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The Taxonomic Composition Changes of Bacteria and Fungi in Plant Residue Composts Induced by Biochar and Calcium Carbonate Application

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Abstract: Plant residues are the main source of humus and some nutrients in soils. The composting of organic waste using modifiers is a promising way to obtain high-quality organic fertilizers. Here, the effect of biochar and calcium carbonate on the abundance and taxonomic composition of bacteria and fungi in mature plant compost has been studied using metagenomic analysis. Plant materials with different initial C:N ratios—low (22, clover), medium (38, rye) and high (68, oats)—served as composting materials in the pot experiment. The plant material mixed with sterile sand was modified by the addition of biochar or calcium carbonate. Both ameliorants increased pH values and humic acid content in composts irrespective of plant material composition. Representatives of the phyla Proteobacteria, Actinobacteriota and Firmicutes dominated among bacteria and representatives of the division Ascomycota dominated among fungi in the mature composts, as in the initial plant samples. The abundances of bacteria and fungi in the cereal composts were higher than in the composts with clover. The effect of biochar and calcium carbonate on the number and taxonomic composition of bacteria and fungi in composts from the same plant material was similar, while the effect of reagents in composts from different raw materials was ambiguous. No one dominant group of bacteria was found to develop in response to biochar or calcium carbonate application in any of the types of composts studied. However, the structure of the fungal community both at the phylum and genus levels changed significantly under the influence of these additives. The addition of calcium carbonate and biochar led to an increase in the abundance of the same groups of fungi, but this increase was different for composts made from different plant materials.

Keywords: biochar; compost; plant residues; bacteria; fungi; metagenomic analysis

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1. Introduction

Organic fertilizers are a promising means of increasing soil fertility and agricultural production sustainability. Systematic use of organic fertilizers increases the soil humus and improves soil physico-chemical and agrochemical properties. Plant residues are one of the main sources of humus replenishment in soils. The main reasons for the humus losses in arable soils are closely interrelated with the reduction in the intake of plant residues into the soil [1–3]. At the same time, when such large quantities are introduced into the soil, this can cause nitrogen immobilization and disturbances in plant nitrogen nutrition. The composting of crop residues is a tool for solving this problem. Composting or fermentation of agricultural organic waste is an important technique in modern agricultural

technologies [4–6]. Biothermal transformation of organic material occurs during composting resulting in products enriched with humus and available forms of nutrients, with a reduced ratio of carbon (C) to nitrogen (N). Such composts are recommended for use as organic fertilizers [7–9].

The composting of organic waste with different modifiers (additives of mineral or organic nature) speeds up the process, improving the quality of the resulting product [10,11]. The addition of modifiers such as biochar to compost can improve substrate aeration, reduce nitrogen losses, accelerate the humification of organic matter and increase the chemical maturity of humic substances [12–14]. Moreover, biochar promotes the formation of a humic acid fraction with a relatively high resistance to mineralization [15–17].

The composting of organic waste is a multistage process involving a variety of microorganisms. The influence of biochar on the composition of the microflora of compost obtained from different animal waste has been studied in detail for bird droppings [18–20] and sheep [21], pig [17,22–25] and cow manure [26]. The microbiota composition transformation during sewage sludge and sawdust composting was examined [27]. Most previous studies have focused on only a single issue related to the integrated application of plant residues in combination with other sources of bioavailable organic C, such as soil organic matter, manure, sewage sludge, etc. [19]. As C and nitrogen (N) are key microbial metabolic nutrients, introducing large amounts of available C and N through composting with plant residues could promote microbial activity. However, little information is available on the impact of plant residue on taxonomic composition changes of microorganisms that are involved in the transformation of plant residues and use them as single sources of organic C. Also, the effect of biochar and other ameliorants (e.g., calcium carbonate) on the community of microorganisms, especially fungi in plant residue composts, has not been sufficiently studied [28,29]. The information is of importance when plant residues are used as a growing medium.

The aim of this work was to study the effect of biochar and calcium carbonate on the taxonomic composition and structure of bacterial and fungal communities in composts prepared from plant residues with different initial C:N ratios.

2. Materials and Methods

2.1. Composts and Their Preparation

An incubation experiment was conducted to study the effect of $CaCO_3$ and biochar on the taxonomic composition of bacteria and fungi in plant residue composts. Plant material used for compost preparation consisted of fresh aboveground biomass of rye (*Secale cereale* L.) and red clover (*Trifolium pratense* L.) and also dry biomass of oat straw (*Avena sativa* L.). These plant residues differed in their starting C:N ratios, which were classed as low (22.1, clover), middle (38.6, rye) and high (68.4, oats) (Table 1). As composting conditioners, biochar and calcium carbonate were used. The biochar was obtained from birch and aspen wood by rapid oxygen-free pyrolysis at a temperature of 550 °C produced in the Research Forestry Institute (Leningrad Region). The composition of the biochar was determined to be: C_{org} (85.6%); N_{tot} (0.43%); pH_{H_2O} (8.1); and ash content (1.8%). The particle size was 0.5–2.0 cm. Despite the high carbon content, biochar is mainly represented by inert, very difficult-to-oxidize forms, with few labile fractions in the organic matter. The content of soluble carbon compounds in biochar was determined to be 0.008%. The properties of the biochar used in the work are described in more detail in a previous study [30]. Calcium (Ca) was added in the form of chemically pure calcium carbonate [$CaCO_3$]. The plant materials were cut into 2 mm thick pieces. Plant material from each species with or without reagents (biochar, $CaCO_3$) was mixed with quartz sand calcined at 700 °C in the ratio 1:1. In the variants with reagents, the content of biochar in the compost was 1%, $CaCO_3$—5% of the dry weight of the compost. The concentrations of the investigated reagents were chosen based on our own preliminary experiments and literature data [8,16]. The experiment was conducted in plastic vessels at a temperature of 25 °C. The weight of plant material was 500 g. The humidity of the compost was maintained

at 60% throughout the composting period (50 days). Periodic stirring of the composted biomass was carried out during the experiment. The experiment was repeated 4 times.

Table 1. Characteristics of the initial plant material used for compost preparation. The average values ± standard deviation are given.

Plant Material t	C	N	Ash	C:N
	%			
Clover	41.6 ± 0.8	1.88 ± 0.02	5.34 ± 0.01	22.1
Rye	43.2 ± 1.6	1.12 ± 0.05	5.88 ± 0.03	38.6
Oats	42.0 ± 1.0	0.61 ± 0.08	5.72 ± 0.04	68.4

2.2. Laboratory Methods

The pH values of samples in water suspensions were assessed by shaking the samples with deionized water at a 1:10 (w/v) ratio for 30 min and then measuring using a pH meter (pH 150 M). The C and N total content in plant materials and composts were determined using an element analyzer (Euro EA3028-HT Analyser, Pavia, Italy). The enrichment of organic substances with nitrogen was characterized in terms of the C:N ratio. Humic substances were extracted from compost samples (1:10 compost/solution mass ratio) with 0.1 M NaOH. Humus substance determination was based on their ability to dissolve in the alkaline aqueous solutions used as extragents [31–33]. Then, humic acids were separated from fulvic acids using 0.5 M H_2SO_4 solution [34–36].

2.3. DNA Extraction and Metagenomic Analysis

To determine the number and taxonomic composition of prokaryotes and fungi, DNA was extracted and purified from compost samples using a set from MACHEREY-NAGEL (NucleoSpin Soil, Düren, Germany). The number of bacteria was estimated by the number of 16SrRNA gene copies and the number of fungi by the number of ITS2 site copies per gram of substrate. To determine the prokaryote taxonomic composition the obtained DNA was used to create libraries of the 16SrRNA marker gene by PCR using universal primers for the variable site V4:F515/R806 (GTGCCAGCMGCCGCG-GTAA/GGACTACVSGGGTATCTAAT) and attaching adapters and unique barcodes from Illumina (USA) [37,38]. The taxonomic composition of the community of fungi and fungus-like organisms was determined by analysis of amplicon libraries of intergenic transcribing ribosomal operon spacer (ITS2) sequences obtained using primer pairs for the ITS2 site ITS3-GCATCGATGAAGAACGCAGC/ITS4-TCCTCCGCTTATTGATGC, followed by addition of service sequences using the Illumina protocol containing linkers and barcodes.

For the initial processing of the data, such as demultiplexing and adapter removal, Illumina software was used (Illumina, Diego, CA, USA). For prokaryotic communities, dada2-1.28.0 [39], phyloseq [40] and DECIPHER [41] software packages in the R environment were used for "denoising", sequence merging, chimera removal, initial phylotype inference (ASV, Amplicon sequence variant) and subsequent ASV taxonomic classification. Fungal microbiome was processed with the QIIME2 [42,43] program package. The number of prokaryotes and fungi in the initial plant raw materials and compost was determined by real-time PCR on a T100 Thermal Cycler (BIO-RAD Laboratories, Inc., Hercules, CA, USA).

To compare the generic composition of bacteria and fungi in compost, the Sorensen similarity coefficient was used [44–46]. The Sorensen similarity index (*SSI*) is equal to the number of genera common to two lists c, expressed as a percentage of the average number of genera in lists a and b:

$$SSI = \frac{2c}{(a+b)} \times 100\% \quad (1)$$

SSI is the most versatile measure when assessing the similarity of two or more data sets. It is convenient because, for its calculation, data can be presented both in the form

of "occurrence" (that is in the form of percentages or parts of a unit, where the sum of the values is 100% or 1.0) and in absolute values.

2.4. Statistical Processing

The experiment consisted of four independent measurements of the parameter investigated with four compost samples being taken from four pots. Data were subjected to analysis of variance procedures (one-way ANOVA). The measures of statistical significance of differences between the mean values were determined by Student–Newman–Keuls test. A correlation analysis of compost chemical indicators (pH, organic substances, humic acids, C:N) and the abundance of microorganisms was also carried out using Pearson (r) and Kendall (t) coefficients. The conclusion about the presence of a significant correlation was made when the absolute value of the correlation coefficient was less than 0.3. Statistical significance was determined at $p < 0.05$. Equality of variances was evaluated using the Levene test. Statistical data processing was performed using IBM SPSS Statistics, Version 28 («IBM», New York, NY, USA).

3. Results and Discussion

3.1. Characteristics of Chemical Properties of Composts

The plant material used for compost preparation differed mainly in nitrogen content and C:N ratio (Table 1). Oat straw was characterized by the lowest N content and the highest C:N ratio; in contrast, the aboveground biomass of clover had the highest nitrogen content and the lowest C:N ratio.

As it is known, the value of the C:N ratio determines the temperature of the compostable material decomposition and the maturation of the compost [47–49]. The rate of decomposition of plant materials and formation of humic substances are in direct correlation with the C:N ratio, with the closer the ratio to 1:1, the faster the process of decomposition and formation of humic substances (10; 15). These patterns were also true in our study. While composting, the C:N ratio of the plant material, especially in the case of oat straw, which initially had the highest values of this indicator, decreased (Table 2).

Table 2. Chemical characteristics of composts from various plant materials. The average values ± standard deviations are given. Different letters denote the average values that differ significantly at $p < 0.05$.

Variants of Experiment		pH	Organic Substances, %	C:N	Humic Acid, % of $C_{tot.}$
Oats	K	7.34 ± 0.04^{a}	37.8 ± 0.69^{d}	39.2 ± 0.38^{ef}	15.8 ± 0.22^{a}
	$CaCO_3$	8.36 ± 0.07^{d}	35.6 ± 0.22^{c}	40.4 ± 0.44^{f}	24.0 ± 1.04^{c}
	BC	7.96 ± 0.02^{c}	36.6 ± 0.34^{cd}	37.5 ± 0.36^{e}	24.8 ± 0.46^{c}
Rye	K	7.28 ± 0.11^{b}	37.0 ± 0.82^{d}	32.2 ± 0.36^{d}	14.6 ± 0.38^{a}
	$CaCO_3$	8.20 ± 0.04^{d}	36.2 ± 0.70^{cd}	34.8 ± 0.85^{d}	19.5 ± 0.66^{b}
	BC	7.68 ± 0.05^{b}	35.4 ± 0.72^{c}	28.8 ± 0.60^{c}	26.4 ± 0.46^{c}
Clover	K	7.35 ± 0.03^{a}	32.6 ± 0.58^{b}	20.2 ± 0.72^{ab}	18.8 ± 0.34^{ab}
	$CaCO_3$	8.30 ± 0.06^{d}	32.2 ± 0.74^{b}	21.0 ± 0.75^{b}	33.5 ± 0.55^{d}
	BC	7.64 ± 0.07^{b}	30.6 ± 0.24^{a}	19.6 ± 0.25^{a}	34.4 ± 0.36^{d}

Note: K—control, BC—biochar.

Such changes in the composition of cereal composts reduce the risk of nitrogen immobilization in the soil and may be favorable for nitrogen nutrition of plants, due to the prolonged release of nitrogen and, accordingly, more complete use of this resource.

Composting plant residues with reagents resulted in increased pH values and humic acid content in all composts. In addition, applying biochar also led to a very significant decrease in the C:N ratio in the cereal compost. And, just as in the initial plant residues,

composts with clover had lower values of the C:N ratio than composts from plant residues of other species.

Previously, we showed that the co-composting of plant residues with biochar or calcium carbonate increased the speed of processes, as well as the volume of formation and stabilization of humic acids (the proportion of stabilized humic acids reaches 40–50%) [16].

Thus, the presence of significant amounts of stabilized humic substances (significantly more resistant to decomposition than organic compounds of plant material) is evidently the reason for the change in the composition of bacteria and greater active synthesis of microbial biomass. In our experiment, these processes took place to the maximum extent in oat straw, the plant material with the highest values of the C:N ratio.

Evidently, calcium carbonate and biochar significantly increase the activity of microorganisms and change the availability of hard-to-immobilize compounds in oat straw, making it more accessible.

3.2. The Number of Bacteria and Fungi

The number of bacteria and fungi in the initial plant material samples was determined by the microflora of their phyllosphere and was lower than in composts based on these samples (Figures 1 and 2).

Only in the fresh aboveground biomass of clover was the number of fungi greater than in the compost made from this material, perhaps, due to the antagonistic effect of bacteria actively developing in the composts. In the control variants, the number of bacteria and fungi in the composts based on cereals was higher than in the composts with clover. Evidently, the difference in the number of microorganisms was due to the difference in the speed of composting: there were fewer microorganisms where the first stages of composting associated with the decomposition of the most available organic substances passed faster. This was indirectly evidenced by the similarity of the action of calcium carbonate and biochar: both of these meliorants were used to accelerate composting [7,12,30].

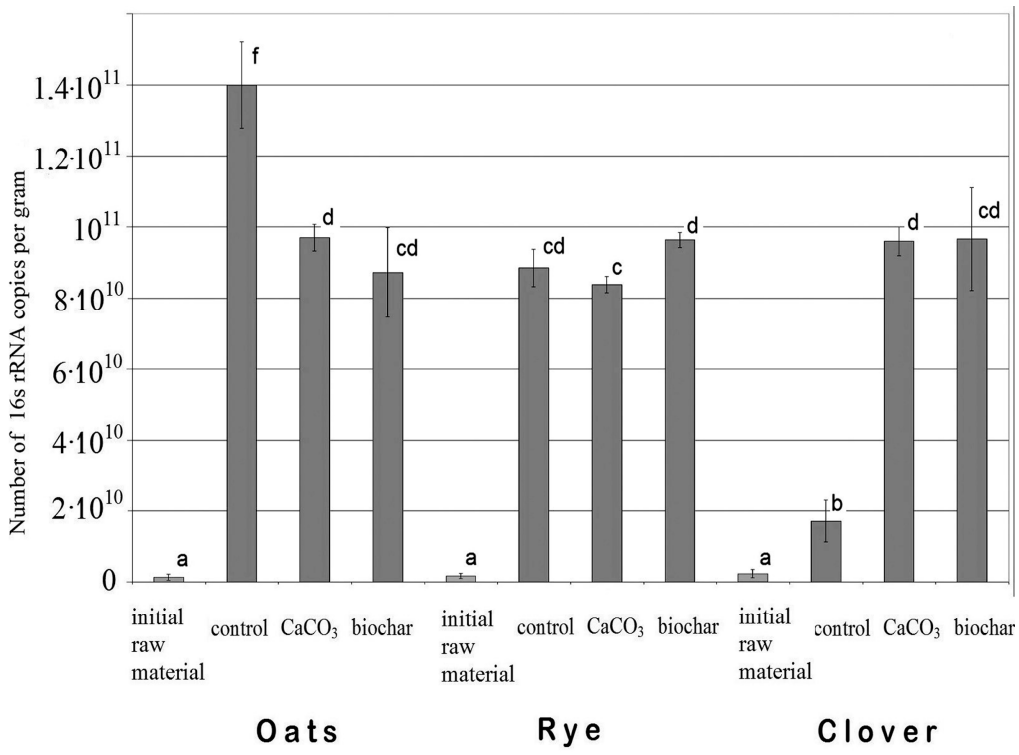

Figure 1. The number of bacteria in the composts in terms of the number of the 16s rRNA gene copies of bacteria. Vertical lines indicate the standard deviation. Different letters indicate the average values that differ significantly at $p < 0.05$.

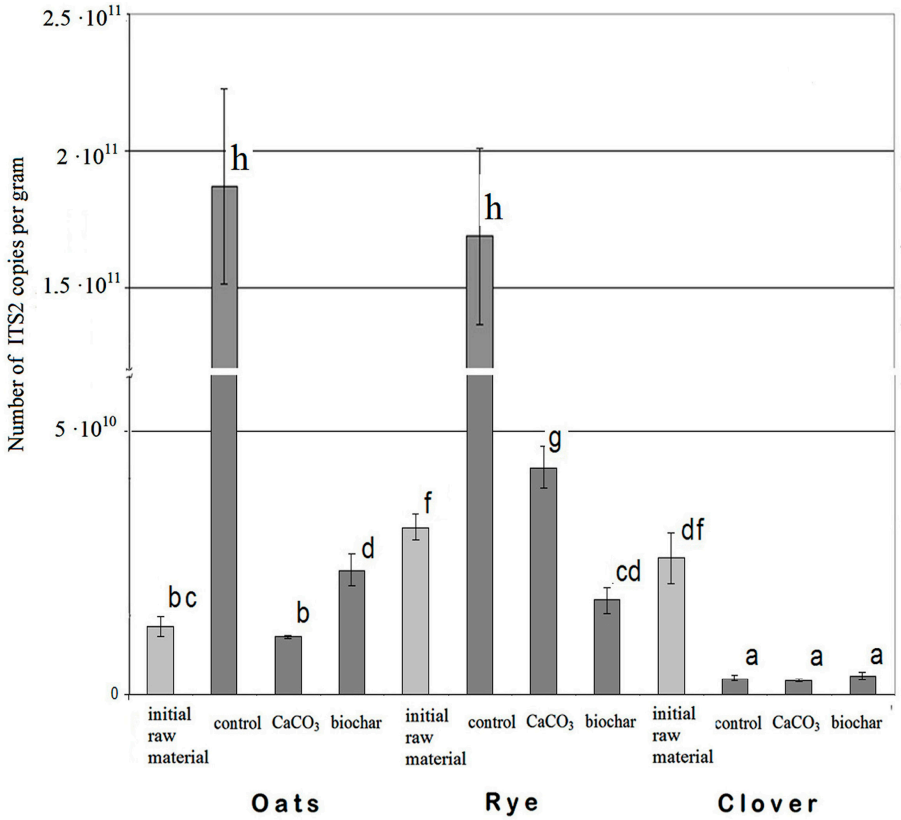

Figure 2. The number of fungi in the composts in terms of the number of the ITS2 rRNA fragment copies of fungi. Vertical lines indicate the standard deviation. Different letters indicate the average values that differ significantly at $p < 0.05$.

The addition of calcium carbonate and biochar had similar effects on the number of microorganisms in the composts. In oat composts, reagents reduced the number of bacteria and fungi, in rye composts, they did not change the number of bacteria, but reduced the number of fungi; and in clover compost, the additives increased the number of bacteria but did not affect the number of fungi.

So the effect of biochar and calcium carbonate on the number of bacteria and fungi was ambiguous and depended on the type of composted material.

4. Dominant and Frequently Encountered Bacteria

4.1. Phylum Level Analysis

In the initial plant material, representatives of the *Proteobacteria* phylum predominated (60–84%). The *Actinobacteriota* phylum (10–27%) and, especially on cereals, *Firmicutes* phylum (10–24%) were also abundantly present (Figure 3).

Representatives of the bacteria phylum *Actinobacteriota* (18–23%), *Firmicutes* (27–38%) and *Proteobacteria* (27–47%) also occupied dominant positions in all the studied composts.

Bacteria phylum *Bacteroidota* (2–9%), *Myxococcota* (3–6%) (in rye composts) and *Planctomycetota* (5–10%) (in rye and oat composts) were present in smaller numbers. There were many hydrolytic and copiotrophic organisms among the representatives of the dominant phyla. This type of bacterial community composition is characteristic of substrates rich in organic matter.

A comparable composition of the dominant phyla (*Firmicutes*, *Actinobacteriota* and *Proteobacteria*) was noted in the composts of rice straw and bird droppings [19,28]. *Firmicutes*, *Actinobacteriota*, *Proteobacteria* and *Bacteroidota* were the dominant phyla in pig-manure-based compost [22,23], and *Firmicutes* and *Proteobacteria* [21] were the dominant phyla in sheep-manure- and tree-branch-based composts.

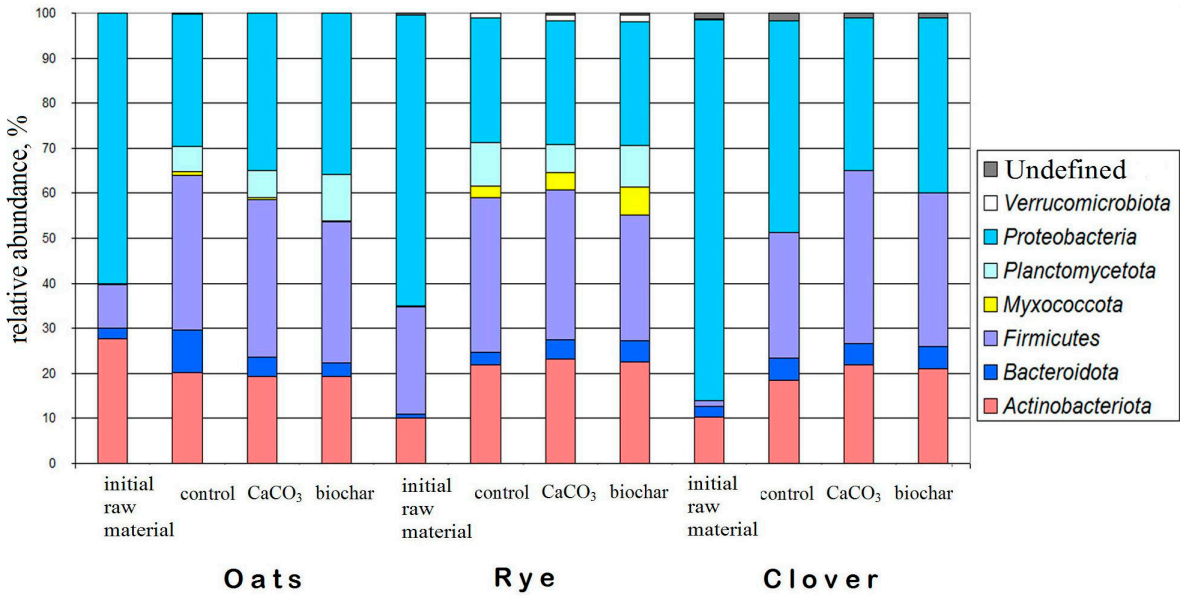

Figure 3. Taxonomic composition of prokaryotes in composts. Only phylum for which the abundance exceeds 1% in one or more variants are given.

The *Planctomycetota* phylum, which stood out somewhat from the general series, was found in significant quantities in the rye and oat composts. The phylum *Planctomycetota* unites mainly oligotrophic bacteria that prefer poor nutrient media; however, bacteria of this phylum can develop also in organic-matter-rich substrates [50].

The appearance of *Planctomycetota* in composts is possibly associated with their ability to decompose chitin [51]. It is likely that the abundant development of fungi in the rye and oat composts was accompanied by fungal hyphae lysis, and as a result, their cell walls become a substrate for chitinolytic microorganisms.

The addition of calcium carbonate or biochar to the composts led to a change in the ratio of bacterial phyla and this change differed depending on the type of composted material. A decrease in the abundance of *Bacteroidota* and an increase in the abundance of *Proteobacteria* and *Planctomycetota* in compost made from oat straw were observed in composts with added biochar (Figure 3). In the case of rye-based composts, the addition of calcium carbonate and biochar increased the abundance of *Bacteroidota* and *Myxococcota* but decreased the abundance of *Planctomycetota* in compost with calcium carbonate and *Firmicutes* in compost with biochar. In clover-based composts, both additives caused a slight increase in the abundance of *Actinobacteriota* and *Firmicutes* and a decrease in the abundance of *Proteobacteria*. These changes are probably due to the different decomposition rates of organic material in composts without and with additives.

4.2. Genus-Level Analysis

Bacteria of genus *Paucibacter* were often found in the initial plant material of all species: oats (8%), rye (44%) and clover (64%). Unclassified actinobacteria of familia *Microbacteriaceae* were also present (4–23%). Unclassified bacteria of the order *Enterobacterales* (23%) and bacteria of the genera *Bacillus* (5%) and *Pseudomonas* (9%), in the case of oats, bacteria of the genera *Bacillus* (7%) and *Brevundimonas* (5%), in the case of rye, and bacteria of the genera *Sphingomonas* (8%) and *Methylobacterium* (6%), in the case of clover, were also present in abundance. Comparison of the bacteria genera composition of the source material (all identified genera were taken into account) using the Sorensen indicator revealed a relatively high similarity between bacterial communities of the different plant residues (67–77%) (Table 3).

Table 3. Sorensen similarity indicator of bacteria communities in different composts.

		Oats				Rye				Clover		
		K	**Ca**	**BC**	**Raw**	**K**	**Ca**	**BC**	**Raw**	**K**	**Ca**	**BC**
Oats	Raw	67	65	66	77	56	54	53	67	65	64	49
	K		28	29	41	30	38	32	40	35	36	43
	Ca			30	42	32	40	33	41	38	36	44
	BC				40	30	38	32	40	35	36	44
Rye	Raw					56	56	55	71	68	68	89
	K						79	79	48	45	40	59
	Ca							39	48	44	43	49
	BC								48	41	38	54
Clover	Raw									57	57	77
	K										83	86
	Ca											87

Note: Raw—Initial NoNote: Raw—Initial material, K—control, Ca—$CaCO_3$, BC—biochar.

In oat composts, the greatest abundance was noted for representatives of the genera *Bacillus* (18–20%), *Paludisphaera* (3–4%), *Azospirillum* (1–6%), *Brevundimonas* (2–4%), *Devosia* (2–5%) or *Mycobacterium* (2–3%), as well as for unclassified bacteria of the familia *Rhizobiaceae* (5–6%). In rye composts, representatives of the genera *Bacillus* (2–8%), *Paenibacillus* (12–20%), *Paludisphaera* (2%), *Devosia* (2–5%) and *Mycrobacterium* (2–4%) and unclassified bacteria of the familia *Rhizobiaceae* (2–4%) showed the greatest abundance. Thus, representatives of *Bacillus* and *Paenibacillus* predominated in cereal composts. These bacteria are characteristic of compost microbial communities and are used to create microbial preparations that accelerate the composting organic waste process [52,53].

In clover composts, the greatest abundance was observed for representatives of the genera *Brevundimonas* (13–16%), *Georgenia* (3–5%) and *Bacillus* (3–8%) and unclassified bacteria of the familia *Microbacteriaceae* (6–8%), *Rhizobiaceae* (2–4%) and *Bacillaceae* (6–10%). The genus *Brevundimonas*, which dominated in the composts with clover, is better known as a participant in the biocomposting process of raw materials of animal origin [53,54]. The relatively high similarity of the bacteria genera composition in the initial material and the control variants (56–67%) is shown by the Sorensen indicator results (Table 3). Evidently, the microbiota of the initial plant material participated in the formation of a community of microorganisms in the resulting composts in our experiments. The composts from fresh aboveground biomass (rye and clover) showed slightly greater similarity in genera composition with each other (38–59%) than with the composts based on oat straw (32–44%) The addition of calcium carbonate and biochar affected the bacteria genera composition in composts with oat straw significantly (28–29% similarity), but hardly changed it in composts with rye and clover (79–86%). Analysis of the dominant and frequent bacteria genera showed that the presence of calcium carbonate or biochar did not change their frequencies.

5. Fungal Community of Composts

5.1. Division and Class Level Analysis

The fungal community of all studied composts consisted of three identified divisional representatives: *Mucoromycota*, *Basidiomycota* and *Ascomycota* (Figure 4). The representatives of *Ascomycota* (49–95%) and among them, representatives of the *Dothideomycetes* class (64–72%), were dominant in all initial plant residues. The *Ascomycota* division was represented primarily by *Sordariomycetes* fungi (48–95%) according to the analysis of microbiota at the class level. *Eurotiomycetes*-class fungi were also abundant in the oat straw composts (7–21%).

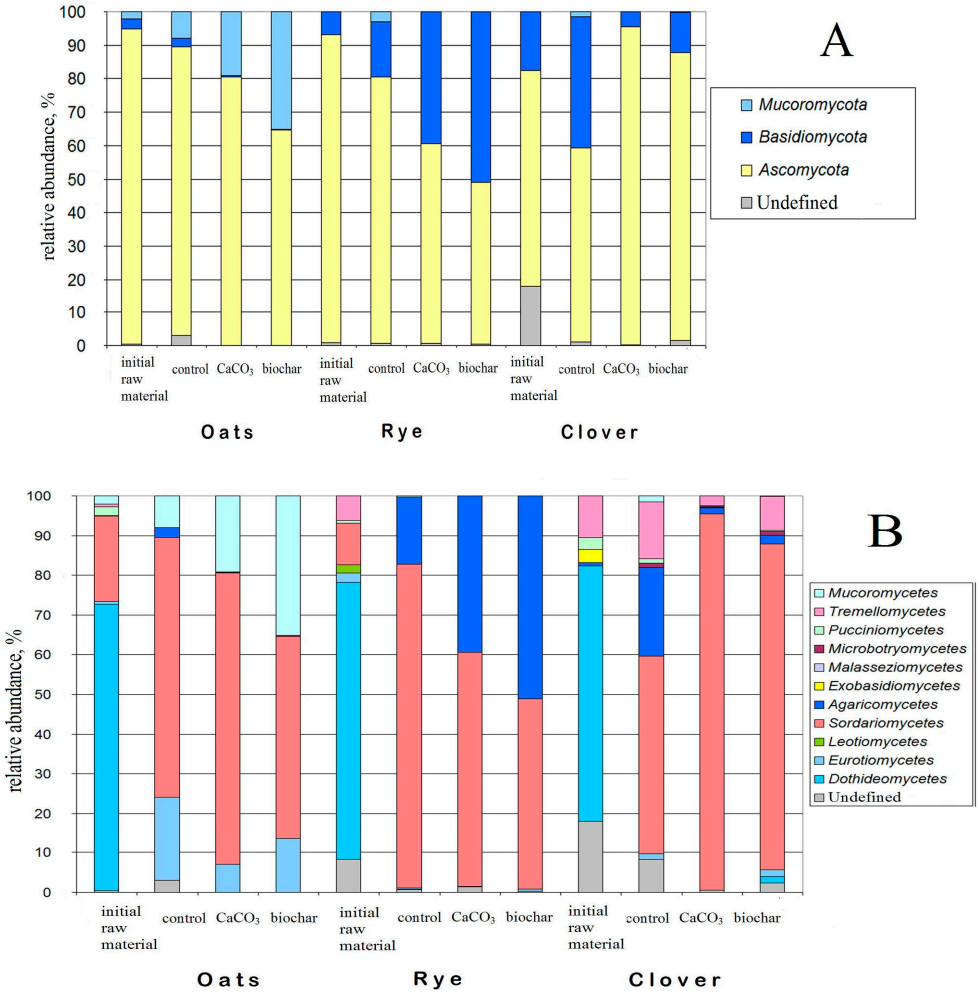

Figure 4. The taxonomic composition of fungi in composts ((**A**)—divisions, (**B**)—classes). The classes whose abundance exceeds 1% in at least one of the variants are given.

The addition of calcium carbonate and biochar led to significant changes in the structure of the fungal communities of the composts. In composts with clover, the abundance of *Ascomycota* increased against the background of a decrease in the abundance of *Basidiomycota*. Conversely, in cereal composts, the addition of reagents reduced the abundance of *Ascomycota* due to an increase in the number of *Mucoromycota* (in oat straw composts) or *Basidiomycota* (in rye composts). However, *Ascomycota* retained their dominant positions in all the studied composts. These changes could be associated with different decomposition rates of organic matter in composts with and without additives. A similar result was obtained when biochar was added to pig-manure- and mulberry-branch-based composts: its addition caused a change in fungal community structure and accelerated compost maturation [17].

5.2. Genus- and Species-Level Analysis

Ascomycota of the genera Pyrenophora (20%), Alternaria (14%), Parastagonospora (28%), Colletotrichum (14%) and Sarocladium (3%) were abundant on oat straw. Ascomycota of the genera Phaeosphaeria (40%), Monographella (4%), Parastagonospora (3%) and Aspergillus fumisynnematus (3%) were dominant on aboveground rye biomass. Fungi of the families Didymellaceae (58%) and Pucciniaceae (3%), the genera Cladosporium (6%), Entyloma (3%) relating to Ascomycota and also Vishniacozyma (8%) relating to Basidiomycota were dominant on clover.

In oat straw composts without the addition of reagents (control) *Aspergillus versicolor* (17%), *Microascus* sp. (12%), *Sarocladium kiliense* (12%), *Penicillium* (4%) and unidentified fungi of the *Nectriaceae* family (40%) were the most abundant. The most represented *Ascomycota* was *Sarocladium kiliense* in the variants with $CaCO_3$ and biochar—its abundance increased markedly compared to the control to 69% and 50%, respectively. Of the *Mucoromycota*, the greatest abundance was noted for *Rhizopus arrhizus*—8% in the control, 16% in the variant with $CaCO_3$ and 31% in the variant with biochar. *Basidiomycota* were represented only sparsely in oat straw composts; nevertheless, the addition of $CaCO_3$ and biochar changed their community structure significantly.

In the rye-based composts, of the *Ascomycota* present, *Zopfiella* sp. (49%), *Remersonia* (9%) and *Mycothermus thermophilus* (18%) were most abundant, while in variants with $CaCO_3$ and biochar, the latter species prevailed (35 and 26% abundance, respectively). The abundance of the genus *Podospora* (19%) in the composts with $CaCO_3$ and of *Zopfiella* sp. (17%) in the composts with biochar were also high. *Basidiomycota* developed likewise very actively in these composts. The most frequent *Basidiomycota* was the agaric fungus of the genus *Coprinopsis*. Its abundance was 17% in the control, 39% in the variant with $CaCO_3$ and 51% in the variant with biochar. At the same time, the proportion of *Mucoromycota* in the rye composts was low. In general, the application of $CaCO_3$ and biochar significantly affected the abundance of the dominant fungi in rye composts and oat composts.

In clover composts, there were also changes in the fungal community structure under the influence of reagents. In these composts, of the *Ascomycota* fungi present, the genera *Plectosphaerella* (12–24%) and *Acaulium* were the most abundant, and the latter was more abundant in variants with $CaCO_3$ and biochar (76% and 62%, respectively) than in the control (9%). There were fewer *Basidiomycota* in these composts than in rye composts but their diversity was slightly higher. In particular, the genus *Coprinopsis* was also found here; however, in composts without additives, its abundance was 18%, while in variants with $CaCO_3$ and biochar, its abundance was less than one percent. The abundance of *Basidiomycota* of the genus *Vishniacozyma* in the control was 12% and in variants with additives was 2 and 4%, respectively. Thus, the application of $CaCO_3$ and biochar significantly affected the abundance of the dominant fungi in all composts.

The comparison of the initial plant residues mycobiota using the Sorensen coefficient showed significant differences (Table 4). The fungal composition on clover and cereal residues particularly differed (14–24% similarity), while those on oat straw and rye biomass showed slightly higher similarity (42%). Mycobiota of the original plant material participated in the formation of the fungal community of the composts. The composting process changed the fungal taxonomic composition of each studied plant residue (24–43% similarity) especially if they were used in a fresh form.

Table 4. Sorensen similarity coefficients of fungal communities in different composts.

		Oats			**Rye**				**Clover**			
		K	**Ca**	**BC**	**Raw**	**K**	**Ca**	**BC**	**Raw**	**K**	**Ca**	**BC**
Oats	Raw	43	39	38	42	25	14	18	14	40	24	29
	K		69	62	13	67	36	33	9	36	27	24
	Ca			86	19	21	27	25	0	31	20	24
	BC				24	13	25	15	0	30	13	27
Rye	Raw					24	21	20	14	27	22	25
	K						58	69	8	34	19	24
	Ca							70	11	34	15	27
	BC								10	33	21	17
Clover	Raw									29	44	61
	K										61	62
	Ca											57

Note: Raw—Initial material, K—control, Ca—$CaCO_3$, BC—biochar.

The similarity of the fungal generic composition between the control and variants with calcium carbonate and biochar in composts was relatively high (58–69%) for all three plants.

Completing the analysis of how the studied reagents affect compost fungal microflora abundance and taxonomic composition, it should be noted that the microbiota of composts with different plant materials react similarly to the addition of calcium carbonate and biochar. The calculation of the Pearson and Kendall correlation coefficients (Tables S1 and S2) showed that no reliable correlation of microorganism abundance with compost pH and humic substance content was observed. Evidently, the slight increase in alkalinity that occurred when calcium carbonate and biochar were added to the compost had no significant effect on the microorganisms. Conversely, a significant correlation was observed between microorganism abundance and organic matter content and C:N ratio.

A positive correlation between microorganism abundance and organic matter content was noted for the bacterial genera *Bosea, Mesorhizobium, Steroidobacter* and *Paludisphaera*. A negative correlation was noted for the abundance of the bacterial genera *Georgenia, Myroides, Pedobacter, Brevundimonas, Sporosarcina* and *Alcaligenes*, for the generic groupings *Allorhizobium—Neorhizobium—Pararhizobium—Rhizobium* and *Methylobacterium—Methylorubrum*, for the unclassified bacteria of the family *Planococcaceae* and the fungal genera *Plectosphaerella, Acaulium* and *Sampaiozyma*.

A positive correlation between microorganism abundance and C:N ratio was noted for the bacterial genera *Fibrisoma, Terribacillus, Devosia* and *Hyphomicrobium*. A negative correlation was noted for the abundance of the bacterial genera *Flavobacterium, Pedobacter, Myroides, Sphingobacterium, Sporosarcina* and *Alcaligenes* and the fungal genera *Cladosporium, Plectosphaerella, Acaulium, Sampaiozyma* and *Vishniacozyma*. The large number of microorganisms showing a significant negative correlation with these indicators is probably related to the gradual replacement of copiotrophic microorganisms by oligotrophic ones, which is typical for mature composts with a significant content of stabilized humus substances.

It is interesting to note that a number of microorganism genera (bacteria *Pedobacter, Myroides, Sporosarcina, Alcaligenes* and fungi *Plectosphaerella, Acaulium, Sampaiozyma*) show a high negative correlation both with organic matter content and C:N ratio. Since it was the introduction of biochar that affected greatly the organic matter content and C:N ratio (Table 2), it was probably the presence of this reagent that changed the abundance of the listed microorganisms.

The ability to enhance humification in composts has been shown for both calcium carbonate and biochar [16]. Since the enzymes of microorganisms participate in the processes of humification [55], we focused on the genera and species that were more abundant in the variants than in the controls. In oat straw composts, these were *Sarocladium kiliense* and *Rhizopus arrhizus*, in rye composts, *Mycothermus thermophilus* and *Coprinopsis* sp. and clover composts, fungi of the genus *Acaulium*. Evidently, the enzymatic activity of these fungi plays a role in the composting processes, activating and enhancing humification. It is known that *Sarocladium kiliense* is an active producer of cellulases and xylanases [56]. Fungi of the genus *Rhizopus*, particularly *Rhizopus arrhizus*, are producers of phenol oxidases [57–59]. Fungi of the genus *Coprinopsis* have shown the ability to decompose lignin [60,61] and, therefore, are also producers of phenol oxidases. Oxidases able to convert aromatic compounds into dimers and polymers with a higher molecular weight have been described for *Mycothermus* fungus [62]. Considering this, it can be assumed that the increase in humification after the addition of $CaCO_3$ and biochar may be associated with the activation of the development of these fungi.

6. Conclusions

The number and taxonomic composition of fungi and bacteria in mature plant composts with oat straw, aboveground rye and clover as raw materials and biochar and calcium carbonate as modifiers have been studied. Representatives of the bacteria phyla *Proteobacteria*, *Actinobacteriota* and *Firmicutes* and the fungal division *Ascomycota* dominated in all initial plant samples and composts. The initial plant material played a significant role

in the bacterial generic composition in the resulting composts but had little effect on the fungal generic composition. The abundance of bacteria and fungi varied depending on the type of plant material. At the same time, the application of calcium carbonate and biochar had similar effects on the number of microorganisms in composts of the same plant material. The abundances of bacteria and fungi were relatively higher in composts based on the carbon-rich cereals compared to those based on clover. At the generic level, the bacterial community in composts was more affected by the addition of biochar and calcium carbonate than the fungal community. However, no single group of either bacteria or fungi was seen to dominate in the communities that developed in response to biochar or calcium-carbonate treatments in different compost types. At the same time, the structure of the fungal community changed significantly in each compost type with additives compared to the control. Moreover, the introduction of calcium carbonate and biochar led to similar changes in terms of the increase in the abundance of the same fungal groups actively involved in the transformation of organic matter. The addition of both ameliorants increased pH values and humic acid content in the mature composts regardless of the composition of the initial plant material. In general, the results of the conducted research show the positive effect of adding the investigated reagents on the properties of the obtained composts, especially in the case of biochar.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy13102521/s1, Table S1: Correlation between chemical parameters of composts and abundance of bacterial genera (only genera that showed a significant correlation with at least one chemical parameter are given); Table S2: Correlation between chemical parameters of composts and abundance of fungi genera (only genera that showed a significant correlation with at least one chemical parameter are given).

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