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Vector analysis of ecoenzyme activities reveal constraints on coupled C, N and P dynamics

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ABSTRACT

The primary goal of this study was to devise a quantitative method of interpreting the simultaneous microbial C, N, and P acquisition through the activities of four, key extracellular enzymes, 1,4-β-glucosidase (BG), leucine aminopeptidase (LAP), 1,4-ß-N-acetylglucosaminidase (NAG) and acid/alkaline phosphatases (AP). To this end, the proportional activity of C vs. N acquiring enzymes (BG/[BG + NAG + LAP]) was plotted against C vs. P acquiring enzymes (BG/[BG + AP]). We then calculated the length and angle of the vector created by connecting a line between the plot origin and point represented by these proportions; the length quantifies relative C vs. nutrient limitation and the angle quantifies the relative P vs. N limitation. Analyses of large data sets obtained from soil, freshwater periphyton and aquatic sediments revealed that logarithmic, arcsine, arcsine-square-root and logit transformations did little to improve the statistical distribution of data over raw proportions. More importantly, the vector characteristics of enzyme activity loci are much easier to interpret for raw proportions than when data have been previously transformed. Analyses also revealed the importance of using consistent methods, i.e., omitting NAG assays for an acidic soil led to overestimates of P limitations, and the importance of understanding the nature of the system, i.e., soils of the Antarctic Dry Valleys had low BG activities, likely because there is little to no local production of cellulose. Further, analyses of four sites in Luquillo Forest, Puerto Rico, showed no differences among sites in relative C demand despite differences in BG activities, and higher P demand in the cloud than lower montane forest, despite higher AP activity at the latter site. Finally, analyses of three decomposing litter types over time revealed contrasting patterns of change in relative C, N and P demand over time, reflecting both stoichiometric and C quality effects on decomposer communities. Thus enzyme activity vectors reflect the simultaneous, relative resource demands of the microbial community independent of variations in total enzyme activity, such as may result from variations in total microbial biomass.

1. Introduction

Heterotrophic microorganisms play a central role in global biogeochemical cycles by mineralizing dead organic matter to its constitutive elements. Because they degrade complex organic compounds by secreting extracellular enzymes into the environment, the activities of these enzymes are often examined for insights to nutrient cycling (Sinsabaugh et al., 2008; Burns et al., 2013). Because most free-living microbial communities are limited or co-limited by energy (usually carbon) or key nutrients (usually nitrogen or phosphorus), Sinsabaugh et al. (2008) developed a means of visualizing the relative C, N and P controls on soil microbial communities by plotting ratios of activities for enzymes associated with C, N and P acquisition. Herein we propose that the vector lengths and angles connecting the origin to individual point loci in these plots can be used to better quantify and visualize these controls.

Four extracellular enzymes, 1,4-β-glucosidase (BG), leucine amino-peptidase (LAP), 1,4-ß-N-acetylglucosaminidase (NAG) and acid/alkaline phosphatases (AP), are most commonly assayed as indicators of C, N, and P acquisition efforts by decomposer

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microorganisms (Sinsabaugh et al., 2008). In reality, microorganisms secrete many different enzymes because dead organic matter, particularly plant tissue is biochemically complex, but only a few account for most of the measurable activity because they target the most abundant substrates in the environment (Sinsabaugh and Follstad Shah, 2012). For example, cellulose is the main structural polysaccharide of plant cell walls and consists of long-chains of glucan molecules; BG is one of the primary enzymes that catalyze cellulose degradation. Similarly, LAP is one of many protease/peptidase enzymes that catalyze the cleavage of amino acids from proteins or other peptide substrates, but leucine and alanine are the most abundant protein amino acids. NAG hydrolyzes oligomers of N-acetyl glucosamine (an amino sugar) in chitin, found in fungal cell walls and invertebrate exoskeletons, and peptidoglycan, the principal component of bacterial cell walls. Finally, AP hydrolyzes phosphomonoesters, liberating phosphate from organic molecules such as phospholipids and nucleic acids. These four enzymes are assumed to be proxy indicators of overall C, N, and P acquisition largely because the activities of other related enzymes are typically lower than- and correlate with them (Sinsabaugh, 1994).

Within this context, Sinsabaugh et al. (2008) suggested that the relative activities of BG/AP and BG/(NAG + LAP) reflect relationships between relative activities of C vs. P and C vs. N acquiring enzymes, respectively, and plotted the ratios ln(BG)/ln(NAG + LAP) vs. ln(BG)/ln(AP) (Fig. 1). Recently, Moorhead et al. (2013) suggested calculating the length and angle of the vector created by connecting a line between the plot origin and point represented by the coordinates BG/AP and BG/(NAG + LAP). The length of the vector quantifies relative C vs. nutrient limitation and the angle quantifies the relative P vs. N limitation. In contrast, Hill et al. (2014) calculated vector lengths and angles from the ratios of log-transformed enzyme activities, ln(BG)/ln(NAG + LAP) and ln(BG)/ln(AP). In either case, simple ratios tend to have skewed distributions, and a zero in the denominator although potentially meaningful in terms of enzyme activities, creates an infinite ratio. An alternative is to plot the proportional enzyme activities BG/(BG + AP) and BG/(BG + LAP + NAG), or perhaps the traditional arcsine or arcsine-square-root transformed values for proportional data. However, Warton and Hui (2011) argue that logit transformations have statistical advantages over arcsine transformations, i.e., ln(p/[1−p]), where p is the proportion of interest. Unfortunately, logit-transformed proportional enzyme activities can also produce undefined values. Nonetheless, the relative strengths and limitations of these various methods have yet to be compared.

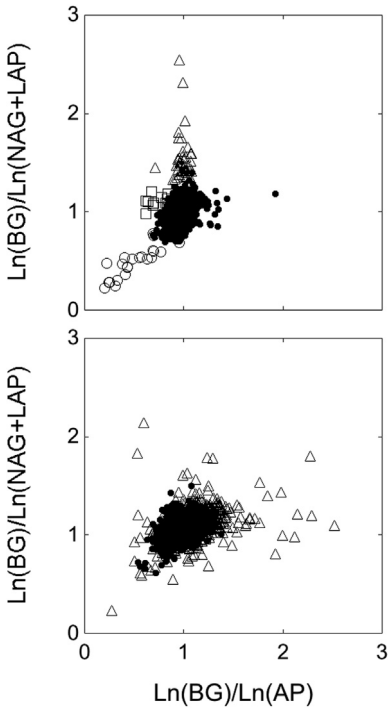

Fig. 1. Relationships between ln(BG)/ln(NAG + LAP) versus ln(BG)/ln(AP) for studies of: top, soil communities at Manistee Forest (triangles), McMurdo Dry Valleys (open circles), Luquillo Forest (squares), all other sites (closed circles), from Sinsabaugh et al. (2008); bottom, freshwater periphyton (closed circles) and sediments (triangles), from Hill et al. (2009). Note that data from all studies cannot be directly compared due to differences between sites and methods (see text for explanations).

The primary goal of this study is to evaluate methods of interpreting microbial C, N, and P acquisition through the activities of extracellular enzymes. Analyses of individual enzymes can reveal differences in the absolute levels of activity between samples but provide little information about the overall behavior or nutritional status of the microbial community (Moorhead et al., 2013). In contrast, the relative activities of C vs. P and C vs. N acquiring enzymes can reveal differences in relative resource allocation toward C, N, and P acquisition, which in turn can be related to carbon use efficiency (Sinsabaugh and Follstad Shah, 2012). Although resource co-limitations cannot be revealed by pairwise comparisons of enzyme activities, two pairwise comparisons can be plotted to simultaneously illustrate relative overall C:N:P activities (Fig. 1). Enzyme activity vectors estimated from these pairwise relationships then can be used to quantify relative patterns of C vs. N vs. P acquisition (Moorhead et al., 2013; Hill et al., 2014). Thus measures of BG, LAP, NAG, and AP can provide different levels of insight to microbial activities, i.e., absolute responses from individual enzymes, relative responses via specific pairs of enzymes, and integral responses through all three relative C, N and P acquiring activities. The objectives of the current study are to compare insights offered by these three levels of interpretation, and to compare different mathematical methods of analyzing the activities of multiple enzymes.

2. Methods

We examined the data presented by Sinsabaugh et al. (2008) and Hill et al. (2009) because both data sets were large, included observations from many sites, and provided thorough discussions of factors explaining the distributions of these data. We also examined a field study of litter decay by Sinsabaugh et al. (2002) to evaluate temporal patterns in enzyme activities during the decomposition process. Our goals herein were not to explain the relationships between environmental factors or litter quality characteristics and enzyme activities, but to focus on relationships between- and interpretations of these activities, including evaluations of their relative strengths and weaknesses. We calculated the activity vector lengths and angles for all data using Microsoft Excel (Office for Mac 2011), based on (1) untransformed activity ratios (e.g., BG/AP), (2) log-transformed ratios (e.g., ln(BG)/ln[AP]), (3) untransformed proportional activities (e.g., BG/[BG + AP]), (4) arcsine-transformed proportions (e.g., asin[BG/(BG + AP)]), (5) arcsine-square-root transformed proportions (e.g., asin[sqrt(BG/[BG + AP])]), and (6) logit transformed proportions (e.g., ln(p/[1−p]) where p = BG/[BG + AP] or BG/[BG + NAG + LAP]). Vector length was calculated as the square root of the sum of the squared values of x and y, where x represents the relative C vs. P acquiring enzyme activities and y represents the relative C vs. N acquiring activities (Moorhead et al., 2013):

$$\text{Length} = \text{SQRT}\left(x^2 + y^2\right) \quad (1)$$

The angle of the vector was calculated as the arctangent of the line extending from the plot origin to point (x, y):