

Article

Farmed Insect Frass as a Future Organic Fertilizer

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Abstract: The aim of this incubation experiment was to evaluate the effect of *Tenebrio molitor* L. frass on selected chemical and microbiological properties of acid peat. The optimal rate of mealworm frass in the substrate for growing ornamental trees and shrubs was determined. Acid peat was fertilized with frass or urea at five nitrogen (N) rates: 0, 50, 100, 200, and 400 mg dm⁻³. Mineral N content and electrical conductivity increased, and calcium content decreased in peat with increasing N rates. Similarly to urea, frass increased the ammonification rate at the beginning of incubation and the nitrification rate from the second week of incubation. Higher frass rates increased the abundance of plant-available nutrients (N, P, Mg, K, and Na) in acid peat. Frass undesirably decreased the counts of bacteria with antagonistic activity against soil-borne plant pathogens. Regarding the abundance of functional genes, the optimal N rate was 100 mg dm⁻³, which promoted the growth of N-fixing and chitinolytic bacteria. Higher N rates promoted the development of aerobic spore-forming bacteria, which produce antibiotics that can be used as biocontrol agents. Moderate fertilizer rates contributed to N accumulation in bacterial biomass. These preliminary findings, which indicate that insect frass can partially replace mineral fertilizers, are promising and can be used in pot and field experiments testing various plant species.

Keywords: acid peat; insect wastes; nutrients; soil bacteriome; urea



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1. Introduction

Insects have played an important role in human life and the economy for centuries. Insect biomass accounts for the majority of biomass from terrestrial animals. Insects have numerous practical applications, including food and feed production. They are an attractive food source due to the vast diversity of insect species with high nutritional value, low production costs, high availability, and environmentally friendly production technologies [1]. According to estimates, insect consumption remains high, with over 2000 species consumed in more than 80 countries around the world [2]. In Europe, edible insects were not considered a significant food source until recently [3], but due to the regulations [4–6] approving insects as food for humans and feed for animals in the European Union, there has been increasing interest in farming insects, particularly mealworms, black flies, and crickets. Farmed insects are high in protein, palatable, and easily digestible, and they have optimal amino acid and fat profiles [7]. Insect farming is more environmentally friendly than livestock production for several reasons. Insect farms generate far less greenhouse gases and ammonia than conventional farms. They occupy less space, they

have lower energy, water, and labor requirements per gram of produced protein, and their carbon footprint is considerably lower than that of conventional farms [1,8,9]. In addition, insect larvae effectively process various waste biomass into nutrients, thus contributing to a closed-loop economy and sustainable development [10–12]. Larvae bioconvert by-products [9], not only organic waste but also waste that is difficult to decompose [13–18]. In some studies [19], insect meal with a high organic nitrogen (N) content was used as fertilizer to improve plant growth.

Insect wastes can be effectively managed in a circular economy. Mealworms (*Tenebrio molitor* L.) generate exuviae and frass. According to Moruzzo et al. [9], frass is a by-product of insect farming that can be used as an organic fertilizer. Insect frass contains many plant-essential nutrients, and its rapid mineralization in the soil leads to the conversion of hard-to-access nutrients into plant-available forms [20]. Frass can improve soil N availability and replace mineral NPK fertilizers [21,22]. Nitrification, namely the microbial oxidation of ammonia from frass, affects the rate of N transformation in soil [23]. Insect diets influence frass quality. In a study by Kagata and Ohgushi [24], cabbage moths, *Mamestra brassicae* L., fed N-rich plants produced frass with a high concentration of N-NH₄. Frass not only enhanced plant growth but also increased plant resistance to abiotic stress [25].

Frass can stimulate the development of soil-dwelling microorganisms. The increased metabolic activity of microorganisms colonizing mealworm frass improved the soil properties and enhanced barley plants' growth and nutrient uptake [21]. Nutrients present in mealworm frass, in particular N and carbon [21,22,26], increase the activity of soil microbes responsible for ammonification, nitrification, denitrification, and N-fixation. At the microbiological level, these processes determine the form, loss, and availability of organic N in soil. Fertilizers should stimulate the development of beneficial bacteria (including antagonistic bacteria) and reduce the counts of phytopathogenic fungi. Beneficial soil microorganisms suppress pests and plant pathogens, induce systemic resistance, and increase crop yields [27,28].

Due to the rapid development of insect farming and the conversion of leftover substrates into fertilizers, the fertilizing potential of frass should be explored to ensure that insect wastes can be safely used [9,29]. Insect feed should be monitored to obtain safe frass for the environment. Frass is a valuable source of N and other macronutrients and micronutrients. Frass is dry, friable, and odorless, and it can be used as an organic fertilizer, particularly in horticultural substrates. Using mealworm frass as a fertilizer in horticulture, pomiculture, and forestry requires further research. Approximately 90% of horticultural growing media in Europe contain peat [30]. Peat has numerous advantages, including a porous structure that retains water and air, low pH, low salinity and nutrient content, low counts of pathogens, pests, and weed diaspores, easy processing, and high availability [31,32]. The demand for high-quality substrates is likely to increase in horticulture, and peat will be the main component of horticultural growing media in the European Union [33]. This substrate is used in the cultivation of horticultural crops and ornamental plants, as well as forest farming. Various peat refining methods are being developed to increase its yield-forming effects, including the addition of calcium (Ca) fertilizers, mineral NPK fertilizers, zeolite, calcined clay, and diatomaceous earth [31,34–36]. Our previous study demonstrated that deacidified peat can be enriched with mealworm frass [37]. The present experiment is part of a comprehensive research study investigating the effect of frass on the chemical and microbiological properties of soils and horticultural substrates, as well as crop yields and quality.

The research hypothesis postulates that acid peat can be enriched with organic substances, such as frass. The aim of the present incubation experiment was to evaluate the influence of *T. molitor* frass on selected chemical and biological properties of acid peat. Frass, a source of nutrients, in particular N, was compared with urea (46% N)—the traditional N source in agriculture. The optimal rate of *T. molitor* frass in the substrate for growing ornamental trees and shrubs was determined, with special emphasis on mineral N levels and the soil microbiome.

2. Materials and Methods

2.1. Experimental Design

Acid peat with the addition of *T. molitor* frass or urea was incubated in a laboratory experiment conducted at the UWM in Olsztyn, Poland, in 2022 (Table 1). Incubation was carried out in tightly closed PET containers with a capacity of 1 dm³ for nine weeks in controlled conditions (at a constant temperature of 26 °C and 60% humidity) in three replicates. During the experiment, substrate samples were taken five times for chemical and microbiological analyses.

Table 1. Design of the laboratory experiment.

Treatments	Rates (mg dm ⁻³ of Substrate)					
	N	0	50	100	200	400
Acid peat with frass	frass *	0	1.32	2.63	5.26	10.52
Acid peat with urea	urea *	0	0.11	0.22	0.44	0.88

*—Nitrogen rates were balanced with the total nitrogen content of frass and urea.

2.2. Characterization and Chemical Analysis of Frass and Acid Peat

Mealworm excrement used in the experiment was obtained from Tenebria Ltd., Lubawa, Poland. Mealworms were fed cereal products supplemented with fresh carrots (at a 1:1 ratio) and yeast. The acidic peat used in the experiment was supplied by Agaris Poland Ltd. (Pasłek, Poland). Selected chemical parameters of the substrate and waste product from *T. molitor* breeding are presented in Table 2.

Table 2. Selected chemical parameters of the substrate and waste product from *T. molitor* breeding.

Parameter	Frass	Acid Peat
pH (distilled H ₂ O)	5.60	5.00
EC (mS cm ⁻¹)	6.92	0.15
C (g kg ⁻¹ DM)	388.10	449.70
N-total (g kg ⁻¹ DM)	38.00	16.95
P (g kg ⁻¹ DM) [†] ; P (mg dm ⁻³ DM) ^{††}	14.70	22.11
K (g kg ⁻¹ DM) [†] ; K (mg dm ⁻³ DM) ^{††}	19.70	45.32
Mg (g kg ⁻¹ DM) [†] ; Mg (mg dm ⁻³ DM) ^{††}	4.27	27.15

[†]—(P, K, Mg) total content in frass; ^{††}—(P, K, Mg) available nutrients in acid peat.

Chemical analyses of acidic peat and the waste product from mealworm farming were carried out in accordance with generally accepted standards and analytical methods. The methods and equipment used in this study were described in detail by Nogalska et al. [37].

2.3. Quantitative PCR of Microbial Functional Genes

DNA was isolated with the Soil DNA Purification Kit. The quantitative PCR setup is presented in Table S1 and was described in previous studies [14,38].

The qPCR standards were prepared based on the appropriate amplicons of *Bacillus subtilis*, *Pseudomonas putida*, and *Fusarium culmorum*. The environmental material was ligated with plasmids and used as the standard for the respective domains. All reactions were performed in samples with a volume of 20 µL each, using the Probe or SybrGreen qPCR Mastermix 2×. Reaction efficiency was determined at 0.93–1.01 (R² = 0.997–1).

2.4. Nanopore Sequencing of the Bacteriome

Pooled samples of total DNA were used for nanopore sequencing and were prepared by combining equal concentrations of genetic material for each repeat in a given variant. The Oxford Nanopore Technology, which allows sequencing of the entire bacterial 16S rRNA region, was used to determine the composition of bacterial communities in the analyzed samples. The procedure applied in this study was similar to that used in the

study of Kosewska et al. [39]. Sequencing and base-calling were performed using the MinKnow software 13.1.6. The obtained data (~45,000 reads) were analyzed using the EPI2ME platform v3.7.3-12305063 (Metrichor™ Ltd., Oxford, UK) using WIMP workflows (version v2023.06.13-1865548, analysis instance ID: 412298).

2.5. Statistical Analysis

Different statistical methods were used to process the results of various analyses due to different distributions of variables and different experimental approaches. Statistical calculations (at a significance level of $\alpha = 0.05$) were performed using STATISTICA 13.3 software [40]. Non-parametric Friedman analysis of variance (ANOVA) with the Iman–Davenport modification was used to evaluate the results of chemical analyses. The Mann–Whitney test was used to determine the significance of statistical differences between frass and urea. Dunn’s test with Bonferroni correction was used to compare the effects of different N rates. The results of the microbiological analyses obtained at the beginning (baseline) and the end of the experiment were processed statistically. In the first stage, 10 microbiological variables were log-transformed. Parametric tests could then be applied. The effects of frass and urea fertilizers and five N rates on the examined microbiological parameters were determined by two-way ANOVA. Tukey’s test was applied to estimate the significance of differences between means. The significance of differences between frass and urea within each N rate was determined by the independent samples *t*-test. This method was applied to analyze the data acquired at the end of the experiment. The significance of differences between mean values obtained at the beginning and the end of the experiment was determined by the dependent samples *t*-test. A total of 629 microbial taxa were split into separate groups (clusters) by the generalized EM cluster analysis with *v*-fold cross-validation. The results of EM clustering were different from those produced by classical *k*-means clustering. The EM algorithm does not compute actual assignments of observations to clusters but rather classification probabilities, i.e., each observation belongs to each cluster with a certain probability. The EM algorithm for clustering was described in detail by Witten [41]. *V*-fold cross-validation was used to find the correct number of clusters. In the next stage, principal component analysis (PCA) was performed, and a dendrogram was created using Ward’s method based on the most significant cluster (No. 1). The values of the Shannon diversity index, the Shannon evenness index, and the Simpson dominance index were computed based on an analysis of microbial taxa. All examined chemical and microbiological parameters were assessed by PCA. Due to the broad scope of the study, various statistical methods were used, including parametric (two-way ANOVA) and non-parametric (Friedman’s repeated measures ANOVA by ranks) tests, PCA, and EM cluster analysis with *v*-fold cross-validation. Such an approach made it possible to analyze the results from different perspectives.

3. Results and Discussion

3.1. Chemical Changes in Acid Peat

Both mealworm frass and urea (46% N), applied at increasing N rates, significantly differentiated the chemical properties of incubated acid peat (Table 3). Irrespective of the applied fertilizer, the concentrations of ammonium nitrogen (N-NH₄) and nitrate nitrogen (N-NO₃) in peat increased significantly and steadily with increasing N rates. Compared with the control treatment, the highest increase (more than six-fold) in the content of mineral N (N-NH₄ + N-NO₃) was found after the application of 50 mg N dm^{−3}, which resulted from a high concentration of N-NO₃. Each of the higher N rates nearly doubled the mineral N content of the substrate relative to the previous rate.

In the first days of incubation, the rate of N ammonification was high in peat fertilized with urea (Figure 1a) and much slower in peat fertilized with frass. The rate of nitrification was high already in the first week of incubation, irrespective of the applied fertilizer (Figure 1b). In the first seven weeks of incubation, the mineral N content of peat fertilized with frass steadily increased and nearly doubled (from 28 to 54 mg dm^{−3}, Figure 1c). A

rapid increase (2.2-fold, to approximately 120 mg dm^{-3}) in mineral N content was also noted in the last two weeks of incubation. Only in the last week of incubation was the content of N-NO_3 (Figure 1b) and mineral N (Figure 1c) in peat fertilized with frass similar to or higher than in peat fertilized with urea. Peat incubated with urea was characterized by a smaller variation in the content of mineral N than peat incubated with frass (Figure 1c), despite the fact that the content of plant-available N, in particular ammonium N (Figure 1a), was much higher after the application of the N fertilizer. In peat fertilized with urea, the mineral N content was high (110 mg dm^{-3} of the substrate) in the first week of incubation and remained stable until week 9 (Figure 1c).

Table 3. Average mineral nitrogen content of acid peat, supplied with different fertilizers and nitrogen rates.

Fertilizer	Nitrogen Rates (mg dm ⁻³)					Mean
	0	50	100	200	400	
N-NH ₄						
Frass	1.92 <i>b</i>	2.09 <i>b</i>	2.16 <i>b</i>	2.81 <i>b</i>	13.30 <i>a</i>	4.47
Urea	2.41 d	5.42 d	15.50 c	47.40 b	142.69 a	42.60
Significant	ns	ns	***	****	****	****
Mean	2.16 D	3.75 D	8.84 C	25.10 B	77.70 A	
N-NO ₃						
Frass	1.59 <i>d</i>	2.60 <i>d</i>	22.30 <i>c</i>	88.00 <i>b</i>	156.00 <i>a</i>	54.00
Urea	1.83 e	37.90 d	68.00 c	105.00 b	127.00 a	67.8
Significant	ns	****	****	****	****	****
Mean	1.71 E	20.30 D	45.10 C	96.50 B	141.00 A	
Mineral N						
Frass	3.51 <i>d</i>	4.69 <i>d</i>	24.50 <i>c</i>	90.80 <i>b</i>	169.00 <i>a</i>	58.50
Urea	4.24 e	43.30 d	83.50 c	152.00 b	269.00 a	110.00
Significant	ns	****	****	****	****	****
Mean	3.88 E	24.00 D	54.00 C	121.00 B	219.00 A	

Significant differences between nitrogen rates in frass and urea groups: *** significant at $p < 0.001$; **** significant at $p < 0.0001$; ns—not significant. Capital letters A, B, . . . , E indicate significant differences between nitrogen rates. Lowercase letters a, b, . . . , e indicate significant differences between nitrogen rates only within the frass group (italics) and only within the urea group (without italics).

Similar mineralization and nitrification rates were described by Houben et al. [21,26], who demonstrated that N mineralization was positively correlated with microbial activity and that nitrification was the dominant process [21]. A considerable decrease in ammonium N content in the first week of soil incubation with frass was also observed by Kagata and Ohgushi [24], who attributed this loss to ammonia volatilization and N immobilization by soil-dwelling microorganisms. In this study, the concentration of mineral N did not decrease in acid peat incubated with frass. Such a decrease was noted in week 7 of incubation in deacidified peat enriched with frass in our previous study [37]. According to Kowalska [42], a drastic decrease in plant-available nitrate N around day 50 of incubation results from excessive growth of cellulolytic bacteria that suppress the development of nitrifying bacteria.

The average content of N-NH_4 and N-NO_3 in peat incubated with frass was 9.5- and 1.3-fold lower, respectively, than that in peat incubated with urea (Table 3). In peat incubated with urea, the content of mineral N increased significantly compared with peat incubated with frass. In treatments supplied with frass, the N-NH_4 content increased significantly only in response to F400, compared with lower rates. The N-NO_3 content increased significantly in response to F100 and higher rates. Urea applied at increasing N rates contributed to a significant and gradual increase in the content of N-NH_4 , N-NO_3 , and mineral N in peat. In the cultivation of ornamental trees and shrubs with low, moderate, and high nutrient requirements, the optimal content of N-NH_4 and N-NO_3 is <20 and $90\text{--}110 \text{ mg N dm}^{-3}$, <25 and $120\text{--}140 \text{ mg N dm}^{-3}$, and <30 and $150\text{--}170 \text{ mg N dm}^{-3}$,

respectively [43]. This incubation experiment met the above requirements when acid peat was fertilized with frass at the two highest rates (200 and 400 mg N dm⁻³). However, when urea was applied at these rates, the maximum permissible level of N-NH₄ exceeded 1.6- and 4.7-fold, respectively, for ornamental trees and shrubs with high nutrient requirements. In contrast to the NO₃⁻ ion, the NH₄⁺ ion can be toxic to plants even at low concentrations, although the nitrate form is more available in an acidic environment.

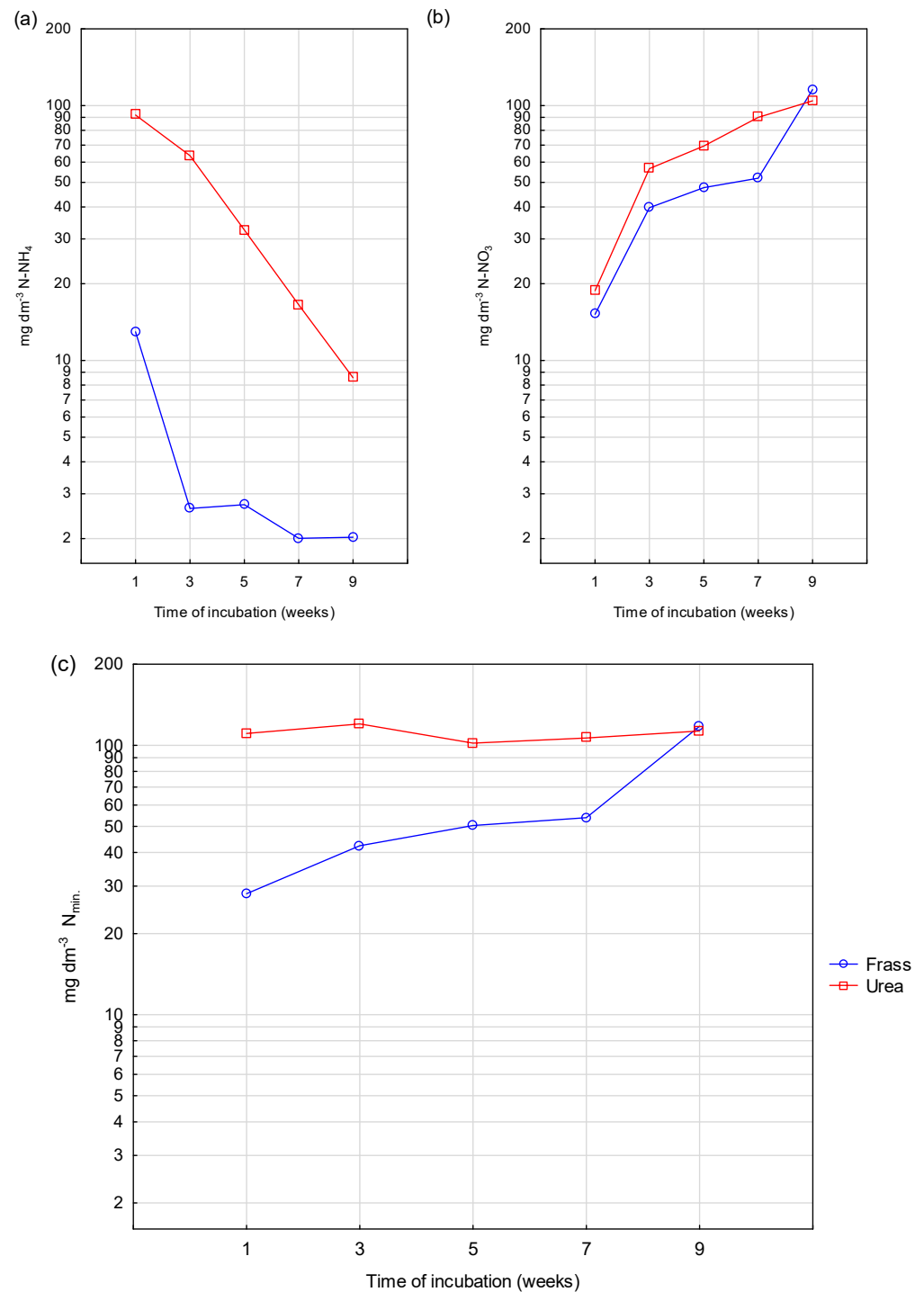


Figure 1. Changes in the content of N-NH₄ (a), N-NO₃ (b), and mineral nitrogen (c) in acid peat incubated with frass and urea. Differences are presented on a logarithmic scale.

High nutrient availability under optimized nutrient management may promote crop growth [44]. In order to improve its fertilizing potential and increase its yield-forming effects, acid peat can be enriched and deacidified with the use of Ca fertilizers, NPK, zeolite, calcined clay, and diatomaceous earth [31,34–36]. However, the demand for acid horticultural substrates has been growing steadily for several reasons. The popularity and production of relatively new fruit species, such as aronia and northern highbush blueberry, have increased in recent years. Coniferous trees and shrubs, heaths and heathers, azaleas, and rhododendrons are widely planted in expanding urban areas [45,46]. They have relatively low soil requirements, play an important ecological role, resist adverse environmental conditions, and are prized for their esthetic appeal. In the cultivation of most ornamental trees and shrubs with low nutrient requirements grown in nursery containers and pots, the recommended pH in H₂O is 4.2–6.5 [47,48].

Before the experiment, acid peat was characterized by highly acidic pH (pH in H₂O = 5.0), and frass was characterized by acidic pH (pH in H₂O = 5.6; Table 2). Irrespective of the applied fertilizer, N rates higher than 100 mg dm^{−3} significantly decreased the pH of peat relative to the control treatment and the lowest N rate (Table 4). Frass had a more acidifying effect on peat than urea, and a significant decrease in pH was noted at F50 and F400. Mealworm frass and urea did not change the pH of peat when applied at moderate rates of 100 and 200 mg N dm^{−3}. Frass had a more acidifying effect on the analyzed substrate than urea, most likely because it contains acid-producing compounds, such as sulfates. Frass has a high content of organic matter that undergoes mineralization with the release of CO₂. Therefore, frass could acidify the substrate to a greater extent than urea since the latter does not contain organic matter. According to Houben et al. [21], the decrease in soil pH is due to the acidic pH of frass and its rapid mineralization, leading to the release of CO₂ and organic acids. Different results were obtained in deacidified peat: urea exerted an acidifying effect, whereas mealworm frass stabilized pH [37].

In addition to the pH of horticultural substrates (which affects the availability of nutrients for plants), EC should also be measured because its value provides information about the concentrations of dissolved nutrients in the soil solution and the salt content of soil. High rates of mineral fertilizers increase soil salinity due to excessive concentrations of the following ions: NO₃[−], K⁺, Na⁺, Cl[−], and SO₄^{2−}. The salinization of horticultural substrates is usually caused by excessive accumulation of K⁺ and SO₄^{2−} ions and, to a lesser extent, Cl[−] and Mg²⁺ ions, followed by Ca²⁺, NH₄⁺, and phosphate ions. Plant tolerance to salt stress varies depending on the species, growth stage, soil moisture content, and cultivation method. Under standard conditions, the EC of peat used in the cultivation of ornamental trees and shrubs with low, moderate, and high nutrient requirements should be <0.8, <1.1, and <1.4 mS cm^{−1}, respectively [47,48]. In this incubation experiment, EC was determined at 0.13 to 0.99 mS cm^{−1}, remaining within the above range (Table 4). Urea contributed to a significantly higher increase in acid peat's EC than frass. The noted increase was caused by higher concentrations of mineral N, particularly N-NH₄, in peat fertilized with urea, which indicates that mineral N was released more rapidly from urea than from frass. It should be noted that the content of the other analyzed nutrients (K, Na, Cl, S-SO₄, Mg, Ca, and P) in peat was lower after the application of urea than frass, and similar results were reported for deacidified peat [37]. The content of these nutrients in acid peat generally increased with increasing N rates. The only exception was Ca, and the decrease in its availability resulted from the fact that this element forms insoluble complexes with phosphate anions.

Table 4. Average content of nutrients and the pH and electrical conductivity (EC) of acid peat supplied with different fertilizers and nitrogen rates.

Fertilizer	Nitrogen Rate (mg dm ^{−3})					Mean
	0	50	100	200	400	
S-SO ₄						
Frass	48.6	45.8	49.6	54.9	75.3	54.8
Urea	48.7	47.1	51.2	50.9	53.1	50.2
Significant	ns	ns	ns	**	****	**
Mean	48.6 D	46.5 C	50.4 BC	52.9 B	64.2 A	
Cl						
Frass	15.3	15.1	17.0	19.3	33.6	20.1
Urea	14.1	10.3	10.6	11.7	12.8	11.9
Significant	ns	****	****	****	****	****
Mean	14.7 C	12.7 E	13.8 D	15.5 B	23.2 A	
P						
Frass	17.3	21.4	31.1	54.3	96.7	44.2
Urea	18.4	17.1	20.1	18.5	20.7	19.0
Significant	ns	****	****	****	****	****
Mean	17.9 D	19.3 D	25.6 C	36.4 B	58.7 A	
Ca						
Frass	705	716	719	707	629	695
Urea	708	656	631	611	562	634
Significant	ns	***	****	****	****	****
Mean	707 A	686 B	675 B	659 C	595 D	
Mg						
Frass	33.9	37.1	40.9	48.2	62.3	44.5
Urea	32.5	38.5	40.9	40.6	38.5	38.2
Significant	ns	ns	ns	****	****	****
Mean	33.2 E	37.8 D	40.9 C	44.4 B	50.4 A	
K						
Frass	37.1	52.2	72.3	107	198	93.3
Urea	37.4	54.5	53.8	58.1	51.8	51.1
Significant	ns	ns	****	****	****	****
Mean	37.2 E	53.4 D	63.0 C	82.5 B	124.9 A	
Na						
Frass	15.8	17.5	21.2	26.1	36.9	23.5
Urea	15.6	13.9	17.8	15.2	14.5	15.4
Significant	ns	****	****	****	****	****
Mean	15.7 D	15.7 D	19.5 C	20.6 B	25.7 A	
pH						
Frass	5.11	4.94	5.00	4.88	4.86	4.96
Urea	5.13	5.28	5.00	4.84	4.96	5.04
Significant	ns	****	ns	ns	****	****
Mean	5.12 A	5.11 A	5.00 B	4.86 D	4.91 C	
EC						
Frass	0.131	0.132	0.270	0.566	0.994	0.419
Urea	0.132	0.296	0.436	0.708	0.830	0.480
Significant	ns	****	****	****	****	****
Mean	0.131 E	0.214 D	0.353 C	0.637 B	0.912 A	

Significant differences between frass and urea groups across nitrogen rates: ** significant at $p < 0.01$; *** significant at $p < 0.001$; **** significant at $p < 0.0001$; ns—not significant. Capital letters A, B, . . . , E indicate the significance of differences between nitrogen rates.

3.2. Quantitative Assessment of Groups of Functional Microorganisms

The incubation of acid peat with different fertilizers and N rates resulted in significant variations in environmental functional gene loads (Table 5). The abundance of total fungi increased significantly in peat treated with frass at lower N rates (F50 and F100 mg dm⁻³) but decreased at higher N rates (F200 and F400). *Clostridium* spp. counts decreased significantly following the application of F400. The incubation time influenced *Pseudomonas* spp. counts, with a significant decrease noted in frass treatments (F100, F200, and F400). The *chiA* gene load increased significantly in F50, F200, F400, U50, and U400 treatments. The *nifH* gene load increased significantly only with urea at the highest N rate. In contrast, the *nosZ* gene load decreased in peat incubated with frass in treatment F100. The *amoA* gene load significantly decreased in treatments F100, F200, F400, U100, U200, and U400, while the *ureC* gene load was significantly lower after applying lower frass rates (F50 and F100) compared with the baseline.

Table 5. Differences in microbial counts and loads of environmental functional genes in acid peat at the beginning (baseline) and the end of the experiment.

Fertilizer	Total Bacteria	Total Fungi	<i>Clostridium</i> spp.	<i>Pseudomonas</i> spp.	<i>Bacillus</i> spp.	<i>chiA</i>	<i>nifH</i>	<i>nosZ</i>	<i>amoA</i>	<i>ureC</i>
Baseline (mean log ₁₀)	10.73	14.15	6.71	5.84	8.52	5.84	9.29	6.56	7.31	8.36
				Difference (log ₁₀)						
0	−0.14	−0.08	0.10	−0.13	0.09	0.07	0.15	−0.03	0.06	−0.23
F50	−0.34	0.75 *	0.17	−0.95 **	0.41	0.46 *	0.03	−0.21	−0.27	−0.47 *
F100	−0.38	0.75 *	0.09	−1.19 ***	0.34	0.05	−0.18	−0.33 *	−0.59 *	−0.55 *
F200	−0.23	−0.81 *	−0.12	−1.38 ***	0.26	0.38 *	0.19	−0.05	−0.81 **	−0.26
F400	−0.09	−0.89 *	−1.22 **	−1.26 ***	0.05	0.43 *	0.40	−0.02	−0.61 *	0.00
U50	−0.08	0.08	−0.08	0.88 **	0.25	0.34 *	0.28	0.01	−0.15	−0.04
U100	−0.18	0.03	0.04	0.93 **	0.21	0.25	0.15	0.01	−0.78 **	−0.05
U200	−0.08	0.06	0.14	1.05 **	0.16	0.21	0.26	0.11	−0.75 **	0.02
U400	0.06	0.23	−0.01	1.45 ***	0.12	0.51 *	0.47 *	0.21	−1.51 ***	0.15

* Significant at $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Functional gene loads in incubated acid peat were influenced by the type of fertilizer and the N rate (Table 6). Total bacterial load was significantly higher after frass application than urea, peaking in F100 and F50 treatments and decreasing in F400. Total fungal load was significantly higher in peat incubated with frass than urea. The load of *Clostridium* spp. increased significantly only in response to the highest N rate. Frass applied at the lowest rate inhibited the growth of *Clostridium*, but the load increased significantly in F200 and reached the highest value in F400. Peat fertilization with urea at U50, U100, and U400 contributed to a significant increase in the load of *Clostridium* spp. The load of *Pseudomonas* spp. varied significantly with different N rates and fertilizer types. *Bacillus* spp. load decreased significantly after applying low N rates (50 and 100 mg dm⁻³) and reached the control treatment level at the highest N rate (400 mg dm⁻³). The abundance of *Bacillus* spp. in peat fertilized with urea at U50 to U200 was significantly higher than that in peat fertilized with frass, while frass showed a more beneficial effect at the highest rate. The *chiA* gene load was significantly lower in peat fertilized with all N rates compared with the control treatment. Frass at F100 ($p < 0.01$) and urea at U50 and U200 ($p < 0.05$) increased the abundance of chitinolytic bacteria. Irrespective of the applied fertilizer, increasing N rates (50, 200, and 400 mg dm⁻³) led to a significant decrease in the number of *nifH* gene copies. The abundance of both *nifH* and *nosZ* genes increased significantly ($p < 0.0001$) after applying frass compared with urea. Frass at F50 and F100 contributed to a significant increase in the number of *nifH* gene copies, relative to the control treatment. The abundance of the *nifH* gene was higher in peat fertilized with N at 50, 100, and 400 mg dm⁻³ than in the control treatment, whereas the 200 mg N dm⁻³ rate significantly decreased its abundance. The abundance of the *nosZ* gene increased after applying frass at all N rates compared with urea (minimum $p < 0.001$). However, frass rates of F50–F200 increased the abundance of the

nosZ gene in peat, whereas urea at higher rates significantly decreased its abundance. The average abundance of the *amoA* gene increased significantly with increasing N rates, and it was significantly higher in acid peat fertilized with urea than frass. Only frass at the lowest N rate significantly increased the number of *amoA* gene copies compared with urea. In turn, the number of *ureC* gene copies increased to 100 mg N dm⁻³ and decreased significantly, even relative to the control treatment. The load of the *ureC* gene was significantly higher in peat incubated with frass than urea ($p < 0.0001$). The abundance of ureolytic bacteria decreased in acid peat incubated with urea in response to increasing N rates.

Table 6. Loads of functional genes in acid peat incubated with frass or urea.

Fertilizer	Nitrogen Rates (mg dm ⁻³)					Mean
	0	50	100	200	400	
Total bacteria (log ₁₀)						
Frass	10.87 c	11.08 ab	11.12 a	10.96 b	10.82 c	10.97
Urea	10.87 a	10.82 ab	10.91 a	10.81 ab	10.67 b	10.82
Significant	ns	**	***	*	*	****
Mean	10.87 B	10.94 AB	11.01 A	10.89 B	10.75 C	
Total fungi (log ₁₀)						
Frass	14.23 b	13.40 c	13.40 c	14.96 a	15.05 a	14.21
Urea	14.23 a	14.07 b	14.13 b	14.09 b	13.92 c	14.09
Significant	ns	***	****	****	****	****
Mean	14.23 B	13.73 C	13.76 C	14.53 A	14.49 A	
Clostridium spp. (log ₁₀)						
Frass	6.61 c	6.54 d	6.62 c	6.83 b	7.93 a	6.91
Urea	6.61 c	6.80 a	6.67 b	6.57 c	6.73 ab	6.68
Significant	ns	*	ns	**	****	****
Mean	6.61 B	6.67 B	6.65 B	6.70 B	7.33 A	
Pseudomonas spp. (log ₁₀)						
Frass	5.97 c	6.79 b	7.04 a	7.23 a	7.10 a	6.83
Urea	5.97 a	4.96 b	4.91 b	4.79 b	4.39 c	5.01
Significant	ns	****	****	****	****	****
Mean	5.97 AB	5.88 B	5.97 AB	6.01 A	5.75 C	
Bacillus spp. (log ₁₀)						
Frass	8.43 ab	8.11 c	8.19 bc	8.26 b	8.47 a	8.29
Urea	8.43 a	8.27 c	8.31 b	8.36 b	8.40 a	8.35
Significant	ns	*	*	*	*	*
Mean	8.43 A	8.19 B	8.25 B	8.31 AB	8.44 A	
chiA (log ₁₀)						
Frass	5.78 a	5.39 b	5.80 a	5.47 ab	5.41 b	5.57
Urea	5.78 a	5.50 c	5.59 bc	5.63 b	5.34 d	5.57
Significant	ns	*	**	*	ns	ns
Mean	5.78 A	5.45 D	5.70 B	5.55 C	5.38 D	
nifH (log ₁₀)						
Frass	9.15 c	9.27 b	9.48 a	9.11 c	8.89 d	9.18
Urea	9.15 a	9.02 b	9.14 a	9.04 b	8.83 c	9.03
Significant	ns	****	****	*	****	****
Mean	9.15 B	9.14 B	9.31 A	9.07 C	8.86 D	
nosZ (log ₁₀)						
Frass	6.59 d	6.77 b	6.89 a	6.61 c	6.58 d	6.69
Urea	6.59 a	6.55 a	6.54 a	6.45 b	6.35 c	6.50
Significant	ns	***	****	***	****	****
Mean	6.59 C	6.66 B	6.72 A	6.53 D	6.47 E	

Table 6. Cont.

Fertilizer	Nitrogen Rates (mg dm ^{−3})					Mean
	0	50	100	200	400	
<i>amoA</i> (log ₁₀)						
Frass	7.25 <i>d</i>	7.58 <i>c</i>	7.91 <i>b</i>	8.12 <i>a</i>	7.92 <i>b</i>	7.76
Urea	7.25 d	7.47 c	8.09 b	8.07 b	8.82 a	7.94
Significant	ns	*	****	*	****	****
Mean	7.25 E	7.53 D	8.00 C	8.09 B	8.37 A	
<i>ureC</i> (log ₁₀)						
Frass	8.58 <i>c</i>	8.83 <i>b</i>	8.91 <i>a</i>	8.61 <i>c</i>	8.35 <i>d</i>	8.66
Urea	8.58 a	8.39 b	8.40 b	8.34 c	8.20 d	8.38
Significant	ns	****	****	****	****	****
Mean	8.58 C	8.61 B	8.65 A	8.48 D	8.28 E	

Significant differences between nitrogen rates in frass and urea groups: * significant at $p < 0.05$; ** significant at $p < 0.01$; *** significant at $p < 0.001$; **** significant at $p < 0.0001$; ns—not significant. Capital letters A, B, . . . , E indicate significant differences between nitrogen rates. Lowercase letters a, b, . . . , d indicate significant differences between nitrogen rates only within the frass group (italics) and only within the urea group (without italics).

A comparison between the baseline and post-incubation microbiological parameters in acid peat revealed diverse effects of the tested fertilizers on the growth of *Pseudomonas* spp. The growth of this bacterial genus was inhibited by frass but stimulated by urea. Lower frass rates also inhibited ureolytic bacteria. In contrast to common observations, frass decreased *Pseudomonas* spp. abundance in acid peat and had no impact on *Bacillus* spp. [49–51]. The activity of ureolytic bacteria, which typically thrives in well-aerated topsoil, was suppressed due to reduced levels of ammonia and oxygen under the experimental conditions. Acidic pH in acid peat negatively affected ureolytic bacteria and *Pseudomonas* spp., potentially enhancing phytopathogenic fungi. Regardless of the fertilizer, N rates above 50 mg dm^{−3} decreased to counts of nitrifying bacteria and oxidation potential, most likely due to the peat's acidic pH. Frass at 100 mg N dm^{−3} positively influenced acid peat's functional genes, increasing bacterial load, decreasing fungal load, and maintaining or altering the abundance of various bacterial groups, including *Pseudomonas* spp., chitinolytic bacteria, nitrous oxide-reducing bacteria, and N-fixing bacteria. According to Wang et al. [52], there is a relationship between the loads of functional genes and N transformations in soil, with modifications of N-NH₄ closely regulated by the *amoA* gene. They found that various genes, such as *narG*, *napA*, and *npxA*, regulated the concentration of N-NO₃. Similar observations were made in this study after the application of frass. Unfortunately, the F100 treatment contributed to a low *Bacillus* spp. load, known for promoting plant growth and producing fungistatic compounds [53,54]. In a study by Przemieniecki et al. [14], mealworm meal with low N content increased the loads of beneficial bacteria in soil, improving plant biometrics.

3.3. Changes in the Bacteriobiome Community and Relationships with the Analyzed Parameters

An analysis of diversity indices revealed higher counts of bacterial species numbers (except in the 200 mg N dm^{−3} treatment) and lower species diversity in acid peat with frass than urea (Table 7). After frass application, the counts of bacterial species increased by 10% on average from the baseline but decreased by 10.3% compared with the control. Urea (especially U50 and U400) significantly decreased the number of taxa (by approximately 37%), and a lower decrease was induced by U100 (17%) and U200 (3.5%). The Shannon diversity index was lower in urea and higher in frass treatments, except for a notable increase at 200 mg N dm^{−3}. Frass-fertilized peat exhibited higher Shannon evenness, peaking at F200, while Simpson's dominance was highest in U400, U50, and U100, and lowest in F200.

Table 7. Bacterial genera with the highest percentage in the bacteriome and dominance classes [†] with diversity indices.

Bacterial Genus	Baseline	0	F50	F100	F200	F400	U50	U100	U200	U400
<i>Candidatus Koribacter</i>	2.8	4.4	3.2	3.0	2.9	2.4	4.9	4.1	4.4	4.6
<i>Bradyrhizobium</i>	3.0	2.4	2.3	3.1	4.0	3.1	2.1	2.9	2.4	3.1
<i>Dokdonella</i>	1.7	2.0	3.0	2.8	1.8	3.8	1.6	2.5	2.9	5.5
<i>Acidobacterium</i>	3.3	2.5	2.1	2.3	2.3	2.2	3.1	4.1	3.5	2.3
<i>Rhodanobacter</i>	6.5	1.4	1.8	1.3	1.5	5.2	2.0	1.7	1.8	3.9
<i>Candidatus Solibacter</i>	2.0	2.7	2.4	3.0	2.6	2.1	4.3	3.0	2.3	2.2
<i>Flavisolibacter</i>	2.6	2.3	2.4	2.1	1.6	1.5	3.1	2.3	2.8	2.9
<i>Pseudolabrys</i>	1.5	1.8	1.3	2.1	2.5	1.4	2.1	3.2	2.5	3.5
<i>Lysobacter</i>	2.7	1.6	2.2	2.1	1.7	2.7	1.8	1.8	2.0	2.9
<i>Granulicella</i>	2.9	2.1	1.3	1.8	2.0	2.4	1.8	2.4	2.3	1.8
<i>Rhodoplanes</i>	1.3	2.3	1.8	2.1	1.9	1.2	2.4	2.6	2.2	2.6
<i>Chitinophaga</i>	1.5	1.2	1.7	1.1	1.3	1.6	1.3	2.2	2.5	2.7
<i>Methylocystis</i>	2.2	1.9	1.3	1.0	2.1	0.8	1.6	1.6	1.5	1.8
<i>Acidisarcina</i>	1.6	1.5	1.3	1.5	1.4	1.5	1.5	2.0	2.0	0.9
<i>Pseudobacter</i>	1.8	1.4	1.5	1.1	0.6	1.4	1.9	1.8	1.7	1.5
<i>Devosia</i>	0.9	1.0	1.1	1.8	3.2	1.8	0.6	1.4	0.9	1.7
<i>Haliangium</i>	0.6	1.4	1.5	1.6	1.1	0.9	1.8	1.8	1.1	0.3
<i>Terriglobus</i>	1.3	1.0	1.1	1.0	1.0	1.3	1.2	1.7	1.5	0.7
<i>Thermomonas</i>	1.2	1.0	1.1	1.3	0.5	1.2	1.2	0.9	0.9	2.0
<i>Mucilaginibacter</i>	3.9	1.7	0.8	0.8	0.5	0.4	0.6	1.1	0.6	0.3
<i>Paludibaculum</i>	0.9	1.2	1.1	1.2	1.1	0.8	0.9	1.3	1.3	0.7
<i>Edaphobacter</i>	1.4	1.1	0.8	0.6	1.2	0.7	1.0	1.4	1.2	0.7
<i>Pseudolysobacter</i>	0.7	0.8	1.0	0.9	0.5	1.0	0.6	1.1	1.1	1.8
<i>Lacunisphaera</i>	0.5	1.1	2.0	2.7	0.9	0.0	0.8	0.7	0.5	0.1
<i>Pseudomonas</i>	0.6	0.5	1.4	1.3	1.3	1.2	0.5	0.5	0.7	0.6
<i>Panacibacter</i>	1.1	1.0	0.9	0.9	0.6	0.3	1.2	1.0	0.9	0.7
<i>Niastella</i>	1.0	1.0	1.3	0.6	0.4	0.6	1.2	0.9	0.8	0.5
<i>Opitutus</i>	0.3	0.9	1.3	2.0	0.8	0.2	0.9	0.8	0.6	0.2
<i>Paenibacillus</i>	0.0	0.1	0.1	0.3	2.2	4.4	0.1	0.1	0.1	0.1
<i>Usitatibacter</i>	0.7	0.9	0.4	0.6	0.3	0.5	1.3	0.7	1.1	1.0
<i>Nitrosospora</i>	0.3	0.4	0.4	0.4	0.3	0.4	0.6	0.9	1.0	2.5
<i>Niabella</i>	0.8	1.2	0.9	0.5	0.6	0.3	0.8	0.7	0.8	0.7
<i>Stenotrophomonas</i>	0.6	0.8	0.7	0.7	0.6	1.0	0.3	0.5	0.9	1.1
<i>Sideroxydans</i>	1.6	1.0	0.3	0.4	0.1	0.1	1.4	0.7	1.3	0.3
<i>Luteimonas</i>	1.1	0.6	0.8	0.6	0.3	0.5	0.8	0.2	0.8	1.2
<i>Nitrospirillum</i>	0.4	0.4	0.6	0.9	0.9	0.8	0.4	0.4	0.7	1.2
<i>Luteitalea</i>	0.4	0.9	1.0	0.9	0.8	0.5	0.8	0.4	0.5	0.3
<i>Steroidobacter</i>	0.5	1.4	2.0	1.4	0.2	0.1	0.2	0.2	0.2	0.3
<i>Filimonas</i>	0.5	0.7	0.6	0.8	0.3	0.4	0.9	0.7	0.8	0.5
<i>Candidatus Nitrotoga</i>	0.7	0.5	0.4	0.4	0.3	0.1	1.4	1.0	1.2	0.2
<i>Thiobacillus</i>	0.4	0.3	0.2	0.9	0.5	0.1	0.5	0.8	1.3	0.8
<i>Candidatus Saccharimonas</i>	0.1	1.5	0.7	0.5	0.4	0.6	0.4	0.5	0.5	0.1
Shannon diversity index	4.82	4.95	4.90	4.83	5.01	4.88	4.57	4.72	4.86	4.48
Shannon evenness index	0.901	0.891	0.899	0.893	0.912	0.898	0.891	0.880	0.880	0.888
Simpson dominance index	0.0132	0.0129	0.0125	0.0136	0.0109	0.0131	0.0181	0.0159	0.0140	0.0202

[†] Dominance classes according to Przemieniecki et al. [38], color intensity, from the darkest: dominant (10.00–5.01%), subdominant (5.00–2.01%), recedent (2.00–1.01%), and sub-recedent (<1.00%).

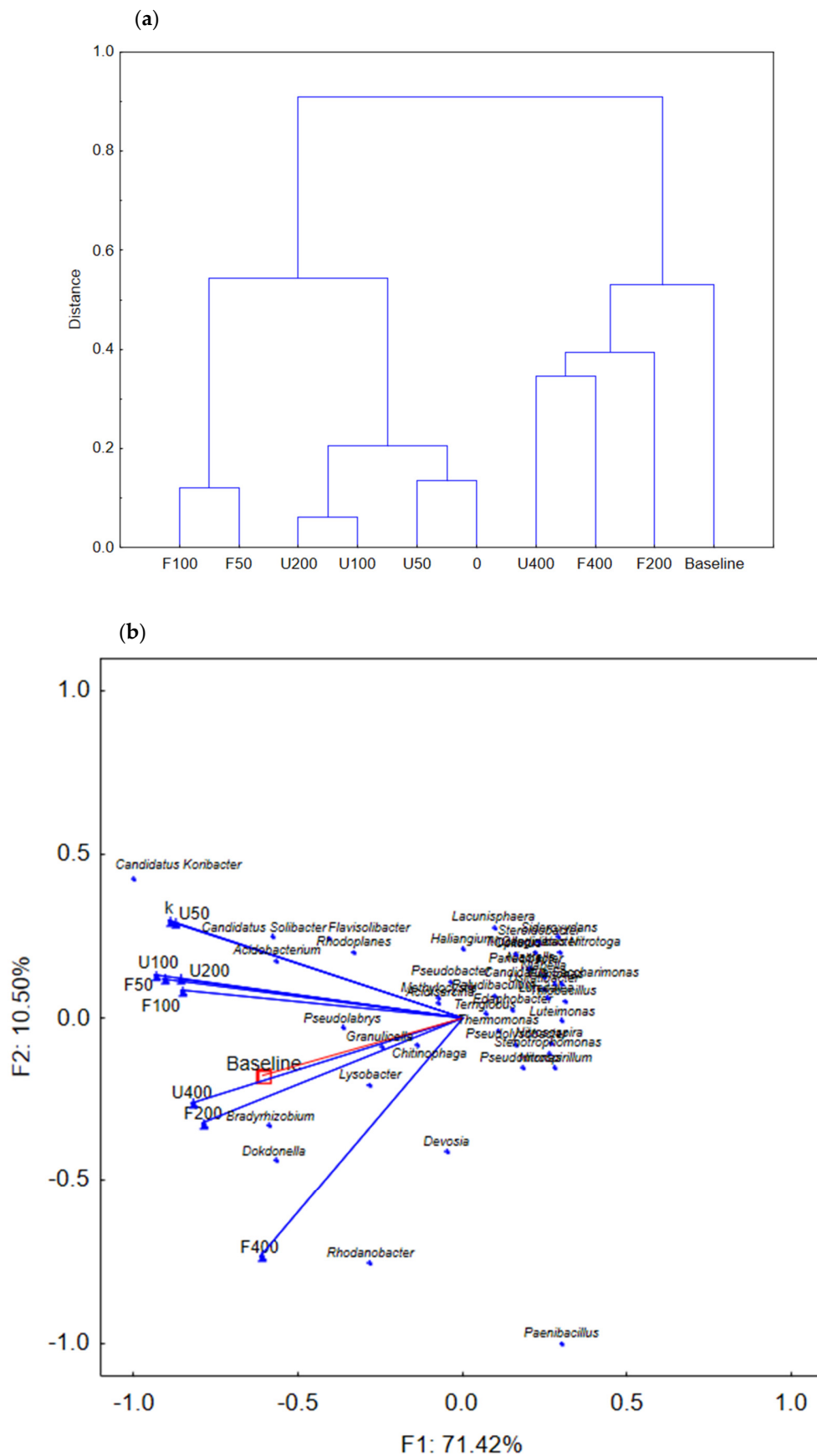
In the hierarchical cluster analysis (Figure 2a), fertilization treatments formed two clades. Subgroups in the first clade included F100 and F50, and the second clade comprised U200, U100, U50, and the control treatment. In the second clade, U400, F400, and F200 formed a subgroup, with baseline data showing low similarity. PCA of the microbiome (Figure 2b) revealed a strong correlation between *Rhodanobacter* and F400. *Dokdonella* and *Bradyrhizobium* correlated with F400, F200, and U400. Baseline values were weakly correlated with principal components. The presence of the genera *Candidatus Koribacter*, *Ca. Solibacter*, *Acidobacterium*, *Flavisolibacter*, and *Rhodoplanes* was characteristic of the remain-

ing fertilization treatments. The generalized EM cluster analysis classified 629 microbial taxa into four groups (Table 7). The first cluster included 42 taxa with the highest overall proportion (50.9–62.7%), averaging 1.21–1.49%. The remaining clusters had decreasing overall proportions and average percentages. The microbial structure analysis at the genus level revealed low diversity, the absence of a dominant genera, and the dominance of three genera: *Ca. Koribacter*, *Bradyrhizobium*, and *Acidobacterium*. The abundance of *Ca. Solibacter*, a subdominant genus, remained stable. *Ca. Koribacter* decreased with frass N rates, especially in F400. *Bradyrhizobium* was 1.7-fold higher in F200. *Acidobacterium* doubled in U100, and *Ca. Solibacter* doubled in U50, compared with the control treatment.

In the metagenomic analysis, the phylum Acidobacteria, including *Ca. Koribacter*, *Acidobacterium*, and *Ca. Solibacter*, emerged as the dominant taxonomic group in all treatments after 60 days of peat incubation. These bacteria degrade simple and complex sugars from organic soils and dead organic matter as carbon sources. Some Acidobacteria produce antimicrobial peptides and siderophores that chelate iron and are drought-resistant. Acidobacteria easily metabolize carbon compounds in nutrient-deficient environments and effectively compete with other bacteria under exposure to environmental pressure [55,56]. In the work of Catania et al. [57], the abundance of Acidobacteria in soil rich in organic matter was significantly correlated with a decreasing pH and cation exchange capacity. In the current study, three genera of the phylum Acidobacteria maintained their dominant status, and *Ca. Solibacter* increased its dominance when the soil pH decreased over two months. The exact roles of *Ca. Solibacter* and *Ca. Koribacter* remain unknown, but the dominant status of these genera could be used as a bioindicator to identify substrates that do not support microbial development. High abundance of these bacterial genera could compromise the total biological activity of soil, nutrient cycling, and symbiotic interactions between plants and other microbial groups.

The genus *Dokdonella* exhibited different dominance patterns, increasing in urea treatments and becoming dominant in U400. *Rhodanobacter* spp., initially dominant, became rare in F50–F200, U100, and U200, and subdominant in U50 and U400, remaining dominant only in F400. *Flavisolibacter* spp., initially subdominant, decreased in F200 and F400. *Pseudolabrys* spp. increased after urea and frass applications. *Lysobacter* spp. and *Granulicella* spp. showed varied dominance patterns near the threshold, with *Granulicella* spp. having lower proportions in all treatments. *Rhodoplanes* spp. remained consistent, except in F400. *Chitinophaga* spp. increased with urea. *Methylocystis* spp. and *Devosia* spp. showed minor dominance pattern variations, rising after the application of F200. Other genera had a relatively low share of the total population, with a single subdominant taxon. A high proportion of *Dokdonella* spp. and *Rhodanobacter* spp., of the family Rhodanobacteraceae, in particular after the application of the highest N rates, indicates that they depend on the availability of oxidized N compounds. Both genera reduce nitrates and are well adapted to environments with a low pH (in particular, *Rhodanobacter*) [58–60].

The order Rhizobiales dominated in all analyzed samples, with a high proportion of the genus *Bradyrhizobium*, which was more prevalent in the rhizosphere than in the soil. Rhizobacteria enter symbiotic relationships with legume plants by forming root nodules that enable atmospheric N fixation. *Bradyrhizobium* bacteria are root symbionts, ubiquitous in the soil and legumes, which have also been found on the surface of sugar beet roots. Research has shown that both diazotrophic and non-diazotrophic *Bradyrhizobium* strains can colonize the root zone of both leguminous and non-leguminous plants. The group of free-living *Bradyrhizobium* spp. includes both diazotrophs and strains incapable of N fixation, most of which are capable of denitrification, H₂ uptake, photosynthesis, and carbon fixation [61–64]. It appears that the above properties can be used to regulate nutrient availability in the rhizosphere, and to modify unfavorable environmental conditions within the root zone.



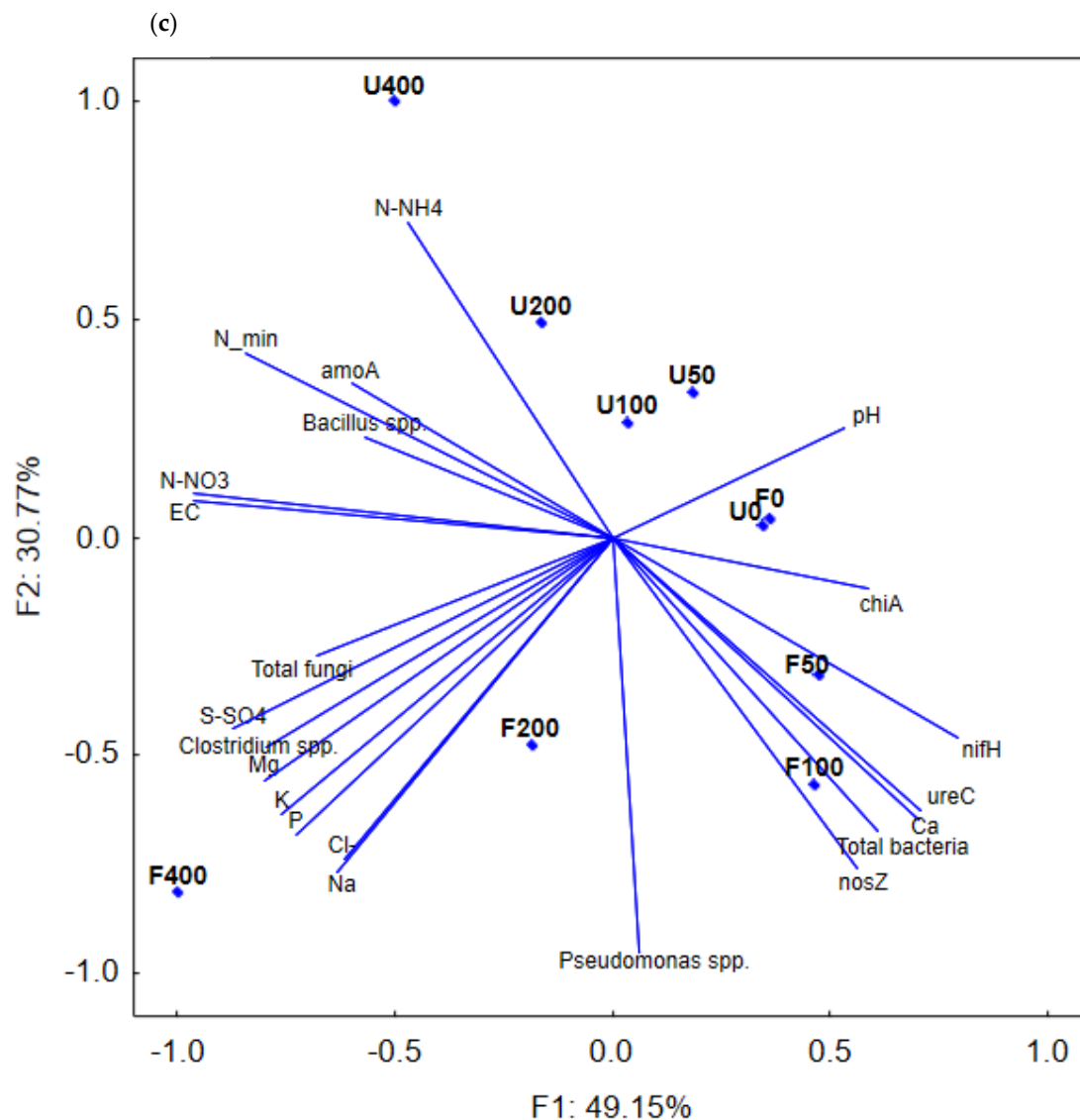


Figure 2. Hierarchical clustering (a), principal component analysis (PCA) of the tested rates of N fertilizer and the bacteriome (b), and PCA of the tested rates of N fertilizer and the chemical and microbiological parameters of acid peat (c).

Nitrospira spp. were subdominant in the treatment supplied with the highest urea rate. This genus contains a complete set of *amo* and *hao* genes required for the oxidation of both NO_2^- and NH_4^+ to NO_3^- , as well as complete ammonia oxidation. The increase in nitrate N levels could also be attributed to increased counts of denitrifying and nitrate assimilation bacteria, such as *Opitutus* spp. [65,66]. *Rhodoplanes* and *Pseudolabrys* are also interesting genera of photosynthetic bacteria, which are plant symbionts and participate in carbon and N cycles. Most photosynthetic bacteria effectively fix molecular N, but *Rhodoplanes* spp. can also utilize N from the decomposition of urea. These bacteria rely on various substrates as carbon sources and readily utilize organic acids [67]. In the present study, the total abundance of *Rhodoplanes* and *Pseudolabrys* increased after urea application. The absence of a light period during peat incubation probably triggered heterotrophic metabolism. It promoted the assimilation of organic carbon, but both bacterial genera could also utilize N from the decomposition of urea. Therefore, the presence of *Rhodoplanes* and *Pseudolabrys* in both urea and frass treatments indicates that organic N was decomposed and acquired through deamination. The genus *Methylocystis* is also highly interesting. In these methanotrophs, methane is oxidized by the methane monooxygenase (MMO) enzyme.

Methylocystis spp. are bioindicators of methanogenic processes in soil. These bacteria were already identified at the beginning of the experiment, which suggests that they utilize various carbon sources. Their presence in the F200 treatment resulted from a small number of subdominants [68,69]. The share of *Devosia* spp. increased in all treatments, and the greatest increase was noted in F200. This observation is difficult to explain because most *Devosia* spp. are diazotrophs that establish symbiotic relationships with legumes. Similarly to the genus *Bradyrhizobium*, *Devosia* spp. are probably free-living bacteria in peat.

The PCA of chemical parameters and microbiological markers (Figure 2c) revealed that F1 and F2 explained 79.92% of the total variance. F1 was correlated with a group of parameters (mainly in F400 and F200) that were negatively correlated with pH. F2 was correlated with *nosZ*, total bacteria, Ca, *ureC*, and *nifH*, which are characteristic of F100 and F50. These parameters were negatively correlated with N-NH₄, mineral N, *amoA*, and *Bacillus* spp. *ChiA* gene abundance was negatively correlated with N-NO₃ and EC. It should also be noted that N-NH₄ content was high in urea treatments, in particular those with the highest urea rates.

In the present study, a higher content of mineral N was positively correlated with high urea rates and negatively correlated with low frass rates. The supply of plant-available N may be limited when N is mineralized slowly. However, rapid N mineralization in urea treatments and high mineral N content can lead to N depletion in the substrate or exert phytotoxic effects. The abundance of N-fixing bacteria, negatively correlated with N-NH₄ content, was highest in peat fertilized with 100 mg N dm⁻³. In addition, a correlation was found between N-fixing bacteria and Ca content. In the rhizosphere and soil, Ca is essential for N fixation—it plays many important roles in N-fixing bacteria, and Ca ions mediate signal transduction pathways during interactions between plants and symbiotic bacteria [70,71].

4. Conclusions

One of the greatest challenges in insect farming for food and feed is adequate management and disposal of waste products, such as exuviae and frass. Sustainable and organic farming has the potential to reduce the use of inorganic fertilizers and increase the utilization of organic residues as a source of N for plant nutrition. The use of mealworm frass as an organic fertilizer is an important pathway for N, P, and K recycling, which contributes to sustainable nutrient management, in line with the European Green Deal. Insect frass can partially or completely replace traditional mineral fertilizers in the production of selected crops. Wider use of mealworm frass as a fertilizer in the cultivation of horticultural crops and ornamental plants, and in forest farming, appears to be beneficial and justified. The slow release of N, a stable pH needed by some crops, and a high content of plant growth-promoting microorganisms in the substrate ensure effective plant growth and development over prolonged periods, which is an important practical consideration. This study makes an original contribution to the existing body of knowledge in the fields of environmental protection, sustainable agriculture, and forestry. The obtained results can be used for designing future studies involving different plant species, and for establishing optimal frass rates. Moreover, the unique spore-forming bacteria whose abundance is stimulated by the addition of frass require further detailed research.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/app14062380/s1>, Table S1: Quantitative polymerase chain reaction setup. References [72–81] are cited in the supplementary materials.

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