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Addition of Chicken Litter Compost Changes Bacteriobiome in Fallow Soil

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Abstract: Composting is an environmentally friendly process, turning animal waste into fertilizer. Chicken litter compost (CLC) improves soil properties, increasing crop yields. However, the CLC effect on the soil microbiome is understudied. This study aimed to compare bacteriobiome diversity in fallow arable Chernozem with and without CLC addition in a field experiment in the Novosibirsk region, Russia, using 16S rRNA gene metabarcoding. *Pseudomonadota*, *Actinomycetota* and *Acidobacteriota* were the most OTU-rich phyla, together accounting for >50% of the total number of sequence reads. CLC-related shifts in the bacteriobiome structure occurred at all taxonomic levels: the *Bacillota* abundance was 10-fold increased due to increased *Bacilli*, both being indicator taxa for the CLC-soil. The main *Actinomycetota* classes were the indicators for the CLC-soil (*Actinobacteria*) and no-CLC soil (*Thermoleophilia*, represented *Gaiella*). Both *Bacillota* and *Actinomycetota* phyla were the ultimate constituents of the CLC added, persisting in the soil for five months of fallowing. The no-CLC soil indicator phyla were *Acidobacteriota* (represented by *Acidobacteria*_Group3) and *Verrucomicrobiota*. Future metabarcoding studies of chicken litter application in agricultural soils, including cropped studies, should address the soil microbiome at the species/strain levels in more detail, as well as how it is affected by specific crops, preferably accompanied by a direct methodology revealing the microbiota functions.

Keywords: 16S rRNA genes; Illumina Miseq; soil bacteria; indicator taxa; West Siberia

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1. Introduction

There is no denying that "sustainable poultry development is vital to global food security and health", as stated by the UN Food and Agriculture Organization [1]. The production of chicken, meat, and eggs has been increasing worldwide: according to the FAO estimates, chicken, meat, and egg production in 2022 increased by 8% each, compared with that in 2018 [2]. This steadily increasing chicken population produces a growing amount of manure: one thousand chicken broilers produces 65 kg of manure per day, whereas the same number of chicken layers produces 150 kg [3]. With the global chicken population reaching $27 \cdot 10^9$ birds in 2022 [2], the amount of manure produced is rather impressive. Chicken manure is rich in nitrogen, phosphorus, potassium and other elements [4], and can be added to soil to improve its fertility and properties [5]. The amount of nitrogen, applied to soil as chicken manure/compost, has also been increasing, reaching $6.3 \cdot 10^9$ t globally in 2021 [6].

Such a large amount of chicken manure requires increasing efforts for its transportation, storage, composting and processing [7,8]. Those countries which can afford land parcels for this purpose resort primarily to composting. Moreover, composting is an environmentally friendly process that turns animal waste into organic fertilizers [9], which were recently found to present more sustainable attributes for agricultural use [10] through the thorough

study of composting [11–13]. The composts of chicken manure/litter from big production facilities are known to increase crop yields [14–16] and improve soil properties [17–19].

As chicken manure, litter, and hence their composts contain diverse gut microbiota [20–23], the application of compost to soil can shift soil ingenious microbiota, affecting soil biotic and abiotic processes to a certain extent [24]. Moreover, the direct land application of chicken litter from big poultry production facilities could be harming animal, human, and environmental health [25,26]. Therefore, in recent years, chicken litter composting has been spreading, while attracting more research attention, as fertilization with composted litter was found to reduce the risk of the transmission of antibiotic resistance genes and enteric bacteria to soil and crops compared with raw manure or litter [27,28], with such research being greatly facilitated by the advance of DNA sequencing technologies. Yet, reports about the effect of adding chicken litter compost are relatively scarce, as many publications describe either chicken manure or chicken litter addition, or the manure droppings of free-range birds. Sometimes, after an addition of chicken litter compost in soil, the field is fallowed (not cropped, with the removing of weeds) to allow the applied compost to be further transformed under the influence of weather and edaphic factors. We hypothesized that, under such conditions, some bacterial markers of the chicken litter compost, mainly chicken gut bacteria, will be markedly increased in the fallow field compared with a field without compost addition.

The aim of the study was to reveal bacteriobiome composition and structure in arable Chernozem under fallow conditions, with and without the addition of chicken litter compost in a field experiment in the south of West Siberia (Novosibirsk region, Russia), by using 16S rRNA gene diversity.

2. Materials and Methods

2.1. Experimental Site and Conditions

The field experiment was carried out in West Siberia, in the south (55°00′2.88″ N, 83°29′7.8″ E), which is largely used for agriculture. In terms of biogeographical zonation, the study area belongs to the forest–steppe zone with a strongly continental climate, with an average annual temperature of 2.7 °C and a precipitation total of 493 mm [29]. According to the international soil classification system [30], the soil is identified as Luvic Phaeozems (Loamic, Albic, Aric), which is one of the most agriculturally important soils of the region.

2.2. Experimental Setup

The field trial was started in 2009. Until that time, the field was in conventional agricultural use, i.e., cropped to spring wheat with moderate rates of mineral nitrogen and low rates of phosphorus fertilization, with mouldboard plowing in the autumn and disking in the spring. No livestock manure, litter, or composts were applied. In 2009, the area was abandoned, and the natural succession of spontaneous restoration started, resulting in the development of a dry meadow dominated by the smooth brome (*Bromus inermis* Leyss.). In spring 2020, the area was disked three times, down to 15 cm. At the end of June 2020, one parcel (173 ha) of the field received chicken litter compost (CLC, from the local chicken-producing industrial facility) at the rate of 30 t ha^{-1} (dry matter), which was incorporated into 0–20 cm topsoil by disk harrowing. The other parcel (184 ha) received no CLC (no-CLC). Both parts were maintained as fallow until the end of the growing season in 2020.

The CLC represents a litter of wood sawdust that had been spread out on the ground in chicken housing, collecting their droppings; after a while, the litter was removed and placed in the open field, where it underwent aerobic decomposition, i.e., composting, for at least three months at positive temperatures without any special additions, mixed once in a while for better aeration. The chemical characteristics of CLC are presented in Table 1.

Table 1. Chemical properties of chicken litter compost (mean ± standard deviation, $n = 4$).

Property		
Initial Water Content, %	24.5	±0.7
pH_{H2O}	7.14	±0.05
EC, mS/cm	8.98	±0.20
Organic Matter, %	85.0	±0.6
N, %	4.48	±0.39
P, %	0.95	±0.09
K, %	1.75	±0.19

2.3. Soil Sampling and Chemical Analyses

The soil was sampled at the end of October 2020 from the 0–20 cm layer from the no-CLC and CLC-amended fields, prior to the onset of the negative daily temperatures in the region. Both fields were divided into two subplots, and from the center of each subplot, four mixed soil samples, composed from six individual/separate replicates taken by a soil corer, were collected. Thus, eight mixed soil samples represented each of the fields; in total, 16 mixed soil samples were collected. In the laboratory, the roots and other plant parts were removed, the soil was sieved (2 mm), and aliquots were taken for drying, stored in the fridge at +4 °C for the DNA extraction (the latter were kept at −20 °C for two weeks). The soil properties were measured using standard techniques [31]. Briefly, organic carbon content was estimated by dichromate digestion; soil organic matter content was estimated by the amount of soil mass loss on ignition at 550 °C for 12 h; nitrate content was determined potentiometrically in 0.1% $AlKSO_4$ solution (soil–solution ratio 1:5 *w/v*); and readily available P and available P were extracted by 0.015 M K_2SO_4 solution (1:5 *w/v*) and 0.1 M $(NH_4)_2C_2H_4O$ $(COO)_2$ solution (pH = 5.7; 1:20 *w/v*), respectively, and determined calorimetrically. Exchangeable K was extracted by 1 M CH_3COONH_4 solution (pH = 7.0; 1:10 *w/v*) and estimated by an atomic absorption spectrometer with flame atomization (Kvant-2A, Russia). Soil pH was measured by equilibrating 10 g of field-moist soil with 25 mL of deionized water. Bulk soil density was calculated as the mass/volume ratio after drying a soil core of the known volume at 105 °C for 24 h. All analyses were performed in triplicate, and the data were expressed on an oven-dry (105 °C) basis.

The soil texture of both fields was characterized as silt loam, though it was very close to silty clay loam. The soil organic matter content was similar in both fields; soil pH was somewhat higher in the field with CLC application. The content of all three important mineral macronutrients in the forms of those readily available for plants were 4–9 times higher in the CLC field compared with the no-CLC field, as shown in Table 2.

Table 2. Some properties of the Chernozem of the experimental fields with and without chicken litter compost (CLC) addition (mean ± standard deviation).

Soil Property	No CLC		CLC	
SOM [1], %	7.69	±0.04 b [2]	7.58	±0.07 a
SOC [1], %	3.84	±0.03 b	3.79	±0.04 a
pH_{H2O}	6.03	±0.01 a	6.36	±0.01 b
Nitrates, mg N/kg	12.2	±5.5 a	63.8	±7.4 b
Readily Available P, mg P/kg	0.61	±0.10 a	3.14	±0.33 b
Available P, mg P/kg	10.7	±3.7 a	100.7	±9.6 b
Exchangeable K, mg K/kg	171	±26 a	687	±52 b
Sand, %	6.6	±3.3	7.5	±3.0
Silt, %	68.0	±3.0	66.2	±2.2
Clay, %	25.4	±4.3	26.3	±3.8

[1] SOM and SOC stand for soil organic matter and soil organic carbon, respectively.[2] Different letters in rows indicate that the values are different ($p \leq 0.05$, Fisher's LSD test).

2.4. DNA Extraction, Amplification and Sequencing

The DNA extraction and amplification was performed exactly as we described earlier [32] (Naumova et al., 2023; https://www.mdpi.com/2076-2607/11/10/2431, accessed on 28 August 2024). Sequencing was also performed with the same sequencer at the same facility, i.e., the Genomics Core Facility of the Siberian Branch of the Russian Academy of Sciences (ICBFM SB RAS, Novosibirsk, Russia). The read data reported in this study were submitted to the NCBI Short Read Archive under bioproject accession number PRJNA1115889.

2.5. Bioinformatic Analysis

The bioinformatic analysis was also performed exactly as we described earlier [32] (Naumova et al., 2023; https://www.mdpi.com/2076-2607/11/10/2431, accessed on 28 August 2024), analyzing raw sequences with UPARSE pipeline [33], using Usearch v.11.0.667, the UPARSE-OTU algorithm, and SINTAX [33] for the taxonomic attribution, referenced with the 16S RDP training set v.19 [34].

The OTU datasets were analyzed by individual rarefaction (graphs are not shown) with the help of the PAST v.4.16 software [35]: the numbers of bacterial OTUs detected, reaching a plateau with an increasing number of sequences, showed that the sampling effort was close to saturation for all samples, thus sufficient to compare diversity on the non-rarefied datasets [36].

2.6. Statistical Analyses

Statistical analyses (descriptive statistics, correlation analysis, ANOVA, PCA and PCoA) were performed by using Statistica v.13.3 (TIBCO Software Inc., Palo Alto, CA, USA) and PAST [35] software packages. OTU-based α-biodiversity indices, as well as β-biodiversity (based on the Bray–Curtis dissimilarity distance) and indicator taxa values, were calculated using PAST. The factor effects and mean differences in post hoc comparisons by Fisher's LSD test were considered statistically significant at the $p \leq 0.05$ level.

3. Results

3.1. General Taxonomic Diversity in the CLC Nacteriobiome

After sequence quality control, a total of 55 different OTUs were identified at a 97% sequence identity level, belonging to two phyla, three classes, seven orders, 21 families and 31 genera. Of these OTUs, 16 OTUs were *Actinomycetota*, 36 were *Bacillota* and 3 were *Pseudomonadota*, the latter with just 0.2 ± 0.1% of the relative abundance. Ten genera were dominants, i.e., contributing at least 1% in the total number of sequence reads, as shown in Table 3.

Table 3. Bacteriobiome composition of the chicken litter compost (relative abundance, %, mean ± standard deviation, $n = 3$).

Dominant Phyla	*Bacillota*	*Actinomycetota*
	80.9 ± 0.7	18.9 ± 0.6
Dominant Classes		
Bacilli	80.8 ± 0.7	
Actinobacteria	18.9 ± 0.6	
Gammaproteobacteria	0.2 ± 0.1	
Dominant Genera		
Unclassified Bacillaceae	50.1± 1.2	
Staphylococcus	13.0 ± 1.6	
Brevibacterium		6.2 ± 0.7
Nosocomiicoccus	5.5 ± 1.1	
Corynebacterium		5.4 ± 0.4
Oceanobacillus	5.3 ± 1.7	
Mammaliicoccus	4.1 ± 0.5	
Yaniella		3.2 ± 0.3
Enteractinococcus		2.7 ± 0.7
Brachybacterium		1.0 ± 0.2
Sum	78.1	18.4

3.2. General Taxonomic Diversity in the Soil Bacteriobiome

After quality filtering and chimera removal, 3286 different OTUs were identified at a 97% sequence identity level. We found 23 bacterial phyla-level clusters, containing 84 classes, with 14 of them not explicitly classified.

The majority of the total number of OTUs (786, or 24%) were members of the *Pseudomonadota* phylum, whereas *Actinomycetota* (546 OTUs) and *Acidobacteriota* (322 OTUs) ranked second and third, as OTU-rich phyla, comprising 17 and 10% of the total number of OTUs, respectively. Many OTUs (445 OTUs, or 14%) were not classified below the domain level.

The dominance of *Actinomycetota, Acidobacteriota* and *Pseudomonadota* phyla was also pronounced in terms of relative abundance; together, they accounted for more than half of the total number of sequence reads, as shown Table 4. As is often found in soils, *Actinomycetota*, with about 1/3 of the relative abundance, was dominant in both fields. The relative abundance of reads that could not be taxonomically attributed below the domain level accounted for ca. 7% of the total number of sequence reads in the soil bacteriobiome of the experimental fields. The *Verrucomicrobiota* phylum was a moderate dominant, whereas such phyla as *Gemmatimonadota* and *Chloroflexota* were minor dominants. As for the *Bacillota* and *Bacteroidota* phyla, they had a markedly differential relative abundance in the studied fields, discussed in detail in Section 3.2 below.

At the class level, the dominance of *Actinomycetota* phylum translated into the dominance of its *Thermoleophilia* and *Actinobacteria* classes, as shown in Table 4, whereas the dominance of the *Acidobacteriota* phylum mostly resulted from the dominance of its Group_1, Group_3, Group_6 and Group_16 classes. The *Pseudomonadota* phylum in the dominating group was represented by its *Alpha*, *Beta- and Deltaproteobacteria* classes.

3.3. Bacterial Taxonomic Diversity as Related to the CLC Addition in Soil

3.3.1. Bacteriobiome Composition and a Comparison of the Relative Abundance

The CLC addition drastically increased *Bacillota* abundance, mostly due to the substantial increase in *Bacilli* relative abundance, shown in Table 4, with *Clostridia* also contributing. At the class level, seven dominating classes were higher in the no-CLC soil (such as *Thermoleophilia*, *Spartobacteria*, *Acidobacteria* (Gp1, Gp3, Gp6, Gp16) and *Acidimicrobiia*, whereas *Actinobacteria*, *Gammaproteobacteria*, *Gemmatimonadia*, *Bacilli*, *Sphingobacteriia*, *Clostridia* and *Thermomicrobia* were the seven dominating classes with relative abundance higher in the CLC-soil.

Table 4. Relative abundance (%, mean ± standard deviation) of the dominant bacterial phyla and classes in the Chernozem of the experimental fields with and without chicken litter compost (CLC) addition.

Taxon	No CLC		CLC		*p*-Value
Actinomycetota	38.7	±7.0	31.2	±5.3	0.031
Pseudomonadota	25.4	±1.8	26.9	±4.9	0.420
Acidobacteriota	13.3	±2.8	6.5	±3.6	0.000
Verrucomicrobiota	6.1	±1.7	2.1	±1.8	0.001
Bacteroidota	2.0	±0.8	4.2	±1.9	0.007
Gemmatimonadota	1.9	±0.6	1.4	±0.7	0.076
Chloroflexota	1.7	±0.7	1.0	±0.4	0.030
Bacillota	1.3	±1.0	18.6	±10.3	0.000
	Class				
Alphaproteobacteria	18.7	±1.2	18.0	±4.2	0.661
Thermoleophilia	18.5	±4.7	5.9	±4.4	0.000
Actinobacteria	13.5	±5.3	20.5	±5.6	0.022
Spartobacteria	5.3	±1.6	2.0	±1.6	0.001
*Acidobacteria*_Gp6	4.7	±2.5	2.0	±1.5	0.022
Acidimicrobiia	3.2	±0.6	1.7	±0.7	0.001
Betaproteobacteria	3.0	±0.7	3.5	±1.1	0.165

Table 4. *Cont.*

Taxon	No CLC		CLC		*p*-Value
Gammaproteobacteria	0.8	±0.4	3.2	±1.3	0.000
*Acidobacteria*_Gp16	2.9	±0.8	1.9	±0.6	0.012
Deltaproteobacteria	2.8	±0.2	2.1	±1.0	0.052
*Acidobacteria*_Gp3	1.9	±0.6	0.5	±0.3	0.000
Gemmatimonadia	1.6	±0.5	3.3	±1.3	0.001
Chitinophagia	1.3	±0.6	0.9	±0.9	0.002
*Acidobacteria*_Gp1	1.3	±0.8	0.6	±0.5	0.065
Bacilli	1.2	±0.9	16.9	±10.6	0.001
Sphingobacteriia	0.2	±0.2	2.0	±1.4	0.002
Clostridia	0.1	±0.1	1.4	±1.2	0.012
Thermomicrobia	0.2	±0.2	1.0	±0.8	0.011

At the dominant genus level, there were several with a differential abundance due to the CLC addition, as shown in Table 5.

Table 5. Relative abundance (%, mean ± standard deviation) of the dominant bacterial genera in the Chernozem of the experimental fields with and without chicken litter (CLC) addition.

Genus	No CLC		CLC		*p*-Value
Gaiella	12.1	±2.9	3.8	±3.4	0.000
Bradyrhizobium	5.8	±0.7	2.0	±1.9	0.000
Spartobacteria_gis *	5.3	±1.6	1.7	±1.6	0.001
un. # *Acidobacteria*_Gp6	4.7	±2.5	1.8	±1.7	0.022
Mycobacterium	3.7	±1.0	2.5	±1.3	0.069
un. *Acidobacteria*_Gp16	2.7	±0.7	1.5	±0.8	0.006
un.*Acidobacteria*_Gp3	1.9	±0.6	0.4	±0.3	0.000
un.*Acidobacteria*_Gp1	1.3	±0.8	0.6	±0.5	0.065
Streptomyces	0.1	±0.4	3.6	±3.2	0.001
Oceanobacillus	0.0	±0.0	3.1	±4.3	0.034
Nocardiopsis	0.0	±0.0	3.0	±6.9	0.040
Devosia	0.1	±0.2	2.3	±1.7	0.001
Peribacillus	0.2	±0.4	1.8	±1.9	0.016
Neobacillus	0.2	±0.4	1.4	±1.2	0.002
Cerasibacillus	0.0	±0.0	1.0	±1.9	0.080

* gis stands for genus incertae sedis; # un. stands for unclassified.

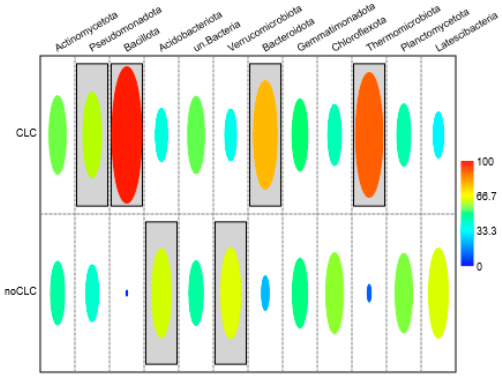
The dominant OTUs, contributing ≥ 1.0% to the total number of sequence reads in a sample, averaged 12 in the no-CLC field and 14 in the CLC one, together accounting for 26% and 23% of the total number of sequence reads, respectively. The total set of dominant OTUs in the study comprised 25. Only four OTUs were common for both fields, namely (1) *Pseudomonadota/Alphaproteobacteria/Hyphomicrobiales/Bradyrhizobiaceae/Bradyrhizobium*; (2) *Pseudomonadota/Alphaproteobacteria/*un. *Hyphomicrobiales*; (3) *Actinomycetota/Thermoleophilia/Gaiellales/Gaiellaceae/Gaiella*; and (4) *Actinomycetota/Actinobacteria/Mycobacteriales/Mycobacteriaceae/Mycobacterium*.

One-way PREMANOVA showed the statistically significant (with *p*-values lower than 0.001) effect of the CLC addition at all taxonomic levels.

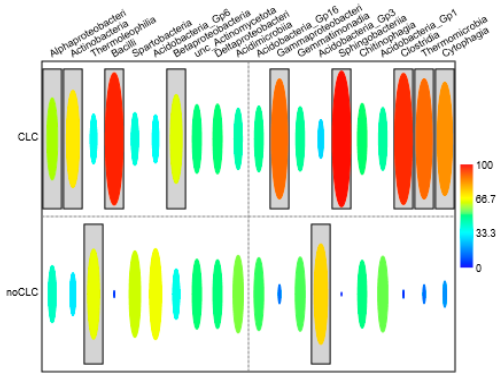
3.3.2. The Indicator Taxa

The indicator taxa (among the dominant ones at the corresponding taxonomic levels, calculated using the number of taxon-specific reads) for each field are shown in Figure 1; there were quite a few at each taxonomic level, indicative of the CLC addition, and several that were indicative of the no-CLC soil. Nine OTUs were indicative of the CLC addition in soil; the *Bacilli* and *Actinobacteria* classes accounted for four of these OTUs each, whereas only one represented *Alphaproteobacteria*. Only one of these

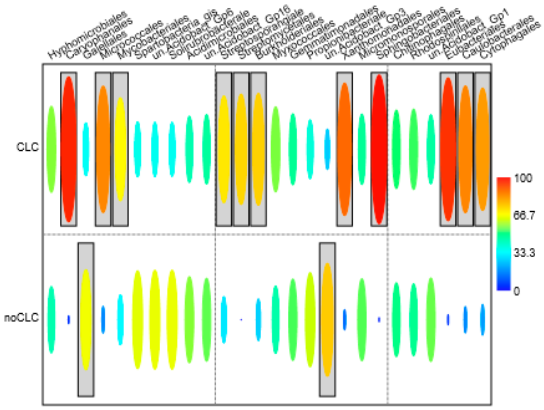
indicator OTUs was explicitly identified to the species level, namely *Oceanobacillus luteolus/Bacillaceae/Caryophanales/Bacilli/Bacillota*. As for the indicator OTUs for the no-CLC soil bacteriobiome, there were five of them: the *Alphaproteobacteria* accounted for two of these OTUs, whereas *Actinobacteria*, *Thermoleophilia* (both *Actinomycelota*), and the *Spartobacteria* class of *Verrucomicrobiota* each contributed one of the indicator OTUs.



(**a**)

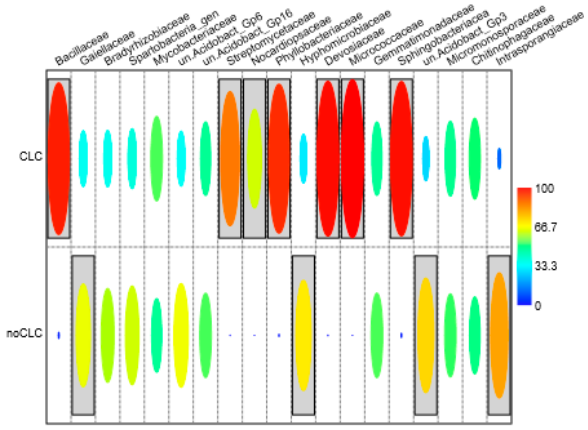


(**b**)

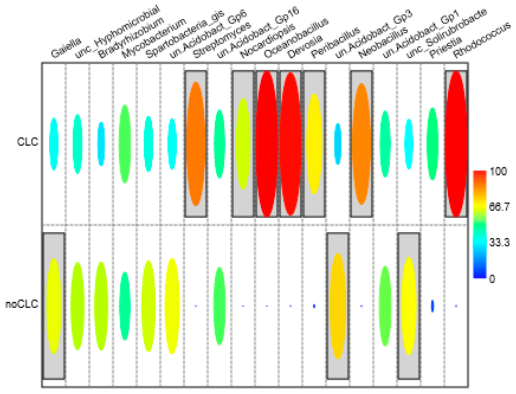


(**c**)

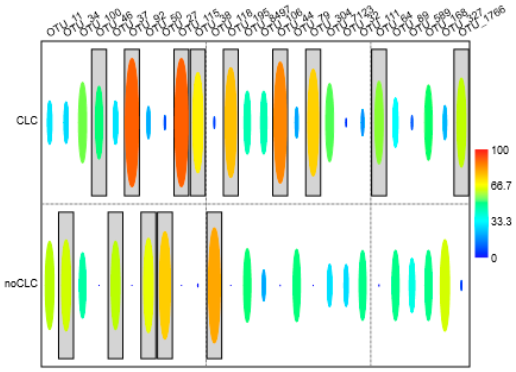
Figure 1. *Cont.*



(**d**)



(**e**)



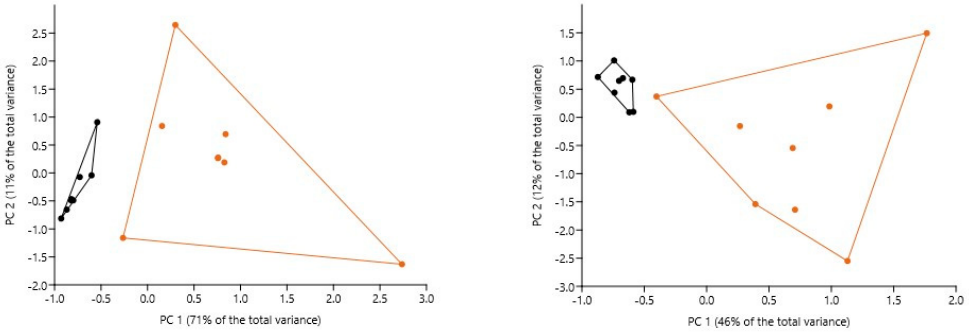
(**f**)

Figure 1. Indicator taxa in the soil bacteriobiome of the fields with CLC) and without (no-CLC) chicken litter compost addition: phyla (**a**), classes (**b**), orders (**c**), families (**d**), genera (**e**) and OTUs (**f**).

3.3.3. Bacteriome Similarity

The location of soil samples in the plane of the first two principal components facilitates the straightforward visualization of the relationship between the fields, shown in Figure 2. Most of the variance was associated with CLC addition, whereas the apparent within-field

variance accounted for just 11–12% of the total one. Notably, the variance between the CLC-field samples was much more pronounced compared with the variance between the no-CLC-field samples.



(**a**) (**b**)

Figure 2. Principal component analysis (based on covariance) of the data matrix, with soil samples as rows and bacterial orders (**a**) and OTUs' (**b**) relative abundance as variables for analysis, indicating the location of soil samples in the plane of principal components 1 and 2, with convex hulls grouping the no-CLC (black circles) and CLC (light brown circles) fields.

3.3.4. Bacteriobiome α- and β-Biodiversity

The α-biodiversity indices did not differ significantly, being practically similar in both studied fields, as shown in Table 6. The number of OTUs per sample was about 1000; bearing in mind that on average only slightly more than 10 OTUs dominated the bacteriobiome in each field, the minor or rare species' sequences accounted for the overwhelming 99% of both bacteriobiomes.

Table 6. Alpha-biodiversity indices (calculated on the OTU's basis; mean ± standard deviation) of bacteriobiomes in the Chernozem of the experimental fields with and without chicken litter compost (CLC) addition.

Taxon	No CLC		CLC		*p*-Value
OTU Richness	937	±128	900	±336	0.769
Chao-1	1321	±142	1158	±414	0.310
Simpson (S)	0.987	±0.002	0.982	±0.017	0.387
Shannon's	5.4	±0.2	5.2	±0.6	0.354
Equitability	0.80	±0.01	0.78	±0.04	0.222
Berger–Parker	0.06	±0.01	0.07	±0.06	0.546
Dominance (1-S)	0.013	±0.002	0.018	±0.017	0.396

As for the β-biodiversity, samples from the CLC field were distant from the no-CLC one (Figure 3).

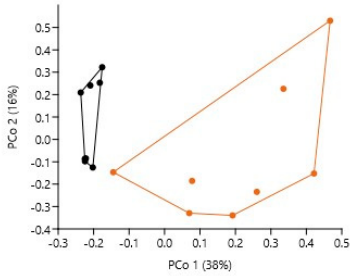


Figure 3. Principal coordinates analysis of the soil bacteriobiome composition (OTU level, Bray–Curtis dissimilarity distance) under different chicken litter compost (CLC) addition, indicating the location of samples in the plane of the first two coordinates. Symbols: black circles denote samples without CLC addition, light brown circles denote soil samples with CLC addition.

4. Discussion

Our study unequivocally showed that CLC addition in soil significantly changed the composition and structure of the soil bacteriobiome. The fact that the relative abundance of *Bacilllota* was an order of magnitude higher than in the no-CLC soil is consistent with the CLC bacteriobiome structure being overwhelmed by the *Bacillota* sequence reads. This finding, being especially remarkable in view of the fact that the soil for the study was sampled more than 5 months after the addition (the fields were kept fallow throughout this time), suggests that the bacteriobiome shifts due to the CLC addition can be rather enduring.

4.1. The Indicator Taxa for the CLC Soil

Indicator taxa can be more responsive to agronomical manipulations, such as fertilization and organic residue addition, and can better predict future crop yields [37]. The indicator phyla for the CLC soil, namely *Bacillota*, *Thermomicrobiota*, *Bacteroidota* and *Pseudomonadota,* are known as the main constituents of the gut bacteriobiome of living organisms, including birds [38,39], with *Bacillota* (formerly referred to as the *Firmicutes* phylum) and *Bacteroidota* (formerly known as *Bacteroidetes)* being the core phyla. The six genera, indicative of the CLC addition, belonged to *Actinomycetota*/*Actinobacteria* (*Streptomyces* and *Nocardiopsis*), *Bacillota*/(*Oceanobacillus*, *Peribacillus* and *Neobacillus*) and *Pseudomonadota/Alphaproteobacteria* (*Devosia*). The CLC-indicator actinomycetal genera here, i.e., *Streptomyces* and *Nocardiopsis*, both natural soil dwellers, are well known for their ability to produce bioactive products [40] and to show antimicrobial [41,42] and plant growth-promoting effects [43]; they also were reported among the indicator genera in soil with organic residue amendment [37]. As for *Devosia*, some of its representatives were reported as promoting plant growth [44], most likely by enhancing nitrogen uptake, and mycorrhization [45]. Representatives of *Peribacillus* and *Neobacilllus* were found as members of plant-growth-promoting rhizobacterial assemblages of many crops [46–49], possessing properties that are highly promising in agricultural and environmental applications. The group of CLC-indicator genera in this study after five months of fallowing post CLC addition seemed to have the potential to benefit future crops' growth and development in fields treated in the same manner; therefore, the crop, soil and environmental consequences of CLC application should be studied in more detail.

Although most of the OTU-level clusters in this study (3053 of 3286) were not identified to the species level, one of the CLC-indicator OTUs was classified as *Oceanobacillus luteolus.* This species was first isolated more than ten years ago from a paddy and a forest soil [49]. We did not manage to find any reports about the bacterium's physiology, ecology and environmental occurrence since that time. Other representatives of the genus are usually found in saline environments [50]. Recently, some reports about the beneficial properties of the *Oceanobacillus* genus, for example, its phosphate solubilizing ability [51] and the ability to synthesize PGP substances [50], were published.

4.2. Bacteriobiome Composition and the Indicator Taxa in the no-CLC Soil

The fallow soil without CLC addition harbors a bacteriobiome developed over five months with minimal organic matter input (with aerial deposition and occasional weeds that managed to emerge and grow between fallow-maintaining treatments), mostly on indigenous soil organic matter. Therefore, such a bacterial profile, as a background for the soil per se, is of great interest ecologically and agronomically in terms of its structural and indicator taxa aspects.

The phyla which were found to be indicators of the soil without CLC, namely *Acidobacteriota* and *Verrucomicrobiota*, are ubiquitous soil dwellers and common dominants in Chernozems and Phaeozems (both undisturbed and cropped) in the south of West Siberia [32,52], as well as similar soils in other regions [53–55]. Notably, the *Actinomycetota* phylum as a single whole was not a CLC or no-CLC indicator phylum, because its two major classes, i.e., the *Thermoleophilia* and *Actinobacteria* class, were indicator classes

for different treatments: the former for the no-CLC soil, and the latter for the CLC soil, as shown in Figure 1b.

At the lower taxonomic levels, the no-CLC indicator taxa were represented by *Gaiella (Gaiellaceae/Gaiellales/Thermoleophilia/Actinomycetota)*, i.e., an actinomycetal genus, widely distributed in the soils of the region [32,52] and similar soils elsewhere [56,57]. The *Gaiellaceae* family was shown to be among the prevailing taxa in the unplanted soil [58], thus indirectly confirming that these actinomycetes can live well solely on soil organic matter. The representatives of the family were members of the core bacteriobiomes in several soil types in Russia, Crimea and Kazakhstan [59]. However, we did not manage to find reports on the bacteriobiome structure in fallow soils with which to compare our results. As for the indicator families in this study, i.e., the *Intrasporangiaceae* (*Micrococcales*, belonging to *Actinobacteria/Actinomycetota*), and *Hyphomicrobiaceae*, belonging to *Hyphomicrobiales/Alphaproteobacteria*, we also could not find reports about their presence in a fallow soil bacteriobiome, although *Hyphomicrobiaceae* was reported as having a highly significant positive correlation with soil organic matter and nutrient content [60]. As for a genus-level cluster of unclassified *Solirubrobacteriales*, the representatives of the order were found in the core bacteriobiome of several soil types [59], as well as among the major constituents of soil bacteriobiome [52,56].

The finding that the no-CLC indicator *Acidobacteria* phylum at the lower taxonomic levels was represented by its Group_3 clusters is consistent with other reports about the *Acidobacteria*_Group_3 taxa being important members of soil bacteriobiomes (for example, see [32]). As for the *Verrucomicrobiota* phylum, we could not find information about its abundance in fallow soils, although it is almost always encountered as a dominant in soils under natural or agricultural phytocenoses [32,52,59].

4.3. Bacteriobiome α- and β-Biodiversity

We found that the α-biodiversity indices did not differ significantly between the CLC- and no-CLC fields. There are few studies reporting changes in α-biodiversity indices due to composted chicken litter addition in soil, and none at all under fallow circumstances. For example, the application of chicken litter compost did not have any effect on α-biodiversity estimates of soil bacteriobiome in an apple rhizosphere [24]. More frequent studies about the effect of chicken manure or its derivatives, and litter without composting, sometimes report confusing results. For instance, a study with chicken manure droppings found increased bacteriobiome α-biodiversity in the soil with chicken manure compared with the soil free from it [61]. Similarly, the application of organic chicken manure fertilizer significantly increased the taxa relative abundance and α-biodiversity of the bacterial community of *Cinnamomum camphora* rhizosphere soil [62]. Other researchers, concluding that the application of chicken litter was a major factor in shaping the soil bacterial communities, found just the opposite effect, i.e., that α-biodiversity parameters were higher in soil without chicken litter addition compared with the litter-modified soil [63]. Apparently, in our study, the drastic shifts in bacteriobiome composition due to the addition of chicken litter compost (especially a 15-fold increased relative abundance of *Bacillota*) did not manifest themselves in α-biodiversity estimates, although these notable shifts in bacteriobiome composition and structure resulted in the markedly different β-biodiversity.

The change in soil properties due to CLC addition was in agreement with the results of other studies: for instance, composted chicken litter was reported to improve soil pH, the content of organic carbon, nitrogen, phosphorus, potassium, calcium, sodium, microbial carbon, and soil respiration [64].

5. Conclusions

The finding that the major bacterial constituents still could persist in high abundance, even over five months of fallowing after the addition of chicken litter compost to soil, implies that such a shift in the soil microbial properties can affect the crop yield of the following year. This conclusion is supported by the beneficial potentiality of the differentially

abundant and/or CLC-soil indicator taxa, as well as by the positive CLC-related changes in the soil chemical properties. In a broader biological context, manure droppings and plant residues have always been part of naturally developing ecosystems; therefore, it stands to reason that the addition of chicken litter compost can be beneficial for promoting plant growth in cropped arable ecosystems.

The characterization of the no-CLC soil bacteriobiome over five months of fallowing may be of special interest to soil microbiologists and ecologists, as it relates to the bacterial community thriving almost solely on indigenous soil organic matter, with scarce additions from aerial deposition [65] and occasional weed plants. Future metabarcoding studies of chicken litter application in agricultural soils, including cropped studies, should address the soil microbial groups at the species/strain levels in more details, as well as how it is affected by specific crops, preferably accompanied by a direct methodology revealing the functional potentiality of microbiota.

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