

Iron availability modulates the effects of future CO₂ levels within the marine planktonic food web

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Marine Ecology Progress Series 565: 17–33 (2017)

Methods S1.

Total iron analyses (tFe)

Total iron concentration consists of both dissolved and particulate iron pools (dFe and PFe). Methodology for dFe analyses is described in M&M section in the main document. For particulate iron analyses (PFe) seawater samples (1-3.5 L) were gently filtered onto 0.45 μm Supor®-450 filters. Filters were precleaned with 10% trace metal hydrochloric acid (Fisher, trace metal grade) at 60°C overnight, rinsed with Milli-Q water, dried and stored until further analysis. Filters were digested in 7 mL acid-washed Teflon vials (Teflon, Rochester, NY, USA) (pre-cleaned with 10% trace metal hydrochloric acid and nitric acid -trace metal grade- at 70 °C during 2-3 days each step). Samples were digested in 3 mL of HNO₃ and 0.5 mL of HF (Fisher, trace metal grade) for 1 h at 200 °C in closed vials and HF was evaporated afterwards at the same conditions. One-and-a-half mL of HNO₃ was to the samples and incubated at 150 °C overnight. Samples were then mixed with 2.25 mL of HClO₄ (Fisher, Optima grade) and heated for 4 h at 200 °C. After complete digestion, samples were evaporated at 200°C until dry, dissolved in 1% nitric acid with 1 ppb indium as internal standard, and analyzed by using a high-resolution inductively coupled plasma-mass spectrometer (ICP-MS, Element XR, Thermo Scientific). Filter blanks were subjected to the same process than samples and blank values were subtracted from sample measurements. Trace metal clean techniques were used throughout all the process when collecting and manipulating samples for both dissolved and particulate metals analyses.

Table S1. Fold change in particulate and dissolved iron between days 12 and 21 under the different treatments during the experiment (SD in brackets).

Treatment	Particulate Fe (PFe)	Dissolved Fe (dFe)
LC-DFB	-7.69 (2.01)	0.06 (1.87)
LC+DFB	-12.23 (1.81)	4.35 (1.28)
HC+DFB	-6.76 (0.52)	1.13 (2.07)
HC-DFB	-7.98 (4.59)	2.71 (0.58)

Table S2. Iron demand calculated for each group to meet their Fe quotas at the highest cell numbers reached during the experiment (SD in brackets)..

Iron demand	nM
<i>Emiliania huxleyi</i> (5-10 μm)	10.14 (1.7)
<i>Synechococcus sp</i> (0,6-2 μm)	2.40×10^{-03} (1.58×10^{-10})
Picoeukaryotes (0.1-2 μm)	1.47×10^{-03} (4.7×10^{-07})
Dinoflagellates (30-75 μm)	9.60×10^{-07} (7.33×10^{-11})
Small nanoeukaryotes (2-7 μm)	2.74×10^{-02} (8.6×10^{-03})
Big nanoeukaryotes (6-20 μm)	2.13×10^{-02} (6.4×10^{-03})
Long chain diatoms (>30 μm)	1.80×10^{-06} (2.5×10^{-07})

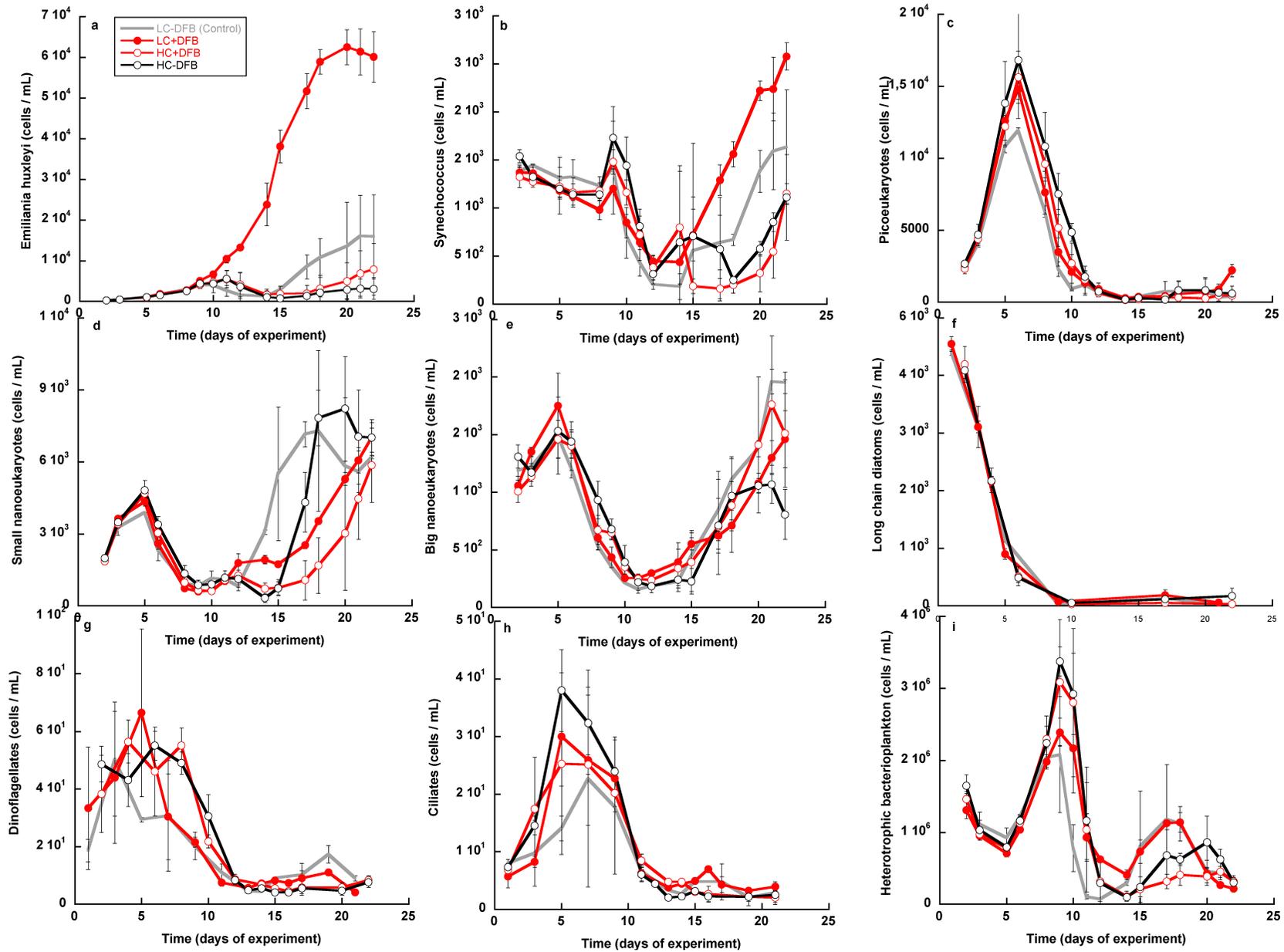


Figure S1. Temporal development of phytoplankton, microzooplankton and heterotrophic bacterioplankton abundances within the mesocosms in the different treatments. Ambient pCO₂ and ambient dFe (LC-DFB, grey); ambient pCO₂ and increased dFe (LC+DFB, red filled circle); increased pCO₂ and increased dFe (HC+DFB, red open circle), increased pCO₂ and ambient dFe (HC-DFB, black open circle). Symbols indicate means of measurements in three independent mesocosms (n=3) except for LC-DFB where n=2. Error bars indicate standard deviations.

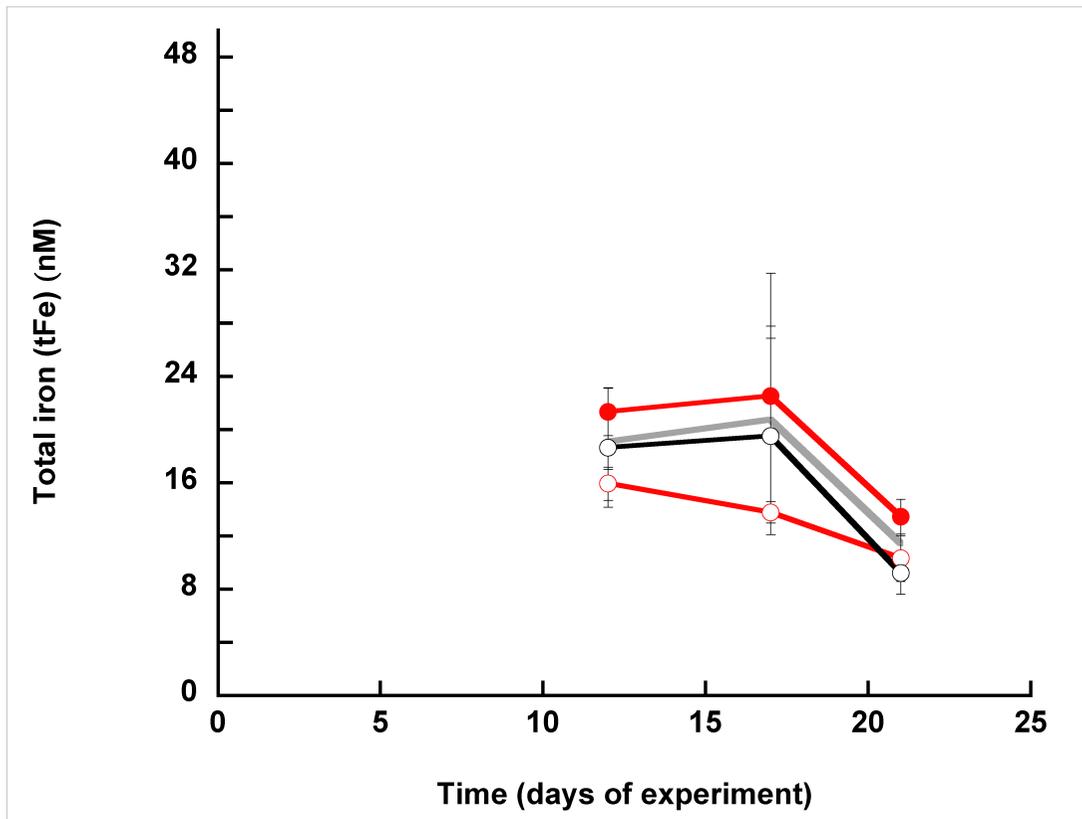


Figure S2. Temporal development of total iron (tFe) within the mesocosms in the different treatments. Ambient pCO₂ and ambient dFe (LC-DFB, grey); ambient pCO₂ and increased dFe (LC+DFB, red filled circle); increased pCO₂ and increased dFe (HC+DFB, red open circle), increased pCO₂ and ambient dFe (HC-DFB, black open circle). Symbols indicate means of measurements in three independent mesocosms (n=3) except for LC-DFB where n=2. Error bars indicate standard deviations.