Ocean acidification and high irradiance stimulate the photophysiological fitness, growth and carbon production of the Antarctic cryptophyte *Geminigera cryophila*

Scarlett Trimborn[1,2], Silke Thoms[1], Pascal Karitter[1], Kai Bischof[2]

[1]EcoTrace, Biogeosciences section, Alfred Wegener Institute, Bremerhaven, 27568, Germany
[2]Marine Botany, Department 2 Biology/Chemistry, University of Bremen, Bremen, 28359, Germany

Correspondence to: Scarlett Trimborn (scarlett.trimborn@awi.de)

Abstract.

Ecophysiological studies on Antarctic cryptophytes to assess whether climatic changes such as ocean acidification and enhanced stratification affect their growth in Antarctic coastal waters in the future are lacking so far. This is the first study that investigated the combined effects of increasing availability of pCO_2 (400 and 1000 µatm) and irradiance (20, 200 and 500 µmol photons m^{-2} s^{-1}) on growth, elemental composition and photophysiology of the Antarctic cryptophyte *Geminigera cryophila*. Under ambient pCO_2, this species was characterized by a pronounced sensitivity to increasing irradiance with complete growth inhibition at the highest light intensity. Interestingly, when grown under high pCO_2 this negative light effect vanished and it reached highest rates of growth and particulate organic carbon production at the highest irradiance compared to the other tested experimental conditions. Our results for *G. cryophila* reveal beneficial effects of ocean acidification in conjunction with enhanced irradiance on growth and photosynthesis. Hence, cryptophytes such as *G. cryophila* may be potential winners of climate change, potentially thriving better in more stratified and acidic coastal waters and contributing in higher abundance to future phytoplankton assemblages of coastal Antarctic waters.

1 Introduction

Even though Antarctic coastal waters comprise a relatively small area relative to the open ocean, these waters are highly productive due to the constant supply of macronutrients and iron (Arrigo et al., 2008). Shelf waters adjacent to the Western Antarctic Peninsula (WAP) are currently undergoing rapid physical changes, exhibiting the most rapid warming rates than anywhere in Antarctica over the last decades (Ducklow et al., 2007, 2013). Rising air temperature resulted in shorter sea ice seasons (Smith and Stammerjohn, 2001) with contrasting effects on phytoplankton biomass, composition and productivity between the northern and southern WAP. For the latter, the earlier retreat of sea ice together with the observed increase in surface water temperature led to shallow water column stratification, which favored phytoplankton growth and productivity. In the northern part of the WAP on the other hand, the earlier disappearance of sea ice was associated to greater wind activities and more cloud formation. As a consequence, a deepening of the upper mixed layer was found, providing less favorable light conditions. Next to reduced chlorophyll *a* accumulation (Montes-Hugo et al., 2009) and primary production (Moreau et al., 2015), a decline of large phytoplankton such as diatoms relative to the whole community was observed (Montes-Hugo et al., 2009; Rozema et al., 2017). Accordingly, a recurrent shift from diatoms to cryptophytes and small flagellates was reported for waters north of the WAP, with important implications for food web dynamics (Moline et al., 2004; Ducklow et al., 2007; Montes-Hugo et al., 2009; Mendes et al., 2017). The frequent occurrence of cryptophytes was previously reported after diatom blooms (Moline and Prezelin, 1996) and was related to surface melt water

stratification (Moline et al., 2004). As a result from rising global air temperatures, surface water freshening is expected to shallow the upper water layer, exposing phytoplankton to higher light intensity (Moreau et al., 2015). Relative to diatoms and the prymnesiophyte *Phaeocystis antarctica*, cryptophytes were found to be the main contributors to biomass in stratified and warm WAP coastal waters potentially resulting from their high tolerance to withstand high irradiances (Mendes et al., 2017). Considering the lack in ecophysiological studies carried out with Antarctic ecologically relevant cryptopyte species it remains yet unclear whether the projected climatic changes could promote cryptophyte growth in Antarctic coastal waters in the future. Hence, higher abundances of cryptophytes could have important implications for the biogeochemistry of these waters, as they are considered to be inefficient vectors of carbon and thus could reduce the efficiency of the biological carbon pump.

Light availability strongly influences the rate of growth and carbon fixation of phytoplankton (Falkowski and Raven 2007). With increasing irradiance, Antarctic phytoplankton species exhibited increased growth and carbon fixation, but only until photosynthesis was saturated (Fiala and Oriol, 1990; Heiden et al., 2016). When exposed to excessive radiation, phytoplankton cells can get photoinhibited and even damaged (Falkowski and Raven, 2007). Cryptophytes are exceptional among eukaryotic microalgae as they contain similar to diatoms chlorophyll a/c proteins, the carotenoid alloxanthin and phycobiliproteins homologous to red algal phycobiliproteins (Gould et al., 2008). This pigment composition allows cryptophytes to cope well under limited irradiance. Different to diatoms and prymnesiophytes, cryptophytes have no photoprotective de-epoxidation/epoxidation cycling of xanthophyll pigments (e.g. diadinoxanthin, diatoxanthin), instead they rely on chlorophyll a/c proteins, which function to dissipate excess light energy, as another type of nonphotochemical quenching (NPQ; Funk et al., 2011; Kana et al., 2012). While laboratory studies so far mainly have concentrated to disentangle the physiological response of Southern Ocean key species of diatom and prymnesiophytes to different environmental factors almost nothing is known on Antarctic cryptophytes. Apart from field studies (Moline et al., 2004; Ducklow et al., 2007; Montes-Hugo et al., 2009), almost nothing is known on how climate change could influence the ecophysiology of Antarctic cryptophytes. Due to the increased solubility of CO_2 in cold water, ocean acidification (OA) is anticipated to strongly affect polar waters (Orr et al., 2005; IPCC Report, 2014). For various Antarctic diatoms and the prymnesiophyte *P. antarctica*, growth and/or carbon fixation remained unaltered by OA alone (Riebesell et al., 1993; Boelen et al., 2011; Hoogstraten et al., 2012; Trimborn et al., 2013; Hoppe et al., 2015; Heiden et al., 2016). Recent studies suggest that Southern Ocean diatoms are more prone to OA especially in conjunction with high light than the prymnesiophyte *Phaeocystis antarctica* (Feng et al., 2010; Trimborn et al., 2017a,b; Beszteri et al. 2018; Heiden et al., 2018; Koch et al., 2018, Heiden et al., 2019). The response of cryptophytes to OA is hitherto almost unexplored. The few studies on temperate phytoplankton communities suggest that cryptophytes were not affected by OA (Schulz et al., 2017), even when exposed in combination with increased ultraviolet radiation (Domingues et al., 2014) or warming (Sommer et al., 2015). Similarly, no discernible OA effect on cryptophyte abundance was found in subantarctic (Donahue et al., 2019) and Antarctic (Young et al., 2015) natural phytoplankton communities composed of diatoms, cryptophytes, and *Phaeocystis* spp..

Based on previous studies on the single effects of light or CO_2 alone, we hypothesize that cryptophytes are able to cope well with OA and high light conditions. Due to the limited information available on Antarctic cryptophyte physiology, this study assessed OA effects and their interaction with increasing irradiance on the physiology of the Antarctic cryptophyte *Geminigera cryophila*. To this end, *G. cryophila* was grown under two

pCO$_2$ levels (400 and 1000 µatm) in combination with three irradiance levels (20, 200 and 500 μmol photons m^{-2} s^{-1}) and their interactive effects on growth, elemental composition and photophysiology were assessed.

2 Material and Methods

2.1 Culture conditions

Triplicate semi-continuous cultures of the Antarctic cryptophyte *Geminigera cryophila* (CCMP 2564) were grown in exponential phase at 2 °C in sterile-filtered (0.2 μm) Antarctic seawater (salinity 30.03). This seawater was enriched with phosphate (final concentration of 100 μmol L^{-1}), nitrate (final concentration of 6.25 μmol L^{-1}) (N:P ratio of 16:1, Redfield, 1963) as well as trace metals and vitamins according to F/2 medium (Guillard and Ryther, 1962). *G. cryophila* cells were grown under a 16h light: 8h dark cycle at three constant light intensities (LL = 20, ML = 200 and HL = 500 μmol photons m^{-2} s^{-1}) using light-emitting diodes (LED) lamps (SolarStinger LED SunStrip Marine Daylight, Econlux). Light intensities were adjusted using a LI-1400 datalogger (Li-Cor, Lincoln, NE, USA) with a 4π-sensor (Walz, Effeltrich, Germany). The three light treatments were further combined with two CO$_2$ partial pressures (pCO$_2$) of 400 (ambient pCO$_2$ treatment) or 1000 μatm (OA treatment, Table 1). All pCO$_2$ treatments and the respective dilution media were continuously and gently bubbled through a frit with humidified air of the two pCO$_2$ levels, which were generated from CO$_2$-free air (< 1 ppmv CO$_2$; Dominick Hunter, Kaarst, Germany) and pure CO$_2$ (Air Liquide Deutschland ltd., Düsseldorf, Germany) with a gas flow controller (CGM 2000, MCZ Umwelttechnik, Bad Nauheim, Germany). The CO$_2$ gas mixtures were regularly monitored with a nondispersive infrared analyzer system (LI6252; Li-Cor Biosciences) calibrated with CO$_2$-free air and purchased gas mixtures of 150 ± 10 and 1000 ± 20 ppmv CO$_2$ (Air Liquide Deutschland). Dilutions with the corresponding acclimation media ensured that the pH level remained constant (±0.07 units, Table 1) and that the cells stayed in the exponential growth phase. *G. cryophila* cells were acclimated to the matrix of three light intensities (LL = 20, ML = 200 and HL = 500 μmol photons m^{-2} s^{-1}) and two pCO$_2$ levels (ambient = 400 and OA = 1000 µatm) for at least two weeks prior to the start of the main experiment. Despite several attempts, *G. cryophila* did not grow at ambient pCO$_2$ in conjunction with HL. For the main experiments, cells grew in exponential phase and final sampling took place between 7,280 and 17,161 cells per mL.

2.1 Seawater carbonate chemistry

The pH of the different cultures and the culture medium was measured every other day and at the final sampling day using a pH-ion meter (pH-Meter 827, Metrohm), calibrated (3 point calibration) with National Institute of Standards and Technology-certified buffer systems. The pH remained constant at 8.13 ± 0.07 and 7.82 ± 0.06 for the ambient pCO$_2$ and OA treatments, respectively (Table 1). Dissolved inorganic carbon (DIC) samples were sterile-filtered (0.2 μm) and stored at 4 °C in 5 mL gas-tight borosilicate bottles without headspace until analysis. DIC was measured colourimetrically in duplicates with a QuAAtro autoanalyzer (Seal Analytical, Stoll et al., 2001). The carbonate system was calculated based on DIC, pH, silicate (97 µmol kg^{-1}), phosphate (6.1 µmol kg^{-1}), temperature (2.0 °C) and salinity (30.03) using the CO2Sys program (results shown in Table 1, (Pierrot et al., 2006) choosing the equilibrium constant of Mehrbach et al. (1973) refitted by Dickson and Millero (1987).

2.2 Growth, elemental stoichiometry and composition

Cell count samples of every *G. cryophila* treatment were taken on a daily basis at the same time of day and were determined immediately after sampling using a Coulter Multisizer III (Beckmann-Coulter, Fullerton, USA). Cell-specific growth rate (μ, unit d^{-1}) was calculated as

$$\mu = (\ln N_{fin} - \ln N_0)/\Delta t, \tag{1}$$

where N_0 and N_{fin} denote the cell concentrations at the beginning and the end of the experiments, respectively, and Δt is the corresponding duration of incubation in days.

Particulate organic carbon (POC) and particulate organic nitrogen (PON) were measured after filtration onto precombusted (12 h, 500 °C) glassfibre filters (GF/F, pore size ~0.6 μm, Whatman). Filters were stored at -20 °C and dried for >12 h at 64 °C prior to sample preparation. Analysis was performed using an Euro Vector CHNS-O elemental analyzer (Euro Elemental Analyzer 3000, HEKAtech GmbH, Wegberg, Germany). Contents of POC and PON were corrected for blank measurements and normalized to filtered volume and cell densities to yield cellular quotas. Production rates of POC and PON were calculated by multiplication of the cellular quota with the specific growth rate of the respective treatment.

2.3 Chlorophyll *a* fluorescence

The efficiency of photochemistry in photosystem II (PSII) was assessed in all treatments using a Fast Repetition Rate fluorometer (FRRf, FastOcean PTX; Chelsea Technologies Group ltd., West Molesey, United Kingdom) in combination with a FastAct Laboratory system (Chelsea Technologies Group ltd., West Molesey, United Kingdom). Cells of the respective treatment were dark-acclimated for 10 min, before minimum chlorophyll *a* (Chl *a*) fluorescence (F_o) was recorded. Subsequently, a single turnover flashlet (1.2 x 10^{22} photons m^{-2} s^{-1}, wavelength 450 nm) was applied to cumulatively saturate photosystem II (PSII), i.e. a single photochemical turnover (Kolber et al., 1998). The single turnover saturation phase comprised 100 flashlets on a 2 μs pitch and was followed by a relaxation phase comprising 40 flashlets on a 50 μs pitch. This sequence was repeated 24 times within each acquisition. The saturation phase of the single turnover acquisition was fitted according to Kolber et al. (1998). From this measurement, the minimum (F_o) and maximum (F_m) Chl *a* fluorescence was determined. Using these two parameters, the dark-adapted maximum PSII quantum yield (F_v/F_m) was calculated according to the equation ($F_m - F_o$) / F_m. During the fluorescence light curve (FLC), cells were exposed for 5 min to eight actinic light levels ranging from 35 to 1324 μmol photons m^{-2} s^{-1}. From these measurements, the light-adapted minimum (F') and maximum (F_m') fluorescence of the single turnover acquisition was estimated. The effective PSII quantum yield under ambient light (F_q'/F_m') was derived according to the equation ($F_m' - F'$) / F_m' (Genty et al., 1989). From this curve, absolute electron transport rates (ETR, e$^-$ PSII^{-1} s^{-1}) were calculated following Suggett et al. (2004, 2009):

$$\text{ETR} = \sigma_{PSII} \text{ x } (F_q'/F_m' \text{ / } F_v/F_m) \text{ x E}, \tag{2}$$

where σ_{PSII} is the functional absorption cross section of PSII photochemistry and E denotes the applied instantaneous irradiance (photons m^{-2} s^{-1}). Light-use characteristics were analyzed by fitting irradiance-dependent ETRs according to Ralph and Gademann (2005), including maximum ETR (ETR$_{max}$), minimum saturating irradiance (I_K) and maximum light utilization efficiency (α). Using the Stern-Volmer equation, nonphotochemical quenching (NPQ) of chlorophyll *a* fluorescence was calculated as F_m / F_m' − 1. From the single turnover measurement of dark-adapted cells, σ_{PSII}, the energy transfer between PSII units (i.e. connectivity, *P*), the re-oxidation of the electron acceptor Q_a (τ) and the concentration of functional photosystem II reaction centers ([RII]) were assessed from iterative

algorithms for induction (Kolber et al., 1998) and relaxation phase (Oxborough, 2012). [RII] represents an estimator for the content of PSII in the sample and was calculated according to the following equation:

$$[RII] = (F_o / \sigma_{PSII}) \times (K_R / E_{LED}),\tag{3}$$

where K_R is an instrument specific constant and E_{LED} is the photon flux from the FRRf measuring LEDs. After the completion of the FLC curve, an additional dark-adaptation period of 10 min was applied, followed by a single turnover flashlet to check for recovery of PSII. Using the F_v/F_m measured before and after the FLC-curve, the yield recovery was calculated and given as % of the initial F_v/F_m (before the FLC-curve). All measurements (n = 3) were conducted at the growth temperature of 2 °C.

2.4 Pigments

Samples for the determination of pigment concentration were filtered onto GF/F filters and immediately frozen at -80 °C until further analysis. Pigments samples were homogenized and extracted in 90% acetone for 24h at 4 °C in the dark. After centrifugation (5 min, 4 °C, 13000 rpm) and filtration through a 0.45 μm pore size nylon syringe filter (Nalgene®, Nalge Nunc International, Rochester, NY, USA), concentrations of chlorophyll *a* (Chl *a*) and c₂ (Chl c_2), and alloxanthin (Allo) were determined by reversed phase High Performance Liquid Chromatography (HPLC). The analysis was performed on a LaChromElite® system equipped with a chilled autosampler L-2200 and a DAD detector L-2450 (VWR-Hitachi International GmbH, Darmstadt, Germany). A Spherisorb® ODS-2 column (25 cm x 4.6 mm, 5 *μ*m particle size; Waters, Milford, MA, USA) with a LiChropher® 100-RP-18 guard cartridge was used for the separation of pigments, applying a gradient according to Wright et al. (1991). Peaks were detected at 440 nm and identified as well as quantified by co-chromatography with standards for Allo, Chl *a* and c2 (DHI Lab Products, Hørsholm, Denmark) using the software EZChrom Elite ver. 3.1.3. (Agilent Technologies, Santa Clara, CA, USA). Pigment contents were normalized to filtered volume and cell densities to yield cellular quotas.

2.5 Statistics

Combined effects of the two pCO₂ (ambient and OA) and light (LL, ML, and HL) treatments on all experimental parameters were statistically analyzed using two-way analyses of variance (ANOVA) with Bonferroni's multiple comparison post tests. To test for significant differences between light treatments of the OA-grown cells of *G. cryophila* cells one-way ANOVAs with additional Bonferroni's multiple comparison post tests were applied. All statistical analyses were performed using the program GraphPad Prism (Version 5.00 for Windows, Graph Pad Software, San Diego California, USA) and the significance testing was done at the $p < 0.05$ level.

3 Results

3.1 Seawater carbonate chemistry

The two target pCO₂ levels of 400 and 1000 μatm were successfully achieved for abiotic control (abiotic, bubbled seawater without cells) and culture bottles (biotic, 1-*way ANOVA*: $p < 0.0001$, Table 1). As the pCO₂ of the abiotic control and culture bottles of the same pCO₂ treatment were similar, this indicates that final cell numbers of *G. cryophila* did not alter the pCO₂ of the culture bottles relative to the culture medium. Similar trends as for the pCO₂ were also apparent for the measured pH values, which yielded 8.13 ± 0.07 and 7.82 ± 0.06 in the culture bottles of the ambient and OA treatments, respectively (Table 1). While DIC concentrations were significantly enhanced for

OA relative to the ambient pCO_2 treatments (*1-way ANOVA*: $p < 0.0001$), they also significantly differed between abiotic control (abiotic, bubbled seawater without cells) and culture bottles (biotic, Table 1).

3.2 Growth, elemental stoichiometry and composition

Growth rates were significantly affected by light (*2-way ANOVA*: $p < 0.01$), but not by OA or the interaction of both factors (Fig. 1A). In response to increasing irradiance, growth rates of cells grown under ambient pCO_2 remained unchanged between LL and ML, but were negatively influenced by HL as they were unable to grow. Under OA, however, growth rates significantly increased between LL and ML by 89% (posthoc: $p < 0.05$) and between ML and HL by 32% (posthoc: $p < 0.05$), respectively. Irrespective of changes in irradiance, pCO_2 or their combination, cellular POC contents did not change (Fig. 1B). Daily POC production rates, were, however, significantly altered by increasing irradiance (*2-way ANOVA*: $p < 0.01$), but not by OA or the interaction of both factors (Fig. 1C). While increasing light intensity did not affect POC production rates of the ambient pCO_2 treatments, there was a significant OA-dependent enhancement by 69% between LL and ML (posthoc: $p < 0.01$) and by 39% between ML and HL, respectively (posthoc: $p < 0.05$). Molar C:N ratios were only significantly influenced by the interaction of both factors together (*2-way ANOVA*: $p < 0.01$; Fig. 1D). From LL to ML C:N ratios did not change for both pCO_2 treatments whereas from ML to HL the ratio declined by 12% for the OA treatment (*1-way ANOVA*: $p < 0.05$). In response to increasing pCO_2, C:N decreased by 10% when grown under LL (posthoc: $p < 0.05$), but remained unaltered at ML.

3.3 Pigments

For all pCO_2 treatments, cellular concentrations of the measured pigments (Allo, Chl *a* and c$_2$) showed a strong and significant decline between LL and ML (*2-way ANOVA*: $p < 0.0001$, Table 2). Between ML and HL, however, different effects were seen, with a significant enhancement for Chl *a* (posthoc: $p < 0.01$) and Allo (posthoc: $p < 0.01$) and no effect for Chl c$_2$. Increasing pCO_2 had generally no effect on cellular pigment quotas (Allo, Chl *a* and c$_2$) except for the Allo quotas of the LL treatments, which displayed a significant OA-dependent decline by 26% (posthoc: $p < 0.01$). A significant interaction between light and CO_2 was only found for Allo quotas (*2-way ANOVA*: $p < 0.05$).

3.4 Chlorophyll *a* fluorescence

The dark-adapted maximum quantum yield of PSII (F_v/F_m) was strongly influenced by irradiance (*2-way ANOVA*: $p < 0.0001$) and CO_2 (*2-way ANOVA*: $p = 0.0012$) and their interaction (*2-way ANOVA*: $p < 0.05$; Fig. 2A). With increasing irradiance F_v/F_m generally declined whereas OA increased it at LL (17%, posthoc: $p < 0.01$) or did not change it at ML. Noticeably, the interaction of HL and OA resulted in the lowest F_v/F_m value. Comparing the F_v/F_m measured before and after the FLC-curve, the F_v/F_m recovery was calculated and given as % of the initial F_v/F_m. Neither high pCO_2 nor the interaction of light and CO_2 affected F_v/F_m recovery whereas the increase in irradiance had a significant effect (*2-way ANOVA*: $p < 0.01$, Fig. 2B), being increased by 11% between LL and ML under ambient pCO_2 (posthoc: $p < 0.05$).

The increase of CO_2 or light alone had no effect on cellular concentrations of functional photosystem II reaction centers ([RCII]) while the interaction of both factors strongly altered [RCII] (*2-way ANOVA*: $p < 0.0001$; Fig. 3A). From LL to ML [RCII] decreased under ambient pCO_2 (39%, posthoc: $p < 0.001$) while the combination of ML with OA synergistically increased it (44%, posthoc: $p < 0.01$, Fig. 3). [RCII] was reduced by OA at LL (37%

posthoc: $p < 0.01$) whereas the combined effect of OA and ML led to an increase (49%, posthoc: $p < 0.01$). While CO_2 and the interaction of CO_2 and light together did not change the energy transfer between PSII units (i.e. connectivity, P), only the increase in irradiance had a significant effect (*2-way ANOVA*: $p < 0.05$), reducing P by 22% between LL and ML under OA (posthoc: $p < 0.05$, Figure 3B). While the increase in CO_2 or light did not alter

5 the functional absorption cross-sections of PSII (σ_{PSII}), the interaction of both factors, however, had an effect (*2-way ANOVA*: $p < 0.05$; Figure 3C). σ_{PSII} values were similar under LL and ML at ambient pCO_2. The interaction of OA and ML, however, lowered them (posthoc: $p < 0.05$, Table 3). On the other hand, when grown under OA σ_{PSII} was larger under HL than under ML (*1-way ANOVA*: $p < 0.01$). Re-oxidation times of the primary electron acceptor Q_a (τ_{Qa}) significantly changed with increasing irradiance (*2-way ANOVA*: $p < 0.05$), but not by high CO_2 or the

10 interaction of both factors together (Figure 3D). τ_{Qa} of OA-acclimated cells was much shorter at HL than at ML (*1-way ANOVA*: $p < 0.05$).

Absolute ETRs differed in amplitude and shape in response to the applied changes in irradiance and pCO_2 (Fig. 4A-C). Both maximum absolute electron transport rates (ETR_{max}) and minimum saturating irradiances (I_K) followed the same trend and were significantly changed by CO_2 (*2-way ANOVA*: $p < 0.05$), but not by light or the

15 interaction of both factors (Table 3). OA significantly enhanced both parameters under LL (ETR_{max}: posthoc: $p < 0.05$, I_K: posthoc: $p < 0.05$), but not under ML. The maximum light utilization efficiency (α) was significantly affected by light (*2-way ANOVA*: $p < 0.01$) and the interaction of CO_2 and light (*2-way ANOVA*: $p < 0.01$), but not by CO_2 alone (Table 3). α significantly increased from LL to ML at ambient pCO_2 (53%, posthoc: $p < 0.01$) while such effect was absent under ML and OA. Between ML and HL, α did not differ when grown under OA.

20 Nonphotochemical quenching (NPQ) generally went up with increasing actinic irradiance during the FLC (Fig. 4D-F). Compared with the LL treatments, NPQ values of the ML and HL treatments were as twice as high. There were no differences in the NPQ pattern between ML and HL treatments observed. Much higher NPQ values were determined in the ambient pCO_2 relative to the OA treatment under LL while such pCO_2 effect was absent under ML.

25 **4 Discussion**

Ecophysiological studies on Antarctic cryptophytes to assess whether climatic changes such as ocean acidification and enhanced stratification affect their growth in Antarctic coastal waters in the future are lacking so far. This study can show that the Antarctic cryptophyte *G. cryophila* may be a potential winner of such climatic conditions as it reached highest rates of growth and particulate organic carbon production when grown under HL and OA.

30

4.1 *Geminigera cryophila* is sensitive to increasing irradiance under ambient pCO_2

The cryptophyte *G. cryophila* was well adapted to grow under LL and ML at ambient pCO_2, yielding similar growth, POC quotas and production rates as well as C:N ratios (Fig. 1). In line with this, the exposure of the cryptophyte *Rhodomonas salina* to 30 up to 150 μmol photons m^{-2} s^{-1} did not lead to any changes in growth rate at 5

35 °C (Hammer et al., 2002). Even though growth and biomass remained unchanged in *G. cryophila* between LL and ML, acclimation to the even higher light intensity of 500 μmol photons m^{-2} s^{-1} was indicated by the reduction in F_v/F_m and [RCII] (Figs. 2A, 3A). Such decline in the number of photosystems is a typical photoacclimation response of most microalgae to increasing light and is usually accompanied by a decrease in cellular concentrations of light harvesting pigments (MacIntyre et al., 2002), as seen here for cellular Chl a and c$_2$ quotas (Table 2). Even though

most studies on temperate cryptophytes report a photoprotective function of Allo, with higher amounts of this carotenoid toward high irradiance (Funk et al., 2011; Laviale and Neveux, 2011), the reduction in cellular Allo quotas from LL to ML in our tested species (Table 2) rather suggests its role in light absorption. Similarly, cellular Allo contents also declined between 40 and 100 μmol photons m^{-2} s^{-1} in the temperate cryptophyte *Rhodomonas marina* (Henriksen et al., 2002). In this study, various photophysiological parameters (P, σ_{PSII}, τ_{Qa}, Fig. 3) did not change between LL and ML in *G. cryophila* while other photoacclimation processes such as higher ETR$_{max}$, I_k and α took place (Table 3). Such light-dependent apparent higher electron flow was accompanied by similar high POC quotas and production rates between LL and ML (Fig. 1B, C) and suggests saturation of the Calvin cycle and therewith the requirement for alternative electron cycling to dissipate the excessive light energy. In the temperate *R. salina*, the onset of NPQ was induced after saturation of the Calvin cycle and found to be located in the chlorophyll a/c proteins and not in the phycobiliproteins (Kana et al., 2012). In fact, NPQ was strongly enhanced in *G. cryophila* between LL and ML at ambient pCO$_2$ (Fig. 4). As ML- relative to LL-acclimated cells also exhibited a higher potential of F_v/F_m recovery after the FLC curve (Fig. 2B), it appears that all these adjustments allowed a reduction of the excitation pressure on the photosynthetic apparatus and protected *G. cryophila* well against short-term high light exposure. Unexpectedly, *G. cryophila* was, however, unable to grow at ~~the highest light intensity of~~ 500 μmol photons m^{-2} s^{-1} under ambient pCO$_2$, pointing towards its vulnerability to cope with HL under present day pCO$_2$. On the other hand, long-term field observations have shown that cryptophytes mainly occur under stratified conditions along the WAP (e.g. Moline and Prézelin, 1996; Moline et al., 2004; Mendes et al., 2013). A connection of this group with high illuminated conditions was first suggested by Mendes et al. (2017), but lacks information which cryptophyte species were present and their photosynthetic responses. The reason for this difference could be related to species- or strain-specific differences. On the basis of our results, the here tested *G. cryophila* strain was indeed able to cope well with medium, but not high irradiances. More tests with other cryptophytes are certainly required for being able to better evaluate cryptophytes' abilities to cope with high irradiances.

4.2 OA alters the physiological response of *G. cryophila* to high irradiance

In line with previous studies on Antarctic diatoms and *P. antarctica* (Hoogstraten et al., 2012; Heiden et al., 2016; Trimborn et al., 2017b), OA in conjunction with low irradiance did not alter growth, cellular contents or production rates of POC in *G. cryophila* (Fig. 1A-C). There was, however, an OA-dependent decline in C:N under LL (Fig. 1D), resulting from a significant enhancement of PON quotas between ambient and high pCO$_2$ (23.1 ± 1.4 and 29.0 ± 1.6 pg N cell^{-1}, respectively~~, posthoc: *p* < 0.05~~). Hence, *G. cryophila* cells most probably benefitted from lower energy investments to acquire inorganic carbon under high pCO$_2$. Whereas the two temperate cryptophytes *Rhodomonas* sp. and *Chroomonas* sp. (Burns and Beardall, 1987; Camiro-Vargas et al., 2005), similar to other Antarctic phytoplankton taxa (Trimborn et al., 2013), were able to actively take up CO$_2$ and HCO$_3$$^-$, the operation of a carbon concentrating mechanism in *G. cryophila* has not been tested so far. As typically observed for temperate phytoplankton (Hopkinson et al, 2011; McCarthy et al., 2012; Yang and Gao, 2012), the higher pCO$_2$ was not used to fix more POC per cell by *G. cryophila* at LL (Fig. 1B, C), but instead fuelled protein build-up through conversion of carbohydrate skeletons to proteins. N assimilation is energetically costly due to the reduction steps involved (Sanz-Luque et al., 2015), therefore the finding of elevated PON buildup under OA and LL is surprising. In line with this, ETR$_{max}$ per PSII increased by 121% from ambient to high pCO$_2$ (Table 3), likely used to reduce nitrite to ammonium. Calculating overall maximum ETRs per cell (cETR$_{max}$ = ETR$_{max}$ x [RCII], given in amol cell^{-1} s^{-1}), there

was also an OA-dependent increase in cETR$_{max}$ by 41% (mean value of 76 and 107 amol cell^{-1} s^{-1} under ambient and high pCO$_2$, respectively), but this increase was comparably lower relative to ETR$_{max}$ per PSII. The reason for this comes from the strong reduction of [RCII] between ambient and high pCO$_2$ at LL (Fig. 3). Next to the positive OA effect on N metabolism, also the photochemical efficiency (F_v/F_m) of *G. cryophila* was significantly increased by 17% by OA under LL (Fig. 2A). Unexpectedly, this effect was not the result of reduced cellular quotas of the light harvesting pigments (Chl *a* and c$_2$), as they remained the same under these conditions. Instead a significant OA-dependent decrease by 26% in the carotenoid Allo was found (Table 2), which could explain the positive OA effect on F_v/F_m. This is in line with the OA-dependent reduction in NPQ observed in LL-acclimated *G. cryophila* cells (Fig. 4), pointing towards a reduced need to dissipate excess light energy following short-term high light exposure. Overall, OA in conjunction with LL was beneficial for *G. cryophila*, with positive effects in particular on N metabolism.

The beneficial OA effect on N assimilation under LL, however, vanished at ML (Fig. 1D, 23.2 ± 3.2 and 23.2 ± 2.3 pg N cell^{-1}, respectively~~, posthoc: *p* < 0.05~~), probably as a result from the higher N metabolism cost to maintain photosynthesis under these conditions (Li et al., 2015). Based on our results, the physiology of the cryptophyte *G. cryophila* remained more or less unchanged between ambient and high pCO$_2$ at ML (Figs.1, 2, 4, Tables 2 and 3). At the highest irradiance (HL), *G. cryophila* could not grow under ambient pCO$_2$, but surprisingly grew well under the same light intensity in conjunction with OA, displaying highest growth rates compared to all other treatments (Fig. 1A). Similarly, this species also showed highest production rates of POC and PON (Fig. 1). Compared with the tested Antarctic diatoms and *P. antarctica* so far, which exhibited either negative or neutral effects in response to OA and high irradiance on growth and/or photosynthesis (Feng et al., 2010; Heiden et al. 2016, Trimborn et al. 2017a,b; Heiden et al. 2019; Koch et al. 2019), growth and photosynthesis of the cryptophyte benefitted synergistically from OA and HL. Looking at the significantly higher amounts of Chl *a* and Allo per cell and its larger σ_{PSII} between ML and HL under OA (Table 2, Figure 3C), this species even reinforced its capacity to absorb light. Faster electron drainage into downstream processes was evident by the shorter Qa re-oxidation time between ML and HL under OA (Figure 3D), supporting that this species indeed managed well to cope with these conditions.

4.3 Implications for the ecology of *G. cryophila* in future coastal Antarctic waters

Along the coast of the Western Antarctic Peninsula, diatoms, prymnesiophytes and cryptophytes represent the main phytoplankton groups, which form prominent blooms and therefore strongly contribute to carbon biomass build-up (Garibotti et al., 2005; Trimborn et al., 2015). Occurrence of cryptophytes in this region was associated with low salinity and warm stratified surface waters (Moline and Prézelin, 1996; Moline et al., 2004; Mendes et al., 2013; Mendes et al., 2017). Only recently, it was suggested that a high tolerance of cryptophytes to withstand high irradiances could potentially explain their occurrence in well illuminated surface waters by Mendes et al. (2017). Our results from a short-term CO$_2$-light experiment point towards a high ability of *G. cryophila* to acclimate to such conditions and to cope well with medium, but not high irradiances, whether this applies for other Antarctic cryptophyte species as well needs further testing. Also it remains unclear whether similar responses would be found when exposed on a longer term. In general, with respect to the projected climatic changes little is known about the potential CO$_2$ sensitivity of cryptophytes. Previous studies mainly assessed the response to OA on cryptophytes at the community level and showed no discernible effects on their abundance (Domingues et al., 2014; Sommer et al.,

2015; Young et al., 2015; Schulz et al., 2017; Donahue et al., 2019). This study is the first at the species level to show that the combination of OA and high irradiance promoted growth and biomass production in the Antarctic cryptophyte *G. cryophila*. In fact, while HL conditions inhibited growth of this species under ambient pCO_2, the combination of OA and HL, on the other hand, enabled it to grow and to cope even better with the applied environmental conditions, reaching highest growth and POC production rates (Fig. 1). This was also accompanied with a high photophysiological capacity of this species when exposed on the short-term to increasing irradiances. The beneficial effect of OA and HL for *G. cryophila* is opposed to previous observations, where growth and/or photosynthesis was inhibited in several diatoms, but no effect for the prymnesiophytes *P. antarctica* (Feng et al., 2010; Trimborn et al., 2017a,b; Beszteri et al., 2018; Heiden et al., 2018; Koch et al., 2018, Heiden et al., 2019). Hence, *G. cryophila* could be a potential winner of climate change, with higher abundances and increased contribution to the productivity of future stratified, acidified and well illuminated coastal Antarctic waters. This study further confirms previous results (Moline et al., 2004; Ducklow et al., 2007; Montes-Hugo et al., 2009; Mendes et al., 2017), which point towards a stronger importance of flagellates in the future. A functional shift away from efficient carbon sinkers such as diatoms to less efficient carbon vectors such as flagellates including cryptophytes and prymnesiophytes could, however, diminish the strength of the biological carbon pump of future Antarctic coastal waters.

Author contributions

ScTr designed the study. PK conducted the experiment. ScTr, SiTh and PK analysed the data. ScTr prepared the paper with contributions from SiTh, PK and KB.

References

Arrigo, K. R., van Dijken, G., and Long, M.: Coastal Southern Ocean: A strong anthropogenic CO_2 sink, Geophys. Res. Lett., 35, 1-6, doi:10.1029/2008GL035624, 2008.

Beszteri, S., Thoms, S., Benes, V., Harms, L., and Trimborn, S.: Acclimation to ocean acidification and high light in three Southern Ocean phytoplankton species: A transcriptomic study, Protist, 169, 958-975, doi:10.1016/j.protis.2018.08.003, 2018.

Boelen, P., van de Poll, W. H., van der Strate, H. J., Neven, I. A., Beardall, J., and Buma. A. G. J.: Neither elevated nor reduced CO_2 affects the photophysiological performance of the marine Antarctic diatom *Chaetoceros brevis,* J. Exp. Mar. Biol. Ecol., 406, 38-45, doi:10.1016/j.jembe.2011.06.012, 2011.

Burns, B. D., and Beardall, J.: Utilization of inorganic carbon by marine microalgae. J. Exp. Mar. Biol. Ecol., 107, 75-86, 1987.

Camiro-Vargas, T. K., Hernandez-Ayon, J. M., Valenzuela-Espinoza, E., Delgadillo-Hinojosa, F., and Cajal-

Medrano, R.: Dissolved inorganic carbon uptake by *Rhodomonas* sp. and *Isochrysis aff. galbana* determined by a potentiometric technique, Aquacultural Engineering, 33, 83-95, 2005.

Dickson, A. G., and Millero, F. J.: A comparison of the equilibrium constants for the dissociation of carbonic acid in seawater media, Deep Sea Res., 34, 1733-1743, doi:10.1016/0198-0149(87)90021-5, 1987.

Domingues, R. B., Guerra, C. C., Barbosa, A. B., Brotas, V., Galvao, H. M., and Notes, A..: Effects of ultraviolet radiation and CO_2 increase on winter phytoplankton assemblages in a temperate coastal lagoon, J. Plankton Res., 36, 672-684, doi:10.1093/plankt/fbt135, 2014.

Donahue, K., Klaas, C., Dillingham, P.W., and Hoffmann, L.J.: Combined effects of ocean acidification and increased light intensity on natural phytoplankton communities from two Southern Ocean water masses, J. Plankton Res., 41(1), 30-45, doi:10.1093/plankt/fby048, 2019.

Ducklow, H. W., Baker, K. , Martinson, D. G., Quetin, L. B., Ross, R. M., Smith, R. C., Stammerjohn, R. C., Vernet, M., and Fraser W.: Marine pelagic ecosystems: the West Antarctic Peninsula. Phil. Trans. Roy. Soc. B: Biol. Sci., 362, 67-94, 2007.

Ducklow, H.W., Fraser, W.R., Meredith, M.P., Stammerjohn, S.E., Doney, S.C., Martinson, D.G., Sailley, S.F., Schofield, O.M., Steinberg, D.K., Venables, H.J., and Amsler, C.D.: West Antarctic Peninsula: An ice-dependent coastal marine ecosystem in transition, Oceanography, 26, 190-203, doi:10.5670/oceanog.2013.62, 2013, 2013.

Falkowski, P. G., and Raven, J. A.: Aquatic Photosynthesis, Princeton University Press, Princeton, New Jersey, 501 pp, 2007.

Feng, Y., Hare, C. E., Rose, J. M., Handy, S. M., DiTullio, G. R., Lee, P. A., Smith, W. O., Peloquin, J., Tozzi, S., Sun, J., Zhang, Y., Dunbar, R. B., Long, M. C., Sohst, B., Lohan, M., and Hutchins, D. A.: Interactive effects of iron, irradiance and CO_2 on Ross Sea phytoplankton, Deep Sea Res. Part I Oceanogr. Res. Pap., 57, 368-383, doi:10.1016/j.dsr.2009.10.013, 2010.

Funk, C., Alami, M., Tibiletti, T., and Green, B. R.: High light stress and the onehelix LHC-like proteins of the cryptophyte *Guillardia theta*. Biochim. Biophys. Acta – Bioenerg., 1807, 841-846, 2011.

Genty, B., Briantais, J.-M., and Baker, N. R.: The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence, Biochim. Biophys. Acta, 990, 87-92, doi:10.1016/S0304-4165(89)80016-9, 1989.

Gould, S. B., Waller, R. F., and McFadden, G. I.: Plastid evolution. Annu. Rev. Plant Biol. 59, 491-517, 2008.

Guillard, R. R. L., and Ryther, J. H.: Studies of marine planktonic diatoms: I. *Cyclotella nana* Hustedt, and *Detonula confervacea* (Cleve) Gran. Can. J. Microbiol., 8, 229-239, doi:10.1139/m62-029, 1962.

Hammer, A., Schumann, R., and Hendrik Schubert Light and temperature acclimation of *Rhodomonas salina* (Cryptophyceae): Photosynthetic performance, Aquat. Microb. Ecol., 29, 287-296, doi:10.3354/ame029287, 2002.

Heiden, J. P., Völkner, C., Jones, E., Van de Poll, W. H., Buma A. G. J., Meredith, M. P., De Baar, H., Bischof, K., Wolf-Gladrow, D., Trimborn, S.: Impact of ocean acidification and high solar radiation on productivity and species composition of a late summer phytoplankton community of the coastal Western Antarctic Peninsula. Limnol. Oceanogr., doi:10.1002/lno.11147, 2019.

Heiden, J. P., Thoms, S., Bischof, K., Trimborn, S.: Ocean acidification stimulates particulate organic carbon accumulation in two Antarctic diatom species under moderate and high solar radiation, J. Phycol., 54, 505-517,

doi:10.1111/jpy.12753, 2018.

Heiden, J. P., Bischof, K., Trimborn, S.: Light intensity modulates the response of two Antarctic diatom species to ocean acidification, Front. Mar. Sci., 3, 260, doi: 10.3389/fmars.2016.00260, 2016.

Henriksen, P. Riemann, B., Sorensen, H. M., and Sorensen, H. L.: Effects of nutrient-limitation and irradiance on marine phytoplankton pigments, J. Plankton Res., 24, 9, 835-858, 2002.

Hoogstraten, A., Timmermans, K. R., and de Baar, H. J. W.: Morphological and physiological effects in *Proboscia Alata* (Bacillariophyceae) grown under different light and CO_2 conditions of the modern Southern Ocean, J. Phycol., 48, 559-568, doi:10.1111/j.1529-8817.2012.01148.x, 2012.

Hopkinson, B. M., Dupont, C. L., Allen, A. E., Morel, F. M. M.: Efficiency of the CO_2-concentrating mechanism of diatoms, Proc. Natl. Acad. Sci. USA, 108, 3830-3837, 2011.

Hoppe, C. J. M., Holtz, L., Trimborn, S., and Rost, B.: Ocean acidification decreases the light-use efficiency in an Antarctic diatom under dynamic but not constant light, New Phytol., 207, 159-171, doi:10.1111/nph.13334, 2015.

Kana, R., Kotabova, E., Sobotka, R., Prasil, O.: Non-photochemical quenching in cryptophyte alga *Rhodomonas salina* is located in chlorophyll a/c antennae, PLoS ONE, 7(1), e29700,doi:10.1371/journal.pone.0029700, 2012.

Kolber, Z. S., Prášil, O., and Falkowski, P. G.: Measurements of variable chlorophyll fluorescence using fast repetition rate techniques: Defining methodology and experimental protocols, Biochim. Biophys. Acta, 1367, 88-106, doi:10.1016/S0005-2728(98)00135-2, 1998.

Koch, F., Beszteri, S., Harms, L., Trimborn, S.: The impacts of iron limitation and ocean acidification on the cellular stoichiometry, photophysiology and transcriptome of *Phaeocystis antarctica*, Limnol Oceanogr,, doi:10.1002/lno.11045, 2018.

Laviale, M., and Neveux, J.: Relationships between pigment ratios and growth irradiance in 11 marine phytoplankton species, Mar. Ecol. Prog. Ser., 425, 63-77, doi: 10.3354/meps09013, 2011.

Li, G., Brown, C. M., Jeans, J. A., Donaher, N. A., McCarthy, A., and Campbell, D. A.: The nitrogen costs of photosynthesis in a diatom under current and future pCO_2. New Phytol., 205, 533-543, doi: 10.1111/nph.13037, 2015.

MacIntyre, H. L., Kana, T. M., Anning, T., and Geider, R. J.: Photoacclimation of photosynthesis irradiance response curves and photosynthetic pigments in microalgae and cyanobacteria, J. Phycol., 38, 17-38, doi:10.1046/j.1529-8817.2002.00094.x, 2002.

McCarthy, A., Rogers, S. P., Duffy, S. J., and Campbell, D. A.: Elevated carbon dioxide differentially alters the photophysiology of *Thalassiosira pseudonana* (Bacillariophyceae) and *Emiliania huxleyi* (Haptophyta), J. Phycol., 48, 635-646, 2012.

Mehrbach, C., Culberson, C. H., Hawley, J. E., and Pytkowicz, R. M.: Measurement of the apparent dissociation constants of carbonic acid in seawater at atmospheric pressure, Limnol. Oceanogr., 18, 897-907, doi:10.4319/lo.1973.18.6.0897, 1973.

Mendes, C. R. B., Tavano, V. M., Leal, M. C., de Souza, M. S., Brotas, V., and Garcia, C. A. E.: Shifts in the dominance between diatoms and cryptophytes during three late summers in the Bransfield Strait (Antarctic Peninsula), Polar Biol., 36, 537-547, doi:10.1007/s00300-012-1282-4, 2013.

Mendes, C. R. B., Tavano, V. M., Dotto, T. S., Kerr, R., De Souza, M. S., Garcia, C. A. E. and Secchi, E. R.: New insights on the dominance of cryptophytes in Antarctic coastal waters: A case study in Gerlache Strait, Deep Sea Res. Part II: Topical Studies Oceanogr., doi:10.1016/j.dsr2.2017.02.010, 2017.

Moline, M. A., and Prézelin, B. B.: Long-term monitoring and analyses of physical factors regulating variability in coastal Antarctic phytoplankton biomass, *in situ* productivity and taxonomic composition over seasonal and interannual timescales, Mar. Ecol. Prog. Ser., 145, 143-160, doi:10.3354/Meps145143, 1996.

Moline, M. A., Claustre, H., Frazer, T. K., Schofield, O., and Vernet, M.: Alteration of the food web along the Antarctic Peninsula in response to a regional warming trend, Glob. Change Biol., 10, 1973-1980, doi: 10.1111/j.1365-2486.2004.00825, 2004.

Montes-Hugo, M., Doney, S.C., Ducklow, H.W., Fraser, W.R., Martinson, D.G., Stammerjohn, S.E., and Schofield, O.: Recent changes in phytoplankton communities associated with rapid regional climate change along the western Antarctic Peninsula, Science 323: 1470-1473, doi:10.1126/science.1164533, 2009.

Moreau, S., Mostajir, B., Bélanger, S., Schloss, I. R., Vancoppenolle, M., Demers, S., and Ferreyra, G. A.: Climate change enhances primary production in the western Antarctic Peninsula, Glob. Change Biol., 21, 2191-2205, doi: 10.1111/gcb.12878, 2015.

Orr, J. C., V. J. Fabry, O. Aumont, L. Bopp, S. C. Doney, R. A. Feely, A. Gnanadesikan, N. Gruber, Ishida, A., Joos, F., Key, R. M., Lindsay, K., Maier-Reimer, E., Matear, R., Monfray, P., Mouchet, A., Najjar, R. G., Plattner, G.-K., Rodgers, K. B., Sabine, C. L., Sarmiento, J. L., Schlitzer, R., Slater, R. D., Totterdell, I. J., Weirig, M.-F., Yamanaka, Y., and Yool, A.: Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms, Nature, 437, 681-686, 2005.

Oxborough, K., Moore, C. M., Suggett, D. J., Lawson, T., Chan, H. G., and Geider, R. J.: Direct estimation of functional PSII reaction center concentration and PSII electron flux on a volume basis: a new approach to the analysis of Fast Repetition Rate fluorometry (FRRf) data, Limnol. Oceanogr. Methods, 10, 142-154, doi:10.4319/lom.2012.10.142, 2012.

Pachauri, R.K., and Meyer, L.A. (Eds.): Climate Change 2014: Synthesis Report., ed. Contribution of workinggroups I, II and III to the fifth assessment report of the intergovernmental panel on climate change, Geneva, Switzerland, 2014.

Pierrot, D., Lewis, E., and Wallace, D. W. R.: MS Excel program developed for CO_2 system calculations, 2006.

Ralph, P. J., and Gademann, R.: Rapid light curves: A powerful tool to assess photosynthetic activity, Aquat. Bot., 82, 222-237, doi:10.1016/j.aquabot.2005.02.006, 2005.

Redfield, A. C.: The biological control of chemical factors in the environment, Am. Sci., 64, 205-221, doi:10.2307/27828530, 1958.

Riebesell, U., Wolf-Gladrow, D. A., and Smetacek, V.: Carbon dioxide limitation of marine phytoplankton growth rates, Nature, 361, 249-251, 1993.

Rozema, P. D., Venables, H. J., van de Poll, W. H., Clarke, A., Meredith, M. P., and Buma, A. G. J.: Interannual variability in phytoplankton biomass and species composition in northern Marguerite Bay (West Antarctic Peninsula) is governed by both winter sea ice cover and summer stratification, Limnol. Oceanogr., 62, 235-252, 2017.

Sanz-Luque, E., Chamizo-Ampudia, A., Llamas, A., Galvan, A. and Fernandez, E.: Understanding nitrate assimilation and its regulation in microalgae, Front. Plant Sci., 6, 899, doi: 10.3389/fpls.2015.00899, 2015.

Schulz, K. G., Bach, L. T., Bellerby, R. G. J., Bermúdez, R., Büdenbender, J., Boxhammer, T., Czerny, J., Engel, A., Ludwig, A., Meyerhöfer, M., Larsen, A., Paul, A. J., Sswat, M. and Riebesell, U.: Phytoplankton blooms at increasing levels of atmospheric carbon dioxide: Experimental evidence for negative effects on prymnesiophytes and positive on small picoeukaryotes, Front. Mar. Sci., 4, 64, doi:10.3389/fmars.2017.00064, 2017.

Smith, R. C., and S. E. Stammerjohn.: Variations of surface air temperature and sea-ice extent in the western Antarctic Peninsula region, Ann. Glaciol., 33, 493-500, doi:10.3189/172756401781818662, 2001.

Sommer, U., Paul, C., Moustaka-Gouni, M. Warming and ocean acidification effects on phytoplankton - From species shifts to size shifts within species in a mesocosm experiment, PLoS ONE, 10(5), e0125239, doi:10.1371/journal.pone.0125239, 2015.

Stoll, M. H. C., Bakker, K., Nobbe, G. H., and Haese, R. R. Continous-flow analysis of dissolved inorganic carbon content in seawater, Analaytical Chem., 73, 4111-4116, doi:10.1021/ac010303r, 2001.

Suggett, D. J., MacIntyre, H. L., and Geider, R. J.: Evaluation of biophysical and optical determinations of light absorption by photosystem II in phytoplankton, Limnol. Oceanogr. Methods, 2, 316-332, doi:10.4319/lom.2004.2.316, 2004.

Suggett, D. J., Moore, C. M., Hickman, A. E., and Geider, R. J.: Interpretation of fast repetition rate (FRR) fluorescence: Signatures of phytoplankton community structure versus physiological state, Mar. Ecol. Prog. Ser., 376, 1-19, doi:10.3354/meps07830, 2009.

Trimborn, S., Brenneis, T., Sweet, E., Rost, B.: Sensitivity of Antarctic phytoplankton species to ocean acidification: growth, carbon acquisition and species interaction, Limnol. Oceanogr., 58, 997-1007, 2013.

Trimborn, S., Hoppe, C. J. M., Taylor, B., Bracher, A., Hassler, C.: Physiological characteristics of phytoplankton communities of Western Antarctic Peninsula and Drake Passage waters, Deep Sea Res. Part I, 98, 115-124, 2015.

Trimborn, S., Brenneis, T., Hoppe, C. J. M., Norman, L., Santos, J., Laglera, L., Völkner, C., Wolf-Gladrow, D., Hassler, C.: Iron sources alter the response of Southern Ocean phytoplankton to ocean acidification, Mar. Ecol. Prog. Ser., 578, 35-50, doi:10.3354/meps12250, 2017a.

Trimborn, S., Thoms, S., Brenneis, T., Heiden, J. P., Beszteri, S., Bischof, K.: Two Southern Ocean diatoms are more sensitive to ocean acidification and changes in irradiance than the prymnesiophyte *Phaeocystis antarctica*, Physiol. Plant., 160, 155-170, doi:10.1111/ppl.12539, 2017b.

Wright, S. W., Jeffrey, S. W., Mantoura, R. F. C., Llewellyn, C. A., Bjornland, T., Repeta, D., and Welschmeyer, N.: Improved HPLC method for the analysis of chlorophylls and carotenoids from marine phytoplankton, Mar. Ecol. Prog. Ser., 77, 183-196, doi:10.3354/meps077183, 1991.

Yang, G., and Gao, K.: Physiological responses of the marine diatom *Thalassiosira pseudonana* to increased pCO$_2$ and seawater acidity, Mar. Environ. Res., 79, 142-151, doi:10.1016/j.marenvres.2012.06.002, 2012.

Young, J. N., Kranz, S. A., Goldman, J. A. L., Tortell, P. D., and Morel, F. M. M.: Antarctic phytoplankton down-regulate their carbon-concentrating mechanisms under high CO$_2$ with no change in growth rates, Mar. Ecol. Progr. Ser., 532, 13-28, 2015.

Table 1 Partial pressures of CO_2 (pCO_2) for the ambient and ocean acidification (OA) treatments were calculated from measured pH, concentrations of dissolved inorganic carbon (DIC), silicate and phosphate, temperature, and salinity using the CO2Sys program (Pierrot et al. 2006). For all parameters, values are given for the abiotic control bottles (abiotic, bubbled seawater without cells) and the culture bottles at the end of the experiment (biotic). Different letters indicate significant differences between treatments ($p < 0.05$). <mark>Different letters indicate significant differences between treatments ($p < 0.05$). Values represent the means ± SD (n = 3).</mark>

Target pCO_2 (µatm)	pCO_2 (µatm)		pH (NBS)		DIC (µmol kg^{-1})	
	abiotic	biotic	abiotic	biotic	abiotic	biotic
Ambient pCO_2, 400	372 ± 18^a	398 ± 66^a	8.14 ± 0.02^a	8.13 ± 0.07^a	2024 ± 7^a	2062 ± 12^b
OA, 1000	986 ± 42^b	865 ± 120^b	7.75 ± 0.02^b	7.82 ± 0.06^b	2160 ± 7^c	2195 ± 16^d

Table 2 Cellular concentrations of chlorophyll *a* and c_2 (Chl *a* and c_2) as well as alloxanthin (Allo) were determined for *Geminigera cryophila* acclimated to ambient or high CO_2 conditions combined with low (LL), medium (ML) or high light (HL). *G. cryophila* did not grow under ambient pCO_2 and HL as indicated by ng. Photosynthetic parameters were derived from at least three independent measurements. <mark>Different letters indicate significant differences between treatments ($p < 0.05$). Values represent the means $\pm$ SD.</mark>

Treatment	Chl *a* (fg cell^{-1})	Chl c_2 (fg cell^{-1})	Allo (fg cell^{-1})
Ambient pCO_2 LL	2358 ± 277^a	172 ± 33^a	81 ± 3^a
OA LL	2300 ± 25^a	131 ± 1^a	60 ± 2^b
Ambient pCO_2 ML	871 ± 225^b	75 ± 24^b	30 ± 8^c
OA ML	662 ± 22^b	48 ± 15^b	27 ± 3^c
Ambient pCO_2 HL	ng	ng	ng
OA HL	772 ± 85^c	41 ± 1^b	36 ± 1^d

Table 3 <mark>Maximum absolute electron transport rates (ETR$_{max}$), minimum saturating irradiances (I_K) and maximum light utilization efficiencies (α)</mark> were determined for *Geminigera cryophila* acclimated to ambient or high CO_2 conditions combined with low (LL), medium (ML) or high light (HL). *G. cryophila* did not grow under ambient pCO_2 and HL as indicated by ng. Photosynthetic parameters were derived from at least three independent measurements. <mark>Different letters</mark> <mark>indicate significant differences between treatments ($p < 0.05$). Values represent the means ± SD.</mark>

Treatment	ETR$_{max}$ (e$^-$ PS^{-1} s^{-1})	I_k (μmol photons m^{-2} s$^-$)	α (rel. unit)
Ambient pCO$_2$ LL	126 ± 28[a]	72 ± 9[a]	1.75 ± 0.17[a]
OA LL	279 ± 52[b]	142 ± 34[b]	1.98 ± 0.11[a]
Ambient pCO$_2$ ML	265 ± 77[b]	102 ± 38[b]	2.67 ± 0.26[b]
OA ML	278 ± 70[b]	138 ± 48[b]	2.06 ± 0.24[a]
Ambient pCO$_2$ HL	ng	ng	ng
OA HL	379 ± 38[b]	156 ± 40[b]	2.50 ± 0.37[a]

Figure legend

Figure 1 Growth rate (A), cellular content (B) and production rate (C) of particulate organic carbon (POC) and the molar ratio of carbon to nitrogen (C:N, D) for *Geminigera cryophila* acclimated to ambient (black bars) or high CO_2 (grey bars) conditions combined with low (LL), medium (ML) or high light (HL). *G. cryophila* did not grow under ambient pCO_2 and HL as indicated by ng. Values represent the means ± SD (n = 3). Different letters indicate significant differences between treatments ($p < 0.05$).

Figure 2 The dark-adapted maximum PSII quantum yield F_v/F_m (A) and the yield recovery after short-term light stress (% of initial) (B) for *Geminigera cryophila* acclimated to ambient (black bars) or high CO_2 (grey bars) conditions combined with low (LL), medium (ML) or high light (HL). *G. cryophila* did not grow under ambient pCO_2 and HL as indicated by ng. Values represent the means ± SD (n = 3). Different letters indicate significant differences between treatments ($p < 0.05$).

Figure 3 Cellular concentrations of functional photosystem II reaction centers [RCII] (A), energy transfers between PSII units (i.e. connectivity, *p*) (B), functional absorption cross sections of PSII photochemistry (σ_{PSII}) (C) and re-oxidation times of the primary electron acceptor Q_a (τ_{Qa}) (D) were determined for *Geminigera cryophila* acclimated to ambient or high CO_2 conditions combined with low (LL), medium (ML) or high light (HL). *G. cryophila* did not grow under ambient pCO_2 and HL as indicated by ng. Different letters indicate significant differences between treatments ($p < 0.05$).

Figure 4 Absolute electron transport rates (ETR, A-C) and nonphotochemical quenching (NPQ, D-F) were measured in response to increasing irradiance in *Geminigera cryophila* acclimated to ambient (black circles) or high CO_2 (white circles) conditions combined with A) low (LL), B) medium (ML) or C) high light (HL). *G. cryophila* did not grow under ambient pCO_2 and HL. ETRs were obtained in three individual measurements. Values represent the means ± SD (n = 3).
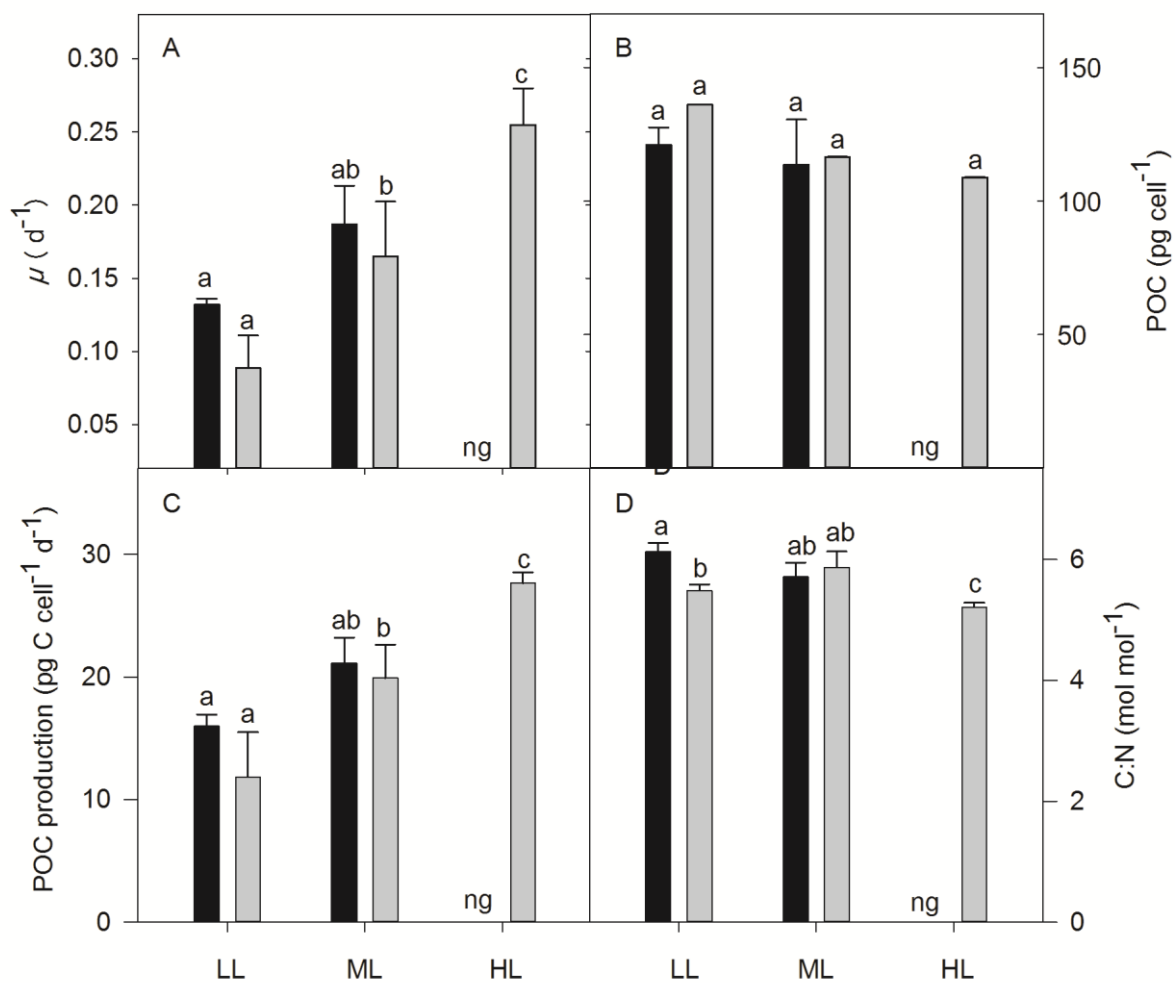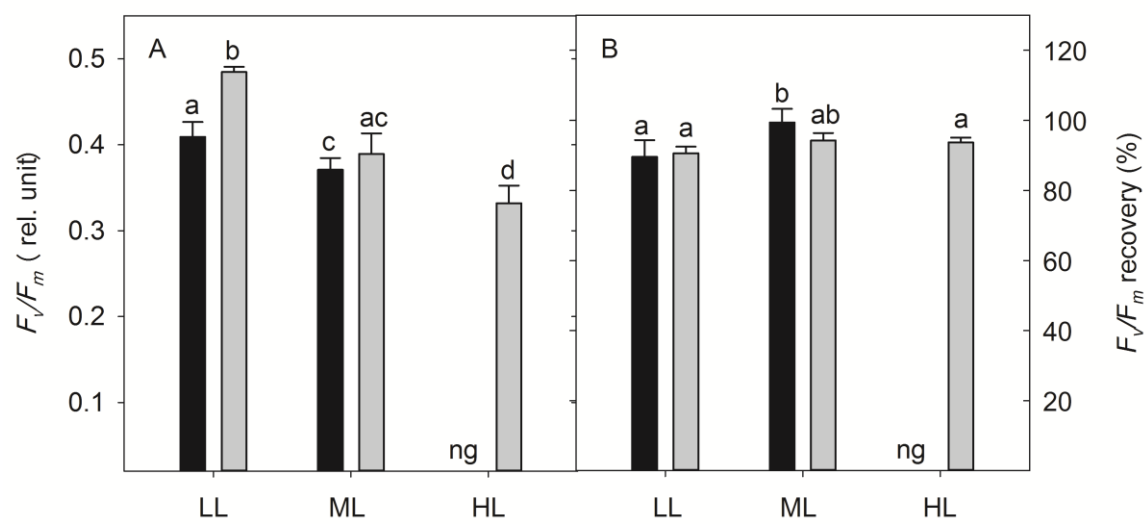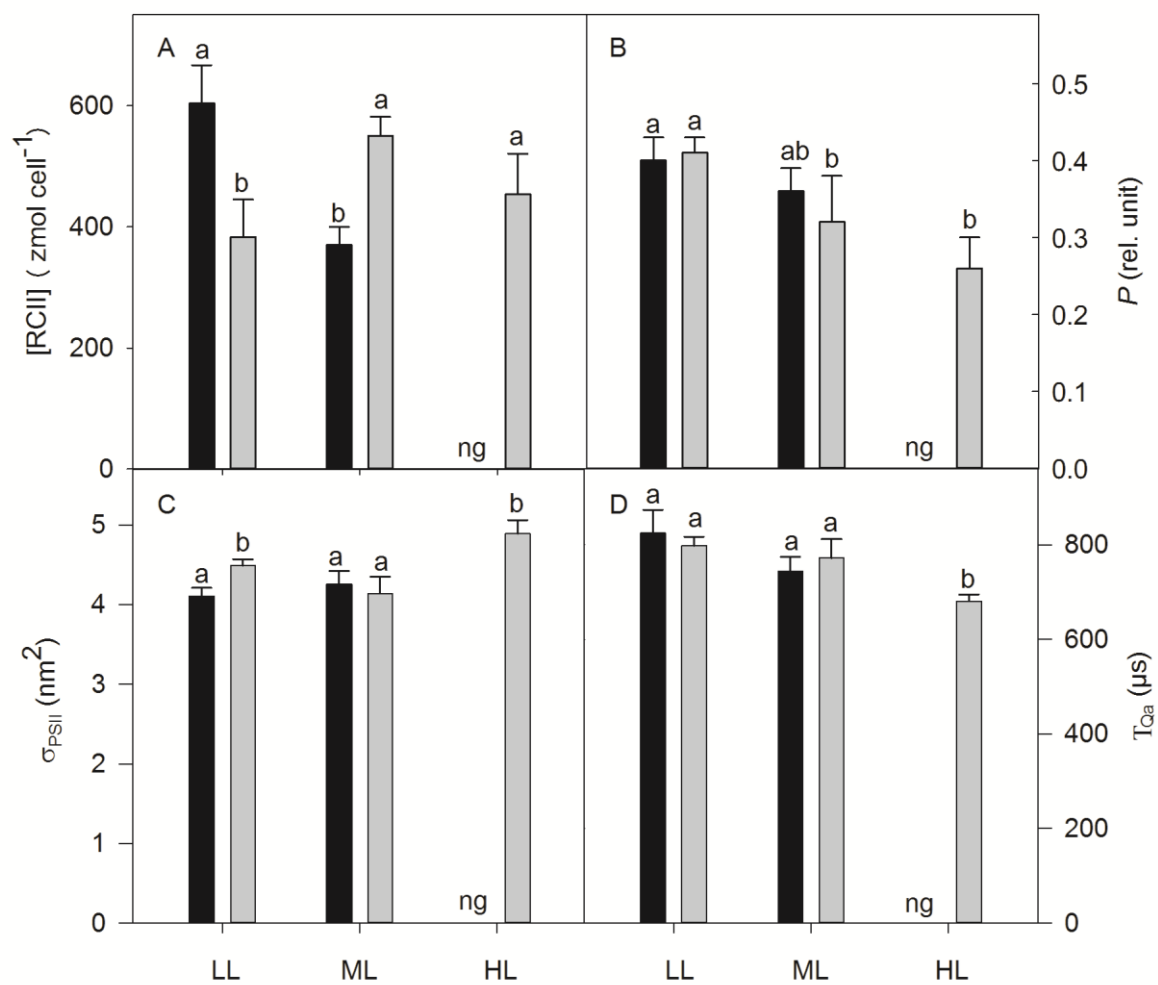
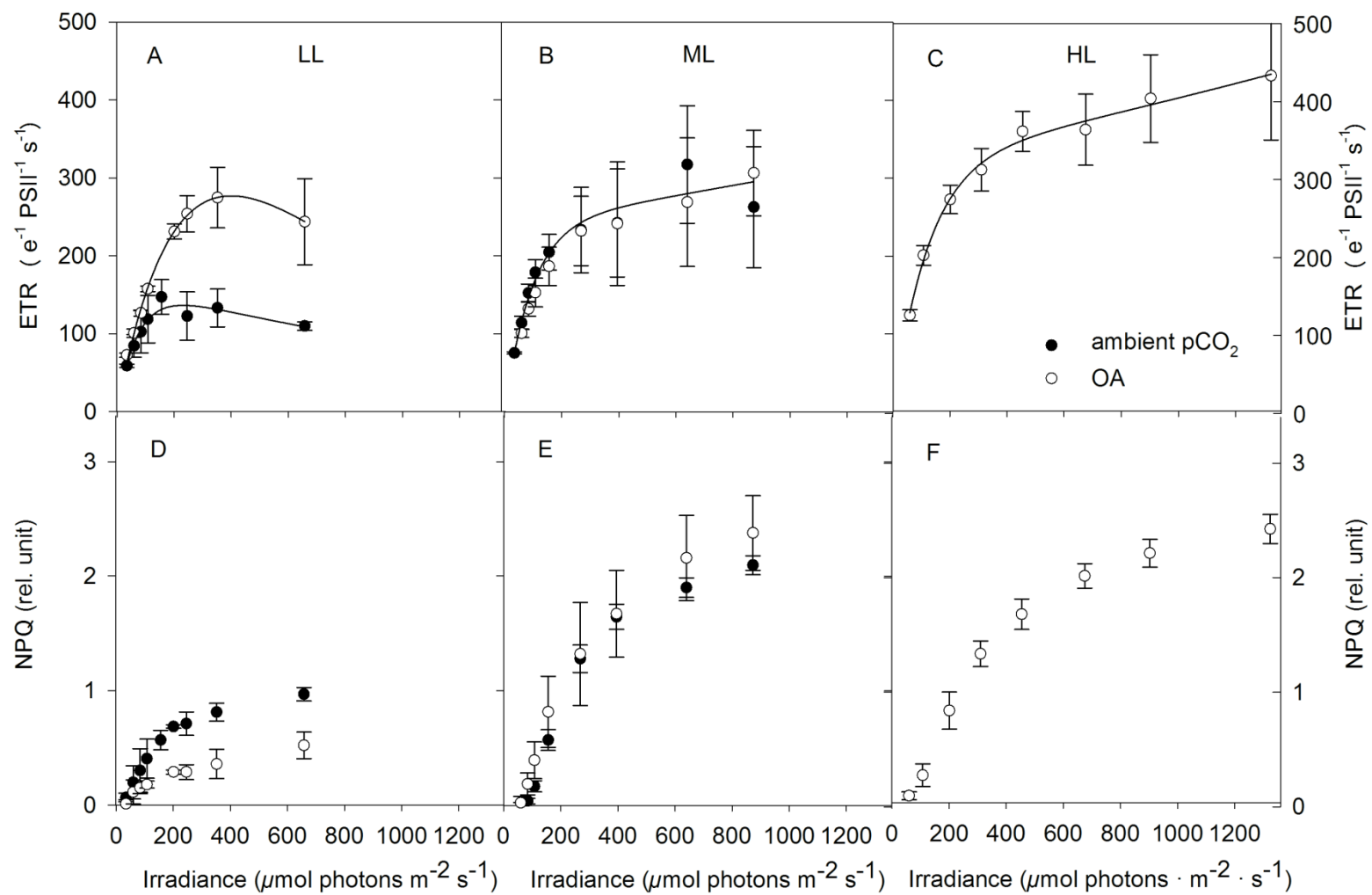Figure 1

Figure 2

Figure 3

Figure 4

Response to the referee comments of John Beardall on "Ocean acidification and high irradiance stimulate growth of the Antarctic cryptophyte *Geminigera cryophila*"

John Beardall (Referee #1)
This is an excellent and novel contribution reporting on experiments of the interaction between light intensity and ocean acidification on aspects of the growth and physiology of an Antarctic cryptophyte. Sine information on Southern Ocean cryptophytes is limited, especially with regard to the effects of elevated CO_2, the current contribution is especially welcome. The main take home message is that elevated CO_2 allows growth at high light, which under present day CO_2 levels would be inhibitory (non-permissive) to growth i.e. increased CO_2 alleviates photoinhibition.
The data presented are thorough and well-presented. The reasoning put forward to explain the data is carefully thought through and although I was surprised that the effects of OA were not apparent under medium high levels, I was persuaded by the arguments of the authors that this might be related to costs of increased N metabolism under those conditions. I have no major criticisms to offer.
AUTHORS: We thank the reviewer for the kind words on our manuscript.

One minor point is on the last line of the results (page 7 line 9) - where the authors state "...much higher NPQ values were determined in the ambient pCO_2 relative to the OA treatment." should this be followed by "in the LL treatment"?
AUTHORS: The reviewer is right, this information was missing. Hence, it is now written in the revised manuscript on P7 L23-25: "Much higher NPQ values were determined in the ambient pCO_2 relative to the OA treatment under LL while such pCO_2 effect was absent under ML."

Response to the referee comments of Marco J. Cabrerizo on "Ocean acidification and high irradiance stimulate growth of the Antarctic cryptophyte *Geminigera cryophila*"

Marco J. Cabrerizo (Referee #2)
Trimborn et al. evaluate in a full-factorial experimental design how the interaction between ocean acidification and high light alter the photophysiology, stoichiometry, production and growth of a model Antarctic Cryptophyte at short-term scales. The topic assessed fits with the Biogeoscience's scope, and is novel as currently information related with the interactive effect of both factors on Antarctic phytoplankton is still scarce. The experiment is well-designed and performed, and the results are appropriate to being published in the Journal.
Below, authors will find a point by point revision with the main issues found over the manuscript, and suggestions which I hope that they find useful.
Respectfully submitted,
Marco J. Cabrerizo
AUTHORS: We thank the reviewer for the kind words on our manuscript.

Title: I suggest including in the title two variables also quantified by authors: production and photophysiology of the species. On potential title could be: "Ocean acidification and high irradiance stimulate the photophysiology, growth and production in the Antarctic cryptophyte Geminigera cryophila".
AUTHORS: To account for the reviewer's comment, we have changed the title to: "Ocean acidification and high irradiance stimulate the photophysiological fitness, growth and carbon production in the Antarctic cryptophyte *Geminigera cryophila*".

Introduction: What are the individual effects of HL and OA on phytoplankton?. I suggest that authors add some general information about the individual effects of light and OA on primary producers, and then they focus such impacts on Antarctic phytoplankton and crytophytes. Through a general view, a potential reader can identify the gaps of knowledge related with the quantification of such impacts on phytoplankton, and the scarcity of experimental studies testing their interaction on this key group.
AUTHORS: According to the reviewers' suggestion we have added information on the individual effect of light and CO_2 availability before pointing out what is known in response to OA and HL for Antarctic phytoplankton. It now reads in the revised manuscript:
   P2, L10-13: "Light availability strongly influences the rate of growth and carbon fixation of phytoplankton (Falkowski and Raven 2007). With increasing irradiance, Antarctic phytoplankton species exhibited increased growth and carbon fixation, but only until photosynthesis was saturated (Fiala and Oriol, 1990; Heiden et al., 2016). When exposed to excessive radiation, phytoplankton cells can get photoinhibited and even damaged."
   P2, L25-30: "For various Antarctic diatoms and the prymnesiophyte *P. antarctica*, growth and/or carbon fixation remained unaltered by OA alone (Riebesell et al., 1993; Boelen et al., 2011; Hoogstraten et al., 2012; Trimborn et al., 2013; Hoppe et al., 2015; Heiden et al., 2016). Recent studies suggest that Southern Ocean diatoms are more prone to OA especially in conjunction with high light than the prymnesiophyte *Phaeocystis antarctica* (Feng et al., 2010; Trimborn et al., 2017a,b; Beszteri et al. 2018; Heiden et al., 2018; Koch et al., 2018, Heiden et al., 2019)."

Lines 27-29: If it was found a contrasted response pattern in southern WAP, it means that there were both a positive as a negative effect. In such case, it partially agrees with those results reported in the northern part. Please rewrite.
AUTHORS: In fact, a contrasted response in chlorophyll a biomass and primary production between the northern and the southern WAP regions was observed. To point this out more

clearly, the effects observed in the northern and southern WAP waters are described more clearly to avoid any misunderstanding in this respect on P1, L25-36: "Rising air temperature resulted in shorter sea ice seasons (Smith and Stammerjohn, 2001) with contrasting effects on phytoplankton biomass, composition and productivity between the northern and southern WAP. For the latter, the earlier retreat of sea ice together with the observed increase in surface water temperature led to shallow water column stratification, which favored phytoplankton growth and productivity. In the northern part of the WAP on the other hand, the earlier disappearance of sea ice was associated to greater wind activities and more cloud formation. As a consequence, a deepening of the upper mixed layer was found, providing less favorable light conditions. Next to reduced chlorophyll *a* accumulation (Montes-Hugo et al., 2009) and primary production (Moreau et al., 2015), a decline of large phytoplankton such as diatoms relative to the whole community was observed (Montes-Hugo et al., 2009; Rozema et al., 2017). Accordingly, a recurrent shift from diatoms to cryptophytes and small flagellates was reported for waters north of the WAP, with important implications for food web dynamics (Moline et al., 2004; Ducklow et al., 2007; Montes-Hugo et al., 2009; Mendes et al., 2017)."

Lines 29-30: *Break this sentence to separate both ideas.*
AUTHORS: This section has been rewritten on P1 L25-36.

Line 45: *Reference?*
AUTHORS: To account for the reviewer's comment the reference Falkwoski and Raven (2007) has been added on P2 L10.

Line 69: *I think that it would be nice that authors include a hypothesis work about what do they expect based on the previous information known?.*
AUTHORS: As suggested by the reviewer, we have included a hypothesis about what we expect based on previous information, it now reads on P2 L36-37: "Based on previous studies on the single effects of light or CO_2 alone, we hypothesize that cryptophytes are able to cope well with OA and high light conditions. Due to the limited information available on Antarctic cryptophyte physiology, this study assessed…".

Material and Methods:
Culture conditions: *This subsection is confusing at the present state. I propose you modifying it as follow: "Before to being used in experimentation (two weeks), triplicate semi-continuous cultures of the Antarctic cryoptophyte G. cryophyla (CCMP 2564) were grown (and maintained in mid-exponential growth) at 2ºC in sterile -filtered (0.2 um) Antarctic seawater (salinity 30.03) enriched with phosphate (final concentration 100 umol L-1) and nitrate (final concentration 6.25 umol L-1) (N:P ratio of 16:1, Redfield 1963), as well as, trace metals and vitamins according to F/2 medium (Ref). Cells were exposed under a 16h L: 8h D cycle and three constant light intensities (20, 200 and 500 µmol m-2 s-1) (LL, ML and HL, respectively)".*
Once you have already described the pre-acclimation phase, you can describe your experimental setup, and finally, all the details related with the CO_2 enrichment. For example, "To assess the interactive effect of light and OA on the photophysiology, metabolism and growth of G. cryophila, a 3x2 full factorial matrix (in triplicate) was implemented with: (a) three light levels (LL, ML and HL) and two pCO_2 treatments (ambient and OA)"…
AUTHORS: We have integrated most of the suggested changes by the reviewer in the section dealing with the culture conditions, it now reads:
        P3, L5-10: "Triplicate semi-continuous cultures of the Antarctic cryptophyte *Geminigera cryophila* (CCMP 2564) were grown in exponential phase at 2 °C in sterile-

filtered (0.2 μm) Antarctic seawater (salinity 30.03). This seawater was enriched with phosphate (final concentration of 100 μmol L^{-1}), nitrate (final concentration of 6.25 μmol L^{-1}) (N:P ratio of 16:1, Redfield, 1963) as well as trace metals and vitamins according to F/2 medium (Guillard and Ryther, 1962). *G. cryophila* cells were grown under a 16h light: 8h dark cycle at three constant light intensities (LL = 20, ML = 200 and HL = 500 μmol photons m^{-2} s^{-1})…"

P3, L12-17: The three light treatments were further combined with two CO_2 partial pressures (pCO$_2$) of 400 (ambient pCO$_2$ treatment) or 1000 μatm (OA treatment, Table 1). All pCO$_2$ treatments and the respective dilution media were continuously and gently bubbled through a frit with humidified air of the two pCO$_2$ levels, which were generated from CO_2-free air (< 1 ppmv CO_2; Dominick Hunter, Kaarst, Germany) and pure CO_2 (Air Liquide Deutschland ltd., Düsseldorf, Germany) with a gas flow controller (CGM 2000, MCZ Umwelttechnik, Bad Nauheim, Germany).

P3, L21-22: "*G. cryophila* cells were acclimated to the matrix of three light intensities (LL = 20, ML = 200 and HL = 500 μmol photons m^{-2} s^{-1}) and two pCO$_2$ levels (ambient = 400 and OA = 1000 μatm)…"

Line 15: *Cells in exponential or mid-exponential phase. Please clarify.*
AUTHORS: Cells were in exponential growth phase, this has been clarified on P3 L24.

Statistics: *Were ANOVA assumptions checked?. Was the interaction light and OA significant in all response variables? Please clarify. Over the results section, I cannot see if Light×OA was or not significant.*
AUTHORS: Of course the ANOVA assumptions were checked. Further, we have added the requested information *throughout* the results section and point now out more clearly whether the interaction of CO_2 and light had a significant influence.

Results: *I propose authors to change the ordering of the results. First, they should show PSII variables, then pigments, metabolism and stoichiometry and finally growth. The rationale behind my proposal is related with the fact that changes at PSII level occurs in temporal scales ranging mseg to minutes, whereas those related with the other variables need more time.*
AUTHORS: Please note that the discussion section mainly focuses on the ecophysiological response und thus concentrates first on the CO_2-Light responses on growth, elemental stoichiometry and composition and then relates these results to the underlying physiological mechanism such as electron transport rates (ETRs) and their influence on the Calvin cycle through comparison of the ETRs with the POC/N quotas and considers photoacclimation responses incl. chlorophyll a fluorescence characteristics and pigment contents. Hence, the order of the presented results is linked to the order of their presentation in the discussion. As this order is also reflecting the temporal scales as suggested by the reviewer, but rather starting from the long-term towards the short-term responses, we would like to keep the order of the results section as it is.

Line 30: *In my humble opinion, I think that authors should consider the effect of light under ambient pCO2. Authors state that "growth rates remained unchanged in cells grown under ambient pCO2", however, according to the figure 1, increasing light had a negative effect on cells. In fact, they did not grow.*
AUTHORS: The reviewer is completely right and we agree that this negative HL-effect on growth under ambient pCO$_2$ needs to be mentioned. Accordingly, we have modified this section on P6 L6-9: "In response to increasing irradiance, growth rates of cells grown under ambient pCO$_2$ remained unchanged between LL and ML, but were negatively influenced by

HL as they were unable to grow. Under OA, however, growth rates significantly increased between LL and ML by 89% (posthoc: $p < 0.05$) and between ML and HL by 32% (posthoc: $p < 0.05$), respectively."

Line 38: Change significantly influenced by negatively influenced
AUTHORS: Agreed and done.

Chl a fluorescence (Lines 12-13): I would rewrite this paragraph in a more direct style. For example, it could be rewritten as follow: "Regarding to the individual effects of both factors on PSIImax, it was found that whereas light conditions decreased it (particularly at HL), OA increased (ca. 17% at LL) or did not affect it (i.e. at ML). Noticeably, lowest values were found under the LightxOA interaction (ca. 0.35) (Fig. 63 2A)".
AUTHORS: As suggested, we have rewritten this part and it now reads on P6 L29-32: "The dark-adapted maximum quantum yield of PSII (F_v/F_m) was strongly influenced by irradiance (*2-way ANOVA*: $p < 0.0001$) and CO_2 (*2-way ANOVA*: $p = 0.0012$) and their interaction (*2-way ANOVA*: $p < 0.05$; Fig. 2A). With increasing irradiance F_v/F_m generally declined whereas OA increased it at LL (17%, posthoc: $p < 0.01$) or did not change it at ML. Noticeably, the interaction of HL and OA resulted in the lowest F_v/F_m value."

Chl a fluorescence (Lines 20-21): This paragraph is a little bit confusing. To avoid any misunderstanding I would rephrase this section as follow: "Fv/Fm recovery was not influenced neither by light nor pCO2 treatment, excepting under ambient pCO2 and ML treatments in which the quantum yield's recovery was maxima (Fig. 2A)".
AUTHORS: We agree with the reviewer that this section can evoke easily misunderstanding. Considering that increasing light indeed significantly altered F_v/F_m recovery (*2-way ANOVA*: $p < 0.01$), it is now written on P6 L34-36: "Neither high pCO_2 nor the interaction of light and CO_2 affected F_v/F_m recovery whereas the increase in irradiance had a significant effect (*2-way ANOVA*: $p < 0.01$, Fig. 2B), being increased by 11% between LL and ML under ambient pCO_2 (posthoc: $p < 0.05$)."

Chl a fluorescence (Lines 22-25): Similarly than mentioned in the previous paragraphs, such idea could be simplified as: "For [RCII], increasing light conditions reduced a 39% the functional RC; however, OA did not show a clear response pattern, as it both decreased (~XX% at LL) as increased (~44% at ML) the functional RC (Fig. 3A)".
AUTHORS: To simplify this section, it is now written in the revised manuscript on P6 L37-P7 L1: "The increase of CO_2 or light alone had no effect on cellular concentrations of functional photosystem II reaction centers ([RCII]) while the interaction of both factors strongly altered [RCII] (*2-way ANOVA*: $p < 0.0001$; Fig. 3). From LL to ML [RCII] decreased under ambient pCO_2 (39%, posthoc: $p < 0.001$) while the combination of ML with OA synergistically increased it (44%, posthoc: $p < 0.01$, Fig. 3). [RCII] was reduced by OA at LL (37%, posthoc: $p < 0.01$) whereas the combined effect of OA and ML led to an increase (49%, posthoc: $p < 0.01$)."

Chl a fluorescence (Lines 26-34): In my humble opinion, in this paragraph is difficult extracting the main finding, and what is the effect of light, OA and their interaction. Also, I sincerely think that authors should present this dataset at the same way than for figure 1-3, in a bar chart. If I see the table 3 presented, I would summarize the findings as follow: "In relation to the individual effects of light and OA on the PSII photophysiology it was found that increasing light conditions exerted a mostly stimulatory effect, excepting on connectivity (i.e. P) where it was inhibitory. OA had a significant positive effect at LL on σPSII, ETRmax and Ik, but significantly inhibitory on α at ML. Light and OA, as a single factors, did not exert any

significant effect on P and τQa. At the same way than mentioned above, the LightxOA interaction had a contrasting effect on the PSII-photosphysiology, being stimulatory on σPSII, ETRmax and Ik, and inhibitory on P and τQa. The interactive effect of both factors did not alter α".

AUTHORS: As suggested by the reviewer, throughout the whole result section we have added the requested information for significant effects by light, CO_2 and their combination for each parameter. Furthermore, we have put together a table summarizing the statistical outcome from the two-way ANOVA, which tested for significant differences in response to light, CO_2 and their combination:

Parameter	Significant light effect	Significant CO_2 effect	Significant light/CO_2-Effect
μ	$p < 0.01$		
POC content			
POC production	$p < 0.01$		
C:N ratio			$p < 0.01$
F_v/F_m	$p < 0.0001$	$p < 0.01$	$p < 0.05$
F_v/F_m recovery	$p < 0.01$		
Chl a	$p < 0.0001$		
Chl c_2	$p < 0.0001$	$p < 0.05$	
Allo	$p < 0.0001$	$p < 0.01$	$p < 0.01$
[RCII]			$p < 0.0001$
P	$p < 0.05$		
σ_{PSII}			$p < 0.05$
τ_{Qa}	$p < 0.05$		
ETR_{max}		$p < 0.05$	
I_K		$p < 0.05$	
α	$p < 0.01$		$p < 0.01$

We agree with the reviewer that the overall effects on photophysiology (P, σ_{PSII}, τ_{Qa}, ETR_{max}, I_K and α) by CO_2, light and their combination should be presented in a less confusing way and more clearly. Considering, however, that no simple and overarching effect was found that applies for all factors (light, CO_2, CO_2-light, please see table above), we prefer to keep presenting each parameter individually instead of describing them together as proposed by the reviewer, which we belief could create confusion, too. To, however, account for the reviewer's point, which is definitely justified, we instead tried to be more clear and to simplify the description of the photophysiological results (P, σ_{PSII}, τ_{Qa}, ETR_{max}, I_K and α). It now reads on P7 L1-11: "While CO_2 and the interaction of CO_2 and light together did not change the energy transfer between PSII units (i.e. connectivity, P), only the increase in irradiance had a significant effect (*2-way ANOVA*: $p < 0.05$), reducing P by 22% between LL and ML under OA (posthoc: $p < 0.05$, Figure 3B). While the increase in CO_2 or light did not alter the functional absorption cross-sections of PSII (σ_{PSII}), the interaction of both factors, however, had an effect (*2-way ANOVA*: $p < 0.05$; Figure 3C). σ_{PSII} values were similar under LL and ML at ambient pCO_2. The interaction of OA and ML, however, lowered them (posthoc: $p < 0.05$, Table 3). On the other hand, when grown under OA σ_{PSII} was larger under HL than under ML (*1-way ANOVA*: $p < 0.01$). Re-oxidation times of the primary electron acceptor Q_a (τ_{Qa}) significantly changed with increasing irradiance (*2-way ANOVA*: $p < 0.05$), but not by high CO_2 or the interaction of both factors together (Figure 3D). τ_{Qa} of OA-acclimated cells was much shorter at HL than at ML (*1-way ANOVA*: $p < 0.05$)."

P7 L13-19: "Both maximum absolute electron transport rates (ETR_{max}) and minimum saturating irradiances (I_K) followed the same trend and were significantly changed by CO_2 (*2-*

way ANOVA: $p < 0.05$), but not by light or the interaction of both factors (Table 3). OA significantly enhanced both parameters under LL (ETR$_{max}$: posthoc: $p < 0.05$, I_K: posthoc: $p < 0.05$), but not under ML. The maximum light utilization efficiency (α) was significantly affected by light (*2-way ANOVA*: $p < 0.01$) and the interaction of CO_2 and light (*2-way ANOVA*: $p < 0.01$), but not by CO_2 alone (Table 3). α significantly increased from LL to ML at ambient pCO_2 (53%, posthoc: $p < 0.01$) while such effect was absent under ML and OA. Between ML and HL, α did not differ when grown under OA."

As proposed by reviewer the parameters [RCII], *P*, σ_{PSII} and τ_{Qa} are now presented in the same figure showing bar charts. Please see Figure 3.

***Chl a fluorescence (Lines 37):** I suggest that figure 4 could be moved to supplementary information, as the core information related with this section/variable is contained in Table 3. Thus, and similarly than mentioned above to authors, Table 3 should be presented as figure instead table. Regarding to the Fig.4, authors can highlight into the text, the stimulatory effect of OA on ETR under LL, and the absence of effect by OA under ML. Also, that such stimulatory effect was coupled with significant? lower NPQ values (i.e. LL) or with not significant differences between amb and OA treatments (i.e. ML). Maxima ETR and NPQ values were found under HL.*
Regarding to the figure 5, I suggest that it could be also moved to supplementary information together with figure 4, and merge both figures into a single one but with 6 panels.
Finally, despite the main goal of this study was assess the interactive effect of HL and OA, authors did not explicitly quantified such impacts on the response variables assessed. Considering that only the OA effect could be calculated, as cells did not grow under HL, I suggest authors calculating the size effect in percentage (or the log response ratio) at least for the HLxOA interaction and for all variables, and show them together in a final figure. Using this approach could strengthen the message of your work, and easily showing to a potential reader if the HLxOA interaction was synergistic/antagonistic (or stimulatory/inhibitory), and on what variable(s).

AUTHORS: The reviewer suggested to "move figure 4 to supplementary information, as the core information related with this section/variable is contained in Table 3". As in our opinion it is important to show on which basis the curve fitting results (shown in Table 3) were derived, we find it indeed important to show the shape of the PI curves and whether the fitting fits well to the curves. Hence, we would like to keep Figure 4 in the main manuscript.

Further the reviewer asks to include *P*, σ_{PSII} and τ_{Qa} into figure 3, this was done. Please see Figure 3.

The reviewer also proposed to highlight in the text the stimulatory effect of OA on ETR and NPQ under LL and the absence of effect by OA under ML". This was done and it is now written on P7 L15-16: "OA significantly enhanced both parameters under LL (ETR$_{max}$: posthoc: $p < 0.05$, I_K: posthoc: $p < 0.05$), but not under ML."
On P7 L22-24: "Much higher NPQ values were determined in the ambient pCO_2 relative to the OA treatment under LL while such pCO_2 effect was absent under ML"

Moreover, the reviewer suggests to move figure 5 to the supplementary material and also to combine Figure 4 and 5. As suggested we combined Figure 4 and 5 into one figure, which is now Figure 4, but refrain from moving this figure into the supplementary material as the content of this figure is critical for the discussion of the whole data set.

As suggested by the reviewer we have calculated the log response ratio (RR) for the OA HL treatment relative to the ambient pCO_2 ML treatment according to Lajeunesse (2015, *Ecology* 96: 2056-2063). The obtained results are shown in the following table:

Parameter	RR
μ	0.41

POC content	-0.07
POC production	0.33
C:N ratio	-0.12
F_v/F_m	-0.16
F_v/F_m recovery	-0.01
Chl a	0.08
Chl c_2	-0.12
Allo	0.28
[RCII]	-0.20
P	-0.22
σ_{PSII}	0.17
τ_{Qa}	-0.12
ETR_{max}	0.33
I_K	0.14
α	0.19

It is true that a positive RR was found for growth and POC production for the OA HL treatment. Considering, however, that negative responses were observed for instance for τ_{Qa}, this does not necessarily mean a negative effect *per se* on phytoplankton physiology. In fact ETR_{max}, I_k and α were positive. On the other hand, re-oxidation times of $_{Qa}$ were shorter and thus indicating faster electron drainage into downstream processes. Hence, a negative RR value does not necessarily indicate a negative response in phytoplankton physiology. Due to the latter finding and the potential for misinterpretation of the results, we refrain from adding this table to the manuscript.

Discussion: What is the main finding of your study? It would be nice highlighting to a potential reader in a sentence or a paragraph what is the gap that this study fills (or contributes).
AUTHORS: As suggested by the reviewer, we have added the following at the beginning of the discussion on P7 L26-29: "Ecophysiological studies on Antarctic cryptophytes to assess whether climatic changes such as ocean acidification and enhanced stratification affect their growth in Antarctic coastal waters in the future are lacking so far. This study can show that the Antarctic cryptophyte *G. cryophila* may be a potential winner of such climatic conditions as it reached highest rates of growth and particulate organic carbon production when grown under HL and OA."

Lines 28-29: Based on the fact that significant higher ETRmax, Ik and alpha values were not coupled with higher POC, and ultimately growth, could it be plausible that cells were less efficient than under LL? Or more damaged?. Thus, this lower photosynthetic efficiency (or higher damage) would be consistent with a 2-fold increase in the NPQ, and ultimately, could support the higher Fv/Fm recovery (%).
AUTHORS: Please note that ETR_{max} and POC production are not necessarily linked by a 1:1 ratio. Linear electron transport is directly coupled to the Calvin cycle, nitrite reduction and other electron accepting processes. In our data set, POC production remained the same between LL and ML under ambient pCO_2, this indicates saturation of the Calvin cycle and thus induction of alternative electron transport pathways. ETRs on the hand do not only reflect linear electron flow, but also comprise alternative electron pathways. Therefore the increase in ETR_{max} between LL and ML under ambient pCO_2 did not yield higher POC production and rather points towards alternative electron transport pathways. Hence, cells photosynthesize as efficiently under LL as under ML, but additionally at ML more electrons are fed into alternative pathways such as xanthophyll cycling as observed by the increase in NPQ. In fact, cells are not damaged, but rather are characterized by a higher F_v/F_m recovery,

supporting a lack of damage. Due to the latter observations, we would like to keep this part of the discussion as it is.

Line 34: *I think that "high light" should be modified to avoid any misunderstanding with the HL treatment.*
AUTHORS: To be more clear, it is now written on P8 L15: "Unexpectedly, *G. cryophila* was, however, unable to grow at 500 μmol photons m^{-2} s^{-1}....".

Line 40: *As the inability for growing of the target specie is a surprising result, and previous results have found that such phytoplankton group grow under HL and stratified conditions, I suggest that authors discuss the potential mechanism(s) or reason(s) that impeded that cell grown.*
AUTHORS: We agree with the reviewer and therefore have now written on P8 L18-21: "A connection of this group with high illuminated conditions was first suggested by Mendes et al. (2017), but lacks information which cryptophyte species were present and their photosynthetic responses. The reason for this difference could be related to species- or strain-specific differences."

Lines 46 and 69: *Statistical information can be omitted in discussion section*
AUTHORS: Done as suggested.

Implications: *On one hand, it is true that the interaction HLxOA stimulated all assessed processes in G. cryophila, however, such responses were based on a short-term scale. It could be plausible that such beneficial effects would be accentuated at long-term scales by an adaptation of the populations, or by contrast, reduced. Authors could briefly discuss such issue in the context of their study.*
On the other hand, in my humble opinion, I think that this idea presented at the end of the manuscript should be also highlighted into the Introduction section, as it represents the core about why assess the interactive effects of both global-change factors on Antarctic phytoplankton communities. Currently, we do not know how communities will respond to such changes, if such changes will lead to a diatoms or flagellates-dominated community, and as a consequence, if it will boost or reduce the C-sink capacity of this area.
AUTHORS: The reviewer points out that the observed responses were obtained through performance of a short-term experiment, while responses could be different on the longer term. To account for this point, we have added the following sentence in the Implications section on P9 L35-38: "Our results from a short-term CO_2-light experiment point towards a high ability of *G. cryophila* to acclimate to such conditions and to cope well with medium, but not high irradiances, whether this applies for other Antarctic cryptophyte species as well needs further testing. Also it remains unclear whether similar responses would be found when exposed on a longer term."

The other suggestion by the reviewer was to point out already in the introduction the implications on carbon biogeochemistry from a shift from diatoms to flagellates. This has been done on P2 L7-9: "Hence, higher abundances of cryptophytes could have important implications for the biogeochemistry of these waters, as they are considered to be inefficient vectors of carbon and thus could reduce the efficiency of the biological carbon pump ".

Minor comments:
Tables 1-3: *Please, include what lowercase letters mean. Are they representing posthoc comparisons? Data represent means and SD? And OA?*
AUTHORS: The reviewer is right, the asked information was missing and has been added. Thanks for pointing this out.

Introduction (line 54): *Change almost nothing by little is known*
AUTHORS: Agreed, it now reads on P2 L20-22: "While laboratory studies so far mainly have concentrated to disentangle the physiological response of Southern Ocean key species of diatom and prymnesiophytes to different environmental factors almost nothing is known on Antarctic cryptophytes."