Environmental Science & Technology

Article

pubs.acs.org/est

Cable Bacteria Control Iron–Phosphorus Dynamics in Sediments of a Coastal Hypoxic Basin

Fatimah Sulu-Gambari,*[,†] Dorina Seitaj,[‡] Filip J. R. Meysman,[‡] Regina Schauer,[§] Lubos Polerecky,[†] and Caroline P. Slomp[†]

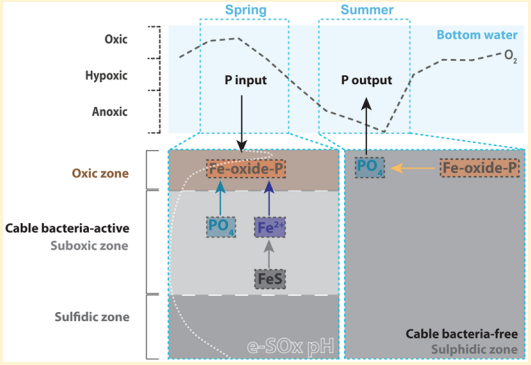
[†]Department of Earth Sciences, Geochemistry, Faculty of Geosciences, Utrecht University, Utrecht, The Netherlands

[‡]Department of Ecosystem Studies, Royal Netherlands Institute for Sea Research, Yerseke, The Netherlands

[§]Center for Geomicrobiology and Section for Microbiology, Department of Bioscience, Aarhus University, Aarhus, Denmark

Supporting Information

ABSTRACT: Phosphorus is an essential nutrient for life. The release of phosphorus from sediments is critical in sustaining phytoplankton growth in many aquatic systems and is pivotal to eutrophication and the development of bottom water hypoxia. Conventionally, sediment phosphorus release is thought to be controlled by changes in iron oxide reduction driven by variations in external environmental factors, such as organic matter input and bottom water oxygen. Here, we show that internal shifts in microbial communities, and specifically the population dynamics of cable bacteria, can also induce strong seasonality in sedimentary iron–phosphorus dynamics. Field observations in a seasonally hypoxic coastal basin demonstrate that the long-range electrogenic metabolism of cable bacteria leads to a dissolution of iron sulfides in winter and spring. Subsequent oxidation of the mobilized ferrous iron with manganese oxides results in a large stock of iron-oxide-bound phosphorus below the oxic zone. In summer, when bottom water hypoxia develops and cable bacteria are undetectable, the phosphorus associated with these iron oxides is released, strongly increasing phosphorus availability in the water column. Future research should elucidate whether formation of iron-oxide-bound phosphorus driven by cable bacteria, as observed in this study, contributes to the seasonality in iron–phosphorus cycling in aquatic sediments worldwide.



INTRODUCTION

Phosphorus (P) is a necessary constituent of organic biomolecules such as DNA and RNA, phospholipids, and ATP[1] and may limit phytoplankton growth in both freshwater and marine ecosystems. In coastal waters, river input is typically the primary P source, while burial in sediments is the major sink. In systems subject to excessive nutrient input, which are often characterized by hypoxic bottom waters (O_2 <63 μM), P is readily remobilized from sediments.[2−4] This "internal" source of P may fuel a high primary productivity in surface waters, which then can sustain bottom water hypoxia, even when riverine P inputs are reduced.[5] Phosphorus recycling from sediments has been shown to hamper recovery of coastal "dead zones" that have developed worldwide over the past decades due to anthropogenic eutrophication.[6,7]

The release of P from sediments during seasonal hypoxia is typically assumed to be the combined result of the release of P upon reductive dissolution of iron (Fe) (oxyhydr)oxides[2,3] (henceforth referred to as iron oxides) and of P stored as polyphosphate in microbial cells during oxic conditions[8] and from degrading organic matter (OM). Conventionally, changes in sediment Fe and sulfur (S) redox chemistry are thought to be driven by seasonal variation in factors external to the sediment. In this model, increased inputs of OM, temperature-dependent stratification of the water column, and a subsequent decline in bottom water oxygen in summer induce high sulfate-reduction rates and the conversion of sediment iron oxides to iron sulfides, leaving fewer iron oxides to bind P.[4,9] Upon reoxygenation of the surface sediment in fall and winter, the iron sulfides are thought to be oxidized mostly by oxygen, creating iron oxides that can sequester P.[1,10,11] Furthermore, large sulfur-oxidizing bacteria, like *Thiomargarita* and *Beggiatoa*, are known to accumulate considerable amounts of P as polyphosphate inclusions.[11] The release of such intracellular reserves has been suggested to enhance benthic release of P during summer in seasonally hypoxic sediments.[10]

Recently, a novel mode of electrogenic sedimentary sulfide oxidation was described involving filamentous bacteria of the Desulfobulbaceae family.[12] These cable bacteria are able to link the oxidation of free sulfide in deep anoxic sediment layers to the reduction of oxygen[12−15] or nitrate (NO_3^-)[16] in surface

Received: September 8, 2015
Revised: December 23, 2015
Accepted: December 31, 2015
Published: December 31, 2015

DOI: 10.1021/acs.est.5b04369
Environ. Sci. Technol. 2016, 50, 1227−1233

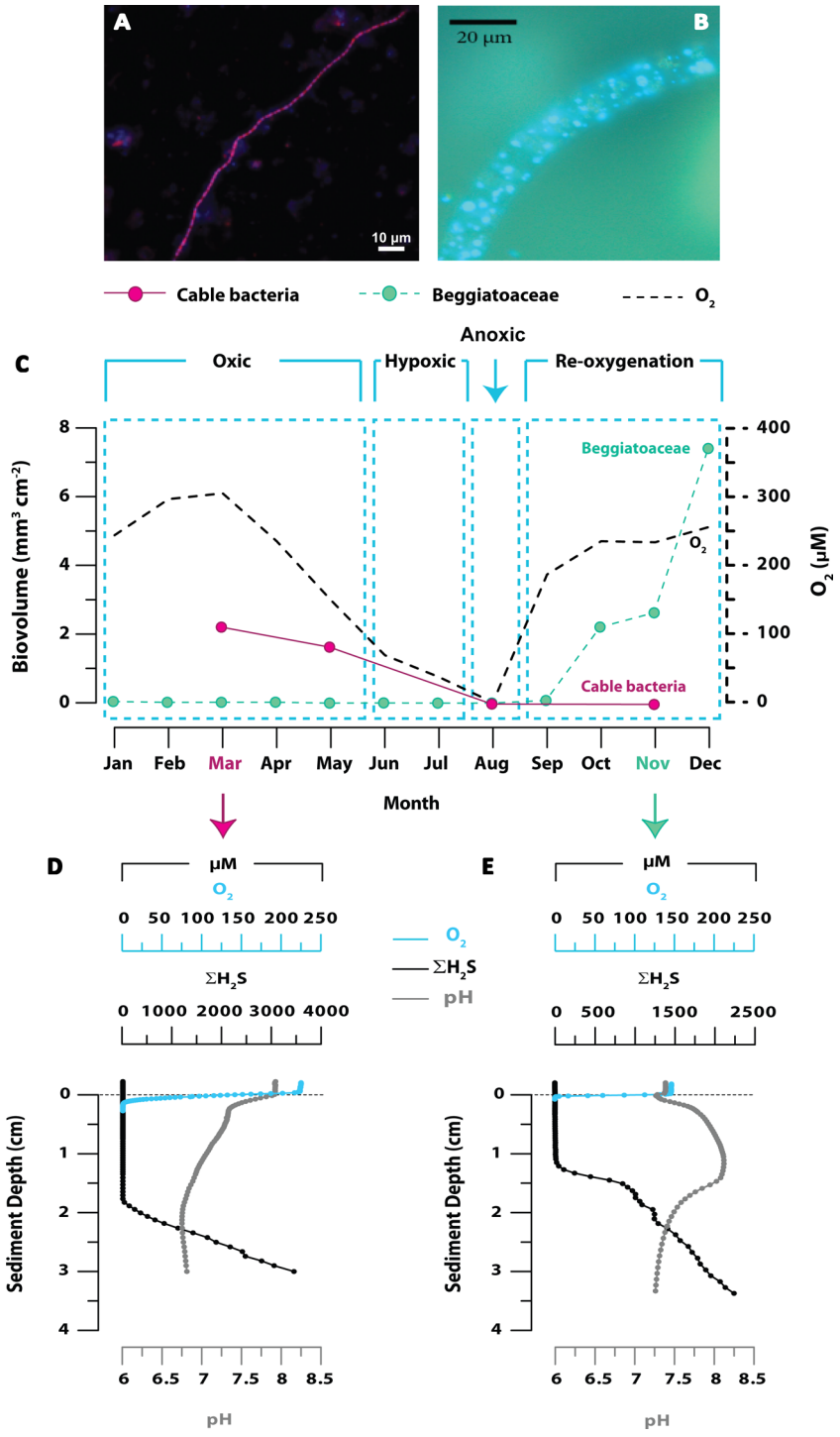


Figure 1. (A) FISH image of filamentous cable bacteria (probe DSB706). (B) Beggiatoaceae filament stained with DAPI as viewed with a fluorescent light microscope (Leica DM4500). (C) Temporal changes in oxygen concentrations in the bottom water and bacterial succession at the sediment surface in 2012. The abundance of cable bacteria (pink dots) was determined in March, May, August, and November only, whereas for Beggiatoaceae (green dots), data were obtained for each sampling month. Microsensor profiles of oxygen, hydrogen sulfide, and pH in sediment pore water in March (D) and November (E). Cable bacteria fingerprints are characterized by a broad subsurface pH minimum while Beggiatoaceae create a broad pH maximum in the suboxic zone, reflecting the effect of bacterial succession on sediment pore water chemistry, as both bacteria induce the formation of oxygen- and sulfide-free suboxic zones.

sediments, by shuttling electrons over centimeter-scale distances. Electrogenic sulfur oxidation by cable bacteria occurs in a wide range of marine sediments,[17] where it has the potential to strongly impact the sediment geochemistry.[14,18] Laboratory experiments demonstrate that electrogenic sulfur oxidation induces acidification of the pore water, resulting in dissolution of iron monosulfide (FeS) and calcium carbonate ($CaCO_3$) in deeper sediment horizons.[14] In these experiments, the released Fe^{2+} partly diffused upward to the oxic zone where it was oxidized as iron oxides [$Fe(OH)_3$], and partly to deeper sulfidic layers, where it reprecipitated as FeS. Cable bacteria hence have the potential to substantially alter iron and sulfur

DOI: 10.1021/acs.est.5b04369
Environ. Sci. Technol. 2016, 50, 1227−1233

cycling in natural sediments. However, the extent to which this occurs in the field and the possible impact on sediment P dynamics are still completely unknown.

Here we present field data from a coastal marine basin, which demonstrate a direct link between seasonal changes in cable bacteria abundance, the formation and dissolution of FeS, and sedimentary P dynamics.

MATERIALS AND METHODS

Location. Our study site is a 34 m deep basin in a marine lake (salinity ~32) in The Netherlands, with rapidly accumulating organic-rich sediments (~2 cm/year).[17] The water column in Lake Grevelingen is seasonally stratified,[19] which induces bottom water O_2 depletion in summer. In 2012, oxygenated bottom waters prevailed from January to May, followed by hypoxia from June to July, anoxia in August, and the return of oxygen from September onward (Figure 1).

Sediment and Pore Water Sampling. Sediment cores were collected monthly on the RV *Luctor* in 2012 with a gravity corer (UWITEC, Austria), using transparent PVC core liners with 60 mm inner diameters. Each month, one core was sliced at high resolution (0.5 cm slices over 10 cm) in a N_2-purged glovebag. Bottom water samples were collected from the overlying water in each core. The pore water was extracted from the sediment using centrifugation (15 min at 4500*g*). Bottom water and pore water samples were filtered (0.45 μm) and subsampled under N_2. Subsamples for total dissolved P, Fe, Mn, and Ca were acidified (37% HCl, 10 μL/mL) and analyzed with inductively coupled plasma optical emission spectrometry (ICP-OES) (PerkinElmer Optima 3000). Pore water analyses of P with ICP-OES and colorimetrically with a nutrient autoanalyzer (Bran and Luebbe)[20] were compared for one sampling outing and found to be nearly identical, indicating most pore water P is present as dissolved inorganic P. Sulfate was measured using a Dionex ion chromatograph. The relative accuracy and precision of the analyses above, as established from standards and duplicates, was always <5%.

Centrifuged sediment samples were freeze-dried, then ground in a N_2-purged glovebox. Total sediment Mn was determined by ICP-OES, following acid destruction with HF–HNO_3.[21] Sediment P was fractionated into exchangeable P, iron-bound P, authigenic Ca–P, detrital P, and organic P using a modified sequential extraction method (SEDEX) extraction procedure.[21,22] The relative error in the P speciation, based on duplicate analyses, was generally <5% with the exception of the detrital P and iron-bound P, which had an error <15%. Sediment sulfur fractions were separated using the extraction method by Burton et al.[23] and modified as described in Kraal et al.[24] Acid-volatile sulfur (AVS) and chromium-reducible sulfur (CRS) fractions were quantified using iodometric titrations, where duplicate samples varied less than 10% for AVS and less than 8% for CRS.

Pore water microprofile measurements were conducted on intact sediment cores (n = 3) within a few hours of retrieval. Profiling was conducted using commercial microsensors operated with a motorized micromanipulator (Unisense A.S., Denmark). Depth profiles of O_2 (25 or 50 μm tip diameter; detection limit <1 μM), H_2S (50 μm tip diameter; detection limit <1 μM), and pH (100 or 200 μm tip diameter electrode) were recorded following standard calibration procedures [for O_2 a two-point calibration in air-saturated seawater (100% saturation) and at depth in anoxic sediment (0% saturation); for H_2S, a five-point standard curve using freshly prepared Na_2S standards; for pH, 3 NBS standards and Tris buffer to correct for salinity, where pH values were reported on a total scale]. Total H_2S ($\sum H_2S = H_2S + HS^-$) was calculated as in Malkin et al.[17]

Bacterial Characterization. Microscopic identification of cable bacteria filaments (March, May, August, and November 2012) was performed by fluorescence in situ hybridization (FISH), using a Desulfobulbaceae-specific oligonucleotide probe[25] (DSB706; 5′-ACC CGT ATT CCT CCC GAT-3′), after staining with DAPI (1 mg/mL), as described in Schauer et al.,[15] and references therein.

To determine the biovolume of Beggiatoaceae filaments, intact sediment cores were sectioned within 24 h of retrieval, at 5 mm depth for the first 4 cm and subsampled (20–30 mg) as described by Seitaj et al.[26] The biovolume was determined from the length (×10) and width (×40) of all filaments found in the sample.[27] Cable bacteria biovolumes (mm^3 cm^{-3}) were calculated from measured filament lengths and diameters and integrated over all eight sediment layers. Polyphosphate inclusions in DAPI-stained cable bacteria and Beggiatoaceae filaments were visualized with a fluorescent light microscope (Leica DM4500) and identified based on visual appearance.[27]

Water Column Sampling. Discrete bottom water samples were collected with a 12 L Niskin bottle at 32 m water depth and transferred from the bottle using Tygon tubing. Bottom water O_2 concentrations were measured by an automated Winkler titration procedure with potentiometric end point detection (Mettler Toledo DL50 titrator and a platinum redox electrode) as described in Knap et al.[28]

Water column subsamples were analyzed for dissolved inorganic phosphate (PO_4) on a nutrient autoanalyzer (Seal QuAAtro). Additional discrete water samples (2.5 L) were filtered (0.7 μm Whatman glass microfiber GF/F filters) for suspended particulate matter (SPM) using a setup that was adjusted (vacuum-sealed) when the bottom water became anoxic. SPM samples were then freeze-dried and fractionated for P phases according to the modified SEDEX procedure discussed above.

Flux Measurements and Calculations. Benthic fluxes of PO_4 from the sediment into the overlying water were measured in intact cores (triplicates) in shipboard closed-chamber incubations. The depth of the overlying water in each core was adjusted immediately upon retrieval to 18–20 cm above the sediment, and the water was subsequently replaced with ambient bottom water. Replacement was conducted with minimal disturbance to the sediment, via a gastight tube, to prevent gas exchange with the atmosphere. Cores were promptly sealed with gastight lids, transferred to a temperature-controlled container, and incubated at in situ temperature. Lids contained two sampling ports and a central stirrer to homogenize the water. Six hour and 18 h incubation periods were applied during summer and winter, respectively. Water samples were collected from each core at regular intervals (5 times), and fluxes were calculated as the change in [PO_4] during the incubation, accounting for the enclosed sediment area and overlying water volume. Diffusive PO_4 fluxes were calculated using Fick's first law, using the dissolved [PO_4] in the first 0.5 cm sediment depth interval and bottom water [PO_4] (Figure S8).

Polyphosphate Quantification. Intracellular P content of individual cable bacteria cells was estimated using nanometer-scale secondary ion mass spectrometry (NanoSIMS). The analysis was performed as described by Vasquez-Cardenas et

DOI: 10.1021/acs.est.5b04369
Environ. Sci. Technol. 2016, 50, 1227–1233

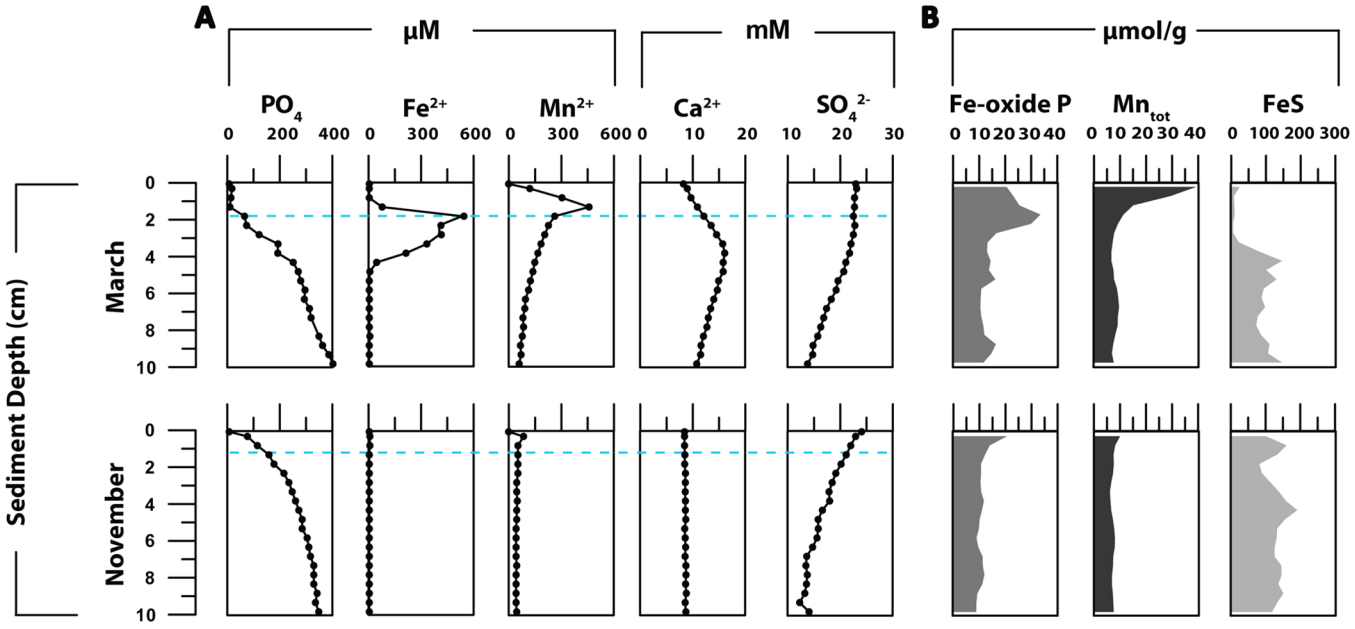


Figure 2. (A) Pore water [PO_4], [Fe^{2+}], [Mn^{2+}], [Ca^{2+}], and [SO_4^{2-}] for March 2012, when cable bacteria are present, and November 2012, when Beggiatoaceae are abundant in the sediment. Dashed lines indicate the depths below which hydrogen sulfide is detectable. (B) Solid-phase iron-bound P, total Mn (Mn_{tot}), and FeS for March and November 2012.

al.,[29] using a NanoSIMS 50L instrument (Cameca, France). Filaments, hand-picked from sediment cores treated with ^{13}C-labeled bicarbonate and propionate, were analyzed for counts of secondary ions and subsequently used to calculate the P/C ratio and the intracellular P content of the cells. Overall, 3−8 different filaments were analyzed from each treatment and zone (Supporting Information, 1.8; Figure S9).

RESULTS AND DISCUSSION

Biogeochemical Signals of Bacteria. In both spring and fall, a suboxic zone devoid of oxygen and free sulfide developed in the surface sediment. The suboxic zone extended from 1.5 to ca. 18 mm and 0.4 to ca. 10 mm in March and November, respectively. Microscopic examination of the sediment using FISH revealed the presence of cable bacteria in spring, down to 40 mm depth.[26] Microsensor depth profiles of O_2, $\sum H_2S$, and pH showed the characteristic geochemical signature of electrogenic sulfur oxidation[14,18] with a high pH near the sediment surface due to proton consumption via the reduction of oxygen ($O_2 + 4e^- + 4H^+ \rightarrow 2H_2O$), and a low pH in deeper, suboxic sediment due to proton production by the oxidation of sulfide ($0.5H_2S + 2H_2O \rightarrow 0.5SO_4^{2-} + 4e^- + 5H^+$). The absence of a distinct pH peak is consistent with high electrogenic activity in sediments characterized by a shallow oxygen-penetration depth.[26] The abundance of active cable bacteria, as reflected by pore water sulfate (SO_4^{2-}) profiles (Figure S1) and biovolumes (Figure 1C), declined from June onward with the onset of hypoxia. The geochemical response to shallow cutting of the sediment confirmed that cable bacteria were metabolically active and the dominant sulfur oxidizers in spring.[29] After summer hypoxia, tufts of large motile sulfur-accumulating sulfur-oxidizing bacteria (Beggiatoaceae) were present at the sediment surface from September to December, with extensive mat formation from October onward (Figure 1). Beggiatoaceae filaments were detected down to a depth of ca. 20 mm in the sediment.[26] Microsensor profiles in fall were consistent with the metabolic activity of Beggiatoaceae, with a suboxic zone that is characterized by a broad pH maximum, which is most likely the result of H_2S oxidation with nitrate ($4HS^- + NO_3^- + 6H^+ \rightarrow 4S^0 + NH_4^+ + 3H_2O$).[12,30] Seasonal surveys over the period 2011−2015 show that cable bacteria are replaced by Beggiatoaceae in fall nearly every year. The reasons for this population shift, however, are not yet fully understood.[26]

Whether cable bacteria or Beggiatoaceae are the dominant sulfur-oxidizing micro-organisms has major implications for sediment Fe, S, and P dynamics at the field site. In March, profiles of pore water Fe^{2+}, Ca^{2+}, and SO_4^{2-} (Figure 2A) and sediment FeS (Figure 2B) are in line with strong dissolution of FeS and $CaCO_3$ by cable bacteria.[14,18] In contrast to previous laboratory experiments,[14] the dissolved Fe^{2+}, which is remobilized and diffuses upward, does not reach the oxic sediment surface (Figure 2A), and so, iron oxidation cannot proceed aerobically. Although there is NO_3^- in the bottom water (30 μM),[19] iron oxidation coupled to nitrate reduction[31] is unlikely, given that the potential downward-diffusive flux of nitrate can only explain ∼27% of the iron removal (Supporting Information, 1.4). Instead, we propose that upward-diffusing Fe^{2+} is largely oxidized by manganese oxides (MnO_2), which are abundant in the surface sediment in March (ca. 40 μmol/g, Figure 2B; $Fe^{2+} + 0.5MnO_2 + H_2O + H^+ \rightarrow Fe(OH)_3 + 0.5Mn^{2+}$). Consequently, Mn^{2+} accumulates in the pore water (Figure 2A) and MnO_2 is lost from the sediment (Figure S2). Upward-diffusing PO_4, produced deeper in the sediment, is removed from the pore water through the association of P with the newly formed iron oxides in the suboxic zone (Figure 2B; Figures S3, S4, and S5). In November, the dominance of Beggiatoaceae results in pore water profiles typical for anoxic, sulfidic sediments, with a gradually increasing phosphate (PO_4) concentration. Profiles of dissolved Fe^{2+}, Mn^{2+}, and Ca^{2+} show little change with depth in the sediment. Sulfate concentrations decrease gradually with depth, indicating sulfate reduction. Iron sulfides are present throughout the sediment profile.

DOI: 10.1021/acs.est.5b04369
Environ. Sci. Technol. 2016, 50, 1227−1233

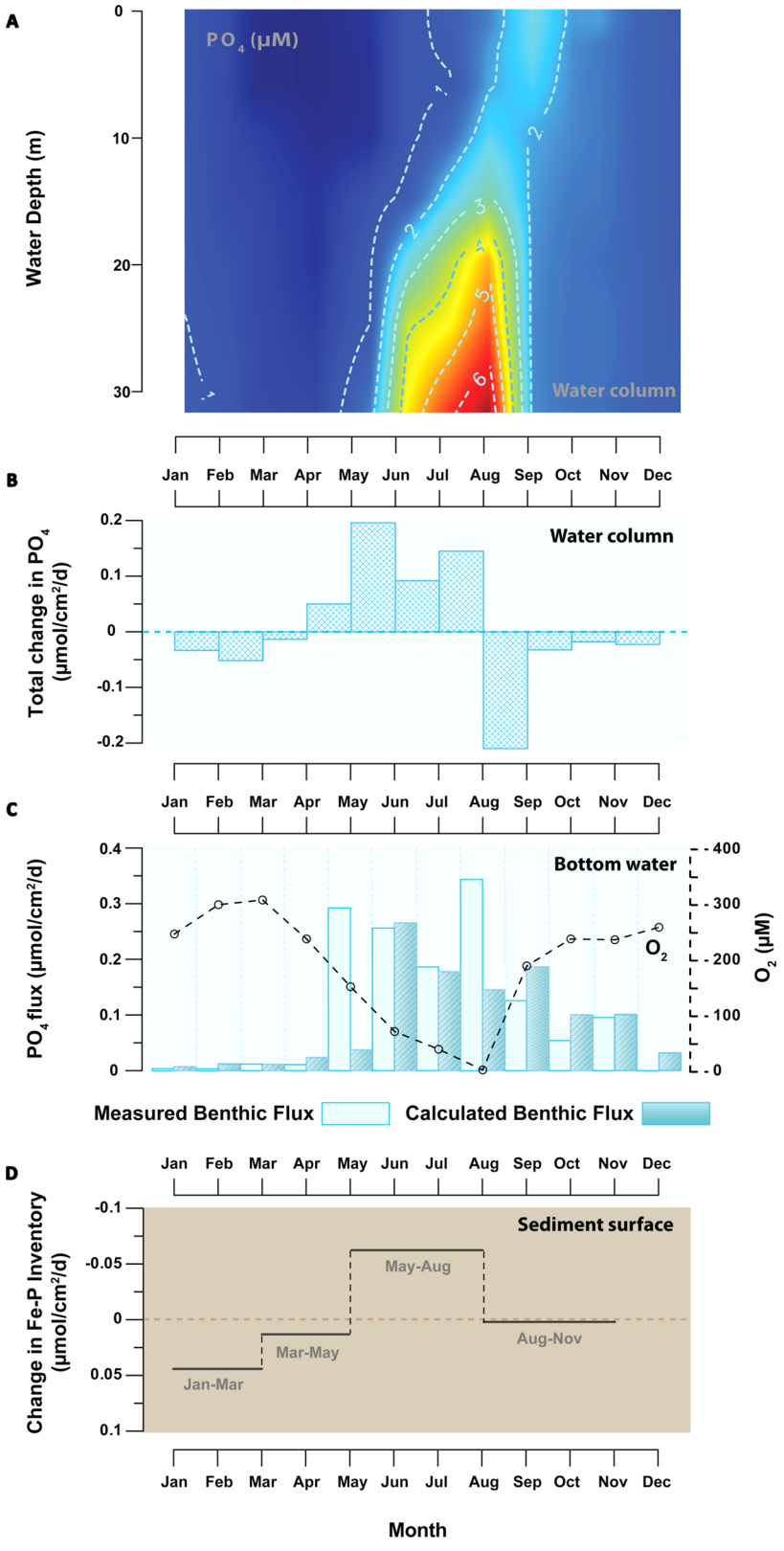


Figure 3. (A) Seasonal change in water column phosphate (in μM) as a function of water depth. (B) Change in phosphate in the water column in 2012 (integrated with depth; μmol cm^{-2} day^{-1}). (C) Flux of phosphate from the sediment to the water column as measured in incubations (also see Table S2) and calculated from pore water profiles (in μmol cm^{-2} day^{-1}). (D) Change in sediment Fe–P inventory (in μmol cm^{-2} day^{-1}) in the surface sediment (0–4 cm) during 4 periods in 2012 (N.B., *y*-axis is inverted).

Impact of Cable Bacteria on Fe–P Cycling. Our results demonstrate that cable bacteria have a major impact on P dynamics in Lake Grevelingen by promoting the formation of iron oxides and removal of pore water P over a much broader zone than expected based on the penetration of oxygen in such organic-rich sediments. In spring, all P released to the pore

DOI: 10.1021/acs.est.5b04369
Environ. Sci. Technol. 2016, 50, 1227–1233

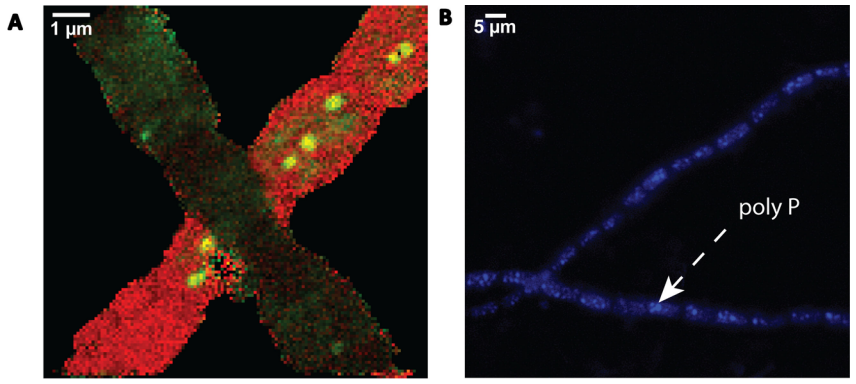


Figure 4. (A) Composite NanoSIMS image of cable bacterial cells treated with ^{13}C-labeled propionate. Intensities of the red and green channels correspond to the values of the $^{13}C/C_{tot}$ and $^{31}P/C_{tot}$ ratios, respectively. The brighter red cells have incorporated propionate and are therefore active, while the darker cells are inactive. The bright-yellow spots correspond to phosphate-rich inclusions. (B) DAPI-stained image of cable bacterial cells from the same core, examined for polyphosphate.

water at depth is sequestered in the sediment as iron-oxide-bound P (Figure 2). As a consequence, there is little sediment-water exchange of phosphate (Figure 3). From late spring onward, bottom-water deoxygenation during seasonal stratification coincides with increased release of phosphate from the sediment and an increase in water-column phosphate (Figure 3A–C). A significant proportion of the phosphate efflux (~20% for the measured flux, 40% for the calculated flux) in late spring and summer (May–August) can be explained by changes in the sedimentary pool of iron-oxide-bound P (Figure 3, parts C and D). In fall, when stratification ceases, oxic conditions in the bottom water are re-established and the surface sediment is colonized by Beggiatoaceae. However, little buildup of iron-oxide-bound P is observed, and critically, the efflux of phosphate from the sediment remains significant (Figure 3C). A similar link between cable bacteria and P sequestration was observed at a second site in the basin (Figures S6 and S7).

Release of P from intracellular polyphosphates in bacteria can also modulate sedimentary P dynamics,[10,11] yet our data suggest that this does not play a major role at the field site. Microscopic analysis of individual filaments of cable bacteria with nanoSIMS indicates the presence of intracellular polyphosphate accumulations (Figure 4), but the amount of accumulated P is negligible compared to that associated with $Fe(OH)_3$ (0.3 mmol P m^{-2} vs 47.9 mmol P m^{-2}; Supporting Information, 1.8). Although Beggiatoaceae also contain intracellular polyphosphates (Figure 1), there is no evidence for a major impact on either the pore water or solid-phase P profiles (Supporting Information, 1.9).

Biogeochemical Implication. Traditionally, the seasonal buildup of iron-oxide-P observed in aquatic sediments (e.g., refs 3, 4, 32, and 33) is assumed to be controlled by changes in the input of reactive OM and bottom water oxygenation. Our data show that cable bacteria are additional key drivers of sediment iron-oxide-bound P formation, allowing for the conversion of FeS to iron oxides over a broad sediment horizon (affecting the upper 20–40 mm). In the absence of cable bacteria, this same Fe conversion process would be primarily governed by seasonal changes in the oxygen penetration depth, which only affects the top 1–2 mm of the sediment. Therefore, at our field site, we observe at least a 10-fold increase in conversion of FeS to iron oxides when compared to a situation without cable bacteria (Supporting Information, 1.4). As a consequence, in sediments where cable bacteria are abundant, P retention is highly efficient. Equally, when their activity ceases, for example due to the establishment of anoxia in the water column, the release of P from the sediment to the overlying water is amplified. Recent work shows that cable bacteria are present in a wide range of sediments, both in marine[17] and freshwater environments.[34] This suggests a potential role for cable bacteria in seasonal changes in sediment Fe–P biogeochemistry in such environments and highlights the necessity for further detailed studies on the impact of cable bacteria, especially within the context of the continued worldwide expansion of areas suffering from eutrophication and bottom water hypoxia.[6]

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.5b04369.

Impact of cable bacteria (Figure S1), seasonal variation in solid-phase manganese (Figure S2), seasonality in sediment phosphorus forms and iron sulfide (Figure S3), alternate oxidants (Figure S4), iron oxide data (Figure S5), impact of cable bacteria on sedimentary phosphorus cycling at a second site in the basin (Figures S6, S7, and Table S1), quantification of polyphosphate in cable bacteria (Figure S9), and impact of Beggiatoaceae on sedimentary P cycling (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*Phone: +31 30 253 5037; e-mail: f.sulu-gambari@uu.nl.

Author Contributions

This study was conceived by C.P.S., F.J.R.M., F.S-G., and D.S. Field sampling: F.S.-G., D.S., R.S., F.J.R.M., and C.P.S. Microbial analyses: D.S. and R.S. NanoSIMS imaging: L.P. Microsensor profiling and flux chamber incubations: D.S. Geochemical analyses of sediments: F.S.-G. F.S.-G. and C.P.S. wrote the manuscript with contributions from all coauthors.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank P. van Rijswijk, S. Hidalgo, M. Hagens, I. Kocken, the crew of the RV *Luctor* (P. Coomans and M. Kristalijn), and various members of the NIOZ and UU teams for their support during the sampling campaigns. We are also grateful to J. Sinke,

DOI: 10.1021/acs.est.5b04369
Environ. Sci. Technol. 2016, 50, 1227–1233

A. Tramper, T. Zalm, and D. van de Meent for analytical support. This research was financially supported by the Darwin Centre for Biogeosciences, the European Research Council, under the European Community's Seventh Framework Programme (ERC Starting Grants 278364 to C.P.S. and 306933 to F.J.R.M.), the Danish Council for Independent Research/Natural Sciences (R.S.), and The Netherlands Organisation for Scientific Research (NWO Vici 865.13.005 to C.P.S.). The NanoSIMS facility at Utrecht University is funded by the large infrastructure subsidy awarded to Jack B. M. Middelburg by The Netherlands Organisation for Scientific Research (NWO).

REFERENCES

(1) Ruttenberg, K. C. *The Global Phosphorus Cycle*; Elsevier: Oxford, U.K., 2014; Vol. *10*, pp 499−558.
(2) Einsele, W. Über die Beziehungen des Eisenkreislaufs zum Phosphorkreislauf 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.* **1942**, *30* (1), 147−201.
(4) Rozan, T. F.; Taillefert, M.; Trouwborst, R. E.; Glazer, B. T.; Ma, S.; Herszage, J.; Valdes, L. M.; Price, K. S.; Luther, G. W., III Iron-Sulfur-Phosphorus Cycling in the Sediments of a Shallow Coastal Bay: Implications for Sediment Nutrient Release and Benthic Macroalgal Blooms. *Limnol. Oceanogr.* **2002**, *47* (5), 1346−1354.
(5) Gustafsson, B. G.; Schenk, F.; Blenckner, T.; Eilola, K.; Meier, H. E.; Muller-Karulis, B.; Neumann, T.; Ruoho-Airola, T.; Savchuk, O. P.; Zorita, E. Reconstructing the development of Baltic sea eutrophication 1850−2006. *Ambio* **2012**, *41* (6), 534−48.
(6) Diaz, R. J.; Rosenberg, R. Spreading Dead Zones and Consequences for Marine Ecosystems. *Science* **2008**, *321* (5891), 926−929.
(7) Middelburg, J. J.; Levin, L. A. Coastal hypoxia and sediment biogeochemistry. *Biogeosciences* **2009**, *6* (7), 1273−1293.
(8) Gächter, R.; Meyer, J. S.; Mares, A. Contribution of bacteria to release and fixation of phosphorus in lake sediments. *Limnol. Oceanogr.* **1988**, *33*, 1542−1558.
(9) Jensen, H. S.; Mortensen, P. B.; Andersen, F. Ø.; Rasmussen, E.; Jensen, A. Phosphorus cycling in a coastal marine sediment, Aarhus Bay, Denmark. *Limnol. Oceanogr.* **1995**, *40* (5), 908−917.
(10) Dale, A. W.; Bertics, V. J.; Treude, T.; Sommer, S.; Wallmann, K. Modeling benthic−pelagic nutrient exchange processes and porewater distributions in a seasonally hypoxic sediment: evidence for massive phosphate release by Beggiatoa? *Biogeosciences* **2013**, *10* (2), 629−651.
(11) Schulz, H. N.; Schulz, H. D. Large Sulfur Bacteria and the Formation of Phosphorite. *Science* **2005**, *307* (5708), 416−418.
(12) 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), 1071−1074.
(13) Pfeffer, C.; Larsen, S.; Song, J.; Dong, M.; Besenbacher, F.; Meyer, R. L.; Kjeldsen, K. U.; Schreiber, L.; Gorby, Y. A.; 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* (7423), 218−221.
(14) 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* (0), 1−13.
(15) Schauer, R.; Risgaard-Petersen, N.; Kjeldsen, K. U.; Tataru Bjerg, J. J.; B Jorgensen, B.; Schramm, A.; Nielsen, L. P. Succession of cable bacteria and electric currents in marine sediment. *ISME J.* **2014**, *8* (6), 1314−1322.
(16) Marzocchi, U.; Trojan, D.; Larsen, S.; Louise Meyer, R.; Peter Revsbech, N.; Schramm, A.; Peter Nielsen, L.; Risgaard-Petersen, N. Electric coupling between distant nitrate reduction and sulfide oxidation in marine sediment. *ISME J.* **2014**, *8* (8), 1682−1690.
(17) Malkin, S. Y.; Rao, A. M. F.; Seitaj, D.; Vasquez-Cardenas, D.; Zetsche, E.-M.; Hidalgo-Martinez, S.; Boschker, H. T. S.; Meysman, F. J. R. Natural occurrence of microbial sulphur oxidation by long-range electron transport in the seafloor. *ISME J.* **2014**, *8*, 1843−1854.
(18) Meysman, F. J. R.; Risgaard-Petersen, N.; Malkin, S. Y.; Nielsen, L. P. The geochemical fingerprint of microbial long-distance electron transport in the seafloor. *Geochim. Cosmochim. Acta* **2015**, *152* (0), 122−142.
(19) Hagens, M.; Slomp, C. P.; Meysman, F. J. R.; Seitaj, D.; Harlay, J.; Borges, A. V.; Middelburg, J. J. Biogeochemical processes and buffering capacity concurrently affect acidification in a seasonally hypoxic coastal marine basin. *Biogeosciences* **2015**, *12* (5), 1561−1583.
(20) Strickland, J. D.; Parsons, T. R. *A Practical Handbook of Seawater Analysis*, 2nd ed.; Bulletin (Fisheries Research Board of Canada): Ottawa, Canada, 1972; Vol. *167*, pp 310−310.
(21) Kraal, P.; Slomp, C. P.; Forster, A.; Kuypers, M. M. M.; Sluijs, A. Pyrite oxidation during sample storage determines phosphorus fractionation in carbonate-poor anoxic sediments. *Geochim. Cosmochim. Acta* **2009**, *73* (11), 3277−3290.
(22) Ruttenberg, K. C. Development of a Sequential Extraction Method for Different Forms of Phosphorus in Marine Sediments. *Limnol. Oceanogr.* **1992**, *37* (7), 1460−1482.
(23) Burton, E. D.; Sullivan, L. A.; Bush, R. T.; Johnston, S. G.; Keene, A. F. A simple and inexpensive chromium-reducible sulfur method for acid-sulfate soils. *Appl. Geochem.* **2008**, *23* (9), 2759−2766.
(24) Kraal, P.; Burton, E. D.; Bush, R. T. Iron monosulfide accumulation and pyrite formation in eutrophic estuarine sediments. *Geochim. Cosmochim. Acta* **2013**, *122* (0), 75−88.
(25) Manz, W.; Amann, R.; Ludwig, W.; Wagner, M.; Schleifer, K.-H. Phylogenetic Oligodeoxynucleotide Probes for the Major Subclasses of Proteobacteria: Problems and Solutions. *Syst. Appl. Microbiol.* **1992**, *15* (4), 593−600.
(26) Seitaj, D.; Schauer, R.; Sulu-Gambari, F.; Hidalgo-Martinez, S.; Malkin, S. Y.; Burdorf, L. D. W.; Slomp, C. P.; Meysman, F. J. R. Cable bacteria generate a firewall against euxinia in seasonally hypoxic basins. *Proc. Natl. Acad. Sci. U. S. A.* **2015**, *112* (43), 13278−13283.
(27) Jørgensen, B. B.; Dunker, R.; Grünke, S.; Røy, H. Filamentous sulfur bacteria, Beggiatoa spp., in arctic marine sediments (Svalbard, 79°N). *FEMS Microbiol. Ecol.* **2010**, *73* (3), 500−513.
(28) Knap, A.; Michaels, A.; Close, A. *JGOFS Protocols*; U.S. JGOFS Planning Office: Woods Hole, MA, 1994; p 198.
(29) Vasquez-Cardenas, D.; van de Vossenberg, J.; Polerecky, L.; Malkin, S. Y.; Schauer, R.; Hidalgo-Martinez, S.; Confurius, V.; Middelburg, J. J.; Meysman, F. J. R.; Boschker, H. T. S. Microbial carbon metabolism associated with electrogenic sulphur oxidation in coastal sediments. *ISME J.* **2015**, *9* (9), 1966−1978.
(30) Sayama, M.; Risgaard-Petersen, N.; Nielsen, L. P.; Fossing, H.; Christensen, P. B. Impact of Bacterial NO_3^- Transport on Sediment Biogeochemistry. *Applied and environmental microbiology* **2005**, *71* (11), 7575−7577.
(31) Straub, K. L.; Benz, M.; Schink, B.; Widdel, F. Anaerobic, nitrate-dependent microbial oxidation of ferrous iron. *Appl. Environ. Microbiol.* **1996**, *62* (4), 1458−1460.
(32) Klump, J. V.; Martens, C. S. Biogeochemical cycling in an organic rich coastal marine basin-II. Nutrient sediment-water exchange processes. *Geochim. Cosmochim. Acta* **1981**, *45* (1), 101−121.
(33) Scicluna, T. R.; Woodland, R. J.; Zhu, Y.; Grace, M. R.; Cook, P. L. M. Deep dynamic pools of phosphorus in the sediment of a temperate lagoon with recurring blooms of diazotrophic cyanobacteria. *Limnol. Oceanogr.* **2015**, *60* (6), 2185−2196.
(34) Risgaard-Petersen, N.; Kristiansen, M.; Frederiksen, R. B.; Dittmer, A. L.; Bjerg, J. T.; Trojan, D.; Schreiber, L.; Damgaard, L. R.; Schramm, A.; Nielsen, L. P. Cable Bacteria in Freshwater Sediments. *Appl. Environ. Microbiol.* **2015**, *81* (17), 6003−6011.

DOI: 10.1021/acs.est.5b04369
Environ. Sci. Technol. 2016, 50, 1227−1233