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# Characterization of plant-derived carbon and phosphorus in lakes by sequential fractionation and NMR spectroscopy

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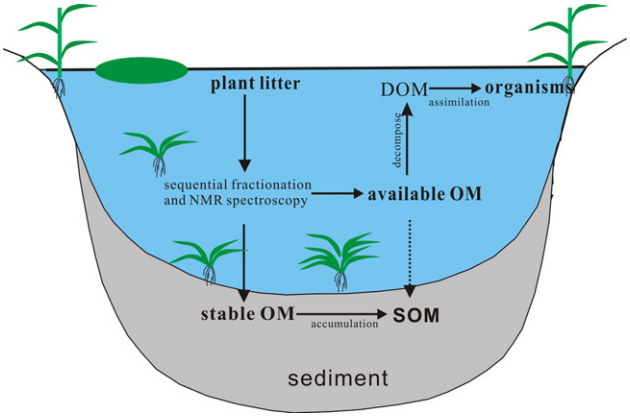
## HIGHLIGHTS

- Sequential fractionation combined with NMR analysis was applied on aquatic plants.
- Labile and stable C and P forms in aquatic plants were characterized.
- 54.7% of OM and 96.2% of P in aquatic plants are potentially available.
- 45.3% of OM and 3.8% of P in aquatic plants would be preserved in sediments.
- Debris of aquatic plants would be an important source for nutrients in lakes.

## GRAPHICAL ABSTRACT

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## ABSTRACT

Although debris from aquatic macrophytes is one of the most important endogenous sources of organic matter (OM) and nutrients in lakes, its biogeochemical cycling and contribution to internal load of nutrients in eutrophic lakes are still poorly understood. In this study, sequential fractionation by $H_2O$, 0.1 M NaOH and 1.0 M HCl, combined with $^{13}C$ and $^{31}P$ NMR spectroscopy, was developed and used to characterize organic carbon (C) and phosphorus (P) in six aquatic plants collected from Tai Lake (Ch: *Taihu*), China. Organic matter, determined by total organic carbon (TOC), was unequally distributed in $H_2O$ (21.2%), NaOH (29.9%), HCl (3.5%) and residual (45.3%) fractions. For P in debris of aquatic plants, 53.3% was extracted by $H_2O$, 31.9% by NaOH, and 11% by HCl, with 3.8% in residual fractions. Predominant OM components extracted by $H_2O$ and NaOH were carbohydrates, proteins and aliphatic acids. Inorganic P ($P_i$) was the primary form of P in $H_2O$ fractions, whereas organic P ($P_o$) was the primary form of P in NaOH fractions. The subsequent HCl fractions extracted fewer species of C and P. Some non-extractable carbohydrates, aromatics and metal phytate compounds remained in residual fractions. Based on sequential extraction and NMR analysis, it was proposed that those forms of C (54.7% of TOC) and P (96.2% of TP) in $H_2O$, NaOH and HCl fractions are potentially released to overlying water as labile components,

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while those in residues are stable and likely preserved in sediments of lakes. These results will be helpful in understanding internal loading of nutrients from debris of aquatic macrophytes and their recycling in lakes.



## 1. Introduction

Debris from aquatic macrophytes is one of the most important endogenous sources of organic matter (OM) and nutrients in lakes (Wu and Xing, 2009; Wu, 2009). In lakes, death and decomposition of aquatic macrophytes play an important role in biogeochemical cycling of nutrients including carbon (C), nitrogen (N) and phosphorus (P) (Kuehn and Suberkropp, 1998; Chinney and Pietro, 2006; Xie et al., 2004; Kuehn et al., 2011). Therefore, biogeochemical cycling of nutrients derived from aquatic plants is an important factor determining concentrations of nutrients in overlying waters of lakes as well as preservation of organic matter (OM) in sediments (Shilla et al., 2006; Zhu et al., 2015). Although not strictly defined, natural organic matter can be differentiated as labile organic matter with a half-life between days to years, stable organic matter with a half-life between years and decades, and inert (or refractory) organic matter with a half-life between decades and centuries (He and Wu, 2015). Whereas labilities of organic matter fractions can be evaluated by laborious microbial growth and incubation (Amon and Benner, 1994; Hunt et al., 2007), their relative labilities, especially the C and P components, could also been estimated by chemical and sized-based fractionations and relevant characterization (Cuss and Guéguen, 2015, He et al., 2015b). For example, *O*-alkyl characterized by solid $^{13}C$ NMR, which can be immediately released, hydrolyzed and then re-assimilated by organisms, was assigned to be labile functional groups. Whereas some refractory groups such as aromatic and alkyl C are less easily decomposed and released, and thus tend to accumulate in sediments for longer periods (Baumann et al., 2009; Kögel-Knabner, 2000; Liu et al., 2016). Similarly, liability of P in debris from plants biomass depends mainly on P association with OM. Phosphorus associated with chemically stable OMs can be precipitated and preserved in sediments of lakes (Hamdan et al., 2012; Liu et al., 2016; Zhu et al., 2015). Especially, interactions of phytate or phytate-like P with various metal ions to form insoluble compounds such as phytate salts, is one possible mechanism for preservation of P in sediments. Therefore, characterization of C and P derived from aquatic plants could be helpful to predict their stability and availability when aquatic plants in lakes die.

Water as an extractant has been widely used to extract organic matter (WEOM) derived from various plants (He et al., 2009a; Liu et al., 2016; Qu et al., 2013). WEOM includes several classes of labile OMs such as carbohydrates and carboxylic acid, and labile organic P such as monoesters and diesters (Liu et al., 2016; He et al., 2009a). Based on these previous studies (He et al., 2009a; Liu et al., 2016; Qu et al., 2013), the important role of plant-derived OM on the biogeochemical cycling of nutrients in lakes has been recognized. However, extraction efficiencies of C and P were relatively lower when extracted only by water. For example, there were only an average value of 21% and 53% for C and P, respectively, in the water-extractable fraction of plants from Tai Lake (Liu et al., 2016). Thus, the molecular composition and relative availability of the OM fraction that cannot be extracted by $H_2O$ was still unknown.

Recently, better efficiencies of extraction were obtained by use of a combination of NaOH and the chelator, ethylenediaminetetraacetic acid (EDTA) to extract P from plants (Cheesman et al., 2010; Noack et al., 2012). Extraction with NaOH-EDTA followed by characterization with $^{31}P$ NMR would significantly enhance knowledge of forms of P derived from plants. However, due to introduction of EDTA that contains functional groups of carbon, this extraction method could not be applied to the characterization and quantification of C in OMs derived from plants. Solutions containing NaOH could be preferentially applied to extract alkaline-soluble OMs due to fewer paramagnetic ions in plants. Whereas extraction by NaOH alone could not be used to provide good estimates of the mobility, solubility or availability of P derived from plants in environments such as lakes.

In a typical fractionation scheme, P in an environmental sample, such as, soil, manure or biosolids, was separated into water extractable labile P, 0.5 M $NaHCO_3$ extractable labile P, 0.1 M NaOH extractable P, 1.0 M HCl extractable P, and residual P (Hedley et al., 1982; Dou et al., 2000, 2002; Yu et al., 2006). This sequential fractionation can also be used in studies of P in sediment (Zhu et al., 2013). In addition to distribution of P Zhu et al. (2013) also determined organic carbon contents of the four fractions of sediments collected from Lake Dianchi, and found that distributions of organic carbon were similar to those of organic P ($P_o$) in the order of NaOH > $NaHCO_3$ > HCl > $H_2O$. Contents of hydrolysable $P_o$ in labile $H_2O$-$P_o$ and $NaHCO_3$-$P_o$ were significantly correlated with those of organic constituents (Zhu et al., 2015). However, there are no reports on application of sequential fractionation to investigate OM and P in plant biomass, which constitute organic-matter dominated matrices. Previously, He et al. (2003) made a comparative study of P from soil and manure by use of extraction with $H_2O$, 0.5 M $NaHCO_3$, 0.1 M NaOH, and 1.0 M HCl, which showed that inorganic mineral-based soil fractionation is appropriate to apply to organic residue-based manure. However, differences exist between manure and plants in terms of the amount and species of C and P since manure consists of products derived from digestion of plants. Thus, application of a sequential fractionation for P in plant biomass required further evaluation.

Solid $^{13}C$ NMR and solid/solution $^{31}P$ NMR can provide specific information on structures of C and P in OMs (Gressel et al., 1996; Möller et al., 2000). Carbon functional groups such as *O*-alkyl, aromatic-C, carboxyl, and methoxyl/*N*-alkyl in WEOM derived from plants could be characterized by the use of solid $^{13}C$ NMR (Qu et al., 2013; Liu et al., 2016). Forms of P including phosphonate, *ortho*-phosphate (*ortho*-P), monoester P, diester P, pyrophosphate, and polyphosphate in WEOM or NaOH-EDTA extracts derived from plants could be characterized by solution $^{31}P$ NMR spectroscopy (Noack et al., 2012, 2014; Cheesman et al., 2010; Feng et al., 2015; Liu et al., 2016). Solid $^{31}P$ NMR could detect P forms without breaking inherent state of samples regardless of its poor resolution, and solid $^{31}P$ NMR is a valuable tool for characterization of non-extracted P after sequential extraction (Hunger et al., 2004; He et al., 2009b). Thus, much more structural information on C and P could be obtained by use of sequential fractionation combined with NMR analysis.

In this study, a three-step fractionation procedure was developed to separate C (organic matter) and P of aquatic plants into four pools, which were then characterized by use of chemical analyses and $^{31}P$ and $^{13}C$ NMR spectroscopy (Fig. 1). The objectives of this study were to: (i) evaluate extractability of organic matter and P in aquatic plant biomass by sequential fractionation; (ii) characterize and compare compositions of organic matter and P in different sequential extracts; and (iii) discuss the lability of C and P derived from various plants and their roles in biogeochemical cycling of nutrients in lakes.

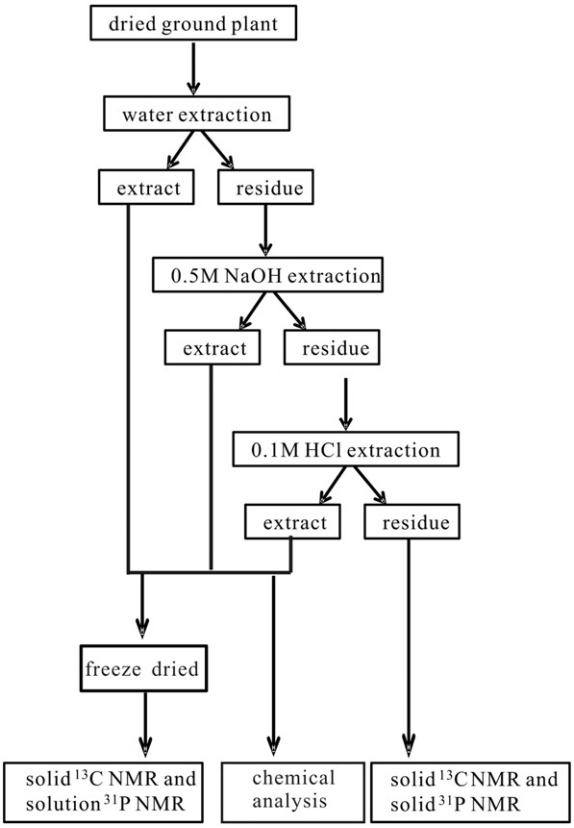


**Fig. 1.** Flow diagram of the sequential extraction and analysis procedure for chemical fractionation and NMR spectroscopy.

## 2. Materials and methods

### 2.1. Sample collection and treatment

Six living aquatic macrophyte species were collected from Tai Lake (China: *Taihu*), a large, eutrophic lake in Jiangsu Province, China. They were i) two emergent plants-water oats (*ZC*, *Zizania caducifloria* Turcz, Gramineae) and branch smartweed (*PD*, *Polygonum divaricatum* Linn), ii) two floating plants-water caltrop (*TB*, *Trapa bispinosa* Roxb.) and water poppy (*NP*, *Nymphoides peltata* (Gmel.) O. Kuntze), and iii) two submerged plants-water milfoil (*MV*, *Myriophyllum verticillatum* Linn.) and water thyme (*HV*, *Hydrilla verticillata* (Lf) Royle). Whole, fresh plants including stems, leaves and roots were washed with deionized water, killed in an oven (90 °C) for 15 min and then dried at 60 °C until a constant mass was obtained. After drying, plants were ground to pass through a 1-mm sieve and stored in an air-tight desiccator until analysis.

### 2.2. Sequential fractionation

OMs derived from aquatic plants were sequentially extracted by use of $H_2O$, 0.1 M NaOH and 1.0 M HCl. Briefly, six ground and dried samples (1.0 g) were extracted with 30 mL of water using an end-over-end shaker (250 rpm) at 22 °C for 18 h. The mixture was then centrifuged at 8000 *g* for 30 min at 4 °C and filtered through Whatman GF/C filter (47 mm in diameter, England). The supernatant was saved as $H_2O$ extracts. A similar extraction procedure was applied to the residual pellets for sequential fractionation with 30 mL of 0.1 M NaOH then 1.0 M HCl for 16 h each to separate the NaOH and HCl fractions (extracts) (Fig. 1). Then, 5 mL of these extracts were used to quantify total organic carbon (TOC), total P (TP) and organic P ($P_o$). The remaining extracts were freeze-dried for solid $^{13}$C NMR and solution $^{31}$P NMR analysis. Residues after sequential extraction were freeze-dried for solid $^{13}$C NMR and solid $^{31}$P NMR analyses. Loss of mass at each step was calculated by the difference between freeze-dried mass of samples before and after extraction by $H_2O$, NaOH and HCl.

### 2.3. Chemical analyses

Contents of C and N in plant biomass were determined by use of an elemental analyzer (Elemental vario macro EL, Germany). Concentrations of TP and inorganic P ($P_i$) in original samples (ground plants) were measured by use of the SMT method described by Ruban et al. (1999, 2001). Briefly, 0.2 g ground powders of aquatic plants were calcined at 550 °C for 3 h in a muffle furnace. After cooling, they were transferred to a centrifuge tube with 20 mL 3.5 M HCl. Then they were shaken for 16 h and centrifuged for 10 min. The supernatant was then used to analyze TP (Murphy and Riley, 1962). To determine concentrations of $P_i$, 0.2 g of samples were extracted with 20 mL 1 M HCl, for 16 h on an end-over-end shaker, then centrifuged for 20 min. Concentrations of $P_i$ in supernatant were determined directly (Murphy and Riley, 1962). Concentrations of $P_o$ were calculated as the difference between TP and $P_i$. Contents of C, N and P in original plants biomass are given in Table 1. Concentrations of TOC in extracts were determined by use of a TOC analyzer (multi N/C 3100, Analytic Jenam Germany). Concentrations of TP (after digestion by $K_2S_2O_8$) and $P_i$ in extracts were determined by use of molybdate colorimetry (Murphy and Riley, 1962), and $P_o$ was calculated by the difference. Total concentrations of Ca, Mg, Al, Fe and Mn in sequential fractions were measured by use of an inductively coupled plasma optical emission spectroscopy (ICP-OES) (Perkin-Elmer, Optima 7000DV at 214.97 nm) following digestion with concentrated $HNO_3$.

### 2.4. Solid $^{13}$C NMR analysis

Solid-state CP/MAS $^{13}$C NMR spectroscopy of ground plant biomass samples, their freeze-dried extracts and residues were acquired by use of a Bruker AV-300 spectrometer at 12.5 kHz and standard, 4-mm, double-bearing probe. Recycle delay time was set to 5 s, pulse width was 1.0 μs, and acquisition time was 0.0492 s. Number of scans acquired varied among samples with a mean number of scans of 2048. Spectra were divided into eight regions, according to chemical shifts reported previously (Cade-Menun and Paytan, 2010; Nelson and Baldock, 2005): I, 0–45 ppm (alkyl C); II, 45–60 ppm (*N*-alkyl and methoxyl-C); III, 60–95 ppm (*O*-alkyl C); IV, 95–110 (di-*O*-alkyl C); V, 110–145 ppm (aromatic C); VI, 145–165 ppm (aromatic *C*—O); VII, 165–190 ppm (carboxyl C/amide C); and VIII, 190–220 ppm (carbonyl C). Relative proportions of carbon groups were determined by integrating intensities of signals over defined chemical shift widows using MestReNova10.0.

### 2.5. Solution and solid $^{31}$P NMR analysis

Freeze-dried extracts (from 100 to 200 mg) were ground, re-dissolved in 1.0 mL 1 M NaOH-0.1 M EDTA by ultrasonic vibration until

**Table 1**
Chemical analysis of plant biomass.

| Aquatic plant | Common name | C (g $kg^{-1}$) | TP (g $kg^{-1}$) | $P_o$ (g $kg^{-1}$) | N (g $kg^{-1}$) | C:N[a] |
|---|---|---|---|---|---|---|
| *ZC* | Water oats | 406 | 2.0 | 0.7 | 21.6 | 22 |
| *PD* | Branch smartweed | 422 | 1.3 | 0.7 | 19.2 | 26 |
| *TB* | Water caltrop | 380 | 2.1 | 1.6 | 24.8 | 18 |
| *NP* | Water poppy | 443 | 2.7 | 1.5 | 27.5 | 19 |
| *MV* | Watermilfoil | 347 | 2.3 | 1.4 | 26.9 | 15 |
| *HV* | Water thyme | 335 | 3.5 | 1.4 | 30.5 | 13 |
| Average | | 389 | 2.3 | 1.2 | 25.1 | 19 |
| SD | | 42.5 | 0.8 | 0.4 | 4.1 | 5 |

[a] Expressed as molar ratio.

completely dissolved, and centrifuged at 8000 *g* for 30 min. Then, 0.5 mL supernatant added with 0.1 mL $D_2O$ was transferred to a 5-mm NMR tube. Solution $^{31}P$ NMR spectra were acquired at 161.98 MHz by use of a Bruker 400 MHz spectrometer equipped with a 5-mm BBO probe. The pulse width was 15 μs, with a pulse delay of 4.32 s, and acquisition time of 0.2102 s. Between 10,000 and 20,000 scans were acquired per sample at 20 °C. Processing of spectra and integrations were carried out by use of MestReNova10.0 software. Assignment of peaks was performed based on results of spiking experiments and data reported in the literature (Cade-Menun et al., 2015; Giles et al., 2015).

Freeze-dried residues remaining after sequential fractionations were analyzed further by use of solid-state $^{31}P$ MAS NMR (Hunger et al., 2004; He et al., 2009b). Briefly, spectra were collected on a Bruker AV300 spectrometer operating at 121.49 MHz using a standard 4 mm double-bearing probe. The relaxation delay time was 5.0 s with a pulse width of 0.8 μs, and an acquisition time of 0.0421 s. The MAS speed was 7.5 kHz, and between 7000 and 20,000 scans were collected.

### 2.6. Statistical analyses 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 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.

## 3. Results

### 3.1. Distribution of C, P and selective metals in sequential fractions

Losses of masses and amounts of C, P varied among the four fractions derived from aquatic plants (Fig. 2). Mean loss of mass of six aquatic macrophytes after sequential extraction was 37.3% with values of 17.9% by $H_2O$, 12.8% by NaOH, and 6.6% by HCl, respectively (Fig. 2). Loss of mass of *NP* was greatest among these aquatic plants. In these aquatic plants, 21.2% (mean) of TOC was extracted by $H_2O$, 29.9% by NaOH, and only 3.5% by HCl, while 45.4% was left in residues after extraction (Fig. 2). The greatest proportion (81.9%) of TOC extracted by $H_2O$, NaOH and HCl was from *NP*. For TP content, 53.3% was extracted by $H_2O$, 31.9% by NaOH, and 11% by HCl, while 3.8% was un-extractable by the sequential fractionation. For $P_i$, 95.5% was extracted by $H_2O$, while only 0.4% by NaOH, 0.6% by HCl, and 3.5% remained in residues. Organic phosphorus extracted sequentially decreased in the following order: NaOH-$P_o$ (61.9%) > HCl-$P_o$ (20.9%) > $H_2O$-$P_o$ (13.7%) > Residual-$P_o$ (9.0%). The molar ratio of C:P, which ranged among plants from 251 to 786 was greatest in the NaOH fraction (Fig. 3). Molar ratios of C:P in emergent macrophytes (*ZC* and *PD*) in $H_2O$ fractions were less than those in HCl fractions, while the order of ratios in floating and submerged plants (*TB*, *NP*, *MV*, and *HV*) were reverses. Also, molar ratios of C:P in submerged plants were least in $H_2O$, NaOH and HCl fractions.

Concentrations of selective metals (Al, Ca, Fe, Mg, Mn) derived from aquatic plants were less than those of C and P in $H_2O$ and NaOH fractions, but not in HCl fractions (Table 2, Fig. 2). Ca and Mg were the predominant metals in the sequential fractions, followed by Fe, Mn and Al. Concentrations of Mg, Ca and Mn in HCl fractions were greater than those in $H_2O$ fractions, and least in NaOH fractions. Greatest concentrations of Fe and Al were observed in HCl fractions and the least were observed in $H_2O$ fractions.

### 3.2. Solid $^{13}C$ NMR spectroscopy of freeze-dried sequentially extracted fractions and the residues

Distributions of C-containing functional groups in sequentially extracted fractions and residues varied among aquatic macrophytes (Table 3, Figs. 4 and 5). In $H_2O$ fractions, the relative abundance of total *O*-alkyl was 54.4% (50.8 mg $g^{-1}$), followed by the proportion of alkyl-C (16.4%, 12.4 mg $g^{-1}$) and COO/N—C═O (15.5%, 11.6 mg $g^{-1}$). Only a small proportion (<10%) of other C-containing functional groups including NCH/$OCH_3$ at 45–65 ppm and aromatic-C at 110–165 ppm were observed (Liu et al., 2016). Proportions of alkyl-C, NCH/$OCH_3$,

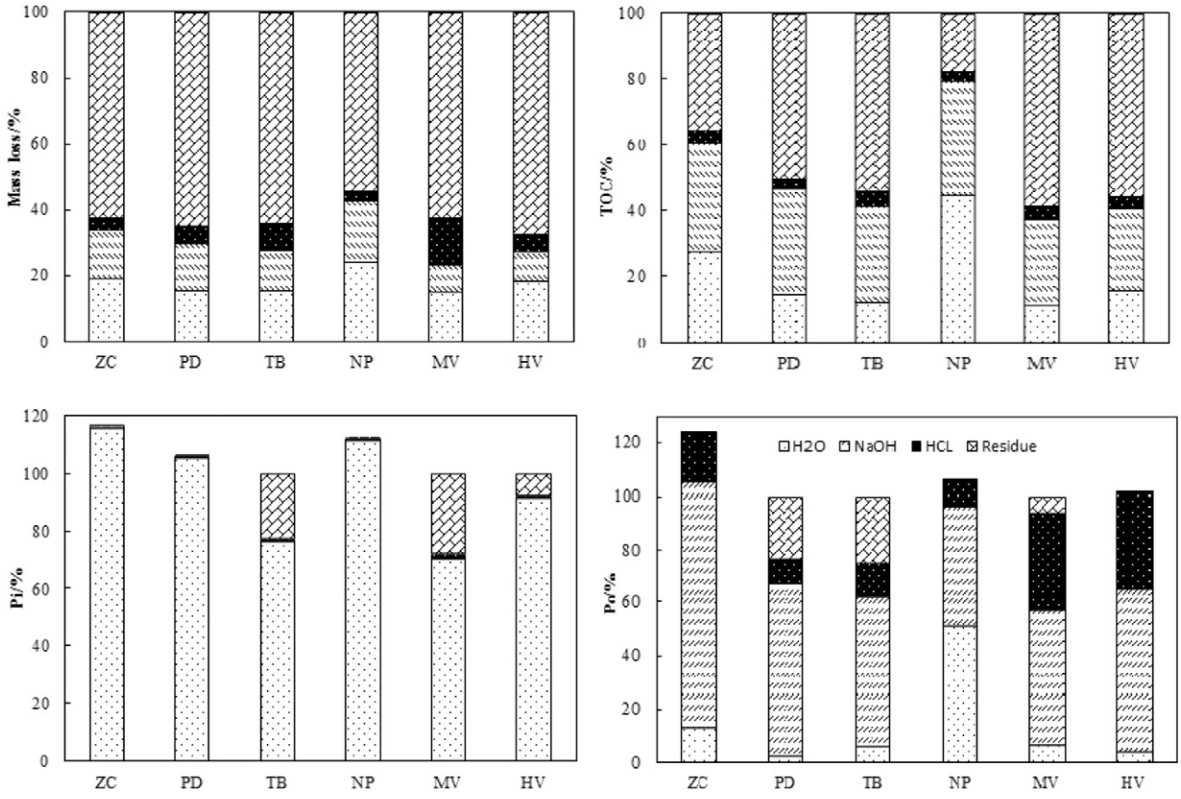


**Fig. 2.** Mass loss and distribution of C and P in squential fractions and residues.

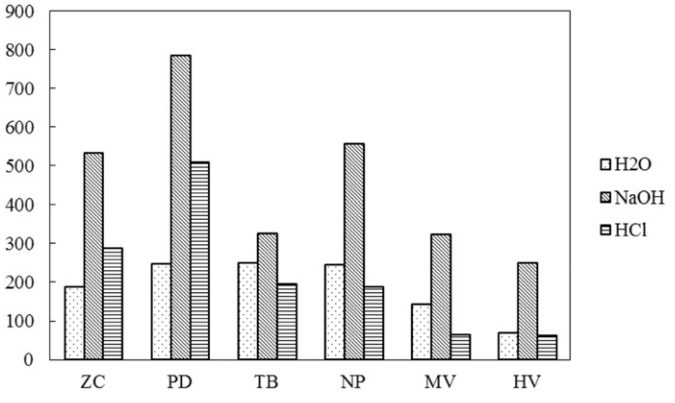


**Fig. 3.** Molar ratios of C:P in $H_2O$, NaOH and HCl fractions.

Ar—C and COO/N—C═O in NaOH fractions were greater than those in $H_2O$ fractions while proportions of *O*-alkyl and O—C—O were less. A small proportion of carbonyl was detected in NaOH fractions. Total *O*-alkyl (33.2%, 40.3 mg $g^{-1}$) was also the predominant groups extracted from OMs by NaOH, followed by alkyl-C (22.3%, 25.1 mg $g^{-1}$) and COO/*N*—C═O (20.1%, 23.1 mg $g^{-1}$). Proportions of NCH/$OCH_3$ and Ar—C were 7.8% (9.3 mg $g^{-1}$) and 13.8% (16.8 mg $g^{-1}$), respectively. Molecular composition was less aliphatic, more aromatic and less polar in NaOH fractions compared with that in $H_2O$ fractions. Also, only one detectable sharp peak at 168 ppm was observed in HCl fractions, which could be assigned to the region of COO/N—C═O.

Functional groups containing carbon in sequential fractions were all detected in the residues after sequential extraction (Fig. 4, Table 3). Proportions of $OCH_3$/NCH (13.6%, 22.8 mg $g^{-1}$) and total *O*-alkyl (57.2%, 99.2 mg $g^{-1}$) in residues were greater than those in sequential fractions, while proportions of Ar—C (8.3%, 14.0 mg $g^{-1}$) and COO/N—C═O (7.5%, 12.9 mg $g^{-1}$) were less in residues. Thus, molecular composition was more aliphatic, less aromatic and more polar in residues.

### 3.3. Solution $^{31}P$ NMR spectroscopy of sequential fractions

Based on solution $^{31}P$ NMR spectra, concentrations of various forms of P in sequential fractions varied among the six aquatic plants (Fig. 6 and Table 4). *Ortho*-phosphate (*ortho*-P) was the predominant form of P in $H_2O$ fractions; concentrations ranged from 0.39 to 1.55 g $kg^{-1}$, and accounted for 59.1% to 90.4% of TP in $H_2O$ fractions. Concentrations of monoesters P (mono-P) ranged from 0.07 to 0.85 g $kg^{-1}$, and accounted for 9.6% to 40.9% of TP in $H_2O$ fractions. Trace amounts of diester P(<2%) were detected in $H_2O$ fractions (Liu et al., 2016). In NaOH fractions, concentractions of *ortho*-P ranged from 0.09 to 0.33 g $kg^{-1}$, and accounted for 10.2% to 50.6% of TP in NaOH fractions, which was less than that in $H_2O$ fractions. Reversely, mono-P was the predominant form of P in NaOH fractions, with proportions ranging from 39.7% to 77.2% (0.26–0.68 g $kg^{-1}$). There were trace amounts of diesters observed in NaOH fractions. In subsequent HCl fractions, still trace amounts of *ortho*-P and mono-P were detected. Concentrations of *ortho*-P and mono-P in HCl fractions varied greatly among species of macrophytes. Maximum contents (0.37 g $kg^{-1}$) of *ortho*-P in HCl fractions were observed in *HV*, and maximum contents (0.55 g $kg^{-1}$) of mono-P were observed in *MV*. There were no deteactable diesters in HCl fractions. Additionally, condensed P such as pyrophophate and polyphosphate was not detected in all these extracts.

Monoesters P were the predominant form of $P_o$ in sequential fractions, however, the detailed composition of mono-P identified from $^{31}P$ NMR spectra varied among $H_2O$, NaOH and HCl fractions (Table 4). Two peaks at 4.31 ppm and 3.97 ppm in $H_2O$ extracts, 4.22 ppm and 3.88 ppm in NaOH extracts, 4.27 ppm and 3.93 ppm in HCl extracts were respectively assigned to the hydrolysis products of phospholipids including α- and β-glycerophosphate. A series of minor peaks at 3.8–3.4 ppm in three sequential fractions were identified as hydrolysis products of RNA. Peaks at 4.85, 3.95, 3.58 and 3.48 ppm in $H_2O$ fractions, and 4.91, 4.0, 3.64, and 3.52 ppm in HCl fractions from floating macrophytes of *NP* were assigned to phytate. Other unassigned peaks in the mono-P region of $^{31}P$ NMR spectra in $H_2O$ frations were defined as "other monoesters". Products of hydrolysis of phospholipids and RNA were detected in all the three sequential fractions from these six plants. Generally, concentrations of $P_o$ forms in NaOH extracts were greater than those in $H_2O$ and HCl fractions. Mean concentrations of α- and β-glycerophosphate were 32.3 and 49.2 mg $kg^{-1}$ in $H_2O$ fractions, 76.8 and 192.1 mg $kg^{-1}$ in NaOH fractions, and 38.0 and 117.8 mg $kg^{-1}$ in HCl fractions, respectively. Concentrations of mononucleotide in $H_2O$, NaOH and HCl fractions were the average value of 6.2, 182.1 and 18.2 mg $kg^{-1}$, respectively. Phytate was only detected in $H_2O$ and HCl fractions from *NP*, at concentrations of 644.8 and 86.4 mg $kg^{-1}$, respectively.

### 3.4. Solid $^{31}P$ NMR spectroscopy of the residues after sequential fractionation

In the major chemical shift region from −20 to 20 ppm, a broad peak centered at −0.51 to −2.51 ppm with several minor peaks was detected in solid $^{31}P$ NMR spectra of residues remaining after sequential fractionation (Fig. 7). Five peaks were detected in the residue of *MV*, whereas only one peak was found in floating macrophytes *TB* and *NP*. One sharp peak with a chemical shift of −1.25 ppm to −0.51 ppm was present in all residues except *NP*. Another sharp peak of 5.5 ppm to 6.6 ppm was found in residues of two emergent macrophytes (*ZC* and *PD*). Peaks between 3.0 ppm to 4.0 ppm were found in residues of *ZC* and *MV*. Residues of *NP* and *MV* had one special peak at −2.35 ppm. One broad peak between −4.2 ppm and −5.7 ppm was found in residues of submerged macrophytes (*MV* and *HV*).

**Table 2**
Concentrations (mg $kg^{-1}$ dry matter) of selected metals in sequentially extracted fractions.

| Aquatic plant | Fraction | Al | Ca | Fe | Mg | Mn |
|---|---|---|---|---|---|---|
| *ZC* | $H_2O$ | 13 | 20 | 68 | 718 | 67 |
| | NaOH | 2 | 39 | 4 | 18 | 2 |
| | HCl | 197 | 3020 | 292 | 496 | 67 |
| *PD* | $H_2O$ | 36 | 33 | 64 | 1065 | 131 |
| | NaOH | 44 | 63 | 160 | 185 | 34 |
| | HCl | 298 | 11,172 | 821 | 1457 | 299 |
| *TB* | $H_2O$ | 3 | 1693 | 6 | 1822 | 20 |
| | NaOH | 55 | 296 | 235 | 374 | 55 |
| | HCl | 510 | 27,820 | 995 | 2635 | 360 |
| *NP* | $H_2O$ | 19 | 130 | 127 | 820 | 30 |
| | NaOH | 22 | 63 | 72 | 512 | 21 |
| | HCl | 170 | 3958 | 220 | 652 | 46 |
| *MV* | $H_2O$ | 1 | 2097 | 1 | 854 | 29 |
| | NaOH | 44 | 639 | 146 | 83 | 38 |
| | HCl | 459 | 68,105 | 1249 | 1798 | 416 |
| *HV* | $H_2O$ | 22 | 114 | 71 | 679 | 175 |
| | NaOH | 41 | 296 | 89 | 35 | 59 |
| | HCl | 549 | 13,227 | 2105 | 2419 | 1186 |
| Mean ± SD[a] | $H_2O$ | 15 ± 13 | 681 ± 949 | 56 ± 47 | 993 ± 428 | 75 ± 64 |
| | NaOH | 35 ± 19 | 233 ± 231 | 118 ± 80 | 201 ± 201 | 35 ± 21 |
| | HCl | 364 ± 164 | 21,217 ± 24,640 | 947 ± 694 | 1576 ± 884 | 396 ± 416 |

[a] SD, standard deviation.

**Table 3**
Percentage of C functional groups in original materials, three extracts and the residues (mean proportions calculated based on the data from six aquatic macrophytes).

| C-type | Alkyl-C | $OCH_3$/NCH | *O*-alkyl C | O—C—O | Aromatic—C | Aromatic C—O | COO/N—C=O | Carbonyl | Al—C[a] | Ar—C[b] | Polar-C[c] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| $H_2O$ | 16.4 ± 3.4 | 4.2 ± 1.0 | 45.8 ± 8.7 | 8.6 ± 2.5 | 6.2 ± 2.3 | 3.4 ± 1.7 | 15.5 ± 4.0 | nd | 75.0 ± 6.7 | 9.5 ± 3.6 | 77.5 ± 5.0 |
| NaOH | 22.3 ± 6.6 | 7.8 ± 1.5 | 27.2 ± 4.4 | 6.0 ± 2.1 | 9.9 ± 3.0 | 3.9 ± 2.4 | 20.1 ± 3.1 | 2.8 ± 2.1 | 63.4 ± 2.4 | 13.7 ± 5.0 | 67.9 ± 5.7 |
| HCl | nd | nd | nd | nd | nd | nd | 100 | nd | nd | – | – |
| residue | 13.5 ± 4.1 | 13.6 ± 2.2 | 45.2 ± 3.4 | 12.0 ± 1.8 | 5.7 ± 2.5 | 2.6 ± 2.2 | 7.5 ± 2.7 | nd | 84.1 ± 3.3 | 8.4 ± 4.7 | 80.8 ± 2.9 |

*nd, not detected.
[a] Al—C (total aliphatic C): 0–110 ppm.
[b] Ar—C (total aromatic C): 110–165 ppm.
[c] Polar-C: 50–110 ppm + 145–220 ppm.

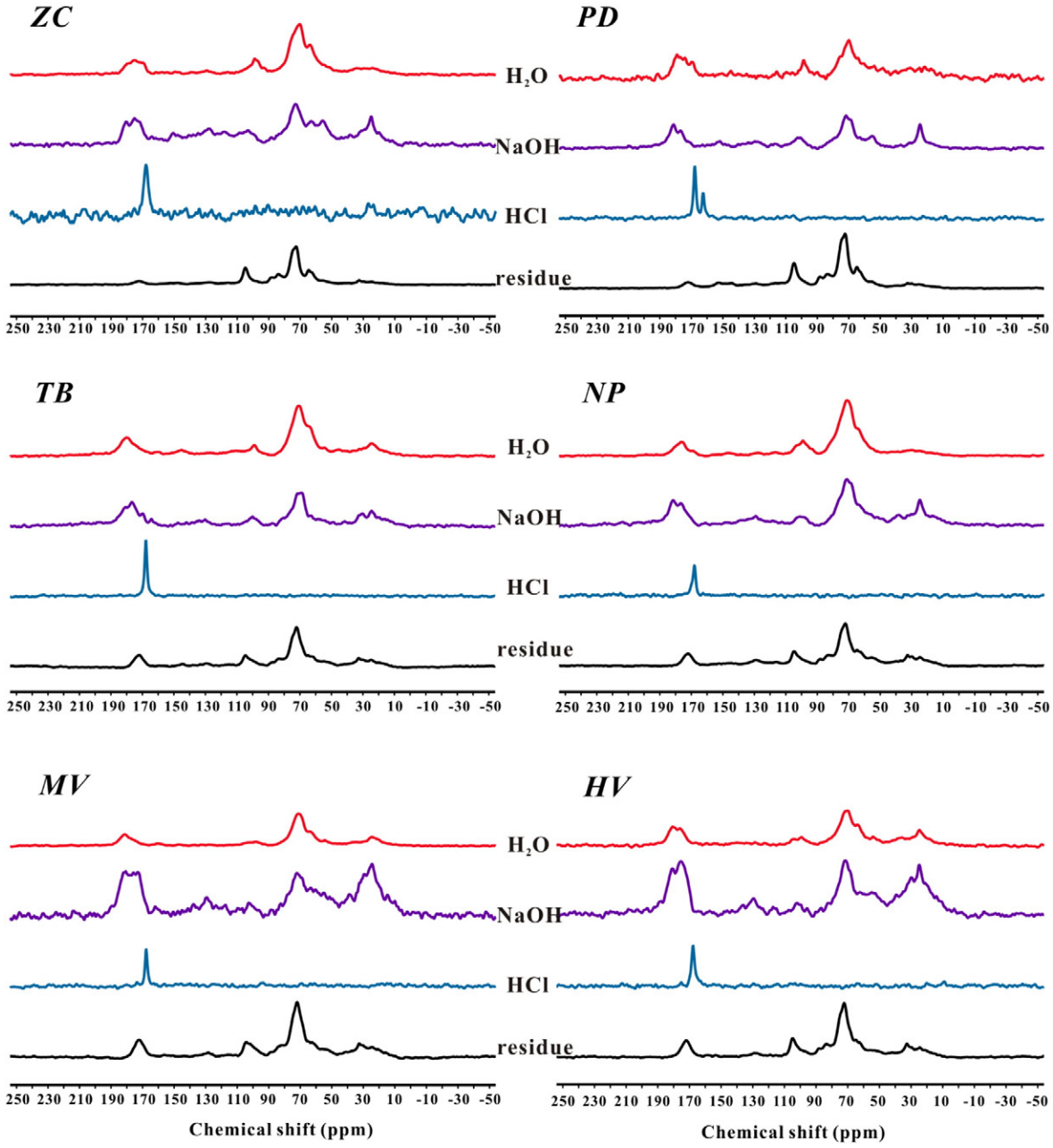


**Fig. 4.** Solid $^{13}C$ NMR spectra of three sequentially extracted fractions by $H_2O$, NaOH and HCl, and residues after extraction in six aquatic plants.

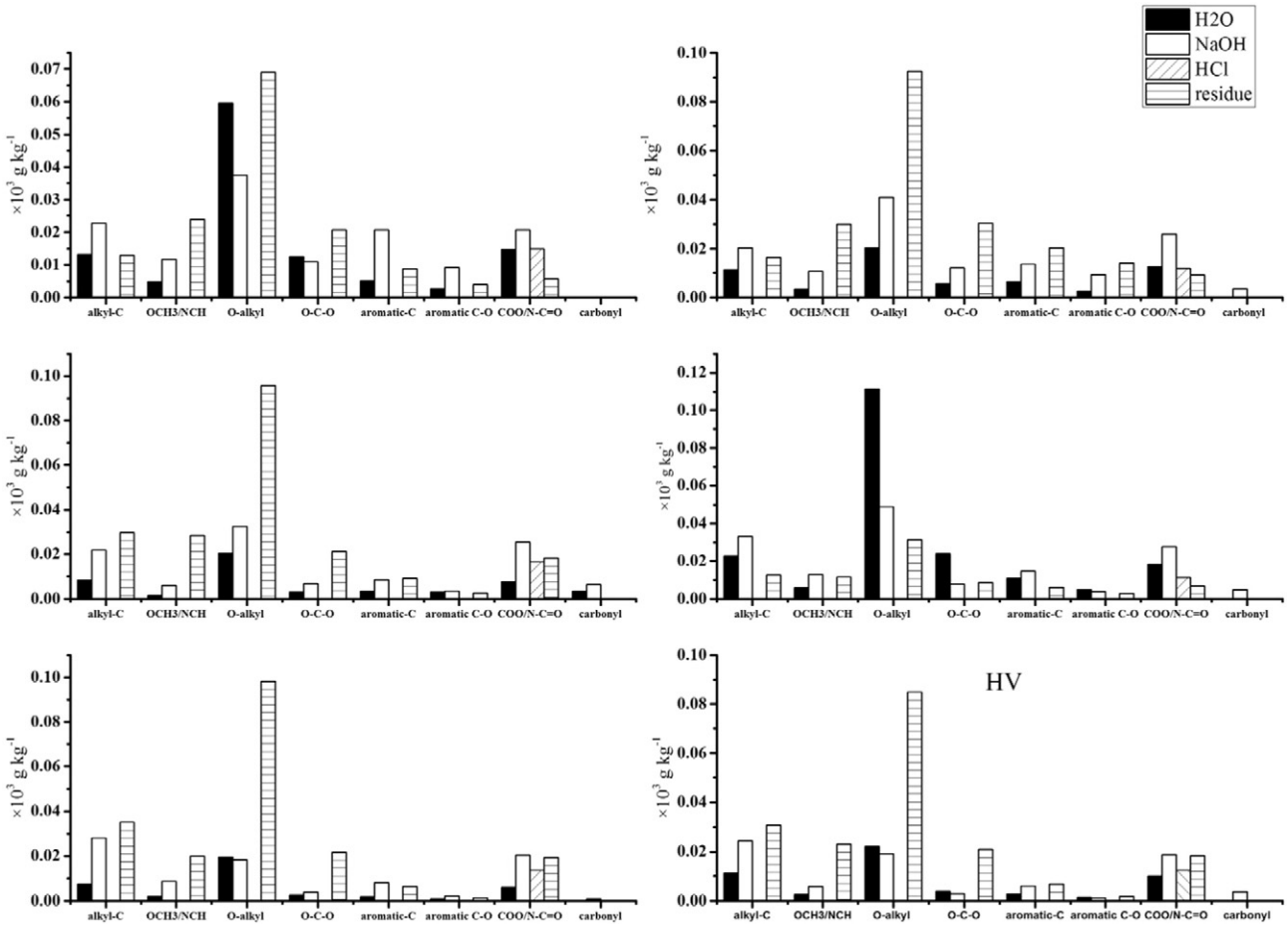


**Fig. 5.** Distributions of C functional groups of three sequentially extracted fractions by $H_2O$, NaOH and HCl, and residues after extraction in six aquatic plants. Concentations of carbon are normalized for mass of plants.

## 4. Discussion

### *4.1. Properties of sequential fractions and residues derived from aquatic plants*

Efficiencies of extraction of C and P in the proposed sequential fractionation was greater than that extracted by water alone, which indicated that the sequential fractionation would be favorable in the characterization of OMs derived from plants. Approached 54.7% of the TOC in the original materials could be extracted by sequential fractionation, which indicated that half of OMs derived from aquatic plants were potentially available. Almost all $P_i$ can be dissolved or released by water extraction, which indicated that much of $P_i$ derived from aquatic plants are soluble and readily released into the overlying water. Thus, debris from aquatic plants was likely an important internal load of P in lakes. In alkaline conditions, $P_o$ derived from the debris of aquatic plants would be released into overlying water, such as large of $P_o$ compounds could be mainly extracted by NaOH extractant. These $P_o$ compounds might be hydrolyzed in biotic processes such as enzymatic hydrolysis, or abiotic processes, and then the released phosphate would be available for organisms (Quiquampoix et al., 2005; Turner et al., 2002; Zhu et al., 2015). Trace amounts of $P_o$ or $P_i$ detected in HCl fractions were likely attributed to the hydrolysis of P bound with acid-soluble OMs or metals in the debris of aquatic plants.

Based on the distribution of P in sequential fractions derived from plants (Fig. 2), forms of P in plants were similar to that in manure (He et al., 2009b) (Table 5). Debris from aquatic plants and manures from grass-feed cattle were both organic residue-based environmental samples. They contain considerable amounts of soluble chemicals including OMs, P compounds, and metals, thus a large proportion of C and P could be extracted into $H_2O$ fractions. However, results of sequential fractionation of aquatic plants were different with those of soils or sediments (Table 5). Thus, the mechanisms of sequential fractionation in organic residue-based materials were likely different with those of mineral-based materials.

Greater concentrations of Mg and Ca extracted by water implied metals in plants exist as ion pairs or complexes that are easily released. The distribution patterns of Ca, Mg and Mn were similar in the order NaOH < $H_2O$ < HCl, while Fe and Al in the order $H_2O$ < NaOH < HCl. This order differed from the distribution patterns of C and P components which were generally lower in HCl fractions than that in NaOH fractions. The observation implied that these metals were mainly present in either the soluble forms or associated with acid-soluble organic matter but not necessarily associated with P. Additionally, the distribution of metals among fractions was inconsistent with results from soils and sediments, but was consistent with that observed in manures (Table 5). This result indicated that it might not be appropriate to apply soil based fractionation interpretations to aquatic plants and manures, which assign NaOH-extractable P to Al- and Fe-bound P, and HCl-extractable P to Ca-bound P. The results may be involved in structural decomposition of aquatic plants in alkaline or acid extractants. Therefore, interpretation of the application of sequential fractionation on matrixes should be done with caution due to their special physico-chemical properties.

### *4.2. Characterization of C and P in sequential fractions and residues*

Dissolution of C functional groups by sequential fractionation could be important for understanding degradation of plant-derived OMs in

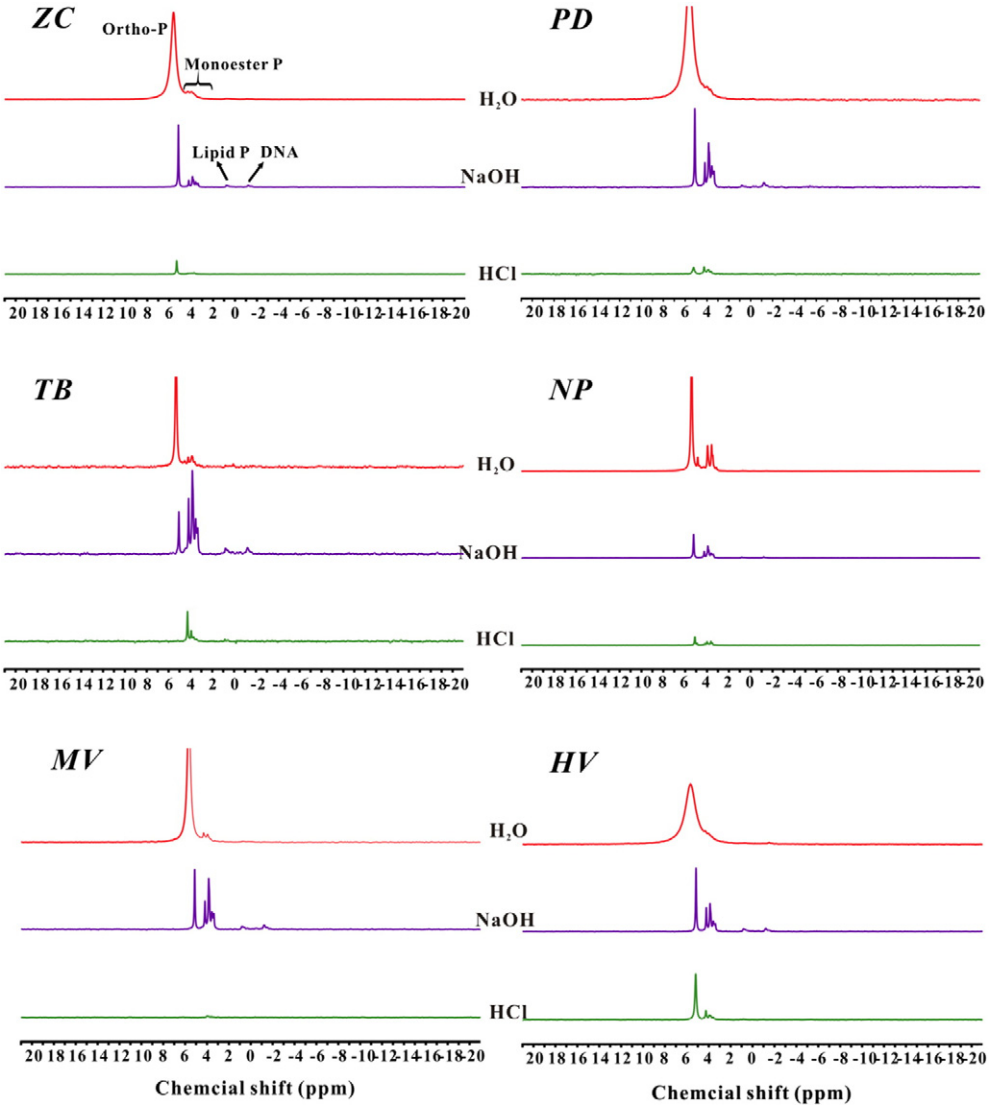


**Fig. 6.** Solution $^{31}P$ NMR spectra of three sequentially extracted fractions by $H_2O$, NaOH and HCl from six aquatic plants in Tai Lake.

lakes (He et al., 2015a; Liu et al., 2016). Large amounts of *O*-alkyl compounds were indicative of polysaccharides extracted by $H_2O$, which implied that polysaccharides were easily soluble OM in debris from aquatic plants. Co-existence of alkyl-C and COO/N—C═O in $H_2O$

**Table 4**
Compositions of P (mg $kg^{-1}$ dry matter) in sequential fractions from aquatic plants of Tai Lake.

| | | | | Mono-P | | | | | | Diester P | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Aquatic plants | Extracts | TP | Ortho-P | Phytate | α-Gly | β-Gly | Mononucleotide | Other mono-P | Total mono-P | Phospholipid | DNA | Total diester P |
| *ZC* | $H_2O$ | 1551 | 1242 | nd | 91.8 | 192.5 | nd | nd | 284.2 | 5.7 | 19.1 | 24.8 |
| | NaOH | 644.6 | 326.2 | nd | 39.6 | 63.8 | 152.4 | nd | 255.7 | 38.3 | 24.4 | 62.7 |
| | HCl | 133.5 | 75.7 | nd | 8.8 | 26.9 | 22.2 | nd | 57.9 | nd | nd | nd |
| *PD* | $H_2O$ | 648.6 | 566.0 | nd | 22.0 | 28.1 | 32.5 | nd | 82.6 | nd | nd | nd |
| | NaOH | 447.2 | 121.2 | nd | 38.6 | 127.9 | 135.1 | nd | 301.6 | 3.9 | 20.5 | 24.4 |
| | HCl | 59.9 | 0.3 | nd | 26.2 | 19.5 | 13.9 | nd | 59.6 | nd | nd | nd |
| *TB* | $H_2O$ | 487.9 | 387.0 | nd | 17.8 | 40.8 | nd | 34.3 | 93.0 | 8.0 | nd | 8.0 |
| | NaOH | 880.7 | 90 | nd | 124.6 | 312.6 | 243.1 | nd | 680.3 | 55.3 | 55.1 | 110.4 |
| | HCl | 219.5 | nd | nd | 125.8 | 55.5 | 38.2 | nd | 219.5 | nd | nd | nd |
| *NP* | $H_2O$ | 2083 | 1230 | 644.8 | 31.5 | nd | nd | 176.4 | 852.7 | nd | nd | nd |
| | NaOH | 713.3 | 227.1 | nd | 63.7 | 210.2 | 181.8 | nd | 455.7 | 9.3 | 21.2 | 30.5 |
| | HCl | 154.3 | 55.9 | 86.4 | 12 | nd | nd | nd | 98.4 | nd | nd | nd |
| *MV* | $H_2O$ | 721.1 | 651.6 | nd | 31.1 | 34.0 | 4.5 | nd | 69.5 | nd | nd | nd |
| | NaOH | 721.7 | 168.7 | nd | 82.4 | 232 | 191.1 | nd | 505.4 | 12.6 | 34.9 | 47.5 |
| | HCl | 554.3 | nd | nd | nd | 554.3 | nd | nd | 554.3 | nd | nd | nd |
| *HV* | $H_2O$ | 2033 | 1552 | nd | nd | nd | nd | 467.7 | 467.7 | nd | 13.7 | 13.7 |
| | NaOH | 843.8 | 281.6 | nd | 111.9 | 206.4 | 189 | nd | 507.2 | 19.7 | 35.3 | 55 |
| | HCl | 511.1 | 370.3 | nd | 55.5 | 50.7 | 34.7 | nd | 140.9 | nd | nd | nd |

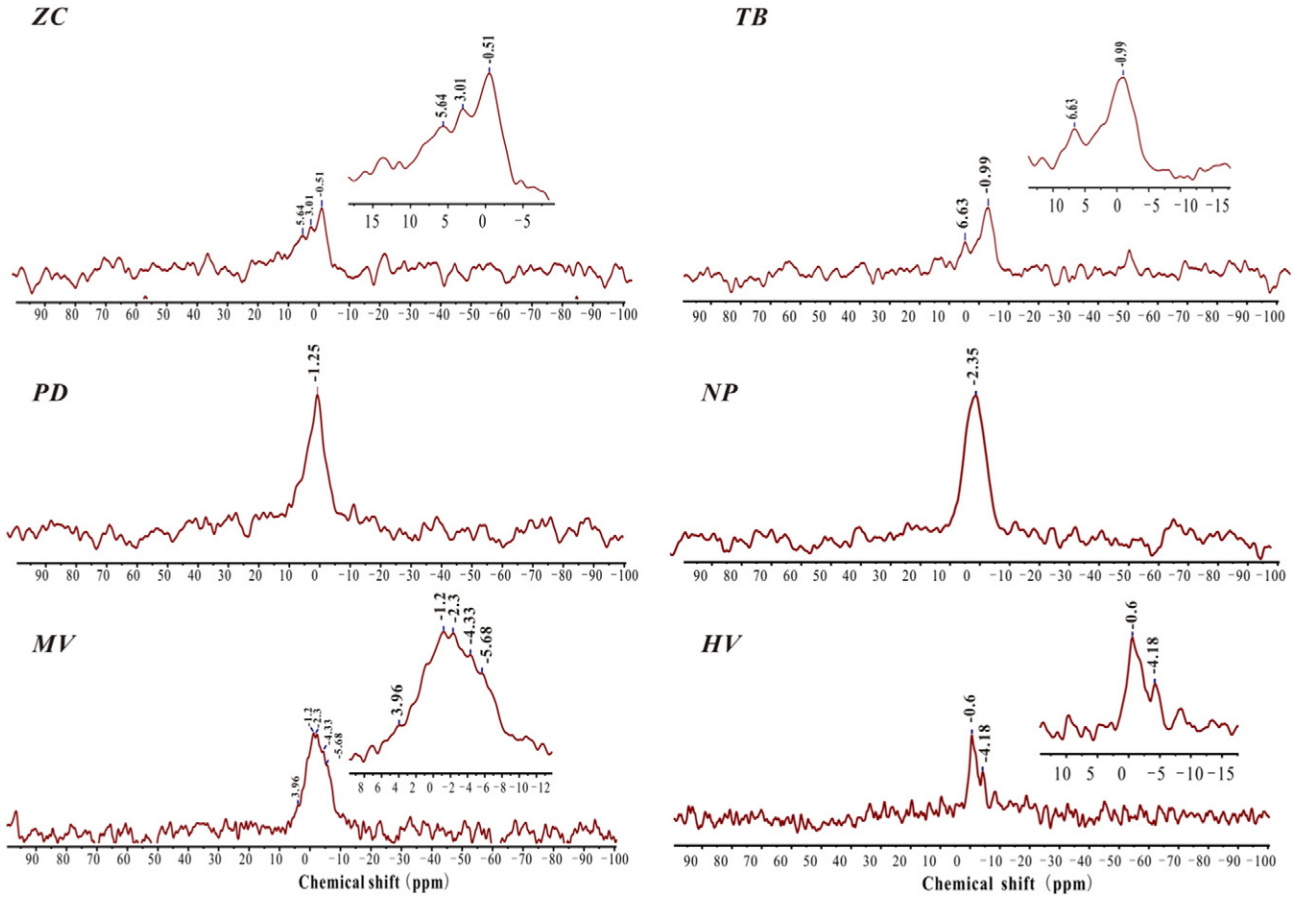


**Fig. 7.** Solid $^{31}$P MAS NMR spectrum of residues from six aquatic macrophytes after sequential extraction by $H_2O$, NaOH, and HCl.

fractions implied that moderate amounts of carboxylic acids of lesser molecular weight or amino acids/proteins could be dissolved by $H_2O$. The small proportion of aromatic-C in $H_2O$ fractions was likely due to the decomposition of phenolic compounds of lesser molecular masses. The greater proportions and concentrations of alkyl-C, $OCH_3$/N—C═O, Ar—C and COO/N—C═O may indicate more lignins, carboxylic acids, or proteins were extracted by NaOH than $H_2O$. The greater concentrations of *O*-alkyl in NaOH fractions were likely attributed to the dissolution of hemicellulose (Sjöström, 1993). Only COO/N—C═O group was detected in HCl fractions, which suggested that a large amount of polypeptides/amino acid dissolved in acid solutions rather than alkaline solutions. Types of C functional groups in the residues after sequential extraction were similar to those in $H_2O$ fractions, which implied that only parts of C functional groups decomposed or released easily. Some non-extracted C functional groups such as *O*-alkyl that considered being readily dissoluble might combine with other

**Table 5**
Contents (mg $kg^{-1}$ dry matter) of P and selected metals in soil, sediment and manure.

| | TP | $P_i$ | $P_o$ | Ca | Mg | Al | Fe | Mn |
|---|---|---|---|---|---|---|---|---|
| Soil[a] | | | | | | | | |
| $H_2O$ | nd | | | | | | | |
| $NaHCO_3$ | 118.4 | 2.56 | nd | 84 | 11.52 | nd | 0.62 | 1.98 |
| NaOH | 601.6 | 83.2 | 35.2 | 76 | 12.72 | 35.1 | 0.66 | 2.365 |
| HCl | 172.8 | 486.4 | 115.2 | 120 | 17.76 | 3456 | 5.8 | 18.7 |
| Residual | 163.2 | 137.6 | 35.2 | 660 | 508.8 | 3024 | 171 | 533.5 |
| Sediment[b] | | 0 | 0 | 552 | 4440 | 10,557 | 280 | 4493.5 |
| $H_2O$ | 2.1–5.3 | | | | | | | |
| $NaHCO_3$ | 67.5–254.6 | 0.01–1.42 | 1.4–5.1 | | | | | |
| NaOH | 289.4–856.2 | 50.9–213.4 | 16.6–45.8 | | | | | |
| HCl | 571.8–1093.2 | 232.2–635.7 | 45.8–220.4 | | | | | |
| Residue | 391.1–661.7 | 574.0–1067.5 | 0–25.7 | | | | | |
| Manure[b] | | | | | | | | |
| $H_2O$ | 1792 | | | | | | | |
| $NaHCO_3$ | 726.4 | 1273.6 | 518.4 | 2252 | 1036.8 | nd | 8.1 | 13.2 |
| NaOH | 694.4 | 489.6 | 236.8 | 1832 | 405.6 | nd | 1 | 9.9 |
| HCl | 464 | 275.2 | 419.2 | 232 | nd | 237.6 | 0.63 | 5.5 |
| Residual | 118.4 | 358.4 | 105.6 | 5720 | 189.6 | 81 | 71 | 48.4 |

"nd" = less than detection limit.
[a] Refer to He et al., 2003.
[b] Refer to Zhu et al., 2013.

insoluble complex OMs, such as condensed tannin or lignin, derived from aquatic plants (Kögel-Knabner, 2002; Webster and Benfield, 1986). For example, the structural polysaccharides—cellulose and hemicelluloses of plant cell walls are physically and chemically bound to lignin in the form of ligno–cellulose complex or lignin–polysaccharide complex, a resistant complex that renders cellulose and hemicelluloses less accessible (Fengel and Wegener, 1984; Sjöström, 1993).

Forms of P observed in sequential fractions derived from aquatic plants were consistent with P extracted from plant materials by water (He et al., 2009a) and NaOH-EDTA (Noack et al., 2012) derived from plant materials. In the $H_2O$ fractions of aquatic plants, *ortho*-P was the predominant form of P, followed by a small proportion of monoesters and diesters. This result was consistent with the results of $H_2O$ fractions derived from debris of crop by Noack et al. (2012, 2014) and He et al. (2009a). The fact that contents and proportions of *ortho*-P were much less in NaOH fractions, while proportions of mono-P were greater, suggested that some P, especially $P_o$ derived from aquatic plants were likely combined with metal or alkali-soluble OMs that could not be extracted by $H_2O$. In subsequent, trace amounts of *ortho*-P and mono-P were also extracted by HCl. *Ortho*-P in HCl fractions was also likely due to hydrolysis of $P_o$ and other condensed $P_i$ in acidic conditions, which was widely supported by previous studies (Turner et al., 2006; Noack et al., 2014). Some *ortho*-P incorporated into acid-soluble OMs to form recalcitrant complexes was likely dissolved by more acidic solutions of HCl (Zhu et al., 2013, 2015).

After sequential fractionation by $H_2O$, NaOH, and HCl, several weak distinguishable peaks appeared in solid $^{31}P$ NMR spectra of the residues after sequential fractionation, which indicated that some forms of P could not be extracted by the sequential extraction. Although it was difficult to identify specific forms of P by use of solid $^{31}P$ NMR, peaks at 6.6–5.5 ppm were likely to be assigned to remaining portion of the sequentially extracted soluble Ca and Mg phosphate (He et al., 2009b). The presence of other peaks from −0.5 ppm to −2.5 ppm might be due to calcium phytate (He et al., 2007; Hunger et al., 2004). Strong peaks in this region indicated that these peaks might also belong to other stable divalent metal phytate or multimetal (i.e., Al/Ca/Mg) phytate compounds. This result is consistent with the dominant metals observed in plants. Additionally, chemical shift between −4.18 ppm and −5.68 ppm might be due to pyrophosphate, which is always below detectable limit due to strong chemical hydrolysis in solutions.

### 4.3. Implications for hydrolysis of P-containing OMs based on sequential fractionation

Relative abundances of C functional groups are indicative of degradation and humification of OMs (Krull et al., 2003; Baldock et al., 1992; Kalbitz et al., 2003). Correlation analysis of abundances of various C functional groups and forms of P in sequential extracts can provide information on hydrolysis of P in OMs derived from aquatic plants. Relationships between C and P in water extracts derived from these aquatic plants have been discussed previously (Liu et al., 2016). The positive correlation of alkyl-C with COO/N—C═O ($p < 0.05$) and negative correlation between alkyl-C and O—C—O ($p < 0.05$) were attributed to selective release and degradation of OMs from aquatic macrophytes. In addition, the abundance of mono-P was 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, $NCH/OCH_3$ and COO/N—C═O. This might be attributed to the hydrolysis and dissolution of RNA and membranes in the process of water extraction, which can also be supported by the positive relationship between loss of mass, after extraction and O—C—O, mono-P ($p < 0.05$).

Relationships between C and P in NaOH extracts differed with that in $H_2O$ extracts (Table 6). Relative abundance of alkyl-C was negatively correlated with *O*-alkyl, O—C—O and aromatic C—O ($p < 0.05$). O—C—O was positively related with aromatic C—O ($p < 0.01$), and aromatic C was negatively correlated with carboxyl ($p < 0.05$). These relationships might be attributed to that greater proportions of aromatic substances, such as hydrolysable tannin and lignin, and less carbohydrates were extracted by NaOH than that extracted by $H_2O$. The abundance of mono-P increased with the proportion of carboxyl increasing, whereas an opposite relationship could be found between mono-P and aromatic-C (Table 6). This is possibly due to hydrolysis of OMs including COO and predecessor of mono-P such as nucleic acid by NaOH extraction.

**Table 6**
Correlation coefficients between C functional groups and P species identified by solid $^{13}C$ NMR and solution $^{31}P$ NMR spectroscopy.

| | Alkyl C | $NCH/OCH_3$ | *O*-alkyl | O—C-O | Aromatic C | Aromatic C—O | Carboxyl | Carbonyl | Ortho-P | Mono-P | Diester P |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | $H_2O$ fractions[a] | | | | | | |
| $NCH/OCH_3$ | 0.554 | | | | | | | | | | |
| *O*-alkyl | −0.759 | −0.585 | | | | | | | | | |
| O—C—O | −0.858* | −0.286 | 0.528 | | | | | | | | |
| Aromatic C | 0.220 | 0.201 | −0.761 | −0.047 | | | | | | | |
| Aromatic C—O | 0.253 | −0.299 | −0.484 | −0.478 | 0.597 | | | | | | |
| Carboxyl | 0.834* | 0.674 | −0.964** | −0.629 | 0.577 | 0.396 | | | | | |
| Ortho-P | 0.563 | 0.760 | −0.562 | −0.631 | 0.198 | 0.198 | 0.676 | | | | |
| Mono-P | −0.548 | −0.729 | 0.552 | 0.635 | −0.186 | −0.229 | −0.673 | – | −0.997** | | |
| Diester-P | −0.175 | −0.379 | 0.108 | −0.098 | −0.168 | 0.434 | 0.002 | – | 0.034 | −0.106 | |
| Mass loss | −0.714 | −0.568 | 0.691 | 0.829* | −0.323 | −0.504 | −0.774 | – | −0.925** | 0.931** | −0.137 |
| | | | | | NaOH fractions | | | | | | |
| $NCH/OCH_3$ | 0.232 | | | | | | | | | | |
| *O*-alkyl C | −0.838* | −0.351 | | | | | | | | | |
| O—C—O | −0.923** | −0.003 | 0.573 | | | | | | | | |
| Aromatic- C | −0.512 | 0.468 | 0.220 | 0.649 | | | | | | | |
| Aromatic C—O | −0.826* | 0.164 | 0.417 | 0.973** | 0.751 | | | | | | |
| Carboxyl | 0.657 | −0.430 | −0.557 | −0.661 | −0.881* | −0.706 | | | | | |
| Carbonyl | 0.074 | −0.892* | 0.224 | −0.345 | −0.786 | −0.514 | 0.639 | | | | |
| Ortho-P | −0.158 | 0.542 | 0.043 | 0.255 | 0.794 | 0.415 | −0.768 | −0.681 | | | |
| Mono-P | 0.216 | −0.419 | −0.048 | −0.314 | −0.852* | −0.463 | 0.753 | 0.643 | −0.973** | | |
| Diester | 0.361 | −0.420 | −0.316 | −0.395 | −0.182 | −0.334 | 0.322 | 0.383 | 0.212 | −0.276 | |
| Mass loss | −0.706 | 0.032 | 0.907* | 0.479 | 0.411 | 0.381 | −0.761 | −0.096 | 0.300 | −0.260 | −0.443 |

Significance of a coefficient at $^{*}p \le 0.05$ and $^{**}p \le 0.01$ ($n = 6$).
[a] Data cited from Liu et al., 2016.

### 4.4. Implications for biogeochemical cycling of plant-derived C and P based on sequential extraction

The combination of sequential fractionation with NMR spectroscopy may provide more information on lability and stability of different forms of OMs and nutrients in aquatic plants, and thus would provide more information for predicting their biogeochemical cycling in lakes. WEOMs contain more labile fractions of OMs or nutrients derived from these aquatic plants, which would readily dissolve and then release into the overlying water. These WEOMs mainly including carbohydrates, carboxyl acids and *ortho*-phosphate are ready to hydrolyze and thus immediately available for algae, living aquatic plants and bacteria in the overlying water of lakes. Thus, water extractable components are mainly bioavailable C and P derived from aquatic plants. NaOH extractable OMs and nutrients including some lignin, hemicellulose, carboxyl acids and monoester $P_o$ compounds are moderately labile components, which would more easily release under alkaline conditions, such as overlying water in an algal blooming lake. These moderately labile components could then be hydrolyzed by enzymes or assimilated by organisms directly. Organic matters or nutrients extracted by HCl including polypeptides/amino acid and $P_o$ are moderately labile components, which would be release under slightly acid conditions, such as in contaminated acid water bodies or acid microenvironments caused by bacterial secretion (Rudrappa et al., 2008). Those OMs and nutrients remaining in residues after sequential fractionation by $H_2O$, NaOH, and HCl are likely more chemically refractory and stable, and was likely to be accumulated and preserved in sediments for longer periods. Also, other processes such as biological processes, photo degradation or flocculation may further influence the degradation or preservation of those extractable and non-extractable OMs and P in lakes. Thus, more information on biogeochemical processes of plant-derived OMs in lake ecosystems should be investigated further.

Aquatic plants can synthesize OM through assimilating N, P and C from the atmosphere, water bodies or sediments (Wu et al., 2001). Meanwhile, the nutritional elements would be recycled to water in the process of metabolism or degradation after their death, which would even promote algae blooming. Aquatic plants, serve as an important endogenous source of OMs, the contribution of plant-derived C and P on OMs and nutrients in water and sediment of lakes were rarely evaluated. Here, information provided by sequential fractionation and NMR would be a valuable tool for evaluating the internal load of plant-derived C and P in lakes. Taking Tai Lake as an example, assuming the average biomass of aquatic plants was 3.39E + 03 g $m^{-2}$ (Zhao, 2013), water areas was 2338 $km^2$, and plant water content was 90%, respectively, and then the total plant-derived C and P could be calculated as $3.08 \times 10^8$ kg and $1.82 \times 10^6$ kg, respectively (Table 7). Among these, $1.68 \times 10^8$ kg C and $1.75 \times 10^6$ kg P are labile based on sequential extraction and NMR analysis, which can be released into the water column and available by organisms. Additionally, approached $1.40 \times 10^8$ kg C and $6.93 \times 10^4$ kg P were attributed to stable components, which was likely to be accumulated and preserved in sediments for a long time. Total amounts of C and P in aquatic plants were far more than those in water of Tai Lake (Table 7), which indicated that plant-derived C and P, especially labile C and P, was an important internal load of OMs and nutrients.

**Table 7**
Concentrations and amounts of C and P in aquatic plants and water body in Tai Lake.

| | Data | Reference or annotation |
|---|---|---|
| | Aquatic plants | |
| Mean density/(g/$m^2$) | 3.39E + 03 | Zhao (2013) |
| Water area/$km^2$ | 2.34E + 03 | Zhu (2008) |
| Wet biomass/Mg | 7.93E + 06 | Density × water area |
| Dry biomass/Mg | 7.93E + 05 | Water content was 90% |
| TOC/(g $kg^{-1}$) | 389 | |
| TP/(g $kg^{-1}$) | 2.3 | |
| TP/kg | 1.82E + 06 | |
| TOC/kg | 3.08E + 08 | |
| Available C/kg | 1.68E + 08 | |
| Stable C/kg | 1.40E + 08 | |
| Available P/kg | 1.75E + 06 | |
| Stable P/kg | 6.93E + 04 | |
| | Water | |
| Volume/$m^3$ | 4.42E + 10 | Water level was 2.99 m |
| TP/(mg/L) | 10.4 | |
| TOC/(mg/L) | 5.45 | |
| TP/kg | 4.60E + 03 | |
| TOC/kg | 2.41E + 05 | |
| TOC(plant)/TOC(water) | 1278 | |
| TP(plant)/TP(water) | 397 | |

## 5. Conclusions

- Sequential fractionation, combined with NMR spectroscopy, can be applied to characterize C and P derived from plant biomass. However, interpretation of the application of sequential fractionation should be done with caution due to their special physico-chemical properties.
- Based on characterization of C in debris of aquatic plants, 54.7% of OMs was considered to be released into the overlying water, whereas other OMs was likely to be accumulated and preserved in sediments for a long time. Among these, carbohydrates, proteins and aliphatic acids in the sequential fractions of aquatic plants are labile OMs, which could be released into the overlying water under neutral, alkaline or acid conditions.
- Based on characterization of P in debris of aquatic macrophytes, up to 96.2% of P could be released into the overlying water. *Ortho*-P can be completely released into the water. Whereas, more $P_o$ would be released into the overlying water in alkaline conditions, which indicated that $P_o$ derived from debris of aquatic plants may become an important source of P for supporting algae blooming in eutrophic lakes.
- Some non-extractable carbohydrates, aromatics and metal phytate compounds remained in residual fractions after extraction were relatively stable components in plants, thus they tend to be preserved in lake sediments for a long time.
- Based on sequential fractionation combined with NMR spectroscopy, available nutrients derived from debris of aquatic plants could amount to $1.68 \times 10^8$ kg C and $1.75 \times 10^6$ kg P in Tai Lake. Thus, the debris of aquatic plants can play a key role as a large source of nutrients in the overlying water of lakes, especially in eutrophic lakes.

## Acknowledgments

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