

Phosphorus dynamics in sediments of a eutrophic lake derived from ^{31}P nuclear magnetic resonance spectroscopy

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Abstract. The determination of organic phosphorus (P) compounds in lake sediments can provide information on the potential for internal P loading. Settling seston and vertical sediment core samples from highly eutrophic Lake Okaro, New Zealand, were collected during a mixed winter and stratified summer period, representing, respectively, when the water column was well oxygenated and when the bottom waters were anoxic. Samples were analysed with ^{31}P nuclear magnetic resonance (^{31}P NMR) spectroscopy, which showed that both bottom sediments and settling seston contained orthophosphate, orthophosphate monoesters and diesters, pyrophosphates, polyphosphates and phosphonates. Phosphorus concentration in settling seston increased ~ 2.5 -fold in winter as a result of seasonally induced changes in phytoplankton biomass, with a marked increase in the concentration of orthophosphate. Several potentially bioavailable P compounds in the bottom sediments were identified that were likely to contribute to recycling of P from the sediment to the water column. An ‘apparent half-life’ was used to quantify the time scales on which these compounds were recycled to the overlying water column. Orthophosphate monoesters that include inositol phosphates were the most persistent P compound. On the basis of half-lives, high internal P loadings may persist for more than 20 years, potentially hindering restoration efforts in Lake Okaro.

Additional keywords: internal loading, Lake Okaro, sedimentation, temporal variability.

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Introduction

Phosphorus (P) has long been recognised as a key nutrient in many aquatic ecosystems because of its potential to limit primary productivity (Vollenweider 1976; Carpenter 2008). In addition to contributions from surface and ground water and, to a lesser extent, atmospheric deposition (Reed-Andersen *et al.* 2000), bottom sediments are a key source of P, especially in eutrophic lakes (Søndergaard *et al.* 2003). Whereas the interactions among physical, chemical and biological processes influencing sediment P concentrations have been intensively investigated (Smolders *et al.* 2006), few studies have investigated the compounds and the reactivity of organic P compounds in lake sediments (e.g. Hupfer *et al.* 2004; Ahlgren *et al.* 2005; Shinohara *et al.* 2012).

Since the first use of ^{31}P nuclear magnetic resonance (NMR) to investigate organic P extracted from New Zealand grassland soils in 1980 (Newman and Tate 1980), this method has become a valuable tool to determine either specific compounds (e.g. orthophosphate) or differentiate organic P-compound classes (e.g. orthophosphate monoesters or diesters) in soils

and sediments worldwide (Cade-Menun 2005). Although several studies have discussed organic P groups in agricultural soils (e.g. McDowell *et al.* 2005), fewer studies have examined speciation of organic P in marine sediments and fewer still in freshwater lake sediments (Cade-Menun 2005). Lake sediment studies have identified several classes of organic P compounds similar to those found in terrestrial soils, including orthophosphate monoesters, orthophosphate diesters and polyphosphates. The relative proportion of these compounds varies, however, suggesting variations among sediment types and prevailing environmental conditions (Hupfer *et al.* 1995; Ahlgren *et al.* 2006).

Differences among the proportions of organic P-compound classes in lake sediments have been related to the oxidation–reduction status of the sediments. Carman *et al.* (2000) described the presence of pyro- and polyphosphates in oxic surface sediments, noting that these compounds were absent in anoxic conditions. Similarly, McDowell (2009) found more bioavailable P-compound classes, particularly orthophosphate diesters, in dry (oxic) sediment than in wet (anoxic) stream sediments of agricultural catchments. Changes in redox

chemistry appear to play an important role in the diagenesis of phosphate diesters (Ingall and Jahnke 1994). Very little work has used the added insight available by ^{31}P NMR to further understanding of P compounds and compound classes for both settling seston and bottom sediments of lakes (Shinohara *et al.* 2012).

In New Zealand, water quality of many of the Te Arawa lakes of the Rotorua region has declined significantly with development of surrounding catchments for pastoral agriculture (Hamilton 2005; Paul *et al.* 2012). Several of the lakes have high internal nutrient loadings as a result of organic enrichment of the bottom sediments and remobilisation of bottom-sediment nutrients with eutrophication-driven depletion of dissolved oxygen in bottom waters (White *et al.* 1978; Burger *et al.* 2007; Trolle *et al.* 2008). Lake Okaro, the focus site of the present study, is the most eutrophic of the Te Arawa lakes. It is a monomictic lake with a hypolimnion that becomes anoxic approximately 1 month after the onset of seasonal stratification, which lasts for approximately 9 months (Özkundakci *et al.* 2010). The concentration of P compounds in settling material in lakes can vary substantially with season, and therefore may have an important role in changing the composition of bottom sediments (Pettersson 2001). For the present study, we chose periods of seasonal stratification, when bottom waters were anoxic, as well as periods of mixing, when the entire water column was well oxygenated, to test for the effects of seasonal variations caused by mixing patterns and redox status on speciation of sediment P.

We hypothesised that sediment organic P is an important source of bioavailable P in Lake Okaro. Because seasonal changes in the water column of Lake Okaro (oxic and anoxic hypolimnion) could lead to changes in rates of P sedimentation and mobilisation, we further hypothesised that the supply and bioavailability of the organic P would change with season. To test these hypotheses, we used ^{31}P NMR to determine the P compounds and compound classes in settling seston and at

different depths and in different seasons in the bottom sediments of Lake Okaro.

Materials and methods

Study site

Lake Okaro is a small lake (0.32 km^2) with a maximum depth of 18 m (Fig. 1). The catchment land-use area (3.89 km^2) is now mostly in dairy farming. The lake has been eutrophic since the early 1960s (Jolly 1977), with regular cyanobacterial blooms and an anoxic hypolimnion during summer stratification (Forsyth *et al.* 1988; Paul *et al.* 2008). Two unnamed streams enter the lake from the north-west. In 2006, these streams were diverted through a 2.3-ha constructed wetland as part of a lake restoration strategy (Tanner *et al.* 2007). Further, on 16 and 17 December 2003, 13 m^3 of alum, equivalent to a dose rate of $0.6\text{ g aluminium m}^{-3}$ in the epilimnion, was applied to the lake to attempt to control internal P loads (Paul *et al.* 2008). In September 2007, 110 t of aluminium-modified zeolite (Z2G1, Blue Pacific Minerals, Tokoroa, New Zealand) was applied to Lake Okaro to act as a sediment capping agent to further decrease internal loading of P (Özkundakci *et al.* 2010).

Sedimentation rates

Sedimentation rates of seston in Lake Okaro were measured with cylindrical sediment traps (PVC, 0.065 m in diameter, 0.65 m in height). Traps were placed at 3- and 9-m depths, deployed at three sites where bottom depths were 11 m (Site S1), 14 m (Site S2) and 18 m (Site S3) (Fig. 1). Traps were deployed when the water column was thermally stratified between 26 January 2007 and 28 February 2007 and fully mixed between 23 May and 22 June 2007. No inhibitors or preservatives were added *a priori* to the traps; so, P compounds analysed in the study potentially include the effects from degradation over

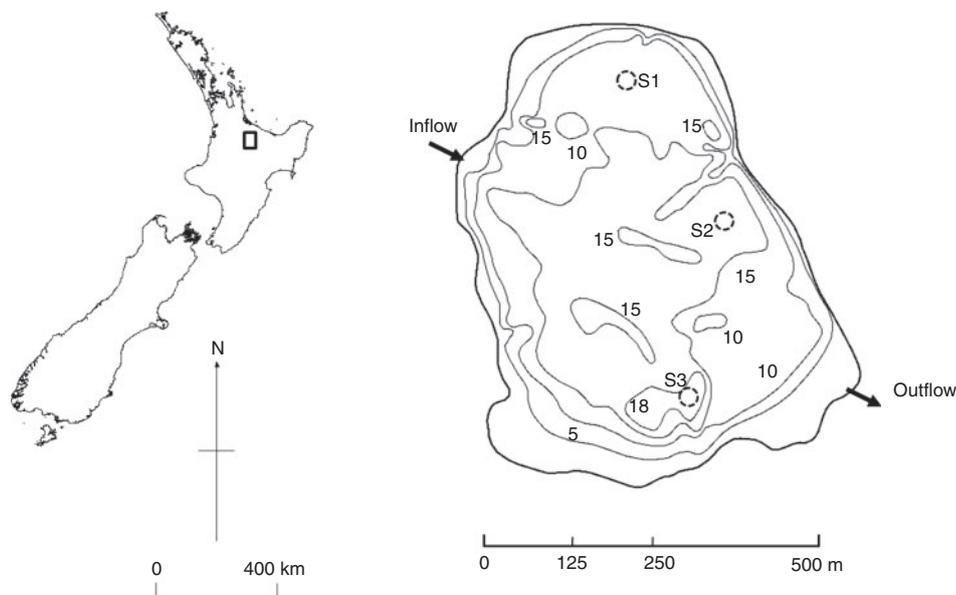


Fig. 1. Location map of Lake Okaro, with depth contours 5, 10, 15 and 18 m, and showing the location of sediment trap and sediment sampling sites (dashed circles marked S1, S2 and S3).

the deployment period. At each trapping depth, four replicate traps were supported by a wooden frame and attached to a central rope suspended vertically in the water column between a bottom anchor and a subsurface buoy (1-m depth). Replicate traps were separated by a distance of at least three times the trap diameter (0.065 m) to minimise effects of trap interactions on water movement and sedimentation. Once retrieved, traps were placed in a chilled container for 30 min before the supernatant was siphoned off and the remaining ~500 mL of fluid was capped and placed on ice for return to the laboratory. This material was stirred immediately on return to the laboratory and subsamples were taken for analysis of total P (TP) and total particulate matter (TPM). Samples for the analysis of TP were first digested using persulphate (Ebina *et al.* 1983), followed by analysis as molybdate-reactive P on a Lachat QuickChem Flow Injection Analyzer (FIA+ 8000 Series, Zellweger Analytics, University of Waikato, New Zealand). Subsamples were filtered through pre-weighed Whatman GF/C filters (GE Healthcare, Australia), which were then dried at 105°C for 24 h to determine TPM, and then combusted at 550°C for 4 h to determine loss on ignition (LOI). Subsamples from trapped material were taken from each depth at Site S2 for ³¹P NMR analysis, on each sampling occasion. Because of the expense of conducting ³¹P NMR, P speciation of settling seston was conducted only at Site S2.

Sediment sampling

An undisturbed sediment core was collected with a gravity corer from each of the three sampling sites (S1, S2 and S3; Fig. 1) on 28 February 2007 (stratified water column) and 22 June 2007 (mixed water column). In the field, sediment cores were sliced into 2-cm intervals down to 30-cm depth, and stored on ice in 50-mL plastic tubes until return to the laboratory where each tube was centrifuged for 40 min at 66.6 Hz to separate pore waters from solid material. Sediment dry weight (DW) was measured by drying sediment samples at 105°C over ~24 h or until constant weight was obtained. Organic content was measured as LOI, by combusting the dried sample for 4 h at 550°C. Analysis of TP, iron (Fe), manganese (Mn) and aluminium (Al) was carried out on aqua regia digests of dried sediment samples that had been homogenised by grinding beforehand, following the procedure of Trolle *et al.* (2008). Sediment core samples from Site S2 were sliced horizontally at 2-cm intervals down to 10 cm for ³¹P NMR analysis.

³¹P NMR analysis

A subsample from bottom sediments and seston of ~10-g wet weight was shaken with 30 mL of 0.25 M NaOH + 0.05 M Na₂-EDTA (1:15 dry sediment:extractant ratio) for 16 h (the subsample of sediment-trap material consisted of pooled samples from four replicate sediment traps; Cade-Menun 1996). Samples were then centrifuged (15 000 rpm) before filtering supernatant through Whatman GF/C filters. Subsamples of the extract were analysed by inductively coupled plasma-mass spectrometry (ICP-MS) for TP. Each extract was pre-concentrated (~10-fold) by rotary evaporation (Büchi Rotavapor R110, BUCHI Labortechnik AG, Flawil, Switzerland) at 30°C and then frozen. This process has been shown to yield a wider range of P compounds and compound classes than the resuspension and analysis of freeze-dried samples

(McDowell 2003; Hupfer *et al.* 2004). Prior to analysis, samples (3 mL) were centrifuged at 66.6 Hz, transferred to a NMR (5-mm-diameter) tube and 1 mL of deuterium oxide (D₂O) was added to the samples to obtain a stable signal lock. The ³¹P NMR spectra were obtained on solutions at a frequency of 161.97 MHz on a Bruker Avance DPX 400 spectrometer fitted with a 5-mm dual inverse board-band (H-X) probe (Bruker, Billerica, MA, USA). An identical number of scans (10 000) was used for each sample by using a pulse angle of 90° with a pulse delay of 5 s and an acquisition time of 0.34 s. The delay between pulses was sufficient to exceed five times the spin-lattice relaxation time (*T*₁), as examined by an inversion recovery sequence, thereby generating quantitative spectra (McDowell *et al.* 2006). Chemical shifts were recorded relative to an external phosphoric acid standard ($\delta = 0$ ppm). Spectra were processed with a 10-Hz exponential line broadening by using Mestre-C software (V. 2.3a, Santiago de Compostela, Spain). Peak areas were calculated by integration on processed spectra and, subsequently, grouped into distinct P compounds (orthophosphate, pyrophosphate and polyphosphate) or compound classes (orthophosphate monoesters and diesters and phosphonates) by using peak assignments derived from the literature (Cade-Menun 2005; Hupfer *et al.* 2004; Ahlgren *et al.* 2006). Peak assignments (chemical shifts) to identify P compounds and compound classes were 19.58 ppm for phosphonates, 6.68–5.20 ppm for orthophosphate, 5.21–3.37 ppm for orthophosphate monoesters, 3.34 to –3.43 ppm for orthophosphate diesters, –3.87 to –4.97 ppm for pyrophosphates, and –18.85 to –21.67 ppm for polyphosphates. The concentration of each P compound group was calculated via the percentage of the total area under each peak occupied in the spectra and the TP in the original extract.

Data analysis

Sedimentation rates of TP and TPM from traps were examined for spatial (i.e. among sites and between depths) and temporal (i.e. between sampling times) differences by using a one-way ANOVA with *post hoc* analysis using a Newman–Keuls test at a confidence interval of 95%. Before analysis, data were first tested for normality and log-transformed if necessary.

Statistical relationships of the elemental composition of lake bed sediments were tested among TP, Fe, Mn, Al and LOI by using Pearson correlation coefficients.

The decay of P compounds and compound classes was modelled as $P(t) = P_0 \times e^{-kt}$, where $P(t)$ is the amount of the P compound that has not yet decayed at time t , P_0 is the initial amount of the P compound that will decay and k is the decay constant (year⁻¹). Half-lives (τ) of P compounds in sediments were then calculated as $T_{1/2} = \ln(2)/k$ (Reitzel *et al.* 2007). Half-lives were determined from the slope of a significant ($P < 0.05$) regression fit to the exponential decay of P concentration with sediment profile depth, using averaged values of the corresponding 2-cm intervals between February and June 2007 to account for potential short-term variations in half-lives. Sediment age for each 2-cm layer was calculated according to the net sedimentation rate in Lake Okaro (Trolle *et al.* 2008). They derived sedimentation rates from a volcanic eruption of nearby Mount Tarawera in 1886, which dispersed volcanic ash over much of the Rotorua district, including Lake Okaro, and

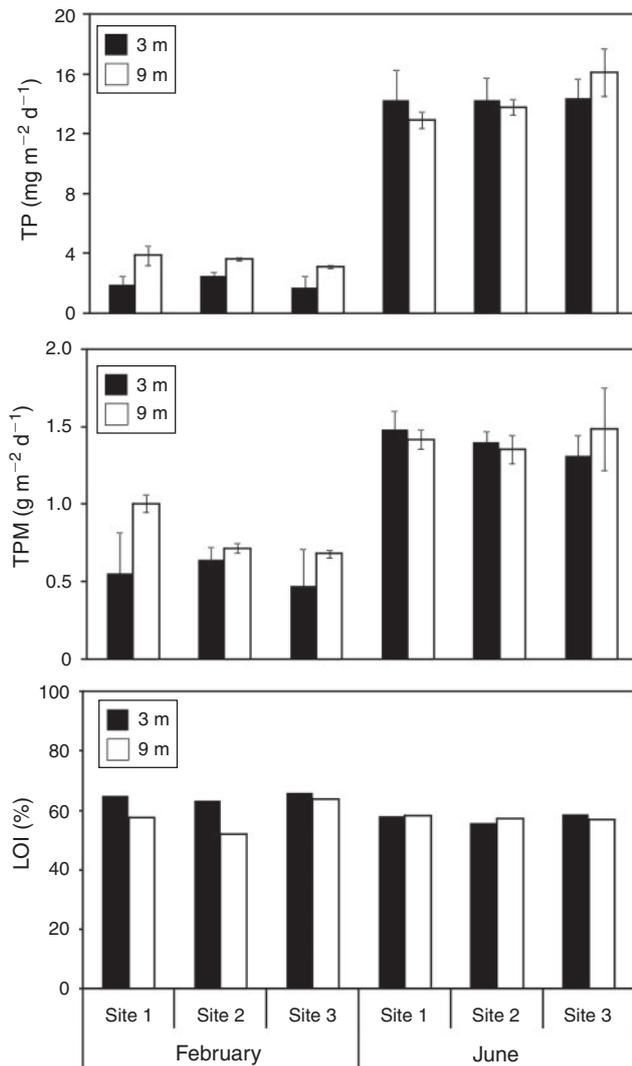


Fig. 2. Sedimentation rates of total phosphorus (mean \pm s.d., mg TP $m^{-2} day^{-1}$) and total particulate material (mean \pm s.d., g TPM $m^{-2} day^{-1}$), and loss on ignition (LOI) as a percentage of TPM, for traps at 3- and 9-m depths at Sites S1, S2 and S3 for stratified and mixed periods.

provided a well delineated tephra in the lake sediments. Therefore, the net sedimentation rate (2.3 mm year^{-1} , Trolle *et al.* 2008) for the lake represents averages for the entire period 1886–2007, but is likely to have varied within this period, especially between pre- and post-1960, when the lake has undergone eutrophication (Forsyth *et al.* 1988). The upper 10 cm of the sediment (the focus of this part of the analysis), however, is assumed to represent a time period during which the lake was highly eutrophic, with no significant land-use change in the catchment, and may therefore reflect sediment deposition of relatively consistent sedimentation rates.

Results

Sedimentation rates

Mean sedimentation rates of TP across the three sites for the period from 26 January 2007 to 28 February 2007, when the

Table 1. Matrix summary of results of *post hoc* Newman–Keuls test to determine differences among sites (S1–S3), depths (3 and 9 m) and sampling times (February and June) for sedimentations rates of total phosphorus (TP) and total particulate material (TPM)
n.s. = not significant ($P > 0.05$)

Parameter	S1	S2	S3	3 m	9 m
February					
TPM	9 m > 3 m	n.s.	n.s.	n.s.	n.s.
TP	9 m > 3 m	9 m > 3 m	9 m > 3 m	(S2 > S3) = S1	n.s.
June					
TPM	n.s.	n.s.	n.s.	n.s.	n.s.
TP	n.s.	n.s.	n.s.	n.s.	(S3 > S1) = S2
3 m					
TPM	Jun > Feb	Jun > Feb	Jun > Feb		
TP	Jun > Feb	Jun > Feb	Jun > Feb		
9 m					
TPM	Jun > Feb	Jun > Feb	Jun > Feb		
TP	Jun > Feb	Jun > Feb	Jun > Feb		

water column was stratified, were $2.06 \text{ mg m}^{-2} \text{ day}^{-1}$ at 3-m depth and $3.52 \text{ mg m}^{-2} \text{ day}^{-1}$ at 9 m (Fig. 2). Rates were approximately five-fold greater from 23 May 2007 to 22 June 2007, when the water column was mixed, at $14.30 \text{ mg m}^{-2} \text{ day}^{-1}$ and $14.26 \text{ mg m}^{-2} \text{ day}^{-1}$ at 3- and 9-m depths, respectively. Rates of sedimentation of TPM and TP for individual stations at 3 and 9 m were, in several instances, significantly ($P < 0.05$) lower for the stratified period than for the mixed period (Table 1). Differences in sedimentation rates among sites and between depths occurred less often than between the two time periods of June and February (Table 1) corresponding to thermally mixed and stratified conditions, respectively. Sedimentation rates of both TP and TPM were significantly ($P < 0.05$) greater at 9-m depth than at 3 m during the stratified period but not during the mixed period. The sedimentation rate of TPM averaged across sites during stratification was $0.56 \text{ mg m}^{-2} \text{ day}^{-1}$ at 3 m and $0.80 \text{ mg m}^{-2} \text{ day}^{-1}$ at 9 m compared with $1.40 \text{ mg m}^{-2} \text{ day}^{-1}$ at 3 m and $1.42 \text{ mg m}^{-2} \text{ day}^{-1}$ at 9 m during mixing (Table 1). On average, LOI constituted 61% of TPM across all sites and depths during stratification and 56% of TPM during mixing (Fig. 2).

Vertical sediment profiles

Phosphorus concentrations in bottom-sediment slices at all three sites and both sampling occasions ranged from $1965 \text{ mg P kg}^{-1} \text{ DW}$ (0–2 cm, S1, mixed period) to $366 \text{ mg P kg}^{-1} \text{ DW}$ (28–30 cm, S3, mixed period; Fig. 3). Generally, P concentrations in the sediment decreased with depth. Across all samples, concentrations of Fe, Mn and Al in sediment slices ranged from $24\,901$ to $11\,208 \text{ mg Fe kg}^{-1} \text{ DW}$, from 600 to $244 \text{ mg Mn kg}^{-1} \text{ DW}$, and from $16\,833$ to $6711 \text{ mg Al kg}^{-1} \text{ DW}$, respectively. LOI generally decreased with depth and ranged from 29.5% (0–2 cm, S1, stratified period) to 5.1% (28–30 cm, S1, stratified period). The concentration of TP was significantly correlated with LOI on the basis of data for all slices (S1: $r = 0.71$; S2: $r = 0.82$; S3: $r = 0.81$; $P < 0.001$). However, sediment P was

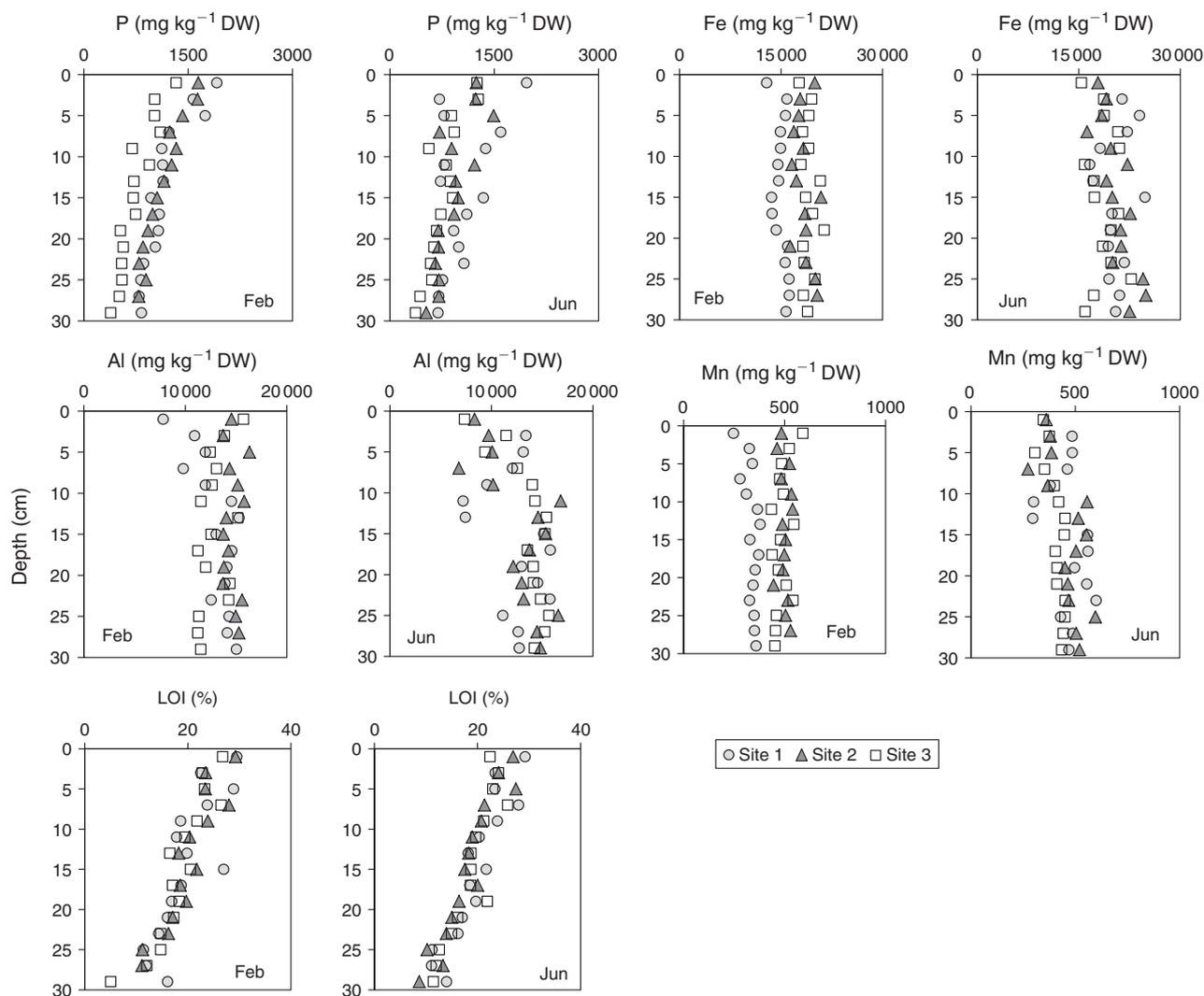


Fig. 3. Vertical profiles of sediment concentrations of total phosphorus ($\text{mg P kg}^{-1} \text{DW}$), iron ($\text{mg Fe kg}^{-1} \text{DW}$), aluminium ($\text{mg Al kg}^{-1} \text{DW}$), manganese ($\text{mg Mn kg}^{-1} \text{DW}$) and loss on ignition (LOI; %) at Sites S1, S2 and S3 for February and June samplings, corresponding to stratified and mixed periods, respectively.

correlated with Fe only at S2 ($r = 0.45$; $P \leq 0.05$) and showed no correlation with either Al or Mn ($P > 0.05$).

^{31}P NMR analysis

Eight different P compounds or compound classes were identified in the bottom sediment extracts at S2; however, only seven were found in the sediment trap samples at S2 (Fig. 4). The eight P compounds included phosphonates, orthophosphate, orthophosphate monoesters, pyrophosphates, polyphosphates, and three different compounds in the orthophosphate diester region. The latter compounds were suggested by Ahlgren *et al.* (2006) and others to be DNA P (-0.83 ppm), microbial P lipids (from 2.66 to -0.15 ppm) and teichoic acid P (2.97 ppm).

The total extracted P using NaOH + EDTA was compared to the extracted TP using aqua regia and the difference is referred to as extraction efficiency. The average percentage of TP extracted from the bottom sediments was 75%, but extraction of TP appeared to become less efficient with depth (Table 2).

Orthophosphate was the most abundant P compound in the bottom sediment extracts from S2, contributing up to 71.9% of the total extracted P, whereas orthophosphate monoesters contributed up to 37.2%, orthophosphate diesters up to 21.7%, polyphosphates up to 11.9%, pyrophosphates up to 6.7% and phosphonates only as much as 3.2%, on average, of the total P extracted. Orthophosphate concentrations generally increased with increasing depth, whereas all other identified P compounds or classes decreased with depth.

The signal to noise ratio of the ^{31}P NMR signal was 133 in the mixed period (June 2007), which was lower than in the stratified period (February 2007), when it was 148. During both periods, the signal to noise ratio was mostly lower in the surface sediments, with less well developed peaks for microbial-P lipids and teichoic-acid P in particular. Although polyphosphates were present throughout the profile in the stratified period, this group was not observed in sediment layers of 4–6 and 8–10 cm in June 2007. Because of the low concentrations of

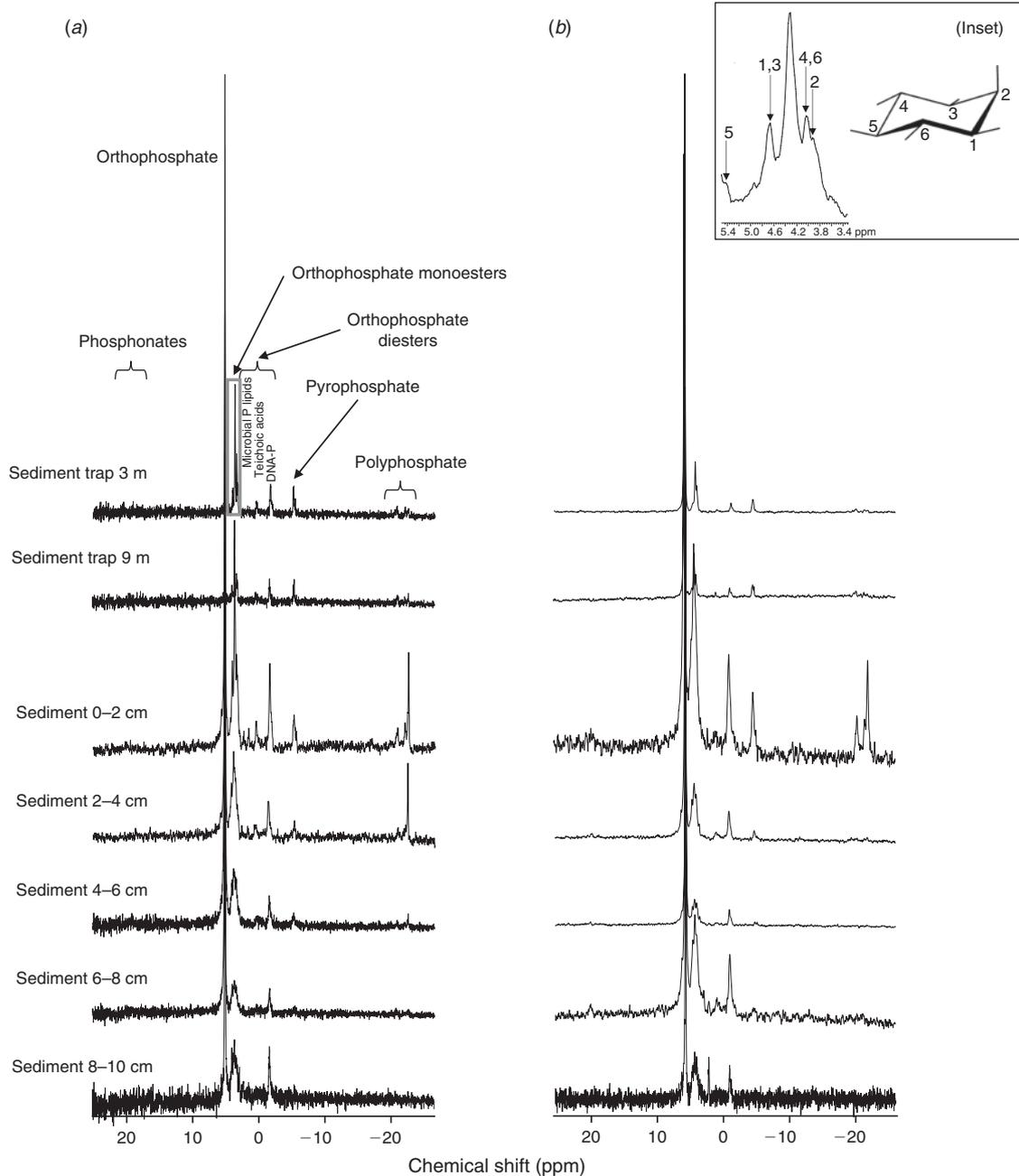


Fig. 4. ^{31}P nuclear magnetic resonance (NMR) spectra of depth profiles of sediment samples down to 10-cm depth from Site S2, collected in (a) February and (b) June 2007, corresponding to stratified and mixed periods, respectively. The inset shows the expanded orthophosphate monoester region (exemplary illustration for sediment sample from 0 to 2 cm, taken in February) with four distinct resonances that have previously been identified as *myo*-inositol hexakisphosphate (Cade-Menun 2005; Turner *et al.* 2005).

phosphonates through the profiles, it is not possible to distinguish whether differences between sampling days were due to the limitations in analytical sensitivity or a distinct change with time.

The model used to determine the decay of P compounds and compound classes resulted in a significant function for orthophosphate monoesters, orthophosphate diesters, pyrophosphates and polyphosphates (Fig. 5). These functions were used to calculate the half-lives for the different P compounds and

compound classes, which ranged from 8 to 23 years, with the half-life being longest for orthophosphate monoesters and shortest for polyphosphates.

The mean extraction efficiency across all depths in the sediment trap material increased from 50% in February 2007 to 83% in June 2007 (Table 3). The mean percentage of orthophosphate in the sediment-trap extract was 35% across all depths in February and 69% in June, whereas other P compound classes decreased.

Table 2. Concentration (mg kg⁻¹ DW) and relative contribution (in parentheses) of phosphorus (P) compounds and compound classes in sediment extracts at different depths from Lake Okaro at Site S2, for February and June 2007

Sediment age refers to the estimated average time since initial sediment deposition (after a volcanic eruption of nearby Mount Tarawera in 1886) at different layer depths. Extraction efficiency refers to the total extracted P using NaOH + EDTA, compared with the extracted total P using aqua regia

Month	Sediment depth (cm)	Sediment age (years)	Phosphorus extraction efficiency (%)	Orthophosphate (mg kg ⁻¹ DW)	Orthophosphate monoesters (mg kg ⁻¹ DW)	Orthophosphate diesters (mg kg ⁻¹ DW)	Pyrophosphates (mg kg ⁻¹ DW)	Polyphosphates (mg kg ⁻¹ DW)	Phosphonates (mg kg ⁻¹ DW)
February	0–2	4.3	99.8	573.4 (34.9)	589.5 (35.8)	235.2 (14.3)	77.1 (4.7)	169.7 (10.3)	0.0 (0.0)
	2–4	13.0	78.9	670.9 (52.0)	403.7 (31.3)	109.0 (8.4)	25.8 (2.0)	81.1 (6.3)	0.0 (0.0)
	4–6	21.7	61.3	493.5 (56.8)	220.4 (25.4)	78.7 (9.1)	27.2 (3.1)	34.0 (3.9)	15.5 (1.8)
	6–8	30.4	82.8	685.2 (66.9)	206.7 (20.2)	86.7 (8.5)	13.3 (1.3)	22.1 (2.2)	10.5 (1.0)
	8–10	39.1	45.7	303.3 (50.0)	181.5 (29.9)	87.8 (14.5)	0.0 (0.0)	15.3 (2.5)	19.3 (3.2)
June	0–2	4.3	54.5	257.8 (38.1)	203.1 (30.0)	78.3 (11.6)	29.3 (4.3)	80.7 (11.9)	8.5 (1.3)
	2–4	13.0	99.7	675.4 (54.7)	349.0 (28.3)	131.0 (10.6)	27.8 (2.3)	25.5 (2.1)	25.6 (2.1)
	4–6	21.7	92.0	986.4 (71.9)	270.0 (19.7)	76.9 (5.6)	20.1 (1.5)	0.0 (0.0)	14.1 (1.0)
	6–8	30.4	60.5	205.2 (47.5)	145.3 (33.7)	65.0 (15.1)	0.0 (0.0)	9.4 (2.2)	6.7 (1.5)
	8–10	39.1	42.8	223.3 (58.9)	112.5 (29.7)	17.4 (4.6)	25.6 (6.7)	0.0 (0.0)	0.0 (0.0)

Discussion

Sedimentation and supply of P to sediments

Sedimentation rates of TPM in Lake Okaro were within the same range as those observed in other eutrophic lakes (Koski-Vähälä *et al.* 2000; Chalar and Tundisi 2001; Pettersson 2001). Rates were greatest during mixing when the P concentration of TPM increased ~2.5-fold, whereas the concentration of organic matter in the trapped material varied little between the mixed and stratified periods. On average, particulate matter in traps in Lake Okaro was enriched in P (7.1 mg TP g⁻¹ TPM) compared with that in other eutrophic lakes (Pettersson 2001, 1.3–5.8 mg TP g⁻¹ TPM). Seasonality of sedimentation rates can be strongly influenced by the discharge of the inflows to the lake (Fuentes *et al.* 2013). Precipitation in the Rotorua Region is highly seasonal, with a total amount of 167 and 47 mm, respectively, during the mixed (i.e. June) and stratified (February) sediment trap deployment period. The resulting increase in inflow discharge to Lake Okaro during winter may explain the increase in sedimentation rates of TPM. A disproportional increase in TP content of settling seston suggests high mobilisation rates of particulate P through erosion during high-flow events, as has been observed in other streams in the same region (Abell *et al.* 2013), and/or seasonality of in-lake phytoplankton biomass (Paul *et al.* 2008). The continuous supply of P-rich organic material to the surficial sediments and its resulting decomposition may enhance sediment release of P with the prolonged periods of anoxia in Lake Okaro, but will also be dependent on the bioavailability of P forms in seston (Søndergaard *et al.* 2003; Smolders *et al.* 2006).

Forms and bioavailability of phosphorus detected by ³¹P NMR in sediment

Central to our hypothesis that organic P compounds may regulate internal P loading in Lake Okaro is that the sediment contains and/or produces bioavailable organic P compounds. Most ³¹P NMR studies have found that organic P compounds such as orthophosphate diesters are more bioavailable than are orthophosphate monoesters (e.g. McDowell and Stewart 2005;

Turner *et al.* 2005). To further examine the bioavailability, we also determined half-lives for each P compound and compound class detected by ³¹P NMR. Half-lives could not be established for orthophosphate because there was no significant trend in concentration with sediment depth, presumably because there is a balance of its consumption and production in the bottom sediments. The apparent half-lives calculated in the present study should be interpreted as indicative, representing the net effect of concurrent P decay and generation processes for one site in the lake.

The P compounds and compound classes detected in the sediments of Lake Okaro, with the exception of phosphonates, have also been detected in sediments of other eutrophic lakes (Reitzel *et al.* 2006). The relative proportions of each are mostly comparable to those in less eutrophic lakes (Ahlgren *et al.* 2005; Reitzel *et al.* 2007). However, care should be taken when making direct comparisons among different studies because of differences in the treatment of the sample, extractants, spectrometers, and whether or not delay times between pulses were sufficient to exceed T_1 and generate quantitative spectra (Cade-Menun 2005; McDowell *et al.* 2006). Although the extractant used for preparation of ³¹P NMR sediment material in our study was similar to those used in other studies (Ahlgren *et al.* 2005; Turner *et al.* 2005; Reitzel *et al.* 2007), any extraction procedure for organic P is likely to induce some hydrolysis (Cade-Menun 2005). The combination of NaOH + EDTA appears to be the most universally accepted extraction technique and decreases degradation of polyphosphates as a result of the complexation of free metals with EDTA (Turner *et al.* 2003, 2005). However, pH stress of the alkaline extraction procedure can alter extracted compounds from their original form (McDowell and Stewart 2005). For example, altering the pH of lake water samples, or soil extracts to >13 has been shown to decrease the proportion of phosphonates, orthophosphate diesters and polyphosphates through hydrolysis or precipitation (McDowell and Stewart 2005). Hence, it is possible that some of these compound classes may be under-represented in our NMR spectra, whereas orthophosphate and orthophosphate monoesters may be over-represented.

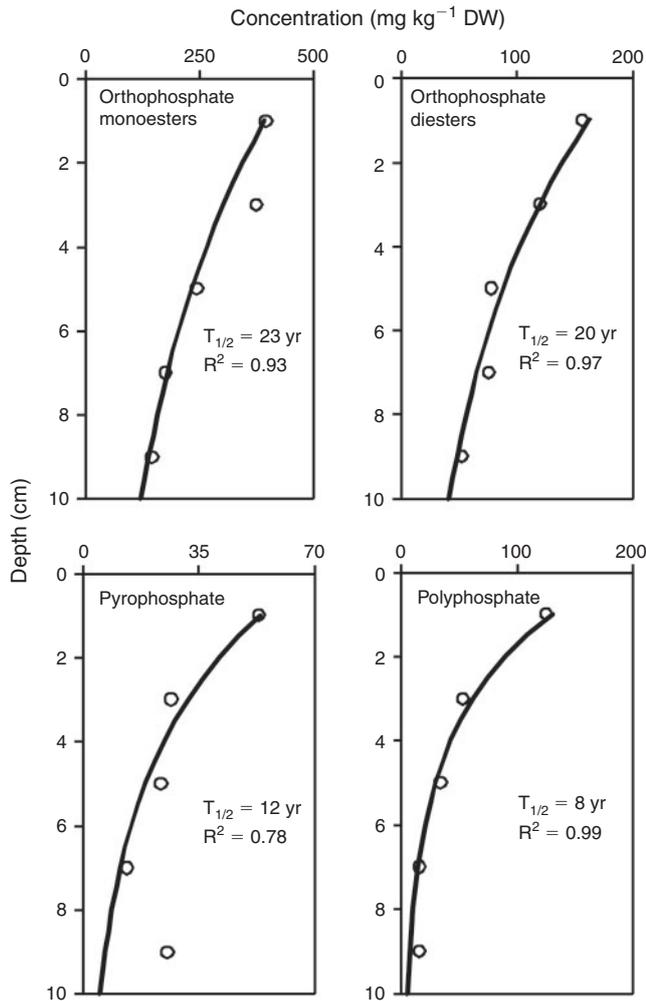


Fig. 5. Average concentrations of phosphorus compounds and compound groups (mg P kg^{-1} DW) between samples taken at Site 2 in February and June, identified using ^{31}P nuclear magnetic resonance (NMR), in the Lake Okaro sediment profiles, and exponential regression (solid line) fit to averaged measured concentrations of the corresponding 2-cm intervals (best fit of R^2 significant at $P = 0.05$).

Orthophosphate monoesters are usually the major organic P compound in soils and sediments (Turner *et al.* 2003) and were anticipated to be the most abundant in Lake Okaro. This group comprises several compounds with varying lability, of which the inositol phosphates (phytate) have often been found to be the most recalcitrant and are therefore usually dominant (DeGroot and Golterman 1993). However, Doolette *et al.* (2011) argued that the abundance of phytate in soil extracts can often be overestimated in the presence of broad humic P signals in the monoester region and that phytate is far less stable than was previously assumed. The expanded view of the orthophosphate monoester region in Fig. 4 shows four distinct resonances, which have previously been identified as phytate (Cade-Menun 2005; Turner *et al.* 2005); however, we were unable to quantify the abundance of phytate directly. It is likely that other P forms within monoesters were dominant in the present study, which is reflected in our half-life value of 23 years, being longer than for all other P compounds and compound classes. Given the presence and potential lability, phytates may provide a continuous low-level source of internal P loading in Lake Okaro during prolonged periods of anoxia of bottom waters in this lake (Özkundakci *et al.* 2010).

Orthophosphate diesters derived from soil organic P tend to be more labile and readily mineralised than are monoesters (Makarov *et al.* 2002). However, the apparent half-life of orthophosphate diesters found in our study was relatively long at 20 years, and similar to what has been found in other studies (Ahlgren *et al.* 2005). The presence of two orthophosphate diester compounds, phospholipids and teichoic acids, may indicate low rates of microbial activity (Makarov *et al.* 2002), which may partially explain the relatively long half-life of the diesters. The greatest proportion of orthophosphate diesters was occupied by DNA-P, which arises mostly from bacterial DNA and to some extent decomposing phytoplankton and has been found to have a half-life of less than a decade (Ahlgren *et al.* 2006). Given the high decay rate of this major constituent of a comparably large P pool in the sediment, DNA-P may be an important contribution to internal P loading relative to monoesters at 23 years (Ahlgren *et al.* 2005).

Pyrophosphate, a short-chain version of polyphosphates, is bioavailable and can support the growth of heterotrophic bacteria from different environments, even in orthophosphate-rich environments (Liu *et al.* 1982; Sundareshwar *et al.* 2001).

Table 3. Sedimentation rate of total phosphorus (TP) at Site S2 (mean \pm s.d.), loss on ignition (LOI), and relative sedimentation, and contribution of identified P compounds and compound classes (in parentheses) in the extracts from sediment traps at Site S2, for February and June 2007 samples. Extraction efficiency refers to the total extracted P using NaOH + EDTA, compared with the extracted TP using aqua regia. Sedimentation of TP was calculated as the mean of four replicate sediment traps at each depth, whereas the sedimentation rate of P compounds and compound classes was based on a pooled sample from four replicate sediment traps at each depth.

Month	Depth (m)	TP ($\text{mg m}^{-2} \text{day}^{-1}$)	LOI (%)	Phosphorus extraction efficiency (%)	Orthophosphate ($\text{mg m}^{-2} \text{day}^{-1}$)	Orthophosphate monoesters ($\text{mg m}^{-2} \text{day}^{-1}$)	Orthophosphate diesters ($\text{mg m}^{-2} \text{day}^{-1}$)	Pyrophosphates ($\text{mg m}^{-2} \text{day}^{-1}$)	Polyphosphates ($\text{mg m}^{-2} \text{day}^{-1}$)
February	3	2.50 \pm 0.27	64.7	44.5	0.36 (32.7)	0.39 (34.9)	0.15 (13.7)	0.09 (7.8)	0.12 (10.9)
	9	3.61 \pm 0.10	57.8	53.6	0.75 (38.9)	0.58 (30.0)	0.22 (11.6)	0.13 (6.9)	0.24 (12.6)
June	3	14.26 \pm 1.49	57.5	82.8	8.33 (70.6)	2.16 (18.3)	0.48 (4.1)	0.48 (4.0)	0.35 (3.0)
	9	13.78 \pm 0.52	57.6	84.3	7.88 (67.8)	2.11 (18.1)	0.53 (4.6)	0.48 (4.1)	0.62 (5.3)

We found an apparent half-life for pyrophosphate of 12 years, which is similar to the 13-year half-life of pyrophosphate found in mesotrophic Lake Erken by Ahlgren *et al.* (2005), but much longer than the 3-year half-life found for sediments from the Baltic Sea (Ahlgren *et al.* 2006). There may be several possible reasons for the longer half-life in our study and in Ahlgren *et al.* (2005) than in deep-sea sediment and it could be related to low concentrations or activity of specific pyrophosphatase enzymes and high mineralisation rates in seasonally anoxic hypolimnia of lakes that could cause degradation of substantial fractions of labile P in the settling seston before it reaches the sediment surface.

Phosphonates (direct C–P bond) were detected in the bottom sediment of Lake Okaro. These compounds are thought to be a by-product of protozoan metabolism, which is common to almost all aquatic habitats with high primary productivity (Carman *et al.* 2000; Ahlgren *et al.* 2005). They also accumulate in a wide range of soils as a result of bacterial activity (Tate and Newman 1982; Hawkes *et al.* 1984). Phosphonates are considered to be very stable compounds that are not readily hydrolysed even after long exposure to acidic and alkaline conditions (Ahlgren *et al.* 2006). Hence, their role in the internal cycling of P within lakes is thought to be minimal.

The half-life of polyphosphates was only 8 years. Polyphosphates contribute significantly to P-release following diagenesis, at rates comparable to P-release from iron oxyhydroxides (Hupfer *et al.* 2004). The short half-life of polyphosphates found in our study suggests that they may play a significant role in internal P loading in Lake Okaro. These results are not consistent with those of Kenney *et al.* (2001) who considered that polyphosphates are not geochemically reactive.

Vertical sediment profiles

The interpretation of vertical sediment P concentration profiles in lakes is inherently difficult because of complex interactions of diagenetic processes (Trolle *et al.* 2010). The correlation analysis between vertical profiles of TP and LOI, Fe, Al and Mn used in the present study may, however, provide some insight into the drivers affecting the vertical distribution of TP in Lake Okaro, albeit causation may not be inferred. Nonetheless, given a consistently strong relationship between TP and LOI in the sediments of Lake Okaro, it is reasonable to assume a causal relationship between these two variables. A lack of relationships between vertical sediment concentration profiles of TP and Fe, Al and Mn, conversely, does not imply that sediment P in Lake Okaro is not associated with these elements because they may be influenced by a disproportionate allochthonous supply and pH and redox conditions (Davison 1993).

Forms of phosphorus detected by ³¹P NMR in seston

The range of P compounds and compound classes in the settling seston in Lake Okaro was similar to that in the top layer of the sediment, except that no phosphonates were present in the settling seston, although there were differences in the relative concentrations and proportions of each compound class. Care must be taken, however, when directly comparing P compounds and compound classes in settling seston and surface sediments, because surface sediment P composition is influenced by both

settled material and P that migrates upward from deeper sediment layers. Seasonally induced changes as a result of mixing and the resulting changes in oxygen status in bottom waters appear to affect not only P concentrations, but also the relative contributions of P compounds in the settling seston. These changes were largely evident as an enriched P concentration of the settling seston and increased recovery as orthophosphate, compared with the surface sediments of Lake Okaro (Table 3). There appeared to be no discernible difference in polyphosphate concentrations in the surface sediments of Lake Okaro during periods of stratification and mixing, corresponding to anoxic and oxic periods, respectively, in bottom waters. In contrast, Hupfer *et al.* (2004) found a general decrease of polyphosphate concentrations in surface sediments during mixing periods, which they attributed to changes in accumulation and hydrolysis (i.e. uptake and release, respectively) by the different communities of microorganisms prevailing under oxic and anoxic conditions. Although the present study did not prove that there was no such synthesis of polyphosphate by microorganisms, it is possible that either a suitable carbon substrate was not available to facilitate luxury uptake and storage of P as polyphosphate (Khoshmanesh *et al.* 2002) or that changes in the communities of sediment microorganisms as a result of mixing may be less important during the early diagenesis of polyphosphates in Lake Okaro.

It has been suggested that pyrophosphates and polyphosphates in settling seston originate from the bacterial community colonising and mineralising settling particles (Hupfer *et al.* 2004; Reitzel *et al.* 2007). Such organisms accumulate and store phosphate as cellular polyphosphates under oxic conditions and these polyphosphates are then hydrolysed during anoxia, and released as orthophosphates (Hupfer *et al.* 2004). The reactivity and the relatively high abundance of polyphosphates in the settling seston in Lake Okaro suggested that this compound is likely to significantly contribute to enrichment of P in the water column before P reaches the bottom sediments, particularly during summer stratification when the hypolimnion is anoxic for several months (Özkundakci *et al.* 2010).

In conclusion, the deposition of seston in Lake Okaro was ~ five-fold higher in the mixing period (June) than in the stratified period (February). Concentration of P compounds and compound classes in the surface sediments of Lake Okaro showed a much lower seasonal variability and mostly reflected the deposition of P composition during stratification. The exponential decrease of organic P in the bottom sediments indicated that organic P is an important long-term source of internal P loading in Lake Okaro. The approach used in the present study, however, only partially identified the source of the organic P compounds in the sediment from the water column. Future work should include the analysis of catchment soils, which will contribute to a better understanding of the origin of different P compounds and compound classes in the lake sediment and, ultimately, provide for a targeted management approach for reducing external and/or internal P loadings.

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