Biogeosciences Discuss., 11, 13739–13774, 2014
www.biogeosciences-discuss.net/11/13739/2014/
doi:10.5194/bgd-11-13739-2014

Biogeosciences
Discussions
Open Access

Effect of ammonium input over the distribution of iron in the seawater and the phytoplankton in a mesocosm experiment in a North Patagonian fjord

N. Sanchez[1], M. V. Ardelan[1], N. Bizsel[2], and J. L. Iriarte[3]

[1]Norwegian University of Science and Technology (NTNU), Department of Chemistry, Trondheim 7491, Norway
[2]Institute of Marine Sciences and Technology, Dokuz Eylul University, H. Aliyev Bulv., No. 10, Inciralti, Izmir, Turkey
[3]Instituto de Acuicultura and Centro de Investigación en Ecosistemas de la Patagonia-CIEP, Universidad Austral de Chile, Puerto Montt. COPAS Sur-Austral, Universidad de Concepción, Concepción, Chile

Received: 30 July 2014 – Accepted: 11 August 2014 – Published: 22 September 2014

Correspondence to: N. Sanchez (nicolas.sanchez@ntnu.no)

Published by Copernicus Publications on behalf of the European Geosciences Union.

Abstract

The distribution and concentration of iron in seawater and plankton were studied under different ammonium concentrations along a 22 day mesocosm experiment in order to assess possible effects of aquaculture over the phytoplankton and the biogeochemistry in fjords of Chile. Brackish and marine water were used in two different setups, each one with 1 control and 4 different NH_4^+ concentrations. Total Chelex labile (TFe_{Ch}), dissolved Chelex labile (DFe_{Ch}) and DGT labile (Fe_{DGT}) iron measurements were performed in seawater, wheile the particulate iron content was determined as total (PFe) and fractionated (PFe_{SF}) for the plankton community. Average concentration per treatment showed higher concentrations for both TFe_{Ch} and DFe_{Ch} in the marine system compared to the brackish. TFe_{Ch} showed general increasing trend in time and with increasing ammonium concentration, exhibiting positive correlation to the chlorophyll and particulate organic carbon content, whereas DFe_{Ch} presented an inverse pattern as expected. Fe_{DGT} showed an average lower concentration compare to DFe_{Ch} with final concentrations significantly lower in treatments with artificial ammonium addition. PFe showed an increasing trend in time and with increasing ammonium in both systems. Yet, when normalized to Chlorophyll *a* or particulate organic carbon the trend inverted, showing that at higher ammonium loading the iron per Chlorophyll *a* or particulate organic carbon decreases. PFe_{SF} major changes occurred in the marine system in the ratio between the 20–140 µm and the 2–20 µm fractions, suggesting possible community structure shift. Overall, ammonium input indicated an effect over iron in the seawater and the particulate matter, depending on the iron form and the microbial assemblage. The further changes over the microbial composition due to ammonium addition may affect the cycling of iron, having possible negative or positive feedbacks over major biogeochemical cycles.

1 Introduction

Fjords ecosystem in Chile, constitute nearly pristine environment experiencing a growing anthropogenic influence, being aquaculture one of the main drivers. This industry has seen and increased expansion in the last two decades, causing growing concern over the environmental impact. Salmon farming releases nutrients as dissolved inorganic species through excretion (Ammonium (NH_4^+) and phosphate (PO_4^{3-})), particulate organic nitrogen (PON) and phosphorus (POP) through defecation, and its dissolved components (DON and DOP) through resuspension from the particulate fractions (Olsen and Olsen, 2008). Oxygen depletion and decreased biodiversity among others are well documented effects for the marine sediments and benthic fauna. However, current knowledge of how waste release affects the structure and function of the pelagic ecosystems is still scarce (Cloern, 2001; Olsen et al., 2006). It has been proposed that this waste release may alter nutrient stoichiometry in the seawater determining to some extent how the marine environment responds to increasing anthropogenic inputs of nutrients (Arrigo, 2005).

Major biogeochemical cycles (carbon, nitrogen and phosphorus) in the marine environment, are strongly dependent on marine microbes as this group is directly responsible for approximately half of the earth's primary production (Field et al., 1998). In coastal areas like fjord regions, the macronutrient nitrogen is mainly contributed as nitrate NO_3^- with the incoming deep water (nutrient-rich) and/or the surface layers through the run-off of inland fertilizers. After phytoplankton uptake, a series of metabolic process occur in order to be finally assimilated as NH_4^+. Within these steps of the nitrogen cycle, as well as for that of major elements, are involved metabolic processes that are dependent on the availability of certain "micro-nutrients". Trace metals such as Mn, Fe, Co, Ni, Cu, Zn and Cd are involved in several biological processes capable of influencing the biochemical cycling in aquatic systems such as of carbon (e.g. carbon-concentrating mechanism involves the Zn metalloenzyme carbonic anhydrase) or nitrogen (e.g. Fe requirements for metalloenzymes in nitrogen cycle) (Morel and

Title Page
Abstract Introduction
Conclusions References
Tables Figures
◀| |▶
◀ ▶
Back Close
Full Screen / Esc
Printer-friendly Version
Interactive Discussion

Price, 2003). As most of these elements are continuously exported out the photic zone to depth as settling organic biomass, biological processes (uptake, trophic transfer, regeneration, excretion and decomposition) are critical in controlling the fate of these bioactive metals in the ocean (Wang et al., 2001). In the case of iron, the key role it plays on major biochemical cycles in the marine environment is well acknowledged (Martin et al., 1991; Martin, 1991; Morel et al., 1991). Specifically related to nitrogen, Fe is involved in the nutrient uptake by diatoms as NO_3^- undergoes sequential reduction to nitrite and ammonium, each step involving the assimilatory nitrate reductase and assimilatory nitrite reductase enzymes respectively (Zehr and Ward, 2002).

Through this feedback control mechanism between so-called "macro" and "micro" nutrients, it can be expected that a species shift in the macronutrient load (e.g. NO_3^- to NH_4^+) in a given environment, may affect in the long term the cycling of trace elements. The biologically "New" vs. "Regenerated" production, based on the $NO_3^- : NH_4^+$ ratio in the water column, is a determinant factor favoring growth rates of certain groups of primary producers (Thompson et al., 1989). Therefore, increased input of dissolved inorganic nutrients (NH_4^+ and PO_4^{3-}) by aquaculture activities may have a direct effect on the phytoplankton community structure (Olsen and Olsen, 2008). The general response from marine pelagic ecosystems to nutrient enrichment is reflected in increase nutrient uptake by phytoplankton and bacteria and hence growth rate, with the consequent increased autotrophic biomass transfer to higher trophic levels (Olsen and Olsen, 2008). However, knowledge on the capacity for phytoplankton to biologically uptake and metabolize these surplus of nutrients, strongly link to the bioavailability of certain trace metals, is still scarce. For instance, changes in nitrogen supply (e.g. NH_4^+), could potentially affect the cycling of iron, perhaps modifying the Fe requirements of the phytoplankton community.

Base on the above, it can be expected that an environment following nutrient enrichment through NH_4^+ may modify nitrogen cycling and the stoichiometry of trace elements linked to it. Therefore, it is likely that NO_3^- to NH_4^+ shift achieved through progressive addition of nutrients would imply in the long term, changes in Fe requirements by phytoplankton, with a consequent effect on trophic transfer to higher trophic levels. In order to asses probable feedbacks process between nitrogen and iron marine cycles and implications in the base of the pelagic marine food web, a mesocosm experiment was carried out to simulate the nutrient enrichment occurring in fjords ecosystems product of salmon farming, to determine the concentration and variation in time of different fractions of Fe in the seawater and plankton community under different NH_4^+ concentrations.

2 Materials and methods

2.1 Study area

Experiments were carried out during the austral summer season between January and February 2011 at the facilities of the Huinay Scientific Field Station (42°22′46″ S–72°25′12″ W) in the Comau fjord, Northern Patagonia (Fig. 1). The fjord hydrography features a two layer system with the presence of a permanent low salinity layer (LSL) between the surface and ~ 5 m, product of the mixing of freshwater (precipitation and river runoff) with oceanic water where the salinity regulates the formation of the pycnocline. The latter determined the reason for using two types of water to perform the experiments.

2.2 Mesocosms set up

A total of 10 (1 m^3) tanks were filled with water collected at depths of 2 (brackish) and 10 (marine) meters respectively, each one representing one treatment (Fig. 1). Water pumped into the tanks was collected with a peristaltic pump (Multifix type M80), placed in a peer and using plastic hose (35 mm diameter) projected 30 m offshore. Flowing water were pumped into a 33 L plastic collector, then afterwards equally distributed to each of the five tanks. The water was not prescreened, in order to contain the different taxonomic groups at the various trophic levels of the natural plankton assemblage.

2.3 Nutrient additions

In order to simulate nutrient enrichment occurring in the water column product of salmon aquaculture waste, tanks were supplied with four different concentration (treatments) of macronutrients (nitrogen, phosphorus and silicon) as ammonium chloride (NH_4Cl), sodium dihydrogen phosphate monohydrate ($NaH_2PO4{\cdot}H_2O$) and sodium metasilicate enneahydrate ($Na_2SiO_3 9{\cdot}H_2O$) every third day at a fix ratio. Although salmon aquaculture does not add silicon into the marine environment, it was supplied due to fjords ecosystem in southern Chile has continuous and in excess natural input of it, thus preventing potential nutrient limiting specifically for diatoms. The five units used per system, were denominated as Control, Natural, Conc 1, Conc 2 and Conc 3, where “Control” corresponded to the unit with no addition of nutrients, whereas “Natural”, received a nutrient input at the average ratio for N : P : Si, occurring in the natural environment (González et al., 2010, 2011; Iriarte et al., 2013). The three other units received the experimental nutrient concentrations from the lowest (Conc1) to the highest (Conc3) (Table 1).

2.4 Laboratory work

Processing of the samples collected in the field as performed under Class-100 laminar flow hood (AirClean 600 PCR Workstation) minimizing possible contamination, whereas laboratory worked was carried out in a class 1000 clean laboratory at the Department of Chemistry at NTNU. After processing, all samples were analyzed by High Resolution Inductive Coupled Plasma Mass Spectrometry (HR-ICP-MS) Element 2 (Thermo-Finnigan) with PFA-Schott type spray chamber and nebulizer.

2.5 Chelex samples

Samples for total Chelex labile (TFe_{Ch}) and dissolved Chelex labile (DFe_{Ch}) iron were collected in acid washed PE bottles, where a volume (∼ 150 mL) of water was collected and added 0.8 mL of Chelex-100 slurry (Bio-Rad Laboratories) (previously treated with Ammonium Acetate buffer ($C_2H_4O_2 \cdot NH_3$)). For the DFe_{Ch}, 0.2 µm acid washed filters (0.45 + 0.2 µm Sartorious Sartobran 300) and all-plastic syringes (PE) were used to filter the water. All samples were double bagged and placed in a shaker (65–80 rpm) for 48–72 h. After this period, each sample was transferred to an acid-washed plastic PE column (Bio-Rad Laboratories), where the water was washed out through the column, and the Chelex-100 containing the material was restrained by the resin present at the end of the column. Columns were first rinsed with Milli-Q water and secondly with ~ 10 mL of 0.1 M Ammonium Acetate buffer to remove the residue of seawater matrix, then afterwards packed and stored at 4 °C. In the clean laboratory, extraction of trace metals was done in a two-step acidifying process, obtaining a final 5 mL 0.6 M HNO_3 sample (Ardelan et al., 2010; Öztürk et al., 2002).

2.6 DGT samples

Diffusive gradient in thin films (DGT) (Zhang and Davison, 1995) samples were collected for labile iron (Fe_{DGT}), placing three DGT samplers in acid washed plastic containers with a known volume (~ 2000 mL) of water. Containers were placed in a shaker (60–80 rpm) for 48–72 h, then after emptied and samplers stored at 4 °C. In the clean laboratory, all DGT samplers were processed over a Teflon sheet, where the first two layers (filter and gel) were removed. The third layer, containing the gel with the resin was transferred to an acid washed PE tube and 4 mL 3 M UP HNO_3 was added. PE tubes containing the resin were put on a shaker at (60–80 rpm) for a 12 h period. Afterwards, content in the PE tubes were transferred to new acid-washed PE tubes, keeping out the gel with resin. To assured total transfer of material, 4 mL of Milli-Q water were used to rinse old tubes and then poured into the new ones, obtaining a final 5 mL 0.6 M HNO_3 sample (Ardelan et al., 2009).

2.7 Size fraction filtration

To determine the concentration and distribution of the particulate iron within the plankton community as a total (PFe) and in different size fractions (PFe_{SF}) present in the mesocosms, simple and sequential fractionation filtration were performed respectively. The former, implied filtration only through 0.2 µm, while the later, encompassed a range of five size classes: 0.8–2 µm (picoplankton), 2–10 µm (nanoplankton), 10–20 µm (larger nanoplankton), 20–140 µm (microplankton) and >140 µm (mesozooplankton). Filtration up to the 10 µm was performed with a simple filtration system fitted to a peristaltic pump and using acid washed filters (Isopore Membrane, polycarbonate, Hydrophilic, 0.2, 2, 10 µm, 47 mm), whereas filtration from 20, 140 and 220 µm fractions were performed with acid washed meshes (Nitex) with different pore size, with the retained material then afterwards washed into 0.2 µm filters. Filtration volumes ranged from ≥ 2000 mL for bigger fractions to a 100 mL for the smaller ones. Samples were kept frozen until further processing.

In the clean laboratory, samples went under High Performance Microwave Reactor (Ultra Clave UC Milestone) digestion, by placing the filters into Teflon tubes, adding 5 mL of 7 M UP HNO_3 and then inside the UC for two hours. After digestion process, samples were set to final dilution of 61 ± 0.3 mg. Original volume was recalculated (density of ultra-pure water 0.998 $gr\,cm^{-3}$ at room temperature), using initial volume filtered to obtain final concentration.

2.8 Blanks and detection limits

The detection limit used here is three times the standard deviation calculated from the measured method blank values. All values reported here, lie above the blank value determined, first subtracted from the blanks obtained from HR-ICP-MS values and then calculated to the appropriate concentration. Blanks and detection limits of the analysis performed in HR-ICP-MS for each technique are presented (Table 2) (Ardelan et al., 2010).

3 Results

3.1 Iron variability in the mesocosms seawater

Measurements for TFe_{Ch}, DFe_{Ch} and Fe_{DGT} along the experiment presented high variability between treatments and among the two systems (Fig. 2). Overall, TFe_{Ch} and DFe_{Ch} concentrations presented higher values on the marine compared to the brackish system. In the other hand, average Fe_{DGT} concentration for all treatments showed no difference between the brackish ($4.0 \pm 2.4\,\mathrm{nmol\,L^{-1}}$) and marine ($3.9 \pm 2.3\,\mathrm{nmol\,L^{-1}}$) system (Table 3). TFe_{Ch} distribution for every treatment in both systems, tended to follow the same distribution pattern in time, with an initial increase followed by a maximum and a posterior decrease (Fig. 2a and b). Mean TFe_{Ch} concentrations for the Control and Natural treatments in both systems presented the lowest values (brackish: $7.7 \pm 2.9\,\mathrm{nmol\,L^{-1}}$ and $11.9 \pm 2.8\,\mathrm{nmol\,L^{-1}}$ and marine: $12.7 \pm 5.4\,\mathrm{nmol\,L^{-1}}$ and $13.5 \pm 2.8\,\mathrm{nmol\,L^{-1}}$ respectively) among all five treatments (Table. 3). DFe_{Ch} distribution in time, exhibited lower range of variability compared to TFe_{Ch}, with a more consistent decreasing trend in time for both systems (Fig. 2c and d). Except for the Natural treatment in the marine system, final concentrations for all treatments in both systems presented lower values than initial ones (Table 3).

Conversely to TFe_{Ch} and DFe_{Ch}, Fe_{DGT} distribution in time showed no clear trend neither in the brackish nor in the marine system (Fig. 2e and f).Values in the brackish system ranged from very low $0.3 \pm 0.1\,\mathrm{nmol\,L^{-1}}$ up to $6.6 \pm 2.2\,\mathrm{nmol\,L^{-1}}$, while those in the marine system presented a broader range (up to $9.5 \pm 2.0\,\mathrm{nmol\,L^{-1}}$). In both systems the final concentrations in the Control and Natural treatments presented the highest values, being above initial values, whereas the final concentrations for the other treatments were below initial values. A comparison between treatment's final concentrations showed significant differences between the Natural and the three artificial nutrient addition treatments (Conc 1, Conc 2 and Conc 3) (1-way ANOVA; DF: 14, Holm–Sidak test), for both brackish and marine mesocosms.

3.2 TFe_{Ch}, DFe_{Ch}, Fe_{DGT} and NH_4^+ loading

No significant relationship was found between TFe_{Ch} and DFe_{Ch} and between DFe_{Ch} and Fe_{DGT} for all 5 treatments neither for the brackish nor the marine system. Yet, comparing TFe_{Ch} and DFe_{Ch} only for treatments with artificial nutrient addition (Conc 1, Conc 2 and Conc 3), a negative correlation ($R^2 = 0.535$) appears for these variables in the brackish system (Fig. 3a). Relative to the NH_4^+ loading, concentrations for TFe_{Ch} presented positive correlation for both the brackish ($R^2 = 0.606$) and marine ($R^2 = 0.839$) systems, reflecting the increasing trend time observed with increasing NH_4^+ concentration. Contrary to TFe_{Ch}, the DFe_{Ch} exhibited a negative trend in both systems, but not significant (Fig. 3b and c).

3.3 Iron variability in the mesocosms plankton community

PFe in all treatments presented a higher mean value ($116.3 \pm 75.1\,\text{nmol}\,\text{L}^{-1}$) in the marine compare to the brackish system ($80.6 \pm 44.6\,\text{nmol}\,\text{L}^{-1}$). Average in time for each treatment with artificial NH_4^+ addition in both systems, showed higher values compare to those of the Control and the Natural treatments. The trend exhibited by PFe content in the plankton biomass followed an increase along time and with increased NH_4^+ loading (Conc 3 treatment values in both systems, showed an noticeable decrease at the end of the experiment) (Fig. 4a). However, when standardized by the total Chlorophyll (Chl *a*) content, PFe : Chl *a* reflected an inverse trend with a decreasing concentration with increasing NH_4^+ loading along time in both systems (Fig. 4b). Same change in trend occurred when PFe was standardized by the particulate organic carbon (PFe : POC) (Fig. 4c). Furthermore, when standardized by and plotted against either Chl *a* or POC, the PFe the three artificial addition treatments showed a negative exponential correlation (R^2: 0.507 to 0.960), supporting the trend observed (Fig. 5).

Likewise PFe in the Control treatments, PFe_{SF} showed a decreasing trend from an initial concentration (all fractions added) of $95.9\,\text{nmol}\,\text{L}^{-1}$ to $32.9\,\text{nmol}\,\text{L}^{-1}$ (65.7 % reduction), and from 173.8 nmol L^{-1} to 70.1 nmol L^{-1} (59.7 % reduction) for the brackish and for the marine mesocosms respectively (Fig. 6). For the Natural treatment, no trend in time for any of the systems was observed. In the same way as PFe, all treatments with artificial nutrient addition showed higher total mean concentration than Control and Natural treatments. Mean total PFe$_{SF}$ concentrations for Conc 1, Conc 2 and Conc 3 of 168.8 ± 54.4, 176.0 ± 40.9 and 230.9 ± 85.6 nmol L^{-1} (mean ± SD) for the brackish and 210.2 ± 19.3, 219.6 ± 74.2 and 183.0 ± 116.4 nmol L^{-1} (mean ± SD) for the marine system, showed increasing trend with increased NH_4^+ loading (Fig. 6).

PFe$_{SF}$ in the 0.8–2 µm fraction as percentage of the total, remained in the same range with no significant changes among treatments in the brackish system. Yet, 0.8–2 µm fraction in the marine system, for all treatments with artificial nutrient addition showed a lower proportion (Conc 1: 10.9 % Conc 2: 17.5 % and Conc 3: 13.7 %) compare to Control (34.8 %) and Natural (35.3 %) treatments. In the case of the PFe$_{SF}$ in the fraction > 140 µm, it was appreciable an increase in the proportion in all treatments with artificial nutrient addition in the brackish (Conc 1: 24.9 %, Conc 2: 46.7 % and Conc 3: 16.7 %) compare to Control (11.6 %) and Natural (14.9 %)), and in the marine ((Conc 1: 20.8 % Conc 2: 35.9 % and Conc 3: 39.8 %) compare to Control (10.3 %) and Natural (12 %)) yet not significant in either case.

3.4 PFe$_{SF}$ in the 20–140 and 2–20 µm fraction

To compare PFe$_{SF}$ contained in the plankton community in the size spectrum from 2 to140 µm, the fractions 2–10 µm and 10–20 µm (containing the nanoplankton) were added and then compare against the 20–140 µm fraction (containing the microplankton) (Fig. 8a and c). In the brackish system, the microplankton – nanoplankton ratio (µ/n ratio) presented no significant differences between treatments (1-way ANOVA, DF: 14 P: 0.589). In the other hand, in the marine system the Control and Natural treatments presented mean ratios of 0.8 ± 0.2 and 0.8 ± 0.4 respectively, while Conc 1, Conc and Conc 3 mean ratios values were 2.4±0.4, 1.9±0.3 and 0.9±0.4 respectively.

Mean ratio for Conc 1 and Conc 2 presented significant differences respect to the Control and Natural (1-way ANOVA, DF: 14 P: < 0.002; Holm–Sidak method). Significant higher ratios imply that the $\mathrm{PFe_{SF}}$ in the 20–140 µm fractions increased its proportion relative to the 2–20 µm.

3.5 Phytoplankton assemblages

The total abundance of phytoplankton presented average higher values in the brackish systems compare to the marine, being proportional to the $\mathrm{NH_4^+}$ concentration in both cases i.e. Control and Natural presented the lowest values, while Conc 2 and Conc 3 presented the highest (Fig. 8b and d). Abundance by main groups revealed initial high values ($> 2 \times 10^6$ cell mL^{-1}) and dominance of nanoplankton in the brackish system, while diatoms and nanoplankton were both present in low numbers (3–7$\times 10^5$ cell mL^{-1}) in the marine system. A long time, for the brackish system it was observed a rapidly decline of the nanoplankton, maintaining low abundance in all treatments (except for some peaks), while in the marine system the nanoplankton remained low throughout the experiment in all treatments. On the contrary, diatoms in the treatments with $\mathrm{NH_4^+}$ addition grew steadily until the abrupt decline in both systems despite of the low initial values and the higher nanoplankton abundance in the brackish system (Fig. 8b and d). Concerning the diatom composition, no significant trend was found related to the water type or for $\mathrm{NH_4^+}$ concentration. Both systems showed the dominance of chain-forming centric diatoms as *Chaetoceros* spp. (medium and small sizes) and *Guinardia delicatula*. By the middle of the experiment, medium size *Chaetoceros* spp. represented $> 70\,\%$ in all treatments, both in the brackish and marine mesocosms.

4 Discussion

4.1 Iron variability in the mesocosms

The Fe distribution pattern appeared to be correlated to the POC and Chl *a* trends observed along the experiment. This implies a direct biological role on the changes in distribution of the different fractions of the element in the seawater (Fig. 7). TFe_{Ch} and DFe_{Ch} followed this pattern with opposite trends, particularly that of TFe_{Ch} exhibiting a remarkably similar trend to that followed by the POC. Given the observed decreasing concentration in DFe_{Ch}, the increase of TFe_{Ch} is presumed as a product of an increase in the particulate iron, via physical-chemical changes and/or uptake by the biota. A possible explanation could be that enhanced biological activity (e.g. release of organic ligands, exudates etc.) might have induced chemical changes in the speciation in a fraction of iron previously not available (e.g. complexed) to the chelex resin, forming newly dissolved iron (including colloids) that afterwards progressively transformed in time into TFe_{Ch}.

A relevant factor related to the observed results, was the high biomass production attained in the mesocosms. Compare to the natural productivity of the region (Iriarte et al., 2007, 2013; González et al., 2010, 2011), the concentrations of Chl *a* and POC observed in the treatments, were higher (due to artificial nutrient addition) and thus contributing to the formation of aggregate material at high rate (noticeable at simple observation). Possible results of this type of particle aggregation could have been enhanced adsorption of the dissolved form onto the aggregates and coagulation of colloids, therefore removing portion of the dissolved fraction and transforming it into particulate. Wong et al. (2006) in a mesoscale experiment reported a quick transformation of the dissolved iron to particulate forms, with as much as 70 % of the added iron transformed in the non-dissolved form after less than 24 h. The trend described lower colloidal iron percentages as the experiment progressed while the particulate fractions increased. The mechanism alleged to be involved, could be a combination of biological uptake (Chen et al., 2003; Nodwell and Price, 2001) or simply adsorption of dissolved (including colloidal) iron to the plankton cell surfaces as well as aggregation of oxyhydroxides (Wong et al., 2006). Accordingly, the decrease observed in TFe_{Ch} towards the end of the experiment, coupled to that of Chl *a* and POC, seems to point to the settlement of the dying phytoplankton, and therefore suggesting that the PFe attached was eventually exported to the bottom of the mesocosm tanks.

Among other factors that could account for the poor correlation between different fractions of iron, mechanical artifact could be an important one. It is known that colloids encompasses a size range from 1 to 1000 nm in diameter (Wells, 1998), lying within the boundaries of the dissolved and particulate matter, consequently subjected to possible bias via artificial manipulation. Moreover, colloid production rates could be enhanced by biological action, presumably through a combination of cell exudation and lysis, microbial degradation of particulate organic matter, sloppy feeding and excretion by zooplankton (Wells and Goldberg, 1994), all factors which were gradually increased in the experiment by the NH_4^+ input. The factors above combined, could have modulated the increase of TFe_{Ch}, and therefore the significant positive linear correlation with NH_4^+ input and TFe_{Ch}. The rather poor negative correlation for DFe_{Ch} and NH_4^+ could be related to the mismatch between newly DFe_{Ch} made available and the uptake by phytoplankton, making an expected decreasing DFe_{Ch} trend less evident.

In the case of the Fe_{DGT}, the fact that the average concentration for all treatments was lower than that of DFe_{Ch}, would suggest that the chelex labile dissolved fraction, was probably not all readily bioavailable. Nevertheless, when looking at the trends in time between Fe_{DGT} and DFe_{Ch}, it was evident the high variation, exhibiting at some points opposite trends even with Fe_{DGT} values higher than the DFe_{Ch} (Fig. 7a and c). The average ratio between DFe_{Ch} and Fe_{DGT}, higher for the marine (1.44) compared to the brackish (1.27), would suggest differences in the proportion of the bioavailable fraction between the systems. Yet, given such variability the latter could not prove significant, but rather to establish a gross pattern. Observing the trends in time, it can be argued that the mechanism behind Fe_{DGT} high variability, might be the response to a dynamic system in which biological (release of organic ligands) and chemical (kinetics and equilibrium) forcing determine changes in the iron speciation in short periods of time. Nonetheless, despite all the variation in time, at the end of the experiment the significant lower Fe_{DGT} concentrations for all the treatments with NH_4^+ compared to the Natural one, reflected the decrease of the bioavailable fraction of iron as consequence of the increased uptake by the growing phytoplankton biomass.

Further sources of variability affecting the results could be invoked. Although carefully performed, the fieldwork carried out was not under 100 % ultra clean conditions, leaving room for possible contamination. Indeed, the high peaks obtained for both TFe_{Ch} and DFe_{Ch} samples appeared to be clear sample artifact. Moreover, provided methodological errors were ruled out, other source of high variation could have been the enclosed system and the conditions set up. Such case could be the NH_4^+ rate supply ($4.6\,\mu mol\,L^{-1}\,d^{-1}$) applied in treatment Conc 3. A significant high concentration (Olsen et al., 2006) was applied to expect for possible toxic effects, in that way exposing the plankton community to drastic changes in short periods of time that would rather not occurred in natural environment.

4.2 Iron content in the planktonic community

Determination of the PFe and PFe_{SF}, aimed to provide an estimation of the particulate iron relative to the particulate carbon pool in the system and hence within the planktonic community revealed changes occurred in time of the concentration and distribution. The results, presumably reflects the effects of the NH_4^+ input over the planktonic composition in both systems. PFe standardized both by POC and Chl *a*, showed a negative exponential relation with NH_4^+, for both the brackish and marine systems, while PFe_{SF} (although not standardized due to lack of fractionated data for POC and Chl *a*) showed for two treatments in the marine system a significant change of the ratio of the absolute iron content between two fractions (micro and nano) of the plankton community. The observed differences for the brackish and marine systems, can be attributed to a great extent to the biological features (different microbial assemblages) under constant physical forcing (i.e. presence of permanent LSL) and other hydrographic parameters proper of fjord ecosystems (Pickard, 1971). The LSL particularly affects the water column features, reducing light penetration, nutrient exchange and limiting wind-induced mixing, during period of strong water column stratification (Gibbs, 2001). The result is an environmental partitioning, with an often less productive (primary productivity) brackish layer based on nutrient recycling, dominated regularly by the nano-plankton size class, whereas below the halocline a marine layer with peak productivity (10–15 m) based on constant nutrient input (deep nutrient-rich waters) and with micro-phytoplankton as the dominant component (Sánchez et al., 2011).

Base on the higher Fe µ/n ratio observed in the marine system, could be suggested that micro-phytoplankton (diatoms in particular), took advantage of the NH_4^+ input at less energy expense, with a consequent probable lesser preference over nitrate. Therefore, a plankton community dominated by big diatoms could be expected to be resembled in the marine system. Yet this was the case, it was also evident that diatoms outweighed in abundance and certainly biomass the nanoplankton fraction in both systems. The evidence of successful growth of diatoms based on NH_4^+ is substantial. The diatom *Thalassiosira pseudonana* was exhibited an 8 % increase in growth based on NH_4^+ compared to NO_3^-, under saturating light and Fe-replete conditions (Thompson et al., 1989), while another study found higher growth rates for ammonium-grown cells than for nitrate-grown cells of several species under the same conditions (Levasseur et al., 1993). In the other hand, an oceanic diatom isolated from the subarctic Pacific was found to have no difference in the growth rates of nitrate- and ammonium-grown cells under Fe-replete conditions (Muggli et al., 1996). It is worth noting that NO_3^- (not added as nutrient), concentrations in the marine system showed a decreasing trend in the Control and Natural, while in the treatments with artificial addition NO_3^- concentration remained constant along the experiment (data not shown), thus supporting the idea of preferential ammonium uptake. Nevertheless, Price et al. (2005) found contrasting results when growing diatoms with NO_3^- and NH_4^+ under high and low Fe-mediums. While the iron quota (Q) was higher for NO_3^- under low Fe, at high Fe, was higher for

NH_4^+ grown cells. Main reasons pointed out referenced that the growth conditions and species differences may be responsible for the contrasting results.

As the Fe µ/n ratio here discussed, the Fe : C ratio is determined mainly by the differences in the community species composition and its physiological state. Contrast to relatively constant Redfield ratios of C : N : P, the cellular Fe : C ratios vary markedly (by a factor of 30) as a function of the iron available (Bruland et al., 2001). Hence, most of the data available on estimation of trace element quotas come from laboratory cultures (e.g. Sunda and Huntsman, 1995), with still few data from natural environments (Cullen and Sherrell, 1999). In such a way, in the scenario with the natural community studied here, it could be expected a high variability but with a tendency to reducing iron uptake, due to the conditions of NH_4^+ excess supply and diatoms dominance (Sunda and Huntsman, 1995). Accordingly, the POC based Fe : C ratio obtained per treatment on both systems, followed a rather decreasing trend on time with increasing NH_4^+ concentration (Fig. 8b and d). Yet, it is observed a more pronounced reduction for the brackish mesocosms despite that initial Control and Natural values were higher compared to marine ones. Given the dominance of diatoms in both systems, it could be argued these would reflect species-specific differences (e.g. diatom species with different Fe requirements). Nevertheless, the diatom composition found (at the genus level) in both systems did not differ significantly to account for the different slopes observed in Fe : C in time.

On the other hand, looking on the composition within the nanoplankton size fraction it is observed that nanoflagellates made up in average double (5 % versus 10 %) of the total abundance in the brackish system compare to the marine. Pico- and nanoplankton with higher surface to volume ratios and a more efficient uptake at low nutrient concentrations (Chisholm, 1992; Price et al., 1994), thrive in environments were the main source of nitrogen is recycled like NH_4^+, hence having lower Fe requirements. Conversely, diatoms in general have a higher requirement on iron to satisfy certain metabolic demands (Bruland et al., 2001), even more so for coastal assemblages since have been shown to have an order-of-magnitude higher iron requirement (on a Fe : C basis) than oceanic species (Sunda and Huntsman, 1995). For this reason, could be argued that nanoflagellates by having a higher contribution to the carbon pool to the brackish system, would have a consequently decrease in the iron to carbon proportion (Schmidt and Hutchins, 1999; Sunda and Huntsman, 1997), whereas diatoms (higher contribution in the marine system), needing to meet minimum Fe requirements, could account for the rather smoothly decrease in Fe : C ratio observed in the marine system.

Despite the reduction on Fe : C ratio, estimated ratios as a proxy for the Fe content of plankton community in Comau fjord during the experiment covered a broad range (265 up to 4224 µmol mol^{-1}). Medians values (more representative here) were between 419 to 1430 and 753 to 1765 µmol mol^{-1} for the brackish and marine mesocosms respectively, situating these values above to what is reported on the literature for phytoplankton (2.3 to 370 µmol mol^{-1}) (Sunda and Huntsman, 1995; Ho et al., 2003; Sarthou et al., 2005). Values reported here account for the potential total cellular iron, not distinguishing between external and intracellular iron content. Reported values range from 63 to 90 % of intracellular iron of total cellular iron under Fe limiting conditions for different cell diameter and phytoplankton class species (Sunda and Huntsman, 1995). Moreover, the study reports intracellular max concentrations of 1700 µmol mol^{-1} for diatom species at high Fe conditions, 30 times higher than needed to support maximum growth rate. Therefore, the values reported here could be reduced by at least 20 %. For further comparisons, a range of carbon to chlorophyll ratios from literature and the Fe : Chl found here, to estimate Fe : C by other means (Table 4). Values obtained were higher than the POC based Fe : C yet, the trend was consistent, thus seeing a decrease in the Fe : C at higher NH_4^+.

When addressing the iron to carbon ratio, it was considered that Chl *a* only account for the autotrophic component of the plankton community (i.e. diatoms, autotrophic dinoflagellates and autotrophic nanoflagellates), whereas POC render the complete amount of organic carbon in the whole community (i.e. including bacteria and protists). Likewise, POC measurements include all organic carbon whether of autochthonous or allochthonous origins, which might be of particular relevance in fjords ecosystem subjected to constant input of terrigenous origin (Syvitski et al., 1987; Vargas et al., 2011). Northern Patagonia fjords, where the Comau fjord is located, are not influenced by glaciers and thus have significantly lower loads of fine inorganic sediments than most fjords further south (Pickard, 1971), for which the input of marine-derived organic carbon on surface sediments varied widely and accounted for 13–96 % (average 61 %) (Sepúlveda et al., 2011). Yet, a study for Comau fjord reports allochthonous contributions to the sediments of 23.6 to 89.9 % at depths of 35 and 475 m respectively (Silva et al., 2011). In this sense, it is important to highlight that this high variability could have a significant effect on the composition of the water collected for the experiments, in particular the brackish system. The pycnocline formed by fresh water input act as barrier, therefore retarding the water mixing, with the probable consequence of temporary accumulation of organic carbon of allochthonous origin in the first meters of water column. As observed in the initial POC content, the brackish water showed a three-fold difference ($263.9 \pm 23.3\,\mathrm{mg\,m^{-3}}$) compare to the marine ($70.1 \pm 11.8\,\mathrm{mg\,m^{-3}}$) mesocosm, hence increasing the carbon to iron ratio and simulating a lower Fe : C in the plankton community for the brackish mesocosms. Regardless the above, and given that both Chl and POC related to the iron showing same trend (exponential decrease), the POC based Fe : C ratio estimation was still considered a reliable approach.

5 Summary

The addition of NH_4^+ in our experimental set up showed that there was an effect on the distribution of the different forms of iron measured in the seawater as well as in the particulate matter representing the content of iron in the plankton community.

The response for both type of water was different in magnitude; the effect depending on the iron form measured and was correlated either positively or negatively with increased NH_4^+ concentration, as seen from the TFe_{Ch} and DFe_{Ch} correlation in the brackish water. The physical-chemical changes observed in Fe measured in the sea water, suggest that apart from dissolvable Fe, TFe (non-filtered) is related to the biological uptake, and that under certain conditions should not be neglected as potential bioavailable source of Fe.

In presence of excess nutrients was observed dominance of microphytoplankton and decrease in Fe : C in both water types. However, the fact that Fe µ/n and Fe : C ratios, species composition and parameters like Chl *a* and POC were considerably different, highlights the key role of the microbial assemblage within each type of water and therefore in the natural environment. Determining *Q* of certain key species is important to infer over the physiological state, adaptation to environment or possible growth limitation in phytoplankton and therefore useful to understand possible ecosystem changes (e.g. a phytoplankton community with a low Fe : C but rather high Chl *a* yield, as seeing here, could result in higher efficiency in carbon export).

Likewise microcosm essays, these results are product of enclosed manipulated systems and therefore interpretations must take this into account. Natural systems are complex and this could be seeing from the differences observed in the brackish and marine systems. Consequently, to better understand the impact that anthropogenic influence (e.g. increase input of NH_4^+ via salmon aquaculture) can cause over the cycling of iron and presumably in a similar way to the biogeochemical cycling of major elements (C, N, and P) in the pelagic ecosystem such as in the fjords of Chile, more emphasis needs to be allocated to study the links between macro and micro-nutrients cycles and he role of the marine biota within.

Acknowledgements. This work was part of the project “CAN WASTE EMISSION FROM FISH FARMS CHANGE THE STRUCTURE OF MARINE FOOD WEBS (WAFOW)? – A comparative study of coastal ecosystems in Norway and Chile (project 193661), and the project “A Cross-disciplinary Integrated Eco-systemic Eutrophication Research and Management Approach – CINTERA” (project 216607), both funded by NTNU – Norwegian Research Council. This work was partially supported by CONICYT of the Chilean government, through the FONDECYT research project (1110614).

We would like to thank the scientists and staff of the Fundacion San Ignacio del Huinay science station for their valuable support and the logistics provided during sampling. We also greatly appreciate the comments of the anonymous reviewer, who help us to considerably improve the manuscript. This is publication 116 of the Huinay Scientific Field Station.

References

Ardelan, M. V., Steinnes, E., Lierhagen, S., and Linde, S. O.: Effects of experimental CO_2 leakage on solubility and transport of seven trace metals in seawater and sediment, Sci. Total Environ., 407, 6255–6266, doi:10.1016/j.scitotenv.2009.09.004, 2009.

Ardelan, M. V., Holm-Hansen, O., Hewes, C. D., Reiss, C. S., Silva, N. S., Dulaiova, H., Steinnes, E., and Sakshaug, E.: Natural iron enrichment around the Antarctic Peninsula in the Southern Ocean, Biogeosciences, 7, 11–25, doi:10.5194/bg-7-11-2010, 2010.

Arrigo, K. R.: Marine microorganisms and global nutrient cycles, Nature, 437, 349–355, doi:10.1038/nature04159, 2005.

Bruland, K. W., Rue, E. L., and Smith, G. J.: Iron and macronutrients in California coastal upwelling regimes: implications for diatom blooms, Limnol. Oceanogr., 46, 1661–1674, 2001.

Chen, M., Dei, R. C. H., Wang, W. X., and Guo, L.: Marine diatom uptake of iron bound with natural colloids of different origins, Mar. Chem., 81, 177–189, 2003.

Chisholm, S. W.: Phytoplankton Size, Primary Productivity and Biogeochemical Cycles in the Sea, Plenum, 213–237, 1992.

Cloern, J. E.: Our evolving conceptual model of the coastal eutrophication problem, Mar. Ecol.-Prog. Ser., 210, 223–253, 2001.

Cullen, J. T. and Sherrell, R. M.: Techniques for determination of trace metals in small samples of size-fractionated particulate matter: phytoplankton metals off central California, Mar. Chem., 67, 233–247, 1999.

Field, C. B., Behrenfeld, M. J., Randerson, J. T., and Falkowski, P.: Primary production of the biosphere: integrating terrestrial and oceanic components, Science, 281, 237–240, 1998.

Gibbs, M. T.: Aspects of the structure and variability of the low-salinity-layer in Doubtful Sound, a New Zealand fiord, New Zeal. J. Mar. Fresh., 35, 59–72, doi:10.1080/00288330.2001.9516978, 2001.

González, H. E., Calderón, M., Castro, L., Clément, A., Cuevas, L., Daneri, G., Iriarte, J., Lizárraga, L., Martínez, R., and Menschel, E.: Primary production and plankton dynamics in the

Reloncaví Fjord and the Interior Sea of Chiloé, Northern Patagonia, Chile, Mar. Ecol.-Prog. Ser., 402, 13–30, 2010.

González, H. E., Castro, L., Daneri, G., Iriarte, J., Silva, N., Vargas, C., Giesecke, R., and Sánchez, N.: Seasonal plankton variability in Chilean Patagonia fjords: carbon flow through the pelagic food web of Aysen Fjord and plankton dynamics in the Moraleda Channel basin, Cont. Shelf Res., 31, 225–243, 2011.

Ho, T. Y., Quigg, A., Finkel, Z. V., Milligan, A. J., Wyman, K., Falkowski, P. G., and Morel, F. M. M.: The elemental composition of some marine phytoplankton, J. Phycol., 39, 1145–1159, 2003.

Iriarte, J. L., González, H., Liu, K., Rivas, C., and Valenzuela, C.: Spatial and temporal variability of chlorophyll and primary productivity in surface waters of southern Chile (41.5–43 S), Estuar. Coast. Shelf S., 74, 471–480, 2007.

Iriarte, J. L., Pantoja, S., González, H., Silva, G., Paves, H., Labbé, P., Rebolledo, L., Ardelan, M., and Häussermann, V.: Assessing the micro-phytoplankton response to nitrate in Comau Fjord (42° S) in Patagonia (Chile), using a microcosms approach, Environ. Monit. Assess., 185, 5055–5070, doi:10.1007/s10661-012-2925-1, 2013.

Levasseur, M., Thompson, P. A., and Harrison, P. J.: Physiological acclimation of marine phytoplankton to different nitrogen source, J. Phycol., 29, 587–595, 1993.

Martin, J. H.: Iron, Liebig's law, and the greenhouse, Oceanography, 4, 52–55, 1991.

Martin, J. H., Gordon, M. R., and Fitzwater, S.: The case for iron, Limnol. Oceanogr., 36, 1793–1802, 1991.

Morel, F. M. M. and Price, N.: The biogeochemical cycles of trace metals in the oceans, Science, 300, 944–947, 2003.

Morel, F. M. M., Rueter, J., and Price, N.: Iron nutrition of phytoplankton and its possible importance in the ecology of ocean regions with high nutrient and low biomass, Oceanography, 4, 56–61, 1991.

Muggli, D. L., Lecourt, M., and Harrison, P.: Effects of iron and nitrogen source on the sinking rate, physiology and metal composition of an oceanic diatom from the subarctic Pacific, Mar. Ecol.-Prog. Ser., 132, 215–227, 1996.

Nodwell, L. M. and Price, N. M.: Direct use of inorganic colloidal iron by marine mixotrophic phytoplankton, Limnol. Oceanogr., 46, 765–777, 2001.

Öztürk, M., Steinnes, E., and Sakshaug, E.: Iron speciation in the Trondheim Fjord from the perspective of iron limitation for phytoplankton, Estuar. Coast. Shelf S., 55, 197–212, doi:10.1006/ecss.2001.0897, 2002.

Olsen, Y., Agustí, S., Andersen, T., Duarte, C. M., Gasol, J. M., Gismervik, I., Heiskanen, A. S., Hoell, E., Kuuppo, P., and Lignell, R.: A comparative study of responses in planktonic food web structure and function in contrasting European coastal waters exposed to experimental nutrient addition, Limnol. Oceanogr., 51, 488–503, 2006.

Pickard, G.: Some physical oceanographic features of inlets of Chile, J. Fish. Res. Board Can., 28, 1077–1106, 1971.

Price, N. M., Ahner, B., and Morel, F.: The equatorial Pacific Ocean: grazer-controlled phytoplankton populations in an iron-limited ecosystem, Limnol. Oceanogr., 39, 520–534, 1994.

Sánchez, N., González, H. E., and Iriarte, J. L.: Trophic interactions of pelagic crustaceans in Comau Fjord (Chile): their role in the food web structure, J. Plankton Res., 33, 1212–1229, doi:10.1093/plankt/fbr022, 2011.

Sarthou, G., Timmermans, K. R., Blain, S., and Tréguer, P.: Growth physiology and fate of diatoms in the ocean: a review, J. Sea Res., 53, 25–42, 2005.

Schmidt, M. and Hutchins, D.: Size-fractionated biological iron and carbon uptake along a coastal to offshore transect in the NE Pacific, Deep-Sea Res. Pt. II, 46, 2487–2503, 1999.

Sepúlveda, J., Pantoja, S., and Hughen, K. A.: Sources and distribution of organic matter in northern Patagonia fjords, Chile ($\sim$ 44–47° S): a multi-tracer approach for carbon cycling assessment, Cont. Shelf Res., 31, 315–329, 2011.

Silva, N., Vargas, C. A., and Prego, R.: Land–ocean distribution of allochthonous organic matter in surface sediments of the Chiloé and Aysén interior seas (Chilean Northern Patagonia), Cont. Shelf Res., 31, 330–339, 2011.

Sunda, W. G. and Huntsman, S. A.: Iron uptake and growth limitation in oceanic and coastal phytoplankton, Mar. Chem., 50, 189–206, doi:10.1016/0304-4203(95)00035-p, 1995.

Sunda, W. G. and Huntsman, S. A.: Interrelated influence of iron, light and cell size on marine phytoplankton growth, Nature, 390, 389–392, 1997.

Syvitski, J. P., Burrell, D. C., and Skei, J. M.: Fjords: Processes and Products, 1987.

Thompson, P. A., Levasseur, M. E., and Harrison, P. J.: Light-limited growth on ammonium vs. nitrate: what is the advantage for marine phytoplankton?, Limnol. Oceanogr., 34, 1014–1024, 1989.

Vargas, C. A., Martinez, R. A., San Martin, V., Aguayo, M., Silva, N., and Torres, R.: Allochthonous subsidies of organic matter across a lake-river-fjord landscape in the Chilean Patagonia: implications for marine zooplankton in inner fjord areas, Cont. Shelf Res., 31, 187–201, 2011.

Wang, W. X., Dei, R. C. H., and Xu, Y.: Cadmium uptake and trophic transfer in coastal plankton under contrasting nitrogen regimes, Mar. Ecol.-Prog. Ser., 211, 293–298, 2001.

Wells, M. L.: Marine colloids: a neglected dimension, Nature, 391, 530–531, 1998.

Wells, M. L. and Goldberg, E. D.: The distribution of colloids in the North Atlantic and Southern Oceans, Limnol. Oceanogr., 39, 286–302, 1994.

Wong, C. S., Johnson, W. K., Sutherland, N., Nishioka, J., Timothy, D. A., Robert, M., and Takeda, S.: Iron speciation and dynamics during SERIES, a mesoscale iron enrichment experiment in the NE Pacific, Deep-Sea Res. Pt. II, 53, 2075–2094, doi:10.1016/j.dsr2.2006.05.037, 2006.

Zehr, J. P. and Ward, B. B.: Nitrogen cycling in the ocean: new perspectives on processes and paradigms, Appl. Environ. Microb., 68, 1015–1024, 2002.

Zhang, H. and Davison, W.: Performance characteristics of diffusion gradients in thin films for the in situ measurement of trace metals in aqueous solution, Anal. Chem., 67, 3391–3400, 1995.

Table 1. Rate supply ($\mu mol\,m^{-3}\,d^{-1}$) and ratio for the different macronutrients added as NH_4Cl for Nitrogen (N), $NaH_2PO4 \cdot H_2O$ for Phosphorus (P) and $Na_2SiO_3 \cdot 9H_2O$ for Silicon (Si) in the different treatments for the brackish (6–10) and marine systems (1–5), in the mesocosm experiment. Control units had no nutrient addition.

Treatment	Mesocosm	N	P	Si	N : P	N : Si
Control	1–6	–	–	–	–	–
Natural	2–7	296.0	19.4	146.8	15.3	2
Conc 1	3–8	1199.7	49.7	594.7	24.2	2
Conc 2	4–9	2991.3	123.8	1483.0	24.2	2
Conc 3	5–10	4674.0	193.5	2317.2	24.2	2

Table 2. Concentration (nmol L^{-1}) relative standard deviation (RSD %), for the blanks analyzed in HR-ICP-MS for the chelex, DGT and fractionation samples in the mesocosm experiment Filter: filter pore size. Std: standard deviation. C. Int 95 %: confidence interval 95 %. C. Int. 95 % (%): confidence interval 95 % percentage.

Technique	Chelex		DGT		Fractionation		
Replicate	nmol L^{-1}	RSD	nmol L^{-1}	RSD	nmol L^{-1}	RSD	Filter
1	0.02	0.01	0.04	0.05	0.02	0.15	0.8
2	0.01	0.10	0.02	0.08	0.01	0.06	0.8
3	0.02	0.01	0.02	0.03	0.02	0.14	0.8
4	0.03	0.09	0.03	0.05	0.01	0.02	2
5	0.04	0.05	0.03	0.08	0.02	0.12	2
6			0.04	0.08	0.01	0.01	2
7			0.03	0.05	0.01	0.03	10
8			0.03	0.09	0.02	0.01	10
9					0.01	0.08	10
Average	0.02	0.05	0.03	0.06	0.02	0.07	
Std	0.01	0.04	0.01	0.02	0.00	0.06	
Rsd (%)	39.07	80.89	23.79	34.52	30.86	80.44	
C. Int. 95 %	0.01	0.04	0.01	0.02	0.00	0.04	
C. Int. 95 % (%)	39.07		17.98		21.82		
Number	5	5	8	8	9	9	

Table 3. Concentration (nmol L^{-1}) for total Chelex labile (TFe_{Ch}), dissolved Chelex labile (DFe_{Ch}) and DGT labile (Fe_{DGT}) iron for all treatments in both the brackish and marine system in the mesocosm experiment.

		TFe_{Ch}					DFe_{Ch}					Fe_{DGT}				
	Sday	Con	Nat	Conc1	Conc2	Conc3	Con	Nat	Conc1	Conc2	Conc3	Con	Nat	Conc1	Conc2	Conc3
Brackish	1	8.3	8.3	8.3	8.3	8.3	8.2	8.2	8.2	8.2	8.2	4.7 ± 1.8	4.7 ± 1.8	4.7 ± 1.8	4.7 ± 1.8	4.7 ± 1.8
	2	9.4	13.3	9.3	7.1	15.1	8.0	3.5	4.9	6.9	5.4	2.6 ± 1.5	0.3 ± 0.1	3.4 ± 2.6	0.3 ± 0.0	2.1 ± 2.9
	3	10.3	12.1	14.9	14.5	23.6	5.2	4.6	4.7	6.4	4.3				3.7 ± 2.6	2.3 ±1.0
	5	7.6	15.6	24.2	25.6	20.2	1.4	6.9	2.0	3.7	2.8	6.3 ± 2.4	6.3 ± 1.7	6.6 ± 2.2	6.2 ± 0.1	5.7 ± 1.7
	8	2.9	10.0	15.9	24.6	9.6	1.0	5.4	4.6	2.8	2.7	4.3 ± 1.9	6.3 ± 0.5	2.4 ± 0.4	2.4 ± 0.8	0.9 ±0.8
Marine	1	13.1	13.1	13.1	13.1	13.1	6.1	6.1	6.1	6.1	6.1	4.1 ± 1.4	4.1 ± 1.4	4.1 ± 1.4	4.1 ± 1.4	4.1 ± 1.4
	2		11.7				6.2	6.3	12.1					1.5 ± 0.5	2.5 ± 3.2	
	3	12.0	15.1	16.2	21.0	19.9	7.5	7.7	6.8	6.4	4.4				2.6 ± 0.5	4.3 ± 3.5
	5	9.4	17.4	31.7	30.4	23.3	6.2	6.8	4.2	4.8	7.4	2.4 ± 0.9	2.1 ± 1.1	4.3 ± 1.3	8.2 ± 1.7	1.8 ±1.1
	8	7.5	10.4	24.3	14.1	9.2	6.6	3.5	2.9	2.8	3.6	9.5 ± 2.1	6.3 ± 2.6	2.9 ± 0.5	2.8 ± 0.9	2.1 ± 0.4

Table 4. Chlorophyll to carbon ratio (Chl : C) (mol mmol^{-1}) and iron to carbon ratio (Fe : C) (µmol mol^{-1}) for a range of values for *T. pseudomona* (Sunda and Huntsmann, 1995). Fe : C (µmol mol^{-1}) based on Fe : Chl (µmol mmol^{-1}) and Chl : C from literature, for all the treatments in both the brackish and marine system in the mesocosm experiment.

Reference			Control		Natural		Conc 1		Conc 2		Conc 3	
Chl : C	Fe : C		Fe : Chl	Fe : C	Fe : Chl	Fe : C	Fe : Chl	Fe : C	Fe : Chl	Fe : C	Fe : Chl	Fe : C
0.127	12.5	Brackish	59968.7	7616.0	121318.2	15407.4	24971.3	3171.4	28578.5	3629.5	9552.5	1213.2
0.258	1770.0			15471.9		31300.1		6442.6		7373.2		2464.6
		Marine	53320.7	6771.7	43888.3	5573.8	62126.1	7890.0	47562.2	6040.4	13315.0	1691.0
				13756.7		11323.2		16028.5		12271.0		3435.3

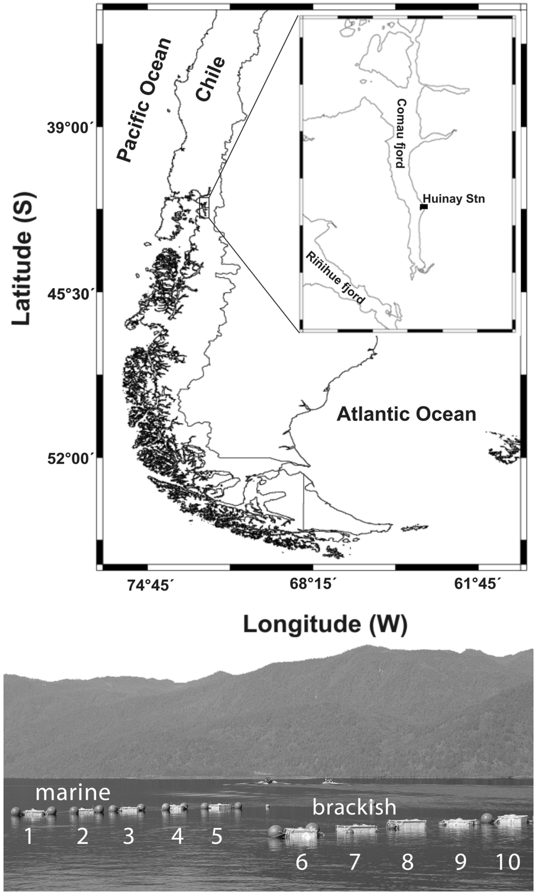

Figure 1. Study area, sampling site (Huinay Stn) and deployment of the brackish and marine systems for the mesocosm experiment in the Comau Fjord, Chile during January–February 2011.

Title Page
Abstract
Introduction
Conclusions
References
Tables
Figures
I◄
►I
◄
►
Back
Close
Full Screen / Esc
Printer-friendly Version
Interactive Discussion

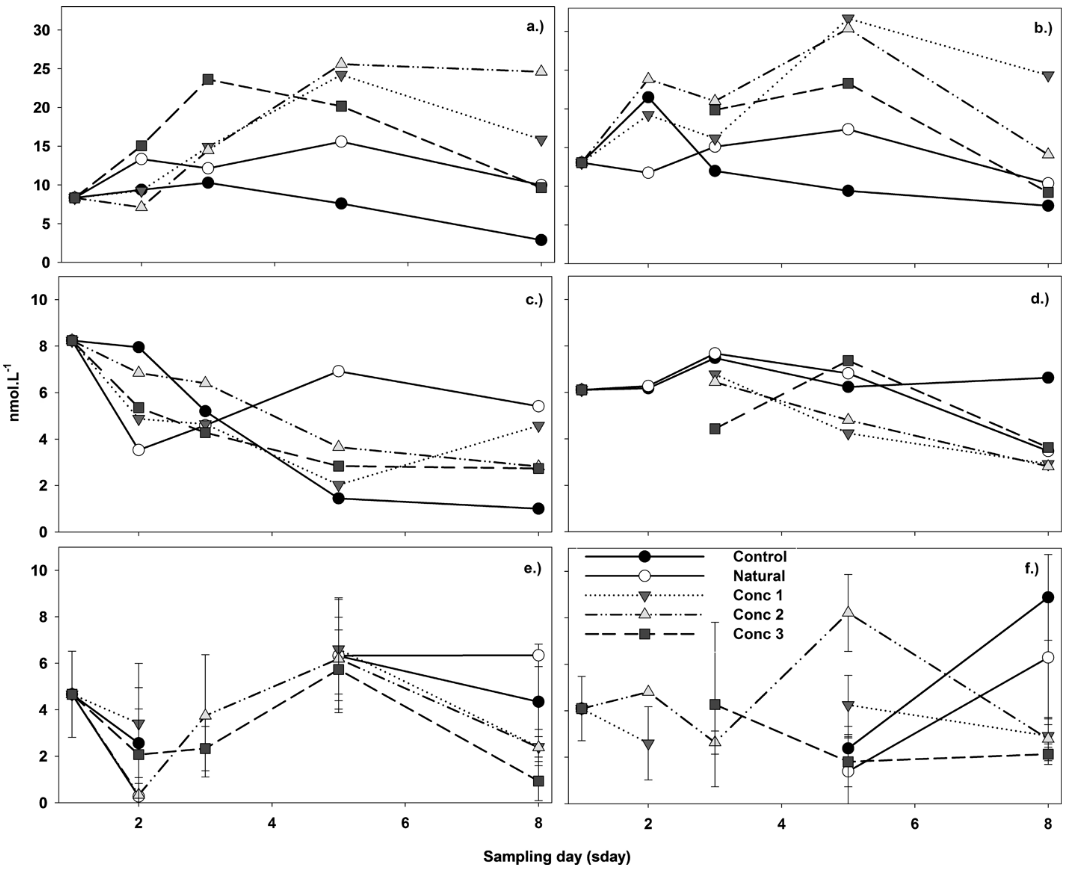

Figure 2. Total Chelex labile iron (TFe_{Ch}) **(a, b)**, dissolved Chelex labile iron (DFe_{Ch}) **(c, d)** and DGT labile iron (Fe_{DGT}) **(e, f)** (nmol L^{-1}), in the brackish (left) and marine (right) systems for all treatments in the mesocosm experiment. Gap between points: missing data. Error bars: standard deviation ($n = 3$).

Title Page
Abstract | Introduction
Conclusions | References
Tables | Figures
◂| |▸
◂ ▸
Back | Close
Full Screen / Esc
Printer-friendly Version
Interactive Discussion

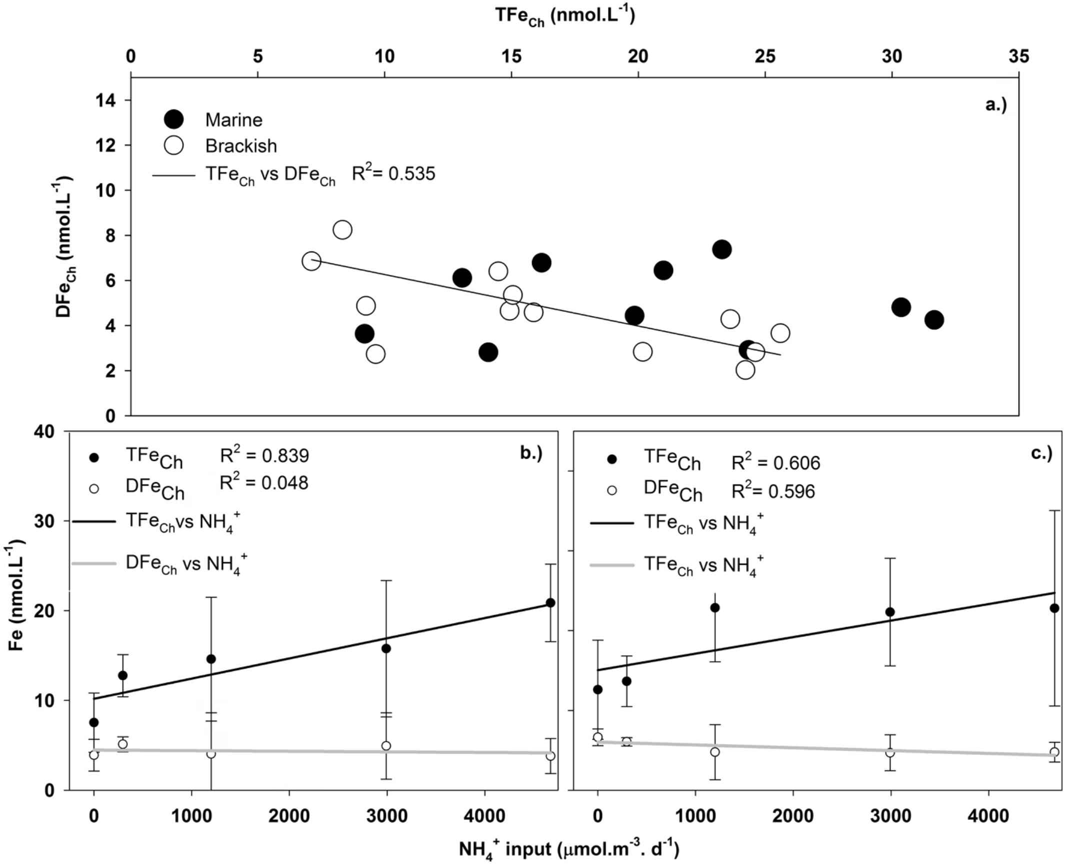

Figure 3. Relation between **(a)** TFe$_{Ch}$ (nmol L^{-1}) vs. DFe$_{Ch}$ (nmol L^{-1}) for both systems (Conc 1, Conc 2, Conc3), TFe$_{Ch}$ and DFe$_{Ch}$ vs. NH$_4^+$ loading in the **(b)** brackish and **(c)** marine systems for all treatments in the mesocosm experiment. Error bars: standard deviation (*n* = 5).

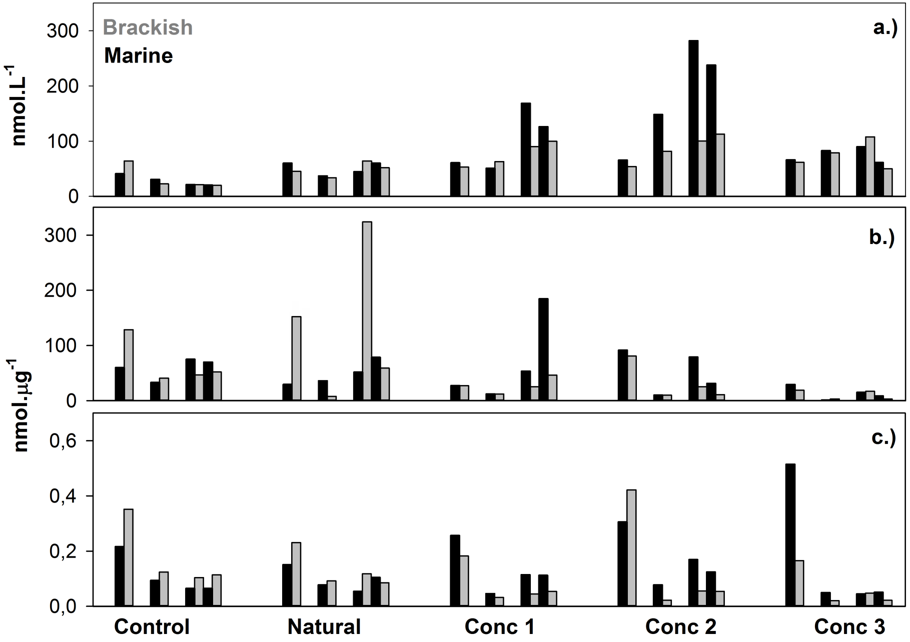

Figure 4. Distribution of **(a)** PFe (nmol L^{-1}), standardized by **(b)** chlorophyll *a* (Chl *a*) (nmol ugr^{-1}) (PFe : Chl *a*) and **(c)** particulate organic carbon (POC) (nmol ugr^{-1}) (PFe : POC), for all treatments along time in the brackish and marine systems in the mesocosm experiment.

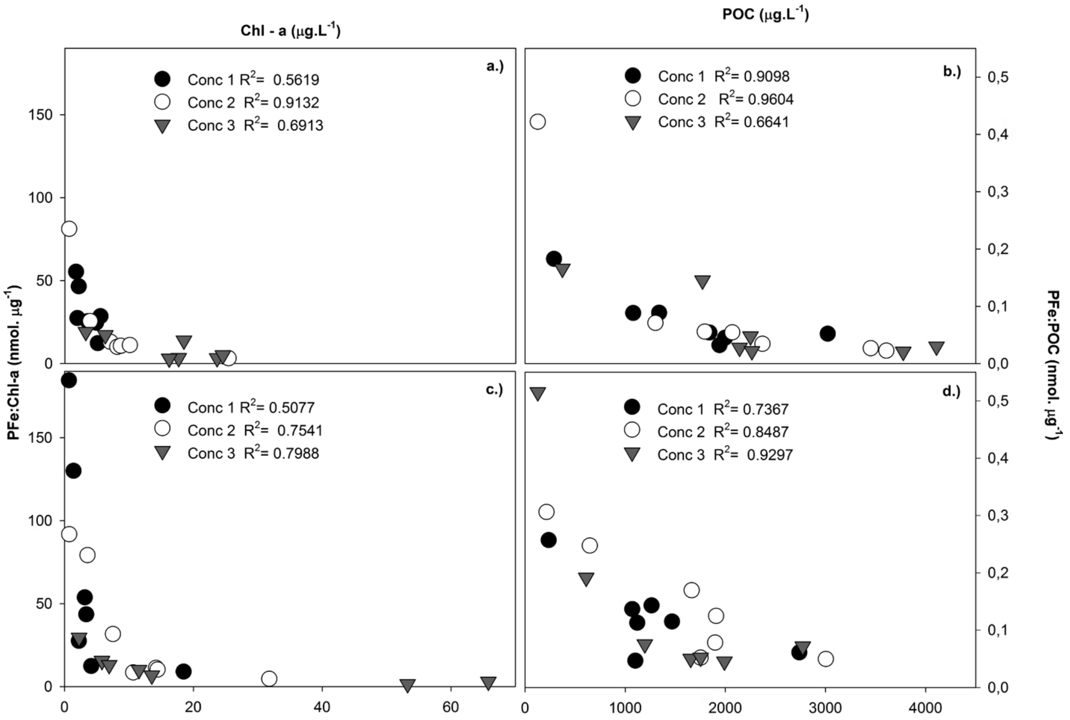

Figure 5. PFe standardized by Chlorophyll *a* (Chl *a*) (nmol µg^{-1}) and by particulate organic carbon (POC) vs. chlorophyll (ugr L^{-1}) and particulate organic carbon (ugr L^{-1}) concentration in the **(a, b)** brackish and **(c, d)** marine systems, for treatments with artificial nutrient addition (Conc 1, Conc 2 and Conc 3) in the mesocosms experiment.

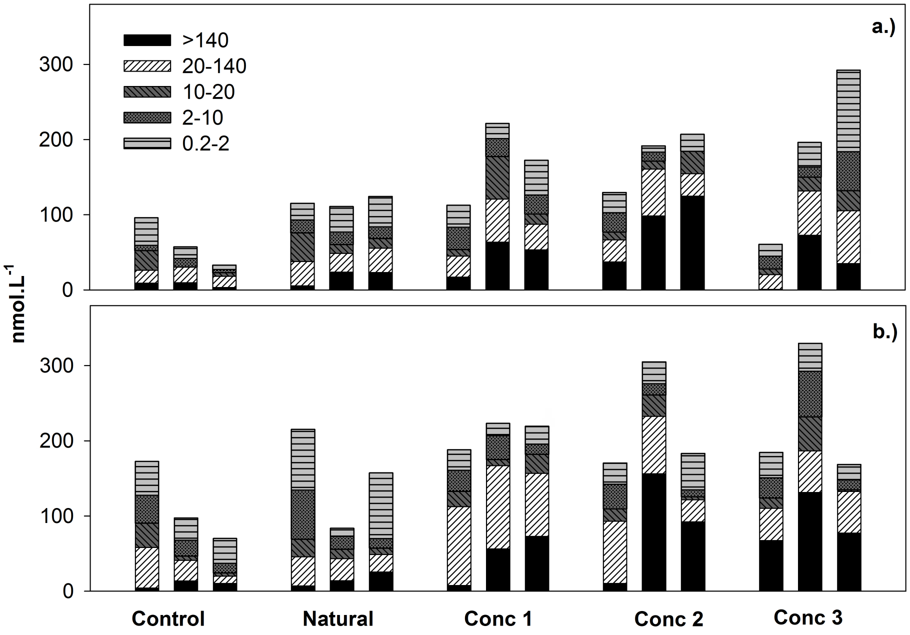

Figure 6. Distribution of the PFe_{SF} (nmol L^{-1}), in the plankton community, contained within the different size classes (µm) in the **(a)** brackish and **(b)** marine systems for all treatments along time in the mesocosm experiment.

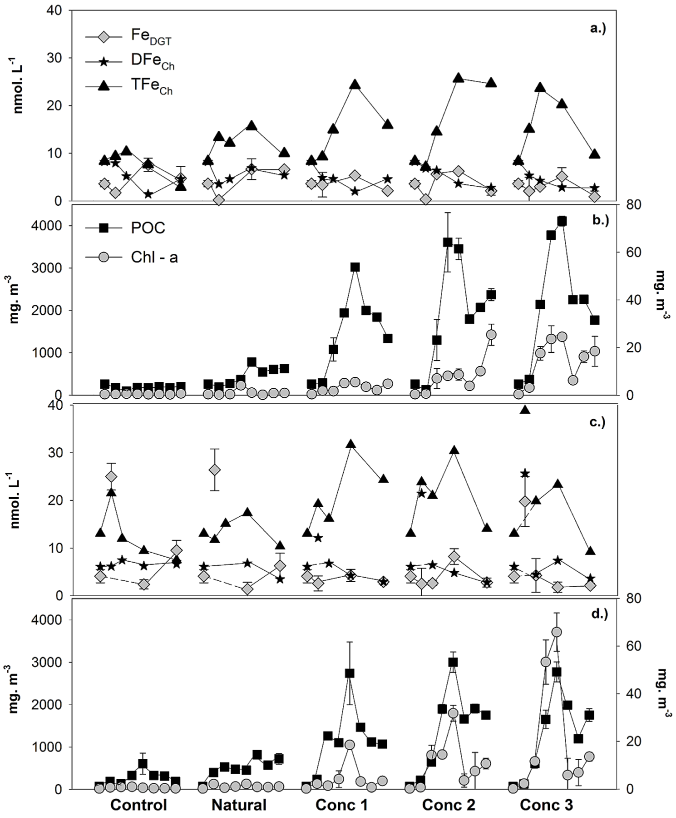

Figure 7. Total Chelex labile iron (TFe_{Ch}), dissolved Chelex labile iron (DFe_{Ch}), DGT labile iron (Fe_{DGT}) (nmol L^{-1}), Chlorophyll (Chl *a*) (mg m^{-3}) (right axis) and Particulate Organic Carbon (POC) (mg m^{-3}) (left axis) concentrations in the **(a, b)** brackish and **(c, d)** marine systems for all treatments in the mesocosm experiment. Isolated points: contamination outliers. Dash line: missing data. Error bars: standard deviation ($n = 3$).

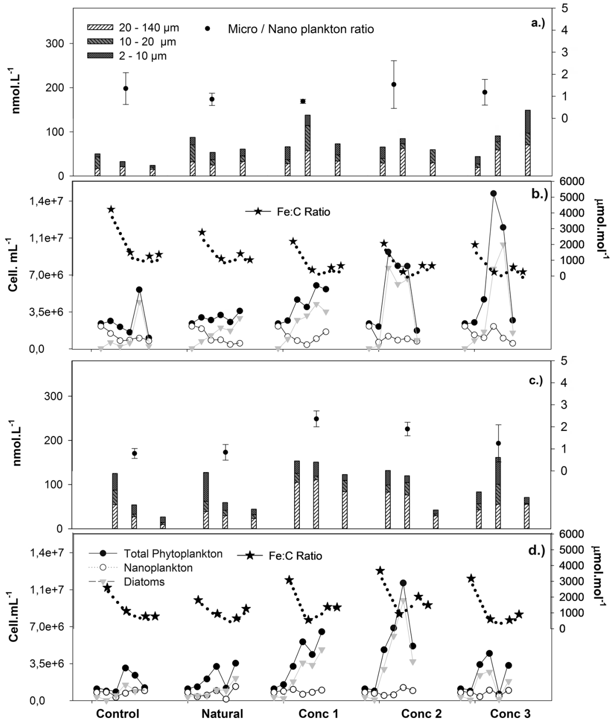

Figure 8. Distribution of PFe_{SF} (nmol L^{-1}) in three fractions (2–10 µm, 10–20 µm and 20–140 µm) of the plankton community (left axis), ratio between PFe_{SF} in the 20–140 µm and 2–10 + 10–20 µm (encompassing the microplankton and nanoplankton fractions respectively) (right axis), abundance (cell L^{-1}) of total phytoplankton, diatoms (microplankton) and nanoplankton and Iron to Carbon ratio (Fe : C) (µmol mol^{-1}) (right axis) in the **(a, b)** brackish and **(c, d)** marine systems for all treatments along time in the mesocosms experiment. Error bars: standard deviation ($n = 3$).