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Shifts in phytoplankton community structure modify bacterial production, abundance and community composition

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ABSTRACT: In recent decades, the phytoplankton community in parts of the Baltic Sea has shifted from diatom dominance to co-occurrence of diatoms and dinoflagellates during the spring bloom. We investigated whether this shift affects bacterial production (BP), abundance and community composition (BCC). Two mesocosm experiments were carried out with water from the SW coast of Finland during the winters of 2012 and 2013. The water was collected before the onset of the spring bloom. Natural seawater was used as a control, and various inocula of diatom and dinoflagellate cultures were used as treatments. After the phytoplankton bloom development, BP (thymidine: BPT; leucine: BPL) was significantly higher in the diatom treatments than in the controls and dinoflagellate treatments (BPT and BPL in 2012 and BPL in 2013). In 2013, the BCC was significantly different between the diatom and dinoflagellate treatments and there was a temporal shift in both experiments. *Alphaproteobacteria* predominated in all treatments at the beginning of the experiments and shifted to flavobacterial (2012) and betaproteobacterial predominance (2013) during the chlorophyll *a* peak. Towards the end of the experiment, *Actinobacteria* and *Betaproteobacteria* predominated in the diatom treatment in 2012, whereas in 2013 *Flavobacteriia* (all treatments) predominated together with *Gammaproteobacteria* and *Cytophagia* (diatom treatments). The results demonstrated that bacterial physiology and community structure are affected by relatively small changes in the phytoplankton community. Thus, the ongoing changes in the phytoplankton community resulting from co-occurrence of diatoms and dinoflagellates may decrease pelagic remineralization of carbon and reduce organic matter fluxes through the microbial loop.

KEY WORDS: Diatom · Dinoflagellate · Bacterial production · Bacterial community · Biogeochemical cycles · Microbial loop · Baltic Sea

INTRODUCTION

The spring bloom is the most important phase of the year in terms of primary production (PP) and carbon fixation in temperate waters (Harris 1986). In contrast to most other temperate coastal areas, where diatoms are typically the dominant group during this period, the phytoplankton spring bloom in several parts of the Baltic Sea is dominated by both diatoms and dinoflagellates, but with spatial and temporal differences in their relative contribution to the biomass (Wasmund et al. 1998, Klais et al. 2011, Lips et al. 2014).

Among the dinoflagellates, at least 4 cold-water species may predominate during the spring bloom

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period in the Baltic Sea (*Peridiniella catenata, Biecheleria baltica, Gymnodinium corollarium*, and *Apocalathium malmogiense*). Furthermore, long-term datasets have demonstrated an increase in the abundance of these dinoflagellate species during the spring bloom, most conspicuously in the Gulf of Finland (Klais et al. 2011). In a climate change scenario with mild winters (decrease in ice expansion and thickness, earlier stratification, etc.), dinoflagellate abundance may increase at the expense of diatoms (Wasmund et al. 2011, 2013, Klais et al. 2013). The potential consequences of such changes in ecosystem functioning are still poorly understood.

Diatoms and dinoflagellates have different sedimentation patterns (Heiskanen 1998). Diatoms settle more rapidly than dinoflagellates and transport more organic material with higher carbon:nitrogen:phosphorus (C:N:P) ratios to the seafloor (Arrigo et al. 2012, Spilling et al. 2014), whereas dinoflagellates either lyse before reaching the sediment (Heiskanen 1998) or settle as dormant resting cysts not readily available for remineralization (Spilling & Lindström 2008). Therefore, diatoms are likely more important than dinoflagellates in terms of input of organic matter to the benthic food web (Heiskanen 1998, Höglander et al. 2004).

Phytoplankton also shape the biochemistry (e.g. the inorganic C:N:P ratio) of the seawater by the uptake of inorganic nutrients, carbon fixation, and release of dissolved organic matter (DOM). For example, the quantity and/or quality of the DOM released by phytoplankton cells during their growth may differ, depending on species-specific properties and the physiological status of the algae (Biddanda & Benner 1997, Meon & Kirchman 2001, Thornton 2014). Some diatoms release large proportions (>20%) of fixed carbon as dissolved organic carbon (DOC), but this is highly species-specific (Wetz & Wheeler 2007). Some dinoflagellates show higher percentages of extracellular release than diatoms (Castillo et al. 2010, López-Sandoval et al. 2013). However, during the spring bloom in the Baltic Sea, diatom-dominated communities excrete more DOC than mixed or dinoflagellate-dominated communities (Spilling et al. 2014).

In addition to the release of DOM by phytoplankton cells, other processes, such as viral lysis, sloppy feeding, and cell death, may also contribute to the production of the DOM pool. Hence, this pool is highly diverse, consisting of labile and refractory parts, which can also be divided into low- (LMW) and high-molecular-weight (HMW) compounds (Amon & Benner 1996). For example, Thornton (2014) classified the cellular DOM released, based on its chemical composition, as amino acids, carbohydrates, fatty acids and lipids, nucleic acids, glycolate, dimethylsulfoniopropionate, dimethyl sulfide, and isoprene. Of these, carbohydrates (mono- and polysaccharides) are most commonly released by the algae (Myklestad 1995, Urbani et al. 2005, Thornton 2014) and more specifically by diatoms, as well as amino acids and proteins (Myklestad 2000). Fatty acids and lipids are excreted by both diatoms and dinoflagellates (Parrish et al. 1994). The labile part of DOC is either respired or transformed into biomass and rapidly channeled to higher trophic levels through the microbial loop (Azam et al. 1983, Smith et al. 1995). Another part of the carbon biomass transformed through microbial metabolism is released and contributes to the refractory DOC pool, which can resist bacterial degradation (Jeong et al. 2010).

The proportion of carbon released or leaked as DOC under stressful conditions (e.g. high temperature and nutrient limitation) may increase as a consequence of climate warming (Thornton 2014). This would have a bottom-up effect on ecosystem functioning by increasing the amount of organic carbon going through the microbial loop. However, since DOC release is highly species-specific, further empirical studies are needed at the phytoplankton and bacterioplankton community levels to improve our understanding of the effect of plankton community structure on carbon cycling through the microbial loop.

The close association between primary producers and heterotrophic bacteria, defined as bacteria–phytoplankton coupling, has been observed for decades (Cole et al. 1988, Baines & Pace 1991, Morán et al. 2002). Phytoplankton cells produce a successive availability of various algal-derived products as DOM, which provides a series of ecological niches for bacteria and leads to changes in bacterial growth rate and community composition (Biddanda & Benner 1997, Riemann et al. 2000, Buchan et al. 2014). For example, *Alphaproteobacteria* are typically abundant in the phytoplankton pre-bloom phase, whereas *Bacteroidetes* and *Gammaproteobacteria* are abundant during and/or after the bloom (Cottrell & Kirchman 2000, Pinhassi et al. 2004, Teeling et al. 2012). In addition to these predominant groups, *Actinobacteria* and *Betaproteobacteria* are also common members of the bacterial community in the brackish Baltic Sea during or after phytoplankton blooms (Riemann et al. 2008, Herlemann et al. 2011, Bunse et al. 2016). A recent study using metagenome-assembled genomes linked these phylogenetic lineages with

Table 1. Inorganic nutrient concentrations of nitrite + nitrate nitrogen (NO_2+NO_3-N), ammonium nitrogen (NH_4-N), phosphate phosphorus (PO_4-P), and dissolved silica (DSi) in µg l^{-1} (mean ± SE, n = 3) at the start of the experiment (Day 0) and on the day of the chlorophyll *a* (chl *a*) peak in 2012 (Day 10 for DIATOM and DINOF treatments, Day 19 for CONTR1 treatment) and 2013 (Day 15). CONTR: control, DIATOM: diatom addition, DINOF: dinoflagellate addition (see 'Materials and methods' for species details); AT: *Achnanthes taeniata*, TB: *Thalassiosira baltica*, BB: *Biecheleria baltica* additions

	2012			2013			
	CONTR1	DIATOM	DINOF	CONTR2	AT	TB	BB
Day 0							
NO_2+NO_3-N	97.47 ± 1.68	108.57 ± 1.33	98.53 ± 0.92	80.20 ± 3.29	91.80 ± 4.10	89.03 ± 2.54	86.40 ± 1.28
NH_4-N	1.93 ± 0.26	1.80 ± 0.38	1.77 ± 0.07	0.03 ± 0.00	0.10 ± 0.00	0.00 ± 0.00	0.13 ± 0.00
PO_4-P	31.21 ± 0.09	32.07 ± 0.09	30.33 ± 0.17	27.40 ± 0.43	27.23 ± 0.60	27.83 ± 0.77	27.50 ± 0.42
DSi	623.83 ± 0.33	628.0 ± 4.59	620.70 ± 3.06	500.20 ± 0.79	506.23 ± 1.80	505.70 ± 4.42	500.03 ± 2.48
Day chl *a* peak							
NO_2+NO_3-N	0.97 ± 0.20	1.10 ± 0.0	2.15 ± 2.92	1.70 ± 0.10	2.17 ± 0.21	1.97 ± 0.03	2.03 ± 0.03
NH_4-N	1.70 ± 0.32	2.06 ± 0.32	0.63 ± 0.16	1.33 ± 0.27	0.63 ± 0.34	0.83 ± 0.49	0.53 ± 0.39
PO_4-P	0.99 ± 0.13	2.89 ± 0.34	1.74 ± 0.35	2.23 ± 0.48	1.53 ± 0.21	1.80 ± 0.17	2.03 ± 0.52
DSi	17.7 ± 1.50	162.87 ± 37.87	553.90 ± 10.89	62.67 ± 23.89	102.20 ± 5.80	103.27 ± 21.04	171.93 ± 10.73

functionality, based on the proportion of various genes involved in metabolic processes (Hugerth et al. 2015).

Environmental factors such as salinity, seasonality, temperature, and inorganic nutrients can shape the structure of the bacterial community composition (BCC) (Lindh et al. 2015, Herlemann et al. 2016, Kirchman et al. 2017). Among them, temperature and light are important environmental factors during the development of the spring bloom (Andersson et al. 1994) that can also affect bacterial activity and, thus, the bacteria–phytoplankton coupling (Lindh et al. 2013, von Scheibner et al. 2014, Landa et al. 2016). For example, an increase in temperature from the *in situ* temperature (2.5°C) to 6–8°C boosted bacterial production (BP) and decreased the time lag between PP and secondary production peaks in several experiments (Hoppe et al. 2008, von Scheibner et al. 2014).

The aim of this study was to determine the effect of the phytoplankton community structure (diatoms vs. dinoflagellates) on the BCC, bacterial abundance (BA), and BP in the Baltic Sea. For this purpose, 2 mesocosm experiments were performed in consecutive years (winters of 2012 and 2013), using seawater collected from the ice edge on the SW coast of Finland. Small inocula of various cultured diatoms and dinoflagellates were used to shift the natural phytoplankton community in an attempt to simulate the spring bloom conditions.

MATERIALS AND METHODS

Experimental setup

Two mesocosm experiments were carried out at Tvärminne Zoological Station, University of Helsinki: one starting in early March 2012 and the other in late February 2013. The water was collected with a 5 l Niskin bottle from the ice edge at Storfjärden (59° 50′ N, 23° 15′ E) at a depth of 20 m, to avoid any influence of fresh water from a nearby river. At the time of water collection, the phytoplankton spring bloom had not yet developed, and the water showed high concentrations of inorganic nutrients (Table 1) and low phytoplankton biomass (Table 2). The water

Table 2. Total carbon biomass (phyto-microzooplankton and nanoflagellates, mg C l^{-1}, mean ± SE, n = 3) at the start of the experiment (Day 0) and on the day of the chlorophyll *a* (chl *a*) peak and diatom:dinoflagellate index (Dia:Dino index) during the phytoplankton bloom phase in 2012 and 2013. On Day 0, the values of the total carbon biomass are from samples collected before (CONTR1 and CONTR2 for 2012 and 2013, respectively) and after addition of the various phytoplankton inocula; see Table 1 for treatment details

	2012			2013			
	CONTR1	DIATOM	DINOF	CONTR2	AT	TB	BB
Day 0	2.69 ± 1.11	23.59 ± 2.47	72.62 ± 50.75	1.35 ± 0.61	2.40 ± 0.28	3.22 ± 0.39	4.44 ± 3.89
Day chl *a* peak	649.23 ± 123.16	1894.94 ± 1111.93	698.56 ± 96.84	370.96 ± 17.35	430.39 ± 18.20	361.76 ± 29.12	562.86 ± 27.40
Dia:Dino index	0.96	0.99	0.15	0.94	1.00	0.98	0.69

was filtered through a 200 µm mesh to exclude large particles and mesozooplankton before filling the experimental containers (20 l square, transparent, polycarbonate containers). The containers were covered with black plastic bags to avoid photoinhibition of the sampled organisms until placed in a climate-controlled room at 4°C and a 12:12 h light:dark cycle. The irradiance was 70 µmol photons m^{-2} s^{-1} measured at the surface of the containers with a cosine collector connected to a Walz ULM 500 light meter. Filtered (0.2 µm) air was gently bubbled through the containers to avoid particle settling and to provide CO_2. No inorganic nutrients were added.

The experimental setup comprised 3 different treatments in 2012 and 4 treatments in 2013, achieved by inoculating the seawater collected with various monocultures of diatoms and dinoflagellates. Each treatment consisted of 3 replicates. In 2012, the treatments were: natural community = no addition (CONTR1), diatom addition (DIATOM), and dinoflagellate addition (DINOF). The phytoplankton species added were a community consisting of *Thalassiosira baltica* and *Chaetoceros wighamii* in the DIATOM treatment and a mix of *Apocalathium malmogiense*, *Gymnodinium corollarium*, and *Biecheleria baltica*, constituting a dinoflagellate complex, and *Peridiniella catenata* in the DINOF treatment. The aim of the cultured community addition was to change the phytoplankton community with minimal increase in the initial biomass. The biomass addition, measured as chlorophyll *a* (chl *a*), was 0.7 and 0.4 µg l^{-1} in the DIATOM and DINOF treatments, respectively. These additions represented 1.4 and 0.8% of the chl *a* maximum in the DIATOM and DINOF treatments, respectively, and <0.2% of the total volume at the time of the addition.

In 2013, the containers were inoculated with monocultures: natural community = no addition (CONTR2); *Achnanthes taeniata* (AT) and *T. baltica* (TB) as the treatments with DIATOM addition; and *B. baltica* (BB) as the treatment with DINOF addition. The inoculum was 0.2–0.3 µg l^{-1} chl *a* in the AT, TB, and BB treatments and represented 0.06% of the chl *a* maximum and <0.05% of the total volume at the time of the addition. All species added are common spring bloom species in the Baltic Sea, and originated from a nonaxenic culture collection or started by isolation of single cells. The cultures were provided by the Marine Research Center, Finnish Environment Institute (SYKE), Finland.

The containers were mixed by stirring prior to each sampling, and subsamples were collected by gentle suction into pre-cleaned bottles. Sampling was carried out 1 to 3 times wk^{-1}, depending on the measurement and the growth phase in the experimental containers. After the inorganic nutrients were depleted in the containers, the temperature was increased to 10°C. At this point (Day 19 in 2012, Day 22 in 2013), part of the biomass (~20–30% of the particulate organic carbon [POC]) was removed by allowing particulate material to settle for 1 h, and 1 l of the settled material was collected by gentle suction through a glass tube. In 2012, an additional removal (~55% of the POC) was done on Day 27. The rationale behind the temperature increase and removal of settled material was to simulate the spring natural environmental conditions with warming of the surface water and settling of POC following the phytoplankton spring bloom. The experiment lasted in total for 41 d in 2012 and 32 d in 2013.

Inorganic, particulate, and dissolved organic nutrients and chl *a*

The inorganic nutrients nitrite + nitrate nitrogen (NO_2+NO_3-N), ammonium nitrogen (NH_4-N), phosphate phosphorus (PO_4-P), and dissolved silica (DSi) were measured according to standard colorimetric methods (Grasshoff et al. 1983). To determine the concentration of POC, particulate organic nitrogen (PON), and particulate organic phosphorus (POP), samples (100 ml) were filtered onto acid-washed, pre-combusted (450°C, 4 h) GF/F filters. The dried POC/PON filters were stored at room temperature until measurement with an ANCA-MS 20-20 mass spectrometer (Europa Scientific). The POP was determined according to Solorzano & Sharp (1980). Samples for biogenic silicate (BSi) were filtered onto 0.8 µm polycarbonate filters and measured using the method of Krausse et al. (1983). The DOC and dissolved organic nitrogen (DON) concentrations were measured from 0.2 µm filtered samples by the high-temperature catalytic oxidation method, using a Shimadzu TOC-V CPH carbon and nitrogen analyzer (Benner et al. 1993). The DON was measured only in 2013. To determine chl *a* concentrations, subsamples (25–100 ml) were filtered onto GF/F filters and the chl *a* was extracted in 10 ml of 96% ethanol (Jespersen & Christoffersen 1987). The samples were stored in darkness at –20°C and placed at room temperature for 24 h before measurement with a Varian Eclipse fluorescent spectrophotometer calibrated against chl *a* standards (Sigma-Aldrich). All filtrations were done in duplicate.

Phytoplankton and microzooplankton community composition and biomass

Samples (100 ml) for the analysis of nano- and microplankton composition were fixed with acid Lugol's solution. At least 400 cells were counted according to Utermöhl (1958), using a Leitz Diavert inverted light microscope. The biovolume and carbon biomass were estimated from microscopy counts, according to Olenina et al. (2006) and Menden-Deuer & Lessard (2000) for phytoplankton, and Olenina et al. (2006), auf dem Venne (1994), and Putt & Stoecker (1989) for ciliates. The cysts of dinoflagellate origin were counted from the settled material that was removed and were identified according to Kremp & Anderson (2000) and Kremp et al. (2005). A modified diatom:dinoflagellate (Dia:Dino) index (using carbon biomass rather than biovolume) was calculated for the phytoplankton bloom phase according to Wasmund et al. (2017). This Dia:Dino index indicates the ratio of diatom to dinoflagellate biomass.

Primary production

PP was determined by measuring the incorporation of ^{14}C-labeled sodium bicarbonate (Nielsen 1952, Gargas 1975). Three 4 ml samples were placed in glass vials and incubated with additions of 10 µl of Na $^{14}CO_3$ (0.2 µCi final activity) for 2 h in the climate room (4 or 10°C). Two samples per treatment were exposed to 70 µmol photons m^{-2} s^{-1}, and one was kept in darkness. After the incubation, 100 µl of 1 M HCl were added to the samples and left under a fume hood overnight. The following day (24 h), 7 ml of liquid scintillation cocktail were added, and the activity was measured in a liquid Wallac Winspectral 1414 scintillation counter. The amount of total dissolved inorganic carbon (DIC) was measured with a Unicarbo high-temperature combustion infrared gas analyzer (Electro-Dynamo Oy). The dark incorporation of ^{14}C was subtracted from the values obtained from the light incubations, and the PP was calculated from the uptake of $Na^{14}CO_3$, knowing the concentrations of the isotope and DIC added. Due to the relatively short incubation period, the PP measured was assumed to represent gross PP (Sakshaug et al. 1997).

Bacterial production

BP was measured by determining the simultaneous incorporation of ^{3}H-thymidine (TdR) for DNA synthesis (BPT) and ^{14}C-leucine (Leu) for protein syntheis (BPL). Three subsamples of 10 or 1 ml (in 2012 and 2013, respectively) were taken from each container. One of the subsamples was fixed with formaldehyde (final concentration 1.85%) and served as a blank of non-biological adsorption of the radioisotopes. All samples were spiked with [methyl-^{3}H]-thymidine and [^{14}C (U)]-leucine (PerkinElmer) at final concentrations of 14–20 and 100–166 nM, respectively. The concentrations were saturating in this region in winter–early spring. The samples were incubated at the experimental temperature (4 or 10°C) for 2 h in darkness. The incubations were stopped by adding formaldehyde to a final concentration of 1.85%. The unincorporated radioactive substrates were removed, using the standard cold trichloroacetic acid (TCA) extraction method (Fuhrman & Azam 1982). In 2012, the samples (10 ml) were filtered onto 0.2 µm mixed cellulose ester filters (Advantec), whereas in 2013 the samples (1 ml) were centrifuged (Smith & Azam 1992). The filters/pellets were dissolved in Insta-Gel Plus scintillation cocktail (PerkinElmer), and the radioactivity was determined with a Wallac WinSpectral 1414 counter. The TdR incorporation was converted to carbon production (µmol C l^{-1} h^{-1}), using a cell conversion factor of 1.4 × 10^{9} cells $nmol^{-1}$ (HELCOM 2008) and a carbon conversion factor of 0.12 pg C × (0.06 μm^3 $cell^{-1}$)$^{0.7}$ (Norland 1993). The ^{14}C-Leu incorporation was converted to carbon production, using the Leu:protein ratio of 0.073 and carbon:protein ratio of 0.86 (Simon & Azam 1989).

Bacterial abundance

BA was determined by flow cytometry according to Gasol & del Giorgio (2000). Sample volumes of 1.2–1.5 ml were fixed with paraformaldehyde (final concentration 1%) for 10–15 min in darkness and stored at −80°C until further processing. Flow cytometry counts were done, staining the cells with SYBR Green I (Sigma-Aldrich). In 2012, an LSRII flow cytometer (BD Biosciences) was used. A known amount of Count bright counting beads (Thermo Fisher Scientific) was added to the sample (~10% of the bacterial counts) to estimate the volume measured. The sample was run for 90 s to obtain ~20 000 events, and the gating of bacterial populations was done using FACS Diva software (BD Biosciences). In 2013, the counts were achieved with a Partec-CUBE flow cytometer equipped with an autosampler, using a counting volume of 25 µl and a flow rate of

0.5 µl s^{-1}. Prior to counting, the samples were diluted 1:10 in 1× Tris-ethylenediaminetetraacetic acid (TE) buffer, pH 8 (AppliChem). Five runs of each sample were counted to cover the potential internal variation of the flow cytometer. For this set of samples, various suspensions of beads with standard concentrations (Partec) were counted daily before and after the counts for quality control. Gating of bacterial populations was done using the Flow Cytometry Standard Express 4 Flow Research Edition software (DeNovo Software).

Bacterial community composition

The BCC samples were collected on Day 0 (start), Day 19 (chl *a* peak), and Day 41 (end) of the experiment in 2012 and on Day 0 (start), Day 13 (chl *a* peak), and Day 27 (BP peak) of the experiment in 2013. In 2012, the aliquots taken from the 3 replicate containers were pooled by treatment, whereas in 2013 the bacterial community in each container was analyzed separately. Water samples (500 ml) were filtered onto 0.2 µm pore-size Whatman cellulose ester filters, which were then stored at −80°C for further processing. DNA extraction was carried out using a Power Soil DNA isolation kit (Mo Bio Laboratories). The 16S ribosomal RNA (rRNA) gene region from V1 to V3 was amplified with a polymerase chain reaction (PCR), using the universal bacterial primers F8 (Chung et al. 2004) and R492 (Edwards et al. 1989). A 2-step PCR and Illumina MiSeq paired-end multiplex sequencing were performed at the Institute of Biotechnology, University of Helsinki, Finland.

Approximately 13 million raw reads of the 16S rRNA gene were obtained. Primer removal was done with Cutadapt (Martin 2011). The paired-end reads were merged with a paired-end read merger (Zhang et al. 2014). Quality filtering (>400 bp, maximum expected error 1), chimera checking (Edgar et al. 2011), and operational taxonomic unit (OTU) clustering (Edgar 2013) were done according to the UPARSE pipeline (Edgar 2013). In total, 1.8 million sequences passed the quality filtering. Taxonomic classification of the OTUs was done with Silva (Quast et al. 2013) in Mothur (Schloss et al. 2009). Chloroplasts, mitochondria, and singletons were removed with Silva, based on the phylogenetic classification, and the libraries were normalized with metagenomeSeq (Paulson et al. 2013), using R (R Development Core Team 2011). In total, 1720 OTUs including 1 million sequences were obtained for further analyses.

Statistics

Differences between the treatments were tested for BPT, BPL, BA, and DOC in both years. In 2013, tests were also run for DON and BCC. The BPT, BPL, BA, DOC, and DON were analyzed by 1-way analysis of variance (ANOVA), using regression coefficients (n = 3) obtained from linear regression analysis run on cumulative sums of each container as the dependent variable and treatment as the factor. The regression analysis and the subsequent ANOVA were run separately for the phytoplankton bloom phase (4°C) and bacterial bloom phase (10°C) in each year. The homogeneity of variances was tested with Levene's test, and in case of unequal variances, the data were transformed or ranked. For post hoc testing, Tukey's *b*-test was used. All statistical analyses were done using IBM SPSS 23 software.

A nonmetric multidimensional scaling (NMDS) plot was constructed to visualize the bacterial community dynamics. To determine whether the BCC differed significantly between treatments in 2013 (2012 omitted due to pooling of replicates), repeated measures permutational ANOVA (PERMANOVA) (fixed-factor treatment; AT: n = 9, TB: n = 9, BB: n = 9, CONTR2: n = 9 and time: Day 0, chl *a* peak, and BP peak) with pairwise comparisons (Anderson 2001, McArdle & Anderson 2001) was done. A total of 9999 permutations, using unrestricted permutation of raw data (Manly 2006), were performed, which is recommended when sample sizes are small (Anderson et al. 2008). The homogeneity of dispersion (i.e. homogeneity of variance) was tested with permutational multivariate analysis of dispersion (Anderson 2006), using the distance to the centroids. All multivariate analyses and NMDS plots were performed on the Bray-Curtis dissimilarity matrix derived from square-root-transformed normalized values. For the multivariate analyses, PRIMER v. 6 software (Clarke & Gorley 2006) with the add-on package permutational ANOVA/multivariate ANOVA+ (MANOVA+) (PERMANOVA+) (Anderson et al. 2008) was used.

RESULTS

The experiments were separated into 2 phases, based on the inorganic nutrient depletion and temperature setup: (1) the phytoplankton bloom phase at 4°C with rapid increase in autotrophic biomass (from Day 0 to Day 19) and (2) the bacterial bloom phase at 10°C with rapid increase in BP (from Day 19 to Day 41 in 2012 and to Day 32 in 2013).

Inorganic nutrients and chl *a* development

The concentrations of the inorganic nutrients (NO_2 +NO_3-N, NH_4-N, PO_4-P, and DSi) were high at the beginning of the experiment in both years (Table 1). There was a drawdown of these nutrients concomitant with the increase in phytoplankton growth. At the chl *a* peak, NO_2+NO_3-N and PO_4-P were almost depleted (Table 1). The NH_4-N concentration was low or below the detection limit in both years and increased slightly after the chl *a* peak in 2013. The DSi concentration decreased, particularly in the treatments with diatom addition, but was never completely depleted. In 2012, chl *a* peaked on Day 10 in 1 replicate of the DIATOM treatment and in all replicates of the DINOF treatment. In the remaining replicates of the DIATOM and CONTR1 treatments, chl *a* peaked on Day 19 (Fig. 1A). The difference among the DIATOM replicates resulted in larger standard errors (SEs) on Days 10 and 19. Hence, the days selected to define the parameters NO_2+NO_3-N, NH_4-N, PO_4-P, DSi, total carbon biomass, and Dia:Dino index on the day of the chl *a* peak were set on Day 10 in the DIATOM and DINOF treatments and on Day 19 in the CONTR1 treatment. The maximum (±SE) concentrations of chl *a* in the DIATOM and CONTR1 treatments were similar (20.60 ± 6.12 and 26.03 ± 1.59 µg l^{-1}, respectively), whereas in the DINOF treatment, the chl *a* peak was markedly lower (9.48 ± 0.48 µg l^{-1}). In 2013, the chl *a* peaks were considerably higher than in 2012 (Fig. 1B). The highest chl *a* value was observed in the BB treatment (54 ± 0.40 µg l^{-1}), whereas in the remaining treatments (CONTR2, AT, TB) the peaks were similarly lower (43 ± 0.80 µg l^{-1}). The chl *a* peak was reached on Day 13 in the AT treatment and on Day 15 in the other treatments. In 2013, Day 15 was selected to define the values of inorganic nutrient concentrations, total carbon biomass, and Dia:Dino index at the chl *a* peak.

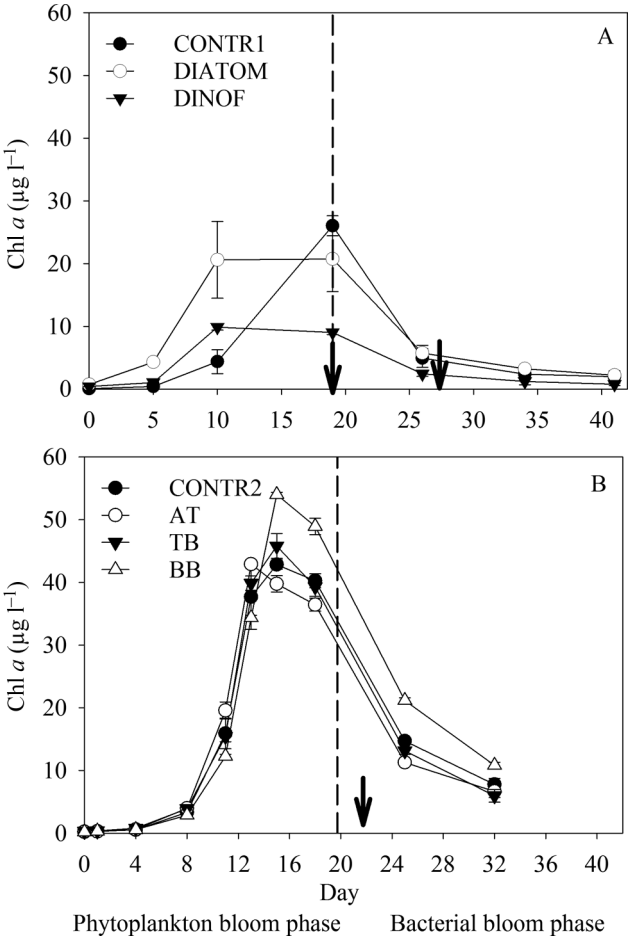

Fig. 1. Chlorophyll *a* concentration (mean ± SE, n = 3) during the experiments in (A) 2012 and (B) 2013. CONTR1 and CONTR2 (controls for 2012 and 2013, respectively) consisted of no addition to the natural plankton community; DIATOM and DINOF treatments consisted of initial addition of diatom and dinoflagellate communities (see 'Materials and methods' for species details); and the AT, TB, and BB treatments consisted of initial additions of *Achnanthes taeniata*, *Thalassiosira baltica*, and *Biecheleria baltica*, respectively. Dashed lines indicate the shift in temperature from 4 to 10°C and the separation between the phytoplankton bloom phase (from Day 0 to Day 19) and the bacterial bloom phase (from Day 19 to the end). Arrows depict the sediment removal on Days 19 and 27 in 2012 and on Day 22 in 2013

Dissolved organic carbon and nitrogen

The DOC concentration was initially high (~6 mg C l^{-1}) and increased to ~8 mg C l^{-1} during the experiment in both years (Fig. 2A,B), but no significant differences were found between the treatments in the phytoplankton bloom phase or the bacterial bloom phase in either of the years (ANOVA, $p < 0.05$; see Table S1 in the Supplement at www.int-res.com/articles/suppl/a081p149_supp.pdf). Nevertheless, the DIATOM treatment showed the biggest increase in the experiment from 2012, with values almost significantly higher (ANOVA, $p = 0.055$) compared with the other 2 treatments.

The DON concentration, measured only in 2013, decreased from Day 0 (333 µg l^{-1}) until Day 13, after which it remained stable at ~250 µg l^{-1} in all treatments (Fig. 2C). However, the decrease in DON in the BB treatment was delayed between Days 8 and 13, which was significantly higher than in the CONTR2 and AT treatments during the phytoplankton bloom phase (Tukey's *b*, $p < 0.05$). In the bacterial

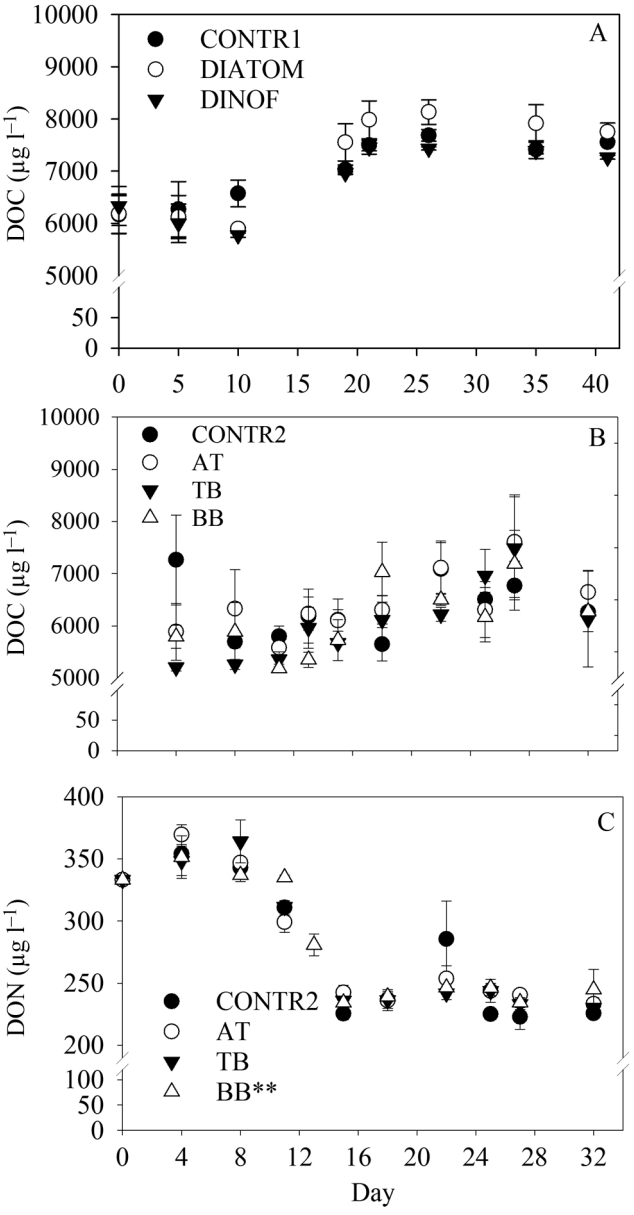

Fig. 2. Dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) concentrations (mean ± SE, n = 3) during the experiments in (A) 2012 and (B,C) 2013. Symbols (**) indicate the treatment with higher significant differences (ANOVA: $p < 0.05$) compared to the the controls (CONTR1 and CONTR2 for 2012 and 2013, respectively) in both the phytoplankton and bacterial bloom phase. No symbol indicates no significant differences. DIATOM: diatom addition, DINOF: dinoflagellate addition (see 'Materials and methods' for species details); AT: *Achnanthes taeniata*, TB: *Thalassiosira baltica*, BB: *Biecheleria baltica* additions

bloom phase, the DON concentration was also the highest value shown in the BB treatment, but was significantly different only when compared with the CONTR2 treatment (Tukey's *b*, $p < 0.05$).

Plankton community composition and biomass

The initial plankton community collected was dominated by ciliates in both years (43% in 2012 and 81% in 2013; Fig. 3), which had the highest carbon biomass of the plankton groups, followed by the flagellates in 2012 and the diatoms in both years. At the chl *a* peak, ciliates accounted for <7% of the carbon biomass in both experiments. The ciliates observed were *Lohmanniella oviformis, Strombidium* sp., *Strobilidium* sp., and tintinnids (data not shown). Unidentified flagellates constituted ~17% of the total biomass in the water collected in 2012, whereas in 2013 their contribution was ~1%. At the chl *a* peak, ~7% of the carbon biomass was flagellates in both years. The species richness in the diatom group was higher in 2012 (6 taxa) than in 2013 (3 taxa), constituting 11.56 and 6.67% of the total carbon biomass, respectively (Fig. 3A,B). Furthermore, the initial plankton carbon biomass was higher in 2012 (2.69 ± 1.11 µg C l^{-1}, ±SE) than in 2013 (1.35 ± 0.61 µg C l^{-1}, Table 2). Dinoflagellate cysts were counted as the percentage of the total carbon biomass of cells encysted. The results demonstrated that cyst formation was independent of dinoflagellate abundance and was highly variable among replicates within each treatment (data not shown).

The addition of the inoculum cultures shifted the phytoplankton community in both years, most conspicuously in 2012 when the inoculated biomass was higher than in 2013 (Table 2). In 2012, some of the species added became dominant in their respective treatments: *Chaetoceros wighamii*, 92% of the carbon biomass in the DIATOM treatment and the dinoflagellate complex, and 79% of the carbon biomass in the DINOF treatment, respectively. These species predominated in the DIATOM and DINOF treatments (Dia:Dino index = 0.99 and 0.15, respectively) on the day of the chl *a* peak (Fig. 3A, Table 2). The maximum carbon biomass in the DIATOM treatment was 3.5-fold higher than in the CONTR1 and 3-fold higher than in the DINOF treatment on that day. In 2013, diatoms were the dominant phytoplankton group, with a Dia:Dino index >0.68. The naturally occurring diatom *Thalassiosira levanderi* (not added) became the dominant species in all treatments at the chl *a* peak (Fig. 3B, Table 2). The contribution of *T. levanderi* was ~43% of the carbon biomass, followed by *Skeletonema marinoi* (~17%) in the CONTR2 and in the treatments with diatom addition (AT and TB). *Achnanthes taeniata* formed 10% and *T. baltica* 13% of the biomass in their respective treatments (AT and TB). The dinoflagellate added, *Biecheleria baltica*,

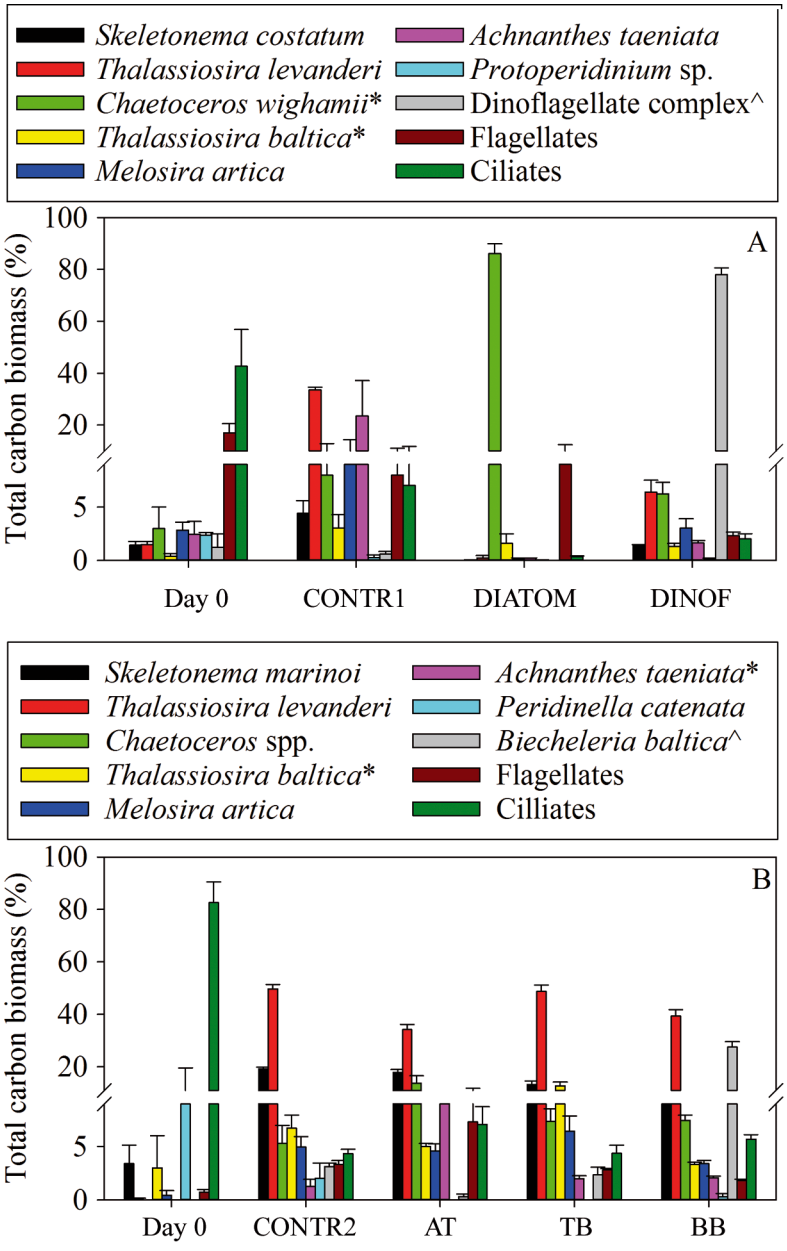

Fig. 3. Composition of the phytoplankton and microzooplankton measured as carbon biomass (mean ± SE, n = 3) in (A) 2012 and (B) 2013 on Day 0 (water collected with algal addition) and on the day of the chlorophyll *a* peak (see Table 1). The phytoplankton community contributed more than 1% of the total community. Symbols indicate the species added in each treatment for diatoms (*) and dinoflagellates (^): DIATOM (*Chaetoceros wighamii** and *Thalassiosira baltica**), DINOF (dinoflagellate complex^ and *Peridiniella catenata*^), AT (*Achnanthes taeniata**), TB (*Thalassiosira baltica**), and BB (*Biecheleria baltica*^). The dinoflagellate complex is formed by *Apocalathium malmogiense*, *Gymnodinium corollarium*, and *Biecheleria baltica*. CONTR1 (CONTR2): controls for 2012 (2013)

showed the second highest biomass contribution (28%) in the corresponding treatment (BB). The carbon biomass was highest in the BB treatment (562.86 ± 27.40 µg C l^{-1}), whereas in the remaining treatments, the biomass was ~24–36% lower than in this treatment (Table 2). The controls (CONTR1 and CONTR2) were diatom-dominated during the phytoplankton bloom phase in both years (Dia:Dino index 0.96 and 0.94 in 2012 and 2013, respectively, Table 2).

Primary production

In general, PP followed the chl *a* development (Fig. 4). In 2012, the highest PP was observed on Day 10 in the DIATOM treatment (~50 µg C l^{-1} h^{-1}). In the DINOF and CONTR1 treatments, the PP peak was lower (Day 10: ~22 µg C l^{-1} h^{-1} and Day 19: ~35 µg C l^{-1} h^{-1}, respectively) than in the DIATOM treatment (Fig. 4A). In 2013, 2 PP maxima were observed in all of the treatments (Fig. 4B). The first peak was on Day 13, with the highest value in the CONTR2 treatment (~70 µg C l^{-1} h^{-1}), and the second peak was on Day 20 after the temperature increase, with the highest value in the BB treatment (~50 µg C l^{-1} h^{-1}). The second PP peak in the BB treatment was similar to the first peak, whereas in the other treatments the first peak was 1.4–3 times higher than the second peak.

Bacterial production

The BP, measured as both cell production (TdR-BPT) and protein production (Leu-BPL), followed similar patterns in both years: they increased towards the end of the experiment. During the phytoplankton bloom phases, the BP was <1 µg C l^{-1} h^{-1} in both years, whereas during the bacterial bloom phases, the BP reached maximum values of BPT (~3 µg C l^{-1} h^{-1}) and BPL (5 µg C l^{-1} h^{-1}) in the DIATOM treatment (2012) and BPT (~1–5 µg C l^{-1} h^{-1}) and BPL (~2.5 µg C l^{-1} h^{-1}) in the AT treatment (2013) (Fig. 5A–D). In 2012, no significant differences were observed in the BP during the phytoplankton

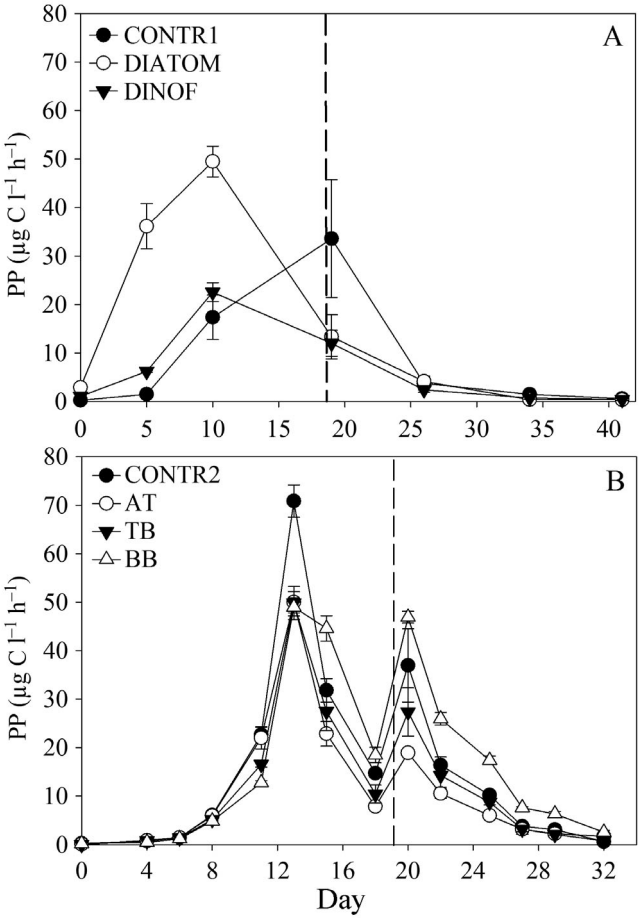

Fig. 4. Primary production (PP) (mean ± SE, n = 3) in (A) 2012 and (B) 2013. Dashed lines indicate the shift in temperature from 4 to 10°C. Abbreviations as in Fig. 3

bloom phase, but there was a difference during the bacterial bloom phase (Table S1). The BP was significantly higher in the DIATOM treatment than in the CONTR1 and DINOF treatments during the bacterial bloom phase (Tukey's *b*, BPT and BPL $p < 0.05$). The differences between the CONTR1 and DINOF treatments were statistically significant for BPL (Tukey's *b*, $p < 0.05$), but not for BPT (Tukey's *b*, $p > 0.005$). In 2013, the BP differed significantly between treatments already in the phytoplankton bloom phase (Table S1). BP was significantly higher in the AT treatment than in the CONTR2 and BB treatments (Tukey's *b*, BPT and BPL $p < 0.05$). The BP was second highest in the TB treatment, but no significant differences were found between the TB and the other treatments (Tukey's *b*, $p > 0.05$). During the bacterial bloom phase, the BP also differed significantly between treatments (Table S1): it was significantly higher in the AT treatment and significantly lower in the BB treatment than in the CONTR2 treatment (Tukey's *b*, BPT-L $p < 0.05$), whereas the BPT levels in the TB and CONTR2 treatments were similar (Tukey's *b*, BPT $p > 0.05$). The BPL was also similar in the AT and TB treatments (Tukey's *b*, BPL $p > 0.05$).

The Leu:TdR incorporation ratio was different in both years (Fig. 5E,F). In 2012, the ratio remained relatively constant in the CONTR1 and DINOF treatments at ~7–13, whereas in the DIATOM treatment the Leu:TdR ratio was more variable, reaching a value of ~25 on Days 19 and 35 (Fig. 5E). In 2013, the Leu:TdR ratio increased in all treatments at the start of the experiment, reaching a peak of ~25 on Day 6 and then dropping to ~15 on Day 8 (Fig. 5F). Later, the Leu:TdR ratio fluctuated in each treatment and decreased toward the end of the experiment.

Bacterial abundance

The response of the BA during the phytoplankton bloom phase differed, depending on the year: in 2012 it increased, whereas in 2013 it decreased. However, during the bacterial bloom phase, the BA increased in most of the treatments in both years (Fig. 6).

In 2012, the BA peaked on Day 24 (~7 × 10^6 cells ml^{-1}) in the DIATOM treatment and decreased thereafter, whereas in the CONTR1 treatment the BA increased throughout the experiment, with highest values on Day 41 (Fig. 6A). In the DINOF treatment, the BA increased only slightly (Fig. 6A). During the phytoplankton bloom phase, no significant differences were found between the treatments (Table S1), whereas in the bacterial bloom phase, the BA showed significant differences between treatments (Table S1), but no significant differences were found in the post hoc test (Tukey's *b*, $p > 0.05$).

In 2013, the BA decreased at the end of the phytoplankton bloom phase, but increased in all the treatments after the temperature was increased to 10°C (Fig. 6B). No significant differences were found during the phytoplankton bloom phase, whereas in the bacterial bloom phase, the treatments differed significantly (Table S1). The highest BA was observed in the AT treatment (~3 × 10^6 cells ml^{-1}) on Day 32, which was approximately half of the abundance of the year before. In contrast, the lowest BA was observed in the BB treatment (maximum 1.5 × 10^6 cells ml^{-1}). The BA in the AT treatment was significantly higher than in the TB and BB treatments (Tukey's *b*, $p < 0.05$), but similar to the CONTR2 treatment (Tukey's *b*, $p > 0.05$). The BA was significantly lower in the BB treatment than in the CONTR2 (Tukey's *b*, $p < 0.05$).

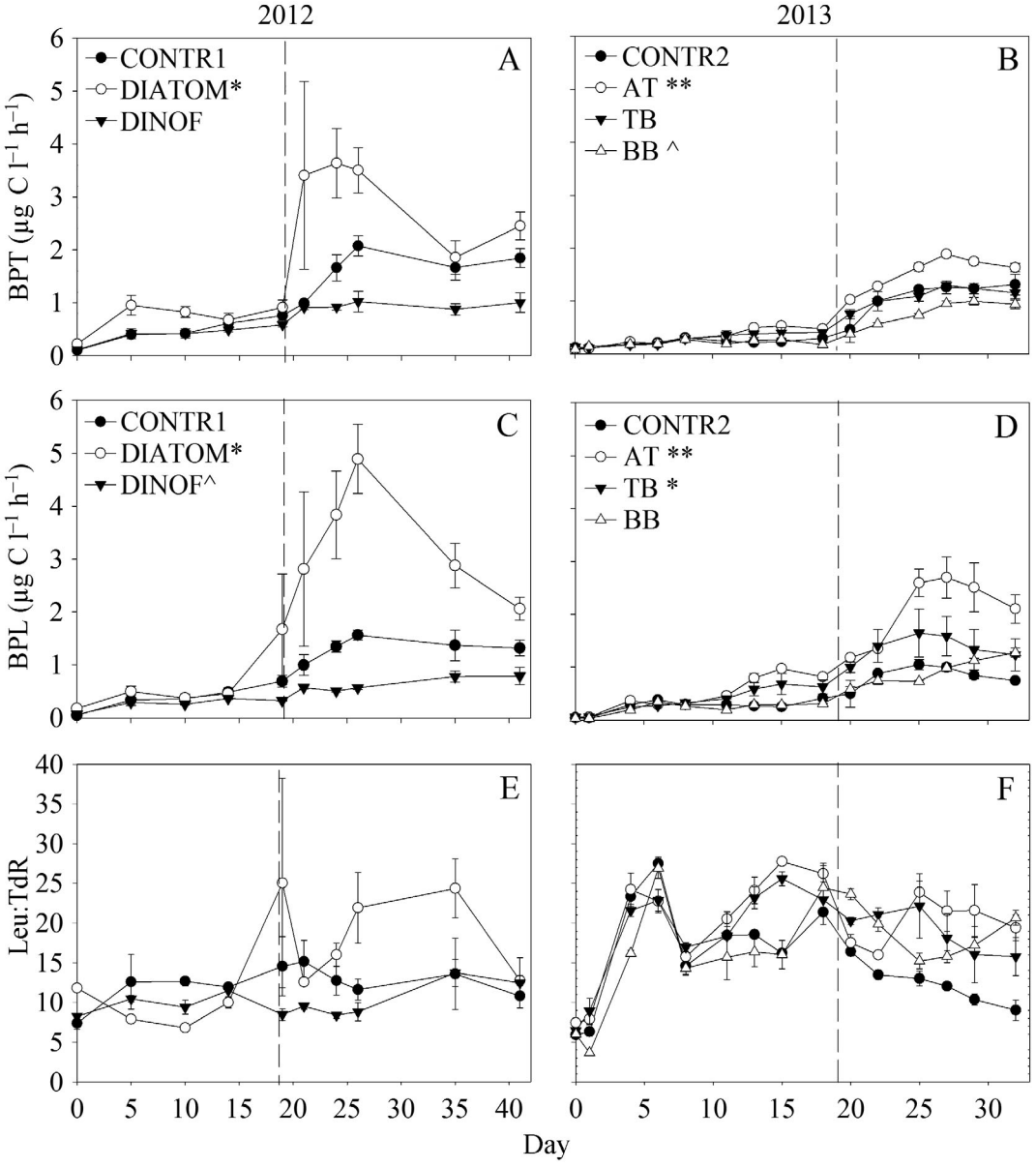

Fig. 5. Bacterial production (mean ± SE, n = 3) measured as thymidine (TdR) and leucine (Leu) incorporation (BPT and BPL, respectively) and the Leu:TdR incorporation ratio in (A,C,E) 2012 and (B,D,F) 2013. Symbols (*, ^) indicate treatments with significant differences (Tukey's *b*, $p < 0.05$), higher (*) and lower (^), compared with the controls (CONTR1 and CONTR2 for 2012 and 2013, respectively). One symbol indicates significant differences in the bacterial bloom phase, 2 symbols indicate significant differences in both the phytoplankton and bacterial bloom phases. Dashed lines indicate the shift in temperature from 4 to 10°C. Abbreviations as in Fig. 3

Bacterial community dynamics and composition

The NMDS plots showed that the bacterial community dynamics were structured by the various treatments, becoming more dissimilar with time (Fig. 7A,B). At the start of the experiments, the bacterial communities in the water collected were similar in 2012 and 2013 (Figs. 7A,B & 8A,B): *Alphaproteobacteria* (SAR11 and family *Rhodobacteraceae*) dominated the community (~50% of the OTUs), while *Actinobacteria* (hgcl clade) and *Acidimicrobiia* (CL500-29 marine group) were the second most common classes, constituting ~10 and ~13% of the OTUs, respectively. The relative abundance of the class *Gammaproteobacteria* was higher in 2013 than in 2012, with dominance of the genera *Methylobacter* (~7%) and *Pseudomonas* (~3%), which were absent in 2012.

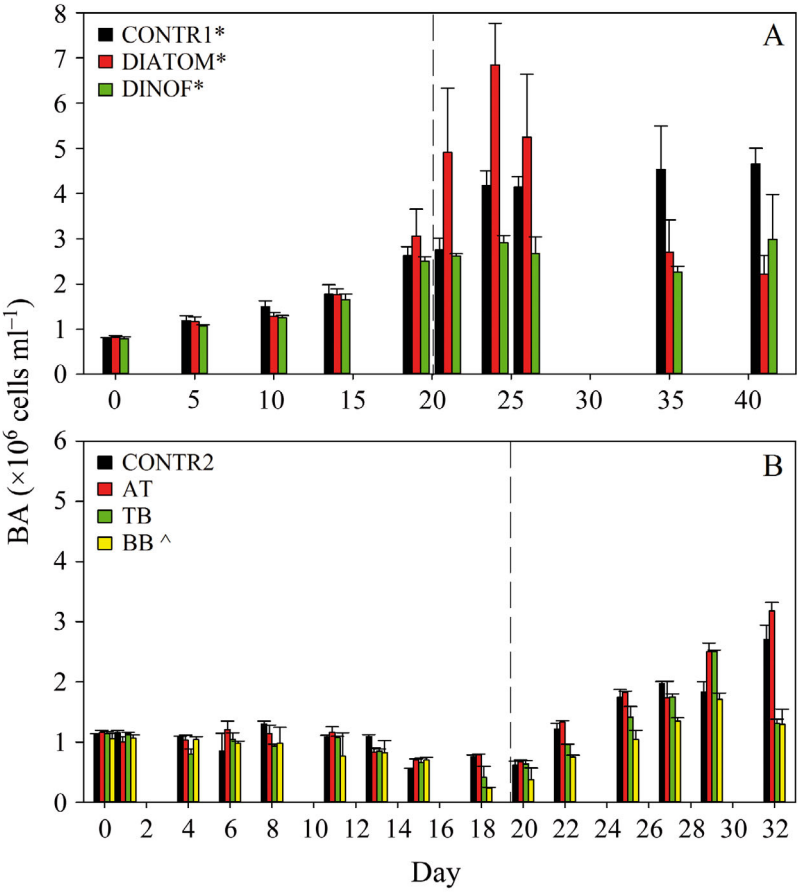

Fig. 6. Bacterial abundance (BA, mean ± SE, n = 3) during the experiments in (A) 2012 and (B) 2013. Symbols (*, ^) indicate treatments with significant differences ($p < 0.05$) in the bacterial bloom phase. The results are based on ANOVA (*) and Tukey's test (^) compared with the controls (CONTR1 and CONTR2 for 2012 and 2013, respectively). Dashed lines indicate the shift in temperature from 4 to 10°C. Abbreviations as in Fig. 3

In 2012, the succession pattern in the DIATOM treatment differed from those in the CONTR1 and the DINOF treatments throughout the experiment (Figs. 7A & 8A), but no statistical tests were conducted, due to the lack of replicates. The class *Flavobacteriia* dominated the bacterial community during the chl *a* peak. In the DIATOM treatment, the genus *Flavobacterium* constituted ~43% of the total relative abundance, whereas in the CONTR1 and DINOF treatments, *Flavobacterium* and the NS3a marine group constituted ~34 and 28%, respectively, of the relative abundance. By the end of the experiment (Day 41), the class *Betaproteobacteria* (genus *Hydrogenophaga* ~20% of the OTUs) increased in the DIATOM treatment, whereas in the CONTR1 and DINOF treatments, the alphaproteobacterial (SAR11) predominance was reverted, accompanied by an increase in the proportion of *Cyanobacteria* (genus *Synechococcus* ~10%). In addition, the relative abundance of the class *Actinobacteria* (genus Candidatus *Aquiluna*) increased in all the treatments towards the end of the experiment, especially in the CONTR1 and DINOF treatments (genus hgcl clade). Within the

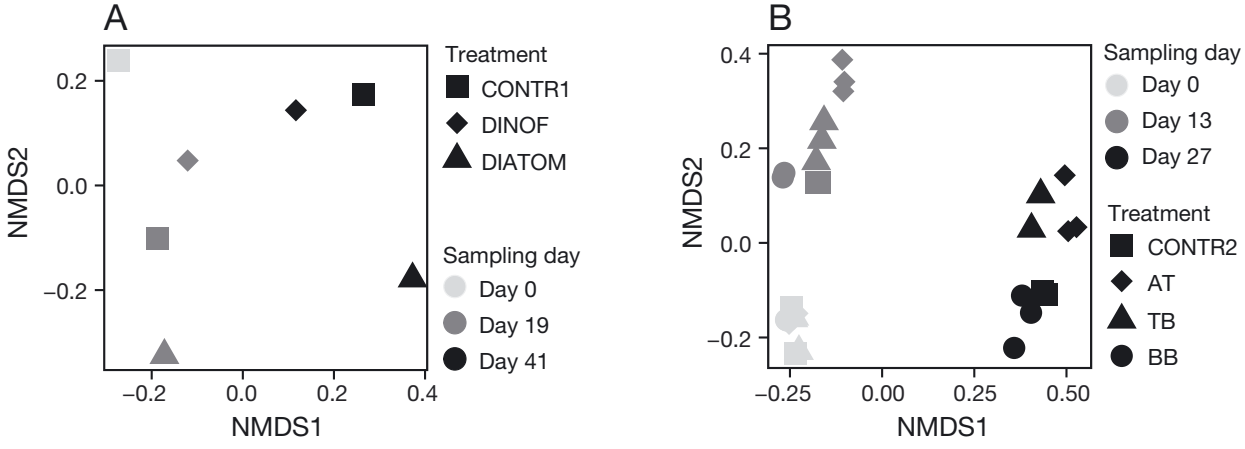

Fig. 7. Nonmetric multidimensional scaling (NMDS) plots of the bacterial community composition on the 3 sampling days (A) in 2012: Day 0 (start), Day 19 (chl *a* peak), and Day 41 (end); and (B) in 2013: Day 0 (start), Day 13 (chl *a* peak), and Day 27 (bacterial production peak). Abbreviations as in Fig. 3. There was no replication in 2012 (n = 1) and replicate samples were collected in 2013 (n = 3). Stress values are 0.019 in 2012 and 0.046 in 2013

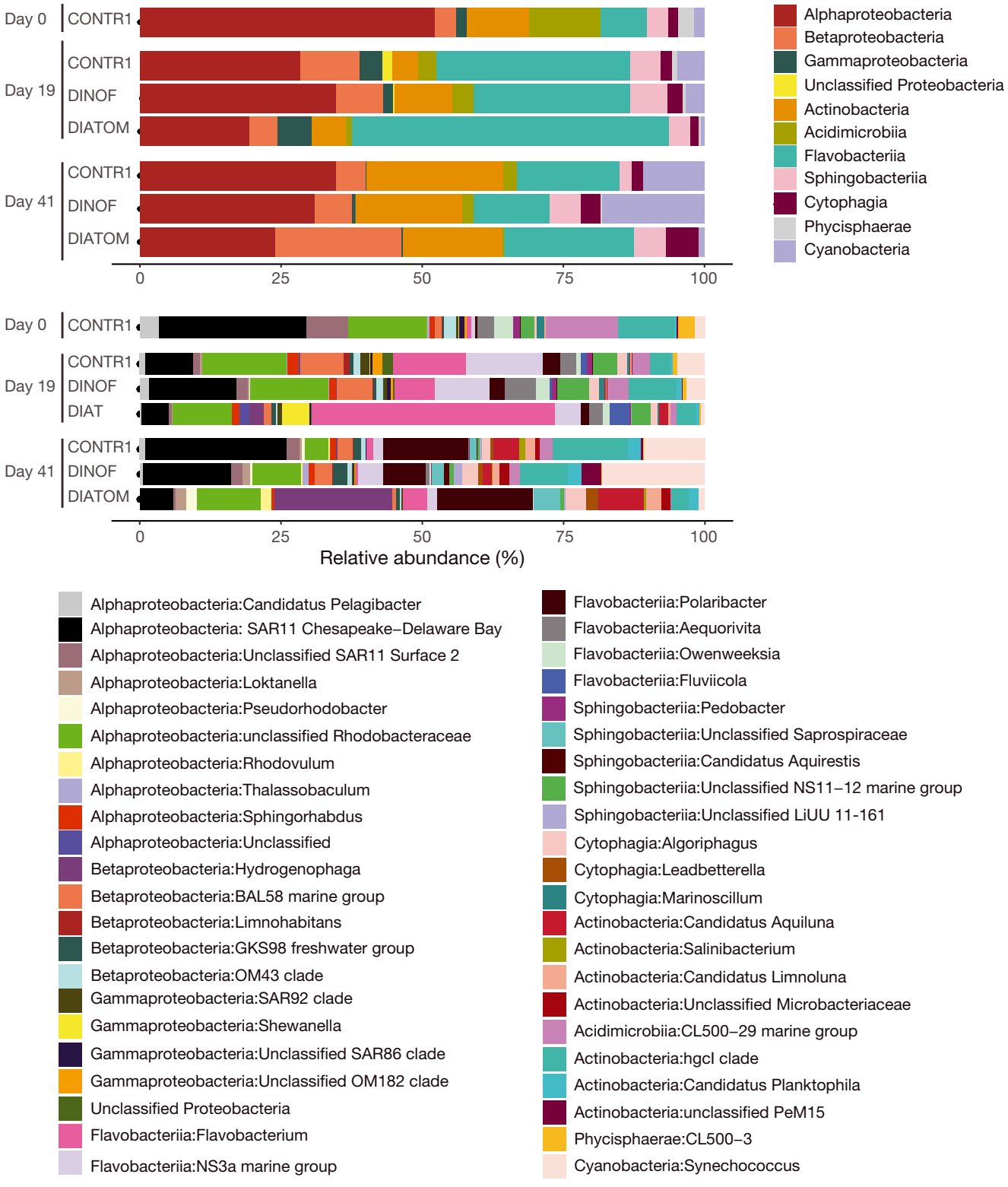

Fig. 8. (Above and following page.) Class- and genus-level bacterial diversity of 16S ribosomal RNA (rRNA) gene sequences (~450 bp) representing >0.1% of all normalized operational taxonomic units (OTUs) on (A) Day 0 (start), Day 19 (chl *a* peak), and Day 41 (end) in 2012, and on (B) Day 0 (start), Day 13 (chl *a* peak), and Day 27 (bacterial production peak) in 2013. Abbreviations as in Fig. 3. No replicates were set in 2012 (n = 1). Significant differences were tested in 2013 (n = 3). *: treatments with significant differences (PERMANOVA, $p < 0.05$) compared to the control

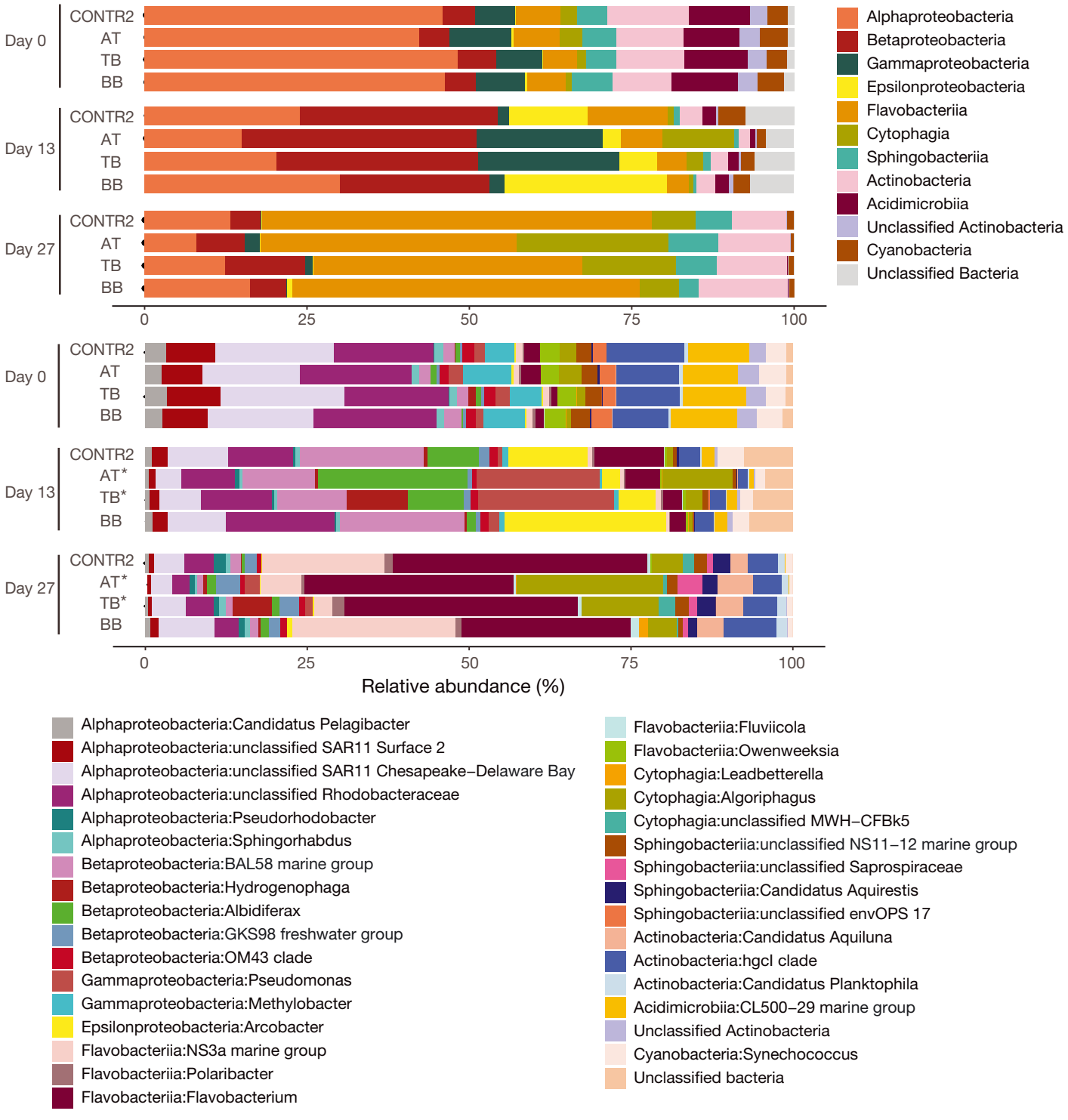

Fig. 8 (continued)

Flavobacteriia clade, *Flavobacterium* and the NS3a marine group were replaced by the genus *Polaribacter* in all of the treatments at the end of the experiment.

In 2013, the bacterial communities were clustered (Fig. 7B) and differed significantly between treatments over time (PERMANOVA, treatment × time: df: 6, pseudo-*F*: 2.41, p = 0.011). Using pairwise tests

for the statistical analysis of the treatments, significant differences were observed between the CONTR2 and both treatments with diatom addition (PERMANOVA, CONTR2 vs. AT: $p = 0.027$ and CONTR2 vs. TB: $p = 0.021$) and between the treatments with diatom and dinoflagellate addition (PERMANOVA, TB vs. BB: $p = 0.006$ and AT vs. BB: $p = 0.013$). No significant differences were found between the CONTR2 and the BB treatment (PERMANOVA, $p > 0.05$). In 2013, patterns similar to those in 2012 were observed in terms of bacterial community changes: *Alphaproteobacteria* (SAR11 and family *Rhodobacteraceae*) decreased and *Flavobacteriia* (*Flavobacterium* and the NS3a marine group) increased during the experiment in all treatments (Fig. 8B). However, in 2013, a decrease in the proportion of *Flavobacteriia* was not observed. *Betaproteobacteria* (BAL58 marine group in all of the treatments; *Albidiferax* in the CONTR2, AT, and TB treatments; and *Hydrogenophaga* in the TB treatment) exhibited transient dominance in the bacterial community (accounting for ~30% of the OTUs) at the chl *a* peak. In addition to *Betaproteobacteria*, transient gammaproteobacterial (genus *Pseudomonas*) dominance was also observed in the AT and TB treatments (~20% of the OTUs), whereas *Epsilonproteobacteria* (genus *Arcobacter*) increased in the CONTR2 (~12% of the OTUs) and BB treatments (~25% of the OTUs). At the BP peak (Day 27), the *Flavobacteriia* clade predominated in all of the treatments (genus *Flavobacterium* ~25–40% of the OTUs), and the relative abundance of the *Actinobacteria* clade increased again (hgcl clade and Candidatus *Aquiluna*). In addition to *Flavobacterium*, the proportion of the NS3a marine group increased in the CONTR2 and BB treatments (~18–25% of the OTUs), whereas the genus *Algoriphagus* (*Cytophagia*) increased in the AT and TB treatments (~12–22% of the OTUs).

DISCUSSION

Two mesocosm experiments were conducted in consecutive years to determine whether the shift in phytoplankton community composition (diatom vs. dinoflagellate) affects BP, BA, and BCC. Based on the temperature setup and the inorganic nutrient concentrations, the experiments were divided into 2 different phases: the phytoplankton bloom phase, characterized by the development of autotrophic organisms with the corresponding chl *a* and PP peaks, and the bacterial bloom phase, characterized by the increase in bacterial growth.

Phytoplankton bloom phase

The high inorganic nutrient concentrations in the water collected and the improved light conditions initiated the phytoplankton bloom phase. The phytoplankton community composition in the water collected was largely formed by diatom species in both years. The addition of the cultures shifted the naturally occurring phytoplankton community; the diatom and dinoflagellate species added predominated in 2012 (Dia:Dino index = 0.95 and 0.15, respectively), but not in 2013 (Dia:Dino index ~ 0.69–1.00), likely because the inoculum was 3.5-fold higher in 2012 than in 2013. This higher addition in 2012 promoted the more rapid development of the phytoplankton bloom in the DIATOM and DINOF treatments than in the CONTR1 treatment and resulted in increased variation between the replicates in the DIATOM treatment.

The dominant cold-water diatoms in the Baltic Sea generally have higher maximum growth rates than competing dinoflagellates (Spilling & Markager 2008). However, dinoflagellates can dominate the phytoplankton community when their initial cell abundance is high enough to build up bloom-level biomass before the diatoms catch up (Kremp et al. 2008). This is what occurred in our DINOF treatment in 2012; the inocula of the dinoflagellate cultures were high enough to retain the dominance throughout the phytoplankton bloom phase. Ice thickness and stratification patterns are other crucial factors affecting dinoflagellate development in the natural environment (Klais et al. 2013), but this was not considered in our experimental setup. In 2013, the naturally occurring (not added) diatom *Thalassiosira levanderi* predominated in all the treatments, even in the BB treatment (Dia:Dino index = 0.69), despite having a biomass lower than that of the other diatom species initially and the maximum growth rate being similar to that of the diatoms added (Spilling 2007). *T. levanderi* is typically abundant during the initial phase of the spring bloom (Wasmund et al. 1998), forming dense blooms under the ice (Arrigo et al. 2012). This species probably benefitted from being acclimated to the initial water conditions at the start of the experiment and managed to increase its relative abundance, compared with competing phytoplankton.

The phytoplankton bloom phase ended with the depletion of nitrate, which is typical of this N-limited region of the Baltic Sea (Tamminen & Andersen 2007). The collapse of phytoplankton blooms is typically followed by an increase in bacterial activity and

BA (Riemann et al. 2000, Fandino et al. 2001, Buchan et al. 2014), which was also observed in our experiments. Primary producers provide an adequate environment for bacterial assemblages, since phytoplankton cells release DOM, which fuels bacterial growth and promotes the coupling between phytoplankton and bacteria (Biddanda & Benner 1997, Buchan et al. 2014, Bunse & Pinhassi 2017). We observed this bacteria–phytoplankton coupling, but with a time lag of 1 wk, most likely due to the carbon limitation and the low temperature (4°C) in our experiments during the phytoplankton bloom phase. In addition, the BA decreased towards the end of this phase in 2013, which could have been caused by competition with phytoplankton cells for inorganic nutrients (Buchan et al. 2014) or increased grazing pressure. Furthermore, high grazing pressure was observed on bacterioplankton at low temperatures in an experiment by von Scheibner et al. (2014). Low temperature, inorganic and organic nutrient limitation, and grazing pressure are crucial factors regulating bacterial growth (Lignell et al. 1993, Lindh et al. 2013, Bunse et al. 2016). Grazing pressure on phytoplankton is low during the spring bloom in the Baltic Sea (Lignell et al. 1993). However, grazing can control the bacterial biomass following the spring bloom (Lignell et al. 1992). In our experiments, we found a clear increase in BA following the phytoplankton bloom phase, suggesting that the grazing pressure at that time did not prevent bacterial biomass growth.

Depending on the growth rate and/or physiological status of the bacterial assemblage, the bacteria invest their cellular resources in either cell division (TdR incorporation) or biomass synthesis and/or maintenance costs (Leu incorporation) (Chin-Leo & Kirchman 1990, Hoppe et al. 2006). The unfavorable conditions of low temperature and chl *a* are often characterized by over 10× higher Leu incorporation than TdR incorporation (Shiah & Ducklow 1997). In 2013, we observed an increase in the Leu:TdR ratio at the start of the experiment, suggesting that increased Leu incorporation was channeled to maintenance, which decreased again after the chl *a* began to increase, most likely reflecting higher carbon availability.

Despite the unfavorable conditions in 2013, there was a significant increase in BP in the AT treatment during the phytoplankton bloom phase. There were also changes in the BCC that were already observed at the chl *a* peak in both years. The bacterial community was dominated initially by *Alphaproteobacteria* (SAR11 and family *Rhodobacteraceae*), which commonly occur under pre-bloom conditions (Andersson et al. 2010, Herlemann et al. 2011, Laas et al. 2015). During the phytoplankton bloom phase, the alphaproteobacterial predominance changed to flavobacteriial predominance (*Flavobacterium* and/or the NS3a marine group) in 2012, whereas in 2013 the community changed to beta- and gammaproteobacterial predominance (*Albidiferax* and the BAL58 marine group-*Pseudomonas*) in the AT and TB treatments and to beta- and epsilonproteobacterial predominance (BAL58 marine group-*Arcobacter*) in the BB and CONTR2 treatments. Both *Flavobacteriia* and *Gammaproteobacteria* are common groups during and/or after phytoplankton blooms (Teeling et al. 2012, Laas et al. 2015, Bunse et al. 2016), and the most abundant genera within these classes (*Flavobacterium* and *Pseudomonas*) are associated with diatom blooms (Amin et al. 2012). *Flavobacteriia* are able to degrade complex HMW substrates (Cottrell & Kirchman 2000, Kirchman 2002, Buchan et al. 2014), whereas *Gammaproteobacteria* are opportunistic bacteria that are able to exploit elevated concentrations of LMW substrates (Eilers et al. 2000, Pinhassi & Berman 2003, Gómez-Consarnau et al. 2012). The bacterial community patterns observed in *Flavobacteriia* and *Gammaproteobacteria* indicate resource partitioning consistent with previous studies (Teeling et al. 2012). However, *Gammaproteobacteria*, as is typical for copiotrophs, may behave as generalists, due to their capability for using various algal-derived DOC sources (Sarmento et al. 2016) and enrichment treatments (Teira et al. 2010). *Betaproteobacteria* are similar to *Gammaproteobacteria* in their DOM consumption (Cottrell & Kirchman 2000, Hoikkala 2012 and references therein) and are positively associated with increased DOC concentrations in estuarine environments (Bouvier & del Giorgio 2002).

The treatments with diatom additions likely produced ample amounts of labile substrates, boosting bacterial growth and causing the pronounced flavobacteriial predominance in the DIATOM treatment in 2012, as well as a peak in *Gamma-* and *Betaproteobacteria* in 2013. *Gammaproteobacteria* may also have occurred in 2012, but the peak may have been missed, due to the long gap between sampling days (Days 0 and 19).

Bacterial bloom phase

The high phytoplankton biomass in the phytoplankton bloom phase and the increase in temperature to 10°C after nutrient depletion were the main

triggers for the development of the bacterial bloom phase following the chl *a* peak. There was a more pronounced effect of the phytoplankton community composition on bacterial activity during the bacterial bloom phase than in the phytoplankton bloom phase.

The BP was significantly higher in the treatments with diatom addition (DIATOM, AT, TB-BPL) and lower in the treatments with dinoflagellate addition (DINOF-BPL, BB-BPT) than in the treatments with no addition (CONTR1 and CONTR2). Thus, the treatments with diatom addition fueled the bacterial growth more efficiently than the treatments with dinoflagellate addition, even before the collapse of the phytoplankton bloom (AT treatment, Fig. 4B in 2013).

In 2012, the inoculum in the DIATOM treatment was larger than in the DINOF treatment and, thus, the PP and total phytoplankton carbon biomass were greater in the DIATOM treatment, suggesting increased carbon fixation in this treatment, compared with the CONTR1 and DINOF treatments. The DOC concentration (Fig. 3A) was slightly higher in the DIATOM treatments than in the DINOF and CONTR1 treatments, but this result was not unambiguous ($p = 0.055$). The DIATOM treatment was dominated by *Chaetoceros* sp. This taxon releases higher concentrations of DOM, measured as percentage of extracellular release (Wetz & Wheeler 2007), and also produces more extracellular polysaccharides than other diatom species (Myklestad 1995), enhancing bacterial activity (Lekunberri et al. 2012, Sarmento & Gasol 2012). The DOM released in the DIATOM treatment was likely translated into 2-fold higher BP, since diatoms can produce highly labile substrates, such as polysaccharides (Myklestad 2000).

In 2013, we investigated whether smaller changes (lower concentrations of culture addition) in the phytoplankton community produce similar effects in the bacterial community. In that year, the chl *a* and first PP peaks were higher than in 2012, but this could have been due to the higher sampling frequency than in 2012. We also observed a second PP peak in 2013, most likely a sign of DOM utilization by the bacterioplankton and consequent nutrient remineralization, which can boost phytoplankton carbon fixation (Sarmento & Gasol 2012, Bunse et al. 2016). However, the chl *a* peak and second PP production peak were highest in the BB treatment, which resulted in the lowest BP, the lowest BA, and also presented a significant delay in the DON utilization, compared with the other treatments. This decoupling between PP and BP in this treatment could have been due to a specific response of the bacterial activity to the DOM by the present phytoplankton community, or alternatively to competition for inorganic nutrients with phytoplankton cells (Sebastián & Gasol 2013, Buchan et al. 2014, Landa et al. 2014). There were also clear differences between the treatments with diatom additions (AT and TB), also suggesting differences in the bacterial response between different diatom-dominated communities, perhaps driven by species-specific release of DOM.

We observed no significant differences in the DOC concentrations between treatments, suggesting rapid turnover and incorporation of the carbon released into the bacterial biomass, which is supported by the difference in BA. The labile DOC release by the algae was quickly taken up by the bacterioplankton, which would explain why there was no quantitative effect of the phytoplankton community on the DOC pool. Another factor is the very high concentration of DOC in the Baltic Sea (6–8 mg l^{-1}), which is caused by the high input of terrestrially derived organic matter (Hoikkala et al. 2015). Any difference in the DOC concentration caused by phytoplankton would have been difficult to detect against this large background signal.

There could also have been other mechanisms affecting bacterial activity. In 2013, the dominant species in both the CONTR2 and BB treatments was the diatom *T. levanderi* (~50 and ~40% of the biomass, respectively), which presumably produced and released DOM relative to its biomass. However, BA was significantly lower in the BB treatments than in the CONTR2 treatment, a difference that was larger than what could have been expected from the relatively small biomass of *Biecheleria baltica* in the BB treatment (Fig. 6). This suggests an indirect effect of *B. baltica* on bacterial activity other than lower quantity/quality of the DOM release. Dinoflagellates produce and release a range of compounds with inhibitory effects on other planktonic organisms (e.g. allelochemicals or toxins; Granéli & Hansen 2006), which may also have antibacterial effects (Trick et al. 1984). Alternatively, bacterial loss processes, such as grazing, could have been elevated in the BB treatment, since many dinoflagellates are mixotrophic, consuming food particles in addition to fixing carbon through photosynthesis (Jeong et al. 2010). Further studies are required to disentangle any indirect effect of *B. baltica* on the bacterial community. In addition, a major proportion (~50% in 2012 and ~30% in 2013) of the POC was removed by taking out the settling material, but there was no difference in the amount of biomass removed between the treatments, so this could not explain the large difference

in bacterial activity between treatments. Disintegration or lysis of dinoflagellate vegetative cells can be a major loss process in a natural environment (Heiskanen 1998), but we observed no dinoflagellate cells lysing, which would likely have increased BP.

The bacterial community dynamics during the bacterial bloom phase differed between 2012 and 2013. In 2012, the predominance of *Flavobacteriia* during the chl *a* peak was reverted back to alphaproteobacterial predominance, whereas in 2013 *Flavobacteriia* predominated in all treatments, with more than 50% of the OTUs. In addition, a shift within the *Flavobacteriia* clade was observed in both years with the occurrence of *Flavobacterium*, the NS3a marine group, and *Polaribacter.* A similar succession pattern was previously observed in the North Sea (Teeling et al. 2016). *Polaribacter* is a recurrent genus in the Baltic Sea during phytoplankton blooms (Laas et al. 2015, Bunse et al. 2016), while the NS3a marine group (and *Flavobacteriia* in general) have been observed during dinoflagellate blooms (Fandino et al. 2001, Yang et al. 2015).

Further differences between treatments were observed in both years. In 2012, the proportion of *Betaproteobacteria* (genus *Hydrogenophaga*) increased in the DIATOM treatment, whereas in the CONTR1 and DINOF treatments, increased numbers of actinobacterial and cyanobacterial groups were observed during the experiment. In 2013, the proportion of the genus *Algoriphagus* (*Cytophagia*) increased in the AT and TB treatments, differing significantly from the CONTR2 and BB treatments. This genus may assimilate N-acetyl glucosamide, a monosaccharide that probably increases during the decay of the diatom blooms (Cottrell & Kirchman 2000, Eckert et al. 2012). The proportion of *Actinobacteria* (hgcl clade and Candidatus *Aquiluna*) increased towards the end of the experiment in this year. *Actinobacteria* is a common clade observed in the Baltic Sea, due to the low salinity. They are considered slow-growing and predator-resistant (Eckert et al. 2012), and are associated with decaying phytoplankton blooms and *Cyanobacteria*-derived DOC (Stepanauskas et al. 2003, Hugerth et al. 2015, Bunse et al. 2016).

The distinct occurrence and predominance of the main bacterial taxa (*Flavobacteriia*, *Beta*- and *Gammaproteobacteria*, *Actinobacteria*) in our experiments could have been due to the difference in sampling days in both years: in 2012, the bacterial community was collected at the end of the experiment (Day 41) and in 2013 at the BP peak (Day 27). Thus, we probably captured different stages of the phytoplankton bloom development. The bacterial communities in the various treatments with diatom additions also became more dissimilar than their respective controls and treatments with dinoflagellate additions. Thus, the composition and/or the quantity of the DOM released may have differed not only between years, but also between the phytoplankton species present in the various treatments. An indication of the differences in quality of the DOM released can be seen in our experiment of 2013, in which we found significant differences between the BB treatment and CONTR2 in the DON concentration, as well as in BPT. However, we found no significant differences in the BCC between the CONTR2 and BB treatment. Shifts in the bacterioplankton composition may be caused by changes in the quality of the DOM (Pinhassi et al. 2004, Landa et al. 2016), but the quantity of the released DOM may also be important (Sarmento et al. 2016). We can make no further conclusions about this topic, based on our data, because we did not characterize the DOM pool in detail.

Nevertheless, the increase in bacterial activity during the bacterial bloom phases and the increased relative abundance of bacteria associated with phytoplankton blooms (*Flavobacteriia*, *Beta*- and *Gammaproteobacteria*) revealed a link between ecosystem functioning and diversity. These results confirm the importance of the proper identification of the phytoplankton community, due to their differences in the quality and quantity of the DOM released (Bunse et al. 2016, Kirchman et al. 2017).

CONCLUSIONS

The differences in the bacterioplankton, in terms of BP, BA, and BCC, in our study were clearly driven by the phytoplankton community composition. The presence of the various phytoplankton species likely produced differences in the quality and/or quantity of the DOM pool, which affected bacterial physiology and the BCC. In particular, the diatom-dominated communities were characterized by higher BA and BP and differed in BCC, suggesting that the diatom-dominated communities excreted more carbon than the communities with a co-occurrence of dinoflagellates. The results suggest that the long-term shift towards higher dinoflagellate abundance during the spring bloom in the Baltic Sea may cause reduction in BP and changes in the BCC, which may reduce pelagic remineralization of organic matter and alter the material fluxes in the microbial loop.

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