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Carbon versus iron limitation of bacterial growth in the California upwelling regime

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Abstract

The importance of iron versus dissolved organic carbon (DOC) in limiting the growth of heterotrophic bacteria is unresolved, even though iron, DOC, and heterotrophic bacteria are recognized to be critical components of oceanic biogeochemical cycles and food web dynamics. We used enrichment experiments to examine the roles of organic carbon and iron in limiting bacterial growth in the California upwelling regime, where phytoplankton can be iron limited depending on the time and location of the upwelling-induced phytoplankton bloom. In no-addition controls and in incubations with added Fe, bacterial production and abundance did not change substantially over time. In contrast, in all eight experiments, addition of glucose alone stimulated bacterial production and growth rates as much as tenfold. In Fe-replete areas (>1 nM), bacterial production and growth rates in glucose plus Fe treatments were similar to those incubations receiving only glucose. However, in low-Fe regions, addition of glucose plus Fe enhanced bacterial production and growth rates significantly more than glucose alone. We were unable to detect any impact of iron on glucose and amino acid catabolism, and the data offer no support for the hypothesis that bacteria are colimited by Fe and DOC. Rather, these results suggest that growth of heterotrophic bacteria is limited primarily by organic carbon even in Fe-poor waters, but when DOC limitation is relieved Fe may rapidly become a limiting factor.

It is now well known that iron limits primary production in high-nutrient, low-chlorophyll (HNLC) regimes, including the subarctic Pacific, the equatorial Pacific, and the Southern Ocean (e.g., Martin and Fitzwater 1988; de Baar et al. 1990; Martin and Fitzwater 1990; Coale et al. 1996). More recent work has demonstrated that phytoplankton communities can become iron limited in the upwelling regime off California (Hutchins and Bruland 1998; Hutchins et al. 1998). Depending on the location and timing of the phytoplankton bloom, this coastal region becomes an HNLC-like system because inputs of Fe from rivers and resuspended sediments are low and because Fe introduced by upwelling is depleted from surface waters before other plant nutrients. The impact of iron on heterotrophic bacterial communities is less clear.

The few studies examining iron limitation of heterotrophic bacteria have reached contradictory conclusions. Pakulski et

al. (1996) found that biomass production of heterotrophic bacteria in the Southern Ocean (Gerlache Strait) was stimulated by addition of iron, whereas Church et al. (2000) found that organic carbon but not iron additions stimulated bacterial production in experiments conducted along a transect south of Tasmania to the Antarctic Polar Front. Organic carbon additions also consistently stimulated bacterial growth rates in the subarctic Pacific (Kirchman 1990) and the equatorial Pacific (Kirchman and Rich 1997), although the effects of Fe additions were not examined. In contrast, Tortell et al. (1996) suggested that heterotrophic bacteria in the subarctic Pacific may be iron limited since Fe:C ratios of bacterial assemblages in this HNLC region were similar to those of iron-limited bacteria in pure cultures. Other studies have observed stimulation of bacterial production with iron enrichments (Price et al. 1994; Hutchins et al. 1998; Cochlan in press), but because the experimental incubations were illuminated or were in situ measurements, these studies cannot rule out indirect effects. Iron could have stimulated phytoplankton production, leading to higher production of dissolved organic carbon (DOC) and in turn stimulation of DOC-limited heterotrophic bacteria.

Iron and DOC may interact and colimit heterotrophic bacterial growth. Bacteria may require more DOC in low iron

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waters because of the effect of iron on DOC catabolism, specifically the growth efficiency of bacterial assemblages. Tortell et al. (1996) found in pure culture experiments that growth efficiencies of some iron-limited bacteria are lower than those of iron-replete cells. Church et al. (2000) provide some support for the colimitation hypothesis. In some experiments they observed the highest stimulation of bacterial production when both iron and DOC were added. In other experiments, however, bacterial production was not simulated by any addition (Church et al. 2000). The role of iron versus DOC in limiting bacterial production and its impact on DOC catabolism are still unclear.

The central California coastal upwelling regime is perhaps an ideal test system for examining controls of heterotrophic bacterial growth because iron concentrations, primary production, and thus the supply of DOC all vary greatly over relatively short time and space scales (Hutchins and Bruland 1998; Hutchins et al. 1998). We conducted enrichment experiments with otherwise untreated water from several stations occupied during the upwelling in June. We found that biomass production and growth rates of heterotrophic bacteria appeared to be primarily limited by organic carbon and that iron could impact bacterial processes only when iron concentrations were low and the supply of organic carbon high.

Methods and materials

Our experiments used surface water samples taken with a trace-metal clean Teflon pump system from various locations between Point Sur and Point Arena, California on the RV *Point Sur* during June 1999. The experiments used darkened 1-liter acid-washed polycarbonate bottles that were filled cleanly with seawater pumped from 10 m and incubated at surface seawater temperatures in deck incubators. Additions of DOC and Fe and sampling of the bottles were carried out in a class-100 laminar flow hood. Contaminating metals were removed from the glucose stock solution using Chelex ion-exchange resin. Iron was added as ferric chloride in 0.01 N HCl. The glucose addition, which was 1 μM in all experiments, was large compared to expected in situ concentrations (<100 nM), but we needed to add even more glucose to match the added Fe (2 nM); the Fe:C ratio of the enrichments was much greater than the Fe:C ratio of natural marine bacterial assemblages (Tortell et al. 1996; Schmidt and Hutchins 1999).

Bacterial production was estimated by the leucine incorporation method in triplicate (Kirchman 1993). The added leucine concentration was 20 nM, and the incubation volume was 10 ml. After incubating for 1 h at the in situ temperature in the dark, samples were filtered through 0.22- μm mixed cellulose ester filters (Sartorius) and rinsed twice with ice-cold 5% trichloroacetic acid (TCA) and twice with ice-cold 80% ethanol. The filters were dissolved in ethyl acetate before addition of scintillation cocktail (Ultima Gold) and radioassaying. Controls were treated similarly but were first killed with TCA before the incubation; these killed control values were subtracted from the values measured in live samples. An index of bacterial growth rates was obtained by

Table 1. Summary of basic parameters in surface waters used for enrichment experiments. The Fe concentrations are the in situ values before the Fe additions.

Station*	In situ Fe (nM)	Temperature (°C)	Chlorophyll† ($\mu\text{g L}^{-1}$)	$^{14}\text{CO}_2$ uptake ($\mu\text{gC L}^{-1} \text{d}^{-1}$)	Leucine incorporation (pM h $^{-1}$)
5	0.2	12.2	0.18	ND‡	105 \pm 14
8	1.1	9.0	2.05	320 \pm 24	609 \pm 33
13	0.66	9.3	1.01	87 \pm 1.0	155 \pm 8.8
14	0.48	9.2	1.23	59 \pm 0.63	201 \pm 10
21	1.5	11.3	0.05	15 \pm 2.0	23 \pm 0.94
22	0.13	11.3	0.47	67 \pm 5.4	114 \pm 9.4
24	0.06	11.8	0.38	49 \pm 1.2	161 \pm 2.1
27	0.38	11.8	0.84	97 \pm 15	188 \pm 4.9

* Stations 13 and 14 are off Point Arena (38°55'N 124°0'W), Sta. 8 is off Point Reyes (37°53'N 123°6'W), and the remaining stations are off Point Sur (36°18'N 122°18'W), California.

† Values are means of two replicates that differed by 10% on average.

‡ ND; not determined.

dividing leucine incorporation rates by bacterial abundance measured by epifluorescent microscopy of DAPI-stained samples (Porter and Feig 1980). This index, which has units of leucine incorporation rates per cell, is sufficient to address the questions posed here and avoids problems in converting leucine incorporation rates and cell abundance into biomass units. Standard errors of the growth rate index were calculated from propagation of error equations (Bevington 1969). $^{14}\text{CO}_2$ fixation rates by phytoplankton were measured in surface waters incubated on deck for 24 h (Hutchins and Bruland 1998).

Respiration and uptake of uniformly labeled ^{14}C -glucose (NEN) and ^{14}C -amino acids (Amersham) were measured in triplicate by standard methods (Hobbie and Crawford 1969). In brief, 50 nM of added radiolabeled compounds were incubated for 1 to 4 h (usually about 3 h) in the dark. After incubation, samples were acidified and the evolved $^{14}\text{CO}_2$ was collected in a base trap. The remaining liquid was filtered through a cellulose acetate filter (0.22 μm pore size), which was radioassayed to determine the amount of incorporated radioactivity. The composition of the ^{14}C -amino acid mixture was similar to that of algal protein according to the manufacturer.

Iron concentrations were measured by flow-injection analysis using a modification of the method described by Measures et al. (1995). Briefly, samples were filtered through a Teflon cartridge filter (Millipore) with a pore size of 0.4 μm and then acidified to pH 1.7–1.8 with HCl. The filtrate was microwaved and then allowed to cool to less than 35°C before analysis. This treatment provides a measurement of total dissolved iron in the sample.

Results

We performed eight enrichment experiments along a transect in the California upwelling system in June 1999. Dissolved iron concentrations varied from 0.06 nM to 1.5 nM along the transect, as the upwelling-induced phytoplankton bloom progressed and advected off shore (Table 1). Like-

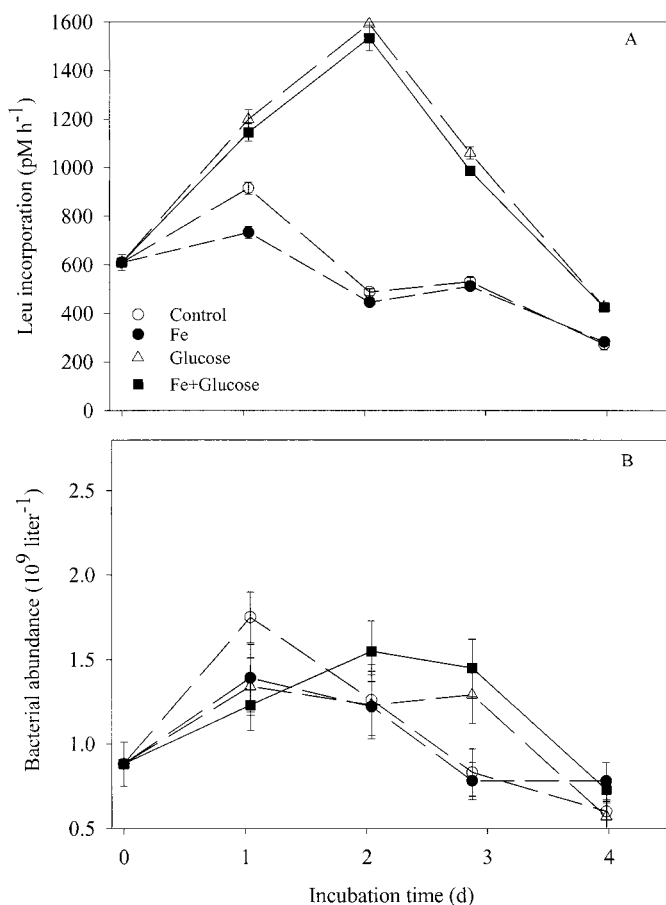


Fig. 1. Bacterial production and abundance in incubations with glucose, Fe, or glucose + Fe added to Fe-rich surface waters of the California upwelling regime. Nothing was added to the controls. In situ dissolved Fe was 1.1 nM (Sta. 8). (A) Bacterial production (leucine incorporation) and (B) bacterial abundance. The mean and standard error are given.

wise, ¹⁴CO₂ fixation rates by phytoplankton and bacterial production varied by 20 to 30-fold (*see below*), and chlorophyll concentrations in surface waters varied by more than tenfold (Table 1). As observed previously (Hutchins and Bruland 1998; Hutchins et al. 1998), Fe addition experiments indicate that phytoplankton production was limited by Fe in the low-Fe surface waters used for our experiments but not in high Fe areas (D. Hutchins, unpubl. data).

Effect of DOC and iron on bacterial growth—In marked contrast to the response of phytoplankton, heterotrophic bacterial production was not affected by the addition of iron alone in any of our dark incubations; production rates in incubations enriched with 2 nM Fe did not change substantially over time and did not differ from rates in no-addition controls. We did not expect Fe limitation in the iron-rich (>1 nM) waters (Fig. 1A), but Fe limitation was not observed even in Fe-poor (<1 nM) waters (Figs. 2A and 3A). In all eight experiments conducted during our study, addition of Fe alone to surface water incubations had no effect on bacterial production.

Unlike the Fe treatments, addition of labile DOC (glucose)

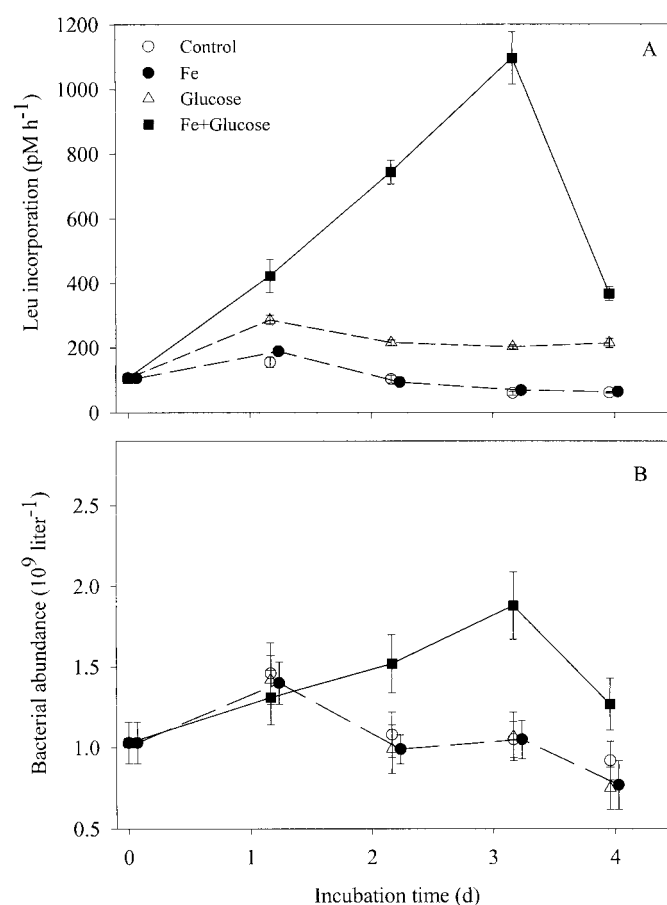


Fig. 2. Bacterial production and abundance in incubations with glucose, Fe, or glucose + Fe added to Fe-depleted surface waters of the California upwelling regime. Nothing was added to the controls. In situ dissolved Fe was 0.2 nM (Sta. 5). (A) Bacterial production (leucine incorporation) and (B) bacterial abundance. The mean and standard error are given. The values for the Fe treatment in the graphs were offset in time for clarity.

substantially increased bacterial production by the first sample point (ca. 1 d) in all experiments. In iron-rich waters, the glucose addition enhanced bacterial production by threefold (Fig. 1A) to more than tenfold (results not shown) over the no-addition controls and the incubations with only Fe added. Bacterial production was similarly stimulated (three to fivefold) by the glucose addition in the iron-poor waters (Figs. 2A and 3A). Glucose affected production rates by the first sample point (as quickly as 7 h) in all eight experiments (Table 2).

Addition of Fe plus glucose stimulated bacterial production more than just the glucose amendment alone, but only in waters with low in situ dissolved Fe concentrations. When dissolved Fe was >1 nM, bacterial production in the glucose + Fe and the glucose-alone treatments was similar; Fig. 1 is from one of two experiments showing no difference between glucose + Fe and glucose-alone treatments (*see also Table 2*). In contrast, when in situ Fe was <1 nM, bacterial production was highest by more than fivefold in the glucose + Fe treatments (Figs. 2A and 3A). Occasionally the additional Fe effect was measurable by the first sample point

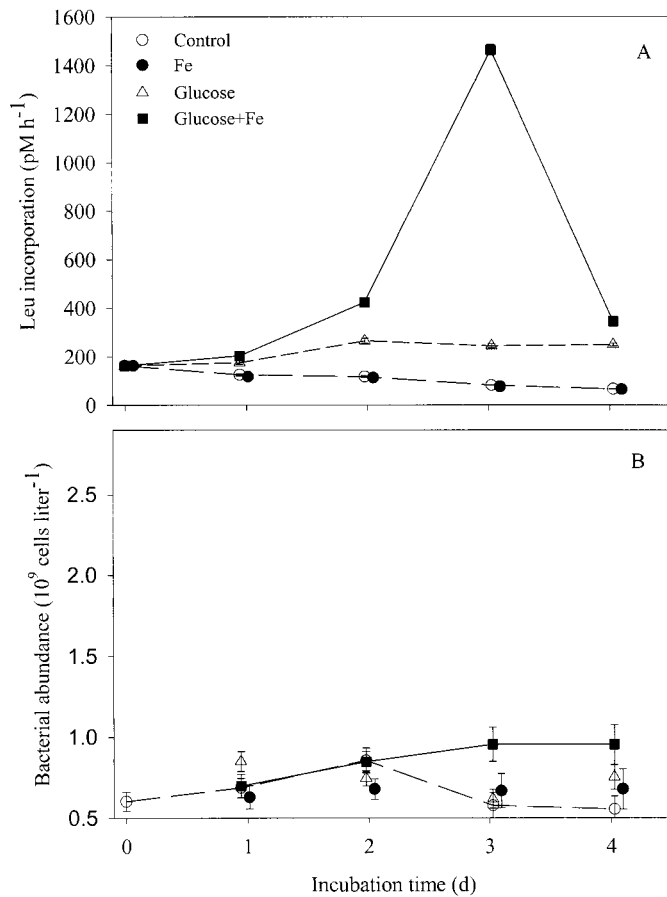


Fig. 3. Bacterial production and abundance in incubations with glucose, Fe, or glucose + Fe added to Fe-poor surface waters of the California upwelling regime. Nothing was added to the controls. In situ dissolved Fe was 0.06 nM (Sta. 24). (A) Bacterial production (leucine incorporation) and (B) bacterial abundance. The mean and standard error are given. The values for the Fe treatment in the graphs were offset in time for clarity.

(Fig. 2A), but often it was not observed until after several days (Fig. 3A), unlike the immediate effect of the glucose-alone treatment (Table 2). These results support our hypothesis that organic carbon is the proximate factor limiting bacterial production but that Fe can quickly become limiting when the DOC supply is adequate and Fe concentrations are low.

Changes in bacterial abundance were much smaller than the large increases in bacterial production induced by the DOC-alone or DOC + Fe additions. The change in bacterial abundance ranged from about twofold (Figs. 1B and 2B) to threefold for the experiment at Point Arena (data not shown). In contrast, bacterial production increased by threefold (Fig. 1A) to tenfold (Figs. 2A and 3A). Unlike the effects on bacterial production, changes in bacterial abundance were more erratic and harder to interpret (Figs. 1B, 2B, and 3B), which is not unexpected, given that variation in abundance is due to complex interactions between bacterial growth and mortality.

Growth rates of heterotrophic bacteria, which were estimated by dividing bacterial production by cell abundance,

Table 2. Summary of enrichment experiments in California upwelling system. Bacterial production (leucine incorporation) was measured daily in experiments lasting as long as four days. The first data set for each experiment is from the first sample after time zero. Data are also given at a second sample point when rates in the glucose plus iron treatment were significantly greater than the glucose-alone treatment. Bacterial production in the iron-alone treatments did not differ significantly from the controls (no addition) in any experiment.

Leucine incorporation (pM h^{-1})						
Station	Fe (nM) [†]	Incubation time (d)	Control	Glucose	Glucose + Fe	Different? [‡]
24	0.06	1.0	125 ± 5	175 ± 7	203 ± 6	*
		2.0	117 ± 5	265 ± 11	423 ± 14	**
22	0.13	1.5	192 ± 10	311 ± 25	344 ± 72	NS
		3.3	125 ± 6	33 ± 3	1125 ± 55	**
5	0.2	1.2	155 ± 16	286 ± 15	423 ± 52	*
27	0.38	0.3	222 ± 2	271 ± 4	298 ± 4	**
14	0.48	0.9	288 ± 8	413 ± 10	384 ± 3	NS
		2.9	288 ± 4	741 ± 12	1381 ± 2	*
13	0.66	1	232 ± 12	335 ± 6	337 ± 17	NS
		3	223 ± 7	639 ± 13	1724 ± 47	**
8	1.1	1	914 ± 25	1198 ± 41	1144 ± 36	NS
21	1.5	0.9	57 ± 1	100 ± 2	95 ± 2	NS

[†] The concentrations are of the in situ Fe before any additions.

[‡] Student's *t*-test was used to compare incorporation rates in the glucose-alone treatment with rates in the glucose plus iron treatment.

* $p < 0.05$; ** $p < 0.001$; NS, not significant.

appeared to be enhanced by the glucose additions (in all experiments) or additionally by glucose + Fe amendments (only in Fe-poor waters). As observed for bacterial production, bacterial growth rates were substantially higher for glucose-amended incubations in both iron-rich (Fig. 4A) and iron-poor (Fig. 4B) waters than in the no-addition controls. It is worthwhile to emphasize that the Fe-alone treatment had no effect on bacterial growth rates in all eight experiments we conducted. But Fe did affect bacterial growth rates when added together with glucose at locations with < 1 nM Fe (Fig. 4B). The stimulation of growth rates ranged from fivefold to tenfold, much greater than experimental errors.

Impact of Fe on DOC catabolism—The additional enhancement of biomass production and growth rates by adding iron along with DOC is consistent with a Fe-C colimitation hypothesis. Previous work suggested that Fe and organic carbon could interact and control bacterial growth by affecting bacterial growth efficiencies and catabolism of DOC (Tortell et al. 1996). To test this hypothesis, we measured uptake and respiration of ^{14}C -glucose and amino acids in both our incubation experiments and in unmanipulated water samples from various locations in the California upwelling regime. We found no significant correlation between dissolved in situ Fe concentrations and ^{14}C -DOC metabolism (Fig. 5A) and no consistent effect of Fe additions (Fig. 5B). Figure 5 shows the ratio of uptake to the sum of uptake and respiration (analogous to growth efficiency), but we also did not observe any relationship between iron concentrations

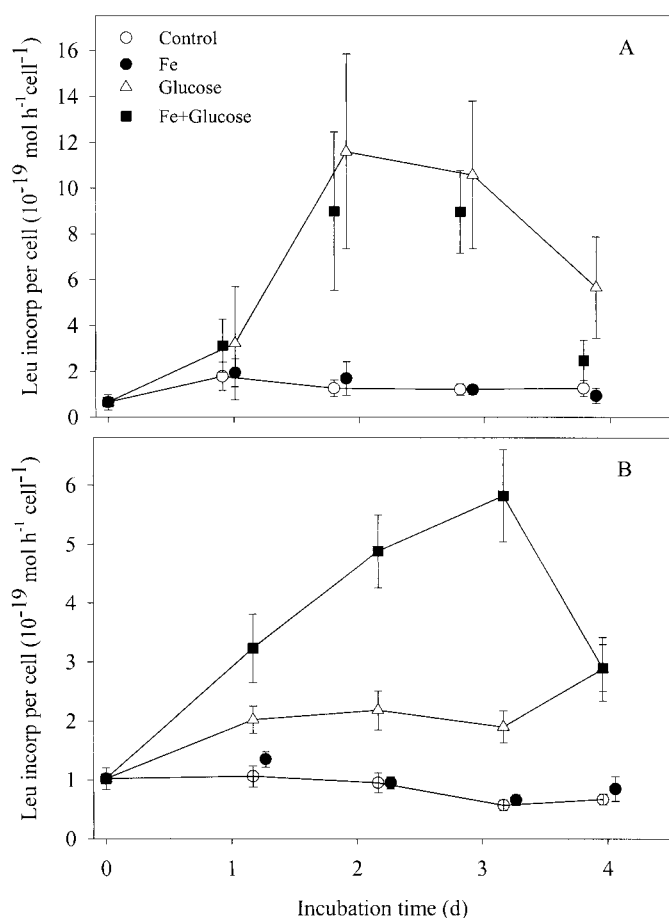


Fig. 4. Index of bacterial growth rates (leucine incorporation per cell) in incubations with glucose, Fe, or glucose + Fe. Nothing was added to the controls. (A) Fe-replete waters (dissolved Fe of 1.5 nM, Sta. 21) and (B) Fe-depleted waters (0.2 nM, Sta. 5). The mean and standard error are given. The values for the glucose (incorporation per cell) and Fe treatments (both graphs) were offset in time for clarity.

and rates of uptake and respiration or any effect of iron additions on these rates (data not shown).

Another, simpler explanation for our results is that bacterial growth became Fe-limited when Fe concentrations were low (at least <1 nM) but only after C-limitation was alleviated by the glucose additions. Consistent with the hypothesis that bacteria are primarily organic carbon limited in surface waters, we observed that in situ bacterial production was highly correlated ($r = 0.89$, $n = 21$) with $^{14}\text{CO}_2$ fixation rates (primary production) in surface waters during our cruise (Fig. 6). Such a high correlation is rather uncommon when bacterial and primary production are compared over relatively short time and space scales (Ducklow and Carlson 1992), such as during our study. In contrast, there was no significant correlation between bacterial production and in situ dissolved Fe concentrations ($r = 0.17$, $n = 21$, $p > 0.05$), nor was there one between $^{14}\text{CO}_2$ uptake in surface waters and Fe concentrations ($r = 0.054$, $n = 22$, $p > 0.05$). Although correlations cannot rule out alternative mechanisms, the most parsimonious explanation for the high cor-

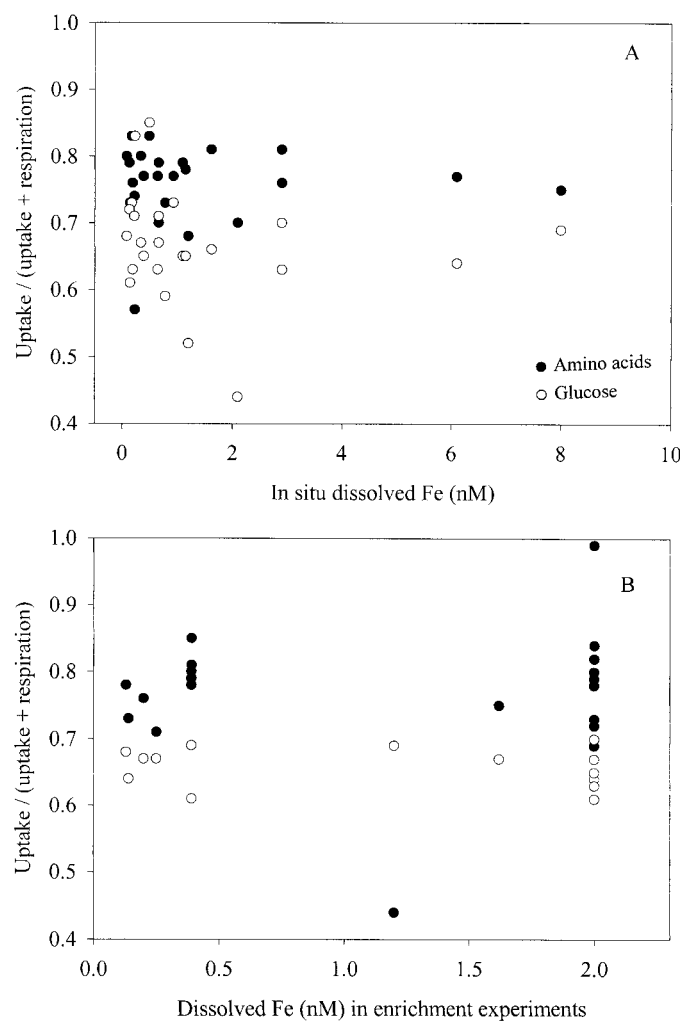


Fig. 5. Ratio of uptake to the sum of uptake and respiration of glucose and amino acids versus dissolved iron concentrations. (A) Samples from surface waters and (B) samples from initial time point of the enrichment experiments.

relation we observed is that $^{14}\text{CO}_2$ fixation is a good index for DOC production, which in turn regulates bacterial production.

Discussion

Our results indicate that biomass production and growth rates of heterotrophic bacteria are primarily limited by organic carbon even in iron-depleted regimes such as the coastal upwelling system we examined. Iron could have a role, however, in affecting bacterial growth and metabolism when the supply of DOC is adequate and iron concentrations are low. Data on iron concentrations proved useful in explaining why iron failed to have any effect on bacterial production in two experiments. It was somewhat surprising to see that iron, added with glucose, had an impact on bacterial growth in two other experiments with relatively high dissolved iron concentrations (0.48 and 0.66 nM), but Hutchins et al. (1998) did observe some signs of iron limitation of

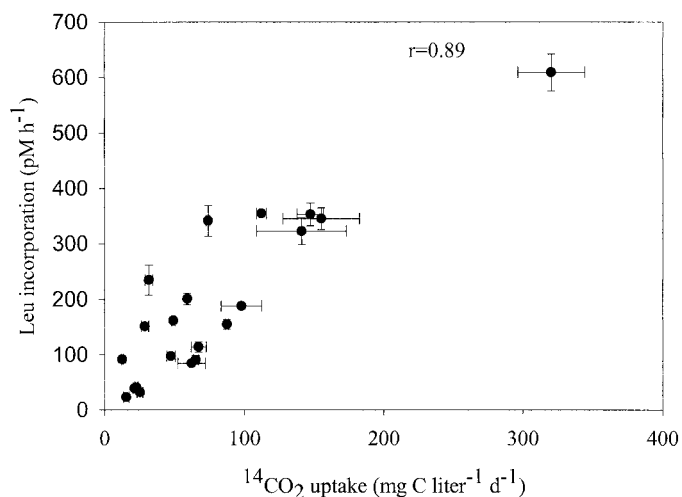


Fig. 6. Bacterial production (leucine incorporation) and $^{14}\text{CO}_2$ fixation in surface samples along the transect in the California upwelling. Means of three replicates \pm SD. Where a bar is not evident it was smaller than the symbol.

diatom growth when dissolved iron was 0.5–1.0 nM. Differences in concentrations and availability of both iron and DOC probably account for why two previous studies (Pakulski et al. 1996; Church et al. 2000) arrived at different conclusions about the role of iron in limiting bacterial production.

Our data showing the greatest stimulation of bacterial growth rates by Fe and DOC additions could be used to argue for bacterial growth being colimited by both DOC and iron (Kirchman 1996; Tortell et al. 1996). Low Fe could impair DOC catabolism leading to higher DOC requirements for bacterial growth and apparent DOC limitation. Catabolism of DOC relies on several iron-containing enzymes in the electron transport system (ETS) and the Krebs cycle (Tortell et al. 1999). If DOC catabolism were impacted by iron more than anabolism, one would expect bacterial growth efficiencies to vary with iron concentrations or cellular iron status. In fact, Tortell et al. (1996) showed that growth efficiencies of three of the five bacteria they examined were lower in iron-limited pure cultures. However, in iron-limited California waters, iron additions had no impact on the uptake and respiration of amino acids and glucose, nor did use of these compounds vary with in situ iron concentrations. These data do not support the hypothesis that growth efficiencies of natural bacterial assemblages are lower in HNLC, iron-limited regimes.

For several reasons, bacterial growth efficiency probably cannot be estimated from the use of compounds like amino acids and glucose. Still, one would expect some effect of iron on respiration of these compounds if bacterial assemblages in HNLC regions were in fact colimited by iron and DOC. Respiration of organic compounds such as glucose begins with glycolysis and the production of acetyl-CoA and then continues in the Krebs cycle where acetyl-CoA is oxidized to CO_2 , coupled to the reduction of NAD^+ to NADH. Iron additions could directly affect organic carbon oxidation, since three Krebs cycle enzymes, aconitase, succinate de-

hydrogenase (Tortell et al. 1999), and fumarase (Flint et al. 1992), contain iron. In addition, any enhancement of the iron-rich ETS may lead to stimulated NADH oxidation and in turn to changes in oxidation of organic compounds. The impact of iron on organic carbon oxidation is not well understood, but extensive work on bacterial isolates has revealed tight feedback mechanisms between ETS activity (NADH oxidation) and organic carbon catabolism (e.g., Riondet et al. 2000). Thus, it was reasonable to expect some effect of iron on glucose and amino acid respiration even if these compounds cannot be used to estimate bacterial growth efficiencies.

Rather than colimitation, a more parsimonious explanation for our results is that heterotrophic bacteria are limited by organic carbon and are only stressed by iron deficiency in iron-depleted regimes. If so, then changes in iron availability alone would have little impact on heterotrophic bacterial growth, as illustrated by our experiments. But molecular studies may reveal more subtle impacts of iron and other elements on the metabolism and community structure of oceanic bacterial assemblages. In addition, bacteria-iron interactions in other HNLC regimes may differ from what we observed. Although the oceanic regimes examined by previous studies and our work do share some common traits (e.g., limitation of phytoplankton production by iron), they also differ greatly in other biogeochemical parameters. For example, coastal HNLC regions like the waters off central California have higher diatom biomass and much lower prokaryotic phototrophic populations than open ocean HNLC regimes (Hutchins et al. 1998). The impact of these and other differences in biogeochemical parameters on bacteria-DOC-iron relationships is not clear.

Our results provide another example of the tight control of standing stocks of heterotrophic bacteria in marine systems. The relatively small changes in bacterial abundance over time in our incubations suggest that mortality of bacteria increased and roughly matched even very large increases in bacterial production caused by the additions. This control of bacterial population levels has been observed in other enrichment experiments in HNLC regions (Kirchman 1990; Kirchman and Rich 1997) and in other oceanic regimes (e.g., the Sargasso Sea; Carlson and Ducklow 1996). However, tight control is not always observed (e.g., Carlson and Ducklow 1996; Pakulski et al. 1996; Church et al. 2000), for reasons that are not entirely clear. Similar to the experiments, seasonal changes in bacterial biomass have been observed in some oceanic regimes (e.g., Ducklow et al. 1993), whereas in others standing stocks do not vary greatly and changes in heterotrophic bacterial production are apparently matched by mortality (e.g., Ducklow et al. 1995; Carlson et al. 1996). Some of these temporal changes can be explained by seasonal fluctuations in phytoplankton communities, but other variation in bacterial biomass is less explicable. The unexplained differences in bacterial responses indicate that we do not fully understand the mechanisms controlling bacterial standing stocks in the oceans.

Tight control of biomass levels has also been observed for phototrophic picoplankton in enrichment experiments. The abundance of small unicellular cyanobacteria changes little, especially compared to the change in the biomass of larger

phytoplankton, after additions of iron to deck incubations (Price et al. 1994; Wells et al. 1994) or after in situ fertilization with iron (Cavender-Bares et al. 1999). In contrast to large phytoplankton, population sizes of both heterotrophic and phototrophic picoplankton can be kept in check by mortality (grazing and viral lysis) even when growth rates are greatly enhanced by DOC or iron additions (Mann and Chisholm 2000).

This tight control of microbial standing stocks also implies that Fe and other elements in microbial biomass are recycled rapidly. Since microbial biomass often dominates the plankton of oligotrophic oceans (e.g., Fuhrman et al. 1989; Campbell et al. 1994), recycling of elements from heterotrophic and phototrophic bacterial biomass is likely to account for much of the regeneration of essential inorganic nutrients. We hypothesize that this rapid recycling, which is probably mostly due to grazing in oligotrophic waters (Strom 2000), is one reason why heterotrophic bacteria do not appear to be primarily limited by iron even in iron-poor surface waters.

It was necessary to conduct our enrichment experiments in the dark in order to examine direct effects of Fe on heterotrophic bacteria. We think that normal DOC production was not disrupted immediately in our dark incubations and that organic carbon limitation was not an artifact of our experimental design. If DOC production directly depended on light and on active photosynthesis, bacterial production and growth rates should have decreased in our no-addition controls. In fact, bacterial growth did not change greatly in the controls or in the iron-amended incubations. These results are consistent with current hypotheses that grazers, not phytoplankton, are the main direct producers of DOC in the oceans (*see* review by Nagata 2000), although direct excretion of DOC by phytoplankton can be substantial in the dark (Mague et al. 1980). Viral lysis, another light-independent process, could also contribute much DOC (Fuhrman 1999; Wilhelm and Suttle 1999). A final argument against our results being an artifact is that we were able to observe organic carbon limitation by the first sample point (<1 d) before DOC production was likely affected by the dark incubations.

One consequence of dark incubations is the lack of labile Fe production via photochemical reactions (Wells and Mayer 1991; Johnson et al. 1994). Even without this supply route of labile Fe, however, heterotrophic bacteria were not primarily limited by Fe in our experiments. Perhaps some of the additional stimulation of adding Fe with glucose was due to the dark incubations; heterotrophic bacteria may have been more stressed by iron deficiency in the dark, especially after addition of glucose. But we suspect that the dominant supply of labile Fe is due to grazing activity on both picoplankton and larger plankton groups and that the lack of photolysis in our dark incubations was not critical. Indeed, recently Barbeau and Moffett (2000) argued that phagotrophic grazers were as important if not more important than photolysis in producing labile Fe in the oceans.

Our results lead to the hypothesis that DOC fluxes should affect Fe cycles and concentrations in the oceans. Heterotrophic bacteria can account for a large fraction of total Fe uptake (Tortell et al. 1996; Schmidt and Hutchins 1999) and are effective competitors for Fe, in part because they produce

high affinity Fe-binding organic ligands (siderophores). Production of siderophores and thus Fe uptake by heterotrophic bacteria is likely limited, like bacterial growth, by the supply of organic carbon in surface waters and probably in deep waters as well. Heterotrophic bacteria are probably the dominant producers of Fe-binding ligands, especially in deep waters where the abundance of microbes other than heterotrophic bacteria is quite low. In both surface and deep waters, the supply of organic material and the form of this material (particulate versus dissolved) should have major impacts on Fe cycles and Fe concentrations in the world's oceans.

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