agronomy

MDPI

Article

Applying Hydrochar Affects Soil Carbon Dynamics by Altering the Characteristics of Soil Aggregates and Microbes

Ting Yan [1,2], Zherui Zhang [2], Zhe Zhang [2], Wenzan Wang [2], Dong Li [3], Tao Zhang [1] and Zhiping Zhu [2,*]

[1] College of Resources and Environmental Sciences, China Agricultural University, Beijing 100193, China; b20203030248@cau.edu.cn (T.Y.); taozhang@cau.edu.cn (T.Z.)
[2] Institute of Environment and Sustainable Development in Agriculture, Chinese Academy of Agricultural Sciences, Beijing 100081, China; 18101089263@163.com (Z.Z.); 82101232144@caas.cn (Z.Z.); wangwenzan0317@163.com (W.W.)
[3] Bureau of Agriculture and Rural Development, Beichuan Qiang Autonomous County, Mianyang 622750, China; lql1016yt@163.com
* Correspondence: zhuzhiping@caas.cn; Tel.: +86-10-8210-6840

Abstract: Hydrochar as a carbon-based fertiliser is hypothesised to permanently improve soils by modifying soil carbon quality through the regulation of soil organic carbon dynamics, aggregation properties and microbial diversity. However, the interactions between soil organic carbon (SOC) molecular structure, soil aggregates and soil microbial communities as a result of hydrochar application have not been fully elucidated. In this study, the use of hydrochar derived from duck farm biomass waste for a maize cultivation experiment verified that hydrochar had a promoting effect on maize growth, effectively increasing the nutrient supply to the soil. The application of hydrochar increased the soil organic carbon content by 78 to 253 per cent, which was dominated by CHON-type lignin, carbohydrates and condensed aromatic structural compounds. Meanwhile, hydrochar had a significant effect on both soil aromatic structures and oxygenated functional groups, forming more soil macroaggregates. In addition, hydrochar had a positive effect on soil bacterial abundance. This study suggests that the key mechanism by which hydrochar regulates soil carbon dynamics is mainly through the stabilising effect of hydrochar on macroaggregates while increasing the abundance of carbon-related microscopic bacteria. These results will help to elucidate the potential effects of aqueous carbon on the biogeochemical cycling of carbon in soils.

Keywords: hydrochar; organic carbon; soil aggregates; microbial community structure

Citation: Yan, T.; Zhang, Z.; Zhang, Z.; Wang, W.; Li, D.; Zhang, T.; Zhu, Z. Applying Hydrochar Affects Soil Carbon Dynamics by Altering the Characteristics of Soil Aggregates and Microbes. *Agronomy* **2024**, *14*, 1015. https://doi.org/10.3390/agronomy14051015

Academic Editor: Zhengqin Xiong

Received: 26 March 2024
Revised: 20 April 2024
Accepted: 7 May 2024
Published: 10 May 2024

1. Introduction

It is widely acknowledged that approximately one-quarter of all anthropogenic greenhouse gas emissions are attributable to the global food system. The Intergovernmental Panel on Climate Change (IPCC) in its Fourth Assessment Report highlighted that up to 90% of the potential for reducing agricultural greenhouse gas emissions could be achieved through carbon sequestration in soils. However, with the progression towards large-scale operations, a significant disconnect has emerged between crop cultivation and livestock husbandry in China, with the proportion of farms integrating these practices plummeting from 71% in 1986 to 12% by 2017 [1]. Commercial duck farming constitutes a vital segment of China's livestock industry, with its annual production of meat ducks accounting for approximately 70% of the global total [2], generating around 76.83 million tons of biomass waste annually from litter and duck manure. This waste, a typical agricultural biomass carbon resource, if left to decompose or rot indiscriminately, can produce substantial amounts of methane, adversely affecting ecosystems and human health.

Processing duck farming biomass waste into carbon-rich materials, such as hydrochar, facilitates the recovery and sequestration of carbon. The application of hydrochar in soil amendments not only aids in soil carbon sequestration but also reduces carbon emissions.

The performance of hydrochar varies significantly due to the diverse range of raw materials used in its production [3]. The stability of hydrochar is a critical factor in its ability to enhance soil carbon stocks, and the carbon components of hydrochar can interact and bond with soil organic matter, becoming part of the stable carbon [4]. The timescale of carbon sequestration may depend on the interactions between carbon undergoing dissolution or microbial transformation and the surfaces of soil minerals. Of particular importance is the formation of soil aggregates, which, as the fundamental units of soil structure, are crucial for controlling and stabilising soil carbon storage [5]. Biomass char (biochar, hydrochar) can influence the distribution and turnover of different organic matter components within various aggregates by affecting soil aggregation. Firstly, the input of biomass char can alter carbon quality by changing the soil organic carbon content and aromaticity. Secondly, biomass char can physically protect soil organic carbon by influencing the accessibility of substrates, such as through the formation of soil aggregates and mineral-organic binding interactions [6]. Additionally, changes in soil carbon quality and physicochemical properties can further affect soil carbon availability and the microbial habitat, thereby influencing the microbial mineralisation of soil organic carbon [7]. Microbial communities play a key role in the composition of soil organic carbon by mediating the decomposition process of organic material in soil. However, there are currently only limited studies have been conducted to elucidate the effects of hydrochar applications on soil microbial communities [8,9].

Based on the above, we can reasonably assume that the hydrochar derived from duck farming biomass waste, as a carbon-based fertiliser, can achieve an improved soil carbon quality by changing soil aggregates and microbial communities to affect soil organic carbon in order to elucidate the complex interactions between hydrochar derived from duck farming biomass waste and the dynamics of soil organic carbon, soil aggregates and soil microbial communities. It is expected to provide a basis for the potential effects of hydrochar on the biogeochemical cycle of carbon in soil ecosystems.

2. Materials and Methods

2.1. Materials

In this study, biomass waste from duck farming, specifically rice husks, sawdust and duck manure, collected from a duck farming cooperative in Cangzhou, Hebei, China, were used as raw materials for hydrochar. The materials were dried at 105 °C for 24 h until there was no change in moisture content. Humic acid (chemical formula $C_9H_9NO_6$) was purchased from Shanghai Yuan Ye Biological Technology Co, Ltd., Shanghai, China. The raw materials were mixed according to the mass ratios of rice husk to sawdust (1:1), duck manure to rice husk to sawdust (1:1:1), and humic acid to duck manure to rice husk to sawdust (1:3:3:3) to produce the mixed raw materials for litter-derived hydrochar (L-HC), duck manure–litter-derived hydrochar (DL-HC), and duck manure–litter-derived hydrochar + humic acid (DLH-HC), respectively. These materials were then adjusted to a solid content of 16% and fed into a reactor, where they were subjected to hydrothermal carbonisation at a reaction temperature of 240 °C and a residence time of 180 min. The target hydrochar was then collected using vacuum filtration and drying.

Potting soil was collected from the Nankou Base of the Chinese Academy of Agricultural Sciences (Beijing, China) from the arable layer at a depth of 0–20 cm inside an institutional greenhouse at various points. The soil was sieved to remove non-soil particles, dried in a shaded area, sieved (2 mm) and thoroughly homogenised to form a composite soil sample for analysis of its physicochemical indicators, as shown in Table 1. The maize seeds used were of the variety Zhengdan 985, and the inorganic compound fertiliser contained nutrients in the ratio $N:P_2O_5:K_2O$ = 1:1:1.

Table 1. Basic properties of soils and hydrochar (on a dry basis).

Samples	EC (us/cm)	pH	TN (%)	P_2O_5 (%)	K_2O (%)	Organic Matter (%)
Soil	670.32	6.56	0.02	0.11	2.08	0.20
L-HC	1006.78	6.41	0.36	0.11	0.28	80.06
DL-HC	1245.36	6.54	1.30	1.61	0.49	79.00
DLA-HC	1193.47	6.23	1.34	1.50	0.66	80.11

Note: The L-HC, DL-HC and DLH-HC represent litter-derived hydrochar, duck manure–litter-derived hydrochar and duck manure–litter–humic acid-derived hydrochar, respectively.

2.2. Experimental Design

The experiment was conducted to assess the bioeffects of incorporating hydrochar into the soil via potted maize. A total of five treatment groups were set up: chemical fertiliser alone (CF) and chemical fertiliser mixed with duck litter (FM) served as controls, while the application of chemical fertiliser mixed with litter-derived hydrochar (FH1), chemical fertiliser mixed with duck manure–litter-derived hydrochar (FH2) and chemical fertiliser mixed with duck manure–litter–humic acid-derived hydrochar (FH3) served as fertilisation treatment groups with different types of hydrochar. Each treatment group was replicated three times.

Each rhizobox (20 cm long, 18 cm wide and 23 cm high) contained 2 kg of soil. As studies such as Khosravi et al. [3] have found that higher application rates of hydrochar (3%) can increase the level of labile carbon available to methanogenic microbes, leading to increased methane production, a more appropriate soil mass ratio of 1% was chosen for the addition of hydrochar in this study. Following the recommended commercial application rate, each rhizobox was treated with 1.5 g of mixed fertiliser. Maize seeds were germinated and grown in the box for 10 days before transplanting to ensure that each rhizobox contained three maize seedlings of similar vigour. All treatment groups were arranged in a completely randomised block design within the greenhouse. The water content was adjusted by weighing all treatment groups every 3–4 days to maintain 60% of the maximum water capacity.

2.3. Analytical Methods

Forty days after the maize seedlings were planted, the entire maize plants were harvested, and the fresh weight of all plant parts, including leaves, stems and roots, was recorded. Soil samples for microbial analysis were taken around the root zone before the maize plants were removed to avoid disturbing the soil microbial composition by removing the plants. A 1 cm diameter mini soil auger was used to collect samples from around the rhizosphere area of each replicate group, and 5 g of the sample was mixed into a sample bag, which was then immediately stored at −80 °C until microbial analysis. After carefully uprooting the maize plants, fresh soil was sampled again, visible plant material and stones were sifted out and the soil was air-dried for assessment of various indicators.

Soil pH and electrical conductivity (EC) were measured using a portable pH meter and glass electrode for a soil:water suspension of 1:5. Soil organic carbon (SOC) was determined using the H_2SO_4-$K_2Cr_2O_7$ wet oxidation method. The presence of surface organic groups in the soil was detected by using a Perkin Elmer Spectrum 400 FT-IR/NIR spectrometer (Perkin Elmer Inc., Tres Cantos, Madrid). Soil available potassium (K_2O) was determined using an atomic absorption spectrophotometer, while soil available phosphorus (P_2O_5) was measured using a UV–visible spectrophotometer, and soil nitrogen (N) was estimated using a semi-micro Kjeldahl titration method. The molecular composition of soil dissolved organic matter (DOM) was analysed using a Bruker SolariX FT-ICR MS (Bruker Daltonik, GmbH, Bremen, Germany). Before performing ESI-FT-ICR-MS analysis, the FA fraction of all of the samples was acidified to pH 2 with HCl (32%, analytical reagent) and pretreated with solid-phase extraction (SPE). Then, ultrahigh-resolution mass spectra were obtained using a Bruker Solari X FT-ICR-MS equipped with a 15.0 T superconducting magnet and a dual-mode electrospray ionisation/matrix-assisted laser desorption ionisation ion source.

The proportion of water-stable aggregates in the soil was measured using a wet sieving device with a sieve size of less than 2 mm, the soil was sieved for 5 min and after correction for coarse material < 0.002 mm, 0.002~0.02 mm and 0.02~2 mm, the weight of each aggregate size (as a percentage of soil weight) was recorded. The Illumina high-throughput sequencing platform was used for double-end sequencing of the ITS region and 16S rRNA genes for bacteria and fungi from CF and FH3 samples, respectively, to analyse changes in soil microbial abundance. Next-generation sequencing library preparations and Illumina MiSeq sequencing were conducted at GENEWIZ Inc. (Beijing, China). DNA samples were quantified using a Qubit 2.0 Fluorometer (Invitrogen, Carlsbad, CA, USA), and DNA quality was checked on a 0.8% agarose gel. Between 30 and 50 ng of DNA was used to generate amplicons using a MetaVx Library Preparation Kit (GENEWIZ Inc., South Plainfield, NJ, USA). For bacterial 16S rDNA amplicon library construction, a panel of proprietary primers was designed to anneal to the relatively conserved regions bordering the V3 and V4 hypervariable regions. The V3 and V4 regions were amplified using forward primers containing the sequence CCTACGGRRBGCASCAGKVRVGAAT and reverse primers containing the sequence GGACTACNVGGGTWTCTAATCC. For fungal ITS rDNA amplicon library construction, the hypervariable ITS2 region was amplified using the primers containing the sequence GTGAATCATCGARTC and reverse primers containing the sequence TCCTCCGCTTATTGAT. In addition to the 16S and ITS target-specific sequences, the primers also contained adapter sequences, allowing for uniform amplification of the library with high complexity ready for downstream NGS sequencing on Illumina MiSeq.

2.4. Data Analysis

Triplicate measurements for each treatment are represented as the mean and standard deviation. Data were analysed using SPSS version 19.0 and Origin Pro 2023. A *p*-value of <0.05 was considered to indicate statistical significance. RDA calculations were performed using Canoco 5.0 software.

3. Results and Discussion

3.1. The Effect of Hydrochar on Soil Properties and Crop Growth

The imposition of both duck farming biomass wastes showed improvements in the electrical conductivity (EC) of the topsoil compared to CF, with increases ranging from 16% to 44%, as shown in Table 2. Compared to the direct application of duck manure and litter (FM), the EC values were generally higher when hydrochar was applied, except in cases where hydrochar without duck manure had lower EC values. Among the treatments, the FH2 treatment showed the most significant increase. Although it has been reported that an increase in EC may lead to higher salt content in crops [10], usually it does not inhibit crop growth in a shorter cultivation period, and the more severe the soil salinisation, the higher the pH, whereas in the present study, no change in soil pH was observed in the treatment groups. Due to the nutrient retention and slow-release properties of biomass charcoal, it can be effectively used to fertilise and manage crops with long growth cycles [11]. Compared to CF and FM, the application of hydrochar increased the levels of total nitrogen, available phosphorus and exchangeable potassium in the soil. The gradient of total nitrogen, available phosphorus and exchangeable potassium content across the different hydrochar treatments followed the order FH1 < FH3 < FH2, indicating that the use of char-based fertilisers can improve soil nutrient availability compared to conventional fertilisers or the direct application of duck manure and litter.

The different treatments showed a consistent trend in biomass accumulation in both maize leaves and stems, as shown in Figure 1a, with the order CF < FH1 < FM < FH2 < FH3. Visual differences were also observed in the early growth stages of the maize plants (Figure 1b). Compared to CF and FM, the application of hydrochar as a charcoal-based fertiliser increased maize biomass, except in cases where hydrochar without duck manure components did not show a significant promoting effect on maize leaf and stem biomass. Research by Khosravi et al. [12] indicated that the application of sewage sludge-

derived hydrochar had minimal effect on soybean growth, while chicken manure-derived hydrochar significantly increased soybean yield by up to 66.2%. Therefore, the type of feedstock used for hydrochar production significantly influences crop productivity when used as a charcoal-based fertiliser, and selecting the appropriate type of hydrochar can effectively serve as a soil amendment to promote crop growth.

Table 2. Changes in soil chemical properties under different hydrochar treatments.

Targets	Treatment Group (Mean ± Standard Deviation)				
	CF	FM	FH1	FH2	FH3
EC (us/cm)	704.42 ± 265.53	886.67 ± 259.29	819.17 ± 213.23	1016.17 ± 173.18	993.50 ± 311.42
pH	6.51 ± 0.22	6.40 ± 0.06	6.42 ± 0.09	6.41 ± 0.07	6.34 ± 0.04
N (mg/kg)	49.08 ± 19.90	63.58 ± 19.04	67.17 ± 3.54	76.17 ± 15.62	71.00 ± 22.19
P_2O_5 (mg/kg)	69.00 ± 27.69	89.17 ± 27.18	96.83 ± 6.13	107.92 ± 22.23	99.83 ± 31.46
K_2O (mg/kg)	137.25 ± 53.86	177.92 ± 53.79	195.58 ± 12.44	216.17 ± 47.08	200.17 ± 62.34

Note: The CF, FM, FH1, FH2, FH3 represent chemical fertiliser alone, chemical fertiliser mixed with duck litter, chemical fertiliser mixed with litter-derived hydrochar, chemical fertiliser mixed with duck manure–litter-derived hydrochar and chemical fertiliser mixed with duck manure–litter–humic acid-derived hydrochar treatment groups, respectively.

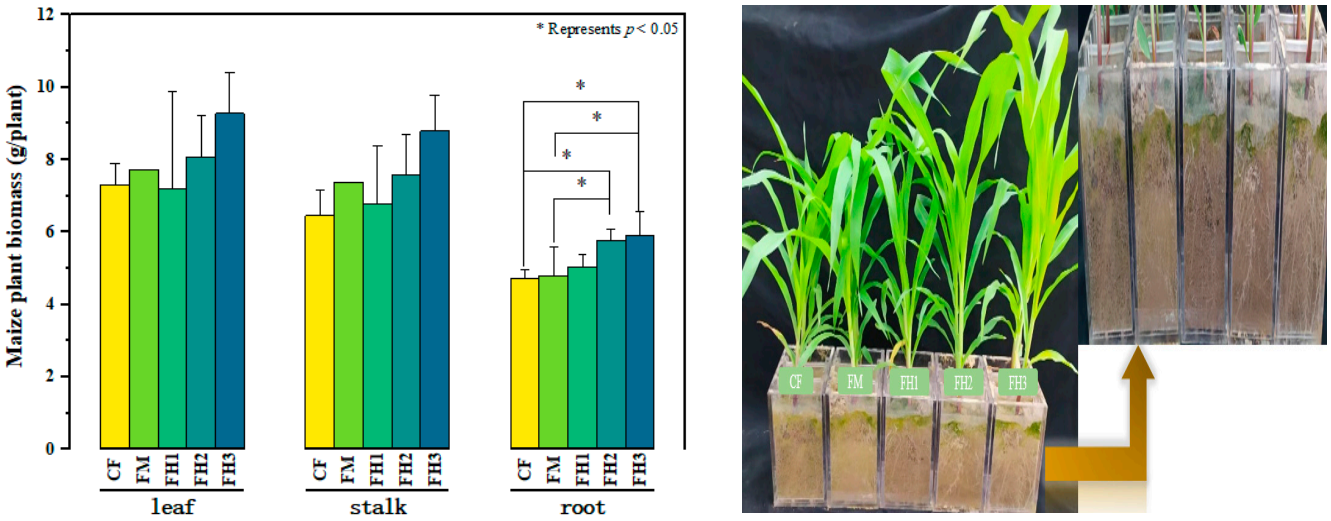

Figure 1. Changes in maize biomass (**a**) and maize seedling growth (**b**) under different hydrochar fertilisation treatments.

Observations of maize seedling roots revealed a positive interactive effect between hydrochar and plant root systems. Significant differences in maize root biomass between treatment groups were observed at the 0.05 significance level ($F = 3.536$, $p = 0.048$). Specific contrasts indicated that FH2 > CF, FH3 > CF, FH2 > FM and FH3 > FM, highlighting the significant promoting effect of duck manure–litter-derived hydrochar on maize root growth, especially when the hydrochar contained duck manure, litter and humic acid components. From a nutrient perspective, although the direct application of duck manure and litter to the soil increased nitrogen and phosphorus nutrient levels, dynamic nutrient uptake should also be considered. Research has shown that straw biochar application can increase both total inorganic and organic phosphorus in the soil, whereas direct straw application only increases total organic phosphorus [13]. In addition, the direct application of organic matter may not sustainably increase soil nitrogen supply capacity in the long term. This may explain why the enhancing effect of the direct application of duck manure and litter

on maize biomass is lower than the effect of using duck manure and litter carbonisation as a charcoal-based fertiliser.

3.2. Effects of Hydrochar on Soil Carbon Dynamics and Carbon Composition

The addition of hydrochar can alter soil carbon quality by changing organic carbon content and aromaticity. The incorporation of hydrochar into the soil has shown a significant effect in increasing soil organic carbon (SOC) content (Figure 2a). Due to the high organic matter content of biomass waste from duck farming, when mixed with fertiliser in the soil, it promotes plant growth and root exudation, thereby increasing soil carbon inputs [14]. Compared to fertiliser-only treatments, increases in soil organic carbon ranged from 78% to 253%. The direct application of duck manure and litter has limited potential to increase soil organic carbon compared to its carbonisated application. Over time, the unstable carbon components in duck manure and litter are gradually consumed and absorbed by microorganisms, promoting soil respiration and leading to a decrease in the rate of SOC increase [15]. Furthermore, the increase in organic carbon with different hydrochar treatments follows the order FH2 < FH1 < FH3, which is consistent with the different organic matter content in the different types of hydrochar used. Hydrochar with a stable internal structure can increase soil SOC content, reduce the SOC mineralisation rate and increase stability against surface oxidation and microbial degradation. This effect is attributed to organic molecules that enhance adsorption and polymerisation to form stable organic matter through surface catalytic activity [16]. In rice cultivation, Sun et al. [17] found that hydrochar reduced the proportion of unstable SOC in the soil by 33.6% to 15.6% while increasing the proportion of stable SOC by 10.3% to 27.0%. This is due to the interaction and association of hydrochar with soil organic matter components of different decomposition stabilities, which also affect the effectiveness of carbon sequestration in the soil [18]. Further understanding of the molecular composition of organic matter following the addition of hydrochar to soil is therefore required.

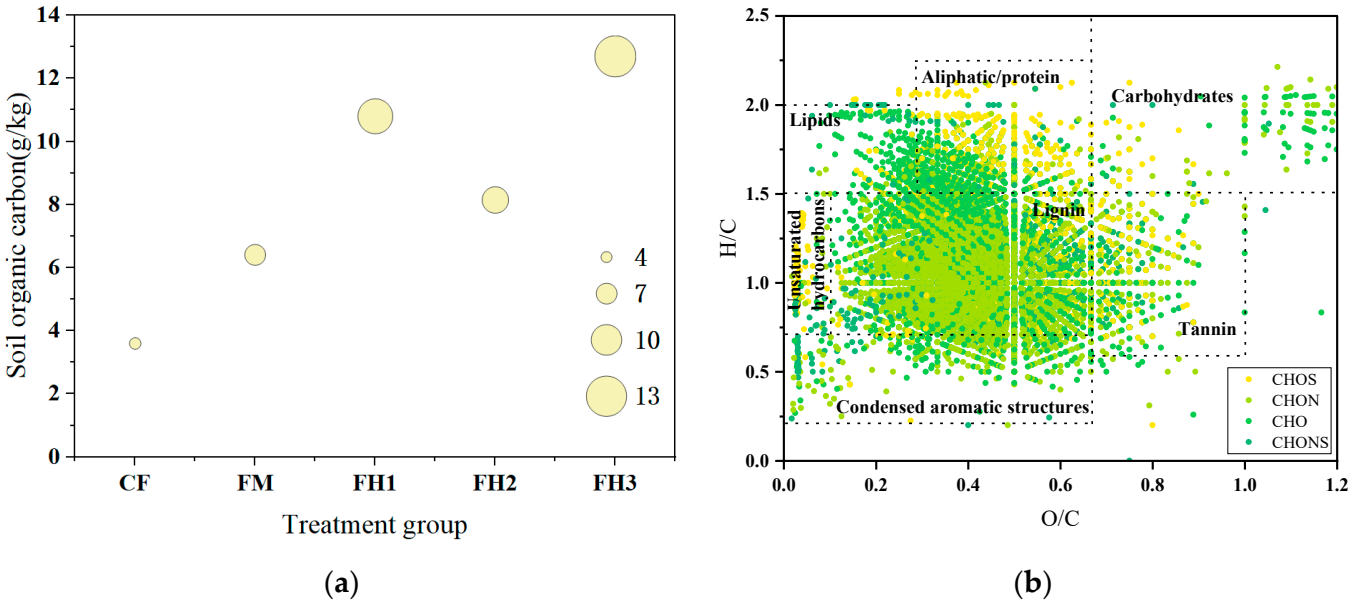

Figure 2. Changes in soil organic carbon (**a**) and the molecular composition of its DOM (**b**) under different hydrochar fertilisation treatments. The size of the circle represents the level of organic carbon content, all corresponding to the vertical coordinate and legend.

Dissolved organic matter (DOM) is a major component of soil organic matter and the most active carbon fraction in soil. Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) provides the highest resolution and mass accuracy to characterise the complex molecular composition of DOM, which was further used in the present

study to investigate organic carbon-related composition and structure after the addition of hydrochar derived from duck farm biomass waste to the soil. As shown in Figure 2b, the molecular composition of DOM is dominated by CHON-type molecular compounds, mainly distributed in regions containing lignin, carbohydrates and condensed aromatic structures, providing a good source of carbon and nitrogen for the soil. Sulphur-containing organic carbon compounds (CHOS-type) are mainly present in the soil as minor components of aliphatic/protein and unsaturated hydrocarbon compounds. Research by Ling et al. [19] indicates that Gemmatimonadetes, Bacteroidia, Alphaproteobacteria and Gammaproteobacteria are the dominant species in biomass char-amended soils, capable of utilising recalcitrant condensed aromatic and tannin compounds in DOM, while *Ramlibacter* are primarily involved in lignin utilisation. Bacilli and Clostridia mainly utilise easily degradable polysaccharides, amino acids and other DOM components. Therefore, the diverse composition of DOM after the addition of hydrochar derived from duck farm biomass waste can provide different soil microbial communities with the necessary materials to promote soil organic carbon transformation.

3.3. Effect of Hydrochar on the Particle Size Distribution of Soil Functional Groups and Agglomerates

The stimulatory effect of biomass char on soil carbon mineralisation is another important factor influencing soil carbon storage and sequestration functions. The potential inhibitory effect of hydrochar on soil organic carbon may involve mechanisms such as the adsorption of organic matter, organic–mineral interactions and aggregate formation [7], which are related to changes in functional groups and the distribution of soil aggregates.

The FTIR spectra of the soil under different treatments are shown in Figure 3. Peaks in the range of 3300–3600 cm^{-1}, attributed to -OH stretching vibrations, are commonly found in carbohydrates, proteins and amide compounds. It can be observed that the peak in the FM treatment, where duck manure and litter was applied directly to the soil, overlaps with the CF treatment without significant differences. In contrast to the CF and FM treatments, the addition of hydrochar derived from duck manure significantly increases the peak intensity in this region. However, the peak intensity in the soil treated with hydrochar derived only from litter shows a significant decrease, indicating that the organic matter in such hydrochar is easily degradable, leading to a reduction in hydroxyl groups. The same trend among the treatment groups extends to the peak at 2920 cm^{-1}, attributed to symmetrical stretching vibrations of O-H, alkyl C-H and ammonium N-H stretching vibrations, indicating an increase in hydroxyl and amine groups in the soil with the addition of hydrochar derived from duck manure. Similarly, peaks in the 1600–1700 cm^{-1} range show consistent trends in intensity across treatments, mainly due to the symmetric stretching of C=C in aromatic hydrocarbons and alkenes and the asymmetric stretching of C=O in amides, suggesting that duck manure-derived hydrochar enhances the aromatic structure of the soil by increasing carboxyl and aldehyde groups. The characteristic peak representing aromatic groups at 1458 cm^{-1} is also present in the soil of all treatment groups. Unlike the previous peaks, the direct application of duck manure and litter also increases the intensity of the peak at this point, possibly due to the promotion of soil humification by the application of duck manure and litter. The transmission peak at 1100 cm^{-1} is attributed to polysaccharides with C-O-C oxygen functional groups and the C-peak of aromatic C-H. Compared to the CF control group, both the FM group, in which the duck manure and litter was applied directly to the soil, and the FH1 group, in which hydrochar derived from the litter was added to the soil, show enhanced peak intensities at this point, with the FH3 treatment group showing the most significant enhancement. The peak at 750 cm^{-1}, attributed to aromatic C-H stretching vibrations, also indicates a higher carbon content in the soil due to the addition of duck manure-derived hydrochar. Overall, the addition of hydrochar derived from litter alone does not increase the abundance of functional groups in the soil, whereas hydrochar incorporating duck manure significantly enhances the aromatic

structure and oxygen functional groups in the soil, especially when both humic acid- and duck manure-derived hydrochar are present as soil amendments.

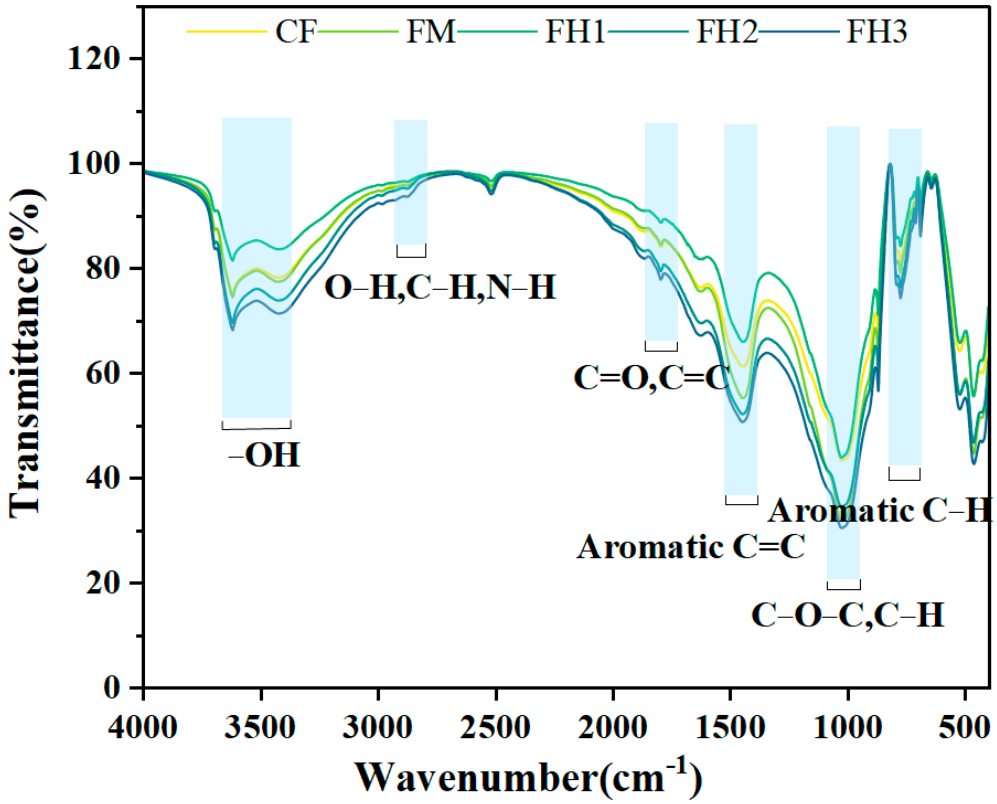

Figure 3. FTIR spectra of each soil under different hydrochar fertilisation treatments.

Soil aggregates are fundamental components of soil structure. Due to the absence of sticky duck manure components and the lower nutrient content, both the CF and FH1 treatments exhibit a sandy loam texture with an extreme distribution of aggregate particle sizes, consisting predominantly of microaggregates (Figure 4). Compared to the FM group, where the duck manure and litter was applied directly to the soil, the addition of hydrochar facilitated the formation of more macroaggregates in the soil, with the proportion of aggregates in the 0.02–2.0 mm particle size range showing the trend FH3 > FH1 > FH2 (Appendix A), consistent with the trends in soil organic carbon content across the groups. In general, an increase in soil aggregate particle size leads to an increase in soil organic carbon, and the increase in organic carbon content is beneficial for improving the soil aggregate structure [20], suggesting an interdependent relationship. On the one hand, organic carbon itself acts as a binding agent that can bind microaggregates into macroaggregates. On the other hand, the addition of hydrochar can indirectly promote the formation of soil humus, carbon compounds, aromatic hydrocarbons and other organic macromolecules, thereby increasing soil organic carbon [21]. Therefore, it can be inferred that the humic acid macromolecules present in the FH3 group may have contributed to some extent to the formation of macroaggregates. Furthermore, the introduction of hydrochar enriches the soil with more functional groups, thereby increasing the stability of soil aggregates. Functional groups on the surface of hydrochar, such as hydroxyl (-OH) and carboxyl (-COOH) groups, can induce cation–bridging interactions, which are primarily responsible for the formation of macroaggregates in soil [22].

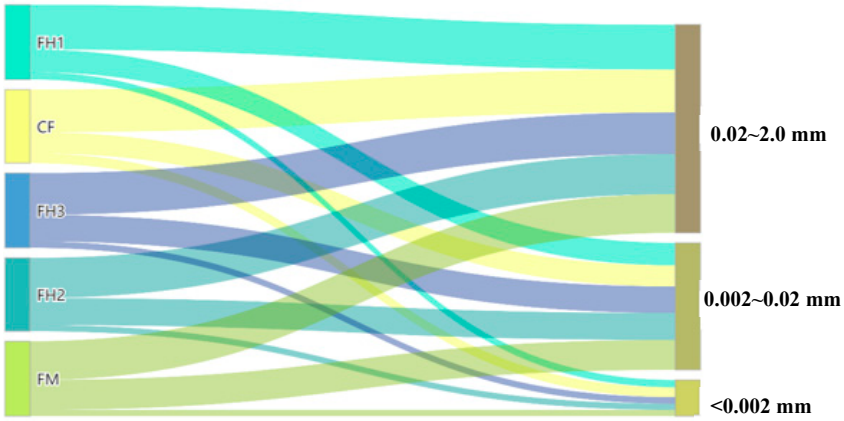

Figure 4. Distribution of soil aggregates under different hydrochar fertilisation treatments.

3.4. Effect of Hydrochar on the Distribution and Composition of Soil Microorganisms

The addition of hydrochar provides a degradable organic carbon source for soil microbes, which regulates microbial growth by enhancing substrate effectiveness, thereby altering soil microbial diversity [7]. In previous studies, the improvement in soil aggregation may be attributed to the addition of hydrochar, which enhances various organic substances produced by soil bacteria, fungi and plant roots, such as organic acids and lipids [23]. Further microbial sequencing analysis was therefore carried out. The coverage of soil samples with and without a hydrochar addition reached 100%, indicating that the 16S and ITS gene sequences were sufficient to accurately describe the diversity and richness of the bacterial and fungal microbial communities in the soil. The Chao1 and ACE (observed species) indices were primarily used to describe the quantity of microbial communities, with higher values, indicating greater species richness, while the Simpson and Shannon indices reflect the diversity and evenness of microbial community distribution. The specific assessment indices are shown in Table 3. For bacteria, the Simpson and Shannon indices were slightly lower in soils with added hydrochar than in soils without hydrochar, but the difference was not significant. However, for Chao1 and ACE, the values in soils with hydrochar were significantly higher than those without hydrochar, showing a clear advantage. Bacteria play a crucial role in the formation of soil macroaggregates and microaggregates, where cyanobacteria, as part of the surface soil biological crust community, can promote soil stability by producing EPS that act as aggregate binders [24]. In contrast, for fungi, all four indices were lower in the FH3 treatment group with added hydrochar than in the control soil. These results suggest that while hydrochar has a positive effect on bacterial abundance, it has a negative effect on bacterial diversity and both fungal abundance and diversity. This is consistent with reports on the effects of biomass char on soil bacterial and fungal diversity, such as the negative effect of hydrochar on AM fungi reported by [23], and the findings of Hu et al. [25] that soil bacterial diversity was higher in biochar amended soils, while biochar reduced soil fungal diversity.

Table 3. Bacterial and fungal abundance and diversity of soils without and with hydrochar application.

Treatment Group	Goods_Coverage	Richness Index		Diversity Index	
		Chao 1	ACE	Simpson	Shannon
		Bacteria			
CF	1.00	2168.79	2115.80	0.98	8.32
FH3	1.00	2533.12	2529.80	0.96	8.25
		Fungi			
CF	1.00	162.16	162.00	0.88	4.05
FH3	1.00	145.03	145.00	0.83	3.40

Note: The CF, FH3 represent chemical fertiliser alone and chemical fertiliser mixed with duck manure–litter–humic acid-derived hydrochar treatment groups, respectively.

A redundancy analysis (RDA) can better show the influence of one set of variables on another set of variables and then find the main factors of soil environment affecting soil microorganisms. As shown in Figure 5a, axis 1 explains 94.12% of the correlation between the 5 variables and bacterial communities; SOC explained 93.7% of the variation and was followed by the aggregate explaining 5.5% of the variation. According to RDA, SOC was the main factor affecting the relative abundance of soil bacteria. Further, observing that soil aggregates, the microbial community and organic carbon are more closely linked in RDA compared to other factors, it can be inferred that the application of hydrochar affects soil organic carbon by altering soil aggregates and the microbial bacterial community.

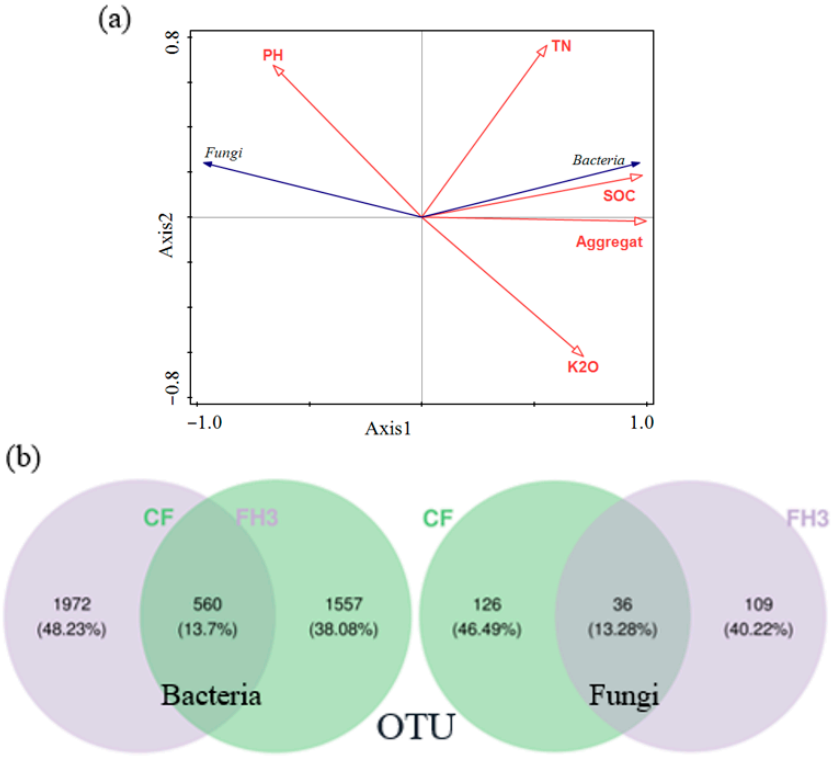

Figure 5. (**a**) RDA ranking map of correlation between soil microorganisms and soil physicochemical properties; (**b**) OTU Venn diagram of bacteria and fungi.

Appendices B and C show the abundance clustering dendrograms of operational taxonomic units (OTUs) for bacteria and fungi in soils with and without a hydrochar addition. Consistent with the microbial abundance data, the bacterial abundance values in the tested soil samples were much higher than the fungal abundance values, and the bacterial OTU dendrogram branches were denser than those of the fungi. In most branches of the bacterial clustering, both soils with and without added hydrochar shared common branches. Bacteria in hydrochar-amended soil were more concentrated in the terminal branches, whereas the proportion of bacteria in the terminal branches of soil without hydrochar was lower, confirming the role of hydrochar in increasing bacterial abundance in the soil. In contrast to bacteria, the out abundance clustering dendrograms for fungi in soil with and without hydrochar showed significant differences. Both treatments had distinct branches that participated separately, especially in the secondary branches, where soil without hydrochar had unique branches. In the tertiary branches with four forks, the fungal abundance in soils with added hydrochar disappeared in two of the branches. This suggests that the addition of hydrochar to the soil affects both bacteria and fungi, with fungi being more affected by hydrochar. Using Venn diagrams to compare the number of shared or unique OTUs between soil samples with and without hydrochar (Figure 5b), it was found that there were significant differences in the community abundance of bacteria

and fungi between soils with and without hydrochar, with only 13.7% and 13.28% of OTUs shared for bacteria and fungi, respectively.

In addition to changes in the abundance and diversity of bacteria and fungi in the soil, the addition of hydrochar also leads to changes in the composition of the microbial community structure. Figure 6 shows the relative abundance of bacterial and fungal community composition at the phylum and genus level in soils with and without a hydrochar addition. Looking at the bacterial phylum classification, the soil microbial community composition is mainly dominated by the phyla Proteobacteria, Actinobacteriota, Bacteroidota and Chloroflexi, with relatively high abundance. The addition of hydrochar to the soil had the most significant effect on the relative abundance of the phylum Actinobacteriota, which increased from 23.6% to 36.9%. In addition, the relative abundance of the phylum Bacteroidota increased by 1.6%, while the phylum Chloroflexi was compressed, reducing its relative abundance to 1.1%. This is consistent with the results of Sun et al. [17], where the phylum Chloroflexi was significantly reduced in soil after a hydrochar addition ($p < 0.05$). Overall, the addition of hydrochar to the soil resulted in relatively uniform changes in the abundance distribution of bacterial phyla, although the relative abundances tended to become more uneven. Figure 6b further illustrates the changes in bacterial composition at the genus level. At the genus level, the addition of hydrochar to the soil promoted a more even distribution of dominant bacterial genera, with most genera showing increased relative abundances, except for genera such as Sphingomonas and Herpetosiphon, which were adversely affected by hydrochar, leading to a decrease in their relative abundances.

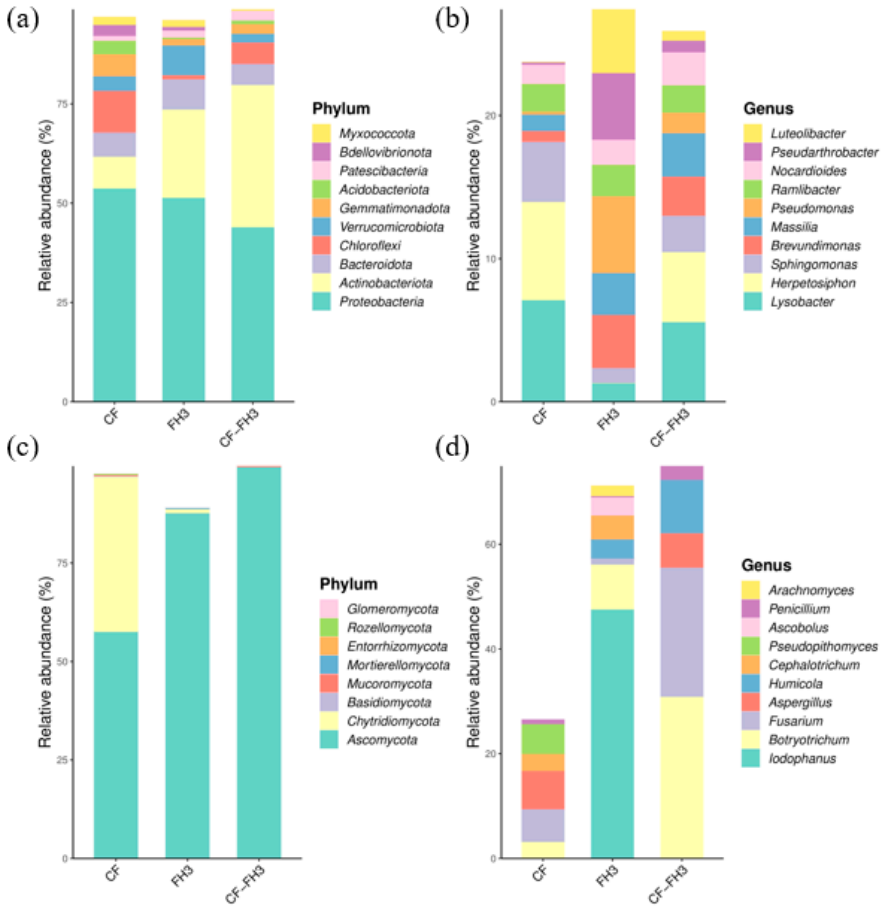

Figure 6. The relative abundance of microorganisms in soils without hydrochar (CF) and with hydrochar (FH3). (**a**) The relative abundance of bacteria at the phylum level; (**b**) The relative abundance of bacteria at the generic level; (**c**) The relative abundance of fungi at the phylum level; (**d**)The relative abundance of fungi at the genus level.

In the fungal realm, the relative abundance distribution at the phylum level is quite extreme, as shown in Figure 6c, with the phyla Ascomycota and Chytridiomycota accounting for 97.4% to 98.3% of the total phylum relative abundance. Hydrochar had a significant effect on the relative abundance distribution of these two phyla, increasing the relative abundance of Ascomycota from 85.4% to 97.2%, while decreasing the relative abundance of Chytridiomycota from 12.9% to 0.2%. Thus, the addition of hydrochar made Ascomycota the dominant phylum among soil fungi. Furthermore, at the genus level of fungal classification, as shown in Figure 6d, it is evident that the addition of hydrochar to the soil led to a significant reduction in the diversity of fungal genera, confirming the earlier conclusion that hydrochar has a negative effect on fungal abundance and diversity in the soil. It is worth noting that due to the humic acid composition of the added hydrochar, the presence of the genus *Humicola* was observed in the hydrochar-amended soil, together with significant increases in the relative abundance of genera such as *Botryotrichum* and *Iodophanus*.

4. Conclusions

In the hydrochar field-returning efficiency and utilisation system, hydrochar, as a carbon-based fertiliser, can greatly increase the supply of nutrients to the soil to promote crop growth, significantly increase soil organic carbon and improve the presence of aromatic structure and oxygen-containing functional groups in the soil, which are dominated by lignin, hydrocarbons and the condensed aromatic structure of the CHON type. At the same time, hydrochar favoured the formation of more macroaggregates and microbial bacterial community in the soil.

Author Contributions: Conceptualization, Z.Z. (Zhiping Zhu); Data curation, T.Y.; Formal analysis, T.Y.; Investigation, T.Y., Z.Z. (Zherui Zhang), Z.Z. (Zhe Zhang) and W.W.; Methodology, T.Y. and Z.Z. (Zhiping Zhu); Resources, Z.Z. (Zhiping Zhu); Supervision, Z.Z. (Zhiping Zhu); Validation, T.Y.; Writing—original draft, T.Y.; Writing—review and editing, D.L., T.Z. and Z.Z. (Zhiping Zhu). All authors have read and agreed to the published version of the manuscript.

Funding: This study was funded by the grant from the National Key Technology Research and Development Program of China [Grant number 2022YFE0115600], the National Waterfowl Industry Technology System of China [Grant number CARS-42-23], the National Key Technology Research and Development Program of China [Grant number 2023YFE0104700] and the National Natural Science Foundation of China [Grant Number 31401944].

Data Availability Statement: Research data are available upon request to the authors.

Conflicts of Interest: The authors declare no conflicts of interest.

Appendix A

Table A1. Soil aggregate content under different hydrochar fertilization treatments.

Treatment Groups	<0.002 mm (%)	0.002~0.02 mm (%)	0.02~2.0 mm (%)	Texturegrading
CF	12.51	26.79	54.42	Sandy loam
FM	8.00	38.05	49.24	Loam
FH1	9.39	28.65	52.11	Sandy loam
FH2	7.92	34.35	51.07	Loam
FH3	8.28	33.77	57.94	Loam

Appendix B

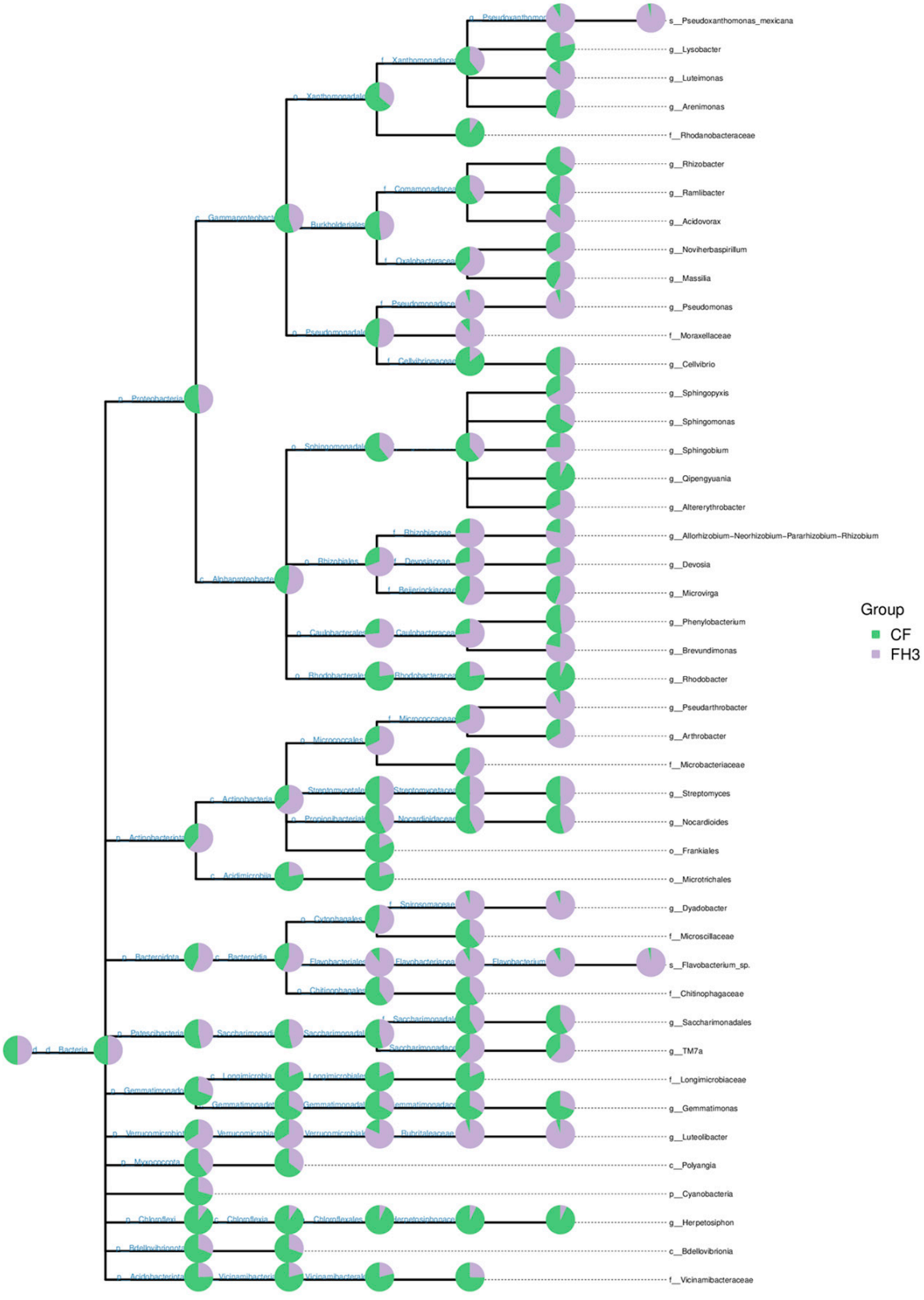

Figure A1. Clustered tree maps of bacteria in soils with and without hydrochar-applied soils.

Appendix C

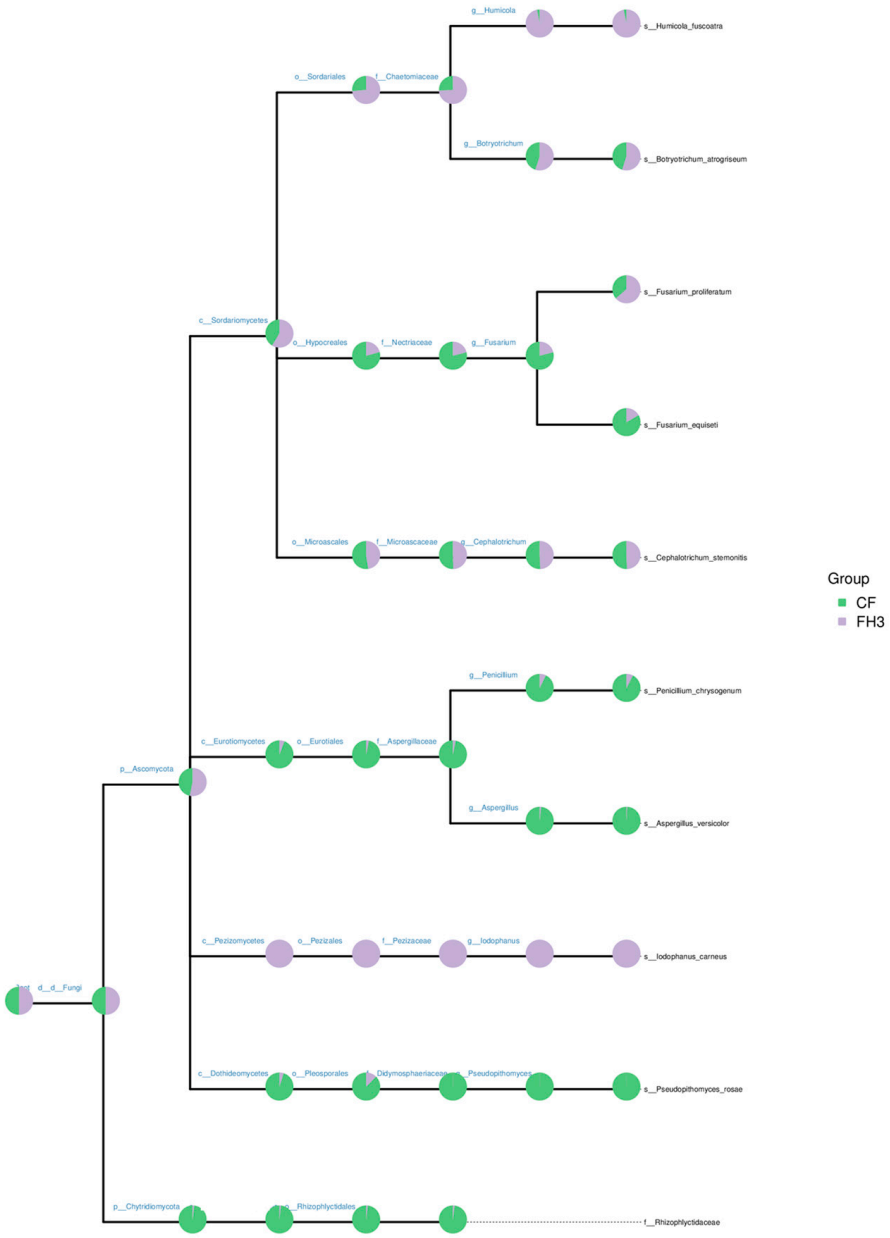

Figure A2. Clustered tree maps of fungi in soils with and without hydrochar-applied soils.

References

1. Jin, S.; Zhang, B.; Wu, B.; Han, D.; Hu, Y.; Ren, C.; Zhang, C.; Wei, X.; Wu, Y.; Mol, A.P. Decoupling livestock and crop production at the household level in china. *Nat. Sustain.* **2021**, *4*, 48–55. [CrossRef]
2. Qu, L.; Chai, T.; Guo, Z.; Zhang, Z.; Huang, Z.; Li, N. Studies on the airborne bacterial communities and antimicrobial resistance genes in duck houses based on metagenome and pcr analysis. *Poult. Sci.* **2024**, *103*, 103365. [CrossRef]
3. Khosravi, A.; Zheng, H.; Liu, Q.; Hashemi, M.; Tang, Y.; Xing, B. Production and characterization of hydrochars and their application in soil improvement and environmental remediation. *Chem. Eng. J.* **2022**, *430*, 133142. [CrossRef]
4. Haddix, M.L.; Paul, E.A.; Cotrufo, M.F. Dual, differential isotope labeling shows the preferential movement of labile plant constituents into mineral-bonded soil organic matter. *Glob. Change Biol.* **2016**, *22*, 2301–2312. [CrossRef]
5. Ngaba, M.J.Y.; Bol, R.; Hu, Y. Stable isotopic signatures of carbon and nitrogen in soil aggregates following the conversion of natural forests to managed plantations in eastern china. *Plant Soil* **2021**, *459*, 371–385. [CrossRef]
6. Chen, C.; Liu, W.; Jiang, X.; Wu, J. Effects of rubber-based agroforestry systems on soil aggregation and associated soil organic carbon: Implications for land use. *Geoderma* **2017**, *299*, 13–24. [CrossRef]

7. Chen, Y.; Sun, K.; Yang, Y.; Gao, B.; Zheng, H. Effects of biochar on the accumulation of necromass-derived carbon, the physical protection and microbial mineralization of soil organic carbon. *Crit. Rev. Environ. Sci. Technol.* **2024**, *54*, 39–67. [CrossRef]
8. Bento, L.R.; Castro, A.J.R.; Moreira, A.B.; Ferreira, O.P.; Bisinoti, M.C.; Melo, C.A. Release of nutrients and organic carbon in different soil types from hydrochar obtained using sugarcane bagasse and vinasse. *Geoderma* **2019**, *334*, 24–32. [CrossRef]
9. Puccini, M.; Ceccarini, L.; Antichi, D.; Seggiani, M.; Tavarini, S.; Hernandez Latorre, M.; Vitolo, S. Hydrothermal carbonization of municipal woody and herbaceous prunings: Hydrochar valorisation as soil amendment and growth medium for horticulture. *Sustainability* **2018**, *10*, 846. [CrossRef]
10. Fernández-Rodríguez, M.J.; Mancilla-Leytón, J.M.; Jiménez-Rodríguez, A.; Borja, R.; Rincón, B. Reuse of the digestate obtained from the biomethanization of olive mill solid waste (omsw) as soil amendment or fertilizer for the cultivation of forage grass (*Lolium rigidum* var. *Wimmera*). *Sci. Total Environ.* **2021**, *792*, 148465. [CrossRef]
11. Ibrahim, M.M.; Guo, L.; Zhang, H.; Wu, F.; Zou, S.; Xing, S.; Mao, Y. Comparative impact of bacillus spp. On long-term N supply and N-cycling bacterial distribution under biochar and manure amendment. *J. Soil Sci. Plant Nutr.* **2022**, *22*, 882–895. [CrossRef]
12. Khosravi, A.; Yuan, Y.; Liu, Q.; Zheng, H.; Hashemi, M.; Tang, Y.; Xing, B. Hydrochars as slow-release phosphorus fertilizers for enhancing corn and soybean growth in an agricultural soil. *Carbon Res.* **2024**, *3*, 7. [CrossRef]
13. Cao, D.; Lan, Y.; Liu, Z.; Yang, X.; Liu, S.; He, T.; Wang, D.; Meng, J.; Chen, W. Responses of organic and inorganic phosphorus fractions in brown earth to successive maize stover and biochar application: A 5-year field experiment in northeast china. *J. Soils Sediments* **2020**, *20*, 2367–2376. [CrossRef]
14. Chew, J.; Zhu, L.; Nielsen, S.; Graber, E.; Mitchell, D.R.G.; Horvat, J.; Mohammed, M.; Liu, M.; van Zwieten, L.; Donne, S.; et al. Biochar-based fertilizer: Supercharging root membrane potential and biomass yield of rice. *Sci. Total Environ.* **2020**, *713*, 136431. [CrossRef] [PubMed]
15. Sagrilo, E.; Jeffery, S.; Hoffland, E.; Kuyper, T.W. Emission of co 2 from biochar-amended soils and implications for soil organic carbon. *Gcb Bioenergy* **2015**, *7*, 1294–1304. [CrossRef]
16. Zhang, M.; Riaz, M.; Zhang, L.; El-Desouki, Z.; Jiang, C. Biochar induces changes to basic soil properties and bacterial communities of different soils to varying degrees at 25 mm rainfall: More effective on acidic soils. *Front. Microbiol.* **2019**, *10*, 1321. [CrossRef]
17. Sun, K.; Han, L.; Yang, Y.; Xia, X.; Yang, Z.; Wu, F.; Li, F.; Feng, Y.; Xing, B. Application of hydrochar altered soil microbial community composition and the molecular structure of native soil organic carbon in a paddy soil. *Environ. Sci. Technol.* **2020**, *54*, 2715–2725. [CrossRef] [PubMed]
18. de Jager, M.; Schröter, F.; Wark, M.; Giani, L. The stability of carbon from a maize-derived hydrochar as a function of fractionation and hydrothermal carbonization temperature in a podzol. *Biochar* **2022**, *4*, 52. [CrossRef]
19. Ling, L.; Luo, Y.; Jiang, B.; Lv, J.; Meng, C.; Liao, Y.; Reid, B.J.; Ding, F.; Lu, Z.; Kuzyakov, Y. Biochar induces mineralization of soil recalcitrant components by activation of biochar responsive bacteria groups. *Soil Biol. Biochem.* **2022**, *172*, 108778. [CrossRef]
20. Sun, Z.; Zhang, Z.; Zhu, K.; Wang, Z.; Zhao, X.; Lin, Q.; Li, G. Biochar altered native soil organic carbon by changing soil aggregate size distribution and native soc in aggregates based on an 8-year field experiment. *Sci. Total Environ.* **2020**, *708*, 134829. [CrossRef]
21. Liu, S.; Kong, F.; Li, Y.; Jiang, Z.; Xi, M.; Wu, J. Mineral-ions modified biochars enhance the stability of soil aggregate and soil carbon sequestration in a coastal wetland soil. *Catena* **2020**, *193*, 104618. [CrossRef]
22. Heikkinen, J.; Keskinen, R.; Soinne, H.; Hyväluoma, J.; Nikama, J.; Wikberg, H.; Källi, A.; Siipola, V.; Melkior, T.; Dupont, C. Possibilities to improve soil aggregate stability using biochars derived from various biomasses through slow pyrolysis, hydrothermal carbonization, or torrefaction. *Geoderma* **2019**, *344*, 40–49. [CrossRef]
23. George, C.; Wagner, M.; Kücke, M.; Rillig, M.C. Divergent consequences of hydrochar in the plant–soil system: Arbuscular mycorrhiza, nodulation, plant growth and soil aggregation effects. *Appl. Soil Ecol.* **2012**, *59*, 68–72. [CrossRef]
24. Philippot, L.; Chenu, C.; Kappler, A.; Rillig, M.C.; Fierer, N. The interplay between microbial communities and soil properties. *Nat. Rev. Microbiol.* **2024**, *22*, 226–239. [CrossRef]
25. Hu, L.; Cao, L.; Zhang, R. Bacterial and fungal taxon changes in soil microbial community composition induced by short-term biochar amendment in red oxidized loam soil. *World J. Microbiol. Biotechnol.* **2014**, *30*, 1085–1092. [CrossRef]