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# Biotechnological Potential of Native Thermophilic Microorganisms for Creating a Soil Biomeliorant from Poultry Manure

**Amiran Khabidovich Zanilov, Zalim Islamovich Dudarov *, Milana Radievna Aznaeva, Rustam Kharunovich Taov and Dinara Gumarbievna Dudarova**

Center for Decarbonization of the Agro-Industrial Complex and the Regional Economy, Federal State Budgetary Educational Institution of Higher Education "Kabardino-Balkarian State University named after H.M. Berbekov", 360004 Nalchik, Russia; amiran78@inbox.ru (A.K.Z.); miazn@mail.ru (M.R.A.); rustam_taov77@mail.ru (R.K.T.); dudarova.dinara00@mail.ru (D.G.D.)

* Correspondence: zalim-dudar@yandex.ru

**Abstract**

The aim of the study was to evaluate the biotechnological potential of thermophilic microorganisms isolated from chernozem soil during composting of poultry manure. The efficiency of the strains was determined by their effect on organic matter degradation, humification intensity, and nitrogen accumulation. The correlation between the quality indicators of the composting process was assessed with the gross values, taking into account the proportion of compost fractions. The strains were identified as: *Aeribacillus pallidus* KCTC 3564$^T$ (cellulolytic), *Neobacillus sedimentimangrovi* FJAT-2464$^T$, *Aeribacillus composti* N.8$^T$, *Caldifermentibacillus hisashii* N-11$^T$ (nitrogen fixers), *Acinetobacter pittii* CIP 70.29$^T$, and *Pseudomonas plecoglossicida* NBRC 103162$^T$ (nitrifies). It was found that all the bacteria increase the proportion of small fractions by 19.0–19.9%. The gross content of humic acids increases under the influence of nitrifiers (15.5%) and nitrogen fixers (5.5%). The total nitrogen content increases under cellulolytics (13.8%) and nitrogen fixers (20.2%). The smallest fraction ($\leq$0.25 mm) in nitrogen fixers and nitrifying variants has the greatest bioreclamation properties, by 16.4% ($p < 0.001$) and 12.9% ($p < 0.001$). Targeted microbial strains provide the direction of the transformation processes during biocomposting. It can also be concluded that assessing the quality of composting based on the fraction distribution can be a promising element of the biofermentation process monitoring.

**Keywords:** thermophilic bacteria; nucleotide sequence of genes; 16S rRNA; biofermentation; poultry manure

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## 1. Introduction

When discussing organic raw materials obtained in the livestock industry, definitions should reflect their status. Until recently, the term "livestock waste" was widely used in relation to manure, which, given its agrobiotechnological potential, was not objective. Poultry manure is a particularly valuable by-product of animal husbandry from an agronomic perspective. Approximately 20 million tons of poultry manure are generated annually in the Russian Federation [1]. The fertilizing potential of manure (natural moisture) in terms of mineral components with a nitrogen, phosphorus, and potassium content of 2.1%, 1.44%, and 0.64%, respectively [2] is comparable to 420 thousand tons of the active substance of mineral fertilizers for nitrogen, 288 thousand tons for phosphorus, and

128 thousand tons for potassium. Manure is also rich in mesoelements that are important for plant nutrition. Thus, according to [3], manure contains 0.67% magnesium oxide, 1.92% calcium, and 0.38% sulfur, which is equivalent to 134, 384, and 76 thousand tons of the active substance, respectively.

The value of poultry manure is not limited to its mineral nutrient content. As carbon-containing substances, they provide energy for both native soil microflora and those introduced with commercial biopreparations. Microorganisms are a significant factor in soil formation, which is essential for restoring the carbon sequestration capacity of the land surface, which has significantly decreased since 2023 and is possibly responsible for the continued rise in atmospheric temperature. According to [4], June 2023, 2024, and 2025 recorded the highest total temperatures in the history of meteorological observations.

The climate effect of organic fertilizers from poultry manure also includes reducing the carbon footprint of crop production by partially or completely replacing mineral fertilizers. The contribution of the mineral fertilizers to the overall carbon footprint of grain crops can reach 25% [5], and in the structure of the carbon footprint of the final product (wheat bread), it accounts for up to 15% [6].

The effectiveness of using poultry manure as fertilizer is largely determined by the processing technology [7]. The choice of technology must be consistent with both environmental and economic considerations. One of the most accessible technologies for processing poultry manure is solid-state aerobic fermentation (composting) [8,9]. The "noble manure" technologies [10] have significant potential for further modernization. Improvements of this technology are largely associated with the selection of specialized microbial strains to produce materials that meet sanitary and epidemiological requirements [11–15] and to improve their quality [16–18].

The use of consortia is aimed at achieving a controlled composting process, depending on the goals and characteristics of the raw material. For example, a bacterial consortium with an index of 5842, consisting of lactic acid bacteria and streptococci (*Streptococcus thermophilus*, *Streptococcus bovis*, *Lactobacillus salivarus* var. *salicinicus*, *Lactobacillus salivarus* var. *salivarius*, *Lactobacillus x* (*acidophilus*)), has been proposed for the safe processing of poultry manure [19]. To reduce unpleasant odors and accelerate the decomposition of poultry manure, it is recommended to apply lactic acid bacteria, photosynthetic bacteria, and yeast locally [20]. A consortium of microorganisms (*Anoxybacillus kamchatkensis* AS-GOS-2, *Bacillus amyloliquefaciens* PL-1, *Aneurinibacillus thermoaerophilus* PL-5, *Brevibacillus brevis* PM-3, and *Pseudoxanthomonas taiwanensis* PM-cell) isolated from animal waste and activated sludge has been created for the joint composting of organic waste from poultry farming and wood processing [10].

The concept of creating microbial consortia for biofermentation of poultry manure in order to create soil fertilizers should take into account the main stages of transformation of organic raw materials and the biophilic elements they contain. The most important stages in composting material are the destruction of primary organic matter and its humification; therefore, it is logical to select bioagents that accelerate these processes. Such bioagents can include specific strains of thermophilic microorganisms, the use of which meets economic interests by shortening the cycle time for creating the final fertilizer. During the humification process, nitrogenous compounds are actively transformed, and therefore, the risk of losing valuable plant nutrients in gaseous form can be reduced by selecting specific microorganisms involved in the nitrogen cycle. The concept should take into account quantitative indicators of the final product: the degree of product degradation (proportion of fine fraction (%)), the degree of humification of the material (proportion of humic substances, mg/kg), the content of total nitrogen (%) and its mineral forms (g/kg), as well as the bioreclamation properties of the final product.

The aim of the study was to evaluate the biotechnological potential of thermophilic microorganisms of various physiological groups isolated from ordinary chernozem during the composting of poultry manure with aerobic solid-state fermentation.

## 2. Materials and Methods

Soil samples for the isolation of target strains were collected from ordinary chernozem in the steppe zone of the Kabardino-Balkarian Republic from a long-term observation site included in the Geographic Network of Long-Term Experiments (Station No. 037, 1948) for monitoring the effects of various types of fertilizers.

Thermophilic Cellulolytic Bacteria ($CB^T$) were isolated using the Koch plate method, followed by cultivation under different temperature conditions on Hutchinson-Clayton growth medium with the following composition: glucose—10 g/L; $NaNO_3$—2.5 g/L; $K_2HPO_4$—1.0 g/L; $MgSO_4 \times 7H_2O$—0.3 g/L; NaCl—0.1 g/L; and $CaCl_2 \times 4H_2O$—0.1 g/L; $FeCl_3 \times 6H_2O$—0.01 g/L.

Thermophilic nitrogen-fixing microorganisms were isolated on Ashby's selective medium with the addition of Fedorov's trace elements of the following composition: sucrose—20.0 g/L; $K_2HPO_4$—0.2 g/L; $MgSO_4 \times 7H_2O$—0.2 g/L; NaCl—0.2 g/L; $K_2SO_4$—0.1 g/L; $CaCO_3$—5.0 g/L; agar—2.0 g/L; 1 mL of stock solution containing $H_3BO_3$ in an amount of 5.0 g/L; $(NH_4)MoO_4 \times 2H_2O$—5.0 g/L; $ZnSO_4 \times 7H_2O$—0.2 g/L; KI and NaBr—0.5 g/L each; and $Al_2(SO_4)_3 \times 18H_2O$—0.3 g/L in 1000 mL of distilled water. The suspension was incubated for 24 h at 47 °C in a thermoshaker (160 rpm). Pure cultures were isolated by subsequent transfer to a solid nutrient medium—fish protein hydrolysate [21].

Nitrifying Bacteria ($NB^T$) used to limit nitrogen losses in gaseous form during fermentation of poultry manure, isolated under different temperature conditions on S.N. Vinogradsky's selective nutrient medium consisting of: $(NH_4)_2SO_4$—2.0 g/L; $K_2HPO_4$—1.0 g/L; $MgSO_4$—0.5 g/L; $FeSO_4$—0.4 g/L; NaCl—2.0 g/L; and tap water. Pure cultures were isolated by subsequent transfer to solid media [21]. The manufacturer of micronutrient components for nutrient media is "Khimreaktivsnab" JSC, Ufa, Russia. The manufacturer of organic nutrient media is "Agat-Med" LLC, Balashikha, Russia.

The thermophilic status of the bacteria was confirmed by transferring them to solid nutrient media (GMF-agar) and incubating them at a wide range of temperatures: 20–65 °C. It was found that four strains ($NFB^T2$, $NFB^T9$, $NFB^T10$, $CB^T$) show active growth at temperatures between 45 and 60 °C, with no growth below 40 °C. The optimal range is 47–50 °C. Nitrifying strains ($NB^T1$, $NB^T2$) were less resistant to elevated temperatures, with no growth above 50 °C, and the ability to grow at 20 °C. Nitrifying strains ($NB^T1$, $NB^T2$) were less resistant to increased temperatures, did not grow above 50 °C, and were capable of growing at 20 °C.

The strains were identified based on the nucleotide sequence of the 16 rRNA genes. For composting poultry manure with the isolated microorganisms, an experimental compost pile was placed on a concrete pad with the following dimensions: length—36 m, width—1.5 m, and height—1 m (Figure 1a). Microorganisms were added with a knapsack sprayer at a rate of 1 L of culture liquid ($2–4 \times 10^8$) per 1 ton of dry poultry manure. Given the bulk density of fresh poultry manure of 0.3 $t/m^3$, 4.5 m of stack was allocated for each treatment. Cellulose-degrading microorganisms were added as a monoculture. Three strains of nitrogen-fixing microorganisms were added together—0.35 L/t of each strain, and two strains of nitrifying microorganisms were added at 0.5 L/t of each one. Samples were taken from each segment and analyzed in three replicates. Manure pile turning was carried out using a STEVIMAN SX-61 (LLC «Small-Scale Mechanization», Moscow, Russia) snow blower (Figure 1b). The turning frequency was 110 h, in accordance with recommendations [22]. The pile moisture content was maintained at 60–65% and monitored

using a professional MS-7828 (AZ Instrument Corp, Taichung, Taiwan) soil moisture meter. The ambient temperature during the experiment (June–July 2025) ranged from 29 to 38 °C during the day to 22–26 °C at night. The composting period lasted 21 days. An average biomass sample for analysis was collected by cutting across the entire height of the pile (Figure 1c). The composted material was located in the Kabardino-Balkarian Republic, Urvan District, Kakhun village, on the territory of the former collective farm "Kakhunsky".




**Figure 1.** View of the experimental pile (**a**), turning over the pile (**b**), and sampling for analysis by cutting along the entire height of the pile (**c**).

The rate of organic matter destruction was determined by sifting air-dried compost through sieves of ≤0.25 mm, 0.5 mm, 1.0 mm, and ≥2.0 mm. The compost was first physically destroyed in a Stegler LM-250 (Shenzhen Bestman Instrument CO., LTD, Shenzhen, China) laboratory mill for 20 s. The degree of mineralization of the organic matter in poultry manure under the influence of the soil microbial strains was determined by measuring the proportion of organic carbon in the fractions. Organic carbon was determined using the Tyurin method [23].

The degree of humification was determined by measuring the humic acid (HA) content spectrophotometrically in each fraction differentially [24]. The nitrogen status of the compost was determined based on the total nitrogen content and the proportion of mineral nitrogen compounds. The total nitrogen content was determined in accordance with [25] using an AKV-10 semiautomatic Kjeldahl apparatus using the titrimetric method. The nitrate content was determined using the method of V.B. Zamyatina [26,27]. The content of the ammonium form of nitrogen [28] was determined using the Nessler reagent [26].

The bioreclamation properties of the compost samples were determined fractionally. For this purpose, a laboratory experiment was conducted with 2.3 g of each fraction (≤0.25 mm, 0.5 mm, 1.0 mm, and ≥2.0 mm) added to trays containing 500 g of ordinary chernozem in 3-fold repetition. The change in the biological response of the soil to the application of each compost fraction after 14 days was assessed by the carbon content of microbial biomass and the invertase activity of the soil.

Statistical analysis was performed using Python 3.10 software with SciPy and Statsmodels libraries. Due to the small sample size ($n$ = 3), the normality of the dis-

tribution was checked using the Shapiro–Wilk test, where the normality criterion was $p > 0.05$. The homogeneity of the variances between the compared groups was assessed using the Levene test, where the assumption of homogeneity of variances was made at $p > 0.05$. When all the necessary conditions are met, a one-way analysis of variance (One-way ANOVA) was performed to assess the effect of various microorganisms (CBT, NBT, and NFBT) on all the studied indicators. When the conditions were violated, the non-parametric Kruskal–Wallis test was performed. The significance levels were as follows: $p < 0.001$ for highest significance, $p < 0.01$ for high significance, $p < 0.05$ for significance, and $p \geq 0.05$ for non-significance. Tukey's test (Tukey HSD) was used for multiple comparisons with the control. The significance level was set at $p = 0.05$. The data were presented as the mean ± standard deviation of three replicates. Graphical data that do not include standard errors reflect the result of recalculating the primary data based on the fraction data. Pearson correlation analysis was performed to study the relationship between the indicators of total nitrogen and humic acids, total nitrogen and mineral forms of nitrogen in the compost, where the results were classified according to the Chaddock scale. The statistical significance of the correlation coefficients was determined by comparing the critical value of Student's *t*-test for a significance level of $p = 0.05$ with the empirical value of Student's *t*-test.

## 3. Results and Discussions

### *3.1. Cultural and Physiological-Biochemical Properties of Isolated Microorganisms*

All isolated microorganisms used in the study were Gram-positive bacteria belonging to five different genera (Table 1).

**Table 1.** Phylogenetic analysis of nucleotide sequences of 16S rRNA gene fragments of soil microorganism strains.

| Strains | Closely Related Type Strain | No GenBank | Similarity, % | Number of Nucleotides, Units |
|---|---|---|---|---|
| $CB^T$ | *Aeribacillus pallidus* KCTC $3564^T$ | CP017703 | 100 | 1272 |
| $NFB^T2$ | *Neobacillus sedimentimangrovi* FJAT-$2464^T$ | MN963926 | 100 | 1384 |
| $NFB^T9$ | *Aeribacillus composti* $N.8^T$ | LT594972 | 100 | 1293 |
| $NFB^T10$ | *Caldifermentibacillus hisashii* N-$11^T$ | AB618491 | 100 | 814 |
| $NB^T1$ | *Acinetobacter pittii* CIP $70.29^T$ | APQP01000001 | 100 | 792 |
| $NB^T2$ | *Pseudomonas plecoglossicida* NBRC $103162^T$ | BBIV01000080 | 100 | 790 |

$CB^T$ are spore-forming, solitary, round bacteria that are urease-, phosphatase-, and amylase-positive, catalase-negative, not proteolytically active, and utilize nitrogen from nitrate salts and are capable of degrading cellulose. Their optimal growth temperature is 45–55 °C, with growth occurring at temperatures up to 65 °C and no growth at 30 °C.

$NFB^T2$ are spore-forming, rod-shaped bacteria that are catalase-, phosphatase-, urease-, and amylase-negative, possessing proteolytic activity, atmospheric nitrogen-fixers, and able to utilize ammonium salts. Their optimal growth temperature is 47–50 °C, with no growth at 30 °C.

$NFB^T9$ are spore-forming, solitary, elongated bacteria that are urease-negative, catalase-, phosphatase-, and amylase-positive, lacking proteolytic activity, and assimilate nitrogen from atmospheric air. Optimum growth temperature is 47–50 °C; no growth occurs at 30 °C.

$NFB^T10$ are spore-forming, solitary, round-ended bacteria that are urease-negative, catalase-, phosphatase-, and amylase-positive, lacking proteolytic activity, and assimilate

nitrogen from atmospheric air. Optimum growth temperature is 47–50 °C; no growth occurs at 30 °C.

NB$^T$1 are thermotolerant, non-spore-forming, round, solitary bacteria that are urease- and catalase-positive, phosphatase- and amylase-negative, and possess proteolytic activity. They assimilate nitrogen from atmospheric air, nitrates, and ammonium salts, and possess the ability to mobilize phosphates. The optimal growth temperature is 30–40 °C, with a maximum temperature of up to 50 °C.

NB$^T$2 are thermotolerant, non-spore-forming, solitary, elongated bacteria that are urease-, catalase-, and phosphatase-positive, and amylase-positive, possessing proteolytic activity. They assimilate nitrogen from nitrates and ammonium salts and possess the ability to mobilize phosphates. The optimal growth temperature is 30–40 °C, with a maximum temperature of up to 50 °C.

### *3.2. Degree of Organic Matter Destruction*

Accelerating the decomposition of organic matter is a fundamental element of composting technologies. However, the authors did not find any use of the proportion of individual fractions (fragmentation) in assessing the composting process of poultry manure in the scientific literature. It is logical to assume that an increase in the proportion of the smallest fractions reflects the intensity of biochemical reactions in the compost. The mechanism of accelerated fractionation of manure under the influence of the studied thermophilic microorganisms may be the same as that of thermophilic actinobacteria, which can use recombinant hydrolases to degrade even synthetic polymers [29–31]. At the same time, the authors propose separating the concepts of "decomposition," which reflects deeper processes of biochemical transformation of organic matter, such as humification, from "destruction," which reflects the quantitative expression of physical parameters such as particle size of the composted material. It can also be expected that the particles reduced by the enzymatic apparatus of microorganisms have a greater surface area in contact with the compost microflora, which participates in the further transformation of carbon and nitrogen-containing compounds.

The fractions presented in the table can be roughly divided into small ($\leq$0.25 mm and 0.5 mm), reflecting the intensity of decomposition, and large (1.0 mm and $\geq$2.0 mm), the proportion of which reflects the advisability of continuing the composting process. The analysis results demonstrate the influence of the studied microorganisms on the destruction of organic matter with varying degrees of intensity (Table 2).

One-factor analysis of variance revealed statistically significant differences between the experimental options for all the studied fractions. The most pronounced differences were observed for the 0.5 mm ($p < 0.001$) and $\geq$2.0 mm ($p < 0.001$) fractions, indicating the impact of the studied microorganisms on the degradation of poultry manure. For the $\leq$0.25 mm and 1.0 mm fractions, the differences were also statistically significant ($p < 0.05$), but less pronounced.

Considering the effect of the isolated strains on the destruction of organic matter in poultry manure, it should be noted that, based on the sum of fractions $\leq$ 0.25–0.5 mm, the strains of nitrifying thermotolerant microorganisms were the most effective, increasing the fragmentation rate of organic matter by 9.7%. A cellulose-degrading strain and a consortium of thermophilic nitrogen-fixing microorganisms increased this rate by 7.9% and 8.8%, respectively. It was expected that cellulolytic microorganisms would be more aggressive in increasing the proportion of degraded matter. The greater effectiveness of nitrifying microorganisms may be due to their ability to release mineral nitrogen, which acts as an activator of the native cellulolytic microflora of poultry manure. The proportion of undestroyed or slightly destroyed organic material (fraction $\geq$ 2.0 mm) was observed in the

control variant (20.8%), which turned out to be 18.8–30.8% higher than under the influence of the studied microorganisms. The proportion of undisturbed or slightly decomposed organic material (fraction ≥ 2.0 mm) was noted in the control variant (20.8%), which turned out to be 18.8% ($p = 0.001$), 29.7% ($p < 0.001$), and 30.7% ($p < 0.001$) higher than under the action of nitrifying, nitrogen-fixing, and cellulosolitic microorganisms, respectively.

**Table 2.** Degree of decomposition of poultry manure after biocomposting.

| Fractions, mm | Variants | Degree of Decomposition, % | SD, % | Variation in Relative Control, % | *p*-Value | Statistical Significance ($p < 0.05$) |
|---|---|---|---|---|---|---|
| ≤0.25 | Start | 16.0 | 0.63 | -* | - | - |
| ≤0.25 | Control | 24.4 | 0.93 | - | - | - |
| ≤0.25 | $CB^T$ | 22.8 | 1.03 | −6.6 | 0.1136 | No |
| ≤0.25 | $NB^T$ | 26.3 | 1.27 | 7.8 | 0.1068 | No |
| ≤0.25 | $NFB^T$ | 24.4 | 0.93 | 0 | 1.0000 | No |
| 0.5 | Start | 16.3 | 0.70 | - | - | - |
| 0.5 | Control | 19.9 | 0.84 | - | - | - |
| 0.5 | $CB^T$ | 25.0 | 0.99 | 25.6 | 0.0024 | Yes |
| 0.5 | $NB^T$ | 22.3 | 1.04 | 12.1 | 0.0370 | Yes |
| 0.5 | $NFB^T$ | 23.8 | 0.44 | 19.6 | 0.0021 | Yes |
| 1.0 | Start | 28.7 | 1.57 | - | - | - |
| 1.0 | Control | 34.9 | 1.24 | - | - | - |
| 1.0 | $CB^T$ | 37.8 | 0.91 | 8.3 | 0.0307 | Yes |
| 1.0 | $NB^T$ | 34.5 | 1.56 | −1.2 | 0.7420 | No |
| 1.0 | $NFB^T$ | 37.1 | 0.90 | 6.3 | 0.1700 | No |
| ≥2.0 | Start | 39.0 | 0.85 | - | - | - |
| ≥2.0 | Control | 20.8 | 0.59 | - | - | - |
| ≥2.0 | $CB^T$ | 14.4 | 0.58 | −30.7 | 0.0002 | Yes |
| ≥2.0 | $NB^T$ | 16.9 | 0.33 | −18.8 | 0.0006 | Yes |
| ≥2.0 | $NFB^T$ | 14.6 | 0.33 | −29.7 | 0.0001 | Yes |

* Comparisons are referenced to the Control value. The initial mean value (Start) serves as a baseline to demonstrate changes over time. The "-" symbol in the table indicates that no direct comparison was performed between the Start and Control conditions.

### *3.3. Organic Matter Mineralization by Fraction*

During composting, active decomposition of organic matter occurs, and the degree of mineralization is estimated by the proportion of total carbon in the biomass. The analysis data (Figure 2a) demonstrate that the introduction of the studied strains into the composted material can accelerate the mineralization of organic matter. The results of the analysis of variance showed that statistically significant differences between the options were established in all fractions ($p < 0.001$).

The minimum degree of mineralization (43.2% on average) was observed in the control variant, in which the proportion of organic carbon increased by 33.4–51.9% as the fraction size increased. Under the influence of nitrifiers, the reduction in organic carbon in all fractions varies between 21.7% ($p < 0.001$) and 36.9% ($p < 0.001$), while the action of cellulolytic enzymes results in a degradation of 9.1% ($p = 0.028$) to 29.9% ($p < 0.001$). The content of organic carbon in the 0.5 mm, 1.0 mm, and ≥2.0 mm fractions decreases by 29.3% ($p < 0.001$), 23.3% ($p = 0.003$), and 45.3% ($p < 0.001$), respectively, under the influence of nitrogen fixators.

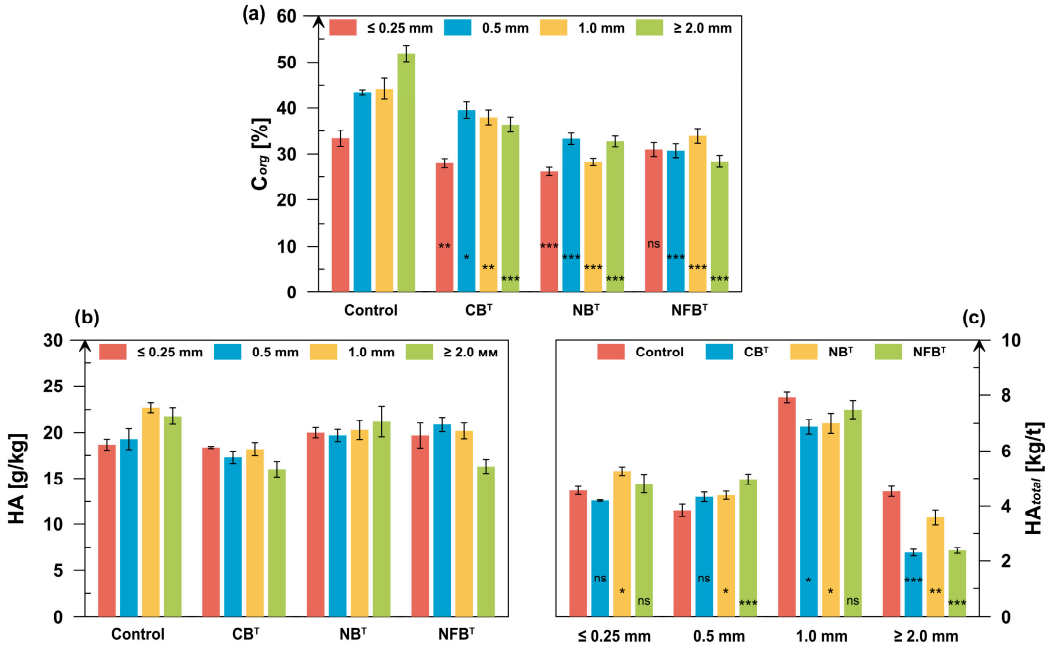



**Figure 2.** Content of (**a**) organic carbon by fractions, (**b**) humic acids, and (**c**) total of humic acids in different fractions of compost. Designations in the figures: $C_{org}$—organic carbon, %; HA—humic acids, g/kg; $HA_{total}$—total humic acid content, taking into account the fraction, kg/t; Control—control variant; $CB^T$—variant with cellulolytic bacteria; $NB^T$—variant with nitrifying bacteria; $NFB^T$—variant with nitrogen-fixing bacteria. The compost fractions studied were: ≤0.25 mm, 0.5 mm, 1.0 mm, and ≥2.0 mm. Levels of *p*-values: very high (***, $p < 0.001$), high (**, $p < 0.01$), significant (*, $p < 0.05$), not significant (ns, $p \geq 0.05$).

### *3.4. Organic Matter Humification by Fraction*

The influence of microorganisms on humic substance accumulation varies by fraction (Figure 2b). The maximum amount of HA in the smallest fraction (≤0.25 mm) is formed under the influence of nitrifiers (20.0 g/kg) and nitrogen fixers (19.7 g/kg), which is 7.0% and 5.3% higher than in the control variant. In the 0.5 mm fraction, nitrogen fixers increase the HA content by 8.3%.

As fractions increase (1.0 and ≥2.0 mm), the maximum HA content is observed in the control variant—22.7 and 21.8 g/kg, respectively. The impact of cellulose-degrading microorganisms on the humification process is minimal.

At the same HA value in the ≤0.25 mm fraction as in the control, a decrease of 10.4%, 19.8%, and 26.6% was observed in the 0.5 mm, 1.0 mm, and ≥2.0 mm fractions, respectively. These data may serve as the basis for a hypothesis on revising the concept of using cellulolytics in the processing of animal byproducts. Thus, they can be added to the composted mass in combination with microbial strains of other physiological groups or used at later stages of fermentation. A more objective assessment of the humus status of fractions can be made based on their total HA content, calculated based on the HA concentration (g/kg) and the fraction's proportion (%) in the compost (Figure 2c). For greater reliability, statistical processing was carried out with data obtained after recalculation to the fraction share. This approach is also proposed for the indicators of total and mineral nitrogen.

The figure shows that the smallest fraction (≤0.25 mm) in 1 ton of compost accumulates between 4.2 and 5.26 kg of HA, depending on the microorganisms used. The maximum effect is achieved with the addition of nitrifying 5.26 kg/t microorganisms. This is 15.4% ($p = 0.01$) higher than in the control. In the next-largest fraction, 0.5 mm, the maximum accumulation of HA occurs under the influence of nitrogen-fixing microorganisms—4.97 kg/t, which is 29.4% ($p < 0.001$) higher than in the control (4.56 kg/t). The effects of

nitrifiers were equal to 4.39 kg/t, which is 14.3% ($p = 0.03$), higher than the control value (3.84 kg/t).

As the fraction size increases, the total HA content in the experimental variants ($CB^T$ and $NB^T$) decreases relative to the control value on 13.1% ($p = 0.01$) and 11.6% ($p = 0.02$). This trend persists in the ≥2.0 mm fraction. The maximum content (4.53 kg/t) was found in the control variant, which is 26.5% ($p = 0.001$) –96.7% ($p < 0.001$) higher than in the variants using microorganisms.

### *3.5. Total Nitrogen Content in Compost Fractions*

The preservation of nitrogen in compost during the processing of waste by-products is an important quality indicator. The analysis results (Figure 3a) indicate variations in compost nitrogen content by fraction depending on the physiological characteristics of the microbial strains used in biofermentation. In the ≤0.25 mm fraction, the nitrogen content was higher than in the control by 13.8% ($CB^T$), 4.8% ($NB^T$), and 20.2% ($NFB^T$). In the 0.5 mm and 1.0 mm fractions, the difference between treatments narrowed. The maximum total nitrogen content (1.98%) relative to the control (1.77%) was observed with the use of nitrogen fixers in the 0.5 mm fraction, with a relative difference of 11.9%. In the 1.0 mm fraction, the difference was 10.7%.

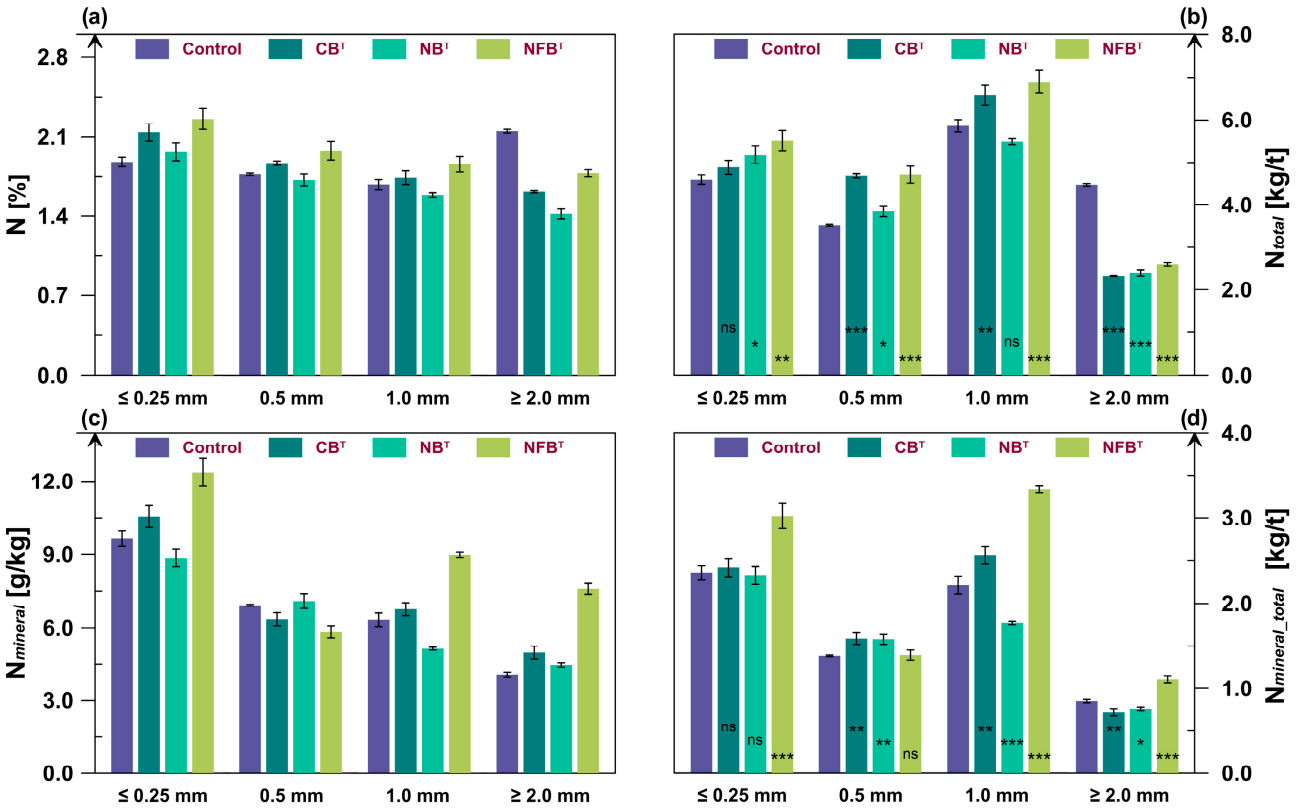



**Figure 3.** Relative content of (**a**) total nitrogen by fractions, (**b**) total content of total nitrogen in compost, (**c**) mineral forms of nitrogen by fractions, and (**d**) total content of mineral forms of nitrogen, taking into account the proportion of fraction. Designations in the figures: N—nitrogen in compost fractions, %; $N_{total}$—total nitrogen content, taking into account the fraction of fractions in kg/t; $N_{mineral}$—mineral forms of nitrogen ($NO_3 + NH_4$), g/kg; $N_{mineral_total}$—total content of mineral forms of nitrogen ($NO_3 + NH_4$), taking into account the proportion fractions, kg/t; Control—control variant; $CB^T$—variant with cellulolytic bacteria; $NB^T$—variant with nitrifying bacteria; $NFB^T$—variant with nitrogen-fixing bacteria. The compost fractions studied were: ≤0.25 mm, 0.5 mm, 1.0 mm, and ≥2.0 mm. Levels of *p*-values: very high (***, $p < 0.001$), high (**, $p < 0.01$), significant (*, $p < 0.05$), not significant (ns, $p \geq 0.05$).

The effect of cellulolytics in the 0.5 mm and 1.0 mm fractions was noted by a slight increase in total nitrogen, by 5.7% and 3.6%. Under the influence of nitrifiers, there was a tendency for the total nitrogen content to decrease as the fraction size increased. Thus, compared to the control variant, in the 0.5 mm, 1.0 mm, and ≥2.0 mm fractions, nitrogen decreased by 2.8%, 5.4%, and 34.0%, respectively. The control variant relative to the experimental variants shows an excess of total nitrogen content in the ≥2.0 mm fraction. Compared to cellulolytics, nitrifiers, and nitrogen fixers, the difference is 32.7%, 51.4%, and 20.8%, respectively. The fertilizer value for total nitrogen in individual fractions, as in the case of HA, should be assessed taking into account their share in the final compost (Figure 3b).

The total nitrogen content in the compost under the influence of the studied microorganisms varies within the range of 9.1–7.0%, where the minimum content was noted in the variant with nitrifying microorganisms (16.9 kg/t). The maximum (19.73 kg/t) was found in the variant with nitrogen-fixing thermophiles. The total nitrogen content of cellulolytics does not differ from that of the control variant. At the same time, differentiation of nitrogen content by fractions demonstrates more significant differences. The effect of microorganisms on the accumulation of total nitrogen is more pronounced in the smallest fractions (≤0.25 mm and 0.5 mm). Thus, the nitrogen content under the influence of cellulolytics, nitrifiers and nitrogen fixers increases by 6.3% ($p$ = 0.283 (not statistically significant)), 12.9% ($p$ = 0.02) and 20.0% ($p$ = 0.001), respectively, for the fraction ≤ 0.25 mm and by 33.0%, 9.1% and 33.8% for the fraction 0.5 mm.

A large amount of nitrogen accumulates in the 1.0 mm fraction. For this fraction, the maximum effect was observed in the treatments with cellulolytics, 6.58 kg/t, and nitrogen fixers, 6.90 kg/t, which is 12.3% ($p$ = 0.009) and 17.8% ($p$ < 0.001) higher than in the control. Nitrifiers were less effective for this fraction (5.49 kg/t), representing a 6.3% ($p$ = 0.158) reduction. In the largest fraction (≥2.0 mm), the maximum total nitrogen content (4.47 kg/t) was observed in the control, which can be attributed to its lower degree of mineralization. Under the influence of microorganisms, 2.33–2.60 kg ($p$ < 0.001) of nitrogen is accumulated in this fraction per 1 ton of compost.

The loss of total nitrogen from the composted mass under the action of nitrifiers is obviously related to the increased denitrification, which is the subsequent stage of nitrogen transformation into gaseous forms. This requires finding ways to limit the loss, such as using nitrification inhibitors, but it is unclear how these substances will affect the overall nitrogen cycle in the compost and the biodegradation process.

The importance of the method for differentiated compost quality assessment by fractions is associated not only with the ability to monitor the intensity and direction of biofermentation processes but also with the ability to more reliably interpret the obtained data. As an example, the correlations between total nitrogen and HA values based on the initial analysis results and after their conversion to gross values, taking into account the proportion of each individual fraction (Table 3), can be seen.

As can be seen from the table, the correlations between total nitrogen and HA values based on relative values do not show a consistent pattern in their content across fractions. Correlation coefficients vary and range from r = −0.765 to r = 0.716.

When converting relative values to absolute values and then taking into account the proportion of each fraction, we obtain highly positive values. The correlation coefficients range from r = 0.902 to 0.976.

**Table 3.** Correlations between nitrogen and HA content.

| Factions | Control (%) | | $CB^T$ (%) | | $NB^T$ (%) | | $NFB^T$ (%) | |
|---|---|---|---|---|---|---|---|---|
| | N | HA | N | HA | N | HA | N | HA |
| Options (Based on the analysis results) | | | | | | | | |
| ≤0.25 | 1.88 | 1.87 | 2.14 | 1.84 | 1.97 | 2.00 | 2.26 | 1.97 |
| 0.5 | 1.77 | 1.93 | 1.87 | 1.73 | 1.72 | 1.97 | 1.98 | 2.09 |
| 1.0 | 1.68 | 2.27 | 1.74 | 1.82 | 1.59 | 2.03 | 1.86 | 2.02 |
| ≥2.0 | 2.15 | 2.18 | 1.62 | 1.60 | 1.42 | 2.12 | 1.78 | 1.63 |
| r | 0.041 | | 0.716 | | −0.765 | | 0.468 | |
| Options (In terms of absolute units, taking into account the proportion of fractions) | | | | | | | | |
| ≤0.25 | 4.59 | 4.56 | 4.88 | 4.20 | 5.18 | 5.26 | 5.51 | 4.81 |
| 0.5 | 3.52 | 3.84 | 4.68 | 4.33 | 3.84 | 4.39 | 4.71 | 4.97 |
| 1.0 | 5.86 | 7.92 | 6.58 | 6.88 | 5.49 | 7.0 | 6.9 | 7.49 |
| ≥2.0 | 4.47 | 4.53 | 2.33 | 2.3 | 2.4 | 3.58 | 2.6 | 2.38 |
| r | 0.943 | | 0.976 | | 0.902 | | 0.969 | |

### *3.6. The Impact of Microorganisms on the Content of Mineral Nitrogen*

One of the main goals of biological fermentation of livestock byproducts is to increase nitrogen availability to plants through the intensive mineralization of nitrogen-containing organic compounds. At the same time, the risk of significant nitrogen loss as ammonia must be considered. The study of various microbial strains is aimed at finding ways to limit non-productive nitrogen losses while improving the overall nitrogen status of compost.

The expediency of introducing selected strains into compostable material is confirmed by test results (Figure 3c).

The proportions of compost fractions when calculating the mineral nitrogen content by physical weight (kg per ton of compost) introduce certain adjustments that more accurately reflect the nitrogen status of each fraction (Figure 3d). In terms of the overall accumulation of mineral nitrogen forms in compost, nitrogen fixers are the most effective, with an accumulation rate of 8.85 kg per ton of compost. This is more than 30% higher than the control (6.79 kg/t). The increase in nitrogen due to cellulolytics is 7.2% (7.28 kg/t). Nitrifiers contribute to a decrease in mineral nitrogen content by 5.3% (6.43 kg/t).

Thermophilic nitrogen-fixing microorganisms had the greatest impact on the accumulation of mineral nitrogen. Thus, with the exception of the 0.5 mm fraction, in the fractions ≤ 0.25 mm, 1.0 mm, and ≥2.0 mm, the increase was 28.1% ($p < 0.001$), 51.1% ($p < 0.001$), and 30.6% ($p < 0.001$), respectively. The cellulolytic thermophilic strain also proved effective, but to a lesser extent. For fractions ≤ 0.25 mm and 1.0 mm fractions, an increase was observed at 15.2% ($p = 0.006$) and 15.9% ($p = 0.002$), respectively, while in the ≥2.0 mm fraction, a decrease of 15.7% ($p = 0.002$) was observed. No significant changes were observed in the ≤0.25 mm fraction under the influence of $CB^T$ and $NM^T$.

The efficiency of nitrifying microorganisms in terms of mineral nitrogen accumulation is observed in the 0.5 mm fraction, with a change of 14.7% ($p = 0.007$), while in the low-decomposed part, there is a decrease in mineral nitrogen forms by 19.5% ($p < 0.001$) in the 1.0 mm fraction and 11.0% ($p = 0.015$) in the ≥2.0 mm fraction.

Given the narrow functionality of nitrifying microorganisms, it is more accurate to evaluate their effect not through their influence on the total mineral nitrogen content, but on its nitrate form in the compost. In this case, for individual fractions, we see results consistent with their function, specifically the accumulation of nitrate nitrogen and a significant reduction in the ratio with ammonium nitrogen (Figure 4).

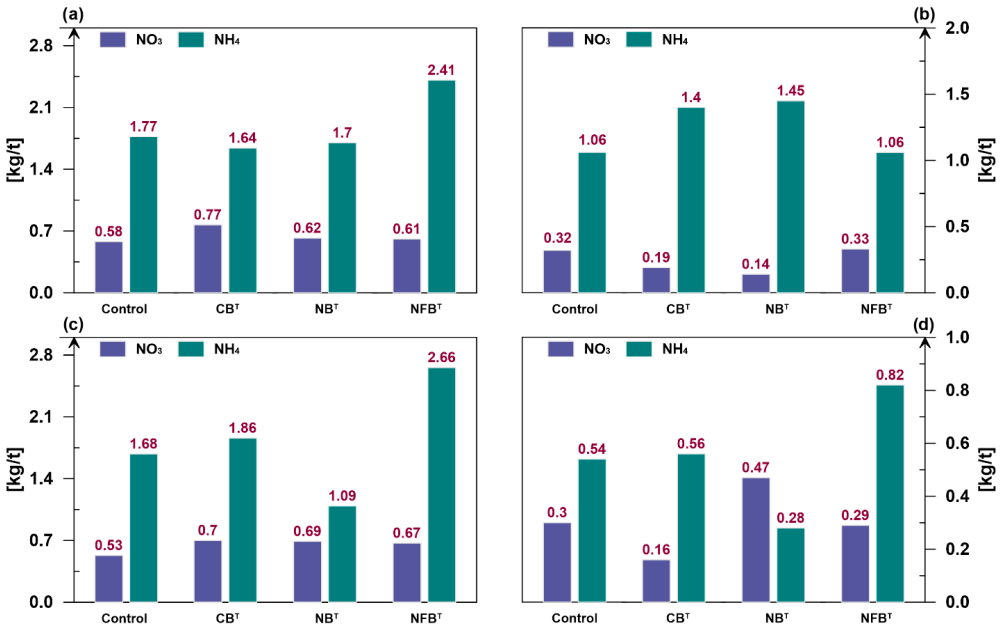



**Figure 4.** Distribution of mineral forms of nitrogen in compost by fractions: (**a**) ≤0.25 mm; (**b**) 0.5 mm; (**c**) 1.0 mm, and (**d**) ≥2.0 mm. Designations in the figures: $NO_3$—nitrate and ammonium; $NH_4$—ammonium; Control—control variant; $CB^T$—variant with cellulolytic bacteria; $NB^T$—variant with nitrifying bacteria; $NFB^T$—variant with nitrogen-fixing bacteria.

Looking at the figure, it should be noted that the ammonium nitrogen content is higher than the nitrate nitrogen content in most variants. On average, nitrogen-fixing microorganisms have the greatest influence on the total ammonium content across fractions. Under their influence, 6.95 kg/t accumulates in the compost, which is 37.6% higher than in the control variant (5.05 kg/ha). Ammonium nitrogen, 5.46 kg/t, accumulates in the compost under the influence of cellulolytic bacteria (+8.1%). The minimum accumulation of ammonium nitrogen (4.52 kg/t) was observed in the variant with nitrifying bacteria, which is 10.5% lower than in the control variant. In terms of nitrate nitrogen, nitrifying microorganisms proved to be the most effective, producing 1.92 kg/t of NO3 in the compost, which is 9.7% higher than the control (1.75 kg/t) and 5.5% higher than the cellulolytic-based treatment (1.82 kg/t). Nitrogen fixers were slightly inferior to nitrifiers in their ability to accumulate nitrate nitrogen in the compost (1.90 kg/t).

A distinctive feature of the nitrifying strain was its activity in larger compost fractions. For example, in the 1.0 mm and ≥2.0 mm fractions, $NO_3$ accumulation was higher than in the control by 30.2% and 51.6%. In the largest fraction, nitrifiers were significantly more effective than cellulolytics and nitrogen fixers by 193.8% and 62.1%, respectively. It can be assumed that large fractions of compost mass provide a favorable ecological niche for nitrifying activity. This thesis is supported by the narrow ratio of nitrate to ammonium nitrogen. This ratio in the fraction ≥ 2.0 mm is 1.68, while for other variants it is 0.57 (Control), 0.29 ($CB^T$), and 0.35 ($NFB^T$).

The influence of nitrifying microorganisms in this case can be assessed as climatically favorable, as it is accompanied by a decrease in the proportion of ammonium, a key predictor of nitrous oxide flux into the atmosphere [32].

Fractional differentiation of the nitrogen status of the compost demonstrates closer correlations when comparing nitrate, ammonium forms, and their sums with the total nitrogen content (Figure 5). The figure shows that recalculating the mineral nitrogen content of compost by fractions more accurately reflects their relationship with the total nitrogen content.

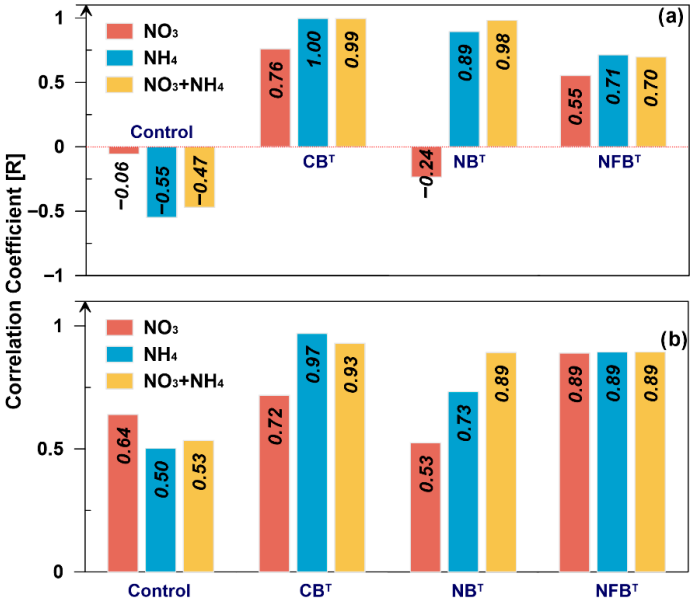



**Figure 5.** Correlation relationships between mineral forms of nitrogen and total nitrogen in compost (**a**) without and (**b**) with the proportion of fractions. Control—control variant; $CB^T$—variant with cellulolytic bacteria; $NB^T$—variant with nitrifying bacteria; $NFB^T$—variant with nitrogen-fixing bacteria.

### *3.7. Bioreclamation Properties of Individual Compost Fractions*

Biological indicators are increasingly being used as indicators of soil fertility. Given the subject of the study—the influence of microorganisms on the transformation of organic matter from livestock byproducts—microbial biomass carbon and invertase activity were used as bioindicators. Microbial biomass carbon represents an important part of the labile fraction of soil organic matter, responsible for effective fertility [33]. Invertase, in turn, indicates the intensity of organic matter decomposition [34].

The application of compost treated with different microbial strains to soil has different effects on the accumulation of microbial biomass carbon (Figure 6a).

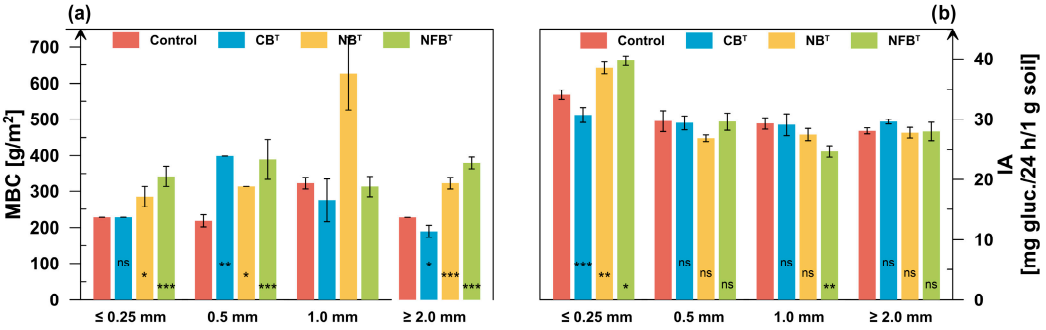



**Figure 6.** Content of microbial biomass carbon (**a**) and invertase activity in soil fertilized with different fractions of compost (**b**). Designations in the figures: MBC—microbial biomass carbon, $g/m^2$; IA—invertase activity, mg glucose/24 h/1 g of soil; Control—control variant; $CB^T$—variant with cellulolytic bacteria; $NB^T$—variant with nitrifying bacteria; $NFB^T$—variant with nitrogen-fixing bacteria. The compost fractions studied were: ≤0.25 mm, 0.5 mm, 1.0 mm, and ≥2.0 mm. Levels of *p*-values: very high (***, $p < 0.001$), high (**, $p < 0.01$), significant (*, $p < 0.05$), not significant (ns, $p \geq 0.05$).

Thus, under the influence of the smallest compost fraction (≤0.25 mm), the maximum accumulation of microbial biomass carbon is observed in the soil variant using thermophilic nitrogen fixers (342 $g/m^2$), which is 50.0% ($p < 0.001$) higher than in the control variant (228 $g/m^2$). Nitrifiers increase this indicator by 25.0% (285 $g/m^2$) ($p = 0.03$). The Microbial

Biomass Carbon (MBC) content under the influence of cellulolytics is equivalent to that in the control variant.

Under the influence of the 0.5 mm compost fraction, all studied strains ($NFB^T$, $NB^T$, $CB^T$) increase the MBC content by 78.4% ($p = 0.001$), 43.6% ($p = 0.04$), and 83.0% ($p < 0.001$), respectively.

Under the influence of the 1.0 mm compost fraction, there are no statistically significant changes. The coarse compost fraction ($\geq$2.0 mm) obtained with nitrogen fixers and nitrifiers increased MBC by 66.7% ($p < 0.001$) and 41.7% ($p < 0.001$), respectively. Cellulosolytics, on the other hand, reduced microbial carbon content by 16.7% ($p = 0.04$).

Summarizing the assessment of compost bioreclamation properties based on microbial biomass carbon, it can be concluded that nitrogen fixers and nitrifiers in compost, in most cases, act as activators of the accumulation of labile organic matter in the soil.

Changes in soil invertase activity under the influence of biofermented compost are observed mainly with the addition of the smallest fraction (Figure 6b). The highest invertase activity is observed in soil samples fertilized with the smallest compost fraction ($\leq$0.25 mm). This trend applies to all experimental variants, but with different degrees. When examining the effects of the treatments within a single fraction of $\leq$0.25 mm, it can be noted that the use of nitrogen-fixing and nitrifying strains in biofermentation increases the bioremediation properties of compost by 16.4% ($p < 0.001$) and 12.9% ($p < 0.002$), respectively. Conversely, the treatment with cellulolytics reduces soil invertase activity by 10.0% ($p = 0.011$).

Differences in the effects of microorganisms on invertase activity in larger fractions of 0.5 mm, 1.0 mm, and $\geq$2.0 mm narrow and range from 26.9 to 29.7, 24.7–29.3, and 27.8–29.6 mg glucose/24 h/1 g soil, respectively.

## 4. Conclusions

The results of the study show that the use of bioagents (target microbial strains) can influence the direction of transformation processes in biocompostable materials, improving the quality of the final product—the degree of fragmentation (destruction), humification, and nitrogen balance, which ultimately impacts the bioreclamation properties of the compost.

The study also suggests that compost fractionation can be considered as a promising method for assessing the quality of poultry manure biofermentation, where the proportion of the smallest fractions reflects the rate of degradation of the primary raw material. Thus, the rate of degradation (fragmentation) of the compost depended on the microorganisms used. In the 0.5 mm fraction, all compared strains were effective, with the maximum degradation properties, 25.6% ($p = 0.0024$), demonstrated by cellulolytic microorganisms.

The total HA content in the $\leq$0.25 mm fractions increased by 15.4% ($p = 0.01$) under the influence of nitrifiers. Under the influence of nitrifies, the increase in 0.5 mm was 14.3% ($p = 0.03$). Nitrogen-fixers were more effective only in the 0.5 mm fraction, whose share increased by 29.4% ($p < 0.001$).

The studied microorganisms also had a positive effect on the nitrogen balance in the compost. Thus, with a relatively small spread in total nitrogen content in the compost across treatments: 18.44 kg/t (Control), 18.47 kg/t ($CB^T$), 16.91 kg/t ($NB^T$), and 19.27 kg/t ($NFB^T$), its redistribution into the fine fractions was observed under the influence of the microorganisms. Thus, in the $\leq$0.25 mm fraction, the total nitrogen content increases by 6.3% ($CB^T$) ($p = 0.283$), 12.9% ($NB^T$) ($p = 0.02$), and 20.0% ($NFB^T$) ($p = 0.001$). Under the influence of the same microorganisms, the total nitrogen content in the 0.5 mm fraction increases by 33.0% ($p < 0.001$), 9.1% ($p = 0.04$), and 33.8% ($p < 0.001$), respectively.

Methodologically, it was more appropriate to calculate the total content of HA and various forms of nitrogen, taking into account the fraction distribution. This was established

by the correlation coefficients between the analytical parameters expressed in g/kg compost and the total content, recalculated per kg/t compost, taking into account the fraction proportions. Thus, in the first case, the spread of the correlation coefficients across the variants was r = 0.041 (Control), r = 0.716 ($CB^T$), r = −0.765 ($NB^T$), and r = 0.468 ($NFB^T$). When taking into account the total nitrogen content, the correlation coefficients in the same variants were: r = 0.943, r = 0.976, r = 0.902, and r = 0.969, respectively. A similar pattern was found when comparing the sum of mineral nitrogen forms with their total nitrogen content. In the first case, the correlation coefficients did not follow a consistent pattern and were: r = −0.06 (Control), r = 0.760 ($CB^T$), r = −0.24 ($NB^T$), and r = 0.55 ($NFB^T$). When considering the total nitrogen content, the correlation coefficients were positive, with medium and high correlation levels: r = 0.64 (Control), r = 0.720 ($CB^T$), r = 0.530 ($NB^T$), and r = 0.890 ($NFB^T$).

The use of microorganisms in the fermentation of poultry manure also increased the bioremediation potential of the fine fractions of the compost. The increase in soil invertase activity and microbial biomass carbon accumulation increased by 16.4% ($p < 0.001$) and 50% ($p < 0.001$), respectively, with the addition of the minimum fraction (≤0.25 mm) of compost processed with nitrogen fixers. These parameters increased by 12.9% ($p = 0.02$) and 25.0% ($p = 0.03$), respectively, with nitrifiers. The maximum effect of cellulolytics—83.0% ($p < 0.001$) on the growth of MBC in soil was achieved with the addition of the 0.5 mm compost fraction.

Prospects for further research include choosing the sequence for adding the isolated strains to the composted mass and determining the optimal time intervals between microbial applications.

**Author Contributions:** All the authors have participated sufficiently in the intellectual content, conception, and design of this work or the analysis and interpretation of the data, as well as the writing of the manuscript. Conceptualization, supervision, writing—review and editing A.K.Z.; data curation, visualization, Z.I.D.; methodology, writing—original draft preparation, M.R.A.; resources, validation, R.K.T.; investigation, D.G.D. All authors have read and agreed to the published version of the manuscript.

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## Abbreviations

The following abbreviations are used in this manuscript:

| | |
|---|---|
| $CB^T$ | Thermophilic Cellulolytic Bacteria |
| $NB^T$ | Nitrifying Bacteria |
| $NFB^T$ | Nitrogen-Fixing Non-Associative Bacteria |
| rRNA | Ribosomal ribonucleic acid |
| HA | Humic acids |
| MBC | Microbial biomass carbon |

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