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Benthic hotspots in the pelagic zone: Light and phosphate availability alter aggregates of microalgae and suspended particles in a shallow turbid lake

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Abstract

Limnetic aggregates from a turbid delta lake with low dissolved nutrient availability were studied in relation to light and dissolved nutrient availability. Quick light-attenuation restricts the euphotic zone to the top surface layer of the water column, whereas mineralization processes in the sediment specifically provide dissolved nutrients near the lakebed. This suggests neither the pelagic nor the benthic zone provides the combination of resources required for microalgal growth. Nutrient mineralization in aggregates could bridge this apparent spatial gap in light and nutrients by providing dissolved nutrients in the euphotic zone, promoting microalgal growth. To explore this, aggregates obtained from turbid and phosphate-limited lake Markermeer (The Netherlands) were exposed in the laboratory to phosphate-replete and phosphate depleted conditions, at high-light and low-light availability. Confocal microscopy revealed that aggregates exhibited alkaline phosphatase activity and contained microalgae, other microbes, and extracellular polymeric substances. The spatial distribution of the phosphatase activity in aggregates largely matched that of chlorophyll *a* (Chl *a*)-lacking microbes, suggesting that these microbes were responsible for the activity. Colorimetric quantification revealed that aggregates exhibited over 1.9-fold higher phosphatase activity than surrounding water. Two-day exposure to different light and phosphate availabilities affected aggregate composition. Phosphate depleted conditions resulted in more Chl *a*-lacking microbes and more phosphatase activity than phosphate-replete conditions. Low-light intensity resulted in higher abundance of extracellular polymeric substances than high-light intensity. In contrast to aggregates from deep stratified systems, Markermeer aggregates were not enriched with dissolved phosphorus. These results suggest that P-cycling in aggregates differs between shallow turbid and deep stratified ecosystems.

Microalgae occurring in shallow turbid lakes in delta regions can face sharp opposing gradients in essential resources. The construction of dams and dykes in these areas to enhance flood protection, land reclamation, and water storage (e.g., Van Eck 1982; Okamura et al. 1996; Lie et al. 2008), has led to increased retention of large deposits of fine sediments in lakes (Noordhuis et al. 2010; Ding et al. 2017). As a result of wind-activity, high concentrations of suspended particles quickly attenuate incident light, restricting the euphotic zone to the top surface layer of the water column for elongated periods of time (Haffner and Evans 1974; Umehara et al. 2012; De Lucas Pardo et al. 2015; Storlazzi et al. 2015). Additionally, re-oligotrophication efforts such as the isolation of delta lakes from direct riverine inflow, and the reduction of external nutrient loading, have resulted in low phosphate availability in some delta lakes (Jeppesen et al. 2005; De Leeuw et al. 2008; Noordhuis 2010).

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Consequently, mineralization processes localized near the (anoxic) sediment layer of the lake bed have likely become the main source of dissolved nutrients for microalgae in these lakes (Smolders and Roelofs 1993). The combination of a light-limited benthic zone and a nutrient-limited pelagic zone, suggests that delta lakes with low nutrient availability comprise a spatial gap between the resources that are essential for microalgal growth. Nevertheless, microalgae can thrive in the turbid water column of shallow delta lakes with low dissolved nutrient availability (Noordhuis 2010; Ding et al. 2017).

In the water column of deep, stratified waters, large (> 500 μm) amorphous aggregates termed marine snow (Suzuki and Kato 1953) or lake snow (Grossart and Simon 1993) have been found to act as local nutrient hotspots. Similar aggregates have been identified in rivers and shallow lakes with higher hydrodynamic forcing (Böckelmann et al. 2000; Neu 2000; Simon et al. 2002; Tang et al. 2010). The activity of extracellular hydrolytic enzymes, produced by microbes in marine and lake snow aggregates, provides dissolved nutrients and organic solutes in aggregates, partly extending in trails from aggregates into the surrounding water (Kiørboe and Jackson 2001; Simon et al. 2002). These hydrolytic enzymes include extracellular alkaline phosphatases (ALPases) (Kaltenböck and Herndl 1992; Grossart and Simon 1998), which release phosphate from organic compounds by hydrolysis of monophosphate ester bonds (Hoppe 2003). Extracellular polymeric substances (EPS), including polysaccharides, proteins, glycoproteins, glycolipids, and extracellular DNA (Flemming et al. 2007), can enhance the formation of aggregates (Kiørboe et al. 1990; Passow et al. 1994; Chow et al. 2015). Various marine and freshwater microalgae have been found to increase the production of EPS under nutrient limitation (Guerrini et al. 1998; Magaletti et al. 2004; Urbani et al. 2005; Abdullahi et al. 2006; Boonchai et al. 2015). In general, this is interpreted as the release of excess photosynthate when the production of carbohydrates by microalgae exceeds the consumption of carbohydrates by microalgae due to nutrient-limited growth requirements (Fogg 1983; Wood and Van Valen 1990). Therefore, nutrient-limited conditions in the euphotic zone of delta lakes could stimulate algal EPS production, promoting the formation of microalgal aggregates. Considering the release of nutrients from marine and lake snow aggregates, the formation of such aggregates could support phytoplankton growth in the water column of turbid, phosphate-limited delta lakes.

This study sets out to analyze whether phosphate-limited and high-light conditions stimulate the formation of pelagic aggregates in the euphotic zone of shallow turbid waters, providing phosphate to microalgae in the water column. For this purpose, we collected aggregates from lake Markermeer, a large delta lake in The Netherlands characterized by turbid conditions during most of the year and low (< 0.5 μM) phosphate availability in the water column (Noordhuis 2010). We aimed to examine: (1) if aggregates in the euphotic zone of the turbid and phosphate-limited water column of lake Markermeer exhibit ALPase activity, and contain microalgae and EPS; (2) if light and phosphate availability affect aggregate composition; and (3) if lake Markermeer aggregates are enriched with ALPase activity and phosphate compared to surrounding water.

We hypothesized that (1) Markermeer aggregates exhibit ALPase activity and comprise microalgae and EPS; (2) low phosphate availability increases the production of EPS by aggregate microalgae under high-light conditions, and increases aggregate ALPase activity irrespective of light availability; and (3) Markermeer aggregates are hotspots of both ALPase activity and phosphate availability compared to surrounding water. To test this, we exposed Markermeer aggregates in the laboratory to the full factorial setup of high-light and low-light intensity, with and without the addition of phosphate. We measured the rate of net aggregate mass gain to infer whether overall microbial growth was limited at these light and phosphorus conditions. Furthermore, we applied confocal laser scanning microscopy (CLSM) to uncover the composition of limnetic aggregates in terms of extracellular ALPase activity microalgae and EPS. In addition, we quantified ALPase activity, dissolved phosphorus and particulate phosphorus in aggregates and in surrounding water in order to infer whether the aggregates are hotspots of ALPase activity and dissolved phosphorus.

Materials and methods

Study lake and sampling

Lake Markermeer is a 3–5 m deep (mean depth 3.6 m), 680 km^2 delta lake located in the center of The Netherlands (Fig. 1). This freshwater lake with marine clay sediment used to be connected to the North Sea until the completion of the Afsluitdijk (Fig. 1A) in 1932. Since the closure of the Houtribdijk (Fig. 1B) in 1975, lake Markermeer has also become disconnected from influences of the IJssel river (Fig. 1C). This resulted in year-round low dissolved inorganic phosphorus concentrations. Less than 0.5 μM phosphate was available in the water column of the lake during the last decades (Noordhuis 2010). Furthermore, wind-induced resuspension of fine marine deposits from the lakebed sediment resulted in year-round low light penetration. In the past decade, the median concentration of suspended particles was 35.0 mg·L^{-1} (interquartile range (IQR) = 5.0–59.0 mg·L^{-1}) in the water column of the lake, and the median Secchi-depth was 0.20 m (IQR = 0.20–0.40 m; 2006 to 2016, http://live.waterbase.nl, accessed 25 July 2017).

Aggregates were collected in triplicate from lake Markermeer (Fig. 1) on 03 July 2016 by casting a bucket from the dike bordering the eastern side of the lake. South-western wind (242°) averaging 5.8 m·s^{-1} on the day prior to the sampling date (http://projects.knmi.nl/klimatologie/daggegevens/selectie.cgi, accessed 01 November 2017) resulted in a well-mixed water column. Surface water with aggregates was bottled and stored

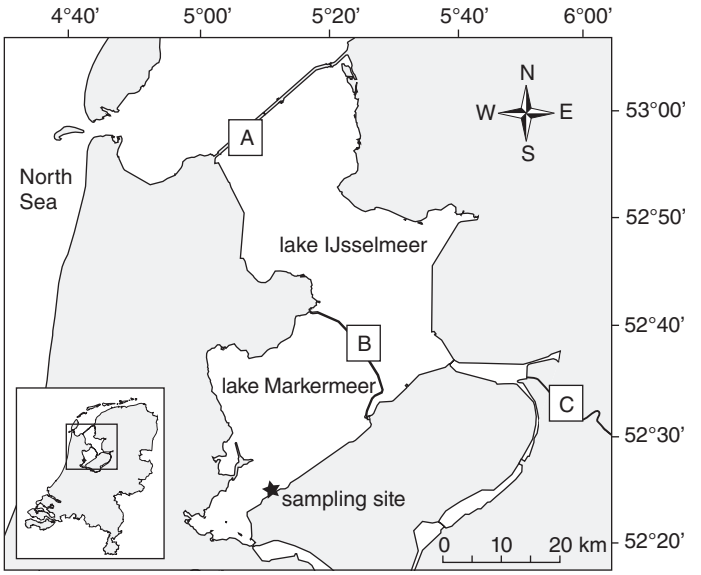

Fig. 1. Sampling site at lake Markermeer, The Netherlands (52°25′5.4″N, 5°12′39.3″E). Capital letters indicate A, Afsluitdijk; B, Houtribdijk; and C, the IJssel river.

in the dark at 15°C until the start of incubations on 04 July 2016. The total phosphorus concentration in sampled surface water averaged 2.0 ± 0.2 μM. This is below the threshold of 3.2 μM total phosphorus, which has been related to successful re-oligotrophication in shallow Danish lakes (Jeppesen et al. 2000).

Experimental treatment

Water with aggregates (2 × 350 mL per replicate) was incubated in sterile 500 mL Erlenmeyer flasks and—in a full factorial setup—exposed to high-light (30–40 μmol·s^{-1}·m^{-2}), low-light (5–10 μmol·s^{-1}·m^{-2}), phosphate-replete (175 μM K_2HPO_4) and phosphate depleted (without the addition of K_2HPO_4) conditions for 2 d at 20°C (n = 3). These conditions were selected based on a pilot experiment in which photosystem II maximum quantum yield was measured for monoalgal cultures *Staurosira* sp., one of the most abundant microalgae in lake Markermeer (Supporting Information Fig. S1). SoLux halogen lamps (prod. code 35003, Eiko, Shawnee) illuminated flasks from above. The emission spectrum of these lamps resembles that of sunlight. The intensity of photosynthetic active radiation was adjusted using neutral density LEE filters and was verified using a light quantum meter (Li-Cor Li 1400). During incubations, flasks were gently aerated through the water using sterile compressed air via glass needles fixed into cellulose plugs closing the flasks.

Aggregate concentration and mass gain

Aggregate concentration was estimated based on dry weight of suspended aggregates both prior to and following the exposure to the experimental treatment. GF/C filters (1.2 μm-pores; 47 mm-Ø; VWR, Radnor, Pennsylvania) were preheated (4 h at 450°C) to combust possible organic contamination. The filters were allowed to cool down in a vacuum desiccator and weighed. A total volume of 50 mL suspended aggregates was filtered over preheated GF/C filters using a vacuum manifold to collect aggregates on the filters. Given the pore size of the GF/C filters, all aggregates larger than 1.2 μm were collected. After filtration, filters were dried (12 h at 60°C) and weighed again.

The rate of net aggregate mass gain μ (d^{-1}) was calculated from the change in aggregate dry weight over the 2-d incubation period by Eq1:

$$\mu = \frac{\ln\left(\frac{DW_f}{DW_i}\right)}{t} \quad \text{(Eq1)}$$

where DW_i and DW_f represent the initial and final aggregate dry weight, and t the incubation time in days. Aggregate organic content was determined by loss on ignition. To this end, the filters were combusted (4 h at 450°C) and weighed again.

Aggregate composition

The spatial distribution of microalgae, other chlorophyll *a* (Chl *a*)-lacking microbes, EPS and ALPase activity within aggregates was studied prior to the experimental treatment (n = 8) and following the experimental treatment (n = 4 for each condition) by way of confocal laser scanning microscopy (CLSM). Prior to microscopy, suspended aggregates (600 mL) were exposed for 30 min to a laminar flow in a Couette flocculator in order to restore possible disaggregation induced by sampling and transportation. The Couette flocculator consisted of a 600 mL-chamber with a total length of 17 cm, a fixed inner cylinder with radius r_1 = 4.4 cm, and a rotatable outer cylinder with radius r_2 = 5.5 cm (Verspagen et al. 2006). The angular velocity ω of the outer cylinder was set to 10.5 radians s^{-1}, resulting in a mean shear rate G_M of 47 s^{-1}, calculated after Van Duuren (1968). After flocculation, the inner cylinder of the Couette flocculator was detached and aggregates were allowed to settle inside the flocculator. Settled aggregates (four or eight samples of 1 mL) were transferred out of the Couette flocculator by suction using a syringe with silicon tubing with an inner radius of 0.3 cm.

Aggregates were stained at room temperature in Nunc™ Lab-Tek II™ chambered coverglasses (prod. code 155409, ThermoFisher Scientific, Waltham, Massachusetts) with a total volume of 1 mL per chamber and 10 mm spacer height. For each replicate a separate chamber was used. Chl *a* autofluorescence was used to identify microalgae. A mixture of three different fluorophores was used to detect other microbes without Chl *a*, EPS, and ALPase activity. Syto-9 (S34854, Molecular Probes, Eugene, Oregon) was used to stain DNA and RNA of live and dead, prokaryotic and eukaryotic microbes. Tetramethylrhodamine (TRITC) labeled *Concanavalin A* (*ConA*) lectins (C860, Molecular Probes), binding α-D-mannose and α-D-glucose groups of EPS with broad species-specificity (Neu and Lawrence 1999; Bahulikar and

Kroth 2007), were used to label EPS. ELF-97 substrate (E6588, Molecular Probes) was used to target extracellular ALPase activity. Hydrolysis of the phosphate-group from this water-soluble substrate produces a fluorescent alcohol that is water-insoluble at pH < 8. As such, the ELF alcohol precipitates on top of or near active ALPases at pH < 8 (Paragas et al. 2002; ELF-97 (E6601, Molecular Probes) Product Information, 2004). Bright-field microscopy was used to discern the outlines of aggregates.

The fluorophores were added to the chambers in the following order. At t = 0 min, 500 μL 30 μM ELF-97 substrate was added and incubated for 60 min. At t = 30 min, seven drops 0.1 g·L^{-1} *ConA* were applied and incubated for 30 min. At t = 55 min, seven drops 0.835 μM Syto-9 were added to the mixture and incubated for 5 min. Fluorophores were diluted to the correct concentration using 10 mM Tris–HCl buffer (pH 7.5) to prevent dissolution of the ELF alcohol precipitate. Following the total incubation time of 60 min, chambers were washed once by replacing the fluorophore mixture with 10 mM Tris–HCl.

Immediately upon the 60-min staining procedure, aggregates were scanned using an inverted LSM510 microscope (Zeiss, Oberkochen, Germany) equipped with four lasers (λ = 351 and 364 nm, 488 nm, 543 nm, and 633 nm) and a 20×/0.75 NA Plan-Apochromat objective (Zeiss). Two 60-μm thick scans were acquired for each replicate, starting at the bottom surface of the chambered coverglass. Datasets were acquired sequentially to prevent spectral bleed-through. The resolution in the z-direction was set to 1 μm. ELF-97 was excited at 351 nm and 364 nm, and detected using a 505–550 BP filter. Syto-9 was excited at 488 nm and detected using a 505–550 BP filter. *ConA* was excited at 543 nm and detected using a 560–615 BP filter. Chl *a* was excited at 633 nm and detected using a 650 LP filter.

Image stacks were imported into MATLAB using the TIFFStack class developed by Muir and Kampa (2015) and processed using the DIPimage toolbox (v. 2.8; www.diplib.org/dipimage). Image processing involved four steps. First, noise was reduced in the EPS and ALPase channels by adding an elliptical median filter with a size of three pixels. Second, background signal was removed from the 8-bit Chl *a* and ALPase channel by subtracting an intensity of 10 and 70, respectively at all pixel locations. Third, 8-bit images were converted to binary images using a fixed threshold, accounting 150, 40, 40, and 100 for the Chl *a*, EPS, Chl *a*-lacking microbes and ALPase channels, respectively. Fourth, to correct for Syto-9 labeling of Chl *a*-comprising microbes, all pixels at which Chl *a* was detected were set to 0 in the channel for Chl *a*-lacking microbes. Pixel cover was calculated as the average percentage of pixels at which a signal was detected from 15 μm to 26 μm depth, measured from the side of the objective. Below 15-μm within aggregates, EPS signal cover was high in two to four subsequent z-slices, possibly due to the presence of a film of settled lectins on the bottom of the coverglass. Deeper than 26 μm within aggregates, the signal of Chl *a*-lacking microbes attenuated. Therefore, these z-slices were excluded from image analyses.

Extracellular ALPase activity and P-content

Prior to ALPase activity and P-content analyses, aggregates were exposed for 30 min to a laminar flow in a Couette flocculator as described in "Aggregate composition" section. At the end of this exposure, settled aggregates were transferred out of the flocculator for analyses of extracellular ALPase activity (1 mL) and P-content (45 mL). Additionally, the water layer was sampled (1 mL) to determine ALPase activity in the surrounding water of the aggregates.

Extracellular ALPase activity was quantified in suspended aggregates and in surrounding water prior to and following the experimental treatment. The colorimetric method described by Sayler et al. (1979) was applied. Briefly, 1 mL of suspended aggregates or 1 mL of surrounding water was diluted with 3 mL 1 M Tris–HCl (pH 8.6). Subsequently, 1 mL of 1 mg·mL^{-1} p-nitrophenyl phosphate (BDH Laboratory Supplies, currently Merck, Kenilworth, New Jersey) in 0.2 M Tris–HCl (pH 7.6) was added to the diluted aggregate suspensions or surrounding water. This reaction mixture was vortexed and incubated for 1 h at 37°C. Cleavage of the phosphate group from p-nitrophenyl phosphate by phosphatases produces the yellow product p-nitrophenol. The reaction was terminated by adding 1 mL 1 M NaOH. Samples were vortexed and centrifuged at 2500 × g for 10 min. Absorption of 200 μL supernatant was measured spectrophotometrically at 418 nm in a microtiter plate. A standard curve was prepared using 0–200 μM p-nitrophenol (Merck). ALPase activity was expressed as μmol P·g^{-1} aggregate dry weight·h^{-1}. Dry weight was estimated from 30 mL suspended aggregates as described above.

Total particulate and total dissolved phosphorus was quantified prior to and following the experimental treatment. To this end, total phosphorus was quantified in suspended aggregates (15 mL) and in filtrate (30 mL) of GF/C filtered aggregates. Suspended aggregates were concentrated by freeze-drying and were destructed subsequently in 3 mL of 1 M H_2SO_4 by autoclaving for 20 min at 121°C and 103.4 kPa. Total phosphorus of destructed aggregates was measured using ICP-OES spectrometry (Optima 8000, PerkinElmer, Waltham, Massachusetts) with 1 ppm Yttrium as internal standard. Total dissolved phosphorus was estimated from the filtrate using Hach LCK349 cuvette tests for total phosphorus (Hach Company, Loveland, Colorado) with a detection limit of 0.5 μM. Total particulate phosphorus in aggregates was calculated by subtracting total dissolved phosphorous concentrations from total phosphorus concentrations of destructed aggregates.

Statistical analyses

Statistical analyses were performed in R (v. 3.2.1; www.r-project.org). Results are reported as the mean ± standard error of the mean, calculated using the bear package (v. 2.6.4; pkpd.

kmu.edu.tw/bear). If data did not follow a normal distribution (as tested by the Shapiro–Wilk test for normality), and $n > 3$, median and IQR are presented.

The effects of light and phosphate availability on the rate of net aggregate mass gain, final concentration, organic content, composition (final microalgae, Chl *a*-lacking microbes and EPS abundance), ALPase activity and particulate P-content were tested in a two-way ANOVA design with Tukey's HSD post-hoc test with light and phosphate availability as factors. The interaction between light and phosphate availability was only included in the model if it was significant ($p < 0.05$). This was only the case for the two-way ANOVA for ALPase activity within aggregates. Diagnostic plots were inspected to verify that variance was equally distributed and to confirm that there were no outliers with a Cook's distance > 0.5. The Shapiro–Wilk test for normality was used to check if residuals followed a normal distribution. Only in the two-way ANOVA test for the analysis of final ALPase abundance, residuals did not follow a normal distribution. Logarithmic transformation of ALPase abundances did not result in a normal distribution of ANOVA residuals. For this reason, the (non-parametric) Wilcoxon rank-sum test with continuity correction was performed instead of the (parametric) two-way ANOVA, to analyze the effects of light and nutrient availability on final ALPase abundance. Since the Wilcoxon rank-sum test cannot deal with multiple explanatory variables, the effects of light and nutrient availability on ALPase abundance were tested separately. The dissolved P-concentrations of samples that were not enriched with phosphate remained below the detection limit of our analyses (0.5 μM). Therefore, these data were omitted from statistical analyses. The effect of light availability on median dissolved P-concentrations in phosphate-enriched samples was tested using the Wilcoxon rank-sum test.

Aggregate variables that were neither affected by light availability, nor by phosphate availability, were compared prior to and following the incubation period using the two-sample *t*-test. In order to verify if variance was equal prior to and following incubation, the F-test for equality in variances was used. If variance was unequal (i.e., for aggregate particulate P-content), the Welch's *t*-test was used. The Shapiro–Wilk test for normality was used to check if data, prior to and following the incubation, followed a normal distribution. The Wilcoxon rank-sum test was used as an alternative to *t*-tests for non-parametric data (i.e., for aggregate abundance of Chl *a*-lacking microbes and for dissolved P-concentration).

Results

Aggregate concentration and mass gain

Prior to the experimental treatment, the concentration of lake Markermeer aggregates averaged 38.0 ± 1.2 mg·L^{-1} with an organic content of 23.1% ± 2.4%. Irrespective of light availability, the addition of phosphate resulted in higher rates of net aggregate mass gain (0.18 ± 0.02 d^{-1}) compared to incubations without the addition of phosphate (0.12 ± 0.02 d^{-1}, $F_{1,9} = 6.50$, $p = 0.03$; Fig. 2). The rate of net aggregate mass gain did not differ between high-light and low-light exposed aggregates ($F_{1,9} < 0.01$, $p = 0.96$). After 2 d of incubation, dry weight of aggregates was higher in phosphate-enriched (54.7 ± 1.43 mg·L^{-1}) than in phosphate depleted (48.0 ± 1.63 mg·L^{-1}) lake water ($F_{1,9} = 8.49$, $p = 0.02$), but did not differ between high-light and low-light exposed aggregates ($F_{1,9} < 0.01$, $p > 0.99$). Final organic content of aggregates averaged 22.7% ± 2.7% and did neither differ between phosphate-replete and phosphate depleted incubations ($F_{1,9} = 0.063$, $p = 0.81$), nor between high-light and low-light incubation ($F_{1,9} = 0.066$, $p = 0.80$). The initial and final mean organic content of aggregates were similar ($t = -0.076$, df = 13, $p = 0.94$).

Aggregate composition

Microalgae (Chl *a*), EPS, Chl *a*-lacking microbes, and extracellular ALPase activity were observed in all aggregates studied by CLSM prior to the experimental treatment ($n = 8$, Fig. 3). The mean Chl *a* cover was 1.19% ± 0.36% of the scanned aggregate surface, (Fig. 4A), the mean EPS cover was 1.63% ± 0.49% (Fig. 4B), the median cover of Chl *a*-lacking microbes was 0.20% (IQR = 0.10–0.26%; Fig. 4C), and the median ALPase activity cover was 0.18% (IQR = 0.13–0.51%; Fig. 4D). EPS were detected on the surface of microalgae and in clouds near clusters of Chl *a*-lacking microbes. Extracellular ALPase activity mainly occurred in spots that matched the spatial distribution of Chl *a*-lacking microbes. In total, 105 of the 144 detected ALPase activity spots overlapped with Chl *a*-lacking microbes, while only eight spots overlapped with microalgae. Bright-field microscopy indicated that no ALPase activity was detected outside of aggregates. The final Chl *a* cover, averaging 1.63% ± 0.21%, neither differed between phosphate-enriched

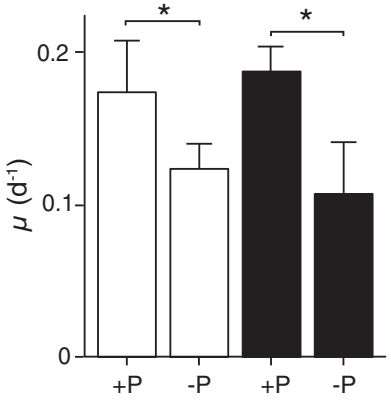

Fig. 2. The effect of phosphate enrichment on the rate of net aggregate mass gain (μ) at high-light and low-light conditions, based on dry weight. Bars depict the average rate of net aggregate mass gain at high-light (white bars) or low-light (black bars) conditions with phosphate (+P) or without the addition of phosphate (–P). Error bars indicate the standard error of the mean ($n = 3$). Asterisks present statistical differences ($p < 0.05$).

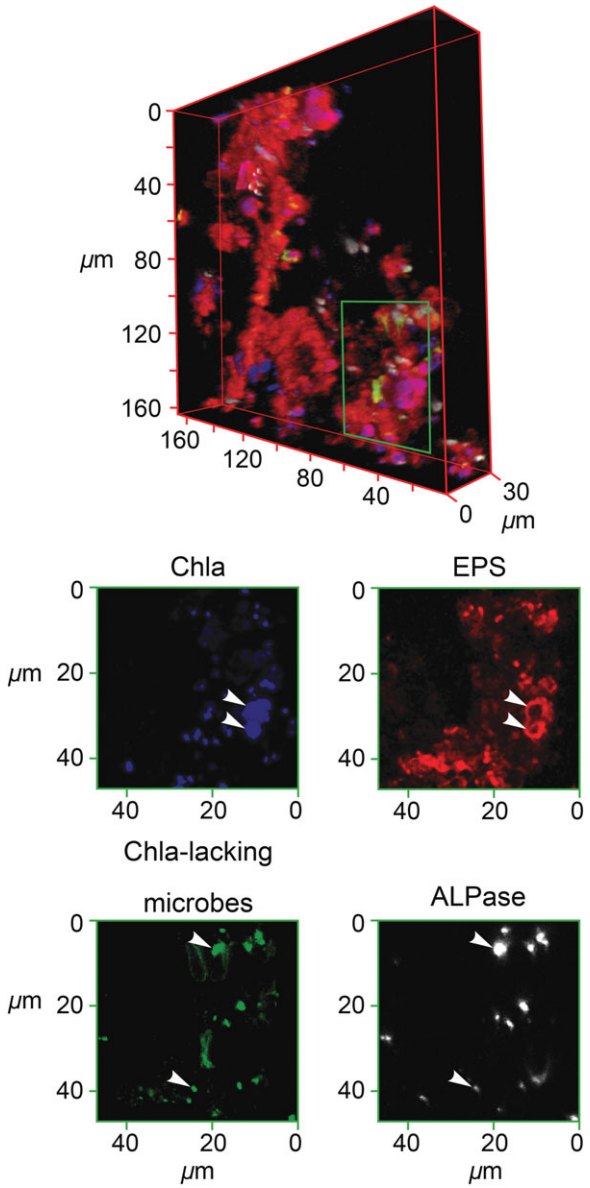

Fig. 3. Slice through a limnetic aggregate of lake Markermeer. Colors represent Chl *a* (blue), extracellular polymeric substances (EPS; red), Chl *a*-lacking microbes (green), and alkaline phosphatase activity (ALPase; white). Projections of the inset are shown below the 3D image for each channel separately. The arrows point to examples of algae (Chl *a*) covered with EPS (upper two projections), and of Chl *a*-lacking microbes overlapping with ALPase activity (lower two projections).

and phosphate depleted, nor between high-light and low-light incubations ($F_{1,13} = 0.63$, $p = 0.44$; and $F_{1,13} = 0.18$, $p = 0.68$, respectively; Fig. 4A). The mean Chl *a* cover was similar prior to and following the 2-d incubation ($t = -1.16$, df = 22, $p = 0.26$). At the end of the incubation, the mean EPS cover was higher in aggregates exposed to low-light (5.43% ± 1.05%) than in aggregates exposed to high-light conditions (2.53% ± 0.68%; $F_{1,13} = 5.06$, $p = 0.04$; Fig. 4B). Phosphate-enrichment did not result in differences in mean EPS cover ($F_{1,13} = 0.09$; $p = 0.77$). The final mean cover of Chl *a*-lacking microbes in aggregates was higher after 2 d of incubation in phosphate depleted conditions (0.61% ± 0.16%), than in phosphate-replete conditions (0.20% ± 0.07%; $F_{1,13} = 5.95$, $p = 0.03$; Fig. 4C). The different light treatments did not influence the final mean cover of Chl *a*-lacking microbes in aggregates ($F_{1,13} = 1.32$, $p = 0.27$). The median cover of ALPase activity was lower following phosphate-enriched incubations (0.0001%, IQR = 0.000–0.001%), than following incubation without the addition of phosphate (0.27%, IQR = 0.091–0.38%; $W = 62$, $p = 0.002$; Fig. 4D). Exposure to the different light intensities did not result in different median cover of ALPase activity in aggregates ($W = 33$, $p = 0.96$).

Extracellular ALPase activity and P-content

Before the incubation treatment, ALPase activity in aggregates averaged 24.6 ± 4.0 μmol P·g^{-1} aggregate·h^{-1} (Fig. 5). Given the concentration of aggregates in the field (38.0 ± 1.2 mg·L^{-1}), this activity corresponds to a potential phosphate release of 0.95 ± 0.18 μmol P·L^{-1}·h^{-1}. This was at least 1.9 times higher than ALPase activity in surrounding water, which was below the detection limit of 0.5 μmol P·L^{-1}·h^{-1}. At high-light intensity, incubation without the addition of phosphate resulted in higher mean ALPase activity in aggregates (95.9 ± 7.6 μmol P·g^{-1} aggregate·h^{-1}) than incubation with the addition of phosphate (33.0 ± 3.0 μmol P·g^{-1} aggregate·h^{-1}; $F_{1,8} = 38.36$, $p < 0.001$). At low-light, mean ALPase activity of aggregates (54.0 ± 6.0 μmol P·g^{-1} aggregate·h^{-1}) did not differ between phosphate-enriched and phosphate depleted incubations ($F_{1,8} = 2.58$, $p = 0.29$).

In phosphate-enriched aggregate suspensions, the final median dissolved phosphorus concentration (155.6 μM, IQR = 138.1–159.1 μM) did not differ between high-light and low-light exposed samples ($W = 2$, $p = 0.4$). The mean dissolved phosphorus concentration in phosphate-enriched aggregate suspensions was similar prior to (159.6 ± 0.9 μM) and following (148.5 ± 6.6 μM) the 2-d incubation ($t = 1.67$, df = 5, $p = 0.15$). In aggregate suspensions without the addition of phosphate, the concentration of dissolved phosphorus remained below the detection limit of 0.5 μM. The final mean particulate phosphorus concentration in aggregates (70.2 ± 77.3 μmol P·g^{-1} aggregate) neither differed between incubations with and without the addition of phosphate ($F_{1,9} = 0.03$, $p = 0.87$), nor between high-light and low-light conditions ($F_{1,9} = 0.16$, $p = 0.70$). The mean particulate phosphorus concentration was similar prior to and following the 2-d incubation ($t = -0.23$, df = 11, $p = 0.82$).

Discussion

In this study, we identified limnetic aggregates in the euphotic zone of a turbid delta lake. Considering the sharp contrasting gradients in nutrients and light that occur in turbid delta lakes, we investigated how changes in phosphate and light availability affect aggregate composition.

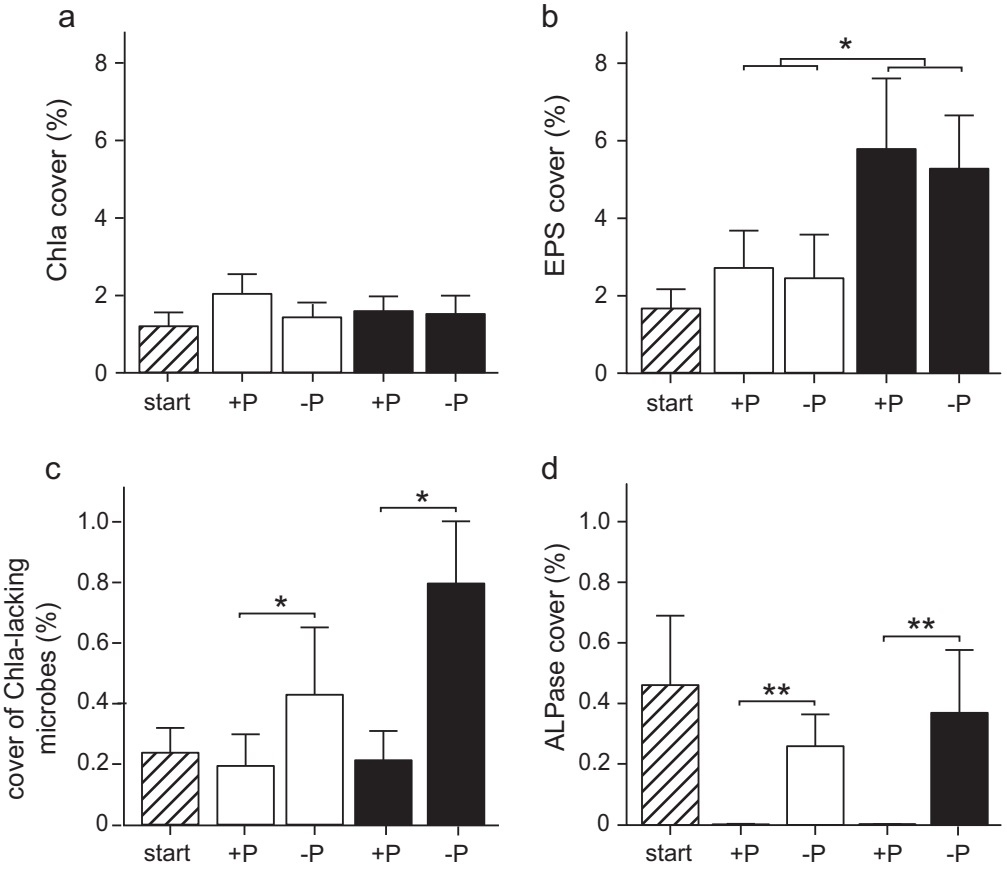

Fig. 4. Effects of light and phosphate availability on aggregate composition. Bars represent average volume (%) of CLSM scans covered by (a) Chl *a*; (b) extracellular polymeric substances (EPS); (c) Chl *a*-lacking microbes; and (d) alkaline phosphatase (ALPase) activity prior to the incubation (start, hatched bars; $n = 8$) and following the incubation ($n = 4$) at high-light (white bars) or low-light (black bars) conditions with phosphate (+P) or without phosphate enrichment (−P). Error bars depict the standard error of the mean. Asterisks indicate significance: *$p < 0.05$; **$p < 0.01$.

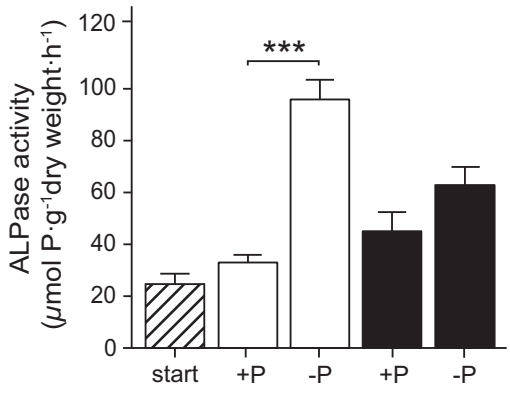

Fig. 5. Effects of light and phosphate availability on alkaline phosphatase (ALPase) activity in aggregates. Bars present average ALPase activity prior to the incubation (start, hatched bar), and following the incubation at high-light (white bars) or low-light (black bars) conditions, with phosphate (+P) or without phosphate enrichment (−P). Error bars depict the standard error of the mean ($n = 3$). Asterisks represent significant differences ($p < 0.001$).

Effects of phosphate on aggregate composition

In three ways, changes in Markermeer aggregates that were induced by the addition of phosphate indicated that aggregate microbes experienced phosphate limitation. Therefore, it was particularly relevant to find that Markermeer aggregates are hotspots of ALPase activity, exhibiting over 1.9-fold higher ALPase activity than in surrounding water. First, phosphate enrichment increased the rate of net aggregate mass gain, suggesting that the overall growth of microbes in Markermeer aggregates was limited by phosphate. Second, in all aggregates prior to the addition of phosphate, ALPase activity was detected. Among other factors, microbial P-demand can induce such extracellular ALPase activity (Hoppe 2003). The spatial distribution of this activity particularly matched that of Chl *a*-lacking microbes, suggesting these microbes had released ALPases into the aggregate matrix. Third, the abundance of Chl *a*-lacking microbes was higher following incubation without phosphate, than following incubation with phosphate. As increased C : P ratios due to low phosphate availability can increase the competitive advantage of heterotrophic bacteria over algae (Grover 2000), this could indicate

that aggregates were limited by phosphate in incubations without the addition of phosphate. It moreover suggests that bacteria in aggregates consume algal EPS that is released due to phosphate-limitation (Currie and Kalff 1984).

Despite considerable aggregate-associated ALPase activity, we neither detected dissolved phosphorus in aggregates, nor in aggregate-surrounding water, both prior to and following the 2-d incubation without the addition of phosphate. This is in contrast to the enrichment of phosphate in aggregates from stratified systems (Simon et al. 2002). For example, Grossart and Simon (1993) showed that lake snow aggregates collected from lake Constance (Germany) comprised over 1000 times more phosphate (72–318 μM) than the phosphate depleted surrounding water (< 0.05–0.1 μM). The contrasting low availability of dissolved phosphorus in aggregates from lake Markermeer could result from efficient phosphate uptake in these aggregates, for example due to higher abundance of living instead of dead microalgae. Alternatively, given the high inorganic content of Markermeer aggregates (77.3% ± 2.7%), the low dissolved phosphorus concentration in Markermeer aggregates could result from sorption of phosphate to aggregate constituents such as clay (Goyne et al. 2008). Furthermore, the bacterial community associated with limnetic aggregates from shallow and turbid systems, dominated by *β-Proteobacteria* and *Cytophaga/Flavobacteria*, could indicate that these aggregates are composed of rather refractory organic material (Simon et al. 2002). Thus, low phosphorus solubilization rates due to the small (22.7% ± 2.7%) and potentially refractory organic fraction of Markermeer aggregates could explain why no dissolved phosphorus could be detected in these aggregates.

Effects of light availability on aggregate composition

Our measurements of net aggregate mass gain show that low-light intensities of 5–10 μmol·s^{-1}·m^{-2}, as compared to high-light conditions of 30–40 μmol·s^{-1}·m^{-2}, did not limit net growth of aggregate-associated microbes in lake Markermeer. In agreement with this finding, Ding et al. (2017) showed that photosystem II maximum quantum yield (F_v/F_m) of microalgae from shallow turbid lake Taihu (China) correlated positively with sudden changes in turbidity. This suggests that microalgae in turbid delta lakes can quickly adapt to low-light availability by increasing their light-sensitivity under turbid conditions caused by sediment resuspension.

Furthermore, our results indicate that the low-light availability applied in the incubations limited the uptake of phosphate by aggregate-associated microbes. Only in the high-light exposed aggregates, phosphate depleted conditions resulted in higher ALPase activity than phosphate-replete conditions. This could result from higher uptake of phosphate by aggregate-associated microbes, decreasing the phosphate availability within aggregates at high-light as compared to low-light conditions. Light-enhanced phosphate uptake has been demonstrated for microalgae in cultures and for natural microalgae populations and has been suggested to increase the competitive advantage of microalgae over bacteria (Smith 1966; Healey 1973; Lemasson et al. 1980; Nalewajko et al. 1981; Nalewajko and Lee 1983).

The different light conditions of our study also affected the abundance of EPS in aggregates. In contrast to our hypothesis that microalgae release EPS in aggregates under high-light and phosphate depleted conditions, low-light conditions resulted in higher EPS abundance in aggregates than high-light conditions irrespective of phosphate availability, as detected by CLSM. Possibly, non-phototrophic microorganisms had produced this relatively high abundance of EPS under low-light intensity. This agrees with literature reviewed by Simon et al. (2002), who noted that bacteria may be of greater importance in aggregate polysaccharide production than generally assumed. Non-phototrophic microbes including bacteria, fungi, and archaea have already been found to produce EPS in mixed biofilm consortia (Chandra et al. 2001; Flemming and Wingender 2010; Zolghadr et al. 2010; Flemming et al. 2016). Accordingly, CLSM imaging of Markermeer aggregates showed that EPS did not only coat microalgae, but also surrounded clusters of Chl *a*-lacking microbes. It is still unclear how the increased EPS abundance in aggregates under low-light conditions affects aggregate functioning. In biofilm consortia, functions attributed to EPS include microbe-biofilm adhesion, biofilm cohesion, water and enzyme retention, sorption of organic and inorganic compounds, protection against grazing, antibiotics and infection and photon transmission to microalgae (Flemming and Wingender 2001, 2010; Vu et al. 2009; Ghafoor et al. 2011; Nwodo et al. 2012). Functions of EPS in suspended aggregates could be similar.

Possible contributions of aggregates to turbid lake productivity

In stratified marine systems, aggregate primary productivity has been found to constitute a considerable fraction of the total water column primary productivity, accounting about 25% of the total volume-specific productivity (Knauer et al. 1982; Prézelin and Alldredge 1983; Gotschalk and Alldredge 1989; Kaltenböck and Herndl 1992). Differences between aggregates from shallow turbid and from stratified deep systems, e.g., in aggregate abundance (marine snow, typically 5–15 mg·L^{-1}; and Markermeer, 38.0 ± 1.8 mg·L^{-1}), aggregate organic content and microbial colonization (Simon et al. 2002), complicate the extrapolation of these results to turbid delta lakes. However, the high extracellular ALPase activity detected in Markermeer aggregates suggests that these aggregates enable microalgal growth in these systems, by providing phosphate in phosphate depleted water. The limited light penetration in turbid water columns does not necessarily restrain this, since the rate of net aggregate mass gain in our experiments was not limited by low light intensities ranging from 5 μmol photons s^{-1}·m^{-2} to 10 μmol photons s^{-1}·m^{-2}.

It is still unclear to what extent the aggregate-associated biomass is passed on to higher trophic levels in turbid delta lakes. In lake Markermeer, both fish and mussel abundance have been low during the last decades, despite year-round high Chl *a* concentrations (fluctuating around 30–60 $\mu g \cdot L^{-1}$; Noordhuis 2010). The relatively high abundance of small microalgae species (< 5 μm) could indicate that the zooplankton grazing pressure is low in lake Markermeer (Noordhuis 2010). This suggests that the primary production associated with Markermeer aggregates is part of a rather closed food web dominated by microalgae and bacteria. Penning et al. (2013) found that increasing concentrations of inorganic particles (up to 750 $mg \cdot L^{-1}$) require zebra mussels to invest more energy in separating (edible) microalgae from (inedible) inorganic particles. As such, the high inorganic content of Markermeer aggregates (constituting > 70% of the total aggregate dry weight) could limit consumption of aggregates by higher trophic levels. In contrast, marine snow constituents have been found to be consumed by diverse micro- and mesoplankters, micronecton and juvenile fish (Bochdansky and Herndl 1992; Larson and Shanks 1996; Dilling et al. 1998; Kiørboe et al. 2003; Dilling and Brzezinski 2004). Part of these constituents would be too small for consumption in non-aggregated form (Lampitt et al. 1993; Wilson and Steinberg 2010). In this way, aggregates in stratified marine systems could constitute a shortcut to higher trophic levels, while transfer of aggregate constituents to higher trophic levels in shallow and turbid lake Markermeer appears to be limited.

Conclusion

In the present study, we identified microalgae in pelagic aggregates in the turbid, phosphate-limited water column of a large and shallow delta lake. The aggregates exhibited higher ALPase activity than surrounding water. Due to this activity, and considering the benthic origin of aggregate constituents, we suggest that these aggregates are "benthic hotspots in the pelagic zone" that fuel microalgal growth in turbid, phosphate depleted water. In striking contrast to lake and marine snow aggregates, no dissolved phosphorus could be detected in the aggregates. This underscores that aggregates in shallow and turbid waters differ from aggregates in deep and stratified waters. Therefore, and specifically in the light of the emergence of shallow turbid lakes in delta regions, impacts of aggregates on microalgal productivity and food web structure in shallow, phosphate-limited lakes warrant further in-depth study.

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Conflict of Interest

None declared.

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