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4 tables and 5 figures (2 tables and 1 figure in supplementary materials)

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

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Abstract

Aboveground primary productivity is widely considered to be limited by phosphorus (P) availability in lowland tropical forests and by nitrogen (N) availability in montane tropical forests. However, the extent to which this paradigm applies to belowground processes remains unresolved. We measured indices of soil microbial nutrient status in lowland, sub-montane and montane tropical forests along a natural gradient spanning 3400 m in elevation in the Peruvian Andes. With increasing elevation there were marked increases in soil concentrations of total N, total P, and readily-extractable P, but a decrease in N mineralization determined by *in situ* resin bags. Microbial carbon (C) and N increased with increasing elevation, but microbial C:N:P ratios were relatively constant, suggesting homeostasis. The activity of hydrolytic enzymes, which are rich in N, decreased with increasing elevation, while the ratios of enzymes involved in the acquisition of N and P increased with increasing elevation, further indicating a shift in the relative demand for N and P by microbial biomass. We conclude that soil microorganisms shift investment in nutrient acquisition from P to N between lowland and montane tropical forests, suggesting that different nutrients regulate soil microbial metabolism and the soil carbon balance in these ecosystems.

1. Introduction

Tropical forests have a major influence on the global carbon (C) cycle, being the most productive ecosystems on Earth and containing 34–55% of the C in forests worldwide (Beer et al., 2010; Pan et al., 2011). The exchange of C between the atmosphere and forests is mediated by the availability of mineral nutrients, so there is widespread interest in understanding how plant or microbial metabolic processes are constrained by the deficiencies of specific 'limiting' nutrients (Cleveland et al., 2011; Wright et al., 2011), and how human alteration of these nutrient cycles may impact tropical ecosystems (Hietz et al., 2011; Townsend et al., 2011). Our understanding of nutrient limitation to the tropical forest C cycle is based largely on the responses of aboveground production. In contrast, belowground processes remain relatively under-studied, despite evidence that belowground processes are limited by different nutrients to those limiting aboveground productivity in some ecosystems, including tropical forests (Sundareshwar et al., 2003; Turner and Wright, 2014). It is important to identify nutrient constraints to soil microbial process in tropical forests to understand how anthropogenic alteration of biogeochemical cycles will impact C storage in these ecosystems.

Primary productivity is commonly constrained by nitrogen (N) and phosphorus (P) availability in ecosystems globally (Elser et al., 2007). In lowland tropical forests, primary productivity is widely considered to be limited by P availability (Reed et al., 2011; Vitousek et al., 2010), in part because lowland forests are dominated by strongly weathered soils that contain low concentrations of biologically available P and high apparent N availability (Hedin et al., 2009; Reed et al., 2011). In contrast, primary productivity in tropical montane forests is often considered to be limited by the availability of N rather than P (Tanner et al., 1998). This is because soil P depletion in montane environments is countered by the actions of tectonic uplift, erosion and landslide activity (Porder and Hilley, 2010), while N inputs via litter mineralization and biological N fixation can be

reduced by low temperatures and fewer legumes (Bruijnzeel et al., 2011). Overall, these processes appear to reinforce the pattern of P deficiency in lowland forests and N deficiency in montane tropical ecosystems.

The notion that there is switch from predominantly P to N limitation of primary productivity between lowland and montane tropical forests is supported by experimental studies in forest communities (Tanner et al., 1998). Also, the widespread existence of P-limitation of primary production in lowland tropical forests (Hedin et al., 2009; Vitousek and Sanford, 1986) is supported by studies in which P fertilization increased the growth of trees and seedlings (Alvarez-Clare et al., 2013) and increased litter production (Mirmanto et al., 1999; Wright et al., 2011). Although co-limitation by N, P and K of seedling and sapling growth (Santiago et al., 2012; Wright et al., 2011) and N and P co-limitation of tree growth (Fisher et al., 2013) have also been reported. In contrast, above-ground productivity in montane forests appears to be constrained primarily by N, based on responses to N fertilization in growth rates and litter production (Fisher et al., 2013; Tanner et al., 1992).

It remains unclear whether the pattern of nutrient limitation in montane and lowland forests holds for belowground organisms as it does for plants. The activity of heterotrophic soil microbes is primarily limited by the availability of labile C, but N and P exert important constraints (Wardle, 1992). In lowland tropical forests, there is evidence to suggest that P limits microbial growth (Turner and Wright, 2014) and microbial C mineralization during decomposition (Cleveland et al., 2006; Kaspari et al., 2008), although other nutrients can also limit soil microbial processes (Hattenschwiler et al., 2011; Kaspari et al., 2009; Waring, 2012). In contrast, studies in tropical montane forest have shown a stimulation of soil microbial biomass or respiration by N fertilization (Corre et al., 2010; Cusack et al., 2011b; Fisher et al., 2013; Li et al., 2006), although conclusions remain tentative because many of these montane forest experiments included N additions but not

P or K. For example, high phosphatase activity in one of these studies suggests potential P limitation of the microbial community in a lower montane forest (Cusack et al., 2011b). We therefore lack conclusive evidence to demonstrate the extent to which soil microbial processes are constrained by nutrients across gradients of tropical lowland and montane forests.

Soil microbial nutrient limitation is often experimentally defined as a response of microbial growth, metabolism or respiration to nutrient addition (e.g. Cleveland et al., 2006; Cusack et al., 2011b; Turner and Wright, 2014). However, the establishment of fertilization experiments at multiple sites and across large environmental gradients is challenging. An alternative approach, more easily replicated across multiple sites, is the indirect assessment of nutrient limitation by measuring the stoichiometry of nutrients in organisms (Vitousek et al., 2010). Nutrient limitation to plant growth in tropical forests has, for example, been inferred from measurements of nutrient stoichiometry in fresh leaves and litterfall (McGroddy et al., 2004; Vitousek and Sanford, 1986). Elemental stoichiometry can similarly be used to indirectly assess nutrient limitations to microbial C metabolism by evaluating the stoichiometry of nutrients in the soil microbial biomass (Cleveland and Liptzin, 2007). The consistent amounts of N and P required to build and maintain different cellular structures gives rise to the hypothesis that, under optimal growth conditions, the C:N:P ratio in organisms is constrained, while a limiting resource supply will be reflected in an altered C:N:P ratio (Elser et al., 2003; Redfield, 1958). Elemental stoichiometry within organisms can indicate a growth limiting resource, provided that the elemental composition of the organism is non-homeostatic (passive regulation; elemental composition reflects resource availability) rather than homeostatic (active regulation; fixed elemental composition) (Sterner and Elser, 2002).

The stoichiometry of enzyme activities can provide further indirect evidence of nutrient limitations to microbial C metabolism by indicating investment into resource acquisition (Sinsabaugh et al., 2008). The activities of enzymes involved in nutrient degradation indicate the

allocation of microbial resources to the acquisition of specific nutrients, which is often in response to a deficiency of the mineral form of that nutrient (Allison et al., 2010; Sinsabaugh and Moorhead, 1994). For example, deficiencies in soil N or P are reflected by higher activity of *N*-acetyl β-glucosaminidase or phosphomonoesterase, respectively (Allison et al., 2007; Olander and Vitousek, 2000; Sinsabaugh and Moorhead, 1994; Treseder and Vitousek, 2001). A deficiency in soil N can also reduce the activity of enzymes in general, because proteins are rich in N (Allison and Vitousek, 2005; Allison et al., 2010). The activity and stoichiometry of nutrient-degrading enzymes can therefore indicate the relative strength and nature of microbial nutrient demand.

We tested the hypothesis that the nutrient status of the soil microbial biomass switches from greater relative demand for P in lowland tropical forest to greater relative demand for N in montane tropical forest. To do this, we measured soil nutrient availability, soil microbial nutrient stoichiometry, and the activity and stoichiometry of soil enzymes along a 3400 m elevation gradient under tropical forest in the Peruvian Andes. We estimated microbial nutrient status using three approaches. First, we determined soil N and P availability along the gradient. Second, we assessed relative differences in the stoichiometry of C, N and P in the microbial biomass, whereby a greater C-to-nutrient ratio indicates increased limitation to microbial growth. Third, we determined the relative differences in the stoichiometry of C, N and P- degrading enzyme activities, whereby a decreased C-to-nutrient enzymatic ratio indicates increased nutrient limitation to microbial metabolism and microbial investment into enzymes for acquisition of that nutrient. This approach allowed indirect assessment of microbial nutrient limitation across a large geographic gradient, but was limited by the assumption that nutrient limitation to microbial growth and metabolism is the sole constraint on elemental stoichiometry in the microbial biomass (assuming non-homeostasis) and on the stoichiometry of enzyme activities. We hypothesized that increasing P availability and decreasing N availability with increasing elevation would lead to changes in indicators of microbial

nutrient stress, including: (1) increased concentrations of extractable inorganic phosphate, but decreased concentrations of N turnover; (2) decreased N:P ratio in the soil microbial biomass; (3) increased activity of enzymes involved in the degradation of compounds containing N relative to P (increased N:P enzymatic ratio); and (4) decreased activities of all enzymes (indicating increasing N limitation).

2. Methods

2.1 Study Sites

We used thirteen study sites situated along an elevation gradient on the Eastern flank of the Peruvian Andes (Whitaker et al., 2014). The sites range in elevation from 194 to 3400 m a.s.l. (above sea level) and have continuous forest cover, which ranges from lowland Amazonian rainforest to upper montane cloud forest. The transect from 1000 to 3400 m a.s.l. is 35 km in length and the two lowland sites are a further 230 km down the valley. Mean annual temperature decreases with increasing elevation (26 to 8 ^{o}C) and mean annual precipitation ranges from 1700 to 3199 mm yr^{-1}, peaking at 1000 m a.s.l. near the base of the mountains, then decreasing with elevation. Although mean annual precipitation is generally lower at higher elevation (Table 1), evidence to date indicates that soils at higher elevation are rarely moisture limited over the seasonal cycle, due in part to limited evapotranspiration and fog deposition within the cloud immersion zone (between 1500 and 3400 m a.s.l.) (van de Weg et al., 2014; van de Weg et al., 2009; Zimmermann et al., 2010).

The sites are situated predominantly on Paleozoic (~450 Ma) meta-sedimentary mudstones, with plutonic intrusions (granite) underlying the sites between 1500 and 2020 m a.s.l. (Carlotto et al., 1996; Clark et al., 2013). The soils at sites above 2520 m have been classified as Umbrisols

according to FAO World Reference Base classification (Inceptisols according to USDA Soil Taxonomy). In contrast, the soils from 1000 to 2020 m have been classified as Cambisols (Inceptisols) and the soils at the two lowland sites have been classified as Haplic Allisols (Ultisols) (194 m a.s.l.) and Haplic Cambisols (Inceptisols) (210 m a.s.l.) (Quesada et al., 2010). The soils at higher elevations are shallower and have a deeper organic layer (e.g. 22.8 cm at the 3030 m a.s.l. site compared to 0.7 cm at the 194 m a.s.l. site; Table 1). Further descriptions of the soils (Quesada et al., 2010; Whitaker et al., 2014), climate (Rapp and Silman, 2012), aboveground productivity and floristic composition (Asner et al., 2013; Feeley et al., 2011; Girardin et al., 2010) are reported elsewhere.

2.2 Soil sampling and analyses for total nutrients

Soils were sampled in December 2010 from five systematically distributed sub-plots within a 1 ha permanent sample plot at each study site at a standardized 0-10 cm depth. For each sub-plot, soil was removed from a 40 × 40 cm area. Soils were sealed in plastic bags and stored at 4 ^{o}C for up to 4 weeks until analysis. Given that temperature does not seasonally vary in our study sites, any seasonal variation in our measured soil and microbial properties would most likely be driven by seasonality of rainfall (Turner and Wright, 2014). However, December is in the rainy season for all of these sites (Rapp and Silman, 2012), therefore our assessments were made during a relatively constant period of active decomposition when moisture was not limiting. Furthermore, soil moisture measurements have shown that none of the sites appear to suffer significant seasonal moisture stress (Zimmermann et al., 2010), suggesting that our sampling is representative of the prevailing conditions at other times of the year.

Total C and N were determined on dried (at 105 ^{o}C) and ground soil samples using a TruSpec CN Elemental Analyzer (LECO, USA). Total P was determined by ignition (550 °C, 1 h) followed by

193 extraction in 1 M H_2SO_4, with phosphate detection in neutralised extracts at 880 nm by automated
194 molybdate colorimetry using a Lachat Quikchem 8500 (Hach Ltd, Loveland, CO). Soil pH was
195 determined in water in a 1:2 soil to solution ratio using a calibrated glass electrode. Bulk density
196 was determined by drying a known volume of soil (taken in a cylinder) for 24 h at 105 ^{o}C to constant
197 mass. Gravimetric moisture content at the time of sampling and water holding capacity (in
198 saturated soils) were calculated according to the amount of water remaining in the soil after being
199 left to drain for 12 hours (Whitaker et al., 2014).
200
201 ***2.3 Soil microbial biomass and extractable nutrients***
202
203 Soil microbial biomass C and N were measured by fumigation-extraction (Brookes et al.,
204 1985; Vance et al., 1987), using ethanol-free chloroform as the fumigant followed by extraction
205 with potassium sulphate (K_2SO_4). Extracts of fumigated and unfumigated soil were analyzed for
206 extractable organic C using a Shimadzu 5000A TOC analyzer (Shimadzu, Milton Keynes, UK). The
207 extracts were analysed for microbial biomass N by colorimetry on a continuous flow stream
208 autoanalyzer (Bran and Luebbe, Northampton, UK), following oxidation with potassium persulphate
209 ($K_2S_2O_8$), by mixing 1.5 ml filtrate with 4.5 ml of 0.165 M $K_2S_2O_8$ then autoclaving for 30 min at 121
210 ^{o}C (Ross, 1992). Microbial C and N were calculated as the difference in the respective nutrient
211 between fumigated and unfumigated extracts, and corrected for unrecovered biomass using *k*
212 factors of 0.35 for microbial C (Sparling et al., 1990) and 0.54 for microbial N (Brookes et al., 1985).
213 Readily-exchangeable phosphate (extractable P) and microbial biomass P were determined
214 by hexanol fumigation and extraction with anion-exchange membranes (Kouno et al., 1995).
215 Phosphate was recovered from anion-exchange membranes by shaking for 1 h in 50 ml of 0.25 M
216 H_2SO_4, with detection in the acid solution by automated molybdate colorimetry using a Lachat

217 Quikchem 8500 (Hach Ltd, Loveland, CO, USA). Extractable P was determined on unfumigated
218 samples and microbial P was calculated as the difference between the fumigated and unfumigated
219 samples, with correction for unrecovered biomass using a k_p factor of 0.4 (Jenkinson et al., 2004).
220 Nitrogen mineralization was derived by extraction with *in situ* cation and anion-exchange
221 resins (Templer et al., 2005). We used the resin-bag method to determine extractable NH_4 and NO_3
222 because standard methods of extraction of NH_4 and NO_3 from soils (e.g. with KCl) should be
223 performed within 24 hours of soils sampling (Turner and Romero, 2009), which was not possible
224 given the remote location of these sites. The remote location of sites also meant we were only able
225 to determine mineralized N in five of the 14 plots, which were distributed across the gradient (210,
226 1000, 1500, 1750, 3025 m a.s.l.). Mixed-bed cation/anion exchange resin were placed inside nylon
227 bags (4 g resin in each) and installed at 10 cm soil depth in systematically distributed locations in
228 each 1 ha plot (n = 15). Resin bags were deployed for one month during November-December 2011
229 and stored at room temperature until extraction. Resin bags were shipped to the University of
230 Aberdeen, UK, extracted using 2 M KCl (Templer et al. 2005) and concentrations of NH_4 and NO_3
231 determined colorimetrically using a Burkard SFA2 continuous-flow analyzer (Burkard Scientific Ltd.,
232 Uxbridge, UK). Extractable NH_4 and NO_3 (total mineralized N) were calculated from the difference
233 between extracted N from resin deployed in the field and resin not deployed (blanks) and
234 expressed as extractable NH_4-N and NO_3-N per g resin per day.
235
236 ***2.4 Soil enzymes***
237 Three enzymes involved in C, N and P cycling were measured using microplate fluorimetric
238 assays with 200 µM methylumbelliferone (MU)-linked substrates as described in Turner and
239 Romero (2010): β-glucosidase (degradation of β-1,4-glycosidic bonds between glucose molecules),
240 *N*-acetyl β-glucosaminidase (degradation of *N*-glycosidic bonds in chitin), and

241 phosphomonoesterase (degradation of monoester-linked simple organic phosphates). The activities
242 of these three enzymes have been used to indicate the stoichiometry of microbial C, N and P
243 nutrition in global ecosystems (Sinsabaugh et al., 2008). For each soil sample, five replicate micro-
244 plates were prepared and incubated at 2, 10, 22, 30 and 40 ^{o}C respectively for each enzyme, to
245 allow calculation of enzyme activity at mean annual temperature for each site.

246 For the fluorimetric assays, 2 g soil (dry weight basis) was added to 200 ml of 1 mM NaN_3
247 solution and dispersed by stirring on a magnetic stir plate. After 5 min and while stirring, 50 µl
248 aliquots of soil suspension were removed using an 8-channel pipette and dispensed into a 96-well
249 microplate containing 50 µl modified universal buffer solution (Tabatabai, 1994) adjusted to pH 4
250 (approximately equivalent to soil pH in all sites; Table 1). Each microplate included assay wells (soil
251 solution plus 100 µl MU substrate), blank wells (soil solution plus 100 µl of 1 mM NaN_3) and quench
252 wells (soil solution plus 100 µl MU standard). For a sub-set of samples we measured enzyme
253 activities using substrate concentrations ranging from 10 – 1000 µl MU to check that the substrate
254 remained in excess at the end of the incubation in our main analyses. A further control plate was
255 prepared with the MU substrates and standards with no soil solution to determine fluorescence
256 from substrates and quenching by soil solution in assay plates. There were eight analytical replicate
257 wells for each assay. Microplates were incubated at each specified temperature in the range 2, 10,
258 20, 30 and 40 ^{o}C for a time period of approximately 4, 3, 2, 1.5 and 1 hour(s), respectively.
259 Following incubation, 50 µl of 0.5 M NaOH was added to terminate the reaction and plates were
260 immediately analyzed on a Fluostar Optima spectrofluorometer (BMG Labtech, Offenburg,
261 Germany) with excitation at 360 nm and emission at 450 nm.

262

263 ***2.5 Calculations and statistics***

264

265 *2.5.1 The stoichiometry of enzyme activities and microbial biomass*

266 Enzyme activities were expressed on the basis of soil organic C (nmol MU g C^{-1} min^{-1}), to
267 allow for direct comparisons among our sites with widely different organic C concentrations.
268 Enzyme activities were determined at standard temperatures (2, 10, 20, 30 and 40 ^{o}C) and
269 calculated for the mean annual temperature at each site (Table 1) by fitting a linear model of
270 activity vs. assay temperature. Hydrolytic enzyme activities, determined using MU substrates, were
271 expressed in nmol MU g C^{-1} min^{-1}. We determined ratios of C, N and P degrading enzymes to detect
272 relative differences in N and P limitations to microbial activity between the sites (Sinsabaugh et al.,
273 2008). Enzyme activity ratios for C:N, C:P and N:P were determined, where C = β-glucosidase, N =
274 *N*-acetyl β-glucosaminidase and P = phosphomonoesterase. Microbial C, N and P and their
275 elemental ratios were expressed as molar values (mmol kg^{-1}), which allowed direct comparison of
276 values with a global meta-analysis (Cleveland and Liptzin, 2007).

277 The indirect assessment of microbial nutrient demand according to variation in enzyme
278 activity requires the assumption that substrate availability is the major influence on variation in
279 enzyme activity, rather than mean annual temperature, soil moisture, soil physical structure and
280 plant community composition. This assumption is supported by our data and elsewhere in the
281 literature (Sinsabaugh et al., 2008) (see supplementary materials for further discussion).

282 Changes in soil properties and enzyme activities with elevation were analysed using one-
283 way ANOVA, with 'elevation' as the factor and 'soil properties' or 'enzyme activities' as the
284 response variable. Further effects of elevation on soil properties, enzyme activities and enzyme
285 ratios were examined using linear models with soil property, microbial ratio or enzyme
286 activity/ratio as the response variable and elevation as the predictive variable.
287 To account for the variability along the transect in organic horizon depth and parent material,
288 which may have confounding influences on microbial nutrient cycling, we further examined the

effects of elevation on microbial and enzymatic elemental ratios among sites where organic horizon only was sampled (1500 – 3400 m) and among sites on constant parent material (sites on Paleozoic shales-slates; 2020 – 3400 m). Pair-wise comparisons were performed using Tukey post-hoc analyses. Correlations among normally distributed soil properties and enzyme activities were examined using Spearman's correlations. Data were log-transformed when model residuals were non-normally distributed. Significant interactions were determined at $p \leq 0.05$. All statistical analyses were performed using R version 2.15 (R Development Core Team 2012).

3. Results

3.1 Soil carbon and nutrients

Total soil C, N and P concentrations all increased with elevation across all sites (Fig. 1, Table 2; total C and N: $p \leq 0.001$; total P: $p = 0.05$). Total C ranged from 1.70% (at 220 m a.s.l.) to 46.54% (at 3030 m a.s.l.), total N ranged from 0.35% (at 194 m a.s.l.) to 2.49% (at 3400 m a.s.l.) and total P ranged from 0.18 mg g^{-1} (210 m a.s.l.) to 1.44 mg g^{-1} (1750 m a.s.l.). The increase in C was relatively greater than for N or P, resulting in increased C:N (ranging from 6.7 to 19.6) and C:P ratios (ranging from 49 to 521) with elevation (Figs. 1-2; Table 2). Similarly, the increase in total N was relatively greater than the increase in total P, resulting in increased N:P ratios with elevation (ranging from 6.7 to 28.2) (Figs. 1-2). Ratios of C:N, C:P and N:P increased significantly with elevation ($p < 0.001$, $p < 0.01$, $p < 0.05$, respectively), although with higher variation for C:P and N:P than C:N (Fig. 2). Total soil C and N across all sites were closely correlated ($R^2 = 0.93$, $p < 0.001$), in contrast to marginal relationships between total C and P ($R^2 = 0.07$, $p < 0.05$) and total N and P ($R^2 = 0.10$, $p = 0.01$) (Fig. 1). Soil pH ranged from 3.8 to 4.6 among sites, but did not vary significantly with elevation (Table 2).

There were major contrasts in the concentrations of total mineralized N and extractable PO_4 with elevation (Table 2, Fig. 3). Mineralized NO_3 decreased with elevation ($p < 0.001$) from 24.21 µg N g resin^{-1} d^{-1} (210 m a.s.l.) to 0.33 µg N g resin^{-1} d^{-1} (1750 m a.s.l.), whereas mineralized NH_4 increased with elevation ($p < 0.01$). However, total mineralized N (NO_3+NH_4) decreased with elevation ($R^2 = 0.61$, $p < 0.001$; Fig. 3). In contrast, extractable PO_4 increased with elevation ($p < 0.001$) from 0.7 mg P kg^{-1} (at 1000 m a.s.l.) to 223.5 mg P kg^{-1} (at 3400 m a.s.l.) (Table 2; Fig. 3).

3.2 Soil microbial nutrients and C:N:P ratios

Soil microbial C, N and P all increased with elevation and ranged ten-fold among sites (Table 3), which approximately corresponded with the increase in organic matter and soil C with elevation (Table 2). The increase was linear and highly significant for microbial C ($R^2 = 0.61$, $p < 0.01$) and microbial N ($R^2 = 0.35$, $p < 0.05$), but not for microbial P ($R^2 = 0.16$, $p = 0.18$), which peaked in mid-elevation sites (1850, 2020 m a.s.l.). Microbial C and N were closely correlated among all sites ($R^2 = 0.62$, $p < 0.001$), in contrast to the less well constrained relationships between microbial C and microbial P ($R^2 = 0.24$, $p < 0.001$), and microbial N and microbial P ($R^2 = 0.22$, $p < 0.001$) (Fig. 1).

Despite the large differences in microbial nutrients, ratios of microbial C:N, C:P and N:P did not vary with elevation across the entire transect ($R^2 = 0.04$, $p = 0.51$; $R^2 = 0.07$, $p = 0.39$; $R^2 < 0.01$, $p = 0.77$) (Table 3; Fig. 2). However, among sites where only organic horizons were sampled, there was a slight increase in microbial C:N and N:P ratios with elevation, and a greater increase for microbial C:P (Table 4). Similarly, among sites on the same parent material there was an elevation-related increase in microbial C:P (Table 4).

3.3 Enzyme activities

All enzyme activities decreased significantly with elevation, when determined at standard assay temperature ($p < 0.001$ for all comparisons; Figure S1 for activity determined at assay temperatures 10 ^{o}C and 30 ^{o}C) and when determined at the mean annual temperature for each site (Fig. 4). To determine enzyme activity at the site mean annual temperature we used linear models of enzyme activity against assay temperature; all of the 42 models (for 3 enzymes and 13 sites) were significant ($p < 0.05$) and the average R^2 of all fitted models was 0.80 (SE = 0.01, $n = 39$; Table S1). After accounting for differences in soil C content among sites, enzyme activities decreased approximately 100-fold with elevation (Fig. 4). The largest decline in enzyme activity with elevation was for phosphomonoesterase and the smallest decline was for *N*-acetyl β-glucosaminidase (Fig. 4; note log scale for enzyme activity).

The enzymatic C:P and N:P ratios increased with elevation (Fig. 5), but not for C:N. The relatively large decrease in phosphomonoesterase activity with elevation compared to other enzymes was reflected by increasing ratios for enzymatic C:P ($R^2 = 0.18$, $p < 0.001$) and N:P ($R^2 = 0.13$, $p < 0.01$) but not for C:N ($R^2 = 0.04$, $p = 0.13$) (Fig. 5). Among sites where only the organic horizon was sampled the pattern of an elevation related increase for enzymatic C:P and N:P, but not C:N, was also observed (Table 4). Among sites on constant parent material, there was an elevation-related increase for enzymatic N:P and a marginal increase for enzymatic C:P (Table 4).

4. Discussion

It has been proposed that tropical forest elevation gradients are gradients of nutrient limitation to plant productivity, with P-limitation prevalent in lowland forests (Vitousek and Sanford, 1986) and N-limitation prevalent in montane forests (Tanner et al., 1998). The major drivers of this shift are considered to be differences in soil nutrient availability along elevation

gradients, caused by changes in rates of soil weathering and turnover, and temperature constraints on decomposition and biological N fixation (Hedin et al., 2009; Reed et al., 2011; Tanner et al., 1998). Therefore, it is reasonable to hypothesize that soil microbial processes are constrained by N and P in the same manner, which is supported for some lowland (Cleveland et al., 2002; Turner and Wright, 2014) and montane tropical forests sites (Corre et al., 2010; Cusack et al., 2011a). Our findings from a 3400 m tropical forest elevation gradient in the Peruvian Andes provide evidence that this paradigm also applies to soil microorganisms, with a gradual transition in investment into nutrient acquisition from P to N between lowland and montane tropical forests.

Evidence that relative microbial investment in nutrient acquisition shifts from P towards N along a tropical elevation gradient can be inferred from differences in nutrient availability and enzyme activity. An increasing P constraint on microbial metabolism with decreasing elevation is supported by the significantly lower concentrations of total and extractable P in low elevation soils (Table 2). Phosphomonoesterase activity was strongly correlated with extractable P (Fig. 3), suggesting that increased microbial synthesis of phosphatases at lower elevations was a direct response to low available phosphate. This apparent strong P constraint on microbial processes in low elevation forests is consistent with increased rates of litter decomposition (Kaspari et al., 2008), C mineralization (Cleveland and Townsend, 2006), greater microbial biomass and decreased phosphomonoesterase activity (Turner & Wright 2014) following P addition to lowland tropical forests.

Evidence of increasing N constraints on microbial metabolism with increasing elevation included a strong reduction in total mineralized N (the sum of resin NO_3 + NH_4; Table 2, Fig. 3) and increase in the ratio of N:P-degrading enzymes at higher elevations, coupled with an overall decline in the activity of all enzymes, presumably because of the high N requirement for building proteins (Allison et al., 2010; Loladze and Elser, 2011) (Fig. 4). Given that microbial N requirements are

largely determined by the rates of protein synthesis (Loladze and Elser, 2011), there must be a threshold at which N scarcity begins to limit the synthesis of *N*-acetyl β-glucosaminidase and other N-acquiring enzymes (Olander and Vitousek, 2000). Other studies of tropical montane forests, including these sites in Peru, provide evidence that low N availability constrains microbial processes at higher elevation. For example, N-limitation of microbial metabolism was indicated by increased heterotrophic soil CO_2 efflux following N-fertilization at the 3030 m elevation site studied here (Fisher et al., 2013). In other montane tropical forests, N-fertilization stimulated microbial biomass (Corre et al., 2010; Cusack et al., 2011b) and increased the activity of hydrolytic enzymes (Cusack et al., 2011b), which supports our finding of N-limitation of microbial synthesis of hydrolytic enzymes in tropical montane forests (Figs. 3, 5).

In contrast, microbial nutrient ratios did not vary over the entire gradient (Fig. 2), which does not support the hypothesis of a shift in nutrient constraints on microbial biomass from P towards N with increased elevation. There were slight elevation-related increases in microbial C:N and C:P ratios in organic soils (Table 4), which can be explained by increased dominance of the microbial biomass at higher elevation by fungi (Whitaker et al., 2014), which have wider C:nutrient ratios compared to bacteria (Six et al., 2006). The overall pattern of relatively constant elemental ratios in the microbial biomass despite large differences in nutrient availability (Fig. 2) can be explained by microbial stoichiometric homeostasis. Although non-homeostatic patterns have been found in marine, freshwater and terrestrial autotrophs (Elser et al., 2009; Elser et al., 2007; Redfield, 1958), homeostasis of microbial nutrition has been demonstrated in cultured bacteria (Makino et al., 2003) and is supported in field studies and observations of constrained soil microbial elemental ratios across ecosystems worldwide (Cleveland and Liptzin, 2007; Hartman and Richardson, 2013). For example, despite an order-of-magnitude shift in soil P relative to soil N concentrations across the 120,000 year Franz Josef temperate rainforest chronosequence,

408 microbial N:P ratios remained relatively constant throughout the majority of the sequence (5.9 ±
409 0.7, compared to 3.3 ± 0.7 in this study) (Turner et al., 2013), while a decade of nutrient addition
410 had no effect on microbial N:P ratios in lowland tropical forest in Panama (Turner and Wright,
411 2014). The list of possible mechanisms by which heterotrophs maintain homeostasis includes their
412 capacity to alter soil nutrient availability by synthesising extracellular enzymes (Sinsabaugh et al.,
413 2009) and to immobilize large amounts of N and P, resulting in low C:N and C:P ratios compared to
414 total soil nutrients and leaf litter (Cleveland and Liptzin, 2007; McGroddy et al., 2004; Sterner and
415 Elser, 2002; Turner and Wright, 2014). For example, it appears that relatively high microbial P
416 immobilization occurred in these tropical soils, because microbial C:P ratios were low when
417 compared to a global dataset (Fig. 1) (Cleveland and Liptzin, 2007).
418 The major drivers of this shift in microbial investment in nutrient acquisition from P towards
419 N appear to be differences in soil weathering, bedrock turnover and temperature. Evidence for a
420 role of pedogenic processes comes from the consistent pattern of increased enzymatic N:P ratios in
421 sites on the same parent material (Table 4) and the greatest P constraints on the microbial biomass
422 in the strongly-weathered lowland forest soils, which were depleted of primary minerals (e.g.
423 Haplic Allisols relative to Umbrisols) (Quesada et al., 2010; Reed et al., 2011; Vitousek, 1984) (Table
424 2). The significant tectonic uplift in the upper Andes (Garzione et al., 2008), together with
425 significant landslide activity and erosion rates reported for this gradient (Clark et al., 2013) likely
426 decrease P constraints in soils on steeper slopes at high elevation by replenishing P and other rock-
427 derived minerals (Porder and Hilley, 2010). Evidence for a role of low temperatures in promoting N
428 constraints at higher elevation comes from studies suggesting a reduction in biological N fixation
429 and N mineralization in montane forests (Bruijnzeel et al., 2011) (Table 2). Low rates of N
430 mineralization have been reported in montane tropical forests in Costa Rica (Marrs et al., 1988),

Panama (Corre et al., 2010), Hawaii (Hall and Matson, 2003) and Ecuador (Arnold et al., 2009; Wolf et al., 2011).

As with any natural environmental gradient, there are a number of other co-varying factors that may influence our conclusions, including differences in parent material, soil development, rainfall patterns and plant community composition (Körner, 2007). In our study we constrained the co-varying influences of organic soil depth and parent material in separate analyses, showing that they did not influence our main finding of a shift from P to N constraints on microbial acquisition with elevation (Table 4). Although mean annual rainfall does not vary linearly with elevation, it is highest in the lowland forest sites (Table 1), which may have strengthened the weathering rate and leaching of available P from these soils. The interactions between plant communities and soils along this gradient more likely re-enforce the shift in nutrient constraints through feedbacks between plant productivity, leaf litter quality and decomposition rates. For example, lower productivity of montane forest plants (Girardin et al., 2010) with lower leaf N:P ratios (van de Weg et al., 2009, 2014), may further slow decomposition rates and the supply of bioavailable soil N (Wardle et al., 2004).

Our understanding of how nutrients may regulate the C cycle in lowland and montane tropical forest is largely based on the responses of aboveground production, whereas the responses of belowground processes remain relatively unknown. Along a 3400 m elevation transect in the Peruvian Andes we provide evidence to support the hypothesis that soil microbial activity, and by inference heterotrophic decomposition and respiration of organic matter, is predominantly constrained by P in lowland forests but by N in montane forests. Despite these constraints, our results suggest that the microbial biomass is relatively homeostatic with respect to nutrients, given the major changes in N and P availability along the elevation gradient. Extrapolating our findings to other sites requires careful consideration of the multiple factors that influence nutrient availability

455 and co-vary with elevation, including differences in parent material and rainfall. Nevertheless these
456 results have important implications for C cycling in tropical ecosystems, because nutrient
457 constraints are important factors in determining how these ecosystems respond to perturbations in
458 climate, atmospheric CO_2 and nutrient enrichment.
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Table legends

Table 1

Summary of site characteristics along the elevation gradient, spanning lowland rainforest (194-210 m a.s.l.), premontane (1000 m a.s.l.), lower montane (1500-2020 m a.s.l.) and upper montane cloud forest (2520-3400 m a.s.l.) (Aragao et al., 2009; Asner et al., 2013; Clark et al., 2013; Girardin et al., 2010; Quesada et al., 2010). na = data not available.

Table 2

Soil nutrients and pH along the elevation gradient. Linear model results (elevation ~ property) are given at the bottom of the table. Values are means with 1 SE (n = 5).

Table 3

Carbon, nitrogen and phosphorus and their ratios in soil microbial biomass along the elevation gradient. Linear model results (elevation ~ property) are given at the bottom of the table. Values are means with 1 SE (n = 5).

Table 4

Relationships between elevation and microbial (**A**) and enzymatic (**B**) carbon, nitrogen and phosphorus ratios, in organic soils only (sites 1500 – 3400 m a.s.l.) and in soils on constant parent material (sites 2020 – 3400 m a.s.l.). The relationships between elevation and microbial and enzymatic carbon, nitrogen and phosphorus ratios for all sites across the gradient are shown in figures 3 and 5, respectively. Significant relationships are in bold ($p \leq 0.05$).

503 **Figure headings**

504

505 **Figure 1**

506 The stoichiometry of total soil C, N and P, and soil microbial C, N and P (molar ratios). The points are
507 coloured according to the elevation gradient (194 – 3400 m a.s.l.), with darker points for lower
508 elevation sites and lighter points for higher elevation sites. The solid lines are linear regressions
509 between total and microbial elements (model parameters are reported in the top-right of each
510 panel). The shaded areas represent ± 1 SE. The dashed lines represent the stoichimetric scaling
511 between C:N:P from a recent global meta-analysis of forests (212:15:1 in soils and 74:9:1 in
512 microbial biomass) (Cleveland and Liptzin, 2007).

513

514 **Figure 2**

515 The relationships between soil and microbial C:N:P stoichiometry with elevation (194 – 3400 m
516 a.s.l.). Total soil C:N, C:P and N:P all significantly varied with elevation (R^2 = 0.45, 0.36, 0.28,
517 respectively $p < 0.05$; see Table 2). Microbial C:P, C:N and N:P ratios did not vary with elevation.
518 Values are means with 1 SE (n =5 replicates, which represents the spatial variation within a 1 ha
519 plot).

520

521 **Figure 3**

522 The decline in phosphomonoesterase activity and incline in resin-extractable P with elevation; and
523 the decline in *N*-acetyl β-glucosaminidase activity and decline in total mineralized N (NO_3 + NH_4)
524 with elevation. The decline and incline of all properties with elevation were significant. Spearman
525 correlation coefficients are reported in Table 4. Values are means with 1 SE (n =5).

526

Figure 4

Enzyme activities 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 the mean annual temperature (MAT) for each site (Table 1). Enzyme activity at MAT was determined using linear regression of temperature and enzyme activities determined at 2, 10 , 22 and 30 ^{o}C (Table S1). Linear regressions are shown among the forest sites (the grassland site is included in the figure but not in linear model). Values are means with 1 SE (n = 5 replicates, which represents the spatial variation within a 1 ha plot).

Figure 5

The stoichiometry of C (β-glucosidase), N (*N*-acetyl β-glucosaminidase) and P (phosphomonoesterase) - degrading enzyme activity along a tropical forest 3400 m elevation gradient. Enzymes activities were determined at the mean annual temperature for each site. Linear models (including all 13 sites) explained the variation in enzymatic ratios with elevation for C:P_{en} (R^2 = 0.18, p <0.001) and N:P_{en} (R^2 = 0.13, p <0.01), but not C:N_{en} (R^2 = 0.04, p =0.13). Values are means with 1 SE (n =5).

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Table 1 Summary of site characteristics along the elevation gradient, spanning lowland rainforest (194-210 m a.s.l.), premontane (1000 m a.s.l.), lower montane (1500-2020 m a.s.l.) and upper montane cloud forest (2520-3400 m a.s.l.) (Aragao et al., 2009; Asner et al., 2013; Clark et al., 2013; Girardin et al., 2010; Quesada et al., 2010). na = data not available.

Site name	Site code	Elevation (m a.s.l.)	Lat	Long	Mean annual temp (^{o}C)	Annual precipita-tion (mm yr^{-1})	Soil organic horizon (cm)	Aspect (deg)	Slope (deg)	Parent material	Soil classification
Explorer's Inn plot 4 (TP4)	TAM-06	194	-12.839	-69.296	26.4	2730	1	169.4	4	Holocene alluvial terrace	Haplic Alisol
Explorer's Inn plot 3 (TP3)	TAM-05	210	-12.830	- 69.271	26.4	3199	2	186.2	6.9	Pleistocene alluvial terrace	Haplic Cambisol
Villa Carmen	VC	1000	-12.866	-71.401	20.7 ± 0.02	3087	4	na	na	na	na
San Pedro 2	SPD-2	1500	-13.049	- 71.537	17.4 ± 1.5	2631	16	143.5	39	Plutonic intrusion (granite)	Cambisol
San Pedro 1	SPD-1	1750	-13.047	- 71.543	15.8 ± 1.3	2631	10	141.9	40.1	Plutonic intrusion (granite)	Cambisol
Trocha Union 8	TRU-08	1850	-13.071	-71.555	16.0 ± 1.3	2472	16	137.0	41.8	Plutonic intrusion (granite)	Cambisol
Trocha Union 7	TRU-07	2020	-13.074	-71.559	14.9 ± 1.1	1827	17	na	na	Paleozoic shales-slates /Granite intrusion	Cambisol
Trocha Union 5	TRU-05	2520	-13.094	-71.574	12.1 ± 1.0	Na	14	na	na	Paleozoic shales-slates	na
Trocha Union 4	TRU-04	2720	-13.107	- 71.589	11.1 ± 1.0	2318	21	189.8	28.6	Paleozoic shales-slates	Umbrisol
Trocha Union 3	TRU-03	3020	-13.109	-71.600	9.5 ± 1.0	1776	17	129.3	37.6	Paleozoic shales-slates	Umbrisol
Wayqecha	WAY-01	3025	-13.190	-71.587	11.1 ± 1.2	1706	23	na	na	Paleozoic shales-slates	Umbrisol
Trocha Union 2	TRU-02	3200	-13.111	-71.604	8.9 ± 1.0	Na	12	na	na	Paleozoic shales-slates	Umbrisol
Trocha Union 1	TRU-01	3400	-13.114	-71.607	7.7 ± 1.1	2555	14	144.3	34.3	Paleozoic shales-	Umbrisol

Table 2

Soil nutrients and pH along the elevation gradient. Linear model results (elevation ~ property) are given at the bottom of the table. Values are means with 1 SE (n = 5).

Site code	elevation (m a.s.l.)	Total C (%)	Total N (%)	Total P (mg P g^{-1})	Total C:N	Total C:P	Total N:P	Resin NO_3 (µg N g^{-1} d^{-1})	Resin NH_4 (µg N g^{-1} d^{-1})	Extractable PO_4 (mg P kg^{-1})	Soil pH
TAM-06	194	2.38 (0.32)	0.35 (0.03)	0.49 (0.07)	6.7	48.6	7.1	-	-	3.3 (0.8)	4.6 (0.1)
TAM-05	210	1.70 (0.25)	0.23 (0.03)	0.18 (0.03)	7.1	94.4	12.8	24.21 (2.94)	3.38 (0.45)	2.7 (0.2)	3.8 (0.1)
VC	1000	16.2 (1.6)	1.34 (0.12)	0.73 (0.05)	11.5	222.3	18.4	14.25 (1.94)	9.64 (1.23)	0.7 (0.1)	3.8 (0.1)
SPD-2	1500	10.3 (1.8)	0.91 (0.12)	1.36 (0.37)	11.2	76.0	6.7	14.11 (3.22)	13.06 (0.68)	44.7 (20.1)	4.0 (0.1)
SPD-1	1750	26.0 (10.0)	1.56 (0.50)	1.44 (0.09)	14.7	180.3	10.8	0.33 (0.08)	13.91 (1.02)	19.0 (3.0)	3.9 (0.1)
TRU-08	1850	31.1 (4.6)	1.86 (0.21)	0.76 (0.06)	16.5	409.2	24.5	-	-	14.4 (3.7)	3.9 (0.1)
TRU-07	2020	37.0 (4.8)	2.00 (0.24)	0.71 (0.10)	18.6	520.6	28.2	-	-	16.3 (4.7)	4.0 (0.1)
TRU-05	2520	25.8 (5.7)	1.73 (0.34)	0.98 (0.14)	14.7	263.6	17.7	-	-	53.1 (8.6)	3.9 (0.1)
TRU-04	2720	28.6 (5.0)	1.64 (0.25)	0.87 (0.19)	17.0	329.0	18.9	-	-	56.0 (12.8)	3.9 (0.1)
TRU-03	3020	27.1 (5.5)	1.57 (0.21)	0.92 (0.13)	16.6	294.6	17.1	-	-	59.7 (20.7	3.8 (0.1)
WAY-01	3025	46.5 (2.1)	2.39 (0.12)	1.09 (0.08)	19.6	427.0	21.9	0.47 (0.21)	11.87 (0.88)	82.0 (23.3)	4.1 (0.1)
TRU-02	3200	44.8 (1.8)	2.42 (0.20)	0.91 (0.02)	18.9	492.6	26.6	-	-	72.8 (12.9)	4.1 (0.7)
TRU-01	3400	42.1 (3.1)	2.49 (0.17)	1.09 (0.09)	17.0	386.1	22.9	-	-	223.5 (33.0)	4.0 (0.2)
	R^2	0.79	0.80	0.30	0.80	0.55	0.38	0.73	0.12	0.51	0.13
	F	40.76	42.98	4.61	45.21	13.27	6.75	154.92	8.06	11.3	1.66
	P	<0.001	<0.001	0.05	<0.001	<0.01	0.03	<0.001	<0.01	<0.01	0.22

Table 3

Carbon, nitrogen and phosphorus and their ratios in soil microbial biomass along the elevation gradient. Linear model results (elevation ~ property) are given at the bottom of the table. Values are means with 1 SE (n = 5).

Site code	Elevation (m a.s.l.)	Microbial C (mmol kg^{-1})		Microbial N (mmol kg^{-1})		Microbial P (mmol kg^{-1})		Microbial C:N		Microbial C:P		Microbial N:P	
TAM-06	194	32.1	(3.5)	2.6	(0.9)	1.18	(0.24)	31.1	(20.1)	33.7	(9.1)	2.9	(1.1)
TAM-05	210	20.5	(3.3)	2.2	(0.7)	1.40	(0.28)	13.8	(5.1)	21.5	(8.9)	2.6	(1.5)
VC	1000	13.8	(2.2)	8.9	(0.9)	0.94	(0.10)	1.6	(0.2)	16.2	(3.9)	10.1	(1.7)
SPD-2	1500	66.2	(9.8)	11.6	(1.2)	7.50	(1.26)	5.9	(0.8)	11.5	(4.4)	1.8	(0.5)
SPD-1	1750	103.7	(35.4)	18.9	(7.7)	7.98	(1.78)	6.1	(0.9)	18.1	(7.4)	2.8	(0.9)
TRU-08	1850	159.3	(27.7)	21.9	(6.7)	11.26	(0.47)	8.7	(1.8)	13.9	(2.0)	1.9	(0.6)
TRU-07	2020	138.6	(17.5)	21.1	(3.9)	11.89	(0.69)	6.9	(0.9)	11.6	(1.8)	1.8	(0.4)
TRU-05	2520	94.2	(14.9)	16.2	(4.7)	8.37	(0.70)	10.9	(4.8)	11.5	(1.8)	2.0	(0.6)
TRU-04	2720	98.5	(32.8)	13.2	(4.9)	5.87	(1.00)	7.8	(0.7)	23.9	(12.5)	3.2	(1.8)
TRU-03	3020	114.8	(12.3)	16.0	(3.5)	5.74	(0.95)	10.4	(4.2)	22.9	(5.0)	3.3	(1.0)
WAY-01	3025	188.4	(26.7)	26.6	(2.9)	8.81	(1.04)	7.1	(0.8)	24.0	(6.5)	3.4	(0.9)
TRU-02	3200	114.3	(17.0)	7.0	(1.7)	5.85	(0.71)	22.9	(7.1)	20.4	(3.0)	0.9	(0.3)
TRU-01	3400	151.5	(18.8)	17.1	(4.8)	2.71	(0.45)	11.1	(2.4)	62.4	(10.7)	6.5	(1.6)
	R^2	0.61		0.35		0.16		0.04		0.07		<0.01	
	F	16.95		5.88		2.06		0.46		0.80		0.09	
	p	*< 0.01*		*< 0.05*		*0.18*		*0.51*		*0.39*		*0.77*	

Table 4

Relationships between elevation and microbial and enzymatic carbon (C), nitrogen (N) and phosphorus (P) ratios, in organic soils only (sites 1500 – 3400 m a.s.l.) and in soils on constant parent material (sites 2020 – 3400 m a.s.l.). The relationships between elevation and microbial and enzymatic carbon, nitrogen and phosphorus ratios for all sites across the gradient are shown in figures 3 and 5, respectively. Significant relationships are in bold ($p \leq 0.05$).

	Microbial C:N	Microbial C:P	Microbial N:P	Enzymatic C:N	Enzymatic C:P	Enzymatic N:P
1500 – 3400 m	*Constant organic horizon*					
Slope	**28**	**576**	**78**	-142	**730**	**990**
R^2	**0.09**	**0.22**	**0.07**	-0.02	**0.09**	**0.15**
F	**5.70**	**14.33**	**4.63**	0.13	**5.82**	**9.52**
P	**0.02**	**< 0.001**	**0.04**	0.72	**0.02**	**< 0.01**
2020 – 3400 m	*Constant parent material*					
Slope	12	**10**	49	-212	427	**757**
R^2	0.06	**0.28**	0.10	0.02	0.11	**0.25**
F	1.97	**12.30**	3.51	0.56	3.97	**11.02**
p	0.17	**0.001**	0.07	0.46	0.055	**< 0.01**

Figure 1

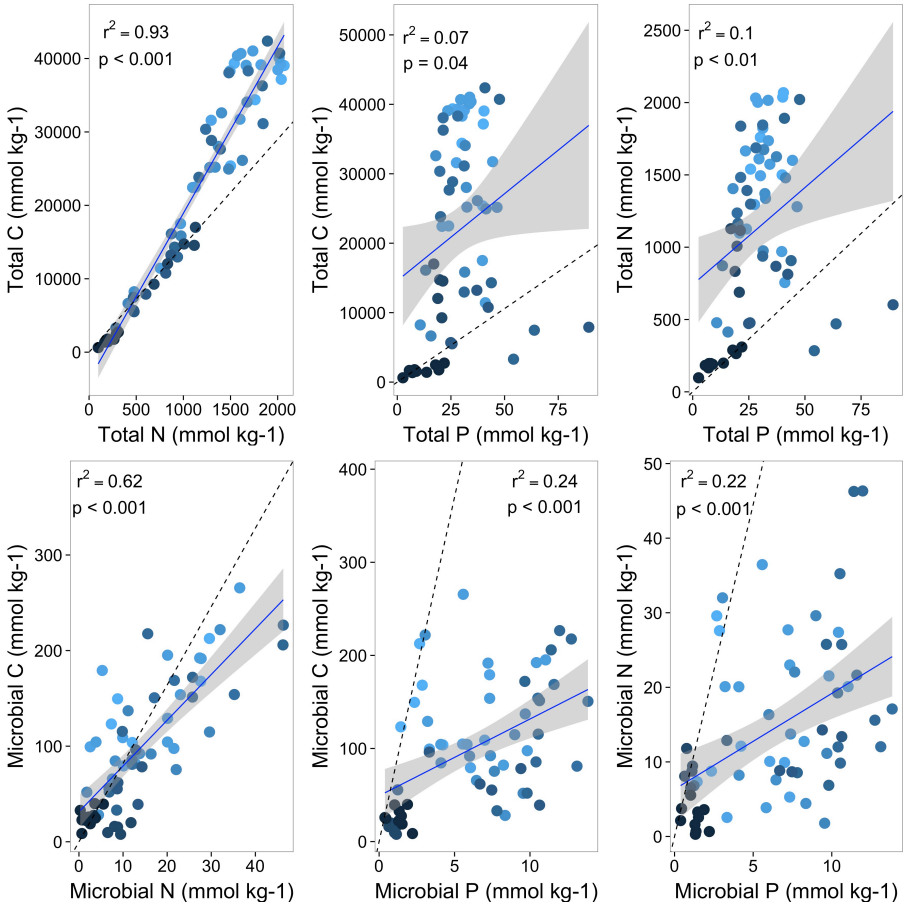

r2 = 0.93
p < 0.001
Total C (mmol kg-1)
Total N (mmol kg-1)
r2 = 0.07
p = 0.04
Total C (mmol kg-1)
Total P (mmol kg-1)
r2 = 0.1
p < 0.01
Total N (mmol kg-1)
Total P (mmol kg-1)
r2 = 0.62
p < 0.001
Microbial C (mmol kg-1)
Microbial N (mmol kg-1)
r2 = 0.24
p < 0.001
Microbial C (mmol kg-1)
Microbial P (mmol kg-1)
r2 = 0.22
p < 0.001
Microbial N (mmol kg-1)
Microbial P (mmol kg-1)

Figure 2

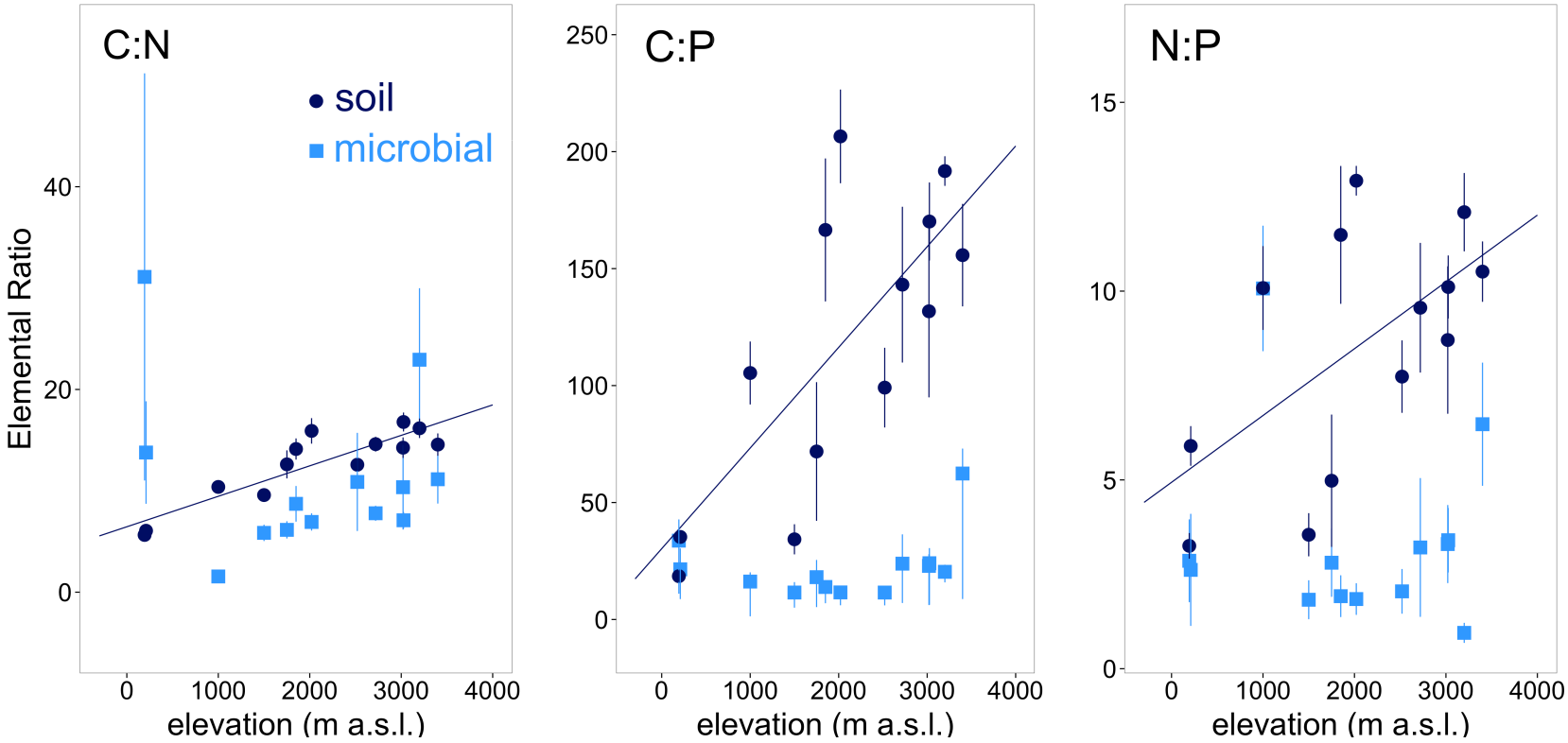

C:N
soil
microbial
Elemental Ratio
0
20
40
0
1000
2000
3000
4000
elevation (m a.s.l.)
C:P
0
50
100
150
200
250
0
1000
2000
3000
4000
elevation (m a.s.l.)
N:P
0
5
10
15
0
1000
2000
3000
4000
elevation (m a.s.l.)

Figure 3

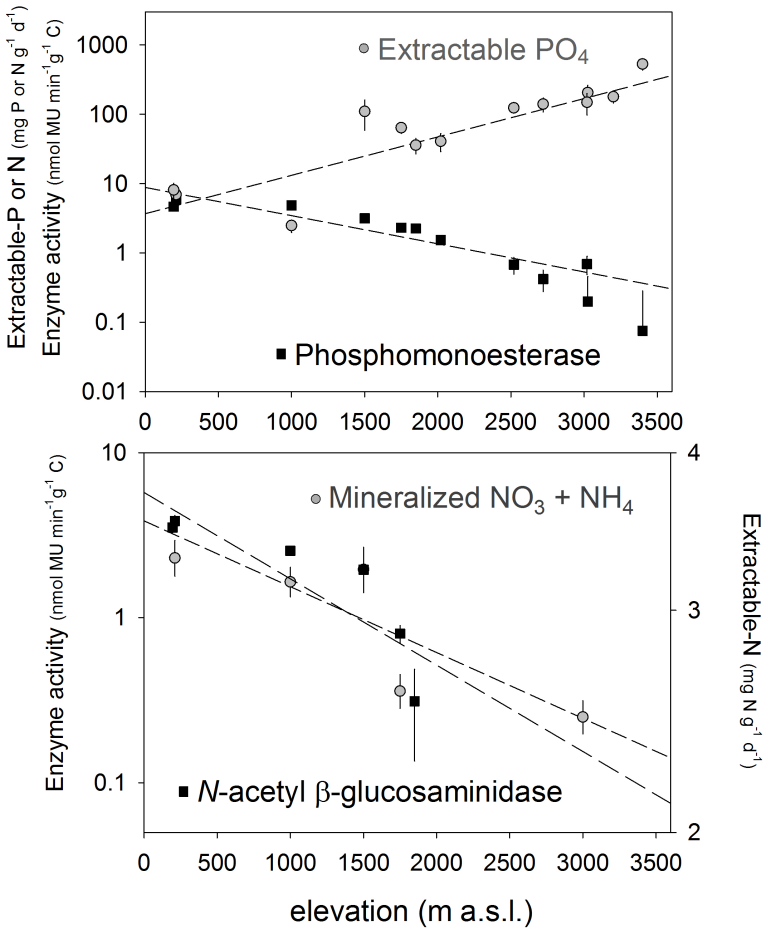

Extractable-P or N (mg P or N g-1 d-1)
Enzyme activity (nmol MU min-1 g-1 C)
Extractable PO4
Phosphomonoesterase
1000
100
10
1
0.1
0.01
0
500
1000
1500
2000
2500
3000
3500
Enzyme activity (nmol MU min-1 g-1 C)
Mineralized NO3 + NH4
N-acetyl β-glucosaminidase
Extractable-N (mg N g-1 d-1)
10
1
0.1
4
3
2
elevation (m a.s.l.)

Figure 4

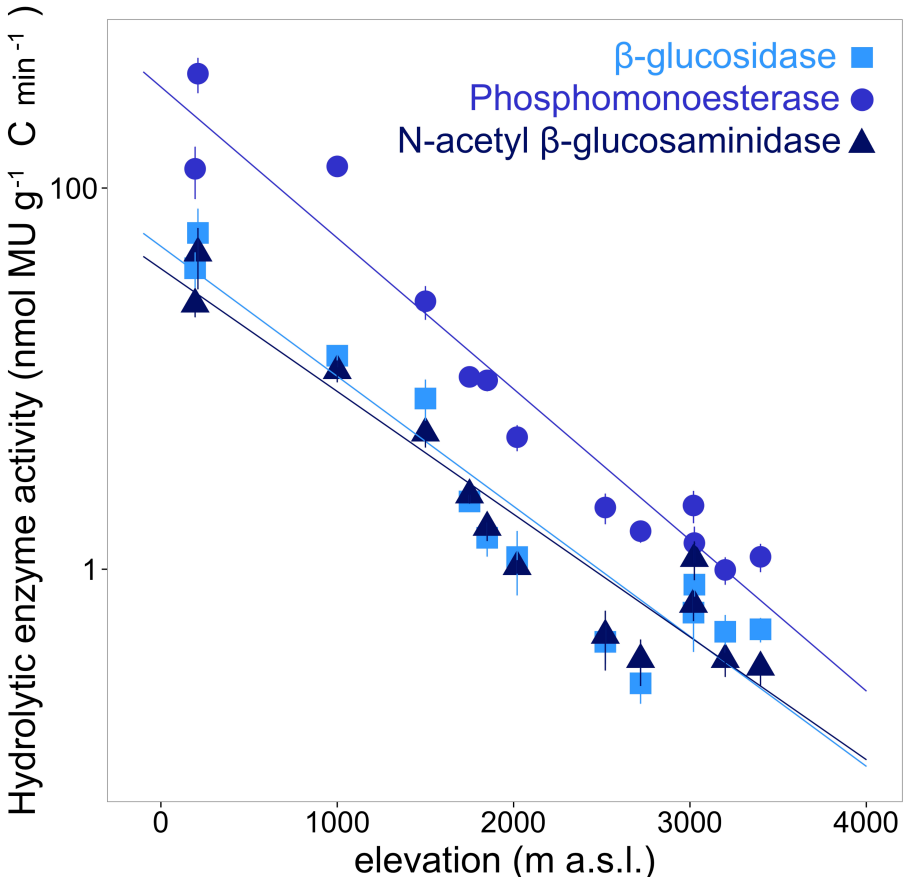
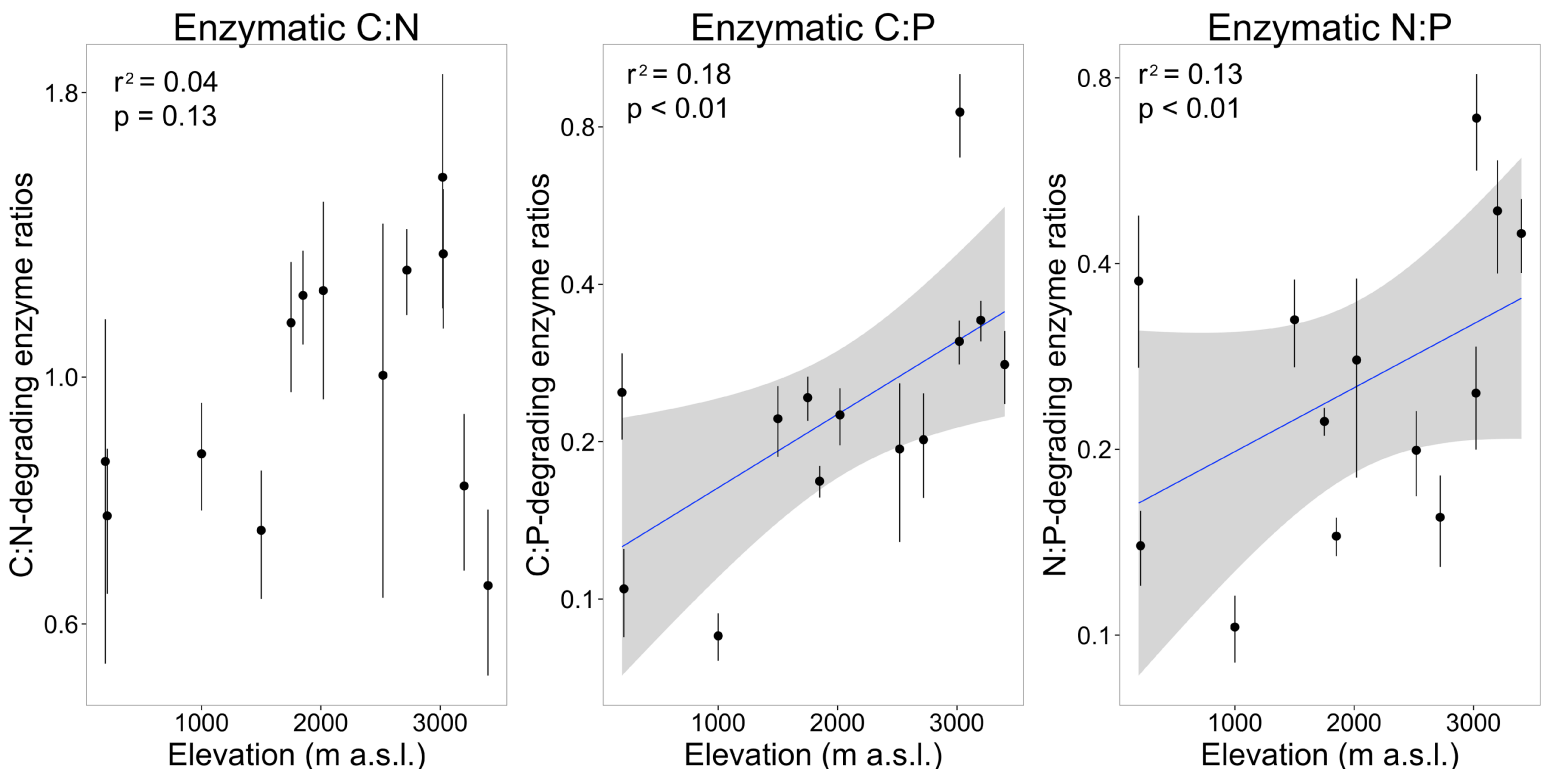

β-glucosidase
Phosphomonoesterase
N-acetyl β-glucosaminidase
Hydrolytic enzyme activity (nmol MU g-1 C min-1)
100
1
0
1000
2000
3000
4000
elevation (m a.s.l.)

Figure 5


Enzymatic C:N
r² = 0.04
p = 0.13
C:N-degrading enzyme ratios
1.8
1.0
0.6
1000
2000
3000
Elevation (m a.s.l.)
Enzymatic C:P
r² = 0.18
p < 0.01
C:P-degrading enzyme ratios
0.8
0.4
0.2
0.1
1000
2000
3000
Elevation (m a.s.l.)
Enzymatic N:P
r² = 0.13
p < 0.01
N:P-degrading enzyme ratios
0.8
0.4
0.2
0.1
1000
2000
3000
Elevation (m a.s.l.)