



# Characteristics and degradation of carbon and phosphorus from aquatic macrophytes in lakes: Insights from solid-state $^{13}\text{C}$ NMR and solution $^{31}\text{P}$ NMR spectroscopy



Shasha Liu <sup>a,b</sup>, Yuanrong Zhu <sup>b,\*</sup>, Wei Meng <sup>b,\*</sup>, Zhongqi He <sup>c</sup>, Weiyong Feng <sup>a,b</sup>, Chen Zhang <sup>b</sup>, John P. Giesy <sup>b,d</sup>

<sup>a</sup> College of Water Sciences, Beijing Normal University, Beijing 100875, China

<sup>b</sup> State Key Laboratory of Environment Criteria and Risk Assessment, Chinese Research Academy of Environmental Sciences, Beijing 100012, China

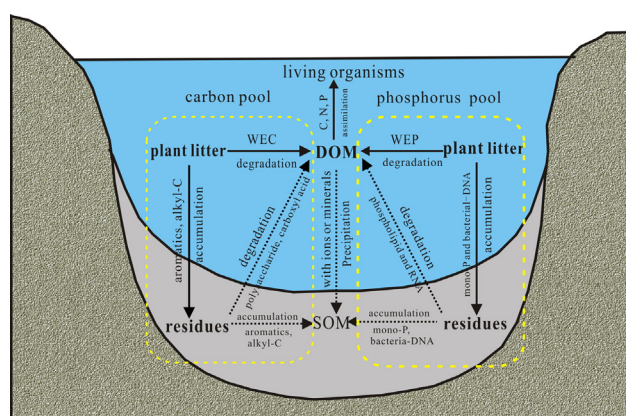
<sup>c</sup> USDA-ARS Southern Regional Research Center, 1100 Robert E Lee Blvd, New Orleans, LA 70124, USA

<sup>d</sup> Department of Biomedical and Veterinary Biosciences and Toxicology Centre, University of Saskatchewan, Saskatoon, Saskatchewan, Canada

## HIGHLIGHTS

- WEOM derived from aquatic macrophytes was characterized.
- C and P in WEOM were characterized by solid  $^{13}\text{C}$  NMR and solution  $^{31}\text{P}$  NMR.
- Degradation and transformation of macrophyte-derived C and P were investigated.
- Macrophyte-derived WEOM are important source for bioavailable nutrients in lakes.

## GRAPHICAL ABSTRACT



## ARTICLE INFO

### Article history:

Received 11 September 2015

Received in revised form 16 November 2015

Accepted 16 November 2015

Available online xxx

Editor: F.M. Tack

### Keywords:

Plant-derived water extractable organic matter

Nuclear magnetic resonance spectroscopy

Phosphorus

## ABSTRACT

Water extractable organic matter (WEOM) derived from macrophytes plays an important role in biogeochemical cycling of nutrients, including carbon (C), nitrogen (N) and phosphorus (P) in lakes. However, reports of their composition and degradation in natural waters are scarce. Therefore, compositions and degradation of WEOM derived from six aquatic macrophytes species of Tai Lake, China, were investigated by use of solid-state  $^{13}\text{C}$  NMR and solution  $^{31}\text{P}$  NMR spectroscopy. Carbohydrates were the predominant constituents of WEOM fractions, followed by carboxylic acid. Orthophosphate (ortho-P) was the dominant form of P (78.7% of total dissolved P) in the water extracts, followed by monoester P (mono-P) (20.6%) and little diester P (0.65%). The proportion of mono-P in total P species increased with the percentage of O-alkyl and O-C-O increasing in the WEOM, which is likely due to degradation and dissolution of biological membranes and RNA from aquatic plants. Whereas the proportion of mono-P decreased with alkyl-C, NCH/OCH<sub>3</sub> and COO/N-C=O increasing, which may be owing to the insoluble compounds including C functional

**Abbreviations:** WEOM, water extractable organic matter; NMR, nuclear magnetic resonance spectroscopy; ZC, *Zizania caduciflora* Turcz., Gramineae; PD, *Polygonum divaricatum* Linn; TB, *Trapa bispinosa* Roxb; NP, *Nymphoides peltata* (Gmel.) O. Kuntze; MV, *Myriophyllum verticillatum* Linn; HV, *Hydrilla verticillata* (Lf) Royle.

\* Corresponding authors.

E-mail addresses: [zhuyuanrong07@mails.ucas.ac.cn](mailto:zhuyuanrong07@mails.ucas.ac.cn) (Y. Zhu), [mengwei@caes.org.cn](mailto:mengwei@caes.org.cn) (W. Meng).

groups of alkyl-C, NCH/OCH<sub>3</sub> and COO/N—C=O, such as aliphatic biopolymers, lignin and peptides. Based on the results of this study and information in the literature about water column and sediment, we propose that WEOM, dominated by polysaccharides, are the most labile and bioavailable component in debris of macrophytes. Additionally, these WEOMs would also be a potential source for bioavailable organic P (e.g., RNA, DNA and phytate) for lakes.

© 2015 Elsevier B.V. All rights reserved.

## 1. Introduction

Aquatic macrophytes are important components of ecological systems in lakes, and can be involved in biogeochemical cycling of nutrients, such as carbon (C), nitrogen (N) and phosphorus (P). They can remove nutrients by assimilation and re-synthesis of N, P, and improve the quality of overlying water by adsorbing heavy metals and organic pollutants in lakes (Clabeaux et al., 2011; Dwivedi et al., 2008; Nirmal-Kumar et al., 2008; Brisson and Chazarenc, 2009). Whereas degradation and release of nutrients from the debris of dead plant biomass could also be an important source of internal nutrients in oligotrophic lakes, even support blooming of algae in eutrophic lakes (Chinney and Pietro, 2006; Xie et al., 2004).

Water extractable organic matter (WEOM) derived from aquatic macrophytes is the most dynamic component in biogeochemical cycling, due to its quick migration and transformation when released from plant litter (Qu, 2013). The dynamics and reactivity of WEOM in the environment depend mainly on their chemical structure and external environment factors (Wu, 2009; He and Wu, 2015). The proportion of alkyl-C and aromatic C in organic matter (OM) would be difficult to decompose, whereas OMs containing more polysaccharides would be degraded and released into the overlying water more easily (Kögel-Knabner, 2002; Krull et al., 2003). Thus, characterization of OM is of great significance in studying degradation processes. Although WEOM from soil, sediment or crop residues have been widely investigated using solid-state <sup>13</sup>C NMR, solution <sup>31</sup>P NMR and enzymatic hydrolysis (Wu et al., 2010; Zhu et al., 2013; Xu et al., 2012; Noack et al., 2012), information on WEOM derived from aquatic macrophytes is very limited (Qu et al., 2013; He et al., 2009). The C functional groups in several aquatic macrophytes were characterized (Qu et al., 2013; He et al., 2009). They revealed that more polysaccharides, but less aromatic distributed in macrophyte-derived WEOM compared with that in exogenous OM. However, information on characteristics of water-extractable C and P, and their roles in nutrient cycling in eutrophic lakes is still poorly understood. Therefore, the overall goal of this study was to gain such information.

Nuclear magnetic resonance (NMR) spectroscopy has been widely used to characterize organic matter in environmental and agricultural samples (Wu and Xing, 2009; Zhang et al., 2009; Qu et al., 2013). Solution <sup>31</sup>P NMR and solid-state <sup>13</sup>C NMR spectroscopy have some advantages over other spectral analyses, such as ultraviolet and infrared spectroscopy. They can generally provide more detailed information on C and P in WEOM (Qu et al., 2013; Zhu et al., 2015). Indeed, solution <sup>31</sup>P NMR and solid-state <sup>13</sup>C NMR were mostly used to analyze soil, sediment, animal manures, humic acid or fulvic acid (Gélinasa et al., 2001; Turner et al., 2003; Doolette et al., 2009; Bai et al., 2013). However, few studies have focused on WEOM from aquatic macrophytes in eutrophic lakes (Qu et al., 2013; Cheesman et al., 2010).

Thus, in this work, we characterized C and P in water extracts derived from aquatic macrophytes of Tai Lake (Ch: *Taihu*), using solution <sup>31</sup>P NMR and solid-state <sup>13</sup>C NMR spectroscopic techniques. Relationships between the distribution of C and P forms in WEOM fractions were further analyzed. Finally, implications on the degradation or transformation of plant-derived C and P in biogeochemical cycling of nutrients in lakes were discussed.

## 2. Materials and methods

### 2.1. Aquatic plant collection and WEOM preparation

Six living aquatic macrophytes species were collected from the macrophyte-dominated zone in the eastern section of Tai Lake, a large, eutrophic lake in Jiangsu Province, China (Fig. 1). The water quality in the eastern section of Tai Lake, termed East Tai Lake, is good due to less influence by human activities. Three types of aquatic plants were selected: a) emergent plants; water oats (*ZC*, *Zizania caduciflora* Turcz., Gramineae) and branch smartweed (*PD*, *Polygonum divaricatum* Linn.); b) floating plants; water caltrop (*TB*, *Trapa bispinosa* Roxb.) and water poppy (*NP*, *Nymphaoides peltata* (Gmel.) O. Kuntze); and c) submerged plants; watermilfoil (*MV*, *Myriophyllum verticillatum* Linn.) and water thyme (*HV*, *Hydrilla verticillata* (Lf) Royle). The whole fresh plants were washed, killed in an oven (90 °C) for 15 min and then dried at 60 °C until a constant mass. After drying, ground plants pass through a 1-mm sieve. Then, these samples were extracted by use of a 30:1 (mL/g) water to sample ratio, and the mixtures were shaken for 18 h (Brown and Sposito, 1991). Suspensions were then centrifuged (8000 × g) for 30 min, and filtered through 0.45-µm glass fiber filters (Hunt et al., 2007). Aliquots of water extracts were used for the qualification of dissolved C, N and P. The remaining water extracts were freeze dried, and then preserved in a desiccator at room temperature until solid <sup>13</sup>C NMR and solution <sup>31</sup>P NMR analysis.

### 2.2. Chemical analysis

Contents of total carbon (TC) and total nitrogen (TN) in ground aquatic macrophytes were determined using an elemental analyzer (Elemental vario macro EL, Germany). Total phosphorus (TP) and inorganic phosphorus (IP) were determined by SMT method described by Ruban et al. (1999, 2001). Organic phosphorus (OP) in samples was calculated by the difference between TP and IP. Dissolved organic carbon (DOC) and dissolved total nitrogen (DTN) in water extracts were measured by a multi N/C 3100 analyzer (Jena, Germany). Dissolved total phosphorus (DTP) and Molybdate-Reactive P (MRP) were measured using molybdenum blue method (Murphy and Riley, 1962), and dissolved organic phosphorus (DOP) calculated by the difference.

### 2.3. Solid-state <sup>13</sup>C NMR analysis

Solid-state CP/MAS <sup>13</sup>C NMR spectroscopy of freeze-dried water extracts was performed in a Bruker AV-300 spectrometer at 12.5 KHz, using a standard 4-mm double-bearing probe. Approximately 2048 scans were accumulated using a pulse width of 1.0 µs, a recycle delay time of 5 s, and a 0.0492 s acquisition time. 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, 0–45 ppm (alkyl-C); II, 45–60 ppm (NCH/OCH<sub>3</sub>); III, 60–95 ppm (O-alkyl C); IV, 95–110 (O-C-O); V, 110–145 ppm (Aromatic C); VI, 145–165 ppm (Aromatic C-O); and VII, 165–190 ppm (COO/N—C=O) (Cade-Menun and Paytan, 2010; Nelson and Baldock, 2005).

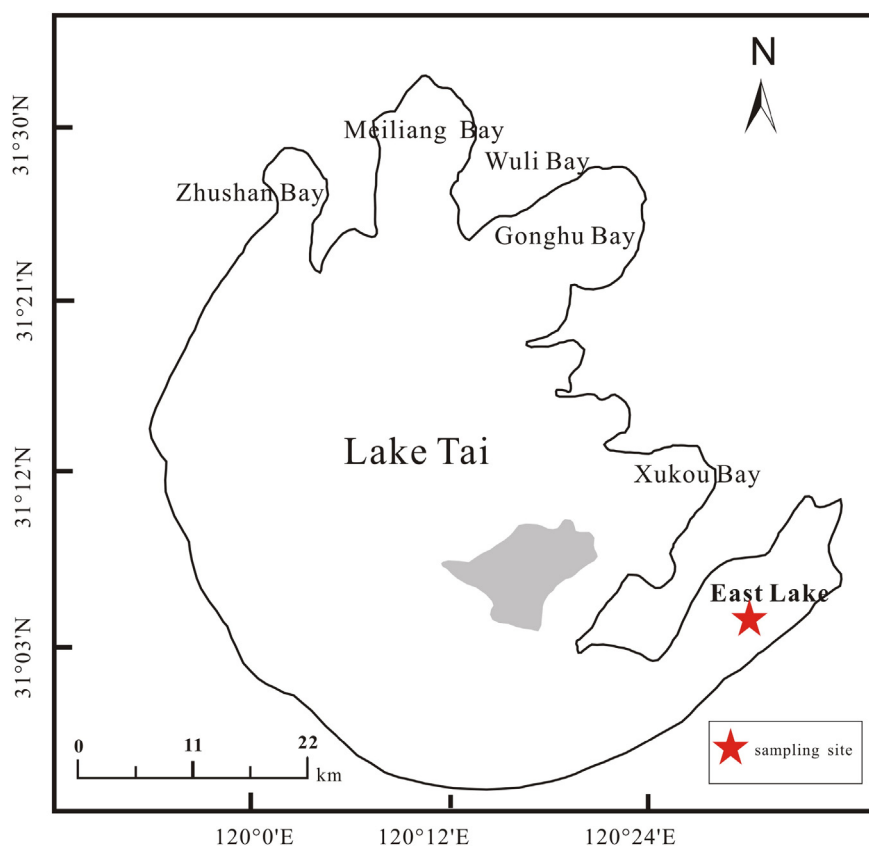


Fig. 1. Map showing sampling sites in Tai Lake (Ch: Taihu).

#### 2.4. Solution $^{31}\text{P}$ NMR analysis

Freeze-dried water extract powders (100 mg) were re-dissolved in 0.5 ml 10 M NaOH and 0.4 ml 1 M NaOH-0.1 M EDTA, and left to stand for 30 min after vortexing. Mixtures were then centrifuged at  $8000 \times g$  for 30 min, and 0.5 ml supernatant were transferred to a 5-mm NMR tube, and then 0.1 ml  $\text{D}_2\text{O}$  was added to lock the signal. Solution  $^{31}\text{P}$  NMR spectra were acquired at 161.98 MHz on a Bruker 400 MHz spectrometer equipped with a 5-mm broad band probe, using a 15- $\mu\text{s}$  pulse width, 4.32-s pulse delay, and 0.21-s acquisition time. Between 10,000 and 20,000 scans were acquired per sample, with temperature controlled to 20 °C. Processing of spectra and integrations was carried out using MestReNova10.0 software. Assignment of peaks was performed by comparison with literature (Cade-Menun et al., 2015; Giles et al., 2015; Cade-Menun and Paytan, 2010), and the spectra obtained during spiking experiments conducted in this study.

#### 2.5. Statistical analysis and plotting

Before performing statistical analyses, data were checked for deviations from normality and homogeneity of variance. To check whether there was a significant linear relationship between the relative abundances of water-extracted C and P, Pearson correlation coefficients ( $r$  values, two-tailed) at  $p < 0.01$  and  $p < 0.05$  were determined using SPSS 19.0. Figures were processed using CorelDraw Graphics Suite X4 (Corel Corp.) and OriginPro 8.0 (OriginLab Corp.).

### 3. Results and discussion

#### 3.1. Properties of aquatic plants and their WEOM

Contents of C, N and P in ground plants and their water extracts are shown in Table 1. Contents of TC the six aquatic macrophytes ranged from 335 to 443  $\text{mg g}^{-1}$  dry mass (dm) with a mean of 389  $\text{mg g}^{-1}$ . The greatest concentration of TC was observed in NP, while the concentration was less in HV. The concentration of TN was 19.2–30.5  $\text{mg g}^{-1}$  with a mean of 25.1  $\text{mg g}^{-1}$ . Content of TP ranged from 1.3 to 3.5  $\text{mg g}^{-1}$  with a mean of 2.3  $\text{mg g}^{-1}$ . Among the six plants, PD had the lowest N and P, while the greatest concentration was observed in HV. Mean ratios of C:N, C:P and N:P were 16, 188 and 11, respectively. Contents of C, N and lignin, as well as the ratios of C:N and C:P are all good predictors of degradation of plants, which has been reported by many researchers (Fog, 1988; Taylor et al., 1989; Melillo et al., 1982; Berg and Staaf, 1980). Generally, lesser contents of lignin and greater contents of N, P are benefit for the degradation of aquatic macrophytes. Therefore, the smaller ratios of C:N and C:P and greater contents of N and P indicated the readily degradation of debris derived from submerged plants of HV and MV. In contrast to debris derived from MV and HV, debris derived from emergent plants of ZC and PD are likely more resistant to decompose for emergent plants ZC and PD due to their high rates of C:N and C:P, as well as less contents of N and P in ZC and PD. This was also supported by results of previous studies on WEOM derived from plants of Lake Dianchi (Qu, 2013).

Mean contents of DOC and DTN in water extracts were 85.7 and 3.1  $\text{mg g}^{-1}$  dm, respectively, which accounted for 21.2% of TC and 13.2% of TN in original plant biomass. Contents of DTP and DOP in

**Table 1**  
Contents of C, N and P in ground plants and their water extracts in Tai Lake.

Sample	Original plant powder							WEOM							
	Contents (mg g <sup>-1</sup> )				Mass ratio			Contents (mg g <sup>-1</sup> ) <sup>b</sup>				Extraction efficiency <sup>c</sup> (%)			
	TC	TP	OP	TN	C:N	C:P	N:P	DOC	DTP	DOP	DTN	C	N	P	OP
ZC <sup>a</sup>	406	2.0	0.7	21.6	18.8	208	11	113	1.6	0.1	4.3	27.8	19.7	79.7	12.9
PD	422	1.3	0.7	19.2	22	331	15	62.2	0.7	0.0	5.1	14.7	26.5	50.9	2.0
TB	380	2.1	1.6	24.8	15	182	12	47.4	0.5	0.1	0.8	12.5	3.1	23.3	6.1
NP	443	2.7	1.5	27.5	16	164	10	198	2.1	0.8	2.9	44.7	10.5	77.1	50.7
MV	347	2.3	1.4	26.9	13	149	12	40.2	0.7	0.1	2.0	11.6	7.6	31	6.7
HV	335	3.5	1.4	30.5	11	95	9	54	2.0	0.1	3.6	16.1	11.8	57.8	4.0
Average	389	2.3	1.2	25.1	16	188	11	85.7	1.3	0.2	3.1	21.2	13.2	53.3	13.7
SD	42.5	0.8	0.4	4.1	4	80	2	60.6	0.7	0.3	1.6	12.9	8.5	23.2	18.5
PD-stem	431	1.2	0.5	17.2	25	365	15	79.8	0.8	0	7.0	18.5	40.8	64.3	2.4
PD-leave	464	1.9	1.2	28.4	16	239	15	56.8	0.8	0.1	1.8	12.3	6.4	43.2	7.9
PD-root	388	1.3	0.7	12.2	32	308	10	23.6	0.3	0.1	0.5	6.1	4.0	26.2	16.7

<sup>a</sup> ZC, *Zizania caduciflora*; PD, *Polygonum divaricatum*; TB, *Trapa bispinosa*; NP, *Nymphoides peltatum*; MV, *Myriophyllum verticillatum*; HV, *Hydrilla verticillata*; PD-stem, PD-leave and PD-root, the stems, leaves and roots of *Polygonum divaricatum*, respectively.

<sup>b</sup> Expressed as the amounts of dry biomass.

<sup>c</sup> The proportion of DOC, DTP, DOP, DTN in WEOM to TC, TP, OP, TN in original plant powder.

extracts were 1.3 and 0.2 mg g<sup>-1</sup>, respectively, accounting for 53.3% and 13.7% of the TP and OP in these samples of macrophyte. These results indicated that a greater proportion of P in aquatic macrophytes tend to be dissolved and released into the water than C or N in these debris. Aquatic macrophytes with greater concentrations of C and P would release more dissolved C or P into the water column upon their death. For example, among the six water extracts, water extraction efficiency of C and P was greatest in NP, a floating plant with greatest C and P concentrations. Distributions of C, N and P were significantly different in stems, leaves and roots (Table 1). The greatest concentrations of C, N and P were observed in leaves, while concentrations were less in roots. However, both the ratios of C:N and C:P was least in leaves. Among water extracts derived from stems, leaves and roots, the extraction efficiency of C, N and P was greatest in stems and lowest in roots.

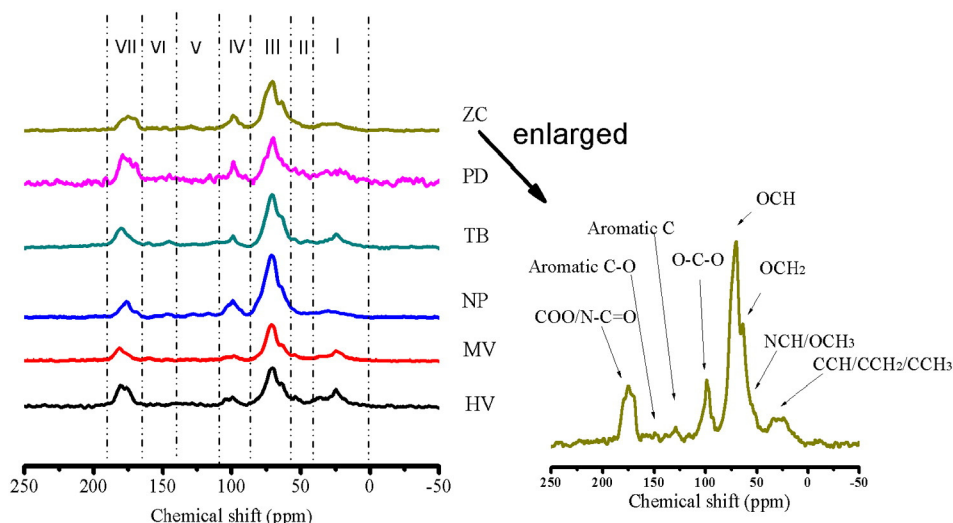
### 3.2. Solid-state <sup>13</sup>C NMR analysis for water extracts

For the six freeze-dried water extracts, solid CP/MAS <sup>13</sup>C NMR spectra showed a dominant band of OCH at 70 ppm, nonpolar alkyl-C of CCH, CCH<sub>2</sub> and CCH<sub>3</sub> at 24 ppm, O–C–O at 98 ppm and COO/N–C=O at 175 ppm (Fig. 2). Other peaks were identified, such as OCH<sub>2</sub> at 64 ppm, and broad signals at 54 ppm due to presence of NCH/OCH<sub>3</sub>. Simultaneous presence of OCH at 70 ppm, OCH<sub>2</sub> at 62 ppm and O–C–O at 98 ppm suggested the presence of carbohydrates, such as starch and

cellulose. NCH at 54 ppm, COO at 175 ppm and alkyl at 0–50 ppm indicated the presence of amino acids, peptides and proteins. The presence of peaks associated with sugar rings, N–C=O and alkyl-C, was most likely associated with the presence of nucleic acids (DNA or RNA). Peaks associated with COO and aromatic C, plus the presence of CCH, CCH<sub>2</sub> and CCH<sub>3</sub>, indicated the presence of carboxylic acid or phenolic acids of lesser molecular masses.

Although the locations of predominant absorption peaks observed by solid <sup>13</sup>C NMR were similar for the six plant-derived WEOM materials, abundances of various C-containing functional groups were varied among these WEOM (Table 2). The maximum abundance of C-containing functional groups with a mean value of 45.8% was observed at 65–90 ppm, which is associated with O-alkyl functional groups. It was followed by a region at 0–45 ppm, indicative of alkyl-C (mean, 16.4%) and at 165–190 ppm (mean, 15.5%) associated with COO/N–C=O. These results indicated that carbohydrates were the predominant components in WEOM fractions extracted from the six plant samples. Also, carboxylic acids (COO) were present in these samples (Fig. 2 and Table 2). Additionally, the small proportion (<10%) of NCH/OCH<sub>3</sub> at 45–65 ppm and aromatic C at 110–165 ppm indicated that trace amounts of lignin and aromatic substances in plant biomass were likely a composition of WEOM derived from these plants (Wilson, 1987).

Abundances of C-containing functional group present at 0–45 ppm were greater in water extracts of MV and HV than other extracts,



**Fig. 2.** Solid CP/MAS <sup>13</sup>C NMR spectra of six freeze-dried water extracts.

**Table 2**  
Relative proportions of carbon functional groups in plant-derived WEOM from solid  $^{13}\text{C}$  NMR analysis.

Sample	Alkyl-C	NCH/OCH <sub>3</sub>	O-alkyl	O-C-O	Aromatic-C	Aromatic C-O	COO/N-C=O	Al-C <sup>a</sup>	Ar-C <sup>b</sup>
	0–45	45–65	65–90	90–110	110–145	145–165	165–190		
ZC	11.7	4.2	52.9	11.1	4.5	2.4	13.2	79.9	6.9
PD	18.2	5.3	32.7	8.9	10.4	4.2	20.4	65.0	14.6
TB	17.5	3.1	43.1	6.2	7.2	6.6	16.5	69.8	13.7
NP	11.5	2.9	56.4	12.0	5.5	2.3	9.3	82.9	7.9
MV	18.6	4.9	48.7	6.3	4.1	2.4	15.0	78.5	6.5
HV	20.9	4.6	40.8	7.3	5.1	2.5	18.8	73.7	7.6
Average	16.4	4.2	45.8	8.6	6.1	3.4	15.5	74.9	9.5
SD	3.9	1.0	8.7	2.5	2.3	1.7	4.0	6.7	3.6

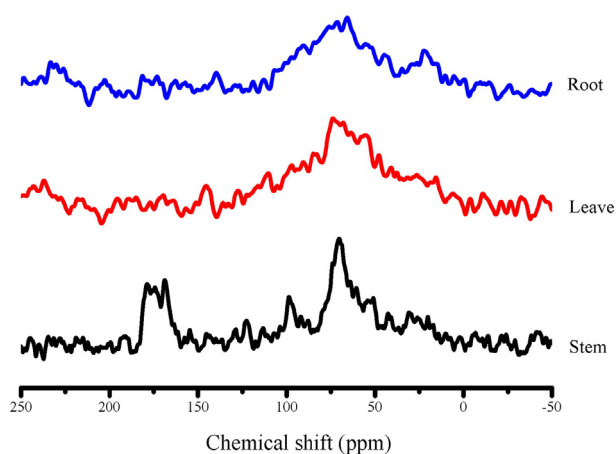
<sup>a</sup> Al-C (total aliphatic C): 0–110 ppm.

<sup>b</sup> Ar-C (total aromatic C): 110–165 ppm.

which suggested that more chemical structure of long-chain aliphatic biopolymers, such as cutans, waxes and cutins (He et al., 2015) would be the composition of WEOM derived from MV and HV. The integral areas at 65–90 ppm, a peak indicative of the presence of polysaccharide, were greater in WEOM derived from ZC and NP. Whereas the percentage of aromatic-C (110–145 ppm) was relatively small in their water extracts. The sharp peaks appearing at approximately 145 ppm, 126 ppm and 116 ppm in floating plants, including TB and NP, were likely owing to the presence of crystallized aromatic substances (Wilson, 1987). The percentage of COO/N-C=O (165–190 ppm) was greater in WEOM fractions from HV (18.8%) and PD (20.4%) than others, which indicated that more organic acids distributed in their water extracts. The CP/MAS  $^{13}\text{C}$  NMR spectra varied among stems, leaves and roots of PD (Fig. 3). The strong absorption peaks at 70 ppm, 99 ppm and around 178 ppm and a weak peak at 120 ppm were observed in stems, while leaves and roots had a broad weak peak at 70 ppm. These results indicated that more polysaccharides and carboxylic acids, while less phenolic acid distributed in WEOM derived from stems than that from leaves and roots. Whereas, more long-chain alkanes and less aromatics and organic acids in WEOM derived from leaves and roots than that from stems. The observation is consistent with previous results reported by Qu, 2013, who also found more polysaccharides in stems and more refractory alkyl-C in leaves. This can also support our observations that the extraction efficiency of C, N and P by water was greater in stems and lesser in leaves and roots.

### 3.3. Solution $^{31}\text{P}$ NMR analysis for WEOM

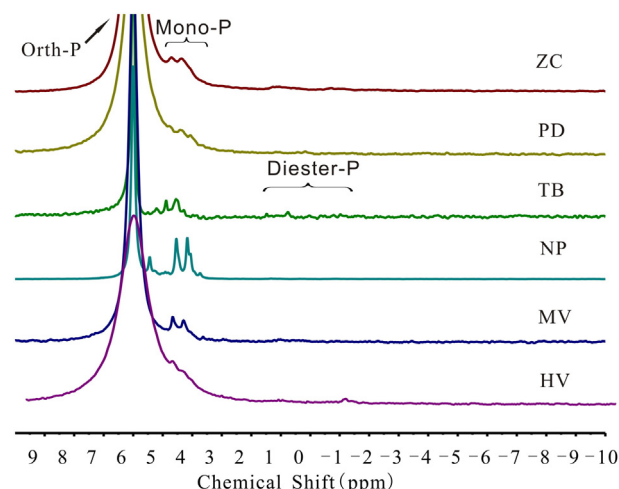
Results of  $^{31}\text{P}$  NMR spectroscopy of WEOM derived from aquatic plants are shown in Fig. 4 and Table 3. The mean concentration of ortho-P (peaks at 5.32–5.67 ppm) was  $9.4 \times 10^2 \text{ mg kg}^{-1} \text{ dm}$ , which



**Fig. 3.** Solid CP/MAS  $^{13}\text{C}$  NMR spectra of water extracts derived from stems, leaves and roots of PD.

accounted for 78.7% of the DTP in water extracts of the plant biomass. This result indicates ortho-P was the predominant form of P in water extracts. Other forms of P observed by  $^{31}\text{P}$  NMR included mono-P (peaks at 3.15–5.00 ppm) and unhydrolyzed diesters (peaks at 1.0 to –1.5 ppm). Mean concentrations were  $3.1 \times 10^2 \text{ mg kg}^{-1}$  (20.6% of TDP) and  $7.7 \text{ mg kg}^{-1}$  (0.65% of TDP), respectively. Unhydrolyzed diesters consisted mainly of incompletely degraded phospholipids (at 0.08–0.83 ppm) and stable DNA (peaks at –1.52 to –1.11 ppm) (Turner et al., 2003). Pyrophosphate and polyphosphates were not detected in these WEOM fractions.

Details of P compounds in the mono-P regions of  $^{31}\text{P}$  NMR spectra were further investigated by spiking experiments (Fig. 5). Four peaks at 4.85, 3.95, 3.58 and 3.48 ppm associated with phytate (dissolved in 1 M NaOH–0.1 M EDTA) were identified with a classic 1:2:2:1 area configuration (designated as C1, C2, C3, and C4 in Fig. 5a) (Turner et al., 2003; Cade-Menun et al., 2015). The WEOM from NP exhibited significantly greater signals at 4.85, 3.95, 3.58 and 3.48 ppm when phytate was added. Phytate was detected only in water extracts derived from NP, which could be accounted for 31.0% of TDP. The addition of a mixture of  $\alpha$ - and  $\beta$ -glycerophosphate (1:1) increased the signals at 4.31 ppm and 3.97 ppm (Fig. 5b). The chemical shift at 3.15 ppm corresponded to phosphocholine (Fig. 5c). The chemical shift at 3.69 ppm was due to AMP (Fig. 5d). The chemical shifts of several mononucleotides overlapped, and thus were difficult to distinguish. According to the results of these spiking studies and chemical shifts previously reported (Turner et al., 2003; Cade-Menun et al., 2015), the peak at 4.53 ppm was identified as Glucose-6-phosphate. Thus, based on results of spiking studies and previous studies,  $\alpha$ - and  $\beta$ -glycerophosphates in WEOM fractions were identified and mean contents were  $32.4 \text{ mg kg}^{-1}$  and  $49.2 \text{ mg kg}^{-1}$ , which accounted for 3.1% and 5.0% of the TDP, respectively. Mononucleotide was only detected in PD and MV, with a mean percentage of 0.9%,



**Fig. 4.** Solution  $^{31}\text{P}$  NMR spectra of six freeze-dried water extracts from plants.

**Table 3**Contents (mg kg<sup>-1</sup>) and relative peak area (%) (shown in parentheses) of solution <sup>31</sup>P NMR spectra in nine WEOM fractions.

P species	ZC	PD	TB	NP	MV	HV	Average	PD-stem	PD-leave	PD-root
Ortho-P	1242 (80.1)	566 (87.3)	387 (79.3)	1230 (59.1)	652 (90.4)	1552 (76.3)	938(78.7)	712.65(93.8)	692.8(82.6)	288.2(87.3)
monoesters										
G6P	nd*	nd	21.0 (4.3)	nd	nd	468(23.0)	81.5 (4.6)	nd	nd	nd
Phytate	nd	nd	nd	644.8 (31.0)	nd	nd	107 (5.2)	nd	nd	nd
α-Glyc	91.8 (5.9)	22.0 (3.4)	17.8 (3.8)	31.5 (1.5)	31.1 (4.3)	nd	32.35 (3.1)	14.3 (1.9)	nd	30.9 (9.4)
β-Glyc	193 (12.4)	28.1 (4.3)	40.8 (8.4)	nd	34.0 (4.7)	nd	49.2 (5.0)	17.8 (2.4)	77.7 (9.3)	11.2 (3.4)
Mononucleotides	nd	32.5 (5.0)	nd	nd	4.5 (0.6)	nd	6.2 (0.9)	10.7 (1.4)	68.4 (8.2)	nd
Phosphocholine	nd	nd	nd	35.0 (1.7)	nd	nd	5.8 (0.28)	4.3 (0.6)	nd	nd
Unknow	nd	nd	13.32 (2.7)	141.4 (6.8)	nd	nd	25.8 (1.6)	nd	nd	nd
Total	284.2 (18.3)	82.6 (12.7)	93.0 (19.1)	852.7 (40.9)	69.6 (9.7)	467.7 (23.0)	308(20.6)	47.0 (6.2)	146.1 (17.4)	42.1 (12.8)
Diesters										
phospholipid	5.7 (0.4)	nd	8.0 (1.6)	nd	nd	nd	2.3 (0.33)	nd	nd	nd
DNA	19.1 (1.2)	nd	nd	nd	nd	13.7 (0.7)	5.5 (0.32)	nd	nd	nd
Total	24.8 (1.6)	nd	8.0 (1.6)	nd	nd	13.7 (0.7)	7.7 (0.65)	nd	nd	nd

G6P, glucose 6 phosphate; α-Glyc, α-glycerophosphate; β-Glyc, β-glycerophosphate.

\* "nd" = "no detected".

while phosphocholine was 0.28%. Glucose-6-phosphate was identified in *TB* and *HV* and the mean content was 81.5 mg kg<sup>-1</sup> (4.6% of TDP). Additionally, there were also some unknown mono-P accounting for 1.6% (Table 3).

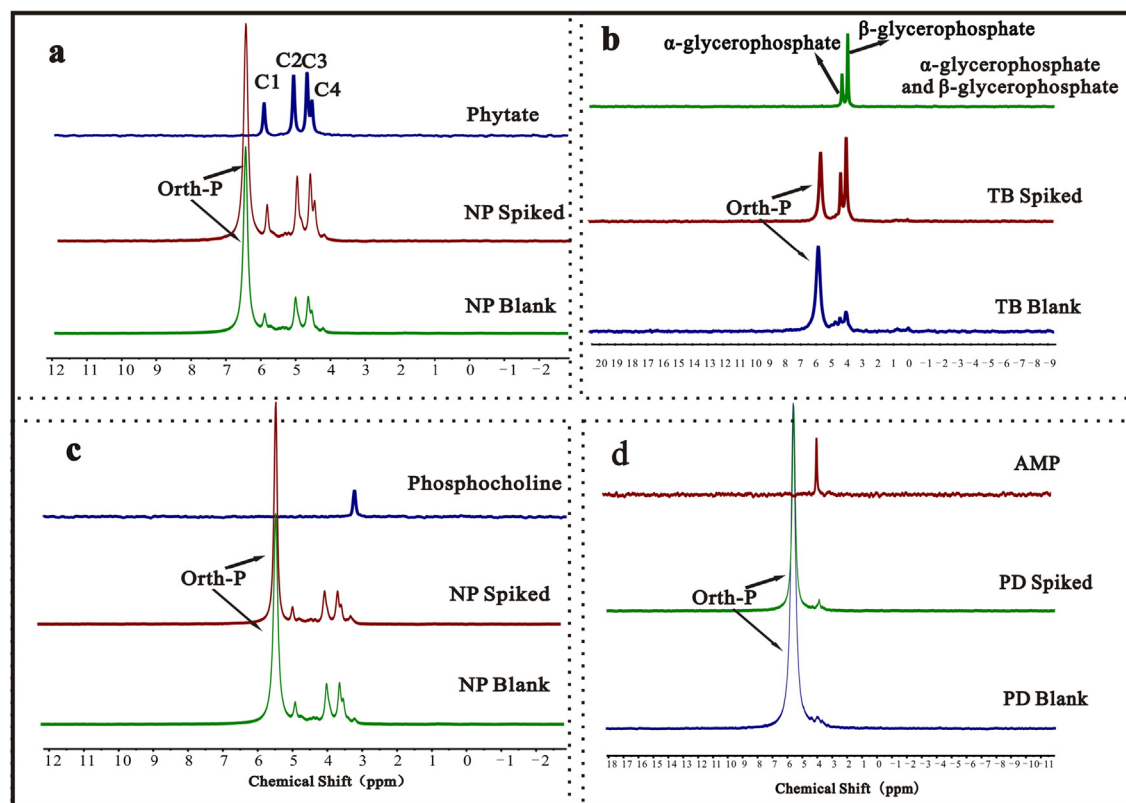
There were significant differences among proportions of different forms of P in WEOM derived from stems, leaves and roots of *PD*. The percentage of ortho-P in WEOM derived from stems was greater than that from leaves and roots, while monoester P was two or three times less in WEOM derived from stems than that from leaves and roots (Fig. 6 and Table 3).

#### 3.4. Correlations of C functional groups and P compounds in WEOM

Relative abundances of C functional groups are indicative of the degradation and humification processes of OM (Krull et al., 2003; Kalbitz

et al., 2003). In addition, correlation analysis of the abundances of different C functional groups can provide information on the internal cross-linking structures of OM (He et al., 2013).

Thus, relationships between different C functional groups in WEOM derived from six plants were analyzed (Table 4). The relative abundance of alkyl-C was positively correlated with COO/N-C=O ( $p < 0.05$ ), suggesting alkyl-C and COO/N-C=O groups in the WEOM perhaps had the similar origins or source dissolved from the samples, such as aliphatic acid, a common OM in aquatic plants. Additionally, the relative abundance of alkyl-C was negatively correlated with O-C-O ( $p < 0.05$ ) in the WEOM, which is likely implied that the OM containing more O-C-O groups and less alkyl-C would be easily released into the water. The relative abundance of aliphatic C (Al-C) was positively correlated with *o*-alkyl ( $p < 0.01$ ) and negatively correlated with aromatic-C and COO/N-C=O ( $p < 0.05$ ). The relationships implied that



**Fig. 5.** Solution <sup>31</sup>P NMR spectra from spiking experiments. NP spiked spectra were obtained after the addition of model into NP sample. NP blank spectra were obtained from macrophyte sample without model. Other spectra were in the same way like NP. Labeled peaks are for phytate (a), 1:1 α-glycerophosphate and β-glycerophosphate (b), phosphocholine (c), and AMP (d).

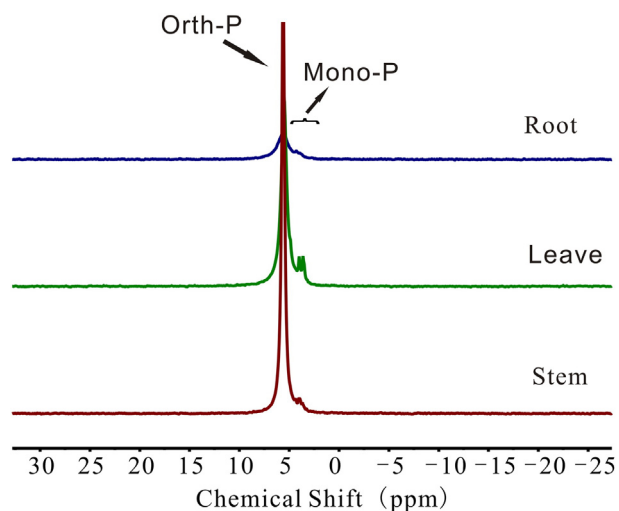


Fig. 6. Solution  $^{13}\text{P}$  NMR spectra of WEOM fractions derived from stems, leaves and roots of PD.

O-alkyl dominated in the Al-C groups, and Al-C competed with aromatic C and COO/N-C=O to be composed of the backbone C structures in WEOM of these plant biomass. This conclusion is also supported by the negative correlation between O-alkyl and COO/N-C=O ( $p < 0.01$ ).

These relationships between C functional groups can be attributed to the selective release and degradation of OM from aquatic macrophytes. Decomposition of aquatic macrophytes involves an initial loss of carbohydrates, including cellulose and hemicellulose, and then the slower transformation of aromatic structures in lignin molecules, followed by the decomposition of more recalcitrant carbon (alkyl-C) (Kögel-Knabner, 2000). In the initial phases of decomposition of aquatic macrophytes, the relative contents of alkyl-C in plant litter increases, while the absolute and relative amounts of O-alkyl decrease (Baumann et al., 2009). This observation can be further confirmed by the negative correlation of DOC concentrations in extracts with the relative abundance of alkyl-C ( $p < 0.05$ ) and COO/N-C=O ( $p < 0.05$ ), and the positive correlation with the total percentage of O-alkyl C and O-C-O ( $p < 0.05$ ). Organic matter consisting of more refractory alkyl-C, COO/N-C=O and aromatic C constituents would be more difficult to be degraded. Thus, less of these compounds than others (e.g., cellulose, hemicellulose) would be expected to be released into water.

Other components of WEOM, such as P, would also release into the water column during the dissolution and degradation of OM in aquatic macrophytes. Relationships between the proportion of different P compounds and C functional groups can provide insights into the internal structure information of WEOM released from aquatic macrophytes.

The positive correlation between the abundance of mono-P in total P species and DOC concentration ( $p < 0.05$ ) can be explained by preferential dissolution of monoester P (e.g.,  $\alpha$ - and  $\beta$ -glycerophosphate, phosphocholine) with the autolysis of WEOM (Table 4). The relationships between mono-P and alkyl-C, NCH/OCH<sub>3</sub>, COO/N-C=O and O-C-O functional groups were analyzed further. Although the correlations between them were not significant, the variation of mono-P with the C functional groups still could be found in Fig. 7. Abundance of mono-P is likely directly proportional to the abundance of O-alkyl and O-C-O, while an opposite trend was observed between abundance of mono-P and alkyl-C, NCH/OCH<sub>3</sub> and COO/N-C=O. The fact that there was no correlation between concentrations of diesters of P and C functional groups might be due to the lability of some diesters, such as RNA and phospholipids.

Monoesters in WEOM were mainly composed of degradation products of RNA and phospholipids, such as mononucleotide, glycerophosphate and phosphocholine. The abundance of mono-P increased with O-alkyl or O-C-O indicated that mono-P in the WEOM mainly originated from the degradation of the components of plants containing O-C-O or O-alkyl groups. RNA contains sugar groups, such as ribose. Thus, degradation of RNA would result in an increase in mono-P and some C functional groups, such as OCH and OCH<sub>2</sub>. Also, degradation products of biological membranes are always associated with phospholipid and glycolipids including structures of OCH<sub>2</sub> and O-C-O groups, as well as mono-P degraded from phospholipid. Phospholipid and glycolipids are the main components of biological membranes, which include the cuticle, middle lamella and cellular membranes (Hance and Anderson, 1963; Salisbury and Ross, 1995; He et al., 2015). Therefore, mono-P would also increase with the dissolution and degradation of biological membranes in WEOM. Inconsistent trend between mono-P and alkyl-C, NCH/OCH<sub>3</sub> and COO/N-C=O may be owing to the insoluble compounds including C functional groups of alkyl-C, NCH/OCH<sub>3</sub> and COO/N-C=O, such as aliphatic biopolymers, lignin and peptides (Kögel-Knabner, 2002) in aquatic macrophytes. This conclusion can be further confirmed by the relationships observed between O-alkyl and alkyl-C and COO/N-C=O, as discussed above. The results presented here are inconsistent with those of Gressel et al. (1996), who found a positive relationship between mono-P and alkyl-C signal intensities in liquid-state NMR spectra obtained from extracts of forest floor and topsoil horizons. The organic floor layers of the forest contain organics of a high degree of humification, which will accumulate more refractory alkyl-C derived from plants. Whereas, mono-P derived from plants are relatively stable in the humification processes of OM. Thus, decomposition of structural components of plant litter during the process of humification or diagenesis may explain the relationship between mono-P and alkyl-C. However, samples examined during the study, the results of which are presented here, were fresh WEOM, without humification. Therefore, soluble components would be preferentially released

Table 4  
Correlation coefficients between C functional groups and P species identified by  $^{13}\text{C}$  NMR and  $^{31}\text{P}$  NMR spectroscopy.

	Alkyl-C	NCH/OCH <sub>3</sub>	O-alkyl	O-C-O	Aromatic C	Aromatic C-O	COO/N-C=O	Al-C	Ar-C	DOC	Ortho-P	Mono-P
NCH/OCH <sub>3</sub>	.554											
O-alkyl	-.759	-.585										
O-C-O	-.858*	-.286	.528									
Aromatic C	.220	.201	-.761	-.047								
Aromatic C-O	.253	-.299	-.484	-.478	.597							
COO/N-C=O	.834*	.674	-.964**	-.629	.577	.396						
Al-C	-.636	-.394	.960**	.512	-.842*	-.696	-.894*					
Ar-C	.261	-.011	-.719	-.256	.926**	.856*	.558	-.870*				
DOC	-.850*	-.576	.694	.906*	-.178	-.408	-.821*	.653	-.307			
Ortho-P	.563	.760	-.562	-.631	.198	.198	.676	-.521	.221	-.854*		
Mono-P	-.548	-.729	.552	.635	-.186	-.229	-.673	.522	-.227	.862*	-.997**	
Diester-P	-.175	-.379	.108	-.098	-.168	.434	.002	-.053	.096	-.168	.034	-.106

Significance of a coefficient at \*  $p \leq 0.05$  and \*\*  $p \leq 0.01$  ( $n = 6$ ).

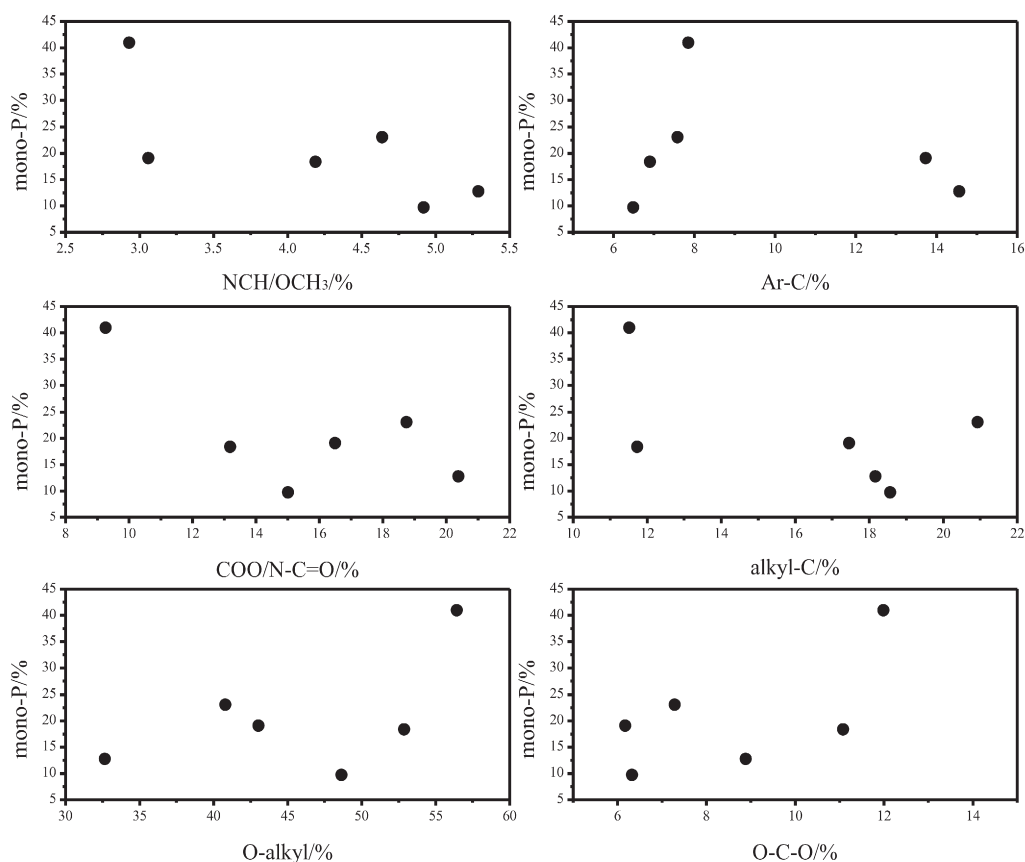


Fig. 7. Relationships between mono-P and C functional groups in WEOM derived from aquatic plants of Tai Lake.

into water and result in coupling of mono-P and sugar groups of *O*-alkyl and *O*-C-O.

### 3.5. Implications for biogeochemical cycling of plant-derived C and P

WEOM derived from aquatic macrophytes would be directly released into the water column when the death of these plants. Also, some WEOM would be precipitated into the sediments by interaction with metal ions (e.g., Fe, Al, and Ca) or minerals (Helms et al., 2013; Riedel et al., 2012; Kalbitz et al., 2005; Kaiser and Guggenberger, 2000), lake sediments could thus be the ultimate “sink” of some WEOM and water unextractable OM in aquatic macrophytes. Therefore, comparison and discussion of P species and C functional groups among

macrophyte-derived WEOM, water column and sediments would help our understanding of the contribution and transformation of plant-derived OM in lake ecosystems.

To our knowledge, there has been no <sup>13</sup>C NMR analyses of dissolved organic matter (DOM) in overlying water of Tai Lake, but several studies focused on the DOM by using other spectral analyses, such as three-dimensional fluorescence and ultraviolet spectra (Yao et al., 2011; Hu et al., 2011). The composition of DOM from Tai Lake water was investigated by use of three-dimensional fluorescence and ultraviolet spectra (Hu et al., 2011). Results of that study showed that proteins and polysaccharides were predominate fractions of DOM from macrophytes-dominant East Tai Lake with few aromatics was observed. This is consistent with the composition of WEOM derived

Table 5

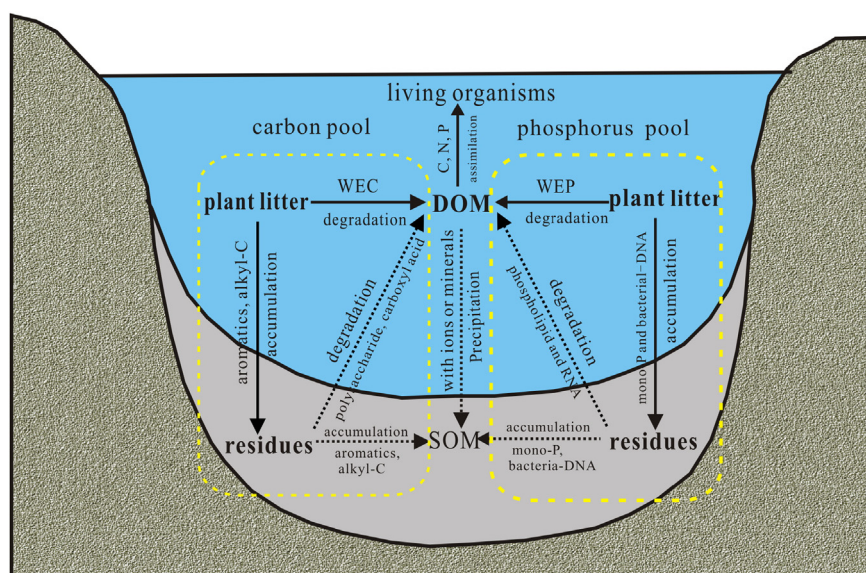
Comparison of C functional groups characterized by solid <sup>13</sup>C NMR between WEOM derived from aquatic macrophytes and FA, HA and Humin derived from sediments of Tai Lake.

Sample	C-types Chemical shift/ppm	Relative abundance of C functional group/(%)							Alkyl-C/Ar-C	
		Alkyl-C	NCH/OCH <sub>3</sub>	O-alkyl	O-C-O	Aromatic-C	O-aromatic	COO/N-C=O		Carbonyl C
		0–45	45–65	65–90	90–110	110–145	145–165	165–190		190–220
WEOM	ZC	11.7	4.2	52.9	11.1	4.5	2.4	13.2	nd	1.7
	PD	18.2	5.3	32.7	8.9	10.4	4.2	20.4	nd	1.3
	TB	17.5	3.1	43.1	6.2	7.2	6.6	16.5	nd	1.3
	NP	11.5	2.9	56.4	12.0	5.5	2.3	9.3	nd	1.5
	MV	18.6	4.9	48.7	6.3	4.1	2.4	15.0	nd	2.9
	HV	20.9	4.6	40.8	7.3	5.1	2.5	18.8	nd	2.8
	Average	16.4	4.2	45.8	8.6	6.1	3.4	15.5	nd	1.9
Sediment <sup>a</sup>	FA	27.7	10.8	10.5	8.6	15.0	5.8	16.6	5.0	1.3
	HA	55.2	13.3	6.1	0.6	6.1	1.7	8.8	8.3	7.1
	Humin	73.5	2.2	1.5	2.2	15.4	2.2	2.9	nd	4.2
	Average	52.1	8.8	6.0	3.8	12.2	3.2	9.4	4.4	4.2

“nd” = not detect.

<sup>a</sup> Data cited from Lin et al., 2011.





**Fig. 8.** Schematic of degradation and accumulation of plant-derived C and P in lake ecosystems. WEC: water extractable C, mainly including polysaccharides and carboxyl acid. WEP: water extractable P, mainly including degradation products of RNA and phospholipid.

from aquatic macrophytes, which may provide new evidence that debris of aquatic macrophytes would be an important source for DOM in water of East Tai Lake.

The relative abundances of C functional groups, characterized by  $^{13}\text{C}$  NMR spectroscopy in WEOM derived from aquatic macrophytes and OM (e.g., FA, HA and Humic) in sediments from Tai Lake (Lin et al., 2011), are shown in Table 5. The percentage of refractory alkyl-C and aromatic-C groups were greater in sediments than that in WEOM, while proportions of O-alkyl and O-C-O were significantly lower in sediment than that in WEOM. It can be assumed that the WEOM derived from aquatic macrophytes would be released into the overlying water, and the unextractable OM remaining in the debris of aquatic macrophytes would be precipitated in the sediments. Thus, these differences could be attributed to degradation and dissolution of lability of compounds containing O-alkyl and O-C-O such as polysaccharides from aquatic macrophytes, and those refractory OM such as lignin and aromatics remaining in the residues would be accumulated in sediments. In addition, the ratio of refractory alkyl-C to aromatic-C in WEOM was similar to that in sediments from Tai Lake, which indicated that some WEOM derived from endogenous aquatic plants would also be the origin of OM in sediments.

A schematic of predominant C functional groups and P compounds in WEOM derived from aquatic macrophytes, water column and sediments of East Tai Lake is shown in Fig. 8. For P compounds, the ratio of diesters to

monoesters is a good indicator for degradation of P-containing materials (Paytan et al., 2003). Quantitative results of different P species in concentrated and freeze-dried water and NaOH-EDTA extracted sediments of East Tai Lake in previous studies (Sun, 2013; Zhu et al., 2015; Zhang et al., 2009) were compared with P-containing compounds in WEOM derived from aquatic macrophytes in this study. The proportion of mono-P and diester-P was significantly greater in the water column than that in plant-derived WEOM and sediments, but ortho-P was less in the water column (Table 6). This is probably owing to the requirement for ortho-P during the growth of plants in the overlying water of lakes. The ratio of diester to monoester was significantly greater in sediments than that in WEOM or water column (Table 6). The result suggested that P compounds identified as monoesters and diesters could undergo substantial transformations in water and sediments. Monoesters, including  $\alpha$ -glycerophosphate,  $\beta$ -glycerophosphate, phosphocholine, mononucleotide, detected by NMR, were mainly degradation products of labile diesters, such as RNA and phospholipids. Therefore, some peaks identified in the  $^{31}\text{P}$  NMR spectra within the monoester range are an indication of the presence of diesters, particularly RNA and phospholipids. The ratio of diester to monoester was greater in the sediment than that in WEOM derived from either aquatic macrophytes or water column. This is likely because degradation of labile diesters detected in the monoester range (phospholipids and RNA), and the production of stable diesters (DNA) by bacteria when some WEOM precipitated into the sediments. The

**Table 6**

Comparison of P characterized by  $^{31}\text{P}$  NMR in the water column, sediments and plant-derived WEOM from East Tai Lake.

Sample		Relative abundance of P species/(%)						Diester-P/mono-P
		Ortho-P	Mono-P	Diester-P	phosphonate	Pyro-P	Poly-P	
WEOM	ZC	80.1	18.3	1.6	nd	nd	nd	0.09
	PD	87.3	12.7	nd	nd	nd	nd	–
	TB	79.3	19.1	1.6	nd	nd	nd	0.09
	NP	59.1	40.9	nd	nd	nd	nd	–
	MV	90.4	9.7	nd	nd	nd	nd	–
	HV	76.3	23.0	0.67	nd	nd	nd	0.03
	Average	78.7	20.6	0.65	nd	nd	nd	0.03
Water column <sup>a</sup>		23.2	60.3	5.98	22.3	3.08	2.82	0.10
Sediment		74.6 <sup>b</sup>	18.9	5.50	nd	1.0	nd	0.29
		88.0 <sup>c</sup>	9.0	2.4	nd	0.6	nd	0.26

<sup>a</sup> "nd" = not detected.

<sup>b</sup> Data cited from Sun, 2013.

<sup>c</sup> From Zhu et al., 2015.

<sup>d</sup> From Zhang et al., 2009.

increase in the proportion of stable diesters, such as DNA, is also consistent with previous reports (Reitzel et al., 2012; Reddy and DeLaune, 2008), where it was found that microbial activity increases diester concentrations owing to the large amounts of diesters in bacteria. Thus, diesters may play a significant role in the biogeochemical cycles of P in the macrophyte-dominated eastern region of Tai Lake.

Phytate is always thought to be derived from exogenous sources, such as soils and plants in land, in both lakes and rivers (Turner et al., 2002; Giles and Cade-Menun, 2014). However, it was detected in WEOM fractions from the floating plant of NP, and accounted for a large proportion (31.0%) of TDP in WEOM. This result indicated that aquatic macrophytes are important sources of phytate in sediments of lakes, especially macrophyte dominated lakes. This conclusion is also supported by large concentrations and proportions of phytate-like P in sediments from East Tai Lake (Zhu et al., 2015). Phytate-like P, including free inositol phosphates and those bound to proteins and fulvic acids (Herbes et al., 1975) constitute significant proportions of phosphate monoester in sediments of lakes (Reitzel et al., 2007; Jørgensen et al., 2011).

#### 4. Conclusions

Carbohydrates were predominant constituents of WEOM derived from macrophytes of Tai Lake, followed by a small proportion of carboxylic acid. There were few contents of lignin and aromatic substances in these WEOMs. Ortho-P was the dominant form of P in water extracts, followed by mono-P and diester P. The forms of C and P in the WEOM derived from aquatic macrophytes were related to structural components of plant biomass. Degradation products of phospholipid and RNA, production of DNA by bacteria as well as plant-derived phytates may play an important role in biogeochemical cycling of P in East Tai Lake. Thus, more attention should be paid on biological factors in cycling of P. The WEOM of aquatic macrophytes was dominated by polysaccharides, which were likely the most labile and bioavailable nutrients for life in lakes. Additionally, the residual OM, except WEOM, in the debris derived from aquatic macrophytes would be degraded by microorganisms or even resistant to degradation and accumulation in the sediment. Thus, the role of these residual OMs derived from aquatic macrophytes should be clarified in further studies.

#### Acknowledgments

This research was jointly supported by National Natural Science Foundation of China (No. 41403094, 41130743 and 41261140337).

#### References

- Bai, Y., Wu, F., Shi, G., 2013. Characterization of Chinese Standard Fulvic Acid Fractions Obtained by Sequential Extractions with Pyrophosphate Buffer from Forest Soil. *Functions of Natural Organic Matter in Changing Environment*. Springer, Netherlands, pp. 239–243.
- Baumann, K., Marschner, P., Smernik, R.J., Baldock, J.A., 2009. Residue chemistry and microbial community structure during decomposition of eucalypt, wheat and vetch residues. *Soil Biol. Biochem.* 41 (9), 1966–1975.
- Berg, B., Staaf, H., 1980. Decomposition rate and chemical changes of Scots pine needle litter. II. Influence of chemical composition. *Ecol. Bull.* 373–390.
- Brisson, J., Chazarenc, F., 2009. Maximizing pollutant removal constructed wetlands: should we pay more attention to macrophyte species selection. *Sci. Total Environ.* 407 (13), 3926–3930.
- Brown, A.D., Sposito, G., 1991. Acid–base chemistry of dissolved organic matter in aqueous leaf extracts: application to organic acids in throughfall. *J. Environ. Qual.* 20, 839–845.
- Cade-Menun, B.J., Paytan, A., 2010. Nutrient temperature and light stress alter phosphorus and carbon forms in culture-grown algae. *Mar. Chem.* 121, 27–36.
- Cade-Menun, B.J., He, Z., Dou, Z., 2015. Comparison of phosphorus forms in three extracts of dairy feces by solution  $^{31}\text{P}$  NMR analysis. *Commun. Soil Sci. Plant Anal.* 46, 1698–1712.
- Cheesman, A.W., Turner, B.L., Inglett, P.W., Reddy, K.R., 2010. Phosphorus transformations during decomposition of wetland macrophytes. *Environ. Sci. Technol.* 44, 9265–9271.
- Chinney, M.J., Pietro, K.C., 2006. Decomposition of macrophyte litter in a subtropical constructed wetland in south Florida (USA). *Ecol. Eng.* 27 (4), 301–321.
- Clabeaux, B.L., Navarro, D.A., Aga, D.S., Bisson, M.A., 2011. Cd tolerance and accumulation in the aquatic macrophyte, *Chara australis*: potential use for Charophytes in phytoremediation. *Environ. Sci. Technol.* 45 (12), 5332–5338.
- Doolittle, A.L., Smernik, R.J., Dougherty, W.J., 2009. Spiking improved solution phosphorus-31 nuclear magnetic resonance identification of soil phosphorus compounds. *Soil Sci. Soc. Am. J.* 73, 919–927.
- Dwivedi, S., Srivastava, S., Mishra, S., Dixit, B., Kumar, A., Tripathi, R.D., 2008. Screening of native plants and algae growing on fly ash affected areas near National Thermal Power Corporation, Tanda, Uttar Pradesh, India for accumulation of toxic heavy metals. *J. Hazard. Mater.* 158 (2–3), 359–365.
- Fog, K., 1988. The effect of added nitrogen on the rate of decomposition of organic matter. *Biol. Rev.* 63, 433–462.
- Gélinasa, Y., Baldock, J.A., Hedges, J.L., 2001. Demineralization of marine and freshwater sediments for CP/MAS  $^{13}\text{C}$  NMR analysis. *Org. Geochem.* 31, 677–693.
- Giles, C.D., Cade-Menun, B.J., 2014. Phytate in animal manure and soils: abundance, cycling and bioavailability. *Applied Manure and Nutrient Chemistry for Sustainable Agriculture and Environment*. Springer, Netherlands, pp. 163–190.
- Giles, C.D., Lee, L.G., Cade-Menun, B.J., Hill, J.E., Isles, P.D., Schroth, A.W., Druschel, G.K., 2015. Characterization of organic phosphorus form and bioavailability in lake sediments using P Nuclear Magnetic Resonance and enzymatic hydrolysis. *J. Environ. Qual.* 44 (3), 882–894.
- Gressel, N., McColl, J.G., Preston, C.M., Newman, R.H., Powers, R.F., 1996. Linkages between phosphorus transformations and carbon decomposition in a forest soil. *Biogeochemistry* 33 (2), 97–123.
- Hance, R.J., Anderson, G., 1963. Identification of hydrolysis products of soil phospholipids. *Soil Sci.* 96 (3), 157–161.
- He, Z., Cao, X., Mao, J., Ohno, T., Waldrip, H.M., 2013. Analysis of carbon functional groups in mobile humic acid and recalcitrant humate extracted from eight US soils. *Pedosphere* 23, 705–716.
- He, Z., Mao, J., Honeycutt, C.W., Ohno, T., Hunt, J.F., Cade-Menun, B.J., 2009. Characterization of plant-derived water extractable organic matter by multiple spectroscopic techniques. *Biol. Fertil. Soils* 45, 609–616.
- He, Z., Wu, F., 2015. Labile organic matter – chemical compositions, function, and significance in soil and the environment. *SSSA Special Publication*. Soil Sci. Soc. Am., Madison, Wisconsin, p. 62.
- He, Z., Zhang, M., Cao, X., Li, Y., Mao, J., Waldrip, H.M., 2015. Potential traceable markers of organic matter in organic and conventional dairy manure using ultraviolet–visible and solid-state  $^{13}\text{C}$  nuclear magnetic resonance spectroscopy. *Org. Agric.* 5, 113–122.
- Helms, J.R., Mao, J., Schmidt-Rohr, K., Abdulla, H., Mopper, K., 2013. Photochemical flocculation of terrestrial dissolved organic matter and iron. *Geochim. Cosmochim. Acta* 121, 398–413.
- Herbes, S.E., Allen, H.E., Mancy, K.H., 1975. Enzymatic characterization of soluble organic phosphorus in lake water. *Science* 187 (4175), 432.
- Hu, C., Zhang, Y., Yu, T., Yao, B., Hu, D., 2011. Spectroscopic characteristics of dissolved organic matter in water from typical area of Taihu Lake. *Spectra Anal.* 31 (11), 3022–3025 (in Chinese).
- Hunt, J.F., Ohno, T., He, Z., Honeycutt, C.W., Dail, D.B., 2007. Inhibition of phosphorus sorption to goethite, gibbsite, and kaolin by fresh and decomposed organic matter. *Biol. Fertil. Soils* 44, 277–288.
- Jørgensen, C., Jensen, H.S., Andersen, F.Ø., Egemose, S., Reitzel, K., 2011. Occurrence of orthophosphate monoesters in lake sediments: significance of myo- and scyllo-inositol hexakisphosphate. *J. Environ. Monit.* 13 (8), 2328–2334.
- Kalbitz, K., Schwesig, D., Rethemeyer, J., Matzner, E., 2005. Stabilization of dissolved organic matter by sorption to the mineral soil. *Soil Biol. Biochem.* 37 (7), 1319–1331.
- Kalbitz, K., Schmerwitz, J., Schwesig, D., Matzner, E., 2003. Biodegradation of soil-derived dissolved organic matter as related to its properties. *Geoderma* 113 (3), 273–291.
- Kaiser, K., Guggenberger, G., 2000. The role of DOM sorption to mineral surfaces in the preservation of organic matter in soils. *Org. Geochem.* 31 (7), 711–725.
- Kögel-Knabner, I., 2000. Analytical approaches for characterizing soil organic matter. *Org. Geochem.* 31, 609–625.
- Kögel-Knabner, I., 2002. The macromolecular organic composition of plant and microbial residues as inputs to soil organic matter. *Soil Biol. Biochem.* 34 (2), 139–162.
- Krull, E.S., Baldock, J.A., Skjemstad, J.O., 2003. Importance of mechanisms and processes of the stabilisation of soil organic matter for modeling carbon turnover. *Funct. Plant Biol.* 30 (2), 207–222.
- Lin, Y., Wu, F., Bai, Y., Xie, F., Cao, Z., Su, H., 2011. Isolation and characterization of standard fulvic acids from soil and sediments in China. *Res. Environ. Sci.* 24 (10), 1143–1148 (in Chinese).
- Melillo, J.M., Aber, J.D., Muratore, J.F., 1982. Nitrogen and lignin control of hardwood leaf litter decomposition dynamics. *Ecology* 63 (3), 621–626.
- Murphy, T., Riley, J.P., 1962. A modified single solution method for the determination of phosphate in natural waters. *Anal. Chim. Acta* 27, 31–36.
- Nelson, P.N., Baldock, J.A., 2005. Estimating the molecular composition of a diverse range of natural organic materials from solid-state  $^{13}\text{C}$  NMR and elemental analysis. *Biogeochemistry* 72, 1–34.
- Nirmal-Kumar, J.L., Soni, H., Kumar, R.N., Bhatt, I., 2008. Macrophytes in phytoremediation of heavy metal contaminated water and sediments in Pariyej Community Reserve, Gu-jarat, India. *Turk. J. Fish. Aquat. Sci.* 8, 193–200.
- Noack, S.R., McLaughlin, M.J., Smernik, R.J., McBeath, T.M., Armstrong, R.D., 2012. Crop residue phosphorus: speciation and potential bio-availability. *Plant Soil* 359, 375–385.
- Paytan, A., Cade-Menun, B.J., McLaughlin, K., Faul, K.L., 2003. Selective phosphorus regeneration of sinking marine particles: evidence from  $^{31}\text{P}$ -NMR. *Mar. Chem.* 82, 55–70.
- Qu, X.X., 2013. Chemical properties, Decomposition Behavior and Environmental Significance of Dissolved Organic Matter (DOM) From Aquatic Macrophytes. Beijing Normal University, Beijing.

- Qu, X., Xie, L., Lin, Y., Bai, Y., Zhu, Y., Xie, F., Giesy, J.P., Wu, F., 2013. Quantitative and qualitative characteristics of dissolved organic matter from eight dominant aquatic macrophytes in Lake Dianchi, China. *Environ. Sci. Pollut. Res.* 20, 7413–7423.
- Reddy, K.R., DeLaune, R.D., 2008. *Biogeochemistry of Wetlands: Science and Applications*. CRC Press, Boca Raton, FL.
- Reitzel, K., Ahlgren, J., Rydin, E., Egemose, S., Turner, B.L., Hupfer, M., 2012. Diagenesis of settling seston: identity and transformations of organic phosphorus. *J. Environ. Monit.* 14, 1098–1106.
- Reitzel, K., Ahlgren, J., DeBrabandere, H., Waldebäck, M., Gogoll, A., Tranvik, L., Rydin, E., 2007. Degradation rates of organic phosphorus in lake sediment. *Biogeochemistry* 82 (1), 15–28.
- Riedel, T., Biester, H., Dittmar, T., 2012. Molecular fractionation of dissolved organic matter with metal salts. *Environ. Sci. Technol.* 46 (8), 4419–4426.
- Ruban, V., López-Sánchez, J.F., Pardo, P., Rauret, G., Muntau, H., Quevauviller, P., 2001. Harmonized protocol and certified reference material for the determination of extractable contents of phosphorus in freshwater sediments — a synthesis of recent works. *Fresenius J. Anal. Chem.* 370 (2), 224–228.
- Ruban, V., López-Sánchez, J.F., Pardo, P., Rauret, G., Muntau, H., Quevauviller, P., 1999. Selection and evaluation of sequential extraction procedures for the determination of phosphorus forms in lake sediment. *J. Environ. Monit.* 1 (1), 51–56.
- Salisbury, F.B., Ross, C.W., 1995. *Plant Physiology*. 3rd ed. Wadsworth Pub. Co, Belmont, CA.
- Sun, J., 2013. *Spatial Distribution of Organic Phosphorus and Relationship With Water Environment Factors of Taihu Lake*. Graduate School of Henan University, Henan.
- Taylor, B.R., Parkinson, D., Parsons, W.F.J., 1989. Nitrogen and lignin content as predictors of litter decay rates: a microcosm test. *Ecology* 70 (1), 97–104.
- Turner, B.L., Mahieu, N., Condron, L.M., 2003. Phosphorus-31 nuclear magnetic resonance spectral assignments of phosphorus compounds in soil NaOH–EDTA extracts. *Soil Sci. Soc. Am. J.* 67 (2), 497–510.
- Turner, B.L., Papházy, M.J., Haygarth, P.M., McKelvie, I.D., 2002. Inositol phosphates in the environment. *Philos. Trans. R. Soc. B* 357 (1420), 449–469.
- Wilson, M.A., 1987. *NMR Techniques and Applications in Geochemistry and Soil Chemistry*. Pergamon Press, Oxford.
- Wu, F., 2009. *Natural Organic Matters and Their Interaction with Pollutants*. Science Press, Beijing.
- Wu, F., Xing, B., 2009. *Natural Organic Matter and Its Significance in the Environment*. Science Press, Beijing.
- Wu, F., Jin, X., Zhang, R., Liao, H., Wang, S., Jiang, X., Wang, L., Guo, J., Li, W., Zhao, X., 2010. Effects and significance of organic nitrogen and phosphorus in the lake aquatic environment. *J. Lake Sci.* 22 (1), 1–7.
- Xie, Y., Qi, D., Ren, B., 2004. Nutrient limitation to the decomposition of water hyacinth (*Eichhornia crassipes*). *Hydrobiologia* 529 (1/2/3), 105–112.
- Xu, X., Luo, X., Jiang, S., Xu, Z., 2012. Biodegradation of dissolved organic carbon in soil extracts and leachates from a temperate forest stand and its relationship to ultraviolet absorbance. *Chin. Sci. Bull.* 57, 912–920.
- Yao, X., Zhang, Y., Zhu, G., Qin, B., Feng, L., Cai, L., Gao, G., 2011. Resolving the variability of CDOM fluorescence to differentiate the sources and fate of DOM in Lake Taihu and its tributaries. *Chemosphere* 82 (2), 145–155.
- Zhang, R., Wu, F., He, Z., Zheng, J., Song, B., Jin, L., 2009. Phosphorus composition in sediments from seven different trophic lakes, China: phosphorus-31 NMR study. *J. Environ. Qual.* 38, 353–359.
- Zhu, Y., Wu, F., He, Z., Giesy, J.P., Feng, W., Mu, Y., Feng, C., Zhao, X., Liao, H., Tang, Z., 2015. Influence of natural organic matter on the bioavailability and preservation of organic phosphorus in lake sediments. *Chem. Geol.* 397, 51–60.
- Zhu, Y., Wu, F., He, Z., Guo, J., Qu, X., Xie, F., Giesy, J.P., Liao, H., Guo, F., 2013. Characterization of organic phosphorus in lake sediments by sequential fractionation and enzymatic hydrolysis. *Environ. Sci. Technol.* 47, 7679–7687.