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Productivity Drives Leaf Mycobiome Diversity Patterns at Global and Continental Scales

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ABSTRACT

Aim: Studies assessing large-scale patterns of microbial diversity have predominantly focused on free-living microorganisms, often failing to link observed patterns to established theories regarding the maintenance of global diversity patterns. We aimed to determine whether foliar fungi on two closely related grass hosts—*Heteropogon contortus* and *Themeda triandra*—display a commonly observed latitudinal gradient in species richness and determine whether host identity, energy (temperature and precipitation), climate seasonality, fire frequency and grass evolutionary history drive the observed patterns in species richness and composition.

Location: Paleotropical.

Time Period: Contemporary.

Major Taxa Studied: Foliar fungi.

Methods: Foliar fungal diversity was quantified from 201 leaf samples of *T. triandra* and *H. contortus* collected across the distributional range of these species. Mixed effects models were used to quantify patterns of diversity and their correlates among and within continents. Ordinations were used to assess drivers of composition.

Results: Foliar fungi displayed consistent latitudinal diversity gradients in richness. Energy was a strong driver of richness at inter-continental and continental scales, while other factors had inconsistent impacts on richness among scales, hosts and guilds.

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Globally, richness was higher in regions of higher growing season temperatures and where hosts were present for longer periods. Composition was primarily structured by geographic region at the global scale, indicating that distance was a dominant driver of community composition. Within Australia, temperature and rainfall seasonality and the amount of growing season rainfall, were the dominant drivers of both richness and composition.

Main Conclusions: We find some support for the idea that foliar fungal species diversity is governed by the same factors as many macro-organisms (energy availability and evolutionary history) at inter-continental scales, but also that fungal diversity and composition in the highly seasonal continent of Australia were driven by factors that shape tropical grassy ecosystems, namely climate seasonality and fire.

1 | Introduction

Elucidating the factors that determine the abundance, distribution and diversity of organisms across spatial and temporal scales remains a fundamental challenge in ecology, particularly for microbial ecology (Dickey et al. 2021). Arguably, the most studied pattern in ecology is the latitudinal diversity gradient (LDG), where species richness decreases from low to high latitudes (Hillebrand 2004). This pattern is pervasive for most macro-organism taxa, with patterns commonly explained by factors relating to energy availability, evolutionary history or other environmental variables (e.g., environmental heterogeneity) (Hawkins et al. 2003; Gillman et al. 2015; Qian et al. 2015). For example, the species-energy hypothesis claims that energy availability generates and maintains observable richness gradients (Brown 1981). Energy is the product of both incoming solar radiation and water availability, which determines an area's productivity, with productivity controlling richness via trophic cascades such that high energy/productivity areas generally support greater species richness (Hawkins et al. 2003; Gillman et al. 2015). The evolutionary time hypothesis postulates that regional species assemblages which have been present in a location for longer periods of time are more species rich as they have had more time to diversify in situ (Qian et al. 2015). Environmental heterogeneity has been implicated as an important driver of species richness, as more heterogeneous environments have more niches and thus can support a greater diversity of species that utilise these different niches (Kerr and Packer 1997).

Studies assessing large-scale patterns and drivers in the diversity of microbes have predominantly focused on free-living soil and aquatic microorganisms (Hendershot et al. 2017; Shoemaker et al. 2017; Dickey et al. 2021). In contrast, patterns and drivers of diversity in microbial symbionts have been poorly explored. This is unsurprising as microbial symbionts have hosts as an additional constraint regulating their spatial distributions (Härer and Rennison 2023), while it is relatively straightforward to sample free-living microorganisms in a repeatable manner across space to quantify free-living microbial diversity and composition (Ramirez et al. 2018; Baldrian et al. 2022; Cowan et al. 2022). Furthermore, few hosts have a geographic distribution wide enough to decouple the effect of host identity from abiotic and spatial drivers on microbial diversity and composition at global scales. The question that arises is whether congruent diversity patterns for macro-organisms, free-living and host-associated microbes exist, considering that the mechanisms generating macroecological patterns of diversity are theoretically the same for all biological organisms—namely: dispersal, niche-based filtering, speciation, biotic interactions and local extinctions (Saupe 2023).

In microbes, the linear LDG is not found as consistently as in macro-organisms (Peay et al. 2016; Dickey et al. 2021). Microbial diversity can increase, decrease or display hump-shaped patterns with latitude. While microbial diversity patterns show relationships with a multitude of factors, including climate (e.g., precipitation), edaphic (e.g., pH) or biotic factors (e.g., host abundance) (Arnold and Lutzoni 2007; Tedersoo et al. 2014; Davison et al. 2015; Větrovský et al. 2019), findings are rarely linked to established mechanisms of richness patterns that have been proposed for macro-organisms (e.g., Hawkins et al. 2003; Field et al. 2009).

Foliar endophyte and pathogen richness display some of the strongest LDGs in species richness of any microbes; but such global patterns have only been assessed for fungi associated with multiple different hosts, with drivers of richness differing between endophytes and plant pathogens (Arnold and Lutzoni 2007; Makiola et al. 2022). The factors driving diversity patterns may differ between functional groups, as each fungal guild differs fundamentally in its resource drivers (Peay et al. 2016), dispersal capabilities (Bowman and Arnold 2021) and environmental sensitivities (Suzzi et al. 2023). Moisture availability appears to be an important driver of large-scale endophyte richness (Arnold and Lutzoni 2007; Peay et al. 2016), whereas host diversity often predicts pathogen richness due to the high degree of host specificity displayed by plant pathogens (Bever et al. 2015; Rutten et al. 2021).

Microbial composition provides further insight into community assembly. Indeed, understanding the relative contributions of host identity, environmental and spatial factors in structuring the composition of host-associated microbial communities across spatial scales is considered to be one of the most important questions in microbial ecology and biogeography (Antwis et al. 2017). Especially as it has become apparent that host-associated microbiomes can control the reproduction, behaviour and disease susceptibility of their hosts (Busby et al. 2016; Henry et al. 2021). Additionally, understanding how microbial communities are structured across various spatial scales is fundamental to predicting the fate of both microbial communities and their hosts in the face of global change, including climate change, species loss and habitat destruction (Busby et al. 2017; Ngubane et al. 2023; U'Ren et al. 2024).

In summary, continued efforts to elucidate macroecological patterns of microbial diversity of different microbial kingdoms or guilds, and the drivers of the observed patterns, are essential to establishing a common framework of what maintains the diversity of microbes. Additionally, understanding how microbial diversity and composition varies with environmental drivers across spatial scales can help generate reliable

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estimates of global fungal diversity, a number which is hotly debated and partly extrapolated from host-diversity studies (Hyde 2022).

The aim of this study was to determine whether foliar fungi within two closely related and widespread Andropogoneae grass species—*Heteropogon contortus* (L.) Beauv. Ex Roem. and *Themeda triandra* Forssk.—displayed consistent LDGs of species richness. These two C_4 grass species were selected because they are widespread across tropical and subtropical savannas, they are abundant where they occur, and they play important roles in regulating the dynamics of the savannas in which they occur (Snyman et al. 2013; Arthan et al. 2021). We assess whether the effects of climate, evolutionary history and repeated disturbances that commonly drive richness patterns in macro-organisms also explain large-scale diversity patterns of grass-associated fungal species at inter-continental and continental scales. Additionally, we determined whether factors known to shape the composition and structure of tropical grassy ecosystems, for example, climate, seasonality and repeated disturbances (Edwards et al. 2010; Greve et al. 2012; Bianconi et al. 2020) also structured C_4 grass-associated fungal communities. Our first objective was to test if total foliar fungal richness, pathogen richness and endophyte richness within our grass hosts followed the classical LDG of species richness across a wide latitudinal range. Our second objective was to determine if energy availability (high temperatures and rainfall), host lineage age (i.e., the length of time since the host species colonised the respective region of its current distributional range) or the factors responsible for structuring and delineating tropical grassy ecosystems from which the host species were collected (climate seasonality, low minimum temperatures and fire frequency) could explain patterns of foliar fungal diversity and composition across large geographic areas. Thirdly, we determined if the mechanisms generating richness patterns were consistent across different fungal guilds, that is, all fungal Amplicon Sequence Variants (ASVs) vs. pathogens vs. endophytes. Lastly, we compared if the factors driving richness and composition patterns differed across scales, that is, inter-continental vs. continental.

(1) We expected that abiotic factors would be most important in shaping foliar fungal richness patterns, because foliar fungi are generally not as dispersal-limited (compared to other symbiotic, for example, ectomycorrhizal or free-living soil associated microbes) at regional scales when compatible hosts are available, but are sensitive to changes in local abiotic environmental conditions (Peay et al. 2012; Bowman and Arnold 2021; Suzzi et al. 2023). (2) We hypothesised that energy availability, that is, temperature and rainfall, would be among the most important abiotic drivers of species richness, with higher productivity areas supporting more fungal species, because energy availability is the most consistent mechanism generating richness gradients in macro-organisms (Hawkins et al. 2003). (3) Additionally, we expected that each fungal guild (i.e., pathogens vs endophytes) would display nuanced responses to different abiotic factors, as each guild differs in its sensitivities to different abiotic conditions (Zimmerman and Vitousek 2012; Chaloner et al. 2021), and because the effects of abiotic conditions on richness may vary in a host-specific manner (U'Ren et al. 2024). (4) We hypothesised that foliar fungal communities would display higher species richness in regions where the host species have been present over longer evolutionary time periods, as the symbionts in these areas would have had more time to diversify in situ (Qian et al. 2015). (5) We expected lower fungal richness in regions with higher seasonality, as seasonality imposes a strong physiological filter on horizontally transmitted foliar fungi that must endure a part of their lives outside their host (Tedersoo et al. 2014; Oita et al. 2021).

2 | Materials and Methods

2.1 | Study System

Grassy ecosystems, which cover ~40% of the global surface, cannot be predicted by some of the long-established paradigms of modern ecology, namely the deterministic relationship between vegetation and abiotic conditions (Lehmann et al. 2019; Pausas and Bond 2019). Tropical grasslands and savannas are not solely shaped by climate; natural disturbances, especially fire, are major drivers of the diversity, structure and boundaries of these systems (Edwards et al. 2010; Greve et al. 2012; Bianconi et al. 2020). In tropical grassy ecosystems, highly seasonal rainfall and the traits of associated grass lineages facilitate repeated disturbances by fire and herbivory, which in turn are critical for maintaining the dominance of grasses in a climatic zone where climatic factors would predict trees to prevail (Pausas and Bond 2019). The extent to which these factors also affect grass-associated fungi is unknown.

2.2 | Host Selection

Foliar fungal species richness and composition were quantified in the two most widely distributed and abundant species of the two closely related grass genera *Themeda* and *Heteropogon*, namely *Themeda triandra* and *Heteropogon contortus*. Previous studies show that the genera *Heteropogon* and *Themeda* are each other's closest relatives (Arthan et al. 2021). The range of each of these two grass species encapsulates the range of the respective genera: *T. triandra* has a paleotropical distribution and *H. contortus* has a pantropical distribution (Dunning et al. 2017; Arthan et al. 2021). Both *T. triandra* and *H. contortus* are important perennial species, as they are abundant where they occur and play major roles in regulating the dynamics of savannas in which they are found (Snyman et al. 2013). *Themeda triandra* and *H. contortus* both thrive with intermediate levels of two common disturbances in tropical savannas and grasslands—fire and herbivory (Archibald et al. 2019), which allows the species to dominate grassy vegetation across such large geographic scales (Snyman et al. 2013; Arthan et al. 2021).

2.3 | Sampling

To quantify fungal species diversity from *T. triandra* and *H. contortus* plants, individual grasses were sampled from as many locations throughout these species' natural range as possible (Table S1). Five to ten mature green leaves with no visible signs of infection or herbivory damage were clipped from the grass tussock and immediately stored on silica gel. In total, we obtained 221 silica-dried (115 *Themeda* and 106 *Heteropogon*) samples from six countries (Table S1).

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2.4 | Sample Preparation, Homogenisation, DNA Extraction and Sequencing

Grass sample preparation, tissue homogenisation, DNA extraction and fungal sequencing followed Harris, Kemler, et al. (2023) (Methods S1). Briefly, samples were washed to remove the epiphytic burden before being homogenised in a laboratory mixing mill. DNA from each sample was extracted using the my-Budget plant DNA extraction kit. Extracted DNA was used to prepare an Illumina amplicon library using a nested PCR approach (following Harris, Kemler, et al. 2023). During Illumina amplicon library preparation, unique barcode-tag indices were added to each sample (Table S2) together with an overlap region (Table S3) for downstream sample identification. The DNA concentration of each sample was calculated and groups of ~15 samples with similar DNA concentrations were combined. Combined samples were cleaned, and DNA concentrations adjusted. This process was repeated until all 221 samples were combined into one final pooled sample with equal DNA concentrations.

The final combined amplicon library with all 221 samples was sequenced at the Genomics Service Unit of the Ludwig Maximilians University (LMU) Biocenter on an Illumina MiSeq sequencer (Illumina Inc., San Diego, CA, USA) using the MiSeq Reagent Kit v3 Chemistry, for 2 × 300 bp paired-end sequencing. All raw sequences were deposited on the NCBI portal under the following accession codes: BioProject—PRJNA1114630; BioSample (SAMN41489108—SAMN41489334). The metadata of the data can be found on figshare (https://doi.org/10.6084/m9.figshare.28830938.v2).

2.5 | Bioinformatics

Bioinformatic processing was performed using the QIIME 1 (Caporaso et al. 2010) and QIIME 2 bioinformatic pipelines (Bolyen et al. 2019). Samples were demultiplexed according to their unique barcode-tag/index combinations, which were removed during demultiplexing together with their identifying primer sequences (Table S3). Since reverse reads often exhibit lower PHRED quality, coupled with length differences in the ITS gene region, which limits the merging of both forward and reverse reads, only forward reads were used for subsequent analyses. Eleven samples were lost during bioinformatic processing (Tables S1 and S2) because the unique barcode-tag/index combinations of these samples failed to amplify, precluding sample recovery (Tables S1 and S2). A further nine *Heteropogon* samples were excluded as they were not from our two target grass species (Tables S1 and S2). Using QIIME 2, the raw sequences from the remaining 201 samples (109 *T. triandra* and 92 *H. contortus*) were passed through deblur (Amir et al. 2017) to assign raw sequence reads to ASVs. All reads were trimmed at 180 bp and the UNITE database (version 9; https://unite.ut.ee/) was used as a reference sequence database to assign taxonomy to the recovered ASVs (Kõljalg et al. 2013; Abarenkov et al. 2024). All ASVs not assigned to the Kingdom Fungi were discarded. The remaining fungal ASVs were written into an ASV feature table, which was used for all statistical analyses. All statistical analyses were performed in R-4.3.1 (R Core Team 2023).

2.6 | Assignment to Fungal Guilds

ASVs were assigned to their corresponding fungal guilds using the *FUNGuildR* package *v* 0.2.0.9 (Nguyen et al. 2016). The total number of ASVs from each sample assigned to the guild's pathogen, endophyte or endophyte pathogen, and which had a confidence ranking of 'highly probable' or 'probably' were summed. Since the number of ASVs assigned to the guild 'endophyte' was low (average 0.25 endophytes ASVs per sample), all ASVs assigned to the guild 'endophyte' were combined with the guild 'endophyte pathogen', as many endophytes can be considered opportunistic pathogens (Slippers and Wingfield 2007). These are henceforth referred to as 'endophyte'. Importantly, the ecological lifestyles of many fungi are still unknown; therefore, assigning ASVs to fungal guilds has a degree of uncertainty and thus should be interpreted cautiously (Nguyen et al. 2016).

2.7 | Predictor Variable Selection

The emergence and rapid spread of C_4 grassy biomes during the Late Miocene was caused by the adaptation of C_4 grasses to fire, grazing, aridity and increased seasonality (Edwards et al. 2010; Lehmann et al. 2019). Therefore, we speculated that these factors could also drive associated endophyte communities. From the possible 19 bioclimatic variables available within the WorldClim dataset (Fick and Hijmans 2017), we selected six variables which are determinants of C_4 grass distribution, dominance and disturbance regimes: BIO4 (temperature seasonality), BIO6 (minimum temperature of the coldest month), BIO8 (mean temperature of the wettest quarter), BIO12 (annual precipitation), BIO15 (precipitation seasonality) and BIO18 (precipitation of the warmest quarter). The climatic data were downloaded from WorldClim version 2.1, at 30″ resolution (Fick and Hijmans 2017). We extracted fire frequency between 2001 and 2020 from the MODIS Fire_cci Burned Area Pixel Product collection 6.1 version 1.1 at 500 m resolution (Giglio et al. 2018) using Google Earth Engine (Gorelick et al. 2017). Lastly, we hypothesised that greater fungal diversity would be found in older grass lineages, that is, in regions where the host grass lineage has occupied a location for longer. We extracted for our *T. triandra* samples the estimated time period that lineages have occupied each region (henceforth 'lineage age') (in Mya) from the subcontinental age estimates estimated by Dunning et al. (2017). More specifically, our Thai samples were assigned the south Asian age estimates, Australian samples the Australian age estimates, Madagascar samples the Madagascan age estimates, the Zambian and Tanzanian samples the East African age estimates, and the South African samples assigned the southern African age estimates (Dunning et al. 2017). Age estimates were not available for *H. contortus* lineages.

We tested for multi-collinearity of predictor variables prior to analyses (Table S4). Pairs of variables with $r > |0.75|$ were considered highly correlated. No pair of variables displayed $r > |0.75|$; therefore, all eight variables were retained for *T. triandra* and all seven variables for *H. contortus* (no lineage age estimate was available for the latter).

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2.8 | Species Richness Measure

We considered both raw species (i.e., measured ASV richness) and estimated ASV richness (i.e., breakaway) as response variables. Differences in read counts between samples can influence the observed richness of a given sample (Willis 2019). Alpha diversity (i.e., local richness) is, therefore, often calculated after adjusting for differences in sample abundances (i.e., read counts) through rarefaction (Gotelli and Colwell 2001). However, for microbial metabarcoding datasets, which are representative of relative abundances, rarefaction is an inappropriate statistical technique (McMurdie and Holmes 2014). An alternative method is to estimate alpha diversity by correcting richness values for unobserved and rare taxa (Willis et al. 2017). We thus calculated breakaway estimated richness (Willis and Bunge 2015) in the *breakaway* package v 4.8.4 (Willis et al. 2022) for all samples, measured the correlation between raw and estimated ASV richness and ran all analyses using both raw and estimated richness for comparison.

2.9 | Patterns and Drivers of Foliar Fungal Richness

To assess whether foliar fungal richness displayed a LDG of species richness, we constructed generalised linear models with a Poisson distribution and a log-link function using the *glm2* package v 1.2.1 (Marschner 2011), where ASV richness was fit in response to the interaction between host and absolute latitude. This was done for the total foliar fungal ASV richness and repeated for each of the fungal guilds (pathogens and endophytes).

The influence of annual precipitation, temperature and precipitation seasonality, minimum temperature of the coldest month, mean temperature of the wettest quarter, precipitation of the warmest quarter and number of fires (2001–2020) on total ASV richness, pathogen richness and endophyte-pathogen richness was tested using random intercept generalised linear mixed effects models (GLMMs) with a Poisson distribution and a log-link function, using the *lme4* package v 1.1–34 (Bates et al. 2015). Interaction terms between host species identity and all variables were fit and geographic zone was included as a random effect (Zuur et al. 2009). Geographic zone represented the broad geographic area where samples were collected (i.e., southern Africa, Madagascar, Thailand and Australia). We tested for overdispersion using the *blmeco* package v 1.4 (Korner-Nievergelt et al. 2015). An observation-level random effect was included to reduce overdispersion to an acceptable level (McCulloch 1997; Harris et al. 2019). We tested models for spatial autocorrelation. When significant spatial autocorrelation was detected, we utilised a spatial filtering approach (Griffith 2003). Briefly, spatial eigenvectors were included as predictor variables in the models to partial out spatial autocorrelation (following a similar approach as Blach-Overgaard et al. 2010). Spatial eigenvectors were constructed using the *spdep* v 1.2–8 and *spatialreg* v 1.2–9 packages (Bivand 2022). Best subset modelling, based on the lowest AIC-value, was used to determine which variables from the global model should be retained (Burnham and Anderson 2002), which was determined using the *MuMIn* package *v* 1.47.5. Since the drivers of species richness are known to vary as a function of spatial scale (Field et al. 2009) and because Australia represented the most consistently sampled continent across many degrees of latitude, we repeated the above analyses for Australia alone, to assess how the drivers of foliar fungal richness differed between inter-continental vs. continental scales.

We calculated the semi-partial R^2 values for each variable or set of variables retained in the final model using the *partR2* package *v* 0.9.1 (Stoffel et al. 2021). Semi-partial R^2 decomposes the variance of the total model R^2 into components uniquely explained by individual predictors, interactions between predictors or groups of predictors, for example, host vs space vs climate (Stoffel et al. 2021).

Since we wanted to test whether the length of time since grasses had colonised various parts of their range influenced the observed ASV richness and because we lacked lineage age estimates for *H. contortus*, we ran separate models for each of the two host species to assess drivers of total ASV richness: only the *T. triandra* model included lineage age.

2.10 | Drivers of Foliar Fungal Community Composition

To determine whether host identity, geographic distance or other factors known to shape the composition and structure of tropical grassy ecosystems, for example, climate, seasonality and repeated disturbances by fires, were the dominant factors shaping the composition of C_4 grass-associated fungal communities at continental and inter-continental scales, we performed Non-metric Multidimensional Scaling analyses on each sample's Bray–Curtis dissimilarities using the *vegan* package v 2.6–10 (Oksanen et al. 2023). To determine which variables to retain as predictors of community composition at both the continental and inter-continental scales, we used the *AICcPermanova* package *v 0.0.2* (Corcoran 2023).

3 | Results

The 201 leaf samples from the two different host species yielded 761,162 demultiplexed sequence reads. On average, each sample had 3787 ± 1455 sequence reads (Table S5). A total of 3904 unique ASVs were recovered, with an average of 120.71 ± 39.9 ASVs per sample. The number of ASVs recovered from all *H. contortus* and *T. triandra* samples was 2488 and 2727, respectively.

Of the 3904 unique ASVs recovered, 83% were named to the family level, 71% to the genus level and 41% to the species level. Only 17 (0.44%) of the recovered ASVs were assigned to the guild endophyte, whereas 282 (7.22%) and 153 (3.92%) of the ASVs were assigned to the fungal guilds endophyte-plant pathogen and plant pathogen, respectively. Comparatively, 1718 (44%) ASVs could not be assigned to any guild. Most of the ASVs belonged to Ascomycota (75%) and Basidiomycota (19%); 6% of the ASVs could not be assigned to a phylum. Within the dataset, the most abundant genera recovered were: *Ramichloridium* (7% of ASVs), *Toxicocladosporium* (5%), *Paraphaeosphaeria* (3%), *Phaeosphaeria* (3%), *Curvularia* (3%) and *Hyweljonesia* (2%).

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3.1 | Latitudinal Diversity Gradient

Themeda triandra and *H. contortus* showed different total foliar fungal ASV richness patterns with latitude, as supported by the interaction term in our model [χ^2 (1, $N=201$) = 9.24, $p=0.002$]: richness decreased with latitude for *T. triandra*, but increased for *H. contortus* (Figure S3A). However, when ASV richness of the two hosts was pooled, total foliar ASV richness decreased significantly from low to high latitudes [χ^2 (1, $N=201$) = 25.95, $p=3.51\times10^{-7}$, $R^2=0.1186$]. Additionally, both fungal guilds, 'pathogen' [χ^2 (1, $N=201$) = 15.26, $p=9.36\times10^{-5}$, $R^2=0.0927$] and 'endophyte pathogen' (including all ASVs classified as endophytes) [χ^2 (1, $N=201$) = 9.4, $p=0.0022$, $R^2=0.0509$], showed significant declining richness from lower to higher latitudes (Figure S3B,C). For both models, the interaction effect between host and absolute latitude was not significant and, therefore, dropped from the models. However, the amount of variation explained by the above models was low, and LDGs seemed to primarily be driven by patterns observed in Australia for total ASV richness [χ^2 (1, $N=51$) = 3.95, $p=0.0468$, $R^2=0.197$] (Figure 1) and endophyte-pathogen richness [χ^2 (1, $N=51$) = 72.7, $p=2.2\times10^{-16}$, $R^2=0.673$] (Figure S4), but not pathogen richness [χ^2 (1, $N=51$) = 0.044, $p=0.8334$, $R^2=0.001$] (Figure S5).

3.2 | Drivers of Richness

3.2.1 | Inter-Continental Foliar ASV Richness

Raw species richness and the estimated species richness values were highly correlated with one another ($r=0.901$). Accordingly, models built on the raw and the estimated richness values yielded similar results (see Figure 2 vs. S1 and Figure 5 vs. S2), and because diversity estimates themselves introduce their own set of biases (Willis 2019), we present and discuss only the results from the raw richness in the main manuscript. We do provide the results based on estimated richness in the Supporting Information.

The best subset GLMM explaining total foliar fungal ASV richness in *H. contortus* at the inter-continental scale retained minimum temperature of the coldest month, mean temperature of the wettest quarter, mean precipitation of the warmest quarter and two spatial eigenvectors (Figure 2). Total ASV richness in *H. contortus* decreased with minimum temperatures of the coldest month, whereas richness increased as mean temperature of the wettest quarter and mean precipitation of the warmest quarter increased. The climatic variables

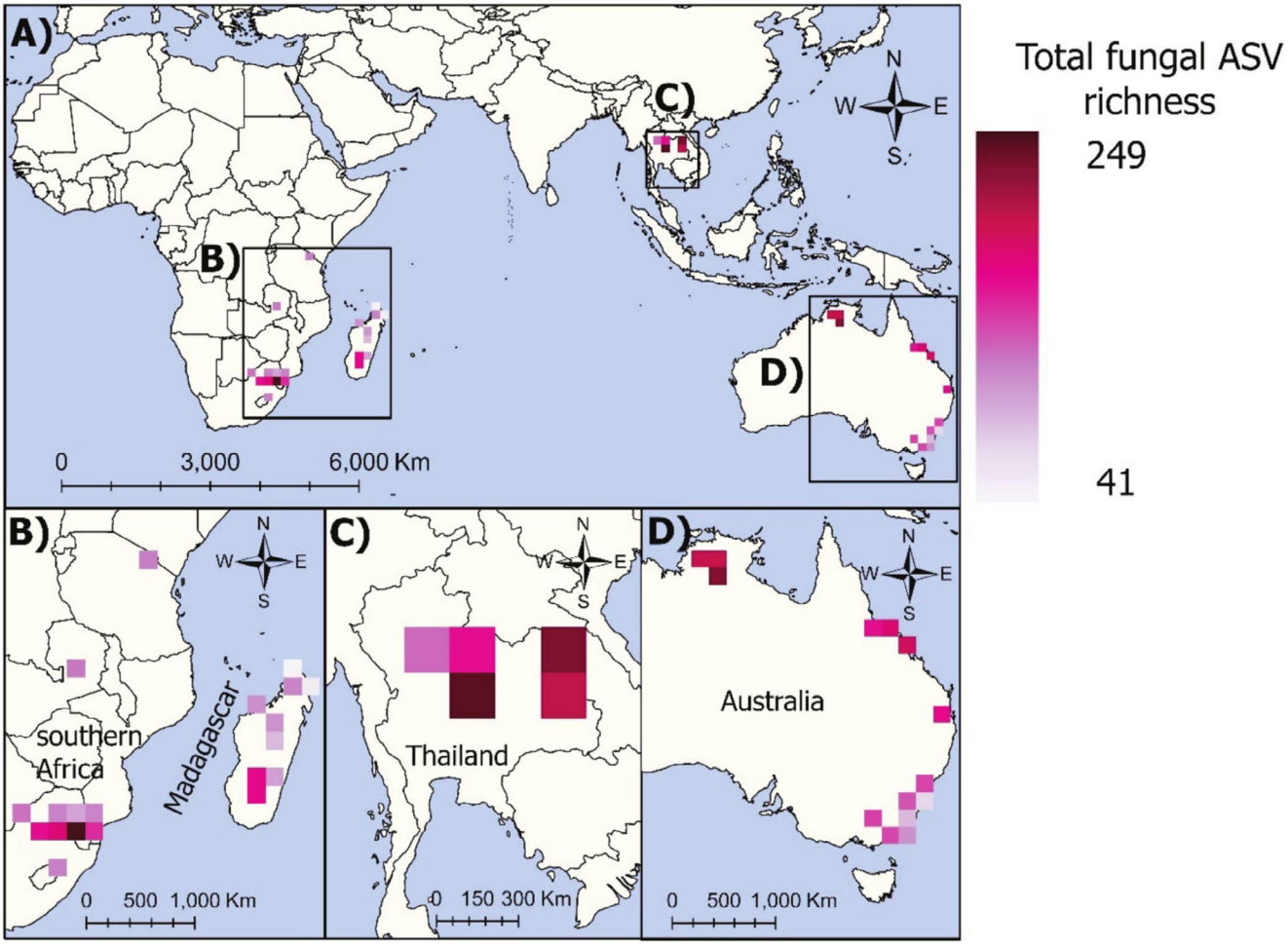

FIGURE 1 | Map showing the average total foliar fungal ASV richness per 1.5×1.5° grid cell, for both *H. contortus* and *T. triandra* combined, from locations where grass leaves were sampled. (A) Displaying the inter-continental overview of the average ASV richness. (B) Inset showing the average ASV richness across southern Africa and Madagascar, (C) inset showing the average ASV richness within Thailand and (D) inset showing the average ASV richness across Australia.

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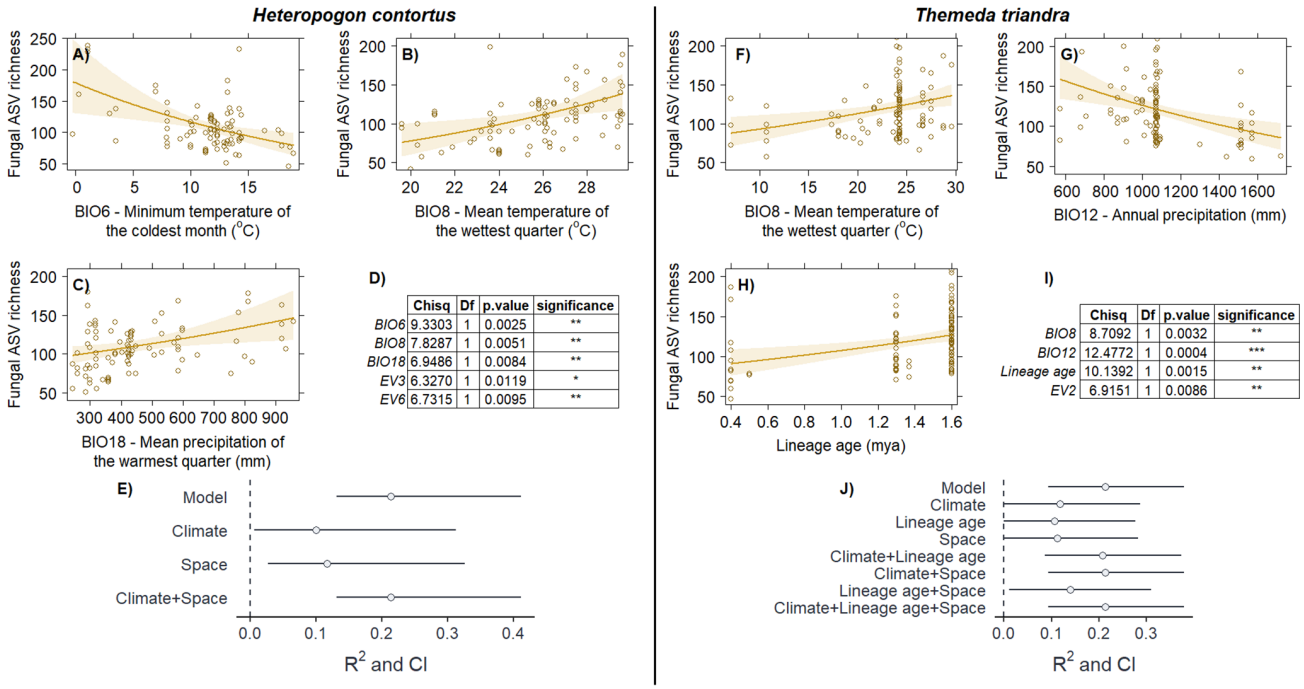

	Chisq	Df	p.value	significance
BIO6	9.3303	1	0.0025	**
BIO8	7.8287	1	0.0051	**
BIO18	6.9486	1	0.0084	**
EV3	6.3270	1	0.0119	*
EV6	6.7315	1	0.0095	**

	Chisq	Df	p.value	significance
BIO8	8.7092	1	0.0032	**
BIO12	12.4772	1	0.0004	***
Lineage age	10.1392	1	0.0015	**
EV2	6.9151	1	0.0086	**

FIGURE 2 | Left hand panel—effect plots (A–C), ANOVA summary statistics (D) and semi-partial R^2 (E) results from the best subset inter-continental model showing the relationship between total foliar fungal ASV richness in *H. contortus* and (A) BIO6—minimum temperature of the coldest month, (B) BIO8—mean temperature of the wettest quarter and (C) BIO18—average precipitation of the warmest quarter. (D) The type II Wald chi-squared ANOVA statistics for the final best subset model assessing drivers of richness in *H. contortus*. Here, EV3 and EV8 represent spatial eigenvector 3 and 8, respectively, which were the two spatial eigenvectors required to reduce spatial autocorrelation to an acceptable level. (E) the semi-partial R^2 results for individual predictors and combinations of predictors and their 95% confidence intervals. Right hand panel—effect plots (F–H), ANOVA summary statistics (I) and semi-partial R^2 (J) results from the best subset inter-continental model showing the relationship between total foliar fungal ASV richness in *T. triandra* and (F) mean temperature of the wettest quarter, (G) BIO12—annual precipitation and (H) *T. triandra* intra-specific lineage age. (I) the type II Wald chi-squared ANOVA statistics for the final best subset model assessing drivers of richness in *T. triandra*. Here, EV2 represents the spatial eigenvector, which reduced spatial autocorrelation to an acceptable level. (J) The semi-partial R^2 results for individual predictors and combinations of predictors and their 95% confidence intervals. Light yellow shading represents the 95% confidence interval for the relationship between total foliar fungal ASV richness and the retained predictor variables (A–C & F–H). The hollow yellow circles represent the partial residuals for their respective effect plots (A–C left hand panel & F–H right hand panel).

and the spatial component explained similar amounts of variation.

Foliar fungal richness of *T. triandra* was best explained by mean temperature of the wettest quarter, annual precipitation, *T. triandra* lineage age and a single spatial eigenvector (Figure 2). Total ASV richness increased with mean temperature of the wettest quarter and *T. triandra* lineage age. Fungal ASV richness decreased with annual precipitation. Climate, lineage age and space all explained similar amounts of variation.

3.2.2 | Inter-Continental Richness Patterns of Fungal Guilds

The best subset GLMM of pathogen richness at the inter-continental scale retained the interaction between host species and temperature seasonality, the interaction between host species and annual precipitation, mean temperature of the wettest quarter, precipitation seasonality and three spatial eigenvectors (Figure 3). The best subset GLMM assessing endophyte-pathogen richness similarly retained the interaction between host identity and temperature seasonality, the interaction between host identity and annual precipitation, mean temperature of the wettest quarter and three spatial eigenvectors, but also retained the interaction between host identity and precipitation seasonality, precipitation of the warmest quarter and the number of fires between 2001 and 2020 (Figure 4).

In *H. contortus*, pathogen richness was unaffected by temperature seasonality, but endophyte-pathogen richness increased with temperature seasonality; whereas in *T. triandra*, pathogen richness decreased and endophyte-pathogen richness increased with temperature seasonality (Figures 3 and 4). Endophyte-pathogen richness decreased, and pathogen richness was unaffected by annual precipitation in *H. contortus*; and pathogen richness decreased and endophyte-pathogen richness was unaffected by annual precipitation in *T. triandra* (Figures 3 and 4). Although annual precipitation was retained in the pathogen model, its effect was small and is, therefore, not discussed. Both pathogen richness and endophyte-pathogen richness increased with mean temperature of the wettest quarter. Pathogen richness of both hosts decreased as precipitation seasonality increased, whereas endophyte-pathogen richness in the two host species showed contrasting responses: in *H. contortus*, richness decreased with precipitation seasonality whereas richness was unaffected in *T.*

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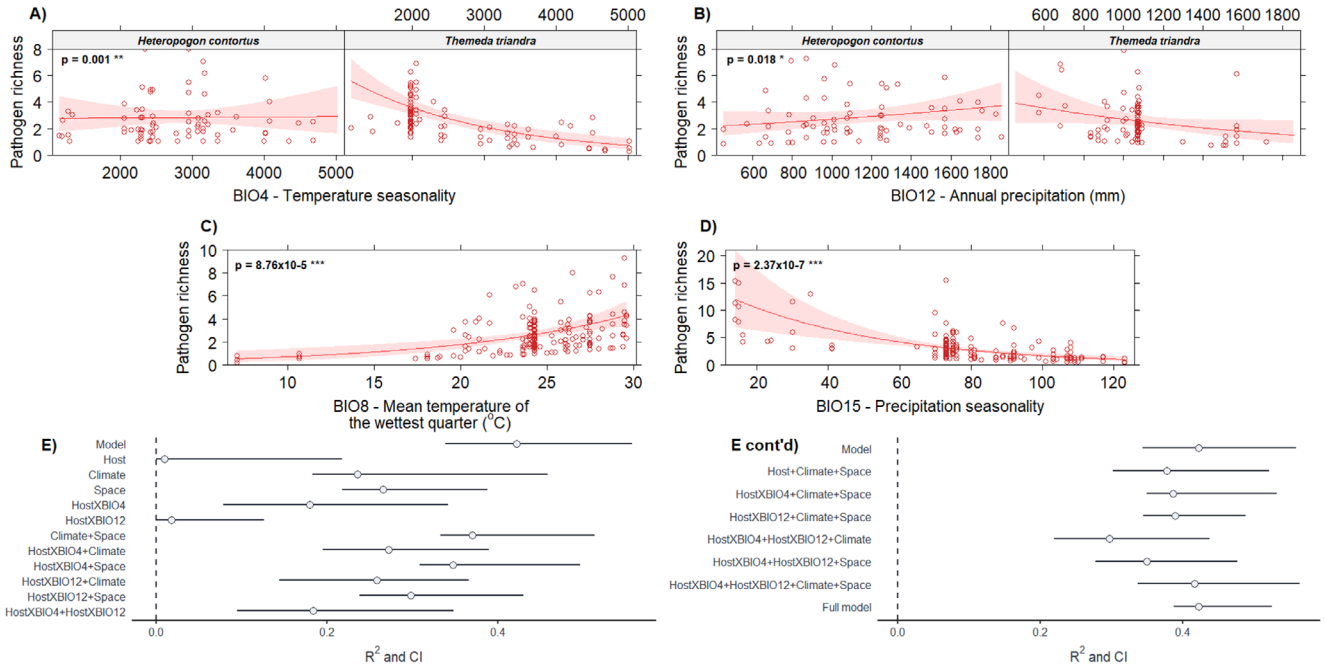

FIGURE 3 | Effect plots (A–D) and semi-partial R^2 (E) results from the best subset inter-continental model showing the relationship between foliar fungal pathogen richness and (A) the interaction between host and BIO4—temperature seasonality, (B) the interaction between host and BIO12—annual precipitation, (C) BIO8—mean temperature of the wettest quarter and (D) BIO 15—precipitation seasonality. (E) The semi-partial R^2 results for individual predictors, interaction terms (e.g., HostXBIO4 is the interaction between grass host and temperature seasonality) and combinations of predictors and their 95% confidence intervals. Light red shading represents the 95% confidence interval for the relationship between total foliar fungal pathogen richness and the retained predictor variables (A–D). The hollow red circles represent the partial residuals for their respective effect plots (A–D).

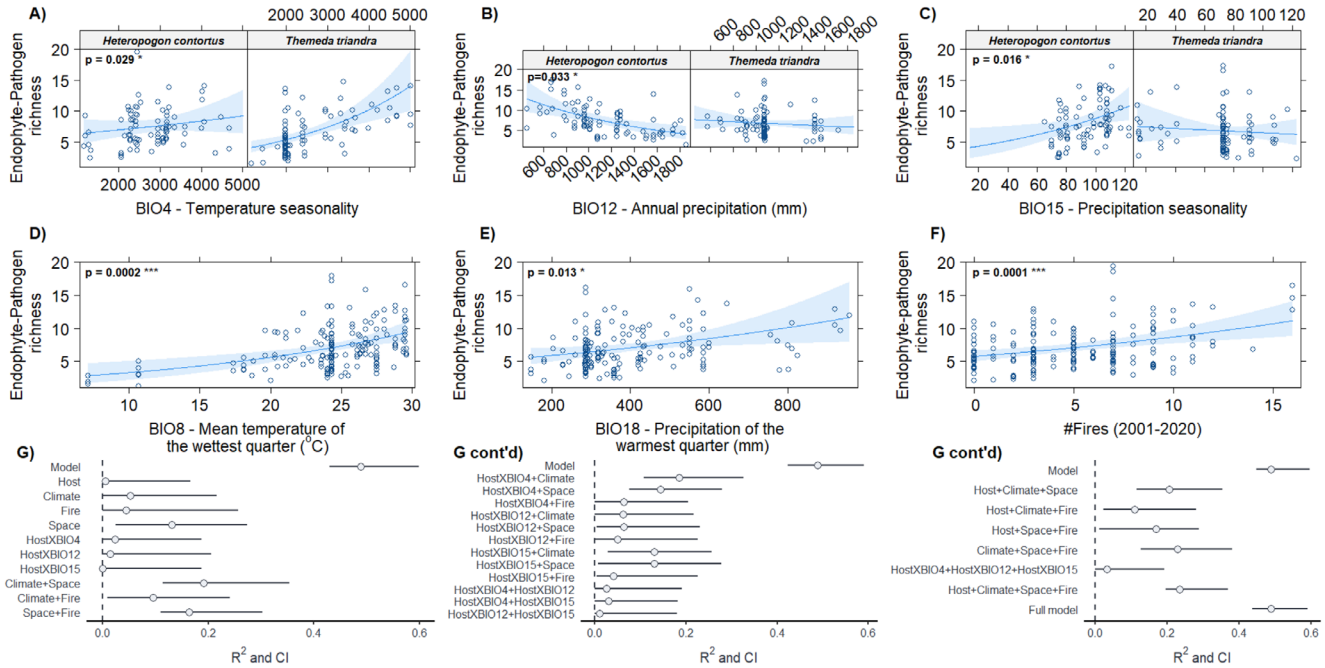

FIGURE 4 | Effect plots (A–F) and semi-partial R^2 (G) results from the best subset inter-continental model showing the relationship between foliar fungal endophyte richness and (A) the interaction between host and BIO4—temperature seasonality, (B) the interaction between host and BIO12—annual precipitation, (C) the interaction between host and BIO15—precipitation seasonality, (D) BIO 8—mean temperature of the wettest quarter, (E) BIO18—mean precipitation of the warmest quarter and (F) the number of fires each sample experienced between 2001 and 2020. (G) The semi-partial R^2 results for individual predictors, interaction terms (e.g., HostXBIO12 is the interaction between grass host and annual precipitation) and combinations of predictors and their 95% confidence intervals. Light blue shading represents the 95% confidence interval for the relationship between total foliar fungal endophyte richness and the retained predictor variables (A–F). The hollow blue circles represent the partial residuals for their respective effect plots (A–F).

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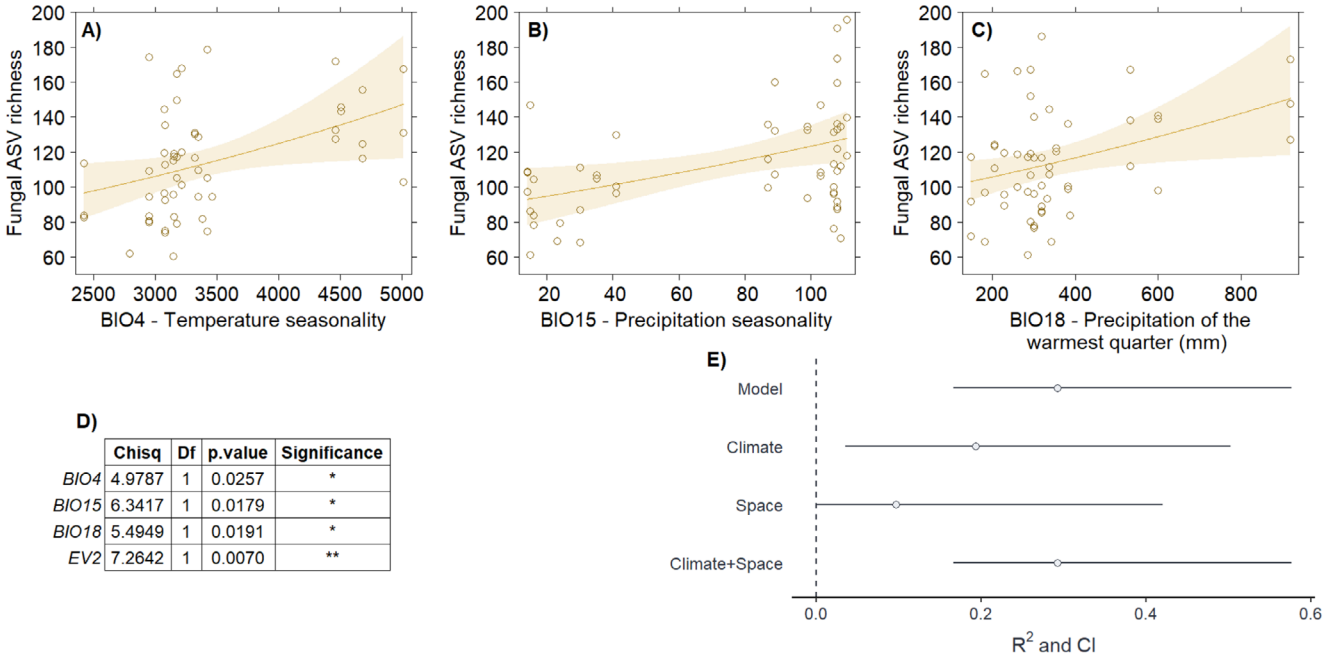

	Chisq	Df	p.value	Significance
BIO4	4.9787	1	0.0257	*
BIO15	6.3417	1	0.0179	*
BIO18	5.4949	1	0.0191	*
EV2	7.2642	1	0.0070	**

FIGURE 5 | Effect plots (A–C), ANOVA summary statistics (D) and semi-partial R^2 (E) results from the best subset model showing the relationship between total foliar fungal ASV richness across Australia and (A) BIO4—temperature seasonality, (B) BIO15—precipitation seasonality and (C) BIO18—precipitation of the warmest quarter. (D) The type II Wald chi-squared ANOVA statistics for the best subset model. Here, EV2 represents the spatial eigenvector, which reduced spatial autocorrelation to an acceptable level. (E) The semi-partial R^2 results for individual predictors and combinations of predictors together with their 95% confidence intervals. Light yellow shading represents the 95% confidence interval for the relationship between total foliar fungal ASV richness and the retained predictor variables (A–C). The hollow yellow circles represent the partial residuals for their respective effect plots (A–C).

triandra. Endophyte-pathogen richness increased as precipitation of the warmest quarter and fire frequency increased. Climate and the spatial component explained similar amounts of variation in pathogen richness, whereas the spatial component contributed more than double the model variance of climate and fire frequency in explaining endophyte-pathogen richness.

3.2.3 | Continental Richness Patterns (Australia)

When the drivers of total ASV richness were assessed for Australia, the GLMM retained temperature seasonality, precipitation seasonality, precipitation of the warmest quarter and one spatial eigenvector (Figure 5). Total ASV richness increased with temperature seasonality, precipitation seasonality and precipitation of the growing season. The three retained climatic variables explained double the variance of the spatial component represented by a single eigenvector. These results were almost identical when breakaway estimated richness was assessed across Australia (Figure S2).

3.3 | Drivers of Community Composition

3.3.1 | Inter-Continental Composition Patterns

The best subset model assessing the drivers of foliar fungal community composition at the inter-continental scale retained geographic zone, the interaction between host and annual precipitation, minimum temperatures of the coldest month, precipitation seasonality and fire frequency (Figure S6). Geographic zone had the strongest effect on foliar fungal community composition ($R^2 = 0.111$), followed by host identity ($R^2 = 0.0299$) and minimum temperature of the coldest month ($R^2 = 0.0257$) (Table S5). All other variables had a small but significant effect on foliar fungal community composition (with R^2 ranging from 0.0138 to 0.0097) (Table S5). Overall, model performance was modest, with ~22% of the variation in composition being explained (Table S5).

3.3.2 | Continental Composition Patterns (Australia)

When the drivers of foliar fungal community composition were assessed for the samples from Australia alone, the best subset model retained temperature and precipitation seasonality, fire frequency and precipitation of the warmest quarter (Figure S7). Although the fungal communities on the two host grasses appeared distinct, host identity was not retained in the final model (Figure S7, Table S6). Precipitation seasonality and temperature seasonality were the strongest predictors, explaining a combined 16.8% of the variation (Table S6). Fire frequency and precipitation of the warmest quarter explained 7.6% and 5.9% of the variation in foliar fungal composition, respectively (Table S6). The model assessing the composition of foliar fungal communities across Australia performed better than the intercontinental model, explaining just over 30% of the variation.

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4 | Discussion

4.1 | Consistent LDGs Displayed by All Foliar Fungal Guilds

Weak LDGs of total foliar, pathogen and endophyte-pathogen richness were mostly observed across a wide latitudinal gradient that encompassed a large part of these two grass species' natural range. Only ASV richness in *Heteropogon contortus* did not increase with latitude. These LDGs were largely driven by richness patterns across Australia. Evidence for LDGs in fungal pathogens is ubiquitous (Peay et al. 2016; Makiola et al. 2022), but for other fungal guilds, for example, endophytes, evidence is equivocal and context-dependent (Arnold and Lutzoni 2007; U'Ren et al. 2024) or non-existent when total fungal richness is assessed (Baldrian et al. 2022). Evidence for LDGs in microbes more generally is uncommon, with less than a third of studies finding support for this macroecological pattern (Dickey et al. 2021). However, tropical regions, particularly in Africa, are vastly undersampled and our views on microbial diversity patterns may change as more samples are taken from these regions (Harris, Slippers, et al. 2023). Here, we find largely consistent support for the LDG of species richness for foliar fungi associated with the same widespread hosts sampled across multiple continents. This demonstrates that at least some microbial communities within certain hosts may display stronger sensitivities to changes in environmental gradients. Importantly, environmental gradients like temperature and precipitation, which are commonly invoked as drivers of LDGs in species richness, are not as strongly correlated with latitude across African savannas as they are across much of the rest of the world (Greve et al. 2012), possibly explaining why observed LDG patterns were significant but weak.

4.2 | Drivers of Total Fungal ASV Richness at the Global Scale

As hypothesised, energy availability was the strongest and most consistent driver of richness patterns across both spatial scales and all fungal guilds, with growing season temperature and rainfall being consistently retained individually or simultaneously across all models. Global foliar fungal ASV richness increased with growing season temperatures in both *H. contortus* and *T. triandra*, and high growing season rainfall resulted in greater ASV richness in *H. contortus*. This was expected as C_4 grasses grow rapidly under high light intensities and temperatures when water becomes available (Still et al. 2003). Plant-derived carbon is a major determinant of fungal richness (Peay et al. 2016) and the rapid growth rates of Andropogoneae grasses during the warm and wet growing season likely provide an abundant food source and rapidly increasing niche space, supporting increased diversity of foliar fungi. This finding lends support to the idea that high-energy availability, which increases the host's productivity is an important factor driving foliar fungal species richness (Hawkins et al. 2003; Field et al. 2009). In general, when microbial communities do follow LDGs, these patterns are usually correlated with temperature gradients (Dickey et al. 2021), similarly to what we find here.

ASV richness in *H. contortus* increased with colder minimum winter temperatures, and richness in *T. triandra* decreased with annual precipitation. We anticipated that cold winter temperatures would reduce richness by imposing a constraint on the survival of fungal hyphae and spores. Our minimum monthly temperature gradient did not extend far into negative temperatures (coldest site = −0.2°C); had the gradient been larger, we may have seen a different pattern: endophyte diversity can display a humped-shaped pattern with minimum temperatures, where richness increases to a point before declining above a critical threshold (Wang et al. 2023). One of our strongest a priori expectations was that endophyte richness would increase with precipitation, as moisture availability is often the strongest determinant of fungal diversity (Peay et al. 2016), especially for foliar endophytes (Zimmerman and Vitousek 2012; Giauque and Hawkes 2016); and precipitation is an important factor controlling energy availability (Hawkins et al. 2003). We found some, but not consistent support for richness increasing with precipitation (with richness only increasing with growing season precipitation). This may be because C_4 Andropogoneae grasses do not require much precipitation to maintain high growth rates (Snyman et al. 2013; Wang et al. 2016). Alternatively, few fungal species could dominate the community at high precipitation, which has been observed in tropical endophyte communities Vincent et al. (2016).

Our results suggest that the evolutionary history of a host affects diversity patterns of its associated mycobiome. In regions where *T. triandra* had been present for longer time periods, fungal diversity was higher, providing support for the evolutionary time hypothesis (Qian et al. 2015). Hosts which have been present in a location for longer could have had more time to form specialised associations (Apigo and Oono 2018) or taxa more time to speciate in situ (Roncal et al. 2011; Qian et al. 2015), increasing the regional species pool. Alternatively, generalist fungi could have had more time to acquire an increased arsenal of secondary metabolite gene clusters, which enabled them to speciate within their hosts (Franco et al. 2022). If such a finding is consistent across other widely distributed hosts, this will be an important consideration for future host-associated microbial diversity studies as it may account for differences in large-scale fungal diversity patterns as well as for extrapolating global fungal diversity estimates.

4.3 | Drivers of Fungal ASV Composition at the Global Scale

At the global scale, fungal community composition was most strongly structured by geographic region, explaining nearly four times the amount of variation as compared to the next most important factor: host identity. The strong effect of geographic region on foliar fungal community composition indicates that distance decay is an important driver of turnover of foliar fungal communities, as in other taxa (Nekola and White 1999; Oono et al. 2017; Bowman and Arnold 2021). It has been suggested that distance decay patterns displayed by foliar endophyte communities are driven by environmental differences between localities and not because of dispersal limitation (Bowman and Arnold 2021), which may explain why the retained environmental variables explained two and a half times the variation explained by host identity, which is otherwise often the strongest predictor of foliar fungal composition (Harris, Kemler, et al. 2023).

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4.4 | Drivers of Pathogen and Endophyte-Pathogen Richness at the Global Scale

There was little consistency between guilds and between host plants in how abiotic factors related to endophyte or pathogen richness. This was expected, as different fungal guilds (e.g., foliar endophytes vs ectomycorrhizal fungi) or microbes from different habitats (e.g., host-associated vs free-living soil associated microbes) show varying sensitivities to different abiotic factors (Bowman and Arnold 2021; Suzzi et al. 2023). The only factor which had a consistent positive and strong effect on the richness of all ASVs and the two fungal guilds was high growing season temperature, which resulted in an increase in richness; this reiterates the importance of energy inputs driving host productivity, which indirectly controls global foliar fungal richness patterns (Hawkins et al. 2003).

The richness of both pathogen and endophyte-pathogen guilds was additionally affected by temperature and precipitation seasonality (a measure of temperature and rainfall variability over the year) and annual precipitation. However, the way in which seasonality and annual rainfall affected the richness of the two guilds mostly differed in a guild- and host-specific manner. U'Ren et al. (2024) similarly found host-specific responses to climatic factors across a large latitudinal gradient within boreal forests.

Both temperature and rainfall seasonality led to increased richness of endophytes, but decreased richness of pathogens. Based on previous work, we expected that seasonality would lead to the decreased richness of both endophytes and pathogens (Milici et al. 2020; Oita et al. 2021). Pathogen richness often reaches its highest levels in aseasonal tropical environments where conditions are stable (Milici et al. 2020; Makiola et al. 2022). Why endophyte richness increased with seasonality may be understood by considering the way C_4 grasses adapt to fluctuating temperatures and inconsistent rainfall. First, both *H. contortus* and *T. triandra* produce longer leaves and generally redistribute carbon into aboveground tissues when growing in highly seasonal areas (Downing and Groves 1985; Novelly 1986). Second, seasonality can promote a high degree of leaf turnover in C_4 grasses, whereby new leaves are flushed in response to sporadic rainfall events (Swemmer et al. 2006). Increased leaf size could provide more niche space, and the redistribution of carbon aboveground provides more resources for newly colonising endophytes, increasing richness. The continual turnover of leaves may increase richness by providing a variety of young, mature and cured leaves on the same grass tussock, which could allow different endophyte species that specialise in the different types of leaves to colonise and coexist within the same host, and even the same leaf, thereby increasing richness. It is also possible that old cured leaves act as a reservoir of fungal inoculum to readily colonise young newly flushed leaves with these founder endophytes shaping richness patterns through priority effects (Fukami et al. 2010). Additionally, the increased endophyte diversity may then help limit pathogen richness through increased competition for space or the production of antimicrobials. Here, we find some evidence that pathogen richness does indeed decline as endophyte richness increases [χ^2 (1, $N = 201$) = 3.667, $p = 0.055$].

Endophyte diversity increased with precipitation of the warmest quarter, that is, rainfall in the growing season, but decreased with annual rainfall (after controlling for other predictors, including rainfall of the warmest quarter). This suggests that rainfall which falls outside the growing season has limited influence on endophyte richness in these C_4 grasses or that areas receiving high annual rainfall may be dominated by a few generalist taxa (Vincent et al. 2016).

Fire frequency was one of the strongest drivers of endophyte richness patterns at the global scale. Some work has shown that endophyte richness increases in hosts after fires (Huang et al. 2016) or that fire more generally can be seen as a driver of fungal diversity (Fox et al. 2022) and biodiversity more generally (Kelly et al. 2020). The mechanisms by which fire promotes endophyte diversity in flammable systems are largely unknown, although it has been suggested that fire promotes richness by increasing habitat heterogeneity and thus the availability of niches (Hopkins et al. 2025).

4.5 | Drivers of Foliar Fungal Richness and Composition Across Australia

At the continental (Australian) scale, the same three factors (temperature and rainfall seasonality, and growing season precipitation) were retained as the dominant drivers of both richness and composition, with fire frequency additionally predicting composition. The finding that richness increased with growing season precipitation supports the hypothesis that energy availability controls host productivity, which indirectly drives foliar fungal richness (Hawkins et al. 2003; Field et al. 2009). We expected that foliar fungal richness across the highly seasonal continent of Australia would decrease with seasonality as highly seasonal climates should impose a strong physiological filter on foliar fungi that must survive for part of their lives outside their hosts (Oita et al. 2021). However, we found the opposite pattern, with richness increasing with both temperature and precipitation seasonality. We hypothesise that climatic seasonality could create a more heterogeneous environment that created a variety of niches for more species to exploit, ultimately increasing richness; this hypothesis requires further testing. Australia is a pyrophytic continent (Archibald et al. 2013) and fire frequency was a dominant factor shaping community composition, most likely through fire frequency effects on vegetation age and structure, and thus promoting habitat heterogeneity and the turnover of fungal taxa (Hopkins et al. 2025).

5 | Conclusions and Future Directions

Since the diversity of foliar fungi exceeds that of all other groups of plant-associated fungi (Blackwell 2011), appreciating how and why their diversity and composition change over large geographic scales represents a fundamental research goal. Even though foliar fungi are buffered from the outside world by growing within their plant host, climate appears the dominant factor shaping foliar fungal diversity and composition patterns over large geographic scales (Bowman and Arnold 2021). The most important climatic factor, however, differs in a host-specific manner (U'Ren et al. 2024).

Here we showed that factors which are important in driving the diversity, structure and boundaries of tropical grassy

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ecosystems, for example, seasonality and disturbance by fires, were also important in shaping foliar fungal richness and composition, at inter-continental and continental scales, often in a host- or guild-specific manner. The concentration of secondary metabolites, for example tannins, can determine both the propensity of C_4 grasses to burn or to be eaten by mammalian herbivores, respectively (Archibald et al. 2019). As foliar fungal symbionts are known to produce secondary metabolites that are important determinants of vegetation flammability or palatability (Grootemaat et al. 2015; Chen et al. 2021), an exciting prospect is to explore whether foliar fungal symbionts influence the propensity of C_4 grasses to burn or be eaten by herbivores through the increased production of secondary metabolites.

Much effort has gone into elucidating which secondary metabolites are produced by fungal endophytes and their potential roles within their plant hosts (Kusari et al. 2012; Ludwig-Müller 2015; Naik et al. 2019). Comparatively little work has been conducted on determining what factors affect the concentration of secondary metabolites produced by foliar fungal symbionts. Compared to dicotyledons, most Poaceae lack the capabilities of producing secondary metabolites and instead rely on microorganisms to perform this task (Kuldau and Bacon 2008). If the concentration of secondary metabolites produced by foliar fungi is governed by their *in planta* richness or composition, this could help explain why certain grass lineages, for example, the Andropogoneae, have come to dominate such a large geographic area. This would have implications in the way we understand how C_4 grassy vegetation spread during the Pliocene aridification (Edwards et al. 2010).

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data and Code Accessibility Statement:All raw sequencing data and the associated metadata were deposited on the NCBI portal under the following accession codes: BioProject - PRJNA1114630; BioSample (SAMN41489108 - SAMN41489334). A copy of the ASV feature table with the assigned fungal taxonomy, all metadata associated with the samples and the R scripts used in this study can be found on figshare https://doi.org/10.6084/m9.figshare.28830938.v2.

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