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Phospholipids fatty acids of drinking water reservoir sedimentary microbial community: Structure and function responses to hydrostatic pressure and other physico-chemical properties

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Abstract

Microbial communities in three drinking water reservoirs, with different depth in Xi'an city, were quantified by phospholipids fatty acids analysis and multivariate statistical analysis was employed to interpret their response to different hydrostatic pressure and other physico-chemical properties of sediment and overlying water. Principle component analyses of sediment characteristics parameters showed that hydrostatic pressure was the most important effect factor to differentiate the overlying water quality from three drinking water reservoirs from each other. NH_4^+ content in overlying water was positive by related to hydrostatic pressure, while DO in water-sediment interface and sediment OC in sediment were negative by related with it. Three drinking water reservoir sediments were characterized by microbial communities dominated by common and facultative anaerobic Gram-positive bacteria, as well as, by sulfur oxidizing bacteria. Hydrostatic pressure and physico-chemical properties of sediments (such as sediment OC, sediment TN and sediment TP) were important effect factors to microbial community structure, especially hydrostatic pressure. It is also suggested that high hydrostatic pressure and low dissolved oxygen concentration stimulated Gram-positive and sulfate-reducing bacteria (SRB) bacterial population in drinking water reservoir sediment. This research supplied a successful application of phospholipids fatty acids and multivariate analysis to investigate microbial community composition response to different environmental factors. Thus, few physico-chemical factors can be used to estimate composition microbial of community as reflected by phospholipids fatty acids, which is difficult to detect.

Key words

Drinking water reservoir, Hydrostatic pressure, Microbial community structure, Multivariate analysis

Introduction

Nowadays, more and more metropolitans are using surface water, such as reservoirs and lakes, as main water supply sources. Therefore, reservoirs and lakes being of prime importance in urban water supply must be carefully managed. Internal pollution is the primary cause of water pollution if external pollution effectively controlled. Internal pollution, caused by pollutants released from sediments, mainly occurs on the multi-phase interface of water-sediment-biofacies.

Thus, this interface is the most important environmental boundary layer for mass exchange between water and sediment. In freshwater sediment ecosystem, microbial communities harbored in sediment and overlying water play an important role in biogeochemical process of nutrients cycling and decomposition, such as organic matter demineralization and biochemical degradation, transformation of nitrogen, phosphorus and sulphur (Zhao *et al.,* 2011; Zhang *et al.,* 2013; Wu *et al.,* 2012). Microbes living in sediments and overlying water can trigger the release of phosphorus and also enhance the adsorption of phosphorus

(Huang *et al.,* 2011). Meanwhile, sediment microbial diversity and composition are strongly affected by environmental factors (Zeglin *et al.,* 2011) and organic carbon and nitrogen input process (Bai *et al.,* 2012). A lot of studies have been conducted to determine bacterial communities living in sediments of various freshwater ecosystems (Zhao *et al.,* 2011; Zeglin *et al.,* 2011; Ionescu *et al.,* 2012; Wang *et al.,* 2012). Previous reports suggest that different freshwater environmental conditions harbor dramatically distinct microbial community composition. Drinking water reservoir is considerably different from these aquatic environmental ecosystems with distinguishing hydrological regime (Lymperopoulou *et al.,* 2012). Drinking water reservoirs are usually found at different depth and easily become thermally and dynamically stratified (Gray, 2006; Humborg *et al.,* 2000; Wetzel 2001). Hydrostatic pressure at bottom is accordingly different. Microbial community structure and function interact with hydrostatic pressure and other physico-chemical factors of sediment and overlying water.

Pressure is an important environmental parameter for microbial life (Aude and Isabelle, 2013). Some scholars have reported effect of high hydrostatic pressure (HHP) on enzyme (Michael and Reyes, 2009), gene expressions (Xu and Ma, 2007; Philippe *et al.,* 2010) and microbial alteration (Tamburini *et al.,* 2009; Rivalain *et al.,* 2010). Influence of high hydrostatic pressure on iron reduction bacterium was conducted (Aude *et al.,* 2012; Wu *et al.,* 2013). But all these research only focused on deep sea environments, where hydrostatic pressures were usually larger than 10MPa. However for most drinking water reservoirs, their depth usually range from several m and 100 m (0.1MPa~1MPa). The one with depth > 100m is rarely seen. Thus, the microbial community structure and function response to hydrostatic pressure, ranging from 0.1MPa to 1MPa, is not clear. Lipid membranes are among the most pressure-sensitive biological structures that exist. As a consequence, with increasing pressure, lipid bilayer loses fluidity and rapidly becomes impermeable to water and other molecules and protein lipid interactions essential for optimal function of membrane are weakened (Winter and Jeworrek, 2009). Low temperature and high hydrostatic pressure have related and synergistic effects on biological membranes (Winter and Jeworrek, 2009), reducing their fluidity by increasing the packing of fatty acyl chains.

Some recent studies have evaluated sediment microbial diversity and composition using CARDFISH, bar-coded pyrosequencing, Biolog, PCR-DGGE AND metabolic and molecular analyses (Röske *et al.,* 2012; Du *et al.,* 2012; Bushaw *et al.,* 2012). Phospholipids are found exclusively in cell membranes, not in other parts of cell (Hill *et al.,* 2000; Syakti *et al.,* 2006), and are rapidly metabolized after cell death (Zhao *et al.,* 2011; Macalady *et al.,* 2000; Mallet *et al.*, 2004). For these reasons, phospholipids can be used to characterize community structure of viable microorganisms. Phospholipids fatty acid analysis (PLFA) is an established method for estimating microbial biomass and analyzing microbial community structure in complex environmental samples (Zhao *et al.,* 2011; Sundh *et al.,* 2005; Dong *et al.,* 2006; Zink *et al.,* 2008). Moreover, PLFA analysis can provide insights into nutritional status or physiological stress response of microorganisms (Hill *et al.,* 2000). However, there are several limitations such as intact phospholipids in sediments may partly derive from membrane remnants; the concept of 'cell death' and 'viability' in natural systems are both biologically and chemically unclear, mainly due to various states of dormancy and starvation that bacteria can adopt (Syakti, et al., 2006); Ratio of specific PLFAs have also been used to indicate stress or starvation, like trans/cis (e.g. 16:1u7t/16:1u7c) or cyclo/mono-unsaturated precursor (cy17:0/ 16:1u7c and cy19:0/18:1u7c) should also be highlighted (Frostegård *et al.,* 2011). In this case, it is presumed that bacteria alter the composition of their cell membrane in response to environmental conditions. Although it may be correct, the change may equally be due to shift in species composition of microbial community. The idea that PLFA patterns always change rapidly in response to altered environmental conditions should be viewed with caution. Detection and interpretation of decrease in PLFA abundance is difficult since it reveals death of microorganisms and subsequent degradation of their PLFAs (Frostegård *et al.,* 2011). Multivariate statistical analysis, including principal components analysis (PCA) and redundancy analysis (RDA), has proved as a promising approach for investigating the profile of fatty acids (Zhao *et al.,* 2011).

Additionally, most of the previous studies have focused only on the occurrence and distribution of pollutants in drinking water reservoirs and sediments (Bu *et al.,* 2013; Anja *et al.,* 2013; Florian *et al.,* 2013). The effect of physico-chemical factors, especially high hydrostatic pressure, of drinking water reservoir sediment on the microbial community structure needs more attention. Investigating the response of microbial community structure and function to hydrostatic pressure and other physico-chemical factors of sediment and overlying water can deduce the microbial community structure and functional changes, based on simple analysis of sediment and overlying water. Then, the release characteristics of endogenous pollutants and its influence on overlying water qualities combined with microbial community function with time flying can be deduced. In the present study, variations in sediment and overlying water physico-chemical factors and phospholipids fatty acid composition in three drinking water reservoirs, with different depth in Xi'an city, were investigated to determine the microbial community structure and function response to different hydrostatic pressure and other physico-chemical properties.

Materials and Methods

Study area : Heihe Jinpen reservoir (HR), Shibianyu reservoir (SR) and Tangyu (TR) reservoir are drinking water reservoirs located in southern part of Xi'an city, Shanxi province, China (Fig. 1). Xi'an is a famous modern city with long history, having a

population of 0.847 billion. Three reservoirs supply 1.26 million steres water to Xi'an each day, covering more than 80% of the total water supply. These drinking water reservoirs are the main source of water supply in Xi'an city with mean depth of 90m (HR), 40m (SR) and 20m (TR) respectively. Because of different depth, the hydrostatic pressure at bottom of each reservoir is diverse.

Collection of sediment and overlying water samples and estimation of physico-chemical parameters : Undisturbed surface sediment samples from each sampling station of three reservoirs were collected with a stainless steel core sampler (100cm length and 15cm diameter) in July 2011. The top of each core (0-5cm) was extruded and sampled. Any debris and fragments of macrofauna were removed manually. Sediment samples for analysis of physico-chemical properties, including sediment total nitrogen (sediment TN), sediment total phosphorus (sediment TP) and sediment organic carbon (sediment OC) were dried with a Freeze Dryer (FD-1D-50, Boyikang, China). TN was measured according to zinc cadmium reduction method after digested by alkaline potassium persulfate. TP was measured following the method of Li (1983). OM was measured according to Standard Methods for the Examination of Water and Wastewater (2002). Each sample had three replicates. Overlying water samples were collected 5cm above the water-sediment interface and finally obtained through the microporous filtering film (0.45 µm pore size). Ammonia nitrogen (NH_4^+), total nitrogen (TN) and phosphate (PO_4^{3-}) was estimated according to Standard Methods for the Examination of Water and Wastewater (2002). Dissolved oxygen (DO) , pH value and water depth was estimated *in situ* (at the bottom of reservoir water body) using a multifunction water quality monitoring instrument Hydrolab DS5 made by HACH company(USA).

Lipid extraction and gas chromatography mass spectrometry (GC-MS) analysis : Sediment samples (4 g) were extracted overnight by the modified method described by Bligh and Dyer (1959). Phospholipids were converted to fatty acid methyl esters (FAME) by heating with 3 ml of 0.5 % methanolic hydrochloric acid (HCl). Hexane/chloroform (4:1 v/v) was used to extract FAME and the solution was evaporated under a stream of nitrogen. Internal standards of C 19:0 methyl ester was employed and FAMEs were dissolved in hexane for chromatographic analysis. FAMEs' were stored in GC vials at -20 °C until GC-MS analysis. Samples (1µl) were injected by auto sampler AOC-20I of a QP2010 plus GC/MS (GCMS-QP2010 plus, Shimadzu, Japan) The column flow was 1.05ml min^{-1}. Full scan-selected ion monitoring mode (Scan-SIM) was used for quantification of FAMEs. Helium was used as carrier gas at a constant flow of 1.0

Fig. 1 : Location of sediment sampling sites at three drinking water reservoirs in Xi'an city (A: HR; B: SR; C:TR)

ml min^{-1}. The ion source temperature was maintained at 200 °C. Temperature program for FAME analysis: injector temperature 260°C; temperature program, 50ºC (2 min), 50-200ºC (3ºC min^{-1}), 200–240°C (5°Cmin^{-1}), and 240°C (10 min). Data collection was initiated after hexane solvent was eluted (3.0 min) and continued until no further peaks were observed. All phospholipids ester-linked FAME were identified based on the relative percentage of ions scanned and retention times compared to standard qualitative bacterial acid methyl ester mix (Supelco) that ranged from C11 to C20. For each sample, abundance of individual fatty acid methyl-esters was expressed on dry weight basis per unit dry weight. Fatty acid nomenclature was used as described by Frostegård *et al.* (1993a, 1993b) and Kandeler *et al.* (2000). The fatty acids i15: 0, a15:0, 15:0, i16: 0, 17: 0, i17:0, cy17:0, 18:1ω7 and cy19:0 were chosen to represent bacterial PLFAs (bactPLFAs) (Frostegård *et al.,* 1993a; Kandeler *et al.,* 2000; Tunlid *et al.,* 1989 and 18:2v6 was used as an indicator of fungal biomass (Kandeler *et al.,* 2000). The ratio of 18:2ω6: bactPLFAs was taken to represent the ratio of fungal: bacterial biomass in sediment (Kandeler *et al.,* 2000; Bardgett and Saggar, 1994). Concentrations of individual FAME were determined by calibration with internal standard (methyl ester C19:0).

Multivariable statistics analysis : PCA was performed using CANOCO 4.5 (Biometris, Wageningen, Netherlands) to study the physico-chemical parameters of different sediments and overlying water samples of three reservoirs.

Relationship between phospholipids fatty acid composition and sediment and overlying water physico-chemical properties was also investigated. The initial detrended correspondence analysis (DCA) results demonstrated that the data exhibited linear rather than unimodal response to the environmental variables (most length of gradient was 0.224, <2) (Zhao *et al.,* 2011; Lepc and Smilauer, 2003; Sapp *et al.,* 2007), so RDA was performed to explain the data by CANOCO 4.5 (Biometris, Wageningen, Netherlands) (Zhao *et al.,* 2011; Lepc and Smilauer, 2003). Ordination biplots including phospholipid fatty acid composition and environmental variables were used to explain the data. The detailed interpretation of ordination plots could be referred to Ter Braak (Ter, 1987).

Results and Discussion

The physico-chemical properties of sediments and overlying water from these three reservoirs are shown in Fig. 2. Attributed to different depth, the hydrostatic pressure at the bottom of each reservoir varied. Accordingly other properties showed more or less regular distinctions. DO concentrations decreased gradually with depth, thus DO value of HR (0.12mgl^{-1}) was much lower than TR (3.88 mgl^{-1}) and SR (2.12 mgl^{-1}). Rasmussen *et al.* (2004) showed that strong oxygen depletion was surprisingly favored by hydrostatic pressure variations and radical changes in pressure act as a mechanism that provokes the release of oxygen from the bottom. There were no distinct discrepancies of pH value among the overlying water from three reservoirs. Sediment OC (2.29%), sediment TN (0.63mgg^{-1}) and sediment TP (0.65 mgg^{-1}) values from HR sediment were lower than TR and SR, but NH_4^+ (2.89 mgl^{-1}) and TN (2.94 mgl^{-1}) values in HR overlying water were higher than TR and SR. This was due to release of NH_4^+ from sediment which was much more sensitive to DO and NH_4^+ and covered 98.3% of TN in HR overlying water. In rough, from the sediment pollution load point of view, the order was TR>SR>HR. But, from the overlying water quality point of view, the order was HR>TR and SR, respectively. Their crucial difference was hydrostatic pressure. As hypolimnion has no source of oxygen to replace that already used, its water may completely be devoid of oxygen. On the other hand, microbial respiration also commonly results in oxygen depletion in the lower portion of water column, especially in eutrophic waters; anoxia near the sediments, in turn, can lead to increased release of nutrients from the sediments. Thus, under anaerobic conditions iron, manganese, ammonia, sulphides, phosphates and silica are released from the sediments into the overlying water. Sediment is biological chemical bomb, while suitable environmental condition is the fuse which can trigger the pollutant release and make the overlying water quality poor. Accordingly it can be assumed that different hydrostatic pressure attributed to different depth, resulting in some changes in environmental factors (such as DO) and ultimately triggered the release of endogenous pollution that deteriorated the overlying water.

PCA ordination of three sediment samples collected from these three reservoirs in Xi'an city is shown in Fig. 3.The first and second axis revealed 80.403 % and 19.597 % of the total variance, respectively. Variables such as pressure, DO, sediment OC, NH_4^+, pH, sediment TP and sediment TN weighed most heavily on the first axis, while second axis was better correlated with PO_4^{3-} and TN. Especially, hydrostatic pressure was the most important effect factor to differentiate the overlying water quality from three drinking water reservoirs from each other. NH_4^+ content in overlying water was positively related with hydrostatic pressure; while DO in water-sediment interface and sediment OC in sediment were negatively related with hydrostatic pressure.

Cellular membrane lipids and their associated fatty acids are useful biomarkers of viable bacterial biomass, as they are essential components of every living cell. Phospholipids ester-linked fatty acids (PLFA) have proved to be of great value in describing bacterial community structure in sediments and in understanding bacterial phylogenic and taxonomic classifications (Guezennec and Fiala, 1996; Paraskevi *et al.,* 2005).

Phospholipids ester-linked fatty acids (PLFA) extracted from each sediment sample were analyzed to obtain information on microbial community composition that accounts for both culturable and nonculturable microorganisms (Paraskevi *et al.*, 2005; Balkwill *et al.,* 1998). Individual PLFA was quantified (Fig. 4).

Seventeen kinds of fatty acids containing 13~28carbon atoms, including saturated (odd and even), chain branched (iso and anteiso) and monounsaturated fatty acids were detected. PLFA content ranged between 1494.22 and 2622.23 nmolg^{-1} d.wt. (Table 2). The main sediment phospholipids-linked fatty acids detected in the present study were 16:0, 16:1w7, 15:0, a15:0 and 14:0, which were all common bacterial signatures, covering 78.50%, 81.57% and 79.12% of the total PLFA from TR, SR and HR respectively. Major PLFA components also included 18:0, 18:1w9 and 18:1w10t. In addition, the total microbial biomass could be calculated by summing all detectable PLFAs. Abundance of total PLFAs, saturated fatty acid (SFA), monounsaturated fatty acid (MUFA), Gram-positive (G^+) bacteria, Gram-negative (G^-) bacteria, their percentages and ratios of Gram-positive to Gram-negative bacteria calculated in each sample and the results are shown in Table 2. Bacterial PLFA profiles were categorized in four groups: Gram-positive bacterial PLFAs: iC14:0 (ammonia-oxidizing bacteria), C16:0 (methane utilizing bacteria), C17:0 (ammonia-oxidizing bacteria), C18:0 (methane utilizing bacteria), 10meC16:0 (actinomycetes) and C20:0 (microeukaryokes); Gram-negative bacterial PLFAs: cyc17:0 (sulphate reducing bacteria) and C16:1ω7c; Fungal indicators of PLFAs: C18:1ω9t (saprotrophic fungi), C18:3ω3c and C18:2ω6c and C16:1ω5c (mycorrhizal (AM) fungi) and Protozoa: C20:4ω6c. The ratio of Gram-positive to Gram-negative bacteria was significantly higher in HR sediment as compared to other two sediments, although total PLFAs content was smaller than the other two. Thus, it is suggested that high hydrostatic pressure and low dissolved oxygen concentration stimulated Gram-positive bacterial population in drinking water reservoir sediment.

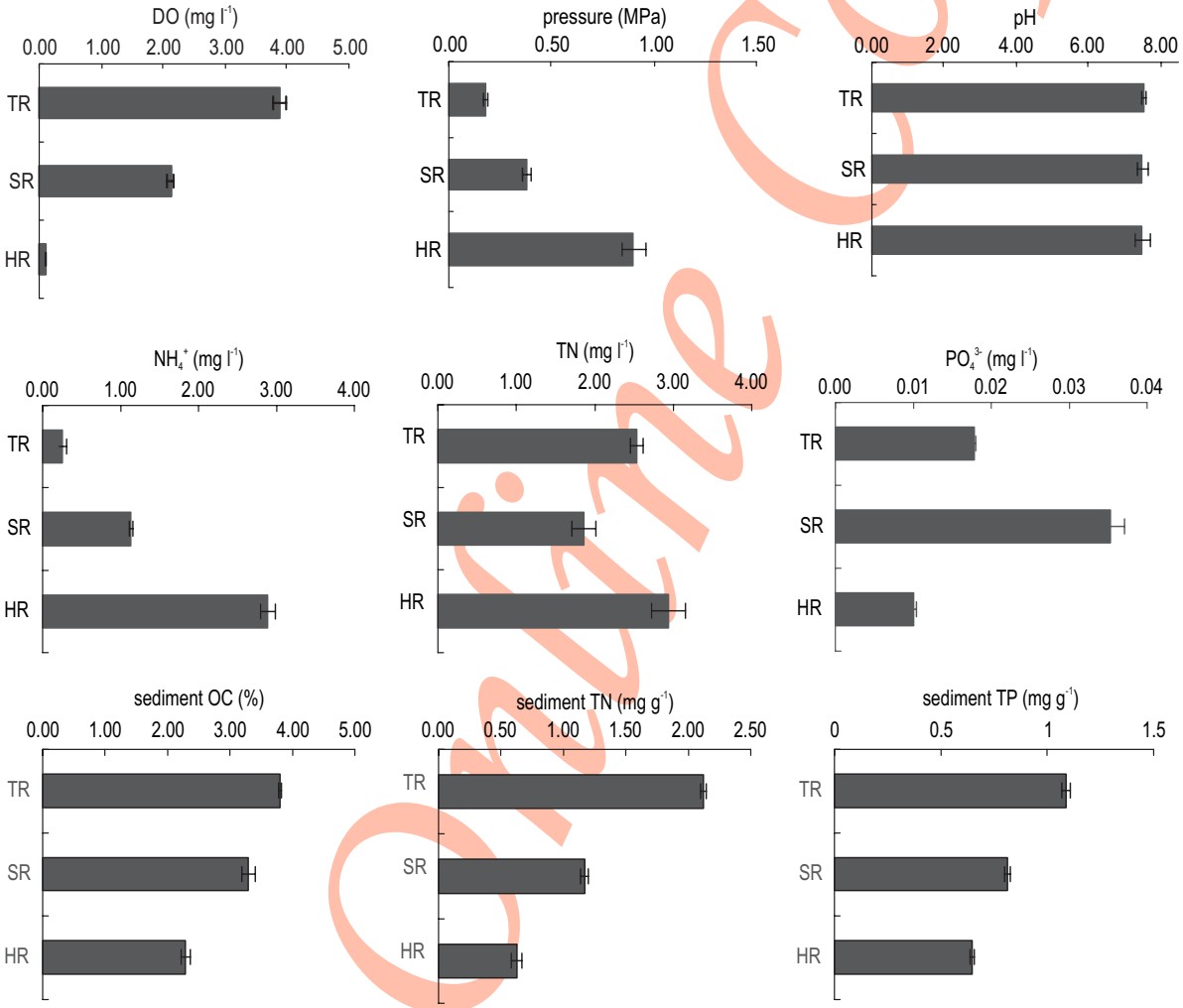

Fig. 2 : Physico-chemical parameters of sediments and overlying water samples of three reservoirs. Error bars represent standard deviation of three replicates

Table 2 : PLFA content in three reservoir sediment samples

	TR	%	SR	%	HR	%
Total PLFAs (nmol g^{-1})	1616.31	-	2622.23	-	1494.22	-
"SFA* (nmol g^{-1})	1213.39	75.07	1888.36	72.01	1188.12	79.51
"MUFA** (nmol g^{-1})	402.92	24.93	733.87	27.99	306.11	20.49
G^+ (nmol g^{-1})	1197.95	74.12	1865.39	71.14	1178.74	78.89
G^- (nmol/g)	418.36	25.88	756.84	28.86	315.49	21.11
G^+/G^-	2.86	-	2.46	-	3.74	-

*"SFA: Saturated fatty acid; **"MUFA: Monounsaturated fatty acid

Mucha *et al*. (2004) also reported that similar environmental parameters in sediment resulted in similar macro benthic community structures. The results of sediments and overlying water physico-chemical parameter PCA (Fig. 3) showed that the environmental parameters of three drinking water reservoirs were significantly different. Accordingly, microbial community structure in three reservoir sediments must be correspondingly different (Fig. 5). Principal components analysis also identified fatty acids that were important in explaining the variability in PLFA profiles. PCA ordinations of PLFAs from three reservoir sediment samples in Xi'an city and each PLFA correlations to the first and second eigen vector is shown in Fig. 5. Three sediment samples were distinct by different based on PLFAs. The first and second axis explained 73.97 % and 26.03 % of the total variance, respectively. Variables such as 18:1w9, a15:0, 17:0, 15:0, cy17:0 and so on weighed most heavily on first axis, while second axis was better correlated with 18:0, 18:2w3,9 and 19:1w9t.

Additionally, occurrence of mid-chain branched fatty acid 10Me,16:0 (SR 0.22% and HR 0.32%), which is considered as a sulfate-reducing bacteria (SRB) biomarker only when 10Me18:0 is absent was detected. These results are in agreement with these authors (Syakti *et al.,* 2006; Parkes *et al.,* 1992; Spring *et al.,* 2000). But it was absent in TR sediment sample. SRB is a rigorous anaerobic bacteria. It was found that fatty acid 10Me, 16:0 content increased with depth. Therefore, it was assumed that SRB preferred low dissolved oxygen and high hydrostatic pressure (in the range between 0.1MPa and 1.0MPa) to high DO and low hydrostatic pressure.

In the present study, variations is physico-chemical factors of sediment and overlying waters and phospholipids fatty acid composition in sediments of three drinking water reservoirs were investigated to determine which environmental parameter had strongest effect on microbial community structure. RDA is a suitable ecological statistic technique to investigate how microbial community structure varies along gradients of environmental variables. Its advantage is that it can calculate contribution of each environmental variables to the microbial community structure changes independently. It can also evaluate the relation between one or a group of variables and another group of variables from statistics point of view. However, relationship between microbial community structure in drinking water reservoir sediments and environmental factors especially, hydrostatic pressure (0.1~1.0MPa) was rarely reported.

Biplots consisting of environmental variables and phospholipids fatty acid samples were chosen to analyze relationship between sediment and overlying water physico-chemical properties and microbial community composition (Fig. 6). In ordination plot, first and second RDA axes accounted for 100% of total variance and first axis alone explained 72.1 %. TR and SR were distributed in fourth and third quadrant respectively. HR located near the edge between the second and first quadrant was in second quadrant. Environmental factors such as pressure, DO, NH_4^+, sediment OC had greatest bearing on PLFA profiles (i.e., microbial community structure). The foregoing analysis showed that both DO and NH_4^+ varied with hydrostatic pressure, and sediment OC was basic physico-chemical characteristic of sediment sample itself. Thus, it can be concluded that hydrostatic pressure and sediment basic physico-chemical factors (such as

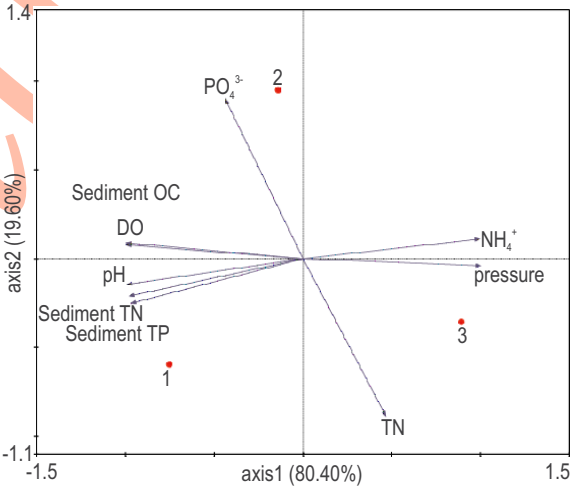

Fig. 3 : PCA of physico-chemical parameters in sediments and overlying water of three drinking water reservoirs (1:TR; 1:SR; 3:HR)

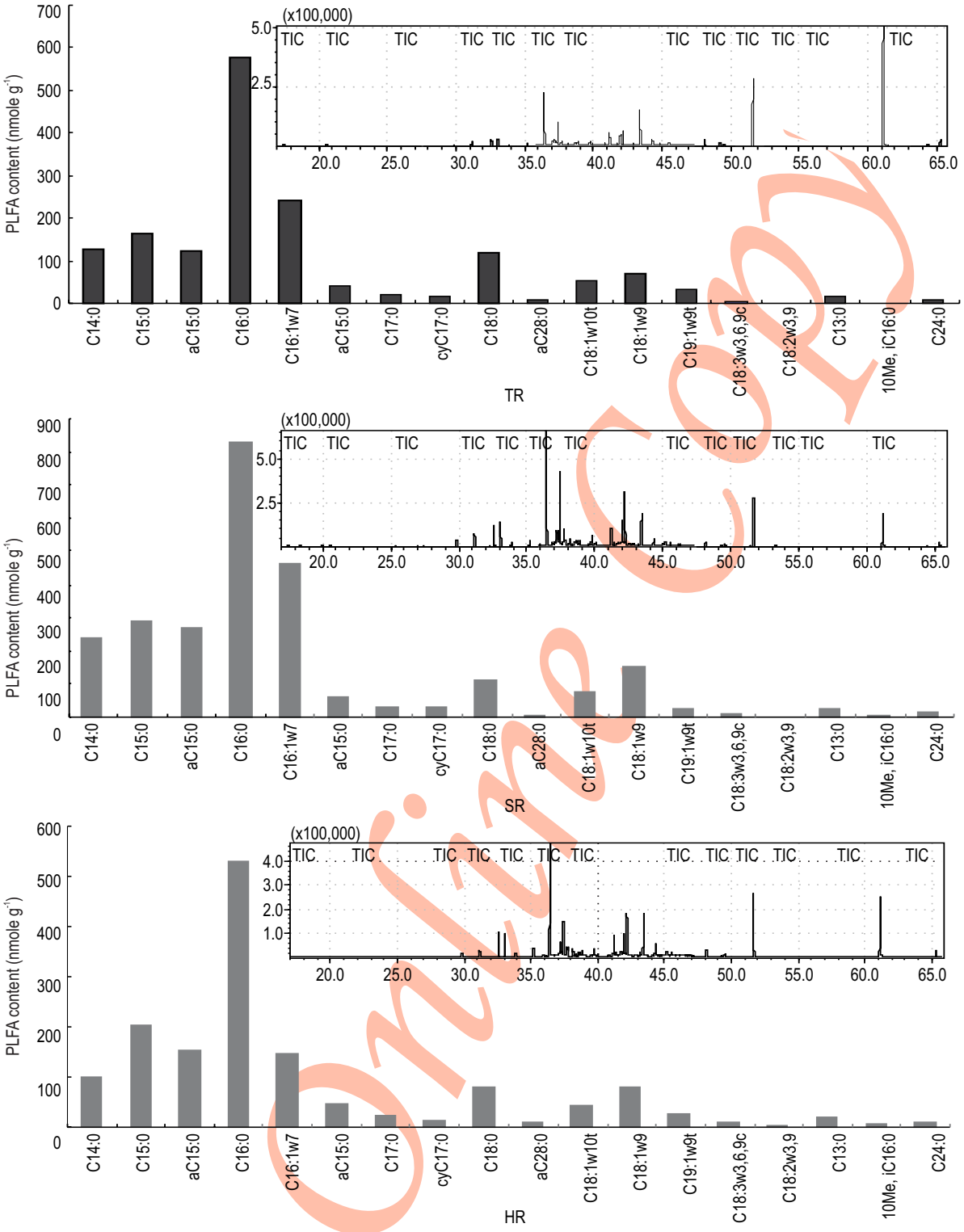

Fig. 4 : Different PLFAs content (arranged according to the time of each peak appeared from left to right) and GC/MS chromatogram of PLFAs in three drinking water reservoir sediments

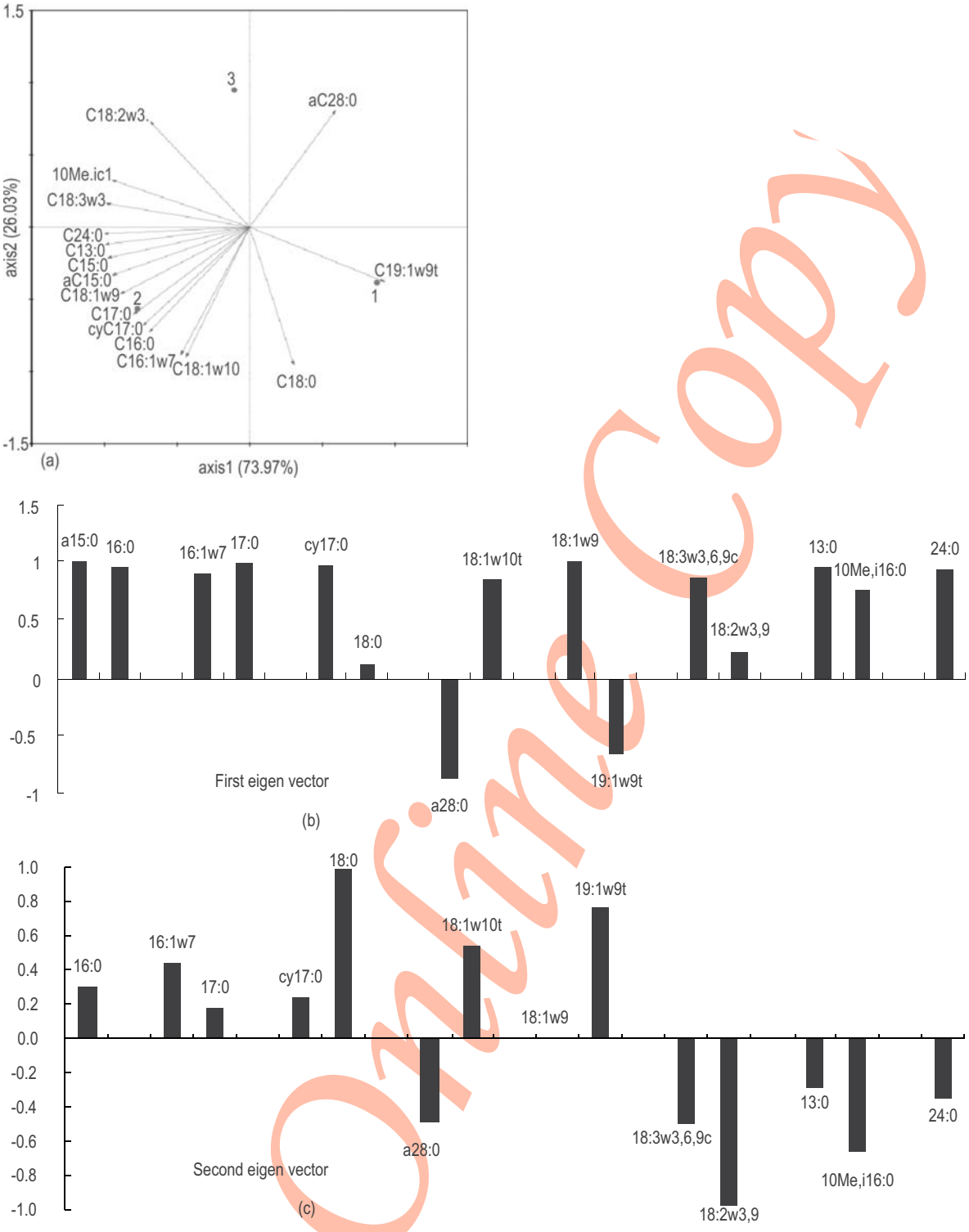

Fig. 5 : PCA of PLFAs in three sediment samples and bar plot of first and second eigenvector of PCA. The height of each bar gives correlation between a PLFA peak and corresponding PCA axis (1:TR; 1:SR; 3:HR)

Table 1 : Measurement uncertainty of each sample replicate for each parameter showed in Fig.2

(Relative standard deviation %) reservoirs									
	Parameters								
	DO	**pressure**	**pH**	**NH_4^+**	**TN**	**PO_4^{3-}**	**Sediment OC**	**Sediment TN**	**Sediment TP**
TR	2.89	5.81	0.80	21.33	3.24	1.23	0.59	1.01	1.82
SR	2.75	5.31	1.74	2.14	8.40	5.16	3.26	2.59	1.51
HR	1.08	6.73	2.76	3.21	7.39	4.30	3.14	6.94	1.55

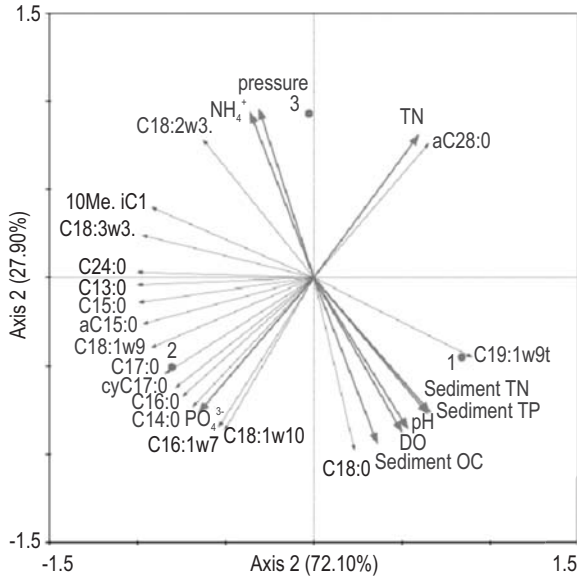

Fig. 6 : Ordination diagram of phospholipids fatty acid composition associated with environmental variables of sampling pressure, DO, pH, NH_4^+, TN, PO_4^{3-}, sediment TN, sediment TP and sediment OC. Environmental variables were indicated as arrows. Phospholipids fatty acid samples were indicated as circle (○, 1:TR; 1:SR; 3:HR).

sediment OC, sediment TN and sediment TP) were important effect factors to microbial community structure, especially hydrostatic pressure. This conclusion was consistent with the previous reports that lipid membranes are among the most pressure-sensitive biological structures that exist (Aude *et al.,* 2012; Aude *et al.,* 2013; Winter and Jeworrek, 2009). As a consequence with increasing pressure, the lipid bilayer loses fluidity and becomes rapidly impermeable to water and other molecules and protein lipid interactions essential to optimal function of membrane are weakened (Philippe *et al.,* 2010; Winter and Jeworrek, 2009). Further studies are needed to investigate the origin and formative process of microbial communities and how sediment physico-chemical characteristic influence microbial community structure in drinking water reservoir sediments.

It can be concluded that hydrostatic pressure was the most important effect factor to differentiate the overlying water quality. NH_4^+ content in overlying water was positively related to hydrostatic pressure, while DO and sediment OC were negatively related to hydrostatic pressure. High hydrostatic pressure and low dissolved oxygen concentration stimulated Gram-positive bacterial population in drinking water reservoir sediment.

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