Fungal Ecology 64 (2023) 101249

Contents lists available at ScienceDirect

Fungal Ecology

journal homepage: www.elsevier.com/locate/funeco

Deterministic processes have limited impacts on foliar fungal endophyte communities along a savanna-forest successional gradient

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ARTICLE INFO

Handling Editor: Prof. Tom Bultman
Corresponding Editor: Prof. L. Boddy

Keywords:
Community assembly
Ecology
Fungi
Microbiome
Succession
Microbes
Trees
Diversity
Savanna
Woody encroachment

ABSTRACT

Patterns and drivers of succession provide insight into the mechanisms that govern community assembly, but remain poorly understood for microbial communities. We assess whether successional trends of trees are mirrored by foliar endophyte communities of three tree species across a deterministic woody successional gradient. Additionally, we test the relative contribution of abiotic predictors, biotic factors, and spatial distance between sites in predicting composition and richness of endophyte communities. Unlike the tree community, endophyte communities showed no consistent evidence of deterministic succession. Host identity was the most important factor structuring endophyte community composition; within hosts, spatial distance from the indigenous forest and between samples was important, while environmental predictors had small and inconsistent effects. Much variation in endophyte composition remained unexplained. In contrast, endophyte richness was well-explained by predictor variables. Host identity was most important in predicting endophyte richness, while the effect of other predictors on richness differed between host species. We conclude that deterministic succession in trees did not result in deterministic succession in endophyte communities; instead community assembly was most strongly influenced by host identity; while within hosts, neutral processes may be more important for endophyte assembly than deterministic factors.

1. Introduction

Understanding the relative contribution of deterministic and stochastic processes during community assembly represents a major unresolved issue in microbial ecology (Dini-Andreote et al., 2015; Antwis et al., 2017; Zhou and Ning, 2017; Tripathi et al., 2018). Unravelling the mechanisms that drive shifts in community composition is crucial for understanding the functions and ultimately the ecosystem processes that microbial communities are able to deliver (Salles et al., 2009; Crowther et al., 2014; Laforest-Lapointe et al., 2017). Succession, the study of how biological communities reorganise through time after disturbances (Johnson, 1979; Chang and Turner, 2019), provides valuable insight into community assembly (Chang and HilleRisLambers, 2016). Understanding succession in microbial communities can, therefore, help to disentangle the relative importance of deterministic and stochastic processes on community assembly (Tripathi et al., 2018).

The composition of communities during succession can be driven by deterministic mechanisms (environmental conditions and biotic filters) and stochastic processes (dispersal, drift, speciation and priority effects) (Hubbell, 2001; Vellend, 2010; Nemergut et al., 2013; Chang and HilleRisLambers, 2016). For microbial communities it has been hypothesised that during the early stages of succession, communities are largely governed by stochastic events, and only as succession continues does the relative strength of deterministic processes begin to cause directional changes in community composition (Dini-Andreote et al., 2015, 2016). However, if stochastic processes such as dispersal are not limiting, they

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https://doi.org/10.1016/j.funeco.2023.101249
Received 29 August 2022; Received in revised form 20 February 2023; Accepted 18 April 2023
Available online 6 June 2023

may overpower deterministic processes and the system remains ecologically neutral (Hubbell, 2001).

Studies on fungal succession have mostly focused on belowground soil and root-associated communities (Blaalid et al., 2012; Brown and Jumpponen, 2014; Davey et al., 2015; Dini-Andreote et al., 2016; Dong et al., 2016; Turner et al., 2019). These studies have repeatedly shown that fungal community assembly during later successional stages is deterministic (Brown and Jumpponen, 2014; Dini-Andreote et al., 2016; Gao et al., 2019; Turner et al., 2019). Both abiotic (e.g. carbon, nitrogen, pH and phosphorus) and biotic (e.g. plant species richness, composition, root exudates and microbial competition) factors are drivers of belowground microbial community composition patterns (Dini-Andreote et al., 2016; Dong et al., 2016; Turner et al., 2019). While some evidence exists for both linked (Davey et al., 2015) and decoupled (Turner et al., 2019) trends of succession between plant communities and belowground fungal communities, generalisable patterns across different fungal guilds are still unclear.

Disentangling the relative influence that host-associated and environmental factors play in determining host-endophyte composition represents a major theme in endophyte ecology (Antwis et al., 2017; Harrison and Griffin, 2020), as these fungi are an important aspect in plant performance and health (Compant et al., 2019). Host identity appears to be one of the most important drivers of foliar fungal endophyte community composition (Terhonen et al., 2019). Host-specific defence mechanisms, the host-specific production of various enzymes, secondary metabolites and concentration differences of nutrients and molecules within their leaves directly influence the composition of foliar fungal endophyte communities (Kembel and Mueller, 2014; Cordovez et al., 2019; Darlison et al., 2019; Tellez et al., 2020). Geographic distance is another important factor influencing community composition, with community dissimilarity generally increasing with geographic distance due to dispersal limitation (Soininen et al., 2007). Evidence for distance decay in foliar fungal endophytes is mixed. Some evidence points to the absence of such a relationship at both small (Cordier et al., 2012; Oono et al., 2017) and large scales (Vincent et al., 2016; Barge et al., 2019; U'Ren et al., 2019), while other studies show evidence of distance decay, especially for rare foliar fungal endophyte taxa (Vaz et al., 2014; David et al., 2016; Koide et al., 2017; Oono et al., 2017). Climate, a major factor driving the community composition of plants, seems to have less influence on determining the composition of fungal endophytes, particularly at fine scales (Compant et al., 2010; Santoyo et al., 2017). Water availability in particular affects some fungal endophytes' ability to germinate and persist (Arnold, 2007; Peay et al., 2016). The effect of climate on endophyte composition may also be indirect: through its effect on host composition and physiology fungal endophyte community composition may be affected (Compant et al., 2010; Terhonen et al., 2019).

Plant community composition structures soil and root-associated fungal communities due to the strong biotic filter imposed by plant hosts (Carney and Matson, 2006; Hausmann and Hawkes, 2009; Hoch et al., 2019; Hu et al., 2019). However, how plant community composition structures fungal endophyte composition is yet to be directly assessed (Griffin and Carson, 2018; Griffin et al., 2019). Therefore, linking plant community composition to foliar fungal endophyte composition may help to disentangle the factors responsible for structuring these fungal communities and ultimately help to understand how this scales-up to mediate plant microbial ecosystem functioning relationships (Laforest-Lapointe et al., 2017; Griffin et al., 2019; Harrison and Griffin, 2020).

In contrast to community composition, species richness considers only the number of species. A number of factors affect foliar fungal endophyte species richness (Arnold and Lutzoni, 2007; Unterseher et al., 2007; Lau et al., 2013; Griffin et al., 2019; Harrison and Griffin, 2020). Host identity appears to be one of the most important drivers (Lau et al., 2013; Peay et al., 2016; U'Ren et al., 2019; Yao et al., 2019). Other factors, including host age or height, microhabitat (e.g. moisture, light intensity and temperature) and plant richness, can also shape foliar fungal endophyte richness (Bernstein and Carroll, 1977; Unterseher et al., 2007; Zimmerman and Vitousek, 2012; Scholtysik et al., 2013; Oono et al., 2015; Griffin et al., 2019). Yet, few studies have simultaneously assessed how multiple factors drive patterns of foliar fungal endophyte richness.

The aim of this study is to assess patterns and drivers of foliar fungal endophyte community assembly in a system undergoing deterministic vegetation succession with associated directional changes in microhabitat conditions. Our first objective was to test whether foliar fungal endophyte communities follow a deterministic successional trend as displayed by the woody tree communities in the same study system. We expected that altered microhabitat conditions would lead endophyte communities to follow deterministic successional trajectories, because disturbances experienced by the tree host and its associated microbes cannot be considered independently of each other (Russell et al., 2014), and because the majority of foliar fungal endophytes are horizontally transmitted, with their composition being strongly influenced by local environmental conditions (Christian et al., 2016). The second objective was to determine the relative contribution of host species identity, surrounding tree composition, geographic distance and abiotic variables to endophyte composition and richness. We expected that host identity would be an important determinant of richness and composition (U'Ren et al., 2019; Harrison and Griffin, 2020), and that wetter areas would result in higher endophyte richness (Zimmerman and Vitousek, 2012).

2. Materials and methods

2.1. Study area

The study was conducted at Buffelskloof Private Nature Reserve (25° 19′ 22.21″ S, 30° 29′ 15.41″ E), Mpumalanga, South Africa. Buffelskloof supports vegetation types from three biomes: Afromontane forest, mid-altitude savanna and montane grassland (Mucina and Rutherford, 2011). The reserve is approximately 1500 ha in size with an altitudinal variation of 1000–1800 m (Buffelskloof Nature Reserve, 2019). Samples were collected between 1314 and 1477 m elevation.

Within the grassland and savanna vegetation on Buffelskloof, bush clumps (BCs) have been establishing and expanding for some decades (Jamison-Daniels et al., 2021). A BC is defined as an association of one or more trees (>1.2 m) with tree saplings (<1.2 m) growing beneath the canopy, that are separated from each other by grassy vegetation (Jamison-Daniels et al., 2021). Bush clumps usually begin as a single founder tree which subsequently aids in the establishment and persistence of additional trees beneath the founder; therefore, larger BCs are older (Barnes and Archer, 1996; Jamison-Daniels et al., 2021). In this system, BCs constitute woody encroachment by indigenous species, often forest-associated species, into grassy ecosystems; and is likely caused by changes in both local (e.g. fire and herbivory management) and global (i.e. rising carbon dioxide levels) drivers (Stevens et al., 2017). In a space-for-time substitution study of the study system, in which the woody vegetation of 40 BCs ranging in size from 10.05 m^2 to 1342.99 m^2 (mean = 266.41 m^2, SE = 347.75 m^2) was characterised, it was found that the formation of BCs follows a deterministic trend of succession in woody species, with a turnover from open-habitat savanna trees to shade-tolerant forest trees as the BCs increase in size (Fig. 1) (Jamison-Daniels et al., 2021). These directional changes in tree community composition are driven by directional changes in microclimatic conditions (temperature, soil moisture and humidity) and light availability (Jamison-Daniels et al., 2021).

2.2. Sampling

Endophyte communities were sampled from the same 40 BCs that were surveyed by Jamison-Daniels et al. (2021). Three tree species that are widely distributed and common across BCs of different sizes (and

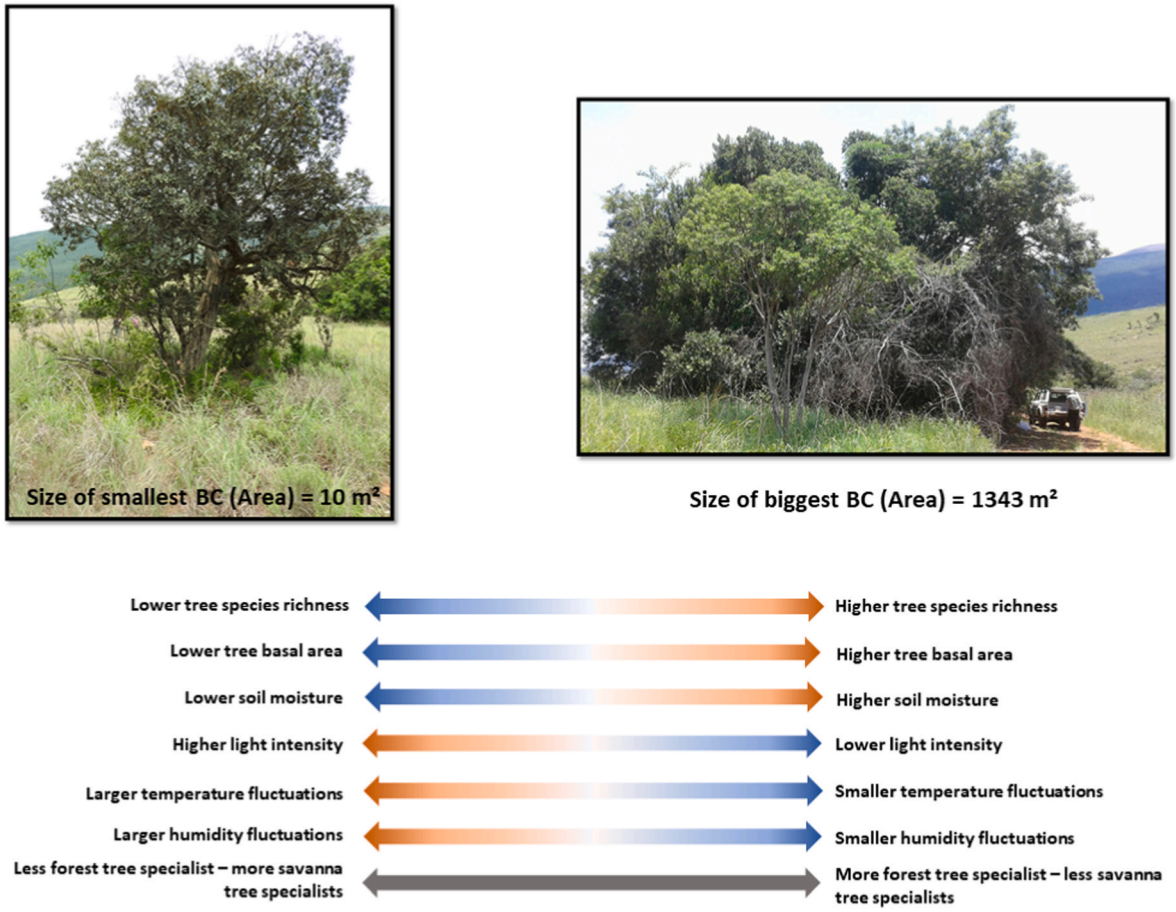

Fig. 1. Image showing the size difference between the smallest and largest BCs from which foliar fungal endophytes from three tree host species were sampled. How various abiotic and biotic factors change with BC is indicated below the photos.

thus different successional stages) were selected: *Euclea crispa* subsp. *ovata* (Burch.) F.White, *Searsia chirindensis* (Baker f.) Moffett and *Canthium inerme* Kuntze. Trees of these species were present in 38 of the 40 BCs.

Field sampling took place between 20 and 23 November 2018, characterising the summer leaf endophyte communities. Leaves were sampled during the early summer months as this corresponded to the growing season. By this time the first rains have fallen, and leaves have emerged and matured, but the season is also early enough that leaves will have minimal herbivory or physical damage. In every BC containing the host species, up to four trees (>1.2 m) per host species were selected. The coordinates of each tree that was sampled were recorded using a Garmin Etrex GPS (Garmin Ltd., Olathe, KS, USA) and the height of each tree was estimated using a 1.2 m dowel stick. All hosts did not occur in all BCs, and BCs did not always contain four individuals from each host. For BCs that had more than four individuals of a host, the first four individuals that were encountered were sampled. *E. crispa*, *C. inerme* and *S. chirindensis* trees were present in 27, 21 and 28 of the BCs, and 84, 50 and 55 individual trees were sampled from each host species, respectively. From each tree, five leaves from each of the four cardinal directions were collected half-way between the highest and lowest leaves. Only fully unrolled leaves that had reached maturity and had no visible signs of infection or insect damage were selected. Leaves were stored in envelopes within a cooler box and processed within 8 h of collection: endophyte communities were sequenced for each tree.

Microclimatic conditions were characterised for each of the 40 BCs, to determine whether differences in microclimate influenced endophyte community composition and richness (see Jamison-Daniels et al., 2021 for full details). Light intensity, temperature (average, minimum, maximum and standard deviation measured over 130 days), relative humidity and soil moisture were all previously measured and subsequently extracted (from Jamison-Daniels et al., 2021). Tree basal area was calculated at 30 cm height (Jamison-Daniels et al., 2021).

The species richness (count of tree species per BC), Shannon-Wiener diversity, species composition and the tree basal area of all trees >1.2 m per BC were obtained from Jamison-Daniels et al. (2021). Only trees >1.2 m were considered, as these represent established vegetation within the BCs. Additionally, proximity of each sampled tree to the large indigenous forest which acted as a source population for most BC trees (Jamison-Daniels et al., 2021) was calculated to represent a proxy for potential endophyte inoculum pressure. Within Google Earth Pro (v7.3.2.5776) a polygon was drawn around the indigenous forest at Buffelskloof Nature Reserve and subsequently extracted as a shape file. The distance from each tree sampled to the edge of the indigenous forest within the reserve was obtained using the points to polygon function in Esri™, ArcMap (Esri™, Redlands, CA, USA).

Leaves underwent surface washing (Arnold and Herre, 2003) to reduce and possibly eliminate the epiphytic burden from each sample. All leaves sampled from one tree were washed together successively in 70% EtOH (30 s), 2% NaOCl (60 s), 70% EtOH (60 s) and autoclaved dH_2O (60 s). The leaves were then placed on paper towel and left to dry. Leaf disks from the dried leaves were cut using a 6 mm cork-borer (sterilised between each sample) and subsequently stored on silica gel in falcon tubes until DNA extraction. For *E. crispa* and *C. inerme* 12–18 leaf disks were cut per leaf, while 22–25 disks were cut from larger *S. chirindensis* leaves.

2.3. Homogenisation, DNA extraction and sequencing

Prior to homogenisation of each sample a metal cylinder and ball bearing were sterilised in a 6% sodium hypochlorite (NaOCl) solution for 3 min, followed by immersion in 70% ethanol (EtOH) for 1 min. All leaf disks from one sample, i.e. from each tree, were homogenised together in the metal cylinder fastened to a Retsch® MM2000 laboratory mixer mill (Retsch® GmbH, Haan, Germany) for 1 min at 70% of the maximum oscillation frequency and subsequently stored at −20 °C until DNA extraction. DNA of 60 mg dried homogenised leaf material per sample was extracted using the my-Budget plant DNA extraction kit (Bio-Budget Technologies GmbH, Krefeld, Germany) following manufacturer's instructions. The ITS region of all extracted samples was amplified using the ITS1-F (Gardes and Bruns, 1993) and ITS4 (White et al., 1990) primer combination and was subsequently visualised on a 0.8% agarose gel with a 100bp ladder, together with positive and negative controls to ensure that we had indeed extracted fungal DNA without contamination.

Illumina amplicon library preparation utilised a nested PCR approach (Unterseher et al., 2016) (Supporting Information Fig. S1, Methods S1). The ITS region was amplified using the ITS1-F (Gardes and Bruns, 1993) and ITS4 (White et al., 1990) primer combination. The final amplicons were subsequently visualised on a 0.8% agarose gel with added ethidium bromide (Supporting Information Methods S1). Concentration of DNA for pooling was quantified from the band intensity of the gel images using ImageJ (Schneider et al., 2012) (Supporting Information Methods S1). The amplicon pools were cleaned using the CleanPCR magnetic bead kit (CleanNA, Waddinxveen, Nertherlands) (Supporting Information Methods S2). In total three samples were lost during homogenisation or due to low DNA concentration resulting in 186 samples that were sent for sequencing.

The final pooled amplicon library 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 × 250 bp paired-end sequencing. All raw sequences were deposited on the NCBI portal under the following accession codes: Sequence Read Archive – SRP291161; BioProject – PRJNA674320; BioSample (SAMN16634781 – SAMN16634966).

2.4. Bioinformatics

Bioinformatic processing was performed in QIIME1 (Caporaso et al., 2010) and QIIME 2 (Bolyen et al., 2019). Samples were demultiplexed according to their unique tag/index combinations (Supporting Information Table S1), which were removed during the process, together with the primer sequences (Supporting Information Table S2). For subsequent analyses only forward reads were used, as reverse reads often suffer from a lower PHRED quality, and due to length differences within the ITS gene region, which often prevents merging of both reads. Three *S. chirindensis* samples which contained less than 10 000 sequences were removed prior to downstream bioinformatic analyses. The raw sequences from the remaining 183 samples were subsequently passed through deblur (Amir et al., 2017) implemented in the QIIME 2 pipeline, which assigns raw sequence reads to Amplicon Sequence Variants (ASVs). Reads were trimmed at 180 bp. The UNITE database was used as reference sequences (version 8; 020219) (https://unite.ut.ee/). Only ASVs which were classified as belonging to the Kingdom Fungi were retained. Fungal ASVs were written into a feature table, which was used for subsequent downstream analyses. The full ASV feature table and all metadata relating to the manuscript can be obtained from figshare: 10.6084/m9.figshare.14518200 – ASV feature table and 10.6084/m9.figshare.14518218 – metadata.

2.5. Statistical software

All analyses were conducted in R, v 3.6.0 (R Core Team, 2019) using the packages *vegan* v2.5-6 (Oksanen et al., 2019), *lme4* v1.1-23 (Bates et al., 2015), *MuMIn* v1.43.17 (Barton, 2019), *car* v3.0-9, *gdm* v1.4.2.2 (Fitzpatrick et al., 2021), *effects* v4.2-0 (Fox, 2003; Fox and Weisberg, 2019), *hillR* v0.5.1 (Li, 2018), *metacoder* v0.3.5 (Foster et al., 2017), *FUNGuildR* v0.2.0.9 (Furneaux and Song; Nguyen et al., 2016), and *phyloseq* v1.2-0 (McMurdie and Holmes, 2013).

2.6. Analyses

All analyses were performed on the full, unrarified, ASV table. This was done for two reasons. First, rarefying the ASV table to the smallest sample size to account for differences in read abundances between samples made no qualitative difference to the interpretation of the results (results not shown). Second, from a statistical point of view, rarefaction is inept for the comparison of relative abundances (McMurdie and Holmes, 2014; Willis, 2019).

As predictor variables that are highly correlated can lead to spurious effects on analyses, all continuous predictor variables were tested for multi-collinearity prior to analyses (Supporting Information Table S3). When two variables were highly correlated, i.e. $r > |0.75|$, one of these variables was removed (Supporting Information Table S3). Bush clump area, BC tree basal area and BC tree species richness were highly correlated. Therefore, only BC tree basal area was retained for analyses on endophyte composition and richness, as it gives a good representation of available woody tree host density within individual BCs. Bush clump area was only retained in the analyses on successional trends, as BC area was a good proxy for BC maturity and woody vegetation successional stage (Jamison-Daniels et al., 2021).

2.7. Taxonomic and functional composition

To assess how the taxonomic and functional composition of foliar fungal communities differed between the three host species, three approaches were used. Firstly, fungal ASVs were assigned to trophic modes and fungal guilds using the function funguild_assign(). Subsequently the relative abundance of fungal phyla, classes, orders, families, genera, trophic modes, and guilds per host tree species were calculated using the functions merge_samples(), tax_glom(), transform_sample_counts(), subset_taxa() and psmelt(). Secondly, heat trees representing the number of samples a particular family of fungi occurred in and the abundance of read counts per family were created per host using the function heat_tree() (Supplementary Information Figs. S2–S4). Lastly, to determine whether the abundance of fungal branches, i.e. fungal families, differed significantly between hosts, every pairwise combination of host and fungal families were compared using the function compare_groups (). The p-value of every pairwise combination was adjusted using the Benjamini-Hochberg correction, and all insignificant pairwise combinations were removed before visualisation.

2.8. Successional trend

A two-step approach was used to determine the processes of endophyte community assembly and to evaluate whether these fungal communities followed the deterministic successional trend observed for their woody tree hosts (Jamison-Daniels et al., 2021).

First, we assessed whether the amount of variation in community composition of our actual endophyte assemblages, explained by a number of predictor variables, was greater than (implying deterministic processes), less than (implying deterministic processes) or not different (implying stochastic processes) to what could be expected in randomly generated communities (Dini-Andreote et al., 2015). Initially, a canonical correspondence analysis (CCA) was conducted using the function cca(), to test which factors, namely host identity, spatial distance

between sampled trees, abiotic conditions (maximum and minimum temperature, temperature standard deviation, light intensity, soil moisture measured per BC) and biotic factors (tree basal area per BC, tree height and distance to forest edge), best explained fungal community composition. Weighted linear regression was performed on the constraining variables (Ter Braak, 1986). To incorporate the effect of spatial distance on community composition, a weighted principal coordinates of neighbourhood matrix (PCNM) analysis (created using the geographic coordinates of every sampled tree) was performed using the function pcnm() with each sample weighted by ASV abundance. This analysis transforms coordinates to a rectangular distance matrix that is acceptable for constrained ordination techniques like CCA (Borcard and Legendre, 2002; Legendre and Borcard, 2008). Backward and forward stepwise permutation tests, for 1000 permutations, were used to determine the best fitting model, based on the model with the lowest AIC score by using the function step() (Venables and Ripley, 2002). Rare species contribute heavily to the chi-squared distance used to plot site and species scores in CCA analysis (Legendre and Legendre, 2012). Therefore, to reduce the spurious effects of rare species within the CCA, an eigenvalue decomposition approach was used to determine in how many samples a particular ASV must have occurred for it to be retained when performing the final ordination (Legendre and Legendre, 2012). The eigenvalue decomposition was completed by writing a loop function. When a considerable drop in inertia for one of the first five eigenvalues is detected, it indicates in how many samples an ASV must have occurred to be retained for the final CCA analysis (Supporting Information Fig. S5) (Legendre and Legendre, 2012). The eigenvalue decomposition detected a drop in the inertia for the third eigenvalue after dropping ASVs that occurred in less than 20 samples (Supporting Information Fig. S5). Therefore, all ASVs (n = 4905) that occurred in 20 samples or less were removed before the final CCA analysis (N = 411) and randomisations.

The amount of variation explained by the best model with the retained predictor variables (host identity, light intensity and four spatial eigenvectors (spatial eigenvector 1, spatial eigenvector 2, spatial eigenvector 13 and spatial eigenvector 17)) was calculated. Then, 10 000 randomly assembled abundance-weighted community data matrices were constructed based on the true community identity, richness and abundance using a loop function written for this purpose (following an approach used in Greve et al., 2008). The randomly assembled matrices conserved species richness per sample as observed in the true community, and set the probability of species being selected proportional to ASV read abundance (Gotelli and Graves, 1996; Gotelli, 2000). For each of the 10 000 randomly assembled communities, a new CCA was performed with the predictors from the best CCA model (see above) again using the function cca(). The 2.5% and 97.5% quantiles of the percentage variation explained by the CCAs were calculated for the 10 000 random communities, and it was assessed whether the percentage variation explained by the CCA of the true community was larger than (>97.5% quantile), smaller than (<2.5% quantile) or not significantly different (between 2.5% and 97.5% quantiles) to the percentage variation explained by the randomly generated communities using a z-test (Greve et al., 2008).

Because CCA randomisation analyses provided evidence for deterministic community assembly (see Results), a second analysis was conducted to test whether changes in community composition could be explained by BC size, as BC size increases with tree succession (Jamison-Daniels et al., 2021). A significant directional change in endophyte species composition with BC size would indicate deterministic succession, while no predictable change in composition with BC size would suggest stochastic succession (Dini-Andreote et al., 2015). We tested this for the fungal communities extracted from each of the three host species using two different pair-wise similarity indices (Morisita and Raup-Crick indices). Pairwise dissimilarity was calculated using the function vegdist (), and converting to similarity by using the formula 1-dissimilarity. The Morisita index is weighted towards assessing similarity in common taxa, while the Raup-Crick index gives more weight to co-occurring rare taxa (Morisita, 1962; Raup and Crick, 1979). Pair-wise similarity in fungal community composition was calculated between all possible combinations of each of the smallest ¼ of the BCs and each of the largest ¾ of the BCs for each host species (following Jamison-Daniels et al., 2021), to establish if there was directional change in community composition as BCs increase in size. For each of the largest ¾ BCs, similarity values with the smallest ¼ of the BCs were averaged. Generalised linear mixed effects models (GLMMs) were used to model the effect of BC area (of the largest ¾ BCs) on foliar fungal endophyte community similarity for both Raup-Crick and Morista similarity indices, using the function glmer(). Bush clump identity of the largest ¾ BCs was included as a random effect in the model (McCulloch, 1997). Since the similarity values for both the indices are scaled between 0 and 1, models were fitted using a binomial distribution and logit link function (Zuur et al., 2009). If the foliar fungal community similarity between the largest and the smallest BCs decreased or increased significantly with BC area of the largest BCs, it was interpreted as an indication of deterministic succession, while no relationship was taken as an indication of communities being ecologically neutral and primarily governed by stochastic processes that structure community composition (Hubbell, 2001; Dini-Andreote et al., 2015; Jamison-Daniels et al., 2021).

2.9. Drivers of assemblage composition

We tested which factors, namely host identity, spatial distance between sampled trees, abiotic conditions (maximum and minimum temperature, temperature standard deviation, light intensity and soil moisture measured per BC) and biotic factors (tree basal area per BC, tree height, distance to forest edge and the tree composition per BC), affected fungal endophyte community composition.

To do this we used a generalised dissimilarity modelling (GDM) approach. This flexible approach enabled us to simultaneously incorporate categorical (i.e. host), linear (e.g. climate), compositional (i.e. tree compositional dissimilarity), and spatial data (i.e. geographic distance) predictors of endophyte composition into one analysis (Ferrier et al., 2007). GDM is a nonlinear extension of matrix regression, which has specifically been designed to deal with two types of nonlinearity commonly encountered in biological data: 1) the curvilinear relationship between ecological or spatial separation and the observed compositional dissimilarity, and 2) non-stationarity, i.e. differences in the rate of compositional turnover along environmental or spatial gradients (Ferrier et al., 2007; Fitzpatrick et al., 2013).

The default of three I-spline basis functions (knots) per predictor variable was used in all GDM analyses (Ferrier et al., 2007); and backwards selection was used to determine how many variables to retain in each of the final models (Williams et al., 2012). The sum of the coefficients per I-spline represents the maximum amount of variation explained by a particular variable, and can be used to determine variable importance (Ferrier et al., 2007). Since host identity explained most of the endophyte compositional dissimilarity for the full dataset (see Results), we repeated the GDM analyses per individual host species using the same approach as above. Models were run on the ASV abundance data. Confidence intervals for significant predictors were generated with 1000 bootstrap iterations, based on 99% of sample sites and taking geographic distance into consideration, using the function plotUncertainty().

2.10. Drivers of endophyte richness

To test the effects of host identity (i.e. host species), abiotic variables (maximum and minimum temperatures, temperature standard deviation, light intensity, relative humidity and soil moisture) and biotic variables (tree basal area per BC, tree height and distance to the forest edge) on ASV richness, random intercept GLMMs with a Poisson distribution and a log-link function were used (Zuur et al., 2009). Interaction

terms between host identity and all abiotic and biotic variables were fit within the model and BC identity was included in the model as a random variable (McCulloch, 1997). Best subset modelling, based on the lowest AIC-value, was used to assess which predictor variables from the global model should be retained by using the function dredge() (Burnham and Anderson, 2002). The model was overdispersed, therefore over-dispersion was corrected by employing the observation-level random effects approach (Lawson et al., 1999; Elston et al., 2001). Marginal and conditional R^2-values were calculated using r.squaredGLMM() (Nakagawa et al., 2017).

Additionally, Shannon entropy and inverse Simpson's diversity were calculated for each endophyte sample using the function hill_taxa(), and GLMMs repeated using Shannon or Simpson's index as response variables (Supplementary Information Figs. S6 and S7; Supplementary Information Tables S4 and S5). Despite being widely used, both these diversity variables have shortcomings that make their interpretation difficult; these have been discussed extensively in the ecological literature (Jost, 2010; Cao and Hawkins, 2019). Therefore, while we present results from different diversity indices, we restrict all further discussions on the richness analyses as it is the best measure of alpha diversity (Sanjit and Bhatt, 2005; Cao and Hawkins, 2019).

3. Results

The 183 leaf samples from three native host species yielded 7 355 098 demultiplexed sequences. On average there were 40 192 sequences per sample; the highest number of sequences from one sample was 103 727 and the lowest 12 533 (Supporting Information Table S6). In total 5 326 unique ASVs were recovered with an average of 193.80 ± 101.82 ASVs per sample. The number of ASVs recovered from *C. inerme*, *E. crispa* and *S. chirindensis* was 1 473, 2 221 and 1 847, respectively.

3.1. Taxonomic and functional composition

Across all three host species we mostly recovered fungi from the Dikarya and a few reads which could only be assigned to "fungi". Most of the fungal reads belonged to the Ascomycota (Fig. 2A). The Dothideomycetes were the dominant fungal class on all three hosts (Fig. 2B), being particularly well represented by the orders Capnodiales and Pleosporales (Fig. 2C). In general, a large proportion of ASVs could not be assigned to a fungal class or higher taxonomic rank – particularly for the host *E. crispa* (Fig. 2). One host – *C. inerme* – had a greater relative abundance of fungi from the Basidiomycota than the other two hosts (Fig. 2A and 3) and Basidiomycota classes, including the Cystobasidiomycetes, Tremellomycetes and Exobasidiomycetes, were all significantly more abundant on *C. inerme* than the other two hosts (Fig. 3). Other significant differences included a greater abundance of Capnodiales and Hypocreales on *C. inerme* than the other two hosts; greater abundance of Didymellaceae, Pleosporaceae and Aureobasidiaceae on *S. chirindensis* than *C. inerme* and *E. crispa*; and more Neodevriesiaceae and Treatosphaeriaceae found on *C. inerme* and *E. crispa* than on *S. chirindensis* (Fig. 3). The few ASVs (approximately 20–40% per host species) that could be assigned to both a trophic mode and fungal guild highlighted that 'pathotroph-saprotrophs' and 'pathotroph-symbiotrophs' were the most abundant across all three hosts (Fig. 2D), while the guilds 'animal pathogen-plant pathogen-undefined

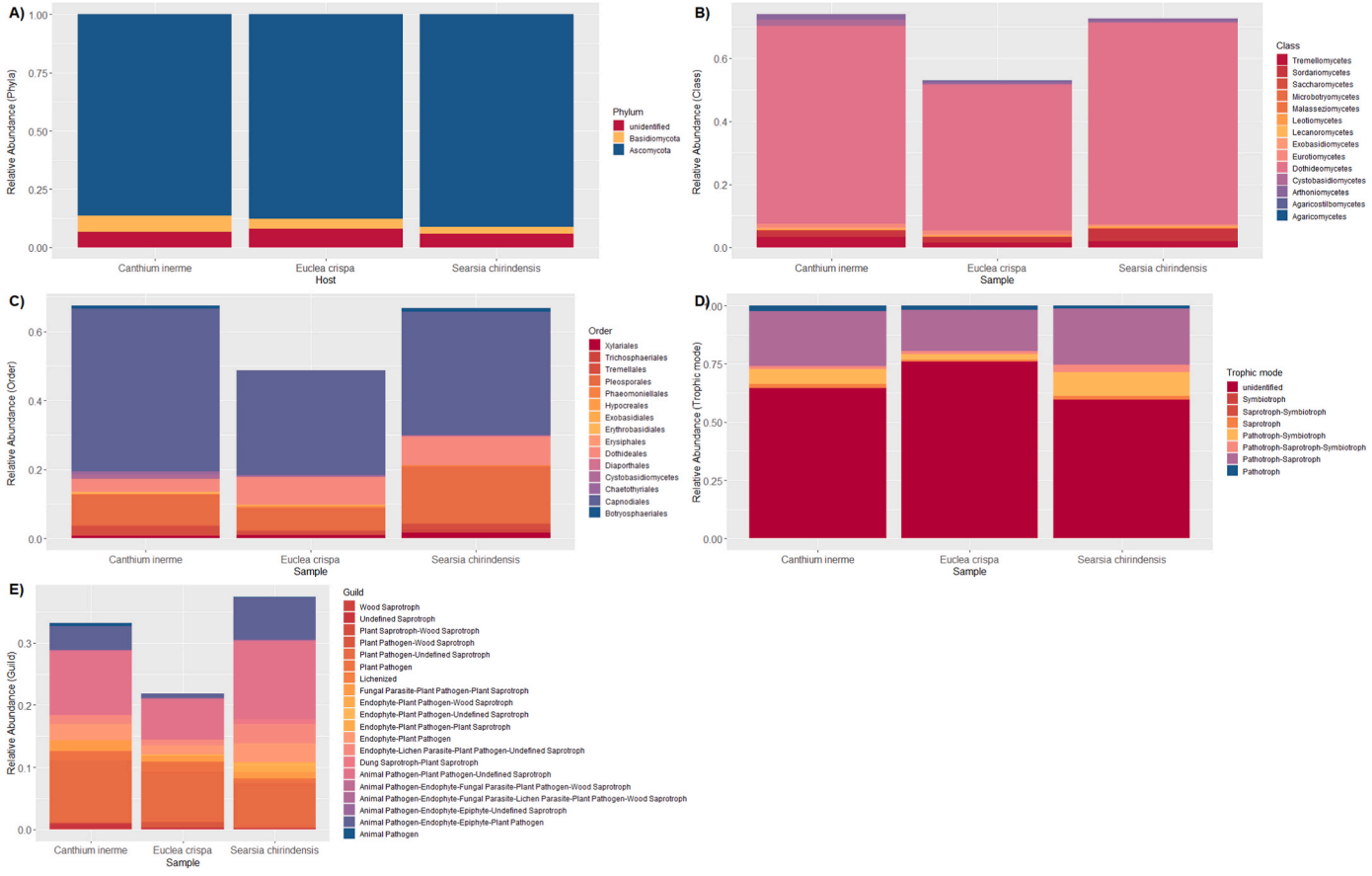

Fig. 2. A) Relative abundance of fungal phyla per host tree species. B) The relative abundance of the 10 most abundant fungal classes per host tree species; unidentified fungal classes are not displayed. C) Relative abundance of the 10 most abundant fungal orders per host tree species; unidentified fungal orders are not displayed. D) The relative abundance of fungal trophic modes per host tree species. E) Relative abundance of the 15 most abundant fungal guilds per host tree species; unidentified guilds are not displayed.

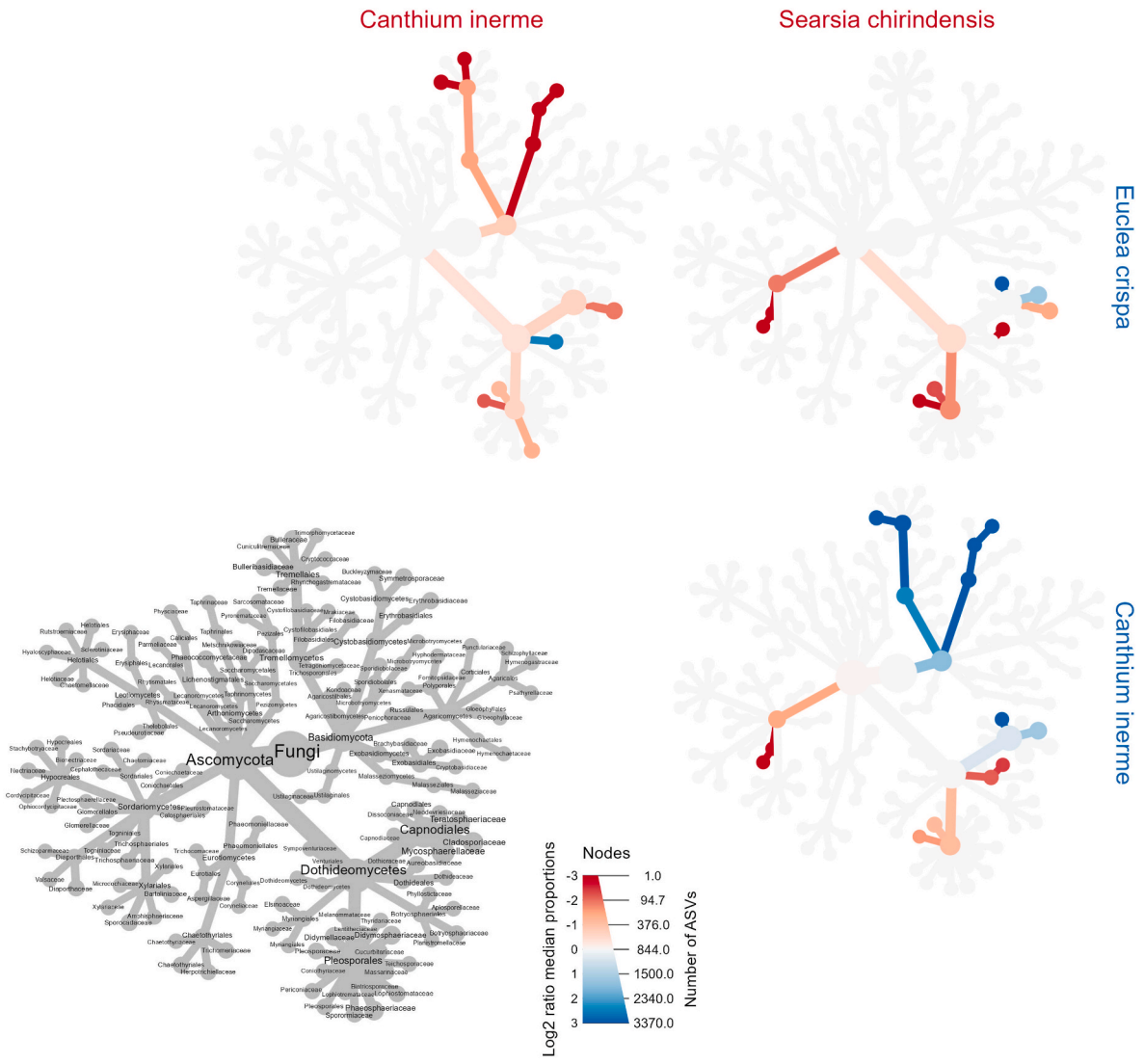

Fig. 3. A heat tree matrix showing which fungal families differ significantly between the three host tree species. Branches in red represent fungal taxa which are significantly more abundant for the host tree species displayed in columns, while branches which are blue represent fungal taxa which are significantly more abundant for host tree species displayed in rows.

saprotroph', 'plant pathogen' and 'animal pathogen-endophyte-epiphyte-plant pathogen' constituted most of the assigned fungal guilds across the three host species (Fig. 2E).

3.2. Successional trend

Variables retained in the CCA (host identity, spatial distance between samples and light intensity) explained 11.76% of the variation of the endophyte community (Supporting Information Fig. S8 and Table S7). In contrast, between 4.27% and 4.75% of the variation in ASV composition of randomly generated communities could be explained by these same predictor variables. Therefore, the predictor variables predicted significantly (2.5 times) more variation in community composition of the true than the randomly generated communities (z-value = 56.812, *p*-value =

Table 1
Results from the generalised linear mixed effects models assessing the effect of bush clump area on the similarity of fungal communities. Pair-wise similarity values are calculated for all samples taken from each of the three different host species. Raup-Crick index is the probabilistic similarity of co-occurrences between rare and common ASVs, while the Morisita similarity gives weighting to more abundant ASVs.

Host species	Similarity index	Fixed effects	Estimate	SE	z-value	*p*-value	Conditional R^2	Random effect SD	Random effect variance	AIC
Euclea crispa	Raup-Crick	Area	−1.3301	0.4968	−2.677	**0.0074**	0.2185	0.7947	0.6315	301.8
Euclea crispa	Morisita	Area	−0.16368	0.1528	−1.071	0.284	0.00133	0	0	243.7
Canthium inerme	Raup-Crick	Area	−0.09949	0.594	−0.167	0.867	0.0613	0.4622	0.2136	104.2
Canthium inerme	Morisita	Area	−0.6755	0.3599	−1.877	0.0605	0.0323	0.2181	0.04758	143.4
Searsia chirindenis	Raup-Crick	Area	0.0064	0.00051	1.248	0.212	0.1082	0.5749	0.3306	148.1
Searsia chirindenis	Morisita	Area	0.0001	0.00031	0.323	0.747	0.0005	0	0	113.9

$<16x10^{-16}$). This indicated that endophyte communities are non-randomly assembled and that deterministic selective forces structure fungal endophyte communities.

However, when compared within hosts, the similarity of foliar fungal communities showed no consistent trends across the gradient of BC size (Table 1). The average Raup-Crick similarity decreased significantly with increasing BC area for endophyte communities of *E. crispa* (Fig. 4 & Table 1), as expected under deterministic succession. However, no significant trends in community similarity with BC area could be observed for rare communities of the other two host species (Table 1). Similarly, the Morisita similarity index showed no host-specific successional trends across BC area (Table 1). Therefore, five of the six analyses provided no evidence of deterministic succession.

3.3. Assemblage composition

The final GDM for the full dataset retained two host species (*E.crispa* and *S. chirindensis*), tree basal area, distance to the indigenous forest edge, geographic distance between samples, light intensity and tree compositional dissimilarity as predictor variables (Fig. 5). The final model for the full dataset was significant (Null Deviance = 691.996, GDM Deviance = 491.635, *p*-value = 0.000001), as were all the predictor variables, except light intensity (Fig. 5; Table 2). The final model explained 28.95% of the deviance in the turnover of foliar fungal endophyte community composition, with host identity alone explaining ~23% of this variance. Of the remaining predictors, distance to the forest and the difference in tree basal area were the most important, both having variable rates of turnover along the gradients and displaying the quickest turnover at short distances from the forest and small differences in tree basal area between bush clumps (Fig. 5C and D). The rate of turnover in fungal endophyte composition along the tree compositional dissimilarity gradient was the steepest of any of the gradients, with the maximum magnitude of turnover reached when the difference in surrounding tree composition was only slightly dissimilar, i.e. samples not collected from the same BC (Fig. 5F).

The GDM and CCA analyses indicated that host identity was the most important variable followed by factors relating to spatial distance (Fig. 5; Supporting Information Fig. S8). However, the GDM analysis was able to explain nearly three times the amount of variation in endophyte composition than the CCA analysis (Fig. 5; Supporting Information Fig. S8). This could be due to the GDM analysis' assumptions regarding different rates of compositional turnover across gradients (non-stationarity), or the fact that the GDM analysis was able to incorporate tree composition into the analysis.

When GDMs were run separately for each host species, different predictors were retained as important in explaining the composition of endophytes within different hosts. Only geographic distance between samples was retained and significant in all models (Figs. 5 and 6). The final GDM model for *E. crispa* was significant (Null Deviance = 147.69, GDM Deviance = 122.65, *p*-value = 0.000001) and explained 16.96% of the deviance in turnover of foliar fungal endophyte community composition. It retained five variables (Supporting Information Table S8), of which distance to the forest edge, geographic distance between samples and the difference in tree basal area were significant (Fig. 6A–C). Distance to the forest was the most important predictor, with the rate of turnover being approximately linear (Fig. 6A). The rate of turnover in endophyte composition along the geographic distance and tree basal area gradients were non-linear with the highest rates of turnover occurring at larger geographic separation between samples and small differences in tree basal area (Fig. 6B & C).

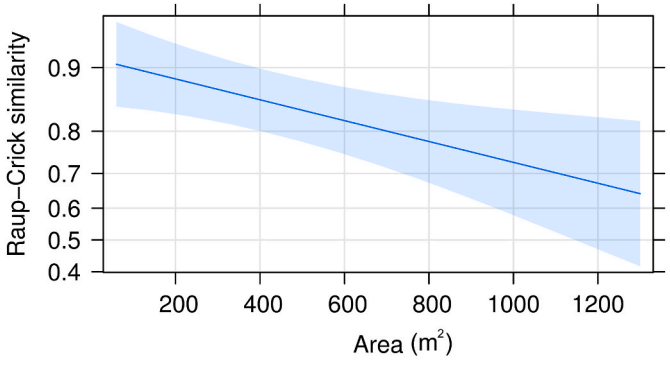

Fig. 4. Effect plot showing the relationship between Raup-Crick similarity values of foliar fungal endophyte communities on *Euclea crispa* and BC area as a proxy for age of the BCs. Light blue shading represents the 95% confidence interval for the fitted model. Only significant trends are shown.

For *C. inerme*, the final GDM model retained five predictor variables (Supporting Information Table S9); however, only minimum temperature and geographic distance between samples were significant (Fig. 6D & E). Minimum temperature was the most important predictor with both larger differences in minimum temperature and geographic distance between samples resulting in higher rates of turnover. The magnitude and rates of turnover in endophyte composition was almost non-existent at low differences in minimum temperatures and short geographic distances between samples but increased dramatically at the higher end of these gradients (Fig. 6D & E). The final model was significant (Null Deviance = 52.85, GDM Deviance = 45.92, *p*-value = 0.00503), and explained 13.12% of the deviance in turnover of foliar fungal endophyte community composition.

The final GDM model for *S. chirindensis* fungal communities retained five predictor variables (Supporting Information Table S10); but the only retained variable which was significant was geographic distance between samples (Fig. 6E). The rate of turnover in endophyte composition increased with distance between samples; this increase was most rapid at short geographic distances (Fig. 6E). The final *S. chirindensis* GDM model was significant (Null Deviance = 42.78, GDM Deviance = 38.622, *p*-value = 0.000001), and explained 9.52% of the deviance in turnover of foliar fungal endophyte community composition. No obvious differences in the degree and rate of turnover between different host species were detected (Supplementary Information Figure S9 – Full model; Figure S10 – *E. crispa*; Figure S11 – *C. inerme* & Figure S12 – *S. chirindensis*).

3.4. ASV richness

Host was the most important predictor of ASV fungal richness, explaining ~55% of the variation alone, with one host – *S. chirindensis* – consistently supporting fewer ASVs (mean = 83.94 ± 52.79) than *C. inerme* (mean = 218.5 ± 95.8) and *E. crispa* (mean = 245.85 ± 70. 82) (Fig. 7). The best subset GLMM model testing the effect of abiotic, biotic and spatial factors on ASV fungal endophyte richness retained light intensity, the interaction between host species and tree height, and the interaction between host species and maximum temperature. Both the interactions between host and maximum temperature, and between host and tree height were significant (Fig. 7, Table 3). ASV endophyte richness decreased in *S. chirindensis* individuals that experienced higher maximum temperatures (Fig. 7A) but was unaffected by temperature differences experienced by individuals of *C. inerme* and *E. crispa* (Fig. 7A). Additionally, ASV endophyte richness decreased with tree height for *S. chirindensis* but did not change with tree height in *C. inerme* and *E. crispa* (Fig. 7B). Fungal ASV richness increased as light intensity decreased (Fig. 7C). Fixed effects explained 63% of variation in ASV richness (Table 3).

4. Discussion

Foliar fungal endophyte communities did not display consistent patterns of deterministic succession within individual host species, despite the surrounding plant community undergoing deterministic

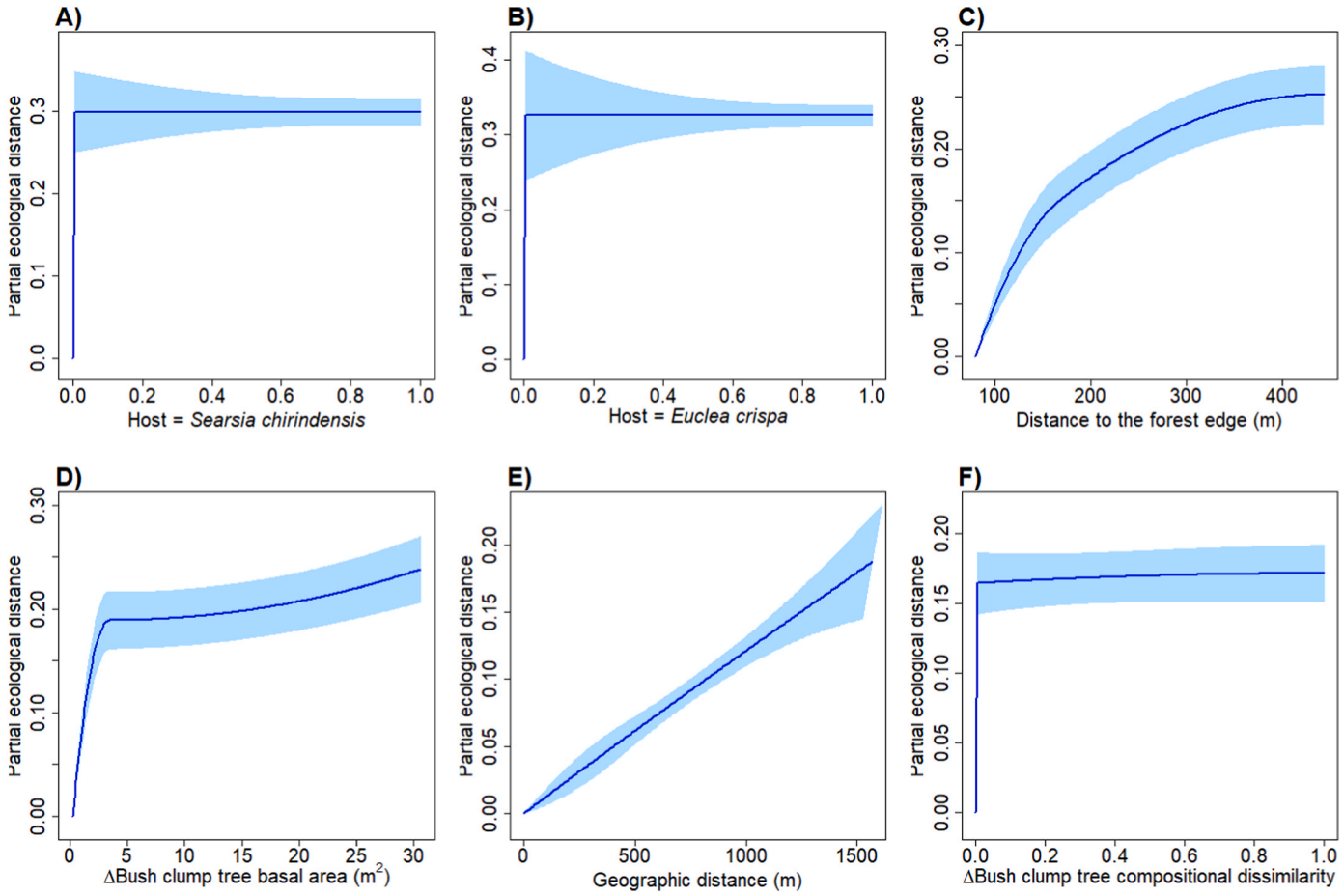

Fig. 5. A-F) Significant retained predictor variables for the GDM performed on the full dataset. Each panel (A–F) represents the fitted I-splines (partial regression fits), and 95% confidence intervals (blue shading), of each retained predictor variable associated with the compositional turnover in foliar fungal endophyte communities. The maximum height reached by each curve represents the total amount of compositional turnover explained by that variable, holding all other variables constant.

Table 2
Relative importance of the retained predictor variables for fungal endophyte compositional turnover for the full dataset. Estimates are determined by summing the coefficients of the I-splines from the GDM. Significant *p*-values of retained predictor variables are indicated in bold.

Gradient	estimate	*p-value*
Searsia chirindensis	0.364518	**<0.001**
Euclea crispa	0.325869	**<0.001**
Distance to forest edge	0.251447	**<0.001**
Δ Tree basal area	0.234689	**<0.001**
Geographic distance	0.174214	**<0.001**
Δ Tree compositional dissimilarity	0.154513	**<0.001**
Inverse light intensity	0.154031	0.075

successional changes. Instead, host species identity was the most important factor shaping community composition and richness patterns for endophytes. After host identity, the one stochastic factor considered here (distance from the forest source) was the next most important predictor of community composition, while the other deterministic forces showed little to no effect on community composition. In contrast, species richness patterns were well-predicted by deterministic factors, but also primarily host identity.

4.1. Taxonomic and functional composition

The fungal communities across all three host trees were dominated by the Ascomycota, particularly members from the class Dothideomycetes – orders Capnodiales, Pleosporales and Dothideales (Figs. 2 and 3). The level of Dothideomycete dominance was unexpected as temperate and tropical trees are often more dominated by fungi in the class Sordariomycetes (Arnold and Lutzoni, 2007; U'Ren et al., 2012). Many of the significant taxonomic differences observed between the three hosts were caused by varying abundances of Basidiomycota taxa which belong to known endophytic and/or phylloplane yeast groups – namely Agaricomycotina (Class Tremellomycetes), Pucciniomycotina (Class Cystobasidiomycetes) and Ustilaginomycotina (Class Exobasidiomycetes) (Arnold and Lutzoni, 2007; Kemler et al., 2017). Other significant taxonomic differences observed between the three host trees were caused by Ascomycete taxa often recovered as plant pathogens or endophytes e.g. Hypocreales, Xylariales, Teratosphaeriaceae and Pleosporaceae. Such findings may explain why the largest proportion of fungi which could be assigned to a trophic mode or fungal guild were pathogens and pathogen-endophytes (Fig. 2); or it may be that pathogens are the more widely studied group due to their economic importance, and that the large percentage of unassigned guilds are from taxa less well studied.

4.2. Successional patterns

Across the common and rare endophyte assemblages of three host tree species, evidence for deterministic succession was only found for the rare endophyte communities of one of the three woody host species (*E. crispa*) and in none of the three assemblages of common endophyte species. Therefore, we find no consistent evidence of deterministic succession of endophyte communities within individual hosts across a

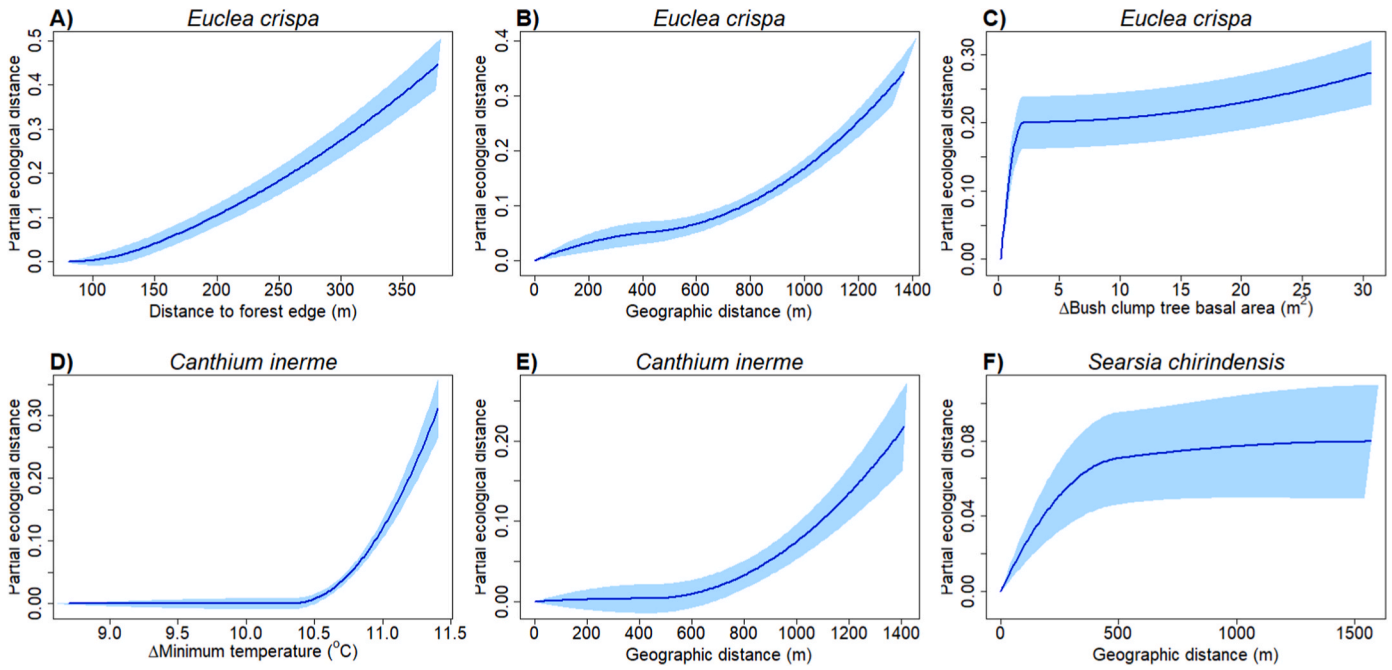

Fig. 6). The fitted I-splines (partial regression fits) of each significant retained predictor variable from GDM analyses modelling the drivers of endophyte community compositional turnover in foliar fungal endophyte communities. Significant predictors are shown for each host species: A-C) *Euclea crispa*; D & E) *Canthium inerme*; F) *Searsia chirindensis*. The maximum height reached by each curve represents the total amount of compositional turnover explained by that variable, holding all other variables constant. Light blue shading represents the 95% confidence interval for the fitted model.

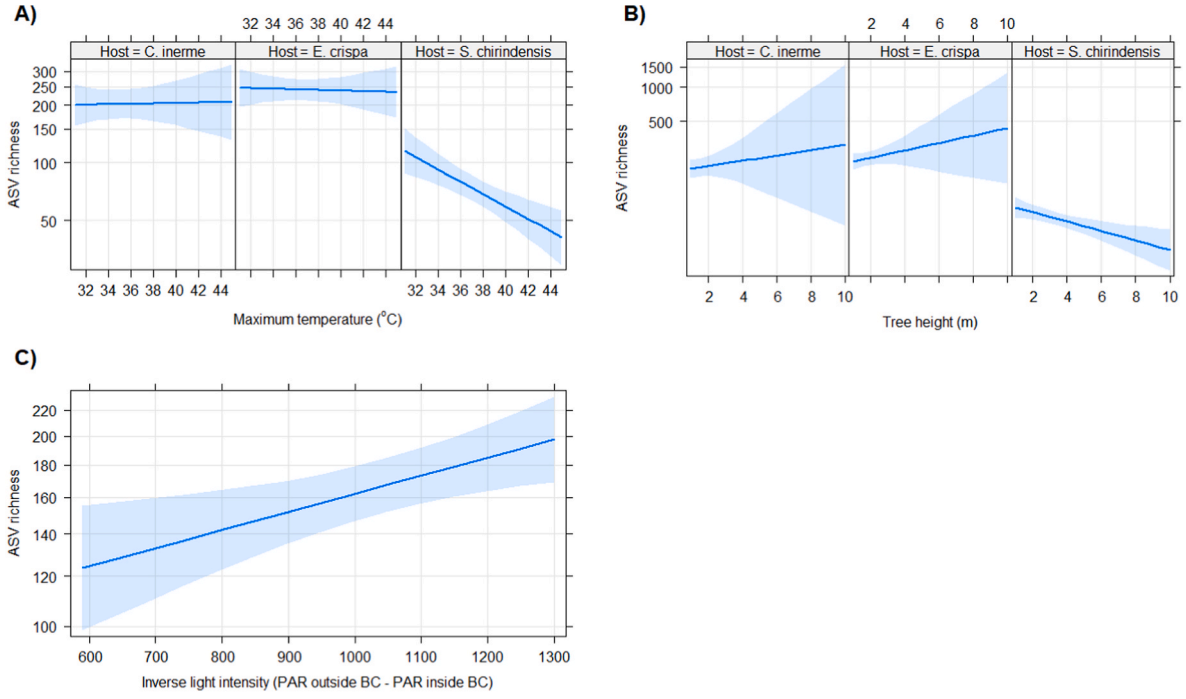

Fig. 7. Effect plots for the significant predictors from the best subset GLMM model for ASV richness of foliar fungi. A) Maximum temperatures experienced within the BCs had no effect on ASV richness in *C. inerme* and *E. crispa*, but decreased with maximum temperature in *S. chirindensis*. B) Height of the trees from which samples were taken had no effect on ASV richness in *C. inerme* and *E. crispa*, whereas ASV richness decreased with maximum temperature in *S. chirindensis*. C) ASV richness decreased with *increasing* light intensity: the x-axis represents inverse light intensity measured as photosynthetic active radiation (PAR) outside a BC minus PAR inside the BC. Light blue shading represents the 95% confidence interval for the fitted model.

woody-plant successional gradient. Under a scenario of deterministic succession, similar directional shifts in fungal endophyte community composition would have been observed since abiotic environmental conditions changed fairly consistently with BC size (Jamison-Daniels et al., 2021). The lack of consistent directional changes in endophyte composition with BC size, but significant effects of the host identity on species composition, suggest that the effect of the plant host on endophyte composition trumps the impact that the consistent changes of

Table 3
Type III Wald chi-square test of the best subset GLMM assessing which factors affect ASV foliar fungal endophyte richness across the bush clump tree successional gradient. The retained variables from the best subset model, and their interactions between each other are fixed effects. Bold p-values represent significant effects at $p < 0.05$. Bush clump identity and sample identity were included as random variables.

Fixed Effects	Df	Chi-square	*p*-value	Marginal R^2
Host	2	6.1462	**0.0463**	0.62705
Maximum temperature	1	0.0113	0.9154	
Tree height	1	0.2964	0.5861	
Inverse light intensity	1	7.9094	**0.0049**	
Host X Maximum temperature	2	12.6551	**0.0018**	
Host X Tree height	2	6.3607	**0.0416**	
Random Effects	**Variance**	**Standard deviation**	**AIC**	**Conditional R^2**
(1|Bush clump ID)	0.0323	0.1797	2079.2	0.98934
(1|Sample ID)	0.1394	0.3733		

environmental variables has on fungal endophyte communities. A large percentage of variation in the community assembly models remain unexplained, suggesting that unmeasured or random processes may contribute to the assembly of endophyte communities (Hubbell, 2001).

The lack of strong evidence for deterministic succession within endophyte communities does not preclude succession of endophyte communities of all trees per bush clump. Our study specifically assessed changes in endophyte composition within single host species across a tree successional gradient. Because tree composition changed in a directional manner with BC size (Jamison-Daniels et al., 2021), and host identity is an important determinant of endophyte composition and richness (Liu et al., 2019; U'Ren et al., 2019; Harrison and Griffin, 2020) (Fig. 5; Fig. 7; Table 2; Table 3), endophyte communities of entire BCs (i.e. across different tree species or within tree genera or families) could show stronger evidence of deterministic changes in endophyte composition. As a certain suite of tree species are associated with a specific successional stage in this system (Jamison-Daniels et al., 2021), their associated endophyte communities may also be associated with the associated tree successional stage. Under this scenario, the succession of endophyte communities would be driven by changes in host species composition with successional stage, and not changes in environmental factors with successional stage.

4.3. Drivers of community composition

Although no consistent evidence of deterministic succession in endophyte communities was observed; one deterministic process – host identity – primarily shaped the community assembly of foliar fungal endophyte communities (Fig. 5, Table 2). Host identity alone explained ~23% of the total ~29% variation in turnover of endophyte composition explained by the final GDM model. Host identity is known to be a major determinant of the composition of foliar fungal endophyte communities (Christian et al., 2016; David et al., 2016; Vincent et al., 2016; Liu et al., 2019; Yao et al., 2019). The specificity of endophytes to a particular host or group of taxonomically related hosts ranges from extremely narrow to relatively wide (Arnold and Lutzoni, 2007; Põlme et al., 2018). This may explain why, even though host identity was the most important factor structuring foliar fungal endophyte communities, the identity of only two host species was retained in the final GDM model, with *C. inerme* consistently being removed as one of the variables which explained the least turnover in endophyte community composition.

Geographic distance between samples was the only significant variable consistently retained in every GDM (i.e. the full dataset and each of the three hosts individually) (Figs. 5 and 6). This indicates that the similarity in composition of foliar fungal endophytes decays with increasing distance, a pattern commonly observed in ecological communities (Nekola and White, 1999; Soininen et al., 2007). Indeed, others have shown that foliar fungal endophytes are dispersal limited over distances from 10 km to >100 km (David et al., 2016; Koide et al., 2017; Oono et al., 2017). However, here we show that even over a shorter spatial distance i.e. <1500m, there is consistent evidence for distance decay driving turnover in endophyte community composition.

The GDMs performed for each host individually, showed that the factors responsible for the turnover in foliar fungal endophyte community composition within a host is variable as is the rate and magnitude of this turnover (Fig. 6 Supporting information Tables S8–S10). Within all tree hosts, either distance from other samples or distance from the forest edge were significant drivers of endophyte community composition, indicating the importance of the stochastic process in determining endophyte community composition. Others have shown how the distance to a potential inoculum source, in our case a large indigenous forest, can be important factor shaping compositional differences in fungal communities (Glassman et al., 2017). In only two of the species, was one out of the many deterministic drivers retained and significant. It has been shown how abiotic conditions, like temperature differences, can shape endophyte community composition due to differences in the physiological performance of endophyte species in dealing with the prevailing conditions (Arnold and Herre, 2003; Peay et al., 2016; Unterseher et al., 2016). Here, abiotic factors seemed to play a relatively small role in explaining fungal composition within host species, perhaps the abiotic gradients used in our study were too short to detect major differences in foliar fungal composition.

4.4. Endophyte richness

Endophyte richness was most strongly influenced by host identity, with endophyte richness of one host (*S. chirindensis*) being consistently lower than that of the other two hosts. Only one abiotic variable showed a consistent effect on endophyte richness across the three host species: reduced endophyte richness at higher light intensity may be caused by the negative impact of UVB on endophyte persistence. High UVB increases leaf desiccation and can activate the plant's defence responses, ultimately leading to lower endophyte richness (Arnold, 2007; Unterseher et al., 2007, 2012).

Other abiotic effects on endophyte richness differed between host species which may shed some light on how such factors drive endophyte richness in individual host species. Lower endophyte richness in *S. chirindensis* occurred in warmer BCs. Temperature differences within BCs may drive differences in chemistry and secondary metabolite production produced by the leaves of *S. chirindensis* that experience high temperatures (Reich et al., 1999; Veteli et al., 2002), potentially leading to lower ASV richness (Arnold and Herre, 2003; Unterseher et al., 2012, 2013). High temperatures can activate the plant defence response, thereby increasing the production of secondary metabolites with potential anti-microbial properties (Unterseher et al., 2016) which decreases the observed endophyte richness by reducing the number of successful endophyte colonisations. Tree height also resulted in lower endophyte richness in *S. chirindensis*. Taller trees are usually older, and older hosts have been shown to support decreased endophyte richness compared to their younger conspecifics (Oono et al., 2015), possibly due to heightened defence mechanisms which resist endophyte colonisation; alternatively established groups of endophytes in older trees may outcompete newly arriving endophytes, ultimately leading to lower endophyte richness in older trees (Unterseher et al., 2007; Oono et al., 2015).

5. Conclusion

This work is, to our knowledge, the first attempt to assess whether

endophyte communities and their hosts display linked or decoupled trends of succession, and ultimately helps us understand whether long standing theories of ecology, e.g. succession, applies to foliar fungal communities within individual host species. Endophyte community assembly within this natural system was primarily structured by one deterministic force – host identity – and there was no consistent evidence of deterministic successional trends in endophyte communities within the three different hosts, despite the fact that the tree community within which these host species were growing was undergoing deterministic succession. Spatial distance between communities, a stochastic factor, played a more important role in determining endophyte community assembly within different host species compared to the many deterministic factors we considered. Also, much of the variation in species composition between sites remained unexplained. This suggests that stochastic processes of community assembly may be more important than deterministic processes in structuring foliar fungal endophyte composition (Hubbell, 2001; Chave, 2004). In stochastic communities, dispersal limitation, speciation and extinction shape the observed community composition within individual hosts (Vellend, 2010; Vellend et al., 2014; Dini-Andreote et al., 2015; Zhou and Ning, 2017). This is in agreement with findings that some microbial communities show weaker or different biogeographical patterns than most macroorganisms (Peay et al., 2016; U'Ren et al., 2019). What was not considered here was the role of endophytes in driving tree fitness and success, which in turn could be contributing to vegetation succession with the BCs (Schlaeppi and Bulgarelli, 2015; Vandenkoornhuyse et al., 2015; Compant et al., 2019). This presents an important question for future consideration. Continued work to gain a deeper understanding of how fungal endophyte communities organise themselves through space and across time within natural systems, and especially the role of deterministic *vs*. stochastic processes, will be essential to appreciate the functions and ultimately the ecosystem services these microbes are able to deliver.

Author contributions

MAH, MK, BS & MG contributed to the design of the research, performance of the research, interpretation of the data and writing of the manuscript. MAH, S-L.J-D, MB & MG contributed to the data collection and data analyses of the research. MAH, MK, FW, DB & AB contributed to the sequencing of the fungal communities.

Declaration of competing interest

We know of no conflicts of interest that are associated with this potential publication. Additionally, we declare that there has been no significant financial support for conducting this research that could have influenced its overall outcome. As the corresponding author, I confirm that the entire manuscript with all supplementary materials has been read and approved for submission by all authors named above.

Acknowledgements

The authors thank Buffelskloof Private Nature Reserve, more specifically Mr John Burrows, for providing them with access to the research site, and to Mpumalanga Tourism and Parks Agency for permits to collect plant material. A special thanks to Mandy Messal and Laura Milne for their help during the collection and processing of samples collected. Luis Pertierra provided helpful comments on the manuscript. The authors would also like to thank the Tree Protection Co-operative Program (TPCP), the centre of excellence in plant health and biotechnology (CPHB) and the South African National Research Foundation (Grant numbers 98889 and 116333) for funding this research.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.funeco.2023.101249.

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