HAL open science

Microbial community structure is stratified at the millimeter-scale across the soil–water interface

Yu-Jia Cai, Zi-Ao Liu, Sha Zhang, Hao Liu, Graeme W. Nicol, Zheng Chen

To cite this version:

Yu-Jia Cai, Zi-Ao Liu, Sha Zhang, Hao Liu, Graeme W. Nicol, et al.. Microbial community structure is stratified at the millimeter-scale across the soil–water interface. ISME Communications, 2022, 2 (1), pp.53. 10.1038/s43705-022-00138-z . hal-03721059

HAL Id: hal-03721059

https://hal.science/hal-03721059

Submitted on 3 Dec 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Distributed under a Creative Commons Attribution 4.0 International License

ISME

www.nature.com/ismecomms

BRIEF COMMUNICATION OPEN

Microbial community structure is stratified at the millimeter-scale across the soil–water interface

Yu-Jia Cai[1,2], Zi-Ao Liu[1], Sha Zhang[1,2], Hao Liu[1,2], Graeme W. Nicol[3] and Zheng Chen[1]✉

© The Author(s) 2022

Soil–water interfaces (SWI) are biogeochemical hotspots characterized by millimeter-scale redox gradients, indicating that parallel changes are also present in microbial community structure and activity. However, soil-based analyses of microbial community structure typically examine bulk samples and seldom consider variation at a scale relevant to changes in environmental conditions. Here we presented a study that aimed to describe millimeter-scale variance in both microbial community structure and physicochemical properties in a lab flooded soil. At this fine-scale resolution, the stratification of biogeochemical properties (e.g., redox potential, nitrate concentration) was consistent with the structure of the active microbial community with clear shifts in the relative abundance of transcriptionally active populations associated with changing redox conditions. Our results demonstrate that spatial scale should be carefully considered when investigating ecological mechanisms that influence soil microbial community structures.

ISME Communications; https://doi.org/10.1038/s43705-022-00138-z

The soil–water interface (SWI) is a biogeochemical hotspot where aerobic microbes are enriched at the top and respire oxygen from the surface water, with microbes capable of anaerobic respiration residing toward the bottom [1]. Physicochemical gradients across the SWI create a range of niches for the dwelling microbes [2] and therefore make the SWI an ideal model system to investigate links between microbial community structure and ecosystem processes. However, the microbial community structure across the SWI remains poorly understood.

Environmental filtering, i.e., the shaping of soil microbial community structure by abiotic factors, is considered an important factor influencing microbial community assembly in highly heterogeneous environments [3, 4]. Although the physicochemical gradients across the SWI usually occur at the µm or mm scale, a stratified microbial community is rarely observed, largely limited by the sampling approaches and analytical methods used. In many cases, the interface is sliced into ~1 cm layer to obtain sufficient material for microbiome analysis [5, 6], but these layers are typically too thick to allow determination of physicochemical gradients with any precision. We, therefore, propose that typical centimeter-scale or bulk sampling hinders attempts to adequately characterize patterns of microbial community structure across the SWI. Furthermore, considering that not all microbes are active in any given environment, microbial community structure profiled by the analysis of genomic DNA does not necessarily reflect the active microbes or community assembly processes [7, 8]. We hypothesized that stratification of active microbial community structures would be observed across the SWI if sampled and analyzed appropriately i.e., at the mm-scale using an RNA-based approach.

We conducted a soil incubation experiment for mm-scale sampling (Fig. S1) using analysis of rRNA content to profile the metabolically active members of the microbial community. Specifically, soil was flooded with sterilized deionized water and incubated for 20 days at 25 °C in the dark before a 50 mm soil core was taken and sampled at 2 mm intervals with prokaryote communities profiled using amplicons derived from reverse-transcribed 16S rRNA (detailed in Supplementary Information). After processing and taxonomic assignment of sequences, distance decay relationships (DDR) describing the similarity in community structures over distance were performed [9, 10]. We detected a strong DDR with the bacterial community similarity (1- Canberra dissimilarity) [9] significantly decreasing with distance through the SWI (Mantel $r = 0.547$, $p < 0.001$) (Fig. 1A). While DDR has been extensively used in microbiome research, patterns vary depending on the sampling scale. Microbial DDR patterns are assumed weak due to the high abundance, short life cycle, and dispersal abilities of microbes [9, 11]. In this study, we observed a DDR that was stronger than that of larger sampling scales (e.g., m or km) [9], but comparable to the cm scale [10]. This demonstrates that the spatial organization of soil microbes with relatively high community dissimilarity can be observed with appropriate spatial scales of analysis [9, 12, 13].

Distinct patterns of active bacterial communities were observed at the phylum level across the SWI (Fig. 1B). For example, the relative abundance of rRNA reads from the *Betaproteobacteriales* within the *Gammaproteobacteria* class (a modification in the SILVA 132 database) decreased greatly (27-fold difference at the extremes of the SWI), while *Bacteroidetes* increased with soil depth (13-fold difference at the extremes). We then examined the active bacterial community composition at the family and genus level for a higher taxonomic

[1]Department of Health and Environmental Sciences, Xi'an Jiaotong-Liverpool University, 111 Ren'ai Road, Suzhou, Jiangsu 215123, China. [2]Department of Geography & Planning, School of Environmental Sciences, University of Liverpool, Brownlow Hill, Liverpool L697ZX, UK. [3]Univ Lyon, CNRS, INSA Lyon, Université Claude Bernard Lyon 1, Ecole Centrale de Lyon, Ampère, UMR5005, 69134 Ecully cedex, France. ✉email: Zheng.Chen@xjtlu.edu.cn

Received: 6 March 2022 Revised: 25 May 2022 Accepted: 14 June 2022
Published online: 30 June 2022

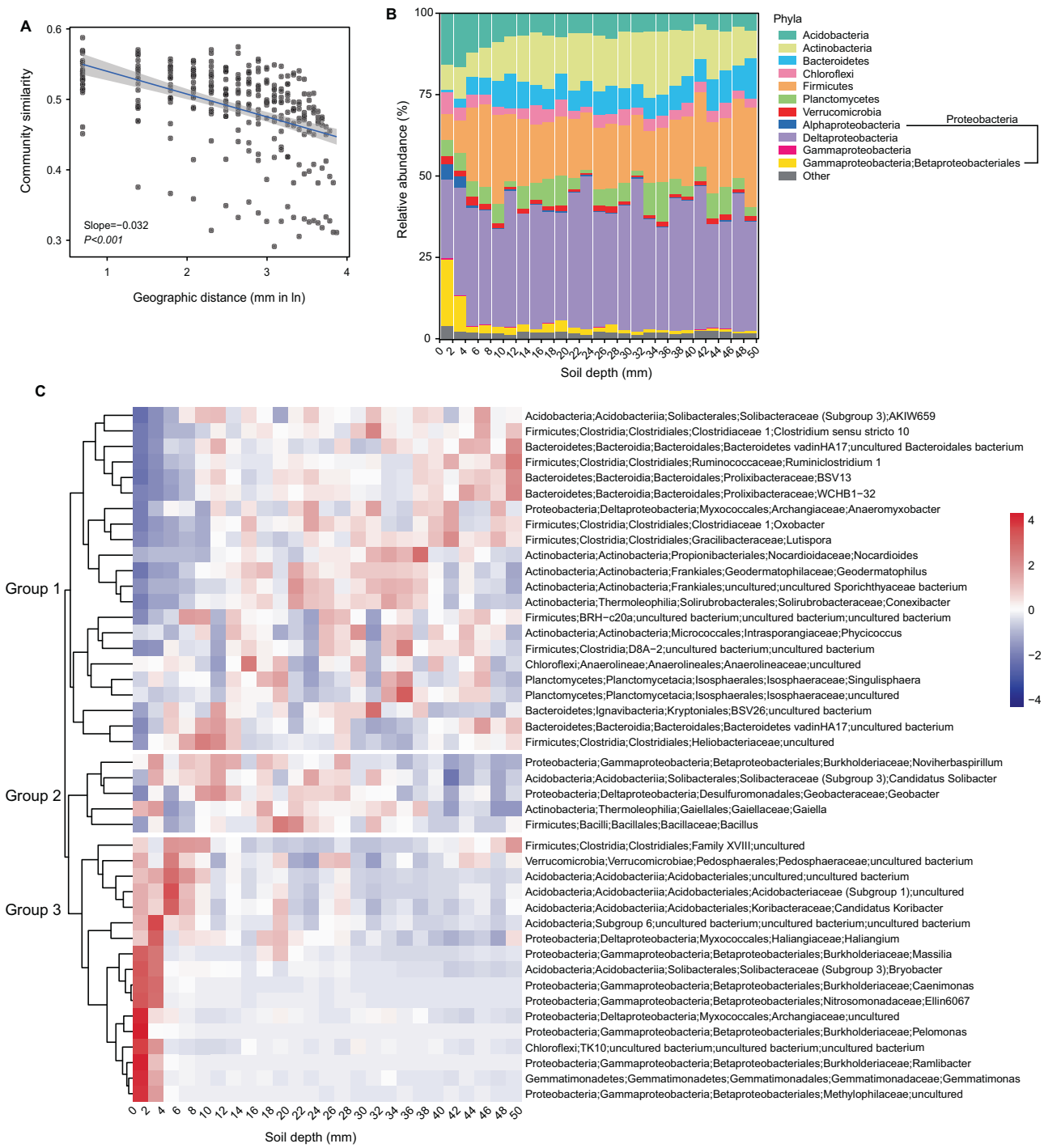

Fig. 1 Community composition of the active microbial communities based on relative abundance of 16S rRNA gene transcripts through a 50 mm water–soil interface. A Changes in Bray-Curtis similarities of microbial communities with spatial distance. A linear trend was determined using ordinary least-squares linear regression with the shaded area representing 95% confidence intervals. **B** Relative abundance of phyla. Only those with a relative abundance ≥1% were included, while those <1% categorized as "Other". **C** Heatmap showing the relative abundance difference of the selected 44 bacterial genera (relative abundance ≥1%). An increase in abundance tends toward red, while a decrease tends toward blue.

resolution (Figs. S2 and 1C). Sequences representing 44 bacterial genera (each with a relative abundance ≥1%) accounted for 77% of all total sequence and were clustered into three different groups reflecting different distribution patterns (Fig. 1C). Bacterial genera within group 1 generally had their lowest relative abundance of 16S rRNA transcripts in the top 0–6 mm and were dominated by strict or facultative anaerobic bacteria such as representatives of the *Ruminococcaceae* family, *Anaeromyxobacter* and *Oxobacter* genera. Bacteria in group 2 were more active from 2 to 40 mm, generally showed low relative activity only at the deepest layers of 40–50 mm and consisted of aerobic and facultative anaerobic bacteria including *Bacillus* and *Gaiella* representatives. In contrast to groups 1 and 2, bacteria in group 3 exhibited a clear decrease in their relative abundance from the surface to 50 mm in the flooded soil profile. Most of the bacteria in this group had the highest relative abundance of 16S rRNA transcripts in the first 4 mm topsoil layer, indicating a

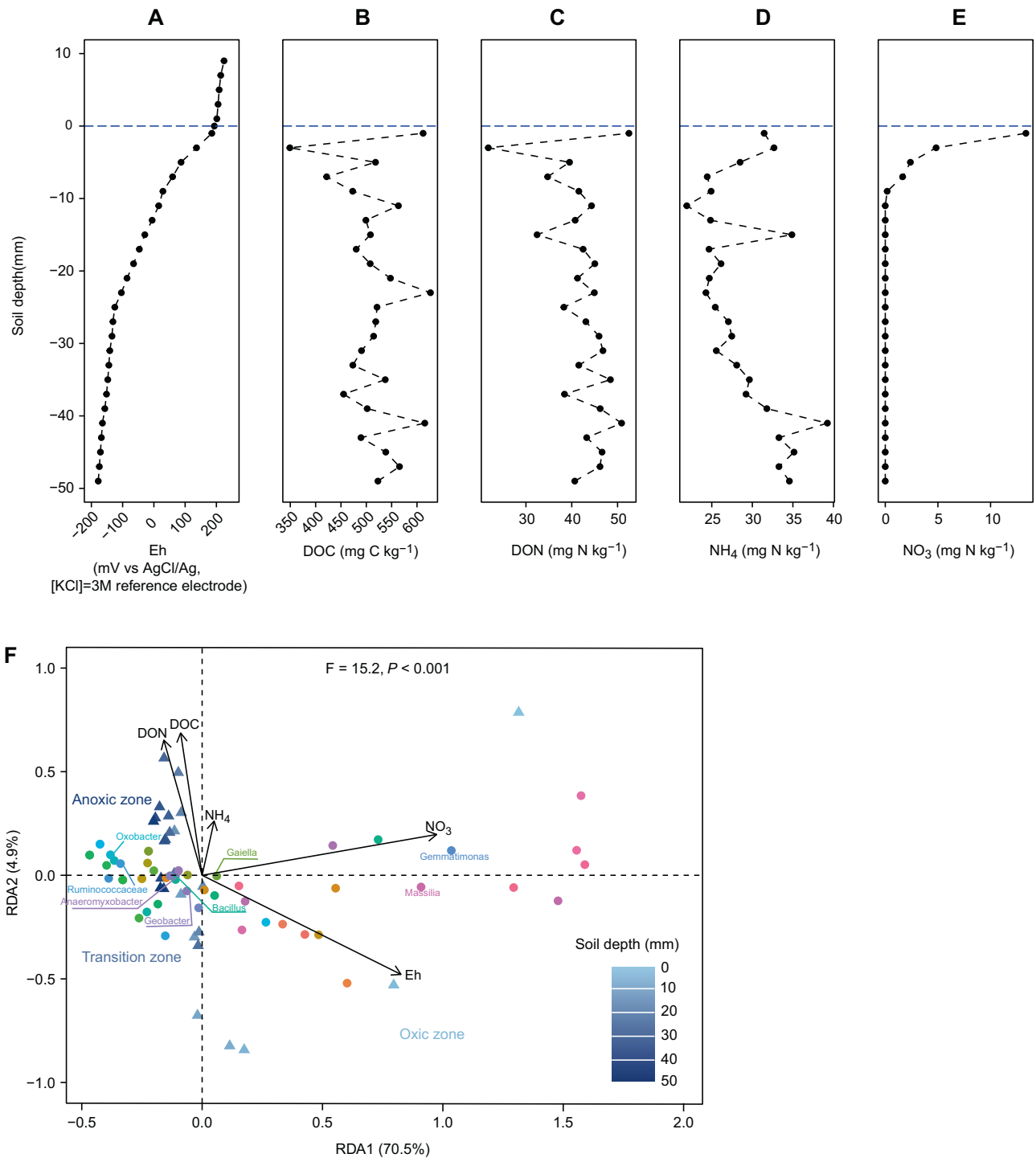

Fig. 2 Depth profiles of soil physicochemical properties and microbial community structure through the SWI depth profile. A Redox potential. **B** Dissolved organic carbon (DOC). **C** Dissolved organic nitrogen (DON). **D** Ammonium-N (NH_4-N). **E** Nitrate-N (NO_3-N). **F** Distance based-redundancy analysis (dbRDA) triplot based on the selected 44 bacterial genera (relative abundance ≥1%) across the SWI. Triangles, bacterial genera; circles, soil samples across SWI; arrows, environmental factors.

cluster of strictly aerobic organisms including representatives of the genera *Massilia* and *Gemmatimonas*.

We measured a range of soil physicochemical properties (Fig. 2A–E) and performed tbRDA analysis based on the selected 44 bacterial genera (Fig. 2F). The measured physicochemical properties correlated with 80.0% of the variance of the selected taxa (Fig. 2F). The main correlating factors were redox potential ($F = 29.6$, $P < 0.001$) and nitrate concentration ($F = 50.3$, $P < 0.001$). Consistent with the distribution of bacteria across the SWI (Figs. 1C and 2), variance in redox potential could be divided into oxic, transition, and anoxic zones (Fig. 2A). The vertical stratification of the microbial community was primarily driven by the availability of oxygen rather than that of other common electron acceptors, as generally observed in lake and marine sediments [6, 14–16].

Microbial communities were functionally annotated using FAPROTAX [17]. While the prediction of metabolic attributes from 16 S rRNA

sequences must be interpreted with caution, the results of functional group distribution were nevertheless consistent with the gradient of biogeochemical processes across the SWI. For example, transcripts associated with organisms performing oxygen-dependent nitrification processes of ammonia and nitrite oxidation were most abundant within the first 4 mm and decreased as soil depth increased (Fig. S3a). This decrease was consistent with the nitrate profile in which nitrate was not detectable below 8 mm (Fig. 2E). Similarly, the abundance of transcripts associated with organisms performing aerobic methanol oxidation and methylotrophy decreased rapidly as soil depth increased (Fig. S3). The methanotrophic populations demonstrated high activity at oxic–anoxic interface [18, 19], and was consistent with the changes in the transcript number of particulate methane monooxygenase sub-unit A (*pmoA*) gene transcripts, a functional marker for methane-oxidizing bacteria) (Fig. S3b). Other metabolic activities such as iron respiration and fermentation were mainly distributed in the sub-oxic areas as these metabolic processes are performed in the absence of oxygen.

In summary, a stratified active microbial community could be observed through the SWI at the mm-scale. These findings could direct future biogeochemical research and identification of corresponding microbial populations across the SWI as these processes occur at very narrow zones and further affect large-scale ecosystem responses (e.g., methane fluxes) [1, 20]. "Who is doing what" remains a major challenge in microbial ecology. We believe the isolation of the specific strains corresponding to a targeted process would be more successful if the inoculum was sampled from hot spots of activity identified via a high resolution, mm-scale profiling of the microbial community. The method could further be applied to study microbial responses to environmental stress (e.g., pollutants, toxics) and allows characterization and enrichment of uncultivated strains with previously unknown metabolic functions by creating artificial gradients. We conclude that the analysis of microbial communities at an appropriate spatial scale should facilitate the identification of the contributors to specific functions processed in microbiome research.

REFERENCES

1. McClain ME, Boyer EW, Dent CL, Gergel SE, Grimm NB, Groffman PM, et al. Biogeochemical hot spots and hot moments at the interface of terrestrial and aquatic ecosystems. Ecosystems. 2003;6:301–12.
2. Borch T, Kretzschmar R, Kappler A, Van Cappellen P, Ginder-Vogel M, Voegelin A, et al. Biogeochemical redox processes and their impact on contaminant dynamics. Environ Sci Technol. 2010;44:15–23.
3. Stegen JC, Lin XJ, Konopka AE, Fredrickson JK. Stochastic and deterministic assembly processes in subsurface microbial communities. ISME J. 2012;6:1653–64.
4. Dini-Andreote F, Stegen JC, van Elsas JD, Salles JF. Disentangling mechanisms that mediate the balance between stochastic and deterministic processes in microbial succession. Proc Natl Acad Sci USA. 2015;112:E1326–32.
5. Behrendt L, Larkum AWD, Trampe E, Norman A, Sorensen SJ, Kuhl M. Microbial diversity of biofilm communities in microniches associated with the didemnid ascidian *Lissoclinum patella*. ISME J. 2012;6:1222–37.
6. Becker KW, Elling FJ, Schroder JM, Lipp JS, Goldhammer T, Zabel M, et al. Isoprenoid quinones resolve the stratification of redox processes in a biogeochemical continuum from the photic zone to deep anoxic sediments of the Black Sea. Appl Environ Microbiol. 2018;84:e02736–17.
7. Locey KJ, Muscarella ME, Larsen ML, Bray SR, Jones SE, Lennon JT. Dormancy dampens the microbial distance-decay relationship. Phil Trans R Soc B. 2020;375:20190243.
8. Blagodatskaya E, Kuzyakov Y. Active microorganisms in soil: critical review of estimation criteria and approaches. Soil Biol Biochem. 2013;67:192–211.
9. Meyer KM, Memiaghe H, Korte L, Kenfack D, Alonso A, Bohannan BJM. Why do microbes exhibit weak biogeographic patterns? ISME J. 2018;12:1404–13.
10. Xue R, Zhao KK, Yu XL, Stirling E, Liu S, Ye SD, et al. Deciphering sample size effect on microbial biogeographic patterns and community assembly processes at centimeter scale. Soil Biol Biochem. 2021;156:108218.
11. Morriss A, Meyer K, Bohannan B. Linking microbial communities to ecosystem functions: what we can learn from genotype-phenotype mapping in organisms. Phil Trans R Soc B. 2020;375:20190244.
12. Armitage DW, Jones SE. How sample heterogeneity can obscure the signal of microbial interactions. ISME J. 2019;13:2639–46.
13. Dini-Andreote F, Kowalchuk GA, Prosser JI, Raaijmakers JM. Towards meaningful scales in ecosystem microbiome research. Environ Microbiol. 2021;23:1–4.
14. Meyerhof MS, Wilson JM, Dawson MN, Beman JM. Microbial community diversity, structure and assembly across oxygen gradients in meromictic marine lakes, Palau. Environ Microbiol. 2016;18:4907–19.
15. Zhou ZC, Meng H, Liu Y, Gu JD, Li M. Stratified bacterial and archaeal community in mangrove and intertidal wetland mudflats revealed by high throughput 16S rRNA gene sequencing. Front Microbiol. 2017;8:02148.
16. Gutierrez-Preciado A, Saghai A, Moreira D, Zivanovic Y, Deschamps P, Lopez-Garcia P. Functional shifts in microbial mats recapitulate early Earth metabolic transitions. Nat Ecol Evol. 2018;2:1700–8.
17. Louca S, Parfrey LW, Doebeli M. Decoupling function and taxonomy in the global ocean microbiome. Science. 2016;353:1272–7.
18. Murase J, Frenzel P. A methane-driven microbial food web in a wetland rice soil. Environ Microbiol. 2007;9:3025–34.
19. Reim A, Lüke C, Krause S, Pratscher J, Frenzel P. One millimetre makes the difference: high-resolution analysis of methane-oxidizing bacteria and their specific activity at the oxic-anoxic interface in a flooded paddy soil. ISME J. 2012;6:2128–39.
20. Peiffer S, Kappler A, Haderlein SB, Schmidt C, Byrne JM, Kleindienst S, et al. A biogeochemical–hydrological framework for the role of redox-active compounds in aquatic systems. Nat Geosci. 2021;14:264–72.

ACKNOWLEDGEMENTS

This work was supported by the National Natural Science Foundation of China (No. 41977320). We thank Prof. John Boyle (School of Environmental Sciences, University of Liverpool, Liverpool, United Kingdom) and Dr. Xue-Ping Chen (School of Environmental and Chemical Engineering, Shanghai University, Shanghai, China) for the valuable comments on this paper. We also thank our lab technicians Ms. Xiao-Ping Xie, Ms. Wen Jiang, and Ms. Xiao Zhou for their assistance in the lab.

AUTHOR CONTRIBUTIONS

Y-JC, Z-AL, and ZC designed the research. Y-JC and Z-AL conducted the experiments and carried out all the chemical and biological analyses with the help of SZ and HL. Y-JC wrote the paper with critical reviews and comments from SZ, GWN, and ZC.

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s43705-022-00138-z.

Correspondence and requests for materials should be addressed to Zheng Chen.

Reprints and permission information is available at http://www.nature.com/reprints

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.

© The Author(s) 2022