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An experimental study on the effects of nutrient enrichment on organic carbon persistence in the western Pacific oligotrophic gyre

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Table S1. A list of previous studies revelant to the present study.‘C’means carbon source; ‘N’means nitrogen source; ‘P’means phosphate source.

Published year	Authors	Study site	Main results relative to this study (Liu et al.)	Treatments	Incubation volume	Dilution	Incubation time
1987	Goldman et al.	Vineyard Sound, Massachusetts	1) The efficiency of NH_4^+ regeneration and also of the carbon gross growth efficiency generally was independent of the sourse of C and N, but increased as the C:N ratio of the substrate (C:N), decreased relative to the C:N ratio of the bacterial biomass(C:N). 2) Inorganic source of both N and P were taken up only in stoichiometric quantities during this phase of growth. 3) Considering that amino acid frequently do not provide all of the N required and that carbohydrates often are the major C source for growth of marine bacteria, we speculate that C:N of available substrates in marine waters is > 10 : 1. Hence, actively growing bacteria may be inefficient remineralized of N.	Experiment A: C/N=1.5:1; 3:1; 6:1; 10:1;—Arainine either singly or supplemental with glucose Experiment B: C/N=0.5:1; 10:1;—by urea alone or by combination of NH_4^+ and glucose and various amino acid	900mL medium in 1L glass container	non dilution	6 to 7 days
1993	Zweifel et al.	Baltic Sea & the Northeast Mediterranean	1) In both Baltic and the Northeast Mediterranean, the least available component for bacterial growth was phosphorus. 2) In the Baltic Sea (salinity, 3~6‰), carbon was available in excess for bacterial growth on all sampling occasion. Compared to the controls, additions of non limiting concentrations of inorganic nitrogen and phosphorus increased the yield of bacteria compared to the control with 156% and the degradation of DOC by 64%. 3) Bacterial carbon content decreased as a result of nutrient additions from 51 ± 7 to 32 ± 5 fg C $cell^{-1}$ 4) Growth efficiencies varied from11 to 54% in untreated cultures compared to 14 to 58% in cultures supplemented with nitrogen and phosphorus.	C—sucrose N—NH_4Cl P—Na_2HPO_4 Treatments: Station-1: control, N, P, N+P Station-2:control, C, N, P, N+P	Station-1: 50L Station-2: 10L	10 fold dilution	Station-1: 192 hours Station-2: 12 hour
1995	Pomeroy et al.	Gulf of Mexico	1) Enrichment experiment in July showed that phosphate to be the primary limiting factor for bacterial production and microbial community respiration, and organic carbon substrate to be the secondary limiting factor. 2) Respiratory rate and bacterial secondary production increased when phosphate was added to water samples. Ammonium, iron and other trace metals, vitamins and chelators had no effect. Glucose was utilized only when supplemented with phosphate. 3) The observed rates of bacterial respiration and production imply the utilization of multiple source of organic and recycled inorganic nutrients in a complex and inefficient food web.	C—glucose N—NH_4^+ P—$H_2PO_4^-$ M—iron and other trace metals Treatments: control, N, M, P, N+P+M, C+N+P+M	125mL incubation system	non dilution	12 hours incubation
1996	Cherrier et al.	Eastern North Pactific	1) PE-DOM (phytoplankton-derived DOM) always stimulated bacterial production and DOM utilization, and the primary nitrogen source supporting this bacterial production was dissolved organic nitrogen (DON). 2) Of the model compounds tested (glucose et al.), net bacterial biomass production was observed only in samples amended with glucose, glucose plus ammonium (glucose+NH_4^+), and dissolved free amino acids (DFAA). 3) We suggest that bacterioplankton biomass production in eastern North Pacific surface waters is primarily energy limited. As a result of this energy limitation, bacterial production appears to be additionally constrained by the quality of the nutrients available for assimilation. 4) Thus, the quality of the DOM substrate, specifically the DOC:DON ratio, can be a major determinant of bacterial production in pelagic marine systems.	C—glucose or Plankton extract-DOM N—NH_4^+ , urea and DFAA P—PO_4^{3-} Treatments: control, C+N, Urea, P, DFAA	1L	non dilution	about 3 days

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1996	Carlson et al.	Sargasso Sea (near Bermuda)	1) In all experiments bacterial growth rates, bacterial carbon production, and BGE increased with the addition of organi carbon supplements. 2) There were no enhancements of bacterial production or DOC utilization above the control when inorganic nutrients were added, indicating that at the time these experiments were conducted bacterial growth was limited by available carbon. 3) Our findings indicate that labile carbon in the form of glucose and DFAA was initially utilized as a carbon source, resulting in an elevated growth rate, and that upon exhaustion of a growth-limiting nutrient, the surplus of labile carbon was utilized as an energy source. 4) The results of this study clearly demonstrate in the 3 separate experiments that, despite unmeasurable concentrations of $NH_4^+$and PO_3^- in surface waters, the addition of labile DOM stimulated bacterial growth rates and biomass yield .	experiment in 1992: Control, glucose, NH_4^+, DFAA experiment in 1992: 0.8 μm filtrate, 90%diluted experiment in 1993: whole water experiment in 1993: Control, glucose, Algal lysate, DFAA experiment in 1994: Control, glucose, NH_4^+, PO_4^{3-}	5L or 10L	0-90% diluted	111 to 216 hours
1997	Kirchman et al.	Equatorial Pacific Ocean (two cruises on transects from 12 ºN to 12 ºS along140 ºW)	1) Addition of glucose, glucose plus ammonium, or free amino acids stimulated bacterial production ([^{3}H] thymidine incorporation), whereas changes in bacterial abundance were either negligible or much less than changes in bacterial production.The average bacterial growth rate also greatly increased following DOM additions,whereas in contrast, addition of ammonium alone never affected production,bacterial abundance, or growth rates. 2) Bacterial production and growth rates appear to be limited by DOM in the equatorial Pacific, and thus bacterial production follows primary production over large spatial and temporal scales in this oceanic regime, as has been observed in other aquatic systems. Although temperature may not limit bacterial growth rates in the equatorial Pacific and similar warm waters, it could still affect how bacteria respond to changes in DOM supply and help set steady-state DOM concentrations.	with and without various additions ,such as: amino acids, ammonium and glucose	1L	non dilution	26 to 80 hours
1997	Rivkin et al.	Gluf Stream, & Sargasso Sea & Caribbean Sea	1) In the Caribbean Sea, additions of NH_4 and PO_4 resulted in a modest increase in growth rate (μ =0.20-0.35 d^{-1}), whereas glucose, either alone or in combination with PO_4 and NH_4, resulted in the largest increase (μ=0.50-0.55 d^{-1}). 2) By contrast, in the Gulf Stream and Sargasso Sea, the addition of glucose, either alone or in combination with NH_4, resulted in the smallest increase in growth rate (μ=0.20-0.4 d^{-1}), whereas the addition of PO_4, either alone or in combination with glucose and NH_4 resulted in the largest increase (μ=0.55-0.60 d^{-1}). 3) Growth rates in the PO_4 amended seawater culture were 5-6-fold greater than the controls, suggesting that ambient concentrations of labile DOC were sufficient to sustain vigorous growth. 4) We propose that PO_4 limitation of bacterial growth may directly influence the accumulation of DOC in the surface layer and thus have a significant impact on carbon cycling in the sea. 5) If bacterial growth were not constrained by inorganic nutrients, more DOC could be assimilated into bacterial biomass and subsequently transferred to protistan and metazoan grazers. 6) At the third trophic step beyond bacteria (bacteria- flagellates -ciliates -mesozooplankton), >90% of the DOC initially assimilated by bacteria would be released as CO_2, and <5% would be transferred to mesozooplankton and hence converted into exportable biomass (in the form of fecal material, soma, or feeding webs). 7) Thus, when bacterial growth is P limited, the quantity of biogenic carbon (POC + DOC) exported to depth may be greater than would occur if DOC were first incorporated into bacterial biomass.	C—glucose N—NH_4^+ P—PO_4^{3-} Treatments: control, N, P, C, N+P, C+P, C+N, C+N+P	900mL inoculum in 1L bottle 4L incubation system	1:5 dilution	36 to 48 hours

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1997	Cotner et al.	Sargasso Sea	1) Bioassays indicated that heterotrophic bacteria may be P limited in the northwestern Sargasso Sea, especially in the spring. 2) Limitation by P and not dissolved organic carbon may explain why dissolved organic carbon accumulates in the water column at that time.	C—glucose N—NH_4Cl P—Na_2HPO_4 Treatments: experiment in 1992: control, C, N, P, N+P experiment in 1993: control, C, N, P, N+P, C+N, C+P, C+N+P		1:1 dilution or whole seawater	48 hours
1998	Thingstad et al.	Northwest Mediterranean	1) Based on high pulse uptake capacity and subsaturated uptake in both the > l μm and in the 0.2 ~ l μm size fractions, P deficiency is suggested for both phytoplankton and heterotrophic bacteria. 2) P limitation of heterotrophic bacteria was also supported by fast positive responses after phosphate addition in both thymidine incorporation in whole-water samples and increased bacterial cell numbers in predator-free water. 3) No effects were found after addition of carbon or nitrogen sources alone. 4) Combined with other published evidence, we suggest that the growth rates of not only phytoplankton, but also of heterotrophic bacteria, are P limited in this environment in summer. 5) The finding has important implications for the dynamics of accumulation of dissolved organic carbon in the photic zone and thus for the carbon cycle of oceans.	C—glucose or sucrose N—NH_4Cl P—Na_2HPO_4 Treatments: control, N, P, C, N+P, C+N, C+P, C+N+P	whole seawater culture: 250mL predator-free seawater culture: 0.5L	non dilution	dark for 82 hours; light for 46 hours
1999	Zweifel U. L.	The Gulf of Riga	1) Nutrient additions had little or no effect on the community turnover time even when glucose, ammonium and phosphate were added together, while a 10 ℃ shift-up in temperature increased the turnover time 10-fold. 2) The results suggest that the underlying mechanism for accumulation of L-DOC was growth rate limitation of the bacterial community caused by low in situ temperature in combination with control by predators.	C—glucose N—NH_4Cl P—Na_2HPO_4 Treatments: control, N, P, C, C+N+P, Temperature	1L	non dilution	3 to 24 hours in 30% light
2000	Caron et al.	Georges Bank(coastal), northwestern of Atlantic Ocean & western Sargasso Sea	1) Georges Bank—Phytoplankton biomass increased significantly in response to nutrient additions in all but 1 experiment, whereas chlorophyll concentrations remained unchanged or decreased in all of the unamended (control) treatments or treatments supplemented with glucose. 2) Georges Bank—Bacterial production increased after 24h in all of the treatments on Georges Bank, and there was little effect of nutrient or glucose addition in unfiltered seawater relative to unamended controls. However, glucose addition to the <1 μm filtrate caused substantial increases in bacterial production relative to controls and N/P-amended treatments in 2 of the experiments from this environment. 3) Sargasso Sea—Glucose had no stimulatory effect (relative to unamended treatments) in 3 of the 4 Sargasso Sea experiments, and only a marginal effect in the fourth. 4) Sargasso Sea—However, the addition of inorganic nitrogen and phosphorus resulted in higher bacterial production (relative to unamended treatments or glucose addition) in 2 of the experiments with unfiltered seawater, and very large increases in 3 of the experiments with 1 μm filtrate. 5) The magnitude of the changes in bacterial production differed greatly between unfiltered and filtered seawater in both ecosystems, indicating an important role for bacterial grazers in controlling bacterial population growth. 6) The results of this study indicated different nutritional restraints on bacterial production in these contrasting environments.	C—glucose N—NH_4Cl P—$Na_2HPO_4 \cdot 7H_2O$ Treatments: control, N+P, C	1 L seawater in 1.25 L bottle	non dilution	24 or 36 hours

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2001	Donachie et al.	Subtropical North Pacific Ocean	1) Bacterial cell production did not change significantly in fsw with glucose (1 μM) or single exogenous N sources (1 μM N) compared to that in fsw alone. 2) Furthermore, there was no significant difference in heterotrophic bacterial cell production in fsw amended with organic or inorganic N, nor between that in fsw with organic N and glucose, or inorganic N and glucose. 3) Cell production did increase significantly, however, in fsw with exogenous glucose (0.38 μM) plus 1 μM inorganic N (NH_4^+) relative to that in fsw only, in fsw with glucose, and in fsw with 1 μM N as amino acids (His, Tyr, Leu). 4) There was no significant difference between heterotrophic bacterial cell production in fsw with glucose, glucose plus amino acids, and that in fsw alone. 5) Our results suggest that heterotrophic activity at Station ALOHA can be regulated by the abundance of particular compounds, regardless of their total concentrations. 6) It appears that auxotrophy and de novo synthesis of cell protein from glucose may coexist among Bacteria at Station ALOHA, and that regulation of ectoenzyme expression is independent of product availability. 7) The results suggest that bacteria at Station ALOHA were limited by both C and N availability.	Treatments: 1. fsw (fitrate seawater) 2. glucose 3. His 4. Tyr 5. Leu 6. NH_4^+ 7. NO_3^-	500mL seawater in PC bottle	non dilution	24 hours
2002	Carlson et al.	Northwestern Sargasso Sea（BATS)	1) In unamended cultures natural assemblages of surface bacterioplankton did not utilize detectable amounts of naturally occurring ‘semi-labile’ DOC over time-scales of days to weeks. 2) Neither bacterial production nor utilization of DOC was enhanced with the addition of inorganic N or P (alone or in combination). 3) Labile DOC amendments stimulated bacterial production and DOC utilization, even in the absence of measurable inorganic nutrients, indicating that the bacterioplankton assemblage was initially energy limited, but did not stimulate utilization of seasonally accumulated DOC. 4) The combination of inorganic N and P with labile DOC enhanced both bacterial production and utilization of ‘semi-labile’ DOC. 5) These data suggest that bacterioplankton community structure, as well as nutrient regime, may be important factors governing the utilization of recalcitrant DOC substrates in the northwestern Sargasso Sea.	C—glucose N—NH_4Cl P—K_2HPO_4 Treatments: experiment in B106: control, N, P, C, N+P, C+N+P experiment in B107: control, N, P, C, C+N+P experiment in HS852: control, C, N+P, C+N+P experiment in B115:control, C, N+P, C+P, C+N, C+N+P experiment in HS875: control, C, N+P, C+N+P	10L	70% dilution	30 or 69 days
2002	Sala et al.	Western Mediterranean Sea	1) The results of the experiments carried out along a coastal-to-open sea transect, with both surface and deep chlorophyll maximum depth waters (DCM), suggest that there is a strong variability in the factors limiting bacteria. While phosphorus was most often the limiting nutrient in the surface samples, at the DCM depths nitrogen or carbon limitation was also found. 2) The surface waters of the open sea station were also studied during the 3 cruises to provide an estimate of seasonal variation of bacterial limitation. Phosphorus limitation was found in the 3 periods: very clear phosphorus limitation in September 1996, possible phosphorus limitation in June 1995 and possible co-limitation with carbon in June 1996. 3) Our results show that phosphorus is the main nutrient limiting bacterioplankton growth in the NW Mediterranean. However, while phosphorus was usually the limiting nutrient in the surface layers, nitrogen and carbon limitation also occurred at other depths. 4) Thus, seasonal and spatial variability in the nutrient limiting bacterial growth should be expected.	C—glucose N—$NaNO_3^-$ P—NaH_2PO_4 Treatments: C-J95-0m/50m: control, N, P, C, C+N+P S-J95-0m/50m: control, N, P, C, C+N+P O-J95-0m/50m: control, N, P, C, C+N+P O-J96-5m: control, N, N+P, C+N+P O-J96-20m: control, N, P, C, N+P, C+N, C+P, C+N+P	Treatments: C-J95-0m/50m: 1L S-J95-0m/50m: 1L O-J95-0m/50m: 1L O-J96-5m/20m: 5L	non dilution	4 to 5 days in light

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2006	Pinhassi et al.	Bay of Blanes	1) Short-term enrichment bioassays (24 h incubation) showed that bacterial P limitation could occur throughout the year, but was most pronounced during spring and summer, coinciding with very low concentrations of dissolved inorganic phosphorus and chlorophyll a, and higher N:P ratios. 2) During the non-stratified period in autumn and winter, bacteria were at times strongly C limited. Inorganic nitrogen limitation was not detected at any time. 3) Long-term bioassays with and without enrichment, where growth was monitored until stationary phase using the seawater dilution culture approach, largely confirmed the results from the short-term bioassays. 4) We conclude that seasonal variability in the type and severity of nutrient limitation can substantially contribute to the regulation of bacterioplankton growth and community composition, and thereby affect the turnover of dissolved organic matter and inorganic nutrients in the sea.	C—glucose N—NH_4Cl P—Na_2HPO_4 Treatments: 1) short term: control, P, C, C+P 2) long term: control, N+P	short term: 250mL long term: 2L	short term: non dilution long term: 20 fold dilution	short term: 24h long term: 68~85h
2009	Hoikkala et al.	Northern Baltic Sea	1) Bacterial production was consistently C limited in the surface layer, with N or both N and P as the secondary limiting nutrients from spring to early summer and in late summer, respectively. 2) In deep water, bacterial growth showed combined temperature and C limitation, and in spring, this also appeared to be true with surface samples.	C—glucose N—NH_4 P—KH_2PO_4 Treatments: control, N, P, C, N+P, C+N, C+P, C+N+P	1L	10 fold dilution	3 days
2014	This study	Western Pacific Ocean	1) The results showed that the dissolved organic carbon (DOC) consumption rates and bacterial community specific growth rates were enhanced by inorganic nutrients enrichment treatments during the initial 48 h incubation. 2) At the end of 14 days incubation, about 1/3 (average 3.3 μmol C kg^{-1}) more organic carbon was respired from the glucose enriched incubation with addition of inorganic nutrients compared to that without addition of inorganic nutrients. 3) In the case no essential nutrients were available, even glucose could not be efficiently used by bacteria and thus remained in the environment. 4) These results suggested that repletion of inorganic nutrients could facilitate microbial consumption of organic carbon and thus has a significant impact on carbon cycling in the environment.	C—glucose EOM—extract from algal derived organic matter N—NO_3^- P—PO_4^{3-} Treatments: control, C+N+P, C, N+P, EOM	20L	non dilution	14 days