Vol. 83: 95–108, 2019
https://doi.org/10.3354/ame01902

AQUATIC MICROBIAL ECOLOGY
Aquat Microb Ecol

Published online June 6

Potential role of oxygen and inorganic nutrients on microbial carbon turnover in the Baltic Sea

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ABSTRACT: Oxygen (O_2) deficiency and nutrient concentrations in marine systems are impacting organisms from microbes to higher trophic levels. In coastal and enclosed seas, O_2 deficiency is often related to eutrophication and high degradation rates of organic matter. To investigate the impact of O_2 concentration on bacterial growth and the turnover of organic matter, we conducted multifactorial batch experiments with natural microbial communities of the central Baltic Sea. Water was collected from suboxic (<5 µmol l^{-1}) depths in the Gotland Basin during June 2015. Samples were kept for 4 d under fully oxygenated and low O_2 conditions (mean: 34 µmol l^{-1} O_2), with or without nutrient (ammonium, phosphate and nitrate) and labile carbon (glucose) amendments. We measured bacterial abundance, bacterial heterotrophic production, extracellular enzyme rates (leucine-aminopeptidase) and changes in dissolved and particulate organic carbon concentrations. Our results show that the bacterial turnover of organic matter was limited by nutrients under both oxic and low O_2 conditions. In nutrient- and glucose-replete treatments, low O_2 concentrations significantly reduced the net uptake of dissolved organic carbon and led to greater accumulation of more labile dissolved organic matter. Our results therewith suggest that the combined effects of eutrophication and deoxygenation on heterotrophic bacterial activity might favor the accumulation of dissolved organic carbon in the Baltic Sea.

KEY WORDS: Oxygen · Heterotrophic bacteria · Baltic Sea · Organic matter · Bacterial heterotrophic production · Extracellular enzymes · Leucine-aminopeptidase

1. INTRODUCTION

Oxygen minimum zones (OMZs) are defined as water masses with oxygen (O_2) concentrations <90 µmol O_2 kg^{-1} (Karstensen et al. 2008, Paulmier & Ruiz-Pino 2009). Based on the O_2 concentration, OMZs are often classified as hypoxic (<60 µmol kg^{-1}), suboxic (<5 µmol kg^{-1}) and anoxic (Karstensen et al. 2008, Gruber 2011). In the Baltic Sea, hypoxia has increased in the last century, mainly due to allochthonous nutrient inputs, subsequent build-up of organic matter and microbial respiration of organic matter (Carstensen et al. 2014, Andersen et al. 2017). Hence, hypoxia in the Baltic Sea is closely coupled to nutrient concentrations. The input of nitrogen and phosphorus from sea, land and atmospheric deposition increased from 1850 to 1980 by 4- and 5-fold, respectively, and to date nutrient concentrations in the Baltic Sea hardly reflect the decrease in anthropogenic nutrient loads achieved in the recent past (Gustafsson et al. 2012, Jutterström et al. 2014). Due to denitrification in suboxic zones, the Baltic Sea is losing some of the allochthonous dissolved inorganic nitrogen (DIN), subsequently leading to DIN deficiency relative to phosphorus. In contrast, nitrogen is fixed by autotrophic cyanobacteria at the surface and by hetero-

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Publisher: Inter-Research · www.int-res.com

trophs at the chemocline in the Baltic Sea, and this process may counteract the loss of nitrogen (Larsson et al. 2001, Voss et al. 2005, Vahtera et al. 2007, Farnelid et al. 2013). The OMZs in the Baltic Sea prevail in the deep basins at 60 to 80 m due to permanent stratification induced by strong salinity gradients (Conley et al. 2009). Inflow events can episodically transport dense and oxygenated water from the North Sea into the Baltic Sea (Mohrholz et al. 2015). The high density of the inflowing North Sea water, however, restricts oxygenation to the bottom water of the basins (from 200 to 232 m). Therefore, intermediate waters (from 70 to 175 m) remain O_2-depleted (e.g. Feistel et al. 2003). Only after strong inflow events (e.g. in 2014) can parts of the intermediate waters become oxygenated (Dellwig et al. 2018).

Within the OMZs, prokaryotes have adapted to low O_2 concentrations via anaerobic respiration pathways (e.g. Lam & Kuypers 2011) and substrate-level phosphorylation (fermentation) (Madigan et al. 2012). Denitrification is the preferred anaerobic respiration pathway because the energy yield is close to that of O_2 reduction. In the OMZs of the Baltic Sea, the availability of nitrate enables significant rates of denitrification (~22.5 nmol N l^{-1} h^{-1}) (Hietanen et al. 2012, Bonaglia et al. 2016). Another important anaerobic respiration pathway, in the Baltic Sea, is dissimilatory nitrate reduction to ammonium (DNRA) (~0.67 nmol N l^{-1} h^{-1}), whereas anaerobic ammonium oxidation (anammox) occurs less frequently (Hietanen et al. 2012, Bonaglia et al. 2016).

A decrease of O_2 concentration and the expansion of OMZs have been predicted for the future ocean (Bopp et al. 2013, Schmidtko et al. 2017). It is still unknown how changes in O_2 concentration affect bacterial cycling of organic matter. This includes the bacterial uptake of dissolved organic matter (DOM), its remineralization and its transformation to more refractory DOM (Jiao et al. 2010). The bacterial cycling of organic matter is related to the hydrolysis of high molecular weight (HMW) organic matter by extracellular enzymes (Hoppe et al. 2002) and the formation of bacterial biomass, which can then be transferred to higher trophic levels (Azam et al. 1983). Since anaerobic respiration has a lower energy yield than aerobic respiration (Lam & Kuypers 2011, Madigan et al. 2012), the overall turnover of organic matter might be reduced within OMZs. Several studies found implications of reduced organic matter degradation under anoxia or suboxia compared to oxic conditions in marine waters and incubation experiments (Nguyen & Harvey 1997, Devol & Hartnett 2001, Van Mooy et al. 2002, Keil et al. 2016, Le Moigne et al. 2017). Others observed similar degradation rates of organic matter in oxic and suboxic or anoxic waters (Lee 1992, Pantoja et al. 2009) and suggested, that the quality of the organic substrate rather than O_2 controls the degradation rate (Pantoja et al. 2009). In the Gotland Deep (Baltic Sea), labile carbon limitation may apply for denitrifying bacteria at the oxic-anoxic interface (Bonaglia et al. 2016). Jørgensen et al. (1999) also showed seasonally alternating carbon and nitrogen limitation of bacteria in the Gulf of Riga. Hence, O_2, carbon and nitrogen may be co-limiting or successively limiting microbial organic matter turnover in the Baltic Sea. However, studies that combine measurements of microbial rates and organic carbon turnover at different O_2 concentrations are scarce.

Here, we tested the hypothesis of reduced bacterial organic matter turnover under low O_2 concentrations compared to fully oxygenated conditions. We investigated bacterial growth, bacterial production, extracellular enzyme rates and the turnover of organic carbon in natural microbial communities under low and saturating O_2 concentrations in a multifactorial experiment.

2. MATERIALS AND METHODS

2.1. Experimental design and treatments

Seawater was sampled in the central Baltic Sea, at the HELCOM stations BY15 in the Gotland Deep (57° 20′ N, 20° 30′ E) and BY21 in the Northern Gotland Basin (58° 26′ N, 20° 19′ E). Samples were taken on 8 and 14 June 2015 during the research cruise AL458 (with RV 'Alkor'). Hydrographic conditions were recorded by CTD (Hydrobios) casts, including measurements of O_2 concentration with the Oxyguard (PreSens) sensor (see below for details) and revealed a permanent stratification at both stations with OMZs below the pycnocline. Seawater was sampled from the O_2 minimum (2–3 µmol l^{-1}) at 110 m to incubate natural microbial communities that had been exposed and adapted to suboxia prior to the incubations. At the sampling depth, bacterial cell abundance was 8.2×10^5 and 8.8×10^5 cells ml^{-1} at Stns BY15 and BY21, respectively. For Stn BY15, the initial nutrient concentrations were 0.1, 0.1, 3.28 and 2.7 µmol l^{-1} for nitrite, nitrate, ammonium and phosphate, respectively. For Stn BY21, nitrite and nitrate were below the detection limit, and ammonium and phosphate concentrations peaked at 2.06 µmol l^{-1} and 2.6 µmol l^{-1}.

At each station (BY15 and BY21), 20 l of seawater were collected with 2 Niskin bottles (12 l) at 110 m depth. Seawater was filtered through a 100 µm mesh to remove larger zooplankton and filled into 12 (Expt BY21) or 18 (Expt BY15) gas-tight serum bottles of 1.12 l (Zscheiler and Klinger). Incubations were performed under oxic and low O_2 conditions (see Fig. 1 for O_2 concentrations) without additions (controls) and with different inorganic nutrient and glucose enrichments. All incubations were performed in triplicate. Expt BY15 included controls and 2 enrichments: (1) GPA, with final concentrations of glucose (100 µmol l^{-1}), sodium phosphate (60 µmol l^{-1}) and ammonium chloride (152 µmol l^{-1}); (2) GPAN, with final concentrations of glucose (100 µmol l^{-1}), sodium phosphate (60 µmol l^{-1}), ammonium chloride (152 µmol l^{-1}) and sodium nitrate (88 µmol l^{-1}). Expt BY21 included controls and glucose (G) enrichment (final concentration: 100 µmol l^{-1}). All enrichment solutions were <0.2 µm filtered prior to addition to avoid prokaryotic contamination. To avoid limitation of the added nutrients and glucose during the duration of the experiments, all enrichments were conducted in excess. The ratio for glucose and ammonium addition was calculated based on a bacterial C:N ratio of 3.5 to 3.9 (Lee & Fuhrman 1987); a ratio for nitrate and ammonium addition of 1.7 was chosen after Hagström et al. (1979).

Low O_2 conditions were obtained by gentle bubbling with a gas mixture containing 0.13% of CO_2 in pure N_2 for 4 h to avoid an increase in pH during O_2 degassing (R. Kiko pers. comm.). Prior to bubbling, the N_2-CO_2 gas mixture was passed through an O_2 reduction column (copper catalyst, Glasgerätebau Ochs) to remove possible O_2 residues. For the oxic treatments, samples were gently bubbled with synthetic air (21% O_2 in N_2 with <1 ppm CO_2) to induce similar perturbations by bubbling. After bubbling, low O_2 bottles were closed with a gas-tight cap and septum, and oxic bottles were covered with gas-permeable parafilm. All bottles were incubated at 10°C on a shaker in the dark for 109 h (BY15) or 94 h (BY21).

During the experiment, 3 replicate incubation bottles per treatment were sampled. Samples for cell abundance were taken daily, for leucine-aminopeptidase (LAPase) rates after 67 h (BY15) or 69 h (BY21) and for bacterial production after 66 h (BY15) or 94 h (BY21). Dissolved organic carbon (DOC), particulate organic carbon (POC), nutrient and amino acid concentrations were determined initially and at the end of the incubation. Due to a limited sampling volume, initial nutrient and amino acid concentrations were determined from a second CTD cast sampling the same water depth immediately after the first cast. The O_2 concentration in all bottles was monitored daily.

2.2. Microbial and biogeochemical methods

2.2.1. Oxygen

O_2 concentration in the field was measured using the Oxygard sensor (PreSens) on the CTD (range: 0 to 437 µmol l^{-1} saturation; accuracy: ±1%), calibrated with Winkler titration triplicates (range: 1 to 500 µmol l^{-1}, precision: ±2 µmol l^{-1}) as described by Le Moigne et al. (2017). In each incubation bottle, O_2 concentration was measured with a calibrated noninvasive optical sensor (PreSens, detection limit: 0.5 µmol l^{-1}; accuracy: ±0.4% at 20.9% and ±0.05% at 0.2%). Values were converted to µmol l^{-1} with the O_2 solubility table from Unisense a/s (Ramsing & Gundersen) using the incubation temperature and salinity data from the CTD.

2.2.2. Cell abundance

Under the assumption that bacteria represent the major fraction of prokaryotic cells (Karner et al. 2001, Thureborn et al. 2013), bacteria were counted by flow cytometry (FACS Calibur, Becton Dickinson) according to Gasol & Del Giorgio (2000). Briefly, 4.5 ml were fixed with 25% glutaraldehyde (1% final concentration) and stored at −20°C until analysis (maximum 1 mo). Immediately before analysis, samples were sonicated for 5 s, filtered through a 50 µm mesh and diluted 10-fold. Then 400 µl of sample were mixed with 10 µl Fluoresbrite® fluorescent beads (Polyscience) and Sybr Green (Invitrogen) (final concentration 1×). The flow of the cytometer was calibrated with 2 standards containing a known amount of reference TruCount Beads™ (BD) and the Fluoresbrite® fluorescent beads (Polyscience), which were added to each sample. The theoretical detection limit was 34 cells ml^{-1} of sample, and the measurement error associated with this method was 2%.

2.2.3. Extracellular enzyme activity

The rate of the extracellular enzyme LAPase was determined using the fluorescent substrate analogue L-leucine-7-Amido-4-Methylcoumarin (Hoppe 1983). Calibration was done with defined concentrations of 7-Amino-4-Methylcoumarin. To determine enzyme kinetics, substrates were added to 5 ml samples,

yielding final concentrations of 1, 5, 10, 20, 50, 80, 100 and 200 µmol l^{-1}. Samples were incubated in the dark at 10°C and room O_2 concentrations for oxic conditions or placed in a gas-tight incubator filled with N_2 for low O_2 conditions. Fluorescence was measured after 6 h on a plate reader fluorometer (FLUOstar Optima, BMG labtech) at 355 nm (excitation) and 460 nm (emission). The measurement error was estimated to 2% using a calibration with triplicates. Michaelis-Menten kinetics were calculated using the simple ligand binding function in SigmaPlot™ 12.0 (Systat Software) to obtain the maximum reaction velocity at saturating substrate concentration (V_{max}) (Chróst & Siuda 2002).

2.2.4. Bacterial heterotrophic production

Heterotrophic prokaryotic production was summarized as bacterial production, assuming that bacterial abundance exceeds archaeal abundance (Karner et al. 2001, Thureborn et al. 2013) and that archaeal production is dominated by autotrophic processes (Ingalls et al. 2006). Bacterial production was estimated from uptake of radioactive labelled (^{3}H) leucine (Kirchman et al. 1985, Smith & Azam 1992). One sub-sample of 1.5 ml per incubation bottle was incubated for 1 h at a final concentration of 20 nmol leucine l^{-1}. Low O_2 samples were bubbled with N_2 and oxic samples with synthetic air. The ^{3}H-leucine uptake was converted into carbon units using the factor 1.5 kg C mol^{-1} leucine (Simon & Azam 1989). An analytical error of 5.2% was estimated using a calibration with triplicates.

2.2.5. Nutrients

From each incubation bottle, 50 ml were filtered through a 0.45 µm pore size syringe filter (glass microfiber GD/X membrane, Whatman) and stored at −20°C until analysis. Nutrient samples from the CTD profiles were measured unfiltered. Ammonium was measured directly on board after Koroleff (1969) and Solórzano (1969). The relative standard deviation (RSD) for this method was 3%, and the detection limit 0.05 µmol l^{-1}. Samples from the incubation experiments were diluted at maximum 1:25 with ultrapure water. Phosphate, nitrate and nitrite concentrations were measured photometrically with continuous flow analysis on an auto-analyzer (QuAAtro; Seal Analytical) after Grasshoff et al. (1999) (detection limit: <0.04 µmol l^{-1}, coefficient of variation: <0.7%).

2.2.6. Dissolved organic carbon and total dissolved nitrogen

For DOC and TDN, 20 ml were filtered through a 0.45 µm syringe filter (glass microfiber GD/X membrane, Whatman), which was rinsed with sample, into a combusted glass ampoule (8 h, 500°C). Samples were acidified with 20 µl of 30% ultrapure hydrochloric acid, sealed and stored at 4°C in the dark. DOC concentrations were determined with a TOC−VCSH (Shimadzu) with 5 to 8 injections after high-temperature catalytic oxidation applying a method modified from Sugimura & Suzuki (1988) and described in more detail by Engel & Galgani (2016).

2.2.7. Dissolved hydrolysable amino acids

Dissolved hydrolysable amino acids were analyzed via high performance liquid chromatography on a 1260 HPLC system (Aglient Technologies) using a C_{18} column (Phenomex Kinetex) after in-line orthophthaldialdehyde derivatization with mercaptoethanol (Lindroth & Mopper 1979, Dittmar et al. 2009). The following amino acids were analyzed: alanine, arginine, glycine, leucine, phenylalanine, serine, threonine, tyrosine, valine, aspartic acid + asparagine (co-eluted), glutamine + glutamic acid (co-eluted), γ-aminobutyric acid and isoleucine. For each sample, 20 ml were filtered through a rinsed Acrodisc® 0.45 µm GHP membrane (Pall) into combusted glass vials (8 h, 500°C) and stored at −20°C. Samples were hydrolyzed at 100°C for 20 h with HCl before analysis according to Lindroth & Mopper (1979). The acid was then evaporated under vacuum at 60°C and washed with Milli-Q water. The detection limit of this method is <1.4 nmol l^{-1}. Using mol% of individual amino acids, the degradation index (DI; Dauwe & Middelburg 1998, Dauwe et al. 1999) of organic matter was calculated as an indicator of organic matter lability using the factor coefficients developed for DOM by Kaiser & Benner (2009). Thereby, fresher and more accessible organic matter is indicated by a higher DI.

2.2.8. Particulate organic carbon and particulate nitrogen

Aliquots (200–400 ml) were filtered (<200 mbar) onto combusted (8 h, 500°C) GF/F filters (~0.7 µm) and stored at −20°C until analysis. Prior to analysis,

filters were acid-fumed (37% hydrochloric acid) (6 h) to remove carbonates and dried (6 h; 60°C). POC and PN concentrations were determined with an elemental analyzer (Euro EA, Hechatech) after Sharp (1974). The instrument was calibrated with acetanilide (0.1 to 0.7 mg) and soil standard (2 to 4 mg). The measurement error of the instrument was 0.4%.

2.3. Turnover of organic carbon

POC formation and DOC uptake were estimated by the difference in POC and DOC concentrations (ΔPOC and ΔDOC) between the start and end of the incubation. ΔPOC:ΔDOC was used as an estimate of the growth efficiency after Kroer (1993). Growth efficiency is defined by the bacterial biomass that is produced relative to the carbon that has been taken up.

2.4. Data analysis

There were always n = 3 replicate incubation bottles. For the oxic control of BY21, only 2 POC:PN filters were analyzed. The impact of O_2 and nutrient concentrations on bacterial activity was statistically tested by a multifactorial ANOVA or a robust ANOVA with the WRS package (Wilcox 2005) applying bootstrapping (2000 replicates) and comparing the median (Field et al. 2012). The differences between the O_2 levels within one nutrient treatment were tested using independent *t*-tests for normally distributed data and the Wilcoxon rank order test for data that were not normally distributed (see Tables S1 & S2 in the Supplement at www.int-res.com/articles/suppl/a083p095_supp.pdf) with a significance level of $p \leq 0.017$, to account for the Bonferroni correction (Field et al. 2012). All statistical tests and plots were conducted in R version 3.4.2 (R Development Core Team 2008).

3. RESULTS

3.1. Oxygen

O_2 concentrations during BY15 differed clearly between low and high O_2 treatments throughout the experiment with differences in O_2 concentrations of at least 107 µmol O_2 l^{-1}, except for 1 incubation bottle (oxic GPAN) on 1 sampling day (Fig. 1A). O_2 concentrations increased over time in the low O_2 incubations, first in the control and subsequently in the enriched incubations. However, after 66 h, low O_2

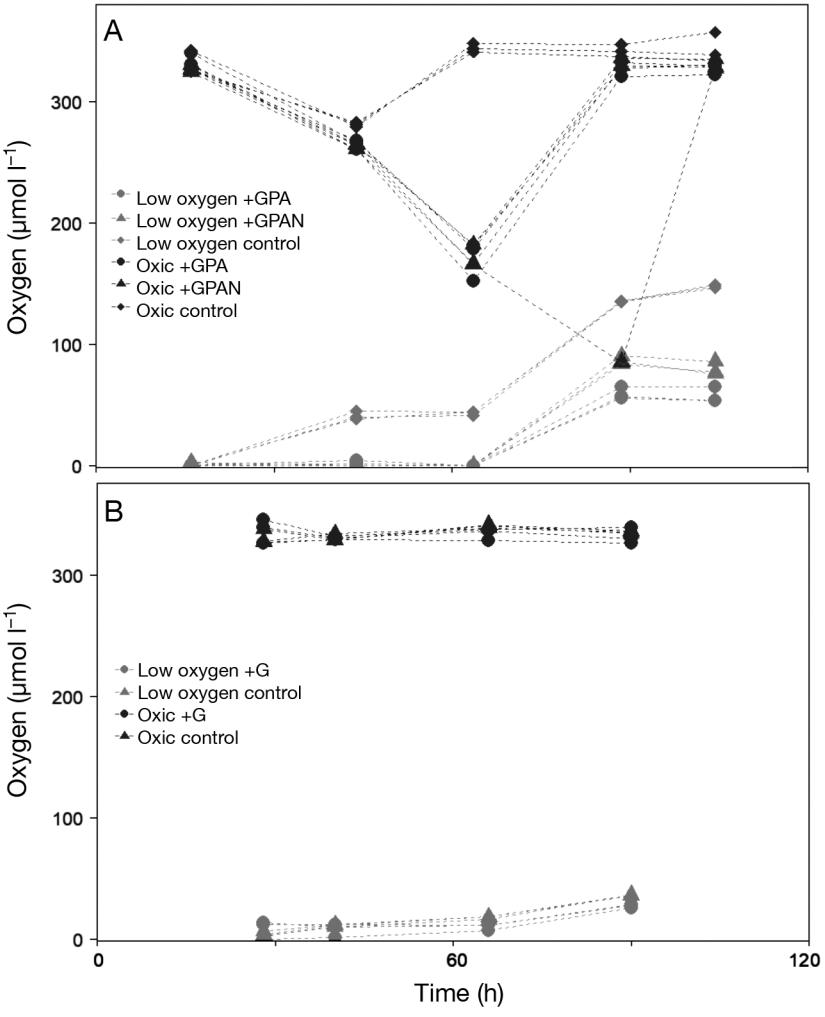

Fig. 1. Temporal development of oxygen (O_2) concentrations in each incubation bottle during (A) BY15 and (B) BY21. Nutrient treatments: +GPA (addition of glucose, phosphate and ammonium), +GPAN (addition of glucose, phosphate, ammonium and nitrate) and +G (addition of glucose). Light grey symbols indicate low O_2 incubations and dark grey the oxic ones. Symbols indicate in (A) the control with ambient nutrient concentrations (diamonds) and the ones with added glucose, ammonium, phosphate (circles) and additionally nitrate (triangles). In (B), symbols indicate the control with ambient glucose concentrations (triangles) and the ones with added glucose (circles)

incubations were still suboxic (GPA and GPAN) or ≤ 44 µmol O_2 l^{-1} (controls). For BY21, O_2 concentrations varied from below the detection limit to 36 µmol O_2 l^{-1} in the low O_2 treatment and ranged from 326 to 346 µmol O_2 l^{-1} in the oxygenated incubations (Fig. 1B).

3.2. Bacterial activity

Bacterial activity was determined by changes in cell abundance as well as by bacterial production and LAPase rates. During BY15, bacterial cell abundance increased significantly 66 h after nutrient addition ($p < 0.01$) (Figs. 2A & 3A, see Table S3 in the Supplement at www.int-res.com/articles/suppl/a083p095_supp.pdf). In the GPA and GPAN treatments, cell abundance peaked 82 h after nutrient addition. At the time of sampling for LAPase and bacterial production (after 66 h), cell abundance differed between O_2 treatments ($p = 0.02$) (Table S3) and was reduced in the low O_2 GPA treatment (GPA: −14% to −140%) compared to the oxic ones. In the GPAN treatment, cell abundance varied, but on average cell abundance was lower (−24%) in the low O_2 than in the oxic incubations (Fig. 3A) (Table 1). Cell abundance in the oxic and low O_2 controls was similar and showed little variation over time (Fig. 2A). In the incubations of BY21, addition of only glucose did not result in significantly higher cell abundance (Fig. 2B). Cell abundance in both control and G increased slowly over time with an average abundance of 2.9 × 10^6 cells ml^{-1} ± 16% for all incubations (Fig. 2B).

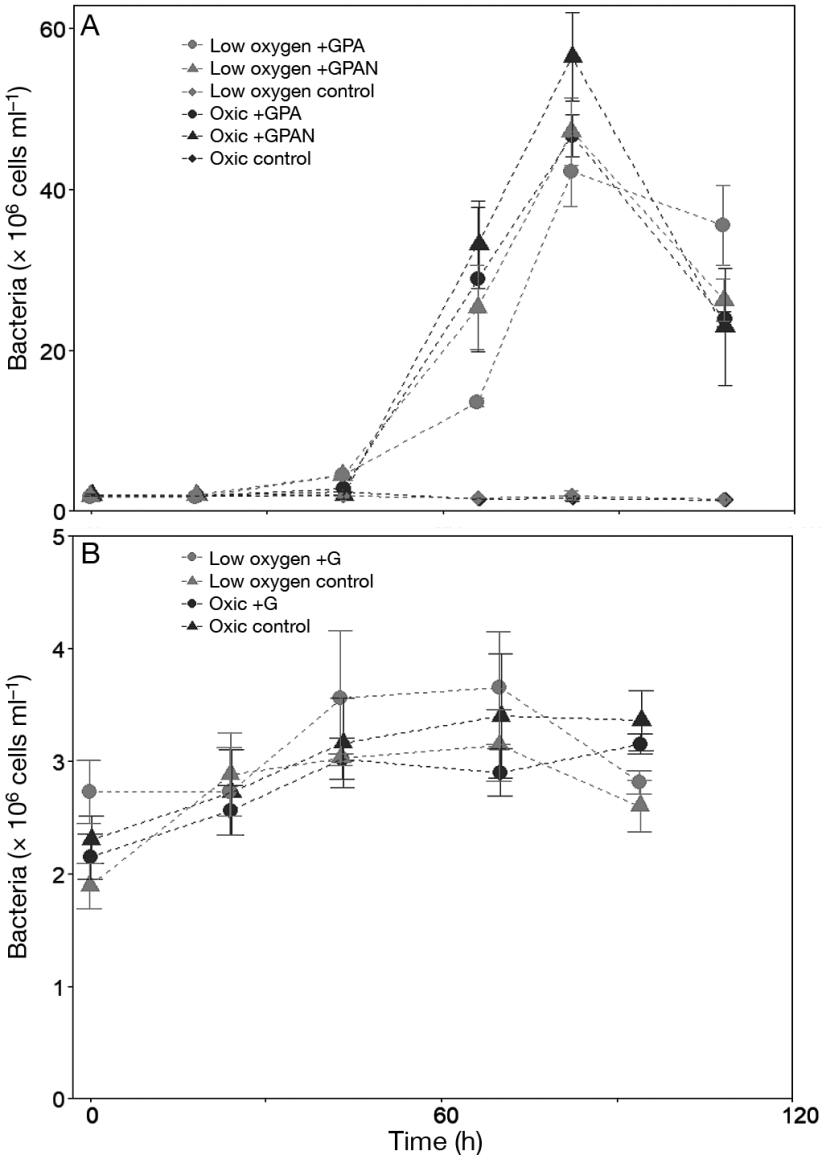

Fig. 2. Changes in average bacterial abundance over time for 3 oxic and low O_2 incubation bottles per treatment (mean ± SD) for the batch experiment (A) BY15 and (B) BY21. See Fig. 1 for symbols and abbreviations

Total and cell-specific bacterial production increased significantly 66 h after nutrient addition during BY15 ($p_{total} < 0.01$, $p_{cell\text{-}specific} < 0.01$) (Fig. 3B, Table S1). Total bacterial production was significantly higher in the oxic compared to the low O_2 treatments ($p < 0.01$), depending on the nutrient and glucose addition ($p < 0.01$) (Table 1, Table S3). With GPA addition, bacterial production was 23 to 32% lower under low O_2 conditions. With GPAN addition, the differences between the O_2 treatments were slightly smaller, with bacterial production being 7 to 32% lower in the low-O_2 incubations (Fig. 3B). In contrast, cell-specific bacterial production was rather lower under oxic conditions, indicating that higher cell abundance in the oxic treatment was responsible for the enhanced total bacterial production (Fig. 3A,B, Table S1). For BY21, bacterial production was significantly higher in incubations with glucose than in the ones without ($p < 0.01$) (Fig. 3B, Table S3). A significant effect of O_2 on bacterial production was only observed in the controls, i.e. without glucose addition, with significantly higher total bacterial production in the oxic treatment than in the low-O_2 one ($p = 0.01$) (Fig. 3B, Table S2, Table 1).

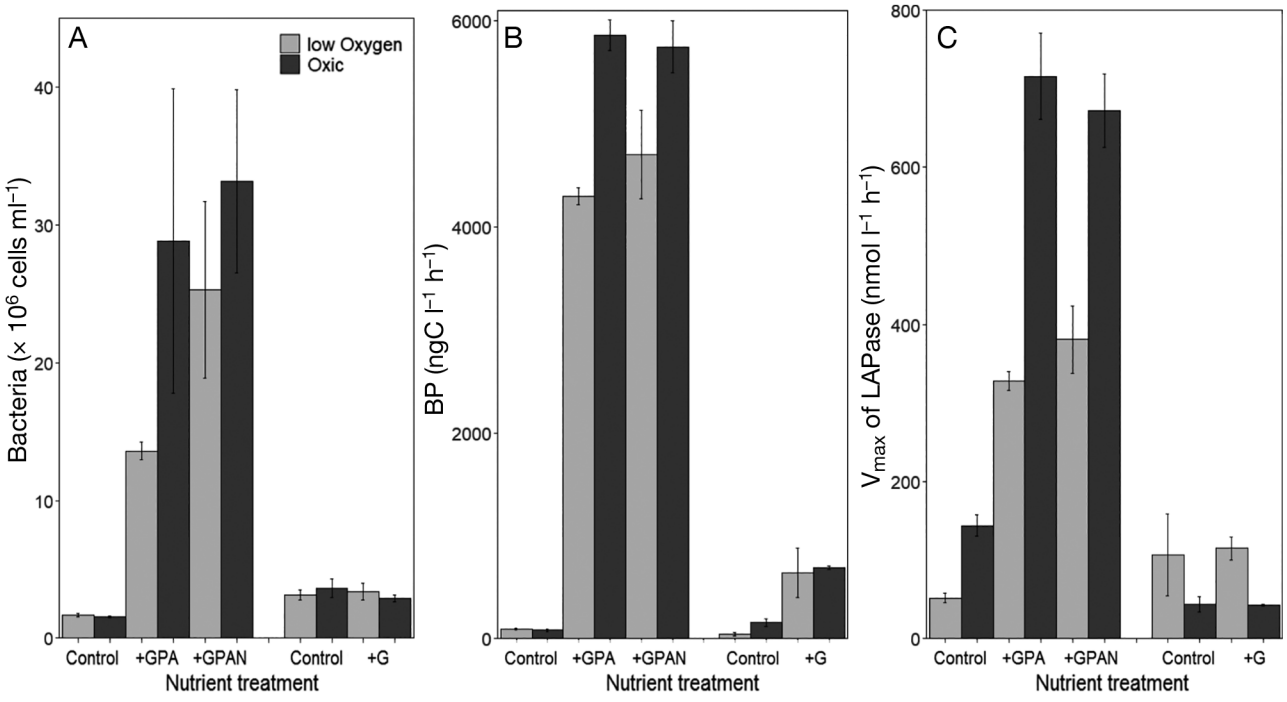

Fig. 3. Effects of nutrient and oxygen treatments on bacterial communities in Expts BY15 and BY21 after 3 to 4 d of incubation: (A) Bacterial abundance, (B) bacterial production (BP) and (C) V_{max} of leucine-aminopeptidase (LAPase). In the control, only the oxygen concentration was modified. See Fig. 1 for abbreviations

LAPase rates differed strongly between treatments with and without nutrient addition during BY15 (Fig. 3C). Total LAPase rates were higher for GPA and GPAN ($p < 0.01$), while cell-specific LAPase rates were higher in the controls ($p < 0.01$), implying that higher total LAPase rates in GPA and GPAN were based on higher cell abundance also (Tables S1 & S3). During BY15, total LAPase rates were significantly higher in the oxic incubations for both GPA and GPAN compared to the low-O_2 treatments ($p < 0.01$) (Fig. 3C, Table 1), while cell-specific LAPase rates showed no difference between the O_2 treatments. In the controls, both total and cell-specific LAPase rates were significantly higher in the oxic than in the low-O_2 incubations ($p < 0.01$) (Table 1). During BY21, LAPase rates did not differ between the controls and the treatments with glucose addition (Fig. 3C). Effects of the O_2 treatments in BY21 differed from those during BY15. In BY21, total and cell-specific LAPase rates were significantly higher in the low-O_2 incubations after glucose addition ($p_{total} < 0.01$, $p_{cell\text{-}specific} = 0.01$) (Fig. 3C, Table 1).

3.3. Biogeochemical transformations

To test whether the turnover of organic matter differed between O_2 and nutrient treatments, we compared oxic and low-O_2 treatments with respect to changes in DIN (i.e. nitrate, nitrite and ammonium), DOC and POC concentrations at the beginning and end of the experiment. At the end of the experiment, no significant difference was observed for nitrite concentrations between the oxic and low-O_2 incubations (Table S1). Nitrate concentrations were significantly lower in the low-O_2 incubations of GPA (0.5 ± 0.0 µmol l^{-1}) and GPAN (0.5 ± 0.0 µmol l^{-1}) than in the oxic GPA (1.0 ± 0.0 µmol l^{-1}) and GPAN (86.4 ± 22.6 µmol l^{-1}) ($p < 0.01$). In the low-O_2 incubations with GPAN, there were 116.4 ± 9.8 µmol l^{-1} of ammonium; thus, concentrations were significantly higher than in the oxic incubations (64.6 ± 0.7 µmol l^{-1}; $p < 0.01$) (Table 1). For BY21, no significant differences between the oxic and low-O_2 incubations were observed for nutrient concentrations, independent of the glucose addition (Table S2).

During BY15, addition of GPA and GPAN resulted in a strong increase in POC formation of ~160 µmol l^{-1} ($p < 0.01$) (Fig. 4A, Tables S1 & S3). Higher POC formation was observed in the oxic incubations of GPAN (5–24%), but differences between the oxic and low-O_2 treatments were not statistically significant due to a high variability within the treatments (Fig. 4A, Table S1). In the controls, POC concentration increased slightly and only in the low-O_2 incuba-

Table 1. Means (standard deviations) of sampled parameters from Expts BY15 and BY21 that differed significantly between oxygen (O_2) treatments. +GPA: added glucose, phosphate and ammonium; +GPAN: added glucose, phosphate, ammonium and nitrate. Incubations were performed in triplicate. The p-values of the pairwise comparison between different O_2 treatments with Wilcoxon or *t*-test and ANOVA results of the O_2 effect are given. The significance (*) level in the pairwise comparison was adjusted to 0.017, to account for the Bonferroni correction. See tables in the Supplement for details

Expt Parameter	Nutrient	Low O_2	Oxic	Pairwise	ANOVA
BY15					
Total bacterial production (µg C l^{-1} h^{-1})	Control	0.1 (0.0)	0.1 (0.01)	0.22	<0.01*
	+ GPA	4.3 (0.1)	5.9 (0.2)	0.10	
	+ GPAN	4.7 (0.4)	5.8 (0.3)	0.02	
Total LAPase (nmol l^{-1} h^{-1})	Control	52 (6)	144 (13)	<0.01*	<0.01*
	+ GPA	328 (12)	716 (54)	<0.01*	
	+ GPAN	381 (43)	672 (47)	<0.01*	
Cell-specific LAPase (amol $cell^{-1}$ h^{-1})	Control	31 (3)	93 (7)	<0.01*	<0.01*
	+ GPA	24 (2)	27 (8)	0.60	
	+ GPAN	16 (4)	21 (6)	0.25	
Bacterial abundance (cell × 10^6 ml^{-1})	Control	1.7 (0.1)	1.6 (0.1)	0.22	0.02*
	+ GPA	13.6 (0.7)	28.8 (11.0)	0.08	
	+ GPAN	25.3 (6.4)	33.2 (6.7)	0.22	
Nitrate (µmol l^{-1})	Control	1.5 (0.2)	1.1 (0.0)	0.05	<0.01*
	+ GPA	0.5 (0.0)	1.0 (0.0)	<0.01*	
	+ GPAN	0.5 (0.0)	86.4 (22.6)	<0.01*	
Ammonium (µmol l^{-1})	Control	2.3 (0.2)	2.1 (0.0)	0.15	0.01*
	+ GPA	59.2 (15.1)	67.3 (4.3)	0.42	
	+ GPAN	116.4 (9.8)	64.6 (0.7)	<0.01*	
DOC consumption (µmol l^{-1})	Control	0(0)	1 (2)	0.24	<0.01*
	+ GPA	−520 (7)	−583 (4)	<0.01*	
	+ GPAN	−540 (4)	−579 (7)	<0.01*	
DI	Control	−1.1 (0.1)	−1.2 (0.2)	0.40	0.01*
	+ GPA	3.1 (0.2)	1.7 (0.4)	<0.01*	
	+ GPAN	2.3 (0.8)	1.3 (1.1)	0.29	
BY21					
Total bacterial production (µg C l^{-1} h^{-1})	Control	0.1 (0.0)	0.2 (0.0)	0.01*	0.5
	+ G	0.6 (0.2)	0.7 (0.0)	0.73	
Total LAPase (nmol l^{-1} h^{-1})	Control	107 (52)	44 (10)	0.11	<0.01*
	+ G	115(14)	43 (1)	<0.01*	
Cell-specific LAPase (amol $cell^{-1}$ h^{-1})	Control	33 (15)	13 (3)	0.08	<0.01*
	+ G	32 (7)	15 (2)	0.01*	
DI	Control	−1.1 (0.2)	−1.3 (0.1)	0.22	0.04*
	+ G	−1.3 (0.2)	−1.7 (0.3)	0.13	

tions. Initial molar POC:PN ratios of BY15 were 6.44. In the controls, molar POC:PN ratios were variable (4.8–10.3) at the end of the incubation, whereas they clearly decreased in GPA and GPAN incubations (4.2–4.5). Addition of glucose during BY21 resulted in an increase of ~5 µmol l^{-1} of POC, at the end of the experiment, whereas POC concentrations decreased slightly in the controls (Fig. 4A, Table S2). There were no significant differences in POC concentrations with respect to the O_2 treatments either. Molar POC:PN ratios varied between 5.7 and 7.6, similar to the initial ratio (5.88).

We estimated the growth efficiency from the ratio of POC formation and DOC consumption (ΔPOC:ΔDOC). The growth efficiency was ~30% (23–37%) in the GPA and GPAN treatments (without a significant difference between nutrient treatments) during BY15 and ~20% (11–26%) during BY21 in the incubations with glucose. There was no significant difference between the O_2 treatments (Tables S1 & S2). Growth efficiency was not estimated for the controls as no net increase in POC concentration was detected.

Similar to POC formation, the uptake of DOC, mainly in form of added glucose, was stimulated by the addition of inorganic nutrients. In the controls during BY15, we observed no significant O_2 effect on DOC consumption or on DOM degradation, as indicated by the DI (Fig. 4B,C). In the GPA and GPAN treatments, the addition of 100 µmol l^{-1} glucose to the initial DOC concentration of 262 µmol l^{-1} should have increased the initial concentration to 862 µmol l^{-1} DOC. Thereby, the consumed DOC (DOC_{start}–DOC_{end}) almost equaled the amount of added glucose (Fig. 4B). Still, in the low-O_2 treatments, significantly more DOC was left over than in the oxic treatments ($p < 0.01$) (Fig. 4B), indicating less uptake or greater release of DOC in the low-O_2 treatments (Table 1). Production of new DOM in the GPA and GPAN treatments, under oxic and low-O_2 conditions, was inferred from changes in organic matter lability as indicated by the amino acid based DI. The final DI was clearly higher in the GPA and GPAN treatment compared to the initial conditions (–0.39) (Fig. 4C), indicating the formation of more labile amino acids. Thereby, the DI was even higher in the low-O_2 treatments, with a significant difference in the GPA treatment ($p < 0.01$) (Fig. 4C, Table 1). For

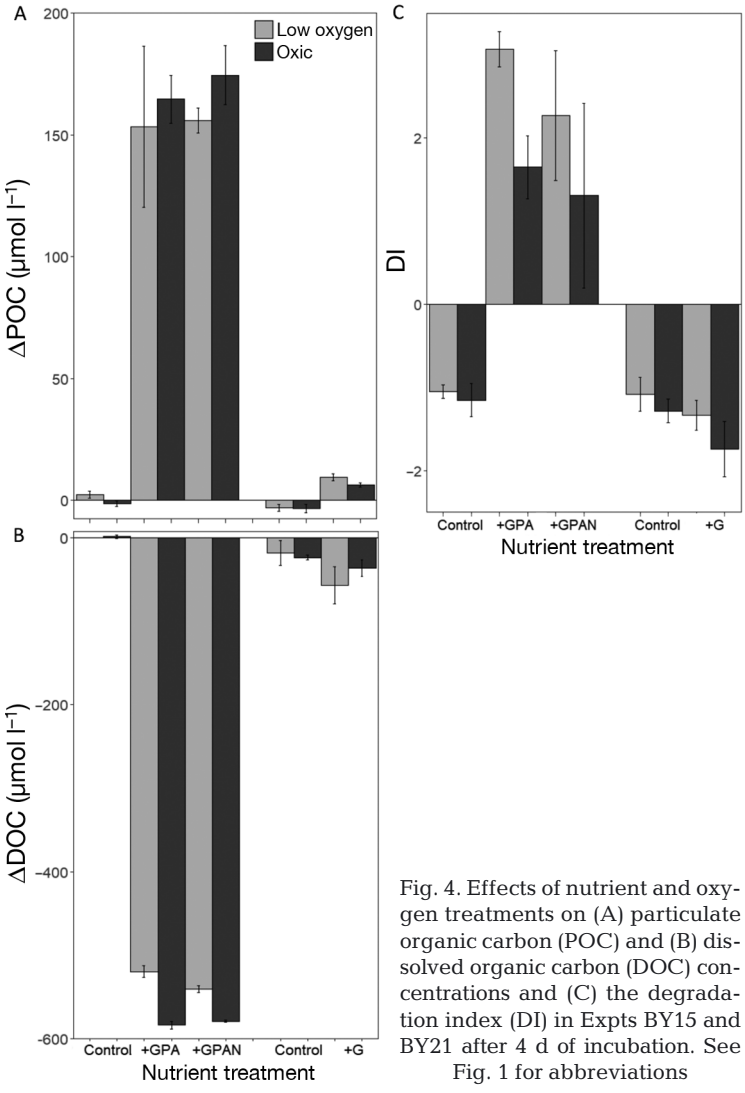

Fig. 4. Effects of nutrient and oxygen treatments on (A) particulate organic carbon (POC) and (B) dissolved organic carbon (DOC) concentrations and (C) the degradation index (DI) in Expts BY15 and BY21 after 4 d of incubation. See Fig. 1 for abbreviations

BY21, the initial DOC concentration was 291 µmol l^{-1}, and the DI was −0.91. At the end of the experiment, DOC consumption was higher in the G treatment ($p = 0.02$) (Table S3). However, only ~25 µmol l^{-1} more DOC was taken up compared to the control (Table S2), suggesting that only a small fraction of the added glucose was consumed without the addition of inorganic nutrients. Between O_2 treatments, there were no significant differences in DOC consumption. Organic matter lability (DI) was similar to the starting conditions.

4. DISCUSSION

We conducted 2 incubation experiments to investigate whether bacterial degradation of organic matter is affected by changes in O_2 concentrations. In the GPA and GPAN treatments, labile organic substrate (glucose) and nutrients, including the potential electron acceptor nitrate, were offered in excess to assure alleviation of limitation throughout the experiment. However, the amount of added nutrients and substrate in the G, GPA and GPAN treatments likely induced changes in the bacterial community composition. For example, it seems likely that fast-growing copiotrophic heterotrophic bacteria could benefit from the high glucose supply, and nitrifiers, which appear in the sampling area (Hietanen et al. 2012), were favored by high levels of ammonium. Thus, we cannot exclude that O_2 effects discussed below will vary with initial biogeochemical and microbial conditions because we did not repeat all nutrient treatments in both experiments and some parameters sampled from the controls did slightly differ between experiments. Extrapolating results from our experiments to other OMZ communities should be done with caution, in particular if the biogeochemical conditions are distinct from those in the Gotland Basin.

Bacterial growth can be limited by several factors, including labile organic carbon, inorganic nutrients and electron acceptors (e.g. Stewart 1988, Jørgensen et al. 1999, Bonaglia et al. 2016). In our study, bacterial production increased after the addition of glucose solely (Fig. 3B), suggesting a limitation of the bacterial community by labile organic carbon. After the addition of glucose and inorganic nutrients, significant amounts of glucose were taken up during the experiments, accompanied by strongly increasing cell abundance and bacterial production (Figs. 3A,B & 4B). Inorganic nutrients therefore were mainly controlling bacterial activity in this experiment.

Under suboxic and anoxic conditions, nitrate is the energetically most favorable electron acceptor for anaerobic respiration (Lam & Kuypers 2011). However, the reduction of nitrate yields less energy than the reduction of O_2, and the conversion of energy into biomass after denitrification was found to be less than expected by the chemical energy yields (e.g. Strohm et al. 2007). In the suboxic water incubated in our experiments, nitrate concentrations were close to the detection limit. Under nitrate and O_2 limitation, bacteria might conduct fermentation, with an even lower energy yield than the reduction of nitrate

(Madigan et al. 2012). We, therefore, expected (1) stronger bacterial growth under low-O_2 conditions after the addition of nitrate as electron acceptor (GPAN) than in the treatment without nitrate (GPA) and (2) enhanced bacterial growth in the oxic treatments, in contrast with the low-O_2 treatments. In our experiments, the addition of nitrate enabled denitrification or DNRA, as indicated by higher ammonium and lower nitrate concentrations observed in the low O_2 compared to the oxic GPAN treatment (Table 1). While cell abundance in the GPA treatment was indeed much lower than in GPAN, bacterial production was rather similar, indicating an efficient glucose utilization by other anaerobic respiration pathways than the reduction of nitrate and by fermenting bacteria (Fig. 3A,B). However, since our incubations were not fully anoxic, microaerobic respiration might have additionally fueled bacterial activity in both GPA and GPAN and weakened potential differences between the oxic and low-O_2 treatments. In our study, bacterial production was reduced at low O_2 concentrations, in both GPA and GPAN treatments (Fig. 3B), corroborating the finding of reduced heterotrophic bacterial growth under low-O_2 conditions (e.g. Stewart 1988). Cell-specific bacterial production, an estimate of the community growth rate, did not show the same trend, suggesting mortality e.g. by viruses and protists or temporal variations in cell-specific bacterial production during the low-O_2 incubation that were not captured by our sampling design. In suboxic waters of the Baltic Sea, protist bacterivory was shown to be a major reason for prokaryotic mortality and exceeded mortality by viral infections (Anderson et al. 2012). However, heterotrophic nanoflagellates, that are known bacterivores, appear near the oxyclines but seem less active at the redoxcline in the Baltic Sea and in anoxic lakes (Weinbauer & Höfle 1998, Anderson et al. 2012). Viruses are assumed to infect up to 62% of the free-living bacteria in marine systems (Proctor et al. 1993), and high cell losses by viral lysis under anoxic conditions were documented for lakes (Weinbauer & Höfle 1998) and in anoxic deep waters of the Cariaco Trench (Taylor et al. 2003). Viruses and protists should have been equally distributed between the incubations but might have developed differently during incubation and influenced bacterial abundance.

To access HMW organic matter, bacteria excrete extracellular enzymes, which can control organic matter degradation in marine systems (Hoppe et al. 2002). O_2 concentrations may affect extracellular enzyme rates in 2 ways: (1) the lower energy yield of anaerobic respiration compared to oxic respiration may result in a decreased production of extracellular enzymes; (2) the need to oxidize more organic substrate to counteract the reduced energy yield by anaerobic respiration enhances the production of extracellular enzymes. In our experiments, the trend of higher LAPase rates in the oxic GPA and GPAN treatments compared to the low-O_2 treatments did not persist after the normalization to cell abundance (Table S1). Moreover, LAPase rates in the controls differed between the experiments (Fig. 3C). Thus, our results do not support the idea of a consistent direct O_2 effect on LAPase rates, being in line with earlier incubation experiments investigating effects of O_2 deficiency (Hoppe et al. 1990). Nevertheless, the reduced abundance of bacteria observed under low-O_2 conditions in the treatments amended with nutrients and glucose may have been responsible for the reduced total LAPase rates in these treatments (Fig. 3C). If applicable to the field, this may suggest a reduced degradation potential of HMW organic matter in low-O_2 systems where substrate and nutrient availability is not limiting.

Elevated bacterial growth under oxic conditions in our experiments implies greater turnover and remineralization of organic matter. Therefore, we analyzed the uptake of DOC, the formation of POC and the change in substrate lability as given by the amino acid based DI (Kaiser & Benner 2009). In all unamended controls, uptake of DOC and formation of POC did not differ between O_2 levels, revealing that carbon turnover of heterotrophic bacteria was not primary limited by O_2 availability. Addition of glucose and inorganic nutrients had a stronger stimulating effect on the turnover of carbon under oxic compared to low-O_2 conditions (Fig. 4A,B). In particular, the net uptake of DOC was co-limited by the availability of O_2 (Fig. 4B). The uptake of DOC was accompanied by the formation of POC. It can be assumed that POC accumulating in the nutrient- and glucose-amended incubations was mainly bacterial biomass, since the POC:PN ratio was close to that of bacteria (Section 3.3) (Goldman et al. 1987, Lee & Fuhrman 1987, Billen et al. 1990, Caron et al. 1995). Therefore, the growth efficiency can be estimated by the ratio ΔPOC:ΔDOC (Kroer 1993), reflecting the ratio between the carbon uptake and the carbon that is incorporated into biomass. Relatively high growth efficiencies of 23 to 36% in the GPA and GPAN treatments were at the upper range of previously reported data for the ocean and may be explained by high labile carbon and nutrient supply (Del Giorgio & Cole 1998). Little is known about the growth efficiency of

aerobic compared to anaerobic microbial communities. In our study, ratios of ΔPOC:ΔDOC did not differ between the O_2 treatments (Tables S1 & S2), suggesting no direct O_2 effect on growth efficiency at low O_2 concentrations. However, growth efficiency may be higher in fully anoxic systems due to chemoautotrophy as suggested by Keil et al. (2016).

Reduced bacterial growth and uptake of organic matter under low O_2 as well as nutrient- and glucose-amended conditions suggest a reduced transport of DOM from bacteria to higher trophic levels in suboxic waters. Reduced degradation of organic matter under hypoxic and anoxic conditions could explain DOC accumulation in the water column (e.g. Devol & Hartnett 2001, Engel et al. 2017, Le Moigne et al. 2017). The higher DI values at the end of the experiment in the nutrient- and glucose-amended treatments compared to the beginning suggest an accumulation of more labile DOM. Additionally, the higher DI values in the low-O_2 incubations (GPA) compared to the oxic ones of our experiments (Fig. 4C) suggest a reduced degradation of DOM or greater production and accumulation of labile DOM. This finding is in line with a previous study showing slower degradation of proteins under anoxia (Nguyen & Harvey 1997) or points towards enhanced extracellular release or viral lysis. The latter might also explain the (on average) lower cell abundance in the low-O_2 treatments (see above). Former studies suggested viral cell lysis as a source of labile DOM that is enhanced in anoxic waters (Proctor et al. 1993, Taylor et al. 2003). The enhanced O_2 effect on DI in combination with the addition of glucose and inorganic nutrients points towards multifactorial environmental control of bacterial organic carbon turnover in suboxic waters.

In the Baltic Sea, the inflow of O_2-rich North Sea water can oxygenate the deep basins (e.g. Mohrholz et al. 2015), whereas at least parts of the intermediate waters remain O_2-depleted (e.g. Dellwig et al. 2018). Oxygenation has a profound effect on nutrient concentrations. Phosphate is bound to the sediment, and ammonium is oxidized to nitrate. The nitrate remains within the water column, since it does not become reduced to dinitrogen gas (Nausch et al. 2003). The results of our incubation experiments suggest that concentrations of labile organic matter would be higher in suboxic or anoxic waters, while the turnover of organic matter would be higher in oxygenated waters, until the bacterial activity in the oxygenated water becomes nutrient-limited. In this case, oxyclines may represent systems, where higher amounts of labile DOM, diffusing from O_2-depleted waters, might fuel the bacterial activity in the more oxygenated waters. This would result in locally high rates of organic matter degradation and bacterial growth and might explain high cell-specific bacterial production measured at oxic-anoxic interfaces in the Baltic Sea (Brettar et al. 2012). Consequently, near oxyclines, more organic matter and subsequently bacterial biomass would enter the food chain and afterwards be respired to carbon dioxide, which might be released into the atmosphere, acting as a greenhouse gas. These potential implications remain to be verified because additional factors such as temperature, the abundance of higher trophic levels and the bacterial community composition can influence the degradation of OM under different O_2 concentrations. For instance, the composition of the bacterial community might favor syntrophy and therefore change the energetic yields of anaerobic degradation processes (Morris et al. 2013 and references within). Consequently, more multifactorial experiments with natural microbial communities are needed to fully identify the influence of O_2 concentrations on bacterial degradation of OM.

5. SUMMARY

The increase of hypoxic and anoxic zones in marine waters requires a better understanding of the microbial organic matter cycling under different O_2 concentrations. In the Baltic Sea, as well as in other enclosed and coastal seas, O_2 and nutrient dynamics are strongly coupled. We showed that nutrient and O_2 concentrations can co-determine bacterial activity and degradation of organic matter. A reduced bacterial activity and organic matter turnover under low O_2 compared to oxic conditions may also be related to differences in cell mortality, e.g. by viral lysis. If our experimental results were applicable to the field, we may speculate that organic matter cycling and carbon transfer to higher trophic levels decline under nutrient-rich hypoxic conditions, resulting in less respiration of organic carbon to carbon dioxide and enhanced storage of carbon in the ocean.

Author contributions: M.M., J.P. and A.E. designed the experiment. M.M. and J.P. performed the experiment and analyzed the data. M.M. and A.E. wrote the manuscript. F.A.C.L.M. and C.C.-N. helped performing the experiment and sampled the experiment and the water column. A.E. and J.P. designed the scientific program of the cruise. All authors assisted writing.

Acknowledgements. We thank Jon Roa for the analysis of dissolved organic carbon, Tania Klüver for the particulate organic carbon measurements, Ruth Flerus for the analysis of amino acids and Bettina Domeyer for the analyses of nutrients. Moreover, we thank Sonja Endres, Alexandra Loginova, Kerstin Nachtigall, Mark Lenz, Birthe Zäncker Angela Stippkugel, Carola Wagner, Clarissa Karthäuser and Moritz Ehrlich for the support conducting the experiment and the data analyses. We thank the crew, officers and the captain of the RV 'Alkor' for the support on board and the successful cruise. We also thank the anonymous reviewers for the helpful comments. This study is a contribution to the Collaborative Research Center 754 / SFB Sonderforschungsbereich 754 'Climate-Biogeochemistry Interactions in the Tropical Ocean'. This research was supported by Helmholtz-Gemeinschaft Deutscher Forschungszentren, a Fellowship of the DFG Excellence Cluster 'The Future Ocean' (CP1403 to F.A.C.L.M.) and by a DAAD short term grant (57130097 to C.C.-N.)

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Editorial responsibility: Gerhard Herndl, Vienna, Austria

Submitted: March 14, 2018; Accepted: March 4, 2019
Proofs received from author(s): June 2, 2019