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Supplement of

Soil microbial nutrient constraints along a tropical forest elevation gradient: a belowground test of a biogeochemical paradigm

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Supplement

Enzyme activity as an indirect assessment of microbial nutrient demand

Our indirect assessments of microbial nutrient demand assume that substrate availability is the major influence on variation in enzyme activity. Our data support this assumption and indicate only minor influences of mean annual temperature, soil moisture, soil physical structure and plant community composition on enzyme activity. First, site MAT alone had a relatively minor direct influence on the comparison of enzyme activities (Fig.4), because the difference in activity between highest and lowest elevation sites (equal to an MAT difference of 18 ^{o}C) was much greater than the effect on activity of an increase of 20 ^{o}C in incubation temperature (Fig. S1). Temperature likely had an indirect influence, however, by affecting plant community composition (Rapp et al., 2012), organic matter inputs to soil and, consequently, substrate availability and microbial community composition (Salinas et al., 2010; Whitaker et al., 2014). Second, soil moisture and diffusional constraints, which have been shown to have strong positive influences on enzyme activities (Steinweg et al., 2012), did not explain the patterns in enzyme activity: enzyme activity decreased and yet soil moisture content increased with elevation (Table 2). Enzyme activity does vary seasonally (Turner and Wright, 2014) and an influence of soil moisture on enzyme activity would be expected during the dry season, but here it was measured during the wet season for all sites when moisture was non-limiting. Third, differences in soil physical properties, due to the transition from mineral to organic surface soils with increasing elevation, can influence enzyme activities. For example, high enzyme activities in clay rich mineral soils could result

from increased sorption of enzymes to clay surfaces; these sorbed enzymes are included in standard assays (Tabatabai, 1994) but may not be biochemically active within the soil matrix (Burns et al., 2013). However, this cannot explain the overall patterns with elevation, which were still present among sites with deep organic horizons (1500 – 3400 m asl). Furthermore, assuming that these effects influence different enzymes similarly, our main conclusions based on the stoichiometry of enzyme activities are unaffected. Finally, the influence of differences in communities of plant roots, which also produce extracellular enzymes, is unlikely to explain the patterns observed in bulk soils here, because the dominant effect of roots on soil enzyme activities occurs in close proximity to root surfaces (Nottingham et al., 2013). Furthermore enzyme activity in bulk soils decreased with elevation but root biomass increased (Girardin et al., 2010). Overall, resource availability appeared to have the greatest direct influence on enzyme activities along this gradient, which has been shown in ecosystems globally (Sinsabaugh et al., 2008).

Supplement Table S1

R^2 values for linear models to determine enzyme activity normalised to site mean annual temperature (MAT); *p* values for linear models are shown where * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Activity at MAT (AT) was determined by AT = (dA/dT) *MAT, where dA/dT is the slope of activity/assay temperature. Values are means with 1 SE (n = 5).

Supplement Table S2

Spearman correlation coefficients between soil properties and stoichiometry of soil nutrients, the microbial biomass and enzyme activities among 13 forest sites.

Supplement Figure S1

Maximum potential enzyme activities (V_{max}) of C (β-glucosidase) N (*N*-acetyl β-glucosaminidase) and P (phosphomonoesterase) - degrading enzymes for 13 sites at elevations ranging from 194 to 3400 m, determined at 10 ^{o}C and at 30 ^{o}C. Values are means with 1 SE (n = 5 replicates, which represents the spatial variation within a 1 ha plot).

Supplement Table S1

Site code	Elevation (m a.s.l.)	β-glucosidase	*N*-acetyl-glucosaminidase	phosphomono-esterase
TAM-06	194	0.75 (0.11) *	0.81 (0.08) *	0.88 (0.01) **
TAM-05	210	0.50 (0.08) *	0.87 (0.10) **	0.87 (0.01) **
VC	1000	0.86 (0.03) **	0.96 (0.02) ***	0.94 (0.01) ***
SPD-2	1500	0.89 (0.03) **	0.97 (0.01) ***	0.96 (0.01) ***
SPD-1	1750	0.68 (0.17) *	0.77 (0.18) *	0.91 (0.02) **
TRU-08	1850	0.76 (0.06) *	0.88 (0.03) **	0.70 (0.04) *
TRU-07	2020	0.68 (0.05) *	0.81 (0.07) *	0.78 (0.05) *
TRU-05	2520	0.54 (0.14) *	0.83 (0.04) **	0.85 (0.06) **
TRU-04	2720	0.65 (0.10) *	0.83 (0.01) **	0.88 (0.02) **
TRU-03	3020	0.72 (0.06) *	0.65 (0.12) *	0.72 (0.08) *
WAY-01	3025	0.72 (0.03) *	0.95 (0.02) ***	0.68 (0.08) *
TRU-02	3200	0.71 (0.03) *	0.77 (0.09) *	0.84 (0.02) **
TRU-01	3400	0.72 (0.13) *	0.88 (0.06) **	0.88 (0.03) **

Supplement Table S2

	Elevation	pH	Tot C	Tot N	Tot P	Tot C:N	Tot C:P	Tot N:P	Ext P	Mic C	Mic C:N	Mic C:P	Mic N:P	C:N-ase	C:P-ase
pH	-0.08	-													
Total C	**0.75**	-0.08	-												
Total N	**0.73**	-0.11	**0.95**	-											
Total P	0.45	0.02	0.43	0.47	-										
Total C:N	**0.73**	-0.06	**0.88**	**0.75**	0.28	-									
Total C:P	**0.65**	-0.13	**0.86**	**0.78**	0.00	**0.88**	-								
Total N:P	**0.52**	-0.20	**0.74**	**0.72**	-0.16	**0.70**	-0.04	-							
Extractable P	**0.84**	0.05	**0.53**	**0.55**	**0.55**	**0.50**	0.35	0.21	-						
Microbial C	**0.67**	0.15	**0.76**	**0.77**	0.47	**0.71**	**0.62**	0.49	**0.53**	-					
Microbial C:N	0.14	0.07	-0.01	0.00	-0.19	0.01	0.04	0.01	0.09	0.12	-				
Microbial C:P	0.23	0.19	0.16	0.21	0.01	0.06	0.08	0.11	0.11	0.37	0.25	-			
Microbial N:P	0.00	0.07	0.04	0.08	0.08	-0.01	-0.04	0.02	-0.09	0.13	**-0.52**	**0.58**	-		
C:N-ase	0.11	-0.08	0.22	0.15	0.16	0.30	0.19	0.12	0.04	0.14	-0.19	-0.20	-0.02	-	
C:P-ase	**0.54**	0.07	0.48	0.41	**0.50**	0.45	0.24	0.09	**0.50**	**0.50**	0.10	0.16	-0.08	0.32	-
N:P-ase	0.42	0.18	0.37	0.36	0.47	0.24	0.11	-0.01	0.44	0.39	0.21	0.28	-0.06	-0.35	**0.72**