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THE USE OF COLONY FORMING UNIT AS INDICATOR OF DECOMPOSITION MICROBIOME – A MODIFIED LITTERBAG APPROACH

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Abstract. *Organic matter decomposition is an important process that assures the recycling of nutrients in agroecosystem soils. This research aimed to assess the presence of decomposition-associated microorganisms on both the organic material and the bag surface, considering them as a buffer between the soil and organic matter. The study of the organic matter decomposition process was conducted using a modified litterbag method, based on tea bags filled with chopped straw, placed in an experimental field exposed to long-term fertilization. Serial dilutions were performed on both the straw material and the surface of the bag that came into contact with the soil and decomposing plant matter. The development of microorganisms increased progressively at 24, 48, and 72 hours, but a maximum CFU (mixed colonies) was recorded in variant 9 for the plant biomass. To this variant intensive soil amendment (5 t/ha) was applied with the addition of phosphorus as a mineral fertilizer, while the other variants ranged from values below 100 to just over 600 colonies. Slightly lower values were recorded in the buffer, where the quantity of fine particles from both soil and straw is lower, which explains the decrease in the number of colonies. The phenomenon of confluence appears in variants 3, 4, and 5, in both the plant biomass and the buffer material in more than half of the plates. This phenomenon led to the unification of microbial colonies, which reduced their visibility and individuality, this process being observed particularly between 48 and 72 hours of incubation. The dynamics of organic matter decomposition are closely associated with the activity of soil microorganisms involved in the transformation and recycling of nutrients. Colony-forming units serve as a useful indicator for evaluating this activity, offering information about the microbial response to various input additions.*

Keywords: *organic matter decomposition, long-term fertilization, microbial colonies, CFU, confluence phenomenon*

INTRODUCTION

In soil, microorganisms (bacteria and fungi) are involved in the decomposition of organic matter, a process that releases nutrients to support plant growth (Vieira & Nahas, 2005). The microbiota composition differs between soil types and depending on soil conditions, with bacterial species being the most numerous (Li et al., 2022).

Chemical fertilizers are important for increasing agricultural production and ensuring food security, but they can have negative effects on the soil and the environment when used in excess (Jote, 2023; Ugwuoke et al., 2024). In the long term, fertilization changes the chemical and physical properties of the soil and affects how microorganisms develop, making it important to understand the mechanisms underlying these changes to protect the biological stability of the soil (Bai et al., 2020). Around 30% of the world's agricultural soil is acidic, which limits the availability of nutrients like nitrogen and phosphorus to plants (Gurmessa, 2021). Adding calcium carbonate can promote microbial decomposition and the release of nutrients in the soil. Soil microorganisms regulate soil structure, energy flow, and nutrient cycling, and changes in microbial communities can affect these processes (Xu et al., 2023).

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Studies by Dou et al. 2023, show that $CaCO_3$ content significantly affects the distribution and stability of soil aggregates.

Microbial biomass develops as organic carbon levels increase, and organic inputs consistently support this development (Prommer et al., 2020). In addition, the use of organic or inorganic fertilizers often causes changes in the structure of the microbial community in the soil (Marschner et al., 2003). In soils, carbon occurs in both organic and inorganic forms and is mainly stored in vegetation, animals, and microorganisms. This process includes four major steps: photooxidation, leaching, fragmentation and mineralization (Almendros & González-Pérez, 2025).

Colony-forming units (CFU) serve as an indicator for assessing the health of agricultural soils (Papp, 2016) especially in the case of lands that have been subjected to intensive treatments and exploitation involving inorganic inputs, especially N, P, K, or combinations of them. This indicator can be used to quantify the level of microbial diversity as well as the activity of microorganisms in the studied soil, which are important factors in maintaining soil fertility and microbiological balance (Rathore & Srinivasulu, 2022). Excessive use of chemical fertilizers over a long period can lead to a microbial imbalance in the soil (Pahalvi et al., 2021). Determining colony-forming units is an accessible and relatively inexpensive method that can be useful for farmers (Sieuwerts et al., 2008).

The decomposition process of straw returned to the field can significantly influence the amount of mineralized organic carbon (Jin et al., 2020). Different fertilization practices can alter the soil microbiome, but their long-term impact on microbial metabolic processes remains poorly understood. Understanding the impact of fertilization on soil microbiomes could help promote the efficient use of fertilizers and the development of sustainable and environmentally responsible agriculture (Verma et al., 2023). Long-term fertilization and the use of soil liming profoundly influence soil microbial communities, which are responsible for nutrient recycling processes and maintaining soil health. Long-term fertilizer experiments provide a unique opportunity to understand how interactions between soil, plants, and agricultural practices shape the structure and function of the soil microbiome (Verma et al., 2023).

The aim of this study is to examine the CFU of microbial colonies in soil that facilitates the decomposition of organic matter in soil subjected to long-term fertilization.

Identifying the phenomenon of confluence is an important step in evaluating this indicator of agricultural soil health. Thus, the comparison between fertilized soils and those liming, both moderately and intensively, with $CaCO_3$, as well as with native soils, demonstrates the relevance of CFU studies in plant matter decomposition, which can supplement or even reduce the use of inputs in agricultural ecosystems.

MATERIALS AND METHODS

The study was conducted in a long-term fertilization experimental field at the ARDS Livada in Satu Mare County. To study the decomposition processes of wheat straw, modified litterbag experiments were used, particularly teabags inoculated with wheat straw, based on the model proposed by Gheorghiță et al. (2025) and Gheorghiță et al. (2026). In this research, the soil used for serial dilutions was replaced with plant material in the decomposition stage, collected after 30 days incubation period from 11 variants (Table. 1). This plant material and buffer material was subsequently homogenized and progressively diluted until a dilution of 10^{-4} was obtained.

Table 1.

Experimental design and factors involved in decomposition of organic matter

	L0	L1 – 2.5 t ha^{-1}					L2 – 5 t ha^{-1}				
F (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

From each dilution, 10 µL was inoculated on Petri dishes containing PDA (Potato Dextrose Agar) culture medium. This type of medium is frequently used due to its nutrient composition, like carbohydrates derived from potato extract and dextrose, which promote the growth of fungi and other microorganisms involved in the decomposition process.

After incubation at 25 degrees Celsius for 24, 48, and 72 hours, individual microbial colonies developed on the surface of the medium, each considered to originate from a colony-forming unit (CFU), a group of cells capable of proliferating and forming visible microbial colonies on the surface of the medium.

The number of CFUs was determined by counting the colonies on the plates. The numbering was performed both manually, by directly observing the colonies and marking them on the back of the Petri dish to avoid double counting and using a manual colony counter. This device facilitates the colony counting process by using a lighting system and a pen that allows each visible colony on the Petri dish to be touched, eliminating the need to count the number as in the manual method.

The colony counting protocol recommends considering only plates containing between 30 and 300 colonies. To expand the analysis and obtain a more detailed picture within a long-term fertilization experiment, we also included in the evaluation plates that exceeded the indicated maximum limit by more than three times, thereby aiming for greater accuracy in the results obtained.

RESULTS AND DISCUSSIONS

The analyzed biomass showed high values at the 24, 48, and 72h in V9, which was subjected to intensive treatment with 5 t/ha $CaCO_3$ and mineral P fertilization (Table 2). This variant shows significant results at all three incubation time points within the microbial community involved in the decomposition of organic matter in agricultural soil. Phosphorus (P) has a significant impact on the decomposition microorganisms, acting as a part that promotes increased diversity, close to the applied soil liming. At the same time, it helps adjust the soil's pH from slightly acidic to neutral, creating more favorable conditions for the development of healthier agricultural crops.

The growth dynamics of microbial colonies at 24 hours indicate intense development activity as early as the first day of incubation. In most of the plates, bacterial colonies appear first and tend to dominate the surface of the culture medium, highlighting a rapid ability to adapt and multiply under experimental conditions. Between 48 and 72 hours, microbial confluence occurs over a large area of the incubated plate, covering more than 50% of it.

This merging or overlapping of microbial colonies makes it difficult to count the viable colonies present on the plates, which can lead to evidently lower values compared to those recorded on the first day, characterized by intense activity and well-defined colonies.

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In the analysis of plant biomass sustaining decomposition, higher values were observed at level L2, characterized by intensive liming equally applied to all variants, from V7 to V11. The acceleration of the decomposition process stimulated microbial activity, thereby contributing to a strong increase in the decomposing microbiome.

The level of liming applied is a decisive factor in shaping the microbial profile of an agricultural ecosystem. A slight neutralization of soil reaction promotes a significant increase in microbial diversity, as evidenced by CFU analysis.

CFU is an indicator used in modern agriculture, holding significant value for the assessment of agricultural ecosystems. Through this analysis, changes in the soil's microbiological balance caused by fertilization practices, the application of amendments, or other environmental factors can be identified. Thus, farmers can make more informed decisions regarding soil management, fertilization optimization, and long-term fertility maintenance. Additionally, this parameter contributes to assessing the soil's capacity to support processes, such as organic matter decomposition and nutrient cycling, that are important in the development of sustainable and productive agricultural systems.

In contrast, the V4 treatment shows low values within 24–48 hours. This treatment is treated with L1, a soil amendment applied at a moderate rate (2.5 t/ha $CaCO_3$) and is also fertilized with P. It is observed that liming appears to have a significant impact, as the average dose proves less effective in supporting microbial diversity in the analyzed soils.

Over the 72-hour period, the lowest value is recorded in variant V5, which is also limed at a medium level (L1) but supplemented with an NP mineral complex. This variant shows slightly lower values compared to the 48-hour period, a decrease in microbial activity over time under these experimental conditions.

The control variant (V1) records high values, comparable to some of the variants treated with various inputs, both organic and inorganic form. The dynamics change positively at each 24-hour interval, with the colonies being well-defined and without the phenomenon of microbial confluence, indicating a more generalist microbiome. On the other hand, the native variant can support a more diverse microbial community, which is not influenced by the addition of external inputs. In the previously analyzed cases, both in situations with high values and in those with low values, a trend toward a more selective microbiome is observed, a factor that favors the occurrence of the confluence phenomenon.

Table. 2

Analysis of biomass on the microbial soil community

B	Var	24h	48h	72h
Control	**V1**	237.75±123.67b	289.50±118.73ab	308±123.74ab
L1	**V2**	209.75±81.97b	231±85.6ab	236.75±84.34ab
	V3	85.75±23.45b	201.50±50.29ab	166.75±53.75b
	V4	24.25±6.8b	90.25±19.92b	89.50±26.47b
	V5	59.50±23.18b	131.75±34.31b	84.25±18.45b
	V6	208.25±80.71b	156±44.65ab	174±40.11b
L2	**V7**	191±29.74b	389.75±183.96ab	290±69.82ab
	V8	209.50±123.97b	182±61.56ab	256.75±126.06ab
	V9	686.50±355.69a	492.75±319.17a	562.50±319.29a
	V10	351.25±62.75ab	388.25±67.61ab	396.50±68.59ab
	V11	303.50±33.28ab	350.25±106.53ab	355.25±106.75ab

The polypropylene bag, placed in the soil along with plant material in a slow-release fertilization system, acts as a buffer material. It comes into contact with both the plant material contained within and the fine soil particles, providing useful information about how the particles adhere to the plant material, as well as about the interaction and transition between it and the soil.

The maximum values recorded at 24 and 48 hours indicate that V6 (Table. 3), fertilized with NPK and subjected to a moderate level of soil amendment, exhibits these peaks. During these two-time intervals, no microbial confluence is observed, suggesting that the microbiome remains well-defined and that the functional community is diverse and dynamic.

At 72 hours, the buffer capacity reached its maximum value in V7, which was treated exclusively with a high dose of organic amendment. Therefore, the transition between fine soil particles and decomposing plant material may lead to increased values in intensively fertilized soils.

On the other hand, sample V5, fertilized with an NP complex and treated with a moderate dose of amendment, exhibits the lowest values across all three analyzed time intervals (24, 48, and 72 hours), though a slight microbial interaction is observed between 48 and 72 hours. This onset of the confluence phenomenon leads to the emergence of a more specialized microbiome, which comes to dominate the analyzed sample. Some microorganisms reach their maximum activity at 48 hours, followed by a transition toward this microbial confluence, in which the adhesion and semi-adhesion processes of the colonies favor the formation of more specialized microbial communities, influenced by the type of inputs added to the soil.

The phenomenon of microbial confluence may occur depending on the microbiome present in the different fertilized soils. This observed phenomenon typically sets in within approximately 48 hours and manifests as the formation of colonies appearing as white, opaque, or slightly translucent films on a well-defined area of the analyzed surface.

Table. 3

Analysis of buffer-material on the microbial soil community

P	Var	24h	48h	72h
Control	**V1**	271.75±60.15ab	273.25±101.08a-c	284.75±99.69a-c
L1	**V2**	194.50±32.83a-d	212±30.61a-d	217.75±30.98a-d
	V3	103.75±35.53b-d	142.25±33.57b-d	129.25±46.29b-d
	V4	80.75±15.6cd	122.75±17.85cd	97.25±12.58cd
	V5	17.25±6.73d	61.25±14.96d	54.75±13.83d
	V6	298.50±82.46a	338.25±85.63a	356.25±88.85a
L2	**V7**	211.75±52.71a-c	367.75±17.23a	422±69.11a
	V8	173.25±76.37a-d	188.25±45.97a-d	255.75±77.26a-d
	V9	232±58.9a-c	308.25±48.53a-c	347±64.87ab
	V10	214.50±55.94a-c	318.75±64.74ab	329.75±67.57ab
	V11	154.50±69.14a-d	187.75±64.2a-d	230.50±71.25a-d

Increasing $CaCO_3$ content contribute to a gradual decrease in aggregate size and stability in calcareous soils. An increase in $CaCO_3$ content affected the structure and quality of soil organic carbon and changed the microbial community. Overall, microbial diversity decreased, and the way microorganisms interact with soil aggregates was altered. This influenced how organic carbon is distributed and stabilized in different soil fractions. Soil functioning is closely linked to how complex and connected microbial networks are. Soil microorganisms engage in intricate interactions, both direct and indirect, positive and negative.

The phenomenon of confluence is frequently observed in CFU-type assays, particularly in Petri dishes where a specific bacterial species comes to dominate the entire surface. A particular species proliferates rapidly and intensively, thus dominating the entire area of the culture medium after a short period of incubation (Davis et al., 2005). As a result of this rapid growth, individual bacterial cultures no longer remain distinct but tend to combine with each other, forming a continuous film with a relative homogeneous aspect, ranging from translucent to opaque depending on the incubation time and period.

Multiple growth scenarios were observed on Petri dishes for both types of experimental substrates (biomass and polypropylene bag). One is represented by gradual appearance of new colonies from one 24 h period to another one. This phenomenon was caused by the different potential of microorganisms to peas from latency to the growing stage. Another scenario showed different confluence potentials which alter the growing pattern along the Petri dish and constantly change the dominance positions. Contrary, the high confluence solely reduces the number of counted colonies but asses the area that is covered by them. A mixed scenario shows both confluence and new colonies appearance, which indicates a different growing potential of microbial species.

Medium liming combined with P and NP (V4, V5) induces a fast confluence of biomass-associated microbiome within the first 48 hours, while an increase in liming dose drastically reduces this potential for the same type of fertilizers. Native microbiomes show a reduced confluence potential, due to its generalist characteristics and there was a 3 times lower confluence potential from 48 to 72 hours. N application, within 72 hours, set the confluence potential up to 20-25% regardless of the amount of liming, while other nutrients act to more than 33% on medium liming (NP, V5) or 23% (P, V9) on high liming.

Table. 4

Mixed analyses (B+P) of microbial communities

		Biomass		**Polyprop**	
	Var	**B1**	**B2**	**P1**	**P2**
Control	**V1**	36.82±15.04b	11.17±6.54ab	38.12±17ab	7.98±5.42ab
L1	**V2**	15.32±4.75b	5.02±3.96b	11.23±4.44b	2.82±0.56b
	V3	158.44±61.63b	21.58±7.29ab	97.8±71.11ab	24.99±4.2ab
	V4	682.37±504.93a	15.5±5.4ab	58.29±15.67ab	24±5.77ab
	V5	252.63±152.56ab	33.69±5.56a	184.41±85.78ab	12.02±6.5ab
	V6	38.63±12.03b	16.54±9.49ab	18.68±8.55b	5.31±1.26b
L2	**V7**	96.02±82.22b	19.6±10.02ab	141.67±94ab	17.13±12.58ab
	V8	40.06±3.15b	25.06±16.51ab	338.66±320.54a	40.7±24.26a
	V9	45.43±15.1b	23.18±16.76ab	58.52±50.05ab	11.72±8.65ab
	V10	10.82±2.03b	2.22±0.79b	83.44±72.51ab	3.32±0.48b
	V11	42.32±16b	2.43±1.49b	35.87±19.29ab	26.65±25.3ab

In Petri dishes, where medium is dominated by fungi, the dynamics are quite different. The powerful growth of the mycelium from the development and extension of the hyphae, contributes to the formation of dense network covering the entire medium. This network can have a limiting effect on bacteria, either by nutrient competition or by acting as a physical barrier. The mycelium inhibits the formation and expansion of bacterial colonies.

A reduction in microbial community and a change of the relationship between microorganisms and soil aggregates were observed, which influenced the distribution and stabilization of organic carbon within different soil fractions (Zhang et al., 2023). Soil functioning is strongly influenced by the organization of microbial networks. Soil microorganisms are implicated in complex, both direct and indirect, positive and negative interactions (Chen et al., 2022). This complexity is not defined by the number of species, but by the way they are connected to one another and support important soil processes, such as the carbon cycle, nutrient storage, and plant resistance. Lime is mainly used to improve the availability of nutrients for plants, but it can also influence soil microorganisms. In turn, changes in microbial activity and community composition can influence plant growth. In most cases, the application of liming increases the levels of organic carbon and other soil nutrients, such as nitrogen.

Organic matter recycling efficiency is directly dependent on both the microbial community composition and the share between fungal and bacterial species (Condron et al., 2010). Due to the ability of soil microorganisms to induce negative interactions toward the species that share the same ecological niche, the decomposition process is directly affected (Zhan, 2024). Bacterial species act to have a more local and in-depth activity, while fungal species can extend their hyphal networks and cover a larger area with the same substrate (Nazir et al., 2009). UFC analysis shows both the number of colonies and their growth dynamics, indicating that species exploit locally the resources or extend their colonies (with confluence potential) to cover a larger area prior to exploitation of substrate. This procedure is useful to determine the generalists from the community based on general culture media or can be extended to specialized cultivation in the presence of organic matter to determine their specialist characteristics (Fried et al., 2010; Ren et al., 2023).

Microbial confluence between the samples analyzed demonstrates that the native variant (Table. 4) shows significant declines between 72 and 48 hours, allowing the specialized microbial community to dominate. V5 fertilized with NP with medium level of liming, recorded a maximum growth of 184 percent, which changes dramatically from 72 to 48 hours with a massive decrease in the result, reaching a value of 12 percent. This radical decrease alters the functioning of the microbiome responsible for the decomposition of organic matter.

An interesting situation is observed in two variants within the 48–24 interval, V3 and V9, both treated with soil lime from medium to intensive and fertilized with phosphorus, achieving a result of 58 percent. No obvious differences in liming levels were observed, and microbial communities were unchanged in both cases.

The V2 sample, tested for 48–24 hours, recorded the lowest value in the entire dataset, demonstrating that simply applying limestone in a moderate dose does not lead to a significant increase in the microbial community.

Analysis of the entire dataset from 72 to 48 indicates a strong influence of the confluence phenomenon across the entire set of variants analyzed, from V1 to V11. This powerful influence of this phenomenon is reflected in a change in microbial activity, which often appears to culminate at 48 hours, followed by a rapid decline or unification at the microbial colony stage.

CONCLUSIONS

In the analysis of plant biomass, V9 (treated with 5 t/ha $CaCO_3$ and phosphorus fertilization) recorded the highest values at all three incubation time points (24, 48, 72 hours), indicating intensive microbial activity and efficient decomposition of organic matter.

The phenomenon of microbial confluence is often observed after 48 hours (V9, biomass) and manifests as the formation of dense colonies and can alter microbial activity, resulting in different functional responses of the microbial species on the incubated plate depending on the applied inputs.

The buffer material shows that V6 (fertilized with NPK and 2.5 t/ha $CaCO_3$) recorded the highest values at 24 and 48 hours without the occurrence of microbial confluence, showing a diverse microbial community. At the 72-hour, the highest values appear in V7 (5 t/ha $CaCO_3$), showing an intense microbial activity.

The application of amendments at various levels and mineral fertilization significantly influences the activity and structure of microbial communities in agricultural soil. The type of fertilization influences the growth dynamics of the microbial communities responsible for the decomposition of organic matter.

BIBLIOGRAPHY

ALMENDROS, G., & GONZALEZ-PEREZ, J. A. (2025). Soil organic carbon sequestration mechanisms and the chemical nature of soil organic matter—A review. Sustainability, 17(15), 6689.

BAI, Y. C., CHANG, Y. Y., HUSSAIN, M., LU, B., ZHANG, J. P., SONG, X. B., ... & PEI, D. (2020). Soil chemical and microbiological properties are changed by long-term chemical fertilizers that limit ecosystem functioning. Microorganisms, 8(5), 694.

CHEN, W., WANG, J., CHEN, X., MENG, Z., XU, R., DUOJI, D., ... & ZHANG, Y. (2022). Soil microbial network complexity predicts ecosystem function along elevation gradients on the Tibetan Plateau. Soil Biology and Biochemistry, 172, 108766.

CONDRON, L., STARK, C., O'CALLAGHAN, M., CLINTON, P., & HUANG, Z. (2010). The role of microbial communities in the formation and decomposition of soil organic matter. In Soil microbiology and sustainable crop production (pp. 81-118). Dordrecht: Springer Netherlands.

DAVIS, K. E., JOSEPH, S. J., & JANSSEN, P. H. (2005). Effects of growth medium, inoculum size, and incubation time on culturability and isolation of soil bacteria. Applied and environmental microbiology, 71(2), 826-834.

DOU, X., ZHANG, J., ZHANG, C., MA, D., CHEN, L., ZHOU, G., ... & DUAN, Y. (2023). Calcium carbonate regulates soil organic carbon accumulation by mediating microbial communities in northern China. Catena, 231, 107327.

FRIED, G., PETIT, S., & REBOUD, X. (2010). A specialist-generalist classification of the arable flora and its response to changes in agricultural practices. BMC ecology, 10(1), 20.

GHEORGHIȚĂ, A., CORCOZ, L., POP, B., PLEȘA, A., STOIAN, V., & VIDICAN, R. (2025). The evolution of microbial functional profile involved in decomposition processes in long-term fertilized experiments. Scientific Papers. Series A. Agronomy, 68(1).

GHEORGHIȚĂ, 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.

GURMESSA, B. (2021). Soil acidity challenges and the significance of liming and organic amendments in tropical agricultural lands with reference to Ethiopia: B. Gurmessa. Environment, Development and Sustainability, 23(1), 77-99.

JIN, Z., SHAH, T., ZHANG, L., LIU, H., PENG, S., & NIE, L. (2020). Effect of straw returning on soil organic carbon in rice–wheat rotation system: A review. Food and Energy Security, 9(2), e200.

JOTE, C. A. (2023). The impacts of using inorganic chemical fertilizers on the environment and human health. Organic and Medicinal Chemistry International Journal, 13(3), 555864.

LI, Y., LI, C., XIN, Y., HUANG, T., & LIU, J. (2022). Petroleum pollution affects soil chemistry and reshapes the diversity and networks of microbial communities. Ecotoxicology and Environmental Safety, 246, 114129.

MARSCHNER, P., KANDELER, E., & MARSCHNER, B. (2003). Structure and function of the soil microbial community in a long-term fertilizer experiment. Soil Biology and Biochemistry, 35(3), 453-461.

NAZIR, R., WARMINK, J. A., BOERSMA, H., & VAN ELSAS, J. D. (2009). Mechanisms that promote bacterial fitness in fungal-affected soil microhabitats. FEMS microbiology ecology, 71(2), 169-185.

PAHALVI, H. N., RAFIYA, L., RASHID, S., NISAR, B., & KAMILI, A. N. (2021). Chemical fertilizers and their impact on soil health. In Microbiota and biofertilizers, Vol 2: Ecofriendly tools for reclamation of degraded soil environs (pp. 1-20). Cham: Springer International Publishing.

PAPP, R. (2016). Soil indicators set up to assess sustainable management in various agricultural and forest ecosystems.

PROMMER, J., WALKER, T. W., WANEK, W., BRAUN, J., ZEZULA, D., HU, Y., ... & RICHTER, A. (2020). Increased microbial growth, biomass, and turnover drive soil organic carbon accumulation at higher plant diversity. Global change biology, 26(2), 669-681.

RATHORE, M. S., & SRINIVASULU, Y. (2022). Soil health sustenance through varied fertility management approaches for increasing microbial load under temperate climatic conditions: a practical approach. In Microbial resource technologies for sustainable development (pp. 319-331). Elsevier.

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.

SIEUWERTS, S., DE BOK, F. A., MOLS, E., DE VOS, W. M., & VAN HYLCKAMA VLIEG, J. E. T. (2008). A simple and fast method for determining colony forming units. Letters in applied microbiology, 47(4), 275-278.

UGWUOKE, C. U., OMEJE, B. A., & EZE, G. E. (2024). Consequences of excessive application of agricultural chemicals on the sustainable environment and food security. International Journal of Agricultural Education and Research, 2 (1) 100, 108.

VERMA, R. C., SINGH, N. K., GANGAVATI, A. R., ASHOKA, P., KESARWANI, A., ALI, I., & PANDEY, S. K. (2023). A review of long-term effects of mineral fertilizers on soil microorganisms. International Journal of Plant & Soil Science, 35(20), 1145-1155.

VERMA, R. C., SINGH, N. K., GANGAVATI, A. R., ASHOKA, P., KESARWANI, A., ALI, I., & PANDEY, S. K. (2023). A review of long-term effects of mineral fertilizers on soil microorganisms. International Journal of Plant & Soil Science, 35(20), 1145-1155.

VIEIRA, F. C. S., & NAHAS, E. (2005). Comparison of microbial numbers in soils by using various culture media and temperatures. Microbiological research, 160(2), 197-202.

YU, X., KEITEL, C., & DIJKSTRA, F. A. (2023). Ameliorating soil acidity with calcium carbonate and calcium hydroxide: effects on carbon, nitrogen, and phosphorus dynamics. Journal of Soil Science and Plant Nutrition, 23(4), 5270-5278.

ZHAN, C. (2024). Microbial decomposition and soil health: Mechanisms and ecological implications. Molecular Soil Biology, 15.

ZHANG, W., MUNKHOLM, L. J., LIU, X., AN, T., XU, Y., GE, Z., ... & WANG, J. (2023). Soil aggregate microstructure and microbial community structure mediate soil organic carbon accumulation: Evidence from one-year field experiment. Geoderma, 430, 116324.