# The Influence of Seasonal Freeze–Thaw in Northeast China on Greenhouse Gas Emissions and Microbial Community Structure in Peat Soil

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**Text S1. Determination of Total Nitrogen, Ammonium Nitrogen, and Nitrate Nitrogen**

Soil total nitrogen (TN) content was determined using a soil carbon and nitrogen analyzer (Elementar Vario EL Ⅲ, Germany). Soil nitrate nitrogen ($NO_3^-$-N) and ammonium nitrogen ($NH_4^+$-N) were determined using the potassium chloride (KCl) extraction method. Briefly, 5 g of soil sample was weighed into a 150 mL triangular flask, 50 mL of 2 M KCl was added, and the mixture was shaken for 1 h at room temperature. The samples were then filtered, calibrated, and analyzed using a SmartChem 450 autoanalyzer (AMS, Italy).

**Text S2. Sequencing Data Analysis**

Sequencing data were processed and analyzed using the analysis platform (https://magic.novogene.com) provided by Beijing NOHZYUAN Technology Co., Ltd. Analyses included microbial diversity assessment, community composition analysis, correlation between environmental factors and community structure, and functional gene prediction.

First, raw sequencing data underwent splicing and quality control. High-quality OTU tables were generated using DADA2 software for sequence clustering/denoising, followed by microbial taxonomic annotation. LEfSe analysis was used to identify microbial taxa with significant differences among freeze–thaw treatments, and Wilcoxon tests were applied to assess significant changes at the phylum and genus levels.

For functional prediction, PICRUSt or FAPROTAX tools were employed to predict microbial functional genes involved in carbon/nitrogen cycling and to examine their potential relationships with greenhouse gas emissions. All analyses were performed using R, SPSS, OriginPro, and GraphPad Prism software: R for diversity, correlation and regression analyses; SPSS for ANOVA and significance testing; OriginPro and GraphPad Prism for visualization; and QIIME2/Mothur for sequence processing and taxonomic annotation.

**Table S1.** Correlation between $CO_2$ and $N_2O$ Emission Rates and Physicochemical Properties of Peatland Soil under Seasonal Freeze-Thaw Cycles.

| emission rate | | DOC | DON | pH | $NH_4^+$ | $NO_3^-$ | SOC | TN | MBC | MBN |
|---|---|---|---|---|---|---|---|---|---|---|
| $CO_2$ emission rate | ST | 0.829* | 0.715 | 0.466 | −0.769 | 0.724 | −0.742 | −0.775 | 0.793 | 0.674 |
| | SB | −0.872* | -0.76 | −0.624 | 0.756 | −0.75 | 0.774 | 0.849* | −0.841* | −0.745 |
| | AT | 0.76 | 0.777 | 0.791 | −0.586 | 0.592 | −0.696 | −0.761 | 0.731 | 0.725 |
| | AB | 0.772 | 0.778 | 0.79 | −0.59 | 0.591 | −0.697 | -0.766 | 0.735 | 0.722 |
| $N_2O$ emission rate | ST | 0.652 | 0.715 | 0.865* | −0.802 | 0.715 | −0.742 | −0.501 | 0.643 | 0.609 |
| | SB | −0.716 | -0.775 | −0.903* | 0.869* | −0.776 | 0.807 | 0.589 | −0.718 | −0.676 |
| | AT | 0.665 | 0.73 | 0.902* | −0.830* | 0.741 | −0.772 | −0.553 | 0.684 | 0.642 |
| | AB | 0.671 | 0.729 | 0.882* | −0.827* | 0.732 | −0.763 | −0.536 | 0.671 | 0.629 |

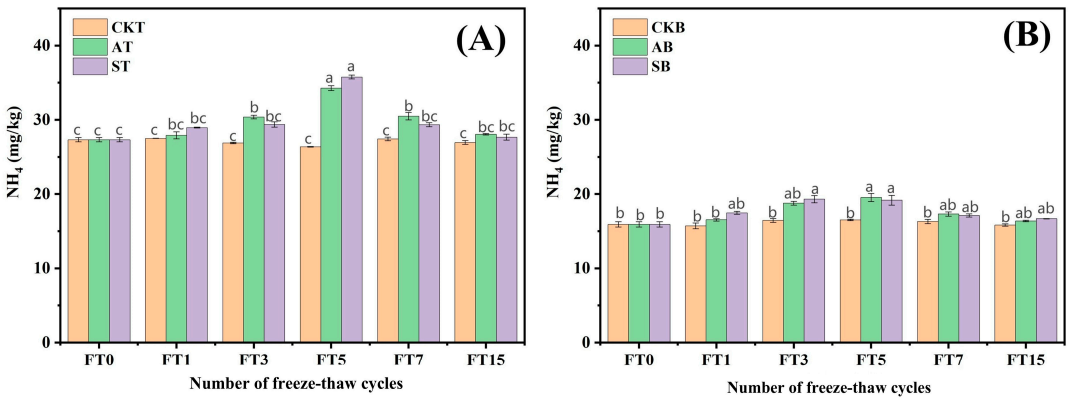



**Figure S1.** Changes in soil ammonium nitrogen ($NH_4^+$) of peatlands under seasonal freeze-thaw cycles.

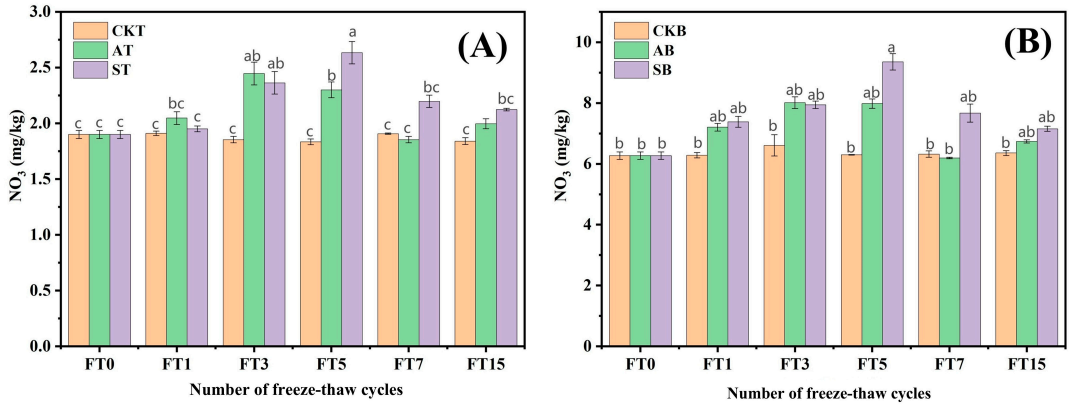



**Figure S2.** Changes in soil nitrate nitrogen ($NO_3^-$) of peatlands under seasonal freeze-thaw cycles.

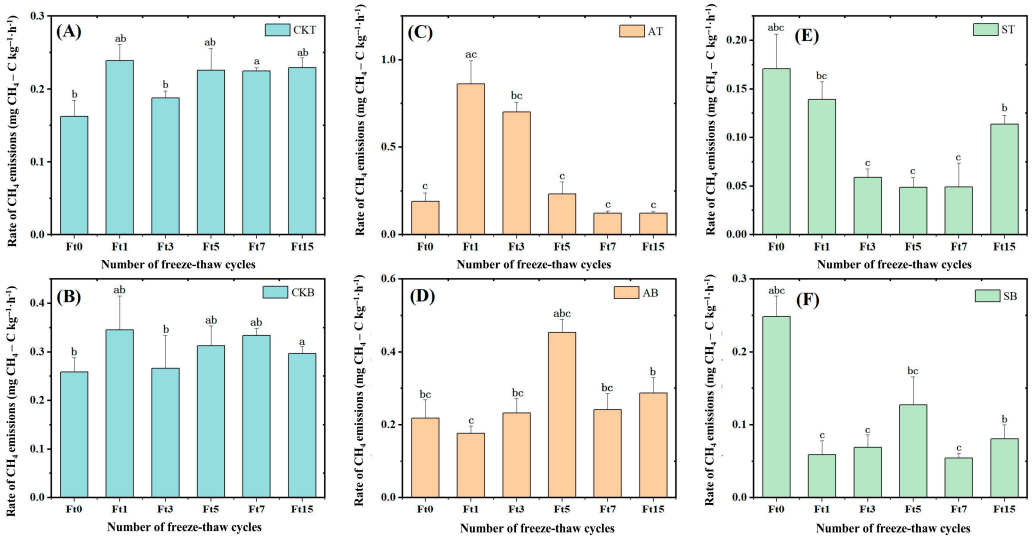



**Figure S3.** The variation of $CH_4$ emission rate in peat soil under seasonal freeze-thaw conditions.

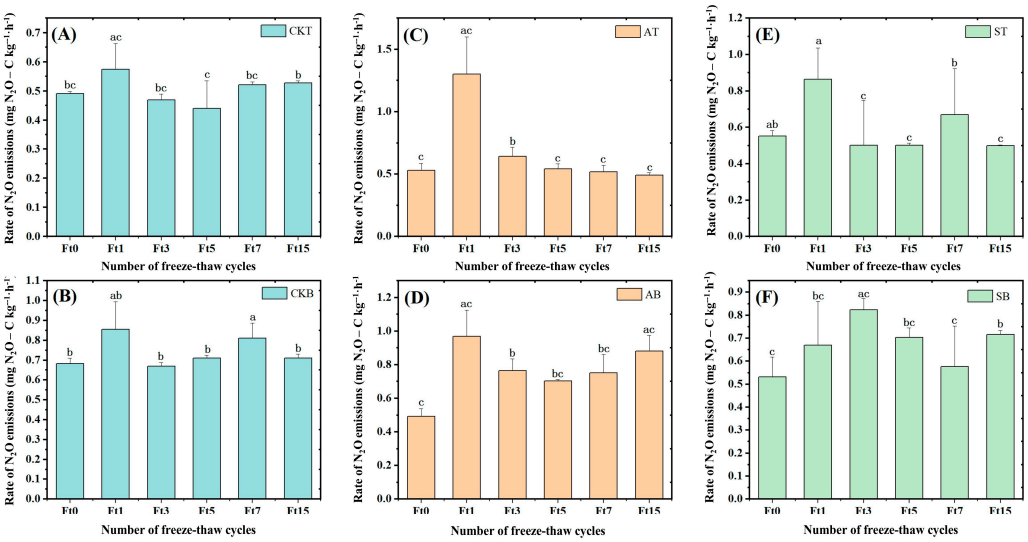



**Figure S4.** The variation of $N_2O$ emission rate in peat soil under seasonal freeze-thaw conditions.

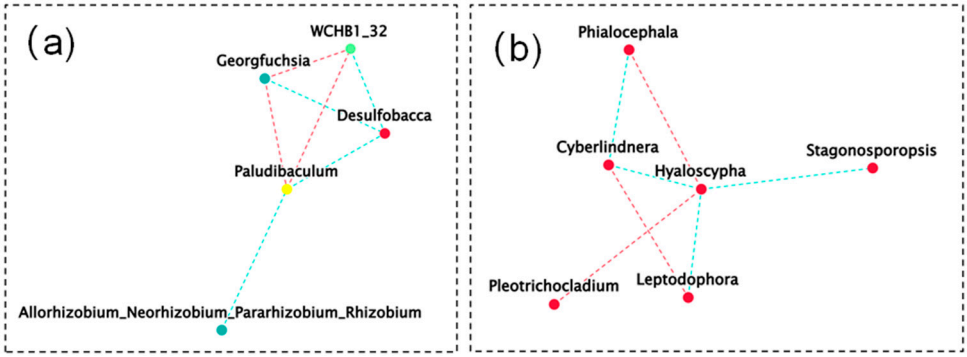



**Figure S5.** (a) Bacterial Co-occurrence Network, (b) Fungal Co-occurrence Network .