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Title page

**Precipitation increases the abundance of fungal plant pathogens in *Eucalyptus*
phyllosphere**

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Originality-Significance Statement

Advancing our knowledge of the current and future distributions of phyllospheric fungal plant pathogens is critical to predict the plant primary productivity. The present study for the first time conducted a large-scale investigation of Eucalyptus phyllospheric microbiome in Australia. Our results suggested that precipitation is the most important driver of fungal taxonomic diversity and abundance. The abundance of fungal plant pathogens exhibited a significant positive linear relationship with precipitation. We built current and future atlases of fungal plant pathogens, which indicate that their abundance probably increases in coastal regions regardless of the climate scenarios considered. These findings improved our ability to predict the patterns of fungal plant pathogens under the climate change, which would be helpful to mitigate their impacts on plant productivity and related economic losses.

Summary

Understanding the current and future distributions of plant pathogens is critical to predict the plant performance and related economic benefits in the changing environment. Yet, little is known about the roles of environmental drivers in shaping the profiles of fungal plant pathogens in phyllosphere, an important habitat of microbiomes on Earth. Here, using a large-scale investigation of *Eucalyptus* phyllospheric microbiomes in Australia and the multiple linear regression model, we show that precipitation is the most important predictor of fungal taxonomic diversity and abundance. The abundance of fungal plant pathogens in phyllosphere exhibited a positive linear relationship with precipitation. With this empirical dataset, we constructed current and future atlases of phyllosphere plant pathogens to estimate their spatial distributions under different climate change scenarios. Our atlases indicate that the abundance of fungal plant pathogens would increase especially in the coastal regions with up to 100-fold increase compared with the current abundance. These findings advance our understanding of the distributions of fungal plant pathogens in phyllospheric microbiomes under the climate change, which can improve our ability to predict and mitigate their impacts on plant productivity and economic losses.

Introduction

Achieving sustainable supply of cereals and timber is challenged by the on-going climate change scenarios, which are predicted to cause more frequent extreme events, such as drought, flooding (Marengo and Espinoza, 2016) and plant disease outbreaks (Chakraborty and Newton, 2011; García - Guzmán and Heil, 2014). It is estimated that ~15% of the global crop production decreased due to biological threats, a significant risk that is expected to exacerbate with the changing environment (Chakraborty and Newton, 2011; Newbery et al., 2016; Delgado-Baquerizo et al., 2020). Moreover, some soil-borne aggressive plant pathogens such as *Alternaria alternata* or *Fusarium oxysporum* are developing resistance to current commercial chemical fungicides, with negative consequences on non-targeted organisms (Khan et al., 2017; Saha et al., 2020). A growing body of experimental and observational studies suggest that emerging fungal pathogens have threatened the global food security (Wei et al., 2019; Corredor - Moreno and Saunders, 2020; Fones et al., 2020). Recently, information on the fungal plant pathogens has become increasingly available at regional and global scales. For instance, a global field survey reported the increasing proportion of plant pathogens in soil under warming, and provided global projections of their distribution under various global change scenarios (Delgado-Baquerizo et al., 2020). Moreover, Delavaux et al. (2021) explored the distribution of root-associated fungal plant pathogens across gradients of precipitation and temperature and found that anthropogenic disturbance reduced the

sensitivity of pathogenic microbes to climate change (Delavaux et al., 2021). These findings are instrumental in formulating calls to reduce the incidence and impacts of pathogens on plant health and yield stability. Nevertheless, researches have mostly focused on the belowground microbes, ignoring the potential impacts of pathogenic microbes in the aboveground habitat – phyllosphere.

Phyllosphere, the aboveground parts of a plant, is a vast habitat for a tremendous diversity of organisms. The total area of phyllosphere on Earth is estimated to be over 10^9 km² when considering the upper and lower leaf surfaces (Vorholt, 2012). Plant phyllosphere attracts and maintains a diverse array of microbes including bacteria, fungi, archaea, and protists (Vorholt, 2012). These microbes extend the host's genomic and metabolic capabilities, and provide a range of essential life-support functions, including immune modulation and stress tolerance, which have far-reaching implications for the host fitness, productivity and ecosystem functioning (Muller et al., 2016; Dini-Andreote and Raaijmakers, 2018; de Vries et al., 2020; Liu et al., 2020; Rosado and Almeida, 2020; Chen et al., 2021; Sun et al., 2021). Owing to its open nature, microbes in the phyllosphere are subject to a variety of biotic and abiotic pressures (Vorholt, 2012). Therefore, phyllosphere is a more dynamic and heterogeneous environment than the soil ecosystem. Soil bacteria (Bahram et al., 2018; Delgado-Baquerizo et al., 2018), fungi (Tedersoo et al., 2014), fungal plant pathogens (Delgado-Baquerizo et al., 2020), protists (Oliverio et al., 2020), and soil fauna (e.g., nematodes (van den Hoogen et al., 2019) and earthworms (Phillips et al., 2019)) have been well characterized at the global scale,

however, the phyllospheric microbes gained much less attention. More importantly, phyllosphere associated plant pathogens may mediate plant responses to climate changes and impact on plant health and productivity. Therefore, a comprehensive understanding of the distribution patterns of plant pathogens in phyllospheric microbiomes is critical to predict their impacts on ecosystem primary productivity under the climate changes.

Here, we conducted a large-scale survey (> 4000 km) of the *Eucalyptus* phyllosphere microbiomes across 112 locations in Australia. The *Eucalyptus* trees comprise for 74% of forested land in Australia (<https://agriculture.gov.au/abares>), which are distributed across a wide range of climatic conditions and are important raw materials for solid wood and pulp products (Fig. S1). Many fungi occurring on the *Eucalyptus* phyllosphere are well-known opportunists or potential pathogens. A classic example of a primary *Eucalyptus* leaf and shoot pathogen is the rust fungus *Austropuccinia psidii* (Crous et al., 2019). In addition, *Neofusicoccum* spp., the best-studied pathogenic species on *Eucalyptus* foliage, could cause dieback symptoms (Crous et al., 2019). Fungal pathogens have been recognised as a serious threat to *Eucalyptus* trees, and this threat has become more prominent as eucalypt plantation areas increase due to the growth of the paper and dissolving pulp industries, particularly over the past 30 years. We hypothesize that phyllosphere-associated fungal pathogens abundance is strongly impacted by climatic factors, e.g., precipitation and temperature. Given the open nature of phyllosphere, microbes in this habitat are more easily affected by the fluctuations of abiotic factors. By employing advanced molecular technologies and modeling, we aimed

to (1) identify the most important predictors for fungal abundance and diversity in phyllosphere; (2) evaluate how these predictors regulate the abundance of phyllospheric fungal plant pathogens; and (3) build atlases for their current and future distributions (year of 2050).

Results

Assemblages and abundance of fungal community in phyllospheric microbiome

Amplicon sequencing yielded a total of 6,957,856 clean sequences, which could be classified into 9,864 fungal phylotypes (OTUs). The number of fungal phylotypes at a given site ranged from 190 to 1,659, with an average of 686 per site. These phylotypes were classified into 10 phyla and 850 genera, with Ascomycota and *Pseudosydowia* being the dominant taxa at the phylum and genus level, respectively. The top 50 genera occupied 98.98% of all the ITS sequences (Fig. S2).

Using the quantitative PCR (qPCR) technique, we quantified the abundance of fungi in phyllospheric microbiome, which ranged from 8.35×10^5 to 1.07×10^{10} copies g^{-1} plant tissue. Ordinary least squares (OLS) regression models showed that fungal abundance ($r^2 = 0.257$, $P < 0.001$, AICc = 246.8) and diversity ($r^2 = 0.068$, $P = 0.003$, AICc = 1,602) were significantly correlated with mean annual precipitation (MAP) (Fig. 1a). A significant second order polynomial relationship was observed between mean annual temperature (MAT) with fungal abundance ($r^2 = 0.095$, $P = 0.002$, AICc = 269.7) and diversity ($r^2 = 0.105$, $P < 0.001$, AICc = 1,598). The abundance and diversity of

phyllospheric fungi peaked at MAT of $\sim 20^{\circ}\text{C}$ and then declined with the increasing MAT (Fig. 1a). Diversity was measured using Chao1 and phylogenetic diversity index and their relationships with MAP and MAT were robust to the choice of index (Fig. S3). Non-metric multidimensional scaling (nMDS) ordinations indicated that MAP and MAT well explained the variations in the overall fungal community profiles (Fig. 1b). Specifically, we found a positive correlation between MAP and fungal nMDS1 ($r^2 = 0.332$, $P < 0.001$, $\text{AICc} = -70.6$), and a negative correlation between MAT and fungal nMDS2 ($r^2 = 0.631$, $P < 0.001$, $\text{AICc} = -173.1$) (Fig. S4). Phyllospheric fungal community compositions were also related to edaphic properties (Table S2). For example, soils with a higher content of dissolved organic matters, e.g., DOC and DON, tended to harbor a higher abundance of phyllospheric fungi. In contrast, negative correlations were observed between fungal diversity and soil dissolved organic nitrogen ($P < 0.001$) and nitrate ($P = 0.042$).

Assemblages of fungal plant pathogens in phyllospheric microbiome

A total of 1,607 phylotypes of fungal plant pathogens were identified based on the FUNGuild database. The relative abundance of pathogenic phylotypes ranged from 0.62 to 86.69% (18.76% on average) (Fig. 2a). The single guild assignment accounted for 54.58% of all pathogenic phylotypes, such as *Neofusicoccum* spp, and the rest are mixed guild assignments including plant pathogens, animal pathogens, endophytes and/or saprotrophic fungi, such as *Teratosphaeria* spp. Only a few genera of fungal plant pathogens dominated the phyllospheric fungal pathogenic phylotypes, including *Neofusicoccum* and *Pestalotiopsis*. We listed the top 10 most abundant genera of fungal

plant pathogens, which together accounted for 66.73% of the retrieved fungal plant pathogens ITS sequences (Fig. 2b).

Predictors of the distribution of fungal plant pathogens

We tested to what the extent environmental filtering explained the distributions of the abundance of fungi and fungal plant pathogens. Spearman correlation analysis (adjusted by FDR) revealed that both soil and climate variables had significant correlations with the abundance of fungi and fungal plant pathogens. The results of Random Forest and OLS models indicated that MAP was a major predictor for fungal plant pathogens (Fig. 3a, b). We then used structural equation modeling (SEM) to generate a system-level understanding of the most important ecological factors controlling the abundance of fungal plant pathogens (Fig. 3c). In line with the Random Forest models, SEM indicated that MAP was the most important factor that positively influenced the abundance of phyllosphere fungal plant pathogens.

Projection of the atlas of fungal plant pathogens

We generated the first atlas in Australia for the distribution patterns of *Eucalyptus* phyllosphere-borne fungal plant pathogens (Fig. 4). This map showed that the higher abundance of plant pathogens can be found in coastal regions with more precipitation (Fig. 4a). Further analyses were performed for the most abundant and frequent fungal pathogens, which revealed that *Neofusicoccum* had a more homogeneous distribution, while *Pestalotiopsis* were more prevalent in coastal regions (Fig. S5).

To estimate the effects of future climate, we modeled the distribution of fungal plant

pathogens in phyllosphere by considering three Representative Concentration Pathways (RCPs). Our projection maps indicated that the abundance of fungal plant pathogens could be increased in coastal regions of Australia in all of the three climate scenarios (RCP 2.6, stringent mitigation scenario, RCP4.5, intermediate scenario and RCP 8.5, very high greenhouse gas emission scenario) (Fig. 4b, c, d). We further calculated the response ratios, which showed that the abundance of all the detected pathogens would increase in slightly over half of all the 112 investigated sites and decrease in the other sites. The highest enrichment could be up to overall 100-fold compared with the present abundance for a single given site. Contrastingly, the abundance of these pathogens would decrease in some inland sites, and the decrease could be up to less than 1% of the present abundance.

Discussion

Response of phyllospheric fungal communities to climatic and edaphic factors

Fungi are one of the most diverse and ancient groups of organisms including an estimated 2–5 million species on Earth (Blackwell, 2011; Hawksworth and Lücking, 2017), and they are integral ecosystem agents that play vital roles in soil nutrient cycling, plant nutrition, and pathology (Heitman et al., 2020; Bahram et al., 2021; Xiong et al., 2021). Biogeographic studies have suggested that the climatic factors (e.g., MAP), edaphic and spatial patterning (e.g., pH, calcium, phosphorus and distance from equator) had the strongest effects soil fungal richness and community composition at the global scale (Tedersoo et al., 2014). In fact, apart from fungal communities, climatic and

edaphic properties have been widely recognized as major drivers in shaping the biogeographic patterns of other soil organisms including bacteria, protists and soil fauna (Bahram et al., 2018; van den Hoogen et al., 2019; Oliverio et al., 2020). By comparison, the potential role of climatic and edaphic properties in phyllosphere microbiome has been relatively less explored.

In the present study, by using the large-scaled investigation, we provided novel insights into the pattern of fungal communities in *Eucalyptus* phyllosphere microbiome and identified their relationships with climatic and edaphic factors. It has been suggested that microorganisms residing on leaves of plants are exposed to highly variable environmental conditions including temperature, humidity, radiation, wind speed and moisture (Vorholt, 2012). Our results showed that phyllosphere fungal abundance and phylogenetic diversity increased with the increasing MAP. Their abundance and diversity peaked at MAT of ~20 °C and then declined with the increasing MAT (Fig. 1). Such unimodal relationships are widely observed, such as the relationships between soil pH – microbial diversity (Fierer and Jackson, 2006), and geographical latitude – soil bacterial functional diversity (Bahram et al., 2018). A meta-analysis of global soil fungal distribution suggested that climate factors such as MAT and MAP, rather than soil and vegetation variables, were the dominant drivers of different aspects of fungal biogeography (Větrovský et al., 2019). Compared with MAT, our results suggested that MAP could be a more critical driver of phyllosphere fungal communities based on the significant linear relationships between fungal abundance and diversity with MAP.

Contrastingly, a previous study based on the Random forest modelling indicated that temperature was the most important predictor of soil fungal biogeography (Větrovský et al., 2019). These differences could be due to the open nature of plant phyllosphere, which is more subjected to water loss than the soil environment.

Furthermore, a higher content of soil DOC and DON, tended to enhance the abundance of phyllospheric fungi (Table S2). Our findings highlighted the roles of edaphic factors in regulating the phyllospheric fungal communities, which simultaneously raises an interesting question that how could the belowground attributes influence the aboveground phyllosphere microbiomes? Although a growing number of studies have provided evidence for the temporal and spatial dynamics of phyllosphere microbiome assembly and functions (Grady et al., 2019; Xiang et al., 2020; Cui et al., 2021), most of these studies were conducted in anthropogenically disturbed croplands, which may complicate the evaluation of the phyllosphere microbiome in response to environmental changes (Delavaux et al., 2021). Although it has been constantly suggested that the plant – soil interactions have broad and far-reaching impacts on the plant health and fitness (Bakker et al., 2018; Berendsen et al., 2018; Yuan et al., 2018), the fundamental knowledge of the mechanisms underlying the cross-talk between above- and below- ground is still in its infancy. For example, some outstanding questions remain to be answered: (1) how does phyllosphere recruit bacteria from a broad scope of origins, such as soil, air, and other plants? And (2) how plant hormones, metabolites and microbial volatile organic compounds (VOCs) mediate the intra- and inter- kingdom

interactions between plant and soil (Fitzpatrick et al., 2020; Preece and Penuelas, 2020; Weisskopf et al., 2021)?

Precipitation facilitates the occurrence of fungal plant pathogens in phyllosphere

In the present study, we further contrasted the resulting taxonomic identities against the FUNGuild database (Nguyen et al., 2016) and identified 1,607 phylotypes of fungal plant pathogens (Fig. 2). Many of these fungal plant pathogens likely affect the productivity of *Eucalyptus* and other crops and plants. For instance, *Neofusicoccum* spp. commonly occurs in *Eucalyptus* bark of branches, stems and leaves, causing stem cankers and dieback (Crous et al., 2019). *Pestalotiopsis* spp. can cause necrotic leaf spots and blights in eucalypts, and lead to leaf spots in various fruits (e.g. strawberry, blueberry, mango, and persimmon) (Yasuda et al., 2003; Ko et al., 2007; Luan et al., 2008; Maharachchikumbura et al., 2014; Zhao et al., 2016) and other plants (Suwannarach et al., 2013). Interestingly, some global dominant fungal plant pathogens in soil, such as *Alternaria*, *Fusarium*, and *Venturia* (Delgado-Baquerizo et al., 2020), accounted for a relatively lower proportion in the phyllosphere. One potential explanation for these discrepancies could be attributed to the contrasting conditions between soil and phyllosphere, for example, the oligotrophic environment of phyllosphere caused by hydrophobic waxy cuticle is subjected to a higher dynamic of abiotic and biotic pressures (Vorholt, 2012). The multipartite interactions among microorganisms and between microorganisms and the plant, especially the plant hormones and VOCs, could have more direct impacts on phyllosphere than soil-borne potential plant pathogens (Holopainen,

2004; Dicke and Baldwin, 2010; Pieterse et al., 2012).

The biogeographic pattern of fungal plant pathogens showed that compared with inland, a higher abundance of fungal plant pathogens was found in coastal regions (Fig. 4). Our Random Forest and SEM models further provide robust evidence that MAP was the best predictor and positively influenced the abundance of phyllosphere fungal plant pathogens. Warming is generally expected to directly affect fungal diseases in plant communities, and a previous study reported that warming influenced the prevalence of fungal disease more than precipitation did in a Tibetan alpine meadow (Liu et al., 2019). However, in the present work, MAT exhibited relatively weaker influences on phyllosphere fungal plant pathogens than MAP based on the Random forest model (Fig. 3). Our results are supported by a recent study of the root-associated fungal pathogens which also found that MAP was positively correlated with phylogenetic species richness of fungal pathogens, especially in undisturbed grasslands (Delavaux et al., 2021). The underlying mechanisms could be explained by the facts that (1) fungal pathogens could benefit from increased moisture, which can facilitate fungal spore germination, growth, and initiation of infection (Strengbom et al., 2006); and (2) Precipitation can affect the dispersal ability of fungal spores through the rain-splash droplets (Gigot et al., 2014). However, in a global survey of soil microbiome, MAT, instead of MAP, had the strongest effects on soil fungal plant pathogens (Delgado-Baquerizo et al., 2020). One explanation for these discrepancies could be related to the relative abundance (percentage) used in the previous work, which did not consider the background fungal biomass. To a large extent,

the impacts of fungal plant pathogens on the host performance is dependent on their absolute abundance rather than their percentage (Tkacz et al., 2018). Moreover, without absolute quantitation data, the physiology and ecology of fungal plant pathogens may be masked by their relative abundance (Tkacz et al., 2018).

Based on the present empirical dataset and the projected future climate data, we generated the first atlas to forecast the patterns of fungal plant pathogens under different under global change scenarios. Our projections provide novel insights into the potential future global distributions for phyllosphere fungal plant pathogens, which suggested that their abundance in coastal regions was predicted to be increased (Fig. 4). These maps are critically important because they can help us to identify the spatial - temporal dynamics of phyllosphere fungal plant pathogens, and to predict potential losses of plant productivity. However, some potential caveats in the interpretation of our findings should be considered. It has been reported that climatic drivers dominated over plant identity in shaping the foliar fungal communities (Faticov et al., 2021), and therefore we did not consider the influences of plant species identity (over 800 eucalyptus species in Australia) on fungal plant pathogens. In addition, we did not measure the direct impacts of pathogens on host fitness or productivity, which to some extent could affect the implications of our prediction. Therefore, systematic, interdisciplinary, and international collaborations are needed to establish a well-connected network among the distribution of fungal plant pathogens, plant infection or the disease, and economic impacts.

Conclusion

Altogether, this empirical study provides detailed and systematic insights into fungal communities in *Eucalyptus* phyllosphere microbiome. Our study shows that phyllosphere is an important reservoir of fungal plant pathogens and precipitation is a major factor that shapes their distributions. Furthermore, we built current and future atlases of phyllosphere fungal plant pathogens, which indicate that their abundance could be increased in coastal regions regardless of the climate scenarios considered. This work is also imperative for predicting ongoing climate change impacts on wood production and forest industries. We envision that broader studies considering more plant species, particularly those staple crops, through interdisciplinary and international collaborations, are required to predict and reduce the negative impacts of plant pathogens on food security, fiber supply and social economics.

Experimental procedures

Field sampling

Eucalyptus foliage and soil samples were collected during May 2019 from a total of 112 locations (50~100 km between two locations) spanning > 4000 km across the eastern and northern Australia (133.42° E to 153.60° E, 19.16° S to 38.19° S) (Fig. S1). On the basis of Interim Biogeographic Regionalization, our sampling regions covered 47 bioregions with minimal anthropogenic impacts (<https://go.nature.com/3e6j21L>). Mean annual precipitation (MAP) and temperature (MAT) ranged from 254 to 1,901 mm and

from 12.93 to 26.54 °C, respectively. At each location, one composite foliage sample was randomly collected from 6 to 10 *Eucalyptus* trees at a height of ~2 m with a hand pruner. In order to minimize variations, we chose trees at the similar age and collected new leaves. To avoid cross-contamination, the pruner was sterilized each time with 75% ethanol. The plant samples were transported on dry ice to the laboratory and frozen at -20 °C for molecular analysis. One composite topsoil sample (0-10 cm) was collected by randomly mixing three soil cores near each plant sample. Soil chemical properties including pH, dissolved organic carbon/nitrogen (DOC/DON), mineral nitrogen (NO₃-N and NH₄⁺-N), total carbon (TC) and total nitrogen (TN) were determined. Climate variables including MAP and MAT were extracted from the WorldClim (<http://www.worldclim.org/>) (Fick and Hijmans, 2017). Soil properties and climate variables of each location can be found in Table S1.

DNA extraction and amplicon sequencing

DNA was extracted from 5 g of each foliage sample using the PowerSoil Kit (Qiagen, Hilden Germany) according to our previous protocol (Chen et al., 2018). The DNA quality was checked with a NanoDrop 2000c spectrophotometer and the DNA concentration was measured using the Qubit™ dsDNA HS Assay kit on a Qubit™ 3.0 fluorometer (Thermo Fisher Scientific Inc., Waltham, USA). Fungal internal transcribed spacer (ITS) region was amplified with the ITS1F/ITS2R (White et al., 1990) using the CFX96 Touch™ PCR Detection System (Bio-Rad, Hercules, USA). The PCR program

was: 95 °C for 5 min, followed by 35 cycles of 95 °C for 30 s, 55 °C for 30 s, 72 °C for 45 s and a final extension at 72 °C for 10 min. Negative controls (template DNA replaced with water) were included to detect any contamination during PCR amplification. The amplicons were purified, quantified, pooled and sequenced on an Illumina MiSeq PE300 platform (Illumina Inc., CA, USA).

Paired-end reads were merged and filtered for quality according to Caporaso et al. (2011) (Caporaso et al., 2011). We used the q-score plugin to filter low-quality reads with average Phred quality score < 20. Chimeric sequences were removed with the USEARCH tool based on the UCHIME algorithm. Quality and barcode filtering resulted in a total of 6,957,856 clean reads. To compared with previous work (Delavaux et al., 2020; Delgado-Baquerizo et al., 2020), the bioinformatic analysis of sequencing data used an operational taxonomic unit (OTU) approach instead of the new developed amplicon sequence variants concept. Open-reference OTU picking using *pick_open_reference_otus.py* at 97% similarity through the QIIME pipeline (Caporaso et al., 2010). Fungal taxonomy was assigned against the UNITE fungal database (V7.2) (Nilsson et al., 2018). To standardize read counts across samples, we rarefied samples to 32,000 reads per sample. For analyses of alpha diversity, observed species, phylogenetic diversity, and Chao1 index were calculated in QIIME. Bray-Curtis distance based non-metric multidimensional scaling (nMDS) ordinations was calculated in R with the ‘vegan’ package (Oksanen et al., 2017) to evaluate the fungal beta diversity. Plant pathogenic lifestyles for fungal communities were predicted and identified using the

FUNGuild database (Nguyen et al., 2016), which was recently used in large scale investigations (Delavaux et al., 2020; Delgado-Baquerizo et al., 2020). Putative plant pathogens within this group were identified based on a Guild assignment that contained “plant pathogen” with confidence of either ‘highly probable’ or ‘probable’ (Nguyen et al., 2016; Delavaux et al., 2020). All raw sequencing data (.fq file) are available at National Center for Biotechnology Information Sequence Read Archive with the accession number PRJNA659980 (Sample name from ITS.P1 to ITS.P112).

Quantification of fungal abundance

In order to show the impacts of background fungal abundances of each location on our results, we transformed the total relative abundance of fungal plant pathogens into absolute abundance. Fungal abundance as represented by the absolute fungal ITS region copy numbers was quantified on a Bio-Rad CFX384 Real-Time PCR Detection system (Bio-Rad, Hercules, USA) with the same primer set and thermal cycling conditions as amplicon sequencing. Plasmids containing a cloned and sequenced ITS region gene fragment were used to generate calibration curves from ten-fold dilutions. All qPCRs were performed in technical triplicates with template-free negative controls.

Statistical analysis

All statistical analyses were performed in R unless otherwise noted. We used the ordinary least squares (OLS) models to determine the relationships between fungal abundance and diversity with climatic parameters. We first identified the shape of the

relationships between fungal abundance/diversity and climatic parameters using the three most common regression models: linear, quadratic and cubic. The degree of polynomial functions (linear, quadratic, cubic) was chosen on the basis of the lowest Akaike information criterion (AICc) values (Burnham et al., 2011). Spearman correlation analyses with FDR correction (Benjamini and Hochberg, 1995) were conducted to evaluate the relationships between soil properties, climate and the abundance of fungi and fungal plant pathogens. These analyses were conducted with ‘psych’ (Revelle, 2017) and results were visualized in a heatmap with the ‘geom_tile’ function in ‘ggplot2’ (Wickham, 2009). The importance (IncMSE%) of climatic factors and soil properties on the total abundance of fungal plant pathogens and the top 10 most abundant pathogens were evaluated by the Random Forest model (ntree = 5000) with the package ‘randomForest’ (Liaw and Wiener, 2002).

We built SEMs to further test the effect of climatic factors and soil properties on the distribution of fungal plant pathogens with the AMOS software (SPSS Inc., Chicago, USA). We assessed the goodness of fit of our model by using multiple fit indices including chi-square test (χ^2) to test the difference between observed and estimated by-model covariance matrices, and root mean square error of approximation (RMSEA) to assess the discrepancy between the observed data and model per degree of freedom (RMSEA < 0.08 shows a good fit) (Schermelleh-Engel et al., 2003).

The mapping (current and future) of the abundance of fungal plant pathogens was done using the kriging interpolation method and cross-validated using the “autoKrige.cv”

function with the "automap" package in R (Hiemstra et al., 2009). For future projections (year of 2050), we used precipitation and temperature predicted from the Coupled Model Intercomparison Project Phase 5 (CIMP 5) dataset (Taylor et al., 2012), and the downscaling and calibration (bias correction) was done with WorldClim (Fick and Hijmans, 2017). The monthly values were averages over a 20-year period. Thus, in this study the climate dataset of 2050 was the average for 2041-2060. We considered three RCPs (a greenhouse gas concentration trajectory adopted by the Intergovernmental Panel on Climate Change), i.e. stringent mitigation scenario (RCP 2.6), an intermediate scenario (RCP4.5) and a very high greenhouse gas emission scenario (RCP 8.5) (CSIRO and Bureau of Meteorology, 2015). In terms of general circulation models, we used the Norwegian Climate Center's Earth System Model (NorESM1-M) (Bentsen et al., 2013). The multiple linear regression model was preceded by all subset regressions, to identify the most important factors associated with the distributions of potential plant pathogens. The identified factors included MAP, MAT and aridity index (AI). Soil properties were not included in the model, because the predicted data for soil properties were not available. After every possible model is inspected based on the AIC, we finally obtained the projection equation for abundance of fungal plant pathogens ($R^2 = 0.334$, $P < 0.001$) :

$$P_{\text{pathogen}(\log)} = 5.6584 + (0.0016 \times \text{MAP}) + (0.0066 \times \text{MAT})$$

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Figure legends

Fig. 1 Mean annual precipitation (MAP) temperature (MAT) exhibit significant impacts on the patterns of phyllosphere fungal communities. (a) Phyllosphere fungal abundance and diversity distribution along with MAP and MAT; The selection of first- and second order polynomial model as the best polynomial fit model was determined on the basis of the corrected Akaike Information Criterion (AICc). (b) Non-metric multidimensional scaling (nMDS) ordinations showing the influence of MAP and MAT on the overall fungal community profiles.

Fig. 2 The relative abundance of plant pathogens across the 112 locations (a). Relative abundance of the most common fungal plant pathogens identified (mean \pm s.e.) (b).

Fig. 3 Climatic and edaphic predictors of the patterns of fungal plant pathogens. (a) Pairwise Spearman's correlation matrix of the main climatic and edaphic determinants and the abundance of fungal plant pathogens. The importance of each determinant was calculated based on the Random Forest model (%IncSME). (b) Distributions of the abundance of fungal plant pathogens along with mean annual precipitation (MAP) and temperature (MAT). (c) Structural equation model (SEM) explored the direct and indirect effects of climatic and edaphic factors on the abundance of fungal plant pathogens. TC: total carbon, TN: total nitrogen, Fungi: fungal abundance based on the qPCR data, Plant pathogen: absolute abundance of plant pathogen.

Fig. 4 Projection of the atlas of fungal plant pathogens. (a) Current map of the

distribution patterns of fungal plant pathogens in phyllosphere; (b-d) The future maps (2050) of distribution patterns of fungal plant pathogens in phyllosphere under different global change scenarios by considering three representative concentration pathways (RCPs). The increase in the abundance and response ratio (fold change) of fungal plant pathogens were also calculated by comparing the future and current abundance of fungal plant pathogens. RCP 2.6, stringent mitigation scenario; RCP 4.5, an intermediate scenario; RCP 8.5, a very high greenhouse gas emission scenario.

Supporting Information

Fig. S1 Mean annual precipitation and temperature of the study sites; and photos of a representative ecosystem type and *Eucalyptus* trees.

Fig. S2 The relative abundance of the top 50 fungal genera in phyllosphere of *Eucalyptus*.

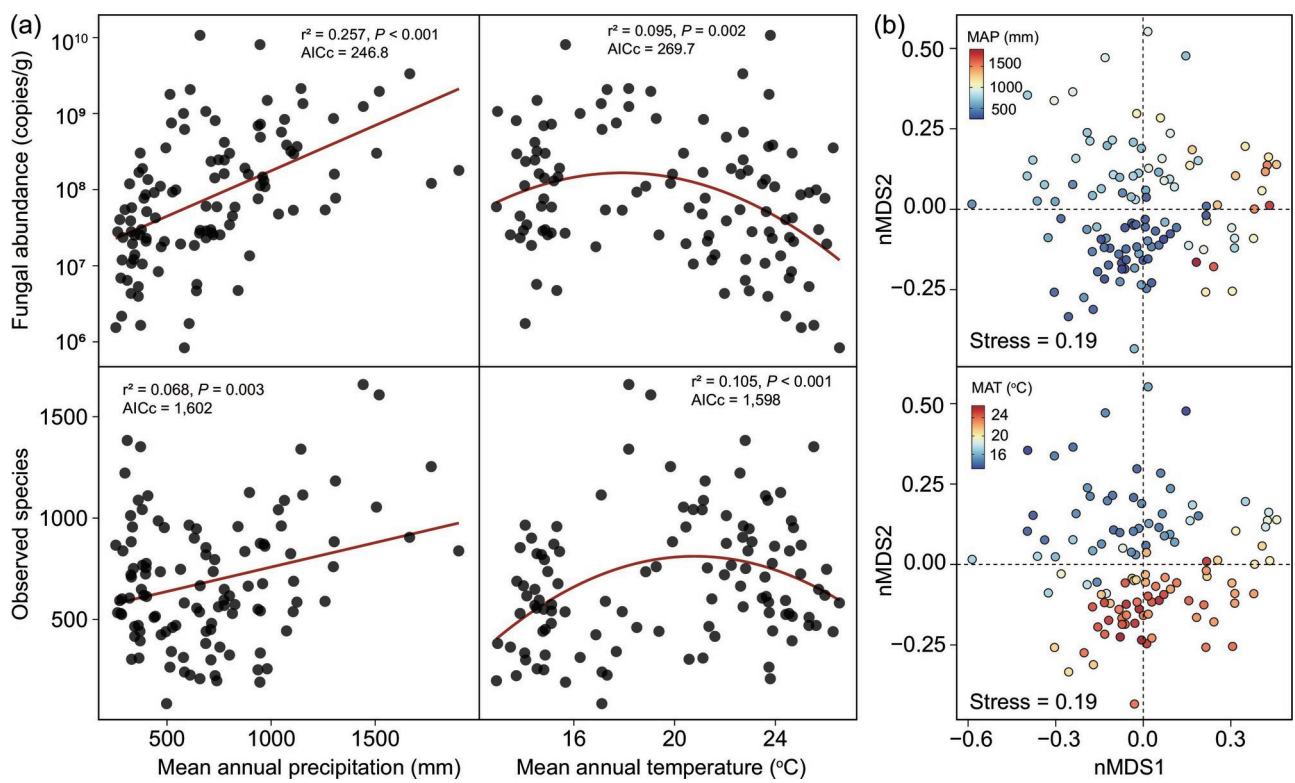
Fig. S3 Phyllosphere fungal diversity (Chao1 and phylogenetic diversity) distribution along with mean annual precipitation and temperature.

Fig. S4 The influence of mean annual precipitation and temperature on the overall fungal community profiles.

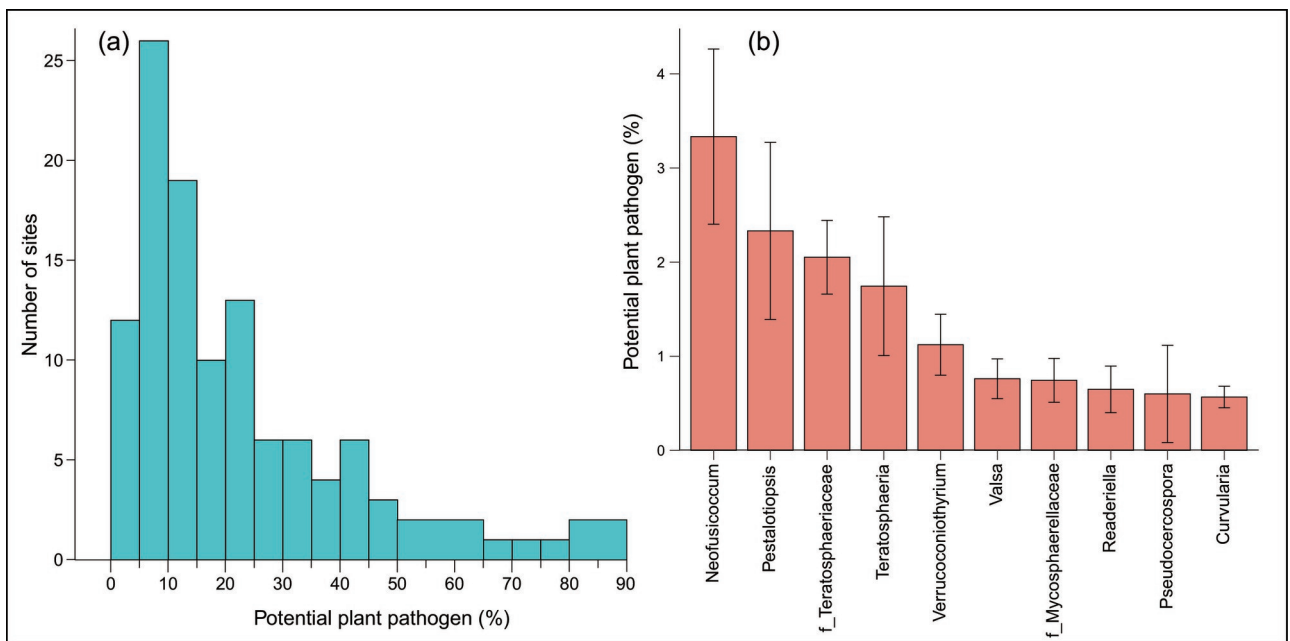
Fig. S5 The atlas of the abundance of *Neofusicoccum* and *Pestalotiopsis* in phyllosphere of *Eucalyptus*.

Table S1 Soil properties and climate variables of each location.

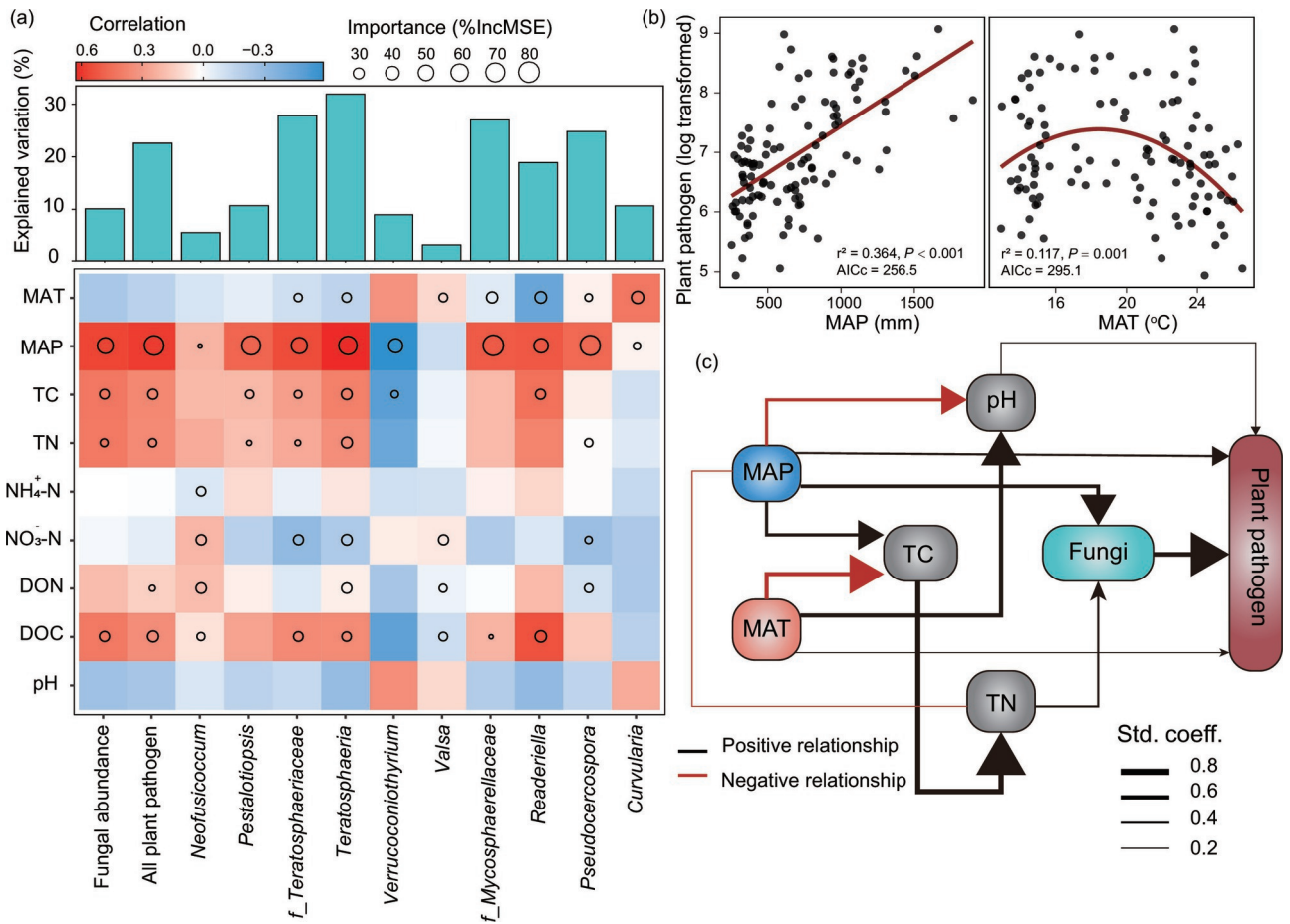
Table S2 Correlation (Spearman) between climatic, edaphic properties and phyllosphere fungal attributes.



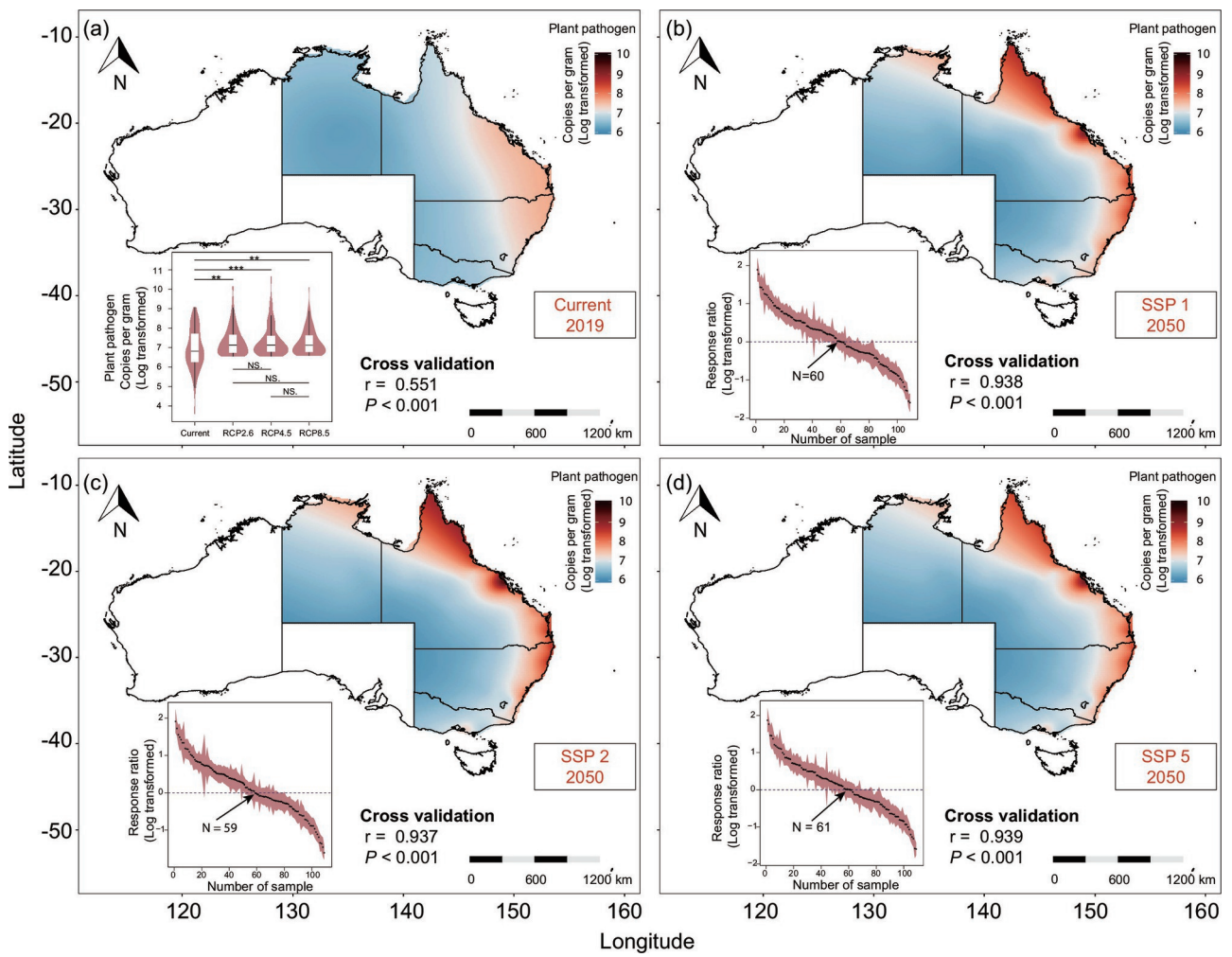
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