Mineral Cycling and pH Gradient Related with Biological Activity under Transient Anoxic−Oxic Conditions: Effect on P Mobility in Volcanic Lake Sediments

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Ⓢ Supporting Information

ABSTRACT: Phosphorus (P) mobility from the sediments to the water column is a complex phenomenon that is generally assumed to be mainly redox sensitive and promoted by anoxic conditions. Thus, artificial aeration of the hypolimnium has been used as a remediation technique in eutrophic water bodies but several times with unexpected disappointing results. To optimize lake restoration strategies, the aim of the present study is to assess the P flux from the sediments under transient anoxic-conditions and to identify the relevant drivers. P sequential extraction, microprofiling (of pH, O_2 and H_2S), and bacterial community identification were performed on a sediment microcosm approach. The results demonstrated that the overall P release from sediments to the water column during transient phase was higher during the oxic phase, mainly from pH sensitive matrixes. The microprofiles signature suggests that the observed pH gradient during the oxic phase can be a result of H_2S oxidation in suboxic layers spatially separated and pared to O_2 reduction in top layers, through an electroactive bacterial network. These findings point to an additional driver to be considered when assessing P mobility under transient anoxic−oxic conditions, which would derive from pH gradients, built on the microbial electrical activity in sediments from freshwaters volcanic lakes.
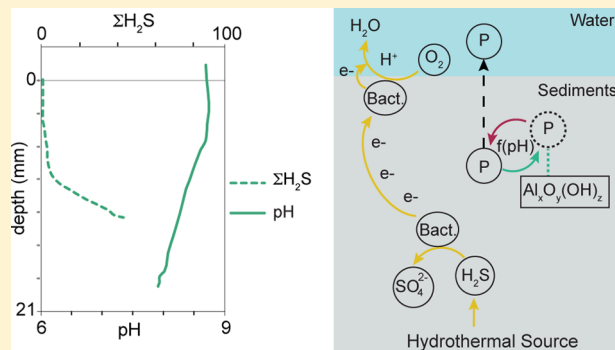
INTRODUCTION

Phosphorus (P) is usually the limiting nutrient of eutrophication and algae blooms in lake ecosystems.[1] Although external P sources are unquestionable pressures leading to eutrophication, the impact of internal inputs from P enriched sediments has been identified as very significant, namely when external loads are eliminated.[1] Several authors state that oxygenated sediments retain P by fixation to Fe (III) minerals while, during anoxic conditions, reduced sediments release phosphorus by reduction of iron and subsequent dissolution of iron−phosphorus solid phase. These statements are based on the model first proposed by Einsele[2] and latter supported by Mortimer.[3−5]

According to such concept, intermittent artificial aeration systems designed to maintain oxic conditions when hypolimnetic anoxic conditions prevail were applied in some lakes (e.g.: Lake Furnas − Portugal, Lake Sempach − Switzerland). However, the chemical pathways of phosphate release from the sediments under those circumstances are controversial.[6,7] Indeed, while oxic condition may favor the stability of P adsorption on iron minerals, several mechanically oxygenated lakes continue to release phosphorus from sediments to the water column with minor or any change in the eutrophication state of the lakes.[8,9] Additionally, it is remarkable that in some lakes with anoxic hypolimnium, the P release rate is not significantly different comparing with the oxic period of the hypolimnium.[10,11] These observations point to other biogeochemical mechanisms involved in P release from sediments.

Lake sediment geochemistry is different from lake to lake and thus, remediation techniques should be designed accordingly to the dominant diagenetic processes.[12] It has been demonstrated that P can be released from vivianite dissolution due to interaction with hydrogen sulfide, a process that is driven in deep sediment layers where oxygen cannot diffuse−and thus having no influence in P retention.[8,13] Therefore, in lakes with

high hydrogen sulfide production, such as volcanic lakes,[14] this process can have a major effect in P release from deep layers, or during anoxic periods.

Besides iron minerals, other nonredox sensitive minerals can release bounded P due to pH shifts.[15] Recently, Nielsen et al. have demonstrated that the development of a pH gradient in marine sediments can be associated with hydrogen sulfide oxidation in deep layers coupled with oxygen reduction present in the top layer.[16] Such electrochemical process spatially separated was thought to be conducted by an electroactive bacterial network.[16,17] Even more recently, filamentous "cable bacteria" were found to be present in the same marine sediments with these electrochemical capabilities.[18,19] While additional experiments are made to understand if a bacterial network or cable bacteria or both are oxidizing H_2S in deep layer with concomitant production of a pH gradient, a question arise: what is the impact of this process on mineral cycling and P mobility?

Therefore, the aim of the present work is to identify drivers of P mobility under transient oxic-anoxic condition in volcanic lakes. Sediments phosphorus sequential extraction (PSE) process and profiling techniques with microsensors to monitor pH, O_2 and ΣH_2S ($[H_2S] + [HS^-] + [S^{2-}]$) concentration in the sediment pore water were carried out. In addition, a screening for iron reducing bacteria with bioelectrical properties was performed.

■ MATERIALS AND METHODS

Sediment Sampling and Microcosm Experiment Setup. The collected sediments belong to the shallow volcanic lake Furnas, located in the archipelago of Azores (Portugal) in S. Miguel Island.[14] Sediment samples were collected at 12 m deep locations of the lake. A gravitational corer (from Uwitec) with tubes of 6 cm in diameter and 60 cm in length penetrated approximately 18−40 cm in the sediments. The first 10 cm of sediments were collected (using an Uwitec cutting apparatus) and preserved at 4 °C, while the rest of the sediments were rejected.

In the microcosm experiment, the sediments were homogenized and placed in three tubes (0.1247 dm^3/tube) inside a parallelepiped reactor filed with the lake's hypolimnetic water (1.412 dm^3/reactor). The water was analyzed for SRP and total P (after acid digestion, explained next) through molybdenum blue/stannous chloride colorimetric method.[22]

Two equal reactors were connected allowing the water to circulate in a closed loop from a reactor to the contiguous one (Figure 1). The water temperature of the reactors was controlled with a Nüve BS302 refrigerating system. The experiment was divided in five different phases as schematized in Figure 1. In the first phase, the three tubes of each reactor were filled with homogenized sediments, leaving approximately 1 cm of headspace in each tube. A small amount of homogenized sediment was taken for PSE setting the initial conditions. The temperature was set to approximately 17 °C. Both reactors were sealed in order to prevent oxygen diffusion to the water, after nitrogen bubbling to complete anoxia. After 24 h, all the sediment settled and the second phase started for a period of 21 days in complete anoxia. At the end of the second phase, the reactors were opened for microprofiling of pH, O_2 and ΣH_2S. During measurements, nitrogen was bubbled in order to restrain oxygen diffusion and keep the reactors in anoxic state.
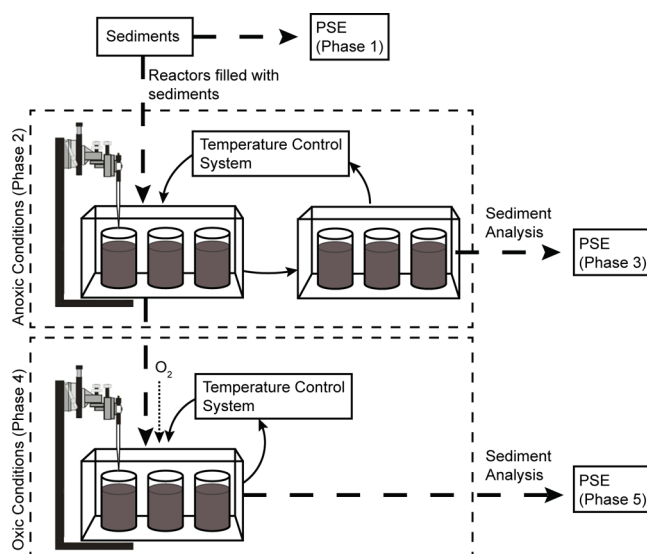

Figure 1. Representation of the different phases (1−5) of the experiment. The P sequential extraction (PSE) is made before the transference of sediments to the reactors (phase 1), before the change in O_2 concentration (phase 3), and at the end of the experiment (phase 5). Measurement of O_2, pH and ΣH_2S was performed at the end of the anoxic (phase 2) and oxic periods (phase 4) through microprofiling technique. Dashed lines indicate the route of the sediments across the five phases. Dotted line indicates the O_2 flux to the reactor in phase four, and solid lines indicate the overlying water flux between the temperature control system and the reactors.

In the third phase, one of the reactors was unplugged from the system for water and sediment analysis. Soluble reactive phosphorus (SRP) in the overlaying water was determined (method described in the Phosphorus Extraction and Analysis section). A sediment sample was taken from the uppermost 2−3 mm from each tube (approximately 2 g of wet sediment) for PSE, just after the microprofiling (at the end of the second phase). The reactor that remained in the experimental system was opened to air allowing the diffusion of oxygen into the water.

The fourth phase was executed exactly the same way as the second phase, with the only difference of keeping the reactor open, with air bubbling in the water to produce oxic conditions at the sediment-water interface (SWI). This phase lasted for 21 days, ending with a microprofiling measurement of pH, O_2 and ΣH_2S. The fifth phase was performed in the same way as the third phase. The SRP in the water was determined and approximately 2 g of wet sediment was collected for PSE.

Microsensor Profiling. Profiles of pH, O_2, and ΣH_2S concentration in the interstitial water of the sediment were made with microsensors. The profiles were made in the first 20 mm (maximum depth) of the sediment. The vertical resolution used in this experiment was 250 μm (80 measured points in the sediments). The microsensors used were OX-50, pH-50 and H_2S-50 for oxygen, pH and H_2S respectively (all with a 50 μm diameter tip) from Unisense.

The total hydrogen sulfide (ΣH_2S) was estimated by measuring H_2S and pH at the same depth simultaneously as ΣH_2S depends on pH[20] (calculations are presented in Supporting Information).

The profile measurements where made at the end phase 2 (anoxic) and 4 (oxic) for each core. To determine the surface of the sediments we have used magnifying lens (10×).

Phosphorus Extraction and Analysis. The phosphorus fractionation method used was based on the Psenner and Pucsko[21] extraction scheme. This extraction method has been used to evaluate the P-binding forms in lake sediments[12,14] (Table 1). After each extraction step, samples were centrifuged

Table 1. Phosphorus Forms Extracted with the Different Solvents in the Sequential Extraction Procedure

step	solvent	P-fraction	P bounding forms
1	NH$_4$Cl (0.1M)	SRP	pore water-soluble phosphate and sediment surface loosely adsorbed phosphate, algal available phosphate.
2	BD (0.11M) (Bicarbonate − Dithionite) 40 °C	SRP	redox-sensitive P, mainly bound to Fe-hydroxide and Mn-hydroxide.
3	NaOH (1M) at 25 °C	SRP	phosphate bound to metallic oxide (mainly Al), and soluble inorganic P-compounds in alkaline solution.
4	HCl (0.5M)	SRP	carbonate and Apatite- P
5	NaOH (1M) at 85 °C	TP	organic refractory P

at 7000 rpm, for 20 min, and the supernatant was filtrated through a 0.45 μm pore diameter membrane. The analysis of SRP was made by the molybdenum blue/stannous chloride method.[22] The total-P (TP) analysis was achieved by an acid digestion of the samples with nitric acid (5 mL) and sulfuric acid (1 mL) at 250 °C for 90 min, as detailed in *Standard Methods of Water and Wastewater*[22] and further determination as SRP.

Bacteria Quantification. The bacterial quantification was made in natural intact sediment core before the experiment started. The amplification of real-time PCR products was carried out with a Chromo4 real-time PCR detector (MJ/Bio-Rad) using SYBR Green as signal dye. PCR amplification was performed in a 25 μL reaction mixture containing 20 μL of master mix (iQ SYBR Green Supermix, Bio-Rad 170-8882), and 5 μL of DNA template (concentrations ranged from 0.10 ng/μL to 1.60 ng/μL). Primers targeting the 16S rRNA were used to quantify total bacteria, 1055f/1392r[23] and *Geobacteraceae* as iron-reducing bacteria, Geo564f Geo840r.[24] The purity of amplified products was checked by the appearance of a single melting peak obtained by increasing temperature from 62 to 95 °C at 0.2 °C/s. Data were analyzed using MJ OpticonMonitor 3.1 (MJ/Bio-Rad). Standard curves were generated from a 10-fold dilution series of positive

controls (10^1 to 10^8 target copies per reaction) included in duplicate in each PCR.

■ RESULTS AND DISCUSSION

As presented in Figure 2, the volcanic lake sediments that were under anoxic conditions released P from the BD and NaOH (at 85 °C) pools when compared with the initial conditions, as a result of reduction of Fe(III) to Fe(II) leading to solubilization of Fe mineral complexes ($Fe_xO_y(OH)_z$) and organic matter mineralization, respectively. When the reactor was exposed to O_2, Fe minerals readsorbed P as observed by the change in P concentration in the BD fraction (compare anoxic/oxic values in BD fraction, Figure 2). This is consistent with the classic paradigm that Fe (III) reduction in anoxic period from oxy-hydroxide minerals will result in dissolution of the Fe−P complex with concomitant P release.[2−4] However, as Fe (III) is converted to Fe (II), two parallel reactions with opposite results will happen: 1. Fe (II) will react with phosphate to produce vivianite ($Fe_3(PO_4)_2$) acting as a P trap; and 2. Fe (II) will react with ΣH_2S creating FeS, which will exhaust the availability of Fe complexes for P binding.[13] The presence and concentration of ΣH_2S in anoxic conditions was identified and quantified through microprofiling technique from the top sediment layers to deep layers (Figure 3).

As vivianite (pKs = 36, at 20 °C)[25] has a lower solubility than FeS (pKs = 2.9, at 20 °C),[26] it will precipitate first, trapping P in the sediments and preventing its dissolution to the water column. However, and as indicated by Gachter and Muller,[13] increasing ΣH_2S production will lead to Fe(II) sequestration and dissolution of Fe(II) minerals including vivianite, that will result in P release. Thus, during the anoxic phase, it is expected that P was first released by the Fe(III) minerals but recaptured by Fe(II) precipitating as vivianite, and then, released again after vivianite reaction with ΣH_2S due to its rising concentration (see conceptual model, Figure 4). This has resulted in a P concentration of 153 μg/L $\pm$ 14 μg/L in the overlying water, at the end of the anoxic phase.

After the shift from anoxic to oxic phase (phase 4), P has been recaptured by Fe oxy-hydroxides as demonstrated by the rising BD fraction in Figure 2. Contrary to what should be expected, P concentration in water duplicated (318 μg/L $\pm$ 21 μg/L, the P mass balance is demonstrated in Supporting Information). PSE results for the oxic phase indicate that P has been released from the aluminum oxy-hydroxide ($Al_xO_y(OH)_z$) fraction (Figure 2 − NaOH). This event was not a result of O_2 concentration change, as Al oxy-hydroxides are not sensible to
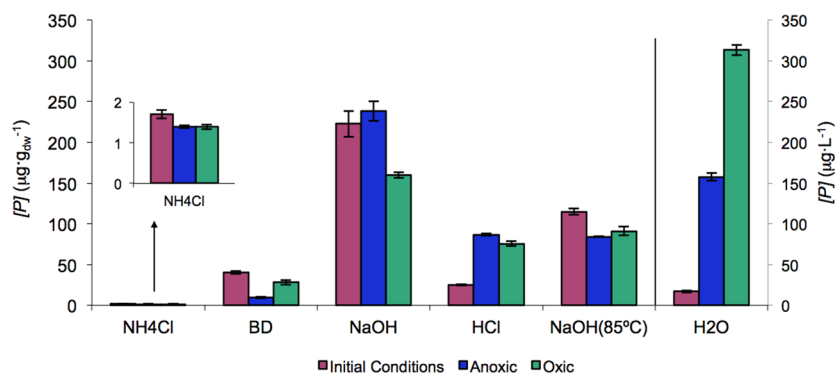

Figure 2. Results of P sequential extraction (left side of the graph) and concentration in water (right side of the graph) for initial (phase 1), anoxic (phase 3) and oxic (phase 5) conditions.
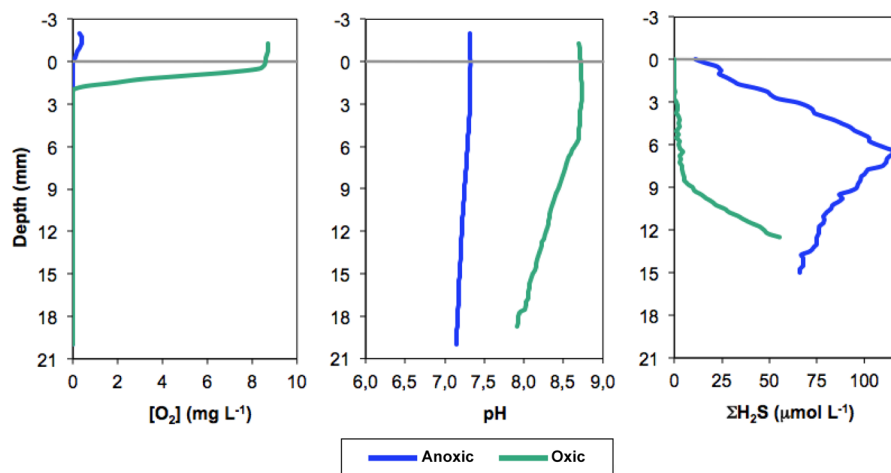
Figure 3. Microprofiling results, for O_2, pH, and ΣH_2S concentration, in oxic and anoxic conditions for the first 20 mm of sediment.
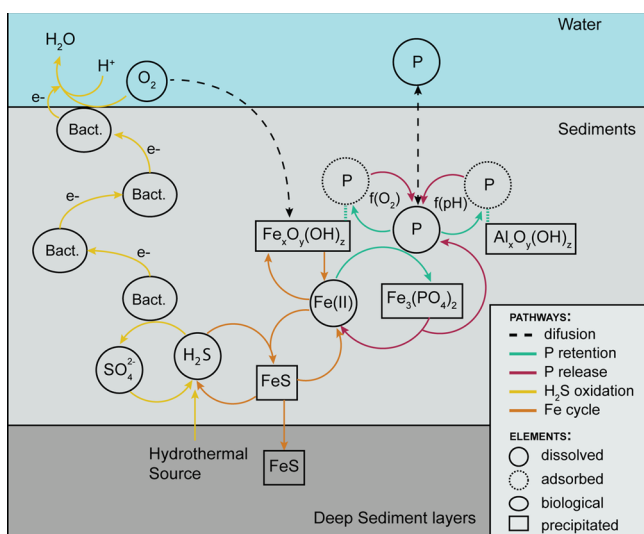

Figure 4. - Simplified conceptual model demonstrating the biologically mediated transport of electrons (e−) from the anoxic sulfidic zone to the oxic zone at sediment water interface (top) with concomitant H^+ consumption resulting in pH rising (adapted from Nielson et al.).[16] Additionally, the model includes two P retention/release pathways base on mineral content: 1. Adsorption/desorption from Fe minerals $(Fe_xO_y(OH)_z)$ as a function of O_2 concentration $[f(O_2)]$ and 2. Adsorption/desorption from Al minerals $(Al_xO_y(OH)_z)$ as a function of pH concentration $[f(pH)]$. Finally, the permanent sink of Fe in deep sediment layers was also considered.

redox potential shift,[15] but to an increase in pH[27] during oxic phase (Figure 3). It has been demonstrated that the pH range between 5 and 8 is optimal for P binding to Al hydroxides.[15,28] The release of P bounded to $Al(OH)_3$ was tested for six lake's sediments as a function of pH.[29] The results indicated high dissolution of $Al(OH)_3$ with concomitant P release at pH above 8.5. During the oxic phase, pH increased above 8.7, which explain the partial P release that was bounded to Al oxy-hydroxides by dissolution. We propose that this pH gradient can be associated with bioelectric currents as explained next.

In marine ecosystems it was indicated that bacteria could drive electrons trough a microbial network connected with nanowires from ΣH_2S in the anoxic layers to oxygen in the upper sediment layers (Figure 4).[16,17] As electrons are driven through this bacterial network and/or as more recently suggested by filamentous bacteria,[18,19] from anoxic layer to the oxic surface layers, O_2 will be the last electron acceptor with concomitant H^+ consumption to produce water (Figure 4). This will result in a pH rise in the upper layers while acidification will occur in anoxic ones, as a result of ΣH_2S oxidation.

The microprofiling signature of ΣH_2S (Figure 3) suggests that this biologically driven electrical mechanism found in marine sediments may also be present in these volcanic sediments. The major evidence that this bioelectrochemical process was active in the present study is revealed by the change in ΣH_2S concentration profile after the O_2 concentration shift (anoxic to oxic period), with concomitant pH gradient formation. The results presented in Figure 3 show that, during the oxic period, ΣH_2S is being consumed in the first 9 mm with concomitant decrease in concentration (compare ΣH_2S concentration between oxic and anoxic period in the first 9 mm of sediments). However, the oxygen profile indicates that it can diffuse only in the first 2 mm of sediment. Thus, ΣH_2S in deeper layer was not being oxidized by direct reaction with oxygen, but possibly through a microbial network capable of driving electrons from ΣH_2S in deep layers (until 9 mm depth) to O_2 in the top layer. This process is additionally confirmed by the change in pH profile that shows a pH gradient formation with higher value in the top sediment layer when compared with the anoxic period. Although a more distinct pH peak in the oxic layer should be expected (when compared with experiences made in marine sediments[16]), there is still a small convex bend in pH profile, which can indicate that the process was active but ceased after 21 days of oxic period.

Moreover, *Geobacteraceae*-like iron reducing bacteria (IRB), which have the ability of driving electrons[30] were identified in Furnas lake sediments and represented around 1.4 ± 0.6% (Figure 5) of total bacteria ($8.48 \times 10^8 \pm 8.93 \times 10^7$ cell/g sediment). The bacterial profiles in the first 10 cm of sediment presented an almost homogeneous distribution (Figure 5). Other previous studies by the authors confirm the presence of these electroactive bacteria in Furnas lake sediments.[31−33] If these *Geobacteraceae*-like bacteria were the main drivers of this mechanism or if other filamentous proteobacteria[18,19] were majorly involved (or both) is still unknown, and will be object of further studies.

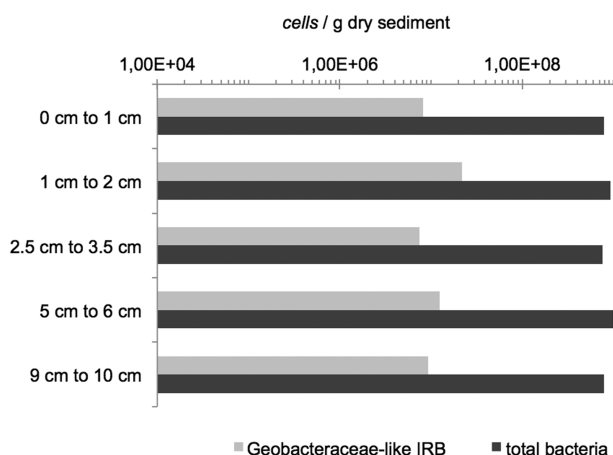*cells / g dry sediment*

Figure 5. Geobacteraceae-like IRB and total bacteria profiles in sediments of the volcanic lake Furnas.

As biological drivers for bioelectrical process exist in Furnas volcanic lake sediments[31−33] and microprofiling results are consistent with this fact, we propose that this is an important process leading to pH rising above 8 and consequently to instability of the Al−P complex.[15] Accordingly, P was released by diffusion through the sediment-water interface, increasing its concentration in the water column (see P release pathway from Al minerals in conceptual model − Figure 4).

This is the first time that ΣH_2S oxidation in suboxic layers spatially separated and pared to O_2 reduction in top layers is being proposed in volcanic lake sediments−specifically relating the effects of this bioelectric process with P mobility. An interesting remark in the subject is that, if in one hand oxic periods will lead to pH gradients due to distant ΣH_2S oxidation and consequently to the dissolution of P bounded to pH-sensitive matrixes, in the other hand the decrease in ΣH_2S concentration by oxidation will inhibit vivianite dissolution and consequently retain P in the sediments.[8] Thus, before the selection of remediation techniques, aiming P retention by increasing O_2 concentration in hypolimnium, both of these antagonist processes concerning P release/retention should be taken into account, and one should make an attempt to understand which of these processes is more pronounced, which can vary from lake to lake. Specifically in Furnas Lake, aeration has been implemented for more than a decade with no improvement in its trophic status (eutrophic lake). Although we cannot directly extrapolate our results (microcosm) to Furnas' diagenetic system, from the present results as well as from other results published elsewhere[31−33] where the same sediments where used, we can conclude that there is bioelectric activity in Furnas' sediments. As hydrothermal sources exist in this lake and H_2S is continuously added to the system,[34] which differentiate this system from other freshwater ones (with low sulfur-based elements, for example, H_2S, $SO_4^{2−}$), biological activity related with the sulfur and iron cycle have an important influence on the diagenetic processes,[30] though from this results we cannot estimate its contribution to pH variation. Other processes, denitrification and sulfate reduction can induce proton (H^+) consumption with concomitant pH rising. Thus, for a direct extrapolation for the lake's diagenetic system, and quantification of the contribution of H_2S oxidation distantly pared with O_2 reduction in pH increase (with concomitant P release), the same experiment should be made assessing the pore water concentration of dissolved $NO_3^−$,

$SO_4^{2−}$, Fe^{2+}, Al^{3+} and dissolved inorganic carbon (DIC) for each anoxic and oxic phase.[17] The same tests should be made with in situ sediments in anoxic and oxic periods.

Finally, Ca minerals stability, similarly to Al minerals is mainly depend on pH[15] rather then redox potential.[21] As expected, bounded P to Ca minerals proved to be a nonredox sensitive fraction as the maximum adsorption occurred during the anoxic phase (Figure 2, HCl). The refractory pool (Figure 2, NaOH 85 °C) remained stable between anoxic and oxic conditions. The initial P content of this fraction was higher comparing with anoxic and oxic phases. Although this fraction represents P in refractory organic matter, the reduction of this fraction suggests that part of it was degradable and thus mineralized with concomitant P release to the water column.

Environmental Implication of Hypolimnium Aeration. This study confirms that O_2 has an impact on Fe minerals stability where P is adsorbed. However, sediments may also release P from other matrixes that are not redox sensitive but are sensible to pH (e.g., Al minerals). In the present case, P extractable by NaOH, corresponding mainly to Al minerals bounded P was almost 3 times higher than the redox sensitive fraction (Fe minerals bounded P−BD fraction). This P release occurred during the oxic period, demonstrating that O_2 variation can have a minor positive effect on P retention by iron minerals when compared with the pronounced negative effect in P release from organic mater (due to increased oxidation) and other metallic mineral matrixes by pH shift (dissolution of Al minerals) or ΣH_2S presence in deeper layers (resulting in vivianite dissolution) of volcanic lakes.

Bioelectrical processes coupling O_2 reduction in top sediment layers with ΣH_2S reduction in deeper layers for almost a centimeter-long, is proposed to be one of the main contributing process for pH rising during oxic phase, thus, enabling P release caused by dissolution of pH-sensitive matrixes. Therefore, aerated hypolimnium in volcanic lakes, aiming P retention, may be counter-productive.

In conclusion, internal P is controlled by a complex biogeochemical system that is lake specific. Therefore, each lake should be assessed for their biological and abiotic sediment components before aeration and destratification processes are implemented. Additionally, we suggest that sediment modeling should be used to assess the dynamics of P mobility of such complex system and to support decision-making in lakes management, namely in eutrophication control.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information
Additional information as noted in the text. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes
The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

■ REFERENCES

(1) Schindler, D. W. The dilemma of controlling cultural eutrophication of lakes. *Proc. Biol. Sci.* **2012**, *279*, 4322–33.

(2) Einsele, W. Über die Beziehungen des Eisenkreislaufs zum Phosphatkreislauf im eutrophen See. *Arch. Hydrobiol.* **1936**, *29*, 664–686.

(3) Mortimer, C. H. The exchange of dissolved substances between mud and water in lakes. *J. Ecol.* **1941**, *29*, 280–329.

(4) Mortimer, C. H. The exchange of dissolved substances between mud and water in lakes II. *J. Ecol.* **1942**, *30*, 147–201.

(5) Mortimer, C. H. Chemical exchanges between sediments and water in the Great Lakes-speculations on probable regulatory mechanisms. *Limnol. Ocean* **1971**, *16* (2), 387–404.

(6) Bostrom, B.; Andersen, J. M.; Fleischer, S.; Jansson, M. Exchange of phosphorus across the sediment-water interface. *Ecosystems* **1988**, *244*, 229–244.

(7) Hupfer, M.; Lewandowski, J. Oxygen Controls the Phosphorus Release from Lake Sediments - a Long-Lasting Paradigm in Limnology. *Int. Rev. Hydrobiol.* **2008**, *93* (4–5), 415–432.

(8) Gächter, R.; Wehrli, B. Ten years of artificial mixing and oxygenation: No effect on the internal phosphorus loading of two eutrophic lakes. *Environ. Sci. Technol.* **1998**, *32* (23), 3659–3665.

(9) Schauser, I.; Chorus, I. Assessment of internal and external lake restoration measures for two Berlin lakes. *Lake Reservoir Manage.* **2007**, *23* (4), 366–376.

(10) Driscoll, C. T.; Effler, S. W.; Auer, M. T.; Doerr, S. M.; Penn, M. R. Supply of phosphorus to the water column of a productive hardwater lake: Controlling mechanisms and management considerations. *Hydrobiologia* **1993**, *02*, 61–72.

(11) Kopácek, J.; Borovec, J.; Hejzlar, J.; Ulrich, K.; Norton, S. a.; Amirbahman, A. Aluminum control of phosphorus sorption by lake sediments. *Environ. Sci. Technol.* **2005**, *39* (22), 8784–9.

(12) Dittrich, M.; Chesnyuk, a; Gudimov, a; McCulloch, J.; Quazi, S.; Young, J.; Winter, J.; Stainsby, E.; Arhonditsis, G. Phosphorus retention in a mesotrophic lake under transient loading conditions: Insights from a sediment phosphorus binding form study. *Water Res.* **2013**, *47* (3), 1433–47.

(13) Gächter, R.; Müller, B. Why the phosphorus retention of lakes does not necessarily depend on the oxygen supply to their sediment surface. *Limnol. Oceanogr.* **2003**, *48*, 929–933.

(14) Ribeiro, D. C.; Martins, G.; Nogueira, R.; Cruz, J. V.; Brito, A. G. Phosphorus fractionation in volcanic lake sediments (Azores-Portugal). *Chemosphere* **2008**, *70*, 1256–1263.

(15) Simmons, J. A. Phosphorus removal by sediments in stream contaminated with acid mine drainage. *Water, Air, Soil Pollut.* **2010**, *209*, 123–132.

(16) Nielsen, L. P.; Risgaard-Petersen, N.; Fossing, H.; Christensen, P. B.; Sayama, M. Electric currents couple spatially separated biogeochemical processes in marine sediment. *Nature* **2010**, *463* (7284), 1071–4.

(17) Risgaard-Petersen, N.; Revil, A.; Meister, P.; Nielsen, L. P. Sulfur, iron-, and calcium cycling associated with natural electric currents running through marine sediment. *Geochim. Cosmochim. Acta* **2012**, *92*, 1–13.

(18) Pfeffer, C.; Larsen, S.; Song, J.; Dong, M.; Besenbacher, F.; Meyer, R. L.; Kjeldsen, K. U.; Schreiber, L.; Gorby, Y.; El-Naggar, M. Y.; Leung, K. M.; Schramm, A.; Risgaard-Petersen, N.; Nielsen, L. P. Filamentous bacteria transport electrons over centimetre distances. *Nature* **2012**, *491*, 218–21.

(19) Schauer, R.; Risgaard-Petersen, N.; Kjeldsen, K. U.; Tataru Bjerg, J. J.; B Jørgensen, B.; Schramm, A.; Nielsen, L. P. Succession of cable bacteria and electric currents in marine sediment. *ISME J.* **2014**, 1–9.

(20) Jeroschewski, P.; Steuckart, C.; Kühl, M. An amperometric microsensor for the determination of H_2S in aquatic environments. *Anal. Chem.* **1996**, *68*, 4351–4357.

(21) Psenner, R.; Pucsko, R. Phosphorus fractionation: Advantages and limits of the method for the study of sediment P origins and interactions. *Arch. Hydrobiol. Beih* **1988**, *30*, 43–59.

(22) APHA, AWWA, and WEF. *Standard Methods of Water and Wastewater*, 20th ed.; American Public Health Association: Washington D.C., 1998; ISBN-10:0875532357.

(23) Harms, G.; Layton, A. C.; Dionisi, H. M.; Gregory, I. M.; Garrett, V. M.; Hawkins, S. A.; Robinson, K. G.; Sayler, G. S. Real-time PCR quantification of nitrifying bacteria in a municipal wastewater treatment plant. *Environ. Sci. Technol.* **2003**, *37*, 343–351.

(24) Bedard, D. L.; Ritalahti, K. M.; Löffler, F. E. The Dehalococcoides population in sediment-free mixed cultures metabolically dechlorinates the commercial polychlorinated biphenyl mixture Aroclor 1260. *Appl. Environ. Microbiol.* **2007**, *73*, 2513–2521.

(25) Al-Borno, A.; Tomson, M. B. The temperature dependence of the solubility product constant of vivianite. *Geochim. Cosmochim. Acta* **1994**, *58*, 5373–5378.

(26) Davison, W. The solubility of iron sulphides in synthetic and natural waters at ambient temperature. *Aquat. Sci.* **1991**, *54* (4), 309–329.

(27) Lake, T.; Jin, X.; Wang, S.; Pang, Y.; Chang, F. Phosphorus fractions and the effect of pH on the phosphorus release of the sediments from different trophic areas. *Environ. Pollut.* **2006**, *139*, 288–295.

(28) Lijklema, L.; Koelmans, A.; Portielje, R. Water quality impacts of sediment pollution and the role of early diagenesis. *Water Sci. Technol.* **1993**, *28* (8–9), 1–12.

(29) Reitzel, K.; Jensen, H. S.; Egemose, S. pH dependent dissolution of sediment aluminum in six Danish lakes treated with aluminum. *Water Res.* **2013**, *47*, 1409–20.

(30) Lovley, D. R.; Holmes, D. E.; Nevin, K. P. Dissimilatory Fe(III) and Mn(IV) Reduction. *Adv. Microb. Physiol.* **2004**, *49*, 219–86.

(31) Martins, G.; Peixoto, L.; Ribeiro, D. C.; Parpot, P.; Brito, A. G.; Nogueira, R. Towards implementation of a benthic microbial fuel cell in lake Furnas (Azores): Phylogenetic affiliation and electrochemical activity of sediment bacteria. *Bioelectrochemistry* **2010**, *78* (1), 67–71.

(32) Martins, G.; Terada, A.; Ribeiro, D. C.; Corral, A. M.; Brito, A. G.; Smets, B. F.; Nogueira, R. Structure and activity of lacustrine sediment bacteria involved in nutrient and iron cycles. *FEMS Microbiol. Ecol.* **2011**, *77*, 666–679.

(33) Martins, G., Ribeiro, D. C., Terada, A., Smets, B. F., Brito, A. G., Nogueira, R. Phylogeny and Activity of *Proteobacteria* in lake sediments. In *Proteobacteria: Phylogeny, Metabolic Diversity and Ecological Effects*; Sezenna, M. L., Ed.; Nova Science Publishers, Inc., 2011; ISBN: 978-1-61761-198-8.

(34) Cruz, J.; Antunes, P.; Amaral, C.; Franca, Z.; Nunes, J. Volcanic lakes of the Azores archipelago (Portugal): Geological setting and geochemical characterization. *J. Volcanol. Geotherm. Res.* **2006**, *156*, 135–157.