The balance of nitrogen forms and number of microorganisms of the nitrogen cycle in vermicomposts based on leaf litter and cow manure

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Abstract. The processes of nitrogen transformation in the vermiculture system by Eisenia fetida using cow manure and leaf litter (poplar with small birch addition) have been investigated. Vermicomposting was carried out for five months in half-cubic meter wooden containers. The Kjeldahl method and potentiometry determined the total nitrogen, NH₄⁺ and NO₃⁻ content in vermicompost. The total nitrogen content in cow manure was 1.83%, in leaf litter – 0.73%. The nitrate and ammonium content in non-composted leaf litter was 351 and 7.3 mEq/kg of dry matter, respectively. The nitrate and ammonium content in non-composted cow manure was 18.2 and 22 mEq/kg, respectively. Both investigated substrates of vermicomposting did not influence total nitrogen content. In cow manure-based vermicomposting system, the ammonium amount decreased by 5.3 times, while the concentration of nitrates increased by 6.5 times. In the leaf litter-based vermicomposting system, the ammonium amount increased by 2.9 times, and the amount of the nitrate increased by 1.6 times. The Azotobacter bacteria actual activity in both vermicomposts was close to 100%. The sum of nitrogen cycle microorganisms in manure vermicompost was 2.4 times higher than in leaf litter vermicompost.

1. Introduction

The present intensive agriculture system results in the increasing demand for organic fertilizers. Among so far insufficiently used resources for the creation of organic fertilizers, an important position is occupied by wood leaf litter, which is annually formed in large quantities and can be considered as a valuable raw material for composting. Leaf litter composts can be obtained using microorganisms and invertebrate animals. Composts made with earthworms are called vermicomposts. Vermicomposts obtained from different wastes can have different qualities. In addition, in processes of vermicomposting, the chemical parameters of initial composting mixtures are changed. Different nitrogen-forms are very important (such as total nitrogen and soluble forms) [1].

The microorganisms of the nitrogen cycle can serve as one of the indicators of the properties of vermicomposts [2]. These groups are free-living nitrogen fixers, ammonifiers and inorganic nitrogen utilizers. The quantitative composition of nitrogen cycle groups microorganisms is determined on nutrient media [3, 4].

We studied Siberian wood leaf litter as an initial substrate for vermicomposting for the last two decades. In particular, we have shown that the vermicompost obtained from the poplar leaf litter processing has several
valuable qualities that distinguish it favourably from manure vermicompost [3]. High calcium content and high calcium/potassium ratio, and some other indicators in vermicompost based on leaf litter in some cases, can be considered more beneficial than in vermicompost based on manure. Thus, in some of the studies, we compared two types of vermicompost – based on leaf litter and manure [3, 5, 6].

The nitrogen transformation in the plant residues vermicomposting is of scientific and practical interest, but it is still poorly understood [7-9]. The work aims to assess the balance of nitrogen-forms and the number of microorganisms of the nitrogen cycle in vermicomposts based on leaf litter and cow manure.

2. Materials and methods

In the experiments, vermicompost samples were used before drying. Vermicompost preparation was carried out according to the method described earlier [5].

Initial components of vermicomposts: greenhouse soil was used as a non-nutritional component (absorbent material), kindly provided by the Siberian Botanical Garden of Tomsk State University. Cow manure was used as the first nutritional component in the form of clean excrement, cleaned of litter. The Ovechkin family's private farm provided manure (Ulu-Yul, Tomsk region, Russia, 57°42’47” N; 85°47’34” E). As the second nutritional component for vermicomposting, fallen leaves of woody plants of Populus nigra L. (black poplar) with a small addition of Betula pendula Roth. (hanging birch), growing on the territory of the Tomsk State University Park, were used (Tomsk, Russia, 56°28′08″ N; 84°56’55” E). Fallen leaves were collected from September 20 to October 20, 2018, at a positive daily temperature until a stable snow cover formed. The collected leaf litter was dried in a farm hangar of Tomsk State University at a temperature of 20-25°C to constant weight and stored in an air-dry state. The initial population of worms Eisenia fetida Savigny was obtained from Dr. Yu B Morey (Institute of Biology, Academy of Sciences of the Kyrgyz SSR) in 1991 and has been supported up to now.

In total, during the experiment, the following variants of the investigated substrates were obtained:

1. Vermicompost based on a mixture of greenhouse soil and cow manure;
2. Vermicompost is based on a mixture of greenhouse soil and wood leaf litter.

Before vermicomposting, the nutritional and non-nutritional components for each option were mixed in a volume ratio of 16:1 – 122 L manure or leaf litter and 7 L greenhouse soil, respectively. Thus, the total volume of the dry mixture was 129 L. After mixing, the substrates were placed in half-cubic meter wood containers with a bottom area of one square meter, mixed again and moistened with settled tap water until a moisture content of 77%. Subsequently, the moisture content of the substrates was regularly monitored and maintained at a level of 75-80%. After one day, 1000 worms were put into the containers with mixtures for vermicomposting. Vermicomposting was carried out in the farm hangar of Tomsk State University at the temperature of 20-25°C for five months.

The extracts of experimental substrates were prepared from dry weighed samples of vermicompost (30 g). Distilled water was added to the weighed samples at a ratio of 1:5 and stirred for 15 min in a magnetic stirrer. The extracts were then filtered and assayed to measure NH₄⁺ and NO₃⁻ concentrations by ionometry [10, 11]. Total nitrogen in dry matter of vermicomposts was determined in the test laboratory of the Tomsk agrochemical service by the Kjeldahl method [12]. All data are calculated on dry matter.

The experiment was conducted in four replicates. For chemical analysis, five samples were taken from each replication (from one container) using the "envelope" method [13]. Data (in tables and in the figure 1) is presented as means. The error bars (in the figure 1) and values after ± (in tables) indicate standard errors of means. We used the W/S-test for normality for the samples under study [14]. Statistical significance at p< 0.05 was estimated using the t-test for independent samples.

The number of ammonifiers was taken into account on a standard medium meat-peptone agar (MPA) [3]. To determine the amount of inorganic nitrogen utilizers, starch-ammonia agar (SAA) medium was used [3, 4]. Ashby medium was used to determine the amount of nitrogen fixers [3].

The preparation of dilutions and crops on nutrient media MPA and SAA were carried out according to standard methods used in soil microbiology [3]. 1 g of the substrate was triturated in a sterile mortar, transferred to a flask with 100 ml of sterile 0.85% NaCl, shaken for 5 min, and made dilutions. Crops were carried out from dilutions 10⁻⁴ and 10⁻⁵. The repetition of the experiment was fourfold.
The actual activity of free-living nitrogen fixers was determined by fouling lumps of the substrate on Ashby medium [3]. The lumps of the substrate were laid out on a nutrient medium in a checkerboard pattern at a distance of about 1 cm from each other. The repetition of the experiment was fourfold.

Also, with the crops, 4 g of each compost and vermicompost sample were taken for moisture determination. The samples were dried to absolutely dry weight at 105°C.

The statistical processing of the results was carried out using Google spreadsheets and MS Excel. Confidence intervals were calculated by the formula (1) [3]:

\[ I_{95} = 2 \sqrt{\frac{\bar{N}}{n}} = \frac{2 \sqrt{T}}{n}, \]

where \( I_{95} \) is the confidence interval, \( \bar{N} \) – the average number of counted colonies, \( T \) – the total number of estimated colonies, and \( n \) – the number of replicates.

Subsequently, the average content of colony-forming units (CFU) in one gram of wet substrate was calculated according to the equation (2):

\[ CFU_w = (\bar{N} \pm I_{95}) \times K \times \frac{1}{V}, \]

where \( CFU_w \) is the number of colony forming units of microorganisms in a gram of wet substrate, \( K \) – the dilution from which the sowing was made with a degree times of 1, and \( V \) – the volume of seed.

The activity of the \textit{Azotobacter} was calculated as the ratio of the number of lumps of soil with the growth of \textit{Azotobacter} to the total number of lumps in percent [3].

A day after sowing, the moisture content of each substrate was determined by the formula (3):

\[ W = \frac{m_w - m_d}{m_d} \times 100, \]

where \( W \) is the moisture content of the substrate in percent, \( m_w \) is the mass of the wet substrate, \( m_d \) is the mass of the dry substrate.

For each substrate, a moisture coefficient was also calculated by the formula (4):

\[ K_w = \frac{100}{100 - W}, \]

where \( K_w \) is the moisture coefficient, \( W \) – the moisture content of the substrate in percent.

The conversion of the number of \( CFU \) in 1 g of wet substrate to \( CFU \) in 1 g of dry substrate was carried out according to the equation (5):

\[ CFU = CFU_w \times K_w, \]

where \( CFU \) is the number of colony forming units in a gram of dry matter.

3. Results and discussion

The chemical parameters of the initial substrates are presented in table 1. The highest content (about 1.8%) of total nitrogen was observed in the cow manure. The total nitrogen content in the initial leaf litter was 2.5 times lower than in manure. The results agree with the literature data, according to which leaf litter is a poor total nitrogen substrate [5]. The high nitrogen content of manure is common knowledge. As for the in the greenhouse soil used in the experiment, we can say that is a good level of nitrogen supply to the soil [15].

Cow manure had the highest ammonium content (over 20 mEq/kg). The ammonium content in leaf litter was three times less than in manure. In the used greenhouse soil, ammonium as a macro-element was practically absent. The maximum nitrate content was found in the initial leaf litter (about 350 mEq/kg). In other initial substrates, this indicator was an order of magnitude lower.
Table 1. The content of nitrogen-forms in initial components before vermicomposting.

| Substrate        | Total N, % | NH$_4^+$, mEq/kg | NO$_3^-$, mEq/kg |
|------------------|------------|-------------------|------------------|
| Greenhouse soil  | 0.95 ±0.10 | 0.04 ±0.004       | 21.60 ±1.31      |
| Cow manure       | 1.83 ±0.10 | 22.04 ±0.790      | 18.15 ±2.52      |
| Leaf litter      | 0.73 ±0.06 | 7.28 ±0.230       | 350.80 ±37.37    |

* mEq – milliequivalent.

In the process of vermicomposting, the total nitrogen content in the initial mixtures practically did not change. The total nitrogen content in vermicompost based on manure was two times higher than that based on leaf litter. Vermicomposting did not any way affect the content of total nitrogen in the studied substrates (figure 1).

![Graphs showing nitrogen balance](image)

Figure 1. The balance of nitrogen-forms in manure-soil and litter-soil mixtures after vermicomposting: total nitrogen, % (a); ammonium, mEq/kg (b); nitrate, mEq/kg (c): ○ – greenhouse soil with cow manure, ● – greenhouse soil with leaf litter; VC – vermicomposting.

The total nitrogen content was the same in the initial litter-soil mixture and in the obtained vermicompost (figure 1a). The total nitrogen content in cow-manure vermicompost was 9% higher than in the initial manure-soil mixture. This difference was statistically insignificant. After vermicomposting the soil-manure mixture (figure 1b), the ammonium content decreased by about three times. It increased after vermicomposting the mixture of soil and litter (by about the same number). Vermicomposting led to an increase in the content of nitrates in both types of substrate. Figure 1c shows that despite the same direction of changes in the content of nitrates in both compostable mixtures, the...
relative increase in the content of nitrates in vermicompost based on manure is an order of magnitude higher than in vermicompost based on leaf litter.

Thus, vermicomposting did not in any way affect the content of total nitrogen in the studied substrates, but it led to significant changes in the content of nitrate and ammonium.

Microorganisms play an essential role in the nitrogen cycle [3, 4]. Table 2 presents data about the content of microorganisms involved in the transformation of nitrogen forms in obtained vermicomposts. Manure vermicompost contained significantly more ammonifiers and utilizers of inorganic nitrogen than vermicompost based on leaf litter. The number of ammonifiers in manure vermicompost was more than twice that of vermicompost based on leaf litter, and the number of inorganic nitrogen utilizers was about 2.5 times higher. The actual activity of free-living nitrogen fixers in both vermicomposts differed insignificantly and approached 100%.

Table 2. Number of some groups microorganisms of nitrogen cycle in vermicomposts based on leaf litter and cow manure.

| Vermicompost | The number of CFU per 1 g of vermicompost on the medium | Actual activity of Azotobacter, % |
|--------------|--------------------------------------------------------|----------------------------------|
|              | Ammonifiers on MPA, \( \cdot 10^8 \) | Inorganic nitrogen utilizers on SAA, \( \cdot 10^8 \) | Total CFU on MPA and SAA, \( \cdot 10^8 \) |                                  |
| Manure       | 72.8 ± 1.91 | 31.0 ± 1.25 | 103.9 | 99.78 |
| Litter       | 30.4 ± 1.23 | 11.7 ± 0.77 | 42.1  | 97.53 |

Thus, the intensity of microbiological processes of nitrogen transformation in manure vermicompost is much higher than that in leaf litter vermicompost. Nitrification begins with ammonium oxidation, leading to the appearance of nitrates in the medium first, which are subsequently oxidized to nitrates [15]. Utilizers of inorganic nitrogen consume ammonium and convert it back into organic matter. But the inorganic nitrogen immobilization process activity is lower than the activity of ammonification. The number of ammonifiers in both vermicompost exceeds the number of utilizers of inorganic nitrogen by about 2.5 times. This difference is slightly higher for leaf litter vermicompost. Hence, it follows that in both vermicompost the rate of ammonium production exceeds the rate of its consumption by utilizers of inorganic nitrogen. Microbiological ammonium oxidation (coupled with subsequent nitrification) is the global biogeochemical nitrogen cycle [16-19]. The intensity of this process is higher in manure vermicompost than in leaf litter vermicompost, (according to the data obtained about the balance of ammonium and nitrates, figures 1b and 1c).

Although the processes of microbiological transformation of nitrogen during the processing of leaf litter are much slower than during the processing of manure, a high initial level of nitrates in poplar litter still leads to the fact that the content of nitrates in vermicompost based on leaf litter is higher than in manure vermicompost (table 1, figure 1c). The ability of poplar to accumulate very high amounts of nitrate-nitrogen in leaves (500 mEq/kg and more in terms of dry matter) was experimentally shown in [20]. Therefore, vermicompost from leaf litter can be considered as potentially valuable fertilizer, and the processes of leaf litter processing in vermiculture themselves require further study.

In the paper some aspects of nitrogen transformation by earthworms *Eisenia fetida* in cow manure and leaf litter was investigated. The total nitrogen content (% in dry matter) was 0.95 in greenhouse soil; 1.83 in cow manure; and 0.73 in leaf litter. The number of ammonium (mEq/kg) was 0.04 in greenhouse soil; 22.04 in cow manure; and 7.28 in leaf litter. The nitrate concentration (mEq/kg) was 21.6 in greenhouse soil; 18.15 in cow manure, and 351 in leaf litter. The total nitrogen content was the same (0.88% in dry matter) in the initial litter-soil mixture and in the obtained from it vermicompost. The total nitrogen content in cow-manure vermicompost was 9% higher than in the initial manure-soil mixture, but this difference was statistically insignificant. The ammonium and nitrate content (mEq/kg) in the mixture of greenhouse soil with cow manure was decreased from 17.64 to 3.32 and increased from 18.84 to 122.59 after
vermicomposting, respectively. The vermicomposting of mixture leaf litter with greenhouse soil induced the ammonium and nitrate concentration increase from 2.21 to 6.3 and from 120.36 to 190.74 mEq/kg, respectively. The \textit{Azotobacter} bacteria actual activity in both vermicomposts was close to 100%. The sum number of ammonifiers and utilizers of inorganic nitrogen in the manure vermicompost and the leaf litter vermicompost was $103.9 \times 10^8$ and $42.1 \times 10^8$ CFU/g, respectively.

4. Conclusion
The results obtained suggest that a higher sum number of ammonifiers and utilizers of inorganic nitrogen causes a higher ammonification and nitrification in substrates based on a mixture of greenhouse soil and cow manure. That is why the relative increase in nitrate content in the mixture of greenhouse soil and cow manure is an order of magnitude higher than the relative increase in the content of nitrates in the mixture of greenhouse soil and leaf litter. However, a high initial nitrate level ensures a high nitrate content in the vermicompost obtained from the conversion of the greenhouse soil and leaf litter mixture. Thus, despite the relatively slow processes of nitrogen transformation in the leaf litter vermicomposting, this substrate can be considered as a valuable raw material for vermicompost production. This raw material can be an alternative to manure and other rich organic matter wastes. The leaf litter vermicompost is a good nitrogen fertilizer not inferior in terms of some manure vermicompost indicators. It opens up perspectives to the rational conversion of large amounts of leaf litter in the Siberian region, where the annually formed fallen leaves are being buried in landfills. At least part of the annual litter can be converted into valuable organic fertilizer. As an extension of our research, we consider that it is necessary to undertake a comparative study of the agrochemical properties of vermicompost obtained by conversion leaf litter from different species of woody plants. In addition, it is of interest to study the possibility of accelerating the vermicomposting processes by pretreating the collected leaf litter with various microbiological preparations.

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References
[1] Broz A P, Verma P Q and Appel C 2016 Nitrogen dynamics of vermicompost use in sustainable agriculture. \textit{Journal of Soil Science and Environmental Management} 7 (11) 173 DOI 10.5897/JSSEM2016.0587
[2] Tereshchenko N N, Yunusova T V and Pisarchuk A D 2012 Microorganisms – unique indicators of vermicompost quality. \textit{Achievements of Science and Technology in Agroindustrial Complex} 5 58
[3] Kornievskaya E, Kurovsky A, Babenko A, Petrochenko K and Sechko O 2020 Microbial structure of nitrogen utilizers in \textit{Populus nigra} L. compost and vermicompost. \textit{IOP C. Ser. Earth Env.} 433 012001 doi:10.1088/1755-1315/433/1/012001
[4] Stakhurlova L D, Shcheglov D I and Svistova I D 2007 Biological activity as an indicator of chernozem fertility in different biocenoses. \textit{Eurasian Soil Sci+.} 40 (6) 694 doi: 10.1134/S1064229307060117
[5] Petrochenko K A, Kurovsky A V, Babenko A S and Yakimov Yu E 2015 Leaf litter-based vermicompost as promising calcium fertilizer. \textit{Tomsk State University Report. Biology} 2 (30) 20 doi: 10.17223/19988591/30/2
[6] Kurovsky A V, Petrochenko K A, Godymchuk A Yu, Babenko A S and Yakimov Yu E 2019 Physicochemical aspects of recycling tree leaf litter in the south of Western Siberia by the Eisenia fetida (Savigny) vermiculture. \textit{IOP C. Ser. Earth Env.} 226 012009 doi:10.1088/1755-1315/226/1/012009
[7] Flores-Sánchez D, Pastor A, Rossing W A H, Kropff M J and Lantinga E A 2016 Decomposition, N contribution and soil organic matter balances of crop residues and vermicompost in maize-based cropping systems in southwest Mexico. \textit{J. Soil Sci. Plant Nut.} 16 (3) 801-817 DOI:
[8] Wako R E 2021 Preparation and characterization of vermicompost made from different sources of materials. *Open J. Plant Sci.* **6**(1) 042-048 DOI: https://dx.doi.org/10.17352/ojps.000031

[9] Lv B, Zhang D, Chen Q and Cui Y 2019 Effects of earthworms on nitrogen transformation and the correpond genes (amoA and nirS) in vermicomposting of sewage sludge and rice straw. *Bioresource Technol.* **287** 121428 https://doi.org/10.1016/j.biortech.2019.121428

[10] Guide to Ion Selective Measurement, Mettler-Toledo GmbH, available at: https://www.mt.com/sg/en/home/library/tips-and-tricks/lab-analytical-instruments/Guide_Ion_meas/jcr:content/download/file/file.res/51300075_Ion_Guide_e.pdf

[11] Yu T R 1992 Electrochemical techniques for characterizing soil chemical properties advances in agronomy **48** 205 https://doi.org/10.1016/S0065-2113(08)60938-4

[12] Bremmer J M 1960 Determination of nitrogen in soil by the Kjeldahl method. *The Journal of Agricultural Science* **55** 1 11 DOI: https://doi.org/10.1017/S0021859600021572

[13] Kapanadze K, Magalashvili A and Imnadze P 2019 Distribution of natural radionuclides in the soils and assessment of radiation hazards in the Khrami Late Variscan crystal massif (Georgia). *Helyon* **5** 01377 doi: 10.1016/j.helyon.2019.e01377

[14] Nosakhare U H and Bright A F 2017 Statistical analysis of strength of W/S test of normality against non-normal distribution using monte carlo simulation. *American Journal of Theoretical and Applied Statistics* **6**(5-1) 62 doi: 10.11648/j.ajtas.s.2017060501.19

[15] Bremmer J M and Mulvaney C S 1983 Nitrogen – total. *Methods of Soil Analysis. Agronomy Monographs* ed A L Page (Madison: American Society of Agronomy, Inc.) chapter 31 pp 595-622 doi:10.2134/agronmonogr9.2.2ed

[16] Lehtovirta-Morley L E 2018 Ammonia oxidation: Ecology, physiology biochemistry and why they must all come together. *FEMS Microbiology Letters* **365** 058 doi: 10.1093/femsle/fny058

[17] Karl D, Letelier R, Tupas L, Dore J, Christian J and Hebel D 1997 The role of nitrogen fixation in biogeochemical cycling in the subtropical North Pacific Ocean. *Nature* **388** 7 533

[18] Ward B B and Jensen M M 2014 The microbial nitrogen cycle *Frontiers in Microbiology* **5** 553 1 https://doi.org/10.3389/fmicb.2014.00553

[19] Lamba S, Bera S, Rashid M, Medvinsky A B, Sun G-Q, Acquisti C, Chakraborty A and Li B-L 2016 Organization of biogeochemical nitrogen pathways with switch-like adjustment in fluctuating soil redox conditions *Royal Society Open Sci.* **4** 160768 https://doi.org/10.6084/m9.figshare.c.3649916.v1

[20] Black B L Fuchigami L H and Coleman G D 2002 Partitioning of nitrate assimilation among leaves, stems and roots of poplar. *Tree Physiol.* **22** 717 DOI: 10.1093/treephys/22.10.717