TROPICAL MONTANE FORESTS IN A CHANGING ENVIRONMENT

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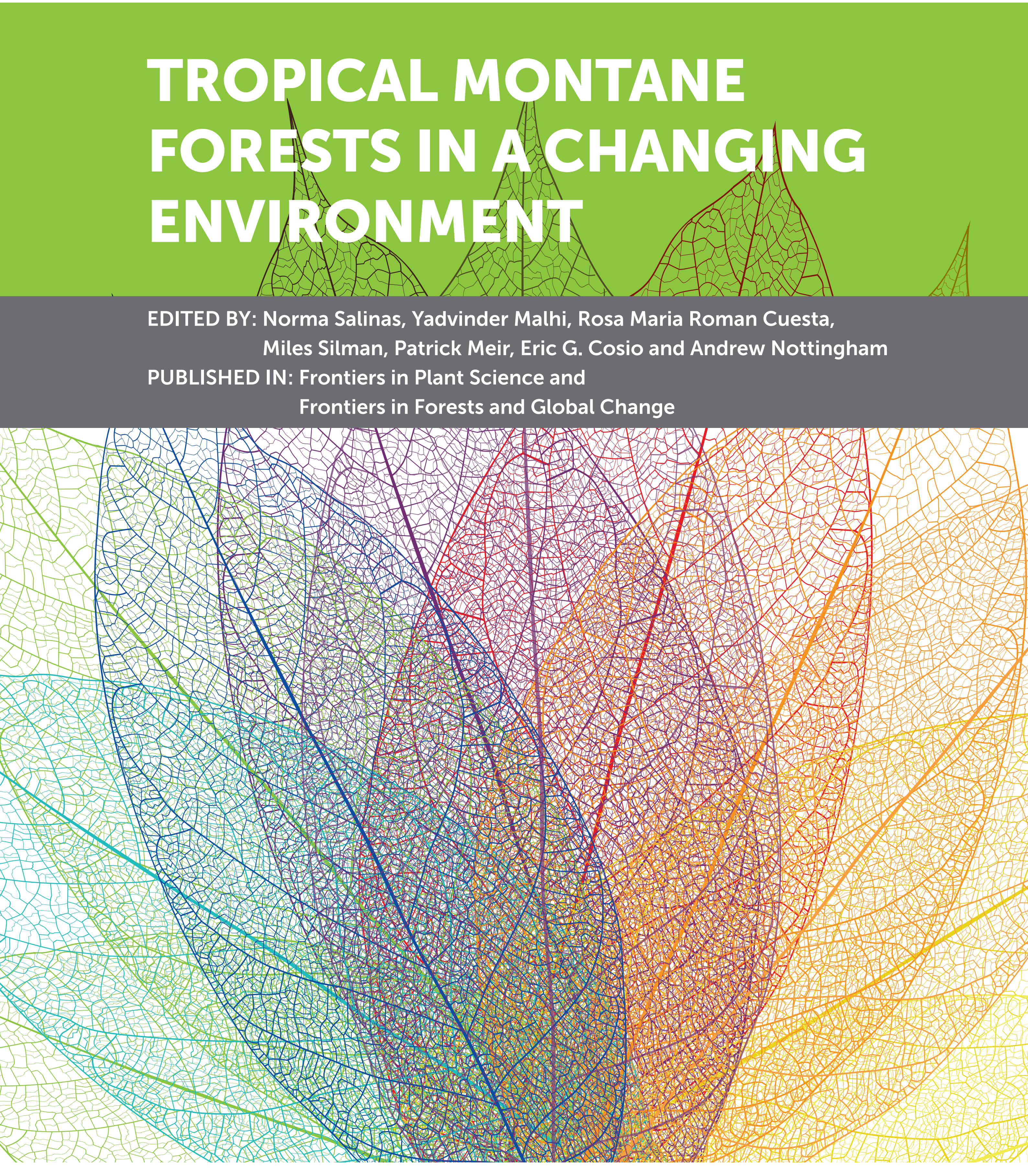

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TROPICAL MONTANE FORESTS IN A CHANGING ENVIRONMENT

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Editorial: Tropical Montane Forests in a Changing Environment

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Editorial on the Research Topic

Tropical Montane Forests in a Changing Environment

Tropical montane forests (TMFs) are found on most of Earth's continents along variable elevation ranges, whose potential upper limits are influenced by cloud condensation heights and minimum temperatures. They are most widespread in South America and in (semi-)humid mountain areas (Richter, 2008). According to FAO and UNEP (2020), the area covered by tropical and subtropical montane forests is around 305 million hectares, about 13% of the area covered by tropical and subtropical forests. Their elevational limits are difficult to establish due to the interactions of the different factors that determine their characteristics. Among these, geomorphology plays a leading role in regulating TMF structure, and provides useful clues on the contributing mechanisms. Most TMFs occur under highly variable topography, including steep slopes (Asner et al., 2014) and landslide-prone terrain (Shreve, 1914; Larsen and Torres-Sánchez, 1998). Also, the latitudinal gradient, orography, and vertical thermal gradients have a direct influence on the fauna and flora of TMFs. The latitudinal pattern is not the same in all TMFs, the temperature and precipitation conditions occur due to seasonality in the climate and are unambiguously linked to species climatic affinity preferences (Ohsawa, 1991; Cuesta et al., 2016; Chu et al., 2019). Another factor is the annual precipitation that generally exceeds c. 1,000–1,200 mm and can be associated with low level cloud cover or mist, which results in a lower incidence of sunlight and lower primary productivity, suggesting that NPP for these forests is driven by changes in photosynthesis. This highlights the importance of variations in solar radiation. Girardin et al. (2010) estimated that NPP values recorded in TMFs range widely between 4 and 7 Mg C ha^{-1} yr^{-1}. Despite this variability, TMFs store significant amounts of carbon in their soils. Malhi et al. (2017) showed that the soil organic layer depth sharply increased with lower mean annual temperatures. Lower temperatures also result in low nutrient inputs through slow mineralization of organic matter (Townsend et al., 1995). Declining temperature appears to be the principal rate-limiting factor for litter decay with increasing elevation on tropical mountains (Schuur, 2001; Salinas et al., 2011). The low temperatures have also been linked to biogeochemical limitations, by reducing nitrogen availability (Nottingham et al., 2015, 2018b) and N_2 fixation (Houlton et al., 2008). However, biogeochemical cycling in TMFs is further affected—returning to our primary driver—by geomorphology via landslide activity and uplift, which increases the supply of rock-derived nutrients such as phosphorus (Tanner et al., 1992; van de Weg et al., 2012; Nottingham et al., 2015). The novel environments TMFs represent are, thus, a product of interconnected geological and climatic forces.

Current global climate models (GCMs) suggest enhanced warming of the tropical mid and upper troposphere (Fu et al., 2011). Consequently, rates of temperature rise are expected to be larger at higher than at lower elevations, as it has already been reported in mountains around the world (Bradley et al., 2006; Roman-Cuesta et al., 2014). There is still uncertainty on the effects that temperature and changing moisture conditions will have on the cloud belt formation in TMFs (Lawton et al., 2001) but upper displacements of the condensation belt are expected (Foster, 2001; Halladay et al., 2012). Moreover, mountain regions are more frequently suffering the impacts of oceanic warming such as El Nino Southern Oscillation ENSO and North Atlantic Oscillation (NAO)/Atlantic Multidecadal Oscillation (AMO), but the effect of their drought and flooding spells on TMFs' flora and fauna are yet under-researched (Foster, 2001; Roman-Cuesta et al., 2014; Oliveras et al., 2017).

Tropical montane forest ecosystems are fragile but exceedingly valuable ecosystems, due to their important role in the provision of ecosystem services, including the regulation of water and the regional climate (Bubb et al., 2004), the capture and storage of carbon (Cuesta et al., 2009; Tejedor Garavito et al., 2012) and—not least—by harboring a vast store of biodiversity (Myers, 1995). The complex spatial and environmental gradients typical of TMFs generate a high diversity of habitats. TMFs are considered among the most biologically diverse and richest ecosystems on Earth (Kessler and Kluge, 2008; Richter, 2008), and recognized as hotspots of species endemism (Gentry, 1993).

These vital ecosystems services are under threat, as climate change is undoubtedly affecting their species composition and metabolic profiles in a myriad of ways. Along an elevation gradient, as global average temperatures rise, elevational shifts in the distribution of species toward regions of lower temperature are to be expected. A major concern is that the speed of climate change appears to be greater than the response capacity (adaptation and migration) for a large number of species in the Andean Amazon. On the one hand, adaptation within species or communities may result in the increased dominance of individuals more tolerant of change. On the other hand, species extinction may occur alongside migration and geographic displacement of susceptible populations toward areas with a more appropriate climate. This implies a high probability of extinction for plant species without this response capacity, which in turn would lead to changes in the carbon cycle, in the dynamics of ecosystems and uncertain impacts on wildlife. But these changes are evidenced not only in animals and plants, soil bacterial, and fungal communities on tropical mountains are also sensitive to temperature (Looby et al., 2016; Nottingham et al., 2018a) and may be affected by rapid climate warming with negative implications for carbon storage (Nottingham et al., 2019) and for plant species composition (Corrales et al., 2016). At the same time, these ecosystems are in a state of global threat due to the dynamics of change in land cover and use (Webster, 1995; Bubb et al., 2004). In many regions, land use patterns have created a mosaic of habitats transformed through the expansion of human activities. These fragmented forests should receive more attention when designing conservation policies. For all these reasons climate change can have severe impacts on montane tropical ecosystems by generating changes in the life zones, increasing the vulnerability of forests to fires, pests, invasive species, and greater deforestation pressure due to the establishment of productive systems with intensive management (Serreze, 2009).

The articles in this special issue aim to fill some of the existing gaps in our knowledge. These studies were conducted in a wide range of TMFs from pristine forests in protected areas to those with varying degrees of human disturbance along South America, Africa, and Asia. These studies have examined TMF responses to environmental cues in forest plots using a variety of tools which include remote sensing, on-site instrumentation, biometrics, and allometry, among others, to model field data and provide us with:

Two contributions to this special issue used leaf chemistry and traits analysis to determine if plant species are sensitive to changes in environmental conditions. Martin et al. assessed differences in 19 foliar traits in paired sun and shade leaves along a humid tropical forest elevation gradient in Peru, to determine if foliar chemical traits, such as photosynthetic pigments, and other leaf traits like LMA differ between them, and if the sources for these variations are environmental or genetic. They found that for most traits (i.e., N, foliar nutrients or defense compounds), there was no significant difference between sun and shade leaves. Other traits for growth, such as LMA and δ13C concentrations, maintain constant offsets, suggesting that the characteristics of shade leaves can be derived from those measured in sun leaves. Their findings also indicate that variation in sunlit canopy foliar traits are controlled primarily by changes in community composition, and secondarily by environmental factors, like elevation or substrate. They conclude that there are significant differences in light-sensitive traits between sun and leaves evaluated, that were maintained across a wide variety of environmental conditions along a 3,500-m elevation gradient suggesting that plasticity associated with light availability is an adaptive change. In contrast, they did not find sun-shade differences in other foliar traits related to defense and metabolism. Gong et al. evaluated the protective function and phylogenetic relationships of the transient red coloration of young leaves in some tropical plant species. They investigated the metabolism, photosynthetic activities, and chemical defenses of leaves from 250 tropical plant species with either red or green young leaves, in a tropical region of southwest China. They found that the occurrence of transient reddening of juvenile leaves in the tropics was coupled with increased levels of both anthocyanins and tannins and that the red coloration protects them from insect herbivory primarily through chemical defense. Also, the red coloration in young leaves is predominantly a result of adaptation to special tropical environmental conditions but without a significant intrinsic phylogenetic relationship between plant species and suggested that the anthocyanins might not function as light attenuators to protect for effects of high light intensity.

Two studies in this collection illustrate photosynthetic plant function related to light and leaf nutrients. Feeley et al. assessed the maximum photosynthetic thermal tolerances of more than 550 individuals of 164 tropical canopy tree species growing in a steep elevation gradient ascending from near sea level to near tree line in the northern Andes mountains of Colombia. They analyzed changes in plant thermal tolerances between elevations at the species and community level and tested the relationship between species' thermal tolerances and their changes in abundance through time in 10 forest inventory plots. They found a high amount of variation in the maximum thermal tolerance (T 50) among species co-occurring within each plot and that this tolerance decreases with plot elevation. However, their results also indicate that the relationship between T 50 and temperature is weak and extremely shallow. Ziegler et al. investigated physiological, chemical, and structural properties of leaves in mature individuals belonging to 12 tree species in a tropical montane rainforest in Rwanda, Central Africa. In this study, they explored the relative importance of area-based total leaf N content and within-leaf N allocation to photosynthetic capacity vs. light-harvesting in controlling the variation in photosynthetic capacity to explore the controls of interspecific variation in photosynthetic capacity and other leaf gas exchange traits. They found that photosynthetic capacity at a common leaf temperature of 25°C was higher in early succession species than in late. However, total leaf N content did not significantly differ between successional groups and there was no significant trade-off between relative leaf N investments in compounds maximizing photosynthetic capacity vs. compounds maximizing light harvesting.

Litton et al. provide information complementary other studies in this issue by examining how litterfall, live foliar nutrient concentration, foliar nutrient resorption efficiency, nutrient return via litterfall, and nutrient use e?ciency vary with mean annual temperature in two dominant tree species in a gradient in Hawaii. Their aim was to understand how increasing mean annual temperature impacts on the availability and ecological stoichiometry of macro and micronutrients. Their findings provide strong evidence that increased mean annual temperature alters the cycling and availability of a broad suite of nutrients in TMFs, with important implications for nutrient limitation to ecosystem processes in a warming world.

de la Cruz-Amo et al. explore the role of Andean TMFs as carbon reservoirs. They calculated the amount of carbon in aboveground and belowground carbon stocks, and in soil organic matter, along two elevation gradients in the southeast of Ecuador and North-Central Peru. They assess how carbon stocks vary along elevation gradients and determine the influence of climate, particularly precipitation seasonality, on the distribution of these stocks across different forest compartments. They report that the combination of annual mean temperature and precipitation seasonality explains the differences in mean total carbon stocks in these three compartments and also show different partitioning patterns along the elevation gradients both in Ecuador and Peru but that total carbon stocks do not change with elevation in either site.

Two studies report results analyzing the literature. Soh et al. systematically mapped all research on the effects of habitat degradation in TMFs globally to identify deficiencies in current knowledge and to guide future research prioritization. After a comprehensive review, they show that habitat degradation in TMFs impacts biodiversity at all ecological levels and is compounded by climate change. However, despite montane species being perceived as more extinction-prone, there are some indications of biotic resilience if disturbance in TMFs is less severe. They confirm that TMFs also provide important ecosystem services being the most important water provision, but that in recent years these ecosystems have come under human pressure manifested in the form of highest rates of deforestation. They highlight the poor research representation of Asian and African TMFs and list the top research priorities which, if addressed, would advance the goals of biodiversity conservation and sustainable use of resources in TMFs.

Tito et al. present an analysis of the natural variation of abiotic and biotic factors along mountain elevation gradients based on two papers that used field experiments conducted along an elevation gradient in the Peruvian tropical Andes. They highlight the potential for use of field experiments in future studies focused on determining the direct and indirect e?ects of climate change. They conclude that, despite abundant research on the effects of global change climate on TMFs, fundamental questions remain unanswered and it will be necessary to apply more fine-scale experimental approaches to help better predict future abundance and distribution patterns of species under altered climate scenarios, and that natural gradients are essential to quickly gain a more complete understanding on the possible impacts of climate change.

To date, there have been few experimental studies using artificial material to explore predation conduct. Murray et al. carried out predation experiments in Peninsular Malaysia at a landscape scale and across a suite of sites of varying disturbance. They used four di?erent prey items—artificial nests, artificial seeds, caterpillar models, and frog models—along a disturbance gradient, from pristine forests to tea plantations. Their purpose was to assess whether predation probability in different habitat types di?ers between mountain ranges, if this probability consistently varies in di?erent habitat types and if predation can be explained by vegetation structure. Their results show that they confirm the first and third hypotheses, but there is no clear trend in predation probability along a habitat disturbance gradient.

Results from this collection of articles show that responses to Global Change can vary greatly among species, ecosystems, and even microsites in TMFs. This suggests that the fate of these forests in response to climate change and greater deforestation pressure due to the establishment of productive agricultural systems with intensive management, can have severe impacts on montane tropical ecosystems by generating changes in vegetation life zones, increasing their vulnerability to fires, pests, invasive species, and having dramatic consequences on downstream ecosystem services. We hope that this selection of papers will stimulate interest and research into those wonderful ecosystems that are TMFs and their elevation gradients, and provide insights into the future of TMF ecosystems in a century of rapid climate change.

AUTHOR CONTRIBUTIONS

NS drafted the first version of the editorial. EC, MS, PM, AN, RR-C, and YM made edits, additions, and revisions. All authors contributed to the article and approved the submitted version.

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Impacts of Habitat Degradation on Tropical Montane Biodiversity and Ecosystem Services: A Systematic Map for Identifying Future Research Priorities

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Tropical montane forests (TMFs) are major centers of evolutionary change and harbor many endemic species with small geographic ranges. In this systematic map, we focus on the impacts of anthropogenic habitat degradation on TMFs globally. We first determine how TMF research is distributed across geographic regions, degradation type (i.e., deforestation, land-use conversion, habitat fragmentation, ecological level (i.e., ecosystem, community, population, genetic) and taxonomic group. Secondly, we summarize the impacts of habitat degradation on biodiversity and ecosystem services, and identify deficiencies in current knowledge. We show that habitat degradation in TMFs impacts biodiversity at all ecological levels and will be compounded by climate change. However, despite montane species being perceived as more extinction-prone due to their restricted geographic ranges, there are some indications of biotic resilience if the impacts to TMFs are less severe. Species richness and key species interactions can be maintained in mildly degraded sites, and gene flow can persist between TMF fragments. As such, minimally degraded areas such as secondary forests and restored areas could play a crucial role in maintaining the meta-community and ecosystem services of TMFs—either via resource provision or by linking patches of pristine forest. Research deficiencies highlighted include poor research representation in Asian and African TMFs, few assessments of population and genetic-level responses to fragmentation, and little assessment of the impacts of habitat fragmentation at *all* ecological levels. To address these concerns, we present a list of the top research priorities to urgently address the growing threat of habitat degradation in TMF.

Keywords: tropical montane forest, cloud forest, habitat degradation, disturbance, deforestation, fragmentation, biodiversity, ecosystem service

INTRODUCTION

In addition to harboring rich biodiversity and endemism (Kessler and Kluge, 2008), tropical montane forests (TMF) provide crucial ecosystem services that include the production of water, carbon sequestration and storage, and prevention of erosion (Martinez et al., 2009; Spracklen and Righelato, 2014; Brenning et al., 2015). The occurrence of TMFs depends on the prevailing climate rather than a defined elevation, and they have lower temperatures and higher humidity relative to tropical lowland forests (Bruijnzeel et al., 1993). As such, TMFs can occur at elevations as low as 500 m above sea level (a.s.l.) on small islands, to 1,200 m a.s.l. on coastal or isolated mountain ridges, and as high as 2,000–3,000 m a.s.l. on large inland mountains (Scatena et al., 2010). Forest structure is similarly variable, with lower-elevation TMF characterized by emergent trees up to 35 m tall, an absence of large buttress roots, two layers of canopy, and many non-vascular epiphytes (Whitmore, 1998; Scatena et al., 2010). Upper TMFs feature shorter trees with high moss cover (Frahm and Gradstein, 1991; Scatena et al., 2010), while sub-alpine TMFs have small gnarled trees and a near absence of epiphytes (Kitayama, 1995; Scatena et al., 2010). About 7% of the 3.2 million square kilometers of TMF is tropical montane cloud forest (TMCF) which lies within the upper and lower bounds of cloud formation (Scatena et al., 2010).

Numerous species endemic to montane regions are threatened with extinction due to deforestation (Long, 1995; Brooks et al., 1999; Renner et al., 2006; Hall et al., 2009). From 2000 to 2010 more than 7 million ha of tropical forest (including forest types beyond TMFs) were lost each year, with a corresponding annual gain in agricultural land of 6 million ha (FAO, 2016). Temperatures in lowland tropical regions have increased by 0.25°C per decade (Malhi and Wright, 2004) and are projected to rise by 2.6–4.8°C (mean 3.7°C) over the twenty first century under the RCP8.5 greenhouse gas concentration trajectory (Cubasch et al., 2013), leading both to species' ranges contracting to higher elevations, and an increased risk of extirpation (Peters and Darling, 1985; La Sorte and Jetz, 2010; McCain and Colwell, 2011). Conversion of lowland regions to agriculture promotes drier conditions in adjacent TMFs due to reduced cloud formation; consequently, forests dependent on humid conditions become restricted to higher elevations, exacerbating habitat loss and fragmentation (Lawton et al., 2001).

Prior efforts to comprehend the collective impacts of habitat degradation on TMFs were focused either on TMCFs or TMFs in the neotropics. The first synthesis of research on TMCFs in 1987 concluded that deforestation, agricultural expansion, roads and human encroachment were major threats (Stadtmüller, 1987). Later, growing interest in the preservation of TMCFs led to the publication of symposium proceedings on TMCF in 1993 and 2004 (Hamilton et al., 1995; Bruijnzeel et al., 2010). Another symposium was conducted in 1993, with a regional focus on neotropical TMFs (Churchill et al., 1995). In Asia, a case-study in Malaysia highlighted the current threats to the local TMCF and proposed multi-sectoral strategies for their conservation (Peh et al., 2011).

Despite these contributions, clear research directions that could support the sustainable use of the world's TMFs are lacking. Research questions for the conservation of TMCF were consolidated in Bruijnzeel et al. (2010), based on a list of one hundred questions of importance to the conservation of global biodiversity by Sutherland et al. (2009). While the questions are valid and well-represented for ecosystem services and species-focused research, none addressed conservation concerns at the community, species-interaction and genetic levels. The Malaysian case-study by Peh et al. (2011) provided a holistic approach to mitigate the threats posed to TMCF; however, the strategies were aimed at tackling inadequacies in various commercial and governmental sectors (i.e., agriculture, forestry, infrastructure, tourism, and policy and legislation) without regard to current research gaps and priorities. To address these shortfalls, we systematically mapped all research on the effects of habitat degradation in TMFs globally to identify deficiencies in current knowledge, and to guide future research prioritization. Specifically, we asked how TMF research is distributed by geographic region, country, degradation type (i.e., deforestation, land-use conversion, habitat fragmentation, and edge effects), ecological level (i.e., ecosystem, community, population, genetic) and taxonomic group. We summarized the impacts of habitat degradation on biodiversity and ecosystem services and examined how degradation of TMFs has affected species richness and composition—two of the most commonly used response metrics for communities. Lastly, we list the top research priorities which, if addressed, would advance the goals of biodiversity conservation and sustainable use of resources in TMF.

METHODS

We performed a search scoping exercise in Web of Science (WoS) to generate a suitable search string to gather relevant articles for a systematic map (Collaboration for Environmental Evidence, 2013) (see **Figure 1** for flow diagram of protocol). Systematic maps or reviews differ primarily from traditional reviews in that the methods for the former show greater transparency (Collaboration for Environmental Evidence, 2013; Berger-Tal et al., 2018). We tested various combinations of keywords that returned a manageable number of hits (<5,000), while not being overly restrictive. Keywords were combined with appropriate Boolean operators (e.g., AND, OR), wildcards (*) to account for alternate word endings and parentheses for nested searches. The eventual search we selected was (trop* monta* forest* OR cloud forest* OR trop* high* elevation* forest* OR trop* mid* elevation* forest*) AND (degrad* OR deforest* OR fragment* OR disturb* OR land convers* OR land change*) which returned 2,495 hits on 23 January 2018. The keywords in the first set of parentheses represented the ecosystem of interest, which broadly covered tropical montane forests, and the keywords in the second set of parentheses were terms related to habitat degradation. Additionally, we also gathered articles from CAB Abstracts using the same search string as the search function allowed for nested searches as in

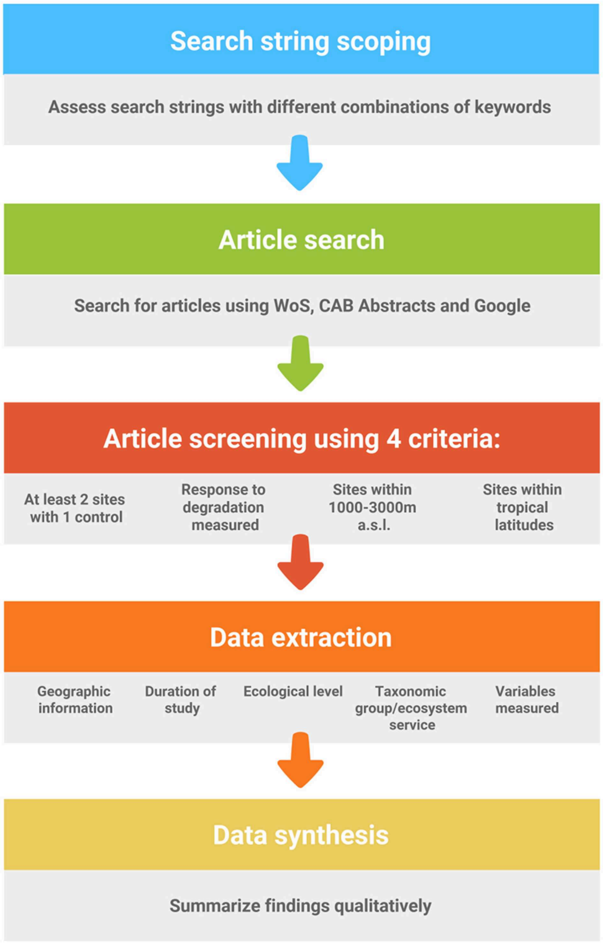

FIGURE 1 | Flow diagram of the systematic mapping protocol. WoS, Web of Science.

WoS. CAB Abstracts returned 458 hits on 5 March 2018. In both cases, the forms of articles accepted included peer-reviewed journal papers, book chapters and conference papers. We also entered the same keywords with wildcard operators in Google Search to find relevant gray-literature that was possibly missed in WoS or CAB Abstracts (but without parentheses) as our original nested search performed poorly with a return of only five hits. Google Search returned 16,600 hits on 24 April 2019, so we assessed the first 100 hits as they were sorted in order of decreasing relevance.

For data extraction, we used four criteria to determine if an article was suited to the purposes of our systematic map. First, at least two study sites were required, where a control (e.g., primary forest) was compared to a site or sites that had undergone some form of anthropogenic impact (e.g., selective logging). Second, a biotic or abiotic (i.e., ecosystem function) response to the impact needed to have been measured. Third, we broadly considered all tropical forests under frequent cloud cover or mist between 1,000 and 3,000 m a.s.l. to be TMFs, in line with other studies (Brooks et al., 1999; La Sorte and Jetz, 2010; Spracklen and Righelato, 2014; Li et al., 2016; Dietrich et al., 2017). Last, study sites needed to occur within tropical latitudes (i.e., between 23°26′13.5″ N and S). However, we made a few exceptions to include studies where TMFs occurred lower than 1,000 m (e.g., montane forests on small islands where cloud base is lower than 1,000 m). We avoided the inclusion of research on forests 3,000 m a.s.l. or higher, as these biomes are regarded as sub-alpine cloud forests and have different floristic structure and lower biodiversity (Scatena et al., 2010). In total, 362 peer-reviewed articles were considered relevant, of which 344 were obtained from our first search using WoS, 16 were added from using CAB Abstracts and two more were included after using Google Search (**Table S1**).

This literature was systematically assessed by reading the title, abstract, and study site descriptions in the body of each article. We rejected studies that did not yield empirical data, such as reviews or studies that performed simulations or meta-analyses. Data extracted from relevant articles included the year of publication, journal/book/conference title, geographic information (i.e., continent and country where the research is conducted; the name, latitude, longitude and elevational range of the study sites), the duration of the study, the ecological level of the response variable (i.e., ecosystem, community, species-interactions, population, genetic), the taxonomic group or ecosystem service (i.e., water regulation (including erosion control) and purification, maintenance of soil fertility, carbon storage and sequestration, and nutrient cycling), the response and explanatory variables measured, and a summary of the key outcomes. We made the distinction between community and species interaction studies by assessing if the study examined symbiotic relationships (i.e., close relationships between species that include predation, competition, mutualism, parasitism and commensalism). For example, research that studied the effect of deforestation on species richness was considered a community study, while a study of the effect deforestation on nest predation was considered a species-interaction study. We collated the conservation status of all studied species, as listed in the International Union for the Conservation of Nature (IUCN) Red List on the 3 August 2018 (IUCN, 2018).

We classified the type of habitat degradation into four categories: deforestation (i.e., any habitat that has lost pristine forest due to anthropogenic causes such as selectively-logged forest, regenerating abandoned clear-cuts, and secondary forests), land-use conversion (i.e., any habitat that has been cleared and converted to pasture or plantations), and habitat fragmentation and edge effects. Species interactions were broadly classified as competition, predation, mutualism, commensalism or parasitism.

As there were several research deficiencies highlighted in our systematic map, it was useful to consider their relative importance, which we did via consensus among the authors. Research priorities in rank order were: (1) ecological level, (2) impact type, (3) geographic region, and (4) focal taxa. At each level, we highlighted sub-topics that deserved greater attention based on the current extent of our knowledge, and on the urgency for conservation action.

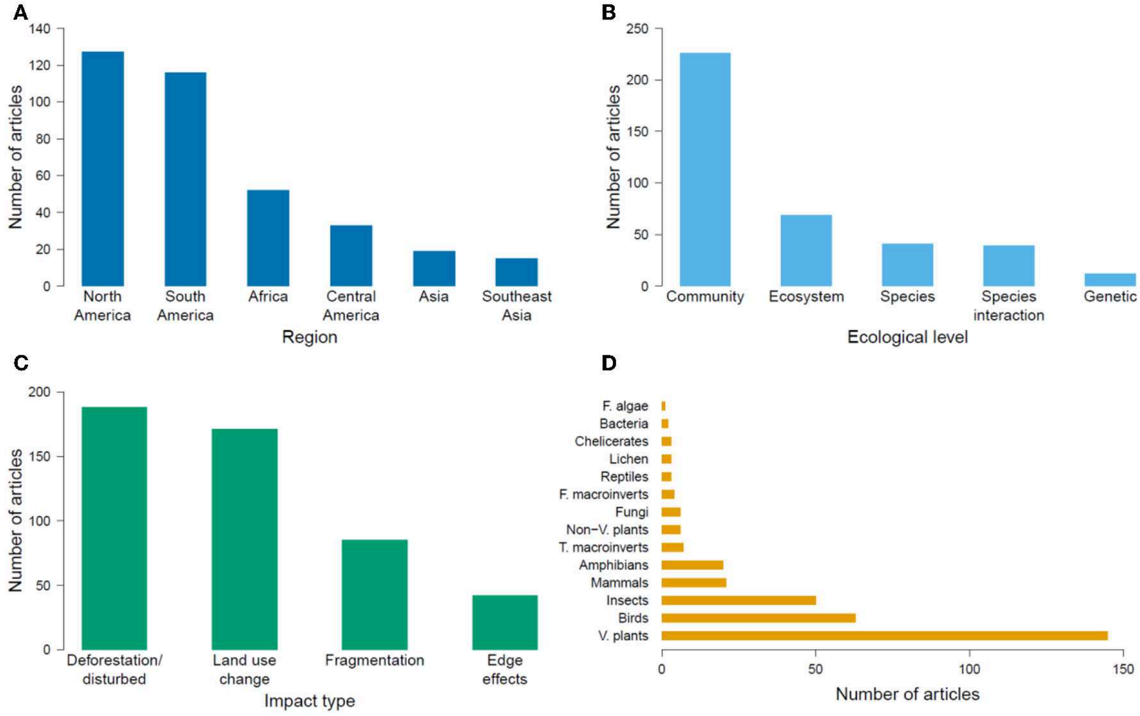

FIGURE 2 | Number of articles published globally from 1991 to 2018 on the impacts of habitat degradation on tropical montane biodiversity in different **(A)** geographical regions, **(B)** ecological levels, **(C)** impact types, and **(D)** taxonomic groups. F, T, and V refer to freshwater, terrestrial, and vascular respectively.

RESULTS

The geographic region with the most number of articles was North America (127 publications, 35% of the global total), and the region with the fewest articles was Southeast Asia (15 publications, 4% of the global total; **Figure 2A**). The number of articles published on TMFs in the Americas more than doubled the number in Africa, and was at least an order of magnitude higher than the number in Asia (**Table 1**). Articles published on Mexican TMFs were the most numerous (121), and constituted 95% of all articles from North America (**Figure 3**). Articles on Columbian and Ecuadorian studies were the next most numerous (43 and 39 articles, respectively). Collectively, these results indicate a very strong research contribution from Latin America, with Mexico, Columbia and Ecuador representing more than 55% of articles considered in this mapping exercise. Among other geographic regions, Costa Rican TMFs were the focus of most articles from Central America (27), Kenyan TMFs were the focus of most articles from Africa (29), and Chinese TMFs were the focus of most articles from Asia (8).

TABLE 1 | Number of published articles on the impact of habitat degradation of tropical montane forests (TMF) in three major regions per unit area of forest.

Region	Number of articles	Area of TMF* (km^2)	Number of articles per 100,000km^2 of TMF
Americas	276	1,150,588	24.0
Africa	52	544,664	9.5
Asia	34	1,562,023	2.2

**Areas of TMFs in each region were obtained from Scatena et al. (2010).*

At the ecological level, most research focused on community ecology, followed by research on ecosystem functioning, species interactions, population ecology and population genetics (**Figure 2B**). Most articles studied the effects of deforestation, followed by land-use change, fragmentation and edge effects (**Figure 2C**). Plants were the most studied taxon, followed by arthropods and birds (**Figure 2D**). When information regarding the duration of a study was available (82% of articles), short-term studies (<4 years) constituted 68% of this subset, while long-term studies (≥4 years) comprised 12%. However, 3% of articles examined long-term effects using historical data collected more than 4 years ago, or included study sites that had been deforested or restored more than 4 years earlier. Many articles yielded no information on the duration of the study (18%).

There were 227 articles focused on community ecology, with most articles from Latin America and the fewest from Southeast Asia. The best studied taxa were vascular plants followed by insects and birds (**Table 2**). Most studies used species richness and species composition (beta diversity) to measure the impacts of environmental change, with only a few measuring functional diversity (**Figure 4A**). Although the impact of deforestation or land conversion of TMF generally resulted in lower species richness (53% of 75 articles), many studies reported no change

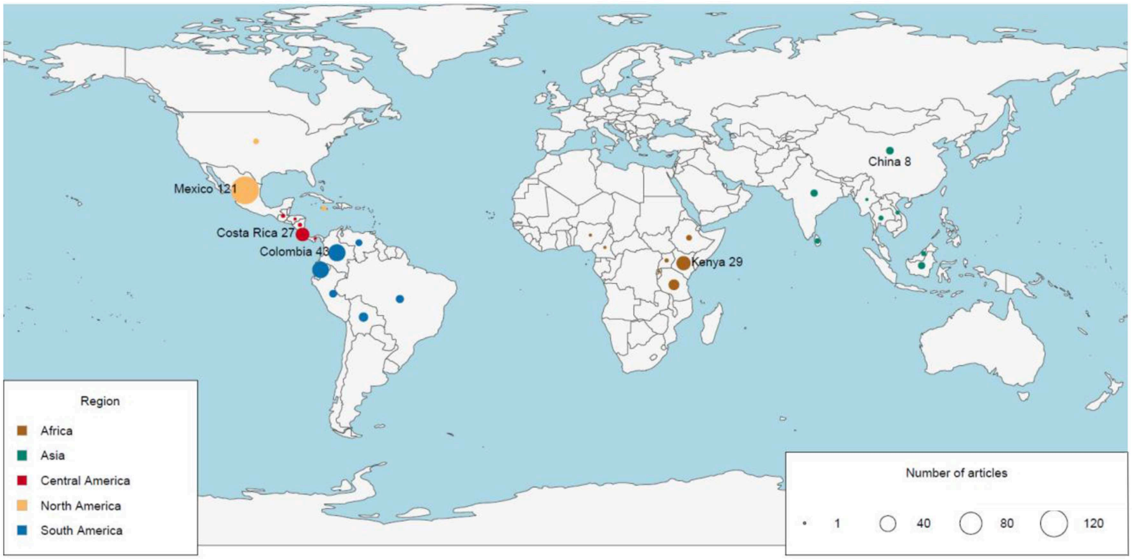

FIGURE 3 | Map of the number of articles published from 1991 to 2018 on the impacts of habitat degradation on tropical montane biodiversity in different countries. The diameter of the circles is directly proportional to the number of published articles. Country names represent the highest number of articles published in the region.

TABLE 2 | Number of articles on the impact of habitat degradation of tropical montane forests at various ecological levels distributed across regions, impact types and taxonomic groups.

Variables	Ecological level				
	Genetic	Population	Species interactions	Community	Ecosystem services
REGION					
North America	3	17	17	77	23
Central America	2	5	5	22	3
South America	2	13	10	77	23
Africa	4	4	6	25	17
Asia	1	3	0	14	2
Southeast Asia	0	0	1	12	2
IMPACT					
Deforestation/disturbance	2	24	16	129	30
Land-use change	0	13	10	107	49
Fragmentation	11	10	10	60	1
Edge effects	1	6	12	27	0
TAXONOMIC GROUP					
Amphibians	–	3	1	17	*na*
Bacteria	–	–	1	1	*na*
Birds	4	8	21	37	*na*
Chelicerates	–	–	–	3	*na*
Freshwater invertebrates	–	–	–	4	*na*
Freshwater algae	–	–	–	1	*na*
Fungi	–	1	2	5	*na*
Insects	2	3	6	40	*na*
Lichen	–	–	–	3	*na*
Mammals	–	7	7	11	*na*
Non–vascular plants	–	–	–	6	*na*
Reptiles	–	–	–	4	*na*
Terrestrial invertebrates	–	–	–	7	*na*
Vascular plants	6	20	28	101	*na*

Dashes indicate no articles were found for the category and "na" is not applicable.

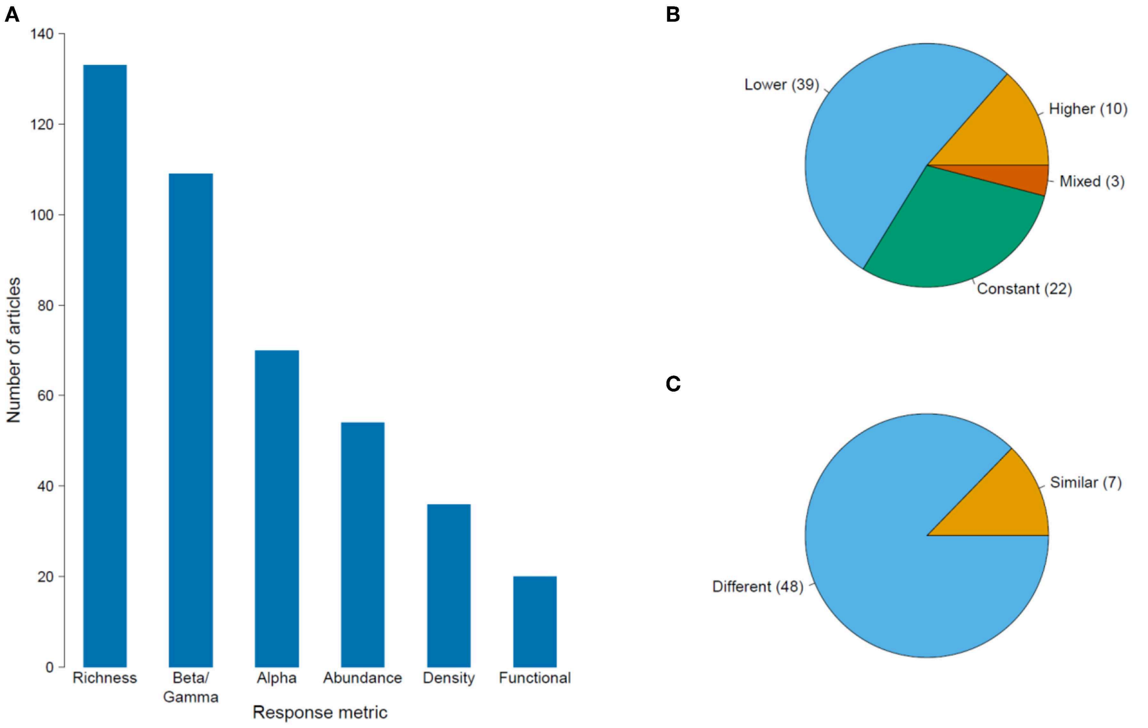

FIGURE 4 | (A) Number of published articles on the impacts of habitat degradation on tropical montane biodiversity using different community response metrics. The proportion of articles showing the community responses to deforestation and habitat disturbance measured using **(B)** species richness; and **(C)** species composition. The numbers in parentheses refer to the number of articles for each type of response. For **(B)**, labels "Lower," "Higher," and "Constant" refer to a decline, increase and no change in species richness in disturbed areas in comparison to pristine sites while "mixed" response refers to a unimodal peak along a disturbance gradient. For **(C)**, labels "Similar" and "Difference" describe if the species composition is the same or different between disturbed and pristine sites.

(29%) or higher species richness (14%) (**Figure 4B**). A few studies (4%) showed peak species richness occurred at an intermediate level of degradation (e.g., the highest number of species was in secondary forest, relative to primary forest and farmland). Species composition showed a more definitive pattern; in 87% of 55 papers, species composition changed in response to deforestation or land-use change (**Figure 4C**).

Of 69 articles on ecosystem services, most were from North America (largely from Mexico; **Table 2**). Most articles (68%) focused on the impacts of land conversion on ecosystem services, while one article focused on fragmentation (**Table 2**). Ecosystem services were broadly classified as water regulation (including erosion control) and purification, maintenance of soil fertility, carbon storage and sequestration, and nutrient cycling. Each category was well-represented, with a slight bias toward articles exploring hydrological impacts and soil fertility (**Figure 5A**).

Species interactions were most commonly studied in Latin America (80% of 39 articles), with seven articles from Africa and one from Asia (**Table 2**). The impacts measured were almost equally represented, with edge effects slightly more studied than deforestation, land conversion or fragmentation (**Table 2**). The most studied species interaction was predation, followed by mutualism, which collectively accounted for 85% of articles (**Figure 5B**). Studies on predation often lacked identification of the predators, due to the rarity of documenting such events.

Population ecology (i.e., species-level processes) was the focus of 41 articles, with (80%) being from Latin America, largely studying vascular plants (**Table 2**). Vertebrates were also well-represented (19% birds, 17% mammals, and 7% for amphibians). Most articles investigated impacts of deforestation and land-use change (73%), with fewer (27%) measuring fragmentation and edge effects (**Table 2**). The demographic parameters measured varied, with population size, density and fecundity being most frequently recorded (61%) (**Figure 6A**). Survival rates and fitness-related traits (e.g., body condition and growth) were also often assessed (30%). To assess the conservation statuses of species in the population studies, we omitted two articles that measured demographic parameters but with a greater focus on community level responses (Hitimana et al., 2004; Cuervo and Restrepo, 2007). Thus, of the 41 remaining papers, 64 species were represented, of which 40% have not had their conservation status assessed by the IUCN, and 30% were considered threatened (Critically Endangered, Endangered or Vulnerable; **Figure 6B**).

Only 12 articles described genetic studies (**Table 2**). The earliest study from 2005 explored the impacts of habitat degradation (logging) on the genetics of an endemic species of oak in China (Zheng et al., 2005). Other genetic studies were published from 2011 to 2015, with six from Latin America, four from Africa and one from Asia (**Table 2**). Most explored

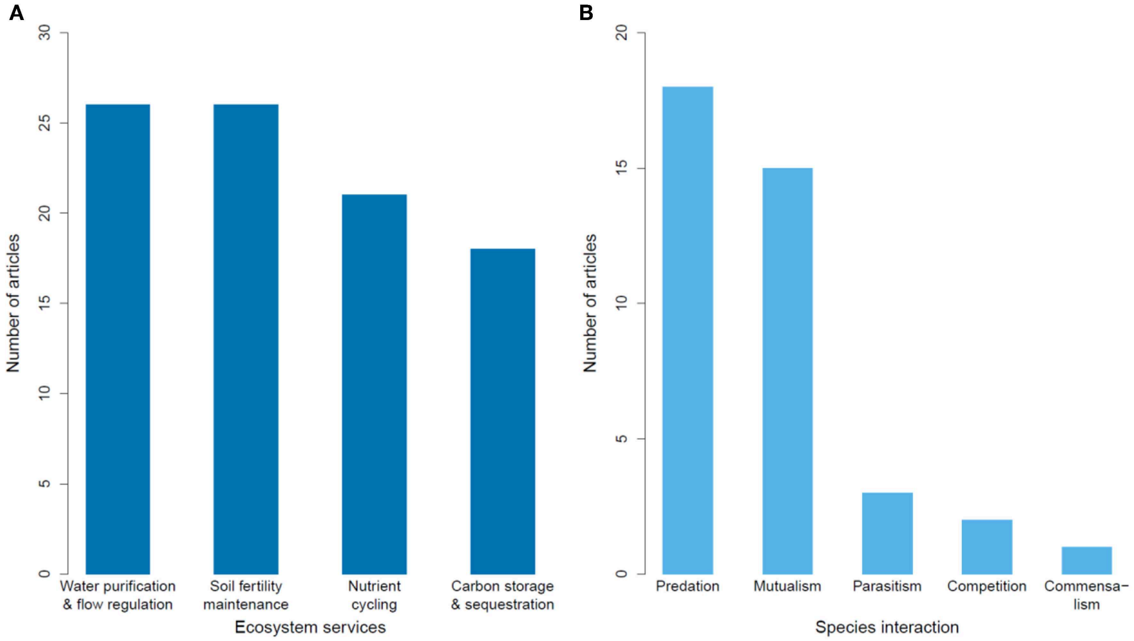

FIGURE 5 | Number of articles published on the impacts of habitat degradation on tropical montane biodiversity studying different types of **(A)** ecosystem services, and **(B)** species interactions.

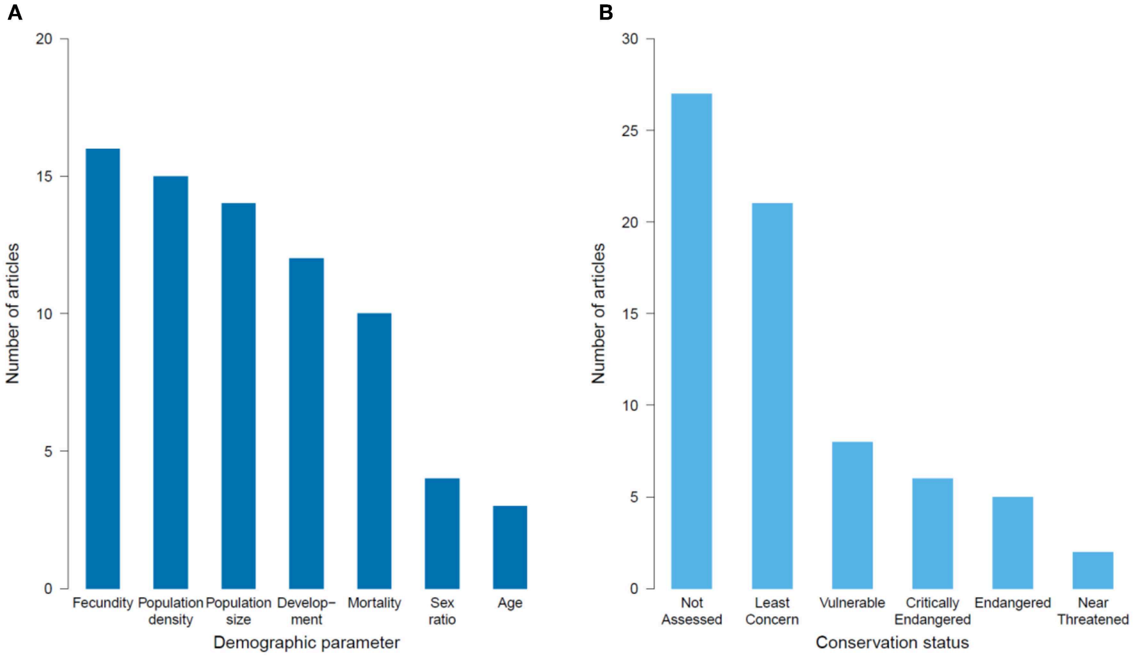

FIGURE 6 | Number of population ecology articles published on the impacts of habitat degradation on tropical montane biodiversity that: **(A)** measured different demographic parameters, and **(B)** studied species listed in the various categories of the IUCN Red List.

the impacts of habitat degradation on a single focal species, but two drew comparisons between two species (Winkler et al., 2011; Quevedo et al., 2013), and another compared seven species (Callens et al., 2011). Most articles examined the impact of fragmentation or edge effects on genetic diversity or gene flow, while a few studied the effects of deforestation or land-use change (**Table 2**). In most cases (75%), deforestation and fragmentation adversely affected genetic variability and gene flow.

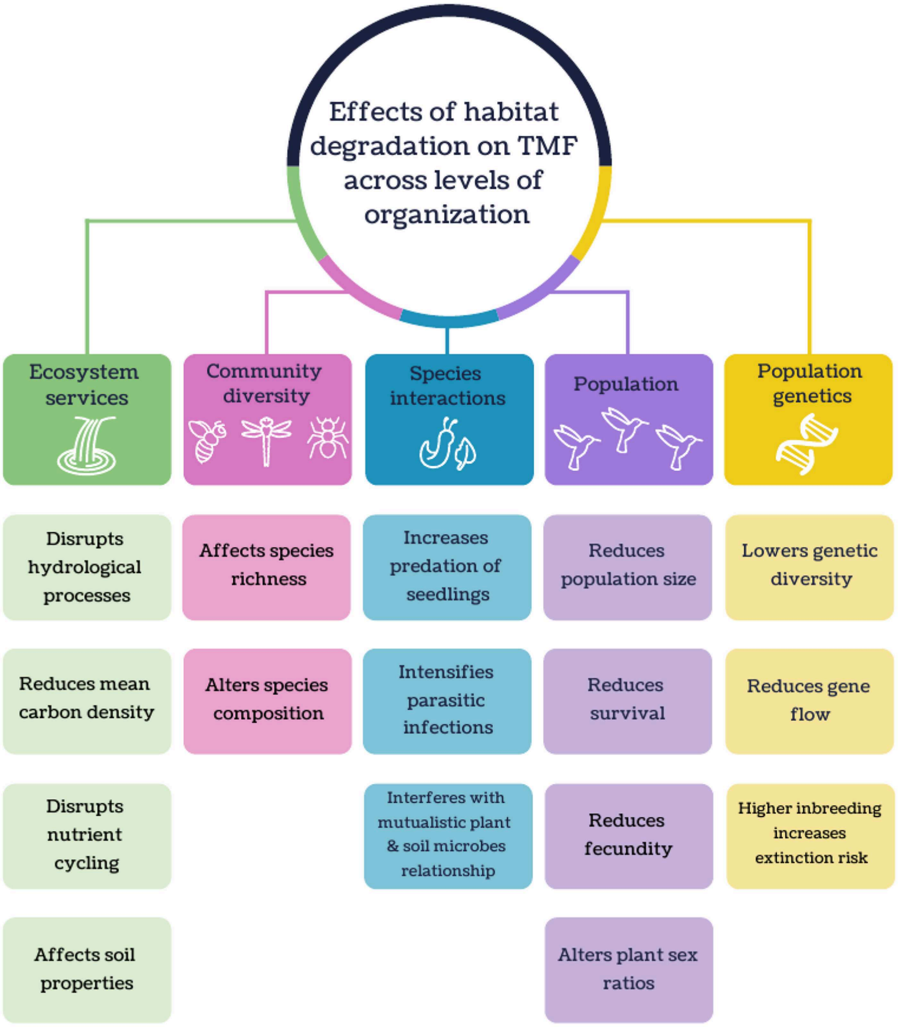

FIGURE 7 | Schematic of the major effects of habitat degradation on tropical montane forest across levels of organization.

Effects of Habitat Degradation on TMF Biodiversity

A myriad of habitat degradation effects on biodiversity and ecosystem services in TMFs were reported (**Figure 7**). Equivocal or inverse responses in species richness may be due to sampling in habitats with intermediate levels of degradation, which often show higher species richness than pristine environments (e.g., Deloya et al., 2007; Vazquez et al., 2011; Mendez-Castro and Rao, 2014; Abella-Medrano et al., 2015; Badillo-Saldana et al., 2016; Gomez-Diaz et al., 2017). The main cause cited was higher resource availability (e.g., food abundance, breeding habitat or light availability) that attract resource-generalist species (Vazquez et al., 2011; Mendez-Castro and Rao, 2014; Abella-Medrano et al., 2015; Badillo-Saldana et al., 2016). Changes in species composition across a disturbance gradient were often reported, with resilient species more likely to be generalists (e.g., Pattanavibool and Dearden, 2002; Gove et al., 2013; Diaz-Garcia et al., 2017; Gomez-Diaz et al., 2017), not threatened (Basham et al., 2016), have broad elevational distributions (Escobar and de Ulloa, 2000), introduced (Gomez-Diaz et al., 2017), and adaptable to climatic change (Ariyanti et al., 2008).

The degradation of TMF can be detrimental to some species interactions such as predator-prey (e.g., Goldsmith et al., 2007; Kumar and O'Donnell, 2007; Anthelme et al., 2014) and mutualistic (e.g., Maldonado et al., 2000; Lehouck et al., 2009; Pizano et al., 2017) relationships. Increased predation of seedlings was observed in deforested areas due to a lack of concealment from predators (Anthelme et al., 2014). Habitat degradation also interfered with mutualistic relationships between plant and soil microbes. For example, plant growth showed a positive response to soil filtrate from TMF due to the presence of beneficial soil microbes, but was negatively affected by soil filtrate from pastures (Pizano et al., 2017). Parasitic infections generally intensified with increased habitat disturbance. Amphibian chytrid fungus (*Batrachochytrium dendrobatidis*) was most prevalent in agroforests (Murrieta-Galindo et al., 2014), and mistletoe (*Psittacanthus schiedeanus*) infection of host trees was more common at the forest edge than the interior (Lopez de Buen et al., 2002).

There were a small number of TMF articles that have investigated how populations respond to habitat degradation. From these studies, habitat fragmentation was shown to reduce the population size of birds and foxes (Husemann et al., 2015; Gallina et al., 2016). For example, the effective of population sizes of the mountain white-eye (*Zosterops poliogaster*) in East Africa were higher in larger and interconnected forest patches (Husemann et al., 2015). Habitat fragmentation can also decrease fecundity in plants (Somanathan and Borges, 2000; Franceschinelli et al., 2015), which may be caused by a decrease in pollinator visits and an increase in seed predation (Franceschinelli et al., 2015). Fragmentation also resulted in reduced plant survival which could be attributed to higher desiccation and seedling predation (Alvarez-Aquino et al., 2004). Trees in isolated patches were also found to be to have altered plant sex ratios apart from natural populations due to a lack of pollinator visits to female trees (Somanathan and Borges, 2000).

While genetic studies conducted in TMFs were rare, most revealed that populations in isolated forests had lower genetic diversity due to inbreeding and reduced gene flow (Cascante-Marin et al., 2014; Habel et al., 2014; Husemann et al., 2015). For example, a lack of genetic variation in epiphytic bromeliad (*Guzmania monostachia*) populations in Costa Rican forest patches was attributed primarily to anthropogenic barriers to gene flow but could also be influenced by life history traits such as its selective breeding system and limited seed dispersal ability (Cascante-Marin et al., 2014). Higher inbreeding and genetic bottlenecks within a small TMF fragment can also increase the extinction risk of a population (Juárez et al., 2011).

Effects of Habitat Degradation on Ecosystem Services Provided by TMFs

Habitat degradation in TMFs has been shown to disrupt several hydrological processes like affecting water conduction in soils, with reduced hydraulic conductivity in secondary forests and plantations (Marin-Castro et al., 2016, 2017). In turn, this likely contributed to increased surface runoff in cultivated land (Lorup and Hansen, 1997; Munoz-Villers and McDonnell, 2013; Suescun et al., 2017). With increasing surface water runoff, streamflow in degraded landscapes can be higher following rainfall (Munoz-Villers et al., 2012; Ramirez et al., 2017) but in the dry season, stream flow from cultivated catchments can be drastically reduced (Castillo et al., 2012). Water storage was also lower in agricultural areas (Guardiola-Claramonte et al., 2010; Schrumpf et al., 2011), and may subsequently lead to a reduction in watershed discharge (Caballero et al., 2013).

Land conversion in TMFs can lead to declines in mean carbon densities due to biomass loss (De Jong et al., 1999; Restrepo et al., 2012). Impacts of land conversion on soil organic carbon (SOC) are less conclusive; most studies reported lower SOC in cultivated land relative to montane forest (e.g., Rhoades et al., 2000; Mendoza-Vega et al., 2003; Omoro et al., 2013; Tesfaye et al., 2016), but two showed weak or no effects (De Jong et al., 1999; Chacon et al., 2015) and another showed no clear trend (Twongyirwe et al., 2013). Such conflicting results may be attributed to variation in soil properties, age since disturbance, the type of cultivated land, and altitude (Twongyirwe et al., 2013; Chacon et al., 2015).

Habitat degradation in TMF can lead to marked changes in N storage and conversion rates. Conversion of TMFs to cultivated land led to a reduction in soil N (Rhoades and Coleman, 1999; Tesfaye et al., 2016) and decreased net N mineralization rates (Campos et al., 2014). Total dissolved nitrogen was higher in plantations than in TMF catchments, probably due to more leaching (Jacobs et al., 2017). Additionally, the rate of N decay from leaf litter in plantations, or in streams within pastures, was slower compared to natural TMFs (Encalada et al., 2010; Loaiza-Usuga et al., 2013; Ramirez et al., 2014).

Land use change alters the properties of montane soils, such as decreasing soil moisture (Schrumpf et al., 2011; Ramirez et al., 2017) and macroporosity (Pinto et al., 2016). Soil microbial biomass generally declines as land disturbance intensifies (Campos et al., 2007, 2014; Mganga et al., 2015, 2016). Macroinvertebrate diversity in soils is lower in deforested sites (Yankelevich et al., 2006; Negrete-Yankelevich et al., 2007) but can peak in secondary forests (De la Rosa and Negrete-Yankelevich, 2012).

DISCUSSION

Potential Biases and Limitations of Our Systematic Map

Our method of gauging research effort per country, by aggregating the number of studies stemming from the country of interest, is likely biased by the extent of TMFs available. Estimating the number of studies per area unit of TMF in each country will provide better resolution. Another caveat to note is that community responses were tabulated without accounting for the intensity of disturbance, beyond broad classifications of habitat types. Also, our classifications did not consider spatial differences among studies, and the impacts of habitat degradation at a site, country or regional scale will vary. Last, our assessment of articles covering ecosystem services mainly focused on those that provided supporting and regulation services, with less emphasis on provisioning and cultural services (Alcamo and Bennett, 2003). A separate systematic review on the impacts of habitat degradation on the ecosystem services provided by TMFs is recommended by using additional search terms (e.g., "ecotourism" and "education") to obtain relevant articles.

Regional Comparisons

Given that more than half the world's remaining TMFs occur in Asia (Scatena et al., 2010), the scant research in this region highlighted in this systematic map is of concern. Our results corroborate an earlier review that found only 5% of published biodiversity studies in Southeast Asia from 1990 to 2010 were conducted in montane forests (Peh et al., 2011). Average annual deforestation rates from 2000 to 2010 for montane forests in Southeast Asia ranged from 0.2 to 0.4% compared to 1.2% for lowland and 2.2% for peat swamp forests

(Miettinen et al., 2011), but deforestation rates in the highlands are increasing due to recent expansion of roads (Peh et al., 2011; Margono et al., 2014). While roads are essential for economic development, they are a major threat to biodiversity (Laurance et al., 2009). In Peninsular Malaysia, the construction of the second East-West highway, completed in 2010, has led to rampant deforestation in the Lojing Highlands despite regulations that restrict logging above 1,000 m (Singh, 2013). Much of the cleared land has been converted to agricultural farms (Omar and Hamzah, 2010). Alarmingly, nearly half of montane primary forest loss in Indonesia has occurred within protected areas (Margono et al., 2014). To tackle illegal logging, Malaysian and Indonesian governments have implemented schemes that award certification to producers that promote sustainable logging practices (e.g., Malaysian Timber Certification Council and the Timber Legality Assurance System in Indonesia) (Peh et al., 2011; Margono et al., 2014). However, such initiatives have not stopped deforestation of protected areas (Peh et al., 2011; Chitra and Cetera, 2018). Imposing sanctions on non-compliant timber producers, and stricter assessments to gain certifications are needed to secure the remaining TMFs in Asia (Chitra and Cetera, 2018).

Research in African TMFs was also poorly represented globally, yet much of Africa's TMF is threatened from overexploitation through illegal logging and poaching, and habitat loss via land conversion to agriculture and charcoal burning (Cronin et al., 2014; Jacobs et al., 2017). Although there are designated protected areas in Africa, their coverage is inadequate and many protected sites are poorly managed (Cronin et al., 2014; Jacobs et al., 2017). Further, the heavy reliance of fertilizers in farms increases nutrient loads in streams that lead to a deterioration in water quality and eutrophication (Jacobs et al., 2017). Political unrest in countries such as Sudan also affect the state of natural resources, such as those in the Imatong Mountains and surrounds which are part of the Eastern Afro-montane ecosystem—considered to be one of Africa's biodiversity hot spots (Uma, 2016). Two decades of civil war have decimated large swathes of forest, particularly on Mount Dongotomea, with two-thirds of the forest lost since 1986 (Gorsevski, 2012; African Wildlife Foundation, 2014). A lack of livelihoods for returning refugees and strong dependence on natural resources has led to increased poaching for bushmeat, illegal logging and fires set deliberately for shifting agriculture (Gorsevski, 2012; African Wildlife Foundation, 2014).

Country Comparisons

Articles describing research conducted in Mexico comprised a third of all relevant papers in this mapping exercise. Although TMFs in Mexico occupy only 1% of the country's land area, they have exceptionally high biodiversity owing to the diversity of habitats and the contribution of biota from Holarctic and Neotropical origins (González-Espinosa et al., 2011; Ponce-Reyes et al., 2012). More than half of the TMFs in Mexico have been converted to crops (Toledo-Aceves et al., 2011) and consequently 70% of forest trees in this ecosystem are threatened (González-Espinosa et al., 2011). Deforestation rates in the highlands of Mexico have also intensified sharply (Cayuela et al., 2006a; Gómez-Mendoza et al., 2006). In the oak and mountain cloud forests of Sierra Norte of Oaxaca, the annual deforestation rates from 1980 to 2000 was 1–2% (Gómez-Mendoza et al., 2006). In the Chiapas highlands, the annual rate increased from 1.3% during 1975 to 1990, to 4.8% during 1990 to 2000 (Cayuela et al., 2006a). More recent estimates of TMF deforestation rates are lacking in Mexican TMF, and considering that the last reported deforestation rates were rising nearly two decades ago, an updated estimate is crucial to assess the current extent of forest loss and re-valuate its impact to montane biota. Additionally, only 12% of Mexico's cloud forests are protected (Ponce-Reyes et al., 2012), and if unprotected forests are cleared, the combined effects of habitat loss and climate change could lead to the extinction of up to 70% of Mexico's endemic vertebrates (Ponce-Reyes et al., 2012).

Integrating the Impacts of Climate Change

Several studies in our systematic map explored the synergistic effects of climatic/microclimatic change and habitat degradation on biotic and abiotic responses. These included changes in species distribution and population sizes, germination and seedling development, community structure, food webs and nutrient availability. For example, the altitudinal distribution of several dung beetle species was higher in deforested areas (where it was hotter and drier) than in intact landscapes (Larsen, 2012). When projected changes in both climate and forested areas were combined, declines in range and population sizes of montane birds were amplified, resulting in 19–42 and 19–62% reductions, respectively (Harris et al., 2014). Harsher microclimates in altered habitats negatively affected germination rates, seedling development and recruitment, which in turn hampered recolonization rates (Werner and Gradstein, 2008; Anthelme et al., 2011; Hietz et al., 2012). Epiphyte species richness declined due to warmer and drier microclimates in disturbed forests (Barthlott et al., 2001) but recovered in restored sites if microclimatic conditions simulated those in primary forests (Diaz-Garcia et al., 2017). The interaction of habitat degradation and climate can also affect nutrient and pollutant deposition (Ponette-González et al., 2010) and is more pronounced in drier seasons, affecting processes such as plant transpiration (Ramirez et al., 2017). Collectively, these studies indicate that the effects of habitat degradation on biodiversity and ecosystem functioning are compounded when climatic/microclimatic change is also considered.

Research Priorities for Habitat Degradation in TMF

Clearly, the most urgent research priority with regards the impacts of habitat degradation on TMF is to understand its effects on population genetics (**Figure 8**). Aside from there being so few genetic studies of species occurring in this forest type, the preservation of genetic diversity is fundamental in maintaining viable populations that have adaptive potential. We suggest applying next-generation sequencing (NGS) in future genetic research, as inferences from NGS are drawn genome-wide (Angeloni et al., 2011). This is unlike the studies identified

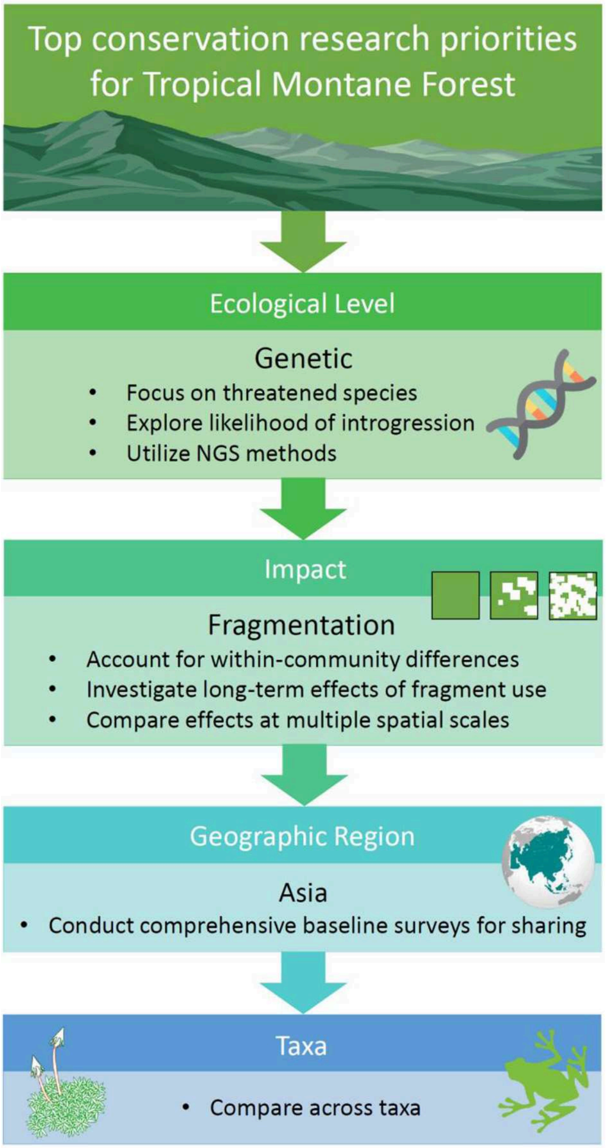

FIGURE 8 | Research priorities for conserving the world's tropical montane forest ecosystems, indicated in order of importance.

via our systematic map, where traditional methods like Sanger sequencing or microsatellites targeted only a few genes.

A logical group for thorough evaluation of their genetic structure are threatened or endemic species, many of which are likely to exist as small populations that are vulnerable to the effects of genetic drift. The findings from the limited number of studies conducted in TMF have shown expected results: (1) habitat fragmentation can impede gene flow and lead to a loss of genetic variation, and (2), improving fragment connectivity can help reserve this trend. Where possible, drawing inferences on gene flow and genetic diversity from multiple species within an ecosystem is ideal, as species responses to fragmentation can differ in concert with variation in species traits. For instance, a study of montane forest birds in Kenya revealed tighter genetic clustering among sedentary species compared to more mobile species (Callens et al., 2011). Further, generalist species are often more robust to the impacts of habitat fragmentation (Janecka et al., 2016).

Not yet documented in our mapping exercise are studies that examine the role played by habitat degradation (and likely interactions with climate change) on introgression in TMF biota. Introgression is the hybridization of closely related species accompanied by repeated back-crossing of the hybrid with a parent species (Anderson, 1949). It is pervasive in natural populations and can accelerate the loss in genetic diversity (Harrison and Larson, 2014). A study in the Ethiopian highlands found that the wild gene pool for *Coffea arabica* had admixed with cultivars grown in close proximity to natural populations (Aerts et al., 2013). This example illustrates the threat posed by non-native species, and it is not inconceivable that increasing habitat loss and/or climate change in TMFs could broaden hybrid zones and increase the likelihood of introgression between related lowland and montane species. If so, montane endemics may experience genetic swamping (i.e., replacement of local genotypes by hybrids) and increased risk of extinction (Ellstrand and Rieseberg, 2016).

Research that investigates the impacts of habitat fragmentation and edge effects on montane biota should also be prioritized, as the results will have profound implications for sustainable land-use planning (e.g., implementing green corridors and evaluating land-sparing vs. land-sharing options) (**Figure 8**). Although fragmentation is well-known for reducing gene flow, it has far-reaching consequences at all ecological levels, including ecosystem services. In general, fragmentation has a negative impact on communities; resulting in a decline in species richness (e.g., Borgella et al., 2001; Kumar and O'Donnell, 2007; Hundera et al., 2013; Mohandass et al., 2014; Rovero et al., 2014), and changes in species composition in response to fragmentation has also been consistently reported (e.g., Larrea and Werner, 2010; Mohandass et al., 2014; Rueda-Hernandez et al., 2015; García-Martínez et al., 2017; Herrera-Paniagua et al., 2017). However, some studies have highlighted that certain spatial characteristics such as fragment area and isolation have no effect on abundance, density or diversity (Muriel and Kattan, 2009; Ulrich et al., 2016). While the extent of degradation may lead to conflicting results, deeper examination of species functional traits, which are indicators of habitat use, reveal that some groups within a community are more affected than others. For instance, two avian studies independently concluded that understory insectivores and canopy frugivores were more sensitive to fragmentation than other functional groups (Kattan et al., 1994; Rueda-Hernandez et al., 2015). Thus, within-community differences should be accounted for in future fragmentation research in TMF.

Our current understanding of the long-term effects of fragmentation is also limited by the scarcity of relevant historical data. In the short term, diversity may not be adversely affected by fragmentation and may even increase (Rey-Benayas et al., 2007). However, the effects are generally negative over the long term, with studies in TMFs showing a decline in plant diversity (>20 years) (Rey-Benayas et al., 2007; Koester et al., 2009), loss of plant biomass and change in species composition (>40 years) (Zahawi et al., 2017), and local extinction of birds (31% loss in over 80 years) (Kattan et al., 1994). Elucidating the environmental factors driving colonization and extinction patterns will allow better comprehension of community dynamics in a fragmented landscape. As such, a multi-season or dynamic occupancy models (DCMs), which are variants of more widely applied multi-species occupancy models, are well-suited for the task (Fernández-Chacón et al., 2014). Both are hierarchical models, but the dynamic model accounts for changes in occupancy over time by including sub-models of colonization and persistence that affect the previous occurrence state. Crucially, DCM models do not assume perfect detection among species, which can lead to misconstrued interpretations of occupancy dynamics.

A key recommendation from our mapping exercise is that effects of fragmentation should be examined at multiple spatial scales, as scaling dependencies in fragmented landscapes are vital for conservation planning (Cushman and McGarigal, 2004). While broad spatial effects have been documented to affect biotic responses (Chiavacci et al., 2018; Froehly et al., 2019) fine scale features such as vegetation heterogeneity were found to be better predictors in other studies (Cayuela et al., 2006b; Banks and Gagic, 2016; Michael et al., 2017). For example, research on golden-cheek warblers, *Setophaga chrysoparia*, showed that landscape composition best predicted species density, but vegetation characteristics was the best predictor of nesting success (Reidy et al., 2017).

Region-wise, research in Asian TMF is under-represented at all ecological levels, impact type and taxa (**Figure 8**). The future discovery of new species is particularly likely in Southeast Asia, given the regions' unique biogeographical history (Holloway and Hall, 1998). In the last 5 years, several new species were discovered in TMFs in Southeast Asia, including amphibians and reptiles from Vietnam (Grismer et al., 2013, 2015; Rowley et al., 2013, 2014, 2015). In these regions, attaining baseline ecological data such as a country-by-country species inventory represents a crucial first step in efficient data-sharing, and accumulation of large databases that facilitate multidisciplinary ecological research. Although we encourage further research of poorly studied taxa, available data for better represented groups such as vascular plants and birds are already useful for comparative studies, and to gain perspective on the overall response of the biome to habitat degradation (**Figure 8**). Comparisons between the responses of endotherms and ectotherms will be of interest, as the latter are more likely to be adversely affected by degradation due to their sensitivity to microclimatic change and generally lower dispersal abilities.

Suggestions for Future Research

Greater attention should be given to how functional diversity, which characterizes the range of ecological roles played by species in a community, may be affected by anthropogenic change in TMFs (Petchey and Gaston, 2006). Species richness estimates and related indices assume all species perform the same roles, but high species richness does not necessarily beget high functional diversity (Stuart-Smith et al., 2013). For example, while bird species richness and density in medium-sized fragments were higher than larger fragments in Mexican TMFs, larger fragments had a distinct functional composition, with a greater proportion of understory insectivore species and canopy frugivores (Rueda-Hernandez et al., 2015). Furthermore, functional diversity may be a better predictor of ecosystem function than species richness or abundance indices (Gagic et al., 2015).

Applying an ecological network is a useful approach to test the effects of degradation on species interactions at the community-level. An ecological network is a collection of nodes represented by species that are joined by links that either represent antagonistic or mutualistic interactions (Pascual and Dunne, 2006). The resilience of ecological networks can be compared along a disturbance gradient (Harvey et al., 2017; Tylianakis and Morris, 2017), and networks that are more resilient to environmental perturbations are generally characterized by having a greater number of nodes (e.g., species or functional diversity) and links (interactions) (Pascual and Dunne, 2006). In one study, seed-dispersal networks of birds in the montane forest interior and edge were compared, revealing that functional and interaction diversity were higher at forest edges (Saavedra et al., 2014). In contrast, parasitoid–host interaction networks were more homogenized in agricultural sites than in lowland forests (Laliberté and Tylianakis, 2010). Such conflicting responses to habitat degradation may relate to different levels of disturbance in these networks (i.e., edge vs. forest interior compared to open agriculture vs. forested habitats). As montane species are highly sensitive to disturbance (Long, 1995; Brooks et al., 1999; Soh et al., 2006), exploring the sensitivity of ecological networks in TMFs across varying disturbance intensities, while factoring the extent of disturbance and time passed since degradation began, will deepen our understanding of how species assemblages change with continued habitat modification.

The lack of population studies of TMF species in Southeast Asia is troubling given that the number of montane species threatened with extinction in this region may be underestimated (Brooks et al., 1999). Further, many species studied in TMFs have not been assessed against IUCN Red List criteria, which may indicate how little is known of their population dynamics. Unsurprisingly, most species without Red List assessments ("Not Evaluated," NE) were vascular plants (>95%, $n = 40$), but included one insect and one fungus. While "NE" are often species new to science, they can potentially be under serious threat, particularly if their habitats are rapidly being lost or are unprotected (Tapley et al., 2018). Indeed, some NE species may be included in national threatened species lists, however, evaluation against IUCN Red List criteria is crucial for formulating policy and prioritizing management of threatened species at multiple scales (e.g., from site-specific environmental impact assessments, to national plans for conservation, to global initiatives like the Convention on

International Trade in Endangered Species of Wild Fauna and Flora, Rodrigues et al., 2006).

Disappointingly, only a few articles investigated the effects of habitat fragmentation or edge effects on ecosystem services in tropical montane environments (**Table 2**). The potential impact of habitat fragmentation cannot be underestimated given that 70% of forest remaining globally is within 1 km of the forest's edge (Haddad et al., 2015). Factors such as fragment size and isolation can influence ecosystem services; for example, regulation of crop pests is more effective next to a forest, but crop production is greatest at intermediate distances from a forest (Mitchell et al., 2014). Careful consideration of the ecosystem services affected by fragmentation is crucial, as trade-offs between different services can be expected. For instance, in a study of montane heathlands, decreasing fragment size reduced biodiversity and recreational value, but increased carbon storage and timber value (Cordingley et al., 2015). Lastly, most studies investigating fragmentation effects on ecosystem services consider "supply" aspects (the potential of a natural resource to provide a benefit to people), without regard for their realization. This realization is affected by the "demand" (the desire for the service) and the "flow", which represents the delivery of the service to the people. The framework provided by Mitchell et al. (2015) considers all three aspects, and will be useful in testing hypotheses on the realization of ecosystem services in forest fragments.

CONCLUSION

Our systematic map has shown that habitat degradation in TMFs has had discernible impacts on biodiversity and ecosystem services. While the impacts of this degradation are fairly well-studied at the community level, and adequate data may be available for meta-analysis, the impacts on genetic diversity and gene flow are less well-understood. Thus, population genetic studies should be prioritized for endemic species that are extinction-prone. We also advocate studying the long-term impacts of habitat degradation (particularly habitat fragmentation) on TMF at multiple spatial and taxonomic scales, and greater support for research based in Asia and Africa.

DATA AVAILABILITY STATEMENT

The dataset generated for this study are included in the article/**Supplementary Material**.

AUTHOR CONTRIBUTIONS

MS and KP conceived the systematic mapping. MS and CB performed the literature search and data extraction. MS wrote the manuscript and all co-authors reviewed the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/ffgc.2019.00083/full#supplementary-material

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Covariance of Sun and Shade Leaf Traits Along a Tropical Forest Elevation Gradient

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Foliar trait adaptation to sun and shade has been extensively studied in the context of photosynthetic performance of plants, focusing on nitrogen allocation, light capture and use *via* chlorophyll pigments and leaf morphology; however, less is known about the potential sun-shade dichotomy of other functionally important foliar traits. In this study, we measured 19 traits in paired sun and shade leaves along a 3,500-m elevation gradient in southern Peru to test whether the traits differ with canopy position, and to assess if relative differences vary with species composition and/or environmental filters. We found significant sun-shade differences in leaf mass per area (LMA), photosynthetic pigments (Chl ab and Car), and δ^{13}C. Sun-shade offsets among these traits remained constant with elevation, soil substrates, and species compositional changes. However, other foliar traits related to structure and chemical defense, and those defining general metabolic processes, did not differ with canopy position. Our results suggest that whole-canopy function is captured in many traits of sun leaves; however, photosynthesis-related traits must be scaled based on canopy light extinction. These findings show that top-of-canopy measurements of foliar chemistry from spectral remote sensing approaches map directly to whole-canopy foliar traits including shaded leaves that cannot be directly observed from above.

Keywords: canopy chemistry, sun-shade adjustment, plant functional traits, community assembly, Andes-Amazon, Peru, Spectranomics

INTRODUCTION

Solar radiation is one of the most limiting resources in tropical forests (Denslow, 1987; Chazdon et al., 1996; King et al., 2005; Smith et al., 2019). Evolution by natural selection has resulted in a diverse set of strategies within and across tropical tree species to maximize light interception and utilization. One strategy to achieve this goal is the partitioning of resources between sun and shade leaf layers and configuring these leaves with different traits. Sun leaves are grown to minimize carboxylation limitations, while shade leaves are adapted to minimize light limitation. For example, sun leaves often have higher leaf mass per area (LMA) and lower nitrogen (N) concentration, thicker palisade parenchyma tissues, and more mesophyll cells (Björkman, 1981; Anderson et al., 1988; Hikosaka and Terashima, 1996), which combined, supports higher photosynthetic rates on an area basis compared to shade leaves (Evans et al., 1988; Evans and Poorter, 2001; Niinemets and Valladares, 2004; and others). In contrast, shade leaves often have lower LMA and similar N on a mass basis, but a larger proportion of N is allocated to chlorophyll to enhance light capture, compensating for lower irradiance to achieve photosynthetic capacity similar to that of sun leaves (Boardman, 1977; Hikosaka and Terashima, 1996; Poorter et al., 2009). How additional leaf traits differ between sun and shade positions is less well known.

Forest canopy studies demonstrate that sun-shade leaf differences in LMA, N, chlorophylls (the combined value of chlorophyll a and b, as Chl ab), and photosynthetic rates are strongly correlated with the vertical light gradient within tree canopies (Farquhar, 1989; Poorter et al., 1995; Niinemets et al., 1999), supporting the optimal resource partitioning hypothesis to maximize canopy photosynthetic production (Shipley et al., 2006). However, maximum photosynthetic rates are rarely achieved within the canopy (Meir et al., 2002; Lloyd et al., 2010; Dewar et al., 2012). Numerous studies have shown that photosynthesis-trait relationships are constrained by physical limitations of leaf architecture (Sack and Scoffini, 2013; Blonder et al., 2017), whole-plant structure or canopy height (Wright et al., 2007; Brodribb and Feild, 2010; Cavaleri and Oberbauer, 2010), and within canopy temperature and/or humidity (Chazdon et al., 1996; Meinzer, 2003), but little is known about the light response of other foliar chemical traits within canopies.

The natural abundance of δ^{13}C (the isotopic ratio of ^{13}C/^{12}C expressed on a $^{0}/_{00}$ relative to a standard) in leaf tissue is a time-integrated measure of CO_2 assimilation by the plant has served as a surrogate for water use efficiency (WUE, the ratio of carbon gained to water lost during gas exchange (Farquhar et al., 1989). Foliar δ^{13}C is determined by the internal and external concentration of CO_2 in leaves, and is sensitive to environmental factors influencing stomatal conductance (i.e., water stress (Ehleringer, 1991), internal resistance in high LMA leaves (Cordell et al., 1998), and decreasing partial pressure of CO_2 with elevation (Körner et al., 1991). Additionally, δ^{13}C has been shown to differ between overstory (direct sunlight) and understory plants (Medina and Minchin, 1980; Ehleringer et al., 1986), and in the source air for plants in different positions within the canopy (Sternberg et al., 1989; Buchmann et al., 1996); however, the effect of different light regimes within the same canopies has had little study (Garten and Taylor, 1992; Holtum and Winter, 2005).

Beyond foliar traits involved in light capture and growth, there are a number of additional chemicals known to be functionally important in leaves, and which are predictors of plant adaptation to environmental conditions (Díaz et al., 1998). We group these traits into three additional categories: defense (phenols, tannins, lignin and cellulose), metabolic regulation, macronutrients (phosphorous P, calcium Ca, potassium K, magnesium Mg), and other micronutrients (boron B, iron Fe, manganese Mn, zinc Zn). Polyphenols encompass a wide array of phenolic and tannic compounds (measured here as bulk phenols and condensed tannins), and are synthesized for chemical defense against pest and pathogens, which are particularly abundant in tropical forests, (Coley and Barone, 1996), and are also used for protection against high solar radiation (e.g., anthocyanins), antioxidants, and other foliar protections (Grace, 2005). In addition, lignin and cellulose affect leaf digestibility and toughness as both defense and structural support (Weng and Chapple, 2010). Macronutrients (P, Ca, K, Mg) and micronutrients (B, Fe, Mn, Zn) play key roles in regulating metabolic activities, cellular allocation, and growth. For example, K assists in maintaining stomatal control and osmotic potential necessary for efficient photosynthesis and respiration, while Ca and Mn play roles in generating cell walls and the chloroplast structure (Salisbury and Ross, 1992). Whether these traits differ among sun and shade positions within canopies is not well known.

Understanding how foliar traits vary in sun and shade leaves may be complicated in tropical forest canopies depending upon whether comparisons are made within or between species (Ter Steege et al., 2006; Wright et al., 2010; Asner and Martin, 2016). High species and structural diversity, in addition to multiple environmental filters, can drive trait variation (Messier et al., 2010). Previous studies of foliar chemistry have focused on the effects of soil fertility, elevation and climate on N, P, and base cation (Ca, Mg, K) concentrations, and morphological traits such as LMA and thickness, in upper canopy leaves. For example, global variation in LMA measured across biomes or within humid tropical forests range from 14–1,500 g m^{-2} and 113–446 g m^{-2}, respectively (Wright et al., 2004; Asner and Martin, 2016). A wide range in LMA was even measured within one species (*Metrosideros polymorpha* Gaudich.) growing across a range of elevations and substrates in the Hawaiian islands (Martin and Asner, 2009). Furthermore, a recent study along multiple elevation gradients in the western Amazon greatly expanded the portfolio of canopy foliar traits, and integrated the role of interspecific variation in the list of explanatory factors regulating variation in foliar chemistry in upper-canopy, sunlit leaves (Asner et al., 2014b). Given such broad species and environmental variation in sunlit foliar traits, is it possible to determine variation between sun and shade leaves?

Here we assess differences in 19 foliar traits in paired sun and shade leaves along a 3,500-m humid tropical forest elevation gradient in Peru. We quantify trait variation within and between these differing leaf types to understand whole-leaf adaptation to both local light and large-scale climatic (elevation) conditions. We focused on traits that: (i) mediate or are indicative of photosynthesis and carbon uptake (Chl ab, carotenoids and

$\delta^{13}C$); (ii) are related to structure and chemical defense; and (iii) are related to metabolism including macronutrient and micronutrients. The elevation gradient, combined with the great diversity of canopy species included in our study, affords a means to compare and contrast foliar trait responses in sunlit and shade leaves across a range of forest structural and compositional contexts.

Using this elevation gradient, we ask: Do foliar chemical traits differ between sun and shade leaves, and do genetic and/or environmental filters, such as climate and soils, limit the variation between sun and shade leaf traits? We expect that light-sensitive traits, such as LMA and photosynthetic pigments, will differ between sun and shade leaves, and that this plasticity, associated with decreasing light availability within canopies, may be coupled with adaptive sensitivity to changing environmental conditions such as incoming solar radiation along the elevation gradient. However, we do not know if other foliar traits, such as those related to defense and nonphotosynthetic metabolism, will follow a similar pattern.

METHODS

Field Sampling

We measured foliar traits from top-of-canopy, fully expanded sun and paired within-canopy shade leaves in 385 tropical trees in ten sites arrayed along an Andes-to-Amazon elevation gradient. This gradient stretches from 200 m elevation in the Amazonian lowlands to 3500 m at the Andean tree line (**Table 1**, **Figure S1**). Changing environmental conditions along this gradient include decreasing temperature with increasing elevation, as well as a U-shaped pattern of incoming solar radiation, with lowest radiation levels in the submontane region (Fyllas et al., 2017; Malhi et al., 2017). Along the gradient, mean annual precipitation (MAP) varies from 1,600–5,300 mm yr^{-1}, high enough to classify all sites as moist or wet tropical forest. The 1-ha forest sites were installed by the Andes Biodiversity Ecosystems Research Group (ABERG, http://www.andesconservation.org) and are part of the ForestPlots (https://www.forestplots.net/) and Global Ecosystems Monitoring Network (GEM; http://gem.tropicalforests.ox.ac.uk/projects/aberg) networks. Mean annual temperature (MAT) ranges from 9.0°C at the highest elevation site to 24.4°C at the lowland sites. A comparison of MAT and elevation measured at the individual sites indicate a nearly one-to-one linear relationship (MAT = 25.7-0.005 x Elevation, R^2 = 0.99, $p <$ 0.05), therefore we chose to analyze environmental influences on trait relationships in terms of elevation rather than presume a causal relationship with temperature.

Soils at sites above 1,500 m are classified in the FAO soil system as Cambisols. The two lowland sites (<500 m above sea level), are a Cambisol located on *terra firme* clay substrate of late Pleistocene age (TAM-05) and an Alisol of very low nutrient concentration on an inactive high-fertility floodplain of late Holocene age (TAM-06; Quesada et al., 2009). The two submontane sites (500–1,000 m elevation) are located on a highly weathered, mineral rich Alisol soil (PAN-03) and a weathered, clay-rich Plinthosol (PAN-02) on the Pantiacolla front range of the Andes, both soils supporting lower nutrient concentrations compared to higher fertility Cambisols. The highest elevation site, ACJ-01, is located at treeline on extremely thin soils. This site is steeply sloping and the plant community is dominated by individuals in the genus Melastomataceae, indicating very poor nutrient soils.

Foliar sampling was undertaken between April and November 2013 as part of the CHAMBASA (Challenging Attempt to Measure Biotic Attributes along the Slopes of the Andes) project. Based on the most recently available census and diameter data for each plot, a sampling protocol was adopted wherein species were sampled that maximally contributed to plot basal area (a proxy for plot biomass or crown area). We aimed to collect the minimum number of species that contributed to 80% of basal area; however, in the diverse lowland forest plots, we only sampled species comprising 60%–70% of plot basal area. For each selected species, 3–5 individual trees were chosen for sampling (five trees in submontane and montane plots; three trees in lowland plots). If three trees were not available in the plot, we sampled additional individuals of the same species from an area immediately surrounding the plot. The collected samples were comprised of 134 species from 89 genera in 49 families. At a given site, between 9 and 26 unique species were sampled (**Table S1**).

Leaf collections were conducted using tree-climbing techniques to ensure that mature leaf samples were collected

TABLE 1 | Site characteristics including soil type, location, elevation, mean annual precipitation (MAP), mean annual temperature (MAT), ambient solar radiation for ten plots sampled for canopy foliar traits along the Andes-Amazon elevation gradient.

Site	Soil Type	Latitude	Longitude	Elevation (m)	MAP (mm yr^{-1})	MAT (°C)	Solar radiation (GJ m^{-2} yr^{-1})
Tambopata; TAM-06	Alisol	–12.8386	–69.2960	215	1900	24.4	4.80
Tambopata; TAM-05	Cambisol	–12.8303	–69.2706	223	1900	24.4	4.80
Pantiacolla; PAN-02	Plintosol	–12.6496	–71.2627	595	2366	23.5	3.82
Pantiacolla; PAN-03	Alisol	–12.6383	–71.2745	848	2835	21.9	3.82
San Pedro; SPD-02	Cambisol	–13.0491	–71.5366	1494	5302	18.8	4.07
San Pedro; SPD-01	Cambisol	–13.0474	–71.5424	1713	5302	17.4	4.35
Trocha Union; TRU-04	Umbrisol	–13.1059	–71.5893	2719	2318	13.5	3.49
Esperanza; ESP-01	Umbrisol	–13.1759	–71.5948	2868	1560	13.1	3.51
Wayquecha; WAY-01	Cambisol	–13.1907	–71.5875	3045	1560	11.8	3.51
Acjanaco; ACJ-01	Cambisol	–13.1469	–71.6323	3537	1980	9.0	4.23

from accurate sun and shade locations within each canopy. Sun leaves are considered leaves found on the outermost layer of the canopy and are exposed to full sunlight at least 80% of daylight hours. If multiple layers were present in the canopy, leaves were collected from the lowest suitable layer and were designated as shade leaves. Relative light levels at these locations were usually < 10% of top of canopy but precise light measurements are still forth coming (Shenkin *pers comm*). Once acquired, each sample was immediately packed in plastic bag and stored on ice in the dark until being transported to a local site for processing within 30 min following collection. Samples were cryo-cooled or dried on site immediately after measurements of fresh weight and leaf area were made. A foliar profile, including 18 chemical traits and LMA, was developed for each sample, sun and shade.

Laboratory Assays

Branches (generally 1–2 m in size with multiple branchlets) of mature leaves were sealed in polyethylene bags in the field to maintain moisture, stored on ice in coolers, and transported to a local site for processing within 3 h. A subset of leaves was selected from the branches for scanning and weighing. Leaf area was determined on a 600 dots-per-inch flatbed top-illumination optical scanner, using enough leaves to fill one scan area of 21 cm × 25 cm (up to about 35 leaves per sample depending on leaf size). Petioles were removed from each leaf prior to scanning, and midveins were removed when they exceeded 1 mm in diameter. Leaves exceeding the surface area of the scanner were cut into sections until 1–2 full scan areas were completed. The scanned leaves were dried at 70°C for 72 h before dry mass (DM) was measured. LMA was calculated as g DM m^{-2}. From a subset of leaves, leaf discs (at least 30 per leaf) were immediately taken from approximately 6–12 randomly selected leaves and transferred to −80°C cryogenic shipping containers. The remaining leaves were detached from the branches and subsamples were selected to represent the range of colors and conditions found among all leaves collected from the branches (such as leaf size or slight variation in age within mature leaves). When epiphylls were encountered, they were removed, along with dust and debris, prior to drying in mobile ovens at 70°C for 72 h followed by vacuum sealing for transport.

Detailed chemical analysis protocols, along with instrument and standards information are downloadable from the Spectranomics Program website (https://gao.asu.edu/spectranomics-protocols), and are summarized here. Dried foliage was ground in a 20-mesh Wiley mill, and subsets were analyzed for a variety of elements and carbon fractions. Total element concentration of macro- (P, Ca, K, Mg) and micronutrients (B, Fe, Mn, Zn) were determined in 0.4 g dry leaf tissue by inductively coupled plasma spectroscopy (ICP-OES; Therma Jarrel-Ash, Waltham MA) after microwave digestion in 10 ml concentrated (~70%) nitric acid solution (CEM MARSXpress; Matthews NC). One blank and two reference standards (Peach NIST SRM 1547 and internal lemon leaf) were digested and measured with each set of 40 foliar samples to track the reproducibility and accuracy of the method.

Carbon fractions including nonstructural carbohydrates (NSC), cellulose and lignin were determined in 0.5 g dry ground leaf tissue through using sequential digestion of increasing acidity in a fiber analyzer (Ankom Technology, Macedon NY). Carbon fractions are presented on an ash-free DM basis following ignition of the remaining sample at 500°C for 5.5 h. A lemon leaf standard was used as a reference with each run to ensure consistency across runs. A subset of the ground material was further processed to a fine powder for determination of total C and N concentration by combustion-reduction elemental analysis (Costec Analytical Technologies Inc. Valencia, CA). Following combustion, a portion of the gas is routed through a mass spectrometer (Picarro Inc. Santa Clara, CA, USA) where the separate isotopes of C^{12} and C^{13} are measured. The isotopic ratio $\delta^{13}C$ is calculated against a reference standard as

$$\delta^{13}C({}^{0}/_{00}) = [((C^{13}/C^{12}sample)/(C^{13}/C^{12}standard)) - 1] \times 1000\,.$$

Frozen leaf disks were used for the chl ab, carotenoid, phenol and tannin determinations. For phenols and tannins, disks were ground in 95% methanol on the high throughput tissue homogenizer. A portion of the solution was further diluted and incubated on an orbital shaker at room temperature (15°C–18°C) in the dark for 48 h to ensure proper phenol extraction (Ainsworth and Gillespie, 2007). A second portion of the solution was further diluted in a 2-ml centrifuge tube containing 10 mg Polyvinylpyrrolidone (PVP) and incubated on ice for 30 min after vortexing. Following centrifugation, 75% of the supernatant was placed in a new centrifuge tube containing another 10 mg PVP for a second precipitation step (Toth and Pavia, 2001). The total phenolic concentration in solution was determined colorimetrically using the Folin-Ciocalteau method. Phenol concentrations were measured in Gallic Acid Equivalents (GAE) relative to an eight-point Gallic acid standard curve. Chlorophyll (chl ab) and carotenoid concentrations were quantified using two frozen leaf disks (total area 1.54 cm^2). These disks were rapidly ground in 1.5 ml centrifuge tubes containing 0.75 ml 100% acetone on a high throughput tissue homogenizer (Troemner, Thorofare, NJ) with a small amount of $MgCO_3$ to prevent acidification. Following dilution and centrifugation for 3 min at 3,000 rpm, the absorbance of the supernatant was measured using a dual-beam scanning UV-VIS spectrometer (Lambda 25, Perkin Elmer, Beaconsfield, UK).

Statistical Analyses

Because sun and shade leaves were collected across sites differing in elevation, climate (i.e. MAP, MAT) and geology (**Table 1**), canopy position at the tree level was effectively nested within site and could not be compared in a fully randomized way; therefore, nested analyses of variance (ANOVA) was used to first define sources of variation in canopy position and site effects in each of the foliar traits to determine if sun leaves differed from shade leaves within canopies at each site. Mass-based foliar traits at the tree level including LMA, N, Chl ab, carotenoids, P, K, and Mg were $\log_{10}$-transformed to meet the assumptions of normality. Ca was transformed by taking the square root to account for the large number of near-zero values. Mass-based foliar traits were converted to area units by dividing by LMA for analysis. For analyses at the individual tree level, all area-based measures except chlorophylls, carotenoids and δ^{13}C were $\log_{10}$-transformed to meet the assumptions of normality. For analyses across sites at the landscape-scale, foliar traits were averaged by site and canopy position. We used ordinary least squares regression to assess relationships between site-level mean values for each canopy trait between sun and shade canopy layers, elevation and their interaction. If there was no interaction between site and canopy position, relationships among sun or shade leaves and elevation were calculated using linear least squares regression, and the offset between sun and shade position was determined with matched pairs t-tests for each trait.

With the goal of examining how the magnitude of variation in chemical traits among sun or shade leaves is distributed within and across species, we assessed within tree variation as well as intraspecific and interspecific coefficients of variation (CV) calculated with untransformed data regressed against elevation. The magnitude of variation among sun and shade leaves within trees at each site was calculated as the CV of the standardized difference between sun and shade leaves within the trees at each site. Intraspecific CV including sun and shade leaves was calculated for each species within a site as the standard deviation in the trait value divided by the mean trait value. The mean of the intraspecific CV including sun and shade leaves values was then used for the analysis. Interspecific CV was calculated as the standard deviation across the species mean values for sun or shade leaves standardized by the mean trait value.

In addition, to determine how the total variance is distributed among taxonomic grouping, canopy position, and site, we developed nested ANOVA models with random effects using the Residual Maximum Likelihood method using SAS JMP 10.0 statistical software package (SAS Institute Inc. Cary NC). For intraspecific and interspecific variation we included the taxonomic levels of genus (g), and species nested within genus (s), as well as canopy position (P) and landscape-level environmental components incorporated as site (T). All effects were treated as random. In each model, *y* is any chemical trait for each canopy sample. This value was modeled as the sum of the mean value for the entire dataset *μ*, the nested genetic effects (genus *j*, and species *i* within genus *j*), the canopy position (P) nested within genus and species, all within the site effect (T; sensu Fyllas et al., 2009; Messier et al., 2010), and the residual error of the measurement *e*:

$$y = \mu + \mathrm{g}_{jl} + s_{ijl} + \mathrm{P}_{kijl} + \mathrm{T}_l + e_{ijkl}$$

The total variance about the mean for a given trait was therefore quantitatively parsed into the variance explained by genera (σ_g^2), species within genera (σ_s^2), canopy position (σ_P^2), site (σ_T^2), and specimens within species (σ_e^2):

$$\sigma_{\mathrm{total}}^2 = \sigma_{\mathrm{g}}^2 + \sigma_{\mathrm{s}}^2 + \sigma_{\mathrm{P}}^2 + \sigma_{\mathrm{T}}^2 + \sigma_{\mathrm{e}}^2 \,.$$

If in a given model, the last term (σ_e^2) accounted for a high percentage of the total variance, then we concluded that site characteristics and taxonomy did not explain the data well. We refer to this as the model residual.

One limitation of this analysis is that it describes the overall variation explained by each input variable. We acknowledge that not all taxa have equal variance; some may have tightly clumped chemical signatures whereas others may vary widely. This analysis will not pick up such trends. Instead, the method quantifies the entire pattern of taxonomic grouping or lack thereof relative to canopy position and site and residual effects. Previous work successfully tested the validity of nested random effects modeling for analysis of taxonomic partitioning of foliar chemical traits (Fyllas et al., 2009; Asner et al., 2014b) but has not determined if there is an effect of shade leaves beyond light sensitive traits such as LMA or leaf dry matter content (Messier et al., 2010).

RESULTS

Growth Traits

Foliar traits displayed a high degree of variation among 385 tropical tree canopies in the 10 sites along the elevation gradient (**Figure 1**, **Table S2**), including some differences between sun and shade leaves within each crown. We found that LMA and δ^{13}C concentrations in sun and shade leaves increased with elevation (**Figures 1A, F**, **Tables 2**, **3**). Mean LMA increased 64% from the lowest- to highest-elevation site while maintaining a near-constant offset in LMA of 19.2 g m^{-2} between sun and shade leaves (**Figure 1A**, **Table 3**). Foliar δ^{13}C was 1.42$^0/_{00}$ less negative in sun than shade leaves, while monotonically increasing by 100% across the length of the gradient (**Figure 1G**, **Tables 2**, **3**). Differences in these traits were consistent across elevation therefore elevation-based relationships for LMA and δ^{13}C were determined separately for sun and shade canopy positions (**Table 2**). Chlorophyll ab (Chl ab) and carotenoid (Car) concentrations averaged 1.4 and 0.2 mg g^{-1} higher in shade compared to sun leaves (**Figures 1B, C**, **Table 3**), but did not vary systematically with elevation. Mean foliar N (mass %) decreased substantially with increasing elevation in the communities along the gradient, but were not different between sun and shade leaves (**Figure 1D**, **Table 3**). When converted to an area-basis (**Tables S3–S5**), dividing by LMA, N, NSC, and δ^{13}C concentrations differed between sun and shade leaves, while Chl ab and carotenoid concentrations were similar in both canopy positions (**Tables S3–S5**). Photosynthetic pigments and NSC displayed positive relationships with elevation on an area-basis whereas δ^{13}C decreased with increasing elevation and N did not vary consistently across elevation (**Tables S3–S5**).

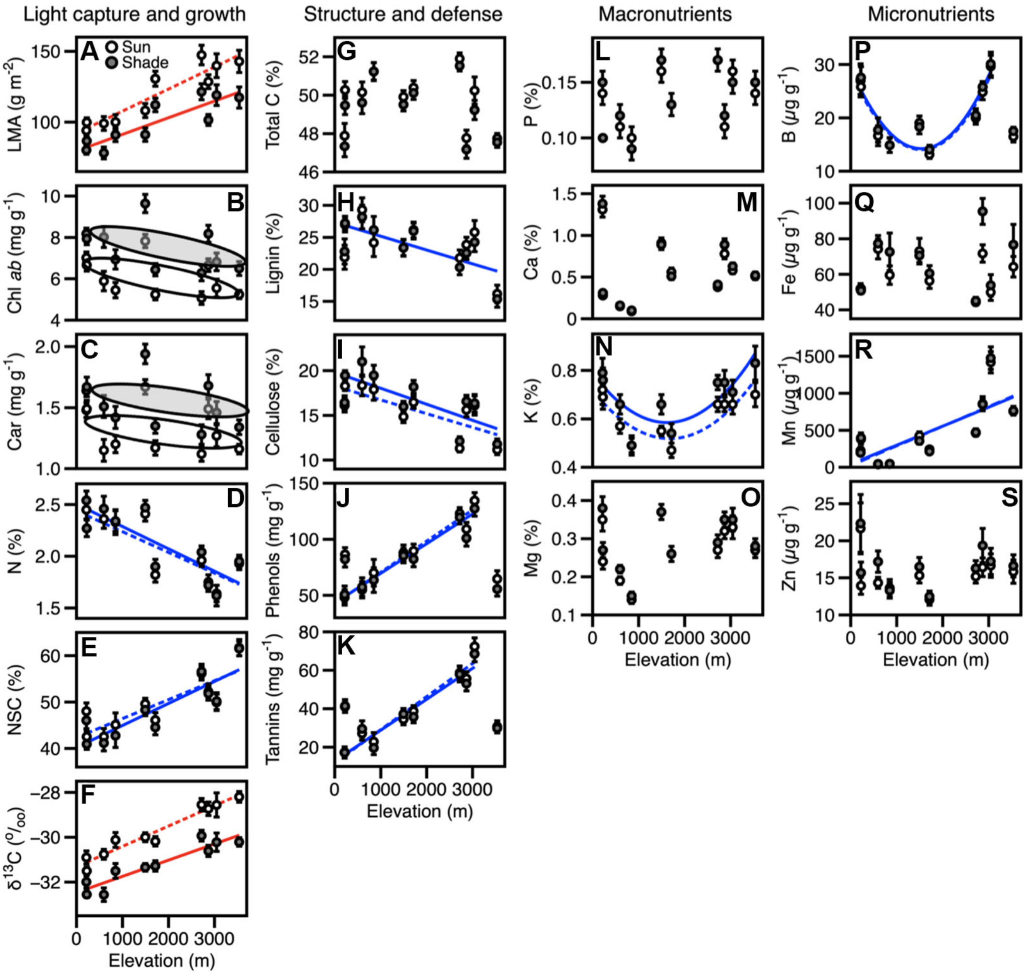

FIGURE 1 | Site mean values for foliar traits, expressed on a mass-basis in sun (open circles) and shade (closed circles) along an elevation gradient are shown. **(A–S)** Variation in foliar traits corresponding to sun-shade canopy positions along an Andes-Amazon elevation gradient in Peru. Error bars represent standard errors. Solid and dotted lines connect trait values across the sites for shade and sun leaves respectively. Red lines indicate significant relationships with elevation. Blue lines show significant trends with elevation for the values of sun and shade when these trait values did not differ between canopy positions. Grey and white ovals indicate significant differences in traits between sun and shade leaves that are not correlated with elevation. Number of individuals per site are given in **Table S1**. Equations for trait-elevation relationships are given in **Table S4**.

Other Traits

Foliar carbon components NSC (lignin, cellulose, and total C) and defense traits were similar in sun and shade leaves, but differed relative to elevation. Total C was invariant with respect to elevation, while lignin and cellulose decreased with elevation (**Figures 1G–I**, **Table S2**). On the other hand, foliar NSC increased with increasing elevation (**Figure 1E**). In contrast, phenols and tannins increased with elevation up 3,045 m (WAY-01), where average concentrations were 130 mg g^{-1} and 70 mg g^{-1}, respectively, after which concentrations of these defense compounds decreased by approximately 50% at the highest elevation site (AJC-01). Phenols and tannins were significantly correlated with elevation only when the extremely nutrient-poor sites (TAM-05 and ACJ-01) were not included in the relationship (**Figures 1J–K**).

Wide ranging values in macronutrients and micronutrients within crowns and among canopies contributed to a lack of distinction between sun and shade leaves, and limited elevation-dependent trends with a few exceptions (**Figures 1L–S**). Mn concentrations increased from 45 µg g^{-1} to almost 1500 µg g^{-1} up to 3,000 m, but decreased by nearly 30% to 850 µg g^{-1} at the highest elevation site (**Figure 1R**). Differing from other traits, K and B displayed a U-shaped pattern, with higher values in the lowlands and montane sites (0.66%–0.72% and 26–30 µg g^{-1}, respectively; **Figures 1N, P**), and substantially lower values in the submontane sites (0.47%–0.54% and 13–14 µg g^{-1}). Different from K, foliar concentrations of B were much lower at the highest elevation site (ACJ-01; 16–17 µg g^{-1}).

Expressed on an area-basis, only total C, lignin, and P concentrations were higher in sun compared to shade leaves and these offsets were maintained across the elevation gradient (**Tables S3–S5**). Additionally, nearly all foliar traits beyond growth related traits showed significant trends with elevation

TABLE 2 | Relationships between site-level mean leaf traits on a mass basis from the sun or shade layer of the canopy and elevation.

Trait	Sun layer		Shade layer	
	R^2	Equation	R^2	Equation
		Light capture and growth		
LMA	0.87 (7.9)***	15.6 x Elevation + 92.1	0.78 (8.3)***	11.8 x Elevation + 79.6
N	0.68 (0.2)**	−0.2 x Elevation + 2.4	0.67 (0.2)**	−0.2 x Elevation + 2.5
Chlorophyll *ab*	NS		NS	
Carotenoids	NS		NS	
NSC	0.70 (3.5)**	4.1 x Elevation + 42.4	0.76 (3.6)**	4.7 x Elevation + 40.2
δ^{13}C	0.95 (0.3)***	0.9 x Elevation + −31.3	0.88 (0.4)***	0.7 x Elevation + −32.5
		Structure and defense		
C	NS		NS	
Lignin	NS		0.51 (2.8)*	−2.2 x Elevation + 27.3
Cellulose	0.55 (1.8)*	−1.5 x Elevation + 18.3	0.54 (2.2)*	−1.8 x Elevation + 19.8
Phenols^1	0.96 (7.0)***	27.8 x Elevation + 42.7	0.93 (7.8)***	25.1 x Elevation + 43.6
Tannins^1	0.94 (5.1)***	17.2 x Elevation + 11.9	0.92 (5.8)***	16.1 x Elevation + 12.7
		Macronutrients		
P	NS		NS	
Ca	NS		NS	
K^1	0.69 (0.06)**	0.07*elev^2-0.24*elev+0.72	0.61 (0.08)**	0.08*elev^2-0.25elev+0.79
Mg	NS		NS	
		Micronutrients		
B^1	0.79 (3.25)**	6.2*elev^2-18.9*elev+28.5	0.84 (2.82)**	6.4*elev^2-19.6*elev+29.3
Fe	NS		NS	
Mn	0.57 (295.1)*	257.1 x Elevation + 41.7	0.56 (312.0)*	265.3 x Elevation + 24.1
Zn	NS		NS	

*Asterisks indicate significant levels as *$p < 0.001$, **$p < 0.01$, and ***$p < 0.05$. ^{1} or NS (nonsignificant) sites ACJ-01 and TAM-05 excluded from regressions due to unusual soil properties interacting with these traits. Elev is elevation. See methods for details.*

Correlation value (R^2) is provided with root-mean-squared error (RMSE) in parentheses. The equations are reported relative to elevation in km. LMA is leaf mass per area. NSC are nonstructural carbohydrates.

when expressed on an area-basis (**Table S5**). The exceptions were lignin, cellulose, and Ca.

Sources of Trait Variation

Multiple canopy foliar traits exhibited variation associated with elevation; however, only growth-related traits differed between sun and shade leaves. On a mass basis, canopy position accounted for a significant portion of the variation among the sites for the light-sensitive traits including Chl ab, carotenoids and δ^{13}C, as well as LMA (**Table 3**). Chl ab and Car were 19% and 13% higher, respectively, in shade than in sun leaves (**Tables 3**, **S2**; t = 13.9 and 10.8 respectively; $p < 0.001$). Foliar concentrations of δ^{13}C were 4.5% less negative in shade than sun foliage (t = -22.4, $p < 0.001$). LMA was 19% higher (t = −18.5; $p < 0.001$) in sun than in shade leaves, resulting in similar values for photosynthetic pigments (Chl ab, Car) in sun and shade leaves when concentrations were calculated on a leaf-area basis (**Tables S3**, **S4**). Higher LMA in sun leaves also resulted in significantly higher area-based N, soluble C, δ^{13}C, P, and lignin, in sun compared to shade leaves (**Tables S3**, **S4**). Because the differences between sun and shade leaves calculated on an area basis are almost entirely due to the changes in LMA (Lloyd et al., 2013), we focus most of our remaining analyses on mass-based traits. There was substantial variation in macronutrient and micronutrient concentrations within and among some sites, but these traits did not show sun-shade differences of sufficient magnitude to separate them on a mass or area basis (**Tables 3**, **S2–S5**).

The high degree of species turnover along this elevation gradient makes disentangling taxonomic versus site effects difficult, and with only one plot per site, this was not the focus of this study. We used a nested approach to partition the variation to determine if, once other sources of variation are accounted for, if canopy position might emerge as an important factor. Analysis of the partitioning of the trait variation by canopy position (sun or shade), site (MAT, MAP, geology, topography, elevation), genetic (intraspecific and interspecific), and residual (measurement error and other nonsite related sources) components, indicated that canopy position figured into the measured variation in LMA, Chl ab, and Car (12%, 14%, and 7%, respectively, **Figure 2**). Also, a larger proportion of the variance in foliar δ^{13}C (25%) was explained by canopy position than any other factor other than the model residual. On the other hand, canopy position accounted for less than 5% of the variance in other traits. Concentrations of photosynthetic pigments (Chl ab and carotenoids) in sun and shade leaves were similar on an area-basis, compensated for the most part by changes in LMA.

As has been found previously (Asner et al., 2015; Asner et al., 2017), genetics (intraspecific/interspecific) explained at least 50% of the variation in most leaf traits (**Figure 2**). However, the degree of partitioning between intraspecific and interspecific components of variation was not consistent among the foliar traits. For many traits the variance attributed to intraspecific or interspecific differences was less than 15%, but interspecific variation was more than 20% higher than intraspecific variation for some nutrients (K, Mg, B, and Zn). In contrast, intraspecific variation was higher in phenols and Fe, 19% and 24% respectively. LMA, δ^{13}C and total C showed almost no

TABLE 3 | Results of nested ANOVA testing for differences among leaf traits on a mass-basis between sun and shade leaves and site[1].

Response variable	Source of variation				
	Site		Canopy position (site)		Offset
	F	P	F	P	μ ± STERR
Light capture and growth					
LMA	22.97	<0.01	7.70	<0.01	−19.18 ± 1.04*
N	31.63	<0.01	0.30	NS	
Chlorophyll *ab*	19.26	<0.01	8.33	<0.01	1.43 ± 0.10*
Carotenoids	19.01	<0.01	4.35	<0.01	0.21 ± 0.02*
Soluble C	30.90	<0.01	0.35	NS	
δ^{13}C	35.22	<0.01	16.56	<0.01	−1.42 ± 0.06*
Structure and defense					
Total C	23.98	<0.01	0.75	NS	
Lignin	11.34	<0.01	0.26	NS	
Cellulose	28.65	<0.01	1.46	NS	
Phenols	24.58	<0.01	0.30	NS	
Tannins	37.85	<0.01	0.20	NS	
Macronutrients					
P	36.95	<0.01	0.35	NS	
Ca	132.97	<0.01	0.46	NS	
K	11.93	<0.01	1.70	NS	
Mg	25.31	<0.01	0.61	NS	
Micronutrients					
B	23.99	<0.01	0.24	NS	
Fe	23.39	<0.01	1.63	NS	
Mn	68.02	<0.01	0.08	NS	
Zn	5.01	<0.01	0.52	NS	

[1]Sample collection at sites varying in elevation, light environment, and geology meant that canopy position was effectively nested within site.

LMA, leaf mass per unit area; LMA, N, chlorophyll ab*, carotenoids, P, K, and Mg were log-transformed before analysis. Ca was transformed by square root.*

STERR is standard error.

** indicates significant offset at* $p < 0.01$*. NS is nonsignificant.*

Mean offset among canopy position (shade to sun) across all sites is also shown (Matched pairs t-test, $p < 0.001$*). LMA is leaf mass per area. NSC are nonstructural carbohydrates.*

difference (<5%) in the variance attributed to intraspecific or interspecific variation. Site explained 22%–62% of the variation in most macronutrient and micronutrient as well as defense compounds (phenols, tannins). A large portion of the total variation in Mn was also explained by site (49%). However, less than 10% of the total variation in K, lignin, and Zn was attributable to site. Variation in these traits was dominated by intra and interspecific variation (65%–80%).

The degree of trait variation between sun and shade leaves was less than or similar to the variation within species at a site and did not change with elevation across the gradient with the exception of defense chemicals (phenols and tannins) and δ^{13}C concentration (**Figure 3**). Variation in these defensive traits was highest among all traits, ranging between 17% and 80%, and was negatively correlated with elevation in canopy position and within and across species. Variation in δ^{13}C concentration also changed along the elevation gradient but the magnitude of change was relatively low: 25% among species and 15% within species. Variation in δ^{13}C within species and between sun and shade leaves increased slightly with increasing elevation (**Figure 3**, **Table S6**). Variation in macronutrients and micronutrients was similar to other foliar traits (6%–21%, 9%–30%, and 21%–90% within tree, intraspecific, and interspecific respectively). A small number of these were significantly correlated with elevation; however, there was no systematic pattern (**Figure 3**). Intraspecific variations in Fe and Mn concentrations were positively correlated with elevation, while intraspecific variation in K and B decreased with increasing elevation.

DISCUSSION

We found that taxonomic and environmental controls on variation in shade leaf trait patterns mirror or match the variation observed in sun leaves. For most traits (i.e. N, foliar nutrients or defense compounds), there was no significant difference between sun and shade leaves. Significant within-canopy differences between sun and shade leaves were measured among certain growth-related traits including LMA, photosynthetic pigments, and δ^{13}C (**Figure 1**, **Tables 2**, **3**), but these traits maintain constant offsets, suggesting characteristics of shade leaves can be derived from those measured in sun leaves. In this discussion we first review data from the few studies examining foliar traits in sun and shade leaves that are not directly associated with photosynthesis. We then discuss the response of sun and shade leaves to environmental variation and compositional turnover along this elevation gradient. We conclude by explaining how this study benefits landscape-scale remote sensing.

Our observed lack of sun-shade differentiation in foliar chemical traits beyond those related to photosynthesis and growth is poorly studied in tropical evergreen species. Grubb (1977) found little difference between sun and shade leaves for C,

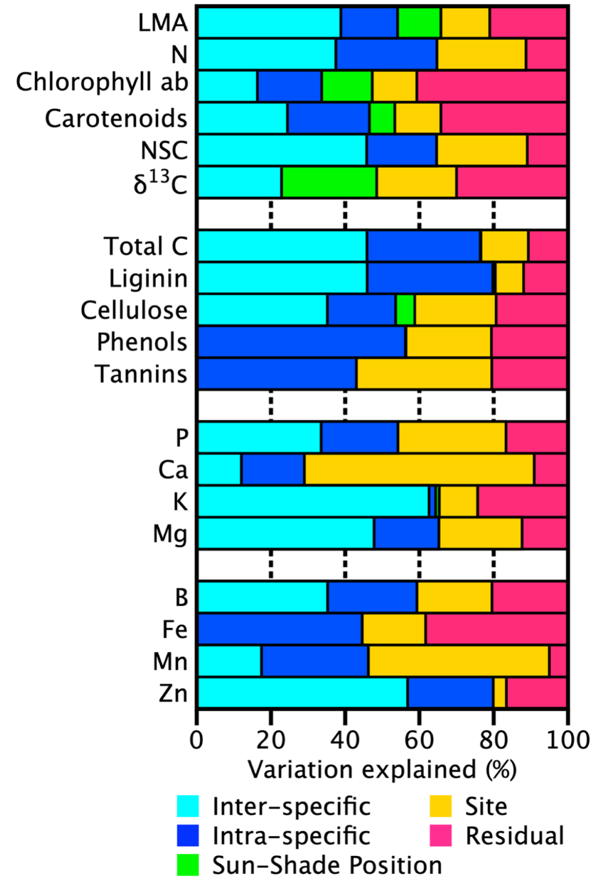

FIGURE 2 | Partitioning of the variance for each tree canopy chemical trait into site, canopy position (sun or shade), phylogenetic variation, and unexplained residual variation for 10 sites along an Andean-Amazon gradient in Peru. The site component incorporates environmental variation in geology, topography, elevation, temperature, precipitation, radiation. Here, phylogenetic variation is separated into intraspecific and interspecific components (species and genera + family partitions respectively). Unexplained residuals are comprised of measurement error and other nonsite-related sources of uncertainty such as tree and foliage selection.

Fe, N, P and slightly higher concentrations of K and Mg in shade leaves in a number of tropical forests. This was also shown more recently for N and P in a large number of species in moist (Poorter and Rozendaal, 2008) and dry tropical forests (Markesteijn et al., 2007). Foliar concentrations of phenols and NSC were lower, and lignin was higher, in shade leaves of gymnosperms and deciduous trees among a number of species (Poorter et al., 2006). In conifer needles, N, P, K and cellulose concentrations were higher in shade leaves, while Ca concentrations were lower (Richardson, 2004).

The role of compositional turnover in driving changes in foliar traits of tree species in the ten sites along this elevation gradient was presented in Asner et al. (2017) and generally follows patterns on elevation gradients found globally (Asner and Martin, 2016). To what extent shade leaves might adhere to the same pattern was not previously explored. Growth-related traits are known to adapt to their light environment and vary in relation light extinction within canopies. The consistency with which sun-shade trait differences are maintained over the large environmental and compositional gradient sampled here might be surprising. However, light levels of the shade leaves were generally less than 10% of the ambient solar radiation at all sites, likely setting a consistent low light limit for foliar adjustment. The consistent offset between sun and shade leaves in 134 species across varying environmental conditions suggests the plasticity in these traits may be genetically coordinated with maximum low light modification set relative to high light and vice versa.

Our findings show that the portion of light-sensitive trait variability attributed to sun-shade position ranged from 7%–25% with the remaining variation split between taxonomic, site, and residual components (**Figure 2**). The proportions changed only minimally if analyses were done without taxonomic and canopy position nested within a site, indicating the convolved effect of species turnover and site along this gradient. Like past studies, our findings indicate that variation in sunlit canopy foliar traits are controlled primarily by changes in community composition, and secondarily by environmental factors, elevation and substrate (Fyllas et al., 2009; Asner et al., 2014a; Blonder et al., 2017). Variation among the other leaf traits followed patterns previously found, with approximately 50% of the variation was found in the taxonomic fraction (intraplus interspecific), and the remainder split between site and residual effects (**Figure 2**). Moreover, interspecific variation was up to three times higher than intraspecific variation, and was generally constant along the elevation gradient, pointing to the dominating role of species turnover along the elevation gradient (**Figure 3**). The large portion of variation in macronutrients and micronutrients found in the site and residual components, coupled with very low values in these chemical traits measured in the highest elevation site (ACJ-01), are likely related to the overall dystrophic soil conditions throughout the elevation gradient.

As is well documented, leaf structural changes represented by LMA were inversely related to foliar N in sun and shade leaves when calculated on an area basis, but concentrations of photosynthetic pigments converged in value on an area basis (**Tables S3**, **S4**). This finding supports our understanding of increasing leaf area in shaded foliage to increase light interception and photosynthetic capacity at lower subcanopy light intensities. These LMA decreases generally come with increased foliar N content per unit area (Chen et al., 1993; Niinemets, 2007; and many others), which is positively related to maximum photosynthetic rate on an area basis throughout the canopy.

LMA differences (16%) measured among multiple species and a large environmental gradient were far lower than the > 60% recently reported for tropical tree leaves by Keenan and Niinemets (2016) after they scaled traits to a uniform light intensity for comparison. They also reported a 40% difference in mass-based N, where we found none. Whether plasticity between sun and shade leaves of 15%–16% in LMA, and thus

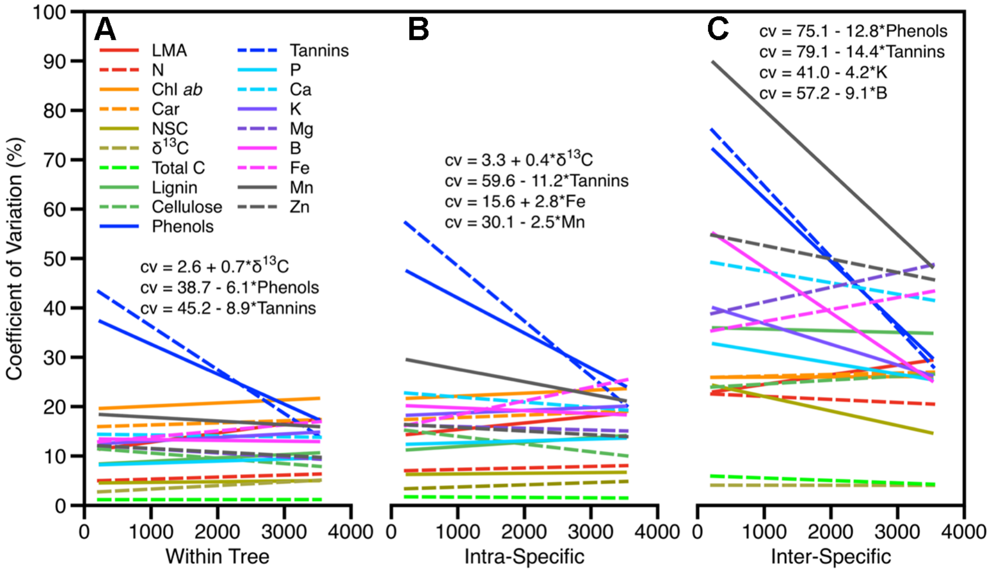

FIGURE 3 | Degree of variation in foliar traits within all sites as they vary across the elevation gradient for **(A)** within trees due to canopy position (sun and shade), **(B)** within species, and **(C)** among species. Significant relationships ($p < 0.05$) between trait variation and elevation are given.

area-based N, has a significant effect on canopy photosynthetic capacity in these trees in under investigation (Bentley, L. *pers comm*); however, differences in the $\delta^{13}C$ concentrations between sun and shade leaves may provide insight into foliar function. Lower $\delta^{13}C$ values in shade leaves is thought to be related to decreased stomatal resistance in thinner, lower-LMA leaves and/or to subcanopy environments that are cooler and more humid, promoting stomatal opening and enhanced rates of CO_2 uptake (Niinemets and Valladares, 2004). Both of these conditions should boost CO_2 uptake and reduce photosynthetic limitations. Lower $\delta^{13}C$ values from soil respiration may also contribute to lower $\delta^{13}C$ values in leaves at lower canopy levels (Medina and Minchin, 1980; Sternberg et al., 1989).

CONCLUSION

We found significant differences in light-sensitive traits between sun and leaves in 385 canopies of 189 species of tropical rainforest trees. These offsets were maintained across a wide variety of environmental conditions along a 3,500-m elevation gradient suggesting this plasticity associated with light availability is an adaptive change. In contrast, we did not find sun-shade differences in 15 other foliar traits related to defense and metabolism.

These findings of parallel patterns, whether as constant offset or close similarity between many of the canopy sun and shade leaf traits, bear on the effort to scale leaf measurements to landscape and regional levels. For example, ongoing work to map canopy traits using optical remote sensing, particularly imaging spectroscopy, has yielded an understanding of sunlit foliar trait responses to soil fertility, climate, and topography (Ustin et al., 2004; Kokaly et al., 2009; Asner et al., 2011; Asner et al., 2016). Such mapping of sunlit canopy traits has been accomplished over millions of hectares of temperate and tropical forest. However, the relation between what can be mapped at the upper portion of the canopy, also known as the top-of-canopy or canopy skin, and the subcanopy or shaded foliage has left remote sensing with uncertain connection to the remainder of the mapped forest. We found that photosynthesis-related traits such as N, LMA, pigments and $\delta^{13}C$, exhibited constant offsets across environmental conditions enabling them to be mapped to upper canopy traits based on principles of light extinction. These principles are well known, and can be applied using other remote sensing techniques that are sensitive to leaf area index and other canopy foliar volumetric properties (Ollinger, 2011). This is not true for shaded leaf traits that are not directly linked to photosynthesis, including numerous macronutrients and defense compounds. However, all of these were of similar magnitude and varied in parallel to their counterparts in sunlit canopy positions. This indicates that top-of-canopy remotely sensed measurements of multiple key foliar chemical traits link directly to whole-canopy foliar properties, including shaded leaves that cannot be directly observed from above. This information is timely because it provides evidence that the rapidly growing area of spectral remote sensing can represent both upper- and lower-canopy foliage, which has been a missing link that can facilitate more robust estimates of canopy function from airborne and satellite platforms.

DATA AVAILABILITY STATEMENT

The datasets generated and analyzed for this study can be found in the ForestPlots (https://www.forestplots.net/) and Global Ecosystems Monitoring Network (GEM; http://gem.tropicalforests.ox.ac.uk/projects/aberg) networks.

AUTHOR CONTRIBUTIONS

The study was conceived and designed by GA, BE, SD, and YM. Field data were collected by RM, LB, AS, NS, KQH, MMP, FCA, and YM. RM carried out laboratory assays. Data analysis was performed by RM. The manuscript was written by RM with contributions from GA, LB, AS, BE, and YM.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2019.01810/full#supplementary-material

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Why Are There so Many Plant Species That Transiently Flush Young Leaves Red in the Tropics?

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Delayed greening of young leaves is a ubiquitous and visually striking phenomenon in the tropics. Here, we investigated the potential ecological functions of red coloration patterns in young leaves. To detect any protective function of the red coloration on the young leaves, leaf damage by insect herbivores was recorded in the field. To determine capacity for chemical defense, the concentrations of tannins and anthocyanins were measured in both young and mature leaves. To test the hypothesis that anthocyanins function as photo-protective molecules, chlorophyll content, maximum photochemical efficiency of *PSII* (F_v/F_m), non-photochemical quenching (*NPQ*), and effective quantum yield of *PSII* (Φ_{PSII}) were measured in the field. Phylogenetic relationships were analyzed to test the relationary significance of the occurrence of redness in young leaves. Compared to the coloration in non-red leaves, young red leaves had significant higher anthocyanins and tannins content and lower herbivore damages. Young, red leaves had the lowest F_v/F_m values, which were significantly lower than those of non-red leaves. *NPQ* values in young red leaves were comparable to those of other groups. Although young red leaves had high Φ_{PSII}, these values were significantly lower than those of the other three groups. The results suggest that the red coloration of young leaves protects them from insect herbivory primary by chemical defense through high concentrations of tannins and anthocyanins. Additionally, low F_v/F_m values in young red leaves indicate that anthocyanins might not be functioning as light attenuators to compensate for insufficient photo-protection mediated by *NPQ*. And finally, red coloration in young leaves is predominantly a result of adaptation to heavy herbivory stress but without significant intrinsic phylogenetic relationship of plant species.

Keywords: red coloration, young leaves, tropics, plant defense, anthocyanins, tannins

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INTRODUCTION

Plant species are sensitive to changes in environmental stimuli and shift their physiological development to adapt to their specific surroundings (Niu et al., 2014). Environmental changes can alter the availability of resources and other conditions crucial to plant performance (Nicotra et al., 2010). Plant leaves are not only the most important photosynthetic organs but also the parts of the plant that can most sensitively respond to environmental changes (Nicotra et al., 2010; Niu et al., 2014). In the field, leaf size, shape, thickness, and folding behavior (Parkhurst and Loucks, 1972; Givnish, 1987; Turner, 1994; Smith et al., 1997; Beerling et al., 2001; Cornelissen et al., 2003; Huang et al., 2012), and even secondary metabolites or leaf coloration (Coley and Aide, 1989; Gould et al., 1995; Westoby, 1998; Nicotra et al., 2010; Chen and Huang, 2013; Niu et al., 2014; Tellez et al., 2016; Wang et al., 2016) can all be altered to respond to abiotic environmental changes (e.g., UV light) or biotic herbivory stresses.

In the tropics, certain plant species have evolved species-specific leaf development patterns, such as delayed greening (Kursar and Coley, 1992). Young leaves in tropical regions worldwide often flush red synchronously during expansion (Dominy et al., 2002; Manetas et al., 2003). Red flushing can be a widespread and visually striking phenomenon in the tropics, with between 20%–40% of the woody species showing red flushed young leaves at a single site (Opler et al., 1980). Coley and Barone (1996) reported that approximately one third of the plant species in tropical forests delayed greening of their young leaves until full expansion. Kursar and Coley (1992) found that 36% of tree species exhibited red coloration of young leaves at Barro Colorado Island (BCI), Panama. Furthermore, Chapman et al. (1999) reported that nearly 49% of forest trees in Kibale National Park, Uganda exhibited redness of young leaves at some point over the years. The delayed greening phenomenon in tropical rainforests of Southeast Asia included as much as 62% of the total studied plant species (Dominy, 2002).

Young red leaves generally contain high levels of anthocyanins, which are the primary contributors to the red coloration of plant leaves (Field et al., 2001; Trojak and Skowron, 2017; Cooney et al., 2018). Anthocyanins are produced not only in response to seasonal, spatial or developmental factors but can also be induced by a number of disparate abiotic (e.g., ultraviolet (UV) light) and biotic (e.g., insect herbivores, folivorous mammals, pathogens) stimuli (Hammerschmidt, 1977; Hipskind et al., 1996; Merzlyak and Chivkunova, 2000; Gould et al., 2002a; Trojak and Skowron, 2017). During leaf expansion, anthocyanins are frequently present in mesophyll and epidermal cells and then disappear from the tissues or decrease in concentration after full leaf expansion (Trojak and Skowron, 2017). In plants, anthocyanins function to screen against harmful reactive oxygen species (ROS), herbivore attacks, fungal attacks, and high light stress (Coley and Aide, 1989; Gould et al., 1995; Gould et al., 2002b; Tellez et al., 2016). However, anthocyanins do not serve as photosynthetic pigments (Trojak and Skowron, 2017). Since a primary function of plant leaves is photosynthesis, why do so many plant species not show an optimized pigment pool, (in terms of the absorption spectrum required for efficient light capture for photosynthesis) but instead accumulate high concentrations of anthocyanins and delay greening at the juvenile stage?

The ecological significance of delayed greening in young leaves has been disputed (Coley and Kursor, 1996). Coley and Aide (1989) found that leaf-cutter ants preferentially pick up leaves without anthocyanins. These authors then proposed the hypothesis that anthocyanins are associated with against invasions by leaf-attacking fungal pathogens. This view was shared by Tellez et al. (2016). Stone (1979) reported that the redness of young leaves may make them invisible to herbivores or may warn potential herbivores of the presence of toxic compounds. Other researchers have found that anthocyanins may function as light attenuators to compensate for insufficient photo-protection mediated by non-photochemical quenching (*NPQ*) (Demmig-Adams and Adams, 1992; Gould et al., 1995; Hughes et al., 2005; Zhu et al., 2018). However, these hypotheses of delayed greening in young leaves were put forward or verified based on studies considering only a few plant species, and whether these mechanisms have widespread adaptive significance for tropical plants is still unclear.

Plant-herbivore interactions constitute an important component of tropical rainforest biodiversity. Reciprocal selection has led to greater investment in defenses in tropical trees than in temperate plant species (Levin and York, 1978; Coley and Aide, 1989; Basset, 1994; Marquis and Braker, 1994). Although plant leaves are easily damaged by generalist or specialist herbivores (Cates, 1980; Coley and Aide, 1989; Broadway and Colvin, 1992; van Dam et al., 1995; Coley and Barone, 1996; Blüthgen and Metzner, 2007), young leaves, especially those with red phenotypes, can effectively decrease herbivore damage (Kursar and Coley, 1991; Karageorgou and Manetas, 2006). However, Chen and Huang (2013) reported that young red leaves have fewer mechanical physical defenses than do green leaves. Thus, the mechanism by which red coloration of young leaves helps them avoid harm from herbivores in the tropics is an important area of phenotypic study.

The tropics usually experience greater natural selection than temperate areas. The strong intensity of natural selection processes in tropical areas, which can be either continuous or intermittent processes consisting of exacerbations and remissions, makes phylogenetic relatedness of plant species decreased in tropical areas. Moreover, these ecological factors and their evolutionary relationships with plant species in tropical rainforests have resulted in an impressive variety of adaptations and interactions (Coley and Barone, 1996).

In the present study, we investigate the metabolism, photosynthetic activities and chemical defenses of leaves from 250 tropical plant species with either red or green young leaves. Our major aim is to investigate why there are so many plant species with transiently red young leaves in the tropics. Additionally, we attempt to highlight any ecological or evolutionary significance of delayed greening in young leaves of plants in the tropics. Our study involved systematic data collection in a tropical region of southwest China, through which we aim to answer the following questions:

1. Is red coloration in young leaves associated with the phylogenetic relationship of plant species?
2. Do young red leaves have better protection against herbivores than green leaves?
3. Does red coloration play a role in photosynthesis and photo-protection?

MATERIALS AND METHODS

Study Sites and Leaf Damage Investigation

This work was carried out between January 2016 and March 2017 in Southern Yunnan, China, with most plant samples coming from Xishuangbanna Tropical Botanical Garden (21° 41´N and 101°25´E, 570 m a.s.l.) and a small number of samples, used for field observation of natural leaf damage, from the botanic garden of Honghe University (23°21´N and 103°25´E, 1331 m a.s.l).

To differentiate the effect that the hydrolysable tannin content in plants had on food plant preference in insect herbivores, an extensive field investigation *in situ* into the feeding patterns of insect herbivores feeding on leaves between early and late stages of maturation were carried out. Our field survey involved 83 red-flushing woody species from 36 families (here called delayed-greening leaves, DGL) and 167 woody species without red young leaves (here called green leaves, GL) from 70 families. Prior to the experiment, three to five trees from each taxon were selected. Herbivore damage to young and mature leaves in each tree was assessed. For each plant, 10 branches from different parts of the tree were selected, and a total of approximately 200 young and mature leaves were assessed for herbivore damage. Irregularly shaped or incomplete leaves were recorded as damaged. The third and the fourth expanding leaves from the apex of each branch were treated as young leaves. The other leaves after the seventh from the apex of each branch were regarded as mature leaves.

Anthocyanins and Chlorophyll Content

We then determined the relative content of anthocyanins and the total chlorophyll content in both mature and young leaves, in order to investigate potential differences in anthocyanin and chlorophyll content between young and mature leaves. From the 250 plant species used in the field investigation into herbivory damage, we selected a total of 210 plant species (66 families, 150 genera), including DGL plant taxa (124 taxa, 43 families, 89 genera) and GL taxa (86 taxa, 42 families, 74 genera) to measure their concentrations of both anthocyanins and chlorophyll.

Determination of Concentration of Anthocyanins

The relative concentration of anthocyanins was determined following Christie et al. (1994) and Zhu et al. (2015) with minor modifications. Pulverized leaf blade tissue (1.0 g fresh weight) was homogenized in 12 ml of methanol containing 0.1 N HCL and maintained at 4°C for 4 h. Three repetitions were performed for each taxon. Particulates were removed by centrifugation at 1000 r/min for 10 min, and the absorption of the anthocyanin extracts was measured by a spectrophotometry (UV 5100B, Shanghai Metash Instrument Co., Ltd., Shanghai, China) at 530 nm. The relative concentration of anthocyanins was then calculated as $10\times A_{530}\times B$, where A is the measured absorption at 530 nm, and B is the dilution ratio of the experimental supernatant.

Chlorophyll Determination

Chlorophyll was extracted by using N, N-dimethylformamide, following Niu et al. (2014). The appropriate leaf area (0.2 cm^2, fresh) of material for each species was punched from fresh leaves (avoiding major leaf veins where possible) through a circular leaf disc puncher (ø = 5 mm). Six 0.2 cm^2 leaf disks were collected from six different leaves for each taxon, weighed fresh (to within 0.1 mg) were used and were then immersed in N, N-dimethylformamide for one night (~12 h) in the dark. Three repetitions were performed for each taxon. The resulting solutions were then centrifuged at 1000 r/min for 10 min, the supernatant was collected and the absorption values were measured using ultraviolet-visible spectrophotometry at 664.5 and 647 nm. The concentration of chlorophyll was calculated as equivalents per fresh weight of the leaves following Inskeep and Bloom (1985) and Niu et al. (2014).

Measurement of Total Tannin Content

Tannic acid is regarded as a major component of the total phenolics within the leaf. Total phenolic content was determined using the Folin-Ciocalteau method, following Singleton and Rossi (1965) and Greer et al. (2014). Pulverized leaf blade tissue (0.5 g freeze-dried weight) was homogenized in 10 ml of ethyl alcohol (60%) and then heated in a water bath (60°C) for 3 h. Samples were weighed (± 0.1 mg) to obtain their fresh weight (FW) before extraction. Three repetitions were completed for each taxon. Particulates were removed by centrifugation at 1000 r/min for 10 min and then filtered through a Buchner funnel. 1.0 ml of the solution was then combined with 60 ml of dH_2O water, 5.0 ml of Folin-Ciocalteau reagent and 15 ml of sodium carbonate in a 100 ml volumetric flask. A phenolic concentration standard curve was generated with tannic acid (concentrations from 0 to 50 mg/L). To better fit the standard curve, leachates were diluted to 8× their original concentrations. Both standard and samples were analyzed in a spectrophotometer at 765 nm. Total phenols are therefore reported as milligrams of tannic acid (gallotannin, Sigma) equivalents per gram fresh tissue (mg TAE/g FW).

Chlorophyll a Fluorescence Activity

Chlorophyll a fluorescence parameters of young and mature leaves were investigated using an Li-6400XT portable photosynthesis system (LiCOR Inc., USA) with an integrated leaf chamber fluorometer (LCF). For this experiment, of the total 250 plant species mentioned, 133 plant species were used, including 71 DGL species and 62 GL species. Ten leaves (five young and five mature leaves) from each taxon were used separately for the assessment of chlorophyll a fluorescence.

The maximum photochemical efficiency of *PSII* (F_v/F_m), which can be used to quantify photo-inhibition (Redondo-Gómez et al., 2006), was then calculated following Figueroa et al. (1997) and Lima et al. (2018). On the following day (9:00–11:00), after light adaptation for half an hour, chlorophyll a fluorescence traits of the same leaves were estimated with the Li-6400XT portable photosynthesis system. Minimum fluorescence (F_0) was measured under a continuous measuring light (1200 μmol quanta $m^{-2}s^{-1}$), and then F_m was measured following a pulse of actinic light of 5000 μmol $m^{-2}s^{-1}$. Maximum quantum yield of *PSII* was calculated as

$$F_v/F_m = (F_m - F_0)/F_m$$

After 30 min to allow for dark adaptation, the chlorophyll fluorescence parameters F_0, F_m, and F_s of each of the young and mature leaves were measured. F_0 is the minimum chlorophyll a fluorescence after dark adaptation; F_m is the maximal fluorescence level in the dark-adapted state, and F_s is the steady-state fluorescence yield (Lima et al., 2018).The effective quantum yield of *PSII* (Φ_{PSII}), which can be used for routine assays of plant heath performance and the quantification of environmental stress, was calculated following Redondo-Gómez et al. (2006) and Lima et al. (2018):

$$\Phi_{PSII} = (F_{m'} - F_s)/F_{m'}$$

Non-photochemical quenching (*NPQ*), which is an important photo-protective process in plants, was also calculated as follows (Redondo-Gómez et al., 2006; Ware et al., 2015; Lima et al., 2018):

$$NPQ = (F_m - F_{m'})F_{m'}$$

Phylogenetic Relationships Between DGL and GL Taxa

The family and genus names of all the studied species (210 species in total) in the APG III system were obtained using the R package plantlist (Zhang, 2018). The phylogenetic relationships between these species were examined using the online tool Phylomatic (Webb and Donoghue, 2005) (www.phylodiversity.net/phylomatic/) based on the Angiosperm consensus tree from Davies et al. (2004). The results were visualized uisng the iTOL (itol.embl.de) online tool for the display and annotation of phylogenetic trees (Letunic and Bork, 2006; Letunic and Bork, 2016). A total of 204 species were shown in the tree, and six species (*Balakata baccata*, *Lagerstroemia siamica*, *Lithocarpus microspermus*, *Nyssa sinensis*, *Parakmeria yunnanensis*, *Stixis suaveolens*) were not because of no record in database. Branch lines and the background of the terminal nodes were colored, displaying DGL and GL plant species. Ten different color strings outside of the tree illustrated the family subtrees, including more than five species. The numerators and denominators of the fractions behind the family names give the number of DGL species and the total number of species studied in this family, respectively.

Statistical Analyses

For each measured parameter, the mean values for each species were used for further calculation and for presentation in figures. A list of all the plant species used in the measurement of photosynthesis physiology or determination of defense chemical content is supplied in the supporting information (**Table S1**). Nonparametric Kruskal–Wallis ANOVAs were conducted to test for differences in the percentage variation of leaf area damaged by insect herbivores between the young red and mature green leaves. GL plant species were compared in the same way. The effects of the color of the young leaf and species on the chlorophyll fluorescence parameter which shown the photosynthetic ability and potential protection mechanism, including F_v/F_m, effective *PSII* efficiency, and non-photochemical dissipation (*NPQ*) were evaluated using two-way analysis of variance (ANOVA). Differences in anthocyanin, chlorophyll and tannin content between young and mature leaves were assessed using an *F* test after all sample data had been tested for normality. A general Pearson linear regression and a Spearman's Rho non-parametric correlation were used to examine any potential relationships between the contents of anthocyanin and tannin in leaves, respectively. All of the statistical analyses were performed with the SPSS v.16.0 statistical package (SPSS, Chicago, IL). As well as the phylogenetic correlation analysis described above, we used two-tailed *U*-tests to compare the proportions of DGL and GL plant species in each family of the phylogenetic branch, to test the null hypothesis that of the color of the young leaves was not associated with their phylogenetic placement. The numbers present and absent in green species were set as the expected numbers. Figures were generated using SigmaPlot software (Version 14.0, Systat Software, San Jose, CA).

RESULTS

Field Investigation of Leaf Damage

Herbivore damage to young and mature leaves of plant species was investigated in the field (**Figure 1**). Young leaves of both DGL and GL species showed significantly lower herbivore damage proportions than did the mature leaves on the same plants ($P < 0.001$; $P < 0.001$, total n = 250). The young GL leaves were more attacked by insect herbivores (26.89 ± 2.72%, n = 167) than young DGL leaves (17.75 ± 3.30%, n = 83) ($P < 0.01$). Approximately 39% (n = 83) of the green mature leaves from DGL plant species were damaged by herbivores, and 38% (n = 167) of the green mature leaves of GL plant species were damaged. There was no significant difference between DGL and GL groups ($P > 0.05$) (**Figure 1**).

Leaf Chlorophyll, Anthocyanin and Tannin Characteristics

We determined the levels of anthocyanins, tannin and chlorophyll characteristics in young and mature leaves (**Figure 2**). Both young leaves from DGL (n = 124) and from GL (n = 86) were investigated.

Anthocyanins in young and mature leaves were also evaluated, as shown in **Figure 2A**. Young leaves with red coloration had the greatest anthocyanin concentration, Around 6.45 ± 0.66 A_{530nm}/g FW anthocyanins were detected in young DGL leaves. This value was significantly higher than those from the other three groups ($F_{3,\ 417} = 38.78$, $P < 0.001$). In contrast, both young and mature GL leaves had relatively low levels of anthocyanins, ranging from 1.76 ± 0.23 to 2.14 ± 0.38 A_{530nm}/g FW (**Figure 2A**). There were no significant differences between these three groups ($P > 0.05$).

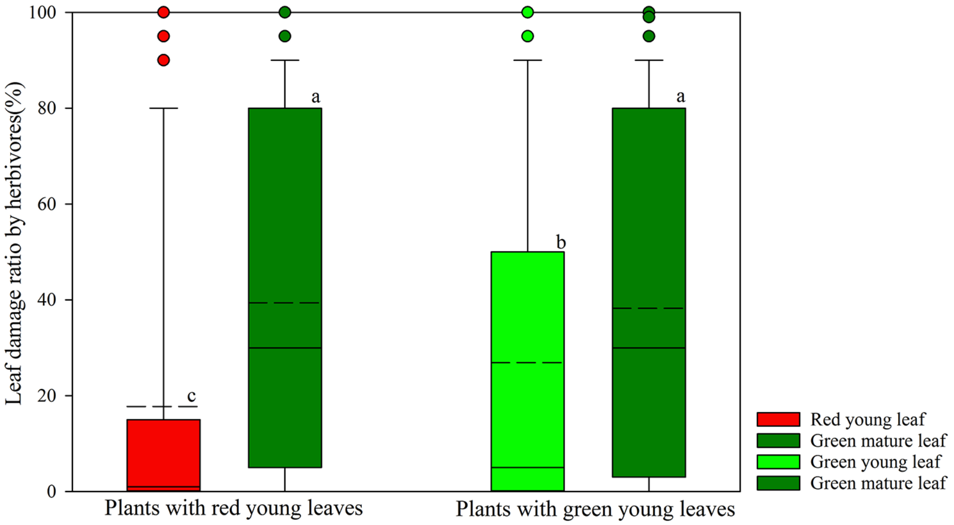

FIGURE 1 | Differences of leaf damage by herbivores in young and mature leaves in red- and green-young leaf taxa. Box and whisker plots illustrate the 5th, 25th, 50th (median), 75th, and 95th percentiles, and the means are given as a short dashed line. Different letters indicate significant differences ($P < 0.05$; Nonparametric Kruskal–Wallis ANOVAs test). Black small circles represent outliers.

The tannin content of young and mature leaves is presented in **Figure 2B**, and shows the same trend as the leaf anthocyanin (**Figure 2A**). The highest tannin content was observed in young DGL leaves (13.81 mg TAE/g FW), which was significantly greater than values from the three non-red groups ($F_{3,\ 417} = 34.6$, $P < 0.001$). The mature leaves from DGL taxa showed the second highest level of tannin (7.86 mg TAE/g FW) (**Figure 2B**). The leaf tannin concentrations in GL taxa were significantly lower than those in DGL taxa. No significant differences between young and mature leaves were found (*t*-test, $P > 0.05$).

The total chlorophyll content varied significantly between the four groups ($P < 0.001$; **Figure 2C**). Mature leaves from all studied species had greater total chlorophyll content than did young leaves from the same species (**Figure 2C**). The total chlorophyll content was 0.14 mg/g FW in GL taxa. Moreover, mature leaves of DGL plant taxa contained more chlorophyll than young leaves from those two groups ($P < 0.01$). Additionally, the young DGL leaves had the lowest chlorophyll content, 0.09 mg/g (**Figure 2C**).

A significant correlation between anthocyanin and tannin contents of young DGL leaves was found ($r = 0.20$, $P < 0.05$, Pearson linear correlation; $r = 0.30$, $P < 0.01$, Spearman's *rs* correlation; n = 96, **Figure 3A**). However, this relationship was not observed in any of the other three groups ($P > 0.05$, **Figure 3**).

Chlorophyll Fluorescence Activity

The chlorophyll a fluorescence of young and mature leaves was evaluated in the field (**Figure 4**). Young leaves from DGL (n = 71) and GL (n = 62) species were used. Both young and mature GL leaves had high F_v/F_m values, ranging from 0.74 to 0.79 (**Figure 4A**). Young DGL leaves had the lowest F_v/F_m values, 0.74, which were significantly lower than the values of the other three non-red leaf groups ($P < 0.001$). There were significant differences ($P < 0.05$) between young and mature leaves in GL leaf taxa.

The differences in Φ_{PSII}, the quantum efficiency of *PSII* photochemistry, between young and mature leaves was assessed (**Figure 4B**). All studied taxa showed relatively high Φ_{PSII} values, ranging from 0.70 to 0.78. Young DGL leaves exhibited the lowest Φ_{PSII} values, which were significantly different from the other three non-red groups ($P < 0.001$). A significant difference among GL leaf taxa was found ($P < 0.05$).

Differences in the *NPQ* values between young and mature leaves were evaluated, ranging from 0.48 to 0.59 (**Figure 4C**). There were significant differences among the four groups ($P < 0.05$). In red-young leaf taxa, *NPQ* values in young red leaves were significant greater than in their mature leaves ($P < 0.05$). However, there were no significant differences between red- and green-young leaves ($P > 0.05$) (**Figure 4C**).

Phylogenetic Reconstruction

The phylogenetic relationships between the 204 studied species were reconstructed (**Figure 5**). The occurrence of redness in young leaves was not closely associated with their phylogenetic relationships. Plants with young leaves that flush red can be allied closely to other DGL taxa, or can belong to genera with many taxa with different coloration. However, the occurrence of redness in young leaves is a universal phenomenon. For example, 8 out of 14 studied species in the Euphorbiaceae showed young red leaves, 16 out of 23 in the Fabaceae, 11 out of 20 in the Moraceae and 6 out of 7 in the Myrtaceae. Additionally, all studied taxa from the Dipterocarpaceae, Fagaceae, Lythraceae, Myrtaceae, Rosaceae, and Salicaceae

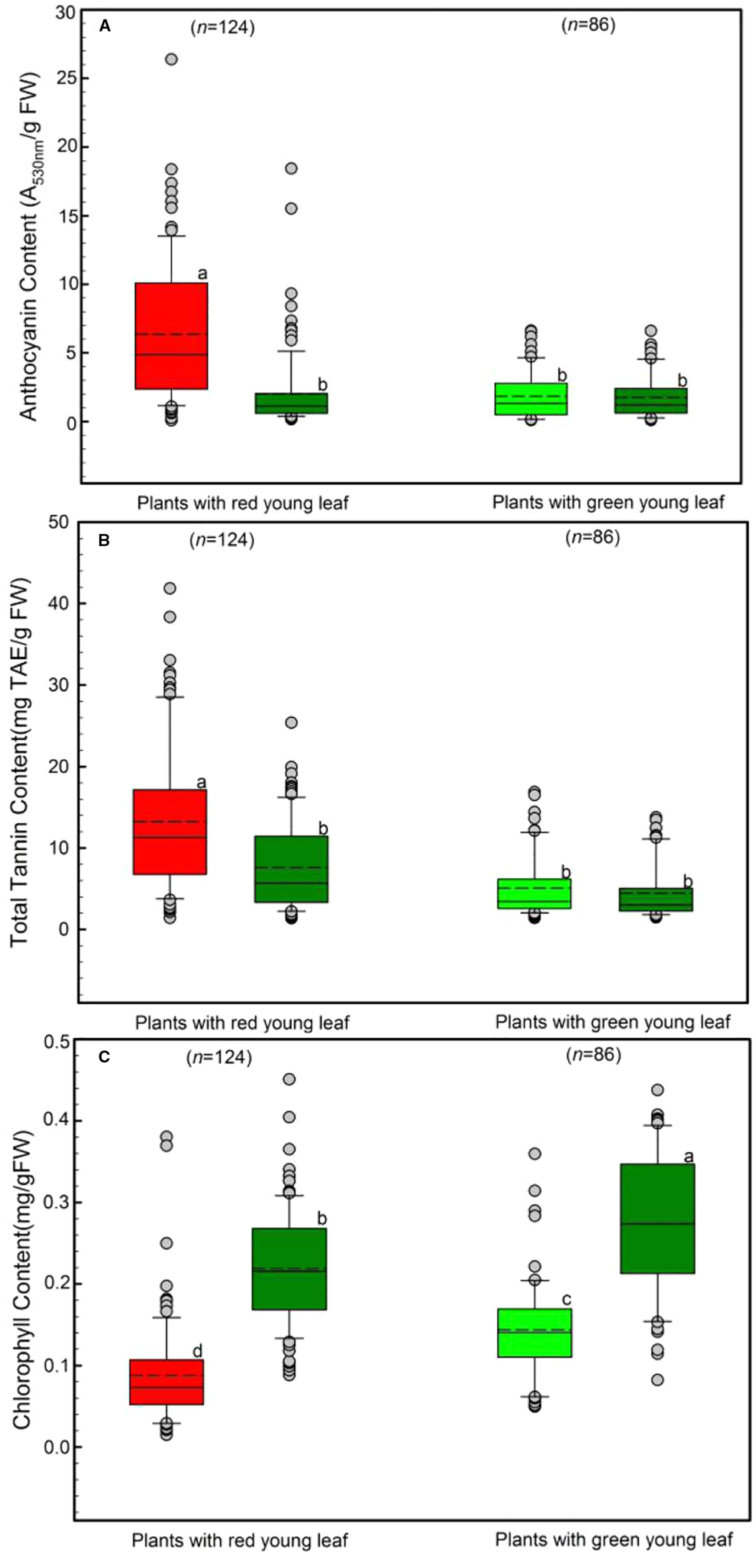

FIGURE 2 | Differences in leaf anthocyanin **(A)**, tannin **(B),** and chlorophyll **(C)** concentrations of young and mature leaves in red- and green-young leaf taxa. Box and whisker plots illustrate the 5th, 25th, 50th (median), 75th, and 95th percentiles, and the means are given as a short dashed line. Different letters indicate significant differences ($P < 0.05$; *F*-test). Grey small circle represent outliers.

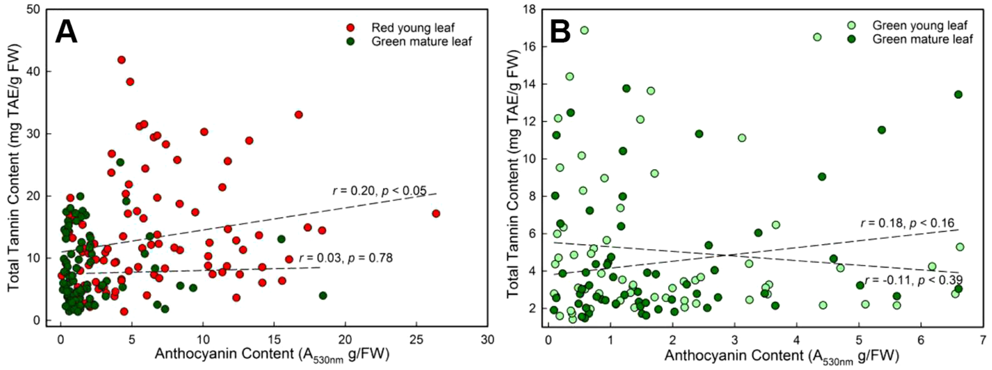

FIGURE 3 | The general Pearson linear correlation between leaf anthocyanin and tannin contents of young and mature leaves in red- and green-young leaf taxa. **(A)** red-young leaf taxa (n = 96); **(B)** green-young leaf taxa (n = 61).

exhibited red-colored young leaves. Conversely, we also found that all studied taxa in the Achariaceae and Piperaceae have green young leaves.

DISCUSSION

The anthocyanin pigments and the tannins, which are defense chemicals, are known to share the same biosynthetic pathway, the phenylpropanoid pathway (Ishikura et al., 1984). It is therefore not surprising that both have a relatively higher accumulation within red young leaves in the present research. Recent literature suggests that high levels of hydrolysable tannins [mainly galloylglucoses/ellagitannins (GGs/ETs)] in the young leaves of plants can inhibit POD mediated anthocyanin degradation and can increase stability and color intensity of anthocyanins by copigmentation activity (Luo et al., 2019). Previous studies have also reported that the delayed greening strategy is widespread in the tropics (Coley and Kursor, 1996; Dominy et al., 2002), and that young tropical leaves with visually striking red coloration have a greater increase in chemical defenses compared with mature leaves than do their temperate counterparts (Coley and Aide, 1989). The ecological function of anthocyanins is hotly debated. Our field observations show that young leaves from DGL plants can more efficiently escape insect herbivory with low leaf damage (17.75 ± 3.30%) compared to young GL leaves (26.89 ± 2.72%, **Figure 1**). We were also able to confirm that young DGL leaves are better able to defend against insect herbivory than are mature leaves. We predict that a higher concentration of anthocyanins and tannins also may plays a role in screening against harmful UV radiation, and can enhance chemical defenses separately based on the generalization of functional ecological specificity.

Theoretically, the low toughness and high nutritive value of young leaves may mean that they are preferred by grazers. Chen and Huang (2013) reported that young red leaves have fewer mechanical defenses than green leaves. Moreover, some studies showed that insect herbivores caused higher leaf area damage to young leaves (Coley and Aide, 1989; Coley and Barone, 1996). These results do not contradict our findings from the Asiatic tropics. Generalist herbivores prefer mature leaves, but they show lower performance than specialist herbivores with specific secondary metabolites (Blüthgen and Metzner, 2007). Therefore, the young DGL leaves may selectively protect themselves through chemical defenses because they have not got physical defense mechanisms.

In the present study, DGL plant taxa produced high amounts of tannins and anthocyanins in those leaves, 13.81 mg TAE/g FW, and 44 A_{530nm}/g FW, respectively (**Figure 2**). Tannins have been widely used as an index of plant chemical defense, as they considerably lower the palatability of leaf tissue (Lowman and Box, 1983). They can defend leaves against insect herbivores by deterrence or toxicity. At the same time, tannin-rich leaves contain high concentrations of anthocyanins because these components share the initial steps of the same synthetic pathway (Winkel-Shirley, 2002). Anthocyanins not only contribute to the redness of young leaves but also can effectively scavenge super-oxide and hydrogen peroxide generated by insect-mediated or mechanical damage through the browning reaction (Gould et al., 2002a).

In the tropical wild, plants face not only selection pressure from herbivory, but also abiotic environmental stresses, such as high light exposure, especially in the tropical rainforest zone. Excessive light can cause photo-inhibition, accompanied by damage to the photosynthetic apparatus and a decrease in the photosynthetic rate and can even cause photo-oxidation (Demmig-Adams and Adams, 1992).

A typical plant taxa has an F_v/F_m that ranges from 0.75 to 0.85 (Björkman and Demmig, 1987; Hogewoning et al., 2012). In this study, young DGL leaves had the lowest F_v/F_m ratio (0.70) and all non-red leaves had relatively high F_v/F_m values (0.77 ~ 0.79) (**Figure 3A**). Red leaves exhibited strikingly lower F_v/F_m ratios than did non-red leaves (**Figure 3A**). Thus, our results suggest the occurrence of photo-inhibition to some extent in young DGL leaves.

Plants can dissipate excessive light energy absorbed by light-harvesting complexes through non-photochemical quenching

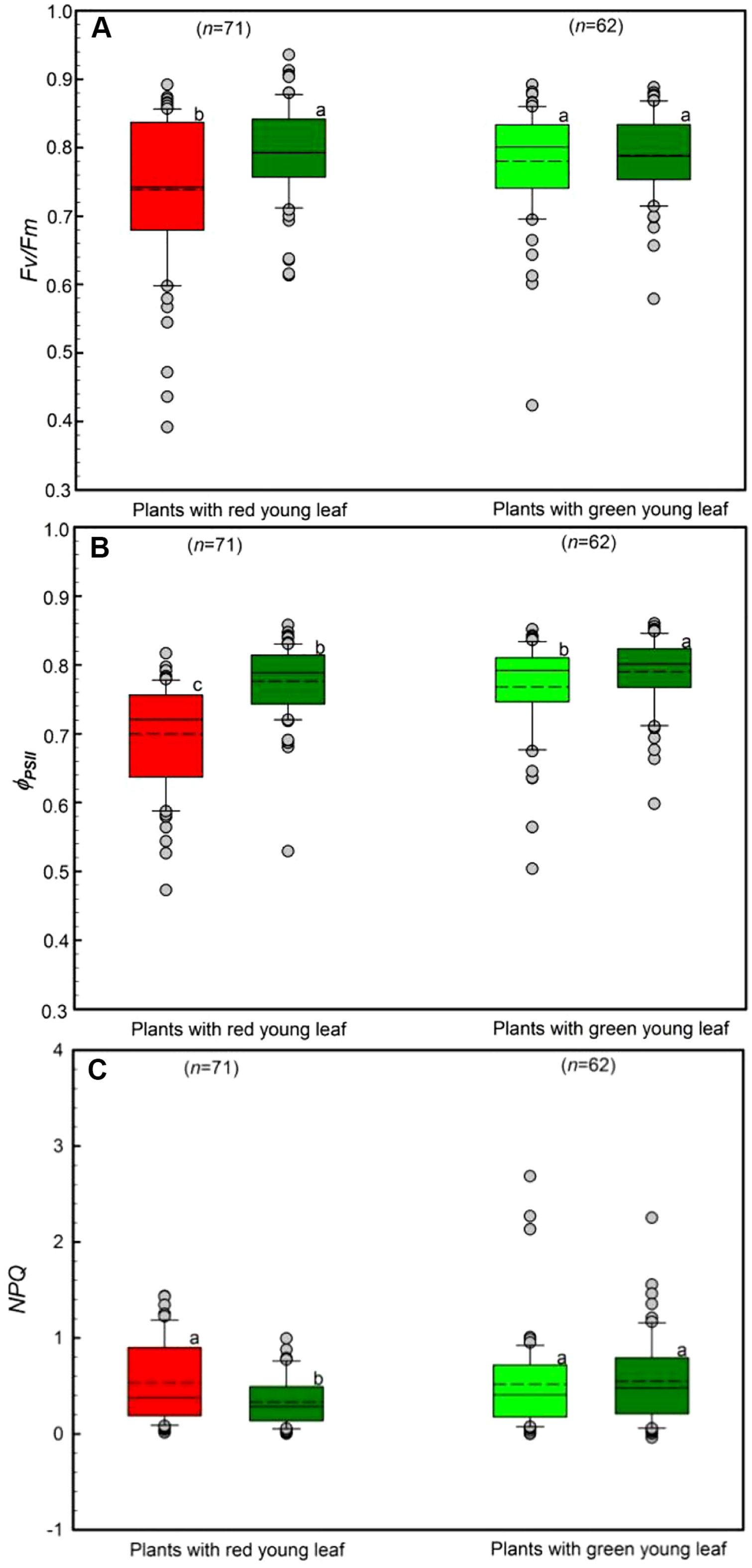

FIGURE 4 | Chlorophyll a fluorescence parameters including F_v/F_m **(A)**, Φ_{PSII} **(B)** and *NPQ* **(C)** of young and mature leaves in red- and green-young leaf taxa. Box and whisker plots illustrate the 5th, 25th, 50th (median), 75th, and 95th percentiles, and the means are given as a short dashed line. Different letters indicate significant differences ($P < 0.05$; two way ANOVAs test). Grey small circle represent outliers.

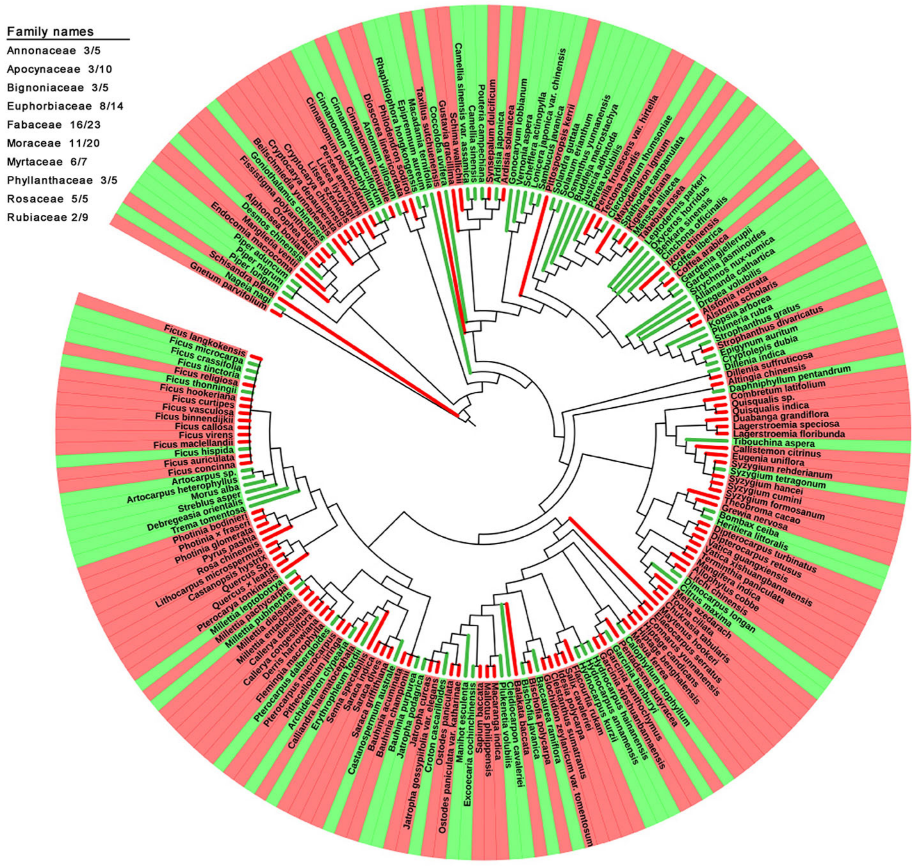

FIGURE 5 | Phylogenetic development tree map of 205 studied plant species with red and green young leaves.

(*NPQ*), which is based on the xanthophyll cycle (Müller-Moulé et al., 2002; Tietz et al., 2017). *NPQ* as measured in this study does not represent the total capacity for non-photochemical quenching of the samples, but it is rather a measure of the instantaneous level of this process depending on the light incident on the sample surface, light absorptivity, energy pressure on the photosystems and photo-protective potential (i.e., xanthophyll pool size). The Φ_{PSII} calculated was also an instantaneous measurement of the operational efficiency of the *PSII* photochemistry, and as such has little to do with the potential capacity for photo protection. In this study, young DGL leaves exhibited significantly greater *NPQ* levels than mature green leaves from DGL plants, but displayed no significant differences from GL plant species (**Figure 4C**). This suggests that *NPQ* could provide photo protection for red-colored young leaves to some extent. The young DGL leaves exhibited significantly lower Φ_{PSII} efficiency (0.74) than did GL leaves (**Figure 4B**). This result was closely associated with lower efficiency of light energy utilization, and suggests that the photosynthetic apparatus of young DGL leaves was not damaged by normal photosynthesis and that red leaves can most probably dissipate excess light energy through other mechanisms.

In addition to their role in *NPQ*, anthocyanins may also be able to function as ideal photo-protective agents because of their spectral absorption characteristics and anti-oxidative properties (Hughes et al., 2005; Zhu et al., 2018) although this is still in dispute. *NPQ* and anthocyanins are likely to be two important tools that can provide photo-protective function in plant leaves through different mechanisms. Anthocyanins-mediated photo-protection always occurs at species-specific developmental stages (Zhu et al., 2018). Combined with the high anthocyanin concentrations of young red leaves, anthocyanins may function as light attenuators and ameliorate the effects of excess light energy. However, the lowest F_v/F_m values observed at young DGL leaves in this study showed that photo-inhibition to some extent occurred. Thus, the high concentrations of anthocyanin in young DGL leaves might not have compensated for the insufficient photo-protection mediated by *NPQ* in these leaves but may enhance the fitness of young plants in tropical rainforests against herbivory damage.

CONCLUSION

In this interspecific, ecophysiological study, the occurrence of transient reddening of juvenile leaves in the tropics was coupled with increased levels of both anthocyanins and tannins. These two classes of compound were found to co-existed in transiently red young leaves and may have an intrinsic quantitative correlation mediated through POD enzyme activities.

Young leaves that flush red in tropical rainforest plants enhance plant defense and increase plant fitness. Moreover, the occurrence of red coloration in young leaves has arisen many times independently and is present in a variety of unrelated families (**Figure 5**). This indicates that red coloration in young leaves is predominantly a result of adaptation to special tropical environmental conditions but without significant intrinsic phylogenetic relationship of plant species. Our findings suggest that the red coloration of young leaves, which contain high concentrations of tannin and anthocyanin, mainly functions as an anti-herbivore defense strategy with chemical components. Furthermore, anthocyanins, which are principally responsible for the redness of young leaves, might not function as light attenuators to compensate for insufficient photo-protection mediated by *NPQ* because of the lowest F_v/F_m values, but may enhance the fitness of young plants in tropical rainforests to defense herbivory damage.

DATA AVAILABILITY STATEMENT

All datasets for this study are included in the article/**Supplementary Material**.

AUTHOR CONTRIBUTIONS

W-CG and L-ZM planned and designed the research. W-CG, Y-HL, C-MW, Y-QC, and L-ZM collected the data. W-CG and L-ZM contributed to data compilation. W-CG analyzed the data with support from L-ZM and C-M-W. W-CG, KM, and L-ZM wrote the manuscript with contributions from C-MW. All authors gave final approval for publication.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2020.00083/full#supplementary-material

SUPPLEMENTARY TABLE 1 | Name list of plant species used in analysis of photosynthesis physiology and defense chemical content.

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Trade-Offs Among Aboveground, Belowground, and Soil Organic Carbon Stocks Along Altitudinal Gradients in Andean Tropical Montane Forests

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Tropical montane forests (TMFs) play an important role as a carbon reservoir at a global scale. However, there is a lack of a comprehensive understanding on the variation in carbon storage across TMF compartments [namely aboveground biomass (AGB), belowground biomass (BGB), and soil organic matter] along altitudinal and environmental gradients and their potential trade-offs. This study aims to: 1) understand how carbon stocks vary along altitudinal gradients in Andean TMFs, and; 2) determine the influence of climate, particularly precipitation seasonality, on the distribution of carbon stocks across different forest compartments. The study was conducted in sixty 0.1 ha plots along two altitudinal gradients at the Podocarpus National Park (Ecuador) and Río Abiseo National Park (Peru). At each plot, we calculated the amount of carbon in AGB (i.e. aboveground carbon stock, AGC), BGB (i.e. belowground carbon stock, BGC), and soil organic matter (i.e. soil organic carbon stock, SOC). The mean total carbon stock was 244.76 ± 80.38 Mg ha^{-1} and 211.51 ± 46.95 Mg ha^{-1} in the Ecuadorian and Peruvian plots, respectively. Although AGC, BGC, and SOC showed different partitioning patterns along the altitudinal gradient both in Ecuador and Peru, total carbon stock did not change with altitude in either site. The combination of annual mean temperature and precipitation seasonality explained differences in the observed patterns of carbon stocks across forest compartments between the two sites. This study suggests that the greater precipitation seasonality of colder, higher altitudes may promote faster turnover rates of organic matter and nutrients and, consequently, less accumulation of SOC but greater AGC and BGC, compared to those sites with lesser precipitation seasonality. Our results demonstrate the capacity of TMFs to store substantial amounts of carbon and suggest the existence of a

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trade-off in carbon stocks among forest compartments, which could be partly driven by differences in precipitation seasonality, especially under the colder temperatures of high altitudes.

Keywords: aboveground biomass, allometric equations, Andes, belowground biomass, climatic gradients, precipitation seasonality, soil organic carbon

INTRODUCTION

Tropical forests are the most important terrestrial carbon sink (Pan et al., 2011). During the last century, however, both the increase in temperatures and the loss of tropical forest cover may have diminished their effectiveness in mitigating the effect of climate change (Gibbs et al., 2007; Saatchi et al., 2011; Liu et al., 2017; Mitchard, 2018). In this context, it is paramount to make accurate estimations of the carbon content of tropical forests. Because programs such as REDD+ (Reducing Emissions from Deforestation and Forest Degradation; http://theredddesk.org/) are intended to provide economic rewards for developing countries that reduce their carbon emissions (Saatchi et al., 2011; Sills et al., 2017), knowing the amount of carbon stored in ecosystems is also key in political and economic terms—not just ecological—when designing effective policies.

Tropical montane forests (TMFs) in the Andes range across broad gradients, both altitudinal (typically from *ca*. 1000 to over 3600 m; Spracklen and Righelato, 2014) and environmental, making them unique for understanding the influence of climate on carbon stocks (Malhi et al., 2010). They are important by providing ecosystem services and as biodiversity hotspots (Bruijnzeel et al., 2011), but in comparison to moist lowland tropical forests, their role as a carbon sink is still poorly understood (e.g. Baker et al., 2004; Gibbs et al., 2007; Malhi et al., 2009; Malhi et al., 2010; Paulick et al., 2017). So far we know that TMFs can store substantially more aboveground biomass (AGB) per unit area than previously believed (global AGB average of 271 Mg ha^{-1}), although their contribution to AGB per unit area is lesser than that of lowland tropical forests (global AGB average of 423 Mg ha^{-1}; Spracklen and Righelato, 2014). Alternatively, TMFs could be important for the storage of carbon as belowground biomass (BGB, Leuschner et al., 2007; Girardin et al., 2010) and as soil organic matter (Raich et al., 2006; Leuschner et al., 2007; Leuschner et al., 2013). This fact can be particularly relevant at higher altitudes, where hydromorphic processes (i.e. water saturation in the soil associated under conditions of reduction) can prevail over podsolisation processes (i.e. downward migration of aluminium, iron and organic matter, and their accumulation in lower layers), thus resulting in lower rates of organic matter decomposition (Schawe et al., 2007) and larger concentration of soil organic carbon (SOC).

In contrast to SOC, aboveground (AGC) and belowground carbon (BGC) are expected to decline with altitude (Kitayama and Aiba, 2002; Raich et al., 2006; Girardin et al., 2010; Girardin et al., 2013; Phillips et al., 2019). However, other patterns have been also reported for the relationship between AGC and altitude: positive monotonic (Tashi et al., 2016), unimodal (Weaver and Murphy, 1990; Lieberman et al., 1996; Raich et al., 1997; Moser et al., 2008; Alves et al., 2010; Larjavaara and Muller-Landau, 2012; Marshall et al., 2012; Ensslin et al., 2015; Phillips et al., 2019), bimodal (Venter et al., 2017), or null (i.e. no relationship; Culmsee et al., 2010; Unger et al., 2012; Peña and Duque, 2013; Peña et al., 2018). There are fewer studies investigating changes in SOC along altitudinal gradients in TMFs. In addition to those reporting an increase of SOC with altitude (Townsend et al., 1995; Schrumpf et al., 2001; Kitayama and Aiba, 2002; Raich et al., 2006; Graefe et al., 2008; Girardin et al., 2010; Moser et al., 2011; Dieleman et al., 2013), some studies report no change at all (Soethe et al., 2007; Zimmermann et al., 2010; Phillips et al., 2019). Few studies have quantified carbon stocks in different TMF compartments (AGB, BGB, soil organic matter) simultaneously or total carbon stocks (Girardin et al., 2010; Phillips et al., 2019).

This study aims to fill some of the existing gaps of knowledge by investigating the role of Andean TMFs as carbon reservoirs. The specific goals were: (1) to understand how carbon stocks from the different forest compartments (AGB, BGB, and soil organic matter) and total carbon stock vary along the altitudinal gradient; and (2) to determine the influence of climate—particularly temperature and precipitation seasonality—on carbon stocks. Understanding these carbon trade-offs should be a priority when formulating policies and designing conservation and management plans aimed at mitigating consequences of environmental change (Mathez-Stiefel et al., 2017).

MATERIAL AND METHODS

Study Area and Climatic Characterization

The study was carried out along two altitudinal gradients of well preserved TMFs: Podocarpus National Park (Ecuador) and Río Abiseo National Park (Peru). These sites extend along wide altitudinal ranges (*ca*. 2000 m) of continuous forest cover, each within a single river basin: the Bombuscaro river in Ecuador and the Montecristo–Abiseo rivers in Peru. Three altitudinal belts were studied in each site: low (800–1,100 m), middle (1,900–2,100 m), and high (2,700–2,900 m). Sixty 0.1 ha (50 × 20 m) plots were established between 2015 and 2017: 10 plots within each belt at each site, at least 300 m apart (coordinates in **Appendix S1**) and avoiding natural disturbances (e.g., tree-fall gaps or landslides). A detailed description of the study area can be found in Bañares-de-Dios et al. (2020).

We retrieved bioclimatic variables from the CHELSA climatological dataset (Karger et al., 2017) for each gradient and selected two variables representing the main axis of climatic variability in our study sites: annual mean temperature (°C) and precipitation seasonality (%). The first was selected since TMFs extend along a broad thermal range (*ca.* 9 and 12 °C in Ecuador and Peru, respectively). Annual mean temperature was highly correlated with the rest of temperature-related bioclimatic variables (Pearson's correlation, r > 0.8). Precipitation seasonality was selected because it can have an important effect on soil mineralization rates and nutrient availability for plants, even though moist TMFs are not subjected to long periods of water deficit and thus its vegetation does not display adaptations to such conditions, like deciduousness. Precipitation seasonality was calculated as the standard deviation of the monthly precipitation estimates expressed as a percentage of the mean of those estimates (i.e. the annual mean). Thus, a higher value of precipitation seasonality means that the total monthly precipitation is more heterogeneously distributed across time. For instance, 50% of precipitation seasonality means that this variability represents 50% of the mean monthly precipitation. Because of the properties of the normal distribution, this implies that differences between the rainiest and driest months could be—with 95% confidence interval—as high as twice (i.e. 200%) the mean monthly precipitation value, whereas if precipitation seasonality were 10% this difference would be only of 40% of the mean monthly precipitation value.

Field Sampling, Floristic, and Functional Data

In each plot, all standing woody stems (including trees, palms, tree ferns and lianas) ≥ 2.5 cm in diameter at breast height (DBH, at 130 cm from the ground) were recorded. For each stem, height was estimated visually since the use of precise instruments proved impractical. For individuals with multiple stems, we used the height of the tallest stem as the individual's height and the square root of the sum of the squares of DBH of each stem as the overall individual's DBH (Arellano et al., 2016). Field measurements were taken by multiple surveyors, who were trained at the beginning of each field campaign. Tree branch samples were collected for taxonomic determination and to estimate wood density. In total, 19,127 individuals were inventoried excluding tree ferns: 9,847 in Ecuador and 9,280 in Peru. Vouchers were identified at regional herbaria. In total, 826 different taxa were recognized from the Ecuadorian plots and 543 taxa from the Peruvian plots. The most abundant families in Ecuador were Melastomataceae (12.1% of total individuals), Lauraceae (10.8%), and Rubiaceae (9.0%) at low altitudes; Rubiaceae (16.5%), Melastomataceae (14.0%), and Lauraceae (12.7%) at mid altitudes; and Melastomataceae (21.5%), Cunoniaceae (16.8%), and Primulaceae (10%) at high altitudes. In Peru, the most abundant families were Leguminosae (8.9%), Rubiaceae (8.2%), and Malvaceae (8.2%) at low altitudes; Piperaceae (21.6%), Lauraceae (9.5%) and Rubiaceae (8.3%) at mid altitudes; and Rubiaceae (16%), Piperaceae (15.7%), and Chlorantaceae (14.6%) at high altitudes.

Branch wood density (WD) was measured based on Cornelissen et al. (2003) and used as a proxy for stem wood density, since both are strongly and positively correlated (Swenson and Enquist, 2008). Sections of branches (as cylindrical as possible) of *ca.* 10 cm long were stripped of cortex, and their volumes calculated as cylinders by measuring their diameter and length fresh. Branch wood density was calculated dividing fresh volume by dry mass after drying at 80°C for 48–72 h. Mean WD and its standard deviation (SD) were calculated for each species using sample values of all conspecifics. For species with just one sample, SD was estimated by multiplying WD by the SD/mean ratio averaged across all the samples in the entire dataset, where SD/mean $\text{ratio}_{\text{Ecuador}} = 0.200$ and SD/mean $\text{ratio}_{\text{Peru}} = 0.234$. Individuals for which WD values were lacking (e.g. emergent tall trees with inaccessible branches or lost samples) were assigned the mean WD of their family and their SD estimated as above. The latter included 611 individuals in 57 taxa from Ecuador (6.2% of the total individuals) and 735 in 59 taxa from Peru (8.5%). Finally, the 63 individuals from Ecuador (0.6% of the total individuals) and 117 from Peru (1.3% of the total individuals) with no WD measurement that could not be identified to family or belonged to families with no WD data (Cyclanthaceae, Dioscoreaceae, and Icacinaceae in Ecuador; Arecaceae and Humiriaceae in Peru), were removed from the analyses.

Carbon Stocks

We used Chave's three-variable pantropical allometry model (Chave et al., 2014) to estimate aboveground biomass (AGB) for each individual tree:

$$AGB = 0.0673 \cdot \left(WD \cdot DBH^{2} \cdot H\right)^{0.976}$$

where *WD* wood density ($g{\cdot}cm^{-3}$), *DBH* diameter at breast height (cm), and *H* is height (m). We then calculated the plot AGB as the sum of the AGB of all trees within each plot. We used the R package "BIOMASS" (Réjou-Méchain et al., 2017) to calculate the uncertainty associated to the estimation of AGB at the tree level, both as a result of measurement error and intraspecific variability in the case of WD. For the estimation of height and DBH, we assumed that the error was normally distributed, with the SD being 5% of the estimated value, thus reflecting that larger trees were likely to produce larger errors in the estimation of height and DBH. For WD, the error followed a normal distribution parametrized by the mean WD and its SD. Then the measurement errors of H, WD and DBH were included into Chave's equation using a Bayesian inference procedure (1000 iterations; Chave et al., 2014). The AGB of lianas was calculated separately through the following equation (Schnitzer et al., 2006):

$$AGB = e^{-1.484+2.657 \cdot ln(DBH)}$$

and we added it to each plot's estimated AGB.

Belowground biomass (BGB) was estimated using Kachamba et al. (2016)'s equation:

$$BGB = 0.285 \cdot DBH^{1.993}$$

We assumed that the aboveground carbon stock (AGC) of a plot accounted for the 50% of its AGB, following Chave et al. (2005).

We replicated this assumption for belowground carbon stock (BGC) from BGB.

Soil samples were collected from the surface (0-15 cm) below the decomposing organic layer (e.g., foliage, small twigs, fruits, seeds) in each plot. Soil samples, consisting of five different subsamples from five different points in the plot, were collected, mixed, air-dried and sifted through a 2-mm sieve (Arellano et al., 2016). Soil pH was determined in 1:2.5 pH-deionized water, soil texture was determined by the Bouyoucos hydrometer method (Bouyoucos, 1962), and total C and N concentrations were measured through dry combustion using a LECO CHNS-932 elemental auto-analyzer. Bulk density was determined by core sampling at three points of each plot. Soil samples were dried at 105 °C for 24 h. Total oven-dry mass of the soil samples were weighted, and then the coarse fraction (>2mm) was separated and weighted to determine the gravimetric coarse fraction content. The plant residue (mainly coarse roots) included in the coarse fraction (>2 mm) was weighted separately in order to determine the percentage present in the whole sample. Bulk density (BD) was calculated as $Mt/\sum_{i=1}^{n} Vi$ where Mt is the total oven-dry mass of the three cores (g), and Vi the volume of each core i (cm^3). Finally, the soil organic carbon stock (SOC) (Mg ha^{-1}) was calculated following Schrumpf et al. (2011):

$$SOC = \frac{C \cdot BD \cdot LTH \cdot FE\ content}{10}$$

where C is soil organic carbon concentration (g kg^{-1}) in the soil layer, BD bulk density (g cm^{-3}), LTH layer thickness (cm), and $FE\ content$ the relative contribution of fine earth fraction (i.e. all soil particles smaller than 2 mm, thus excluding gravel, stones and coarse plant residue) to total soil mass. Despite important variations in BD across sites and altitudinal ranges (see **Appendix S3**) that make seemingly reasonable to correct SOC estimates by an equivalent soil mass, SOC has been historically quantified to a fixed depth. This method has been employed in the vast majority of publications comparing SOC between treatments or over time periods (Wendt and Hauser, 2013), it is designated as good practice by the Intergovernmental Panel on Climate Change (IPCC, 2003), and has been subsequently used in protocols of global importance to assess SOC, such as that of the European Joint Research Centre (JRC-EU; Stolbovoy et al., 2007).

Relation Between Carbon Stocks, Altitude, and Climate

Generalized linear models (GLMs) with a Gamma error distribution were fitted to determine how carbon stocks vary in the different compartments of the forest and along the altitudinal gradient separately for Ecuador and Peru. Quadratic terms of the explanatory variables were included to account for non-linear relationships. To understand the role of climate on the carbon stocks of the different compartments, we fitted another set of GLMs with a Gamma error distribution to relate each of the three carbon stock response variables, as well as total carbon stock, to annual mean temperature (°C) and precipitation seasonality, as well as their interaction, using data from both sites. The significance of predictors was tested using the Chi-squared statistic ($\alpha \leq 0.05$), and the explained deviance (D^2) was used to assess the goodness of fit of the model:

$$D^2 = \frac{(nulldeviance - residualdeviance)}{nulldeviance}$$

All analyses were conducted with the R environment (R Development R Core Team, 2018).

RESULTS

Climatic Characteristics and Soil Properties

Annual mean temperature and temperature seasonality decreased consistently as altitude increased at both sites. Temperature range was broader in the Peruvian (11.5–23.9 °C annual mean) than in the Ecuadorian site (12.2–21.1 °C annual mean) (**Appendix S2**). Patterns of annual precipitation differed between sites: while in the Ecuadorian site precipitation increased with altitude (from 957 to 1,614 mm/year), in the Peruvian site it ranged from 1,019 to 2,007 mm/year, and was lowest at the mid altitudinal belt (**Appendix S2**). The range of precipitation seasonality in Ecuador was much narrower (24.3–32.9%) than in Peru (31.8–50.8%), where it increased from low to middle and high altitudes (**Appendix S2**).

Soil pH ranged from 2.31 to 5.98 in Ecuador and from 3.64 to 6.83 in Peru. Soils from Ecuador showed consistently lower pH values and higher organic carbon concentration, C/N ratio, and bulk density than those from Peru at each altitudinal belt (**Appendix S3**). There was an overall decrease in pH and an increase in organic carbon concentration and C/N ratio with altitude (**Appendix S3**). Soils from Ecuadorian plots showed a similar sandy loam texture along the altitudinal gradient, while in the Peruvian plots soil texture ranged from loam, at low altitude, to sandy loam at middle altitude, and loamy sand at high altitude (**Appendix S3**). In both sites, bulk density gradually decreased with altitude. Soils from Ecuador showed consistently lower pH values and higher organic carbon concentration, C/N ratio, and bulk density than those from Peru at each altitudinal belt (**Appendix S3**).

Carbon Stocks Along the Altitudinal Gradient

Mean diameter at breast height (DBH) and tree height increased in Ecuador from low to mid altitudes and then decreased again at high altitude, whereas in Peru these values did not change much across altitudinal belts (**Appendix S4**). Wood density remained mostly constant across altitudinal belts in Ecuador, with a slight average increase at higher altitude, whereas in Peru average wood density values decreased monotonically with altitude (**Appendix S4**).

AGC ranged from 16.82 to 222.07 Mg ha^{-1} among Ecuadorian plots, and from 37.14 to 160.17 Mg ha^{-1} among Peruvian plots (**Figure 1**). BGC ranged from 26.79 to 126.65 Mg ha^{-1} in Ecuador, and from 46.62 to 119.94 Mg ha^{-1} in Peru. SOC ranged from 26.65 to 268.09 Mg ha^{-1} in Ecuador, and from 12.87 to 100.38 Mg ha^{-1} in Peru. Total carbon stock ranged from 86.85

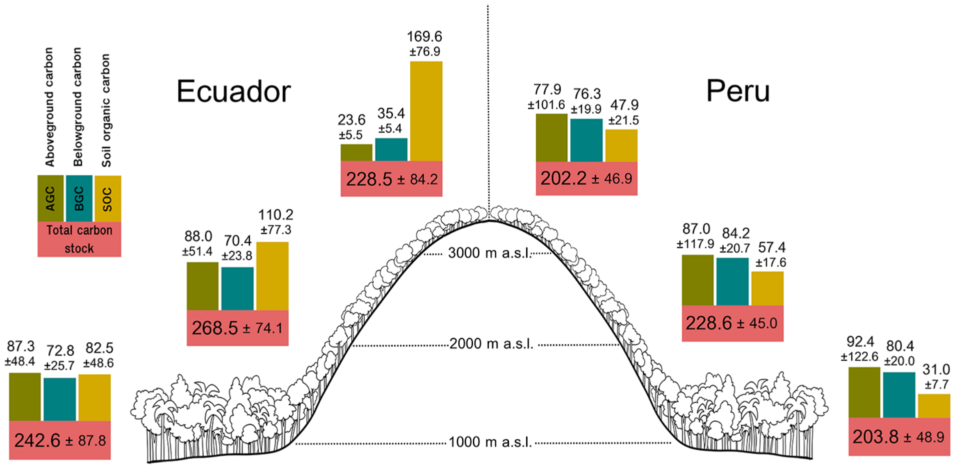

FIGURE 1 | Carbon stocks (mean ± sd) for each forest compartment (above and belowground biomass, and soil organic carbon) and total carbon stock per altitudinal belt (drawing by María Medel).

to 406.35 Mg ha^{-1} in Ecuador (mean value 244,76 ± 48.40 Mg ha^{-1}), and from 116.73 to 318.52 Mg ha^{-1} in Peru (mean value 211.51 ± 80.38 Mg ha^{-1}). The mean total carbon stock found across all study sites was 229.02 ± 68.06 Mg ha^{-1}. All plot-level estimations of AGC, BGC, and SOC are available in **Appendix S1**.

The largest mean AGC was found at low (87.34 ± 48.40 Mg ha^{-1}) and middle altitudes (88.03 ± 51.39 Mg ha^{-1}) from Ecuador, and decreased by *ca.* 73.2% at high altitude (23.56 ± 5.47 Mg ha^{-1}; **Figure 1**); whereas in Peru, the greatest mean AGC was found at low altitude (92.44 ± 122.62 Mg ha^{-1}), decreasing by *ca.* 6% at mid altitude (86.97 ± 117.86 Mg ha^{-1}) and *ca.* 15.7% at high altitude (77.90 ± 101.56 Mg ha^{-1}; **Figure 1**). In Ecuador, the best model for aboveground carbon (AGC) contained the quadratic term of altitude (**Table 1A**), and the predicted response curve revealed a unimodal relationship, reaching a maximum estimated AGC at *ca.* 1500 *m* (**Figure 2A**). In Peru, however, AGC did not show any relationship with altitude (**Figure 2B**, **Table 1A**).

Regarding BGC, the largest mean value in Ecuador was found at low (72.75 ± 25.74 Mg ha^{-1}); and middle altitudes (70.37 ± 23.85 Mg ha^{-1}) and decreased by 49.7% at high altitude (35.38 ± 5.43 Mg ha^{-1}; **Figure 1**); whereas in Peru, the largest mean BGC was found at middle altitude (84.21 ± 20.66 Mg ha^{-1}; **Figure 1**). Overall, BGC followed the same relationship with altitude as AGC, both in Ecuador and Peru (**Figures 2C, D**, respectively; **Table 1**).

At each altitude, SOC in Ecuadorian plots were in general higher than in Peruvian plots. In Ecuador, the largest mean SOC

TABLE 1 | Analysis of deviance tables for the generalized linear models testing the effect of altitude (linear and quadratic terms) on different carbon stocks, namely aboveground carbon (AGC) in Mg/ha, belowground carbon (BGC) in Mg/ha, soil organic carbon (SOC) in Mg/ha, and total carbon stocks, in a) Ecuador and b) Peru, respectively.

Response variable	Term	Deviance	d.f.	p-value	D^2	Estimate	Std. Error
a) Ecuador							
AGC	**Linear**	**4.700**	**1**	**<0.001**	**0.333**	**$-7.757\ 10^{-5}$**	**$1.356\ 10^{-5}$**
	Quadratic	**6.220**	**1**	**<0.001**	**0.441**	**$2.481\ 10^{-8}$**	**$4.014\ 10^{-9}$**
BGC	**Linear**	**1.947**	**1**	**<0.001**	**0.381**	**$-3.683\ 10^{-5}$**	**$8.902\ 10^{-6}$**
	Quadratic	**1.524**	**1**	**<0.001**	**0.298**	**$1.174\ 10^{-8}$**	**$2.436\ 10^{-9}$**
SOC	**Linear**	**2.400**	**1**	**0.008**	**0.192**	**$-8.584\ 10^{-6}$**	**$1.137\ 10^{-5}$**
	Quadratic	0.682	1	0.654	0.005	$1.237\ 10^{-9}$	$2.746\ 10^{-9}$
Total carbon stock	Linear	0.025	1	0.627	0.007	$-4.980\ 10^{-6}$	$2.711\ 10^{-6}$
	Quadratic	0.385	1	0.058	0.110	$1.330\ 10^{-9}$	$6.943\ 10^{-10}$
b) Peru							
AGC	Linear	0.075	1	0.462	0.022	$1.472\ 10^{-6}$	$7.106\ 10^{-6}$
	Quadratic	0.002	1	0.915	0.000	$-2.117\ 10^{-10}$	$1.977\ 10^{-9}$
BGC	Linear	0.002	1	0.852	0.001	$-2.432\ 10^{-6}$	$5.076\ 10^{-6}$
	Quadratic	0.017	1	0.611	0.010	$7.167\ 10^{-10}$	$1.405\ 10^{-9}$
SOC	**Linear**	**1.031**	**1**	**0.004**	**0.180**	**$-3.739\ 10^{-5}$**	**$1.222\ 10^{-5}$**
	Quadratic	**0.849**	**1**	**0.008**	**0.148**	**$8.573\ 10^{-9}$**	**$3.222\ 10^{-9}$**
Total carbon stock	Linear	0.007	1	0.717	0.005	$-1.647\ 10^{-6}$	$1.711\ 10^{-6}$
	Quadratic	0.043	1	0.360	0.030	$4.334\ 10^{-10}$	$4.716\ 10^{-10}$

Deviance, degrees of freedom (d.f.), p-values and explained deviance (D^2) is shown for each term in the models. The estimated coefficients and their standard errors are also shown. Statistically significant terms (p-value ≤ 0.05) are highlighted in bold.

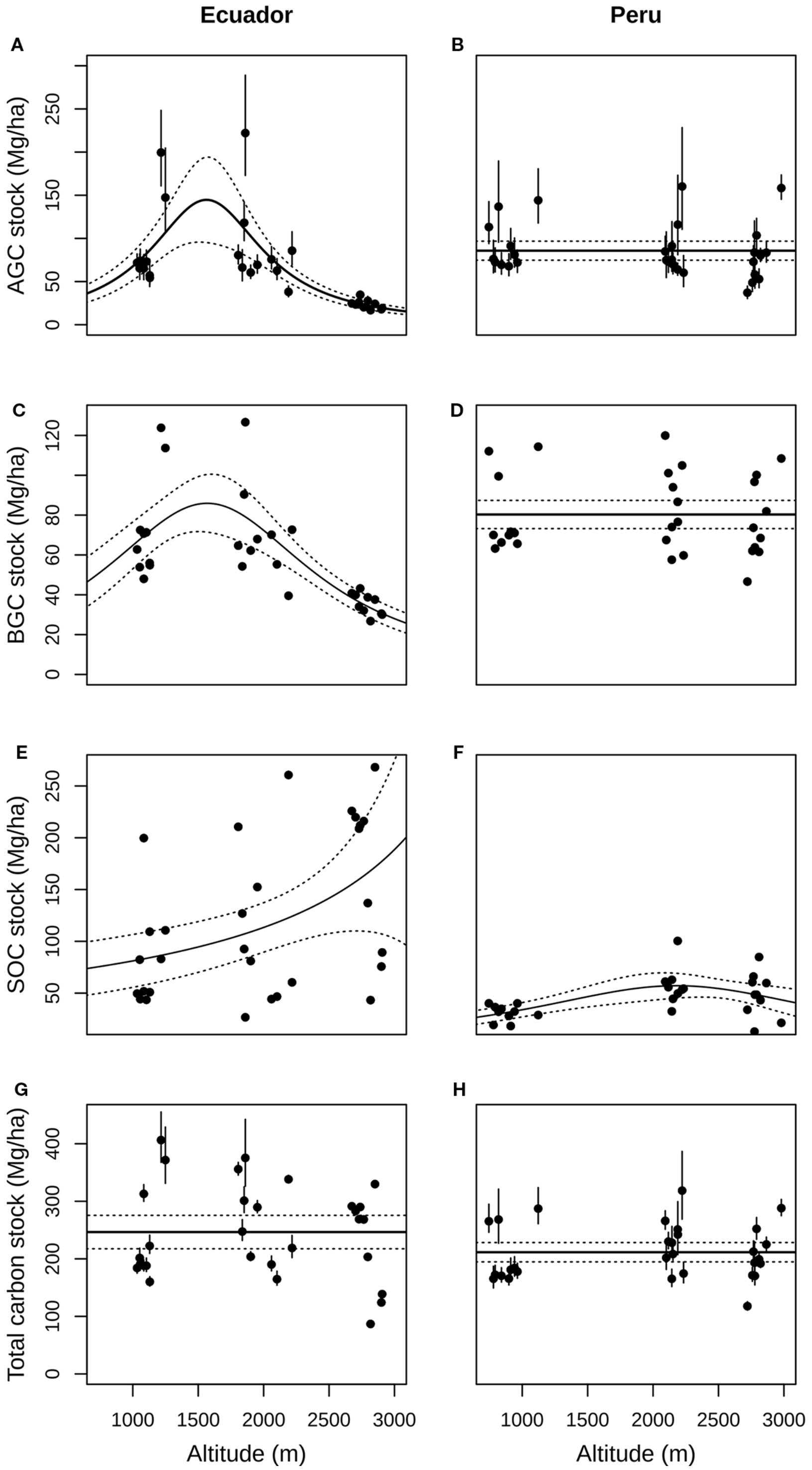

FIGURE 2 | Relationship between aboveground **(A, B)**, belowground **(C, D)**, soil organic **(E, F)** and total carbon stocks **(G, H)** and altitude (m a.s.l), both in Ecuador (left charts) and Peru (right charts), with its 95% confidence intervals (dotted lines). AGC, Aboveground carbon; BGC, Belowground carbon; SOC, Soil organic carbon.

was found at high (169.6 ± 76.9 Mg ha^{-1}) and middle altitudes (1,10.2 ± 77.3 Mg ha^{-1}) and the lowest mean SOC at low altitude (82.5 ± 48.6 Mg ha^{-1}; **Figure 1**). Nevertheless, in Peru, the largest mean SOC was found at middle altitude (57.4 ± 17.6 Mg ha^{-1}), though the differences across altitudinal belts were not as large as in Ecuador (**Figure 1**). SOC increased with altitude in Ecuador, while in Peru slightly increased from low to middle altitude and then stabilizing from middle to high altitude, with the relationship being quadratic (**Figures 2E, F**; **Table 1**).

The resulting total carbon stock (the sum of AGC, BGC and SOC) did not vary with altitude, neither in Ecuador nor in Peru (**Figures 2G, H**, respectively; **Table 1**).

Carbon Stocks and Climate

Annual mean temperature and precipitation seasonality, but not their interaction, had a statistical significant effect on AGC, BGC, and SOC. There was, however, no effect of either climate variable or their interaction on total carbon stocks (**Table 2**). Precipitation seasonality had a positive effect on both AGC and BGC at low (i.e. warm temperatures, represented by red lines in **Figures 3A, B**) and at high altitudes (i.e. cold temperatures, represented by blue lines in **Figures 3A, B**). Temperature also had a positive effect on both AGC and BGC. Whereas the effect of precipitation seasonality on SOC was almost negligible when annual mean temperature was warm, there was a very marked negative effect when cold (**Figure 3C**). Finally, the total carbon stock did not show any difference with precipitation seasonality, either under warm or cold annual mean temperature (**Figure 3D**).

DISCUSSION

Our results show that carbon storage at each compartment of the forest (AGC, BGC, and SOC) follows a distinct pattern along the altitudinal gradient that differs between sites.

Although AGC is expected to decline with altitude as a result of lower-statured trees (Kitayama and Aiba, 2002; Raich et al., 2006; Girardin et al., 2010; Girardin et al., 2013), in Ecuador our model shows maximum AGC at middle altitude (*ca.* 1500 m). This result is similar to the unimodal patterns reported in other studies (Weaver and Murphy, 1990; Lieberman et al., 1996; Raich et al., 1997; Moser et al., 2008; Alves et al., 2010; Larjavaara and Muller-Landau, 2012; Marshall et al., 2012; Ensslin et al., 2015; Phillips et al., 2019), and it has been suggested to be caused by a complex combination of factors that occur at mid altitudes, including optimal balance of respiration (with respiration costs being lower at mid altitudes) and photosynthesis (which is not yet inhibited by low air temperatures) and being less prone to disturbance compared to low and high altitudes (Marshall et al., 2012). Conversely, in Peru there was no relationship between AGC and altitude. No change of AGC with altitude has been also reported in other studies, whether caused by the dominance of Fagaceae that can reach very large sizes at high altitudes (Culmsee et al., 2010; Peña et al., 2018), or by increased soil fertility with altitude, which would have a positive effect on plant growth compared to poorer lowland soils, thus compensating the negative effect that lower temperatures have on AGC (Unger et al., 2012). The latter is more likely to explain the lack of an AGC pattern with altitude seen in Peru.

The same pattern of variation of AGC with altitude was found for BGC in both sites. This is not surprising as the equations for estimating AGC and BGC both relied on tree DBH. In general, coarse-root biomass contains the largest fraction of BGC, even if carbon stored in fine-root biomass can, in relative terms, be particularly high in upper TMFs (up to 46.0% of total BGC; Girardin et al., 2010), probably as a consequence of limited soil nutrients available (Leuschner et al., 2007; Girardin et al., 2010). Thus, it seems reasonable to assume that coarse root biomass, and therefore BGC, will be positively correlated with AGC because taller trees (more abundant at lower TMFs) would invest more into coarse-root anchoring—particularly in windy or erosion-prone sites—whereas smaller trees (more abundant at upper TMFs) will not be able to develop a large deep root system (Girardin et al., 2010).

TABLE 2 | Analysis of deviance tables for the generalized linear models testing the effect of annual mean temperature (T, in °C), precipitation seasonality (PS, in %), and their interaction (T:PS) on different carbon stocks, namely aboveground carbon (AGC), belowground carbon (BGC), soil organic carbon (SOC), and total carbon stocks. Deviance, degrees of freedom (d.f.), p-values and explained deviance (D^2) is shown for each term in the models.

Response variable	Term	Deviance	d.f.	p-value	D^2	Estimate	Std. Error
AGC	**T**	**1.921**	**1**	**0.017**	**0.104**	**$-3.606\ 10^{-4}$**	**$1.574\ 10^{-4}$**
	PS	**2.231**	**1**	**0.010**	**0.121**	**$-1.524\ 10^{-3}$**	**$7.103\ 10^{-4}$**
	T:PS	0.954	1	0.093	0.052	$7.555\ 10^{-6}$	$4.438\ 10^{-3}$
BGC	**T**	**0.728**	**1**	**0.008**	**0.089**	**$-2.195\ 10^{-4}$**	**$9.217\ 10^{-5}$**
	PS	**2.285**	**1**	**<0.001**	**0.280**	**$-9.662\ 10^{-4}$**	**$4.158\ 10^{-4}$**
	T:PS	0.219	1	0.145	0.207	$3.884\ 10^{-6}$	$2.644\ 10^{-6}$
SOC	**T**	**4.463**	**1**	**<0.001**	**0.139**	**$2.037\ 10^{-4}$**	**$1.659\ 10^{-4}$**
	PS	**9.283**	**1**	**<0.001**	**0.290**	**$9.664\ 10^{-4}$**	**$8.058\ 10^{-4}$**
	T:PS	0.087	1	0.615	0.003	$-2.770\ 10^{-6}$	$5.547\ 10^{-6}$
Total carbon stock	T	0.000	1	0.997	0.000	$1.397\ 10^{-5}$	$2.501\ 10^{-5}$
	PS	0.124	1	0.223	0.023	$7.666\ 10^{-5}$	$1.165\ 10^{-4}$
	T:PS	0.018	1	0.646	0.003	$-3.549\ 10^{-7}$	$7.765\ 10^{-7}$

The estimated coefficients and their standard errors are also shown. Statistically significant terms (p-value ≤ 0.05) are highlighted in bold. Precipitation seasonality is calculated as the standard deviation of the monthly precipitation estimates expressed as a percentage of the mean of those estimates (i.e. the annual mean).

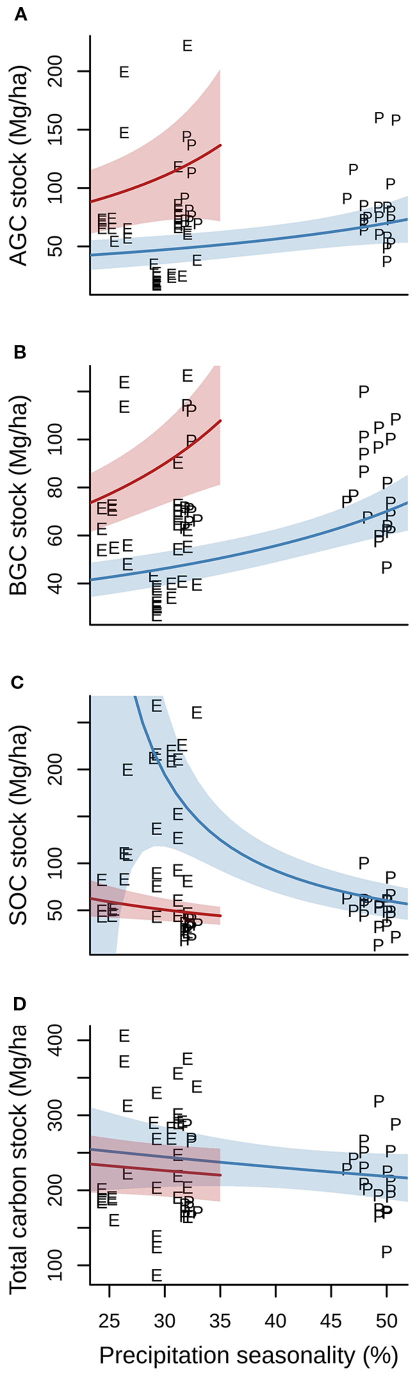

FIGURE 3 | Relationship between aboveground **(A)**, belowground **(B)**, soil organic **(C)** and total carbon stocks **(D)** and precipitation seasonality (%) both under warm (24°C annual mean temperature, red lines) and cold (12°C, blue lines) conditions. E, Ecuadorian plots; P, Peruvian plots. Precipitation seasonality is calculated as the standard deviation of the monthly precipitation estimates expressed as a percentage of the mean of those estimates (i.e. the annual mean). The range of predictions for increasing values of precipitation seasonality (x-axis) represent the observed values in our two study sites, with lower variation at low altitude (i.e. warm conditions) and higher variation at high altitude (i.e. cold conditions). Shaded areas represent 95% confidence intervals upon model predictions.

In general, our results confirm that TMFs soil properties such as acidity, soil organic matter accumulation and C/N ratio increase with altitude, as reported in prior studies (Grieve et al., 1990; Schawe et al., 2007; Schrumpf et al., 2011). Yet, there were differences in the patterns of SOC along the altitudinal gradient between the two sites. Upper TMFs in Ecuador can store large amounts of SOC compared to forests at low altitudes, which is in agreement with patterns reported in previous studies for TMFs (Townsend et al., 1995; Schrumpf et al., 2001; Kitayama and Aiba, 2002; Raich et al., 2006; Graefe et al., 2008; Girardin et al., 2010; Moser et al., 2011; Dieleman et al., 2013). Contrastingly, in Peru, SOC slightly increased from low to middle altitude and remained unchanged above. Similarly, Schawe et al. (2007) observed in Bolivian TMFs a continuous increase of SOC up to 2000–2400 m that remained constant above that altitude. The increase in SOC at higher altitude in the Ecuadorian gradient might be partly explained by an increased C/N ratio as a consequence of low temperature and high precipitation that may cause soil acidification and subsequent low biological activity. However, this reasoning is not valid for explaining the lower SOC at the high altitude TMF in Peru. It is interesting to note that, on average, upper TMF soils in Peru showed higher organic carbon concentration (**Appendix S3**), but proportionally lesser bulk density, which ultimately explains the decrease in SOC from middle to high altitude. Typically, soils with higher organic content have lesser bulk density, although the bulk density of organic horizons also depends on the degree of decomposition, the make-up of plant residue in the soil and the void ratio. A greater amount of fiber or coarse roots in the core soil create a more open structure that results in more voids and therefore lower bulk density. The coarse-root (>2mm in diameter) fraction present in soils at high altitude in Peru constitute a much higher percentage of total soil weight than those from Ecuador, which may partly explain the lower value of bulk density of soils from Peru at high altitude, and will ultimately have a great influence on the calculation of the carbon stock of these soils.

Overall, regardless of the individual patterns of AGC, BGC, or SOC along the altitudinal gradient, we did not observe any variation with altitude at either study site when carbon stocks from all compartments were combined. This pattern has been also found by other studies (Raich et al., 2006; Girardin et al., 2010; Selmants et al., 2014; Phillips et al., 2019). It is likely the result of a trade-off among carbon stocks stored at different forest compartments. The key question, thus, is why different carbon stocks (AGC, BGC, and SOC) show diverging patterns of change with altitude between sites, but when combined they result in similar total carbon stocks that remain constant across altitude. It has been suggested that the lack of response to altitude of total carbon stocks is the result of changes in the prevailing atmospheric temperatures, which would trigger opposite trends in AGC and SOC, thus equalising total carbon stocks along the altitudinal gradient (Selmants et al., 2014; Phillips et al., 2019). However, this does not explain the disparity in the patterns of AGC, BGC and SOC reported in various studies of TMFs. We believe that there are other climatic drivers that may be playing an important role in determining the observed trade-offs, allowing for different patterns of change of the carbon stocks stored in the individual forest compartments—as reported in a number of studies—while maintaining total carbon stocks in a plot constant across altitude. Our analyses suggest that at low altitudes, warmer temperatures may promote nutrient cycling and plant growth (Kitayama and Aiba, 2002; Girardin et al., 2010) that contribute to high AGC and BGC, but also result in low SOC values. At those low altitudes, little variation in precipitation seasonality was observed between Ecuador (average value of 32.2%) and Peru (average value of 25.5%; **Appendix S2**). Higher precipitation seasonality therefore contributed to higher AGC and BGC, but showed little effect on SOC. Conversely, at high altitudes we found different patterns of precipitation seasonality: whereas our forest plots in the upper belt in Ecuador showed similar values of precipitation seasonality to those found at low altitudes (average value 29.7%), these were much higher for our plots in Peru (average value 50.2%; **Appendix S2**). This fact could explain the differences in the observed patterns of carbon stock among forest compartments between Ecuador and Peru. At high altitude in Ecuador, low precipitation seasonality results in 1) an accumulation of organic matter in the soil that renders high SOC values (Schawe et al., 2007) and, since available nutrients subsequently become scarce (due to low nutrient cycling), 2) a decrease in plant growth, as shown by the low AGC and BGC values. Conversely, the higher precipitation seasonality at greater altitude in Peru allows for drier periods during which mineralisation rates increase. The latter may result in 1) less accumulation of organic matter in the soil—as indicated by the decrease in SOC values—that allows for a richer pool of nutrients available to plants, and thus 2) favor an increase in plant growth, as shown by greater AGC and BGC values. Yet, we acknowledge that our conclusions should be taken with caution, as biotic drivers (e.g. soil food-web, plant competition aboveground, etc.) and variables influencing soil dynamics (e.g. soil type, plant residue quality and quantity, amount and quality of soil organic matter, microbial activity and composition) are also likely to contribute significantly to carbon stocks.

Ultimately, TMFs can make an important contribution to global carbon stocks. Our estimates of AGC (80.42 Mg ha^{-1} on average) are somewhat lower than those reported for other Andean TMFs (106.04 Mg ha^{-1} on average; Spracklen and Righelato, 2014). This could be partly a consequence of using branch wood density as a proxy for stem wood density, which could result in lower AGB and AGC estimated values, particularly in species with very hard wood species and/or large individuals, that could ultimately account for an important portion of a community's biomass. There is, however, enormous variation among studies, with estimates ranging from 77.20 Mg ha^{-1} (Girardin et al., 2010) to 409.07 Mg ha^{-1} (Grimm and Fassbender, 1981), proving the need for further studies. In general terms, although evergreen tropical lowland forests can store larger AGC than Andean TMFs (e.g. Baker et al., 2004; Malhi et al., 2009; Spracklen and Righelato, 2014), the latter—especially those at higher altitudes—can store a substantial amount of carbon in the form of SOC (Raich et al., 2006), a component of the system that has been often neglected.

The mean total carbon stock found in our study sites (229.02 ± 68.06 Mg ha^{-1}) was within the low to middle range (87–754 Mg ha^{-1}) reported in a meta-analysis of TMFs by Raich et al. (2006), and similar to the results recently reported for Colombian TMFs (241.3 ± 37.5 Mg ha^{-1}; Phillips et al., 2019). Yet, according to our study, and considering that the extent of TMFs in the Neotropics has been recently estimated to be 750,000 km^2 (Kappelle and Brown, 2001), New World TMFs could store 16–19 10^9 Mg C, and up to 48–58 10^9 Mg C, for the estimated *ca*. 2.2 10^6 km^2 of TMF worldwide (Mulligan, 2010). Although there is considerable uncertainty in these predictions—as is often the case when it comes to standing biomass estimation—they can be used to illustrate how large amounts of carbon in TMFs from both biomass and soils, are as much at risk of emission through deforestation and land use change as tropical lowland forests, with the consequent dramatic impact on global climate.

Conclusions

Our study emphasizes the important role that TMF plays in storing carbon. It points out the existence of trade-offs among carbon stocks when partitioning the three TMFs compartments—AGB, BGB, and soil organic matter. The result is that plot overall carbon stock along the altitudinal gradient remains constant, although patterns of variation differed between sites when looking at individual components of the system. Precipitation seasonality may partly explain these differences, as the occurrence of drier periods in the year would increase mineralization rates, particularly at higher altitudes. This would result in less accumulation of organic matter in the soil and, consequently, an increase in plant growth and AGB. Such trade-offs reflect the potential of TMFs to store substantial amounts of carbon that contribute significantly to global carbon stocks.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/**Supplementary Material**.

AUTHOR CONTRIBUTIONS

VC, LC and MM conceptualized the research. GB-D-D, CE, IG-C and NS contributed to field sampling and data collection. VC conducted all soil analyses and estimations of soil carbon stocks. AL and LC-A conducted estimations of aboveground and belowground biomass and carbon stocks. LC-A, GB-D-D and LC performed all statistical analyses and drafted the manuscript. All authors reviewed and contributed to the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2020.00106/full#supplementary-material

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The Thermal Tolerances, Distributions, and Performances of Tropical Montane Tree Species

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Due to global warming, many species will face greater risks of thermal stress, which can lead to changes in performance, abundance, and/or geographic distributions. In plants, high temperatures above a species-specific critical thermal maximum will permanently damage photosystem II, leading to decreased electron transport rates, photosynthetic failure, and eventual leaf and plant death. Previous studies have shown that plant thermal tolerances vary with latitude, but little is known about how they change across smaller-scale thermal gradients (i.e., with elevation) or about how these thermal tolerances relate to species' local performances and geographic distributions. In this study, we assess the maximum photosynthetic thermal tolerances (T_{50}) of nearly 200 tropical tree species growing in 10 forest plots distributed across a >2,500 m elevation gradient (corresponding to a 17°C temperature gradient) in the northern Andes Mountains of Colombia. Using these data, we test the relationships between species' thermal tolerances and (1) plot elevations and temperatures, (2) species' large-scale geographic distributions, and (3) changes in species' abundances through time within the plots. We found that species' T_{50} do in fact decrease with plot elevation but significantly slower than the corresponding adiabatic lapse rate (-0.4 vs. $-5.7°C\ km^{-1}$) and that there remains a large amount of unexplained variation in the thermal tolerances of co-occurring tree species. There was only a very weak association between species' thermal tolerances and their large-scale geographic distributions and no significant relationships between species' thermal tolerances and their changes in relative abundance through time. A potential explanation for these results is that thermal tolerances are adaptations to extreme leaf temperatures that can be decoupled from regional air temperatures due to microclimatic variations and differences in the species' leaf thermoregulatory properties.

Keywords: climate change, tropical cloudforests, thermophilization, leaf temperatures, microclimate, leaf traits

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INTRODUCTION

With anthropogenic climate change driving rapid increases in global temperatures, many plant species will face greater risks of thermal stress (Saxe et al., 2001; Parmesan and Hanley, 2015). If severe enough, thermal stress can limit the performance of individuals and populations, eventually leading to the retraction of some species' ranges (or shifts in species' ranges if local extinctions are offset by simultaneous invasions into cooler areas) and local extinctions (Chen et al., 2011; Feeley, 2012; Lenoir and Svenning, 2015). These local extinctions can in turn lead to decreased diversity through biotic attrition (Colwell et al., 2008; Wiens, 2016), changes in community composition (Feeley et al., 2011, 2013; Duque et al., 2015; Fadrique et al., 2018) and potential changes in important ecosystem services such as carbon sequestration/storage (Clark et al., 2003; Brienen et al., 2015), regional climate regulation (Cox et al., 2000; Luo, 2007), and food production (Tito et al., 2018).

In plants, thermal stress can be caused by the increase in respiration and/or the decrease in photosynthesis due to decreased efficiency or damage to leaf metabolic processes. At high temperatures, photosystem II (PSII) can be permanently damaged leading to decreased electron transport rates, photosynthetic failure, and eventual leaf death (Baker, 2008). The temperature (generally 40-60°C) at which irrecoverable damage occurs to PSII, can be referred to as a plant's maximum physiological thermal tolerance. The heat tolerances of plants can vary markedly between species and previous studies have found that they are generally correlated with large-scale latitudinal gradients in temperature, such that plants growing in hot equatorial habitats (e.g., rainforests) have higher heat tolerances on average compared to plants from colder, higher latitude habitats (e.g., temperate or boreal forests) (O'sullivan et al., 2017; Zhu et al., 2018). However, the change in thermal tolerances across latitude is not as steep as the corresponding change in air temperature. This suggests that many tropical species may have reduced thermal safety margins (difference between temperatures and tolerances) compared to temperate species and thus will be at elevated risk of thermal stress due to global warming (Perez et al., 2016; O'sullivan et al., 2017).

A similar relationship between plant thermal tolerances and temperature should also exist along smaller-scale environmental gradients, for example across elevational gradients. In other words, plant species at lower, hotter elevations should have higher tolerances than species at higher, colder elevations (e.g., O'sullivan et al., 2013). However, this hypothesis remains almost entirely untested (but note that measurements of tree species in a single highland plot in Peru do show lower thermal tolerances than species in lowland forests at similar latitudes; O'sullivan et al., 2017). Consequently, we do not know if tropical plant species in general are at elevated risk of thermal stress or if highland tropical species have relatively greater thermal safety margins that can help protect them against the negative effects of rising temperatures. Likewise, it is unknown how leaf thermal tolerances relate to plant species' performances across large-scale spatial and temporal thermal gradients. For example, are the ranges of species with lower thermal tolerances spatially restricted to areas with lower regional temperatures? And, within specific sites, are species with higher thermal tolerances experiencing performance advantages, and hence increasing in their relative abundances compared to less-tolerant species, as temperatures rise under global climate change?

To answer these questions, we assess the thermal tolerances of >550 individuals of 164 tropical canopy tree species growing in 10 forest inventory plots, each 1-hecatare in area (= 189 species × site populations). Study plots are distributed across a steep elevation gradient ascending from near sea level to near treeline (∼3,000 m asl) in the northern Andes Mountains of Colombia (Duque et al., 2015; Peña et al., 2018; Agudelo et al., 2019). We analyze changes of plant thermal tolerances between elevations at the species and community level (intraspecific patterns were not analyzed but will be investigated in future studies). We conduct two sets of analyses to investigate the relationship between the thermal tolerances of species and their ability to tolerate high temperatures. In the first, we test the relationship between species' thermal tolerances and their observed geographic distributions in relation to regional-scale mean annual and maximum temperatures. In the second set of analyses, we test the relationship between species' thermal tolerances and their changes in abundance through time as recorded in each of the study plots. These analyses are used to test the hypotheses that (1) species with higher thermal tolerances will have geographic distributions including hotter areas than species with lower thermal tolerances, and that (2) due to rising temperatures, species with higher thermal tolerances will experience performance advantages over co-occurring species with lower thermal tolerances and thus will increase in relative abundance over time. Together these analyses provide valuable insight into the importance of temperature and thermal tolerances in setting current and future species distributions.

METHODS

Study Area

This study was conducted using data collected from 10 permanent forest inventory plots in the northwest Andes Mountains of Colombia, located between 5°50′ and 8°61′ N Latitude and 74°61′ and 77°33′ W Longitude. Plot elevations range from 192 to 2,880 m asl. The average total annual rainfall recorded in the plots varies from ∼ 1,750 to 3,500 mm year^{-1}, and mean annual temperature (MAT, °C) varies from 10 to 27°C (**Table 1**; **Figure 1**) with an adiabatic lapse rate of ∼5.7°C km^{-1} elevation. Mean Annual Temperatures in this area have been increasing steadily at the rate of approximately 1°C per century since the early 1900s (Rohde et al., 2013).

Plot Data

The study plots are all 1-ha in area and were initially established between 2006 and 2009. In the initial plot censuses, all trees, palms and tree ferns with stem diameter at breast height (DBH) ≥ 10 cm were tagged, mapped, measured, and identified. In all cases, the point of DBH measurement was painted on the stem to ensure that re-measurements in subsequent censuses were at the same stem location. The study plots were all recensused multiple

TABLE 1 | Location and climate of study plots.

Plot name	Latitude (°N)	Longitude (°W)	Elevation (m asl)	Total annual precipitation (mm)	Mean annual temperature (°C)	Mean max temp of warmest month (°C)
Puerto Triunfo	6.01	−74.61	192	2,250	27	32
Támesis	5.78	−75.67	550	1,750	26	28
Maceo	6.46	−74.79	1,024	2,250	24	27
Porce	6.78	−75.08	1,033	1,920	22	25
Anorí	6.99	−75.14	1,794	2,400	25	21
Angelópolis	5.78	−75.67	2,118	2,435	18	28
Ventanas	7.08	−75.48	2,120	3,500	16	19
Jardín	5.49	−75.90	2,550	2,274	15	18
Caicedo	6.38	−76.03	2,635	1,892	15	16
Belmira	6.61	−75.65	2,800	2,100	10	15

times. During each plot recensuses, DBH growth, individual recruitment, and mortality were recorded. In cases where the recorded diameter growth was either $<$-0.1 cm year^{-1} or larger than 7.5 cm year^{-1}, the DBH of the second census was adjusted to avoid growth values out of this range (Condit, 1998; Condit et al., 2004). The Angelópolis, Anorí and Belmira plots were each recensused three times (4 total censuses including initial) with the last census completed in 2017. The Caicedo, Jardín, Maceo, Porce, Puertro Triunfo, Támesis, and Ventanas plots were each recensused twice. In all censuses, all plants were identified to the lowest possible taxonomic level using voucher samples deposited at the University of Antioquia's Herbarium (HUA) in Medellin, Colombia.

Previous studies have indicated that these forests are undergoing a process of compositional change called "thermophilization," possibly due to rising temperatures (Duque et al., 2015). In thermophilization, species with distributions centered on lower, hotter elevations (i.e., more-thermophilic species) increase in relative abundance and species with distributions centered on higher, cooler elevations (i.e., less-thermophilic or cryophilic species) decrease in relative abundance through time. This pattern mirrors that observed at other neotropical montane sites (Feeley et al., 2011, 2013; Fadrique et al., 2018).

Study Species

Thermal tolerances were estimated for a total of 15 to 22 species in each of the 10 study plots (**Table S1**). Species were chosen to include the 10 most-abundant canopy tree species in each plot as well as an additional ∼10 species spanning a wide range of geographic-based thermal optima and observed changes in abundance through time (see below). To estimate thermal tolerances, we collected sun-exposed leaves from three mature individuals of each species. Leaf samples were obtained using specialized pruning equipment and/or professional tree climbers. Once collected, the leaf samples were placed immediately in plastic "ziplock" bags with moistened paper towels and transported in coolers to laboratories at the National University of Colombia in Medellin for testing. In total, we sampled 164 species. The majority of species were sampled from just one plot each; 21 species were sampled from two plots each and two species were each sampled from three plots (**Table S1**). Hereafter, all species by plot combinations are treated as "species."

Estimating Photosynthetic Thermal Tolerances

We estimated the maximum thermal tolerance (T_{50}) of each of the study species to be the temperature at which photosystem II suffers $\geq$50% irrecoverable damage as determined through measurements of leaf fluorescence (F_V/F_M) in leaf samples exposed to different temperature treatments. Methods for estimating T_{50} were based on standard protocols modified from Krause et al. (2010, 2013). Prior to tests, we first measured the initial status of each leaf based on their pre-treatment F_V/F_M. Leaves were dark adapted for ∼20 min. After dark adaptation, we measured initial fluorescence emission (F_0) and maximum total fluorescence (F_M) using an OS30p$^+$ handheld fluorometer (Opti-Science, Hudson, NH USA). F_V/F_M was then calculated as the ratio of maximum variable ($F_V = F_M$-F_0) to maximum total fluorescence. Subsequently, we cut 2 cm disks from each leaf (avoiding major veins). Leaf disks were placed in Miracloth fabric pouches (abaxial and adaxial leaf surfaces covered by three and one layer of fabric, respectively) to prevent anaerobiosis (Krause et al., 2010) and placed inside waterproof plastic bags. Air was removed from the bags and bags were completely submerged for 15 min in preheated circulating water baths maintained at set temperatures of 22.0, 40.0, 42.2, 44.5, 47.0, 49.3, 51.5, 53.2, and 56.5°C. During heat treatments, leaves were kept under dim light and thus were not likely exposed to adequate quantities of light needed to induce the production of violaxanthin and zeaxanthin, which can ameliorate damage at high temperatures and possibly lead to lower estimates of heat tolerances (Krause et al., 2015). After 15 min, the leaf disks were removed from the water baths and bags, and placed in petri dishes lined with moist paper towels where they were left under dim light (∼1 μmol photons m^{-2} s^{-1}) at room temperature (20–23°C) and allowed to recover for ∼24 h. After this recovery period, the leaf disks were dark-adapted for 20 min and F_V/F_M was measured.

To estimate the T_{50} for each species, we modeled the relationship of F_V/F_M vs. treatment temperature for each species

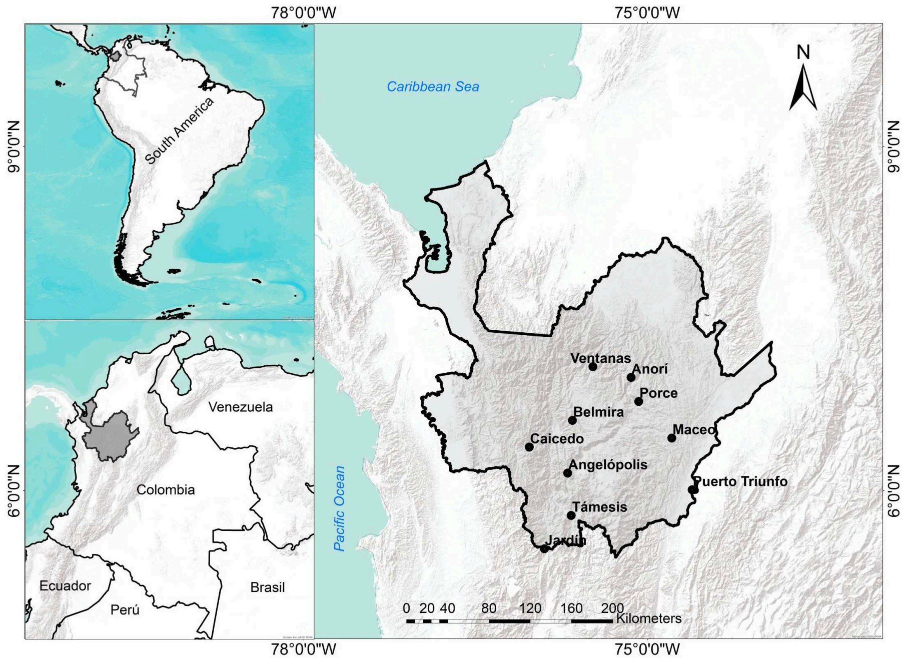

FIGURE 1 | Map of study area showing the locations of the 10 study plots (1-ha each) in the Antioquia department of Colombia. Plot elevations are Puerto Triunfo: 192 m; Támesis: 550 m; Maceo: 1,024 m; Porce: 1,033 m; Anorí: 1,794 m; Angelópolis: 2,118 m; Ventanas: 2,120 m; Jardín: 2,550 m; Caicedo: 2,635 m; Belmira: 2 800 m.

using a logistic non-linear least squares model with the "nls" function in the R *stats* package [i.e., nls($F_V/F_M \sim \theta_1/(1 + \exp(-(\theta_2 + \theta_3 \times \text{Temperature})))$] where θ_1 is the control value of F_V/F_M (≈ 0.8) and θ_2 and θ_3 are the intercept and slope coefficients of the logit(Fv/Fm) $\sim$ Temperature relationship, respectively; R Core Team, 2018). T_{50} was then calculated as the temperature modeled to cause a 50% reduction in F_V/F_M compared to the control (**Figure S1**). To generate bootstrapped mean and 95% confidence level estimates of T_{50} for each species, we reiterated the model of F_V/F_M vs. temperature 100 times. During each iteration, F_V/F_M and temperature data were sampled randomly with replacement to recalculate T_{50}. For some species ($n = 18$), estimates of T_{50} were deemed unreliable due to unreasonable values (i.e., $T_{50} > 55$ or $< 40°C$) or very large 95% confidence intervals (i.e., 95% CI $> 5°C$; **Table S1**, **Figure S1**). These species were excluded from any subsequent analyses that used T_{50}.

Species Abundances and Changes in Abundance

We calculated the relative abundance (BA_{rel}) of each study species in each plot census as the total basal area of the focal species (= summed cross sectional area of all conspecific stems in a plot at breast height [1.33 m above ground]) relative to the total basal area for all stems in the plot (including stems of species not assessed for T_{50}; **Table S2**). We then estimated the annualized rate of change in relative abundance through time for each species as the slope of the linear regression of BA_{rel} vs. census date (ΔBA_{rel}, % year^{-1}; **Table S1**). Using the ΔBA_{rel} estimates, we also classified each species as either increasing or decreasing in relative abundance through time.

Geographic-Based Thermal Niches

In order to assess how the study species' T_{50} relate to the species' large-scale geographic distributions, we used natural history observation records to estimate the range of temperatures over which each study species is known to occur. Specifically, for each of the study species, we downloaded all available collection and observation records using the BIEN data portal (queried via R package *BIEN* in March 2019). The BIEN data portal provides access to a number of different public data sources including the Global Biodiversity Information Facility (GBIF), SpeciesLink and various inventory plot networks, and records have undergone a base level of cleaning to minimize taxonomic and georeferencing errors (Maitner et al., 2018). For each georeferenced observation, we extracted the mean annual temperature (MAT, BIOCLIM1) and mean maximum daily temperatures of the warmest month (MTWM, BIOCLIM5) from the CHELSA extrapolated climate database (Karger et al., 2017) at a spatial resolution of 30 arc seconds ($\sim$1-km resolution at the equator). Any records with obvious georeferencing errors (e.g., falling in large bodies of water or outside of the neotropics) were discarded. We also masked the records to include only those from within the "Tropical & Subtropical Moist Broadleaf Forests" and "Tropical & Subtropical Dry Broadleaf Forests" (Dinerstein et al., 2017) in the Andean countries of Colombia, Venezuela, Ecuador, Peru, and Bolivia, in order to minimize potential errors and to reduce the influence of non-forest or non-Andean congeners when working at the genus level (see below). For each of the study species with $\geq$10 "clean"

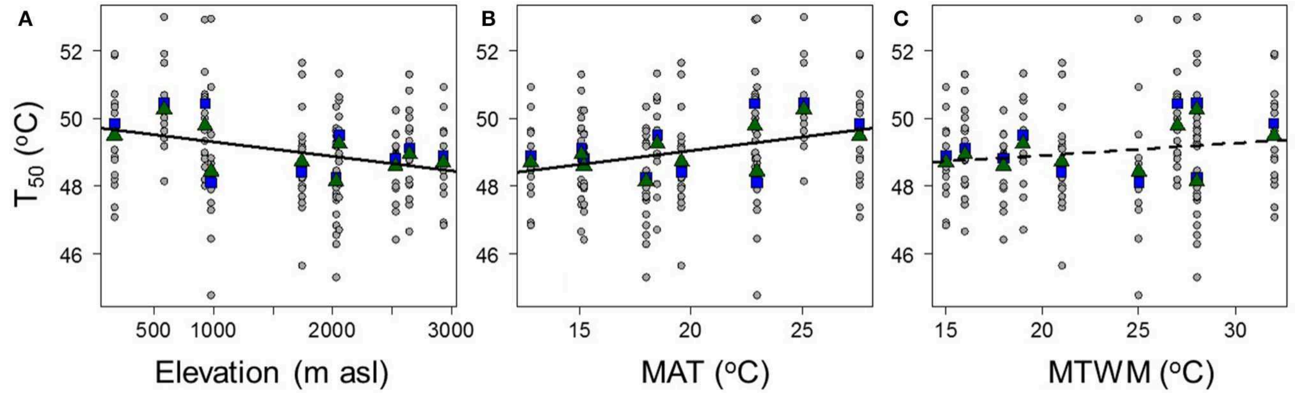

FIGURE 2 | The relationships between species' thermal tolerances (T_{50}) and **(A)** plot elevation, **(B)** mean annual temperature (MAT), and **(C)** mean maximum temperature of the warmest month (MTWM). Circle points and lines show species-level data; square points show plot-level averages with the species weighted by their relative abundances, and triangle points show unweighted plot averages. $T_{50} = 49.72^{\circ}\text{-}0.0004 \times \text{Elevation}$, $R^2 = 0.07$, $P = 0.00101$; $T_{50} = 47.39^{\circ} + 0.0827 \times \text{MAT}$, $R^2 = 0.07$, $P = 0.00115$; $T_{50} = 48.16^{\circ} + 0.0372 \times \text{MTWM}$, $R^2 = 0.02$, $P = 0.0701$.

georeferenced records from Andean forests, we then calculated the geographic-based thermal optimum (GT_{opt}) as the median of the extracted MAT values (analyses were repeated using the mean MAT values but results did not differ qualitatively). We calculated the geographic-based thermal maximum (GT_{max}) as the 95% quantile of the extracted BIOCLIM5 values (the 95% quantile was used rather than the absolute maximum in order to help minimize the influence of outliers or remaining georeferencing errors). For morphospecies ($n = 37$) and species with <10 records ($n = 52$), we estimated the GT_{opt} and GT_{max} at the genus-level using all available records for congeners (**Table S1**). For a small number of species ($n = 8$), there were not sufficient records available from Andean forests to estimate GT_{opt} and GT_{max} at either the species- or genus-level; these species were excluded from relevant analyses. We also conducted all analyses using just the species for which GT_{opt} and GT_{max} could be estimated at the species-level but the results were not markedly changed (**Table S3**).

Relationships between variables (elevation, temperature, T_{50}, GT_{opt} and GT_{max}, ΔBA_{rel}) were assessed using ordinary linear least squares regressions. In the analyses of ΔBA_{rel}, species were weighted by the strength of the coefficient of determination (R^2) of their BA_{rel} vs. census date relationship. We also used logistic regression to test if the probability of increasing in relative abundance ($\Delta BA_{rel} > 0$) was related to T_{50}, GT_{opt}, or GT_{max}. All analyses were conducted in R version 3.6.1 (R Core Team, 2018).

RESULTS

In accord with *a priori* predictions, the photosynthetic thermal tolerances (T_{50}) of tree species were positively related to plot temperature and elevation, such that the species sampled from hot lowland plots tended to have higher T_{50} than species sampled from cold highland plots ($T_{50} = 49.72^{\circ}\text{-}0.0004 \times \text{Elevation}$, $R^2 = 0.07$, $P = 0.00101$; $T_{50} = 47.39^{\circ} + 0.0827 \times \text{MAT}$, $R^2 = 0.07$, $P = 0.00115$; $T_{50} = 48.16^{\circ} + 0.0372 \times \text{MTWM}$, $R^2 = 0.02$, $P = 0.0701$; **Figure 2**). At the plot level, there was a tendency for the average T_{50} (with species weighted by their relative basal area) to vary with temperature and elevation, but the relationships were not significant (mean $T_{50} = 49.92^{\circ}\text{-}0.0004 \times \text{Elevation}$, $R^2 = 0.24$, $P = 0.149$; mean $T_{50} = 47.47^{\circ} + 0.0866 \times \text{MAT}$, $R^2 = 0.23$, $P = 0.156$; mean $T_{50} = 48.04^{\circ} + 0.0501 \times \text{MTWM}$, $R^2 = 0.12$, $P = 0.331$; **Figure 2**).

Similarly, tree species sampled from the lowland plots tended to have geographic distributions that were centered on hotter areas (i.e., higher GT_{opt}) and that included hotter areas (i.e., higher GT_{max}) than species from highland plots ($GT_{opt} = 25.72^{\circ}\text{-}0.0026 \times \text{Elevation}$, $R^2 = 0.45$, $P < 2\ e^{-16}$; and $GT_{max} = 31.29^{\circ}\text{-}0.0012 \times \text{Elevation}$, $R^2 = 0.2$; $P = 1.07e^{-10}$; **Figure 3**). The plot-level average of GT_{opt} and GT_{max} both decreased significantly with elevation (mean $GT_{opt} = 26.14^{\circ}\text{-}0.0028 \times \text{Elevation}$, $R^2 = 0.82$, $P = 0.000282$; and mean $GT_{max} = 31.64^{\circ}\text{-}0.0016 \times \text{Elevation}$, $R^2 = 0.64$, $P = 0.00567$; **Figure 3**). Plot-level relationships did not change if the species were not weighted by their abundances.

There was a significant positive relationship between T_{50} and GT_{opt} ($GT_{opt} = -2.93^{\circ} + 0.4931 \times T_{50}$, $R^2 = 0.05$, $P = 0.00858$; **Figure 4A**). However, there was not a significant relationship between T_{50} and GT_{max} ($GT_{max} = 20.95^{\circ} + 0.1680 \times T_{50}$, $R^2 = 0.01$, $P = 0.165037$; **Figure 4B**).

Contrary to predictions, there was no overall pattern for tree species with higher relative T_{50} ($T_{50,relative} = T_{50}$-Plot Average T_{50}) to increase in relative abundance through time ($\Delta BA_{rel} = -3.506\ e^{-06} + 1.046\ e^{-05} \times T_{50,relative}$, $R^2 = 0.01$, $P = 0.218$; **Figures 5A**, **6**). A significant positive relationship between thermal tolerances and changes in relative abundance was observed in only three of the plots (Maceo: $\Delta BA_{rel} = -3.125\ e^{-03} + 6.368\ e^{-05} \times T_{50}$, $R^2 = 0.36$, $P = 0.009$; Porce: $\Delta BA_{rel} = -2.835\ e^{-03} + 5.843\ e^{-05} \times T_{50}$, $R^2 = 0.60$, $P = 0.005$; Ventanas: $\Delta BA_{rel} = -3.994\ e^{-03} + 8.320\ e^{-05} \times T_{50}$, $R^2 = 0.37$, $P = 0.021$; **Figure 6D**). Likewise, while there were positive relationships between species' changes in relative abundance through time and both their relative GT_{opt} ($GT_{opt,relative} = GT_{opt}$-Plot Average GT_{opt}) and their relative GT_{max} ($GT_{max,relative} = GT_{max}$-Plot

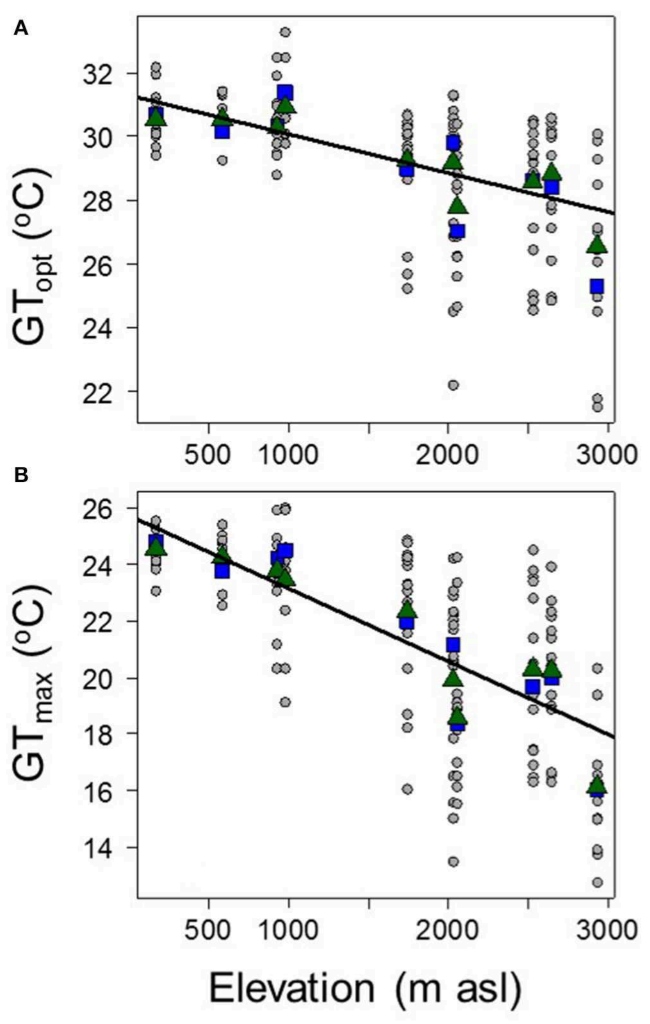

FIGURE 3 | The relationships between plot elevation and species' **(A)** geographic-based thermal optima (Gt_{opt}), and **(B)** geographic-based thermal maximum (GT_{max}). Circle points and lines show species-level data; square points show plot-level averages with the species weighted by their relative abundances, and triangle points show unweighted plot averages. GT_{opt} = 25.72°-0.0026 × Elevation, $R^2 = 0.45$, $P < 2e^{-16}$; GT_{max} = 31.29°-0.0012 × Elevation, $R^2 = 0.2$, $P = 1.07\ e^{-10}$.

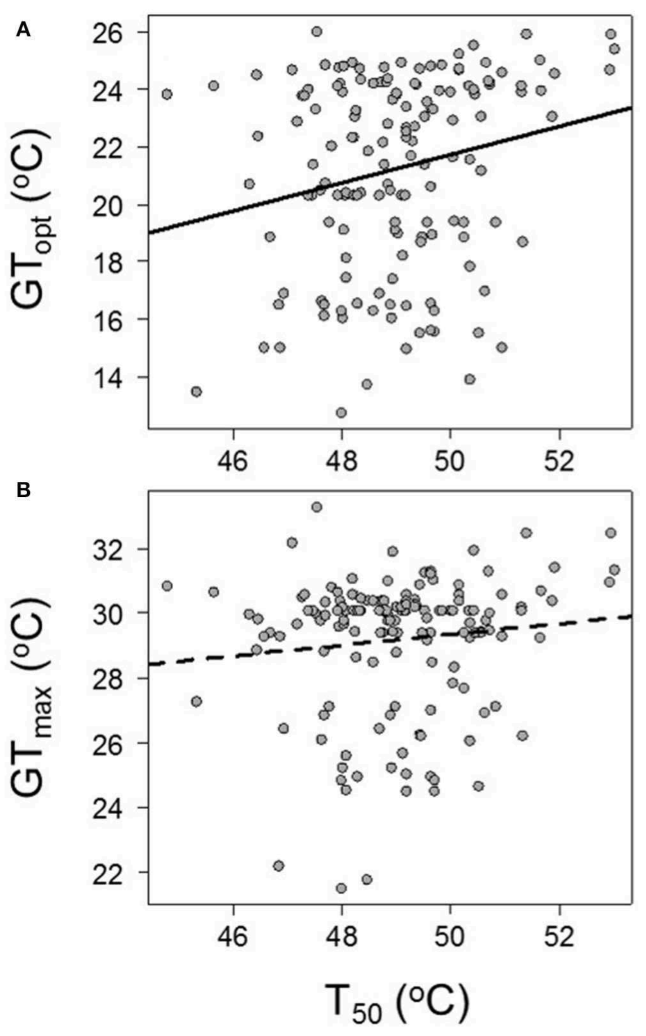

FIGURE 4 | The relationships between species' physiological maximum thermal tolerance (T_{50}) and **(A)** GT_{opt} and **(B)** GT_{max}. $GT_{opt} = -2.93° + 0.4931 \times T_{50}$, $R^2 = 0.05$, $P = 0.00858$; $GT_{max} = 20.95° + 0.1680 \times T_{50}$, $R^2 = 0.01$, $P = 0.165037$.

Average GT_{max}), these relationships were not significant ($\Delta BA_{rel} = -5.300\ e^{-06} + 9.521\ e^{-07} \times GT_{opt,relative}$, $R^2 = 0.02$, $P = 0.0677$; and $\Delta BA_{rel} = -7.945\ e^{-06} + 9.9960\ e^{-07} \times GT_{max,relative}$, $R^2 = 0.02$, $P = 0.120$; **Figures 5B,C**). The probability of species increasing in abundance was not significantly associated with any measure of thermal tolerance [$\text{logit}(\Delta BA_{rel} > 0) = 0.166 + 0.00268 \times T_{50,relative}$, $P = 0.982$; $\text{logit}(\Delta BA_{rel} > 0) = 0.172 + 0.0100 \times GT_{opt,relative}$, $P = 0.169$; and $\text{logit}(\Delta BA_{rel} > 0) = 0.137 + 0.0162 \times GT_{max,relative}$, $P = 0.0821$].

DISCUSSION

In this study, we tested several hypotheses about how the thermal tolerances of tropical montane tree species relate to their geographic distributions and performances under rising temperatures. First, we tested the hypothesis that species from cold, highland forest communities have lower thermal tolerances compared to species from lowland forests where ambient air temperatures are reliably hotter. Our results support this hypothesis since T_{50}, GT_{opt}, and GT_{max} all increased with plot temperature and decreased with plot elevation. Likewise, the plot-level average T_{50} tended to decrease with elevation (and increased with plot MAT), and plot-level averages of both GT_{opt} and GT_{max} both decreased significantly with elevation.

We found a very high amount of variation in the T_{50} among species co-occurring within each plot such that elevation or temperature only explained a small, albeit highly significant, proportion of interspecific variation. A potential explanation for these patterns is that thermal tolerances are evolved in response to leaf temperatures and that leaf temperatures can

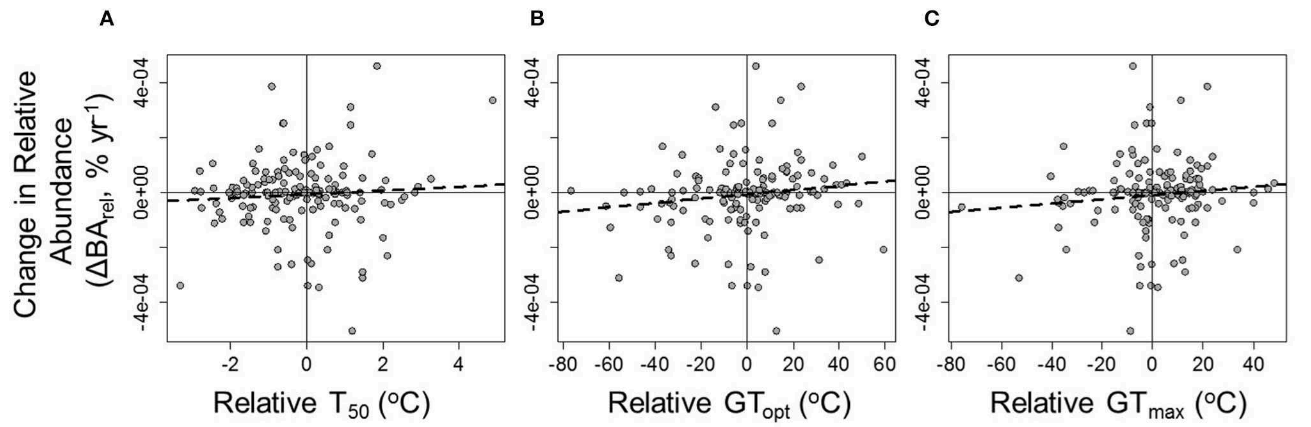

FIGURE 5 | The relationships between annual changes in species' relative abundance (ΔBA_{rel}) and **(A)** relative T_{50}, **(B)** relative GT_{opt}, and **(C)** relative GT_{max} (see text). $\Delta BA_{rel} = -3.506e^{-06} + 1.046e^{-05} \times T_{50,relative}$, $R^2 = 0.01$, $P = 0.218$; $\Delta BA_{rel} = -5.300\ e^{-06} + 9.521\ e^{-07} \times GT_{opt,relative}$, $R^2 = 0.02$, $P = 0.0677$; and $\Delta BA_{rel} = -7.945\ e^{-06} + 9.9960\ e^{-07} \times GT_{max,relative}$, $R^2 = 0.02$, $P = 0.120$.

be decoupled from regional air temperatures due to the fact that (1) even within a single plot, different species can occur predominantly in different thermal or moisture microhabitats and (2) co-occurring species can have different thermoregulatory behaviors and mechanisms.

Even over small areas (e.g., within single hectare inventory plots), there may exist many different microhabitats that create different thermal regimes for the leaves of co-occurring species. For example, leaves of canopy species will be exposed to more direct sunlight and thus reach higher temperatures than leaves of understory species (Smith, 1978; Rey-Sánchez et al., 2016; Slot et al., 2019). Air and leaf temperatures can also be strongly influenced by small-scale changes in topography (Geiger et al., 2009; Dobrowski, 2011; Sears et al., 2011; Curtis et al., 2016; Graae et al., 2018; Lembrechts et al., 2019). Many studies have found that tropical species are non-randomly distributed within plots. These studies, which have focused primarily on the relationships between the distributions of species and patterns of soil nutrients and soil water availability, have found good evidence that more drought-tolerant species occur in dryer microhabitats and that drought-intolerant species occur in wetter microhabitats (Harms et al., 2001; Valencia et al., 2004; Engelbrecht et al., 2007; Condit et al., 2013; Cosme et al., 2017; Zuleta et al., 2018). It is possible that species with higher or lower T_{50} are likewise selectively distributed in hotter or cooler microhabitats, respectively. Additional studies are needed to investigate the distribution of tropical tree species in relation to thermal microhabitats.

Another factor that can influence leaf temperatures is the thermal regulatory strategies of the plants themselves. Different leaf sizes, shapes, morphologies, and behaviors can all lead to markedly different leaf temperatures of co-occurring plants (Parkhurst and Loucks, 1972; Meinzer and Goldstein, 1985; Leigh et al., 2012; Fauset et al., 2018). For example, all else being equal, larger leaves will reach higher maximum temperatures than will smaller leaves (Leigh et al., 2017; Wright et al., 2017). Likewise, leaves that absorb more solar radiation (rather than reflecting or transmitting the light) will reach higher maximum temperatures (Smith, 1978; Rey-Sánchez et al., 2016). Another important leaf thermoregulatory mechanism is evapotranspiration, which cools leaves through latent heat loss (Nobel, 1999; Lambers et al., 2008). Evapotranspirative cooling requires access to water and thus is generally most effective in species that have developed mechanisms for the rapid uptake and transport of water (both to the leaf via xylem transport and out of the leaf via stomata) and/or species that occur in wetter microhabitats with abundant soil water availability (Gates, 1968; Oren et al., 1999; Meinzer et al., 2008). Because of habitat selection and thermoregulatory mechanisms, leaf temperatures can be decoupled from air temperature and may differ significantly between species under identical air temperatures (Michaletz et al., 2015). By extension, this also means that species under markedly different air temperatures can have similar or identical leaf temperatures (Helliker and Richter, 2008; Michaletz et al., 2016). If thermal tolerances are indeed adapted or acclimated to leaf temperatures rather than air temperatures, then this decoupling may explain the high amount of variation between species within plots as seen in our study, as well as in other studies looking at patterns of thermal tolerances across latitude (O'sullivan et al., 2017).

Another hypothesis that we tested in this study was that tropical tree species with higher thermal tolerances have geographic ranges that encompass hotter regional air temperatures (Zhu et al., 2018). In accord with this hypothesis, we did find a significant positive relationship between species' T_{50} and their thermal optima as based on their large-scale geographic distributions. However, T_{50} explained very little variation between species' GT_{opt} and there was not a significant relationship between T_{50} and species' geographic-based thermal maximum (GT_{max}). As discussed above, microhabitat selection and thermoregulatory strategies may decouple leaf temperatures from air temperatures—especially when air temperatures are estimated at the scale of 1-km^2 pixels. If this is true, then

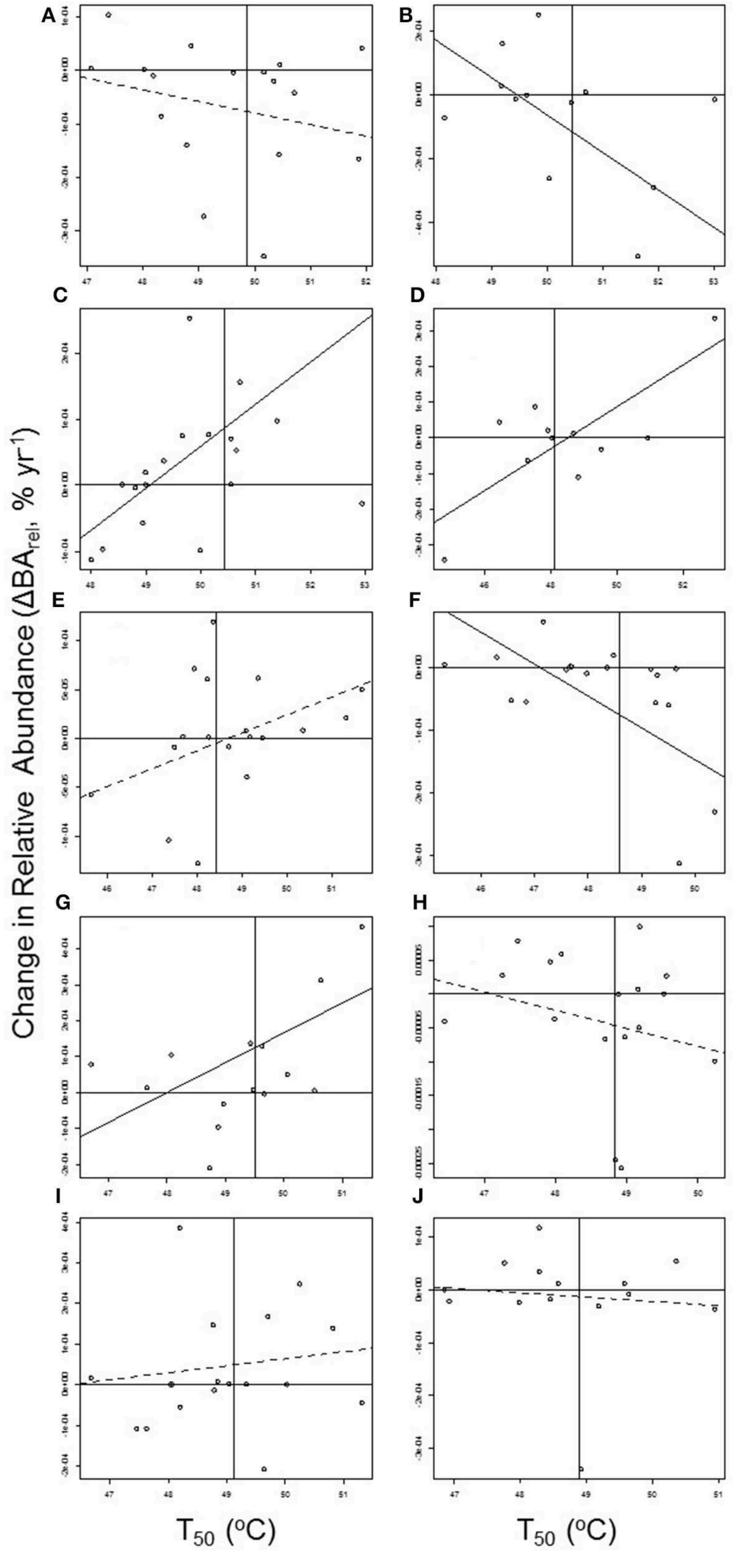

FIGURE 6 | The within-plot relationships between annual changes in species' relative abundance (ΔBA_{rel}) and species' T_{50}. Horizontal lines indicate no change in abundance through time and vertical lines indicate plot-average T_{50}. Solid regression lines indicate significant ($p \leq 0.05$) relationships (weighted regressions with

(Continued)

FIGURE 6 | species weighted by the coefficient of determination of their BA_{rel} vs. census date relationship). Plots are ordered by increasing elevation: **(A)** Puerto Triunfo: $\Delta BA_{rel} = 1.001\ e^{-03}\text{-}2.161\ e^{-05} \times T_{50}$, $R^2 = 0.06$, $P = 0.335$; **(B)** Támesis: $\Delta BA_{rel} = 5.779\ e^{-03}\text{-}1.169\ e^{-04} \times T_{50}$, $R^2 = 0.47$, $P = 0.339$; **(C)**, Maceo: $\Delta BA_{rel} = -3.125\ e^{-03} + 6.368\ e^{-05} \times T_{50}$, $R^2 = 0.36$, $P = 0.009$; **(D)** Porce: $\Delta BA_{rel} = -2.835\ e^{-03} + 5.843\ e^{-05} \times T_{50}$, $R^2 = 0.60$, $P = 0.005$; **(E)** Anorí: $\Delta BA_{rel} = -8.976\ e^{-04} + 1.844\ e^{-05} \times T_{50}$, $R^2 = 0.13$, $P = 0.150$; **(F)** Angelópolis: $\Delta BA_{rel} = 2.400\ e^{-03}\text{-}5.094\ e^{-05} \times T_{50}$, $R^2 = 0.42$, $P = 0.004$; **(G)** Ventanas: $\Delta BA_{rel} = -3.994\ e^{-03} + 8.320\ e^{-05} \times T_{50}$, $R^2 = 0.37$, $P = 0.021$; **(H)** Jardín: $\Delta BA_{rel} = 1.261\ e^{-03}\text{-}2.678\ e^{-05} \times T_{50}$, $R^2 = 0.06$, $P = 0.345$; **(I)** Caicedo: $\Delta BA_{rel} = -7.950\ e^{-04} + 1.718\ e^{-05} \times T_{50}$, $R^2 = 0.02$, $P = 0.567$; and **(J)** Belmira: $\Delta BA_{rel} = 3.782\ e^{-04}\text{-}8.006\ e^{-06} \times T_{50}$, $R^2 = 0.01$, $P = 0.766$.

it may explain how some species with relatively low T_{50} are able to include areas with high mean annual or maximum temperatures in their distributions and likewise why some species with high T_{50} are geographically restricted to cooler areas. In order to better assess how thermal tolerances relate to geographic distributions, finer-scale climate models will need to be coupled with leaf biophysical models to map the distributions of leaf temperatures across species ranges (Kearney and Porter, 2017). Another possibility is that thermal tolerances are locally adapted or acclimated to plot conditions, which would obscure any relationships between T_{50} and large-scale species-wide distributions (Zhu et al., 2018).

The final hypothesis that we tested was that because of global warming, tree species that are more-thermophilic should increase in abundance through time within each plot relative to less-thermophilic species. Our results did not support this hypothesis. We did not find consistent or significant relationships between the direction or rates of changes in species' relative abundances (BA_{rel}) and any measure of the species' thermal tolerances or preferences (T_{50}, GT_{opt}, GT_{max}). As above, the lack of a strong relationship between thermal properties and their changes in abundance may be due at least in part to the decoupling of air temperature and leaf temperatures. In other words, some species may be better able to regulate their leaf temperatures and thus may experience less changes in leaf temperatures over time than other co-occurring species. It is also possible that other factors could be influencing changes in species' abundances besides rising temperatures, thus obfuscating patterns. More specifically, there is large stochastic component to tree mortality, especially in montane forests where landslides and other local disturbances are very common (Clark et al., 2016). These stochastic events lead to a large amount of demographic "noise." To overcome this noise, large-scale spatial or temporal datasets are required (Wagner et al., 2010). It is possible that this study was simply too short of duration to detect a directional signal in abundance changes, especially since we were only looking at a small subset of focal species within individual 1-ha plots.

CONCLUSIONS

Global warming makes it imperative that we understand the ability of species to tolerate and perform at hotter temperatures. Here we assessed the maximum physiological thermal tolerance of photosystem II (T_{50}) for nearly 200 species of tropical montane trees growing at different elevations along the flanks of the Colombian Andes Mountains. We used our estimates of T_{50} to test if thermal tolerances vary predictably across an elevation gradient of more than 2,500 m (corresponding to a *ca* 20°C gradient in mean annual and maximum temperatures). Our results showed that there is in fact a negative relationship between elevation and T_{50} such that species occurring at lower elevations are generally capable of tolerating hotter temperatures than species occurring at higher elevations. However, our results also indicate that the relationship between T_{50} and temperature is weak and extremely shallow (**Figure 2**). Indeed, the slope of the T_{50} vs. MAT relationship was only 0.08, and the slope of the T_{50} vs. MTWM relationship was only 0.05. Our findings mirror those of O'sullivan et al. (2017) who likewise found a shallower-than-expected relationship between plant thermal tolerances and temperature (specifically, $T_{max} = 49.155 + 0.264 \times MTWM$; unfortunately, differences in the methods used to estimate thermal tolerances prohibit a direct comparison of results).

The shallow relationships between plant thermal tolerances and plot temperatures across both elevational and latitudinal gradients suggest that species from hot low-elevation and low-latitude forests will have smaller average thermal safety margins (difference between temperature and thermal tolerance) than either high-latitude or high-elevation species, and consequently that lowland tropical plants may be at especially high risk of damage from rising global temperatures (Perez et al., 2016). However, we also found a large amount of variation in the T_{50} of species co-occurring in any given site (i.e., some species growing in low-elevation forests where MAT = 27°C can have lower T_{50} than species from high elevation forests where MAT = 10°C, and vice versa) and also very little relation between species' thermal tolerances and their large-scale geographic distributions (i.e., some species with low T_{50} have ranges that include hotter areas than species with higher T_{50}). Consequently, many lowland species may potentially have larger thermal safety margins than highland species, making it hard to make generalized predictions about risk.

One possible explanation for the high variation in thermal tolerances of co-occurring species is that T_{50} (and other comparable measures) is evolved in response to extreme leaf temperatures that can be decoupled from air temperatures. A decoupling of air and leaf temperatures, and the potential ability of some plants to maintain relatively constant leaf temperatures despite global warming, could also explain why there was no significant relationship between species' T_{50} and changes in species abundances over time. The thermal regulatory behaviors of tropical plants, and the ability of these behaviors to respond to climate change, remains poorly studied and deserves further attention.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/**Supplementary Material**.

AUTHOR CONTRIBUTIONS

KF and AD designed the study. JM-V, AS, and DT collected the field data. KF and TP analyzed the data. KF wrote the manuscript. KF, TP, and AD edited the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/ffgc.2020.00025/full#supplementary-material

Figure S1 | Species-level changes in F_V/F_M recorded in leaves exposed to different temperature treatments and model fits used to estimate T_{50}.

Table S1 | Study species, measures of thermal tolerance (T50, GTopt, GTmax), changes in abundance.

Table S2 | Results of analyses using only species for which GT_{opt} and GT_{max} could be estimated at the species-level.

Table S3 | Basal area (cm^2) of focal species and total tree basal area recorded in each census of the 10 study plots.

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PERSPECTIVE
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Mountain Ecosystems as Natural Laboratories for Climate Change Experiments

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Experimental studies are needed to empirically examine the effects of climate change on terrestrial organisms and to serve as the basis for predictions and management practices. As such, designing and implementing experimental systems that can simulate complex changes in the natural environment is currently a major area of interest of climate change science. Most climate change experiments (e.g., infrared heaters, open-top chambers) are typically performed within small, controlled environments and often manipulate just temperature and/or CO_2 concentration. Other factors are more difficult to control (e.g., wind speed, soil moisture) or are frequently ignored (e.g., biotic interactions), leading to uncertainties in the results and limiting our ability to make realistic predictions about species' responses to future environmental changes. We examined the natural variation of abiotic and biotic factors along mountain elevational gradients in order to highlight the potential for using these systems as natural laboratories for climate change research and experiments. The high variability of different abiotic and biotic factors along elevational gradients provides a good opportunity to carry out field transplant/translocation experiments aimed at answering some critical questions, including: How will new biotic assemblages affect key interactions and processes? What are the factors that influence species assemblages under novel climates? How do local abiotic factors influence the establishment of species migrating into novel and climatically suitable habitats? Based on empirical evidence, we strongly encourage researchers to take advantage of the natural environmental gradients found in mountains to study the potential direct and indirect impacts of climate change on species, communities and biodiversity as a whole.

Keywords: altitudinal gradients, common garden experiments, experimental warming, field experiments, global warming, latitudinal gradients, novel interactions, transplant experiments

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INTRODUCTION

As the global climate continues to change and the effects of these changes become more pronounced, we need to prioritize studies that can provide a solid understanding of how complex environmental changes will directly and indirectly impact ecological processes. Experimental studies are critical for empirically examining the effects of climate change on terrestrial systems and organisms, and these studies may serve as the basis for many predictions and management practices. However, designing and implementing experiments that can fully simulate multifactorial changes in the natural environment remains a daunting problem.

Most climate change experiments have been carried out in controlled environments. Heating experiments such as infrared heaters, open-top chambers, soil heating cables or glasshouses are typically employed over small spatial scales and often manipulate just temperature and/or CO_2 concentrations (Bokhorst et al., 2011; Pelini et al., 2011; Elmendorf et al., 2015; Kimball, 2016; Wang et al., 2017). Although a specific variable(s) of interest can be controlled in this type of experiments, other factors are more difficult to control (e.g., wind speed, radiation, relative air humidity, soil moisture content). In addition, simulating changes in some factors, such as biotic interactions, may be difficult or impossible within laboratory or mesocosm experiments. Several recent studies have highlighted the limitations in the results from controlled experiments that can arise due to these and other problems. For example, a large-scale comparative analysis (for 1,634 plant species) of warming experiments and observational studies concluded that heating experiments underestimate the effects of climate change on plant phenological responses (Wolkovich et al., 2012) – although a subsequent analysis suggested that this is not always true for tundra species (Elmendorf et al., 2015). Likewise, Yang et al. (2018) compared three different methods (transplants, open top chamber and field long-term monitoring) and showed that the influence of altered competition under warmer conditions was only detected in the transplant experiments. Another recent study (Ettinger et al., 2019) evaluated the alteration of microclimates within experimental warming plots (15 studies) and showed that heating experiments produce changes in soil conditions (soil drying) that can have important biological consequences, but that this indirect effect of warming was often overlooked. These limitations decrease our ability to predict species' responses to climate change, and consequently our capability to plan and effectively implement conservation actions (Pressey et al., 2007; Reside et al., 2018; Ettinger et al., 2019).

Conducting experiments within natural environments is one promising option for more realistic assessments of how species and communities will respond to climate change. There is a growing number of studies taking advantage of natural environmental gradients – including latitudinal (Frenne et al., 2013; Nooten and Hughes, 2014; Nooten et al., 2014), elevational (Alexander et al., 2015; Tito et al., 2018), geothermal (Valdés et al., 2018) or forest successional gradients (Agosta et al., 2017) – in climate change studies. The use of elevational gradients can be an especially powerful and logistically feasible tool for studying how climate and other factors influence species' distributions and physiologies (Malhi et al., 2010; Silveira et al., 2019). Field experiments using elevational gradients can reveal effects that cannot be easily detected with experiments in controlled or artificial settings (Alexander et al., 2015; Nooten and Hughes, 2017; Tito Leon, 2017; Tito et al., 2018).

One of the most important advantages of field experiments is the possibility of detecting changes in biotic interactions and their effects (Alexander et al., 2015; Nooten and Hughes, 2017; Tito et al., 2018). There is a broad consensus that climate change will modify ecological communities by driving some species locally extinct and/or by creating new assemblages among species whose ranges did not previously overlap (Hobbs et al., 2006; Lavergne et al., 2010; Lurgi et al., 2012). Indeed, some field experiments have shown that the indirect effects of altered interactions are the most important drivers of plant responses to climate change (Alexander et al., 2015; Tito et al., 2018). However, despite the clear need to understand how the combined direct and indirect effects of climate change will affect species assemblages, field studies that allow for altered biotic interactions remain scarce. Revising more than half a million studies that focused on the possible impacts of climate change on terrestrial organisms, Nooten and Hughes (2017) found only 47 that were based on field experiments.

Here, we present a brief analysis of the natural variation of abiotic and biotic factors along mountain elevation gradients in order to highlight the potential for using these systems in climate change studies. Many of the ideas presented here are based on a set of field experiments conducted along an elevation gradient in the Peruvian tropical Andes (Tito Leon, 2017; Tito et al., 2018). Our goal in presenting this perspective article is to encourage the use of field experiments in future studies focused on determining the direct and indirect effects of climate change.

ENVIRONMENTAL VARIATION ALONG MONTANE ELEVATIONAL GRADIENTS

During last few decades, there were many efforts to define mountainous areas and to map the occurrence of these areas globally (Kapos et al., 2000; Körner et al., 2011, 2017; Elsen and Tingley, 2015; Antonelli et al., 2018; Price et al., 2019). The resulting classification system, which has been employed in many studies (Elsen and Tingley, 2015; Price et al., 2019), is based on elevation (>300 m asl) and ruggedness criteria, and indicates that mountain areas occupy approximately 21–24% of the global land area (Kapos et al., 2000; Meybeck et al., 2001). However, because the criteria previously used to define mountain areas allowed the inclusion of areas (e.g., plateaus, hilly lowland terrain) that seem inappropriate in a mountain biodiversity context, the methodology was improved and refined by applying a finer spatial resolution and considering local elevation range (maximal elevation difference among neighboring grid points: 200 m across 3 × 3 grid points of 30′′ within each grid cell; see Körner et al., 2011 for more detail) (Körner et al., 2011, 2017; Price et al., 2019). Using this new approach, it is now acknowledged that mountainous regions represent ∼12% of the Earth's terrestrial land surface outside of Antarctica (Körner et al., 2011, 2017). One of the most outstanding characteristics of mountainous regions is the high altitudinal variability of many climatic and non-climatic factors (Körner, 2007; Rapp and Silman, 2012). The high heterogeneity of abiotic conditions over short spatial scales is also reflected in the elevational variation of the diversity and composition of many montane plant and animal communities (Körner, 2007; Farfan-Rios et al., 2015). As a consequence, mountain regions host especially high levels of diversity and endemism (Barthlott et al., 1996; Myers et al., 2000; Körner, 2004; Rahbek et al., 2019b).

Abiotic Factors

Although the topography of mountains changes considerably from one region to another, there is a universal and steady decrease in mean air temperatures as elevation increases (Körner, 2007; Kessler et al., 2011; Rapp and Silman, 2012; **Figure 1A**). However, the rate that temperature decreases with elevation can vary depending on the slope and location of the mountain. For instance, along an elevation gradient in the southeastern slope of the Peruvian Andes, temperature decreases with an adiabatic lapse rate of approximately 5.2°C for every 1,000 m of elevation (Rapp, 2010), while on a southwestern Andean slope temperature decreases at a rate of 7.4°C per 1,000 m (Tito et al., 2018). Nonetheless, the temporal variation in temperature follows similar patterns on both the eastern (Rapp and Silman, 2012) and western slopes of the Andes (Tito et al., 2018). Several other studies also report an adiabatic decrease in temperature with elevation. For example, Kessler et al. (2011) show elevational temperature decrease for 20 other tropical and temperate regions.

Soil physical and chemical properties usually vary within mountain ranges and there is no consistency in patterns between mountain areas (Körner, 2007; Rahbek et al., 2019a). The heterogeneity of soil properties along mountain slopes depends on local characteristics such as topography, vegetation and geographic location. For example, soil organic matter increases with elevation in the Peruvian Andes (Zimmermann et al., 2010; Tito et al., 2018), in the mountains of Hawaii (Townsend et al., 1995), and in Mt. Rainier in the western United States (Zimmermann et al., 2010; Ettinger and Hillerislambers, 2017). In contrast, in the Himalayas, soil organic matter decreases at higher elevations (Sheikh et al., 2009). In the southwestern Peruvian Andes, high concentrations of phosphorus and potassium are found at middle elevations (∼3,500 m asl), whereas calcium content increases and magnesium content decreases strongly with elevation (Tito et al., 2018). Nitrogen and phosphorus content show no trend with elevation on Mt. Rainier (Ettinger and Hillerislambers, 2017) and on the arid Tianshan Mountain in northwest China (Xu et al., 2019). In the Andes, nitrogen content decreases with elevation and is limiting for plants at high elevations (Zimmermann et al., 2010). Studies argue that many tropical mountain soils are younger and more nitrogen-limited at high elevations, and that soils are older and more phosphorus-limited at low elevations (Vitousek and Sanford, 1986; Vitousek and Farrington, 1997). In short, the existing studies indicate that soil properties vary strongly with elevation (but not necessarily in the same way in different regions) and that future studies on species' responses to climate change should take this into account.

Precipitation, relative humidity, and solar radiation are other important abiotic factors that vary in mountains, but without unidirectional elevational trends (Körner, 2007). For example, photosynthetically active radiation (PAR) on Mt. Rainier increases with elevation (Ettinger and Hillerislambers, 2017); in contrast, in tropical montane cloud forests, PAR decreases with elevation due to increased cloud cover and cloud immersion (Rapp and Silman, 2012). There is no general pattern for precipitation changes in relation to elevation (Körner, 2007). In some mountains (e.g., in the southern Peruvian Andes) the elevational variation in precipitation is not significant due to steep slopes and rapid changes in elevation over very small spatial scales (Condom et al., 2011). Humidity increases with elevation in some mountains (e.g., in the western United States) (Körner, 2007; Ettinger and Hillerislambers, 2017) but decreases with elevation in others (e.g., eastern of Neotropical Andes)

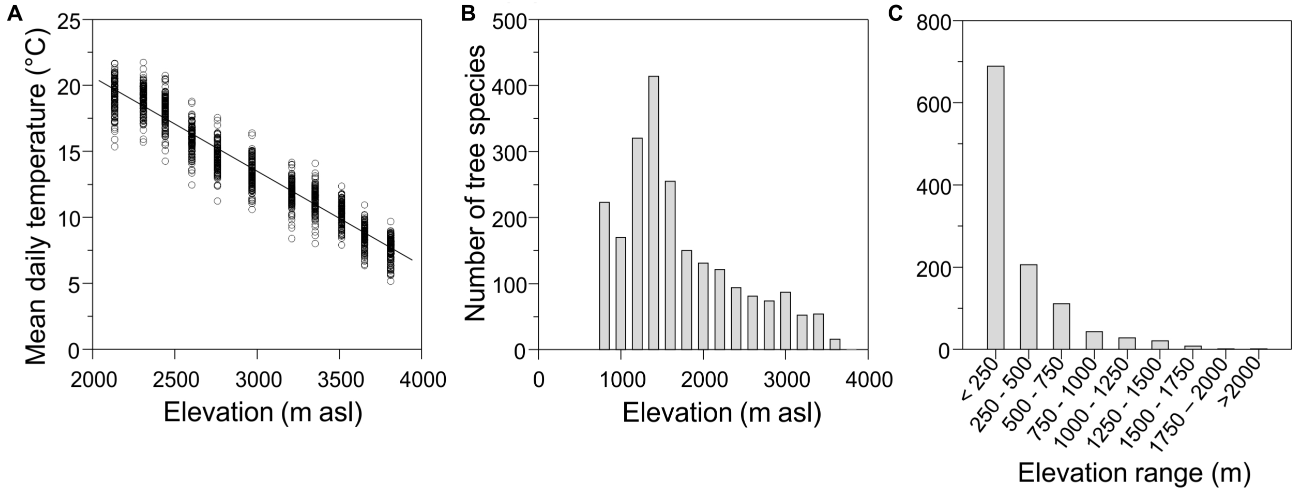

FIGURE 1 | (A) Mean daily temperature along an elevational gradient (2,135–3,812 m asl) in the southwestern region of the Peruvian Andes. Eleven temperature sensors (iButton DS1922L) were installed approximately every 150 m of elevation from 2,135 to 3,812 m asl. Each sensor was installed at a height of 1.5 m from the ground and remained in the field from February to July 2015, recording the temperature every 2:30 h. **(B)** Number of tree species in relation to elevation and **(C)** in relation to the extent of the distribution range. Panels **(B,C)** are based on data reported by Farfan-Rios et al. (2015) for tree plot censuses along along an elevation gradient from 800–3625 m asl in Manu National Park, Peru.

(Rapp and Silman, 2012) and is greatest at mid-elevations in others (e.g., Mount Kilimanjaro) (Körner, 2007). Because of their low temperatures, high elevations are generally more susceptible to frost, snow and hail.

Current Variation in Biotic Factors

There are elevated levels of species richness and endemicity in most mountain regions, and thus these systems are clear priority areas for conservation (Myers et al., 2000; Körner, 2004, 2007; Rahbek et al., 2019b). While covering just 12% of the land surface, mountains host more than 85% of the world's bird, amphibian and mammal species (Körner et al., 2011; Rahbek et al., 2019b), and at least one third of the terrestrial plant species (Barthlott et al., 1996; Körner, 2004, 2007). Most montane species have narrow elevational ranges (Rahbek et al., 2019b; **Figure 1C**).

Richness, composition and structure of plant and animal communities change along elevational gradients. Farfan-Rios et al. (2015) recorded over 1,000 species of trees, palms and lianas along an elevation gradient of ∼2,800 m, from submontane forests at 800 m asl up to the tree line at 3,625 m asl in Manu National Park, Peru. From the distributional ranges reported in this study, we observe that plant species richness generally decreases with elevation but has a marked peak at ∼1,400 m asl (**Figure 1B**), consistent with previous studies (Rahbek, 1995; Girardin et al., 2010).

Similar to the elevational variation of plant species richness, the diversity of herbivores (Hodkinson, 2005; Metcalfe et al., 2014; Rasmann et al., 2014; but see Galmán et al., 2018), frugivorous birds (Dehling et al., 2014; Bender et al., 2019), seed predators (Hargreaves et al., 2019), and soil bacteria and fungi (Nottingham et al., 2018) also decreases with elevation. In the case of geometrid moths (Beck et al., 2017), salamanders (McCain and Sanders, 2010), tropical ferns (Kessler et al., 2011) and non-volant small mammals (McCain, 2005), mid-elevational peaks in species richness are reported, but with sharp declines in richness at higher elevations. These elevational patterns of species richness and abundance can cause changes in the biotic pressures that local populations endure at different elevations within their ranges. For example, studies show that herbivory (Metcalfe et al., 2014), seed predation (Hargreaves et al., 2019), and predation rates on model caterpillars (Roslin et al., 2017) are greater at lower elevations. In addition, immigrant lowland plant species are expected to have increased competitive abilities (Yang et al., 2018). It is generally hypothesized that biotic interactions (e.g., competition, herbivory) set the lower limit of species' elevational ranges while harsh climatic conditions set species' upper elevational range limits (Ettinger et al., 2011; Hillerislambers et al., 2013).

PREDICTED SPECIES RESPONSE AND FUTURE RESEARCH DIRECTIONS

Many plant and animal species are migrating to higher elevations and latitudes in response to ongoing changes in climate (e.g., Chen et al., 2011; Feeley et al., 2011b; Lenoir and Svenning, 2015; MacLean and Beissinger, 2017; Fadrique et al., 2018; Freeman et al., 2018). However, studies suggest that migration rates are commonly slower than the rates required to track concurrent warming (Feeley et al., 2011a; Fadrique et al., 2018). Furthermore, several species appear unable to migrate due to different biological (e.g., dispersal capacity) or geographical (e.g., area availability) barriers (Hobbs et al., 2006; Rehm and Feeley, 2015, 2016; Alexander et al., 2018). However, several species may not need to migrate and may be able to persist under altered climates due to phenotypic plasticity and acclimation (MacLean and Beissinger, 2017). Overall, species vary widely in both their ability to change their geographic distributions due to climate change, but additional work is required to develop a more detailed understanding of climate-driven range shifts. For example, phytophagous insects are mobile organisms and can "migrate" faster than their associated host plants (Berg et al., 2010). Therefore, in this system, asynchronous species migrations could eventually lead to disrupted or reorganized interaction networks (**Figure 2B**).

In this context, several fundamental questions remain unanswered, including: How will new biotic assemblages affect key interactions and processes? What are the factors that influence species assemblages under altered climates? And, how do local abiotic factors (e.g., new soil conditions) influence the establishment of species migrating into novel and climatically suitable habitats? (Ibañez et al., 2006). The pronounced elevation and temperature gradients in mountains offer an ideal natural system to carry out field experiments aimed at answering these and other critical questions. Downslope translocation and transplant experiments can simulate future scenarios of climate change in the natural environment and can be used to determine the potential direct and indirect impacts of global warming on individuals, populations and communities. We summarize below some of the field transplant experiments that have used natural environmental gradients to simulate future conditions.

In order to assess the impacts of natural enemies (herbivores and pathogens) that can occur under future warming on potato and maize varieties in the Andes, Tito et al. (2018) used downslope transplants of seeds and tubers (with their home soil) to warmer areas (+1.3 and +2.6°C). This experiment revealed that the survival and productivity of both potato and maize varieties under warmer climates was strongly affected by novel pest and diseases. Other studies using latitudinal gradients have likewise shown that warming can lead to new suites of herbivore species colonizing the host plants (Nooten et al., 2014) and cause increased damage in some species (Nooten and Hughes, 2014). Beyond the obvious need for similar studies in more species and systems, these transplant studies reveal some new hypotheses that need to be addressed. For example, generalist interactions among species will likely be less constrained by the identity of the host plants (Dunn et al., 2009; Lurgi et al., 2012), while specialist enemies or mutualists with obligate interactions may be limited to asynchronous migration with their host plants (Dunn et al., 2009; Lurgi et al., 2012). Theoretical studies suggest that the risk of species extinctions consequently increases with the level of specialization (Binzer et al., 2011; Lurgi et al., 2012). Furthermore, warming also likely influences the phenology of insects and their host plants and may lead to temporal mismatches between species

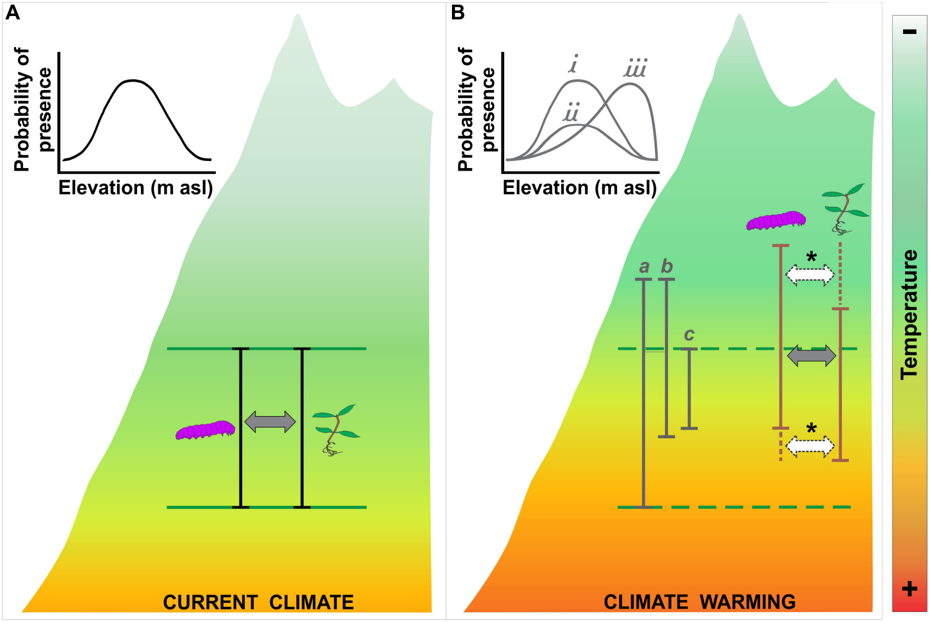

FIGURE 2 | Schematic representation of possible scenarios of species distribution changes in response to warming. The mountain is represented by the background figure and the color gradients represent the altitudinal variation in temperature. **(A)** Current conditions showing the range of distribution of a species (black bar and delimited by green lines) and the hypothetical probability of occurrence. **(B)** Possible changes in distribution and abundance patterns under climate change scenarios. Some hypotheses are: warming can lead to range expansion (*a*), shifts (*b*) or contractions (*c*), and probability of occurrence will likely remain stable (*i*), decrease over the range (*ii*) or move to the range margin (*iii*) (Feeley et al., 2012; Feeley, 2015; Peterson et al., 2019). On the right side, potential plant-animal (e.g., herbivores, pollinators, predators; represent by caterpillar) interaction partners mismatches (*) under asynchronous species migration scenarios are indicated.

and alter trophic interactions (Renner and Zohner, 2018; Boukal et al., 2019). Overall, little is known about how climate change will impact the role of consumers on host plants or as prey for predators (Tylianakis et al., 2008; Lurgi et al., 2012).

To explore how the responses of plants to climate change depend on the migration of other species, Alexander et al. (2015) transplanted alpine plant communities along an elevational gradient in the Swiss Alps. This study showed that the performance of plants growing under warmer climates (simulated through downslope transplants) is strongly reduced by new competitors that emerge through upward migrations. On the other hand, high-elevation competitors had little or no effect on the plants migrating upslope. Further similar experimental studies could enable us to better understand plant community responses to altered competitive interactions under climate change (Alexander et al., 2016) and address additional areas of uncertainty, such as priority effects (Fadrique and Feeley, 2016).

In the studies of Tito et al. (2018) and Tito Leon (2017), upslope migrations were simulated by growing plants in soils translocated from higher elevations (i.e., upward migrating plants will grow under their current climate but at higher elevations and thus in different soils) allowing them to test the effect of changing soil conditions on plant performance. The productivity of maize varieties significantly decreased in high-elevation soils due to changes in soil nutrients (including altered Ca:Mg concentrations) (Tito et al., 2018); in contrast, the survival and growth of seedlings of a dominant cloud forest tree species (*Weinmannia bangii*) were not affected by changes in soil (Tito Leon, 2017). Understanding the potential effects of climate change on soil biota and on plant microbe interactions are other important areas of study where soil translocation experiments could provide valuable information. The vast majority of plant species are associated with soil microorganisms that are critical for the growth and establishment of plants, and for dictating plant community composition (Collins et al., 2016; Sayer et al., 2017). A greenhouse warming experiment revealed that changes in soil biota due higher temperatures negatively affected the competitive capacity of *Poa trivialis*, although in the absence of competitors it favored their growth (Cardinaux et al., 2018). Another recent study (Nottingham et al., 2019) that translocated soil monoliths along an elevation gradient in the Andes showed that warming caused shifts in microbial community composition and changes in microbial physiology leading to increased carbon use efficiency.

Several authors have suggested that local adaptation may play a key role in determining species' responses to warming (e.g., Peterson et al., 2018, 2019; Solarik et al., 2018); despite this, the possibility of local adaptation is often ignored or overlooked (Feeley, 2015; Peterson et al., 2019). Transplant experiments from different local populations within species' ranges can be used to study the influence of local adaptation on species' response to climate change. In their study of *W. bangii*, Tito Leon (2017) transplanted seedlings collected from the upper edge, middle and lower edge of the species' elevational/thermal range. These transplants revealed that the responses to warming varied between populations such that the mid-range populations were generally more sensitive to warming than the range-edge populations. This same study also showed that seedling survival increased with elevation. This suggests that the most favorable conditions for seedling survival of this species occur in areas near the upper limit of the current elevational range, contrary to expectation areas near to the middle of species' range should be the most favorable and that conditions should be more stressful near range limits (Holt, 2003; Sexton et al., 2009; **Figure 2A**). Overall, fine-scale experimental approaches such as these could help us to better predict the future abundances and distributions of species under altered climate scenarios, as well as the potential evolutionary responses of species (Verheyen et al., 2019). The possibility that the abundance patterns within species ranges could remain constant or shifted as species' ranges move, expand or contract (**Figure 2B**) under climate change (Feeley et al., 2012; Feeley, 2015; Peterson et al., 2019) also needs to be tested empirically.

CONCLUSION

There are many knowledge gaps and areas of uncertainty about how species will respond to current and future climate change. In particular, we know very little about how biotic interactions (e.g., between plants and their competitors, herbivores, pollinators, pathogens, and soil microbes) will be affected by altered climates and how these interactions will influence individual species and communities. The field experiment studies that have been conducted to date indicate that transplant and translocation experiments along natural environmental gradients in mountain systems offer a very valuable, but underutilized, approach to study species' response to climate warming. We encourage future studies to take advantage of natural gradients to help us gain a more complete understanding of the possible impacts of climate change on species, communities and biodiversity.

DATA AVAILABILITY STATEMENT

Publicly available datasets were analyzed in this study. This data can be found here: http://www.scielo.org.pe/scielo.php?script=sci_arttext&pid=S1727-99332015000200004.

AUTHOR CONTRIBUTIONS

RT conceived the idea. All authors contributed to the writing and editing of the manuscript.

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Impact of Mean Annual Temperature on Nutrient Availability in a Tropical Montane Wet Forest

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Despite growing understanding of how rising temperatures affect carbon cycling, the impact of long-term and whole forest warming on the suite of essential and potentially limiting nutrients remains understudied, particularly for elements other than N and P. Whole ecosystem warming experiments are limited, environmental gradients are often confounded by variation in factors other than temperature, and few studies have been conducted in the tropics. We examined litterfall, live foliar nutrient content, foliar nutrient resorption efficiency (NRE), nutrient return, and foliar nutrient use efficiency (NUE) of total litterfall and live foliage of two dominant trees to test hypotheses about how increasing mean annual temperature (MAT) impacts the availability and ecological stoichiometry of C, N, P, K, Ca, Mg, Mn, Fe, Zn, and Cu in tropical montane wet forests located along a 5.2°C gradient in Hawaii. Live foliage responded to increasing MAT with increased N and K concentrations, decreased C and Mn concentrations, and no detectable change in P concentration or in foliar NRE. Increases in MAT increased nutrient return via litterfall for N, K, Mg, and Zn and foliar NUE for Mn and Cu, while decreasing nutrient return for Cu and foliar NUE for K. The N:P of litterfall and live foliage increased with MAT, while there was no detectable effect of MAT on C:P. The ratio of live foliar N or P to base cations and micronutrients was variable across elements and species. Increased MAT resulted in declining N:K and P:K for one species, while only P:K declined for the other. N:Ca and N:Mn increased with MAT for both species, while N:Mg increased for one and P:Mn increased for the other species. Overall, results from this study suggest that rising MAT in tropical montane wet forest: (i) increases plant productivity and the cycling and availability of N, K, Mg, and Zn; (ii) decreases the cycling and availability of Mn and Cu; (iii) has little direct effect on P, Ca or Fe; and (iv) affects ecological stoichiometry in ways that may exacerbate P–as well as other base cation and micronutrient – limitations to tropical montane forest productivity.

Keywords: ecological stoichiometry, Hawaii, litterfall, macro- and micronutrients, mean annual temperature, nutrient resorption efficiency, nutrient use efficiency

INTRODUCTION

Rising global temperatures are predicted to increase biogeochemical process rates including the cycling of carbon (C) (Boisvenue and Running, 2006; Heimann and Reichstein, 2008; Litton and Giardina, 2008; Lin et al., 2010) and nitrogen (N) (Hart and Perry, 1999; Rustad et al., 2001; Bai et al., 2013). Cross-site syntheses (Raich et al., 2006; Litton and Giardina, 2008), eddy flux networks spanning global temperature gradients (Baldocchi et al., 2001; Luyssaert et al., 2007), and elevation gradients (Moser et al., 2011; Giardina et al., 2014; Malhi et al., 2017), generally show that in the absence of water limitations, rising mean annual temperature (MAT) increases forest C cycling. *In situ* soil warming, greenhouse and open-top chamber studies (Rustad et al., 2001; Bai et al., 2013) and soil core replacement studies (Hart and Perry, 1999; Hart, 2006) also collectively show that rising temperatures increase N cycling and availability. Despite this growing body of work, insights into how long-term and whole forest warming affect the suite of essential and potentially limiting nutrients are lacking, and tropical forests are especially understudied despite accounting for a significant proportion of global terrestrial C storage and productivity (Saugier et al., 2001; Bonan, 2008).

Methodological constraints associated with warming an entire forest are the major driver of this knowledge gap. For example, soil warming manipulation experiments (e.g., Melillo et al., 2017) warm the belowground environment, but tree stems and the forest canopy are not subjected to warming, thereby decoupling above- and belowground ecophysiological processes that regulate the supply and acquisition of C, N, other essential nutrients and water (Chapin III et al., 2011). Other studies have addressed this methodological concern by warming single small trees or even very small statured forests (Chung et al., 2013), but these studies have been limited to temperate regions (Cavaleri et al., 2015) and unavoidably represent short-term (<10 year) and small-scale (<0.1 ha) insights into whole forest response to rising temperatures. Moreover, nutrient responses to warming, which are critical to understanding feedbacks to forest C budgets and terrestrial C balance, remain understudied in temperate and especially tropical regions (Melillo et al., 2011).

As a result, confidence in model-based forecasts of the role of forests in mitigating rising atmospheric CO_2 levels is constrained by the lack of whole ecosystem warming studies. Classic expectations point to increased productivity in a warmer world, but warming can create or exacerbate other limitations to productivity. For example, Free Air CO_2 Enrichment (FACE) experiments have shown that macro- and micronutrients can respond to CO_2 enrichment in complex ways (Liu et al., 2007), with initial CO_2-driven increases in productivity sometimes disappearing as N becomes immobilized and progressively limiting to productivity (Norby et al., 2010; Feng et al., 2015; Terrer et al., 2019). Further, nutrient availability can influence decomposition rates and the release of nutrients from decomposing litter (Liu et al., 2007). Together, these studies highlight that a more complete understanding of the response of forest C cycling to rising temperatures should include assessments of impacts on the cycling and availability of essential macro- and micronutrients.

Evidence from elevation gradient studies indicates that increasing MAT elevates N cycling rates and availability. Marrs et al. (1988) found that field net N mineralization and nitrification rates increased with rising MAT across a 2,600 m elevational gradient in tropical wet forests of Costa Rica. Cardelús and Mack (2010) documented enrichment in canopy tree foliage $\delta^{15}N$ with increasing MAT along this same gradient, indicating increased N cycling rates (Craine et al., 2009). Across an Ecuadorian gradient, Moser et al. (2011) found that N limitations to productivity increased with elevation, while in an Arizona, United States reciprocal soil core transplant experiment, a 2.5°C increase in MAT resulted in an 80% increase in net N mineralization and nitrification rates (Hart and Perry, 1999; Hart, 2006). Results from gradient studies are supported by many manipulative soil warming studies. The first meta-analysis of 12 studies Rustad et al. (2001) reported that experimental warming increased net N mineralization on average by 46%. In a more recent meta-analysis of 528 observations from 51 studies, Bai et al. (2013) reported that experimental soil warming increased net N mineralization (52%), net nitrification (32%), and pools of soil inorganic N (20%) and leaf N (3%). Neither meta-analysis included tropical sites, but a tropical mesocosm soil warming study documented more than a doubling of extractable soil NO_3^- with soil warming (Liu et al., 2017). The combined results of elevation gradient and manipulative studies demonstrate that in the absence of moisture limitations, warming causes a near universal increase in N cycling and availability. Liu et al. (2017) also reported that soil warming increased soil P availability by ∼20%, but the hypothesis that warming accelerates biogeochemical process rates for other elements has yet to be tested in a whole forest warming study, and the response of essential nutrients other than N and P to whole forest warming is largely unknown (Vitousek et al., 1992; Tanner et al., 1998).

Numerous methods are used to assess nutrient limitation in forests including manipulative (e.g., fertilization experiments), indicator-based (e.g., foliar stoichiometry, nutrient resorption efficiencies), laboratory assay, and nutrient depletion-based methods (Sullivan et al., 2014). None are problem free or equally suited for all ecosystem types, but all have shown utility for assessing nutrient limitation, particularly when multiple techniques are applied in a single study (Sullivan et al., 2014). Aboveground litterfall and nutrient return via litterfall are robust indicators of ecosystem metabolism (Vitousek, 1984; Litton et al., 2007) because aboveground litterfall is a major avenue for C and nutrient transfer between vegetation and soils (Vitousek, 1984; Uriarte et al., 2015), with litterfall varying strongly by species and forest type (Binkley and Giardina, 1998; Tang et al., 2010), soil type (Vitousek, 2004), over time (Chave et al., 2010), and in response to changes in atmospheric CO_2 concentrations (Norby et al., 2005) and climate (Sayer and Tanner, 2010). Litterfall is also strongly linked to total above- and belowground C flux in forests, accounting for approximately 10% of gross primary production and showing little variation across a global range in forest types (Raich and Nadelhoffer, 1989; Litton et al., 2007).

Elevation gradients in MAT offer an observational approach to monitoring the effects of rising temperatures on whole ecosystems. While avoiding the issues discussed above, gradient-based approaches are often poorly constrained with respect to covariation of other variables that drive ecosystem processes (e.g., moisture availability, soils, vegetation, land-use and disturbance history). We established a highly constrained MAT gradient spanning 13.0 to 18.2°C in mature tropical montane wet forest on Hawai'i Island in which substrate age and soil type, vegetation composition, disturbance history, soil water availability, and solar radiation are all relatively constant (Litton et al., 2007; Giardina et al., 2014; Selmants et al., 2014). We view this MAT gradient as a model study system (distinct from a mesocosm) for testing hypotheses about the effects of rising MAT on ecosystem processes because these fully functioning ecosystems are not constrained by unnecessary variation (Vitousek, 2004). Further, this model study system is: (i) tractable because the entire gradient occurs over a spatial scale of <15 kilometers, greatly facilitating the logistics of conducting regular intensive measurements; (ii) simple because tree diversity in all plots is low and homogenous for tropical forests (Ostertag et al., 2014; Lutz et al., 2018), with the same two species accounting for 84–97% of stand tree basal area in all plots (Litton et al., 2011); and (iii) representative because these forests are structurally and functionally similar to other wet tropical forests despite low tree diversity (Ostertag et al., 2014; LaManna et al., 2017; Johnson et al., 2018; Lutz et al., 2018). For example, Lutz et al. (2018) showed that across the Forest Global Earth Observatory (ForestGEO) network of forest dynamics plots, the tropical montane wet forests from our MAT gradient (Laupāhoehoe) are structurally representative of moist and wet tropical forests with respect to: stand density (Laupāhoehoe = 3925 stems ha^{-1}; global mean = 5276; global range = 1692–8956); stand biomass (Laupāhoehoe = 241 Mg ha^{-1}; global mean = 305; global range = 111–495); large diameter threshold above which half of total biomass in a forest is contained (Laupāhoehoe = 63 cm; global mean = 47; global range = 29–72); and percentage of total biomass contained in the largest 1% of trees (Laupāhoehoe = 58%; global mean = 57; global range = 17–83). With respect to function, total belowground C flux (TBCF) for our plots (1200 to 1800 g C m^{-2} yr^{-1}; Giardina et al., 2014), a robust indicator of belowground function (Litton et al., 2007), is comparable to the range of TBCF reported for tropical forests globally (600 to 1600 g C m^{-2} yr^{-1}; Litton and Giardina, 2008).

We used this model study system to examine how aboveground litterfall, live foliar nutrient concentration, foliar nutrient resorption efficiency (NRE), nutrient return via litterfall, and nutrient use efficiency (NUE) of a suite of macronutrients (C, N, P, K) and micronutrients (Ca, Mg, Mn, Cu, Zn, Fe) vary with MAT. To understand MAT effects on ecological stoichiometry, we examined C:N, C:P, and N:P, and the amounts and ratios of K, Ca, Mg, Mn, and Fe, in stand-level litterfall and live foliage of the two tree species that dominate all plots across this gradient. From global data analyses (Raich et al., 2006; Luyssaert et al., 2007; Litton and Giardina, 2008; Giardina et al., 2014) and prior work from our MAT gradient, we know that rising MAT increases: (i) the flux of C in litterfall by increasing net primary production; and (ii) soil NO_3^- availability via increased ammonia oxidizer activity (Pierre et al., 2017).

Based on this prior work, we hypothesized that rising MAT would: (***H1***) increase live foliage concentrations of a suite of macro- and micronutrients due to temperature-driven increases in N cycling and availability (Rustad et al., 2001; Bai et al., 2013; Pierre et al., 2017); (***H2***) decrease NRE of foliar N and P, as increased live foliage nutrient concentrations (i.e., ***H1***) drive decreased foliar resorption of nutrients (Kobe et al., 2005; Vergutz et al., 2012), macronutrient NRE appears to be negatively correlated with MAT across broad latitudinal gradients (Vergutz et al., 2012), and forest productivity appears to be co-limited by N and P in this study system (Vitousek and Farrington, 1997); (***H3***) increase nutrient return through litterfall and decrease NUE for the suite of macro- and micronutrients examined based on meta-analyses results demonstrating temperature-driven increases in the cycling and availability of N (Rustad et al., 2001; Bai et al., 2013); (***H4***) given ***H1*** and ***H3***, not alter ecological stoichiometry in litterfall and live foliage C:N:P (McGroddy et al., 2004); and (***H5***) decrease N:element and P:element for K, Ca, Mg, Mn, Cu, Zn, and Fe based on previous findings that N:element and P:element generally decrease with experimental warming and with increasing MAT across continental scale gradients (Tian et al., 2019).

MATERIALS AND METHODS

Site Description

The study was conducted within nine 20 × 20 m permanent plots arrayed across a 5.2°C MAT gradient ranging from 800 (18.2°C) to 1600 (13°C) m above sea level in the Laupāhoehoe Unit of the Hawaii Experimental Tropical Forest (19°56′41.3″ N, 155°15′44.2″ W) and the Hakalau Forest National Wildlife Refuge (19°50′31.3″ N, 155°17′35.2″ W) on the Island of Hawaii. Mean annual precipitation ranges from ~3 m at the top of the elevation gradient to ~4.5 m at the bottom (**Supplementary Table S1**); because potential evapotranspiration also increases with MAT (**Supplementary Table S1**), we found very little variation (CV = 10.5%) in mean monthly soil water content across plots – a critical index of water availability to plants (**Supplementary Table S2**). Because all plots are located below the average base height of the trade wind inversion, the above-canopy light environment varies by <5% across this MAT gradient (**Supplementary Table S1**). All plots are defined as tropical montane wet forests and classified as mature and mildly aggrading *Metrosideros polymorpha* Gaudich.-*Acacia koa* A. Gray forests (Litton et al., 2011), typical of windward forests on Hawaii Island (Asner et al., 2009; Jacobi et al., 2017). The canopy tree species *M. polymorpha* and the mid-canopy tree species *Cheirodendron trigynum* (Gaudich.) A. Heller comprise 84 to 97% of tree basal area (BA) across all plots (Litton et al., 2011; Selmants et al., 2014).

We used high resolution (<2 m horizontal and <0.2 m vertical) light detection and ranging (LiDAR) to select the nine plots with maximum basal area for a given elevation within the same soil type. These plots were selected from 10 to 20 candidate plots per elevation that were identified as being centered within larger stands of similarly sized forest. Consequently, stand level increases in BA and decreases in stand density with MAT represent real effects of MAT (for a detailed description of plot selection methods see Litton et al., 2011; Selmants et al., 2014). The mildly aggrading, mature condition of plots and the larger surrounding forest was determined using repeat high resolution LiDAR imagery to examine biomass change over time (Kellner and Asner, 2009).

Soils across the gradient are classified as tephra-derived, moderate to well-drained hydrous, ferrihydritic/amorphic, isothermic/isomesic Acrudoxic Hydrudands of the closely related Akaka, Honokaa, Maile, and Piihonua soil series (**Supplementary Table S2**). These soil series are moderately common across Hawaii Island covering ∼40,000 ha[1], with the larger Andisol soil order comprising ∼50% of all soils on Hawaii Island (Deenik and McClellan, 2007). Based on radiocarbon analyses of soils to 1 m depth, soil age across all plots is ∼20,000 years (Giardina et al., 2014), one of three common soil ages across windward Hawaii Island. Soil pH, cation exchange capacity, base saturation and bulk density are all relatively constant across plots (**Supplementary Table S2**).

Aboveground Litterfall

Fine aboveground litterfall (foliage, reproductive tissue, and branches and twigs < 2.54 cm diameter) was collected monthly from eight replicate 0.174 m^2 litter traps in each of the nine plots for one year from April 2009 to March 2010 (Giardina et al., 2014). Litterfall from traps within a given plot was composited, resulting in a single litterfall sample per MAT plot for each of 12 months. Litterfall was placed in a forced-air drying oven within 12 h of collection, dried at 70°C to a constant mass, and sorted and weighed by species and component: tree leaf litterfall, tree fern litterfall, reproductive tissue (fruits and flowers), woody material, and other material too fine to identify. Component litterfall for each month and plot was ground on a Wiley-mill and then processed on a ball mill until the entire sample could be passed through a #40 mesh.

Live Foliage Nutrient Concentration and Nutrient Resorption Efficiency

Live foliage in each plot was collected in 2010 from three individuals of each of the two dominant tree species (*M. polymorpha* and *C. trigynum*), which together comprise ≥84% of stand tree basal area (Litton et al., 2011). All samples were taken from the most recent fully expanded cohort of leaves from the middle (*M. polymorpha*) or upper (*C. trigynum*) third of the canopy that were exposed to full sunlight at least part of the day. Individual leaves (3–5 from each tree) were oven-dried at 70°C in a forced-air oven to a constant mass and ground on a ball mill to pass a #40 mesh. All nutrient analyses (C, N, P, Mg, K, Ca, Mn, Zn, Cu, and Fe) were conducted at the University of Hawaii at Hilo Analytical Lab. Carbon and N concentrations were measured on a Costech 4010 Elemental Combustion system; other nutrients were measured on a Varian Vista MPX ICP-OES after approximately 0.25 g of sample was dry-ashed (5 h at 500°C) and resuspended in 0.5 M HCl.

Nutrient resorption (or retranslocation) efficiency (NRE), defined as the proportional (%) withdrawal of a nutrient during senescence, was calculated for N and P in both *M. polymorpha* and *C. trigynum* on a mass basis following Vergutz et al. (2012) as:

$$\text{NRE} = 1 - (\text{Mass of Nutrients in Foliar Litterfall}/ \\ \text{Mass of Nutrients in Live Foliage}) \times 100$$

Live leaf mass was estimated as the average value for each species at each MAT plot from the live foliage samples (see above). To account for leaf mass loss during senescence, a necessary step for estimates of resorption (van Heerwaarden et al., 2003), we used a global mass loss correction factor of 0.78 for evergreen angiosperms from Vergutz et al. (2012). This approach assumes that leaching of nutrients from litterfall traps between monthly litterfall collections is minimal. We limited this analysis to N and P because prior studies have shown that resorption of these two nutrients is orders of magnitude higher than leaching losses (see Vergutz et al., 2012). Nonetheless, reported NRE values for N and P are likely overestimates. This limits our ability to compare our NRE values with those of other studies, but still allows for exploration of relationships with MAT within our study system, with the assumption that tissue mass loss resulting from senescence does not vary along the MAT gradient – a reasonable assumption given the constrained nature of the gradient.

Nutrient Return via Litterfall

Litterfall nutrient concentrations of C, N, P, K, Mg, Ca, Mn, Zn, Cu, and Fe were analyzed separately for each plot, month and litterfall component as described above. Across plots and months, nutrient concentrations were determined directly for 89% of all litterfall samples (range of 85–92% for individual plots). For the other ∼10% of litterfall (range of 7–14% across plots), there was not enough tissue available to analyze nutrient concentrations directly, so nutrient concentrations were based on the average of all available values for that plot and litterfall component from the remaining time periods. In so doing, we assumed that there was no variability in litterfall nutrient concentration for a given component at a given MAT over the course of a year. The remaining 1% of total litterfall (range of 0.3–2.4% across plots) was not directly analyzed or estimated for nutrient concentration, and was only included in the analysis of litterfall mass.

Ideally, nutrient use efficiency (NUE; production of organic matter per unit of nutrient taken up) is estimated from total production per total amount of a nutrient acquired by a plant over a given amount of time. Alternatively, nutrient return via litterfall can be calculated as the product of the mass and the nutrient concentration of a given litterfall component for a given MAT and month and summed across components and months for a given plot to calculate NUE following Vitousek (1982) as: NUE = $\text{Mass}_{\text{litterfall}}$ / Nutrient return via litterfall. This approach defines $\text{Mass}_{\text{litterfall}}$ ($g\ m^{-2}\ yr^{-1}$) as the total litterfall mass for a given plot, with nutrient concentration of litterfall ($g\ m^{-2}\ yr^{-1}$) calculated as above. This alternative approach may

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[1] https://soilseries.sc.egov.usda.gov/

overestimate NUE for reasons relating to nutrient resorption, such that estimations are sensitive to NRE. Because NRE did not vary with MAT for any nutrient examined (see section "Results" below), we viewed this approach as providing a reasonable index of the response of NUE to MAT (Vitousek, 1982).

Ecological Stoichiometry

We examined MAT effects on mass-based ecological stoichiometry of C:N:P, as well as the ratios of N and P to K, Ca, Mg, Mn, and Fe, for total annual litterfall and live foliage of the canopy and mid-story dominant trees (*M. polymorpha* and *C. trigynum*, respectively). Results were used to index relative nutrient availability and nutrient limitation (Sullivan et al., 2014).

Data Analyses

We used simple linear regression analyses (SPSS Statistics Ver. 25.0; IBM Corp.) to examine MAT effects on the various independent variables. Because most variables met assumptions of normality and homogeneity of variance and these tests are difficult to interpret with small sample sizes, all analyses were conducted on untransformed data. Individual plots were the experimental unit for all analyses ($n = 9$); litterfall collections were composited across traps within months and a given plot to give a single representative subsample of each litterfall component for each plot and month for nutrient content analysis. This approach does not allow for examination of within-plot variability, but a prior study along this MAT gradient showed that the within-plot coefficient of variation (CV) for soil-surface CO_2 efflux, a highly spatially variable C flux, ranged from 17 to 46%, with an average CV of 33% across all plots and increasing within-plot variability with increasing MAT (Litton et al., 2011). As a result of these small sample sizes, $\alpha = 0.10$ was used to determine significance, with a $P \leq 0.10$ cutoff used to present regression lines.

RESULTS

Aboveground Litterfall

Aboveground litterfall mass ranged from ∼450 to 900 g m^{-2} yr^{-1} across all plots, and increased linearly and positively with MAT (**Figure 1**; $r^2 = 0.50$; $P = 0.03$). For every 1°C increase in MAT, litterfall increased by 47.3 g m^{-2} yr^{-1}. *M. polymorpha* foliage was the primary litterfall component across the MAT gradient, accounting for an average of 47% (range of 33–57%) of total annual litterfall across plots. Overall, tree foliage was the primary component of litterfall across plots (47–78% of total litterfall), followed by woody material (10–20%), *Cibotium* spp. fronds (2–19%), unidentified material (3–9%), and reproductive tissues (1–9%).

Live Foliage Nutrient Concentration and Nutrient Resorption Efficiency

Nitrogen concentration of live foliage increased with MAT for both *M. polymorpha* ($r^2 = 0.46$; $P = 0.05$) and *C. trigynum* ($r^2 = 0.33$; $P = 0.10$) (**Figure 2**). Live foliar K concentration also increased with MAT for *C. trigynum* ($r^2 = 0.50$; $P = 0.03$), but no detectable effect was observed for *M. polymorpha* ($r^2 = 0.09$; $P = 0.44$). In contrast, live foliar C and Mn concentrations decreased with MAT for both *M. polymorpha* (C: $r^2 = 0.88$ and $P < 0.01$; Mn: $r^2 = 0.71$ and $P = 0.01$) and *C. trigynum* (C: $r^2 = 0.84$ and $P < 0.01$; Mn: $r^2 = 0.59$ and $P = 0.02$). There was no detectable effect of MAT on live foliar nutrient concentrations of the other elements for either species (**Figure 2**), or on foliar nutrient resorption efficiencies for N or P in either *M. polymorpha* ($r^2 \leq 0.15$) or *C. trigynum* ($r^2 \leq 0.15$) (**Figure 3**).

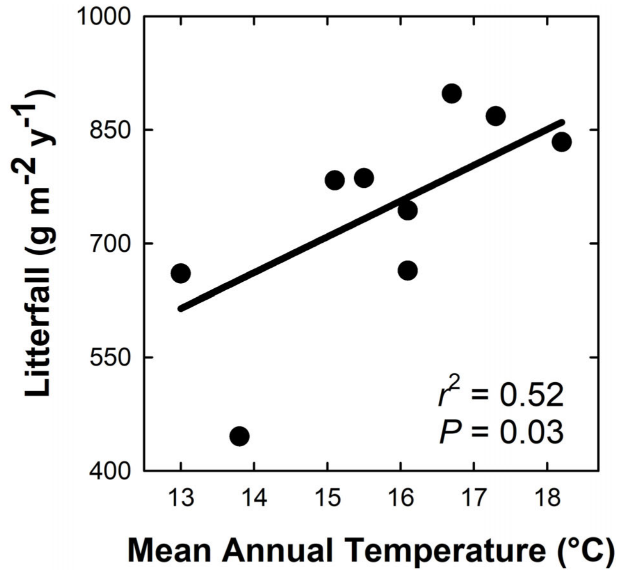

FIGURE 1 | Aboveground litterfall mass (g biomass m^{-2} yr^{-1}) increases with mean annual temperature across a 5.2°C gradient in a Hawaiian tropical montane wet forest.

Nutrient Return via Litterfall

Nutrient return via litterfall increased with MAT for N ($r^2 = 0.40$; $P = 0.07$), K ($r^2 = 0.60$; $P = 0.02$), Mg ($r^2 = 0.52$; $P = 0.03$), and Zn ($r^2 = 0.58$; $P = 0.02$) but decreased with MAT for Cu ($r^2 = 0.64$; $P < 0.01$) (**Figures 4**, **5**). There were no detectable effects of MAT for P, Ca, Mn and Fe ($P > 0.12$). In turn, we found that MAT decreased NUE for K ($r^2 = 0.43$; $P = 0.06$) and marginally for Zn ($r^2 = 0.33$; $P = 0.10$), and increased NUE for Mn ($r^2 = 0.72$; $P < 0.01$) and Cu ($r^2 = 0.65$; $P < 0.01$). There was no detectable effect of MAT on NUE for the remaining elements examined ($P > 0.14$) (**Figures 4**, **5**).

Ecological Stoichiometry

We detected no effect of MAT on litterfall C:N ($r^2 = 0.22$; $P = 0.21$), but C:N of live foliage decreased with MAT for both *M. polymorpha* ($r^2 = 0.51$; $P = 0.03$) and *C. trigynum* ($r^2 = 0.54$; $P = 0.02$). Litterfall N:P increased with MAT ($r^2 = 0.37$; $P = 0.08$), as did live foliage N:P for both *M. polymorpha* ($r^2 = 0.60$; $P = 0.01$) and *C. trigynum* ($r^2 = 0.52$; $P = 0.03$) (**Figure 6**). There was no detectable effect of MAT on either C:P of litterfall ($r^2 = 0.01$; $P = 0.81$) or of live foliage for either *M. polymorpha* ($r^2 = 0.27$; $P = 0.16$) or *C. trigynum* ($r^2 = 0.11$; $P = 0.39$).

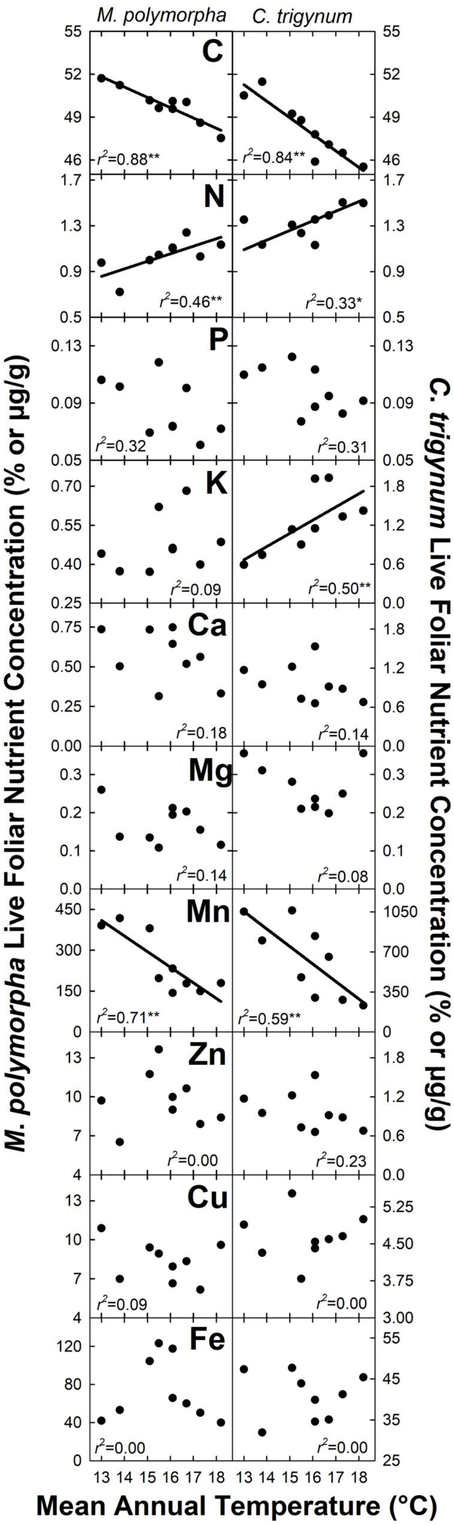

FIGURE 2 | Nutrient concentration of live foliage biomass for the two dominant trees (*Metrosideros polymorpha* and *Cheirodendron trigynum*) in a Hawaiian tropical montane wet forest across a 5.2°C mean annual temperature gradient. Values are displayed as % for macronutrients (C, N, P, K, Ca, and Mg) and ug g^{-1} for micronutrients (Mn, Zn, Cu, and Fe). Significance of regression is indicated as $^{*}P < 0.10$ and $^{**}P < 0.05$.

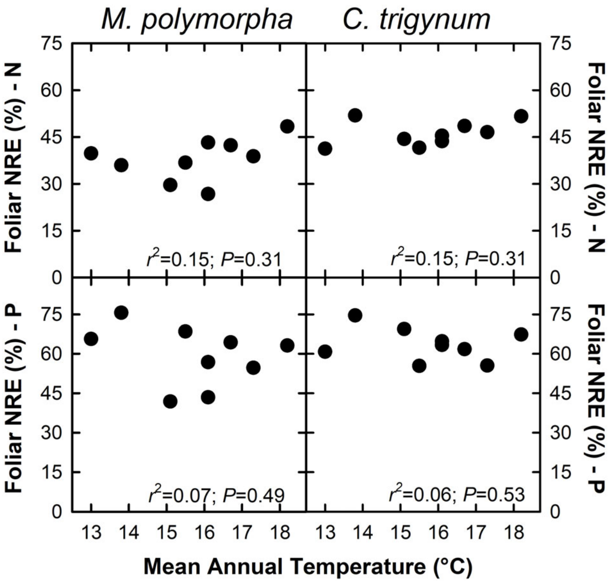

FIGURE 3 | Foliar nutrient resorption efficiency (NRE; %) of nitrogen (N) and phosphorus (P) in the two dominant trees (*Metrosideros polymorpha* and *Cheirodendron trigynum*) does not vary across a 5.2°C mean annual temperature gradient in Hawaiian tropical montane wet forest.

The ratio of N or P to base cations and micronutrients for live foliage was variable across elements, and to some extent between species (**Figure 7**). Increased MAT resulted in declining N:K and P:K for *C. trigynum* (N:K $r^2 = 0.55$, $P = 0.02$; N:P $r^2 = 0.82$, $P < 0.01$), while P:K declined for *M. polymorpha* ($r^2 = 0.81$; $P < 0.01$). Both N:Ca and N:Mn increased with MAT for *M. polymorpha* (N:Ca $r^2 = 0.34$, $P = 0.10$; N:Mn $r^2 = 0.65$, $P = 0.01$) and *C. trigynum* (N:Ca $r^2 = 0.36$, $P = 0.09$; N:Mn $r^2 = 0.60$, $P = 0.01$), while N:Mg increased with MAT for *M. polymorpha* ($r^2 = 0.35$; $P = 0.09$) and P:Mn increased with MAT for *C. trigynum* ($r^2 = 0.42$; $P = 0.06$). The MAT response of these element ratios in litterfall generally aligned with live foliage results, but were more variable and weaker.

DISCUSSION

Results from our highly constrained MAT gradient demonstrate that warming can: (i) accelerate ecosystem processes; (ii) variably impact the availability of different elements; and (iii) alter ecological stoichiometry. Collectively, these findings provide strong evidence that increased MAT alters the cycling and availability of a broad suite of nutrients in tropical montane forests, with important implications for nutrient limitations to ecosystem processes in a warming world.

Live Foliage Nutrient Concentration and Nutrient Resorption Efficiency

We found limited support for the hypothesis that increasing MAT would increase live foliage macro- and micronutrient concentrations (***H1***). Only live foliar N concentrations (for both

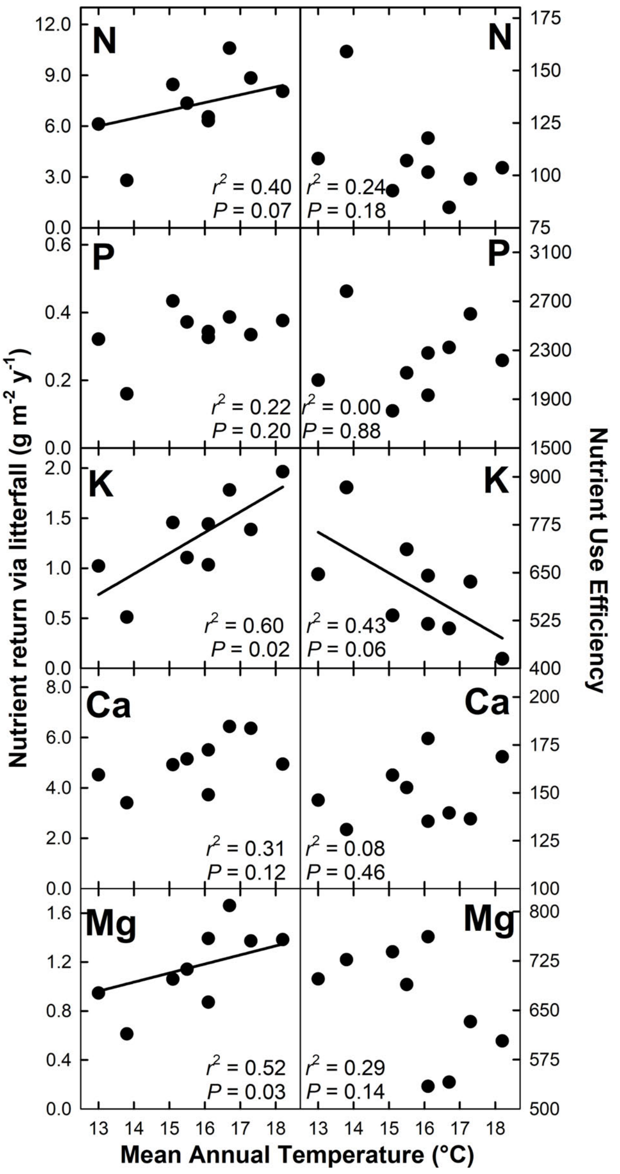

FIGURE 4 | Macronutrient return via total litterfall and macronutrient use efficiency for a Hawaiian tropical montane wet forest across a 5.2°C mean annual temperature gradient.

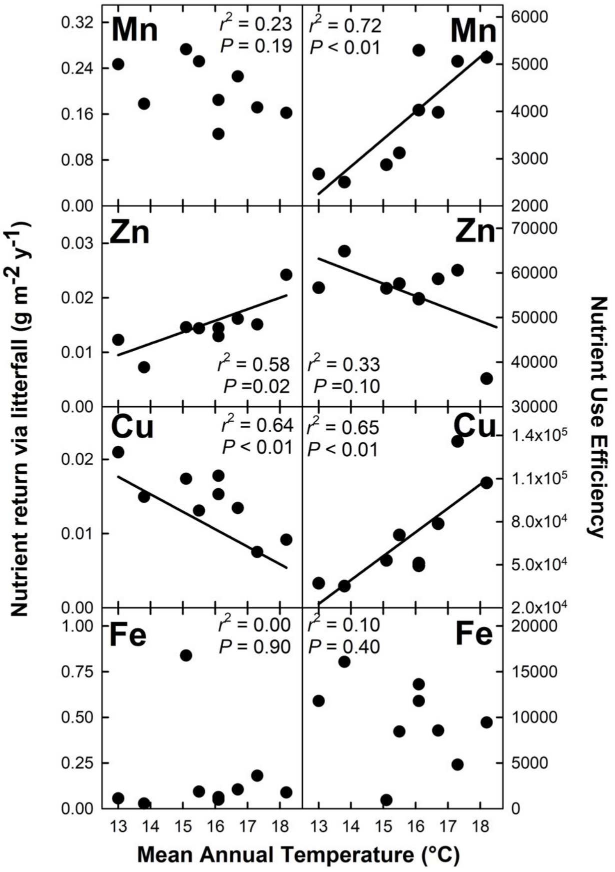

FIGURE 5 | Micronutrient return via total litterfall and micronutrient use efficiency for a Hawaiian tropical montane wet forest across a 5.2°C mean annual temperature gradient.

species) and K concentration (for one species) increased with MAT, while C and Mn concentrations showed the opposite pattern, and other nutrients showed no detectable pattern. Across this MAT gradient, increased foliar N concentrations with MAT are likely the result of increased N supply driven by temperature related increases in: (i) litter decomposition rates (Bothwell et al., 2014; Giardina et al., 2014), (ii) NO_3^- cycling and availability via increased ammonia oxidizer activity (Pierre et al., 2017), and (iii) total belowground C investment for nutrient acquisition (Giardina et al., 2014). Accelerated litter decomposition and increased belowground C supply could also explain the higher live foliage K concentration at warmer MATs. While less studied than N or P, fertilization with K has been shown to increase tree growth and plant tissue K concentrations. For example, Tripler et al. (2006) examined a wide diversity of boreal and temperate forests, as well as two tropical mangrove forests, and found that K fertilization increased tree growth in 69% of the forests examined.

Tanner et al. (1998) reported increased live foliar concentrations of N, P, and K with increasing MAT for individual tropical elevation gradients. However, when examined collectively across gradients, foliar N, P and K concentrations were unrelated to MAT. Vitousek (1998) found that for younger soils foliar N was higher at warm compared with cool forests, while Vitousek et al. (1992) found that foliar concentrations of N, P, and K in wet sites generally increased with rising MAT but foliar Ca and Mg showed little variation. Similarly, across a Malaysian elevation gradient, MAT increased leaf litterfall concentrations of N but not those of P, K, Ca, or Mg (Proctor et al., 1989). Bateman et al. (2019) found that for soils in Hawaii with similarly high water balance, total and exchangeable Ca and Mg were low and varied little with MAT. In a global survey, foliar N and P concentrations decreased with increasing MAT (Reich and Oleksyn, 2004). The discrepancy between our results and those of global scale syntheses could be explained by global

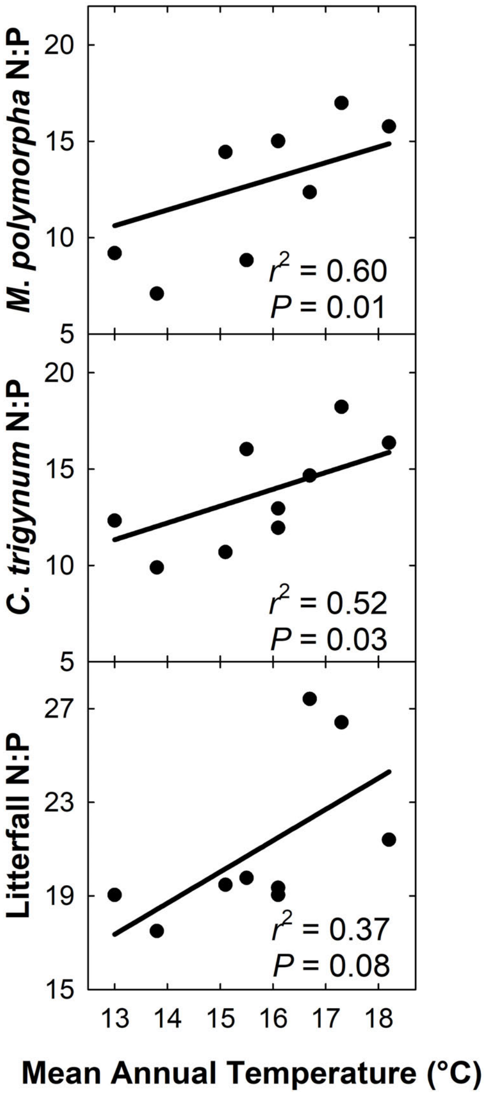

FIGURE 6 | N:P of litterfall and live foliage of both *M. polymorpha* and *C. trigynum* increased across a 5.2°C mean annual temperature gradient in Hawaiian tropical montane wet forest.

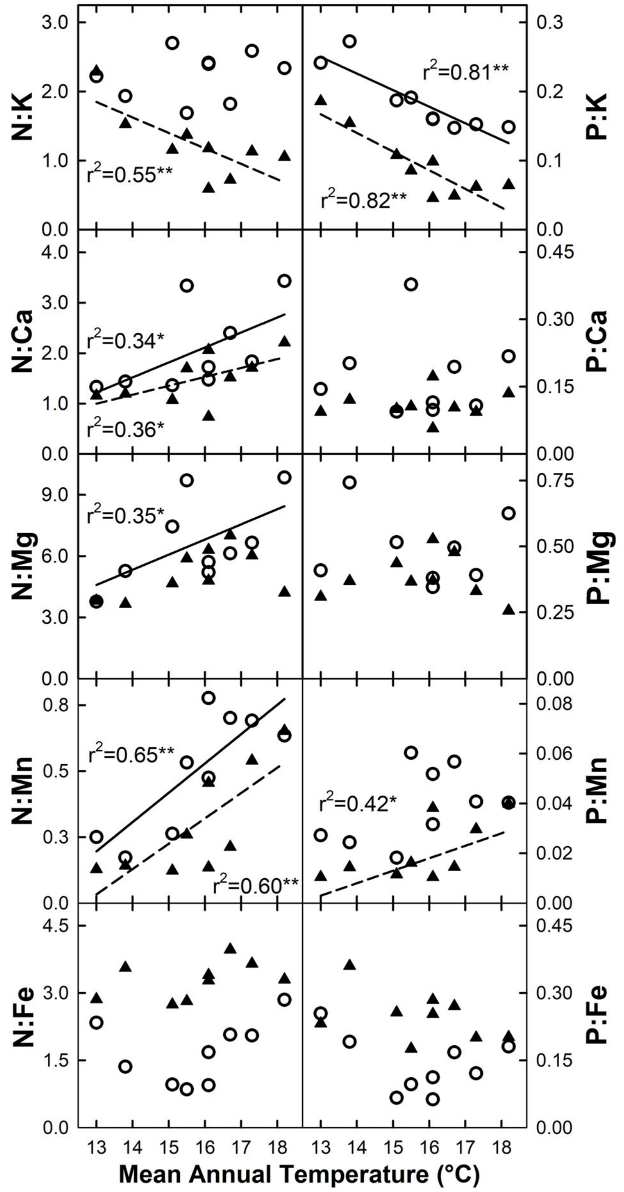

FIGURE 7 | Stoichiometric responses of live foliage nitrogen (N) and phosphorus (P) ratios with micro- and macronutrients for *M. polymorpha* (open circles and solid regression lines) and *C. trigynum* (closed triangles and dashed regression lines) across a 5.2°C mean annual temperature gradient in Hawaiian tropical montane wet forest. Significance of regression is indicated as $^*P < 0.10$ and $^{**}P < 0.05$.

comparisons capturing much greater variation in environmental drivers of nutrient dynamics other than MAT – for example, soil moisture, substrate and soils, disturbance history, and phylogenetic constraints are difficult to constrain across large scale gradients. Results from global, cross-site syntheses and meta-analyses provide important hypotheses about the impact of rising MAT on biogeochemical processes but may not align with the responses to MAT observed across individual gradients such as ours. As such, our study represents an important test of hypotheses about biogeochemical responses to rising MAT, with results providing much needed local scale data to inform modeling efforts.

We documented a strong, negative, linear response of live foliar Mn concentration to increasing MAT for both species examined (∼14% decrease for every 1°C increase in MAT). Litter decomposition may be strongly controlled by litter Mn content, particularly during late stages of decomposition (Berg et al., 2007, 2010), suggesting that Mn could play a critical role in the cycling and availability of other essential nutrients. However, across our gradient, litter decomposition increased with MAT (Bothwell et al., 2014; Giardina et al., 2014), despite: (i) a steep decline in live foliar Mn concentration; (ii) a trend of decreasing Mn return via litterfall; and (iii) increased nutrient use efficiency for Mn with increasing MAT (see below). These results indicate that Mn availability did not override other controls on litter decomposition rates. Further, while a global analysis indicated that Mn may have a role in controlling plant productivity (Ågren and Weih, 2012), declines in Mn with rising MAT were matched by increased litterfall and TBCF (Litton et al., 2011; Bothwell et al., 2014; Giardina et al., 2014).

We interpret the lack of a detectable response in live foliage nutrient concentration for other elements examined as evidence that either they are not limiting to primary production across our MAT gradient or their availability is not governed by MAT. While productivity appears to be co-limited by N and P in the middle of our MAT gradient (Vitousek and Farrington, 1997), our results support the idea that nutrient limitations are influenced by MAT. In line with these findings, Augusto et al. (2017) concluded that across a global range of sites, N limitations are driven by climate while P limitations are driven by soil parent material–explaining why P availability may be largely unresponsive to climate.

Our NRE estimates for N (38–46%) and P (59–64%) are similar to previously reported values for forests: 47% for N and 54% for P from a global synthesis of woody species (Yuan and Chen, 2009); 56% for N and 58% for P for evergreen woody angiosperms (Vergutz et al., 2012). However, the lack of variation in NRE we observed with MAT contrasts with Vergutz et al. (2012) who reported declining N and P NRE with increasing MAT, and with Yuan and Chen (2009) who reported that N NRE declined while P NRE increased with MAT. While increases in foliar nutrient content appear to drive decreases in foliar resorption (Kobe et al., 2005; Vergutz et al., 2012), Sullivan et al. (2014) found that foliar nutrient resorption was a poor indicator of nutrient limitation along a well-constrained substrate age gradient. Regardless, our results do not support the hypothesis that rising MAT will reduce foliar NRE for N and P (***H2***). Collectively, these findings highlight that because of confounding variation in other drivers of forests processes, global-scale patterns may not predict local responses to rising MAT.

Nutrient Return via Litterfall

We hypothesized that increased MAT would increase nutrient return through litterfall while reducing nutrient use efficiency (***H3***), which was supported for N, K, Mg, and Zn, but not for Mn and Cu, which showed opposite patterns, or for P, Ca, and Fe, which showed no detectable patterns. This variation across nutrients has important implications for assessing nutrient limitations to productivity and for modeling ecological stoichiometry (see below). Our gradient-based N cycling (Pierre et al., 2017) and litter decomposition (Bothwell et al., 2014) results align with those of short-term warming experiments (Rustad et al., 2001; Bai et al., 2013), and a recent analysis showing a strong effect of MAT on foliar N content across the Hawaii Experimental Tropical Forest where seven of our nine plots are located (Balzotti and Asner, 2018). In contrast, rising MAT had a more variable effect on the biogeochemical cycling of other essential macro- and micronutrients. Tripler et al. (2006) found that among base cations, K cycling was uniquely sensitive to biotic processes. However, we also found that the cycling of Mg and Zn, the later an essential micronutrient involved in a variety of enzymatic reactions (Broadley et al., 2007), both increased with MAT, indicating that elements other than K may be sensitive to warming which is in line with Ågren and Weih (2012) who reported that environmental factors can drive variation in the concentration of foliar macro- and micronutrients.

In our study, Mn and Cu had contrasting responses to MAT compared with N, K, Mg, and Zn, with the availability of each declining with increasing MAT. These declines drove increases in NUE. In contrast, there was no detectable MAT response for P, Ca, and Fe. We speculate that the decreased availability of Mn with rising MAT could indicate that warming accelerates Mn transformation by fungi into insoluble forms (Keiluweit et al., 2015). In contrast we have no interpretation for the decline in Cu availability with rising MAT, nor for the consequences of this response. The lack of detectable response in P availability to MAT can be interpreted as the result of: (i) the stability of P availability with warming, which constrains P uptake as N availability increases with MAT; or (ii) declining availability with rising MAT that is offset by increasing investment by trees belowground (i.e., TBCF) to secure P (Treseder and Vitousek, 2001). The lack of a pattern for Ca and Fe could indicate that declining availability is offset by increasing TBCF, or simply that these elements are not limiting to ecosystem processes.

Nutrient use efficiency (NUE) can serve as a proxy for nutrient availability, and has been shown to be nutrient specific and to vary among plant species (Vitousek, 1982; Tang et al., 2010), and with temperature (Raich et al., 1997; Unger et al., 2010). Our NUE estimates were larger than those found by Tang et al. (2010), but comparable to values reported by Vitousek (1984) and Inagaki et al. (2011). In our study, NUE largely reflected patterns in nutrient return via litterfall, indicating that K, Mg, and Zn availability increase with MAT, whereas Cu and Mn availability decrease with MAT, the later perhaps relating to increased plant demand in response to faster photosynthetic rates (Cu) or decreased solubility with decomposition (Mn).

Ecological Stoichiometry

Finally, we predicted that increased MAT would not alter ecological stoichiometry of live foliage and litterfall (***H4*** and ***H5***), which was supported only for litterfall and live foliage C:P – likely representing a shift in the potential of N and P to limit productivity. Litter and live foliage C:N and N:P both increased linearly and positively with MAT. These findings point to potential N limitations in the coolest plots, P limitations in the warmest plots and, as previously observed by Vitousek and Farrington (1997), N and P co-limitation in plots in the middle of the MAT gradient. The stronger N:P relationships with MAT for *C. trigynum* and *M. polymorpha* live foliage versus senesced litter could relate to: (i) collected litter was composed of diverse tissues (foliage, fruits, small stems) from all species occurring in the plots; (ii) nutrient resorption from live foliage during senescence varies across species and canopy position; (iii) nutrient mobility in response to leaching varies among elements, species and canopy position; and/or (iv) senesced leaves remain attached to stems in the canopy for weeks to months, during which leaching or other forms of decomposition occur.

While interpreting the significance of N:P for nutrient limitations can be complicated (Güsewell, 2004), foliar N:P < 14 generally corresponds with N-limitation, foliar N:P > 16 with P-limitation, and intermediate foliar ratios with N and P co-limitation (Aerts and Chapin, 1999). In our study live foliage N:P for the two study species averaged 9.6 in the two coolest plots, suggesting N limitation to productivity, while N:P for the two warmest plots averaged 16.8, indicating P-limitations. N:P for intermediate plots ranged from 12.4 to 14.0, indicating either weak N-limitation or co-limitation by both N and P. Increased N:P with increasing MAT was also documented in global analyses by Reich and Oleksyn (2004) and Yuan and Chen (2015), the latter of which was interpreted as a decoupling of the N and P biogeochemical cycles under global change. Importantly, ecosystems can adjust to changes in stoichiometry. For example, in Hawaii, Treseder and Vitousek (2001) found that excess N can be used by plants to accelerate phosphatase production and activity under P limiting conditions.

Spatial and temporal variation in macro- and micronutrient availability is an important driver of variation in plant stoichiometry (Ågren and Weih, 2012), with plant stoichiometry arising from physiological constraints that appear to be invariant to factors impacting ecosystem metabolism (McGroddy et al., 2004). While McGroddy et al. (2004) found that forests globally are characterized by relatively well-constrained C:N:P in foliage and litterfall, even small changes in stoichiometry can reflect shifting nutrient limitations. For example, in a global analysis that included data from McGroddy et al. (2004), Zechmeister-Boltenstern et al. (2015) observed that climate exerts a strong influence on the stoichiometry of leaves, roots, and leaf and root litter, with increasing MAT broadly increasing N:P of all four categories. In a more recent synthesis, Augusto et al. (2017) found that climate regulates N limitations, with N availability increasing with warming, but that P limitations are regulated by soil parent material. Working across a highly constrained four million-year geological chronosequence in Hawaii where N and P limitations to productivity vary with soil age, Treseder and Vitousek (2001) found that fertilization altered the mechanisms by which plants acquire soil N and P. For example, N additions stimulated P-tase activity in soil while additions of P suppressed P-tase activity but also reduced mycorrhizal colonization and P uptake capacity. Conversely, Herbert and Fownes (1999) found that variation in soil fertility across the same Hawaii chronosequence had little effect on net primary productivity or allocation of photosynthate to leaves, fine roots and wood.

Interpreting MAT driven changes in the stoichiometry of base cations and micro-nutrients is constrained by the low number of studies that have examined these elements. Tian et al. (2019) identified a negative effect of MAT on foliar N:K, N:Fe and P:Fe across natural climate gradients. Of these, only the decline in N:K aligns with our results, in partial support of ***H5***. Across our plots, foliar N content for *C. trigynum* and N return via litterfall both increased with rising MAT, but the increases for foliar and litterfall K were steeper. Because N and K are important macronutrients, their increased availability with warming would indicate that changes to N:K will be less impactful on forest productivity than stoichiometric changes involving P. In contrast to Tian et al. (2019), we also observed increasing K driving declines in foliar P:K for both species examined, also supporting ***H5***. Along with increasing N:P, declining P:K further supports the view that warming may cause or exacerbate P limitations to productivity in these forests.

We showed rising MAT increases N:Ca and N:Mn for both species and N:Mg for *M. polymorpha*, which for Ca and Mg were caused by increasing N, and for Mn by increasing N and declining Mn. We also observed increasing P:Mn with rising MAT for *C. trigynum,* which was driven solely by a decline in Mn as live foliar P concentrations and P return through litterfall did not vary with MAT. Overall, litter nutrient ratios responded less clearly to increased MAT, perhaps for reasons discussed above. However, litterfall patterns in N:Mn and to a lesser extent P:K and P:Mn were strong, in part because of strong MAT related patterns for N and K (increasing) and Mn (decreasing). Notably, the role of Ca, Mg, and especially Mn in limiting productivity of tropical forests is poorly understood, but across our sites their live foliage concentrations and nutrient return via litterfall declined, remained unchanged, or increased slightly with increased MAT. More information is needed on ecosystem level controls of base cation and micronutrient availability in tropical wet forests.

Taken together, our results indicate that warming in the absence of moisture limitations or altered disturbance regimes has the potential to increase tropical montane wet forest productivity and the cycling and availability of N and K (strong support) and Mg and Zn (moderate support), but will decrease the cycling and availability of Mn and Cu (moderate support). We found little evidence that warming will affect the cycling and availability of P, Ca or Fe, but warming-related increases in the supply of other elements may cause P, or possibly base cation, micronutrient limitations to ecosystem processes. The results presented here provide an enhanced picture of the response of a variety of micro- and macronutrients to future increases in temperature in tropical montane wet forests, which can be used to refine and parameterize ecosystem models needed to forecast ecosystem response to global environmental change.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

AUTHOR CONTRIBUTIONS

CL and CG designed the research, secured the funding, and collected the data. CL, KF, and PS performed the data analyses. CL and CG led interpretation and writing of the manuscript, with assistance from all other authors.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2020.00784/full#supplementary-material

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Predation on Multiple Prey Types Across a Disturbance Gradient in Tropical Montane Forests of Peninsular Malaysia

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Predation plays a critical role in animal and plant survivorship, and can be highly sensitive to habitat loss and disturbance. Tropical montane forests in Southeast Asia are being modified rapidly by land-use change, and the consequences of this on predation likelihood are poorly understood. In Peninsular Malaysia, we conducted predation experiments at eight tropical montane forest sites along a disturbance gradient. We investigated whether (1) predation pressure in primary forests differs between different mountains; (2) predation probability is linked to habitat degradation; and (3) vegetation variables explain predation occurrence. At each forest site, we placed artificial nests with real and model quail eggs, dishes with real and artificial seeds of the cempedak (*Artocarpus champeden*), models resembling four-lined tree frogs (*Polypedetes leucomystax*) and models of the late instar caterpillar of the common Mormon (*Papilio polytes*) at points 100 m apart for three nights. Using Bayesian binomial simulations, we showed that predation likelihood in primary forests from different mountains can vary (e.g., probability of the difference in predation rate of artificial caterpillars between two primary forests was estimated at 82–100%). We also found that higher predation was not linked to habitat degradation for all artificial prey and seeds (e.g., comparing forests of varying degrees of disturbance from the same mountain, the probability that predation of an artificial caterpillar is lower at the primary forest was estimated at 2–20% only). Model selection and hierarchical partitioning showed that vegetation variables can explain predation occurrence, suggesting microhabitat characteristics may be influential. Conducting predation experiments by using artificial prey and seeds is useful for comparing predation likelihood at different sites, making ecological comparisons, and for informing conservation decisions. This novel approach of using multiple prey items also showed that predation for each can vary and thus caution against deploying a single prey type to draw broad inferences of predation in degraded systems.

Keywords: artificial prey, Southeast Asia, tropical cloud forest, habitat structure, statistical modeling

INTRODUCTION

Predation is a highly influential ecological process (Sih et al., 1985; Schmitz et al., 2010), affecting animal reproductive strategies (Martin, 1995; Fontaine and Martin, 2006), plant distribution (Bazzaz and Pickett, 1980; Chapman et al., 2016), vegetation recovery and forest succession (Doust, 2011). Predators and their associated ecosystem functions can be highly sensitive to habitat degradation due to anthropogenic pressures (Dobson et al., 2006; Schmitz et al., 2010). Generally, disturbed or degraded forests experience a higher incidence of avian nest predation than their pristine counterparts (Stephens et al., 2003; Vetter et al., 2013); caterpillar predation has been shown to be higher in forest fragments compared to continuous forest, although season and prey density have a great deal of influence (Posa et al., 2007; Ruiz-Guerra et al., 2012; Tvardikova and Novotny, 2012); and seed predation may be higher in forest fragments (Guariguata et al., 2002).

"High order effects" may be responsible for the higher predation in disturbed forests. For example, the absence of mega-predators may increase the populations of some medium- to small-size macropredators, which in turn could be detrimental to their prey species (Turner and Corlett, 1996). Another mechanism that increases predation in degraded forests are "negative edge effects" whereby the altered internal microclimate of forest near the edge may increase the density of disturbance-associated opportunistic predators (Laurance, 1991; Turner and Corlett, 1996).

However, the relationship between predation and habitat disturbance in tropical montane forests remains unclear. For example, studies of the edge effect on avian nest predation in montane forests have equivocal findings: Carlson and Hartman (2001) found no edge effect on nest predation rate in an Eastern Arc montane forest in Tanzania and Spanhove et al. (2014) reported from a montane forest in Kenya that nest predation increased from the edge toward the forest interior. Conversely, Sedláček et al. (2014) provided evidence for an edge effect on nest predation (i.e., higher predation) in a montane forest in the Bamenda-Bano Highlands, Cameroon.

Disturbance is a major driver of plant species diversity in both ecological and evolutionary time. However, little is understood about how this affects the incidence of seed predation in tropical montane forests (Chapman et al., 2016). Chinchilla (2009) reported that seed predation of four native tree species was not different between a primary tropical montane forest and its fragments in Mexico, and Myster (2015) found that seed predation in an undisturbed primary Ecuadorian forest was higher than a nearby secondary forest recovered from agriculture.

As hotspots of endemism and sources of major river systems, Southeast Asia's tropical montane forests have high conservation value (Peh et al., 2011). Despite this biological and geological importance, montane forests in Southeast Asia are threatened by increasing encroachment from agriculture, commercial forestry, and infrastructure development (Peh et al., 2011). Some case studies imply that degradation of these forests can have detrimental effects on predator-prey relationships (Goldsmith et al., 2007) and reduce population viability of range-restricted species (Sedláček et al., 2014). Therefore, investigating this critical ecological process in both pristine and disturbed forests in tropical mountains is key to our understanding of the current and future state of these habitats (Soh et al., 2019).

In this study, we carried out predation experiments in tropical montane forests in Peninsular Malaysia at a landscape scale and across a suite of sites of varying disturbance. We used four different prey items—artificial nests, artificial seeds, caterpillar models and frog models—along a disturbance gradient, from pristine forests to tea plantations. Based on this novel approach (i.e., using multiple prey types), we assessed whether predation probability in a given montane habitat type (e.g., pristine forest or rural area) differs between mountain ranges. We hypothesized that predation probability between mountain ranges may differ even within the same habitat type as predator distribution can be heterogenous (see Emmering and Schmidt, 2011). We also investigated whether predation probability consistently varies in different habitat types, and hypothesized that more disturbed land uses face greater predation. Lastly, we investigated whether predation can be explained by vegetation structure.

MATERIALS AND METHODS

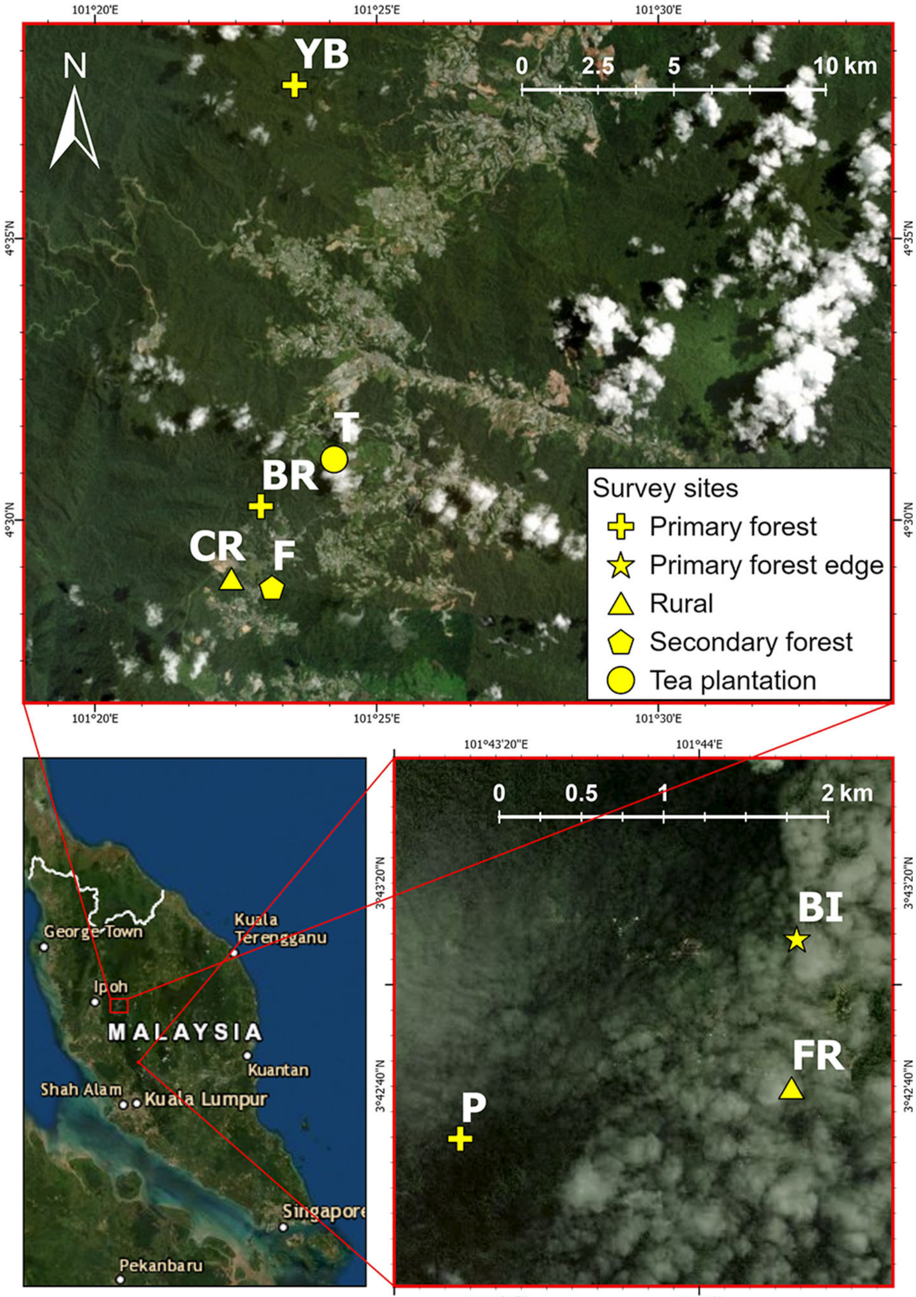
Study Areas

We surveyed a total of eight study sites across three locations (Cameron Highlands, Lojing Highlands, and Fraser's Hill) along the Titiwangsa mountain range in Peninsular Malaysia to represent a range of tropical montane habitats with varying degrees of human disturbance (**Figure 1**). These included primary forests (Pine, hereafter "Fraser primary"; Brinchang, "Cameron primary"; Yong Belar, "Lojing primary"), a primary forest edge (Bishop, "Fraser edge"), secondary forests (Forestry, "Cameron secondary"), a tea plantation ("Cameron tea") and rural areas in Fraser's Hill ("Fraser rural") and Cameron Highlands ("Cameron rural"). A summary of the habitat types, location, and background information of the eight sites is given in **Table 1**.

Predation Experiments

We carried out the predation experiments from 30 July to 22 August 2016 (**Table 1**). Models were made using Sculpey III non-toxic modeling clay and painted with non-toxic paint to mimic real quail eggs, four-lined tree frogs (*Polypedetes leucomystax*), late instar caterpillars of the common Mormon (*Papilio polytes*) and cempedak (*Artocarpus champeden*) seeds. We used quail eggs because of their small size, which provided a close representation of shrub-nesting babbler species (see Sodhi et al., 2003). The chosen tree frog, butterfly, and plant species for our experiments were appropriate as they are commonly found in the montane forests of Peninsular Malaysia.

We set up 16 experimental stations along on a transect within each site 100 m apart. Each transect started randomly on an existing trail, at least 150 m from the edge of the forest. Each experimental station was a circular plot of 5 m radius off the trail, where we randomly installed our artificial prey items at least 2 m apart from each other (**Supplementary Figure 1**). For the egg experiments, we placed one real egg and one artificial egg



Map prepared by Kieran Murray (2020).
Esri, HERE, Garmin, (c) OpenStreetMap contributors, and the GIS user community, Source: Esri, DigitalGlobe, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AeroGRID, IGN, and the GIS User Community

FIGURE 1 | Satellite map of study sites of varying habitat disturbance levels within Cameron and Lojing Highlands (top panel) and Fraser's Hill (bottom right panel), Peninsular Malaysia. For site abbreviations and detailed descriptions, refer to **Table 1**.

in an artificial nest secured at a height of 1 m onto a shrub or tree sapling, as per Sodhi et al. (2003). We placed one real seed and one artificial seed on a petri dish for seed experiments. Petri dishes were placed directly on the forest floor near the buttress of trees to facilitate relocation (Sodhi et al., 2003).

For the caterpillar model experiments, we attached one artificial caterpillar onto a wooden stick, ~2 cm from the end and attached it at the height of 1 m using thin wire to a branch, as per Posa et al. (2007). Lastly, we placed one artificial frog on a cardboard square (8 cm^2) for the frog model experiments, which were placed directly onto the forest floor. Following Sodhi et al. (2003), we adjusted the vegetation so that the percentage of cover by the vegetation directly above all prey items was about 50% when viewed at a height of 1.70 m.

TABLE 1 | Summary of information on study sites for predation experiments from July to August 2016 including the names of the mountain ranges and sites and abbreviation used in the text; description of the habitat type; the geographical coordinates of the site, the median canopy cover of the sites; and the dates of the predation experiments.

Name of mountain range	Name of site and abbreviation used in text	Description	Coordinates	Median canopy cover (%)	Dates of predation experiment (2016)
Fraser's Hill	Fraser primary (Pine, P)	Pristine primary rainforest	3°42′29.7“N, 101°43′13.0”E	81.9	3–6 Aug
	Fraser edge (Bishop, BI)	Primary rainforest near to edge (<100 m)	3°43′09.0“N, 101°44′19.2”E	88.1	31 Jul–3 Aug
	Fraser rural (FR)	Suburban roadside with forest edge	3°42′40.0“N, 101°44′18.2”E	56.1	30 Jul–2 Aug
Cameron Highlands	Cameron primary (Brinchang, BR)	Pristine primary rainforest	4°30′14.8“N, 101°22′56.7”E	91.3	9–12 Aug
	Cameron secondary (Forestry, F)	Secondary forest	4°28′48.9“N, 101°23′08.6”E	80.9	14–17 Aug
	Cameron tea (T)	Plantation extending into patchy primary forest	4°31′04.8“N, 101°24′14.7”E	46.3	15–18 Aug
	Cameron rural (CR)	Roadside with forest edge	4°28′59.3“N, 101°22′25.2”E	7.8	10–13 Aug
Lojing Highlands	Lojing primary (Yong Belar, YB)	Pristine primary rainforest	4°37′43.6“N, 101°23′32.6”E	91.4	19–22 Aug

We checked each experimental station after 3 days to determine any predation events. Nests were considered preyed upon if an egg was missing, broken, pecked, cracked or smashed, or if bite-marks were found on the artificial egg. Seeds were considered preyed upon if a seed was missing, or bite-marks were found on either real or artificial seed. Similarly, we considered predation to have occurred if a caterpillar or frog model was missing, or bite-marks were found. We examined the bite-marks imprinted on the artificial prey items or real seed to determine the potential predators (Sodhi et al., 2003; Posa et al., 2007). Predation by small mammals was determined by the presence of incisor marks ($\leq$2 mm wide; i.e., evidence of gnawing); birds were identified by peck marks, long thin streaks or pinches; arthropods by small scratches, boreholes or bites; and reptiles by small, equally spaced teeth marks (Sodhi et al., 2003; Posa et al., 2007). Any prey item that could not be located or that was suspected to have been tampered with by human passers-by was omitted from analysis.

Vegetation Sampling

To determine if vegetation structure affects predation probability, we measured eight vegetation structure variables within each of the 16 experimental stations (i.e., circular plot of 5 m radius, each 100 m apart). These parameters included (1) aboveground biomass per ha; (2) canopy cover; (3) number of dead trees; (4) number of saplings; (5) ground cover; (6) leaf litter depth; (7) shrub cover; and (8) understorey volume.

To estimate the aboveground biomass, we measured the diameter at breast height (DBH) of all trees with DBH $\geq$ 2 cm within the experimental station; and used the allometric equation for tropical montane trees from Edwards and Grubb (1977). We used a densiometer to measure the canopy cover by averaging four readings from four cardinal directions. We counted the number of dead trees, and saplings (trees with DBH < 2 cm) within the experimental stations. In four cardinal directions 5 m from the center of each experimental station, we measured the average leaf litter depth using a ruler, and the average percentage of shrub cover (i.e., vegetation under 2 m in height) by visual estimation from two independent observers. At these four locations within each experimental station, we took photographs of the ground and overlayed these photographs with a grid of 48 squares. The number of squares with vegetation were tallied for each photograph and then used to obtain the average percentage of ground cover. Last, we also estimated understorey vegetation volume by erecting a 6-m telescopic pole marked at every 20 cm interval and counting the number of intervals the vegetation contacted the pole at four random positions (Soh et al., 2006).

Caveats

Our study design, admittedly, could yield some potential biases or erroneous estimates of natural predation. First, our experiments did not consider specific prey-predator interactions. The traits of prey can affect the behavior of predators and predation occurrence (Sih et al., 2010; Sih, 2013). For example, seeds with caloric content are more frequently hoarded by rodents and thus less preyed upon, forming a mutualistic relationship with rodents (Wang et al., 2014). Second, like other studies with artificial predation, our results might not reflect directly natural predation, but instead, a predation risk. This is due to several limitations of using artificial prey and seeds: (1) both artificial and quail eggs lacked thermal cues, that attract reptilian predators (Thompson and Burhans, 2004), and parental activity, which can increase predation likelihood (Martin et al., 2000; Robinson et al., 2005); (2) the cardboard on which the frog models were placed may influence predator's recognition; (3) caterpillar and frog models lacked locomotion—an important signifying cue for many predators (Paluh et al., 2014); and (4) our use of relatively large cempedak seeds might not be representative of other tree species, as large seeds may have a lower predation likelihood due to low densities of large seed dispersers (Doust, 2011). Also, seeds partially damaged by predators may not affect viability as they may still survive and germinate (see Chapman et al., 2016). Nevertheless, since all experimental stations in this comparative study used the same artificial prey and seeds, the effect of the absence of natural markers was, therefore, controlled across all sites.

Data Analysis

All statistical analyses were performed using R 3.2.2 (R Core Team, 2017). Chi-squared tests were used to determine if predation on each prey item category (e.g., seeds) was associated with a particular taxonomic group (e.g., mammals). To determine if the probability of predation differed between sites, we conducted Bayesian analysis using the "Bbinom" (Bayesian binomial simulation) function from the "wiqid" package (Meredith, 2017) to compare two sets of binomial data (presence and absence of predation). Admittedly, our sample size for each study site was relatively small ($n = 16$ for each prey type). Nevertheless, a Bayesian approach is suitable for analyzing small data sets for comparative studies (Hox et al., 2012). This approach simulates a sample (50,000 Markov Chain Monte Carlo [MCMC] simulations) from the posterior for a binomial likelihood (i.e., probability of predation of one site being lower than the other) with an informative prior. However, we used a uniform prior in our analyses instead, as we did not have any prior information about the predation probability in a Malaysian montane forest (see Tuyl et al., 2009). For each prey item, we compared binomial likelihood of predation between (1) different localities of pristine forest along the same mountain range; (2) different localities of rural area along the same mountain range; and (3) habitats of varying degree of anthropogenic disturbance within the same mountain.

We performed non-metric multidimensional scaling (NMDS) for each prey item to determine the relationship between predation events and vegetation structure. All variables were transformed to achieve normality if necessary, using Shapiro-Wilk tests of normality. Variables expressed in terms of percentage (i.e., canopy cover, shrub cover, and ground cover) were arcsine square-root transformed. Leaf litter depth and the number of trees with DBH < 2 cm strongly correlated with canopy cover ($r_s > 0.7$) (**Supplementary Table 1**); these variables were therefore excluded from the analysis. NMDS was performed using the "metaNMDS" function from the "vegan" package (Oksanen et al., 2017).

We applied binary logistic regression and used MuMIn package (Barton, 2016) to run model selection based on Akaike Information Criterion (AIC; Akaike, 1973), comparing models for all possible parameter subsets in terms of parsimony and prediction. Due to the small sample size (number of samples/number of parameters < 40), we used AICc. The difference in the AICc values between the best model (one with the lowest AICc value) and other models (Δ_i) was calculated. Models were then ranked in order of increasing Δ_i. Finally, we used the hier.part package (Walsh and Nally, 2013) to run hierarchical partitioning regression analysis to determine the contribution of each variable toward predation.

TABLE 2 | Number of artificial eggs, artificial seeds, caterpillar models, and frog models (excluding those tampered by humans) used in the predation experiments, and number of artificial prey items predated by birds, mammals, reptiles, and arthropods.

	Artificial egg (pair)	Artificial seed (pair)	Caterpillar model	Frog model
Total	124	120	120	121
Absence of predation	90	68	89	103
Presence of predation	34	52	31	18
Bird	16	5	23	2
Mammal	4	22	0	3
Reptile	6	1	2	5
Arthropod	1	8	1	0
Missing/not determined	7	16	5	8

Set-ups where the prey items were not recovered or their bite-marks were unidentified were considered missing/not determined.

RESULTS AND DISCUSSION

General Patterns

A total of 124 artificial nests–each containing one artificial egg and one quail egg; 120 pairs of seeds–one artificial seed and one cempedak seed; 120 caterpillar models; and 121 frog models were used in this study. We observed that 27% of artificial nests, 43% of seed stations, 26% of caterpillar models, and 15% of frog models were preyed upon (**Table 2**).

Avian predation was observed at artificial nests across all sites (**Figure 2A**). There was a significant association of artificial nest predation with avian predators ($\chi^2 = 9.76$; $P = 0.02$). Seed predation was significantly associated with mammalian predators (**Figure 2B**) ($\chi^2 = 14.31$; $P < 0.01$). Caterpillar models were preyed upon by all predator types, except mammals (**Figure 2C**; **Table 2**); but their predation was significantly associated with avian predators ($\chi^2 = 8.53$; $P = 0.04$). Lastly, frog models were preyed upon by all predator types, except arthropods (**Figure 2D**; **Table 2**), and there was no significant association of frog model predation with a particular predator type ($\chi^2 = 3.56$; $P = 0.31$).

Our experiments broadly distinguished four groups of predators based on the characteristics of their bite marks. As many bite marks were unidentified, we did not have an adequate sample size to determine if there was a shift in predator taxa for each prey item or seed across the disturbance gradient. This phenomenon of a predator taxa shift from invertebrate to vertebrate coincided with land use intensity in one tropical lowland forest predation experiment (Boyle, 2012). Admittedly, the method of analyzing bite marks proved to be challenging: (1) some bite marks were not easily distinguishable, for example those of reptiles and non-rodent mammals; and (2) some invertebrate predators may leave inconspicuous or even no mark on clay models (Tvardikova and Novotny, 2012).

Incorporating camera trapping into predation experiments should improve the data quality for future investigations. This

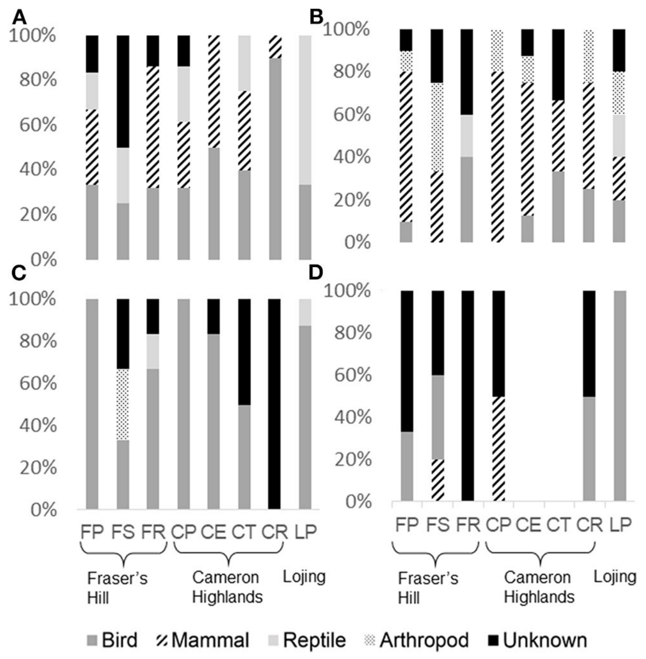


FIGURE 2 | Proportion of each predator type for **(A)** artificial nests; **(B)** artificial seeds **(C)** caterpillar models; and **(D)** frog models. Sites are clustered together according to their respective mountain range and presented in an order of increasing canopy cover (see **Table 1**). For abbreviations, refer to **Table 1**.

approach would enable a rapid assessment of whether predation is a conservation issue, for example if non-native predators are present (Pender et al., 2013). Future work should also estimate the richness and abundance of natural potential predators in order to control for any species-area relationship, as predator abundance could be correlated with occurrence of predation.

Hypothesis 1: Predation Probability Differs Between Mountains

Our results showed that predation probability differs between different primary forests. The probability of a difference in artificial nest predation rate between Lojing primary and Cameron primary being greater than zero is low, estimated at 33% only (**Supplementary Table 2**). However, the probability that Lojing primary had a lower artificial nest predation rate than Fraser primary was estimated at 78% (**Supplementary Table 2**). Lojing primary had a lower artificial seed predation rate than Fraser primary and Cameron primary, estimated at a probability of 98 and 78%, respectively (but the probability of the difference between Cameron primary and Fraser primary was low, estimated at 8% only; **Supplementary Table 2**). Lojing primary also had a lower artificial caterpillar predation rate than Fraser primary and Cameron primary, estimated at a probability of 82 and 100%, respectively (conversely, the probability of the difference between Cameron primary and Fraser primary was low, estimated at only 3%; **Supplementary Table 2**). For artificial frogs, the probability of the predation difference between Lojing primary and Cameron primary being greater than zero is low, estimated at 66% only (similarly, that between Fraser primary and Cameron primary was also low, estimated at 3%; **Supplementary Table 2**). However, Lojing primary had a lower artificial frog predation rate than Fraser primary, estimated at 85% probability (**Supplementary Table 2**).

Similarly to observations in primary forests, rural areas also had different predation probability. Between Cameron rural and Fraser rural, the probability of the difference in artificial nest predation rates being greater than zero was estimated at 0% (i.e., their predation rates were the same; **Supplementary Table 3**). The predation rates of the artificial seeds and caterpillar models between these sites being greater than zero was low as well, estimated at 40 and 5%, respectively (**Supplementary Table 3**). However, the probability of the difference for the frog models was relatively high, estimated at 70% (**Supplementary Table 3**).

Our predation tests using different artificial prey and seeds therefore consistently reveal that the primary forests, even along the same mountain range, could have different predation likelihood. Likewise, predation likelihood could be different between the rural areas in separate locations. These observations fit the concept of site-dependent spatial heterogeneity in predation risk (see Rodenhouse et al., 1997). Differences in the local predator abundance and dynamics are possible mechanisms driving this (Emmering and Schmidt, 2011).

However, there are also some interesting patterns: predation at Lojing primary was constantly lower than Fraser primary for nests (probability = 78%), seeds (98%), caterpillars (82%) and frogs (85%), whereas predation between Fraser primary and Cameron primary did not differ across all prey types. Overall, our results indicate that predation likelihood may be site-specific, influenced by the structure of surrounding vegetation (see Seibold et al., 2013; for further discussion see section Hypothesis 3: Vegetation Structure Explains Predation Probability).

Hypothesis 2: Association of Predation Probability With Habitat Disturbance

Our artificial avian nest experiment showed that there is no association between predation probability and habitat disturbance. Across the different land uses in Fraser's Hill, Fraser primary had a lower artificial nest predation rate than Fraser edge and Fraser rural with the probability estimated at 79 and 98%, respectively (**Supplementary Table 4**). In Cameron Highlands, Cameron primary had a lower artificial nest predation pressure than Cameron tea (probability that the difference is greater than zero = 91%). However, the probability that the artificial nest predation in Cameron primary was lower when compared to Cameron secondary and Cameron rural was estimated at only 30% each (**Supplementary Table 4**). Therefore, understorey bird nests in primary forest do not consistently show a lower predation pressure compared to nests in the more disturbed habitats.

Like the artificial avian nest, the artificial seeds did also not show a correlation between predation probability and disturbance intensity. In Fraser's Hill, the probability that Fraser primary had a lower artificial seed predation rate than Fraser edge and Fraser rural was low, estimated at 68 and 1%, respectively

(**Supplementary Table 5**). Similarly, we estimated the probability that Cameron primary had a lower predation pressure than Cameron secondary, Cameron rural and Cameron tea at only 15, 10, and 5%, respectively (**Supplementary Table 5**). Hence, the seeds in primary forests may not have a lower predation pressure compared to seeds in more disturbed habitats.

Results of artificial avian nest and seeds experiments were also observed in the caterpillar models. In Cameron Highlands, the caterpillar models had a lower predation pressure in the Cameron primary compared to the Cameron secondary (probability that the difference being greater than zero = 98%), Cameron rural (probability = 97%) and Cameron tea (89%) (**Supplementary Table 6**). However, there is no clear evidence that the predation of caterpillar models in Fraser primary was lower than Fraser edge (probability that the difference being greater than zero = 29%) and Fraser rural (probability = 40%) (**Supplementary Table 6**). Hence, it remains equivocal that the caterpillars may have a lower predation pressure in primary forest than more disturbed habitats.

In agreement with other prey types, the predation probability of frog models was also not associated with habitat disturbance. In Cameron Highlands, there is no clear evidence that the frog models in primary forest had a lower predation pressure than those in secondary forest (probability that the difference being greater than zero = 2%), rural (probability = 20%) and tea (probability = 2%) (**Supplementary Table 7**). Likewise, in Fraser's Hill the predation of frog models in primary forest was not lower than in forest edge (probability of the difference being greater than zero = 30%) and Fraser rural (probability = 3%) (**Supplementary Table 7**). Showing similar results as other preys and seeds, the frogs in primary forest may not have a lower predation pressure compared to frogs in more disturbed habitats.

This study finds no conclusive evidence that predation likelihood is linked to habitat degradation. Our experiments using different artificial prey and seeds show that primary tropical montane forests may not have a lower predation probability than more disturbed systems. Conversely, the disturbed or degraded montane forests may not necessarily have increased predation. Hence, our observations contrasted with the general view that predation increases with disturbance intensity (e.g., Guariguata et al., 2002; Huhta et al., 2003; Stephens et al., 2003; Posa et al., 2007; Ruiz-Guerra et al., 2012; Tvardikova and Novotny, 2012; Vetter et al., 2013).

Our results, however, also yield two repeatable patterns: First, artificial nest predation at Fraser primary was consistently

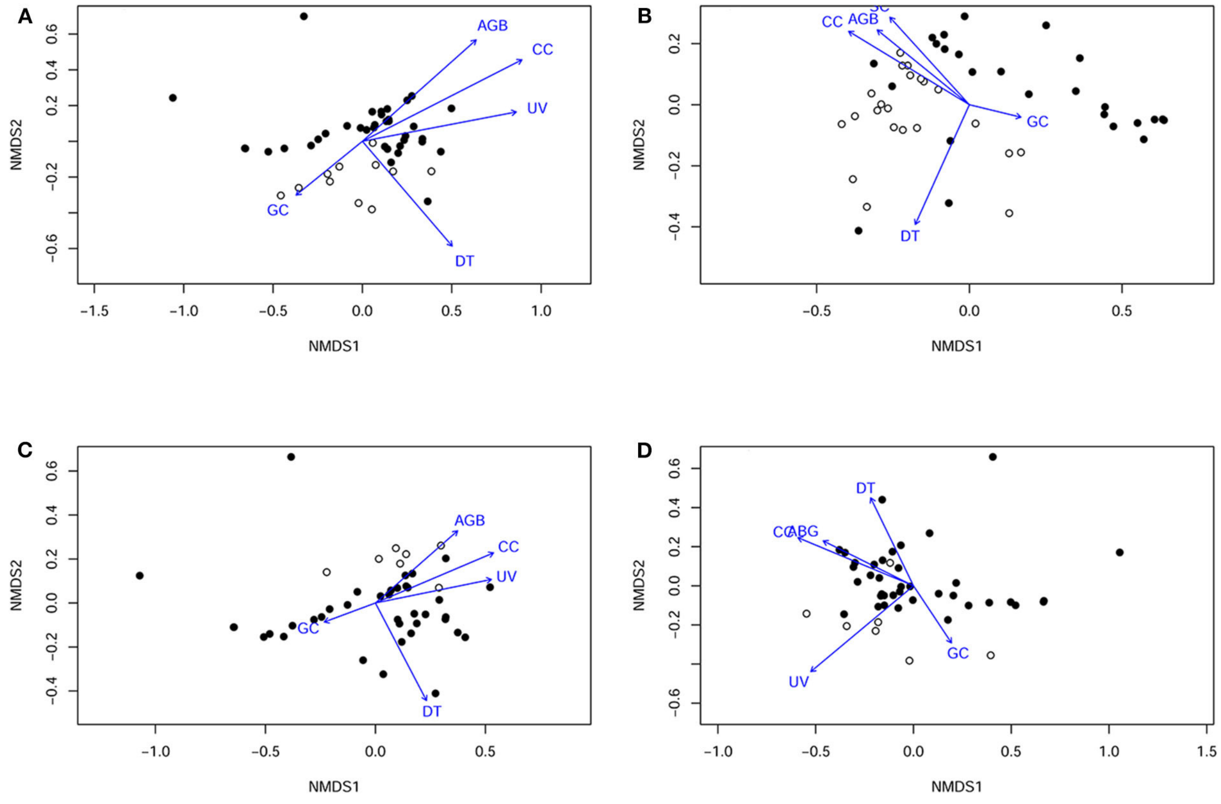


FIGURE 3 | Non-metric multidimensional scaling plots showing predation across of different prey items–**(A)** artificial nests; **(B)** artificial seeds **(C)** caterpillar models; and **(D)** frog models—across all sites, with sites where predation occurred and did not occur indicated, and each variable included in the analysis plotted accordingly. AGB, aboveground biomass; CC, canopy cover; DT, dead trees; GC, ground cover; SC, shrub cover; and UV, understorey volume. Open circles are sites where predation occurred, and black circles where artificial prey models were left untouched.

TABLE 3 | Standardized binary logistic regression coefficients and AICc values for top models with $\Delta_i < 2.0$ predicting predation events of (A) artificial nest; (B) artificial seed; (C) caterpillar model; and (D) frog model.

	Intercept	Aboveground biomass	Canopy cover	Dead trees	Ground cover	Understorey volume	df	logLik	AICc	Δ_i	Weight
A	2.201	−1.659	NA	NA	NA	NA	2	−23.677	51.626	0	0.163
	−1.186	NA	NA	NA	NA	NA	1	−25.573	53.236	1.609	0.073
	0.761	−1.518	NA	NA	1.846	NA	3	−23.393	53.344	1.718	0.069
	2.349	−1.820	NA	0.298	NA	NA	3	−23.394	53.346	1.719	0.069
	−0.482	NA	−0.608	NA	NA	NA	2	−24.624	53.520	1.894	0.063
B	−5.747	2.468	NA	0.603	NA	NA	3	−25.837	58.232	0	0.155
	−5.524	2.529	NA	NA	NA	NA	2	−27.145	58.562	0.330	0.131
	−6.602	2.120	NA	0.620	NA	0.618	4	−25.138	59.229	0.100	0.094
	−7.852	2.700	NA	0.739	2.534	NA	4	−25.299	59.551	1.319	0.080
	−6.135	2.152	NA	NA	NA	0.554	3	−26.526	59.610	1.379	0.078
C	−15.829	2.738	NA	−2.294	7.375	1.099	5	−13.136	37.810	0	0.126
	−8.321	NA	NA	−1.906	5.248	1.065	4	−14.904	38.809	0.999	0.076
	−3.527	NA	NA	−1.753	NA	0.668	3	−16.177	38.940	1.131	0.072
	−5.686	2.063	NA	−1.834	NA	NA	3	−16.194	38.973	1.163	0.070
	−7.307	1.856	NA	−1.971	NA	0.586	4	−15.063	39.126	1.316	0.065
	−10.943	2.867	NA	−2.145	5.464	NA	4	−15.071	39.142	1.333	0.065
	−17.220	3.803	−1.110	−2.457	6.826	1.339	6	−12.674	39.558	1.749	0.053
	−10.102	3.392	−1.450	−2.093	NA	0.950	5	−14.132	39.802	1.993	0.047
D	−4.182	NA	NA	NA	NA	0.680	2	−17.568	39.421	0	0.155
	−4.181	NA	−0.930	NA	NA	1.008	3	−16.750	40.085	0.664	0.111
	−6.962	NA	NA	NA	3.208	0.885	3	−17.009	40.603	1.182	0.086
	−1.692	NA	NA	NA	NA	NA	1	−19.450	40.993	1.573	0.071
	−4.055	NA	NA	−0.438	NA	0.707	3	−17.258	41.101	1.680	0.067
	−7.267	1.702	−1.627	NA	NA	1.082	4	−16.110	41.220	1.799	0.063

lower than its disturbed counterparts (Fraser edge, probability = 79%; and Fraser rural, 98%). Second, artificial caterpillar predation at Cameron primary was repeatedly lower than its disturbed counterparts (Cameron secondary, probability = 98%; Cameron rural, 97%; and Cameron tea, 89%). These patterns imply that the association of predation likelihood with habitat degradation may be context dependent, influenced by the interaction between prey type and locality; and they warrant further investigation. Varied responses of predator species in each area to degradation, as well as other complex dynamics which influence predators, could also be drivers of some of these patterns (see Laurance, 1991; Turner and Corlett, 1996; Schmitz et al., 2010; Doherty et al., 2015). With respect to statistical power, these clear predation patterns—supported by their relatively high probabilities—indicate that our sample size was sufficient.

Hypothesis 3: Vegetation Structure Explains Predation Probability

We plotted an NMDS ordination of the experimental stations (with presence and absence of predation) and vegetation vectors, with the stress value of 0.12 for the final solution on 2 dimensions, for all artificial prey and seeds (**Figure 3**).

The NMDS ordination of artificial nests showed a distinct cluster of experimental stations with predated nests broadly formed in both lower quadrants, in relation to the vegetation variables (**Figure 3A**). There was evidence of collinearity between shrub cover and understorey volume. Model selection revealed that only the aboveground biomass was included in the most parsimonious model ($\Delta_i = 0$). The coefficient of aboveground biomass in this model estimated at −1.66 suggests that artificial nest predation was associated with lower aboveground biomass. Other vegetation variables such as canopy cover (coefficient = −0.61), dead trees (coefficient = 0.30) and ground cover (coefficient = 1.85) appeared in other robust models with $\Delta_i < 2$ (**Table 3**), indicating that predation was lower at experimental stations with less canopy cover; and may be associated with more dead trees and greater ground cover. Hierarchical partitioning confirmed that aboveground biomass was the most important vegetation variable, independently explaining 51% of artificial nest predation (**Figure 4A**).

The ordination of artificial seed predation shows a distinct cluster of experimental stations, with predated seeds broadly occupying the lower and upper left quadrants in relation to vegetation variables (**Figure 3B**). Model selection revealed that only aboveground biomass and dead trees were included in the most parsimonious model ($\Delta_i = 0$). The coefficients of aboveground biomass and dead trees in this model were estimated at 2.47 and 0.60, respectively, suggesting that artificial seed predation was associated with higher aboveground biomass and more dead trees. Ground cover (coefficient = 2.53) and understorey volume (coefficient = 0.55–0.62) appeared in some robust models with Δ_i <2 (**Table 3**), indicating that artificial seed predation may be associated with greater ground cover and understorey volume. Hierarchical partitioning showed that the aboveground biomass was the most important vegetation variable, independently accounting 42% of artificial seed predation (**Figure 4B**).

The ordination of caterpillar models shows a distinct cluster of experimental stations with predated seeds broadly occupying the upper right quadrant, in relation to the vegetation variables (**Figure 3C**). Model selection revealed that aboveground biomass, dead trees, ground cover, and understorey volume were included in the most parsimonious model (Δ_i = 0). The estimated coefficients of aboveground biomass (2.74), ground cover (7.38); and understorey volume (1.10) were all positive in this model. Dead trees were an exception to this (coefficient = −2.29). This suggests that caterpillar model predation was associated with higher aboveground biomass, ground cover, and understorey volume, with fewer dead trees. Canopy cover did not appear in any of the robust models with Δ_i <2 (**Table 3**). These indicate that the caterpillar model predation may not have any association with canopy cover. Hierarchical partitioning showed that the number of dead trees is the important vegetation variable, independently accounting for 33% of the artificial caterpillar predation outcome (**Figure 4C**); while understorey volume and aboveground biomass accounted for 25 and 23%, respectively.

Lastly, the ordination of frog models shows that the experimental stations with predated artificial frogs were distinctly clustered in the lower left quadrant (**Figure 3D**). Model selection revealed that only understorey volume (coefficient = 0.68) was include in the most parsimonious model (Δ_i = 0), suggesting that artificial frog predation was associated with higher understorey volume. Other vegetation variables such as aboveground biomass (coefficient = 1.70), canopy cover (coefficient = −0.93 to −1.63), dead trees (coefficient = −0.44), ground cover (coefficient = 3.21) and understorey volume (coefficient = 0.71 to 1.08) appeared in other robust models with Δ_i <2 (**Table 3**), indicating that artificial frog predation was higher at experimental stations with less canopy cover and fewer dead trees; and may be associated with greater vegetation cover. Hierarchical partitioning showed that the understorey volume was the most important vegetation variable, independently explaining 70% of the artificial frog predation outcome (**Figure 4D**).

Different artificial prey and seeds demonstrated idiosyncratic vegetation characteristics associated with their predation, which may be explained by the microhabitat requirements of their predators during hunting (Martin, 1995; Seibold et al., 2013; Dagan and Izhaki, 2020). Model selection and hierarchical partitioning showed that vegetation cover may be the most influential variable for predation. Specifically, higher nest predation in tropical montane forest may be associated with lower aboveground biomass and canopy cover—a biotic environment which could increase the exposure and visibility of the nests to predators (Martin, 1995). On the other hand, higher predation of seeds, caterpillars and frogs may be associated with higher vegetation cover, which potentially provides predators with protection (Seibold et al., 2013; Dagan and Izhaki, 2020).

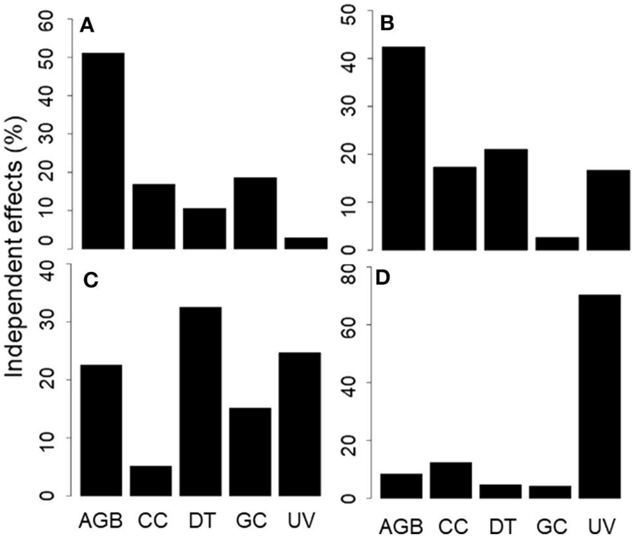


FIGURE 4 | Hierarchical partitioning graphs, showing the independent effect (in terms of percentage) of each vegetation structure variable on the likelihood of predation occurring for **(A)** artificial nests; **(B)** artificial seeds; **(C)** caterpillar models; and **(D)** frog models. Scales differ between each graph. AGB, aboveground biomass; CC, canopy cover; DT, dead trees; GC, ground cover; and UV, understorey volume.

CONCLUSION

Our study showed that (1) predation probability differs between primary forests of different mountains, despite that they belong to the same mountain range; (2) there is no clear trend of predation probability along a habitat disturbance gradient; and (3) the predation probability of a prey item or seed may be influenced by the vegetation variables of its immediate surroundings. The use of multiple prey items clearly showed that predation risk for each can vary considerably and thus cautions against deploying a single prey type to draw a broad inference of predation in degraded systems. Investigating patterns using both microhabitat and the landscape scale broadened the inferences we could make about the drivers of predation in the study area. Future research should be directed toward understanding how vegetation structure can affect predator behaviors and their hunting efficiency in different montane habitats. Incorporating habitat fragmentation as a factor into the future study design will also help us to gauge the potential conservation value of human-modified landscape in a tropical mountain forest.

DATA AVAILABILITY STATEMENT

The data is already available online on the Knowledge Network for Biocomplexity. https://knb.ecoinformatics.org/#view/knb.1380.1.

AUTHOR CONTRIBUTIONS

KP and MS conceived the research. KM and MS carried out the field experiments. KM did the analyses and wrote the paper. All authors commented on the drafts.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/ffgc.2020.00080/full#supplementary-material

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Contrasting Dependencies of Photosynthetic Capacity on Leaf Nitrogen in Early- and Late-Successional Tropical Montane Tree Species

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Differences in photosynthetic capacity among tree species and tree functional types are currently assumed to be largely driven by variation in leaf nutrient content, particularly nitrogen (N). However, recent studies indicate that leaf N content is often a poor predictor of variation in photosynthetic capacity in tropical trees. In this study, we explored the relative importance of area-based total leaf N content (N_{tot}) and within-leaf N allocation to photosynthetic capacity versus light-harvesting in controlling the variation in photosynthetic capacity (i.e. V_{cmax}, J_{max}) among mature trees of 12 species belonging to either early (ES) or late successional (LS) groups growing in a tropical montane rainforest in Rwanda, Central Africa. Photosynthetic capacity at a common leaf temperature of 25°C (i.e. maximum rates of Rubisco carboxylation, V_{cmax25} and of electron transport, J_{max25}) was higher in ES than in LS species (+ 58% and 68% for V_{cmax25} and J_{max25}, respectively). While N_{tot} did not significantly differ between successional groups, the photosynthetic dependency on N_{tot} was markedly different. In ES species, V_{cmax25} was strongly and positively related to N_{tot} but this was not the case in LS species. However, there was no significant trade-off between relative leaf N investments in compounds maximizing photosynthetic capacity versus compounds maximizing light harvesting. Both leaf dark respiration at 25°C (+ 33%) and, more surprisingly, apparent photosynthetic quantum yield (+ 35%) was higher in ES than in LS species. Moreover, R_{d25} was positively related to N_{tot} for both ES and LS species. Our results imply that efforts to quantify carbon fluxes of tropical montane rainforests would be improved if they considered contrasting within-leaf N allocation and photosynthetic N_{tot} dependencies between species with different successional strategies.

Keywords: photosynthesis, nitrogen, allocation, early successional, late successional, tropical montane forests

INTRODUCTION

Tropical forests play an important role in controlling the global carbon cycle and, thus, the rate of ongoing climate change (Lewis, 2006; Stocker et al., 2014). They store more than half of the carbon in the world's forests (Pan et al., 2011), and provide roughly one-third of the global terrestrial primary production (Beer et al., 2010). Reliable quantification of the carbon uptake of tropical forests across time and space thus requires understanding of how carbon fluxes (carbon gain through photosynthesis and carbon loss through respiration) vary among environmental conditions and tree functional types. More specifically, Dynamic Global Vegetation Models (DGVMs) and Earth System Models (ESMs) require accurate representation of the factors controlling variation in the maximum rates of photosynthetic carboxylation (V_{cmax}) and electron transport (J_{max}), as well as leaf respiration (Rogers, 2014; Walker et al., 2014). For tropical forests in general and African rainforests and tropical montane forests in particular, much remains to be explored regarding these controls.

Most DGVMs and ESMs employ the photosynthesis model by Farquhar et al. (1980), which represents the variation in V_{cmax} and J_{max} (at a reference temperature) as either fixed values for different plant functional types or as linear functions of area-based total leaf nitrogen content (N_{tot}; Kattge et al., 2009; Thornton et al., 2009; Zaehle et al., 2010; Rogers, 2014; Walker et al., 2014). However, a recent global meta-analysis found that interspecific variation in V_{cmax} and J_{max} was much more closely related to photosynthetic N use efficiency than to N_{tot} (Ali et al., 2015). Moreover, several studies in tropical rainforests have found that area-based leaf nutrient content (i.e. N, phosphorous) is often a poor predictor of the large interspecific variation in photosynthetic capacity (Coste et al., 2005; van de Weg et al., 2012; Houter and Pons, 2014; Dusenge et al., 2015; Bahar et al., 2016; Hasper et al., 2017). Some of these studies have indicated that the fractional investment of leaf N into compounds maximizing photosynthetic capacity (i.e. V_{cmax} and J_{max}) is a considerably stronger determinant of interspecific variation in V_{cmax} and J_{max} than N_{tot} (Coste et al., 2005; Dusenge et al., 2015; Hasper et al., 2017). Yet, more research is needed to confirm this pattern since these studies were conducted on seedlings in a greenhouse (Coste et al., 2005) or on a rather small number of rainforest tree species (six in Dusenge et al., 2015; five in Hasper et al., 2017). The strength of the relationship between photosynthesis and N_{tot} may depend on leaf phosphorus (P) content (Reich et al., 2009). However, V_{cmax} – N relationships were similarly weak at both high and lower altitude in Rwanda, in spite of leaf P content being twice as high at the higher site (Dusenge et al., 2015). Furthermore, leaf P content and photosynthetic N use efficiency (V_{cmax} per unit leaf N) were not correlated in a large study of Andean and Amazonian rainforest species (Bahar et al., 2016).

Part of the reason for why interspecific variation in photosynthetic capacity is often poorly related to total leaf nutrient content may be that species with different successional strategies differ in within-leaf N allocation. Fast-growing and short-lived early-successional (ES) tree species usually make greater investments in N-rich molecules involved in photosynthesis and respiration than slow-growing and long-lived late-successional (LS) tree species, regenerating in low light under tree canopies (Raaimakers et al., 1995; Valladares and Niinemets, 2008; Xiao et al., 2018). In contrast, leaves of LS species are often more long-lived and make larger fractional investments in rather N-poor structural compounds and pigmentation. However, these patterns do not always hold true for tropical tree species. A study with seedlings of 14 rainforest species found that while leaf mass per unit leaf area (LMA) increased with species' shade tolerance, photosynthetic capacity and N_{tot} content did not systematically change (Coste et al. (2005). Another study on 17 rainforest tree species, reported that photosynthetic capacity decreased with increasing species' shade tolerance while LMA and N_{tot} did not change (Houter and Pons, 2014). These studies thus suggest that interspecific variation in photosynthetic capacity in tropical trees is often controlled by within-leaf N allocation, but more research is needed to explore the link between within-leaf N allocation strategies and other plant traits (e.g., other leaf traits, life history traits).

A recent study on six tropical montane rainforest tree species indicated that there may be a trade-off involved in within-leaf N allocation, such that ES species with high fractional N investments into compounds that maximize photosynthetic capacity (i.e. V_{cmax} and J_{max}) invest less N into compounds involved in light-harvesting (i.e., chlorophyll and photosystems), and vice versa for LS species (Dusenge et al., 2015). Such differences between ES and LS species are in line with the "carbon gain hypothesis" put forward to explain plant shade-tolerance. It states that shade-tolerant LS species have plant traits that maximize carbon gain under low light conditions (e.g., low respiration and LMA, high chlorophyll content and quantum yield of photosynthesis; Valladares and Niinemets, 2008). However, the study by Dusenge et al. (2015) found that LS species, in spite of indications of higher chlorophyll content, actually had significantly lower quantum yield than ES species. Clearly, more research is needed to better understand the roles played by different plant traits in controlling shade-tolerance in tropical forests (Valladares et al., 2016; Poorter et al., 2019).

Africa harbors 27% of all tropical forests (Scatena et al., 2010) and 13% of all tropical montane forests (elevation > 1000 m a.s.l; Spracklen and Righelato, 2014). However with respect to ecological and biogeochemical understanding of carbon dynamics, the available data on African tropical forests is scarce, mainly due to the lack of an extensive long-term observation network (Lewis et al., 2009). This is particularly the case for mountainous ecosystems (Mountain Research Initiative EDW working group; Pepin et al., 2015). Here, we investigated physiological, chemical and structural properties of leaves in mature individuals belonging to 12 tree species—five ES and seven LS species—growing in one of Africa's largest remaining tropical montane rainforests, Nyungwe forest in Rwanda. The overall aim of this study was to explore the controls of interspecific variation in photosynthetic capacity and other leaf gas exchange traits in tropical montane rainforest tree species. Based on previous research, the following predictions were tested:

1. ES species have higher photosynthetic capacity (higher V_{cmax}, and $J_{\max}$) than LS species;
2. Area-based total leaf N content is a poor predictor of photosynthetic capacity;
3. Successional groups differ in their within-leaf N allocation;
4. There is a trade-off in the allocation of leaf N between investments into compounds maximizing photosynthetic capacity versus compounds maximizing light harvesting;
5. Key predictions of the “carbon-gain hypothesis” do not apply to montane rainforest tree species.

A previous study in Nyungwe forest showed that neither intra- nor interspecific variation in photosynthetic capacity was related to leaf P content (Dusenge et al., 2015), which was not investigated here.

MATERIALS AND METHODS

Study Site and Plant Species

Data were collected on mature trees (**Table 1**) in Nyungwe National Park (2°17—2°49’ S, 29°03—29°29’ E; elevation 1600–2950 m, investigated plots at 1950–2500 m). Nyungwe National Park (hereafter called “Nyungwe”) is located in the southwestern part of Rwanda, Central Africa, within the Albertine Rift ecoregion (Plumptre et al., 2007). Nyungwe covers 1013 km^2 and forms, together with the contiguous Kibira national park in Burundi, the largest block of tropical mi-elevation montane forest remaining in Africa, with large areas encompassing a mixture of primary and secondary forest due to its disturbance history (Plumptre et al., 2002).

At a meteorological station located at Uwinka (2° 28’43”S, 29° 12’00” E, 2465 m altitude; Nsabimana, 2009; Nyirambangutse et al., 2017), the average day and night air temperatures during 2007–2015 were 15.8°C and 13.5°C, respectively, and the difference between the warmest and coldest month was 1.1°C. The mean relative humidity was 84% and annual rainfall was 1855 mm.

Nyungwe harbors more than 260 tree and shrub species, with 24 recorded as endemic to the Albertine rift (Plumptre et al., 2002). The 12 species investigated in this study were selected to represent common ES and LS species, according to data from 15 half ha monitoring plots recently established in the forest (**Table 2** in Nyirambangutse et al., 2017). The most abundant ES and LS species in Nyungwe are *Macaranga kilimandscharica* and *Syzigium guineense*, respectively, each accounting for 18% of the total number of trees with a diameter at breast height ≥ 30 cm according to a forest-wide survey (Plumptre et al., 2002). The other ES species co-occurred with *M. kilimandscharica*, except *H. abyssisnica* which was found at edges and gaps, while the five of the six other LS species clearly co-occurred with *S. guineense*. *Ocotea kenyensis* occurred together with both *M. kilimandscharica* and *S. guineense* but has been described as a LS species in the literature (Tesfaye et al., 2002). It was present mostly as rather large trees in our plots, indicating that when co-occurring with *M. kilimandscharica* it might be a survivor of earlier disturbance events. The 12 studied species together account for 76% of the total basal area of all trees with diameter at breast height ≥5 cm in the 15 monitoring plots (**Table 1**).

Leaf Gas Exchange Measurements

Field measurements of leaf gas exchange in mature trees were conducted from late February to early August 2015 between 9:00 and 17:00 h, using two portable leaf gas exchange instruments (LI6400; LI-COR Inc., Lincoln, NE, USA) with the standard 2 cm × 3 cm leaf chamber and a light source (6400-02B LED Light Source). Fully expanded newly mature sun leaves without visible damage were selected and measured for responses of net photosynthetic rate (A_{n}) to eight CO_2 concentrations (range 60–2000 µmol mol^{-1}; so called A-C_{i} curves) at a photosynthetic photon flux density (PPFD) of 1800 µmol m^{-2} s^{-1}. Then, A_{n} was measured at five different levels of PPFD (0, 25, 50, 75, and 100 µmol m^{-2} s^{-1}; so called light-response curves) at a CO_2 concentration of 400 µmol mol^{-1} of air entering the leaf chamber. The relative air humidity was kept between 60% and 80% during the measurements to

TABLE 1 | Description of early-successional (ES) and late-successional (LS) tree species investigated in this study.

Species	Family	Successional group[a]	Diameter at breast height (cm)	%BA in plots	Tree height (m)
Hagenia abyssinica (Bruce) J.F.Gmel.	Rosaceae	ES	28 ± 14	0.3	8 ± 5
Harungana montana Spralet	Clusiaceae	ES	41 ± 21	2.3	20 ± 2
Macaranga kilimandscharica Pax	Euphorbiaceae	ES	22 ± 5	24.8	15 ± 4
Prunus africana (Hook. f.) Kalkm.	Rosaceae	ES	35 ± 13	0.6	19 ± 6
Polyscias fulva (Hiern.) Harms	Araliaceae	ES	52 ± 13	3.4	20 ± 3
Carapa grandiflora Sprague	Meliaceae	LS	40 ± 22	2.6	19 ± 5
Cleistanthus polystachyus Hook.f. ex Planch.	Euphorbiaceae	LS	31 ± 15	2.6	18 ± 4
Faurea Saligna Harv.	Proteaceae	LS	53 ± 21	6.1	25 ± 7
Ficalhoa laurifolia Hiern.	Theaceae	LS	37 ± 12	2.5	22 ± 4
Ocotea kenyensis (Chiov.) Robyns & Wilczek	Lauraceae	LS	40 ± 18	3.0	22 ± 5
Strombosia scheffleri Engl.	Olacaceae	LS	31 ± 10	1.2	20 ± 6
Syzygium guineense (Willd.) DC.	Myrtaceae	LS	50 ± 21	26.6	20 ± 5

Means ± SE are represented for n = 5–7 species per successional group and 7–15 trees per species.

[a] The classification of the species into successional groups was based on information in the following publications in combination with our own observations of abundance in plots dominated by Macaranga kilimandscharica (main ES species) and Syzygium guineense (main LS species) trees: Bloesch et al., 2009; Fischer and Killmann; Bussmann, 2002; Tesfaye et al., 2002; Fashing, 2004; Fashing et al., 2004; Eilu and Obua, 2005; Kindt et al., 2014; Rutten et al., 2015.

TABLE 2 | Summary report with results of a two-factor mixed-effects ANOVA and a linear mixed-effects model (see *Statistical Analysis* section).

Parameter	Factor	F-value	p-value
Mixed-effects ANOVA			
V_{cmax25}	Succ	9.8	**0.011**
J_{max25}	Succ	11.6	**0.009**
J_{max25}/V_{cmax25}	Succ	0.2	0.66
A_{280}	Succ	9.4	**0.012**
AQY	Succ	10.7	**0.008**
R_{d25}	Succ	6.9	**0.025**
N_{tot}	Succ	0.4	0.54
LMA	Succ	1.4	0.26
Chl	Succ	1.6	0.23
Linear mixed-effects model			
V_{cmax25}	N_{tot}	0.67	0.41
	Succ	8.7	**0.015**
	N_{tot}*Succ	5.8	**0.018**
	N_{LH}	11.7	**0.001**
N_{R+B}	Succ	4.2	0.067
	N_{LH}*Succ	0.5	0.5
	N_{tot}	7.14	**0.009**
R_{d25}	Succ	7.14	**0.023**
	N_{tot}*Succ	0.15	0.7
N_{tot}	LMA	72.2	**<0.001**
	Succ	0.0083	0.93
	LMA*Succ	1.5	0.22
	N_{LH}	5.7	**0.019**
AQY	Succ	4.8	0.052
	N_{LH}*Succ	2	0.16

Bold numbers represent $p < 0.05$. Traits analyzed were: maximum rates of Rubisco carboxylation capacity (V_{cmax25}, μmol m^{-2} s^{-1}) and electron transport (J_{max25}, μmol m^{-2} s^{-1}) at 25°C; photosynthetic rates at a constant intercellular (C_i) CO_2 concentration of 280 ppm (A_{280}, μmol m^{-2} s^{-1}); the ratio of J_{max25} to V_{cmax25} (J_{max25}:V_{cmax25} ratio); leaf mass per unit leaf area (LMA, g m^{-2}) and area-based total leaf nitrogen content (N_{tot}, g m^{-2}); leaf dark respiration measured at 25°C; apparent quantum yield of photosynthesis (AQY); fractional investments of total leaf N content into compounds maximizing photosynthetic capacity (N_{R+B}) and compounds maximizing photosynthetic light-harvesting (N_{LH}).

avoid stomatal closure. Measurements of the response of A_n to C_i were performed only if the starting value of stomatal conductance (g_s) was above a minimal threshold of 0.03 mol m^{-2} s^{-1}. Measurements of dark respiration (R_d) were conducted on a neighboring leaf, which had been covered by tinfoil and acclimated to darkness for a least 30 min prior to the measurement, to avoid post-illumination CO_2 burst (Atkin et al., 1998). Most measurements were conducted at a leaf temperature of 20°C, but 12 measurements conducted under unusually warm conditions were made at 25°C.

Leaf gas exchange was measured on one leaf per tree in at least eight trees per species. The trees were selected from as many of the 15 forest plots as possible (some species were, however, present in only a few plots) to account for possible differences among plots (e.g., fertility). The total number of measured leaves was 116. Sun leaves were made accessible by cutting 1 to 2 m branches using a saw mounted on a 20 m long telescopic pole. The branches were immediately placed into a water-bucket prior to gas exchange measurements. The short-term effect of cutting was previously evaluated for Nyungwe tree species, showing no significant effect on V_{cmax} and a quite small negative effect (−8%; $p < 0.05$) on J_{max} (Dusenge et al., 2015). It was therefore unlikely that branch excision caused a sufficient disruption of xylem water continuity to substantially affect gas exchange measurements, as it may occur in some tropical tree species (Santiago and Mulkey, 2003).

After the measurement campaign from late February to April, it was found that a leak had been present in one of the two instruments used. The conductance of the leak was quantified and used to recalculate A_n and C_i data on the assumption that the CO_2 concentration around the leaf chamber was 400 μmol mol^{-1}. Species-specific V_{cmax} values determined for the adjusted data were very similar to the V_{cmax} values determined for data from the instrument without a leak (on average 1% difference). However, we refrain from reporting J_{max} data for the measurements affected by the leak since the leak correction was considerably larger at high compared to low CO_2 concentrations inside the leaf chamber (e.g., about five times as large at 2000 μmol mol^{-1} than at 60 μmol mol^{-1}, at an ambient outside CO_2 concentration of 400 μmol mol^{-1}). As result, we present no J_{max} data for two out of 12 species.

Leaf Gas Exchange Data Analyses

The photosynthesis model by Farquhar et al. (1980), with modifications of photosynthetic temperature dependencies by Bernacchi et al. (2001), was used to parameterize V_{cmax} and J_{max} from A-C_i curve data by the least squares method. The rates of carboxylation-limited (A_c) and electron transport-limited net photosynthesis (A_j) were calculated as:

$$A_c = \frac{V_{cmax}(C_i - \Gamma^*)}{C_i + K_c\left(1 + \frac{O}{K_o}\right)} - R_l \qquad \text{(Eqn 1)}$$

and

$$A_j = J\frac{C_i - \Gamma^*}{4 \quad C_i + 8\Gamma} = R_l \qquad \text{(Eqn 2)}$$

where C_i is the leaf intercellular CO_2 concentration, K_c and K_o are Michaelis-Menten constants for CO_2 and O_2, respectively; Γ^* is the CO_2 compensation point in the absence of mitochondrial respiration; R_l is the non-photorespiratory CO_2 release in the light; and J is the rate of electron transport. For K_c, K_o, and Γ^*, the values at 25°C as well as the temperature sensitivities were taken from Bernacchi et al. (2001). The internal leaf conductance for CO_2 was not estimated and therefore "apparent" V_{cmax} and J_{max} values are reported, based on C_i rather than on the CO_2 concentration at the chloroplast. The parameterization of V_{cmax} and J_{max} were done based on partial pressure units (P_a) of CO_2 (C_i and Γ^*) and O_2; not on mole-based units.

Values of V_{cmax}, J, and R_l were determined simultaneously with the only *a priori* restriction made to the A-C_i fitting that data points with C_i below 100 μmol mol^{-1} were forced to be V_{cmax}-limited. Values of J_{max} were estimated from J as in Medlyn et al. (2002). The uncertainty of the values of the curvature of the light-response (0.9) and quantum yield of electron transport (0.3 mol electrons mol^{-1} photons) used when calculating J_{max} from J has only a minor effect on the estimated value of J_{max} (Medlyn et al., 2002). Values of J_{max} were reported only if the A_j limited part of the A-C_i curve had at least two data points, or from one single data point if C_i > 1000 μmol mol^{-1} and/or A_j was at least 10% lower than A_c at the C_i value of that data point. These criteria caused the exclusion of only two J_{max} values. Light-saturated net photosynthesis at a common C_i of 280 µmol mol^{-1} (A_{280}; assuming the intercellular to ambient CO_2 concentration to be 0.7) was calculated based on the fitted photosynthesis model for each leaf. Values of V_{cmax}, J_{max}, and A_{280} are reported for a reference leaf temperature of 25°C using temperature response equations from Bernacchi et al. (2001), although most measurements were conducted at 20°C. Reported values of R_d were standardized to a leaf temperature of 25°C (R_{d25}) using a Q_{10} value of 2.14, as suggested for tropical species (Atkin and Tjoelker, 2003).

The apparent (i.e. based on incident rather than absorbed radiation) quantum yield of photosynthesis was determined as the slope of the light-response curve in the PPFD range 25–50 µmol m^{-2} s^{-1}.

Leaf Structural and Chemical Traits

After gas exchange measurements, leaves were collected and the dry mass of leaf discs of known area was recorded after drying at 70°C until constant weight in order to calculate leaf mass per unit leaf area (LMA, g m^{-2}). Discs were then ground to fine powder in a ball mill, which was weighed and analyzed for N concentration using an elemental analyzer (EA 1108; Fison Instruments, Rodano, Italy).

Leaves were also measured for SPAD values, a proxy of leaf chlorophyll content (Uddling et al., 2007) optically measured using a SPAD meter (SPAD model 502; Minolta corporation, Ltd., Osaka, Japan). Ten evenly distributed readings were made across each leaf, again avoiding major veins. Leaf chlorophyll content was estimated from SPAD measurements using an equation for tropical tree species provided by Coste et al. (2010).

Within-Leaf N Allocation

The leaf N investments were determined for the following components of the photosynthetic apparatus: Rubisco (N_R); bioenergetics, including coupling factors, electron carriers except for photosystems, and Calvin-Benson cycle enzymes except for Rubisco (N_B); and light-harvesting complexes and photosystems (N_{LH}).

The N_R was estimated using the equation and parameters provided by (Niinemets and Tenhunen, 1997):

$$N_R = \frac{0.160\ V_{cmax}}{V_{cr}} \qquad \text{(Eqn 3)}$$

where V_{cmax} is the maximum rate of carboxylation, 0.160 converts Rubisco to N [g N in Rubisco (g Rubisco)$^{-1}$] and V_{cr} the specific activity of Rubisco at 25°C [20.78 µmol CO_2 (g Rubisco)$^{-1}$ s^{-1}].

The N_B was estimated as:

$$N_B = \frac{J_{max}}{156 \times 8.06} \qquad \text{(Eqn 4)}$$

where it is assumed that N in bioenergetics is proportional to J_{max}, that 156 is the ratio of electron transport to cytochrome f content in mol mol^{-1} s^{-1} and that 8.06 is the amount of cytochrome f per unit N in bioenergetics in µmol g^{-1} (Niinemets and Tenhunen, 1997). The sum of N_R and N_B (N_{R+B}) was used as a measure of leaf N in compounds determining the maximum photosynthetic rate, i.e. photosynthetic capacity

The N_{LH} was estimated according to Evans and Poorter (2001) as:

$$N_{LH} = 41 \times 0.0155 \times Chl \qquad \text{(Eqn 5)}$$

where *Chl* is the area-based chlorophyll content (g m^{-2}), 41 is the N content per unit chlorophyll in light-harvesting complexes and photosystems in sun exposed leaves in mol mol^{-1}, and 0.0155 is the molar mass ratio of N to chlorophyll. We divided N_{R+B} and N_{LH} by N_{tot} to get the fractional investments (g g^{-1}) to compounds maximizing photosynthetic capacity and light harvesting, respectively.

For leaves lacking J_{max} data (see *Leaf Gas Exchange Measurements* section above), N_B was estimated by assuming that these leaves had the same J_{max}/V_{cmax} ratio as other leaves of the same species for which J_{max} data were available. In two species lacking J_{max} data altogether, the J_{max}/V_{cmax} ratio was assumed to be the mean of all other species (which did not significantly differ among the other species or between ES and LS species). The fraction of the total leaf N was markedly smaller for N_B (4%) than for N_R (21%), causing small uncertainty in the estimation of N_{R+B} introduced by this N_B data gap filling.

Statistical Analysis

To analyze the effect of successional identity on photosynthetic capacity (V_{cmax25}, J_{max25} and J_{max25}/V_{cmax25} ratio), R_{d25}, AQY, LMA, chlorophyll content, and N_{tot}, we used a two-factor mixed-effects ANOVA, with successional identity as a main factor and species as a random factor nested within successional group. The relationship between V_{cmax25} and N_{tot} was analyzed with a linear mixed-effects model following Zuur et al. (2009) with V_{cmax25} as response variable, successional identity as a categorial factor, N_{tot} as a covariate, and species as a random factor with trees as replicates. We had five and seven species for early- and late-successional (**Table 1**), respectively, and for each species 7–15 trees were measured. Differences were considered statistically significant if $p < 0.05$. All analyses were performed in R (version 3.5.2), and the following packages were used: *lme4* (for mixed-effects modelling), *dplyr* (for data manipulation), and *ggplot2* and *cowplot* (for graphing).

RESULTS

Basal rates of photosynthetic capacity (i.e. V_{cmax25} and J_{max25}) differed between ES and LS species. V_{cmax25} was 58% higher in ES (71 ± 9 µmol m^{-2} s^{-1}) than in LS (45 ± 3 µmol m^{-2} s^{-1}) species (**Figure 1A**; **Table 2**). Similarly, J_{max25} was 68% higher in ES (171 ± 21 µmol m^{-2} s^{-1}) than in LS (102 ± 6 µmol m^{-2} s^{-1}) species (**Figure 1B**). Values of A_{280} were 58% higher in ES (14 ± 2 µmol m^{-2} s^{-1}) than LS (9 ± 1 µmol m^{-2} s^{-1}) species (**Figure 1C**). The J_{max25}/V_{cmax25} ratio (2.4 across all species) was not statistically significant between ES and LS species, despite the relatively larger difference in J_{max25} compared to V_{cmax25} between ES and LS species (**Figure 1D**). Leaf dark respiration at 25°C (R_{d25}) was 33% higher in ES (1.6 ± 0.1 μmol m^{-2} s^{-1}) compared to LS species (1.2 ± 0.1 μmol m^{-2} s^{-1}) (**Figure 2A**, **Table 2**). Similarly, apparent photosynthetic quantum yield (AQY) was 35% higher in ES (0.042 ± 0.003 mol mol^{-1} photon) than in LS (0.031 ± 0.003 mol mol^{-1} photon) species (**Figure 2B**). Additionally, neither total leaf nitrogen (N_{tot}, 2.4 g m^{-2} across all species), nor LMA (128 g m^{-2}), nor chlorophyll content (0.84 g m^{-2}) differed between ES and LS groups (**Figure 3**; **Table 2**; **Supplementary Table 2**).

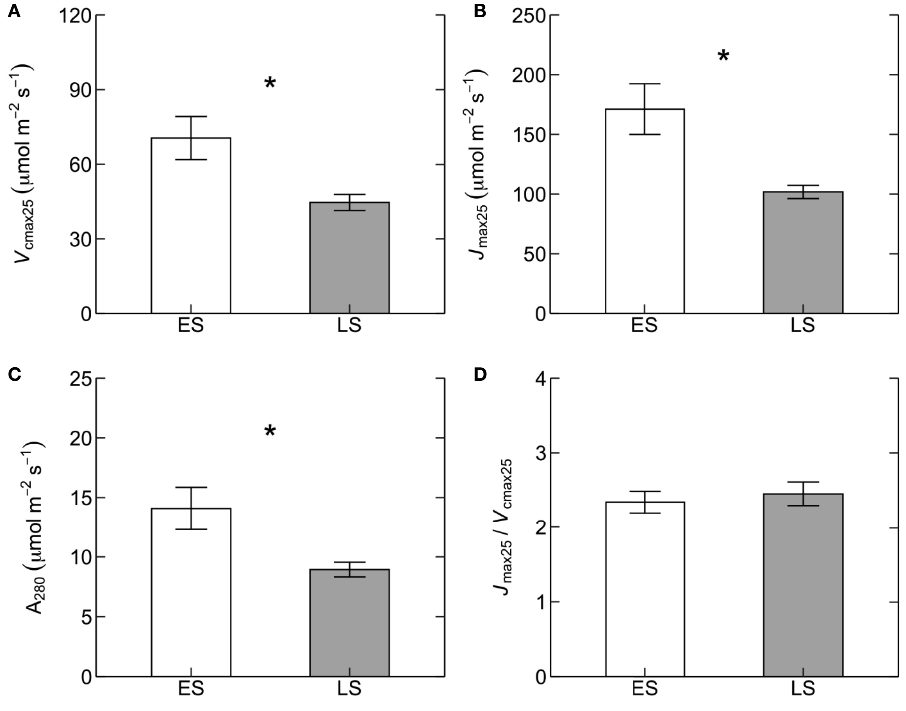

FIGURE 1 | Photosynthetic traits at 25°C. Maximum rates of **(A)** Rubisco carboxylation capacity (V_{cmax25}, μmol m^{-2} s^{-1}) and **(B)** electron transport (J_{max25}, μmol m^{-2} s^{-1}), **(C)** photosynthetic rates at a constant intercellular CO_2 concentration (C_i) of 280 ppm (A_{280}, μmol m^{-2} s^{-1}), and **(D)** the ratio of J_{max25} to V_{cmax25} (J_{max25}/V_{cmax25}) in early-successional (ES, white) versus late-successional (LS, gray) tree species in Nyungwe forest. The asterisks (in **A–C**) indicate statistical significance ($p < 0.05$). Error bars represent SE with n = 5–7 species per successional group and 7–15 trees per species.

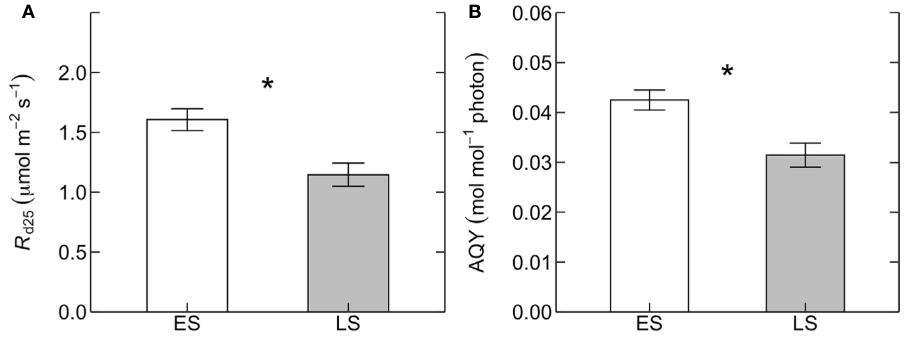

FIGURE 2 | **(A)** Leaf dark respiration measured at 25°C (R_{d25}, μmol m^{-2} s^{-1}) and **(B)** apparent quantum yield of photosynthesis (AQY, mol mol^{-1} photon) for early-successional (ES, white) and late-successional (LS, gray) tree species in Nyungwe forest. The asterisks (in **A, B**) indicate statistical significance ($p < 0.05$). Error bars represent SE with n = 5–7 species per successional group and 7–15 trees per species.

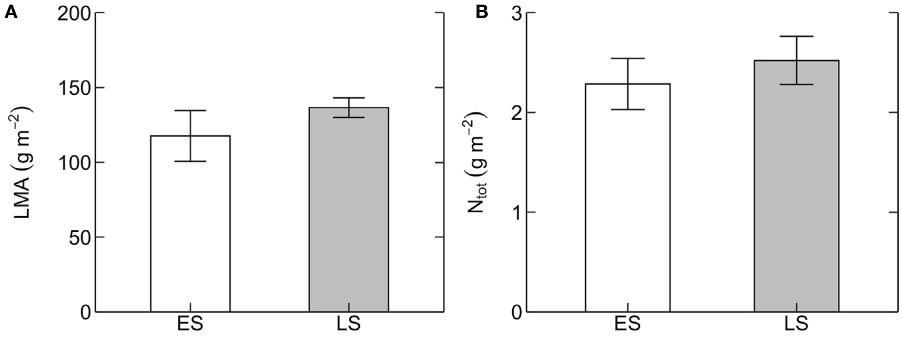

FIGURE 3 | Leaf structural and chemical traits. **(A)** Leaf mass per unit leaf area (LMA, g m^{-2}) and **(B)** area-based total leaf nitrogen content (N_{tot}, g m^{-2}) in early-successional (ES, white) and late-successional (LS, gray) tree species in Nyungwe forest. Error bars represent SE with n = 5–7 species per successional group and 7–15 trees per species.

The relationship between V_{cmax25} and N_{tot} differed between ES and LS species (**Figure 4**; **Table 2**). In ES species, V_{cmax25} increased with N_{tot}, while in LS species there was no such dependency at all. At low N_{tot} (~ 1 g m^{-2}), V_{cmax25} was similar in both groups. At higher N_{tot}, however, ES species had considerably higher V_{cmax25} than LS species and this difference increased progressively with the magnitude of N_{tot}. However, R_{d25} was positively related with N_{tot} for both ES and LS species, with similar slopes but different intercepts (**Figure 5**; **Table 2**).

There was a positive relationship between the fraction of total leaf N investments into compounds maximizing photosynthetic capacity (N_{R+B}) versus compounds involved in light-harvesting (N_{LH}), with both ES and LS species displaying similar slopes (**Figure 6**, **Table 2**).

For both ES and LS species, N_{tot} was positively related to LMA (**Supplementary Figure 1**; **Table 2**). There was also a negative relationship between N_{LH} and AQY for both groups, with similar slopes and a nearly significantly lower intercept ($p = 0.052$) for LS species (**Supplementary Figure 2**; **Table 2**).

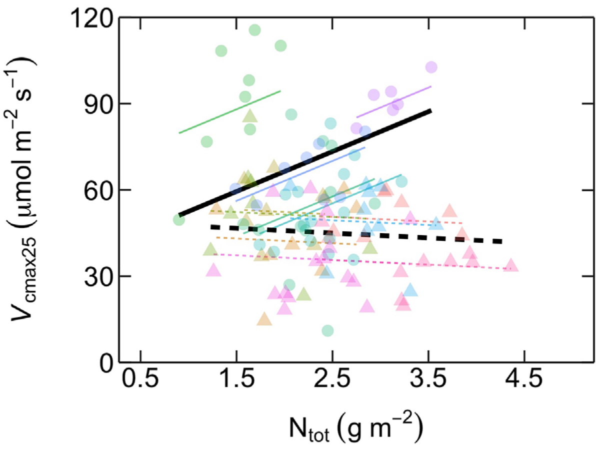

FIGURE 4 | Relationship between maximum rates of Rubisco carboxylation capacity at 25°C (V_{cmax25}, μmol m^{-2} s^{-1}) as a function of area-based total leaf nitrogen content (N_{tot}, g m^{-2}) in early-successional (ES) and late-successional (LS) tree species in Nyungwe forest. Different symbol colors represent each of the 12 studied species, and symbol shapes represent successional groups (ES = circle; LS = triangle). Black solid (ES: $V_{cmax25} = 13.9\ N_{tot} + 38.6$) and dashed (LS: $V_{cmax25} = -1.6\ N_{tot} + 49.1$) lines are overall regression lines for ES and LS groups, respectively. Colored lines represent regression lines for each species belonging to ES (solid) and LS (dashed) groups, respectively, but with common successional group-specific slopes. n = 5–7 species per successional group and 7–15 trees per species.

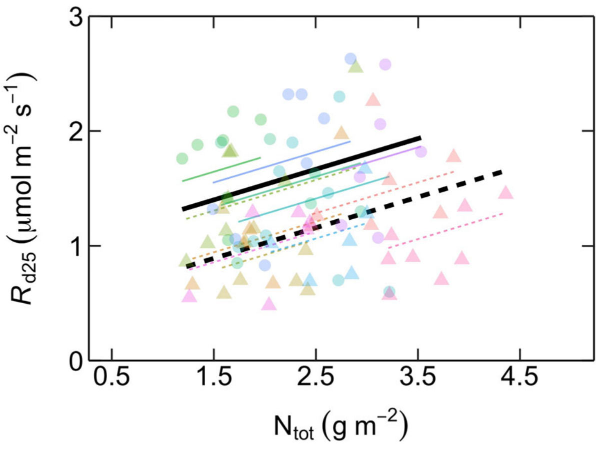

FIGURE 5 | Relationship between dark respiration at 25°C (R_{d25}, μmol m^{-2} s^{-1}) as a function of area-based total leaf nitrogen content (N_{tot}, g m^{-2}) in early-successional (ES) and late-successional (LS) tree species in Nyungwe forest. Different symbol colors represent each of the 12 studied species, and symbol shapes represent successional groups (ES = circle; LS = triangle). Black solid (ES: $R_{d25} = 0.27\ N_{tot} + 0.99$) and dashed (LS: $R_{d25} = 0.27\ N_{tot} + 0.5$) lines are overall regression lines for ES (solid) and LS (dashed) groups, respectively, but with common slopes since these did not significantly differ. Colored lines represent regression lines for each species belonging to ES (solid) and LS (dashed) groups. n = 5–7 species per successional group and 7–15 trees per species.

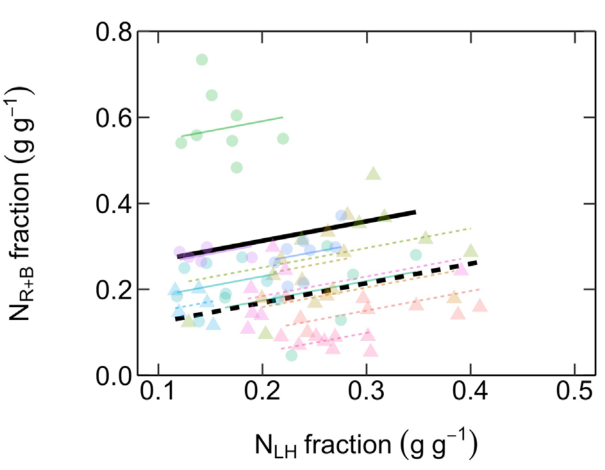

FIGURE 6 | Relationship between fractional investments of total leaf N content into compounds maximizing photosynthetic capacity (N_{R+B}, g g^{-1}) and compounds maximizing photosynthetic light-harvesting (N_{LH}, g g^{-1}). Different symbol colors represent each of the 12 studied species, and symbol shapes represent successional groups (ES = circle; LS = triangle). Black solid and dashed lines are overall regression lines for ES ($N_{R+B} = 0.45\ N_{LH} + 0.22$) and LS ($N_{R+B} = 0.45\ N_{LH} + 0.08$) groups, respectively, but with common slopes since these did not significantly differ. Colored lines represent regression lines for each species belonging to ES (solid) and LS (dashed) groups. n = 5–7 species per successional group and 7–15 trees per species.

DISCUSSION

With the overall aim to explore the controls of interspecific variation in photosynthetic capacity in tropical montane rainforest trees, we investigated physiological, morphological and chemical leaf traits in mature trees of 12 Central African montane rainforest tree species with contrasting light requirements. These species together represent about 60% of large trees (stem diameter at breast height ≥ 30 cm) in Nyungwe forest—Africa's largest remaining mid-elevation montane rainforest block (Plumptre et al., 2002; Chao et al., 2011). The results highlight a marked difference in photosynthetic N dependency between different successional groups, with photosynthetic capacity strongly related to total area-based leaf N content (i.e. N_{tot}) in ES species but not in LS species.

Photosynthetic capacity was markedly higher in ES compared to LS species (**Figure 1**). This difference was caused by strongly contrasting relative investments of leaf N to compounds maximizing photosynthetic capacity, since N_{tot} was similar in the two successional groups (**Figure 3**). These results confirm our first and third predictions. The second prediction, of poor dependency of photosynthetic capacity on N_{tot}, was true for LS species but not for ES species (**Figure 4**). The lack of significant relationship in LS species is in line with other recent studies on tropical rainforest tree species (e.g. Coste et al., 2005; van de Weg et al., 2012; Houter and Pons, 2014; upland species: Bahar et al., 2016; Hasper et al., 2017; Gvozdevaite et al., 2018). However, it contrasts with a study in lowland forests of subtropical China showing a positive relationship between N_{tot} and photosynthetic capacity in LS species but not in ES species (Zhang et al., 2018). Our results on LS species also differ from those of global meta-analyses demonstrating a significant positive relationship between N_{tot} and photosynthetic capacity in tropical trees (Kattge et al., 2009; Reich et al., 2009; Walker et al., 2014).

There are several possible reasons for why meta-analyses report positive relationships between N_{tot} and photosynthetic capacity while specific field studies do not. First, as we show here, photosynthetic N dependency seems strongly linked to species successional strategy (**Figure 4**). A field study focusing primarily on LS species may therefore not detect any significant relationship between N_{tot} and photosynthetic capacity while a meta-analysis including both ES and LS species would do so. Second, the meta-analyses included several studies that examined the canopy vertical variation in N_{tot} and photosynthetic capacity (Porté and Loustau, 1998; Carswell et al., 2000; Kumagai et al., 2001; Meir et al., 2002; Sholtis et al., 2004; Calfapietra et al., 2005; Domingues et al., 2005; Tissue et al., 2005). Since both N_{tot} and photosynthetic capacity are typically higher in sun leaves than in shade leaves this contributes to the overall relationship between the two variables in these studies (e.g. Carswell et al., 2000). Third, meta-analyses included data from tropical areas with large variation in soil fertility (both N and P) while such variation is considerably lower in most specific field studies. This is well illustrated in a recent field study on 210 tree species from lowland Amazonian (lower soil fertility) and upland Andes (higher soil fertility) tropical rainforests, in which a significant relationship between photosynthetic capacity and leaf N_{tot} was found when data from both sites were pooled together (not necessarily a causal relationship since leaf N and P co-varied; Bahar et al., 2016). However, when trees from upland sites with high and fairly homogenous soil fertility were analyzed alone, no relationship between photosynthetic capacity and N_{tot} was found.

Our findings are in line with a recent global meta-analysis covering all types of plants and ecosystems which showed that within-leaf N allocation was a crucial determinant of variation in photosynthetic capacity (Ali et al., 2015). It further showed that about half of the variation in photosynthetic capacity could be attributed to environmental factors influencing photosynthetic N use efficiency (i.e. V_{cmax} or J_{max} divided by N content). Our study suggests that successional group is another factor, not included in the meta-analysis of Ali et al. (2015), which may explain a significant part of variation in photosynthetic capacity. Our results suggest ES and LS species allocate equal fractions of leaf N into compounds maximizing photosynthetic capacity at low N_{tot}, but that at higher N_{tot} ES species gradually increase their absolute N investments to photosynthetic capacity while LS species do not (**Figure 4**). These results are in agreement with the general understanding of how shade-intolerant ES species and shade-tolerant LS species differ with respect to leaf physiological traits related to carbon assimilation, i.e. that ES species prioritize high photosynthesis and rapid growth (Raaimakers et al., 1995; Hikosaka, 2004; Valladares and Niinemets, 2008; Reich, 2014). They provide novel insight by showing that the typical assumption of N_{tot} as a key determinant of photosynthetic capacity seems to hold for ES species but not for LS species, at least in tropical montane forests.

In contrast to the different relationships between V_{cmax25} and N_{tot} in ES and LS species, R_{d25} was positively related to N_{tot} in both groups (**Figure 5**). This may reflect that, as N_{tot} increases, LS species invest the additional N at high N_{tot} into maintenance and secondary metabolism (i.e. defense) rather than into increased photosynthetic capacity (which did not increase; **Figure 4**).

The fourth prediction tested—that there is a trade-off in the allocation of leaf N between investments into compounds maximizing photosynthetic capacity versus compounds maximizing light harvesting—was not supported by our results (**Figure 6**). This hypothesis, proposed by Dusenge et al. (2015) and corroborated by Hasper et al. (2017), was based on their observations of a negative relationship between photosynthetic capacity (i.e. V_{cmax25} and J_{max25}) and SPAD values (a proxy for area-based leaf chlorophyll content). In the current study, we further tested the hypothesis by explicitly investigating the possibility of a trade-off between fractional leaf N investments into Rubisco and bioenergetics (N_{R+B}) and light harvesting compounds (N_{LH}). Strikingly, we found the opposite trend, suggesting that the hypothesis of Dusenge et al. (2015) may not be a general trade-off explaining species successional strategy. It is likely that there are other within-leaf N allocation trade-offs involved which were not investigated here. A recent meta-analysis (Onoda et al., 2017) revealed that the trade-off between photosynthetic N and structural N in cell walls, the two major leaf N pools, underlies the "leaf economics spectrum" (Wright et al., 2004; Hikosaka, 2004). However, this type of structure-function trade-off in N allocation is unlikely to explain the differences in the V_{cmax25}-N_{tot} relationships between ES and LS species found in our study (**Figure 4**), since they did not differ in LMA or N_{tot} (**Figure 3**) and shared a common positive LMA-N_{tot} relationship (SI **Figure 1**).

The fifth prediction tested—that key predictions of the "carbon-gain hypothesis" do not apply to montane rainforest tree species—was corroborated by our study. While photosynthetic capacity and R_{d25} (as also seen in Baltzer and Thomas, 2007) differed in a way predicted by the carbon gain hypothesis (both lower in LS species), AQY, N_{tot}, chlorophyll content, and LMA did not (**Table 2**). In complete contradiction with that hypothesis, AQY was even lower in LS compared to ES species, as also observed in a previous study on tropical montane trees species (Dusenge et al., 2015). Furthermore, our findings showed a negative relationship between AQY and N_{LH} for both groups, implying that increased allocation of leaf N to light harvesting compounds does not necessarily improve light use efficiency, but rather the opposite. The lack of difference in LMA between LS and ES species was not surprising as it agrees with several studies on both mature and young tropical rainforest trees (e.g. Coste et al., 2005; Houter and Pons, 2014; Dusenge et al., 2015; Mujawamariya et al., 2018; Ntawuhiganayo et al., 2020). Some caution should be taken when interpreting observations on sun-exposed leaves of mature trees with respect to species shade tolerance in the understorey. However, since species ranking of leaf traits potentially linked to shade tolerance appears to be similar in sun and shade leaves of juvenile as well as mature tress (Rozendaal et al., 2006; Coste et al., 2009; Dusenge et al., 2015) our results likely have relevance for trees growing in the shade as well.

Implications

Most DGVMs and ESMs represent the variation in V_{cmax} and J_{max} (at a reference temperature) as either fixed values for different plant functional types or as linear functions of area-based leaf N, i.e. N_{tot} (Kattge et al., 2009; Thornton et al., 2009; Zaehle et al., 2010; Rogers, 2014). Our finding of contrasting photosynthetic dependencies on N_{tot} in ES versus LS species suggests that both these approaches are problematic. Constant values for different plant functional types fail to account for factors that control variation in photosynthetic variation within each group, e.g. the variation in N_{tot} of ES species in the present study (**Figure 4**). The N_{tot} function concept, on the other hand, fails to recognize the lack of photosynthetic N dependency found for LS species. Our findings suggest that future model approaches would benefit from introducing a plant trait like within-leaf N allocation or photosynthetic N use efficiency. Such traits may be linked to environmental conditions, as reported earlier (Ali et al., 2015), and also to successional strategy, as found here. Our findings also provide important knowledge to improve the accuracy of smaller-scale process-based models developed to estimate gross and net primary production in tropical montane rainforests. Recent work has supplied these models with a better understanding of the climatic variables and functional traits driving forest productivity, but they still currently suffer from large and unaccounted between-species variation in photosynthetic capacity—leaf N relationships (van de Weg et al., 2014; Fyllas et al., 2017). Additionally, our results suggest that a better understanding of the controls of within-leaf nutrient allocation would contribute to a deeper understanding of plant strategies related to successional status and their position in the "fast-slow" plant economic spectrum (Reich, 2014).

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

AUTHOR CONTRIBUTIONS

CZ, MED, GW, and JU designed the study, CZ, MED, EZ, and BN collected the data, and CZ, MD, and JU analyzed the data. CZ, MED, and JU drafted the article using feedback from all co-authors. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2020.500479/full#supplementary-material

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