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SHORT COMMUNICATION

Aquatic heterotrophic bacteria have highly flexible phosphorus content and biomass stoichiometry

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Bacteria are central to the cycling of carbon (C), nitrogen (N) and phosphorus (P) in every ecosystem, yet our understanding of how tightly these cycles are coupled to bacterial biomass composition is based upon data from only a few species. Bacteria are commonly assumed to have high P content, low biomass C:P and N:P ratios, and inflexible stoichiometry. Here, we show that bacterial assemblages from lakes exhibit unprecedented flexibility in their P content (3% to less than 0.01% of dry mass) and stoichiometry (C:N:P of 28: 7: 1 to more than 8500: 1200: 1). The flexibility in C:P and N:P stoichiometry was greater than any species or assemblage, including terrestrial and aquatic autotrophs, and suggests a highly dynamic role for bacteria in coupling multiple element cycles.

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Terrestrial ecosystems are an important source of nutrients and organic carbon (C) to freshwater rivers and lakes as well as the coastal ocean. Past work has shown that heterotrophic bacteria, a group of organisms that process terrestrial inputs of organic carbon, nitrogen (N) and phosphorus (P) (Biddanda *et al.,* 2001), are C-poor and P-rich (Makino *et al.,* 2003) relative to terrestrial inputs characterized by high C:P ratios. As a result, bacterial assemblages in freshwater ecosystems should experience elemental imbalance and act as efficient exporters of organic carbon to downstream ecosystems. However, freshwater ecosystems metabolize most of the organic C they receive from terrestrial ecosystems (Cole *et al.,* 2007) and it has been shown recently that strains (Scott *et al.,* 2012) and assemblages (Godwin and Cotner, 2014) of bacteria from lakes can be P-poor and stoichiometrically flexible. Here, we demonstrate that bacterial assemblages from lakes exhibit unprecedented plasticity in their stoichiometry and discuss the implications of flexible composition to ecosystem processes.

To determine the extent of stoichiometric flexibility within assemblages, we performed two experiments in which we cultured the bacteria-sized fraction of plankton from a northern temperate lake under varying C:P supply ratios and measured their biomass composition (Supplementary Methods). We created C:P_{supply} ratios from 31.6:1 to more than 2 20 000:1 by manipulating the supply of phosphate in a defined medium, with all other nutrients in excess of C and P. At each level of C:P_{supply}, we enriched the lake assemblages in batch cultures and used these enrichments to inoculate chemostats at the same C:P_{supply}. The chemostats were maintained at a dilution rate (0.33 d^{-1}) that is low relative to assemblage growth rates measured in lakes (Cotner *et al.,* 2001).

The bacterial P content decreased from a mean of 3.55% of dry mass when the assemblage was C-limited to 0.006–0.05% when the assemblage was most P-limited (Figure 1). The range of P content measured in the assemblage cultures was nearly equal to the range of existing data in the literature, particularly for P relative to dry mass (Supplementary Table 6). Single-cell measurements from plankton environments indicated the potential for even lower phosphorus quotas (Norland *et al.,* 1995; Cotner *et al.,* 2010), although many of those cells may not be actively growing, potentially decreasing their demand for P-rich RNA, where much of the P resides in bacterial cells (Makino *et al.,* 2003). The P relative to dry mass values measured here were lower than those reported for a bacterium grown in the absence of added phosphate and high concentrations of arsenate (0.012% of dry mass as P, Wolfe-Simon *et al.,* 2010). The results presented here clearly demonstrate that bacteria can have P content less than 0.01% of dry mass when growing at low levels of P.

The C:$P_{biomass}$ and N:$P_{biomass}$ of the bacterial assemblages increased from 28:1 and 6:1, respectively, when C-limited to a maximum of >8500:1 and >1200:1 when P-limited (Figure 1). The ranges of C:$P_{biomass}$ and N:$P_{biomass}$ observed in this study cover nearly the entire range of measurements recorded in previous studies for bacterial cultures and assemblages (Figure 2; Supplementary Table 6) and nearly

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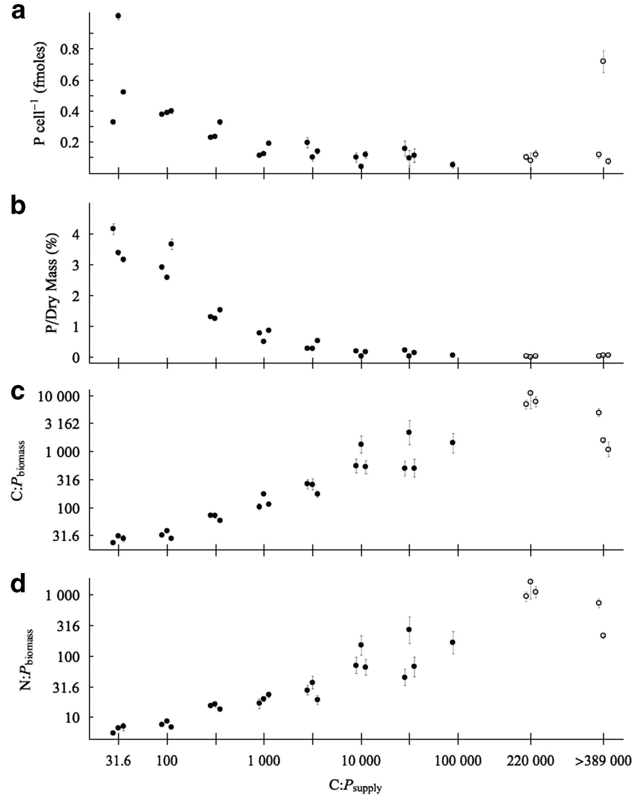

Figure 1 Effect of C:P_{supply} ratio on biomass P/cell (**a**), P/dry mass (**b**), C:$P_{biomass}$ (**c**) and N:$P_{biomass}$ (**d**) ratios in chemostats diluted at 0.33 d^{-1}. Data from Experiment 1 are displayed as solid circles, and open circles denote data from Experiment 2. At each level of C:P_{supply}, the data from replicate chemostat are staggered to improve clarity. The error bars represent the s.e. of the ratio for each chemostat, following propagation of errors from the numerator and denominator. In Experiment 1, C:$P_{biomass}$ and N:$P_{biomass}$ (analysis of variance, $P<1\times10^{-5}$) increased and P/dry mass and P/cell decreased ($P<0.005$) significantly with increasing C:P_{supply}. On the basis of changes in C:$P_{biomass}$, the assemblage was defined as P-sufficient at C:P_{supply} of 31.6:1 and P-limited at C:P_{supply} of 10 000:1 and greater. At C:P_{supply} of 1 00 000:1, only one chemostat had P content above the analytical detection limit and only two chemostats without added P had N above the detection limit.

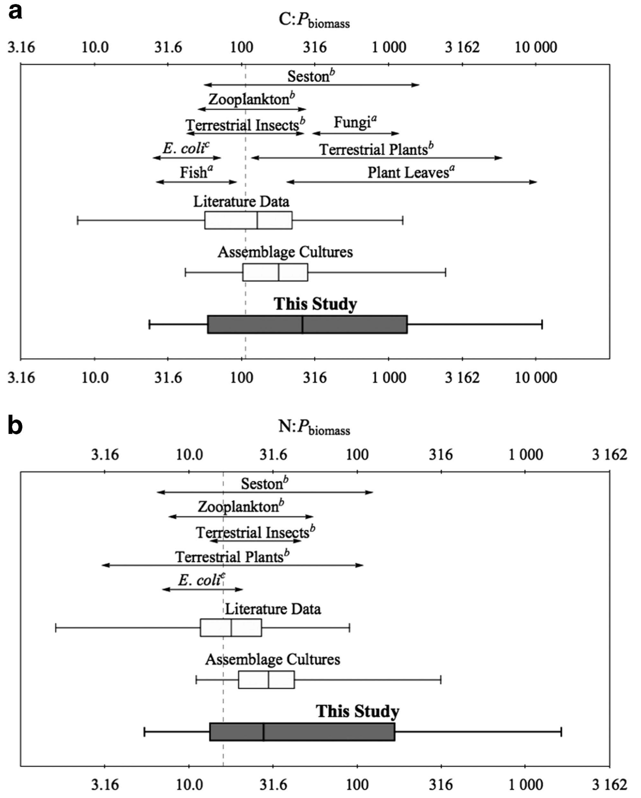

Figure 2 Ranges of C:$P_{biomass}$ and N:$P_{biomass}$ for bacterial cultures and other organisms, with separate panels for C:$P_{biomass}$ (panel **a**) and N:$P_{biomass}$ (panel **b**). Data for heterotrophic bacteria are separated by sources: literature data (Supplementary Table 7), assemblage chemostat cultures (Godwin and Cotner, 2014) and this study. Ranges for other organisms were from [a] (Cross *et al.,* 2005) and [b] (Elser *et al.,* 2000). [c]Ranges for *E. coli* were compiled from multiple studies (Supplementary Table 7). Seston refers to suspended particulate matter (phytoplankton, heterotrophs and detritus). The boxplots display data for individual replicate cultures where data are available, with the centerline representing the median, the edges of the box representing the 25% and 75% quantiles, and the whiskers representing the maximum and minimum values. Dashed lines indicate the Redfield ratio (C:N:P = 106:16:1).

match the ranges of C:$P_{biomass}$ and N:$P_{biomass}$ observed in vascular plant tissues (Elser *et al.,* 2000; Sterner and Elser, 2002; Reich and Oleksyn, 2004). Furthermore, the bacterial assemblage (of multiple strains) exhibited greater stoichiometric plasticity than has been documented in any other species or assemblage, including terrestrial and aquatic primary producers (Sterner and Elser, 2002; Persson *et al.,* 2010). These experiments demonstrate that previous assumptions of low and invariant C:$P_{biomass}$ (Tanaka *et al.,* 2009; Fanin *et al.,* 2013) and high relative P content for bacteria (Wolfe-Simon *et al.,* 2010) do not represent the physiological flexibility of bacteria in natural assemblages. Although mean cellular P content decreased under P limitation, much of the flexibility in C:$P_{biomass}$ was due to a substantial increase in cellular C content (Supplementary Figure 1), likely owing to the accumulation of C-rich storage molecules (Thingstad *et al.,* 2005).

The range of stoichiometric flexibility present in natural assemblages is critical to understanding homeostasis within ecosystems. Strict homeostasis of assemblage C:N:$P_{biomass}$ leads to the prediction that the ratio of regenerated C:P increases dramatically with increasing resource C:P (Sterner, 1990), but flexible biomass stoichiometry allows tight coupling and negative feedback between bacterial biomass stoichiometry and resource stoichiometry, facilitating the inherent resilience of ecosystems to nutrient perturbations (Scheffer *et al.,* 2001). It is increasingly recognized that much of the organic matter metabolized in rivers and lakes originates in terrestrial ecosystems where C:P and N:P ratios can be much higher than for organic matter originating in aquatic ecosystems (Lennon and Pfaff, 2005). The observations in this study of extreme flexibility in bacterial biomass stoichiometry are consistent with observations of higher and more variable biomass C:P and

N:P in the seston (suspended particulate matter) in freshwaters than in pelagic (offshore) marine systems where terrestrial influences and nutrient gradients are less profound (Cotner *et al.,* 2010).

The bacteria in inland waters and the coastal ocean experience stoichiometric imbalance when they process terrestrial inputs of dissolved and particulate organic matter with high C:P ratios. Compared with bacteria with low and invariant $C:P_{biomass}$, assemblages that increase their $C:P_{biomass}$ in response to this imbalance will remineralize less 'excess' C through respiration and could decrease the export of organic matter to downstream ecosystems. In ecosystems where internal nutrient cycling processes are dominant and bacteria regenerate a large fraction of available nutrients (for example, offshore marine systems), flexible bacterial stoichiometry likely stabilizes dissolved inorganic nutrient concentrations and inhibits fluctuations.

The capacity of heterotrophic bacteria to continue to buffer C and nutrient feedbacks in ecosystems is likely challenged by the use of inorganic fertilizers that decrease the exported C:N and C:P ratios to aquatic systems (Arbuckle and Downing, 2001) and anthropogenic warming that increases both the export of organic carbon and the C:N:P stoichiometry of that material (Freeman *et al.,* 2001; Urban *et al.,* 2011). Additionally, because stoichiometric flexibility decreases with increasing relative growth rates (Makino and Cotner., 2004; Hillebrand *et al.,* 2013), bacterial assemblages in low-temperature environments could become less flexible as the result of anthropogenic warming. By examining the capacity of aquatic bacterial assemblages to respond to C:N:P imbalance, we can evaluate the influence of stoichiometric flexibility on aquatic ecosystem productivity and the extent and periodicity of nutrient fluctuations.

Conflict of Interest

The authors declare no conflicts of interest.

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