Molecular characterization of macrophyte-derived dissolved organic matters and their implications for lakes

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HIGHLIGHTS

- Multiple spectroscopy methods were combined to characterize macrophyte-derived DOM.
- Floating and submerged plants have greater potential to accumulate and return N and P.
- Macrophyte-derived DOM has less aromatic than natural waters and soil leachates.
- Shoots have more protein-like and carbohydrate materials, while leaves are rich in aromatics.
- Nutrients released from macrophytes should be paid much attention in P regulation.

GRAPHICAL ABSTRACT
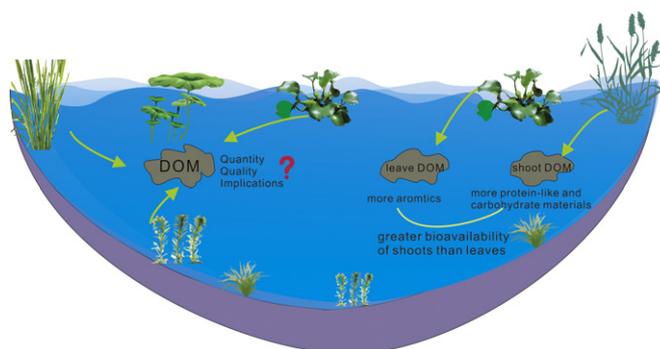
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ABSTRACT

Chemical properties of whole organic matter (OM) and its dissolved organic matter (DOM) extracted from three types of dominant macrophytes in Lake Dianchi were comparatively characterized using elemental analysis, UV, 3D-EEM and ^{13}C NMR spectroscopy and their implications for lakes were discussed. Ratios of C/N and C/P were least in the floating water hyacinth and submerged sago pondweed, while total dissolved nitrogen (TDN) and phosphorus (TDP), dissolved organic nitrogen (DON) and phosphorus (DOP) were greatest in those species. In emergent species, C/N, C/P, DON and DON/TDN were less in leaves than in their corresponding shoots. The specific UV absorbance at 254 nm (SUVA$_{254}$) and 280 nm (SUVA$_{280}$) of extracts were in the range of 0.50–1.96 L/mg C·m and 0.40–1.48 L/mg C·m. Both SUVA values were greater in leaves than those in shoots. 3D-EEM spectra showed only a single fulvic-like fluorescence in leaves of emergent macrophytes. In contrast, protein-like peak were observed in spectra of floating and submerged species, as well as the shoot DOM of emergent species. Solid-state ^{13}C NMR demonstrated that leaves had greater percentage of recalcitrant alkyl C and aromatic C, while shoots were rich in labile carbohydrates. The overall characterization works suggested that macrophyte-derived DOM has less aromatic constituents than do DOM in natural waters and soil leachates. Also OM and DOM derived from shoots had greater contents of protein-like and carbohydrate materials, while leaves were rich in aromatics. Floating and submerged plants possessed potential to not only accumulate excess N and P, but also for returning them to the lake. Shoots of riparian and emergent species were also an important source of nutrients. Thus, macrophyte biomass should be a great concern in nutrient regulation in Lake Dianchi.

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1. Introduction

Organic matter (OM) is an important component in the biogeochemical cycles of terrestrial and aquatic ecosystems (Wu and Xing, 2009). It is the major constituent in cycling of elements and flow of energy through ecosystems, and a primary factor influencing biochemical compositions of soils and/or sediments. OM serves as a substrate for binding pollutants, and is closely related to the carbon cycle and global warming (Wu and Xing, 2009). Thus, OM in both terrestrial and aquatic ecosystems is a significant area of research in biogeochemistry.

Dissolved organic matter (DOM) is the most easily released and reactive component of OM, and plays an important ecological role especially in aquatic systems (He and Zhang, 2015). Chemical composition of DOM is a key factor in determining its fate and environmental behaviors in lakes, such as reactions with pollutants, rates of degradation and turnover, and humification (Oades, 1988; Wu and Xing, 2009). Chemical properties of allochthonous DOM composed of humic substances have been more thoroughly studied (He et al., 2006; Kögel-Knabner, 1997; Senesi et al., 2003). However, limited research has focused on non-humic DOM freshly released from autochthonous plants, which is characterized by lesser molecular-weight organic matter (Liu et al., 2016; Zhang et al., 2013).

In lacustrine ecosystems, OM materials are mainly derived from aquatic macrophytes, phytoplanktons, bacteria, and terrestrial inputs from riparian plants or soils (Croué et al., 2003). As one of the primary producers in lake systems, aquatic macrophytes are major autochthonous sources of OMs to most lakes, but are more important in shallow lakes with well-developed littoral zones. Macrophyte OM includes a portion that is more easily dissolved and another fraction that is not soluble in water but participates in biogeochemical cycles of lakes after being decomposed by microbes. In aquatic systems, DOM reacts with inorganic and organic substances more frequently and faster than DOM in terrestrial systems (Findlay and Sinsabaugh, 2003). Thus, the types, quantities and possible environmental behaviors of DOM released by autochthonous macrophytes in lakes needed to be addressed.

For better understanding of the quantities and features of DOM released by autochthonous macrophytes, in this study, one riparian species, three emergent macrophytes, one floating species and one submerged species were collected from Lake Dianchi. OM and DOM fractions were characterized by use of ultraviolet-visible (UV–vis), three-dimensional excitation-emission fluorescence matrix (3D-EEM), and solid-state ^{13}C nuclear magnetic resonance (^{13}C NMR) spectroscopic analysis. The objectives of this study were to: 1) characterize OM and DOM from macrophytes of different life forms and also the shoots and leaves of emergent and riparian species; 2) explore relationships between the chemical properties of macrophyte-derived OM and DOM on both qualitative and quantitative aspects; 3) estimate the ratios of N and P derived from macrophytes to total N or P in lake water; and 4) further discuss the degradation of OM/DOM from different species and possible environmental implications on contaminants based on their chemical compositions.

2. Materials and methods

2.1. Study site and sample collection

Lake Dianchi is located in the southwestern plateau of China (24°40′ ~ 25°02′ N, 102°36′ ~ 102°47′ E) (Fig. 1). The lake is at an elevation of 1886 m and has a surface area of 330 km². The volume of the lake is approximately 1.2×10^{11} m³. The mean and maximum depths are 4.4 m and 15.1 m, respectively. The whole lake is divided into two parts: the Caohai section in the north and the Waihai section in the south by the natural dike in northeast of the lake. Dianchi has a subtropical plateau monsoon climate. Vegetation around Lake Dianchi has the typical characteristics of sub-tropical evergreen broad-leaved forest. Annual precipitation is 1036 mm, which is concentered in the latter half of the year.

Water temperature ranges from 9.8 °C to 24.5 °C, with an annual average of about 16.0 °C. Lake Dianchi is adjacent to Kunming City, the capital of Yunnan Province, Therefore, large quantities of industrial wastewater and municipal sewage have been discharged into the lake. Consequently, a significant amount of nitrogen and phosphorus nutrients enter the lake from the catchment. At present, the lake water is heavily polluted and suffers from eutrophication (Li et al., 2002; Liu et al., 2015). According to data publicly available from the Dianchi management administration, in 2010, concentrations of total phosphorus (TP) and total nitrogen (TN) were 0.28 and 1.06 mg/L, respectively (Table S1).

The distribution of aquatic plant communities in the lake is extremely uneven, and the majority of aquatic plants are concentrated along the coast. Dominant aquatic plants are emergent and submerged plants in the littoral zone. Total biomass of aquatic plants in Dianchi reached up to 41,019.65 Mg (Yu et al., 2000). Based on the dominant species of aquatic plants reported previously (Zhou et al., 2013; Song et al., 2011), six species of macrophytes including riparian, emergent, floating, and submerged types were collected at their maturation stage, from the Waihai section, during October 2011 (Fig. 1). They were one riparian species—crofton weed (*Eupatorium adenophorum* Spreng., Asteraceae), three emergent macrophytes—water oats (*Zizania caduciflora* Turcz., Gramineae), common reed (*Phragmites australis* Trin., Gramineae) and oriental pepper (*Polygonum orientale* L., Polygonaceae), one floating species—water hyacinth (*Eichhornia crassipes* (Mart.) Solms, Pontederiaceae) and one submerged species—sago pondweed (*Potamogeton pectinatus* L., Potamogetonaceae). At least five specimens of each species were collected. In the laboratory, leaves and shoots were separated and washed with deionized water, then killed by heating at 90 °C for 1–2 h and followed by 12–24 h of complete drying at 60 °C. Dry materials were then ground to pass through a 1-mm sieve.

2.2. Extraction of DOM

DOM was extracted by water (ultra-pure, mili-Q) with a 35:1 (*ml/g*) water to sample mass ratio (He et al., 2009). The suspension was then shaken at 4°C for 18 h (Brown and Sposito, 1991). After centrifugation for 20 min, extracts were filtered under vacuum through 0.45-μm pore size polycarbonate filters (He et al., 2009). Aliquots of the supernatant were used directly for quantification of carbon (C), nitrogen (N), phosphorus (P) and measurement of UV–vis and 3D-EEM spectra. The remaining supernatant was freeze-dried and kept in a desiccator at room temperature for solid-state ^{13}C NMR analysis.

2.3. Chemical analysis

Before extraction of DOM, total carbon (TC), total nitrogen (TN) and hydrogen (H) in ground dry materials were determined using an elemental analyzer (Elementar vario macro EL, Germany) following an in situ HCl acidification procedure (Nieuwenhuize et al., 1994). Total phosphorous (TP) in ground dry samples was determined by methods described previously (Pardo et al., 2003). In plant extracts, concentrations of total dissolved carbon (TDC), dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) were determined by a multi N/C 3100 analyzer (Analytic Jena, German). NH_4-N in extracts was measured by Nessler's reagent photometry (Demutskaya and Kalinichenko, 2010). NO_3-N in extracts was quantified by use of a UV–vis spectrophotometer at 220 nm and corrected at 275 nm (Agilent Technologies, Wilmington, DE, USA). Dissolved organic nitrogen (DON) in plant extracts was then determined by differences between TDN and the sum of NH_4-N and NO_3-N. Total dissolved P (TDP) (after digestion with potassium persulfate ($K_2S_2O_8$) in an autoclave at 121 °C for 30 min) and total dissolved inorganic P (DIP) in plant extracts were determined by use of molybdenum blue method (Murphy and Riley, 1962). Concentrations of dissolved organic P (DOP) were then determined as the difference between TDP and DIP. Triplicate samples of each species were used in all tests.
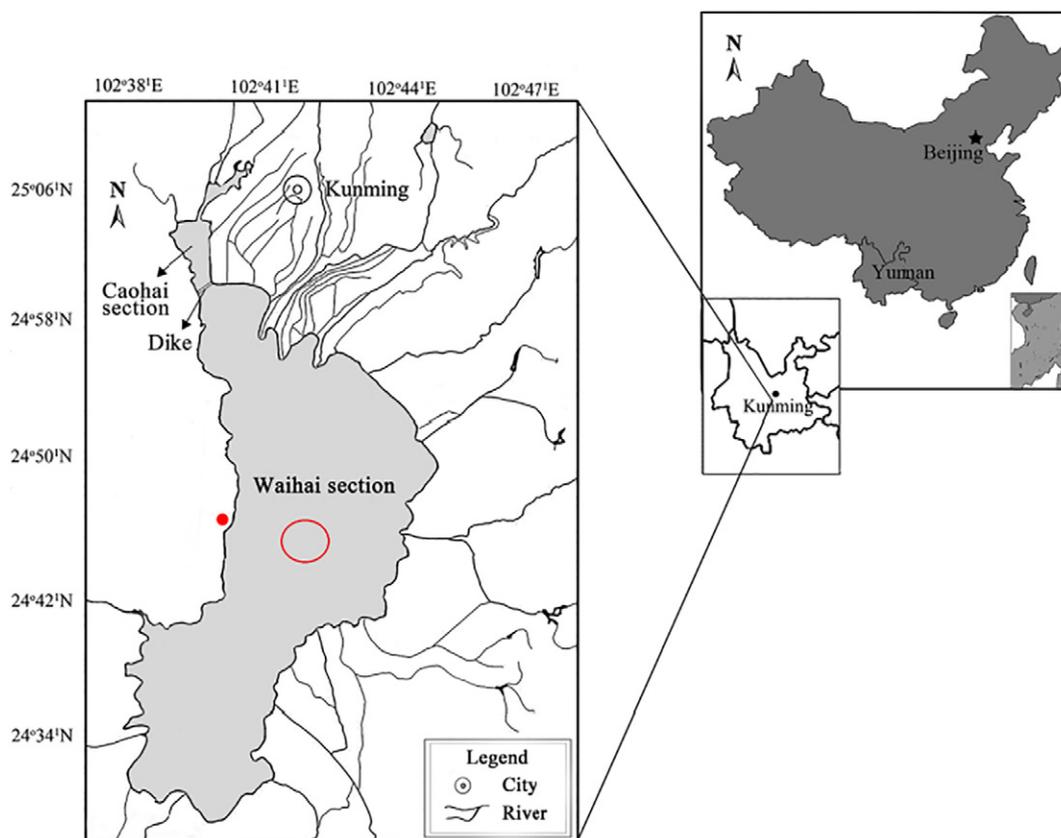
Fig. 1. Map of the sampling region of Dianchi Lake. All aquatic macrophytes were sampled in the region of Waihai section. The sampling region of aquatic macrophytes was within the red circle in the map. One riparian species crofton weed was collected in the lake shore shown with solid red dot in the map.

2.4. Spectroscopic characterization

Chemical features of DOM were characterized by UV–visible, 3D-EEM and solid-state CP/MAS ^{13}C NMR spectroscopies. UV–visible spectra were measured in the range of 200–700 nm by use of a UV–vis 8453 DAA spectrophotometer (Agilent Technologies, USA) in a 1.0 cm quartz cuvette, and ultra-pure (Milli-Q) water was used as a blank. Specific UV absorbance at 254 nm and 280 nm (SUVA$_{254}$, SUVA$_{280}$, L / (mg C·m)) were calculated from absorbance at 254 and 280 nm divided by DOC concentration, respectively (Zhang and He, 2015).

3D-EEM spectra of DOM were performed with a PerkinElmer Precisely LS55 Fluorescence Spectrometer (PerkinElmer, USA) equipped with a 150-W continuous output xenon arc lamp. The bandpass was set to be 10 nm for both excitation and emission wavelengths. The excitation wavelength (Ex) was in the range of 200–550 nm and the emission wavelength (Em) was from 250 to 600 nm. The responding time was set automatically and the scanning rate was 1500 nm/min. In order to prevent any inner filter effect, solutions were diluted to UV absorbance at 260 nm to 0.1 using purified water (Ohno and He, 2011).

Solid-state CP/MAS ^{13}C NMR spectra of freeze-dried DOM samples were performed on a Bruker AV-300 spectrometer at 12.5 kHz by using a standard 4 mm double-bearing probe. The recycle delay was 5 s, the pulse width was 2.4 μs, and the contact time was set as 2000 μs (He et al., 2013). The relative distribution of C functional groups in different structures was determined by integrating the signal intensities over defined chemical shift windows using MestReNova10.0. The defined spectral windows and corresponding structures were: I, 5–50 ppm (alkyl-C); II, 50–60 ppm (NCH/OCH$_3$); III, 60–90 ppm (O-alkyl C); IV, 90–108 (O—C—O); V, 108–145 ppm (aromatic C); VI, 145–165 ppm (aromatic C—O); VII, 165–187 ppm (COO/N—C=O); and VIII, 187–220 ppm

(C=O) per the literature with minor adjustments for better resolutions (Nelson and Baldock, 2005; Ranatunga et al., 2017).

2.5. Statistical analysis

Differences of C, N, P among plants, as well as their dissolved components in DOM derived from macrophytes were evaluated by *t*-test (*p* < 0.05) derived from analysis of one-way ANOVA using Origin 8.0 software (OriginLab, Inc., USA). To check whether there was a significant linear relationship of C, N and P, as well as C functional groups in whole OM and the DOM fractions of the macrophytes, Pearson correlation coefficients (*r* values) were performed at α = 0.05 level of significance using Origin 8.0 software.

3. Results and discussion

3.1. Elemental composition characteristics

Elemental components of ground, dry samples of macrophytes varied among species (Table 1). Floating water hyacinth and submerged sago pondweed exhibited the least C/N (10.4 and 12.9, respectively) and C/P (62.1 and 100.5, respectively) ratios. When comparing C/N and C/P in leaves and shoots of the riparian and emergent species, C/N and C/P were less in leaves (14.7–22.6 and 148.7–227.1, respectively) than in shoots (29.3–54.8 and 184.7–615.7, respectively). These results indicated that leaves have a greater content of N and P per unit of C than do shoots, especially those of water hyacinth and sago pondweed. C/N and C/P in OM of macrophytes were from 10.4 to 54.8 and 62.1 to 615.7, which is less than those previously reported for deciduous and coniferous trees (62.1–106.6 and 1140.2–3867.8) (Lorenz et al., 2004), but

Table 1
Element composition in the ground dry samples of the macrophytes.

	TC	TN	H	TP	C/H	C/N	C/P
	(%)	(%)	(%)	(%)	Ratio	Ratio	Ratio
Crofton weed (l)	43.87 ± 0.18[a¶]	2.27 ± 0.03[a]	5.84 ± 0.03[a]	0.68 ± 0.02[a]	0.63	22.55	166.66
Crofton weed (s)	43.61 ± 0.29[a]	0.96 ± 0.01[b]	5.96 ± 0.11[a]	0.61 ± 0.01[ab]	0.61	52.99	184.69
Water oats (l)	41.31 ± 0.07[b]	2.67 ± 0.02[a]	5.71 ± 0.05[a]	0.47 ± 0.02[b]	0.60	18.05	227.06
Water oats (s)	41.72 ± 0.20[b]	1.66 ± 0.02[c]	6.18 ± 0.05[a]	0.32 ± 0.09[c]	0.56	29.32	336.80
Common reed (l)	44.60 ± 0.21[ac]	3.54 ± 0.05[d]	5.99 ± 0.27[a]	0.51 ± 0.06[b]	0.62	14.70	225.92
Common reed (s)	45.28 ± 0.18[c]	0.99 ± 0.02[b]	5.95 ± 0.10[a]	0.19 ± 0.03[d]	0.63	53.36	615.65
Oriental pepper (l)	44.10 ± 0.17[a]	2.91 ± 0.01[e]	5.59 ± 0.07[ab]	0.77 ± 0.01[a]	0.66	17.76	148.66
Oriental pepper (s)	43.18 ± 0.09[a]	0.92 ± 0.01[b]	5.58 ± 0.06[a]	0.22 ± 0.05[bd]	0.65	54.77	507.16
Water hyacinth (l)	37.97 ± 0.26[d]	4.25 ± 0.06[f]	5.15 ± 0.02[c]	1.58 ± 0.03[e]	0.61	10.42	62.08
Sago pondweed (l)	38.18 ± 0.02[d]	3.45 ± 0.01[g]	5.38 ± 0.09[bc]	0.98 ± 0.01[f]	0.59	12.89	100.51
Several deciduous and coniferous trees					0.8–1.4[#]	62.1–106.6[*]	1140.2–3867.8
Algae						4.8–5.3[†]	
Redfield ratio						6.6[‡]	106.0[‡]

Data are presented as the mean ± SE (n = 3).

l: leaf; s: stem.

  [¶] The values with different letters in the same column significantly different at $p ≤ 0.05$.

  [*] Cited from Lorenz et al. (2004).

  [†] Cited from Hedges et al. (2002).

  [#] Cited from Berg and McClaugherty (2013).

  [‡] Cited from Redfield (1963).

greater than those of algae/phytoplankton (4.8–6.6 and 106.0) (Hedges et al., 2002; Redfield, 1963). These results showed that the macrophytes studied exhibited lesser accumulation of N and P than algae/phytoplankton, but they still had a relatively greater accumulation of nutrients comparing with terrestrial plants. Additionally, C/N ratios have often been used to distinguish origins of organic matters in environment (Meyers, 1994). In this study, C/N ratios in OM of macrophytes were between values of algae/phytoplankton and terrestrial plant, which arises from the absence of cellulose in algae and its abundance in vascular plants. The obvious distinction observed in the study further confirmed that C/N ratio can be used to distinguish sources of OM either from algae, aquatic plants or terrestrial plants in lakes. The ratio of C/H which indicates aromaticity of the organic matters was greatest in leaves of oriental pepper and least in that of sago pondweed. In riparian and emergent species, ratios of C/H were similar or slightly greater in leaves (Table 1), which implied a greater content of aromatic substances in leaves.

For distributions of C, N and P, correlations between their dissolved fractions (TDC, TDN, and TDP) and those in their parent OM (C, N, and P) were least for C ($r = 0.4$, $p > 0.05$), and greatest for P ($r = 0.95$, $p < 0.01$) (Fig. S1). The correlation between N and TDN was also significant ($r = 0.7$, $p < 0.05$) (Fig. S1). The poor correlation between TDC (DOC) and TC ($r = 0.4$, $p > 0.05$) could be due to difference in solubility of organic matters, which is determined by their polarity, percentage of hydrophobic and hydrophilic components. The significant correlation between TN and TDN ($r = 0.7$, $p < 0.05$) could be due to the complete solubility of inorganic N and greater polarity of amides in organic N. The greatest correlation ($r = 0.95$, $p < 0.01$) between TP and TDP among C, N, and P might be due to the greatest percent of DIP in TDP in all samples (65.0–90.8%) (Table 2). Carpenter and Lodge (1986) also reported that 60–90% of TDP released through leaching was DIP. The significant relationship between dissolved components and parent OM suggested that nutrient releases (N and P, especially) were closely related to those accumulated in tissues of macrophytes. Based on the greatest contents of N, P, TDN and TDP being in floating and submerged plants (Tables 1, 2), they are shown to have the greatest abilities in both accumulating excess N and P in their tissues and also for returning N and P to the water column via release concurring with DOM.

Releases of dissolved C, N and P from macrophytes varied according to the contents of dissolved components and their ratios in total contents (Table 2). Ratios of TDP/TP (15.0–33.6) were greatest when compared with DOC/TC (6.0–15.6) and TDN/TN (4.4–37.3), which indicated that a greater proportion of P in aquatic macrophytes tended to be dissolved and released into the water than C or N in plant debris. In plant extracts,

ratios of DON/TDN ranged from 23.5 to 94.9 (with a mean of 70.5), while the ratios of DOP/DTP ranged from 9.1to 35.0 (with a mean of 19.0). This result revealed that organic N is the dominant form of N released from macrophytes, while inorganic P dominated forms of releasable P in macrophytes. Among leaves of the species, TDN/TN, DON and DON/TDN were greatest in floating water hyacinth (17.9%, 174.3 mg/L and 80.0%, respectively) and submerged sago pondweed (15.6%, 144.3 mg/L and 93.9%, respectively). Shoots of all riparian and emergent species had a greater DON/TDN (48.0–94.9%) than did their corresponding leaves (23.5–75.2%). TDN/TN ratios were also greater in shoots of emergent species than that in leaves. Ratios of DON/TDN were least in both leaf and shoot of the annual oriental pepper (23.5% and 48.0%, respectively). Based on distribution of DON and DON/TDN, it is reasonable to assume that there was more biomass containing organic nitrogen, such as amino acids/peptides/proteins in the floating water hyacinth, the submerged sago pondweed, and shoot of the emergent species. This observation was consistent with earlier reports that the floating and submerged species always have a greater content of organic N (Howard-Williams, 1981; Polomski et al., 2009). Similar to DON/TDN, DOP/TDP ratios were greatest in the floating water hyacinth (32.2%) and the submerged sago pondweed (35.0%). Meanwhile, water hyacinth and sago pondweed also had relatively greater TDP/TP ratios among leaves of collected macrophytes. Except for annual oriental pepper, ratios of TDP/TP and DOP/TDP were similar or greater in shoots (28.7–33.6% and 12.6–17.9%, respectively) than in their corresponding leaves (15.0–28.3% and 9.1–18.4%, respectively) (Table 2). All these results further demonstrated that floating and submerged plants are easier to release nutrients into the lake water. Furthermore, leaves of water hyacinth, sago pondweed, and shoots of the riparian and emergent species should be an important source of the bioavailable nutrients (N and P) for the microbes. Consequently, nutrients released from floating and submerged macrophytes should be a great concern in eutrophic lakes.

3.2. UV–vis spectral features

UV–vis spectroscopy is an important tool used to semi-quantitatively evaluate DOM sources, composition, and structure of natural organic matter due to their sensitivity, small quantities of sample needed for measurement and no need to sophisticate preparation (Kalbitz et al., 2003). The absorbance in UV–Vis spectra of macrophyte-derived DOM decreased exponentially with increasing wavelength over the range of 200–600 nm. Absorbance shoulders near 200–380 nm regions were detected in all UV–Vis spectra of macrophyte-derived DOM, which is

Table 2
Contents of C, N, and P in the DOM fraction of the macrophytes.

Sample	DOC mg/L	TDN mg/L	DON mg/L	NH_4-N mg/L	NO_3-N mg/L	TDP mg/L	DOP mg/L	DIP mg/L	DOC/TC (%)	TDN/TN (%)	DON/TDN (%)	TDP/TP (%)	DOP/TDP (%)
Crofton weed (l)	1478.32 ± 34.17[a]	40.10 ± 0.34[a]	29.76 ± 3.09[a]	8.56 ± 2.78	1.77 ± 0.02	39.28 ± 0.90[a]	7.21 ± 0.35[a]	32.07 ± 0.90	11.8	6.2	74.2	20.2	18.4
Crofton weed (s)	1941.84 ± 95.69[b]	15.11 ± 0.55[b]	12.06 ± 1.96[b]	2.69 ± 1.44	0.34 ± 0.03	49.95 ± 0.64[b]	7.97 ± 2.06[b]	41.99 ± 2.69	15.6	5.5	79.8	28.7	16.0
Water oats (l)	1014.29 ± 19.71[cd]	105.13 ± 1.42[c]	79.01 ± 1.67[c]	17.18 ± 1.96	8.94 ± 0.08	38.04 ± 0.09[a]	3.48 ± 0.56[b]	34.55 ± 0.50	8.6	13.8	75.2	28.3	9.1
Water oats (s)	1172.73 ± 10.67[d]	91.02 ± 0.46[d]	86.41 ± 1.57[ce]	4.17 ± 1.34	0.42 ± 0.09	27.62 ± 0.41[c]	3.50 ± 0.36[b]	24.12 ± 0.25	9.8	19.2	94.9	30.2	12.6
Common reed (l)	1421.33 ± 26.97[a]	77.95 ± 1.13[e]	34.45 ± 2.25[d]	10.85 ± 1.12	32.69 ± 0.11	26.96 ± 0.86[c]	2.89 ± 0.64[b]	24.06 ± 0.71	11.2	7.7	44.2	18.5	10.7
Common reed (s)	830.83 ± 13.38[de]	105.48 ± 2.17[c]	95.79 ± 2.42[e]	1.36 ± 0.07	8.34 ± 0.31	18.26 ± 0.37[c]	3.27 ± 0.06[b]	14.99 ± 0.31	6.4	37.3	90.8	33.6	17.9
Oriental pepper (l)	865.32 ± 12.06[de]	36.88 ± 0.68[e]	8.67 ± 1.06[b]	9.14 ± 0.82	19.08 ± 0.28	33.01 ± 0.93[e]	7.17 ± 0.59[a]	25.85 ± 0.36	6.9	4.4	23.5	15.0	21.7
Oriental pepper (s)	744.90 ± 6.61[e]	25.81 ± 0.15[f]	12.39 ± 0.70[b]	3.15 ± 0.73	10.25 ± 0.18	18.92 ± 0.37[d]	3.10 ± 0.37[b]	15.82 ± 0.07	6.0	9.8	48.0	30.1	16.4
Water hyacinth (l)	892.00 ± 12.06[de]	217.90 ± 1.70[g]	174.34 ± 3.67[e]	10.70 ± 1.87	32.85 ± 0.12	112.38 ± 1.76[f]	36.24 ± 0.51[c]	76.13 ± 1.34	8.2	17.9	80.0	24.9	32.2
Sago pondweed (l)	713.50 ± 8.47[h]	153.61 ± 1.33[h]	144.34 ± 3.79[f]	8.85 ± 0.14	0.40 ± 0.08	61.23 ± 0.21[g]	21.41 ± 0.41[d]	39.81 ± 0.62	6.5	15.6	93.9	21.9	35.0

Data are presented as the mean ± SE.
l: leaf; s: stem.
[a] The values with different letters in the same column significantly different at $p \leq 0.05$.

consistent with that observed in DOM derived from phytoplankton and macrophytes reported previously (Zhang et al., 2013). The absorption peak near 200–380 nm regions is due to π–π^* electron transition in unsaturated structures, such as those in aniline derivatives, phenolic arenes, polyenes, and polycyclic aromatic hydrocarbons with two or more rings (Duarte et al., 2003; He et al., 2009). This indicated the presence of conjugated alkenes, unsaturated carbonyl compounds, or aromatic substances in constituents of DOM.

Specific UV absorbance (SUVA) is defined as the UV absorbance of a water sample at a given wavelength normalized for dissolved organic carbon (DOC) concentration. The specific UV absorbance at 254 nm ($SUVA_{254}$) has been shown to be strongly correlated with the hydrophobic organic acid fraction of DOM (Spencer et al., 2012) and is a useful proxy for DOM aromatic content (Weishaar et al., 2003) and molecular weight (Chowdhury, 2013). Another commonly cited DOC-normalized absorbance wavelengths $SUVA_{280}$ was also examined in extracts of macrophytes. $SUVA_{254}$ and $SUVA_{280}$ of extracts were in the range of 0.50–1.96 L/mg C·m and 0.40–1.48 L/mg C·m, respectively (Table 3), which is comparable to crop and aquatic plant leachate values reported by Pellerin et al. (2010). However, $SUVA_{254}$ in DOM derived from macrophytes was less than those for soil leachates (Hansen et al., 2016), surface water (Jaffé et al., 2008) and ground water (Chen et al., 2003). The significant difference revealed that macrophyte-derived DOM contains a lesser percentage of aromatic materials than do natural water and soil leachates. Either for shoots or leaves, both the values of $SUVA_{254}$ and $SUVA_{280}$ in DOM extracted from terrestrial plant crofton weed were greater than those from aquatic macrophytes, which indicated that aquatic macrophytes-derived DOM also have less aromatics than terrestrial plants. $SUVA_{254}$ and $SUVA_{280}$ were least in sago pondweed (0.86 and 0.67, respectively) and water oats (1.02 and 0.65, respectively) among the leaves of six macrophytes. For leaves and shoots in riparian and emergent species, both of the two values for SUVA were greater in leaves (0.86–1.96 and 0.65–1.48, respectively) than those in shoots (0.50–0.82 and 0.40–0.90, respectively) for all the species (Table 3). This indicated that leaves contain a greater percentage of aromatic substances or have a greater molecular weight than the DOM from shoots, which is consistent with that observed from C/H ratios.

3.3. 3D-EEM fluorescence spectroscopy

Since 3D-EEM spectroscopy is an analytic technology with a great sensitivity and selectivity on DOM analysis (Li et al., 2014; Wu et al., 2003, 2004a, 2004b), it was widely used to investigate the presence of aromatic substances, conjugate chromophores and/or the unsaturated aliphatic chains in organic matters (Croué et al., 2003; Weishaar et al., 2003). Fluorophores in DOM were classified as: humic-like (peak C, E_x/E_m = 350–440 nm/430–510 nm; peak D, E_x/E_m = 280–288 nm/420–455 nm), visible fulvic-like (Peak A, E_x/E_m = 310–360 nm/370–

Table 3
SUVA values of DOM derived from macrophytes in Lake Dianchi.

Samples	$SUVA_{254}$ (L/(mg C·m))	$SUVA_{280}$ (L/(mg C·m))
Crofton weed (l)	1.96	1.48
Crofton weed (s)	0.82	0.90
Water oats (l)	1.02	0.65
Water oats (s)	0.50	0.40
Common reed (l)	1.52	1.04
Common reed (s)	0.59	0.58
Oriental pepper (l)	1.63	1.18
Oriental pepper (s)	0.63	0.60
Water hyacinth (l)	1.10	0.91
Sago pondweed (l)	0.86	0.67

l: leaf; s: stem.
$SUVA_{254}$ (specific UV absorbance at 254 nm), the UV absorbance at 254 nm per unit of dissolved organic carbon (DOC) concentration.
$SUVA_{280}$ (specific UV absorbance at 280 nm), the UV absorbance at 280 nm per unit of dissolved organic carbon (DOC) concentration.

450 nm), ultraviolet fulvic-like (Peak B, $E_x/E_m = 240$–270 nm/370–440 nm), protein-like (tryptophan-like fluorescence peak E, $E_x/E_m = 260$–290 nm/300–350 nm; tyrosine-like fluorescence peak F, $E_x/E_m = 260$–290 nm/300–320 nm) (Cuss and Guéguen, 2015; Stedmon and Markager, 2005).

Based on the traditional EEMs 'peak picking' technique, three fluorescence peaks including peak A, peak C and peak E were observed in macrophyte-derived DOM (Fig. 2). This observation suggests that both humic-like and protein-like materials could be rapidly released from macrophytes. Spectral characteristics of the three components (peaks)
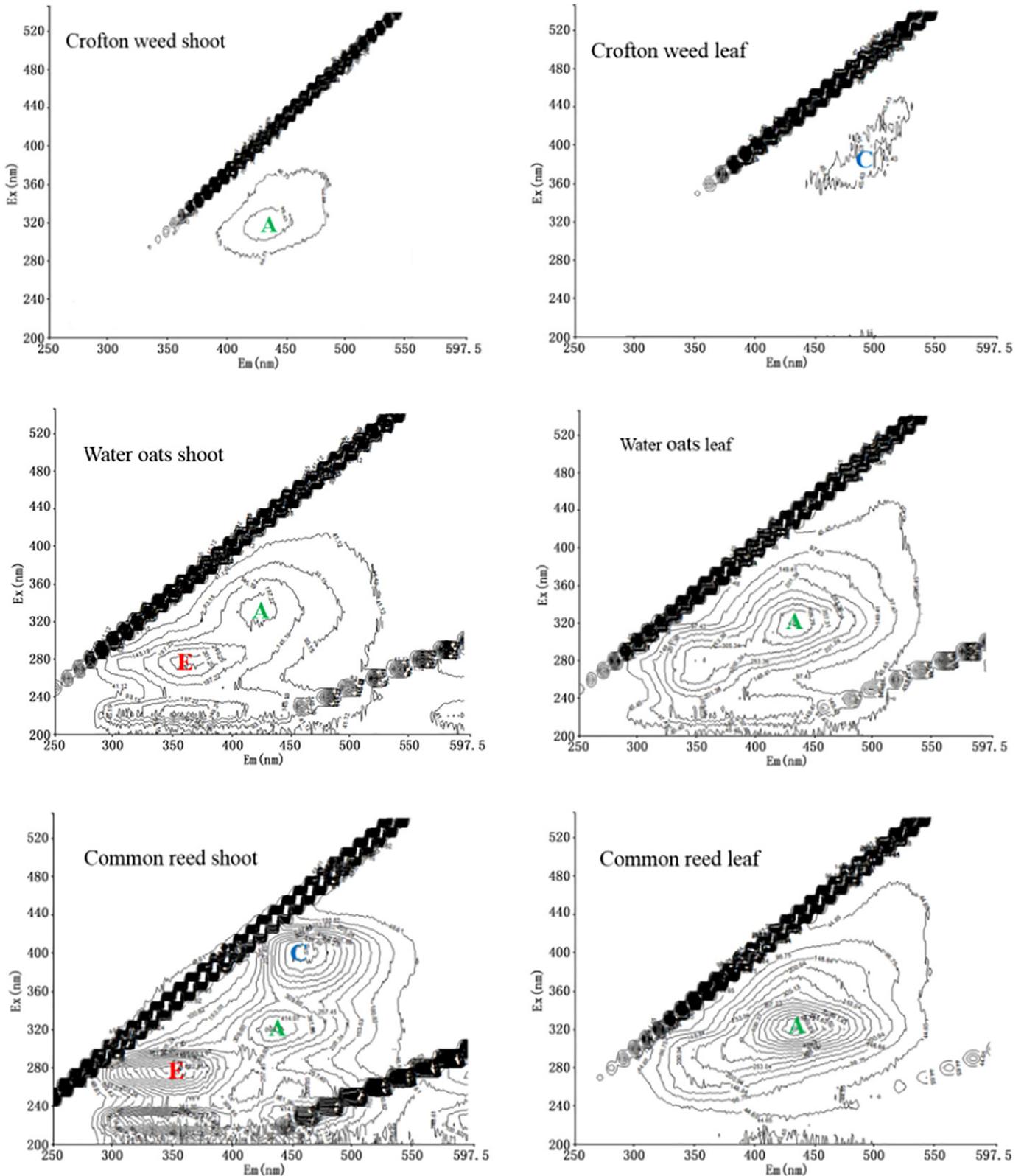
Fig. 2. 3D-EEM spectra of the macrophyte-derived DOM. Peak A, C, and E correspond to fulvic-like fluorescence, humic-like fluorescence and protein-like fluorescence peak, respectively.
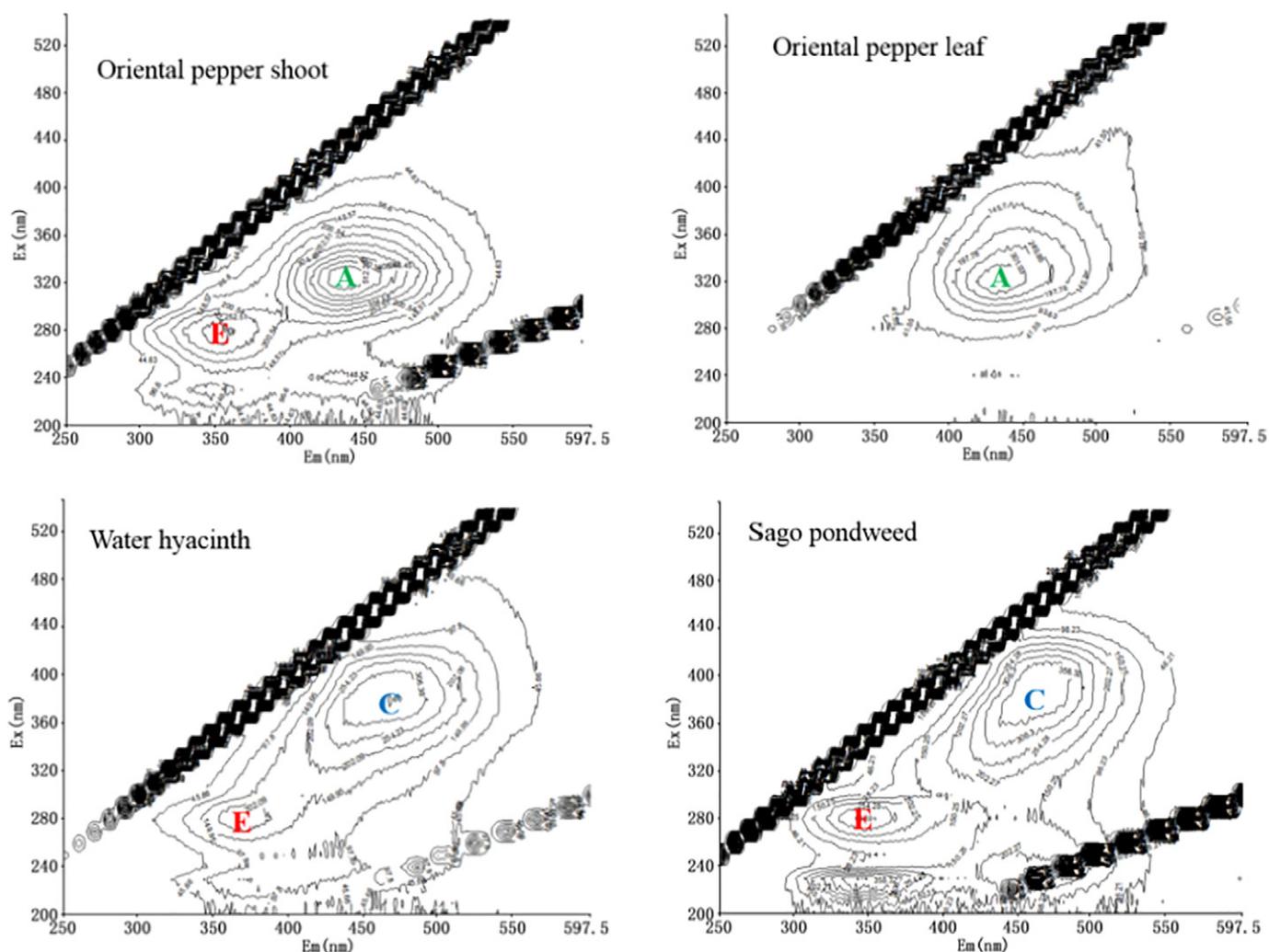
Fig. 2 (*continued*).

observed in DOM from macrophytes were similar to those of DOM previously reported in other aquatic environments (Gao et al., 2010; Wu et al., 2001; Zhang et al., 2013). On the other hand, differences in fluorescence signals were observed among species of macrophyte and also between shoots and leaves. The peak A was observed in all DOM from shoots and leaves of three species of emergent plants (including water oats, oriental pepper, and common reed) and also the shoot of terrestrial plant crofton weed. This observation revealed that fulvic-like fluorescence components existed in these DOM extracts, which is mainly due to phenols and/or carboxyl/carbonyl groups (Senesi, 1990). The additional protein-like peak was observed in spectra of DOM derived from leaves of the floating and submerged species, and shoots of all the emergent species, which indicated a possibility of greater contents of protein-like materials in these samples. This result is consistent with greater ratios of DON/TDN in floating and submerged plants and in shoots than leaves observed through elemental analysis. Additionally, peak C was detected in leaf-derived DOM of water hyacinth, sago pondweed, and crofton weed, and shoot-derived DOM of common weed, which indicated the existence of humic-like components in these plant extracts.

3.4. Solid-state ^{13}C NMR spectroscopy

The solid-state ^{13}C NMR spectra can provide detailed information on C functional groups in organic matters (Möller et al., 2000; Nelson and Baldock, 2005). A chemical shift of 5–50 ppm in ^{13}C NMR was attributed to alkyl-C in lipids, cutins or waxes (Kögel-Knabner, 1997; Perminova et al., 1999). Among the samples, OM of the floating water hyacinth leaves and DOM extracted from leaves of water hyacinth and sago pondweed had the greatest percent of C in the range of 5–50 ppm (Table S2, 4). When comparing peaks in this range between leaves and shoots, peaks appear more frequently and signals are stronger in DOM from leaves than those from shoots of each species.

The spectral interval of 50–108 ppm was traditionally attributed to the presence of polysaccharides. The resonance peaks between 50 and 60 ppm indicated N-substituted alkyl C in amino acids and methoxyl C in lignin or alcoholic structures (Conte et al., 2010; He et al., 2009). The resonance peaks between 60 and 90 ppm were due to the presence of carbohydrates in celluloses and hemicelluloses (He et al., 2009), and peaks in the region of 90–108 ppm are attributed to O—C—O anomerics. Percentages of total carbohydrate C (50–108 ppm) were from 57.1–74.1% in OM and 37.6–56.5% in DOM of leaves, respectively, in which OM in leaves of oriental pepper and DOM of water hyacinth had the least percentage of carbohydrate C (57.1% and 37.6%, respectively). On the other hand, water oats and sago pondweed had greater percentages of carbohydrate C, both in their OM and DOM derived from leaves. For both OM and DOM samples, shoots exhibited stronger peaks and greater percentage of carbohydrates (76.5–86.3% and 61.3–83.8%, respectively) than did their corresponding leaves. These observations suggested that shoots are a more important storing and releasing source of polysaccharides when comparing with leaves. Distribution of polysaccharide C (50–108 ppm) in OM was consistent with that in their corresponding DOM.

Table 4
Solid-state ^{13}C NMR identified C functional groups assignments and relative abundance (%) of the DOM fraction from macrophytes in Lake Dianchi.

Sample	Chemical shift (ppm)								Al-C[a]	Ar-C[b]	Carbohydrate[c]	145–220	A + AR[d]	A/A-O[e] (ratio)	∑Ar/∑Al[f] (ratio)
	5–50	50–60	60–90	90–108	108–145	145–165	165–187	187–220							
Crofton weed (l)	16.11	3.72	46.69	6.08	13.12	3.70	8.96	1.62	72.60	16.82	56.49	14.28	32.93	0.29	0.23
Crofton weed (s)	4.22	8.25	59.94	15.56	7.10	2.02	2.65	0.27	87.97	9.11	83.75	4.94	13.34	0.05	0.10
Water oats (l)	17.31	7.42	36.50	9.79	10.53	4.57	12.54	1.33	71.02	15.10	53.71	18.44	32.41	0.32	0.21
Water oats (s)	10.91	8.47	54.96	12.27	2.87	1.93	7.76	0.83	86.62	4.79	75.71	10.52	15.71	0.14	0.06
Common reed (l)	25.97	7.76	25.92	6.73	12.12	4.03	15.63	1.84	66.38	16.15	40.41	21.5	42.12	0.64	0.24
Common reed (s)	15.00	9.86	42.61	9.72	4.19	1.98	15.70	0.94	77.19	6.17	62.19	18.62	21.17	0.24	0.08
Oriental pepper (l)	18.71	4.59	33.47	5.48	14.68	4.70	15.58	2.80	62.25	19.38	43.54	23.08	38.09	0.43	0.31
Oriental pepper (s)	11.26	4.67	41.42	15.23	11.28	4.66	10.41	1.07	72.58	15.94	61.32	16.14	27.20	0.18	0.22
Water hyacinth (l)	21.68	4.71	29.36	3.52	13.61	3.43	17.29	6.40	59.27	17.03	37.59	27.12	38.71	0.58	0.29
Sago pondweed (l)	26.50	5.92	33.50	6.46	7.45	2.19	15.91	2.06	72.38	9.64	45.88	20.16	36.14	0.58	0.13
Blue-green algae[i]															
Humic DOM		1–13[h]		1–9[h]		3.3	15.0		14–33[h]	22–58[h]					0.5–0.8[g]

l: leaf; s: stem.
[a] Al-C% was calculated by the Al-C region (0–108 ppm)/C in the region of 0–220 ppm.
[b] Ar-C% was calculated by the Ar-C region (108–165 ppm)/C in the whole region 0–220 ppm.
[c] Carbohydrate (50–108 ppm).
[d] A + AR was calculated by the sum of C% in the region of 5–50 ppm and 108–165 ppm.
[e] A/A-O was calculated by C% in the region of 5–50 ppm divided by C% in the region of 50–108 ppm.
[f] ∑Ar/∑Al was calculated by C% in the region of 5–108 ppm divided by C% in the region of 108–165 ppm.
[g] Cited from Perminova et al. (1999).
[h] Cited from Thorn et al. (1989).
[i] Cited from Nguyen et al. (2005).

However, the abundance of C from 50 to 60 ppm in OM was different from that in their corresponding DOM. This might be due to different contents of N-substituted alkyl C in the macrophytes and their different abilities in accumulation and release of organic N (Table S2, 4).

Peaks from 108 to 165 ppm are usually assigned to olefinic carbons, aromatic C—H carbons, guaiacyl C2 and C6 in lignin (108–145 ppm), and aromatic COR or CNR groups (145–165 ppm) (Leifeld and Kögel-Knabner, 2005). In particular, peaks at 115, 128, 144, 147, 154 ppm are usually attributed to unsaturated structures such as the protonated C, C-substituted aromatic C, and phenolic C (Kögel-Knabner, 2002). Peaks at 150 ppm suggested the presence of tannins. In the spectra of both OM and DOM, it is apparent that there are more peaks in this range in leaves than in their corresponding shoots (Fig. 3), suggesting the complicated features of C functional groups in leaves. In the ranges of both aromatic C (108–145 ppm) and phenolic C (145–165 ppm), leaves showed greater percentages of C (7.4–14.7% and 2.8–5.0%, respectively) than their corresponding shoots (3.3–10.0% and 0.9–3.7%, respectively) (Table S2, 4). Among the leaves, sago pondweed had the least aromaticity (sum of aromatic C and phenolic C) in both its OM (9.2%) and DOM (9.64%), which is consistent with that observed by C/H ratios.

Peaks in the range of 165–187 ppm and 187–220 ppm were attributed to the carboxyl/amide and carbonyl groups, respectively. Among the leaves, water hyacinth, sago pondweed and crofton weed had the greatest percentage of C from 165 to 187 ppm in OM (10.1, 8.4 and 8.5%, respectively) (Table S2). Water hyacinth and sago pondweed also showed the greatest content of C in this range in their DOM samples (Fig. 3, Table 4). Further, for every species, the leaves exhibited a greater percent of C in this range in both their OM and DOM. These results indicated that leaves of water hyacinth and sago pondweed might have greater proportions of substances which contain carboxyl/amide groups.

For OM of leaves, the relative abundance of the recalcitrant components A + AR (sum of alkyl C and aromatic C) decreased in the order: oriental pepper > crofton weed > sago pondweed > water hyacinth > common reed > water oats (Table S2). In every riparian and emergent species, the percentages were greater in leaves than in their corresponding shoots. Ratios of A/A-O (alkyl C/O-alkyl C), an indicator of the relative abundance of recalcitrant component over the labile portion (He et al., 2015), were also greater in leaves than in their shoots. Sago pondweed and water oats had the least A/A-O ratios (0.14 and 0.19) among leaves of all the species. Although distributions of various type of C in OM and DOM were not the same, there were significant correlations between carbohydrates in OM and DOM ($r = 0.82$, $p < 0.01$), A + AR in OM and DOM ($r = 0.66$, $p < 0.05$), and A/A-O in OM and DOM ($r = 0.79$, $p < 0.01$) (Fig. S2). Such positive internal relationships indicated that for these major C functional groups were closely associated with each other and the whole OM and the DOM fraction possessed similar backbone C chains or matrix. In DOM derived from macrophytes, A + AR, A/A-O, and C% in 145–220 ppm which represents phenols/carboxyl/amides/carbonyls were also less in shoots (13.3–27.2%, 0.05–0.24, and 4.9–18.6%, respectively) than that in leaves (32.4–42.1%, 0.29–0.64, and 14.3–27.1%, respectively), but relative abundance of carbohydrates was greater in shoots (61.3–83.8%) than that in leaves (40.1–56.5%). The ratio of ∑Ar/∑Al (ratio of the total aromatic C and total aliphatic C) ranged from 0.13 to 0.31 in DOM of leaves while the range in shoots was 0.06–0.22 (Table 4).

3.5. Implications for decomposition behaviors of aquatic macrophytes in lakes

DOM is not a static constituent. It not only varies among sources, but also follows a continuous dynamic during degradation of DOM and parent OM (Kalbitz et al., 2003). Degradation of OM has traditionally been divided into two stages, the rapid one (physical leaching) and the following slow (biological/chemical) stage (Berg and McClaugherty, 1989). Chemical properties of litters were identified as the most important factor determining their decomposition behaviors. Lesser C/N and
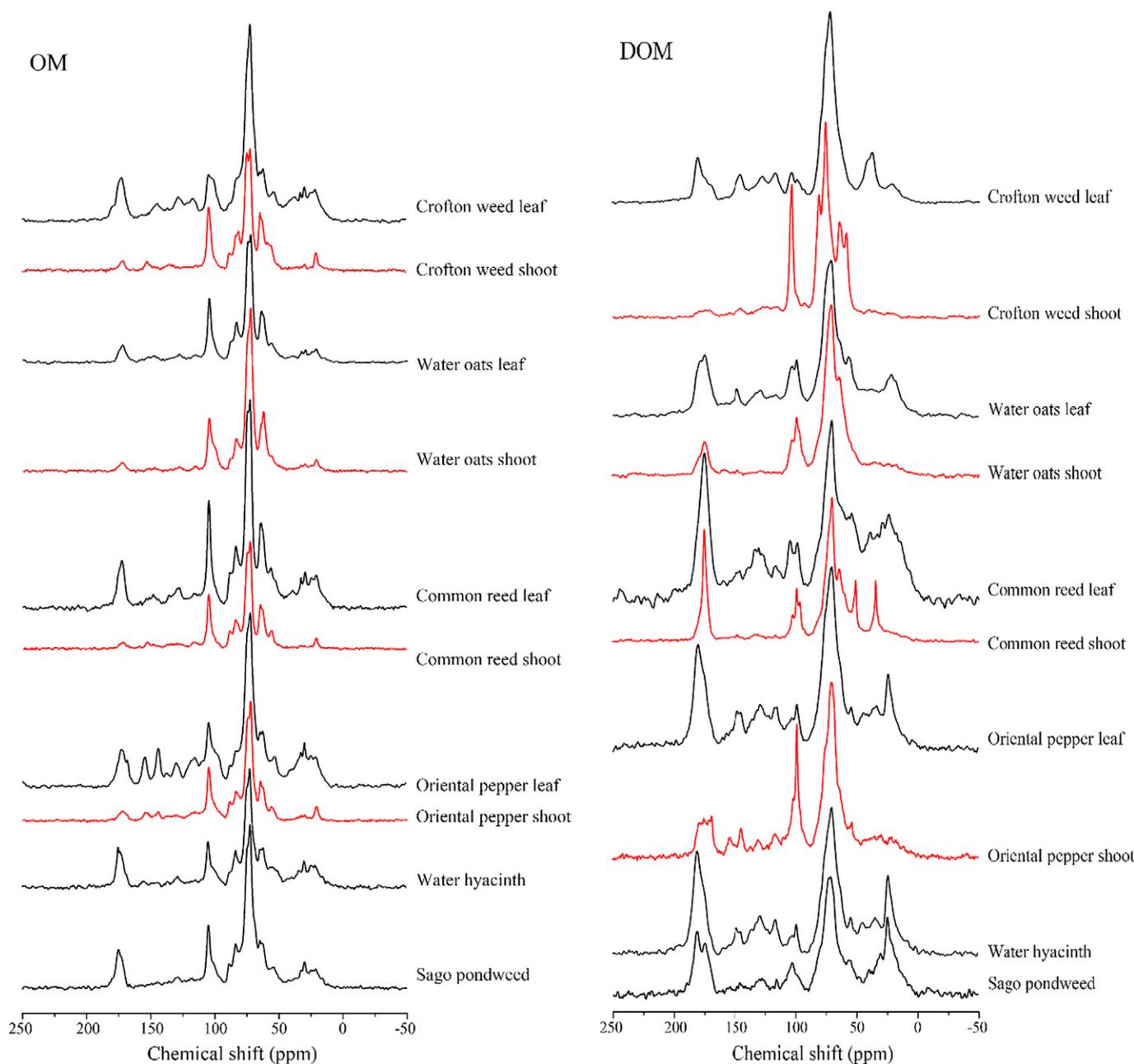
Fig. 3. Solid-state ^{13}C NMR spectra of whole OM and the freeze-dried DOM fraction of the macrophytes.

C/P indicate prior utilization of labile N and P by bacteria during their population growth stage, and substrates with lesser C/N, C/P had greater rates of decomposition during early stages of decomposition (Berg and McClaugherty, 2013). Thus, the lesser C/N and C/P ratios in water hyacinth, sago pondweed and leaves of all the riparian and emergent species indicated their rapid decomposition and release into the lake water in the early stages of degradation. Additionally, chemical composition revealed by ^{13}C NMR are closely related to decomposition rates of litter (Hunt and Ohno, 2007; Krull et al., 2003). The alkyl and aromatic structures related to fatty acids, cutins, waxes, and lignin are resistant to microbial attack and more stable during decomposition whose degradation take from decades to hundreds of years (Hunt and Ohno, 2007; Krull et al., 2003). Alternatively, carbohydrates, as shown by the O-alkyl peaks in the ^{13}C NMR spectra were identified as the most labile and active type of C, which turns over with rates of months to years (Kalbitz et al., 2003; Marschner and Kalbitz, 2003). Qu (2013) has found that the more rapid decomposition stage of OM was mainly

dominated by C/N and C/P, while the subsequent decomposition rate was mainly determined by contents of A + AR, A/A-O in OM. Thus, DOM released during this stage should be firstly composed of soluble sugars which are from decomposition of the easily-degradable hemicellulose and cellulose (Berg and McClaugherty, 2013), then followed by decomposed soluble alkyl and aromatic C. In this study, A + AR and A/A-O were greater in OM derived from leaves than that from shoots of emergent and riparian species, while contents of carbohydrates were greater in shoots than in leaves of these species (Table S2). These results indicated that shoots contain more labile components, and OM in leaves was more recalcitrant. Hence, even after decomposition of the most labile components, the remaining OM derived from shoots should decompose more easily so that the shoot part was a more important contributor of the degraded soluble substances.

DOM represents the most soluble part of OM and is the first part to be released from parent OM. Although there were significant correlations between A + AR, A/A-O, and percent carbohydrate in OM and DOM

(Fig. S2), DOM might have a different decomposition pattern from their parent OM. In natural aquatic environments, decomposition of DOM is determined by both biological and photolytic degradation. However, based on the greater content of organic N, as indicated by concentration of DON, great DON/TDN (Table 2), stronger C—N peaks around 48 ppm in the ^{13}C NMR spectra (Fig. 3) and protein-like peaks in their 3D-EEM spectra (Fig. 2), in leaves of water hyacinth, sago pondweed and shoots of the emergent species, these samples seemed to have greater contents of nitrogenous compounds such as amino acids, peptides, or proteins than did leaves of the emergent and riparian species. Combining with the greater carbohydrate and less A + AR percentage in shoots, DOM of water hyacinth, sago pondweed and shoots of the emergent species were a more important bioavailable nutrient source for microbes than were leaves.

3.6. Chemical characteristics of DOM as indicators for its environmental implications

It has been reported that binding affinity of PAHs to organic matter (K_{oc}) was positively correlated with aromaticity and molecular weight of OM, but negatively correlated with their polarity (Chen et al., 2005; Kang and Xing, 2005; Tanaka et al., 2005). Based on the combined information from the greater C/H and SUVA values (SUVA$_{254}$ and SUVA$_{280}$), the stronger fulvic-like fluorescence and the greater percentage of unsaturated structures in leaves, leaf-derived DOM seems to own a greater aromaticity than DOM derived from shoots. $\sum Ar/\sum Al$ was the best predictor for binding affinity of PAHs to humic substances, which had a positive linear correlation with K_{oc} of three PAHs (pyrene, fluoranthene, anthracene) (Perminova et al., 1999). In this study, leaves of all samples except sago pondweed had similar $\sum Ar/\sum Al$ (0.21–0.31), which were greater than did extracts of DOM from shoots (0.06–0.22) (Table 4), and they were less than those in the humic substances (0.5–0.8) (Perminova et al., 1999). Based on the positive correlation between K_{oc} and $\sum Ar/\sum Al$, the non-humic DOM from macrophytes should have lesser binding abilities with PAHs, and that of shoots should be even less than that of leaves. Hence, DOM from macrophytes should have a less influence on dynamics of PAHs than do humic substances. Since most of the species were perennials and decomposition mainly occurs in leaves, DOM derived from leaves would be predicted to have a stronger effect on the dynamics of PAHs in lakes than DOM from shoots.

Besides aromaticity, DOM from leaves had greater percentages of aromatic phenolic-C, carboxyl/amide groups, and carbonyl groups (Table 4). These functional groups are important binding sites for metal ions (Croué et al., 2003). Among the species, leaves of water hyacinth, oriental pepper and sago pondweed had a greater percentage of these C functional groups than other species (Table 4). It has been reported that DOM with lesser molecular weights have more binding sites for metal ions than those with greater molecular weight, and hence have greater binding capacity for metals (Wu and Xing, 2009). Normally, DOM from plants has a molecular weight of <1000 Da, which suggests possible greater effects on dynamics and toxicity of metals in lakes (Hunt and Ohno, 2007). As the more binding sties in DOM, the greater binding constant (lg$K$) will be (Wu and Xing, 2009). Thus, results presented indicated that DOM from leaves, especially those of water hyacinth, oriental pepper and sago pondweed might have significant influence on heavy metal pollution in the lake such as reported with leaf-litter leachate (Cuss and Guéguen, 2012).

3.7. Estimation of macrophyte-derived nutrients and suggestions on the management of lake eutrophication

Emergent species can accumulate P via their roots from sediments, while floating and submerged species absorb P from both water and sediment (Granéli and Solander, 1988). Thus, macrophytes serve as a pathway for transfer of P in lake systems. Different from N which can be depleted from lakes through denitrification to the atmosphere, P can

only be transferred among fractions and ultimately released into the water (Søndergaard, 2007). Total N and P contents in the water bodies itself and carried by inflow to Lake Dianchi were estimated according to the concentrations of N and P in water and the volume of total lake water or inflow volume in 2010 to the lake (parameters shown in Table S1). TN and TP in macrophytes in Lake Dianchi were also evaluated by the mean concentrations of N and P in macrophytes and the total dry mass of macrophytes in the whole lake. Thus, various ratios and contributions could be calculated from values provided (Table S3). According to the evaluation results (Table S3), the ratio of P accumulated in macrophytes to total P of the water column of the lake was three times more than the ratio of N. Compared with inputs of N and P to the lake via inflows in 2010, P accumulated in macrophytes was approximately 1.6-fold more than that in inflows. However, the ratio of N accumulated in macrophytes to that in inflow was only 0.3. The percentage of P released (TDP) from macrophytes in the TDP of lake water was seven-fold greater than that of TDN. The ratio of TDP in macrophyte to that carried by inflow was almost 10-fold more than that of TDN. These results showed that macrophytes in the lake are important stores of P, while external loading is the major source of N in the lake. Additionally, the greater percentage of DIP released showed that macrophytes not only have an ability to accumulate P, but also be ready to release P back to the lake. The release of P through DIP which is the most easily used P fraction by algae (Carpenter and Lodge, 1986) would further support blooms of phytoplankton in the lake. With this concern, it is important to use the accumulation capability of macrophytes to remove P from the lake by reasonable harvesting at an appropriate time so as to avoid release of P back to the lake, especially for those species that have strong capabilities to accumulate and release P such as common reed, oriental pepper, water hyacinth and so on.

4. 5. Conclusions

In this study, chemical properties of whole OM and its DOM fraction from six dominant macrophytes in Lake Dianchi were characterized by use of elemental analysis, UV, 3D–EEM and solid ^{13}C NMR spectroscopy. Quantitative and qualitative results revealed that OM derived from macrophytes is an important store and source of nutrients, especially for floating and submerged macrophytes. Compared to leaves, shoots of emergent and riparian macrophytes had a greater bioavailability during plant senescence due to its greater percentage of labile carbohydrates. Additionally, macrophyte-derived DOM was predicted influencing less on PAHs but more on heavy metals based on the results from ^{13}C NMR spectroscopy. In summary, macrophyte-derived DOM play an important role both in contribution to eutrophication and implication on contaminants in lakes. Thus, naturally occurred DOM released from aquatic macrophytes in lakes should be given great concerns in eutrophication regulation. It is important to use the accumulation capability of macrophytes to remove P out of the lake by reasonable harvesting at an appropriate time so as to avoid release of P back to the lake. The characterization of macrophyte-derived DOM and possible implication on contaminants would provide more reference for eutrophication regulation and removal of hazard materials in freshwaters.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2017.10.289.

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Molecular characterization of macrophyte-derived dissolved organic matters and their implications for lakes

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Supplementary Figures
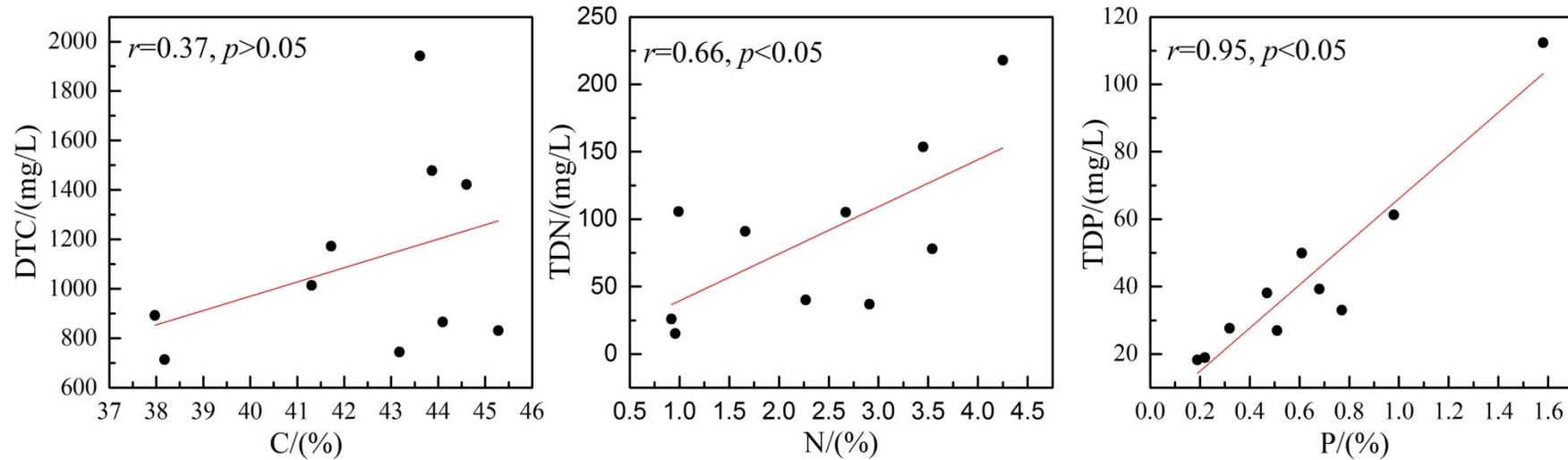
Fig. S1. Correlations between C, N, P in whole organic matters and their corresponding dissolved components in macrophyte extracts. TDC, TDN, and TDP were total dissolved carbon, nitrogen, and phosphorus, respectively in extracts. C, N and P were total carbon, nitrogen and phosphorus in ground macrophyte biomass, respectively.
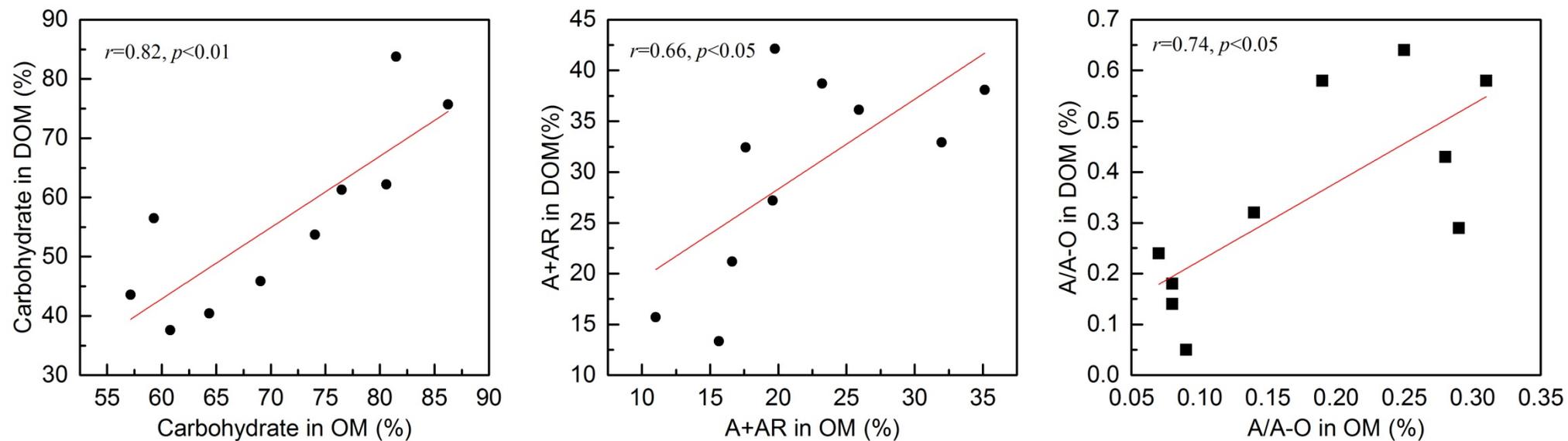
Fig. S2. Correlations between carbohydrates, A+AR, and A/A-O identified by solid ^{13}C NMR in whole OM and their corresponding values in freeze-dried DOM fractions. A+AR was calculated by the sum of C% in the region of 5-50 ppm and 108-165 ppm; A/A-O was calculated by C% in the region of 5-50 ppm divided by C% in the region of 50-108 ppm.

Supplementary Tables

Table S1 Basic parameters in Lake Dianchi.

	Data	References
Total volume of the lake	$1.2*10^{11}\,m^3$	Wang et al., (2012)
Total plant biomass (dry weight)	41019 Mg	Yu et al., (2000)
TN in water	2.14 mg/L	Chen et al., (2010a); Su (2011)
TP in water	0.17 mg/L	Chen et al., (2010b); Su (2011)
TDN in water	1.17 mg/L	Chen et al., (2010a)
TDP in water	0.008 mg/L	Chen et al., (2010b)
Volume of total inflow in 2010	$3.7\times10^8\,m^3$	Dianchi Management Administration (2010)
TN in inflow of 2010	8.87 mg/L	Dianchi Management Administration (2010)
TP in inflow of 2010	0.433 mg/L	Dianchi Management Administration (2010)

Table S2 Percent distribution (%) of characteristic carbons in the solid-state ^{13}C NMR spectra of whole OM from macrophytes in Lake Dianchi.

Sample	Chemical shift (ppm)								Al-C[a]	Ar-C[b]	Carbohydrates[c]	A+AR[d]	A/A-O[e] (ratio)
	5-50	50-60	60-90	90-108	108-145	145-165	165-187	187-220					
Crofton weed (l)	16.97	5.52	43.95	9.82	11.87	2.92	8.49	0.46	76.26	14.79	59.29	31.99	0.29
Crofton weed (s)	7.04	8.11	59.80	13.58	6.06	2.55	2.70	0.15	88.53	8.61	81.49	15.66	0.09
Water oats (l)	10.71	6.05	54.77	13.24	7.43	2.76	4.93	0.11	84.77	10.19	74.06	17.62	0.14
Water oats (s)	6.84	5.98	66.22	14.06	3.25	0.92	2.46	0.27	93.09	4.18	86.25	11.02	0.08
Common reed (l)	15.82	5.63	47.49	11.25	9.2	2.96	7.55	0.10	80.2	12.16	64.37	19.76	0.25
Common reed (s)	5.31	6.22	60.27	14.10	8.25	3.04	2.69	0.12	85.89	11.30	80.58	16.61	0.07
Oriental pepper (l)	16.19	5.50	40.09	11.55	14.71	5.01	6.93	0.02	73.34	19.71	57.14	35.13	0.28
Oriental pepper (s)	5.94	4.58	57.23	14.69	9.99	3.66	3.74	0.18	82.44	13.64	76.50	19.59	0.08
Water hyacinth (l)	18.56	6.69	43.91	10.17	7.38	2.38	10.14	0.78	79.32	9.76	60.77	23.21	0.31
Sago pondweed (l)	13.33	5.57	52.13	11.37	7.77	1.43	8.38	0.03	82.39	9.2	69.06	25.92	0.19
Deciduous/coniferous tress[*]												32.1-35.6[*]	0.30-0.37[*]
Algae[†]												47.7-55.1[†]	1.1-1.3[†]

l: leaf; s: stem;

[a] Al-C% was calculated by the Al-C region (0-108ppm)/C in the region of 0-220 ppm;

[b] Ar-C% was calculated by the Ar-C region (108-165 ppm)/C in the whole region 0-220 ppm;

[c] Carbohydrate (50-108 ppm);

[d] A+AR was calculated by the sum of C% in the region of 5-50 ppm and 108-165 ppm;

[e] A/A-O was calculated by C% in the region of 5-50 ppm divided by C% in the region of 50-108 ppm;

[*] Cited from Lorenz et al (2004);

[†] Cited from Hedges et al (2002).

Table S3 Estimated N and P from macrophytes and their ratios to that in lake water and inflow of Dianchi.

	Macrophytes (Mg)	Lake water in Dianchi (Mg)	Macrophyte/Lake water (%)	Inflow (Mg/yr)	Macrophyte/Inflow
N	968.9	256800	0.4	3280	0.3
P	259.7	20400	1.3	160.4	1.6
TDN	133.7	140400	0.1	-	0.041
TDP	65.4	960	6.8	-	0.408

TND, Total dissolved nitrogen; TDP, Total dissolved phosphorus. Total N and P contents in the Lake water were estimated according to the concentrations of N and P in Lake water multiply by the volume of total lake water; Total N and P contents in inflow were calculated by the concentrations of N and P in inflow multiply by inflow volume in 2010 to the lake. Total N and P in macrophytes in Lake Dianchi were also evaluated by the mean concentrations of N and P in macrophytes multiply total dry mass of macrophytes in the whole lake. TND and TNP were also calculated in the same method.

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