Microphytobenthos and chironomid larvae attenuate nutrient recycling in shallow-water sediments

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Nutrient retention in estuarine sediments

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Summary

1. In shallow-water sediments, the combined action of microphytobenthos and bioturbating fauna may differentially affect benthic nutrient fluxes and exert a bottom-up control of pelagic primary production. In many cases, the effects of microphytobenthos and macrofauna on nutrient cycling were studied separately, ignoring potential synergistic effects.

2. We measured the combined effects of microphytobenthos and chironomid larvae on sediment-water fluxes of gas (O_2, TCO_2 and N_2) and nutrients (NH_4^+, NO_3^-, NO_2^-, PO_4^{3-} and SiO_2) in shallow-water sediments of a hypertrophic freshwater lagoon. Fluxes were measured in the light and in the dark in reconstructed sediments with low (L=600 ind m^{-2}), high (H=1800 ind m^{-2}) and no (C) addition of chironomid larvae, after 3 weeks preincubation under light/dark regime to allow for microalgal growth. Besides flux measurements, pore water nutrient (NH_4^+, PO_4^{3-} and SiO_2) and dissolved metal concentrations (Fe^{2+} and Mn^{2+}) were analyzed and diffusive fluxes were calculated.

3. Chironomid larvae increased sediment heterotrophy, by augmenting benthic O_2 demand and TCO_2 and N_2 dark production. However, on a daily basis, treatments C and L were net O_2 producing and N_2 sinks while treatment H was net O_2 consuming and N_2 producing. All treatments were net C sink regardless chironomid density.

4. Microphytobenthos always affected benthic nutrient exchange, as significantly higher uptake or lower efflux were measured in the light compared to dark incubations. Theoretical inorganic N, P and Si demand by benthic microalgae largely exceeded both dark effluxes of NH_4^+, PO_4^{3-} and SiO_2 and their net uptake in the light, suggesting the relevance of N-fixation, water column NO_3^-, and solid-phase associated P and Si as nutrient sources to benthic algae.

5. Chironomid larvae had a minor effect on inorganic N and P fluxes while they significantly stimulated inorganic Si regeneration. Their bioturbation activity, significantly altered pore water chemistry, with a major reduction of nutrients (highest for NH_4^+ and lowest for SiO_2) and metals concentration. Underlying mechanisms are combinations of burrow ventilation and bioirrigation with stimulation of element-specific processes as coupled nitrification-denitrification, co-precipitation, and inhibition of anaerobic paths such as Fe^{3+} or Mn^{4+} reduction or re-oxidation of their end-products.

6. The combined activity of benthic algae and chironomid larvae may significantly attenuate internal nutrient recycling in shallow eutrophic ecosystems, and contribute to the control of pelagic primary production.

Introduction

Shallow, bioturbated sediments are sites of intense biogeochemical processes, including carbon fixation, nutrient uptake and organic matter mineralization (MacIntyre *et al.*, 1996; Miller *et al.*, 1996). They are also sites of multiple interactions among trophic levels: macrofauna may keep elevated the growth potential of microphytobenthos through feeding, excretion and bioturbation activities. This results in a positive feedback between the biomass and growth of benthic algae and the biomass and growth of primary consumers (Herren *et al.*, 2017). Macrofauna may also stimulate the activity of bacteria as well as different chemical processes (Kristensen, 1984; Aller, 1994). Mutualistic interactions in benthic communities are poorly studied but they may produce important effects on nutrient dynamics (Herren *et al.*, 2017). Among these, the minimization of losses (i.e. large effluxes to the water column) or imports (i.e. N-fixation) are expected (Magri *et al.*, 2017). The transition between transparent and turbid status in shallow aquatic ecosystems and its reversal has been traditionally interpreted in terms of nutrient excess or deficiency and of pelagic or benthic grazing pressure (Scheffer *et al.*, 1993; Caraco *et al.*, 1997; Scheffer, 1998; Carpenter *et al.*, 2003). Turbid waters characterize systems with high nutrient levels and low or inefficient grazing pressure, sustaining high phytoplankton biomass (Malone, 1992; Scheffer, 1998). However, the benthic compartment may promote water clarity in the presence of large densities of filter-feeding macrofauna (Caraco *et al.*, 1997) or when sediments retain nutrients and minimize internal recycling (Sundbäck *et al.*, 2000; Hölker *et al.*, 2015). While the role of zooplankton grazing on algal abundance is well studied, the role of benthos on pelagic production in shallow ecosystems is poorly investigated (Officer *et al.*, 1982; Stadmark & Conley, 2011; Hölker *et al.*, 2015). Photosynthesis by benthic microalgae may represent an important fraction of total primary production in shallow water ecosystems (MacIntyre *et al.*, 1996; Underwood, 2008). Microphytobenthos may form thick mats on the sediment surface, where the availability of light and nutrients may result in high rates of primary production, biomass turnover and nutrient uptake, translocation and retention (Revsbech *et al.*, 1981; Underwood & Kromkamp, 1999). Active mats of benthic algae, deeply affecting surface sediment biogeochemistry, are described in lakes (Herren *et al.*, 2017), shallow bays (Bartoli *et al.*, 2003), coastal areas (Sundbäck *et al.*, 2006) and estuaries (Cabrita & Brotas, 2000). The benthic carbon flux driven by microphytobenthos may support the activities of bacteria, meiofauna and macrofauna (Miller *et al.*, 1996). In the light period, benthic algae may significantly enhance sediment oxygen penetration and assimilate dissolved nutrients from both the water column and the pore water (Revsbech *et al.*, 1981; Rysgaard *et al.*, 1995). A thicker oxic layer produces cascade effects on a number of processes in the proximity of the water-sediment interface, which is a critical zone due to its steep redox gradients and intense microbial

and geochemical activity (MacIntyre *et al.*, 1996; Sundbäck *et al.*, 2000; Risgaard-Petersen, 2003). Some of these effects have been analyzed in detail, for example the stimulation or competitive inhibition of nitrification and denitrification, which ultimately depend on the background nitrogen level and on the net autotrophy or heterotrophy of the benthic system (Risgaard-Petersen, 2003). Different works report how active mats of benthic algae act as a trap for nutrient regenerated from the sediment, and prevent their diffusion to the water column (Bartoli *et al.*, 2003; Sundbäck *et al.*, 2004). Net autotrophic sediments may exert a bottom-up control on pelagic primary production via nutrient uptake and retention and promote clear water status (Cerco & Seitzinger, 1997; Sundbäck *et al.*, 2000). Heterotrophic sediments may instead regenerate large nutrient amounts to the water column and stimulate or maintain elevated rates of pelagic primary production (Risgaard-Petersen, 2003; Bartoli *et al.*, 2009; Pinardi *et al.*, 2009).

Benthic fauna may exert a top-down control of pelagic primary production but it may simultaneously excrete large amounts of nutrients and increase the organic matter content of sediment via biodeposition, stimulating microbial activity and mineralization at the interface (Caraco *et al.*, 1997; Stief, 2013; Ruginis *et al.*, 2014; Benelli *et al.*, 2017). Bivalves may remove phytoplankton (Newell *et al*., 2007; Higgins & Vander Zanden, 2010) but they also may stimulate its growth in transparent water enriched with excreted nutrients, resulting in an unclear net effect on pelagic production (Stadmark & Conley, 2011). As filter-feeding burrowers, some chironomid larvae may, on the contrary, displace particles from the water column to their burrows and, simultaneously, favor N loss and P retention via burrows ventilation and bioirrigation (Svensson & Leonardson, 1996; Lewandowski & Hupfer, 2005; Shang *et al.*, 2013; Hölker *et al.*, 2015). High densities of filter-feeding sediment dwelling invertebrates may therefore exert a top-down and bottom-up control of phytoplankton growth and promote a clear-water status (Hölker *et al.*, 2015). Such control may be further increased by the mutualistic interactions between microphytobenthos and tube-dwelling invertebrates, resulting in even larger nutrient uptake and retention (Herren *et al.*, 2017). In addition, bioturbating fauna can affect the physical and chemical processes within sediments. Bioturbation includes particles reworking, ventilation of burrows and bioirrigation by animals living on or in the substratum (Kristensen *et al.*, 2012). Such kind of activity may alter sediment-water fluxes and change vertical and horizontal gradients of pore water chemical compounds (Shull *et al.*, 2009). Burrow ventilation, that is the pumping of oxygen (O_2), nitrate (NO_3^-) and particles-rich waters within the sediment for respiration and feeding purposes (Kristensen *et al.*, 2012; Hölker *et al.*, 2015), has many consequences on the biogeochemistry of sediments including the increases of the oxidized sediment volume (Kristensen, 1984). The structure and the metabolic activity of the microbial communities that are living along the burrow

walls could also change and biogeochemical microniches may form (Stief & de Beer, 2002; Bertics & Ziebis, 2009; Laverock *et al.*, 2010).

While the role of benthic algae and macrofauna on sediment fluxes was analyzed separately in multiple studies, little is known about their combined effects (Miller *et al.*, 1996; Tang & Kristensen, 2007; Lohrer *et al.*, 2010; Herren *et al.*, 2017). As both microphytobenthos and tube-dwelling organisms may exert a strong control of regenerated nutrients, their synergistic effect may be quantitatively important in shallow eutrophic ecosystems, limiting internal recycling.

In this study, we incubated sediments with and without chironomid larvae in the dark and in the light to evaluate the effects on benthic metabolism of macrofauna and microphytobenthos, when they are co-occurring. We performed this study using sediments collected from a shallow eutrophic lagoon, where chironomid larvae represent the dominant macrofauna (Zettler & Daunys, 2007). Chironomids improve P retention, stimulate denitrification and remove phytoplankton via filtration (Svensson, 1997; Lewandowski *et al.*, 2007; Hölker *et al.*, 2015). We hypothesized that the mutualism between chironomid larvae and microphytobenthos (*sensu* Herren *et al.*, 2017) may stimulate nutrient retention within sediments, minimize recycling to the pelagic environment and may favor water clarity.

Materials and methods

Sampling procedure and microcosms set up

In March 2016, chironomid larvae (*Chironomus plumosus*), sediment and water were collected from a muddy site (55°17.2388’ N, 21°01.2898’ E, depth 2.5 m) within a confined area in the Lithuanian part of the Curonian Lagoon. The sediment sampled for the experiment had a density of 1.28 g cm^{-3}, a porosity of 0.94 and a water content of 93.6%; the C/N molar ratio of the upper 0-10 cm horizon was 7.6. Muddy sediments from the sampling site host large numbers of chironomid larvae (Markiyanova, 2016*)*, with reported densities averaging 1700 ± 1300 ind m^{-2} (Zettler & Daunys, 2007). In the laboratory, nearly 15 L of the collected sediment were sieved (0.5 mm mesh) in order to remove large debris, chironomid larvae and other occasional macrofauna, and gently mixed to a slurry. Then, the sediment homogenate was transferred into 12 bottom capped Plexiglass liners (height=30 cm, inner diameter=8 cm), to reconstruct a 15 cm thick sediment column with an overlying 14 cm thick water column. Thereafter *in situ* water was gently added, after positioning on the sediment surface a floating polystyrene disc (height=1 cm, inner diameter=7.5 cm) to avoid sediment resuspension. At the end of this procedure, 12 identical reconstructed sediment cores were obtained. Three treatments, each with 4 replicates, were realized: control sediment without macrofauna (C), sediment with low (L) and sediment with high (H) density of chironomid larvae. Treatments with low and high densities were prepared by adding 3 and 9 larvae per core,

respectively, corresponding to 600 and 1800 ind m^{-2} and within *in situ* densities (Zilius *et al*., 2014; Markiyanova 2016). All added chironomids burrowed deep inside sediments within 15 min, as suggested by ramified light halos along the vertical sediment profile (Fig. 1). In each core, a magnetic bar was fixed 10 cm above the sediment interface to stir water avoiding sediment resuspension. Then, all the cores were submerged with the top open in a temperature-controlled (14 ± 0.2 °C) tank (100 L), containing aerated and well-stirred lagoon water. The tank was provided with a central magnet rotating at 40 rpm and driving all magnets inside the cores in order to ensure water exchange with the tank and to supply phytoplankton to chironomid larvae. The tank cover was provided with 6 halogen lamps (Osram Decostar, 35W), positioned above each core and producing an irradiance of 96 ± 12 µE $m^{-2}s^{-1}$ measured at the sediment-water interface with an underwater quantum sensor (LI-COR 192s) and over a 16 hours light and 8 hours dark period (Fig. 1). The cores were preincubated for a period of three weeks, in order to have a) stable vertical and horizontal chemical gradients after sediment sieving and homogenization and chironomid larvae addition, b) established bacterial communities and microphytobenthos along burrows and on the sediment surface, respectively. During the preincubation period all microcosms were regularly checked for the development of light brown halos along chironomid larvae burrows and of benthic algal mats on the sediment surface, which occurred within 10 days (Fig. 1). A methodological work reports that bacterial communities recover after the stress of sediment sieving within 25 days and that defaunation with this technique is less impacting than sediment freezing or anoxia induction (Stocum & Plante, 2006). Nearly 30 % of the water volume in the tank was renewed every 2 days in order to maintain *in situ* conditions in term of suspended matter, nutrient concentrations and chemical gradients across the sediment-water interface.

Benthic flux measurements

After the preincubation period, light and dark fluxes of dissolved gas and nutrients were measured via short-term batch incubations, as detailed in Ruginis *et al.* (2014). Incubations lasted 5 hours, in order to keep initial O_2 concentration (330 µM) within 20 % of the initial value. Lowest O_2 concentration at the end of the incubation was 258 µM, measured in a microcosm with high chironomid density. At the beginning and at the end of the incubation, dissolved O_2 concentration was measured with a microelectrode (Unisense A/S, DK) and an initial and a final water sample (100 ml) was collected with plastic syringes from each core from a one-way valve located in transparent, gas-tight top lids fixed in each liner at the beginning of the incubation. The collected volume was replaced with an equivalent amount of tank water entering the core from another valve. Each water sample underwent the same processing: an aliquot of 40 ml was transferred to 12 ml exetainers (Labco, UK) for total inorganic carbon (TCO_2), dissolved O_2 and N_2 analyses. The last

two were added with 100 µl of 7 M $ZnCl_2$ to stop microbial activity while TCO_2 was immediately titrated (see analytical methods below). An aliquot of 20 ml was filtered (GF/F glass-fiber filters) and transferred into 20 ml plastic vials in order to analyze dissolved inorganic N compounds: ammonium (NH_4^+), nitrite (NO_2^-) and nitrate (NO_3^-) and dissolved inorganic silica (SiO_2). DIN was calculated as the sum of ammonium, nitrite and nitrate (DIN = NH_4^++NO_2^-+NO_3^-). Another aliquot was filtered and transferred into 5 ml glass vials to measure soluble reactive phosphorus (PO_4^{3-}). Details on the analytical techniques are reported below. Dissolved gas and nutrient fluxes were calculated according to the equation below:

$$Flux_x = \frac{([x]_f - [x]_i) \times V}{A \times t}$$

where $[x]_f$ and $[x]_i$ are the concentrations (µM or mM) of the solute x at the end and at the start of the incubation, respectively, V (L) is the volume of the core water phase, A (m^2) is the area of the sediment and t (h) is the incubation time.

Daily fluxes (µmol $m^{-2}d^{-1}$ or mmol $m^{-2}d^{-1}$) were calculated according to the following equation:

$$Daily\ flux_x = (hourly\ dark\ flux \times h_D) + (hourly\ light\ flux \times h_L)$$

where h_D and h_L are the numbers of dark and light hours during incubation, respectively.

Net and gross O_2 fluxes were converted into theoretical net and gross nutrient uptake by benthic algae. Rates were multiplied by a photosynthetic quotient of 1.2 to convert O_2 production into C uptake (Sundbäck *et al.*, 2004). We used oxygen data instead of measured TCO_2 fluxes as microbial processes (i.e. nitrification) may results in overestimation of C-fixation rates by benthic algae. Calculated net and gross C-fixation were divided by the Redfield ratio 106:16:15:1 and converted into inorganic N, Si and P uptake (Redfield, 1958; Sigmon & Cahoon, 1997).

Pore water extraction and diffusive fluxes calculation

At the end of light/dark incubations, the cores (n=12) were sliced in 5 layers at 0–1, 1–2, 2–3, 3–5 and 5–10 cm intervals in order to analyze the vertical distribution of dissolved inorganic nutrients (NH_4^+, PO_4^{3-} and SiO_2) and metals (Fe^{2+} and Mn^{2+}). Briefly, the sediment was extruded and sliced in a N_2 filled glow bag. Pore water were obtained by gently squeezing with N_2 (1.5–3 bar) of discrete sediment slices through GF/F filters with a squeezer bench (KC-Denmark, DK). An aliquot for metal analysis was immediately transferred into 6 ml exetainers, containing 100 µl of concentrated ultrapure HCl. Another aliquot (5 ml) was frozen for later NH_4^+, PO_4^{3-} and SiO_2 analyses.

Diffusive fluxes of all solutes across sediment layers were calculated from nutrient profiles in the sediment by applying the Fick's First Law (Berner, 1980):

$$J = \frac{\delta C}{\delta z} \cdot Ds$$

where J is the diffusive flux of the solute (µmol $m^{-2}h^{-1}$), $\frac{\delta C}{\delta z}$ is the concentration gradient of the solute between adjacent sediment layers (µmol m^{-4}) and Ds is the diffusion coefficient of the solute in the sediment (m^2h^{-1}), which was calculated from the following equation (Lerman, 1979):

$$Ds = D_w^0 \cdot \theta^2$$

where D_w^0 is the diffusion coefficient (m^2h^{-1}) of the solute in the water (Wollast & Garrels, 1971; Li & Gregory, 1974) and θ^2 is the sediment tortuosity, calculated according to Boudreau (1997):

$$\theta^2 = 1 - 2ln\phi$$

where ϕ is the sediment porosity. Diffusion coefficients of nutrients and metals were corrected for *in situ* temperature (14 °C) according to the Stokes-Einstein relation (Li & Gregory, 1974). Excess sediment from all slices was pooled and homogenized and a subsample of 5 ml was dried at 60 °C for 48 h in order to measure porosity.

Analytical methods

Dissolved O_2 was measured by means of polarography with a microelectrode (90 % response time in <5 s, 50 µm tip; Unisense, Denmark). Dissolved N_2 was analyzed by membrane inlet mass spectrometer (MIMS, Bay instruments, USA). Dissolved N_2 concentrations were calculated from obtained N_2:Ar ratio and theoretical Ar concentration derived from Weiss (1970). TCO_2 was measured via 6 end points 0.1 N HCl microtitration (Anderson *et al*., 1986). Dissolved nutrients (NO_x^-=NO_3^-+NO_2^-, NO_2^-, PO_4^{3-} and SiO_2) from incubations and pore water samples were measured with a continuous flow analyzer ($Scan^{++}$, Skalar, sensitivity 0.3 µM) using standard colorimetric methods (Grasshoff *et al*., 1983). NO_3^- was calculated as the difference between NO_x^- and NO_2^-. NH_4^+ was analyzed spectrophotometrically using salicylate and hypochlorite with nitroprussiate as catalyst (Bower & Holm-Hansen, 1980).

Statistical analysis

Two-way analysis of variance (ANOVA) was used to test the effects of the factors illumination (dark/light measurements) and chironomid larvae density (including their interactive effects) on benthic fluxes of dissolved gas and nutrients. The same analysis was used to test the effects of chironomid larvae density and sediment depth, including their interactions, on pore water solute concentrations. Homogeneity of variance was checked using the Levene Median test and data were transformed if significant heteroscedasticity was found. Only ammonium fluxes were log-transformed. For significant factors, a pairwise multiple comparison of means was carried out with

the post-hoc Holm-Sidak test. Statistical significance was set at *P* level lower than 0.05. All the analyses were performed using Sigma Plot 11.0.

Results

Benthic metabolism

Both factors illumination and chironomid larvae density produced a significant effect on the fluxes of dissolved O_2, TCO_2 and N_2 (Fig. 2 and Table 1). In the light, net O_2 production and TCO_2 consumption along the three treatments C, L and H were measured, suggesting the development of an active algal mat and the occurrence of benthic photosynthesis. During the light incubation, sediments displayed negative N_2 fluxes (i.e. N-fixation > denitrification) that became less negative in the H treatment, with the highest density of chironomid larvae. In the dark, increasing density of chironomid larvae stimulated aerobic (O_2, from -1.03 ± 0.07 to -1.96 ± 0.15 mmol $m^{-2}h^{-1}$), total (TCO_2, from 0.86 ± 0.56 to 2.33 ± 0.19 mmol $m^{-2}h^{-1}$) and anaerobic (NO_3^- reduction to N_2, from 0.40 ± 0.02 to 1.01 ± 0.18 mmol $m^{-2}h^{-1}$) sediment respirations, which nearly doubled in H as compared to C (Fig. 2). The respiratory quotient of sediments (data not shown) was close to unity and not significantly different among treatments. On a daily basis, the C and L treatments were net O_2 producing (i.e. net autotrophic) while H was net O_2 consuming (i.e. net heterotrophic). Daily N_2 fluxes were negative in the net autotrophic conditions C and L and positive in the heterotrophic treatment H. Daily TCO_2 fluxes were negative in all treatments.

Benthic nutrient fluxes

All forms of inorganic N were mostly net consumed by the sediment, regardless of the density of chironomid larvae (Fig. 3 and Table 1). Fluxes tended to be more negative in the light than in the dark, but differences were significant only for NO_2^- (Table 1). Fluxes of NO_3^- were nearly two orders of magnitude higher than those of NH_4^+ and NO_2^-, reflecting different concentrations of the three ions in the water column (0.9, 0.8 and 109 µM for NH_4^+, NO_2^- and NO_3^-, respectively). On a daily basis, DIN fluxes were all negative and mostly driven by NO_3^-, with values averaging -22.3 ± 3.0 mmol $m^{-2}d^{-1}$ (Fig. 3).

Fluxes of PO_4^{3-} were low and within ±1 µmol $m^{-2}h^{-1}$ (Fig. 3). Differences between light and dark incubations were significant, with small regeneration in the dark and uptake in the light; differences among the three treatments C, L and H were not significant (Table 1). As for DIN, also dissolved inorganic P daily fluxes were all negative and not statistically different among treatments, with values averaging -10.6 ± 3.5 µmol $m^{-2}d^{-1}$.

Reactive silica was the only macronutrient with chironomid larvae-dependent fluxes (Fig. 3 and Table 1). Increasing density of chironomids significantly stimulated the regeneration of SiO_2 in both light and dark conditions. SiO_2 fluxes tended to be more negative or less positive in the light as compared to dark incubations, with differences close to significant level ($P = 0.078$). SiO_2 daily fluxes were negative only in the C treatment (-98 ± 19 µmol $m^{-2}d^{-1}$) while they were increasingly positive in L (190 ± 108 µmol $m^{-2}d^{-1}$) and in H (378 ± 104 µmol $m^{-2}d^{-1}$).

Net and gross theoretical inorganic N, P and Si uptake, calculated from rates of O_2 production, were similar to NO_3^- uptake or N-fixation rates but they were much higher than NH_4^+, NO_2^-, SiO_2 and PO_4^{3-} nutrient fluxes measured in the light and in the dark or calculated from pore water chemical gradients in the upper sediment layer (Table 2 and Figs. 3 and 4).

Pore water vertical profiles and diffusive fluxes

Pore water nutrient profiles and diffusive fluxes calculated across the interface and across adjacent sediment layers are plotted in Fig. 4. The two-way ANOVA suggests that differences among treatments C, L and H were highly significant (Table 3) but that they depended upon the sediment layer (treatment x depth significant interaction). Pooling depths, NH_4^+ concentrations were not statistically different in L and H treatments (Table 3) while they were higher in C (Holm-Sidak pairwise comparison, $P < 0.001$). Regarding PO_4^{3-} and SiO_2, all treatments were different with concentrations in C > L > H (Holm-Sidak pairwise comparison, $P < 0.001$). The comparison of layers suggested important nutrient-specific differences among treatments. In the control treatment, all the nutrients displayed increasing concentrations with depth, with values significantly higher from the top to the bottom layers. Values approached 206 ± 11, 17 ± 3 and 349 ± 32 µM at 7.5 cm depth for NH_4^+, PO_4^{3-} and SiO_2, respectively (Fig. 4). Such increase with depth was not occurring for the three nutrients in L and H treatments, where concentrations were not statistically different along the vertical profile (NH_4^+, PO_4^{3-}) or displayed an increase only in the upper layers (SiO_2)(Fig. 4).

Calculated diffusive fluxes across the sediment-water interface in C treatment were 8 ± 3, 0.4 ± 0.1 and 31 ± 3 µmol NH_4^+, PO_4^{3-} and SiO_2 $m^{-2}h^{-1}$, respectively, while they were much lower in L and H treatments (Fig. 4). In C, diffusive fluxes of the three nutrients across adjacent sediment layers peaked at 2-2.5 cm depth while they were strongly reduced, in particular for NH_4^+ and PO_4^{3-}, in L and H treatments.

Vertical pore water profiles of Fe^{2+} and Mn^{2+} in the three treatments are shown in Fig. 5. There were significant differences among treatments along sediment horizons (two-way ANOVA, treatment x depth interaction, Table 3). Pooling all depths, the three treatments resulted statistically different with concentrations in C > L > H (Holm-Sidak pairwise comparison, $P < 0.005$). The

highest diffusive fluxes of Fe^{2+} and Mn^{2+} in control sediments were calculated at 2.5 and 2 cm depth, respectively (Fig. 5). In H treatment, there was virtually no accumulation of Fe^{2+} in pore water and diffusive fluxes were set to zero while a peak accumulation of Mn^{2+} was measured at 2.5 cm depth, coinciding with the layer of maximum diffusion upwards.

Discussion

Macrofauna and microphytobenthos activities promote benthic N and P retention and loss

Results from this work suggest how the combined activity of benthic microalgae and chironomid larvae contributes not only to the net retention of inorganic N and P within the sediment, but also to their net uptake from the water column. Inorganic Si was net retained and assimilated by the benthic system only in control sediments without macrofauna, presumably due to uptake by a mat of benthic diatoms (Sigmon & Cahoon, 1997), while a chironomid larvae density-dependent net regeneration was found. Macrofauna, including chironomid larvae, may excrete large amounts of inorganic N, P and Si or favor their mineralization, which may fuel large sediment efflux (Gallepp, 1979; Devine & Vanni, 2002). However, in the analyzed shallow sediments, processes retaining nutrients exceeded rates of N and P mineralization and excretion. These results are different from what reported on the net effect of macrofauna, in particular for benthic N cycling, where recycling generally exceeds denitrification (Stief, 2013). They therefore suggest that changes induced by bioturbation on benthic biogeochemistry are species, element- and likely sediment-specific (Michaud *et al.*, 2006).

Our results may be important for shallow eutrophic aquatic environments, where the combined activity of benthic algae and chironomid larvae may significantly attenuate the internal recycling, which is the flux of nutrients that sustain pelagic primary production. The dependency of phytoplankton on sediment regeneration may be quantitatively relevant in dry periods, when external loads decrease and the removal of nutrient at the sediment-water interface may promote water clarity. Such bottom-up control adds to the demonstrated active filtration of phytoplankton that chironomid larvae perform via burrow ventilation, by pumping large water volumes within sediments (Hölker *et al.*, 2015).

Results of this study show also that pore water concentrations of inorganic N and P, and to a minor extent Si, decrease in bioturbated as compared to non-bioturbated sediments, likely due to coupled nitrification-denitrification (N) and co-precipitation (P). The latter is supported by significantly different accumulation of Fe^{2+} and Mn^{2+} in pore water of both low and high chironomid larvae density treatments, due to macrofauna-mediated sediment oxidation (Lewandowski *et al.*, 2007). Results from flux measurements exclude a chironomid larvae-dependent efflux of NH_4^+ and PO_4^{3-} resulting from bioirrigation, that was evident only for SiO_2 efflux, significantly stimulated by

chironomid larvae. Our results align with those of Lewandowski *et al.* (2007), suggesting that the burrowing, ventilation and bioirrigation activities of chironomids change the chemistry of pore water, setting to zero the vertical gradients and the regeneration of N and P to the water phase. The present study confirms also the importance of dark and light measurements when studying bioturbation in shallow sediments, as daily budgets allow to produce a more realistic picture of benthic functioning, which integrates the dark, heterotrophic period with the light, autotrophic one. We acknowledge that results from our study were obtained from manipulated and reconstructed sediments, but sediment sieving was demonstrated to produce a minor impact on bacterial communities and their recovery as compared to other defaunation techniques as the induction of anoxia and freezing (Stocum & Plante, 2006). Furthermore, we preincubated our microcosms for 3 weeks before starting the measurements, which is close to the period of recovery of the bacterial community (25 days) reported in the Stocum & Plante (2006) experiment. Incubations performed shortly after the addition of burrowing macrofauna to reconstructed sediment may result in large efflux of pore water solutes, due to active burrowing, with overestimation of the role of macrofauna in natural sediments (Bartoli *et al.*, 2000).

The light period and the role of benthic algae

Fluxes of dissolved oxygen and nutrients (Si in particular) measured in the light strongly support the development of benthic algae, including diatoms, on the sediment surface during the preincubation period. The occurrence of an homogeneous and a patchy light brown mat on control and bioturbated cores sediment surface, respectively, was also noticed by visual inspection. Benthic primary production measured in C, L and H treatments (500-3000 µmol C $m^{-2}h^{-1}$) falls within the range reported for shallow sediments (Nedwell & Raffaelli, 1999). Such photosynthetic activity had a clear effect on nutrient dynamics, resulting in net daily uptake of inorganic N and P in all treatments, largely exceeding rates of mineralization and excretion. The presence of macrofauna reversed the sink role of sediments only for inorganic Si, which was net regenerated in L and H treatments. Our results align with previous investigations on the role of benthic algae as nutrient traps (Bartoli *et al.*, 2003; Sundbäck *et al.*, 2004 and 2006).
Estimated N requirements by benthic algae (Table 2) were much higher than the measured fluxes of NH_4^+ and NO_2^- in both light and dark incubations, while they were comparable with N_2 and NO_3^- fluxes measured in the light. This suggests that neither dark regeneration nor light uptake of NH_4^+ or NO_2^- satisfied microphytobenthos N requirements. Negative N_2 fluxes were always measured in the light in the three treatments; however, rates decreased from the most autotrophic condition C to the most heterotrophic condition H, in agreement with decreasing net O_2 production and calculated N demand. Such results conform to those reported by Sundbäck *et al.* (2006), demonstrating that in

autotrophic sediments assimilation prevails over denitrification. Comparable rates of N-fixation are reported for estuarine sediments by Fulweiler *et al.* (2007), but in heterotrophic sediments incubated in the dark, and in oligotrophic lagoons in the light (Charpy *et al.*, 2007). Also Scott *et al.* (2008), investigating microbial N–cycling along a gradient of NO_3^- availability, measured negative N_2 fluxes and N-fixation rates comparable to those reported in the present study. They concluded that cyanobacteria may be responsible for such rates.

Nitrate fluxes were always negative, with higher uptake during light as compared to dark incubations, suggesting NO_3^- as possible N source to microphytobenthos. In general, pelagic and benthic algae display a preference for NH_4^+ assimilation (Boyer *et al.*, 1994; Nedwell & Raffaelli, 1999). However, algae may switch to NO_3^- uptake when NH_4^+ is limiting or when the NO_3^- to NH_4^+ ratio is elevated, as in our experiment (50:1; McCarthy *et al.*, 1977). Unfortunately, with our data we cannot quantify the fraction of NO_3^- consumption that goes to microphytobenthos, to denitrifiers or that may sustain bacterial growth. We also do not know the rates of N-fixation and denitrification in the light, as our method measures the net N_2 flux, which is the sum of the two opposite processes (Kana *et al.*, 1994). It remains therefore unknown the fraction of NO_3^- uptake and N-fixation that is shared by bacteria and benthic algae. Such information would clarify why the sum of NO_3^- and N_2 fluxes in the light always exceeds calculated gross N uptake by microphytobenthos. In the Neuse River estuary, similar results were explained in terms of heterotrophic bacterial DIN uptake (Boyer *et al.*, 1994).

Net and gross calculated Si uptake rates were much higher than fluxes measured at the sediment-water interface, suggesting that pore water represented the major source of SiO_2 to benthic algae. This speculation is based on the assumption that the mat of algae developed on the sediment surface was mainly composed of diatoms, requiring large amounts of inorganic Si in relation to other nutrients to synthesize their frustules (Sigmon & Cahoon, 1997). This is plausible, as Si concentrations in pore water were comparatively higher than those of N and P. Alternatively¸ the comparison between theoretical and measured fluxes may suggest that benthic algae were not siliceous. However, there are differences between measured light and dark Si fluxes, supporting diatoms uptake at sediment-water interface. Similar arguments are true for inorganic P: calculated net and gross uptake largely exceeded fluxes measured in the light, suggesting that benthic algae extracted inorganic P from the pore water or may cope with limited P availability. Overall, N and P requirements by microphytobenthos resulted in fluxes to the sediments of both nutrients regardless of the presence of chironomid larvae. Concerning Si, only control sediments without chironomids were net Si sinks while benthic uptake only smoothed Si regeneration in L and H treatments.

Net autotrophic sediments were demonstrated to inhibit the activity of nitrifiers and denitrifiers, likely due to competition for N, alteration of O_2 and pH values in the upper sediment layer or

production of specific inhibitory substances (Risgaard-Petersen, 2003). In the surface sediments of the Curonian Lagoon photosynthesis at the interface resulted in negative N_2 fluxes, which were reversed in the dark, when denitrification prevailed. This is likely due to large availability of NO_3^- and active denitrification when benthic algae are less active, even if uptake by benthic algae was demonstrated also in the dark (Rysgaard *et al.*, 1995).

The activity of benthic algae is limited by light penetration to the upper sediment microlayer and we may speculate that pore water solutes, diffusing from the sediment to the water column, are assimilated and retained in the algal biomass. This is always true in control sediments, where gradients from the sediment to the water are elevated for all the considered nutrients (Bartoli *et al.*, 2003; Sundbäck *et al.*, 2004). In the bioturbated sediments, on the contrary, chemical gradients are modified by the activity of larvae, with element-specific mechanisms. If microphytobenthos operates at the interface, the activity of chironomid larvae operates deeper into the sediments, within the upper 10-15 cm.

Benthic metabolism and pore water features in sediments bioturbated by chironomid larvae

A large number of studies analyzed the effect of chironomids on sediment biogeochemistry over a wide range of water temperatures (10-20 °C) and larvae densities (up to 12,000 ind m^{-2}); chironomids were generally demonstrated to stimulate aerobic and anaerobic benthic respiration, with a few exceptions (Stief & Hölker, 2006). Our results align with previously published rates of oxygen uptake in chironomid larvae bioturbated ($-0.53<x<-2.5$ mmol $m^{-2}h^{-1}$) versus not bioturbated ($-0.40<x<-1.0$ mmol $m^{-2}h^{-1}$) sediments (Pelegrí & Blackburn, 1996; Svensson, 1997; Hansen *et al.*, 1998; Shang *et al.*, 2013; Soster *et al.*, 2014). In the present work chironomids stimulated benthic oxygen consumption by a factor of 2, that falls within the range reported in the literature, between 1.2 and 3.6. This means that chironomid larvae have a deep impact on surface sediment metabolism. Such impact includes the larvae respiratory needs (6-8 µg O_2 $mg^{-1}_{AFDW}h^{-1}$ at 20 °C, reported in Baranov *et al.* (2016)), together with the stimulation of microbial or chemical processes by larvae bioturbation.

Comparatively, the effects of chironomids bioturbation on benthic TCO_2 fluxes were less studied. Stief and Hölker (2006) demonstrated that predatory fish may induce changes in the behavior of chironomid larvae, that burrow deeper and reduce surface foraging, resulting in significantly lower rates of carbon mineralization and CO_2 fluxes. Hansen *et al.* (1998) determined a stimulation of TCO_2 production by a factor of ~2 in the presence of chironomids, comparable to that measured for O_2 and similar to the results of our study. Our TCO_2 and O_2 data suggest that chironomid larvae did not alter the respiratory quotient, meaning that they produced a similar stimulation of aerobic and anaerobic metabolism.

In our experiment, we did not measure appreciable CH_4 fluxes in any of the experimental conditions; in the dark rates varied between 0.7 ± 0.6 (C) and 1.9 ± 1.2 (H) µmol $m^{-2}h^{-1}$, while in the light rates varied between 0.4 ± 0.7 (C) and 1.1 ± 0.8 (H) µmol $m^{-2}h^{-1}$ (data not shown), without significant differences among light and dark rates and among treatments. On the contrary, high rates of N_2 production were measured in the dark. Denitrification, in excess of N-fixation, varied from 395 ± 17 (C) to 1,012 ± 182 (H) µmol N $m^{-2}h^{-1}$, suggesting a stimulation of this process in the presence of chironomid larvae by a factor of 2.6. Shang *et al.* (2013) measured with the IPT much smaller rates and a stimulation of D_w (denitrification of water column nitrate) by a factor of ~12 and of D_n (coupled nitrification-denitrification) by a factor of ~3 with 2,264 ind m^{-2}. Svensson (1997) measured denitrification with the IPT under increasing NO_3^- concentrations in bioturbated vs not bioturbated sediments (2,000 ind m^{-2}) and found a large stimulation of D_w (by a factor of 3), with rates from ~120 to ~380 µmol $m^{-2}h^{-1}$ at NO_3^- concentration of 300 µM. Also Pelegrí & Blackburn (1996) demonstrated a slight increase of coupled nitrification-denitrification as compared to denitrification of water column nitrate, due to burrow ventilation, injection of nitrate-rich water into the sediments and to low-density of nitrifiers in the organic sediments inhabited by chironomids. Their reported rates (20-60 µmol N $m^{-2}h^{-1}$) are much lower than those reported in this study.
We can say that denitrification rates in the Curonian Lagoon sediments with chironomid larvae are among the highest reported in the literature and we can speculate that they are mainly sustained by high NO_3^- concentrations in the water column (100 µM in the study period). The nitrate consumption we have measured in fact has similar rates as those of N_2 efflux.
In eutrophic shallow aquatic systems, chironomid larvae are abundant and their sediment reworking, burrow ventilation and bioirrigation activities may alter the biogeochemistry of the whole benthic system (Lewandowski *et al.*, 2007; Morad *et al.*, 2010). Bioturbation may influence aerobic and anaerobic processes and the abundance of some microbial communities (Nogaro *et al.*, 2008; Bertics & Ziebis, 2009). Ventilation and bioirrigation activities introduce O_2 and NO_3^--rich water inside the sediments, and these solutes diffuse through the burrow walls augmenting the oxidized sediment volume (Lewandowski *et al.*, 2007; Hölker *et al.*, 2015). Pore water NH_4^+ decreased in presence of chironomids, likely due to its oxidation to NO_2^- and to NO_3^- by nitrifying bacteria, as hypothesized also by Lewandowski *et al.* (2007). As a result, in chironomid larvae bioturbated sediments the calculated NH_4^+ diffusive fluxes across the sediment-water interface were almost zero (Svensson, 1997; Stief & De Beer, 2002). Pore water concentration of PO_4^{3-} was also significantly reduced in sediments with chironomids, likely due to co-precipitation with ferric iron and retention within sediments as insoluble Fe-hydroxides (Gunnars *et al.*, 2002; Lewandowski *et al.*, 2007). Contrarily to N, P, Fe and Mn, the concentrations of Si in pore water were affected by a much lower degree by chironomids, as this nutrient dynamics are much less redox-dependent, and

Si mobility is more dependent upon physical and chemical processes (Schelske *et al.*, 1986). This result is in agreement with the net fluxes we measured during incubations, where Si was the only nutrient regenerated to the water column in presence of chironomids, probably due to the flushing of Si-rich pore water.

Benthic processes in Curonian Lagoon shallow and bioturbated sediments: do they matter?

Results from the present study confirm the potential role of shallow and bioturbated sediments as bottom-up controllers of pelagic primary production, as evidenced by Hölker *et al.* (2015). Chironomid larvae are demonstrated to actively pump water within sediments and have the potential to filter the whole water column in shallow aquatic ecosystems (Morad *et al.*, 2010). They may perform active control of phytoplankton, with a clearance rate comparable to that of filter-feeding pelagic zooplankton (Roskosch *et al.*, 2010; Hölker *et al.*, 2015). This means that in hypertrophic shallow system as the Curonian Lagoon they may contribute to control pelagic primary production. In a recent paper, Lesutiene *et al.* (2014) demonstrated that chironomid flesh displays a drop in ^{15}N signature after a cyanobacterial bloom, due to active incorporation of algal biomass with low ^{15}N and rapid turnover. In the same work, this effect was not evident in zebra mussel flesh, even of small individuals, suggesting much slower biomass turnover of these bivalves (Lesutiene *et al.*, 2014). Moreover, our study suggested that the combined action of chironomids and algae may retain regenerated nutrients within the sediments, with Si as only exception. This is also an interesting output for a hypertrophic freshwater lagoon that in summer is cyanobacteria-dominated, due to a number of different reasons. First of all, internal nutrient recycling may support blooms in the summer, when external loads from the basin are at their minimum level, due to sudden drop of discharge and nutrient concentration in the spring-summer transition (Lubiene *et al.*, 2017). If sediments retain nutrients, and P in particular, while Si is regenerated, cyanobacteria lose the competitive advantage that they have in comparison with other, not harmful algal groups as diatoms (Pilkaityte & Razinkovas, 2007). Other aspects acting in opposite direction should be taken into account. First, chironomid larvae density peaks in spring, when the experiment was performed, and when riverine nutrient concentrations and loads are still very high. This may result in large stimulation of water column denitrification, as shown in this study, but may not affect significantly the elevated N background. In the summer, chironomid larvae become flying insects that leave the sediments resulting in decreasing densities and associated ecosystem services. We may speculate that burrows turn anoxic in a short while after flying out of insects and that trapped P may be suddenly regenerated to the water column, proportionally more than N, with a positive feedback for cyanobacteria (Zilius *et al.*, 2015; Petkuviene *et al.*, 2016). These aspects were hypothesized and discussed by Hölker *et al*. (2015) and require some laboratory experiments under controlled

conditions to verify whether such redox-dependent P efflux occurs or not. Ultimately, such efflux and its intensity depend upon the reactivity of the sedimentary P pools associated to chironomid larvae burrows. In the Curonian Lagoon sediments, we measured a significant decrease of pore water Fe and Mn in the presence of chironomids, suggesting that the establishment of anoxia may reverse iron and manganese oxidation and favor P efflux. Another important aspect is that the Curonian Lagoon, despite shallow, is a turbid system with limited light penetration, and shallow sediments represent a minor fraction of the total surface. The Nemunas River, together with nutrients, transports to the Curonian Lagoon large amounts of phytoplankton and it is unlikely that pelagic or benthic filter feeders are able to control such load (Lubiene *et al.*, 2017). Transparent water periods occur in the Lagoon, but they are short and confined into specific areas where for example densities of bivalves (or chironomids) are locally abundant. Excess nutrient delivery from the Nemunas watershed and unbalanced stoichiometry have resulted into extremely high pelagic primary production and cyanobacterial blooms, ultimately affecting the functioning of the benthic system through anoxia, organic enrichment and loss of macrofauna. If this trend will be reversed, recolonization of macrofauna and increased light penetration may favor the growth of microphytobenthos and the benthic system may contribute to maintain water clarity (Herren *et al.*, 2017).

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Tables

Table 1 Results of two-way ANOVA testing the effects of the factors incubation condition (illumination) and treatment (control, low and high chironomid density) on gas (O_2, TCO_2 and N_2) and nutrient (NH_4^+, NO_3^-, NO_2^-, DIN, PO_4^{3-} and SiO_2) fluxes.

	Source of variation	Df	SS	MS	F	P
O_2 flux	Illumination	1	53.9	53.9	373.1	**<0.001**
	Treatment	2	8.0	4.0	27.7	**<0.001**
	Interaction	2	1.0	0.5	3.3	0.059
	Error	18	2.6	0.1		
	Total	23	65.5	2.8		
TCO_2 flux	Illumination	1	107.8	107.8	87.7	**<0.001**
	Treatment	2	11.5	5.8	4.7	**0.023**
	Interaction	2	4.1	2.1	1.7	0.215
	Error	18	22.1	1.2		
	Total	23	145.6	6.3		
N_2 flux	Illumination	1	6.7	6.7	234.3	**<0.001**
	Treatment	2	1.2	0.6	21.7	**<0.001**
	Interaction	2	0.1	0.0	1.0	0.378
	Error	18	0.5	0.0		
	Total	23	8.5	0.4		
NH_4^+ flux	Illumination	1	0.3	0.3	0.7	0.422
	Treatment	2	2.1	1.1	2.4	0.118
	Interaction	2	1.9	0.9	2.1	0.148
	Error	18	8.0	0.4		
	Total	23	12.3	0.5		
NO_3^- flux	Illumination	1	1.3	1.3	2.3	0.143
	Treatment	2	0.0	0.0	0.0	0.965
	Interaction	2	0.3	0.2	0.3	0.745
	Error	18	10.0	0.6		
	Total	23	11.6	0.5		
NO_2^- flux	Illumination	1	83.1	83.1	4.5	**0.049**
	Treatment	2	5.0	2.5	0.1	0.875
	Interaction	2	24.4	12.2	0.7	0.531
	Error	18	334.5	18.6		
	Total	23	447.0	19.4		
DIN flux	Illumination	1	1.3	1.3	2.4	0.139
	Treatment	2	0.0	0.0	0.0	0.969
	Interaction	2	0.3	0.2	0.3	0.742
	Error	18	10.1	0.6		

	Total	23	11.8	0.5		
PO_4^{3-} flux	Illumination	1	7.4	7.4	22.3	**<0.001**
	Treatment	2	0.2	0.1	0.3	0.747
	Interaction	2	0.1	0.0	0.1	0.910
	Error	18	5.9	0.3		
	Total	23	13.6	0.6		
SiO_2 flux	Illumination	1	340.2	340.2	3.5	0.078
	Treatment	2	2052.3	1026.1	10.5	**<0.001**
	Interaction	2	385.4	192.7	2.0	0.167
	Error	18	1751.6	97.3		
	Total	23	4529.4	196.9		

Df, degree of freedom; SS, sum of squares; MS, mean of squares; F, F-statistics; P, P-value

Table 2 Theoretical net and gross nutrient uptake by benthic microalgae calculated from O_2 fluxes in light incubations of reconstructed sediment cores with no (C), low (L) and high (H) density of chironomid larvae.

	N-uptake		P-uptake		Si-uptake	
Treatment	**Net**	**Gross**	**Net**	**Gross**	**Net**	**Gross**
	(µmol N $m^{-2}h^{-1}$)		(µmol P $m^{-2}h^{-1}$)		(µmol Si $m^{-2}h^{-1}$)	
C	492 ± 45	695 ± 47	31 ± 4	43 ± 3	522 ± 48	738 ± 50
L	325 ± 53	561 ± 55	20 ± 3	35 ± 4	345 ± 57	596 ± 59
H	124 ± 48	508 ± 56	8 ± 2	32 ± 3	132 ± 51	540 ± 59

Table 3 Results of two-way ANOVA testing the effects of the two factors treatment (control, low and high chironomid density) and depth on dissolved nutrient (NH_4^+, PO_4^{3-} and SiO_2) and metal (Fe^{2+} and Mn^{2+}) pore water concentrations.

	Source of variation	Df	SS	MS	F	P
NH_4^+	Treatment	2	104737.1	52368.5	451.2	**<0.001**
	Depth	4	41635.9	10409.0	89.7	**<0.001**
	Interaction	8	63352.4	7919.0	68.2	**<0.001**
	Residual	45	5222.9	116.1		
	Total	59	214948.3	3643.2		
PO_4^{3-}	Treatment	2	566.4	283.2	95.0	**<0.001**
	Depth	4	340.1	85.0	28.5	**<0.001**
	Interaction	8	331.6	41.4	13.9	**<0.001**
	Residual	45	134.1	3.0		
	Total	59	1372.2	23.3		
SiO_2	Treatment	2	139176.7	69588.4	100.2	**<0.001**
	Depth	4	358587.9	89647.0	129.0	**<0.001**
	Interaction	8	27659.4	3457.4	5.0	**<0.001**
	Residual	45	31264.7	694.8		
	Total	59	556688.8	9435.4		
Fe^{2+}	Treatment	2	3607.6	1803.8	68.5	**<0.001**
	Depth	4	3836.5	959.1	36.4	**<0.001**
	Interaction	8	3294.6	411.8	15.6	**<0.001**
	Residual	45	1185.2	26.3		
	Total	59	11923.9	202.1		
Mn^{2+}	Treatment	2	1648.0	824.0	170.7	**<0.001**
	Depth	4	3158.2	789.6	163.5	**<0.001**
	Interaction	8	452.8	56.6	11.7	**<0.001**
	Residual	45	217.3	4.8		
	Total	59	5476.3	92.8		

Df, degree of freedom; SS, sum of squares; MS, mean of squares; F, F-statistics; P, P-value

Figure legends

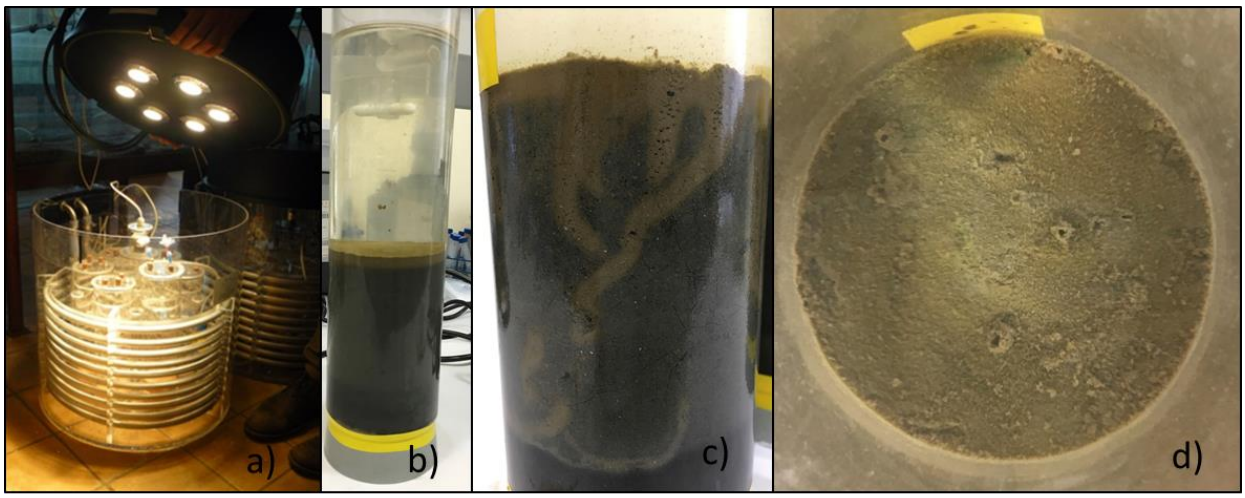
Fig. 1 The images display the incubation tanks we used and in particular the water temperature control and the core illumination systems (a), a control core (b), a core with chironomid larvae (c) and a patchy mat of benthic algae developed at the sediment-water interface during the preincubation period (d).

Fig. 2 Benthic fluxes of dissolved oxygen (O_2), total inorganic carbon (TCO_2) and dissolved molecular nitrogen (N_2) measured in light (white bars) and dark (black bars) incubations of C, L and H treatments (n=4). Grey bars in O_2 and TCO_2 represent gross primary production. Averages ± standard errors are reported. All fluxes are expressed in mmol $m^{-2}h^{-1}$. Dots represent daily averages ± standard errors expressed in mmol $m^{-2}d^{-1}$.

Fig. 3 Light and dark benthic fluxes of ammonium (NH_4^+), nitrite (NO_2^-), nitrate (NO_3^-), dissolved inorganic nitrogen (DIN), soluble reactive phosphorus (PO_4^{3-}) and dissolved reactive silica (SiO_2) measured in C, L and H treatments (n=4). Averages ± standard errors are reported. All fluxes are expressed in µmol $m^{-2}h^{-1}$. Dots represent daily averages ± standard errors of nutrient fluxes expressed in µmol or mmol $m^{-2}d^{-1}$.

Fig. 4 Vertical profiles of pore water and diffusive fluxes of dissolved inorganic nutrients (NH_4^+, PO_4^{3-} and SiO_2) in C, L and H treatments. Dots represent nutrient concentrations (µM) in the different layers (averages ± standard errors) and the horizontal bars represent diffusive fluxes (µmol $m^{-2}h^{-1}$) across sediment layers (averages ± standard errors).

Fig. 5 Vertical profiles of pore water dissolved reduced metals (Fe^{2+} and Mn^{2+}) and their diffusive fluxes in C, L and H treatments. Dots represent metal concentrations (µM) in the different layers (averages ± standard errors) and the horizontal bars represent the diffusive fluxes (µmol $m^{-2}h^{-1}$) across sediment layers (averages ± standard errors).



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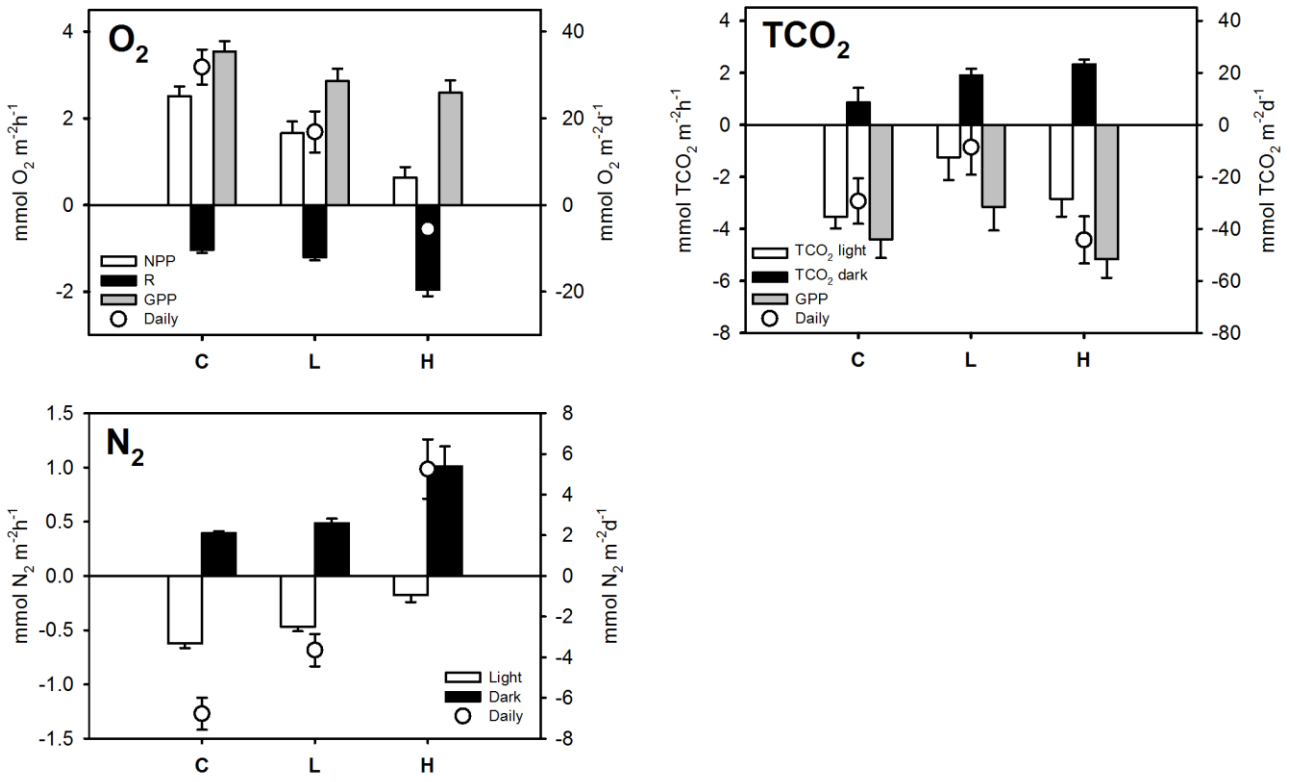

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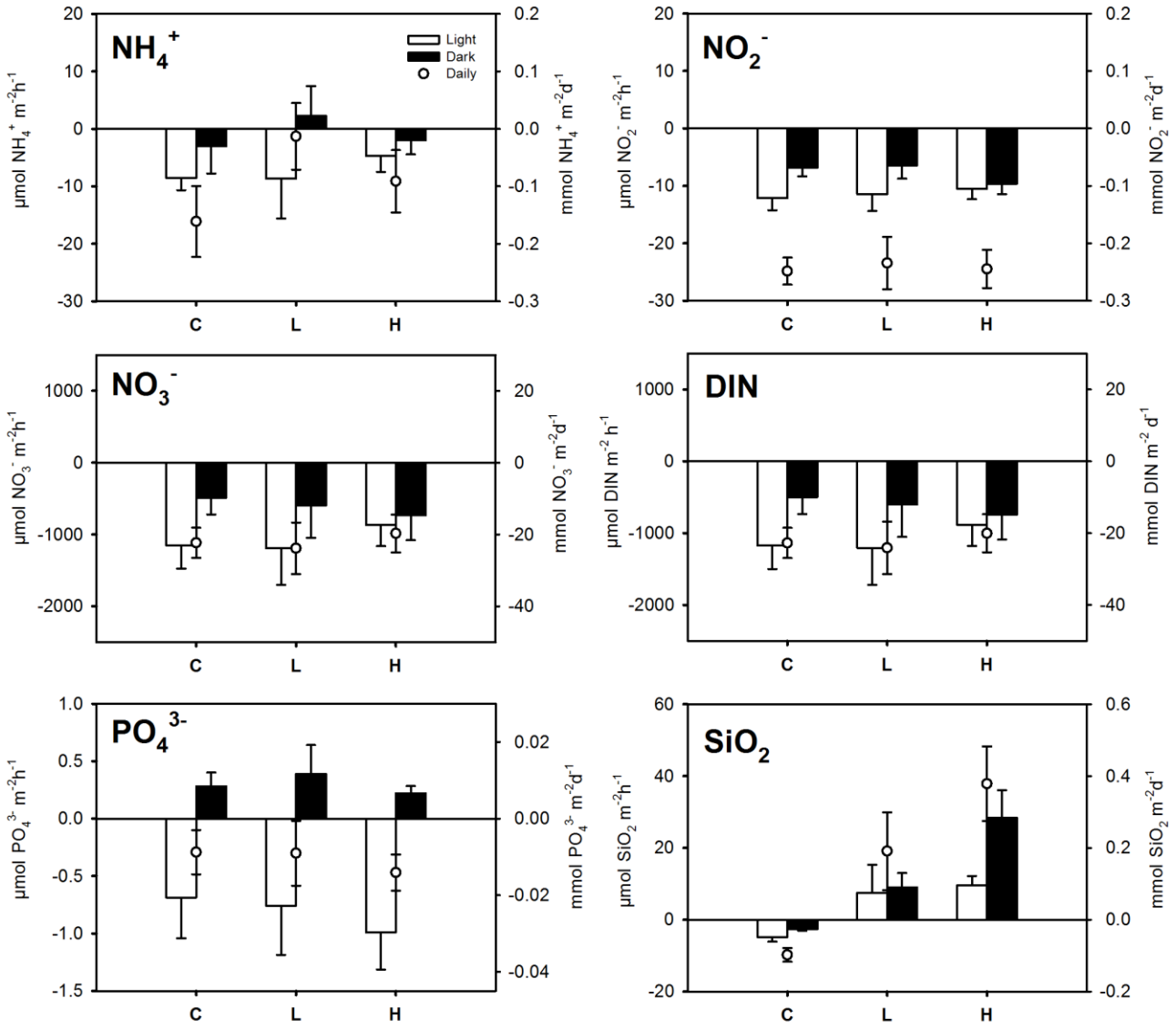

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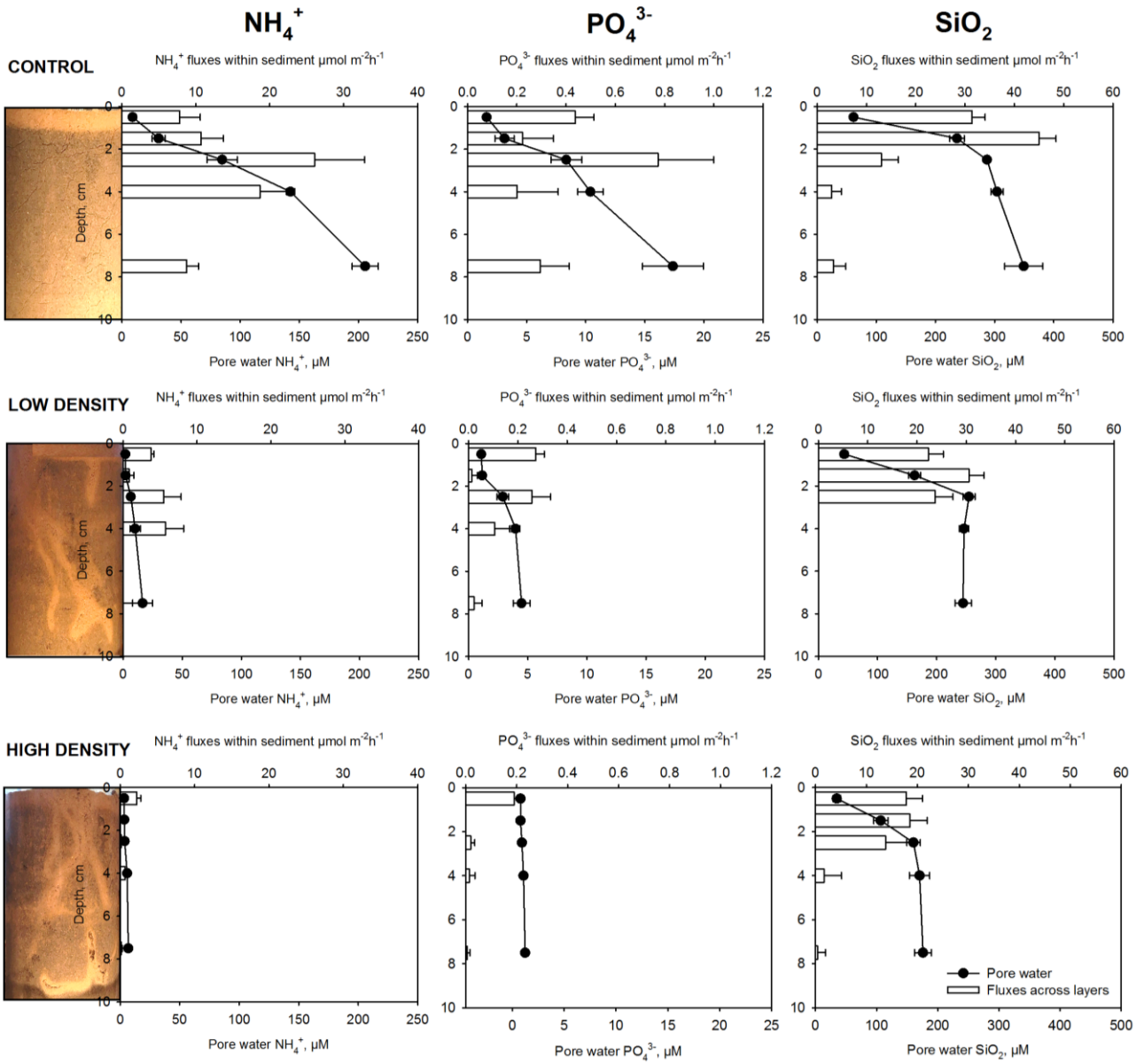

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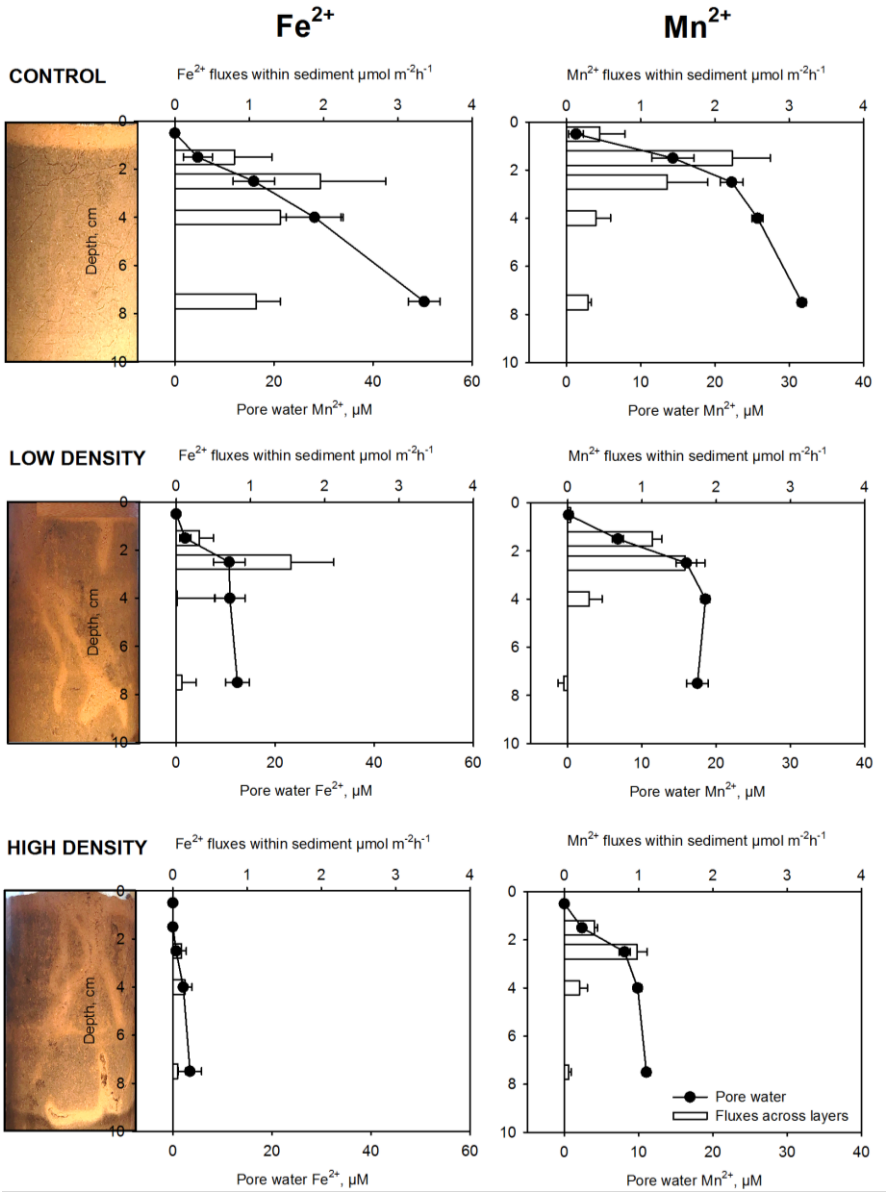

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