Note: line numbers in referee comments refer to original BGD discussion paper.

Reply to Referee #1

All referee comments are in bold and our answers in normal font. Changes in the manuscript text are kept in italics.

General comments: This paper investigates the impacts of dust or iron additions on phytoplankton community with and without CO2 enrichment in the northeast Pacific, which is known as a HNLC region. The experimental design is unique, and the incubation is conducted appropriately.

Reply: We thank the referee for this positive comment.

The authors present a novel dataset regarding the effects of dust addition and/or increased CO2 on natural phytoplankton community in the HNLC area. However, the introduction and material and methods are insufficiently constructed to explain the importance of this work. For example, it is not clear from the manuscript why the authors use CJ-2 dust as Fe source. It would be better to cite previous works regarding Fe solubility and, if available, effects of dust addition on phytoplankton. The method of statistical analysis should also be described in sufficient detail with the threshold value (e.g., $p<0.05$) to determine statistical significance. In addition, some of the discussions are lack of adequate referencing.

I recommend that the authors make a greater attempt to improve the manuscript. This paper can be accepted in the Biogeosciences after revision considering the comments below.

Specific comments:

Page 12284, lines 24-25. Is this statement correct? I believe that diatoms possess highly efficient CCMs than coccolithophores as described in the previous review by Reinfelder (2011, Annu. Rev. Mar. Sci.). Please revise this section.

Reply: The reviewer is right. The words were inverted. The end of the paragraph, p 12284, starting line 24, has been changed.

New sentence: *Coccolithophores and diatoms generally exhibit low- and high-efficiency CCMs, respectively (Reinfelder, 2011). Alternatively, fertilisation with FeSO4 usually favors the growth of diatoms in HNLC waters (Boyd et al., 2007 and references therein). It is not known whether FeDust deposition will favor diatoms or coccolithophores in the context of OA. OA could profoundly modify the structure and functioning of a phytoplankton community typically dominated by calcifying haptophytes.*

Pages 12284-12285, Introduction. The topic of duct should be described and it should be separated from that of Fe, because it is unclear whether Asian duct might serve as a Fe source or not.

Reply: The following sentences have been added p 12284 starting line 9, to explain the importance of dust.

New sentence: *Sources of iron to the northeast subarctic Pacific include vertical mixing, eddies, tidal currents and convection (Cullen et al., 2009; Royer et al., 2010), volcanic ash (Mélançon et al., 2014; Olgun et al., 2011), and desert dust (Boyd et al., 1998; Jickells et al., 2005). Dust, which is considered one of the most important sources, is deposited sporadically mostly in the spring during occasional dust storms originating from the deserts of northern Asia (Duce and Tindale, 1991). A natural strong dust deposition event has been shown to nearly double particulate organic carbon (POC) concentration in the northeast subarctic Pacific in 2001 (Bishop et al., 2002).The importance of eddies and vertical diffusion in the Gulf of Alaska was recently reviewed and found to be greater than previously thought (Crawford et al., 2007; Johnson et al., 2005; Lam and Bishop, 2008).*

Pages 12286-12287, Experimental setting and location. Please give the temperature and salinity in the sampling site.

Reply: The following sentence with the requested information was added p. 12286 starting line 23:

New sentence: *A CTD profile conducted at the same station 2h after all bags were filled showed a temperature of 13.5oC and a salinity of 32.6 at 10 m depth*

Page 12288, lines 2-4. It is difficult to understand experimental conditions without the previous paper by Nishikawa et al. (2000) due to the lack of information about CJ-2 dust in the manuscript. Please describe the properties (e.g., size, chemical composition, and iron solubility) of CJ-2 dust in more detail in this section or discussion. Ooki et al. (2009, J. Geophys. Res. Atom.) might serve as a useful reference for the iron dissolution property.

Reply: The following sentence with additional information was added p. 12288 line 2:

New sentence: *Briefly, CJ-2 dust was collected from the Tengger desert surface soil, roughly sieved and blown through a wind tunnel designed to collect fine particles. Median diameter of CJ-2 dust is 24.1 µm. CJ-2 dust is characterized by a Fe content of 3.02 ± 0.12% and Fe solubility of 0.33% (Ooki et al., 2009).*

Page 12290, lines 19-21. In this sentence, the authors described that the 6 groups were quantified by CHEMTAX. However, table 3 shows 8 algal groups in the pigment ratio matrix for CHEMTAX analysis. Which is correct? In addition, the authors should provide the reference or method used to determine the initial pigment:chl a ratios in the table 3.

Reply: The matrix ratio used in the CHEMTAX analysis of the data is the same that is regularly used in northeast subarctic Pacific waters and was described in Royer et al. (2010). We agree that more information will be welcomed, especially considering the

importance of speciation in the paper (see below). Regarding the question related to the algal groups, 8 were measured by the ratio matrix but 2 of them were below detection limit at P26, which resulted in the confusion between whether 6 or 8 groups have been identified. We are now clearly explaining that 8 groups were measured but that prasinophytes and cryptophytes were below detection limit at our sampling station. The end of the paragraph on page 12290 was changed (starting line 19).

New sentence: *The initial pigment ratio matrix loaded into the CHEMTAX program (Table 3) was obtained by averaging the minimum and maximum values of pigment : Chl a ratios given in Table 1 of Mackey et al. (1996) and is similar to that used by Suzuki et al. (2002) and Royer et al. (2010) for samples collected in the subarctic North Pacific. Eight algal groups were quantified using the chemotaxonomy program CHEMTAX (Mackey et al., 1996): cyanobacteria, pelagophytes, haptophytes (including coccolithophores), diatoms, dinoflagellates, prasinophytes, cryptophytes and chlorophytes. For a description of the pigment types, see Zapata et al. (2004).* And the following sentence was added as the second sentence of the Taxonomy section, p 12294:

New sentence: *Prasinophyte and cryptophyte biomarkers were not detectable during our experiments.*

Page 12292, Statistical analysis: The threshold value (e.g., p<0.05) for determining statistical significance should be described in this section.

Reply: The following sentence was added to the section New sentence: *The threshold value for determining statistical significance was $p < 0.05$.*

Page 12294, lines 14-16. "The highest POC concentration..." Is this statement indicates that the POC concentration in the Dust treatment was significantly higher than that of Dust+Acid treatment and the other treatments?

Reply: Yes. The point we want to make here is that POC concentrations were higher in the Dust treatment than in all other treatments, including the Dust+Acid treatment. The sentence was reworded to clarify our message

New sentence : *The highest POC concentration was measured in the Dust treatment (287 ± 30 µg L-1) and lowering the pH resulted in a 24% decrease in POC concentration at T4 (Dust+Acid treatment: 217 ± 2 µg L-1) (Fig. 2c).*

Page 12295, lines 20-21. "These results suggest a. . ." This statement would be described in the discussion.

Reply: It was deleted from the results sections as it is already referred to in the discussion p12300, lines 27-29.

Page 12295, lines 22-28. "Phytoplankton can acquire. . .is likely to be." These statements seem better placed in the discussion.

Reply: As requested by referee #2, more details on what FeDFB uptake means in term of iron uptake was added to the method section 2.4.4. Consequently, the lines referred above were removed from the manuscript as the information is given elsewhere in the new version of the manuscript.

Page 12297, line 25 (and elsewhere). It is better to use "µatm" instead of "ppm" for the unit used to express partial pressure.

Reply: Done

Page 12297, line 26. The period can be omitted.

Reply: Done.

Page 12297, lines 25-27. "The abrupt decrease. . .(Figs. 2a and 3c)" In this statement, why can the authors say that the community composition was unaffected by CO2? Since the effects of acidification on the community composition were not described statistically in the results, it is hard to accept the statement. Rather, in the figure 3, chl a biomass of diatoms, haptophytes, and pelagophytes seems to have increased in the Control+Acid treatment relative to Control treatment.

Reply: These groups indeed look like they have a higher biomass, but due to a low number of replicates and poor statistical power, they were not significantly different at the threshold level of $p<0.05$. We do agree that statistical results should be described in the results section, so we added the following sentence to the end of the taxonomy section in the results:

New sentence: *The only treatment to show a statistically significant difference with the Control is the Dust treatment, which had significantly higher concentrations of chl a attributable to diatoms and cyanobacteria than the Control at T4. No statistical difference between any treatment and its acidified counterpart could be detected.*

Page 12298, line 11-14. In the discussion regarding the CO2 effect on the HNLC water, it should be noted that the other experiment conducted using HNLC water demonstrated strong negative effects on diatoms at elevated CO2 levels (Sugie et al. 2013, Biogeosciences; Endo et al. 2015, Biogeosciences).

Reply : The following sentence has been added to the paragraph :

New sentence: *Another experiment conducted with water from the diatom-dominated Fe-limited Bering Sea has shown a negative effect of elevated CO2 on diatoms (Sugie et al., 2013, Endo et al., 2015), which was not observed in the Fe-enriched treatments.*

Page 12299, lines 5-7. This statement requires supporting references regarding the response of DOC and/or TEP productions to ocean acidification. For example,

Yoshimura et al. (2014, Deep Sea Res. I) reported minimal effects of increased CO2 on DOC production in the Fe-limited phytoplankton communities.

Reply: This statement was supported by Riebesell et al., 2007 but we also added a reference to the paper of Engel et al. (2014) who also observed a stimulation of DOC production at elevated CO2. In addition, as suggested, we mention the paper by Yoshimura et al. (2014) in order to give a broader view of the types of responses observed so far.

New sentence: *Similar significant stimulation of DOC production at elevated CO2 was reported by Engel et al. (2014) in coastal waters but only a weak and inconsistent CO2 induced decrease in DOC production was observed by Yoshimura et al. (2014).*

Page 12300, lines 26-29. How did the authors make sure that phytoplankton growth was limited by Fe availability? I feel that the decrease in carbon fixation rate is insufficient to explain Fe limitation for phytoplankton without references. Given that Fe was released and re-adsorbed by the dust, DFe concentration might have remained constant by a balance between dissolution and re-adsorption. This paragraph needs to be reconsidered.

Reply: Decrease in the carbon fixation rate is only one of the supporting data for Fe-limitation. The high FeDFB uptake rates measured at that time represent a stronger indicator of Fe limitation. The paragraph has been changed to put more emphasis on the FeDFB uptake rate.

Modified paragraph: *Our results suggest that after a period of active growth, phytoplankton in the Dust treatment became Fe-limited 4 days into the experiment. This conclusion is supported by the very low absolute and chl a normalized C fixation rates in the Dust treatment as compared to the Control and FeSO4 treatments at T4 (Fig. 4a), as well as by the FeDFB uptake rates normalized to C uptake rates which were higher in the Dust treatment than in the Control (Fig. 4b). Dust particles are known to efficiently adsorb Fe (Ye et al., 2011). The rapid return to Fe deficiency in the Dust treatment may*

thus result from a combination of increased Fe demand and re-adsorption of Fe onto dust particles. This explanation implies however that the re-adsorbed Fe becomes less prone to desorption, which needs to be demonstrated. These results suggest that the influence of the Fe released from dust lasted less than 4 days during our experiments.

Pages 12301-12302, "In order to further explore. . .was detected with the two factors". These statements seem better placed in the material and methods or results.

Reply: This part of the text was moved to the results section and a new sentence was added to the paragraph from which the text was taken out to improve fluidity.

New sentence: *The two-factor ANOVA allowed us to increase the statistical power and detect a negative effect of acidification on chl a concentration after 4 days.*

Page 12302, lines 18-19. This statement is inconsistent with Fig. 3c, which indicates that chl a concentration of haptophytes increased in the Fe and Dust treatments relative to control treatment.

Reply: These increases are note statistically significant due to our small number of replicates. Therefore, we cannot state that there were more haptophytes in the Fe and Dust treatments.

The sentence has been reworded to: *This can be explained by the lack of significant difference in the abundance of DMSP-rich haptophytes in the Control, FeSO4 and Dust treatments at T4.*

Figures 1-5. Sample size should be described in the figure captions

Reply: Done

References cited:

Endo, H., Sugie, K., Yoshimura, T., and Suzuki, K: Effects of CO2 and iron availability on rbcL gene expression in Bering Sea diatoms. Biogeosciences, 12, 2247-2259, 2015.

Ooki, A., Nishioka, J., Ono, T., and Noriki, S: Size dependence of iron solubility of Asian mineral dust particles. J. Geophys. Res. Atmos., 114, D3202, 2009.

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Boyd, P. W., Jickells, T., Law, C. S., Blain, S., Boyle, E. A., Buesseler, K. O., Coale, K. H., Cullen, J. J., de Baar, H. J. W., Follows, M., Harvey, M., Lancelot, C., Levasseur, M., Owens, N. P. J., Pollard, R., Rivkin, R. B., Sarmiento, J., Schoemann, V., Smetacek, V., Takeda, S., Tsuda, A., Turner, S., and Watson, A. J.: Mesoscale iron enrichment experiments 1993-2005: Synthesis and future directions, Science, 315, 612-617, 2007.

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Crawford, W. R., Brickley, P. J., and Thomas, A. C.: Mesoscale eddies dominate surface phytoplankton in northern Gulf of Alaska, Prog. Oceanogr., 75 (2), 287-303, 2007.

Cullen, J. T., Chong, M., and Ianson, D.: British Columbian continental shelf as a source of dissolved iron to the subarctic northeast Pacific Ocean, Global Biogeochem. Cy., 23, 2009.

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Engel, A., Piontek, J., Grossart, H.-P., Riebesell, U., Schulz, K. G., and Sperling, M.: Impact of CO2 enrichment on organic matter dynamics during nutrient induced coastal phytoplankton blooms, J. Plankton Res., 36(3), 641-657, 2014.

Jickells, T. D., An, Z. S., Andersen, K. K., Baker, A. R., Bergametti, G., Brooks, N., Cao, J. J., Boyd, P. W., Duce, R. A., Hunter, K. A., Kawahata, H., Kubilay, N., laRoche, J., Liss, P. S., Mahowald, N., Prospero, J. M., Ridgwell, A. J., Tegen, I., and Torres, R.: Global iron connections between desert dust, ocean biogeochemistry, and climate, Science, 308, 67-71, 2005.

Lam, P. J. and Bishop, J. K. B.: The continental margin in a key source of iron to the HNLC North Pacific Ocean, Geophys. Res. Lett., 35(2), 10.1029/2008GL033294, 2008.

Olgun, N., Duggen, S., Croot, P. L., Delmelle, P., Dietze, H., Schacht, U., Oskarsson, N., Siebe, C., Auer, A., and Garbe-Schoenberg, D.: Surface ocean iron fertilization: The role of airborne volcanic ash from subduction zone and hot spot volcanoes and related iron fluxes into the Pacific Ocean, Global Biogeochem. Cy., 25, 2011.

Reply to Referee #2

All referee comments are in bold and our answers in normal font. Changes in the manuscript text are kept in italics.

This manuscript contributes important empirical evidence and debate on the influence that ocean acidification may have on biogeochemistry in the oceans. Iron availability is episodic in the NE Pacific and plays an important part in determining pelagic community composition and levels of productivity. Investigating how an

increase in pCO2/decrease in pH influences the response to episodic iron availability in natural oceanic communities is ambitious and unsurprisingly, not altogether easy to interpret. Nonetheless, the authors have designed an effective and achievable experiment, applied a suite of relevant measurements and provided, in general, a balanced interpretation of the results. Several points could be clarified to improve the manuscript:

1. Title: The title fails to capture the essence of the study, in my view. It puts a large emphasis on the influence of dust additions but the manuscript is presented very much as an ocean acidification+dust experiment. It also does not capture the impact on community composition, which is a much larger component of the paper than the DMS response.

Reply: We thank the reviewer for pointing that out. The title has been reworded to: *Impact of ocean acidification on phytoplankton assemblage, growth and DMS production following Fe-Dust additions in the NE Pacific HNLC waters*

2. Iron treatments and bioavailability: The application of two different versions of iron addition is useful and adds a great deal to the interest of the paper. However, this is not reflected in the stated objective of the research (L12286, L12+) and should be (1). How do differences in the form of iron used for previous comparable experiments influence those results (Introduction, L12285, L3+)? (2) Although the reader is directed to Nishikawa et al (2000) and Hwang and Ro (2006) for more information on the specific dust added, it would be useful to present more details on the dust in this context. This should include total iron content and possibly the form of the iron present in the dust (3). If DFe is not a good measure of the bioavailability of iron (Section 4.3) what should be measured? (4) Please discuss why the Fe contained in the dust is more available than the added FeSO4 (5).

Reply: (1) A reference to FeSO4 was added to the objective.

New paragraph: *The objective of this study was to determine how a decrease in pH by 0.2 units could influence the impact of Fe delivered as FeSO4 or Asian dust on the growth and taxonomic composition of the phytoplankton assemblage of the Fe-limited northeast*

subarctic Pacific in summer, and to explore how pH-induced changes could affect the production of the climateactive gas DMS.

(2) To our knowledge, this is the first study using a natural form of iron, i.e. dust, in the context of a HNLC region. Studies intercomparison is not possible. A comparable protocol was used in a study conducted in Atlantic where the addition of Sahara dust stimulated nitrogen fixation (Mills et al., 2004). In this study, the addition of FeSO4 and dust had similar effect on growth. But this last study is not directly relevant to our study.

(3) As requested, more information has been added on CJ-2 dust (see reply to referee #1).

(4) This question does not have a simple answer, since iron bioavailability doesn't have a universally accepted definition and methods used to quantify it also vary amongst laboratories. However, in a previous study, we showed that TDFe concentrations had a better predictive capacity than DFe values. We are referring to this previous work in the revised version of the manuscript.

New sentence: *Similar conclusions were reached in a previous study (Mélançon et al., 2014), where TDFe was also measured and shown to be a better indicator of Fe bioavailability than DFe.*

(5) Unfortunately, we can only speculate on that. A new sentence has been added in section 4.3 to explain the possibilities.

New sentence: *We can only speculate as to why Fe contained in dust stimulated more growth than FeSO4. Part of the answer may be related to the speed at which the Fe is released from dust, a relatively low release rate allowing a more efficient utilization of Fe. In comparison, and as can be seen in Fig. 1, FeSO4 is rapidly utilized and probably scavenged by particles and ligands following fertilization.*

3. Phytoplankton taxonomy and pigments (Section 2.4.3): The study uses HPLC based pigment analysis and Chemtax to assign class-specific contributions to total chlorophyll. This requires more explanation. In particular, how is the contribution of diatoms differentiated from that of haptophytes or pelagophytes? As Table 3 illustrates, these classes share several of the biomarker pigments and differentiating them is far from exact. At the last, the authors should make clear the uncertainty in

the class-specific chlorophyll assignment and take it into account in their conclusions that increased pCO2 increased the fitness of diatoms over other taxa.

Reply: Fucoxanthin is the main biomarker of diatoms but, as shown in Table 3 and pointed out by the reviewer, fucox is also present in haptophytes and pelagophytes. In contrast, 19'- butanoyloxyfucoxanthin (but) is present in haptophytes and pelagophytes but not in diatoms. Thus, an increase in the concentration in "fucox" that is not accompanied by an increase in "but" can be used to differentiate the contribution of diatoms from that of haptophytes or pelagophytes. The method has been described in more detail (see reply to referee #1, and modifications in the text), however, we do not feel it is necessary to present raw pigment data. Our result do not show increased fitness of diatoms over other taxa linked to increased pCO2 as there is no significant difference in the concentration of diatoms between acidified and nonacidified treatments. The increase in diatom biomass is more likely caused by iron fertilization.

4. Increased respiration is provided as one explanation for why increased 14C assimilation is not reflected in increased biomass in Control+Acid (P12298, L23+) and Dust+Acid (P12301, L19+). However, using 24h 14C incubations to determine carbon assimilation is generally thought to measure something closer to net production than gross production; meaning that any increase in the rate of respiration would be captured by that measurement.

Reply: Good point. This explanation was deleted from the list.

5. DMSP and DMS. Given the generally high DMSP content of dinoflagellates, it is puzzling that the almost immediate loss of dinoflagellates is not reflected in the trends in DMSP? What/who were the main contributors to the DMSP pools? Are the high initial DMS:DMSP ratios (roughly 1:4) a product of the experimental set-up, i.e. cell disruption/ negative impact on dinoflagellates, or were in situ DMS concentrations similar at the time the water was sampled? Reply: We cannot ascertain how fast the loss of dinoflagellates took place, since measurements were made only at T0 and T4. The DMSPt (which also includes dissolved DMSP) could decrease as dinoflagellates lysed and DMSPd got consumed by bacteria. It is likely that dinoflagellates and haptophytes were the main contributors to the DMSP pool, and the

DMSP decrease was caused by the loss of dinoflagellates. The DMS concentration is not unusually high. The northeast subarctic Pacific is known for very high DMS concentrations and high DMS:DMSP ratios. In situ DMS concentration at the same station and depth 17h prior to our water collection was ~15 nmol L-1, a value relatively close to the value of 10 nmol L-1 measured at the beginning of the incubation. A note on the usually high concentration of DMS in this region has been added in the section 4.2 of the revised manuscript.

New sentence: *DMS concentrations were high, but usual for this region in the summer (Wong et al., 2005).*

6. Iron uptake rate. Figure 4C. I am not sure this shows Fe uptake rate. This was measured as the incorporation of added 55FeDFB. A clear explanation (Section 2.4.4) of how (and why) Fe uptake rates are calculated from the assimilation of the 55FeDFB complex is required.

Reply: We have amended the paragraph to provide an explanation for examining Fe uptake from DFB. Since uptake of Fe from DFB makes up a small portion of the manuscript, and is not the primary focus of it, we refer to previously published work that provides a thorough explanation for how the Fe uptake rates are calculated (in section 2.4.4; Maldonado and Price 1999; Semeniuk et al. 2009). However, we have provided a few more details to clarify how the rates were calculated.

Revised section 2.4.4 *The siderophore desferrioxamine B (DFB) has been used as a model ligand for studying the bioavailability of strongly organically complexed Fe in seawater (e.g. Maldonado and Price 1999; Hutchins et al. 1999; Shi et al. 2010). Iron uptake from DFB occurs through a high affinity Fe transport system, and can reflect the Fe nutritional status of laboratory phytoplankton strains (Maldonado and Price 1999; Maldonado et al. 2006) and natural phytoplankton assemblages (Semeniuk et al. 2009; Taylor et al. 2013; Semeniuk et al. in press). Thus, we examined whether uptake rates of Fe complexed to DFB varied among our treatments with varying Fe bioavailability. Uptake of 55Fe bound to DFB was performed as previously described (Maldonado and Price 1999; Semeniuk et al. 2009). The 0.5 nM 55Fe (Perkin Elmer) was complexed with 5% excess DFB in pH 3.5 Milli Q for 30 minutes (Maldonado and Price, 1999). The*

resulting 55FeDFB complex was subsequently equilibrated in 0.22 µm filtered seawater for 2 h. Approximately 250 mL of seawater was subsampled from each incubation bag into acid-cleaned polycarbonate bottles. Just before dawn, the equilibrated 55FeDFB complex and 10 µCi of H14CO3- (Perkin Elmer) were added to each 250 mL bottle. From each assay bottle, 1 mL of sample was taken in order to determine the initial total added activities of 55Fe and 14C. To prevent inorganic 14C from offgassing in the initial activity sample vial, 500 µL of 5 M NaOH was added. After 24 h, the content of each bottle was gently filtered onto a 47 mm diameter, 1 µm porosity polycarbonate filter (AMD) under low vacuum pressure (≤70 mm Hg). Just before going dry, the filters were immersed in 5 mL of Titanium-ethylenediaminetetraacetic acid (Ti-EDTA) wash to remove extracellular Fe (Hudson and Morel, 1989). The filters were then rinsed with 10 mL of filtered seawater to remove any loosely associated tracer. Filters were placed into 7 mL borosilicate scintillation vials, immersed in 5 mL Scintisafe 50% scintillation cocktail, and conserved in the dark until analysis on a Beckman LS65005514 scintillation counter. Volumetric Fe uptake and C-fixation rates were calculated as described elsewhere (Maldonado and Price 1999; Semeniuk et al. 2009). Previous work with phytoplankton assemblages along Line P has demonstrated that uptake of Fe from DFB by natural phytoplankton communities is linear over 24 h (Maldonado and Price 1999). Thus, Fe uptake rates were calculated assuming the accumulation of 55Fe by cells was linear during the assay. In order to compare Fe uptake rates among treatments, volumetric rates were normalized to the amount of C fixed during the assay. The 14C uptake rates normalised to chl a is used here as an indicator of the growth status of the autotrophic assemblage.

7. In general, the table and figure legends would benefit from a greater level of explanation.

Reply: Sample size was added as requested by reviewer 1, and more information was added to the legendsof tables and figures (see below).

Here are the modified table/figure titles:

Table 1: Description of Fe/dust additions and acidification status of experimental conditions for each treatment. All treatments were conducted in triplicate.

Table 3: Biomarker pigment initial ratio matrix for CHEMTAX: Chl a ratios for eight algal groups.

Figure 1: Average concentration of DFe in each treatment during the incubations measured at T0, T2 and T4. (a) Control and Control+Acid. (b) Fe and Fe+Acid. (c) Dust and Dust+Acid. Error bars indicate standard deviations. n = 3 except for Acid, T0, T2, Dust+Acid, T0, and Control (all times) where n = 2.

Figure 2: Average concentration of chl a (left axis) during the incubations and POC at T4 (right axis) in each treatment. (a) Control and Control+Acid. (b) Fe and Fe+Acid. (c) Dust and Dust+Acid. Error bars indicate standard deviations. Dashed line indicates POC concentration at T0. Chl a: n = 3 except for Acid, T0, Dust+Acid, T4, Fe+Acid, T0 and Control (all times) where n=2 because of missing/unreliable data or contamination (Control 1). POC: n=3 except Control where n=2.

Figure 3: Average chl a concentration (µg L-1) attributable to each of the measured groups of phytoplankton initially (T0: white bar) and for each of the treatments after 4 d (T4) of incubation (Control, Control+Acid, Fe, Fe+Acid, Dust, Dust+Acid; gray bars). (a) Diatoms. (b) Dinoflagellates. (c) Haptophytes. (d) Pelagophytes. (e) Chlorophytes. (f) Cyanobacteria. Error bars indicate standard deviations. n = 2 except for T0 (all groups but dinoflagellates) where n = 3 and Acid, diatoms, Control, haptophytes and pélagophytes, Dust+Acid, cyanobacteria and Fe+Acid, haptophytes and cyanobacteria where n = 1.

Figure 4: Average (a) Absolute C assimilation rates. (b) C assimilation rates normalized to chl a concentration at T4 and (c) Fe uptake rates normalized to chl a concentration at T4. Error bars indicate standard deviations. Absolute C assimilation rates: n = 3 except Control and Acid where n = 2, C assim norm to chl a: n = 3 except Control, Acid and Dust+Acid where n=2, Fe uptake rates normalized to chl a: n = 3 except Control and Dust+Acid where n = 2.

Figure 5: DMSPt (a, b and c) and DMS (d, e and f) concentrations (nmol L-1) in the Control and Control+Acid treatments (a and d), the Fe and Fe+Acid treatments (b and e), and the Dust and Dust+Acid treatments (c and f). Error bars indicate standard deviations.. n = 3 except Control where n = 2.

Minor points: P12287, L19, CO2SYS needs to be consistent.

Reply: corrected to CO2SYS.

P12291, L16. What size of filter was used?

Reply: 47 mm diameter. The information has been added to the text.

P12294, L3 etc. the levels of precision, reflected in the number of decimal places, should be consistent.

Reply: Done.

P12295, L10. Maybe refer to the Control in this sentence.

Reply: Done.

New references cited

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Impact of ocean acidification on phytoplankton assemblage, growth and DMS production following Fe-Dust additions in the NE Pacific HNLC waters ~~Effects of dust additions on phytoplankton growth and DMS production in high CO_2 northeast Pacific HNLC waters~~

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Abstract

Ocean acidification (OA) is likely to have an effect on the fertilizing potential of desert dust in high-nutrient, low-chlorophyll oceanic regions, either by modifying Fe speciation and bioavailability, or by altering phytoplankton Fe requirements and acquisition. To address this issue, short incubations (4 days) of northeast subarctic Pacific waters enriched with either $FeSO_4$ or dust, and set at pH 8.0 (in situ) and 7.8 were conducted in August 2010. We assessed the impact of a decrease in pH on dissolved Fe concentration, phytoplankton biomass, taxonomy and productivity, and the production of dimethylsulfide (DMS) and its algal precursor dimethylsulfoniopropionate (DMSP). Chlorophyll *a* (chl *a*) remained unchanged in the controls and doubled in both the $FeSO_4$-enriched and dust-enriched incubations, confirming the Fe-limited status of the plankton

assemblage during the experiment. In the acidified treatments, a significant reduction (by 16-38%) of the final concentration of chl *a* was measured compared to their non-acidified counterparts, and a 15% reduction in particulate organic carbon (POC) concentration was measured in the dust-enriched acidified treatment compared to the dust-enriched non-acidified treatment. $FeSO_4$ and dust additions had a fertilizing effect mainly on diatoms and cyanobacteria. Lowering the pH affected mostly the haptophytes, but pelagophyte concentrations were also reduced in some acidified treatments. Acidification did not significantly alter DMSP and DMS concentrations. These results show that dust deposition events in a low-pH iron-limited Northeast subarctic Pacific are likely to stimulate phytoplankton growth to a lesser extent than in today's ocean during the few days following fertilization and point to a low initial sensitivity of the DMSP and DMS dynamics to OA.

1 Introduction

The northeast subarctic Pacific is a high-nutrient, low-chlorophyll (HNLC) region characterized by a phytoplankton assemblage dominated in summer by calcifying coccolithophores and extremely high concentrations of the biogenic climate-active gas dimethylsulfide (DMS) (Levasseur et al., 2006; Wong et al., 2006). Several studies have demonstrated that iron (Fe) addition in these Fe-poor waters stimulated phytoplankton growth, in which diatoms often outcompeted other phytoplankton groups, including coccolithophores, and resulted in a decrease in DMS concentrations (Boyd et al., 2005; Hamme et al., 2010; Mélançon et al., 2014). Sources of iron to the northeast subarctic Pacific include vertical mixing, eddies, tidal currents and convection (Cullen et al., 2009; Royer et al., 2010), volcanic ash (Mélançon et al., 2014; Olgun et al., 2011), and desert dust (Boyd et al., 1998; Jickells et al., 2005). Dust, which is considered one of the most important sources, is deposited sporadically mostly in the spring during ~~few~~occasional dust storms originating from the deserts of northern Asia (Duce and Tindale, 1991). A natural strong dust deposition event has been shown to nearly double particulate organic carbon (POC) concentration in the northeast subarctic Pacific in 2001 (Bishop et al., 2002). The importance of eddies and vertical diffusion in the Gulf of Alaska was recently reviewed and found to be greater than previously thought (Crawford et al., 2007; Johnson

et al., 2005; Lam and Bishop, 2008) Ongoing ocean acidification (OA) is however likely to compromise our current understanding of the ecosystem's response to Fe addition by potentially altering Fe bioavailability (Breitbarth et al., 2010; Shi et al., 2010) and phytoplankton physiology and community composition (Tortell et al., 2002).

OA is currently in progress, is measurable and is caused by CO_2 emissions to the atmosphere that end up in the ocean (Gattuso et al., 2013). The ocean has taken up one third of the CO_2 emissions since the beginning of the industrial era. The dissolution of CO_2 in seawater increases the concentration of bicarbonate (HCO_3^-), protons (H^+) (thereby decreasing pH) and decreases the concentration of carbonate (CO_3^{2-}), leading to calcite and aragonite undersaturation. Studies have shown that calcifiers growing in acidified conditions generally present lower net calcification, although their growth, synthesis and abundance are not generally affected (Kroeker et al., 2013 and references therein). On the other hand, the increase in dissolved CO_2 in seawater could favor the growth of phytoplankton groups with low surface area to volume ratios (S/V) that are limited by the diffusion of CO_2 across their surface or with low-efficiency carbon concentrating mechanisms (CCMs) by reducing the energetic cost of carbon (C) assimilation. Coccolithophores and diatoms generally exhibit ~~high~~low- and ~~low~~high-efficiency CCMs, respectively (Reinfelder, 2011). Alternatively, fertilisation with $FeSO_4$ usually favors the growth of diatoms in HNLC waters (Boyd et al., 2007 and references therein). It is not known ~~If~~whether Fe-Dust deposition will favor diatoms or coccolithophores in the context of OA. ~~OA favors diatom growth in the northeast subarctic Pacific, then it~~OA could profoundly modify the structure and functioning of a phytoplankton community typically dominated by calcifying haptophytes.

Studies examining the effects of acidification on the bioavailability of Fe in HNLC regions have provided contrasting results. Breitbarth et al. (2010) observed a significant increase in Fe(II) half-life and concentration in response to CO_2 enrichment, suggesting that a lower pH could increase Fe bioavailability. However, Fe bioavailability could also decrease during acidification due to changes in dissolved Fe speciation. Shi et al. (2010) observed that complexation of Fe(III) by organic ligands containing acidic, unprotonated functional groups (e.g. carboxylic acid) is strengthened in response to small decreases in

surface water pH, resulting in decreased inorganic Fe concentrations - the more bioavailable form of Fe. Furthermore, Fe uptake rates decrease when acquiring organically complexed Fe – such as Fe(III) bound to desferrioxamine B (DFB) – because the enzymatic reduction of Fe(III) at the cell surface may release protons (Shi et al., 2010). Experimental studies with natural communities also yield inconsistent results. A study combining CO_2 and Fe manipulations of a natural northwest subarctic Pacific community showed a decrease in coccolithophore abundance at higher CO_2 levels (750 and 1000 ~~ppm~~µatm) regardless of the Fe status, but no effect of the CO_2 level on diatoms nor on total chlorophyll *a* (chl *a*) concentrations (Endo et al., 2013). A similar study conducted in HNLC waters of the Weddell Sea, Antarctica, showed an increased C-specific primary productivity with increasing CO_2 concentrations in Fe-enriched treatments but not in Fe-depleted treatments (Hoppe et al., 2013).

By altering algal physiology and community composition, OA is likely to influence dimethylsulfoniopropionate (DMSP) and DMS production. DMS originates mostly from the enzymatic cleavage of DMSP, an osmolyte produced by several groups of phytoplankton. DMSP quotas in phytoplankton vary by three orders of magnitude, with coccolithophores and diatoms known as strong and poor producers, respectively (Keller et al., 1989). Results from the few studies which have looked at the impact of OA on DMS production are inconsistent. Several of them have reported a decrease in DMS production in acidified waters (Archer et al., 2013; Arnold et al., 2013; Avgoustidi et al., 2012; Hopkins et al., 2010b). However, an increase in the concentration of DMS at high CO_2 was measured in five bioassays conducted in northwest European waters (Hopkins and Archer, 2014), during the first ten days of the Third Pelagic Ecosystem CO_2 Enrichment Study (PeECE III) (Vogt et al., 2008; Wingenter et al., 2007), and during a mesocosm study conducted in the coastal waters of Korea (Kim et al., 2010). The effect of acidification on DMSP concentration is usually smaller than on DMS, and a greater variability in responses is generally observed: particular or total DMSP increases in some studies (Archer et al., 2013; Arnold et al., 2013), decreases in others (Avgoustidi et al., 2012; Hopkins and Archer, 2014; Hopkins et al., 2010b) or shows no response to increased pCO_2 (Lee et al., 2009; Vogt et al., 2008). The causes for this variability are not well known.

The objective of this study was to determine how a decrease in pH by 0.2 units could influence the impact of Fe delivered as $FeSO_4$ or Asian dust ~~deposition (i.e. Fe bioavailability)~~ on the growth and taxonomic composition of the phytoplankton assemblage of the Fe-limited northeast subarctic Pacific in summer, and to ~~further~~ explore how these pH-induced changes could affect the production of the climate-active gas DMS.

2 Material and Methods

2.1 Experimental setting and location

On deck incubations were conducted during a cruise along the Line-P transect aboard the Canadian Coast Guard Ship John P. Tully. Water was collected at Ocean Station Papa (OSP) (50°N, 145°W) from 10 m depth on 27 August 2010 using a Teflon® diaphragm compressed air activated pump with Teflon® tubing and filtered through a 200 µm nylon mesh to remove large zooplankton. A CTD profile conducted at the same station 2h after all bags were filled showed a temperature of 13.5°C and a salinity of 32.6 at 10 m depth. Water was incubated in 5 L collapsible bags (Hyclone® Labtainers ™). A flow of surface water was continuously pumped through the incubators to keep the temperature at in situ levels. Measured transmittance shows that the incubation bags filtered 55 % of ultraviolet A (UVA) radiation, 70 % of ultraviolet B (UVB) radiation, and 33 % of photosynthetically active radiation (PAR), irradiance corresponding roughly to a depth of 10 m for the 400-600 nm wavelengths (Sasaki et al., 2001). The incubations lasted 4 days and subsampling took place at T0 (0-20 min after acidification and enrichment), T2 (after 2 days), and T4 (at the end of the incubation). All materials in contact with seawater were cleaned to prevent trace-metal contamination according to protocols established by the international GEOTRACES program and available in GEOTRACES' Methods Manual (Cutter et al., 2010).

2.2 Treatments and acidification protocol

Incubation bags were submitted to six treatments (in triplicate) representing the following combination of dust or Fe addition and acidification: Control, Control+Acid, Fe,

Fe+Acid, Dust, Dust+Acid (Table 1). The carbonate system parameters and methods used for acidification were based on the recommendations of Riebesell et al. (2010). The technique chosen was the addition of a strong acid (HCl) and bicarbonate ($NaHCO_3$). The target value of 750 ~~ppm~~µatm CO_2 was chosen to reproduce the concentration of CO_2 expected in 2100 following the "business as usual" scenario IS92a by the Intergovernmental Panel on Climate Change (IPCC) (Meehl et al., 2007). Target values of the carbonate system parameters (DIC, pCO_2, alkalinity and pH) were calculated using the MS Excel macro ~~CO_2sys~~ CO2SYS (Pierrot et al., 2006) (sets of constants: K1, K2 from Mehrbach et al. (1973) refitted by Dickson and Millero (1987), $KHSO_4$: Dickson (1990), pH scale: seawater scale (mol kg^{-1} – SW)) and are presented in Table 2. To reach these target values, a final concentration of 122.4 µmol kg^{-1}_{sw} of trace-metal clean 6 mol L^{-1} Seastar™ Baseline HCL solution and a final concentration of 115.1 µmol kg^{-1}_{sw} of a trace-metal clean solution of $NaHCO_3$ were added to each acidified treatment bag through the Luer lock port using a syringe. The piston was activated several times to ensure proper mixing.

2.3 Fe and dust addition

Two sources of Fe were used for the fertilization: $FeSO_4$ and standardized Asian dust CJ-2. Briefly, CJ-2 dust was collected from the Tengger desert surface soil, roughly sieved and blown through a wind tunnel designed to collect fine particles. Median diameter of CJ-2 dust is 24.1 µm. CJ-2 dust is characterized by a Fe content of 3.02 ± 0.12% and Fe solubility of 0.33% (Ooki et al., 2009). ~~(~~For more information on CJ-2 dust, see Nishikawa et al. (2000) and Hwang and Ro (2006)~~)~~. $FeSO_4$ was added at the final concentration of 0.6 nmol L^{-1} and CJ-2 dust was added at the final concentration of 2 mg L^{-1}. Twenty hours prior to their addition in the bags, dust and Fe solutions were prepared by adding CJ-2 dust samples or $FeSO_4$ to MilliQ water. Proper quantities of Fe-enriched solutions were added to each bag with a syringe through the Luer lock port. The piston was pushed and pulled several times to ensure proper mixing. Then, each bag was gently shaken for 5 minutes to homogenize its content prior to sampling.

2.4 Chemical and biological variables

2.4.1 Carbonate system

Dissolved inorganic carbon (DIC) and alkalinity were measured at T0 and T4. DIC was measured by a coulometric method using a Single operator multi-parameters metabolic analyzer (SOMMA) (Johnson et al., 1993) coupled to a UIC 5011 coulometer, according to standard protocols (Dickson et al., 2007). Samples were calibrated against Andrew Dickson (Scripps) CRM water batch 101. Alkalinity was measured using an open cell method consisting of a Brinkmann Dosimat 665 an Alpha PHE-4841 glass body combination electrode according to standard protocols outlined in the Guide to best practices for ocean CO_2 measurements (Dickson et al., 2007). Samples were calibrated against Andrew Dickson (Scripps) CRM water batch 101. Partial pressure of CO_2 (pCO_2) and pH were calculated using the MS Excel macro CO2SYS as described above.

2.4.2 Macronutrients and dissolved Fe

For macronutrients, at each subsampling time, ~10 mL of each bag was filtered in an in-line syringe filter to remove particles (Polycarbonate 0.8 Micron, 25 mm filters) and placed in polystyrene test tubes. The test tubes were immediately frozen at -20^{o}C in an aluminium freezer block. Concentrations of nitrate, silicic acid and phosphate were measured ashore using a Technicon AA autoanalyzer II following the methods described in Barwell-Clarke and Whitney (1996).

Dissolved Fe (DFe) samples were collected at each subsampling time by filtering ~125 mL (0.22 µm) into acid-cleaned low density polyethylene bottles under a clean laminar flow hood or in the trace-metal clean positive pressure plastic tent (the "bubble") constructed in the main lab as described in Johnson et al. (2005). DFe samples were placed in trace-metal clean, low-density polyethylene bottles and acidified to pH 1.7 for 20-30 h. Samples were then buffered to pH 3.2 using a formic acid-ammonium formate buffer. DFe was quantified according to GEOTRACES protocols available in the Methods Manual (Cutter et al., 2010). All DFe samples were analyzed on board in the "bubble" using a flow injection analysis (FIA) method where Fe is concentrated from the seawater matrix onto a chelating resin and detected by chemiluminescence as first

described by Obata et al. (1993) with modifications presented in Obata et al. (1997). Samples were pre-concentrated on a resin column of 8-hydroxyquinoline immobilized on silica gel. The eluent was then combined with ammonia, hydrogen peroxide and luminol. A Hamamatsu photomultiplier tube quantified the light emitted by the reaction of Fe and luminol as it passed through the detection cell. Fe concentration was determined using an external standard curve. Accuracy of the system was checked by regular measurements of the standard reference seawaters SAFe D1 and D2 (Johnson et al., 2007). Our average values of the SAFe D1 and two SAFe D2 standards were 0.63 ± 0.05 nmol L^{-1} Fe (n = 7), 0.91 ± 0.05 nmol L^{-1} Fe (n = 6) and 0.90 ± 0.03 nmol L^{-1} (n = 4), consistent with the community consensus value of 0.67 ± 0.04 nmol L^{-1} (D1) and 0.93 ± 0.02 nmol L^{-1} (D2).

2.4.3 Pigments and particulate organic carbon

For chl *a* determination, 305 mL of water was withdrawn from each bag at each subsampling time. The water was filtered through 25 mm GF/F filters at ≤9.33 kPa vacuum and filters were frozen at -20°C until analysis. Acetone (90 %) was added to extract the pigments 24 h prior to analysis. Pigments were quantified with a Turner 10 AU fluorometer as described in Strickland and Parsons (1972). To characterize the initial pigment composition by High Performance Liquid Chromatography (HPLC), two samples of ca. 2 L were taken directly from the pump when treatment bags were filled at T0. At T4, ca. 1 L was sampled from two incubation bags. Samples were filtered at ≤9.33 kPa vacuum on 47 mm GF/F filters and filters were frozen at -80˚C until analysis onshore. Pigments were extracted by placing the filters in 95 % methanol at -20˚C in the dark for 24 h prior to analysis. The extracts were filtered through 25 mm diameter polytetrafluoroethylene (PTFE) syringe filters (0.2 µm pore size) and analyzed using a Waters Alliance 2695 (HPLC) system equipped with a 2996 Photodiode Array Detector (PDA) and a reverse phase C8 column (Waters Symmetry), with a pyridine-containing mobile phase (Zapata et al., 2000). Analysis was performed on a 200 µl injection of sample mixed with water in the autosampler at a ratio of 5:1 immediately prior to injection. Pigment concentrations were quantified using commercially available standards (Danish Hydraulic Institute). The initial pigment ratio matrix loaded into the CHEMTAX program (Table 3) was obtained by averaging the minimum and maximum values of

pigment : Chl a ratios given in Table 1 of Mackey et al. (1996) and is similar to that used by Suzuki et al. (2002) and Royer et al. (2010) for samples collected in the subarctic North Pacific. Eight algal groups were quantified using the chemotaxonomy program CHEMTAX (Mackey et al., 1996): cyanobacteria, pelagophytes, haptophytes (including coccolithophores), diatoms, dinoflagellates, prasinophytes, cryptophytes and chlorophytes. For a description of the pigment types, see Zapata et al. (2004).~~Six algal groups were quantified using the chemotaxonomy program CHEMTAX (Mackey et al., 1996): cyanobacteria, pelagophytes, haptophytes (including coccolithophores), diatoms, dinoflagellates and chlorophytes. The initial pigment ratio matrix is presented in Table 3. For a description of the pigment types, see Zapata et al. (2004).~~

Particulate organic carbon (POC) concentrations were measured at T0 and at T4. At T0, three samples of 500 mL of seawater were pumped from the sampling station. At T4, 500 mL were sampled from each one of the incubation bags. Samples were filtered on pre-combusted 25 mm GF/F and the filters were placed in open cryovials and allowed to dry in an oven at 60^{o}C for 48 h. The cryovials were then capped and kept in drierite until onshore analysis. POC and particulate nitrogen were measured using a mass spectrometer (Delta Plus, Thermo Finnigan Mat) coupled with an elemental analyzer (CE Instrument model 1110).

2.4.4 C and Fe uptake rates

The siderophore desferrioxamine B (DFB) has been used as a model ligand for studying the bioavailability of strongly organically complexed Fe in seawater (e.g. Maldonado and Price 1999; Hutchins et al. 1999; Shi et al. 2010). Iron uptake from DFB occurs through a high affinity Fe transport system, and can reflect the Fe nutritional status of laboratory phytoplankton strains (Maldonado and Price 1999; Maldonado et al. 2006) and natural phytoplankton assemblages (Semeniuk et al. 2009; Taylor et al. 2013; Semeniuk et al. in press). Thus, we examined whether uptake rates of Fe complexed to DFB varied among our treatments with varying Fe bioavailability.

Uptake of 55Fe bound to DFB was performed as previously described (Maldonado and Price 1999; Semeniuk et al. 2009). The 0.5 nM 55Fe (Perkin Elmer) was complexed with 5% excess DFB in pH 3.5 Milli Q for 30 minutes (Maldonado and Price, 1999). The

resulting 55FeDFB complex was subsequently equilibrated in 0.22 µm filtered seawater for 2 h. Approximately 250 mL of seawater was subsampled from each incubation bag into acid-cleaned polycarbonate bottles. Just before dawn, the equilibrated 55FeDFB complex and 10 µCi of H14CO3- (Perkin Elmer) were added to each 250 mL bottle. From each assay bottle, 1 mL of sample was taken in order to determine the initial total added activities of 55Fe and 14C. To prevent inorganic 14C from off-gassing in the initial activity sample vial, 500 µL of 5 M NaOH was added.

After 24 h, the content of each bottle was gently filtered onto a 47 mm diameter, 1 µm porosity polycarbonate filter (AMD) under low vacuum pressure (≤70 mm Hg). Just before going dry, the filters were immersed in 5 mL of Titanium-ethylenediaminetetraacetic acid (Ti-EDTA) wash to remove extracellular Fe (Hudson and Morel, 1989). The filters were then rinsed with 10 mL of filtered seawater to remove any loosely associated tracer. Filters were placed into 7 mL borosilicate scintillation vials, immersed in 5 mL Scintisafe 50% scintillation cocktail, and conserved in the dark until analysis on a Beckman LS65005514 scintillation counter.

Volumetric Fe uptake and C-fixation rates were calculated as described elsewhere (Maldonado and Price 1999; Semeniuk et al. 2009). Previous work with phytoplankton assemblages along Line P has demonstrated that uptake of Fe from DFB by natural phytoplankton communities is linear over 24 h (Maldonado and Price 1999). Thus, Fe uptake rates were calculated assuming the accumulation of 55Fe by cells was linear during the assay. In order to compare Fe uptake rates among treatments, volumetric rates were normalized to the amount of C fixed during the assay. The 14C uptake rates normalised to chl a is used here as an indicator of the growth status of the autotrophic assemblage.~~Uptake of ^{55}Fe bound to DFB was performed as previously described (Semeniuk et al., 2009). The 0.5 nM ^{55}Fe (Perkin Elmer) was complexed with 5% excess DFB in pH 3.5 Milli Q for 30 minutes (Maldonado and Price, 1999). The resulting ^{55}FeDFB complex was subsequently equilibrated in 0.22 µm filtered seawater for 2 h. Approximately 250 mL of seawater was subsampled from each incubation bag into acid-cleaned polycarbonate bottles. Just before dawn, the equilibrated ^{55}FeDFB complex and 10 µCi of $H^{14}CO_3^-$ (Perkin Elmer) were added to each 250 mL bottle. From each assay~~

bottle, 1 mL of sample was taken in order to determine the initial total added activities of ^{55}Fe and ^{14}C. To prevent inorganic ^{14}C from off-gassing in the initial activity sample vial, 500 µL of 5 M NaOH was added. In order to account for diurnal fluctuations in Fe uptake and C fixation, the assay bottles were incubated for 24 h alongside the experimental bags.

After 24 h, the content of each bottle was gently filtered onto a 1 µm polycarbonate filter under low vacuum pressure (≤9.33 kPa). Just before going dry, the filters were immersed in 5 mL of Titanium ethylenediaminetetraacetic acid (Ti-EDTA) wash to remove extracellular Fe (Hudson and Morel, 1989). The filters were then rinsed with 10 mL of filtered seawater to remove any loosely associated tracer. Filters were placed into 7 mL borosilicate scintillation vials, immersed in 5 mL Scintisafe 50% scintillation cocktail, and stored in the dark until analysis on a Beckman LS65005514 scintillation counter. Volumetric Fe uptake and C fixation rates were calculated as described elsewhere (Semeniuk et al., 2009). In order to compare Fe uptake rates among treatments, volumetric rates were normalized the amount of C fixed during the assay. The ^{14}C uptake rates normalized to chl *a* is used here as an indicator of the growth status of the autotrophic assemblage.

2.4.5 DMS and DMSP concentration [MGS1]

DMS and DMSP concentrations were measured following the techniques described in Royer et al. (2010). Briefly, for DMS, water samples were withdrawn from the bags at every subsampling time in 50-mL serum bottles and analyzed on board using a purge and trap system coupled to a gas chromatograph following methods described in Scarratt et al. (2000). Total DMSP ($DMSP_t$) was measured in an unfiltered water sample of 3.5 mL. The samples were acidified with 50 µL of 50% H_2SO_4 and conserved at 4ºC in the dark until analysis.

2.5 Statistical Analysis

All statistical analyses were run on Statistical Analysis System (SAS) software. The threshold value for determining statistical significance was $p < 0.05$. Repeated-measures ANOVA were used to test the difference between treatments and the changes in time for the means of biological and chemical variables. Normality of the data was determined

using the Shapiro-Wilk test. ANOVA on ranks was used when normality of the data could not be assumed. Differences between the mean concentrations of phytoplankton groups, as measured by HPLC, were assessed using one factor ANOVA. Two-way ANOVAs were used to isolate the effect of one factor (acid, Fe addition).

3 Results

3.1 State of the carbonate system, macronutrients and Fe concentrations

Table 2 presents the average and standard deviation of the four parameters of the carbonate system measured (DIC and alkalinity) and calculated (pCO_2 and pH) for each treatment at T0 and T4. Target values of pH and pCO_2 in the acidified treatments were reached with averages of 7.80 ± 0.01 and 740 ± 23 ~~ppm~~µatm CO_2 respectively, at T0. DIC values in acidified treatments reached an average of 2139 ± 4 µmol kg^{-1}_{sw} , a value 1.5% higher than the target value but consistent among acidified treatments. Alkalinity values in the acidified treatment were 2243 ± 7 µmol kg^{-1}_{sw}, a value 2.5% higher than the in situ (and target) value of 2187 µmol kg^{-1}_{sw}. DIC, alkalinity and pH values all remained stable during the 4-d incubations. From T0 to T4, pCO_2 values varied by 3-12% in the acidified treatments and by 2-15% in the non-acidified treatments, but values of the acidified versus non-acidified treatments remained different from each other.

Initial concentrations of nitrate, silicate and phosphate were 8.0 ± 0.2 µmol L^{-1}, 14.2 ± 0.2 µmol L^{-1} and 0.88 ± 0.02 µmol L^{-1}. Nutrient concentrations remained high during the course of the experiments, with nitrate, silicate and phosphate decreasing by less than 6%, 4% and 13%, respectively (data not shown).

DFe, operationally defined as the fraction that passes through a 0.22 μm filter, includes soluble and colloidal Fe (de Baar et al., 2005). DFe concentrations started at 0.41 ± 0.09 nmol L^{-1} and 0.21 ± 0.02 nmol L^{-1} in the Control and Control+Acid treatments, respectively, and decreased to 0.07 ± 0.01 nmol L^{-1} and 0.04 ± 0.01 nmol L^{-1} over the time course of the experiment (Fig. 1a). In the Fe and Fe+Acid treatments, DFe started at 0.65 ± 0.32 nmol L^{-1} and 0.47 ± 0.23 nmol L^{-1}, respectively. The DFe concentration decreased to ca. 0.11 nmol L^{-1} on day 2 and to ca. 0.06 nmol L^{-1} on day 4 in both treatments (Fig. 1b). In the Dust treatment, DFe started at 0.28 ± 0.10 nmol L^{-1}, decreased

to 0.12 ± 0.01 nmol L^{-1} at T2 and remained at this level at T4. In the Dust+Acid treatment, DFe started at 0.18 ± 0.05 nmol L^{-1}, increased to 0.28 ± 0.01 nmol L^{-1} at T2 and decreased slightly to 0.21 ± 0.07 at T4 (Fig. 1c).

3.2 Plankton biomass

Average initial chl *a* concentration in all treatments was 0.39 ± 0.03 µg L^{-1} (Fig. 2). In the Control and Control+Acid treatments, chl *a* concentration remained stable for the length of the experiment (Fig. 2a). In the Fe treatment, chl *a* concentrations reached 0.~~8~~76 ± 0.~~2~~16 µg L^{-1} after 4 days, a value significantly higher than measured in the control at the same time (p-value = 0. 0269; Fig. 2b). In the Fe+Acid treatment, chl *a* concentrations increased to 0.~~6~~58 ± 0.~~2~~15 µg L^{-1} after 4 days, a value not significantly different from the one reached at the end of the Fe treatment. The addition of dust also had a significant stimulating effect on phytoplankton growth compared to the Control (p-value: 0.0071) with chl *a* reaching 0.~~9~~88 ± 0.23 µg L^{-1} at T4 (Fig. 2c). The chl *a* concentration reached at T4 in the Dust treatment was not statistically different than the one reached in the Fe treatment. In the Dust+Acid treatment, chl *a* concentration reached 0.74 ± 0.01 µg L^{-1} at T4, a value again not significantly different from the concentrations reached at the end of the Dust treatment (Fig. 2c). Although the difference is not significant, a trend appears in chl *a* concentrations: chl *a* concentrations are always slightly lower in the acidified vs non-acidified treatment. In order to detect any interactive effect of Fe or acidification on chl *a* and to further explore these apparent trends in chl *a*, all treatments were grouped and tested with a two-factor ANOVA. The first factor, enrichment, had three possible states (Fe, Dust, nil) and the second factor, acid, had two possible states (+ acid, control). This analysis showed a significant effect of the Fe enrichment (p-value = 0.0060) and a significant effect of the acidification (p-value = 0.0385) on chl *a* concentration. However, no combined effect (synergic or antagonistic) was detected with the two factors.

Initial POC concentration was 75.3 ± 11.2 µg L^{-1} (not shown) and increased in all treatments including the Control. After 4 days, POC concentrations were similar in the Control (125.5 ± 0.3 µg L^{-1}) and Control+Acid (122.5 ± 17.6~~8~~ µg L^{-1}) treatments (Fig. 2a). The average POC concentration at T4 in the Fe treatment (169.2 ± 55.8~~6~~ µg L^{-1}) was not statistically different than in the Control. Final POC concentration in the Fe+Acid

treatment (~~219~~ 189.3 ± 29.2 µg L^{-1}) was not significantly different than in the Fe treatment, but significantly higher than in the Control (Fig. 2b). The highest POC concentration~~s were~~ was measured in the Dust treatment (286.7~~7~~ ± 39.7~~30~~ µg L^{-1}) and lowering the pH resulted in a 24% decrease in POC concentration at T4 (Dust+Acid treatment: 217.4 ± 2.0 µg L^{-1}) (Fig. 2c).

3.3 Taxonomy

The initial phytoplankton biomass (T0) was dominated by chlorophytes (37% of total chl *a*), followed by haptophytes (31%), pelagophytes (19%) and dinoflagellates (13%) (from Fig. 3). Prasinophyte and cryptophyte biomarkers were not detectable during our experiments. Dinoflagellates were present in low concentrations at T0 and became undetectable at T4 in the Control and in all treatments (Fig. 3b). In contrast with the dinoflagellates, diatoms and cyanobacteria were below the detection limit at T0 and became detectable at T4 in the Control and in all treatments (Fig. 3a, f). These changes in community composition in the control show that the sampling and/or incubation conditions had a negative effect on the growth of dinoflagellates and a positive effect on the growth of diatoms and cyanobacteria. Figure 3 shows that diatoms were responsible for most of the increases in chl *a* measured in the Fe, Fe+Acid, Dust and Dust+Acid treatments compared to the control. The only treatment to show a statistically significant difference with the Control is the Dust treatment, which had significantly higher concentrations of chl *a* attributable to diatoms and cyanobacteria than the Control at T4. No statistical difference between any treatment and its acidified counterpart could be detected.

3.4 Carbon and Fe uptake rates at T4

At T4, C assimilation rate was 92 ± 50 nmol C L^{-1} h^{-1} in the Control (Fig. 4a). In the Control+Acid treatment, C assimilation was 195 ± 21 nmol C L^{-1} h^{-1}, a value significantly higher than in the Control. C assimilation rates in the Fe and Fe+Acid were significantly higher than the Control, but not different from each other with values of 189 ± 23 nmol C L^{-1} h^{-1} and 243 ± 66 nmol C L^{-1} h^{-1}, respectively. C assimilation rate in the Dust treatment was similar to the Control with 59 ± 24 nmol C L^{-1} h^{-1}. Lowering the pH

significantly increased C assimilation rate in the Dust+Acid treatment (145 ± 61 nmol C L^{-1} h^{-1}) compared to the Dust treatment.

The chl *a*-specific C assimilation rate (size fraction > 1 µm) in the Control at T4 was 0.23 ± 0.01 µmol C µg chl a^{-1} h^{-1} (Fig. 4b). Lowering the pH increased significantly the chl *a*-specific C assimilation rate to 1.03 ± 0.13 µmol C µg chl a^{-1} h^{-1} in the Control+Acid treatment. A similar albeit less pronounced pH-induced increase in chl *a*-specific C assimilation was observed between the Fe (0.25 ± 0.03 µmol C µg chl a^{-1} h^{-1}) and the Fe+Acid treatments (0.43 ± 0.15 µmol C µg chl a^{-1} h^{-1}). The Dust and Dust+Acid treatments had chl *a*-specific C assimilation rates of 0.07 ± 0.04 and 0.17 ± 0.10 µmol C µg chl a^{-1} h^{-1}, respectively. It is noteworthy that although comparable biomasses were achieved after 4 days in the $FeSO_4$ or Dust treatments, the chl *a*-specific C assimilation rate was significantly lower in the Dust treatment as compared to the Fe treatment and in the Dust+Acid compared to the Fe+Acid treatment. ~~. These results suggest a faster return to Fe-deficiency with dust addition than with $FeSO_4$.~~.

~~Phytoplankton can acquire Fe(III) from within strong organic complexes via cell surface reductases associated with a high affinity Fe transport system (Kustka et al., 2007; Maldonado and Price, 2001; Shaked et al., 2005). The reduction of Fe(III) increases during Fe-limitation (Maldonado and Price, 2001; Shaked et al., 2005), and thus uptake of Fe from DFB may reflect the degree of Fe-limitation experienced by a phytoplankton community (Taylor et al., 2013). Thus, the higher the FeDFB uptake rate normalized to C assimilation rate, the more Fe-limited the phytoplankton community is likely to be.~~ At T4, FeDFB uptake rates normalized to C assimilation were 0.94 ± 0.55 µmol Fe mol C^{-1} in the Control, 0.34 ± 0.07 in the Control+Acid treatment, 0.33 ± 0.16 in the Fe treatment and 0.39 ± 0.20 in the Fe+Acid treatment (Fig. 4c). In the dust treatment, FeDFB uptake rate normalized to C assimilation was high (1.44 ± 0.66 µmol Fe mol C^{-1} h^{-1}), also suggesting a rapid return to Fe-limiting conditions in this treatment. Lowering the pH decreased the FeDFB uptake rates normalized to C in the Dust+Acid treatment (0.55 ± 0.39 µmol Fe mol C^{-1}), but had no effect on the Fe+Acid treatment (0.39 ± 0.20 µmol Fe mol C^{-1}).

3.5 $DMSP_t$ and DMS

Initial average $DMSP_t$ concentration was 39.8 ± 3.6 nmol L^{-1} and decreased between 10 and 21 nmol L^{-1} at T4 in all treatments (Fig. 5a, b, c). Lowering the pH resulted in no significant change in $DMSP_t$ concentrations in the control and in the Fe and Dust treatments. Initial average DMS concentration in all treatments was 10.0 ± 1.1 nmol L^{-1} and decreased in all treatments to reach concentrations varying between 3.4 – 6.6 nmol L^{-1} at T4 (Fig. 5d, e, f). Neither the addition of Fe/dust nor the decrease in pH had an effect on DMS concentrations

4 Discussion

4.1 Considerations on the experimental protocol

The experimental approach used in this study has limitations, some of which deserve to be addressed forefront in order to avoid misinterpretations of the results. First, the sampling and incubation procedures negatively affected the growth of dinoflagellates. For this reason, no conclusion could be drawn on the effect of the treatments on dinoflagellates. For all other taxa, the influence of the treatments could only be addressed by comparing the samples collected at T4. Second, the abrupt acidification rate imposed to the plankton assemblage during our study is not representative of the slow process that is currently taking place in the ocean. Hence, acclimation and adaptation to acidification which will most probably take place in the natural system cannot take place during our transient and short experiment. Transient experiments, in the manner conducted here, are nevertheless useful to characterize the direct impact of OA on Fe bioavailability and to observe short-term resistance/sensitivity of organisms to OA. It is likely that organisms capable of withstanding rapid decreases in pH will also display tolerance to a more gradual decrease in pH.

4.2 Initial in situ conditions and impact of acidification

Oceanic conditions encountered during the experiments were typical of this part of the northeast Pacific and time of year. Macronutrients and chl *a* concentrations were high and low, respectively, indicative of the HNLC conditions characterizing the Gulf of Alaska in

summer (Harrison et al., 1999; Hopkinson et al., 2010). DMS concentrations were high, but usual for this region in the summer (Wong et al., 2005). The DFe concentration of 0.4 nmol L^{-1} measured in the Control at T0 was higher than expected, but the Fe-limited status of the plankton community was confirmed by the absence of chl *a* accumulation in the Control and the increase in chl *a* induced by the addition of $FeSO_4$ (Fig. 2a, b). Also as expected for this time of the year, the greatest contributors to total chl *a* included chlorophytes, haptophytes and pelagophytes (Fig. 3), while diatoms represented minor contributors. Hence, the combined influences of dust and pH on phytoplankton growth, taxonomy, and DMS production reported in this study can be extrapolated to the northeast subarctic Pacific summer conditions. However, for the reason mentioned above, our protocol does not allow us to draw conclusions on how dinoflagellates, which represented ca. 13 % of the autotrophic biomass in situ, respond to OA.

The abrupt decrease in pH (by 0.2 units) and increase in pCO_2 (by 335 µatm~~ppm~~) had no detectable effect on the Fe-limited phytoplankton biomass and community structure~~.~~ (Figs. 2a and 3c). During a comparable experiment conducted in the same region and under similar oceanographic conditions (HNLC waters, phytoplankton dominated by haptophytes and chlorophytes), Hopkinson et al. (2010) showed that increasing CO_2 to 760-1204 ~~ppm~~µatm had little effect on chl *a*, nutrient drawdown, or phytoplankton growth rates after 5 days in Fe-limited conditions. In their experiment, they did not observe a decrease in the biomass of haptophytes as during our study, but the absence of present-day CO_2 control (observations restricted to low and high CO_2 treatments) limits the comparison between the two studies. Another similar experimental study conducted in the northwest subarctic Pacific revealed a small decrease in haptophyte relative biomass at high CO_2 levels (1000 ~~ppm~~µatm) compared to low CO_2 level (180 ~~ppm~~µatm) after 6 days and an increase in diatoms biomass in all treatments but no pCO_2-related statistical change in the abundance of diatoms and total chl *a* after 5 days (Endo et al., 2013). Another experiment conducted with water from the diatom-dominated Fe-limited Bering Sea has shown a negative effect of elevated CO2 on diatoms (Sugie et al., 2013, Endo et al., 2015), which was not observed in the Fe-enriched treatments. Thus, albeit the differences mentioned above, results from these three experiments suggest that pH

expected toward the end of this century will only have a small negative impact, if any, on total autotrophic biomass in the HNLC waters of the subarctic North Pacific.

Acidification resulted in the up-regulation of C assimilation in the control (Fig. 4a,b). Such pH-induced increases in C assimilation have previously been reported in pH manipulation experiments (Riebesell et al., 2007; Tortell et al., 2008). During their study in the same oceanic region, Hopkinson et al. (2010) reported an increase in photosynthetic efficiency in their low pH treatment, an increase they attributed to energy savings from down-regulation of the CCMs. Increasing CO_2 concentrations (lowering pH) could have resulted in a down-regulation of this costly mechanism, freeing energy for other metabolic pathways such as C assimilation.

Unexpectedly, the low pH-induced up-regulation of C assimilation measured at day 4 did not directly translate into an increase in POC in the non-Fe-enriched waters. In point of fact, acidifying non-Fe-enriched waters (Control+Acid treatment) had no effect on chl *a* or POC concentrations but resulted in a 2-fold and 4-fold increase in absolute and chl *a*-normalized C assimilation rates, respectively, after 4 days (Fig. 4). The absence of a higher POC concentration in the Control+Acid treatment during our study suggests that the newly assimilated C was not converted into biomass or that a loss mechanism would impede the buildup of POC. Such mechanisms could include increased ~~respiration,~~ grazing by micrograzers or~~,~~ DOC exudation and subsequent transparent exopolymer particle (TEP) formation. An increase in C uptake without biomass accumulation under acidified conditions has previously been observed during a similar 12-day experiment (Riebesell et al., 2007). This unexpected result was attributed to the release of DOC and subsequent formation of TEPs which are known to accelerate particle aggregation and sinking. Similar significant stimulation of DOC production at elevated CO_2 was reported by Engel et al. (2014) in coastal waters but only a weak and inconsistent CO_2 induced decrease in DOC production was observed by Yoshimura et al. (2014).In the absence of sinking as in our experiment, the aggregation of DOC into TEP may only explain the absence of increase in POC if a large proportion of the TEP produced adsorbed on the walls of the incubation bags. Even though our measurements do not allow identifying the fate of the increased assimilated C in the high pCO_2 treatment, they point toward a

perturbation of the C cycling, either by an increase in DOC exudation~~,~~ or grazing ~~or respiration rate~~. If confirmed, such pH-induced modification of C cycling and pools in HNLC waters could have important impacts on microbial dynamics and C export.

Overall, our results show that OA in the HNLC waters of the northeast subarctic Pacific may initially negatively impact the growth of haptophytes but stimulate phytoplankton C assimilation by the Fe-limited cells. In spite of these effects at the cellular and taxonomic levels, lowering the pH had little effect on the net accumulation of biomass (chl *a* and POC) after 4 days.

4.3 Dust fertilization in a high CO_2 northeast subarctic Pacific

During the 4 days of the experiment, dust fertilization had the same stimulating effect on net chl *a* production as the addition of $FeSO_4$. This similarity confirms that the phytoplankton assemblage was Fe limited when the study was conducted, and that Fe was responsible for the stimulating effect of dust on phytoplankton growth (Fig. 2). However, the average final POC concentration in the Dust treatment was 69% higher than in the $FeSO_4$ treatment, suggesting a more efficient Fe stimulation of phytoplankton C assimilation in the former. These results suggest that 2 mg L^{-1} of CJ-2 dust releases at least as much bioavailable Fe during the first 4 days of the experiment as the addition of 0.6 nmol L^{-1} $FeSO_4$. We can only speculate as to why Fe contained in dust stimulated more growth than FeSO4. Part of the answer may be related to the speed at which the Fe is released from dust, a relatively low release rate allowing a more efficient utilization of Fe. In comparison, and as can be seen in Fig. 1, $FeSO_4$ is rapidly utilized and probably scavenged by particles and ligands following fertilization. Diatoms, cyanobacteria and, to a lesser extent pelagophytes, benefited the most from the dust enrichment, reaching higher group-specific chl *a* concentrations than the Control incubation at T4. Except dinoflagellates, which did not thrive in the Control, all other groups maintained their biomass in the Dust treatment. This response to Fe addition is comparable to the one reported for previous small and large-scale Fe fertilization experiments conducted in the Gulf of Alaska showing an initial increase in the abundance of major taxa and a dominance of diatoms (Boyd et al., 1996; Levasseur et al., 2006; Marchetti et al., 2006; Martin and Fitzwater, 1988; Mélançon et al., 2014). We saw no clear difference in the

structure of the phytoplankton assemblage whether dust or $FeSO_4$ was used as fertilizer. In that regard, $FeSO_4$ seems to be a good substitute for dust in studies of the early response (< 4 days) of plankton communities to dust deposition in the northeast subarctic Pacific.

DFe measurements were poor indicators of Fe bioavailability following dust deposition in our study. In contrast with the $FeSO_4$ treatment where almost all the added Fe was still present and measured in the dissolvable pool at T0 (ca. 20 min. following the addition), DFe concentrations remained low and near *in-situ* levels during the entire length of the dust-addition experiments (Fig. 1b, c). Considering that the addition of Dust did stimulate algal growth, the low and constant concentration of DFe in the Dust treatment suggests that the release of bioavailable Fe from dust was matched by bacterial and phytoplankton Fe acquisition. Re-adsorption of the released Fe by the dust particles may also be responsible for the low levels of DFe measured during the experiment (Ye et al., 2011). These results also show that DFe may not be a good indicator of dust deposition events in the oceanic environment where the release of DFe from the low concentrations of dust deposited is unlikely to exceed bio-uptake and re-adsorption on particles. Similar conclusions were reached in a previous study (Mélançon et al., 2014), where total dissolvable Fe (TDFe)[MGS2] was also measured and shown to be a better indicator of Fe bioavailability than DFe.

Our results suggest that after a period of active growth, phytoplankton in the Dust treatment became Fe-limited 4 days into the experiment. This conclusion is supported by the very low absolute and chl *a* normalized C fixation rates in the Dust treatment as compared to the Control and $FeSO_4$ treatments at T4 (Fig. 4a), as well as by the FeDFB uptake rates normalized to C uptake rates which were higher in the Dust treatment than in the Control (Fig. 4b). Dust particles are known to efficiently adsorb Fe (Ye et al., 2011). The rapid return to Fe deficiency in the Dust treatment may thus result from a combination of increased Fe demand and re-adsorption of Fe onto dust particles. This explanation implies however that the re-adsorbed Fe becomes less prone to desorption, which needs to be demonstrated. These results suggest that the influence of the Fe released from dust lasted less than 4 days during our experiments. In natural

environments, this period of influence may be even shorter due to fast sinking of larger dust particles. Based on Stoke's Law and assuming our dust particles were spherical, CJ-2 dust particles may sink at an average speed of 32 m/day, which would take particles out of a 60 m-deep euphotic zone in ~2 days. Due to the combined effect of both Fe re-adsorption on particles and fast sinking, the impact of natural dust deposition may thus be of relatively short duration in the environment, similar to the time-frame of our *in vitro* study.

Decreasing the pH resulted in a slightly lower biomass (chl *a* by 16% and POC by 15%) in the Dust+Acid treatment than in the Dust treatment (Fig. 2c). The decrease in biomass corresponded to decreasing trends in the contribution of haptophytes and to a lesser extent, of pelagophytes and cyanobacteria to total chl *a* concentrations. As discussed above, a likely explanation for the lower biomass reached after 4 days is the negative effect of acidification on the growth of the coccolithophores (Engel et al., 2005; Harvey et al., 2013; Kroeker et al., 2010). C assimilation rates (absolute and chl *a*-normalized) were ca. 2-fold higher in the Dust+Acid treatment than in the Dust treatment at T4, probably reflecting the stimulating effect of higher CO_2 concentrations on diatom C assimilation. These results reinforce the aforementioned hypothesis that the up-regulation of C assimilation was paired with an increased particulate C loss via enhanced C exudation from the cells~~,~~ or increased grazing~~ or respiration~~.

It is obvious from Figure 2 that the natural variability among the bags of a same treatment as well as the short length of the incubations limited our capacity to statistically distinguish trends resulting from the two treatments (i.e. Fe/dust addition and acidification). The two-factor ANOVA allowed us to increase the statistical power and detect a negative effect of acidification on chl *a* concentration after 4 days. ~~In order to further explore these apparent trends in chl *a*, all treatments were grouped and tested with a two-factor ANOVA. The first factor, enrichment, had three possible states (Fe, Dust, nil) and the second factor, acid, had two possible states (+ acid, control). This analysis showed a significant effect of the Fe enrichment (p-value = 0.0060) and a significant effect of the acidification (p-value = 0.0385) on chl *a* concentration. However, no combined effect (synergic or antagonistic) was detected with the two factors.~~ This

suggests that OA will not increase Fe bioavailability to natural HNLC phytoplankton communities. However, it does not preclude the possibility that acidification may have exacerbated Fe limitation in our experiment. If the effect of acidification on the growth of calcifying haptophytes was expected, it is not expected in the case of diatoms, pelagophytes and cyanobacteria. Since these non-calcifying organisms also presented a lower biomass in acidified treatments, it is likely that Fe bioavailability has been reduced via interactions between pH, ligands and Fe speciation.

4.4 Impact of acidification and dust deposition on $DMSP_t$ and DMS

The general decrease in $DMSP_t$ and DMS concentrations measured in all treatments likely reflects the loss of DMSP-rich dinoflagellates due to sampling and/or bottle effect and their replacement by diatoms with low DMSP quotas. In spite of the increase in chl *a* and POC measured in the $FeSO_4$ and Dust treatments compared to the Control, the alleviation of Fe limitation had no impact on the concentrations of $DMSP_t$ or DMS. This can be explained by the ~~similar abundance~~lack of significant difference in the abundance of DMSP-rich haptophytes in the Control, $FeSO_4$ and Dust treatments at T4. Indeed, the addition of $FeSO_4$ or dust mostly stimulated the growth of DMSP-poor diatoms, which would have little effect on $DMSP_t$ concentrations.

Our results show no statistical differences between DMSPt concentrations between the acidified and non-acidified treatments after 4 days. Although the short duration of our incubations may explain this lack of response, other studies conducted over longer period of time have reported similar results. For example, lowering the pH had no effect on $DMSP_t$ concentrations during the nutrient-stimulated bloom of a community from a Norway fjord (Vogt et al., 2008). In their 24-day experiment the absence of effect was attributed to the resistance of the planktonic community considering that similar increases in chl *a* concentration and species succession were observed in all CO_2 treatments. Studies reporting pH-induced changes in $DMSP_t$ concentrations are usually associated with alterations of the structure of the phytoplankton assemblage after several days. For example, a low pH-induced increase in dinoflagellates after 13 days resulted in higher $DMSP_t$ concentrations during a mesocosm study with Arctic water (Archer et al., 2013).

Lowering the pH had no measurable effect on DMS concentrations, a result probably also related to the short duration of the experiment. Previous studies showed either an increase or decrease in DMS concentrations with acidification, but these differences become measurable late in the experiment at or after the peak of the blooms. For example, during the experiment PeECE III in Raunefjorden, Norway, Wingenter et al. (2007) observed an increase in time-integrated average amount of DMS at high-CO_2, but the pH-related differences could only be observed after 6 days. In this case, the authors attributed the pH effect to a difference in viral attack and phytoplankton lysis at the chl *a* peak, a situation that is not likely to have occurred in our short incubations. Contrastingly, a decrease in DMS concentrations with high CO_2 was observed during other mesocosm studies conducted in the same fjord (Hopkins et al., 2010; Avgoustidi et al. 2012). These authors suggested that the dominance of flagellates and picoeucaryotes during their study as compared to coccolithophores during PeECE III could explain the divergent responses observed in regards of DMS production.

Archer et al. (2013) also measured a decrease in DMS concentration under high CO_2 conditions in Arctic waters, a decrease they attributed to an increase in bacterial production and decrease in DMS yield. It should be noted that none of the experiments described above reports an effect of CO_2 on DMS in the first 4 days. The sole study so far showing a rapid effect of a decrease pH is by Hopkins and Archer (2014) who measured a decrease in DMSP and an increase in DMS after 4 days during shipboard bioassays experiments with NW European waters. In that case, the changes in DMSP and DMS were associated with a rapid decline in the abundance of small cells in the acidified treatments and a possible cellular release and cleavage of DMSP to DMS. Their results show nonetheless a regional variability of the responses. The lack of response of DMS concentration to pCO_2 in our incubations might reflect this natural variability and a particular resistance of our initial community to acidification.

5 Conclusion

This study demonstrates that the degree of OA expected to occur by the end of the century is likely to have a detectable but minor impact on the short-term response of Fe-

limited planktonic communities to sporadic atmospheric Fe-dust depositions. The addition of $FeSO_4$ and Asian dust stimulated the growth of all major phytoplankton groups during our 96-h incubations, thereby confirming the Fe-depleted status of the community and the potential of natural dust deposition for stimulating phytoplankton growth in this HNLC region. In the acidified treatments, Fe in the form of $FeSO_4$ or Asian dust still had a fertilizing effect on the algal assemblage, but to a lesser extent than in the non-acidified treatment. The trends reported here suggest that OA could moderate the growth response of pelagophytes and haptophytes to dust deposition. The fact that non-calcifying taxa were also affected by acidification suggests that the lower pH possibly interferes with ligands, Fe speciation and transporters to reduce Fe bioavailability in these HNLC waters. Finally, our results suggest a low sensitivity of the DMS dynamics to acidification, both under Fe-limited and Fe-replete conditions, in the northeast subarctic Pacific. In order to understand the mechanisms behind this apparent resistance, studies on DMSP and DMS phytoplankton and bacterioplankton metabolisms during longer incubation periods are advisable.

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Table 1: Description of Fe/dust additions and acidification status of ~~experimental conditions for~~ each treatment. All treatments were conducted in triplicate.

Treatment	Addition of Fe	Acidification
Control	No	No
Control+Acid	No	Yes
Fe	$FeSO_4$ (+ 0.6 nmol L^{-1})	No
Fe+Acid	$FeSO_4$ (+ 0.6 nmol L^{-1})	Yes
Dust	CJ2 dust (+ 2.0 mg L^{-1})	No
Dust+Acid	CJ2 dust (+ 2.0 mg L^{-1})	Yes

Table 2: Values of DIC, alkalinity, pH and pCO_2 in each treatment at T0 and T4.

		DIC (µmol kg^{-1}_{sw})		Alkalinity (µmol kg^{-1}_{sw})		pH		pCO_2 (~~ppm~~µatm)	
Target values (acidified)		2107		2187 (*in situ*)		7.78		750	
		T0	T4	T0	T4	T0	T4	T0	T4
Control	Avg	1998	1998	2184	2180	8.01	7.99	410	439
	SD	1	2	8	16	0.02	0.04	16	41
Control+Acid	Avg	2141	2147	2244	2248	7.79	7.77	745	799
	SD	5	8	3	7	0.01	0.03	17	67
Fe	Avg	1992	1989	2186	2187	8.03	8.02	390	400
	SD	2	3	2	5	0.01	0.01	8	14
Fe+Acid	Avg	2137	2134	2240	2233	7.80	7.77	743	802
	SD	5	5	11	10	0.02	0.02	29	30
Dust	Avg	1991	1991	2190	2187	8.04	8.02	381	411
	SD	2	5	5	3	0.02	0.02	16	19
Dust+Acid	Avg	2138	2135	2245	2248	7.80	7.80	731	734
	SD	3	1	9	4	0.02	0.01	29	21

Table 3: Biomarker pigment initial ratio matrix for CHEMTAX: Chl *a* ratios for eight algal groups. ~~Initial ratio matrix~~

Class / Pigment	**Chl c_3**	**Chl c_2**	**Peri**	**19'-but**	**Fucox**	**Prasinox**	**Violax**	**19'-hex**	**Diadinox**	**Allox**	**Zeax**	**Chl_*b***	**Chl_*a***
Prasinophytes	0	0	0	0	0	0.360	0.114	0	0	0	0.142	0.888	1
Cryptophytes	0	0.126	0	0	0	0	0	0	0	0.136	0	0	1
Diatoms	0	0	0	0	0.457	0	0	0	0.239	0	0	0	1
Dinoflagellates	0	0.285	0.532	0	0	0	0	0.192	0.121	0	0	0	1
Haptophytes	0.238	0	0	0.261	0.583	0	0	0.680	0.196	0	0	0	1
Pelagophytes	0.125	0.127	0	0.933	0.625	0	0	0	0.438	0	0	0	1
Chlorophytes	0	0	0	0	0	0	0.028	0	0	0	0.059	0.285	1
Cyanobacteria	0	0	0	0	0	0	0	0	0	0	0.334	0	1

Abbreviations: Chl c_3, chlorophyll c_3; Chl c_2, chlorophyll c_2; Peri, peridinin; 19'-but, 19'-butanoyloxyfucoxanthin; Fucox, fucoxanthin; Prasinox, prasinoxanthin; Violax, violaxanthin;19'-hex, 19'-hexanoyloxyfucoxanthin; Diadinox, diadinoxanthin; Allox, alloxanthin; Zeax, zeaxanthin; Chl *b*, chlorophyll *b*; Chl *a*, chlorophyll *a*.

Figure 1: Average concentration of DFe in each treatment during the incubations measured at T0, T2 and T4. (a) Control and Control+Acid. (b) Fe and Fe+Acid. (c) Dust and Dust+Acid. Error bars indicate standard deviations. n = 3 except for Acid, T0, T2, Dust+Acid, T0, and Control (all times) where n = 2.

Figure 2: Average concentration of chl *a* (left axis) during the incubations and POC at T4 (right axis) in each treatment. (a) Control and Control+Acid. (b) Fe and Fe+Acid. (c) Dust and Dust+Acid. Error bars indicate standard deviations. Dashed line indicates POC concentration at T0. Chl *a:* n = 3 except for Acid, T0, Dust+Acid, T4, Fe+Acid, T0 and Control (all times) where n=2 because of missing/unreliable data or contamination (Control 1). POC: n=3 except Control where n=2.

Figure 3: Average chl *a* concentration (µg L^{-1}) attributable to each of the measured groups of phytoplankton initially (T0: white bar) and for each of the treatments after 4 d (T4) of incubation (Control, Control+Acid, Fe, Fe+Acid, Dust, Dust+Acid; gray bars). (a) Diatoms. (b) Dinoflagellates. (c) Haptophytes. (d) Pelagophytes. (e) Chlorophytes. (f) Cyanobacteria. Error bars indicate standard deviations. n = 2 except for T0 (all groups but dinoflagellates) where n = 3 and Acid, diatoms, Control, haptophytes and pélagophytes, Dust+Acid, cyanobacteria and Fe+Acid, haptophytes and cyanobacteria where n = 1.

Figure 4: Average (a) Absolute C assimilation rates. (b) C assimilation rates normalized to chl a concentration at T4 and (c) Fe uptake rates normalized to chl *a* concentration at T4. Error bars indicate standard deviations. Absolute C assimilation rates: n = 3 except Control and Acid where n = 2, C assim norm to chl *a*: n = 3 except Control, Acid and Dust+Acid where n=2, Fe uptake rates normalized to chl *a*: n = 3 except Control and Dust+Acid where n = 2.

Figure 5: $DMSP_t$ (a, b and c) and DMS (d, e and f) concentrations (nmol L^{-1}) in the Control and Control+Acid treatments (a and d), the Fe and Fe+Acid treatments (b and e), and the Dust and Dust+Acid treatments (c and f). Error bars indicate standard deviations.. n = 3 except Control where n = 2.