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Community structure and photosynthetic physiology of phytoplankton in the northwest subarctic Pacific during an in situ iron fertilization experiment (SEEDS-II)

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ABSTRACT

Temporal changes in the abundance, community composition, and photosynthetic physiology of phytoplankton in surface waters were investigated during the second in situ iron (Fe) fertilization experiment in the NW subarctic Pacific (SEEDS-II). Surface chlorophyll *a* concentration was 0.75 mg m^{-3} on the day before the first Fe enrichment (i.e. Day 0), increased ca. 3-fold until Day 13 after two Fe additions, and thereafter declined with time. The photochemical quantum efficiency (F_v/F_m) and functional absorption cross-section (σ_{PSII}) of photosystem II for total phytoplankton in surface waters increased and decreased inside the Fe-enriched patch through Day 13, respectively. These results indicate that the photosynthetic physiological condition of the phytoplankton improved after the Fe infusions. However, the maximum F_v/F_m value of 0.43 and the maximum quantum yield of carbon fixation (ϕ_{max}) of 0.041 mol C (mol photon)$^{-1}$ during the development phase of the bloom were rather low, compared to their theoretical maximum of ca. 0.65 and 0.10 mol C (mol photon)$^{-1}$, respectively. Diatoms, which were mainly composed of oceanic species, did not bloom, and autotrophic nanoflagellates such as cryptophytes and prasinophytes became predominant in the phytoplankton community inside the Fe-enriched patch. In ferredoxin/flavodoxin assays for micro-sized (20–200 μm in cell length) diatoms, ferredoxin was not detected but flavodoxin expressions consistently occurred with similar levels both inside and outside the Fe-enriched patch, indicating that the large-sized diatoms were stressed by Fe bioavailability inside the Fe-enriched patch even after the Fe enrichments. Our data suggest that the absence of a Fe-induced large-sized diatom bloom could be partly due to their Fe stress throughout SEEDS-II.

1. Introduction

In the metabolic processes of phytoplankton, iron (Fe) plays crucial roles in electron transport both in photosynthesis and respiration, nitrogen assimilation via nitrate reductase, nitrite reductase, and nitrogenase, and chlorophyll synthesis (Raven et al., 1999; Sunda, 2001). In some algal groups, Fe-containing superoxide dismutase (FeSOD) and ascorbate peroxidase (APX) protect their cellular components from photo-oxidative stress induced by reactive oxygen species (ROS) (Ishikawa et al., 2003; Wolfe-Simon et al., 2005). Since Fe functions in these important cellular metabolic processes, Fe availability significantly regulates the growth activities of phytoplankton.

The results of mesoscale in situ Fe fertilization experiments confirm that Fe controls the photosynthetic physiology of phytoplankton and that its supply is of importance to regulate marine ecosystems and biogeochemical cycles in high-nitrate, low-chlorophyll (HNLC) waters (de Baar et al., 2005; Boyd et al., 2007). The most prominent biological responses among the mesoscale in situ Fe fertilization experiments were observed in the Subarctic Pacific iron Experiment for Ecosystem Dynamics

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Study (SEEDS) conducted in the Western Subarctic Gyre (WSG) of the NW subarctic Pacific during the summer of 2001. A single Fe enrichment was followed by a massive phytoplankton bloom composed mainly of the chain-forming diatom *Chaetoceros debilis* with concomitant decreases in macronutrient concentrations and CO_2 fugacity (fCO_2) in surface waters (Tsuda et al., 2003). In addition, the community structure of phytoplankton (Suzuki et al., 2005) and the species composition of diatoms (Tsuda et al., 2005) were dramatically altered as a result of Fe enrichment. These were consistent with the past in situ Fe fertilization experiments where the algal community shifted from pico- ($<2\,\mu m$) and nanoplankton (2–10 μm) to microplankton ($>10\,\mu m$) (de Baar et al., 2005). In contrast, the size composition of phytoplankton did not change during an Fe-induced algal bloom formation in the second mesoscale in situ Fe fertilization experiment in the NW subarctic Pacific (SEEDS-II) conducted in the summer of 2004 (Tsuda et al., 2007).

In this paper, we describe changes over time in the abundance and community composition of phytoplankton during SEEDS-II as estimated from algal pigment signatures and light microscopy. Phytoplankton pigment analyses using high-performance liquid chromatography (HPLC) and the CHEMTAX program provide quantitative estimates of the community structure of phytoplankton at the class level (MacKey et al., 1996). Although microscopy enables the identification and direct count of algal cells at the species level, tiny and/or fragile cells may be difficult to identify due to lack of morphological characteristics and difficulty in their fixation. Therefore these two techniques were used complementarily and would be useful for gross characterization of algal populations during SEEDS-II. In addition, in order to investigate the effect of Fe enrichment on the photosynthetic physiology of phytoplankton in SEEDS-II, measurements of fast repetition rate (FRR) fluorometry (Kolber et al., 1998) and photosynthesis–irradiance (*P–E*) curves (Sakshaug et al., 1997) were also carried out. It is known that Fe limitation leads to a decrease in the photochemical quantum efficiency (F_v/F_m) of algal photosystem II (PSII) and a concomitant increase in the functional absorption cross-section (σ_{PSII}) of this photosystem (e.g., Greene et al., 1992). According to Raven et al. (1999), a non-heme Fe^{2+} is involved in D1/D2 heterodimer of a PSII reaction center and plays a key role in electron transport between the primary and secondary electron acceptors (i.e. Q_A and Q_B, respectively) of PSII. Furthermore, one or two Fe atoms are contained in cytochrome b_{559}, which is strongly connected to the D1/D2 heterodimer in phytoplankton cells (Raven et al., 1999). As a result, primary charge separation of the radical pair of PSII reaction center is inhibited strongly by lack of Fe, and that leads to a decrease in F_v/F_m ratio. The increase in σ_{PSII} under Fe deficiency can be explained by the classical lake model (Knox, 1975) and the loss of the functional PSII reaction centers. F_v/F_m is considered to be an index of the quantum yield for carbon fixation (Falkowski et al., 1994), and was compared to the estimates obtained from the *P–E* curve experiment. Furthermore, to evaluate Fe deficiency among micro-sized diatoms (20–200 μm) having potentially high carbon export in the water column (Kawakami and Honda, 2007), the accumulation of their photosynthetic redox proteins ferredoxin and flavodoxin was examined using immunological probes. Under conditions of Fe deficiency, the Fe-S protein ferredoxin, which is located on the acceptor side of PSI, can be replaced by the non-Fe-containing flavoprotein flavodoxin in some prokaryotic and eukaryotic algae including diatoms (La Roche et al., 1996). Previous studies reported that flavodoxin abundance and the ratio of ferredoxin to the sum of ferredoxin and flavodoxin (i.e. Fd index) could be a diagnostic marker for estimating Fe deficiency (e.g., La Roche et al., 1996; Doucette et al., 1996; McKay et al., 1999).

2. Materials and methods

The SEEDS-II experiment was conducted in the WSG of the northwest subarctic Pacific (48°N, 166°E) from 20 July to 22 August 2004, onboard the R/V *Hakuho Maru* (JAMSTEC, Japan) and R/V *Kilo Moana* (University of Hawaii, USA). After an observation on Day 0 (i.e. 20 July 2004), 332 kg Fe (1800 kg $FeSO_4 \cdot 7H_2O$) were added to an 8×8 km patch of surface waters with sulfur hexafluoride (SF_6) as a tracer of the water mass. Furthermore, second Fe infusion (950 kg $FeSO_4 \cdot 7H_2O$) was carried out in the Fe patch on Day 6 without SF_6 tracer. An overview of the SEEDS-II experiment is described in Tsuda et al. (2007). Water samples except for a few of the HPLC pigment and FRRf measurements and the photosynthetic protein analyses (see below) were collected with acid-cleaned 12-L Teflon-coated X-Niskin bottles mounted on a CTD-CMS (carousel multi-sampling system) with a titanium hydrowire. Seawater temperature data were obtained from the cruise reports. Changes over time in the concentrations of Fe and macronutrients inside and outside the Fe patch were reported elsewhere (see Nishioka et al., 2009; Saito et al., 2009). Photosynthetic active radiation (PAR) was measured on deck using quantum sensors and data loggers throughout the experiment.

2.1. HPLC pigment analysis

Water samples (1 L each) collected at 5 m depth (except 2 m on Day 0) inside and outside the Fe-enriched patch were filtered onto Whatman GF/F filters (25 mm diameter) under gentle vacuum (<100 mmHg) or onto 20-μm nylon mesh filters (47 mm diameter) without vacuum (i.e. gravity filtration). Size-fractionation using the 20-μm nylon mesh was carried out only during the R/V *Hakuho Maru* cruise (i.e. Days 0–12 and 23–32). The GF/F filters were blotted with a filter paper and the GF/F and nylon mesh filters were stored in a deep-freezer (−80 °C) or in liquid nitrogen until analysis on land. The analysis of phytoplankton pigments using HPLC was carried out following Suzuki et al. (2005).

To estimate the temporal changes in the community structure of phytoplankton in surface waters inside and outside the Fe-enriched patch using pigment signatures, the CHEMTAX program (MacKey et al., 1996) was used. The initial pigment ratios of Suzuki et al. (2002a, 2005), who estimated the community composition of phytoplankton in the NW subarctic Pacific during summer, were used to compare the results of the present study with their outputs. Prymnesiophytes, pelagophytes, and prasinophytes in our CHEMTAX analysis are synonymous with the haptophytes type 3, chrysophytes type 2, and prasinophytes type 3 of Mackey et al. (1996), respectively. Phytoplankton pigment data obtained following the GF/F filtration inside and outside the Fe-enriched patch during SEEDS-II were treated separately for the CHEMTAX analysis. The pigment data on Day 0 were included in the data from outside the Fe patch. In addition, pigment data at 10 m depth (data not shown but these data were very similar to those at 5 m) were also incorporated into the CHEMTAX analyses in order to enhance the statistical reliability (see Mackey et al., 1996).

2.2. Light microscopy

One liter of the water samples collected at 5 m depth inside the Fe-enriched patch was preserved with Lugol's iodine solution. The samples were concentrated to 20–30 ml by settling, and an aliquot of the concentrated samples were used for identification and enumeration of phytoplankton cells with an inverted or a standard microscope following the method of Tsuda et al. (2005).