlight functional microbial diversity and activity in long term fertilized soil.

CONCLUSIONS

Fertilization and liming influence soil microbiome functioning, generating variations in metabolic activity reflected in quartile distributions. Moderate liming stimulates the diversity and density of microbial activity, particularly in Q1 and Q2, while intensive applications reduce activity in Q3 and Q4 and cause negative values, showing a decrease in the functionality of microorganisms involved in the decomposition of organic matter.

Moderate amendment (L1) stimulates the expansion and intensification of soil microbial communities, leading to higher functional diversity and more active decomposition processes. Soil pH correction supports a stable and balanced microbiome, while intensive amendment (L2) tends to select specialized microorganisms.

Application of mineral fertilizers (N, P, NP, NPK) leads to narrowing and contraction of microbial diversity. Changes in nutrient availability, along with modifications in soil chemistry, encourage the selection of dominant microbial groups, causing a decline in overall functional diversity.

The DEMSA model shows how long-term fertilization changes the microbiome, shifting from generalist microbes, which can adapt to various conditions, to specialist microbes, which are better suited to a specific environment. This transition indicates that inputs not only affect which species live in the soil, but also how they adapt their metabolism to the available resources.

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REFERENCES

- Bhattacharjee, A., Dubey, S., & Sharma, S. (2022). Storage of soil microbiome for application in sustainable agriculture: prospects and challenges. *Environmental Science and Pollution Research*, 29(3), 3171-3183.
- Chang, C. Y., Bajić, D., Vila, J. C., Estrela, S., & Sanchez, A. (2023). Emergent coexistence in multispecies microbial communities. *Science*, 381(6655), 343-348.
- Chen, Y. J., Leung, P. M., Wood, J. L., Bay, S. K., Hugenholtz, P., Kessler, A. J., ... & Greening, C. (2021). Metabolic flexibility allows bacterial habitat generalists to become dominant in a frequently disturbed ecosystem. *The ISME Journal*, 15(10), 2986-3004.
- Coban, O., De Deyn, G. B., & van der Ploeg, M. (2022). Soil microbiota as game-changers in restoration of degraded lands. *Science*, 375(6584), abe0725.
- de Mendiburu, F. (2023). Statistical Procedures for Agricultural Research. *R package version 1.3-7*, <https://CRAN.R-project.org/package=agricolae>.
- Dincă, L. C., Grenni, P., Onet, C., & Onet, A. (2022). Fertilization and soil microbial community: a review. *Applied Sciences*, 12(3), 1198.
- Ge, G., Li, Z., Fan, F., Chu, G., Hou, Z., & Liang, Y. (2010). Soil biological activity and their seasonal variations in response to long-term application of organic and inorganic fertilizers. *Plant and Soil*, 326(1), 31-44.
- Gessner, M. O., Swan, C. M., Dang, C. K., McKie, B. G., Bardgett, R. D., Wall, D. H., & Hättenschwiler, S. (2010). Diversity meets decomposition. *Trends in ecology & evolution*, 25(6), 372-380.
- Gheorghită, A., Pleșa, A., Pop, B., Stoian, V., & Vidican, R. (2026). Shifts in Straw-Associated Functional Microbiomes Under Long-Term Soil Management. *Microbiology Research*, 17(3), 51.
- Gheorghită, A., Vidican, R., Corcoz, L., & Stoian, V. (2024). Kinetics of the microbial functional profile involved in decomposition shaped by long-term application of inputs. *Scientific Papers. Series A. Agronomy*, 67(1).
- Gonzalez, J. M., & Aranda, B. (2023). Microbial growth under limiting conditions-future perspectives. *Microorganisms*, 11(7), 1641.
- Hartmann, M., & Six, J. (2023). Soil structure and microbiome functions in agroecosystems. *Nature Reviews Earth & Environment*, 4(1), 4-18.
- Islam, W., Noman, A., Naveed, H., Huang, Z., & Chen, H. Y. (2020). Role of environmental factors in shaping the soil microbiome. *Environmental Science and Pollution Research*, 27(33), 41225-41247.
- Khatoon, H., Solanki, P., Narayan, M., Tewari, L., Rai, J. P. N., & Hina Khatoon, C. (2017). Role of microbes in organic carbon decomposition and maintenance of soil ecosystem. *International Journal of Chemical Studies*, 5(6), 1648-1656.
- Liao, J., Cao, X., Zhao, L., Wang, J., Gao, Z., Wang, M. C., & Huang, Y. (2016). The importance of neutral and niche processes for bacterial community assembly differs between habitat generalists and specialists. *FEMS Microbiology Ecology*, 92(11), fiw174.
- Liu, M., Hu, F., Chen, X., Huang, Q., Jiao, J., Zhang, B., & Li, H. (2009). Organic amendments with reduced chemical fertilizer promote soil microbial development and nutrient availability in a subtropical paddy field: The influence of quantity, type and application time of organic amendments. *Applied Soil Ecology*, 42(2), 166-175.
- Luo, G., Rensing, C., Chen, H., Liu, M., Wang, M., Guo, S., ... & Shen, Q. (2018). Deciphering the associations between soil microbial diversity and ecosystem multifunctionality driven by long-term fertilization management. *Functional Ecology*, 32(4), 1103-1116.
- Macias-Benitez, S., Garcia-Martinez, A. M., Caballero Jimenez, P., Gonzalez, J. M., Tejada Moral, M., & Parrado Rubio, J. (2020). Rhizospheric organic acids as biostimulants: monitoring feedbacks on soil microorganisms and biochemical properties. *Frontiers in plant science*, 11, 633.
- Malard, L. A., & Guisan, A. (2023). Into the microbial niche. *Trends in Ecology & Evolution*, 38(10), 936-945.
- Maron, P. A., Sarr, A., Kaisermann, A., Lévêque, J., Mathieu, O., Guigue, J., ... & Ranjard, L. (2018). High microbial diversity promotes soil ecosystem functioning. *Applied and Environmental Microbiology*, 84(9), e02738-17.
- Oksanen J, Simpson G, Blanchet F, Kindt R, Legendre P, Minchin P, O'Hara R, Solymos P, Stevens M, Szoecs E, Wagner H, Barbour M, Bedward M, Bolker B, Borcard D, Carvalho G, Chirico M, De Caceres M, Durand S, Evangelista H, FitzJohn R, Friendly M, Furneaux B, Hannigan G, Hill M, Lahti L, McGlinn D, Ouellette M, Ribeiro Cunha E, Smith T, Stier A,

- Ter Braak C, Weedon J (2022). Community Ecology Package. *R package version 2.6-4*, <https://CRAN.R-project.org/package=vegan>
- Ortega, R., Miralles, I., Domene, M. A., Meca, D., & Del Moral, F. (2024). Ecological practices increase soil fertility and microbial diversity under intensive farming. *Science of The Total Environment*, 954, 176777.
- Phale, P. S., Basu, A., Majhi, P. D., Deveryshetty, J., Vamsee-Krishna, C., & Shrivastava, R. (2007). Metabolic diversity in bacterial degradation of aromatic compounds. *Omic: a journal of integrative biology*, 11(3), 252-279.
- Poyraz, N., Sezen, S., & Mutlu, M. B. (2021). Potential of enzymatic methods and Biolog ecoplate analysis for investigation of microbial functionality in agricultural soils. *Journal of Scientific Reports-A*, 047, 218-234.
- R Core Team (2024). R: A Language and Environment for Statistical Computing. *R Foundation for Statistical Computing*, Vienna, Austria. <https://www.R-project.org/>.
- Revelle, W. (2024). Procedures for Psychological, Psychometric, and Personality Research. Northwestern University, Evanston, Illinois. *R package version 2.4.3*, <https://CRAN.R-project.org/package=psych>
- Soares, M., & Rousk, J. (2019). Microbial growth and carbon use efficiency in soil: Links to fungal-bacterial dominance, SOC-quality and stoichiometry. *Soil Biology and Biochemistry*, 131, 195-205.
- Ren, Y., Ge, W., Dong, C., Wang, H., Zhao, S., Li, C., ... & Han, Y. (2023). Specialist species of fungi and bacteria are more important than the intermediate and generalist species in near-urban agricultural soils. *Applied Soil Ecology*, 188, 104894.
- Stoian, V., Vidican, R., Florin, P., Corcoz, L., Pop-Moldovan, V., Vaida, I., ... & Pleșa, A. (2022). Exploration of soil functional microbiomes—A concept proposal for long-term fertilized grasslands. *Plants*, 11(9), 1253.
- Vidican, R., Stoian, V., Corcoz, L., Pleșa, A., Pop, B., & Gheorghită, A. (2025). Increased activity of specialized functional microbiomes in historically polluted soils after bioremediation. *Scientific Papers. Series A. Agronomy*, 68(1), 225-232.
- Vidican, R., Stoian, V., Șandor, M., Ozunu, A., Nistor, I. A., & Petrescu, D. C. (2017). Fertilization and Pesticides as Elements of Pressure on Microbial Communities. *Resilient Society*, 229. 229-241.
- Wang, X., Feng, J., Ao, G., Qin, W., Han, M., Shen, Y., ... & Zhu, B. (2023). Globally nitrogen addition alters soil microbial community structure, but has minor effects on soil microbial diversity and richness. *Soil Biology and Biochemistry*, 179, 108982.
- Yang, Y., Long, Y., Li, S., & Liu, X. (2023). Straw return decomposition characteristics and effects on soil nutrients and maize yield. *Agriculture*, 13(8), 1570.
- Zhang, N., Nunan, N., Hirsch, P. R., Sun, B., Zhou, J., & Liang, Y. (2021). Theory of microbial coexistence in promoting soil-plant ecosystem health. *Biology and Fertility of Soils*, 57(7), 897-911.
- Zhang, S., Zhu, Q., de Vries, W., Ros, G. H., Chen, X., Muneer, M. A., ... & Wu, L. (2023). Effects of soil amendments on soil acidity and crop yields in acidic soils: A world-wide meta-analysis. *Journal of Environmental Management*, 345, 118531.