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ORIGINAL ARTICLE

Response of sedimentary processes to cyanobacteria loading

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ABSTRACT

Sedimentation of pelagic cyanobacteria in dystrophic freshwater and oligohaline lagoons results in large inputs of labile organic matter (OM) to the benthos. We used an experimental approach to study the short-term impact of such phenomena on the benthic microbial community metabolism and on the nitrogen (N) fluxes across the sediment-water interface. We hypothesized an increase of respiratory activity, including N loss via denitrification and its recycling to the water column. Our results show that the incorporation within sediments of the settled bloom increases benthic bacterial activities. This is coupled to large DON and NH_4^+ effluxes, and to a comparatively smaller increase of N_2 production, while no significant effects were detected for the benthic fluxes of NO_x^-. We constructed flow schemes for N compounds, which show that while denitrification was significantly stimulated by amending cyanobacterial biomass to the sediments, it represented less than 1% of total OM mineralization. Interestingly, we observed that total released nitrogen (DIN+DON+N_2 efflux) was dominated by DON, which contributed 75-80% of the net N efflux, suggesting incomplete mineralization of OM. With the measured total N mobilization rate of about 15 mmol N m^{-2} d^{-1} it would take more than 4 months to regenerate the total organic N input to sediments (2031 mmol N m^{-2}), which represents the post-bloom deposited particulate organic N. These results suggest limited losses to the atmosphere and slow diffusive recycling of N buried into sediments, mostly as DON. Such regenerated N may eventually be flushed to the open sea or sustain pelagic blooms within the estuarine environment, including cyanobacteria, with a negative feedback for further import of atmospheric nitrogen via N-fixation.

Key words: Cyanobacteria; organic matter; sediments; benthic metabolism; DON; denitrification.

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INTRODUCTION

Planktonic cyanobacterial blooms are spreading worldwide (Conley *et al*., 2009; Qin *et al.,* 2010) and are particularly common in the hypertrophic and shallow freshwater lagoons along the coast of the Baltic Sea (Wasmund, 2002; Nawrocka and Kobos, 2011; Bresciani *et al.,* 2012). For example, the phytoplankton in the Curonian lagoon (SE Baltic Sea) is dominated by cyanobacteria, representing up to 90% of total chlorophyll *a* during the summer months, with a high proportion of the nitrogen (N) fixer *Aphanizomenon flos-aquae* (Pilkaityte and Razinkovas, 2007). Dramatic concentrations, exceeding 200 µg Chl *a* L^{-1}, are frequent and spread over 70% of the lagoon surface (Bresciani *et al.,* 2012; Zilius *et al.*, 2014). Cyanobacterial biomass accumulates exponentially during calm weather conditions, and exhibits patterns similar to those of opportunistic macroalgae, with fast growth, light-limitation, sudden collapse of the production, and dystrophy (Viaroli *et al*., 2001).

During blooms, a major part of the cyanobacteria looses the ability to float, and becomes trapped in the hypoxic-to-anoxic, dark water mass overlying sediments (Zilius *et al*., 2014). Here, it may settle and represent an important input of labile organic matter (OM) to the sediments. Cyanobacterial biomass may be incorporated within upper sediment horizons during resuspension events, occurring when wind exceeds 2 m s^{-1} (Zilius *et al*., 2014).

There are numerous experimental studies on the responses of sediments to OM loadings. OM represents a source for mineralization and may thus fuel the consumption of electron acceptors and the regeneration of mineral nutrients. Fresh OM additions may also exert a priming effect by stimulating the benthic catabolism of the original more refractory OM in the sediment (Van Nugteren *et al*., 2009; Guenet *et al*., 2010). The latter mechanism may be sustained by the lability of the newly deposited organic matter, and therefore by its macromolecular composition (low cellulose and high N content) and by physical processes (*i.e*., bioturbation or resuspension) that displace fresh OM from the surface to subsurface sediments. Diatoms, dinoflagellates, macrophytes or terrestrial material have been widely used for experimental OM additions to the sediment (Hansen and Blackburn, 1992; Tuominen *et al*., 1999; Kristensen and Holmer, 2001; Garcia-Robledo *et al*., 2013). These studies have shown that the macromolecular composition of the OM determines whether it is more or less refractory or labile to microbial metabo-

lism. The addition of labile OM is expected to stimulate microbial activities within sediments (Kristensen and Holmer, 2001), comprising an initial conversion of both fresh and old buried OM into soluble small organic compounds and subsequently into inorganic forms. Elevated inputs of labile OM to sediments may lead to the rapid exhaustion of energy yielding electron acceptors as O_2, NO_3^-, Mn(IV) and Fe(III) and stimulate low energy yielding microbial transformations as sulfate reduction or methanogenesis (Canfield, 1993; Kristensen and Holmer, 2001). The relative importance of the latter processes depends on salinity, with sulfate reduction as dominant path for carbon oxidation in anoxic marine sediments and methanogenesis as dominant path in freshwater and oligohaline sites (Capone and Kiene, 1988; Canfield, 1993; Holmer and Storkholm, 2001).

The elemental stoichiometry of settled OM may regulate the relative amount of dissolved inorganic nitrogen (DIN) regenerated from sediments. However, the expected efflux from sediment may become uncoupled from that of the original OM inputs. DIN effluxes may be reduced by coupled nitrification-denitrification and by anammox, the anaerobic ammonium oxidation (Rysgaard *et al*., 2004). At high levels of OM loading, oxygen penetration within sediments decreases due to enhanced aerobic respiration and slow diffusional transport (Meysman *et al*., 2006). Oxygen shortage may limit ammonium oxidation and, as a consequence, denitrification coupled to nitrification. Simultaneously, it promotes denitrification of water column nitrate due to thinner diffusional path to reach the denitrification zone in sediments (Rysgaard *et al*., 1994). Under specific circumstances (*i.e.* sulfidic sediments) denitrification can, however, be replaced by dissimilative reduction of nitrate to ammonium, resulting in the recycle of inorganic N (Christensen *et al*., 2000; Gardner *et al*., 2006; Algar and Vallino, 2014).

Burial or mineralization of pelagic OM into sediments may affect the variety of N-related microbial processes, with implications for the net nutrient fluxes across the sediment-water interface. In cascade, the ecological stoichiometry of regenerated nutrients may potentially feedback on the growth of phytoplankton (Eyre and Ferguson, 2009). Nevertheless, little is known about the specific impact of settled pelagic cyanobacteria on the microbial N processes in the sediments of shallow coastal environments.

In the present study we experimentally simulated a sudden settling and burial of cyanobacteria OM into the sediments of the Curonian lagoon, while considering the highly dynamic situation of this estuarine system. While sedimentation of cyanobacteria is expected to occur when they loose their ability to float, under calm weather conditions, the occurrence of strong winds may result in sediment resuspension, mixing and redistribution of new and old sediment particles along a thick sediment layer (Zilius *et al*., 2014).

The aim of the present study is to assess the short-term impact of a post-bloom burial of pelagic cyanobacteria into the sediment upon reestablishment of calm, well-mixed water column conditions. We specifically hypothesize that stimulation of benthic metabolism is followed by increased N recycling. We considered that enhanced denitrification, resulting in N loss, may impair the ecological stoichiometry of regenerated nutrients, with implications for pelagic primary producers. Therefore, we paid special attention to how cyanobacteria OM incorporation within sediments affect N cycling processes and attempted to quantify the main flow paths of N-cycling after the bloom settling, to provide a benthic N budget.

METHODS

Sediment and cyanobacteria collection and microcosms set-up

We simulated the burial of increasing amount of pelagic cyanobacteria into the upper sediment horizon and analyzed the short-term effects on benthic metabolism and N recycling. For this purpose, portions of homogenized sediments were amended with two levels of OM, reproducing a small and a large cyanobacterial settling events. Homogenized sediments without amendments were used as the control treatment.

In July 2012, during a cyanobacterial bloom, surface sediments (the upper 10 cm) were collected with a hand corer in the Curonian lagoon at a confined deep site (55°17.2388' N, 21°01.2898' E, 3.5 m depth). During the last five years benthic metabolism and nutrient fluxes have been intensively studied in this lagoon (Zilius, 2011; Zilius *et al*., 2012, 2014). The sampling site that was selected for this study is representative of 45% of the lagoon surface and lays within a depositional area with fluffy, inconsistent and frequently resuspended sediments (Zilius *et al*., 2014). The collected material (about 10 L) was sieved (0.5 mm mesh) to remove large debris and occasional chironomid larvae, and gently mixed to prepare homogenized sediments. This procedure is widely employed in laboratory experiments where the effects of specific factors on sedimentary processes are tested. In fact, it decreases the small-scale variability that is associated to intact sediments (Glud and Blackburn, 2002). However, sediment sieving and homogenization alter chemical gradients and the vertical distribution of the OM, which are relevant and driving factors for benthic microbial metabolism. At the investigated site the combined wind and wave action resuspend and homogenize the upper sediment layer, frequently resetting redox and concentration gradients, as well redistributing recently settled and old OM along the upper sediment horizon.

During the bloom event, phytoplankton, dominated by *Aphanizomenon flos-aquae* (Vaičiūtė, pers. communication), was collected from the water column at the sampling site with a 60 µm planktonic net. Such net selects large colonies of cyanobacteria, so that the combination of the community composition and of the sampling strategy has resulted in the dominant collection and use of N-fixers. In the laboratory, the collected cyanobacterial suspension was centrifuged at 1000 rpm for 5 min in 50 mL tubes to concentrate the particulate material and remove water.

The homogenized, sieved sediments were transferred into 3 large glass beakers (1.75 L) to create the different treatments. The first served as the control treatment and contained only sieved sediment without OM-cyanobacteria addition. Treatments with a low and a high level of OM-cyanobacteria enrichment were prepared by adding and mixing 10 and 50 mL of the plankton concentrate to the slurry, respectively. These corresponded to a low (20%) and high (100%) addition of the total particulate OM suspended at the sampling site during the summer bloom of 2011 (77.8 mg L^{-1} in a 3.4 m deep water column, Zilius *et al*., 2014), representing 53.3 and 266.5 g dry mass of OM per m^2 surface. The homogenized sediments from the 3 beakers were thereafter transferred each into three transparent cylindrical bottom capped liners (internal diameter 8 cm, 30 cm length) in order to reconstitute three replicates of 10 cm high sediment cores for each treatment. All microcosms were then carefully filled with filtered lagoon water (salinity <1), wrapped in aluminium foil, provided with a magnetic bar suspended 10 cm above the sediment and fully submerged with the top open in a 75 L tank, containing aerated and well stirred lagoon water. This allowed mixing of the tank water with the water inside the cores during the preincubation period. After this procedure, the reconstructed cores had a transparent water phase overlying a 10 cm thick homogeneous sediments; the sediment-water interface was clearly recognizable and did not present a fluffy layer. The preincubation (48 h) and incubations for net flux and denitrification measurements described below were performed at the *in situ* temperature (22°C) in darkness, which reproduced the conditions at the 3.5 m depth due to strong light attenuation in the water column (Zilius *et al*., 2014). An external magnet rotating at 40 rpm ensured water stirring within each core and its exchange with the tank, thus maintaining 100% of oxygen saturation of overlying water while avoiding sediment resuspension. This simulates well-mixed conditions at intermediate wind velocities ($2< x <5$ m s^{-1}) that are most common during the summer period (Zilius *et al*., 2014). The 48 h preincubation was set in order to establish chemical gradients between pore and overlying water, and perform measurements under stable conditions after the disturbance and non-steady state created by sieving and homogenization procedures. We considered to this purpose two days as sufficient and a good compromise to avoid excessive aging of such small microcosms.

Flux measurements

After preincubation, all cores were closed and the benthic net fluxes of dissolved gases (O_2 and CH_4), inorganic carbon (TCO_2) and nutrients (NH_4^+, NO_x^- and DON) were measured in the dark (see above). Incubation procedure was standard, with initial and final sampling from each core water phase, as detailed in Dalsgaard *et al*. (2000). Incubations lasted for 4 h in order to keep the variation of oxygen within 20-30% of its initial concentration (Zilius *et al*., 2014). At the beginning and at the end of the incubations three aliquots of 20 mL each were transferred to 12 mL exetainers (Labco, UK) and fixed with Winkler reagents for O_2 and with 50 µL of 4 $HgCl_2$ for CH_4 and TCO_2 measurements, respectively. An aliquot of 20 mL was filtered (Whatman GF/C filters) and transferred into glass tubes for nutrient analysis. Solute exchange across the water-sediment interface was calculated according to the general flux equation:

$$F_x = \frac{(C_f - C_i)\times V}{A\times t}$$

where F_x (mmol or µmol m^{-2} h^{-1}) is the flux, C_i and C_f (mM or µM) are the concentrations of the *x* solute at the beginning and at the end of incubation, respectively, V (L) is volume of the core water phase, A (m^2) is the sediment surface area and t (h) is the incubation time. The contribution of O_2 to the total carbon catabolism was calculated by comparing TCO_2 production and O_2 uptake assuming a respiratory coefficient for aerobic respiration (CO_2/O_2) of 1 (mol/mol).

Denitrification and dissimilative nitrate reduction to ammonium measurements

A second dark incubation was performed after flux measurements for the estimation of denitrification rates according to the isotope paring technique (IPT; Nielsen, 1992) and the simultaneous evaluation of dissimilative reduction of nitrate to ammonium (DNRA). As the IPT technique requires to enrich the nitrate water pool with $^{15}NO_3^-$, this second incubation was performed 2 h after the flux measurements. Therefore, the cores were submerged again in the tank to refill and renew the overlying water in the sediment cores. Denitrification and DNRA were measured by adding labelled potassium nitrate ($K^{15}NO_3$ 99.8% atom, Cambridge Isotope Laboratories, MA, USA) to a final concentration of 30 µM in each core water phase. *In situ* $^{14}NO_3^-$ concentration was about one order of magnitude lower (2.9 µM). A detailed description of the incubation procedure is reported in Ruginis *et al*. (2014). $^{14}N^{15}N$ and

$^{15}N^{15}N$ abundance in N_2 in the slurried core were analyzed by mass spectrometry at the National Environmental Research Agency, Silkeborg, Denmark. An additional subsample of the slurried sediment was collected and treated with 1 M KCl for the determination of the exchangeable ammonium pool and the $^{15}NH_4^+$ fraction.

Rates of denitrification of water column nitrate (Dw) and of nitrate produced within sediments via nitrification (Dn) were obtained according to the equations and assumptions reported in Nielsen (1992); total denitrification is defined as the sum of Dw and Dn. The contribution of total denitrification in OM mineralization was estimated assuming a molar ratio 1.25 between TCO_2 and N_2 production rates (Richards, 1965). Methodological concerns have been raised about the IPT, mainly due to the concurrence of anammox, which cannot be discriminated from denitrification as a source of N_2 and make invalid the IPT assumptions. However, anammox represents a minor fraction of N_2 production in organic-rich freshwater ecosystems (Koop-Jakobsen and Giblin, 2009). A recent theoretical study predicts low anammox rates under elevated DOC/nitrate ratio (Algar and Vallino, 2014), which is typical for the summer in the Curonian lagoon sediments. Therefore, we adopted the IPT, assuming that the anammox contribution to N_2 fluxes was negligible. Rates of DNRA are calculated from the production of $^{15}NH_4^+$, according to the equations reported in Risgaard-Petersen and Rysgaard (1995).

Analytical methods

Salinity was measured by an YSI multiple probe (Pro 1030) with sensitivity 0.1. Dissolved O_2 concentration was measured by the Winkler method (sensitivity 1 µM, APHA, 1975), TCO_2 was determined via 0.1 N HCl microtitration (sensitivity 3 µM; Anderson, 1986). CH_4 was measured via gas chromatography (FISONS 9000 GC, equipped with a flame ionization detector and a sensitivity of 0.1 µM). Dissolved inorganic nutrients (NH_4^+, NO_2^- and NO_x^-) were measured with a continuous flow analyzer (San^{++}, Skalar) using standard colorimetric methods (sensitivity 0.3 µM, Grasshoff, 1983). Nitrate (NO_3^-) was calculated as the difference between NO_x^- and NO_2^-. Total dissolved N was analyzed by the high temperature (680°C) combustion catalytic oxidation/NDIR method using a Shimadzu TOC 5000 analyzer provided with a TN module (sensitivity 2 µM). Dissolved organic nitrogen (DON) was calculated as a difference between total dissolved N and DIN (NH_4^++NO_2^-+NO_3^-). The proportion of $^{15}NH_4^+$ to $^{14}NH_4^+$ was determined following the protocol described in Risgaard-Petersen and Rysgaard (1995) with a set of Roboprep-G-Plus GC in combination with Tracermass spectrometer equipped with triple collector at the National Environmental Research Agency, Silkeborg, Denmark. The detection limit was 2 nmol of $^{15}NH_4^+$ in 2 mL sample (Risgaard-Petersen and Rysgaard, 1995). Organic carbon (C_{org}) and total N (TN) content of the sediments and of the concentrated planktonic material added to sediments was analysed with a Perkin-Elmer CHN analyzer, before measurements samples was acidified in order to remove carbonates.

Data analysis

One-way analysis of variance (ANOVA) was employed to test whether OM additions (3 treatments -no, 20% and 100% addition-, each with 3 replicates) affected sediment metabolism and nutrient exchange across the sediment-water interface. Homogeneity of variance was checked using Cochran's test and data were transformed if significant heteroscedasticity was found. A pair-wise comparison of means were carried with *post-hoc* Tukey HSD test ($P<0.05$).

RESULTS

Reconstructed sediment characteristics

In the control microcosms, comprising the reconstructed sediment without OM-cyanobacteria addition, the C/N ratio of the organic matter was 12.7, similar to *in situ* values (12.5; Zilius unpublished data) at the time of sampling (Tab. 1). The concentrated cyanobacterial slurries, which were employed in this study, contained C_{org}=47.91% and TN=10.67% of their dry weight. This OM had a molar C/N ratio of 5.2, similar to that of *Aphanizomenon sp*. (C/N=4.9; Karlson *et al*., 2008). Such C/N ratio was lower than other sources of organic matter used in previous studies (Caffrey *et al*., 1993; Conley and Johnstone, 1995; Holmer, 1996), which were 7.5, 7.2 and 7.6 for yeast, diatoms and flagellates, respectively. Nevertheless, the addition of the cyanobacteria had only a minor impact on the element ratios bulk sediment as it represented a low quantity to the OM present before addition, despite its strongly different element ratios. Elemental analyses of the sediments in fact showed that in both 20% and 100% treatments the C and N content and their ratio displayed a modest increase, probably not significant considering the uncertainty of the measurements.

Tab. 1. Organic carbon (C_{org}) and total nitrogen (TN) pools measured in reconstructed sediment cores.

Treatment	C_{org} (%)	TN (%)	C/N (molar)
Control	9.01	0.71	12.7
20%	9.60	0.74	13.0
100%	10.82	0.77	14.1

Control, sieved and slurried sediment without OM addition; 20%, sieved and slurried sediment added with 20% of particulate organic matter in the water column during a cyanobacteria bloom; 100%, sieved and slurried sediment added with 100% of particulate organic matter in the water column during a cyanobacteria bloom.

Benthic metabolism

After 2 days from the addition, cyanobacterial matter input resulted in increased aerobic and anaerobic microbial processes (Fig. 1). This was shown by a significant (ANOVA, $P<0.05$) impact of enrichment treatments on O_2 and TCO_2 fluxes, denitrification rates and CH_4 efflux from the sediment (Tab. 2). In most cases, the 20% addition was not significantly different either from the control or from the 100% addition, while the 100% addition was significantly different from the control in all cases (Fig. 1). Hence, when comparing the 100% addition to the control it was observed that O_2 uptake increased 5.4-fold, TCO_2 efflux increased 2.7-fold while denitrification and CH_4 efflux increased ~2 and 14-fold, respectively. The respiratory quotient, which is the ratio between TCO_2 and O_2 flux, was significantly >1 in all treatments, suggesting the accumulation of anaerobic metabolism end-products within sediments.

Calculated rates of denitrification were below 35 µmol N m^{-2} h^{-1} in all microcosms. The main pathway of N_2 production, about two-thirds, was based on the reduction of NO_3^- diffusing from the bottom water into the sediment, while the remaining one-third was sustained by denitrification coupled to nitrification (Fig. 1B). Both Dw and Dn were stimulated by additions of OM-cyanobacteria (Tab. 2). It was not possible to calculate the rates of dissimilative reduction of NO_3^- to NH_4^+ in our control and organic enriched sediments as $^{15}NH_4^+$concentrations were below the detection limits of the method in all analyzed samples, suggesting low DNRA activity.

Nutrient fluxes

Ammonium and DON fluxes were significantly impacted by treatments, while we did not observe a significant impact for the NO_x^- fluxes (Fig. 2 and Tab. 2 for ANOVA). The 20% addition was never significantly different from the control, while the 100% addition was significantly different from the control for the NH_4^+ and DON fluxes (Figs. 2 A,B). Comparing the 100% addition to the control it was observed that both NH_4^+ and DON fluxes increased approximately ~3-fold. Thus, DON followed the same trend as NH_4^+, however the DON efflux in all treatments was on average ~5-fold higher than that of NH_4^+ (Figs. 2 A,B). Treatment effects on NO_x^- fluxes, if present, were masked by high variability of fluxes.

DISCUSSION

Limits and ecological relevance of the experimental simulation

This experiment was designed to analyze the short-term response of benthic microbial activity and N-cycling to the burial of pelagic cyanobacteria biomass into sediments. The strong and significant differences observed for the metabolic rates and benthic N fluxes between the control and the 100% addition confirmed that degradation of the cyanobacterial matter started just after its addition to sediments. As the present experiment was limited to three days, we may assume that diffusion-control of transport rates was re-established very quickly while the diagenetic

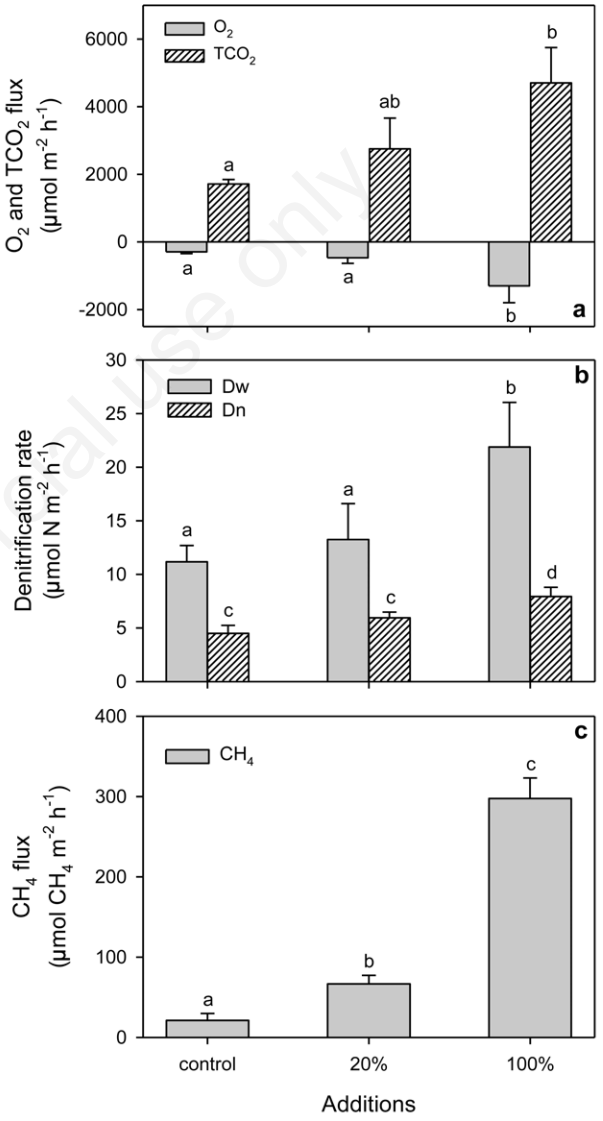

Fig. 1. Net fluxes of dissolved oxygen and inorganic carbon (a), rates of coupled nitrification-denitrification (Dn) and denitrification due to NO_3^- diffusion (Dw) from overlying water (b) and net flux of methane (c) to overlying water at different additions of cyanobacterial matter; bars represent mean values and error bars represent standard deviation (n=3); labeling by letters indicates the outcome of post-hoc pair-wise comparisons, *i.e.*, bars sharing the same letter are not significantly different (Tukey HSD, n.s. $P>0.05$), while bars that have different letters are statistically different (Tukey HSD, $P<0.05$).

process rates might be changing due to succession phenomena among the microbial populations. The approach we adopted, with some limitations, mimic what occurs in shallow dystrophic estuaries where physical processes redistribute settled blooms within the upper sediment horizons. To this purpose, we have sieved and homogenized sediments, after adding increasing amounts of cyanobacteria biomass, simulating an input of 20 and 100% of a summer bloom. This manipulation may alter some relevant sediment properties.

Sediment sieving and homogenization may reduce the amount of the organic carbon pool and set to zero steep vertical gradients in pore water chemistry, that drive the entity and direction of benthic fluxes (Berg *et al*., 1998; Day *et al*., 1995). Furthermore, in homogenized sediments fresh and old OM is mixed along the vertical profile, which is unlikely in most systems, unless strong bioturbation or resuspension. Sieving may also oxidize reduced metal pools or chemical forms as sulfides, introducing artifacts in redox-dependent cycles as that of phosphorus. On the other hand, the creation of homogeneous microcosms is a widely used approach, for example in bioturbation studies, that allows to address the effects of specific factors (Henriksen *et al*., 1983; Bartoli *et al*., 2000; Svensson *et al*., 2001). In our experiment this manipulation allowed to target the effect of labile OM-cyanobacteria incorporation on benthic features, removing other sources of variability of intact sediments (Glud and Blackburn, 2002). The limitations of our experimental procedure should be taken into account when attempting to translate laboratory results to *in situ* conditions. Nonetheless, metabolic (O_2 uptake and denitrification) and nutrient flux rates (NH_4^+ and DON) measured in this experiment are comparable to those carried out with intact cores (Zilius, 2011; Zilius *et al*., 2012, 2014). This suggests that the manipulation we performed did not alter benthic metabolism and that after two days of preincubation the gradients between pore and bottom water were similar to those *in situ*. Svensson *et al*. (2001) compared process rates in undisturbed and sieved sediments and did not find significant effects of the manipulation procedure on rates of denitrification, which was a key process also in our work. We therefore consider the differences we have found between unamended and cyanobacteria amended sediments reliable, and providing new insights on what occurs *in situ*. Our procedure may also represent a way to simulate frequently resuspended, mixed and homogenized *in situ* sediments. With this respect, the Curonian lagoon is shallow

Tab. 2. One-way ANOVA results. Square root transformation was applied to data when significant heteroscedascity was found.

Measure	Factor	df	SS	MS	F	P
O_2	OM additions	2	2.9	1.45	116.5	<0.001
	Residual	6	0.1	0.01		
	Total	8	3.0			
TCO_2	OM additions	2	13.8	6.9`	10.7	<0.05
	Residual	6	3.9	0.65		
	Total	8	17.7			
Dw	OM additions	2	193.5	96.7	9.4	<0.05
	Residual	6	61.6	10.3		
	Total	8	255.1			
Dn	OM additions	2	17.9	9.0	16.8	<0.01
	Residual	6	3.2	0.5		
	Total	8	21.1			
CH_4	OM additions	2	256.8	128.4	205.2	<0.001
	Residual	6	3.8	0.6		
	Total	8	260.5			
NH_4^+	OM additions	2	7599.8	3799.9	20.7	<0.01
	Residual	6	1099.7	183.3		
	Total	8	8699.5			
NO_x^-	OM additions	2	2.8	1.4	0.8	0.494
	Residual	6	10.6	1.8		
	Total	8	13.4			
DON	OM additions	2	206922.1	103461.1	6.7	<0.05
	Residual	6	93279.9	15546.7		
	Total	8	300303.0			
DIP	OM additions	2	224.9	112.4	3.7	0.088
	Residual	6	179.9	30.0		
	Total	8	404.8			

df, degrees of freedom; SS, sum of squares; MS, mean square; F, F value; P, significance level, where P< 0.05.

and impacted by strong wind-wave action, with wind speed exceeding 5 m s^{-1} (Bresciani *et al*., 2012). In the summer, transient calm weather periods with wind speed below 2 m s^{-1} occur, resulting in temporary stratification of the water column, rapid build up and subsequent deposition of cyanobacterial bloom (Zilius *et al*., 2014). The sampling site we have chosen, representative of nearly half the lagoon surface, is a site of phytodetritus accumulation and has fluffy sediments frequently respuspended (Zilius *et al*., 2012, 2014). The mixing of surficial sediment may redistribute settled phytoplanktonic material along the upper vertical profile and stimulate aerobic and anaerobic bacterial activity along a thick horizon and DON regeneration to the water column, as our results suggest. In our experiment we simulated the resuspension of the upper 10 cm horizon, which likely overestimates the *in situ* processes. However the vertical profiles of porosity are very homogeneous at the study sites and typical of fluffy sediments (Zilius *et al*., 2012).

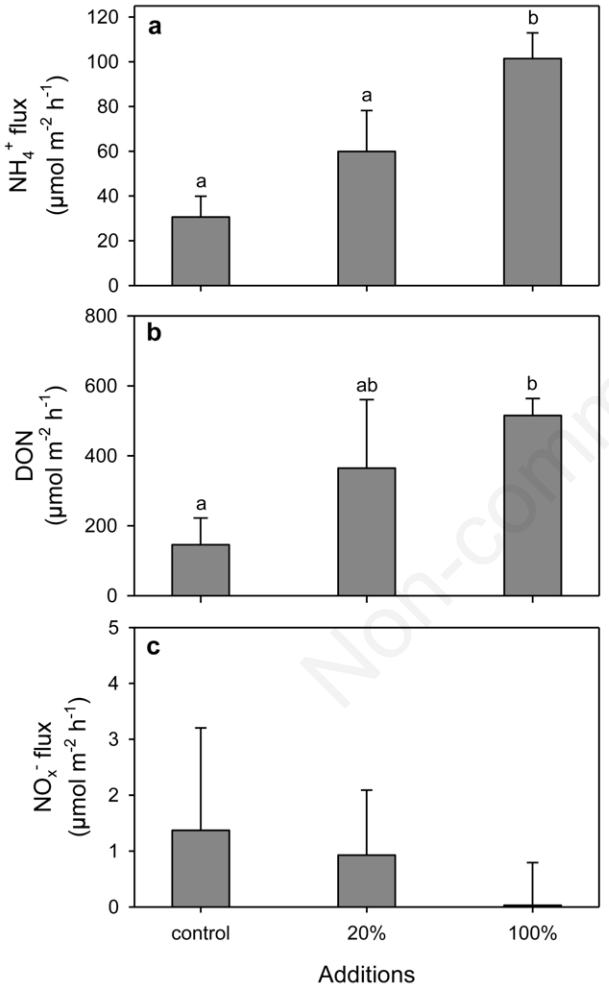

Fig. 2. Net fluxes of ammonium (a), dissolved organic nitrogen (b) and sum of nitrites and nitrates (c) across sediment-water interface at different additions of cyanobacterial matter; bars represent mean values and error bars represent standard deviation (n=3); labeling by letters indicates the outcome of post-hoc pairwise comparisons, *i.e.,* bars sharing the same letter are not significantly different (Tukey HSD, n.s. P>0.05), while bars that have different letters are statistically different (Tukey HSD, P<0.05).

Resuspension and destruction of the vertical zonation and redox profile is likely to occur and interrupt long-term stable zonation in shallow estuaries. This justifies the experimental homogenization of the sediment with the cyanobacterial addition, as deposited OM may be mixed into sediments. Our approach was short-term: we maintained the microcosms for 48 hours before measurements as we considered this preincubation time enough to have gradients established after the manipulation and not too long to lose the effect of the added OM or to have artifacts associated to the small microcosms we have used (Engström-Öst *et al*., 2013). Other studies analyzing the impact of OM addition to sediments were based on longer preincubation periods, since less labile organic matter (*e.g*., yeasts, diatoms, macroalgae) was used (Hansen and Blackburn, 1992; Enoksson, 1993; Conley and Johnstone, 1995; Tuominen *et al*., 1999; Garcia-Robledo *et al*., 2013). They also addressed deeper (>5 m depth) and probably less dynamic systems. Most of these experiments were carried out under steady state conditions. In some cases, they followed a time-series approach, and the tendency to achieve stable fluxes across the interface was observed (Hansen and Blackburn, 1992). We acknowledge that our study lack of a temporal analysis of processes and is limited to a single set of measurements, so our data provide limited information on the temporal evolution of processes. However, our results are relevant to understand how changes in microbial catabolism associated to labile OM inputs may impact, on a short-term, the biogeochemical process and nutrient fluxes across the sediment water interface. These may potentially feedback on the growth of the phytoplankton, particularly when it is limited by nutrients in summer (Eyre and Ferguson, 2009; Garcia-Robledo *et al*., 2013). Besides the temporal issue, future studies should address also the relevance of resuspension as mechanism coupling the benthic and pelagic compartments that release in the water column the solutes accumulated in the pore water.

Nitrogen cycling in the sediments enriched by cyanobacteria biomass

Collectively, the measured or calculated net flux and process rates for the different N compounds allow us to realize a tentative benthic nitrogen budget and propose coherent daily flow schemes for the post-bloom period (Fig. 3). These schemes also enable us to speculate about the rates of ammonification and release of DON shortly

after the burial (*i.e*., 2 days), the initial breakdown and/or mineralization of cyanobacteria biomass within sediments. Fig. 3 shows the schemes for the control and the 100% treatment, which significantly differed for all rates (except for NO_x^- flux) in the post-hoc comparisons.

In our study, total released nitrogen (DIN+DON+N_2 efflux) was dominated by DON, which contributed 75-80% of the net N efflux from the sediment into the water column (Fig. 3 and Tab. 3). Still, these measurements can be underestimated, due to rapid bacterial uptake in near-bottom water (Middleboe *et al*., 1998). Our findings are in agreement with observations of Lomstein *et al*. (1998), Middleboe *et al*. (1998) and Alkhatib *et al*. (2013), who also measured proportionally higher benthic fluxes of DON than DIN in shallow and deep coastal environments. High DON release from sediment has been suggested to represent an intermediate phase and its release from the sediment could be related to sediment OM reactivity (Lomstein *et al*., 1998; Alkhatib *et al*., 2013). In general, it seems that elevated DON efflux is a temporal phenomenon (Hansen and Blackburn, 1992; Enoksson, 1993; Lomstein *et al*., 1998) since initial hydrolysis, fermentation or cell lysis could liberate a large amount of it while its conversion into NH_4^+ is lacking behind. In windy areas as the Curonian lagoon, we may expect that frequent perturbations may explain the high DON effluxes, as observed during nutrient flux monitoring programs.

The very high DON flux compared to DIN and O_2 fluxes suggests that death and lysis of added cyanobacteria sustained a major portion of the total N flux if compared to microbial activity. However, O_2 uptake was much lower than TCO_2 production, suggesting a large proportion of anaerobic processes in total benthic respiration. The 14-fold increased CH_4 efflux in the 100% treatment with respect to the control is clearly due to enhanced methanogenesis. However, the magnitude of this diage-

Tab. 3. Equations used to calculate the nitrogen flows in Fig. 3.

No.	Pathway of N	Determination	Equation
1	NH_4^+ efflux	measured	-
2	NO_x^- flux	measured	-
3	DON efflux	measured	-
4	Exch+pw NH_4^+ pool	measured	-
5	Total sed NH_4^+ pool	calculated	(1)+(4)+(6)
6	Nitrification	calculated	(2)+(7)
7	Dtot	measured	-
8	Dw	measured	-
9	Dn	calculated	(7) - (8)

Exch+pw NH_4^+ pool*, ammonium pool in sediment comprising both the exchangeable fraction (*i.e.*, ions weekly attached to solid particles) and freely dissolved ammonium in pore water (pw);* total sed NH_4^+ pool*, total available ammonium pool in sediments;* Dtot*, total denitrification rates;* Dw*, denitrification due to NO_3^- diffusion from overlaying water;* Dn*, coupled nitrification-denitrification.*

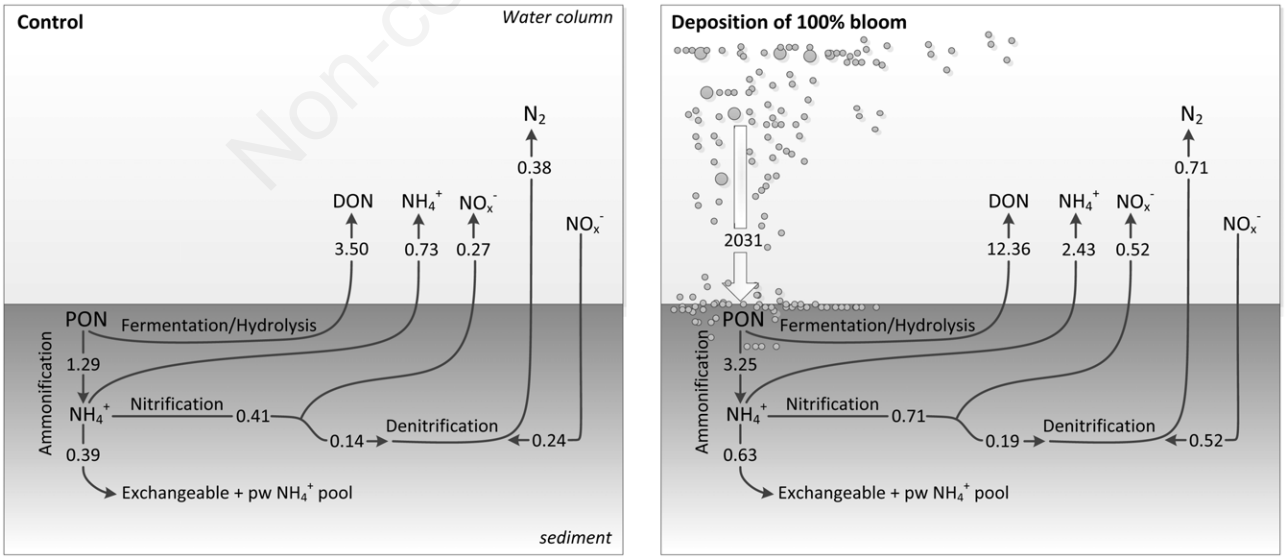

Fig. 3. Flow schemes for the benthic pathways of nitrogen for the control and 100% cyanobacterial addition, which have been calculated combining measured flux and process rates. The net rates are expressed on a daily basis per unit of sediment surface (mmol m^{-2} d^{-1}). Nitrification rates were estimated as the sum of the NO_x^- efflux, the nitrification which was coupled to denitrification and denitrification of water column NO_x^-. Total ammonium pool in sediment comprises both the exchangeable fraction (*i.e*., ions attached to solid particles) and freely dissolved ammonium in pore water (pw). Dissimilative NO_3^- reduction to NH_4^+ (DNRA) is not shown as rates were negligible.

netic process is uncertain and may be largely underestimated due to two main reasons. The first deals with an unknown percentage of the produced CH_4 that is consumed by methanotrophic bacteria. Such percentage, however, could be no higher than about 70% when comparing diffusive CH_4 and O_2 fluxes (Fig. 1). The second deals with the formation of CH_4 bubbles in the fluffy sediments we have employed, that escape to the atmosphere and may represent up to 80% of the total CH_4 production (Wassman *et al*., 1992). The possible importance of CH_4 bubbles is also highlighted by the fact that in the 100% addition we were only able to explain 35% of the TCO_2 efflux, of which 28% by aerobic respiration, 6% by dissolved CH_4 (assuming CO_2/CH_4 of 1 mol/mol) and 1% by denitrification. The difference may thus be attributed to the CH_4 bubbles formed by methanogenesis. However, other possible sources of CO_2 production in the sediment include CO_2 production by fermentation and H_2-producing acetogenesis and dissolution of calcium carbonate favoured by the acidification due to catabolic processes. While mixing sediments, at the end of the second incubation for denitrification measurements, gas ebullition was clearly observed, in particular in OM amended microcosms. The formation of methane bubbles has implication also for denitrification, as some N_2 can be stripped and not properly quantified, resulting in underestimated rates.

With a total N mobilization rate of about 15 mmol N m^{-2} d^{-1} it would take more than 4 months to regenerate and release the initial input of 2031 mmol N m^{-2}, which represents all deposited particulate organic N from the bloom. Together with the limited N loss to the atmosphere via denitrification, these data suggest that sediments are an efficient mid-term trap for settled N, at least if only diffusive processes are accounted for. Future studies should also address sediment resuspension, a common event in shallow estuaries, as conduit for nutrient transport back and forth between water and sediment.

In all cases, mineralisation of OM yielded less NH_4^+ than DON liberation; thus, NH_4^+ production represented 27% and 21% of the total N-mineralisation in the control and 100% treatments, respectively. Part of it enriched the exchangeable NH_4^+ pool, while 13% and 6% of NH_4^+ went into nitrification in the control and 100% treatments, respectively. The bulk of this nitrification was coupled to denitrification. In sediments containing black carbon pools (*i.e*., refractory to microbial degradation) as those considered in the present study, any addition of labile OM would provide fresh substrate for remineralization and may thus favour nitrification and coupled nitrification-denitrification. Therefore in our experiments nitrification was weakly, albeit significantly (Fig. 1B), affected by OM additions, which is in contradiction with observations reported by Sloth *et al*. (1995) and Tuominen *et al*. (1999) where they employed yeast and diatoms, respectively. These authors found that increased OM addition would tend to inhibit nitrification and thus contribute to limit N removal through coupled nitrification-denitrification. In contrast, our study describes a positive effect on denitrification, both for Dn and on Dw. The unmeasurable rates of DNRA, even in OM amended treatments, suggest that denitrification was the main pathway for dissimilative NO_3^- reduction in the Curonian lagoon sediment. This confirms seasonal measurements of *in situ* rates of DNRA that was never measurable in this system (Zilius, *unpublished data*). The relative importance of denitrification and DNRA along freshwater to oligohaline sediments with a high OM loading is related to salinity, likely via sulfide effects, and C/N ratio in sediments (Christensen *et al*., 2000; Gardner *et al*., 2006; Burgin and Hamilton, 2007). It was recently demonstrated that denitrification could be more competitive process if buried OM meets nutritional requirements of denitrifiers where sedimentary carbon quality and/or NH_4^+ supply is limited (Fulweiler *et al*., 2013).

Implications for shallow estuarine systems along the Baltic Sea coasts

In the Curonian Lagoon massive inputs of labile OM to the benthic system stimulated sediment respiration with maximum effects on CH_4 net efflux and O_2 uptake, with a 16 and 5 fold increase, respectively. Denitrification increased but rates were low due to low water column NO_3^- concentrations in the summer and limited O_2 penetration controlling nitrification rates (Zilius *et al*., 2012). This process was not quantitatively relevant as respiration path and with respect to the input of N to the benthic system, which was (slowly) recycled. Increased aerobic and anaerobic benthic respiration and of methanogenesis in particular, is an expected effect after the settling of blooms in other similar shallow, hypertrophic and oligohaline ecosystems in the Baltic Sea area. Implications at the ecosystem level deal with a large production of a powerful greenhouse gas and the establishment of hypoxic conditions that are unlikely in shallow aquatic systems but may be favoured by calm weather conditions and water stratification. This was demonstrated in a 3.5 m deep water column in the Curonian Lagoon and had cascade effects on inorganic P recycling (Zilius *et al*., 2014).

The large release of DON from sediments is an interesting finding. Future studies should analyse whether these high DON fluxes are related to the transient situation simulated in this study, or if in the Curonian lagoon DON is exceeding DIN systematically. They should address also how relevant is the released DON as N source for pelagic primary producers, in particular during summer, when inorganic N loads from the main lagoon tributary, the Nemunas River, are at their seasonal minimum. Assuming constant rates in time and space, we upscaled

measured fluxes to nearly half the lagoon surface, and calculated that in the summer sediment may release approximately 9000 kmol DON month^{-1} to water. This corresponds to nearly 15% of the average DON monthly input in the summer by the Nemunas River (Stepanauskas *et al*., 2002). However, it is suggested that only 30% of the DON entering the lagoon is potentially bioavailable, suggesting that the relevance of benthic DON might increase (Stepanauskas *et al*., 2002). Such pool may be assimilated in the lagoon or transported with outflow to the sea (Seitzinger and Sander, 1997).

In the water column DON can be photomineralizated to NH_4^+ and assimilated by bacteria and/or phytoplankton (Berman, 1987; Middleboe *et al*., 1998; Aarnos *et al*., 2012; Korth *et al*., 2012). Assimilation by bacteria or phytoplankton of DON is an understudied but potentially relevant N pathway in estuarine systems (Berman and Bronk, 2003). It is likely depending upon the reactivity of this large and heterogeneous N pool, as well as by the adaptation of phytoplankton to utilize an array of compounds alternative to NH_4^+ (Seitzinger *et al*., 2002; Stepanauskas *et al*., 2002; Korth *et al*., 2012). Pelagic microalgae are demonstrated to utilize DON derived from phytoplankton decomposition (Korth *et al*., 2012).

Therefore, we speculate that DON released from sediment, due to lysis or decomposition of buried microalgal biomass, may represent a N source to cyanobacteria when DIN is limiting in summer. In previous studies it was shown that blooming N-fixers (*e.g*., *Aphanizomenon* sp.) are able to take up mainly DON, avoiding an energy costly process as N fixation (Sörensson and Sahlsten, 1987; Berman, 1997, 2001). However, turnover rates of DON and its interplay with N-fixation is still poorly understood in oligohaline estuaries as the Curonian Lagoon, and remains a perspective for future studies.

CONCLUSIONS

Shortly after its incorporation within sediments, cyanobacteria-OM enhances aerobic and anaerobic bacterial activities and fuels a large regeneration of dissolved N, mostly in organic forms. The proportion of N imported from the atmosphere to the sediment via pelagic N-fixation and post-bloom settling which is converted back to N_2 is small. This suggests N recycling and a positive feedback for eutrophication phenomena and cyanobacterial blooms, at least in the short-term. Future studies should address the mechanisms involved in the stimulation of benthic activity and investigate the dynamics of post-bloom benthic processes over longer time scales.

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