https://doi.org/10.5194/bg-2020-419
Preprint. Discussion started: 13 November 2020

Biogeosciences Discussions

Spatiotemporal patterns of N_2 fixation in coastal waters derived from rate measurements and remote sensing

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Abstract. Coastal lagoons are important sites for nitrogen (N) removal via sediment burial and denitrification. Blooms of heterocystous cyanobacteria may diminish N retention as dinitrogen (N_2) fixation offsets atmospheric losses via denitrification. We measured N_2 fixation in the Curonian Lagoon, Europe's largest coastal lagoon, to better understand the factors controlling N_2 fixation in the context of seasonal changes in phytoplankton community composition and external N inputs. Temporal patterns in N_2 fixation were primarily determined by the abundance of heterocystous cyanobacteria, mainly *Aphanizomenon flosaquae*, which became abundant after the decline in riverine nitrate inputs associated with snowmelt. Heterocystous cyanobacteria dominated the summer phytoplankton community resulting in strong correlations between chlorophyll-a (Chl-a) and N_2 fixation. We used regression models relating N_2 fixation to Chl-a, along with remote sensing-based estimates of Chl-a to derive lagoon-scale estimates of N_2 fixation. N_2 fixation by pelagic cyanobacteria was found to be a significant component of the lagoon's N budget based on comparisons to previously derived fluxes associated with riverine inputs, sediment-water exchange and losses via denitrification. To our knowledge, this is the first study to derive ecosystem-scale estimates of N_2 fixation by combining remote sensing of Chl-a with empirical models relating N_2 fixation rates to Chl-a.

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1 Introduction

Biological dinitrogen (N_2) fixation plays an important role in the nitrogen (N) budget of aquatic ecosystems as it transforms gaseous N_2 into reactive forms, which are available for assimilation by microorganisms, algae and plants (Gruber, 2004; Hayes et al., 2019). Coastal ecosystems contain diverse diazotrophic communities, comprised of unicellular cyanobacteria, colonial heterocystous cyanobacteria, and heterotrophic bacteria (Bentzon-Tilia et al., 2014; Riemann et al., 2010; Zilius et al., 2020). Unicellular cyanobacteria and heterotrophic diazotrophs dominate in tropical and oligotrophic marine systems (Farnelid et al., 2016; Riemann et al., 2010; Zehr et al., 2003), whereas colonial heterocystous cyanobacteria dominate N_2 fixation in temperate systems (Klawonn et al., 2016). The dominant colonial heterocystous cyanobacteria in the Baltic Sea and its coastal areas are *Aphanizomenon*, *Nodularia* and *Dolichospermum* (Olofsson et al., 2020b). Their proliferation has the potential to alter N cycling and thereby influence N export to coastal waters.

Eutrophic lagoons, like those in the Baltic region (Asmala et al., 2017), undergo a shift from phosphorus (P) limitation in spring to N and silica limitation in summer and fall (Patuszak et al., 2005; Vybernaite-Lubiene et al., 2017). Such changes, which largely depend on the timing and magnitude of riverine nutrient inputs, create a temporal niche for diazotrophic cyanobacteria that are capable of overcoming N limitation, and thus often dominate the summer phytoplankton community (Paerl and Otten, 2013; Vybernaite-Lubiene et al., 2017). Large blooms of cyanobacteria are associated with high oxygen demand, which results in transient (night-time) oxygen depletion and enhances the release of dissolved inorganic phosphorus (DIP) from sediments (Petkuviene et al., 2016; Zilius et al., 2014). Thus, a positive feedback is established whereby surplus P favors N-limitation and further proliferation of cyanobacteria that are capable of fixing N_2. Although there has been progress in understanding the causes and expansion of cyanobacterial blooms in eutrophic coastal ecosystems (e.g. Bartoli et al., 2018; Paerl et al., 2001; Paerl and Paul, 2012 and references therein), seasonal patterns and environmental controls of N_2 fixation associated with cyanobacterial blooms are not well understood. Particularly challenging is the estimation of N_2 fixation at larger scales, owing to the patchy distribution of cyanobacteria.

In the present study, we analysed spatiotemporal patterns of pelagic N_2 fixation in relation to plankton community characteristics in the Curonian Lagoon. The lagoon is characterized by massive summer blooms of cyanobacteria, with chlorophyll a (Chl-a) concentrations as high as 400 μg L^{-1} (Bresciani et al., 2012). Our prior work showed that the occurrence of cyanobacteria blooms had a large effect on N cycling and retention in this system (Zilius et al., 2018). Cyanobacteria blooms reduced annual N retention in the lagoon because summer N_2 fixation offsets winter denitrification. In addition, N contained in cyanobacteria biomass was more likely to be exported from the lagoon to the Baltic Sea, rather than buried in sediments, due to their positive buoyancy. A limitation of the prior study was that the patchy and dynamic nature of cyanobacteria blooms resulted in considerable uncertainty in estimating N_2 fixation at larger spatiotemporal scales (e.g., for monthly, lagoon-scale N balances). In this follow-up study, we focus on seasonal changes in phytoplankton abundance and community composition and their utility in predicting N_2 fixation. We derive models relating N_2 fixation to heterocyst density, abundance of

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heterocystous cyanobacteria and Chl-a, and use remote sensing of Chl-a to derive estimates of N_2 fixation at larger spatiotemporal scales. We consider N_2 fixation in the context of other, previously measured inputs and losses of N from the lagoon.

2 Material and Methods

2.1 Study site

The Curonian Lagoon is located along the southeast coast of the Baltic Sea (Fig. 1). It is the largest coastal lagoon in Europe (area = 1584 km^2). The lagoon is a shallow waterbody (mean depth = 3.8 m) that discharges to the Baltic Sea through the narrow Klaipeda strait and occasionally receives inputs from the Baltic during periods of wind-driven forcing (Zemlys et al., 2013). These events are typically of short duration and result in small increases in salinity (typically by 1–2, maximum = 7) in the northern portion of the lagoon. During sampling carried out for this study, salinity in the lagoon was below < 0.5, suggesting limited brackish water intrusions. There was little difference in temperature between surface and the bottom layers (< 2 ^oC) indicating well-mixed conditions (Zilius et al., 2020).

The Nemunas River is the principal tributary (mean annual discharge = 16.4 km^3) and main source of nutrient inputs to the lagoon (Vybernaite-Lubiene et al., 2018; Zilius et al., 2018). The inflow of the Nemunas enters near the mid-point along the north-south axis of the lagoon. Hydrodynamic modelling studies suggest that the bulk of riverine inputs travel north toward the Klaipeda Strait resulting in shorter water residence time in the northern lagoon (Umgiesser et al., 2016). Longer water residence time in the southern lagoon provides favorable conditions for cyanobacteria bloom development (Bartoli et al., 2018). Patterns of phytoplankton seasonal succession are similar throughout the lagoon, transitioning from diatom and chlorophyte dominance in spring to cyanobacteria-dominated blooms in summer and fall (Zilius et al., 2018). Spatially-extensive blooms of heterocystous cyanobacteria (*Aphanizomenon* and *Dolichospermum*) are occasionally observed, particularly during low-wind conditions (Bartoli et al., 2018).

2.2 Sample collection

We measured pelagic N_2 fixation and characterized bacterioplankton and phytoplankton communities at stations located in northern and central regions of the lagoon during April–November 2018. Water samples were collected monthly at two depth layers (0–1.5 m, and 2.0–3.5 m) in the deeper, central area (southern site; mean depth = 3.5 m), and at one depth layer (0–1.5 m) in the shallow northern site (mean depth = 1.5 m). Water samples were transferred to 1) sterilized amber borosilicate bottles (0.5 L) for bacteria counting, 2) to opaque HPDE bottles (2 L) for nutrient analyses, and 3) 20 L jars for N_2 fixation measurements. All samples were transported on ice (except for N_2 fixation experiment) within half an hour after collection for subsequent laboratory processing and analyses. During each sampling, water temperature, salinity and dissolved oxygen were measured *in situ* at the surface (0.5 m depth) and bottom (0.5 m above the sediment) using a YSI 460 multiple probe (Xylem).

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Vertical profiles of photosynthetically active radiation (PAR) were measured with a LI-192 underwater quantum sensor (LI-COR®). We also monitored total nitrogen (TN) concentrations in the Nemunas River to compare riverine N loads with N inputs via N_2 fixation (as described in Zilius et al., 2018).

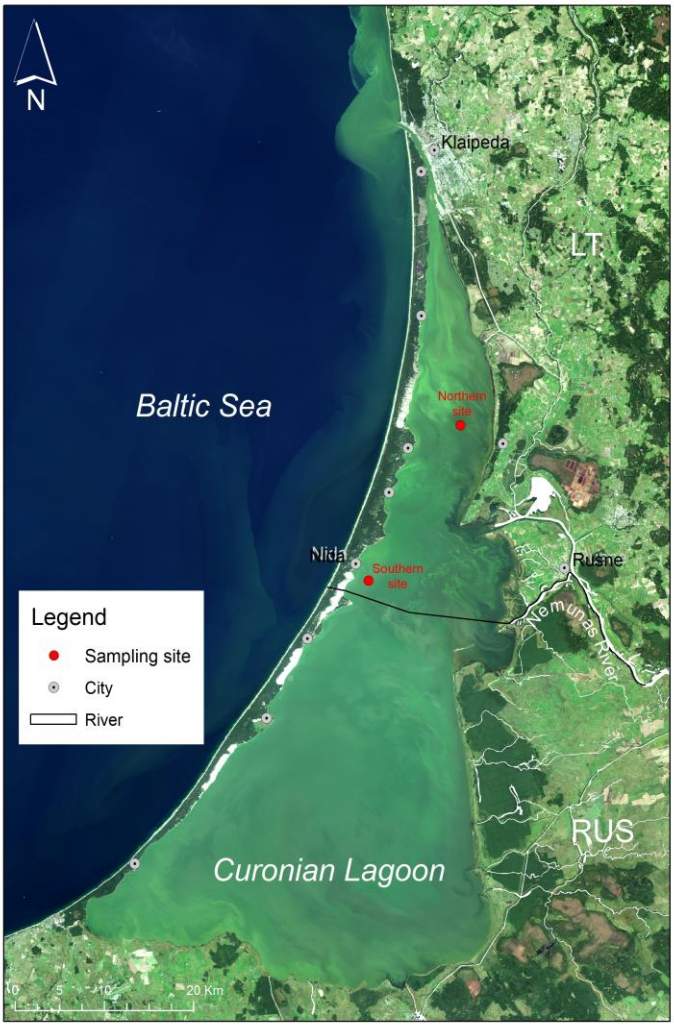

Figure 1: Satellite image by OLI/Landsat-8 (18/09/2014) showing summer blooms in the Curonian Lagoon with the sampling sites (red circles) representing the northern and south-central regions.

2.3 Chemical analysis

Water samples were GF/F filtered in triplicate for inorganic and organic nutrient analysis as previously described by Vybernaite-Lubiene et al. (2017). Dissolved inorganic nutrients (NH_4^+, NO_2^-, NO_3^-, and DIP) were determined

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colorimetrically using a continuous flow analyser (San^{++}, Skalar) following the methods described in Grasshoff et al. (1983). Total dissolved nitrogen (TDN) was analyzed by the high temperature (680 ºC) combustion, catalytic oxidation/NDIR method using a Shimadzu TOC 5000 analyzer with a TN module. Dissolved organic nitrogen (DON) was calculated as difference between TDN and DIN ($NH_4^+ + NO_2^- + NO_3^-$). Dissolved organic carbon (DOC) was determined with a Shimadzu TOC 5000 analyzer using an acetanilide dilution series as a standard (Cauwet, 1999). Total dissolved phosphorus (TDP) was determined after digestion and oxidation of the organic P forms with alkaline peroxodisulphate acid digestion (Koroleff, 1983). Dissolved organic phosphorus (DOP) was calculated as difference between TDP and DIP. Water samples for Chl-a were filtered through Whatman GF/F filters (nominal pore size 0.7 µm). Pigments were extracted with 90% acetone (24 h at 4 °C) and measured by spectrophotometry (Jeffrey and Humphrey, 1975; Parsons et al., 1984). Particulate matter was collected on pre-ashed (4 h at 550 ºC) Advantec GF75 filters (nominal pore size 0.3 µm) for particulate nitrogen (PN, river water) and isotopic signature (δ^{15}N-PN, lagoon) analysis. Prior to analysis, filters were dried at 60 ºC to constant weight and later analysed with an Elementar Vario EL Cube (Elementar Analysen systeme GmbH) interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd) at the Stable Isotope Facility of UC Davis, USA. TN was calculated as a sum of dissolved and particulate fractions.

2.4 Phytoplankton and bacteria counts

Samples for phytoplankton counting were immediately preserved with acetic Lugol's solution and examined at magnifications of ×200 and ×400, using a LEICA DMI 3000 inverted microscope. Phytoplankton community composition was determined using the Utermöhl method (Utermöhl, 1958) according to HELCOM recommendations (HELCOM, 2017). Phytoplankton biomass (mg L^{-1}) was calculated according to the methodology described in Olenina et al. (2006) and converted into carbon units (µg C cell L^{-1}) following Menden-Deuer and Lessard (2000). The number of heterocysts was also determined (cells L^{-1}).

The abundance of heterotrophic bacteria and picocyanobacteria was determined in filtered samples (50 µm size mesh), which were preserved in 0.25% glutaraldehyde (final concentration) and stored at −80 °C until analysis (Marie et al., 2005). Samples for determination of heterotrophic bacteria biomass were stained with SYBR Green I (Invitrogen) to a final concentration of 1:10000 (Marie et al., 2005), diluted with Milli-Q water, and analysed with a flow cytometer (BD Accuri™C6, DB Biosciences) at a medium flow rate (35 µL min^{-1}) for 2 min. Microspheres of 1 µm (Fluoresbrite plain YG, Polysciences) were added to samples as an internal standard. Samples for picocyanobacteria (≤ 3 µm) were analysed at a flow rate of 66 µL min^{-1} with an acquisition time of 2 min. Microspheres of 3 µm (Fluoresbrite plain YG, Polysciences) were used as an internal standard. A factor of 20 fg C per cell was used to convert bacteria counts to carbon biomass (Lee and Fuhrman, 1987).

2.5 Nitrogen fixation

Monthly measurements of N_2 fixation in the water column were performed using the $^{15}N_2$ technique described in Montoya et al. (1996). Rates were measured in two depth layers (0–1.5 m, and 2.0–3.5 m) at the deeper, southern site, and for the whole

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water column (0–1.5 m) at the shallow northern site. The samples were filled (avoiding air bubbles) into 500 ml transparent HDPE bottles. Each sample received 0.5 ml $^{15}N_2$ (98% $^{15}N_2$, Sigma-Aldrich) injected by syringe through a gas-tight septum (Zilius et al., 2018). As the isotopic equilibration takes up to several hours (Mohr et al., 2010), we incubated the samples for 24 h, thus minimizing equilibration effects which can lead to underestimation of rates (Mulholland et al., 2012; Wannicke et al., 2018) but prevents potential contamination through the production of isotopically enriched water. Surface water samples were incubated outdoors at ambient irradiance, while samples from 2.0–3.5 m were wrapped in aluminium foil as *in situ* irradiance was below 1% of surface PAR at these depths. After incubation, suspended material was collected on pre-ashed (4 h at 450 ºC) Advantec GF75 filters (nominal pore size 0.3 µm). This nominal pore size filter was used instead the conventional (0.7 µm) as it allows for quantitative collection of smaller cells comprising the active diazotrophic community (Bombar et al., 2018). All samples were stored frozen until analysis with a continuous-flow isotope ratio mass spectrometer (Thermo-Finnigan, Delta S, Bremen) at the Leibniz Institute for Baltic Sea Research Warnemünde (IOW). Volumetric rates of N_2 fixation were calculated following Eq. 1 (Montoya et al., 1996):

$$N_2 \text{ fixation rate } (\text{nmol } l^{-1}h^{-1}) = \frac{V}{2}\overline{PN} \approx \frac{V}{2} \times \left(\frac{[PN]_0 - [PN]_f}{2}\right) \quad (1)$$

Where V (the specific rate of N_2 uptake) is derived from Eq. 2:

$$V = \frac{1}{\Delta t} \times \frac{\left(A_{PN_f} - A_{PN_0}\right)}{\left(A_{N_2} - A_{PN}\right)} \quad (2)$$

A_{PN} is the ^{15}N atom % enrichment of the PN pool at the beginning (t_0) and end (t_f) of an incubation, A_{N2} is the ^{15}N atom % enrichment of the dissolved N_2 gas in the incubated water, and PN is the concentration of PN at the beginning (t_0) and end (t_f) of the incubation. Volumetric N_2 fixation rates were converted to areal rates taking into account the depth of the water column and the thickness of each depth layer (see above).

2.6 Remote sensing

Prior studies have developed and validated techniques for remote sensing of Chl-a in the Curonian Lagoon (Bresciani et al., 2014; INFORM, 2016; Riddick et al., 2019). We obtained satellite images for 6 dates spanning the period when phytoplankton communities were dominated by heterocystous cyanobacteria and water temperatures exceeded 15 ºC (July–September). Chlorophyll-a concentrations were obtained from optical satellite images using an on-board Multispectral Instrument (Sentinel-2: September 7 and 20) and an on-board Ocean and Land Colour Instrument (Sentinel-3: July 4 and 24, August 8 and 23). Satellite images were resampled to a nominal pixel size of 300 m resulting in a grid matrix of ~17,000 cells comprising the area of the lagoon. Cells adjacent to the shoreline were excluded from analyses due to potential interference from aquatic vegetation and benthic algae. Atmospheric correction was carried out using the Second Simulation of the Satellite Signal in the Solar Spectrum-Vector code (6SV; Vermote et al., 1997) previously used in other satellite applications for the Curonian Lagoon (Bresciani et al., 2014). The parametrization of the 6SV code was performed using the Maritime model. Values of Aerosol Optical Thickness (AOT) were obtained from AERONET sites and using MODIS-derived AOT values available from the NASA Giovanni application (https://giovanni.gsfc.nasa.gov/giovanni/). Chl-a concentrations were derived after the

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application of a semi-empirical band-ratio model that uses reflectance in the red and near-infrared spectral regions (De Santi et al., 2019; Gitelson et al., 2007).

Equation 3 used for Sentinel-2 data:

$$Chl-a, mg\ m^{-3} = (76.36 \pm 2.29) \times \frac{Ref_{705}}{Ref_{665}} - (51.57 \pm 0.26), \quad (3)$$

Equation 4 used for Sentinel-3 data:

$$Chl-a, mg\ m^{-3} = (52.19 \pm 1.81) \times \frac{Ref_{708}}{Ref_{665}} - (32.07 \pm 0.57), \quad (4)$$

where Ref_x indicates the reflectance of the band with central wavelength x.

Prior work validating satellite-derived Chl-a against *in situ* observations in the Curonian Lagoon showed good agreement for both Sentinel-2 (R^2 = 0.91, root-mean-square error (RMSE) = 18.6 mg m^{-3}) and Sentinel-3 R^2 = 0.95, RMSE = 7.4 mg m^{-3}) images (INFORM, 2016; Riddick et al., 2019). Estimates of N_2 fixation were derived for each of the grid cells based on satellite-derived Chl-a and regression models relating measured N_2 fixation to Chl-a (regressions provided in Results).

2.7 Statistical analysis

Principal coordinates analysis (PCoA) was performed to visualize spatiotemporal patterns in plankton community variables (*Aphanizomenon*, *Dolichospermum*, non N_2-fixing cyanobacteria, and heterotrophic bacteria biomass) and their relationship to nutrient concentrations. This analysis was performed using Primer 7 software (Primer-E Ltd., v.7; Plymouth, United Kingdom; Clarke and Gorley, 2015) on Euclidean distances of normalized and forth-root transformed variables. The relationship between Chl-a and N_2 fixation was examined using linear regression. In addition, variance analysis (two-way ANOVA) was used to test differences in Chl-a concentration between process surface and bottom. The assumptions, data normality, and homogeneity of variance, were checked using Shapiro–Wilk test and Cochran's test, respectively. For significant factors, post hoc pairwise comparisons were performed using the Student-Newman-Keuls (SNK) test. The significance level was set at $\alpha = 0.05$. Analyses were performed in SigmaPlot 14.0 software.

3 Results

3.1 Phytoplankton and bacteria communities

Seasonal patterns in phytoplankton biomass and community composition followed expected trends based on prior work at this site (Fig. 2). Surface Chl-a during April–July ranged from 25 to 57 μg L^{-1} at the southern site and from 14 to 26 μg L^{-1} at the northern site. Higher Chl-a was observed in late summer with values ranging from 52 to 286 μg L^{-1} at the northern site and from 96 to 256 μg L^{-1} at the southern site. Phytoplankton biomass showed corresponding changes, increasing from ~1000 to 4000 μg C L^{-1} during spring to late summer. Diatoms dominated the spring phytoplankton community (April–May) accounting

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for up to 94% of total biomass. During June–July, diatoms were replaced by non- and N_2-fixing cyanobacteria; the later accounted for up to 36% of total phytoplankton biomass. Between August and November, N_2-fixing cyanobacteria dominated the community (86% of total biomass). The main N_2-fixing cyanobacteria were *Dolichospermum* spp. (formerly *Anabaena*) and *Aphanizomenon flosaquae*. Heterotrophic bacteria accounted for ~30% of the total plankton biomass (bacteria + phytoplankton) during the early successional (diatom-dominated) phase. Heterotrophic bacteria biomass was higher during the cyanobacterial bloom (June–October), increasing from ~80 to 250 µg C L^{-1}, however, their relative contribution to total plankton biomass decreased to ~17%. Phytoplankton biomass and community composition were generally similar between surface and bottom layers, except in October–November when the abundance of N_2-fixing cyanobacteria was greater in the surface layer (2500–3500 µg C L^{-1}) relative to the bottom layer (<100 µg C L^{-1}). Picocyanobacteria were not detected during the study period at either site.

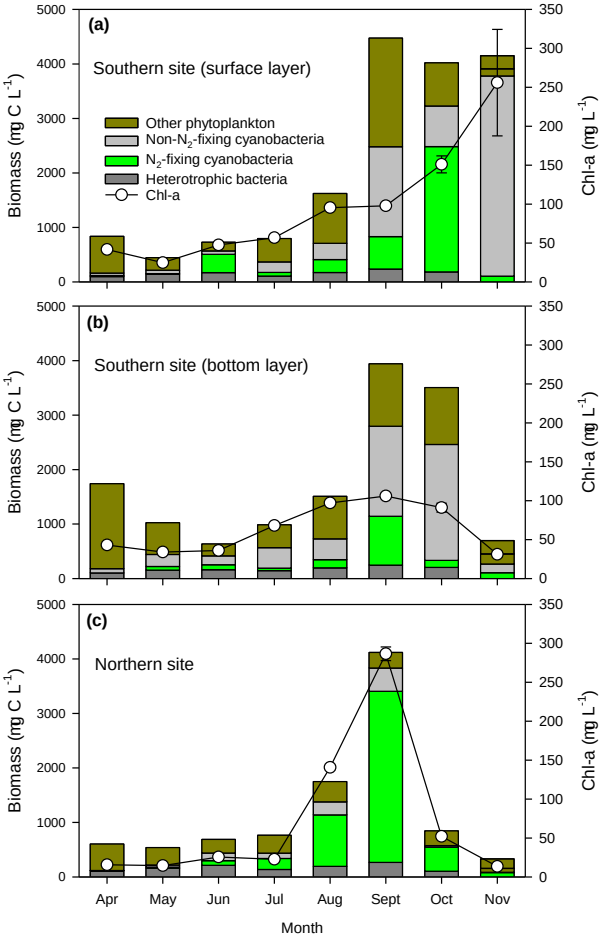

Figure 2: Phytoplankton and bacteria biomass at southern (a, b) and northern (c) sites in the Curonian Lagoon during 2018. Chlorophyll-a concentrations are mean values and standard error (some error bars not visible).

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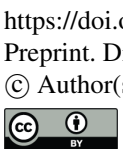

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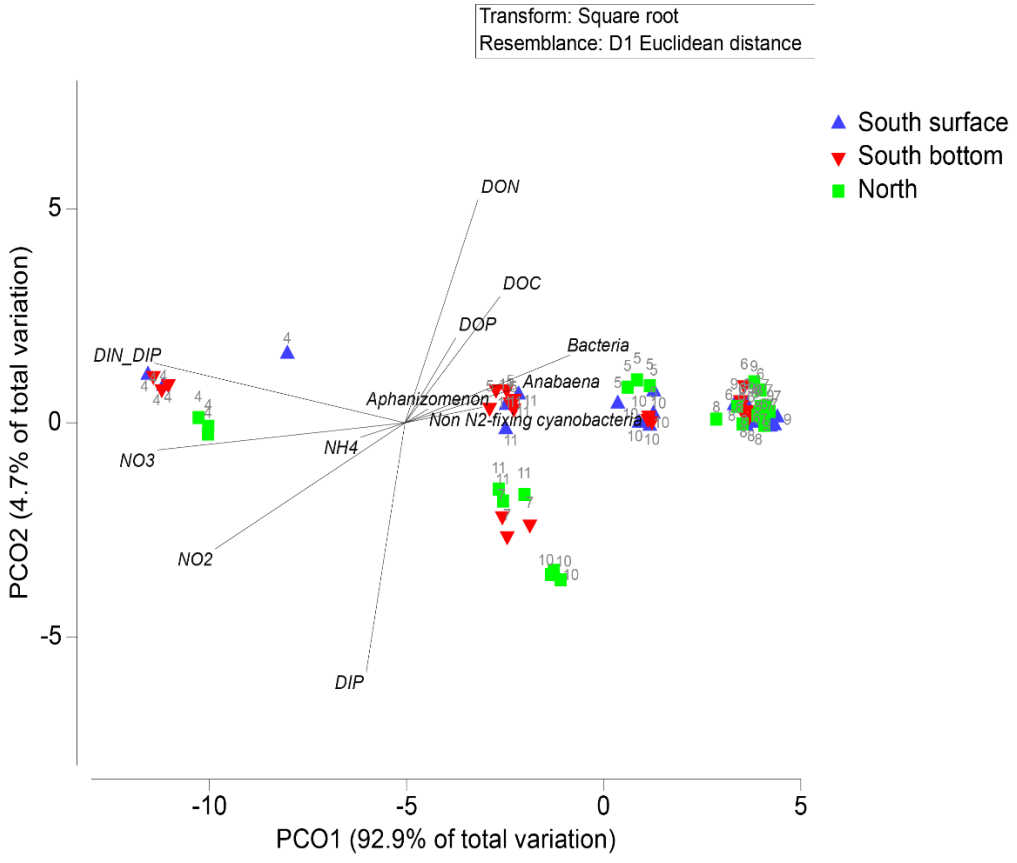

Figure 3: Principal coordinate biplots generated on Euclidean distances of normalized and forth-root transformed nutrient concentrations (DOC, NH_4^+, NO_2^-, NO_3^-, DON, DIP, DOP, and DIN:DIP). Overlaid vectors show individual chemical variables (those significantly correlating with either of the two primary axes, with Pearson correlations > 0.5) and plankton community biomass (*Aphanizomenon*, *Anabaena (in text Dolichospermum)*, non N_2-fixing cyanobacteria and heterotrophic bacteria).

Principal coordinates analysis revealed spatiotemporal differences in nutrient concentrations and plankton community characteristics (Fig. 3). The first principal coordinate axis explained 93% of the total variation by differentiating April samples on the basis of high NO_3^- (~80 µM) and high DIN:DIP (~200 molar) relative to samples collected in May–November (NO_3^- mean = 2.5 µM; DIN:DIP mean = 14 molar; Fig. 3, 4). Biomass of *Aphanizomenon*, *Dolichospermum*, non N_2-fixing cyanobacteria and heterotrophic bacteria were positively associated with axis 1 indicating their dominance during low N and low DIN:DIP conditions. The second principal coordinate axis accounted for 5% of variation and separated samples collected in July from the bottom layer (southern site) and during October–November at the northern site. These samples were characterized by higher DIP (up to 2 µM) and lower DON (< 40 µM) relative to samples collected at other sites and dates (DIP

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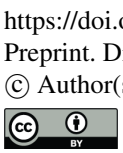

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< 0.5 µM; DON 40–60 µM), but did not reveal differences in community characteristics. Overall, NO_3^- was the dominant fraction of dissolved N in spring, whereas DON was the main fraction in summer and fall. Seasonal variation in DIP and DOP was small (< 2 µM) in comparison to N, such that changes in the relative availability of N vs. P were largely determined by N concentrations.

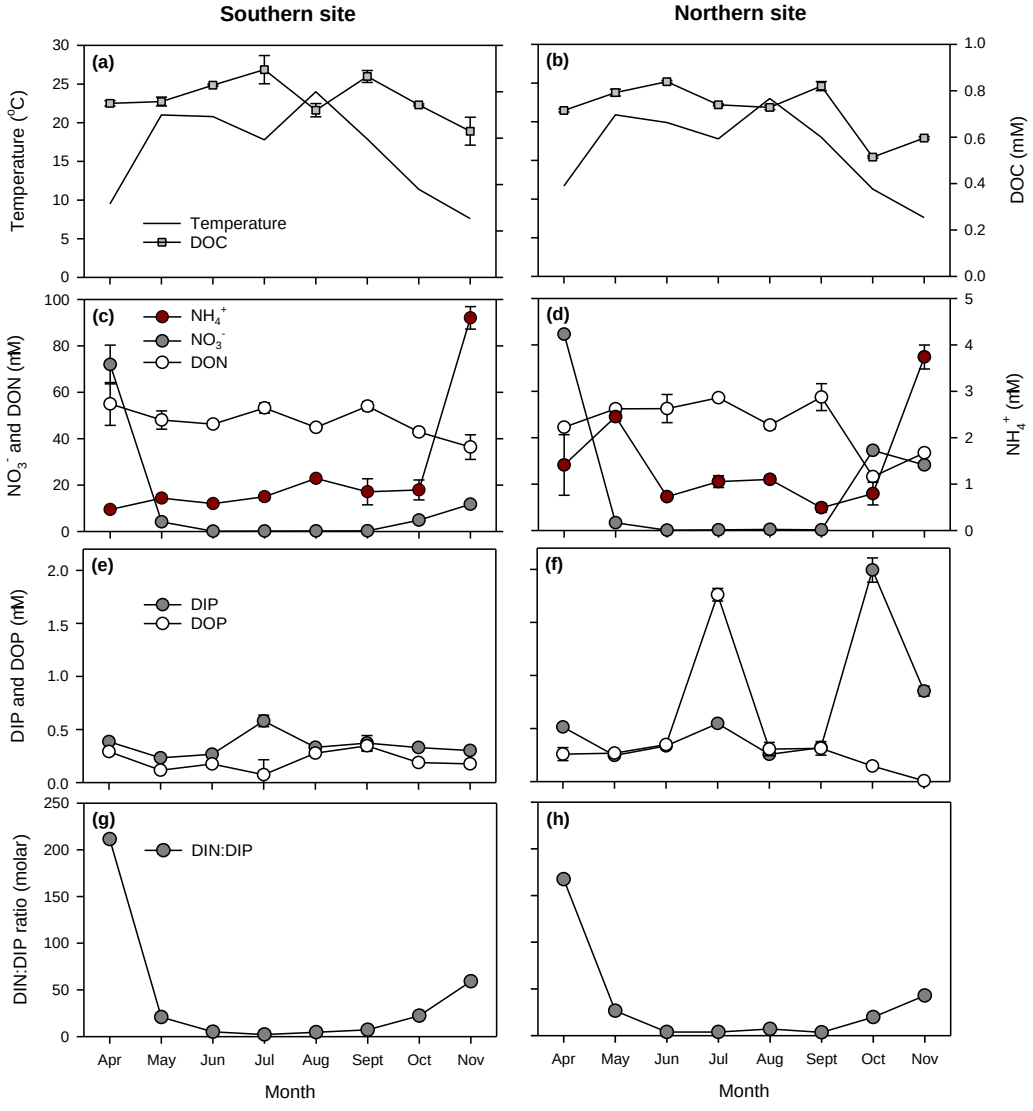

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Figure 4: Temporal patterns in temperature, dissolved organic carbon (a, b), dissolved and organic nitrogen (c, e), phosphorus (e, f), and DIN:DIP ratios (g, h) at southern (left panel) and northern (right panel) sites in the Curonian Lagoon during 2018 (error bars denote standard error; some not visible).

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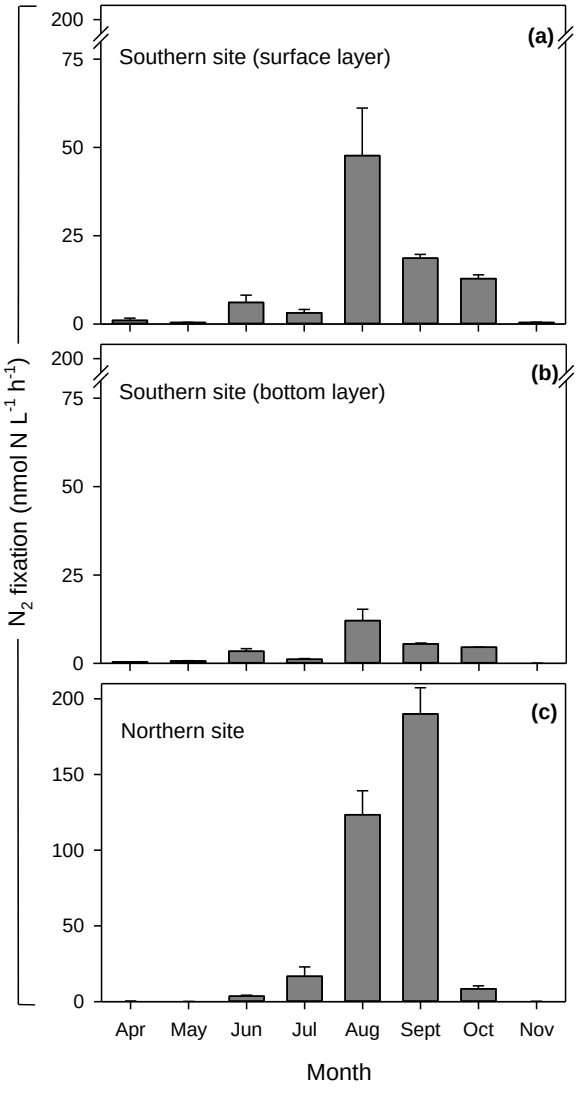

230 **Figure 5: Rates of N_2 fixation at northern (a) and southern (b, c) sites in the Curonian Lagoon in 2018 (data are mean values and standard error based on 3 replicates).**

3.2 N_2 fixation

Rates of N_2 fixation varied by over two orders of magnitude (< 1 to > 200 nmol N L^{-1} h^{-1}) with the highest rates measured at the northern site in August and September (123 ± 16 and 190 ± 17 nmol N L^{-1} h^{-1}, respectively; Fig. 5). A comparison of N_2
235 fixation in the surface and bottom layers at the southern site showed that rates were consistently lower (<15 nmol N L^{-1} h^{-1}) in the deeper layer. The abundance of heterocysts varied seasonally with lowest values less than 2000 cells L^{-1} and peak values exceeding 2 million cells L^{-1} in late summer (Fig. 6). Heterocysts of *Aph. flosaquae* accounted for > 95% of total heterocysts

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as contributions from *Dolichospermum* spp. were small by comparison (< 150,000 cells L^{-1}). Peak heterocyst densities were higher at the northern site (3,380,000 cells L^{-1}) relative to the southern site (2,877,000 cells L^{-1}). The N isotopic signature of PN declined with increases in heterocyst densities and rates of N_2 fixation (Fig. 6). Prior to the cyanobacteria bloom, $\delta^{15}N$ values were 6–9 ‰ and declined to less than 1 ‰ by August before rebounding in October–November. Smaller declines in $\delta^{15}N$ values were observed at the southern site.

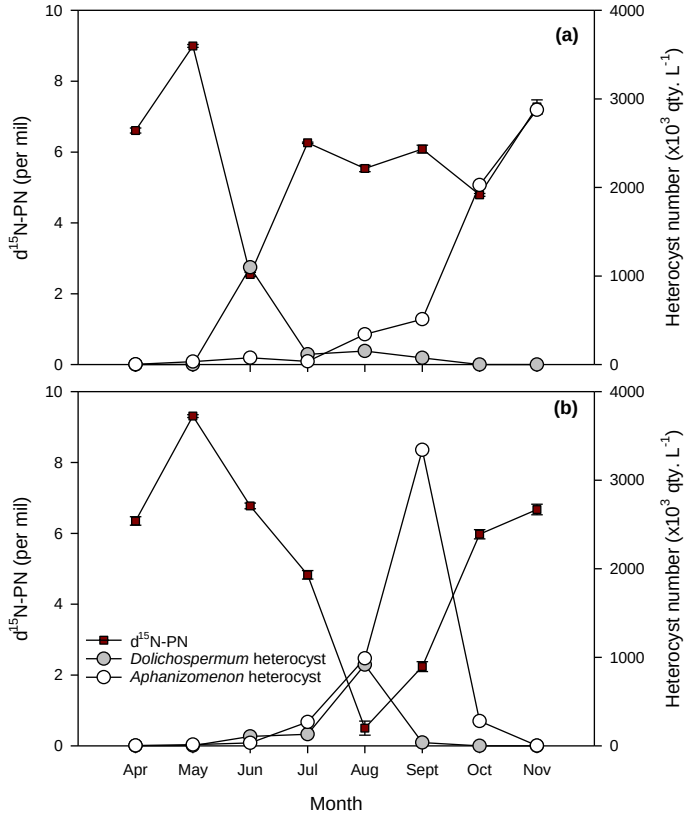

Figure 6: Abundance of heterocysts of *Dolichospermum* spp. and *Aphanizomenon flosaquae*, and stable isotope composition of particulate nitrogen ($\delta^{15}N$-PN) at southern (a) and northern sites (b) in the Curonian Lagoon during 2018. $\delta^{15}N$-PN values are mean and standard error (some error bars not visible).

N_2 fixation in the surface layer was significantly and positively correlated with Chl-a (R^2 = 0.91), *Aph. flosaquae* biomass (R^2 = 0.83) and *Aph. flosaquae* heterocysts (R^2 = 0.88 all $p < 0.001$; Fig. 7). N_2 fixation in the bottom layer was weakly correlated with Chl-a (R^2 = 0.52, p =0.07), but not *Aph. flosaquae* biomass or heterocysts. Chlorophyll-specific N_2 fixation was considerably lower in the deeper layer (0.102 ± 0.049 nmol N μg^{-1} Chl-a h^{-1}) relative to the surface layer (0.737 ± 0.076 nmol N μg^{-1} Chl-a h^{-1}) (Fig. 7).

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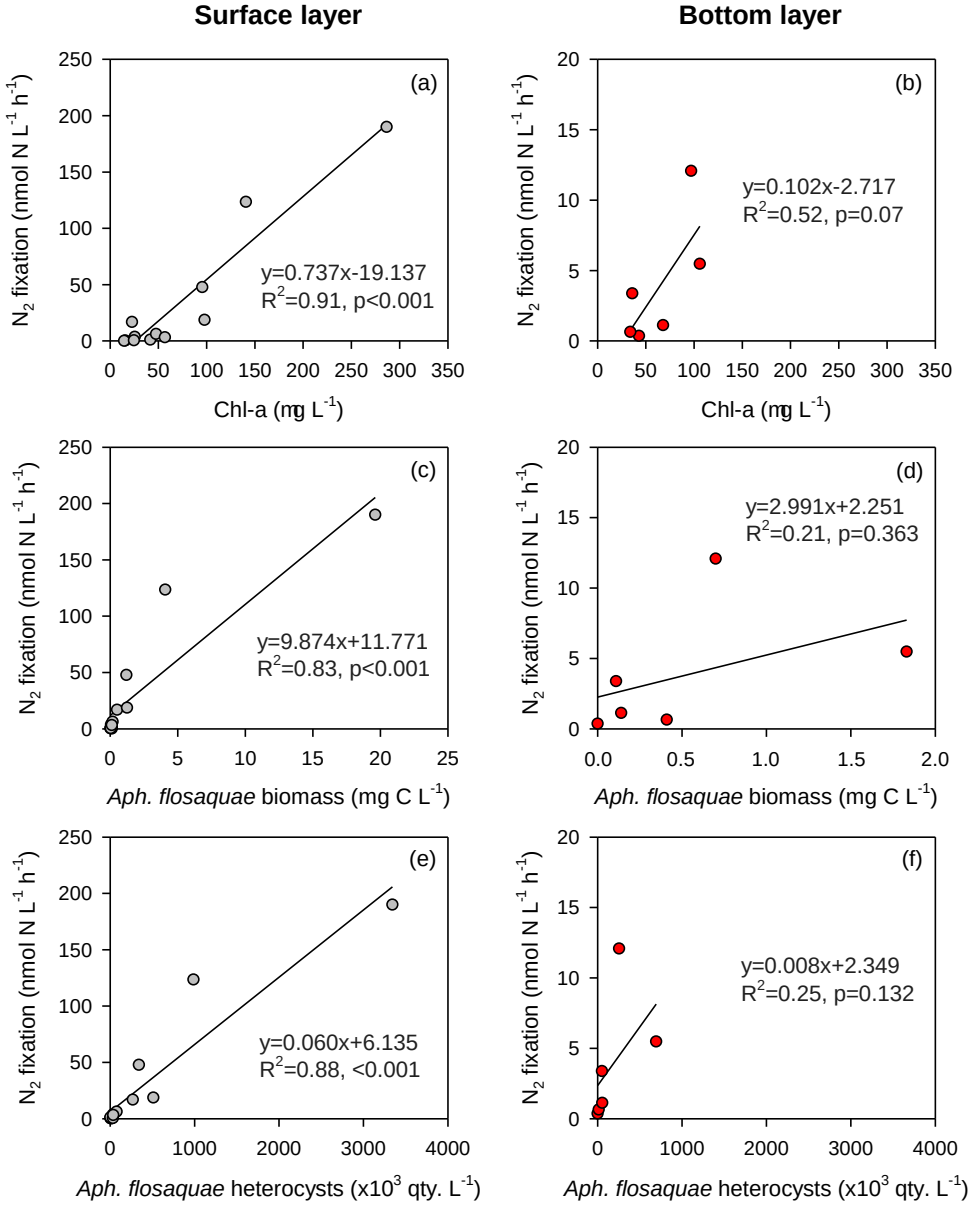

Figure 7: Relationships between N_2 fixation and Chlorophyll-a (a, b), *Aphanizomenon flosaquae* biomass (c, d) and their heterocysts (e, f) in surface (northern and southern sites) and bottom (southern site) layers of the Curonian Lagoon.

Remote sensing-based estimates of lagoon-wide Chl-a increased from 65.4 ± 0.9 µg L^{-1} (July 4) to 88.7 ± 0.2 µg L^{-1} (August 8), and thereafter remained relatively stable throughout August and September (means = 84 to 89 µg L^{-1}). Satellite-derived Chl-a values for each grid cell were used along with the regressions relating N_2 fixation to Chl-a (Fig. 7) to derive estimates

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of N_2 fixation for each cell. We used the relationship between N_2 fixation and Chl-a for the surface layer to derive estimates of N_2 fixation for the upper water column (0–2 m). For deeper areas, we used the relationship between N_2 fixation and Chl-a for the bottom layer. We assumed that surface Chl-a (from remote sensing) was representative of Chl-a in the deeper layer as we did not find significant differences between the two layers sampled at the southern site (SNK test, $p < 0.05$). The impact of the bloom on N_2 fixation can be visualized from the relatively low and uniform values throughout the lagoon during July, and the subsequent development of localized hotspots in the southern lagoon during August and September (Fig. 8). Lagoon-wide average values of N_2 fixation increased from 1559 (July 4), to 2467 (August 8) and thereafter ranged from 2324 to 2352 µmol m^{-2} d^{-1} through the end of September.

4 Discussion and Conclusion

We characterized seasonal variation in phytoplankton communities in relation to nutrient conditions to better understand the mechanisms regulating pelagic N_2 fixation in the Curonian Lagoon. Findings based on this study and our prior work (Zilius et al., 2018) suggest that the decline in riverine NO_3^- inputs following spring snowmelt, and the subsequent depletion of DIN in the lagoon, provides favourable conditions for an active diazotrophic community during summer and fall. Stoichiometric ratios of dissolved inorganic nutrients are frequently used to identify potential limiting elements and their role in driving community succession (Perez et al., 2011; Ptacnik et al., 2010). The occurrence of elevated NO_3^- concentrations and high DIN:DIP after spring runoff was followed by an extended period (8 months) of persistent low N availability, creating a temporal niche for heterocystous cyanobacteria (Supplement, Fig. S1). In a related study, we used molecular techniques to document the diversity of diazotrophs of the Curonian Lagoon and found that the community shifted from N_2-fixing heterotrophic bacteria in spring to photosynthetic heterocystous cyanobacteria in summer–fall (Zilius et al., 2020). Here, we show that spatial and temporal variation in rates of N_2 fixation were primarily determined by the abundance of heterocystous cyanobacteria. The maximum abundance of heterocysts occurred during the bloom of *Aph. flosaquae* and corresponded to the peak in N_2 fixation rates and the decline in δ^{15}N-PN to values similar to atmospheric N. Heterocyst formation is triggered by inorganic N depletion (e.g. Kumar et al., 2010) and in the Curonian Lagoon we observed the first peak in heterocyst abundance in early summer as DIN was depleted to < 1 µM. Our estimates of *Aph. flosaquae* abundance were an order of magnitude higher than those previously reported for temperate and boreal estuarine systems (Bentzon-Tilia et al., 2015; Klawonn et al., 2016; Olofsson et al., 2020a). The proliferation of heterocystous cyanobacteria in this system is favoured by P release from sediments, particularly when bloom conditions result in high water column respiration and transient (night-time) depletion of oxygen (Petkuviene et al., 2016; Zilius et al., 2014). These results support prior work showing that *Aph. flosaquae* is the principal contributor to N_2 fixation in the brackish Baltic Sea and its adjacent coastal areas (Klawonn et al., 2016; Olofsson et al., 2020a).

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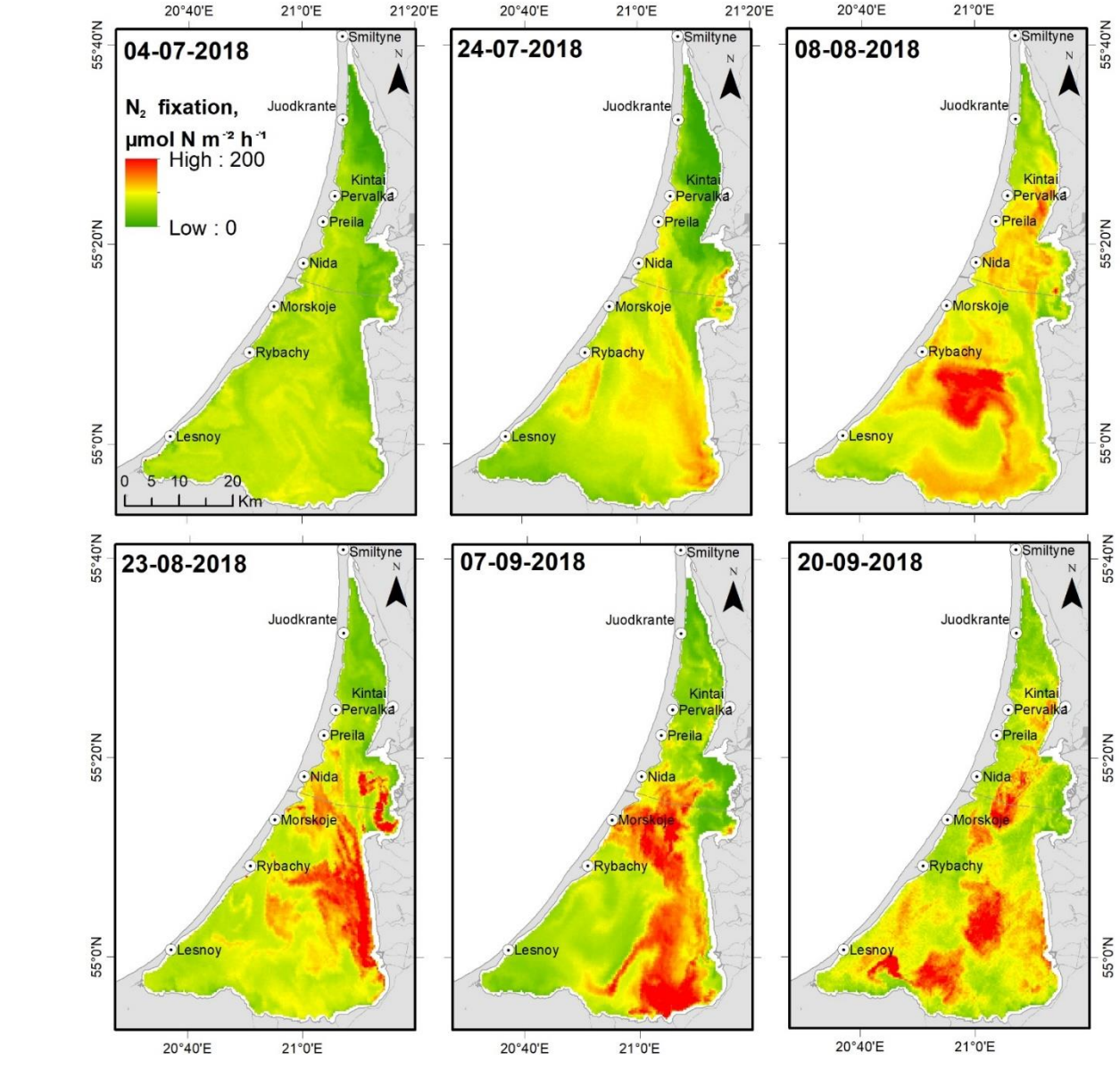

Figure 8: Estimates of pelagic N_2 fixation in the Curonian Lagoon derived from remote sensing of Chlorophyll-a.

The patchy distribution of cyanobacteria poses a significant challenge to reliably extrapolating results from site-specific measurements to the ecosystem scale (Zilius et al., 2014; 2018). Surface accumulation of positively-buoyant cyanobacteria and subsequent wind dispersion adds a dynamic component to biogeochemical processes in eutrophic lakes and estuarine systems (e.g. Gao et al., 2014; Klawonn et al., 2015; Zilius et al., 2014). Our previous work describing N fluxes in the Curonian Lagoon relied on a simple extrapolation of N_2 fixation rates measured at the two stations also used in this study. Here, we

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improve on our ability to scale up these measurements by using remote sensing of Chl-a to infer spatial and temporal variation in N_2 fixation. We benefitted from prior work deriving Chl-a estimates from satellite images and their calibration to *in situ* measurements (Bresciani et al., 2014), but the success of the approach largely relied on the fact that heterocystous cyanobacteria dominated the summer–fall phytoplankton community of the lagoon, which provided a significant correlation between N_2 fixation and Chl-a. The transferability of this approach to other systems would likely depend on this facet; in systems where heterocystous cyanobacteria account for a small and variable fraction of Chl-a, the utility of Chl-a as a predictor of N_2 fixation may be limited. Prior studies have used remote sensing to infer N_2 fixation, though by less direct means. For example, Hood et al. (2002) used SeaWiFS-derived estimates of *Trichodesmium* Chl-a, and modelled relationships between N_2 fixation and underwater irradiance to infer N_2 fixation in the tropical Atlantic Ocean. Coles et al. (2004) used remote sensing of Chl-a to estimate phytoplankton production in the North Atlantic and infer rates of N_2 fixation needed to support production. Other studies have related taxa-specific N_2 fixation to *in situ* measurements of Chl-a or algal biomass, including recent work in the Baltic Sea (Olofsson et al., 2020a). To our knowledge, ours is the first study to derive ecosystem-scale estimates by combining remote sensing of Chl-a with empirical models relating rates of N_2 fixation to Chl-a.

Our remote sensing-based estimates of N_2 fixation for the Curonian lagoon ranged from 1559 to 2467 μmol m^{-2} d^{-1} (mean = 2140 ± 147 μmol m^{-2} d^{-1}) during July–September. By comparison, *in situ* estimates scaled using our prior method (based on the proportion of lagoon area represented by the two stations) yielded estimates ranging from 360 to 3590 μmol m^{-2} d^{-1} (mean = 1930 ± 870 μmol m^{-2} d^{-1}) for corresponding dates. The two approaches yielded similar mean values, though with lower variability among those based on remote sensing. We attribute this to the shifting spatial distribution of cyanobacteria in the lagoon, which results in greater variability in site-specific measurements relative to the lagoon-scale assessments captured by remote sensing. These new estimates confirm our prior findings regarding the importance of N_2 fixation to the N balance of the lagoon. During periods of low river discharge, rates of N_2 fixation were twofold higher compared to monthly TN loads from the Nemunas River (June–October 2018 range = 830 to 1120 μmol m^{-2} d^{-1}) (Supplement, Fig. S1). N_2 fixation during summer and fall largely offset annual average denitrification (3190 μmol m^{-2} d^{-1}) and was equivalent to half of the measured sediment-water TDN exchange (3790 μmol m^{-2} d^{-1}; Zilius et al., 2018). Our prior work also showed enhanced PN export to the Baltic Sea during periods when the lagoon was dominated by cyanobacteria. Positive buoyancy allows *Aph. flosaquae* and other cyanobacteria to remain suspended in the water column, which favours export in lagoon outflow, rather than retention via sedimentation (Bukaveckas et al., 2019). Overall, these findings suggest that the occurrence of heterocystous cyanobacteria blooms has substantially diminished the potential for the lagoon to attenuate N fluxes to the Baltic Sea. As blooms of N_2-fixing cyanobacteria are common among the three large Baltic lagoons (Curonian, Oder, and Vistula), their effect in diminishing lagoon N retention may be regionally important (Bangel et al., 2004; Dmitrieva and Semenova, 2012). Our approach using remote sensing combined with local, empirical models relating N_2 fixation to Chl-a may provide a useful means for assessing the role of cyanobacteria blooms in the context of N budgets for the Baltic Sea (e.g. Savchuk, 2018).

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Our research has allowed us to better understand the environmental conditions that favour the occurrence of heterocystous cyanobacteria blooms and their contributions to the N budget of the lagoon. Important questions remain regarding the factors that regulate rates of N_2 fixation and the fate of atmospherically-derived N. Underwater irradiance is likely an important factor influencing biomass-specific N_2 fixation given its energetic costs. The Curonian Lagoon is a relatively turbid system in which the photic zone typically occupies less than 30% of the water column (Zilius et al., 2014). Our no-light incubations simulating the deeper layer of the southern lagoon showed that N_2 fixation was occurring, but at biomass-specific rates that were 7-fold lower in comparison to the surface layer. Low N_2 fixation rates during dark incubations were also observed in cyanobacteria filaments collected from other coastal sites of the Baltic Sea (Sveden et al., 2015). Though heterocystous cyanobacteria continue to fix N_2 in the dark, it remains unknown for how long due to the high energetic costs. Previous measurements of N_2 and carbon fixation in *Aphanizomenon* from the Baltic Sea suggest that respiration of stored cell products may provide energy for N_2 fixation under low light conditions (Sveden et al., 2015). Our study, as well as prior work, is based on 24-h incubations, simulating conditions at a fixed depth, which may not be indicative of rates that could be sustained by diazotrophs circulating over a range of depth and light conditions. Positive buoyancy and periodic mixing toward the surface may allow cyanobacteria to capture sufficient light energy to sustain N_2 fixation (Stal and Walsby, 2000). In addition to light availability, water temperature is likely an important constraint on seasonal patterns of N_2 fixation in temperate systems. Results from this, and a prior study (Zilius et al. 2018), show that despite a high abundance of *Aph. flosaquae* heterocysts at the end of fall, N_2 fixation rates declined substantially when water temperature dropped below 15 ºC. Zakrisson et al. (2014) suggested that temperature controls the enzymatic activity of nitrogenase, which directly regulates the intensity of N_2 fixation in filaments.

Recent work has shown that N fixed by diazotrophs is subsequently distributed to the planktonic food web (Adam et al., 2016), which likely involves a variety of mechanisms including grazing, leakage of DON and remineralization of N following algal senescence (Eglite et al., 2018). The relative importance of these pathways is not well known, though our data for the Curonian Lagoon suggests that heterotrophic bacteria, non-N fixing cyanobacteria and a diverse group of grazers benefit from the activities of heterocystous cyanobacteria. The biomass of heterotrophic bacteria increased during the bloom of heterocystous cyanobacteria to levels (250 µg C L^{-1}) that were appreciably higher than other coastal (Gulf of Finland and Archipelago Sea = 30–55 µg C L^{-1}) or open areas of the Baltic Sea (Bothnian Sea ~80 µg C L^{-1}; Baltic Proper = 16–44 µg C L^{-1}; Heinänen, 1991). It is likely that heterocystous cyanobacteria release dissolved organic matter which stimulates the growth of heterotrophic bacteria (Berg et al. 2018; Berner et al. 2018; Bertos-Fortis et al. 2016; Hoikkala et al. 2016). There is also evidence that non-N_2-fixing cyanobacteria benefitted from the bloom of heterocystous cyanobacteria as indicated by higher abundance of *Microcystis* spp. and *Planktotrix agardhii*. Measured low $\delta^{15}N$ values (0.5 ± 0.2 ‰) in suspended living material suggest that fixed N can temporally support most of the nutritional needs for plankton (bacteria + phytoplankton) growth. Lastly, our prior work using stable isotopes tracked atmospherically-derived N from cyanobacteria to a diverse group of consumers and suggested that 50–80% of secondary production was supported by cyanobacteria during bloom events (Lesutiene et al., 2014).

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In conclusion, our study contributes to a better understanding of the activity of coastal diazotrophs and their seasonal dynamics in eutrophic estuarine systems. The use of remote sensing allowed us to estimate N_2 fixation rates at the ecosystem scale and to show that these rates are high and relatively stable despite the dynamic and patchy distribution of cyanobacteria. The propensity for cyanobacteria to form dense, localized aggregates may influence the efficiency with which by-products of their carbon and N_2 fixation are disseminated by creating biogeochemical hotspots (Klawonn et al., 2015, 2019). Since intensifying blooms of cyanobacteria have already been observed in coastal areas of the Baltic Sea (Olofsson et al., 2020b), we may expect their stronger effect on ecosystem functioning in future. Therefore, further work combining remote sensing and *in situ* studies may provide greater insights as to the fate of atmospherically-derived N and its implications for ecosystem energetics.

Authors contribution

MZ, PAB and DV conceived the ideas and designed methodology. MZ, IVL, SB and TB led the field survey and experimental activities. IV-L, DV, DO, EV, IL, SBr, AA and AZ assisted with analysis and data collection and analysis. AZ assisted with statistical analyses. MZ and SB secured funding for the investigation. MV provided use of specialized facilities, MZ and PAB wrote the first draft of the paper, and all co-authors contributed to writing review and editing.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgment

We are in debt for the Coast Guard District of the State Border Guard Service for logistic support. We thank Jolita Petkuviene and Adele Mačiūtė for assistance in field sampling and laboratory analysis.

Financial support

This study has been supported by LMT grant “The role of atmospheric nitrogen fixation in the largest eutrophicated European lagoon (NitFix)” (Agreement No. P-MIP-17-126).

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