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**BALANCE OF NITROGEN AND MICROBIOLOGICAL CHARACTERISTICS
OF VERMICOMPOSTS OBTAINED FROM THE PROCESSING OF COW MANURE,
LEAF LITTER, AND MOWN GRASS**

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Abstract

The mean total nitrogen content in vermicomposts based on cow manure, a mixture of willow–poplar leaf litter, and mown grass amounted to 1.92, 1.64, and 2.15%, respectively. The increase in total nitrogen content during vermicomposting of cow manure and leaf litter was 39% and 50%, respectively. During vermicomposting of mown grass, the total nitrogen content decreased by 26%. The actual activity of *Azotobacter* bacteria was highest in vermicomposts based on mown grass (98%). For vermicomposts based on leaf litter, this indicator was 85%, while manure-based vermicompost was 70%. Vermicomposts obtained from mown grass surpassed other variants in the content of ammonifiers (10^{10} CFU/g), nitrifiers (6×10^6 cells/g), and inorganic nitrogen utilizers (1.4×10^{10} CFU/g). A very strong positive correlation ($r = 0.9999$) was established between the total nitrogen content and the content of ammonifiers in vermicomposts. The balance of total nitrogen content (percentage difference between nitrogen content in vermicomposts and initial mixtures) correlated negatively with the content of inorganic nitrogen utilizers in vermicomposts ($r = -0.97$).

Keywords: cow manure, willow–poplar leaf litter, mown grass, vermicompost, total nitrogen, ammonifiers, nitrifiers, inorganic nitrogen utilizers, free-living nitrogen-fixing microorganisms.

**ІРІ ҚАРА МАЛДЫҢ КӨҢІН, ЖАПЫРАҚ ТӨСЕНІШІН ЖӘНЕ ШАБЫЛҒАН
КӨҒАЛ ШӨБІН ӨНДЕУ АРҚЫЛЫ АЛЫНҒАН ВЕРМИКОМПОСТТАРДЫҢ
АЗОТ БАЛАНСЫ ЖӘНЕ МИКРОБИОЛОГИЯЛЫҚ СИПАТТАМАЛАРЫ**

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Аңдатпа

Сиыр көңі, тал-терек жапырақтарының аралас төсеніш қалдықтары және шабылған көгал шөбі негізінде алынған вермикомпосттардағы азоттың орташа жалпы мөлшері тиісінше 1,92; 1,64 және 2,15% құрады. Сиыр көңі мен түскен жапырақтарды вермикомпосттау барысында жалпы азот мөлшерінің артуы тиісінше 39% және 50% болды. Шабылған көгал шөбін вермикомпосттау кезінде жалпы азот мөлшері 26%-ға

төмендеді. *Azotobacter* бактерияларының белсенділігі ең жоғары деңгейде шабылған шөп негізіндегі вермикомпосттарда байқалып, 98% құрады. Жапырақ төсеніштері негізіндегі вермикомпосттарда бұл көрсеткіш 85%, ал көң негізіндегі вермикомпосттарда шамамен 70% болды. Шабылған көгал шөбін өңдеу нәтижесінде алынған вермикомпосттар аммонификаторлар (10^{10} КОЕ/г), нитрификаторлар (6×10^6 жасуша/г) және бейорганикалық азотты пайдаланушылар ($1,4 \times 10^{10}$ КОЕ/г) мөлшері бойынша басқа нұскалардан жоғары болды. Вермикомпосттардағы жалпы азот мөлшері мен аммонификаторлар мөлшері арасында өте күшті оң корреляция анықталды ($r = 0,9999$). Жалпы азот мөлшерінің балансы (вермикомпосттағы азот мөлшері мен бастапқы қоспалардағы азот мөлшері арасындағы пайыздық айырмашылық) вермикомпосттардағы бейорганикалық азотты пайдаланушылар мөлшерімен теріс корреляцияда болды ($r = -0,97$).

Кілт сөздер: сиыр көні, тал мен теректің түскен жапырақтары, шабылған көгал шөбі, вермикомпост, жалпы азот, аммонификаторлар, нитрификаторлар, бейорганикалық азотты пайдаланушылар, еркін тіршілік ететін азотфиксациялаушы микроорганизмдер.

БАЛАНС АЗОТА И МИКРОБИОЛОГИЧЕСКИЕ ХАРАКТЕРИСТИКИ ВЕРМИКОМПОСТОВ, ПОЛУЧЕННЫХ ПРИ ПЕРЕРАБОТКЕ НАВОЗА КРС, ЛИСТОВОГО ОПАДА И СКОШЕННОЙ ГАЗОННОЙ ТРАВЫ

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Аннотация

Среднее общее содержание азота в вермикомпостах на основе коровьего навоза, смешанного ивово-тополиного листового опада и скошенной газонной травы составило 1,92, 1,64 и 2,15% соответственно. Увеличение общего содержания азота в процессе вермикомпостирования коровьего навоза и опавших листьев составило 39% и 50% соответственно. При вермикомпостировании скошенной газонной травы общее содержание азота упало на 26%. Актуальная активность бактерий *Azotobacter* была выше всего в вермикомпостах на основе скошенной травы – 98%. Для вермикомпостов на основе листового опада этот показатель составил 85%, для вермикомпостов на основе навоза – около 70%. Вермикомпосты, полученные при переработке скошенной травы превосходили другие варианты по содержанию аммонификаторов (10^{10} КОЕ/г), нитрификаторов (6×10^6 кл./г) и утилизаторов неорганического азота ($1,4 \times 10^{10}$ КОЕ/г). Установлена очень сильная положительная корреляция ($r=0,9999$) между содержанием общего азота и содержанием аммонификаторов в вермикомпостах. Баланс содержания общего азота (процентная разница между содержанием азота в вермикомпостах и исходных смесях) отрицательно коррелировал с содержанием утилизаторов неорганического азота в вермикомпостах ($r = -0,97$).

Ключевые слова: коровий навоз, опавшая листва ивы и тополя, скошенная газонная трава, вермикомпост, общий азот, аммонификаторы, нитрификаторы, утилизаторы неорганического азота, свободноживущие азотфиксирующие микроорганизмы.

Introduction

Vermicomposting is a natural decomposition process in which earthworms and microorganisms jointly mineralize organic waste, transforming it into a nutrient-rich organic fertilizer [1, 2]. It involves a bio-oxidative process wherein earthworms act in synergy with

bacterial, fungal, and actinomycete communities to stabilize organic waste by altering its physical and biochemical characteristics [3]. The greater part of the organic matter is decomposed, while the residues are converted into a stabilized vermicompost that is rich in humic compounds, hormones, and minerals [4, 5]. Unlike conventional mineral fertilizers, vermicompost provides a multifaceted approach to crop nutrition, being extensively adopted in large-scale organic production systems [6, 7]. Accumulated evidence indicates that its application positively influences seed vigor, plant ontogenesis, and overall productivity, all while preserving the soil's intrinsic quality. Given that intensive agricultural practices often degrade soil structure and biological activity, the use of vermicompost serves as a restorative measure, concurrently enhancing the soil's physical framework, chemical buffering capacity, and microbial diversity [2, 4, 8]. From a technological standpoint, vermicomposting aligns with the principles of a circular economy, offering a low-cost, energy-efficient strategy for the remediation of diverse non-toxic organic residues, with reduced greenhouse gas emissions relative to traditional composting [2, 9, 10]. Beyond its well-recognized role as a complete macronutrient supplier for crops, vermicompost also exhibits bioprotective properties, suppressing soil-borne pathogens and pests, while simultaneously modulating key soil fertility indices, including nitrogen availability and the carbon-to-nitrogen equilibrium.

Among the essential macronutrients, nitrogen warrants particular attention due to its unique biogeochemical behavior within closed vermicomposting systems (e.g., containers isolated from the underlying soil). In such setups, the gross (total) pools of phosphorus and potassium remain largely conserved, as their quantities are inherently determined by the initial substrate composition, with only minor fluctuations driven by microbial turnover. In contrast, the total nitrogen pool is subject to substantial quantitative modification, primarily through the biological reduction of atmospheric dinitrogen – a process catalyzed by specialized diazotrophic microorganisms. It is crucial to emphasize that these observations pertain to the gross elemental reserves, quantified via complete oxidative mineralization of the sample, which represent the theoretical nutrient reservoir available for plant uptake.

Furthermore, nitrogen often acts as a yield-limiting factor in agroecosystems, largely due to the high mobility of its oxidized mineral forms (nitrates), which are prone to leaching beyond the rhizosphere. This inherent instability complicates the selection of fertilizers capable of sustaining a controlled, prolonged release of nitrogen in the root zone. Emerging evidence suggests that certain organic amendments, including vermicompost, may offer a more gradual and synchronized nitrogen supply compared to their synthetic counterparts, thereby mitigating nutrient losses and enhancing use efficiency.

From a biotechnological perspective, the feedstock for vermicomposting can be broadly categorized into two classes based on their recalcitrance. The first comprises readily decomposable, "soft" agricultural residues (e.g., fruit and vegetable waste), characterized by elevated moisture content, low lignocellulose complexity, and a high proportion of soluble saccharides, which facilitate rapid microbial and earthworm activity [11]. The second encompasses lignified, cellulosic materials that are inherently more resistant to degradation. Livestock manure typifies the first category, while woody leaf litter exemplifies the second. Notably, mown grass occupies an intermediate position, harboring both fibrous structural components and nitrogen-/potassium-rich parenchymatous tissues, thus exhibiting features of both groups.

Collectively, fallen foliage and grass clippings constitute the bulk of urban green waste, a voluminous byproduct of municipal and agroecosystem maintenance. The disposal of tree leaf litter, in particular, poses a significant logistical and financial burden; its incineration, still

practiced in some regions, not only squanders a valuable nutrient resource but also contributes to atmospheric particulate pollution [12–15]. In response to these challenges, vermicomposting has emerged as a robust, ecologically sound biotechnology capable of converting this heterogeneous organic stream into a marketable soil conditioner, thereby delivering tangible agro-ecological and economic returns [16, 17].

The primary objective of this investigation was to assess the key agrochemical parameters and microbial community dynamics governing nitrogen turnover during the vermicomposting of willow-poplar leaf litter and grass mowings under the climatic constraints of Western Siberia's southern regions.

Materials and methods

While diverse organic feedstocks have been explored in vermicomposting research globally, livestock manure—particularly from cattle—remains the standard reference substrate [18–27]. Over the past decade, our team has systematically investigated the vermiprocessing of woody leaf litter, a major urban landscaping residue, consistently benchmarking its performance against that of traditional manure-based systems.

This study was designed to compare the physicochemical and microbiological profiles of vermicomposts derived from three distinct feedstocks: cow manure, leaf litter, and mown grass. The nitrogen status of the substrates was evaluated based on total nitrogen concentration in both initial mixtures and final products, and the nitrogen balance, expressed as the percentage change in total nitrogen content post-vermicomposting. Concurrently, the abundance of key nitrogen-cycling trophic groups – including ammonifiers, inorganic nitrogen utilizers, free-living diazotrophs, and nitrifiers – was enumerated using solid and liquid selective media.

For vermicomposting, we used a culture of *Eisenia fetida* earthworms, maintained by the Head of the Department of Agrobiotechnology of Tomsk State University, Professor Andrey Babenko.

The feedstocks utilized comprised air-dried cattle manure (bedding-free), leaf litter, and grass mowings sourced from the Tomsk University Grove. The leaf litter fraction consisted of a composite mixture from *Populus nigra* L. and *Salix fragilis* L., species selected for their elevated foliar mineral nutrient content, with the rationale for their use detailed in our prior publication [28].

Litter collection was conducted annually over a nine-year period between late September and mid-October, under positive mean daily temperatures and prior to permanent snow cover. The harvested material was desiccated at ambient conditions to a stable weight and stored dry. Prior to use, the litter was milled to a 10 × 10 mm particle size. A low-mineralized, air-dried sphagnum peat served as the bulking/sorption agent. The feeding component (manure or litter) and peat were mixed in a 1:1 ratio (100 g dry weight each), yielding a 200 g (approx. 2 L) mixture per experimental unit. The substrates were hydrated with distilled water to 75% moisture content five days prior to inoculation and equilibrated to pH 5.5–5.7. Subsequently, 20 mature *E. fetida* individuals (totaling ~8 g biomass) were introduced into each 7-L ventilated container. The vermicomposting process was conducted in darkness at 21 ± 3°C for a duration of 3.5 to 4 months.

Mown grass (standard cereal grass mixture) was vermicomposted in outdoor piles from June to September for 5 years. The size of the piles was 1 × 1.5 m, with a side height of 15–20 cm. The bottom of the piles was lined with polyethylene film. The same film was used to cover the piles to prevent over-moistening of the substrate during rains. The mown grass was placed in the pile. At the pre-composting stage, the moisture content of the grass was brought to 70–75% with regular tap water and kept for about two weeks. After that, worms were introduced

into the piles at a rate of 400 mature individuals per square meter. The duration of vermicomposting was 2.5–3.5 months.

Post-vermicomposting, the substrates were manually cleared of earthworms and cocoons. For chemical assays, samples were oven-dried at 105°C, and total nitrogen was quantified via spectrophotometric analysis of sulfuric acid (H₂SO₄) mineralizates using a selenium catalyst, as described previously [28–30]. Microbiological analyses were performed on fresh (non-dried) material. Populations of ammonifiers and inorganic nitrogen utilizers were estimated via the limiting dilution technique on meat-peptone agar and starch-ammonia agar, respectively, with results expressed as colony-forming units (CFU) per gram of absolute dry matter. The actual activity of free-living *Azotobacter* spp. was assessed as a percentage using the lump overgrowth method on Ashby's medium. Detailed protocols for these microbiological assays are available in our earlier studies [30, 31].

For each substrate, 12 independent vermicomposting units (biological replicates) were established. From each unit, 3 subsamples were taken and analysed separately (analytical replicates); the reported standard errors reflect the combined variation across all biological and analytical replicates.

Data aggregation and statistical evaluation across the multi-year study involved the Kolmogorov–Smirnov test for normality assessment, followed by one-way analysis of variance (ANOVA) and Pearson's correlation analysis. All tabulated results are expressed as arithmetic means accompanied by the standard error of the mean (SEM).

Results and Discussion

Table 1 presents data on the nitrogen content in three types of substrates subjected to vermicomposting. The highest values of total nitrogen content in the initial mixtures for vermicomposting were recorded in unprocessed mown grass (2.5% of dry mass). The lowest values were characteristic of the total nitrogen content in mixtures of peat and fallen leaves – at the level of one percent. The total nitrogen content in mixtures of peat and cow manure was slightly less than one and a half percent.

The total nitrogen content in the finished vermicomposts, as in the initial mixtures, preserved the order of increasing values across the substrate series: leaf litter – cow manure – mown grass. However, the degree of difference between the variants decreased somewhat (Table 1). This pattern – the reduction in the difference in nitrogen content between variants – is well explained and illustrated by the third indicator presented in the table – the balance of total nitrogen in the substrates.

Table 1. Total nitrogen content in three types of substrates during vermicomposting

Substrate	Initial	Vermicompost	Total nitrogen balance, %
Cow manure	1.38 ± 0.05*	1.92 ± 0.09	38.91 ± 2.47
Leaf litter	1.10 ± 0.01*	1.64 ± 0.07	49.77 ± 6.57
Mown grass	2.38 ± 0.09*	2.15 ± 0.38	– 26.02 ± 2.86*

*Significant difference in comparison with all other groups: * – p < 0.05 (from here on)

All values are derived from 12 independent biological replicates, each measured in 3 analytical replicates.

As can be seen from Table 1, the total nitrogen content changed most strongly during vermicomposting in the mixtures of peat and leaf litter – an increase of 50%. For manure, this indicator was somewhat lower – about 40%. After vermicomposting of mown grass, the total

nitrogen balance turned out to be negative – the content of this macronutrient in the vermicomposts was 26% lower than in the initial substrate.

Table 2 presents data on the content of microorganisms of the nitrogen cycle in the vermicomposts under study.

Table 2. Abundance of nitrogen-cycle microorganisms in vermicomposts obtained from the processing of three substrate types

Substrate	Actual activity of <i>Azotobacter</i> , %	Ammonifiers, CFU per 1 g, $\times 10^9$	Nitrifiers, cells per 1 g, $\times 10^6$	Inorganic nitrogen utilizers, CFU per 1 g, $\times 10^9$
Cow manure	70.45 $\pm$ 5.0*	7.57 $\pm$ 0.2	0.88 $\pm$ 0.3*	6.99 $\pm$ 0.37*
Leaf litter	85.01 $\pm$ 2.3*	4.68 $\pm$ 0.3*	3.01 $\pm$ 0.6*	3.54 $\pm$ 0.33*
Mown grass	97.75 $\pm$ 1.1*	10.04 $\pm$ 1.2	6.30 $\pm$ 0.6*	13.8 $\pm$ 1.20*

*Significant difference compared with all other groups: * – $p < 0.05$ (same hereafter).

All means and activities are based on 12 independent biological replicates, each assayed in 3 replicates.

From table 2, it is evident that the actual activity of free-living nitrogen-fixing bacteria of the genus *Azotobacter* in vermicomposts obtained from the processing of mown grass approached 100%. In vermicomposts based on cow manure and leaf litter, this indicator was at the level of 70% and 85%, respectively. In the same order, an increase in the number of nitrifiers was observed – from 9×10^5 cells per gram in manure-based vermicomposts to 6×10^6 in vermicomposts based on mown grass.

The lowest content of ammonifiers and inorganic nitrogen utilizers was recorded in vermicomposts based on leaf litter – 4.7×10^9 and 3.5×10^9 , respectively. In vermicomposts based on cow manure, the values of these indicators were one and a half to two times higher than in leaf-litter-based vermicomposts, while in vermicomposts based on mown grass, they were an order of magnitude higher than in both leaf-litter-based and manure-based vermicomposts.

Having performed a correlation analysis between the microbiological indicators and the nitrogen content values in the studied substrates, we established a pronounced correlation for two pairs of indicators: "ammonifier content – total nitrogen content in finished vermicomposts, $r = 0.9999$ ", which indicates an almost functional relationship between the traits, and "inorganic nitrogen utilizer content – total nitrogen balance after vermicomposting, $r = -0.97$ ".

It should be noted, however, that the high (close to unity) correlation coefficients obtained in this study were calculated on a limited number of substrate variants ($n = 3$). Therefore, they are valuable primarily as indicators of the direction and strength of the relationship under the specific conditions of this experiment, but they cannot be regarded as universal quantitative dependencies. Their biological interpretation requires caution, and the conclusions we have drawn are tentative and need further verification on a wider range of substrates and under more diverse vermicomposting conditions.

How might one attempt to interpret the results obtained in this work? It is obvious that a significant increase in total nitrogen content during the vermicomposting process can only be attributed to the activity of nitrogen-fixing microorganisms. If we compare this indicator in vermicomposts based on leaf litter and manure, such a logical pattern is clearly evident: in substrates where the activity of nitrogen fixers is higher, the increase in total nitrogen content is characterized by higher values. Previously, we demonstrated the same relationship in a comparative study of vermicomposting of three substrate types: manure, poplar leaf litter, and

willow leaf litter [32]. The strong correlation between the number of ammonifiers and the total nitrogen content in vermicomposts is also quite logical, since ammonification is the first obligatory link in the mineralization of organic nitrogen in any substrates and environments.

However, vermicomposts based on mown grass fall out of the general picture. This refers to the negative balance of total nitrogen during vermicomposting. At present, the reasons for this phenomenon remain unclear. Nevertheless, we can make certain assumptions in this regard. We believe that the strong negative correlation between the increase in total nitrogen content during vermicomposting and the number of microorganisms that utilize inorganic nitrogen does not reflect the real negative influence of microorganisms of this group on the nitrogen balance. Most likely, this relationship is indirect. That is, inorganic nitrogen utilizers are probably more abundant in those substrates where there is a greater number of mineral forms of nitrogen – ammonium and nitrates (substrate-stimulated growth of microbial numbers). Even without additional chemical analysis of mobile forms of nitrogen, it is quite obvious that, based on the highest values of the number of ammonifiers and nitrifiers, the highest concentrations of nitrates and ammonium should be present in the processed mown grass. However, these forms of nitrogen (especially nitrates) are very mobile and extremely poorly retained in soil or other substrates. The technology of vermicomposting of mown grass in our experiments was maximally approximated to industrial tasks – obtaining a finished product directly at the sites of waste generation, in outdoor piles. Although these piles were equipped with insulating materials, it is most likely completely impossible to prevent the leaching of nitrates from the piles into the surrounding soil under real (non-laboratory) conditions. One cannot also exclude the mechanism of nitrogen loss resulting from the fact that under certain conditions (unfavorable for vermicomposting) in substrates in outdoor piles, the activity of denitrifying microorganisms may increase. These organisms act as antagonists to nitrogen fixers in terms of shifting the total nitrogen balance either toward an increase (when nitrogen fixers prevail) or toward a decrease (when denitrifiers prevail). However, it should be emphasized that the proposed explanations (i.e. both nitrate leaching and enhanced denitrification) remain working hypotheses and require rigorous experimental verification. In addition to monitoring important physicochemical parameters like pH and substrate moisture content, a thorough examination of the dynamics of mineral nitrogen forms (ammonium and nitrate) during the whole vermicomposting process is required to validate these hypotheses. To make firm conclusions on the causes of nitrogen losses, direct measurements of gaseous nitrogen emissions (such as N_2O and N_2) from grass windrows under actual conditions would be ideal.

Conclusion

Overall, the results obtained in this work allow us to state that all types of the studied food substrates (cow manure, leaf litter, mown grass) are good sources of total nitrogen for vermicomposting. The total nitrogen content in all vermicomposts obtained in our experiments falls within the generally accepted ranges for this indicator [33–36]. However, the observed disparity in nitrogen balance between the substrates underscores the critical role of initial feedstock quality, particularly the carbon-to-nitrogen ratio and the bioavailability of labile organic fractions, in steering the direction of nitrogen transformation. Woody leaf litter is the most promising substrate in terms of increasing the total nitrogen content in finished vermicomposts, which, in combination with the enormous volumes of this type of waste, allows us to consider vermiprocessing of leaf litter as an ecological biotechnology for the return of nitrogen to agroecosystems. Mown grass is advisable to use for obtaining vermicomposts with the highest content of total and (presumably) mineral nitrogen. The exceptionally high abundance of nitrifiers and inorganic nitrogen utilizers in grass-derived vermicomposts, while

advantageous for rapid mineralization, simultaneously creates a potential vulnerability to nitrate leaching under open-air processing conditions. Addressing this trade-off through the optimization of bulking agents, moisture regimes, or the introduction of specific microbial consortia could represent a promising avenue for enhancing nitrogen conservation in vermicomposting systems. To effectively address these challenges, future research must shift toward a deeper understanding of the underlying microbial community dynamics and molecular mechanisms. Utilizing advanced metagenomic sequencing to track the temporal successions of functional microbial groups could reveal how specific operational parameters influence the expression of nitrogen-cycling genes (such as *amoA*, *nirS*, and *nosZ*). This molecular-level insight will provide a predictive framework for nitrogen behavior, enabling the development of customized microbial inoculants tailored to the unique biochemical profiles of mown grass and leaf litter. Furthermore, transforming these findings into viable industrial biotechnologies requires a broader focus on ecological sustainability and process optimization. Evaluating the total carbon footprint and gaseous emissions—specifically nitrous oxide and ammonia—through comprehensive life cycle assessments (LCA) is essential for validating the green credentials of the process. On a practical level, investigating the co-composting of these distinct matrices represents a vital frontier; strategically blending high-nitrogen grass with carbon-rich leaf litter could engineer a self-buffering system that maximizes nutrient retention, balances the carbon-to-nitrogen ratio, and minimizes environmental risks on a commercial scale. However, further improvement of vermicomposting technologies and scientific research aimed at minimizing nitrogen losses during the processing of mown grass in vermiculture are necessary.

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