

## **Supporting information**

for

### **Hydrodynamic-driven microbial community assembly and coalescence shape nitrogen transformation in river confluence**

Cizhang Hui <sup>1,2</sup>, Hongwu Tang (✉)<sup>1,2,3</sup>, Qihua Ran <sup>1,2,4</sup>, Dongfang Liang <sup>5</sup>, Yi Li  
(✉)<sup>4</sup>, Saiyu Yuan <sup>1,2</sup>

1 College of Water Conservancy and Hydropower Engineering, Hohai University,  
Nanjing 210098, China

2 Key Laboratory of Hydrologic-Cycle and Hydrodynamic-System of Ministry of  
Water Resources, Hohai University, Nanjing 210098, China

3 School of Civil Engineering and Transportation, South China University of  
Technology, Guangzhou 510641, China

4 State Key Laboratory of Water Cycle and Water Security, Hohai University, Nanjing  
210098, China

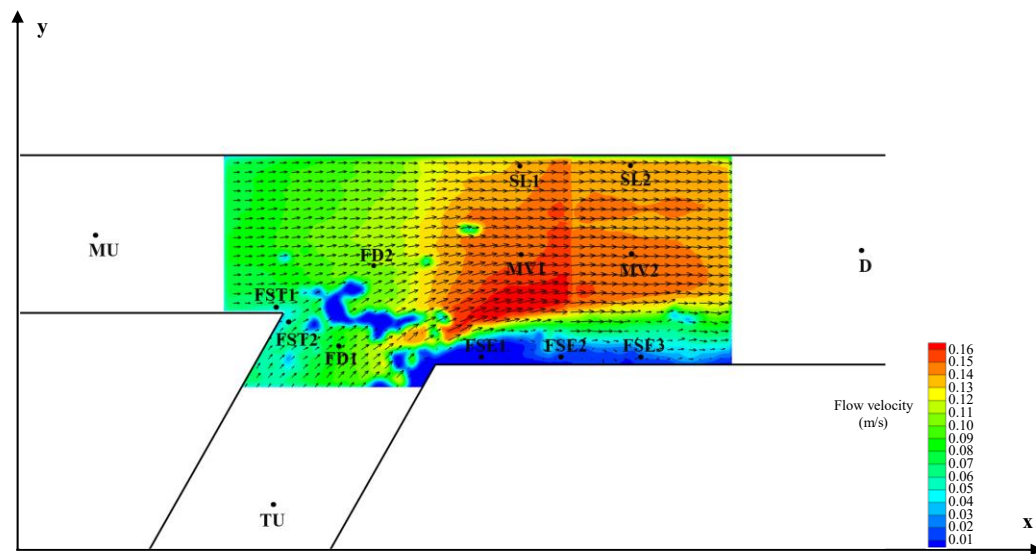
5 Department of Engineering, University of Cambridge, Cambridge CB2 1TN, UK

The following supporting information appendix consists of seven pages, including  
two supplementary figures and three supplementary tables.

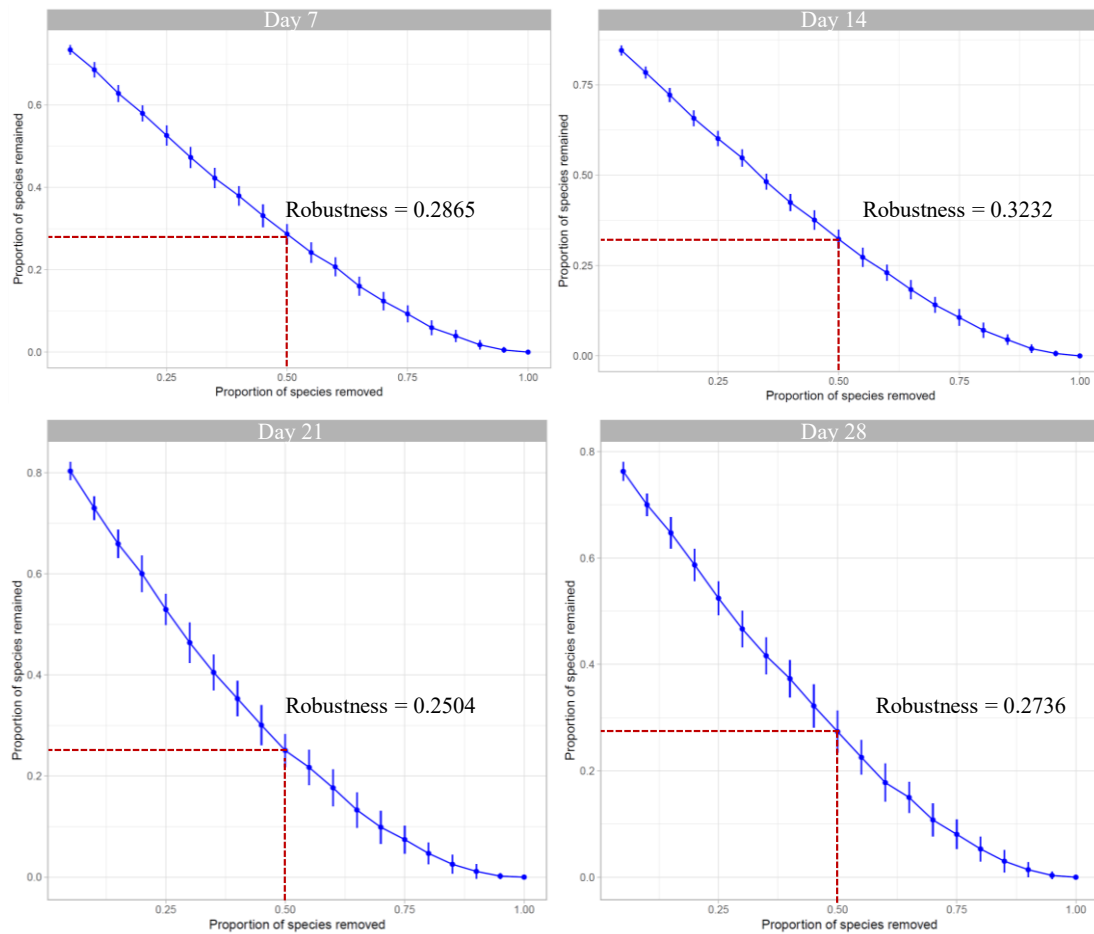
---

✉ Corresponding authors  
E-mail: hwtang@hhu.edu.cn (H. Tang); envly@hhu.edu.cn (Y. Li)

## 1. SUPPLEMENTARY FIGURE



**Fig. S1 Flow characteristics in the confluence area.** The arrows represent the velocity vectors, and the colors of contour illustrate the magnitude of velocity. The names of sampling points were related to hydrodynamic zones, i.e., main stream upstream (MU), tributary upstream (TU), flow stagnation zone (FST), flow separation zone (FSE), flow deflection zone (FD), maximum velocity zone (MV), and shear layer zone (SL).



**Fig. S2** The effects of species removal on microbial community co-occurrence structure at different days. The proportion of the remaining nodes after removing 50% of nodes from the network was considered as the network robustness.

## 2. SUPPLEMENTARY TABLES

**Table S1** Sequences of primers used for absolute fluorescence quantitative PCR

Target gene	Primer name	Sequences (5' to 3')	Reference
amoA	amoA 1f	GGGGTTTCTACTGGTGGT	Rotthauwe et al., 1997
	amoA 2r	CCCCTCKGSAAAGCCTTCTTC	
narG	narG-F	TCGCCSATYCCGGCSATGTC	Bru et al., 2007
	narG-R	GAGTTGTACCAGTCRGCSGAYTCSG	
nirK	Copper583F	TCATGGTGCTGCCGCGYGANGG	Yan et al., 2003
	Copper909R	GAACTTGCCGGTKGCCCAGAC	
nxA	nxA-F	CAGACCGACGTGTGCGAAAG	Louca et al., 2016
	nxA-R	TCCACAAGGAACGGAAGGTC	

**Table S2** Settings of sources and sinks for the SourceTraker analysis

<b>Sources</b>		<b>Sinks</b>
<b>Main stream upstream</b>	<b>Tributary upstream</b>	
MU	TU	FD1, FD2, FSE1, FSE2, FSE3, FST1, FST2, MV1, MV2, SL1, SL2

The sources and sinks are different sampling points in the confluence flume.

**Table S3** Results of permutational multivariate analysis of variance (PERMANOVA) testing the effects of time, hydrodynamic zone, and their interaction on microbial community composition based on Bray-Curtis dissimilarity.

<b>Source</b>	<b>df</b>	<b>SS</b>	<b>R<sup>2</sup></b>	<b>F</b>	<b>p-value</b>
Time	7	1.9519	0.14413	1.3021	0.001
Hydrodynamic zones	3	1.3463	0.09941	2.0955	0.001
Time × Hydrodynamic Zone	21	5.1052	0.37696	1.1352	0.001
Residual	24	5.1397	0.37951		
Total	55	13.5432	1		

Note: df is degrees of freedom, representing the number of independent values for each factor. SS is sum of squares, indicating the variance explained by each factor. R<sup>2</sup> is the proportion of total variance explained by each factor, representing its effect size. F is the F-statistic, calculated as the ratio of mean squares ( $MS = SS/df$ ), testing the significance of the factor. p-value is the probability that the observed effect is due to random chance, with values < 0.05 indicating statistical significance.

## References

- Bru, D., Sarr, A., Philippot, L., 2007. Relative Abundances of Proteobacterial Membrane-Bound and Periplasmic Nitrate Reductases in Selected Environments. *Appl. Environ. Microb.* 73, 5971-5974.
- Louca, S., Hawley, A.K., Katsev, S., Torres-Beltran, M., Bhatia, M.P., Kheirandish, S., Michiels, C.C., Capelle, D., Lavik, G., Doebeli, M., Crowe, S.A., Hallam, S.J., 2016. Integrating biogeochemistry with multiomic sequence information in a model oxygen minimum zone. *Proc. Natl. Acad. Sci. U. S. A.* 113(40), E5925-E5933.
- Rotthauwe, J.H., Witzel, K.P., Liesack, W., 1997. The ammonia monooxygenase structural gene *amoA* as a functional marker: molecular fine-scale analysis of natural ammonia-oxidizing populations. *Appl. Environ. Microb.* 63, 4704-4712.
- Yan, T., Fields, M.W., Wu, L., Zu, Y., Tiedje, J.M., Zhou, J., 2003. Molecular diversity and characterization of nitrite reductase gene fragments (*nirK* and *nirS*) from nitrate- and uranium-contaminated groundwater. *Environ. Microbiol.* 5, 13-24.