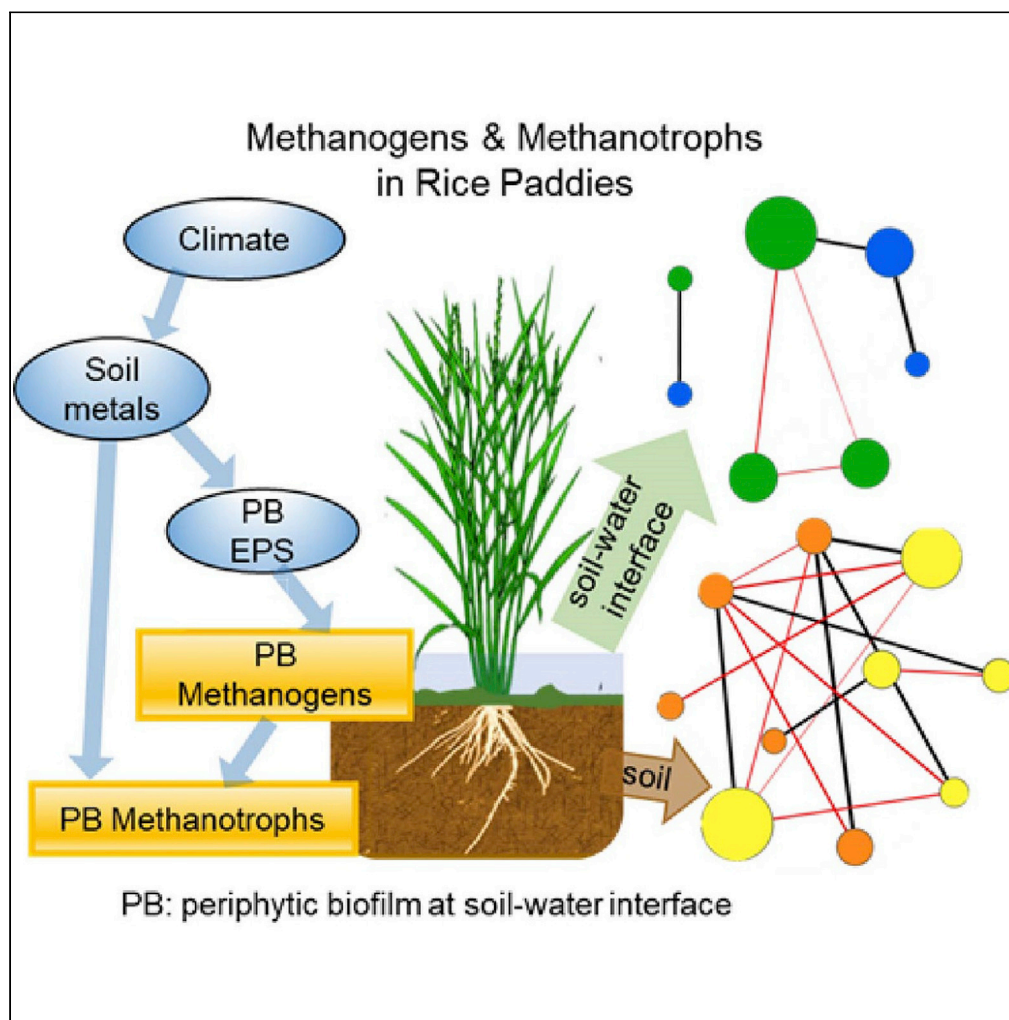


Article

Distribution of methanogenic and methanotrophic consortia at soil-water interfaces in rice paddies across climate zones



Sichu Wang,
Pengfei Sun,
Junzhuo Liu, Ying
Xu, Jan Dolfing,
Yonghong Wu

yhwu@issas.ac.cn

Highlights

PB and soil from 66 rice paddies across three climate zones were sampled

Interactions of CH₄-related microbes in PB were less complex than in soil

Climate indirectly drove changes in composition of methanogens and methanotrophs

EPS and soil metal were most important in influencing their relative abundances

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Article

Distribution of methanogenic and methanotrophic consortia at soil-water interfaces in rice paddies across climate zones

Sichu Wang,^{1,2,3} Pengfei Sun,^{1,3} Junzhuo Liu,^{1,3} Ying Xu,^{1,3} Jan Dolfing,⁴ and Yonghong Wu^{1,3,5,*}

SUMMARY

Periphytic biofilms (PB) at the soil-water interface contributes 7–38% of the methane emission from rice paddies, yet the biogeographical mechanism underlying and affecting the process remain elusive. In this study, rice fields along an edapho-climatic gradient were sampled, and the environmental drivers affecting distribution of methanogenic and methanotrophic communities were evaluated. The methanogenic and methanotrophic communities at soil-water interface showed less complex inter/intra-generic interactions than those in soil, and their relative abundances were weakly driven by spatial distance, soil organic carbon, soil total nitrogen and pH. The nutrient supply and buffering capacity of extracellular polymeric substance released by PB reduced their interaction and enhanced the resilience on edaphic environment changes. Climate affected soil metal content, extracellular polymeric substance content, and thus the methane-related communities, and caused geographical variation in the impacts of PB on methane emissions from rice paddies. This study facilitates our understanding of geographical differences in the contribution of PB to methane emission.

INTRODUCTION

Rice paddies are an important source of atmospheric methane (CH₄), accounting for 10% of the total anthropogenic CH₄ emission.¹ Recent research has shown that 7–38% of the CH₄ emitted from rice paddies is contributed by the periphytic biofilms (PB) growing at the soil-water interface.² The amount and proportional contribution of the PB varied with the geographical location with the largest contribution observed for the subtropics.² PB in rice paddies consist of an intricate mixture of phototrophic, autotrophic and heterotrophic microorganisms,³ including both methanogens and methanotrophs. Geographical environment appears to play a major role in the dynamics of these microorganisms and the resulting uptake and emission of CH₄ by the PB. Insight into the geographical distribution and environmental drivers of methanogens and methanotrophs in PB facilitates our understanding of geographical differences in the contribution of PB to CH₄ emission.

Spatial distance and niche theory have been effectively applied to explain variation in the abundance and diversity of microbes.^{4–6} Methanogens in rice paddies are thought to inhabit anoxic compartments especially the waterlogged soil at depths of 3–20 cm, whereas aerobic methanotrophs thrive preferentially in oxic surface soil and oxic microhabitats of the rhizosphere.^{7,8} An investigation in methanotrophs in paddy soils across China indicated a significant variation of the relative abundance of *Methylococcus*, and higher rates of nitrogen fertilization could decrease methanotrophic activity.⁹ Wu et al. found that moderately thermophilic methanogens are common in temperate soils,¹⁰ but the dominant methanogen group varied with soil type.^{10,11} Climate, soil texture, nutrient contents, salinity and pH are also considered to be critical drivers of methanogenic and methanotrophic communities in rice paddies.^{9,12} However, these critical drivers are largely hypothetical proposed based on analyses of rhizosphere and bulk soil, as factors influencing methanogens and methanotrophs at the soil-water interface have been little explored.

Methanogens and methanotrophs at the soil-water interface exist as multispecies aggregates with other bacteria, algae, fungi, protozoa, and metazoan. These microorganisms produce a matrix of extracellular

¹State Key Laboratory of Soil and Sustainable Agriculture, Institute of Soil Science, Chinese Academy of Sciences, 71 East Beijing Road, Nanjing 210008, China

²Institute of Agricultural Resources and Environment, Jiangsu Academy of Agriculture Sciences (JAAS), 50 Zhongling Road, Nanjing 210014, China

³Zigui Three Gorges Reservoir Ecosystem, Observation and Research Station of Ministry of Water Resources of the People's Republic of China, Shuitianba Zigui, Yichang 443605, China

⁴Faculty of Energy and Environment, Northumbria University, Newcastle upon Tyne NE1 8QH, UK

⁵Lead contact

*Correspondence: yhwu@issas.ac.cn

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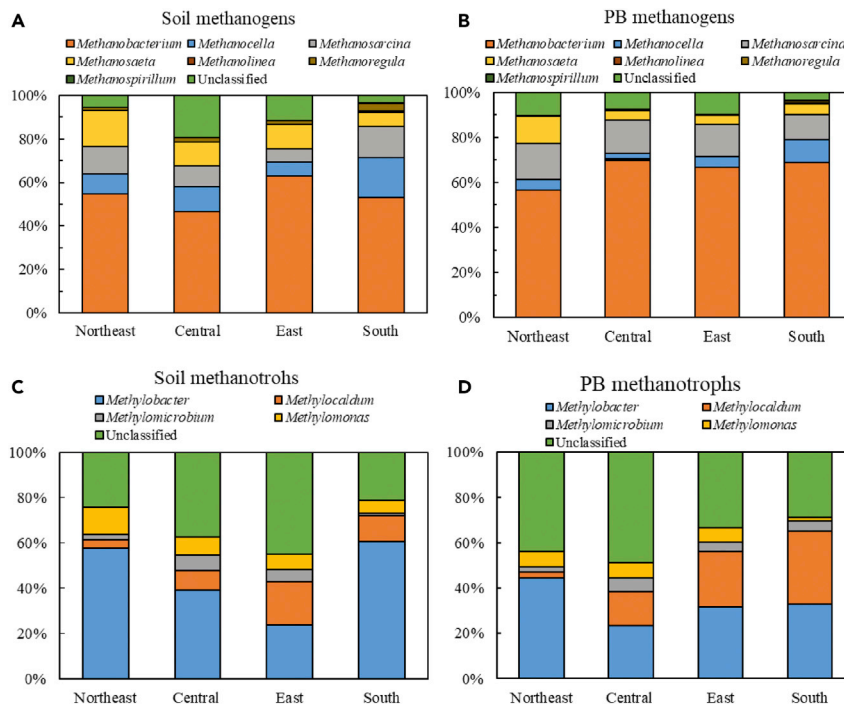


Figure 1. Composition of methanogens and methanotrophs in paddy soils and periphytic biofilm (PB) in different rice zone of China

(A) methanogens in soils; (B) methanogens in PB; (C) methanotrophs in soils; (D) methanotrophs in PB. Number of PB and soil samples taken in northeast, central, east and south China was 12,15, 21 and 18 each, respectively.

polymeric substances (EPS) to fix themselves as well as minerals and nutrients, forming biofilms at the soil-water interface. The EPS enables biosorption of trace minerals and sequestration of organic matter and nutrients.^{13,14} The distinct physical structure of EPS acts as a buffer for microbes in the interior of aggregates to modulate against environmental change and resist adverse environmental effects.^{13,15,16} This buffering and resistance capacity of EPS raises the questions whether the current conjectures of microbial distribution also apply to the methanotrophs and methanogens at soil-water interface, and whether and to what extent the abundance and diversity of these microbes are driven by the environmental factors, such as temperature, soil organic carbon (SOC), soil total nitrogen (STN), metal ions and pH. Furthermore, microbes in PB mostly originate from the underlying soil and constantly exchange and interact with soil microbes,³ which leads to the question whether there is any association between CH₄-related microbes in soil and at soil-water interface.

Geographical locations have shown quantitatively different impacts of PB on CH₄ emissions from rice paddies.² To evaluate whether these differences are reflected in the methanogenic and methanotrophic communities and compare the communities at soil-water interface from that in soil, rice fields across the temperate, subtropic and tropic climate zones were sampled, and the composition, diversity, interaction and environmental drivers of methanogenic and methanotrophic communities were studied in detail in this study.

RESULTS

Composition and diversity of methanogens and methanotrophs

Methanogens and methanotrophs were detected in both PB and soil at all the sampling sites, and their community compositions in soil and PB were similar (Figure 1). Genus *Methanobacterium* was the most abundant methanogen in both PB and soil, followed by the genus *Methanosarcina* (Figures 1A and 1B). The relative abundance of *Methanosaeta* was higher in northeastern rice paddies, whereas *Methanocella* (Rice Cluster I) were more abundant in the southern rice planting region. The relative abundances of *Methanosaeta*, *Methanocella* and *Methanoregula* were larger in soil than in PB. *Methylobacter* and

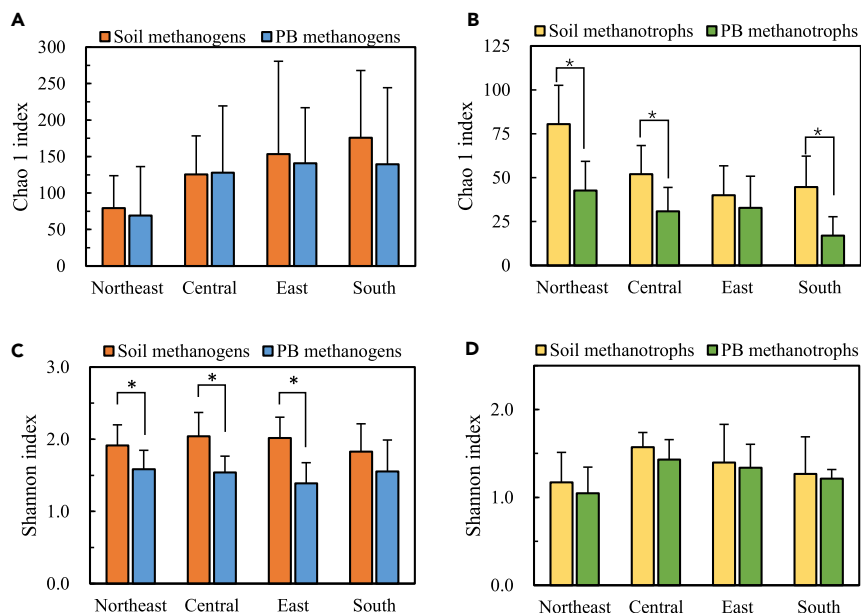


Figure 2. Species richness and diversity of methanogenic and methanotrophic community in soil and PB

(A) Chao 1 index of methanogens in soil and PB, (B) Chao 1 index of methanotrophs in soil and PB, (C) Shannon index of methanogens in soil and PB, and (D) Shannon index of methanotrophs in soil and PB. Sample numbers of PB or soil in northeast, central, east and south China were 12, 15, 21 and 18, respectively. The significant differences between treatments were tested by one-way ANOVA analysis (LSD) and indicated by asterisks: *, $p < 0.05$; **, $p < 0.01$. Data are represented as mean + SD.

Methylocaldum were the two most common genera of methanotrophs in PB and soil (Figures 1C and 1D). The relative abundance of *Methylocaldum* increased from north to south in both PB and soil. *Methylomicrobium* and *Methylomonas* were also present in PB as well as in soil.

Though similar in community composition, PB and soil were different in richness and diversity of methanogens and methanotrophs (Figure 2). Chao 1 index of methanotrophic community in PB was significantly higher than the index of soil methanotrophs and the Shannon-Weiner index of soil methanotrophs was higher than that of PB methanotrophs ($p < 0.05$). Species diversity and richness also varied with rice planting regions. Chao 1 richness of methanogens increased from northeast to south, for both of the PB and soil, and the reverse phenomenon was observed for that of methanotrophs. Shannon diversity of methanotrophs was highest at central rice planting regions and diversity of methanogens exhibited little difference among the four rice planting regions.

Co-occurrence networks of methanogens and methanotrophs

Co-occurrence patterns of methanogenic and methanotrophic communities in soil and PB are shown in Figure 3. The network of co-occurrences among methanogens and methanotrophs in soil was more complex than that in PB, indicated by the number of nodes and links. A total of six positive and ten negative significant interactions was detected in soil (Figure 3A). Specifically, one positive and three negative intra-generic interactions were among methanogens, three negative interactions were among soil methanotrophs, and there were five positive and four negative inter-generic co-occurrence relations between methanogens and methanotrophs. The most abundant methanogenic genus, *Methanobacterium*, was not observed in the soil network, indicating that competitive and mutualistic interactions between *Methanobacterium* and other microbes were very weak. In the contrast, the dominant methanotrophs *Methylobacter*, as well as methanogenic genera *Methanosarcina* and *Methylocaldum* were key genera in the interaction network illustrating their rich interplay with other genera. Weak co-occurrence relations were observed between methanogens and methanotrophs in PB (Figure 3B), encompassing three positive and three negative interactions involving two methanogenic genera and three methanotrophic genera. Strong positive co-occurrence relationships were observed between the methanogens *Methanocella*

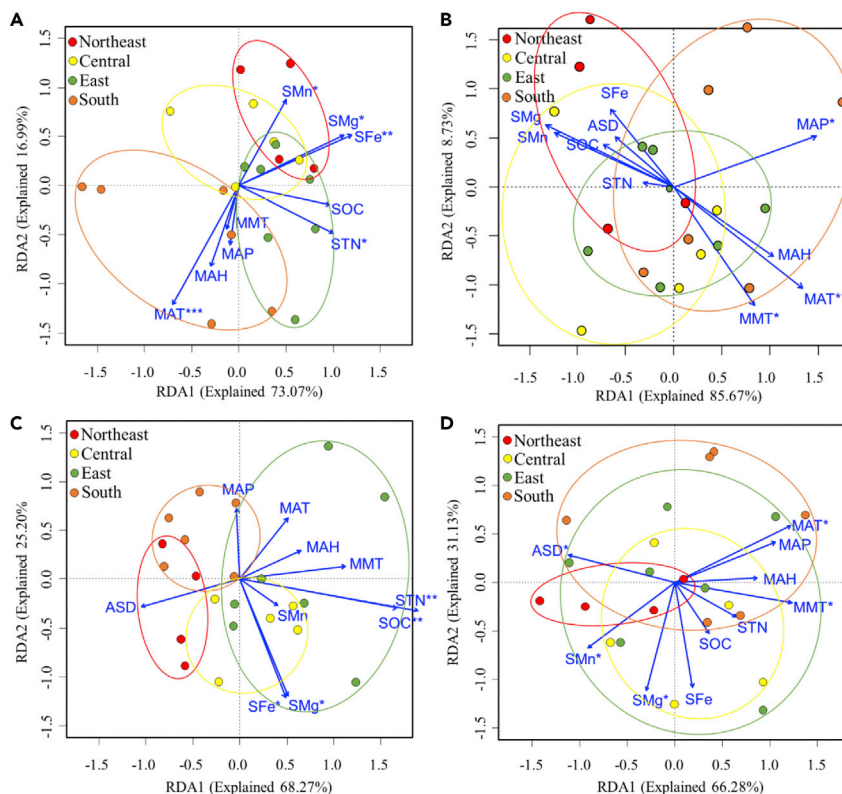


Figure 4. Redundancy analysis (RDA) of environmental variables with the composition of (A) soil methanogens, (B) PB methanogens, (C) soil methanotrophs and (D) PB methanotrophs in different rice planting zones of China (n = 22)

The arrowed lines refer to the environmental variables. The red, yellow, green and orange points indicate sampling sites from northeast, central, east and south rice zone of China, respectively, and the sampling sites from the same zone are circled by the corresponding colors. The longer the arrowed line, the more likely the factors influenced the community composition. The significance was indicated by asterisks: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. The closer a point is to another, the more similar their microbial composition.

structure of methanogens and methanotrophs in PB were mostly influenced by climatic variables MAT, MMT, MAP and ASD (Figures 4B and 4D). In Figures 4A and 4C, points from the same rice-planting region tend to gather together, contrast to the points in Figures 4B and 4D which were mixed thoroughly.

Pathway by which environmental variables influence abundance of methanogens and methanotrophs

To further identify the direct and indirect effects of the environmental variables on the relative abundance of methanogenic and methanotrophic communities a partial least squares path modeling (PLS-PM) model was constructed and the significant variables identified above were incorporated into the models. EPS content of PB was included in the models because it responds quickly to environmental change and affects community composition of PB. EPS content was established to be the most important driver of methanogens in PB, with the strongest effect on their relative abundances (Figure 5A). Its direct path coefficient was 0.559, whereas the direct contributions of soil CN (SOC and STN), climate and soil metals had relatively weak path coefficients of -0.178 , 0.116 and 0.003 , respectively. The variable climate could also indirectly influence the methanogenic community via altering soil environment and EPS content, and consequently had a high total effect (total path coefficient = 0.342).

Soil metal was the variable with the highest explanatory power to the relative abundance of methanotrophs in PB, with direct and indirect path coefficients of 0.415 and 0.085 , respectively (Figure 5B). Although its direct path coefficient was lower than that of EPS (-0.212), climate (-0.168) was the second most important

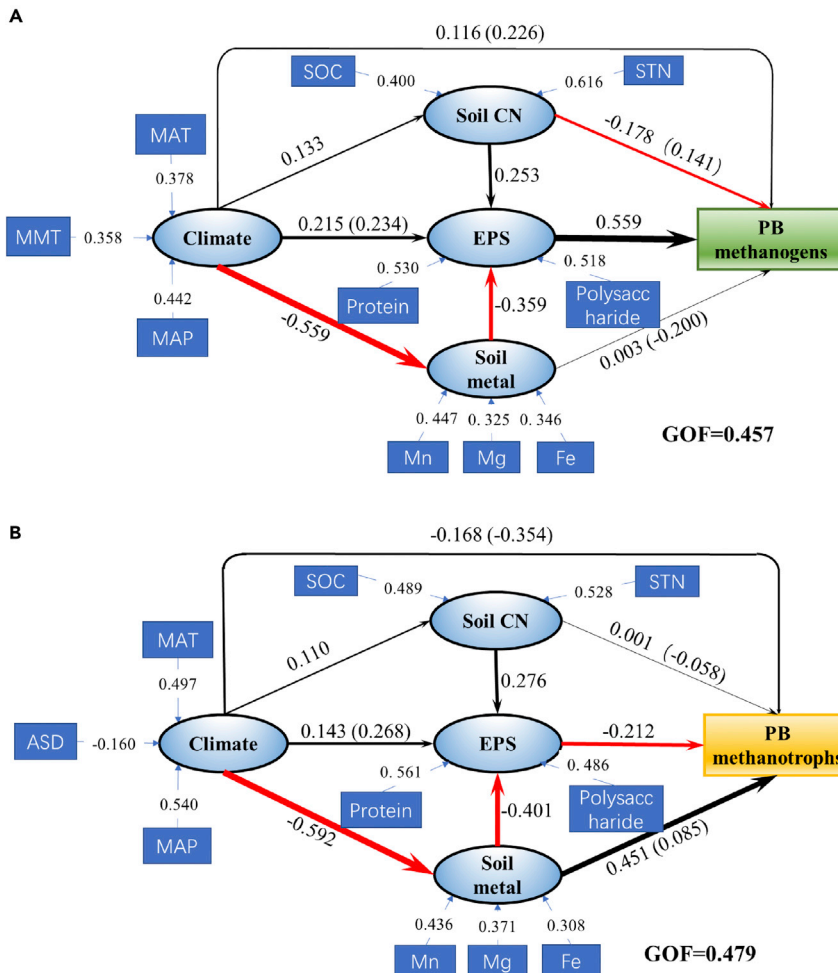


Figure 5. Partial least squares path modeling (PLS-PM) displaying the effect of environmental variables on the relative abundance of (A) methanogenic and (B) methanotrophic communities (n = 22)

In each panel, variables in boxes are observable variables; variables in circles are latent variables representing relevant observable variables and the value on the arrow from blue box to circle indicates the weight of the observable variable. The black and red arrows refer to positive and negative relationships, respectively. Numbers adjacent to these arrows are direct (and indirect) standardized path coefficients, and the width of the arrow is in proportion with the strength of the direct path coefficients. Models were assessed using the goodness of fit (GOF) statistic.

driver, as it indirectly influenced periphytic methanotrophs through changing the contents of soil metals and EPS. The indirect path coefficients of climate through soil metals and EPS were -0.267 and -0.087 , respectively. Surprisingly, the contribution of soil CN in explaining methanotrophic community traits in PB was negligible ($p < 0.001$).

The mechanism by which climate affects the methanogenic and methanotrophic communities in the PBs showed clear differences. For both communities the climate effects observed are mediated by the effect of climate on the soil metal content. However, for the methanogens the effect was primarily modulated via the effect of soil metal on EPS, whereas in the case of the methanotrophs the soil metal had a strong direct effect.

DISCUSSION

Comparison of methanogenic and methanotrophic communities at soil-water interface with those in soil

Despite living in different niches modulated by different oxygen concentrations, methanogens and methanotrophs coexist in the PB. Autotrophs, such as microalgae, dominate the PB during most of its

existence and release oxygen (O₂) through photosynthesis,^{17,18} making PB an oxidative habitat. Methanogens, which have long been considered strictly anaerobic,¹⁹ were found in PB, suggesting these methanogens may not be exposed and adapted to oxygenic conditions but instead inhabit anoxic niches inside the PB matrix. Catabolic processes of certain species capable of fermentative carbon metabolism and N₂ fixation create anaerobic micro zones in PB, providing anaerobes, including methanogens, with additional niches shielded from oxygen.²⁰

As the most abundant genus in both soil and PB, *Methanobacterium* has a broader substrate spectrum, producing CH₄ not only using CO₂, but also methylated compounds,^{21–24} which should allow it to adapt to and dominate in both soil and periphytic biofilm. The hydrogenotrophic methanogens *Methanocella* and *Methanoregula*, which use CO₂ to produce CH₄,²⁵ were less abundant in PB than in paddy soil (Figures 1A and 1B), which might be because photosynthetic microalgae in PB compete for CO₂ with hydrogenotrophic methanogens. Furthermore, *Methanocella* has a distinct preference for plant photosynthates in rice rhizosphere under flooded conditions,²⁶ and could therefore be more abundant in soil than in PB.

The Genera *Methylobacter*, *Methylocaldum*, *Methylomicrobium* and *Methylomonas* are all Type I methanotrophs (*Gammaproteobacteria*), which are specialists and grow rapidly under optimal conditions,^{24,27} in contrast to the more generalist type II methanotrophs (*Alphaproteobacteria*) which tolerate a wider range of environmental conditions and utilize a variety of substrates.^{28,29} Type I methanotrophs tend to be more active in environments with higher oxygen and lower CH₄ levels compared to type II methanotrophs surviving well in anoxic bulk soil.³⁰ The PB and soil samples in this study were collected at about the 20th day after rice transplantation, when the flood duration was not yet long enough to make the topsoil a thoroughly anaerobic environment. Therefore, the high-affinity type I methanotrophs thrived, whereas type II methanotrophs (e.g., *Methylocystis* and *Methylosinus*) were not detected.

Interaction between methanogens and methanotrophs

There is considered to be a symbiotic interaction between methanogens and methanotrophs.³¹ Methanogens use simple substrates like formate, ethanol, acetate and CO₂/H₂ to produce CH₄, which is consumed by methanotrophs as their only carbon and energy source. The interaction network are modulated by resource and substrate availability,^{32,33} which in turn shape microbial community assembly.³⁴ In this study, the less complex microbial co-occurrence network of PB (Figure 2B) implied the availability of more diverse energy sources from the environment.^{35,36} In addition to immobilization and uptake of dissolved organic carbon (DOC) from floodwater,³⁷ the carbohydrate-rich EPS itself is also an emphatic source of carbon and energy for the microbes in PB.^{38,39}

Utilizing diverse substrates, *Methanobacterium* was dominant in methanogenic communities with little competition or cooperation with other methanogens and methanotrophs. The higher proportions of negative correlations among methanotrophs indicated strong competition among methanotrophs in both soil and PB,⁴⁰ especially between *Methylobacter* and other methanotrophs. However most methanogens in both PB and soil were positively and strongly correlated, suggesting extensive mutualistic interaction.³² In Figures 4A and 4C, aggregated distribution of points from the same rice-planting region indicated that methanogens and methanotrophs in the soils of the same region shared similar community compositions. In contrast, for PB (Figures 4B and 4D), the points were mixed thoroughly, indicating limited spatial dependence in the composition of methanogens and methanotrophs in PB.

Relative abundance and community structure of methanogens and methanotrophs along an edapho-climatic gradient

Temperature is one of the most important factors to define structure and diversity of the methanogenic microbial community.⁴¹ The *Methylocaldum* methanotroph and *Methanocella* methanogen are potential thermophiles,^{42–44} so their relative abundances were positively correlated with temperature (Table S1). On the contrary, the relative abundance of *Methanosaeta* increased with decreasing temperature.⁴⁴ Previous studies reported that increasing temperature could reduce the diversity of soil methanotrophs.⁴⁵ In this study, the Chao 1 and Shannon-Weiner indexes of methanotrophs followed a decreasing trend from north to south rice planting regions (Figure 2), following the increasing temperature gradient.

Table 1. Spearman correlations between environmental variables and the relative abundance of methanogens and methanotrophs in PB

	WpH	SOC	STN	E_polysaccharide	E_protein
P_Methanogens	NS	NS	NS	0.439*	0.407*
P_Methanotrophs	NS	NS	NS	-0.590**	-0.618**

Note: SOC, soil organic carbon content; STN, soil total nitrogen content; WpH, floodwater pH; E_polysaccharide, polysaccharide content in extracellular polymeric substance (EPS) of PB; E_protein, protein content in EPS of PB. NS, not significant ($p > 0.05$); *, $p < 0.05$; **, $p < 0.01$. $n = 22$.

The pH has long been recognized as a critical driver of microbial communities, including methanogenic and methanotrophic community composition.^{12,40,46} Surprisingly, in this study, only a weak correlation was observed between pH and the relative abundances of methanogens and methanotrophs in PB (Table 1). This may be attributed to the buffering of EPS surrounding the microbes. The major component of EPS, polysaccharide, contains multiple uronic acid moieties with numerous carboxylic acid ($-\text{COOH}$) groups, which acts as a weak acid and dissociates partially ($\text{RCOOH} \rightleftharpoons \text{RCOO}^- + \text{H}^+$), buffering against pH variation and maintaining required osmotic level for the cells.¹³

Meanwhile, EPS is a high-density organic carbon pool,⁴⁷ consisting of polymeric components especially polysaccharides and proteins.¹⁴ Bacteria in PB convert EPS into dissolved organic matter; hence, EPS serves as a source of carbon and nutrients to other microbes.⁴⁸ Thus, methanogens and methanotrophs in PB were little limited by environmental carbon and nutrients availability (i.e., SOC, STN). This is indicated by the positive correlations between the contents of polysaccharide and protein and the relative abundance of methanogens and the negative correlations with that of methanotrophs (Table 1).

As indicated by the PLS-PM (Figure 5), EPS content and soil metal were the most important direct drivers influencing the relative abundance of methanogens and methanotrophs, respectively, in PB. Metal ions play important roles in the synthesis of enzymes involved in the oxidation of CH_4 , such as Fe-containing soluble methane monooxygenase (sMMO) located in the cytoplasm of some methanotrophs.^{49,50} Owing to the buffering and nutrient supply of periphytic EPS,^{13,38,39} methanogens and methanotrophs in PB were less sensitive to the edaphic factors SOC, STN and pH, which are known critical drivers of soil methanogens and methanotrophs.^{12,51,52}

Climate and soil metal induced changes in soil environment and EPS content indirectly influence the abundance of methanogens and methanotrophs in PB (Figure 5). Rice paddies in south China have lowest metal contents and a warm and humid climate (Table S2), which promote expansion of methanogenic community and reduce methanotrophic community in PB, and as a result, the potential to emit the most CH_4 . High metal contents and low temperature in northeast China are favorable to methanotrophs and unfavorable to methanogens in PB, resulting in the lowest contribution of PB to CH_4 emissions.² The geographical variation of the impacts of PB on CH_4 emissions from rice paddies is at least partly attributed to the differences of methanogenic and methanotrophic communities driven by the climatic and edaphic variables.

In conclusion, the EPS and soil metal contents were the most important factors influencing their relative abundances along the edapho-climatic gradient. Climatic variables, especially mean annual temperature, indirectly drove changes in community composition. Low temperature, low EPS contents and high soil metal content were favorable to methanotrophs but unfavorable to methanogens. This work enriches understanding of the geographical distribution and diversity of microbes at the soil-water interface. Our results indicate that enhancing soil metal content offers opportunities to promote methanotrophs at the soil-water interface, thus providing prospects for CH_4 mitigation.

Limitations of the study

PB and soil samples were collected once during the tillering stage of rice in this study. A monitoring of methanogenic and methanotrophic microbe dynamics in different growth stages of rice was lacking. The abundance and community composition of methanogens and methanotrophs were influenced by the dissolved oxygen, redox potential, substrate and mineral availability, which vary with flooding time

and rice growth. In addition, the absolute abundance of methanogens and methanotrophs should be quantified by quantitative polymerase chain reaction (qPCR) which was not included in this study. Thus, periodic sampling and quantitative characterization of methanogens and methanotrophs are needed in future work.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at <https://doi.org/10.1016/j.isci.2022.105851>.

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AUTHOR CONTRIBUTIONS

Y.W. designed the study, S.W., P.S., J.L., and Y.X. collected and analyzed samples, S.W. performed data analyses and visualization, S.W., Y.W., and J.D. wrote the manuscript, and all authors contributed substantially to revisions.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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REFERENCES

1. Sun, M., Zhang, H., Dong, J., Gao, F., Li, X., and Zhang, R. (2018). A comparison of CH₄ emissions from coastal and inland rice paddy soils in China. *Catena* 170, 365–373. <https://doi.org/10.1016/j.catena.2018.06.035>.
2. Wang, S., Sun, P., Zhang, G., Gray, N., Dolfig, J., Esquivel-Elizondo, S., Peñuelas, J., and Wu, Y. (2022). Contribution of periphytic biofilm of paddy soils to carbon dioxide fixation and methane emissions. *Innovation* 3, 100192. <https://doi.org/10.1016/j.xinn.2021.100192>.
3. Wu, Y. (2016). Periphyton: Functions and Application in Environmental Remediation (Elsevier). <https://doi.org/10.1016/b978-0-12-801077-8.00008-9>.
4. Ramette, A., and Tiedje, J.M. (2007). Multiscale responses of microbial life to spatial distance and environmental heterogeneity in a patchy ecosystem. *Proc. Natl. Acad. Sci. USA* 104, 2761–2766. <https://doi.org/10.1073/pnas.0610671104>.
5. Liu, D., Wang, H., An, S., Bhople, P., and Davlatbekov, F. (2019). Geographic distance and soil microbial biomass carbon drive biogeographical distribution of fungal communities in Chinese Loess Plateau soils. *Sci. Total Environ.* 660, 1058–1069. <https://doi.org/10.1016/j.scitotenv.2019.01.097>.
6. Martiny, J.B.H., Bohannan, B.J.M., Brown, J.H., Colwell, R.K., Fuhrman, J.A., Green, J.L., Horner-Devine, M.C., Kane, M., Krumins, J.A., Kuske, C.R., et al. (2006). Microbial biogeography: putting microorganisms on the map. *Nat. Rev. Microbiol.* 4, 102–112. <https://doi.org/10.1038/nrmicro1341>.
7. Lee, H.J., Jeong, S.E., Kim, P.J., Madsen, E.L., and Jeon, C.O. (2015). High resolution depth distribution of Bacteria, Archaea, methanotrophs, and methanogens in the bulk and rhizosphere soils of a flooded rice

- paddy. *Front. Microbiol.* 6, 639. <https://doi.org/10.3389/fmicb.2015.00639>.
8. Vaksmaa, A., van Alen, T.A., Ettwig, K.F., Lupotto, E., Valè, G., Jetten, M.S.M., and Lüke, C. (2017). Stratification of diversity and activity of methanogenic and methanotrophic microorganisms in a nitrogen-fertilized Italian paddy soil. *Front. Microbiol.* 8, 2127. <https://doi.org/10.3389/fmicb.2017.02127>.
 9. Yang, W., Wang, W., Shen, L., Bai, Y., Liu, X., Tian, M., Wang, C., Yang, Y., and Liu, J. (2022). Biogeographical distribution and regulation of methanotrophs in Chinese paddy soils. *Eur. J. Soil Sci.* 73. <https://doi.org/10.1111/ejss.13200>.
 10. Wu, X.L., Friedrich, M.W., and Conrad, R. (2006). Diversity and ubiquity of thermophilic methanogenic archaea in temperate anoxic soils. *Environ. Microbiol.* 8, 394–404. <https://doi.org/10.1111/j.1462-2920.2005.00904.x>.
 11. Conrad, R., Klose, M., Noll, M., Kemnitz, D., and Bodelier, P.L.E. (2008). Soil type links microbial colonization of rice roots to methane emission. *Glob. Change Biol.* 14, 657–669. <https://doi.org/10.1111/j.1365-2486.2007.01516.x>.
 12. Nazaries, L., Karunarathne, S.B., Delgado-Baquerizo, M., Campbell, C.D., and Singh, B.K. (2018). Environmental drivers of the geographical distribution of methanotrophs: insights from a national survey. *Soil Biol. Biochem.* 127, 264–279. <https://doi.org/10.1016/j.soilbio.2018.08.014>.
 13. Nagar, S., Antony, R., and Thamban, M. (2021). Extracellular polymeric substances in Antarctic environments: a review of their ecological roles and impact on glacier biogeochemical cycles. *Polar Sci.* 30, 100686. <https://doi.org/10.1016/j.polar.2021.100686>.
 14. Xu, Y., Wu, Y., Esquivel-Elizondo, S., Dolfing, J., and Rittmann, B.E. (2020). Using microbial aggregates to entrap aqueous phosphorus. *Trends Biotechnol.* 38, 1292–1303. <https://doi.org/10.1016/j.tibtech.2020.03.012>.
 15. Tang, J., Wu, Y., Esquivel-Elizondo, S., Sørensen, S.J., and Rittmann, B.E. (2018). How microbial aggregates protect against nanoparticle toxicity. *Trends Biotechnol.* 36, 1171–1182. <https://doi.org/10.1016/j.tibtech.2018.06.009>.
 16. Ma, W.-J., Zhang, J.-T., Wang, Y., Li, G.-F., Wu, X.-X., Yao, Y.-X., Cheng, Y.-F., Huang, B.-C., and Jin, R.-C. (2021). Extracellular polymeric substances excreted by anammox sludge act as a barrier for As(III) invasion: binding property and interaction mechanism. *Chemosphere* 278, 130414. <https://doi.org/10.1016/j.chemosphere.2021.130414>.
 17. Wu, Y., Liu, J., Lu, H., Wu, C., and Kerr, P. (2016). Periphyton: an important regulator in optimizing soil phosphorus bioavailability in paddy fields. *Environ. Sci. Pollut. Res. Int.* 23, 21377–21384. <https://doi.org/10.1007/s11356-016-7363-0>.
 18. Mori, T., Miyagawa, Y., Onoda, Y., and Kayaba, Y. (2018). Flow-velocity-dependent effects of turbid water on periphyton structure and function in flowing water. *Aquat. Sci.* 80, 6. <https://doi.org/10.1007/s00027-017-0552-1>.
 19. Yuan, Y., Conrad, R., and Lu, Y. (2011). Transcriptional response of methanogen *mcrA* genes to oxygen exposure of rice field soil. *Environ. Microbiol. Rep.* 3, 320–328. <https://doi.org/10.1111/j.1758-2229.2010.00228.x>.
 20. Xia, Y., She, D., Zhang, W., Liu, Z., Wu, Y., and Yan, X. (2018). Improving denitrification models by including bacterial and periphytic biofilm in a shallow water-sediment system. *Water Resour. Res.* 54, 8146–8159. <https://doi.org/10.1029/2018wr022919>.
 21. Thauer, R.K., Kaster, A.K., Seedorf, H., Buckel, W., and Hedderich, R. (2008). Methanogenic archaea: ecologically relevant differences in energy conservation. *Nat. Rev. Microbiol.* 6, 579–591. <https://doi.org/10.1038/nrmicro1931>.
 22. Malyan, S.K., Bhatia, A., Kumar, A., Gupta, D.K., Singh, R., Kumar, S.S., Tomer, R., Kumar, O., and Jain, N. (2016). Methane production, oxidation and mitigation: a mechanistic understanding and comprehensive evaluation of influencing factors. *Sci. Total Environ.* 572, 874–896. <https://doi.org/10.1016/j.scitotenv.2016.07.182>.
 23. Wang, C., Shen, J., Liu, J., Qin, H., Yuan, Q., Fan, F., Hu, Y., Wang, J., Wei, W., Li, Y., and Wu, J. (2019). Microbial mechanisms in the reduction of CH₄ emission from double rice cropping system amended by biochar: a four-year study. *Soil Biol. Biochem.* 135, 251–263. <https://doi.org/10.1016/j.soilbio.2019.05.012>.
 24. Kong, D., Li, S., Jin, Y., Wu, S., Chen, J., Hu, T., Wang, H., Liu, S., and Zou, J. (2019). Linking methane emissions to methanogenic and methanotrophic communities under different fertilization strategies in rice paddies. *Geoderma* 347, 233–243. <https://doi.org/10.1016/j.geoderma.2019.04.008>.
 25. Alpana, S., Vishwakarma, P., Adhya, T.K., Inubushi, K., and Dubey, S.K. (2017). Molecular ecological perspective of methanogenic archaeal community in rice agroecosystem. *Sci. Total Environ.* 596–597, 136–146. <https://doi.org/10.1016/j.scitotenv.2017.04.011>.
 26. Conrad, R., Erkel, C., and Liesack, W. (2006). Rice Cluster I methanogens, an important group of Archaea producing greenhouse gas in soil. *Curr. Opin. Biotechnol.* 17, 262–267. <https://doi.org/10.1016/j.copbio.2006.04.002>.
 27. Gao, D., Sheng, R., Whiteley, A.S., Moreira-Grez, B., Qin, H., Zhang, W., Zhan, Y., and Wei, W. (2020). Effect of phosphorus amendments on rice rhizospheric methanogens and methanotrophs in a phosphorus deficient soil. *Geoderma* 368, 114312. <https://doi.org/10.1016/j.geoderma.2020.114312>.
 28. Shiau, Y.-J., Cai, Y., Jia, Z., Chen, C.-L., and Chiu, C.-Y. (2018). Phylogenetically distinct methanotrophs modulate methane oxidation in rice paddies across Taiwan. *Soil Biol. Biochem.* 124, 59–69. <https://doi.org/10.1016/j.soilbio.2018.05.025>.
 29. Ho, A., Kerckhof, F.M., Luke, C., Reim, A., Krause, S., Boon, N., and Bodelier, P.L.E. (2013). Conceptualizing functional traits and ecological characteristics of methane-oxidizing bacteria as life strategies. *Environ. Microbiol. Rep.* 5, 335–345. <https://doi.org/10.1111/j.1758-2229.2012.00370.x>.
 30. Mayumi, D., Yoshimoto, T., Uchiyama, H., Nomura, N., and Nakajima-Kambe, T. (2010). Seasonal change in methanotrophic diversity and populations in a rice field soil assessed by DNA-stable isotope probing and quantitative real-time PCR. *Microbes Environ.* 25, 156–163. <https://doi.org/10.1264/jsm2.me10120>.
 31. Wang, L., Ge, J., Feng, L., Liu, Y., Li, Y., Wang, J., Xiao, X., and Zhang, Z. (2022). The synergism between methanogens and methanotrophs and the nature of their contributions to the seasonal variation of methane fluxes in a wetland: the case of dajiuhe subalpine peatland. *Adv. Atmos. Sci.* 39, 1375–1385. <https://doi.org/10.1007/s00376-021-1255-z>.
 32. Shi, S., Nuccio, E.E., Shi, Z.J., He, Z., Zhou, J., and Firestone, M.K. (2016). The interconnected rhizosphere: high network complexity dominates rhizosphere assemblages. *Ecol. Lett.* 19, 926–936. <https://doi.org/10.1111/ele.12630>.
 33. Zhou, J., Deng, Y., Zhang, P., Xue, K., Liang, Y., Van Nostrand, J.D., Yang, Y., He, Z., Wu, L., Stahl, D.A., et al. (2014). Stochasticity, succession, and environmental perturbations in a fluidic ecosystem. *Proc. Natl. Acad. Sci. USA* 111, E836–E845. <https://doi.org/10.1073/pnas.1324044111>.
 34. Bai, R., Wang, J.-T., Deng, Y., He, J.-Z., Feng, K., and Zhang, L.-M. (2017). Microbial community and functional structure significantly varied among distinct types of paddy soils but responded differently along gradients of soil depth layers. *Front. Microbiol.* 8, 945. <https://doi.org/10.3389/fmicb.2017.00945>.
 35. Tu, Q., Yan, Q., Deng, Y., Michaletz, S.T., Buzzard, V., Weiser, M.D., Waide, R., Ning, D., Wu, L., He, Z., and Zhou, J. (2020). Biogeographic patterns of microbial co-occurrence ecological networks in six American forests. *Soil Biol. Biochem.* 148, 107897. <https://doi.org/10.1016/j.soilbio.2020.107897>.
 36. Gaston, K.J. (2000). Global patterns in biodiversity. *Nature* 405, 220–227. <https://doi.org/10.1038/35012228>.
 37. Battin, T.J., Butturini, A., and Sabater, F. (1999). Immobilization and metabolism of dissolved organic carbon by natural sediment biofilms in a Mediterranean and temperate stream. *Aquat. Microb. Ecol.* 19, 297–305. <https://doi.org/10.3354/ame019297>.
 38. Gilan, I., and Sivan, A. (2013). Effect of proteases on biofilm formation of the plastic-degrading actinomycete *Rhodococcus ruber* C208. *FEMS Microbiol. Lett.* 342, 18–23. <https://doi.org/10.1111/1574-6968.12114>.

39. McKew, B.A., Dumbrell, A.J., Taylor, J.D., McGenity, T.J., and Underwood, G.J.C. (2013). Differences between aerobic and anaerobic degradation of microphytobenthic biofilm-derived organic matter within intertidal sediments. *FEMS Microbiol. Ecol.* **84**, 495–509. <https://doi.org/10.1111/1574-6941.12077>.
40. Luan, L., Liang, C., Chen, L., Wang, H., Xu, Q., Jiang, Y., and Sun, B. (2020). Coupling bacterial community assembly to microbial metabolism across soil profiles. *mSystems* **5**, e00298-20. <https://doi.org/10.1128/mSystems.00298-20>.
41. Lee, H.J., Kim, S.Y., Kim, P.J., Madsen, E.L., and Jeon, C.O. (2014). Methane emission and dynamics of methanotrophic and methanogenic communities in a flooded rice field ecosystem. *FEMS Microbiol. Ecol.* **88**, 195–212. <https://doi.org/10.1111/1574-6941.12282>.
42. Medvedkova, K.A., Khmelenina, V.N., Suzina, N.E., and Trotsenko, Y.A. (2009). Antioxidant systems of moderately thermophilic methanotrophs *Methylocaldum szegediense* and *Methylococcus capsulatus*. *Microbiology* **78**, 670–677. <https://doi.org/10.1134/S0026261709060022>.
43. Sherry, A., Osborne, K.A., Sidgwick, F.R., Gray, N.D., and Talbot, H.M. (2016). A temperate river estuary is a sink for methanotrophs adapted to extremes of pH, temperature and salinity. *Environ. Microbiol. Rep.* **8**, 122–131. <https://doi.org/10.1111/1758-2229.12359>.
44. Conrad, R., Klose, M., and Noll, M. (2009). Functional and structural response of the methanogenic microbial community in rice field soil to temperature change. *Environ. Microbiol.* **11**, 1844–1853. <https://doi.org/10.1111/j.1462-2920.2009.01909.x>.
45. Shrestha, P.M., Kammann, C., Lenhart, K., Dam, B., and Liesack, W. (2012). Linking activity, composition and seasonal dynamics of atmospheric methane oxidizers in a meadow soil. *ISME J.* **6**, 1115–1126. <https://doi.org/10.1038/ismej.2011.179>.
46. Liu, J., Zang, H., Xu, H., Zhang, K., Jiang, Y., Hu, Y., and Zeng, Z. (2019). Methane emission and soil microbial communities in early rice paddy as influenced by urea-N fertilization. *Plant Soil* **445**, 85–100. <https://doi.org/10.1007/s11104-019-04091-0>.
47. Arrigo, K.R. (2014). Sea ice ecosystems. *Ann. Rev. Mar. Sci.* **6**, 439–467. <https://doi.org/10.1146/annurev-marine-010213-135103>.
48. Nichols, C.A.M., Guezennec, J., and Bowman, J.P. (2005). Bacterial exopolysaccharides from extreme marine environments with special consideration of the southern ocean, sea ice, and deep-sea hydrothermal vents: a review. *Mar. Biotechnol.* **7**, 253–271. <https://doi.org/10.1007/s10126-004-5118-2>.
49. Kenney, G.E., Sadek, M., and Rosenzweig, A.C. (2016). Copper-responsive gene expression in the methanotroph *Methylosinus trichosporium* OB3b. *Metallomics* **8**, 931–940. <https://doi.org/10.1039/c5mt00289c>.
50. Chidambarampadmavathy, K., Obulisamy, P.K., and Heimann, K. (2015). Role of copper and iron in methane oxidation and bacterial biopolymer accumulation. *Eng. Life Sci.* **15**, 387–399. <https://doi.org/10.1002/elsc.201400127>.
51. Hu, H.-W., Zhang, L.-M., Yuan, C.-L., and He, J.-Z. (2013). Contrasting Euryarchaeota communities between upland and paddy soils exhibited similar pH-impacted biogeographic patterns. *Soil Biol. Biochem.* **64**, 18–27. <https://doi.org/10.1016/j.soilbio.2013.04.003>.
52. Aronson, E.L., Dubinsky, E.A., and Helleker, B.R. (2013). Effects of nitrogen addition on soil microbial diversity and methane cycling capacity depend on drainage conditions in a pine forest soil. *Soil Biol. Biochem.* **62**, 119–128. <https://doi.org/10.1016/j.soilbio.2013.03.005>.
53. Webster, R. (2008). *Soil Sampling and Methods of Analysis* (CRC Press).
54. Lu, R. (2000). *Analytical Methods of Soil Agrochemistry (In Chinese)* (Chinese Agricultural Science and Technology Press).
55. Hernández, M., Klose, M., Claus, P., Bastviken, D., Marotta, H., Figueiredo, V., Enrich-Prast, A., and Conrad, R. (2019). Structure, function and resilience to desiccation of methanogenic microbial communities in temporarily inundated soils of the Amazon rainforest (Cunia Reserve, Rondonia). *Environ. Microbiol.* **21**, 1702–1717. <https://doi.org/10.1111/1462-2920.14535>.
56. Wang, S., Ma, L., Xu, Y., Wang, Y., Zhu, N., Liu, J., Dolfig, J., Kerr, P., and Wu, Y. (2020). The unexpected concentration-dependent response of periphytic biofilm during indole acetic acid removal. *Bioresour. Technol.* **303**, 122922. <https://doi.org/10.1016/j.biortech.2020.122922>.

STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Critical commercial assays		
Power Soil DNA Isolation Kit	Mo Bio	12888-100
Deposited data		
Raw Data for 16s rRNA sequencing	This study	NCBI: RJNA854289

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Yonghong Wu (yhwu@issas.ac.cn).

Materials availability

This study did not generate new unique materials.

Data and code availability

The raw sequences data have been deposited at the NCBI and are publicly available as of the date of publication. Accession numbers are listed in the [key resources table](#). This paper does not report original code. Any additional information required to reanalyze the data reported in this paper is available from the [lead contact](#) upon request.

METHOD DETAILS

In-situ sample collection

A total of 66 sampling sites in 22 counties ([Figure S1](#)) were selected in the main rice planting regions of China; the sites were divided into four groups according to their locations (Northeast, Central, East and South China). The environmental characteristics of the four groups of sampling sites were shown in [Table S2](#). In each county, three sampling sites were randomly chosen within an area of 10 km², and the PB, bulk soil and floodwater were collected from each site at ~ the 20th day after rice transplantation (tillering stage) in 2019. Specifically, fresh PB with adherent soil particles was peeled off from soil surface by a razor knife, and ~200 g underlying soil (0-5 cm) was collected by shovels. The PB and bulk soil samples were sealed in sampling bags. 100-mL plastic bottles were filled with floodwater. Then the PB, soil and water samples were kept on ice and immediately transported to the laboratory for analyses.

Chemical analyses

About 100g of each soil sample was air dried, grinded, and then the chemical analyses were carried out according to standard methods.⁵³ After grinding, passing through a 0.149-mm sieve, and removing the carbonate by adding 0.1 M hydrochloric acid (HCl), the SOC content of the soil samples was determined by an elemental analyzer (Vario MAX CN, Elementar, Germany). The STN contents were measured using the elemental analyzer without pretreatment with HCl. The total Mg, Fe and Mn contents were analyzed using an inductively coupled plasma optical emission spectrometer (Avio 200 ICP-OES, PerkinElmer, USA) after acid digestion according to Lu's method.⁵⁴ The pH of floodwater was measured by a Seven Excellence™ digital pH meter (Mettler-Toledo, Switzerland) after filtration. The EPS was extracted from fresh PB samples. Specifically, 1 g PB (dry weight, by converting fresh weight and moisture content) was re-suspended in 2 mL 2M-NaOH solution, diluted to 10 mL, shaken (20°C, 120 rpm, 2.5 h), and centrifuged for 20 min (4°C, 10000 rpm). Then the supernatant was filtered by a filter membrane (0.45 μm), and the filtrate was mixed with ethanol (v/v = 1:4), placed (4°C, 12 h), centrifuged again for 10 min (10000 rpm). The supernatant was discarded and the precipitate obtained was the EPS. Protein content in EPS was measured using the Coomassie brilliant blue staining method, and polysaccharose content was estimated using the Anthrone method.

Molecular analyses

PCR amplification and sequencing were carried out to quantify the microbial composition of soil and PB. After the PB and soil samples were freeze dried, DNA was extracted from 0.1 g PB and 0.25 g soil, respectively, using a DNA isolation kit (Power Soil DNA Isolation Kit, MoBio, USA). The primer pair of 515F/806R (5'-GTGCCAGCMGCCGCGGTAA-3' and, 5'-GGACTACVSGGGTATCTAAT-3') targeting the V4-V5 region of the 16S rRNA gene were used for amplification.⁵⁵ The PCR products were assessed by electrophoresis on a 1% agarose gel, purified using a gel extraction kit (EZNA Gel Extraction Kit, Omega, USA), and then sequenced on an Illumina HiSeq 2500 platform. The QIIME 1.6.0 pipeline software was used to analyze the sequences. Sequences with similarity $\geq 97\%$ were assigned to the same operational taxonomic units (OTUs) by the Uparse software.

Meteorological data collection

Meteorological data including ASD, MAT, MMT, MAP and MAH of sampling sites were collected from the website of China National Meteorological Data Services Center (<http://data.cma.cn/site/index.html>).

QUANTIFICATION AND STATISTICAL ANALYSIS

The relative abundances of methanogens and methanotrophs at the genus level were counted based on taxonomy of methanogens and methanotrophs.²² Significance differences (p -value) between treatments was tested by one-way ANOVA analysis using the SPSS statistics software v22.0 (International Business Machines Corp., Armonk, USA). The Spearman's correlation coefficients between the relative abundance of methanotrophs and methanogens and environmental variables were calculated by the SPSS statistics software. The Chao 1 richness and Shannon diversity of methanogenic and methanotrophic communities were calculated according to the method described before.⁵⁶ Generic co-occurrence patterns were calculated by R software and visualized on the Gephi software (<http://gephi.org>). Co-occurrence was defined as a correlation between genera that was statistically significant ($p < 0.05$). The effect of environmental variables on microbial relative abundances was visualized by RDA by R software using the "vegan" package. The PLS-PM was constructed also in R using the "plsrm" package. Specifically, an empirical model was established based on the Pearson's correlation analysis. Then the significantly correlated variables were put into the empirical model to calculate the standardized path coefficients among these variables. The latent variable Climate represents MAT, MAP and MMT/ASD, Soil metal represents soil total Mg, Fe and Mn contents, and EPS represents polysaccharide and protein contents of PB. Observable variables PB methanogens and PB methanotrophs are the relative abundance of these communities. The structure of the empirical model was adjusted using the best goodness of fit (GOF) statistic. Sample numbers (n) and the significance (p) were indicated in the figure legends. The significance is indicated by asterisk: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.