

## LETTER

## Effects of sea surface warming on marine plankton

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### Abstract

Ocean warming has been implicated in the observed decline of oceanic phytoplankton biomass. Some studies suggest a physical pathway of warming via stratification and nutrient flux, and others a biological effect on plankton metabolic rates; yet the relative strength and possible interaction of these mechanisms remains unknown. Here, we implement projections from a global circulation model in a mesocosm experiment to examine both mechanisms in a multi-trophic plankton community. Warming treatments had positive direct effects on phytoplankton biomass, but these were overcompensated by the negative effects of decreased nutrient flux. Zooplankton switched from phytoplankton to grazing on ciliates. These results contrast with previous experiments under nutrient-replete conditions, where warming indirectly reduced phytoplankton biomass via increased zooplankton grazing. We conclude that the effect of ocean warming on marine plankton depends on the nutrient regime, and provide a mechanistic basis for understanding global change in marine ecosystems.

### Keywords

Climate warming, mesocosm experiment, phytoplankton, POTSMOM-C.

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### INTRODUCTION

Marine phytoplankton contribute approximately half of the global primary production, form the basis of marine food webs, and strongly influence biogeochemical processes in the ocean (Field *et al.* 1998). Recently, there has been growing evidence that global phytoplankton biomass and productivity are changing over time. Despite increasing trends in some regions (Gregg *et al.* 2003; Chavez *et al.* 2011), most observations and physical models suggest that at large scales, average phytoplankton biomass and productivity are declining, a trend which is predicted to continue over the coming century (Behrenfeld *et al.* 2006; Boyce *et al.* 2010; Henson *et al.* 2010; Steinacher *et al.* 2010; Hofmann *et al.* 2011; Sommer *et al.* 2012). While the exact magnitude of past and possible future phytoplankton declines is uncertain, there is broad agreement across these studies that marine phytoplankton biomass declines constitute a major component of global change in the oceans. Multiple lines of evidence suggest that changes in phytoplankton biomass and productivity are related to ocean warming (Behrenfeld *et al.* 2006; Polovina *et al.* 2008; Boyce *et al.* 2010). At least two distinct mechanisms influence this trend: a physically mediated effect of upper-ocean warming on vertical stratification that indirectly affects phytoplankton by limiting nutrient supply rates, and a direct effect of warming on plankton metabolic rates.

The physically mediated effects of temperature are primarily associated with enhanced vertical stratification, reduced mixed layer depth (MLD) and consequently reduced nutrient flux into

the upper oceans (Behrenfeld *et al.* 2006; Doney 2006; Boyce *et al.* 2010). This mechanism is believed to explain observed (Polovina *et al.* 2008) and predicted (Polovina *et al.* 2011) spatial expansion of the oligotrophic gyres of the ocean, and reduced phytoplankton biomass and production in the Eastern Central Pacific during El Niño years (Behrenfeld *et al.* 2006). Stratification may also influence the maximum depth of penetrative solar radiation available for phytoplankton growth (Doney 2006; Dave & Lozier 2013). In a global model, simulation progressive warming induced a shoaling of MLD, reduced vertical mixing and weakened deep-water circulation. This led to a projected 50% decline in average phytoplankton chlorophyll *a* concentration from the year 2000 to 2200 (Hofmann *et al.* 2011). In addition to temperature-induced changes in stratification, processes associated with wind mixing and horizontal advection may also be important (Dave & Lozier 2013).

The direct effect of warming on plankton manifests as increasing metabolic rates of both phytoplankton and zooplankton, and a growing imbalance between photosynthesis and respiration rates as temperature increases. Heterotrophic processes are more sensitive to temperature than autotrophic ones (López-Urrutia *et al.* 2006; O'Connor *et al.* 2009), leading to higher grazing rates by zooplankton and consequent reduction in phytoplankton biomass under warmer conditions (Müren *et al.* 2005; Sommer & Lengfellner 2008; O'Connor *et al.* 2009; Sommer & Lewandowska 2011). This mechanism may be partly offset by faster nutrient recycling by bacteria, which increases phytoplankton productivity (Taucher & Oschlies 2011).

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To date, the influences of these two mechanisms on marine phytoplankton have been largely explored in isolation and it remains unclear how they might interact, and what their cumulative effects on marine plankton communities may be. The physical effects of warming on stratification, nutrient supply and plankton abundance are typically explored at large scales, either through compilation of shipboard sampling data (Boyce *et al.* 2010, 2012), satellite observations (Behrenfeld *et al.* 2006; Martinez *et al.* 2009), or model simulations (Henson *et al.* 2010; Hofmann *et al.* 2011). In contrast, the metabolic effects of warming on plankton are typically investigated at smaller scales, through micro- and mesocosm experiments (O'Connor *et al.* 2009; Sommer & Lewandowska 2011; Holding *et al.* 2013). Only one modelling study that we are aware of has attempted to combine these effects, but with large uncertainties due to a limited empirical information (Olonscheck *et al.* 2013). Here we merge large- and small scale approaches to test for the relative effects of both physically- and biologically mediated effects of warming on a marine plankton community at the same time. Specifically, global ocean model simulations were used both to estimate the average rate of warming under the IPCCs A1F1 emissions scenario (Nakicenovic & Swart 2000), but more importantly, to quantify the effects of warming on average nitrogen flux into the photic layer. Based on these model results, experimental mesocosms were established to mimic realistic physical ocean conditions under continued global warming, and to test both the effects of warming, and consequent reductions in nutrient flux on a complex, multi-trophic plankton system. Contrasting our results with previous warming experiments, we discuss how the mechanisms controlling the plankton ecosystem response to warming might vary between different regions and seasons.

## MATERIAL AND METHODS

### General circulation model

To quantify the effects of increasing greenhouse emissions on ocean warming, stratification and nutrient fluxes, we employed a coarse-resolution ocean general circulation model (OGCM), which includes a state-of-the-art model of the ocean carbon cycle and marine ecosystem. While the former derives from an improved version of the Modular Ocean Model version 3.1 (Hofmann & Maqueda 2006) the latter follows the parametrisation of Six and Maier-Reimer (Six & Maier-Reimer 1996) utilising a fixed elementary Redfield ratio between carbon, nitrogen and phosphate. The model was extended to account for the effects of mineral ballast on the vertical export of organic matter (Hofmann *et al.* 2011). The horizontal resolution was  $3.75 \times 3.75$  degrees while the water column was subdivided into 24 vertical layers, increasing in thickness with depth. Primary production was assumed to be constrained to the four uppermost layers with a uniform thickness of 25 m (integrated over the upper 100 m). In the steady state, the model exports about 8.6 Pg of particulate organic carbon (POC) across the 100 m horizon into the deep ocean while an atmospheric  $\text{CO}_2$  partial pressure of 282 atm is established.

The atmospheric forcing of the ocean is provided by the climatological NCEP/NCAR-reanalysis database (Kalnay *et al.* 1996). To consider the transient effects of anthropogenic global warming between years 1800 and 2100 in our simulations, we have utilised the atmospheric anomalies of surface temperature, precipitation and relative humidity from a former model run of the coupled Earth climate system model CLIMBER-3 (Kuhlbrodt *et al.* 2009), as forced by an IPCCs A1F1 emissions scenario. This scenario assumes continuing exponential increase in global carbon emissions until the year 2100, and approximates current trends in emission growth (Nakicenovic & Swart 2000). The procedure is described in detail by Hofmann *et al.* (2011). We derived from the model the average temperature in the photic layer (top 100 m), the average total nitrogen (TN) concentration, and the average vertical flux (sum of vertical convective mixing, advection and diffusion) of dissolved inorganic nitrogen (DIN) into the photic layer, for the years 1800 (pre-industrial baseline), 2000 and 2100.

### Mesocosm experiment

We explored the effects of temperature (2 levels) and nutrient flux (6 levels) in a factorial mesocosm experiment consisting of 12 experimental units. This design followed a regression approach; this was chosen to model the response of plankton communities to a wide gradient in nutrient flux, warming and their interaction. We simultaneously filled all mesocosms (1500 l volume, 1 m depth) with sea water containing natural plankton communities from the Baltic Sea. Mesocosms were placed in temperature-controlled climate chambers and the water was gently and uniformly mixed by propellers. Mesozooplankton dominated by copepods were obtained from net hauls (200  $\mu\text{m}$  mesh) and added to each mesocosm in natural concentrations of 6 ind.  $\text{l}^{-1}$ . Computer-controlled light conditions mimicked daily and seasonal irradiance patterns calculated from astronomic equations for above-cloud irradiance (Brock 1981) at our study site, light reduction by clouds and attenuation coefficient typical for Baltic Sea waters at the time when the experiment was performed. This light scenario mimicked the light dose for 10 m mixing depth (halocline *in situ*). Six mesocosms were cooled by 3 °C and six were warmed by 3 °C above ambient sea temperatures observed in the Baltic Sea, for a total 6 °C temperature gradient as simulated by the above-described model. Initial nutrient concentrations were low and typical for Baltic Sea waters at the onset of summer stratification (see Supporting Information). Because stratification could not be directly manipulated in the mesocosms, nitrate ( $\text{NaNO}_3$ ), phosphate ( $\text{KH}_2\text{PO}_4$ ) and silicate ( $\text{Na}_2\text{O}_3\text{Si}$ ) were added every third day to simulate model-projected changes in nutrient flux. We ran the experiment over 5 weeks from 16 May to 18 June 2012 with six nutrient treatments: 100% (control, no nutrients added), 106, 112, 119, 126 and 134% of total nitrogen (TN). The lowest nutrient addition treatment (106%) corresponded to the average monthly nutrient flux into the photic zone predicted by the model for the year 2100, i.e. a nutrient regeneration rate of about 6% of the total nitrogen pool per month. The higher treatments corresponded to average preindustrial values (112%) and the full

range of nutrient fluxes seen across upwelling, coastal and shelf seas (see Results) including typical summer conditions. The nutrient additions were scaled relative to the initial TN pool and divided over the course of the experiment to sum up to above-mentioned monthly fluxes. Other macronutrients (phosphate, silicate) were added in proportions 16N : 1P : 11Si, as they would during vertical mixing events in the ocean and in the Baltic Sea.

During the experiment, we measured key biological, physical and chemical variables. Water temperature, pH, salinity and fluorescence were measured daily. Mesocosms were sampled three times per week for bacteria, phytoplankton, heterotrophic nanoflagellates (HNF), chlorophyll, TN, total phosphorous (TP), particulate organic carbon, nitrogen and phosphorous (POC, PON, POP), and dissolved inorganic nutrients ( $\text{NO}_2^-$  and  $\text{NO}_3^-$ ,  $\text{NH}_4^+$ ,  $\text{PO}_4^{2-}$ ,  $\text{SiO}_4^{2-}$ ). Zooplankton, primary production and respiration were measured weekly. Bacteria and phytoplankton < 5  $\mu\text{m}$  cell size were counted using a flow cytometer (FACScalibur, Becton Dickinson), larger phytoplankton and microzooplankton were fixed with Lugol's iodine and counted after Utermöhl (1958), using an inverted microscope. Biomass contribution of different phytoplankton and microzooplankton species were estimated from carbon content (Putt & Stoecker 1989; Menden-Deuer & Lessard 2000) after approximation of cell biovolumes to geometric bodies (Hillebrand *et al.* 1999). Copepods were identified to genus level and counted with a binocular microscope. HNF (heterotrophic nanoflagellates) were fixed with formaldehyde (2% final solution), stained by DAPI (1  $\mu\text{g L}^{-1}$  final concentration) and counted using an epifluorescence microscope. For estimation of primary production and community respiration rates, samples (100 mL) were incubated inside the mesocosms at mid depth (light bottles) or in closed containers inside the climate chambers (dark bottles) for 24 h. Subsequently, concentrations of dissolved oxygen were measured (Hansen 1999).

### Statistical analyses

To quantitatively explore the linear relationships between temperature or nutrients and the plankton community an unconstrained multivariate principal component analysis (PCA) was performed (Rao 1964). The PCA was calculated using the nutrient (range 100–134%) and temperature (−3 or 3 °C) treatment levels and the time series of biological and chemical variables. The calculations were made using the correlation matrix, so that the relative strength of treatment effects could be better interpreted.

To test the linear effects of experimental treatments on individual variables (fluorescence, phytoplankton biomass etc.), we used generalised least squares (GLS) models with temperature and nutrients, as well as their interaction, as covariates. GLS models were selected in order to account for time dependence of the response variable within each mesocosm. Models were fit to the data according to,

$$y = X\beta + \varepsilon$$

where  $y$  is the response vector,  $X$  is a matrix of model covariates,  $\beta$  are the model parameters estimated by maximum likelihood and  $\varepsilon$  are the model errors which are specified as,

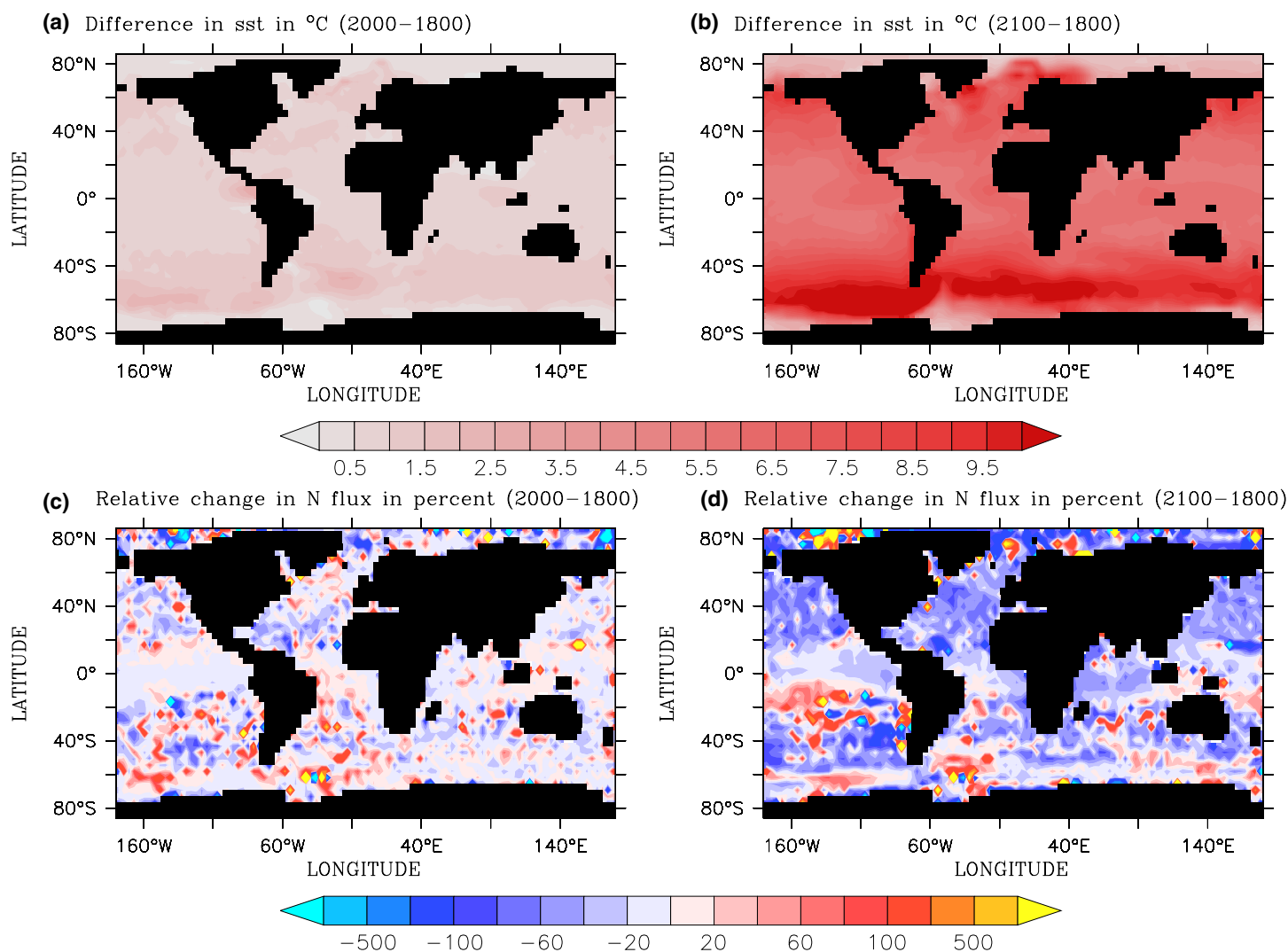
$$\varepsilon \sim N(0, \delta)$$

where 0 is the mean and  $\delta$  is the error-covariance matrix. To account for autocorrelation, the covariance parameters of  $\delta$  were assumed to follow a time-dependent autoregressive process (AR1). All AR1 parameters were estimated from the raw data *a priori* to model fitting. As a sensitivity check, we also estimated AR1 parameters from the model residuals, however, since the model covariates (temperature and nutrient treatment levels) did not vary over time, this procedure had no effect on the model estimation. All model residuals were checked for normality and transformed prior to model re-fitting where required. GLS model assumptions were verified by examination of the residuals. For each dependent variable, the best-fit model was selected using Akaike's Information Criterion (AIC), which takes into account both goodness of fit and model complexity. The GLS models did not account for non-linear response trajectories. To check for non-linearity in the responses we plotted the residuals vs. nutrient treatment for each GLS model. This procedure did not reveal any non-linear patterns in the residuals, and suggested that most variables changed as a linear function of the nutrient treatment level. All statistical analyses were conducted using R version 3.0.1 (64-bit) (R Development Core Team, R Foundation for Statistical Computing, Vienna, Austria).

## RESULTS

### Model simulation

To obtain realistic temperature and nutrient flux predictions for the years 1800, 2000 and 2100, we ran the POTSMOM-C general circulation model under the IPCC SRES A1F1 scenario. Because the mesocosm experiment was conducted using a North Atlantic (Kiel Bay, Baltic Sea) plankton community, we extracted changes in average sea surface temperature (SST, Fig. 1a), as well as average annual nutrient fluxes (Fig. 1b) across the North Atlantic (0–60° N), for the years 1800, 2000 and 2100. Average SST there increased from 23.7 °C in 1800 to 24.6 °C in 2000, and was projected to reach 29.8 °C in 2100 (Fig. 1a). This 6 °C gradient was used to establish the temperature treatments in the experiment. As for nutrient fluxes, average dissolved inorganic nitrogen (DIN) flux into the North Atlantic surface layer (0–100 m) was 1.93  $\mu\text{mol L}^{-1} \text{ year}^{-1}$  in 1800. With increasing SST and stratification this average annual flux of DIN diminished by 13% until the year 2000 (1.68  $\mu\text{mol L}^{-1} \text{ year}^{-1}$ ), and by 44% until 2100 (1.08  $\mu\text{mol L}^{-1} \text{ year}^{-1}$ , Fig. 1b). In relative terms, this DIN flux equates to a renewal of *c.* 12% of the total nitrogen pool (TN, includes both dissolved plus particulate nitrogen) in the surface layer per month in 1800 (average TN: 1.33  $\mu\text{mol L}^{-1}$ ) and 2000 (TN: 1.21  $\mu\text{mol L}^{-1}$ ), and 10% in 2100 (TN: 0.90  $\mu\text{mol L}^{-1}$ ). These values varied by region: total DIN flux into the surface layer can be as high as 30–40% of TN per month in some upwelling and coastal regions (Fig. 1b). Model-simulated SST and nutrient trends in the North Atlantic were similar to those in other oceans, with the exception of polar seas, which showed very large, and possibly unrealistic, projected temperature increases by 2100 (Fig. 1).



**Figure 1** Ocean general circulation model. (a) Absolute annual mean temperature changes projected from 1800 to 2000, and (b) from 1800 to 2100. Colour bar indicates temperature increase in °C. (c) Relative changes in dissolved nitrogen flux into the euphotic zone 1800–2000, and (d) 1800–2100. Colour bar indicates nitrogen flux increases (red) or decreases (blue) in per cent, relative to the baseline year. Results from this model for the North Atlantic (0–60° N) were used to parameterise mesocosm experiments simulating future ocean conditions.

### Mesocosm experiment

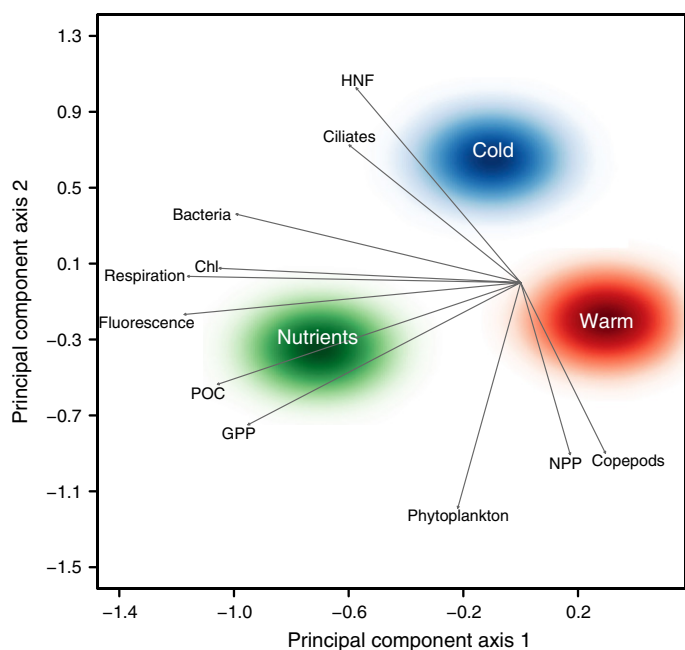
Our mesocosm experiment was initiated during the period of seasonal thermal stratification in summer 2012. Initial conditions were typical for the temperate North Atlantic summer ( $T = 15\text{ °C}$ , low dissolved nutrient concentrations, dominance by small-sized flagellates and copepods, see Appendix S1). Experimental temperature and nutrient treatments covered the range of model outcomes from 1800 to 2100; i.e. a temperature range of  $6\text{ °C}$  (six mesocosms were cooled  $3\text{ °C}$  below and six mesocosms were warmed  $3\text{ °C}$  above ambient temperature), and a monthly enrichment flux of DIN ranging between 0 and 34% of the TN pool.

TN concentrations depended on nutrient treatment and varied little over the course of the experiment with concentrations ranging between 18 and  $43\text{ }\mu\text{mol L}^{-1}$  (see Supporting Information). TP concentrations were below  $1\text{ }\mu\text{mol L}^{-1}$  and slowly decreased with time in all experimental units. N : P

ratios were on average higher in the warm mesocosms than in the cold ones (*c.* 16 and 13 respectively). Silicate concentrations declined precipitously in all experimental units over the course of the experiment (see Supporting Information).

Results suggest that the net response of marine phytoplankton to ocean warming was mainly driven by temperature-driven changes in nutrient availability, and less so by the direct (metabolic) effects of temperature on plankton. Principal component analysis (PCA) was used to explore multivariate response of the community to experimental treatments (Fig. 2). The first two principal component axes explained 57% of the variance, and suggested that changes in nutrient supply were linked to changes in fluorescence, chlorophyll *a* concentration, POC, gross primary production (GPP) and community respiration. In contrast, changes in the abundance of consumers were more strongly related to temperature, but in the opposite direction: copepods increased with warming, while ciliates and HNF were favoured by cool conditions





**Figure 2** Principal component analysis of the experimental plankton community. Positioning of a dependent variable close to coloured regions indicates positive effects of experimental warming (red), cooling (blue) or nutrient enrichment (green). POC = Particulate organic carbon, GPP = Gross primary production, NPP = Net primary production, HNF = Heterotrophic nanoflagellates, Chl = Chlorophyll.

(Fig. 2). Combined effects of nutrients and temperature were observed on phytoplankton biomass and bacteria abundance.

Over the course of the experiment we observed an increase in phytoplankton biomass and a subsequent bloom, especially in treatments with a positive nutrient flux (Fig. 3a,b). Maximum bloom biomass was nutrient-dependent, with a larger peak at higher nutrient supply. Generalised least squares (GLS) analysis accounting for temporal dependence of the individual time series showed a significant positive effect of nutrients on phyto-

plankton biomass, fluorescence, chlorophyll *a* concentration and POC (Fig. 3c, Table 1). Temperature increase also had a positive effect on phytoplankton biomass. The timing of the bloom was temperature dependent, with more rapid development and an earlier peak in warm treatments.

Net primary production (NPP) broadly followed the trajectory of phytoplankton biomass (Fig. 3a,b). However, no statistically significant changes in NPP were observed in response to changes in temperature or nutrient supply (Table 1). Community respiration declined over the experimental period, (Fig. 3) but trended higher in the warm treatments (non-significant trend,  $P = 0.056$ ). Both respiration and gross primary production (GPP) increased significantly with nutrient supply (Table 1).

Copepod abundance also increased over the course of the experiment (Fig. 3a,b), due to new production of copepod nauplii larvae which developed into copepodites. This growth was accelerated in warm mesocosms (Fig. 3b), and likely resulted in periodically increased grazing pressure there. Ciliates, in contrast, trended downwards over the course of the experiment (Fig. 3a,b), and their abundances were strongly negatively correlated with copepods ( $r = -0.46$ , 2-tailed  $P < 0.001$ ). This observation suggests copepods feeding heavily on ciliates, and less so on phytoplankton, which showed a positive correlation with copepod abundance ( $r = 0.42$ , two-tailed  $P = 0.001$ ). In addition to the effects of temperature on plankton abundance and biomass, temperature also increased variability in plankton abundance over time in most groups (Fig. 3a,b), significantly so for phytoplankton biomass, copepods, ciliates and HNF (ANOVA,  $P < 0.05$ , see Supporting Information for details).

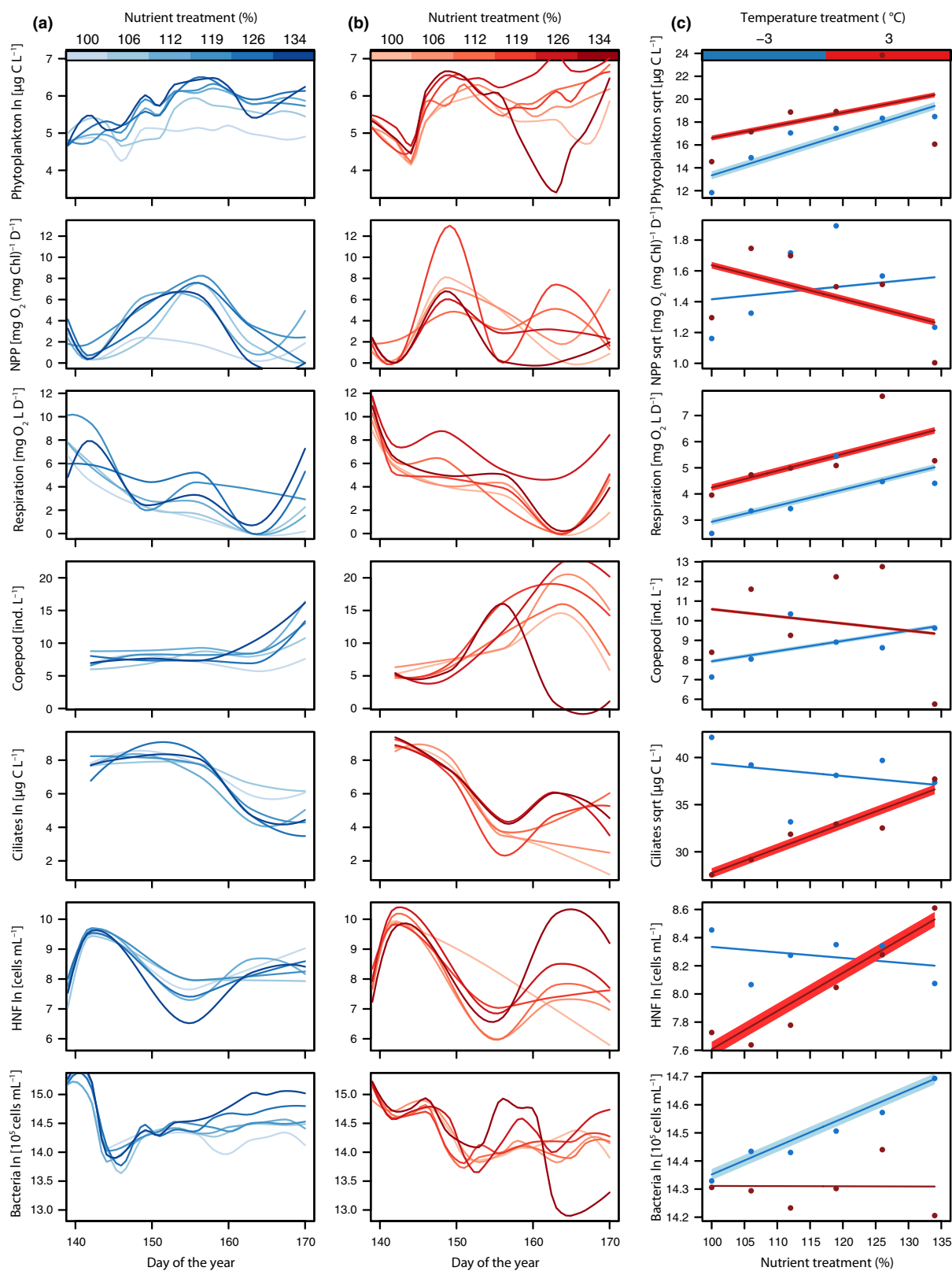
## DISCUSSION

Our OGCM model simulations suggest that DIN flux into the upper ocean have declined from preindustrial times and will continue to decline substantially with rising sea surface temperature over most of the ocean (Fig. 1). This mechanism is

**Table 1** Generalised least squares analysis

Variable	Units	Predictor	Coefficient	SE	<i>P</i>
Phytoplankton biomass	$\ln(\mu\text{g C L}^{-1})$	Nutrients	<b>0.145</b>	<b>0.039</b>	<b>&lt; 0.001</b>
		Temperature	<b>2.175</b>	<b>0.903</b>	<b>0.017</b>
POC	$\ln(\text{mg C L}^{-1})$	Nutrients	<b>0.014</b>	<b>0.002</b>	<b>&lt; 0.001</b>
Chlorophyll <i>a</i>	$\text{sqrt}(\text{mg L}^{-1})$	Nutrients	<b>0.009</b>	<b>0.003</b>	<b>0.005</b>
Fluorescence	$\ln$	Nutrients	<b>0.013</b>	<b>0.004</b>	<b>0.005</b>
NPP	$\text{sqrt}[\text{mg O}_2 (\text{mg Chl})^{-1} \text{D}^{-1}]$	Temperature	-0.003	0.009	0.712
GPP	$\text{sqrt}(\text{mg O}_2 \text{L}^{-1} \text{D}^{-1})$	Temperature	<b>0.032</b>	<b>0.013</b>	<b>0.014</b>
Respiration	$\text{mg O}_2 \text{L}^{-1} \text{D}^{-1}$	Nutrients	<b>0.063</b>	<b>0.030</b>	<b>0.041</b>
		Temperature	0.700	1.941	0.056
Copepod abundance	$\text{ind. L}^{-1}$	Temperature	1.224	1.181	0.304
Ciliate biomass	$\text{sqrt}(\mu\text{g C L}^{-1})$	Temperature	-6.325	7.303	0.390
HNF abundance	$\ln(\text{cells mL}^{-1})$	Nutrients	0.012	0.012	0.338
Bacteria abundance	$\ln(10^5 \text{ cells mL}^{-1})$	Nutrients	<b>0.010</b>	<b>0.004</b>	<b>0.024</b>
		Temperature	0.966	0.727	0.186
		Nut $\times$ Temp	-0.010	0.006	0.108

Results represent the most parsimonious model selected from a base model including the effects of nutrients, temperature and their interaction (Nut  $\times$  Temp). POC = Particulate Organic Carbon. NPP = Net primary production. GPP = Gross Primary Production. HNF = Heterotrophic nanoflagellates. Significant effects ( $P < 0.05$ ) in bold.



**Figure 3** Time trends and responses of plankton community to changes in temperature and nutrient supply. (a,b) Interpolated time series for the main biological variables are plotted for each mesocosm treatment ( $n = 12$ ). Blue trend lines depict cold mesocosm treatments and red lines depict warm treatments. The shading of the trend lines identifies the mesocosm nutrient treatment level (% of total nitrogen). Interpolation of each time series was performed using the Akima method (Akima 1978) (c) Generalised least squares model predicted trends in main biological variables as functions of temperature and nutrient treatment levels (points represent averaged values over time). Shaded areas represent the 95% confidence limits about the trend lines. Generalised least square statistical analysis results are reported in Table 1.

likely contributing to observed global declines in marine phytoplankton biomass, particularly at lower latitudes (Behrenfeld *et al.* 2006; Boyce *et al.* 2010). According to our model there is also a positive trend in DIN flux in some regions, such as the Labrador Sea, Cariaco Basin and Bermuda, where primary production was observed to increase since *c.* 1990 (Chavez *et al.* 2011). On average, however, nutrient flux into the photic layer was projected to decrease by over 40% from the year 1800 to 2100.

Using our model, we were able to implement realistic climate-change predictions of ocean temperature and nutrient changes within our mesocosm experiment. This approach allowed us to test for the relative strength and interaction of both physically- and biologically mediated mechanisms of rising sea surface temperature on plankton. If we consider the mean range in nutrient flux predicted from our model (i.e. 106–112% relative to average TN), physically- and biologically mediated effects on plankton seem to be of similar magnitude (Fig. 3c). However, if we consider the full range of variability in nutrient flux, which covers upwelling and coastal regions (up to 134% relative to average TN, Fig. 3c), the importance of changes in nutrient supply becomes more apparent.

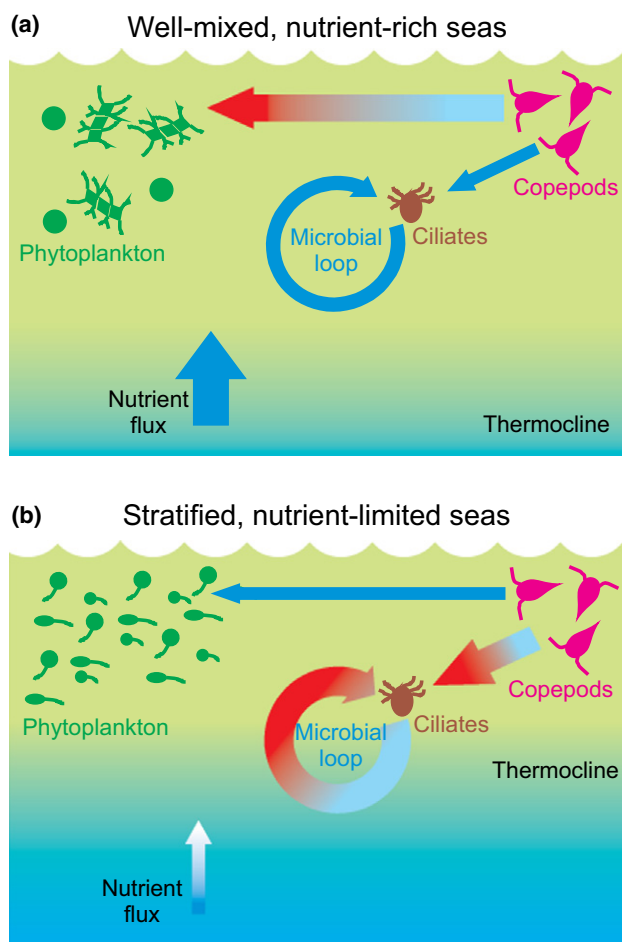
Our experimental results suggest that during Baltic Sea summer conditions (warm, stratified, nutrient poor) plankton biomass is primarily controlled by nutrient supply to the photic zone (Table 1). We observed statistically significant negative effects of reduced nutrient flux on phytoplankton biomass, chlorophyll *a* concentration, fluorescence, POC, respiration and bacteria abundance during the experiment. There was little evidence for increased top-down regulation of phytoplankton biomass in response to warming during our experiment, but some evidence of increased copepod grazing on ciliates, with possible cascading effects on the microbial loop. Typical for stratified conditions, concentrations of silicate were low over much of the experiment. This disadvantaged diatoms; hence the phytoplankton community was largely dominated by small-sized flagellates. Copepods often feed on microzooplankton such as ciliates when large phytoplankton (20–200  $\mu\text{m}$ ), such as diatoms are rare (Stibor *et al.* 2004). Ciliates in turn, prey on HNF which consume bacteria (Azam *et al.* 1983). The countervailing trajectories of these trophic groups over time (Fig. 3a,b) are consistent with a temperature-driven trophic cascade from copepods to bacteria, with ciliates and HNF as intermediary links. Yet, the effects of temperature on the microbial loop were statistically non-significant in the GLS analysis, likely because other pathways were also important. Ciliates are also known to feed on small phytoplankton and bacteria (Bernard & Rassoulzadegan 1990; Johansson *et al.* 2004), both of which were abundant in this experiment. Thus, the observed positive effect of temperature on phytoplankton biomass could be driven by copepods switching to feed on ciliates, and thus reducing microzooplankton grazing pressure on the numerically dominant small phytoplankton. This 'grazer release' hypothesis is indirectly supported by the observed positive relationship between copepods and phytoplankton ( $r = 0.42$ , 2-tailed  $P = 0.001$ ), negative relationship between copepods and ciliates ( $r = -0.46$ , 2-tailed  $P < 0.001$ ), and between ciliates and phytoplankton ( $r = -0.34$ , 2-tailed

$P = 0.008$ ). It has been suggested that the microbial loop might turn over faster with warming, thereby supporting photosynthesis due to the faster turnover of nutrients (Taucher & Oschlies 2011). However, the present results paint a more complex picture: when copepods feed mainly on ciliates instead of phytoplankton, HNFs are released from grazing pressure by ciliates, and hence reduce bacteria abundance. Consequently, nutrients will not necessarily be recycled faster, except if that function is supplied by HNFs rather than by bacteria (Mariottini & Pane 2003). Accordingly, in our experiment we observed a decrease in nutrient content over time with little sign of efficient nutrient recycling (see Supporting Information).

Our results contrast with earlier experiments under identical warming treatments (up to 6 °C temperature range), conducted with plankton of the same geographical origin during nutrient-replete Baltic Sea winter conditions (Sommer *et al.* 2012). Under such conditions (cold, mixed, nutrient-rich) direct effects of warming on plankton metabolism prevailed. Warming was associated with increased copepod grazing rates and top-down regulation of phytoplankton biomass, which was strongly dominated by diatoms. When considered in the context of this previous study (Sommer *et al.* 2012), our current findings suggest that differences in the response of plankton to warming are explained primarily by the large seasonal contrast in nutrient supply under well-mixed vs. stratified conditions. These contrasting results suggest that effects of warming on plankton biomass and the balance of top-down vs. bottom-up forcing vary from the winter (high nutrient) to the summer (low nutrient) season. On the basis of this, we hypothesise that such seasonally varying mechanisms may also explain much of the global spatiotemporal variability in the response of plankton to gradual warming and increased stratification.

In an attempt to summarise results from this and previous warming experiments, we propose a conceptual model of the response of marine plankton to ocean warming contingent upon the prevailing nutrient regime (Fig. 4). In nutrient-replete waters such as most coastal regions or seasonally mixed shelf seas (e.g. Baltic Sea during winter/spring transition), temperature influences plankton mainly through metabolic changes (Fig. 4a). In such systems phytoplankton communities are typically dominated by large diatoms (20–200  $\mu\text{m}$ ), which are the preferred food source for overwintering copepods (Sommer & Sommer 2006). Warming creates a growing imbalance between zooplankton grazing and phytoplankton growth that progressively increases copepod grazing pressure and reduces the standing stock of phytoplankton. Globally, coastal and shelf regions are the most highly productive when scaled to their geographical area, thus increasing the relevance of the mechanisms described above.

In nutrient-limited waters such as the oligotrophic open ocean or seasonally stratified shelf seas (e.g. Baltic Sea in summer), temperature influences plankton mainly through physical mechanisms (related to stratification and nutrient supply); (Fig. 4b). Under these conditions, bottom-up regulation via nutrient supply primarily drives phytoplankton growth, abundance and size structure, with concurrent effects on planktonic consumers. Low-nutrient concentrations favour small



**Figure 4** Trophic interactions in marine pelagic ecosystems in response to sea surface warming. (a) In well-mixed, nutrient-rich waters phytoplankton is dominated by medium to large diatoms; these are heavily grazed by copepods. (b) In stratified, nutrient-limited waters small flagellates dominate phytoplankton biomass; copepods feed mainly on ciliates, increasing the relative importance of the microbial loop for energy flows. Warming (symbolised by gradient arrows) gradually elevates the thermocline, decreases nutrient supply and increases grazing pressure. This always leads to a reduction in phytoplankton biomass, with grazer effects on phytoplankton dominant in (a) and nutrient effects dominant in (b).

phytoplankton ( $< 20 \mu\text{m}$ ), such as flagellates (Edwards *et al.* 2012) and limit phytoplankton growth. Warming increases nutrient limitation, which results in reduction in phytoplankton biomass and a shift toward a phytoplankton assemblage dominated by small phytoplankton (Li *et al.* 2009; Morán *et al.* 2010). Due to the lack of preferred food, such as large diatoms ( $20\text{--}200 \mu\text{m}$ ), copepods switch to feed on ciliates (Stibor *et al.* 2004). As most of the ocean is strongly stratified and our model outcome predicts a large future decrease in nutrient supply (Fig. 1), the physical mechanism will probably explain most of the observed variability in open-ocean plankton biomass and the importance of this mechanism will increase as nutrients become more limited. Simple extrapolation of our results to open ocean communities should be, however, approached with caution. Species composition of such communities can be quite different from our experimen-

tal plankton assemblage, and this might introduce differing responses. However, we point out that the general shape of response functions of different phytoplankton species to changes in temperature and nutrients is universal (Litchman & Klausmeier 2008). Furthermore, we caution that changes in other physical processes like wind mixing or horizontal transport of water masses might be influential in affecting both nutrient supply and distribution of phytoplankton biomass in a warming ocean (Dave & Lozier 2013).

Our proposed model of the plankton response to warming is supported by results from an estuarine study, in which experimental warming caused increased grazing and lower phytoplankton biomass under nutrient-rich, but not under nutrient-poor conditions (O'Connor *et al.* 2009). This suggests that the model discussed here may be more broadly applicable, although more work is needed, particularly in oligotrophic open-ocean communities. It is also yet unclear, however, to which extent this might apply to freshwater ecosystems, because of large differences in plankton community structure between marine and freshwater ecosystems (Stibor *et al.* 2004; Sommer & Sommer 2006). A mesocosm experiment manipulating warming and stratification in a deep temperate lake found that both warming and increasing stratification sped up successional sequences (timing of bloom events), but did not affect the peak bloom biomass of phytoplankton and zooplankton (Berger *et al.* 2010).

Changes in marine plankton may have consequences for biogeochemical cycles, climate patterns, fisheries and the structure and function of marine ecosystems. These important and wide-ranging effects provide a strong motivation to better understand how plankton communities will change under continued ocean warming. At present, predicting the effects of climate change on marine plankton is constrained by the ability to suitably parameterise biological interactions and their strengths. The outcome of the POTSMOM-C model (Fig. 1) provides information on spatiotemporal distribution of low- and high-nutrient waters, whereas this and previous experiments (Stibor *et al.* 2004; O'Connor *et al.* 2009; Sommer *et al.* 2012) provide a mechanistic basis to understand changes in biotic interactions with climate warming. Taken together, these results highlight the importance of both physical and biological mechanisms in understanding the response of marine pelagic ecosystems to ocean warming, and enhance our ability to predict the impact of climate change on marine planktonic biomass and productivity.

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#### STATEMENT OF AUTHORSHIP

All authors contributed to the design of the study, data interpretation and manuscript editing; A.M.L., D.G.B., B.M., U.S. and B.W. performed the experiment; M.H. performed the circulation model work; D.G.B. performed all statistical analyses; A.M.L., D.G.B. and B.W. wrote the manuscript.



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