

## Article

# Functional Characteristics and Cellulose Degradation Genes of the Microbial Community in Soils with Different Initial pH Values

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**Abstract:** Soil pH critically regulates microbial community structure and activity, thereby influencing carbon transformation processes in terrestrial ecosystems. However, the mechanisms underlying pH-mediated shifts in microbial metabolic functions and cellulose-degrading functional genes remain poorly understood. This study investigated the responses of bacterial communities, metabolic profiles, and the abundance of cellobiohydrolase I (cbhI) and glycoside hydrolase family 48 (GH48) genes to varying pH levels in fluvo-aquic and red soils. High-throughput sequencing, PICRUSt-based metabolic prediction, and quantitative PCR were employed to analyze microbial composition, functional traits, and gene dynamics. Network analysis clarified linkages between functional genes, pathways, and taxa. The results revealed that elevated pH significantly increased CO<sub>2</sub> emissions and dissolved organic carbon (DOC) content in both soils. Dominant taxa, including Alphaproteobacteria, Bacteroidetes, Xanthomonadaceae, and Mycoplasma, exhibited pH-dependent enrichment. Metabolic predictions indicated that pH positively influenced genes linked to biodegradation and xenobiotic metabolism in fluvo-aquic soil but suppressed energy-metabolism-related genes. Contrastingly, in red soil, cbhI and GH48 gene abundance declined with rising pH, suggesting that acidic conditions favor cellulolytic activity. Network analysis identified strong positive correlations between CO<sub>2</sub> emissions and Caulobacteraceae, while cbhI and GH48 genes were closely associated with taxa such as Xanthomonadaceae, Comamonadaceae, and Micromonosporaceae, which drive organic matter decomposition. These findings underscore pH as a pivotal regulator of microbial community structure and functional gene expression, with soil-specific responses highlighting the need for tailored strategies to optimize carbon cycling and sequestration in agricultural ecosystems.

**Keywords:** soil pH; metabolic function; microbial community; cellulose degradation; functional genes; network analysis



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## 1. Introduction

Soil pH serves as a critical regulator influencing both physicochemical properties and biological characteristics in agricultural soils. Current research demonstrates that elevated pH levels significantly enhance soil organic carbon (SOC) mineralization processes [1,2]. This

biochemical conversion represents a primary mechanism for atmospheric CO<sub>2</sub> release, with amplified emissions directly contributing to greenhouse effect intensification through altered carbon cycling dynamics. Furthermore, alkaline conditions promote the solubilization of soil organic matter, particularly increasing dissolved organic carbon (DOC) concentrations [3,4]. Such pH-induced solubility changes render organic matter in fluvo-aquic soils more chemically labile compared to acidic soil systems, thereby reducing carbon stabilization potential. The dual regulatory mechanisms of pH—mediating both mineralization rates and substrate solubility—establish its pivotal role in determining soil carbon sequestration capacity and atmospheric carbon fluxes [5–7].

Soil pH exerts profound regulatory effects on microbial ecology through its narrow optimal range for microbial physiological processes [8]. The parameter fundamentally governs both microbial biomass dynamics and community architecture, as evidenced by phospholipid fatty acid profiling studies in the Hoosfield acid strip, which established pH as a determinant of microbial population shifts [9,10]. Although pH-driven bacterial community shifts are well documented [11], methodological limitations hinder definitive conclusions. Current conclusions predominantly rely on comparative analyses of geographically disparate samples with inherent physicochemical variabilities beyond pH differences, thus precluding definitive causal attribution [12,13]. Though pioneering work at the Lausanne experimental station examined microbial responses under uniform soil matrices, these investigations failed to account for confounding factors such as root exudates and pH-mediated variations in plant-derived carbon inputs [14]. This knowledge gap underscores the necessity for controlled experiments isolating pH effects from edaphic and biological covariables.

Beyond community composition analysis, pH-driven functional metabolic adaptations remain poorly characterized. Emerging evidence suggests that pH gradients modulate enzymatic potential and substrate utilization efficiency, thereby altering nutrient transformation kinetics during organic matter decomposition [15]. Recent methodological advances, particularly Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt), enable the predictive modeling of microbial metabolic functions through 16S rRNA sequence extrapolation [16]. While successfully deployed in wastewater treatment and composting systems, this metagenomic prediction tool remains underutilized in deciphering pH-dependent functional shifts in natural soil environments. The systematic application of such omics approaches could unravel mechanistic linkages between pH gradients, microbial catalysis pathways, and biogeochemical cycling dynamics [17].

The advent of molecular biotechnology has intensified the scientific scrutiny of functional gene dynamics in soil ecosystems [18]. While nitrogen-cycling-associated genes have been extensively documented, systematic investigations into genetic determinants governing carbon transformation processes remain scarce. Notably, carbon-fixation-related genes exert critical regulatory effects on organic matter mineralization. Cellulose, constituting the predominant recalcitrant carbon pool in arable soils, undergoes decomposition through a coordinated enzymatic hydrolysis—a rate-limiting step in terrestrial carbon cycling [19]. This biochemical process is mediated by three synergistic enzyme groups: endoglucanases, cellobiohydrolases (e.g., GH48 and cbhI) [20], and  $\beta$ -glucosidases, which collectively depolymerize crystalline cellulose. Recent methodological advances enable the targeted quantification of cellulolytic microorganisms via primers specific to cbhI and GH48 genes in ascomycete, basidiomycete fungi, and actinobacteria. These genetic markers not only reflect cellulolytic microbial abundance, but also elucidate their ecological contributions to carbon flux regulation. Given the pH-sensitive nature of enzymatic activities and microbial consortia, deciphering the tripartite relationship between pH gradients, cellulase-encoding genes, and microbial taxa is imperative for unraveling pH-mediated carbon conversion

mechanisms. Soil pH critically regulates cellulolytic gene expression through direct enzymatic effects (e.g., pH-dependent enzyme stability) and indirect community shifts (e.g., favoring Acidobacteria in acidic soils vs. Proteobacteria in alkaline systems) [21]. Additionally, pH alters DOC availability and ionic toxicity (e.g., aluminum), further modulating microbial investment in cellulase production. These mechanisms highlight pH's dual role in shaping cellulolytic gene dynamics [22]. Complementary network analyses further enable the identification of bacterial co-occurrence patterns and their associations with functional genes and environmental parameters [23,24].

In this study, in order to investigate functional characteristics and cellulose degradation genes of the microbial community in different initial pH, a 28-day incubation was conducted to investigate the succession of bacterial community under different pH gradients. The structure of the microbial community was determined by high-throughput sequencing, and the metabolic function characteristics of bacteria were further predicted by PICRUSt. Meanwhile, quantitative PCR was adopted to analyze the GH48 and cbhI genes' abundance related to cellulose degradation, and the links between metabolic function characteristics and the cellulose degradation genes of the microbial community were established.

## 2. Materials and Methods

### 2.1. Soil Sample

The fluvo-aquic soil [25] samples were obtained from Fengqiu County, Henan Province, China (35°00' N, 114°24' E), following the wheat harvest. This region, characterized by a summer maize–winter wheat rotation system, represents a typical area of the North China Plain. The site experiences an average annual temperature of 13.9 °C and receives 615 mm of precipitation yearly. In contrast, red soil [26] was collected in July from Yingtan City, Jiangxi Province (28°12' N, 116°57' E), subsequent to rice harvesting. This southern location has warmer and wetter climatic conditions, with mean annual temperature and precipitation reaching 18 °C and 1800 mm, respectively.

The fluvo-aquic soil originates from Yellow River alluvial deposits, while the red soil was developed from Quaternary red clay. According to the China Soil Taxonomic Classification, these soils are categorized as semi-hydromorphic soil and Fe-accumulic stagnic Anthrosol, respectively. Surface soil samples (0–20 cm depth) were collected from both sites. Following the removal of stones and visible plant residues, the soils were thoroughly mixed and sieved through a 2 mm mesh. The processed samples were then divided into two portions: one portion was air-dried for physicochemical analysis, while the other was preserved at 4 °C in refrigeration for subsequent incubation experiments.

### 2.2. Soil Experimental Procedure

To establish pH gradients, fluvo-aquic soil was adjusted to four levels (pH 7.0, 7.8, 8.5, and 9.0) through the addition of chemical amendments: 7.5 g  $\text{Al}_2(\text{SO}_4)_3$  for pH 7.0, no amendment for pH 7.8, 0.4 g lime for pH 8.5, and 0.9 g lime for pH 9.0, with corresponding labels of A 7.0, A 7.8, A 8.5, and A 9.0. Similarly, red soil was modified to create a pH gradient (5.8, 6.7, 7.5, and 8.5) by incorporating varying amounts of lime: 0 g, 0.1 g, 0.2 g, and 0.8 g, designated as R 5.8, R 6.7, R 7.5, and R 8.5, respectively. All soil samples underwent a two-week pre-incubation period at 25 °C and 50% water-holding capacity (WHC) in a biochemical incubator to reactivate microbial communities [27,28]. Following this stabilization phase, experimental incubations were conducted using 10 g of fresh soil (triplicates per treatment) in 250 mL conical flasks. The incubation process, maintained at 25 °C for 28 days, involved sealing each flask with a rubber stopper equipped with a central glass tube. Throughout the experimental period, soil moisture was consistently maintained at 50% WHC through periodic additions of deionized water.

### 2.3. Soil Chemical Analysis

Soil chemical analyses were performed using standardized methods [29–31]. Organic carbon content was quantified through wet oxidation with potassium dichromate ( $K_2Cr_2O_7$ ) and sulfuric acid ( $H_2SO_4$ ). For total nitrogen determination, the Kjeldahl digestion method was employed. Soil pH measurements were conducted using a digital pH meter (Ohaus, Brooklyn, NY, USA) with a soil-to-water ratio of 1:2.5. To assess dissolved organic matter, soil samples were extracted with potassium sulfate ( $K_2SO_4$ ) and filtered through 0.45  $\mu m$  membranes, followed by an analysis of dissolved organic carbon (DOC) and nitrogen (DON) concentrations using a TOC analyzer (TOC-VCPH, Shimadzu, Kyoto, Japan).

### 2.4. CO<sub>2</sub> Flux Measurement

CO<sub>2</sub> flux was measured at 1, 3, 5, 7, 14, 21, and 28 days. At each gas sampling time point, 20 mL gas was taken from the headspace of the conical flask by airtight syringe and the gas was removed into a 20 mL vacuum vial for CO<sub>2</sub> flux analysis [32]. The concentration of CO<sub>2</sub> was measured by gas chromatography (Agilent 7890B, Santa Clara, CA, USA). CO<sub>2</sub> emission rates were calculated by the following equation [33]:

$$E = P \times V \times \frac{\Delta c}{\Delta t} \times \frac{1}{RT} \times M \times \frac{1}{m}$$

where E is CO<sub>2</sub> flux ( $\mu g \cdot g^{-1} \cdot h^{-1}$ ), P is the standard atmosphere pressure (Pa), V is the headspace volume of the conical flask ( $cm^3$ ),  $\Delta c$  is the concentration changes in CO<sub>2</sub> (ppm),  $\Delta t$  is the time between gas sample (h), R is universal gas constant ( $8.31 Pa \cdot m^3 \cdot mol^{-1} \cdot K^{-1}$ ), T is absolute gas temperature (K), M is the molecular mass of CO<sub>2</sub> ( $g \cdot mol^{-1}$ ), and m is the mass of incubating soil by dry weight basis (g).

### 2.5. DNA Extraction, High-Throughput Sequencing, and qPCR Amplification

Soil DNA extraction was performed on 0.6 g fresh samples using the FastDNA Spin Kit for Soil (MP Biomedicals, Solon, OH, USA) following manufacturer's protocol. Extracted DNA was quantified and quality-checked using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA) before storage at  $-80^\circ C$  for subsequent analysis.

For bacterial community analysis, the V4 region of 16S rRNA genes was amplified using universal primers F515 (5'-GTGCCAGCMGCCGCGGTAA-3') and R806 (5'-GGACTACVSGGTATCTAAT-3'). The 50  $\mu L$  PCR reaction mixture consisted of 2  $\mu L$  (5  $\mu M$ ) of each primer, 27  $\mu L$  ddH<sub>2</sub>O, 2.5  $\mu L$  template DNA (10 ng), 10  $\mu L$  five-fold Fastpfu buffer, 1  $\mu L$  TransStart Fastpfu polymerase (TransGen, Beijing, China), 5  $\mu L$  dNTPs (2.5 mM), and 0.5  $\mu L$  bovine serum albumin. Following amplification using previously described thermal cycling conditions [34], PCR products were purified using a PCR Clean-up Purification Kit (MP Biomedicals) and quantified with a Qubit 2.0 fluorimeter (Invitrogen, Carlsbad, CA, USA). Equimolar concentrations of purified products were pooled for paired-end sequencing (2  $\times$  250 bp) on an Illumina MiSeq platform [35–37].

Gene quantification of GH48 and cbhI was conducted through quantitative real-time PCR (q-PCR) using an iCycler system (BioRad, Hercules, CA, USA) with Bio-Rad iQ5 v2.0 analysis software. Target genes were amplified using specific primer pairs: fungcbhIF (ACCAAYTGCTAYACIRGYAA) and fungcbhIR (GCYTCCCAIATRTCCATC) for cbhI, and GH48\_F8 (GCCADGHTBGGCGACTACCT) and GH48\_R5 (CGCCCCABGMSWWGTACCA) for GH48. The 20  $\mu L$  reaction mixture contained 0.4  $\mu L$  (10  $\mu M$ ) of each primer, 7.6  $\mu L$  ddH<sub>2</sub>O, 1.6  $\mu L$  diluted DNA (10-fold), and 10  $\mu L$  2 $\times$  SuperMix. Standard curves ( $10^2$ – $10^8$  gene copies) were generated using tenfold serial dilutions of plasmid DNA containing target gene sequences. The primers fungcbhIF (5'-ACCAAYTGCTAYACIRGYAA-3') and fungcbhIR (5'-GCTTCCCAIATRTCCATC-3') for cbhI and GH48\_F8 (5'-GCCADGHTBGGCGACTACCT-3')

and GH48\_R5 (5'-CGCCCCABGMSWWGTACCA-3') for GH48 were selected based on their specificity for targeting cellulolytic genes in ascomycete fungi, basidiomycetes, and actinobacteria, as validated in previous studies by Edwards et al. and de Menezes et al. [38,39]. The qPCR reactions were performed using 2× SYBR Green SuperMix (Bio-Rad, Hercules, CA, USA), which contains SYBR Green I dye, dNTPs, stabilizers, and iTaq DNA polymerase. Each 20 µL reaction mixture included 0.4 µL (10 µM) of each primer, 7.6 µL ddH<sub>2</sub>O, 1.6 µL diluted DNA (10-fold), and 10 µL SuperMix. Thermal cycling conditions followed the protocols described in the aforementioned references, with an initial denaturation at 95 °C for 3 min, followed by 40 cycles of 95 °C for 30 s, 55 °C annealing for 30 s, and 72 °C extension for 45 s.

## 2.6. Statistical Analysis

Before analysis, Raw Illumina PE 250 bp data were first quantity-filtered and trimmed to a consistent length, then the same sequence, chimera, and low-quality sequence were deleted. The OTU (operational taxonomic unit) [40] was identified at a >97% similar sequence clusters level using UCLUST in Quantitative Insights into Microbial Ecology (QIIME) software pipeline. The representative sequence of each OTU was selected and assigned the taxonomy annotation using the Ribosomal Database Project (RDP) classifier. The indicators of Chao1, Shannon, and Observed-OTUs were determined to represent the alpha-diversity of microbial communities. Principal components analysis (PCA) was conducted using R (version 3.4.3 for windows) package to compare microbial community structures of all samples. The bacterial OTUs were uploaded into PICRUSt, which was used to predict the functional characteristics of bacterial OTUs, and the metagenomes prediction was analyzed using the KEGG database after normalizing the OTUs [41]. It should be noted that PICRUSt predictions are based on inferred gene content from reference genomes rather than direct metagenomic data, which may introduce inaccuracies in complex or under-characterized microbial communities. The statistical analysis was performed by one-way analysis of variance (ANOVA) with SPSS 20.0 software, and post hoc tests (Tukey's LSD) was used to assess significance. Graphs were conducted using Origin 8.1.

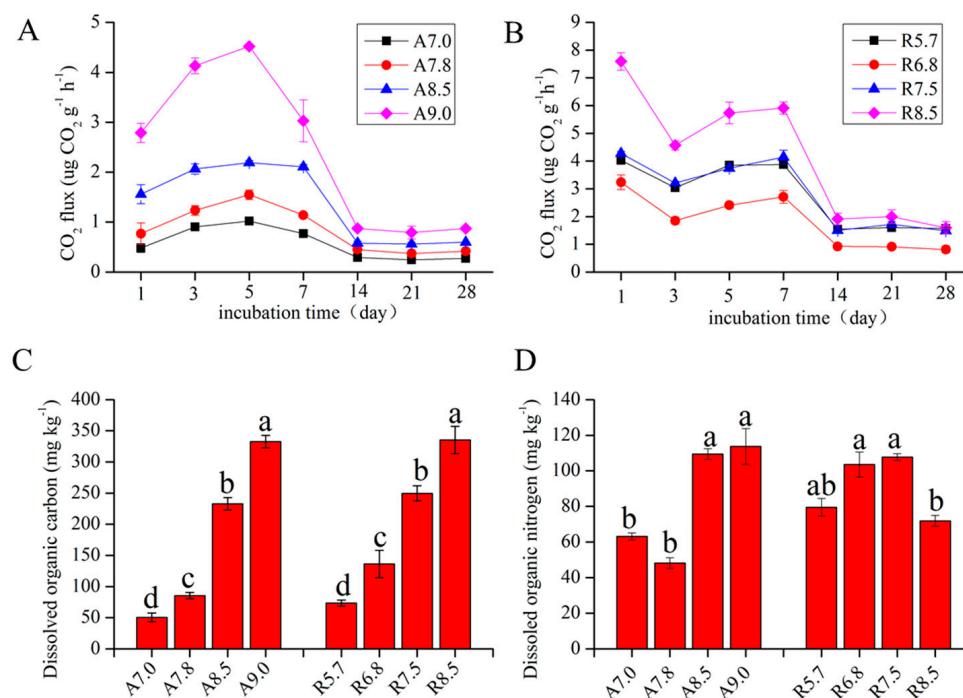
Network analysis [42,43] was conducted on bacterial high throughput sequence data and soil properties using maximal information coefficient (MIC) in MINE software. The MIC was a highly useful score that can reveal the strength of linear and non-linear associations among variables [44]. A total of 143 OTUs with strong positive ( $r > 0.8$ ), strong negative ( $r < -0.08$ ), and strong nonlinear ( $\text{MIC-p}^2 > 0.8$ ) relationships were included in network diagrams in Cytoscape 3.6.1 [45]. The network analyzer tool was used to calculate the network topological characteristics in Cytoscape. Mode application with default parameters was used for analyzing the modular structure of highly interconnected nodes. OTUs with maximum betweenness centrality scores were considered as keystone species [46].

## 3. Results and Discussion

### 3.1. Soil CO<sub>2</sub> Emission and Soil Properties

Soil organic carbon mineralization releases CO<sub>2</sub>, and pH plays an important role in this process [47]. In the present study, the respiration variations of two kinds of soil with different pH values were revealed. As shown in Figure 1A,B, the CO<sub>2</sub> flux is high from the first day to the 14th day, but this decreases and tends to be stable after 14 days in fluvo-aquic and red soil. Labels A 7.0, A 7.8, A 8.5, and A 9.0 denote fluvo-aquic soil treatments adjusted to pH 7.0, 7.8, 8.5, and 9.0, respectively, while R 5.7, R 6.7, R 7.5, and R 8.5 represent red soil treatments at pH 5.7, 6.7, 7.5, and 8.5 (Section 2.2). Subfigures C and D (labeled a–d) illustrate dissolved organic carbon (DOC) and nitrogen (DON) concentrations under each pH treatment, where a: pH 7.0 (fluvo-aquic)/pH 5.7 (red), b: pH 7.8/pH 6.7, c: pH 8.5/pH 7.5, and d: pH 9.0/pH 8.5.





**Figure 1.** (A) Temporal variation of CO<sub>2</sub> flux in fluvo-aquic soil across pH treatments; (B) Temporal variation of CO<sub>2</sub> flux in red soil across pH treatments; (C) pH-dependent dissolved organic carbon (DOC) concentrations in fluvo-aquic and red soils. (D) pH-dependent dissolved organic nitrogen (DON) concentrations in fluvo-aquic and red soils. Note; the labels a–d denote hierarchical levels, where a represents the highest and d the lowest rank.

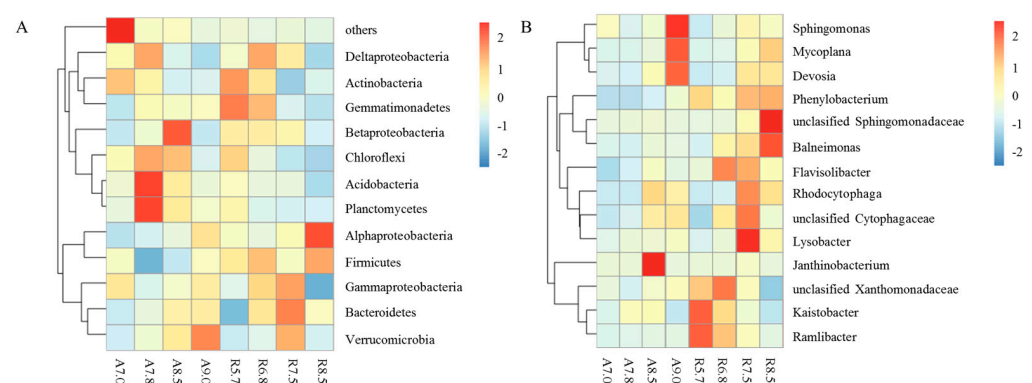
Generally, the obvious result was that CO<sub>2</sub> flux was positively correlated with pH in fluvo-aquic and red soil, except the R5.7. This result was consistent with the results of Xu et al. [48], who found that the highest emissions of CO<sub>2</sub> occurred in relatively high pH soil. Acidity is detrimental to the activity of soil microorganisms, which leads to a reduction in soil respiration [49]. In addition, low pH promoted aluminum activity, which was harmful to microbial activity after releasing aluminum into the soil [50,51]. On the other hand, the addition of lime increases the ability of soil microorganisms to utilize macromolecular substances and the metabolic potential of microorganisms, thus increasing soil respiration.

Moreover, the changes in DOC and DON content in two kinds of soil with different pH gradients are shown in Figure 1C,D. In fluvo-aquic soil, DOC concentrations increased by 208.68 mg·kg<sup>-1</sup> from pH 7.0 to pH 8.5; in red soil, DOC concentrations increased by 240.60 mg·kg<sup>-1</sup> from pH 5.7 to pH 8.5. It was obvious that the DOC concentrations were positively correlated with soil pH (Figure 1C). DON showed the highest contents in pH 9.0 for alkaline soil and the highest in pH 7.5 for red soil, respectively (Figure 1D). The high DOC and DON concentrations in the high pH value were likely to be a result of the stimulation of the solubility of soil organic matter induced by liming [52]. A tentative inference on this result is that the increase in pH likely helps metabolize large molecules [53] and releases more readily available substances for use by the microbes; thus, accelerated soil microbial respiration leads to the loss of soil-dissolved organic carbon.

### 3.2. Bacterial Community Composition

The bacterial community composition of two kinds of soil with different pH values was determined based on the high-throughput sequence. In the present study, Alphaproteobacteria, Bacteroidetes Acidobacteria, and Actinobacteria were the dominant phyla in all treatments in two kinds of soil (Figure 2A). The relative abundance of Alphaproteobacteria increased the most as pH increased in both soils, which was similar to previous results. For

instance, an upward trend has been found in the relative abundance of Alphaproteobacteria toward high pH in arable soil and Craibstone Experimental Farm soil due to different original soils [14,54]. The relative abundance of Bacteroidetes was higher in high-pH soils than those with low pH. Alphaproteobacteria and Bacteroidetes, as eutrophic microorganisms, grow well in the sufficient substrate condition [55]. In our study, the increasing content of DOC with high pH may contribute to the increased abundances of Alphaproteobacteria and Bacteroidetes. As shown in our study, Acidobacteria presented a decreased trend toward high pH (Figure 2A). Many members of the Acidobacteria phylum belong to oligotrophic microorganisms. Thus, the increase in DOC content with high pH increased competition and inhibited the activity of members of Acidobacteria, which was in line with the previous study [12]. As heterotrophic aerobic bacteria, Actinobacteria decreased toward high pH in two kinds of soil (Figure 2A).

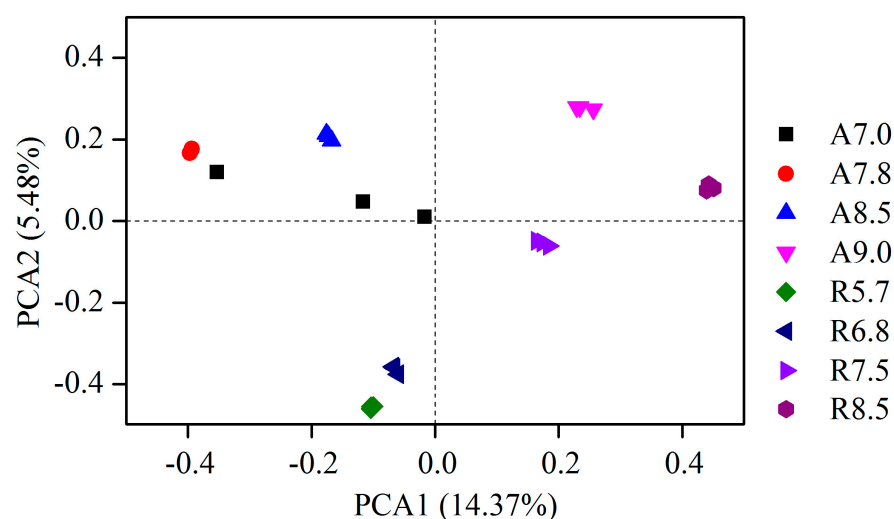


**Figure 2.** Heatmap indicates the relative abundance of major bacterial phyla and genera with different treatment in two soil samples: (A) the relative abundance of dominant bacterial phyla and classes of Proteobacteria; (B) the relative abundance of the 14 most abundant bacterial genera.

The 14 most abundant bacterial genera (Figure 2B) were unclassified Xanthomonadaceae, unclassified Sphingomonadaceae, Mycoplana, and Kaistobacter in two kinds of soil (Figure 2B). Xanthomonadaceae was described previously as a decomposer of hydrocarbon, and it can obtain carbon from co-occurring microbes [56]. Sphingomonadaceae could transform the nutrients and decompose the recalcitrant carbon resource and aromatic compound [57,58]. In our study, the relative abundance of Sphingomonadaceae showed an obvious increase toward high pH in red soil, which indicated that high pH could stimulate soil refractory substance and reduce the stability of soil organic carbon. The abundance of Mycoplana, belonging to the family Caulobacteraceae, showed a significantly positive correlation with pH value in two kinds of soil (Figure 2B). Mycoplana was considered as a rhizospheric microorganism and it was prevalent in fertile soils [59]. Taken together, Mycoplana microorganisms are abundant in high pH because the high pH value improves the nutrient utilization rate of soil. Kaistobacter was more sensitive to pH change in red soil than that in fluvo-aquic soil and its abundance showed a negative correlation with pH in red soil (Figure 2B). It has been demonstrated that Kaistobacter is widely present in an Fe mineral reduction environment; the low pH value can increase the solubility of Fe, increase the activity of  $\text{Fe}^{3+}$  reductase, and reduce  $\text{Fe}^{3+}$  to  $\text{Fe}^{2+}$  to facilitate root absorption [60] (Figure 3).

Principal component analysis (PCA) revealed distinct clustering patterns of bacterial communities across pH gradients. The first two principal components (PC1 and PC2) explained 68% of the total variance (PC1: 52%, PC2: 16%). Fluvo-aquic soil treatments (A 7.0, A 7.8, A 8.5) clustered closely along PC1, indicating minimal structural divergence at moderate pH levels. In contrast, red soil treatments (R 5.7, R 6.7) formed a separate group, reflecting inherent differences in native microbial communities. Notably, high-

pH treatments (A 9.0 and R 8.5) diverged significantly from their respective soil clusters, demonstrating that extreme pH shifts restructured bacterial assemblages [61].



**Figure 3.** Principal component analysis (PCA) of bacterial community from two kinds of soil with different values of pH.

The distribution along PC1 correlated strongly with pH gradients (Mantel  $r = 0.89$ ,  $p < 0.001$ ), confirming pH as the dominant driver of community variation. This axis primarily separated taxa adapted to alkaline conditions (e.g., Alphaproteobacteria) from acidophilic groups (e.g., Acidobacteria). In red soil, the rightward shift of R 8.5 along PC1 mirrored the enrichment of Bacteroidetes and suppression of Actinobacteria, consistent with their pH-dependent metabolic strategies [62].

Mantel tests further quantified pH-dependent associations (Table 1). Significant positive correlations ( $r > 0.6$ ,  $p < 0.01$ ) were observed between pH and Alphaproteobacteria ( $r = 0.76$ ), Bacteroidetes ( $r = 0.69$ ), and Actinobacteria ( $r = 0.78$ ) in fluvo-aquic soil, while Acidobacteria abundance declined with pH ( $r = -0.63$ ). In red soil, Bacteroidetes ( $r = 0.90$ ) and Acidobacteria ( $r = -0.83$ ) exhibited contrasting trends, underscoring soil-specific pH effects. Notably, CO<sub>2</sub> emissions and DOC showed strong pH dependence ( $r > 0.85$ ), aligning with enhanced microbial activity under alkaline conditions [63–65].

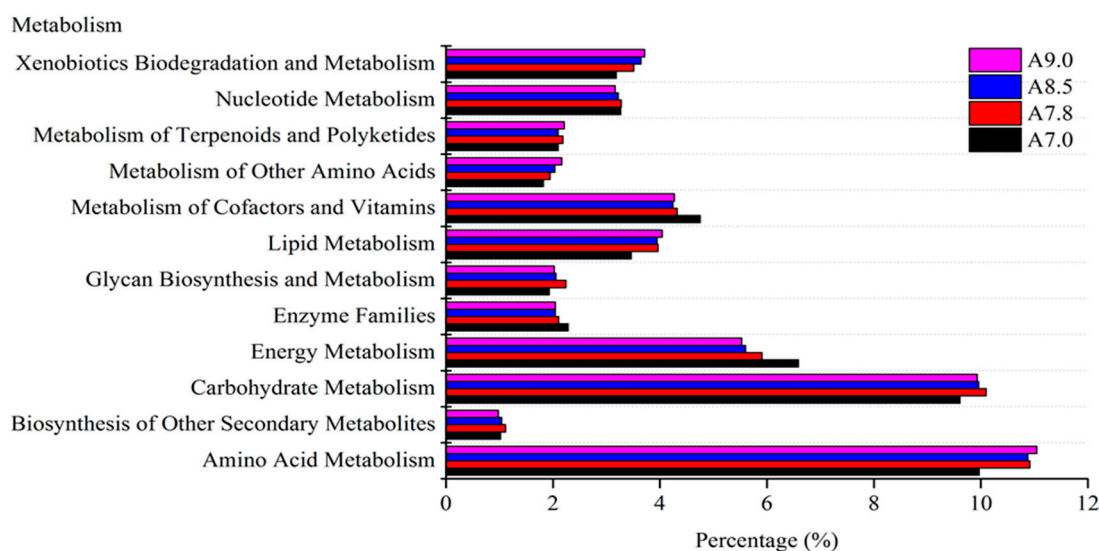
**Table 1.** The correlations ( $r$ ) and significance ( $P$ ) were determined by Mantel tests between pH and bacterial phyla, genus, and environmental variables.

|                      | Alkaline Soil |         | Red Soil |         |
|----------------------|---------------|---------|----------|---------|
|                      | $r$           | $p$     | $r$      | $p$     |
| Dominant Phyla       |               |         |          |         |
| Alphaproteobacteria  | 0.76          | <0.0001 | 0.58     | 0.003   |
| Bacteroidetes        | 0.69          | 0.001   | 0.9      | <0.0001 |
| Actinobacteria       | 0.78          | 0.0001  | 0.63     | 0.001   |
| Acidobacteria        | 0.63          | 0.005   | 0.83     | <0.0001 |
| Functional Genes     |               |         |          |         |
| GH48                 | 0.145         | 0.121   | 0.601    | 0.002   |
| cbhI                 | −0.095        | 0.839   | 0.543    | 0.006   |
| Soil Properties      |               |         |          |         |
| DOC                  | 0.91          | <0.0001 | 0.95     | <0.0001 |
| DON                  | 0.95          | <0.0001 | 0.95     | <0.0001 |
| CO <sub>2</sub> flux | 0.864         | <0.0001 | 0.714    | 0.001   |

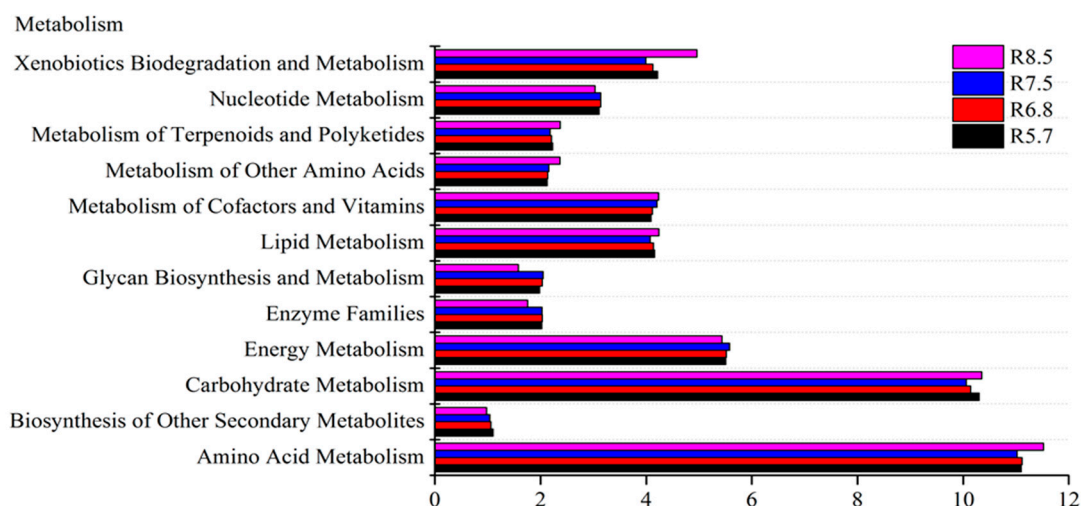


### 3.3. Bacterial Functional Prediction in Response to pH Gradient

In addition, the pH value could also change the metabolism function of the bacterial community [30,66]. The functional characteristics of the bacterial community from a different gradient of pH in two kinds of soil were analyzed by PICRUSt [41]. There are twelve functional groups of the genes in the metabolism category. The dominant groups are amino acid metabolism, carbohydrate metabolism, and energy metabolism in fluvo-aquic and red soil (Figures 4 and 5).



**Figure 4.** Prediction of bacterial metabolism function with different pH values in alkaline soil analyzed by PICRUSts.

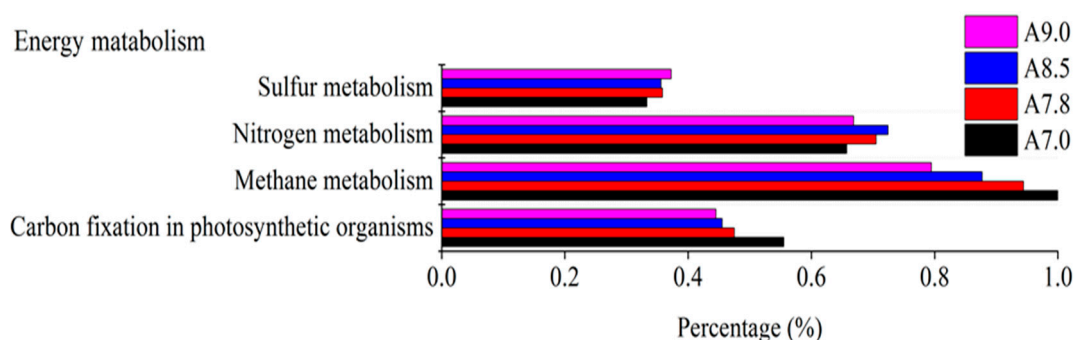


**Figure 5.** Prediction of bacterial metabolism function with different pH values in red soil analyzed by PICRUSts.

For alkaline soil, the relative abundance of genes connected with cofactors, vitamins, enzyme families, and energy metabolism decreased with the increasing pH [67–69]. The genes involved in lipid metabolism, amino acid metabolism, and xenobiotics biodegradation metabolism exhibited a continuous increase toward high pH value (Figure 4). It was concluded that high pH in fluvo-aquic soil inhibited bacterial activity related to energy metabolism, but promoted bacterial capability related to xenobiotics biodegradation and metabolism, amino acid metabolism, and lipid metabolism.

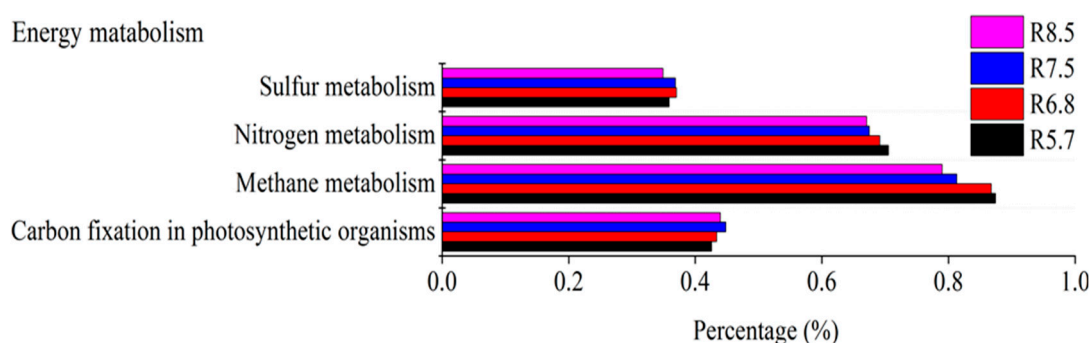
For red soil, the sequences assigned to the biosynthesis of other secondary metabolites decreased gradually between pH 5.7 and pH 8.5. Conversely, the abundance involved in the metabolism of other amino acids, cofactors, and vitamins showed an increase as pH increased, and there was no significant change in the other bacterial metabolism capability in red soil (Figure 5).

Otherwise, the subgroups of energy metabolism were predicted, such as carbon fixation in photosynthetic organisms, methane metabolism, nitrogen metabolism, and sulfur metabolism (Figure 6). The abundance of bacterial carbon fixation in photosynthetic organisms decreased toward high pH in fluvo-aquic soil. The shift in metabolism function of the bacterial community was possibly caused by the utilization of readily decomposable substrates.



**Figure 6.** Subsystem of energy metabolism with different pH values in alkaline soil analyzed by PICRUSTs.

The sequence of bacterial methane metabolism function was more abundant in high pH (Figure 7). Accompanied by hydrolysis, acidogenesis, and acetogenesis, methanogenesis finished the cellulose degradation process. It was speculated that the alkaline environment is conducive to the decomposition of cellulose, which is not good for carbon fixation. The sequence associated with nitrogen metabolism revealed a negative relationship with pH in red soil, which was supported by the study that the increase in pH decreased cumulative  $N_2O$  production [70]. In conclusion, pH influenced the metabolism function of bacteria and the effects differed in two kinds of soil.



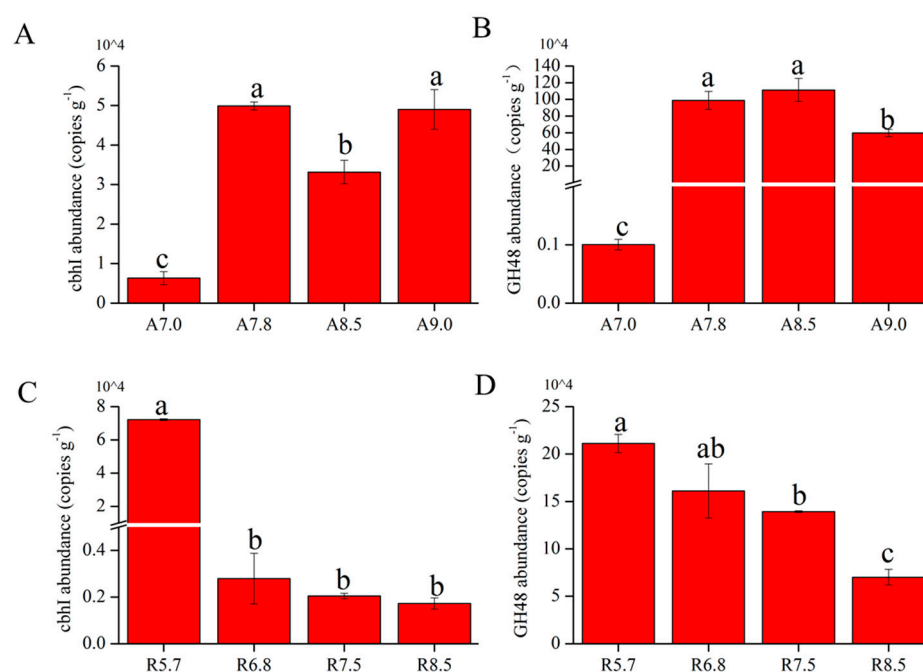
**Figure 7.** Subsystem of energy metabolism with different pH values in red soil analyzed by PICRUSTs.

### 3.4. GH48 and cbhI Gene Abundance Under Different pH Values

Cellulose is the major component of organic carbon in farmland soil; thus, the ecological characteristics of microorganisms in their degradation process are very important. Likewise, it was considered that the cellulolytic enzyme encoded by cbhI gene and GH48 gene could catalyze cellulose decomposition [38]. Therefore, it is of great importance to investigate the change in chhI gene and GH48 gene abundance in response to pH alteration.

In fluvo-aquic soil, the abundance of GH48 gene ranged from  $1.04 \times 10^3$  to  $5.96 \times 10^5$  copies  $g^{-1}$ , and cbhI gene ranged from  $0.6 \times 10^4$  to  $4.9 \times 10^4$  copies  $g^{-1}$ ; all of them were

significantly lower in A7.0 than that in higher pH treatments (Figure 8A). In red soil, the abundance of GH48 gene ranged from  $7.01 \times 10^4$  to  $21 \times 10^4$  copies  $g^{-1}$ , while *cbhI* gene ranged from  $0.17 \times 10^4$  to  $7.2 \times 10^4$  copies  $g^{-1}$ . The abundances of GH48 and *cbhI* gene in red soil gradually decreased with increasing pH value, and it declined by  $1.40 \times 10^5$  copies  $g^{-1}$  soil and  $7.0^3 \times 10^4$  copies  $g^{-1}$  between R 5.7 and R 8.5, respectively (Figure 8B). Exchangeable aluminum was reported to increase as pH increases, and the high contents of exchangeable aluminum were harmful to microbial activity. For fluvo-aquic soil, GH48 and *cbhI* gene abundance were lowest in A 7.0, partly due to the influence of the addition of exchangeable aluminum in regulating pH. Therefore, proper the reduction in soil pH can reduce the abundance of cellulose-related genes, which is beneficial to the carbon fixation of fluvo-aquic soil. As for red soil, pH showed a negative relationship with the cellulose degradation gene (GH48 and *cbhI*). It was reported that enzyme composition containing cellulolytic enzymes, such as endo-glucanase and polysaccharide hydrolases, was contacted better with cellulose-rich material under acidic conditions. Our result likely means that the cellulose degradation gene could play an important role in breaking down cellulose under low pH value in red soil (Figure 8C,D). Generally, pH would regulate the organic matter decomposition by controlling the key gene abundance, but the effect of the key genes on organic matter decomposition is different for different types of soil [71–73].

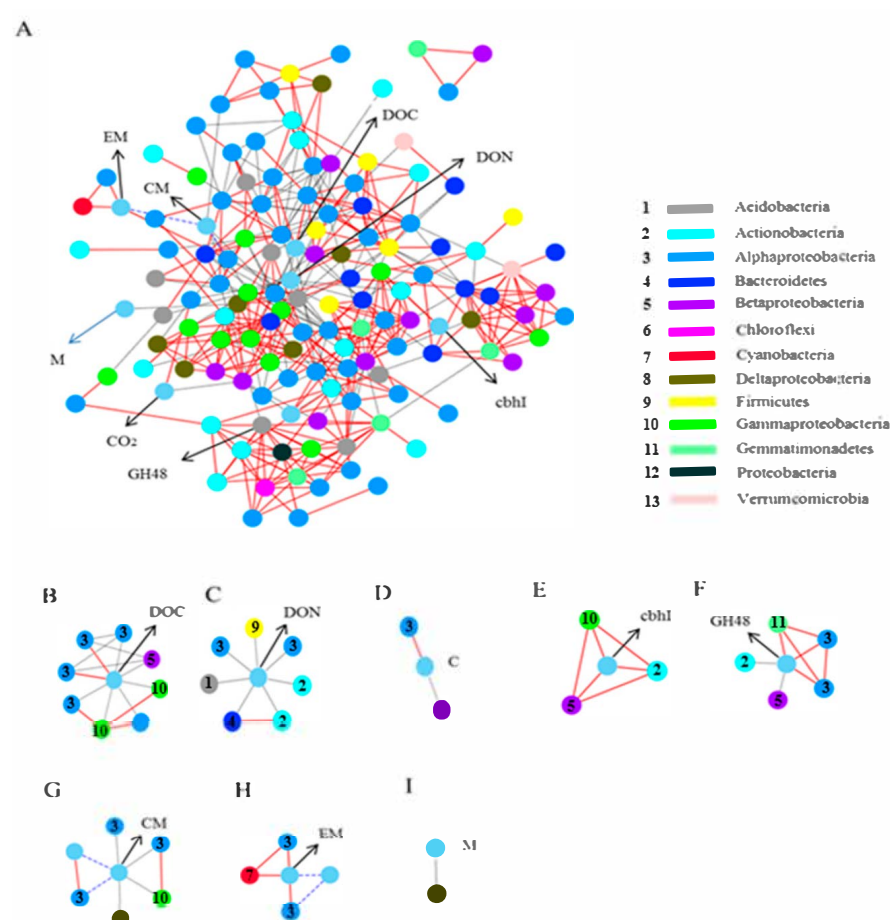


**Figure 8.** (A) GH48 gene abundance under increasing pH gradients in fluvo-aquic soil. (B) *cbhI* gene abundance under increasing pH gradients in fluvo-aquic soil. (C) Decline in GH48 gene abundance with rising pH in red soil. (D) Decline in *cbhI* gene abundance with rising pH in red soil. Note; the labels a–c denote hierarchical levels, where a represents the highest and d the lowest rank.

From above, the observed shifts in GH48 and *cbhI* gene abundance under varying pH levels align with pH-driven microbial community restructuring (Section 3.2). In red soil, the decline in GH48 and *cbhI* gene abundance at higher pH (Figure 8B) corresponds with reduced Acidobacteria (Figure 2A, Table 1), a phylum known to harbor cellulolytic taxa. Conversely, the enrichment of Alphaproteobacteria and Bacteroidetes at elevated pH (Table 1) suggests their potential functional redundancy in cellulose degradation under alkaline conditions, albeit with lower gene abundance. This implies that pH-induced microbial compositional changes directly modulate cellulolytic gene dynamics, with soil-specific taxa driving functional adaptations.

### 3.5. Network Associations Among Bacterial Community and Soil Chemical Characteristics, Functional Genes, and Microbial Metabolism

Network analysis was applied to reflect the associations between bacterial community and environmental factors such as soil chemical characteristics and soil respiration [74–77]. For 418 significant associations (edges), the network contains 127 nodes with an average number of clustering coefficient of 0.44 and neighbors of 8.46 (Figure 9A). Network edges were principally composed of strong positive associations, and the dominate OTUs belonged to Alphaproteobacteria, Deltaproteobacteria, and Actinobacteria (Figure 9A). The change in pH influenced dominant soil microbial community activity, thus affecting soil CO<sub>2</sub> emissions through soil microbial respiration [78]. Network analysis indicated that CO<sub>2</sub> emission showed a strong positive association with Caulobacteraceae and was nonlinear with Comamonadaceae. Caulobacteraceae is adaptive to oligotrophic conditions of low availability of metabolic substrate [57].



**Figure 9.** Network analysis revealing the associations among bacterial community and soil chemical characteristics, functional genes, and microbial metabolism (A). Subnetwork for the microorganism with soil chemical characteristics (B), functional genes (C), and microbial metabolism (D). Red solid and blue dashed lines represent strong positive linear ( $r > 0.8$ ) and strong negative ( $r < -0.08$ ) relationships, while gray lines denote strong nonlinear associations ( $\text{MIC-p}^2 > 0.8$ ). Colored nodes signify corresponding OTUs assigned to major phyla and classes. (E): Temporal dynamics of microbial enzymatic activity (e.g., cellulase,  $\beta$ -glucosidase) across pH gradients. (F): Soil microbial biomass carbon (MBC) and nitrogen (MBN) variations under different pH treatments. (G): pH-dependent shifts in fungal-to-bacterial ratio influencing organic matter decomposition. (H): Correlation heatmap between soil physicochemical properties (e.g., DOC, DON) and microbial taxa abundance. (I): Functional redundancy analysis of cellulolytic genes (GH48, cbhI) across microbial taxa in contrasting pH conditions.

As the heterotrophic bacteria, Comamonadaceae contributes to the decomposition of soil organic compounds and is adaptive to rich nutrient environments [79,80]. The cluster of bacteria related to dissolved organic carbon (DOC), including Mycoplana, Erythrobacteraceae, Sphingomonadaceae, Lysobacter, Thermomonas, Rhodoplanes, and Janthinobacterium, was also shown in the network. Mycoplana and erythrobacteraceae were significantly positively correlated with DOC. Mycoplana was commonly found in the rhizosphere, which contributed to soil nutrient cycling and the decomposition of soil organic pollutants [81]. Therefore, the higher relative abundance of Mycoplana in high pH could accelerate soil organic matter transformation; this could be supported by the fact that higher DOC content occurred in high-pH treatment. Previous research also found that Erythrobacteraceae was capable of degrading the polycyclic aromatic hydrocarbon in soil [82,83].

Additionally, Sphingomonadaceae, Lysobacter, and Thermomonas showed nonlinear relationships with DOC, and Sphingomonadaceae and Thermomonas per formed a significantly positive correlation with Lysobacter, indicating that there was a synergistic effect of the microbial community on soil carbon cycling [84–86]. Furthermore, Bacillus, Phenylobacterium, Flavisolibacter, Phycococcus, Micromonosporaceae, and Acetobacteraceae were nonlinearly related to DON. Acetobacteraceae has been described as a N-fixing bacterium that could incorporate atmosphere N<sub>2</sub> into soil organic matter. Bacteria from the Acetobacteraceae family could generate organic acids as a final metabolic product by incompletely oxidating sugars and alcohols and could survive in very acidic conditions, but the optimum range is close to pH 5.0–6.5. Members of Flavisolibacter and Bacilli were considered as degraders of cellulose and soil organic matter; therefore, they could contribute to soil C cycling [87–89].

To further explore the relationship between bacterial community and carbon-related functional genes, two clusters of bacteria neighboring GH48 and cbhI were constructed. As shown in Figure 9B, Xanthomonadaceae, Comamonadaceae, and Micromonosporaceae exhibited positive associations with the cbhI gene [90,91]. GH48 displayed a nonlinear correlation with Pimelobacter and Comamonadaceae, and a positive correlation with Sphingomonadaceae and Gemmatimonadetes. Xanthomonadaceae and Sphingomonadaceae have been shown to have the ability to degrade complex organic matter. It has been proved that a high abundance of Gemmatimonadetes and Comamonadaceae was beneficial to decompose chitin and soil organic compounds [82,83]. Micromonosporaceae from phylum Actinobacteria have been reported to have the capability of breaking down cellulose [92,93]. Thus, these results indicated that GH48 and cbhI genes showed close associations with microbial groups involved in the decomposition of soil organic matter [94,95].

In order to explore the association between bacterial metabolism and bacterial taxa, subnetworks were further constructed (Figure 9C). There was a nonlinear relationship between Haliangiaceae and metabolism (M). It was obvious that Sphingomonas, Streptophyta, and Agrobacterium had notable positive correlations with energy metabolism (EM). In addition, carbohydrate metabolism (CM) exhibited a nonlinear relationship with four genera, including Rhodoplanes, Sphingomonadaceae, Lysobacter, and Haliangiaceae, while it displayed a negative association with Agrobacterium. Specifically, energy metabolism (EM) was negatively correlated with carbohydrate metabolism (CM). In general, some bacterial taxa were positively related to different biochemical metabolic pathways, indicating that bacterial taxa had a synergistic effect on metabolic pathways [93].

#### 4. Conclusions

The present work revealed that pH greatly influenced the soil mineralization and microbial community composition, and the effect depended on soil types. A high pH value



led to an increase in CO<sub>2</sub> emissions and soil DOC content. The pH-driven restructuring of microbial communities significantly influenced the abundance of cellulose-degrading functional genes (cbhI and GH48) in fluvo-aquic soils, as taxa harboring these genes (e.g., Xanthomonadaceae, Comamonadaceae) exhibited pH-dependent shifts in relative abundance. These compositional changes, in turn, modulated functional gene pools linked to cellulose metabolism. Similarly, pH affected the abundance of functional genes (cbhI and GH48) in red soil, but had less of an effect on carbohydrate metabolism. This study concluded that, for fluvo-aquic soil, the abundance of carbohydrate metabolism and cellulose degradation were largest, and it is conducive to carbon preservation and soil stability. The microbial communities were more vulnerable to pH change in red soil than in fluvo-aquic soil.

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