Phosphorus fractions in sediments and their relevance for historical lake eutrophication in the Ponte Tresa basin (Lake Lugano, Switzerland) since 1959

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Abstract

Lake Lugano is one of several deep lakes in Switzerland that have not yet recovered from eutrophication after large reductions of external phosphorus (P) loadings. Persistent eutrophication has been attributed mainly to internal P loadings from sediments. To achieve the restoration goals, it is critically important to evaluate the sediment P availability and release risk in this lake. In this study, we combined sequential P extraction (four fractions) with enzyme hydrolysis to assess distribution characteristics of P forms and potential bioavailability of organic P in an anoxic sediment profile from the Ponte Tresa basin of Lake Lugano, southern Switzerland. Labile P forms, i.e. mostly redox-sensitive iron bound P and metal oxides bound P (Al/Fe-P), comprised ~70% of total P in the sediment profile (1959-2017 CE), suggesting a high potential for P release from the anoxic sediment. Potentially bioavailable organic P forms (determined by addition of substrate specific enzymes) were considerably higher in the surface sediments (top 5 cm), which is very likely to release P in the near future with early diagenesis. The net burial rates (NBR) of redox sensitive Fe-P fraction and total P in sediments both showed significant decreasing trends from 1959 to 2017 CE, when trophic levels of the lake

increased from mesotrophic to hypertrophic status. We suggest that, in the Ponte Tresa basin, higher eutrophication conditions led to enhanced sediment P release (mainly from redox sensitive Fe-P fraction), thus reducing P-NBR in sediments. This study highlights the concern that in deep monomictic lakes, eutrophication restoration might be hindered by extensive internal P cycling and reduced capacity of sediment P-trapping.

Keywords: Lake sediments; Deep lakes; Organic phosphorus; Labile phosphorus; Internal phosphorus loading.

1. Introduction

Eutrophication of aquatic ecosystems has been a global environmental concern for decades, especially in freshwater lakes (Cao et al., 2016; Hu et al., 2007; Smith et al., 1999). Excessive phosphorus (P) loading is recognized as one of the main causes of eutrophication as P is the limiting element for primary productivity in many freshwater lakes (Rothe et al., 2014; Worsfold et al., 2016). In many cases, however, management efforts focusing on reducing external P loads have resulted in delayed or even failed lake-system recovery from eutrophication (Søndergaard et al., 2001). The main cause for this was found to be the development of internal P loadings (P release from sediments), which recycles P back to the overlying water, thereby enhancing lake primary productivity and adversely affecting the lake trophic status (Gächter, 1987; Horppila et al., 2017; Tammeorg et al., 2016).

Phosphorus accumulated in lake sediments as a potential source of lake eutrophication has received considerable attention over the last decades. Numerous studies have applied sediment P fractionation to evaluate potential P availability and P release risks into lake water (Cavalcante et al., 2018; Jin et al., 2006; Kaiserli et al., 2002; Kangur et al., 2013; Ruttenberg, 1992). The rational of these fractionation procedures is that different P forms in sediments have different labilities and, thus, potentials to release P back into lake water. For example, apatite-P is considered a relatively stable P fraction contributing to permanent P burial in

sediments of most lakes (Zhang et al., 2013), whereas P bound to redox-sensitive Fe and Al/Fe (oxyhydr) oxides can potentially be released under anoxic or varying redox conditions (Burley et al., 2001; Lai and Lam, 2008). While inorganic P (P_i) forms in lake sediments were the focus of most previous studies, organic P (P_o) speciation has been relatively under-studied (Mitchell, 2005). However, in recent years, P_o has been demonstrated to be a significant component in lake sediments and might play the dominant role in sediment P release in some eutrophic lakes (Ahlgren et al., 2005; Torres et al., 2014; Zhang et al., 2008). To estimate potentially bioavailable P_o in lake sediments, enzymatic hydrolysis has been commonly considered as a useful approach (Zhu et al., 2018; Zhu et al., 2013).

In general, the distribution of P fractions in lake-sediment profiles is affected by various factors, such as external P inputs, sedimentation rates, sediment P release, sediment composition, early diagenetic processes, redox conditions, and other environmental conditions in lakes (Carey and Rydin, 2011; Kaiserli et al., 2002; Søndergaard et al., 1996; Trolle et al., 2010). Sedimentary P species also largely depend on the lake trophic status (Carey and Rydin, 2011; Torres et al., 2014; Torres et al., 2016; Wang et al., 2006). Eutrophication is usually associated with changes in physicochemical conditions such as higher pH in lake water and lower redox potentials in the water-sediment interface, which can prompt P mobility and change P forms in sediments (Cao et al., 2016; Zhang et al., 2009). Heretofore, studies mainly have focused on the relationships between surface sediment P-fraction concentrations and various trophic status in different lakes (Carey and Rydin, 2011; Gonsiorczyk et al., 1998; Huo et al., 2011; Jin et al., 2006; Zhang et al., 2009). To our knowledge, few studies have reported P-fraction net burial rates (NBR) in short sediment cores and their temporal responses to the eutrophication history of the lake. Moreover, most of the sediment P-fractionation studies were conducted in rather shallow polymictic eutrophic lakes (Gao et al., 2005; Søndergaard et al., 2003). By comparison, sedimentary P fractions in deep, eutrophic monomictic or even meromictic lakes remain poorly understood, in particular regarding the time series of P-fraction NBR.

In the present study, we examined a short sediment core from the Ponte Tresa basin (Lake Lugano). We have selected this basin because it is a deep, warm monomictic subalpine lake and its eutrophication history since the mid-last century is well documented. Importantly, Lake Lugano is suffering from a delay of eutrophication recovery despite the reduction of external P loads since the 1980s (Lepori and Roberts, 2017). This delay is mainly reflected by presently still enhanced primary productivity as well as phytoplankton assemblage typically found in eutrophic waters (Bechtel and Schubert, 2009; Simona, 2003). The recovery from anthropogenic eutrophication in Lake Lugano has been hindered by sediment P release (Lepori and Roberts, 2017) which is further reinforced by some symptoms of eutrophication (e.g. longer stratification and anoxic periods of up to one year) (Lepori et al., 2018). However, little is known about the P composition and availability in the sediment profiles of the lake, and the question how P fractions NBR in sediments changed with different trophic conditions through time.

The aims of this study were to (1) investigate P fractions and potential bioavailability of P_o in sediment profiles of the Ponte Tresa basin, and (2) explore how the P-fraction NBR in sediments have varied with different trophic levels of the lake over the recent past decades. For these purposes, a sequential extraction scheme and enzyme addition assay were employed to characterize different P forms and potentially bioavailable P_o in sediments. The temporal changes of P-fraction NBR were evaluated in relation to the reconstructed trophic history of the lake.

2. Materials and methods

2.1. Study site

Lake Lugano is a tectonic-glacial lake situated on the border between Switzerland and Italy in the southern Alps (Fig.1a). The lake is fed by a large number of mountain streams and rivers (Cannata et al., 2018), with a total catchment area of 565.6 km^2 (Fig.1a). The Ponte

Tresa basin (45°58'N, 8°52'E; Fig. 1b) is the smallest (surface area 1.1 km^2) and most shallow (maximum water depth at ~51m) basin of Lake Lugano connected to outlet river Tresa (Fig.1a) The direct watershed (land area, 5.6 km^2) of this basin is mainly made up of calcareous rocks, gneiss, and porphyry, and mean water residence time is relatively short (ca. 0.04 year; Simona, 2003). A more detailed description on the morphological and hydrological characteristics of the Ponte Tresa basin is reported in Simona (2003).

The climate in the Ponte Tresa catchment is classified as oceanic with warm summers and temperate winters. The basin water remains mostly ice-free during winter season (Franchini et al., 2017). The vegetation in the catchment is composed of oak (*Quercus petraea* and *Q. robur*) and chestnut (*Castanea sativa*) forests (colline belt) with agricultural fields in the lower parts. Residential and industrial buildings dominate on the Holocene alluvial fans to the north and west, and around the lake (Fig.1b). The Ponte Tresa basin has a warm monomictic mixing regime: it is seasonally stratified between early summer and mid-autumn (Premazzi and Marengo, 1982), and experiences typically one mixing event per year, usually in February or March (Lepori and Roberts, 2017).

Lake Lugano has a long history of nutrient pollution starting as early as in the 1930s-1940s (Lehmann et al., 2004). In the Ponte Tresa basin, nutrient enrichments in the lake water started after the 1940s due to increasing urbanization and industrialization in the watershed, and the first massive algal bloom occurred in 1958 (Schneider et al., 2018). The basin was classified as eutrophic according to the measured water phosphate concentrations during 1972-1982 (mostly between 45-160 $\mu g \cdot L^{-1}$ in the late summer months) (Premazzi and Marengo, 1982; Rimer, 2017). From ca. 1970 onwards, the basin has been eutrophic to hypertrophic as indicated by diatom-assemblages data (Lotter, 2001). The eutrophication in the Ponte Tresa basin has led to permanently or temporarily anoxic conditions on the surface of lake sediments (Züllig, 1982). In Lake Lugano, high productivity during summer also tends to cause an increase of pH and calcite preciptation in the surface waters (Bernasconi et al., 1997).

To manage eutrophication in Lake Lugano, several remediation measures were implemented to reduce external P loadings, such as the installation of wastewater treatment plants in 1976, and a ban on phosphates in detergents in Switzerland in 1986 (Barbieri and Simona, 2001; Span et al., 1994). These lake restoration endeavors have resulted in reductions of external P loads (from 77 to 36 tons per year) and lake-water P concentration during lake mixing (from 113 to 38 $\mu g \cdot L^{-1}$) in the southern basin of the lake from the early 1980s until the last decade (2006-2015) (IST-SUPSI, 2016). Nonetheless, primary productivity in the lake remains at high levels and, in the southern basin of Lake Lugano (connected to Ponte Tresa basin), annual primary production remained as high as > 400 g $C \cdot m^{-2} \cdot y^{-1}$ between 1983 and 2014 (Franchini et al., 2017).

2.2. Sediment coring and sampling

In September 2017, several short sediment cores were collected from the depocenter (i.e. the deepest point where the sediment surface is well preserved) of the Ponte Tresa basin using a UWITEC gravity corer (45°58'00.4''N, 8°51'56.5''E, Fig. 1b). After the cores were collected, they were tightly sealed, kept in a cooling box, and , within 5 hours , stored in a dark cold room (~4 °C).

The sediment core selected for this study (PTRE 17-2) was 105 cm long. It was stored for two months before opening. After opening and splitting lengthwise, one-half core (PTRE 17-2-A) was transferred immediately into a glove box with an anoxic atmosphere. Sampling in the glove box was done in a nitrogen (N_2) atmosphere to protect sediment samples from oxidation and possible changes in P forms (Lukkari et al., 2007a; Lukkari et al., 2007b). The sediments were continuously sampled from top 0 to 37.5 cm in 2.5 cm intervals. The fresh sediments for each sample slice were homogenized. After sampling the sediment for sequential P extraction (Section 2.3), the remaining sediment was freeze-dried for water contents measurements and NaOH-EDTA extraction (Section 2.4). The other half core (PTRE 17-2-B) was stored in the cold (4°C) conditions. After the sediment surface became oxidized and varves were more

visible, core pictures were taken with a Nikon D80 digital camera for visual stratigraphic correlation and chronology (Section 2.6).

2.3. Sequential P extraction

The P-fractionation extraction protocol was slightly modified from the method described by Lukkari et al. (2007a). In our procedure (Fig. 2), P in fresh sediments (corresponding to ~0.4 g dry weight) was sequentially extracted by 50 mL extractants into four different fractions (F1 to F4). The first two steps (F1 and F2) were performed in the absence of oxygen (N_2 atmosphere). Fraction 1 was extracted with 0.46M sodium cloride (NaCl) (ACS grade, Carl Roth GmbH&Co.), fraction 2 with 0.11 M sodium dithionite ($Na_2S_2O_4$) (assay (iodometric) ≥87 %, Merck KGaA) in 0.11 M sodium bicarbonate ($NaHCO_3$) (ACS grade, Merck KGaA) buffer (pH 7.0) which is referred to later as NaBD, fraction 3 with 0.1 M sodium hydroxide (NaOH) (R.G. grade, Hänseler AG), and fraction 4 with 0.5 M hydrochloric acid (HCl) (ACS grade). Extractant solutions were all prepared using deionized and filtered water (Merck MilliQ-water). All extractions were performed in triplicates for each sediment slice. All extraction steps described in Fig. 2 were carried out in Corning® 50 mL centrifuge tubes at room temperature using an orbital shaker table (400 rpm). The extraction time depended on the extractants (from 1 to 18 h, Fig. 2), and all rinse steps were 15 min. After each extraction and rinsing step, supernatant was seperated by centrifugation at 4000 rpm for 15 min at room temperature. We made the following adjustments compared to the originally published extraction method (Lukkari et al., 2007a). Firstly, the extraction procedure was ended after the F4 fraction because, in our study, we are not interested in the residuals after F4 fraction, which is mostly refractory organic P accoring to Lukkari et al. (2007a); Secondly, we did not aerate the supernatants of NaBD-extracted samples prior to colorimetric quantification of soluble reactive P, as recommended in the original method. The aeration procedure was skipped because we used the malachite green method (Ohno and Zibilske, 1991) instead of the molybdenum blue method to determine the soluble reactive P concentrations in all extracts (Lukkari et al., 2007a).

Tests showed that sample aeration between 0 to 4 hours had no effect on the quantified P concentrations in NaBD extracts (Fig. S1a-c in Supplementary data). In the malachite green method, decomposition products of dithionite in aerobic aqueous solutions have no effect on malachite green and the phosphomolybdate complex and thus, no interference with spectrophotometric analysis of soluble reactive P (Barberis et al.,1998). Lastly, P quantification in all obtained extracts (F1 to F4) was perfomed in the unfiltered samples. Pre-tests comparing colorimetrically determined P concentrations in both unfiltered and filtered samples (0.45 µm nitrocellulose membrane) from F1 to F3 fractions showed an average difference of 1.3% (data not shown), suggesting that particulate P (undissolved P) in the extracts was minor. This appears to agree with previous findings in the Ponte Tresa basin that a great part of particulate P is mineralized at the water-sediment interface (Premazzi and Marengo, 1982).

We assigned the four obtained P fraction (F1 to F4) to different P forms, according to the literature. NaCl-P_{tot} (F1) represents loosely adsorbed P on particle surfaces of sediments and in pore water. This P fraction can easily be released from the interstitial water to overlaying waters, and thus be available for algae growth (Jin et al., 2006; Ribeiro et al., 2008). NaBD-P_{tot} (F2) includes redox-sensitive fractions of P bound to hydrated oxides, mainly those of Fe. It has been confirmed that, under anoxic conditions, this fraction is highly labile and can be released from sediments to the lake water where it is available for algae growth (Ding et al., 2016; Rydin, 2000). NaOH-P_{tot} (F3) is partly inorganic P (NaOH-P_i) bound to metal oxides (mainly Al, Fe) and partly organic P compounds (NaOH-P_o). NaOH-P_i is potentially labile and may release P under anoxic or high pH environments (Rydin, 2000). NaOH-P_o contains moderately labile P_o in fulvic acids and refractory P_o bound with humic acids (Zhu et al., 2013). The NaOH-P_o fraction could also be bioavailable when labile parts of P_o are released and subjected to enzymatic hydrolysis (Monbet et al., 2007). HCl-P_{tot} (F4) is usually referred to as apatite-P and P bound to carbonates sourced from either co-precipitation with endogenic carbonates or from allochthonous lithogenic material (Gonsiorczyk et al., 1998; Kaiserli et al., 2002). This fraction is mostly considered as non-bioavailable and “permanently” buried P pool in lake sediments but it can dissolve in acidic environments (Wang and Liang, 2015).

We chose the described procedure because it highlights the redox sensitive Fe-bound P and organic P fractions (Lukkari et al., 2007a) which are crucial P pools in deep lakes with seasonally anoxic conditions in the hypolimnion (Gu et al., 2016). Sediments for extraction were shielded from oxygen until the start of step F3 of the procedure (Fig. 2) to avoid alterations in P and Fe fractionations due to oxidation artifacts (Lukkari et al., 2007a). However, this method only provides information about total P_o in sediments. It is unclear to what extent the total P_o in sediments is potentially bioavailable. Thus, enzymatic hydrolysis (see Section 2.4) was performed to estimate hydrolyzable and, thus, potentially bioavailable P_o in sediment samples.

2.4. NaOH-EDTA extraction and enzymatic hydrolysis

The extraction with 0.25M NaOH and 0.05M ethylenediamine tetraacetic acid (EDTA) was used for total P_o extraction on bulk sediments (Bowman and Moir, 1993; Turner et al., 2005; Zhang et al., 2009). For that, 30 ml NaOH-EDTA (0.25 M-0.05 M) solution was used for total P_o extraction on freeze-dried and homogenized bulk samples (~3 g). Total P_o (NaOH-EDTA P_o) was quantified as the difference between total P and inorganic P in NaOH-EDTA extracts.

The P_o in NaOH-EDTA extracts was further characterised into potentially bioavailable (i.e. enzyme-labile) and potentionally non-bioavailable (i.e. enzyme stabile) P_o. In principle, the NaOH-EDTA extract is treated with a combination of different P_o hydrolysing phosphatase enzymes (Jarosch et al., 2015). The increase in P_i in the extract is indicative on the amount of P_o hydrolysed by the substrate specific enzymes. By using a combination of different phosphatases the potential bioavailabilty of P_o can be estimated. Three different enzymes (an acid phosphatase (Sigma P1146), a phosphodiesterase (nuclease, Sigma N8630), and a phytase (Ronozyme ® HiPhos (M)) were used in combination to determine enzyme-labile P_o in the NaOH-EDTA extract. Enyzme dillutions were chosen to ensure full hydrolysis of added model compounds (glycerol phosphoate (Sigma G6501), deoxyribonucleic acid (Sigma D3159)

and phytic acid (Simga P8810)) during incubation at 37°C for 24h. Incubations were performed on 96-well plates with eight analytical replicates per sediment sample under sterile conditions.

2.5. Extracts and sediment sample analysis

Phosphorus extracted in each fraction (F1-F4, Section 2.3) and NaOH-EDTA extracts (Section 2.4) was divided into inorganic P (P_i) and organic P (P_o). The P_i concentrations were measured in the unfiltered samples colorimetrically by the malachite green method (Ohno and Zibilske, 1991) which quantifies the same P_i as the traditional molybdenum blue mothed (Murphy et al., 1962), yet with a higher accuracy at lower P_i concentrations (Uemura et al., 2010). We used 4 mL polystyrene macro-cuvettes with a 10 mm light path, and determined the absorbance at 610 nm using a UV-1800 UV-VIS spectrophotometer (Shimadzu Europe GmbH, Germany). Calibration curves for all colorimetrial analyes were made including the respective extract matrixes. Total P in extract samples was measured by inductively coupled plasma mass spectroscopy (7700x ICP-MS) (Agilent Technologies, Germany) after the dilution with nitric acid (HNO_3) to reach a final concentration of 1% v/v HNO_3 in Corning® 15 mL centrifuge tubes. The P_o in extracts was determined by the difference between total P and P_i. Total P in sediments was obtained from the sum of total P of the four fractions (F1-F4) in Section 2.3. In the same way, total P_i and total P_o in sediments were calculated by the sum of P_i and P_o of the four fractions, respectively.

Water contents (%), dry bulk density ($g \cdot cm^{-3}$), and sediment mass accumulation rates (MAR; $g \cdot cm^{-2} \cdot y^{-1}$) were determined according to the method of Håkanson and Jansson (1983). The concentrations and net burial rates NBR (fluxes) of P fractions in sediments (Section 2.3-2.4) are expressed as $mg \cdot kg^{-1}$ DW (dry weight) and $\mu g \cdot cm^{-2} \cdot yr^{-1}$, respectively. We calculated NBR of all P fractions by multiplying sediment MAR and P-fraction concentrations in sediments.

2.6. Sample dating

The sample ages of Core PTRE 17-2 were based on careful visual stratigraphic correlation (layer by layer) with the chronology of Core PTRE 15-3 (Schneider et al., 2018) which was dated with varve counting and historically documented flood-layer markers. Characteristic marker layers (e.g. flood layers, algae bloom layers, conspicuously thick varves) were identified in Core PTRE 17-2 and used for precise correlation between the two cores (Fig. 3). The mean age of each sample was obtained by averaging the ages of the top and bottom depth of the sample.

2.7. Data analysis

Statistical analysis was performed using R statistical computing (R Development Core Team, 2017). Significant trends in time series of NBR of different P fractions and TP in sediments were tested using univariate Mann-Kendall's trend test at the 5% significance level (R-package "Kendall"; McLeod, 2005).

3. Results

3.1. Sample ages

The sediment core of PTRE 17-2 correlated well with the core PTRE 15-3-A from Schneider et al. (2018) but was less compacted (Fig. 3). The mean age of each sample with the age uncertainty is shown in the right table of Fig. 3. The average resolution of each sample is approximately 4 years and the sediment sample in the lowest part analyzed (35-37.5 cm) dates back to 1959 CE (Common Era).

3.2. Sequentially extracted P fractions in sediment profiles

Total P concentrations of all P fractions (F1-F4), and P_i and P_o concentrations of each fraction in sediment profiles (0-37.5 cm) are shown in Fig. 4. Total P (TP) concentration in sediments ranged from 972 to 5143 $mg \cdot kg^{-1}$ (Fig. 4a). Sedimentary P was mainly present in the inorganic form (Fig. 4b-e), with total P_i concentrations ranging from 739 to 4527 $mg \cdot kg^{-1}$ (average ~79% of TP in sediments). In the NaBD-P_{tot} and HCl-P_{tot} fractions, most of the P was present as P_i (average 83% and 80%, respectively), whereas in the NaCl-P_{tot} and NaOH-P_{tot} fractions, the proportions of P_i were relatively lower (average 70% and 52%, respectively).

The rank order of P fractions in the whole sediment profile was NaBD-P_{tot} (472-2677 $mg \cdot kg^{-1}$, 36-60% of TP in sediments) > NaOH-P_{tot} (325-2000 $mg \cdot kg^{-1}$, 20-39% of TP) > HCl-P_{tot} (134-613 $mg \cdot kg^{-1}$, 7-38% of TP) > NaCl-P_{tot} (34-209 $mg \cdot kg^{-1}$, 2-10% of TP), as seen in Fig. 4b-e (relative proportions are given in Fig. S2 in Supplementary data).

The vertical variation of TP and P-fraction concentrations in sediment profiles show that TP, NaBD-P_{tot} and NaOH-P_{tot} fractions exhibited a generally similar pattern (Fig. 4a, c, and d). The concentrations of TP, NaBD-P_{tot} and NaOH-P_{tot} sharply increased from the bottom (35-37.5 cm, 1959 CE) to a maximum value at 27.5-30 cm (1969 CE). They had a generally decreasing trend towards the topmost sediments, except for a short but distinct peak at a depth of 5-7.5 cm (2004 CE). In contrast, NaCl-P_{tot} and HCl-P_{tot} showed a different structure (Fig. 4b and 4e). NaCl-P_{tot} increased from the bottom depth to a peak at 17.5-20 cm (1987 CE), and then declined towards the surface sediments. HCl-P_{tot} decreased from the bottom until a depth of 15-17.5 cm (1990 CE) and then, after a sharp peak at 10-12.5 cm (ca. 1998 CE), declined towards the surface sediments.

3.3. Organic P and enzymatic hydrolysis of P_o from NaOH-EDTA extraction on bulk sediments

The average recovery of NaOH-EDTA P_o in all sediment samples was 124%, compared with the sum of P_o from the sequential P extraction (F1-F4, Section 2.3; Table S1 in

Supplementary data), suggesting that a large proportion of sediment P_o was extracted with the NaOH-EDTA procedure. NaOH-EDTA P_o concentrations in sediments ranged from 162 $mg \cdot kg^{-1}$ to 991 $mg \cdot kg^{-1}$, which contributed to 13-57% of NaOH-EDTA TP in sediments (Fig. 5a and Fig. S3b in Supplementary data). The vertical variations of NaOH-EDTA P_o in sediments throughout the profile did not follow a discernable pattern (Fig. 5a). However, the sediments above the depth of 32.5 cm obviously contained more NaOH-EDTA P_o than those in the bottom layers (32.5-37.5 cm).

Enzyme labile P_o concentrations varied between 56 and 730 $mg \cdot kg^{-1}$, with contributions of 9-91% to NaOH-EDTA P_o (Fig. 5b). In the sediment profile (Fig. 5a), enzyme labile P_o also displayed higher contents above the depth of 32.5 cm. The concentrations of enzyme labile P_o remained relatively constant in the middle-section sediments (7.5-27.5 cm), but then showed an increasing trend towards the topmost sediments (0-7.5 cm).

3.4. Phosphorus fractions net burial rates (NBR) in sediments during 1959-2017 CE

We define four stages in the time series of net burial rates (NBR) of all P fractions and sedimentary TP from 1959 to 2017 CE (Fig. 6b), based on the differences of the P data among different stages.

In Stage I (1959-1963 CE; 37.5-32.5 cm), most of the P fractions and TP in sediments had high NBR, except for NaCl-P_{tot}, NaOH-P_o, and enzyme labile P_o with low NBR values. This stage was marked with multiple flood layers in sediments and considerably high mass accumulation rates (MAR) with values exceeding 0.08 $g \cdot cm^{-2} \cdot yr^{-1}$.

In Stage II (1963-1973 CE; 32.5-25 cm), NBR of NaBD-P_{tot}, NaOH-P_i, enzyme labile P_o and TP of sediments remained high, whereas NaCl-P_{tot}, NaOH-P_o, and HCl-P_{tot} had generally low NBR values. The MAR values declined from Stage I, remaining at ~ 0.07 $g \cdot cm^{-2} \cdot yr^{-1}$.

In Stage III (1973-2004 CE; 25-7.5 cm), compared with Stage II, NBR of labile P fractions, enzyme labile P_o and TP of sediments decreased. At the same time, NaCl-P_{tot} and NaOH-P_o

NBR increased. HCl-P_{tot} and enzyme stabile P_o NBR remained mostly at the same levels as in Stage II. After 1994 until 2004 CE, all the P fractions and TP NBR increased, except for enzyme stabile P_o with a decrease at ~ 2000 CE. The MAR in this section was mostly constant except for a sharp increase after 1994 CE.

In Stage IV (2004-2017 CE; 7.5-0 cm), enzyme labile P_o and NaOH-P_o showed enhanced NBR values, whereas NBR of other P fractions and TP of sediments decreased to the lowest levels. The MAR firstly declined but increased again after ~ 2010 CE.

The results from the univariate Mann-Kendall trend test are shown in Table S2 (in Supplementary data). From 1959 to 2017 CE, we observe significantly decreasing trends in the NBR of sediment TP and NaBD-P_{tot} fraction (TP: Kendall Score (S) = −37, p-value = 0.04; NaBD-P_{tot}: S = −38, p-value = 0.04). For other P fractions, no significant trends in NBR were found (p-values > 0.05).

4. Discussion

4.1. Phosphorus composition in sediments of the Ponte Tresa basin (1959-2017 CE)

NaBD-P_{tot} is the overall largest P-fraction in sediments of the Ponte Tresa basin (since 1959 CE). According to Ribeiro et al. (2008) and Ding et al. (2016), this is the most important fraction for sediment P release to lake water under anoxic conditions. Indeed, the recent sediments in the Ponte Tresa basin are anoxic (Schneider et al., 2018; Züllig, 1982), implying that there is still a high potential for sediment NaBD-P_{tot} release back to the lake water supporting continuing eutrophication.

NaOH-P_{tot} is the second largest P form in our sediments. The inorganic part of this fraction (i.e. NaOH-P_i) also constitutes a relatively large part of sediment TP (~24%). The anoxic sediment conditions of the Ponte Tresa basin could also prompt P release from NaOH-P_i. However, P release from NaOH-P_o is more related to organic matter degradation in sediments (Huo et al., 2011; Zhu et al., 2013). When the mineralization process of organic matter in

sediments of Ponte Tresa increases due to a higher trophic level of Lake Lugano (Barbieri and Mosello, 1992), NaOH-P_o might be released from sediments.

HCl-P_{tot} is the third largest P form with generally constant contributions to sedimentary TP (Fig. 4a and Fig. S2 in Supplementary data), which are within the range of HCl-P_{tot} proportions of TP for calcareous lake sediments (Gonsiorczyk et al., 1998; Jin et al., 2006; Kaiserli et al., 2002; Zhang et al., 2013). The calcareous rocks in the watershed (Harloff, 1926; Salmaso et al., 2007) and endogenic Ca-P formation during $CaCO_3$ precipitation in the lake are possibly responsible for the high contents of sediment HCl-P_{tot} in this basin. Nevertheless, this fraction is minor and relatively stable in sediments compared with NaBD-P_{tot} and NaOH-P_{tot}. HCl-P_{tot} is thought to be released only in low pH environments (pH < 6) (Jin et al., 2006). In Lake Lugano, pH in the surface waters was observed to vary from 7 to 9 during the annual cycle (Bernasconi et al., 1997). The pH in the hypolimnion was measured between 6 and 7 during summer stratification of the Ponte Tresa basin (Rimer, 2017), which is unlikely to favor sediment Ca-P release at the water-sediment interface.

NaCl-P_{tot} is the smallest P fraction in the sediments under investigation. NaCl-P_{tot}, NaBD-P_{tot}, and NaOH-P_i fractions are generally considered as potentially reactive or labile P forms in lake sediments and, therefore, critically important P sources for internal P loadings (Cavalcante et al., 2018). As the concentrations of NaCl-P_{tot} were very low (Fig. 4a), NaBD-P_{tot} and NaOH-P_i represent the major labile-P fractions in the present study. Therefore, our results imply that under anoxic conditions, there is still a large proportion of labile P present in the sediments (~70% of TP, 1959-2017 CE) which might, potentially, be released as internal P loads to the overlying water.

4.2. Distribution and potential bioavailability of P_o in sediment profiles

Organic P (P_o) production within Lake Lugano is predominantly derived from autochthonous organic matter (Bechtel and Schubert, 2009; Bernasconi et al., 1997) and

increases, in principle, with higher primary productivity. However, P_o preserved in sediment profiles is, instead, the net balance of P_o sedimentation, which is the result of P_o deposition to the sediment-water interface and P_o recycling back to lake water or diffusion into deeper layers after early diagenesis and P_o degradation over time (Matisoff et al., 2016; Zhu et al., 2018). Microbial phosphatase enzymes have been found in sediments of numerous reported lakes and may play an important role in the P release from anoxic breakdown of sedimentary P_o (Torres et al., 2016; Zhang et al., 2007; Zhu et al., 2018). Additionally, the decomposition of organic matters can alter the pH-redox conditions within the sediments facilitating the P release to pore water (Yuan, 2017).

Because of the natural degradation of P_o during diagenesis (Ahlgren et al., 2006; Zhang et al., 2013), declines of P_o with increasing sediment depth have been widely observed in mesotrophic-eutrophic lakes, such as Lake Erken (Ahlgren et al., 2005; Reitzel et al., 2007), Lake Taihu (Ding et al., 2013), Lake Dianchi (Zhu et al., 2018), and also in the Baltic Sea (Ahlgren et al., 2006). In the Ponte Tresa basin, we observed no clear declines in concentrations of NaOH-EDTA P_o with greater sediment depth (Fig. 5a) and total P_o of sediments from sequential P extraction (Fig. S4 in Supplementary data; Section 2.3). This may suggest that no intense diagenetic changes occurred over time or the mineralization of P_o was coincidently balanced by its sedimentation. Nevertheless, considerably higher P_o concentrations in the top 0 to 32.5 cm sediments can be likely attributed to increased P_o- and OM-sedimentation with eutrophication.

In the Ponte Tresa basin, enzyme labile P_o (i.e. potentially bioavailable P_o) concentrations showed a strong decrease from the surface sediments to a depth of 5 cm (Fig. 5a), which might be associated with the early degradation of fresh organic matters. According to Torres et al. (2016) and Wobus et al. (2003), microbial phosphatase activity in surface sediments was higher than in deeper layers from many reported lakes in the USA and Europe. Therefore, it appears likely that the recently-sedimented labile P_o in sediments of the Ponte Tresa basin would be degraded by microbes and, eventually, diffuse back to the lake water. The relatively

constant enzyme labile P_o in the middle-section sediments (7.5-27.5 cm) is closely related to similarly stable NaOH-EDTA P_o contents (Fig. 5a). This could be explained by the fact that at greater depth, microbial phosphatase activities and corresponding enzyme hydrolysis of P_o have been stabilized in sediments (Reitzel et al., 2007). Interestingly, the maximum contents of enzyme labile P_o existed at greater depths (27.5-32.5 cm). Several studies have suggested that Al/Fe (oxyhydr) oxide minerals in sediments can absorb and stabilize P_o in-situ and, thus, protect P_o from extracellular enzymes hydrolysis (Ruttenberg and Sulak, 2011; Zhu et al., 2018). The high amounts of NaBD-P_{tot} and NaOH-P_i fractions in these layers (27.5-32.5 cm) (Fig. 4c and d) indicated high sedimentary Al/Fe contents, which might help stabilize and preserve labile P_o in sediments. The high Al/Fe contents can possibly explain the high contents of enzyme labile P_o in these deeper layers.

4.3. Relationship between P-fraction NBR in sediments and lake trophic state since 1959 CE

In the Ponte Tresa basin, the eutrophication history since the 1920s is very well documented by sedimentary green pigments data (Chl-*a* and Pheophytin-*a*) at almost annual resolution (Fig. 6a) (Schneider et al., 2018). In our study, the time-series of P-fraction NBR only focused on the period when lake mesotrophic conditions changed to eutrophic or even hypertrophic conditions around 1960 CE. Based on our results, during the last 50-60 years, we observed marked differences in the NBR of different P forms and TP in sediments among Stages I-IV (Fig. 6b), which generally coincided with different lake trophic conditions since 1959 CE.

In Stage I and Stage II (1959-1973 CE), overall increasing green pigments concentrations and fluxes (Fig. 6a) indicate that the lake was transitioning from mesotrophic to eutrophic conditions (i.e., the trophic level was lower than today but increasing). This is also supported by the study from Salmaso and Mosello (2010). During this period, NBR of TP in sediments and labile P fractions (here NaBD-P_{tot}, NaOH-P_i and enzyme labile P_o) were clearly higher.

This might result from high external P loads and relatively less sediment P release. During this time period (1959-1973 CE), large external P-loadings were reported from Lake Lugano (Lehmann et al., 2004), which could have resulted in enhanced P sedimentation to surface sediments. Meanwhile, the Ponte Tresa basin had shorter summer stratification/anoxic periods (compared to Stage III) and oxic conditions in the hypolimnion persisted during winter (Züllig, 1982). This should, in principle, have caused less P release from sediments to lake water, and resulted in less dissolved P accumulation in the hypolimnion. Consequently, more NaBD-P_{tot} and NaOH-P_i were preserved in sediments. Moreover, the high NBR of potentially bioavailable P_o was likely related to Al, Fe oxides/minerals as discussed in Section 4.2. However, during Stage I (1959-1960 CE), high NBR of green pigments and some P fractions (e.g. NaBD-P_{tot}, NaOH-P_i) might be biased through the presence of flood layers and related sediment focusing (including erosion of littoral sediments), both giving rise to very high sediment mass accumulation rates (MAR). High values of MAR, in turn, would result in high NBR of sediment P and green pigments, although the concentrations of both were relatively low (Fig. 4 and Fig. 6a).

In Stage III (1973-2004 AD), the Ponte Tresa basin was in highly eutrophic to hypertrophic conditions, which is reflected by high levels of green pigments fluxes and several algal blooms (the peaks of green pigment fluxes; Fig. 6a). Similar conclusions were reported from studies of Lotter (2001) and Bechtel and Schubert (2009). It is noteworthy that, under severely eutrophic conditions, NBR of NaBD-P_{tot}, NaOH-P_i and TP in sediments were generally lower than before (Stage II). This result may be explained by a combined effect of enhanced P release from sediments due to ongoing eutrophication and progressing hypolimnetic anoxia. In seasonally anoxic lakes (e.g. the Ponte Tresa basin and the southern basin of Lake Lugano), and when lake productivity remains sufficiently high, it is very likely to engender prolonged stratification (up to one year) with more severe anoxia at the water-sediment interface (Lepori et al., 2018). Anoxic conditions favor sediment P release from NaBD-P_{tot} and NaOH-P_i fractions and, as a result, the NBR of these two fractions in sediments declined compared with Stage II. Moreover, Al/Fe oxyhydroxides would have decreased in the sediments of this section

compared with Stage II, given the low amounts of Al/Fe bound P fractions (i.e. NaBD-P_{tot} and NaOH-P_i). Therefore, extracellular phosphatase activity in sediments has been possibly less affected by the protection of P_o by Al/Fe oxyhydroxides. Consequentially, P_o hydrolysis in the sediment layers of Stage III (maximum eutrophication) was likely enhanced. This might be the main reason for lower amounts of enzyme labile P_o preserved in sediments during Stage III. The enhanced NBR of TP and most of the P fractions during the upper part of Stage III (1994 to 2000 CE, 7.5-15 cm depth) is also noteworthy, but it seems to be influenced by increased MAR (possibly not all related with flood layers) whereas the concentrations of P fractions and TP in sediments changed with little variation (Fig. 4).

In Stage IV (2004-2017 CE), lake productivity slightly decreased until 2006 CE (see green pigment fluxes, Fig. 6a), but the basin became again more eutrophic up to the present. In the 1980s until the last decade, external P loadings in the entire Lake Lugano have been reduced by ~ 50% (Barbieri and Simona, 2001; Lepori and Roberts, 2017). In the Ponte Tresa basin, the contrast between higher eutrophic status and low P NBR in sediments would suggest a substantial contribution of internal P loads from sediments under high eutrophic levels, which in turn sustained or augmented on-going eutrophication. This process was possibly further favored by complete mixing events after a long stratification period 2006-2009 CE (Schneider et al., 2018; Veronesi et al., 2002) when P from the hypolimnion was brought up to the photic zone. Such high internal P loads were also observed in the southern basin of Lake Lugano, where they were estimated to contribute about 40% to the turnover TP in the lake water during the last decade (Lepori and Roberts, 2017).

Overall, we found significantly decreasing trends in NBR of sediment TP and NaBD-P_{tot} from 1959 to 2017 CE, the time with increasing eutrophic levels in the Ponte Tresa basin. Our results might indicate that, in such a deep seasonally-stratified lake as the Ponte Tresa basin, higher eutrophication levels would be associated with enhanced sediment P release (mainly from the Fe-P fraction). This can serve as a plausible explanation for the reduced sediment TP and NaBD-P_{tot} NBR in the past few decades. On the other hand, our study suggests an

importance of sediment P release (internal P loadings) for the delayed recovery of eutrophication in Lake Lugano, similarly to the conclusions in Lepori and Roberts (2017). Furthermore, during the last few decades climate warming has influenced Lake Lugano's restoration by affecting the surface water temperature and mixing regime of the lake (Lepori and Roberts, 2015). One implication of this study is that the warming scenario would very likely enhance stratification in the Ponte Tresa basin and more internal P loadings might be expected.

5. Conclusions

The P-fractionation results show that labile P fractions (mainly NaBD-P_{tot} and NaOH-P_i) were the dominant forms (~70% of TP) in sediments of the Ponte Tresa basin (1959-2017 CE). The anoxic sediment environment highlights high potentials for P-release from the labile P fractions sustaining continuing eutrophication of the lake. The potentially bioavailable P_o in deeper layers seems to be stabilized, but high concentrations of enzyme labile P_o in the top-most sediments (0-5 cm) are very likely to degrade and release P in the future.To the best of our knowledge, this study is the first to investigate the relationship between P-fraction NBR in sediments and historical lake eutrophication of the last few decades in a deep, eutrophic lake. It is interesting to observe that NBR of sediment TP and the NaBD-P_{tot} fraction showed significantly decreasing trends under more eutrophic conditions since the 1960s. We further suggest that, in seasonally stratified deep lakes with hypolimnetic anoxia, of which the Ponte Tresa basin is an example, higher eutrophication levels could lead to enhanced sediment P release and, thus, reduce P NBR in sediments. This study calls for the concern that, under such conditions, the recovery from anthropogenic eutrophication might be slow and difficult because of extensive internal P cycling and reduced capacity of sediment P-trapping. This study reveals the importance to adopt effective measures to minimize internal P-fertilization in lake restoration programs. The labile-phosphorus data presented by this study supports the geochemical approaches to reduce P release from sediments by applying aluminum and iron as “capping” materials.

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Appendix A. Supplementary data

Additional tables (Tables S1-S2) and figures (Figures S1-S4) are the supplementary data related to this article.

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Figure captions:

Figure 1. Study site. (a) An overview of the watershed of Lake Lugano, with the maps of Lake Lugano (in the dark blue color), the Ponte Tresa basin (red rectangle), and Switzerland (left inset). (b) Bathymetric map of the Ponte Tresa basin. The black asterisk indicates the coring site (color figure online); Grey areas around the lake indicate dwellings, and green areas forests. The figure was adapted from Schneider et al. (2018).

Figure 2. Sequential P extraction protocol. Sodium dithionite ($Na_2S_2O_4$) dissolved in 0.11 M sodium bicarbonate ($NaHCO_3$) buffer (pH 7.0) henceforth is termed as NaBD. Extraction

steps inside the dashed rectangle were carried out in a nitrogen (N_2) atmosphere (Color figure online).

Figure 3. RGB (red, green and blue) contrast enhanced sediment core picture of Core PTRE-15-3-A (Schneider et al., 2018), high-resolution true color picture of Core PTRE 17-2 (oxidized sediment surface) and pictograph of the top 0-37.5 cm of Core PTRE 17-2. Red lines between two cores indicate the stratigraphic marker layers; yellow colors highlight the detrital layers from the visual comparison with Core PTRE-15-3-A (Color figure online). The table on the right side describes the mean age of each sample including the uncertainties (PTRE 17-2), derived from visual stratigraphic correlation (layer by layer) with the chronology of Core PTRE 15-3 (Schneider et al., 2018).

Figure 4. Vertical profiles of (a) the total phosphorus concentrations with stacked four P fractions, and (b-e) P_i (inorganic P) and P_o (organic P) concentrations of the four P fractions in sediments. Error bars represent one standard deviations of three analysed replicates. The concentration is shown as the mean value of the three replicates in dry weight of sediments (DW); note the different scales for the x-axes (Color figure online).

Figure 5. Vertical profiles of (a) enzyme labile P_o and enzyme stabile P_o concentrations and (b) their average proportions in NaOH-EDTA extracted total P_o in sediments (Color figure online). Error bars show one standard deviation of eight analytical replicates.

Figure 6. (a) Green pigments (Chl-*a* + Phe-*a*) concentrations (green color) and fluxes (blue color) recorded in sediments of the Ponte Tresa basin from 1920 to 2015 CE (Schneider et al., 2018); $RABD_{590\text{-}730}$ represents the hyperspectral scanning image (HSI) inferred green pigments. (b) The sediment mass accumulation rates (MAR) and net burial rates (NBR) of all P fractions and total P (TP) in sediments of the Ponte Tresa basin between 1959 and 2017 CE. All of the P fractions NBR data except the year of 1960 (flood layer at 32.5-35 cm depth) were used. The light yellow shading highlights flood layers in the Core PTRE 17-2. Green horizontal lines separate the four stages (Stage I to IV) (Color figure online).

Figure 1_R1

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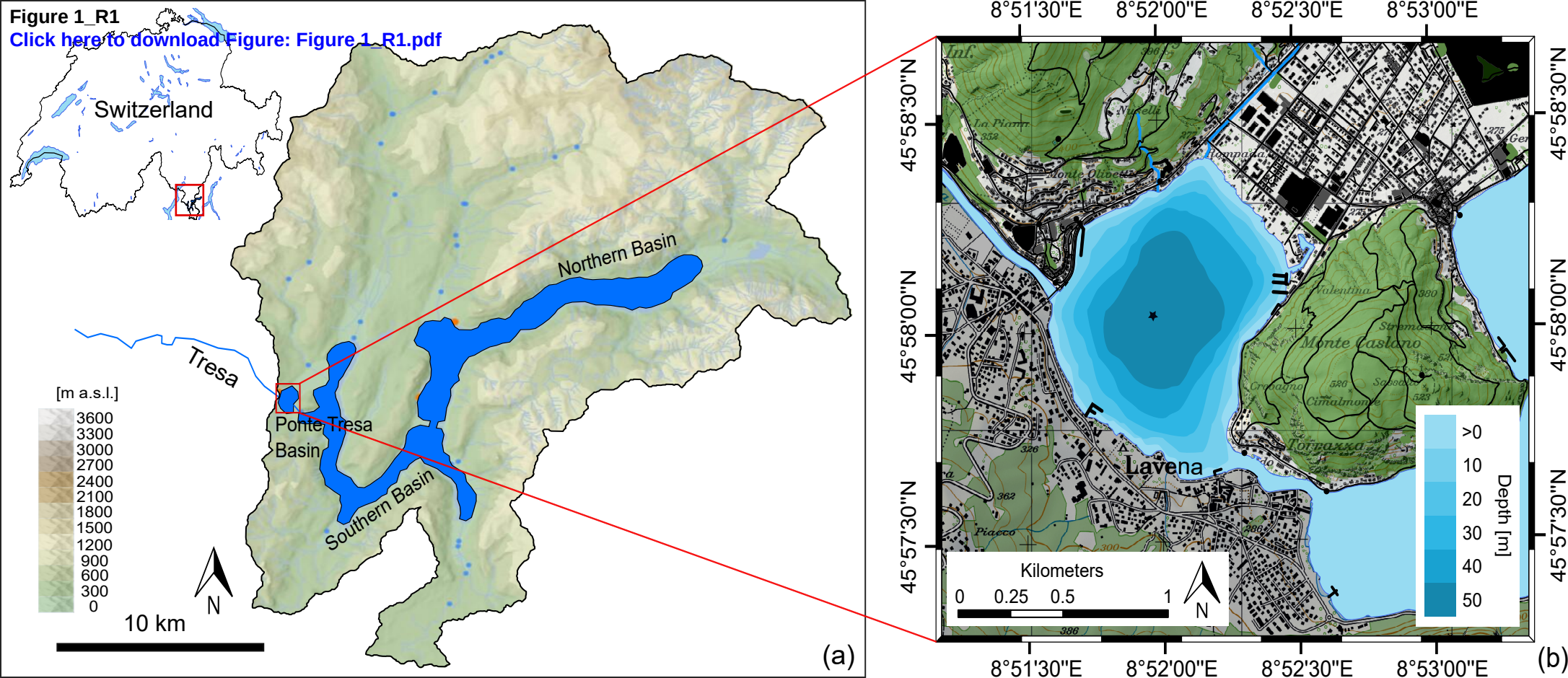

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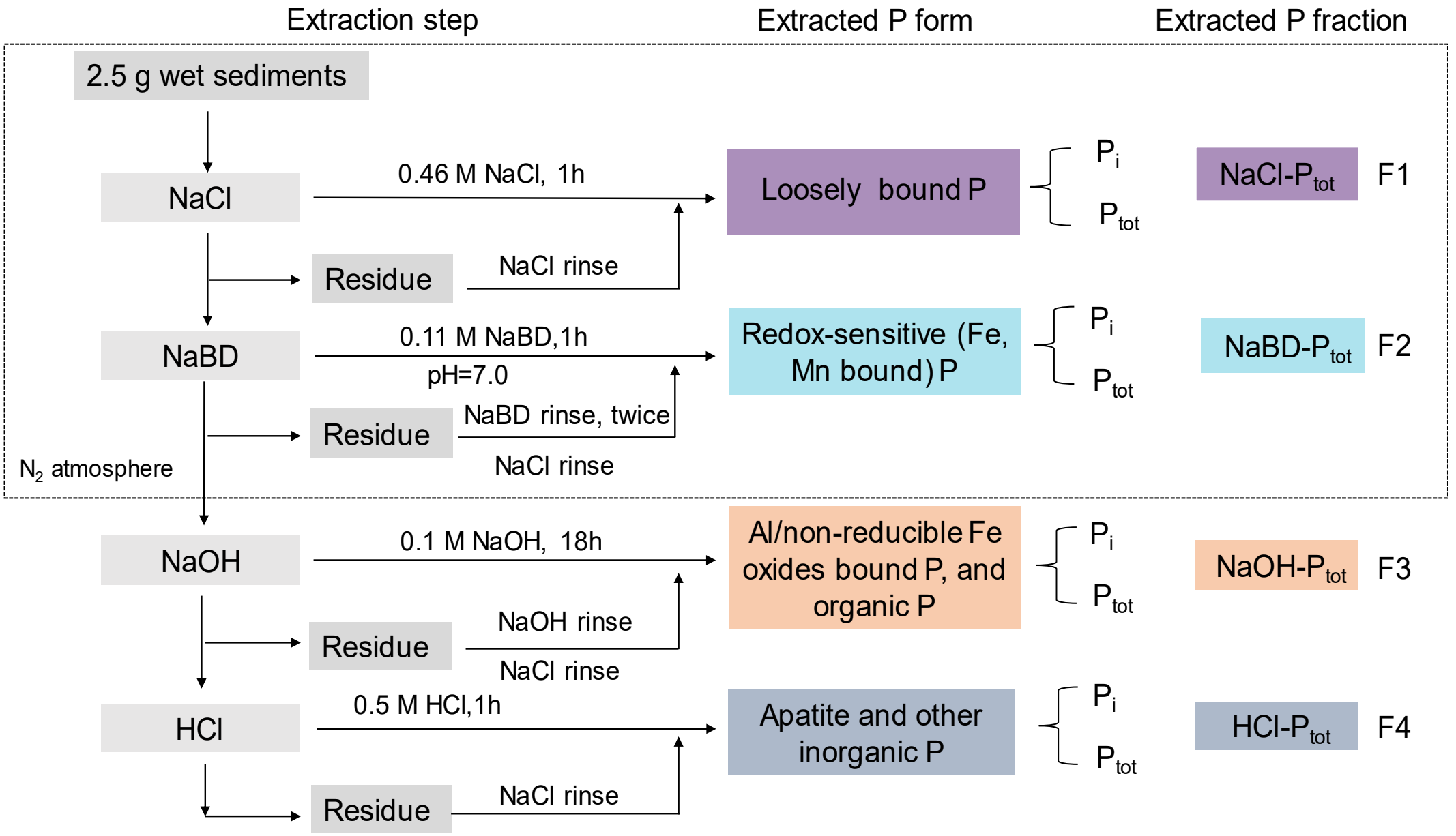

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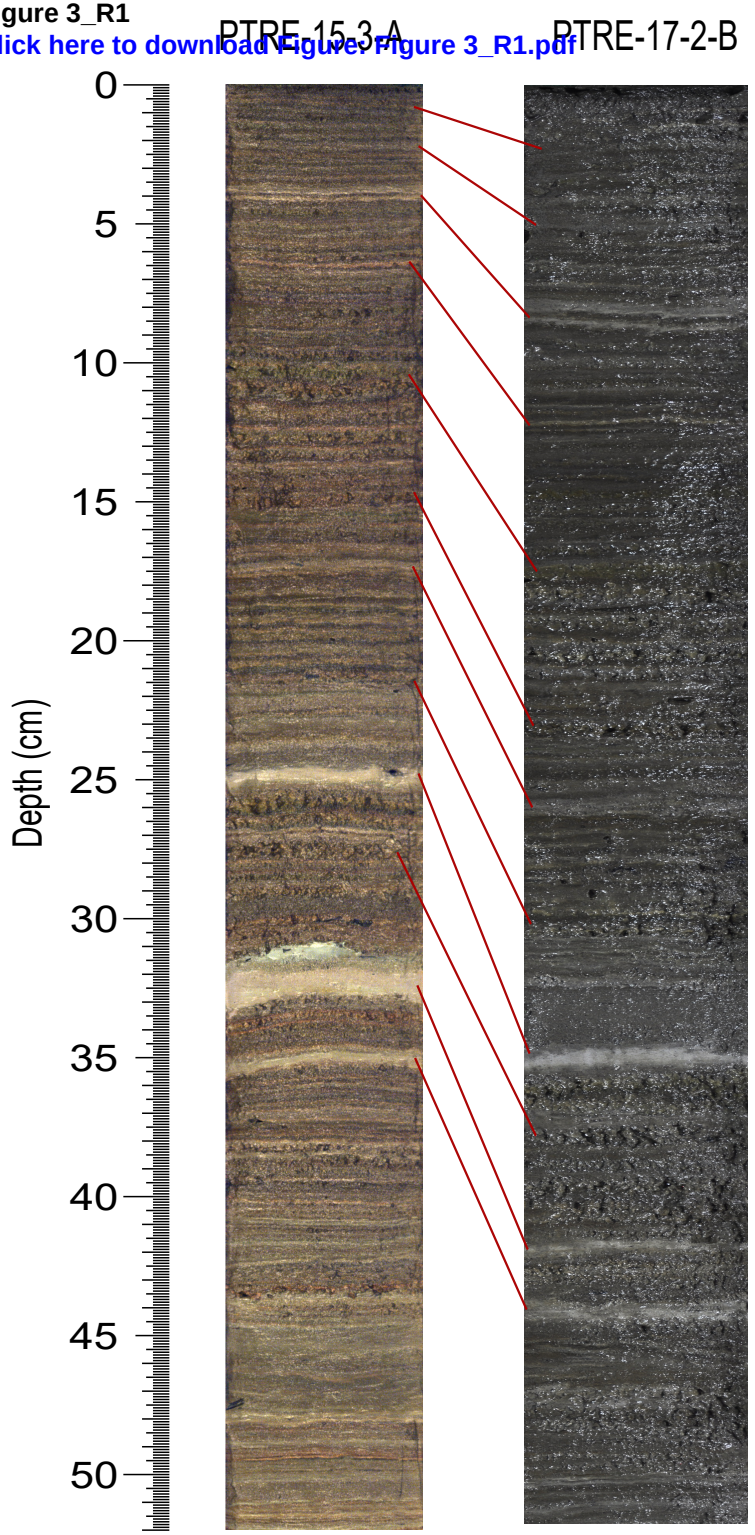

Depth (cm)	Mean age (CE)	Uncertainty (-)	Uncertainty (+)
0-2.5	2014	2014	2015
2.5-5	2009	2008	2009
5-7.5	2004	2004	2005
7.5-10	2001	2001	2001
10-12.5	1998	1998	1999
12.5-15	1994	1994	1995
15-17.5	1990	1990	1991
17.5-20	1987	1987	1988
20-22.5	1983	1983	1985
22.5-25	1978	1977	1981
25-27.5	1973	1972	1976
27.5-30	1969	1968	1971
30-32.5	1963	1963	1965
32.5-35	1960	1960	1960
35-37.5	1959	1959	1959

Figure 4_R1

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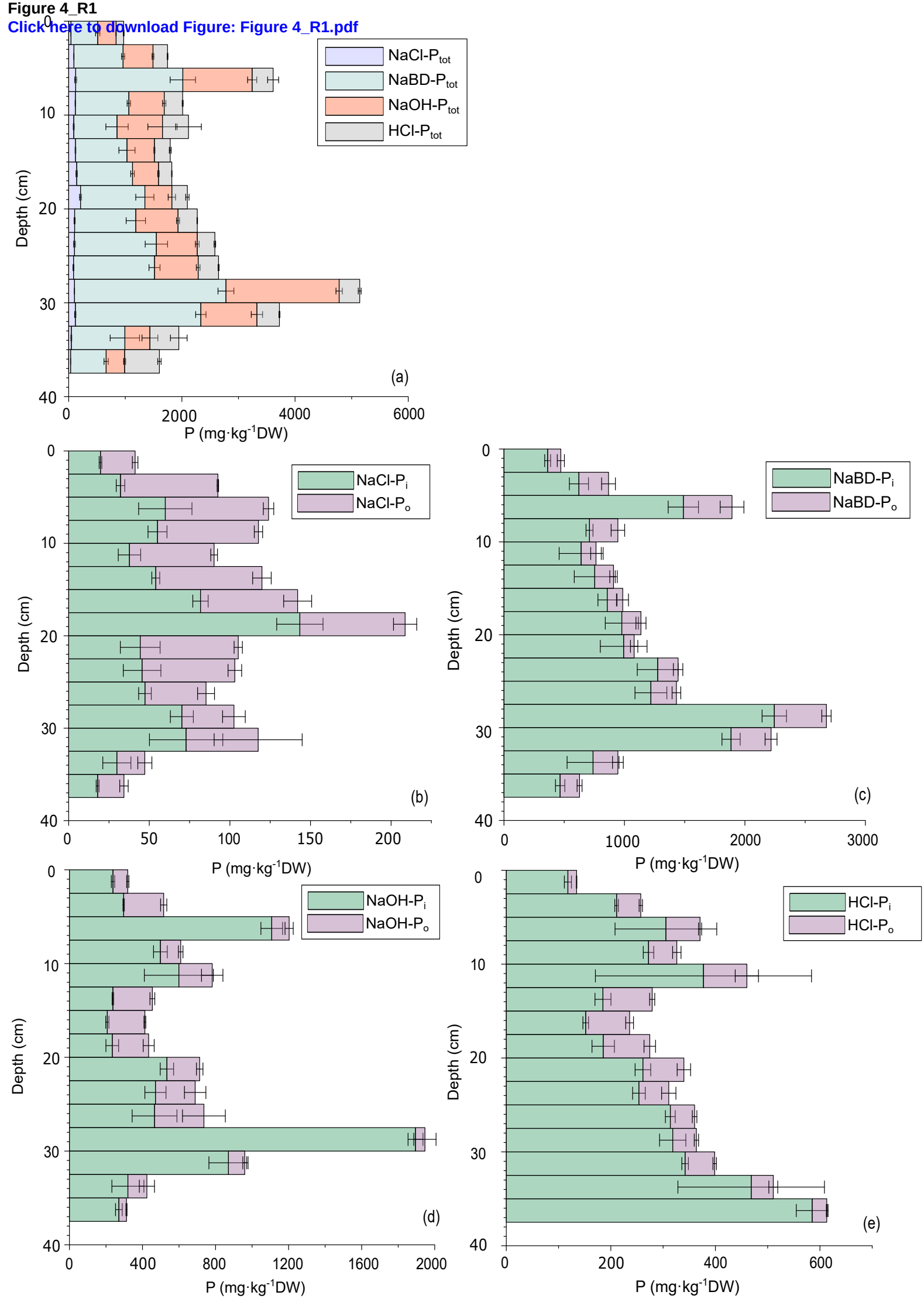

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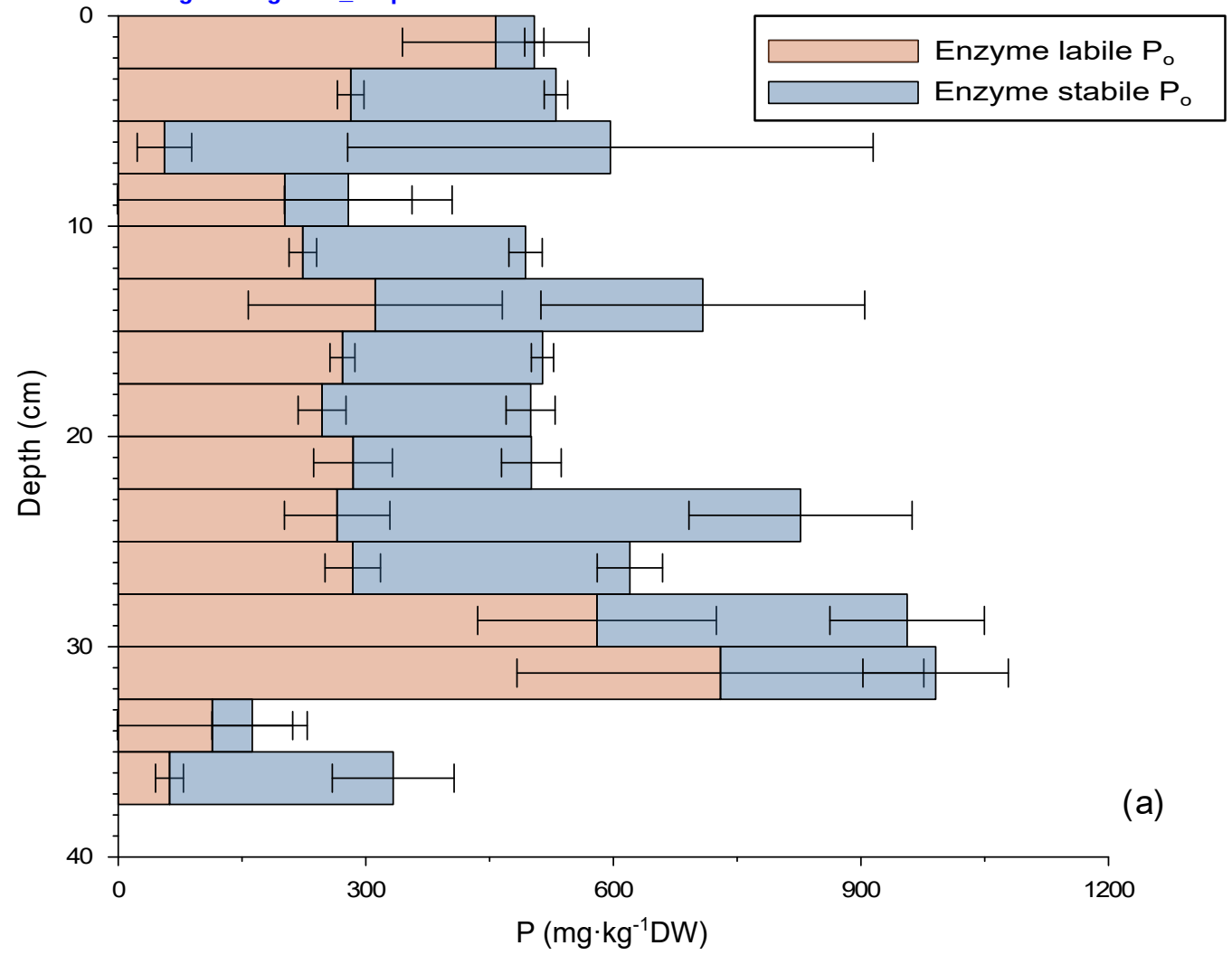

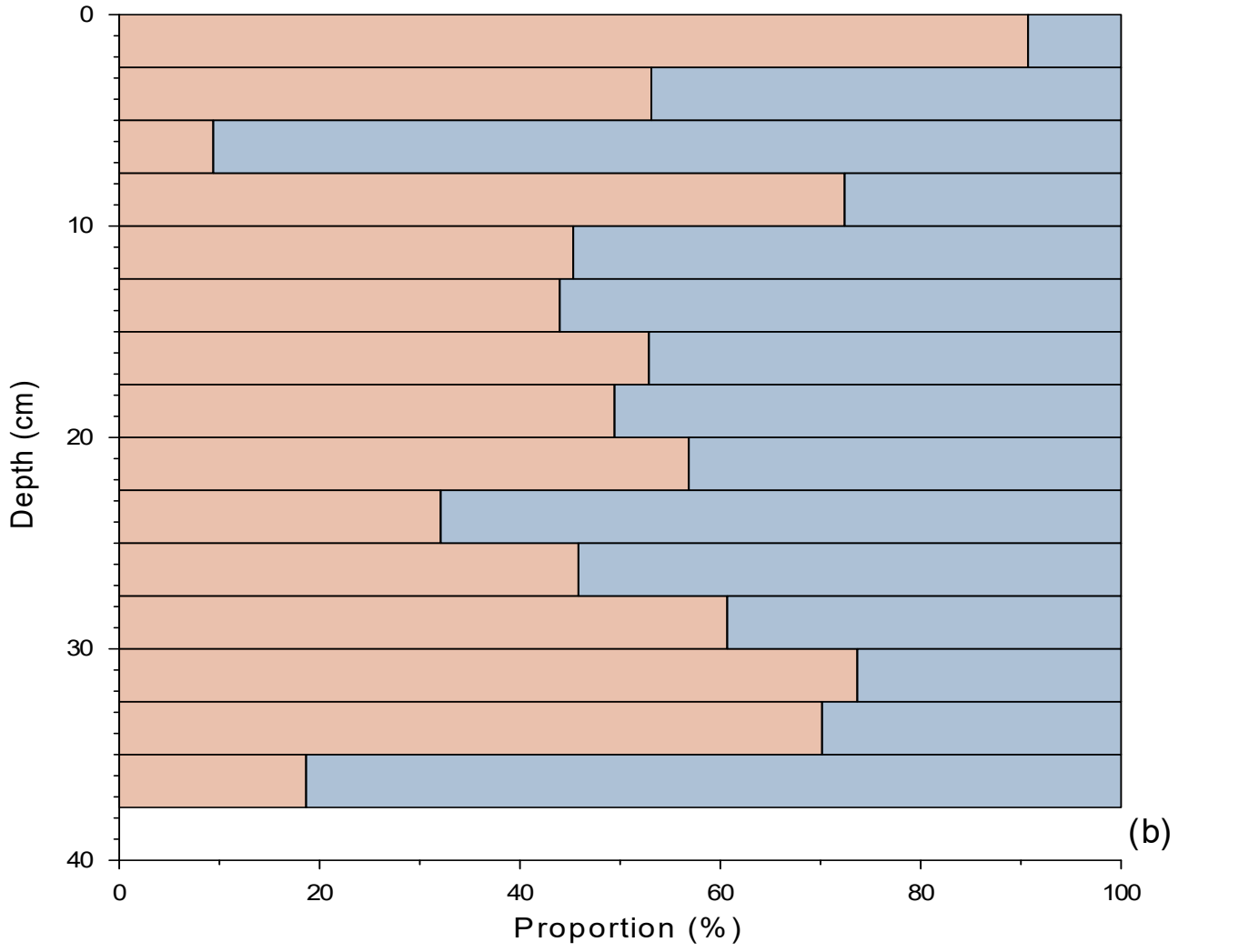

Figure 6_R1

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