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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). Eoenzymatic activities are linked to both microbial metabolism and environmental resource availability. In this paper, we extend the theoretical and empirical framework for eoenzymatic stoichiometry from nutrient availability to carbon composition by relating ratios of β-1,4-glucosidase (BG), acid (alkaline) phosphatase (AP), β-N-acetylglucosaminidase (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\textsubscript{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 eoenzymatic activities into general theories of ecology.
Keywords (separated by ‘-‘) β-glucosidase - β-N-acetylglucosaminidase - Decomposition - Ecological stoichiometry - Extracellular enzyme activity - Leucine aminopeptidase - Phenol oxidase - Phosphatase - Soil organic matter - Threshold element ratio

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

Robert L. Sinsabaugh · Jennifer J. Follstad Shah

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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 $\beta$-1,4-glucosidase (BG), acid (alkaline) phosphatase (AP), $\beta$-N-acetylglucosaminidase (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 $\beta$-glucosidase · $\beta$-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
BG Biomass C:N ratio
$B_{C:P}$ Biomass C:P ratio

Biogeochemistry
DOI 10.1007/s10533-010-9482-x
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 microfibril 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 (Sinsabaugh 2009). 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).
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

\[
\text{TER}_{\text{CP}} = (A_{\text{P}}/GE) \times B_{\text{C:P}}
\]

\[
\text{TER}_{\text{CN}} = (A_{\text{N}}/GE) \times B_{\text{C:N}}
\]

where \(A_{\text{P}}\) and \(A_{\text{N}}\) are assimilation efficiencies for P and N, GE is microbial growth efficiency and \(B_{\text{C:P}}\) and \(B_{\text{C:N}}\) 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

\[
\frac{BG}{AP} \propto \left(\frac{\text{TER}_{\text{CP}}}{B_{\text{C:P}}}\right) = \left(\frac{A_{\text{P}}}{GE}\right)
\]

\[
\frac{BG}{(NAG + LAP)} \propto \left(\frac{\text{TER}_{\text{CN}}}{B_{\text{C:N}}}\right) = \left(\frac{A_{\text{N}}}{GE}\right)
\]

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 \(\text{TER}_{\text{CP}}/B_{\text{C:P}}\) and \(\text{TER}_{\text{CN}}/B_{\text{C:N}}\)) and microbial metabolism (expressed as the ratios of \(A_{\text{P}}/GE\) and \(A_{\text{N}}/GE\)).

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 \(B_{\text{C:P}}\) and \(B_{\text{C:N}}\). 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 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

\[
\text{Biogeochemistry}
\]
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 2). 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 relation-
ships 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 Sin-
sabaugh 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 chromatog-
raphy mass spectroscopy (GCMS) techniques
(Grandy and Neff 2004). 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
ruanged 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 decom-
position 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 com-
position 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-corr-
corrected post hoc tests. Subsequently, ln (BG)/ln (POX)
was standardized by (1) running an ANCOVA in
The 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 $\beta$-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

### Table 1 Data sources

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Additional information on these sites, including descriptions of sampling and analysis methods and original citations were presented by Sinsabaugh et al. (2008)
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, r² = 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 2005).
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.

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.
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; Stursvoda 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 (Moorehead and Sinsabaugh 2006). Because the elemental C:N ratio 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_CN. 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; Courtly 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 (Moorehead 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

$$\text{TER}_{C:P} \propto \frac{\text{BG:POX}}{\text{AP:POX}}$$  \hspace{1cm} (5)

$$\text{TER}_{C:N} \propto \frac{\text{BG:POX}}{[\text{NAG + LAP}]/\text{POX}}$$  \hspace{1cm} (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 \[\frac{d(\text{mass loss})}{d(\text{EEA})}\] 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 \(\ast \) 50 to \(\ast \) 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 \(\text{TER}_{\text{C:P}}\) is on average smaller than the effect of POX normalization on \(\text{TER}_{\text{C:N}}\) (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 \(\text{TER}_{\text{C:N}}\) expression in Eq. 6 declines more rapidly with increasing organic matter recalcitrance than the \(\text{TER}_{\text{C:N}}\) expression in Eq. 4.

The implications of Eqs. 5 and 6 for stoichiometric theory are most easily seen by focusing on \(\text{TER}_{\text{N:P}}\). 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 \(\text{TER}_{\text{N:P}}\) 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 \((\mu)\) is a function of cellular rRNA content (Elser et al. 2003). As the rate of nutrient supply increases, \(\mu\) increases and \(B_{\text{N:P}}\) and \(\text{TER}_{\text{N:P}}\) decrease, principally because of increases in cellular P quota (Sterner and Elser 2002). A larger P quota increases the likelihood of P limitation on \(\mu\). If the magnitude of EEA is a measure of resource supply, the growth rate hypothesis suggests that regressions of \(\ln(\text{NAG} + \text{LAP})\) vs. \(\ln(\text{AP})\) 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 \(\text{TER}_{\text{N:P}}\) 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 for 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 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 (Moorehead 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; Moorehead 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 ratio \(A_{N/GE}\) and \(A_{P/GE}\), to carbon composition and nutrient availability, represented by the ratios \(\text{TER}_{C:N}/B_{C:N}\) and \(\text{TER}_{C:P}/B_{C:P}\), 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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