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SYNTHESIS & EMERGING IDEAS

Ecoenzymatic stoichiometry of recalcitrant organic matter decomposition: the growth rate hypothesis in reverse

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Abstract The flow of carbon and nutrients from plant production into detrital food webs is mediated by microbial enzymes released into the environment (ecoenzymes). Ecoenzymatic activities are linked to both microbial metabolism and environmental resource availability. In this paper, we extend the theoretical and empirical framework for ecoenzymatic stoichiometry from nutrient availability to carbon composition by relating ratios of β-1,4-glucosidase (BG), acid (alkaline) phosphatase (AP), β-N-acetyl-glucosaminidase (NAG), leucine aminopeptidase (LAP) and phenol oxidase (POX) activities in soils to measures of organic matter recalcitrance, using data from 28 ecosystems. BG and POX activities are uncorrelated even though both are required for lignocellulose degradation. However, the ratio of BG:POX activity is negatively correlated with the relative abundance of recalcitrant carbon. Unlike BG, POX activity is positively correlated with (NAG + LAP) and AP activities. We propose that the effect of organic matter recalcitrance on microbial C:N and C:P threshold element ratios (TER) can be represented by normalizing BG, AP and (NAG + LAP) activities to POX activity. The scaling relationships among these ratios indicate that the increasing recalcitrance of decomposing organic matter effectively reverses the growth rate hypothesis of stoichiometric theory by decreasing carbon and nutrient availability and slowing growth, which increases $TER_{N:P}$. This effect is consistent with the narrow difference between the mean elemental C:N ratios of soil organic matter and microbial biomass and with the inhibitory effect of N enrichment on rates of decomposition and microbial metabolism for recalcitrant organic matter. From these findings, we propose a conceptual framework for bottom-up decomposition models that integrate the stoichiometry of ecoenzymatic activities into general theories of ecology.

Keywords β-glucosidase · β-*N*-acetylglucosaminidase · Decomposition · Ecological stoichiometry · Extracellular enzyme activity · Leucine aminopeptidase · Phenol oxidase · Phosphatase · Soil organic matter · Threshold element ratio

Abbreviations

A_N	Assimilation efficiency for nitrogen
A_P	Assimilation efficiency for phosphorus
AP	Acid (alkaline) phosphatase
$B_{C:N}$	Biomass C:N ratio
$B_{C:P}$	Biomass C:P ratio

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BG	β-1,4-glucosidase
EEA	Ecoenzymatic activity
LAP	Leucine aminopeptidase
NAG	β-*N*-acetylglucosaminidase
POX	Phenol oxidase
SOM	Soil organic matter
TER	Threshold element ratio
GE	Growth efficiency

Introduction

Lignocellulose is the principal product of plant production; its decomposition by environmental enzymes (ecoenzymes) is a rate-limiting process that supports detrital food webs and drives macronutrient cycles. Once the crystalline microfiber structure of plant cell walls has been disrupted, cellulose is relatively labile to degradation (Ljungdahl and Eriksson 1985; Sinsabaugh 2005). Lignin, which forms a polymeric cage around cellulose microfibrils, is more recalcitrant because it has a variable structure that includes a variety of monomers linked by C–C and ether bonds that require more energy to break than the glycosidic linkages of cellulose (Eriksson et al. 1990; Higuchi 1990; Hammel 1997). In general, the depolymerization of cellulose is a hydrolytic process involving three classes of enzymes: β-1,4-exoglucanases, β-1,4-endoglucanases and β-1,4-glucosidases. Lignin degradation is a non-specific oxidative process catalyzed by enzymes most generally described as phenol oxidases and peroxidases that use molecular oxygen or peroxide as an electron acceptor (Claus 2003; Rabinovich et al. 2004; Baldrian 2006; Sinsabaugh 2010).

During the early stages of plant litter decomposition, cellulose is degraded preferentially yielding glucose which is readily assimilated and consumed in intermediary metabolism (Moorhead and Sinsabaugh 2006). As decomposition progresses, the depolymerization of lignin becomes increasingly limiting to microbial carbon acquisition (Schimel and Weintraub 2003; Allison 2006; Herman et al. 2008), which drives changes in microbial community composition (Moorhead and Sinsabaugh 2006) and increased production of phenol oxidases and peroxidases. The non-specific activity of these enzymes produces quinones and other reactive phenolic species and that condense to form secondary humic products that become major components of soil organic matter (Grandy and Neff 2008).

In ecological studies, the most commonly measured cellulolytic enzyme is β-1,4-glucosidase (BG). BG catalyzes the final step in cellulose depolymerization, the hydrolysis of cellobiose to glucose. Because of the abundance and ubiquity of cellulose, which comprises approximately half of terrestrial plant production, BG activity is closely linked to soil organic matter (SOM) content such that activity per unit SOM shows less variation on an ecosystem scale than other commonly measured ecoenzymatic activities (Sinsabaugh et al. 2008).

Cellulose does not contain N or P so decomposers also produce enzymes to acquire these nutrients from other sources. The principal organic N sources are amino acids (peptides, proteins) and amino sugars (chitin, peptidoglycan) (Nannipieri and Paul 2009). As with other polymers, several classes of enzymes are needed to degrade proteins and microbial cell wall components like chitin and peptidoglycan, but in ecological studies only the most abundant enzymes that generate terminal products for microbial consumption are typically measured (Sinsabaugh and Foreman 2001; Stursova et al. 2006). The most common of these are leucine aminopeptidase (LAP) which hydrolyzes leucine and other nonpolar amino acids from the N terminus of polypeptides, and β-1,4-*N*-acetylglucosaminidase (NAG) which hydrolyzes glucosamine from chitobiose and other soluble oligosaccharides (Sinsabaugh et al. 2008). The principal organic sources of P are phospholipids and phosphosaccharides, in particular inositol phosphates. Phosphate is mineralized from these molecules by phosphatases (AP) that hydrolyze the ester linkages to carbon. Phosphatases are broadly classed as acid phosphatases and alkaline phosphatases based on their reaction mechanism and optimal pH.

Phenol oxidase (POX) activities in soil are more dynamic than commonly measured hydrolytic activities (Sinsabaugh et al. 2008). This variation may partly reflect its varied functions. Some organisms ("miners", Moorhead and Sinsabaugh 2006) produce these enzymes specifically to degrade lignin and humics to access C, N and P. But more broadly distributed functions include detoxification of phenolics and reactive metals, pigment production, and antimicrobial defense (Burke and Cairney 2002; Claus 2003; Rabinovich et al. 2004; Baldrian 2006).

High spatiotemporal variation in POX activity may also be product of lower environmental stability. Reduced stability relative to hydrolytic enzymes arises because the metal cofactors required for activity are vulnerable to chelation by competing molecules and the products of enzymatic activity are unstable molecules that may react with the enzyme itself. Within ecosystems, POX activity is often related to organic matter composition and decomposition rates but activities often do not correlate with the activities of BG and other hydrolases (Sinsabaugh 2010).

Ecoenzymatic stoichiometry

The threshold element ratio (TER) is defined as the elemental C:N or C:P ratio at which control of microbial metabolism switches from energy supply (C) to nutrient supply (N, P) (Sterner and Elser 2002; Allen and Gillooly 2009; Hladyz et al. 2009; Doi et al. 2010). TER is an intersection of the metabolic theory of ecology, which describes ecological organization in thermodynamic terms and ecological stoichiometry theory, which describes ecological organization in terms of elemental resource availability (Sterner and Elser 2002; Brown et al. 2004; Allen and Gillooly 2009). TER can be modeled as

$$\mathrm{TER_{C:P}} = (\mathrm{A_P}/\mathrm{GE}) * \mathrm{B_{C:P}} \quad (1)$$

$$\mathrm{TER_{C:N}} = (\mathrm{A_N}/\mathrm{GE}) * \mathrm{B_{C:N}} \quad (2)$$

where A_P and A_N are assimilation efficiencies for P and N, GE is microbial growth efficiency and $B_{C:P}$ and $B_{C:N}$ are the elemental C:P and C:N ratios of microbial biomass (Frost et al. 2006; Allen and Gillooly 2009). TER values vary depending on the degree to which elemental homeostasis is maintained by microorganisms and the effects of external resource supply on assimilation and growth efficiencies (Sterner and Elser 2002, Gusewell and Gessner 2009). Sinsabaugh et al. (2009) proposed that ecoenzymatic ratios may be related to the elemental composition of microbial biomass, microbial metabolism, and the TER by

$$\mathrm{BG}/\mathrm{AP} \propto (\mathrm{TER_{C:P}}/\ \mathrm{B_{C:P}}) = (\mathrm{A_P}/\mathrm{GE}) \quad (3)$$

$$\mathrm{BG}/(\mathrm{NAG}+\mathrm{LAP}) \propto (\mathrm{TER_{C:N}}/\ \mathrm{B_{C:N}}) = (\mathrm{A_N}/\mathrm{GE}) \quad (4)$$

In this conception, the stoichiometry of ecoenzymatic ratios is determined by the elemental stoichiometries of resource supply and microbial biomass (expressed as the ratios of $TER_{C:N}/B_{C:N}$ and $TER_{C:P}/B_{C:P}$) and microbial metabolism (expressed as the ratios of A_N/GE and A_P/GE).

Sinsabaugh et al. (2010) evaluated Eqs. 3 and 4 using data from freshwater biofilm and plankton communities. Their analysis showed that the slopes of the regressions ln (BG) vs. ln (AP) and ln (BG) vs. ln (NAG + LAP) were related to mean $B_{C:P}$ and $B_{C:N}$. Specifically, the BG/AP slope for plankton was greater than the slope for biofilm because the mean elemental C:P ratio of plankton (16) was greater than the mean C:P ratio of biofilm (7). Conversely, the BG/(NAG + LAP) slope for plankton was lower than the slope for biofilm because the mean elemental C:N ratio of plankton (6.6) was lower than the mean C:N ratio of biofilm (8.6). In addition, the relationship between N-acquiring and P-acquiring ecoenzymatic activities [(LAP + NAG)/AP] had a slope <1, which was consistent with the growth rate hypothesis that $TER_{N:P}$ decreases with increasing resource supply because faster growth increases cellular P quota relative to N quota (Sterner and Elser 2002; Gusewell and Gessner 2009).

In this study, we extend the analysis of ecoenzymatic stoichiometry to POX. The net effect of POX activity in soil, regardless of its initial function, is the degradation and mineralization of lignin and other polyphenolic molecules. Given that cellulose and lignin are the dominant components of plant production, ratios of BG:POX activities may reflect the stoichiometry of SOM composition analogous to the relationships between ratios of hydrolytic activities and elemental nutrient availabilities described by Sinsabaugh et al. (2009). The relationship between cellulolytic and ligninolytic enzyme activities is also of interest for modeling plant litter decomposition. Many models describe decomposition rates as functions of litter lignin or N content (Meentemeyer 1978; Melillo et al. 1982; Taylor et al. 1989). Decomposition models that proceed directly from microbial or ecoenzymatic activity are limited by the lack of empirical relationships (Schimel and Weintraub 2003; Moorhead and Sinsabaugh 2006). Analyses of the stoichiometry of commonly measured ecoenzymatic activities in

relation to organic matter composition may further development of bottom-up decomposition models.

Methods

Ecoenzymatic analyses

The data analyzed herein are a subset of those presented by Sinsabaugh et al. (2008). In that study, soil ecoenzymatic activity (EEA) from 40 terrestrial ecosystems was analyzed in relation to soil pH, organic matter, mean annual precipitation and mean annual temperature. Data summaries and ecological metadata are presented in the original paper. In this analysis, only cases that included values for BG, AP, NAG, LAP and POX activities were included (Table 1). This reduced the number of ecosystems to 25. To these data, we added 36 new cases from three systems included in the original study (arid grassland, creosote shrubland and juniper savanna within the Sevilleta National Wildlife Refuge in central New Mexico) and 24 new cases from three additional sites (piñon-juniper forest, ponderosa pine forest, spruce forest located in New Mexico that are part of a network of eddy covariance monitoring sites; Tierney Adamson, unpublished). The final data set has 605 cases from 28 ecosystems.

All activities are expressed in units of nmol h^{-1} gOM^{-1}. These activities were $\log_e$ transformed prior to analysis to normalize variance. Bivariate relationships between ecoenzyme activities were determined using standardized major axis (Type II) regression (SMATR v2.0, Warton et al. 2006).

SOM recalcitrance

At a coarse scale, SOM can be considered a mix of labile (L) and recalcitrant (R) components. The traditional Van Soest analysis of organic matter separates non-extractable material into acid soluble (e.g., holocellulose) and acid insoluble (lignin, humus) fractions. This analysis is the basis for the lignocellulose index (LCI) defined as the ratio of lignin/(lignin + cellulose), which has been used as an indicator and predictor of relative decomposition rates (Berg and McClaugherty 2003; Moorhead and Sinsabaugh 2006, Herman et al. 2008). For plant litter, initial LCI values range from about 0.3 to 0.6. For SOM, LCI is typically 0.7–0.8 for coarse particulate fractions (Grandy et al. 2007). A LCI of 0.4 is considered the transition point for lignin control of decomposition (Herman et al. 2008); a LCI value of 0.7 is considered the "end of decomposition", i.e., the point of transition from litter to stabilized soil organic matter (Berg and McClaugherty 2003)

A similar index of relative SOM recalcitrance can also be created based on pyrolysis gas chromatography mass spectroscopy (GCMS) techniques (Grandy and Neff 2008). Using total products derived from lignin as a measure of recalcitrant carbon and total products derived from cellulose as a measure of labile carbon, the index R/(R + L) has a range of values similar to those based on Van Soest analysis. For temperate oak-maple forest soils divided into three particle size fractions this ratio ranged from 0.2 to 0.8 with higher values associated with SOM in coarse soil fractions and lower values found in silt–clay fractions (Grandy et al. 2008). Similarly, Grandy et al. (2007) reported LCI values of 0.7 and 0.3 for light coarse and heavy silt–clay SOM fractions from alpine forest and tundra in Colorado.

We propose that ln (BG)/ln (POX) activity ratio is inversely related to the relative abundance of recalcitrant carbon. From several decades of decomposition studies, the connection between increasing organic matter recalcitrance and increasing oxidative enzyme activities is broadly assumed (Herman et al. 2008). Unfortunately, there are few cases where both enzyme activity potentials and organic matter composition have been measured on the same samples. We found three small examples: (1) a leaf litter decomposition study from which the initial LCI of dogwood, maple and oak litter can be related to the turnover activities for BG and POX (Carreiro et al. 2000); (2) an analysis of SOM composition and enzyme activities for alpine tundra and spruce-fir forest sites in Colorado that used pyrolysis GCMS (Grandy et al. 2007) and (3) a study of SOM composition and enzyme activities for three size ranges of soil particles collected from maple and oak dominated forest sites in northern Michigan that used pyrolysis GCMS (Grandy et al. 2008). For these data, we analyzed the ratio of BG:POX activity in relation to R/(R + L) using ANCOVA and Bonferroni-corrected post hoc tests. Subsequently, ln (BG)/ln (POX) was standardized by (1) running an ANCOVA in SMATR v2.0 (Warton et al. 2006),

Table 1 Data sources

Site	Location	Cover/biome	Soil	Cases
Crested Butte CO	N39 W107	Sagebrush shrubland	Alfisol	45
Konza Prairie LTER KS	N39 W97	Tall grass prairie	Udic arguistoll	24
Kruger National Park SA	S24 E32	Tall Grass prairie	Alfisol	6
Ukulinga SA	S30 E29	Tall Grass prairie	Plinthic paleustalf	5
Niwot Ridge LTER CO	N40 W105	Spruce/Fir forest	Inceptisol	106
Niwot Ridge LTER CO	N40 W105	Lodgepole pine forest	Inceptisol	33
Duke Forest NC	N36 W79	Loblolly Pine Forest	Acidic hapludult	22
Oak Ridge National Lab TN	N36 W84	Sweetgum Forest	Aquic hapludult	14
Sevilleta LTER NM	N34 W107	Grama grassland	Thermic halpocalcid	71
Sevilleta LTER NM	N34 W107	Creosote shrubland	Thermic halpocalcid	29
Sevilleta LTER NM	N34 W107	Juniper shrubland	Mesic halpocalcid	40
Delta Junction AK	N63 W145	Black Spruce forest	Pergelic cryaquepts	2
UT Arboretum, OH	N41 W83	Oak forest	Aeric haplaquept	39
Fuller Preserve, OH	N41 W8	Oak forest	Typic argiaquoll	34
Chicago Botanical Garden, IL	N42 W88	Maple forest	Oxyaquic Hapludalf	8
Luquillo LTER, PR	N18 W66	Montane tropical forest	Aquic tropohumults	1
Luquillo LTER, PR	N18 W66	Palm forest	Aquic tropohumults	2
Luquillo LTER, PR	N18 W66	Cloud forest	Aquic tropohumults	3
Luquillo LTER, PR	N18 W66	Lower montane forest	Aquic tropohumults	3
CAP LTER, AZ	N33 W112	Sonoran desert	Aridisol	5
CAP LTER. AZ	N33 W112	Urban Sonoran desert	Aridisol	5
Kitty Todd, OH	N41 W83	Mesic tallgrass prairie	Typic Haplaquolls	16
Southview savannah, OH	N41 W83	Mesic tallgrass prairie	Typic Udipsamments	22
Secor Metropark, OH	N41 W83	Oak/maple forest	Typic Haplaquolls	40
Ohio University, OH	N39 W82	Oak/maple/ash forest	Hapludalfs	6
Mountainair, NM	N35 W106	Pinyon-Juniper forest	Mesic halpocalcid	12
Valle Caldera, NM	N36 W107	Ponderosa pine forest		6
Valle Caldera, NM	N36 W107	Spruce forest		6

Additional information on these sites, including descriptions of sampling and analysis methods and original citations were presented by Sinsabaugh et al. (2008)

which provided a common slope estimate for all data sets and with intercepts for each data set; (2) calculating ln (BG)/ln (POX) at a median value of R/(R + L) (0.45) using the common slope and data set-specific intercept, and (3) dividing the observed activity ratio by the ratio when R/(R + L) = 0.45. Standardized values of ln (BG)/ln (POX) were then related to R/(R + L) using ordinary least squares regression. Standardization removes variation associated with environmental factors other than the parameters of interest and better allows for comparison across disparate datasets.

Results

Phenol oxidase activity per g OM was not significantly related to β-glucosidase activity and only weakly related to phosphatase activity ($r^2 = 0.013$, $p = 0.006$) (Fig. 1). There was a positive correlation between POX activity and (NAG + LAP) activity ($r^2 = 0.116$). POX-NAG and POX-LAP relationships were stronger but showed inverse trends, negative for NAG and positive for LAP (Fig. 1). Because the slope of the LAP regression was greater than the slope of the NAG regression (0.821 vs. −0.693), the

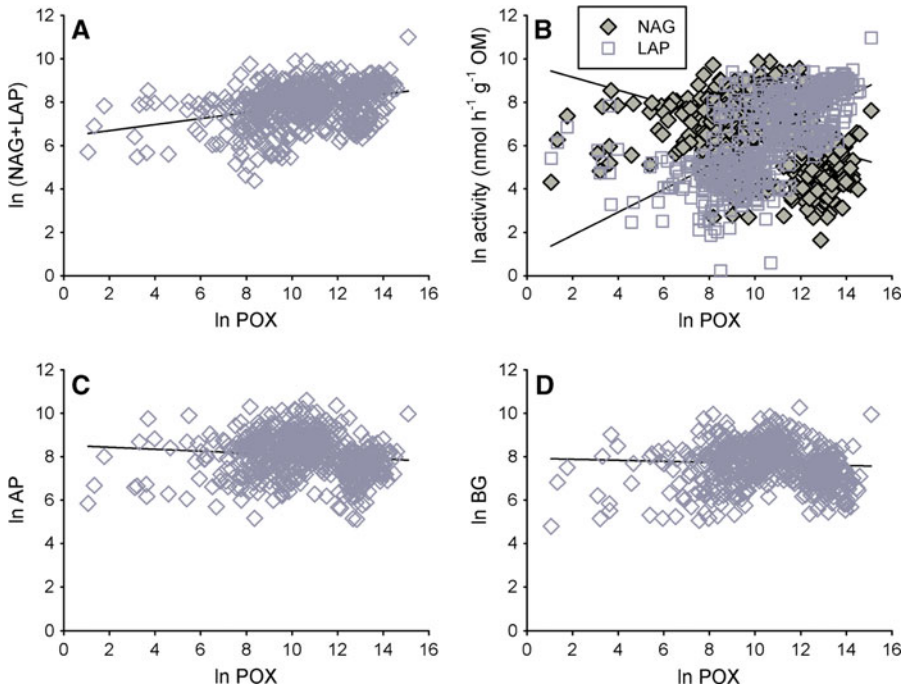

Fig. 1 β-1,4-glucosidase (*BG*), acid/alkaline phosphatase (*AP*), β-*N*-1,4-acetylglucosaminidase (NAG) and leucine aminopeptidase (*LAP*) activities in relation to phenol oxidase (*POX*) activity. POX activity is most closely related to N acquisition. **a** ln (NAG + LAP) = 0.408(ln POX) + 3.55, n = 579, $r^2 = 0.116$, $p < 0.001$. **b** ln NAG = −0.693(ln POX) + 13.93, $n = 605$, $r^2 = 0.186$, $p < 0.001$; ln LAP = 0.821 (ln POX) − 2.30, $n = 579$, $r^2 = 0.417$, $p < 0.001$. **c** AP and POX have a weak relationship (ln AP = −0.411(ln POX) + 12.40, $n = 605$, $r^2 = 0.013$, $p = 0.006$). **d** BG and POX activities are not significantly related ($n = 605$, $p = 0.148$)

net relationship between POX and (NAG + LAP) was positive.

The weak or non-significant regressions between POX activity and the activities of BG, AP and (NAG + LAP) indicate that changes in ratios of hydrolytic to oxidative activity are largely driven by variation in POX activity (Fig. 1). As a result, ln (BG):ln (POX) ratios are more variable (mean 0.781 with a 45% coefficient of variation) than the ratios of ln (BG):ln (AP) (mean 0.958, CV 10%) and ln (BG):ln (NAG + LAP) (mean 0.981, CV 12%) (Fig. 2). Collectively these ratios describe a three dimensional ecoenzymatic space for organic matter decomposition and acquisition of energy and nutrients by heterotrophic microbial communities (Fig. 2).

The relationship between ln (BG)/ln (POX) and R/(R + L) varied among studies. The regressions shared a common slope (−0.64, 95% CI 0.21 to −2.95) but differed in their ln (BG)/ln (POX) intercepts. When ln (BG)/ln (POX) ratio was standardized across datsets and then compared to R/(R + L) using ordinary least squares regression, the slope was −1.055 (95% CI −1.80 to −0.31, $r^2 = 0.53$) (Fig. 3). Using this regression, the mean soil ln (BG)/ln (POX) ratio for our dataset (0.781), corresponded to an R/(R + L) ratio of 0.614.

Discussion

Our analyses confirm findings from ecosystem studies that POX activity potentials often do not correlate with hydrolytic activities in multivariate analyses (Sinsabaugh 2010). For POX and BG, this statistical independence is interesting because lignin and cellulose are not only the most abundant components of plant detritus but are also physically and chemically linked in plant cell walls. Humus, the secondary product of plant litter decomposition and microbial production, is also a physicochemical mix of polyphenolics and carbohydrates, as well as peptides and aliphatic hydrocarbons (Schulten and Leinweber

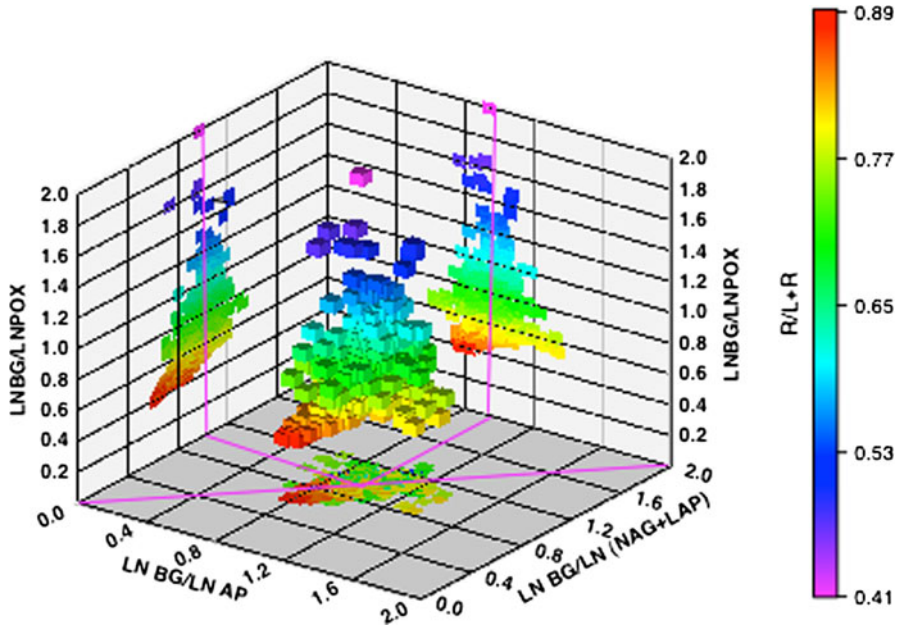

Fig. 2 Distribution of ecoenzymatic carbon and nutrient acquisition activities for soil microbial communities. The mean values for ratios of ln (BG):ln (POX), ln (BG):ln (AP) and ln (BG):ln (NAG + LAP) are 0.781(SD 0.355), 0.958 (0.093) and 0.981 (0.118), respectively ($n = 605$). The ratio R/(R + L) is the fraction of recalcitrant (R) organic carbon within an organic matter pool, L represents labile organic carbon

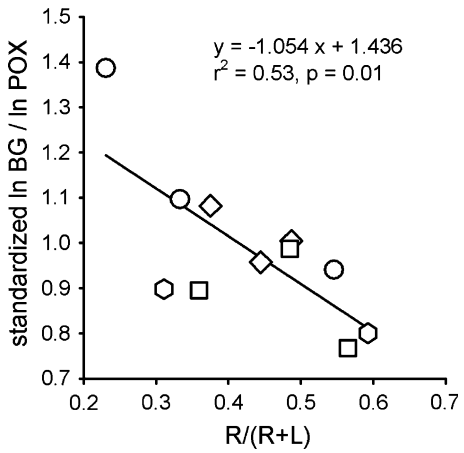

Fig. 3 Ratio of standardized ln (BG):ln (POX) in relation to chemical measures of organic matter composition. The ratio R/(L + R) is the fraction of recalcitrant (R) organic carbon within an organic matter pool, where L represents labile organic carbon. The maple SOM (*filled diamond*) and oak SOM (*filled circle*) data come Grandy et al. (2008); the leaf litter data (*filled square*) are from a decomposition study by Carreiro et al. (2000); the alpine tundra/spruce SOM data (⬢) are from Grandy et al. 2007

2000; Abe and Watanabe 2004; Grandy and Neff 2008). The apparent independence of BG and POX activities suggests that the controls on expression, activity and turnover differ for these two classes of enzyme at the community and ecosystem scale.

A related finding is that POX activity ranges widely while BG activity per unit SOM is relatively constrained (Fig. 1). This pattern suggests that, at the community level, phenol oxidase expression does not come at the expense of BG expression, i.e., the two activities are not inversely related. This is significant because the activities of ubiquitous hydrolytic enzymes involved in C, N and P acquisition do covary, and are often related through resource allocation models that are ultimately tied to microbial metabolism and the elemental stoichiometry of microbial biomass (Allison et al. 2007; Sinsabaugh et al. 2009, 2010). In these models, resources directed toward producing enzymes that increase the acquisition of a scarce nutrient are diverted from enzyme expression pathways for nutrients of higher availability. If the resource supply remains stable, community composition is expected to reach a state where C, N and P are co-limiting (Danger et al. 2008). If resources for POX production are effectively uncoupled from those used for production of BG and other hydrolases, then these resources presumably come at the expense of slower microbial growth. If so, these ecoenzymatic patterns are consistent with models that describe plant litter decomposition as a product of a succession of microbial guilds that exhibit progressively slower

growth rates as the composition of residual organic matter becomes less structurally defined (Moorhead and Sinsabaugh 2006).

Although POX activity varies independently of BG activity and very nearly so for AP activity, there is a statistical link between POX activity and N acquisition. In part, this association reflects the mutual relationships that POX, NAG and LAP activities have with bulk soil pH (Sinsabaugh et al. 2008). As soil pH increases, NAG activity declines while POX and LAP activities increase, generating a positive correlation for POX and LAP and a negative correlation for POX and NAG. This pH effect may be sufficient to account for the positive relationship between POX and (NAG + LAP) activities (Fig. 1). However, experimental manipulations of N availability indicate that regulatory interactions may also contribute to this association.

High N availability suppresses POX activity in many temperate and boreal forest soils, which can affect decomposition and the distribution and composition of SOM (Fog 1988; Michel and Matzner 2003; Knorr et al. 2005; Grandy et al. 2008; Sinsabaugh 2010). In some reports, NAG and LAP activities are also suppressed by experimental N addition (Olander and Vitousek 2000; DeForest et al. 2004; Stursova et al. 2006). For this reason, microbial guild models for decomposition link N acquisition by "miners", i.e., late successional microorganisms with the enzymatic capacity to decompose lignocellulose and humus, to phenol oxidase and peroxidase activities (Moorhead and Sinsabaugh 2006). Because the elemental C:N rato of humus can approach that of microbial biomass (C:N <25) (Gregorich et al. 2006; Cleveland and Liptzin 2007), the oxidative breakdown of humus by miners accesses complexed peptides and other N moieties. Abe and Watanabe (2004) found that peptide N accounted for 66–90% of the N content of humic acids. Artigas et al. (2008) reported that POX and LAP activities were inversely related to $B_{C:N}$. Other studies suggest that ectomycorrhizal basidiomycetes express phenol oxidase, along with proteolytic and chitinolytic enzymes, for the purpose of mining N from humics and supplying it to host plants (Burke and Cairney 2002; Hobbie and Horton 2007; Talbot et al. 2008; Courty et al. 2009). Thus the comparatively strong relationship between POX and LAP ($b = 0.821$, $r^2 = 0.417$) may reflect intersecting regulatory feedbacks as well as a similar pH relationship.

BG activity is strongly linked to AP and (NAG + LAP) activities such that ratios of BG:AP and BG:(NAG + LAP) can be empirically related to the elemental C:P and C:N stoichiometry of environmental resources (Hill et al. 2009; , Sinsabaugh et al. 2008, 2009, 2010). An analogous relationship may exist between the BG:POX activity ratio and indices of SOM composition (Fig. 3), even though these activities are statistically independent at community and ecosystem scales. When elemental C:N and C:P ratios are used to assess nutrient availability, there is an implicit assumption that organic matter composition is not a significant variable.

As plant litter decomposes, labile carbon decreases, aromatic and aliphatic hydrocarbon content increases, and C:N and C:P ratios decline as N and P compounds are incorporated into humic complexes (Grandy and Neff 2008). Enzymatic decomposition, measured as mass loss per unit activity, becomes less efficient (Sinsabaugh et al. 2002; Sinsabaugh 2010). Production of phenol oxidases and peroxidases increases to access complexed C, N and P, but these non-specific activities generate quinones and other reactive phenolic products that can inhibit enzyme activities, increase oxidative stress, and act as antibiotics (Sinsabaugh and Linkins 1987; Nierop et al. 2006; Sinsabaugh 2010). The efficiency of enzymatic degradation is further reduced by non-competitive sorption of enzymes to mineral and organic colloids and the limited availability of effective binding sites (Schimel and Weintraub 2003; Allison and Jastrow 2006; Grandy and Neff 2008). The net effect of these processes is to increase the metabolic effort needed to solubilize a unit of C, N or P (Schimel and Weintraub 2003) and decrease rates of microbial growth (Moorhead and Sinsabaugh 2006).

As SOM becomes more recalcitrant, C, N and P acquisition should become increasingly dependent on POX activity. To represent this effect on ecoenzymatic stoichiometry, we propose that the TER relationships described in Eqs. 3 and 4 be modified to

$$TER_{C:P} \propto [BG/POX] / [AP/POX] \quad (5)$$

$$TER_{C:N} \propto [BG/POX] / [(NAG + LAP)/POX] \quad (6)$$

Algebraically, the EEA terms in Eqs. 5 and 6 are equivalent to those in Eqs. 3 and 4, but ecologically the differences are significant. First, incorporation of POX activity into the numerators and denominators captures

the declining efficiency of enzymatic decomposition [d(mass loss)/d(EEA)] and the increasing metabolic cost of growth observed in decomposition studies as the residual organic material becomes increasingly humified and carbon and nutrients become increasingly difficult to extract. Second, BG/POX, as an indicator of organic matter recalcitrance, reflects the temperature sensitivity of microbial decomposition. The decomposition of labile, i.e., polymeric, substrates has a low activation energy compared to that of recalcitrant substrates, i.e., undefined secondary compounds and humus. As a result, activation energy increases from $\sim$50 to $\sim$80 kJ mol^{-1} as organic matter recalcitrance increases (Bossata and Ågren 1999, Fierer et al. 2005, Conant et al. 2008a, b). Third, POX activity alters the relationship between hydrolytic activities and TER because the numerators and denominators are differentially affected. For Eq. 5, the POX effect on $TER_{C:P}$ is on average smaller than the effect of POX normalization on $TER_{C:N}$ (Eq. 6) because AP and POX activities are weakly ($r = 0.11$) and negatively correlated. The positive and stronger ($r = 0.34$) relationship between (NAG + LAP) and POX activities (Fig. 1) means that the $TER_{C:N}$ expression in Eq. 6 declines more rapidly with increasing organic matter recalcitrance than the $TER_{C:N}$ expression in Eq. 4.

The implications of Eqs. 5 and 6 for stoichiometric theory are most easily seen by focusing on $TER_{N:P}$. The standardized major axis regression for BG and AP activities normalized by POX activity has a slope of 0.917 (95% CI 0.902–0.933); the corresponding regression for BG and (NAG + LAP) has a slope of 0.978 (95% CI 0.958–0.999, Fig. 4). These slopes are significantly different from regressions for the enzymatic relationships presented in Eqs. 3 and 4, which have slopes of 1.162 (95% CI 1.106–1.222) and 1.091 (95% CI 1.028–1.157), respectively (Sinsabaugh et al. 2009). For the POX normalized activities, the $TER_{N:P}$ relationship has a slope of 1.07 (=0.978/0.917) compared to 0.94 (=1.091/1.162) for the hydrolytic ratios.

The growth rate hypothesis predicts that specific growth rate (μ) is a function of cellular rRNA content (Elser et al. 2003). As the rate of nutrient supply increases, μ increases and $B_{N:P}$ and $TER_{N:P}$ decrease, principally because of increases in cellular P quota (Sterner and Elser 2002). A larger P quota increases the liklihood of P limitation on μ. If the magnitude of EEA

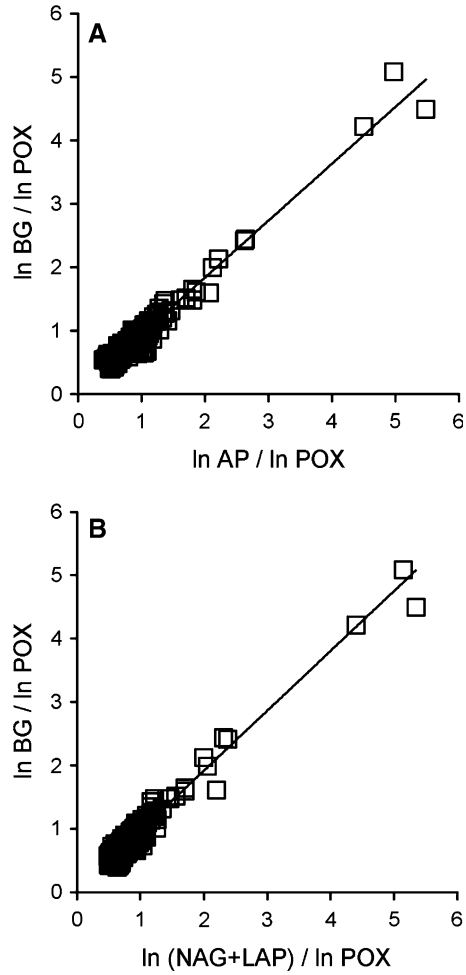

Fig. 4 Standardized major axis regressions for BG and AP activities (**a**) and BG and (NAG + LAP) activities (**b**) normalized to POX activity. The ratios are indicators of relative N, P and C availability for recalcitrant SOM. The slopes of the ln (BG) versus ln (AP) (slope 0.917, 95% CI 0.902–0.933, $n = 605$, r^2 0.956, $p < 0.001$) and ln (BG) versus ln (NAG + LAP) (slope 0.978, 95% CI 0.958–0.999, $n = 605$, r^2 0.931 $p < 0.001$) regressions are <1.0, suggesting that **c** demand increases faster than N and P demand as SOM recalcitrance increases

is a measure of resource supply, the growth rate hypothesis suggests that regressions of ln (NAG + LAP) vs. ln (AP) should have slopes <1. Equations 5 and 6 predict a reversal of this scenario: as organic matter recalcitrance increases, nutrient supply effectively declines, growth rates slow, P quota drops and $TER_{N:P}$ increases. These effects influence (1) the biogeochemical equilibrium between SOM composition and biomass composition and (2) the role of N and P availability on decomposition rates.

The mean molar C:P ratios for SOM and microbial biomass are 186 and 60, respectively, the corresponding values for C:N ratio are 14.3 and 8.6 (Cleveland and Liptzin 2007). The SOM to biomass ratio is 3.1 (=186/60) for C:P and 1.7 for C:N (=14.3/8.6). Equations 5 and 6 suggest that the SOM to biomass ratio for C:N is narrower because soil microorganisms are more N-limited than element ratios suggest because a significant fraction of the N is condensed into recalcitrant molecules, reducing availability. In terms of EEA, the narrower SOM to biomass ratio is the result of the positive correlation between the activity of hydrolytic N-acquiring enzymes, particularly LAP, and phenol oxidase.

The growth rate hypothesis reversal predicted by Eqs. 5 and 6 is also consistent with the "paradoxical" effects of nutrient enrichment on decomposition rates. Gusewell and Gessner (2009) evaluated the growth rate hypothesis using cellulose filters and newly senescent leaf litter. As predicted, increasing the nutrient supply to the decomposer communities reduced $TER_{N:P}$ from 45 to 2, increased mass loss rates and shifted community composition from bacterial dominance to fungal dominance. Many litter decomposition studies show that N enrichment accelerates mass loss rates and increases EEA during early stages (Carreiro et al. 2000; Allison and Vitousek 2004; Knorr et al. 2005). However, for recalcitrant litter and SOM, N enrichment can reduce rates of decomposition and respiration (reviewed by Fog 1988; Knorr et al. 2005; Treseder 2008). This inhibition is associated with decreases in POX activity (reviewed by Sinsabaugh 2010). Suppression of POX activity by N saturation reduces the availability of C to "miners" (Moorhead and Sinsabaugh 2006), which prevents microbial metabolism from moving along the trajectory projected by the growth rate hypothesis. In some cases, this constraint can be

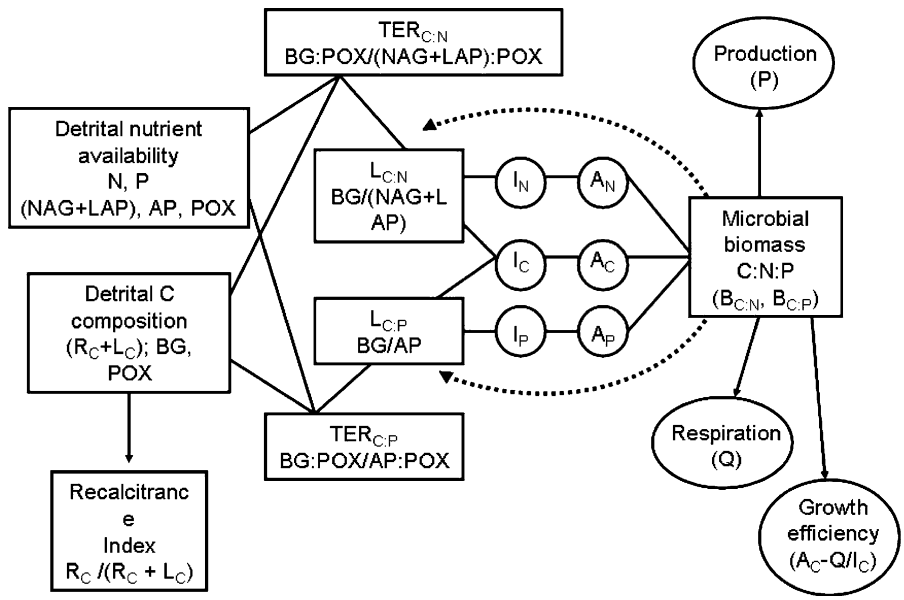

Fig. 5 Ecoenzymatic intersection of the stoichiometric and metabolic theories of ecology. Detrital organic matter composition, defined by ratios of elemental C:N:P and recalcitrant carbon content are related to microbial biomass composition and microbial metabolism. The threshold element ratio (TER) defines the point at which control of community metabolism shifts from energy flow to nutrient flow. Because these flows are mediated by ecoenzymatic activities, ratios of these activities are related to TER and microbial metabolism. *Boxes* include ecoenzyme activities, ratios of ecoenzymatic activities and threshold element ratios (TER) that regulate the generation of labile carbon and nutrients. *Dashed lines* represent ecoenzyme production by microbes. *Ovals* are microbial metabolic processes. Abbreviations: recalcitrant carbon (R_c); labile carbon (L_c); elemental C:N and C:P ratio of labile carbon ($L_{C:N}$, $L_{C:P}$); ingestion rates for N, P and C (I_N, I_P, I_C); assimilation efficiencies for N, P and C (A_N, A_P, A_C); elemental C:N and C:P ratios of microbial biomass ($B_{C:N}$, $B_{C:P}$); microbial respiration (Q); microbial production (P); microbial growth efficiency ($A_C - Q/I_C$); β-glucosidase activity (BG); acid/alkaline phosphatase activity (AP); β-N-acetylglucosaminidase activity (NAG); leucine aminopeptidase activity (LAP); phenol oxidase activity (POX)

alleviated by labile carbon amendments, a phenomenon known as carbon priming. The critical value for the transition from nutrient acceleration of growth to nutrient repression corresponds to the transition from net N immobilization to net N mineralization, which has been defined by various indices: lignin:N ratios of ~24 (Osono and Takeda 2004), molar C:N ratios of ~25–50 (Moore et al. 2006; Parton et al. 2007).) or LCI values of ~0.5 (Moorhead and Sinsabaugh 2006, Herman et al. 2008).

Decomposition models based on microbial activity variously incorporate the effects of changing carbon composition and nutrient availability on microbial metabolism (Schimel and Weintraub 2003; Moorhead and Sinsabaugh 2006). In practice these models are difficult to apply because empirical data on key relationships are lacking. Because ecoenzymes are the proximate agents of decomposition, it may be possible to construct bottom-up decomposition models based on EEA stoichiometry and the relationships represented in Eqs. 3–4 and 5–6. Conceptually, the stoichiometric framework we propose relates microbial metabolism, represented by the ratios A_N/GE and A_P/GE, to carbon composition and nutrient availability, represented by the ratios $TER_{C:N}/B_{C:N}$ and $TER_{C:P}/B_{C:P}$, through EEA ratios (Fig. 5). Because these ecoenzyme activities are easily measured and vary on the same spatiotemporal scale as microbial metabolism, this approach has the potential to facilitate further development and application of these decomposition models in the context of general ecological theory.

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References

Abe T, Watanabe A (2004) X-ray photoelectron spectroscopy of nitrogen functional groups in soil humic acids. Soil Sci 169:35–43

Allen AP, Gillooly JF (2009) Towards and integration of ecological stoichiometry and the metabolic theory of ecology to better understand nutrient cycling. Ecol Lett 12:369–384

Allison SD (2006) Brown ground: a soil carbon analogue for the green world hypothesis? Am Nat 167:619–627

Allison SD, Jastrow JD (2006) Activities of extracellular enzymes in physically isolated fractions of restored grassland soils. Soil Biol Biochem 38:3245–3256

Allison SD, Vitousek PM (2004) Extracellular enzymes and carbon chemistry as drivers of tropical plant litter decomposition. Biotropica 36:285–296

Allison SD, Gartner T, Holland K, Weintraub M, Sinsabaugh RL (2007) Soil enzymes: linking proteomics and ecological process. Manual of environmental microbiology. ASM Press, Washington, pp 704–711

Artigas J, Romani AM, Sabater S (2008) Relating nutrient molar ratios of attached microbial communites to organic matter utilization in forested streams. Arch für Hydrobiol 173:255–264

Baldrian P (2006) Fungal laccases—occurrence and properties. FEMS Microbol Rev 30:215–242

Berg B, McClaugherty C (2003) Plant litter decomposition, humus formation, carbon sequestration. Springer Verlag, Berlin

Bossata E, Ågren GI (1999) Soil organic matter quality interpreted themodynamically. Soil Biol Biochem 31: 1889–1891

Brown JH, Gillooly JF, Allen AP, Savage VM, West GB (2004) Toward a metabolic theory of ecology. Ecology 85:1771–1789

Burke RM, Cairney JWG (2002) Laccases and other polyphenol oxidases in ecto- and ericoid mycorrhizal fungi. Mycorrhiza 12:105–116

Carreiro MM, Sinsabaugh RL, Repert DA, Parkhurst DF (2000) Microbial enzyme shifts explain litter decay responses to simulated nitrogen deposition. Ecology 81: 2359–2365

Claus H (2003) Laccases and their occurence in prokaryotes. Arch Microbiol 179:145–150

Cleveland CC, Liptzin D (2007) C:N:P stoichiometry in soil: is there a "Redfield ratio" for the microbial biomass? Biogeochemistry 85:235–252

Conant RT, Drijber RA, Haddix ML, Parton WJ, Paul EA, Plante AF, Six J, Steinweg JM (2008a) Sensitivity of organic matter decomposition to warming varies with its quality. Global Change Biol 14:868–877

Conant RT, Steinweg JM, Haddix ML, Paul EA, Plante AF, Six J (2008b) Experimental warming shows that decomposition temperature sensitivity increases with soil organic matter recalcitrance. Ecology 89:2384–2391

Courty PE, Hoegger PJ, Kilaru S, Kohler A, Buée M, Garbaye J, Martin F, Kües U (2009) Phylogenetic analysis, genomic organization, and expression analysis of multi-copper oxidases in the ectomycorrhizal basidiomycete *Laccaria bicolor*. New Phytol 182:736–750

Danger M, Daufresne T, Lucas F, Pissard S, Lacroix G (2008) Does Liebig's law of the minimum scale up from species to communities? Oikos 117:1741–1751

DeForest JL, Zak DR, Pregitzer KS, Burton AJ (2004) Anthropogenic NO_3-deposition alters microbial community function in northern hardwood forests. Soil Sci Soc Am J 68:132–138

Doi H, Cherif M, Iwabuchi T, Katano I, Stegen JC, Striebel M (2010) Integrating elements and energy through the

metabolic dependencies of gross growth efficiency and the threshold elemental ratio. Oikos 119:752–765
Elser JJ, Acharya K, Kyle M, Cotner J, Makino W, Markow T, Watts T, Hobbie SE, Fagan W, Schade J, Hood J, Sterner RW (2003) Growth rate-stoichiometry couplings in diverse biota. Ecol Lett 6:936–943
Eriksson K-E, Blanchette RA, Ander P (1990) Microbial and enzymatic degradation of wood components. Springer-Verlag, Berlin
Fierer N, Craine JM, McLauchlan K, Schimel JP (2005) Litter quality and the temperature sensitivity of decomposition. Ecology 86:320–326
Fog K (1988) The effect of added nitrogen on the rate of decomposition organic matter. Biol Rev 63:433–462
Frost PC, Benstead JP, Cross WF, Hillebrand H, Larson JH, Xenopoulos MA, Yoshida T (2006) Threshold elemental ratios of carbon and phosphorus in aquatic consumers. Ecol Lett 9:774–779
Grandy AS, Neff JC (2008) Molecular C dynamics downstream: the biochemical decomposition sequence and its impact on soil organic matter structure and function. Sci Total Environ 404:297–307
Grandy AS, Neff JC, Weintraub MN (2007) Carbon structure and enzyme activities in alpine and forest ecosystems. Soil Biol Biochem 39:2701–2711
Grandy AS, Sinsabaugh RL, Neff JC, Stursova M, Zak DR (2008) Nitrogen deposition effects on soil organic matter chemistry are linked to variation in enzymes, ecosystems and size fractions. Biogeochemistry 91:37–49
Gregorich EG, Beare MH, Mckim UF, Skjemstad JO (2006) Chemical and biological characteristics of physically uncomplexed organic matter. Soil Sci Soc Am J 70: 975–985
Gusewell S, Gessner MO (2009) N:P ratios influence litter decomposition and colonization by fungi and bacteria in microcosms. Funct Ecol 23:211–219
Hammel KE (1997) Fungal degradation of lignin. In: Cadisch G, Giller KE (eds) Driven by nature: plant litter quality and decomposition. CAB International, Wallingford, UK, pp 33–46
Herman J, Moorhead D, Bjorn B (2008) The relationship between rates of lignin and cellulose decay in aboveground litter. Soil Biol Biochem 40:2620–2626
Higuchi T (1990) Lignin biochemistry: biosynthesis and biodegradation. Wood Sci Technol 24:23–63
Hill BH, Elonen CM, Jicha TM, Bolgrien DW, Moffett MF (2009) Sediment microbial enzyme activity as an indicator of nutrient limitation in the great rivers of the Upper Mississippi River basin. Biogeochemistry. doi:10.1007/s10533-009-9366-0
Hladyz S, Gessner MO, Giller PS, Pozo J, Woodward G (2009) Resource quality and stoichiometric constraints on stream ecosystem functioning. Freshw Biol 54:957–970
Hobbie EA, Horton TR (2007) Evidence that saprotrophic fungi mobilise carbon and mycorrhizal fungi mobilise nitrogen during litter decomposition. New Phytol 173: 447–449
Knorr M, Frey SD, Curtis PS (2005) Nitrogen additions and litter decomposition: a meta-analysis. Ecology 86: 3252–3257
Ljungdahl LG, Eriksson K-E (1985) Ecology of microbial cellulose degradation. Adv Microb Ecol 8:237–299
Meentemeyer V (1978) Macroclimate and lignin control of litter decomposition rates. Ecology 59:465–472
Melillo JM, Aber JD, Muratore JF (1982) Nitrogen and lignin control of hardwood leaf litter decomposition dynamics. Ecology 63:621–626
Michel K, Matzner E (2003) Response of enzyme activities to nitrogen addition in forest floors of different C-to-N ratios. Biol Fertil Soils 38:102–109
Moore TR, Trofymow JA, Prescott CE, Fyles J, Titus BD (2006) Patterns of carbon, nitrogen and phosphorus dynamics in decomposing foliar litter in Canadian forests. Ecosystems 9:46–62
Moorhead DL, Sinsabaugh RL (2006) A theoretical model of litter decay and microbial interaction. Ecol Monogr 76: 151–174
Nannipieri P, Paul E (2009) The chemical and functional characterization of soil N and its biotic components. Soil Biol Biochem 41:2357–2369
Nierop KGJ, Preston CM, Verstraten JM (2006) Linking the B ring hydroxylation pattern of condensed tannins to C, N and P mineralization. A case study using four tannins. Soil Biol Biochem 38:2794–2802
Olander LP, Vitousek PM (2000) Regulation of soil phosphatase and chitinase activity by N and P availability. Biogeochemistry 49:175–190
Osono T, Takeda H (2004) Accumulation and release of nitrogen and phosphorus in relation to lignin decomposition in leaf litter of 14 tree species. Ecol Res 19:593–602
Parton W, Silver WL, Burke IC, Grassens L, Harmon ME, Currie WS, King JY, Adair EC, Brandt LA, Hart SC, Fasth B (2007) Global-scale similarities in nitrogen release patterns during long term decomposition. Science 315:361–362
Rabinovich ML, Bolobova AV, Vasilchenko LG (2004) Fungal decomposition of natural aromatic structures and xenobiotics: a review. Appl Biochem Microbiol 40:1–17
Schimel JP, Weintraub MN (2003) The implications of exoenzyme activity on microbial carbon and nitrogen limitation in soil: a theoretical model. Soil Biol Biochem 35:549–563
Schulten HR, Leinweber P (2000) New insights into organic-mineral particles: composition, properties and models of molecular structure. Biol Fertil Soils 30:399–432
Sinsabaugh RL (2005) Fungal enzymes at the community scale. In: Dighton J, Oudermans P, White J (eds) The fungal community, 3rd edn, Chap 17. CRC Press, New York
Sinsabaugh RL (2010) Phenol oxidase, peroxidase and organic matter dynamics of soil. Soil Biol Biochem 42:391–404
Sinsabaugh RL, Foreman CM (2001) Activity profiles of bacterioplankton in a eutrophic river. Freshw Biol 46: 1–12
Sinsabaugh RL, Linkins AE (1987) Inhibition of the *Trichoderma viride* cellulase complex by leaf litter extracts. Soil Biol Biochem 19:719–725
Sinsabaugh RL, Carreiro MM, Repert DA (2002) Allocation of extracellular enzymatic activity in relation to litter composition, N deposition, and mass loss. Biogeochemistry 60:1–24

Sinsabaugh RL, Lauber CL, Weintraub MN, Ahmed B, Allison SD, Crenshaw C, Contosta AR, Cusack D, Frey S, Gallo ME, Gartner TB, Hobbie SE, Holland K, Keeler BL, Powers JS, Stursova M, Takacs-Vesbach C, Waldrop M, Wallenstein M, Zak DR, Zeglin LH (2008) Stoichiometry of soil enzyme activity at global scale. Ecol Lett 11: 1252–1264

Sinsabaugh RL, Hill BH, Follstad Shah JJ (2009) Ecoenzymatic stoichiometry of microbial organic nutrient acquisition in soil and sediment. Nature 462:795–798

Sinsabaugh RL, Van Horn DE, Follstad Shah JJ, Findlay SG (2010) Ecoenzymatic stoichiometry in relation to productivity for freshwater biofilm and plankton communities. Microb Ecol (in press)

Sterner RW, Elser JJ (2002) Ecological stoichiometry: the biology of elements from molecules to the biosphere. Princeton University, Princeton

Stursova M, Crenshaw C, Sinsabaugh RL (2006) Microbial responses to long term N deposition in a semi-arid grassland. Microb Ecol 51:90–98

Talbot JM, Allison SD, Treseder KK (2008) Decomposers in disguise: mycorrhizal fungi as regulators of soil C dynamics in ecosystems under global change. Funct Ecol 22:955–963

Taylor BR, Parkinson D, Parsons WFJ (1989) Nitrogen and lignin content as predictors of litter decay rates: a microcosm test. Ecology 70:91–104

Treseder KK (2008) Nitrogen additions and microbial biomass: a meta-analysis of ecosystem studies. Ecol Lett 11: 1111–1120

Warton DI, Wright J, Falster DS, Westoby M (2006) Bivariate line-fitting methods for allometry. Biol Rev 81:259–291