ISSN: 2181-3337 | SCIENTISTS.UZ
INTERNATIONAL SCIENTIFIC JOURNAL SCIENCE AND INNOVATION
ISSUE DEDICATED TO THE 80TH ANNIVERSARY OF THE ACADEMY OF SCIENCES OF THE REPUBLIC OF UZBEKISTAN

QUANTITATIVE ANALYSIS OF NITROGEN CYCLE MICROORGANISMS IN VERMICOMPOSTS BASED ON WOODY LEAF LITTER (SALICACEAE) AND COW MANURE

[1]Kornievskaya E.V., [2]Gummer Ya.M., [3]Kurovsky A.V., [4]Babenko A.S., [5]Khan I.
[1,2,3,4,5]Biological Institute, Tomsk State University, Tomsk, Russian Federation
https://doi.org/ 10.5281/zenodo.8353181

Abstract. *The quantitative composition of microorganisms – ammonificators, inorganic nitrogen fixers, oligotrophs, and free-living nitrogen fixers – was studied at the beginning, in the middle and at the end of vermicomposting mixtures based on cow manure, black ma leaf litter and brittle willow leaf litter. Free-living nitrogen fixers were not detected in the initial mixture; this group of microorganisms was detected in the middle and at the end of vermiculture. Microorganisms of the other groups were present in the vermicomposts throughout the whole period of vermiculture, their maximum number was found in the vermicompost based on cow manure. Nevertheless, a very high index of the number of nitrogen fixers in vermicomposts based on black poplar and brittle willow enables us to recommend all three types of vermicompost for use as nitrogen fertilizers.*

Keywords: *vermicompost, black poplar, brittle willow, Populus nigra, Salix fragilis, nitrogen cycle microorganisms, ammonifiers, inorganic nitrogen utilizers, oligotrophs, free living nitrogen fixers.*

Introduction. One of the most important types of organic fertilizer, and one that is increasingly in demand, is vermicompost – compost produced by earthworms. However, one of the most important nutrients is nitrogen, which is present in vermicompost in the form of different compounds. Worms and microorganisms are involved in the chemical transformation of compounds in vermicompost [1, 2]. The Department of Agricultural Biology of Tomsk State University is working on the production and research of vermicompost based on woody leaf litter of local tree species. The quantitative composition of nitrogen-cycling microorganisms in vermicomposts based on cow manure and some types of leaf litter was considered in previous studies [3, 4]. However, these papers only describe the results for processed vermicompost. How the number of nitrogen cycle microorganisms varies during the vermicomposting process has not been investigated. The aim of this work is to study the quantitative composition of nitrogen cycle microorganisms: ammonifers, inorganic nitrogen utilizers, oligotrophs, free-living nitrogen fixers in vermicomposts based on leaf litter of Salicaceae trees at the beginning, in the middle and at the end of the closed vermicomposting cycle.

Materials and methods. The non-food component (sorbent, filler) used in the vermicomposting of substrates was high peat with increased organic content OOO Garden Retail Service; Russia, Moscow region, Khimki. Cow manure in the form of clean excrement, cleaned from litter, was used as the first feed component. The manure was provided by the private subsidiary farm of the Gummer family (Ishtan village, Tomsk region, Russia 57°09′24″N 84°07′49″E). As the second and third food components for vermicomposting were used fallen leaves of woody plants growing on the territory of the University Grove, a natural complex that is part of the historical and architectural ensemble of Tomsk State University (Russian Federation, 56°28′08″N; 84°56′55″E). Fallen leaves of plants of the family Salicaceae – *Populus nigra* L. (black poplar) and *Salix fragilis* L. (brittle willow) – were used. The leaves were collected between

20 September and 20 October 2021, when the average daily temperature was positive, until the formation of a stable snow cover. The collected leaves were dried at room temperature in the laboratory and stored in air-dry conditions.

Vermiculture of the worm *Eisenia fetida* Savigny (Lumbricidae) was provided by Professor A.S. Babenko, Head of the Department of Agricultural Biology of Tomsk State University.

In total, the following variants of the vermicomposts under study were obtained during the experiment:

1. Vermicompost based on a mixture of high peat and cow manure.
2. Vermicompost based on a mixture of high peat and black poplar leaves.
3. Vermicompost based on a mixture of high peat and brittle willow leaves.

Before composting, the manure was chopped to a size of 1 to 3 cm and the leaf litter was also chopped to a size of 0.5 to 1 cm. The food component and the peat for each variant were mixed in a 1:1 ratio of 100 g and 100 g. Thus, the total mass of the dry mixture was 200 g and the volume occupied by each mixture was approximately 2.1 liters. After mixing, the substrates were placed in 10-liter plastic buckets, mixed again and moistened with distilled water to a moisture content of 75 % by weight.

After a holding period of 7 days, 20 worms with a total weight of 8 g were added to the buckets of vermicomposting mixtures. The duration of vermicomposting was 122 days.

Samples of vermicompost for microbiological analysis were taken before adding worms, after 65 and 122 days of vermicomposting. Vermicompost was sown on nutrient media on the day of sampling. Methods for determining the concentration of microorganisms of different trophic groups and methods for primary statistical processing of the results have been described in previous publications [3, 4]. Vermicompost samples were obtained in three biological replicates and data in the article are presented as means with standard errors of the mean.

Results and discussion. The microflora indicators of three ecological and trophic groups of the nitrogen cycle in vermicomposts based on woody leaf litter at different periods of vermicomposting differed significantly (Table 1).

Table 1

Microbiological characteristics of mixtures during vermicomposting

Food/nutritional component of the mixture	The number of CFU per 1 g of vermicompost $\cdot 10^9$								
	Ammonifiers			Inorganic nitrogen utilizers			Oligotrophs		
	Star-ting	Mid-dle	End	Star-ting	Mid-dle	End	Star-ting	Mid-dle	End
Cow manure	15,77± 2,92	6,27± 0,40	7,85± 0,70	16,03± 2,48	9,52± 0,56	6,99± 0,64	15,95± 2,57	7,43± 0,56	8,18± 0,42
Populus nigra leaf litter	4,39 ± 0,99	5,87± 0,81	5,02± 0,47	2,88± 0,21	4,02± 0,77	3,54± 0,57	2,92± 0,26	6,13± 0,81	4,71± 0,55
Salix fragilis leaf litter	3,16 ± 0,32	6,95± 0,76	6,26± 0,82	2,43± 0,34	4,79± 0,82	5,77± 0,59	3,48± 0,29	5,51± 0,55	4,73± 0,64

All four groups of microorganisms were present in the middle and at the end of vermicomposting. Free-living nitrogen fixers were not observed in the substrate at the beginning of vermicomposting.

The amount of ammonificators, inorganic nitrogen users and oligotrophs was maximum in the manure-based mixture at the beginning of vermicomposting. The quantitative indices of the mixtures with leaf fall were similar in these parameters (Table 1).

Since the high peat used in the experiment was the same in all variants, the differences in the composition of the microflora of the initial mixture can be justified by the different content of microorganisms in manure and leaf litter. It is also noticeable that the processes of ammonification, inorganic nitrogen utilization and decomposition of organic matter into carbon dioxide and water are more intensive in the manure-based mixture without worms.

In the middle of the vermicomposting period the pattern was different. The number of ammonifiers in the vermicomposts did not vary significantly, but this indicator was slightly higher in the brittle willow leaf litter based vermicompost. The number of inorganic nitrogen users was significantly higher in the manure-based vermicompost and the two leaf litter based vermicomposts did not differ significantly, but was slightly higher in the brittle willow fall based vermicompost. The number of oligotrophs tended to decrease in the series of manure, black poplar and brittle willow based vermicomposts. The number of inorganic nitrogen fixers in vermicompost based on black poplar fall was not significantly different from this indicator in the other vermicomposts studied, but there were significant differences between vermicompost based on manure and vermicompost based on brittle willow.

However, at the end of vermicomposting, the number of ammonifers and inorganic nitrogen fixers was minimal in black poplar leaf litter based vermicompost, maximal in manure based vermicompost, and intermediate in brittle willow leaf litter based vermicompost. Black poplar and brittle willow leaf litter vermicomposts did not differ in the number of oligotrophs. The number of oligotrophs in manure-based vermicompost was almost twice as high as in leaf litter-based vermicompost, indicating more intensive processes of organic matter loss in this vermicompost.

Actual activity of free-living nitrogen fixers was only detected in the middle and at the end of vermicomposting (Table 2). Bacteria of the genus *Azotobacter* were not detected when substrates without earthworms were sown on Ashby's nutrient medium. These results are in agreement with literature data where an associative relationship between free-living nitrogen fixers and earthworms has been reported [5]. It is likely that the process of nitrogen fixation by free-living bacteria coincides with the onset of earthworm activity in the food mixtures, which requires further research. In the middle and at the end of vermiculture, the actual activity of nitrogen fixers was significantly higher in vermicomposts based on leaf litter (Table 2). At the same time, quantitative changes in the actual activity of free-living nitrogen fixers during vermiculture manifested themselves differently in the different vermicomposts. In manure-based vermicompost the actual activity of nitrogen fixers was significantly higher at the end of vermicomposting than in the middle, in poplar-based vermicompost this indicator did not change practically and was close to 100 % at the end of vermicomposting, in vermicompost it decreased from 93,37 % to 86,64 %. Chemical analysis of the gross nitrogen content, which was carried out in an accredited laboratory, showed a statistically significant increase in gross nitrogen at the end of vermicomposting in all vermicompost samples (figure 1). The greatest increase in the amount of nitrogen relative to the initial mixture was observed in the willow leaf-based vermicompost. The greater increase in gross nitrogen in willow-based vermicompost compared to poplar-based vermicompost with almost the same level of actual activity of nitrogen-fixing bacteria can be explained by the fact that in willow-based vermicompost free-living nitrogen-fixing bacteria fixed nitrogen more intensively. The ability of bacteria to fix nitrogen depends on the physical and chemical characteristics of the substrate in which the bacteria live [6]. As the vermicomposting process was carried out in a closed system (plastic buckets), the only source of the increase in gross nitrogen could be nitrogen fixation by microorganisms.

Table 2

Characteristics of nitrogen-fixing activity of microorganisms during vermicomposting

Vermicompost	Actual activity free living nitrogen fixers, %		
	Starting	Middle	End
Cow manure	0	60,11± 5,21	76,48± 2,81
Populus nigra leaf litter	0	90,61± 1,87	89,59± 2,84
Salix fragilis leaf litter	0	93,37± 1,55	86,64± 2,27

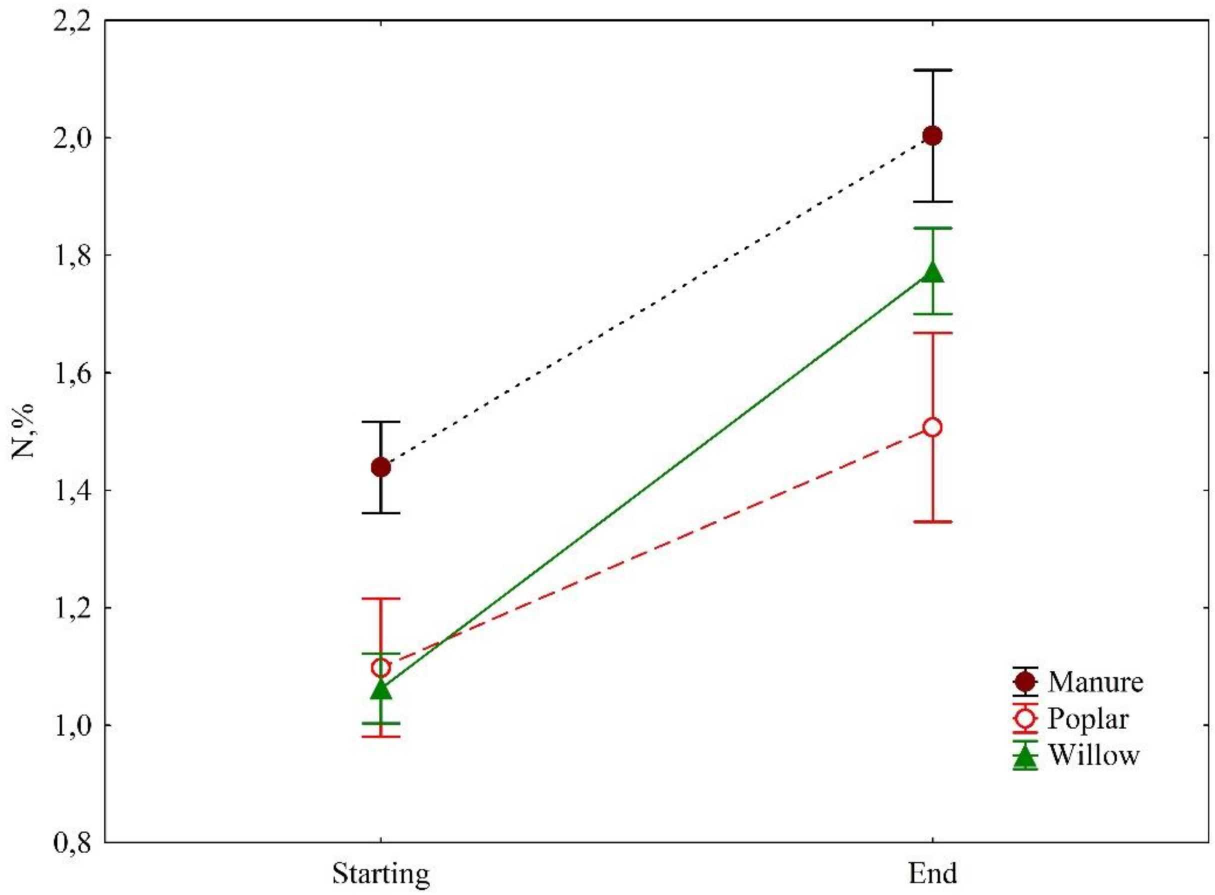

Figure 1. Gross nitrogen balance in vermicomposts based on cow manure, poplar leaf litter, willow leaf litter before the start and at the end of vermicomposting

In the literature describing quantitative indicators of vermicompost microflora using nutrient media, meat peptone agar and special media for groups of microorganisms such as fungi and actinomycetes are used [7, 8, 9]. This approach to quantifying the microflora does not allow us to assess the contribution of microorganisms to the transformation of chemical substances.

According to our data, the highest number of microorganisms of all the trophic groups studied was found in the manure-based vermicompost, which can be evaluated from different points of view. On the one hand, a high number of microorganisms may indicate a good quality vermicompost, since the higher the total number of ammonifiers and inorganic nitrogen fixers, the more mineral forms of nitrogen are present in the soil [10]. However, in addition to the microorganisms that are obviously beneficial to plants, the manure-based vermicompost also contained an increased number of oligotrophs, which cause unproductive losses of nutrient elements. The intensity of atmospheric nitrogen fixation, determined by the index of actual activity of free-living nitrogen fixers, was significantly higher in leaf litter based vermicompost, in agreement with data obtained previously [4].

Conclusion: During the process of vermiculture, changes in the qualitative and quantitative composition of nitrogen cycle microorganisms were observed. In the initial mixture of food and non-food substrate, ammonifiers, inorganic nitrogen utilizers and oligotrophs were present, but free-living fixers of atmospheric nitrogen were absent. During vermicomposting, the number of ammonifiers, inorganic nitrogen fixers and oligotrophs varied, but the number of microorganisms of these groups was highest in the cow dung based vermicompost. Free-living nitrogen fixers were detected in the vermicomposts at the middle and end of vermiculture, and their number increased in the cow dung based vermicompost, remained practically unchanged in the black poplar fall based vermicompost and decreased in the willow fall based vermicompost. At the same time, the actual activity of free-living nitrogen fixers in all three types of vermicompost averaged more than 50%. The duration of the laboratory vermiculture of 122 days can be considered optimal for obtaining vermicompost of good quality. The results of the study of the quantitative composition of the microflora allow to recommend all the studied vermicomposts for use as nitrogen fertilizer.

REFERENCES

1. Lv B, Zhanga D, Chen Q, Cui Y. Effects of earthworms on nitrogen transformation and the correspond genes (amoA and nirS) in vermicomposting of sewage sludge and rice straw. Bioresource Technology 2019 287 121428 doi: 10.1016/j.biortech.2019.121428
2. Broz A P, Verma P Q and Appel C Nitrogen dynamics of vermicompost use in sustainable agriculture. Journal of Soil Science and Environmental Management 2016 7 (11) 173 DOI 10.5897/JSSEM2016.0587
3. Kurovsky A, Kornievskaya E, Gummer Ya, Babenko A and Saratchandra Babu M. The balance of nitrogen forms and number of microorganisms of the nitrogen cycle in vermicomposts based on leaf litter and cow manure // IOP Conference Series: Earth and Environmental Science. 2021. Vol. 935, № 1. P. 012002. DOI: 10.1088/1755-1315/935/1/012002
4. Kornievskaya E, Kurovsky A, Babenko A, Petrochenko K and Sechko O. Microbial structure of nitrogen utilizers in Populus nigra L. compost and vermicompost //IOP Conference Series: Earth and Environmental Science. 2020. Vol. 433. P. 012001. doi:10.1088/1755-1315/433/1/012001

5. Tereshchenko N N, Yunusova T V and Pisarchuk A D. Microorganisms – unique indicators of vermicompost quality. Achievements of Science and Technology in Agroindustrial Complex 2012. Vol. 5. P. 58–60
6. Pandey P. K. and Sreelu N. S. Nitrogen-fixing aerobic bacteria and their nitrogenase activity in North Mumbai coastal waters. J. Indian Fish. Assoc. 2006. Vol. 33. P. 95-102
7. Emperor G N and Kumar K Microbial Population and Activity on Vermicompost of Eudrilus eugeniae and Eisenia fetida in Different Concentrations of Tea Waste with Cow Dung and Kitchen Waste Mixture. Int. J. Curr. Microbiol. App. Sci. 2015. Vol. 4(10). P. 496
8. Ganguly R K and Chakraborty S K Assessment of microbial roles in the bioconversion of paper mill sludge through vermicomposting. J. of Environmental Health Sci. and Eng. 2018. Vol. 16. P. 205 doi: 10.1007/s40201-018-0308-4
9. Karmegam N, Vijayan P, Prakash M, Paul J A J Vermicomposting of paper industry sludge with cowdung and green manure plants using Eisenia fetida: A viable option for cleaner and enriched vermicompost production. J. of Cleaner Production 2019. Vol. 228. P. 718 doi: 10.1016/j.jclepro.2019.04.313
10. Dymova L V, Nesterova L B Interrelation of the number of microorganisms and mineral forms of nitrogen in the soil. Agrochemical Bulletin. 2006. Vol. 4. P. 8