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RESEARCH ARTICLE

Using solid ^{13}C NMR coupled with solution ^{31}P NMR spectroscopy to investigate molecular species and lability of organic carbon and phosphorus from aquatic plants in Tai Lake, China

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Abstract Forms and labilities of plant-derived organic matters (OMs) including carbon (C) and phosphorus (P) were fundamental for understanding their release, degradation and environmental behaviour in lake ecosystems. Thus, solid ^{13}C and solution ^{31}P nuclear magnetic resonance (NMR) spectroscopy were used to characterize biomass of six aquatic plants in Tai Lake, China. The results showed that carbohydrates (61.2% of the total C) were predominant C functional group in the solid ^{13}C NMR spectra of plant biomass, which may indicate high lability and bioavailability of aquatic plants-derived organic matter in lakes. There was 72.6–103.7% of the total P in aquatic plant biomass extracted by NaOH–EDTA extracts. Solution ^{31}P NMR analysis of these NaOH–EDTA extracts further identified several molecular species of P including orthophosphate (50.1%), orthophosphate monoesters (46.8%), DNA (1.6%) and pyrophosphate (1.4%). Orthophosphate monoesters included β-glycerophosphate (17.7%), hydrolysis products of RNA (11.7%), α-glycerophosphate (9.2%) and other unknown monoesters (2.1%). Additionally, phytate, the major form of organic P in many lake sediments, was detected in floating plant water poppy. These inorganic P (e.g. orthophosphate and pyrophosphate) and organic P (e.g. diester and its degradation products) identified in plant biomass were all labile and bioavailable P, which would play an important role in recycling of P in lakes. These results increased knowledge of chemical composition and bioavailability of OMs derived from aquatic plants in lakes.

Keywords Aquatic plants · Nuclear magnetic resonance spectroscopy · Organic matter · Phosphorus · Lability · Lakes

Highlights 1. C and P in aquatic plants were characterized by solid ^{13}C and solution ^{31}P NMR.
2. Labile carbohydrates were predominant OM in debris derived from aquatic plants.
3. Orthophosphate and labile monoesters were dominant species of P in aquatic plants.
4. Phytate was identified to be a significant composition in a floating plant.
5. Macrophyte-derived OMs were important sources for bioavailable nutrients in lakes.

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Introduction

Aquatic plants play significant roles in nutrient cycling in aquatic ecosystems such as lakes due to large quantities of biomass produced by them and their high capacity to accumulate nutrients (Clarke and Wharton 2001; Abdo and Da Silva 2002). They could improve water quality by accumulating nutrients from both water and sediments of lakes (Ozimek et al. 1993; Rodrigo et al. 2013; Lee et al. 2009). The restoration of aquatic macrophytes for improving water quality in shallow eutrophic lakes thus became an eco-friendly and effective strategy that has been widely applied worldwide (Dhote and Dixit 2009; Sun et al. 2009; Iamchaturapatr et al. 2007). However, excessive growth of aquatic plants could also release large amounts of nutrients such as carbon (C), nitrogen (N) and phosphorus (P) into the overlying water in their dying/decomposition process, which may pose a serious threat to eutrophication of lakes (Wetzel 1996; Chinney and Pietro 2006; Xie et al. 2004).

Mass or molar ratios of C/N and C/P have been widely utilized as indicators of potential decomposition of biomass derived from plants (Moore et al. 2011). For example higher ratios of C/N or C/P in organic matters (OMs) imply their refractory and resistance to decompose (Taylor et al. 1989; Berg and McClaugherty 2007). Actually, the activities and fates of nutrients in the biomass derived from aquatic plants are dependent mainly on their existing molecular species (Krull et al. 2003). However, the ratio of C/N or C/P provides only limited information on the molecular structure, and it was hard to gain more knowledge of nutrient forms and their decomposition of litter biomass further. Therefore, it is essential to know more about forms and labilities of nutrients such as C and P in the biomass of aquatic plants and their litters.

Nuclear magnetic resonance (NMR) spectroscopy including solid ^{13}C NMR and solution ^{31}P NMR spectroscopy can be used to characterize the chemical composition of aquatic macrophytes and provide more detailed information on molecular species of C and P. Comparison with other optical analytical methods such as UV–Vis, three-dimensional excitation-emission matrix (3D EEM) and fourier transform infrared spectroscopy (FT-IR), NMR spectroscopy can provide further structural information on functional groups of carbon. A major advantage of NMR technique is the possibility to obtain not only structural information but also their relative abundances which is impossible for UV–Vis and 3D EEM. Thus, NMR spectroscopy (e.g. solid ^{13}C NMR and solution ^{31}P NMR) was a powerful tool, which has been widely applied in investigations of structure of organic matter from various environmental samples such as manures, sediments and soils (e. g. He et al. 2009, 2015; Zhu et al. 2015; Cade-Menun and Paytan 2010; Cade-Menun et al. 2015). However, the application of these techniques in C and P derived from aquatic plant biomass is limited (Qu et al. 2013; Feng et al. 2016). Thus, characterization of functional groups of C and P in plant-derived OMs by combination of solid ^{13}C NMR and solution ^{31}P NMR spectroscopy would enhance our understanding for labilities of plant-derived OMs and nutrients in freshwater ecosystems. In a previous study, water extractable organic matter (WEOM) derived from six aquatic macrophytes species from Tai Lake (Ch: *Taihu*), China was investigated by use of both solid ^{13}C NMR and solution ^{31}P NMR spectroscopy (Liu et al. 2016). The results showed that 54.4% of WEOM was carbohydrates, and only 9.5% was aromatics. >Additionally, orthophosphate was the dominant form of P (78.7% of total dissolved P) in the WEOM. Those observations indicated that plant-derived WEOM was labile, which could play significant roles in biogeochemical cycling of nutrients in freshwater lakes. However, WEOM was only represent 21.2% of total C and 53.3% of total P in the whole plant biomass (Liu et al. 2016). Therefore, as a continuation of that project, in this study, solid ^{13}C NMR and solution ^{31}P NMR spectral features of the whole biomass of the six macrophyte species were investigated. Furthermore, different parts of one species (branch smartweed) were also analysed by solid ^{13}C NMR and solution ^{31}P NMR spectroscopy. Detailed information on forms of organic C and P in whole aquatic plant biomass will be useful in better understanding biogeochemical cycling of aquatic plant-derived nutrients in lakes.

Material and methods

Study site and sample collection

Three types of aquatic macrophytes (six macrophytes) were collected from the eastern section of Tai Lake, a large, shallow, eutrophic lake located in the lower reaches of Yangtze River, China. The plants selected included the following: (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*, *Nymphoides peltata* (Gmel.) O. Kuntze); and (c) submerged plants, watermilfoil (*MV*, *Myriophyllum verticillatum* Linn.) and water thyme (*HV*, *Hydrilla verticillata* (Lf) Royle). Whole fresh plants (including roots, stems and leaves) were rinsed free of adhering particles, cut into 10-cm sections and then killed in an oven (90 °C) for 15 min. They were homogenized, freeze dried, ground to pass 2 mm and stored at −4 °C until use. One species of branch smartweed was separated into stem, leaves and root and pre-treated according above.

Solid CP/MAS ^{13}C NMR spectroscopy

Solid CP/MAS ^{13}C NMR spectra of prepared samples were collected by use of a Bruker AV-300 spectrometer operating at 12.5 kHz and using a standard 4-mm double-bearing probe. The detail procedure and parameters could be found in Liu et al. (2016). Relative abundances of C functional groups in different structures were estimated by integrating signal intensities over defined chemical shift windows using MestReNova10.0. The integrating spectral ranges and corresponding structures were assigned as follows: I, 0–45 ppm (alkyl-C); II, 45–60 ppm (NCH/OCH$_3$); 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).

NaOH–EDTA extraction and solution ^{31}P NMR spectroscopy

Phosphorus was extracted by shaking 0.5 g of ground aquatic plants with 30 mL of 0.5 M NaOH and 25 mM EDTA for 16 h at room temperature. Extracts were then centrifuged (8000×*g*) for 30 min, and filtered through Whatman No. 42 paper. Aliquots of extracts were used for qualification of dissolved total P (TP) (after digestion with $K_2S_2O_8$). Inorganic P (P_i) in extracts was determined by use of the molybdenum blue method. Concentrations of organic P (P_o) in extracts were then calculated as the difference between TP and P_i. Remaining extract was freeze dried, and then ground and preserved in a desiccator at room temperature until used for solution ^{31}P NMR analysis.

Each freeze-dried extract (100 mg) was re-dissolved in 1.0 mL 1 M NaOH–0.1 M EDTA and left to stand for 30 min after vortexing. Mixtures were then centrifuged at 8000×*g* for 30 min, and 0.5 mL supernatant was transferred to a 5-mm NMR tube, and then 0.1 mL D_2O was added to lock the signal. Spectral parameter for solution ^{31}P NMR were set at 161.98 MHz on a Bruker 400 MHz spectrometer equipped with a 5-mm broad band probe, using a 15-μs pulse width, 4.32-s pulse delay and 0.21-s acquisition time. Spectra processing, peak assignment and integration were carried out using MestReNova10.0 software per Liu et al. (2016) and Feng et al. (2016).

Results and discussion

Carbon functional groups in debris of aquatic plants

Characterization and quantification of C functional groups

In solid ^{13}C NMR spectra of whole biomass of the six aquatic plants, five obvious peaks at 30, 62, 72, 104 and 172 ppm were observed (Fig. 1). These peaks were associated with non-polar alkyl-C, NCH/OCH_3, *O*-alkyl C, O–C–O and COO/N–C=O respectively (Kögel-Knabner 1997; Mao et al. 2007; He et al. 2009). The observed signals at 172, 62 and 30 ppm revealed the presence of amino acids, polypeptides and/or proteins. In humic substances, only the ^{13}C NMR-identified non-polar alkyl-C group (0–45 ppm) positively correlated to the protein component identified by ultrahigh resolution electrospray ionization Fourier transform ion cyclotron resonance mass spectrometer (He et al. 2013). This indicates that peaks around 30 ppm in humic substance is more related to protein components. However, in plant-derived OMs, the multiple peaks around 30 ppm are more related to non-polar cutin-related wax materials (He et al. 2015; Preston et al. 2009). The dominant presence of OCH at 72 ppm, OCH_2 at 62 ppm and O–C–O at 104 ppm suggested the presence of carbohydrate functional groups (such as, starch and cellulose, and lignins) as the backbone components of the plant biomass (He et al. 2015). Peaks associated with COO, aromatic C and alkyl C indicated the presence of carboxylic acid, phenolic acids or lignins. Based on the nature of plant biomass, the alkyl C, *O*-alkyl C, aromatic C, aromatic C-C/H and aromatic C-O, NCH could be further assigned to functional groups of waxes, carbohydrates, lignins/tannins and proteins/peptides in debris of these aquatic plants respectively (Li et al. 2015).

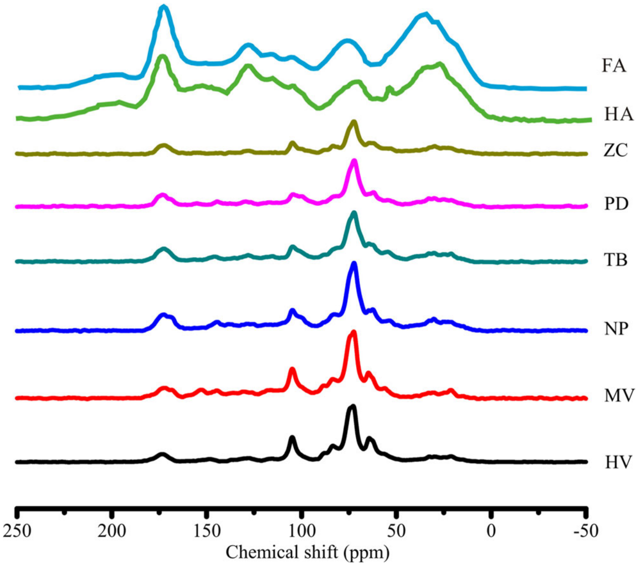

Fig. 1 Solid ^{13}C NMR spectra of the biomass samples of six whole aquatic plants, IHSS humic acid (HA) and fulvic acid (FA). Characterization of HA and FA was referred from Thorn et al. (1991)

Although the solid ^{13}C NMR spectra among these aquatic plants showed similar shapes of resonance peaks, quantitatively integrated peak areas indicated different abundances of these C functional groups in aquatic plants (Table 1). Total *O*-alkyl including *O*-alkyl and O–C–O at 60–110 ppm accounted for 61.2% of total C and was predominant in these functional groups. The following group was alkyl-C, with a percentage of 13.2%. Mean percentage of other C functional groups such as total aromatic C (including aromatic C and aromatic C–O), carboxyl C and NCH/OCH_3 was relatively lesser, with a percentage of 12.3, 8.6 and 4.7% respectively (Table 1). Organic compounds with a simple structure such as polysaccharides followed by proteins and lipids are generally rapidly utilized by microbes under oxic conditions, whereas other compounds with high molecular weight, irregular structure and aromatic structure, which were termed “recalcitrant”, would protect from degradation by microbes for a long time (Krull et al. 2003; Dodla et al. 2012). Thus, the predominant *O*-alkyl and less alkyl and aromatics detected in solid ^{13}C NMR spectra indicated lability of OMs derived from aquatic

Table 1 Relative abundances (%) of C functional groups in ground plants by solid ^{13}C NMR

Type	Sample	Alkyl-C 0–45	NCH/ OCH_3 45–65	*O*-Alkyl C 65–90	O–C–O 90–110	Aromatic-C 110–145	Aromatic C–O 145–165	COO/ N–C=O 165–190	Al–C[a]	Ar–C[b]	A + Ar[c]	A/A–O[d] (ratio)
Emergent	*ZC*	10.4	5.1	56.2	13.2	6.2	2.8	6.2	84.8	9.0	19.4	0.15
	PD	7.5	4.3	50.2	13.5	10.2	7.2	7.0	75.5	17.5	24.9	0.12
Floating	*TB*	12.4	4.8	42.0	18.7	8.2	4.6	9.4	77.9	12.7	25.2	0.20
	NP	13.3	5.7	47.3	10.8	9.2	4.1	9.6	77.1	13.3	26.6	0.23
Submerged	*MV*	14.9	4.0	45.2	12.8	7.9	5.2	10.0	76.9	13.1	28.0	0.26
	HV	20.9	4.2	47.0	10.2	5.8	2.5	9.4	82.3	8.4	29.2	0.37
	Average	13.2	4.7	48.0	13.2	7.9	4.4	8.6	79.1	12.3	25.6	0.22
	SD	4.5	0.7	4.8	3.0	1.7	1.7	1.6	3.6	3.3	3.4	0.09
	PD-stem	1.5	4.0	65.4	16.4	4.9	4.0	3.8	87.3	8.9	10.4	0.02
	PD-leave	12.7	5.5	42.0	14.1	11.0	8.5	6.2	74.2	19.5	32.2	0.23
	PD-root	2.5	5.8	68.7	14.9	4.3	2.6	1.2	91.9	6.8	9.4	0.03
	IHSS HA[e]	29.0		13.0	7.0	31.0		15.0	49.0	31.0	60.0	1.45
	IHSS FA[e]	35.0		16.0	6.0	22.0		17.0	57.0	22.0	57.0	1.59

[a] Al–C (total aliphatic C): 0–110 ppm

[b] Ar–C (total aromatic C): 110–165 ppm

[c] A + Ar: alkyl C (0–45 ppm) + total aromatic C (110–165 ppm)

[d] A/A–O: alkyl-C (0–45 ppm) / total O-alkyl (65–110 ppm)

[e] Referred from Thorn et al. (1991)

plant biomass. The relative abundance of C functional groups in aquatic plants showed remarkably distinct with that in standard humic acid and fulvic acid (Thorn et al. 1991) which contain more alkyl C and total aromatic C and less polysaccharides (Fig. 1; Table 1). The difference revealed that non-humic substances such as aquatic plants, as parental materials of humic substances, may undergo the release of labile polysaccharides and accumulation of refractory alkyl and aromatic C in the process of humification, which is consistent with our previous observations in WEOM derived from aquatic plants (Liu et al. 2016).

There was a significant variation in abundance of C functional groups among these aquatic plants (Table 1). Percentages of total *O*-alkyl (carbohydrates) in emergent plants were higher than those in floating and submerged plants, while relative abundances of alkyl C and carboxyl were lesser. This result might indicate that there are more abundance of carbohydrates such as cellulose and hemicellulose in emergent plants, whereas greater percentage of aliphatic acids or biopolymers in submerged and floating plants. The explanation for the difference is based on the fact that emergent plants are vascular plants with well-developed structural components, which contain large amounts of hemicellulose, whereas floating and submerged plants contain more storage materials and waxy components (Kögel-Knabner 2002). It has been reported previously that long-chain alkanes in sediments may be derived from contributions of floating and submerged macrophytes (Xiong et al. 2010). Based on results here, it was found that relative abundances of non-polar alkyl C groups were in an increasing order: emergent < floating < submerged plants. Apparently, more hydrophobic waxy components are required to protect plants when they are submerged in water for a long time. Such an order of relative intensity of alkyl C peaks among WEOM derived from three types of aquatic plants had not been observed previously (Liu et al. 2016). These results implied that extraction with water yielded alkyl groups of the six plants was not similar among these plants. These results further indicated that WEOM had only water dissolved portions rather than whole characteristics of OMs in aquatic plants.

Further investigation of C functional groups of debris from leaves, stems and roots of emergent *PD* showed that hydrophobic waxy components and aromatics were rich in leaves and negligible in stems and roots (Fig. 2; Table 1). In addition, more aromatics (like tannin) and less carbohydrates were found in leaves than roots and stems (Fig. 2; Table 1), which were associated with high contents of lignocellulose in stems and low-molecular-weight organic acids and tannin in leaves (Kögel-Knabner 2002).

Indicators for the degradation of aquatic plant biomass

Relative abundances of functional groups and their ratios in OMs can be used to evaluate their degradation in the process

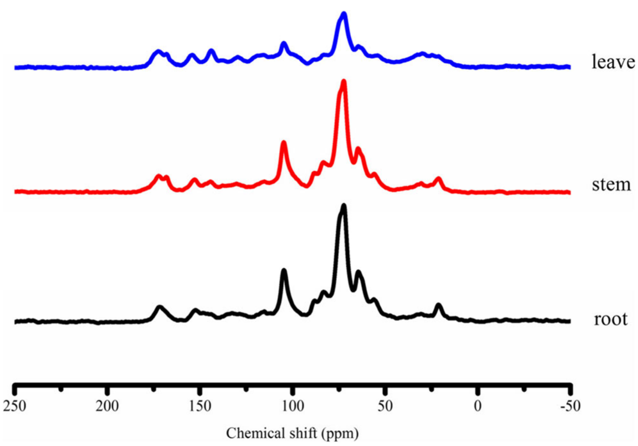

Fig. 2 Solid ^{13}C NMR spectra of the stem, leave and roots of *PD*

of biogeochemical cycling. It was reported that the non-aromatic materials, especially polysaccharides, are prior to decompose, whereas relative percentage of aromatic materials would always increase (Kalbitz et al. 2003; Skjemstad et al. 2001). Carbohydrates are usually considered to be the most labile components as they can be readily broken down by a wide range of organisms, which serve as the initial energy and C source for biological life (Krull et al. 2003). Thus, they are usually assigned to the fast-degrading labile pool, with months or few years degradation period (Krull et al. 2003). Alkyl structures and aromatic ring structure are more resistant to decomposition than carbohydrates and thus are considered as stable forms of OM (Krull et al. 2003). Degradation period of refractory alkyl C and aromatic C was approximately decades or hundreds of years (Coleman and Jenkinson 1995, 1996). In addition, ratios of A/A–O (alkyl C/total aromatic C) and A + Ar (sum of alkyl C and total aromatic C) are good indicators of degradation rate (Baldock et al. 1997; Krull et al. 2003). The greater ratio of A/A–O implies relatively more lipid C and less polysaccharide, which would be difficult to decompose in environment (Baldock et al. 1997). The higher value of A + AR reveals more refractory components of alkyl C and aromatic C in OMs, which would be more difficult to decompose (Krull et al. 2003). In this study, relative abundances of alkyl C were in the order that emergent < floating < submerged plants, while the carbohydrates were in the reverse order that emergent > floating > submerged plants. The ratios of A/A–O were similar to abundance of alkyl C in the order that emergent < floating < submerged plants. Values for A + Ar were in the order that emergent < floating < submerged plants. Based on these orders of relative abundance of C functional groups and their ratios, the potential degradation degree of debris derived from aquatic plants was in the order that emergent > floating > submerged plants. This result was inconsistent with the potential degradation degree of aquatic plants based on other indicators, such as C/N and C/P (Liu et al. 2016). Based on ratios of C/N and C/P, submerged plants tend to be degraded more easily than emergent plants (Liu et al. 2016). This difference was likely due to the fact that emergent plants contain more structural carbohydrates such as cellulose and hemicellulose rather than high soluble carbohydrates. Cellulose can be protected from immediate breakdown because it is often surrounded by hemicellulose and coated with lignin. Additionally, some phenols alike molecular species such as hydrolysable tannin are also easily dissolvable. Although A + Ar and A/A–O were greater in submerged plants than that in emergent plants, it may be difficult to evaluate their degradation due to their special components. This result was also supported by previous studies (Lorenz et al. 2004). Degradation of debris derived from plants in lakes had been studied previous based on simulation experiments (Qu 2013). The results suggested that degradation of these debris evaluated by C functional group in ^{13}C NMR spectra is only valid after leaching stage, whereas C/N or C/P could be valid in the beginning of degradation (Qu 2013). Therefore, degradation degree of debris derived from aquatic plants should be comprehensively evaluated by both information on elemental composition and functional groups in long time scales of degradation experiments.

Forms of P in NaOH–EDTA extracts

Extraction efficiency of P in debris of aquatic plants by NaOH–EDTA

Efficiency of extraction of total P by NaOH–EDTA ranged from 72.6 to 103.7% of total P in aquatic plant biomass, while extraction efficiency of P_o ranged from 72.1 to 135.3% (Table 2). These results were consistent with those observed in previous studies (Noack et al. 2012), who obtained TP extraction efficiency of 73–110% in eight crop residues by use of NaOH–EDTA. Extraction efficiencies of P_o in crop residues by NaOH–EDTA were greater than those of other extractants, such as water and weak acids, which was only 56–63 and 61–64% respectively (Noack et al. 2014). These results demonstrated that extraction with NaOH–EDTA was a valid method to extract P from aquatic plants.

Characterization of P in NaOH–EDTA extracts by solution ^{31}P NMR spectroscopy

Several species of P including orthophosphate, monoesters, diesters and pyrophosphate were detected by use of solution ^{31}P NMR spectra (Fig. 3). These solution ^{31}P NMR spectra were relatively simpler than spectra observed in soils, manure and other environmental samples (He et al. 2007; Cade-Menun et al. 2015; Zhu et al. 2015), but more complicated than those in WEOM samples derived from crops and aquatic plants (He et al. 2009; Liu et al. 2016). The composition of P in extracts of aquatic plants was similar with that in NaOH–

Table 2 Extraction efficiencies of P by NaOH–EDTA in six aquatic plants and different parts of *PD*

Sample	Aquatic plant biomass (mg g^{-1})		NaOH–EDTA extracts (mg g^{-1})		Extraction efficiency (%)	
	TP	P_o	TP	P_o	TP	P_o
ZC	2.0	0.7	2.1	0.9	103.7	135.3
PD	1.3	0.7	1.4	0.8	105.0	116.4
TB	2.1	1.6	1.5	1.2	72.6	72.1
NP	2.7	1.5	2.7	1.6	99.3	109.8
MV	2.3	1.4	2.1	1.3	91.3	94.4
HV	3.5	1.4	3.2	1.5	91.4	103.9
Average	2.3	1.2	2.2	1.2	93.9	105.3
SD	0.8	0.4	0.7	0.3	12.0	21.3
PD-stem	1.2	0.5	1.1	0.5	91.9	100.6
PD-leave	1.9	1.2	1.8	1.2	95.8	98.7
PD-root	1.3	0.7	1.2	0.7	93.7	105.8

EDTA extracts from crop residues characterized by solution ^{31}P NMR (Noack et al. 2012).

Integration of ^{31}P NMR spectra in NaOH–EDTA extracts showed that a large proportion of P was detected as orthophosphate (5.3–5.25 ppm), accounting for 33.0–65.9% of total NaOH–EDTA extractable P (Table 3). Pyrophosphate was identified at −5.0 ppm, accounting for 0.5–2.3% of total NaOH–EDTA extractable P (Table 2). Orthophosphate was reported to be the predominant species of P in aquatic plants such as foxtail, common reed and black plant (Cheesman 2010; Feng et al. 2016). The majority, if not all, of the orthophosphate in these plants might not really be associated with OM of plant biomass, rather than orthophosphate transported and/or stored in the plant tissues (He et al. 2009). The very high proportion of P in aquatic plants existing as orthophosphate has the potential to be returned to the lake waters in a readily available form for plants and microorganisms. The observation of the higher portion of orthophosphate in the stem and root than in the leaves of *PD* (Fig. 4; Table 3) also indicated the bioactive leaves would utilize orthophosphate for biosynthesis. In the viewpoint of environmental concerns of decaying plant biomass, the inorganic P including orthophosphate and pyrophosphate in aquatic plants are labile components in debris from plants, which would readily increase bioavailable P in water column. This is also supported by a previous study (Liu et al. 2016), which showed that orthophosphate could be accounted for 78.7% of the total water extractable P from aquatic plants.

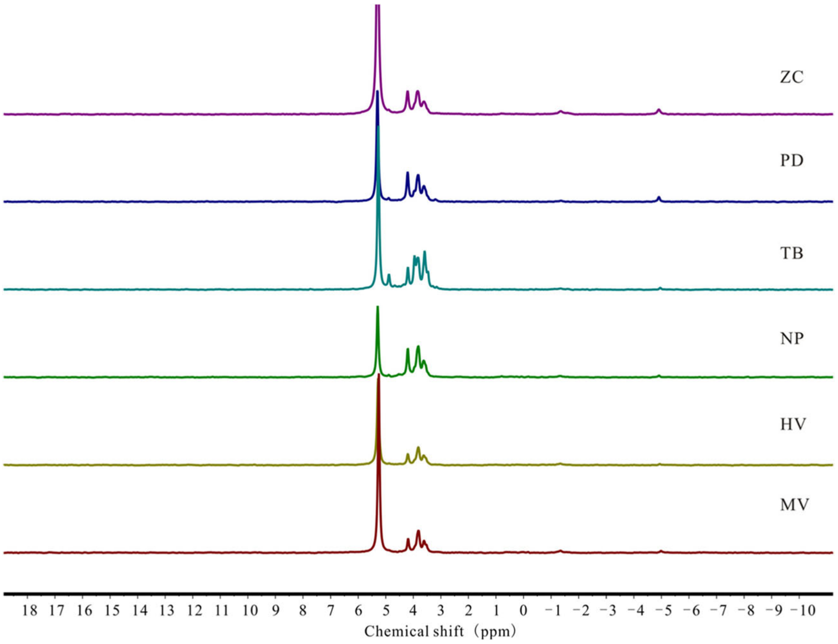

Fig. 3 Solution ^{31}P NMR spectroscopy of NaOH–EDTA extracts of six aquatic plants

Table 3 Species of P and their relative abundances (%) characterized by solution ^{31}P NMR

Sample	Orthophosphate	Phytate	α-Glycerophosphate	β-Glycerophosphate	Hydrolysis product of RNA	Unknown monoesters	Total monoesters	DNA	Pyrophosphate
ZC	64.7	0.0	5.7	15.0	12.0	0.0	32.7	1.8	0.8
PD	53.8	0.0	7.6	18.1	15.8	0.0	41.5	2.5	2.3
TB	33.0	0.0	15.1	27.3	20.3	1.7	64.4	1.7	0.9
NP	41.3	37.3	6.3	13.9	0.0	0.0	57.4	0.8	0.5
HV	42.2	0.0	12.5	18.6	12.9	11.2	55.2	0.6	2.0
MV	65.9	0.0	7.9	13.2	8.9	0.0	30.0	2.2	2.0
PD-stem	65.1	0.0	5.6	14.5	12.6	0.0	32.7	1.6	0.6
PD-leave	39.1	0.0	10.7	21.8	23.3	1.8	57.6	2.8	0.5
PD-root	53.8	0.0	7.2	15.5	16.4	0.0	39.0	2.8	4.4

DNA was the only diester detected in these NaOH–EDTA extracts, which accounted for only 0.6–2.5% of total P in NaOH–EDTA extracts. DNA is relatively chemical stable in natural waters compared with RNA, but they could act as an important source of P in lakes especially when orthophosphate concentrations are low (Pinchuk 2008). Both RNA and phospholipid were not detected in any extracts. It is likely that phospholipid and RNA hydrolysed to monoester P during the process of alkaline extraction and pre-concentration (Cade-Menun et al. 2006), though DNA was relatively stable during alkaline extraction.

Monoesters (5.2–3.2 ppm) accounted for 30.0–64.4% of total P in NaOH–EDTA extracts (Table 3). The compositions of P in the monoesters region of ^{31}P NMR spectra were further analysed based on spiking experiments (Fig. 5). Alkaline degradation products of phospholipids, α- and β-glycerophosphates, at 4.2 and 3.8 ppm respectively, were predominant species in the region of the spectra associated with monoesters, accounting for 20.1–42.4% of total NaOH–EDTA extractable P (Table 3). Two minor peaks at 3.5 and 3.6 ppm were assigned to nucleotide from the alkaline degradation of RNA, with a proportion of 0.0–20.3% of total NaOH–EDTA extractable P. Due to the easily degradation of phospholipid and RNA, their degradation products were detected in extracts of almost all plants. Thus, large proportions of monoesters detected in these aquatic plants were likely assigned to labile diester P, which could be also readily used by biological processes in lakes. Based on aggregative results, large quantities of P derived from debris of aquatic plants were labile and bioavailable P, which may accelerate eutrophication process of lakes. Therefore, large biomass of aquatic plants should be removed in proper situations to prevent their recycling of nutrients into water column in eutrophic lakes.

Phytate, an important monoester P, was detected in a floating plant *NP* (Fig. 5). It comprised 37.3% of the total NaOH–EDTA extractable P from *NP*. The result was

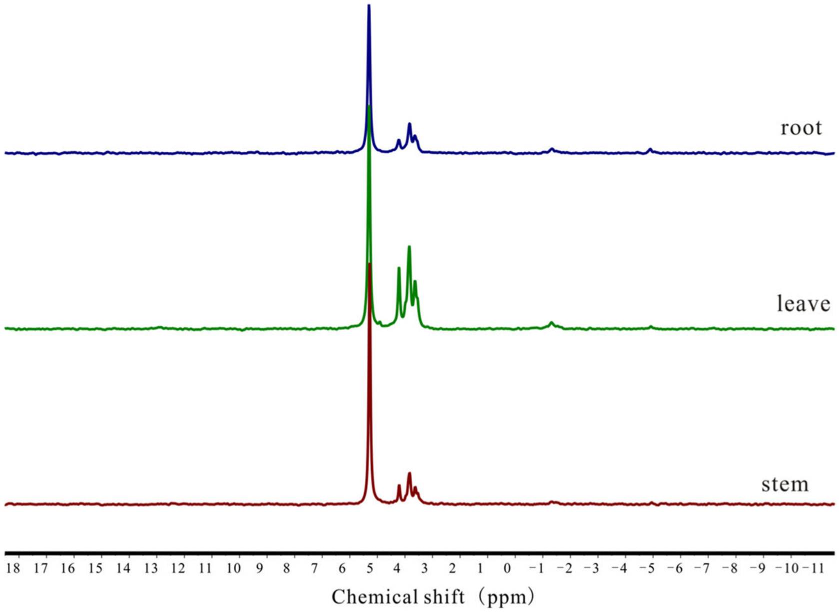

Fig. 4 Solution ^{31}P NMR spectroscopy of NaOH–EDTA extracts of stem, leave and root of *PD*

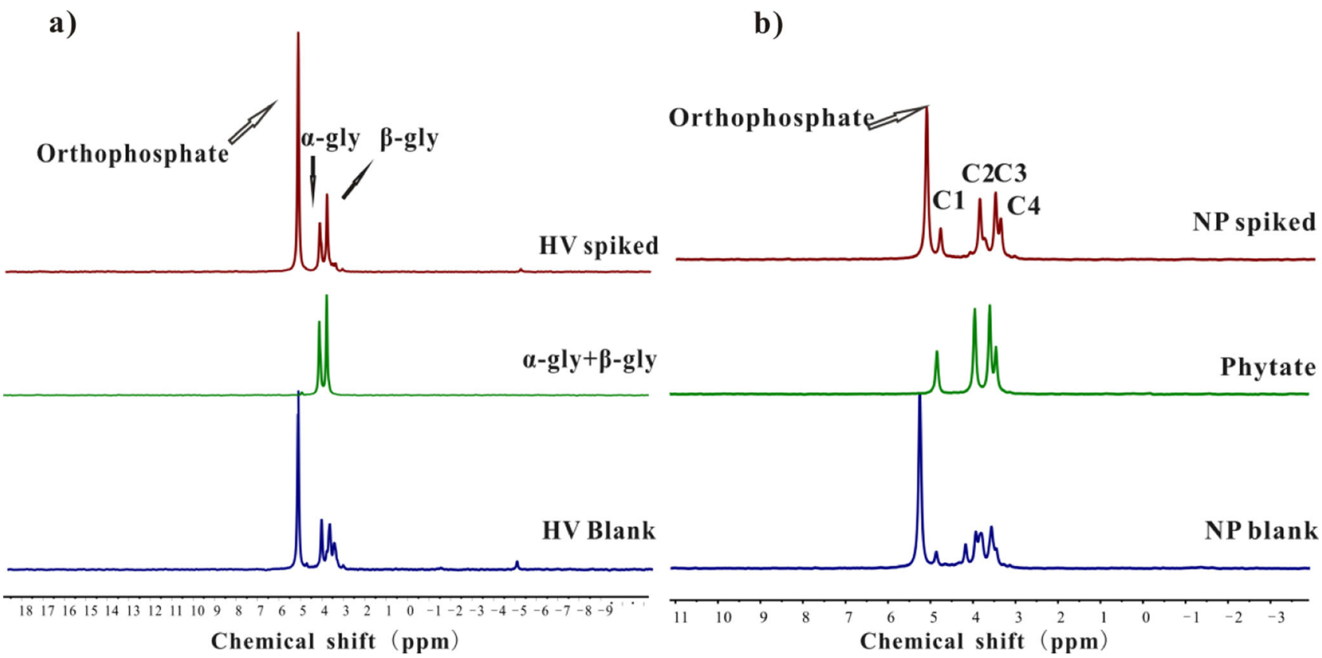

Fig. 5 Identification of special monoester region in spectra of aquatic plant extracted with NaOH–EDTA and analysed with solution ^{31}P NMR (**a**, *HV blank*; **b**, *NP blank*), re-analysed by solution ^{31}P NMR after addition of a spike (**a**, *HV spiked*; **b**, *NP spiked*) and standards (**a**, *phytate*; **b**, *α-gly* + *β-gly*)

consistent with those observed previously in water extracts of the same six plants (Liu et al. 2016). Phytate has been previously detected in tissues including roots, stems and leaves (Brearley and Hanke 1996; Campbell et al. 1991; Weimer and Armstrong 1979). However, none of these previous results were based on ^{31}P NMR spectroscopy and spiking experiments. For example Campbell et al. (1991) applied ferric precipitation, ion-exchange chromatography and high voltage paper electrophoresis to demonstrate that phytic acid is a P-containing compound in roots and crown tissue of alfalfa. Weimer and Armstrong (1979) applied the phytase hydrolysis to identify and quantify phytate in four aquatic plants. More recently, He et al. (2011) have shown by solution ^{31}P NMR spectroscopy with phytate spiking that no phytate is present in their humic fraction samples even though the phytase hydrolysis released organic P in their samples. This was likely due to the fact that phytase possess broad substrate specificity beyond phytate compounds (He and Honeycutt 2001; Zhu et al. 2015). Thus, these earlier observations of the presences of phytate in some plant biomass samples may need more rigorous scrutiny. Generally, the majority of phytate are stored in seeds, with far lower concentrations in other parts of the plant (Noack et al. 2012). Phytate is the primary storage compound of P and energy in seeds accounting for up to 60–89% of the total seed P (Raboy 2007). Phytate itself can be easily hydrolysed by phytase. However, in natural environment, the negatively charged phosphorus in phytate strongly binds to minerals or metallic cations which result in insoluble and unavailable (Turner and Weckström 2009; Zhu et al. 2013, 2015). Thus, phytate can be preserved for a long time in lake ecosystems, such as sediments. Therefore, phytate derived from floating macrophytes may constitute an important source of P_o in sediments. More aquatic plant species need to be further studied on their composition of P in our subsequent work in order to clarify this issue.

Relative abundances of species of P varied among the aquatic plants studied here. The proportion of monoesters was relatively greater in floating plants than that in emergent and submerged plants, while proportions of orthophosphate were the opposite (Fig. 4; Table 3). The greater proportion of orthophosphate would result in more orthophosphate release since almost all of the orthophosphate in plant biomass can be extracted by water (Liu et al. 2016). Thus, these results above may indicate that debris derived from emergent and submerged plants would be more easily released bioavailable orthophosphate, whereas potential bioavailable P_o would be released more in debris from floating plants in lakes. In different parts of *PD*, relative proportions of monoesters in leaves were greater than that in stems and roots, while proportions of orthophosphate in stems were slightly greater than that in leaves and roots (Fig. 4; Table 3). This indicated that majority of P in debris derived from stems was readily bioavailable for microorganisms when released into the overlying water in lakes, while P in debris derived from leaves would be also bioavailable after degradation such as enzymatic hydrolysis.

Summary and conclusions

1. Labile carbohydrates were predominant C functional groups in debris derived from aquatic plants, followed by waxes, lignins/tannins and proteins/peptides. The debris derived from aquatic plants was an important source of bioavailable organic C for life in lakes.
2. Orthophosphate and monoesters including hydrolysis products of diesters were predominant species of P in debris derived from aquatic plants, which are important internal sources of bioavailable P for organisms when recycling again after death in lakes. Additionally, debris derived from floating plant *NP* would be an important source for phytate in sediments of lakes.
3. Debris derived from aquatic plants could be one of the important internal sources of labile OMs and nutrients in lakes; thus, they should be paid more attention in the management and restoration of eutrophic lakes.

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