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Shifts in soil fungi and extracellular enzyme activity with simulated climate change in a tropical montane cloud forest

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ABSTRACT

Tropical montane cloud forests are vulnerable to climate change. The cloud layer is lifting, causing warmer and drier conditions. With climate change, tropical ecosystems have the potential to accentuate global CO_2 emissions because of their significant influence over global C cycling. Unfortunately, we do not know how this will affect belowground communities, like soil fungi, and the vital ecosystem processes they control. We performed a soil translocation experiment along an elevation gradient in Monteverde, Costa Rica to assess how fungal communities, soil decomposition, and extracellular enzyme activity (EEA) of C-degrading enzymes may shift with climate change. Soils were translocated to four lower elevation sites. These sites spanned 4 °C increases in temperature and a 20% decline in soil moisture. We used microbial cages to isolate the fungal community and monitor how soil fungi would respond to warmer, drier conditions. Fungal abundance and diversity increased with warmer and drier conditions. Fungal communities also shifted. Specifically, we found changes in the richness of fungal phyla. Richness of lichen-forming fungi, pathogens, wood saprotrophs, and yeasts increased. In addition, we found that EEA was higher under warmer and drier conditions. Our results suggest that high elevation soils may shift towards an increased capacity to decompose C under future climate conditions. Moreover, with climate change, animals or plants in tropical montane cloud forests may be exposed to a greater richness of fungal pathogens. Overall, our study reveals that the lifting cloud layer may affect the fungal community within these forests, which in turn may affect both the structure and function of these forests.

1. Introduction

Clouds distinguish tropical montane cloud forests from lowland forests. Unfortunately, this cloud layer is lifting because of increased sea surface temperatures, causing warmer temperatures and increased dry days (Karmalkar et al., 2011; Lawton et al., 2001; Still et al., 1999). These changes have implications for global C cycling owing to the disproportionate influence that tropical forests have over C cycling. For instance, tropical forests contain one third of the world's C (Jobbágy and Jackson, 2000). They also exchange more carbon dioxide (CO_2) with the atmosphere than any other ecosystem (Pan et al., 2011). In addition, there is more soil C in montane forests than lowland forests (Grieve et al., 1990; Raich et al., 2006). The fate of that C will depend on decomposition by soil fungi. In tropical montane cloud forests, studies have shown dramatic effects of climate change aboveground: plants and animals are migrating upslope to maintain their optimal climates (Colwell et al., 2008; Feeley and Silman, 2010; Thomas et al., 2004), and biodiversity is declining due to fungal pathogens (Pounds et al., 1999, 2006). But, we have little information regarding how climate change may alter belowground communities—including fungi—and their influence on ecosystem C.

Cloud immersion is vital because it affects the structure and function of these forests (see Dalling et al., 2016; Fahey et al., 2016). These effects extend beneath the soil. The dense cloud layer yields cooler temperatures, more rainfall, less light (reducing photosynthesis), and higher humidity (reducing evapotranspiration) compared to adjacent lowland forests (Schawe et al., 2010). These conditions lead to slower rates of decomposition and nutrient cycling (Bruijnzeel et al., 1993; Grubb, 1977). Decomposition rates are especially low at high elevations where temperatures become even cooler (Vitousek et al., 1994). This pattern is accentuated as soils become waterlogged and anaerobic (Schuur, 2001; Silver et al., 1999). Slower rates of decomposition at high elevations lead to a buildup of soil organic matter (Raich et al., 2006) and soil C pools (Dieleman et al., 2013; Girardin et al., 2010; Schuur et al., 2001).

Fungi are the primary decomposers in soil (de Boer et al., 2006) and are also important pathogens in tropical ecosystems (Gilbert, 2005). The lifting cloud layer could affect soil fungi, because they are sensitive

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to changes in temperature and precipitation (Allison and Treseder, 2008; Hawkes et al., 2011; McGuire et al., 2011). For example, warmer conditions could support faster decomposition of the organic-rich soil at high elevations, providing fungi with greater C and N availability. This increase in resources could promote diversification, and allow fungi to become more abundant. Moreover, richness of free-living filamentous fungi (many of which are decomposer fungi) and yeasts (simple C decomposers) tends to be greater at lower elevations (with warmer and drier soils) in tropical montane cloud forests (Looby et al., 2016). These relationships suggest that richness of these decomposer groups may increase under climate change.

Shifts in the fungal community could affect decomposition through changes in extracellular enzyme activity (EEA). Fungi produce extracellular enzymes that break down and target specific forms of C. Extracellular enzyme activity may also be altered with climate change, because enzymes at high elevations have greater temperature sensitivity (Nottingham et al., 2016). The effects on soil C could be dramatic because decomposition can increase exponentially with temperature (Benner et al., 2010). Overall, warmer temperatures in tropical montane cloud forests may allow certain fungi to proliferate, stimulating decomposition and CO_2 release from the soil.

Fungal pathogens can also influence community dynamics by infecting certain animals, plants, and other fungi. In tropical montane cloud forests, there have already been severe declines in aboveground biodiversity due to the proliferation of fungal pathogens (Pounds et al., 1999, 2006). Moreover, disease in terrestrial organisms is expected to increase with climate change (Harvell et al., 2002). With continued climate change, fungal pathogens could proliferate and aboveground species could be exposed to new pathogens.

Translocation experiments along elevation gradients have become increasingly important in understanding the fate of C under climate change (Malhi et al., 2010; Sundqvist et al., 2013). Translocation studies have shown that soil respiration (Chen, 2012; Zimmermann et al., 2009) and litter decomposition (Salinas et al., 2011; Scowcroft et al., 2000) may increase with changing climate conditions. But, more detailed information is needed on how soil fungal communities and EEA may be altered.

In a previous study, we characterized soil properties and fungal community composition along an elevation gradient on the Pacific slope of the Cordillera de Tilarán in Monteverde, Costa Rica (Looby et al., 2016). Here, we performed a soil translocation experiment along this elevation gradient to determine how fungal communities would change with warmer and drier conditions. High elevations in Monteverde are predicted to be the most vulnerable to climate change (Karmalkar et al., 2008). Thus, we focused on effects of climate change on soils from the highest elevation site. We translocated these high-elevation soils to four lower elevation sites associated with 1, 2, 3, and 4 °C increases in temperature, and a 4–20% decline in soil moisture. Moving soils from high to low elevations simulates the decline in cloud cover associated with the lifting cloud layer; and thus, soils experienced warmer temperatures and drier conditions.

We hypothesized that fungal abundance and diversity would increase in soils moved to lower elevation sites. Furthermore, we hypothesized that fungal community composition would shift in soils moved to lower elevations, because fungal phyla and functional groups may vary in their responses to climate. Finally, we hypothesized that EEA would increase in soils moved to lower elevations sites due to warmer temperatures and drier conditions. To test these hypotheses, we measured fungal abundance, diversity, and community composition, and EEA of C degrading enzymes.

2. Materials and methods

2.1. Study sites

In August 2013, an elevation transect was established along the Pacific slope of the Cordillera de Tilarán within the Monteverde Cloud Forest Reserve (10°18′N, 84°47′W) in Monteverde, Costa Rica (Looby et al., 2016). The transect ranges from 1305 to 1850 m.a.s.l. (meters above sea level), with sites established at approximately every 50 m increase in elevation. In this study, we used field sites located every 100 m increase in elevation, including 1430, 1549, 1656, 1743, and 1850 m.a.s.l. Field sites are all located within primary, undisturbed forest and cover three Holdridge life zones: premontane, lower montane, and montane forests (Holdridge, 1967). All soil along the transect is classified as inceptisols (Centro Científico Tropical, personal communication).

2.2. Soil translocation design and collection

We used a soil translocation experiment to test how increased temperatures and decreased precipitation would affect fungi and extracellular enzyme activity. More specifically, soils were moved to lower elevations so that fungal communities would experience warmer temperatures and drier conditions. We measured soil temperature at two locations at each site from November 25, 2014 to April 18, 2015 using iButton temperature loggers (QA supplies, Norfolk, VA) to verify the temperature range across our translocation sites. We also measured soil temperature at four random locations at each elevation at the time of sample collection. Based on our observations, a change of approximately 100 m in elevation corresponds to a one-degree (°C) temperature change in soil.

To manipulate the fungal community, soil was enclosed in microbial cages. Each cage was 10 cm × 10 cm and made of nylon mesh with a pore size of 0.45 μm (Maine Manufacturing, ME, USA). This pore size prevents new fungi from entering, while allowing exchange of water, nutrients, organic compounds, and some bacteria with the local environment. Microbial cages have been effective in isolating microbial communities in prior studies (Allison et al., 2013; Holden et al., 2015; Reed and Martiny, 2013). By transplanting fungi via these microbial cages, we were able to monitor how the fungal community would change with warmer and drier conditions.

Soil from 1850 m.a.s.l. was translocated to low-elevation sites to simulate warmer temperatures and drier conditions (Table 1). Soil was also placed into microbial cages and kept at 1850 m.a.s.l. as a reference site, and thus represented 0 °C warming and 0% drying. Temperature is presented as increase in soil temperature compared to the reference site. Soil moisture content (%) declined with decreasing elevation. This was determined after collection by taking subsamples of soil from each cage and measuring gravimetric moisture content. Gravimetric moisture content was determined by drying subsamples of soil at 65 °C and then re-weighing them. Soil moisture is presented as decline in soil moisture (%) compared to the reference site.

Twenty soil cores (2 cm diameter by 10 cm deep; mostly O horizon) were collected along an established 20 m straight line at 1850 m.a.s.l. in November 2014. Soils were transported to the lab and homogenized

Table 1
Site characteristics of soil translocation experiment simulating climate change. Soil was placed in microbial cages and were translocated from 1850 m.a.s.l. to four lower elevation sites that warmer and drier. Five replicates were placed at each elevation. Soil was kept at 1850 m.a.s.l. and used as a reference. Thus, these soils represented 0 °C warming and 0% drying.

Elevation (m.a.s.l.)	Latitude (N)	Longitude (W)	Temperature change from 1850 m.a.s.l. (°C)	Moisture change from 1850 m.a.s.l. (%)
1850	10°19′02.02″	84°47′40.03″	0	0
1743	10°18′57.64″	84°47′47.83″	1	−4.17
1656	10°18′48.69″	84°47′57.74″	2	−10.8
1549	10°18′18.40″	84°47′46.36″	3	−20.0
1430	10°17′28.81″	84°47′30.97″	4	−19.9

by hand. We placed 8 g of field moist soil into the microbial cages. Five fungal cages were placed on the ground surface and protected with wire at each field site. These cages were covered with adjacent leaf litter to maintain the natural conditions of soils at these field sites. Moreover, although leachate would be able to enter the cages, this does not disrupt the integrity of the experiment—plant communities are also migrating upslope (Colwell et al., 2008; Lenoir et al., 2008; Wiens, 2016), and soil fungi will be exposed to these new types of leaf litter.

Five replicates at each site were collected after ten months (September 2015) towards the end of the wet season. Average monthly precipitation for the wet season up until collection was 198 mm; 272 mm was recorded during September 2015 (measured at 10.3092°N, 84.8135° W; 1375 m.a.s.l.). Average minimum and maximum daily temperatures for the 2015 wet season were 15.4 °C and 22.8 °C. Although microbial cages have typically been used in drier ecosystems, we found no evidence that these cages became waterlogged. In fact, we observed that soil moisture values were similar to those found in our previous study (Looby et al., 2016). Microbial cages were stored at −20 °C and transported to UC Irvine. All microbial cages were destructively sampled for downstream processing. Soils were stored at −20 °C for all analyses except for extracellular enzyme assays, which were stored at −80 °C (German and Allison, 2015; Holden et al., 2013; Romero-Olivares et al., 2017).

2.3. Fungal abundance

We measured fungal hyphal length as a metric for total soil fungal abundance (Brundrett et al., 1996). Briefly, 4 g (wet weight) soil from each microbial cage was extracted with 1.5 M solution of sodium hexametaphosphate. Soil solutions were passed through 0.2-μm nylon filters to collect hyphae. Filters were stained with acid fuchsin, mounted on a glass slide with polyvinyl lactic acid (PVLG) slide mounting medium, and dried at 65 °C overnight. Hyphal lengths were measured using a gridline intersect method at 200× on a Nikon phase-contrast microscope (Nikon Eclipse e400, AG Heinze, Lake Forest, CA, USA). Hyphal lengths were calculated as cm g^{-1} dry soil.

2.4. Fungal community composition

We extracted soil DNA from each microbial cage with the PowerSoil DNA Isolation kit (MoBio, Carlsbad, CA, USA) following the manufacturer guidelines. DNA quality and concentrations were quantified using a NanoDrop and standardized to 10 ng/μL prior to PCR amplification.

We used modified primers targeting the 5.8S encoding gene to amplify the ITS2 region of fungal ribosomal encoding genes. These primers produce a smaller amplicon than primers targeting the entire ITS region. In turn, this reduces species bias and PCR chimeras, but maintains the same level of fungal diversity (Ihrmark et al., 2012). We amplified a ~340 bp region of the fungal ITS2 gene using a staggered primer design. This included a forward primer (ITS9f; AATGATACGG CGACCACCGAGATCTACAC TC TTTCCCTACA CGACGCTCTTCCGATCT NNNNNGAACGCAGCRAAIIGYGA) and barcoded, reverse primers with the reverse complement of the 3' Illumina adapter (CAAGCAGAAGAC GGCATACGAGAT), a unique 12 base barcode, a pad (AGTCAGTCAG), a linker sequence (CC), and the ITS4 primer (TCCTCCGCTTATTGATA TGC). We used a staggered primer design that included 0, 1, 2 or 3 bases preceding the ITS4 primer (e.g., CC-ITS4, CC-G-ITS4, CC-AG-ITS4, or CC-CAG-ITS4). This design increases the diversity of amplicon sequences across the Illumina Miseq flowcell early in the read (Tremblay et al., 2015). This improves amplicon detection and sequence quality.

Each PCR reaction included: 21.5 μL of Platinum PCR Supermix (Invitrogen, Carlsbad, CA), 0.75 μL of each primer (10 μM), 1 μL of BSA (10 mg mL^{-1}), and 1 μL of DNA (10 ng). Reactions ran with a hot start at 94 °C for 5 min, 35 cycles of 95 °C for 45 s, 50 °C for 1 min, 72 °C for 90 s, and a final extension step of 72 °C for 10 min. PCR reactions from each microbial cage were ran in triplicate, pooled, and purified with Agencourt AMPure XP magnetic beads (Beckman Coulter, Brea, CA). We quantified purified samples using the Qubit dsDNA High Sensitivity Assay Kit (Life Technologies, Grand Island, NY). All samples were then pooled in equimolar concentrations. The pooled sample was sequenced as 2 × 300 bp paired end reads on one lane of an Illumina MiSeq sequencer. Sequencing was performed at the Genomics core in the Institute for the Integrative Genome Biology at the University of California, Riverside. Raw sequence files can be accessed in the NCBI database under the BioProject ID PRJNA357504.

We processed sequences through the Quantitative Insights Into Microbial Ecology (QIIME) pipeline (v. 1.9.1; Caporaso et al., 2010). Sequences were assembled and filtered for quality control. We retained sequences characterized by a minimum Phred score sequence cutoff threshold of 33 and higher. Sequences were discarded if they had less than 80% consecutive high-quality reads and more than two consecutive low-quality base reads. Chimeras were detected and removed using USEARCH 6.1 (v. 6.1.544), and global singleton reads were removed. After quality control, our dataset contained ~3.5 million high-quality sequences that were clustered into operational taxonomic units (OTUs) at a 97% similarity cutoff. One representative sequence from each OTU was chosen, and the closest taxonomic identity was determined via BLAST comparison in the GenBank database. A taxonomic assignment was made for each OTU using nomenclature classification in the UNITE database (v.7; release date 8.1.2015). To avoid bias due to differing library sizes, samples were normalized to 8,242 sequences per sample.

We assigned functional groups to OTUs using the FUNGuild algorithm (Nguyen et al., 2016; database checked February 2016), which is presently the largest database. Functional groups are assigned at the genus level and with confidence levels of "highly probable," "probable," and "possible." Approximately 50% of OTUs were matched to a functional guild within the FUNGuild database. Only assignments with confidence levels of "highly probable" or "probable" were included in analyses (95.3% of assignments). Functional groups were categorized as follows: endophytes (dark septate endophytes and endophytes), lichen-forming fungi, pathogens (animal pathogens, mycoparasites, and plant pathogens), saprotrophs, and wood saprotrophs. Furthermore, any functional guild assignment that was designated with the growth form "yeast" were classified as such in subsequent analyses. Due to the nature of the experimental design (i.e., transplant of soil and not plant hosts) mycorrhizal fungi were not included in analyses.

2.5. Extracellular enzyme activity

Soil EEA for C-degrading enzymes was measured using the microplate fluorometric protocol of (German et al., 2011). These enzymes included: α-glucosidase (AG; starch degrading), β-glucosidase (BG; cellulose degrading), cellobiohydrolase (CBH; cellulose degrading), and β-xylosidase (BX; hemicellulose degrading). Overall, these hydrolytic enzymes target labile to intermediate C.

Briefly, 1 g of soil was homogenized in 125 mL sodium acetate buffer (pH 5.0) using a hand blender. Two hundred microliters of the soil homogenate were added to 50 μL fluorometric substrate solution and incubated for 1 h. For soils at elevations 1, 2, 3, and 4 °C warmer, assays were incubated at 14, 15, 16, 17, and 18 °C. These were the soil temperatures measured at the time of collection. To terminate activity, 10 μL of 1 M NaOH was added. Fluorescence was measured at 365 nm excitation and 450 nm emission. Each microplate included substrate controls, homogenate controls, and 4- methylumbelliferone, and a standard curve was calculated to determine potential activities. Potential EEA was calculated as nmol product released h^{-1} g^{-1} dry soil.

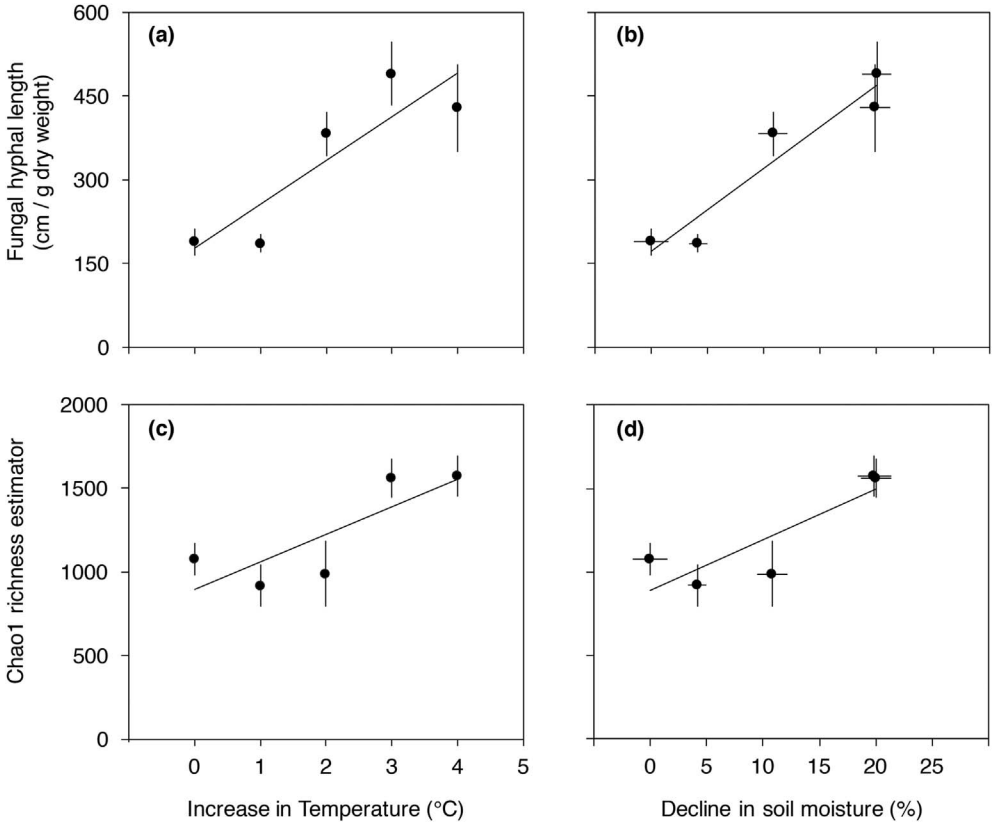

Fig. 1. Fungal abundance and diversity with warmer and drier conditions. Fungal abundance increased with (a) warmer temperatures and (b) drier conditions. Fungal taxonomic richness as determined by the Chao1 richness estimator also increased with (c) warmer and (d) drier conditions. There were no significant differences between temperature and moisture as predictors of fungal abundance and diversity when regression models were compared. Lines are significant best-fit regressions, and symbols represent means ± SE (n = 5). Statistical results are presented in Table 2.

2.6. Statistics

We used linear models to test whether temperature and moisture predicted relationships in dependent variables. Dependent variables included fungal abundance, extracellular enzyme activities, fungal taxonomic richness, richness of fungal phyla, and richness of fungal functional groups. Temperature and moisture were highly correlated in this study (R^2 = 0.873, P < 0.001). These parameters were tested separately to avoid any issues with multicollinearity. Subsamples were nested within site to account for potential lack of independence between samples that were located at the same site. We inspected for normality of the data using q-q plots. In addition, we verified that the residuals and standardized residuals were randomly distributed in relation to fitted values. We also checked that the standardized residuals were normally distributed and inspected the leverage of standardized residuals using Cook's distance. Results for BX activity were transformed as a polynomial function to better fit the data. Results are stated as significant when P < 0.05.

The method described above produced two linear models (one for temperature and one for moisture) for each dependent variable. To determine whether temperature or moisture was a better predictor of each one of the dependent variables we used a combination of metrics and a test following Talbot et al. (2015) to compare both models for each variable. First, we calculated pseudo R^2 values for each model with the r.squaredLR command in the MuMIn package in R. Higher pseudo R^2 values indicate a better fit to the data, and thus show better prediction. Second, we calculated the Akaike Information Critierion (AIC) using the generic AIC function in R. Smaller AIC values indicate a better fit to the data. Finally, we conducted an analysis of deviance with the function “anova” of the package glm in R (using the LRT option) to determine which one of the two models provide a better fit to the data using the likelihood ratio test approach. In the current study, all metrics were in agreement. All analyses were performed in R (v. 3.3.2, R Core Team, 2016).

Taxonomic richness (alpha diversity) was computed for each sample using Chao1 estimator and observed OTUs. In fungi, traits and environmental preferences are highly conserved among phyla (Stajich et al., 2009; Treseder et al., 2014). Thus, we also compared differences in phyla as another metric to assess how the fungal community may shift. To assess richness within fungal phyla, we calculated total number of OTUs within each phyla in each sample. In addition, we calculated richness within each functional group as the total number of OTUs within each functional group in each sample. We used linear models as described above to test whether temperature and moisture predicted relationships in alpha diversity, richness of fungal phyla, and richness of functional groups. We used a PERMANOVA using Bray-Curtis dissimilarity to analyze fungal community composition as a function of temperature change and soil moisture content with the adonis function in the Vegan package of R (Oksanen et al., 2012). Non-metric multidimensional scaling (NMS) plots were constructed in R to visualize fungal community composition.

3. Results

For each measure of fungal abundance, community composition, and extracellular enzyme activity, we determined whether temperature or moisture was a significantly better predictor. When temperature predicted a given variable significantly better than moisture, we display the temperature relationship in a figure. Likewise, when moisture was significantly stronger, we show the moisture relationship only. When temperature and moisture displayed statistically equivalent relationships, we depict both relationships.

3.1. Fungal abundance and diversity

Fungal abundance increased significantly with warmer temperatures and drier conditions (Fig. 1a and b; Table 2). Alpha diversity increased in soils with warmer temperatures and drier conditions (Fig. 1c

Table 2
Statistical tests for effect of temperature and moisture on fungal abundance, alpha diversity, richness of fungal phyla, richness of fungal functional groups, and extracellular enzyme activity. Extracellular enzymes of C-degrading enzymes included: α-glucosidase (AG), β-glucosidase (BG), cellobiohydrolase (CBH), and β-xylosidase. Log likelihood (lnl) and Akaike Information Criterion (AIC) for fit are reported. Asterisks represent significance of regression (***$P < 0.001$, **$P < 0.01$, *$P < 0.05$). Non-significant (NS) denotes $P > 0.10$.

Category	Parameter	Moisture				Temperature				Model comparisons with log likelihood ratio
		Pseudo R^2	F	lnl	AIC	Pseudo R^2	F	lnl	AIC	Moist vs. temp (P)
Fungal abundance	**Hyphal length**	0.737	44.6***	−145	307	0.731	9.28***	−146	311	NS
Alpha diversity	**Chao1**	0.487	12.2**	−161	339	0.619	5.19**	−158	336	0.088
	Observed OTUs	0.391	8.56**	−148	313	0.514	3.46*	−146	311	NS
Richness of fungal phyla	**Ascomycota**	0.542	15.7**	−141	297	0.644	5.84**	−138	296	NS
	Basidiomycota	0.138	1.54	−116	248	0.308	1.46	−113	247	NS
	Chytridiomycota	0.208	1.12	−64.7	145	0.475	2.36	−60.0	140	0.028*
	Cryptomycota	0.542	13.4**	−54.2	124	0.593	4.84*	−52.9	126	NS
	Zygomycota	0.188	2.48	−92.3	201	0.226	0.803	−91.7	203	NS
Richness of fungal functional groups	**Endophytes**	0.083	0.525	−67.8	152	0.378	1.86	−63.4	147	0.036*
	Lichen-forming	0.434	7.31*	−54.8	126	0.631	5.20**	−49.8	120	0.023*
	Pathogens	0.517	11.2**	−87.2	190	0.771	8.67**	−79.0	178	< 0.001***
	Saprotrophs	0.199	2.61	−116	247	0.313	1.40	−114	248	NS
	Wood saprotrophs	0.409	6.01*	−79.3	175	0.524	3.19*	−76.8	174	NS
	Yeasts	0.676	28.0***	−92.5	201	0.809	13.5***	−86.5	193	0.008**
Extracellular enzyme activity	**AG activity**	0.612	20.7***	−61.8	140	0.599	4.90*	−62.2	144	NS
	BG activity	0.560	17.4***	−134	284	0.692	7.50**	−130	280	0.050*
	CBH activity	0.498	19.6***	−96.8	203	0.541	5.21**	−95.8	206	NS
	BX activity	0.118	0.492	−116	248	0.667	6.21**	−105	229	< 0.001***

and d; Table 2) according to the Chao1 richness estimator. Similarly, the observed number of OTUs also increased (Table 2). When regression models were compared using log likelihood ratios, there were no significant differences between temperature and moisture as predictors of abundance or richness estimates.

3.2. Fungal community composition

Fungal community composition shifted with increasing temperatures ($F = 4.31$, $R^2 = 0.170$, $P < 0.001$; Fig. 2a) and decreasing moisture ($F = 4.30$, $R^2 = 0.170$, $P < 0.001$; Fig. 2b). This alteration in community composition was associated with changes in the richness of certain fungal phyla (Fig. 3; Table 2). There was an overall increase in the richness of Ascomycota with warmer temperatures and drier conditions (Fig. 3a and b). In contrast, the richness of Cryptomycota decreased in soils translocated to warmer and drier conditions (Fig. 3c and d). When regression models were compared using log likelihood ratios, there were no significant differences between temperature and moisture as predictors of richness of Ascomycota and Cryptomycota.

Richness within particular fungal functional groups shifted with warmer temperatures and drier conditions (Fig. 4; Table 2). Specifically, the richness of lichen-forming fungi (Fig. 4a), pathogen (Fig. 4b), and yeast (Fig. 4c) all increased. When regression models were compared using log likelihood ratios, temperature was a better predictor than moisture for lichen-forming fungi, pathogens, and yeasts (Table 2). Richness of wood saprotrophs increqasd with warmer and drier conditions (Fig. 5a and b). There were no significant differences between temperature and moisture as predictors of richness of wood saprotrophs (Table 2).

3.3. Extracellular enzyme activity

Potential EEA shifted with climate (Figs. 6 and 7; Table 2). AG activity was significantly higher at warmer temperatures and drier conditions (Fig. 6a and b). Similarly, CBH activity was significantly higher at warmer temperatures and drier conditions (Fig. 6c and d). There were no significant differences between temperature and moisture as predictors of AG and CBH activities. Likewise, BG activity increased in the warmer (Fig. 7a) and drier climate, and peaked at 2 and 3 °C. Unlike the other EEAs, BX activity increased only with warmer temperatures (Fig. 7b) at 1 °C. When regression models were compared using log likelihood ratios, temperature was a better predictor than moisture for BG and BX activities (Table 2).

4. Discussion

Cloud cover in these forests is intimately linked to the structure and function of the ecosystem. Unfortunately, the cloud layer is lifting, and

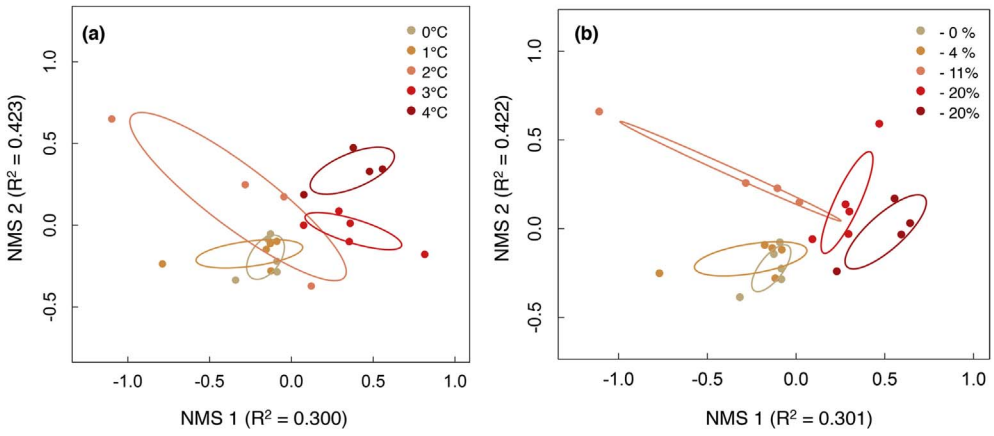

Fig. 2. Nonmetric multidimensional scaling (NMS) ordination showing differences in fungal community composition with (a) warmer temperatures ($P < 0.001$) and (b) drier conditions ($P < 0.001$). In panel (a) symbols represent each microbial cage and are colored by a gradient indicating increasing temperature from 0 (tan) to 4 °C (dark red). In panel (b) Symbols represent each microbial cage and are colored by a gradient indicating decreasing moisture from 0% (tan) to −20% (dark red). Ellipses represent 95% confidence intervals. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

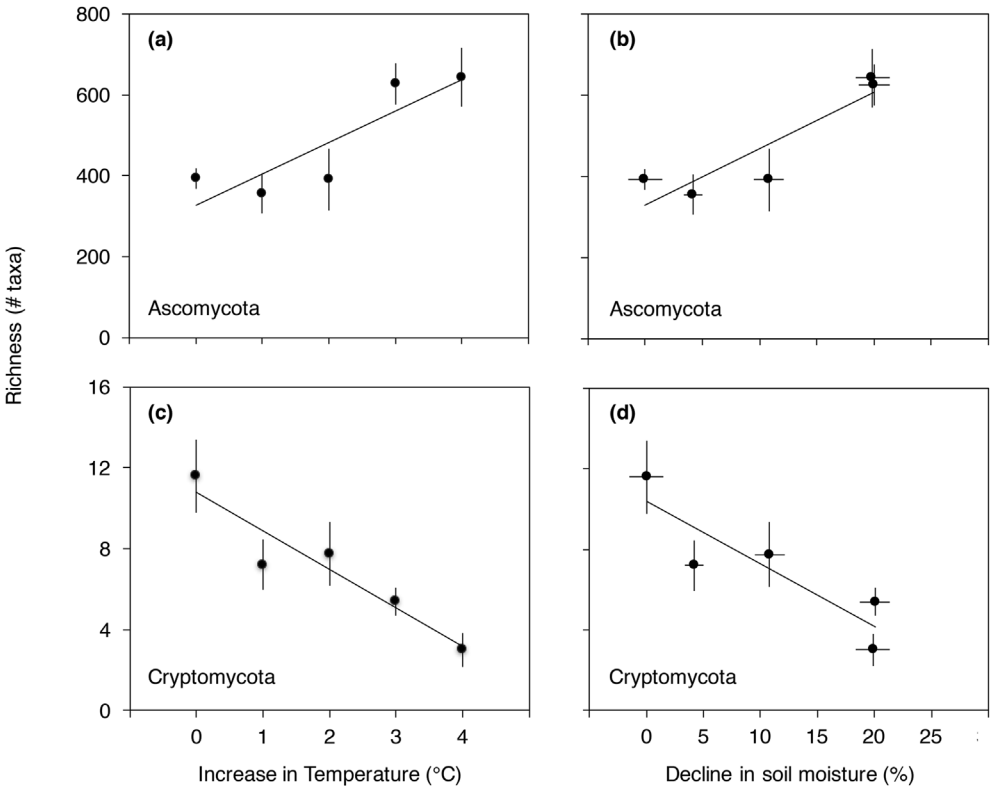

Fig. 3. Richness of Ascomycota and Cryptomycota with warmer and drier conditions. Richness of Ascomycota increased with (a) warmer temperatures and (b) drier conditions. There was a decline in richness of Cryptomycota with (c) warmer and (d) drier conditions. There were no significant differences between temperature and moisture as predictors of richness of Ascomycota and Cryptomycota when regression models were compared. Lines are significant best-fit regressions, and symbols represent means ± SE (n = 5). Statistical results are presented in Table 2. .

these forests are becoming warmer and drier (Karmalkar et al., 2011; Lawton et al., 2001; Still et al., 1999). As these changes occur, the larger pools of soil C that tropical montane cloud forests contain may be broken down and released as CO_2. It is critical to perform *in situ* temperature manipulations in order to understand how tropical ecosystems—like tropical montane cloud forests—may change (Cavaleri et al., 2015). In general, temperatures in tropical regions are predicted to increase by 1.8–5.0 °C by 2100 (IPCC, 2013). More specifically, in montane regions in Costa Rica, temperatures at the highest elevations are predicted to increase approximately 3.0 °C (Karmalkar et al., 2008). Our *in situ* warming manipulation represents the temperature increase predicted with climate change. Overall, our results suggest that climate change in tropical montane cloud forests may alter fungal communities and the breakdown of C.

We acknowledge that this experiment was conducted across one slope of the mountain. Conditions vary immensely across the Atlantic and Pacific slopes of the Cordillera de Tilarán due of rainshadow effects; because of this, these two slopes cannot be compared. Moreover, we chose to conduct this climate change experiment along the Pacific slope because climate change is having a disproportionate effect here due to deforestation in the Pacific lowlands (Lawton et al., 2001; Karmalkar et al., 2011).

4.1. Fungal abundance and diversity

We found support for our hypothesis that fungal abundance and diversity would increase when soils were translocated to lower elevations (Fig. 1). The soils were collected from the highest elevation site, where relatively cool, water-saturated conditions may have slowed decomposition rates and fostered organic matter accumulation (Looby et al., 2016). Once these C-rich soils were exposed to drier conditions at low elevations, oxygen availability may have increased. This change, together with warmer temperatures, could have improved fungal growth. Likewise, rarer fungi may have proliferated under these more amenable conditions, leading to the increase in fungal diversity.

It is important to note that even though fungi were not able to enter the microbial cages, diversity can still increase within them after transplanting. This increase could occur if particular fungal OTUs were present in the reference site, but were so rare that they fell below the detection limit for sequencing. If these OTUs then proliferated at the translocation site, they could become detectable. This mechanism would allow diversity to increase within the microbial cages.

4.2. Fungal community composition

Our hypothesis that fungal community composition would shift with changing climate was supported (Fig. 2). Shifts in the fungal community were driven by changes in the richness of phyla (Fig. 3) and fungal functional groups (Figs. 4 and 5).

Warmer temperatures and drier conditions were associated with a higher richness of Ascomycota, which was one of the most abundant fungal phyla. Ascomycetes have thick-walled spores, which can confer drought tolerance (Treseder et al., 2014). This trait may have allowed ascomycete taxa to become more abundant in the warmer, drier sites. On the other hand, the warmer and drier conditions at lower elevations were associated with a decline in the richness of Cryptomycota. This ancestral phylum seems to be adapted to wet conditions (Jones et al., 2011; Treseder et al., 2014). Cryptomycota have zoospore stage where they use a flagellum for motility. Most likely, Cryptomycota depend on the water-logged conditions present at high elevations. Our results imply that as high-elevation soils become warmer and drier the richness of this phylum may decline.

Fungal functional groups also responded to changing climate conditions; the richness of lichens, pathogens, wood saprotrophs, and yeasts all increased with warmer and drier conditions (Figs. 4 and 5). Greater fungal richness can be associated with faster decomposition (Setälä and McLean, 2004; Hättenschwiler et al., 2005; Bonanomi et al., 2015). Yeasts specialize in decomposing more labile forms of C, like simple sugars (Treseder and Lennon, 2015). Our results suggest that a number of yeast taxa may proliferate under climate change, and this could have consequences for C dynamics within tropical montane cloud forests. An increase in yeast richness could lead an increased

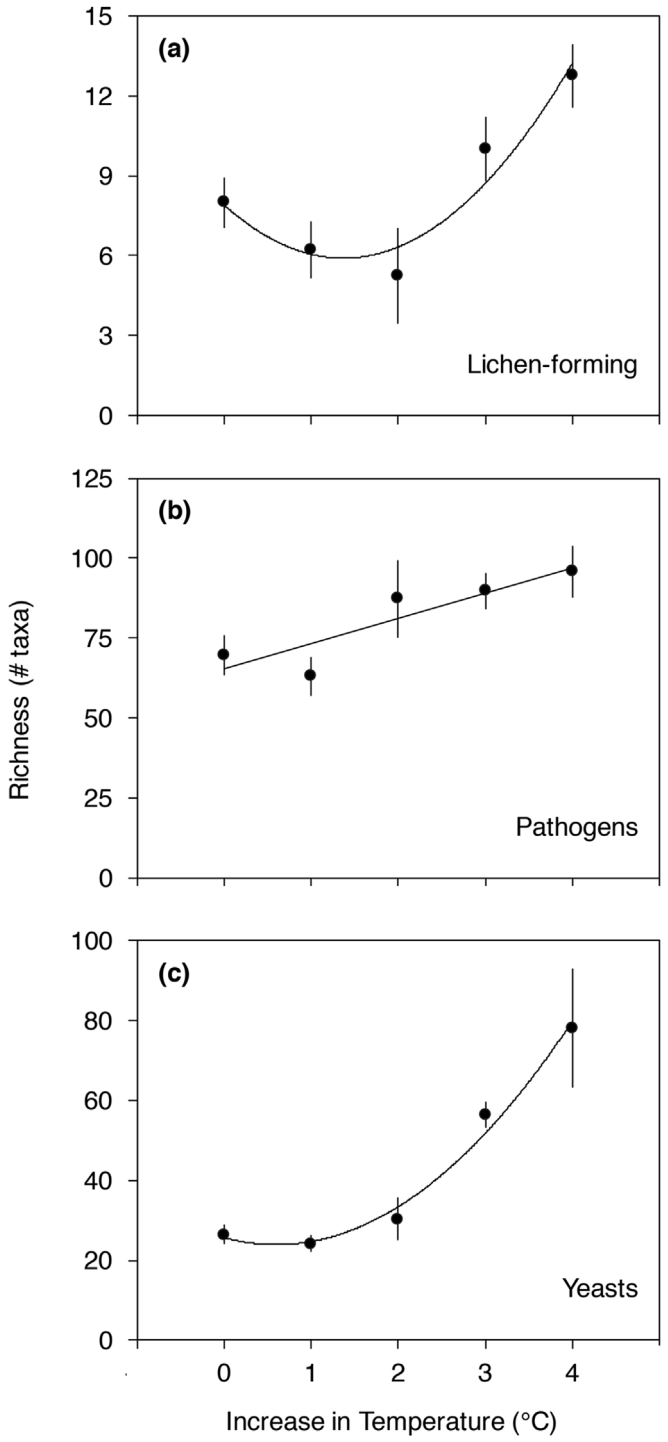

Fig. 4. Taxonomic richness of fungal functional groups with warming. Fungal taxa were identified and grouped as (a) lichen-forming, (b) pathogens, and (c) yeasts. These functional groups increased overall with both warmer and drier conditions. Model comparisons showed that temperature had a greater effect than moisture on lichen-forming, pathogens, and yeasts. Lines are best-fit regressions and symbols represent means ± SE (n = 5). Statistical results are presented in Table 2. .

breakdown of more labile forms of C.

An increase in richness of wood saprotrophs with climate change could have consequences for C dynamics. Wood saprotrophs are responsible for decomposing recalcitrant C, like lignin. Decomposition of recalcitrant C generally increases with warming (Conant et al., 2008). Another study along an elevation gradient suggested that wood saprotrophs were sensitive to temperature (Meier et al., 2010). The increased richness of wood saprotrophs found in our study could lead to a greater capacity of the fungal community to breakdown more recalcitrant C.

Fungi are not only important in the decomposition of organic material, but they also can act as pathogens. Climate change is predicted to increase disease incidence in terrestrial organisms, especially plants (Harvell et al., 2002). Our findings are consistent with this prediction, as warmer, drier conditions caused fungal pathogen richness to increase. For example, we observed more OTUs from plant pathogens in the genera *Cylindrocladiella* and *Pestalotiopsis* in warmer sites. Both genera are common plant pathogens in the tropics. *Pestalotiopsis* causes guava scab on *Psidium guayaba* trees, and tip blight on some forms of *Podocarpus* sp.; both are present in Monteverde. *Cylindrocladiella* causes types of leaf spot, and root and stem rot, including species of oak. This increased pathogen richness could alter plant and animal diversity. As climate continues to change, existing pathogens could spread, and new pathogens could emerge. This mechanism could perpetuate biodiversity loss within tropical montane cloud forests (Bradshaw et al., 2009).

4.3. Extracellular enzyme activity

For three of the four enzymes we measured, we found support for our hypothesis that EEA of C degrading enzymes would increase with warmer temperatures and drier conditions (Figs. 6 and 7). The exception was β-xylosidase, which only increased with warmer temperatures. Increased EEA with warming has been found in other studies (Davidson and Janssens, 2006), but the effects of climate change on enzymes are difficult to generalize and predict (see Burns et al., 2013). Other studies in the tropics have used elevation gradient approaches to determine how decomposition may change with warming. In the Peruvian Andes, extracellular enzymes from high elevations soils were more temperature sensitive (Nottingham et al., 2016). Also, in the Peruvian Andes, Salinas et al. (2011) performed a litter translocation experiment and determined that decomposition may increase with warming. In Hawaii, *Metrosideros polymorpha* leaf litter decomposed faster when translocated to lower elevation sites (Scowcroft et al., 2000). Finally, a soil translocation experiment in Puerto Rico demonstrated that decomposition might increase with warmer temperatures as suggested by lower soil organic C and increased respiration (Chen, 2012). Our study is one of the first to use a soil translocation design along an elevation gradient to determine how EEA may respond to climate change.

Fungi are the primary producers of hydrolytic enzymes (Schneider et al., 2012), and they dominate decomposition of cellulose and hemicellulose (de Boer et al., 2005). Thus, the observed increase in fungal abundance with warmer temperatures could have led to the higher EEA. In addition, shifts in fungal community composition could have contributed. Fungi can specialize in breaking down different forms of C (Hanson et al., 2008; McGuire et al., 2010; Setälä and McLean, 2004). For instance, many ascomycetes prefer cellulose (Osono, 2007), and they were more abundant in the warmer, drier sites. Shifts toward this phylum coincide with the higher CBH and BG activity in warmer, drier conditions.

Overall, our findings suggest that EEA targeting labile-to-intermediate forms of C could increase with the warmer temperatures predicted in tropical montane cloud forests. In a review paper, Bradford et al. (2016) noted that ecosystems harboring organic-rich and water-saturated soils are most vulnerable to soil C losses under climate change. Indeed, the soils translocated in this study possessed relatively high C concentrations, and they were often water-saturated under ambient conditions (Looby et al., 2016). These soil C stocks may be broken down more rapidly under future climate conditions, owing to higher EEA.

5. Conclusions

Cloud cover within tropical montane cloud forests is declining, and the structure and function of these unique forests will most likely

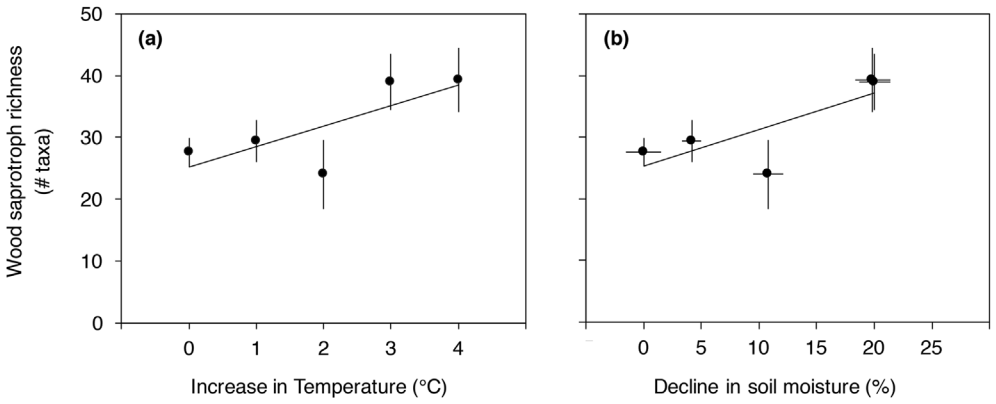

Fig. 5. Taxonomic richness of wood saprotrophs with warming. Wood saprotrophs increased with (a) warmer temperatures and (b) drier conditions. There were no significant differences between temperature and moisture as predictors of wood saprotroph richness when regression models were compared. Lines are best-fit regressions and symbols represent means ± SE (n = 5). Statistical results are presented in Table 2.

change in response. But, will these high elevation forests begin to function like tropical lowlands? Our results suggest that this is possible with respect to the fungal community and EEA. Here, we used a soil translocation experiment to determine whether fungal communities and decomposition of C would change with warmer and drier conditions predicted with the lifting cloud layer.

Our findings indicate that with warming and drought, high-elevation soils may shift towards an increased ability to break down labile to intermediate forms of C. This could be supplemented by a greater capacity of the fungal community to breakdown more complex C through an increase in wood saprotrophs. In total, these changes may lead to increased CO_2 release into the atmosphere. Moreover, pathogens may proliferate under warmer and drier conditions. Biodiversity within these forests is already being affected by climate change as aboveground species are relocating to high elevations. Our results suggest that not only will aboveground communities be relocating, but they may also encounter a greater diversity of pathogens. Consequently, our study indicates that the lifting cloud layer in tropical montane cloud forests may affect how these forests function via changes in the fungal community.

Author contributions

CIL conceived and designed the study, performed research, analyzed data and wrote the manuscript. KKT designed the study, analyzed data, and helped write the paper. Both authors contributed significantly to the current version.

Conflict of interest

The authors declare no conflict of interest.

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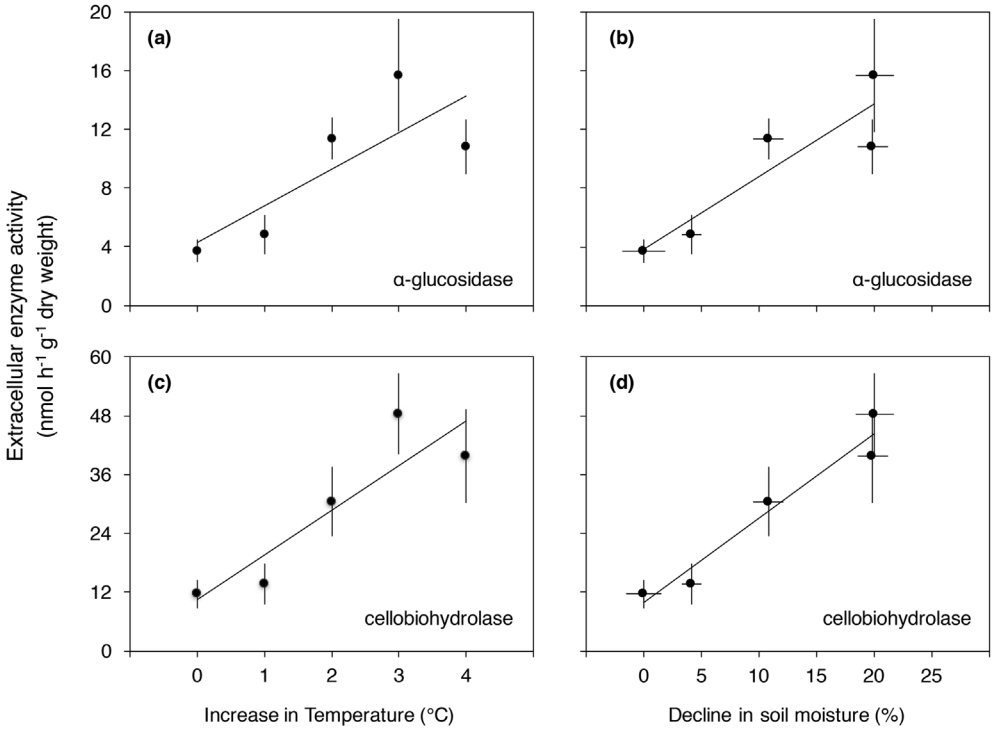

Fig. 6. Potential extracellular enzyme activity (EEA) of α-glucosidase (AG) and cellobiohydrolase (CBH) with warmer and drier conditions. Enzyme activity of AG increased with (a) warmer and (b) drier conditions. Enzyme activity of CBH also increased with (c) warmer and (d) drier conditions. There were no significant differences between temperature and moisture as predictors of enzyme activities of AG and CBH when regression models were compared. Lines are best-fit regressions and symbols represent means ± SE (n = 5). Statistical results for extracellular enzyme activity are presented in Table 2.

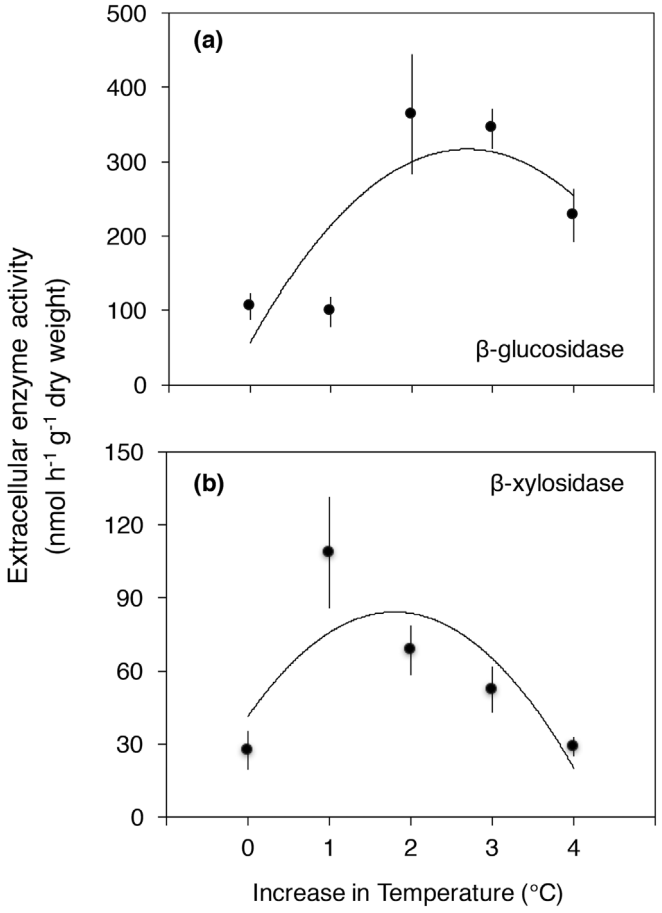

Fig. 7. Potential extracellular enzyme activity (EEA) of β-glucosidase (BG) and β-xylosidase (BX) with warming. Activity of (a) BG increased with warmer temperatures, and peaked at 2 and 3 °C. BG activity also increased drier conditions, but temperature was a better predictor than moisture for BG activity. Activity of BX increased only with (b) warmer temperatures at 1 °C. Lines are best-fit regressions and symbols represent means ± SE (n = 5). Statistical results for extracellular enzyme activity are presented in Table 2. .

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References

Allison, S.D., Treseder, K.K., 2008. Warming and drying suppress microbial activity and carbon cycling in boreal forest soils. Global Change Biology 14, 2898–2909.
Allison, S.D., Ying, L., Weihe, C., Goulden, M.L., Martiny, A.C., Treseder, K.K., Martiny, J.B., 2013. Microbial abundance and composition influence litter decomposition response to environmental change. Ecology 94, 714–725.
Benner, J., Vitousek, P.M., Ostertag, R., 2010. Nutrient cycling and nutrient limitation in tropical montane cloud forests. In: Bruijnzeel, L.A., Scatena, F.N., Hamilton, L.S. (Eds.), Tropical Montane Cloud Forests. Cambridge University Press, New York, pp. 90–100.
Bonanomi, G., Capodilupo, M., Incerti, G., Mazzoleni, S., Scala, F., 2015. Litter quality and temperature modulate microbial diversity effects on decomposition in model experiments. Community Ecology 16, 167–177.
Bradford, M.A., Wieder, W.R., Bonan, G.B., Fierer, N., Raymond, P.A., Crowther, T.W., 2016. Managing uncertainty in soil carbon feedbacks to climate change. Nature 6, 751–758.
Bradshaw, C.J., Sodhi, N.S., Brook, B.W., 2009. Tropical turmoil: a biodiversity tragedy in progress. Frontiers in Ecology and the Environment 7, 79–87.
Bruijnzeel, L.A., Waterloo, M.J., Proctor, J., Kuiters, A.T., Kotterink, B., 1993. Hydrological observations in montane rain forests on Gunung Silam, Sabah, Malaysia, with special reference to the Massenerhebung'effect. Journal of Ecology 81, 145–167.
Brundrett, M., Bougher, N., Dell, B., Grove, T., Malajczuk, N., 1996. Working with Mycorrhizas in Forestry and Agriculture. ACIAR Monograph, vol. 32.
Burns, R.G., DeForest, J.L., Marxsen, J., Sinsabaugh, R.L., Stromberger, M.E., Wallenstein, M.D., Weintraub, M.N., Zoppini, A., 2013. Soil enzymes in a changing environment: current knowledge and future directions. Soil Biology and Biogeochemistry 58, 216–234.
Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Peña, A.G., Goodrich, J.K., Gordon, J.I., Huttley, G.A., Kelley, S.T., Knights, D., Koenig, J.E., Ley, R.E., Lozupone, C.A., McDonald, D., Muegge, B.D., Pirrung, M., Reeder, J., Sevinsky, J.R., Turnbaugh, P.J., Walters, W.A., Widmann, J., Yatsunenko, T., Zaneveld, J., Knight, R., 2010. QIIME allows analysis of high-throughput community sequencing data. Nature Publishing Group 7, 335–336.
Cavaleri, M.A., Reed, S.C., Smith, W.K., Wood, T.E., 2015. Urgent need for warming experiments in tropical forests. Global Change Biology 21, 2111–2121.
Chen, D., 2012. Patterns of Soil Properties and Respiration along an Elevation Gradient in the Luquillo Mountains. Northeastern Puerto Rico, Masters Thesis.
Colwell, R.K., Brehm, G., Cardelús, C.L., Gilman, A.C., Longino, J.T., 2008. Global warming, elevational range shifts, and lowland biotic attrition in the wet tropics. Science 322, 258–261.
Conant, R.T., Steinweg, J.M., Haddix, M.L., Paul, E.A., Plante, A.F., Six, J., 2008. Experimental warming shows that decomposition temperature sensitivity increases with soil organic matter recalcitrance. Ecology 89, 2384–2391.
Dalling, J.W., Heineman, K., González, G., Ostertag, R., 2016. Geographic, environmental and biotic sources of variation in the nutrient relations of tropical montane forests. Journal of Tropical Ecology 32, 368–383.
Davidson, E.A., Janssens, I.A., 2006. Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. Nature 440, 1401–1408.
de Boer, W., Kowalchuk, G.A., van Veen, J.A., 2006. "Root-food" and the rhizosphere microbial community composition. New Phytologist 170, 3–6.
de Boer, W., de Folman, L.B., Summerbell, R.C., Boddy, L., 2005. Living in a fungal world: impact of fungi on soil bacterial niche development. FEMS Microbiology Reviews 29, 795–811.
Dieleman, W., Venter, M., Ramachandra, A., Krockenberger, A.K., Bird, M.I., 2013. Soil carbon stocks vary predictably with altitude in tropical forests: implications for soil carbon storage. Geoderma 204, 59–67.
Fahey, T.J., Sherman, R.E., Tanner, E.V.J., 2016. Tropical montane cloud forest: environmental drivers of vegetation structure and ecosystem function. Journal of Tropical Ecology 32, 355–367.
Feeley, K.J., Silman, M.R., 2010. Biotic attrition from tropical forests correcting for truncated temperature niches. Global Change Biology 16, 1830–1836.
German, D.P., Allison, S.D., 2015. Drying and substrate concentrations interact to inhibit decomposition of carbon substrates added to combusted Inceptisols from a boreal forest. Biology and Fertility of Soils 51, 525–533.
German, D.P., Weintraub, M.N., Grandy, A.S., Lauber, C.L., Rinkes, Z.L., Allison, S.D., 2011. Optimization of hydrolytic and oxidative enzyme methods for ecosystem studies. Soil Biology and Biogeochemistry 43, 1387–1397.
Gilbert, G.S., 2005. Dimensions of plant disease in tropical forests. In: Burslem, D.F.R.P., Pinard, M.A., Hartley, S.E. (Eds.), Biotic Interactions in the Tropics: Their Role in the Maintenance of Species Diversity. Cambridge University Press, Cambridge, pp. 141–164.
Girardin, C.A.J., Malhi, Y., Aragão, L.E.O.C., Mamani, M., Huaraca Huasco, W., Durand, L., Feeley, K.J., Rapp, J., Silva-Espejo, J.E., Silman, M., Salinas, N., Whittaker, R.J., 2010. Net primary productivity allocation and cycling of carbon along a tropical forest elevational transect in the Peruvian Andes. Global Change Biology 16, 3176–3192.
Grieve, I.C., Proctor, J., Cousins, S.A., 1990. Soil variation with altitude on volcan Barva, Costa Rica. Catena 17, 525–534.
Grubb, P.J., 1977. Control of forest growth and distribution on wet tropical mountains: with special reference to mineral nutrition. Annual Review of Ecology and Systematics 8, 83–107.
Hanson, C.A., Allison, S.D., Bradford, M.A., Wallenstein, M.D., Treseder, K.K., 2008. Fungal taxa target different carbon sources in forest soil. Ecosystems 11, 1157–1167.
Harvell, C.D., Mitchell, C.E., Ward, J.R., Altizer, S., Dobson, A.P., Ostfeld, R.S., Samuel, M.D., 2002. Climate warming and disease risks for terrestrial and marine biota. Science 296, 2158–2162.
Hättenschwiler, S., Tiunov, A., Scheu, S., 2005. Biodiversity and litter decomposition in terrestrial ecosystems. Annual Review of Ecology, Evolution, and Systematics 36, 191–218.
Hawkes, C.V., Kivlin, S.N., Rocca, J.D., Huguet, V., Thomsen, M.A., Suttle, K.B., 2011. Fungal community responses to precipitation. BioScience 17, 1637–1645.
Holden, S.R., Berhe, A.A., Treseder, K.K., 2015. Decreases in soil moisture and organic matter quality suppress microbial decomposition following a boreal forest fire. Soil Biology and Biochemistry 87, 1–9.
Holden, S.R., Gutierrez, A., Treseder, K.T., 2013. Changes in soil fungal communities, extracellular enzyme activities, and litter decomposition across a fire chronosequence in Alaskan boreal forests. Ecosystems 16, 34–36.
Holdridge, L.R., 1967. Life Zone Ecology. Tropical Science Center, Costa Rica.
Ihrmark, K., Bödeker, I.T.M., Cruz-Martinez, K., Friberg, H., Kubartova, A., Schenck, J., Strid, Y., Stenlid, J., Brandström-Durling, M., Clemmensen, K.E., Lindahl, B.D., 2012. New primers to amplify the fungal ITS2 region–evaluation by 454-sequencing of artificial and natural communities. FEMS Microbiology Ecology 82, 666–677.
IPCC, 2013. Climate Change 2013: the Physical Science Basis. Cambridge University Press, Cambridge.
Jobbágy, E.G., Jackson, R.B., 2000. The vertical distribution of soil organic carbon and its relation to climate and vegetation. Ecological Applications 10, 423–436.
Jones, M.D.M., Forn, I., Cadelha, C., Egan, M.J., Bass, D., Massana, R., Richards, T.A., 2011. Discovery of novel intermediate forms redefines the fungal tree of life. Nature

474, 200–203.
Karmalkar, A.V., Bradley, R.S., Diaz, H.F., 2011. Climate change in Central America and Mexico: regional climate model validation and climate change projections. Climate Dynamics 37, 605–629.
Karmalkar, A.V., Bradley, R.S., Diaz, H.F., 2008. Climate change scenario for Costa Rican montane forests. Geophysical Research Letters 35, 1–5.
Lawton, R.O., Nair, U.S., Pielke, R.A., Sr Welch, R.M., 2001. Climatic impact of tropical lowland deforestation on nearby montane cloud forests. Science 294, 584–587.
Lenoir, J., Gégout, J.C., Marquet, P.A., de Ruffray, P., Brisse, H., 2008. A significant upward shift in plant species optimum elevation during the 20th century. Science 320, 1768–1771.
Looby, C.I., Maltz, M.R., Treseder, K.K., 2016. Belowground responses to elevation in a changing cloud forest. Ecology and Evolution 6, 1996–2009.
Malhi, Y., Silman, M., Salinas, N., Bush, M., Meir, P., Saatchi, S., 2010. Introduction: elevation gradients in the tropics: laboratories for ecosystem ecology and global change research. BioScience 16, 3171–3175.
McGuire, K.L., Bent, E., Borneman, J., Majumder, A., Allison, S.D., Treseder, K.K., 2010. Functional diversity in resource use by fungi. Ecology 91, 2324–2332.
McGuire, K.L., Fierer, N., Bateman, C., Treseder, K.K., Turner, B.L., 2011. Fungal community composition in Neotropical rain forests: the influence of tree diversity and precipitation. Microbial Ecology 63, 804–812.
Meier, C.L., Rapp, J., Bowers, R.M., Silman, M., Fierer, N., 2010. Fungal growth on a common wood substrate across a tropical elevation gradient: temperature sensitivity, community composition, and potential for above-ground decomposition. Soil Biology and Biogeochemistry 42, 1083–1090.
Nguyen, N.H., Song, Z., Bates, S.T., Branco, S., Tedersoo, L., Menke, J., Schilling, J.S., Kennedy, P.G., 2016. FUNGuild: an open annotation tool for parsing fungal community datasets by ecological guild. Fungal Ecology 20, 241–248.
Nottingham, A.T., Turner, B.L., Whitaker, J., Ostle, N., Bardgett, R.D., McNamara, N.P., Salinas, N., Meir, P., 2016. Temperature sensitivity of soil enzymes along an elevation gradient in the Peruvian Andes. Biogeochemistry 127, 217–230.
Oksanen, J., Kindt, R., Legendre, P., O'Hara, R.B., Simpson, G.L., Solymos, P., Henry, M., Stevens, H., Wagner, H., 2012. Vegan: Community Ecology Package. R Package.
Osono, T., 2007. Ecology of ligninolytic fungi associated with leaf litter decomposition. Ecological Research 22, 955–974.
Pan, Y., Birdsey, R.A., Fang, J., Houghton, R., 2011. A large and persistent carbon sink in the world's forests. Journal of Ecology 333, 988–993.
Pounds, J.A., Bustamante, M.R., Coloma, L.A., Consuegra, J.A., Fogden, M.P.L., Foster, P.N., La Marca, E., Masters, K.L., Merino-Viteri, A., Puschendorf, R., Ron, S.R., Sánchez-Azofeifa, G.A., Still, C.J., Young, B.E., 2006. Widespread amphibian extinctions from epidemic disease driven by global warming. Nature 439, 161–167.
Pounds, J.A., Fogden, M., Campbell, J.H., 1999. Biological response to climate change on a tropical mountain. Nature 398, 611–615.
R Core Team, 2016. A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria v.3.3.2.
Raich, J.W., Russell, A.E., Kitayama, K., Parton, W.J., Vitousek, P.M., 2006. Temperature influences carbon accumulation in moist tropical forests. Ecology 87, 76–87.
Reed, H.E., Martiny, J.B.H., 2013. Microbial composition affects the functioning of estuarine sediments. The ISME Journal 7, 868–879.
Romero-Olivares, A.L., Allison, S.D., Treseder, K.K., 2017. Decomposition of recalcitrant carbon under experimental warming in boreal forest. PLoS One 12, e0179674.
Salinas, N., Malhi, Y., Meir, P., Silman, M., Roman Cuesta, R., Huaman, J., Salinas, D., Huaman, V., Gibaja, A., Mamani, M., Farfan, F., 2011. The sensitivity of tropical leaf litter decomposition to temperature: results from a large-scale leaf translocation experiment along an elevation gradient in Peruvian forests. New Phytologist 189, 967–977.
Schawe, M., Gerold, G., Bach, K., Gradstein, S.R., 2010. Hydrometerological patterns in relation to montane forests types along an elevational gradient in the Yungas of Bolivia. In: Bruijnzeel, L.A., Scatena, F.N., Hamilton, L.S. (Eds.), Tropical Montane Cloud Forests. Cambridge University Press, New York, pp. 90–100.
Schneider, T., Keiblinger, K.M., Schmid, E., Sterflinger-Gleixner, K., Ellersdorfer, G., Roschitzki, B., Richter, A., Eberl, L., Zechmeister-Boltenstern, S., Riedel, K., 2012. Who is who in litter decomposition? Metaproteomics reveals major microbial players and their biogeochemical functions. The ISME Journal 6, 1749–1762.
Schuur, E.A.G., 2001. The effect of water on decomposition dynamics in mesic to wet Hawaiian montane Forests. Ecosystems 4, 259–273.
Schuur, E.A.G., Chadwick, O.A., Matson, P.A., 2001. Carbon cycling and soil carbon storage in mesic to wet Hawaiian montane forests. Ecology 82, 3182–3196.
Scowcroft, P.G., Turner, D.R., Vitousek, P.M., 2000. Decomposition of Metrosideros polymorpha leaf litter along elevational gradients in Hawaii. Global Change Biology 6, 73–85.
Setälä, H., McLean, M.A., 2004. Decomposition rate of organic substrates in relation to the species diversity of soil saprophytic fungi. Oecologia 139, 98–107.
Silver, W.L., Lugo, A.E., Keller, M., 1999. Soil oxygen availability and biogeochemistry along rainfall and topographic gradients in upland wet tropical forest soils. Biogeochemistry 44, 301–328.
Stajich, J.E., Berbee, M.L., Blackwell, M., Hibbet, D.S., James, T.Y., Spatafora, J.W., Taylor, J.W., 2009. The fungi. Current Biology 19, 840–845.
Still, C.J., Foster, P.N., Schneider, S.H., 1999. Simulating the effects of climate change on tropical montane cloud forests. Nature 398, 608–610.
Sundqvist, M.K., Sanders, N.J., Wardle, D.A., 2013. Community and ecosystem responses to elevational gradients: processes, mechanisms, and insights for global change. Annual Review of Ecology, Evolution, and Systematics 44, 261–280.
Talbot, J.M., Martin, F., Kohler, A., Henrissat, B., Peay, K.G., 2015. Functional guild classification predicts the enzymatic role of fungi in litter and soil biogeochemistry. Soil Biology and Biogeochemistry 88, 441–456.
Thomas, C.D., Cameron, A., Green, R.E., Bakkenes, M., Beaumont, L.J., Collingham, Y.C., Erasmus, B.F.N., De Siqueira, M.F., Grainger, A., Hannah, L., Hughes, L., Huntley, B., Van Jaarsveld, A.S., Midgley, G.F., Miles, L., Ortega-Huerta, M.A., Peterson, A.T., Phillips, O.L., Williams, S.E., 2004. Extinction risk from climate change. Nature 427, 145–148.
Tremblay, J., Singh, K., Fern, A., Kirton, E.S., He, S., Woyke, T., Lee, J., Chen, F., Dang, J.L., Tringe, S., 2015. Primer and platform effects on 16S rRNA tag sequencing. Frontiers in Microbiology 6, 1–15.
Treseder, K.K., Lennon, J.T., 2015. Fungal traits that drive ecosystem dynamics on land. Microbiology and Molecular Biology Reviews 79, 243–262.
Treseder, K.K., Maltz, M.R., Hawkins, B.A., Fierer, N., Stajich, J.E., McGuire, K.L., 2014. Evolutionary histories of soil fungi are reflected in their large-scale biogeography. Ecology Letters 17, 1086–1093.
Vitousek, P.M., Turner, D.R., Parton, W.J., Sanford, R.L., 1994. Litter decomposition on the Mauna Loa environmental matrix, Hawai'i: patterns, mechanisms, and models. Ecology 75, 418.
Wiens, J.J., 2016. Climate-related local extinctions are already widespread among plant and animal species. PLoS Biology 14. http://dx.doi.org/10.1361/journal.pbio/2001104.
Zimmermann, M., Meir, P., Bird, M.I., Malhi, Y., Ccahuana, A.J.Q., 2009. Climate dependence of heterotrophic soil respiration from a soil-translocation experiment along a 3000 m tropical forest altitudinal gradient. European Journal of Soil Science 60, 895–906.