Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 14 December 2022 doi:10.20944/preprints202212.0246.v1

Article

The Succession of the Cellulolytic Microbial Community from the Soil during Oat Straw Decomposition

Anastasiia K. Kimeklis [1,2,*,†], Grigory V. Gladkov [1,†], Olga V. Orlova [1], Alexey M. Afonin [1,3], Emma S. Gribchenko [1], Tatiana S. Aksenova [1], Arina A. Kichko [1], Alexander G. Pinaev [1] and Evgeny E. Andronov [1,4,*]

[1] All-Russian Research Institute of Agricultural Microbiology, 196608 Saint Petersburg, Russia; ruginodis@gmail.com (G.V.G.); falenki@hotmail.com (O.V.O.); afoninalexeym@gmail.com (A.M.A.); egribchenko@arriam.ru (E.S.G.); tsaksenova@mail.ru (T.S.A.); 2014arki@gmail.com (A.A.K.); ag.pinaev@gmail.com (A.G.P.)
[2] Department of Applied Ecology, Saint-Petersburg State University, 199034 Saint Petersburg, Russia
[3] A.I. Virtanen Institute for Molecular Sciences, University of Eastern Finland, FI-70211 Kuopio, Finland
[4] Dokuchaev Soil Science Institute, 119017 Moscow, Russia
* Correspondence: kimeklis@gmail.com (A.K.K.); eeandr@gmail.com(E.E.A.)
† These authors contributed equally to this work.

Abstract: The decomposition of straw is a dynamic process, which is reflected in the decomposing microbial consortia, however, this process is poorly understood. Here we performed an experiment on the gradual colonization of oat straw by the soil microbial community. The results showed that soil respiration and bacterial diversity were negatively correlated – while the first decreased, the latter increased. In accordance with the dynamics of diversity and respiration, succession was divided into early, middle, and late decomposition phases. Analysis of 16S rRNA and ITS2 amplicon sequencing data revealed three jointly changing groups of phylotypes, corresponding with distinguished phases of decomposition. The presence of cellulolytic members of Proteobacteria, Bacteroidota, Firmicutes, and Actinobacteriota for bacteria and Ascomycota for fungi had been detected since the early phase, but most of the initial phylotypes were gone by the end of the phase. The second phase marked the functional core with a constant set of other phylotypes from these phyla, persisting in the community. Full metagenome sequencing of the microbial community from the end of the middle phase confirmed that major members of this consortium had GH genes, connected to cellulose and chitin degradation. The appearance of non-cellulolytic members from Bdellovibrio, Myxococcota, Chloroflexi, and Crenarchaeota characterized the last phase of the mature community.

Keywords: oat straw; chernozem; cellulolytic community; succession; glycoside hydrolases; metagenome sequencing; amplicon sequencing

1. Introduction

In agriculture, the production of grain is accompanied by the production of straw, whose yield surpasses the target product [1-3]. There are ways of handling excessive straw quantities, differing in their economic and labor costs. One of the most cost-effective ways of utilizing excessive straw is burning, but it wastes potentially valuable resources and results in severe environmental consequences, including gas emissions and the negative impact of heat on soil fertility [2,4]. Other ways of straw usage include biofuel production [5], the investigation of which is a promising research direction. However, it requires straw transportation, which induces extra costs. So, processing straw at the origin site can be a solution to multiple problems. The reintroduction of straw into the field solves both the problems of transportation costs and nutrient loss. It prevents soil erosion and involves plant residues in the global carbon cycle [6,7]. However, this method has some disadvantages to overcome. Straw provides some easily digestible carbohydrates, pro-

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 14 December 2022 doi:10.20944/preprints202212.0246.v1

teins, lipids, and minerals, but it mostly consists of recalcitrant lignocellulose. Additionally, the introduction of bare straw into the soil shifts the ratio of carbon to nitrogen, which must be compensated for its effective assimilation by microorganisms. Thus, the search for ways of the effective processing of straw is still an acute problem for agriculture.

Soil from different environments can serve as a source of cellulolytic microorganisms. A number of studies focused on the isolation of single strains from various soil types [8-12], but this approach has several flaws. It was reported that cellulolytic bacteria may take up to a fifth part of the total soil community [13]. Additionally, it has been shown that many families of enzymes are simultaneously involved in the decomposition of straw, and different functions are distributed between different members of the microbial community, making it impossible to isolate a single “most important” member [14]. So, the complex task of straw degradation is achieved by the association of microorganisms acting together. Thereby, there is still an ongoing search for cellulolytic microorganisms which would facilitate straw decomposition.

Since straw is a complex raw substrate, its decomposition requires the work of multiple enzyme systems, found in a variety of bacteria and fungi. Cellulose, as the main component of straw, is decomposed by enzymes, most of which are listed in the Carbohydrate-Active EnZymes database (CAZy) [15]. The biggest class of enzymes in CAZy comprises Glycoside hydrolases (GH), which are currently divided into 173 families based on the amino acid sequence similarity [16]. GH class encompasses enzymes, aimed at the glycosidic bond between carbohydrates or a carbohydrate and a non-carbohydrate moiety [17]. Consequently, cellulose decomposition is carried out by multiple, but not all enzyme families across the GH classes. Different families include enzymes, aimed mainly at the β-1,4 links in the polysaccharide chain of the recalcitrant cellulose (β-glucosidases, exo-β-glucanases, and endo-β-glucanases) and hemicellulose molecules (β-xylosidase, β-mannanase; β-mannosidase, β-xylanase, etc.), gradually breaking it into more accessible compounds [18]. The main families, containing these enzymes, are GH1, GH3, GH5, GH6, GH7, GH9, GH10, GH30, GH43, and others [18,19]. In natural habitats, these enzyme systems are distributed among different members of the microbial community [20]. Understanding the principles of formation and functioning of the cellulolytic microbial consortium is essential knowledge for the formulation of highly effective preparations for straw decomposition.

Multiple studies have shown that during the composting of untreated straw with a natural epiphytic microbiome, the microbial community undergoes taxonomic and functional succession [21,22]. The aim of this study was to isolate the cellulolytic community from the soil and study its full succession stages in sterile straw. For this, we chose chernozem, a soil type common in the southern regions of Russia. Cellulolytic capabilities of the chernozem microbiome were reported earlier [23]. As a source of a lignocellulolytic substrate, we chose oat (*Avena*), a widely cultivated forage crop. A model laboratory experiment of colonizing sterile straw by soil microbiota was set up in order to study the succession of the oat straw decomposition community. We analyzed microbial activity by the measurement of soil respiration, taxonomy succession by the sequencing of 16S rRNA gene for prokaryotes and ITS region for fungi on the Illumina Miseq platform, and cellulolytic potential of the resulting community by the search of GH genes in the nontargeted metagenome obtained on the Oxford Nanopore MinION platform.

2. Results

2.1. Microbial activity

During the six months of the experiment, notable decomposition of straw in nylon sachets was observed. Maximum SR values were detected at the beginning of the experiment, and they declined towards the end. According to One-Way ANOVA, carbon dioxide emission rates were separated into three groups with significantly different SR values (p-value ≤ 0,05), from high to low: (a) 3-21 days, (b) 28-35 days, and (c) 42-182 (Figure 1).

The SR experimental values in the first two groups were significantly higher than controls. In the last group, SR experimental values were higher than controls until the 133 days, though not significantly in all measurements except one. According to the dynamics of carbon dioxide emission, three phases of microbial activity were distinguished - (1) early, which lasted for the first month; (2) middle, which lasted until the third month; and (3) late, which lasted until the end of the experiment. In the early phase, activity was the highest and was rapidly decreasing towards the end. In the middle phase activity continues to decrease but at a slower pace. In the late phase stabilization of activity occurs.

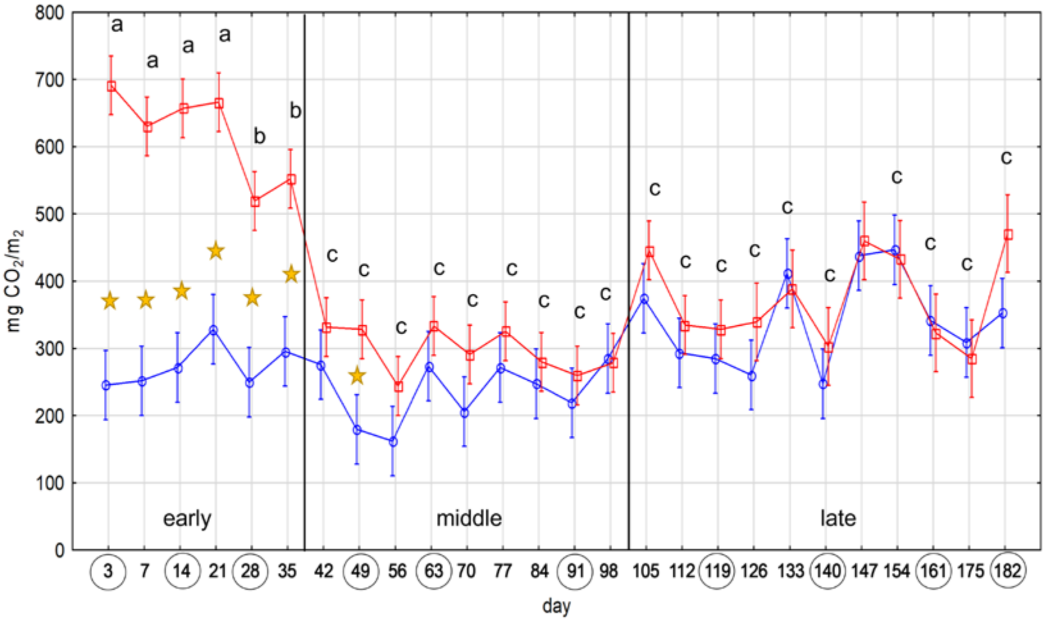


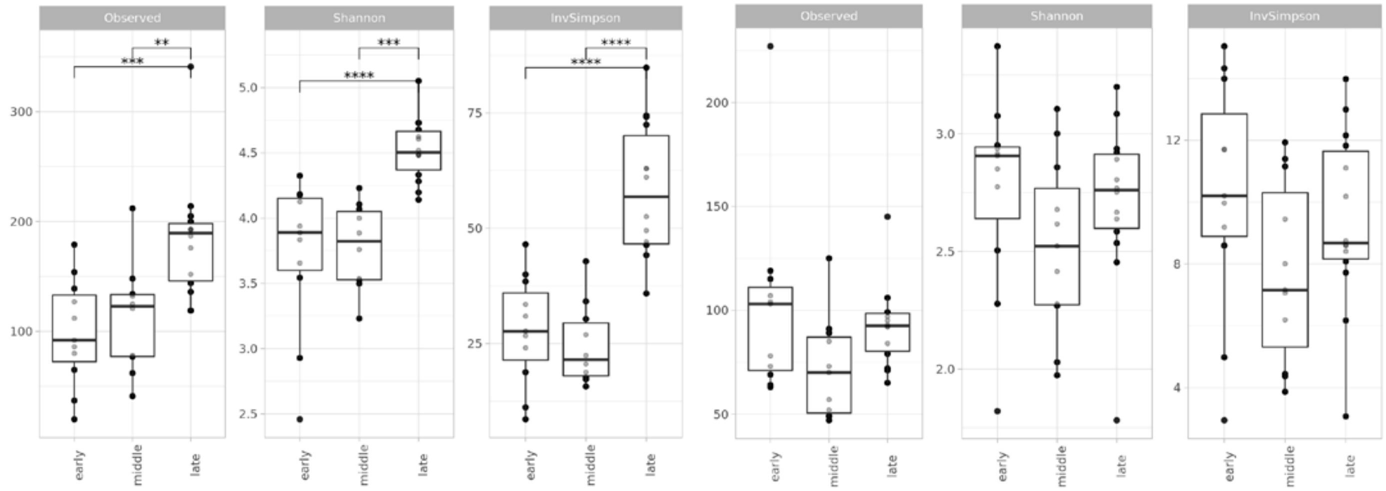
Figure 1. Soil Respiration (SR) data. On the Y axes are SR values in mg CO_2 per m_2, and on the X axis are days of measurement. The blue line corresponds to the SR values of containers with soil without added straw (control). The red line corresponds to SR values of containers with soil with added straw in sachets (experiment). Vertical red and blue bars denote 0.95 confidence intervals. Stars mark significant (p-value ≤ 0.05) differences between the experiment and control values. Groups of experimental SR values without significant (p-value ≤ 0.05) differences are marked with the same letter ('a', 'b', and 'c'). Vertical black bars mark different phases of decomposition: early, middle, and late. Circles around days on the X-axis denote samples chosen for amplicon sequencing.

In accordance with the results of the SR measurement, subsequent taxonomical analysis of the dynamics of microbial colonization of straw was performed on substrates from ten sampling periods, covering the entire experiment and different phases of microbial activity: early (days 3, 14, 28), middle (days 49, 63, 91), and late (days 119, 140, 161, 182).

2.2. Microbial diversity

In total 35 out of 36 libraries of 16S rRNA gene amplicons were left after a quality check. Data from all libraries amounted to 521 643 reads with a median of 12 894, which were attributed to 1 245 phylotypes (Table S1). For the ITS2 fragment amplicons, all 36 libraries passed a quality check. In total 815 134 reads were acquired, with a median of 22 657. Data was attributed to 1 295 phylotypes, but only 43% were assigned to a known kingdom.

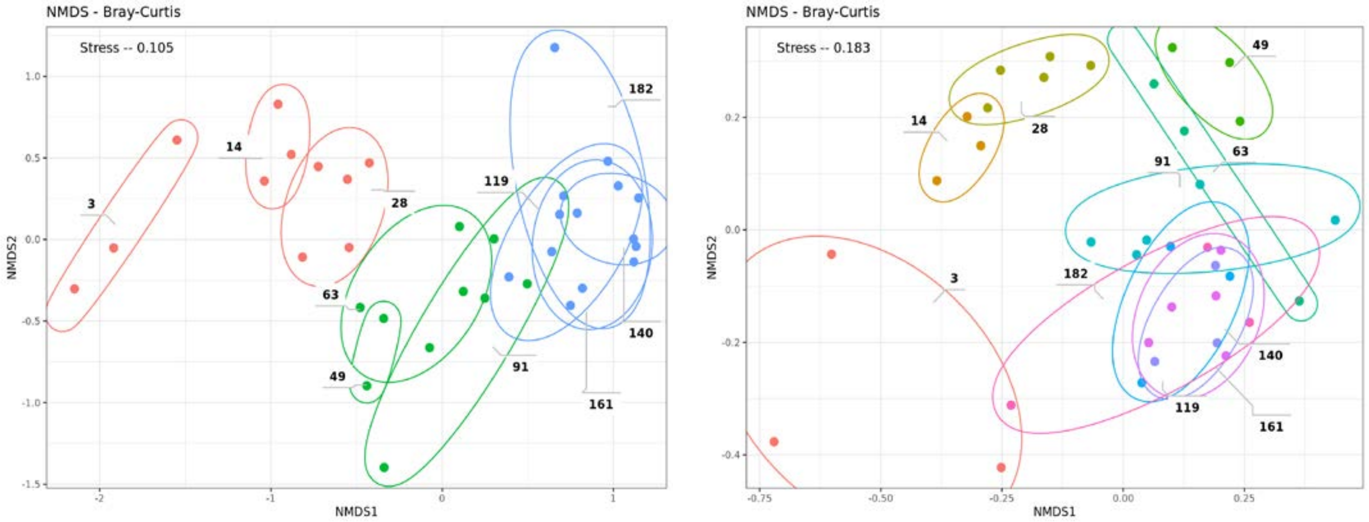
Both bacterial richness and evenness of the straw decomposing community, assessed by three alpha diversity indices (Observed, Shannon, and Inverted Simpson), significantly increased during the experiment (Figure 2a). The lowest values were detected on day 3, which was the earliest time point in the analysis; the highest values were reached on day 119, which marks the beginning of the late phase (Figure S1a). Alpha diversity indices were negatively correlated with SR values, as shown by Pearson's product-moment coefficient (-0.6980158, p-value = 0.02479) (Figure S2). Divided into phases, the alpha diversity indices of samples from the early and middle phases didn't differ significantly from each other but were significantly lower than those from the last phase. At the same time, the measurement of MPD (mean pairwise distances) showed that the early phase was significantly less diverse than the later phases (p-value ≤ 0.001) (Table S2). The early phase was marked by increasing microbial diversity. In the middle phase, the increase slowed down. In the late phase, diversity abruptly reached its maximum values and stayed stable until the end of the experiment.



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

Figure 2. Alpha diversity indices (Observed, Shannon, InvSimpson) for (**a**) prokaryotic, based on 16S rRNA gene amplicon libraries, and (**b**) fungal data, based on ITS2 fragment amplicon libraries; divided on the X-axis into three phases: early, middle, and late. Significant differences were assessed by ANOVA: (**) p-value ≤ 0.01; (***) p-value ≤ 0.001; (****) p-value ≤ 0.0001. .

Beta diversity, which marks differences between different stages of straw colonization, coincided with alpha diversity. According to PERMANOVA, the dispersion between microbial communities of different phases was more than inside phases (F = 8.2033, p-value ≤ 0.001). NMDS plot showed notable dynamics of decomposing prokaryotic microbiota, more pronounced in the early phase than in the latter (Figure 3a). Stepwise comparison of beta diversity between the first sample with the others showed an acceleration of dynamics in the early phase, then a slowdown in the middle with an abrupt increase before the last phase (Figure S3).



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

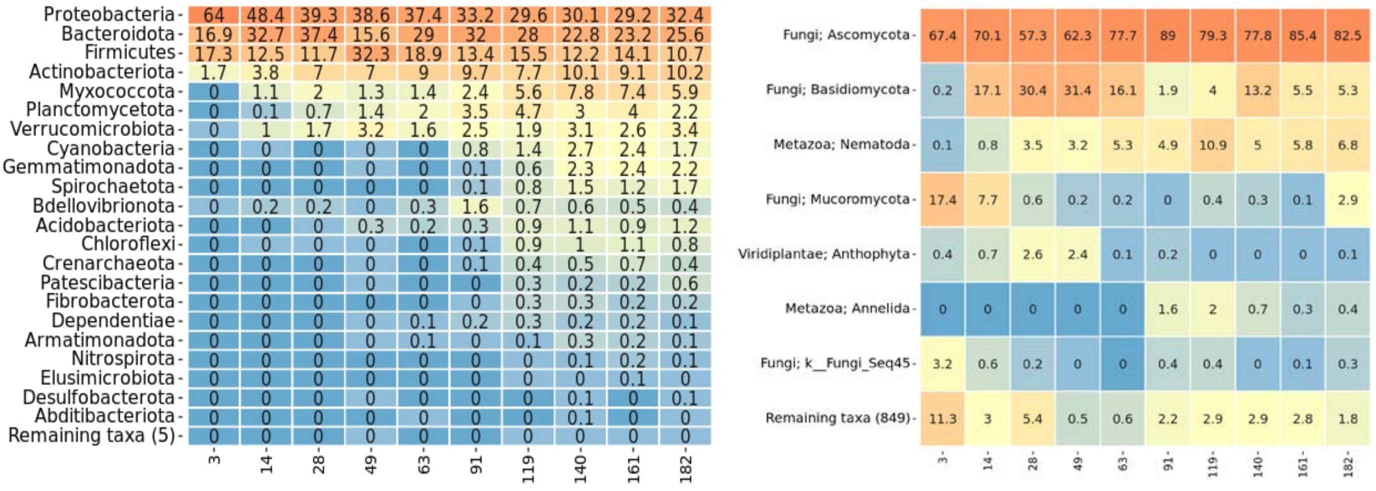
Figure 3. NMDS plot of the beta-diversity of the microbial straw decomposing community from ten sampling points accessed with Bray–Curtis, based on (**a**) prokaryotic and (**b**) fungal amplicon sequencing data. Replicates of each sampling day are surrounded by ellipses. .

For the fungal part of the straw-decomposing community, no such tendencies were revealed as for the bacterial part. The evenness and richness of the fungi, according to the same alpha diversity indices, didn't differ significantly between samples and no phases could be distinguished (Figure 2b). A similar observation can be made of the beta diversity plot (Figure 3b). NMDS shows shifts in diversity between samples, but it was not unidirectional, as for bacteria.

Thus, according to alpha and beta metrics, the straw-decomposing bacterial community accumulated diversity during the early and middle phases and reached its peak by the fourth month of the experiment, when it could be considered a mature microbial consortium. The fungal part of the community didn't show clear dynamics during its succession.

2.3. Taxonomy overview

During prokaryotic succession, the number of represented phyla in the community increased. The first colonizers on the third day were attributed only to four phyla: Proteobacteria, Bacteroidota, Firmicutes, and Actinobacteriota (Figure 4a). On the 14th day Verrucomicrobiota, Myxococcota, Planctomycetota, and Bdellovibrionota appeared. Acidobacteriota appeared on the 49th day. Chloroflexi, Cyanobacteria, Gemmatinomonadota, Spirochaeota, and Crenarchaeota appeared on the 91st day. Since the 119 days, the maximum presence of bacterial phyla was registered, including Armatimonadota, Dependentiae, Fibrobacteriota, Nitrospirota, and Patescibacteria. The most frequent genera among bacterial phylotypes were *Chitinophaga*, *Ohtaekwangia*, *Bacillus*, *Rhizobium*, *Pseudomonas*, and *Inquilinus* (Figure S4a).



	3	14	28	49	63	91	119	140	161	182
Proteobacteria	64	48.4	39.3	38.6	37.4	33.2	29.6	30.1	29.2	32.4
Bacteroidota	16.9	32.7	37.4	15.6	29	32	28	22.8	23.2	25.6
Firmicutes	17.3	12.5	11.7	32.3	18.9	13.4	15.5	12.2	14.1	10.7
Actinobacteriota	1.7	3.8	7	7	9	9.7	7.7	10.1	9.1	10.2
Myxococcota	0	1.1	2	1.3	1.4	2.4	5.6	7.8	7.4	5.9
Planctomycetota	0	0.1	0.7	1.4	2	3.5	4.7	3	4	2.2
Verrucomicrobiota	0	1	1.7	3.2	1.6	2.5	1.9	3.1	2.6	3.4
Cyanobacteria	0	0	0	0	0	0.8	1.4	2.7	2.4	1.7
Gemmatimonadota	0	0	0	0	0	0.1	0.6	2.3	2.4	2.2
Spirochaetota	0	0	0	0	0	0.1	0.8	1.5	1.2	1.7
Bdellovibrionota	0	0.2	0.2	0	0.3	1.6	0.7	0.6	0.5	0.4
Acidobacteriota	0	0	0	0.3	0.2	0.3	0.9	1.1	0.9	1.2
Chloroflexi	0	0	0	0	0	0.1	0.9	1	1.1	0.8
Crenarchaeota	0	0	0	0	0	0.1	0.4	0.5	0.7	0.4
Patescibacteria	0	0	0	0	0	0	0.3	0.2	0.2	0.6
Fibrobacterota	0	0	0	0	0	0	0.3	0.3	0.2	0.2
Dependentiae	0	0	0	0	0.1	0.2	0.3	0.2	0.2	0.1
Armatimonadota	0	0	0	0	0.1	0	0.1	0.3	0.2	0.1
Nitrospirota	0	0	0	0	0	0	0	0.1	0.2	0.1
Elusimicrobiota	0	0	0	0	0	0	0	0	0.1	0
Desulfobacterota	0	0	0	0	0	0	0	0.1	0	0.1
Abditibacteriota	0	0	0	0	0	0	0	0.1	0	0
Remaining taxa (5)	0	0	0	0	0	0	0	0	0	0

	3	14	28	49	63	91	119	140	161	182
Fungi; Ascomycota	67.4	70.1	57.3	62.3	77.7	89	79.3	77.8	85.4	82.5
Fungi; Basidiomycota	0.2	17.1	30.4	31.4	16.1	1.9	4	13.2	5.5	5.3
Metazoa; Nematoda	0.1	0.8	3.5	3.2	5.3	4.9	10.9	5	5.8	6.8
Fungi; Mucoromycota	17.4	7.7	0.6	0.2	0.2	0	0.4	0.3	0.1	2.9
Viridiplantae; Anthophyta	0.4	0.7	2.6	2.4	0.1	0.2	0	0	0	0.1
Metazoa; Annelida	0	0	0	0	0	1.6	2	0.7	0.3	0.4
Fungi; k__Fungi_Seq45	3.2	0.6	0.2	0	0	0.4	0.4	0	0.1	0.3
Remaining taxa (849)	11.3	3	5.4	0.5	0.6	2.2	2.9	2.9	2.8	1.8

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

Figure 4. Heatmap of the most abundant phyla in the microbial straw decomposing community from ten sampling points, based on (a) prokaryotic and (b) fungal amplicon sequencing data. The relative abundance is given in % of the read count of each sampling day, orange for maximal, and blue for minimal values. .

Fungal diversity was presented by three phyla during the whole sampling period (Figure 4b). A major part of fungi phylotypes belonged to Ascomycota. Apart from it, there was a presence of Basidiomycota and Mucoromycota representatives on different sampling days. The most frequent fungi phylotypes were attributed to the genus and species level, including *Chloridium aseptatum*, *Coniochaeta verticillata*, *Coniochaeta canina*, *Schizothecium inaequale*, *Chloridium*, *Coniochaeta*, *Schizothecium*, *Humicola sardiniae*, *Albifimbria verrucaria*, *Conocybe zeylanica*, and *Gibberella intricans* (Figure S4b).

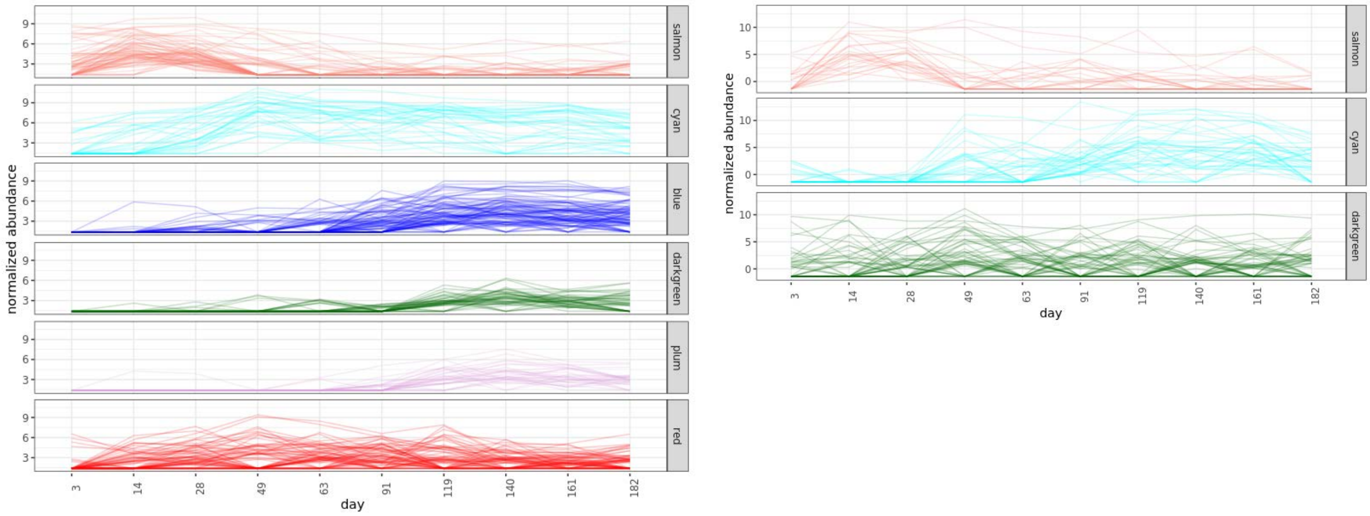
2.4. Community succession

2.4.1. Data filtering

The peculiarity of the experiment design was that we followed the dynamics of the development of the decomposing community in ten physically distant compartments - sachets with straw. In order to identify general patterns in the microbiome development and remove random individual outliers of sachets we left in the analysis only phylotypes with the following characteristic: the presence of at least 10 reads in more than 10% of samples. After this filtering, only 338 out of 1425 bacterial phylotypes were left with an additional 107 “major outliers” (Table S3). For fungi, 116 out of 1295 phylotypes were left in the analysis (Table S4).

Among bacterial representatives in the individual sachets, some unique phylotypes with high read counts were allocated into the “major outliers” group. Dispersion of these phylotypes between days showed that most of them stood not only as outliers of individual sachets but also as technical replicates within one sachet (Figure S5). Among those were representatives of *Pseudomonas*, *Sphingomonas*, *Fructilactobacillus*, *Levilactobacillus*, *Lactiplantibacillus*, and *Escherichia* (Figure S6).

The filtered set of universally represented phylotypes was used to access the microbial succession during the phases of straw colonization. Since the diversity of microorganisms increased, it was incorrect to apply pairwise sample comparison methods or compositional data analysis methods to this dataset. Therefore, the WGCNA method after variance stabilizing transformation (DESeq2) was used to formalize the association of bacteria into groups characteristic of different colonization phases. Analysis separated phylotypes into six clusters, which could be distinguished into four groups of distinct patterns (Figure 5a). Five groups coincided with the earlier established division of the experiment into the three phases of microbial activity.



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

Figure 5. Clusters of jointly changing groups of phylotypes for (a) prokaryotic and (b) fungal amplicon sequencing data, as assessed by WGCNA. (a) Early phase – salmon cluster, middle phase – cyan clyster, late phase – blue, green, and plum clusters, universal – red cluster. (b) Early phase – salmon cluster, late phase – cyan clyster, universal – green cluster. .

2.4.2. Bacterial phases

The first so-called "early" group represented phylotypes, appearing and reaching their maximum in the first month of incubation, disappearing almost completely in later stages. In WGCNA it corresponds with the salmon cluster, consisting of 73 phylotypes (Figure S7a). The most abundant phylotypes in this group, which were not necessarily unique in taxonomy for the whole dataset, belonged to Bacteroidota (*Chitinophaga*, *Dyadobacter*, and *Flavobacterium*) and Proteobacteria (*Cupriavidus*, *Achromobacter*, *Rhizobium*, *Pseudomonas*, and *Lysobacter*). Some of the above and a few more phylotypes from this group were attributed to unique taxa, detected only in this phase, including representatives of Actinobacteriota (*Cellulosimicrobium*, *Glycomyces*, and *Microbacterium*), Bacteroidota (*Chryseobacterium* and *Flavobacterium*), Proteobacteria (*Achromobacter*, *Neorhizobium*, *Cupriavidus*, *Lysobacter*, *Massilia*, *Ensifer*, *Microvirga*, *Pseudoduganella*, *Stenotrophomonas*, and *Xylophilus*).

The second "middle" phase group represented phylotypes, which reached their maximum by the second month of incubation and persisted in the community onwards. By WGCNA these 75 phylotypes were assigned to the cyan cluster (Figure S7b). The most prominent representatives belonged to Bacteroidota (*Chitinophaga*, *Ohtaekwangia*), Firmicutes (*Bacillus*, *Solibacillus*, *Planococcaceae*, and *Terribacillus*), Proteobacteria (*Inquilinus*, *Rhizobium*, *Bradyrhizobium*, *Luteibacter*, *Starkeya*, and *Luteimonas*), and Planctomycetota (*Singulisphaera*).

The third, most diverse, group represented phylotypes, appearing at the late phase, after three months of incubation. These included three clusters: blue (86), green (44), and plum (30) (Figures S7cde). Here and later the number in brackets stands for the number of phylotypes observed in a group. In this cluster, major representatives also belonged to Bacteroidota (*Ohtaekwangia* and Microscillaceae). Many representatives of Acidobacteriota (4), Actinobacteriota (*Conexibacter*, *Galbitalea*, *Dactylosporangium*, *Iamia*, and *Solirubrobacter*), Verrucomicrobiota (9), Myxococcota (9), Cyanobacteria (3), Chloroflexi (6), Bdellovibrionota (4), Spirochaeota (3), Planctomycetota (12), Crenarchaeota (4), Gemmatimonadota (1) and others appeared at this stage.

The last group, corresponding to the red cluster, contained 75 phylotypes, consistently or without apparent patterns appearing in all samples (Figure S7f). Its most universally abundant phylotypes were attributed to *Paenibacillus*, *Starkeya*, *Pseudoflavitalea*, *Niastella*, and *Lysinibacillus*. Sporadic appearance of phylotypes from Bacteroididota (*Ohtaekwangia*, *Chitinophaga*), Actinobacteroidota (*Conexibacter* and *Actinocorallia*), Verrucomicrobiota (*Terrimicrobium*) and others was noted.

To conclude, representatives of Bacteroidota (*Chitinophaga*, *Ohtaekwangia*) were persistent in all phases of bacterial succession, but each phase had its own phylotypes, attributed to these genera. The early phase was characteristic of Gammaproteobacteria representatives, which disappeared later from the community. The middle phase was specific to a wide variety of Firmicutes and Alphaproteobacteria, appearing and persisting in the community. The last phase marked the burst of bacterial diversity from different phyla.

2.4.3. Fungal phases

The WGCNA analysis managed to separate fungi phylotypes into three clusters, one correlating with the early phase of microbial activity (salmon), one corresponding with the middle phase (cyan), and one dispersed across all days (green) (Figure 5b). Coinciding with alpha and beta diversity analyses, many fungal phylotypes were detected at all phases of the experiment, with only some species demonstrating differences according to the day of sampling (Figures S8abc). *Coprinellus flocculosus* and *Schizothecium inaequale* were characteristic of the fungal community during the early phase, while *Chloridium aseptatum*, *Colletotrichum sidae*, and *Scytalidium* were appearing mainly after the second month of the experiment. Phylotypes belonging to Ascomycota (*Metarhizum*, *Albifimbria*, *Chaetomium*, *Coniochaeta*, *Staphylotrichum*, *Gibberella humicola*), Basidiomycota (*Conocybe*, *Occultifur*, *Waitea*), and Mucoromycota (*Actinomucor*) were periodically encountered in the dataset.

doi:10.20944/preprints202212.0246.v1

2.5. Glycoside hydrolases content

Three-month sample, the borderline between the middle and late phases of straw decomposing microbial community succession, was chosen for the search of the GH genes. The resulting yield of full metagenome sequencing of DNA from the 91-day sample representing this phase was 10.9 Mbp, with N50 of 4886. The metagenome was polished and annotated, and only genes annotated as belonging to the CAZy database were investigated further.

According to the CAZy database, the metagenome of the decomposing microbial community contained 1388 GH genes, 1194 of which belonged to Bacteria and 193 to Fungi (Table S5). As assigned by EggNogg, the most abundant CAZy genes were attributed to Proteobacteria (Xanthomonadales, Sphingomonadales, Bradyrhizobiaceae, Rhizobiaceae) (455 genes), Bacteroidetes (Sphingobacteriales, Cytophagales) (339 genes), Actinobacteriota (Streptosporangiales) (156 genes) and Firmicutes (60 genes) phyla for bacteria and Ascomycota (Sordariomycetes) (191 genes) phylum for fungi. So, out of the four most major phyla in the bacterial part of the decomposing microbial community, detected by Illumina sequencing, all were also represented by the highest quantities of GH genes. However, according to 16S rRNA gene sequencing data, the relative abundance of Firmicutes was always higher than Actinobacteriota in all analyzed days of the experiment, while the relative content of GH genes for these phyla was reversed.

According to the CAZy classification, the most represented GH families in the metagenome of the three-month-old straw decomposing community were attributed to GH3 (227), GH31 (117), GH18 (114), and GH20 (91). Analysis of the main functions of the presented GH families allowed distinguishing three major groups in the metagenome: connected to cellulose degradation (“cellulose” group), connected to metabolism of simple carbohydrates (“carbohydrate” group), and connected to chitin degradation (“chitin” group) (Table 1). The main representatives of the “cellulose” group in this dataset belonged to GH3, GH5, GH9, GH30, GH43, and GH94 families. Families from the “carbohydrates” group included GH31, GH95, GH15, and GH77. A notable presence was detected in the families from the “chitin” group, including GH18, GH19, and GH20. All these GH families from all three groups were found in almost all phyla, detected by 16S rRNA and ITS2 amplicon sequencing, and their relative abundance coincided with the taxonomy data. Proteobacteria had all groups present, and the “cellulose” group was the most abundant, followed by the “carbohydrate” and then the “chitin”. For Bacteroidota, Actinobacteriota, Firmicutes, Acidobacteriota, and Planctomycetota phyla “cellulose” and “carbohydrate” groups were equally represented, while the “chitin” group was less present than the other two. The “cellulose” and “carbohydrate” groups were also detected in minor quantities in Verrucomicrobiota, Cyanobacteria, and Chloroflexi. As for the fungal part of the decomposing community, in Ascomycota “the chitin” group of GHs had more matches than the “cellulose” and “carbohydrate” groups. For Basidiomycota only one gene was found, attributed to the “chitin” group.

To conclude, according to the search of GH genes in the mature straw decomposing microbial consortia, functionally they were represented by GH, involved in cellulose, simple carbohydrates, and chitin utilization. The main carriers of these genes coincided with bacterial and fungal phyla, appearing in the community from the first days of straw colonization.

Table 1. The distribution of the main GH families found in the metagenome of the cellulolytic community between phyla. Three groups of GH families were distinguished: “cellulose” (GH3, GH5, GH9, GH43, GH94, GH30, etc.), “carbohydrates” (GH31, GH95, GH15, GH77, GH38, GH32, etc.), and “chitin” (GH18, GH19, GH20).

Kingdom	Phylum	“cellulose” group	“carbohydrates” group	“chitin” group

	Euryarchaeota	0	1	0
Bacteria	Proteobacteria	239	113	67
	Bacteroidota	126	123	67
	Actinobacteriota	60	52	34
	NA	46	27	14
	Firmicutes	19	22	3
	Acidobacteriota	14	13	4
	Planctomycetota	8	11	1
	Verrucomicrobiota	1	8	0
	Cyanobacteria	3	3	0
	Chloroflexi	3	2	0
Fungi	Ascomycota	55	56	68
	Basidiomycota	0	0	1
	NA	0	1	0
	Total	574	432	259

3. Discussion

Soil is a complex substrate containing nutrients in a variety of forms: from easy-to-digest to recalcitrant. Moreover, this environment is under constant biotic and abiotic stress. All this forms a complex soil microbiota, consisting of a plethora of microorganisms, adapted to various nutritional and climatic conditions. Straw introduction into the soil creates a surplus of nutrients, specifically carbon compounds, which facilitates a new path in the microbiota succession [24]. Earlier we demonstrated that chernozem is a potential source of cellulolytic microorganisms by both traditional microbiology and molecular methods [25,26]. Here we attempted to analyze the process of colonization of the lignocellulosic substrate by the chernozem microbiota and identify its most prominent phases.

Due to the design of our experiment, the only measured agrophysical parameter was soil respiration (SR), which is defined as the process of carbon dioxide released by microorganisms. The application of this method has shown its effectiveness in assessing microbial activity in response to anthropogenic agricultural practices [27]. Maximum values of SR were detected on the first measurement on the third day of the experiment, after which a significant decline in SR values, specifically after the second month, was detected. Previously the effect of elevated values of SR during cellulose decomposition was associated with the introduction of additional glucose to the substrate [28]. Thus, our results could be explained by the particular substrate of lignocellulose we used: oat straw has a high content of water-soluble carbon, which is more accessible to microorganisms than cellulose [29,30]. It might have led to a higher microbial activity connected with the catalysis of simple carbohydrates present in the unaltered straw.

Depending on the design and the duration of the experiment on the straw decomposition, two or three phases could be distinguished in a process of microbial succession [21, 22,31]. Our data allowed us to distinguish three phases of bacterial succession during the decomposition of lignocellulosic subtract: early (1st month), middle (2nd month), and late (3rd month). This distinction was supported by the microbial activity assessed by carbon dioxide emission and by the bacterial dynamics assessed by the high-throughput 16S rRNA gene sequencing. Despite the fact that the experiment was laid in multiple separate nylon bags, the pattern of microbial succession turned out to be common, with the exception of several outlier phylotypes. Each phase was characteristic of a group consisting of several dozens of co-variable bacterial phylotypes. These phylotypes included both taxa unique for each phase and common throughout the experiment.

Despite the evidence that the early phase of community formation involved the degradation of simple carbohydrates, early microbial colonizers of straw were potentially cellulose-degrading organisms. Among those appeared representatives of actinomycetes, which are known to be active producers of secondary metabolites [32]. For instance, *Cellulosimicrobium* was reported to be a normal part of soil microbiota [33] and to have cellulase and xylanase activities [34-37]. Some strains of *Microbacterium* were reported to have cellulolytic activities [38]. However, maximum diversity they reached by the late phase. Some minor representatives from different phyla of the early succession phase, including *Streptomyces* [39], *Chryseobacterium* [40], and *Dyadobacter* [41] were reported to be able to degrade lignocellulose. The early stages were also characterized by a high relative representation of Gammaproteobacteria (*Pseudomonas*, *Cupriavidus*, *Massilia*) and Alphaproteobacteria (Rhizobiaceae) - most of them were reported to contain a lot of cellulase-active GHs. It was established by earlier findings that Proteobacteria, specifically Alpha- and Gammaproteobacteria play a major role in cellulose decomposition [42]. In accordance with these data, in this study, about half of the GH found in the metagenome of the community involved in the decomposition of cellulose belongs to the representatives of Proteobacteria.

Firmicutes were present at all phases, but most prominently they populated the microbial community in the middle phase. This is consistent with the findings that Firmicutes appear after the initial stage of lignocellulose decomposition [43]. Many genera of this phylum, detected in this dataset, were reported to have cellulolytic strains, including *Bacillus* [44,45], *Paenibacillus* [46], *Lysinibacillus* [47], A relatively low content of GH genes in the representatives of this phylum was shown, but it could be explained by the differences of annotation bases for 16S rRNA and metagenome data, and low coverage of the metagenome assembly.

The most prominent role of the straw decomposition community in this experiment was played by Bacteroidota. A wide range of microorganisms from this phylum is known to play an important role in the decomposition of various polymers [48]. In our work, it was shown that these microorganisms are present at every succession stage, with the succession of some representatives of this phylum (*Chitinofaga*) by others (*Ohtaekwangia*). Moreover, this phylum accounted for the second largest part of GH genes, found in the metagenome. It is worth noting that according to the Polysaccharide-Utilization Loci (PUL) DataBase, the major representative of the early community *Chitinofaga* is rich in PULs, which is a marker of active cleavage of complex polysaccharide substrates already at the early stages [49].

In spite of the fact that a significant proportion of microorganisms not associated with lignocellulose decomposition appear in the later stages of decomposition, we can assume they are an important part of the stable cellulolytic community. For example, it is known that enzymes associated with sulfur metabolism may play an important role in the decomposition of complex straw components, such as polyphenol compounds [50]. The presence of specific nitrifiers and methylotrophs in the community (*Nitrocosmicus*, *Nitrospira*) can play an important role in the construction of efficient communities. The role of nitrogen exchange in catalytic soil systems is underestimated because, in addition to the competition for carbon sources, the high competition for free nitrogen should also be considered [51]. *Starkeya*, one of the major inhabitants of the middle phase, was described to have a chemolithoautotrophic lifestyle, which allows it to both consume carbon dioxide and produce it [52]. *Conexibacter*, which appears in the late phase, was isolated as a soil bacterium, involved in the carbon and nitrogen cycle [53]. The appearance of predatory microorganisms (obligate - Bdellovibrionta, Vampiriovibrionota, facultative - Myxococcota, *Cytophaga*, *Lysobacter*) at the different phases also indicates the reorientation of the community from simple carbohydrate catalysis, since it is known to be a powerful factor in the dynamics of microbial succession [54,55]. There is also evidence, that some of the genera, detected at various phases of decomposition (*Pseudomonas*, *Planctomyces*, *Vampiriovibrio*, *Luteibacter*) can be accompanying microflora, which acts as secondary consumers [56]. These examples expand the understanding of the complexity of interactions between community members.

We showed an increase in bacterial diversity, its phylogenetic diversity, and succession from a relatively simple cellulolytic community to a complex microbial community of autochthonous microorganisms with variable functions in the community. At the same time, we did not observe an increase in fungal diversity. This may be linked to the difference in the life cycle duration of these groups of microorganisms. In contrast to bacterial succession, only two phases were identified in fungal succession, but many major phylotypes were present in all phases. Many fungi found in the community were described as saprophytic with various enzymatic activities. The early phase one was specific in *Schizothecium inaequale*, which is described both as coprophilous [57] and endophytic fungus [58]. Another endophyte, associated with decaying matter, was *Coprinellus flocculosus*, which is a mushroom-forming fungus [59]. Species associated with the late phase of straw decomposition were reported to be endophytic with high enzymatic activities - *Chloridium aseptatum* [60], *Scytalidium* [61,62], *Colletotrichum sidae* [63]. Other saprophytic fungi, found in the community, usually associated with soil or plants and reported to have high enzymatic activity, were *Humicola sardiniae* [64-66], *Gibberella intricans* [67,68], *Albifimbria verrucaria* [69], *Chaetomium iranianum* [70,71], *Occultifur* [72,73], *Waitea circinata* [74]. *Coniochaeta*, which is widely presented in the later phase, is a genus with well-known lignocellulolytic fungi [75,76]. The fungal consortium in the early phase was also presented by the known food mold *Actinomucor elegans*, which was reported to have high enzyme activity, including protease, lipase, glutaminase, and others [77,78].

The major phylotypes of fungi described above, with the exception of *Coniochaeta*, are not described in the literature as typical cellulolytic bacteria. So, the analysis of the role of the fungal fraction in the community remains unclear. It is known that, in spite of the fact that the fraction of nucleic acids encoding CAZymes belonging to the bacterial component is relatively superior to the fungal component of the community, functionally it is fungal enzymes that can play the main role in the degradation of the lignocellulosic complex [79]. It cannot be excluded that the decrease in diversity and the shift of the fungal community from Mucoromycota and Basidiomycota at early stages to Ascomycota at later stages was the result of the antifungal activity of the microbial community. For instance, *Mucilaginibacter*, found in the late phase, potentially can be a mycophagous bacteria [80]. This assumption is also confirmed by the high level of chitinases we found in part of the microbial community.

4. Materials and Methods

The idea of the experiment was to model the dynamics of the formation of cellulolytic consortium from soil microbiota using straw as a substrate and study its colonization process. To achieve this sterilized straw in nylon sachets was submerged in the soil for six months. Chernozem from the Stone steppe in the Voronezskaya area was chosen as a source of decomposing microbiota. Its characteristics were: C_{total} 4.86 ± 0.12%; pH_{salt} 6.40 ± 0.08; N_{total} 0.533 ± 0.02. As a source of lignocellulose biomass oat (*Avena*) straw was used with the following characteristics: ash 9.98±2.04, N_{total} 1.897±0.012, C:N 23.5, water-soluble carbon 11.8±0.50 g/kg.

The experiment took place in 2018. The soil was ground and sieved at 5 mm, watered to 60% of the full moisture capacity, placed in the 2-liter plastic containers, and left to rest for 2 weeks to eliminate the effect of these manipulations on the CO_2 emission. Straw was shredded into 0-2 mm particles, 1-gram portions were placed in the small nylon sachets and were subjected to E-beam sterilization. Wetted sachets (10 per container) were placed

doi:10.20944/preprints202212.0246.v1

vertically in rows at a depth of 0.5-4 cm inside seven replicate containers with pre-prepared soil. Additionally, five replicate control containers with soil and without straw were laid at the same time. More detailed information about the experiment layout was described earlier [81]. The humidity of the substrates was kept constant at 60% and the temperature was maintained at 28±1°C for the duration of the experiment.

To assess microbial activity linked to straw decomposition during the experiment, soil respiration (SR) in the experimental and control containers was measured weekly for six months using the absorption method [82]. SR data was processed in Statistica 13, using One-Way ANOVA with posthoc Tukey HSD test (TIBCO Software Inc., USA).

Coinciding with some of the SR measurements, sachets with straw were pulled one by one out of five experimental containers once every 1-2 weeks for the first two months, and after that once every 3-4 weeks. Two experimental containers with straw sachets remained intact for all six months for the measurement of unaltered SR. The content of pulled-out sachets was stored in plastic tubes at -20°C for the subsequent molecular analysis. Three to five replications for each time of sampling (36 samples in total) were selected for the DNA extraction using NucleoSpin® Soil Kit (Macherey-Nagel GmbH & Co. KG, Düren, Germany) as described previously (2019).

For the analysis of taxonomic dynamics of straw colonization, libraries of partial 16S rRNA gene (for bacteria and archaea) and of ITS2 (for fungi) were prepared and sequenced on the Illumina Miseq platform (Illumina, Inc., San Diego, CA, USA) as described previously [83,84].

Data from sequenced amplicon libraries were processed using the DADA2 pipeline [85] in the R software environment v. 4.2 [86]. Taxonomic identification was carried out using the Silva 138 database [87] for 16S rRNA gene sequences and the Unite database [88] for ITS sequences. The phylogenetic tree was constructed using the SEPP [89] for 16S rRNA data and IQ-TREE 2.1.2 program [90] for ITS2. Further processing was carried out using the phyloseq [91] and ampvis2 [92] packages. Alpha diversity was accessed by: Observed, Shannon [93], and inverted Simpson [94], MPD from picante [95], with the significance of mean differences between them calculated using ANOVA [96]. Beta diversity was accessed by NMDS [97] with the Bray–Curtis distance matrix [98]. The WGCNA [99] method after variance stabilizing transformation from DESeq2 [100] was used to distinguish the microbial association into groups characteristic of different colonization phases.

To assess the composition of GH genes in the cellulolytic community the DNA isolated from the 3-month composting straw sample was used for the full metagenome sequencing using the MinION platform (Oxford Nanopore Technologies, Oxford, UK) as described previously [83]. The resulting raw reads were basecalled using guppy v. 6.0.6 [101]) with a high-accuracy model, clipping adapter sequences, and were additionally checked for adapter sequences using porechop v. 0.2.4 [102], which were removed. Flye v 2.9 with a --meta flag [103] was used to assemble the metagenome from the reads. Assembly was polished using a single run of medaka v. 1.5.0-rc.2 [104], which was used for subsequent steps. The assembly was annotated using eggNOG-mapper v. 2.1.9, using -m diamond and --dmnd_frameshift [105]. The search for GH genes was conducted using hmm profiles from the PHAM database [106]. The attribution of GH genes to different functional groups was performed using the CAZy database [15].

The code is available at https://crabron.github.io/manuals/straw_wgcna.html, accessed on 9 December 2022.

5. Conclusions

In our work, we demonstrated the dynamics of microbial colonization of straw substrate by soil microbiota during a six-month period. The process of bacterial succession led to an increase in diversity during the experiment. However, no increase in diversity was shown for the fungal community. Bacterial succession was divided into three phases, corresponding to microbial activity and the predominance of different taxa. The early phase was characterized by the appearance of representatives of Bacteroidetes, Alpha-and Gammaproteobacteria, which were shown as potential active decomposers of lignocellulose substrate, but which disappear by the end of the phase. The middle phase can be considered the constant core of the emerging cellulolytic community, most of which contain GH genes, connected with cellulose decomposition, according to the metagenome sequencing, including bacteria (Chitinophaga, Bacillus, Ohtaekwangia, Rhizobiaceae) and fungi (Chloridium and Coniochaeta). The prevalence of genes of GH families connected to the cellulolytic activity for major groups of both bacteria and fungi communities in the metagenome of this phase was shown. The last phase marks the functional diversification of the community, when predatory microorganisms and bacteria, involved in the cycling of other non-carbon substrates, released as a result of the activity of other microorganisms, appear. All this may suggest that we should not consider cellulosic communities only as a source of GH-rich microorganisms; a comprehensive approach is required to construct stable and effective decomposing communities.

Supplementary Materials: The following supporting information can be downloaded at: www.mdpi.com/xxx/s1, Figure S1-S8: title; Table S1-S5.

Author Contributions: Conceptualization, Anastasiia Kimeklis and Grigory Gladkov; Data curation, Grigory Gladkov; Formal analysis, Grigory Gladkov, Olga Orlova and Alexey Afonin; Funding acquisition, Evgeny Andronov; Investigation, Anastasiia Kimeklis and Grigory Gladkov; Methodology, Grigory Gladkov and Olga Orlova; Project administration, Evgeny Andronov; Resources, Emma Gribchenko, Tatiana Aksenova, Arina Kichko and Alexander Pinaev; Software, Grigory Gladkov and Alexey Afonin; Supervision, Evgeny Andronov; Validation, Anastasiia Kimeklis, Grigory Gladkov and Alexey Afonin; Visualization, Anastasiia Kimeklis and Grigory Gladkov; Writing – original draft, Anastasiia Kimeklis and Grigory Gladkov; Writing – review & editing, Anastasiia Kimeklis, Grigory Gladkov and Alexey Afonin.

Funding: This research was funded by the Russian Science Foundation, grant number 18-16-00073.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data are available at the NCBI Project PRJNA841641, BioSamples: SAMN28605563, SAMN31919897-SAMN31919906.

Acknowledgments: We thank the Centre for Genomic Technologies, Proteomics and Cell Biology (ARRIAM, Russia) for performing the preparation and sequencing of gene and genomic libraries.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

References

1. Mani, S.; Sokhansanj; Stumborg, S.; Samson, R.; Fenton, J. Production and Distribution of Cereal Straw on the Canadian Prairies. *Can. Biosyst. Eng.* / Le Genie des biosystems au Canada 2006, 48, 39–44.
2. Seglah, P.A.; Wang, Y.; Wang, H.; Bi, Y.; Zhou, K.; Wang, Y.; Wang, H.; Feng, X. Crop Straw Utilization and Field Burning in Northern Region of Ghana. *J. Clean. Prod.* 2020, 261, 121191, doi:10.1016/j.jclepro.2020.121191.
3. Huang, Y.; Zhou, F.; Nan, Z. Comparative Grain Yield, Straw Yield, Chemical Composition, Carbohydrate and Protein Fractions, In Vitro Digestibility and Rumen Degradability of Four Common Vetch Varieties Grown on the Qinghai-Tibetan Plateau. *Animals (Basel)* 2019, 9, 505, doi:10.3390/ani9080505.
4. Romasanta, R.R.; Sander, B.O.; Gaihre, Y.K.; Alberto, Ma.C.; Gummert, M.; Quilty, J.; Nguyen, V.H.; Castalone, A.G.; Balingbing, C.; Sandro, J.; et al. How Does Burning of Rice Straw Affect CH4 and N2O Emissions? A Comparative Experiment of Different on-Field Straw Management Practices. *Agric. Ecosyst. Environ.* 2017, 239, 143–153, doi:10.1016/j.agee.2016.12.042.
5. Ren, J.; Yu, P.; Xu, X. Straw Utilization in China—Status and Recommendations. *Sustainability* 2019, 11, 1762, doi:10.3390/su11061762.
6. Chen, L.; Yang, S.; Gao, J.; Chen, L.; Ning, H.; Hu, Z.; Lu, J.; Tan, X.; Zeng, Y.; Pan, X.; et al. Long-Term Straw Return with Reducing Chemical Fertilizers Application Improves Soil Nitrogen Mineralization in a Double Rice-Cropping System. *Agronomy* 2022, 12, 1767, doi:10.3390/agronomy12081767.
7. Han, Y.; Ma, W.; Zhou, B.; Salah, A.; Geng, M.; Cao, C.; Zhan, M.; Zhao, M. Straw Return Increases Crop Grain Yields and K-Use Efficiency under a Maize-Rice Cropping System. *Crop J.* 2021, 9, 168–180, doi:10.1016/j.cj.2020.04.003.

8. Dobrzyński, J.; Wróbel, B.; Ewa, G. Cellulolytic Properties of a Potentially Lignocellulose-Degrading Bacillus Sp. 8E1A Strain Isolated from Bulk Soil. *Agronomy* 2022, 12, 665, doi:10.3390/agronomy12030665.
9. Ghazanfar, M.; Irfan, M.; Nadeem, M.; Shakir, H.; Khan, D.-M.; Ali, S.; Saeed, S.; Mehmood, T. Isolation of cellulolytic bacteria from soil and valorization of different lignocellulosic wastes for cellulase production by submerged fermentation. *Cellul. Chem. Technol.* 2021, 55, 821–828, doi:10.35812/CelluloseChemTechnol.2021.55.69.
10. Harindintwali, J.D.; Zhou, J.; Habimana, I.; Dong, X.; Sun, C.; Nwamba, M.C.; Yang, W.; Yu, X. Biotechnological Potential of Cellulolytic Nitrogen-Fixing Klebsiella Sp. C-3 Isolated from Paddy Soil. *Bioresour. Technol. Rep.* 2021, 13, 100624, doi:10.1016/j.biteb.2021.100624.
11. Shrestha, B.G.; Ghimire, S.; Bhattarai, S.; Phuyal, S.; Thapa, B. Isolation and Screening of Potential Cellulolytic and Xylanolytic Bacteria From Soil Sample for Degradation of Lignocellulosic Biomass. *JTLS* 2016, 6, 93192, doi:10.11594/jtls.06.03.06.
12. Wilhelm, R.C.; Cardenas, E.; Leung, H.; Szeitz, A.; Jensen, L.D.; Mohn, W.W. Long-Term Enrichment of Stress-Tolerant Cellulolytic Soil Populations Following Timber Harvesting Evidenced by Multi-Omic Stable Isotope Probing. Front. Microbiol. 2017, 8.
13. Berlemont, R.; Allison, S.D.; Weihe, C.; Lu, Y.; Brodie, E.L.; Martiny, J.B.H.; Martiny, A.C. Cellulolytic Potential under Environmental Changes in Microbial Communities from Grassland Litter. *Front. Microbiol.* 2014, 5.
14. Avellaneda-Torres, L.M.; Pulido, C.P.G.; Rojas, E.T. Assessment of Cellulolytic Microorganisms in Soils of Nevados Park, Colombia. *Braz. J. Microbiol.* 2014, 45, 1211, doi:10.1590/s1517-83822014000400011.
15. Drula, E.; Garron, M.-L.; Dogan, S.; Lombard, V.; Henrissat, B.; Terrapon, N. The Carbohydrate-Active Enzyme Database: Functions and Literature. *Nucleic Acids Res.* 2022, 50, D571–D577, doi:10.1093/nar/gkab1045.
16. Henrissat, B. A Classification of Glycosyl Hydrolases Based on Amino Acid Sequence Similarities. *Biochem. J.* 1991, 280 (Pt 2), 309–316, doi:10.1042/bj2800309.
17. Bourne, Y.; Henrissat, B. Glycoside Hydrolases and Glycosyltransferases: Families and Functional Modules. *Curr. Opin. Struct. Biol.* 2001, 11, 593–600, doi:10.1016/S0959-440X(00)00253-0. 1.
18. Yeoman, C.J.; Han, Y.; Dodd, D.; Schroeder, C.M.; Mackie, R.I.; Cann, I.K.O. Chapter 1 - Thermostable Enzymes as Biocatalysts in the Biofuel Industry. In *Advances in Applied Microbiology*; Academic Press, 2010; Vol. 70, pp. 1–55.
19. Nguyen, S.T.C.; Freund, H.L.; Kasanjian, J.; Berlemont, R. Function, Distribution, and Annotation of Characterized Cellulases, Xylanases, and Chitinases from CAZy. *Appl. Microbiol. Biotechnol.* 2018, 102, 1629–1637, doi:10.1007/s00253-018-8778-y.
20. Cortes-Tolalpa, L.; Jiménez, D.J.; de Lima Brossi, M.J.; Salles, J.F.; van Elsas, J.D. Different Inocula Produce Distinctive Microbial Consortia with Similar Lignocellulose Degradation Capacity. *Appl. Microbiol. Biotechnol.* 2016, 100, 7713–7725, doi:10.1007/s00253-016-7516-6.
21. Wei, H.; Wang, L.; Hassan, M.; Xie, B. Succession of the Functional Microbial Communities and the Metabolic Functions in Maize Straw Composting Process. *Bioresour. Technol.* 2018, 256, 333–341, doi:10.1016/j.biortech.2018.02.050.
22. Zhang, X.; Borjigin, Q.; Gao, J.; Yu, X.; Zhang, B.; Hu, S.; Han, S.; Liu, R.; Zhang, S. Community Succession and Straw Degradation Characteristics Using a Microbial Decomposer at Low Temperature. *PLOS ONE* 2022, 17, e0270162, doi:10.1371/journal.pone.0270162. 1.
23. Shuliko, N.N.; Khamova, O.F.; Timokhin, A.Y.; Boiko, V.S.; Tukmacheva, E.V.; Krempa, A. Author Correction: Influence of Long-Term Intensive Use of Irrigated Meadow-Chernozem Soil on the Biological Activity and Productivity of the Arable Layer. *Sci. Rep.* **2022**, *12*, 19755, doi:10.1038/s41598-022-23711-x.
24. Chen, L.; Sun, S.; Yao, B.; Peng, Y.; Gao, C.; Qin, T.; Zhou, Y.; Sun, C.; Quan, W. Effects of Straw Return and Straw Biochar on Soil Properties and Crop Growth: A Review. *Front. Plant Sci.* 2022, 13.
25. Melnichuk, T.N.; Egovtseva, A.Y.; Abdurashytov, S.F.; Abdurashytova, E.R.; Turin, E.N.; Gongalo, A.A.; Zubochenko, A.A.; Pashtetskiy, V.S. State of Microbiocenosis of Southern Chernozem under the No-till System. *E3S Web Conf.* 2020, 224, 04009, doi:10.1051/e3sconf/202022404009.
26. Evdokimova, E.V.; Gladkov, G.V.; Kuzina, N.I.; Ivanova, E.A.; Kimeklis, A.K.; Zverev, A.O.; Kichko, A.A.; Aksenova, T.S.; Pinaev, A.G.; Andronov, E.E. The Difference between Cellulolytic ‘Culturomes’ and Microbiomes Inhabiting Two Contrasting Soil Types. *PLOS ONE* 2020, 15, e0242060, doi:10.1371/journal.pone.0242060.
27. Wolińska, A. Chapter 2 - Metagenomic Achievements in Microbial Diversity Determination in Croplands: A Review. In *Microbial Diversity in the Genomic Era*; Das, S., Dash, H.R., Eds.; Academic Press, 2019; pp. 15–35.
28. Elmajdoub, B.; Marschner, P. Response of Microbial Activity and Biomass to Soil Salinity When Supplied with Glucose and Cellulose. *Soil Sci. Plant Nutr.* 2015, 15, 816–832, doi:10.4067/S0718-95162015005000056.
29. Cuddeford, D. Oats for Animal Feed. In *The oat crop*; Springer, 1995; pp. 321–368.
30. Ma, B.-L.; Zheng, Z.; Ren, C. Chapter 6 - Oat. In *Crop Physiology Case Histories for Major Crops*; Sadras, V.O., Calderini, D.F., Eds.; Academic Press, 2021; pp. 222–248 ISBN 978-0-12-819194-1.
31. Wahdan, S.F.M.; Ji, L.; Schädler, M.; Wu, Y.-T.; Sansupa, C.; Tanunchai, B.; Buscot, F.; Purahong, W. Future Climate Conditions Accelerate Wheat Straw Decomposition alongside Altered Microbial Community Composition, Assembly Patterns, and Interaction Networks. *ISME J* 2022, 1–14, doi:10.1038/s41396-022-01336-2.
32. Qin, S.; Xing, K.; Jiang, J.-H.; Xu, L.-H.; Li, W.-J. Biodiversity, Bioactive Natural Products and Biotechnological Potential of Plant-Associated Endophytic Actinobacteria. *Appl. Microbiol. Biotechnol.* 2011, 89, 457–473, doi:10.1007/s00253-010-2923-6.

33. Mhatre, P.H.; Karthik, C.; Kadirvelu, K.; Divya, K.L.; Venkatasalam, E.P.; Srinivasan, S.; Ramkumar, G.; Saranya, C.; Shanmuganathan, R. Plant Growth Promoting Rhizobacteria (PGPR): A Potential Alternative Tool for Nematodes Bio-Control. *Biocatal. Agric. Biotechnol.* 2019, 17, 119–128, doi:10.1016/j.bcab.2018.11.009.
34. Huang, S.; Sheng, P.; Zhang, H. Isolation and Identification of Cellulolytic Bacteria from the Gut of *Holotrichia Parallela* Larvae (Coleoptera: Scarabaeidae). *Int. J. Mol. Sci.* 2012, 13, 2563–2577, doi:10.3390/ijms13032563.
35. Oh, H.-W.; Heo, S.-Y.; Kim, D.Y.; Park, D.-S.; Bae, K.S.; Park, H.-Y. Biochemical Characterization and Sequence Analysis of a Xylanase Produced by an Exo-Symbiotic Bacterium of Gryllotalpa Orientalis, Cellulosimicrobium Sp. HY-12. *Antonie Van Leeuwenhoek* 2008, 93, 437–442, doi:10.1007/s10482-007-9210-2.
36. Opazo, R.; Ortúzar, F.; Navarrete, P.; Espejo, R.; Romero, J. Reduction of Soybean Meal Non-Starch Polysaccharides and α-Galactosides by Solid-State Fermentation Using Cellulolytic Bacteria Obtained from Different Environments. *PLOS ONE* 2012, 7, e44783, doi:10.1371/journal.pone.0044783.
37. Villas-Boas, S.; Esposito, E.; Mitchell, D. Microbial Conversion of Lignocellulosic Residues for Production of Animal Feeds. *Anim. Feed Sci. Technol.* 2002, 98, 1–12, doi:10.1016/s0377-8401(02)00017-2.
38. Sadhu, S.; Saha, P.; Mayilraj, S.; Maiti, T.K. Lactose-Enhanced Cellulase Production by Microbacterium Sp. Isolated from Fecal Matter of Zebra (Equus Zebra). *Curr. Microbiol.* 2011, 62, 1050–1055, doi:10.1007/s00284-010-9816-x.
39. Ventorino, V.; Ionata, E.; Birolo, L.; Montella, S.; Marcolongo, L.; de Chiaro, A.; Espresso, F.; Faraco, V.; Pepe, O. Lignocellulose-Adapted Endo-Cellulase Producing Streptomyces Strains for Bioconversion of Cellulose-Based Materials. *Front. Microbiol.* 2016, 7.
40. Tan, H.; Miao, R.; Liu, T.; Yang, L.; Yang, Y.; Chen, C.; Lei, J.; Li, Y.; He, J.; Sun, Q.; et al. A Bifunctional Cellulase-Xylanase of a New *Chryseobacterium* Strain Isolated from the Dung of a Straw-Fed Cattle. *Microb. Biotechnol.* 2018, 11, 381–398, doi:10.1111/1751-7915.13034.
41. Photphisutthiphong, Y.; Vatanyoopaisarn, S. Dyadobacter and Sphingobacterium Isolated from Herbivore Manure in Thailand and Their Cellulolytic Activity in Various Organic Waste Substrates. *Agric. Nat. Resour.* 2019, 89–98, doi:10.34044/j.anres.2019.53.2.01.
42. Gladkov, G.V.; Kimeklis, A.K.; Afonin, A.M.; Lisina, T.O.; Orlova, O.V.; Aksenova, T.S.; Kichko, A.A.; Pinaev, A.G.; Andronov, E.E. The Structure of Stable Cellulolytic Consortia Isolated from Natural Lignocellulosic Substrates. *IJMS* 2022, 23, 10779, doi:10.3390/ijms231810779.
43. Meng, Q.; Yang, W.; Men, M.; Bello, A.; Xu, X.; Xu, B.; Deng, L.; Jiang, X.; Sheng, S.; Wu, X.; et al. Microbial Community Succession and Response to Environmental Variables During Cow Manure and Corn Straw Composting. *Front. Microbiol.* 2019, 10.
44. Kim, Y.-K.; Lee, S.-C.; Cho, Y.-Y.; Oh, H.-J.; Ko, Y.H. Isolation of Cellulolytic Bacillus Subtilis Strains from Agricultural Environments. *ISRN Microbiol.* 2012, 2012, 650563, doi:10.5402/2012/650563.
45. Ma, L.; Wang, X.; Zhou, J.; Lü, X. Degradation of Switchgrass by Bacillus Subtilis 1AJ3 and Expression of a Beta-Glycoside Hydrolase. *Front. Microbiol.* 2022, 13.
46. Yadav, S.; Dubey, S.K. Cellulose Degradation Potential of Paenibacillus Lautus Strain BHU3 and Its Whole Genome Sequence. *Bioresour. Technol.* 2018, 262, 124–131, doi:10.1016/j.biortech.2018.04.067.
47. Duan, H.; Fu, C.; Du, G.; Xie, S.; Liu, M.; Zhang, B.; Shi, J.; Sun, J. Dynamic Microstructure Assembly Driven by Lysinibacillus Sp. LF-N1 and Penicillium Oxalicum DH-1 Inoculants Corresponds to Composting Performance. *Microorganisms* 2022, 10, 709, doi:10.3390/microorganisms10040709. 1.
48. McKee, L.S.; La Rosa, S.L.; Westereng, B.; Eijsink, V.G.; Pope, P.B.; Larsbrink, J. Polysaccharide Degradation by the Bacteroidetes: Mechanisms and Nomenclature. *Environ. Microbiol. Rep.* **2021**, *13*, 559–581, doi:10.1111/1758-2229.12980.
49. Terrapon, N.; Lombard, V.; Drula, É.; Lapébie, P.; Al-Masaudi, S.; Gilbert, H.J.; Henrissat, B. PULDB: The Expanded Database of Polysaccharide Utilization Loci. *Nucleic Acids Res.* 2018, 46, D677–D683, doi:10.1093/nar/gkx1022.
50. Pareek, S.; Azuma, J.I.; Matsui, S.; Shimizu, Y. Degradation of Lignin and Lignin Model Compound under Sulfate Reducing Condition. *Water Sci. Technol.* 2001, 44, 351–358.
51. Halsall, D.M.; Gibson, A.H. Cellulose Decomposition and Associated Nitrogen Fixation by Mixed Cultures of Cellulomonas Gelida and Azospirillum Species or Bacillus Macerans. *Appl. Environ. Microbiol.* 1985, 50, 1021–1026, doi:10.1128/aem.50.4.1021-1026.1985.
52. Kappler, U.; Davenport, K.; Beatson, S.; Lucas, S.; Lapidus, A.; Copeland, A.; Berry, K.W.; Glavina Del Rio, T.; Hammon, N.; Dalin, E.; et al. Complete Genome Sequence of the Facultatively Chemolithoautotrophic and Methylotrophic Alpha Proteobacterium *Starkeya Novella* Type Strain (ATCC 8093T). *Stand. Genomic Sci.* 2012, 7, 44–58, doi:10.4056/sigs.3006378.
53. Seki, T.; Matsumoto, A.; Shimada, R.; Inahashi, Y.; Ōmura, S.; Takahashi, Y. Conexibacter Arvalis Sp. Nov., Isolated from a Cultivated Field Soil Sample. *Int. J. Syst. Evol. Microbiol.* 2012, 62, 2400–2404, doi:10.1099/ijs.0.036095-0.
54. Hungate, B.A.; Marks, J.C.; Power, M.E.; Schwartz, E.; van Groenigen, K.J.; Blazewicz, S.J.; Chuckran, P.; Dijkstra, P.; Finley, B.K.; Firestone, M.K.; et al. The Functional Significance of Bacterial Predators. *mBio* 2021, 12, e00466-21, doi:10.1128/mBio.00466-21.
55. Johnke, J.; Cohen, Y.; de Leeuw, M.; Kushmaro, A.; Jurkevitch, E.; Chatzinotas, A. Multiple Micro-Predators Controlling Bacterial Communities in the Environment. *Curr. Opin. Biotechnol.* 2014, 27, 185–190, doi:10.1016/j.copbio.2014.02.003.

56. Wilhelm, R.C.; Pepe-Ranney, C.; Weisenhorn, P.; Lipton, M.; Buckley, D.H. Competitive Exclusion and Metabolic Dependency among Microorganisms Structure the Cellulose Economy of an Agricultural Soil. *mBio* 12, e03099-20, doi:10.1128/mBio.03099-20.
57. Zheng, X.; Song, K.; Li, S.; Zhang, H.; Bai, N.; Zhang, J.; Zhang, H.; Cai, S.; Lv, W.; Cao, L. Effects of Long-Term Integrated Agri-Aquaculture on the Soil Fungal Community Structure and Function in Vegetable Fields. *Sci. Rep.* 2021, 11, 10813, doi:10.1038/s41598-021-90109-6.
58. Tymon, L.S.; Morgan, P.; Gundersen, B.; Inglis, D.A. Potential of Endophytic Fungi Collected from Cucurbita Pepo Roots Grown under Three Different Agricultural Mulches as Antagonistic Endophytes to Verticillium Dahliae in Western Washington. *Microbiol. Res.* 2020, 240, 126535, doi:10.1016/j.micres.2020.126535.
59. Wood, M. California Fungi: *Coprinellus Flocculosus.* Available online: http://www.mykoweb.com/CAF/species/Coprinellus flocculosus.html (accessed on 24 November 2022).
60. Wei, M.-J.; Zhang, H.; Dong, W.; Boonmee, S.; Zhang, D. Introducing *Dictyochaeta Aquatica* Sp. Nov. and Two New Species of *Chloridium* (Chaetosphaeriaceae, Sordariomycetes) from Aquatic Habitats. *Phytotaxa* 2018, 362, 187, doi:10.11646/phytotaxa.362.2.5.
61. Heinen, P.R.; Betini, J.H.A.; Polizeli, M.L.T.M. Xylanases. In *Encyclopedia of Microbiology (Fourth Edition)*; Schmidt, T.M., Ed.; Academic Press: Oxford, 2019; pp. 604–615.
62. Takahashi, K. Chapter 74 - Aspergilloglutamic Peptidase. In *Handbook of Proteolytic Enzymes (Third Edition)*; Rawlings, N.D., Salvesen, G., Eds.; Academic Press, 2013; pp. 307–310.
63. Damm, U.; Cannon, P.F.; Liu, F.; Barreto, R.W.; Guatimosim, E.; Crous, P.W. The *Colletotrichum Orbiculare* Species Complex: Important Pathogens of Field Crops and Weeds. *Fungal Divers.* 2013, 61, 29–59, doi:10.1007/s13225-013-0255-4.
64. Davies, G.J.; Schülein, M. Structural Studies on Fungal Endoglucanases from Humicola Insolens. In *Progress in Biotechnology*; Petersen, S.B., Svensson, B., Pedersen, S., Eds.; Carbohydrate Bioengineering; Elsevier, 1995; Vol. 10, pp. 225–237.
65. Matsumoto, H.; Koganei, K.; Nishida, N.; Koyama, Y.; Saito, S.; Kataoka, H.; Ogihara, J.; Kasumi, T. Cell Dispersion Culture for the Effective Growth of *Humicola Insolens* and Efficient Enzyme Production. *J. Biosci. Bioeng.* 2014, 117, 257–262, doi:10.1016/j.jbiosc.2013.08.014.
66. Schülein, M. Enzymatic Properties of Cellulases from *Humicola Insolens*. *J. Biotechnol.* 1997, 57, 71–81, doi:10.1016/s0168-1656(97)00090-4.
67. El-Said, A.H.M.; Saleem, A. Ecological and Physiological Studies on Soil Fungi at Western Region, Libya. *Mycobiology* 2008, 36, 1–9, doi:10.4489/MYCO.2008.36.1.001.
68. Sivapalan, A.; Metussin, R.; Harndan, F.; Zain, R.M. Fungi Associated with Postharvest Fruit Rots of Durio Graveolens and D. Kutejensis in Brunei Darussalam. *Australas. Plant Pathol.* 1998, 27, 274–277, doi:10.1071/AP98033.
69. Matić, S.; Gilardi, G.; Gullino, M.L.; Garibaldi, A. Emergence of Leaf Spot Disease on Leafy Vegetable and Ornamental Crops Caused by Paramyrothecium and Albifimbria Species. *Phytopathology* 2019, 109, 1053–1061, doi:10.1094/PHYTO-10-18-0396-R.
70. Blanchette, R.; held, B.; Abdel-Azeem, A. New Record of Chaetomium Iranianum MF787598 (Chaetomiaceae) for the Egyptian and African Mycobiota. *Microbial Biosystems* 2017, 2, 6–9, doi:10.21608/mb.2017.5208.
71. Darwish, A.; Abdel-Azeem, A. Chaetomium Enzymes and Their Applications. In *Recent Developments on Genus Chaetomium*; 2020; pp. 241–249.
72. Khunnamwong, P.; Ribeiro, J.R.A.; Garcia, K.M.; Hagler, A.N.; Takashima, M.; Ohkuma, M.; Endoh, R.; Sugita, T.; Jindamorakot, S.; Limtong, S. Occultifur Plantarum f.a., Sp. Nov., a Novel Cystobasidiomycetous Yeast Species. *Int. J. Syst. Evol. Microbiol.* 2017, 67, 2628–2633, doi:10.1099/ijsem.0.001988.
73. Sampaio, J.; Bauer, R.; Begerow, D.; Oberwinkler, F. Occultifur Externus Sp. Nov., a New Species of Simple-Pored Auricularioid Heterobasidiomycete from Plant Litter in Portugal. *Mycologia* 1999, 91, 1094–1101, doi:10.2307/3761639.
74. de la Cerda, K.A.; Douhan, G.W.; Wong, F.P. Discovery and Characterization of Waitea Circinata Var. Circinata Affecting Annual Bluegrass from the Western United States. *Plant. Dis.* 2007, 91, 791–797, doi:10.1094/PDIS-91-7-0791.
75. López, M.; Vargas García, M.; Suárez-Estrella, F.; Dien, B.; Moreno, J. Lignocellulose-Degrading Enzymes Produced by the Ascomycete Coniochaeta Ligniaria and Related Species: Application for a Lignocellulosic Substrate Treatment. *Enzyme Microb. Technol.* 2007, 40, 794–800, doi:10.1016/j.enzmictec.2006.06.012.
76. Mondo, S.J.; Jiménez, D.J.; Hector, R.E.; Lipzen, A.; Yan, M.; LaButti, K.; Barry, K.; van Elsas, J.D.; Grigoriev, I.V.; Nichols, N.N. Genome Expansion by Allopolyploidization in the Fungal Strain Coniochaeta 2T2.1 and Its Exceptional Lignocellulolytic Machinery. *Biotechnol. Biofuels* 2019, 12, 229, doi:10.1186/s13068-019-1569-6.
77. Han, B.-Z.; Ma, Y.; Rombouts, F.M.; Robert Nout, M.J. Effects of Temperature and Relative Humidity on Growth and Enzyme Production by Actinomucor Elegans and Rhizopus Oligosporus during Sufu Pehtze Preparation. *Food Chemistry* 2003, 81, 27–34, doi:10.1016/S0308-8146(02)00347-3.
78. O'Toole, D.K. Soybean | Soymilk, Tofu, and Okara. In *Reference Module in Food Science*; Elsevier, 2016.
79. Hobdey, S.E.; Donohoe, B.S.; Brunecky, R.; Himmel, M.E.; Bomble, Y.J. New Insights into Microbial Strategies for Biomass Conversion. In *Direct Microbial Conversion of Biomass to Advanced Biofuels*; Elsevier, 2015; pp. 111–127.

80. Tláskal, V.; Voříšková, J.; Baldrian, P. Bacterial Succession on Decomposing Leaf Litter Exhibits a Specific Occurrence Pattern of Cellulolytic Taxa and Potential Decomposers of Fungal Mycelia. *FEMS Microb. Ecol.* 2016, 92, fiw177, doi:10.1093/femsec/fiw177.
81. Orlova, O.V.; Kichko, A.A.; Pershina, E.V.; Pinaev, A.G.; Andronov, E.E. Succession of Bacterial Communities in the Decomposition of Oats Straw in Two Soils with Contrasting Properties. *Eurasian Soil Sc.* 2020, 53, 1620–1628, doi:10.1134/S1064229320090112.
82. Van Cleve, K.; Coyne, P.I.; Goodwin, E.; Johnson, C.; Kelley, M. A Comparison of Four Methods for Measuring Respiration in Organic Material. *Soil Biol. Biochem.* 1979, 11, 237–246, doi:10.1016/0038-0717(79)90068-3. 1.
83. Gladkov, G.; Kimeklis, A.; Zverev, A.; Pershina, E.; Ivanova, E.; Kichko, A.; Andronov, E.; Abakumov, E. Soil Microbiome of the Postmining Areas in Polar Ecosystems in Surroundings of Nadym, Western Siberia, Russia. *Open Agriculture* **2019**, *4*, 684–696, doi:10.1515/opag-2019-0070.
84. Kichko, A.A.; Aksenova, T.S.; Shapkin, V.M.; Zverev, A.O.; Khiutti, A.V.; Andronov, E.E. Analysis of Mycobiome in Damaged Potato (Solanum Tuberosum l.) Leaves by Using Metagenomic Approaches. *S-h. biol.* 2019, 54, 990–1001, doi:10.15389/agrobiology.2019.5.990rus.
85. Callahan, B.J.; McMurdie, P.J.; Rosen, M.J.; Han, A.W.; Johnson, A.J.A.; Holmes, S.P. DADA2: High-Resolution Sample Inference from Illumina Amplicon Data. *Nat. Methods* 2016, 13, 581–583, doi:10.1038/nmeth.3869.
86. R Core Team. R: A Language and Environment for Statistical Computing. Available Online: Https://Www.R-Project.Org/ (Accessed on 9 December 2022).
87. Quast, C.; Pruesse, E.; Yilmaz, P.; Gerken, J.; Schweer, T.; Yarza, P.; Peplies, J.; Glöckner, F.O. The SILVA Ribosomal RNA Gene Database Project: Improved Data Processing and Web-Based Tools. *Nucleic Acids Res.* 2013, 41, D590–D596, doi:10.1093/nar/gks1219.
88. Nilsson, R.H.; Larsson, K.-H.; Taylor, A.F.S.; Bengtsson-Palme, J.; Jeppesen, T.S.; Schigel, D.; Kennedy, P.; Picard, K.; Glöckner, F.O.; Tedersoo, L.; et al. The UNITE Database for Molecular Identification of Fungi: Handling Dark Taxa and Parallel Taxonomic Classifications. *Nucleic Acids Res.* 2019, 47, D259–D264, doi:10.1093/nar/gky1022.
89. Janssen, S.; McDonald, D.; Gonzalez, A.; Navas-Molina, J.A.; Jiang, L.; Xu, Z.Z.; Winker, K.; Kado, D.M.; Orwoll, E.; Manary, M.; et al. Phylogenetic Placement of Exact Amplicon Sequences Improves Associations with Clinical Information. *mSystems* 2018, 3, e00021-18, doi:10.1128/mSystems.00021-18.
90. Minh, B.Q.; Schmidt, H.A.; Chernomor, O.; Schrempf, D.; Woodhams, M.D.; von Haeseler, A.; Lanfear, R. IQ-TREE 2: New Models and Efficient Methods for Phylogenetic Inference in the Genomic Era. *Mol. Biol. Evol.* 2020, 37, 1530–1534, doi:10.1093/molbev/msaa015.
91. McMurdie, P.J.; Holmes, S. Phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. *PLOS ONE* 2013, 8, e61217, doi:10.1371/journal.pone.0061217.
92. Andersen, K.S.; Kirkegaard, R.H.; Karst, S.M.; Albertsen, M. Ampvis2: An R Package to Analyse and Visualise 16S RRNA Amplicon Data 2018, 299537.
93. Shannon, C.E.; Weaver, W. The Mathematical Theory of Communication; University of Illinois Press, 1949.
94. Simpson, E.H. Measurement of Diversity. *Nature* 1949, 163, 688–688, doi:10.1038/163688a0.
95. Picante Package – Rdocumentation. Available online: https://www.rdocumentation.org/packages/picante/versions/1.8.2 (accessed on 1 December 2022).
96. Girden, E.R. ANOVA: Repeated Measures.; Sage Publications, Inc., 1992.
97. Gower, J.C. Principal Coordinates Analysis. In *Wiley StatsRef: Statistics Reference Online*; John Wiley & Sons, Ltd, 2015; pp. 1–7.
98. Bray, J.R.; Curtis, J.T. An Ordination of the Upland Forest Communities of Southern Wisconsin. *Ecol. Monogr.* 1957, 27, 325–349, doi:10.2307/1942268.
99. Langfelder, P.; Horvath, S. WGCNA: An R Package for Weighted Correlation Network Analysis. *BMC Bioinformatics* 2008, 9, 559, doi:10.1186/1471-2105-9-559.
100. Love, M.S.A. DESeq2 2017.
101. Wick, R.R.; Judd, L.M.; Holt, K.E. Performance of Neural Network Basecalling Tools for Oxford Nanopore Sequencing. *Genome Biology* 2019, 20, 129, doi:10.1186/s13059-019-1727-y.
102. Wick, R.R.; Judd, L.M.; Gorrie, C.L.; Holt, K.E.Y. 2017 Completing Bacterial Genome Assemblies with Multiplex MinION Sequencing. *Microbial Genomics* 3, e000132, doi:10.1099/mgen.0.000132.
103. Kolmogorov, M.; Bickhart, D.M.; Behsaz, B.; Gurevich, A.; Rayko, M.; Shin, S.B.; Kuhn, K.; Yuan, J.; Polevikov, E.; Smith, T.P.L.; et al. MetaFlye: Scalable Long-Read Metagenome Assembly Using Repeat Graphs. *Nat. Methods* 2020, 17, 1103–1110, doi:10.1038/s41592-020-00971-x.
104. Medaka: Sequence Correction Provided by ONT Research. Available Online: Https://Github.Com/Nanoporetech/Medaka (Accessed on 9 December 2022).
105. Cantalapiedra, C.P.; Hernández-Plaza, A.; Letunic, I.; Bork, P.; Huerta-Cepas, J. EggNOG-Mapper v2: Functional Annotation, Orthology Assignments, and Domain Prediction at the Metagenomic Scale. *Mol. Biol. Evol.* 2021, 38, 5825–5829, doi:10.1093/molbev/msab293.

106. Blum, M.; Chang, H.-Y.; Chuguransky, S.; Grego, T.; Kandasaamy, S.; Mitchell, A.; Nuka, G.; Paysan-Lafosse, T.; Qureshi, M.; Raj, S.; et al. The InterPro Protein Families and Domains Database: 20 Years On. *Nucleic Acids Res.* 2021, 49, D344–D354, doi:10.1093/nar/gkaa977.