

## 1 **Supplementary Methods**

### 2 Experimental design

3 We analysed the sediment bacteria, but not the water column bacteria, for the  
4 following five reasons. Firstly, the natural aquatic systems are mostly composed of  
5 sediment and water; thus, the sediment is an essential part of aquatic microcosms. By  
6 adding both sediment and artificial lake water, we experimentally assembled whole,  
7 natural aquatic environments. Secondly, sediment acts as a minimal medium with all the  
8 necessary nutrients and mineral elements for the growth of microorganisms that are not  
9 contained in the artificial lake water. Thirdly, we expected that the sediment bacteria  
10 would be less influenced by disturbance effects because the sediment bacteria were in  
11 more stable physical conditions than the water column bacteria. The sediment was below  
12 the ground surface, and thus the sediment bacteria were less exposed to ambient  
13 environmental variations, such as UV radiation, than the bacteria in the water column.  
14 Sediment bacteria may also face less grazing pressure from higher organisms than water  
15 column bacteria, which are prone to grazing. Fourthly, we expected that the sediment  
16 bacteria were less structured by stochastic processes because they were environmentally  
17 selected, first by water column and then by sediment. Our previous study showed that  
18 deterministic processes are more important in structuring the bacterial communities in  
19 sediments than of the overlying water within aquatic environments<sup>1</sup>. Finally, compared  
20 to water column bacteria, especially those with low nutrient supply and thus with low  
21 biomass, sediment bacteria were much easier to collect after siphoning out the overlying  
22 water, preserve in sterile tubes in the field conditions, and examine with molecular  
23 methods. We faced practical issues with sampling the bacteria in the overlying water

24 because of the very large nutrient gradients. For example, the bacterial biomass in the  
25 overlying water at the lowest nutrient treatment (e.g., controls) was too low for the DNA  
26 analyses with the small volume of overlying water left over after the chemical analyses.  
27 Furthermore, we considered a large number of samples from each mountain, and the  
28 logistical issues thus prevented us from efficiently sampling the water-column bacteria.  
29 Due to the remote field conditions, we could not manually the filter water samples in situ  
30 for bacteria within a reasonable time period, and it was not possible to carry the water  
31 back to the laboratory. Even if the water had been transferred to the laboratory, the  
32 experimental delay (e.g., in days) would likely to happen for additional water-filtering  
33 manipulations.

34 In the one-month-incubation experiments, the sediment bacterial species were  
35 selected by two environmental filters, the overlying water and sediments. The simplified  
36 experimental ecosystems were highly suitable for the examination of the relative  
37 importance of temperature and nutrient factors on bacterial communities. Because  
38 bacterial species are mainly dispersed through the air, the colonized communities allowed  
39 us to capture the most natural local or regional species pools possible. Due to the multiple  
40 dispersal sources of bacterial species at local, regional, and global scales <sup>2,3</sup>, we could not  
41 identify the exact origins of sediment bacteria in the microcosms. This challenge was  
42 especially prevalent after the bacteria in the sediment were further filtered by the local  
43 environments of the overlying water and sediment in the microcosms. Thus, we did not  
44 examine bacterial communities of the samples from other local habitats, such as air dust,  
45 soil, streams, and lakes, to track the sources of sediment bacteria in the microcosms.

46 We did not use any specific natural aquatic bacterial communities from ponds or  
47 lakes in the current experiments, but established new communities via post-dispersal  
48 effects. By using sterile microcosms, we simplified the underlying processes for the  
49 observed bacterial communities by partly eliminating the legacies of historical events that  
50 affect the communities in natural ponds or lakes. This is because, together with  
51 contemporary environmental filtering, historical contingencies are one of the main forces  
52 affecting present-day microbial communities <sup>4</sup>. Compared to the microcosms, natural  
53 aquatic environments such as ponds or lakes are influenced by numerous covariant  
54 variables that are difficult to control for but that are important for organisms <sup>5,6,7</sup>. (1) The  
55 origin of natural aquatic environments affects the evolution and dispersal history of  
56 species in their biological communities. For instance, the selected lakes might have  
57 different origin and formation histories that affect their morphology and hydrological  
58 conditions. (2) Catchment area, surrounding vegetation, and local physical-chemical  
59 conditions also vary spatially and affect the aquatic biota. (3) The initial communities  
60 based on natural communities of ponds or lakes can differ spatially thus resulting in very  
61 heterogeneous initial communities, and the biological interactions with other organisms  
62 are probably more complex in nature than in our experimental microcosms. (4) To have  
63 consistent initial communities by inoculating microcosms with natural communities of  
64 ponds or lakes (i.e., the sediment bacteria in Taihu Lake) is less feasible for such broad-  
65 scale experiments due to the difficulties in preserving and transporting these natural  
66 aquatic communities for large distances and long periods of time. (5) Most importantly,  
67 using natural communities from ponds or lakes as the initial community does not  
68 eliminate the dispersal effects from the air, and thus the potential for strong priority

69 effects<sup>8</sup> that would likely occur. This is because the air is one of the main dispersal  
70 routes for bacterial species in nearly all surface aquatic environments<sup>9, 10, 11</sup>.

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72 Physicochemical analyses

73 The concentrations of ammonium ( $\text{NH}_4^+$ ), nitrate ( $\text{NO}_3^-$ ), nitrite ( $\text{NO}_2^-$ ), and dissolved  
74 inorganic phosphorus ( $\text{PO}_4^{3-}$ ) in the overlying water were measured with a flow injection  
75 analyser (Skalar SA1000, Breda, Netherlands). The sediment total organic carbon (TOC)  
76 was determined using a solid TOC analyser (SSM-5000A, Shimadzu, Japan). The  
77 sterilized sediments before the field experiments were measured for the above variables  
78 in three replicates.

79 We did not measure all the potentially important variables for the bacterial  
80 communities in the sediment, such as oxygen concentration or UV radiation. This is  
81 largely because we have no evidence about the potential importance of these variables on  
82 sediment microbial communities. Further, we do not expect these potential effects to be  
83 stronger than nutrient and temperature effects considered in our study. This is because the  
84 bottles were buried in the local soil to approximately 10% of the total bottle height, and  
85 the sediment in each bottle was below the ground surface. Therefore, it is unlikely that  
86 UV radiation would have had strong effects on these communities that would mask the  
87 temperature or nutrient effects on the bacteria. In addition, we added 15 g sterilized  
88 sediment to the bottom of each bottle, which created a sediment layer of approximately 5  
89 mm. Based on our previous studies on the oxygen concentrations at the sediment-water  
90 interface in eutrophic lakes, e.g., Wang et al.<sup>12, 13</sup>, the 5-mm sediment layer would be less  
91 likely to be anoxic during the experimental period, especially with the shallow overlying

92 water column (approximately 18 cm) and low total organic carbon ( $0.587 \pm 0.390\%$ ).

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94 Bacterial community analyses

95 Genomic DNA was extracted from freeze-dried sediments using the phenol  
96 chloroform method<sup>14</sup>. Real-time qPCR quantification of bacterial 16S rRNA genes in  
97 sediments was performed on an iCycler iQ5 thermocycler (Bio-Rad, Hercules, USA) as  
98 described previously<sup>15</sup>. Bacterial 16S rRNA genes were sequenced using MiSeq  
99 (Illumina, San Diego, USA) as described previously<sup>16</sup>. We amplified 16S rRNA genes in  
100 triplicate using the universal bacterial primers 515F, 5'-GTGCCAGCMGCCGCGGTAA-  
101 3' and 806R, 5'-GGACTACHVGGGTWTCTAAT-3', targeting the V4 region. Positive  
102 PCR products were confirmed with agarose gel electrophoresis. PCR products from  
103 triplicate reactions were combined and quantified with PicoGreen. PCR products from  
104 samples sequenced in the same MiSeq run were pooled at equal molality. The pooled  
105 mixture was purified with a QIAquick Gel Extraction Kit (QIAGEN Sciences,  
106 Germantown, USA) and re-quantified with PicoGreen. Sample libraries were prepared  
107 according to the MiSeq<sup>TM</sup> Reagent Kit Preparation Guide (Illumina, San Diego, USA).

108 We processed the sequences primarily using the QIIME pipeline (v1.8)<sup>17</sup> following  
109 recent references e.g.,<sup>16,18</sup>. Briefly, overlapped paired-end sequences from MiSeq were  
110 assembled using FLASH<sup>19</sup> and poorly overlapped and poor quality sequences were  
111 filtered out before de-multiplexing based on barcodes. Then, the sequences were  
112 clustered into OTUs at 97% pairwise identity with the seed-based uclust algorithm<sup>20</sup>.  
113 After chimeras were removed via Uchime, and representative sequences from each OTU  
114 were aligned to the Greengenes imputed core reference alignment V.201308<sup>21</sup> using

115 PyNAST<sup>22</sup>. The taxonomic identity of each representative sequence was determined  
116 using the RDP Classifier<sup>23</sup> and chloroplasts were removed.

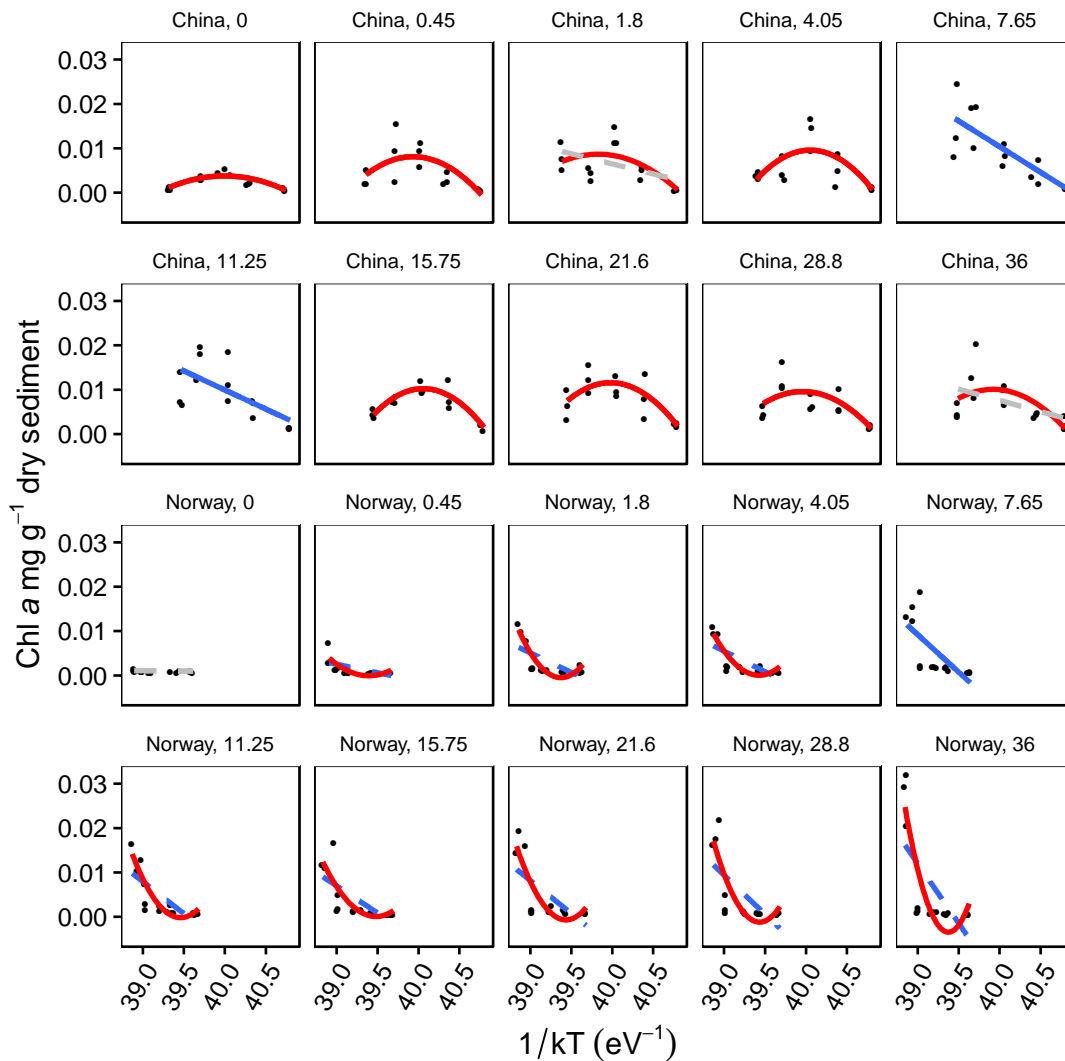
117 We removed singletons before the subsequent analyses, which is a common  
118 procedure in microbial studies, and has been highly recommended in popular microbial  
119 analysis pipelines even before the generation of OTU tables (Supplementary Fig. 12). We  
120 found that the removal of singletons and rare species (e.g., those occurring in less than 6  
121 samples) did not notably affect the patterns in species richness in our data  
122 (Supplementary Fig. 12). To keep the reported results as simple as possible, we used the  
123 community OTU table without singletons for all analyses. For Archaea, we obtained 102  
124 sequences from 42 out of 300 total samples. These archaeal sequences can be classified  
125 into 13 OTUs related to the families Nitrososphaeraceae, Nitrosocaldaceae,  
126 Methanocellaceae, Methanobacteriaceae, Methanomicrobiaceae, Methanosaetaceae,  
127 Sulfolobaceae, and DHVEG-1. Due to the insufficient sequence number for Archaea, we  
128 excluded all archaeal sequences from the data.

129 **Supplementary Tables**

130 **Supplementary Table 1.** Regression analyses of biodiversity with environmental  
 131 variables. Multiple regression analyses of bacteria diversity and community composition  
 132 were performed as a function of environmental variables. For diversity and community  
 133 composition, species richness and the first axis of non-metric multidimensional scaling,  
 134 respectively, were used as the response variables. The best models were identified using  
 135 Akaike’s information criterion, and variables were selected in multiple regressions only if  
 136  $P < 0.05$ . All of the variables were standardized (mean = 0; SD = 1) and are displayed  
 137 with increasing  $P$ -values. Temp: measured water temperature. Temp.2: the squared value  
 138 of measured water temperature. ADD.NO3: the initially added  $\text{NO}_3^-$ .  $\text{NO}_3$ ,  $\text{NO}_2$ , and pH:  
 139 water  $\text{NO}_3^-$  and  $\text{NO}_2^-$ , and pH, respectively. Chl  $a$ : sediment Chl  $a$  ( $\text{mg}\cdot\text{g}^{-1}$ ). Copies: copy  
 140 number of 16S rRNA genes qualified by real-time qPCR (copy number per gram dry  
 141 sediment).  
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| Response    | Mountains | Model $r^2$ | Explanatory variables and Beta-weights <sup>§</sup> |            |         |           |           |          |
|-------------|-----------|-------------|---|------------|---------|-----------|-----------|----------|
| Diversity   | Norway    | 0.595       | Temp ***  | Temp.2 *** | NO2 *** | pH *      |           |          |
| Diversity   | China     | 0.397       | Temp ***  | Temp.2 *** | NO2 *** | Chl $a$ * |           |          |
| Composition | Norway    | 0.605       | Temp ***  | Temp.2 *** | NO2 *** | pH ***    |           |          |
| Composition | China     | 0.880       | Temp.2 ***  | pH ***     | NH4 *** | NO3 **    | ADD.NO3 * | Copies * |

143 <sup>§</sup> Standardized partial regression coefficients, \*\*\* $P < 0.001$ ; \*\* $P < 0.01$ ; \* $P < 0.05$ .  
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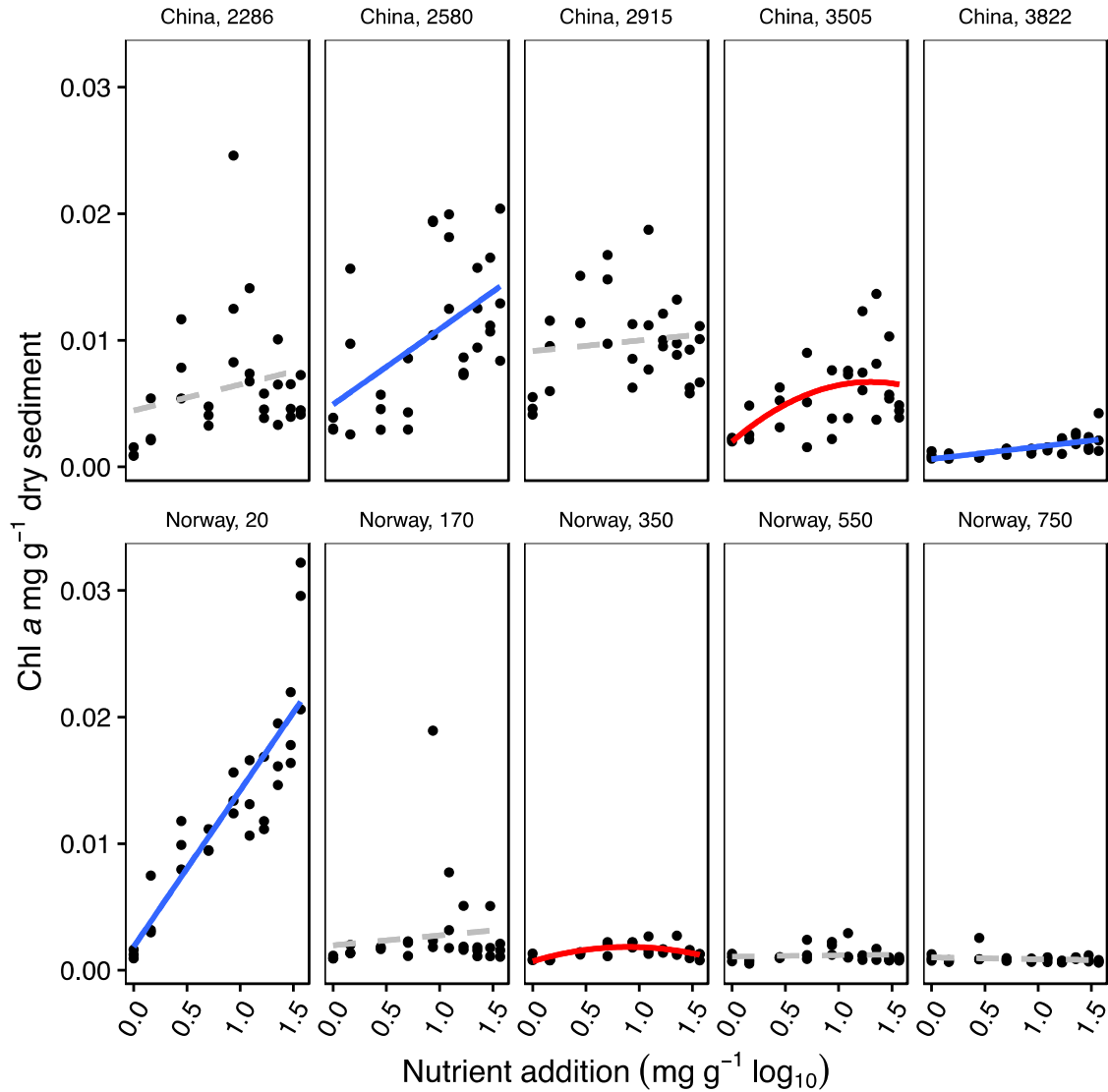
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172 **Supplementary Figure 1.** The relationships between sediment Chlorophyll *a* and  
 173 temperature. The relationships were analyzed for each nutrient enrichment level and  
 174 location, and we used the inverse absolute temperature ( $1/kT$ ), where  $k$  is Boltzman's  
 175 constant  $8.62 \times 10^{-5} \text{ eV K}^{-1}$ , and  $T$  is absolute temperature in Kelvin. These relationships  
 176 are fitted by linear and quadratic models, the significance of which ( $P < 0.05$ , F-test) are  
 177 shown with blue and red lines (solid or dotted). The better model, shown in solid line,  
 178 was selected based on lower value of Akaike's information criterion. Gray dotted lines  
 179 indicate non-significant relationships of a linear model ( $P > 0.05$ , F-test).

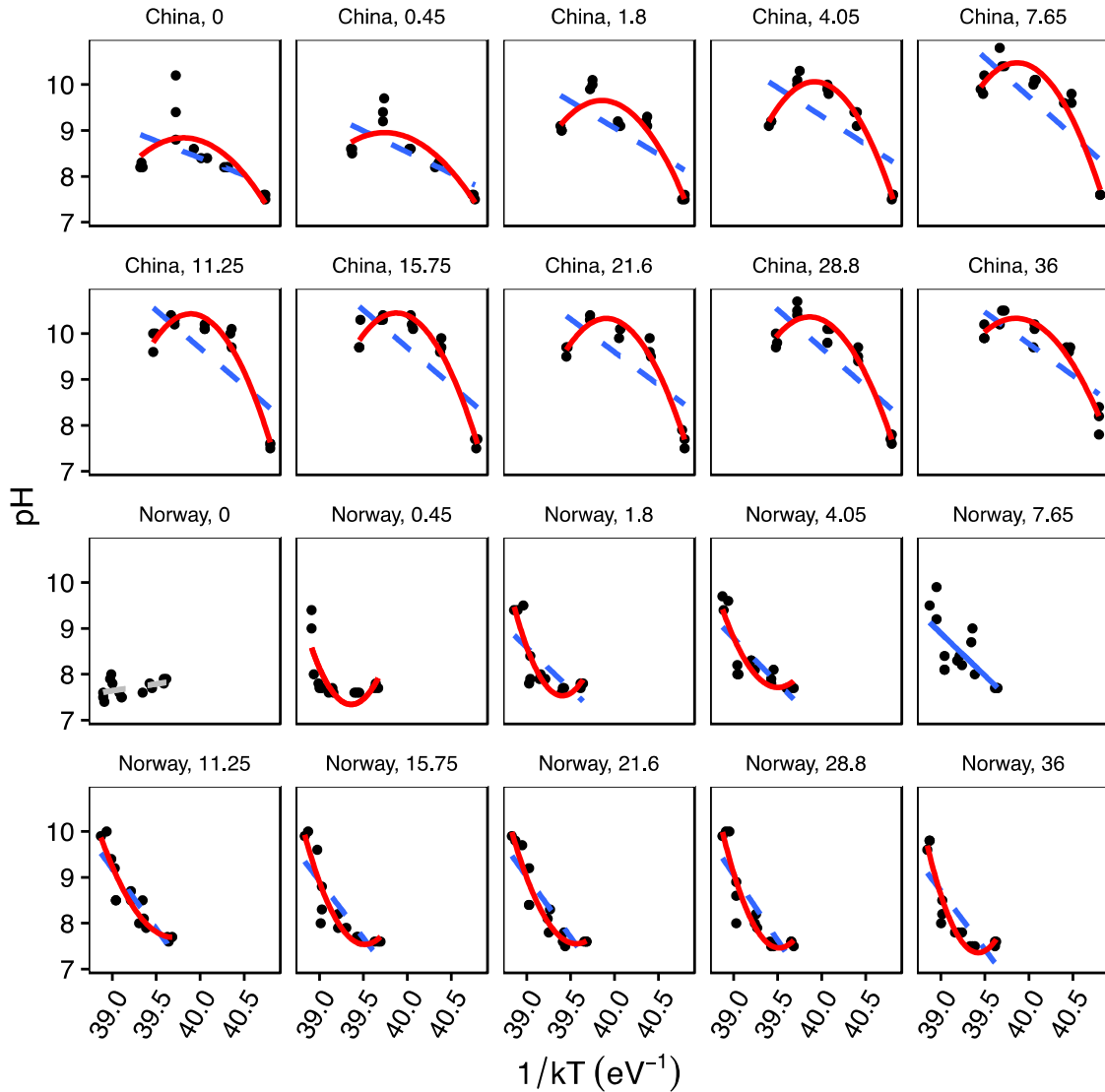
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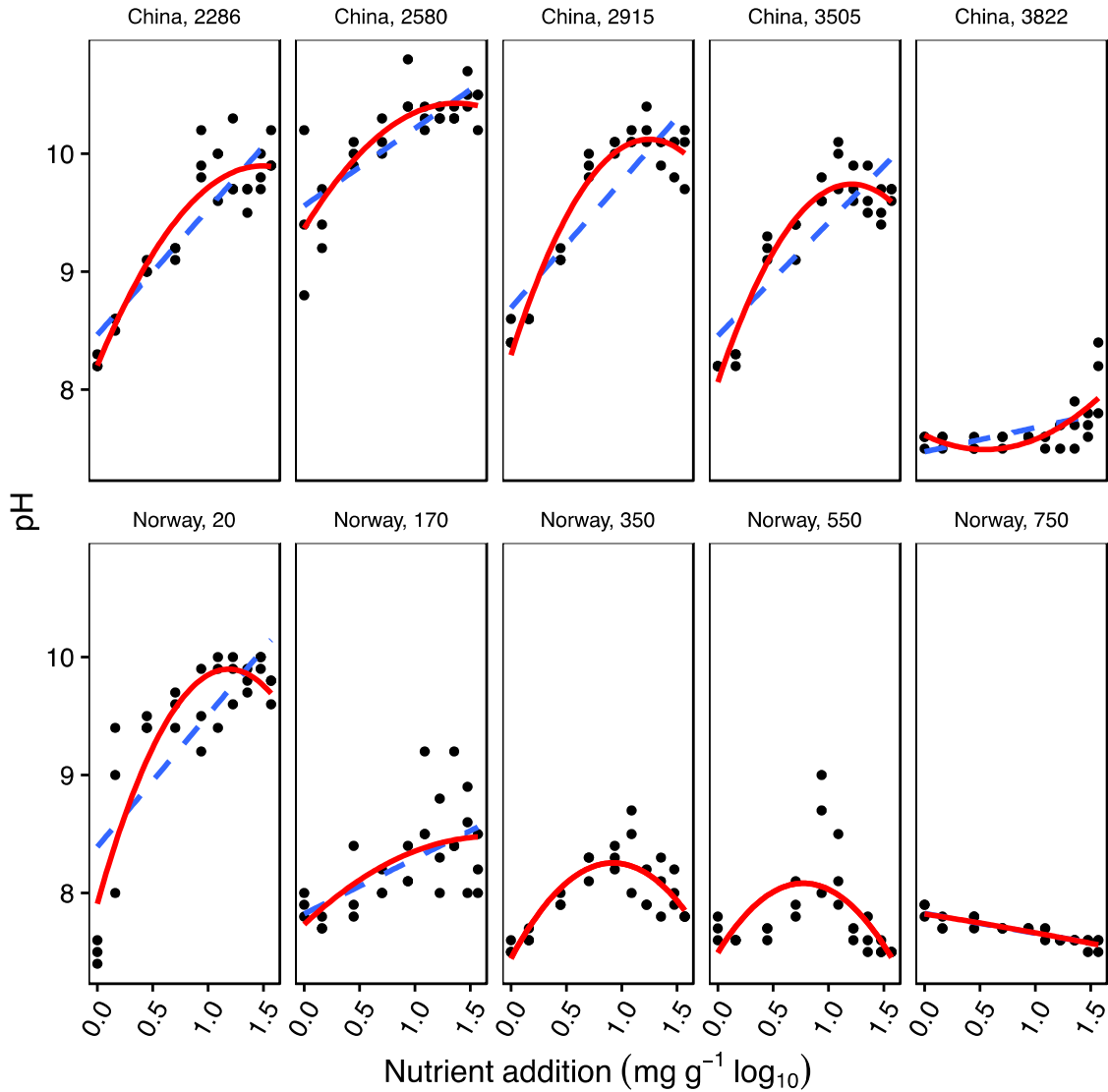
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**Supplementary Figure 2.** The relationships between sediment Chlorophyll *a* and nutrient enrichment. These relationships for each elevation band were fitted by linear and quadratic models, the significance of which ( $P < 0.05$ , F-test) are shown with blue and red lines (solid or dotted). The better model, shown in solid line, was selected based on lower value of Akaike's information criterion. Gray dotted lines indicate non-significant relationships of a linear model ( $P > 0.05$ , F-test).



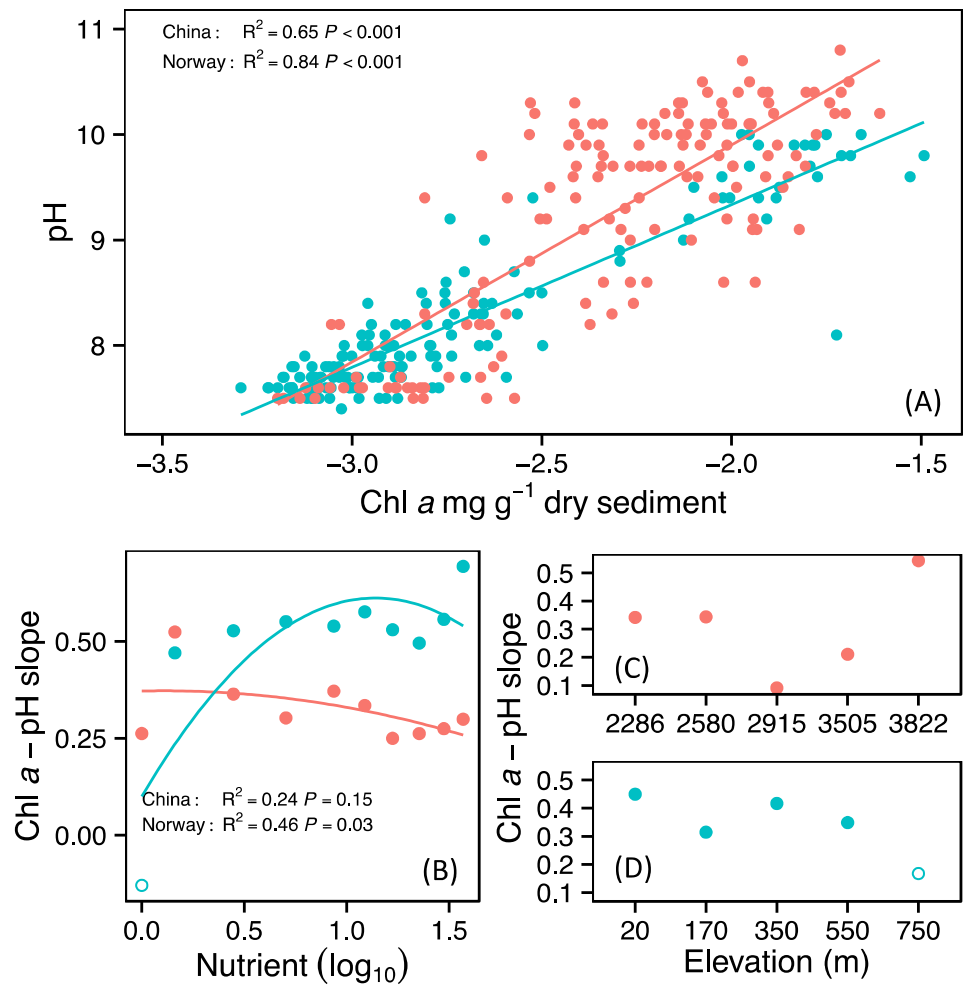
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**Supplementary Figure 3.** The relationships between overlying water pH and temperature. These relationships for each nutrient enrichment and location were fitted by linear and quadratic models, the significance of which ( $P < 0.05$ , F-test) are shown with blue and red lines (solid or dotted). The better model, shown in solid line, was selected based on lower value of Akaike's information criterion. Gray dotted lines indicate non-significant relationships of a linear model ( $P > 0.05$ , F-test).



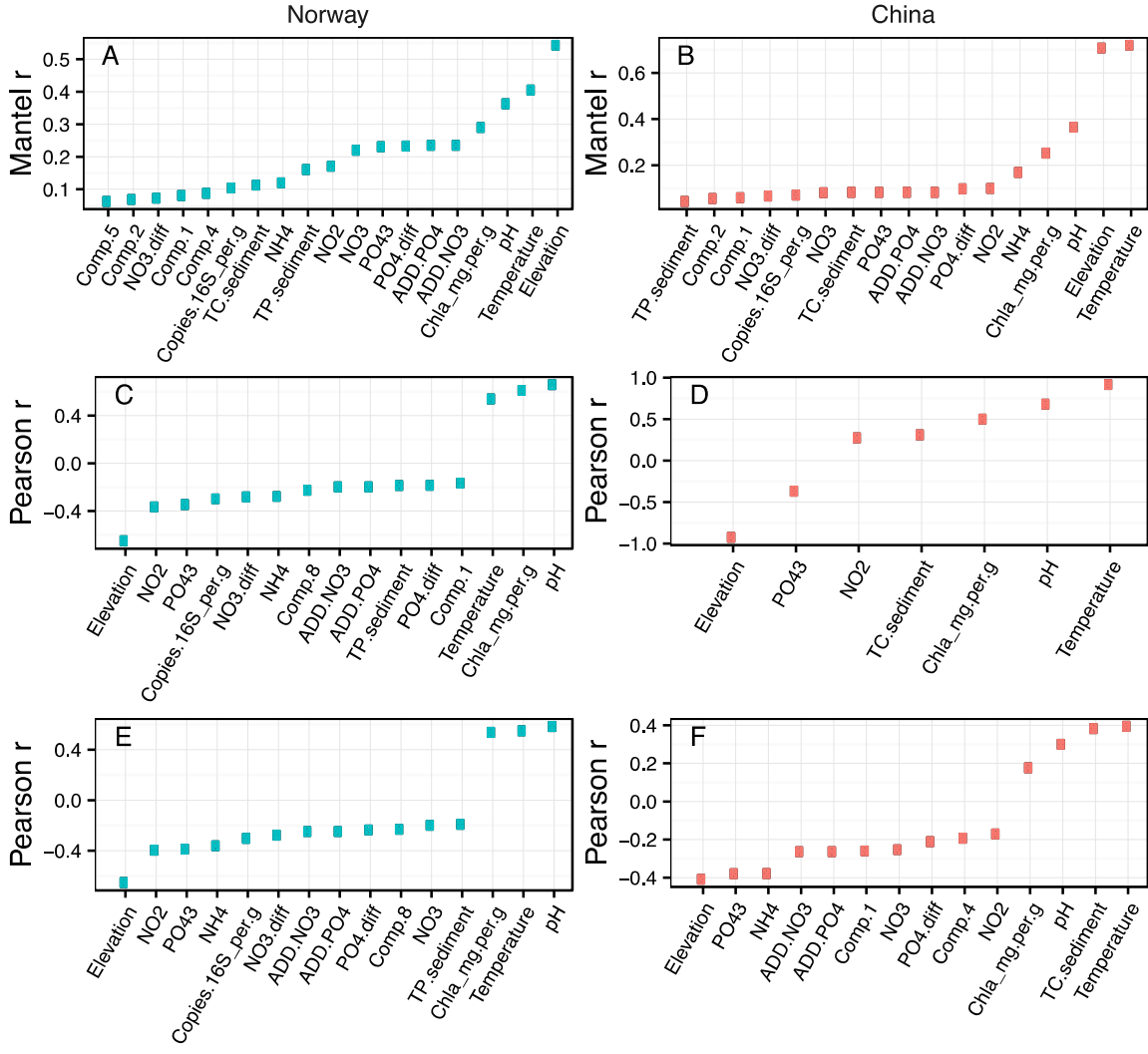
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**Supplementary Figure 4.** The relationships between overlying water pH and nutrient enrichment. These relationships for each elevation band were fitted by linear and quadratic models, the significance of which ( $P < 0.05$ , F-test) are shown with blue and red lines (solid or dotted). The better model, shown in solid line, was selected based on lower value of Akaike's information criterion. Gray dotted lines indicate non-significant relationships of a linear model ( $P > 0.05$ , F-test).

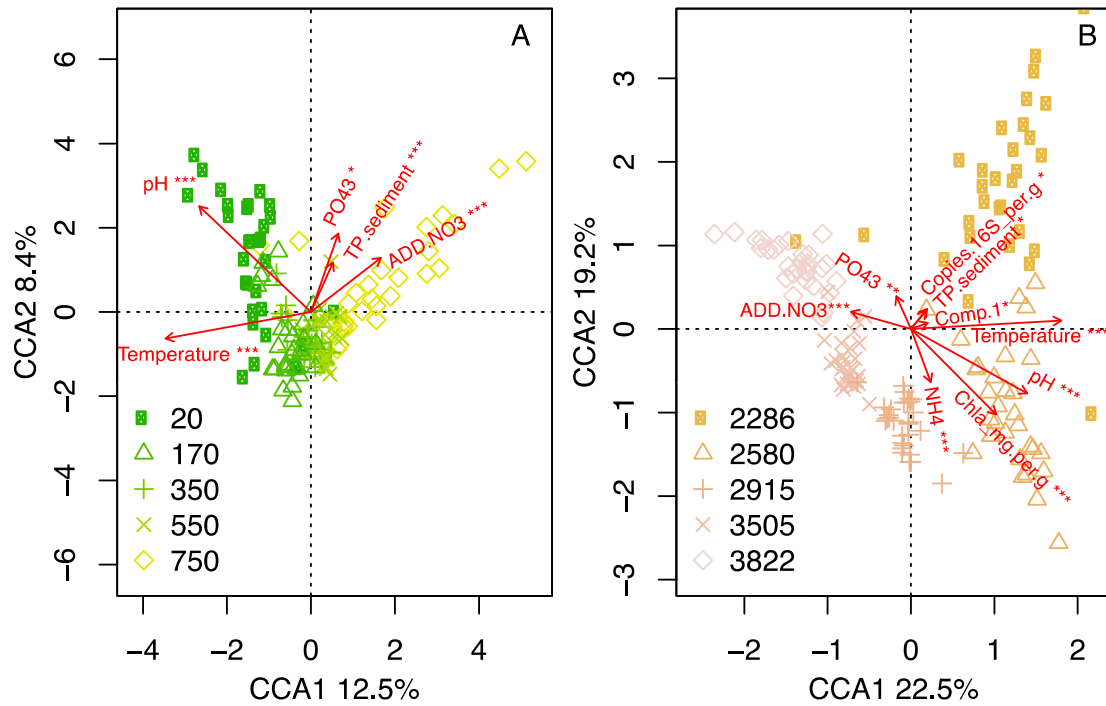


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**Supplementary Figure 5.** The relationships between sediment Chlorophyll *a* and overlying water pH. The relationships were fitted with linear models and significances were examined with F-test. (A) The linear relationship between Chlorophyll *a* and pH for each region. (B) The slopes of Chlorophyll *a*-pH relationships along nutrient enrichment. (C, D) The Chlorophyll *a*-pH relationships along elevational gradients. Solid dots indicate the significant ( $P < 0.05$ , F-test) relationships (B-D). Red colour for China and blue colour for Norway.

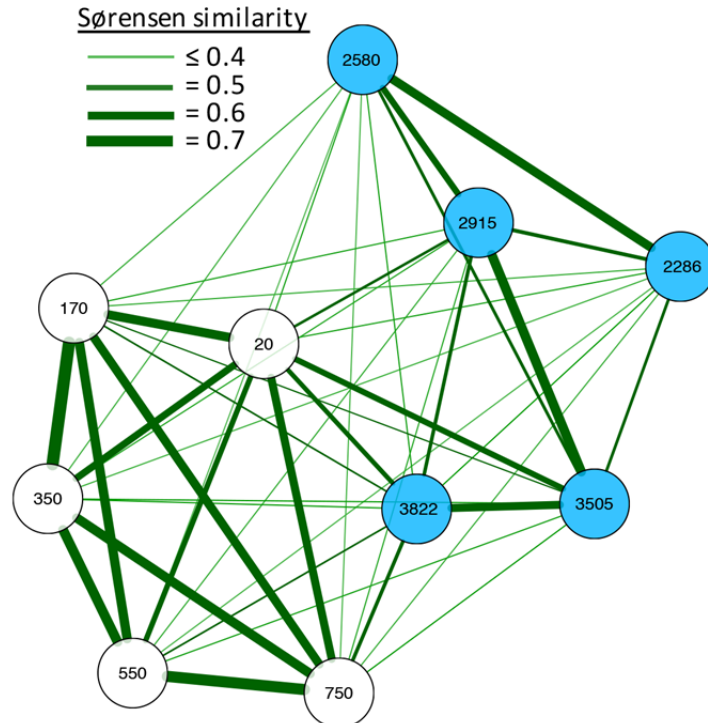


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 215 **Supplementary Figure 6.** Correlations between environmental variables and bacterial  
 216 biodiversity. We only included the significant ( $P < 0.05$ ) environmental variables related  
 217 to community similarity based on Mantel test (A, B), the first axis of non-metric  
 218 multidimensional scaling of the communities with Pearson correlations (C, D) and  
 219 species richness with Pearson correlations (E, F). Temperature: Water temperature in situ.  
 220 pH: Water pH. Chla\_mg.per.g: Chl *a* in the sediments ( $\text{mg}\cdot\text{g}^{-1}$ ). NH<sub>4</sub>, NO<sub>2</sub>, and NO<sub>3</sub>:  
 221 NH<sub>4</sub><sup>+</sup>, NO<sub>2</sub><sup>-</sup>, and NO<sub>3</sub><sup>-</sup> in the water at the end of experiments. ADD.NO<sub>3</sub> and ADD.PO<sub>4</sub>:  
 222 initial NO<sub>3</sub><sup>-</sup> and PO<sub>4</sub><sup>3+</sup> added to the microcosms. NO<sub>3</sub>.diff and PO<sub>4</sub>.diff: NO<sub>3</sub><sup>-</sup> and PO<sub>4</sub><sup>3+</sup>  
 223 differences between the start and end of the experiments. TC.sediment and TP.sediment:  
 224 total organic carbon and total phosphorus in the sediments at the end of experiments.  
 225 Copies.16S\_per.g: copy number of 16S rRNA genes qualified by real-time qPCR (copy  
 226 number per gram dry sediments). Comp.1, Comp.2, Comp.3, Comp.4 and Comp.5:  
 227 principal components of metal concentrations (such as K, Na, Ca, Mg, Al, Fe, Zn, Cu, Cr  
 228 and Ni).  
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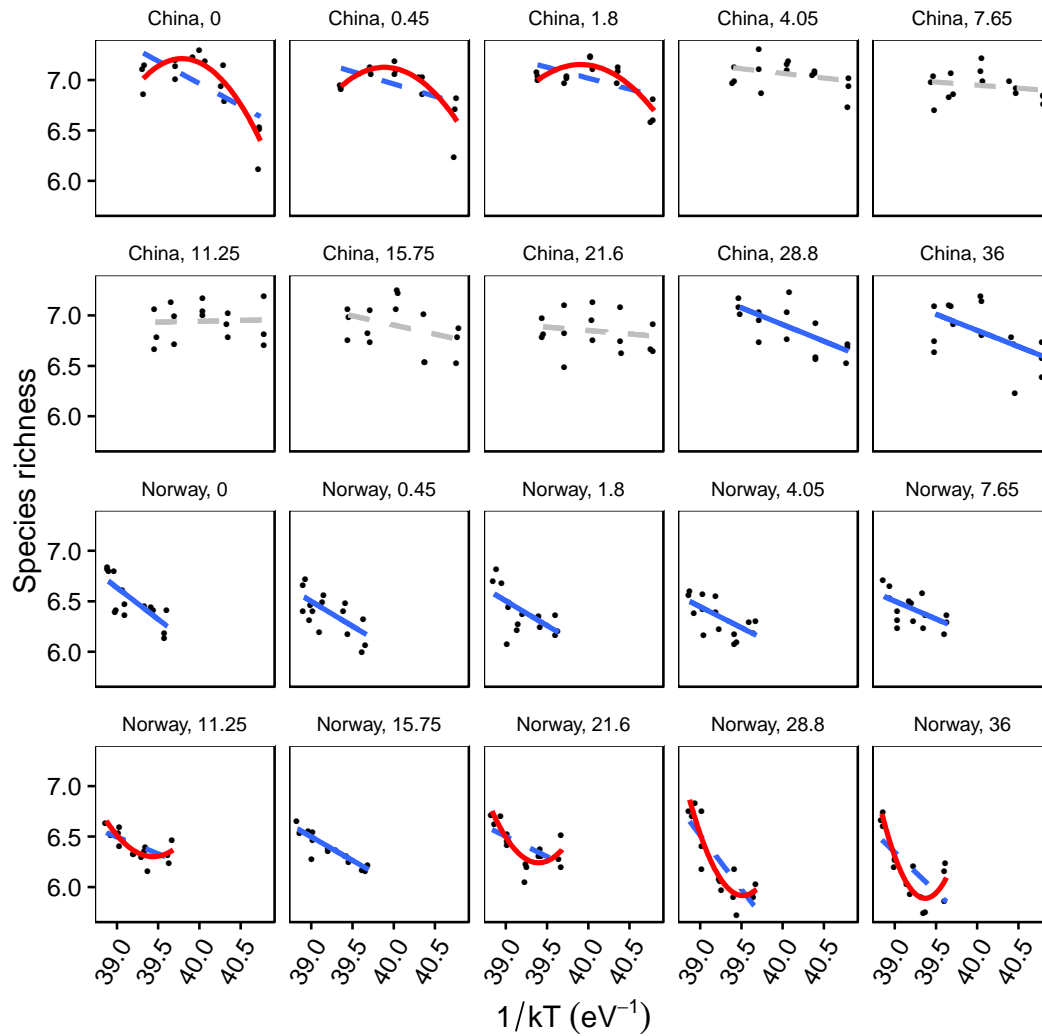
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**Supplementary Figure 7.** Canonical correspondence analysis for the bacterial communities. A is for Norway, and B is for China. Detrended correspondence analysis was used to determine that the gradient length of the species abundance matrix is larger than 2.0 along the first axis, thus implying a unimodal species responses. Thus, considering the long environmental gradients (especially for nutrients), we applied canonical correspondence analysis (CCA) to examine the relationships between bacterial community composition and explanatory variables. All environmental variables, except pH, were log-transformed [by  $\log(X + 1)$  or  $\log(1000X + 1)$ ]. The abbreviations of environmental variables are given in Supplementary Figure 6. The elevations (units: m a.s.l) were shown with different colors. The significance of variables was assessed with permutation test ( $n = 1,000$ ). \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ .



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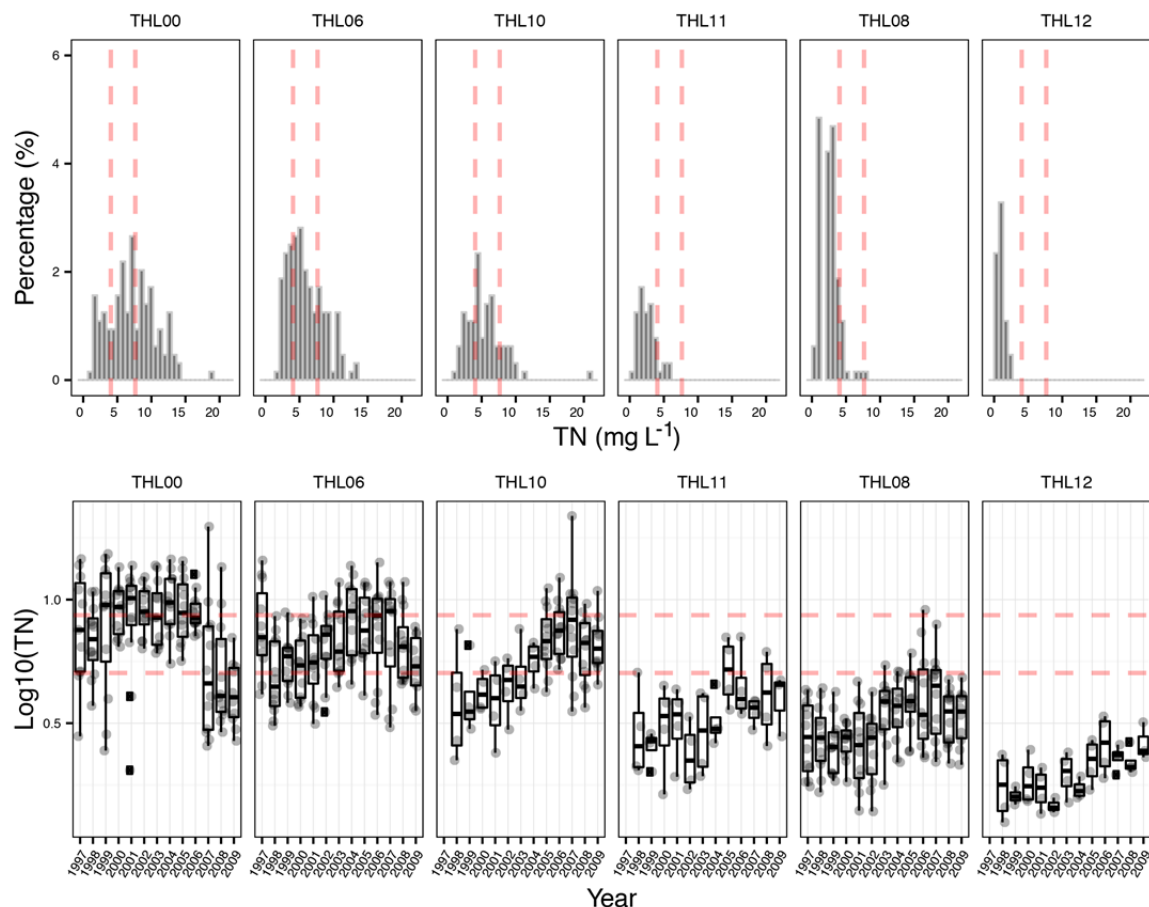
**Supplementary Figure 8.** The community Sørensen similarity between elevations of the two mountains. Blue: The Laojun Mountain, China. White: The Balggesvarri Mountain, Norway. The elevations (unit: m a.s.l.) of each site are shown in the nodes. Thicker edges indicate high Sørensen similarity in bacterial composition between elevations within and between regions.



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**Supplementary Figure 9.** The relationships between temperature and bacterial species richness. These relationships for each nutrient enrichment and location were fitted by linear and quadratic models, the significance of which ( $P < 0.05$ , F-test) are shown with blue and red lines (solid or dotted). Species richness was log-transformed, and we used the inverse absolute temperature ( $1/kT$ ), where  $k$  is Boltzman's constant  $8.62 \times 10^{-5} \text{ eV K}^{-1}$ , and  $T$  is absolute temperature in Kelvin. The better model, shown in solid line, was selected based on lower value of Akaike's information criterion. Gray dotted lines indicate non-significant relationships of a linear model ( $P > 0.05$ , F-test).



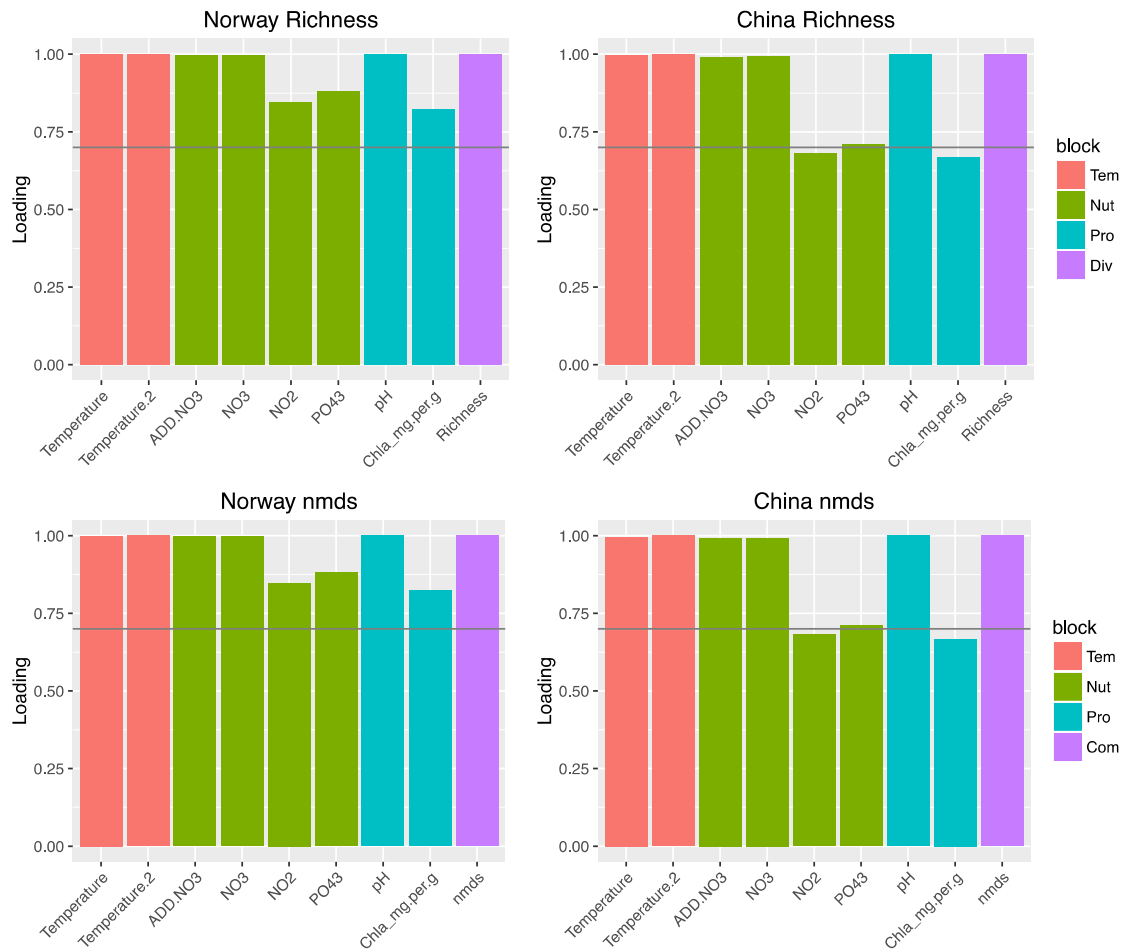


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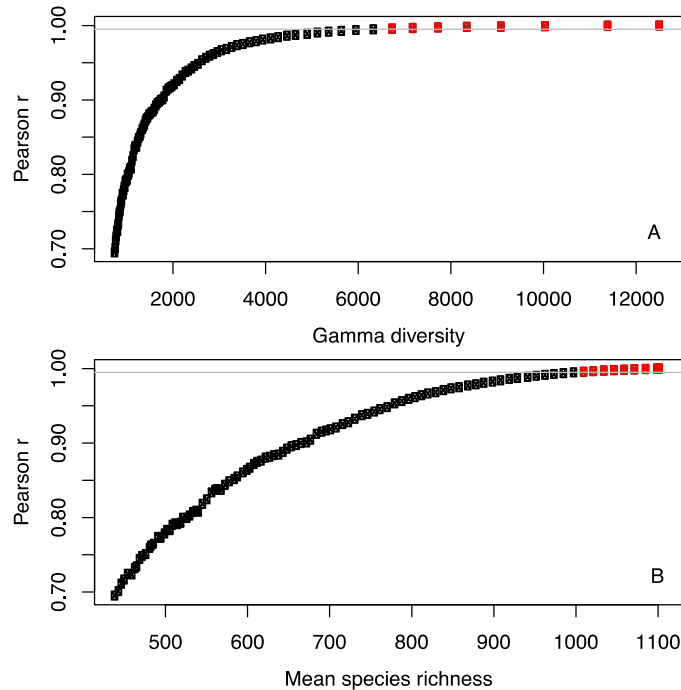
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**Supplementary Figure 10.** The nutrient concentrations in Taihu Lake, China, in the  
 262 years 1997-2015. We considered total nitrogen (TN), which was measured monthly by  
 263 CNERN, Taihu Laboratory for Lake Ecosystem Research. We selected six monitoring  
 264 sites from river mouth (THL00, THL06, THL10, and THL11), lake center (THL08), and  
 265 macrophyte-dominated region (THL12), which present clear nutrient variations  
 266 geographically. Upper panel: The relative frequency histogram of TN concentrations for  
 267 each site. Lower panel: The boxplot of TN through the years for each site. The red dotted  
 268 lines indicate the intermediate nutrient enrichment observed in this study: 4.05 and 7.65  
 269  $\text{mg N L}^{-1} \text{NO}_3^-$ , respectively. These results showed that observed TN concentrations in  
 270 Taihu Lake, exhibiting a high spatial heterogeneity within the lake, are frequently higher  
 271 than the intermediate nutrient enrichment values in our experiments (i.e.,  $\sim 4.05\text{-}7.65 \text{ mg}$   
 272  $\text{N L}^{-1} \text{NO}_3^-$ ).

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275 **Supplementary Figure 11.** The loadings of observed variables for the latent variables in  
 276 path models. The observed variables were used for the partial least squares path modeling  
 277 (Fig. 4), and we showed their loadings for the latent variables. Most of the loadings were  
 278 selected with the threshold 0.7, as suggested in previous studies<sup>24</sup>. The observed  
 279 variables included for each latent variable were shown in difference colors. There latent  
 280 variables are Temperature (Tem), Nutrient (Nut), Productivity (Pro) and Diversity (Div)  
 281 or Community composition (Com). Temperature: measured water temperature.  
 282 Temperature.2: the squared value of measured water temperature. ADD.NO3: the initially  
 283 added  $\text{NO}_3^-$ . NO3, NO2, and pH: water  $\text{NO}_3^-$  and  $\text{NO}_2^-$ , and pH, respectively.  
 284 Chla\_mg.per.g: sediment Chl *a* ( $\text{mg}\cdot\text{g}^{-1}$ ).  
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287 **Supplementary Figure 12.** The effects of removal of singleton or other rare species on  
 288 species richness.

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290 We removed the singletons or other rare species from the full community data set  
 291 (that is, with singletons and 300 total samples). Singleton was defined as a read with a  
 292 sequence that is present exactly once, i.e. is unique among the all sequence reads. The  
 293 rare species was defined continuously as the species occurring in only 1 sample, 2, 3, ..., ,  
 294 or 100 samples. For each community with species removal, gamma diversity (i.e., x-axis  
 295 in panel A) is the total OTU number of full data set, and mean species richness (i.e., x-  
 296 axis in panel B) is the average species richness of the 300 samples. Gamma diversity and  
 297 mean species richness were highest with singletons included, followed by the removal of  
 298 the rare species (i.e., occurring in only 1 sample, 2, 3, ..., , or 100 samples).

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299 For the obtained diversity (A: gamma diversity; B: mean species richness), we  
 300 calculated the Pearson correlations (i.e., y-axis in panels A and B) between diversity of  
 301 the communities without singletons and that of the communities with singletons or the  
 302 communities without rare species. All Pearson correlations were significant ( $P < 0.01$ ),  
 303 and Pearson  $r$  decreased continuously along the gradients of both gamma diversity (A) and  
 304 mean species richness (B). For the community data (in red color) with the removal of  
 305 singletons and rare species occurring in less than 6 samples, the Pearson correlations were  
 306 quite similar, and all higher than 0.995, indicated by gray horizontal lines (A, B).

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307 These results showed that the effects of OTU removal on the values of species  
 308 richness were low when the singletons and other rare species were excluded.  
 309 Furthermore, it is common in microbial studies to remove singletons before detailed  
 310 ecological analyses, and it is also highly recommended in microbial analysis pipelines  
 311 (such as usearch, <http://www.drive5.com/usearch/manual/singletons.html>; Qiime,  
 312 [http://qiime.org/tutorials/open\\_reference\\_illumina\\_processing.html](http://qiime.org/tutorials/open_reference_illumina_processing.html)). Thus, to keep our  
 313 reported results as simple as possible, we finally used the OTU tables of bacterial  
 community without singletons.

314 **Supplementary References**

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