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Effects of Canopy Damage and Litterfall Input on CO_2-Fixing Bacterial Communities

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Abstract: Extreme weather events often cause canopy disturbance and litter deposition. To study the CO_2-fixing bacterial response to forest damage, we simulated the canopy damage caused by extreme weather with four different treatments: control (CN), canopy trimming + removal of branches and leaves debris (TR), canopy trimming + retaining of branches and leaves debris (TD), and undamaged + transplantation of branches and leaves debris (UD). We used the *cbbL* gene, which encodes ribulose-1,5-biphosphate carboxylase/oxygenase (RubisCO), for Miseq sequencing to analyze the dynamics of community composition of soil CO_2-fixing bacteria for five consecutive years after canopy damage. Double treatments of canopy damage and litterfall inputs (TD) facilitate forest restoration better than single treatments (TR or UD). Most soil CO_2-fixing bacteria are facultative autotrophic bacteria, and *Nitrosospira*, *Streptomyces*, and *Saccharomonospora* are the main carbon-fixing microorganisms, which have significant differences during the restoration of damaged forest canopy. The forest ecosystem restoration after canopy damage lasted 4–5 years. Rainfall and pH showed a significant negative correlation with most soil CO_2-fixing bacteria communities. This study provides a theoretical basis for improving the carbon sequestration capacity of forest soil CO_2-fixing bacteria after extreme weather and also provides guidance for forest ecosystem management.

Keywords: extreme weather events; canopy openness; litterfall decomposition; CO_2-fixing bacteria; *cbbL* gene

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1. Introduction

Extreme weather events caused by climate warming frequently occur, such as typhoons and ice storms, and have an enormous impact on forest ecosystems [1]. In January and February 2008, prolonged and widespread ice storms occurred in southern China, causing severe damage to 18.6 million hectares of forest, accounting for 1/10 of the total forest area in China [2]. While some trees were collapsed and died, the canopies of most trees were damaged during the ice storms, resulting in abnormal litterfall [3]. The effects of the ice storm on the forest were canopy reduction and litter debris deposition [4]. After the disaster, the respiration of the forest increased, leaf biomass decreased sharply, and carbon fixation of photosynthesis decreased, which might be converted from a carbon sink to a carbon source [3].

Canopy damage causes changes in environmental factors such as light intensity and precipitation in the forest, and leads to litter decomposition and soil nutrient content changes, increasing the exposure of litter to sunlight, thus greatly accelerating the carbon cycle [4]. Litterfall is the primary carbon source and nutrient recycling for forest soil, releasing nutrients and organic compounds after decomposition that contribute to the composition of soil organic matter and maintain the microbiome [5]; further, litter input leads to changes in soil carbon content. The mechanisms of litterfall decomposition into the soil layer are fundamental processes for ecosystem functions, which regulate soil organic

matter circulation, carbon dioxide emission into the atmosphere, and carbon sequestration into the soil [6]. Soil organic carbon (SOC) is defined as a positive or negative priming effect, which means that the input of exogenous carbon can accelerate or inhibit the decomposition of SOC [7]. Humid tropical forests have the highest rates of litterfall production globally and have the fastest litter decomposition rates, leading to rapid turnover of litterfall carbon and nutrient stocks [4]. Soil microorganisms regulate the defoliated material decomposition and the soil organic matter transformation [8]. The composition of most soil microbial communities is sensitive to disturbance rather than immediate recovery [9].

It was widely believed that carbon fixation mainly depends on plant photosynthesis, and soil microorganisms promote carbon cycling by participating in the degradation of organic matter. The role of microorganisms in carbon sequestration has been underestimated [10]. It is speculated that global terrestrial soil microorganisms fix 0.5%–4.1% of atmospheric CO_2 per year, with an annual carbon sequestration of 0.3 to 3.7 Pg [11]. Soil autotrophic bacteria play an important role in atmospheric CO_2 sequestration and affect the regeneration and circulation of organic matter [12]. Bacteria can convert CO_2 into organic carbon through biochemical reactions and then use CO_2 to form their own cell materials [12]. These biochemical reactions can be divided into bacterial photosynthesis, chemical synthesis, and heterotrophic CO_2 fixation [13]. The Calvin cycle is the predominant pathway for CO_2 fixation and is performed by bacteria and algae [14]. Ribulose 1,5-bisphosphate carboxylase/oxygenase (RubisCO) acts as a key CO_2 assimilation enzyme, catalyzing the first reaction step in the Calvin cycle [12]. RubisCO exists in four forms (Form I-IV) with different structures, catalytic functions, and O_2 sensitivities [12]. RubisCO I plays a significant role on soil [14]. The *cbbL* gene encoding the large subunit of RubisCO I has been used as a functional marker for molecular research of CO_2-fixing bacteria [14]. The phylogenetic study of *cbbL* gene sequence shows that the *cbbL* gene encoding I RubisCO can further belong to one of the four evolutionary clades of IA–ID [12]. These clades include obligate autotrophic bacteria (form IA RubisCO), green plants and some cyanobacteria sequences (form IB), facultative autotrophic bacteria (form IC), and chromophytic algae (form ID) [13]. Some aerobic oxidizing bacteria, such as *Bradyrhizobium japonicum*, *Mycobacterium* sp., and *Burkholderia* sp., also contain the *cbbL* gene and can thus fix CO_2 via the Calvin cycle combined with CO oxidation [15]. The key role of these bacteria in the C cycle makes it possible that the same bacterial group would also be the first biome to re-establish in soil during the early recovery stage after disturbance [16]. To date, the molecular ecology of CO_2-fixing bacteria in forest [17] and agriculture systems [12] has received increasing attention. The community structure and diversity of the CO_2-fixing bacteria are affected by plant types, land management, and edaphic factors [18], especially soil available N, soil pH, and soil organic carbon (SOC).

The study aims to investigate the effects of canopy damage and abnormal litterfall on the diversity and community structure of CO_2-fixing bacteria and their dynamics during the ecological restoration process. Specifically, we were interested in the following questions. First, what are the effects of canopy damage and abnormal litterfall input on soil CO_2-fixing bacterial diversity and community structure? Does canopy damage or abnormal litterfall input have a more significant effect? Second, do environmental factors have significant effects on the community structure of soil CO_2-fixing bacteria? Third, what role does soil CO_2-fixing bacteria play in litter degradation and carbon sequestration during forest ecosystem restoration? The study simulated the restoration process after canopy damage and used the Illumina MiSeq sequenced technology to analyze the community structure of CO_2-fixing bacteria during canopy damage restoration. It reflected the response of soil CO_2-fixing bacteria to forest restoration and clarified the importance of environmental factors on soil CO_2-fixing bacteria. The study provides a theoretical basis for studying the carbon sequestration potential of forest soil microorganisms and further understanding the importance of soil CO_2-fixing bacteria in forest ecosystems.

2. Materials and Methods

2.1. Site Area and Experimental Design

The experimental sites were located in the southeast of Xiaoken Forest Farm, Qujiang District, Shaoguan City, Guangdong Province in China (113°49′08″–113°52′12″ E, 24°39′42″–24°42′33″ N). A humid subtropical monsoon climate dominated the location. The sampling terrain was hilly with red soil, average elevation ranging from 550 to 580 m, and an average gradient of 33°. The site is characterized by a typical subtropical evergreen and deciduous broad-leaved mixed forest. The dominant species in the 25-year-old tree layer are *Castanopsis fissa*, *Castanopsis carlesii*, *Cinnamomum camphora*, *Pinus massoniana*, and *Machilus kwangtungensis* (Figure S1).

The forest canopy simulated ice and snow damage experiment was performed according to the historical registers collected by Wang et al. [19] in 2008. In December 2010, the canopy density and stand of the Xiaokeng Forest Farm were investigated. Four experimental quadrats of 30 × 30 m were set up on the same hilltops with three similar hilltops replicates. The spacing between the four quadrats was greater than 10 m, and the spacing between replicates was greater than 100 m. The canopy damage treatment was conducted from December 2010 to February 2011. Four quadrats per sampling site were divided into four different treatments: control (CN), canopy trimming + removal of branches and leaves debris (TR), canopy trimming + retaining of branches and leaves debris (TD), and undamaged + transplantation of branches and leaves debris (UD) [20,21]. For sample areas with TD and TR canopy damage, trees with a diameter at breast height of 5–10 cm were treated by breaking the top under the first level of bifurcation; trees with diameter at breast height > 10 cm were treated by pulling off their branches with diameter < 10 cm [19]. The damaged branches and leaves of the TD sample area remained in the TD sample area while those of the TR sample area were removed and evenly scattered in the UD sample area [19]. The CN sample was the undamaged sample area. The sample areas were treated by cutting open the notches and manually pulling them off. The sample areas were well maintained, and the mortality rate of damaged trees was low.

From 2011 to 2015, between July and August of each year, about 200 g of 0–10 cm soil samples were taken from 12 fixed sites through five sampling points. The samples were collected from four experimental quadrats with three replicates per quadrat for five consecutive years, and a total of 60 samples were obtained. After removing impurities (stones, litter, plant roots), the samples were divided into two fractions: one was air-dried and sieved using a 2 mm mesh for physicochemical analysis; the other was stored in a refrigerator at −80 °C for molecular experiments. Samples were coded as follows: CNm.n, where CN represents the control, m represents the year the sample was collected, and n represents the replicate (i.e., CN12.1 is replicate 1 of the control sample collected in 2012).

2.2. Measurements of Physicochemical and Biological Parameters

Soil pH was determined by the acidimeter method with a soil:water ratio of 1:2.5 (w/v) [22]. Soil organic carbon (SOC) was determined by potassium dichromate oxidation titration [23]. Total nitrogen (TN) was determined by the semi-micro Kjeldahl method [24]. Nitrate-nitrogen (NO_3^--N) was determined by phenol disulfonic acid colorimetry [24], total phosphorus (TP) was determined by sodium hydroxide alkali melting molybdenum antimony colorimetry [25], and available phosphorus (AP) was determined by sodium hydrogen carbonate solution-Mo-Sb anti spectrophotometric [26].

Rainfall and temperature data between December 2010 and August 2015 were collected from an automatic weather station, established by the Nanling Ecological Station of the Chinese Academy of Forestry (CAF), about 2 km from the samples. Considering that the soil samples were collected in August of each year, only the rainfall and the average temperature in July and August provided by the Qujiang Meteorological Bureau from 2011 to 2015 were analyzed. The rainfall range was 97.50–187.85 mm, and the temperature range was 24.1–29.0 °C (Figure S2). From 2011 to 2014, the average temperature in July to August changed little, and the temperature was slightly lower in 2015. In 2013, the rainfall

increased significantly to 187.85 mm, and in other years, the rainfall fluctuated between 100–120 mm (Figure S2).

Canopy openness (CO) was calculated using a full-frame digital camera (Nikon D700S) and 180° fisheye lens (Nikon F = 8 mm) from December 2010 to August 2015. The camera was situated 1 m above the ground at the intake of the stand perpendicular to the image, and the Gap Light Analyzer software (v2.0) was used to analyze the photographs to calculate canopy openness [27]. From December 2010 to August 2015, four 1.0 × 1.0 × 0.2 m (length × width × height) litterfall (LI) collection net frames with a 1.0 cm mesh aperture were placed at the center of each quadrat. The litterfall of four collection nets per quadrat sample site was collected and combined to form one composite sample. The composite samples were dried at 65 °C and weighed at the end of each month. The litterfall volume was collected for 5 years from December 2010 to July in 2011 and from August to July in 2012–2015. The amount of litterfall is the sum of the amount of litterfall collected annually.

2.3. DNA Isolation and PCR Amplification

Soil DNA was extracted from the frozen samples (0.5 g wet weight) using an Ezup Column Soil DNA Kit (Sangon Biotech, Shanghai, China) according to the manufacturer's instructions [28]. DNA quality was checked using a BioPhotometer plus (Eppendorf, Germany) to ensure the concentration was higher than 10 ng/μL and stored at −20 °C before sequencing. For the amplification of the *cbbL* gene (489–495 bp) of CO_2-fixing bacteria, the primers of K2f (5′-ACCAYCAAGCCSAAGCTSGG-3′) and V2r (5′-GCCTTCSAGCTTGCCSACCRC-3′) were chosen [12]. The reaction conditions were 95 °C for 3 min; followed by 35 cycles of 94 °C for 30 s, 52 °C for 30 s, and 72 °C for 30 s; followed by an extension at 72 °C for 7 min. All PCR reactions were performed in 20 μL total mixture containing 10 μL 2× Taq PCR Master Mix, 1.0 μL of 10 μM each K2f and V2r primers, 2.0 μL of DNA template (50 mg/L), 2.0 μL of BSA (2.0 mg/mL Bovine Serum Albumin), and 4 μL of ultrapure water.

The PCR amplicons were electrophoresed on 2% (*w/v*) agarose gel and purified using the GeneJET Gel Extraction Kit (Thermo Scientific, Belmont, MA, USA). Sequencing libraries were generated using the NEB Next1 UltraTM DNA Library Prep Kit for Illumina (New England Biolabs, Ipswich, MA, USA) following the manufacturer's recommendations. The library quality was assessed on a Qubit 2.0 Fluorometer (Thermo Scientific, Belmont, MA, USA) and Agilent Bioanalyzer 2100 system. Following quality analysis, the quality-filtered library was sequenced on an Illumina MiSeq platform, and 2 × 300 bp paired-end reads were generated. The *cbbL* gene amplification was MiSeq sequenced and analyzed at Honor-Tech, Beijing, China.

2.4. Bioinformatics Analysis

Raw data were demultiplexed, quality-filtered, and merged by the following criteria: (1) truncated 300 bp reads with an average quality score < 20 over 50 bp were included, and read codes less than 50 bp were discarded; (2) nucleotide mismatches or reads that contain ambiguity in the primer match were removed; (3) only sequences exceeding overlapping 10 bp were assembled; (4) unassembled reads were discarded.

After optimization, the clean tags were clustered into operational taxonomic units (OTUs) for species classification by Usearch (v8.0.1623) at a sequence similarity level of 97%. The representative sequence of each OTU was screened for further annotation. These representative sequences were translated into amino acid sequences using Perl programming, clustered using the Uclust (v1.2.22q) software aligned with the Greengenes (v13_8) reference database [29] and the NCBI (National Center for Biotechnology Information) database with a similarity threshold of 0.9–1, the species were annotated, and the colony composition of all samples at each classification level was counted. The subsampling was based on the lowest sequencing depth [30]. Chao1 [31] and Shannon diversity indexes measured community richness and community diversity, respectively, using Qiime (version 1.9.0), where higher indexes reflect higher community richness and diversity. The

rarefaction curve reflects the microbial diversity of each sample at different sequenced quantities, indicating the sequenced data volume of the sample was reasonable.

2.5. Statistical Analysis

Firstly, the Kolmogorov–Smirnov test was used to analyze the normality of the data. Secondly, the effects of different treatments on soil physicochemical properties and the relative abundance of soil CO_2-fixing bacteria were statistically analyzed by a one-way ANOVA. The means of treatments were compared using Duncan's multiple range tests ($p < 0.05$) [32]. The data were described by average ± standard error, in which different lowercase or uppercase letters showed a significant difference between different groups ($p < 0.05$). Different lowercase letters indicate a significant difference between different treatments in the same year, and different uppercase letters indicate a significant difference between different years in the same treatment, with a significant difference at the 0.05 level.

Since the length of the first axis in the detrended correspondence analysis (DCA) of species was less than 3, redundancy analysis (RDA) was selected to analyze the relationship between soil CO_2-fixing bacteria, sample comprehensively, and environmental factors; the analysis was carried out using the vegan package in R (v4.1.0) [33]. An envfit analysis (envfit function used with 999 permutations) identified a significant difference in soil CO_2-fixing bacteria community composition and environmental factor among treatments in R's vegan package [33].

The top 50 representative OTUs sequences with the most abundance were selected and compared using Mafft (v7.149) [4]. The tree topology was obtained by Neighbor-Joining method with 1000 bootstrap replicates to estimate the stability of tree topologies [34] using MEGA (v7.0.14) software. Heatmap analysis was conducted on the top 50 OTUs in the sample using R's pheatmap package [35]. Spearman's correlation analyses among the top 50 soil CO_2-fixing bacteria genera and environmental factors were conducted and displayed as a heatmap in R (v4.1.0).

2.6. Nucleotide Sequence Accession Number

The Miseq sequencing raw reads were deposited into the NCBI Sequence Read Archive (SRA) database (Accession Number: PRJNA757792).

3. Results

3.1. Dynamics of Environmental Factors

A study on different treatments in the same year found that the canopy openness of TD and TR was significantly higher than that of CN and UD during 2011–2012 ($p < 0.001$) (Figure 1A). In 2011, the canopy openness of TD and TR plots increased by 72.93% and 64.21%, respectively, compared with CN plots. In 2012, the canopy openness of TD and TR plots increased by 71.59% and 69.48%, respectively, compared with CN plots. With the restoration of damaged forests, the canopy openness of TD and TR was slightly higher than that of CN and UD in 2013 ($p = 0.037$) (Figure 1A), and the canopy openness of TD and TR plots increased by 16.19% and 7.77%, respectively, compared with CN plots. Due to the impact of rainfall in 2013, the canopy openness of TD and TR was slightly lower than that of CN and UD from 2014 to 2015 (Figure 1A). In 2011, the litterfall input of UD and TD was significantly higher than that of CN and TR ($p < 0.001$) due to the addition of litterfall removed from TR to UD, with 60.39% and 59.21% increases in litter input by UD and TD compared with the control (Figure 1B). The litterfall input of TD and TR was significantly lower than that of CN and UD from 2012 to 2014 ($p < 0.01$) but there was no significant difference in 2015 (Figure 1B). In the fifth year of canopy damage, there was little difference in canopy openness and litter input among different treatment plots (Figure 1).

The interannual change study of the same treatment showed that in the process of ecological restoration after forest canopy damage, the canopy openness of TD plots and TR plots showed a gradually decreasing trend (Figure 1A). In the third year after destruction, compared with the control, the canopy openness of TD plots and TR plots was close to

the pre-destruction level (Figure 1A). In the second to fifth year of forest canopy damage, the trend of forest canopy openness (Figure 1A) and litter input (Figure 1B) in TD and TR samples, and in CN and UD samples, was consistent.

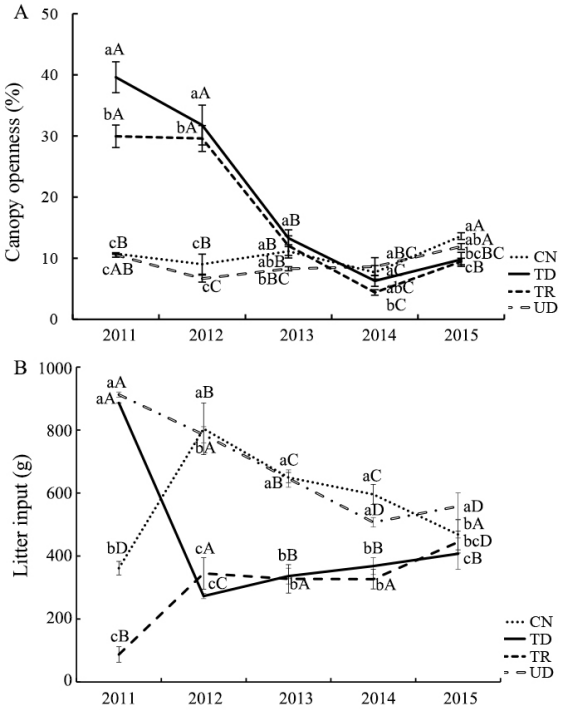

Figure 1. Dynamics of canopy openness and litterfall input. (**A**) Canopy openness. (**B**) Litterfall input. The data in the diagram are average ± standard error. Letters a, b, and c indicate a significant difference between the four treatments in the same year as analyzed by Duncan's test ($p < 0.05$). Letters A, B, C, and D indicate a significant difference between different years in the same treatment as analyzed by Duncan's test ($p < 0.05$).

3.2. Soil Physicochemical Properties

Canopy damage and litterfall input have a significant effect on the physicochemical properties of soil (Table 1). The variation trend of SOC content and TN (total nitrogen) content in different treatments in the same year was consistent (Table 1). Through the analysis of soil physicochemical properties in different treatments for 5 years, it was found that there was no significant difference in SOC and TN content by adding litter input alone (CN vs. UD). In the first year after canopy damage, the SOC content increased by 43.20% with the increase in canopy opening (TD vs. UD) (Table 1). When litter was added after canopy damage (TD vs. TR), SOC content increased by 19.63% in the first year and decreased by 35.38% in the second year. In 2013, the SOC content of TD sample was the highest ($p < 0.001$), and there was no significant difference in other samples. In 2014, the TR sample had the highest SOC content, while the TD sample had the lowest SOC content ($p = 0.006$). The TN content of TR plots increased by 19.76% and 9.55% compared with the control CN plots two years before canopy damage. The TN content of TD plot increased by 17.37% compared with the control CN plot in the first year before canopy damage and decreased by 24.20% in the second year. The TN content of TD was higher than other samples in 2013 ($p = 0.001$) and lower than other samples in 2014 ($p = 0.025$). In the third year of treatment, the content of SOC and TN in the dual-treatment TD plot increased, which may be related to rainfall. In the fourth year of treatment, the highest levels of SOC and TN were observed in the single-treatment TR plot and UD plot of forest canopy destruction, which may be different from the previous carbon and nitrogen sources. Compared with the control, the TD and TR plots in the first year of canopy damage showed a 4.68% and 6.96% decrease in soil pH, respectively. After adding litter for the second year,

compared with the control, the soil pH of the UD plot significantly increased, and there was no significant difference in the fifth year (Table 1).

Table 1. The physicochemical properties of the soils from the four experimental stations.

Sample	pH	Soil Carbon (g/kg)	Total Nitrogen (g/kg)	Total Phosphorus (g/kg)	Available Phosphorus (mg/kg)	Nitrate Nitrogen (mg/kg)
CN11	4.92 ± 0.01 aA	21.65 ± 0.21 cAB	1.67 ± 0.01 bA	0.30 ± 0.00 abA	3.63 ± 0.12 cBC	7.72 ± 2.45 bA
TD11	4.70 ± 0.03 bA	31.39 ± 0.32 aA	1.96 ± 0.03 aA	0.29 ± 0.01 bA	6.13 ± 0.09 bA	9.13 ± 1.62 bA
TR11	4.60 ± 0.03 cA	26.24 ± 0.60 bB	2.00 ± 0.06 aA	0.29 ± 0.00 bAB	7.02 ± 0.10 aB	27.51 ± 0.51 aA
UD11	4.88 ± 0.01 aA	21.92 ± 0.31 cA	1.66 ± 0.02 bA	0.32 ± 0.01 aAB	6.07 ± 0.06 bB	10.11 ± 0.21 bB
CN12	4.2 ± 0.04 bC	21.83 ± 1.96 abAB	1.57 ± 0.08 abA	0.32 ± 0.01 aA	0.92 ± 0.07 bC	2.05 ± 0.76 aB
TD12	4.44 ± 0.05 aB	18.41 ± 4.78 bC	1.19 ± 0.21 bC	0.29 ± 0.03 aC	0.77 ± 0.12 bB	1.58 ± 0.59 aB
TR12	4.34 ± 0.10 abB	28.49 ± 0.70 aAB	1.72 ± 0.06 aA	0.29 ± 0.01 aAB	0.75 ± 0.07 bE	1.68 ± 0.86 aC
UD12	4.43 ± 0.06 aC	23.87 ± 1.69 abA	1.44 ± 0.1 abA	0.34 ± 0.02 aA	1.46 ± 0.17 aC	3.78 ± 0.17 aC
CN13	4.82 ± 0.01 bA	22.07 ± 0.45 bAB	1.53 ± 0.02 bA	0.25 ± 0.00 cB	4.92 ± 0.09 bB	2.31 ± 0.33 cB
TD13	4.78 ± 0.02 bA	27.80 ± 0.24 aAB	1.73 ± 0.04 aAB	0.27 ± 0.01 bAB	5.30 ± 0.10 bA	9.80 ± 1.99 aA
TR13	4.57 ± 0.01 cAB	21.18 ± 0.49 bB	1.59 ± 0.00 bA	0.30 ± 0.00 aAB	5.14 ± 0.12 bC	4.01 ± 0.52 bcBC
UD13	4.89 ± 0.03 aA	21.40 ± 0.26 bA	1.56 ± 0.02 bA	0.29 ± 0.01 aAB	6.49 ± 0.16 aB	8.31 ± 2.44 abBC
CN14	4.55 ± 0.01 cB	20.23 ± 0.12 bcB	1.49 ± 0.04 aA	0.28 ± 0.00 aB	8.96 ± 2.12 aA	10.57 ± 0.16 bA
TD14	4.72 ± 0.01 aA	19.03 ± 0.24 cC	1.33 ± 0.03 bC	0.24 ± 0.00 cB	7.41 ± 1.47 aA	2.06 ± 0.08 dB
TR14	4.59 ± 0.02 bcA	23.1 ± 0.25 aB	1.60 ± 0.02 aA	0.26 ± 0.00 bB	11.34 ± 0.14 aA	6.94 ± 0.16 cB
UD14	4.64 ± 0.02 bB	21.9 ± 1.15 abA	1.52 ± 0.09 aA	0.28 ± 0.01 aB	7.48 ± 0.25 aA	16.78 ± 0.81 aA
CN15	4.67 ± 0.08 aB	25.64 ± 2.43 aA	1.63 ± 0.12 aA	0.33 ± 0.03 aA	1.07 ± 0.17 aC	6.67 ± 1.98 aAB
TD15	4.4 ± 0.07 aB	22.73 ± 2.69 aBC	1.44 ± 0.04 aBC	0.31 ± 0.03 aA	1.50 ± 0.23 aB	7.98 ± 1.21 aA
TR15	4.41 ± 0.12 aAB	34.62 ± 5.42 aA	1.93 ± 0.28 aA	0.33 ± 0.04 aA	1.35 ± 0.28 aD	6.46 ± 2.05 aB
UD15	4.57 ± 0.10 aBC	28.35 ± 4.20 aA	1.51 ± 0.10 aA	0.34 ± 0.03 aAB	1.53 ± 0.20 aC	7.48 ± 2.63 aBC

Note: The data in the table are average ± standard error. Letters a, b, c, and d indicate a significant difference between the four treatments in the same year as analyzed by Duncan's test ($p < 0.05$). Letters A, B, C, D, and E indicate a significant difference between different years in the same treatment as analyzed by Duncan's test ($p < 0.05$).

Comparing different years in the same area, it was found that the SOC content of single-treatment TR and UD in the sample plot showed an upward trend in the first two years of canopy damage, a decrease in the third year of canopy damage, and an upward trend in the fourth to fifth years of canopy damage. The SOC content of the dual-treatment TD sample was the highest in the first year of canopy damage and the lowest in the second and fourth years after canopy damage ($p = 0.016$) (Table 1). The SOC content of the TR sample was the highest in the fifth year of canopy damage ($p = 0.025$). There was no significant difference in SOC content in the CN and UD samples during canopy damage restoration.

3.3. Soil CO_2-Fixing Bacteria Diversity

The total reads ranged from 23,697 to 39,227, the total OTU numbers ranged from 420 to 881, total Chao1 indexes ranged from 712.14 to 1048.72, and the Shannon indexes ranged from 4.58 to 7.78 (Figure 2). The rarefaction curves indicate that the depth of the sequence was sufficient to reflect the community composition of the soil CO_2-fixing bacteria (Figure S3). Chao1 and Shannon indexes of treated and CN samples showed similar trends (Figure 2). In 2013, the sudden drop of Chao1 and Shannon indexes may have been caused by excessive rainfall rather than canopy damage and litter input.

The analysis of soil CO_2-fixing bacteria diversity of different treatment samples in the same year found that the Chao1 indexes of treatment samples were significantly lower than CN ($p = 0.042$) (Figure 2A) but the Shannon index was higher than that of CN (Figure 2B) in 2011. In 2012, the Shannon index of UD was significantly higher than that of TR and TD, and the TD sample's Shannon index was the lowest ($p < 0.001$) (Figure 2B). The Chao1 index of TD was the highest and those of TR and UD were the lowest in 2013 ($p = 0.044$) (Figure 2A). In 2014 and 2015, there were no significant differences in the diversity indexes of the four treatments (Figure 2). These results showed that the richness of the treatments decreased and the diversity increased after one year of canopy damage. The Chao1 index

and Shannon index of TR and UD plots showed no significant difference except for the Shannon index in the second year of canopy damage (Figure 2B). The annual change trend of Chao1 and Shannon indexes in the sample with the dual treatment of canopy damage and litterfall input (TD) was gentler than in single treatments (TR and UD) (Figure 2).

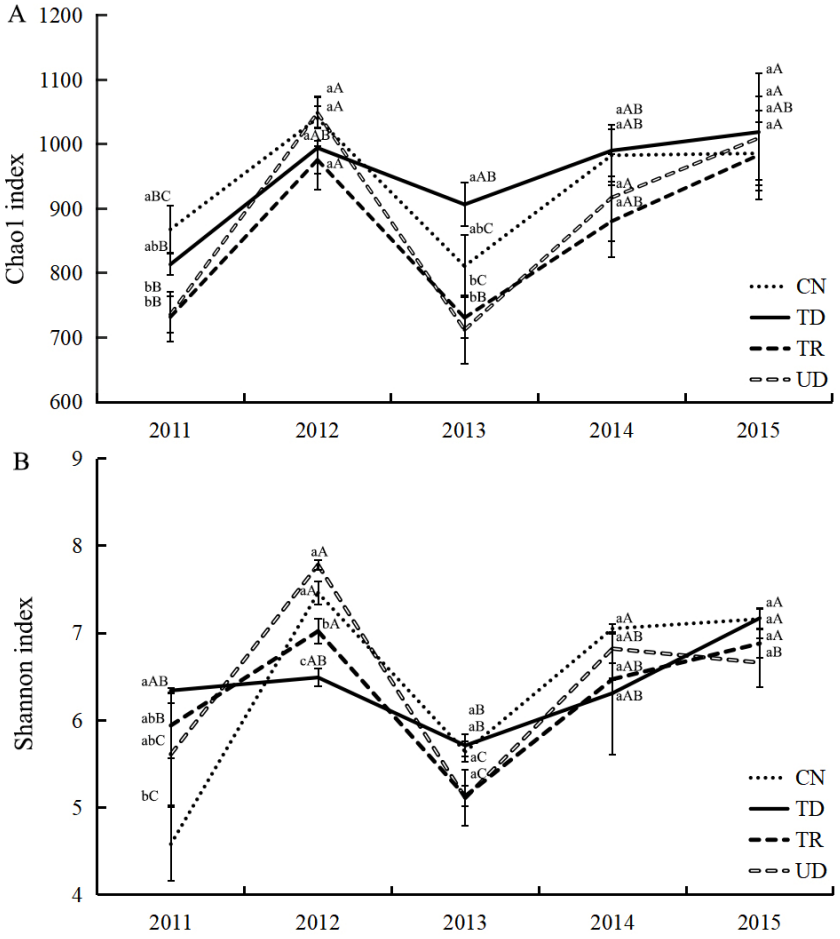

Figure 2. The soil CO_2-fixing bacteria diversity of samples. (**A**) Chao1 index. (**B**) Shannon index. The data in the diagram are average ± standard error. Letters a, b, and c indicate a significant difference between the four treatments in the same year as analyzed by Duncan's test ($p < 0.05$). Letters A, B, and C indicate a significant difference between different years in the same treatment as analyzed by Duncan's test ($p < 0.05$).

Comparing different years in the same region, it was found that the trends of the Chao1 index and Shannon index of soil CO_2-fixing bacteria in the four sample plots were similar, with a significant decrease in 2013 (Figure 2). Compared with other plots, the changes in the Chao1 index and Shannon index of TD plots in different years were relatively flat. The Chao1 index and Shannon index values of the TD plot reached their highest in 2015 (Figure 2), indicating that the community richness and diversity of CO_2-fixing bacteria in the TD plot reached their maximum values.

3.4. Soil CO_2-Fixing Community Composition

There were five phyla in different treatments, Proteobacteria (57.25%), Actinobacteria (39.67%), Cyanobacteria (1.45%), Chloroflexi (0.16%), and Firmicutes (0.007%) (Figure 3A). The Proteobacteria of the three treatments was significantly higher than that in CN ($p = 0.030$) (Figure 3B), but Cyanobacteria and other bacteria were significantly lower than in CN ($p = 0.001$), in 2011 (Figure 3A). In the first year of damaged forest canopy, compared with the control, the increase in forest canopy openness and litter input both caused the increase in Proteobacteria and the decrease in Actinobacteria (Figure 3), and the change caused by single

treatment (TR plot and UD plot) was more obvious than that by double treatment (TD plot). In the second year after canopy damage, Actinobacteria in TR and TD plots with canopy openness increased and Proteobacteria decreased compared with control plots. In the third year of damaged canopy, Proteobacteria in the TR sample was significantly higher than that in TD and UD ($p = 0.048$) (Figure 3B). However, Actinobacteria was significantly lower in TR than in TD and UD in 2013 ($p = 0.034$). In 2014, Proteobacteria in TR was significantly higher than in the other three samples and CN was the least prevalent ($p = 0.001$) (Figure 3B). Actinobacteria in the treated samples was significantly lower than in CN ($p = 0.002$) in 2014 (Figure 3C). In 2015, Proteobacteria in CN, TR, and UD was significantly higher than in the TD sample ($p = 0.037$) (Figure 3B).

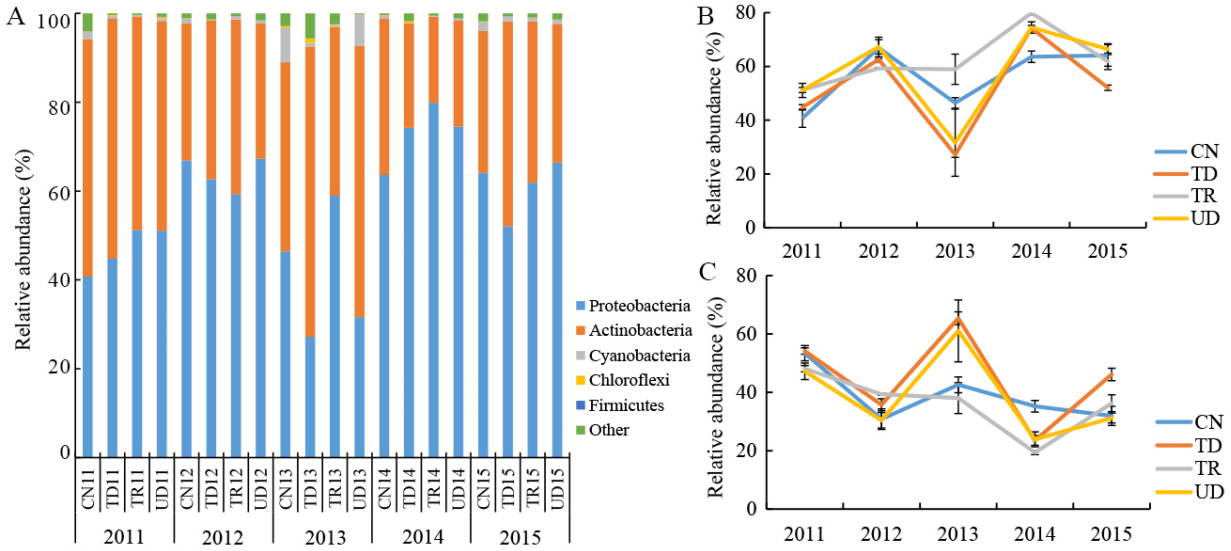

Figure 3. Soil CO_2-fixing bacterial communities at phylum level. (**A**) All phyla. (**B**) Proteobacteria. (**C**) Actinobacteria. The data in the diagram are average ± standard error.

The analysis of soil CO_2-fixing bacteria in different years of the same treatment showed that the trend of the four plots' sites was similar. In 2012 and 2014, Proteobacteria increased and Actinobacteria decreased in the four sample plots ($p < 0.01$) (Figure 3). In 2013, Proteobacteria decreased and Actinobacteria increased in the four sample plots ($p < 0.01$), indicating that Actinobacteria is related to rainfall, with higher rainfall leading to an increase in Actinobacteria content (Figure 3).

At the genus level, there were 69 identifiable genera of soil CO_2-fixing bacteria. Among the six genera with an average relative abundance higher than 5%, in 2011, *Nitrosospira*, *Streptomyces* ($p = 0.023$), *Mycobacterium* ($p = 0.014$), and *Nocardia* ($p = 0.038$) were higher than the control in the three treatments, *Saccharomonospora* ($p < 0.001$) and *Bradyrhizobium* ($p < 0.001$) were lower than the control (Figure 4), and the *Streptomyces* of the dual-treatment TD plot were significantly greater than those of the single-treatment (TR plot and UD plot) and control plots. In 2012, *Nitrosospira* and *Streptomyces* ($p = 0.023$) of the three treatments were higher than the control, *Nocardia* and *Bradyrhizobium* ($p = 0.001$) were lower than the control, and *Nitrosospira* of the dual-treatment TD plot was higher than that of the single-treatment (TR plot and UD plot) and control plots. In 2013, *Nitrosospira* ($p < 0.001$) of the three treatments was significantly lower than the control, while *Nocardia* ($p = 0.008$) was significantly higher than the control (Figure 4). *Streptomyces* ($p = 0.023$) and *Bradyrhizobium* ($p < 0.001$) of the UD plot were significantly highest, while *Saccharomonospora* ($p < 0.001$) of the TD plot was significantly highest in 2013. Compared with the control, *Streptomyces* and *Mycobacterium* were significantly lower in TR and TD plots in 2013, while *Saccharomonospora* was significantly higher, indicating that these bacteria groups were closely related to canopy openness. In 2014, the *Saccharomonospora* ($p = 0.006$), *Mycobacterium* ($p = 0.009$), and *Nocardia* ($p = 0.008$) of the three treatment groups were significantly lower than those of

the control group. In 2015, *Saccharomonospora* ($p < 0.001$) in TD was significantly increased, *Mycobacterium* ($p = 0.012$) was higher in TD and TR samples, and *Bradyrhizobium* ($p < 0.001$) in the three treatment groups was significantly lower than in the control group (Figure 4). As the genus with the highest genera, *Nitrosospira* showed no significant change between 2014 and 2015 (Figure 4). The differences in soil CO_2-fixing bacteria among different treatments are shown in Table S1.

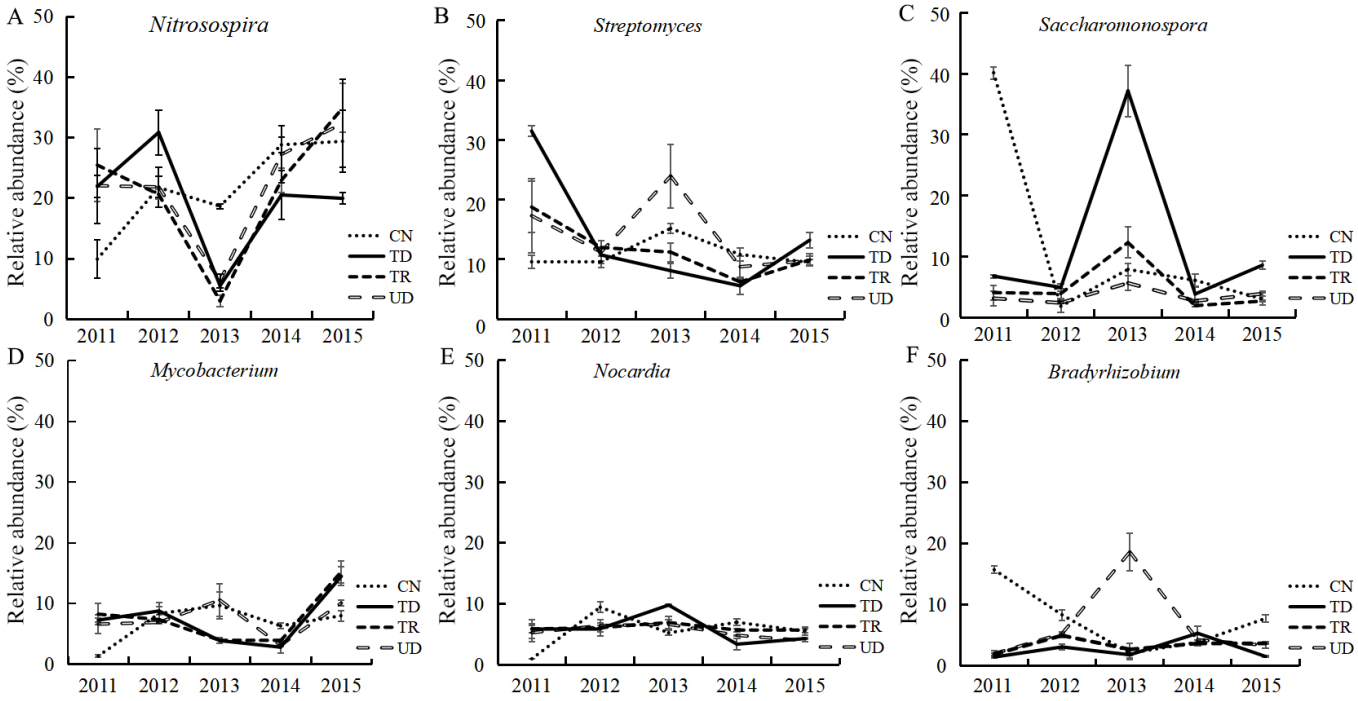

Figure 4. Comparison of the top six genera of soil CO_2-fixing bacterial in different samples. The data in the diagram are average ± standard error. (**A**) *Nitrosospira*; (**B**) *Streptomyces*; (**C**) *Saccharomonospora*; (**D**) *Mycobacterium*; (**E**) *Nocardia*; (**F**) *Bradyrhizobium*.

At the genus level, *Nitrosospira* from both TR and UD plots decreased in the first three years of treatment, increased significantly in the fourth year, and reached the highest value in the fifth year ($p < 0.05$) (Figure 4A). The *Streptomyces* and *Mycobacterium* of TR plots decreased in the first four years of treatment and increased in the fifth year. The *Nitrosospira* of TD plots was significantly highest in 2012 and lowest in 2013 ($p = 0.001$) (Figure 4A). The *Streptomycetes* of TD plots decreased in the first four years of treatment, increased in the fifth year, and were significantly highest in 2011 ($p = 0.006$) (Figure 4B). The content of *Nitrosospira* in the UD plot was significantly lowest in 2013, while the content of *Mycobacterium* was significantly highest in 2013 (Figure 4). *Saccharomonospora* was highest in the treated plots in 2013 (Figure 4C), which may be related to rainfall. *Bradyrhizobium* shows a dynamic trend in different years under the same treatment (Table S1).

The soil CO_2-fixing bacteria *cbbL* phylogenetic tree was constructed with the top 50 OTUs' corresponding sequences from soils, 10 published *cbbL* reference sequences, and a *cbbM* reference sequence as the outgroup. The most abundant 50 OTUs accounted for 57.91% of the total number of sequences. The *cbbL* gene sequence of soil CO_2-fixing bacteria could be divided into two branches, type IA (obligate autotrophic bacteria) and type IC (facultative autotrophic bacteria) (Figure 5), among which IC type accounted for 96% of the top 50 OTUs' corresponding sequences obtained by sequencing. These IC-type OTUs belong mainly to *Nitrosospira*, *Streptomyces*, *Mycobacterium*, and *Saccharomonospora* (Figure 5).

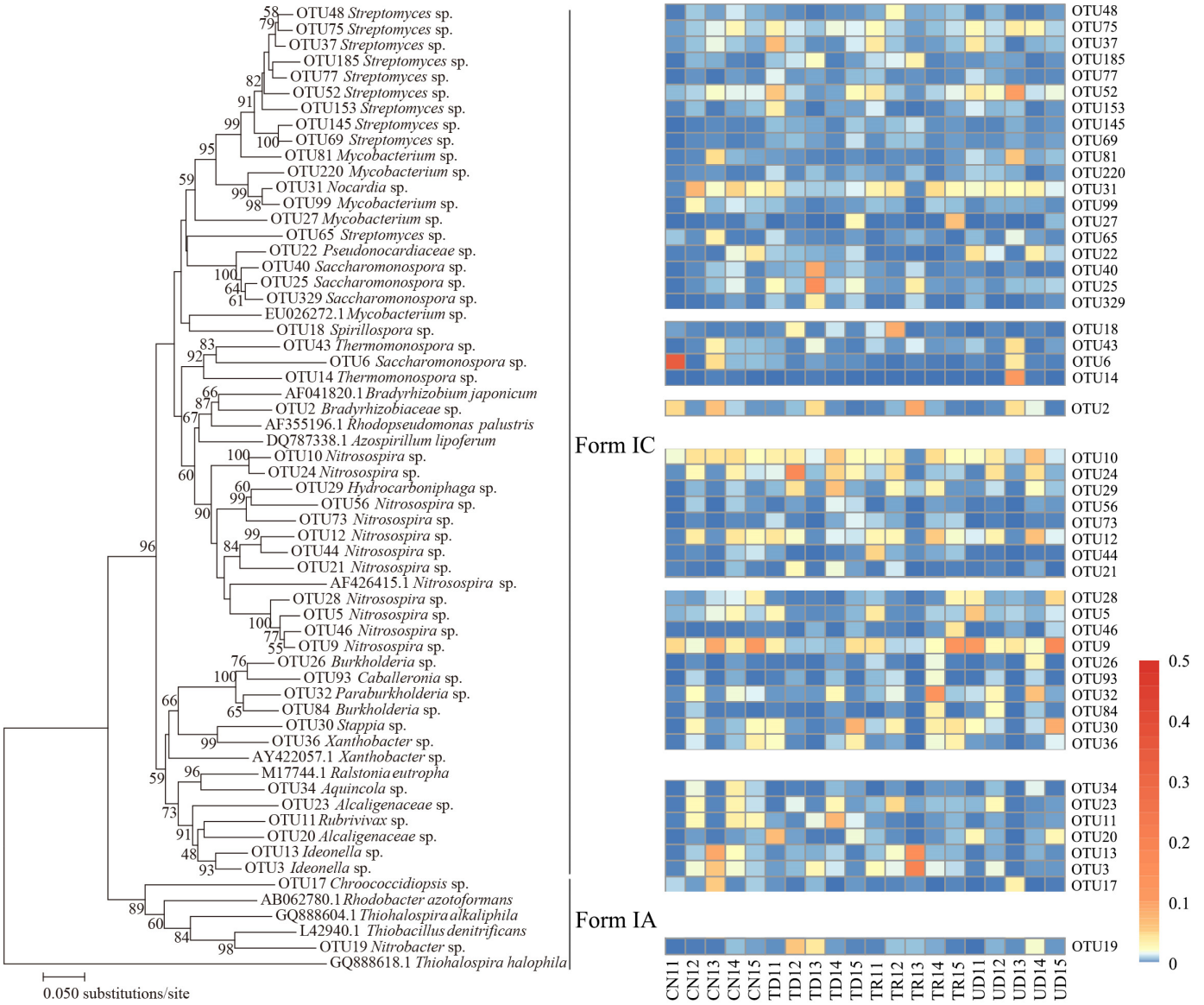

Figure 5. Phylogenetic tree with heatmap of soil CO_2-fixing bacterial at OTUs level. The bootstrap frequencies (≥50%) are shown at the supported branches. The data in the diagram are averages.

3.5. Environmental Factors Influence the Soil CO_2-Fixing Bacteria Communities

The RDA demonstrated that soil CO_2-fixing bacteria communities at the genera level correlated with canopy openness (r^2 = 0.5795, p = 0.013), SOC (r^2 = 0.6860, p = 0.007), and AP (r^2 = 0.8141, p = 0.006) in 2011 (Figure 6A). In 2012, soil CO_2-fixing bacteria communities at the genera level had a significant relationship with canopy openness (r^2 = 0.7484, p = 0.003), litterfall input (r^2 = 0.8091, p = 0.001), AP (r^2 = 0.6923, p = 0.008), and NO_3^--N (r^2 = 0.5495, p = 0.034) (Figure 6B). Soil CO_2-fixing bacteria communities at the genus level had a significant relationship with litterfall input (r^2 = 0.8518, p = 0.001), SOC (r^2 = 0.8178, p = 0.007), TN (r^2 = 0.8025, p = 0.002), and TP (r^2 = 0.7652, p = 0.003) in 2013 (Figure 6C). Soil CO_2-fixing bacteria communities at the genera level correlated with litterfall input (r^2 = 0.7476, p = 0.002), pH (r^2 = 0.5425, p = 0.039), SOC (r^2 = 0.5654, p = 0.032), TP (r^2 = 0.8142, p = 0.001), and NO_3^--N (r^2 = 0.7997, p = 0.004) in 2014 (Figure 6D). In 2015, there were no significant differences between environmental factors and soil CO_2-fixing bacteria communities at the genus level (Figure 6E).

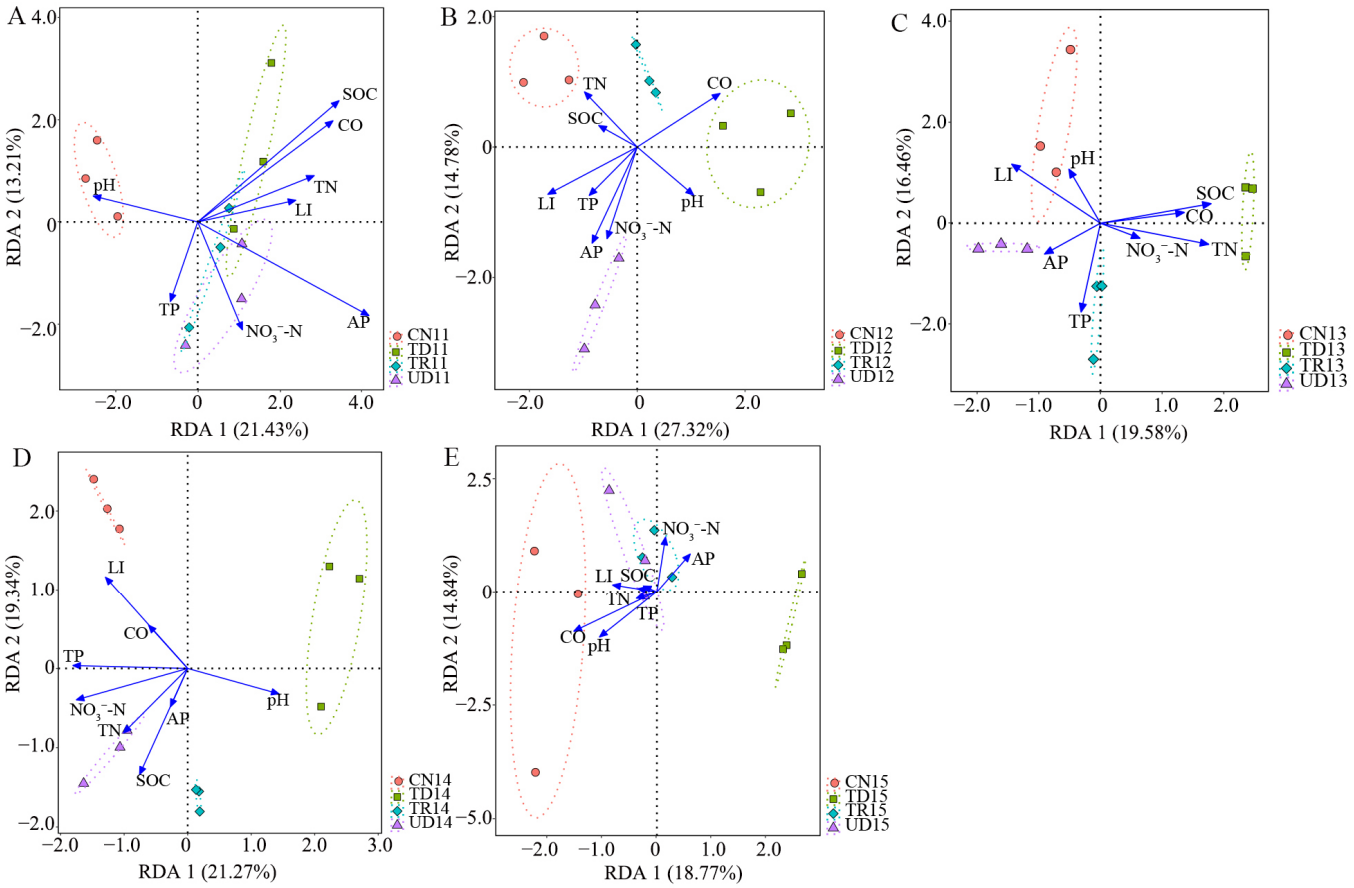

Figure 6. RDA analysis of the soil CO_2-fixing bacteria communities and environmental factors. (**A**) 2011; (**B**) 2012; (**C**) 2013; (**D**) 2014; (**E**) 2015. SOC: soil organic carbon; TN: total nitrogen; NO_3^--N: nitrate-nitrogen; TP: total phosphorus; AP: available phosphorus; CO: canopy openness; LI: litterfall input.

At the genus level, the soil CO_2-fixing bacteria community in the CN sample was correlated with rainfall (r^2 = 0.8150, p = 0.005) and pH (r^2 = 0.7827, p = 0.002) (Figure S4). The soil CO_2-fixing bacteria communities in the soil of TD sample were correlated with temperature (r^2 = 0.7889, p = 0.002), rainfall (r^2 = 0.6300, p = 0.002), and NO_3^--N (r^2 = 0.7117, p = 0.001) (Figure S4). The soil CO_2-fixing bacteria communities in TR plots were correlated with temperature (r^2 = 0.8724, p = 0.001), rainfall (r^2 = 0.7205, p = 0.001), and SOC (r^2 = 0.5899, p = 0.006) (Figure S4). The relationship between soil CO_2-fixing bacteria communities was correlated with temperature (r^2 = 0.6557, p = 0.003), rainfall (r^2 = 0.7612, p = 0.001), pH (r^2 = 0.6735, p = 0.001), and AP (r^2 = 0.4407, p = 0.032) (Figure S4).

Spearman's correlation analysis examined the correlation between relative abundance of the top 50 genera and soil/site characteristics. The heatmap showed that pH and rainfall clustered together, and AP, TN, and NO_3^--N clustered together, while others were further apart on the ordination. Spearman's correlation analyses showed that canopy openness was significantly positively correlated with *Saccharomonospora*, *Thermomonospora*, *Acidovorax*, *Aquabacterium*, and *Inquilinus* and negatively correlated with *Bradyrhizobium*, *Paraburkholderia*, *Methyloferula*, and *Caballeronia* (Figure 7). Litterfall input was significantly positively correlated with *Oscillatoria*, *Synechococcus*, *Cupriavidus*, and *Aminobacter* and negatively correlated with *Nitrobacter*, *Spirillospora*, *Acidovorax*, and *Rhodopseudomonas* (Figure 7). SOC was significantly positively correlated with *Mycobacterium*, *Stappia*, *Xanthobacter*, and *Mesorhizobium* (Figure 7). With the exception of *Saccharomonospora* and *Thermomonospora*, most genera showed a significant negative correlation with pH and rainfall (Figure 7).

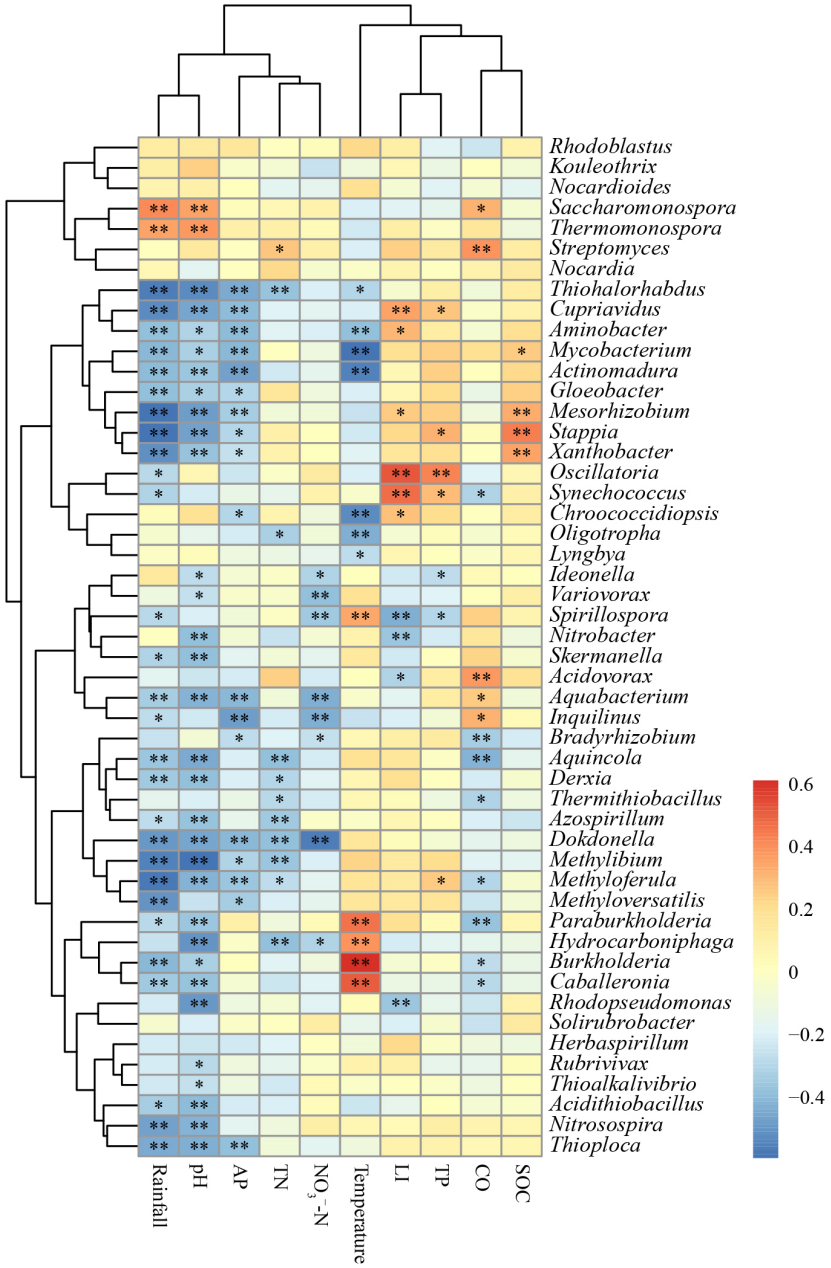

Figure 7. Spearman's correlation between environmental factors and soil CO_2-fixing bacteria at genus level. SOC: soil organic carbon; TN: total nitrogen; NO_3^--N: nitrate-nitrogen; TP: total phosphorus; AP: available phosphorus; CO: canopy openness; LI: litterfall input. Significance levels: ** $p < 0.01$; * $p < 0.05$.

4. Discussion

In this study, canopy openness of TD and TR was lower than that of CN and UD in 2014 and 2015 after canopy damage in winter 2010, and there was no significant difference in litter volume input for the 2015 sample, showing that the litterfall input was restored to control levels. There was no significant difference in soil physicochemical properties in treatment and control samples in 2015, including pH, SOC, TN, TP, AP, and NO_3^--N. Two important indicators describing the richness and diversity indices of the microbial community [36], the Chao1 and the Shannon indexes, were similar in 2015, showing that the damaged forest was restored to a pre-damaged level in 2014–2015. In other words, 4–5 years are required for forest ecosystem restoration after canopy damage, consistent with previous studies [37].

4.1. Effects of Canopy Damage and Litterfall Input on Soil Physicochemical Parameters

Canopy damage caused gaps of different sizes and influenced litterfall on the ground. Canopy gap can directly or indirectly affect soil pH value, soil temperature, soil humidity, soil physicochemical properties, litter decomposition, nutrient cycling, and reduce competition for light and other resources by increasing light and rainfall, thus promoting the growth of understory [38]. Aboveground litterfall is one of the most critical components of the carbon and nutrient cycle, regulating soil microclimate by forming a buffer interface between the soil surface and the atmosphere [39]. With litter addition alone, there was no significant change in SOC content (CN vs. UD), which is consistent with Castañeda-Gómez's [40] finding that doubled above-ground litter did not increase soil C in any of the forests studied, possibly due to long-term soil excitation effects. In the first year of canopy damage, canopy openness increased, the content of SOC and TN significantly increased, and the content of TP significantly decreased (TD vs. UD) (Table 1). When litter was added after forest canopy damage, soil pH and SOC contents increased, while AP and NO_3^--N contents decreased (TD vs. TR). After canopy damage and litter treatment, soil pH decreased, and SOC and TN contents increased significantly (CN vs. TD). These results indicate that in the early stages of canopy damage, the changes in SOC were mainly influenced by canopy openness, and increases in canopy openness and litter input can increase the content of SOC. Gong [41] found that both light thinning (thinning intensity < 35%) and moderate thinning (thinning intensity 35%–55%) significantly increased SOC stocks, and light thinning and moderate thinning significantly increased SOC stocks by 7.5% and 16.1%, respectively. Zimmerman et al. [42] showed that removal of litter and woody debris generated by hurricanes could improve the soil availability of nitrogen and aboveground productivity in the forest. In this study, we found that in 2012 and 2014, the contents of SOC, TN, and NO_3^--N in TD sample were significantly lower than those of other samples (Table 1), which may be the result of the increase in carbon dioxide concentration caused by soil microbial mineralization [43]. It was speculated that the C/N ratio and its high nutrient content in fresh litter changed the community structure of soil microorganisms, and the different preferences of microorganisms for carbon sources resulted in different dynamic changes of soil C. During soil mineralization, the SOC in the soil is gradually converted to simpler substrates and eventually to CO_2, which reduces organic carbon content in soil [44,45]. During this period, soil microorganisms mainly play the role of mineralization. As a result of soil microbial humification and mineralization in different stages, the dynamic change in SOC caused by abnormal litter decomposition goes from a negative priming effect to a positive priming effect in the early stage; due to the existence of soil CO_2-fixing bacteria, SOC reaches a negative priming effect again; and finally, it reaches a dynamic balance. The dynamic changes in SOC in different treatments had different times for the negative priming effect and positive priming effect by comparing the organic carbon content, and the trend of the negative priming effect and positive priming effect was consistent with that of Yu et al. [7]. The time to reach different priming effects in TD was earlier than in other treatments; in other words, double treatments of canopy damage and litterfall inputs (TD) facilitate forest restoration better than single treatments (TR or UD) after extreme weather events.

4.2. Effects of Canopy Damage and Litterfall Input on Soil CO_2-Fixing Bacterial Communities

Changes in litterfall input and canopy openness lead to differences in soil physicochemical properties [46]. Soil bacteria generally react more quickly to disturbances than other microorganisms [47]. In 2011, the Chao1 indexes of treatment samples were significantly lower than CN but the Shannon index was significantly higher than that of CN (Figure 2). In 2011, *Nitrosospira*, *Streptomyces*, *Mycobacterium*, and *Nocardia* abundances were significantly higher in treatment groups than in control (Figure 4). The previous studies showed that *Streptomyces*, *Mycobacterium*, and *Nocardia* can decompose the lignin in litter by secreting extracellular peroxidases [48]; meanwhile, *Nitrosospira* is an ammonia-oxidizing bacterium and can further convert ammonium nitrogen into an effective form to absorb

nitrate-nitrogen [49]. In 2011, the CO_2-fixing bacterial richness and diversity of the TD sample with the double treatment of canopy openness and litterfall input were higher than those of the single-treatment samples (TR and UD samples), and the ability to degrade litter was also higher than with single treatment, which made the content of SOC higher than that of single-treatment samples. Nitrogen is considered a good predictor of plant litter decomposition, and early litter decomposition is regulated by the concentration of N, P, and other nutrients [21]. Silver et al. [4] found that the N and P concentrations in trimmed litter increased compared with that of the intact canopy. In 2011, soil bacteria mainly undergo humification and participate in C and N cycles in the early stage of soil succession or after disturbance, including simple organic carbon decomposition, nitrogen fixation, and ammonium oxidation [16], thus increasing the content of SOC, TN, NO_3^--N, and AP (Table 1). In 2012, the Shannon indexes of TR and TD samples were significantly lower than those of UD and CN (Figure 2B); *Nitrosospira* and *Streptomyces* abundances of treatments were higher but *Nocardia* and *Bradyrhizobium* were lower in treatments than in control (Figure 4). The content of SOC in TR and UD sample was significantly increased, which may be caused by CO_2-fixing bacteria with degradation abilities. In 2012, the soil CO_2-fixing bacteria with degradation ability and total soil CO_2-fixing bacteria in TD sample decreased, which may because other microorganisms in the soil convert SOC into inorganic matter and CO_2 through mineralization and respiration, thus reducing SOC content [7]. In 2013, the richness and diversity of soil carbon sequestrating microorganisms decreased in the four plots, and the richness and diversity of soil carbon sequestrating microorganisms in the dual-treatment TD plots were higher than those in the single-treatment TR and UD plots, which was due to the heavy rainfall in 2013. When high rainfall enters the soil, the competition among bacterial cells and other nutrient interactions may reduce soil bacterial diversity by reducing community uniformity [50]. Our study found that rainfall was negatively correlated with most CO_2-fixing bacteria; when rainfall was high, the content of most CO_2-fixing bacteria decreased (Figure 7). There was no significant difference in soil CO_2-fixing bacteria in the four samples in 2014 and 2015, which means the soil CO_2-fixing community had restored to the previous level.

4.3. Relationship between Soil CO_2-Fixing Bacterial Communities and Environmental Factors

During restoration after canopy damage, the soil's physicochemical properties were changed, such as pH, SOC, AP, and NO_3^--N, leading to a different change in the richness of soil CO_2-fixing bacteria (Figure 6). Previous studies showed that the increase in soil-available nitrogen leads to soil acidification, which may inhibit the growth of CO_2-fixing bacteria to a certain extent [13]. The low soil pH can inhibit the enzymatic and metabolic activities of bacteria, which does not promote bacterial growth [51]. The increase in soil pH helps release dissolved organic matter, which benefits bacterial diversity [52]. Our study found that most CO_2-fixing bacteria were significantly negatively correlated with rainfall, pH, and AP (Figure 7). RDA revealed that canopy opening and litter input have significant effects on CO_2-fixing bacteria communities (Figure 6) and also showed that interannual changes in rainfall and temperature (Figure S4) are related to temporal and spatial changes in regional heterogeneity. Plant–soil microbial interactions under climate change scenarios are important for understanding the effects of climate change on ecosystem stability and carbon cycling [20]. Temperature increase affects the carbon input, decomposition, and stability of the carbon pool, which, in turn, affects the carbon cycle of the terrestrial ecosystem [53]. Rainfall was also an important driver of soil CO_2-fixing bacterial communities' composition. Rainfall causes soil water changes that directly affect the water use of plants and soil microorganisms [54]. Soil water also affects soil microorganisms' structure and function by affecting the soil's matrix, pH, and temperature [11]. Wang et al. [55] found that precipitation changes have no significant effect on the bacterial community composition of surface soil or rhizosphere soil but affect the bacterial abundance and the response of the dominant community to precipitation changes. The infiltration of organic matter from the surface to deeper soil is a major component of soil carbon flux and represents

a large fraction of the organic matter fresh input to the subsoil and groundwater [56]. As water-bearing microhabitats become interconnected after rainfall moistens the soil, the competition among bacteria and other nutrient interactions may reduce soil bacterial diversity by reducing community uniformity [50,57]. In this study, rainfall in 2013 was higher than in other years, and the richness and diversity of the soil CO_2-fixing bacterial community were the lowest among the examined period (Figure 2). This is consistent with the view that the decrease in humidity decreases bacterial diversity and richness in the 0–5 cm surface soil [55].

Forest ecosystem management has largely overlooked the importance of the ability of terrestrial microorganisms to contribute to ecosystem regeneration after disturbance [20]. Our findings contribute to understanding how soil CO_2-fixing bacteria community structure is affected by disturbances and forest management practices. The short-term changes in CO_2-fixing bacteria communities caused by forest disturbance are important for understanding the dynamics of litter decomposition, soil organic matter accumulation, and nutrient cycling.

5. Conclusions

To study the effects of extreme weather events, specifically frost damage, on soil CO_2-fixing bacteria in the forest ecosystem, we simulated forest canopy damage from the 2008 ice storms in southern China. After damage, the canopy openness and litterfall input significantly affected the diversity and community composition of soil CO_2-fixing bacteria. Restoration of the forest ecosystem was more straightforward in canopy damage and litterfall input (TD) dual treatment than single treatment (TR or UD). Rainfall and pH have a significant negative correlation with soil CO_2-fixing bacteria diversity and community structure, which played decisive factors. The results showed that the forest fully recovered 4–5 years after the canopy damage. These results provide strong evidence for understanding and highlighting the role of soil CO_2-fixing bacteria in forest restoration.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/f14091712/s1, Figure S1: The dominant species in the plot; Figure S2: Dynamics of rainfall and average temperature in July–August; Figure S3: Shannon index rarefaction curves of soil CO_2-fixing bacteria; Figure S4: RDA analysis of the soil CO_2-fixing bacteria communities and environmental factors. (A) CN, (B) TD, (C) TR, (D) UD; Table S1: Comparison of soil CO_2-fixing bacteria at genus level.

Author Contributions: Conceptualization, J.L.; methodology, F.Y., J.L. and H.Z.; software, F.Y.; investigation, F.Y., J.L. and H.Z.; data curation, F.Y. and Z.L.; writing—original draft preparation, F.Y. and Z.L.; writing—review and editing, F.Y. and J.L.; supervision, F.Y. and J.L.; project administration, F.Y. and J.L.; funding acquisition, F.Y. and J.L. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The data are available on reasonable request.

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

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