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ORIGINAL ARTICLE

Bacterioplankton communities in a high-altitude freshwater wetland

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Abstract Microbial communities play a crucial role in various biogeochemical processes in aquatic ecosystems. However, existing knowledge on microbial communities in the waters of wetlands is still very scant. The objective of the present study was to investigate the bacterioplankton community in the Luoshijiang Wetland, a high-altitude freshwater wetland in the Yunnan-Kweichow Plateau. Water samples were collected from different sites. The bacterioplankton community was characterized using 16S rRNA gene clone library analysis. A spatial variation of the diversity and composition of the bacterioplankton community was observed. *Verrucomicrobia* and *Proteobacteria* were the most abundant components. *Proteobacteria* might play an important role in water self-purification, but the significance of *Verrucomicrobia* remained unclear. Moreover, Pearson's correlation analysis showed that *Actinobacteria* and *Gemmatimonadetes* were positively correlated with nitrite nitrogen in waters, while *Alphaproteobacteria* with dissolved phosphorous.

Keywords Microbial community · *Luteolibacter* · *Proteobacteria* · *Verrucomicrobia* · Wetlands · Freshwater

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Introduction

Microbial communities play vital roles in decomposition of organic matter, remineralization of nutrients, and biogeochemical cycling in terrestrial and aquatic environments. Wetlands are unique ecosystems, and can act as ecotones between terrestrial and aquatic systems (Menon et al. 2013). Wetlands are important players in nutrient cycling, sediment accretion, pollution filtration, and erosion control (Qin and Mitsch 2009). Knowledge of microbial communities can greatly contribute to our understanding of the complex processes within wetland ecosystems. To date, little is known about microbial communities in wetland ecosystems. A few previous studies indicated that microbial community structure in wetlands was dependent on soil type (Tang et al. 2012; Peralta et al. 2013), soil depth (Tang et al. 2012), carbon and nitrogen sources (Morrissey et al. 2013), vegetation (Akiyama et al. 2010), and successional stage (Tang et al. 2011). These previous studies focused on soil microorganisms in wetlands. However, the bacterioplankton community is also a key component in aquatic ecosystems, due to its efficient nutrient uptake, high abundance, and large growth potential (Parveen et al. 2013). Unfortunately, information on bacterioplankton communities in the waters of wetlands is still very limited (Dorador et al. 2013).

The Luoshijiang Wetland, a typical freshwater wetland in the Yunnan-Kweichow Plateau, is located in Dali City, Yunnan Province. The fresh wetland is adjacent to the Rrhai Lake, the second largest high-altitude lake in Yunnan Province. The wetland covers an area of 1 km^2 with an elevation of about 2,056 m and is surrounded by farmlands. Annual mean air temperature and annual precipitation in the local region were 15.7 °C, and 1000–1200 mm, respectively. To date, information on microbial communities in high-altitude wetlands is still very scant. Tang et al. (2012) investigated soil bacterial

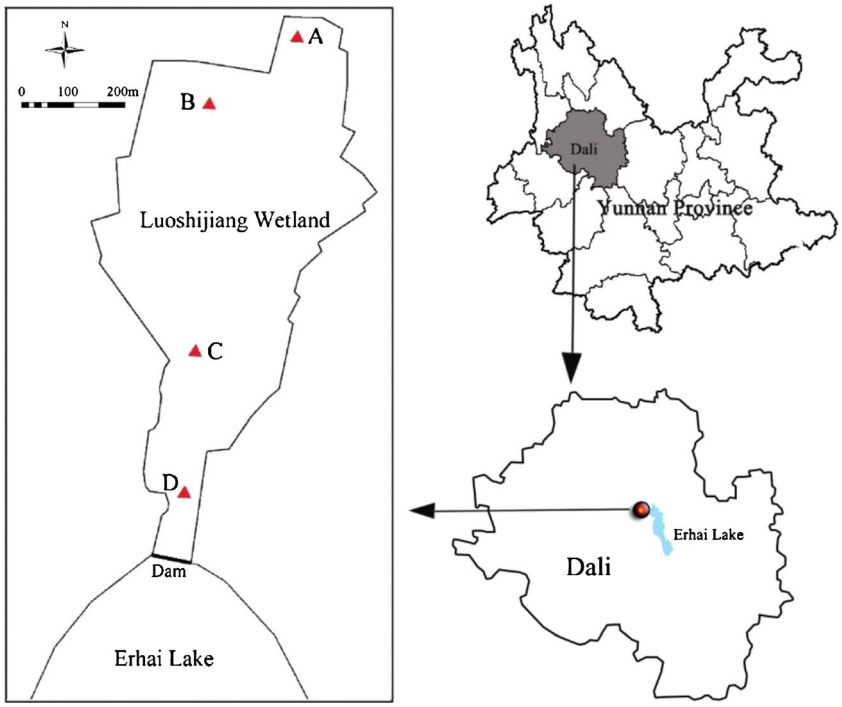

Fig. 1 Schematic representation of the Luoshijiang Wetland and sampling sites.

communities in the Zoige Wetland of the Qinghai-Tibetan Plateau in China. Dorador et al. (2013) reported on microbial diversity of five high altitude wetlands from the Chilean Altiplano. Unfortunately, there has been no report on microbial communities in other high-altitude wetlands. Therefore, the main objective of the current study was to investigate the bacterioplankton community structure of the Luoshijiang Wetland.

Materials and methods

Study sites and water sampling

Surface water samples (0–20 cm) in triplicate from four different sites of the Luoshijiang Wetland were collected in March 2013: A (25°57′25″N–100°06′06″E, no vegetation zone), B (25°57′12″N–100°05′59″E, reed-planted zone), C (25°57′4″N–100°06′00″E, densely water-lily-planted zone), and D (25°56′55″N–100°05′59″E, sparsely water-lily planted zone) (Fig. 1). At the time of sample collection, the temperature and pH of the four samples were about 15 °C and 7.5, respectively. The chemical parameters of the water samples are shown in Table 1.

Bacterial clone library analysis

For analysis of the bacterial community, water samples (250 mL) were filtered through 0.22-μm-pore-size membranes (diameter 50 mm; Millipore). The membrane filter was cut into quarters with a sterile scalpel and was used for further molecular analysis. DNA was extracted using the E.Z.N.A.® Water DNA kit (Omega, USA) according to the manufacturer's protocol. Bacterial clone libraries were constructed according to the literature (Zhang et al. 2012; Lu et al. 2013). Briefly, bacterial 16S rRNA genes were amplified using primers 27 F (5′-GAGTTTGATCMTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′). The purified PCR products were cloned into pGEM-T-easy Vector (Takara Corp, Japan), and clones containing an insert of the correct size were sequenced. Chimera-free bacterial sequences with ≥97 % identity were grouped as operational taxonomic units (OTUs). OTUs, rarefaction curves, and Shannon diversity index were determined using the DOTUR program (Schloss and Handelsman 2005). The Ribosomal Database

Table 1 Chemical parameters of water samples

Parameters	Sample A	Sample B	Sample C	Sample D
TN (mg/L)	3.84	2.62	2.99	1.92
NH_4^+-N (mg/L)	0.27	0.04	0.15	0.07
NO_3^--N (mg/L)	0.16	0.26	0.02	0.01
NO_2^--N (mg/L)	0.01	0.01	0.01	0.26
DOC (mg/L)	28.7	35.0	41.0	34.0
TP (mg/L)	0.19	0.07	0.10	0.09
DP (mg/L)	0.07	0.02	0.01	0.02

TN total nitrogen; *DOC* dissolved organic carbon; *TP* total phosphorous; *DP* dissolved phosphorus

Table 2 OTU-based community richness and diversity indices for Samples A–D

Sample	No. of sequences	OTUs	Shannon index
A	93	26	2.6
B	92	35	3.2
C	95	9	0.7
D	91	20	1.7

Project analysis tool "classifier" (http://rdp.cme.msu.edu/classifier/classifier.jsp) was used to determine the taxonomic identities of the bacterial sequences (Wang et al. 2007). In addition, Pearson's correlation analysis of the bacterial community structure with the determined chemical parameters was performed using SPSS 20.0 software.

Nucleotide sequence accession numbers

The sequences obtained in this study were submitted to GenBank under accession numbers KF443412–KF443504 for Sample A, KF443505 –KF443596 for Sample B, KF443597–KF443691 for Sample C, and KF443692 – KF443782 for Sample D, respectively.

Results

Bacterial diversity

A total of 91– 95 bacterial sequences were retrieved from each sample. Based on the threshold of 3 % difference, OTUs, rarefaction curves, and Shannon diversity index were obtained using the DOTUR program (Schloss and Handelsman 2005). Clone libraries with Samples A, B, C, and D were composed of 26, 35, 9, and 20 OTUs, respectively (Table 2). The Shannon diversity index of bacterial community in Sample C was only 0.7, much lower than that in the other three samples (1.7– 3.2).

The rarefaction curve for Sample C approached a plateau, indicating that the community was well sampled but had a very low bacterial diversity (Fig. 2). However, the rarefaction curves for Samples A, B, and D did not level off completely, suggesting that further sequencing would have resulted in more OTUs in each sample.

Bacterial community composition

The bacterial phylum composition of the four water samples is shown in Fig. 3. In this study, six known phyla were identified in these samples including *Proteobacteria*, *Actinobacteria*, *Bacteroidetes*, *Cyanobacteria*, *Gemmatimonadetes*, and *Verrucomicrobia*. However, only *Proteobacteria*, *Bacteroidetes*, and *Verrucomicrobia* were shared among all the four water samples. A shift in the composition of major bacterial groups was found in the different sampling sites in the Luoshijiang Wetland. For example, the major bacterial groups (with relative abundance no less than 10 %) in both Sample A and Sample D were *Verrucomicrobia* (64.5 % or 65.9 %) and *Gammaproteobacteria* (12.9 % or 15.4 %), while *Verrucomicrobia* (88.4 %) predominated in Sample C. Sample B was mainly represented by *Verrucomicrobia* (43.5 %), *Betaproteobacteria* (25 %), and *Gammaproteobacteria* (17.4 %).

Table 3 shows the Pearson's correlation coefficients for the relationship between the proportion change of the major bacterial groups and water chemical properties. *Actinobacteria* and *Gemmatimonadetes* had a significant positive correlation with nitrite nitrogen ($p<0.05$). *Alphaproteobacteria* also showed a significant positive correlation with dissolved phosphorus ($p<0.05$). However, other major bacterial groups did not show any significant correlation with the determined water

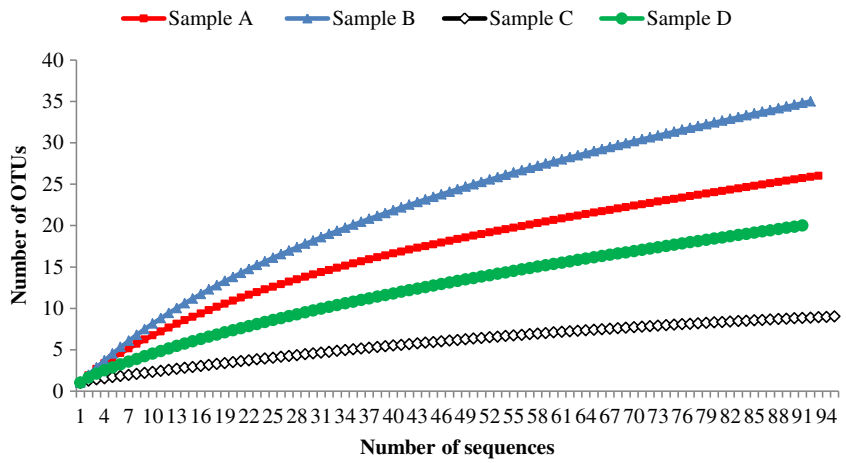

Fig. 2 Rarefaction curves of OTUs in Samples A–D evaluated by 3 % sequence variation

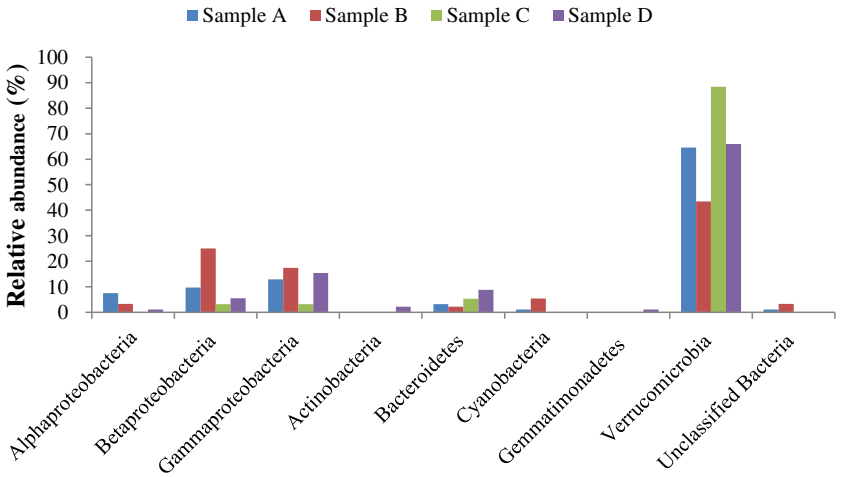

Fig. 3 Comparison of the quantitative contribution of the sequences affiliated with different phyla and classes to the total number of sequences from Samples A–D. Sequences not classified to any known phylum are included as unclassified bacteria.

chemical parameters. Correlation analyses were limited by the very low number of samples analyzed ($n=4$).

Table 4 illustrates the abundance and distribution of the 24 known genera detected in the four samples. At the genus level of taxonomic classifications, variations of bacterial community structure among the four samples were more evident. For Samples A and B, a large portion of sequences (50/93 or 61/92) could be classified at the genus level. In contrast, for Samples C and D, only a small portion of sequences could be related to known bacterial genera. A total of 14 genera were present only in a single sample. Members of genus *Luteolibacter* were dominant in Samples A and B, but were detected with much lower abundance in Samples C and D. *Haloferula* was the third largest component in Sample A, while the minor one in the other samples. These results indicate a large spatial variation of verrucomicrobial communities in the investigated wetland. Moreover, the abundance and composition of proteobacterial communities also differed greatly among the four different samples.

Discussion

Bacterial community in the water body of freshwater ecosystems is usually highly diverse (Humbert et al. 2009; Kwon et al. 2011; Kadnikov et al. 2012). However, to the authors' knowledge, this was among the first reports on diversity of bacterioplankton communities in freshwater wetlands. A spatial variation of bacterioplankton community diversity was observed in the Luoshijiang Wetland.

Verrucomicrobia is a universally distributed phylum and can be present in terrestrial and aquatic habitats (Arnds et al. 2010; Freitas et al. 2012; Parveen et al. 2013) and manmade bioreactors (Feng et al. 2013; Liao et al. 2013a, b). The reported isolates from phylum *Verrucomicrobia* could utilize various carbon compounds (Arnds et al. 2010). Little attention has been paid to the abundance of aquatic *Verrucomicrobia*, although a few previous studies have shown the dominance of *Verrucomicrobia* in the waters of freshwater lakes (Lindström et al. 2004; Arnds et al. 2010; Kolmonen et al. 2011). The

Table 3 Pearson's correlation coefficients describing the relationship between water characteristics and the change of the relative abundance of major bacterial groups

	TN	NH_4^+-N	NO_3^--N	NO_2^--N	DOC	TP	DP
Alphaproteobacteria	0.724	0.627	0.609	-0.372	0.752	0.803	0.953[a]
Betaproteobacteria	-0.021	-0.453	0.930	-0.351	0.861	-0.272	0.025
Gammaproteobacteria	-0.312	-0.396	0.570	0.346	0.619	-0.138	0.246
Actinobacteria	-0.770	-0.378	-0.578	0.999[a]	-0.526	-0.337	-0.246
Bacteroidetes	-0.659	-0.203	-0.876	0.893	-0.842	-0.280	-0.362
Cyanobacteria	-0.021	-0.478	0.915	-0.409	0.826	-0.322	-0.053
Gemmatimonadetes	-0.770	-0.378	-0.578	0.999[a]	-0.526	-0.337	-0.246
Verrucomicrobia	0.156	0.429	-0.822	0.001	-0.820	0.184	-0.190

TN total nitrogen; *DOC* dissolved organic carbon; *TP* total phosphorus; *DP* dissolved phosphorus

[a] Correlation is significant at the 0.05 level.

Table 4 Distribution of the sequences affiliated with the identified genera in Samples A–D

Phylogenetic affiliation	Sample A	Sample B	Sample C	Sample D
Alphaproteobacteria				
Rhodobacter	—[a]	2	—	—
Sphingomonas	—	—	—	1
Betaproteobacteria				
Polynucleobacter	4	1	2	—
Methylotenera	—	2	1	—
Undibacterium	—	4	—	—
Limnohabitans	3	8	—	4
Dechloromonas	—	—	—	1
Hydrogenophaga	—	2	—	—
Malikia		1	—	—
Acidovorax	1	—	—	—
Gammaproteobacteria				
Pseudomonas	8	5	—	8
Shewanella	—	—	1	1
Silanimonas	—	1	—	—
Aeromonas	—	5	2	4
Rheinheimera	3	3	—	—
Bacteroidetes				
Fluviicola	—	—	—	2
Flavobacterium	2	—	—	1
Cyanobacteria				
Cryptomonadaceae	1	—	—	—
Chlorophyta	—	2	—	—
Gemmatimonadetes				
Gemmatimonas	—	—	—	1
Verrucomicrobia				
Luteolibacter	19	23	1	5
Haloferula	7	1	1	—
Prosthecobacter	2	—	—	—
Opitutus	—	1	—	—
Total	50	61	8	28

[a] —, not detected

abundance of *Verrucomicrobia* in lakes might be positively correlated with nutrient-richness and phosphorus availability, and could vary between seasons and between more and less humic basins (Arnds et al. 2010). However, de Figueiredo et al. (2007) found that *Verrucomicrobia* was associated with the most oligotrophic aquatic ecosystems and low pH values. To date, the existing information on the abundance and distribution of *Verrucomicrobia* in the waters of wetlands is very scant. Dorador et al. (2013) revealed a low abundance of *Verrucomicrobia* in high-altitude wetlands of the Chilean Altiplano. To the authors' knowledge, this was the first report on the dominance of *Verrucomicrobia* in the waters of wetlands. In this study, *Verrucomicrobia* was the largest component in the bacterial communities, but its relative abundance varied among the different sampling sites. Surprisingly, no significant correlation was observed between *Verrucomicrobia* and the determined water chemical parameters. Therefore, further efforts are necessary in order to elucidate the links between *Verrucomicrobia* and the environmental parameters in aquatic ecosystems.

Luteolibacter (*Verrucomicrobia*) was the largest genus detected in the Luoshijiang Wetland. To the authors' knowledge, this was the first report on the dominance of *Luteolibacter* in a freshwater ecosystem. Members of genus *Luteolibacter* have been isolated from activated sludge (Park et al. 2013), Arctic tundra soil (Jiang et al. 2012), marine environments (Yoon et al. 2008), and leek rhizosphere (da Rocha et al. 2011). Unfortunately, the role of *Luteolibacter* species in the environment remains largely unclear. Only a recent study indicates *Luteolibacter algae* H18 could assimilate fucoidan as a sole carbon source (Ohshiro et al. 2012). Moreover, information on the other detected verrucomicrobial genera *Haloferula*, *Prosthecobacter*, and *Opitutus* is still very limited, and their ecological roles also remain unclear. Therefore, further study is necessary in order to elucidate the significance of the dominance of phylum *Verrucomicrobia* in wetland.

Phylum *Proteobacteria* might play active roles in biodegradation of organic pollutants and carbon cycling, and various biogeochemical processes in aquatic ecosystems (Cheng et al. 2013). *Proteobacteria* (mostly *Alpha*-, *Beta*-, and *Gammaproteobacteria*) usually predominate in freshwater habitats (Kwon et al. 2011). There have also been a few reports on the abundance and composition of *Proteobacteria* in the waters of high-altitude lakes. Sommaruga and Casamayor (2009) found that *Betaproteobacteria* commonly predominated in high-altitude lakes in the Mount Everest region (Nepal). *Betaproteobacteria* was also the dominant group in Lake Namco, the largest Tibetan lake (Liu et al. 2013a). Wu et al. (2006) revealed that, in 16 high-mountain lakes located on the Tibetan Plateau (China), *Betaproteobacteria* was abundant in all freshwater lakes, while *Alpha*- and *Gammaproteobacteria* gained much higher abundance in saline lakes. However, very limited information exists on the abundance and composition of *Proteobacteria* in the waters of wetlands. Dorador et al. (2013) reported the abundance of *Proteobacteria* (alpha, beta, gamma and delta subgroups) in high-altitude wetlands of the Chilean Altiplano. In this study, *Proteobacteria*, composed of alpha, beta and gamma classes, was the second largest phylum in the Luoshijiang Wetland, but a marked shift in its relative abundance occurred in the different sampling sites. *Alphaproteobacteria* was detected with low abundance in all the water samples and was positively correlated with dissolved phosphorus ($p < 0.05$). *Betaproteobacteria* was one of the major components in Sample B (25 %), but became much less abundant in the other sampling sites. *Gammaproteobacteria* was abundant in Samples A, B and D. However,

Betaproteobacteria and *Gammaproteobacteria* did not show significant correlation with the determined water chemical parameters.

Microorganisms from alphaproteobacterial genera (*Rhodobacter* and *Sphingomonas*), betaproteobacterial genera (*Hydrogenophaga* and *Acidovorax*), and gammaproteobacterial genera (*Pseudomonas*, *Shewanella* and *Aeromonas*) are known for biodegradation of a variety of environmental organic pollutants (Zhang et al. 2011; Ogugbue et al. 2012; Wang et al. 2012; Cheng et al. 2013; Johnson et al. 2013; Liao et al. 2013a; Liu et al. 2013b). Therefore, the presence of these microorganisms might play important roles in reduction of organic pollutants and water self-purification in the Luoshijiang Wetland.

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References

Akiyama M, Shimizu S, Ishijima Y, Naganuma T (2010) Response of microbial community structure to natural and accelerated hydrarch successions in the boreal wetlands in northern Hokkaido, Japan. Limnology 11(3):273–279

Arnds J, Knittel K, Buck U, Winkel M, Amann R (2010) Development of a16S rRNA-targeted probe set for *Verrucomicrobia* and its application for fluorescence in situ hybridization in a humic lake. Syst Appl Microbiol 33:139–148

Cheng W, Zhang JX, Wang Z, Wang M, Xie SG (2013) Bacterial communities in sediments of a drinking water reservoir. Ann Microbiol. doi:10.1007/s13213-013-0712-z

da Rocha UN, van Elsas JD, van Overbeek LS (2011) *Verrucomicrobia* subdivision 1 strains display a difference in the colonization of the leek (Allium porrum) rhizosphere. FEMS Microbiol Ecol 78(2): 297–305

de Figueiredo DR, Pereira MJ, Moura A, Silva L, Barrios S, Fonseca F, Henriques I, Correia A (2007) Bacterial community composition over a dry winter in meso- and eutrophic Portuguese water bodies. FEMS Microbiol Ecol 59(3):638–650

Dorador C, Vila I, Witzel KP, Imhoff JF (2013) Bacterial and archaeal diversity in high altitude wetlands of the Chilean Altiplano. Fundam Appl Limnol 182(2):135–159

Feng S, Chen C, Wang QF, Zhang XJ, Yang ZY, Xie SG (2013) Characterization of microbial communities in a granular activated carbon-sand dual media filter for drinking water treatment. Int J Environ Sci Technol 10(5):917–922

Freitas S, Hatosy S, Fuhrman JA, Huse SM, Welch DBM, Sogin ML, Martiny AC (2012) Global distribution and diversity of marine *Verrucomicrobia*. ISME J 6(8):1499–1505

Humbert JF, Dorigo U, Cecchi P, Le Berre B, Debroas D, Bouvy M (2009) Comparison of the structure and composition of bacterial communities from temperate and tropical freshwater ecosystems. Environ Microbiol 11(9):2339–2350

Jiang F, Li WJ, Xiao MC, Dai J, Kan WJ, Chen L, Li WX, Fang CX, Peng F (2012) *Luteolibacter luojiensis* sp nov., isolated from Arctic tundra soil, and emended description of the genus *Luteolibacter*. Int J Syst Evol Microbiol 62:2259–2263

Johnson RJ, Smith BE, Rowland SJ, Whitby C (2013) Biodegradation of alkyl branched aromatic alkanoic naphthenic acids by *Pseudomonas putida* KT2440. Int Biodeterior Biodegrad 81: SI 3–8

Kadnikov VV, Mardanov AV, Beletsky AV, Shubenkova OV, Pogodaeva TV, Zemskaya TI, Ravin NV, Skryabin KG (2012) Microbial community structure in methane hydrate-bearing sediments of freshwater Lake Baikal. FEMS Microbiol Ecol 79(2):348–358

Kolmonen E, Rantala-Ylinen A, Rajaniemi-Wacklin P, Lepitö LA, Haukka K, Sivonen K (2011) Bacterioplankton community composition in 67 Finnish lakes differs according to trophic status. Aquat Microb Ecol 62:241–250

Kwon S, Moon E, Kim TS, Hong S, Park HD (2011) Pyrosequencing demonstrated complex microbial communities in a membrane filtration system for a drinking water treatment plant. Microbes Environ 26(2):149–155

Liao XB, Chen C, Wang Z, Wan R, Chang CH, Zhang XJ, Xie SG (2013a) Changes of biomass and bacterial communities in biological activated carbon filters for drinking water treatment. Process Biochem 48(2):312–316

Liao XB, Chen C, Wang Z, Wan R, Chang CH, Zhang XJ, Xie SG (2013b) Pyrosequencing analysis of bacterial communities in drinking water biofilters receiving influents of different types. Process Biochem 48(4):703–707

Lindström ES, Vrede K, Leskinen E (2004) Response of a member of the *Verrucomicrobia*, among the dominating bacteria in a hypolimnion, to increased phosphorus availability. J Plankton Res 26:241–246

Liu YQ, Yao TD, Jiao NZ, Liu XB, Kang SC, Luo TW (2013a) Seasonal dynamics of the bacterial community in Lake Namco, the largest Tibetan lake. Geomicrobiol J 30(1):17–28

Liu GF, Zhou JT, Meng XM, Fu SQ, Wang J, Jin RF, Lv H (2013b) Decolorization of azo dyes by marine *Shewanella* strains under saline conditions. Appl Microbiol Biotechnol 97(9):4187–4197

Lu PP, Chen C, Wang QF, Wang Z, Zhang XJ, Xie SG (2013) Phylogenetic diversity of microbial communities in real drinking water distribution systems. Biotechnol Bioprocess Eng 18(1):119–124

Menon R, Jackson CR, Holland MM (2013) The influence of vegetation on microbial enzyme activity and bacterial community structure in freshwater constructed wetland sediments. Wetlands 33(2):365–378

Morrissey EM, Jenkins AS, Brown BL, Franklin RB (2013) Resource availability effects on nitrate-reducing microbial communities in a freshwater wetland. Wetlands 33(2):301–310

Ogugbue CJ, Sawidis T, Oranusi NA (2012) Bioremoval of chemically different synthetic dyes by *Aeromonas hydrophila* in simulated wastewater containing dyeing auxiliaries. Ann Microbiol 62(3):1141–1153

Ohshiro T, Harada N, Kobayashi Y, Miki Y, Kawamoto H (2012) Microbial fucoidan degradation by *Luteolibacter algae* H18 with deacetylation. Biosci Biotechnol Biochem 76(3):620–623

Park J, Baek GS, Woo SG, Lee J, Yang J, Lee J (2013) *Luteolibacter yonseiensis* sp nov., isolated from activated sludge using algal metabolites. Int J Syst Evol Microbiol 63:1891–1895

Parveen B, Mary I, Vellet A, Ravet V, Debroas D (2013) Temporal dynamics and phylogenetic diversity of free-living and particle-associated *Verrucomicrobia* communities in relation to environmental variables in a mesotrophic lake. FEMS Microbiol Ecol 83(1):189–201

Peralta RM, Ahn C, Gillevet PM (2013) Characterization of soil bacterial community structure and physicochemical properties in created and natural wetlands. Sci Total Environ 443:725–732

Qin P, Mitsch WJ (2009) Wetland restoration and ecological engineering: International conference of wetland restoration and ecological engineering. Ecol Eng 35(4):437–441

Schloss PD, Handelsman J (2005) Introducing DOTUR, a computer program for defining operational taxonomic units and estimating species richness. Appl Environ Microbiol 71(3):1501–1506

Sommaruga R, Casamayor EO (2009) Bacterial 'cosmopolitanism' and importance of local environmental factors for community composition in remote high-altitude lakes. Freshw Biol 54(5):994–1005

Tang YS, Wang L, Jia JW, Fu XH, Le YQ, Chen XZ, Sun Y (2011) Response of soil microbial community in Jiuduansha wetland to different successional stages and its implications for soil microbial respiration and carbon turnover. Soil Biol Biochem 43(3):638–646

Tang J, Ding X, Wang LM, Xu QR, Yang ZR, Zhao J, Sun Q, Feng S, Zhang J (2012) Effects of wetland degradation on bacterial community in the Zoige Wetland of Qinghai-Tibetan Plateau (China). World J Microbiol Biotechnol 28(2):649–657

Wang Q, Garrity GM, Tiedje JM, Cole JR (2007) Naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl Environ Microbiol 73(16):5261–5267

Wang Y, Wan R, Zhang SY, Xie SG (2012) Anthracene biodegradation under nitrate-reducing conditions and associated microbial community changes. Biotechnol Bioprocess Eng 17(2):371–376

Wu QL, Zwart G, Schauer M, Kamst-van Agterveld MP, Hahn MW (2006) Bacterioplankton community composition along a salinity gradient of sixteen high-mountain lakes located on the Tibetan Plateau, China. Appl Environ Microbiol 72(8):5478–5485

Yoon J, Matsuo Y, Adachi K, Nozawa M, Matsuda S, Kasai H, Yokota A (2008) Description of *Persicirhabdus sediminis* gen. nov., sp nov., *Roseibacillus ishigakijimensis* gen. nov., sp nov., *Roseibacillus ponti* sp nov., *Roseibacillus persicicus* sp nov., *Luteolibacter pohnpeiensis* gen. nov., sp nov and *Luteolibacter algae* sp nov., six marine members of the phylum '*Verrucomicrobia*', and emended descriptions of the class *Verrucomicrobiae*, the order *Verrucomicrobiales* and the family *Verrucomicrobiaceae*. Int J Syst Evol Microbiol 58:998–1007

Zhang SY, Wan R, Wang QF, Xie SG (2011) Identification of anthracene degraders in leachate-contaminated aquifer using stable isotope probing. Int Biodeterior Biodegrad 65(8):1224–1228

Zhang SY, Wang QF, Xie SG (2012) Bacterial and archaeal community structures in phenanthrene amended aquifer sediment microcosms under oxic and anoxic conditions. Int J Environ Res 6(4):1077–1088