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Article

The Effect of Mineral-Based Soil Conditioners on the Improvement of Acidified Soils and Microbial Communities

Jina Ding [1,2,3], Yaqi Zhang [4,5], Jia Liu [6], Kailou Liu [7], Shuang Wang [2,3], Sang Yoon Kim [8], Yakov Kuzyakov [9], Zhenke Zhu [2,3], Tida Ge [2,3], Zhaofeng Yuan [2,3,*]

1 Ningbo Key Laboratory of Agricultural Germplasm Resources Mining and Environmental Regulation, College of Science and Technology, Ningbo University, Ningbo 315300, China; dingjina@nbu.edu.cn
2. State Key Laboratory for Quality and Safety of Agro-Products, Key Laboratory of Biotechnology in Plant Protection of MARA, Zhejiang Key Laboratory of Green Plant Protection, Ningbo University, Ningbo 315211, China; wangshuang1@nbu.edu.cn, zhuzhenke@nbu.edu.cn, getida@nbu.edu.cn
3 International Science and Technology Cooperation Base for the Regulation of Soil Biological Functions and One Health of Zhejiang Province, Ningbo University, Ningbo 315211, China
4 Department of Resource Science, College of Environmental & Resource Sciences, Zhejiang University, Hangzhou 310058, China; yaqi.zhang2@uq.net.au
5 ZJU-Hangzhou Global Scientific and Technological Innovation Center, Hangzhou 311200, China
6 Institute of Soil and Fertilizer & Resource and Environment, Jiangxi Academy of Agricultural Sciences, Nanchang 330200, China; liujia@jxaas.cn
7 Jiangxi Institute of Red Soil and Germplasm Resources, Nanchang 330046, China; liukailou@163.com
8 Department of Agricultural Chemistry & Interdisciplinary Program in IT-Bio Convergence System, Sunchon National University, Suncheon 57922, Republic of Korea; sykim@scnu.ac.kr
9 Department of Soil Science of Temperate Ecosystems, Department of Agricultural Soil Science, University of Göttingen, Göttingen 37077, Germany; ykuzyakov@yandex.com
*** Corresponding author:** Yuanzhaofeng@nbu.edu.cn

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Abstract: Soil acidification impairs nutrient availability, microbial structure and biogeochemical cycling, particularly in intensively cultivated tropical soils. Mineral-based soil conditioners have emerged as a potential solution, yet their effects on soil microbial networks and greenhouse gas emissions remain underexplored. Here, we evaluated four mineral-derived conditioners—a mineral blend (MB), calcined (MC), water-quenched (WQ), and commercial product (SC)—applied to acidic banana soil (pH 4.76) in a 100-day laboratory incubation. We assessed soil pH, exchangeable acidity, CO_2 and N_2O emissions, extracellular enzyme activities, and microbial community structure using 16S/ITS sequencing and co-occurrence network analysis. All conditioners significantly increased soil pH (to 6.06–7.25) and reduced exchangeable H^+ and Al^{3+} (>85%). MB- and SC-treated soils markedly enhanced CO_2 emissions, while MC- and WQ-treated soils reduced N_2O emissions by 27.00% and 20.44%, respectively. Enzyme activities (β-glucosidase, N-acetylglucosaminidase) and microbial biomass carbon increased at early stages, especially in MB- and WQ-treated soils. Microbial diversity and network complexity were significantly enhanced by MC- and WQ-treated soils. Bacterial communities in these treatments exhibited higher modularity and positive associations, suggesting increased microbial connectivity and ecological resilience. Fungal networks responded more variably across treatments. Our results highlight the potential of mineral-based conditioners to alleviate soil acidity, regulate microbial-driven nutrient cycling, and mitigate N_2O emissions. These findings provide mechanistic insights into microbial responses under acid stress and offer sustainable options for managing degraded soils.

Keywords: soil conditioners; soil microorganisms; high-throughput sequencing; microbial symbiotic network

1. Introduction

Soil acidification is a major cause of soil degradation and crop yield decline, particularly in tropical and subtropical agroecosystems [1]. It disrupts biogeochemical cycling by altering soil physicochemical properties [2], reducing organic matter content [3], increasing Al^{3+} toxicity, and suppressing microbial activity and diversity [4,5]. These changes weaken nitrogen transformation, promote greenhouse gas (GHG) emissions such as CO_2 and N_2O [6], and contribute to feedback loops of soil acidification and biological degradation [7]. The causes of soil acidification include intensive fertilization with ammonium salts, depletion of base cations by crop removal, and organic acid accumulation from residue decomposition [8]. Addressing these effects requires soil amendments that enhance nutrient retention, microbial functionality, and soil structural stability [9,10].

To mitigate acidification, various soil amendments such as lime, straw, and organic matter have been used to raise pH and enhance fertility [11,12]. However, these materials often have short-term buffering capacity and may lead to nutrient leaching or secondary salinization when overapplied [13–15]. Mineral-derived soil conditioners—composed of natural silicate and carbonate-rich materials such as phosphate rock, dolomite, and feldspar—offer an alternative with higher long-term effectiveness [16]. These materials gradually release alkaline ions and nutrients (e.g., Ca^{2+}, Mg^{2+}), thereby improving pH, soil buffering capacity, and microbial microhabitats [17]. Grinding silicate into small particles and dispersing them in the soil to enhance weathering and release nutrients—although these methods are simple, their effectiveness is very limited. Research indicates that olivine has a dissolution rate of only 8% over two years, whereas calcined silicate products can significantly accelerate reaction rates in the soil environment [18].

Although previous research has demonstrated the role of mineral conditioners in improving soil chemical properties and enzyme activity [19,20], their effects on soil microbial community structure, network stability, and functional responses remain inadequately studied, particularly regarding differences in regulatory mechanisms among different thermal processing methods (e.g., calcination and water quenching). Microbial co-occurrence networks reflect ecosystem stability and microbial cooperation under stress [21], yet few studies have investigated how these networks respond to soil amendments in acidified soils.

To address this gap, we evaluated four mineral-derived conditioners—a mineral blend (MB), calcined (MC), water-quenched (WQ), and a commercial product (SC)—in a 100-day incubation experiment using acidified banana soil (pH 4.76). We analyzed soil pH, exchangeable acidity, nutrient dynamics, GHG emissions (CO_2, N_2O), and microbial community structure. Our objectives were to: (1) evaluate the influence of conditioners on pH and GHG fluxes; (2) assess microbial biomass, enzyme activities, and diversity; and (3) explore microbial network complexity as a proxy for functional stability.

2. Materials and Methods

2.1. Preparation and Characterizations of Soil Conditioners

The raw materials for soil conditioning agents, including phosphate rock, dolomite, olivine, potassium feldspar and plagioclase were purchased from Hangjing Biotechnology Company and passed through a 200 mesh sieve. The raw materials, including 35% phosphate rock, 20% dolomite, 21% olivine, 20% potassium feldspar, and 4% plagioclase, were blended in the specified proportions, and then the mixture was divided into three portions. The first portion was directly used as mineral blending of soil conditioner (MB). For the second portion, the mixture was subjected to calcination at 1000 ℃ for 10 minutes in a muffle furnace, namely mineral calcination (MC). The third portion underwent a process where the minerals sintered at 1000 ℃ for 10 minutes were rapidly immersed in water when the temperature naturally cooled to 500 ℃, and subsequently dried in an oven at 60 ℃ (WQ). To assess the fertility of the three prepared conditioners, commercially available soil conditioners are employed as controls (SC).

The surface morphology and microstructure of the resulting samples were investigated by means of field-emission scanning electron microscopy (FE-SEM, Hitachi, Tokyo, Japanese). The particle size distribution was determined using a particle size analyzer (Mastersizer 3000, Malvern Panalytical Limited, Worcestershire, UK). Soil conditioners produced through various processes underwent continuous leaching to analyze their chemical composition. First, 0.5 g of each conditioner sample was placed in a 50 mL centrifuge tube. Subsequently, 40 mL of boiled ultrapure water was added, and the mixture was shaken thoroughly with the lid secured. The samples were then oscillated at 200 rpm for 30 minutes. After natural settling, the tubes were centrifuged at 4000 rpm for 10 minutes. The supernatant was then collected for analysis. Extract the mineral conditioner using ammonium acetate by adding 40 mL of the solution and shaking at 200 rpm for 30 minutes. Centrifuge at 4000 rpm for 10 minutes. Transfer the supernatant to a volumetric flask. Wash the residue three times with water, centrifuging each time. Combine the supernatants in the volumetric flask and mix thoroughly to prepare the solution for measurement. The leaching process for mineral conditioner using a 0.5 mol/L HCl solution mirrors that of ammonium acetate. Potassium, calcium, and magnesium concentrations were measured via atomic absorption spectrometry (ZEEnit700P, Analytik Jena, Jena, Germany), while phosphorus was quantified using a colorimetric method.

2.2. Soil Sampling

The lateritic soil (0–20 cm) was collected from banana plantation land in Jinjiang Village, Jiangxi Town, Jiangnan District, Nanning, Guangxi Province (22° 45′ N, 108° 6′ E). Basic soil physiochemical properties include a pH of 4.76 (1:2.5, soil-to-water), total C of 15.64 $g \cdot kg^{-1}$, total N of 1.75 $g \cdot kg^{-1}$, dissolved organic C (DOC) of 41.14 $mg \cdot kg^{-1}$, total N (TN) of 0.99 $g \cdot kg^{-1}$, total P (TP) of 1.23 $g \cdot kg^{-1}$, and exchangeable acid of 2.53 $cmol \cdot kg^{-1}$. Before the initial experiment, the soil was sieved and homogenized using a 4 mm mesh, then adjusted the water content of soil to 40% of the maximum

water holding capacity in the field and pre-incubated in darkness at 25 °C for 2 weeks to stimulate microbial activity.

2.3. Experimental Design and Soil Incubation

The experiment consisted of five treatments with 3 replicates each. The treatments were as follows: (1) no additions (soil only, CK), (2) MB, (3) MC, (4) WQ and (5) SC. In this study, the water content of soil was adjusted to 60% of maximum water holding capacity in the field, then a mixture of 60 g soil (according to dry soil) and 0.6 g soil conditioner was placed in a 250 mL aseptic sampling bottle. To minimize evaporation, the bottle cap was loosely fitted, and water loss was compensated every two to three days using deionized water, determined by weighing. All bottles were incubated at 25 °C in darkness up to 100 days. Soil samples were destructively collected after incubation for 1, 7, 15, 45, and 100 days to analyze the soil properties.

Another the same treatment experiment, including 20 g soil (according to dry soil) and 0.2 g soil conditioners, were placed in a 500 mL mineralized bottle for gas collection. A group of empty bottles was set as the blank group, and the other experimental treatment conditions were consistent with the soil collection. Headspace gas (30 mL) was sampled after 1, 2, 4, 5, 7, 9, 11, 13, 15, 20, 25, 30, 40, 55, 70, 85 and 100 days of incubation with a gas-tight syringe. After collecting the air, the vacuum pump was used to change the air in the mineralized bottle and placed outdoors for 30 minutes to re-air the mineralized bottle, then installed a rubber plug to seal the mineralized bottle and continued the culture.

2.4. Analysis of Soil Properties and CO_2 and N_2O Sampling

The soil pH was measured at a ratio of 1:2.5 (soil-to-distilled water, w/v). The NH_4^+ and NO_3^- were extracted using 0.05 mol·L^{-1} K_2SO_4 shaken for 0.5 h at 180 rpm and quantified by a flow analyser (AutoAnalyzer 3, SEAL Analytical Ltd., Norderstedt, Germany) [20]. Olsen P was extracted using 0.5 mol ·L^{-1} $NaHCO_3$ and analyzed by a flow analyzer. The microbial biomass carbon (MBC) was determined using the chloroform-fumigation-extraction method [22]. Both the MBC and DOC were determined by a carbon automatic analyzer (TOC 2500, Shimadzu, Kyoto, Japan). Both the total carbon (TC) and total nitrogen (TN) were determined by an element analyzer (FlashSmart, Thermo Fisher, Milan, Italy). The exchangeable acid, exchangeable H^+ and exchangeable Al^{3+} in soil were determined by 1 mol·L^{-1} KCl exchange-neutralization titration [23]. The activities of β-glucosidase (BG) and chitinase (NAG) and phosphatase (AP) in soils were determined by a 96-microporous plate fluorescence assay. The CO_2 and N_2O concentrations were measured by an Agilent 8890A gas chromatograph (Agilent Technologies, Palo Alto, CA, USA).

2.5. DNA Extraction and High-throughput Amplicon Sequencing

DNA was extracted from 0.25 g of fresh soil samples using the Qiagen DNeasy PowerSoil Kit (Mo Bio, Carlsbad, CA, USA) following the manufacturer's protocols. The concentration and quality of genomic DNA were assessed using a Nanodrop ND-100 spectrophotometer (Thermo Scientific, Wilmington, NC, USA). The qualified DNA samples were sent to Guangdong MeGG Gene Technology Co., Ltd. for high-

throughput sequencing. The soil bacterial 16S V4 region and fungal ITS1 region were amplified using the bar-coded, as described in S1.

The QIIME2 platform was employed for data splicing and quality control. Original sequence data underwent quantitative filtering using q2 demux (DADA2). Bacterial species were annotated through 100% similarity clustering with the SILVA 132 database (https://www.arb-silva.de/), while fungal species were annotated using the UNITE database (https://unite.ut.ee/).

2.6. Statistical Analysis

The normality and homogeneity of variance were checked using Shapiro–Wilk's and homogeneity of variance tests. Statistical analysis was performed using IBM SPSS Statistics (SPSS Inc., Chicago, IL, USA) and R version 4.1.2. One-way analysis of variance (ANOVA) was used to assess the difference in CO_2, N_2O emissions, exchangeable acids, chemical elements, soil enzyme activity, richness index and Shannon index.

For the microbial analysis, α-diversity (bacterial and fungal communities for all treatments) was presented by Shannon index and richness index. Principal coordinates analysis (PCoA) was conducted based on the Bray–Curtis distance matrices to illustrate the microbial community structures. Spearman correlation coefficients between the selected OTUs were calculated by 'psych' package in R. A valid co-occurrence was defined as a strong correlation between OTUs when the correlation coefficient ® exceeded 0.8 or was below −0.8, with p-values < 0.01, adjusted using the Benjamini–Hochberg method. The co-occurrence networks were visualized using Gephi 0.9.2 software (The Gephi Consortium, Paris, France). A distance-based redundancy analysis (db-RDA) was performed to analyze the relationship between the microbial community structure, CO_2, N_2O and soil physicochemical characteristics in response to different soil conditioners. The above analyses and insight were all conducted in the 'vegan' and 'ggplot2' package in R.

3. Results

3.1. Morphological and Physicochemical Characteristics of Soil Conditioners

The morphology of mineral raw materials undergoes significant changes after different processing techniques. The MB-treated conditioner exhibits large fragmented pieces with sharp edges (Figure 1a,b). After MC treatment, the particles presented an irregular spherical shape with collapse phenomena and porous structures on the surface compared to MB treatment (Figure 1c,d). The WQ-treated conditioner exhibited thin sheet and wrinkled honeycomb structures, presenting substantial structural changes compared to the MC-treated conditioner (Figure 1e,f). The XRD patterns of both the MC and WQ-treated conditioners exhibit significant distinction compared to those of MB-treated sample (Figure 1g). In addition, it can be found that the CaO, MgO, and Ca and Mg phosphate complexes are produced by the MC or WQ treatment, enhancing their application to soil for crop uptake. The grain size of the

MC-treated conditioner slightly increased compared to the MB-treated conditioner, while the grain size of WQ-treated conditioner significantly decreased (Figure 1h).

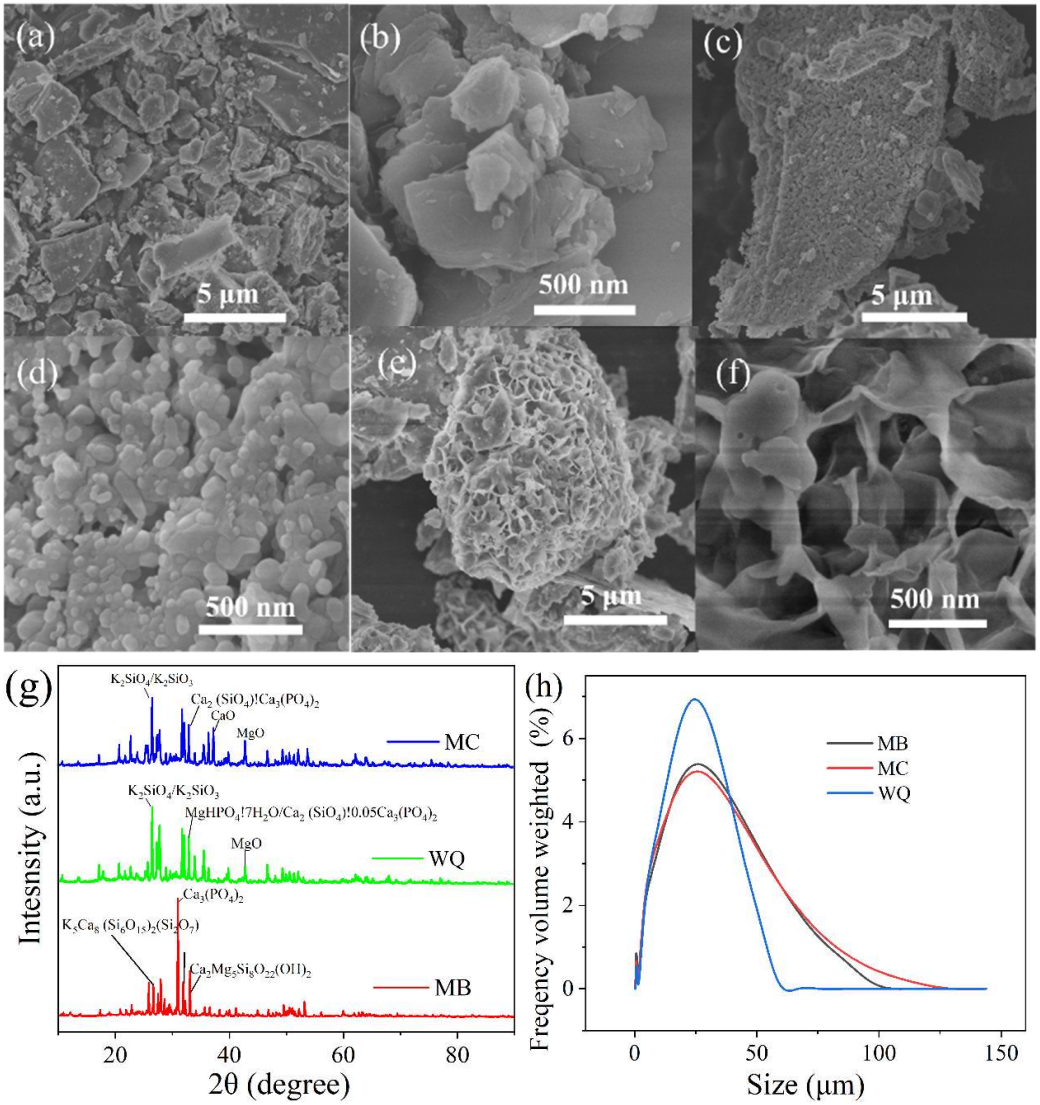

Figure 1. SEM morphology of different soil conditioners, MB treatment **(a,b)**, MC treatment **(c,d)**, WQ treatment **(e,f)**. The XRD patterns of different soil conditioners **(g)**, and particle size distribution of soil conditioners **(h)**.

Different extractants were used to sequentially extract the soil conditioners, with water-soluble extractants removing readily available nutrients, salt solutions extracting potentially available nutrients, and acid solutions extracting nutrients that were less accessible to soil microorganisms and plants. The water-soluble K content from WQ-treated soil conditioner showed a 276.12% increase compared to MB (Table 1). The water-soluble and salt-soluble Ca content from MC and WQ were significantly higher than those from MB. Salt-soluble Mg content increased by 3219% in MC-treated soil conditioner and by 2405% in WQ-treated soil conditioner compared to MB. All soil conditioners showed similar pH values, while the TC content in MB-treated sample was significantly higher than that in other treated samples.

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Table 1. Trace element content of different soil conditioners by continuous leaching and total element content.

	Water-Soluble Element Content ($mg \cdot kg^{-1}$)			**Salt-Soluble Element Content ($mg \cdot kg^{-1}$)**			**Acid-Soluble Element Content ($mg \cdot kg^{-1}$)**			**Total Element Content ($g \cdot kg^{-1}$)**					
	K	Ca	Mg	K	Ca	Mg	K	Ca	Mg	TK	TCa	TMg	TC	TP	pH
MB	29.78±2.36^{b}	324.00±26.7^{c}	90.96±18.43^{a}	211.3±6.35^{a}	567±76.52^{c}	21.32±6.54^{c}	22.72±3.2^{b}	3767±252.99^{a}	1294.00±67.84^{a}	2.62±0.32^{ab}	8.21±0.36^{a}	2.18±0.09^{a}	31.42±1.4a	48.98±1.2^{b}	12.48±0.08^{a}
MC	0.648±0.02^{c}	1722±97.14^{b}	3.464±0.10^{b}	199.7±12.52^{a}	1105±121.3^{a}	707.71±45.52^{a}	37.55±1.5^{a}	3588±307.52^{b}	364.62±83.52^{c}	2.98±0.03^{a}	7.16±0.10^{b}	2.05±0.05^{a}	12.98±0.2^{b}	49.65±2.1^{b}	12.48±0.4^{a}
WQ	112.11±13.0^{a}	2330±196.2^{a}	4.79±0.75^{b}	210.8±21.52^{a}	996±87.83^{b}	534.01±32.14^{b}	21.21±0.6^{b}	1957±138.53^{c}	538.04±18.43^{b}	2.2±0.17^{b}	6.67±0.20^{c}	1.67±0.17^{b}	13.27±0.2b	51.12±3.0^{a}	12.41±0.09^{a}

Error bars represent the standard deviation of the mean (n = 3). Different letters indicate significant differences ($p < 0.05$).

3.2. Gas Emission Dynamics: CO_2 and N_2O

CO_2 and N_2O emission rates varied across treatments during the 100-day incubation period (Figure 1c, Figure 2a). All four soil conditioner treatments exhibited higher CO_2 emission rates than the control (CK) in the early stage of incubation. The CO_2 emission rate peaked on day 1 in MB- and SC-treated soils, and on day 2 in MC- and WQ-treated soils. After the peak, emission rates declined rapidly and stabilized after approximately 25 days. Cumulative CO_2 emissions increased in all treatments compared to CK (Figure 2b). Specifically, MC, WQ, MB, and SC treatments increased cumulative CO_2 by 21.47%, 25.83%, 61.04%, and 92.56%, respectively. The cumulative CO_2 emissions from MB and SC were significantly higher than those from MC and WQ ($p < 0.05$).

N_2O emissions were unstable in the first 20 days of incubation but became relatively stable in the later stages (Figure 2c). From day 15 onward, all conditioner treatments showed lower N_2O fluxes compared to CK. The cumulative N_2O emissions (Figure 2d) decreased by 27.00% in MC-treated soil and by 20.44% in WQ-treated soil. In contrast, MB-treated soil showed a 101.11% increase in cumulative N_2O compared to CK. SC treatment did not significantly differ from the control in cumulative N_2O emissions.

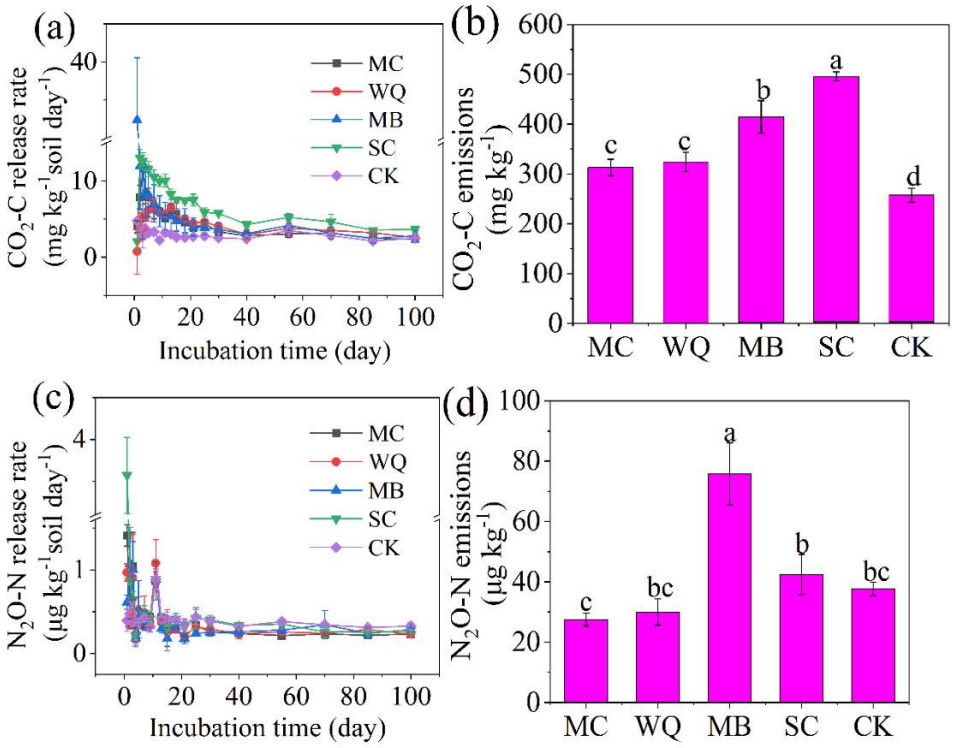

Figure 2. CO_2-C emission fluxes **(a)**, cumulative CO_2-C emissions **(b)**, N_2O-N emission fluxes **(c)**, and cumulative N_2O-N emissions **(d)** from soil during incubation. Different letters indicate significant differences ($p < 0.05$). Error bars represent the standard deviation of the mean ($n = 3$).

3.3. Soil Property

Throughout the culture period, the pH of conditioner-treated soils was consistently higher than that of the CK-treated soil, especially the SC-treated with the

largest increase (Figure 3a). From the starting to day 15, pH in the MC, WQ, MB, and SC-treated soils increased from 4.86 to 6.50, 6.39, 6.06 and 7.25, respectively, and then stabilized. Conversely, the CK-treated soil showed a negligible pH change. The soil conditioner treatments and culture time had significant effects on the soil pH. After 100 days, the substantial reductions can be observed for the conditioner-treated soils in total exchangeable acidity, exchangeable Al^{3+}, and exchangeable H^{+}, decreasing from 79.62% to 89.27%, from 87.84% to 97.97%, and from 64.26% to 89.59%, respectively, with no significant differences among conditioners (Figure 3b). The great reduction in the exchangeable H^{+} and Al^{3+} occurred in the SC-treated soil, significantly lower than the other-treated soils ($p < 0.05$).

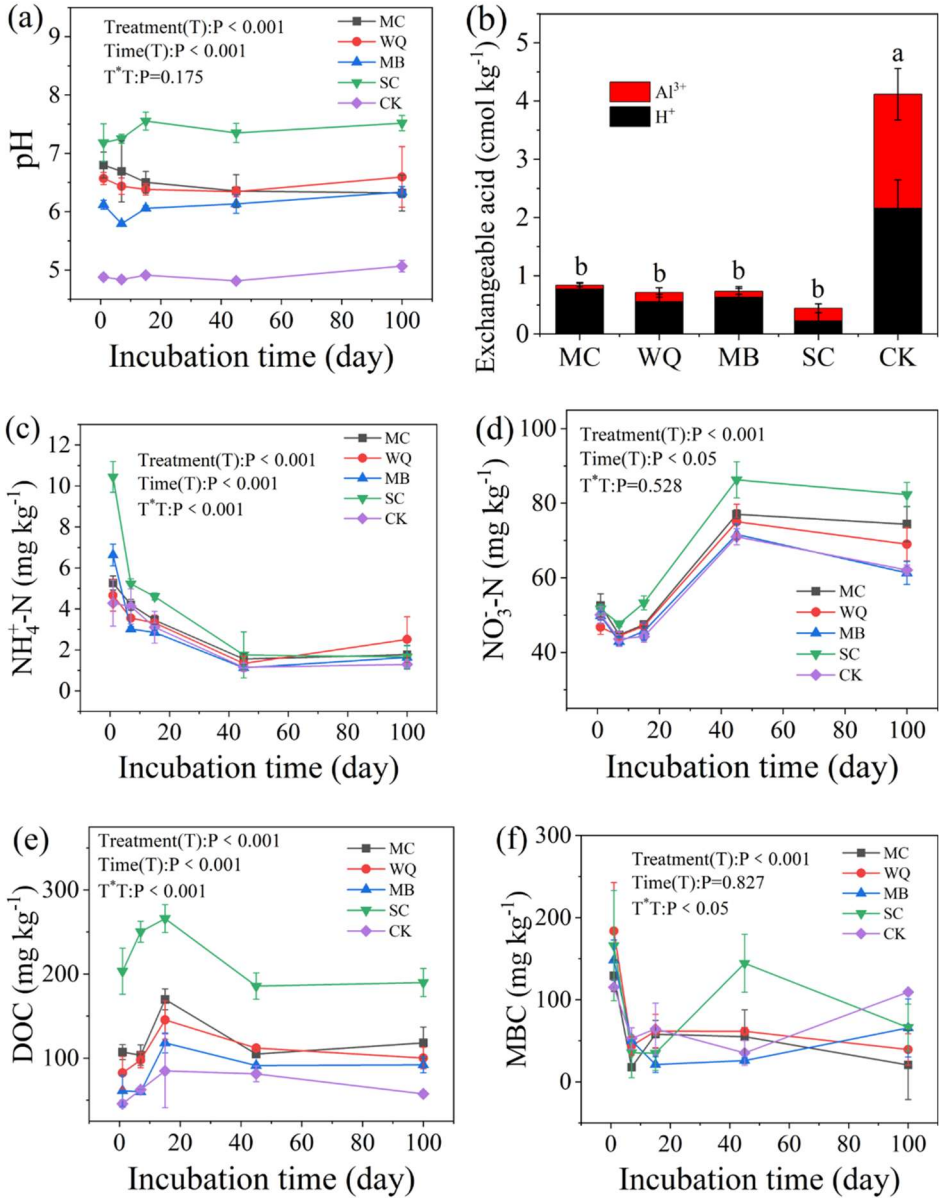

Figure 3. Changes of pH **(a)**, exchangeable acid **(b)**, NH_4^+-N **(c)**, NO_3^--N **(d)**, DOC **(e)**, MBC **(f)** under various treatments over the 100-d incubation period. Error bars represent the standard deviation of the mean (n = 3). Different letters indicate significant differences ($p < 0.05$).

As illustrated in Figure 3c, the NH_4^+-N content in all the treated soils peaked on the first day, followed by a steep decline until the 45th day. Afterward, the NH_4^+-N content slightly increased with prolonging the culture time. The NH_4^+-N content was significantly affected by the soil conditioner type, incubation period and their interaction. The NO_3^--N content increased in the conditioner-treated soils (Figure 3d). From the first to the 7th day, NO_3^--N content slightly decreased, then grew exponentially from the 7th to 45th day. After the 45th day, the NO_3^--N levels gradually declined with extending the culture time. Overall, NO_3^--N content elevated significantly by the application of soil conditioners.

The DOC content of soil significantly increased by the conditioner application (Figure 3e), reaching the maximum on the 15th day of soil incubation, followed by a rapid decline between the 15th and 45th day, and stabilized thereafter. The DOC content ranked as follows: SC > MC > WQ > MB > CK.

The application of various soil conditioners influenced microbial biomass carbon (MBC) content of soil, which peaked on the first day of incubation (Figure 3f). During the initial 15 days, the MBC content of conditioner-treated soils was higher than that of CK-treated soil. Over time, MBC content initially increased and subsequently decreased across all treatments. Notably, the CK-treated soil exhibited significantly higher MBC content (109.36 $mg \cdot kg^{-1}$) compared to the conditioner-treated soils (20.65, 39.46, 65.72, 66.15 $mg \cdot kg^{-1}$). The MBC content was significantly impacted by the conditioner type ($p < 0.001$) and the interaction between conditioner type and incubation duration ($p < 0.05$). Furthermore, the conditioner-treated soils had lower Olsen-P content than the CK-treated soil (Figure S1).

3.4. Soil Carbon, Nitrogen and Phosphorus Hydrolase

The BG, NAG and AP enzyme activity were unstable in the first 15 days but became relatively stable in the later stages (Figure 4a,b, Figure S2). After 7 days, the BG enzyme activity increased by 47.83% and 55.76% in both the WQ and MB-treated soils, respectively, compared to the CK-treated soil. Similarly, the NAG enzyme activity rose by 68.25% and 70.04% in both the WQ and MB-treated soils. The AP enzyme activity in the MB-treated soil was 53.26% higher than that in the CK-treated soil (Figure S2). However, after 15 days, the BG, NAG, and AP enzyme activities in the CK-treated soil were higher than those in the conditioner-treated soils, likely due to reduced microbial activity from the soil conditioner over time, diminishing extracellular enzyme activity. Metrological analysis of soil ecological enzymes revealed significant differences only in the quantitative ratios related to soil carbon and phosphorus cycles (Figure S2b,c).

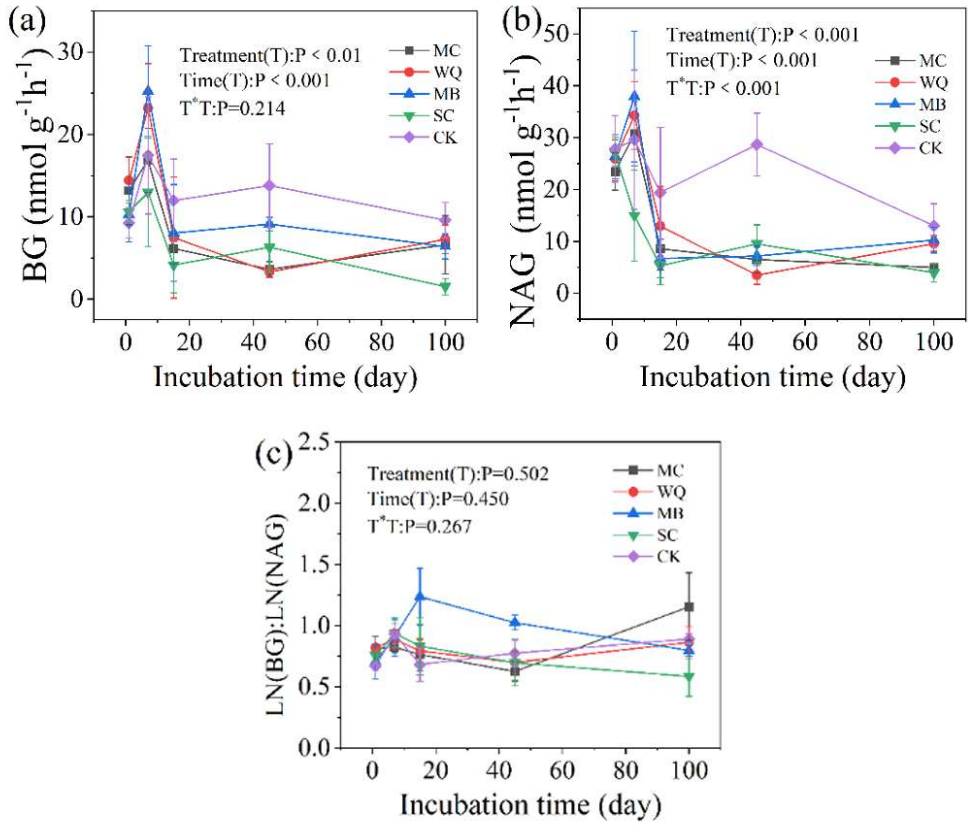

Figure 4. Extracellular enzyme activities of soil BG **(a)**, NAG **(b)** and ecological enzymatic characteristics of soil **(c)** during incubation. BG: β – glucosidase, NAG: chitinase.

3.5. Microbial Community Structure and Diversity

Throughout the culture period, soil bacterial community richness generally exhibited an initial increase, following by a decrease, and then another increase, except for the MB-treated soil with the opposite pattern (Figure 5a). During the first seven days, both the MC- and WQ-treated soils had a higher bacterial community richness compared to the other soils. On the day 7, the MB-treated soil exhibited the highest bacterial community richness. The Shannon index trend for soil bacteria paralleled that of the richness index. Figure 5b presents the fungal community richness under distinct soil treatment types. For both the MC and WQ treatments, the richness initially declined and then increased. By the later stages of culture, no significant differences were observed for the different soils. On the day 7, the fungal community richness in the MC-treated soil was significantly lower than that in the other-treated soils, but on the 45th day, it was significantly higher. Throughout the culture period, the fungal community richness in both the MB and SC-treated soils increased by an average of 6.32% to 16.23%. Significant differences in the bacterial Shannon index among these treatments were observed between the day 1 and day 15 (Figure S3a). On the day 1, the Shannon index for bacteria in both the MC and WQ-treated soils increased by 6.01% and 5.71%, respectively, compared to the CK-treated soil. The bacterial community composition was related with the soil conditioner types.

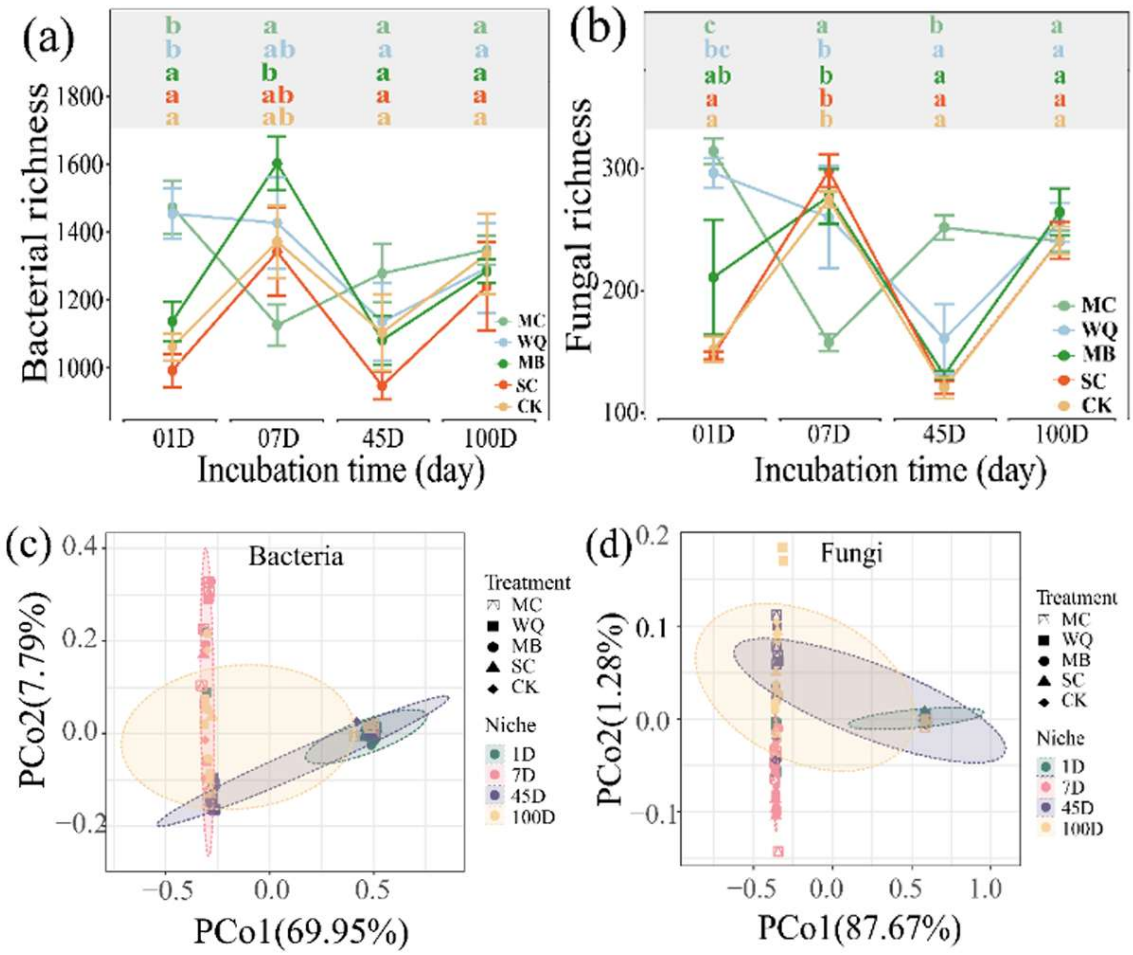

Figure 5. Richess index of soil bacterial and fungal communities bacterial **(a,b)**, Principal coordinates analysis (PCoA) of bacterial and fungal communities **(c,d)**. Different letters indicate significant differences ($p < 0.05$).

In terms of bacterial β-diversity in the soil (Figure 5c), the contribution rates of the PCo1 and PCo2 axes were 69.95% and 7.79%, respectively, with a cumulative contribution rate of 77.74%. On the first day, bacterial communities under different treatments were separated along the PCo1 axis, while on day 100, they were separated along the PCo2 axis. This indicates that both culture time and the application of different soil conditioners jointly significantly influenced the bacterial community characteristics.

For fungal communities, significant differentiation along the PCo1 axis was observed on the first and the seventh days of culture. On the day 7, the fungal community in the WQ-treated soil was clearly separated along the PCo2 axis from the other communities (Figure 5d). The culture time, in combination with the application of different soil conditioners, significantly influenced the fungal community characteristics, and the fungal community characteristics were also affected by different soil conditioners ($p < 0.001$). The Shannon index of soil fungi exhibited a significant variation across treatments on the day 1, day 15, and day 45 of culture. On the day 1, the Shannon index for the MC- and WQ-treated soils increased by 94.68% and 92.67%, respectively, compared to that for the CK-treated soil, as shown in Figure S3b.

In all samples, Proteobacteria, Firmicutes, Actinobacteriota, Chloroflexi, Planctomycetota and Acidobacteriota are dominant, comprising 76.93% to 92.25% of bacterial sequences (Figure 6a). On the day 100, the conditioner-treated soils exhibited higher Proteobacteria levels compared to the CK-treated soil. The relative abundance of Planctomycetota increased in the WQ-treated soils, while the relative abundance of Chloroflexi reduced in the MC-treated soils compared to the CK-treated soil. Firmicutes were significantly abundant in both the MB- and SC-treated soils, Proteobacteria were abundant in the MC-treated soil, and WPS-2 was abundant in the WQ-treated soil, demonstrating that the soil conditioners influence bacterial community structure at the phylum level.

Among the soil samples, the dominant fungal orders were Umbelopsidales, Hypocreales, Mortierellales, Eurotiales, Tremellales, Sordariales and Pleosporales, comprising 72.71% to 99.23% of total fungal abundance (Figure 6b). Compared to those in the CK-treated soil, Umbelopsidales showed a higher relative abundance, while Mortierellales was less abundant. Eurotiales and Tremellales were more prevalent in the WQ- and MC-treated soils than those in the CK-treated soil. Conversely, Sordariales were less abundant in both the WQ- and MC-treated soils. The abundance of Umbelopsidales and Eurotiales notably increased in the MC-treated soil, and Hypocreales were significantly enriched in the WQ-, MB-, and SC-treated soils. Overall, different soil conditioners influenced the fungal community structure, especially the Umbelopsidales, Hypocreales, and Mortierellales.

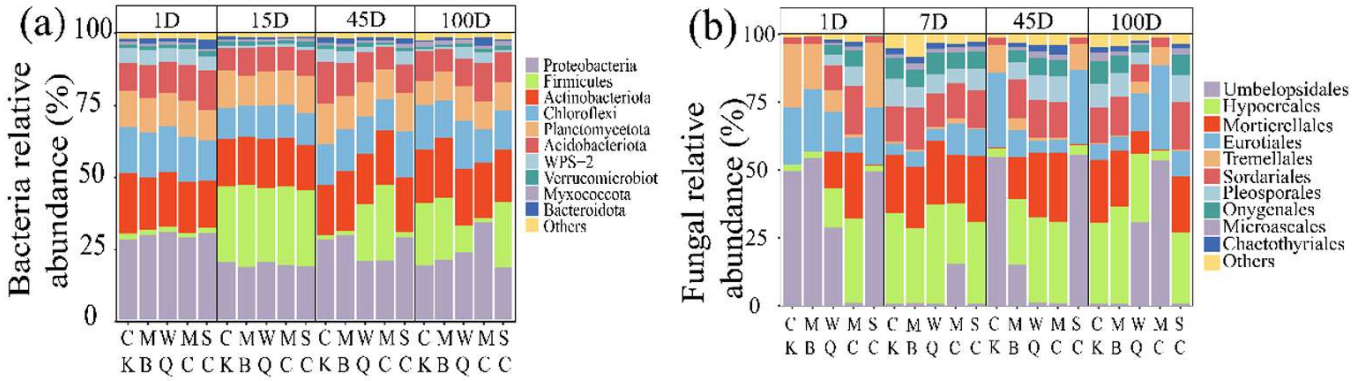

Figure 6. Taxonomic composition of soil bacterial **(a)** and fungi communities **(b)** during incubation.

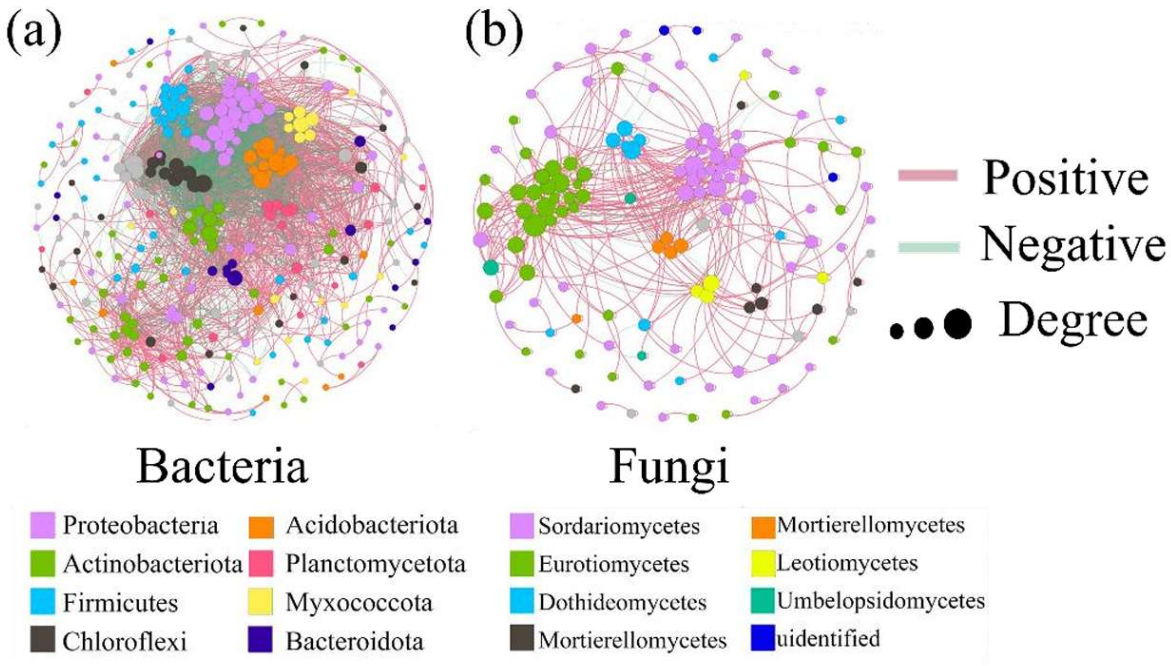

Figure 7. Symbiotic network pattern of bacterial **(a)** and fungi **(b)** in WQ-treated soil during incubation.

Spearman correlation was employed to analyze the bacterial co-occurrence network in the soils with different conditioners (Figure 7a). In the various soils, bacteria formed distinct and intricate microbial networks. These interspecific relationships were predominantly positive, suggesting a higher prevalence of cooperation over competition, albeit with relatively distant associations. The bacterial network was the most complex in both the MB and SC-treated soils, while the most distant associations were observed in the MC-treated soil. The fungal network in the MC, WQ, and MB-treated soils exhibited a simpler structure with a more distant connection between communities (Figure 7b). In contrast, the SC treatment resulted in a more complex fungal network with a stronger inter-community connection (Table 2).

Table 2. Topological characteristics of fungal co-occurrence networks in the different soils

Microorganism	Treatment	Node Number	Edge Number	Positive	Negative	Average Degree	Modularity
Bacterial	MC	305	2377	67.90%	32.10%	15.6	1.8
	WQ	307	2897	64.90%	35.10%	18.9	1.9
	MB	314	2928	66.30%	33.70%	18.7	1.7
	SC	314	2928	69.10%	30.90%	18.7	1.7
	CK	279	3843	66.90%	33.10%	27.6	1.1
Fungi	MC	136	428	95.70%	4.30%	6.3	0.66
	WQ	136	351	91.60%	8.40%	5.2	0.82
	MB	153	1471	69.10%	30.90%	19.2	1.03
	SC	161	2212	78.00%	22.00%	27.5	0.47
	CK	169	2108	76.90%	23.10%	24.9	0.51

3.6. Correlations between Microbial Community and Environmental Variables

Redundancy analysis (RDA) was performed to examine the relationship between microbial community structure and soil physicochemical properties (CO_2 and N_2O emissions, and soil enzyme activities) using bacteria and fungi as response variables. The changes in soil bacterial community structure were significantly associated with DOC ($p = 0.009$), N_2O ($p = 0.024$), pH ($p = 0.027$) and CO_2 ($p = 0.034$) (Figure 7a). The CO_2 emissions from both the MC and SC-treated soils showed a significant negative correlation with the bacterial community at the first day of culture, but a significant positive correlation at both the day 7 and day 100. Conversely, the CO_2 emissions from both the WQ and MB-treated soils were significantly positively correlated with the bacterial community at both the day 1 and day 7, and significantly negatively correlated at the day 100. The bacterial community in both the MC and SC-treated soils on the first day was significantly negatively correlated with pH, DOC and N_2O, whereas it was significantly positively correlated with the bacterial community in both the MB and WQ-treated soils. At both the day 7 and day 100, the bacterial communities in both the MC and SC-treated soils were significantly negatively correlated with pH, DOC, and N_2O, while those in both the MB and WQ-treated soils were also significantly negatively correlated.

The soil fungal community showed a significant correlation with N_2O ($p = 0.001$). The fungal communities in the MC-treated soil exhibited a significant negative correlation with N_2O across all three culture periods. In contrast, the fungal communities in the WQ-treated soil at both the day 1 and day 7, and in both the MB- and SC-treated soils at the 100th day, demonstrated a significant positive correlation with N_2O (Figure 8b).

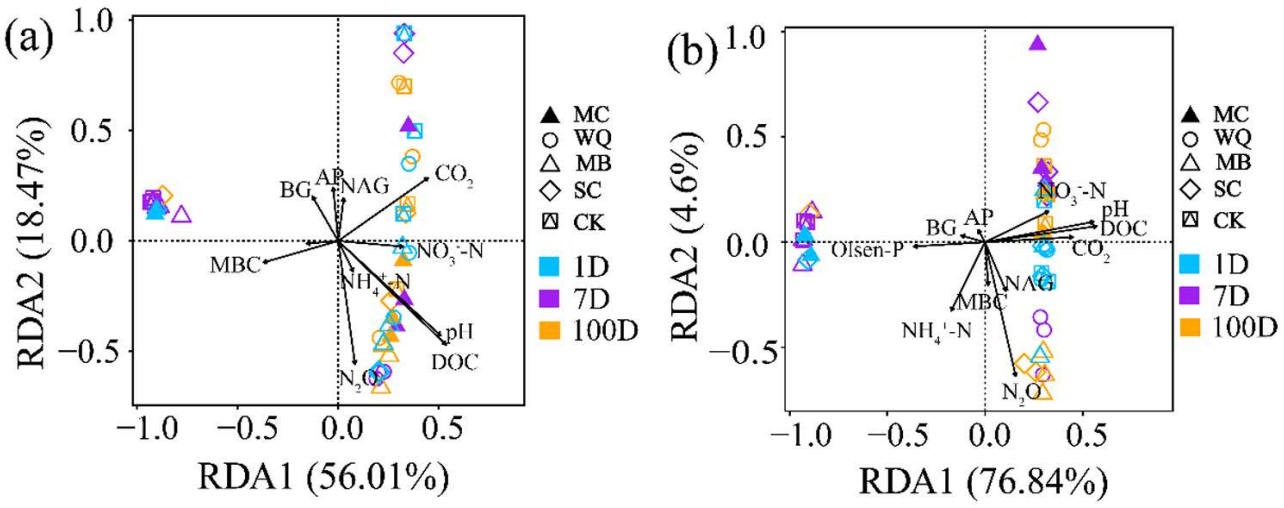

Figure 8. RDA analysis of the soil bacteria **(a)** and fungi **(b)** communities.

4. Discussion

4.1. Morphological and Physicochemical Characteristics of Soil Conditioners

The morphology and chemical compositions of the minerals changed by the distinct treatments. The MB conditioner maintained its original properties, exhibiting primarily an irregular block and fragment structure (Figure 1). This structure consists mainly of layered subparticles, consistent with the inherent characteristics of the mineral [24]. The high-temperature calcination induced fusion between mineral phases, resulting in a spherical and porous structure, accompanied by structural transformation and partial release of carbon dioxide. The honeycomb morphology observed in the WQ-treated sample is attributed to rapid quenching in cold water after high-temperature treatment, which generates substantial gas release and structural modification. Additionally, water quenching may lead to the loss of some soluble mineral elements, reducing the available sodium, calcium, and magnesium content compared to MC treatment (Table 1). Notably, the available K, Ca, Mg contents in MC- and WQ-treated conditioners were higher than those in MB-treated conditioners. This suggests that MC and WQ treatments significantly enhance nutrient availability in the conditioner, demonstrating that MC and WQ treatments effectively activate nutrients in soil conditioners—particularly the WQ treatment. The CaO, MgO and K_2SiO_4 in MC and WQ react readily to form effective forms of calcium, magnesium, potassium and other nutrients under acidic soil conditions.

4.2. Response of Soil CO_2 and N_2O Emissions to Soil Conditioners

Soil organic carbon mineralization is a key metabolic process carried out by soil microorganisms, decomposing organic matter, releasing energy, nutrients and CO_2, and playing a central role in the soil carbon cycle. In this study, the CO_2 emission rate peaked on day 2 and remained elevated during the initial 20 days of incubation before gradually stabilizing. This trend likely reflected the abundance of labile organic matter in the early stage, which promoted microbial activity and accelerated CO_2 release. As incubation progressed, CO_2 emissions declined, likely due to substrate depletion and the weakening of microbial activity caused by nutrient limitation. The application of soil conditioners significantly increased CO_2 emissions (Figure 2b), possibly as a result of pH-induced stimulation of microbial activity [2], which in turn promoted organic carbon mineralization [25]. Significant differences were observed in cumulative CO_2 emissions among these treatments, with SC exhibiting markedly higher emissions than MC, WQ, and MB. This could be attributed to the presence of carbonate ions (CO_3^{2-}) in SC, which rapidly react with protons (H^+) to generate CO_2.

Nitrous oxide (N_2O) is a potent greenhouse gas and contributes to stratospheric ozone depletion. The secondary N_2O peak observed around day 10 (Figure 2c) reflects a crucial transition in nitrogen cycling processes. Initial soil pH elevation (Figure 3a) promoted nitrifying bacteria activity, converting NH_4^+ (Figure 3c) to NO_3^- (Figure 3d) while consuming oxygen. This created localized anoxic zones where denitrifying bacteria utilized accumulated NO_3^- as an alternative electron acceptor, reducing it to N_2O. The temporal accumulation of this intermediate denitrification product

demonstrates how mineral-based conditioners sequentially stimulate different N-cycling microbial groups through rapid modification of soil physicochemical conditions. In this study, the cumulative N_2O emissions were significantly reduced in both MC- and WQ-treated soils (Figure 2d). After day 15, N_2O emissions in conditioner-treated soils were consistently lower than those in the control, and remained stable thereafter. All four conditioner treatments increased soil pH, indicating a possible correlation between elevated pH and reduced N_2O emissions. Vekic et al. reported that soil conditioners reduce N_2O emissions by elevating soil pH and regulating nitrogen transformation processes such as mineralization, nitrification, and denitrification [26]. Similarly, Abdalla et al. observed that liming reduced N_2O emissions by increasing soil pH [27]. These findings are consistent with our results, further supporting that elevated soil pH is associated with reduced N_2O emissions in acidic soils.

4.3. Response of Soil Property to Soil Conditioners

In this study, compared with the CK treatment, all the pH of soil increased by the MC, WQ, MB and SC treatment (Figure 3a). Soil conditioners inherently contain alkaline substances, particularly following high-temperature calcination, which generates compounds like calcium oxide (CaO), magnesium oxide (MgO), potassium silicate (K_2SiO_3), and calcium magnesium phosphate ($Ca_3(PO_4)_2$). These substances neutralize soil H^+, releasing Ca^{2+}, Mg^{2+}, K^+, HPO_4^{2-}, $H_2PO_4^-$, etc., enhancing soil nutrient status, and increasing soil pH. In addition, the soil exchangeable acid contents were significantly reduced by the MC, WQ, MB, and SC treatments (Figure 3b). Studies have shown that SO_4^{2-} increases in the soil by the conditioner application, in which the Al^{3+} were adsorbed by SO_4^{2-} in the soil, generating $AlSO_4^+$ [28], reducing the net charge of both ions, while desorbing OH- into the soil solution [29], reducing the concentration of Al^{3+} and H^+, and increasing the soil pH.

Throughout the culture period, soil pH initially decreased and then increased, likely due to nitrification. The synchronous inflection points for NH_4^+-N and NO_3^--N on day 45 (Figure 3c,d) signify a crucial transition in the N cycle. Initially, nitrification under elevated pH consumed NH_4^+-N and accumulated NO_3^--N. The subsequent shift after day 45 resulted from NH_4^+-N depletion limiting nitrification, while accumulated NO_3^--N stimulated denitrification and microbial immobilization. The slight NH_4^+-N rebound suggests initiating N mineralization. These coupled inflection points—where weakened nitrification reduced NO_3^--N supply and enhanced consumption accelerated its decline—demonstrate the soil N cycle's dynamic self-regulation from single-process dominance to multi-process equilibrium under conditioner influence

Oxidation of each mole of NH_4^+ to NO_3^- releases two moles of protons. The rise in soil NO_3^--N content during this period indicates nitrification. Leggo et al. observed that using NH_4^+-rich zeolite as a soil conditioner can lead to high NO_3^--N concentrations, with a marked increase occurring 7–45 days post-application [30]. This may result from ion exchange between soil Ca ions and NH_4^+ in the conditioner, facilitating immediate utilization by nitrifying microorganisms and thus enhancing nitrification substrates. After 45 days, the NO_3^--N content decreased (Figure 3d), possibly due to the fact that the significant consumption of O_2 under the intense

microbial activity creates an anaerobic microenvironment that reduces NO_3^--N levels [31]. The synchronous inflection points for NH_4^+-N and NO_3^--N on day 45 (Figure 3c,d) signify a crucial transition in the N cycle. Initially, nitrification under elevated pH consumed NH_4^+-N and accumulated NO_3^--N. The subsequent shift after day 45 resulted from NH_4^+-N depletion limiting nitrification, while accumulated NO_3^--N stimulated denitrification and microbial immobilization. The slight NH_4^+-N rebound suggests initiating N mineralization. These coupled inflection points—where weakened nitrification reduced NO_3^--N supply and enhanced consumption accelerated its decline—demonstrate the soil N cycle's dynamic self-regulation from single-process dominance to multi-process equilibrium under conditioner influence. Additionally, the conditioner-treated soils exhibited significantly higher DOC content than the control (Figure 3e). The conditioner altered the physical and chemical properties of soil, boosted the microbial activity, and promoted the organic matter degradation, thereby increasing the soil DOC content [32].

4.4. Response of Soil Hydrolase to Soil Conditioners

Soil enzymes predominantly originate from microorganisms, which secrete extracellular enzymes to break down organic matter into simpler, more accessible forms to absorb energy and nutrients [33]. This decomposition process requires the coordinated action of multiple enzymes and is influenced by limiting factors. In this study, it is found that the application of soil conditioner enhanced microbial activity, metabolism, and enzyme activity. Initially, the enzyme content increased rapidly but decreased gradually over time. Microorganisms primarily utilized active Nutrients, necessitating substantial amounts of available organic matter early on. The conditioner-treated soils exhibited higher dissolved organic carbon (DOC) levels compared to the CK-treated soil. And the β-glucosidase (BG) enzyme activity was great in the CK-treated soils, suggesting that the conditioner-treated soils were less constrained by carbon availability. The changes in microbial biomass carbon (MBC) further indicate that microorganisms secrete extracellular enzymes to acquire nutrients when they are scarce [34].

NAG is an enzyme involved to the nitrogen cycle, primarily catalyzing the hydrolysis of chitosaccharides and glycoproteins to release glucose residues. Rafael et al. demonstrated that the soil conditioner enhances the activities of soil alkaline phosphomonoesterase, phosphodiesterase, and β-glucosidase, thereby increasing phosphorus availability, aligning with the findings in this study [35]. The stoichiometric ratio of ecological enzyme illustrates the link between microbial metabolism and environmental nutrient availability, serving as a metric for soil microbial biomass and nutrient limitation [21]. In this study, the LN(BG): LN(AP) ratio in the SC-treated soil was lower than that in other-treated soils, suggesting that the nutrient availability increased, reducing the need for microorganisms to secrete extracellular enzymes for nutrient acquisition.

4.5. Response of Soil Microbial Community Structure and Diversity to Soil Conditioners

Soil conditioners can mitigate acidity, adjust pH, and alter nutrient content and enzyme activity, thereby influencing the structure and function of soil microbial communities. Microorganisms derive nutrients by decomposing organic matter; thus, nutrient-rich soils facilitate microbial growth and reproduction. The variations in bacterial and fungal community as biomarkers was used to assess soil ecological conditions [36]. The application of soil conditioners modifies the bacterial community structure, enhancing the bacterial abundance and diversity. Bossolani et al. demonstrated that the conditioners significantly alter bacterial and fungal communities in acidic soils, aligning with our findings [37]. In this study, the richness of bacterial and fungal communities increased in both the SC- and MB-treated soils (Figure 5). However, the richness initially increased in both the MC- and WQ-treated soils, followed by a decline. The timing difference in fungal richness nadir between MC (day 7) and WQ (day 45) treatments stems from their distinct modification mechanisms. MC-treated soil highly reactive oxides cause a rapid pH spike and pulse nutrient release, acting as a "chemical bomb" that imposes acute chemical stress on acid-adapted fungi, leading to early richness decline. In contrast, WQ-treated soil transformed minerals provide milder pH adjustment, while its unique honeycomb structure offers microhabitat refugia that initially buffer fungal communities. The delayed WQ-treated soil nadir emerges from prolonged nutrient competition and niche specialization processes within these stable micropores, representing slower biological succession. This demonstrates how conditioner properties—chemical reactivity, physical structure, and nutrient release kinetics—jointly shape microbial successional patterns (Figure 5). In ecosystem research, the syntrophic patterns of microbial communities are primarily analyzed through biological networks. Biological network studies aim to uncover interactions, relationships, and structures among organisms, providing a reliable foundation for ecosystem research. Compared to the control (CK), the bacterial networks in MC, WQ, MB, and SC treatments were more complex, while the fungal networks of the two soils showed opposite results. This indicates that soil conditioners have a better stabilizing effect on soil bacterial communities. The stability of microbial communities is a crucial safeguard for achieving ecological functions. Therefore, the application of soil conditioners can promote a more stable structure in soil bacterial networks, enabling bacteria to play a significant role in ensuring soil functionality.

Soil conditioners significantly affect the species composition of microorganisms, particularly the dominant flora. The abundance of Proteobacteria decreased across all treated soils. Proteobacteria as the dominant nitrogen-fixing microorganisms, highlight their crucial role in soil nitrogen fixation [38]. Chloroflexi and Acidobacteriota are also notable components of the soil bacterial community. After 100 days of culture, the abundance of Chloroflexi and Acidobacteria declined compared to earlier periods (Figure 6). Studies by Sun et al. indicate a positive correlation between Chloroflexi abundance and soil microbial biomass carbon (MBC) [39], with Chloroflexi contributing to MBC production through CO_2 fixation, aligning with these findings (Figure 6). Previous research suggests Acidobacteria's role in the

terrestrial carbon cycle [40], implying that both Chloroflexi and Acidobacteria may enhance the global carbon cycle. The fungal communities in the MB-, WQ-, and SC-treated soils were significantly enriched with Hypocreales, known for their role in controlling crop diseases and pests [41]. These shifts in dominant microbial community structure can serve as biomarkers for ecological environments and offer a theoretical foundation for assessing the ecological effects of soil conditioner applications.

Microbial community symbiosis is primarily assessed through biological networks, which elucidate interactions, relationships, and structures among organisms, providing a robust foundation for ecosystem research. Studies indicate that microbial diversity and complexity significantly influence ecosystem function and stability. Microbial communities comprise various bacteria, fungi, and other microorganisms that interact biologically [42]. The diversity index indicates the number and distribution of microbial species, in which higher values mean greater species variety and more complex community structures. This diversity reflects ecosystem stability and resilience to disturbances, with microbial composition and distribution crucial to overall ecosystem stability [43]. In the conditioner-treated soils, the bacterial network exhibited increased complexity compared to the CK-treated soil, while the fungal network showed reduced complexity, suggesting the enhanced stability of bacterial community. Thus, soil conditioners enhance the stability of bacterial network structures, underscoring the pivotal role of bacteria in maintaining the soil function.

4.6. Correlations between Microbial Community and Environmental Variables

Conditioners significantly impact the physicochemical properties of acidic soils and influence the cycling of carbon, nitrogen, and phosphorus nutrients. Different acidic soil nutrients exhibit varied responses to conditioner applications. Alkaline nutrients in conditioners, particularly those in calcined and quenched forms, interact with exchangeable H^+ ions in the soil, raising soil pH. This elevation in pH facilitates the reaction between carbonate and H^+ to form bicarbonate, contributing to carbon sequestration. Additionally, these alkaline nutrients generate K^+, Ca^{2+}, Mg^{2+}, HPO_4^{2-}, and $H_2PO_4^-$, which are essential for microbial uptake and utilization, while altering soil enzyme activity and affecting soil mineralization and emissions of CO_2 and N_2O. Typically, CO_2 is released through the decomposition of soil organic matter and microbial activity, with mineralization rates increasing alongside pH. This aligns with our study findings, where conditioner application elevated both soil pH and CO_2 emission fluxes.

Soil microorganisms play a crucial role in regulating carbon and nitrogen cycling by decomposing organic matter, fixing nutrients, and synthesizing organic compounds, thereby maintaining soil ecosystem stability [44]. Our findings indicate significant differences in the diversity and composition of soil microbial communities across various ecosystems, closely linked to changes in soil environmental factors [40]. In this study, the application of soil conditioner altered the physical and chemical properties of soil. For instance, pH significantly influenced the availability of nutrients like dissolved organic carbon (DOC) (Figure 8), and the microbial community

composition was strongly associated with soil carbon and nitrogen levels. Organic matter serves as the primary carbon source for microbial communities, providing energy and nutrients through decomposition. Additionally, nitrogen is essential for microbial growth, and microorganisms can convert fixed nitrogen into accessible forms. The structure and abundance of microbial communities not only influence soil carbon and nitrogen content and composition but also affect microbial growth and metabolism.

The application of various soil conditioners significantly influences soil microbial community composition (Figure 8). The abundance of Acidobacteria and Proteobacteria is determined by the physical and chemical properties of soil [38,40]. Acidobacteria are crucial in soil ecosystems, decomposing and utilizing soil organic matter and organic carbon [40], thus representing a key taxon. Proteobacteria are vital for soil nutrient cycling [38]. In fungi, Umelliformes secrete carbohydrate hydrolase enzymes to break down large organic molecules into smaller, absorbable nutrients [45]. Studies indicated that the various soil conditioners can influence enzyme activity and the microbial community structure involved in carbon, nitrogen and phosphorus cycles. RDA (Figure 8) revealed significant correlations between soil bacterial communities and dissolved organic carbon (DOC), N_2O, pH, carbon dioxide (CO_2), as well as fungal communities and N_2O emissions. These nutrient levels were directly linked to the active substances generated by the conditioner treatment. This suggests that the bacterial community is affected by soil physicochemical properties and mineralization, whereas the fungal community is more sensitive to nitrogen nutrients.

5. Conclusions

We hypothesized that the mode of thermal activation determines the capacity of mineral-based conditioners to ameliorate acidified soil and, importantly, to steer microbial networks toward greater stability and lower N_2O emissions. Calcination (MC) and water-quenching (WQ) conditioners converted Ca/Mg-bearing minerals into highly reactive CaO, MgO, and Ca-Mg-phosphate phases; consequently, they raised soil pH to 6.4–6.5, reduced exchangeable Al^{3+} by more than 90%, and lowered cumulative N_2O emissions by 27% (MC) and 20% (WQ), respectively. These treatments increased bacterial network modularity by 60–70%, elevated the relative abundances of Proteobacteria and Planctomycetota, and enriched Hypocreales fungi known for disease suppression. In contrast, the MB- and SC-treated soils produced the largest CO_2 pulses (+61–93%) without suppressing N_2O and generated denser but less modular microbial networks, suggesting lower ecological stability. Across all conditioners, pH, dissolved organic carbon (DOC), and N_2O flux explained 34% of the variation in bacterial community structure, while fungal assemblages responded primarily to N_2O and NH_4^+ dynamics, underscoring the nitrate turnover–fungi nexus. These results provide the first experimental evidence that thermal activation (calcination or quenching) of silicate- or carbonate-containing mixtures can be tuned to mitigate soil acidity and nitrous oxide emissions, while promoting a resilient, disease-suppressive microbiome. This approach offers a scalable, low-cost alternative to lime for resource-poor tropical regions and supports emerging rock-weathering strategies for climate-smart agriculture. The study was conducted under controlled

laboratory incubation without plants; root exudation and rhizosphere priming could alter conditioner dissolution and microbial responses. Field trials are needed to verify N_2O mitigation and yield benefits under contrasting crop rotations and fertilizer regimes.

Supplementary Materials: Figure S1: Changes of Olsen-P after the additions of soil conditioners over the 100-d incubation period; Figure S2: Extracellular enzyme activities of soil AP: phosphatase (a) and ecological enzymatic characteristics of soil (b,c) during incubation.; Figure S3: Shannon index of soil bacterial (a) and fungal (b) communities.

Author Contributions: For research articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used "Conceptualization, J.D. and T.G.; methodology, S.W.; software, S.W.; validation, J.D., Z.Z. and J.L.; formal analysis, K.L.; investigation, Y.K.; data curation, Z.Y.; writing—original draft preparation, J.D.; writing—review and editing, Y.Z., S.Y.K. and Y.K.; visualization, Z.Y.; supervision, J.D.; project administration, Z.Y.; funding acquisition, Z.Y. All authors have read and agreed to the published version of the manuscript." Please turn to the CRediT taxonomy for the term explanation. Authorship must be limited to those who have contributed substantially to the work reported.

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