

1 Point by point reply

2 Page 7, line 5. Change “HCL” to “HCl”.

3 Reply : done

4 Page 7, lines 12-13. This sentence remains somewhat unintelligible to readers. It would be better
5 to give a definition of iron solubility (It is usually given as the ratio of dissolved iron to total
6 iron).

7 Reply : we split the sentence in two parts and modified the part about solubility.

8 New sentence : Ooki et al. (2009) measured a 0.33% Fe solubility, defined as the ratio of
9 dissolved iron to total iron, for CJ-2 dust.

10 Page 9, Line 23 (and elsewhere). This manuscript uses both “chl a” and “Chl a”. Please unify
11 them.

12 Reply : unified as "chl *a*"

13 Page 26, References. Please add the newly cited articles to the reference section.

14 Reply : the referee is right, some new references were forgotten. I added them, and I also
15 changed the position of some references which I noticed were not in proper alphabetical order.

16 Reference added :

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

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

21 Sugie, K., Endo, H., Suzuki, K., Nishioka, J., Kiyosawa, H., and Yoshimura, T.: Synergistic
22 effects of pCO₂ and iron availability on nutrient consumption ratio of the Bering Sea
23 phytoplankton community, *Biogeosciences*, 10, 6309–6321, 2013.

24 Yoshimura, T., Sugie, K., Endo, H., Suzuki, K., Nishioka, J., and Ono, T.: Organic matter
25 production response to CO₂ increase in open subarctic plankton communities: Comparison of six
26 microcosm experiments under iron-limited and -enriched bloom conditions, *Deep-Sea Res. I*, 94,
27 1–14, 2014.

1 **Impact of ocean acidification on phytoplankton assemblage, growth and DMS**
2 **production following Fe-Dust additions in the NE Pacific HNLC waters**

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1 **Abstract**

2 Ocean acidification (OA) is likely to have an effect on the fertilizing potential of desert dust in
3 high-nutrient, low-chlorophyll oceanic regions, either by modifying Fe speciation and
4 bioavailability, or by altering phytoplankton Fe requirements and acquisition. To address this
5 issue, short incubations (4 days) of northeast subarctic Pacific waters enriched with either FeSO₄
6 or dust, and set at pH 8.0 (in situ) and 7.8 were conducted in August 2010. We assessed the
7 impact of a decrease in pH on dissolved Fe concentration, phytoplankton biomass, taxonomy and
8 productivity, and the production of dimethylsulfide (DMS) and its algal precursor
9 dimethylsulfoniopropionate (DMSP). Chlorophyll *a* (chl *a*) remained unchanged in the controls
10 and doubled in both the FeSO₄-enriched and dust-enriched incubations, confirming the Fe-
11 limited status of the plankton assemblage during the experiment. In the acidified treatments, a
12 significant reduction (by 16-38%) of the final concentration of chl *a* was measured compared to
13 their non-acidified counterparts, and a 15% reduction in particulate organic carbon (POC)
14 concentration was measured in the dust-enriched acidified treatment compared to the dust-
15 enriched non-acidified treatment. FeSO₄ and dust additions had a fertilizing effect mainly on
16 diatoms and cyanobacteria. Lowering the pH affected mostly the haptophytes, but pelagophyte
17 concentrations were also reduced in some acidified treatments. Acidification did not significantly
18 alter DMSP and DMS concentrations. These results show that dust deposition events in a low-pH
19 iron-limited Northeast subarctic Pacific are likely to stimulate phytoplankton growth to a lesser
20 extent than in today's ocean during the few days following fertilization and point to a low initial
21 sensitivity of the DMSP and DMS dynamics to OA.

1 1 Introduction

2 The northeast subarctic Pacific is a high-nutrient, low-chlorophyll (HNLC) region characterized
3 by a phytoplankton assemblage dominated in summer by calcifying coccolithophores and
4 extremely high concentrations of the biogenic climate-active gas dimethylsulfide (DMS)
5 (Levasseur et al., 2006; Wong et al., 2006). Several studies have demonstrated that iron (Fe)
6 addition in these Fe-poor waters stimulated phytoplankton growth, in which diatoms often
7 outcompeted other phytoplankton groups, including coccolithophores, and resulted in a decrease
8 in DMS concentrations (Boyd et al., 2005; Hamme et al., 2010; Mélançon et al., 2014). Sources
9 of iron to the northeast subarctic Pacific include vertical mixing, eddies, tidal currents and
10 convection (Cullen et al., 2009; Royer et al., 2010), volcanic ash (Mélançon et al., 2014; Olgun
11 et al., 2011), and desert dust (Boyd et al., 1998; Jickells et al., 2005). Dust, which is considered
12 one of the most important sources, is deposited sporadically mostly in the spring during
13 occasional dust storms originating from the deserts of northern Asia (Duce and Tindale, 1991). A
14 natural strong dust deposition event has been shown to nearly double particulate organic carbon
15 (POC) concentration in the northeast subarctic Pacific in 2001 (Bishop et al., 2002). The
16 importance of eddies and vertical diffusion in the Gulf of Alaska was recently reviewed and
17 found to be greater than previously thought (Crawford et al., 2007; Johnson et al., 2005; Lam and
18 Bishop, 2008) Ongoing ocean acidification (OA) is however likely to compromise our current
19 understanding of the ecosystem's response to Fe addition by potentially altering Fe
20 bioavailability (Breitbarth et al., 2010; Shi et al., 2010) and phytoplankton physiology and
21 community composition (Tortell et al., 2002).

22 OA is currently in progress, is measurable and is caused by CO₂ emissions to the atmosphere that
23 end up in the ocean (Gattuso et al., 2013). The ocean has taken up one third of the CO₂ emissions
24 since the beginning of the industrial era. The dissolution of CO₂ in seawater increases the
25 concentration of bicarbonate (HCO₃⁻), protons (H⁺) (thereby decreasing pH) and decreases the
26 concentration of carbonate (CO₃²⁻), leading to calcite and aragonite undersaturation. Studies have
27 shown that calcifiers growing in acidified conditions generally present lower net calcification,
28 although their growth, synthesis and abundance are not generally affected (Kroeker et al., 2013
29 and references therein). On the other hand, the increase in dissolved CO₂ in seawater could favor
30 the growth of phytoplankton groups with low surface area to volume ratios (S/V) that are limited

1 by the diffusion of CO₂ across their surface or with low-efficiency carbon concentrating
2 mechanisms (CCMs) by reducing the energetic cost of carbon (C) assimilation. Coccolithophores
3 and diatoms generally exhibit low- and high-efficiency CCMs, respectively (Reinfelder, 2011).
4 Alternatively, fertilisation with FeSO₄ usually favors the growth of diatoms in HNLC waters
5 (Boyd et al., 2007 and references therein). It is not known whether Fe-Dust deposition will favor
6 diatoms or coccolithophores in the context of OA. OA could profoundly modify the structure and
7 functioning of a phytoplankton community typically dominated by calcifying haptophytes.

8 Studies examining the effects of acidification on the bioavailability of Fe in HNLC regions have
9 provided contrasting results. Breitbarth et al. (2010) observed a significant increase in Fe(II)
10 half-life and concentration in response to CO₂ enrichment, suggesting that a lower pH could
11 increase Fe bioavailability. However, Fe bioavailability could also decrease during acidification
12 due to changes in dissolved Fe speciation. Shi et al. (2010) observed that complexation of Fe(III)
13 by organic ligands containing acidic, unprotonated functional groups (e.g. carboxylic acid) is
14 strengthened in response to small decreases in surface water pH, resulting in decreased inorganic
15 Fe concentrations - the more bioavailable form of Fe. Furthermore, Fe uptake rates decrease
16 when acquiring organically complexed Fe – such as Fe(III) bound to desferrioxamine B (DFB) –
17 because the enzymatic reduction of Fe(III) at the cell surface may release protons (Shi et al.,
18 2010). Experimental studies with natural communities also yield inconsistent results. A study
19 combining CO₂ and Fe manipulations of a natural northwest subarctic Pacific community
20 showed a decrease in coccolithophore abundance at higher CO₂ levels (750 and 1000 µatm)
21 regardless of the Fe status, but no effect of the CO₂ level on diatoms nor on total chlorophyll *a*
22 (chl *a*) concentrations (Endo et al., 2013). A similar study conducted in HNLC waters of the
23 Weddell Sea, Antarctica, showed an increased C-specific primary productivity with increasing
24 CO₂ concentrations in Fe-enriched treatments but not in Fe-depleted treatments (Hoppe et al.,
25 2013).

26 By altering algal physiology and community composition, OA is likely to influence
27 dimethylsulfoniopropionate (DMSP) and DMS production. DMS originates mostly from the
28 enzymatic cleavage of DMSP, an osmolyte produced by several groups of phytoplankton. DMSP
29 quotas in phytoplankton vary by three orders of magnitude, with coccolithophores and diatoms
30 known as strong and poor producers, respectively (Keller et al., 1989). Results from the few

1 studies which have looked at the impact of OA on DMS production are inconsistent. Several of
2 them have reported a decrease in DMS production in acidified waters (Archer et al., 2013;
3 Arnold et al., 2013; Avgoustidi et al., 2012; Hopkins et al., 2010b). However, an increase in the
4 concentration of DMS at high CO₂ was measured in five bioassays conducted in northwest
5 European waters (Hopkins and Archer, 2014), during the first ten days of the Third Pelagic
6 Ecosystem CO₂ Enrichment Study (PeECE III) (Vogt et al., 2008; Wingenter et al., 2007), and
7 during a mesocosm study conducted in the coastal waters of Korea (Kim et al., 2010). The effect
8 of acidification on DMSP concentration is usually smaller than on DMS, and a greater variability
9 in responses is generally observed: particular or total DMSP increases in some studies (Archer et
10 al., 2013; Arnold et al., 2013), decreases in others (Avgoustidi et al., 2012; Hopkins and Archer,
11 2014; Hopkins et al., 2010b) or shows no response to increased pCO₂ (Lee et al., 2009; Vogt et
12 al., 2008). The causes for this variability are not well known.

13 The objective of this study was to determine how a decrease in pH by 0.2 units could influence
14 the impact of Fe delivered as FeSO₄ or Asian dust on the growth and taxonomic composition of
15 the phytoplankton assemblage of the Fe-limited northeast subarctic Pacific in summer, and to
16 explore how these pH-induced changes could affect the production of the climate-active gas
17 DMS.

1 **2 Material and Methods**

2 **2.1 Experimental setting and location**

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

19 **2.2 Treatments and acidification protocol**

20 Incubation bags were submitted to six treatments (in triplicate) representing the following
21 combination of dust or Fe addition and acidification: Control, Control+Acid, Fe, Fe+Acid, Dust,
22 Dust+Acid (Table 1). The carbonate system parameters and methods used for acidification were
23 based on the recommendations of Riebesell et al. (2010). The technique chosen was the addition
24 of a strong acid (HCl) and bicarbonate (NaHCO₃). The target value of 750 µatm CO₂ was chosen
25 to reproduce the concentration of CO₂ expected in 2100 following the “business as usual”
26 scenario IS92a by the Intergovernmental Panel on Climate Change (IPCC) (Meehl et al., 2007).
27 Target values of the carbonate system parameters (DIC, pCO₂, alkalinity and pH) were
28 calculated using the MS Excel macro CO2SYS (Pierrot et al., 2006) (sets of constants: K1, K2
29 from Mehrbach et al. (1973) refitted by Dickson and Millero (1987), KHSO₄: Dickson (1990),

1 pH scale: seawater scale ($\text{mol kg}^{-1} - \text{SW}$) and are presented in Table 2. To reach these target
2 values, a final concentration of $122.4 \mu\text{mol kg}^{-1}_{\text{sw}}$ of trace-metal clean 6 mol L^{-1} Seastar™
3 Baseline ~~HCL-HCl~~ solution and a final concentration of $115.1 \mu\text{mol kg}^{-1}_{\text{sw}}$ of a trace-metal clean
4 solution of NaHCO_3 were added to each acidified treatment bag through the Luer lock port using
5 a syringe. The piston was activated several times to ensure proper mixing.

6 **2.3 Fe and dust addition**

7 Two sources of Fe were used for the fertilization: FeSO_4 and standardized Asian dust CJ-2.
8 Briefly, CJ-2 dust was collected from the Tengger desert surface soil, roughly sieved and blown
9 through a wind tunnel designed to collect fine particles. Median diameter of CJ-2 dust is 24.1
10 μm . CJ-2 dust is characterized by a Fe content of $3.02 \pm 0.12\%$. Ooki et al. (2009) measured a
11 0.33% and Fe solubility, defined as the ratio of dissolved iron to total iron, for CJ-2 dust, of
12 0.33% (Ooki et al., 2009). For more information on CJ-2 dust, see Nishikawa et al. (2000) and
13 Hwang and Ro (2006). FeSO_4 was added at the final concentration of 0.6 nmol L^{-1} and CJ-2 dust
14 was added at the final concentration of 2 mg L^{-1} . Twenty hours prior to their addition in the bags,
15 dust and Fe solutions were prepared by adding CJ-2 dust samples or FeSO_4 to MilliQ water.
16 Proper quantities of Fe-enriched solutions were added to each bag with a syringe through the
17 Luer lock port. The piston was pushed and pulled several times to ensure proper mixing. Then,
18 each bag was gently shaken for 5 minutes to homogenize its content prior to sampling.

19 **2.4 Chemical and biological variables**

20 **2.4.1 Carbonate system**

21 Dissolved inorganic carbon (DIC) and alkalinity were measured at T0 and T4. DIC was
22 measured by a coulometric method using a Single operator multi-parameters metabolic analyzer
23 (SOMMA) (Johnson et al., 1993) coupled to a UIC 5011 coulometer, according to standard
24 protocols (Dickson et al., 2007). Samples were calibrated against Andrew Dickson (Scripps)
25 CRM water batch 101. Alkalinity was measured using an open cell method consisting of a
26 Brinkmann Dosimat 665 an Alpha PHE-4841 glass body combination electrode according to
27 standard protocols outlined in the Guide to best practices for ocean CO_2 measurements (Dickson
28 et al., 2007). Samples were calibrated against Andrew Dickson (Scripps) CRM water batch 101.

1 Partial pressure of CO₂ (pCO₂) and pH were calculated using the MS Excel macro CO2SYS as
2 described above.

3 **2.4.2 Macronutrients and dissolved Fe**

4 For macronutrients, at each subsampling time, ~10 mL of each bag was filtered in an in-line
5 syringe filter to remove particles (Polycarbonate 0.8 Micron, 25 mm filters) and placed in
6 polystyrene test tubes. The test tubes were immediately frozen at -20°C in an aluminium freezer
7 block. Concentrations of nitrate, silicic acid and phosphate were measured ashore using a
8 Technicon AA autoanalyzer II following the methods described in Barwell-Clarke and Whitney
9 (1996).

10 Dissolved Fe (DFe) samples were collected at each subsampling time by filtering ~125 mL (0.22
11 µm) into acid-cleaned low density polyethylene bottles under a clean laminar flow hood or in the
12 trace-metal clean positive pressure plastic tent (the “bubble”) constructed in the main lab as
13 described in Johnson et al. (2005). DFe samples were placed in trace-metal clean, low-density
14 polyethylene bottles and acidified to pH 1.7 for 20-30 h. Samples were then buffered to pH 3.2
15 using a formic acid-ammonium formate buffer. DFe was quantified according to GEOTRACES
16 protocols available in the Methods Manual (Cutter et al., 2010). All DFe samples were analyzed
17 on board in the “bubble” using a flow injection analysis (FIA) method where Fe is concentrated
18 from the seawater matrix onto a chelating resin and detected by chemiluminescence as first
19 described by Obata et al. (1993) with modifications presented in Obata et al. (1997). Samples
20 were pre-concentrated on a resin column of 8-hydroxyquinoline immobilized on silica gel. The
21 eluent was then combined with ammonia, hydrogen peroxide and luminol. A Hamamatsu
22 photomultiplier tube quantified the light emitted by the reaction of Fe and luminol as it passed
23 through the detection cell. Fe concentration was determined using an external standard curve.
24 Accuracy of the system was checked by regular measurements of the standard reference
25 seawaters SAFe D1 and D2 (Johnson et al., 2007). Our average values of the SAFe D1 and two
26 SAFe D2 standards were $0.63 \pm 0.05 \text{ nmol L}^{-1} \text{ Fe}$ (n = 7), $0.91 \pm 0.05 \text{ nmol L}^{-1} \text{ Fe}$ (n = 6) and
27 $0.90 \pm 0.03 \text{ nmol L}^{-1}$ (n = 4), consistent with the community consensus value of
28 $0.67 \pm 0.04 \text{ nmol L}^{-1}$ (D1) and $0.93 \pm 0.02 \text{ nmol L}^{-1}$ (D2).

1 2.4.3 Pigments and particulate organic carbon

2 For chl *a* determination, 305 mL of water was withdrawn from each bag at each subsampling
3 time. The water was filtered through 25 mm GF/F filters at ≤ 9.33 kPa vacuum and filters were
4 frozen at -20°C until analysis. Acetone (90 %) was added to extract the pigments 24 h prior to
5 analysis. Pigments were quantified with a Turner 10 AU fluorometer as described in Strickland
6 and Parsons (1972). To characterize the initial pigment composition by High Performance Liquid
7 Chromatography (HPLC), two samples of ca. 2 L were taken directly from the pump when
8 treatment bags were filled at T0. At T4, ca. 1 L was sampled from two incubation bags. Samples
9 were filtered at ≤ 9.33 kPa vacuum on 47 mm GF/F filters and filters were frozen at -80°C until
10 analysis onshore. Pigments were extracted by placing the filters in 95 % methanol at -20°C in the
11 dark for 24 h prior to analysis. The extracts were filtered through 25 mm diameter
12 polytetrafluoroethylene (PTFE) syringe filters (0.2 μm pore size) and analyzed using a Waters
13 Alliance 2695 (HPLC) system equipped with a 2996 Photodiode Array Detector (PDA) and a
14 reverse phase C8 column (Waters Symmetry), with a pyridine-containing mobile phase (Zapata
15 et al., 2000). Analysis was performed on a 200 μl injection of sample mixed with water in the
16 autosampler at a ratio of 5:1 immediately prior to injection. Pigment concentrations were
17 quantified using commercially available standards (Danish Hydraulic Institute). The initial
18 pigment ratio matrix loaded into the CHEMTAX program (Table 3) was obtained by averaging
19 the minimum and maximum values of pigment : ~~Chl~~-chl *a* ratios given in Table 1 of Mackey et
20 al. (1996) and is similar to that used by Suzuki et al. (2002) and Royer et al. (2010) for samples
21 collected in the subarctic North Pacific. Eight algal groups were quantified using the
22 chemotaxonomy program CHEMTAX (Mackey et al., 1996): cyanobacteria, pelagophytes,
23 haptophytes (including coccolithophores), diatoms, dinoflagellates, prasinophytes, cryptophytes
24 and chlorophytes. For a description of the pigment types, see Zapata et al. (2004). Particulate
25 organic carbon (POC) concentrations were measured at T0 and at T4. At T0, three samples of
26 500 mL of seawater were pumped from the sampling station. At T4, 500 mL were sampled from
27 each one of the incubation bags. Samples were filtered on pre-combusted 25 mm GF/F and the
28 filters were placed in open cryovials and allowed to dry in an oven at 60°C for 48 h. The
29 cryovials were then capped and kept in drierite until onshore analysis. POC and particulate
30 nitrogen were measured using a mass spectrometer (Delta Plus, Thermo Finnigan Mat) coupled
31 with an elemental analyzer (CE Instrument model 1110).

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1 **2.4.4 C and Fe uptake rates**

2 The siderophore desferrioxamine B (DFB) has been used as a model ligand for studying the
3 bioavailability of strongly organically complexed Fe in seawater (e.g. Maldonado and Price
4 1999; Hutchins et al. 1999; Shi et al. 2010). Iron uptake from DFB occurs through a high affinity
5 Fe transport system, and can reflect the Fe nutritional status of laboratory phytoplankton strains
6 (Maldonado and Price 1999; Maldonado et al. 2006) and natural phytoplankton assemblages
7 (Semeniuk et al. 2009; Taylor et al. 2013; Semeniuk et al. in press). Thus, we examined whether
8 uptake rates of Fe complexed to DFB varied among our treatments with varying Fe
9 bioavailability.

10 Uptake of ^{55}Fe bound to DFB was performed as previously described (Maldonado and Price
11 1999; Semeniuk et al. 2009). The 0.5 nM ^{55}Fe (Perkin Elmer) was complexed with 5% excess
12 DFB in pH 3.5 Milli Q for 30 minutes (Maldonado and Price, 1999). The resulting $^{55}\text{FeDFB}$
13 complex was subsequently equilibrated in 0.22 μm filtered seawater for 2 h. Approximately 250
14 mL of seawater was subsampled from each incubation bag into acid-cleaned polycarbonate
15 bottles. Just before dawn, the equilibrated $^{55}\text{FeDFB}$ complex and 10 μCi of $\text{H}^{14}\text{CO}_3^-$ (Perkin
16 Elmer) were added to each 250 mL bottle. From each assay bottle, 1 mL of sample was taken in
17 order to determine the initial total added activities of ^{55}Fe and ^{14}C . To prevent inorganic ^{14}C
18 from off-gassing in the initial activity sample vial, 500 μL of 5 M NaOH was added.

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

26 Volumetric Fe uptake and C-fixation rates were calculated as described elsewhere (Maldonado
27 and Price 1999; Semeniuk et al. 2009). Previous work with phytoplankton assemblages along
28 Line P has demonstrated that uptake of Fe from DFB by natural phytoplankton communities is
29 linear over 24 h (Maldonado and Price 1999). Thus, Fe uptake rates were calculated assuming
30 the accumulation of ^{55}Fe by cells was linear during the assay. In order to compare Fe uptake

1 rates among treatments, volumetric rates were normalized to the amount of C fixed during the
2 assay. The ^{14}C uptake rates normalised to chl *a* is used here as an indicator of the growth status
3 of the autotrophic assemblage. DMS and DMSP concentration

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

10 2.5 Statistical Analysis

11 All statistical analyses were run on Statistical Analysis System (SAS) software. The threshold
12 value for determining statistical significance was $p < 0.05$. Repeated-measures ANOVA were
13 used to test the difference between treatments and the changes in time for the means of biological
14 and chemical variables. Normality of the data was determined using the Shapiro-Wilk test.
15 ANOVA on ranks was used when normality of the data could not be assumed. Differences
16 between the mean concentrations of phytoplankton groups, as measured by HPLC, were assessed
17 using one factor ANOVA. Two-way ANOVAs were used to isolate the effect of one factor (acid,
18 Fe addition).

19 3 Results

20 3.1 State of the carbonate system, macronutrients and Fe concentrations

21 Table 2 presents the average and standard deviation of the four parameters of the carbonate
22 system measured (DIC and alkalinity) and calculated (pCO_2 and pH) for each treatment at T0
23 and T4. Target values of pH and pCO_2 in the acidified treatments were reached with averages of
24 7.80 ± 0.01 and $740 \pm 23 \mu\text{atm CO}_2$ respectively, at T0. DIC values in acidified treatments
25 reached an average of $2139 \pm 4 \mu\text{mol kg}^{-1}_{\text{sw}}$, a value 1.5% higher than the target value but
26 consistent among acidified treatments. Alkalinity values in the acidified treatment were
27 $2243 \pm 7 \mu\text{mol kg}^{-1}_{\text{sw}}$, a value 2.5% higher than the in situ (and target) value of $2187 \mu\text{mol kg}^{-1}_{\text{sw}}$.
28 DIC, alkalinity and pH values all remained stable during the 4-d incubations. From T0 to T4,

1 pCO₂ values varied by 3-12% in the acidified treatments and by 2-15% in the non-acidified
2 treatments, but values of the acidified versus non-acidified treatments remained different from
3 each other.

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

8 DFe, operationally defined as the fraction that passes through a $0.22 \mu\text{m}$ filter, includes soluble
9 and colloidal Fe (de Baar et al., 2005). DFe concentrations started at $0.41 \pm 0.09 \text{ nmol L}^{-1}$ and
10 $0.21 \pm 0.02 \text{ nmol L}^{-1}$ in the Control and Control+Acid treatments, respectively, and decreased to
11 $0.07 \pm 0.01 \text{ nmol L}^{-1}$ and $0.04 \pm 0.01 \text{ nmol L}^{-1}$ over the time course of the experiment (Fig. 1a).
12 In the Fe and Fe+Acid treatments, DFe started at $0.65 \pm 0.32 \text{ nmol L}^{-1}$ and $0.47 \pm 0.23 \text{ nmol L}^{-1}$,
13 respectively. The DFe concentration decreased to ca. 0.11 nmol L^{-1} on day 2 and to ca. 0.06 nmol
14 L^{-1} on day 4 in both treatments (Fig. 1b). In the Dust treatment, DFe started at $0.28 \pm 0.10 \text{ nmol}$
15 L^{-1} , decreased to $0.12 \pm 0.01 \text{ nmol L}^{-1}$ at T2 and remained at this level at T4. In the Dust+Acid
16 treatment, DFe started at $0.18 \pm 0.05 \text{ nmol L}^{-1}$, increased to $0.28 \pm 0.01 \text{ nmol L}^{-1}$ at T2 and
17 decreased slightly to 0.21 ± 0.07 at T4 (Fig. 1c).

18 **3.2 Plankton biomass**

19 Average initial chl *a* concentration in all treatments was $0.39 \pm 0.03 \mu\text{g L}^{-1}$ (Fig. 2). In the
20 Control and Control+Acid treatments, chl *a* concentration remained stable for the length of the
21 experiment (Fig. 2a). In the Fe treatment, chl *a* concentrations reached $0.76 \pm 0.16 \mu\text{g L}^{-1}$ after 4
22 days, a value significantly higher than measured in the control at the same time (p-value = 0.
23 0269; Fig. 2b). In the Fe+Acid treatment, chl *a* concentrations increased to $0.58 \pm 0.15 \mu\text{g L}^{-1}$
24 after 4 days, a value not significantly different from the one reached at the end of the Fe
25 treatment. The addition of dust also had a significant stimulating effect on phytoplankton growth
26 compared to the Control (p-value: 0.0071) with chl *a* reaching $0.88 \pm 0.23 \mu\text{g L}^{-1}$ at T4 (Fig. 2c).
27 The chl *a* concentration reached at T4 in the Dust treatment was not statistically different than
28 the one reached in the Fe treatment. In the Dust+Acid treatment, chl *a* concentration reached
29 $0.74 \pm 0.01 \mu\text{g L}^{-1}$ at T4, a value again not significantly different from the concentrations

1 reached at the end of the Dust treatment (Fig. 2c). Although the difference is not significant, a
2 trend appears in chl *a* concentrations: chl *a* concentrations are always slightly lower in the
3 acidified vs non-acidified treatment. In order to detect any interactive effect of Fe or acidification
4 on chl *a* and to further explore these apparent trends in chl *a*, all treatments were grouped and
5 tested with a two-factor ANOVA. The first factor, enrichment, had three possible states (Fe,
6 Dust, nil) and the second factor, acid, had two possible states (+ acid, control). This analysis
7 showed a significant effect of the Fe enrichment (p-value = 0.0060) and a significant effect of the
8 acidification (p-value = 0.0385) on chl *a* concentration. However, no combined effect (synergic
9 or antagonistic) was detected with the two factors.

10 Initial POC concentration was $75.3 \pm 11.2 \mu\text{g L}^{-1}$ (not shown) and increased in all treatments
11 including the Control. After 4 days, POC concentrations were similar in the Control (125.5 ± 0.3
12 $\mu\text{g L}^{-1}$) and Control+Acid ($122.5 \pm 17.6 \mu\text{g L}^{-1}$) treatments (Fig. 2a). The average POC
13 concentration at T4 in the Fe treatment ($169.2 \pm 55.8 \mu\text{g L}^{-1}$) was not statistically different than
14 in the Control. Final POC concentration in the Fe+Acid treatment ($189.3 \pm 29.2 \mu\text{g L}^{-1}$) was not
15 significantly different than in the Fe treatment, but significantly higher than in the Control (Fig.
16 2b). The highest POC concentration was measured in the Dust treatment ($286.7 \pm 39.7 \mu\text{g L}^{-1}$)
17 and lowering the pH resulted in a 24% decrease in POC concentration at T4 (Dust+Acid
18 treatment: $217.4 \pm 2.0 \mu\text{g L}^{-1}$) (Fig. 2c).

19 **3.3 Taxonomy**

20 The initial phytoplankton biomass (T0) was dominated by chlorophytes (37% of total chl *a*),
21 followed by haptophytes (31%), pelagophytes (19%) and dinoflagellates (13%) (from Fig. 3).
22 Prasinophyte and cryptophyte biomarkers were not detectable during our experiments.
23 Dinoflagellates were present in low concentrations at T0 and became undetectable at T4 in the
24 Control and in all treatments (Fig. 3b). In contrast with the dinoflagellates, diatoms and
25 cyanobacteria were below the detection limit at T0 and became detectable at T4 in the Control
26 and in all treatments (Fig. 3a, f). These changes in community composition in the control show
27 that the sampling and/or incubation conditions had a negative effect on the growth of
28 dinoflagellates and a positive effect on the growth of diatoms and cyanobacteria. Figure 3 shows
29 that diatoms were responsible for most of the increases in chl *a* measured in the Fe, Fe+Acid,
30 Dust and Dust+Acid treatments compared to the control. The only treatment to show a

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

4 **3.4 Carbon and Fe uptake rates at T4**

5 At T4, C assimilation rate was $92 \pm 50 \text{ nmol C L}^{-1} \text{ h}^{-1}$ in the Control (Fig. 4a). In the
6 Control+Acid treatment, C assimilation was $195 \pm 21 \text{ nmol C L}^{-1} \text{ h}^{-1}$, a value significantly higher
7 than in the Control. C assimilation rates in the Fe and Fe+Acid were significantly higher than the
8 Control, but not different from each other with values of $189 \pm 23 \text{ nmol C L}^{-1} \text{ h}^{-1}$ and 243 ± 66
9 $\text{nmol C L}^{-1} \text{ h}^{-1}$, respectively. C assimilation rate in the Dust treatment was similar to the Control
10 with $59 \pm 24 \text{ nmol C L}^{-1} \text{ h}^{-1}$. Lowering the pH significantly increased C assimilation rate in the
11 Dust+Acid treatment ($145 \pm 61 \text{ nmol C L}^{-1} \text{ h}^{-1}$) compared to the Dust treatment.

12 The chl *a*-specific C assimilation rate (size fraction $> 1 \mu\text{m}$) in the Control at T4 was 0.23 ± 0.01
13 $\mu\text{mol C } \mu\text{g chl } a^{-1} \text{ h}^{-1}$ (Fig. 4b). Lowering the pH increased significantly the chl *a*-specific C
14 assimilation rate to $1.03 \pm 0.13 \mu\text{mol C } \mu\text{g chl } a^{-1} \text{ h}^{-1}$ in the Control+Acid treatment. A similar
15 albeit less pronounced pH-induced increase in chl *a*-specific C assimilation was observed
16 between the Fe ($0.25 \pm 0.03 \mu\text{mol C } \mu\text{g chl } a^{-1} \text{ h}^{-1}$) and the Fe+Acid treatments (0.43 ± 0.15
17 $\mu\text{mol C } \mu\text{g chl } a^{-1} \text{ h}^{-1}$). The Dust and Dust+Acid treatments had chl *a*-specific C assimilation
18 rates of 0.07 ± 0.04 and $0.17 \pm 0.10 \mu\text{mol C } \mu\text{g chl } a^{-1} \text{ h}^{-1}$, respectively. It is noteworthy that
19 although comparable biomasses were achieved after 4 days in the FeSO₄ or Dust treatments, the
20 chl *a*-specific C assimilation rate was significantly lower in the Dust treatment as compared to
21 the Fe treatment and in the Dust+Acid compared to the Fe+Acid treatment..

22 At T4, FeDFB uptake rates normalized to C assimilation were $0.94 \pm 0.55 \mu\text{mol Fe mol C}^{-1}$ in
23 the Control, 0.34 ± 0.07 in the Control+Acid treatment, 0.33 ± 0.16 in the Fe treatment and 0.39
24 ± 0.20 in the Fe+Acid treatment (Fig. 4c). In the dust treatment, FeDFB uptake rate normalized
25 to C assimilation was high ($1.44 \pm 0.66 \mu\text{mol Fe mol C}^{-1} \text{ h}^{-1}$), also suggesting a rapid return to
26 Fe-limiting conditions in this treatment. Lowering the pH decreased the FeDFB uptake rates
27 normalized to C in the Dust+Acid treatment ($0.55 \pm 0.39 \mu\text{mol Fe mol C}^{-1}$), but had no effect on
28 the Fe+Acid treatment ($0.39 \pm 0.20 \mu\text{mol Fe mol C}^{-1}$).

1 **3.5 DMSP_t and DMS**

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

8 **4 Discussion**

9 **4.1 Considerations on the experimental protocol**

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

23 **4.2 Initial in situ conditions and impact of acidification**

24 Oceanic conditions encountered during the experiments were typical of this part of the northeast
25 Pacific and time of year. Macronutrients and chl *a* concentrations were high and low,
26 respectively, indicative of the HNLC conditions characterizing the Gulf of Alaska in summer
27 (Harrison et al., 1999; Hopkinson et al., 2010). DMS concentrations were high, but usual for this
28 region in the summer (Wong et al., 2005). The DFe concentration of 0.4 nmol L^{-1} measured in

1 the Control at T0 was higher than expected, but the Fe-limited status of the plankton community
2 was confirmed by the absence of chl *a* accumulation in the Control and the increase in chl *a*
3 induced by the addition of FeSO₄ (Fig. 2a, b). Also as expected for this time of the year, the
4 greatest contributors to total chl *a* included chlorophytes, haptophytes and pelagophytes (Fig. 3),
5 while diatoms represented minor contributors. Hence, the combined influences of dust and pH on
6 phytoplankton growth, taxonomy, and DMS production reported in this study can be
7 extrapolated to the northeast subarctic Pacific summer conditions. However, for the reason
8 mentioned above, our protocol does not allow us to draw conclusions on how dinoflagellates,
9 which represented ca. 13 % of the autotrophic biomass in situ, respond to OA.

10 The abrupt decrease in pH (by 0.2 units) and increase in pCO₂ (by 335 μatm) had no detectable
11 effect on the Fe-limited phytoplankton biomass and community structure (Figs. 2a and 3c).
12 During a comparable experiment conducted in the same region and under similar oceanographic
13 conditions (HNLC waters, phytoplankton dominated by haptophytes and chlorophytes),
14 Hopkinson et al. (2010) showed that increasing CO₂ to 760-1204 μatm had little effect on chl *a*,
15 nutrient drawdown, or phytoplankton growth rates after 5 days in Fe-limited conditions. In their
16 experiment, they did not observe a decrease in the biomass of haptophytes as during our study,
17 but the absence of present-day CO₂ control (observations restricted to low and high CO₂
18 treatments) limits the comparison between the two studies. Another similar experimental study
19 conducted in the northwest subarctic Pacific revealed a small decrease in haptophyte relative
20 biomass at high CO₂ levels (1000 μatm) compared to low CO₂ level (180 μatm) after 6 days and
21 an increase in diatoms biomass in all treatments but no pCO₂-related statistical change in the
22 abundance of diatoms and total chl *a* after 5 days (Endo et al., 2013). Another experiment
23 conducted with water from the diatom-dominated Fe-limited Bering Sea has shown a negative
24 effect of elevated CO₂ on diatoms (Sugie et al., 2013, Endo et al., 2015), which was not
25 observed in the Fe-enriched treatments. Thus, albeit the differences mentioned above, results
26 from these three experiments suggest that pH expected toward the end of this century will only
27 have a small negative impact, if any, on total autotrophic biomass in the HNLC waters of the
28 subarctic North Pacific.

29 Acidification resulted in the up-regulation of C assimilation in the control (Fig. 4a,b). Such pH-
30 induced increases in C assimilation have previously been reported in pH manipulation

1 experiments (Riebesell et al., 2007; Tortell et al., 2008). During their study in the same oceanic
2 region, Hopkinson et al. (2010) reported an increase in photosynthetic efficiency in their low pH
3 treatment, an increase they attributed to energy savings from down-regulation of the CCMs.
4 Increasing CO₂ concentrations (lowering pH) could have resulted in a down-regulation of this
5 costly mechanism, freeing energy for other metabolic pathways such as C assimilation.

6 Unexpectedly, the low pH-induced up-regulation of C assimilation measured at day 4 did not
7 directly translate into an increase in POC in the non-Fe-enriched waters. In point of fact,
8 acidifying non-Fe-enriched waters (Control+Acid treatment) had no effect on chl *a* or POC
9 concentrations but resulted in a 2-fold and 4-fold increase in absolute and chl *a*-normalized C
10 assimilation rates, respectively, after 4 days (Fig. 4). The absence of a higher POC concentration
11 in the Control+Acid treatment during our study suggests that the newly assimilated C was not
12 converted into biomass or that a loss mechanism would impede the buildup of POC. Such
13 mechanisms could include increased grazing by micrograzers or DOC exudation and subsequent
14 transparent exopolymer particle (TEP) formation. An increase in C uptake without biomass
15 accumulation under acidified conditions has previously been observed during a similar 12-day
16 experiment (Riebesell et al., 2007). This unexpected result was attributed to the release of DOC
17 and subsequent formation of TEPs which are known to accelerate particle aggregation and
18 sinking. Similar significant stimulation of DOC production at elevated CO₂ was reported by
19 Engel et al. (2014) in coastal waters but only a weak and inconsistent CO₂ induced decrease in
20 DOC production was observed by Yoshimura et al. (2014). In the absence of sinking as in our
21 experiment, the aggregation of DOC into TEP may only explain the absence of increase in POC
22 if a large proportion of the TEP produced adsorbed on the walls of the incubation bags. Even
23 though our measurements do not allow identifying the fate of the increased assimilated C in the
24 high pCO₂ treatment, they point toward a perturbation of the C cycling, either by an increase in
25 DOC exudation or grazing. If confirmed, such pH-induced modification of C cycling and pools
26 in HNLC waters could have important impacts on microbial dynamics and C export.

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

1 4.3 Dust fertilization in a high CO₂ northeast subarctic Pacific

2 During the 4 days of the experiment, dust fertilization had the same stimulating effect on net chl
3 *a* production as the addition of FeSO₄. This similarity confirms that the phytoplankton
4 assemblage was Fe limited when the study was conducted, and that Fe was responsible for the
5 stimulating effect of dust on phytoplankton growth (Fig. 2). However, the average final POC
6 concentration in the Dust treatment was 69% higher than in the FeSO₄ treatment, suggesting a
7 more efficient Fe stimulation of phytoplankton C assimilation in the former. These results
8 suggest that 2 mg L⁻¹ of CJ-2 dust releases at least as much bioavailable Fe during the first 4 days
9 of the experiment as the addition of 0.6 nmol L⁻¹ FeSO₄. We can only speculate as to why Fe
10 contained in dust stimulated more growth than FeSO₄. Part of the answer may be related to the
11 speed at which the Fe is released from dust, a relatively low release rate allowing a more
12 efficient utilization of Fe. In comparison, and as can be seen in Fig. 1, FeSO₄ is rapidly utilized
13 and probably scavenged by particles and ligands following fertilization. Diatoms, cyanobacteria
14 and, to a lesser extent pelagophytes, benefited the most from the dust enrichment, reaching
15 higher group-specific chl *a* concentrations than the Control incubation at T4. Except
16 dinoflagellates, which did not thrive in the Control, all other groups maintained their biomass in
17 the Dust treatment. This response to Fe addition is comparable to the one reported for previous
18 small and large-scale Fe fertilization experiments conducted in the Gulf of Alaska showing an
19 initial increase in the abundance of major taxa and a dominance of diatoms (Boyd et al., 1996;
20 Levasseur et al., 2006; Marchetti et al., 2006; Martin and Fitzwater, 1988; Mélançon et al.,
21 2014). We saw no clear difference in the structure of the phytoplankton assemblage whether dust
22 or FeSO₄ was used as fertilizer. In that regard, FeSO₄ seems to be a good substitute for dust in
23 studies of the early response (< 4 days) of plankton communities to dust deposition in the
24 northeast subarctic Pacific.

25 DFe measurements were poor indicators of Fe bioavailability following dust deposition in our
26 study. In contrast with the FeSO₄ treatment where almost all the added Fe was still present and
27 measured in the dissolvable pool at T0 (ca. 20 min. following the addition), DFe concentrations
28 remained low and near *in-situ* levels during the entire length of the dust-addition experiments
29 (Fig. 1b, c). Considering that the addition of Dust did stimulate algal growth, the low and
30 constant concentration of DFe in the Dust treatment suggests that the release of bioavailable Fe

1 from dust was matched by bacterial and phytoplankton Fe acquisition. Re-adsorption of the
2 released Fe by the dust particles may also be responsible for the low levels of DFe measured
3 during the experiment (Ye et al., 2011). These results also show that DFe may not be a good
4 indicator of dust deposition events in the oceanic environment where the release of DFe from the
5 low concentrations of dust deposited is unlikely to exceed bio-uptake and re-adsorption on
6 particles. Similar conclusions were reached in a previous study (Mélançon et al., 2014), where
7 total dissolvable Fe (TDFe) was also measured and shown to be a better indicator of Fe
8 bioavailability than DFe.

9 Our results suggest that after a period of active growth, phytoplankton in the Dust treatment
10 became Fe-limited 4 days into the experiment. This conclusion is supported by the very low
11 absolute and chl *a* normalized C fixation rates in the Dust treatment as compared to the Control
12 and FeSO₄ treatments at T4 (Fig. 4a), as well as by the FeDFB uptake rates normalized to C
13 uptake rates which were higher in the Dust treatment than in the Control (Fig. 4b). Dust particles
14 are known to efficiently adsorb Fe (Ye et al., 2011). The rapid return to Fe deficiency in the Dust
15 treatment may thus result from a combination of increased Fe demand and re-adsorption of Fe
16 onto dust particles. This explanation implies however that the re-adsorbed Fe becomes less prone
17 to desorption, which needs to be demonstrated. These results suggest that the influence of the Fe
18 released from dust lasted less than 4 days during our experiments. In natural environments, this
19 period of influence may be even shorter due to fast sinking of larger dust particles. Based on
20 Stoke's Law and assuming our dust particles were spherical, CJ-2 dust particles may sink at an
21 average speed of 32 m/day, which would take particles out of a 60 m-deep euphotic zone in ~2
22 days. Due to the combined effect of both Fe re-adsorption on particles and fast sinking, the
23 impact of natural dust deposition may thus be of relatively short duration in the environment,
24 similar to the time-frame of our *in vitro* study.

25 Decreasing the pH resulted in a slightly lower biomass (chl *a* by 16% and POC by 15%) in the
26 Dust+Acid treatment than in the Dust treatment (Fig. 2c). The decrease in biomass corresponded
27 to decreasing trends in the contribution of haptophytes and to a lesser extent, of pelagophytes and
28 cyanobacteria to total chl *a* concentrations. As discussed above, a likely explanation for the
29 lower biomass reached after 4 days is the negative effect of acidification on the growth of the
30 coccolithophores (Engel et al., 2005; Harvey et al., 2013; Kroeker et al., 2010). C assimilation

1 rates (absolute and chl *a*-normalized) were ca. 2-fold higher in the Dust+Acid treatment than in
2 the Dust treatment at T4, probably reflecting the stimulating effect of higher CO₂ concentrations
3 on diatom C assimilation. These results reinforce the aforementioned hypothesis that the up-
4 regulation of C assimilation was paired with an increased particulate C loss via enhanced C
5 exudation from the cells or increased grazing.

6 It is obvious from Figure 2 that the natural variability among the bags of a same treatment as
7 well as the short length of the incubations limited our capacity to statistically distinguish trends
8 resulting from the two treatments (i.e. Fe/dust addition and acidification). The two-factor
9 ANOVA allowed us to increase the statistical power and detect a negative effect of acidification
10 on chl *a* concentration after 4 days. This suggests that OA will not increase Fe bioavailability to
11 natural HNLC phytoplankton communities. However, it does not preclude the possibility that
12 acidification may have exacerbated Fe limitation in our experiment. If the effect of acidification
13 on the growth of calcifying haptophytes was expected, it is not expected in the case of diatoms,
14 pelagophytes and cyanobacteria. Since these non-calcifying organisms also presented a lower
15 biomass in acidified treatments, it is likely that Fe bioavailability has been reduced via
16 interactions between pH, ligands and Fe speciation.

17 **4.4 Impact of acidification and dust deposition on DMSP_t and DMS**

18 The general decrease in DMSP_t and DMS concentrations measured in all treatments likely
19 reflects the loss of DMSP-rich dinoflagellates due to sampling and/or bottle effect and their
20 replacement by diatoms with low DMSP quotas. In spite of the increase in chl *a* and POC
21 measured in the FeSO₄ and Dust treatments compared to the Control, the alleviation of Fe
22 limitation had no impact on the concentrations of DMSP_t or DMS. This can be explained by the
23 lack of significant difference in the abundance of DMSP-rich haptophytes in the Control, FeSO₄
24 and Dust treatments at T4. Indeed, the addition of FeSO₄ or dust mostly stimulated the growth of
25 DMSP-poor diatoms, which would have little effect on DMSP_t concentrations.

26 Our results show no statistical differences between DMSP_t concentrations between the acidified
27 and non-acidified treatments after 4 days. Although the short duration of our incubations may
28 explain this lack of response, other studies conducted over longer period of time have reported
29 similar results. For example, lowering the pH had no effect on DMSP_t concentrations during the

1 nutrient-stimulated bloom of a community from a Norway fjord (Vogt et al., 2008). In their 24-
2 day experiment the absence of effect was attributed to the resistance of the planktonic
3 community considering that similar increases in chl *a* concentration and species succession were
4 observed in all CO₂ treatments. Studies reporting pH-induced changes in DMSP_t concentrations
5 are usually associated with alterations of the structure of the phytoplankton assemblage after
6 several days. For example, a low pH-induced increase in dinoflagellates after 13 days resulted in
7 higher DMSP_t concentrations during a mesocosm study with Arctic water (Archer et al., 2013).

8 Lowering the pH had no measurable effect on DMS concentrations, a result probably also related
9 to the short duration of the experiment. Previous studies showed either an increase or decrease in
10 DMS concentrations with acidification, but these differences become measurable late in the
11 experiment at or after the peak of the blooms. For example, during the experiment PeECE III in
12 Raunefjorden, Norway, Wingenter et al. (2007) observed an increase in time-integrated average
13 amount of DMS at high-CO₂, but the pH-related differences could only be observed after 6 days.
14 In this case, the authors attributed the pH effect to a difference in viral attack and phytoplankton
15 lysis at the chl *a* peak, a situation that is not likely to have occurred in our short incubations.
16 Contrastingly, a decrease in DMS concentrations with high CO₂ was observed during other
17 mesocosm studies conducted in the same fjord (Hopkins et al., 2010; Avgoustidi et al. 2012).
18 These authors suggested that the dominance of flagellates and picoeucaryotes during their study
19 as compared to coccolithophores during PeECE III could explain the divergent responses
20 observed in regards of DMS production.

21 Archer et al. (2013) also measured a decrease in DMS concentration under high CO₂ conditions
22 in Arctic waters, a decrease they attributed to an increase in bacterial production and decrease in
23 DMS yield. It should be noted that none of the experiments described above reports an effect of
24 CO₂ on DMS in the first 4 days. The sole study so far showing a rapid effect of a decrease pH is
25 by Hopkins and Archer (2014) who measured a decrease in DMSP and an increase in DMS after
26 4 days during shipboard bioassays experiments with NW European waters. In that case, the
27 changes in DMSP and DMS were associated with a rapid decline in the abundance of small cells
28 in the acidified treatments and a possible cellular release and cleavage of DMSP to DMS. Their
29 results show nonetheless a regional variability of the responses. The lack of response of DMS

- 1 concentration to $p\text{CO}_2$ in our incubations might reflect this natural variability and a particular
- 2 resistance of our initial community to acidification.

1 5 Conclusion

2 This study demonstrates that the degree of OA expected to occur by the end of the century is
3 likely to have a detectable but minor impact on the short-term response of Fe-limited planktonic
4 communities to sporadic atmospheric Fe-dust depositions. The addition of FeSO₄ and Asian dust
5 stimulated the growth of all major phytoplankton groups during our 96-h incubations, thereby
6 confirming the Fe-depleted status of the community and the potential of natural dust deposition
7 for stimulating phytoplankton growth in this HNLC region. In the acidified treatments, Fe in the
8 form of FeSO₄ or Asian dust still had a fertilizing effect on the algal assemblage, but to a lesser
9 extent than in the non-acidified treatment. The trends reported here suggest that OA could
10 moderate the growth response of pelagophytes and haptophytes to dust deposition. The fact that
11 non-calcifying taxa were also affected by acidification suggests that the lower pH possibly
12 interferes with ligands, Fe speciation and transporters to reduce Fe bioavailability in these HNLC
13 waters. Finally, our results suggest a low sensitivity of the DMS dynamics to acidification, both
14 under Fe-limited and Fe-replete conditions, in the northeast subarctic Pacific. In order to
15 understand the mechanisms behind this apparent resistance, studies on DMSP and DMS
16 phytoplankton and bacterioplankton metabolisms during longer incubation periods are advisable.

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- 4

1 Table 1: Description of Fe/dust additions and acidification status of each treatment. All
2 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

3

1 Table 2: Values of DIC, alkalinity, pH and pCO₂ in each treatment at T0 and T4.

		DIC ($\mu\text{mol kg}^{-1}_{\text{sw}}$)		Alkalinity ($\mu\text{mol kg}^{-1}_{\text{sw}}$)		pH		pCO ₂ (μatm)	
Target values (acidified)		2107		2187 (<i>in situ</i>)		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

2
3

4 | Table 3: Biomarker pigment initial ratio matrix for CHEMTAX: ~~Chl~~-chl *a* ratios for eight algal groups.

Class / Pigment	Chl <i>c</i>₃	Chl <i>c</i>₂	Peri	19'-but	Fucox	Prasinox	Violax	19'-hex	Diadinox	Allox	Zeax	Chl <i>b</i>	Chl <i>a</i>
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

5 | **Abbreviations:** Chl *c*₃, chlorophyll *c*₃; Chl *c*₂, chlorophyll *c*₂; Peri, peridinin; 19'-but, 19'-butanoyloxyfucoxanthin; Fucox,
6 | fucoxanthin; Prasinox, prasinoxanthin; Violax, violaxanthin; 19'-hex, 19'-hexanoyloxyfucoxanthin; Diadinox, diadinoxanthin; Allox,
7 | alloxanthin; Zeax, zeaxanthin; Chl *b*, chlorophyll *b*; Chl *a*, chlorophyll *a*.

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

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

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

18 Figure 4: Average (a) Absolute C assimilation rates. (b) C assimilation rates normalized to chl *a*
19 concentration at T4 and (c) Fe uptake rates normalized to chl *a* concentration at T4. Error bars
20 indicate standard deviations. Absolute C assimilation rates: $n = 3$ except Control and Acid where
21 $n = 2$, C assim norm to chl *a*: $n = 3$ except Control, Acid and Dust+Acid where $n=2$, Fe uptake
22 rates normalized to chl *a*: $n = 3$ except Control and Dust+Acid where $n = 2$.

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

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