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Belowground responses to elevation in a tropical montane cloud forest

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Belowground responses to elevation in a tropical montane cloud forest

DISSERTATION

submitted in partial satisfaction of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

in Biological Sciences

by

Caitlin Irene Looby

Dissertation Committee:
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Professor Steven D. Allison
Professor Michael L. Goulden

2017
DEDICATION

To

My parents Robert and Elizabeth, for showing me how to work hard.
My sister Megan and brother-in-law Steve, for showing me love and encouragement.
  My grandma Irene, for showing me how to be strong.
  My Uncle Bobby, for showing me how to stay positive.
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ABSTRACT OF THE DISSERTATION

Belowground responses to elevation in a tropical montane cloud forest

By

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Doctor of Philosophy in Biological Sciences

University of California, Irvine, 2017

Professor Kathleen K. Treseder, Chair

Tropical montane cloud forests (TMCF) are defined by their characteristic cloud cover. This cloud cover endows these forests with unique structural and functional characteristics. However, climate change is reducing cloud cover, and causing warmer temperatures and longer dry periods in TMCF. Studies show that there will be devastating effects on plant and animal species aboveground. But, few studies have investigated soil communities and associated processes in TMCF. The goal of this dissertation was to characterize soil fungal communities and associated properties to determine how they respond to elevation. And in turn, assess how soil fungi and decomposition may respond to projected climate change.

In Chapter 1, I characterized soil fungal communities and associated properties along an elevation gradient in a TMCF in Monteverde, Costa Rica. I found that soil properties, fungal communities, and microbial processes varied with elevation and across seasons. In Chapter 2, I simulated the effects of climate change by performing a soil translocation experiment across this same gradient. I found that fungal decomposers and pathogens may increase under warmer, drier conditions. I also found that decomposition increased under these warmer, drier conditions. The results suggest that decomposition may increase, and plants and animals may be exposed to more pathogens. In Chapter 3, I compared canopy and ground soils along two elevation gradients. I
also assessed if these two soil types differed in how their properties, fungal communities, and extracellular enzyme activity (EEA) varied with elevation. I found that fungal communities differed between canopy and ground soils. Moreover, canopy soils had greater EEA than ground soils, suggesting that canopy soils have a greater decomposition potential and may contribute greatly to C cycling. Carbon dynamics in canopy soil may also be particularly responsive to climate change even more than we might predict based on observations from ground soil. Collectively, the results of my dissertation provide insights into how the structure and function of TMCF may be altered through changes in the fungal community.
INTRODUCTION

The characteristic cloud cover of tropical montane cloud forests (TMCF) distinguishes them from lowland forests. Cloud cover affects the structure and function of TMCF (see Dalling et al., 2016; Fahey and Sherman, 2016). In terms of function, TMCF operate at a slower pace than lowlands. For instance, these forests tend to have slower rates of decomposition causing a buildup of C (Dieleman et al., 2013; Girardin et al., 2013; Schuur et al., 2001). In regards to structure, TMCF support some of the highest biodiversity on the planet—especially canopy epiphytes (Gentry and Dodson, 1987). The high abundance and diversity of epiphytes in the canopy exists because of the added moisture and nutrients delivered by clouds, and retained by canopy soils (Bohlman et al., 1995; Nadkarni et al., 2004). However, climate change is reducing cloud cover, causing warmer temperatures and longer dry periods (Karmalkar et al., 2011; Lawton et al., 2001; Still et al., 1999). The fate of TMCF will depend heavily on the response of soil fungi. Soil fungi are important decomposers, pathogens, and plant symbionts. Therefore, changes in these communities may affect the rate of C breakdown, aboveground biodiversity, and plant recruitment of symbionts in the face of increased environmental stress. But, few studies have characterized soil fungi in TMCF both in the ground and the canopy. And we do not know how climate change may alter soil fungi and rates of decomposition in these forests. Overall, this limits our ability to predict how the structure and function of these forest may change with projected climate change.

Tropical montane cloud forests are high-elevation forests characterized by persistent low-level cloud cover. These clouds affect the structure and function of these forests (Bruijnzeel and Veneklaas, 1998). TMCF have cooler temperatures, more rainfall, horizontal precipitation (via mist), strong winds, less light (decreasing photosynthesis), and higher humidity (decreasing
evapotranspiration) compared to lowland forests (Schawe et al., 2010). Clouds—and the mist they provide—allow these forests to support high-levels of biodiversity. Most TMCF sit in the world’s biodiversity hotspots (Myers et al., 2000). And although they cover less than one percent of the world’s land surface (Bubb et al., 2004), they are one of the most species-rich and species-dense ecosystems on the planet (Bruijnzeel et al., 2010). This is especially true for epiphytes (Gentry and Dodson, 1987)—another defining characteristic of TMCF (Grubb, 1977).

The effects of clouds extend into the soil. TMCF have slower rates of decomposition and nutrient cycling (Bruijnzeel et al., 1993), especially at high elevations where temperatures are cool (Vitousek et al., 1994), and soils are waterlogged (Schuur, 2001) and anaerobic (Silver et al., 1999). Slower rates of decomposition causes a buildup of soil organic matter (Raich et al., 2006) and soil C (Dieleman et al., 2013; Girardin et al., 2010; Schuur et al., 2001). But, there is not only soil on the ground, soil also accumulates in the canopy. Litter and other epiphyte matter collects and decomposes on the branches of canopy trees forming highly organic soil. Epiphytes depend on canopy soils to hold water and nutrients (Bohlman et al., 1995; Nadkarni et al., 2004) so they can survive the environmentally stressful conditions in the canopy.

However, deforestation in lowland forests is causing an increase in land and sea surface temperatures, which is reducing cloud cover in TMCF (Karmalkar et al., 2011; Lawton et al., 2001; Still et al., 1999). In turn, this is causing more variable precipitation, and an increase in the number of days without precipitation, especially during the dry season (Karmalkar et al., 2008; Pounds et al., 1999). These changes will affect the structure of these forests (via changes in biodiversity) and ecosystem function (Martínez et al., 2009). Many of these changes may depend on the response of soil fungi. Because soil fungi are sensitive to shifts in temperature and moisture, elevation gradients can be excellent tools in understanding how soil fungi may shift
under climate change (Malhi et al., 2010; Sundqvist et al., 2013). But, not only do we have a limited understanding of how soil fungi will change under future climate, we also have knowledge gaps in what types of fungi are currently present. This is especially true for canopy soils, and will become increasingly important because epiphyte communities are extremely vulnerable to climate change (Nadkarni, 2010).

Soil fungi have important functional roles in ecosystems. For instance, fungi are the primary decomposers in soil (de Boer et al., 2006), and dominate the decomposition of many forms of C, like cellulose and hemicellulose (de Boer et al., 2005). Because certain fungi target different types of C (Hanson et al., 2008; McGuire et al., 2010; Setälä and McLean, 2004), shifts in temperature and moisture with elevation could affect what fungi are present, and thus what types of C are targeted. It is especially unclear how canopy soils contribute to C cycling TMCF. Climate change in TMCF could lead to increased rates of decomposition in soil, and thus CO₂ release into the atmosphere.

Soil fungi can also affect the structure of TMCF because fungal pathogens are drivers of biodiversity in the tropics (Gilbert, 2005). Local extinctions are widespread as climate change is forcing plants and animals to migrate upslope to maintain their optimal climates (Colwell et al., 2008; Thomas et al., 2004; Wiens, 2016). Not only are species migrating upslope and suffering local extinctions, but also biodiversity is declining due to increased fungal pathogens (Pounds et al., 1999; 2006). It is unclear how fungal pathogens may shift under future climate conditions, and how this might affect aboveground diversity. In addition, fungal symbionts—like endophytes—can help plants alleviate environmental stress, such as drought (Giauque and Hawkes, 2013). Recruitment of these symbionts may become especially important under future climate conditions, especially in the canopy. Overall, it is important to determine what types of
fungal functional groups are present in TMCF soils, and how these communities may change under projected climate change.

In summary, how fungal communities and associated processes, like decomposition, vary with elevation in TMCF is uncertain. Moreover, we do not know how soil fungi and decomposition may change under future climate change, and how these changes will affect the structure and function of these forests. Therefore, the goal of this dissertation was to address the following questions:

1. How do soil properties, fungi, and associated processes vary with elevation? And do these trends vary seasonally?
2. How will soil fungi and decomposition change under projected warmer temperatures and drier conditions in a TMCF?
3. Do canopy and ground soils differ in their soil properties, fungal communities, and decomposition potential? Do these two soil types differ in how they vary with elevation, and thus their sensitivity to future climate change?

This dissertation was conducted along elevation transects on the Cordillera de Tilarán within the Monteverde Cloud Forest Reserve (10°18´N, 84°47´W) in Monteverde, Costa Rica. In Chapter 1, I established an elevation transect on the Pacific slope of the mountain. This transect ranged from 1300 to 1850 m.a.s.l. with sites established every 50 m increase in elevation. This study area is located on the leeward side of the mountain, consists of a strong climatic gradient, and is experiencing rapid climate change. I characterized soil properties, fungal community composition, and microbial properties along this elevation transect.

In Chapter 2, I performed a transplant experiment along this same elevation gradient to mimic future climate conditions. I moved soil from 1850 m.a.s.l. down the mountain to represent
a 1, 2, 3, 4°C increase in temperature, and a corresponding 20% total decline in soil moisture. Soil was kept in “microbial cages”; these cages allow water, nutrients, and some bacteria to pass through, but prohibit local fungi from entering (Allison et al., 2013). Using this approach, I assessed how soil fungi and decomposition—in terms of percent mass loss and EEA—shifted under these warmer and drier conditions.

In Chapter 3, I compared differences in canopy and ground soils along two elevation gradients on the Atlantic (windward) and Pacific (leeward) slopes. I determined how soil properties, fungal communities, and EEA differ between the two soil types. Moreover, I determined differences in elevational trends between canopy and ground soils to assess potential differences in sensitivity to future climate change. Ultimately, this dissertation provides an in depth understanding of what types of soil fungi are present and how these fungi are structured with elevation in TMCF. It delivers new information on how soil fungi and decomposition may be altered due to climate change. Collectively, this dissertation offers insights into how the structure and function of these forests may change.
CHAPTER 1

Belowground responses to elevation in a changing cloud forest

Introduction

Tropical ecosystems have a disproportionate influence on global biodiversity, primary production, and biogeochemical cycling (Townsend et al., 2011). Tropical montane cloud forests (TMCF) are a distinct category of tropical ecosystem. They are high elevation forests characterized by persistent, low-level cloud cover. These clouds alter light, temperature, and precipitation, thereby endowing TMCF with unique structural and functional characteristics (Bruijnzeel and Veneklaas, 1998). For example, TCMF are shorter in stature and exhibit lower productivity, increased epiphytic biomass, and slower nutrient cycling rates compared to the lowland tropics (Grubb, 1977; Hobbie and Vitousek, 2000; Tanner et al., 1998).

Tropical montane cloud forests are extremely important hydrologically, biologically, and ecologically. In terms of hydrology, these ecosystems play a crucial role in local and regional water budgets and atmospheric circulation of water (Lawton et al., 2001; Nair et al., 2003; Ray et al., 2006). In terms of biology, TMCF are biodiversity hot spots. Even though they cover only 0.4 percent of the Earth’s land surface, they contain 20% of the world’s plant species and 16% of its vertebrate species (Myers et al., 2000).

In terms of ecology, TMCF have decreased rates of decomposition due to abiotic factors such as decreased temperatures and water-logged soils (Vitousek and Sanford 1986). Inevitably, decreased rates of decomposition can lead to larger soil C stocks, higher soil C:N ratios, and stronger nutrient limitations present within TMCF (Vitousek et al., 1994). This suite of characteristics, such as soil temperature, moisture, and C:N ratios can affect the composition of microbial communities within the soil.
Few studies have investigated microbial communities within TMCF, and information regarding soil fungal communities is especially limited. Fungal communities are sensitive to changes in temperature and precipitation (McGuire et al., 2011; Rustad and Fernandez, 1998; Schuur, 2001). In addition, soils with high C:N ratios tend to support a greater abundance of fungi (Fierer et al., 2009). Fungal communities are important because they conduct much of the decomposition that occurs within soils. Changes in the fungal community can induce changes in decomposition (Setälä and McLean, 2004), ostensibly because fungal species vary in their role in decomposition (Hanson et al., 2008; McGuire et al., 2010). We know that abiotic factors, such as temperature and moisture, can cause slower rates of decomposition, but biotic factors such as shifts in fungal community might also contribute.

Soil temperature, moisture, and C:N ratios can shape fungal communities tend to shift predictably with elevation. However, shifts in belowground communities with elevation are variable (Bahram et al., 2012; Bryant et al., 2008; Fierer et al., 2011; Lin et al., 2010; Meng et al., 2013; Singh et al., 2014; 2012; Whitaker et al., 2014b). This is especially true for fungi, and most fungal studies have focused on a specific functional group (e.g., Bahram et al., 2012; Coince et al., 2014; Gai et al., 2012; Gorzelak et al., 2012; Gómez-Hernández and Williams-Linera, 2012; Meier et al., 2010; Zimmerman and Vitousek, 2012).

Shifts in temperature and moisture along elevation gradients could elicit changes in the fungal community, which in turn can alter rates of decomposition and nutrient cycling along the gradient. Recently, Pellissier et al. (2014) used pyrosequencing to demonstrate that fungal communities varied across an elevation gradient in Swiss alpine grasslands. Within tropical montane forests, work has been conducted in Ecuador on mycorrhizal fungi (e.g., Camenzind and Rillig 2013; Camenzind et al., 2014; Camenzind et al., 2015). Moreover, Cantrell et al.
(2013) used fatty acid composition and TRFLP analyses to determine how the abundances of microbial functional groups vary with elevation in Puerto Rico. Tedersoo et al. (2014) included TMCF in their global assessment of fungal diversity, but not along an elevation gradient. The current study is one of the first to examine how whole soil fungal communities respond to changes to elevation within TMCF using high-throughput sequencing.

We used an elevation transect on the Pacific slope of the Cordillera de Tilarán in Monteverde, Costa Rica to determine how fungal and soil dynamics vary with elevation and season. We hypothesized that increasing elevation would be associated with increased soil C:N ratios due to decreased temperatures and availability of soil N. Furthermore, we hypothesized that fungal abundance should increase at higher elevations in response to higher soil C:N, which typically favors fungal growth. We also predicted decreased microbial basal respiration at higher elevations due to decreased temperatures and water-saturated soils. Lastly, we hypothesized that fungal community composition would shift with elevation, with a decline in richness at higher elevations due to environmental filters (i.e., temperature and moisture) structuring these communities.

**Methods**

**Field sites**

An elevation transect was established in August 2013 along the Pacific slope of the Cordillera de Tilarán in the Monteverde Cloud Forest Reserve (10°18´N, 84°47´W) near Monteverde, Costa Rica. This study area is located on the leeward side of the mountain range in an undisturbed primary forest. The transect ranges from 1305 m.a.s.l. to 1850 m.a.s.l. with sites located every 50 m increase in elevation (Table 1.1). These 12 sites cover three Holdridge life zones: premontane,
lower montane, and montane forests (Holdridge 1967; Table 1.1). The 50 m intervals in elevation allowed us to examine high-resolution changes in climate. We assessed differences in the dry season (January to May) versus the wet season (June to November). More detailed climatic and physical characteristics of the Monteverde Cloud Forest Reserve are provided by Clark et al., 2000.

Soil Sampling

We collected soils in August 2013 (wet season) and April 2014 (dry season). Average minimum and maximum daily temperatures for the 2013 wet season were 15.6°C and 20.3°C (measured at 10.3092° N, 84.8135° W; 1375 m.a.s.l.). Average monthly precipitation for the wet season was 2471 mm; 1795 mm was recorded during August 2013. Average minimum and maximum daily temperatures for the 2014 dry season were 14.9°C and 21.7°C. Average monthly precipitation was 183 mm during the dry season, with 385 mm recorded during April 2014. Soil sampling occurred at the end of the month for both seasons, and thus precipitation measurements represent the month prior to sampling.

Twelve soil cores (2 cm diameter by 10 cm deep) were taken mainly in the O horizon at random locations along a 20 m line at each of the twelve sites; sampling locations were at least 2 m apart. Although we sampled mainly in the O horizon, we observed that the O horizon was a greater proportion of the sample with increasing elevation. A separate set of samples was collected at each site to determine soil bulk density. We used bulk density measurements to adjust values to a square meter basis for microbial basal respiration and fungal abundance in order to understand potential ecosystem-level effects. We transported soil on ice to UC Irvine within 48 hours of collection. Prior to analysis, soil from each site was composited, homogenized, and sieved at 2 mm. Soils were stored at 18°C for biogeochemical analyses, and
were processed within 72 hours after collection. Soils were stored at -20°C for fungal community analysis.

**Soil properties**

Soil temperature was measured at 10 cm depth in four randomly-selected locations at each site. Subsamples were weighed, dried 65°C for 48 hours, and then re-weighed to determine gravimetric moisture content. Soil pH was determined using a 1:2 ratio (w/v) of soil to DI H₂O. Soil C and N concentrations were measured by combustion on an elemental analyzer (Flash EA 1112, Thermo Scientific, Waltham, MA) and used to determine C:N ratios.

**Fungal abundance**

Fungal hyphal length was measured as a metric for total soil fungal abundance. We measured the length of fungal hyphae during the wet and dry seasons using a modified procedure from Brundrett et al. (1996). Briefly, 3 subsamples of 4 g (wet weight) soil were extracted with 1.5 M solution of sodium hexametaphosphate. This soil solution was passed through a 0.2-µm nylon filter to collect the 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 200x on a Nikon phase-contrast microscope (Nikon Eclipse e400, AG Heinze, Lake Forest, CA, USA).

**Microbial basal respiration**

Microbial basal respiration is the rate of CO₂ respiration in soil originating from the decomposition of organic matter by microbes (Alef and Nannipieri 1995). Microbial basal respiration was measured in a laboratory incubation using an infrared gas analyzer (PP Systems EGM-4, Amesbury, MA, USA) to monitor CO₂ flux using a closed chamber approach.
Measurements were made at 20°C during a 4-hr incubation, which provides the best prediction for initial rates of respiration (Creamer et al., 2014).

**Fungal community composition**

We extracted DNA from three ~0.25 g samples of soil from each elevation using the MoBio PowerSoil kit (MoBio, Carlsbad, CA). DNA concentrations were standardized to 10 ng/µL prior to PCR amplification.

Primers targeting the 5.8S encoding gene were modified to amplify the ITS2 region of the ribosomal encoding genes from fungi. By producing a shorter amplicon than primers targeting the entire ITS region, these primers reduce species bias and PCR chimeras while maintaining the same level of fungal diversity (Ihrmark et al., 2012). A ~340 bp region of fungal ITS DNA was amplified with a staggered primer design. This included a forward primer (ITS9f; AATGATACGGCGACCACCAGATCTACAC TCTTTCCCTACA CGACGCTCTTCCGATCT NNNNGAACGCAGCRAAIIGYGA) and barcoded, reverse primers that contain the reverse complement of the 3’ Illumina adapter (CAAGCAGAAGACGGCATACGAGAT), a unique, 12 bp barcode, a pad (AGTCAGTCAG), a linker sequence (CC), and the ITS4 primer (TCCTCCGCTTATTGATATGC). The staggered design incorporated an additional 0, 1, 2 or 3 bp preceding the ITS4 primer (i.e., CC-ITS4, CC-G-ITS4, CC-AG-ITS4, or CC-CAG-ITS4). This staggered design increases the level of diversity of amplicon sequences across the Illumina Miseq flowcell early in the read, which improves the accuracy of amplicon cluster detection and resolution and overall sequence quality (Tremblay et al., 2015).

Each reaction contained: 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 (10 ng) of DNA. The
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 sample were run in triplicate, pooled, and purified with Agencourt AMPure XP magnetic beads (Beckman Coulter, Brea, CA). Purified samples were quantified with the Qubit dsDNA High Sensitivity Assay Kit (Life Technologies, Grand Island, NY) and pooled in equimolar concentrations. The pooled sample was sequenced as 2 x 300 bp paired end reads on one lane of an Illumina MiSeq sequencer at the Genomics core in the Institute for the Integrative Genome Biology at the University of California, Riverside.

We obtained a total of ~16.9 million sequences, which were processed through Quantitative Insights Into Microbial Ecology (QIIME) pipeline v. 1.9.0 (Caporaso et al., 2010). In QIIME, sequences were quality checked, aligned, and clustered into operational taxonomic units (OTUs) at a 97% similarity cut off. After quality control, our dataset contained ~3.8 million high-quality sequences that were used to generate the OTU table. One representative sequence from each OTU was chosen, and the closest taxonomic identity was determined via BLAST comparison in GenBank and the UNITE (v.7; release date 3.2.2015) database. To avoid bias due to different library sizes, samples were rarefied to the lowest coverage: 14,628 sequences per sample. Dataset wide singletons and non-fungal OTUs were identified and discarded manually prior to statistical analysis.

Finally, we assigned OTUs to functional groups based on taxonomic identity. We used the same designations as (Tedersoo et al., 2014), although we grouped all pathogenic and parasitic taxa into one category, “pathogens”, regardless of host. We also split saprotrophic taxa into “free-living filamentous fungi” and “yeasts” depending on morphotype. Yeasts included
facultative and obligate yeast. We were able to classify 43-61% of OTUs to functional group within each sample.

Statistical analyses

We used linear regressions to test for relationships between the different soil parameters and elevation. We nested subsamples within elevation to account for variances that may be associated in our experimental design. Differences are reported as significant when \( p < 0.050 \). Data for microbial basal respiration and relative abundances of Chytridiomycota and Zygomycota were ranked and analyses were performed to ensure that outliers were not solely driving these relationships. In order to examine differences between these relationships during the wet and dry seasons, an analysis of covariance (ANCOVA) was used (Sokal and Rohlf, 2012). Linear regressions are reported as different when \( p < 0.050 \) indicating that there are differences in seasonality in the relationships between the measured soil parameter and elevation.

A multiple regression analysis was conducted on dry season data to test for the relationship between hyphal length and soil moisture versus microbial basal respiration. All statistical analyses were carried out using the statistical program R (Version 0.98.507, R Development Core Team 2013).

Taxonomic richness (alpha diversity) was computed using observed number of fungal OTUs, Shannon diversity index, and Simpson’s diversity index in QIIME. Relative abundances of fungal phyla were calculated as the proportion of sequences present in each sample. We determined these proportions by calculating the total number of sequences from each phylum within that subsample and dividing by the total number of sequences per subsample. For taxonomic richness of fungal communities and relative abundance of fungal phyla, we used linear regressions and ANCOVA analyses to test for differences between elevations and seasons.
To determine the relationship between fungal community composition and elevation, we used a PERMANOVA analysis using Bray-Curtis dissimilarity and the adonis function in the Vegan package of R (Anderson 2001; Oksanen et al., 2012). In this model, we also tested the relationships between fungal community composition and season, Holdridge life zone, soil temperature, soil moisture content, C:N ratios, and pH independently. Nonmetric multidimensional scaling (NMS) plots were used to visualize fungal community composition.

To examine changes in functional groups with elevation, we counted the number of OTUs (i.e., richness) within each functional group in each sample. We then took the average of the three replicates per site/season and performed linear regressions.

**Results**

**Soil properties**

Soil temperature decreased significantly with elevation \((p < 0.001\) for each season; Fig. 1.2a; Table 1.2), and the extent of the decline differed between seasons \((p < 0.001\). More specifically, soil temperature declined about 5°C during the wet season, but only 3°C during the dry season.

In addition, soil moisture increased significantly with elevation \((p \text{ (wet)} = 0.008, p \text{ (dry)} = 0.003; \) Fig. 1.2b; Table 1.2). As expected, soil moisture was higher during the wet season than the dry season, and there was a significant difference between the wet and dry seasons \((p < 0.001\).

Soil pH decreased significantly with elevation during each season \((p \text{ (wet)} = 0.001, p \text{ (dry)} = 0.011; \) Fig. 1.2c; Table 1.2), and the seasons did not differ significantly from one another \((p = 0.345\).
Moreover, soil C:N ratios increased significantly with elevation during both seasons ($p < 0.001$ for each season; Fig. 1.2d; Table 1.2) with no significant differences between season ($p = 0.462$).

**Fungal abundance**

Fungal hyphal length increased significantly with elevation during the wet and dry seasons ($p < 0.001$ for each season; Fig. 1.3a; Table 1.2), and there was a significant difference between the seasons ($p < 0.001$). There was a stronger correlation between hyphal length and elevation during the wet season ($r^2 = 0.862$) than the dry season ($r^2 = 0.637$).

**Microbial basal respiration**

Rates of microbial basal respiration increased significantly with elevation during the dry season ($p < 0.001$; Fig. 1.3b; Table 1.2). Seasonality was most pronounced in the montane forest sites (1743 to 1850 m.a.s.l.), where microbial basal respiration was notably higher during the dry season than in the wet season. To determine a potential cause of this trend, we performed a further analysis to quantify relationships between this parameter and fungal abundance and soil moisture content during the dry season. Fungal abundance and soil moisture both had a significant effect on microbial basal respiration ($r^2 = 0.559$). Microbial basal respiration increased significantly with increasing fungal abundance ($p < 0.001$) and, to a lesser extent, increasing soil moisture ($p = 0.022$). Dry season data were used for both parameters due to the fact that the trend was only present during the dry season.

**Fungal diversity and community composition**

Taxonomic richness decreased significantly with elevation during the wet season, based on Simpson’s diversity index ($p = 0.008$; Fig. 1.4; Table 1.3) and observed number of fungal OTUs ($p = 0.050$; Table 1.3). Values for Simpson’s diversity index are inversely proportional to
diversity: higher values indicate decreased diversity. The Shannon diversity index did not vary significantly with elevation. In addition, there were no significant relationships of any of the diversity metrics with elevation during the dry season (Table 1.3).

Fungal communities shifted significantly with elevation ($p<0.001$; Fig. 1.5; Table 1.4) and with other soil conditions that co-varied with elevation: temperature, moisture, C:N, and pH ($p<0.001$ in each case). Variations in fungal communities were also explained by Holdridge life zone ($p<0.001$) and, to a lesser extent, by season ($p=0.039$).

Shifts in fungal community composition were associated with shifts in the taxonomic richness within certain fungal phyla. During the wet season, the proportion of sequences representing the phyla Chytridiomycota and Zygomycota increased with elevation ($p$ (Chytridiomycota) =0.018; Fig. 1.6a; $p$ (Zygomycota) = 0.003; Fig. 1.6b; Table 1.5). In contrast, the Glomeromycota decreased with elevation ($p=0.001$; Fig 1.6c; Table 1.5) during the dry season. The taxonomic richness of some functional groups varied significantly with elevation during the wet season (Fig. 1.7; Table 1.6). Specifically, OTU richness of free-living filamentous fungi declined at the highest elevations ($p=0.005$; Fig. 1.7a). Endophytic OTU richness declined markedly with elevation in the lower half of the gradient, and remained consistently low in the upper half ($p=0.005$; Fig. 1.7e). Ectomycorrhizal (ECM) fungi displayed the opposite trend—OTU richness increased with elevation, leveling off at the highest sites ($p=0.006$; Fig. 1.7c). Yeasts and lichens declined linearly with elevation, but only marginally significantly ($p$ (yeasts) =0.060, $p$ (lichens) =0.071; Fig. 1.7d and f). There were no elevational trends in OTU richness in pathogenic, ericoid, and arbuscular mycorrhizal functional groups in the wet season. Moreover, the only functional group that varied significantly with elevation during the dry season was arbuscular mycorrhizal fungi (i.e., Glomeromycota; Fig. 1.6c).
Discussion

Abiotic factors

Elevation gradients can be used to understand the influence of important abiotic factors on belowground communities and associated processes (Malhi et al., 2010). Our study provided an examination of the responses of soil dynamics and fungal communities to changes in elevation. Soils at higher elevations experience lower temperatures and wetter conditions. Results from this study support the notion that there may be abiotic and biotic filtering on belowground communities.

We found that soil C:N ratios increased with increasing elevation. Many elevation studies have reported higher C:N ratios and larger pools of C in soils at higher elevations (Dieleman et al., 2013; Girardin et al., 2010; Moser et al., 2011; Raich et al., 2006; Soethe et al., 2008; Whitaker et al., 2014a). Moreover, tropical montane forests can be N limited (Fisher et al., 2013; LeBauer and Treseder, 2008; Vitousek and Sanford, 1986). Although we did not directly test for N limitation, the increase in C:N ratios with elevation suggest low N availability higher elevations.

Fungal abundance

Elevated soil C:N ratios at the higher elevation sites may have contributed to the observed increases in fungal abundance. Indeed, high soil C:N is often associated with fungal-dominated communities (Fierer et al., 2009). A previous study found no relationship between hyphal respiration and elevation (Fisher et al., 2013). In an elevation gradient in Andean Peru, Whitaker et al. (2014a) likewise found an increase in fungal dominance as well as soil C:N ratios at higher elevations. Nevertheless, few other studies have directly assessed fungal abundance within tropical montane forests. Litter at higher elevations is typically more recalcitrant due to
increased nutrient limitation, leaf thickness, and sclerophylly (Bruijnzeel and Veneklaas, 1998). This pattern could favor the presence of fungi, which can break down more recalcitrant organic matter than many bacteria (de Boer et al., 2006; Floudas et al., 2012; Schneider et al., 2012). Higher amounts of organic matter, typical of higher elevations (Grieve et al., 1990; Tanner et al., 1998) can also support a greater abundance of fungi.

Our results demonstrate that fungal abundance was greater in the dry season than in the wet season. Other studies have shown increased fungal abundance during drier conditions (Guadarrama and Álvarez-Sánchez, 1999; Hawkes et al., 2011; Sigüenza et al., 1996). This could be due to plant phenology. During the dry season, there is increased litterfall, providing more substrate for fungi to decompose. Alternately, fungal turnover may have been slower in these drier conditions.

Microbial activity

The increase in fungi could have contributed to the rise in microbial basal respiration with elevation during the dry season. Other studies have found similar trends with elevation. In a cloud forest in Colombia, (Cavelier and Peñuela, 1990) found that increased soil respiration accompanied increased moisture at higher elevations. In the current study, microbial basal respiration was correlated with soil moisture as well as fungal abundance. It is possible that the greater abundance of fungi at higher elevations—and during the dry season—improved the potential for mineralization of organic material. This potential was then modified by water availability, so that microbial basal respiration was reduced at lower elevations during the dry season, where soil moisture was low.

Studies in Monteverde have shown that the orographic cloud layer is rising due to the increase in sea surface temperatures, causing an increase in dry days, which are days that receive
no precipitation or mist (Karmalkar et al., 2008; Lawton et al., 2001; Pounds et al., 2006; 1999; Still et al., 1999). This trend is especially evident during the dry season and on the Pacific slope of the Cordillera de Tilarán. Ultimately, climate change is exposing this ecosystem to drier and warmer conditions due to the rise of the cloud layer.

Our results suggest that microbial basal respiration could potentially be sensitive to the rising cloud layer. Microbial basal respiration varied with elevation only during the dry season, when clouds typical provide a significant source of water (Goldsmith et al., 2012; Holwerda et al., 2010). Microbial production of CO$_2$ might decrease as the cloud layer rises, since microbial basal respiration was particularly high in the sites exposed to cloud cover. The response of microbial production of CO$_2$ to changes in temperature and moisture may determine whether TMCF accentuate or mitigate greenhouse gas emissions under future climate.

**Fungal community composition**

Our knowledge of how elevation affects belowground communities is limited, and the trends that have been determined are variable, particularly in regards to fungi (Bahram et al., 2012; Bryant et al., 2008; Fierer et al., 2011; Meier et al., 2010; Meng et al., 2013; Singh et al., 2014; 2012; Whitaker et al., 2014b). Most studies have focused on a specific fungal functional group (e.g., Bahram et al., 2012; Coince et al., 2014; Gai et al., 2012; Gorzelak et al., 2012; Gómez-Hernández and Williams-Linera, 2012; Meier et al., 2010; Zimmerman and Vitousek, 2012). This study is one of the first using high-throughput sequencing of whole soil fungal communities and their responses to changes in elevation, especially within TMCF. Our hypothesis that fungal community composition would shift with elevation was supported. Moreover, we found that fungal communities shifted with the environmental parameters that
change with elevation (i.e., temperature, soil moisture content, C:N, pH, and Holdridge life zone). Fungal communities also varied seasonally, but to a lesser extent.

Moreover, we found that taxonomic richness and diversity of fungi declined with elevation according to observed number of fungal OTUs and Simpson’s diversity index during the wet season. Others have found declines in richness of plant, animals, and microbes with elevation. This pattern occurs in about 25 percent of studies, although peaks at mid-elevations are more common (Rahbek, 2005).

Since fungal diversity is lower at higher elevations, some combination of dispersal, environmental, and biotic filters may be operating there. In terms of dispersal, winds are often strong in Monteverde, so soil fungi may move along the gradient relatively easily. Regarding environmental filters, soil fungi are sensitive to changes in temperature and moisture (Allison and Treseder, 2008; Hawkes et al., 2011; McGuire et al., 2011). Our elevation gradient ranged widely in environmental variables such as soil temperature, moisture content, and C:N over a small distance. These steep changes might have filtered taxa at the higher elevations. However, fungal hyphae were more abundant at higher elevations, and microbial basal respiration did not appear to be inhibited there during the wet season. Thus, we did not detect any evidence that the environmental conditions at the upper sites were particularly stressful for fungi during the wet season, when the declines in richness occurred. It is possible that a biotic filter was acting on fungal taxa. Perhaps competition was stronger at higher elevations, leading to the exclusion of some fungal taxa.

These shifts in fungal community composition were not driven by shifts in the most abundant fungal phyla (i.e., Ascomycota and Basidiomycota). Instead, higher elevations were associated with increases in the number of taxa from the phyla Chytridiomycota and
Zygomycota during the wet season. Chytridiomycota and Zygomycota are older phyla that are relatively constrained to regions with higher levels of precipitation (Treseder et al., 2014). An increase in the relative abundance of these phyla with elevation during the wet season may be due to the higher soil moisture content present at high elevation at the end of the gradient.

Functional groups of fungi also responded to elevation, primarily during the wet season. Specifically, the community tended to shift from free-living filamentous fungi and endophytes at the lower sites toward ECM fungi at the higher sites. For ECM fungi, similar trends have been found along some elevational gradients (Gómez-Hernández and Williams-Linera, 2012; Miyamoto et al., 2014), but not in others (Bahram et al., 2012; Kernaghan and Harper, 2001). Soil conditions can drive richness of ECM fungi, since these fungi can be habitat-specific (Peay et al., 2010; 2015). For instance, (Gómez-Hernández and Williams-Linera, 2012) reported that soil moisture was related to ECM richness in their elevational gradient. Nitrogen availability might also be a factor. Ectomycorrhizal fungi absorb and deliver soil organic N to plants in exchange for C (Hobbie and Hobbie, 2006). Plants may have invested more in ECM fungi at higher elevations, where soil C:N ratios are relatively high and N might limit plant growth. Thus, at higher elevations ECM fungi might be outcompeting free-living filamentous fungi for C (Gadgil and Gadgil, 1975; Gadgil and Gadgil, 1971). If so, this process might act as a biotic filter causing decreased richness at higher elevations.

In contrast, richness of endophytic fungi declined with elevation. Other studies have documented a similar trend (Ranelli et al., 2015; Zimmerman and Vitousek, 2012). Endophytes are symbiotic fungi that inhabit the aboveground tissues of plants. Nevertheless, litterfall can deliver endophytic DNA to the soil. Endophytic fungi can confer stress resistance as they reduce plant water loss when water is limiting (Giauque and Hawkes, 2013). Endophyte community
composition can be driven by rainfall along elevation gradients (Zimmerman and Vitousek, 2012). In our gradient, drier conditions at the lower sites could have induced plants to form relationships with more endophytic taxa.

These relationships with elevation—and associated climatic conditions—suggest that fungal communities along this elevation gradient may be vulnerable to climate change. Studies in Monteverde suggest that climate change is exposing this ecosystem to drier and warmer conditions. Our study suggests that both the abiotic (temperature, moisture) and biotic (microbial basal respiration and fungal community composition) properties of soils may be sensitive to the rising cloud layer. CO$_2$ emissions might decline based on our observations of basal microbial respiration. Fungal communities may become more diverse, especially in free-living filamentous fungi. Since this functional group includes strong decomposers (Treseder and Lennon, 2015), the shift in community composition may mitigate somewhat the decrease in CO$_2$ emissions. The balance of these abiotic and biotic responses may determine whether TMCF accentuate or mitigate greenhouse gas emissions under future climate.

Consequently, although this study is observational, it demonstrates that fungal community composition shifts with elevation and with climatic factors that co-vary with elevation (i.e., temperature and moisture). Moreover, this study suggests that there may be both abiotic and biotic filtering occurring across this elevation gradient. For instance, higher elevations may be suitable environments for fungi, as demonstrated by the higher abundance of fungi and increased microbial basal respiration at these elevations. However, there is decreased richness at higher elevations, suggesting that there is stronger competition occurring at these sites. This was further shown by the differing responses of fungal functional groups to elevation. Ectomycorrhizal fungi may be outcompeting other fungal functional groups, such as free-living
filamentous taxa. Thus, there may be abiotic and biotic mechanisms for decreased rates of decomposition at higher elevations.

Implications

This elevation gradient can be used to improve our understanding of the roles that temperature and moisture have in influencing belowground communities and processes, and allow for better predictions of how tropical ecosystems will respond to climate change. Studies in Monteverde have shown that the rising cloud layer is particularly influential during the dry season. However, our study demonstrates that there is important structuring of fungal communities during the wet season. Therefore, any changes in climate could have important ecological consequences by potentially changing how these communities are structured, and altering responses of important belowground processes that may mitigate or accentuate climate change. Nevertheless, this study examines soil properties and fungal communities on one elevation gradient over one year, and thus there is much more to learn within this study system.
Table 1.1. Field sites locations and life zone assignments along the permanent transect established within the Monteverde Cloud Forest Reserve.

<table>
<thead>
<tr>
<th>Elevation (m.a.s.l.)</th>
<th>Latitude (N)</th>
<th>Longitude (W)</th>
<th>Holdridge Life Zone</th>
</tr>
</thead>
<tbody>
<tr>
<td>1305</td>
<td>10°16'41.71&quot;</td>
<td>84°47'01.29&quot;</td>
<td>Premontane Forest</td>
</tr>
<tr>
<td>1352</td>
<td>10°16'41.51&quot;</td>
<td>84°46'59.27&quot;</td>
<td>Premontane Forest</td>
</tr>
<tr>
<td>1399</td>
<td>10°17'26.70&quot;</td>
<td>84°47'34.36&quot;</td>
<td>Premontane Forest</td>
</tr>
<tr>
<td>1430</td>
<td>10°17'28.81&quot;</td>
<td>84°47'30.97&quot;</td>
<td>Premontane Forest</td>
</tr>
<tr>
<td>1501</td>
<td>10°17'43.85&quot;</td>
<td>84°47'36.96&quot;</td>
<td>Premontane Forest</td>
</tr>
<tr>
<td>1549</td>
<td>10°18'18.40&quot;</td>
<td>84°47'46.36&quot;</td>
<td>Lower montane Forest</td>
</tr>
<tr>
<td>1600</td>
<td>10°18'40.73&quot;</td>
<td>84°47'56.37&quot;</td>
<td>Lower montane Forest</td>
</tr>
<tr>
<td>1656</td>
<td>10°18'48.69&quot;</td>
<td>84°47'57.74&quot;</td>
<td>Lower montane Forest</td>
</tr>
<tr>
<td>1698</td>
<td>10°18'54.42&quot;</td>
<td>84°47'52.21&quot;</td>
<td>Lower montane Forest</td>
</tr>
<tr>
<td>1743</td>
<td>10°18'57.64&quot;</td>
<td>84°47'47.83&quot;</td>
<td>Montane Forest</td>
</tr>
<tr>
<td>1797</td>
<td>10°19'01.02&quot;</td>
<td>84°47'43.80&quot;</td>
<td>Montane Forest</td>
</tr>
<tr>
<td>1850</td>
<td>10°19'02.02&quot;</td>
<td>84°47'40.03&quot;</td>
<td>Montane Forest</td>
</tr>
</tbody>
</table>
**Table 1.2.** Changes in soil properties and microbial dynamics with elevation and season.\(^1\)

<table>
<thead>
<tr>
<th>Soil characteristics</th>
<th>Wet season</th>
<th>Dry season</th>
<th>Wet vs. Dry</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(F)</td>
<td>(r^2)</td>
<td>(p)</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>66.5</td>
<td>0.861</td>
<td>(&lt;0.001)</td>
</tr>
<tr>
<td>Moisture (%)</td>
<td>2.73</td>
<td>0.204</td>
<td>(0.008)</td>
</tr>
<tr>
<td>pH</td>
<td>4.27</td>
<td>0.286</td>
<td>(&lt;0.001)</td>
</tr>
<tr>
<td>Soil C:N</td>
<td>26.1</td>
<td>0.710</td>
<td>(&lt;0.001)</td>
</tr>
<tr>
<td>Microbial basal</td>
<td>0.869</td>
<td>0.075</td>
<td>0.139</td>
</tr>
<tr>
<td>respiration (µg CO(_2)-C m(^{-2}) h(^{-1}))</td>
<td>66.3</td>
<td>0.862</td>
<td>(&lt;0.001)</td>
</tr>
</tbody>
</table>

\(^1\)Significant \(p\)-values in bold
Table 1.3. Changes in alpha diversity with elevation.\(^1\)

<table>
<thead>
<tr>
<th>Alpha diversity metric</th>
<th>Wet season</th>
<th>Dry season</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F</td>
<td>r(^2)</td>
</tr>
<tr>
<td>Observed OTUs</td>
<td>1.34</td>
<td>0.112</td>
</tr>
<tr>
<td>Shannon Index</td>
<td>0.495</td>
<td>0.044</td>
</tr>
<tr>
<td>Simpson’s Index</td>
<td>2.87</td>
<td>0.212</td>
</tr>
</tbody>
</table>

\(^1\)Significant p-values in bold.
Table 1.4. PERMANOVA results for fungal community composition as a function of elevation, season, Holdridge life zone, and soil properties.\(^1\)

<table>
<thead>
<tr>
<th>Environmental parameter</th>
<th>(F)</th>
<th>(r^2)</th>
<th>(p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elevation</td>
<td>7.34</td>
<td>0.095</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Season</td>
<td>1.74</td>
<td>0.024</td>
<td>0.039</td>
</tr>
<tr>
<td>Holdridge life zone</td>
<td>5.94</td>
<td>0.146</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Temperature</td>
<td>4.37</td>
<td>0.058</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Soil moisture content</td>
<td>3.91</td>
<td>0.059</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>C:N</td>
<td>9.19</td>
<td>0.116</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>pH</td>
<td>4.55</td>
<td>0.061</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

\(^1\) Significant \(p\)-values in bold.
Table 1.5. Relationships of the relative abundance (proportion of sequences) of fungal phyla with elevation.¹

<table>
<thead>
<tr>
<th>Phylum</th>
<th>Wet season</th>
<th>Dry season</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$F$</td>
<td>$r^2$</td>
</tr>
<tr>
<td>Ascomycota</td>
<td>0.304</td>
<td>0.028</td>
</tr>
<tr>
<td>Basidiomycota</td>
<td>0.472</td>
<td>0.042</td>
</tr>
<tr>
<td>Blastocladiomycota</td>
<td>1.01</td>
<td>0.086</td>
</tr>
<tr>
<td>Chytridiomycota</td>
<td>2.57</td>
<td>0.194</td>
</tr>
<tr>
<td>Glomeromycota</td>
<td>0.496</td>
<td>0.044</td>
</tr>
<tr>
<td>Rozellomycota</td>
<td>1.24</td>
<td>0.104</td>
</tr>
<tr>
<td>Zygomycota</td>
<td>3.56</td>
<td>0.250</td>
</tr>
</tbody>
</table>

¹Significant $p$-values in bold.
Table 1.6. Statistics for taxonomic richness of functional groups by elevation and season.¹

<table>
<thead>
<tr>
<th>Functional group</th>
<th>Wet season</th>
<th></th>
<th>Dry season</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r²</td>
<td>p</td>
<td>r²</td>
<td>p</td>
</tr>
<tr>
<td>Free-living filamentous</td>
<td>0.563</td>
<td>0.005</td>
<td>0.000</td>
<td>0.967</td>
</tr>
<tr>
<td>Pathogenic</td>
<td>0.180</td>
<td>0.168</td>
<td>0.001</td>
<td>0.935</td>
</tr>
<tr>
<td>Ectomycorrhizal</td>
<td>0.542</td>
<td>0.006</td>
<td>0.284</td>
<td>0.074</td>
</tr>
<tr>
<td>Yeast</td>
<td>0.310</td>
<td>0.060</td>
<td>0.004</td>
<td>0.852</td>
</tr>
<tr>
<td>Endophytic</td>
<td>0.565</td>
<td>0.005</td>
<td>0.035</td>
<td>0.561</td>
</tr>
<tr>
<td>Lichen</td>
<td>0.290</td>
<td>0.071</td>
<td>0.178</td>
<td>0.172</td>
</tr>
<tr>
<td>Ericoid</td>
<td>0.063</td>
<td>0.432</td>
<td>0.018</td>
<td>0.679</td>
</tr>
<tr>
<td>Arbuscular mycorrhizal</td>
<td>0.109</td>
<td>0.293</td>
<td>0.433</td>
<td>0.020</td>
</tr>
</tbody>
</table>

¹ Significant p-values in bold.
**Fig. 1.1.** *Lactarius indigo* (Russulaceae) along the elevation transect established in the Monteverde Cloud Forest Reserve (10°18’N, 84°47’W).
Fig. 1.2. Elevational trends were found in (a) soil temperature, (b) soil moisture content, (c) soil pH, and (d) soil C:N ratios during both the wet (●) and dry (□) seasons. Lines are significant best-fit regressions for wet (solid line) and dry (dashed line) seasons. Symbols are mean ± SE (n=3) for each site. Significant differences between seasons are designated with an asterisk. Statistical results are presented in Table 1.2.
Fig. 1.3. Elevational trends were found in (a) fungal abundance during the dry (□) and wet (•) seasons and (b) microbial basal respiration increased during the dry season. Seasonality had a significant effect on the relationships between fungal abundance and elevation. Lines are significant best-fit regressions for wet (solid line) and dry (dashed line) seasons. Symbols represent means ± SE (n=3). Significant differences between seasons are designated with an asterisk. Statistical results are presented in Table 1.2. Microbial basal respiration data were ranked to ensure that outliers were not solely driving the relationship.
Fig. 1.4. Fungal taxonomic richness decreases with elevation during the wet season according to the Simpson’s diversity index. Values for Simpson’s diversity index are inversely proportional to diversity: higher values indicate decreased diversity. Lines are significant best-fit regression, and symbols represent means ± SE ($n=3$). Table 1.3 shows other diversity metrics and associated statistical results.
**Fig. 1.5.** Compositional differences in fungal communities with elevation, as a nonmetric multidimensional scaling (NMS) plot. Fungal communities differed significantly with elevation (Table 1.4); $r^2$ values represent the correlation between the ordination axes and our data. Numbers adjacent to symbols indicate elevation. Open squares signify dry season samples, and closed circles signify wet season samples. Symbols are means ± SE of three replicates. Data are colored by life zone, including premontane forest (red), lower montane forest (purple), and montane forest (blue) representing low, mid, and higher elevation sites.
Fig. 1.6. Relative abundance of (a) Chytridiomycota and (b) Zygomycota with elevation during the wet (●) season and (c) Glomeromycota during the dry (□) season. Lines are significant best-fit regressions for wet (solid line) or dry (dashed line). Symbols represent mean ± SE (n=3). Chytridiomycota and Zygomycota data were ranked to ensure that outliers were not solely driving relationships. Relative abundance of other phyla and statistical results are presented in Table 1.5.
Fig. 1.7. Taxonomic richness of functional groups by elevation during the wet season. Fungal taxa were grouped as (a) free-living filamentous, (b) pathogenic, (c) ectomycorrhizal, (d) yeast, (e) endophytic, (f) lichen, (g) ericoid, or (h) arbuscular mycorrhizal. Lines are best-fit regressions and symbols represent number of taxa found at each elevation. Statistical results are presented in Table 1.6.
CHAPTER 2

Shifts in soil fungi and decomposition with simulated climate change in a tropical montane cloud forest

Introduction

Clouds distinguish tropical montane cloud forests (TMCF) from lowland forests. Unfortunately, this cloud layer is lifting, 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 exchange more carbon dioxide (CO₂) 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 TMCF, 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; Pounds et al., 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 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 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 temperatures and drier soils) in TMCF (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 breakdown 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 due to the exponential relationship between decomposition and temperature (Benner et al., 2010). Overall, warmer temperatures in TMCF may allow certain fungi to proliferate, stimulating decomposition and CO₂ release from the soil.
Fungal pathogens can also influence community dynamics by infecting certain animals, plants, and other fungi. In TMCF, there have already been severe declines in aboveground biodiversity due to proliferation of fungal pathogens (Pounds et al., 1999; Pounds et al., 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 are predicted to be the most vulnerable to climate change (Karmalkar et al., 2011; 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 to 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 extracellular enzyme activity 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 decomposition via percent mass loss of soil and EEA of C degrading enzymes.

Methods

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). This transect ranges from 1305 to 1850 m.a.s.l., with sites established approximately every 50 m increase in elevation. Here, we used field sites at 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).

Soil translocation design and collection

We used a soil translocation experiment to test how increased temperatures and decreased precipitation would affect fungi, decomposition, 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 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 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.

To manipulate the fungal community, soil was enclosed in microbial cages. These cages are 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 2.1). Soil was 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 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. Soil moisture is presented as percent soil moisture within each microbial cage.

Twenty soil cores (2 cm diameter by 10 cm deep; mostly O horizon) were collected along an established 20 m line at 1850 m.a.s.l. in November 2014. Soils were transported to the lab and homogenized 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;
Five replicates at each site were collected after ten months (September 2015) towards the end of the wet season. Upon collection, microbial cages were inspected for tears; we discarded two damaged microbial cages. 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 using standing soil (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.

**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 200x 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.

**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; AATGATACGGCGACCACCGAGATCTACAC TC TTTCCCTACA CGACGCTTTCCGATCT NNNNNGAACGCAGCRAAIIGYGA) and barcoded, reverse primers with the reverse complement of the 3’ Illumina adapter (CAAGCAGAAGACGGCATACGAGAT), a unique 12 base barcode, a pad (AGTCAGTCAG), a linker sequence (CC), and the ITS4 primer (TCCTCCGCTTATTGATATGC). We used a staggered primer design (Tremblay et al., 2015) 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. 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 x 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 singletons 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), which is presently the largest database. Functional groups are assigned at the genus and species 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.

**Decomposition**

We determined changes in decomposition as mass loss of soil. To measure mass loss, we measured the total amount of soil in each cage upon collection. We calculated the dry mass of soil before and after collection. Mass loss was determined as the percent difference between the initial dry mass and the final dry mass of soil.

**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, 1g 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 1h. 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 1M 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.
Statistics

We used linear models to test whether temperature and moisture predicted relationships in dependent variables. 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 was transformed as a polynomial function to better fit the data. Subsamples were nested within site to account for potential lack of independence between samples that were located at the same site. Results are stated as significant when $P < 0.05$. Proportion of variation in dependent variables by temperature and moisture was estimated using modified methods of Talbot et al., (2015). Specifically, we calculated pseudo $R^2$ values for each relationship with the r.squaredLR command in the MuMIn package in R. This uses results of the likelihood-ratio test to estimate improvement from a null model by a fitted model. AIC values were extracted using this package in R. To determine whether temperature or moisture was a better predictor of dependent variables we compared regression models using the log likelihood ratio test. All analyses were performed in R (v. 3.3.2, R Development Core Team 2016).

Taxonomic richness (alpha diversity) was computed for each sample using Chao1 estimator and observed OTUs. To assess differences in fungal phyla, we calculated total number of OTUs within each phyla in each sample. In addition, differences in functional groups were calculated using the total number of OTUs within each functional group in each sample. Differences in alpha diversity, richness of fungal phyla, and richness of functional groups were determined as described above. 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., 2009). Non-metric multidimensional scaling (NMS) plots were constructed in R to visualize fungal community composition.

Results

Fungal abundance and diversity

Fungal abundance increased significantly with warmer temperatures and drier conditions (Fig. 2.1a; Table 2.2). Alpha diversity increased in soils with warmer temperatures and drier conditions (Fig. 2.1b; Table 2.2) according to the Chao1 richness estimator. Similarly, the observed number of OTUs also increased (Table 2.2). 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.

Fungal community composition

Fungal community composition shifted with increasing temperatures (F = 4.31, R² = 0.170, P < 0.001; Fig. 2.2a) and decreasing moisture (F = 4.30, R² = 0.170, P < 0.001; Fig. 2.2b). This alteration in community composition was associated with changes in the richness of fungal phyla (Fig. 2.3; Table 2.2). The richness of Ascomycota increased with warmer temperatures and drier conditions (Fig. 2.3a). The richness of Cryptomycota decreased in soils translocated to warmer and drier conditions (Fig. 2.3b). 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 of fungal functional groups shifted with warmer temperatures and drier conditions (Fig. 2.4; Table 2.2). The richness of lichen-forming fungi (Fig. 2.4a), pathogen (Fig.
2.4b), wood saprotrophs (Fig. 2.4c), and yeast (Fig. 2.4d) all increased. When regression models were compared using log likelihood ratios, temperature was a better predictor than moisture for lichen-forming, pathogens, and yeasts (Table 2.2). There were no significant differences between temperature and moisture as predictors of richness of wood saprotrophs.

**Decomposition**

Percent mass loss increased with warmer temperatures and drier conditions (Fig. 2.5). When regression models were compared using log likelihood ratios, there were no significant differences between temperature and moisture as predictors of soil mass loss.

Potential EEA shifted with climate (Fig. 2.6; Table 2.2). AG activity was significantly higher at warmer temperatures and drier conditions (Fig. 2.6a). Likewise, BG activity increased in the warmer and drier (Fig. 2.6b) climate. Similarly, CBH activity was significantly higher at warmer temperatures and drier conditions (Fig. 2.6c). Unlike the other EEAs, BX activity increased only with warmer temperatures (Fig. 2.6d). When regression models were compared using log likelihood ratios, temperature was a better predictor than moisture for BG and BX activities (Table 2.2). There were no significant differences between temperature and moisture as predictors of AG and CBH activities.

**Discussion**

Cloud cover in TMCF is intimately linked to the structure and function of the ecosystem. However, the cloud layer is lifting, and 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 TMCF contain may be broken down and released as CO₂. It is critical to perform *in situ* temperature manipulations in order to understand how tropical ecosystems—like TMCF—
may change (Cavaleri et al., 2015). In general, temperatures in tropical regions are predicted to increase by 1.8 to 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 TMCF 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 to 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., 2001).

**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. 2.1). 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.

**Fungal community composition**

Our hypothesis that fungal community composition would shift with changing climate was supported (Fig. 2.2). Shifts in the fungal community were driven by changes in the richness of phyla (Fig. 2.3) and fungal functional groups (Fig. 2.4).

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 them 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 (Fig. 2.4). 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 TMCF. An increase in yeast richness could lead an
increased 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). An increase in wood saprotroph richness 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. 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 TMCF (Bradshaw et al., 2009).

**Decomposition**

Our results suggest that decomposition may increase under climate change. We found support for our hypothesis that soil mass loss would increase under warmer and drier conditions (Fig. 2.5). Overall, soil decomposition rates were high, with an as much as 67 percent mass loss in the warmest, driest sites. Nevertheless, these fast turnover rates are consistent with findings in the literature for cooler, tropical regions (Chapin et al., 2011). Increased decomposition—and thus loss of soil C—could increase CO₂ emissions into the atmosphere.
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 
(Fig. 2.6). The exception was β-xylosidase, which only increased with warmer temperatures. 
Other studies in the tropics have used elevation gradient approaches to determine how 
decomposition may change with warming. In the Peruvian Andes, 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 (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 TMCF. 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.

Conclusions

Cloud cover within TMCF is declining, and the structure and function of these unique forests will most likely 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 decomposition. 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 decompose 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 CO₂ release into the atmosphere. Moreover, pathogens may proliferate under warmer and drier conditions. Biodiversity within TMCF 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 TMCF may affect how these forests function via changes in the fungal community.
Tables

Table 2.1. 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.

<table>
<thead>
<tr>
<th>Elevation (m.a.s.l.)</th>
<th>Latitude (N)</th>
<th>Longitude (W)</th>
<th>Temperature change from 1850 m.a.s.l. (°C)</th>
<th>Moisture change from 1850 m.a.s.l. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1850</td>
<td>10°19'02.02&quot;</td>
<td>84°47'40.03&quot;</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1743</td>
<td>10°18'57.64&quot;</td>
<td>84°47'47.83&quot;</td>
<td>1</td>
<td>-4.17</td>
</tr>
<tr>
<td>1656</td>
<td>10°18'48.69&quot;</td>
<td>84°47'57.74&quot;</td>
<td>2</td>
<td>-10.8</td>
</tr>
<tr>
<td>1549</td>
<td>10°18'18.40&quot;</td>
<td>84°47'46.36&quot;</td>
<td>3</td>
<td>-20.0</td>
</tr>
<tr>
<td>1430</td>
<td>10°17'28.81&quot;</td>
<td>84°47'30.97&quot;</td>
<td>4</td>
<td>-19.9</td>
</tr>
</tbody>
</table>
Table 2.2. Statistical tests for effect of temperature and moisture on fungal abundance, alpha diversity, richness of fungal phyla, richness of fungal functional groups, and decomposition. Decomposition included measuring extracellular enzyme activity of C-degrading enzymes including: 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).

<table>
<thead>
<tr>
<th>Category</th>
<th>Parameter</th>
<th>Moisture</th>
<th>Temperature</th>
<th>Model comparisons with log likelihood ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Pseudo R²</td>
<td>F</td>
<td>lnl</td>
</tr>
<tr>
<td><strong>Fungal abundance</strong></td>
<td>Hyphal length</td>
<td>0.737</td>
<td>44.6***</td>
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</tr>
<tr>
<td><strong>Alpha diversity</strong></td>
<td>Chao1</td>
<td>0.487</td>
<td>12.2**</td>
<td>-161</td>
</tr>
<tr>
<td></td>
<td>Observed OTUs</td>
<td>0.391</td>
<td>8.56**</td>
<td>-148</td>
</tr>
<tr>
<td><strong>Richness of fungal phyla</strong></td>
<td>Ascomycota</td>
<td>0.542</td>
<td>15.7**</td>
<td>-141</td>
</tr>
<tr>
<td></td>
<td>Basidiomycota</td>
<td>0.138</td>
<td>1.54</td>
<td>-116</td>
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<tr>
<td></td>
<td>Chytridiomycota</td>
<td>0.208</td>
<td>6.47</td>
<td>-164</td>
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<tr>
<td></td>
<td>Cryptomycota</td>
<td>0.542</td>
<td>13.4**</td>
<td>-54.2</td>
</tr>
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<td></td>
<td>Zygomycota</td>
<td>0.188</td>
<td>2.48</td>
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<td><strong>Richness of fungal functional groups</strong></td>
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<td></td>
<td>Lichen-forming</td>
<td>0.434</td>
<td>7.31*</td>
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<td></td>
<td>Pathogens</td>
<td>0.517</td>
<td>11.2**</td>
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<td></td>
<td>Saprotrophs</td>
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<td>2.61</td>
<td>-116</td>
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<td></td>
<td>Wood saprotrophs</td>
<td>0.409</td>
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<td>-79.3</td>
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<tr>
<td><strong>Decomposition</strong></td>
<td>Yeasts</td>
<td>0.676</td>
<td>28.0***</td>
<td>-92.5</td>
</tr>
<tr>
<td></td>
<td>Soil mass loss</td>
<td>0.946</td>
<td>268.***</td>
<td>-64.7</td>
</tr>
<tr>
<td></td>
<td>AG activity</td>
<td>0.612</td>
<td>20.7***</td>
<td>-61.8</td>
</tr>
<tr>
<td></td>
<td>BG activity</td>
<td>0.560</td>
<td>17.4***</td>
<td>-134</td>
</tr>
<tr>
<td></td>
<td>CBH activity</td>
<td>0.498</td>
<td>19.6***</td>
<td>-96.8</td>
</tr>
<tr>
<td></td>
<td>BX activity</td>
<td>0.118</td>
<td>0.492</td>
<td>-116</td>
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</table>
Fungal abundance and diversity with soil moisture content. Fungal abundance (a) and (b) fungal taxonomic richness as determined by the Chao1 richness estimator increased with 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.
Fig. 2.2. 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.
Fig. 2.3. Richness of (a) Ascomycota and (b) Cryptomycota with 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 2.2.
Fig. 2.4. Taxonomic richness of fungal functional groups with warming. Fungal taxa were identified and grouped as (a) lichen-forming, (b) pathogens, (c) wood saprotrophs, and (d) yeasts. Lines are best-fit regressions and symbols represent means ± SE (n=5). Model comparisons showed that temperature had a greater effect than moisture on lichen-forming, pathogens, and yeasts. Statistical results are presented in Table 2.2.
Fig. 2.5. Percent mass loss of soil with drier conditions. Soils decomposed *in situ* for 10 months. Soil mass loss increased with drier conditions in the field. There were no significant differences between temperature and moisture as predictors of percent mass loss 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.
Fig. 2.6. Potential extracellular enzyme activity (EEA) with warmer and drier conditions. Enzyme activity of (a) α-glucosidase (AG) and (c) cellobiohydrolase (CBH) increased with drier conditions. Activity of (b) β-glucosidase (BG) and (d) β-xylosidase (BX) increased with warmer temperatures. Temperature was a better predictor than moisture for BG and BX activities 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 2.2.
CHAPTER 3

Carbon in the canopy: how soil fungi and extracellular enzymes differ in canopy and ground soils

Introduction

Tropical montane cloud forests (TMCF) are structurally complex ecosystems owing to their characteristic cloud cover. Part of this structural complexity is the high abundance and diversity of canopy epiphytes (Gentry and Dodson, 1987). These epiphytes contain soil—known as canopy soil—that hold water and nutrients allowing them to thrive in the harsh canopy conditions (Bohlman et al., 1995; Nadkarni et al., 2004). In general, tropical forests have a significant influence over global biodiversity and C cycling. However, most of our knowledge comes from soils on the ground. Unfortunately, we do not know how canopy soils contribute to important ecosystem processes, like decomposition. Because of the large amount of organic matter (Ingram and Nadkarni, 1993) and nutrients (Nadkarni, 1984) in canopy soil, they may play a large role in ecosystem nutrient dynamics. In addition, they may be serving as reservoirs for soil fungi that are important in decomposition and alleviating environmental stress for plants. Determining how canopy soils differ from ground soils will provide a better understanding of how natural environmental stress affects soil communities and decomposition. This is especially important as canopy communities in TMCF that are experiencing less cloud cover and longer dry periods with climate change (Karmalkar et al., 2011; Lawton et al., 2001; Still et al., 1999).

The high abundance and diversity of epiphytes contribute to the complex structure of TMCF (see Fahey and Sherman, 2016). In some forests, epiphytes consist of one third of the foliar biomass (Nadkarni, 1984), about one third of the vascular species, and about half of the total plants at the stand level (Gentry and Dodson, 1987). Along the leeward slope of the
Talamanca range in Costa Rica, old growth forests have an estimated 12 kg of epiphytes per tree, and 2,820 kg of epiphytes per hectare (Kohler et al., 2010).

Since epiphytes are disconnected from the ground, they depend on canopy soil to retain water and nutrients. Canopy soils consist of dead organic matter from epiphytes, and other plant material intercepted by branches in the canopy (Nadkarni et al., 2004). These soils have a high water storage capacity (Bohlman et al., 1995), and can consist of up to 45 percent of the nutrient capital in some forests (Nadkarni, 1984). These characteristics help alleviate the environmentally stressful conditions in the canopy. Canopy communities experience high winds, drier conditions (Cardelús and Chazdon, 2005), and higher UV radiation compared to the understory. Canopy soils may play important ecosystem roles in these forests, but we lack an in depth knowledge of their ecosystem-level contributions (see Gotsch et al., 2016), especially decomposition and soil diversity.

Tropical forests have a disproportionate influence over global biodiversity and biogeochemical cycling (Townsend et al., 2011). Many predictions regarding the influence of tropical ecosystems on biodiversity and C cycling are based on ground soils. But, there is a large amount of organic matter within canopy soils that is being decomposed. Canopy soils can consist of 60 percent dead organic matter (Ingram and Nadkarni, 1993). In addition, canopy soils have large nutrient capital, and may play a large role in ecosystem dynamics (Nadkarni, 1984; Nadkarni et al., 2004). Inevitably, these soils may be serving as large reservoirs for soil fungi—the main players in plant breakdown (de Boer et al., 2006).

Few studies have quantified decomposition in canopy soils. One study found that canopy soils have slow turnover, while other components of epiphytic matter decompose quickly (Nadkarni and Matelson, 1991). However, increased organic matter concentrations can lead to
increased activity of some extracellular enzymes responsible for decomposition (Sinsabaugh et al., 2008). Canopy soils also have similar microbial activity compared to soils on the ground (Vance and Nadkarni, 1990). Thus, higher amounts of organic matter within canopy soils could lead to increased extracellular enzyme activity (EEA).

High organic matter in canopy soil also correlates with higher fungal growth compared to ground soils (Rousk and Nadkarni, 2009). But, because of inaccessibility, few studies have investigated fungal communities and decomposition in canopy soils (eg. Inselsbacher et al., 2007; Orlovich et al., 2013; Pittl et al., 2010). Changes in the fungal community can affect decomposition through changes in EEA. Specifically, fungi produce extracellular enzymes that target particular types of C. But, we do not know what types of fungi are present in canopy soils and what functional roles they are fulfilling. This is especially important in the context of decomposition of C and stress resistance.

Soil diversity is frequently influenced by stress, and low species richness can result from harsh abiotic conditions (Decaëns, 2010). Changes in diversity can affect the types of functional groups present in canopy soils. For instance, yeasts decompose simple C, and proliferate in more stressful environments (Treseder and Lennon, 2015). Moreover, other types of fungi—like endophytes—can abate pressures from environmental stress, like drought (Giauque and Hawkes, 2013).

In Costa Rica, TMCF are experiencing decreased cloud cover and increased variability in rainfall (Karmalkar et al., 2011; Lawton et al., 2001; Still et al., 1999); the frequency of mist has also declined (Pounds et al., 1999). Because they are disconnected from the ground, canopy communities are extremely vulnerable to climate change, and may serve as biotic indicators of change (Gotsch et al., 2016; Nadkarni 2010). Despite evidence that epiphyte matter can store
large amounts of water and nutrients, the water potential of some epiphytes is similar to plants on the ground (Gotsch et al., 2015).

Because of this, epiphytes are extremely dependent on canopy soils. Future changes may become especially important with future climate change as conditions become more variable in these forests. Elevation gradients have become increasingly important in investigating how microbial communities may shift and understanding the fate of C under future climate change (Malhi et al., 2010; Sundqvist et al., 2013). Fungi are sensitive to changes in temperature and precipitation (Allison and Treseder, 2008; Hawkes et al., 2011; McGuire et al., 2011). If fungal communities shift, the organic-rich canopy soils may experience faster rates of decomposition.

In a previous study, we demonstrated that soil properties and fungal functional groups shift with elevation in ground soils (Looby et al., 2016). Here, we compared soil properties, extracellular enzyme activity, and fungal communities in canopy and ground soils. Soils were collected along elevation transects on the Atlantic and Pacific slopes of the Cordillera de Tilarán in Monteverde, Costa Rica. Canopy soils house a large amount of organic material, and experience environmentally stressful conditions. Canopy soils are also vulnerable to climate change. It is important to begin to unravel how canopy soils differ from ground soils in the microbial communities that reside there, and their decomposition potential. Moreover, as conditions in the canopy become more stressful with climate change, we need to understand how sensitive these soils may be to these changes. Therefore, we asked these questions: 1. Do canopy soils differ from ground soils in their properties, EEA, and fungal communities? and 2. Do the two soil types differ in how they respond to elevation? Varying responses to elevation can help understand differences in sensitivity to future climate change.
For the first question, we hypothesized that EEA of C degrading enzymes would be higher in canopy soils because of increased C. Furthermore, we hypothesized that canopy soils would harbor distinct fungal communities and have lower levels of diversity, due to selection for a subset of taxa that are adapted to harsher environmental stress. Finally, fungal functional groups would differ between canopy and ground soils. For the second question, we hypothesized that EEA and fungal community composition of canopy soils would be more sensitive to changes in elevation compared to ground soils. To test these hypotheses, we measured soil properties, decomposition via EEA of C degrading enzymes, and fungal diversity and community composition.

Methods

Field sites and collection

Two elevational transects were established in preserved forest in Monteverde, Costa Rica in the Monteverde Cloud Forest Reserve (10°18´N, 84°47´W) and nearby private land. Eight sites in total spanned these transects from 850 to 1400 m.a.s.l. along the Atlantic slope and from 1150 to 1810 m.a.s.l. along the Pacific slopes (Table 3.1). The slopes vary in their climate, and were analyzed separately throughout the study. Briefly, the Atlantic slope is on the windward side of the mountain range, and receives more rainfall, more water inputs from clouds (horizontal precipitation), and more wind than the Pacific slope (Häger and Dohrenbusch, 2010). The Pacific slope—on the leeward side—has a steeper temperature and soil moisture gradient across elevation. The Pacific slope also experiences greater precipitation seasonality than the Atlantic, with greater distinction between seasons increasing at lower elevations.
Canopy and ground soil were collected in February and March 2014 during the dry season. The same trees were sampled at both time points. Three trees were sampled at each site, with a few exceptions. For instance, due to equipment failure only two trees were ascended at 1200 m.a.s.l. (Atlantic). Only two climbable trees were present at 1760 m.a.s.l. (Pacific). Near the divide at 1810 m.a.s.l., high winds and frequent branch and tree falls make conditions dangerous for climbing, so soil was sampled from two partially fallen trunks resting approximately 1 m above the ground, with two samples per trunk taken 1 m apart. Since the “canopy” is stunted and disturbed in the elfin forest, the low trunks yield a decent approximation of the canopy soil found above reach. Although 1760 and 1810 m.a.s.l. are similar elevations, trees at 1760 m.a.s.l. experience much less wind and mist due to their position; thus, samples were analyzed separately.

Trees were climbed using standard Single Rope Technique. At each tree, a total of five soil samples were collected, two from the canopy and three from the ground. Canopy samples were collected from main branches. On the ground, three collections were made at a distance of 5 to 10 m from the main trunk and at least 5 m from each other. Ground soil samples were taken using a trowel (approximately 5 cm deep) to obtain the top organic layer of soil. Canopy soil was collected using a knife and small shovel described in Nadkarni et al., (2004). Soil samples were stored at -20°C within 8 hours post collection.

Soil properties

Soil temperature was measured on each branch and adjacent to soil samples collected on the ground for each tree and site. Subsamples were weighed, dried 65°C for 48 hours, and then reweighed to determine gravimetric moisture content. Soil pH was determined using a 1:2 ratio (w/v) of soil to DI H2O. Soil C and N concentrations were measured by combustion on an
elemental analyzer (Flash EA 1112, Thermo Scientific, Waltham, MA) and used to determine percent C and soil C:N ratios.

Extracellular enzyme assays

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

In summary, 1g of soil was homogenized in 125 mL sodium acetate buffer (pH 5.0) using a hand blender. Two hundred microliters of soil homogenate was added to 50-µL fluorometric substrate solutions, and incubated for 1h. Soils were incubated at temperatures measured at the time of collection. Activity was terminated by adding 10 µL of 1M NaOH. Fluorescence was measured at 365 nm excitation and 450 nm emission. Microplates included substrate controls, homogenate controls, and 4- methylumbelliferone. A standard curve was calculated to determine potential activities. Potential EEA was calculated as nmol product released h\(^{-1}\) g\(^{-1}\) dry soil.

Fungal community composition

Soil DNA was extracted from each sample 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. Description of these primers and PCR parameters are explained in detail in Looby et al., (2016). PCR reactions from each sample were run 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). Purified samples were pooled separately in equimolar concentrations. The pooled samples were sequenced on two lanes of an Illumina MiSeq sequencer as 2 x 300 bp paired end reads. Sequencing was performed at the Genomics core in the Institute for the Integrative Genome Biology at the University of California, Riverside.

We processed sequences using the Quantitative Insights Into Microbial Ecology (QIIME) pipeline (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 82% 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 singletons were removed. After quality control, our high-quality sequences 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 11-20-2016). To avoid bias due to differing library sizes, samples were normalized to 2,416 sequences per sample.

We assigned functional groups to OTUs using the FUNGuild algorithm (Nguyen et al., 2016). Functional groups are assigned at the genus and species level and with confidence levels of “highly probable,” “probable,” and “possible.” Approximately 58% and 56% of OTUs from the wet and dry seasons respectively were matched to a functional guild within the FUNGuild database. Only assignments with confidence levels of “highly probable” or “probable” were included in analyses. Fungal functional groups were categorized as follows: decomposers (saprotrophs and wood saprotrophs), endophytes (dark septate endophytes and endophytes),
lichen-forming, mycorrhizal (arbuscular mycorrhizal fungi and ectomycorrhizal fungi), and pathogens (animal pathogens, mycoparasites, and plant pathogens). Furthermore, any functional group assignment that was designated with the growth form “yeast” were classified as such.

Statistics

To determine differences in soil properties and EEA between canopy and ground soils, we used a mixed effects model. The model determined the effect of soil type and the interaction between slope and soil type (fixed effects), and tree was included as a random effect. We calculated $P$-values using parametric bootstrapping as part of the “mixed” function in the package afex of R (Singmann et al., 2016). We used 1000 simulations to calculate $P$-values. Differences were considered significant when $P < 0.050$. When the interaction between slope and soil type was significant, we performed pairwise comparisons for each slope separately using the “contrast” function in the R package lsmeans (Lenth, 2016). To determine if elevation affects canopy and ground soils differently, we performed a mixed model using the nlme package in R (Pinheiro et al., 2016). We included elevation and soil type as fixed effects, and tree as a random effect. Atlantic and Pacific slopes were analyzed separately. All statistical analyses were carried out using R (v. 3.3.2, R Development Core Team 2016).

Taxonomic richness (alpha diversity) was computed for each sample using Shannon diversity index and observed OTUs. To assess differences in functional groups, we calculated relative abundances as proportion of OTUs in each sample. Proportions were determined for each sample by calculating the number of OTUs from each functional group in that sample, divided by the total number of OTUs found within that sample. We used the linear mixed effects model, as described above, to determine differences in alpha diversity and relative abundance of functional groups between canopy and ground soils. In addition, we used a PERMANOVA using
Bray-Curtis dissimilarity to analyze fungal community composition as a function of soil type and properties measured (i.e., elevation, temperature, moisture, pH, C:N, and C) with the adonis function in the Vegan package of R (Oksanen et al., 2017). The PERMANOVA was run with each term added last in the model to evaluate variance explained when all other factors were accounted for (Kivlin et al., 2014). Soil properties are reported and stated as significant ($P < 0.050$) if values were significant when added last. Non-metric multidimensional scaling (NMS) plots were constructed in R to visualize fungal community composition.

**Results**

**Soil properties**

Soil properties differed between the two soil types (Fig. 3.1a and b; Table 3.2). Soil moisture content was lower in canopy soils compared to ground soils ($\chi^2 = 8.21, P = 0.005$). Soil temperature was higher in canopy soils ($\chi^2 = 6.75, P = 0.010$). Canopy soils were also more acidic than ground soils ($\chi^2 = 63.1, P < 0.001$). Percent C was higher in canopy soils ($\chi^2 = 172, P < 0.001$). In addition, soil C:N was higher in canopy soils ($\chi^2 = 74.7, P < 0.001$).

In several cases, canopy and ground soils differed in how soil properties varied with elevation, based on significant elevation*type interactions (Fig. 3.2; Table 3.3). On the Atlantic slope, soil moisture content in canopy soils had a larger increase with increasing elevation than did ground soils ($P < 0.001$). The Pacific slope displayed a similar trend, although it was non-significant. Soil temperature in canopy soils declined more rapidly with increasing elevation than did ground soils (Atlantic: $P = 0.002$; Pacific: $P < 0.001$). The pH in canopy soils varied differently with elevation compared to that of ground soils, but without a clear linear trend (Atlantic: $P = 0.005$; Pacific: $P = 0.004$). Ground soils had a significantly larger change in
percent C with elevation than canopy soils in the Pacific slope ($P = 0.007$), but not in the Atlantic slope ($P = 0.354$). There was no significant difference in soil C:N with elevation between the soil types (data not shown, $P > 0.05$).

**Extracellular enzyme activity**

Potential EEA of all enzymes was higher in canopy soils than ground soils (Fig. 3.3a and b, Table 3.4). Specifically, canopy soils had significantly higher activities of AG ($\chi^2 = 25.9, P < 0.001$), BG ($\chi^2 = 24.3, P < 0.001$), CBH ($\chi^2 = 26.6, P < 0.001$), and BX ($\chi^2 = 33.5, P < 0.001$).

Canopy soils differed in how BX activities responded to elevation compared to ground soils; this interaction was significant on the Pacific slope and marginally significant on the Atlantic slope (Fig. 3.4c,d; Table 3.3). On the Pacific slope, activity of BX declined markedly with increasing elevation in canopy soils, but more subtly in ground soils across elevations (Fig. 3.4c, $P < 0.001$). On the Atlantic slope, BX activity was more variable across elevations in the canopy soils than in the ground soils, but with non-linear trends (Fig. 3.4d). For AG, the interaction between elevation and soil type was significant on the Pacific slope (Fig. 3.4a, $P < 0.001$), but not the Atlantic slope (Fig. 3.4b, $P = 0.332$). In particular, on the Pacific slope, AG activity declined strongly with elevation in canopy soils but not ground soils (Fig. 3.4a). For BG and CBH, interactions between soil type and elevation were not significant (data not shown, Pacific: BG: $P = 0.103$; CBH: $P = 0.066$).

**Fungal diversity and community composition**

Fungal diversity was lower in canopy soils as shown by Shannon diversity (Table 3.5; $\chi^2 = 18.3, P < 0.001$) and the number of observed OTUs (Table 3.5; $\chi^2 = 13.6, P < 0.001$). Fungal diversity did not differ between canopy and ground soils in their relationship with elevation ($P > 0.050$).
Fungal community composition significantly differed between canopy and ground soils along both slopes (Fig. 3.5a and b; Table 3.6). Fungal communities were structured by soil type, elevation, slope, temperature, moisture content, pH, percent C, and soil C:N.

**Fungal functional groups**

Relative abundance of fungal functional groups also differed between canopy and ground soils (Fig. 3.6; Table 3.5). Canopy soil had a lower abundance of decomposer fungi ($\chi^2 = 73.4$, $P < 0.001$) and mycorrhizal fungi ($\chi^2 = 69.2$, $P < 0.001$). Canopy soils had a higher abundance of endophytes ($\chi^2 = 23.1$, $P = 0.002$), lichen-forming fungi ($\chi^2 = 24.3$, $P < 0.001$), and yeasts ($\chi^2 = 30.0$, $P < 0.001$). There was no significant difference between abundance of pathogens between the two soil types ($P > 0.05$).

Canopy soils differed in how relative abundance of decomposers responded to elevation compared to ground soils (Fig. 3.7a,b and 3.8a and b; Table 3.3). Specifically, the relative abundance of decomposers increased more dramatically in canopy soils with increasing elevation, compared to ground soils (Fig. 3.7a and b; Atlantic: $P = 0.050$; Pacific: $P = 0.049$). Endophyte relative abundance displayed a similar trend on the Pacific slope (Fig. 3.7c, $P = 0.041$) but not the Atlantic slope (Fig. 3.7d, $P = 0.727$). Lichen-forming fungi varied more widely with elevation in canopy soils than ground soils, but with inconsistent trends across slopes (Fig. 3.8a and b; Atlantic: $P = 0.020$; Pacific: $P = 0.041$). The relative abundance of yeasts was associated with a significant elevation by soil type interaction on just the Pacific slope (Fig. 3.7e,f; Pacific: $P = 0.012$; Atlantic: $P = 0.707$). There were no clear linear trends in yeast relative abundance on either slope.
Discussion

Soil properties

We found overall support for our hypotheses that canopy soils and ground soils would differ in soil moisture, temperature, pH, percent C, and C:N ratios. Although canopy soils have a high water holding capacity (Bohlman et al., 1995; Köhler et al., 2010), they experience extremes in drying and rewetting (Coxson and Nadkarni, 1995). Variable precipitation levels in the dry season may allow these soils to dry, and become especially stressful for soil fungi.

In addition, we found that canopy soils were warmer. In Monteverde, there is greater microclimate variability (Clark et al., 2000) and less cloud cover (Goldsmith et al., 2012) in the dry season. Less cloud cover—and thus more light—may cause a greater temperature difference between the canopy and the ground. In Monteverde, TMCF are projected to experience decreased cloud immersion (Nair et al., 2003; Still et al., 1999) and longer periods without rain in the dry season (Karmalkar et al., 2011; Pounds et al., 1999; 2006). Decreased cloud immersion may allow more light to hit these forests, and increase environmental stress in the canopy. We also found that soil temperature declined more dramatically with increasing elevation in canopy soils compared to ground soils. Potentially, canopy soils may be more sensitive to future temperature changes than ground soils.

Moreover, canopy soils in these forests are highly organic (Clark et al., 2000). This is most likely why they are more acidic, have higher percent C, and have higher C:N ratios compared to ground soils. Nadkarni et al., (2002) also found that canopy soils in these forests were more acidic and had higher percent C. Nevertheless, Nadkarni et al., (2002) also found no significant difference in soil C:N between the two soil types. Higher C:N ratios in canopy soils
also suggests lower N availability in these soils. It also suggests that fungi may be especially important in canopy soils (Fierer et al., 2009).

**Extracellular enzyme activity**

We found support for our hypothesis that EEA of C degrading enzymes would be higher in canopy soils. Other studies have found differing patterns of decomposition between canopy and ground soils. One study found that decomposition of a common substrate was slower in the canopy (Cardelús, 2009), while another found high activity of N-degrading enzymes in bromeliad tanks (Inselsbacher et al., 2007). Our results suggest that there are high rates of decomposition occurring in canopy soils.

Soils with higher organic matter concentrations can lead to increased EEA (Sinsabaugh et al., 2008). More substrate—as shown by higher percent C—is most likely contributing to the higher EEA found in canopy soils. Moreover, another study in Monteverde found that canopy soil had similar microbial activity and biomass compared to ground soil (Vance and Nadkarni, 1990). Fungi are the main producers of hydrolytic enzymes (Schneider et al., 2012), and they dominate decomposition of cellulose and hemicellulose (de Boer et al., 2005). Shifts in fungal community composition could have contributed to differences in decomposition between soil types because fungi specialize in breaking down different forms of C (Hanson et al., 2008; McGuire et al., 2010; Setälä and McLean, 2004).

In canopy soils, BX activities varied dramatically with increasing elevation, but were relatively constant in ground soils. This suggests that hemicellulose breakdown by this enzyme may be more sensitive to climate change in canopy soils. Other studies along tropical elevation gradients have suggested that decomposition may increase with warmer and drier conditions (Salinas et al., 2011; Scowcroft et al., 2000; Looby et al., in review). Our study suggests that
decomposition of hemicellulose C in canopy soils may change even more than in ground soils.

**Fungal diversity and community composition**

We found overall support for our hypothesis that diversity would be lower in canopy soils, and that fungal community composition would differ between canopy and ground soils. Canopy soils experience warmer temperatures, long periods without water or nutrient inputs, strong winds, and high UV radiation (Bohlman et al., 1995). The greater abiotic stress occurring in the canopy is most likely selecting for different fungal communities than in ground soils. Moreover, soil diversity is frequently influenced by stress, and low species richness can result from harsh abiotic conditions (Decaëns, 2010). Stressful conditions could be the reason diversity is lower in canopy soils.

**Fungal functional groups**

Our results support the overall hypothesis that fungal functional groups would differ between soil types. There was a higher abundance of decomposer fungi in ground soils. These fungi consist of wood saprotrophs that specialize in decomposing more recalcitrant forms of C, like lignin. Thus, there may be less fungi that specialize in breaking down recalcitrant C in canopy soils, and instead are replaced by yeasts. Yeasts specialize in decomposing simple C, like sugars, and are often found in more stressful environments (Treseder and Lennon, 2015). One study indicate that organic matter in canopy is more labile than on the ground (Vance and Nadkarni, 1990). Simpler forms of C, and stressful conditions in the canopy may be selecting for a higher abundance of yeasts.

The relative abundance of decomposers tended to increase non-linearly with increasing elevation in canopy soils. However, in ground soils decomposer abundance was less variable across elevation. In a previous study in this TMCF, richness of free-living filamentous fungi
(which are primarily decomposers) declined with increasing elevation in ground soils (Looby et al., 2016). A study also suggests that some decomposer fungi may increase with future warming and drying in these forests (Looby and Treseder, in review). Our results suggest that decomposers in canopy soils may be more sensitive to future change compared to ground soils. However, decomposer fungi are less abundant at warmer and drier elevations. Thus, fungi that decompose more recalcitrant material may decline under warmer and drier conditions projected with future climate change.

Our results also suggest that yeasts represent a greater portion of the soil fungal community in the canopy than the ground. Yeasts may proliferate in canopy soils where conditions become more stressful and variable. Because they are more relatively abundant in canopy soils, they may contribute more to breakdown of labile C there.

In addition, endophytes were more abundant in canopy soils. Endophytes are symbiotic fungi that reside in the aboveground tissues of plants. Endophytes confer stress resistance by reducing plant water loss if water is limiting (Giauque and Hawkes, 2013), and can be important for canopy communities. Canopy conditions are variable and environmentally stressful, and can experience long periods without water. Endophytes are most likely abating these pressures.

Conclusions

The cloud cover that defines TMCF is lifting, and these forests are becoming warmer and drier. The structure and function of these forests will change, and these changes may be driven by the response of soil fungi. For instance, as conditions become warmer and drier, rates of decomposition may speed up through increased fungal abundance, richness of wood saprotrophs, and EEA (Looby et al., in review). These changes could cause TMCF to start operating at a
faster pace. Moreover, the structure of these forests could change as existing fungal pathogens could spread and new fungal pathogens could emerge (Looby et al., in review). This could perpetuate biodiversity loss within TMCF.

The current study provides a deeper understanding of how the structure and function of these forests may change from the perspective of canopy soil. Canopy and ground soils contain different types of soil fungi. Most likely, these differences are driven by increased environmental stress in the canopy. If cloud cover is reduced and precipitation becomes more variable, conditions in the canopy may become more stressful. Decomposers may become less abundant, and EEA of hemicellulose-targeting enzymes may vary more strongly with future climate change. Consequently, C dynamics in canopy soil may be particularly responsive to climate change—more so than we might predict based on observations from ground soil.
Tables

**Table 3.1.** Field sites along two elevation transects where canopy and ground soils were collected. Elevation transects were on the Atlantic and Pacific slopes of the Cordillera de Tilarán in Monteverde, Costa Rica. Field sites spanned various forest types.

<table>
<thead>
<tr>
<th>Slope</th>
<th>Elevation (m.a.s.l.)</th>
<th>Holdridge Life zone</th>
<th>Qualitative forest description</th>
</tr>
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<td>Premontane wet forest</td>
<td>Seasonal moist forest</td>
</tr>
<tr>
<td></td>
<td>1450</td>
<td>Lower montane wet forest</td>
<td>Seasonal, transitional cloud forest</td>
</tr>
<tr>
<td></td>
<td>1600</td>
<td>Lower montane rainforest</td>
<td>Cloud forest</td>
</tr>
<tr>
<td></td>
<td>1760</td>
<td>Lower montane rainforest</td>
<td>Cloud forest; elfin forest</td>
</tr>
<tr>
<td></td>
<td>1810</td>
<td>Lower montane rainforest</td>
<td>Cloud forest; elfin forest</td>
</tr>
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<td>Premontane rainforest</td>
<td>Wet, cloud forest</td>
</tr>
<tr>
<td></td>
<td>1200</td>
<td>Premontane rainforest</td>
<td>Wet, transitional cloud forest</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>Premontane rainforest</td>
<td>Wet, lower montane forest</td>
</tr>
<tr>
<td></td>
<td>850</td>
<td>Premontane rainforest</td>
<td>Wet, lower montane forest</td>
</tr>
</tbody>
</table>
Table 3.2. Statistical results for differences in soil properties between canopy and ground soils. To determine differences in soil properties we used a mixed effects model that determined the effect of soil type and the slope by type interaction (slope*type). We report effect estimates, $\chi^2$, and $P$-values. Effect estimates for type are units difference from ground soils, and for slope are units difference from Pacific slope. $P$-values were calculated using parametric bootstrapping. When slope*type was significant, we used pairwise comparisons to test each slope separately.¹

<table>
<thead>
<tr>
<th>Category</th>
<th>Parameter</th>
<th>Effect</th>
<th>$\chi^2$</th>
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<td></td>
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<td>Slope*Type</td>
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</tr>
<tr>
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<td>Type, Pacific only</td>
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¹Significant $P$-values in bold
Table 3.3. Statistical results for differences in elevational responses between canopy and ground soils (i.e., elevation*type interactions). We tested differences in soil properties, extracellular enzyme activity (EEA) of C-degrading enzymes, fungal diversity, and relative abundance of fungal functional groups. Extracellular enzymes included: α-glucosidase (AG), β-glucosidase (BG), cellobiohydrolase (CBH), and β-xylosidase. Differences were determined using a mixed model ANCOVA. Analyses for Atlantic and Pacific slopes were carried out separately.\(^1\)

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\(^1\) Significant \(P\)-values in bold.
Table 3.4. Statistical results for differences in potential extracellular enzyme activity (EEA) of C-degrading enzymes between canopy and ground soils. EEA of C-degrading enzymes included: α-glucosidase (AG), β-glucosidase (BG), celllobiohydrolase (CBH), and β-xylosidase. To determine differences in soil properties we used a mixed effects model that determined the effect of soil type and the slope by type interaction (slope*type). We report effect estimates, $\chi^2$, and $P$-values. Effect estimates for type are units difference from ground soils, and for slope are units difference from Pacific slope. $P$-values were calculated using parametric bootstrapping. When slope*type was significant, we used pairwise comparisons to test each slope separately.

<table>
<thead>
<tr>
<th>Extracellular enzyme activity</th>
<th>Effect</th>
<th>$\chi^2$</th>
<th>$P$</th>
</tr>
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<tbody>
<tr>
<td>AG</td>
<td>Type</td>
<td>-119</td>
<td>25.9</td>
</tr>
<tr>
<td></td>
<td>Slope*Type</td>
<td>79.3</td>
<td>7.13</td>
</tr>
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<td></td>
<td>Type, Atlantic only</td>
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</tr>
<tr>
<td></td>
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</tr>
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<td>BG</td>
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<td>Slope*Type</td>
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<td>6.74</td>
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<td>n.a.</td>
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<tr>
<td></td>
<td>Type, Pacific only</td>
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<td>n.a.</td>
</tr>
<tr>
<td>CBH</td>
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<tr>
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<td>1.91</td>
</tr>
<tr>
<td></td>
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<tr>
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<td>Type, Pacific only</td>
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<td>n.a.</td>
</tr>
<tr>
<td>BX</td>
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<td>-179</td>
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</tr>
<tr>
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<td>Slope*Type</td>
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<td>5.04</td>
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<tr>
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</tr>
<tr>
<td></td>
<td>Type, Pacific only</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
</tbody>
</table>

1Significant $P$-values in bold.
**Table 3.5.** Statistical results for differences fungal diversity and relative abundance of fungal functional groups between canopy and ground soils. To determine differences in soil properties we used a mixed effects model that determined effect of soil type and the slope by type interaction (slope*type). We report effect estimates, $\chi^2$, and $P$-values. Effect estimates for type units difference from ground soils, and for slope are units difference from Pacific slope. $P$-values were calculated using parametric bootstrapping. When slope*type was significant, we used pairwise comparisons to test each slope separately.\(^1\)

<table>
<thead>
<tr>
<th>Category</th>
<th>Parameter</th>
<th>Effect</th>
<th>$\chi^2$</th>
<th>$P$</th>
</tr>
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<td><strong>Fungal diversity</strong></td>
<td>Shannon diversity</td>
<td>Type</td>
<td>2.05</td>
<td>18.3</td>
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<tr>
<td></td>
<td></td>
<td>Slope*Type</td>
<td>-1.61</td>
<td>8.11</td>
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<tr>
<td></td>
<td></td>
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<td>n.a.</td>
</tr>
<tr>
<td><strong>Observed OTUs</strong></td>
<td></td>
<td>Type</td>
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<td>13.6</td>
</tr>
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<td></td>
<td></td>
<td>Slope*Type</td>
<td>-84.5</td>
<td>3.69</td>
</tr>
<tr>
<td><strong>Functional groups</strong></td>
<td>Decomposers</td>
<td>Type</td>
<td>0.091</td>
<td>73.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Slope*Type</td>
<td>0.046</td>
<td>4.14</td>
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<td></td>
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</tr>
<tr>
<td></td>
<td></td>
<td>Type, Pacific only</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Type</td>
<td>-0.013</td>
<td>23.1</td>
</tr>
<tr>
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<td></td>
<td>Slope*Type</td>
<td>-0.006</td>
<td>0.890</td>
</tr>
<tr>
<td><strong>Lichen-forming</strong></td>
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<td>Type</td>
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</tr>
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<td></td>
<td></td>
<td>Slope*Type</td>
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<td>9.26</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Type, Atlantic only</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Type, Pacific only</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
<tr>
<td><strong>Mycorrhizal</strong></td>
<td></td>
<td>Type</td>
<td>0.042</td>
<td>69.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Slope*Type</td>
<td>-0.005</td>
<td>0.320</td>
</tr>
<tr>
<td><strong>Pathogens</strong></td>
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<td>Type</td>
<td>-5.92</td>
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</tr>
<tr>
<td></td>
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<td>Slope*Type</td>
<td>7.82</td>
<td>0.00</td>
</tr>
<tr>
<td><strong>Yeasts</strong></td>
<td></td>
<td>Type</td>
<td>-0.023</td>
<td>30.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Slope*Type</td>
<td>-0.001</td>
<td>0.01</td>
</tr>
</tbody>
</table>

\(^1\) Significant $P$-values in bold.
Table 3.6. PERMANOVA results for fungal community composition as a function of soil type (canopy, ground), elevation, slope (Atlantic, Pacific), and soil properties.

<table>
<thead>
<tr>
<th>Environmental parameter</th>
<th>R²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil type</td>
<td>0.688</td>
<td>0.014</td>
</tr>
<tr>
<td>Elevation</td>
<td>0.718</td>
<td>0.015</td>
</tr>
<tr>
<td>Slope</td>
<td>0.742</td>
<td>0.015</td>
</tr>
<tr>
<td>Moisture content</td>
<td>0.564</td>
<td>0.012</td>
</tr>
<tr>
<td>Temperature</td>
<td>0.459</td>
<td>0.009</td>
</tr>
<tr>
<td>pH</td>
<td>0.640</td>
<td>0.013</td>
</tr>
<tr>
<td>Percent C</td>
<td>0.479</td>
<td>0.010</td>
</tr>
<tr>
<td>Soil C:N</td>
<td>0.451</td>
<td>0.010</td>
</tr>
</tbody>
</table>

1 Significant P-values in bold.
Fig. 3.1. Canopy soils differed from ground soils in soil moisture content, soil temperature, and soil C:N ratios along the (a) Pacific and (b) Atlantic slopes. Canopy soils are shown as dark bars, and ground soils are shown as white bars. Asterisks represent significance (*** $P < 0.001$, ** $P < 0.01$, *$P < 0.05$, † $P < 0.1$). Data are presented as mean ± SE (number samples = Pacific: 26 canopy, 39 ground; Atlantic: 22 canopy, 33 ground). Detailed statistical results are presented in Table 3.2.
Fig. 3.2. Soil properties by elevation in canopy (○) and ground (●) soils. Along the Atlantic slope, (b) soil moisture content changed more dramatically with elevation in canopy compared to ground soils, as indicated by a significant elevation*type interaction. Similarly, temperatures of canopy soils were more sensitive to elevation than were temperatures of ground soils along the (c) Pacific and (d) Atlantic slopes. There was a slight increase soil pH in canopy soils with elevation, and a slight decline in soil pH in ground soils along the (e) Pacific and (f) Atlantic slopes. Along the Pacific slope, (g) percent C was more sensitive to elevation in ground soils than canopy soils. Lines are significant best-fit regressions for canopy (dashed line) and ground (solid line) soils. Symbols are mean ± SE. Detailed statistical results are presented in Table 3.3.
Fig. 3.3. Potential extracellular enzyme activity (EEA) in canopy and ground soils in the (a) Pacific and (b) Atlantic slopes. Enzyme activity of α-glucosidase (AG), β-glucosidase, cellobiohydrolase (CBH), and β-xylosidase (BX) was higher in canopy soils (black bars) compared to ground (white bars) soils. Note that activities for AG and BG on the Pacific slope were only marginally significant, and are designated. Data are presented by slope because of significant slope by type interactions. Asterisks represent significance (*** $P < 0.001$, ** $P < 0.01$, *$P < 0.05$, † $P < 0.1$). Data are presented as mean ± SE (number samples = Pacific: 25 canopy, 39 ground; Atlantic: 22 canopy, 33 ground). Detailed statistical results are presented in Table 3.4.
Fig. 3.4. Canopy and ground soils differed in the elevational responses of potential extracellular enzyme activity (EEA) along the Pacific slope, as indicated by significant elevation*type interactions. In contrast, these interactions were not significant for the Atlantic slope. Enzyme activity of (a) $\alpha$-glucosidase (AG) and (c) $\beta$-xylosidase (BX) declined more dramatically with elevation in canopy ($\circ$) compared to ground (●) soils ($P < 0.001$). Lines are significant best-fit regressions for canopy (dashed line) and ground (solid line) soils. Symbols are mean ± SE (number samples = Pacific: 25 canopy, 39 ground; Atlantic: 22 canopy, 33 ground). Detailed statistical results are presented in Table 3.3.
Fig. 3.5. Nonmetric multidimensional scaling (NMS) ordination showing differences in fungal community composition between canopy (○) and ground (●) soils along the (a) Pacific and (b) Atlantic slopes. Canopy and ground soils differed significantly in fungal community composition ($P = 0.014$) across slopes. Fungal communities also structured based on elevation, slope, and soil properties (Table 3.6). Each symbol represents one sample.
Fig. 3.6. Relative abundances of fungal functional groups in canopy and ground soils along both slopes. Relative abundance of decomposers, endophytes, lichen-forming, mycorrhizal, pathogens, and yeasts differed between the canopy (black bars) and ground (white bars) soils. Difference in relative abundance of pathogens between soil types was not significant. Asterisks represent significance (*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, † $P < 0.1$). Data are presented as mean ± SE (number samples = canopy: 46; ground: 69). Detailed statistical results are presented in Table 3.5.
Fig. 3.7. Relative abundance of fungal functional groups by elevation in canopy versus ground soils. Responses to elevation were significantly different in canopy (○) versus ground (●) soils for (a) decomposers, (c) endophytes, and (e) yeasts on the Pacific slope, and for (b) decomposers on the Atlantic slope, as indicated by significant elevation*type interactions. Lines are significant best-fit regressions for canopy (dashed line) and ground (solid line) soils. Symbols are mean ± SE (number samples = canopy: 46; ground: 69). Detailed statistical results are presented in Table 3.3.
Fig. 3.8. Relative abundance of lichen-forming fungi varied more dramatically with elevation in canopy (○) versus ground (●) soils along the (a) Pacific and (b) Atlantic slopes, as indicated by a significant elevation*type interaction. Relative abundances of decomposers, endophytes, and yeasts also varied more dramatically with elevation in canopy compared to ground soils. Lines are mean ± SE (number samples = canopy: 46; ground: 69). Detailed statistical results are presented in Table 3.3
I was sitting on the patio of the Café Caburé looking across a dirt road into the densely forested Bajo del Tigre Reserve. A car drove by, kicking up a dust cloud. These are not the clouds you look for in the damp, verdant cloud forests of Monteverde, Costa Rica. But it had been a really dry year.

A gust of wind traveled upslope toward the cafe, and the brown cloud dissipated. I reached for my sweatshirt. Although this was the tropics, I was 4,600 feet up the mountain. The air was comfortably cool.

Then a clear, metallic call cut through the wind. A Froot Loops-colored beak protruded from the tree line. Tourists and birders jumped out of their chairs, eyes pressed to their binoculars.

“Look! To the left, it’s the keel-billed toucan. Can you see it?”

I heard the shouts but was focused on something else: the shocked and distressed faces of locals and other scientists like myself.

The toucan was not supposed to be there.

For the last decade, I have made regular visits to Monteverde to study the soil in the surrounding cloud forest, situated on the Pacific side of the Cordillera de Tilarán.

Clouds have a significant effect on what happens here, high up in the mountains. Less sunlight hits these forests, and in the cooler, wetter conditions that prevail, processes like decomposition operate at a slower pace than in lowland rain forests. These forests are packed with species found nowhere else.

But things are changing. The cloud layer is moving up the mountain. Warmer temperatures in the lowlands are causing clouds to form higher up than they should, and the forests that were once enveloped in these mists now suffer long dry spells. This is affecting not only the animals and plants, but also the soils I study. As the damp ground dries, dead plants break down faster, releasing carbon dioxide into the atmosphere.

In these changing situations, species either adapt, move or die. That’s why the keel-billed toucan we saw was so high on the mountain. The toucan was moving up as the climate below warmed.

Of course, this is not just a problem in Costa Rica. Uphill migrations are happening throughout the tropics right now. Tropical plants and animals tend to tolerate only very narrow temperature ranges. Small deviations are a big deal. So mountains can provide temporary relief for lowland
species as temperatures warm. That is, until they get to the top and there is nowhere else to go.

This can lead to local extinctions, as species vanish from certain areas. And although these local extinctions do not always lead to global eradication, they do give us a good indication of how a species might fare overall in the future.

We don’t just have studies that indicate such movement is taking place, you can see it happening, even during a meal on a patio.

My interrupted lunch was three years ago. Now the keel-billed toucan hangs out 460 feet higher up the mountain. There is only 1,000 more feet to the top.
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