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REVIEW AND SYNTHESIS

Evolutionary trade-offs among decomposers determine responses to nitrogen enrichment

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Abstract

Evolutionary trade-offs among ecological traits are one mechanism that could determine the responses of functional groups of decomposers to global changes such as nitrogen (N) enrichment. We hypothesised that bacteria targeting recalcitrant carbon compounds require relatively high levels of N availability to support the construction costs of requisite extracellular and transport enzymes. Indeed, we found that taxa that used more recalcitrant (i.e. larger and cyclic) carbon compounds were more prevalent in ocean waters with higher nitrate concentrations. Compared to recalcitrant carbon users, labile carbon users targeted more organic N compounds, were found in relatively nitrate-poor waters, and were more common in higher latitude soils, which is consistent with the paradigm that N-limitation is stronger at higher latitudes. Altogether, evolutionary trade-offs may limit recalcitrant carbon users to habitats with higher N availability.

Keywords

Database synthesis, labile organic carbon, Micrococcineae, nitrogen, phylogenetic independent contrasts, *Pseudomonas*, recalcitrant organic carbon.

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INTRODUCTION

Microbes are often sensitive to global changes, with frequent alterations in abundance, community composition, or prevalence of functional genes in response to anthropogenic nitrogen (N) enrichment, elevated atmospheric CO_2 and warming (Allison & Martiny 2008). Since microbes perform critical biogeochemical functions such as decomposition and greenhouse gas production, they could potentially mediate feedbacks between global change and ecosystem dynamics. However, evolutionary trade-offs, in which one physiological or ecological trait is gained at the cost of another (Darwin 1859; Stearns 1989), may constrain the extent to which these feedbacks occur. Here, we focus on trade-offs in heterotrophic bacteria between two ecological traits: tolerance of low N availability and ability to use recalcitrant carbon compounds for energy. To our knowledge, this is the first microbial study to explicitly address trade-offs that link sensitivity to a global change with shifts in ecosystem processes.

Allocation of finite resources is a mechanism that can lead to evolutionary trade-offs, as resources invested in one trait cannot always be simultaneously invested in others (Levins 1968). These trade-offs limit the ability of taxa to be generalists, both in terms of environmental conditions and biogeochemical functions. For instance, N is required to construct enzymes for catalysis and uptake of organic compounds, especially large, complex recalcitrant compounds (Schimel & Bennett 2004). Thus, we hypothesised that taxa that invest in the use of recalcitrant compounds may have N demands that cannot be met at lower N availabilities. In addition, we hypothesised that taxa adapted to low N conditions may invest in procurement of organic N compounds (to supplement inorganic N sources) (Allison *et al.* 2010), which could occur at the expense of uptake of recalcitrant carbon compounds. If these trade-offs exist, we expect that the breakdown of recalcitrant carbon compounds – leading to release of CO_2 – by bacteria should be inhibited in N-poor habitats, and that exposure to anthropogenic N sources may alleviate that inhibition.

We classified 519 bacterial taxa for traits related to use of labile vs. recalcitrant carbon substrates, use of organic vs. inorganic N, co-variation with ocean nitrate concentrations, and latitudinal distributions in the soil (see Table S1 in Supporting Information). The degree of recalcitrant carbon use was indicated by substrate use profiles (SUPs) obtained *in vitro*, based on proportions of targeted organic substrates with relatively high molar weights or cyclic (including aromatic and aliphatic) structures (Table S2). These characteristics – in particular, high molar weights – are typical of long-lived compounds in the environment (Muir & Howard 2006). Use of recalcitrant carbon compounds (from SUPs) was cross-checked with relevant genes from 65 taxa for which complete genomes were available (Table S3). Specifically, we noted the prevalence of genes related to use of organic substrates that were cyclic, polymeric and non-microbial in origin. Organic N use was indicated by SUPs (Table S1). Finally, to examine the responses of taxa to N availability *in situ*, we used spatial distributions of taxa identified via DNA sequences obtained from environmental samples in the global ocean sampling (GOS) survey (Table S4) (Rusch *et al.* 2007) and a global synthesis of soils (Table S5). Nitrate concentration was used as an indicator of inorganic N availability in the oceans, as nitrate is a dominant form of N in ocean waters, especially near areas of upwelling and river inputs (Eppley & Peterson 1979; Janke 1990). Analogous indices of N availability in soil were difficult to obtain at a global scale. We examined latitude of soil site instead, as N-limitation tends to be stronger at higher latitudes in terrestrial systems (LeBauer & Treseder 2008).

METHODS

Substrate use profiles

We used the GEN III MicroPlate database (Biolog, Inc., Hayward, CA, USA) of bacteria to determine SUPs. The GEN III MicroPlate

database reports phenotypic information collected by Biolog Inc. regarding the use of organic substrates by laboratory-isolated bacteria. Each plate contains 71 substrates, with one substrate per well. The substrates include 35 cyclic and 36 non-cyclic compounds that range in molar weight between 46.03 and 1302.47 g mol^{-1} and in N content between 0 and 32% mass (Table S2). A plate is inoculated with a single strain of bacteria at a standardised cell density (90–98% turbidity) in commercial inoculating fluid (IF-A, Biolog Catalog #72401) and then incubated aerobically for 22 h at 33 °C, or at optimal incubation conditions as determined by preliminary tests. The formulations of the Biolog media are proprietary, but they are similar to that of Bochner *et al.* (2001), which include sodium chloride, triethanolamine HCl, sodium pyruvate, ammonium chloride, sodium phosphate, sodium sulfate, magnesium chloride, potassium chloride, ferric chloride and tetrazolium violet. Substrate use is indicted colorimetrically by development of a tetrazolium redox dye in comparison to a negative control that contains no added substrate. Altogether, we used SUPs from 519 bacterial taxa ('study taxa') (Table S1). Study taxa were those that used at least one substrate in the Gen III MicroPlate, and for which a 16S sequence was available in a curated database (see below). For each taxon, we determined three traits: first, the average molar weight of compounds used; second, the proportion of compounds used that were cyclic; and third, the average N content of compounds used. Recalcitrant substrates tend to be of relatively high molar weight and cyclic (Muir & Howard 2006). We matched taxa with corresponding 16S sequences obtained from the curated GreenGenes database (DeSantis *et al.* 2006), constructed an alignment, and estimated a phylogeny using SATé under the default Center Tree 5 decomposition model (Liu *et al.* 2009). After each alignment process, a maximum likelihood tree was created with RAxML v7.0.4 under the GTR + gamma model for 100 iterations (Stamatakis *et al.* 2005) (Fig. 1).

Genomics

As an independent means of assessing traits, we surveyed taxa for the presence of relevant functional genes. The National Center for Biotechnology Information (NCBI) Microbial Genomes Resources contained annotated, complete genomes of 65 of the study taxa (http://www.ncbi.nlm.nih.gov/genomes/MICROBES/microbial_taxtree.html, accessed 7/11/2010). We used the NCBI Clusters of Orthologous Groups (COG) tool to search the chromosome(s) of each genome for proteins putatively related to uptake or hydrolysis of relatively large, cyclic (i.e. recalcitrant) carbohydrates (Table S3). We focused on compounds that are uncommon in microbial biomass, to avoid genes related to maintenance or conversion of the microbe's own tissues. We expected that the proportion of genes encoding for these proteins would be correlated with the phenotypes determined by

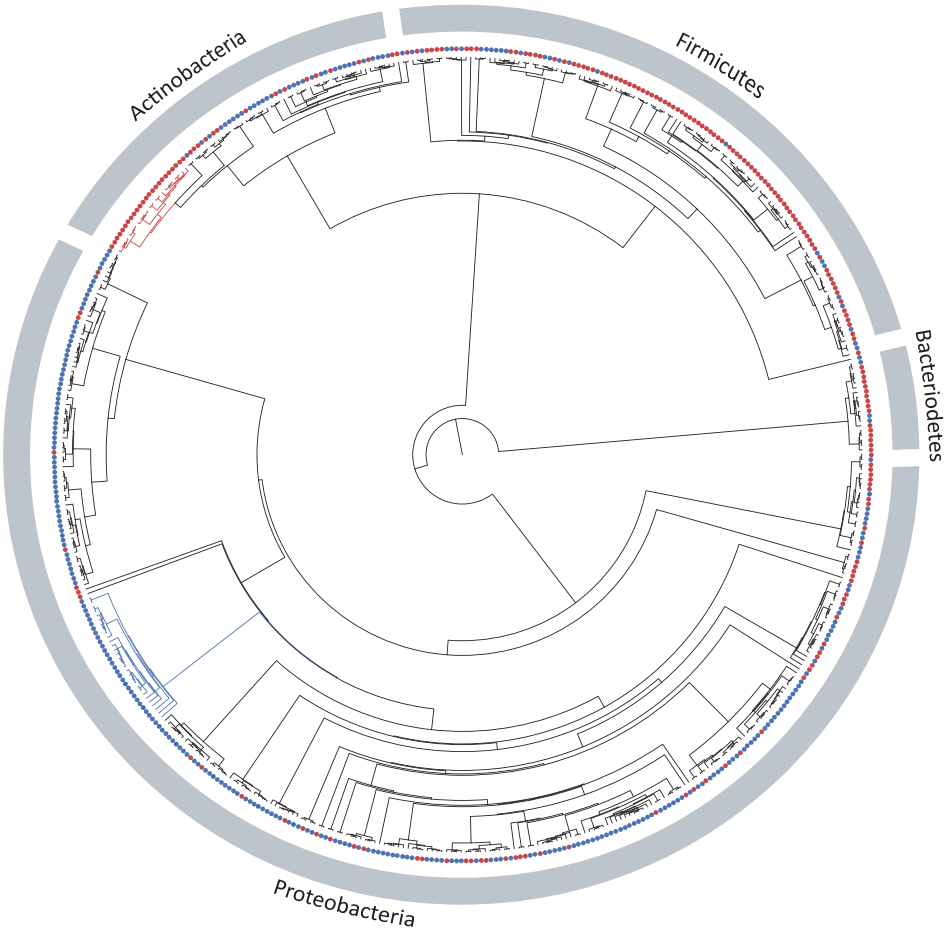

Figure 1 Maximum likelihood tree of all 519 study taxa. Circle colour for each taxon indicates average molar weight of organic compounds used. Red circles: molar weight > 186.74 g mol^{-1}, blue: < 186.74 g mol^{-1}. Red lines highlight Micrococcineae taxa; blue lines, *Pseudomonas* taxa. Proteobacteria and Bacteroidetes are gram-negative; Firmicutes and Actinobacteria, gram-positive.

SUP. Although ideally we would also search for genes related to acquisition and use of organic N from the environment, it was difficult to identify those that targeted external vs. internal sources of organic N. For example, amino acids are a common form of bioavailable organic N in oceans and soils (Eppley & Peterson 1979; Janke 1990; Schulten & Schnitzer 1997), but microbes also produce, transport and recycle these compounds internally. Thus, we did not consider functional genes for organic N use.

Distributions in oceanic habitats

We used the advance BLAST tool in the Community Cyberinfrastructure for Advanced Microbial Ecology Research and Analysis (CAMERA) platform to identify clones in the GOS survey that matched 16S sequences of the study taxa by ≥ 97% similarity (Table S4). This level of 16S similarity is generally associated with a ≥ 70% similarity of total genomic DNA between strains, which suggests that other genes are shared as well (Stackebrandt & Goebel 1994). In the GOS survey, Rusch *et al.* (2007) used shotgun sequencing to characterise bacteria filtered from 40–200 L surface sea water from globally-distributed ocean sites. Sequences of the study taxa matched 107 taxa in 29 sites. Although additional matches were found for the Sargasso Sea sampling site, we did not include this site in our analyses, as that site was the subject of disproportionately intensive sampling compared to the others. We used the World Ocean Atlas (http://www.nodc.noaa.gov/OC5/SELECT/dbsearch/dbsearch.html, accessed 06/07/2010) to obtain annual averages of nitrate concentrations in surface waters of the relevant sampling sites. For each taxon, we calculated a weighted average nitrate concentration for inhabited sampling sites, weighted by the abundance of clones per site (Table S1). We also calculated a weighted average latitude of inhabited sites for each taxon, for comparison with latitudinal distribution in soils.

Distributions in soil habitats

Soil bacteria were ascertained from the primary literature by E. Miller, S. Kivlin, and C. Hawkes. Briefly, they searched literature published from September 2006 until May 2007 and listed in either the ISI Web of Science or Agricola databases, using the keywords 16S rRNA, 16S rDNA and soil, and soil microb* comm* comp*. They included only papers with unfertilized, uncontaminated soil sites that contained more than one sequence. They downloaded full-length (> 1200 bp) 16S sequences and eliminated chimeras identified by Greengenes Bellerophon version 3 (DeSantis *et al.* 2006). We used an internal BLAST algorithm in BioEdit to define 108 study taxa that matched sequences in the soil database at ≥ 97% similarity (Hall 1999). We collected the geographic coordinates and the biome of each collection site, either from the literature or by personal correspondence with the authors (Table S5). We calculated the average latitude at which each taxon resided in the soil (Table S1).

Statistics

We conducted phylogenetic independent contrasts between traits using the analysis of traits (AOT) module in Phylocom (Webb *et al.* 2008), and then checked for clustering of traits by using non-metric multidimensional scaling (SPSS 2002). We used AOT to confirm contrasts in traits between gram positive and gram negative taxa.

RESULTS

Consistent with our hypotheses, taxon traits formed two clusters based on phylogenetic independent contrasts (Fig. 2, Table S5), with the exception of average ocean latitude. One cluster consisted of positive relationships between molar weight of substrates used, proportion cyclic substrates, recalcitrant carbohydrate genes and average ocean nitrate concentrations. The positive relationship between average molar weight used (or proportion cyclic substrates used) and average ocean nitrate concentration is consistent with our first hypothesis, that taxa targeting recalcitrant carbon require higher N availability to produce associated extracellular and transport enzymes. Moreover, we found support for our second hypothesis, given that average molar weight and proportion cyclic substrates used were negatively related to average substrate N content (Fig. 2, Table S6).

Among study taxa detected in ocean sites, the genus *Pseudomonas* (22% of clones) and the sub-order Micrococcineae (18%) were most dominant. Moreover, study taxa displayed fairly consistent SUPs within each of these two clades. Specifically, taxa from *Pseudomonas* (gram-negative Proteobacteria) targeted relatively low-molar weight, non-cyclic organic compounds (Fig. 1, Table 1). In contrast, taxa from Micrococcineae (gram-positive Actinobacteria) tended to use relatively high-molar weight, cyclic organic substrates. Compared to the Micrococcineae, *Pseudomonas* were present in ocean sites with lower nitrate concentrations and they used higher concentrations of organic N (Table 1). In addition, *Pseudomonas* generally occupied mid-latitude soils, while Micrococcineae tended to be found in tropical soils. Differences between *Pseudomonas* and the Micrococcineae were mirrored by contrasts between all gram-positive bacteria and all gram-negative bacteria within the study; gram-positive bacteria targeted higher-molar weight compounds, more cyclic compounds, fewer N-rich compounds and were present at higher ocean nitrate concentrations and more southern latitudes (Table 1).

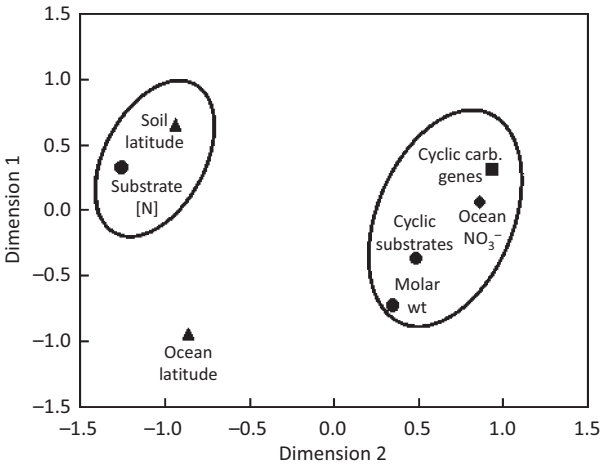

Figure 2 Non-metric multidimensional scaling plot of relationships among traits of study taxa. Each symbol represents one trait. Symbols that are closer to one another are more strongly positively correlated (Table S1). Symbol shapes indicate data source. Circles = substrate use profiles, squares = genomes, diamonds = ocean samples, triangle = soil samples. The final stress was 0.071, and the proportion of variance explained by the two dimensions was 0.967.

Table 1 Traits of selected clades

Trait	Micrococcineae	*Pseudomonas*	Independent contrasts: Gram-positive minus gram-negative taxa*	Independent contrasts: *P*-value
Average molar weight of substrates used (g mol^{-1})	242.7 ± 6.6	146.4 ± 3.6	43.0 ± 4.9	< 0.001
Cyclic substrates used (% of substrates assayed)	76.5 ± 3.0	22.7 ± 2.3	17.1 ± 2.2	< 0.001
Average organic N concentration of substrates used (%)	0.9 ± 0.3	4.8 ± 2.3	−1.7 ± 0.2	< 0.001
Recalcitrant carbohydrate genes (% of genome)	–‡	–‡	0.11 ± 0.03	0.002
Average NO_3^- concentration of ocean sites (μM)	4.9 ± 0.6	0.7 ± 0.1	2.7 ± 0.5	< 0.001
Average latitude of soil sites (°)	5.3 ± 5.7	27.8 ± 5.0	−31.9 ± 3.4†	< 0.001

*Means ± 1 SE.

†More negative values are more southern.

‡Not calculated due to low number of whole genome sequences within clades.

DISCUSSION

We found suggestions of an evolutionary trade-off between recalcitrant carbon use and tolerance of low N conditions. Specifically, recalcitrant carbon users were less common than labile carbon users in marine waters with lower nitrate concentrations and in soils at higher latitudes, where N-limitation is typically stronger (Fig. 2, Table S6). One mechanism that could lead to this trade-off is a higher demand for N by recalcitrant users, as this group must invest N in the production of extracellular enzymes that break down complex compounds, and in diverse transport proteins to take up the products. Extracellular enzyme production, for instance, can require 4–6% of a microbe's N budget (Frankena *et al.* 1988; Allison *et al.* 2010). Extracellular enzyme production appears to be limited where N availability is low, given that N additions often increase the production of extracellular enzymes in these ecosystems (Allison *et al.* 2010). Transport proteins might also represent a significant N expense, as these proteins can account for up to 50% of the mass of microbial cell membranes (Gooday 1994). A recent study found that yeast exposed to ammonium- and urea-limiting conditions for multiple generations acquired deletions of the GAP1 locus, which encodes for a transporter that targets all 20 protein amino acids (Gresham *et al.* 2010). The loss of this transporter is consistent with down-regulation of N investment in membrane transporters under N stress. Labile carbon users need fewer extracellular enzymes, and can invest this N instead in transport proteins that target organic N sources. This strategy would allow labile users to better-tolerate habitats with low availability of inorganic N. Indeed, use of recalcitrant compounds (i.e. average molar weight and proportion cyclic substrates) was negatively related to use of organic N among taxa (Fig. 2, Table S6).

Organic N users in the soil were more abundant at latitudes where N is typically more limiting (LeBauer & Treseder 2008), given that we found a positive relationship between average substrate N concentration and average latitude of soils at which those taxa resided (Fig. 2, Table S6). In contrast, the average latitude of inhabited ocean sites was not strongly related to organic N use, which is not surprising given that nitrate concentrations did not vary significantly by latitude in the ocean sites (Pearson correlation, $r = 0.03$, $P = 0.900$). The contrasting latitudinal distributions of organic N users in soils vs. oceans were consistent with differences in latitudinal distributions in N availability between the two environments.

Labile C users should need fewer extracellular enzymes, and might invest their resources instead in transport proteins that target organic N sources. This strategy would allow labile C users to better-tolerate habitats with low availability of inorganic N. Nitrogen fixation is another means of augmenting N availability in N-poor ecosystems, and labile C users might be relatively well-positioned to invest N and energy in this process (Vitousek & Hobbie 2000). Only five of the study taxa, however, contained nitrogenase genes (*Pectobacterium atrosepticum*, *Burkholderia vietnamiensis*, *Bradyrhizobium japonicum*, *Pseudomonas stutzeri*, and *Klebsiella pneumoniae* subsp. *ozaenae*). This sample size is too small to formally test for relationships between N fixation capacity and the other traits, although the N-fixers tended to use a lower proportion of cyclic compounds (21 ± 7% SE) with a smaller molar weight (153.9 ± 13.8 g mol^{-1}) than did non-N fixers (cyclic: 47 ± 3%; molar weight: 203.9 ± 9.5).

The observed trade-offs among traits are not likely to be an artifact of correlations in substrate traits or ocean chemistry. For instance, neither cyclic compounds ($H = 0.635$, $P = 0.528$) nor larger molar weight compounds (Pearson correlation, $r = 0.098$, $P = 0.418$) possessed significantly lower N contents than non-cyclic or smaller compounds, respectively (Table S2). In addition, nitrate concentrations were not significantly related to sea floor depth in the ocean sites in this study (Table S4, Pearson correlation, $r = 0.058$, $P = 0.38$). This pattern is germane because near-shore (i.e. shallower) areas generally contain higher concentrations of dissolved organic carbon (including recalcitrant carbon) than do off-shore areas (Guo *et al.* 1995). Thus, the positive relationship between recalcitrant carbon use and ocean nitrate concentrations was probably not due to greater availability of recalcitrant carbon in ocean sites with higher nitrate concentrations.

We highlighted two clades, *Pseudomonas* and the Micrococcineae, because the majority of study taxa in the *Pseudomonas* targeted relatively labile carbon compounds, whereas those in the Micrococcineae used more recalcitrant carbon compounds. Thus, these clades can serve as examples for each functional type. We found that their traits were consistent with evolutionary trade-offs suggested for the 519 taxa as a whole. Specifically, labile carbon use (by *Pseudomonas*) occurred concurrently with prevalence in low NO_3^- ocean water and higher soil latitudes, and with greater use of organic N. Recalcitrant carbon use (by Micrococcineae) was associated with the opposite traits. These results suggest that the hypothesised evolutionary trade-offs may be operating within clades as well as across the broader phylogenetic range represented in this study.

Other examples of evolutionary trade-offs in microbes have been reported that have implications for biogeochemical processes. For instance, algae that evolve a tolerance of low nutrient conditions can concurrently lose defences against predation by rotifers (Yoshida *et al.* 2003). In this case, algal taxa that populate low-nutrient areas may also display higher mortality rates – and faster microbial turnover – owing

to predation. These results imply that evolutionary trade-offs may lead bacterial strains to specialise on certain temperature ranges. As a result, the ability of individual taxa to respond immediately to climate change may initially be constrained, with time lags dependent on rates of adaptation. Nevertheless, trade-offs need not always influence microbial responses to environmental conditions. In thermal adaptation of *E. coli*, selection for fitness at cooler temperatures only sometimes results in a decline in fitness at warmer temperatures (Bennett & Lenski 2007). In addition, Velicer & Lenski (1999) found little evidence that adaptation to abundant energy and nutrient resources led to a concomitant decrease in fitness under scarce resources, and vice-versa.

Our findings should be considered within the context of several caveats. First, SUPs were determined *in vitro*, under conditions quite different from marine and soil environments. Nevertheless, genomic traits of taxa were consistent with SUPs in that prevalence of recalcitrant carbohydrate-related genes was positively related to use of larger, cyclic substrates. Second, our analyses were restricted to bacterial taxa that could be cultivated aerobically and in monoculture, even though most bacterial taxa are not culturable by current methods. However, the study taxa consisted of 107 of the 811 taxa from the GOS survey (Rusch *et al.* 2007) and 108 of 1245 taxa from the soil database. Third, this analysis was restricted to bacteria. It remains to be seen whether decomposer fungi display similar trade-offs. Fourth, we essentially tested for correlations between traits, which limits our ability to identify causal mechanisms (Bennett & Lenski 2007). A more robust test would involve experimental selection for recalcitrant substrate use, for instance, combined with assays for sensitivity to N availability and use of organic N. This would be a valuable future endeavour.

An evolutionary trade-off between recalcitrant carbon use and tolerance of low N conditions has important implications for carbon storage under global change. Human activities are leading to N enrichment in soils via anthropogenic N deposition (Vitousek *et al.* 1997), and to increases in nitrate concentrations in oceans via terrestrial runoff (Howarth *et al.* 1996). If recalcitrant users are constrained to habitats with relatively high N availability, they may proliferate under anthropogenic N enrichment. As a result, decomposition rates of recalcitrant carbon may increase, leading to greater fluxes of CO_2 to the atmosphere. Indeed, increases in decomposition of organic matter – including large cyclic compounds such as cellulose – in response to N additions have been documented in marine waters (Kirchman *et al.* 1991; Zweifel *et al.* 1993; Martinez-Garcia *et al.* 2010).

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AUTHORSHIP

KKT conceived the conceptual framework and performed the database syntheses. SNK constructed the phylogeny and conducted the soil database analyses. CVH contributed to construction of the soil database. KKT wrote the first draft of the manuscript, and all authors contributed to the revisions.

REFERENCES

Allison, S.D. & Martiny, J.B.H. (2008). Resistance, resilience, and redundancy in microbial communities. *Proc. Natl. Acad. Sci. USA*, 105, 11512–11519.

Allison, S.D., Weintraub, M.N., Gartner, T.B. & Waldrop, M.P. (2010). Evolutionary-economic principles as regulators of soil enzyme production and ecosystem function. In: *Soil Enzymology* (eds Shukla, G.C. & Varma, A.). Springer, Heidelberg, pp. 245–258.

Bennett, A.F. & Lenski, R.E. (2007). An experimental test of evolutionary trade-offs during temperature adaptation. *Proc. Natl. Acad. Sci. USA*, 104, 8649–8654.

Bochner, B.R., Gadzinski, P. & Panomitros, E. (2001). Phenotype MicroArrays for high-throughput phenotypic testing and assay of gene function. *Genome Res.*, 11, 1246–1255.

Darwin, C.R. (1859). *On the Origin of Species by Means of Natural Selection, or the Preservation of Favoured Races in the Struggle for Life*. John Murray, London.

DeSantis, T.Z., Hugenholtz, P., Larsen, N., Rojas, M., Brodie, E.L., Keller, K. *et al.* (2006). Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. *Appl. Environ. Microbiol.*, 72, 5069–5072.

Eppley, R.W. & Peterson, B.J. (1979). Particulate organic matter flux and planktonic new production in the deep ocean. *Nature*, 282, 677–680.

Frankena, J., Vanverseveld, H.W. & Stouthamer, A.H. (1988). Substrate and energy costs of the production of exocellular enzymes by *Bacillus licheniformis*. *Biotechnol. Bioeng.*, 32, 803–812.

Gooday, G.W. (1994). Cell membrane. In: *Growing Fungus* (eds Gow, N.A.R. & Gadd, G.M.). Chapman & Hall, London, pp. 43–62.

Gresham, D., Usaite, R., Germann, S.M., Lisby, M., Botstein, D. & Regenberg, B. (2010). Adaptation to diverse nitrogen-limited environments by deletion or extrachromosomal element formation of the GAP1 locus. *Proc. Nat. Acad. Sci.*, 107, 18551–18556.

Guo, L., Santschi, P.H. & Warnken, K.W. (1995). Dynamics of dissolved organic carbon (DOC) in oceanic environments. *Limnol. Oceanogr.*, 40, 1392–1403.

Hall, T.A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucl. Acids Symp. Ser.*, 41, 95–98.

Howarth, R.W., Billen, G., Swaney, D., Townsend, A., Jaworski, N., Lajtha, K. *et al.* (1996). Regional nitrogen budgets and riverine N & P fluxes for the drainages to the North Atlantic Ocean: natural and human influences. *Biogeochemistry*, 35, 75–139.

Janke, R.A. (1990). Ocean flux studies: a status report. *Rev. Geophys.*, 28, 381–398.

Kirchman, D.L., Suzuki, Y., Garside, C. & Ducklow, H.W. (1991). High turnover rates of dissolved organic carbon during a spring phytoplankton bloom. *Nature*, 352, 612–614.

LeBauer, D.S. & Treseder, K.K. (2008). Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed. *Ecology*, 89, 371–379.

Levins, R. (1968). *Evolution in Changing Environments*. Princeton University Press, Princeton.

Liu, K., Raghavan, S., Nelesen, S., Linder, C.R. & Warnow, T. (2009). Rapid and accurate large-scale coestimation of sequence alignments and phylogenetic trees. *Science*, 324, 1561–1564.

Martinez-Garcia, S., Fernandez, E., Calvo-Diaz, A., Maranon, E., Moran, X.A.G. & Teira, E. (2010). Response of heterotrophic and autotrophic microbial plankton to inorganic and organic inputs along a latitudinal transect in the Atlantic Ocean. *Biogeosciences*, 7, 1701–1713.

Muir, D.C.G. & Howard, P.H. (2006). Are there other persistent organic pollutants? A challenge for environmental chemists. *Environ. Sci. Technol.*, 40, 7157–7166.

Rusch, D.B., Halpern, A.L., Sutton, G., Heidelberg, K.B., Williamson, S., Yooseph, S. *et al.* (2007). The *Sorcerer II* Global Ocean Sampling Expedition: Northwest Atlantic through Eastern Tropical Pacific. *PLoS Biol.*, 5, e77.

Schimel, J.P. & Bennett, J. (2004). Nitrogen mineralization: challenges of a changing paradigm. *Ecology*, 85, 591–602.

Schulten, H.R. & Schnitzer, M. (1997). The chemistry of soil organic nitrogen: a review. *Biol. Fertil. Soils*, 26, 1–15.

SPSS (2002). *Systat version 10.2*. SPSS Inc., Chicago, IL.

Stackebrandt, E. & Goebel, B.M. (1994). A place for DNA-DNA reassociation and 16S ribosomal-RNA sequence analysis in the present species definition in bacteriology. *Int. J. Syst. Bacteriol.*, 44, 846–849.

Stamatakis, A., Ludwig, T. & Meier, H. (2005). RAxML-III: a fast program for maximum likelihood-based inference of large phylogenetic trees. *Bioinformatics*, 21, 456–463.

Stearns, S.C. (1989). Trade-offs in life history evolution. *Funct. Ecol.*, 3, 259–268.
Velicer, G.J. & Lenski, R.E. (1999). Evolutionary trade-offs under conditions of resource abundance and scarcity: experiments with bacteria. *Ecology*, 80, 1168–1179.
Vitousek, P.M. & Hobbie, S. (2000). Heterotrophic nitrogen fixation in decomposing litter: patterns and regulation. *Ecology*, 81, 2366–2376.
Vitousek, P.M., Aber, J.D., Howarth, R.W., Likens, G.E., Matson, P.A., Schindler, D.W. *et al.* (1997). Human alteration of the global nitrogen cycle: sources and consequences. *Ecol. Appl.*, 7, 737–750.
Webb, C.O., Ackerly, D.D. & Kembel, S.W. (2008). Phylocom: software for the analysis of phylogenetic community structure and trait evolution. *Bioinformatics*, 24, 2098–2100.
Yoshida, T., Jones, L.E., Ellner, S.P., Fussmann, G.F. & Hairston, N.G. (2003). Rapid evolution drives ecological dynamics in a predator-prey system. *Nature*, 424, 303–306.
Zweifel, U.L., Norrman, B. & Hagstrom, A. (1993). Consumption of dissolved organic carbon by marine bacteria and demand for inorganic nutrients. *Mar. Ecol.-Prog. Ser.*, 101, 23–32.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

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Table S2 Characteristics of compounds in substrate use profile assay.
Table S3 Genes related to use of recalcitrant carbohydrates.
Table S4 Global ocean sampling sites included in analyses.
Table S5 Soil sites included in analysis.
Table S6 Phylogenetic independent contrasts of taxa traits.

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