

Differential effects of iron additions on organic and inorganic carbon production by phytoplankton

Abstract—Bottle and mesoscale experiments have demonstrated that iron additions enhance phytoplankton growth and reduce surface $p\text{CO}_2$ in high-nutrient, low-chlorophyll (HNLC) regions of the world oceans. Here we show that iron additions specifically stimulate organic but not inorganic carbon production in the HNLC Subarctic Pacific. Five-hour ^{14}C labeling experiments performed during incubation of surface water samples demonstrated a large increase in the rate of organic carbon produced but no change in the rate of inorganic carbon production. The same result was obtained on two different dates: one when coccolithophores formed a relatively large proportion of total autotrophic biomass; the other when coccolithophores were less abundant. Together with previous taxonomic observations, our results imply that iron fertilization may be particularly effective in drawing down CO_2 in surface waters by stimulating primary production but not calcium carbonate precipitation, which augments CO_2 .

Iron enrichment experiments in HNLC regions have consistently shown that phytoplankton growth and nutrient use are stimulated by Fe (Martin et al. 1989; Coale et al. 1996). In addition, it has generally been observed that large diatoms benefit particularly from Fe additions and that the structure of the phytoplankton community, not just primary production, is affected by iron (Price et al. 1994; Cavender-Bares et al. 1999). The net export of CO_2 to deep seawater (“the biological CO_2 pump”) of course increases with particulate organic carbon export from the surface but decreases with particulate inorganic carbon export. This is because precipitation of CaCO_3 decreases the alkalinity and pH of seawater and thus increases its CO_2 concentration. The effectiveness of the biological pump thus depends ultimately on the rate of inorganic carbon fixation into organic carbon, which removes CO_2 , and also on the rate of precipitation of CaCO_3 , which produces CO_2 . For example, it has been shown that a global decrease in calcification as a result of atmospheric CO_2 increase (Riebesell et al. 2000) leads to a more efficient sequestration of CO_2 by the oceans (Archer and Maier-Reimer 1994). The possible role of iron in controlling the efficiency of the biological CO_2 pump, during modern times as well as during glacial/interglacial cycles, thus depends on both its overall effect on organic carbon production and its differential effects on calcifying and noncalcifying phytoplankton. Here we examine this question by comparing directly the effect of iron enrichment in an HNLC region on organic and inorganic carbon production (i.e., CO_2 fixation by photosynthesis and CaCO_3 precipitation).

Iron enrichment experiments were conducted on two cruises in the iron-limited region of the Subarctic Pacific in June 1998 and September 1999. For both cruises, water was collected at Station PAPA (50°N, 145°W) with trace-metal-clean Go-Flo bottles and incubated in a flow-through incubator on deck in acid-cleaned polycarbonate bottles. In 1998,

experimental bottles containing 5 nM unchelated Fe and unamended controls were incubated for 6 d. In 1999, experimental bottles containing 1 nM of chelated Fe (1:1.5 EDTA:Fe) were incubated for 9 d in one experiment or for only 24 h in another experiment. All multiday incubations were conducted in 2- or 4-liter bottles, and 24-h incubations were conducted in 250-ml bottles. Incubations were run in quadruplicate for 1998 experiments, and in triplicate (controls) or duplicate (Fe-amended) for 1999 experiments, with one sample collected from each bottle for measurements of carbon production rates.

Samples for total chlorophyll ($>0.4 \mu\text{m}$) were taken periodically from all multiday incubation bottles to monitor growth. Chlorophyll *a* samples were prepared by extracting filters for 12 h at -4°C in 90% acetone. Chlorophyll was determined fluorometrically according to Parsons et al. (1984) using a Turner Designs fluorometer, calibrated against a pure chlorophyll *a* standard. Samples for carbon production rates were taken at the end of the experiment in June 1998 (day 6) and on days 2 and 7 of the September 1999 experiment. Carbon production rates were determined by incubating 200-ml subsamples for 5 h during midday with 30 $\mu\text{Ci H}^{14}\text{CO}_3^-$ and measuring the incorporation of ^{14}C into the $>0.4 \mu\text{m}$ fraction. All filters and filter rims were rinsed three times with filtered seawater to wash off unincorporated label. Inorganic carbon was separated from organic carbon according to a modification of the microdiffusion technique (Paasche and Brubak 1994), in which particulate inorganic carbon is measured by trapping $^{14}\text{CO}_2$ liberated from acid-treated filters. Particulate organic carbon is measured as the acid-stable fraction left on the filter. Samples were counted using Scintisafe Plus Scintillation cocktail in a Beckman LS 1801 scintillation counter. All rates of carbon production were calculated based on the short-term ^{14}C incubations and represent daytime production rates. Based on laboratory studies (Paasche and Brubak 1994), the microdiffusion technique has a precision of 6.3% of the mean. Our field measurements have an additional error due to slight population differences between the samples.

In June 1998, the coccolithophorid abundance was relatively high and amounted to 37% of the total autotrophic biomass (A. Peña, pers. comm.). Our measurement of inorganic carbon production in the control experiment after 6 d amounted to 33% of the total (Table 1). After 6 d of incubation with 5 nM Fe addition, the rate of organic C production increased by about 60% compared to the control (t -test $P < 0.05$), whereas there was no significant effect on the rate of inorganic C production (Fig. 1).

In September 1999, we noted a reduced presence of calcifying phytoplankton, with coccolithophores making up 14% of the total autotrophic biomass (A. Peña, pers. comm.). We conducted both long-term (9 d) and short-term (24 h)

Table 1. Carbon production rates of Fe addition experiments at Station PAPA in the Subarctic Pacific.

Experiment			Organic C* (mg C m ⁻³ hr)	Inorganic C* (mg C m ⁻³ hr)
June 1998	Day 6	Control	1.53 ± 0.32	0.76 ± 0.24
Multiday incubation		5 nM Fe	2.35 ± 0.18, <i>P</i> = 0.02	0.75 ± 0.07, <i>P</i> = 0.98
September 1999	Day 2	Control	0.93 ± 0.04	0.034 ± 0.003
Multiday incubation		1 nM Fe	1.16 ± 0.07, <i>P</i> = 0.03	0.023 ± 0.011, <i>P</i> = 0.31
	Day 7	Control	0.77 ± 0.07	0.029 ± 0.008
		1 nM Fe	3.15 ± 1.76, <i>P</i> = 0.04	0.046 ± 0.018, <i>P</i> = 0.28
September 1999	24 h	Control	0.58 ± 0.05	0.010 ± 0.001
24-h incubation		1 nM Fe	0.80 ± 0.06, <i>P</i> = 0.02	0.010 ± 0.002, <i>P</i> = 1.0

* Errors are standard errors of four (June 1998), three (September 1999, 24-h incubation and controls for September 1999, multiday incubations) and two (+Fe for September 1999, multiday incubation) replicates. *P* values are probabilities that means are the same, as determined with a Student's *t*-test assuming equal variances. A one-tailed *t*-test was performed for the organic C uptake rates; two-tailed *t*-tests were performed for the inorganic C uptake rates.

incubations with and without added Fe to investigate the timing of the Fe-induced stimulation of organic and inorganic ¹⁴C production. For the long-term incubations, we measured organic and inorganic C production after 2 and 7 d. The measured rate of inorganic carbon production was only 4% of the total in the control bottles at both times (Table 1), indicating a much lower rate of calcification by coccolithophores compared to June 1998. As previously seen in such long-term incubations (Martin et al. 1989), the Fe-enriched treatments showed a dramatic stimulation of phytoplankton growth, with the control bottles exhibiting very little change (Fig. 2). On day 2, the Fe-amended samples had 25% more chlorophyll than the control and also showed about a 25% increase in the rate of organic C production,

but no significant change in the inorganic C production (Table 1). By day 7, the Fe-amended samples exhibited an increase of over 300% in the rate of organic C production relative to the control, whereas inorganic C production remained unchanged (Table 1). After just 24 h of incubation with Fe in the short-term experiment, well before any major changes in species composition could have occurred, there was a significant increase in the rate of production of organic C (*t*-test *P* < 0.05) and no change in the rate of production of inorganic C (Table 1). All increases in organic C production in +Fe treatments were significantly different than the controls (*P* < 0.05). In contrast, inorganic C production was not significantly affected by the Fe treatment in any of the incubations (Table 1). The preferential stimulation of organic

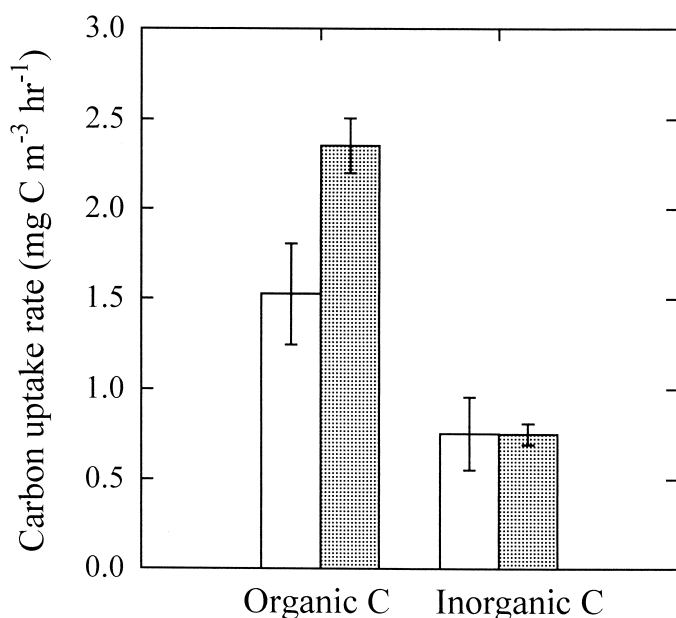


Fig. 1. Rates of organic and inorganic carbon production on day 6 of a multiday Fe addition experiment in the Subarctic Pacific, June 1998. Clear bars represent unamended controls, shaded bars are experimental bottles containing 5 nM Fe. Error bars represent standard error of four replicates.

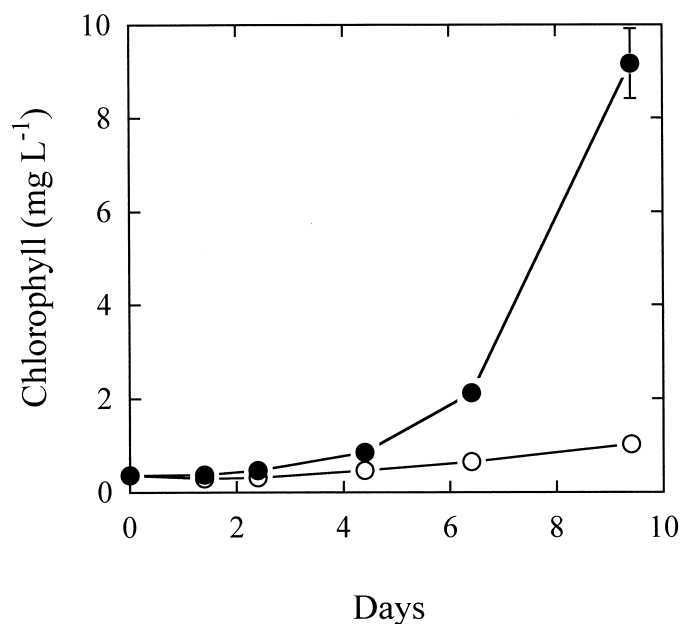


Fig. 2. Chlorophyll growth curves for multiday Fe incubations in the Subarctic Pacific, September 1999. Open symbols are control treatments, shaded symbols are Fe-enriched (1 nM) treatments. Error bars represent standard error of triplicate 4-liter bottles for controls and duplicate 2-liter bottles for Fe-enriched treatments.

over inorganic C production was observed in both cruises, regardless of the initial importance of calcifying phytoplankton in the species assemblage. Culture experiments have shown that coccolithophores can calcify at night when photosynthesis is not occurring (Balch et al. 1996), which allows the possibility that C production rates calculated over 24 h would give different results than our daytime measurements.

The relative increase in organic C production over inorganic C production caused by iron may reflect either a relative decrease in the calcification rate of calcifying organisms or a preferential stimulation of noncalcifying phytoplankton. Previous observations strongly imply that the latter is the case. As noted above, several investigators have remarked that Fe addition preferentially stimulates large diatoms in experiments ranging from bottle enrichments to mesoscale fertilizations (Price et al. 1994; Cavender-Bares et al. 1999). Further, experiments using low-Fe seawater on deck (Martin et al. 1989) and in the lab (Muggli and Harrison 1997) have shown that coccolithophores respond little to Fe enrichments. This has been confirmed with taxon-specific pigment analyses of Fe addition experiments (Hutchins et al. 1998). To our knowledge, however, the effect that Fe-induced changes in phytoplankton community structure has on the inorganic versus organic carbon production rates and hence on the efficiency of the biological carbon pump has not been noted previously.

The question of why coccolithophores do not respond to Fe enrichments remains unanswered. It is possible that coccolithophores have adapted to living in low-Fe waters by reducing their Fe requirements and thus already grow maximally at oceanic levels of Fe. The few laboratory experiments examining Fe requirements of oceanic coccolithophores have shown that they are indeed well adapted to low-Fe conditions (Brand et al. 1983; Muggli and Harrison 1997). Whether this is due to reduced cellular Fe requirements, more efficient Fe transport systems, or a reduction in size under Fe-stress is still unclear. As of yet, the role of iron in coccolithophore growth and ecology has not been adequately studied and it is impossible to distinguish between these and other possible hypotheses.

The original iron hypothesis (Martin 1990) posits that natural iron fertilization caused the roughly 80 % decrease in CO₂ during the last ice age by stimulating primary production. Our data indicate that such an increase in production may be particularly effective in drawing down CO₂ since it may occur without a concomitant increase in inorganic carbon production and the attendant formation of CO₂. A quantitative examination of this effect would require a detailed model of ocean production taking into account the recycling of nutrients from various depths at various latitudes. A qualitative estimate may be obtained by examining the possible response of the three major HNLC regions to iron fertilization. The Southern ocean has low inorganic carbon production, and the differential responses of calcifying and noncalcifying phytoplankton to iron addition in this region is thus of little consequence. The same is true, to a somewhat lesser extent, of the North Pacific. It is thus in the Equatorial Pacific, where calcification rates are high, that the consequences of the nonresponse of calcifying phytoplankton to Fe addition would be most important.

If our data (obtained in a few short-term incubations on samples from the Subarctic Pacific) can be extrapolated to the long term and generalized to other HNLC regions, iron fertilization of the Equatorial Pacific could lead to complete use of nutrients there, without an increase in calcification (if we ignore the limitation posed, for example, by silica concentration). In this scenario, total organic production in the Equatorial and Subtropical Pacific would change little because the nutrients presently unused at the equator would not be available to augment production in the gyres as occurs today. But the lowered organic production in the gyres would presumably be accompanied by a proportional decrease in inorganic production. The net effect of Fe fertilization of the Equatorial Pacific would thus be, indirectly, an overall decrease in the total inorganic carbon production of low and middle latitude regions.

Suppose for the sake of argument that this indirect mechanism caused a 10% decrease in the global ratio of inorganic to organic export production. Then, according to the calculations of Sigman et al. (1998), the resulting increase in atmospheric CO₂ draw-down would be about 15 ppm. This represents only a modest fraction of the glacial/interglacial CO₂ change. Thus, in order to explain a sizeable fraction of the glacial/interglacial fluctuation in CO₂ as a consequence of changes in inorganic carbon production, one would have to assume that long-term Fe fertilization would result directly in a widespread decrease in the precipitation of CaCO₃ during glacial times, perhaps even in (non-HNLC) oligotrophic regions.

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Evidence for tight coupling between active bacteria and particulate organic carbon during seasonal stratification of Lake Michigan

Abstract—Suspended particulate organic matter (POM) plays a critical role in the planktonic ecology of Lake Michigan during seasonal thermal stratification. We show, based on stable isotope and ribosomal RNA (rRNA) data, that the relationship between suspended POM and active biomass changes as thermal stratification persists. Stable isotope data indicated that sources of suspended POM change between July and October, moving from primary production at a deep chlorophyll layer to recycling-based production in surface waters. Concomitant change in the distribution of active bacterial and eukaryotic biomass was observed as indicated by rRNA abundances. Active bacterial and eukaryotic biomass were highly correlated throughout the year. However, the correlation between suspended POM and active bacterial biomass varied seasonally and reflected the transitions in planktonic ecology. Suspended POM from depths >60 m was primarily of sedimentary origin. The combined application of stable isotope and rRNA analysis of suspended POM indicated a dynamic relationship between the bulk POM reservoir and living planktonic biomass.

The release of bioactive elements via the cycling of suspended particulate organic matter (POM) is a fundamental control on photosynthesis and, hence, the planktonic ecology of thermally stratified lakes. However, suspended POM is not a passive reservoir composed entirely of detrital material, but encompasses most of the active plankton including phytoplankton, zooplankton, and bacteria. Few studies have specifically addressed the relative contribution and ecologi-

cal role of active particulate biomass to the cycling of POM as a whole.

Phytoplankton, via the photosynthetic fixation of carbon and nutrients, represents a source of suspended POM on which zooplankton feed. Another source of POM is heterotrophic bacteria; they consume dissolved organic matter (DOM) of planktonic origin and convert it to particulate biomass. Protozoa and larger zooplankton, in turn, consume phytoplankton, bacteria, and other POM and excrete bioactive elements metabolized by phytoplankton and bacteria. Thus, phytoplankton, zooplankton, and heterotrophic bacteria are not only the active biological components of suspended POM, they are also the primary agents of POM cycling.

The roll of heterotrophic bacteria in the cycling of POM is a function of the abundance and quality of organic carbon and the trophic structure of the lake. These factors may vary seasonally. Pace and Cole (1994) have developed a model to predict the response of heterotrophic bacteria to resource availability as inferred from heterotrophic bacterial biomass or production (Fig. 1). When bacterial mortality by grazing is low, a tight relationship is expected between bacteria and available resources. Alternatively, when grazing is dominant, only a weak relationship is expected. This model provides a conceptual framework with which to evaluate possible linkages between bacteria and POM.

In Lake Michigan, there is evidence that heterotrophic bacterial growth may be limited by DOM availability (Gard-