Vertical partitioning of freshwater bacterioplankton community in a deep mesotrophic lake with a fully oxygenated hypolimnion (Lake Biwa, Japan)

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Running title: Bacterial community in oxygenated hypolimnion

Originality-Significance Statement

Despite its ecological importance, an oxygenated hypolimnion is a largely understudied system in freshwater microbial ecology compared with the extensively studied epilimnion. This study revealed the previously unreported diversity of prokaryotic communities in deep oxygenated waters of a freshwater lake (Lake Biwa, Japan). We used high-throughput sequencing of the 16S rRNA gene amplicon from samples taken from the entire water column over a period of 15 months. Such a comprehensive investigation in a pelagic freshwater system is unprecedented, thereby enabling us to identify the number of previously understudied bacterioplankton groups that were hypolimnion habitat specialists.

SUMMARY

In freshwater microbial ecology, extensive studies are attempting to characterize the vast majority of uncultivated bacterioplankton taxa. However, these studies mainly focus on the epilimnion and little is known regarding the bacterioplankton inhabiting the hypolimnion of deep holomictic lakes, despite its biogeochemical importance. In this study, we investigated the bacterioplankton community composition in a deep freshwater lake with a fully oxygenated hypolimnion (Lake Biwa, Japan) using high-throughput 16S rRNA gene amplicon sequencing. Sampling at a pelagic site over 15 months throughout the water column revealed that the community composition in the hypolimnion was significantly different from that in the epilimnion. The bacterial community in the hypolimnion was composed of groups dominating in the whole water layer (e.g., bacI-A1 and acI-B1) and groups that were hypolimnion habitat specialists. Among the hypolimnion specialists, members of *Chloroflexi* and *Planctomycetes* were highly represented (e.g., CL500-11, CL500-15, and CL500-37), followed by members of *Acidobacteria*, *Chlorobi*, and nitrifiers (e.g., *Ca.* Nitrosoarchaeum, *Nitrosospira*, and *Nitrospira*). This study identified the number of previously understudied taxa dominating the deep aerobic freshwater habitat, suggesting that the biogeochemical cycling there is driven by the microbial community that are different from that in the

epilimnion.

INTRODUCTION

With the development of cultivation-independent molecular tools, our understanding of the ecology of freshwater bacterioplankton has experienced unprecedented growth. In particular, constructing comprehensive and consensus knowledge of globally distributed phylogenetic groups (Glöckner *et al.*, 2000; Zwart *et al.*, 2002) has facilitated the identification of the vast majority of uncultured taxa. In the latest taxonomic framework, a fine taxonomic unit named tribe, which contains >97% sequence identity over the full length of the 16S rRNA gene, was proposed (Newton *et al.*, 2011). Following this nomenclature, studies are attempting to reveal the eco-physiology of individual uncultivated tribes to understand their roles in biogeochemical cycling and microbial food webs (Eckert *et al.*, 2012; Eiler *et al.*, 2012; Salcher *et al.*, 2013; Tada and Grossart, 2014). Such a fine phylogenetic resolution is necessary, because ecophysiological characteristics are often different even among closely related tribes, as was found among members of acI (Garcia *et al.*, 2012; Ghylin *et al.*, 2014), *Polynucleobacter* (Hahn *et al.*, 2012; Watanabe *et al.*, 2012), and *Limnohabitans* (Kasalický *et al.*, 2013; Šimek *et al.*, 2014).

In lakes with deep, holomictic and oligo- to mesotrophic conditions, the hypolimnion can remain oxygenated throughout the stratified period. In such lakes,

62 the oxygenated hypolimnion constitutes a large portion of the lake volume and serves
63 as an important layer for the microbial remineralization of organic matter derived from
64 the epilimnion (Wetzel, 2001). Despite its importance, available knowledge on
65 freshwater bacterioplankton largely focuses on the epilimnetic community (Newton *et*
66 *al.*, 2011). While there is growing evidence for bacterioplankton groups that exclusively
67 occur in the oxygenated hypolimnion (Urbach *et al.*, 2001; Pollet *et al.*, 2011; Okazaki
68 *et al.*, 2013; Callieri *et al.*, 2015), only few studies have conducted comprehensive
69 community analysis (i.e., the Sanger sequencing of environmental 16S rRNA gene
70 clones) in the realm (Glöckner *et al.*, 2000; Urbach *et al.*, 2001; Bel'kova *et al.*, 2003;
71 Humbert *et al.*, 2009). The available knowledge is currently insufficient to reach a firm
72 understanding of bacterioplankton inhabiting the oxygenated hypolimnion.
73 The present study aimed to acquire an overview of the bacterioplankton
74 community composition in an oxygenated hypolimnion using high-throughput
75 sequencing of the 16S rRNA gene amplicon. Samples were spatiotemporally taken in a
76 mesotrophic, holomictic (monomictic) deep lake with a fully oxygenated hypolimnion
77 (Lake Biwa, Japan), and the sequences were analyzed with a fine phylogenetic
78 resolution (i.e., distinguishing <97% identical sequences). This allowed us to
79 demonstrate that the bacterioplankton community in the hypolimnion is significantly

different from that in the epilimnion and to characterize groups showing hypolimnion-specific distribution patterns, which have eluded researchers due to their absence in the epilimnion.

RESULTS AND DISCUSSION

The high-throughput sequencing produced 133,266 reads of the V4 region of the 16S rRNA gene amplicon from the 45 samples taken in 15 months at three different depths (5, 50, and 72 m) at a pelagic site of Lake Biwa (Fig. 1). Our analysis generated 859 OTUs that are affiliated with bacteria and four OTUs that are affiliated with archaea. The list of representative sequences of each OTU and the read proportion of each OTU in all 45 samples are available in Supplementary Table S1 and S2, respectively. The details of the sampling, sequencing, and data analysis procedures are described in the Supplementary Experimental Procedures. Nucleotide sequence data reported in this study are available in the Sequence Read Archive database under accession numbers DRX048052–DRX048095 (BioProject: PRJDB4503).

Analysis of the Shannon diversity index in each sample (Fig. 2A) indicated that the alpha diversity between the epilimnion and hypolimnion samples during the stratification period (April to December; Fig. 1) was not significantly different ($p = 0.156$; Wilcoxon test). This means that the phylogenetic richness and the evenness of

98 the bacterioplankton community in both water layers were generally comparable.
99 However, an analysis of the beta diversity between the samples by non-metric
100 multidimensional scaling (NMDS) revealed that the community composition in the
101 epilimnion and hypolimnion were different from each other (Fig. 2B). The samples from
102 50 and 72 m were plotted closely together and showed similar succession patterns, and
103 the highest divergence from the epilimnetic samples occurred from October to
104 December (Fig. 2B). In the epilimnion, samples taken from August to October showed
105 the highest divergence, and the samples from the mixing period (January–March) were
106 positioned between the plots from both layers (Fig. 2B). These results indicated that
107 the thermocline separates the bacterioplankton community. In Lake Biwa, the strongly
108 stratified mid-summer epilimnion is characterized as the nutrient deficient (Kim *et al.*,
109 2006) and DOC-rich (Maki *et al.*, 2010; Thottathil *et al.*, 2013) clear water phase. Such
110 a severe condition may have selected groups that were acclimatized to this
111 environment and resulted in characteristic communities in the epilimnion from August
112 to October (Fig. 2B). On the other hand, in the hypolimnion, an accumulation of semi-
113 labile (Maki *et al.*, 2010) or humic-like (Thottathil *et al.*, 2013) refractory DOM
114 throughout the stratification period has been suggested. The selection of the
115 bacterioplankton that is capable of utilizing this less bioavailable DOM that

116 accumulates in the hypolimnion may have resulted in the characteristic communities
117 that were observed at the end of the stratified period (Fig. 2B).
118 At the phylum-level of phylogenetic resolution, *Bacteroidete*s and
119 *Actinobacteria* were predominant in the epilimnion during stratification, accounting for
120 37.3%–59.5% and 8.9%–39.3% of the total amplicon reads, respectively (Fig. 3A). The
121 *Chloroflexi* and *Planctomycetes* became relatively abundant in the hypolimnion during
122 stratification, ranging from 1.3% to 31.5% and 3.4% to 23.1% respectively (Fig. 3A). It
123 should be noted that these data potentially include some biases and should be
124 considered with caution. First of all, the total prokaryotic abundance by season and
125 depth varied more than six fold in this study (Fig. 1). Consequently, the proportion of
126 amplicon reads should not be directly related to the abundance. Furthermore, the value
127 is potentially biased by the processes of DNA extraction and amplification (McCarthy
128 et al., 2015; von Wintzingerode et al., 1997) and by uneven copy numbers of the 16S
129 rRNA gene among target organisms (Farrelly et al., 1995). For example, according to
130 the rrnDB database (Stoddard *et al.*, 2015), the *Chitinophagaceae*, which includes
131 members of bacI, one of the most represented *Bacteroidetes* in the present study, had
132 as high as 3–6 copies of the rrn operon per chromosome, which may have resulted in
133 the high read proportion of *Bacteroidetes* in our data (Fig. 3). Nevertheless, we still

found clear patterns, i.e., the same taxa could be either highly represented (e.g., > 5% of the total reads) or nearly absent (e.g., < 0.5% of the total reads) among different samples. The pattern is particularly clear when we compare data between the epilimnion and hypolimnion during stratification, as shown in the beta diversity analysis (Fig. 2B).

To investigate the difference between the epilimnetic and hypolimnetic communities, data from each layer during the stratified period were pooled (Fig. 3B). Then the 30 predominant OTUs in each layer (a total of 49 OTUs because 11 OTUs were shared by both water layers) were identified (the procedures are described in the Supplementary Experimental Procedures). Consequently, 26 OTUs were identified by following the taxonomic framework by Newton *et al.* (2011), and the other 23 OTUs were named following the name of the BLAST hit sequence that had the highest and >99% identity (Table S2).

The dominant members of the epilimnetic community were affiliated with previously known groups: the acI-B1, acI-C2, acI-A7, and Iluma-A1 tribes (*Actinobacteria*), the bacI-A1 tribe, the bac-II-A clade, and the bacV and bacI lineages (*Bacteroidetes*) (Newton *et al.*, 2011), and the LimC cluster of *Limnohabitans* (*Betaproteobacteria*) (Kasalický *et al.*, 2013) (Fig. 3B). Other well-studied tribes of

LD12 (Salcher *et al.*, 2011a; Heinrich *et al.*, 2013), PnecB of the *Polynucleobacter* (Wu and Hahn, 2006a; Salcher *et al.*, 2011b; Hahn *et al.*, 2012), and LD28 (Salcher *et al.*, 2015) were less abundant but ranked as one of the representative groups of the epilimnetic community (Fig. 3B). In the hypolimnetic community, while bacI-A1, acI-B1, acI-A7, Iluma-A1, and LimC showed high proportion, CL500-11 of the *Chloroflexi* (Urbach *et al.*, 2001) was the most represented (Fig. 3B). In addition, CL500-15, CL500-37, CL500-3 (*Planctomycetes*) (Urbach *et al.*, 2001), LiUU-3-374 (*Acidobacteria*), and LiUU-3-330 (*Chlorobi*) (Eiler and Bertilsson, 2004) were highly represented in the hypolimnetic community (Fig. 3B).

We further inspected the vertical preferences of the individual groups based on their read percentage (Fig. 4) and patterns of appearance in each layer (Fig. 5). Members of the acI lineage were reported to consume the relatively bioavailable DOM, such as amino acids, polyamines, di- and oligopeptides, and carbohydrates (Garcia *et al.*, 2012; Salcher *et al.*, 2013; Ghylin *et al.*, 2014). Other studies have proposed an "opportunistic" nature of *Bacteroidetes* (Eiler and Bertilsson, 2007; Zeder *et al.*, 2009; Salcher, 2013) and *Limnohabitans* (Šimek *et al.*, 2011, 2014; Salcher, 2013), which quickly respond to phytoplankton blooms by rapidly exploiting fresh photosynthetic products. The fact that bacI-A1, acI-A1, acI-A7, and LimC could sustain their

population, even in the dark stratified hypolimnion (Fig. 4, 5), suggests that they are not absolutely dependent on fresh and labile photosynthetic products. It is possible that they are passively transported from the epilimnion as particle-associated bacteria because high sinking fluxes of blooming phytoplankton (Kagami *et al.*, 2006) and cyanobacteria (Takasu *et al.*, 2015) have been reported in the lake. Because we only used the V4 region of the 16S rRNA gene for the analysis, we may not be able to detect the difference of the genotype in the same OTU between the epilimnion and hypolimnion. Nonetheless, our data demonstrated that the groups ubiquitous in the surface freshwater habitat could also be predominant in the deeper (> 50 m) aerobic layers.

The epilimnion-specific distribution patterns of LD12 and PnecB (Fig. 4, 5) were in agreement with previously reported vertical profiles (Wu and Hahn, 2006b; Salcher *et al.*, 2011a, 2011b). The present data demonstrated that several other groups (e.g., acI-C2 and bacII-A) also showed preferences to the epilimnion (Fig. 4, 5). They presumably consume substrate that is only available in the euphotic layer (e.g., labile photosynthetic products from phytoplankton) or prefer the higher temperature in the epilimnion (Fig. 1). It is also possible that they possess a light-driven metabolic pathway and prefer the euphotic surface water. The presence of rhodopsin genes among

188 members of acI, *Bacteroidetes*, LD12, and *Polynucleobacter* has been suggested
189 (Atamna-Ismaeel *et al.*, 2008; Sharma *et al.*, 2009; Martinez-Garcia *et al.*, 2012; Ghylin
190 *et al.*, 2014).

191 The dominance of *Chloroflexi* CL500-11 bacterioplankton in the hypolimnion
192 during stratification in the lake (Fig. 3B, 4) has been previously reported using
193 fluorescent *in situ* hybridization (Okazaki *et al.*, 2013). In this study, we further
194 identified *Planctomycetes* CL500-15, CL500-37, and CL500-3 as highly represented
195 hypolimnion-specific groups (Fig. 3B, 4, 5). All the four groups (with the prefix
196 "CL500-") were originally reported as representative bacterioplankton in the
197 oxygenated hypolimnion (500-m deep) of the ultraoligotrophic Crater Lake (USA)
198 during the stratified period (Urbach *et al.*, 2001, 2007). This suggests that these groups
199 are not endemic but are commonly distributed in the oxygenated hypolimnion of
200 freshwater lakes.

201 CL500-15, a member of an uncultured class OM190, was highly represented in
202 the hypolimnion, with as high as 11.1% of the total amplicon reads in August at 72 m
203 (Fig. 4). To the best of our knowledge, this is the first study of such a high frequency of
204 CL500-15 detection, although small numbers of clone library sequences were found in
205 the oxygenated hypolimnion of Crater Lake (Urbach *et al.*, 2001), Lake Annecy, and

206 Lake Bourget (France) (Pollet *et al.*, 2011) (referred to as OTU45 in the literature).
207 CL500-37 and CL500-3 are affiliated with the *Phycisphaeraceae*, with an 85% identity
208 in 611 bp of the original 16S rRNA gene partial sequence [note that CL500-37 was
209 regarded as a member of the CL500-3 cluster in the studies in Crater Lake (Urbach *et*
210 *al.*, 2001, 2007)]. It is remarkable that neither were found in the oxygenated
211 hypolimnion of Lake Annecy or Bourget (Pollet *et al.*, 2011), while both were present in
212 the winter mixing water of the deep oligotrophic Lake Stechlin (Germany) (Tada and
213 Grossart, 2014) (referred to as OTU22 and OTU10 in the literature, respectively). In
214 addition, four other *Planctomycetes* belonging to the *Planctomycetaceae* were also
215 found as hypolimnion-specific groups: P-OTU1, P-OTU76, P-OTU31, and CL500-52
216 (Fig. 3B, 4, 5). In the oxygenated hypolimnion of Lake Annecy and Bourget, P-OTU1
217 and CL500-52 (referred to as OTU2 in the literature) were predominant members of
218 *Planctomycetes*, while P-OTU76 and P-OTU31 were less represented (Pollet *et al.*,
219 2011). Altogether, *Planctomycetes* may be a phylum that is generally distributed in the
220 oxygenated hypolimnion with different predominating members at different habitats.
221 Their high diversity (covering the three classes) across the phylum supports the idea
222 that their unique physiological characteristics shared among the phylum might enable
223 their successful dominance in the hypolimnion; for example, nucleoid

224 compartmentalization and endocytosis-like protein uptake are thought to be common
225 characteristics of the phylum (Fuerst and Sagulenko, 2011). We propose that members
226 of *Planctomycetes* should receive more attention in future studies given their
227 potentially important roles in deep freshwater ecosystems.
228 The occurrence of *Ca.* Nitrosoarchaeum of ammonia-oxidizing archaea (AOA)
229 in the oxygenated hypolimnion (Fig. 3B, 4, 5) has also been reported in Crater Lake
230 (Urbach *et al.*, 2001), Lake Redon (Spain) (Auguet *et al.*, 2012), Lake Maggiore
231 (Italy/Switzerland) (Coci *et al.*, 2015), and Lake Superior (USA/Canada) (Mukherjee *et*
232 *al.*, 2016). In the previous studies, niche separation of AOA and ammonia-oxidizing
233 bacteria (AOB) was suggested between lakes (Mukherjee *et al.*, 2016) and depths (Coci
234 *et al.*, 2015) but is unlikely between seasons (Auguet *et al.*, 2012). Our data
235 demonstrated that AOA only occurred at the later phase of the stratified period, while
236 AOB (*Nitrosospira*) more continuously occurred in the hypolimnion (Fig. 4, 5). Another
237 group of nitrifiers, *Nitrospira*, were also represented in the hypolimnion during the
238 stratification (Fig. 4, 5), yet only sporadic reports are available on *Nitrospira*
239 inhabiting the oxygenated water columns of freshwater lakes (Bel'kova *et al.*, 2003;
240 Tada and Grossart, 2014; Mukherjee *et al.*, 2016). In Lake Biwa, nitrate accumulation
241 in the hypolimnion throughout the stratified period with a constantly low ammonium

242 concentration (order of nM) was reported (Kim *et al.*, 2006; Thottathil *et al.*, 2013).
243 Although direct evidence is lacking, it is likely that the three nitrifiers were involved in
244 nitrification in the water column of the oxygenated hypolimnion of the lake.
245 In our data, two members of uncommon phyla were also identified as
246 hypolimnion-specific groups: LiUU-3-374 (*Acidobacteria*) and LiUU-9-330 (*Chlorobi*)
247 (Fig. 4, 5), both of which were originally described in Swedish lakes (Eiler and
248 Bertilsson, 2004). Their closest relatives found in the public sequence database were
249 almost exclusively retrieved from natural freshwater environments (data not shown).
250 This and the fact that our samples were from a pelagic lake site (Fig. 1) together
251 indicate that they are indigenous and were not transported from an allochthonous
252 source. In our data, the LiUU-3-374 highly represented from the winter mixing period
253 to the early stratified period (Fig. 4). Considering that the only isolated strain in the
254 same family (*Holophagaceae*) is able to degrade methoxylated aromatic compounds
255 (Liesack *et al.*, 1994), it is plausible that LiUU-3-374 contribute to refractory DOM
256 degradation in the lake ecosystem, as was proposed for members of deep sea
257 *Acidobacteria* (Quaiser *et al.*, 2008). LiUU-9-330 was affiliated with an uncultured
258 class OPB56, which is a deeply branched lineage in the *Chlorobi* phylum (Hiras *et al.*,
259 2015). Although their ecological characteristics remain unknown, it is likely that they

260 are not strictly hypolimnion-specific because many closely related sequences in the
261 public database were reported from the surface waters of mesotrophic (e.g., FN668200
262 by Van den Wyngaert *et al.*, 2011) to even hypereutrophic (e.g., JN371709 by Li *et al.*,
263 2012) lakes. In our data, LiUU-9-330 were not exclusively detected in the hypolimnion
264 but also occurred in the mid-summer epilimnion (Fig. 4).
265 This study is the first comprehensive investigation of the bacterioplankton
266 community composition in the oxygenated hypolimnion of a freshwater lake covering
267 the whole stratification period. We identified many phylogenetic groups that
268 preferentially occurred in the hypolimnion, which were understudied by previous
269 research that is targeting only the epilimnion. Future studies focusing on the eco-
270 physiology of these individual hypolimnion specialists are crucial to further understand
271 the microbial ecology and biogeochemical cycling in the pelagic freshwater ecosystem.

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441

TABLE AND FIGURE LEGENDS

Fig. 1

Basic information on the study site. (A) Location of the sampling site (Lake Biwa, Japan). (B) A spatiotemporal profile of water temperature determined by a CTD profiler. (C) A spatiotemporal profile of total prokaryotic abundance determined by enumeration of DAPI-positive cells. Dots indicate the depths and months from which the samples for the community analysis were taken.

Fig. 2

(A) Alpha (Shannon) diversity of 45 individual samples. Samples from the mixing period (Mix), the hypolimnion (Hypo.), and the epilimnion (Epi.) during stratification, were separately plotted. The differences between them were tested by the Wilcoxon rank sum test. The p values are shown on the right of the panel. (B) Beta diversity of 45 individual samples analyzed by non-metric multidimensional scaling (NMDS). Arrows indicate transitions of sequentially taken samples at each depth that characterizes the epilimnetic and hypolimnetic communities. Plot symbols and colors illustrate sampling depths and seasons.

Fig. 3

(A) Phylum-resolved community composition of individual samples, shown in the

460 percentage to the total amplicon reads. Numbers on the horizontal axis indicate the
461 sampling month. (B) The composition of the epilimnetic (left) and hypolimnetic (right)
462 communities, composed of pooled data of each layer during stratification (highlighted
463 by dashed rectangles in the panel A). A bar graph on the top shows phyla assignment in
464 each community. Bars shown below indicate the proportion of the 30 predominant
465 OTUs in each community. Bar colors indicate phyla to which individual groups were
466 assigned. Asterisks in the group name distinguish the different OTUs assigned to the
467 same group.

468 **Fig. 4**

469 Spatiotemporal distribution patterns of the predominant phylogenetic groups, shown in
470 the proportion (%) to the total amplicon read in each sample. In each panel,
471 abundances of three depths × 15 months were indicated by colors shown in the three
472 rows × 15 columns matrix. Colors in all heat maps are comparable (i.e., drawn to the
473 same scale) while the color range in individual panels is shown on the right side of the
474 matrix with the maximum value recorded in the group. Panels are arranged by
475 phylum, separated by dashed lines. Asterisks in the group name distinguish the
476 different OTUs assigned to the same group. Gray lines in the matrix illustrate the
477 separation of the epilimnion and hypolimnion by a thermocline (April–December).

Fig. 5

Proportion of appearance in each water layer of the 49 predominant groups. The horizontal and vertical axes indicate percentages from 12 epilimnion and 24 hypolimnion samples during stratification, respectively. Data points at the top left side suggest a preference for the hypolimnion and data points at the bottom right side suggest a preference for the epilimnion. Colors of the data points illustrate the phyla to which individual groups were assigned. Asterisks in the group name distinguish the different OTUs assigned to the same group.

Table S1

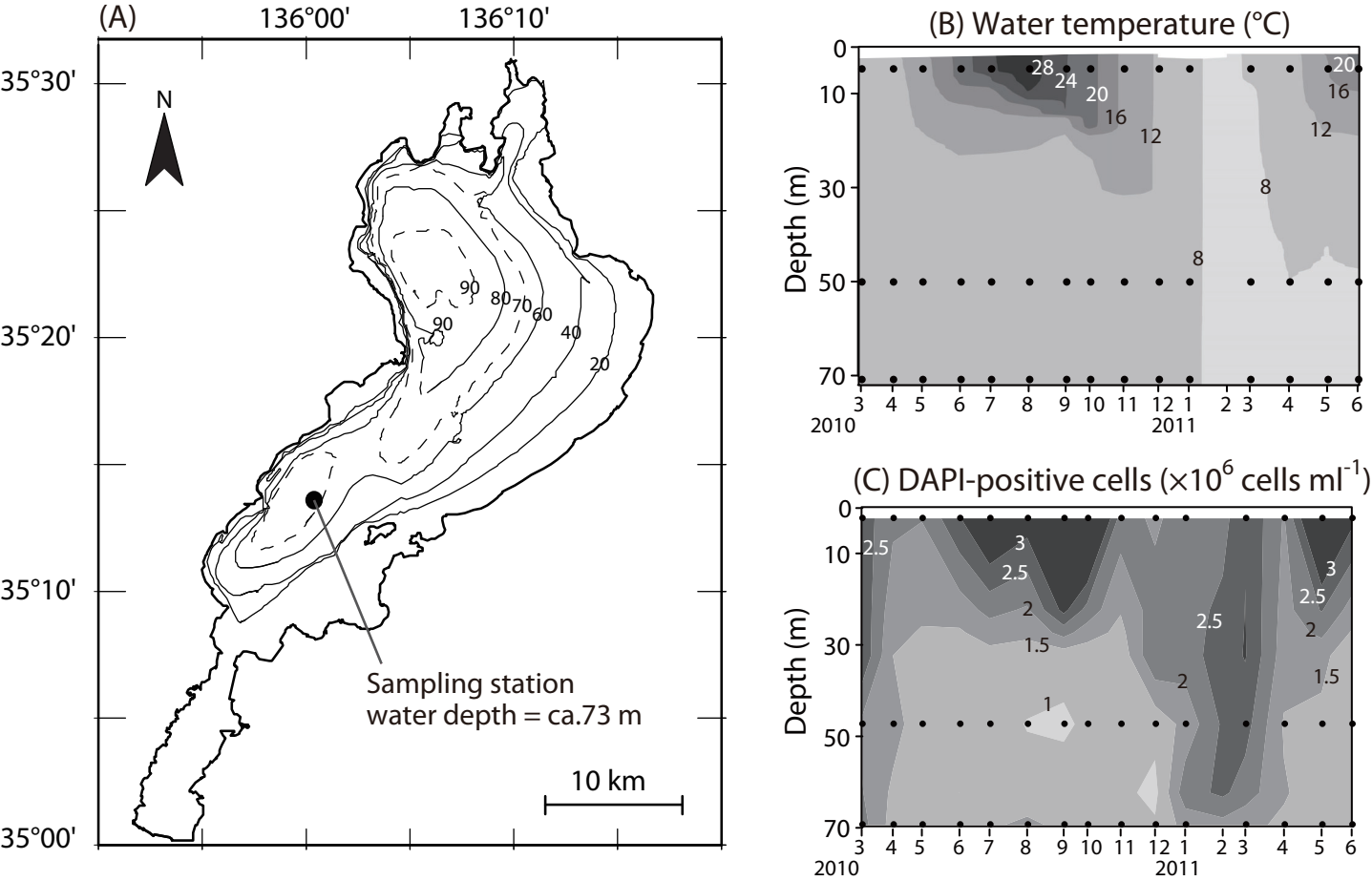
Fasta-formatted representative sequences of each OTUs, created by the UPARSE pipeline. The number of OTU corresponds to that shown in Table S2.

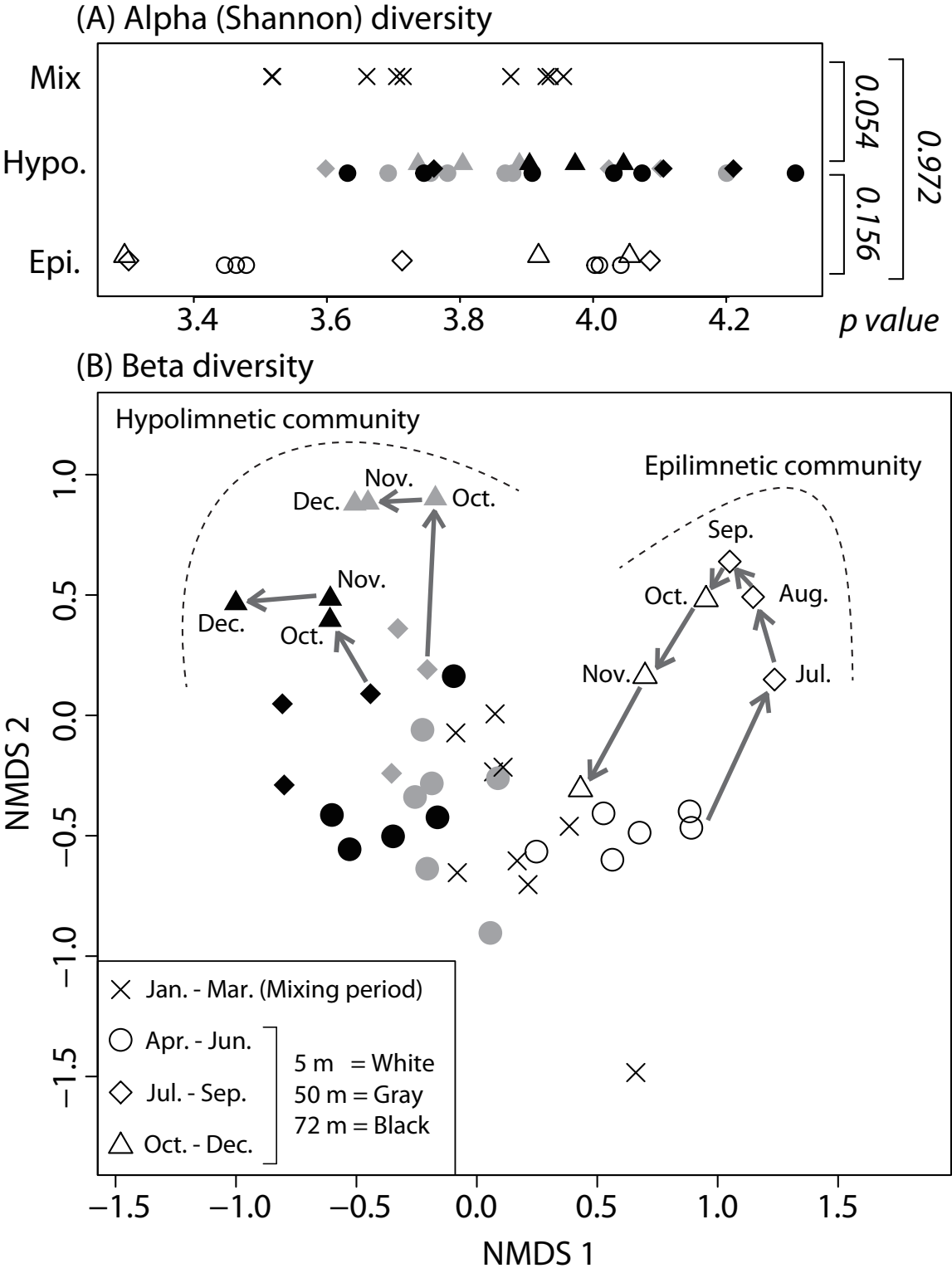
Table S2

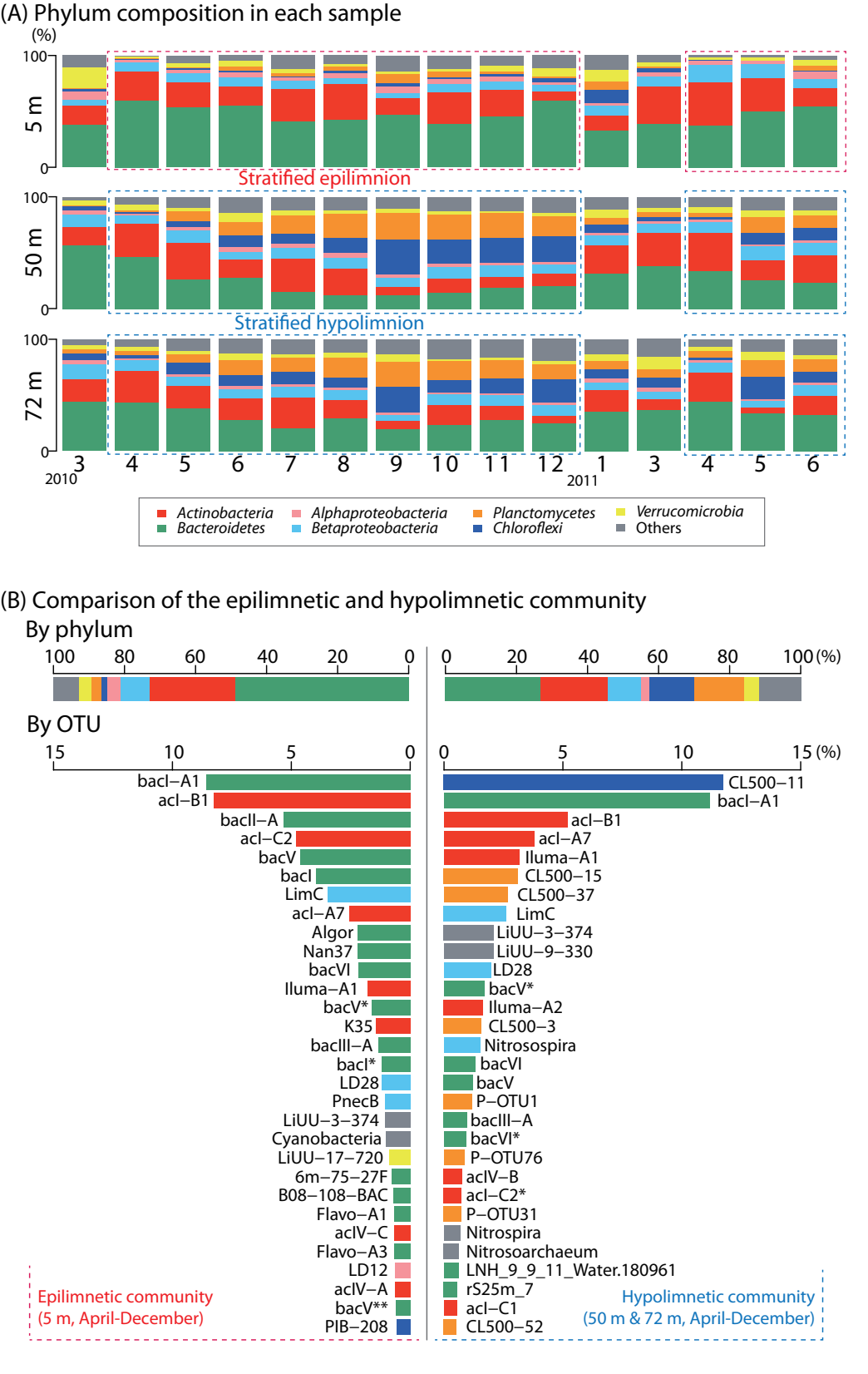
Relative proportion for 863 OTUs in all 45 samples (i.e., raw data for Figures 2–5), with the taxonomic assignment determined by the SILVA classification. For the predominant 49 OTUs, the identified names and accession numbers of reference sequences with which the identification was carried out are also shown.

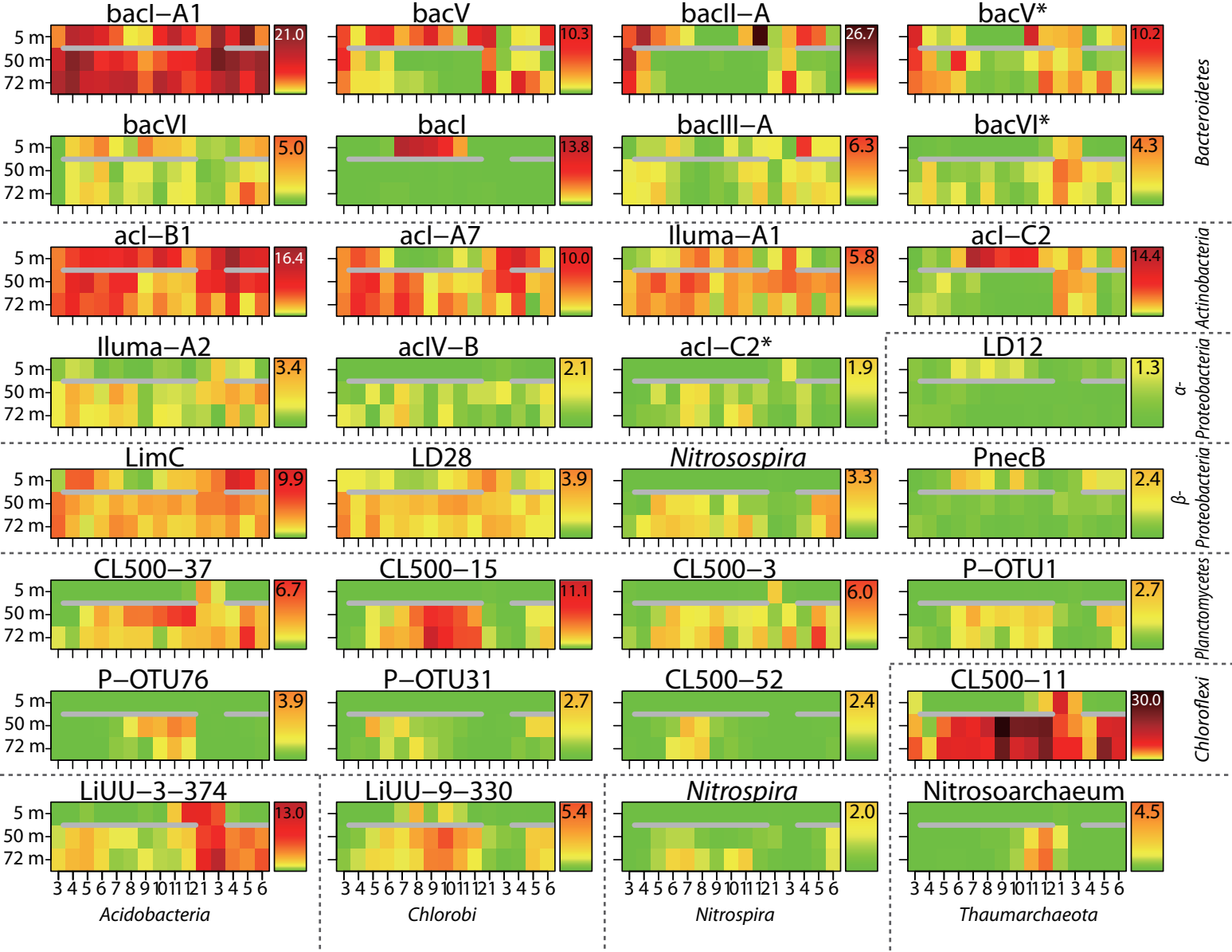
Table S3

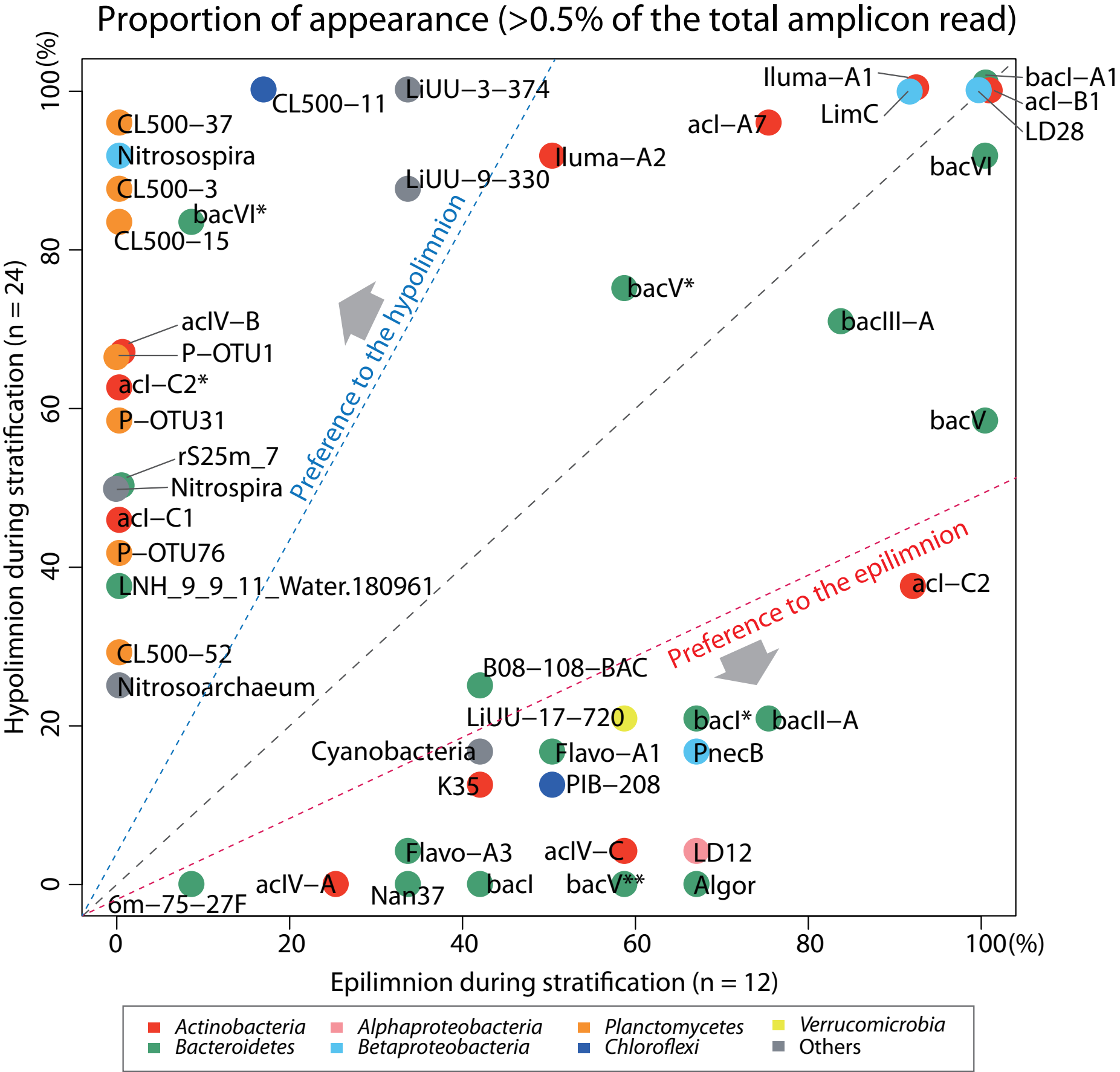
PCR primers and conditions used in this study.


(A)
136°00'
136°10'
35°30'
35°20'
35°10'
35°00'
N
90
90
80
70
60
40
20
Sampling station
water depth = ca.73 m
10 km
(B) Water temperature (°C)
Depth (m)
0
10
30
50
70
28
24
20
16
12
8
8
20
16
12
3 4 5 6 7 8 9 10 11 12 1 2 3 4 5 6
2010
2011
(C) DAPI-positive cells (×10^6 cells ml^-1)
Depth (m)
0
10
30
50
70
2.5
3
2.5
2
1.5
1
2.5
2
3
2.5
2
1.5
3 4 5 6 7 8 9 10 11 12 1 2 3 4 5 6
2010
2011


(A) Alpha (Shannon) diversity
Mix
Hypo.
Epi.
0.054
0.156
0.972
3.4
3.6
3.8
4.0
4.2
p value
(B) Beta diversity
Hypolimnetic community
Epilimnetic community
Nov.
Dec.
Oct.
Sep.
Aug.
Jul.
NMDS 1
NMDS 2
Jan. - Mar. (Mixing period)
Apr. - Jun.
Jul. - Sep.
Oct. - Dec.
5 m = White
50 m = Gray
72 m = Black


(A) Phylum composition in each sample
(%)
100
0
5 m
50 m
72 m
Stratified epilimnion
Stratified hypolimnion
3
4
5
6
7
8
9
10
11
12
1
3
4
5
6
2010
2011
Actinobacteria
Bacteroidetes
Alphaproteobacteria
Betaproteobacteria
Planctomycetes
Chloroflexi
Verrucomicrobia
Others
(B) Comparison of the epilimnetic and hypolimnetic community
By phylum
100 80 60 40 20 0
0 20 40 60 80 100 (%)
By OTU
15 10 5 0
0 5 10 15 (%)
bacI–A1
acI–B1
bacII–A
acI–C2
bacV
bacI
LimC
acI–A7
Algor
Nan37
bacVI
Iluma–A1
bacV*
K35
bacIII–A
bacI*
LD28
PnecB
LiUU–3–374
Cyanobacteria
LiUU–17–720
6m–75–27F
B08–108–BAC
Flavo–A1
acIV–C
Flavo–A3
LD12
acIV–A
bacV**
PIB–208
CL500–11
bacI–A1
acI–B1
acI–A7
Iluma–A1
CL500–15
CL500–37
LimC
LiUU–3–374
LiUU–9–330
LD28
bacV*
Iluma–A2
CL500–3
Nitrosospira
bacVI
bacV
P–OTU1
bacIII–A
bacVI*
P–OTU76
acIV–B
acI–C2*
P–OTU31
Nitrospira
Nitrosoarchaeum
LNH_9_9_11_Water.180961
rS25m_7
acI–C1
CL500–52
Epilimnetic community
(5 m, April-December)
Hypolimnetic community
(50 m & 72 m, April-December)


bacI–A1
bacV
bacII–A
bacV*
bacVI
bacI
bacIII–A
bacVI*
acI–B1
acI–A7
Iluma–A1
acI–C2
Iluma–A2
acIV–B
acI–C2*
LD12
LimC
LD28
Nitrosospira
PnecB
CL500–37
CL500–15
CL500–3
P–OTU1
P–OTU76
P–OTU31
CL500–52
CL500–11
LiUU–3–374
LiUU–9–330
Nitrospira
Nitrosoarchaeum
5 m
50 m
72 m
21.0
10.3
26.7
10.2
5.0
13.8
6.3
4.3
16.4
10.0
5.8
14.4
3.4
2.1
1.9
1.3
9.9
3.9
3.3
2.4
6.7
11.1
6.0
2.7
3.9
2.7
2.4
30.0
13.0
5.4
2.0
4.5
3 4 5 6 7 8 9 10 11 12 1 2 3 4 5 6
Acidobacteria
Chlorobi
Nitrospira
Thaumarchaeota
Bacteroidetes
Actinobacteria
α-Proteobacteria
β-Proteobacteria
Planctomycetes
Chloroflexi


Proportion of appearance (>0.5% of the total amplicon read)
Hypolimnion during stratification (n = 24)
Epilimnion during stratification (n = 12)
0
20
40
60
80
100(%)
Preference to the hypolimnion
Preference to the epilimnion
CL500−11
LiUU−3−374
CL500−37
Nitrosospira
CL500−3
bacVI*
CL500−15
LiUU−9−330
Iluma−A1
LimC
acI−A7
Iluma−A2
bacI−A1
acI−B1
LD28
bacVI
bacV*
bacIII−A
acIV−B
P−OTU1
acI−C2*
P−OTU31
bacV
rS25m_7
Nitrospira
acI−C1
P−OTU76
LNH_9_9_11_Water.180961
acI−C2
CL500−52
Nitrosoarchaeum
B08−108−BAC
LiUU−17−720
bacI*
bacII−A
Cyanobacteria
Flavo−A1
PnecB
K35
PIB−208
Flavo−A3
acIV−C
LD12
acIV−A
Nan37
bacI
bacV**
Algor
6m−75−27F
Actinobacteria
Alphaproteobacteria
Planctomycetes
Verrucomicrobia
Bacteroidetes
Betaproteobacteria
Chloroflexi
Others

SUPPLEMENTARY EXPERIMENTAL PROCEDURES

Sampling procedure

Monthly sampling was conducted from March 2010 to June 2011 (except for February 2011) at a pelagic site of Lake Biwa (35°12′58″N 135°59′55″E; depth ca. 73 m). In each month, the samples were collected from 5 m, 50 m, and 72 m (i.e., 1 m above the bottom), using a 5 L Niskin-X bottle (General Oceanics, FL). Water samples were refrigerated until further processing. The water temperature and total prokaryotic abundance were determined as described previously (Okazaki *et al.*, 2013).

DNA extraction, amplification, and pyrosequencing

For DNA extraction, prokaryotic cells in a 25 mL water sample were collected on a 0.2 μm polycarbonate filter. Samples were maintained at -20°C until DNA was extracted by the PowerSoil DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA, USA), following the manufacturer's instructions. For amplicon sequencing, the V4 and V5 regions of the 16S rRNA gene were amplified with modified 530F and 907R primers, which can target a broad range of bacteria and archaea (Nunoura *et al.*, 2012). A two-step PCR was employed to efficiently obtain amplicons, including an eight-base-pair DNA tag (for post-sequencing sample identification), and the 454 adaptors conjugated on both sides at the end. The primer sequences and PCR conditions used are provided in Table S3. Each PCR step was performed in a 25 μL volume with the buffer system of Blend Taq Plus (TOYOBO, Osaka, Japan), followed by purification with the UltraClean PCR Clean-Up Kit (MoBio Laboratories, Carlsbad, CA, USA).

The final product from each sample was equimolarly pooled according to quantification by a Nanodrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Sequencing was performed in the 1/8 regions of a sequencing reaction of a GS-FLX sequencer (Roche 454 Titanium) (Macrogen Japan Corp. Kyoto, Japan).

Operational Taxonomic Unit (OTU) creation and basic analysis

Sequence data were analyzed using the UPARSE pipeline (Edgar, 2013) by subsequently applying commands and scripts following the author's guideline (http://www.drive5.com/usearch/manual/uparse_cmds.html). We used a fastq_maxee value of 1.0, a truncated length of 200 bp, and an OTU creation identity threshold of 97%. Thereafter, taxonomic assignment of individual OTU was performed by SINA 1.2.11 (Pruesse *et al.*, 2012) referring SILVA 123 classification (Quast *et al.*, 2013). Subsequently, non-prokaryotic OTUs (i.e., chloroplast, eukaryote, and unclassified domain) were removed.

We only used the V4 region (i.e., the truncated length of 200 bp) of the 16S rRNA gene

in our analysis. If the V5 region was included (i.e., a truncated length of 350 bp), the number of total reads was considerably lower (< 40% of a 200 bp dataset), since longer reads are more prone to removal by the quality filtering step in the pipeline.

The alpha and beta diversity analysis were carried out by the phyloseq (McMurdie and Holmes, 2013) and the vegan (Oksanen *et al.*, 2015) packages of the R software (http://www.R- project.org/), respectively.

Identification of the predominant OTUs

Predominant OTUs were further identified following the freshwater bacterioplankton taxonomic framework (Newton *et al.*, 2011). Using NCBI BLAST+ tools 2.2.31 (Camacho *et al.*, 2009), each OTU was mapped against a reference fasta-formatted database (containing 11,587 partial and full 16S rRNA sequences) that was created by the ARB software (Ludwig, 2004) from the original data provided by Newton *et al.* (2011). If an OTU showed a >99% similarity to a reference sequence, the OTU was identified by following the phylogeny of the reference sequence in the original ARB data. We followed the finest naming structure available in the reference sequence, such as "tribe" (>97% identity over the full-length 16S rRNA sequence), "clade" (>95%), or "lineage" (85%–90%), as proposed in the literature (Newton *et al.*, 2011). OTUs that failed to be named by this procedure were identified using the NCBI BLAST online search against the public sequence database (http://www.ncbi.nlm.nih.gov/). Each OTU was named following the name of the hit sequence that had the highest and >99% identity. If there were multiple candidates, the OTU was preferentially named after published sequences with the prefixes CL- (Urbach *et al.*, 2001), LiUU- (Eiler and Bertilsson, 2004), and P-OTU- (Pollet *et al.*, 2011). For members of the genus *Limnohabitans*, we followed the nomenclature proposed by Kasalický *et al.* (2013). For nitrifying bacteria, the more widely accepted nomenclature of genera was used. For *Cyanobacteria*, identification was not carried out, since the 200 bp information is insufficient for taxonomic assignment.

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