LETTERS

Ecoenzymatic stoichiometry of microbial organic nutrient acquisition in soil and sediment

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Biota can be described in terms of elemental composition, expressed as an atomic ratio of carbon:nitrogen:phosphorus (refs 1–3). The elemental stoichiometry of microoorganisms is fundamental for understanding the production dynamics and biogeochemical cycles of ecosystems because microbial biomass is the trophic base of detrital food webs[4–6]. Here we show that heterotrophic microbial communities of diverse composition from terrestrial soils and freshwater sediments share a common functional stoichiometry in relation to organic nutrient acquisition. The activities of four enzymes that catalyse the hydrolysis of assimilable products from the principal environmental sources of C, N and P show similar scaling relationships over several orders of magnitude, with a mean ratio for C:N:P activities near 1:1:1 in all habitats. We suggest that these ecoenzymatic ratios reflect the equilibria between the elemental composition of microbial biomass and detrital organic matter and the efficiencies of microbial nutrient assimilation and growth. Because ecoenzymatic activities intersect the stoichiometric and metabolic theories of ecology[7–9], they provide a functional measure of the threshold at which control of community metabolism shifts from nutrient to energy flow.

Ecological stoichiometric theory uses elemental ratios and the concept of stoichiometric invariance to predict nutrient retention and biomass production on subcellular to ecosystem scales, creating a unifying theory of ecology[5]. The complementary metabolic theory of ecology uses thermodynamics and the concept of energetic invariance to predict metabolic activity across all levels of biological organization[7–10]. Both theories have developed over the course of a century and are supported by large bodies of literature, but evaluations are complicated because ecological systems are controlled by non-equilibrium flows of both materials (nutrients) and energy (carbon) and it is difficult to know which is predominant in a specific context. Ecoenzyme (enzymes not contained by membranes of living cells) activity represents an intersection of ecological stoichiometric theory and metabolic theory of ecology because enzyme expression is a product of cellular metabolism specifically regulated by environmental nutrient availability (Supplementary Fig. 1).

Terrestrial soils and freshwater sediments contain reserves of organic carbon estimated at 1,500 petagrams (ref. 11; $1\ \mathrm{Pg} = 10^{15}\ \mathrm{g}$) and 0.2 Pg (ref. 12), respectively. Mineralization of this organic matter by heterotrophic microorganisms creates the trophic base for detritus food webs, drives global carbon and nutrient cycles, and mediates plant production and atmospheric composition. The proximate agents of organic matter decomposition are ecoenzymes that deconstruct plant and microbial cell walls, depolymerize macromolecules, and ultimately produce soluble substrates for microbial assimilation[13,14]. Many of these enzymes are expressed and released into the environment by microorganisms in response to environmental signals; others enter the environment through cell lysis. The most widely assayed ecoenzymes are those involved in the degradation of lignocellulose, the principal product of primary production. Lignocellulose does not contain N or P so enzymes that hydrolyse proteins, chitin and peptidoglycan (the principal sources of organic N) and mineralize P from nucleic acids, phospholipids and other ester phosphates are also widely measured[15].

The enzymatic degradation of biopolymers requires the synergistic interaction of several classes of enzymes. However, ecological studies generally quantify only the activities of enzymes that catalyse the terminal reactions that produce assimilable products from the principal C, N and P sources. The mostly widely measured activities include β-1,4-glucosidase (BG), β-1,4-N-acetylglucosaminidase (NAG), leucine aminopeptidase (LAP) and acid (alkaline) phosphatase (AP; Table 1). The potential activities of these enzymes are frequently linked to rates of microbial metabolism and biogeochemical processes, and used as indicators of microbial nutrient demand[15–17].

Microbial nutrient demand is determined by the elemental stoichiometry of microbial biomass in relation to environmental nutrient availability. The mean C:N:P ratio of plant litter is about 3,000:46:1 (ref. 2). Microbial respiration and nutrient immobilization increase the N and P content of litter as it decomposes, eventually creating soil organic matter with a mean C:N:P ratio of 186:13:1 (ref. 3). N and P are further concentrated in soil microbial biomass, which has a mean C:N:P ratio of 60:7:1 (ref. 3). Similar elemental ratios have been reported for heterotrophic microbial biomass associated with surface sediments of inland waters[18].

We analysed the stoichiometry of BG, NAG, LAP and AP activities, using data collected in three synoptic studies of terrestrial soils and freshwater sediments (Table 2). These measures of organic C, N and P acquisition show similar scaling relationships that become increasingly correlated along the hydrologic gradient from terrestrial soil, to wetland (lentic) sediment, to river (lotic) sediment (Fig. 1). The slopes of the ecoenzymatic C:N and C:P regressions differ significantly by habitat, except for the C:N activity regressions for soil and lotic

Table 1 | Ecoenzymes included in this study

Enzyme	Abbrev.	EC*	Function
β-1,4-glucosidase	BG	3.2.1.21	Cellulose degradation: hydrolyses glucose from cellobiose
β-1,4-N-acetylglucosaminidase	NAG	3.2.1.14	Chitin and peptidoglycan degradation: hydrolyses glucosamine from chitobiose
Leucine aminopeptidase	LAP	3.4.11.1	Proteolysis: hydrolyses leucine and other hydrophobic amino acids from the N terminus of polypeptides
Acid (alkaline) phosphatase	AP	3.1.3.1	Hydrolyses phosphate from phosphosaccarides and phospholipids

* Enzyme Commission Number.

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Table 2 | Mean ecoenzymatic activities of sediments and soils

	Habitat		
	Lotic	Lentic	Soil
BG	7,580	20,090	4,960
AP	5,080	39,500	7,980
NAG	3,750	6,520	2,570
LAP	1,580	46,400	950
BG:AP	1.49	0.51	0.62
BG:(NAG + LAP)	1.42	0.38	1.41
(NAG + LAP):AP	1.05	1.34	0.44
Sites	445	60	40
Observations	445	60	935

Lotic sediments were collected from the Upper Mississippi, Missouri and Ohio River systems using the US Environmental Protection Agency's EMAP synoptic sampling design; lentic sediments were collected from the Great Lakes Basin using the EMAP synoptic sampling design, and terrestrial soils were collected from 40 ecosystems that span all major biomes. Sediment and soil data from refs 17–19. Units of activity are nmol h^{-1} per g C. BG:AP, BG:(NAG + LAP) and (NAG + LAP):AP are metrics for ecoenzymatic C:P, C:N and N:P acquisition activities, respectively. BG, β-1,4-glucosidase; NAG, β-1,4-N-acetylglucosaminidase; LAP, leucine aminopeptidase; AP, alkaline/acid phosphatase (AP).

sediment (Supplementary Information). Within habitats, C acquisition has similar scaling relationships with N and P acquisition (N/P 1.03/1.03 for lotic sediment, 0.77/0.76 for lentic sediment, 1.09/1.16 for soil, Fig. 1). Consequently, the slope of N:P activity regressions is approximately 1.0 for all habitats (Fig. 1). In all three environments, the mean ecoenzymatic C:N:P activity ratio is approximately 1:1:1 (Fig. 2). The mean C:P activity ratio for freshwater sediments is significantly greater than that for terrestrial soils (1.049 versus 0.959). The mean C:N activity ratio for terrestrial soils is significantly greater than that for freshwater sediments (1.023 versus 0.997). The stoichiometry among the C, N and P acquiring enzymes is more diffuse in terrestrial soils than in aquatic sediments (Fig. 1), probably because soils have greater heterogeneity in organic matter composition, nutrient distribution and edaphic conditions[19] (Supplementary Information).

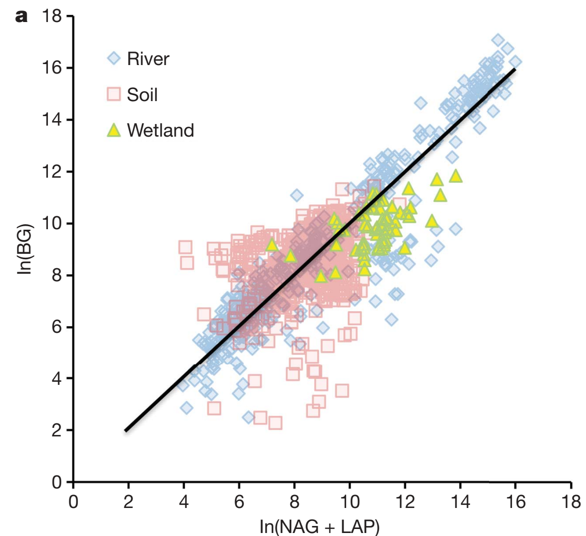
The distribution of ecoenzymatic C:N:P activity ratios identifies the boundaries of microbial community response to disturbances or fluctuations in nutrient availability, and shows that heterotrophic microbial communities of diverse composition share a common pattern of functional organization. The mean C:N:P stoichiometry of these enzyme activities indicates that the rates of supply of assimilable substrates from the respective C, N and P reservoirs are on average similar in magnitude, and thus that proximate limitations on microbial production may readily shift between C, N and P (ref. 20).

The similarity of ecoenzymatic C:N:P stoichiometry across ecosystems is unexpected, because these activities vary widely and experimental manipulations within ecosystems show that C, N and P acquisition activities can be modulated by inorganic nutrient availability[21–23]. These responses are generally interpreted in the context of resource allocation models based on the premise that cellular resources directed towards N and P acquisition reduce resources available for C acquisition[15,17] (Supplementary Information). Our analyses suggest that the capacity of microbial communities to alter relationships among ecoenzymatic C, N and P acquisition activities in response to environmental resource availability is limited, with similar boundaries in all habitats.

The stoichiometry of ecoenzymatic activity can be related to ecological stoichiometric theory and the metabolic theory of ecology through the threshold elemental ratio (TER) concept[9,24]. TER is the critical elemental C:P or C:N ratio at which metabolic control of an ecological system switches from energy flow, represented by C, to limiting nutrient flow, represented by P or N. TER for P and N can be represented as[9]:

$$\mathrm{TER}_{\mathrm{C:P}} = \left(\frac{A_{\mathrm{P}}}{\mathrm{GE}}\right) B_{\mathrm{C:P}} \quad (1)$$

$$\mathrm{TER}_{\mathrm{C:N}} = \left(\frac{A_{\mathrm{N}}}{\mathrm{GE}}\right) B_{\mathrm{C:N}} \quad (2)$$



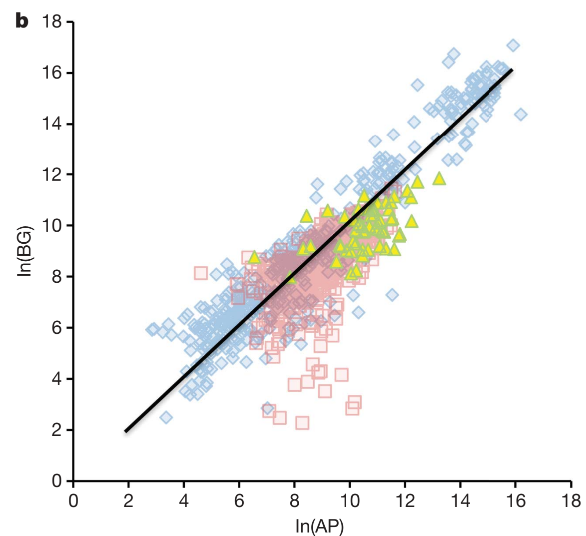


Figure 1 | Organic nitrogen (N) acquisition activity and organic phosphorus (P) acquisition activity in relation to carbon (C) acquisition. N acquisition is measured by the potential activities of β-1,4-N-acetylglucosaminidase (NAG) and leucine aminopeptidase (LAP) (**a**); P acquisition is measured as acid (alkaline) phosphatase (AP) activity (**b**); C acquisition is represented by β-1,4-glucosidase (BG). For lotic sediments, the standardized major axis (type II) regression for C:P acquisition activity is $\ln(\mathrm{BG}) = 1.030 \times \ln(\mathrm{AP}) + 0.149$, $R^2 = 0.897$, $P < 0.001$; the C:N regression is $\ln(\mathrm{BG}) = 1.032 \times \ln(\mathrm{NAG + LAP}) - 0.268$, $R^2 = 0.882$, $P < 0.001$; $n = 445$. For lentic sediments, the type II C:P regression is $\ln(\mathrm{BG}) = 0.763 \times \ln(\mathrm{AP}) + 1.831$, $R^2 = 0.330$, $P < 0.001$; the C:N regression is $\ln(\mathrm{BG}) = 0.767 \times \ln(\mathrm{NAG + LAP}) + 1.520$, $R^2 = 0.323$, $P < 0.001$; $n = 60$. For soils the C:P regression is $\ln(\mathrm{BG}) = 1.162 \times \ln(\mathrm{AP}) - 1.845$, $R^2 = 0.396$, $P < 0.001$; the C:N regression is $\ln(\mathrm{BG}) = 1.091 \times \ln(\mathrm{NAG + LAP}) - 0.660$, $R^2 = 0.157$, $P < 0.001$; $n = 929$). The slopes of enzymatic C:P and C:N regressions differ significantly by habitat ($P < 0.017$) with the exception of C:N regression slopes for soil and lotic sediments. For the global data set, the type II C:P regression is $\ln(\mathrm{BG}) = 1.037 \times \ln(\mathrm{AP}) - 0.481$, $R^2 = 0.761$, $P < 0.001$; the C:N regression is $\ln(\mathrm{BG}) = 1.018 \times \ln(\mathrm{NAG + LAP}) - 0.124$, $R^2 = 0.695$, $P < 0.001$; $n = 1434$); the N:P regression is $\ln(\mathrm{NAG + LAP}) = 1.018 \times \ln(\mathrm{AP}) - 0.351$, $R^2 = 0.694$, $P < 0.001$. Reference lines with a slope of 1.0 are shown on the graphs.

where A_{P} and A_{N} are assimilation efficiencies for P and N, GE is microbial growth efficiency and $B_{\mathrm{C:P}}$ and $B_{\mathrm{C:N}}$ are the C:P and C:N ratios of microbial biomass. Using the mean elemental C:P ratios for soil organic matter (186) and microbial biomass (60; ref. 3) as values for $\mathrm{TER}_{\mathrm{C:P}}$ and $B_{\mathrm{C:P}}$, and 0.9 for A_{P} (ref. 9), mean microbial growth efficiency is 0.29, the same value reported for aquatic invertebrates[9]

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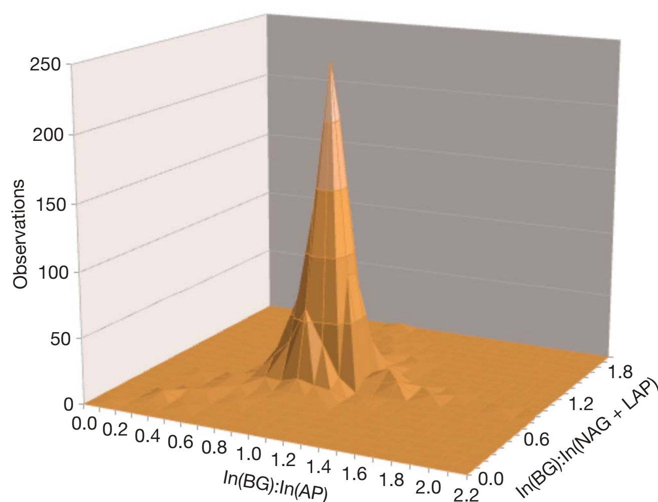

Figure 2 | Frequency distribution of ecoenzymatic ratios. **Distribution of heterotrophic microbial communities in terrestrial soils and freshwater sediments in relation to ecoenzymatic C:N and C:P acquisition activity, as measured by ratios of BG:(NAG + LAP) and BG:AP activities. By these measures, the mean global ratio of C:N:P acquisition is approximately 1:1:1 (mean C:N 1.01 (s.d. 0.15), mean C:P 0.99 (0.14)). Mean values for lotic sediment, wetland sediment and soil C:P activity are 1.063 (0.170), 0.943 (0.098) and 0.959 (0.114), respectively; mean values for C:N acquisition activity are 1.008 (0.130), 0.912 (0.091) and 1.023 (0.163), respectively. The mean ecoenzymatic C:P acquisition ratio for freshwater sediment (1.049) is significantly greater than that for soils (analysis of variance, $F_{1,1440} = 147$, $P < 10^{-31}$). The mean ecoenzymatic C:N acquisition ratio for freshwater sediment (0.997) is significantly lower than that of soils ($F_{1,1440} = 10.6$, $P = 0.011$).**

and similar to mean growth efficiencies for soil[25] and freshwater[26] microorganisms. The calculation suggests that C:P ratios >186 and GE < 0.29 are indicators of P limitation, rather than energy (C) limitation. The parallel calculation using elemental C:N ratios of 14.3 and 8.6 for soil organic matter and microbial biomass[3] yields a GE value of 0.54. Because this value does not correspond with the GE estimate from the $TER_{C:P}$ calculation and lies near the extreme for microbial community GE estimates, we infer either that mean A_N is half that of A_P, or more likely, that the mean $TER_{C:N}$ is greater than the mean C:N ratio of soil organic matter. If $A_N = A_P = 0.9$ and mean GE is 0.29, then $TER_{C:N}$ is approximately 27. This value is consistent with many empirical estimates of $TER_{C:N}$ (refs 16, 27) and with analyses indicating that TER values are 2–3 times greater than elemental biomass ratios[24].

Ratios of ecoenzymatic activities can be related to both resource availability and microbial metabolism:

$$TER_{C:P}/L_{C:P} \propto BG/AP \propto L_{C:P}/B_{C:P} \quad (3)$$

$$TER_{C:N}/L_{C:N} \propto BG/(NAG + LAP) \propto L_{C:N}/B_{C:N} \quad (4)$$

where $L_{C:P}$ and $L_{C:N}$ are the elemental C:P and C:N ratios of labile organic matter, that is, organic matter readily available for microbial consumption. Equations (3) and (4) can be reduced and combined with equations (1) and (2):

$$BG/AP = p_0(TER_{C:P}/B_{C:P}) = p_0(A_P/GE) \quad (5)$$

$$BG/(NAG + LAP) = n_0(TER_{C:N}/B_{C:N}) = n_0(A_N/GE) \quad (6)$$

where p_0 and n_0 are normalization constants (Supplementary Information).

Using the mean C:P value of soil organic matter (186) for $TER_{C:P}$, the mean C:P of microbial biomass (60) for $B_{C:P}$, and a mean BG:AP ratio of 1.0, the $\log_e$ transformed version of equation (5) returns a $\ln(p_0)$ of −1.13. Alternatively, the observed $\ln(p_0)$ of −1.84 from the soil C:P regression (Fig. 1) returns a BG:AP ratio of 0.5 (Table 1, equivalent to ln(BG)/ln(AP) of 0.95). For nitrogen, using a $TER_{C:N}$ estimate of 27, the mean C:N of microbial biomass (8.6) for $B_{C:N}$ and a mean BG:(NAG + LAP) ratio of 1.0, the $\log_e$ transformed version of equation (6) returns a $\ln(n_0)$ of −1.14; using the observed $\ln(n_0)$ of −0.66 from the soil C:N regression (Fig. 1) returns a BG/(NAG + LAP) ratio of 1.6 (Table 2, equivalent to ln(BG)/ln(NAG + LAP) of 1.06).

At a global scale, these relationships show that ecoenzymatic activity ratios can be empirically linked to the biogeochemical equilibrium between microbial growth efficiency and the elemental compositions of soil organic matter and microbial biomass. Because ecoenzymatic activities are related to both resource availability and microbial growth on the same spatiotemporal scale, analyses of these activities may be used to resolve energetic and nutrient constraints on microbial community metabolism in the context of stoichiometric and metabolic theories of ecology.

METHODS SUMMARY

The soil data were obtained from ref. 19; the freshwater sediment data were presented in refs 28 and 29 (Table 2). A common assay protocol using fluorigenic substrates was used in all studies. Cases with missing values for one or more enzyme activity were excluded from our analyses. Where necessary, activities expressed per g organic matter (OM) were converted to units per g C using a conversion of 0.45 g C/g OM. The type II regression results (Fig. 1) were calculated using SMATR v2.0[30] (Supplementary Table 1).

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

Received 3 July; accepted 29 October 2009.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

Acknowledgements J.J.F.S. was supported by the National Science Foundation (DBI-0630558).

Author Contributions R.L.S. provided data from soils. B.H.H. provided data from freshwater sediments. J.J.F.S. and R.L.S. collaborated on data synthesis and interpretation. All authors contributed to manuscript preparation.

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METHODS

Sample collection. Terrestrial soil, excluding surface litter, was collected at each site to depths of 5–20 cm. The samples included in this study are a subsample of those assembled in ref. 19 for a meta-analysis of soil enzyme activities and include all major biomes. Nearly all of the sampling was done in the past ten years. Sampling design varied among ecosystems. Metadata for the individual terrestrial ecosystems included in Table 2 were presented in ref. 19.

River sediment samples were collected as part of the US Environmental Protection Agency's Environmental Monitoring and Assessment Program for Great Rivers (EMAP-GRE), which includes the Upper Mississippi River from Lower St Anthony Falls in Minneapolis-St Paul, Minnesota, to the confluence with the Ohio River at Cairo, Illinois; the Missouri River from Fort Peck Dam in Montana to the confluence with the Mississippi River at St Louis, Missouri; and the Ohio River from the confluence of the Allegheny and Monongahela Rivers in Pittsburgh, Pennsylvania, to the confluence with the Mississippi River[29]. The EMAP-GRE design was spatially balanced and used an unequal probability for selecting sites based on unique river reaches, for example, the unimpounded Upper Mississippi River. The survey design selected 447 sites representing 4,838 km of river channel. Sampling at these sites occurred July–September 2004–06. Surface sediment samples (top 5 cm) at each site were collected at 11 stations equally spaced along a 500 m, longitudinal channel-margin transect and combined for all stations at a site, resulting in a single composite sample per site.

Lentic sediment samples were collected from coastal wetlands within the Laurentian Great Lakes[28]. The sampled wetlands represent the basin-wide range of natural conditions and human impacts on wetlands. Twenty-four wetlands were sampled in 2002, 20 in 2003 and 14 in 2004. Sampling locations within each wetland were selected by dividing the wetted perimeter of the wetland into seven equal interval segments with sampling stations corresponding to segment boundaries. Surface sediment samples (top 5 cm) were collected from emergent vegetation, submerged vegetation and open water zones. Within each zone sediment collections were combined for all stations resulting in three samples per wetland. Cases were included in our analyses only if they had values for all four ecoenzymatic activities (Table 2).

Enzyme assays. Hydrolytic enzyme activities were assayed using substrates linked to a methylumbelliferyl fluor[19,28,29]. Sediment samples were assayed at 20 °C using pH 8 bicarbonate buffer. Soil samples were assayed at 20 °C in either pH 8 bicarbonate buffer or pH 5 acetate buffer to approximate the bulk soil pH of the system from which they were collected. Activities were calculated in units of nmol h^{-1} per g C.

Statistical analyses. Statistical analyses and results are described in the text with additional results included in the Supplementary Information.

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