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Extracellular enzymes in terrestrial, freshwater, and marine environments: perspectives on system variability and common research needs

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Abstract Extracellular enzymes produced by heterotrophic microbial communities are major drivers of carbon and nutrient cycling in terrestrial, freshwater, and marine environments. Although carbon and nutrient cycles are coupled on global scales, studies of extracellular enzymes associated with terrestrial, freshwater, and marine microbial communities are not often compared across ecosystems. In part, this disconnect arises because the environmental parameters that control enzyme activities in terrestrial and freshwater systems, such as temperature, pH, and moisture content, have little explanatory power for patterns of enzyme activities in marine systems. Instead, factors such as the functional diversity of microbial communities may explain varying patterns of enzyme activities observed in the ocean to date. In any case, many studies across systems focus on similar issues that highlight the commonalities of microbial community organization. Examples include the effective lifetime of enzymes released into the environment; the extent to which microbial communities coordinate enzyme expression to decompose complex organic substrates; and the influence of microbial community composition on enzyme activities and kinetics. Here we review the often-disparate research foci in terrestrial, freshwater, and marine environments. We consider the extent to which environmental factors may regulate extracellular enzyme activities within each ecosystem, and highlight commonalities and current methodological challenges to identify

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research questions that may aid in integrating cross-system perspectives in the future.

Keywords Extracellular enzymes · Soil · Aquatic · Terrestrial · Marine · Microbial communities

Introduction

Most primary production in terrestrial, freshwater, and marine environments enters detrital food webs and is consumed by heterotrophic microorganisms. The majority of macromolecular detritus must be degraded initially into assimilable substrates by enzymes released into the environment by secretion, and also released to some extent via cell lysis (Arnosti 2011; Maire et al. 2012; Sinsabaugh and Shah 2012). Enzymes act as an environmental detritus filter, degrading macromolecules into smaller mono- and oligomers for microbial assimilation (Burns 1978). Across systems, the magnitude, nature, and distribution of extracellular enzyme activities reflect differences in environmental nutrient availability and organic matter quantity, composition, and consumption in relation to microbial community diversity and growth. The extent to which enzymes maintain activity is dependent on the intrinsic stability of the enzymes themselves as well as the capacity of the environmental matrix to sorb and stabilize active enzymes through associations with particle surfaces and dissolved organic matter (DOM) (Tietjen and Wetzel 2003; Allison 2006; Nannipieri 2006). As a result, the activity and turnover rate of enzymes may vary across systems; within systems there may be multiple pools of active enzymes with different turnover rates. Thus, enzyme pool sizes, turnover rates and kinetics vary widely across systems, and may require different methods of study.

Partly because of differences in methodology, there has traditionally been little comparison of extracellular enzymes across terrestrial, freshwater, and marine systems. Nonetheless, terrestrial, freshwater, and marine elemental cycles catalyzed by extracellular enzymes are interlinked by transfer of elements across system boundaries. Specific questions and technical issues are common to all systems. An obstacle to conceptualizing cross-system perspectives is the disparate biotic and abiotic mechanisms driving enzyme activities in terrestrial, freshwater, and marine systems. Factors that regulate terrestrial enzyme activities provide relatively little explanatory power within marine environments. For example, small-scale (spatial and temporal) gradients in solid surfaces, temperature, and pH can account for significant variations in terrestrial and freshwater ecosystems, but provide little explanatory power in the vast expanse of ocean waters. Therefore, additional factors are necessary to provide a conceptual basis for understanding the extent and nature of variations in enzyme activities observed across environments.

We begin this review by considering the effects of solid surfaces and particles on extracellular enzymes. We then expand to the effects of spatial and temporal gradients in temperature and pH on enzyme activities. Acknowledging the many potential factors that could influence enzyme activities, for this review we focus on microbial community diversity and metabolic capabilities, because of increasing evidence highlighting their importance in elemental cycling. The following sections outline some of the distinctive features of research within each type of system, and discuss the potential integration of enzyme activities into biogeochemical models. The final section of this review is focused on methodological challenges spanning all systems and future research questions that should be addressed.

Abiotic drivers

Surface interactions as controls on enzymatic activity

At micrometer scales, terrestrial, freshwater, and marine systems are heterogeneous environments, with resources, microorganisms, and ecological processes distributed in a non-uniform manner (e.g. Parkin 1987; Sexstone et al. 1985). This microscale heterogeneity is strongly influenced by the abundance of solid surface areas within a given volume, as shown schematically by the vertical axis in Fig. 1a, which separates environments ranging from ocean waters to soils.

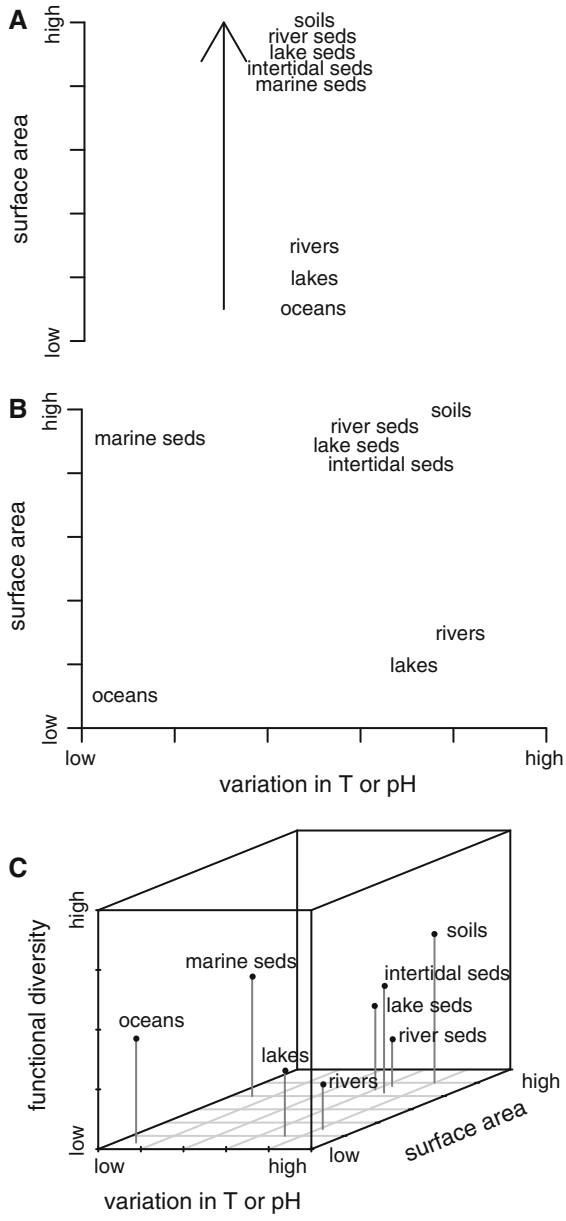

Fig. 1 Conceptual diagram of factors relevant to extracellular enzyme activities that distinguish different environments. **a** Surfaces as a fraction of total volume; **b** surface to volume, variations (spatial and temporal) in temperature or pH; **c** surface to volume, temperature and pH variations, and community functional diversity (see text). *Seds* sediments

Enzymes and their activities are influenced by interactions with surfaces. In soils and sediments, unlike pelagic waters, mineral particle size, distribution, composition, and density strongly regulate enzyme activities. Sorbed enzymes may become less active but may be protected from degradation for much longer than dissolved enzymes, and may regain activity after desorption (Wetzel 1993). Enzyme–surface interactions are dictated by mineralogy, composition of associated organic substances, and thermodynamic properties; these interactions in turn regulate the active enzyme pool-size, access to organic substrates, and turnover rates (Allison 2006; Wallenstein and Weintraub 2008; Sinsabaugh 2010).

In pelagic systems (aquatic environments excluding bottom and shoreline), particle density and composition varies in relation to factors including the source and proximity of terrestrial runoff, phytoplankton production, turbulence, and flocculation processes. DOM can form transient surfaces such as marine snow, lake snow, or river snow (Simon et al. 2002), which increase available surface area and provide "hotspots" for biogeochemical reactions and elevated enzyme activities (Smith et al. 1992; Grossart and Simon 1999; Ziervogel et al. 2010).

Effects of small-scale (spatial and temporal) variations in temperature and pH on enzyme activities

Temperature and pH are dominant controls on enzyme activities, affecting substrate binding and stability, as well as enzyme kinetics. Likewise, water volume and hydraulic residence time influence the rate and magnitude of spatial and temporal shifts in temperature and pH. For example, in marine systems major shifts in temperature take place over much greater spatial and temporal scales than in freshwater and terrestrial environments. In the ocean, pH variations are comparatively small due to high carbonate concentrations; variations in salinity are also generally small over very large spatial scales. By comparison, most freshwater systems lack a carbonate buffer system comparable in capacity to the ocean, and typically exhibit a much wider range of salinities, pH, and temperature excursions relative to the ocean. At large scales, such as across ecosystems, soil pH varies by orders of magnitude. Smaller scale vertical gradients within soil ecosystems may reflect differences in parent material, vegetation, and weathering. At even finer scales, pH gradients may exist within soil aggregates. These spatial and/or temporal variations in temperature or pH are represented by the second axis of our conceptual schematic (Fig. 1b), and

provide significant separation for soil and freshwater environments, but not for marine waters or sediments, with the exception of intertidal zones.

Biotic drivers

Effects of microbial community composition and capabilities on enzyme activities

The low explanatory power of the first two physicochemical axes (Fig. 1a, b) for variations in enzyme activities in marine waters suggests that at minimum, a third axis is needed to differentiate controls on enzyme activities across this range of systems. We have chosen a biotic factor—differences in microbial community composition and capabilities, i.e. community functional diversity—as the third axis (Fig. 1c). The position of a given environment as shown in the figure is arbitrary, since the specific extent to which community composition and capabilities influence enzyme activities in an ecosystem is not yet quantifiable. Nonetheless, inclusion of microbial community functional diversity enables us to develop a conceptual representation of cross-system factors that can affect enzyme activities.

Selection of this factor is supported by a growing number of studies demonstrating the importance of community functional diversity in controlling enzyme activities in marine environments. Indeed, the comparative lack of solid surfaces in ocean waters suggests that the majority of active enzymes in marine waters have a closer connection to producing microbes than do enzymes in soil environments, where abiotic surfaces more strongly regulate enzyme activities and turnover rates. Investigations of organisms isolated from marine waters (Martinez et al. 1996), genomic and biochemical investigations of cultured isolates (Glöckner et al. 2003; Bauer et al. 2006; Weiner et al. 2008; Wegner et al. 2013), and genomic investigations of single cells sorted directly from ocean waters (Martinez-Garcia et al. 2012) have demonstrated specific enzymatic capabilities and substrate specialization among individual organisms. The relationship between compositional and functional differences in microbial communities remains to be defined precisely. That such differences exist in the ocean, however, is supported by metagenomic investigations in ocean waters showing spatial (Gomez-Pereira et al. 2010, 2012) and temporal (Teeling et al. 2012) differences in polysaccharide hydrolase genes associated with specific phylogenetic groupings, which extend to differences in enzymatic function (Arnosti et al. 2012).

Community functional diversity also separates terrestrial and freshwater environments (Fig. 1c). In soils, recent studies show that microbial community composition affects community functional attributes, particularly in response to changes in water and nutrient availability (Stromberger et al. 2011; Lennon et al. 2012; Fierer et al. 2012). In terrestrial environments, in situ enzyme activities are dynamic (Bell and Henry 2011) and responsive to changes in microbial biomass and community structure (i.e. production), declining with stabilization (mineral and organic sorption) and degradation (Waldrop et al. 2000). However, not all changes in enzyme activities are associated with differences in microbial community composition. In a recent study of a freshwater system, for example, large temporal variations in enzyme activities were found along stream flowpaths, but little spatial variation and little correspondence with bacterial community composition was evident (Frossard 2012). The extent to which differences in enzyme activities are driven by changes in microbial community composition, versus changes in metabolic regulation of specific microorganisms, remains to be determined.

Current research foci

Much current research in terrestrial, freshwater, and marine ecosystems is directed towards organic matter cycling processes, but focal points within ecosystems differ considerably. Part of this difference is due to the nature and dynamics of organic matter in these systems. Carbon-rich structural components such as lignin, cellulose, and hemicellulose constitute a major part of (initial) terrestrial particulate organic matter (POM). Freshwater POM reflects contributions from soil and litter-derived terrestrial sources, as well as algal production. In marine systems, the vast majority of actively cycling carbon is in the form of DOM, not POM (e.g. Hedges 1992). Moreover, there is a much wider diversity of initial carbon structures in marine organic matter, and protein constitutes a much larger fraction of POM in the surface ocean (Wakeham et al.

1997) than it does in terrestrial systems. Marine POM thus has a much lower C/N ratio than terrestrial POM. The ability to resolve these differences in organic matter composition, however, rapidly becomes a problem of chemical characterization (Hedges et al. 2000; Lee et al. 2004) in all of these environments. The observation that microbial communities can readily mineralize 'low quality' (high C/N) organic matter (e.g. Arnosti and Holmer 2003), and the persistence of chemically-characterizable organic components over geologic timescales (Cowie et al. 1995), demonstrates the gap between our analytical perspective and the ability of microbial communities to access organic substrates. To provide a comparative framework of reference, in the following section we briefly review major focal points of work within each system, highlighting major research directions and results that are mostly still system-specific.

Terrestrial systems

Soil microbes—bacteria, archaea, and fungi—facilitate the decomposition and transformation of detritus into soil organic matter. As litter decomposes through enzymatic activities and microbial metabolism, the chemistry of decomposition products and remaining litter tend to converge (Wickings et al. 2012; Wallenstein et al. 2012b). While plant material is the original source of soil organic matter, most of the organic compounds found in soils have been transformed or metabolized by microbes (Liang et al. 2011; Miltner et al. 2011; Bradford et al. 2013). However, soil organic matter continues to be susceptible to attack and further enzymatic degradation.

The distribution of specific microbial functional groups in the soil profile is largely attributed to plant litter, roots, and carbon availability (Jobbágy 2000; Posada et al. 2012). Microbial biomass is typically more abundant and has higher fungal:bacterial ratios near the soil surface. Plant communities directly affect soil microbial community composition and activity through alteration of the physical environment during root growth and substrate availability through root exudation (Zak et al. 2003; Johnson et al. 2004; Bird et al. 2011). These effects are strongest in the rhizosphere (de Graaff et al. 2010; Orwin et al. 2010), the soil zone directly impacted by roots, and used by plants to exploit soil organic nutrient pools. The rhizosphere provides a source of labile carbon input during the growing season, and is thought to prime microbial decomposition of more recalcitrant organic matter (Kuzyakov 2002). As a result, root carbon inputs to the soil are increasingly investigated as a driver of microbial nutrient acquisition and enzyme production (Drake et al. 2013).

In addition to being released by microorganisms, extracellular enzymes can also be present on root surfaces or secreted by roots into the rhizosphere (Treseder and Vitousek 2001). In particular, significant root-associated phosphatase production (Tarafdar and Claassen 1988; Dinkelaker and Marschner 1992; Richardson 2001; Araújo et al. 2008) and chitinase activities (Lam and Ng 2001; Faugeron et al. 2006) have been observed. Other nitrogen (N) acquiring extracellular enzymes such as proteases have not been well examined in the context of plant root nutrient acquisition. Overall, little is known about the extent and distribution of root enzyme activity and the degree to which extracellular enzymes enable plants to make use of organic nutrients in the soil.

Soil enzyme assays are useful for assessing microbial community function to answer questions related to soil decomposition (i.e. C-cycling) and nutrient cycling (i.e. N and/or P-cycling). Microbes produce specific enzymes (i.e. C-, N-, or P-degrading enzymes) to meet nutrient demands within their soil environments, also referred to as ecological stoichiometry. Observing the ratios of potential enzyme activities is a robust approach to assess microbial nutrient demands. In brief, the microbial demand for nutrients is determined by the elemental stoichiometry of microbial biomass in relation to environmental nutrient availability. For example, a 1:1 ratio of V_{max} values between two enzyme functional groups (e.g. C:N nutrient acquisition) would suggest that the demand for N is high relative to the demand for C when considering microbial biomass C:N ratios (at the community level) is typically 8:1, and potential C assimilation is usually lower than potential N assimilation (Cleveland and Liptzin 2007).

Freshwater systems

In freshwater ecosystems, enzyme activities per unit organic matter are generally greater than those of soils because of the absence of water stress, as well as nutrient availabilities enhanced by wastewater inputs and agricultural and urban runoff. Analyses of biofilm,

surface sediment, and hyporheic zone (groundwater and/or river water mixing beneath a stream bed) enzyme activities in freshwater systems have typically focused on correlations with microbial substrate consumption and production, or community composition. Because most ecosystem metabolism of lotic (flowing water) systems is associated with sediment microbial communities, there have been fewer studies of enzyme activities in the water column (see summaries of early work in Chróst 1991). Since allochthonous inputs of plant litter account for a large fraction of the organic matter input to many inland water systems, litter decomposition studies have been a part of inland water studies, beginning with measurements of cellulase, β-glucosidase and phosphatase activities associated with deciduous leaf litter decomposing in a woodland stream (Sinsabaugh et al. 1981). Subsequent studies generally show positive correlations between lignocellulose degrading enzyme activities and decomposition rates, relationships that have been used to estimate instantaneous decomposition rates for POM from enzyme measurements (Sinsabaugh et al. 1994; Jackson et al. 1995).

Given the hydrodynamic connectivity of inland water ecosystems, it is much easier to establish statistical connections between enzyme activities, microbial metabolism, resource availability, and landscape attributes in freshwater than in terrestrial or marine systems. Consequently, there are more synthetic analyses of enzyme relationships for freshwater microbial communities. Recent examples include Krauss et al. (2011), who review the ecology and physiology of aquatic fungi in relation to decomposition, nutrient cycling and detoxification, and Sinsabaugh et al. (2012), who compare the activities and ratios of β-glucosidase, phosphatase, leucine aminopeptidase and *N*-acetylglucosaminidase in freshwater sediments and terrestrial soils. One finding from the latter review is that mean ratios of β-glucosidase: phosphatase activity in freshwater sediments are significantly greater than those of terrestrial soils, consistent with the lower elemental C:P ratios of sediments.

Studies of extracellular enzymatic activities associated with decomposing POM led to the development of resource allocation models that link enzyme stoichiometry to nutrient availability, and the first models for estimating decomposition rates from enzyme measurements (Sinsabaugh et al. 1994; Jackson et al. 1995). Planktonic and hyporheic studies linked resource composition and metabolism to analyses of microbial community composition. In turn, these relationships led to development and testing of models that link enzyme activities to ecological stoichiometry (Sinsabaugh et al. 2010) and the carbon use efficiency of microbial communities (Sinsabaugh and Shah 2012).

Marine systems

The study of microbial extracellular enzymes in marine systems includes vast ocean volumes with dilute concentrations of DOM and sparsely-distributed, ephemeral patches of particles; below these waters are compact sediments of variable origin, composition, and depth. Within these environments, the overall theme of most research on extracellular enzymes is to determine the activity of heterotrophic microbial communities in the context of degradation of complex organic substrates.

Two major factors limit our knowledge of the distribution and function of microbial enzymes in marine systems. Access to much of the ocean is limited due to constraints of ship time, as well as difficulties in obtaining samples. Consequently, with few exceptions (e.g. Baltar et al. 2009, 2010, 2013), most investigations of enzyme activities in marine waters have been carried out in surface- and near-surface waters (upper ca. 200 m of the ocean's average 4,000 m depth) or in shallow coastal zones. The second problem—limitations in our means to effectively measure enzyme activities (see below), and problems of substrate sorption—in part explains the comparative paucity of data on enzyme activities in marine sediments. Most investigations of marine sediments come from coastal and near-surface environments; with very few exceptions (Coolen and Overmann 2000; Coolen et al. 2002; Lloyd et al. 2013), all of these studies have been carried out in the upper ca. 20 cm of the sediment column, and only a handful of studies (e.g. Boetius and Lochte 1994, 1996; Boetius et al. 2000; Dell'Anno et al. 2000) come from surface sediments retrieved from the deep ocean. We thus have almost no data (Kobayashi et al. 2008) on enzymatic activities from the microbial biosphere of deep-subsurface sediments of the ocean basins, which contain an estimated 30 % of the earth's microbial biomass (Kallmeyer et al. 2012).

Although most investigations in marine systems have used a few substrate proxies (such as MUF-β-glucose and leucine-MCA; see below) to measure enzyme activities, an increasing number of studies have focused specifically on determining activities of a broader range of enzymes, including those of endo-acting enzymes that hydrolyze polymers mid-chain. These efforts have used fluorescently labeled polysaccharides and plankton-derived extracts (Arnosti 1995, 2003) or spin probes (Steen et al. 2006), as well as larger peptides labeled with (Pantoja et al. 1997; Pantoja and Lee 1999) and without added fluorophores (Liu et al. 2010). These substrates demonstrate the fundamental importance of substrate structure in determining hydrolysis rates: in coastal waters, larger peptides were more rapidly hydrolyzed than smaller peptides of the same chemical structure (Pantoja and Lee 1999); hydrolysis rates among peptides containing the same components in different order were also found to differ (Liu et al. 2010).

These studies also demonstrate that microbial communities in ocean waters frequently hydrolyze only a subset of the polysaccharide substrates that are readily hydrolyzed in the underlying sediments (Arnosti 2000, 2008). These differences in enzymatic capabilities may be linked to differences in microbial community composition between the water column and underlying sediments (Fig. 1c) (Teske et al. 2011). A recent study further demonstrated latitudinal gradients in the abilities of heterotrophic microbial communities to hydrolyze different polysaccharide substrates (Arnosti et al. 2011). The spectrum of substrates hydrolyzed decreases with increasing latitude, reflecting a pattern of latitudinal changes in community diversity and decreasing species richness at higher latitudes observed in other investigations (e.g. Baldwin et al. 2005; Pommier et al. 2007; Fuhrman et al. 2008).

The driving forces behind observed depth- and site-related differences in enzyme activities of heterotrophic microbial communities in ocean waters (Arnosti et al. 2005; Steen et al. 2008, 2012) remain to be established. Ultimately, differences in microbial community composition and large-scale patterns of microbial biogeography, which are now being discerned in ocean environments (Pommier et al. 2007; Zinger et al. 2011; Friedline et al. 2012), must be major factors affecting enzymatic capabilities of whole communities, hence the selection of this factor as the third axis in Fig. 1c.

One of the factors contributing to understanding enzyme activities in freshwater and terrestrial environments is a well-established sequence of organic matter transformations during early diagenesis of plant litter. In ocean waters and sediments, in contrast, establishing a meaningful linkage between the presences of specific substrates and enzyme activities is precluded currently by the challenge of determining the chemical structure of marine POM and DOM (Hedges et al. 2000; Lee et al. 2004) at the level that is relevant for considerations of enzyme structural specificities. We are thus unable at present to determine key aspects of enzyme and community function. These factors greatly limit the development of models of enzyme activities that can be applied to marine systems, as discussed in the next section.

Enzyme-based decomposition models

Several mathematical models have been developed to explore the relationships between extracellular enzymes, organic matter decay, decomposer communities, and environmental factors affecting decomposition. These models can be used to better understand and quantify linkages between organic matter, organisms, and processes, and to explore the potential impact of variations in specific parameters (e.g. organism distribution, substrate characteristics, enzyme characteristics) on environmental processes. Most of these models are based on studies of terrestrial and to a lesser extent, freshwater systems, and reflect biases that do not equally apply to marine environments, such as the dominant input of terrestrial plant litter mentioned above. Structural polysaccharides (e.g., cellulose and hemicellulose) comprise the largest pool of detrital organic matter in terrestrial and freshwater ecosystems and are hydrolyzed by a suite of related enzymes whose activities tend to be correlated with each other and with the cumulative decay of cellulosic substrates (Sinsabaugh et al. 1994; Jackson et al. 1995). Thus a few enzymes (e.g., β-glucosidase) have emerged as proxies for the actions of many. Critical nutrients, including N and P, are typically much less concentrated in plant litter than in microbes, so that N- and P-acquiring enzyme activities can be relatively high compared to C-acquisition in terrestrial systems, and are again represented by a few proxies, i.e., β-*N*-acetylglucosaminidase and leucine

aminopeptidase for N-acquisition and acid or alkaline phosphatase for P-acquisition.

Recent statistical analyses of C-, N- and P-acquiring extracellular enzyme activities in terrestrial soils, aquatic sediments, and freshwater plankton have shown convergence across ecosystems. Regression models of β-glucosidase versus phosphatase (C vs. P acquiring) activities and β-glucosidase vs. β-*N*-acetylglucosaminidase + Leucine aminopeptidase (C vs. N acquiring) have slopes near 1.0 (range 0.85–1.27), but with activity ratios that vary with elemental composition of available organic matter (Sinsabaugh et al. 2008, 2010, 2012). Thus the apparent stoichiometry of enzyme activity integrates the elemental stoichiometry of the microbial biomass and (non-marine) detrital organic matter with the assimilation of energy and nutrients.

These empirical relationships between microbial stoichiometry and metabolism, enzyme activities, and substrate chemistry provide a basis for mechanistic models of soil and freshwater systems (see below), but their applicability to marine systems is uncertain. For example, the range of variation in C- and N-acquiring enzyme activities in marine environments may be narrower, given the comparatively lower fraction of structural polysaccharides produced by algae compared to terrestrial plants. Also, models of terrestrial systems rarely resolve DOM into more than one or two pools, and often do not include DOM at all; in marine systems, DOM is by far the largest pool of actively cycling carbon. Finally, the appropriate proxy enzyme(s) for C-acquisition in marine systems likely varies with depth and location (see above). Thus, a similarly convergent model of marine enzyme stoichiometry seems unlikely, at least with current knowledge.

A mechanistic model of decomposition based on enzyme activities would likely have some similar features across systems, aside from differences in dominant forms of substrates and indicator enzymes. The simplest enzyme models simulate the decomposition of a single substrate by a single pool of enzymes, although the types of substrate and enzymes vary (Vetter et al. 1998; Sinsabaugh and Moorhead 1997; Schimel and Weintraub 2003; Lawrence et al. 2009; Allison et al. 2010; Resat et al. 2012). While these models lack sufficient complexity to evaluate more complex relationships between different types of substrates, enzymes, and microbial activities, they should be generally applicable to all types of systems and require a minimum number of parameters.

More complex models include multiple pools of enzymes that degrade specific types of substrates. For example, Moorhead et al. (2012) simulated the actions of β-glucosidase and β-acetylglucosaminidase as they hydrolyzed cellulose and chitin, respectively. The allocation of C and N resources between these two enzymes in response to microbial demands and substrate qualities approximated patterns of enzyme activities consistent with empirical models (Sinsabaugh et al. 2008, 2010; Sinsabaugh and Shah 2012). However, this kind of approach requires considerable knowledge of key substrate and enzyme characteristics, and the manner in which they interact, which again is not available for marine systems.

Temporal and spatial heterogeneity have many implications for enzyme-based models even at small scales (Fig. 1) because extracellular enzymes work outside the cell. Vetter et al. (1998) found that the kinetic characteristics of enzymes, as well as the diffusivity of both enzymes and hydrolysates, limited the distance over which microorganisms could obtain resources in saturated sediments. This is because it takes time for enzymes to diffuse from microorganisms to substrate, hydrolyze the substrate, and for products to diffuse back to the microorganisms, while microorganisms continue metabolizing resources. Resat et al. (2012) found that the coexistence of free versus membrane-bound cellulose enzymes increased overall cellulose use and reduced variability in a spatially structured soil. Thus multiple microbe–enzyme relationships can increase the efficiency of substrate use in a spatially heterogeneous environment.

Allison (2005, 2012) and Folse and Allison (2012) included C-, N- and P-acquiring enzyme activities in their models to evaluate how relationships among microorganisms differing in enzyme expression affected community structure. Their results showed that a spatial distribution of organisms was sufficient to explain the persistence of microorganisms that produced no extracellular enzymes (Allison 2005), as well as coalitions of different microorganisms that varied in the specific combinations of enzymes they produced. All of these models were devised for soil systems at small spatial scales. The broader depth, latitude and seasonal variations in marine waters are likely to superimpose additional variations in

substrate, enzyme, and microbial interactions rarely considered in soil models.

Many of the uncertainties in enzyme pool dynamics discussed above have important implications for enzyme-based models. To date, enzyme production in models is largely considered to be constitutive and enzymes are considered to have more rapid turnover than the microorganisms that produce them (e.g., Schimel and Weintraub 2003; Allison 2005; Moorhead et al. 2012). Sinsabaugh and Moorhead (1997) found that the ratio of enzyme: microorganism turnover times necessary to maintain a stable model was narrow, and that more persistent enzymes destabilized the system. Spatial heterogeneity may also interact with enzyme persistence, with high persistence favoring fast-growing microorganisms that may not produce enzymes (Allison 2005), leading to another unstable system. Relative enzyme and microbial turnover times have not been a central focus of enzyme models to date, and other uncertainties further complicate this relationship. For example, the increased persistence of enzymes adsorbed to mineral or humic surfaces is probably not as destabilizing if adsorption reduces activity. However, adsorption also obscures the relationship between potential enzyme activity assayed from field samples and the actual investment of microorganisms in their production (see above). In summary, a broad range of questions in terrestrial and freshwater systems have been addressed, but models for these environments have limited explanatory power for marine systems, in particular because of uncertainty in substrate characteristics and appropriate representative enzymes.

Common research needs

Our conceptual framework integrating abiotic and biotic factors (Fig. 1) presents an opportunity to define common research needs in order to integrate and understand the many driving factors controlling enzyme activities within and among terrestrial, freshwater, and marine systems. For example, the nature and properties of surfaces in soil and sediment (Fig. 1a) may be particularly important in determining the effective 'active lifetime' of enzymes in the environment as sorption and/or occlusion can stabilize enzymes as well as inhibit enzyme reactions. Temperature and pH variations (particularly in soils and in freshwater systems; Fig. 1b) may also fundamentally affect enzyme lifetimes, as enzymes produced under one set of conditions may not function optimally when conditions change. The effective lifetime of an enzyme also helps define the extent to which changes in community functional diversity (Fig. 1c) change the enzymatic potential within an environment.

These considerations lead to a number of specific questions:

(1) What are effective 'lifetimes' for active enzymes; how long are they catalytically effective? What are the most important parameters determining these lifetimes? Do these parameters differ among terrestrial, freshwater, and marine environments? What is the fate of most extracellular enzymes released into the environment?
(2) How do enzyme lifetimes compare to timescales of variation of microbial metabolism, hydrolysate uptake, and microbial lifespan?
(3) To what extent do enzymes no longer associated with cells, or adsorbed to particles, contribute to net activity? Does this fraction vary by classes of enzymes? Do enzymes persist long enough in soils and sediments so that most of the extracellular enzyme activity is no longer closely tied to microbial metabolism?

In order to couple models to experimental data, methods to assess effective enzyme pool size (i.e., not only concentration, but activity) as well as to determine enzyme turnover rates are needed. This point also relates to common needs in experimental measurements (see below); current methods cannot distinguish large pools of ineffective and/or slowly-acting enzymes from smaller pools of highly efficient enzymes. Moreover, the presence of isoenzymes (structurally distinct enzymes that catalyze the same reaction) has been documented in marine waters (Arrieta and Herndl 2002). Multiphasic kinetics (K_m, V_{max}, kcat, etc.) resulting from the presence of isozymes further complicate efforts to define kinetic parameters for environmental enzymes, and to measure factors regulating enzyme activities in the environment.

Community functional diversity as a driver of enzyme activities also encompasses a number of common needs. In particular, a better idea of the range across the axis of Fig. 1c, and the relative placement of

different environments on this axis, would enable us to weigh the influence of different controlling factors. Common research needs, however, begin at the level of the individual cell:

(1) What are the pathways that signal environmental substrate availability and regulate gene expression?
(2) What is the functional significance for microbial genomes to contain multiple genes for isozymes catalyzing the same reactions (e.g., Glöckner et al. 2003; Bauer et al. 2006; Wegner et al. 2013)?
(3) Are gene expression, production, and export affected by the same factors across environments?

The basics of enzyme induction are known from studies using model organisms such as *E. coli*. Given the diversity of natural microbial communities, however, these models are not necessarily applicable to all organisms, particularly those in relatively carbon-poor environments such as oligotrophic ocean waters.

Additionally, considerations of the capabilities of microbial communities as a whole, rather than as a collection of disparate organisms, are needed. In some cases numerically minor members of the community may express biogeochemically important enzymes (Beier and Bertilsson 2011); the environmental conditions that favor this situation should be explored. Furthermore, emergent properties of microbial communities may be manifested via communication among organisms (e.g. quorum sensing; Gram et al. 2002; Hmelo et al. 2011; van Mooy et al. 2012). Alternately, differential expression of genes by individuals of a single type has been observed in pure cultures (Baty et al. 2000a, b). Expression of specific enzymes by only a fraction of the members of a species would represent a further level of complexity between the biochemistry of single cells and the ecological function of whole microbial communities.

Direct links between community composition and enzymatic function are currently difficult to establish, given the diversity of uncultured organisms of unknown capabilities that are identified by modern molecular methods (e.g. Campbell et al. 2011). Metagenomic profiling (Mackelprang et al. 2011; Teeling et al. 2012) and single-cell genomic sequencing (Martinez-Garcia et al. 2012) have provided insight into community and individual potential for enzyme production, but the extent to which—and conditions under which—this potential is expressed in the environment are largely unexplored. Determining the relevant taxonomic scale (i.e., degrees of genetic similarity) required to relate community composition to enzyme production should be a focus of future work. Changes in microbial community composition may impact the nature and specificity of microbially-produced enzymes, but the relative importance of community composition versus community metabolic regulation is not yet known. A better understanding of the precise links between community composition and enzymatic function would enable us to more precisely quantify the influence of biotic drivers on enzyme activities (Fig. 1c). This question is particularly relevant to cross-system comparisons, given the differences in community composition between freshwaters and marine waters (e.g. Bouvier and del Giorgio 2002; Crump et al. 2004; Silveira et al. 2011), soils (Lauber et al. 2009; Moora et al. 2011; Rout and Callaway 2012), and the marine water column and sediments (Zinger et al. 2011).

Methodological challenges related to common research needs

Methodological advances will facilitate progress in addressing many of the common research needs. Some questions, particularly those associated with cellular control of enzyme production, response to specific signals, and identification of genes that are activated under specific conditions, can likely be answered through use of genomic and transcriptomic methods, while metaproteomics offers the potential for directly linking enzymes to taxa that produce them (Schneider et al. 2012). Other questions may be answered as more microbial genomes are fully sequenced, and through increased representation of organisms derived from the environment in existing databases of organisms and enzymes (e.g. Cantarel et al. 2008; Chang et al. 2009).

The development of new methods to measure enzyme activities is a need that crosses environmental boundaries. The vast majority of investigations in terrestrial, fresh water, and marine systems use commercially-available substrate proxies to assess potential enzyme activities (Hoppe 1983; Somville and Billen 1983; German et al. 2011). These substrate

proxies consist of a fluorophore, typically MUF (4-methylumbelliferone) or MCA (7-amido-4-methyl coumarin) covalently linked to a monomer such as glucose or leucine. Upon hydrolysis of the fluorophore-monomer bond, the fluorophore increases in fluorescence; hydrolytic activity is measured as an increase in fluorescence over time. Hydrolysis rates measured with a few substrate proxies are often extrapolated to polysaccharide and protein hydrolysis in general (e.g. Christian and Karl 1995; Fukuda et al. 2000; Piontek et al. 2012). This extrapolation is problematic especially in marine environments, since the nature of the enzymes most important for processing of marine organic matter is unclear.

Furthermore, the relationship between hydrolysis rates measured with substrate proxies and the hydrolysis rates of the macromolecules they are intended to represent is tenuous (Warren 1996). Such proxies cannot accommodate extracellular enzymes that contain substrate-binding modules that position macromolecules appropriately for hydrolysis (Boraston et al. 2004). With few exceptions (Obayashi and Suzuki 2005, 2008; Bong et al. 2013; Steen and Arnosti 2013), these enzyme assays typically reflect only exo-acting activities (hydrolysis of a polymer from the terminal position), not the activities of endo-acting enzymes that hydrolyze polymers mid-chain. Extracellular-enzyme-producing organisms, however, frequently produce both exo-and endo-acting enzymes (e.g. Weiner et al. 2008). New efforts to experimentally capture the diversity and activity of enzymes in natural environments, in a manner that would facilitate integration with genomic, metagenomic, and proteomic information, should be a high priority.

Related to these issues is the fundamental question about the size of substrates that are transported across cellular membranes. Implicit in many of our assumptions about substrate hydrolysis is the idea that organisms take up monomeric constituents. Consumption of polymeric organic matter may also occur through the production of oligomers, however, not just monomers. The uptake limit of bacterial porins would permit transport of larger oligomers (Benz and Bauer 1988). Investigations have demonstrated preferential microbial uptake of oligomers relative to monomers (Arnosti and Repeta 1994; Cotta and Zeltwanger 1995). Moreover, recent studies in marine systems have shown that glucose, for example, may be taken up by only a small fraction of heterotrophic bacteria in a broad range of systems (e.g. Nikrad et al. 2012) and that the organisms using polymers are quite distinct from those using monomeric constituents (e.g. Kirchman et al. 2007; Alonso-Saez et al. 2012). Similar patterns apply to terrestrial environments, where functional guilds of opportunists, decomposers, and miners are recognized (Moorhead and Sinsabaugh 2006; Rinkes et al. 2011). Lastly, a primary challenge for researchers studying enzyme activity in soil, freshwater, and marine systems is determining effective pool size and activity—enzyme persistence and turnover rates. New methods must be developed to differentiate between enzymes that are present, those that are actually functional and those that have denatured into available, N-rich organic matter. In parallel, improvements are needed in our ability to distinguish the fraction of organic matter (substrate) that is enzymatically and microbially accessible, as distinct from the fraction that can be chemically measured (e.g. Arnosti and Holmer 2003).

Structuring factors across environments: the same or different?

Heterotrophic microorganisms obtain critical resources through the actions of extracellular enzymes in all ecosystems. Extracellular enzymes are therefore major drivers of carbon and nutrient cycling. Although biogeochemical cycles within these environments are interconnected, few efforts have been made to integrate enzyme studies across the different environments. Marine and terrestrial systems in particular have been viewed as incompatible for comparative studies due to differences in environmental controls, community organization, and the quantity and composition of substrates. Above, we discussed how environmental parameters such as surface area, temperature, pH, and moisture content greatly affect the activities of enzymes and organisms in terrestrial systems (and in many inland waters) but have little explanatory power for patterns of enzyme activities measured in marine systems, where biotic drivers, including microbial community composition and substrate diversity may exert the greatest influence. There has been greater research emphasis on spatial relationships between microbial communities and enzyme activities in marine systems, and greater focus on the effects of solid surfaces and dynamic

environmental properties on their activities in terrestrial systems. Despite historical differences in research foci and actual differences in environmental controls on enzyme activities among habitats, a number of common research needs have been identified.

Increased cross-disciplinary study and interactions would benefit researchers within fields as well. For example, fluorescence-based enzyme assays, which are much more sensitive than the colorimetric assays historically used in terrestrial studies, were first employed in the environment by aquatic researchers. The adoption of the fluorescence-based assays has allowed soil scientists to study enzyme activities under temperature and pH conditions more true to the actual environment. Conversely, enzyme models developed in the context of freshwater and terrestrial systems may provide insight into the dynamics of marine extracellular enzymes if fundamental substrate-enzyme dynamics can be identified. While the relative importance of specific structuring factors (e.g. pH, presence of surfaces, etc.) varies dramatically among terrestrial, freshwater, and marine environments, the underlying process is the same: microorganisms produce extracellular enzymes in order to gain a selective advantage; those enzymes subsequently catalyze biogeochemical cycles. Further collaboration among researchers across systems should lead to a more complete understanding of the ultimate controls and biogeochemical consequences of extracellular enzymes across environments.

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