

<https://doi.org/10.1038/s41522-025-00711-z>

Increasing drying changes the relationship between biodiversity and ecosystem multifunctionality



Chaoran Li¹, Jun Hou¹, Ming Kong², Yu Yao³, Tanveer M. Adyel⁴, Jun Wu¹, Guoxiang You¹, Yue Yu⁵, Songqi Liu⁶, Zijun Yang¹ & Lingzhan Miao¹ ✉

Increased drying of rivers under global climate change is leading to biodiversity loss. However, it is not clear whether biodiversity loss affects river functions. In this study, we investigated the changes in biofilm community diversity and functions in an artificial stream after different drying durations. A critical drying duration of around 60 days was found in the microbial composition and functions. Therefore, different drying durations can be divided into short-term drying (~0–20 days) and long-term drying (~60–130 days) to analyse the effect of biodiversity in terms of ecosystem functions. In summary, the dominant relationship of biodiversity on community stability got uncoupled after long-term drying. Community assembly became dominant in maintaining multifunctionality with increasing drying duration rather than biodiversity as traditionally perceived. This study reveals the importance of community assembly, extending theoretical knowledge of the relationship between biodiversity and ecosystem multifunctionality.

Extreme events (e.g. drying) induced by climate warming and human activities significantly affected river ecosystems and caused degradation of ecosystem functions^{1,2}. River disconnection and drying are becoming more frequent and severe under the influence of extreme drying, such as intermittent rivers and ephemeral streams (IRES) which experience intermittent dryings, and this alteration in river hydrology and resource availability significantly affects the entire benthic ecosystem^{3,4}. As a crucial ecological indicator in river ecosystems, benthic biofilm is paramount in unravelling the biodiversity and ecosystem functions⁵. In recently, Maestre, et al.⁶ found that plant function was positively and significantly related to species richness in drylands, and this relationship became progressively stronger and more positive as environmental stress increased⁷. However, Alvarez, et al.⁸ observed a decoupling of α -diversity from ecosystem function after environmental disturbances in a river ecosystem, possibly due to the community instability caused by rapid biotic turnover during the recovery period. So β -diversity and microbial network structure should be considered to completely analyse biodiversity, which may provide deeper insights than focusing on a single alpha diversity in isolation^{9,10}. Therefore, in response to various

environmental pressures, exploring the relationship between biodiversity and ecosystem function (BEF) has become a priority for ecologists^{11–13}.

In addition, actual ecological communities are undoubtedly governed by rules such as niche-based theory and the neutral theory, and different community assembly mechanisms were demonstrated to also significantly affect ecosystem functions^{14,15}. Based on the niche-based theory and the neutral theory^{16,17}, ecologists broadly accepted that both stochastic ('neutral') and deterministic processes influence community assembly simultaneously¹⁸, which includes five driving mechanisms: homogeneous selection, homogenising dispersal, undominated, dispersal limitation, and heterogeneous selection^{19,20}. Consequently, exploring the determinism and stochasticity in the community assembly process is critical to comprehending the effect of community assembly to ecosystem functions^{12,21}. Ecosystems are highly valued for supporting various ecological functions, yet most research focuses on single processes in isolation. Therefore, the relationship between biodiversity and ecosystem multifunctionality (B-EMf) deserves more research attention^{22–24}. Further to this, microbial community stability should be focus on since the

¹Key Laboratory of Integrated Regulation and Resources Development on Shallow Lakes, Ministry of Education, College of Environment, Hohai University, Nanjing, 210098, People's Republic of China. ²Nanjing Institute of Environmental Sciences, Ministry of Ecology and Environment, Nanjing, 210042, People's Republic of China. ³School of Environment, Nanjing Normal University, Nanjing, 210023, China. ⁴Centre for Nature Positive Solutions, School of Science, RMIT University, Melbourne, VIC, 3000, Australia. ⁵Department of Civil, Environmental, and Geomatic Engineering, ETH Zürich, Zürich, 8092, Switzerland. ⁶School of Environmental Science and Engineering, Suzhou University of Science and Technology, Suzhou, 215009, People's Republic of China. ✉e-mail: lzmiao@hhu.edu.cn; mlz1988@126.com

sustainability of both the multifunctionality and biodiversity rendered by the benthic ecosystem are dependent on a relatively stable microbiome²⁵.

The state of ecosystems during rewetting is more important for investigating the ecological effects of intermittent rivers than focusing on the state during drying. Considering that complex interactions of biofilm communities and multifunctionality can be clearly analysed in controlled environments, experiments were carried out using artificially modified rivers which rewetted after different drying durations. Metabolic functions related to elements such as carbon, nitrogen and phosphorus were considered in the calculation of biofilm multifunctionality, based on their necessity for ecosystem maintenance and energy flow^{10,11}. The partial least squares path models (PLS-PM) were used to analyse the main factors affecting ecosystem multifunctionality after different drying duration, whether biodiversity or community assembly, and whether community stability plays an important role in maintaining multifunctionality.

Results

The responses of biofilm functions during rewetting after different drying duration

The biofilm multifunctionality index increased with increasing drying duration up to 60 days, while no further increase in the index was

observed beyond this duration (Supplementary Fig. 1a). Similar responses were also observed in the nitrogen metabolic index (Supplementary Fig. 1b), carbon metabolic index (Supplementary Fig. 1c), and phosphorus metabolic index (Supplementary Fig. 1d). The cluster analysis between groups after z-score standardisation with different biofilm functions showed that the longer the duration of drying, the stronger the functional difference with control, with a critical drying period of 60 days (Fig. 1a). Specifically, the biofilm metabolic activities of BGLU, NADH, NOS, AMO, LAP, NAR and ACP peaked after 60 days of drying and then decreased following prolonged drying (Fig. 1a), while the responses of ecosystem metabolism were exactly the opposite, with decreases after 60 days of drying followed by an increases (Fig. 1a). Additionally, there was a significant difference between the biofilm multifunctionality after moderate drying duration (control, 20 days, and 60 days) and after prolonged drying duration (100 days and 130 days) (Fig. 1b, Supplementary Table 4, PERMANOVA, $p < 0.05$). Meanwhile, the same difference was also found in the biofilm carbon cycle and the nitrogen cycle after different drying duration (Fig. 1b, Supplementary Table 4, PERMANOVA, $p < 0.05$); and the gradually increased difference with increasing drying between the control with each experimental group was found in phosphorus cycle (Fig. 1b, Supplementary Table 4, PERMANOVA, $p < 0.05$).

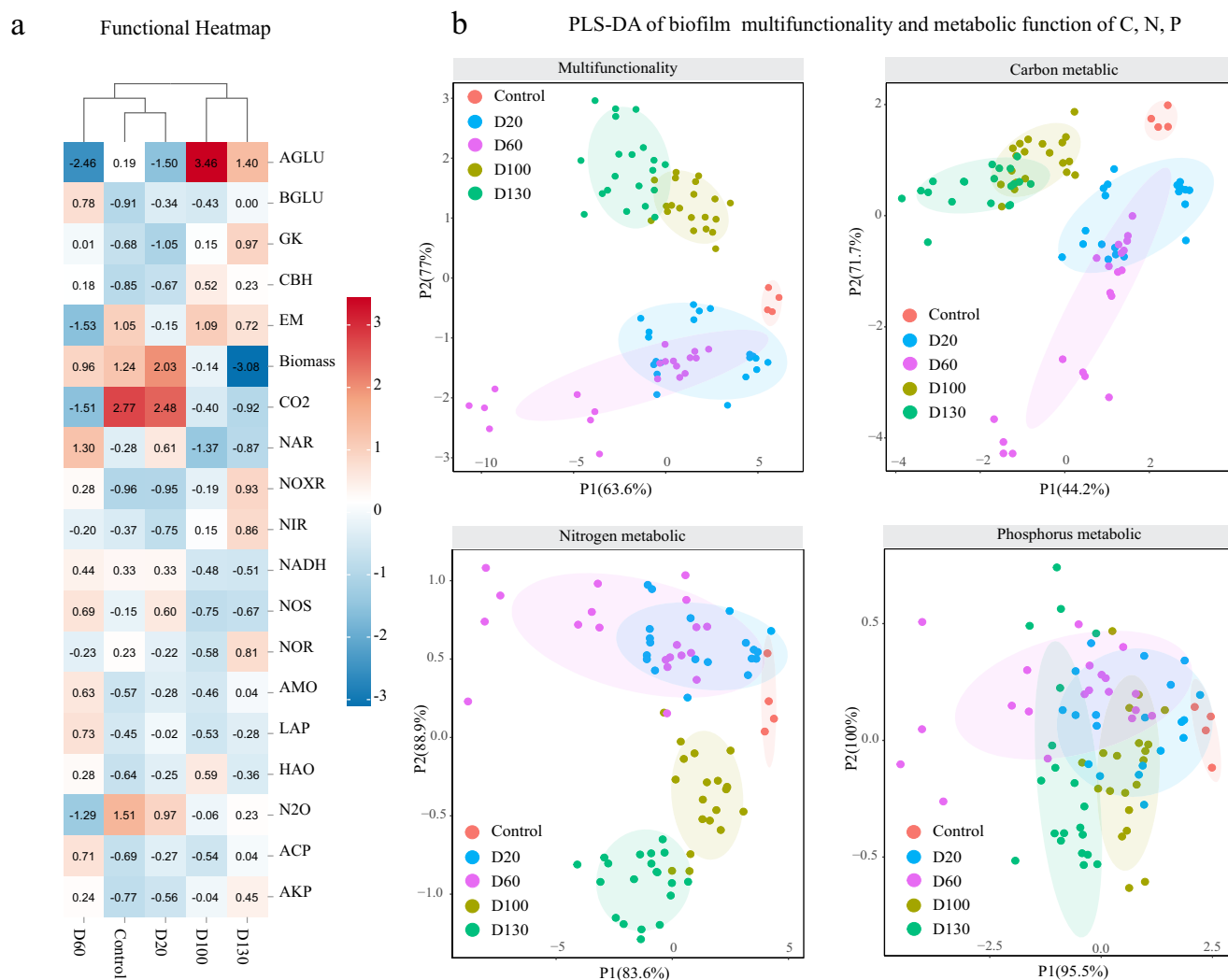


Fig. 1 | The responses of biofilm functions during rewetting after different droughtdrying duration. a Heatmap of all ecosystem functions related to the cycling and storage of carbon, nitrogen, and phosphorus. The values on the colour blocks means the index after z-score standardisation. The cluster tree was

constructed based on PERMANOVA. **b** PLS-DA of biofilm multifunctionality and elemental metabolism (carbon metabolic, nitrogen metabolic and phosphorus metabolic) after different drying duration.

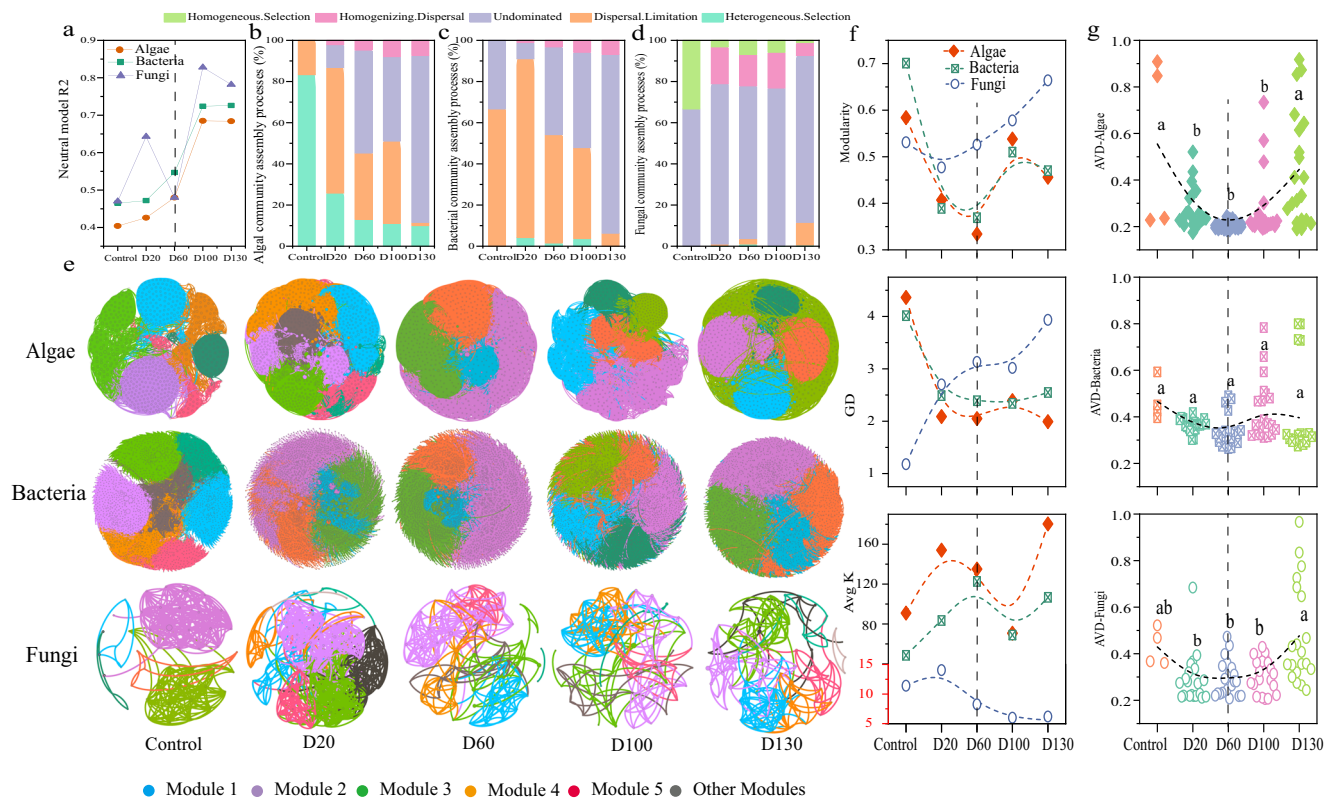


Fig. 2 | The changes of microbial network and community assembly mechanisms. **a** The fitting results of biofilm neutral community model (R^2); (**b–d**). Biofilm community assembly. Deterministic processes include homogeneous and variable selection, and stochastic processes include dispersal limitation, homogenising dispersal, and undominated; (**e**). Network revealing the modular associations among biofilm OTUs; (**f**). Topological nature of the network structure (modality, Avg K and

GD); and (**g**). Biofilm community stability (AVD) of different components after different drying duration. The black dashed line (**f** and **g**) marks the ‘drying inflexion point’. D20 is rewetting after 20 days of drying. Different letters (**g**) indicate significant differences within the biofilm community stability (ANOVA, $p < 0.05$) across different drying duration.

The changes of microbial network and community assembly mechanisms

All samples approached saturation in rarefaction curves analysis, indicating that the sequencing effort was sufficient to estimate the responses of these biofilm microbial communities to drying and rewetting alternation (Supplementary Figs. 2, 3). Neutral community model fits (R^2) showed that the effects of stochasticity gradually dominated in the biofilm community assembly, especially after 60 days of drying (Fig. 2a and Supplementary Figs. 7–9). Meanwhile, the findings of the null model showed that undominated processes and dispersal limitation were the significant drivers of algal and bacterial community assembly, and the undominated processes always dominated the fungal community assembly (Fig. 2b–d). The network modularity of the different biofilm fractions responded consistently to the different drying duration, which decreased and then increased with drying duration, with a significant inflexion point at around 60 days of drying. At the same time, the fungal network were more sensitive with a considerable inflexion point occurring after 20 days (Fig. 2e, f). However, the networks, AvgK and GD of the bacteria and algae were precisely the opposite of the fungal network, as AvgK of the fungal network gradually decreased, and GD gradually increased with increasing drying (Fig. 2f). Nevertheless, they both showed a significant inflexion point at around 20 days of drying (Fig. 2f). In addition, the eukaryotic community stability decreased and then increased significantly with increasing drying, while the stability of bacteria showed no significant change, indicating that eukaryotic microorganisms were significantly affected by drying and unable to recover rapidly during the rewetting period (Fig. 2g).

The effects of different drying duration on the relationship between biodiversity and multifunctionality

Based on the critical drying period of 60 days in the changes in biofilm functions and biodiversity with increasing drying duration, especially the clustering results of biofilm multifunctionality (Fig. 2b), a short-term drying group (control and D20) and a long-term drying group (D60, D100, and D130) can be classified (Fig. 4). Prolonged drying had a significant effect on the BEF relationship and the relationship of the community assembly mechanism with ecosystem functions (Fig. 3a, b, PLS-PM). After long-term drying, biodiversity decoupled with ecosystem functions, and instead community assembly contributes more to functions (Fig. 3a, b), especially to phosphorus cycle (Fig. 3a, b).

The PLS-PM results showed that the increasing drying duration had a significant effect on the relationship of biofilm multifunctionality with community assembly (from -0.289 to 0.446) and biodiversity (from -0.28 to 0.191) during rewetting (Fig. 3c, d). The most important predictor for biofilm multifunctionality was community assembly rather than biodiversity (Fig. 3c, d). In addition, the relationship of multifunctionality with community stability similarly changed from a negative to a positive effect (Fig. 3c, d). With drying increasing, the significant impact of biofilm biodiversity on community stability during rewetting was weakened (Fig. 3c, d).

Discussion

For investigating the mechanism of climate change aggravated river drought on river ecological functions, this study analysed the main factors affecting ecological multifunctionality after different drying durations by the PLS-PM model with biofilm as the object. The research seeks to determine whether the factor is biodiversity or community assembly and if community stability

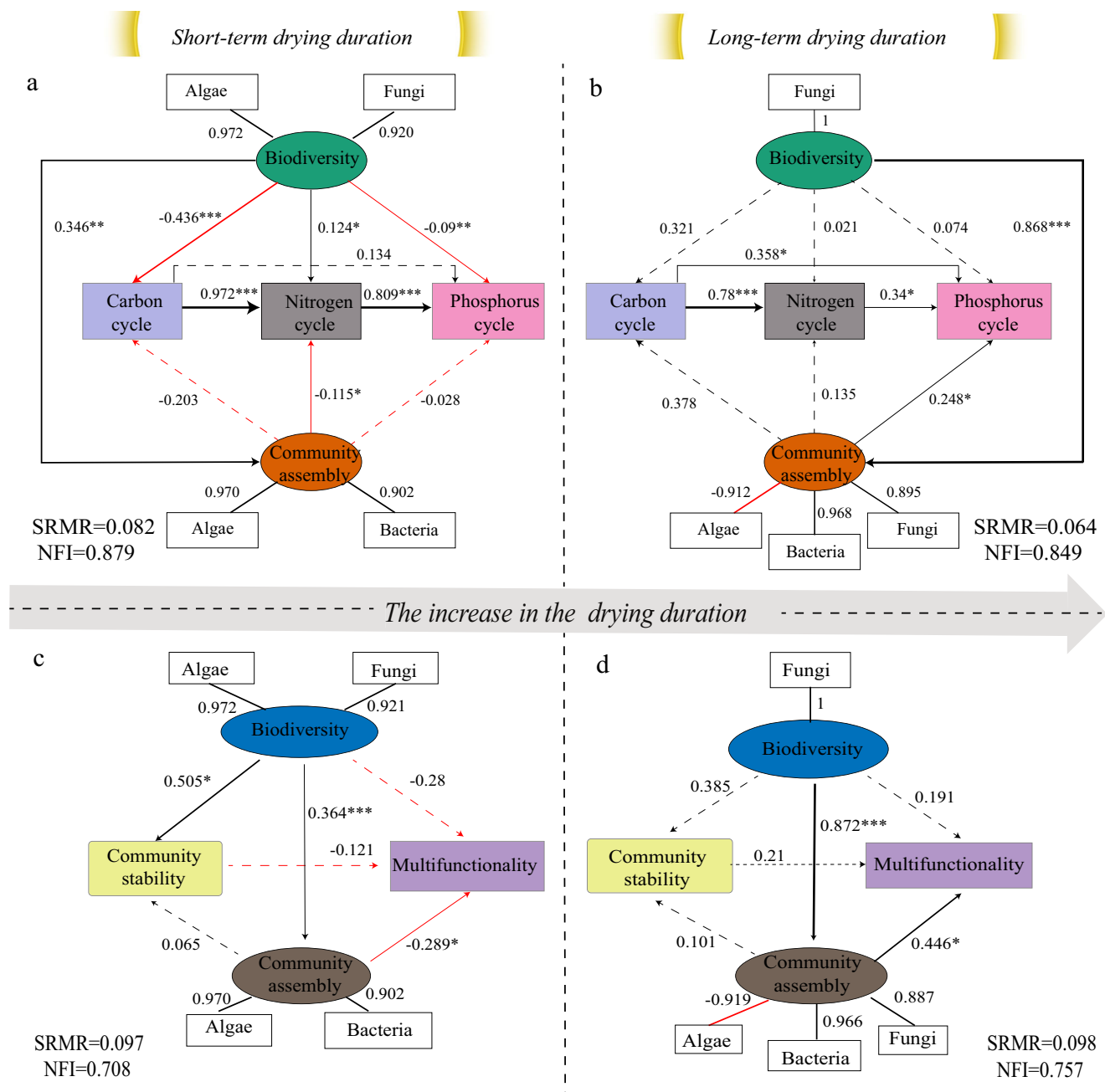


Fig. 3 | The effects of different drought times/drying duration on the relationship between biodiversity and multifunctionality. The relationship between biodiversity and community assembly and the cycling function of different elements during rewetting of different drying durations (a, b) and the relationship with multifunctionality and community stability (c, d). The red and black lines represent

negative and positive correlations, respectively, and the asterisks represent significant effects. Arrow width is proportional to the strength of the relationship (Supplementary Table 6). Insignificant biofilm functional and structural parameters were excluded based on their outer loadings by employing SmartPLS software (Version 3, Boenningstedt, Germany).

plays an important role in maintaining multifunctionality. The results provide evidence regarding the importance of community assembly on multifunctionality in intermittent streams, considering the effect of the drying period during rewetting.

The critical drying duration for biofilm biodiversity and functions

The phosphorus cycle of biofilm showed higher drying resistance than the carbon and nitrogen cycles (Fig. 1b), reflecting the importance of phosphorus to generate energy for microbial metabolism during rewetting^{26,27}. In contrast, the carbon and nitrogen cycles of the biofilm could not maintain the same state with control after a prolonged drying

duration (Fig. 1b). This is because after a prolonged drying there are not enough carbon and nitrogen sources within the biofilm for microbial growth, and it can only use phosphorus to generate energy for survival⁵. Based on the correlative nature of the individual functions²⁸, biofilm multifunctionality was more sensitive to increasing drying than single functions (Fig. 1), reflecting the similar underlying processes to increasing drying²⁹.

The same critical time point of river drying was also observed in the fitting result of the neutral community model (Fig. 3a), and the increasing dominance of stochasticity resulted from the stimulation from the hydrological change perturbations to stochastic factors^{30,31}. Biofilm communities

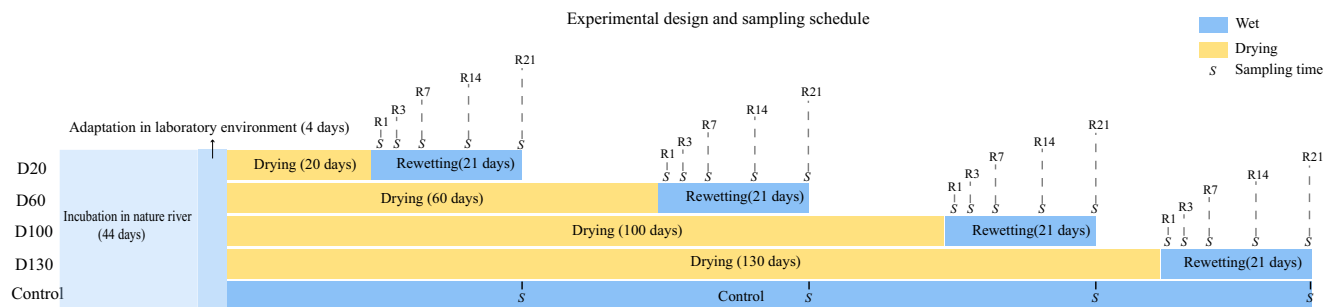


Fig. 4 | The alternation of drying and rewetting experimental design and sampling time axis. D20 is the experimental group that rewetting after 20 days of drying. R1 means the first day after rewetting.

are more susceptible to stochasticity with decreasing diversity^{32,33} (Supplementary Fig. 8). Of the stochastic influence, undominated as the major factor for community assembly mechanisms of all components of biofilms (Fig. 3b–d), and it was especially significant for fungi. This might be due to microorganisms reduce interspecies competition by changing their resource utilisation strategies to increase individual independence with limited habitat resources after a prolonged drought, which may exert a more substantial influence in a low heterogeneous environment and beta diversity (Supplementary Table 5)^{34–36}. The varied influence of dispersal limitation on different components of biofilms may be related to their different survival strategies, as the relative abundance of the microbial community could be the major factor influencing dispersal limitation^{37–39}. Prolonged drying reduce microbial heterogeneity, diminishing ecosystem resilience by rendering microbial communities less equipped to face environmental adversities⁴⁰. This homogenisation disrupts the efficient cycling of key nutrients such as carbon, nitrogen, and phosphorus—elements critical for ecosystem productivity and health. Such disruptions may impair vital ecosystem services, including soil fertility, water purification, and greenhouse gas regulation^{41,42}.

The growing theoretical and empirical evidence suggests that the mechanisms driving spatial variation in diversity to the in-depth study of the BEF relationship are of high theoretical importance⁴³. Apart from the decreased microbial alpha diversity with increasing drought (Supplementary Figs. 4–6), the reduction in beta-diversity (Bray–Curtis dissimilarity between groups) with prolonged drying durations indicated that extended drying periods contributed to decreased microbial community heterogeneity (Supplementary Table 5), which could substantially impact biofilm functions through the “insurance effects” (microorganisms maintain their functions in variable environments by increasing beta diversity)⁴⁴.

Furthermore, the consistent response of the biofilm network modularity of the different components indicated different microbial communities rescaled to form a complex network of interactions following drying disturbance⁴⁵. In this study, the bacterial and algal network’s response mechanism to increasing drying stress reflected a tight and complex network structure⁴⁶, with a critical period of 60 days for rivers to dry up. The response of the fungal network was the opposite (Fig. 3e, f), which might be due to the higher environmental adaptation for fungi under extreme conditions⁴⁷, especially under the more impaired conditions of bacterial and algal communities^{48,49}. There was also a critical time point of river drying at 60 days for eukaryotic (fungi and algae) community stability, corresponding to biofilm functions and confirming the community stability’s critical effect on biofilm function²⁵. Meanwhile, bacteria were more resistant to increasing drying, likely due to its most complex network or resistant phenotypes⁵⁰. Consistent with the cluster analysis of biofilm functions and the result of the PLS-DA from multifunctionality, there was a critical drying period for rivers at around 60 days for biofilm biodiversity (Figs. 1–3). So, a short-term drying group (control and D20) and a long-term drying group (D60, D100, and D130) were classified based on the result.

The relationship between biodiversity and ecosystem functions decoupled after long-term drying

Two possible explanations exist for the decoupling between biodiversity and ecosystem functions after prolonged drying (Fig. 3). The first facet to consider is functional plasticity, defined as the ability of microbial communities to adapt to environmental fluctuations by fine-tuning their performance⁴⁹. For example, BGLU, NOS, LAP, AMO and NAR tended to adapt to environmental changes (increase followed by decrease or a decrease followed by the rise with increasing drying duration) (Fig. 1a). While analysed from the perspective of elemental metabolic cycling, the elemental metabolic cycling functions of the biofilm differed significantly before and after the critical period for rivers to dry up (60 days of drying) (Fig. 1b). This was especially true for the carbon and nitrogen element metabolic functions (Fig. 1b), indicating that functional plasticity is not the only explanation for this decoupling. Hence, functional redundancy might also contribute to the results that some metabolic functions of biofilms could be maintained at a certain level when the biofilm diversity had been irreversibly affected by a prolonged drying, even though they may have behaved differently for a single function⁵¹. In addition, the explanatory power of biodiversity was always vital for community assembly (Fig. 3), since microbial species adapt to environmental change by generating a trade-off between environmental filtering and disposal limitation⁵².

Community assembly is more important for ecosystem multifunctionality than biodiversity after long-term drying

The environment selects microbial functional traits rather than species to maintain essential ecosystem functions⁵³, which is why community assembly contributed more to biofilm ecosystem functions than biodiversity after long-term drying (Figs. 3, 4). The significant effect of community assembly on biofilm ecosystem functions shifted to the phosphorus cycle from the nitrogen cycle, which could be because the phosphorus cycle was more susceptible to being filtered by environmental conditions⁵⁴ (Fig. 3). Notably, the relationship between biofilm multifunctionality and different factors changed from a negative to a positive correlation following prolonged drying (Fig. 4), indicating that coexisting species, following niche partitioning based on various resources, could positively interact and further improve community functional performance¹⁴. There was a relatively significant positive effect between biofilm biodiversity and community stability (Fig. 4), while after long-term drying, the narrow distribution of community functional groups and the depleting redundancy of biofilm functions may have weakened the relationship between biofilm biodiversity and community stability⁵⁵.

Limitations and environmental implications

A critical period for rivers to dry up of around 60 days significantly altered the biodiversity and multifunctionality of biofilm communities. This shift emphasises the resilience of microbial communities to short-term drying conditions, where biodiversity significantly influences multiple ecosystem processes. However, the dependence of ecosystem multifunctionality on community assembly became more pronounced under long-term drying

conditions, suggesting that the structural dynamics of microbial communities play a key role in maintaining ecosystem multifunctionality under environmental stress. Furthermore, this study highlights the importance of stochastic processes in community assembly, especially under prolonged drying conditions, which may lead to reconfiguration of interactions between community assembly and ecosystem multifunctionality.

Understanding the relationship between community assembly and microbial functions is at the forefront of current ecological research^{56,57}. Yet, this paradigm has not been broadly studied in river ecosystems. In this study, we examined the impact of different drying duration on the effect of biodiversity and community assembly on biofilm multifunctionality through indoor simulation experiments. Nevertheless, it is essential to acknowledge that the controlled experimental environment might potentially restrict the extensive extrapolation of our findings, considering the heterogeneous environmental composition in natural IRES. For example, the dynamics of critical variables, including light, temperature, and organic matter within natural river ecosystems, might coincide with dry-to-wet transitions and affect the extent and nature of biofilm responses⁵⁸. More rigorous tests and further field experiments on the generality of this result in different ecosystems are needed for consideration as a fundamental principle of microbial ecology.

Based on this study, increasingly refined insights have been gained into the critical period for rivers to dry up of biofilm multifunctionality and the vital contribution of community assembly in driving it. Addressing the identified research gaps will progressively enhance the future policy relevance of critical periods for rivers to dry up. Implementing timely rewetting replenishment before reaching the critical period for rivers to dry up is likely the most essential and effective strategy. Additionally, biodiversity decoupled from ecosystem functions after long-term drying disturbances, meaning further work to identify the main drivers of ecosystem functions will be critical for ultimately predicting the response of community functions to environmental changes. More importantly, the increasing prominence of stochastic processes becomes more pronounced with longer drying durations, and community assembly is more important for ecosystem multifunctionality than biodiversity.

This study suggests an inaugural demonstration of the dominant role of stochastic assembly in shaping community structure and ecosystem multifunctionality. Elucidating the intricate connections among community assembly, biodiversity, and ecosystem functioning is critical for preserving biodiversity and effectively managing ecosystems.

Methods

Biofilm colonisation and laboratory experiment

Cobbles (2–3 cm in diameter) were colonised in Qin Huai River (32°03' 36.2 N; 118°44' 38.1 E) in May 2023 (mean temperatures is 16–24 °C), and the colonisation and water quality parameters are detailed in the Supporting Information (Supplementary Table 1). After 44 days, these cobbles with biofilm were translocated to acclimatised to the artificial streams (160 cm long, 20 cm wide, and 30 cm high) in a greenhouse at 18 ± 2 °C for four additional days under constant water quality through the addition of a nutrient solution per week (Supplementary Table 2)^{59–62}. The channels were equipped with a pump (BT100-1L, Longer Precision Pump Co., Ltd., Baoding, China) to ensure water recycling and that the velocity of 0.14 m/s was maintained (consistent with the velocity of the Qin Huai River)⁶³.

In order to study the effect of drying duration on river ecosystems, we set up four experimental groups (after 20, 60, 100, and 130 days of drying duration, with measurements of biofilm community composition and ecological functions during the 21 days of rewetting), and a control group without drying^{64,65}. Samples were collected from experimental groups at five different times (after rewetting 1, 3, 7, 14 days and 21 days) and four replicates per time by randomly selecting specific amounts of cobbles from the channels. And sampled from the control group when experimental groups finish rewetting 21 days everytime (Fig. 4). More details are provided in the Supporting Information (Supplementary Note 1).

DNA sequencing and analyses

Four biofilm replicates were collected at five different times from four experimental groups during the rewetting and sampled from the control group at four different rewetting periods (Fig. 4). Detailed descriptions of the DNA extraction, eukaryotic (algal and fungal) 18S and bacterial 16S amplifications, and MiSeq sequencing are available in the Supporting Information and Supplementary Note 2⁴⁶. The MagaBio Soil/Fecal Genomic DNA Purification kit (Bioer, Hangzhou, China) was used to extract the total DNA. The primer pair 515 F (5'-GTGCCAGCMGCCGCGGTAA-3') and 806 R (5'-GGACTACHVGGGTWTCTAAT-3')^{66,67} was chosen to amplify the V4 hypervariable regions of the 16S rRNA gene for bacteria. For eukaryotes, we used 528 F (5' GCGTAATTCAGCTCCAA) and 706 R (5' AATCCRAGAATTTACACCTCT) primer sequences to amplify the V4 region of the 18S rRNA gene⁴⁶. Meanwhile, the fungus and algae were distinguished from each other at the kingdom level for analyse; then the number of effective tags (no. of seqs) and the operational taxonomic units (OTUs) synthesis information table (OTU_table) were obtained for the subsequent analysis.

Microbial community beta diversity (community heterogeneity) was calculated through the dissimilarity between experimental communities based on the Bray–Curtis index³¹. Network analyses of different biofilm components (bacteria, fungi, and algae) were conducted on species abundance data at the OTU level⁶⁸, with Pearson's correlation p -value ≤ 0.05 and $|\text{cor}| > 0.5$. Additionally, biofilm microbial community stability was evaluated in terms of average variation degree, which was calculated using the deviation degree from the mean of the normally distributed OTU relative abundance⁶⁹. We analysed the biofilm community assembly based on the null model using the iCAMP v1.5.12 package⁷⁰ and the neutral community model¹⁸. The R^2 value indicates the goodness of fit to the neutral community model, which means that the community assembly is fully consistent with stochastic processes when R^2 is close to 1⁷¹. The Supporting Information provides the detailed experimental determination, including statistical analyses of sequencing data (Supplementary Note 2).

Biofilm multifunctionality

In order to comprehensively assess functional changes in river ecosystems, we calculated z-scores for all ecosystem functions related to the cycling of carbon, nitrogen, and phosphorus. More detailed experimental determinations are provided in the Supporting Information (Supplementary Note 3 and Supplementary Table 3). At first, we averaged the standardised scores¹³ of all individual ecosystem functions and calculated z-scores of all functions evaluated⁷² as in Eqs. 1–2. Based on related functions, carbon metabolic, nitrogen metabolic and phosphorus metabolic also be calculated in the same way. The multifunctionality index was the average z-score for all functions measured.

$$x_{z\text{-score}} = \frac{x_i - \mu}{\sigma} \quad (1)$$

$$MF = \sum_{i=1}^n X_{z\text{-score}i} \quad (2)$$

Where x_i is each biofilm function, μ refers to the mean of the x_i and σ refers to the standard deviation of the x_i . $X_{z\text{-score}i}$ is the z-score of each biofilm function, and n is the number of all ecological functions monitored.

Partial least squares path models

The PLS-PM was constructed to calculate the relationship of BEF, as well as the impact of community assembly and biodiversity on multifunctionality. We analysed the biodiversity of different biofilm components (algae, bacteria, and fungi) by computing z-scores of their alpha (richness), beta (community heterogeneity), and network diversity (AvgK, AvgCC, GD, and modularity) as in Eqs. 1–2⁷². We took the biofilm biodiversity, carbon cycle index, nitrogen cycle index, phosphorus cycle index, community stability and community assembly of different microbial components (fungi, algae,

and bacteria) as different potential variables in PLS-PM^{73,74}. During the model fitting process, insignificant biofilm functional and structural parameters were excluded based on their outer loadings by employing SmartPLS software (Version 3, Boenningstedt, Germany), with the validity of the fitting results (Model_Fit, Path Coefficients, *p*-values for both direct and indirect effects, and other indicators). D_ULS, D_G and Rms Theta in the model fit indices are all such that smaller values indicate a better fit, while the model is only acceptable when SRMR is <0.1 and NFI is >0.7⁴⁶. The dashed and solid lines are used to distinguish direct and indirect effects between the factors, allowing to focus only on the direct effects when performing impact analyses.

Statistical analyses

The significant differences in biofilm functions and biodiversity among groups was performed by a one-way ANOVA analysis ($n = 4$) after ensuring that all assumptions of ANOVA were met. This included verifying the independence of samples, testing for normality using the Shapiro-Wilk test, and confirming homogeneity of variances with Levene's test. The data will be appropriately transformed (e.g., log-transformed) where necessary to meet these assumptions. The results were considered statistically significant at $p < 0.05$. Partial Least-Squares Discriminant Analysis (PLS-DA) was used to distinguish the biofilm multifunctionalities of different groups based on PERMANOVA (Adonis) analyses (Anderson, 2001), as well as analysed the differences in elemental metabolic (carbon metabolic, nitrogen metabolic and phosphorus metabolic). To study the influence of the biodiversity of different biofilm components (algae, bacteria, and fungi) on biofilm functions, the mantel test was conducted based on Pearson's correlation using the ggcor package in R⁷⁵.

Data availability

The 16S and 18S sequence data that support the findings of this study are available in the NCBI repository (accession code: PRJNA1014953). The remaining data supporting the findings of this study are available within the article and source data file.

Received: 8 October 2024; Accepted: 22 April 2025;

Published online: 06 May 2025

References

- Yan, Z. et al. Asynchronous responses of microbial CAZymes genes and the net CO₂ exchange in alpine peatland following 5 years of continuous extreme drought events. *ISME Commun.* **2**, 115 (2022).
- Satoh, Y. et al. The timing of unprecedented hydrological drought under climate change. *Nat. Commun.* **13**, 3287 (2022).
- Messenger, M. L. et al. Global prevalence of non-perennial rivers and streams. *Nature* **594**, 391–397 (2021).
- He, M. et al. Grazing and global change factors differentially affect biodiversity–ecosystem functioning relationships in grassland ecosystems. *Glob. Change Biol.* **28**, 5492–5504 (2022).
- Sabater, S., Timoner, X., Borrego, C. & Acuña, V. Stream biofilm responses to flow intermittency: from cells to ecosystems. *Front. Environ. Sci.* <https://doi.org/10.3389/fenvs.2016.00014> (2016).
- Maestre, F. T. et al. Plant species richness and ecosystem multifunctionality in Global Drylands. *Science* **335**, 214–218 (2012).
- Jucker, T. & Coomes, D. A. Comment on “plant species richness and ecosystem multifunctionality in Global Drylands”. *Science* **337**, 214–218 (2012).
- Alvarez, S. A. et al. Diversity decoupled from ecosystem function and resilience during mass extinction recovery. *Nature* **574**, 242 (2019).
- Tilman, D., Reich, P. B. & Isbell, F. Biodiversity impacts ecosystem productivity as much as resources, disturbance, or herbivory. *Proc. Natl. Acad. Sci. USA* **109**, 10394–10397 (2012).
- Brun, P. et al. The productivity–biodiversity relationship varies across diversity dimensions. *Nat. Commun.* **10**, 5691 (2019).
- Brauns, M. et al. A global synthesis of human impacts on the multifunctionality of streams and rivers. *Glob. Change Biol.* **28**, 4783–4793 (2022).
- Zhang, Z., Lu, Y., Wei, G. & Jiao, S. Rare species-driven diversity–ecosystem multifunctionality relationships are promoted by stochastic community assembly. *mBio* **13**, e0044922 (2022).
- Byrnes, J. E. K. et al. Investigating the relationship between biodiversity and ecosystem multifunctionality: challenges and solutions. *Methods Ecol. Evol.* **5**, 111–124 (2014).
- Yu, X., Polz, M. F. & Alm, E. J. Interactions in self-assembled microbial communities saturate with diversity. *ISME J.* **13**, 1602–1617 (2019).
- Li, M. et al. Facilitation promotes invasions in plant-associated microbial communities. *Ecol. Lett.* **22**, 149–158 (2019).
- Gause, G. F. Experimental analysis of vito volterra's mathematical theory of the struggle for existence. *Science* **79**, 16–17 (1934).
- Hubbell, S. P. *The Unified Neutral Theory of Biodiversity and Biogeography* (MPB-32), Vol. 392 (Princeton University Press, 2001).
- Stegen, J. C., Lin, X., Konopka, A. E. & Fredrickson, J. K. Stochastic and deterministic assembly processes in subsurface microbial communities. *ISME J.* **6**, 1653–1664 (2012).
- Vellend, M. Conceptual Synthesis in Community Ecology. *Q. Rev. Biol.* **85**, 183–206 (2010).
- Vellend, M. *The Theory of Ecological Communities* (MPB-57), Vol. 248 (Princeton University Press, 2017).
- Xun, W. et al. Diversity-triggered deterministic bacterial assembly constrains community functions. *Nat. Commun.* **10**, 3833 (2019).
- Delgado-Baquerizo, M. et al. Microbial diversity drives multifunctionality in terrestrial ecosystems. *Nat. Commun.* **7**, 10541 (2016).
- Wagg, C., Bender, S. F., Widmer, F. & van der Heijden, M. G. A. Soil biodiversity and soil community composition determine ecosystem multifunctionality. *Proc. Natl. Acad. Sci. USA* **111**, 5266–5270 (2014).
- Soliveres, S. et al. Biodiversity at multiple trophic levels is needed for ecosystem multifunctionality. *Nature* **536**, 456 (2016).
- Griffiths, B. S. & Philippot, L. Insights into the resistance and resilience of the soil microbial community. *FEMS Microbiol. Rev.* **37**, 112–129 (2013).
- Schimel, J., Balser, T. C. & Wallenstein, M. Microbial stress-response physiology and its implications for ecosystem function. *Ecology* **88**, 1386–1394 (2007).
- Timoner, X., Acuña, V., Schiller, D. V. & Sabater, S. Functional responses of stream biofilms to flow cessation, desiccation and rewetting. *Freshw. Biol.* **57**, 1565–1578 (2012).
- Manning, P. et al. Redefining ecosystem multifunctionality. *Nat. Ecol. Evol.* **2**, 427–436 (2018).
- Hu, W. et al. Aridity-driven shift in biodiversity–soil multifunctionality relationships. *Nat. Commun.* **12**, 5350 (2021).
- Houseman, G. R., Mittelbach, G. G., Reynolds, H. L. & Gross, K. L. Perturbations alter community convergence, divergence, and formation of multiple community states. *Ecology* **89**, 2172–2180 (2008).
- Zhou, J. et al. Stochasticity, succession, and environmental perturbations in a fluidic ecosystem. *Proc. Natl. Acad. Sci. USA* **111**, E836–E845 (2014).
- Evans, S., Martiny, J. B. H. & Allison, S. D. Effects of dispersal and selection on stochastic assembly in microbial communities. *ISME J.* **11**, 176–185 (2017).
- Vellend, M. et al. Assessing the relative importance of neutral stochasticity in ecological communities. *OIKOS* **123**, 1420–1430 (2014).
- Nemergut, D. R. et al. Patterns and processes of microbial community assembly. *Microbiol. Mol. Biol. Rev.* **77**, 342–356 (2013).
- Durant, S. M. Competition refuges and coexistence: an example from Serengeti carnivores. *J. Anim. Ecol.* **67**, 370–386 (1998).

36. Barnard, R. L., Osborne, C. A. & Firestone, M. K. Responses of soil bacterial and fungal communities to extreme desiccation and rewetting. *ISME J.* **7**, 2229–2241 (2013).
37. Jousset, A. et al. Where less may be more: how the rare biosphere pulls ecosystems strings. *ISME J.* **11**, 853–862 (2017).
38. Liu, L., Yang, J., Yu, Z. & Wilkinson, D. M. The biogeography of abundant and rare bacterioplankton in the lakes and reservoirs of China. *ISME J.* **9**, 2068–2077 (2015).
39. Hall-Stoodley, L., Costerton, J. W. & Stoodley, P. Bacterial biofilms: from the natural environment to infectious diseases. *Nat. Rev. Microbiol.* **2**, 95–108 (2004).
40. Thomsen, M. S. et al. Heterogeneity within and among co-occurring foundation species increases biodiversity. *Nat. Commun.* **13**, 581 (2022).
41. Daleo, P. et al. Environmental heterogeneity modulates the effect of plant diversity on the spatial variability of grassland biomass. *Nat. Commun.* **14**, 1809 (2023).
42. Chang, Y. et al. Phytodiversity is associated with habitat heterogeneity from Eurasia to the Hengduan Mountains. *N. Phytol.* **240**, 1647–1658 (2023).
43. Mokany, K., Burley, H. M. & Paini, D. R. beta diversity contributes to ecosystem processes more than by simply summing the parts. *Proc. Natl Acad. Sci. USA* **110**, E4057–E4057 (2013).
44. Mokany, K., Burley, H. M. & Paini, D. R. β diversity contributes to ecosystem processes more than by simply summing the parts. *Proc. Natl Acad. Sci. USA* **110**, E4057–E4057 (2013).
45. Olesen, J. M., Bascompte, J., Dupont, Y. L. & Jordano, P. The modularity of pollination networks. *Proc. Natl Acad. Sci. USA* **104**, 19891–19896 (2007).
46. Li, C. et al. Eukaryotes contribute more than bacteria to the recovery of freshwater ecosystem functions under different drought durations. *Environ. Microbiol.* <https://doi.org/10.1111/1462-2920.16370> (2023).
47. Huelsmann, M. & Ackermann, M. Community instability in the microbial world. *Science* **378**, 29–30 (2022).
48. Falasco, E., Bona, F., Rizzo, A. M. & Piano, E. Hydrological intermittency drives diversity decline and functional homogenization in benthic diatom communities. *Sci. Total Environ.* **762**, 143090 (2021).
49. Gionchetta, G., Oliva, F., Romani, A. M. & Bañeras, L. Hydrological variations shape diversity and functional responses of streambed microbes. *Sci. Total Environ.* **714**, 136838 (2020).
50. Luan, L. et al. Coupling bacterial community assembly to microbial metabolism across soil profiles. *Msystems* **5**, e00298–20 (2020).
51. Allison, S. D. & Martiny, J. B. H. Resistance, resilience, and redundancy in microbial communities. *Proc. Natl Acad. Sci. USA* **105**, 11512–11519 (2008).
52. Lawrence, D. et al. Species interactions alter evolutionary responses to a novel environment. *PLoS Biol.* **10**, e1001330 (2012).
53. Burke, C., Steinberg, P., Rusch, D., Kjelleberg, S. & Thomas, T. Bacterial community assembly based on functional genes rather than species. *Proc. Natl Acad. Sci. USA* **108**, 14288–14293 (2011).
54. Fierer, N. et al. Cross-biome metagenomic analyses of soil microbial communities and their functional attributes. *Proc. Natl Acad. Sci. USA* **109**, 21390–21395 (2012).
55. Wertz, S. et al. Decline of soil microbial diversity does not influence the resistance and resilience of key soil microbial functional groups following a model disturbance. *Environ. Microbiol.* **9**, 2211–2219 (2007).
56. In 't Zandt, D., Kolarikova, Z., Cajthaml, T. & Munzbergova, Z. Plant community stability is associated with a decoupling of prokaryote and fungal soil networks. *Nat. Commun.* **14**, 3736–3736 (2023).
57. Zhang, X. et al. Local community assembly mechanisms shape soil bacterial beta diversity patterns along a latitudinal gradient. *Nat. Commun.* **11**, 5428 (2020).
58. Colls, M., Timoner, X., Font, C., Sabater, S. & Acuna, V. Effects of duration, frequency, and severity of the non-flow period on stream biofilm metabolism. *Ecosystems* **22**, 1393–1405 (2019).
59. Li, C., Miao, L., Adyel, T. M., Wu, J. & Hou, J. Transformation of biofilm to carbon sinks after prolonged droughts linked with algal biodiversity change. *Environ. Sci. Technol.* **57**, 15487–15498 (2023).
60. Timoner, X. et al. Does biofilm origin matter? Biofilm responses to non-flow period in permanent and temporary streams. *Freshwater Biol.* **65**, 514–523 (2020).
61. Romani, A. M. et al. Biofilm structure and function and possible implications for riverine DOC dynamics. *Microb. Ecol.* **47**, 316–328 (2004).
62. Battin, T. J., Kaplan, L. A., Denis Newbold, J. & Hansen, C. M. E. Contributions of microbial biofilms to ecosystem processes in stream mesocosms. *Nature* **426**, 439–442 (2003).
63. Liao, K. et al. Integrating microbial biomass, composition and function to discern the level of anthropogenic activity in a river ecosystem. *Environ. Int.* **116**, 147–155 (2018).
64. Marxsen, J., Zoppini, A. & Wilczek, S. Microbial communities in streambed sediments recovering from desiccation. *Fems Microbiol. Ecol.* **71**, 374–386 (2010).
65. Fontana Jr, A. J. D. Minimum water activity limits for growth of microorganism. *Water Activity Foods* <https://doi.org/10.1002/9781118765982.app4> (2020).
66. Caporaso, J. G. et al. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. *Proc. Natl. Acad. Sci. USA* **108**, 4516–4522 (2011).
67. Zhou, Y. Q. et al. Warming reshaped the microbial hierarchical interactions. *Glob. Change Biol.* **27**, 6331–6347 (2021).
68. Wagg, C., Schlaeppi, K., Banerjee, S., Kuramae, E. E. & van der Heijden, M. G. A. Fungal-bacterial diversity and microbiome complexity predict ecosystem functioning. *Nat. Commun.* **10**, 4841 (2019).
69. Xun, W. et al. Specialized metabolic functions of keystone taxa sustain soil microbiome stability. *Microbiome* **9**, 35 (2021).
70. Ning, D. et al. A quantitative framework reveals ecological drivers of grassland microbial community assembly in response to warming. *Nat. Commun.* **11**, 4717 (2020).
71. Ostman, O. et al. Regional invariance among microbial communities. *Ecol. Lett.* **13**, 118–127 (2010).
72. Maestre, F. T., Soliveres, S., Gotelli, N. J., Quero, J. L. & Berdugo, M. Response to comment on “plant species richness and ecosystem multifunctionality in Global Drylands”. *Science* **337**, 214–218 (2012).
73. Zhou, L. et al. Stochastic determination of the spatial variation of potentially pathogenic bacteria communities in a large subtropical river. *Environ. Pollut.* **264**, 114683 (2020).
74. Liao, H. et al. Hyperthermophilic composting accelerates the removal of antibiotic resistance genes and mobile genetic elements in sewage sludge. *Environ. Sci. Technol.* **52**, 266–276 (2018).
75. Bei, Q. et al. Extreme summers impact cropland and grassland soil microbiomes. *ISME J.* <https://doi.org/10.1038/s41396-023-01470-5> (2023).

Acknowledgements

We are grateful for the grants for the project supported by the National Natural Science Foundation of China (No. 52379063), the Fundamental Research Funds for the Central Universities (B240205016), Graduate Research and Innovation Projects of Jiangsu Province (KYCX24_0902), The central government of Lhasa City guides the establishment of local science and technology development funds (LSKJ202438). TMA was supported by an Australian Research Council Discovery Early Career Researcher Award (DECRA, DE240100633) grant and the 2024 Thomas Davies Research Grant for Marine, Soil and Plant Biology from the Australian Academy of Science.

Author contributions

Conceptualisation: J.H., M.K. Methodology: Y.Y., J.W., G.Y. Investigation: S.L., Z.Y., T.M.A. Visualisation: Y.Y., C.L. Supervision: J.H., L.M. Writing—original draft: C.L. Writing—review & editing: C.L., J.H., L.M.

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/s41522-025-00711-z>.

Correspondence and requests for materials should be addressed to Lingzhan Miao.

Reprints and permissions 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-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, 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 licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence 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 licence, visit <http://creativecommons.org/licenses/by-nc-nd/4.0/>.

© The Author(s) 2025