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Article

Reduced Methane Production Is a Crucial Factor Contributing to Reduced Methane Emissions from Degraded Wetlands

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Abstract: Methane (CH₄) is a major greenhouse gas contributing to climate change, and degraded wetlands are both sources and sinks of CH₄. However, little is known about the role of microbial communities in mediating methane cycling during the process of wetland degradation. To investigate the microbial mechanisms influencing CH₄ flux in degraded marsh wetlands, a metagenomic approach was used to study the processes of CH₄ production and oxidation in herbaceous marsh wetlands. In this study, soil samples were collected from four degradation gradients of herbaceous marshes in the lower reaches of the Tumen River in Jilin Province, China. In situ CH₄ fluxes were measured, and the microbial communities involved in CH₄ metabolism, the abundance of key enzymes and KEGG pathways were analyzed. Environmental factors influencing CH₄ flux were also correlated. One-way ANOVA indicated that wetland degradation significantly reduced CH₄ flux and altered environmental factors. Metabolic pathway analysis showed that the CO₂ pathway of methanogenesis was dominant. The abundance of methanogens and the key enzyme (mcrA; EC: 2.8.4.1) decreased, while the number of methanotrophs increased, with no significant change in the abundance of the key enzyme (pMMO; EC: 1.14.18.3). Random forest and partial least squares regression analyses (PLSR) indicated that the key environmental factors affecting CH₄ flux are soil water content (SW), total carbon (TC), and soil pH. Therefore, the decrease in methanogen abundance and metabolic activity due to reduced soil moisture is the primary reason for the rapid decline in methane emissions. The main factor affecting methane flux is the methane production process, rather than methane oxidation. Further research is needed to reduce the significant uncertainty in estimating CH₄ emissions from degraded wetlands.

Keywords: wetland degradation; CH₄ metabolism; methanogens; methanotrophs; metagenomic

1. Introduction

Wetland is a transitional area between terrestrial ecosystems and aquatic ecosystems, which is known as the “cradle of life” and the “kidney of the earth” [1]. Although wetlands cover only 5% to 8% of the global land surface, they contribute approximately one-third of the world’s total CH₄ emissions [2,3]. However, in recent years, soil degradation caused by climate change and human activities has become an increasingly serious problem for wetlands [4]. Changes in vegetation density and composition caused by wetland degradation can lead to variations in the quantity and quality of litter [5,6]. The availability of organic matter for soil microbial communities is consequently affected, altering the natural exchange of greenhouse gases (GHGs) such as methane (CH₄) and nitrous oxide (N₂O) [7,8]. Given that degraded wetlands are significant sources of GHG emissions to the atmosphere [9,10], it is urgently necessary to determine the impacts of widespread wetland degradation on GHG exchanges.

Methane (CH₄) is a potent greenhouse gas with a global warming potential 34 times that of carbon dioxide (CO₂), and its atmospheric concentration has increased 2.5 times since the Industrial

Revolution [11]. Model predictions indicate that global CH₄ emissions from wetlands will increase in the 21st century, resulting in a positive feedback to global warming [12]. Wetland degradation causes changes in the fundamental physical and chemical properties of soil and the biogeochemical cycling of soil nutrients [13]. It also leads to high spatiotemporal variability in the biotic and abiotic factors regulating CH₄ flux [14]. Therefore, studying the mechanisms of CH₄ emissions under the influence of climate and human activities is crucial.

Soil organic matter is metabolized by microorganisms to produce CH₄ as the final product [15]. Generally speaking, the CH₄ flux in wetlands is the net result of production under anaerobic conditions and consumption under aerobic conditions, and it is transported to the atmosphere through molecular diffusion, ebullition, and plant aerenchyma [16,17]. Methanogens utilize simple carbon compounds such as carbon dioxide (CO₂), acetate, and formate to produce methane [18]. In contrast, the CH₄ oxidation process in the soil directly affects the rate of CH₄ oxidation and the flux of CH₄ released into the atmosphere [19]. Identifying the micro-regulatory mechanisms of these two processes on CH₄ metabolism in degraded wetlands is particularly important. Previous studies have explored the factors influencing CH₄ flux in degraded wetlands. Most research suggests that the decline in soil water content is both a cause of wetland degradation and the main driving factor affecting CH₄ flux [14,20]. There are also studies that have confirmed the significant impact of carbon substrates and soil temperature on CH₄ metabolism [21,22]. However, these studies mostly focus on environmental factors affecting CH₄ flux, with relatively little research conducted at the level of microbial community composition and metabolic pathways. Additionally, research in both natural and laboratory systems indicates that methane flux may exhibit compensatory responses due to changes in methanogenic community composition [23], suggesting that solely quantifying physical and chemical variables is insufficient to characterize methane production capacity. Since microorganisms play a central role in CH₄ emissions, determining how the structure and activity of microbial communities support local variability in methane production may further enhance our ability to understand, model, and predict wetland CH₄ flux on a global scale. Therefore, revealing the impact of wetland degradation on carbon metabolism-related microbial communities and metabolic pathways is of great significance for better elucidating the entire carbon cycle process.

Metabolic enzymes released by microorganisms regulate the production and oxidation of CH₄. Under anoxic conditions, methanogens use a series of specialized intracellular enzymes and coenzymes to produce CH₄ through a series of biochemical reactions [24]. The key enzyme in the methanogenesis process is methyl coenzyme M reductase (MCR), which is responsible for reducing the methyl group bound to coenzyme M in the final step of all methanogenesis pathways. It is commonly used as a functional gene marker [25]. Methane monooxygenase (MMO) is the most crucial enzyme in the methane oxidation process. In the first step of the biochemical pathway of CH₄ oxidation, aerobic methanotrophs use methane monooxygenase to catalyze the oxidation of methane to methanol. Methanol is then oxidized to formaldehyde through other pathways, and formaldehyde is converted into intracellular substances via the serine pathway or the ribulose monophosphate pathway [11]. However, the impact of wetland degradation on CH₄ related microbial metabolic enzymes has not yet been fully elucidated.

This paper reports a case study in which in-situ soil CH₄ fluxes across four degradation gradients were measured during the 2023 growing season. The study analyzed the impact of wetland degradation on soil CH₄ emissions and identified soil microbial metabolic processes, including CH₄ production and oxidation, based on Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways. This study aims to determine the response of these two processes to wetland degradation, elucidate how soil microbial communities, detailed metabolic pathways, and related enzymes in these processes regulate changes in CH₄ emissions after wetland degradation, and identify the key environmental factors driving these changes. Based on this, the following hypotheses were proposed: (1) Wetland degradation characterized by moisture loss may inhibit the activity of methanogens and reduce CH₄ flux; (2) Wetland degradation will cause changes in the KEGG pathways of soil CH₄

production and oxidation, and these changes may play an important role in regulating soil CH₄ metabolism.

2. Materials and Methods

2.1. Site Description and In Situ CH₄ Measurement

The sampling site was located at the Jingxin Wetland, Hun Chun, China (42°42′, 130°34′E; elevation: 11 m; Figure 1). The study region has a medium temperate offshore monsoon climate and influenced by the Sea of Japan. The annual average temperature in the study area is 5.6 °C and the annual average precipitation is 823.7 mm. According to the classification method of Xiaoping and Guilin [26], the Jingxin Wetland is a wetland complex with intermittent distribution of riverine wetland, lake wetland, marsh wetland and artificial wetland. The soils are divided into 8 soil types, with white slurry soil and meadow soil as the main ones.

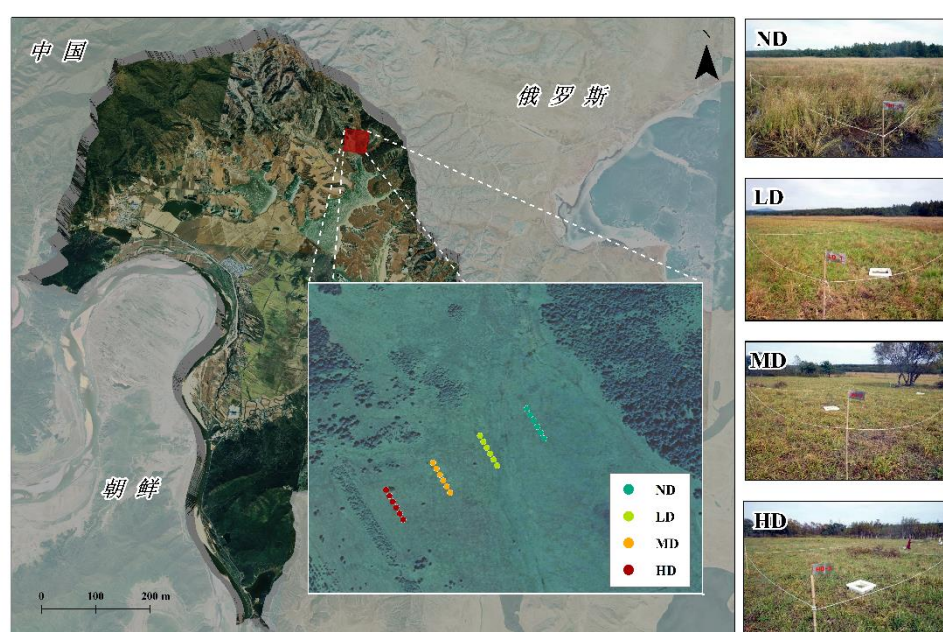


Figure 1. Study region and four degradation gradients in the Jingxin Wetland, Hun Chun, China. ND, LD, MD, and HD represent the four degradation levels: non-degraded (ND), lightly degraded (LD), moderately degraded (MD), and highly degraded (HD), respectively.

This experiment was conducted in September 2022, during the peak growing season. As shown in Figure 1, this experiment was conducted in September 2022, during the peak growing season. Based on the characteristics of plant community structure (proportion of dominant plant species, vegetation coverage, and above-ground biomass) and hydrology (water level), the herbaceous swamp wetlands were divided into four treatments: non-degraded (ND), lightly degraded (LD), moderately degraded (MD), and highly degraded (HD). Six transects (300 meters) provided 6 replicates. For each transect, four plots along approximately 300 meters were randomly selected to represent the four degradation gradients. In total, 24 samples (four degradation levels × 6 replicates) were measured for in-situ CH₄.

This study used the static chamber method to collect CH₄ gas. The static chamber consists of two parts: a movable gas sampling chamber (50 cm × 50 cm × 60 cm) and a base (60 cm × 60 cm × 20 cm). A gas sampling port was installed at the top, which was sealed with a silicone stopper. A sampling point was randomly selected within the experimental plot. One week before gas sampling began, the base was inserted into the soil to a depth of 10 cm. During gas sampling, water was added to 2/3 of the depth of the base's groove to ensure that the gas sampling chamber could be inserted while

maintaining a seal and preventing water overflow. This setup ensured that the gas sampling chamber remained well-sealed during the sampling period. Gas sampling was conducted at 09:00 Beijing time, which represents the optimal time for daily average flux [27]. After sampling, the gas samples were transported to the laboratory for measurement using a gas chromatograph.

2.2. Soil Sampling

At the conclusion of the in-situ CH₄ measurements on August 1, 2022, a soil drill was used to collect the topsoil (0-15 cm) in the center of the PVC ring, and the samples collected from five points in each plot were mixed to form one composite sample. The mixed soil samples were then divided into two subsamples. The first subsample was air-dried for physicochemical property analysis, including soil water content (SW), total carbon (TC), pH, electrical conductivity (EC), soil temperature (ST), carbon-to-nitrogen ratio (C/N), microbial biomass carbon (MBC), and dissolved organic carbon (DOC). The second subsample was stored at -80 °C for metagenomic sequencing.

2.3. Metagenomic Sequencing

Environmental soil samples were collected, and metagenomic sequencing was conducted on the Illumina high-throughput sequencing platform (Shanghai Majorbio Technology Co., Ltd.). Soil samples were subjected to genomic DNA extraction using the Mag-Bind® Soil DNA Kit (Omega Bio-tek, Norcross, GA, U.S.). Concentration and purity of extracted DNA was determined with TBS-380 and NanoDrop2000, respectively. DNA extract quality was checked on 1% agarose gel. DNA extract was fragmented to an average size of about 400 bp using Covaris M220 (Gene Company Limited, China) for paired-end library construction. Paired-end library was constructed using NEXTFLEX® Rapid DNA-Seq (Bioo Scientific, Austin, TX, USA). Adapters containing the full complement of sequencing primer hybridization sites were ligated to the blunt-end of fragments. Paired-end sequencing was performed on an Illumina NovaSeq 6000 (Illumina Inc., San Diego, CA, USA) at Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China) using the NovaSeq 6000 S4 Reagent Kit v1.5 (300 cycles) according to the manufacturer's instructions (www.illumina.com). The following specific procedures are detailed in the Supplementary Methods.

2.4. Statistical Analyses

Soil physiochemical properties and enzyme abundance of four degradation levels (ND, LD, MD, HD) were analyzed via one-way analysis of variance in SPSS (ver. 25.0; IBM, Armonk, NY, USA) with Tamhane's T2, with $p < 0.05$ considered significant. The significance of the effect of each variable on CH₄ production and oxidation was determined through Mantel tests, and the obtained significance level was assessed using the r statistic and p values. Partial least squares regression (PLSR) is an alternative method to ordinary regression for problems with partly or highly co-linear predictor variables. Prior to using PLSR, a preliminary analysis indicated that many of the environmental predictors were co-linear (Table S3). Therefore, we used the R package „pls” to evaluate how environmental factors influence CH₄ flux. In PLSR modeling, the importance of a predictor for both the independent and the dependent variables is given by the variable importance for the projection (VIP). The terms that have large VIP values have the greatest explanatory power of the dependent variable. The cross-validated goodness of prediction (Q^2) and the percentage of variance explained for the response variables (CH₄ flux), as well as the cross-validated root-mean-squared error (RMSECV), which represents the difference between the predicted and observed values of each individual pass, were determined for each model. The regression coefficients (RC) reveal the direction and strength of the impact of each variable in the PLSR model.

3. Results

3.1. Methane Flux and Environmental Factors

Degradation significantly altered wetland CH₄ emissions and soil physicochemical properties. The CH₄ emissions were $6.62 \pm 3.76 \text{ mg m}^{-2}\text{h}^{-1}$, $0.47 \pm 0.91 \text{ mg m}^{-2}\text{h}^{-1}$, $0.16 \pm 0.10 \text{ mg m}^{-2}\text{h}^{-1}$, and $0.05 \pm 0.04 \text{ mg m}^{-2}\text{h}^{-1}$ for the ND, LD, MD, and HD plots, respectively (Table S1). Degradation significantly reduced CH₄ emissions, but differences between degradation gradients were not significant (Figure 2a). Wetland degradation significantly reduced SW (from $337.67 \pm 97.30\%$ in ND to $34.15 \pm 1.85\%$ in HD, Figure 2b), TC (from $154.13 \pm 18.25 \text{ g kg}^{-1}$ in ND to $30.46 \pm 2.51 \text{ g kg}^{-1}$ in HD, Figure 2f), and EC (from $122.20 \pm 39.39 \text{ us cm}^{-3}$ in ND to $46.32 \pm 3.59 \text{ us cm}^{-3}$ in HD, Figure 2d) and decreased continuously with increasing degradation gradient, but did not reach significant levels. Their trends were consistent with those of methane emissions. In contrast, degradation caused significant increases in ST, from $16.08 \pm 0.26 \text{ }^{\circ}\text{C}$ in ND to $19.13 \pm 0.31 \text{ }^{\circ}\text{C}$ in HD. PH increased from 5.05 ± 0.09 in ND to 5.57 ± 0.02 in HD. As the degradation gradient increased, C/N (from 14.66 ± 0.52 in ND to 9.06 ± 0.40 in HD, Figure 2i) decreased significantly (Table S1). However, There were no significant differences in DOC among the four degradation levels (Figure 2g). In addition, MBC were higher in LD than in the others treatments (Figure 2h).

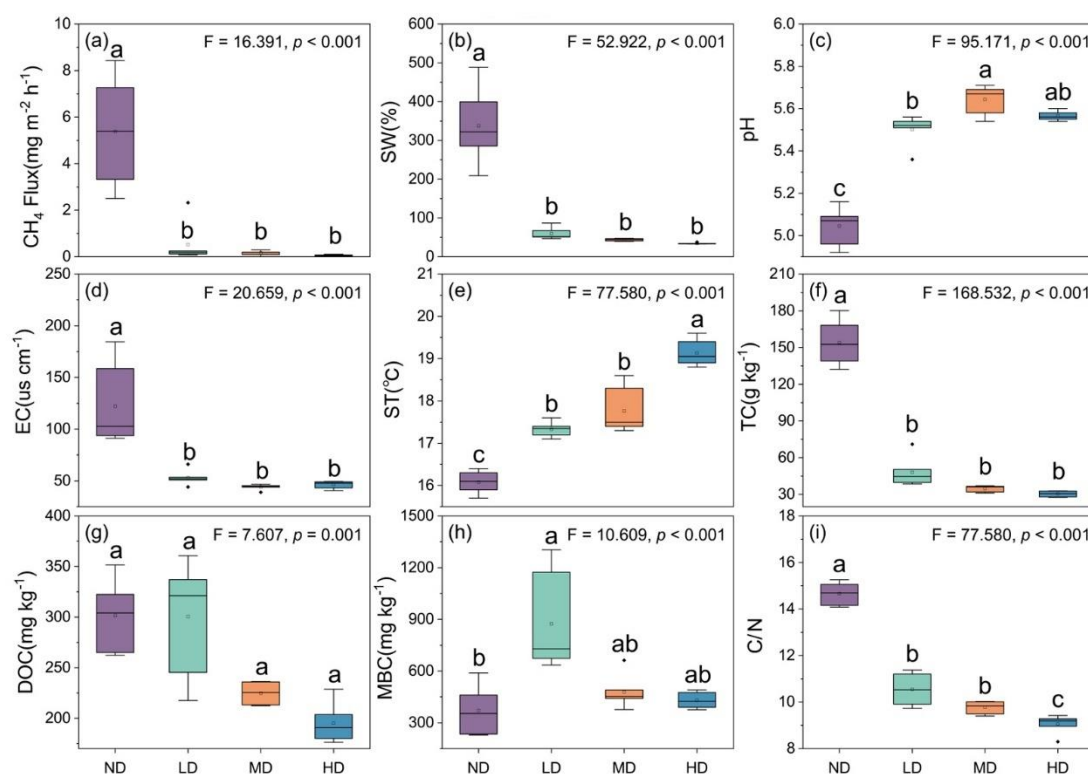


Figure 2. Environmental factors at the different degradation gradient sites. (a-i) Represent the trends of CH₄ Flux, SW, TC, pH, EC, ST, C/N, MBC, DOC with degradation gradient, respectively. Different lowercase letters indicate significant differences at the $p < 0.05$ level (errorbars indicate standard errors). ND, non-degraded; LD, lightly degraded; MD, moderately degraded; HD, highly degraded; SW, soil water content; TC, total carbon; EC, electrical conductivity; ST, soil temperature; C/N, ratios of TC and TN; MBC, microbial biomass carbon; DOC, dissolved organic carbon.

3.2. Effects of Wetland Degradation on Microbial Community

Methanogens are key methane-producing microorganisms and phylogenetically belong to the Archaea domain. Species annotation results based on the NR database indicate that the study area encompasses the three nutritional pathways of methane production: hydrogenotrophic, acetoclastic, and methylotrophic. The dominant methanogens in the four wetland degradation gradients are

hydrogenotrophic methanogens, including the orders Methanocellales , Methanobacteriales, Methanococcales, Methanomicrobiales, and Methanopyrales. Their proportion of the total methanogens increased from 49.51% in ND to 76.99% in HD (Table 1). The second most abundant methanogens are facultative methanogens of the order Methanosarcinales, whose proportion decreased from 37.95% in ND to 16.39% in HD. The third most abundant methanogens are acetoclastic methanogens, including Methanosarcina and obligate aceticlastic methanogens of the genus Methanothrix, whose proportion decreased from 9.83% in ND to 9.03% in HD as the wetlands degraded. Methylophilic methanogens, including Methanomassiliicoccales and Methanonatronarchaeales, had the smallest proportion, increasing from 3.92% in ND to 6.62% in HD. However, wetland degradation led to an overall 99.93% reduction in methanogen abundance, significantly decreasing the abundance of all types of methanogens.

Table 1. Abundance of methanogens with different nutritional types (mean ± standard deviation; n = 6) and results of Kruskal-Wallis H statistical tests. Values represent species abundance normalized as Transcripts Per Million (TPM). TPM accounts for sequencing depth and gene length, calculated as the number of reads per gene divided by its length (in kilobases), normalized across all species within a sample. ND, LD, MD, and HD represent the four degradation levels: non-degraded (ND), lightly degraded (LD), moderately degraded (MD), and highly degraded (HD), respectively.

Category	Methanogens	ND	LD	MD	HD	p value
hydrogenotrophic methanogens	Methanocellales	324.54 ± 80.42 a	1.49 ± 1.01 b	1.44 ± 0.62 b	0.88 ± 0.64 b	<0.01
	Methanobacteriales	396.27 ± 73.90 a	1.64 ± 1.37 ab	0.50 ± 0.33 b	0.69 ± 1.37 b	<0.01
	Methanococcales	29.94 ± 7.99 a	0.00 ± 0.00 b	0.00 ± 0.00 b	0.00 ± 0.00 b	< 0.001
	Methanomicrobiales	183.49 ± 36.05 a	0.79 ± 1.29 ab	0.099 ± 0.15 b	0.06 ± 0.10 b	<0.01
	Methanopyrales	3.94 ± 0.92 a	0.18 ± 0.23 ab	0.00 ± 0.00 b	0.00 ± 0.00 b	< 0.001
methylophilic methanogens	Methanomassiliicoccales	72.94 ± 18.45 a	0.00 ± 0.00 b	0.03 ± 0.05 b	0.14 ± 0.22 b	<0.01
	Methanonatronarchaeales	1.32 ± 0.53 a	0.00 ± 0.00 b	0.00 ± 0.00 b	0.00 ± 0.00 b	< 0.001
Acetate-degrading methanogens	Methanothrix	163.48 ± 50.81 a	0.16 ± 0.24 ab	0.03 ± 0.07 b	0.00 ± 0.00 b	< 0.001
	Methanosarcina	22.73 ± 5.15 a	0.32 ± 0.35 b	0.02 ± 0.05 b	0.19 ± 0.32 b	<0.01
	Methanosarcinales	719.18 ± 149.06 a	0.36 ± 0.40 b	0.19 ± 0.13 b	0.35 ± 0.49 b	<0.01

Aerobic methanotrophs in the study area include the phyla Proteobacteria and Verrucomicrobia. Among them, Proteobacteria include type I and type II methanotrophs. The proportion of type I methanotrophs in the total methanotrophs decreased from 31.78% in ND to 3.22% in HD (Table 2). Type II methanotrophs have consistently been the dominant group in the region, with their proportion in the total methanotrophs increasing from 62.83% in ND to 94.94% in HD. The phylum Verrucomicrobia had the smallest proportion, decreasing from 5.39% in ND to 1.84% in HD. A non-parametric test of methanotrophs abundance at the family level showed that wetland degradation significantly increased the abundance of type II methanotrophs from the family Beijerinckiaceae, excluding Methylocystaceae, from 16.21±4.06 in ND to 38.85±4.29 in MD. However, degradation significantly reduced the abundance of type I methanotrophs and the family Verrucomicrobiaceae, decreasing from 22.13±13.11 and 3.75±1.01 in ND to 1.74±0.72 and 1.00±0.47 in HD, respectively, with no significant differences observed among LD, MD, and HD.

Table 2. Abundance of methanotrophs with different nutritional types (mean ± standard deviation; n = 6) and results of Kruskal-Wallis H statistical tests. Values represent species abundance normalized as Transcripts Per Million (TPM). TPM accounts for sequencing depth and gene length, calculated as the number of reads per gene divided by its length (in kilobases), normalized across all species within a sample. ND, LD, MD, and HD represent the four degradation levels: non-degraded (ND), lightly degraded (LD), moderately degraded (MD), and highly degraded (HD), respectively.

Category	Methanotrophs	ND	LD	MD	HD	p value
Type II methanotrophs	Beijerinckiaceae	16.21 ± 4.06 b	30.02 ± 2.34 ab	38.85 ± 4.29 a	32.17 ± 2.42 ab	< 0.001
	Methylocystaceae	27.53 ± 12.95 a	19.19 ± 5.67 a	19.50 ± 1.71 a	19.24 ± 3.53 a	0.457
Type I methanotrophs	Methylococcaceae	22.13 ± 13.11 a	2.58 ± 0.86 ab	2.38 ± 0.89 b	1.74 ± 0.72 b	<0.01
Verrucomicrobia	Verrucomicrobiaceae	3.75 ± 1.01 a	0.70 ± 0.25 b	1.16 ± 0.20 b	1.00 ± 0.47 b	<0.01

3.3. Differences of CH₄ Metabolism Pathways Under Different Degradation

The study identified four methane production (MP) pathways in the study area: the CO₂ pathway, trimethylamine metabolism, the methanol pathway, and the acetate pathway. Based on their nutritional types, the CO₂ pathway is classified as hydrogenotrophic, trimethylamine metabolism and the methanol pathway as methylotrophic, and the acetate pathway as acetoclastic.

One-way ANOVA of key enzyme abundances revealed that wetland degradation significantly impacted the CO₂ pathway by reducing enzyme levels at multiple stages. In the initial step, the abundance of fwdA (EC: 1.2.7.12), which catalyzes CO₂ reduction to formyl, decreased significantly (Figure 3b (1)). Similarly, enzymes involved in the intermediate conversion of formyl to methyl-coenzyme M—ftr (EC: 2.3.1.101), mtd (EC: 1.5.98.1), and mtrA (EC: 2.1.1.86)—also showed notable declines of 85.26%, 84.48%, 100%, and 96.29%, respectively, in HD compared to ND (Figure 3b (2), (4), (6)). Conversely, the abundance of mer (EC: 1.5.98.2), another enzyme in the intermediate stage, significantly increased by 87.48%, while mch (EC: 3.5.4.27) showed no significant change (Figure 3b (5), (3)). In the acetate pathway, early-stage enzymes involved in acetate activation and metabolism—ackA (EC: 2.7.2.1), pta (EC: 2.3.1.8), and acs (EC: 6.2.1.1)—exhibited significant increases in abundance, rising by 26.57%, 57.34%, and 4.52%, respectively (Figure 3b (8), (7), (9)). Conversely, in the methanol pathway, the key enzymes MtaA (EC: 2.1.1.246) and MtaB (EC: 2.1.1.90), responsible for methanol activation and methyl transfer, showed significant declines with increasing degradation levels, dropping by 93.82% and 79.92%, respectively (Figure 3b (10), (11)). In the trimethylamine metabolism pathway, the abundance of dmd (EC: 1.5.8.1/2), which catalyzes the initial activation of trimethylamine, increased dramatically by 1121.83%. However, the abundances of enzymes involved in methyl transfer—MtbA (EC: 2.1.1.247), MtmB (EC: 2.1.1.248), MtbB (EC: 2.1.1.249), and MttB (EC: 2.1.1.250)—declined significantly by 94.71%, 99.29%, 98.33%, and 77.63%, respectively. But most importantly, wetland degradation significantly reduced the abundance of the key enzyme methyl-coenzyme M reductase α -subunit (McrA; EC: 2.8.4.1), which catalyzes the final step of methane production, with a 99.90% decline compared to ND ($p < 0.05$).

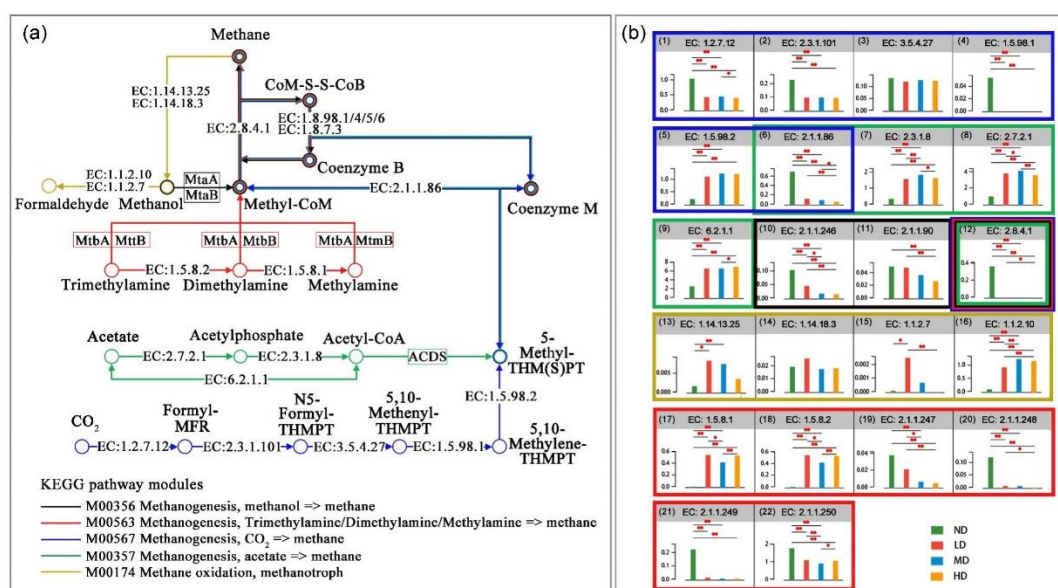


Figure 3. Significant Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways of CH₄ production and oxidation in wetland soils (a). The five related pathways are colored differently, including the CO₂ pathway, trimethylamine metabolism, methanol pathway, acetate pathway and CH₄ oxidation pathway. One-way analysis of variance (ANOVA) was conducted for the relative abundances of genes encoding enzymes involved in CH₄ metabolism (b). ND, LD, MD, and HD represent the four degradation levels: non-degraded (ND), lightly degraded (LD), moderately degraded (MD), and highly degraded (HD), respectively.

The study focused only on the key steps of methane oxidation, where methane monooxygenase (MMO) catalyzes the conversion of methane to methanol, which is subsequently oxidized to formaldehyde by methanol dehydrogenase. The abundance of pmoA (EC: 1.14.18.3), which is involved in the first step of methane oxidation, showed no significant change. However, the abundance of mmoX (EC: 1.14.13.25) significantly increased in LD and MD, rising by 69.58% and 45.43% compared to ND, respectively, while no significant difference was observed between HD and ND. During the conversion of methanol to formaldehyde, the abundance of mdh1 (EC: 1.1.2.7) first increased and then decreased, with no significant difference observed between ND and HD. In contrast, the abundance of xoxF (EC: 1.1.2.10) in degraded wetlands significantly increased compared to ND.

3.4. Factors Determining CH₄ Emission Under Different Degradation

To identify the factors driving methane-metabolizing microbial communities in degraded wetlands, we used Mantel analysis to relate functional composition (based on biochemical KEGG modules) to environmental factors. The methane-producing (MP) functional community showed no significant correlation with environmental factors in ND and HD. In LD, SW was significantly correlated with the methane-producing community ($P < 0.05$, Figure 5), while in MD, pH was significantly correlated with the MP community ($p < 0.05$, Figure 5). The methane-oxidizing (MO) functional community was significantly correlated with ST and pH ($P < 0.05$, Figure 5).

Table 4 presents the individual PLSR models constructed for CH₄ flux in ND and LD-MD-HD soils, including the number of components required for each model to achieve optimal predictive performance (maximum Q² and minimum RMSECV). The first two components explained 74% and 78% of the total variance in ND and LD-MD-HD, respectively. Figure 5 illustrates the Variable Importance in Projection (VIP) and Regression Coefficients (RC) of the environmental factors. In the model constructed for ND (Figure 5a), DOC obtained the highest VIP value, followed by SW. Higher DOC levels corresponded to increased CH₄ flux. Similarly, lower SW contributed to reduced CH₄ flux, as indicated by the positive RC. In the model constructed for LD-MD-HD (Figure 5b), SW had the highest VIP value, followed by pH. Lower SW levels corresponded to reduced CH₄ flux (positive regression coefficient), while higher pH levels corresponded to reduced CH₄ flux (negative regression coefficient).

Table 3. Summary of the PLSR models for methane flux at different stages of degradation.

Treatment	R ²	Q ²	Component	% of explained variability in Y	Cumulative explained variability in Y (%)	RMSECV	Q ² cum
ND	0.74	0.87	1	73.86	73.86	1.095	0.446
			2	22.24	96.10	0.839	0.871
LD-MD-HD	0.78	0.73	1	78.06	78.06	1.029	0.729

4. Discussion

4.1. Effects of Wetland Degradation on Magnitude of Methane Emissions

The most common form of wetland degradation, characterized by water loss, reduces methane emissions. For example, the pristine peatlands in the Zoige region have degraded due to artificial drainage, significantly reducing CH₄ emissions. The degradation of peatland vegetation on the Qinghai-Tibet Plateau has also led to a decrease in CH₄ flux. Research on high-altitude permafrost wetlands in Tibet suggests that wetland degradation and soil moisture loss lead to a decrease in methane production potential. These findings are consistent with the results of this study. Although the degradation of swamp peatlands in the Amazon region only affects CH₄ emissions on a micro scale and is not significant on a macro scale, the main environmental driving factors are also related to moisture [14]. Additionally, a study by Padhy, Bhattacharyya, Dash, Nayak, Parida, Baig and Mohapatra [28] on the Sundarbans mangroves in India found that wetland degradation increased the abundance of enzymes involved in methanogenesis. This implies that mangrove degradation could lead to higher CH₄ emissions because the degradation decreases sediment salinity, reducing competition for substrates between sulfate-reducing bacteria and methanogens. A study on tropical peatlands in Southeast Asia indicated that wetland degradation caused by fires leads to variations in methane emissions corresponding to changes in floodwater levels, resulting in distinct dry and wet periods [29].

4.2. Effects of Wetland Degradation on Environmental Factors

Methane flux is affected by factors such as soil water content (SW), pH, soil temperature (ST), and the availability of carbon substrates. These soil characteristics play a significant role, either directly or indirectly, in regulating soil CH₄ flux. Previous studies have shown that certain soil physicochemical properties undergo significant changes as wetlands degrade. Abulaizi, Chen, Yang, Hu, Zhu and Jia [30] conducted a study on the alpine wetlands of Bayanbulak and found that SW, TC, and TN exhibited a decreasing trend with increasing degradation levels ($p < 0.05$). Yan, Yang, Chen, Zou, Zhao, Yan, Wang and Wu [31] found that SW decreased with increasing degradation. Our findings are consistent with previous studies. We demonstrated that as wetland degradation intensifies, SW significantly decreases (Figure 2b), with a statistically significant difference between ND and other degradation stages ($p < 0.001$). This may be because wetland degradation is typically accompanied by a decline in water table levels, changes in soil drainage properties, and the loss of moisture, all of which directly affect the soil's water retention capacity. As a result, wetland degradation often leads to a reduction in soil water content. Furthermore, water scarcity inhibits plant growth and affects nutrient uptake by plants [32], potentially causing dominant species in native wetlands to lose their dominance. Consequently, lower SW results in suboptimal growth conditions, leading to a decline in soil organic matter [33,34]. Changes in soil water content also affect the stoichiometric C/N balance, the relationship between soil C and N, and other soil properties. Consistent with the changes in surface soil C/N observed in the study by Huo, Chen, Zou, Lu, Guo and Tang [35], we found a significant decrease in C/N along the degradation gradient ($p < 0.001$). Furthermore, wetland degradation significantly affects soil pH. In the study area, the soil in non-degraded (ND) wetlands was mildly acidic. However, the pH values in the low-degraded (LD), medium-degraded (MD), and high-degraded (HD) areas were significantly higher than those in the ND area, indicating that wetland degradation reduces soil acidity. This result is consistent with the findings of Li, Hao, Yan, Kang, Wang, Zhang, Li, Wu and Kang [36], where the increase in pH was attributed to enhanced water evaporation during wetland degradation, causing salts and alkali to rise from the subsoil to the topsoil. The decline in groundwater levels and soil moisture further increased the salinity and alkalinity concentration in the topsoil, which likely exacerbated the rise in soil pH. This study also found a significant decrease in EC, which is consistent with the findings of Wan, Yu, Kong, Zhang, Chen, Zhao and Liu [37] and Zhang, Qi, Tong, Wang, An, Zhang and Lu [38]. This may be due to the reduction in vegetation and organic matter during the degradation process,

leading to a decrease in the concentration of electrolytes (such as calcium, magnesium, potassium, and sodium ions) in the soil, thereby lowering EC.

4.3. Effects of Wetland Degradation on Microbial Communities Associated with Methane Production and Oxidation

Methanotrophy (oxidation of CH_4) and methanogenesis (production of CH_4) are the two important microbial mediated processes that play an important role in methane emission from soil to the atmosphere in wetland ecologies [28,39]. Changes in the community structure of methanogens and methanotrophs may be the primary reason for the decrease in CH_4 flux after the degradation of the Jingxin Wetland. The abundance of methanogens and the abundance of the MCR (EC: 2.8.4.1) enzyme are both inhibited by wetland degradation (Table 1; Figure 3b (12)), while the abundance of dominant methanotrophs and soluble methane monooxygenase (sMMO; EC: 1.14.13.25) are promoted by wetland degradation (Table 2, Figure 3b (13)). This suggests that the strong suppression of methane production and the slight stimulation of methane oxidation in the soil may be the reasons for the decrease in net CH_4 flux [11].

The abundance of methanogens and methanotrophs is often used to reflect the potential for methane production and oxidation [21,40]. Undoubtedly, the observed low abundance of methanogens typically indicates lower methane emissions. Some scholars believe that disrupting the original water balance of wetlands is the essence of wetland degradation, and changes in soil moisture are the primary cause of wetland degradation. It is well known that methane production is a strictly anaerobic microbial process; therefore, when wetlands degrade and soil moisture decreases, the soil's anaerobic conditions are weakened, which in turn inhibits methane production [41,42]. In our study, a consistent conclusion was drawn that in the LD treatment, the methane production functional module was significantly positively correlated with SW (Figure 4b). This may be because the decrease in SW allows oxygen to penetrate the wetland surface, leading to a significant reduction in the abundance of methanogens [43,44].

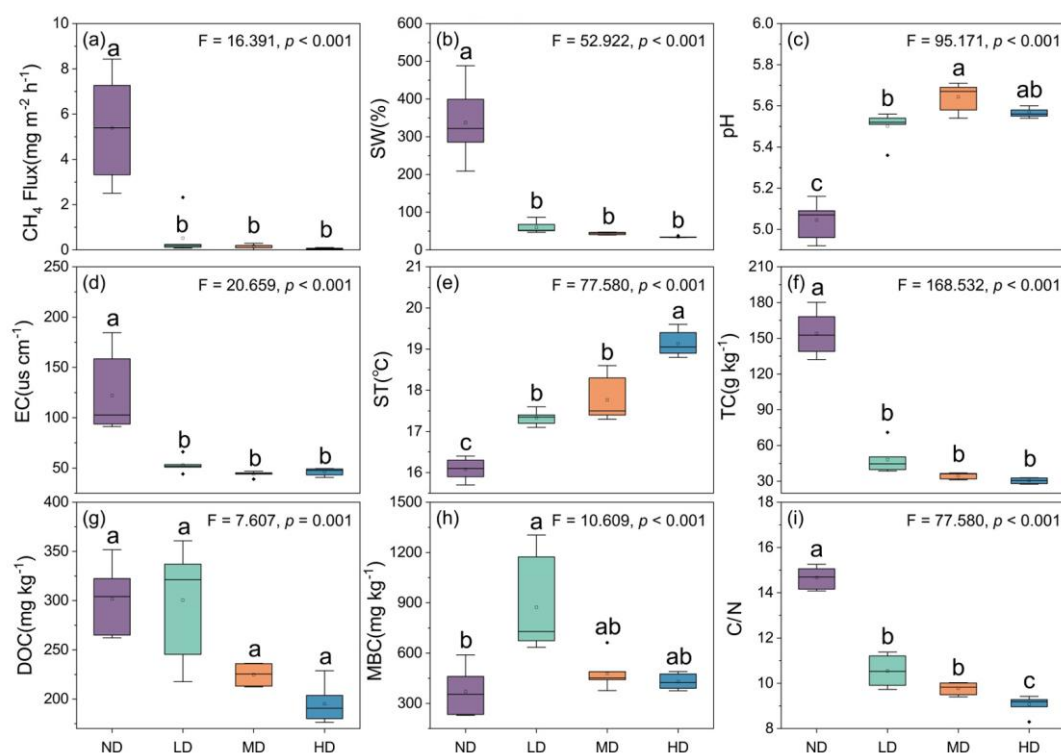


Figure 4. The significance of the effect of environmental factors on the soil microbial community involved in CH_4 production and oxidation was defined using Mantel tests (permutations = 999), and the resulting significance level was tested using the Mantel r statistic and p values. The color gradient denotes Spearman's

correlation coefficients. MP, methane production related microbial subcommunity; MO, methane oxidation related microbial subcommunity. Panels (a), (b), (c), and (d) represent non-degraded (ND), lightly degraded (LD), moderately degraded (MD), and highly degraded (HD), respectively.

Methanotrophs are the only microbial CH₄ sinks in wetlands, and their relative abundance also affects CH₄ flux [45]. Type II methanotrophs have been described as microorganisms that can survive under adverse conditions and tolerate stress [46], and they exhibit a competitive advantage over Type I methanotrophs under low pH conditions [47–49]. Therefore, the weakly acidic conditions in the wetland of this study area may be the main reason why Type II methanotrophs have become the dominant methane-oxidizing microbial community. In addition, we observed a significant increase in the Beijerinckiaceae family of Type II methanotrophs in the MD treatment (Table 2), which may be related to the increase in temperature and pH (Figure 4c). CH₄ uptake often shows very little temperature dependence, generally increasing slightly with increasing soil temperatures [50]. This effect may be associated with the temperature-dependent increase in the enzymatic activity of soil methane-utilizing bacteria and the reduced diffusion resistance of CH₄ in air at higher temperatures [51,52]. The increase in the Beijerinckiaceae family may be due to their optimal growth at neutral pH, while the significant decline of Type I methanotrophs and Verrucomicrobia may be attributed to their acidophilic nature [53]. In an existing study discussing the evolution of Type II methanotrophs in the Beijerinckiaceae family, it was emphasized that they exhibit strong oxidation capabilities in environments with low methane concentrations, effectively degrading methane even at lower concentrations. As a result, they play an important role in environments with low methane concentrations [54]. This may explain why, after wetland degradation, Beijerinckiaceae could significantly increase compared to other types of methanotrophs.

However, a higher relative abundance of Type II methanotrophs does not necessarily imply a higher methane oxidation rate. This can be explained by the C1 assimilation RuMP pathway utilized by Type I methanotrophs, which is more efficient at converting carbon into biomass compared to the serine pathway used by Type II methanotrophs [55]. Additionally, it has been reported that most microbial species in the soil (up to 80%) are in an inactive or dormant state [56].

The first step of the methane oxidation process involves methane monooxygenase (MMO) oxidizing methane to methanol. MMO can be categorized into particulate methane monooxygenase (pMMO; EC: 1.14.18.3) and soluble methane monooxygenase (sMMO; EC: 1.14.13.25). pMMO is more common because it is produced by all methane-oxidizing microorganisms in wetland and upland environments, whereas sMMO can only be expressed by a single genus, primarily in moist, copper-deficient environments [57,58]. Therefore, pMMO is likely to play a more crucial role than sMMO in the consumption of atmospheric CH₄ [11]. In this study, sMMO significantly increased after degradation, while pMMO showed no significant differences among the four treatments (Figure 3b). This may indicate that wetland degradation only slightly stimulates methane oxidation.

4.4. Effects of Wetland Degradation on Methane Metabolism Pathways

We found that SW is the primary environmental factor affecting CH₄ flux (Figure 5). Some researchers believe that disrupting the original water balance of wetlands is the essence of wetland degradation, and changes in soil moisture are the primary cause of wetland degradation. It is well known that methane generation is a strictly anaerobic microbial process. Therefore, after wetland degradation, the decrease in soil moisture can reduce soil anoxia, thereby inhibiting methane production [42,59]. In our study, the decrease in SW allowed oxygen to penetrate the top layer of the wetland, which may be the direct reason for the significant disappearance of methanogens. In contrast, aerobic conditions stimulated the growth of methanotrophs, which subsequently decreased (Table 2). The abundance of type II methanotrophs increased, which is consistent with the findings of Zhang, Naafs, Huang, Song, Xue, Wang, Zhao, Evershed, Pancost and Xie [60] that type II methanotrophs activity may be enhanced during drought periods. The changes in the abundance of

sMMO and pMMO indicate a slight increase in the metabolic activity of the methane-oxidizing community.

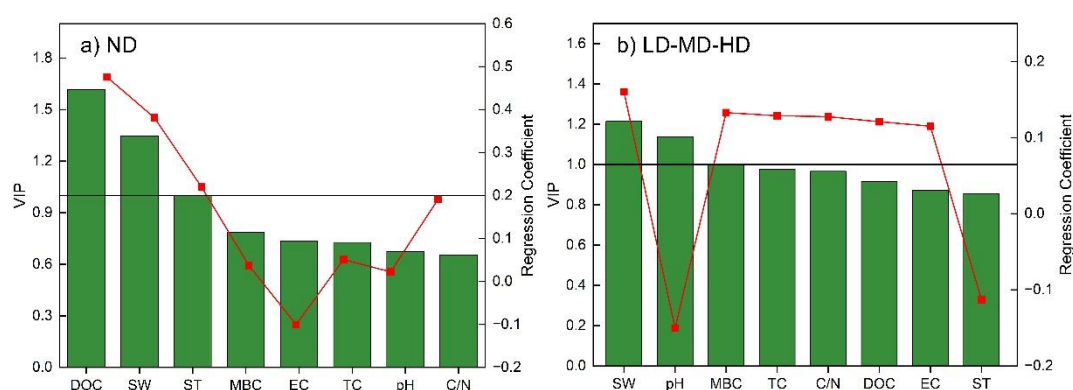


Figure 5. Partial Least Squares Regression (PLSR) analysis results of the impact of environmental factors on CH_4 flux. ND, LD, MD, and HD represent the four degradation levels: non-degraded (ND), lightly degraded (LD), moderately degraded (MD), and highly degraded (HD), respectively.

Soil carbon substrates are an important factor influencing greenhouse gas emissions from wetlands [61]. Plants can provide carbon substrates for methane production through root exudates and litter [22,62]. Changes in soil moisture are closely related to shifts in vegetation community structure. Soil moisture and organic matter content largely determine plant composition and biomass production [63]. Due to the decrease in SW, dominant species in native wetlands may lose their dominance, affecting soil carbon input and organic carbon decomposition, leading to a reduction in organic matter and TC [64]. Previous studies have investigated the effects of soil organic carbon, dissolved organic carbon, total nitrogen, and C/N on methane production [21,22,61]. Our current research highlights the significant role of TC in methane generation (Figure 5), while the impact of soil organic carbon as a carbon substrate on methane flux remains underexplored. Therefore, further research on the specific contribution of SOC to the methane generation process and its influencing mechanisms is of great significance for a comprehensive understanding of the methane cycle.

We also found that pH plays an important role in influencing the microbial community. While the slightly acidic soil in ND limits the growth of certain flora, fauna, and methane-metabolizing microorganisms, it simultaneously creates a unique ecological environment that cultivates a group of specialized acid-tolerant methanogens, supporting the survival of specific species. For example, in acidic bog peatlands ($\text{pH} < 5$), the dominant methanogenesis pathway is hydrogenotrophic methanogenesis [65–67]. According to the experimental results of Li, Sun, Li and Yuan [68], when the pH decreased from 7 to 4.8, the abundance of acetoclastic methanogens decreased with the lowering pH, while the total hydrogenotrophic methanogens increased from 12.8% to 66.5%. This indicates that hydrogenotrophic methanogens are more acid-tolerant than acetoclastic methanogens. The study by [69] also reached the same conclusion: hydrogenotrophic methanogens can grow under low pH conditions ($\text{pH} 6.0 - 4.0$), while acetoclastic methanogens are more sensitive to low pH ($5.5 - 4.0$) than hydrogenotrophic methanogens. This might explain why the hydrogenotrophic pathway is the primary methanogenesis pathway in our study area. However, after wetland degradation, the decrease in soil acidity may render the original methanogenic community unsuitable for survival, leading to a significant loss of hydrogenotrophic methanogens. Methanosarcina and Methanotrix, which belong to acetoclastic methanogens, completely disappeared. After the decrease in soil acidity, no inhibition of methanotrophs was observed. This may be because soil pH shapes the diversity and composition of CH_4 oxidation microbial communities. Methanotrophs seem to thrive in soils ranging from acidic to neutral pH [70].

Methane emissions from wetland soil microorganisms are not only influenced by carbon substrates provided by plants [71,72], but plants also act as conduits for methane transfer from the soil to the atmosphere. The decline in soil moisture may alter the vegetation community, particularly plants with aerenchyma, leading to a reduction in methane emissions [73]. In this study, wetland degradation led to a decrease in the number of *Cyperus* species with aerenchyma in ND [74], which restricted CH₄ transfer to the atmosphere and may be one of the reasons for the decline in CH₄ flux.

5. Conclusion

Based on metagenomic methods, this study investigated the microbial regulatory mechanisms of CH₄ emissions in wetlands at different degradation levels in the Tumen River basin. The following conclusions were drawn:

(1) Wetland degradation significantly altered environmental factors such as SW, TC, and pH. These changes had a notable impact on CH₄ flux, leading to a near disappearance of CH₄ emissions.

(2) The decline in SW and TC content, along with the decrease in soil acidity, led to reduced abundance of methanogens and key enzymes (mcrA; EC: 2.8.4.1) across all pathways, which is the reason for the dramatic reduction in methane production. The increase in methanotrophs and the lack of significant changes in the abundance of key enzymes (pMMO; EC: 1.14.18.3) indicate that methane consumption only slightly increased.

(3) After wetland degradation, the decline in CH₄ production activity far exceeds the increase in CH₄ oxidation activity, leading to the disappearance of methane sources in wetlands. This indicates that under degradation conditions, the primary factor influencing methane flux is the methane production process rather than methane oxidation. Therefore, we believe that the rapid decline in methane emissions is primarily due to the reduced abundance and metabolic activity of methanogens, which is largely caused by the decrease in soil moisture.

Given the complexity of marsh wetlands and the uncertainties of global climate change, further research is needed to explore the mechanisms through which wetland degradation affects CH₄ emissions.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org.

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