

Microbial Heterotrophic Metabolic Rates Constrain the Microbial Carbon Pump

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The respiration of dissolved organic matter by heterotrophic bacteria and Archaea represents the largest sink in the global marine biological carbon cycle, an important constraint on organic carbon supply, and the major driver of global elemental nutrient cycles. Direct measurement of heterotrophic production and respiration is difficult. However, the recent development of methods involving *in vivo* electron transport system activity, bioassay uptake of specific prokaryotic substrates, and nutrient addition incubations are poised to discern the complex interactions between metabolic rate, community structure, and organic and inorganic nutrient availability. In a changing global environment, it is important to understand how increasing sea surface temperature, melting sea ice, ocean acidification, variable dust deposition, and upwelling intensity will impact the metabolism of Bacteria and Archaea and so the balance between carbon sequestration and carbon dioxide evasion to the atmosphere. Continued and improved measures of prokaryotic production and respiration are vital components of this endeavor.

The downward flux of organic carbon from the surface ocean to depth via passive sinking of particles, active transport by animals, and mixing of dissolved organic matter (DOM) is known as the biological carbon pump (BCP). The microbial carbon pump (MCP) is a conceptual component of the BCP, used to describe the microbial production of refractory DOM (RDOM) which can be stored for millennia in the deep sea, rather than being respired to dissolved inorganic carbon and returned to the atmosphere (1). Heterotrophic bacteria and Archaea generate RDOM through degradation and transformation of particulate and dissolved organic matter, exudation, and cell lysis (2, 3). In addition to assimilation and transformation of recently produced DOM, prokaryotes also degrade 'older' DOM (4) derived from photochemically transformed upwelled DOM (5, 6) and potentially from methane seeps (7). Hence, understanding the magnitude and variability of the production and respiration of bacteria and Archaea is important not only for quantifying the efficiency of the BCP (8) and the role of prokaryotes in regulating carbon fluxes (9), but also for constraining the flow of DOM through the MCP.

The composition and lability of DOM affect the prokaryotic carbon demand [$\text{PrCD} = \text{prokaryotic production (PrP)} + \text{prokaryotic respiration (PrR)}$] and the prokaryotic growth efficiency ($\text{PrGE} = \text{PrP}/\text{PrCD}$, the proportion of the prokaryotic carbon demand used for prokaryotic production). PrGE is influenced by the availability of organic and inorganic substrates as well as the energetic costs of growth in a particular environment, and so tends to be low at times of nutrient limitation or environmental stress and higher during increased primary productivity and supply of nutrients (8, 10).

The direct measurement of PrP and PrR and calculation of PrCD and PrGE is technically and interpretatively challenging. This is due to uncertainties associated with factors such as the pre-incubation separation of the heterotrophic bacterioplankton fraction from the rest of the plankton community, the different incubation times required for PrR and PrP measurements, the effect of light on PrP and PrR, the quantification of prokaryotic excretion of DOM, and the conversion factors used to derive rates of carbon production and respiration from radiolabeled thymidine or leucine incorporation and oxygen consumption (8,9). Large uncertainties in PrGE contribute significantly to the

mismatch between measurements of mesopelagic microbial metabolic activity and estimates of the influx of organic carbon that could support this microbial activity (11).

Recent methodological developments have the potential to reduce uncertainties in PrR and PrP determinations. For example, measurements of *in vivo* electron transport system activity estimated from the reduction of the tetrazolium salt INT are linearly related to *in situ* rates of respiration, and avoid problems associated with pre-incubation filtration and relatively long incubation times (24 hours) (12). Additionally, single cell assays that measure incorporation of selected organic compounds by specific prokaryotic groups compare and contrast the components of DOM taken up by bacteria and Archaea (13). Including these assays in time series studies can elucidate the influence of environmental factors such as light on PrP (14, 15).

Climate change will likely affect precipitation, river flow, ice melt, atmospheric deposition, and the timing and strength of along-shore winds that stimulate coastal upwelling, and so may significantly change the supply of inorganic and organic substrates to marine prokaryotes. Concomitant increases in sea surface temperature and decreases in pH and carbonate ion concentration could lead to changes in phytoplankton and zooplankton community structure, subsequently impacting foodweb-derived DOC (16). In short-term experiments, prokaryotic turnover of phytoplankton-derived polysaccharides was increased at the lower pH levels projected to occur with a doubling of atmospheric CO_2 , with the potential to reduce carbon export and enhance respiratory CO_2 production (17). Field studies and inorganic and organic nutrient bioassay experiments show PrR and PrGE in coastal regions to be either mainly controlled by the DOC pool or colimited by organic and inorganic nutrients (18–20). Climate-driven increases in DOC supply may also impact the plankton community photosynthesis to respiration (P:R) ratio. When released from organic carbon limitation, heterotrophic prokaryotes can outcompete phytoplankton for inorganic nutrients, thereby decreasing the overall P:R ratio, increasing the proportion of DOC that is respired, and decreasing the amount that is sequestered (21).

This review aims to highlight our incomplete understanding for the causes of variability in the PrGE and respiratory potential of heterotrophic bacteria and Archaea. As new research supports the pivotal role of these microbes in the present and future ocean (22, 23), the lack of routine measurements of PrP and PrR in relation to phylogenetic composition, as well as to DOM characterization and assimilation potential, becomes increasingly difficult to defend.

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