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Mixing depth and allochthonous dissolved organic carbon: controlling factors of coastal trophic balance

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ABSTRACT: The interacting effects of different mixing depths and increased allochthonous dissolved organic carbon (DOC) on the ratio of heterotrophic to autotrophic production (i.e. trophic balance) was evaluated in a mesocosm study with a stratified water column. An autumn plankton community from the northern Bothnian Sea showed significantly decreased phytoplankton production and somewhat increased bacterial production with added DOC. In addition, increased mixing depth further reduced phytoplankton production. With a deep pycnocline and added DOC, the system became net-heterotrophic, with an average bacteria-to-phytoplankton production ratio of 1.24. With a deep pycnocline without added DOC, the trophic balance was changed to 0.44 (i.e. autotrophic). With a shallow pycnocline, the system remained net-autotrophic irrespective of DOC addition. We propose that increased precipitation in northern Europe due to climate change may result in changed density stratification and increased allochthonous DOC transport to the sea, leading to more heterotrophic coastal aquatic ecosystems. Such a scenario may entail reduced biological production at higher trophic levels and enhanced CO_2 emission to the atmosphere.

KEY WORDS: Trophic · Balance · Phytoplankton · Bacterioplankton · Mixing · Depth · DOC · Mesocosm

INTRODUCTION

Climate change models of global warming indicate increased precipitation for northern Europe, which in turn will increase river transport into the sea (Räisänen et al. 2004, Dore 2005). An estimated 20% increase in precipitation for northern Scandinavia (Meier 2006) would reduce salinity in adjacent seawater, change the vertical stratification in the water column and increase the discharge of nutrients as well as dissolved and particulate material into the sea. Therefore, in the present paper, we seek to experimentally evaluate the potential effects on a plankton community of changes in water-column stratification and in the supply of allochthonous dissolved organic carbon (DOC) and assess their relative importance. We used a unique experimental mesocosm facility in which we simulated a natural stratified water column, with a warmer upper mixed layer and a more stagnant lower layer, and we focused on the balance between heterotrophic and autotrophic production.

The projected increase of allochthonous DOC is of special interest in plankton ecology because DOC may act as a substrate for heterotrophic bacteria and yet may also reduce light penetration into the water column via brownification due to its humic contents. Earlier studies strongly support that allochthonous DOC acts as a key factor for bacterial production, both in the coastal zone and in lakes (Opsahl & Benner 1997, Jansson et al. 2000, Raymond & Bauer 2000, Hessen et al. 2010).

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From field studies in the Baltic Sea, Sandberg et al. (2004) concluded that bacterial production in the Bothnian Bay was not coupled to primary production and was strongly dependent on terrigenous DOC, whereas the bacterial production in the more southern Bothnian Sea was mainly based on DOC from primary producers. Wikner & Andersson (2012) used long-term monitoring data from the northern Bothnian Sea to study the effects of variable freshwater river discharge on the balance between bacterial and primary production, and found that years with unusually high river discharge caused a 2.2-fold increase in the bacteria-to-phytoplankton production ratio at the basin scale. A climate scenario of increased precipitation in northern Europe (Meier 2006) will cause increased microbial heterotrophy, and therefore decreased transfer efficiency to higher trophic levels. These projections are comparable to the known impacts of allochthonous DOC in limnic ecosystems, where shifts between food chains based on heterotrophic production and those based on primary production can take place after moderate changes in allochthonous DOC loading (Jansson et al. 2000).

One factor related to projected climate change is the change in water-column stratification. Increased temperature and freshwater discharge will change the density stratification in the sea, with significant consequences for primary production through altered light conditions. Studies in different lakes, both from field measurements (e.g. Kunz & Diehl 2003) and from mesocosm experiments in the lakes (e.g. Berger et al. 2007) have shown that both the production and temporal succession of phytoplankton is governed by the density stratification of the water column.

As discharge of river water and dissolved organic matter (DOM) would primarily imply increased supply of nutrients, our primary hypothesis was a fertilization response with increased primary production, and negligible effect of light extinction and a deeper mixed layer. The alternative hypothesis, however, was reduced light climate by coloured DOM and a mixed layer exceeding the photic zone, amplified by bacterial growth and competition for mineral nutrients. The latter scenario would result in a shift in trophic balance to marked net heterotrophy.

MATERIALS AND METHODS

The experiment was conducted in the indoor mesocosm facility at Umeå Marine Sciences Centre, University of Umeå, Sweden, situated at the northern Bothnian Sea (63° 34′ N, 19° 50′ E) in the Baltic Sea. The mesocosm facility consists of 12 insulated black cylindrical polyethylene tanks, 0.75 m in diameter and 5 m in height. The tanks were filled with unfiltered brackish water from ca. 4 m depth taken near the laboratory. All 12 tanks were filled in parallel in order to minimize differences between tanks due to variability over time in the inlet water. A temperature control system (Honeywell AB) maintained a constant temperature in each of 3 vertical sections of the tanks. We kept the thermocline at either 1.5 m (shallow pycnocline) or 3.5 m (deep pycnocline). To stabilize the stratification, the salinity of the bottom water was increased by 0.8 g l^{-1} by the addition of scientific grade marine salt (Coralife) 24 h prior to addition of the upper surface water. The temperature in the bottom layer was kept at 10 ± 0.3°C, while the upper mixed layer was maintained at 18 ± 0.4°C.

The 12 mesocosm tanks were divided into 4 experimental groups, each with 3 replicates: (1) 'Deep+ DOC': pycnocline at 3.5 m and DOC added; (2) 'Deep–DOC': pycnocline at 3.5 m and no DOC added; (3) 'Shallow+DOC': pycnocline at 1.5 m and DOC added and (4) 'Shallow–DOC': pycnocline at 1.5 m and no DOC added. The upper water mass was mixed with a simple airlift system with continuous slow bubbling through a polyvinylchloride (PVC) pipe 3 cm in diameter placed in the tank centre and extending from the surface to the desired pycnocline depth (1.5 or 3.5 m). Each mesocosm tank was irradiated on a 16:8 h light:dark cycle by a metal halogen lamp (Philips MH/CDM-T 150 W/930) with an emission spectrum resembling that of natural sunlight, but with lower irradiance than sunlight below 400 nm. The average irradiance at the surface was ca. 400 µmol photons m^{-2} s^{-1}.

Inorganic nutrients and allochthonous DOC were added to the surface water at the start of the experiment and every third day thereafter. The additions were proportional to the volume of the upper mixed layer and represented increases of 0.9 µM ammonia, 5.8 µM nitrate and 0.7 µM phosphate. The addition of DOC corresponded to an increase in DOC of 0.36 mg C l^{-1} for each addition, equivalent to 9.2% of the initial DOC level in the brackish water. A total of 8 DOC additions were made during the month-long experiment, corresponding to a total addition of 2.86 mg C l^{-1}. The amount of DOC added to mesocosms was based on the estimated riverine inputs of DOC to the Öre estuary (Pettersson et al. 1997). The added DOC consisted of 80% (C units) laboratory-grade humic acids with a C content of 48% (Sigma-Aldrich) and 20% yeast extract with a C content of 38.7% (Difco).

This ratio of refractory (humic acid) and labile (yeast extract) DOC was chosen to reflect earlier estimates of the biologically labile fraction of riverine DOC (on average 19%; Søndergaard & Middelboe 1995).

Sampling and *in situ* measurements were performed every third day. Prior to the collection of water, vertical profiles of salinity, temperature and oxygen were measured at each 1 m depth, using an *in situ* probe (Hanna HI 9828 multiparameter instrument with probe HI 769828; see www.hannainst.com). Irradiance profiles of photosynthetically active radiation (PAR) at each 0.5 m interval were obtained using a Licor LI1400 radiometer with a LI-193 spherical sensor (LI-COR®). These data were used to calculate the light attenuation coefficient (k) and the irradiance at 0 m depth (I_0) in the exponential equation: $I_d = I_0 \times \exp(-k \times d)$, where I_d is irradiance at d m depth. The equation was then used to calculate the average irradiance in the upper mixed layer. Water samples were siphoned out through a PVC tube from the middle of the mixed layer (either 0.75 or 1.75 m depth) and the middle of the lower layer (either 3.25 or 4.25 m depth) and collected in a 20 l canister. Subsamples for different analyses were taken from these storage canisters, ensuring that the samples were well mixed. Due to a malfunction of one mesocosm tank (treatment Deep+DOC), this series only included 2 replicates.

After sampling, 2 µm filtered brackish water was added to each mesocosm tank as a replacement for the collected water samples. To this water was added nutrients and to the upper mixed layer of selected treatments, DOC. Chlorophyll *a* (chl *a*) concentration was measured after ethanol extraction of pigments from cells filtered on a Whatman GF/C filter, using a Perkin Elmer LS 30 Luminescence spectrometer (Aminot & Rey 2002). The filtrate was analysed for humic substances by fluorescence spectroscopy at 350/450 nm excitation/emission wavelength according to HELCOM standards (Coble et al. 1990, Wedborg et al. 1994). Dissolved ammonium, nitrite, nitrate, phosphate and silicate were analysed using a 4-channel autoanalyser (QuAAtro marine, Bran & Luebbe®) according to protocols of the Swedish Standards Institute and HELCOM (Grasshoff et al. 1983). For particulate organic carbon (POC) and nitrogen (PON), 100 to 200 ml of water was filtered onto duplicate pre-combusted (450°C for 2 h) GF/F glass-fibre filters, which were analysed with a Carlo Erba elemental analyser.

Phytoplankton production was measured by a 3 h incubation at mid-day of 100 ml water in Nunc culture bottles to which were added 3.2 µCi ^{14}C-bicarbonate. At each sampling occasion, 1 transparent and 1 dark bottle were incubated in the centre of the tank at mid depth of both the upper mixed layer and the stagnant bottom layer. At 3 times, additional incubations were performed at 9 depths in the upper 2.0 m. After incubation, 5 ml subsamples were withdrawn and transferred to scintillation vials. These vials were further treated with addition of 0.3 ml 5 M HCl, bubbling with air for at least 30 min, and addition and mixing with 15 ml Optiphase HiSafe 3 scintillation cocktail before counting in a Beckman scintillation counter. Carbon assimilation was calculated as previously described (Colijn & Edler 1998). To calculate phytoplankton production per m^3 in the mixed layer and per m^2 for the whole water column, we used derived equations for phytoplankton production versus depth from the depth-profile incubations combined with the results from fixed-depth incubations. Production was modelled as an exponential function of depth; from the vertical production profiles, an average exponent (k) for the change in production (P) versus depth (d, in m) was calculated to fit the equation: $P = P_0 \times \exp(-k \times d)$, where P_0 is a constant defining the production at zero depth. At each time point, the result from the 2 fixed depths was used in the equation to find the constant P_0 best fitting these 2 points, using the previously calculated value for k. This adapted equation was used to estimate the average phytoplankton production above the pycnocline and in the whole water column. Daily phytoplankton production was given by multiplying the production per hour by 16, and total production over the experimental period was calculated by integrating daily phytoplankton production over all the days.

Water samples for analyses of bacterial abundance were preserved in formaldehyde (2% final concentration), and the cells were stained with Acridine Orange (Hobbie et al. 1977, Blackburn et al. 1998). The number and volume of bacterial cells were analysed by epifluorescence microscopy (Zeiss Axiovert 100 Inverted Microscope, Carl Zeiss AB) and image analysis (Blackburn et al. 1998). These data was used to calculate the carbon content per cell, as given by Norland (1993):

$$m_b = 0.12 \times v_b^{0.7} \quad (1)$$

where m_b is the bacterial carbon content in pg cell^{-1} and v_b the bacterial volume in µm^3 cell^{-1}.

Bacterial production was measured by a 1 h incubation at *in situ* temperature with ^{3}H-labeled thymidine (Amersham®; 1 mCi ml^{-1}, 80 Ci mmol^{-1}), at a final concentration of 25 nM using a conversion factor of 1.4×10^{18} cells mol^{-1} (Wikner & Hagström

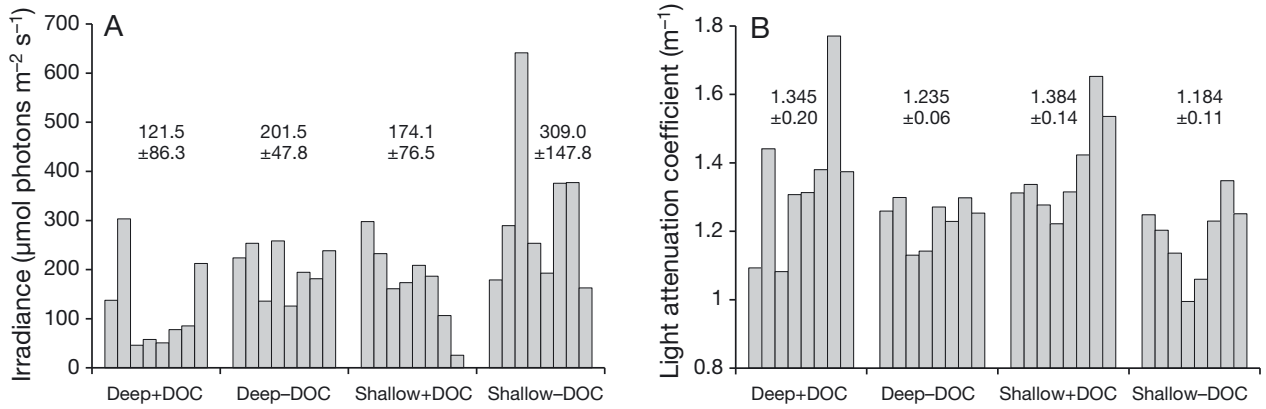

Fig. 1. Temporal development in (A) average irradiance in the mixed layer (shallow = 1.5 m, deep = 3.5 m) and (B) average light attenuation coefficient (*k*) for the 4 treatment groups. Bars represent (in order) Day 4, 7, 10, 13, 16, 23, 26 and 32. Treatment group averages ±SD for the whole experimental period are given above bars. DOC: dissolved organic carbon

1999). Triplicate incubations and 2 controls (cold trichloroacetic acid, TCA added to 5% final concentration) were incubated for each sample. Calculations of bacterial production per m^2 were made by integrating over depth, assuming homogenous production within the upper and lower water columns and uniform production over 24 h of the day. Integration over time was made as for phytoplankton production.

Mesozooplankton composition and abundance were analysed only at Day 22 of the experiment. A 5 l water sample was taken from the upper and lower water layer of each tank, and the mesozooplankton was collected by filtering the water through a 60 µm plankton gauze screen. The animals were rinsed with seawater previously filtered through a 0.2 µm Nuclepore™ filter into a 100 ml screw-cap vial, and formaldehyde was added to a final concentration of 2%. Within 1 wk, the samples were analysed using a Leica MZ 16 stereo microscope, with magnifications between 10 and 50 times, depending on individual body size. All individuals belonging to the dominant taxa Cladocera, Copepoda (copepodites and nauplii separated), and Rotatoria were counted.

For all statistical tests, we used the software package Systat 13 (version 13.1; www.systat.com). The average and standard deviation for the 4 treatment groups were calculated for all time points and presented graphically, together with the mean (or summed production) for the whole experiment of the 3 replicates in each group. As a first test of differences between treatment groups, we used pairwise *t*-tests. We considered differences between 2 groups with Bonferroni-adjusted p-values ≤ 0.05 as statistically significant. To evaluate if pycnocline depth or allochthonous DOC additions or both caused significant effects on the results, we used repeated measures ANOVA, with pycnocline depth (2 levels) and allochthonous DOC addition (2 levels) as independent variables, and sampling day as the repeating factor. The original data were log-transformed to achieve normal distribution of the data, and the critical p-value was set to 0.05.

RESULTS

Average irradiance level in the upper mixed layer varied over time, but usually with highest values in treatment Shallow–DOC (Fig. 1A). Average for the whole period gave lowest value for treatment Deep+DOC, followed by Shallow+DOC. The light attenuation coefficient was highest for treatment Shallow+DOC and lowest for Shallow–DOC (Fig. 1B). The repeated measures ANOVA for light levels at 1, 2, 3, 4 and 4.5 m depth showed a highly significant effect of DOC addition in all depth layers ($p \leq 0.001$). In contrast, pycnocline depth had no significant effect on the light level at any depths (p varying between 0.152 and 0.455). The depth of the 1% light level ranged between 1.8 and 4.4 m, with means (± 95% CI) of 2.6 ± 0.44 and 2.5 ± 0.32 m (deep and shallow pycnocline) for treatments with added DOC and 3.2 ± 0.30 and 3.5 ± 0.41 m for treatments without addition of DOC.

Table 1 presents the range in environmental conditions of the 4 series of treatments. The 2 treatments with deep pycnoclines showed somewhat higher salinity differences between the surface and deep water (15 to 17% higher) than the treatments with shallow pycnocline (7 to 8% higher). Average temperatures were close to the pre-set temperatures of 18°C (range 18.0 to 18.4°C) above the pycnocline and

Table 1. Range in environmental conditions during the experiment. DOC: dissolved organic carbon; QSE: quinine sulphate equivalents; POC: particulate organic carbon; PON: particulate organic nitrogen; upper: upper water layer; lower: lower water layer

Variable	Deep+DOC	Deep–DOC	Shallow+DOC	Shallow–DOC
Salinity (g kg^{-1}) upper	4.41 ± 0.01	4.43 ± 0.01	4.37 ± 0.01	4.38 ± 0.01
Salinity (g kg^{-1}) lower	5.07 ± 0.12	5.18 ± 0.04	4.67 ± 0.07	4.73 ± 0.06
Temperature (°C) upper	18.2 ± 0.24	18.0 ± 0.30	18.4 ± 0.44	18.2 ± 0.24
Temperature (°C) lower	10.0 ± 0.23	10.0 ± 0.26	10.1 ± 0.23	9.8 ± 0.17
O_2 (% saturation) upper	102–142	112–120	106–117	107–130
O_2 (% saturation) lower	75–125	95–110	86–110	91–107
Nitrate (mg N m^{-3}) upper	3–63	2–35	1–228	3–272
Nitrate (mg N m^{-3}) lower	3–136	2–69	1–382	3–388
Ammonium (mg N m^{-3}) upper	5–121	4–75	5–92	4–33
Ammonium (mg N m^{-3}) lower	5–56	4–14	5–68	4–56
Phosphate (mg P m^{-3}) upper	3–27	3–17	3–67	3–73
Phosphate (mg P m^{-3}) lower	3–36	3–19	3–73	3–100
DOC (g C m^{-3}) upper	3.84–4.43	3.79–4.22	3.84–4.43	3.68–4.26
Humic acid (g QSE m^{-3}) upper	13.9–18.0	12.2–14.5	10.2–17.3	7.8–14.4
POC (mg m^{-3}) upper	242–853	327–919	344–582	331–725
PON (mg m^{-3}) upper	14.7–64.8	7.2–53.7	7.2–25.7	10.0–37.4

10°C (range 9.8 to 10.1°C) below the pycnocline. The well-mixed upper water column was always oversaturated with oxygen, whereas the water column below the pycnocline occasionally showed a slight reduction below saturation (i.e. 100%). Due to regular addition of nitrate, ammonium and phosphate, these nutrients were never critically depleted. The addition of DOC (including humic acid) in 2 of the treatments only had marginal effects on the actual measured levels, with overall ranges of DOC of 3.7 to 4.4 g C m^{-3} and 7.8 to 18.0 g m^{-3} of humic acid. POC and PON showed considerably more variability, but with highly overlapping ranges for the 4 treatments.

Silicate was not added and could therefore have been a limiting nutrient for diatom production. Fig. 2 shows that there was a strong reduction in Si over

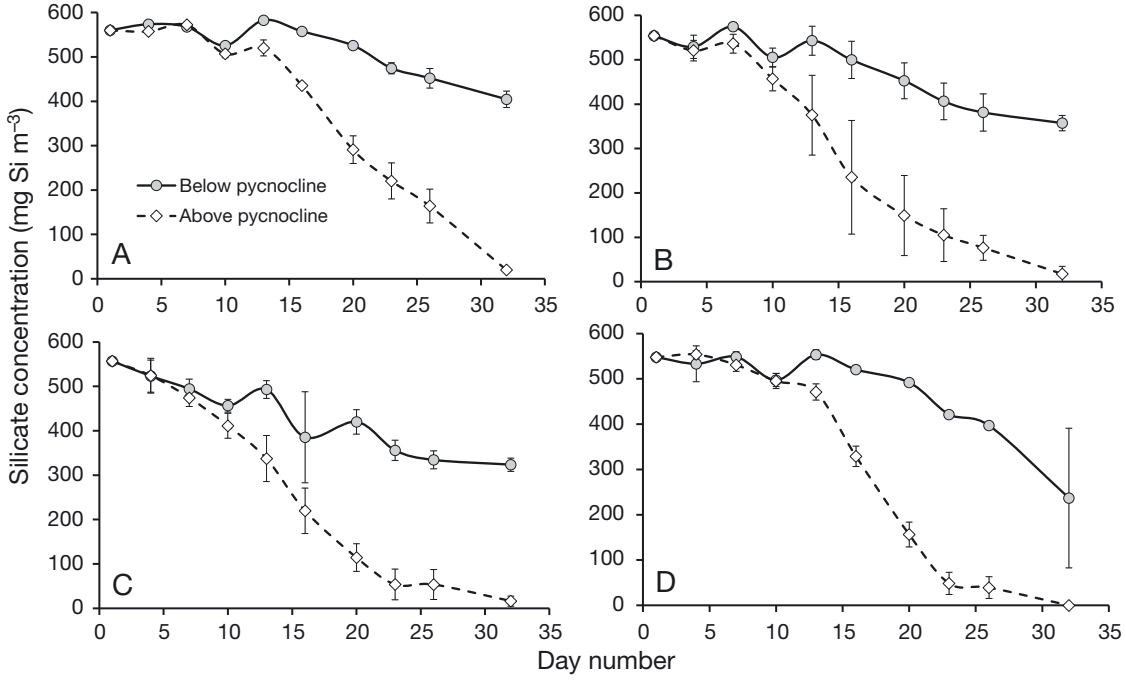

Fig. 2. Temporal development of average silicate concentration in the upper and lower water layer in tanks with the 4 treatments: (A) Deep+DOC; (B) Deep–DOC; (C) Shallow+DOC; and (D) Shallow–DOC. DOC: dissolved organic carbon; vertical lines: ±SD

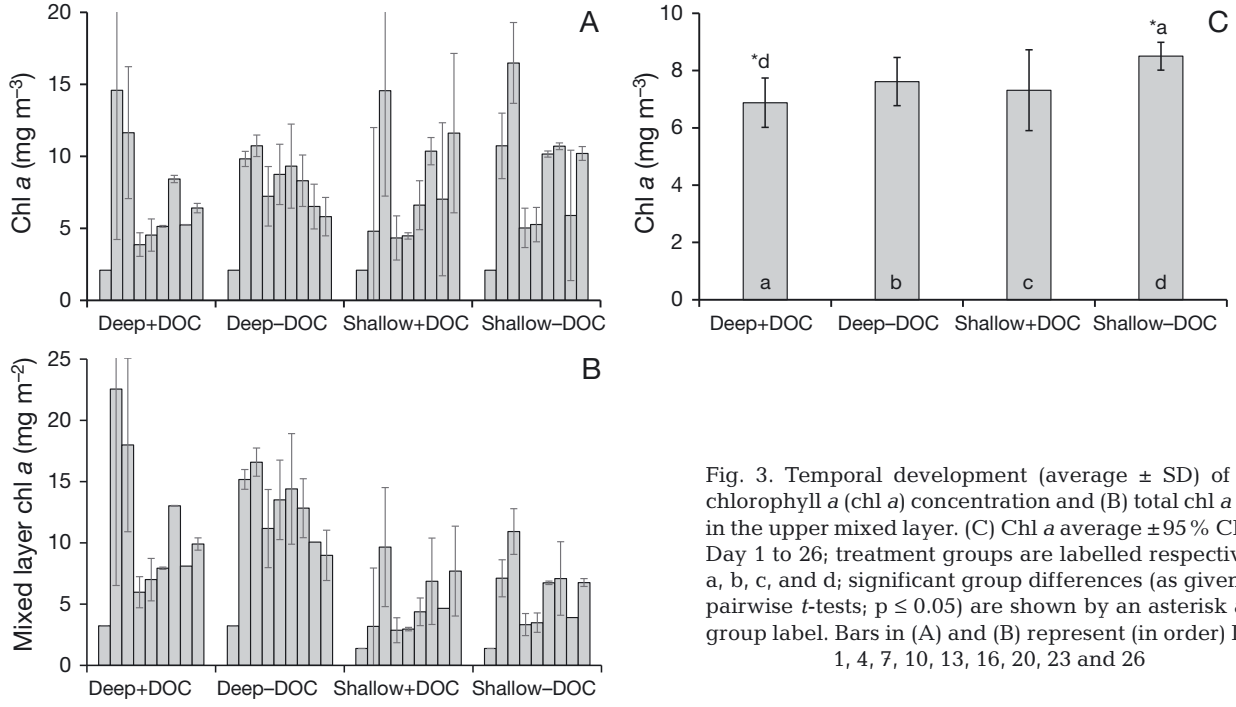

Fig. 3. Temporal development (average ± SD) of (A) chlorophyll *a* (chl *a*) concentration and (B) total chl *a* m^{-2} in the upper mixed layer. (C) Chl *a* average ±95% CI for Day 1 to 26; treatment groups are labelled respectively a, b, c, and d; significant group differences (as given by pairwise *t*-tests; $p \leq 0.05$) are shown by an asterisk and group label. Bars in (A) and (B) represent (in order) Day 1, 4, 7, 10, 13, 16, 20, 23 and 26

time, especially above the pycnocline, with potentially limiting levels from Day 23 onwards in the 2 treatments with a shallow pycnocline. On the final day of the experiment, Si readings in all treatments were close to zero above the pycnocline.

Chl *a* varied over time, with a peak of 11 to 16 µg l^{-1} in all treatments after around 1 wk (Fig. 3A). The average chl *a* concentration in treatments without DOC addition was on average 11% (deep) and 16% (shallow) higher than treatments with added DOC for the whole period (Fig. 3B). Treatment Deep+DOC was significantly lower ($p < 0.05$) than Shallow–DOC (Fig. 3B), but the repeated ANOVA did not reveal any significant effects of either pycnocline depth or DOC additions ($p > 0.05$; Table 2).

Bacterial abundance ranged from 1.4 to 9.4 × 10^9 cells l^{-1} above the pycnocline (Fig. 4A), with mean values (±95% CI) for the 4 treatments of 3.1 ± 1.1 × 10^9 cells l^{-1} for Deep+DOC, 4.7 ± 2.5 × 10^9 cells l^{-1} for Deep–DOC, 3.5 ± 1.6 × 10^9 cells l^{-1} for Shallow+DOC and 4.2 ± 1.3 × 10^9 cells l^{-1} for Shallow–DOC (Fig. 4C). The pairwise *t*-test on mean abundance revealed a significant difference between treatments Shallow+DOC and Shallow–DOC, both above pycnocline and in the whole water column. Repeated ANOVA showed that DOC, but not pycnocline depth, had a significant effect on bacterial abundance (Table 2).

The phytoplankton production per m^3 in the surface water was usually higher in treatments with shallow pycnocline, and peak production occurred towards the end of the experiment (Fig. 5A,B). Production also tended to be higher in treatments without DOC addition, both for deep and shallow pycnocline. Total production per m^2 showed considerable differences between treatment groups, and the pairwise *t*-test showed that the treatment Deep+DOC was significantly lower than the other 3 treatments (Fig. 5C). A repeated measures ANOVA gave strong evidence for significant effects on phytoplankton production of both pycnocline depth and DOC addition (Table 2). The summed phytoplankton production for the measured 26 d was approximately 2500 mg C m^{-2} for Deep+DOC, 5900 mg C m^{-2} for Deep–DOC, 5000 mg C m^{-2} for Shallow+DOC, and 6700 mg C m^{-2} for Shallow–DOC.

Bacterial production per unit volume in the upper mixed layer reached its maximum in the middle of the experimental period, and most of the time, DOC addition caused a slight increase in production (Fig. 6A). Similar results were obtained for bacterial production below the pycnocline (Fig. 6B) and for the whole water column (Fig. 6C). Total bacterial production for the measured 23 d was approximately 1800 mg C m^{-2} for Deep+DOC, 1400 mg C m^{-2} for Deep–DOC, 1600 mg C m^{-2} for Shallow+DOC, and 1200 mg C m^{-2} for Shallow–DOC (Fig. 6D). The pairwise *t*-test of the summed production showed that the treatment Deep+DOC was significantly higher than Shallow–DOC (Fig. 6D). The ensuing repeated measures ANOVA showed that DOC addition had a

Table 2. Repeated measure ANOVA (columns show *F*-values, with p-values in parentheses) with pycnocline (Pycno) depth at 2 levels (deep and shallow), dissolved organic carbon (DOC) additions at 2 levels (no addition and regular addition), and time as the repeating factor (n = 8 or 9). Log-transformation of original data was used for normal distribution. Significant effects ($p \leq 0.05$) of treatments, changes over time and interaction effects are indicated in **bold**. B/P: bacterial to phytoplankton production ratio. Between-group df = 1 in all cases; within-group df = 7 except for bacterial abundance, lower and upper and primary production and upper and whole column, where df = 8

Variable	N	Pycno	DOC	Time	Pycno × DOC	Pycno × Time	DOC × Time	Pycno × DOC × Time
Bacterial abundance, lower water layer	99	0.209 (0.326)	**12.717 (0.012)**	**11.277 (<0.001)**	0.748 (0.42)	**3.149 (0.006)**	0.744 (0.652)	1.252 (0.291)
Bacterial abundance, upper water layer	98	1.144 (0.773)	**7.367 (0.035)**	**5.221 (<0.001)**	0.916 (0.375)	1.846 (0.091)	0.682 (0.705)	0.809 (0.598)
Bacterial production m^{-3}, lower water layer	90	3.64 (0.098)	**6.467 (0.038)**	**38.806 (<0.001)**	1.008 (0.349)	**3.795 (0.002)**	1.135 (0.357)	**2.725 (0.018)**
Bacterial production m^{-3}, upper water layer	91	635 (0.452)	1.564 (0.251)	**20.122 (<0.001)**	0.137 (0.722)	0.165 (0.991)	1.092 (0.383)	0.933 (0.49)
Bacterial production, whole column	90	2.592 (0.151)	5.146 (0.058)	**39.298 (<0.001)**	0.275 (0.616)	0.852 (0.55)	0.929 (0.493)	2.683 (0.088)
Chlorophyll *a*, upper water layer	99	0.121 (0.739)	2.95 (0.13)	**4.245 (0.001)**	0.157 (0.704)	2.092 (0.062)	0.867 (0.539)	1.592 (0.16)
Primary production m^{-3}, upper water layer	105	**180.037 (<0.001)**	**46.919 (<0.001)**	**8.108 (<0.001)**	3.818 (0.092)	**2.309 (0.032)**	1.042 (0.414)	1.388 (0.222)
Primary production, whole column	105	5.396 (0.053)	**44.474 (<0.001)**	**17.964 (<0.001)**	4.49 (0.072)	**7.157 (<0.001)**	1.211 (0.31)	1.11 (0.371)
Primary production per chl *a* unit	95	**32.382 (0.005)**	6.554 (0.063)	**12.407 (<0.001)**	1.883 (0.242)	1.205 (0.333)	1.949 (0.099)	1.326 (0.275)
B/P production ratio m^{-3}	88	**197.866 (<0.001)**	**124.183 (<0.001)**	**34.229 (<0.001)**	**9.001 (0.024)**	1.677 (0.141)	0.988 (0.453)	**3.151 (0.009)**
B/P production ratio m^{-2}	88	**10.952 (0.013)**	**55.126 (<0.001)**	**77.202 (<0.001)**	2.99 (0.127)	**9.884 (<0.001)**	1.873 (0.094)	**4.442 (0.001)**

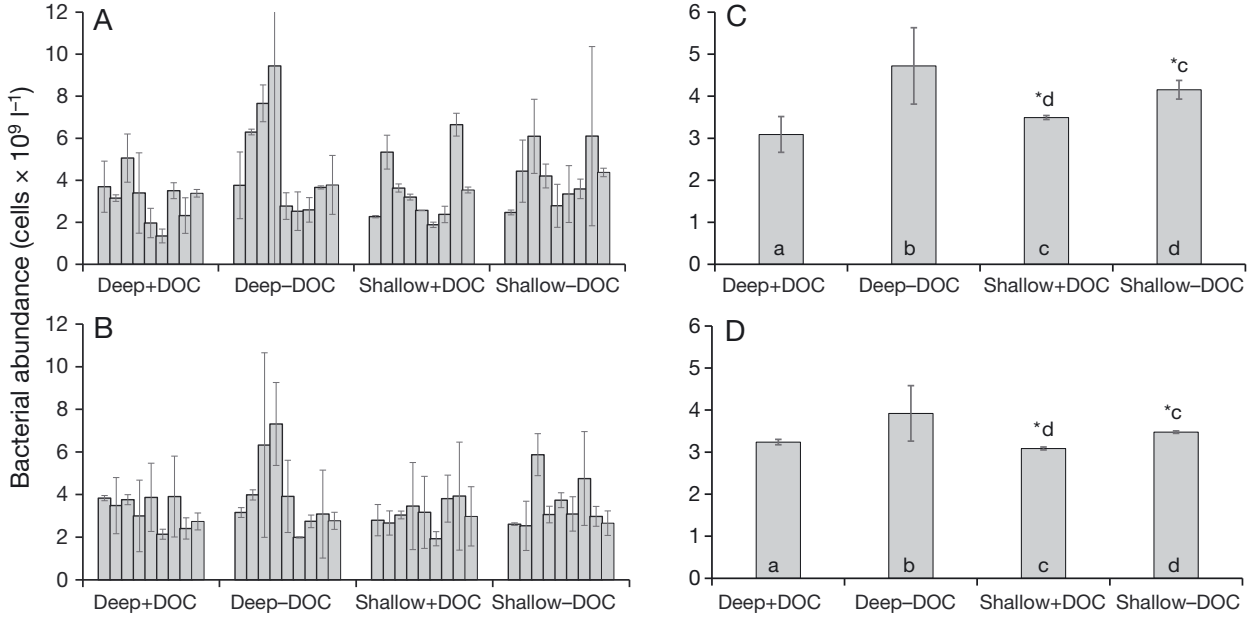

Fig. 4. Temporal development in average bacterial abundance in the (A) upper and (B) lower water layer over time, and average abundance for Day 3 to 31 in the (C) upper and (D) lower water layer. Bars in (A) and (B) represent (in order) Days 4, 7, 10, 13, 16, 20, 23, 26 and 31. Vertical lines: ±SD in (A) and (B) and 95% CI in (C) and (D). Treatment groups in (C) and (D) are labelled respectively a, b, c, and d, and significant differences ($p \leq 0.05$) between means (as given by pairwise *t*-tests) are shown by an asterisk and group label

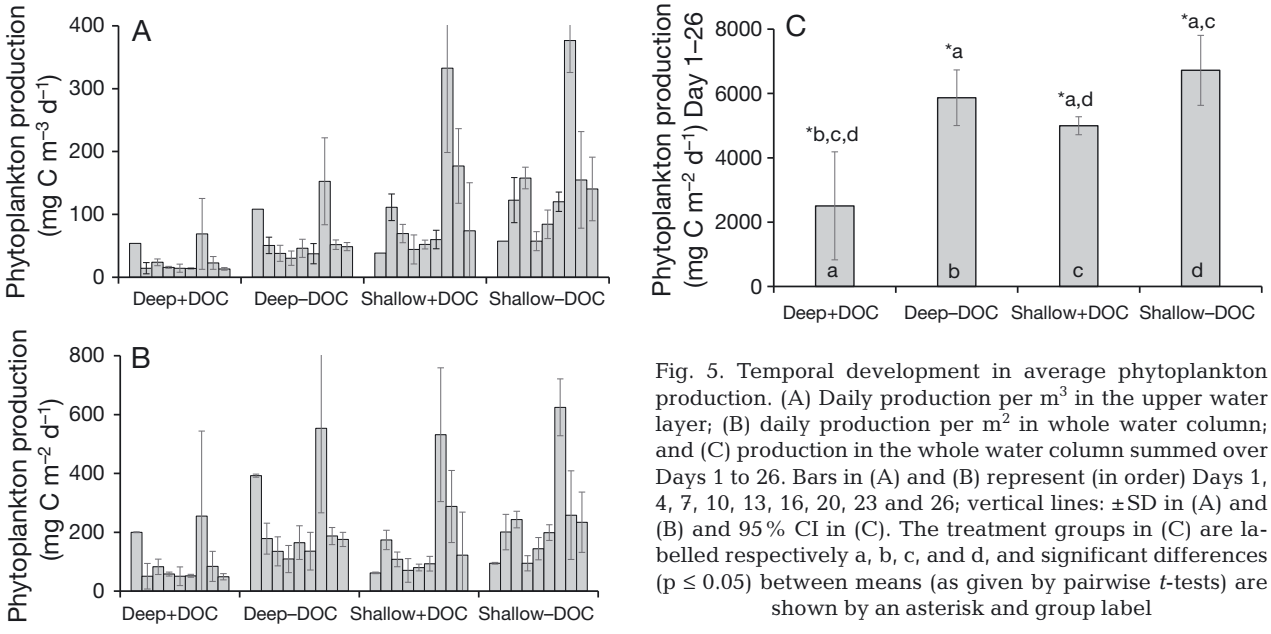

Fig. 5. Temporal development in average phytoplankton production. (A) Daily production per m^3 in the upper water layer; (B) daily production per m^2 in whole water column; and (C) production in the whole water column summed over Days 1 to 26. Bars in (A) and (B) represent (in order) Days 1, 4, 7, 10, 13, 16, 20, 23 and 26; vertical lines: ±SD in (A) and (B) and 95% CI in (C). The treatment groups in (C) are labelled respectively a, b, c, and d, and significant differences ($p \leq 0.05$) between means (as given by pairwise *t*-tests) are shown by an asterisk and group label

significant effect on bacterial production only below the pycnocline, whereas pycnocline depth did not show any significant effect on bacterial production (Table 2).

The ratio between bacterial and phytoplankton production (B/P), indicating the trophic balance in the plankton community, showed a strong trend of increased heterotrophy towards the middle of the experiment for all treatments, both in the mixed surface layer (Fig. 7A) and for the whole water column (Fig. 7B). After the maximum in the middle period, the B/P ratio gradually diminished to its minimum observed at the end of the period. For the whole period, the average (±95% CI) B/P ratio for the whole water column was 1.24 ± 0.41 for Deep+DOC, 0.42 ± 0.16 for Deep–DOC, 0.77 ± 0.29 for Shallow+DOC

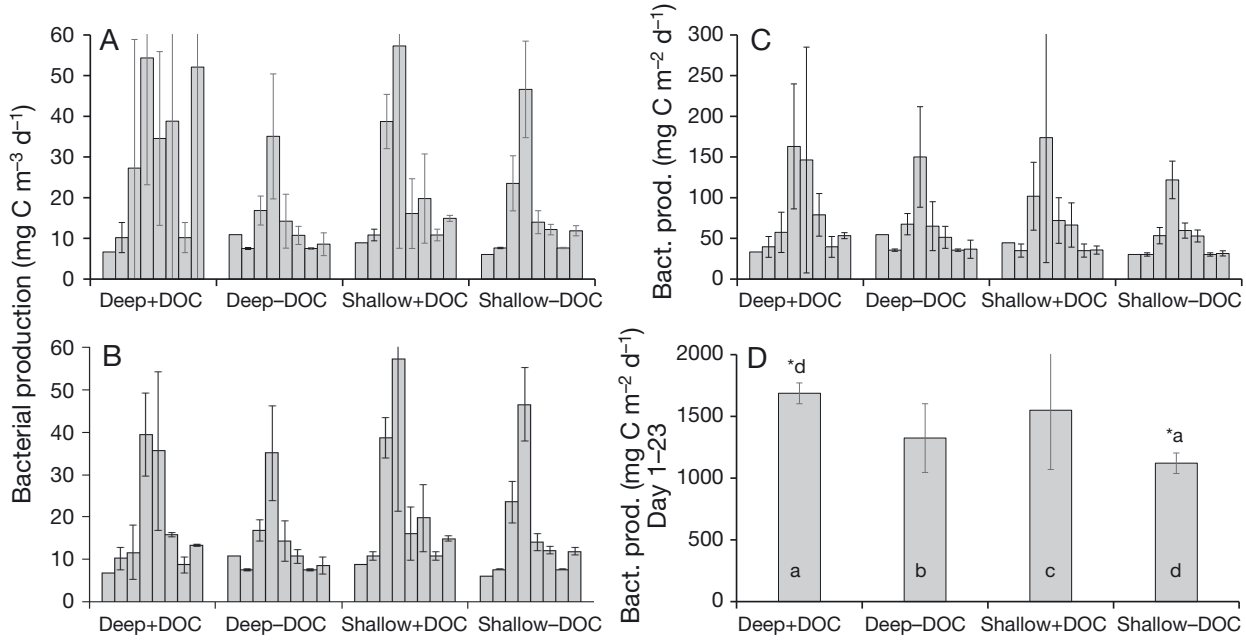

Fig. 6. Temporal development in average bacterial production. Daily production (A) per m^3 above pycnocline; (B) per m^3 below pycnocline; and (C) per m^2 in whole water column. (D) Production per m^2 in whole water column summed for Day 1 to 23. Bars represent (in order) Days 1, 4, 7, 10, 13, 16, 20 and 23. Vertical lines: ±SD in (A–C) and 95% CI in (D). Treatment groups in (D) are labelled respectively a, b, c, and d, and significant differences ($p \leq 0.05$) between means (as given by pairwise *t*-tests) are shown by an asterisk and group label

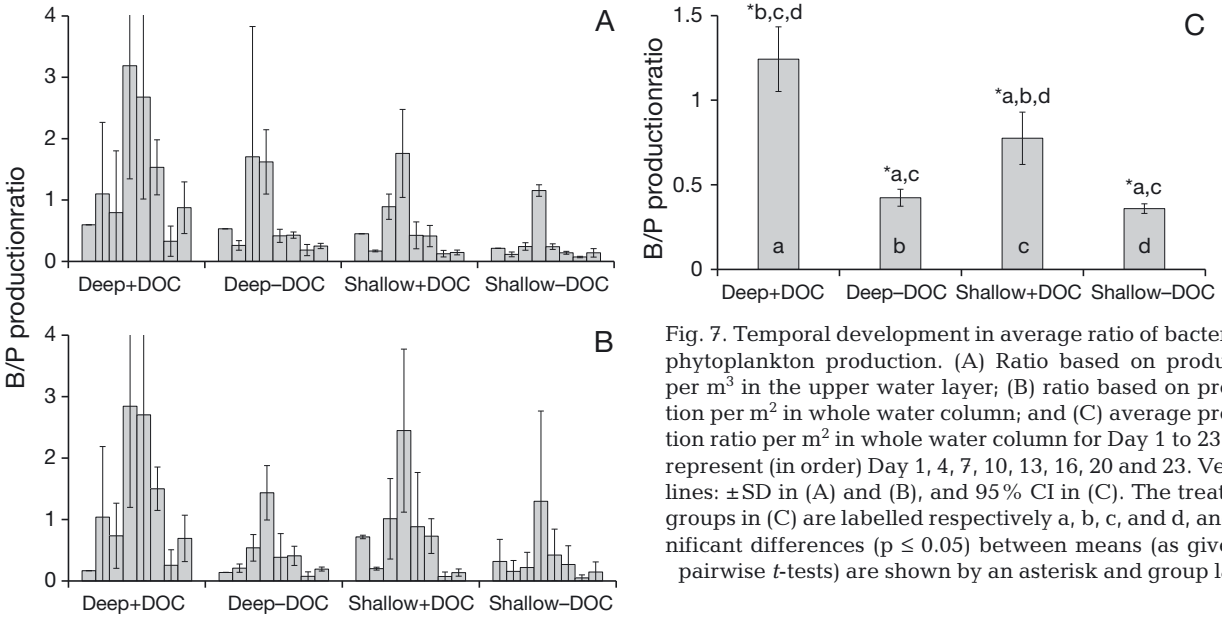

Fig. 7. Temporal development in average ratio of bacterial to phytoplankton production. (A) Ratio based on production per m^3 in the upper water layer; (B) ratio based on production per m^2 in whole water column; and (C) average production ratio per m^2 in whole water column for Day 1 to 23. Bars represent (in order) Day 1, 4, 7, 10, 13, 16, 20 and 23. Vertical lines: ±SD in (A) and (B), and 95% CI in (C). The treatment groups in (C) are labelled respectively a, b, c, and d, and significant differences ($p \leq 0.05$) between means (as given by pairwise *t*-tests) are shown by an asterisk and group label

and 0.36 ± 0.13 for Shallow–DOC. Pairwise *t*-tests showed that the treatment Deep+DOC had a higher ratio than all others, and the 2 treatments without DOC addition were significantly lower in production than those with added DOC (Fig. 7C). The ensuing repeated measures ANOVA showed that both pycnocline depth and DOC addition significantly influenced the B/P ratio in the upper mixed layer and in the whole water column (Table 2).

Zooplankton abundance at Day 22 tended to be lower with DOC addition (Fig. 8), but considerable variability between replicates made the effect not significant ($p > 0.05$; Table 3). All 4 taxa showed highly significant differences in abundance between the upper mixed layer and the bottom layer ($p < 0.011$; Fig. 8, Table 3), but the only significant experimental treatment effect was on Rotatoria abundance, which was strongly negatively affected by DOC addition ($p = 0.001$; Table 3).

DISCUSSION

Theoretically, light-controlled phytoplankton production per m^3 should always decrease with increasing depth of the mixed layer, except when there is photoinhibition involved. Net production per surface area, on the other hand, will increase until the compensation depth is reached, and thereafter decrease; the shape of the vertical production curve depends on the light attenuation coefficient and respiration loss. Chromatic DOC should theoretically act as a

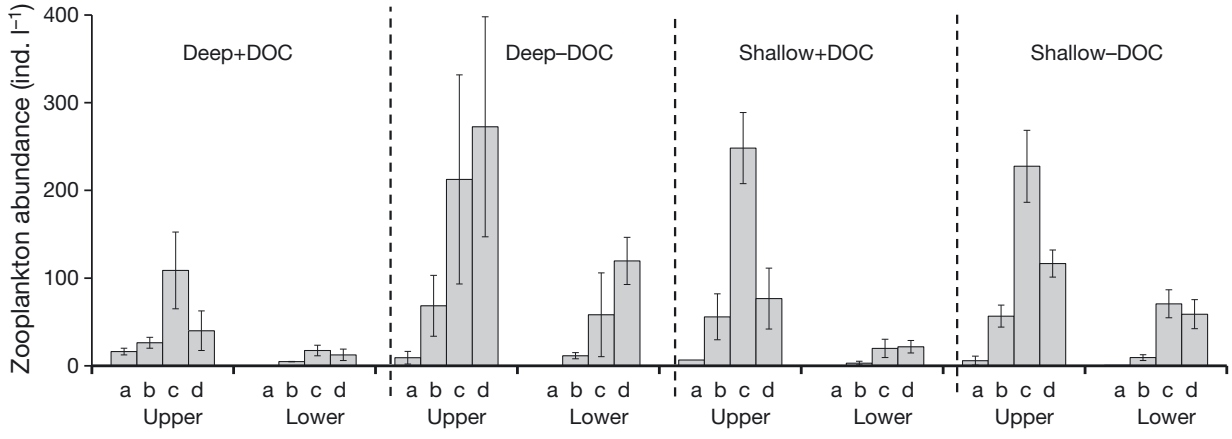

Fig. 8. Zooplankton composition at Day 22 with the 4 treatments. Upper: upper mixed water layer; lower: water layer below pycnocline. a: Cladocera; b: Copepoda; c: copepod nauplii; d: Rotatoria. Vertical lines: ±SD

Table 3. ANOVA results on effect of pycnocline depth (Pycno; 2 levels), dissolved organic carbon addition (DOC; 2 levels) and depth (2 levels; above and below pycnocline) for abundance of dominant zooplankton taxa at Day 22 of the experiment (columns show *F*-values, with p-values in parentheses). Significant ($p \leq 0.05$) main and interaction effects are shown in **bold**. Between-group df = 1 in all cases

Production source	N	Multiple R	Pycno	DOC	Depth	Pycno × DOC	Pycno × Depth	DOC × Depth	Pycno × DOC × Depth
Copepodites	23	0.840	0203 (0.659)	2.775 (0.116)	**27.140 (<0.001)**	1.565 (0.230)	0.343 (0.567)	0.668 (0.427)	1.289 (0.274)
Copepod nauplii	23	0.866	2.517 (0.134)	2.662 (0.124)	**33.222 (<0.001)**	1.185 (0.294)	1.469 (0.244)	0.017 (0.898)	1.382 (0.261)
Cladocerans	23	0.756	1.850 (0.194)	0.660 (0.429)	**16.802 (0.001)**	0.547 (0.471)	2.160 (0.162)	0.850 (0.371)	0.379 (0.538)
Rotatoria	23	0.851	2.632 (0.126)	**16.793 (0.001)**	**8.376 (0.011)**	6.738 (0.020)	0.487 (0.496)	1.457 (0.246)	1.327 (0.267)

source of shade, thereby reducing autotrophic production by light-controlled algae. Phytoplankton production per m^3 in our experiment showed this clear combined effect of mixing depth and DOC, with an average of 4.0 times higher phytoplankton production per m^3 in the shallow mixed layer compared with the lower water layer with added DOC, and 2.6 times higher phytoplankton production in the shallow mixed layer compared to the lower layer without added DOC (Fig. 5). Our results thus indicate responses closely predicted by theory. The phytoplankton production per m^2 in our experiment was, on average, 2.0-fold higher with a shallow than a deep pycnocline for mesocosms with added DOC, and was 1.1-fold higher for mesocosms without added DOC (see Fig. 5C). This indicates that a stronger reduction in average light level occurred when both added chromophoric DOC and a deep mixed layer were present. Thus, increased freshwater discharge as a consequence of climate change may reduce the light level in the coastal zone by the 2 different mechanisms simultaneously.

If we assume the compensation depth to be the depth of 1% surface irradiance (Kirk 2011), the treatments Deep+DOC and Deep–DOC had compensation depths of 2.6 and 3.2 m respectively, corresponding to 0.9 and 0.3 m above the pycnocline depth of 3.5 m. Theoretically, therefore, we would expect a stronger negative effect of a deep compared to a shallow pycnocline when adding DOC, as also was confirmed by our results.

The light climate in the mesocosm tanks with black, light-absorbing inside walls differs from the natural environment. The average effect of DOC addition on the depth of 1% surface irradiance in the mesocosm tanks was a reduction by 19% in tanks with deep pycnocline and 29% in tanks with shallow pycnocline. From a field study in the coastal northern Bothnian Sea between June and December 2013 with 21 stations and 24 time points, the light attenuation coefficient ranged between 0.300 and 1.425, with an average of 0.595 (authors' unpubl. data). The corresponding average depth of 1% surface irradiance was 7.7 m, with a range from 3.2 to 15.4 m, i.e. a reduction from maximum to minimum of 79%. Thus, changes in light climate generated by DOC additions in our experiment are well within the ranges in the natural environment. The average irradiance at a pycnocline depth of 3.5 m in the mesocosms was 0.9 and 1.3% of surface irradiance, with and without DOC addition, respectively. With a pycnocline depth of 1.5 m, Shallow+DOC and Shallow–DOC received on average 12.5 and 16.9%, respectively, of the surface irradiance. Light attenuation data from the Bothnian Sea (see above) show that the light irradiance in the mesocosms at 3.5 m (deep pycnocline) corresponds to an average depth in the field of 7.3 to 7.9 m, but with a range from 3.0 to 15.1 m. Similarly, mesocosm light irradiance at 1.5 m (shallow pycnocline) corresponds to an average depth of 3.0 to 3.5 m in the field, with a range from 1.2 to 6.9 m. Thus, our experimental design corresponded to realistic conditions in the natural environment.

Our experimental results are different from results compiled by Nurnberg & Shaw (1998), based on metadata from more than 600 lakes, most of them in North America. Their results showed higher phytoplankton production, and especially bacterial production, in coloured lakes compared to clear lakes. However, because there was a strong correlation between colour (from DOC), total phosphorus and nitrogen, the functional explanation for the positive effect of DOC on phytoplankton was probably indirect, through the organically bound nutrient supply.

The influx of allochthonous DOC to a system will promote heterotrophic production and activity and change the heterotrophic/autotrophic balance, and therefore also dramatically change the ecological transfer efficiency (Findlay et al. 1991, Wikner et al. 1999, Berglund et al. 2007, Dahlgren et al. 2011). It has been proposed by Blomqvist et al. (2001) that it is solely the carbon component in humic material that governs the structure and function of the pelagic food web in humic lakes. They added sucrose as a form of clear DOC to an oligotrophic clear-water lake and found that it had the same effect as coloured DOC, with a reduction in phytoplankton production and increased bacterial production. That result is in contrast to our study, where a significant difference of added DOC was found on phytoplankton production, more marked in the deep mixed layer compared to the shallow (4.0 and 2.6 times higher respectively), providing strong support for a light-shading effect. In addition, carbon-replete bacteria will become competitors against phytoplankton for limiting inorganic nutrients, further hampering phytoplankton production in a nutrient-limited environment (Pengerud et al. 1987, Mindl et al. 2005). This process may explain the result of Blomqvist et al. (2001), and shows the dual hampering effect of allochthonous DOC on phytoplankton production.

Although allochthonous DOC fluxes to aquatic ecosystems are often large, much of this material is recalcitrant and difficult for organisms to assimilate, whereas autochthonous DOC is used more easily (Pace et al. 2004). The amount and quality of DOC released from the terrestrial environment is governed by the type of soil and by weather conditions (Harrison et al. 2008). Photolysis will, however, transform carbon compounds in river water to become more bioavailable for bacterioplankton (Bertilsson et al. 1999, Tranvik & Bertilsson 2001). Riverine DOC also becomes more bioavailable when entering the saline coastal environment, with a seasonal variation that shows the highest values during the spring flood (Wikner et al. 1999). Our statistical tests showed only partly significant positive effects of added DOC for bacterial production (Fig. 6C, Table 2). The main explanation for this weak response might be the much higher primary production in treatments without added DOC, which probably also included more of the easily assimilated autochthonous DOC for bacterial production. Allochtonous DOC thus maintained bacterial production despite reduction in primary production. In addition, bacterial production may have been hampered by repeated small DOC additions, like we used, as bacteria have been reported to favour a single large pulse of DOC compared to repeated low level additions (Lennon & Cottingham 2008). Morán et al. (2002) further proposed that a strong dependence of bacterial production on phytoplankton extracellular production is only expected in open-ocean environments at great distance from coastal inputs of allochthonous DOC. However, Morán et al. (2013) later found a strong relationship between bacterial production and phytoplankton production in the coastal Waquoit Bay, Massachusetts, USA, where phytoplankton-derived DOC supported 50% of the bacterial carbon demand. Ducklow et al. (2002) used data from the previous North Atlantic Bloom Experiment to show that the DOC produced by phytoplankton could sustain a bacterial production of approximately 15% of the total primary production. They concluded that bacterial production above 20% of total primary production can only be supported by extensive inputs of allochthonous DOC. If we assume that 15% of the primary production in our experiment was transferred to bacterial production, we can subtract that figure from the recorded bacterial production to estimate the bacterial production supported by allochthonous DOC. For the 23 d measured, the Deep+DOC treatment gave 1434 mg C m^{-2}, Deep–DOC gave 601 mg C m^{-2}, Shallow+DOC gave 940 mg C m^{-2}, and Shallow–DOC gave 277 mg C m^{-2}; i.e. 2.4 to 3.4 times higher bacterial production with added DOC.

Our limited results for zooplankton only indicated a significant treatment effect on Rotatoria, with a population reduction related to DOC addition. Previously, Hitchcock et al. (2016) found that estuarine zooplankton (including Rotatoria) from an estuary in New South Wales, Australia, usually showed a positive response to episodic DOC inflow, with between 29 and 56% of copepod biomass based on this episodic DOC inflow. The effects varied over time in their experiment, and since we only made a single measurement, we cannot make any strong conclusions from our results.

We conclude that allochthonous DOC entering a brackish-water microbial community can negatively influence primary producers by reducing light availability, intensifying competition for inorganic nutrients, and simultaneously supporting increased or at least sustained bacterial production. Furthermore, increased mixing depth below the compensation depth will strongly reduce phytoplankton production. In synergy, these effects elevate the ratio of bacterial production to phytoplankton production, forcing the carbon flow through longer food chains at the

expense of production at higher trophic levels. Our results, therefore, support the hypothesis of Wikner & Andersson (2012), based on long-term field data, that the projected future climate-change driven increase in river discharge of organic material should change the balance in biological production at the food base level towards a more heterotrophic system. Increased DOC discharge may therefore result in longer food chains, which, by energy loss between each trophic level, may decrease production at higher trophic levels. Future fisheries of natural fish stocks might then be dramatically reduced. In addition, our results suggest that climate change may boost itself by making coastal sea areas even more important net producers of CO_2 to the atmosphere. This may be especially pronounced following extreme weather events including high precipitation.

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