

DIFFERENCES IN MICROBIAL DIVERSITY AND COMMUNITY STRUCTURE AMONG TWO VERMICOMPOST GRANULATIONS

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Abstract

This paper presents the results of the research concerning the influence of two vermicompost granulations, C1 < 2 mm and C2 > 2 mm, obtained by sieving, on bacterial and fungal diversity and community structure. Both bacteria and fungi have colonized the vermicompost granulation C2 > 2 mm with higher counts as compared with C1 fraction. Global microbial activity was expressed by higher values of soil respiration in C2 granulation (123.809 mg CO₂ x 100 g⁻¹soil) as compared to 116.833 mg CO₂ x 100 g⁻¹soil in C1 granulation. A rich bacteria population with high diversity ($H' = 2.104$ bits) and homogeneity ($\epsilon = 0.662$) was found in C2 granulation. C1 granulation contained a less diverse bacteriobiome, with $H' = 1.750$ bits and $\epsilon = 0.594$. Fungi presented lower biodiversity values than bacteria. Conditions from C1 were more favourable than those from C2 granulation ($H' = 0.921$ bits, $\epsilon = 0.324$ in C1 and $H' = 0.403$ bits, $\epsilon = 0.089$ in C2 fraction). Bacterial microflora from C1 granulation was dominated by Bacillaceae, accompanied by Pseudomonas and actinomycetes and a mix of species (Bacillus, Micrococcus, Pseudomonas, Arthrobacter and actinomycetes) in C2 granulation. In both fractions identified strong cellulolytic species (Fusarium, Scopulariopsis, Penicillium, Cladosporium).

Key words: vermicompost; granulation; bacteria; fungi; microbial communities; biodiversity.

INTRODUCTION

Agricultural intensification reduces microbial network complexity and the abundance of keystone taxa (Banerjee et al., 2019). The restoration of microbial diversity in agricultural soils needs utilization of various natural amendments and fertilizers for improving soil physical, chemical and biological properties. On the other hand, research look for sustainable alternatives for management of enormous quantities of organic wastes, with potential benefits for both the environment and agricultural economics. One of them refers to decomposition of organic wastes by vermicomposting, the final product resulting from this process is the vermicompost, an organic biofertilizer utilisable for its beneficial effects on soil properties and plant growth and health. Vermicomposting is defined as the process of biodegradation and stabilization of organic materials, facilitated by the collaborative efforts of earthworms and

mesophilic microorganisms. While microorganisms play a vital role in the biochemical breakdown of organic matter, earthworms contribute by fragmenting and conditioning the substrate (conferring a good aeration and increasing the surface area), which induces significant changes in its microbiota and its biological activity, thus accelerating substrate decomposition (Madhushani et al., 2024). Epigeic earthworms are generally used for organic waste decomposition and they consume microorganisms specially fungi to satisfy their nitrogen requirement, thus modulating microbial communities and biosynthesis of Plant Growth Promoting Biostimulants (PGPBs). In addition to high macro-nutrient content, the microbial properties of vermicompost providing plant growth-stimulating capability could be attributed to the PGPBs, which include humification substances (HS), particularly fulvic acid, components with plant hormones activity (auxin, cytokinin, gibberellin, etc.), protein hydrolysates,

betaines. Increase in microbial biomass and diversity of beneficial microflora (e.g. plant growth-promoting bacteria, actinomycetes and fungi) indicated that vermicomposting facilitates microbial proliferation in final stabilized product (Esakkiammal et al., 2015).

Another recent study on the vermicompost microbiota and the effect of PGPBs on plants growth and health confirmed the biosynthesis of phytohormones: indolil acetic acid (IAA), jasmonic acid (JA) and salicylic acid (SA), and the production of HS (especially fulvic acid), responsible for auxin-like activity of vermicompost (Zhang et al., 2024).

It is well-known that earthworm castings are enriched in main nutrients and calcium humate, the binding agent that prevents desiccation and contributes to the incubation and proliferation of beneficial microbial species, such as *Trichoderma* spp., *Pseudomonas* spp. and vesicular-arbuscular mycorrhizal species. A wide variety of wastes can be utilized as substrates for degradation with various earthworms and microorganisms. Manyuchi et al. (2012) vermicomposted the waste corn pulp blended with cow dung using *Eisenia fetida*. Rupani et al. (2023) vermicomposted green organic wastes using *Eisenia fetida* under field conditions.

Achsa & Prabha (2013) utilized vermicompost produced from banana waste to improve the growth parameters of tomato plants. Vermicomposting of paper cup wastes is a source of cellulolytic bacteria with high ability to produce lytic enzymes for accelerated cellulose decomposition (Karthika et al., 2020). Research on vermicompost microflora and beneficial effect for plants carried out by Maji et al. (2017) found that humic acid rich vermicompost promotes plant growth by improving soil structure, aeration conditions, diversity and biomass of microbial community, root nodulation and mycorrhizal colonization in the roots of peas. Yao et al. (2023) presented the complex role and mechanisms of action of beneficial fungi from genus *Trichoderma*, frequently identified in vermicomposts. Its biological control mechanism of plant fungal and nematode disease included competition, antibiosis, antagonism and mycoparasitism (Kumar et al., 2016), as well as the mechanism of promoting plant growth, inducing plant

systemic resistance and inactivation of the pathogen's enzymes involved in the infection process (Singh et al., 2018; Yan et al., 2021). *Trichoderma* species were reported to produce volatile organic compounds responsible for antimicrobial activity, antibiotics, various enzymes (Joo & Hussein, 2022). Detritivorous earthworm activity stimulates microbial communities to use more efficiently the available energy, shaping their structure in organic wastes during vermicomposting. Earthworms are able to accelerate the decomposition of organic matter by modulating the decomposer community composition in the short term. Different vermicomposts can be produced by using different earthworm species and different types of organic wastes containing huge and specific microbial composition. Thus, it is possible to obtain specific vermicomposts utilisable for different practical applications.

In a recent study, Manzoor et al. (2024) strongly recommended the vermicompost as potential organic fertilizer for sustainable vegetable cultivation for the multiple benefits, such as: a slow-releasing organic manure rich in humic substances (fulvic acid, humic acid, and humin); enhancing microbial diversity, enzymatic activity; providing a sustained supply of macro and micronutrients; improving their retention and absorption due to its high porosity, water-holding capacity, and aeration; stimulating plant growth and suppressing the phytopathogens. Similar results reported by Iqbal et al. (2024) presented the beneficial effect of vermicompost application in alleviating the Cd toxicity by enriching the soil with beneficial microbes, enzymes, and humic acids, improving soil structure and water retention capacity, enhancing soil health, plant physiological and antioxidant defence mechanisms, in fragrant rice. Vermicomposting may contribute to soil organic carbon (SOC) sequestration in aggregates by bacterial necromass-C and with 43.96% higher values from fungal necromass-C (Zang et al., 2023). Barik et al. (2010) reviewed the results concerning the production of vermicompost from various agricultural wastes, the soils treated with vermicompost presenting higher microbial loads (e. g. nitrogen fixing bacteria in paddy fields) than in untreated plots.

By improving soil physical, chemical and microbial properties, increasing the number and biodiversity of microorganisms, vermicomposts can be utilized in sustainable agriculture and in soil restoration (Trivedi et al., 2021). A recent study in China on the effects of vermicompost application on the soil microbial community structure and fruit quality in melon (*Cucumis melo*) reported enhancement of soil chemical properties, increasing enzyme activity, and diversity of microbial communities, as well as chlorophyll content and melon quality (Tian et al., 2024). Lim et al. (2015) recommended the use of vermicompost as an effective organic fertilizer and biocontrol agent in organic farming for its beneficial effects on soil, yields and economy. The process of vermicomposting is self-sustaining, self-regulating, and self-improving technology. It requires minimal or no energy input, being considered a low-cost process and an environmentally friendly waste management option (Abad & Shafiqi, 2024). Vermicompost obtained is an easily available organic fertilizer comparatively with inorganic fertilizers, and a sustainable green solution for organic waste management problems (Arancon et al., 2006; Madhushani et al., 2024). Few authors (Pocius et al., 2014) investigated the possibility to obtain granulated vermicompost and assessed the influence of granulation process parameters on granulated fertilizer properties.

Research has been carried out aiming to compare the bacterial and fungal diversity, community structure, microbial activity and quality of two vermicompost granulations, C1<2 mm and C2>2 mm, obtained by sieving.

MATERIALS AND METHODS

The experiment was carried out in laboratory conditions at the Faculty of Horticulture from University of Agronomic Sciences and Veterinary Medicine of Bucharest. The biological material represented by food scraps, compost and different organic inputs (coco coir, hay, dead leaves, crushed egg shells, coffee grounds) and 100 red worms from species *Eisenia andrei* (California Red Wigglers) per tray was composted at the room temperature of 20°C over a period of 47 weeks. The study involved testing each variant with

three replicates, to produce vermicompost in trays.

The vermicompost obtained had a neutral pH (7.19) and high humus content (50.12%). A complete description of the experiment and the results of chemical parameters of the vermicompost were presented elsewhere (Urechescu et al., 2024). The values exceeded the target with more than 15 times for humus, 4 times for Calcium, 3 times for Magnesium, 22 times for Phosphorus and 40 times for Potassium. Two granulations, C1<2 mm and C2>2 mm have been obtained by sieving the vermicompost and samples from each were taken in order to assess their microbiological characteristics.

Microbiological analyses were performed according to soil dilution method using specific solid culture media: Nutrient agar (NA) produced by Difco USA for heterotrophic aerobic bacteria and potato-dextrose agar (PDA) produced by Merck KGaA Germany for fungi (Dumitru & Manea, 2011). After 7 days incubation at dark, colonies developed in Petri plates were counted and microbial density (Total Number of Bacteria-TNB and Total Number of Fungi-TNF) was calculated and values reported to gram of dry vermicompost (d.v).

Taxonomic identification was done using morphologic criteria, by examination of microbial characteristics and measurements using a MC 5.A optic microscope. Species were identified according to the determinative manuals for heterotrophic bacteria (Bergey & Holt, 1994) and for fungi (Watanabe, 2002).

The total number of species (S) for each microbial community from C1 and C2 vermicompost granulation, as well as relative abundance (A%) and dominance of species were recorded.

The Shannon index (H') that takes into account both the species richness and evenness (ϵ) was used to evaluate the microbial biodiversity in each vermicompost granulation (Mohan & Ardelean, 1993). According to Morris et al. (2014), the value of diversity index of Shannon increases with the increased number of species and when the species are evenly distributed. Similarity indices (SI) between the lists of microbial species from C1 and C2 granulations were calculated (Tiwari et al., 1994).

The global physiological activities of microflora from each vermicompost granulation were determined by substrate induced respiration method (SIR) and results expressed as $\text{mg CO}_2 \times 100 \text{ g}^{-1} \text{ vermicompost}$ (Matei, 2011). Circular chromatograms have been done according to the procedure recommended by Pfeiffer (1984). Vermicompost extracts have been migrated on filter paper previously impregnated with developing substances in order to obtain information concerning the biological quality of each vermicompost granulation. The image peculiarities referring to shape, dimensions, colour, texture, uniformity indicate the health, vitality, intensity of biotic activity, the level of organic matter complexity and the presence of stable humus in C1 and C2 vermicompost granulations.

RESULTS AND DISCUSSIONS

The results concerning the level of colonization of C1 and C2 vermicompost granulations with the main groups of microorganisms showed the development of high numeric effectiveness of heterotrophic aerobic bacteria and very high for fungi, with intense physiological activities due to the performant and complex enzymatic equipment, reflected by the high levels of soil respiration potential, as indicator of global microbial activities, in both vermicompost granulations (Table 1).

Table1. Total counts of bacteria, fungi and soil respiration from laboratory experiment with vermicompost of red worms, granulations C1 (< 2 mm) and C2 (> 2 mm)

No crt.	Experimental variant	TNB ($\times 10^6$ viable cells $\times \text{g}^{-1} \text{ d.v.}$)	TNF ($\times 10^3$ cfu $\times \text{g}^{-1} \text{ d.v.}$)	Soil respiration ($\text{mg CO}_2 \times 100 \text{ g}^{-1} \text{ vermicompost}$)
1	C1 Vermicompost granulation	53.102	235.414	116.833
2	C2 Vermicompost granulation	106.003	261.880	123.809

Thus, the total counts of heterotrophic bacteria from C1 granulation were 53.102×10^6 viable cells $\times \text{g}^{-1} \text{ d.v.}$ and doubled (106.003×10^6 viable cells $\times \text{g}^{-1} \text{ d.v.}$) at C2 granulation.

The total counts of fungi were also higher in C2 granulation (261.880×10^3 cfu $\times \text{g}^{-1} \text{ d.v.}$) in

comparison with the values registered in C1 granulation (235.414×10^3 cfu $\times \text{g}^{-1} \text{ d.v.}$). Accordingly, the global physiological activity values were more active in C2 vermicompost granulation ($123.809 \text{ mg CO}_2 \times 100 \text{ g}^{-1} \text{ vermicompost}$) than in C1 vermicompost granulation ($116.833 \text{ mg CO}_2 \times 100 \text{ g}^{-1} \text{ vermicompost}$). Results are supported by other research reporting the great increase in total numbers of bacteria and actinomycetes in the earthworm gut compared to the soil (Esakkiammal & Lakshmibai, 2013) and increased bacterial, fungal and actinomycetes counts in the 60th day of vermicomposting with *Eudrilus euginae* (Esakkiammal et al., 2015).

Taxonomic composition of bacterial microflora and species abundance in C1 vermicompost granulation is presented in Figure 1 and in C2 vermicompost granulation, in Figure 2.

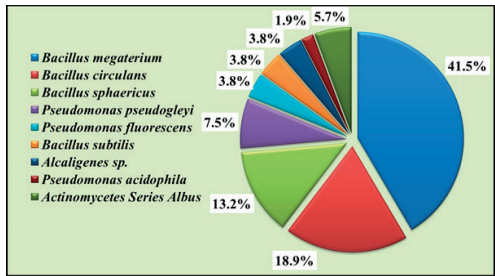


Figure1. Pie chart of percent mean relative abundance of bacterial microflora composition in C1 vermicompost granulation

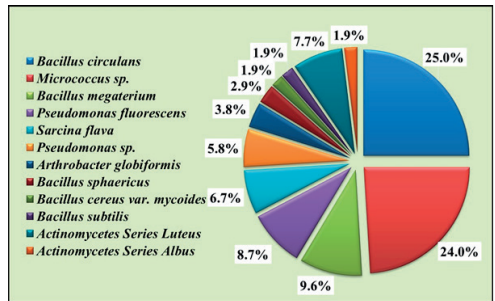


Figure 2. Pie chart of percent mean relative abundance of bacterial microflora composition in C2 vermicompost granulation

Bacterial microflora from C1 granulation consists in 4 species of bacilli (*Bacillus megaterium*, *B. circulans*, *B. sphaericus*, *B. subtilis*) accompanied by pseudomonads,

Alcaligenes and actinomycetes. *Bacillus megaterium*, the dominant species in C1 vermicompost granulation (A=41.5%), but also present with lower abundance at C2 granulation (A=9.6%), is known for the ability to solubilize phosphorus from inaccessible forms. Its presence indicates an advanced phase of organic matter degradation because it doesn't belong to the zymogenous (initial) microflora, its activity being characteristic to the next phase when it intervenes in degradation of more recalcitrant forms of organic matter.

Bacillus subtilis is a species belonging to zymogenous (initial) microflora, with activity oriented toward decomposition of easy degradable organic matter and more humid environments. It was identified with moderate effectiveness in both C1 (A=3.8%) and C2 (A=1.9%) vermicompost granulations. Its proliferation is stimulated by favourable aeration conditions. The presence of O₂ in sufficient quantities stimulates this species to degrade the organic wastes of vegetal origins during the composting process. In C1 granulation it is accompanied by *Pseudomonas acidophila* (A=1.9%) and *Pseudomonas pseudogleyi* (A=7.5%), two species adapted to live in similar (cold and humid) environment conditions. *Bacillus subtilis* is considered as a beneficial species for plants when added in biofertilizer composts/vermicomposts and present important mutual relationship with host plant. It releases different signalling molecules to improve biotic and abiotic stress tolerance or resistance, to promote plant growth, root development and to control the pathogens (Beauregard et al., 2013). Chaurasia et al. (2005) found that diffusible and volatile compounds produced by a *Bacillus subtilis* strain presented antagonistic activity against fungal pathogens by causing structural deformations. Accordingly, Boiu-Sicuia et al. (2023) reported a broad and strong antifungal effect of *B. subtilis* and other species of bacilli mediated by living cells, volatile active metabolites and cell-free supernatants against grape spoilage pathogens. Other data from literature evidenced that plant growth promotion effect was also due to volatile organic compounds produced by *Bacillus subtilis* SYST2 strain (Tahir et al., 2017).

Pseudomonas fluorescens present in both granulations with different abundance in bacteriobiome (A=3.8% in C1 and A=8.7% in C2 vermicompost granulation) belongs to zymogenous microflora acting as degrader of easily degradable organic substrates and considered one of the most important plant growth promotor bacteria from vermicompost (Shiva et al., 2013). It has a crucial role in inducing suppression of plant pathogens in rhizosphere (Matei & Matei, 2024) by producing antimicrobial metabolites (siderophores), extracellular lytic enzymes, fluorescent pigments and activation of plant defence-related mechanisms, as stated in various research from literature (Attitalla et al., 2001; Nagarajkumar et al., 2004).

Boruah & Kumar (2002) reported plant disease suppression and growth promotion by a fluorescent *Pseudomonas* strain. Another non-fluorescent species of *Pseudomonas* identified only in C2 vermicompost granulation with A=5.8%. Results of research carried out by other authors confirm the capacity of various species from genus *Pseudomonas* to produce metabolites responsible for a broad-spectrum of antifungal activity and biofertilizing properties (Kumar et al., 2005). Redouan et al. (2018) demonstrated that beneficial bacteria *Pseudomonas* spp. presented antifungal activity and were efficient agents for the control of grey and green mould. Santoyo et al. (2012) reviewed the mechanisms responsible for biocontrol and plant growth-promoting activity in bacterial species of *Bacillus* and *Pseudomonas*. Singh et al. (2013) reported the involvement of *Trichoderma harzianum* and *Pseudomonas* sp. in biocontrol of *Sclerotium rolfsii* rot in tomato (*Lycopersicon esculentum*). The communities of chitinase-producing bacteria vermicompost-associated showed inhibitory effects against plant pathogens *Rhizoctonia solani*, *Colletotrichum coccodes*, *Pythium ultimum*, *P. capsici* and *Fusarium moniliforme*, as reported in literature (Yasir et al., 2009; Veliz et al., 2017).

Previous research noticed an increase of 3.2 times of fluorescent pseudomonads numeric effectiveness in the variant treated with vermicompost as compared with untreated control and of approximately 4 times increase of actinomycetes counts. Relative abundance of

potential plant pathogenic species in fungal populations from variants treated with vermicompost was lower than 5%, values indicating soil suppressiveness against pathogens (Matei & Matei, 2017). The antimicrobial activity of *Pseudomonas* spp. and other bacteria and fungi was attributed to their capacity to produce siderophores, metabolites efficient in suppression of various phytopathogens and pests (Ahmed & Holmström, 2014). Other biocontrol strain (*Pseudomonas fluorescens* CHA0) presented the capability to produce secondary metabolites helping it to avoid protozoan grazing (Jousset et al., 2006).

Representatives of genus *Micrococcus* (A=24%) were co-dominant along with *Bacillus circulans* (A=25%) at C2 granulation. The spores with thick membrane produced by *B. circulans* confer to this species a high adaptability to heat and hydric stress conditions, favouring its survival. Apart of *B. circulans*, other 4 species of bacilli less abundant were identified in samples from C2 vermicompost granulation: *B. megaterium*, *B. sphaericus*, *B. cereus* var. *mycoides*, *B. subtilis*. Another species belonging to the family of Micrococcaceae, identified at the same granulation, was *Sarcina flava* (A=6.7%), the proliferation of this group of bacteria being connected with the presence of red worms that processed the substrate and turned it into vermicompost. The other bacteria species from microbial community from C2 granulations were represented by fluorescent and non-fluorescent *Pseudomonas* spp., *Arthrobacter globiformis* (A= 3.8%) and Actinomycetes. The last 2 taxa present a high adaptability to a wide range of nutritive substrates, from poor to rich, being favoured especially by the presence of materials previously degraded by pseudomonads from zymogenous microflora.

Actinomycetes from series Albus were present in both granulations, with higher value A= 5.7% in C1 and lower value A=1.9 in C2. Representatives of Series Luteus were identified only in C2 granulation with high relative abundance A=7.7%. Thus, actinomycetes were better represented in C2 vermicompost granulation (as number and taxonomic diversity) than in C1 granulation. Actinomycetes are active cellulase-producers

for organic wastes decomposition and enrich the vermicompost in antibiotic producing agents with antimicrobial role, as resulting from literature (Augustine et al., 2008). Other authors found Proteobacteria and Actinomycetes as the dominant bacteria during earthworms' growth during vermicomposting of two types of agricultural wastes. They reported that abundance of dominant bacteria changed under the influence of earthworm activity and gut transit (Qian et al., 2024).

Taxonomic composition of fungal microflora and species abundance in C1 vermicompost granulation is presented in Figure 3 and in C2 vermicompost granulation, in Figure 4.

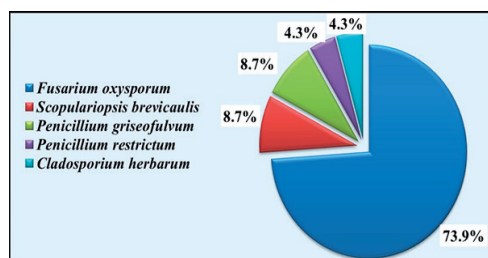


Figure3. Pie chart of percent mean relative abundance of fungal microflora composition in C1 vermicompost granulation

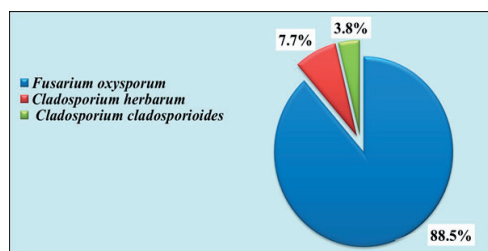


Figure4. Pie chart of percent mean relative abundance of fungal microflora composition in C2 vermicompost granulation

Fusarium oxysporum was present as dominant species in both C1(A=73.9%) and C2 (A=88.5%) vermicompost granulations, well developed and active degrader of organic materials in conditions of good aeration. Species was frequent identified in dung, various composts, including vermicomposts. It acts as antibacterial as well as antifungal agent, with role in the biocontrol of plant pathogens due to his active metabolites and stimulates plants defensive responses. The growth hormones produced by this rhizosphere species

promote plant growth and development, especially in tomato, radish, lettuce and maize.

Cladosporium herbarum, cosmopolitan species, with high ability to produce cellulolytic, amylolytic, pectinolytic enzymes and melanin, has a crucial role in binding particles of substrate in aggregates and in humification process. Species has a high affinity for rhizosphere conditions. The metabolites produced present antibacterial and antifungal activity, stimulate plant immunity, important in biocontrol and promote plant growth.

It was identified with moderate effectiveness in both C1 (A=4.3%) and C2 (A=7.7%) vermicompost granulations. In C2 granulation, it was accompanied by another species belonging to the same genus (*Cladosporium cladosporioides*), but less abundant (A=3.8%).

Other differential species identified only at C2 vermicompost granulation were cosmopolitan, organic waste decomposer using diversified enzymatic equipment, active producers of metabolites with antimicrobial role, belonging to genus *Penicillium*: *P.restrictum* (A=4.3%), *P. griseofulvum* (A=8.7), and *Scopulariopsis brevicaulis* (A=8.7%) with strong cellulolytic, ligninolytic, humifying capacities and specific for rhizosphere where present multiple interactions with plants.

Chaudhary et al. (2018) evidenced the role of *Penicillium* in enhancing plants resistance to abiotic stresses.

Similarity Index value calculated between the lists of bacteria identified meaned that 57.15% were species shared between the two granulations. Also, half of fungal species were common to both list from C1 and C2 vermicompost granulations (SI=50%).

Table 2 presents the comparative values of S indices (measure microbial community richness, with higher values indicating greater community richness) and Shannon indices (measure microbial community diversity, with higher values indicating greater community diversity) from C1 and C2 vermicompost granulations (Qian et al., 2024).

Thus, a rich bacteria population with S=12 species, high diversity ($H'=2.104$ bits) and homogeneity ($\epsilon=0.662$) was found in C2 granulation. C1 granulation contained a less

rich bacteriobiome (S=9 species), with $H'=1.750$ bits and $\epsilon=0.594$.

Table 2. Species richness and Diversity indices of bacterial and fungal communities from laboratory experiment with vermicompost of red worms, granulations C1 (< 2 mm) and C2 (> 2 mm)

Vermicompost granulation	Bacterial Diversity	Fungal Diversity
C1(< 2 mm)	S=9 Shannon $H'=1.750$ bit $E(\epsilon)=0.594$	S=5 Shannon $H'=0.921$ bit $E(\epsilon)=0.324$
C2 (> 2 mm)	S=12 Shannon $H'=2.104$ bit $E(\epsilon)=0.662$	S=3 Shannon $H'=0.403$ bit $E(\epsilon)=0.089$

Fungi presented lower values of richness and biodiversity indices than bacteria. Conditions from C1 were more favourable for fungal microflora than those from C2 granulation (S=5 species, $H'=0.921$ bits, $\epsilon=0.324$ in C1 and S=3 species, $H'=0.403$ bits, $\epsilon=0.089$ in C2 fraction). Pfeiffer chromatograms from Figure 5 conferred supplementary information on the quality of the vermicompost granulations C1 < 2 mm and C2 > 2 mm.

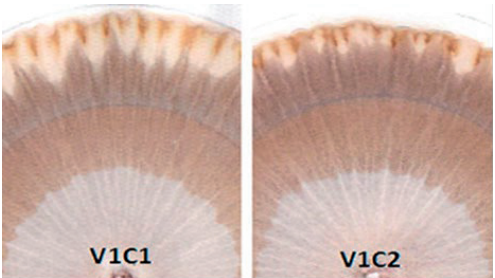


Figure 5. The aspect of sections of Pfeiffer chromatograms from C1 and C2 vermicompost granulations

The general aspect of chromatograms from the two granulations is harmonious, corresponds to a good quality of vermicompost, with distinct central, internal, intermediary and external zones present, clearly visible.

Central and internal (mineral) zones, reflecting a good structure and oxygenation during vermicomposting process, present comparable dimensions and aspect between C1 and C2 granulations.

The image continues with the integration zone of minerals, with organic matter and protein intermediary zone, the latest slightly larger in

C2 granulation. The aspect of external zone highlighted some differences concerning the significance of forms and dimensions that revealed a higher enzyme content as well as bacterial biomass, diversity and activity in C1 than in C2 vermicompost granulation. The dark coffee brown forms from the edges of external zone confirm the presence of humic substances, especially stable forms of humus in both chromatograms, but with specificity concerning their quantity and composition for each vermicompost granulation.

All the aspects of the chromatograms characterize good conditions in both vermicompost granulations, with a great richness and diversity of elements they contain. Results from recent studies (Zhang et al., 2024) attributed the higher nutritional value of vermicompost, as compared to traditional composts, to increased mineralization and humification facilitated by earthworm activity. Vermicompost improves soil characteristics (porosity, aeration, drainage, and water-holding capacity), creating favourable conditions for plant growth.

The results from our experiment with two vermicompost granulations are comparable with other research on the composition of vermicompost microflora carried out on 18 variants of organic wastes. Yampolskaya et al. (2022) reported 6 genera of micromycetes and 3 to 4 taxonomic units/variant, evidencing the importance for the synthesis of humic substances of melanin and pigments - producing genera *Cladosporium*, *Penicillium* and *Fusarium*, identified in our vermicompost granulations, too. Species from *Bacillus* and *Penicillium* genera were also reported by other authors, as part from vermicompost microflora composition. Illanjiam et al. (2019), in their study on microbial diversity of vermicompost and its efficacy on organic vegetables, identified three bacterial strains, such as: *Bacillus* sp., *Klebsiella* sp. and *Azotobacter* sp. and four fungal isolates, such as: *Aspergillus* sp., *Microsporum* sp., *Penicillium* sp. and *Trichophyton* sp. Thus, the vermicompost bacteria presented the ability to produce plant growth promoting substances as well as biosurfactants. Beneficial microbial biomass improved seed germination, seedling length and seed vitality in wheat and recommended

the vermicompost obtained as effective for the improved growth of vegetable crops such as brinjal (*Solanum melongena*), ladies finger (*Abelmoschus esculentus*), chilli (*Capsicum annum*), tomato (*Solanum lycopersicum*). Satpathy et al. (2020) reported the presence of 8 bacterial strains in vermicompost produced from a mixture of cow dung, straw, neem leaf and vegetable wastes, using earthworms (*Eisenia fetida*). All these bacteria were beneficial as they enhanced the nutrient status of vermicompost and improved the soil aeration and fertility. Species from genera *Bacillus*, *Pseudomonas*, *Micrococcus* and actinomycetes were also identified in our study on biodiversity of bacteria from C1 and C2 vermicompost granulations. Kapila et al. (2021) evaluated the microbiological quality of vermicompost prepared from 7 different types of organic wastes using *Eisenia fetida*. Authors presented the taxonomic composition of microbial community in vermicompost and the activity of hydrolytic enzymes associated with bacterial and fungal species. They identified 18 microbial taxa, 6 of them being also identified in our present experiment (phosphate solubilizers like *Bacillus* sp. and PGPBs like *Pseudomonas* sp., *Micrococcus*, actinomycetes, pigment producing actinomycetes; *Penicillium* and *Fusarium*). Microbial variation in the vermicompost was 10 to 20 times higher than in control without earthworms. The high microbial diversity present, supported significant levels of carboxy-methyl-cellulase, exoglucanase, xylanase, β -glucosidase, phosphatase and urease activities. The good fertilizer value of the vermicompost recommended it as green manure and a safe method of organic waste disposal.

Other research on vermicomposting benefits recommended vermicompost containing actinomycetes and their antibiotics for obtaining chemical-free, nutritive and health protective foods in organic horticulture (Sinha et al., 2013). Previous research results (Urechescu et al., 2022) highlighted the beneficial effect of vermicompost as a culture substrate on the quality of lettuce seedlings (*Lactuca sativa*), similarly to those reported by Alper et al. (2017) on plant growth parameters and yields at the same horticultural plant. Also, other authors consider that earthworms and

vermiculture are valuable to sustainable agriculture by using them to ameliorate soils damaged by agrochemicals and other pollutants, to restore soil fertility, to improve yields and to mitigate global warming effects by soil organic carbon sequestration (Sinha et al., 2014). It was demonstrated the crucial contribution of bacterial and fungal microflora to carbon sequestration in agroecosystems (Six et al., 2006). According to Wagg et al. (2019), the high fungal-bacterial diversity and microbiome complexity have a major contribution to ecosystem functioning. The high efficiency of microbial carbon use in vermicomposting process promotes global soil carbon storage (Tao et al., 2023).

Results from present research are in concordance with those reported in a study on the possibility of granulating wet vermicompost into granules using vermiwash for easier storage and packaging. The particle size was 0.6 mm and a distribution recommended for fertilizer granules. The vermicompost NPK content increased by 48.9%, 55.4% and 75% respectively upon granulation, due to the additional fertilizer macronutrients that were supplied by the vermiwash (Manyuchi & Nyamunokora, 2014).

Other authors (Vronskis et al., 2016) obtained vermicompost pellets with 6 mm diameter at high conditioning temperatures 95°C, considered a concentrated fertilizer, by reducing the volume of material more than 10 times by water removal and material thickening while extruding. The vermicompost application conferred tolerance to stress and benefits, such as: seedling vigour and plant health, attributed mainly to high concentrations of plant available macro- and micro nutrients, plant growth promoting organic acids and high microbial activity.

As in our vermicompost granulations, containing a rich microbiota with high biological activity, the beneficial effect of microorganisms in improving soil structure and forming of stable aggregates by binding capacity of fungal hyphae and bacterial exopolysaccharides was already confirmed by Gupta & Germida (2015).

Vermicompost with enriched microflora and gypsum amendments were successfully utilized

for improving aggregate formation in bauxite residue (Zhu et al., 2017).

CONCLUSIONS

Both bacteria and fungi have colonized the vermicompost granulation C2 (> 2 mm) with higher counts in comparison with C1 granulation (< 2 mm).

Global microbial activity was expressed by higher values of soil respiration in C2 granulation as compared with lower values in C1 granulation.

A rich bacteria population with 12 species, high diversity ($H' = 2.104$ bits) and homogeneity ($\epsilon = 0.662$) was found in C2 granulation.

C1 granulation contained a less rich and diverse bacterial community than C2 granulation, with 9 species, $H' = 1.750$ bits and $\epsilon = 0.594$.

Fungi presented lower biodiversity values than bacteria.

Conditions from C1 were more favourable for fungal diversity than those from C2 granulation ($H' = 0.921$ bits, $\epsilon = 0.324$ in C1 and $H' = 0.403$ bits, $\epsilon = 0.089$ in C2 fraction).

Bacterial microflora from C1 granulation was dominated by bacilli, accompanied by *Pseudomonas* and actinomycetes and a mix of species (*Bacillus*, *Micrococcus*, *Pseudomonas*, *Arthrobacter* and actinomycetes) in C2 granulation.

In both fractions identified strong cellulolytic fungal species from genera *Fusarium*, *Scopulariopsis*, *Penicillium*, *Cladosporium*.

Specific paper chromatograms highlighted the good quality of the two vermicompost granulations, revealing a high microbial biomass and metabolic activity, confirmed by the presence of active enzymes and richness of humus compounds.

Further research is needed to assess the effect of the vermicompost granulation as biofertilizers for various plants cultivated in organic horticulture.

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