Final author comments: **Phytoplankton biomass, composition, and productivity along a temperature and stratification gradient in the Northeast Atlantic Ocean**

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The manuscript will undergo a major revision based on the valuable comments of the reviewers. We thank the reviewers for their work, which has improved the manuscript. In short, a supplement will be provided which states model equations, and group specific P vs E parameters. Furthermore, the supplement provides data on microscopy samples that were analyzed in concert with CHEMTAX analysis. Furthermore, pigment ratios used for CHEMTAX are provided in the supplement.

In addition changes were made to the photoacclimation assumptions for the productivity model. For the previous calculations low light acclimation was assumed at Chl-a concentrations in excess of 0.5 mg m^{-3}. This has been reconsidered and changed. The changes are supported by onboard experiments that investigated the photoacclimation state of the phytoplankton, using recovery of photosynthetic efficiency from excess light exposure as a measure for photoacclimation state. These experiments will be included in the revised ms. Due to these changes, productivity values higher latitudes in summer were higher (18%), whereas values for oligotrophic stations in spring were lower (26%). Graphs and correlations have changed accordingly. The changes had minor effects on the overall conclusions drawn from this work.

Please note that one co-author was added to the manuscript (P.D. Rozema), due to his involvement with the photoacclimation experiments.

Furthermore, hypotheses were included in the introduction.

Detailed enquiries are answered below.

Response to referee #3

1)There are statements in the discussion and conclusions that hint at the proposed focus for the study, e.g., the influence of temperature changes on phytoplankton community composition and productivity. The results and conclusions, and discussion, could also do with some level of hypotheses to add structure and order.

Hypothesis will be included in the introduction in ln 19: We hypothesized that SST influences phytoplankton biomass and composition by affecting nutrient concentrations in the upper open ocean. Therefore, relationships between SST and nutrient concentrations can be expected along existing temperature gradients. Furthermore, relationships between SST, phytoplankton biomass, composition and productivity can be expected along existing temperature gradients in the open ocean. Recent studies on temperature and stratification relationships have focused on the oligotrophic open ocean, where nutrient limitation of phytoplankton is a dominant feature (Behrenfeld et al., 2006; Polovina et al., 2008, Dave and Lozier, Lozier et al., 2011). In this context, temperate and higher latitude regions have received less attention and studies that include both oligotrophic and higher latitudes waters on this topic are currently lacking.

2)The use of CHEMTAX to assign chlorophyll biomass between phytoplankton groups is well used in the literature, and there are recognised limitations to this approach which require careful consideration and ground-truthing. The accompanying study by Mojica et al. (submitted to L&O and unavailable for review) potentially holds such information but currently it is not clear whether this paper supports the appropriation of biomass between phytoplankton groups. Neither is it clear whether these two papers contain the same data/information or conclusions.

A comparison of CHEMTAX with light microscopy and flow cytometry data will be included in the supplement:

Comparison of CHEMTAX with light microscopy and flow cytometry

The taxonomic information obtained by CHEMTAX was compared with light microscopy observations on fixed sampled and with flow cytometry data (Mojica et al. submitted). For light microscopy, 100 ml seawater was fixed by 1 ml of Lugol iodine solution, supplemented with 0.5% glutarealdehyde in dark bottles. Based on CHEMTAX, 18 samples (7 and 11 from spring and summer, respectively) were selected for light microscopic analysis and compared with the taxonomic data obtained with CHEMTAX. Fifty ml of fixed sample was concentrated by sedimentation (24 h) and observations were made on an Olympus IMT-2 inverted microscope, using 20 and 40 times magnification for phytoplankton larger and smaller than 20 µm, respectively. The microscopy observations are briefly discussed below.

In summer, the haptophytes *Phaeocystis sp* (free cells, on bladders and in colonies) increased in concentration from low to high latitude (38,000 up to 2,208,000 cells l^{-1}, dominating the phytoplankton biomass at higher latitudes). Diatom concentrations in summer were low and increased from low to higher latitudes (0-2,000 up to 25,000 cells l^{-1}, *Pseudo-Nitzschia delicatessima*, *Nitzschia longissima*). Larger diatoms were found at low concentrations at high latitudes (*Rhizosolenia*, *Proboscia* sp <1,000 cells l^{-1}). Small dinoflagellates (< 15 µm) appeared mostly heterotrophic (concentrations 22,000-100,000 cells l^{-1}). Larger dinoflagellates were observed at higher latitudes in low concentrations (*Ceratium* sp, < 2,000 cells l^{-1}). In spring, *Phaeocystis* was not abundant, but small *Emiliania huxleyi* like cells were abundant at mid-latitudes (7,074,887 cells l^{-1}). However, the presence of this species was not confirmed by flow cytometry. Furthermore, unidentified pico-eukaryotes were abundant (584,000-4,162,433 cells l^{-1}) at low and mid latitudes in spring. Small diatoms (*Chaetoceros* sp and *Nitszchia longissima*) concentrations were around 3,760 cells l^{-1} at stratified stations, whereas small (presumably heterotrophic) dinoflagellates were around 4,000 cells ml^{-1}. Large dinoflagellates (*Ceratium* sp.) were found in concentrations of 40 cells l^{-1}. At non-stratified stations, large diatoms (*Chaetoceros* sp., *Thalassiosira* sp., *Proboscia* sp., *Rhizosolenia* sp., dominated the phytoplankton community at latitude 25 °N. At higher latitudes, large (>20 µm) Prasinophytes (5,600 cells l^{-1}) and Cryptophytes (6,000 cells l^{-1}) were observed, whereas diatom concentrations were lower.

Data obtained by flow cytometry will be presented in detail by Mojica et al. (submitted). Patterns obtained by flow cytometry of *Synechococcus* spp. and *Prochlorococcus* spp. were comparable with those obtained by pigment composition. However, flow cytometry abundance of *Prochlorococcus* spp. in the upper 50 m of oligotrophic stations in summer was higher than the contribution to Chl-a suggested from pigment composition.

Direct comparison of phytoplankton composition between these methods is complicated by the differences in units and by the specific limitations of each method. Flow cytometry provides abundance data of phytoplankton groups that are smaller than 20 µm, including some

groups that are difficult to identify using light microscopy (e.g. small eukaryotes, *Prochlorococcus* spp., and *Synechococcus* spp.). Light microscopy gives detailed information on larger phytoplankton species. In contrast, CHEMTAX provides taxonomic information relative to Chl-a for phytoplankton with a size range > 0.7 µm. In this respect, all methods are complementary to each other. Overall, patterns in phytoplankton composition obtained by light microscopy and flow cytometry were in agreement with CHEMTAX. The dominance of phytoplankton with a haptophytes pigment signature, and the low contribution of diatoms in summer to the phytoplankton community were revealed by light microscopy and CHEMTAX. The dominance of diatoms at higher latitudes in spring was observed by both CHEMTAX and light microscopy. Also the overall low contribution of (photosynthetic) dinoflagellates was shown by CHEMTAX and light microscopy. In oligotrophic waters, increasing dominance of *Prochlorococcus* spp., *Synechococcus* spp. was shown by CHEMTAX and flow cytometry with decreasing latitude.

3) Specific Comments - the abstract lacks a statement of the purpose of the study.
The following will be included in the abstract: The goal of this study was to identify relationships between phytoplankton and physical factors in an existing SST and stratification gradient.

4) pg 7, ln 4: oligotrophic waters are defined as below the detection limits, but what are the detection limits? **Detection limit for nitrate: 0.03 µmol/l, will be included in the method.**

5) pg 8, ln 15: why choose 0.1% as depth of the euphotic zone rather than 1% as often used in other studies? **We choose the 0.1% depth as the limit of the euphotic zone because in our opinion this is a better representation of the depth below which net photosynthesis is negligible. With maximal PAR surface values between 2000-3000 µmol photons m^{-2} s^{-1}, light at the 1% light depth would be between 20-30 µmol photons m^{-2} s^{-1}, which is more than enough for net phytoplankton photosynthesis.**

6) Were the CHEMTAX results ground-truthed in any way? Difficult to access this without access to Mojica et al. (submitted). This is key to the manuscript and modelling of the group specific production and so the lack of information makes it impossible to access the validity of the pigment or model data. **CHEMTAX data were compared with flow cytometry data and with light microscopy data on lugol fixed samples. See supplement section above.**

7) High and low light pigment ratios?
Pigment ratios used for CHEMTAX will be provided in the supplement and in Mojica et al. (submitted).

8) pg 8, ln 23: Why were samples grouped by latitude, and in what way where they? **The data were grouped to minimize the residual error of the CHEMTAX calculations.**

9) pg 9, ln 12: What are the further details on phytoplankton species composition in Mojica et al.? **Mojica et al presents the CHEMTAX data that were used for the productivity calculations of this ms. Furthermore, Mojica et al presents extensive data on flow cytometry from the same cruise.**

10) pg 9, ln 26: How is satellite derived irradiance data in situ? **This is not in situ and this statement will be rephrased.**

11) How valid is a linear relationship between carbon fixation and temperature? How is the slope of a growth versus temperature experiment in units of mg C m-2 d-1? **As stated in the discussion, a linear relationship is in our opinion more valid than an exponential relationship with temperature. There are no data on phytoplankton growth vs temperature responses of individual species that support an exponential response in this temperature range.**

12) pg 10, ln 11: Is Chl-a a valid measure of phytoplankton biomass? **As stated this is used as an indicator of phytoplankton biomass. It is well known and also discussed in this ms that Chl-a has limitations as a biomass indicator.**

13) PvE parameters from nutrient replete cultures, growing under optimum irradiance conditions, do not seem appropriate for use with field samples?
The irradiance conditions of the cultures are provided in the supplement, taken from Kulk et al. 2011. **Due to the high turnover of phytoplankton, nutrient starved algae with low growth rates will be largely removed from the community by grazing. Furthermore, net primary production relative to Chl-a typically does not change during nutrient limited growth.**

What was the light:dark cycle of these cultures? What were their daily photon fluxes and how did they correspond to the in situ conditions?, **Kulk et al. 2011 used a 12h light-12h dark cycle, this will be in the supplement information.**

14) pg 11, ln 2: What is the basis for assuming that where Chl exceeds 0.5 mg m-3, the phytoplankton community was low light adapted? Reference? How does this influence the results? **This part has been reconsidered and this criterion was removed. After evaluation of our measurements the following photoacclimation considerations were included. For the summer, we used data for high light acclimated species up to the irradiance dose of 5.4 mol m^{-2} day^{-1} (corresponding to the cultures that were grown at 125 µmol photons m^{-2} s^{-1}). For depths experiencing lower light doses, phytoplankton was assumed low light acclimated. For spring, we assumed low light acclimated phytoplankton for all depths. We used onboard experiments during which recovery of photosynthetic efficiency of excess light exposed phytoplankton was monitored and used these results as indicator for photoacclimation state. These experiments showed that phytoplankton recovery after excess light exposure was significantly lower in spring compared to summer. Furthermore, differences between samples from the chlorophyll maximum and subsurface were negligible in spring, whereas they were clearly visible in summer. These experiments will be included in the method and results and will be presented in a graph.**

15) pg 12, ln 7: The correlation between SST and stratification is reported, with correlation coefficients, but not p values are reported. Where these statistically significant?
All reported correlations in bold were significant at $P < 0.05$, as stated in the method section.

16) pg14, ln 24: Only 30% of productivity from cyanobacteria: how does this compare with other studies? **References to studies with comparable results are included.**

17) How do the contributions in spring and summer compare with similar studies? **References to studies with comparable results are included.**

18) pg 21, ln 2: Is this the aim of the study - "Overall, this study showed that the model approach can expand the use of phytoplankton pigments and provided useful insight in group specific productivity"??
This sentence is rephrased: Overall, the model approach can expand the use of phytoplankton pigments and provided useful insight in group specific productivity.

19) Tables 1-4: bold values are 'significant' at what level? **P-value are stated in the results section.**