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REVIEW AND SYNTHESIS

Stoichiometry of soil enzyme activity at global scale

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Abstract

Extracellular enzymes are the proximate agents of organic matter decomposition and measures of these activities can be used as indicators of microbial nutrient demand. We conducted a global-scale meta-analysis of the seven-most widely measured soil enzyme activities, using data from 40 ecosystems. The activities of β-1,4-glucosidase, cellobiohydrolase, β-1,4-*N*-acetylglucosaminidase and phosphatase g^{-1} soil increased with organic matter concentration; leucine aminopeptidase, phenol oxidase and peroxidase activities showed no relationship. All activities were significantly related to soil pH. Specific activities, i.e. activity g^{-1} soil organic matter, also varied in relation to soil pH for all enzymes. Relationships with mean annual temperature (MAT) and precipitation (MAP) were generally weak. For hydrolases, ratios of specific C, N and P acquisition activities converged on 1 : 1 : 1 but across ecosystems, the ratio of C : P acquisition was inversely related to MAP and MAT while the ratio of C : N acquisition increased with MAP. Oxidative activities were more variable than hydrolytic activities and increased with soil pH. Our analyses indicate that the enzymatic potential for hydrolyzing the labile components of soil organic matter is tied to substrate availability, soil pH and the stoichiometry of microbial nutrient demand. The enzymatic potential for oxidizing the recalcitrant fractions of soil organic material, which is a proximate control on soil organic matter accumulation, is most strongly related to soil pH. These trends provide insight into the biogeochemical processes that create global patterns in ecological stoichiometry and organic matter storage.

Keywords

C : N : P ratio, cellobiohydrolase, ecological stoichiometry, leucine aminopeptidase, peroxidase, phenol oxidase, phosphatase, soil enzyme activity, soil organic matter, β-1,4-glucosidase, β-1,4-*N*-acetylglucosaminidase.

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INTRODUCTION

Terrestrial soils contain the largest reservoir of organic carbon in the biosphere (*c.* 1800 Pg). Mineralization of this organic matter by heterotrophic microorganisms affects global carbon and nutrient cycles, plant production and atmospheric composition. The proximate agents of soil organic matter (SOM) decomposition are extracellular enzymes that deconstruct plant and microbial cell walls and reduce macromolecules to soluble substrates for microbial assimilation (Burns 1978; Burns & Dick 2002). In the context of global nutrient cycles, these enzymes catalyse processes that are antipodal to C-fixation by ribulose bisphosphate carboxylase and N-fixation by nitrogenase.

Extracellular enzyme activity (EEA) in soils has been studied for more than a century with a goal of understanding the biochemistry of decomposition and nutrient cycling (Skujins 1978). Soil EEA has also been studied in relation to ecosystem responses to global change and other disturbances (e.g. Lipson *et al.* 2005; Sinsabaugh *et al.* 2005; Finzi *et al.* 2006). The most widely assayed enzymes are those involved in the degradation of cellulose and lignin, the most abundant components of plant litter (Allison *et al.* 2007). Other commonly measured enzymes hydrolyze proteins, chitin and peptidoglycan, which are the principal reservoirs of organic N (Caldwell 2005). Extracellular phosphatases are of interest for their role in mineralizing P from nucleic acids, phospholipids and other ester phosphates (Turner *et al.* 2002; Toor *et al.* 2003). The structural heterogeneity of biopolymers requires the interaction of several classes of enzymes to reduce them to constituent monomers available for microbial consumption (Ljungdahl & Eriksson 1985; Kirk & Farrell 1987; Sinsabaugh 2005). However, most studies of soil EEA are limited to enzymes that catalyse the production of the terminal monomers, because the kinetics are easier to study and the reactions produce assimilable products (Allison *et al.* 2007).

Because EEA mediates microbial nutrient acquisition from organic matter, these activities are commonly interpreted as indicators of microbial nutrient demand (Olander & Vitousek 2000; Schimel & Weintraub 2003; Caldwell 2005; Moorhead & Sinsabaugh 2006). This demand is determined by the elemental stoichiometry of microbial biomass in relation to environmental nutrient availability. Stoichiometric constraints on biomass composition are evident for phytoplankton (Redfield 1958), terrestrial plants (McGroddy *et al.* 2004) and animals (Sterner & Elser 2002) as well as soil microbial biomass (Cleveland & Liptzin 2007). However, within all of these groups, there is variation in biomass stoichiometry among ecosystems that can be related to constraints imposed by local nutrient availability. For example, large-scale variation in the C : N : P ratios of plant foliage is consistent with observations that tropical forests are generally more P-limited than high-latitude forests, which tend to be N-limited (McGroddy *et al.* 2004; Reich & Oleksyn 2004). This pattern arises because high rates of weathering in tropical regions lead to the loss of rock-derived nutrients, such as P, while episodic glaciations in high-latitude regions limit the accumulation of N (Walker & Syers 1976; Vitousek & Howarth 1991). Microbial biomass composition does not follow a latitudinal trend but does vary in relation to ecosystem type (Cleveland & Liptzin 2007). Because EEA links environmental nutrient availability with microbial production, large-scale patterns in EEA may reveal the constraints on microbial biomass stoichiometry and enzyme relationships to SOM composition.

Large-scale EEA patterns may also provide insights into the biochemical controls on soil carbon storage. Because EEA catalyses rate-limiting steps in organic matter degradation, correlations between rates of plant litter decomposition, microbial production and EEA are frequently observed (Andersson *et al.* 2005; Sinsabaugh *et al.* 2005; Weintraub *et al.* 2007; Waldrop & Harden 2008). However, the contribution of these relationships to the global distribution of SOM has not been evaluated.

Despite thousands of published studies, technological limitations and lack of standardized protocols have precluded a comparative analysis of the magnitude and distribution of soil EEA in relation to global climatic and edaphic gradients. During the past decade, protocols that combine the use of fluorigenic substrates with high throughput microplate technology have come into general use (Sinsabaugh *et al.* 1997; Marx *et al.* 2001). As a result, we can now assemble a comparative database of soil EEA potentials for 40 ecosystems. These data reveal unexpected stoichiometric constraints on the functional organization of microbial communities and the dynamics of SOM accumulation.

METHODS

Data description

Soil, excluding surface litter, was collected at each site to depths of 5–20 cm, and assayed for the potential activities of one or more extracellular enzymes. Hydrolytic enzymes were assayed using substrates linked to a methylumbelliferyl fluor; oxidative enzymes were assayed colorimetrically using L-3,4-dihydroxyphenylalanine (Table 1). Activities were calculated in units of nmol h^{-1} g^{-1} dry mass and nmol h^{-1} g^{-1} SOM. Samples were incubated at 20 ± 2 °C, except for Niwot Ridge samples, which were incubated at 15 ± 2 °C. To approximate ambient soil pH (Table 2), acidic soils were assayed at pH 5 by suspending

Table 1 Soil enzymes assayed for potential activity

Enzyme	EC	Abbreviation	Substrate
β-1,4-glucosidase	EC 3.2.1.21	BG	4-MUB-β-D-glucoside
Cellobiohydrolase	EC 3.2.1.91	CBH	4-MUB-β-D-cellobioside
β-*N*-acetylglucosaminidase	EC 3.2.1.14	NAG	4-MUB-*N*-acetyl-ß-D-glucosaminide
Leucyl aminopeptidase	EC 3.4.11.1	LAP	L-Leucine-7-amido-4-methylcoumarin
Acid (alkaline) phosphatase	EC 3.1.3.1	AP	4-MUB-phosphate
Phenol oxidase	EC 1.10.3.2	POX	L-3,4-dihydroxyphenylalanine
Peroxidase	EC 1.11.1.7	PER	L-3,4-dihydroxyphenylalanine and H_2O_2

EC, enzyme commission classification; MUB, methylumbelliferyl.

c. 1 g soil in 100 mL of 50 mM sodium acetate buffer; alkaline soils were assayed at pH 8 using 50 mM sodium bicarbonate buffer.

The database includes activities for the seven-most widely measured soil enzymes from 40 ecosystems. The number of cases per ecosystem (the number of locations sampled × the number of sampling dates), ranges from 4 to 169, for a total of 1154 cases (Table 3). Metadata for all sites are appended as Supporting information.

Enzyme description

β-1,4-Glucosidase (BG) and cellobiohydrolase (CBH) are enzymes that contribute to the degradation of cellulose and other beta-1,4 glucans (Ljungdahl & Eriksson 1985). The principal function of BG is hydrolysis of cellobiose to glucose, but many of these enzymes are active against other substrates as well. CBH hydrolyzes cellobiose dimers from the non-reducing ends of cellulose molecules. β-*N*-acetyl-glucosaminidase (NAG) plays a role in the degradation of chitin and other β-1,4-linked glucosamine polymers that are analogous to the role of BG in cellulose degradation (Sinsabaugh 2005). Leucine aminopeptidase (LAP) hydrolyzes leucine and other hydrophobic amino acids from the N terminus of polypeptides. There are other classes of aminopeptidases, but assays of environmental samples generally show the greatest activities towards leucine- and alanine-linked substrates, so LAP activity is broadly used as an indicator of peptidase potential (Sinsabaugh & Foreman 2001; Stursova *et al.* 2006). Phosphatases (alkaline and acid, AP) hydrolyze phosphomonoesters, and in some cases phosphodiesters, releasing phosphate (Turner *et al.* 2002; Toor *et al.* 2003). The degradation of polyphenols (e.g. lignin, tannin and their degradation products) is an oxidative process (Kirk & Farrell 1987). Two classes of enzymes have a large role. Phenol oxidases (POX, e.g. laccases) have Cu-containing prosthetic groups with redox potentials sufficient to extract electrons from phenolic groups (Mayer & Staples 2002). Peroxidases (PER, e.g. lignin peroxidase, Mn peroxidase) have Fe-containing haeme prosthetic groups that use H_2O_2 or secondary oxidants to degrade aromatic compounds (Dorán & Esposito 2000; Hofrichter 2002).

Statistical analysis

Univariate and multivariate (enter-removal) linear regression analyses were used to relate mean ecosystem EEA (Table 3) to variation among ecosystems in mean annual temperature (MAT), mean annual precipitation (MAP), soil pH and SOM concentration (Table 2). A principal components analysis that included data from 24 ecosystems was used to reduce the seven enzyme variables to two factors. The remaining 16 sites had missing data for one or more enzyme activities. Mean factor values with 95% confidence intervals were calculated for each ecosystem to graphically display large-scale patterns in the distribution of soil EEA. Ratios of ln(BG) : ln(AP) and ln(BG) : ln(NAG + LAP) activities were calculated for all cases. These indices, measures of the enzymatic resources directed towards acquisition of organic P and organic N relative to C, were used to test for functional convergence in soil EEA distributions across ecosystems and compare relative nutrient demand in relation to climatic gradients.

RESULTS

The potential activities g^{-1} dry soil of four enzymes, BG, CBH, NAG and AP, varied across ecosystems in relation to SOM concentration (R^2: 0.55, 0.42, 0.49 and 0.60 respectively; Fig. 1, Table 4). Five enzyme activities had significant but weaker univariate relationships with bulk soil pH (R^2: CBH 0.12, NAG 0.31, LAP 0.28, AP 0.36, POX 0.17; Table 4). Links to climate parameters were more tenuous: CBH and NAG were correlated with MAT; BG, LAP and AP were correlated with MAP (Table 4). Multiple regressions that

Table 2 Study locations and ecosystem characteristics

Site	Abbreviation	Location	Cover/Biome	Soil	SOM (%)	pH	MAT (°C)	MAP (mm)
Crested Butte, CO	CB	N39 W107	Sagebrush shrubland	Alfisol	8.4	8.0	1.1	650
Konza Prairie LTER, KS	KNZ	N39 W97	Tall grass prairie	Udic arguistoll	8.2	5.6	13.0	835
Kruger National Park, SA	KRNP	S24 E32	Tall grass prairie	Alfisol	6.5	5.1	22.9	550
Ukulinga, SA	UKL	S30 E29	Tall grass prairie	Plinthic paleustalf	13.0	5.1	17.6	694
Niwot Ridge LTER, CO	NWT/S	N40 W105	Spruce/fir forest	Inceptisol	28.8	5.0	−3.7	930
Niwot Ridge LTER, CO	NWT/P	N40 W105	Lodgepolepine forest	Inceptisol	28.7	5.0	−3.7	930
Duke Forest, NC	DF	N36 W79	Loblolly pine forest	Acidic hapludult	5.0	5.0	15.5	1140
Oak Ridge National Lab, TN	ORNL	N36 W84	Sweetgum forest	Aquic hapludult	2.8	6.0	14.2	1390
Sevilleta LTER, NM	SEV/G	N34 W107	Grama grassland	Thermic halpocalcid	1.8	8.2	13.2	250
Sevilleta LTER, NM	SEV/C	N34 W107	Creosote shrubland	Thermic halpocalcid	2.4	7.5	13.2	250
Sevilleta LTER, NM	SEV/J	N34 W107	Juniper shrubland	Mesic halpocalcid	3.7	7.2	13.2	250
Manistee National Forest, MI	MNF/O	N44 W85	Black/White oak forest	Entic halporthod	2.5	3.9	9.7	890
Manistee National Forest, MI	MNF/MO	N44 W85	Sugar maple/red oak forest	Typic halporthod	2.7	4.1	9.7	890
Manistee National Forest, MI	MNF/M	N44 W85	Sugar maple/basswood forest	Typic halporthod	4.5	5.6	9.7	890
Delta Junction, AK	DJ/A	N63 W145	Aspen forest	Pergelic cryaquepts	26.7	5.3	−1.9	311
Delta Junction, AK	DJ/S	N63 W145	Black Spruce forest	Pergelic cryaquepts	19.7	5.2	−1.9	311
Delta Junction, AK	DJ/H	N63 W145	Herbaceous sere	Pergelic cryaquepts	14.5	5.5	−1.9	311
McMurdo Dry Valley, ANT	MCM	S77 E163	Cold desert	Anhyorthels/Anhyoturbels	0.55	8.7	−22.4	100
Arctic LTER, AK	ARC/T	N69 W149	Tundra/tussock	Typic aquaturbel	94	4.5	−9	330
Arctic LTER, AK	ARC/S	N69 W149	Tundra/shrub	Aquic umborthel	81	4.9	−9	330
Cedar Creek, MN	CDR/H	N45 W93	Forb/grass grassland	Udipsamments	2.0	6.0	6.7	801
Cedar Creek, MN	CDR/M	N45 W93	Sugar maple/basswood forest	Udipsamments	3.3	5.3	6.7	801
Cedar Creek, MN	CDR/A	N45 W93	Bigtooth aspen forest	Udipsamments	2.5	5.6	6.7	801
Cedar Creek, MN	CDR/O	N45 W93	Pin oak forest	Udipsamments	2.0	5.1	6.7	801
Cedar Creek, MN	CDR/P	N45 W93	White pine forest	Udipsamments	1.2	5.6	6.7	801
UT Arboretum, OH	UTA	N41 W83	Oak forest	Aeric haplaquept	12.0	6.4	10	856
Fuller Preserve, OH	FP	N41 W8	Oak forest	Typic argiaquoll	12.0	6.9	10	856
Harvard Forest, MA	HFR	N42 W71	Mixed deciduos forest	Typic Dystrocrept	12.7	4.3	7.6	1100
Chicago Botanical Garden, IL	CBG	N42 W88	Maple forest	Oxyaquic Hapludalf/ Aeric Epiaqualf	11.3	6.4	9.3	935
Barro Colorado Monument, Panama	BCNM	N9 W80	Lowland tropical forest	Oxisol	9.4	5.5	27	2600
Luquillo LTER, PR	LUQ/M	N18 W66	Montane tropical forest	Aquic tropohumults	10.5	5.0	19.6	3137
Luquillo LTER, PR	LUQ/P	N18 W66	Palm forest	Aquic tropohumults	23.6	4.9	19.0	4172
Luquillo LTER, PR	LUQ/C	N18 W66	Cloud forest	Aquic tropohumults	25.9	4.9	18.9	3237
Luquillo LTER, PR	LUQ/LM	N18 W66	Lower montane forest	Aquic tropohumults	14.7	5.0	21.0	3500
CAP LTER, AZ	CAP/D	N33 W112	Sonoran desert	Aridisol	2.2	7.7	17	250
CAP LTER. AZ	CAP/U	N33 W112	Urban Sonoran desert	Aridisol	3.1	7.6	17	250
Kitty Todd, OH	KT	N41 W83	Mesic tallgrass prairie	Typic Haplaquolls	10.4	7.2	10	900
Southview savannah, OH	SS	N41 W83	Mesic tallgrass prairie	Typic Udipsamments	3.3	6.4	10	900
Secor Metropark, OH	SM	N41 W83	Oak/maple forest	Typic Haplaquolls	7.4	6.6	10	900
Ohio University, OH	OU	N39 W82	Oak/maple/ash forest	Hapludalfs	9.8	5.9	10	900

SOM, soil organic matter; MAT, mean annual temperature; MAP, mean annual precipitation.

Table 3 Potential soil EEA across ecosystems shown as mean values (nmol h^{-1} g SOM^{-1}) with coefficients of variation

Site	*n*	BG	CBH	NAG	LAP	AP	POX	PER
CB	45	1850 (33)	NA	377 (36)	763 (94)	988 (74)	414 000 (50)	NA
KNZ	24	3320 (18)	1010 (18)	2530 (25)	205 (32)	6180 (12)	3880 (77)	3120 (149)
KRNP	12	2670 (16)	525 (18)	1210 (18)	62 (20)	4200 (18)	2220 (144)	209 (346)
UKL	12	2650 (24)	794 (47)	2750 (31)	72 (40)	3700 (33)	11 700 (162)	1500 (168)
NWR/S	169	4290 (116)	2290 (149)	3190 (100)	125 (180)	5440 (107)	13 900 (170)	1540 (222)
NWR/P	53	3790 (115)	2120 (105)	3330 (127)	115 (135)	5270 (120)	6150 (188)	3750 (208)
DF	45	3450 (54)	757 (53)	3340 (46)	121 (70)	9460 (77)	12 800 (136)	163 000 (101)
ORNL	18	4490 (24)	1040 (34)	2100 (23)	187 (44)	9130 (27)	17 500 (69)	412 000 (64)
SNWR/G	87	2740 (109)	438 (104)	114 (81)	5730 (44)	2490 (47)	1 016 000 (109)	3 056 000 (119)
SNWR/C	27	2280 (172)	395 (192)	200 (187)	7590 (135)	2140 (184)	383 000 (178)	842 000 (82)
SNWR/J	27	1820 (50)	278 (64)	145 (47)	4380 (56)	2670 (39)	299 000 (70)	807 000 (57)
MNF/O	39	1480 (67)	450 (51)	1450 (58)	131 (69)	2440 (45)	1110 (207)	31 000 (159)
MNF/MO	39	2310 (46)	641 (37)	1110 (73)	192 (47)	4070 (27)	386 (305)	33 300 (147)
MNF/M	39	3690 (41)	1050 (40)	1450 (90)	396 (29)	4110 (29)	40 (323)	9570 (192)
DJ/A	4	3160 (70)	NA	2750 (68)	47 (57)	2550 (33)	ND	11 500 (120)
DJ/S	4	3360 (37)	NA	2760 (59)	34 (76)	3850 (30)	37 700 (129)	6830 (200)
DJ/H	4	4850 (18)	NA	1570 (43)	31 (163)	2820 (20)	ND	ND
MCM	44	119 (139)	NA	NA	3920 (88)	3070 (100)	210 000 (89)	217 000 (107)
ARC/T	40	3940 (93)	2090 (74)	2080 (66)	637 (108)	NA	NA	NA
ARC/S	40	1940 (33)	881 (60)	1670 (56)	340 (56)	NA	NA	NA
CDCR/H	36	6810 (49)	1080 (106)	2920 (66)	NA	9820 (75)	424 (299)	93 400 (61)
CDCR/M	18	4640 (31)	1070 (41)	1870 (31)	NA	5870 (31)	1370 (260)	54 100 (41)
CDCR/A	18	5840 (39)	1190 (59)	2680 (24)	NA	8250 (28)	4780 (184)	69 800 (30)
CDCR/O	36	7080 (48)	1560 (51)	3210 (50)	NA	13 000 (67)	6020 (184)	96 700 (53)
CDCR/P	36	9200 (67)	1960 (92)	3090 (60)	NA	14 200 (80)	2880 (285)	148 000 (36)
UTA	40	4240 (40)	1510 (57)	2340 (57)	2490 (77)	5960 (39)	54 500 (81)	36 000 (96)
FP	30	5940 (20)	2700 (24)	2490 (28)	3930 (38)	5400 (40)	48 600 (89)	69 700 (58)
HF	12	NA	NA	NA	NA	NA	9700 (57)	50 400 (65)
CBG	24	4450 (53)	1540 (49)	2080 (39)	2010 (66)	5120 (48)	8230 (275)	34 200 (81)
BCNM	8	1920 (33)	512 (46)	1560 (40)	158 (108)	11 500 (27)	ND	5200 (55)
LUQ/M	3	367 (27)	41 (53)	189 (36)	42 (20)	6132 (3)	24 000 (87)	44 800 (32)
LUQ/P	3	355 (29)	35 (8)	167 (33)	25 (20)	1210 (38)	5400 (93)	60 600 (50)
LUQ/C	3	176 (11)	33 (14)	108 (44)	11 (30)	3400 (25)	1775 (90)	29 400 (78)
LUQ/LM	3	986 (14)	164 (26)	433 (42)	79 (28)	4720 (20)	17 100 (75)	65 000 (43)
CAP/D	10	1080 (50)	67 (94)	42 (53)	5770 (55)	983 (43)	8140 (129)	71 800 (35)
CAP/U	10	1180 (33)	77 (48)	42 (46)	7360 (44)	1000 (34)	8910 (147)	34 100 (51)
OH/KT	24	1160 (92)	214 (97)	705 (104)	652 (85)	2100 (91)	7 (187)	12 (233)
OH/SS	12	981 (37)	1480 (63)	4820 (50)	777 (69)	15 800 (29)	33 200 (137)	5400 (346)
OH/SM	48	1410 (63)	337 (91)	845 (91)	336 (183)	1850 (61)	16 500 (157)	10 600 (153)
OH/OU	10	4560 (41)	1710 (58)	2320 (25)	666 (52)	5200 (21)	1400 (316)	15 700 (142)
GLOBAL	1154	3320 (70)	942 (78)	1740 (70)	1450 (156)	5300 (71)	70 600 (266)	178 000 (293)

n, number of cases (number of experimental units sampled × number of sampling dates); NA, not assayed; ND, not detected. Enzyme abbreviations given in Table 1.

included edaphic and climatic variables accounted for 50–70% of the variation in activity among ecosystems, except for PER (22%). Within these regression models, soil pH was a significant variable for all enzymes except CBH.

Extracellular enzyme activity potentials are also commonly presented as specific activities (i.e. activity g^{-1} SOM) to analyse and compare the dynamics of decomposition (Table 3). Because of their strong covariance with SOM, the mean-specific activities of BG, CBH, NAG and AP varied by less than an order of magnitude across ecosystems and showed similar coefficients of variation (CV, 70–78%, Table 3). Specific LAP, POX and PER activities, which showed stronger relationships with soil pH (Fig. 2), varied more widely across ecosystems with CVs of 156%, 266% and 293%, respectively (Table 3). On average, spatiotemporal variation in specific EEA within ecosystems was lower than the variation among ecosystems (mean within ecosystem CV: BG 50%, CBH 64%, AP 49%, NAG 57%, LAP 71%,

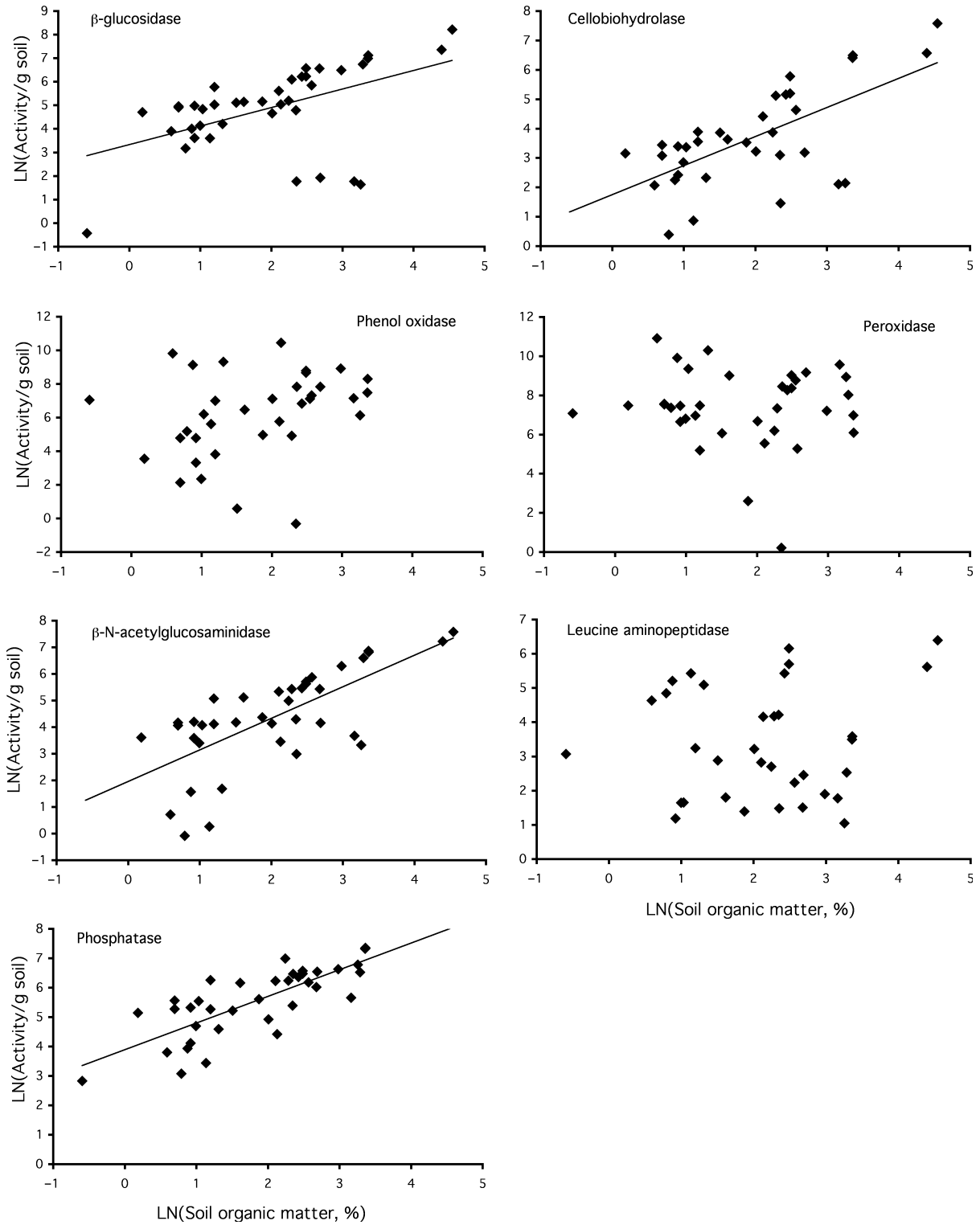

Figure 1 Natural logarithm of mean extracellular enzyme activity g^{-1} dry soil by site in relation to natural logarithm of soil organic matter concentration (%). Linear regressions are shown for the four enzymes with statistically significant relationships ($P < 0.05$). R^2 values for BG, CBH, AP and NAG are 0.55, 0.42, 0.60 and 0.49 respectively. Slopes are 0.98, 0.96, 0.80 and 1.13 respectively. Enzyme abbreviations given in Table 1.

Table 4 Regression statistics relating ln(EEA g^{-1} soil dry mass) to climatic and edaphic variables

	SOM	MAT	MAP	pH	Multiple
BG	0.55*	–	0.22	–*	0.56
CBH	0.42*	0.46*	–	0.12	0.56
NAG	0.49	0.31*	–	0.31*	0.57
LAP	–*	–*	0.20*	0.28*	0.70
AP	0.60*	–	0.18	0.36*	0.63
POX	–*	–	–	0.17*	0.50
PER	–	–	–*	–*	0.22

Values are R^2 statistics for significant ($P < 0.05$) linear regressions. Multiple is R^2 statistics for multiple linear regressions (stepwise removal) of ln(EEA g^{-1} DM) as *f*(SOM, MAT, MAP, pH).
Abbreviations and units given in Tables 1 and 2. POX regressions exclude five sites with anomalous undetectable values; PER regressions exclude two sites with anomalous undetectable values (Table 3).
*Variables that make significant (*t*-test, $P < 0.05$) contributions to the multiple linear regressions.

POX 158%, PER 116%). However, within ecosystems, the CV for hydrolytic activities covaried with the number of observations (R^2: BG 0.29, CBH 0.29, AP 0.23, NAG 0.17, LAP 0.23, $P < 0.05$), so the full magnitude of spatiotemporal variation within many of the ecosystems represented may be underestimated. Variation in oxidative activities, though greater than that of hydrolytic activity, was not correlated with sampling effort (R^2: POX 0.05, PER 0.006, $P > 0.05$).

The specific activities of all seven enzymes had statistically significant relationships with soil pH within multiple linear regression models, and all but BG and CBH also showed significant univariate regressions with pH (Fig. 2, Table 5). Relationships with climate variables were weaker: only three specific activities (BG CBH, LAP) had significant relationships with MAP; two (CBH, NAG) had significant relationships with MAT (Table 5). Multiple regression models that included the two climatic (MAP and MAT) and soil pH captured 17% (AP) to 70% (LAP) of between ecosystem variances in EEA (Table 5).

Principal components analysis (PCA) of data from 24 ecosystems reduced the seven enzyme variables to two factors that captured 80% of the variation. Ordination of ecosystems by these factors showed two discrete distributions (Fig. 3). Arid and semiarid sites, which generally have low SOM and alkaline soil pH, varied primarily in relation to factor 2 (32% of variance, positively correlated with LAP, POX and PER). Wetter ecosystems, which generally have acidic soil pH, varied principally along factor 1 (46% of variance, positively correlated with BG, CBH, NAG and AP). No sites showed high activity for both sets of variables.

Estimates of C : N : P composition for soil and soil microbial biomass converge on 186 : 13 : 1 and 60 : 7 : 1 respectively (Cleveland & Liptzin 2007). However, nutrient acquisition effort, as indicated by the potential activities of the hydrolytic enzymes that generate readily consumed products from the largest soil pools of organic C, N, and P (i.e. cellulose, protein, chitin, peptidoglycan and sugar phosphates), may be more equitably distributed. The ratio ln(BG) : ln(NAG + LAP), an indicator of potential C : N acquisition activity averaged 1.02 ± 0.20 (SD); the corresponding C : P ratio, represented by the ratio of ln(BG) : ln(AP) activity, was 0.95 ± 0.15 (Fig. 4). By these indicators, the ratio of C : N : P acquisition activity is *c.* 1 : 1 : 1. Although lignin, tannin and other aromatic components of plant and microbial biomass are mineralized within the soil profile, they are not primary carbon sources for any major group of soil microorganisms, so POX and PER activities are not included in this acquisition ratio.

At the ecosystem scale, enzymatic indicators of relative nutrient availability showed patterns in relation to climatic gradients. The mean enzymatic C : P acquisition ratio for ecosystems declined in relation to both MAT ($R^2 = 0.33$) and MAP ($R^2 = 0.71$), suggesting that P availability declines relative to C as soil-weathering intensity increases (Fig. 5). Using a multiple linear regression [enzymatic C : P ratio = *f*(MAT, MAP)], the C : P acquisition ratio is predicted to decrease from 1.1 to 0.7 across latitudinal gradients in weathering intensity ($R^2 = 0.74$, $F = 47$, MAT coefficient: −0.00239, MAP coefficient: −0.0000704, intercept: 1.044) The mean C : N acquisition ratio for ecosystems was not related to MAT, but did show a weak positive relationship with MAP ($R^2 = 0.16$, Fig. 5). Because the C : P and C : N acquisition ratios had opposing trends in relation to MAP, the N : P acquisition ratio was negatively related to MAP ($R^2 = 0.58$, Fig. 5).

DISCUSSION

The description of soil EEA on a global scale provides a frame of reference for comparing ecosystems and an opportunity to relate the soil microbial community function to global patterns of microbial biomass composition, nutrient dynamics and SOM storage. Our analysis documents that the most commonly measured extracellular enzyme activities show different ranges of variation and different distributions in relation to ecosystem variables, yet converge on a common pattern linked to the stoichiometry of microbial growth.

For BG, CBH and AP, activity g^{-1} dry soil tracked SOM content. LAP, POX and PER activities varied widely but generally increased with soil pH, while NAG activity was strongly related to both SOM (positively) and soil pH (negatively). When specific activities (i.e. activity g^{-1} SOM)

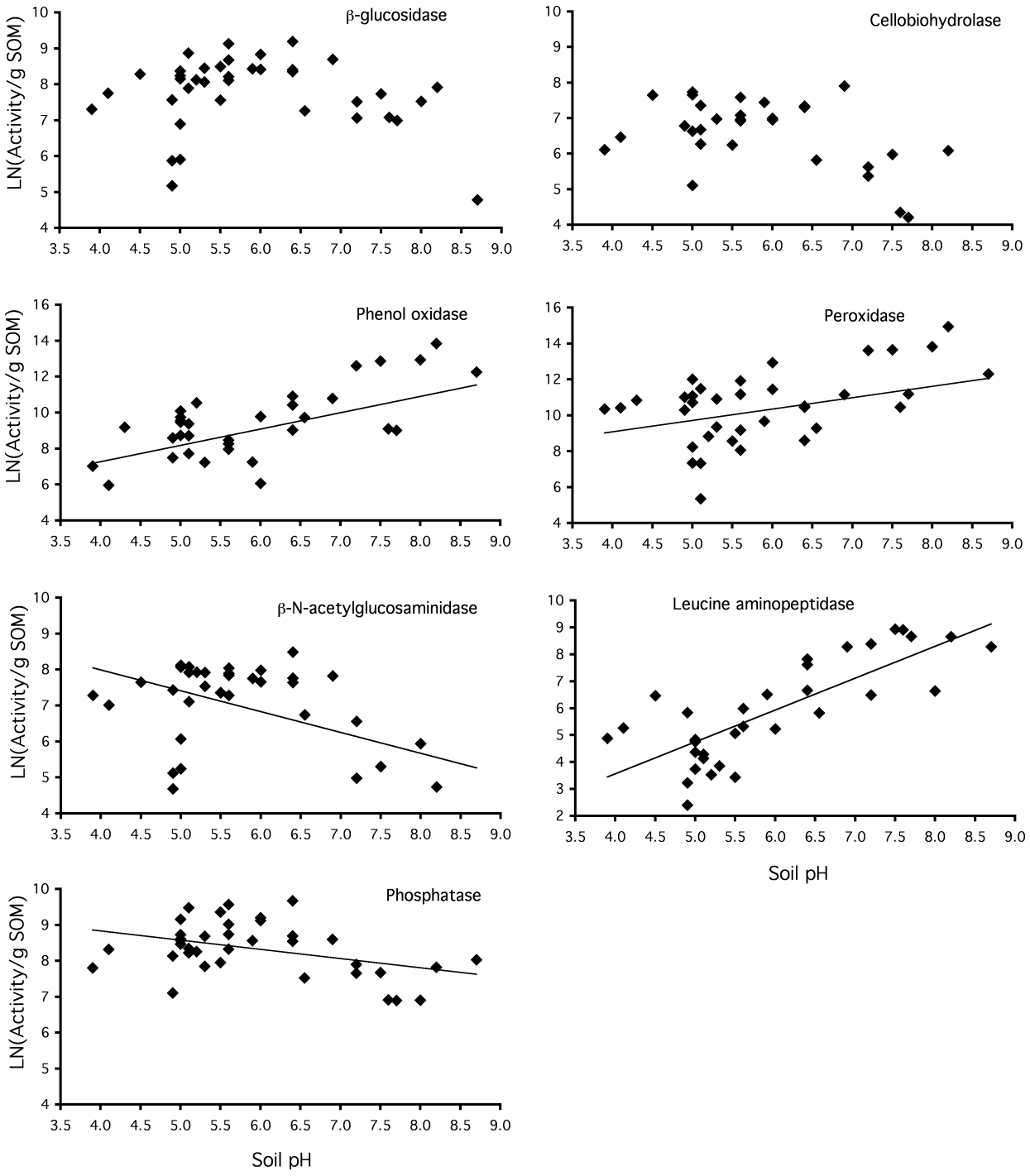

Figure 2 Natural logarithm of mean extracellular enzyme activity g^{-1} soil organic matter by site in relation to soil pH. Linear regressions are shown for the five enzymes with statistically significant relationships ($P < 0.05$). R^2 values for POX, PER, NAG, LAP and AP are 0.21, 0.10, 0.23, 0.62 and 0.16 respectively. Regression slopes are: POX 0.91, PER 0.63, NAG −0.54, LAP 1.25 and AP −0.25. Enzyme abbreviations given in Table 1.

are compared, all enzymes show a statistically significant relationship to soil pH in either univariate or multivariate models with weak negative trends for BG, CBH and AP, a strong negative trend for NAG and strong positive trends for LAP, POX and PER. Whether EEA is expressed g^{-1} of soil or g^{-1} SOM, soil pH emerges as the variable most

Table 5 Regression statistics relating ln(EEA g^{-1} SOM) to climatic and edaphic variables

	MAT	MAP	pH	Multiple
BG	–	0.19*	–*	0.40
CBH	0.39*	0.27*	–*	0.53
NAG	0.21*	–	0.23*	0.50
LAP	–	0.30*	0.62*	0.70
AP	–	–	0.16*	0.17
POX	–	–	0.21*	0.43
PER	–	–	0.10*	0.25

Values are R^2 statistics for significant ($P < 0.05$) linear regressions. Multiple is R^2 statistics for multiple linear regressions (stepwise removal) of ln(EEA g^{-1} SOM) as *f*(MAT, MAP, pH). Abbreviations and units given in Tables 1 and 2. POX regressions exclude five sites with anomalous undetectable values; PER regressions exclude two sites with anomalous undetectable values (Table 3).
*Variables that make significant (*t*-test, $P < 0.05$) contributions to the multiple linear regressions.

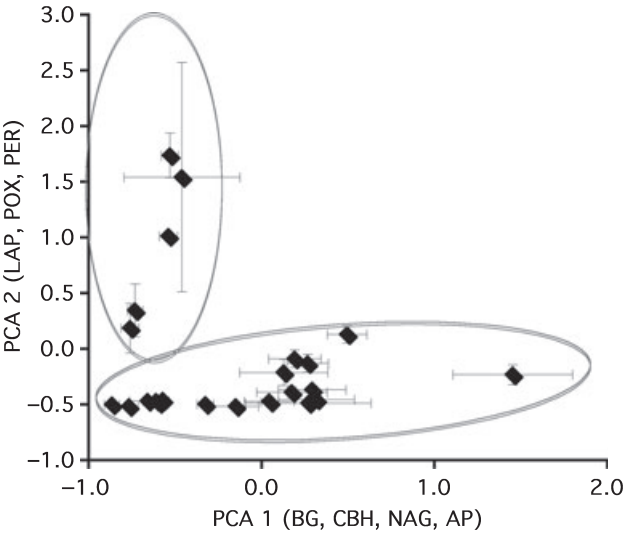

Figure 3 Ordination of 24 ecosystems based on potential soil extracellular enzyme activity g^{-1} organic matter using principal components analysis (varimax rotation). Factor 1 (46% of variance) is correlated with BG ($r = 0.89$), CBH (0.84), NAG (0.92) and AP (0.84). Factor 2 (31% of variance) is correlated with LAP (0.85), POX (0.83) and PER (0.74). The vertical grouping represents arid and semiarid ecosystems with soil pH > 7. The horizontal grouping represent ecosystems with relatively high precipitation and soil pH < 7. Values shown are means with 95% confidence intervals. Enzyme abbreviations given in Table 1.

closely linked to ecosystem variation (Tables 4 and 5). These patterns resemble recent findings that microbial diversity in soil and other systems also follow pH gradients (Baath & Anderson 2003; Fierer & Jackson 2006; Singh *et al.* 2006; Cookson *et al.* 2007).

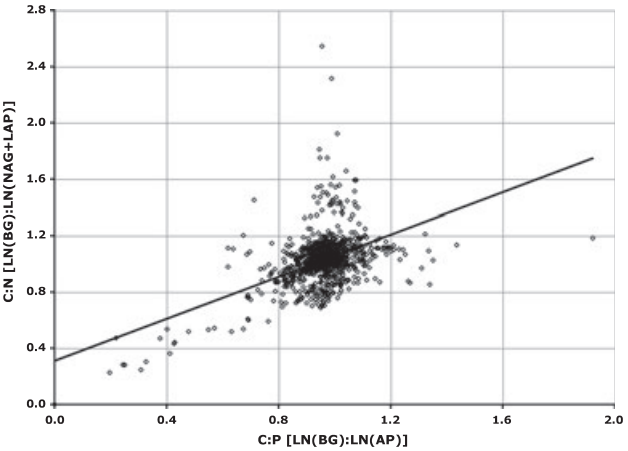

Figure 4 Ratio of ln(BG) : ln(NAG + LAP), an indicator of potential C : N acquisition activity, in relation to the ratio ln(BG) : ln(AP), an indicator of potential C : P acquisition activity. The centroid is 0.95 ± 0.15 (SD) for C : P and 1.02 ± 0.20 for C : N values > 1.2 for either ratio constrain values of the complementary ratio. The regression C : N = 0.75 (C : P) + 0.31 has an R^2 value of 0.28, $n = 929$.

The association of pH and EEA reflects interactions at multiple scales of organization. Soil pH has direct biochemical effects on the activity of extracellular enzymes immobilized in the soil matrix. Glycosidases have pH optima *c.* 5 ± 1. POX, lignin peroxidases and most proteases (metallo-proteases, serine proteases) have optima of 8 ± 1. Extracellular phosphatases are produced in acid and alkaline active forms by various taxa. At the ecosystem scale, soil pH reflects climatic controls on soil weathering and plant community composition, which may affect the large-scale distribution of EEA through changes in nutrient availability and SOM composition, as well as microbial community composition.

These interactions over multiple levels of organization generate global patterns that are not observed at the ecosystem scale. The most conspicuous of these is the distribution of oxidative activity. Basidiomycetes produce a variety of extracellular oxidative enzymes and are generally considered to be the most efficient degraders of lignin (Rabinovich *et al.* 2004; Baldrian 2006). These organisms are most abundant in mid- to high-latitude forests where the dominant plants have high lignin concentrations and the soil is acidic. Within these ecosystems, POX and PER activities tend to increase with secondary succession (Sinsabaugh *et al.* 2005). This trend is evident for the MNF and DJ ecosystems (Table 3). But at the global scale, this biome-specific trend is overwhelmed by the inclusion of arid alkaline soils, which have near optimal pH for POX and PER activities and edaphic conditions that may promote enzyme stability (Stursova & Sinsabaugh 2008), even though basidiomycetes are relatively uncommon (Porras-Alfaro *et al.* 2008).

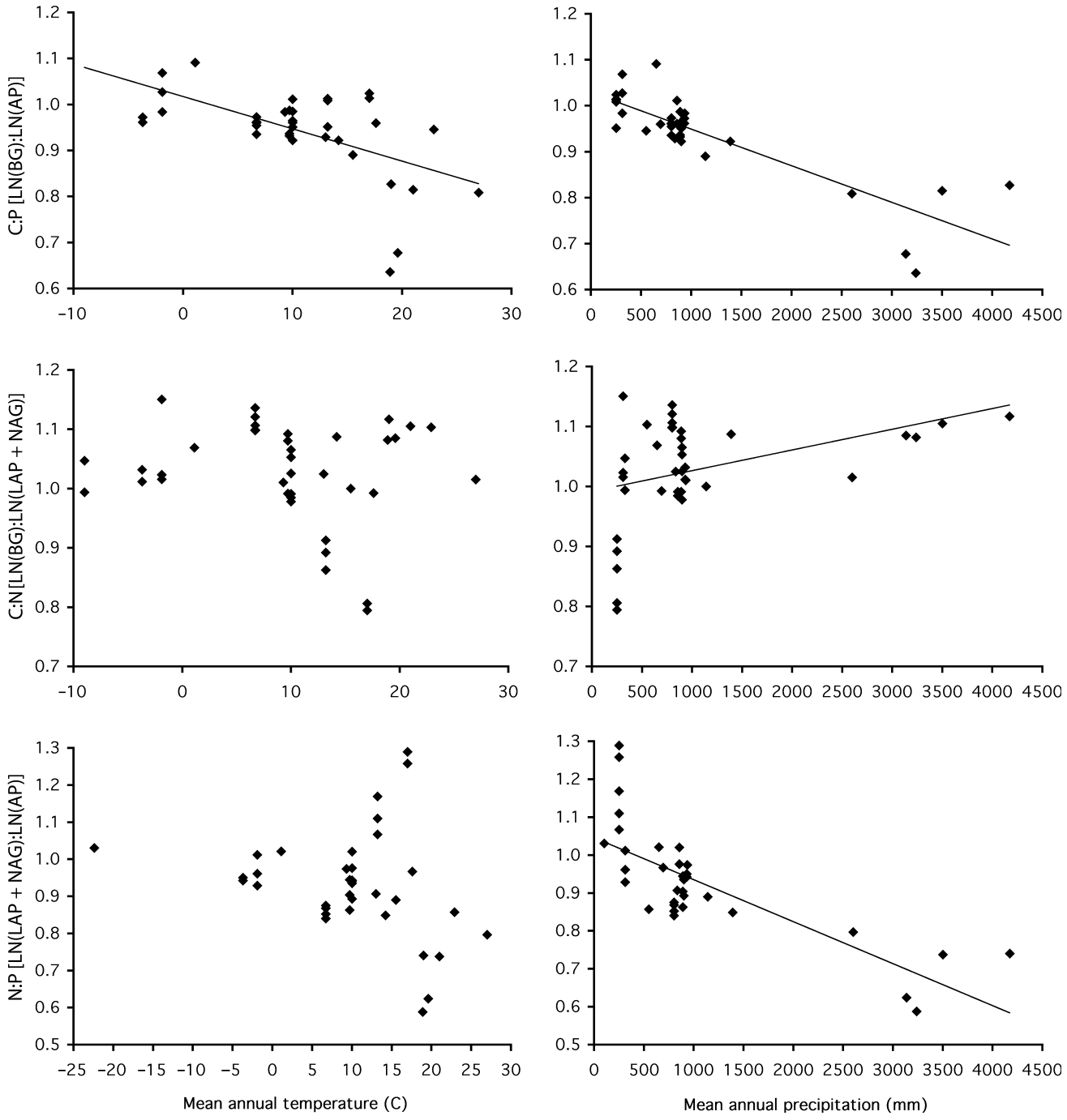

Figure 5 Mean ecosystem ratios of C : P, C : N and N : P acquisition activity, as indicated by ratios of ln(BG) : ln(AP), ln(BG) : ln(LAP + NAG) and ln(LAP + NAG) : ln(AP) respectively, in relation to mean annual temperature and mean annual precipitation. Data from the McMurdo Dry Valleys are excluded from the C : P and C : N graphs because BG activities are extremely low. Enzyme abbreviations are listed in Table 1. Regression statistics for C : P vs. MAT: $n = 36$, $R^2 = 0.33$, $F = 16.7$, $P < 0.001$, $a = -0.0070$; for C : P vs. MAP: $n = 36$, $R^2 = 0.71$, $F = 84.2$, $P < 0.001$, $a = -0.000080$; for C : N vs. MAP: $n = 38$, $R^2 = 0.16$, $F = 6.61$, $P = 0.014$, $a = 0.000035$; for N : P vs. MAP: $n = 37$, $R^2 = 0.58$, $F = 47.5$, $P < 0.001$, $a = -0.00011$. The regressions for C : N and N : P vs. MAT were not statistically significant ($P > 0.1$).

The oxidative degradation of lignin, tannin and other aromatic components of plant litter is generally considered the rate-limiting step in decomposition (Meentemeyer 1978; Fog 1988). Freeman *et al.* (2001), for example, proposed that POX activity was the proximate control on organic matter mineralization, and thereby CO_2 efflux, in high-latitude peats (histosols), and that regional climate warming could release constraints imposed by low oxygen availability on the activity of these immobilized enzymes, leading to net losses of SOM. Our EEA analyses suggest a broader context for POX and PER in SOM storage. Despite low rates of primary production, SOM content is greatest in high-latitude ecosystems where POX and PER activities in soil are physicochemically constrained by low pH, low temperature and low oxygen availability caused by soil flooding. SOM contents are lowest in arid ecosystems, which also have low rates of primary production, where alkaline pH increases the solubility of polyphenols and optimizes POX and PER activities (Collins *et al.* 2008).

Another global pattern in the distribution of EEA is the convergence of C : N : P acquisition potentials, as measured by ln(BG) : ln(LAP + NAG) : ln(AP) activities (Fig. 3). Across ecosystems, BG activity was most strongly correlated with the abundance of SOM. Despite its low pH optimum, specific BG activity varied only weakly with soil pH, presumably because cellulose and other β-1,4-glucan polymers dominate the organic matter inputs of vegetated ecosystems. Declines in specific activity as a result of higher soil pH are counteracted by increased enzyme expression. The role of plant litter in controlling BG activity is suggested by data from the McMurdo Dry Valleys of Antarctica where there are no plants, soil pH is high and the specific activity of BG is only 3% of the global average (Table 3). As indicators of organic N acquisition from amino acids and amino sugars, LAP and NAG showed similar ranges of activity but inverse relationships to soil pH (Fig. 2). As a result, the sum of LAP + NAG was similar across ecosystems. AP activity, like BG, varied across ecosystems largely in relation to SOM abundance. Soil pH had little effect on specific AP activity, presumably because both acid and alkaline active enzymes are produced. Because of these trends, specific C, N and P acquisition potentials generally showed a consistent stoichiometry across ecosystems, even though the component activities had different relationships with environmental variables.

The consistency of stoichiometric relationships across ecosystems is unexpected because experimental manipulations within ecosystems show that C, N and P acquisition activities can be modulated by inorganic nutrient availability (Olander & Vitousek 2000; Sinsabaugh *et al.* 2002; Stursova *et al.* 2006), following resource allocation models based on the premise that cellular resources directed towards N and P acquisition reduce resources available for C acquisition (Sinsabaugh & Moorhead 1994; Allison *et al.* 2007). The convergence of the C : N : P acquisition ratio on a global scale shows that the plasticity of these relationships is constrained. The C : N and C : P acquisition ratios increase colinearly to a maximum value of 1.2 (Fig. 4). Values > 1.2 for either ratio occur only when the other ratio remains < 1.2 and such instances occurred in < 3% of the cases in our dataset. Thus, the C : N : P acquisition ratio appears to be an integral feature of soil microbial community function, linking environmental nutrient availability to the C : N : P stoichiometry of microbial biomass (Cleveland & Liptzin 2007).

Although nutrient acquisition ratios are constrained by stoichiometry, variance at the ecosystem scale follows large-scale biogeochemical patterns. Biogeochemical theory predicts that soil N availability should be highest in tropical ecosystems, while P availability should be greatest in mid- to high-latitude ecosystems (Walker & Syers 1976; Martinelli *et al.* 1999). P is a rock-derived nutrient that may be lost due to leaching or occlusion in mineral particles in highly weathered soils. N, an atmospherically derived nutrient, tends to be scarce in areas that have experienced recent glaciation. These large-scale trends are apparent in the elemental C : N : P ratios of plants (McGroddy *et al.* 2004; Reich & Oleksyn 2004). Our analyses show that they are also reflected in the enzymatic ratios of C : N : P acquisition by soil microbial communities. While individual enzyme activities were not strongly linked to climatic variables, mean ecosystem C : P acquisition ratios declined as MAT and MAP increased, indicating that soil microbial communities direct more effort to acquiring and cycling P relative to processing C in more weathered soils (Fig. 5). The C : N acquisition ratio was much less responsive to climatic gradients. However, the trend towards greater ratios, indicative of higher relative N availability, with increasing MAP is consistent with biogeochemical predictions.

The contrast between the robust C : P acquisition relationship with climate measures and the weak C : N acquisition relationship extends patterns observed at the ecosystem scale. In both aquatic and terrestrial ecosystems, an inverse relationship between extracellular phosphatase activity and relative P availability is a general phenomenon, reflecting the role of P in energy metabolism. Because most extracellular phosphatases will hydrolyze phosphate from a wide range of substrates, it is relatively easy to measure this potential with a single assay.

N acquisition from organic matter is more complex than P acquisition. N is distributed among several classes of polymers as well as humic molecules, so N acquisition strategies are linked to the C-substrate preferences of particular taxa (McGill & Cole 1981; Manzoni *et al.* 2008). In the context of decomposition models, three broad strategies

can be defined, each assigned to a guild of organisms: opportunists consume labile proteins, decomposers need external N inputs to decompose lignocellulose and miners use oxidative enzymes to breakdown humus for C and N (Moorhead & Sinsabaugh 2006). Given the diversity of N acquisition strategies and their conflation with carbon acquisition, it is not surprising that studies that compare soil EEA responses to experimental N amendment produce mixed results. In some ecosystems, POX and PER (e.g. Frey *et al.* 2004; Sinsabaugh *et al.* 2005), LAP (e.g. Stursova *et al.* 2006) or NAG activities (e.g. Olander & Vitousek 2000; DeForest *et al.* 2004) decrease with N amendment, but others studies find no response (e.g. Zeglin *et al.* 2007). As a result, large-scale relationships between particular enzyme activities and measures of N availability are likely to be weaker than relationships between P availability and phosphatase activity.

Our analyses indicate that the enzymatic potential for hydrolyzing the labile components of SOM is tied to substrate availability, soil pH and the stoichiometry of microbial nutrient demand. The enzymatic potential for oxidizing the recalcitrant fractions of soil organic material, which is a proximate control on SOM accumulation, is most strongly related to soil pH. These trends provide insight into the biogeochemical processes that create global patterns in ecological stoichiometry and organic matter storage.

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