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Climate warming dominates over plant genotype in shaping the seasonal trajectory of foliar fungal communities on oak

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Summary

- Leaves interact with a wealth of microorganisms. Among these, fungi are highly diverse and are known to contribute to plant health, leaf senescence and early decomposition. However, patterns and drivers of the seasonal dynamics of foliar fungal communities are poorly understood.
- We used a multifactorial experiment to investigate the influence of warming and tree genotype on the foliar fungal community on the pedunculate oak *Quercus robur* across one growing season.
- Fungal species richness increased, evenness tended to decrease, and community composition strongly shifted during the growing season. Yeasts increased in relative abundance as the season progressed, while putative fungal pathogens decreased. Warming decreased species richness, reduced evenness and changed community composition, especially at the end of the growing season. Warming also negatively affected putative fungal pathogens. We only detected a minor imprint of tree genotype and warming × genotype interactions on species richness and community composition.
- Overall, our findings demonstrate that warming plays a larger role than plant genotype in shaping the seasonal dynamics of the foliar fungal community on oak. These warming-induced shifts in the foliar fungal community may have a pronounced impact on plant health, plant–fungal interactions and ecosystem functions.

Introduction

Almost all plant organs harbour microorganisms (Vorholt, 2012; Reinhold-Hurek *et al.*, 2015). Plant leaves are no exceptions – they are associated with a large number of fungal species, both on their surface and within and between their cells (Jumpponen & Jones, 2009; Redford *et al.*, 2010; Turner *et al.*, 2013; Vacher *et al.*, 2016; Laforest-Lapointe & Whitaker, 2019; U'Ren *et al.*, 2019). Foliar fungi and plants interact in many different ways. Some leaf fungi extract resources and weaken plant defences, whereas others increase plant defence and strengthen the ability to endure abiotic stress (Redman *et al.*, 2002; Arnold *et al.*, 2003; Jaber & Enkerli, 2017). Fungi may also contribute to leaf senescence and decomposition (Voříšková & Baldrian, 2013; Vacher *et al.*, 2016). As such, the foliar fungal community plays an important role in regulating plant fitness, interactions between plants and other species and ecosystem functioning (Clay & Holah, 1999; Bradley *et al.*, 2008; Vandenkoornhuyse *et al.*, 2015).

Climate is one of the major factors that shape microbial communities in nature (Bálint *et al.*, 2015; Ren *et al.*, 2015; Vacher *et al.*, 2016; Zhu & Penuelas, 2020). From a climate perspective, two aspects of the interactions between fungi and the foliage are of particular interest: how climate, in particularly temperature, influences the foliar fungal community (Runion *et al.*, 1994; Penuelas *et al.*, 2002) and whether the response of the foliar fungal community to climate is consistent across plant species and genotypes (Bálint *et al.*, 2013; Kivlin *et al.*, 2019). As different microbial taxa tolerate moisture and temperature differently (i.e. they differ in their climatic niche), a shift in climate may change the microbial community composition (Singh *et al.*, 2010; Peñuelas *et al.*, 2012). Several studies have shown that the fungal community composition changes along latitudinal and elevational gradients, which are used as proxies for temperature

gradients (Cordier *et al.*, 2012; Zimmerman & Vitousek, 2012). However, other environmental factors (e.g. precipitation or soil properties) also change alongside these gradients, which can confound the effect of temperature. Experimental studies of climate warming on fungal communities have mostly focused on root and soil fungi (Frey *et al.*, 2008; Schindlbacher *et al.*, 2011; Treseder *et al.*, 2016; Romero-Olivares *et al.*, 2017; Solly *et al.*, 2017). The handful of studies that used experimental heating to investigate the effect of warming on foliar fungi tended to find a negative or no effect on biomass and diversity (Table 1). These observations suggest that, at least in some cases, warming exposes foliar fungi to stress, either directly or indirectly, by boosting plant defences, which can exclude less adapted taxa. Importantly, the role of temperature in influencing foliar fungal communities may shift across the growing season, causing different imprints during different parts of the season – and thus shaping seasonal community dynamics.

In addition to climatic effects, genetic variation among plants can also shape the associated fungal communities and consequently the eco-evolutionary dynamics between plants and their fungal communities, which has been studied within the framework of community genetics (Gaylord *et al.*, 1996; Whitham *et al.*, 2006; Zytynska *et al.*, 2011; Jousimo *et al.*, 2014; Barker *et al.*, 2018). Specifically, differences in leaf traits, nutrient content and secondary chemistry among genotypes may result in differential colonization of plant genotypes by microorganisms, which consequently leads to variation in microbial community composition (Hoffman & Arnold, 2008; Bálint *et al.*, 2013; Wagner *et al.*, 2016; Hamonts *et al.*, 2018). For example, Sapkota *et al.* (2015) found that plant genotype was an important factor in shaping the fungal community on wheat, barley, oat and rye, and Rajala *et al.* (2013) found strong differences in the needle fungal community among Norway spruce clones. Moreover, the effect of plant genotype on the foliar fungal community may depend on the abiotic environment. For example, Bálint *et al.* (2015) showed that genotype and warming interactively shaped the foliar fungal community composition on balsam poplar (Table 1). The presence of warming × genotype interactions indicates that warming shifts the foliar fungal community, but differently for each plant genotype. Such warming-induced shifts in the fungal community may strongly affect the absolute and relative fitness of plant genotypes, and thereby shape the ecological and evolutionary trajectory of plant populations to climate warming.

While previous studies have shown that the diversity and composition of the foliar fungal community change strongly during the growing season (Collado *et al.*, 1999; Osono, 2008; Jumpponen & Jones, 2010; Gomes *et al.*, 2018; Materatski *et al.*, 2019), we know less about the drivers that shape this seasonal trajectory. The potential factors that can drive seasonal variation in the foliar fungal community are diverse: shifts in community structure can be attributed to temporal changes in climatic conditions (e.g. temperature, relative humidity and UV exposure), leaf surface characteristics (e.g. leaf wax chemistry, nutrient content and cuticle thickness) and fungal life cycles (Kembel *et al.*, 2014; Copeland *et al.*, 2015; Agler *et al.*, 2016). For example, Martins

Table 1 Overview of experimental studies that have investigated the effect of warming on the foliar fungal community associated with plants.

Study	Plant species	Type and total duration of warming	Identification method	Community metrics	Other abiotic factors tested	Effect of warming	Effect of genotype	Interactive effect of warming and genotype
Bálint *et al.* (2015)	*Populus balsamifera*	Passive warming via open-top chambers 6 months (May to September) over two years (2010 and 2011)	Molecular identification	Diversity, evenness and community composition	Latitudinal gradient (reciprocal transplant)	Warming decreased diversity and evenness Warming changed community composition	Community composition differed among host genetic identities	Strong interactive effect of warming and host genetic identity on community composition
Kazenel *et al.* (2019)	Perennial grasses: *Achnatherum lettermanii* *Festuca thurberi* *Poa pratensis*	Infrared heating lamps 23 years	Molecular identification	Diversity and community composition	Altitudinal gradient	No effect of warming on fungal diversity or community composition	Not tested	Not tested
Kivlin & Rudgers (2019)	Perennial grasses: *Achnatherum lettermanii* *Festuca thurberi* *Poa pratensis*	Infrared heating lamps 26 years	Molecular and culture identifications	Community composition	No	No effect of warming on fungal diversity or community composition	Not tested	Not tested
Randriamanana *et al.* (2015)	*Populus tremula*	Infrared heating lamps 5 months (June to October 2012)	Culture identification	Relative abundance, as based on fungal morphotaxa	UV-A and UV-B radiation	Warming decreased the relative abundance of fungi	No effect of genotype on the relative abundance of fungi	No interactive effect of warming (or UV radiation) and genotype on relative abundance of fungi

et al. (2016) showed that fungal endophytes dominate the fungal community on olive leaves in the early season, when leaves are more susceptible to infection. As the season progresses, the abundance and diversity of yeast species increase, which might be related to the fact that ageing leaves release more nutrients for yeasts to grow (Glushakova & Chernov, 2004, 2007; Kemler *et al.*, 2017). The order of species arrival can lead to priority effects, where early-season colonizers influence the ability of later species to colonize the leaves, and thereby affect the seasonal dynamics of the foliar fungal community (Hibbing *et al.*, 2010; Hiscox *et al.*, 2015). Hence, early-season effects of the environment and plant genotype on the foliar fungal community may influence the subsequent seasonal trajectory of the foliar community, as well as leaf senescence and decomposition. The shifts in the foliar fungal community can represent species turnover, but also changes in species richness (Baselga, 2010, 2012; Wang *et al.*, 2017; Campos *et al.*, 2018; Gao *et al.*, 2019).

In this study, we examined the relative influence of elevated temperature and plant genotype on the foliar fungal community of the pedunculate oak (*Quercus robur*) across the full growing season. To this end, we set up a two-factorial field-heating experiment using five oak genotypes, and sampled the foliar fungal community in the early, middle and late season. We expected that:

(**1**) Fungal species richness would increase, with a gradual replacement of endophytic fungi by yeasts towards the end of the growing season.

(**2**) Experimental heating would decrease species richness, reduce evenness and change community composition.

(**3**) Tree genotype and warming × genotype interactions would affect foliar fungal species richness, evenness and community composition.

Materials and Methods

Study system

The pedunculate oak *Quercus robur* grows on a wide range of soil types in forests, wooded pastures and agricultural landscapes in Europe, and reaches its northern limit in central Sweden (Stenberg & Mossberg, 2003). Like the foliage of any woody plant, the leaves of the pedunculate oak host a diverse fungal community that consists of both epiphytic and endophytic fungi (Jumponen & Jones, 2010; Cordier *et al.*, 2012; Jakuschkin *et al.*, 2016). Among fungal pathogens, powdery mildew species from the genus *Erysiphe* are particularly abundant on leaves of the pedunculate oak (Desprez-Loustau *et al.*, 2011, 2018; Jakuschkin *et al.*, 2016).

Experimental setup

To investigate the relative importance of warming and tree genotype on the foliar fungal community across the season, we conducted a heating experiment in cages under open field conditions. In the experiment, we used 3- to 6-yr-old oak trees (*c.* 1.2 m in height) that were grafted from five large mother trees (henceforth referred to as 'genotypes'). The mother trees were randomly selected from a 5 km^2 island in southwestern Finland, and thereby reflect genotypic variation at the population level (Pohjanmies *et al.*, 2015). The grafted trees were produced by inserting a single twig in a bark slit of a randomly selected rootstock in 2011–2013. During the next 2 yr, all branches of the rootstock were successively pruned back, finally resulting in a treelet with branches and foliage exclusively representing the grafted genotype. For more details on grafting, location of the mother trees, and oak genetic differentiation within the island as compared with larger spatial scales, see Pohjanmies *et al.* (2015, 2016), Ekholm *et al.* (2017) and Faticov *et al.* (2020). For the experiment, six cages (5 × 5 × 2.2 m) were built using wooden frames (for details, see Faticov *et al.*, 2020). The temperature in three of the cages was increased by *c.* 2°C above ambient temperature, which matches the mitigation scenarios reaching concentrations of *c.* 500 ppm CO_2-eq by 2100 (IPCC, 2014). To maintain the temperature difference between the two treatments, we installed thermostats in the heated cages. These thermostats automatically turned off the heaters when the temperature difference between the control and heated cages exceeded 2°C (Faticov *et al.*, 2020). The heating started several weeks before bud burst (9 May 2017) and continued until leaf senescence (20 October 2017). We used three ceramic heaters (2000 W, 240 V) placed at 120° angles to each other (Kimball, 2005) to increase temperature in the heated cages. The experiment was run in a pasture of the Swedish Livestock Research Centre at the Swedish University of Agricultural Sciences (SLU) at Lövsta, Uppsala (59°50.14′N, 17°48.78′E). The design was slightly unbalanced, owing to initial variation among the number of replicates per tree genotype. Within each of the six cages, we had 22–26 trees belonging to the five genotypes (three to six replicates per tree genotype in each cage), with a total of 132 trees. The trees were randomly placed in a regular grid, with inter-pot distances of 30 cm. We randomized the position of the trees every other week to avoid positional effects. To keep soil moisture similar in both treatments and through time, trees were watered *ad libitum*. For more details on the experimental design, see Faticov *et al.* (2020).

Leaf sampling and sample preparation

To assess the seasonal dynamics of the foliar fungal community, we sampled leaves at the start, middle and end of the growing season. The sampling dates were 16 June 2017, when 90% of the trees had leaves that were > 2 cm long; 17 July 2017, when all trees had fully developed leaves; and 7 September 2017, when 5% of the leaves on the full set of the trees had turned brown. Leaf samples were collected in individual Ziploc bags and dried with silica gel. Each sample consisted of three randomly selected leaves per tree, which were subsequently pooled during the analysis. In total, this collection resulted in a set of 396 samples (three sampling dates × five genotypes × two heating treatments × three replicate cages per heating treatment × three to six replicate trees per genotype).

To prepare samples for DNA extraction, we used a metal corer to punch four leaf discs with a diameter of 5 mm from each side of the midrib of each of the three replicate leaves per tree. Hence,

each sample consisted of 24 pooled leaf discs. All sampling was done in a laminar flow hood, and the metal corer was sterilized after processing each sample with 95% ethanol and flaming over a Bunsen burner. The leaf discs from each sample were ground into fine powder using a bead mill (TissueLyser II; Qiagen).

Molecular methods and bioinformatics

DNA extractions were performed using NucleoSpin Plant II DNA Kit (Macherey Nagel, Düren, Germany), following the standard protocol. DNA was extracted from 20 mg of each sample. To characterize the foliar fungal community, we used primers targeting the internal transcribed spacer (ITS2) region (Schoch *et al.*, 2012). We used the forward primer fITS7 (Ihrmark *et al.*, 2012) and reverse primer ITS4 (White *et al.*, 1990), which target a 250–450 bp fragment encompassing the entire ITS2 with flanking sequences in the 5.8 and LSU genes. Each of the primers was fitted with 8 bp sample specific sequence tags. For PCR reactions, we followed the protocol of Clemmensen *et al.* (2016). In brief, PCR reactions were run in a volume of 50 µl that included 5 µM fITS7 (CX_8T-GTG ARTCATCGARTCTTTG) and 3 µM ITS4 (CX_8-TCCTCC GCTTATTGATATGC). The final amplicon pool was sequenced at SciLifeLab/NGI (Uppsala, Sweden) on a PacBio RS II system (Pacific Biosciences, Menlo Park, CA, USA). Obtained sequences were analysed with the bioinformatics pipeline SCATA (scata.mykopat.slu.se; Ihrmark *et al.*, 2012), where they were clustered into species hypotheses (SHs) based on single linkage clustering, with a 98.5% sequence similarity to the next neighbour required in order to enter a SH. For full details on the molecular methods and bioinformatics, see Supporting Information Methods S1. Sequences are archived at the Sequence Read Archive (www.ncbi.nlm.nih.gov/sra) with accession no. PRJNA671804.

In total, 713 141 sequences from 346 samples passed quality filtering. After removing plant sequences (36% of the sequences), we ended up with 460 692 reads, which clustered into 946 SHs, including four singletons. On average, fungal communities were represented by 1323 reads per sample, ranging from three to 6442. The most abundant sequence in each SH was used as a representative one. Species hypotheses were identified to species level by comparing representative sequences with SHs in the UNITE (Abarenkov *et al.*, 2010; Kõljalg *et al.*, 2013) and NCBI (Pruitt *et al.*, 2007) databases. Before the analysis, we excluded six samples with less than 10 reads each and 17 SHs that had no association with oak leaves based on current knowledge (rust fungi attacking grasses from the genus *Puccinia* and species of wood decomposers from the genera *Vuilleminia*, *Fomitopsis*, *Fomes* and *Heterobasidium*). For the analysis, we used a set of the 178 most common SHs (out of an original total of 946 SHs), because rare taxa had low contribution to overall signal and to make careful manual annotation practically manageable. The most common SHs made up 90% of the total number of remaining reads once species not associated with the oak leaves had been removed.

To classify SHs into ecologically meaningful categories, we manually assigned putative functional guilds to each of the 178 SHs based on taxonomic identity of the species or the taxonomic identity of closely related foliar fungal species in the UNITE (> 98% sequence similarity) and NCBI (> 97% sequence similarity) reference databases. In this study, we manually assigned fungi to six broad categories: yeasts; putative fungal pathogens; putative saprotrophic or other endophytic fungi; unknown function, Ascomycetes; unknown function, Basidiomycetes; and unidentified fungi. We designated yeasts as a separate guild because of the taxonomic complexity and the fact that many yeast species can belong to several functional guilds (Kemler *et al.*, 2017). The details and description of the assigned putative functional guilds can be found in Table S1. For each sample, the relative abundance of each fungal guild was calculated as the ratio between the summed number of reads of all SHs in a given guild and the total number of reads (with unidentified SHs included in the total number of reads). For the list of species hypotheses, see Table S2. We also calculated fungal species richness (number of SHs per sample), Pielou's evenness (Pielou, 1966) and Shannon diversity (Shannon, 1948).

Statistical analysis

All analyses were conducted in R v.3.6.0 (R Core Team, 2019). To investigate the impact of warming and oak genotype on the community descriptors of the fungal community throughout the growing season, we independently modelled species richness, evenness and diversity as a function of the fixed effects 'warming', 'tree genotype', 'season' and their two- and three-way interactions using linear mixed effects models with the function 'lmer' in the LME4 package. To account for variation among cages, we included the random effect 'cage', and to account for repeated sampling of the same trees, we included the random effect 'tree', nested under 'cage'. As we detected interactions between season and warming, we also fitted separate univariate models to data from the early, middle and late seasons, respectively, in each case modelling the response variables richness, evenness and diversity as a function of the fixed effects 'warming', 'tree genotype' and their interaction. To achieve normality of the residuals, species evenness in the mid-season was log-transformed. To account for differences in sequencing depth, we included the square-root-transformed read count as a covariate in all models (Tedersoo *et al.*, 2014). Sampling effort was assessed using the function 'rarecurve' in the VEGAN package (Oksanen *et al.*, 2020; Fig. S1). To test for significance, we used the function 'Anova' in the CAR package (Bates *et al.*, 2015; Fox & Weisberg, 2019). For each significant fixed effect, we calculated the marginal R^2 using the function 'r.squaredGLMM' in the MuMIn package (Bartoń, 2020), by running separate models with the same random effect structure but only a single fixed effect included. For a detailed overview of the models used, including link functions, see Table S3.

To assess the drivers of the fungal community composition throughout the season, we modelled multivariate fungal community composition as a function of 'warming', 'tree genotype', 'season' and their two- and three-way interactions using a PERMANOVA as implemented in the function 'adonis2' (with the argument *by* = *margin*) in the VEGAN package (Oksanen *et al.*, 2020). All models were run using both presence–absence and absolute count data, with Jaccard metrics for presence–absence

data and Bray–Curtis dissimilarity metrics for the absolute count data. As we detected an interaction between warming and season, we also fitted separate models to data from the early, middle and late seasons, respectively, modelling the community composition as a function of the fixed effects 'warming', 'tree genotype' and their interaction. We included square-root-transformed read count in all models, to account for differences in sequencing depth. To account for our split-plot experimental design and repeated measures structure, we restricted permutations within blocks (i.e. 'cage' or 'tree', nested in 'cage' depending on the model) using the function 'how' of the R package PERMUTE (Simpson, 2019; see Table S3). For each significant factor, we extracted partial-R^2 values from the PERMANOVA model output (Oksanen *et al.*, 2020). The partial-R^2 is the sum of squares associated with the variable (or interaction) of interest divided by the total sum of squares of the data (Oksanen *et al.*, 2020). Because we used marginal models, the value associated to the partial-R^2 of interaction terms only accounts for the contribution of these interactions given all other explanatory variables (including main effects and their interactions) remain constant (Legendre *et al.*, 2011). For this reason, we have to interpret the partial-R^2 associated with these interactions as their independent contribution to the model.

Because PERMANOVA analysis does not separate effects of species turnover from changes in species richness, we disentangled these responses using the functions 'beta.temp' and 'beta.pair' from the package BETAPART (Baselga & Orme, 2012). We first analysed the changes in community composition during the season by computing dissimilarity values for each tree between the early and mid-season and between the mid- and late season. We then disentangled responses to warming during each part of the growing season. In both analyses, we partitioned the total β diversity (Jaccard dissimilarity calculated based on presence–absence data) into two indices, where β_{JTU} is the turnover component of Jaccard dissimilarity and β_{JNE} is the species gain or loss component of the Jaccard dissimilarity.

To investigate which SHs differed in abundance across the season and between the warming and control treatments in the early, middle and late seasons, we conducted differential abundance analysis with the Deseq2 extension (Love *et al.*, 2014) in the PHYLOSEQ package (McMurdie & Holmes, 2013). The Benjamini–Hochberg procedure was used to adjust *P*-values to account for multiple comparisons. To test the effect of warming throughout the season on the relative abundance of functional guilds, we modelled the relative abundance of each guild as a function of the fixed effects 'warming', 'tree genotype', 'season' and their two- and three-way interactions. To account for variation among cages and repeated sampling of the same trees, we included 'cage' and 'tree' (as nested within 'cage') as random effects in the model. We also included square-root-transformed read count into the models, to account for differences in sequencing depth.

Results

Fungal species evenness tended to decrease during the growing season (Fig. 1; Table 2). The seasonal trajectory of species richness, diversity and evenness differed between the warming and control treatments (Fig. 1; Table 2). At the start of the growing season, fungal richness, diversity and evenness did not differ between the control and warming treatments, whereas all three community descriptors were higher in the control treatment than in the warming treatment towards the end of the growing season (Fig. 1; Tables 2, 3). While tree genotype had no independent effect on the richness, diversity and evenness of the fungal community (Table 2), the effect of warming on fungal richness and diversity (but not evenness) differed among tree genotypes at the end of the season (Table 3).

The composition of the foliar fungal community shifted during the growing season, with season explaining 10% of the variation in community composition (Figs 2a, S2; Table 2). Warming explained 2–6% of the variation at the start and middle of the season, and had a larger impact on the fungal community composition at the end of the season (R^2 = 16% for absolute counts; cf. Fig. 2b–d; Tables 3, S5). By contrast, genotype consistently explained only a minor part of the variation during the entire season (3–5% for presence–absence; Tables 3, S5). The shift in community composition between the early, middle and late seasons was mainly a result of species turnover and only to a minor extent a result of species gain ($\beta_{JTU} >> \beta_{JNE}$; Fig. S3). Similarly, the differences in community composition between warming and control treatments in the early, middle and late seasons were mainly attributed to species turnover ($\beta_{JTU} >> \beta_{JNE}$; Fig. S4).

The differential abundance analysis showed that 54 SHs had changed in relative abundance from early to mid-season, 47 from middle to late season, and 64 from early to late season (Table S6). The relative abundance of SHs in the phylum Basidiomycota increased during the growing season (Figs 3, S5). For example, the abundance of SHs in the genera *Filobasidium*, *Vishniacozyma*, *Naganishia* and *Dioszegia* (Tremellomycetes) as well as *Buckleyzyma* (Cystobasidiomycetes), which are all basidiomycete yeasts, increased from the early to late season. The seasonal changes in relative abundance of SHs in the Ascomycota phylum, most of which were endophytes/pathogens, varied strongly among species. For example, *Venturia ditricha* and *Cytospora quercicola* decreased, while SHs in the genus *Taphrina* increased in relative abundance during the growing season (Figs 3, S5). Several SHs had a peak in their relative abundance in the middle of the season, for example *Mycosphaerella tassiana*, *Apiognomonia errabunda* and the genus *Erysiphe* (Fig. S5). The effect of warming varied among SHs. For example, in the early season, warming had a negative effect on the abundance of the endophyte *Ramularia endophylla* (Fig. S6a). In the middle of the season, warming had a negative effect on SHs in the yeast genera *Filobasidium*, *Vishniacozyma* and *Dioszegia*, while it had a positive effect on the abundance of *Buckleyzyma aurantiaca* and *Naganishia* sp. (Fig. S6b). In the late season, *A. errabunda*, *Erysiphe* sp., *Aureobasidium pullulans*, *Naganishia* and *Filobasidium* had a higher relative abundance in the heated cages, while the relative abundances of the SHs in the genera *Dioszegia* and *Vishniacozyma* were lower (Fig. S6c). Overall, the relative abundance of yeasts increased during the growing season, whereas the relative abundance of putative fungal pathogens decreased (Fig. 4; Table S7). Warming decreased the relative abundance of putative fungal pathogens in

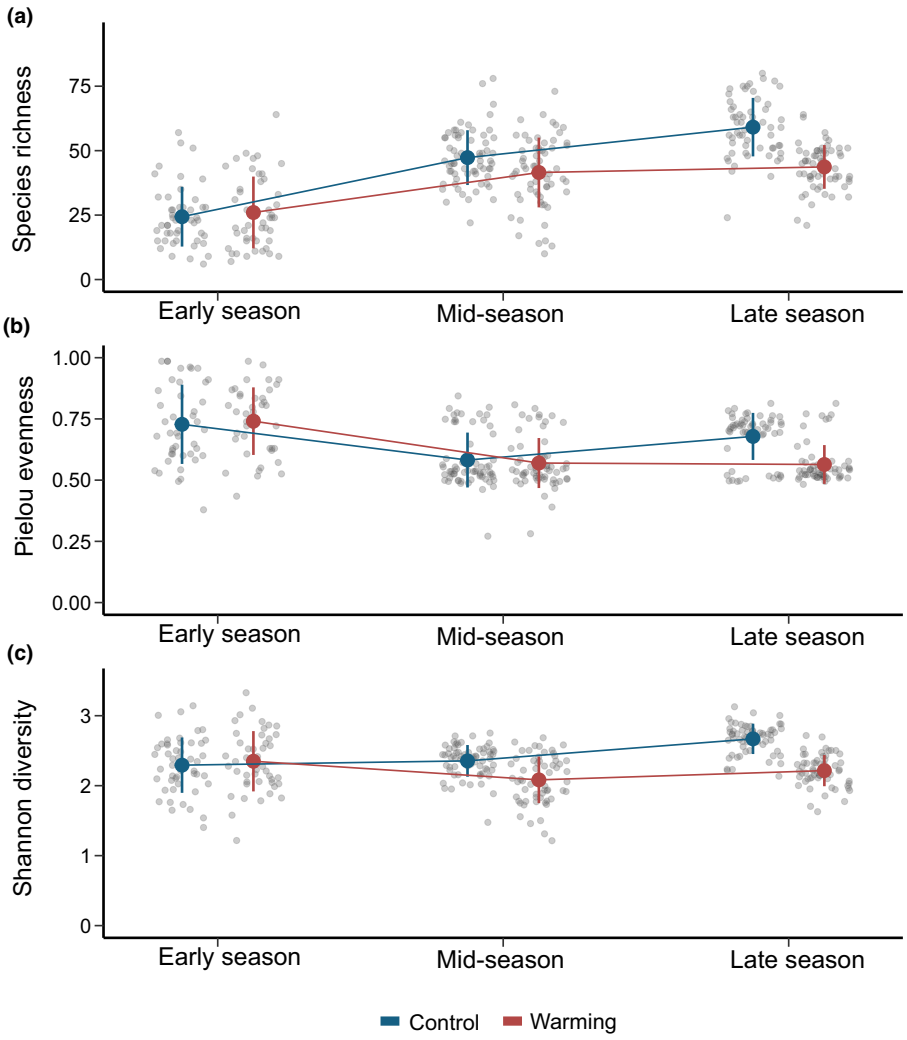

Fig. 1 The impact of warming on the foliar fungal community on the pedunculate oak *Quercus robur* during the growing season. Shown are the effect of warming on species richness (a), Pielou evenness (b) and Shannon diversity (c) in the early, middle and late seasons. The large circles represent the mean values and the error bars represent standard deviations. The small circles represent raw data points (total $n = 340$), which are horizontally jittered to avoid overlap.

the early and late seasons, but not in the middle of the season (Fig. 4; Table S7).

Discussion

In this study, we investigated the impact of warming and tree genotype on the foliar fungal community across one growing season. Fungal species richness increased, evenness tended to decrease, and community composition shifted during the growing season. Yeasts increased, whereas putative fungal pathogens decreased, in relative abundance during the growing season. Warming decreased species richness, reduced evenness and changed community composition, especially towards the end of the growing season. Warming also negatively affected the relative abundance of putative fungal pathogens. When partitioning the total β diversity into its components of turnover and changes in species richness, we found that the differences in the fungal community composition between the early, middle and late seasons, and between the control and warming treatments, were mainly explained by species turnover. We detected a small effect of tree genotype on fungal community composition across the growing season and a weak interactive effect of warming and tree genotype on species richness at the end of the growing season. Overall, warming played a larger role than plant genotype in structuring the leaf foliar community. Based on these findings, we predict that climate warming will decrease fungal species richness and change leaf fungal community composition, especially towards the end of the growing season.

Seasonal dynamics of the foliar fungal community

Our observation that species richness increased and evenness decreased during the growing season is in line with several previous studies (Unterseher *et al.*, 2007; Jumpponen & Jones, 2010), but contrasts with other studies that have observed decreasing fungal species richness towards the late season (Guo *et al.*, 2008; Chen *et al.*, 2020). We also detected a shift in community composition, mainly in the form of species turnover, rather than gain of new species. A strong change in community composition is consistent with previous studies, which demonstrated a pronounced shift in the foliar fungal and bacterial community composition during the growing season (Ercolani, 1991; Ding & Melcher, 2016). Studies on the fungal community composition of bur oak (*Quercus macrocarpa*) and Norway spruce (*Picea abies*)

Table 2 The impact of warming (W), tree genotype (G), season (S) and their interactions on the foliar fungal community of the pedunculate oak *Quercus robur* as modelled using linear mixed models for species richness, evenness and diversity, and PERMANOVA of community composition (presence–absence and absolute count data).

	Warming (W)		Genotype (G)		Season (S)		W × G		W × S		G × S		W × G × S		$\sqrt{\text{Read count}}$	
	P	R^2	*P*	R^2	*P*	R^2	*P*	R^2	*P*	R^2	*P*	R^2	*P*	R^2	*P*	R^2
Richness ($n = 340$)	**< 0.001**	0.04	**0.010**	0.01	**0.012**	0.41	**0.043**	0.07	**< 0.001**	0.50	0.826	-	**0.037**	0.52	**< 0.001**	0.76
Evenness ($n = 340$)	**0.001**	0.02	0.481	-	**< 0.001**	0.27	0.910	-	**< 0.001**	0.32	0.965	-	0.565	-	**< 0.001**	0.23
Diversity ($n = 340$)	**< 0.001**	0.12	**0.041**	0.02	**< 0.001**	0.07	0.225	-	**< 0.001**	0.26	0.295	-	0.447	-	**0.006**	0.03
Community composition (presence–absence, $n = 340$)	**0.001**	0.02	**0.001**	0.01	**0.001**	0.08	**0.001**	0.01	**0.001**	0.02	0.112	-	0.345	-	**0.001**	0.03
Community composition (absolute count, $n = 340$)	**0.001**	0.02	**0.001**	0.01	**0.001**	0.10	**0.001**	0.01	**0.001**	0.02	0.243	-	0.189	-	**0.001**	0.08

Square-root-transformed read count was included as a covariate in all models to account for variation in sequencing depth. Shown are *P*-values and R^2-values for species richness, evenness, diversity and community composition. For the univariate models, we calculated the marginal R^2 using the function 'r.squaredGLMM' in the MuMIn package (Bartoń, 2020), by running separate models with the same random effect structure but only a single fixed effect included. The R^2 for the community composition models are partial-R^2 calculated on marginal models, which keep constant all variables except the one used to calculate the partial-R^2 associated with the variable (or interaction) considered (Legendre *et al*., 2011). Test statistics and degrees of freedom are reported in Supporting Information Table S4. Significant estimates ($P < 0.05$) are shown in bold.

also found that changes in community composition were mainly a result of species turnover (Jumpponen & Jones, 2010; Haas *et al*., 2018).

The strong seasonal changes of the foliar fungal community might be linked to the physical and chemical changes associated with leaf ageing and seasonal changes in weather conditions (Osono, 2008; Jumpponen & Jones, 2010; Fort *et al*., 2016). For example, increased moisture at the leaf surface and damage to the protective cuticle of the leaves may explain the increase of yeast populations during the growing season, as they may benefit from the leakage of nutrients (Inácio *et al*., 2002; Glushakova & Chernov, 2004, 2007; Kemler *et al*., 2017). As one example, *Aureobasidium*, which is known to be involved in the early decomposition of leaves and can grow as a yeast and as filamentous mycelium, increased in abundance during the growing season in this and previous studies (Sadaka & Ponge, 2003; Jumpponen & Jones, 2010). In contrast to yeasts, fungal pathogens decreased in relative abundance from the early to the late season. For example, both the oak powdery mildew *Erysiphe* sp. and the oak leaf spot pathogen *A. errabunda* decreased towards the end of the growing season. Reasons for the decline in pathogens towards the end of the season might include increased competition with yeasts, but also higher amounts of resistance of older leaves (Develey-Rivière & Galiana, 2007). Taken together, our findings show a shift in the fungal community composition, as well as in the relative abundance of functional guilds, across the growing season.

The impact of warming and tree genotype on the foliar fungal community

Our experimental warming decreased fungal species richness and reduced evenness on oaks in the middle and late seasons. Interestingly, Bálint *et al*. (2015), in the only other study experimentally heating the foliar fungal community on trees, also reported a decrease in fungal richness and evenness on balsam poplar under warmer temperatures, and this consistency provides a first indication that the pattern may be general. We further found that warming influenced the fungal community composition, with a particularly strong effect towards the end of the growing season. In line with this finding, several observational studies have reported shifts in the composition of foliar fungal communities along altitudinal and elevational gradients, which are often used as proxies for changes in climate (Hashizume *et al*., 2010; Cordier *et al*., 2012; Davey *et al*., 2013; Coince *et al*., 2014; Whitaker *et al*., 2018; Abrego *et al*., 2020a,b; Cai *et al*., 2020). However, findings along such gradients are often confounded by concurrent changes in abiotic and biotic environmental conditions. From the three experimental warming studies to date that measured community composition, Bálint *et al*. (2015) found a change in the foliar fungal community on balsam poplar with warming, whereas Kazenel *et al*. (2019) and Kivlin & Rudgers (2019) did not find an effect of long-term warming on the foliar fungal community composition of three perennial grass species (*Achnatherum lettermanii*, *Festuca thurberi* and *Poa pratensis*). Among the functional groups that changed in relative abundance, the negative effect of warming on putatively pathogenic fungi appears particularly interesting. While several empirical studies have reported a lower disease intensity with experimental warming (Pautasso *et al*., 2012; Siebold & von Tiedemann, 2013), the current finding contrasts with the general expectation that climate warming will increase pathogen growth and transmission (Harvell *et al*., 2002; Launay *et al*., 2014; Liu *et al*., 2019). Clearly, it is hard to predict how plant pathogens will be affected by climate warming in the future. Nonetheless, such studies, conducted in a range of study systems, may bring us a

Table 3 The impact of warming (W), tree genotype (G) and their interaction on the foliar fungal community of the pedunculate oak *Quercus robur*, estimated separately for the early, middle and late seasons.

	Warming (W)		Genotype (G)		W × G		√Read count	
	P	R^2	*P*	R^2	*P*	R^2	*P*	R^2
Early season (June 16)								
Richness (n = 94)	0.943	-	0.051	-	0.877	-	**< 0.001**	0.68
Evenness (n = 94)	0.761	-	0.888	-	0.951	-	**< 0.001**	0.26
Diversity (n = 94)	0.611	-	0.088	-	0.643	-	0.153	-
Community composition (presence–absence, n = 94)	**0.040**	0.02	**0.011**	0.05	0.273	-	**0.001**	0.07
Community composition (absolute count, n = 94)	**0.001**	0.03	0.128	-	0.421	-	**0.001**	0.18
Mid-season (July 17)								
Richness (n = 128)	**0.002**	0.05	0.150	-	0.131	-	**< 0.001**	0.65
Evenness (n = 128)	0.352	-	0.660	-	0.592	-	**0.003**	0.06
Diversity (n = 128)	**< 0.001**	0.19	0.290	-	0.421	-	**0.013**	0.05
Community composition (presence/absence, n = 128)	**0.001**	0.05	**0.017**	0.03	0.722	-	**0.001**	0.06
Community composition (absolute count, n = 128)	**0.001**	0.05	**0.006**	0.04	0.147	-	**0.001**	0.26
Late season (September 7)								
Richness (n = 118)	**< 0.001**	0.38	0.475	-	**0.006**	**0.20**	**< 0.001**	0.36
Evenness (n = 118)	**< 0.001**	0.30	0.438	-	0.314	-	0.611	-
Diversity (n = 118)	**< 0.001**	0.50	0.683	-	**0.049**	0.12	0.387	-
Community composition (presence–absence, n = 118)	**0.002**	0.10	**0.029**	0.04	0.543	-	**0.001**	0.04
Community composition (absolute count, n = 118)	**0.001**	0.16	0.575	-	0.217	-	**0.001**	0.18

Shown are results from linear mixed models for species richness, evenness and diversity and PERMANOVA of community composition (presence–absence and absolute count data). Square-root-transformed read count was included as a covariate in all models to account for variation in sequencing depth. Shown are *P*-values and R^2-values for species richness, evenness, diversity and community composition. For the univariate models, we calculated the marginal R^2 using the function 'r.squaredGLMM' in the MuMin package (Bartoń, 2020), by running separate models with the same random effect structure but only a single fixed effect included. The R^2 for the community composition models table are partial-R^2 calculated on marginal models, which keep constant all variables except the one used to calculate the partial-R^2 associated with the variable (or interaction) considered (Legendre *et al*., 2011). Test statistics and degrees of freedom are reported in Supporting Information Table S5. Significant estimates ($P < 0.05$) are shown in bold.

step forward in understanding the disease outbreaks and invasions under a warmer climate. Overall, our findings raise at least five major questions for future research:

(**1**) Does the response of the foliar fungal community to warming differ among plant functional types, as suggested by the differences between the few existing studies on trees and grasses (Table 1 and this study)?

(**2**) Is the response of the foliar fungal community to warming a direct effect of temperature on fungal growth and physiology, or is the response mediated by changes in physical leaf traits, nutrient content and secondary chemistry?

(**3**) Do fungal guilds consistently differ in their response to warming?

(**4**) How does the increasing difference in species richness, evenness and community composition between the warming and control treatment during the growing season in a given year affect the seasonal dynamics of the foliar fungal community in the subsequent year?

(**5**) Do other climatic factors, such as soil moisture and precipitation, interact with elevated temperature in shaping foliar fungal communities?

In addition to warming, fungal community composition differed among tree genotypes, explaining ≤ 5% of the variation in the fungal community composition during the early, middle and late growing seasons. Similarly, Qian *et al*. (2018) and Wagner *et al*. (2016) demonstrated empirically that the foliar fungal community composition differed among genotypes of both the shrub *Mussaenda pubescens* and the perennial mustard *Boechera stricta*. We did not detect an interactive effect between warming and tree genotype on the fungal community composition on oak. In contrast to the foliar fungal community, fungal species richness, evenness and diversity did not differ significantly among the oak genotypes during any part of the growing season. However, we detected a weak interactive effect of warming and tree genotype on species richness and diversity during the late season. Contrary to these findings, several studies have shown strong interactive effects of plant genotype and warming on fungal community composition on plants (Table 1). At this stage, it is unclear whether these differences among studies are a result of differences in the experimental setup, host species studied or the spatial scale from which tree genotypes were collected (Tack *et al*., 2012). A promising avenue for future research would be to conduct

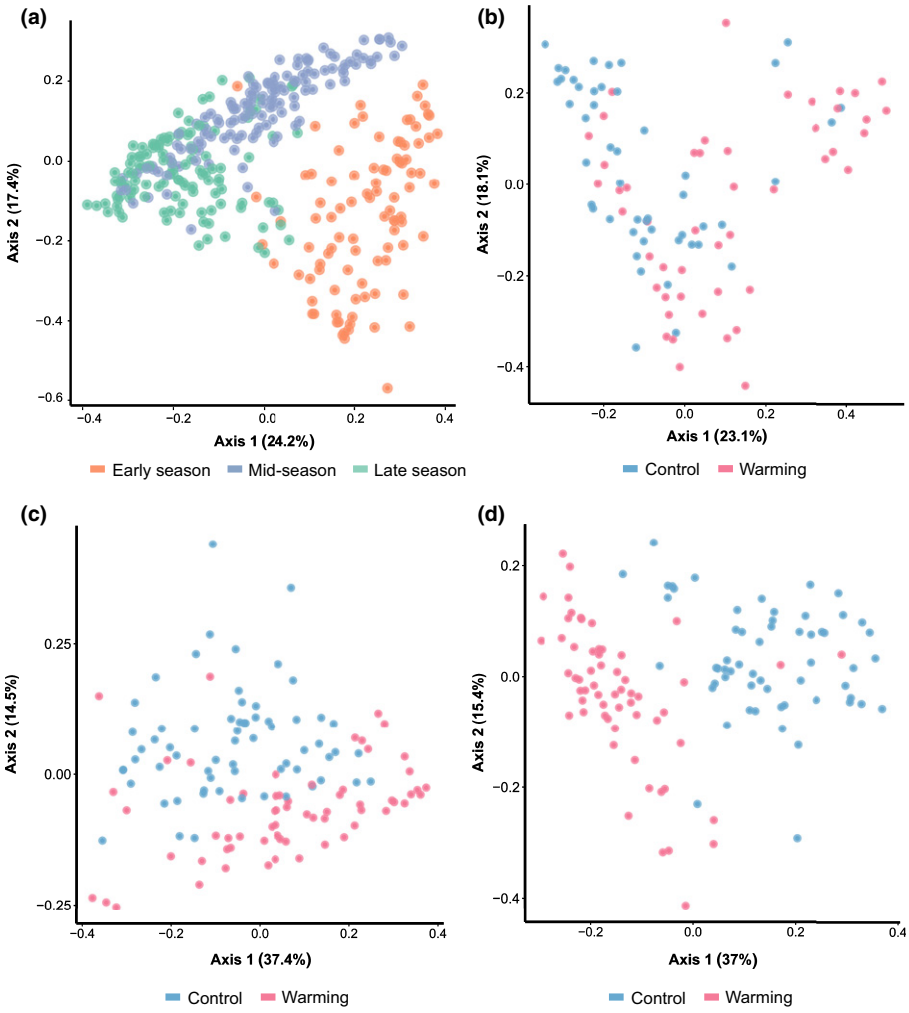

Fig. 2 The impact of warming on the foliar fungal community on the pedunculate oak *Quercus robur* during the growing season. (a) Difference in community composition between the early, middle and late seasons. (b–d) Impact of warming on the fungal community composition in the early, middle and late seasons, respectively. Visualization is based on principal coordinate analysis using Bray–Curtis metrics.

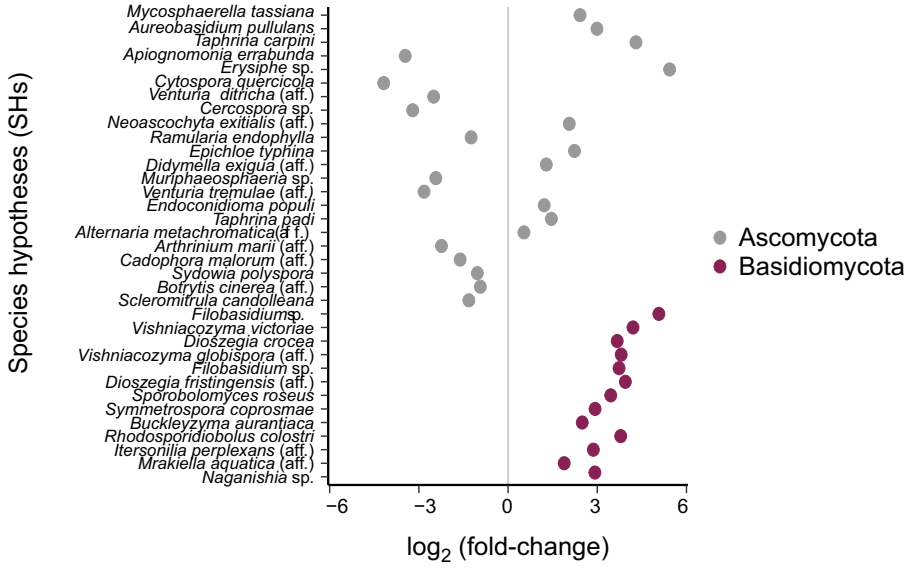

Fig. 3 The change in relative abundance of ascomycetous and basidiomycetous species hypotheses (SHs) between the early and late seasons. A negative $\log_2$(fold-change) shows that SHs were significantly more abundant in the earlier part of the season, while a positive $\log_2$(fold-change) shows SHs that were significantly more abundant in the later part of the season. The SHs are ordered within each phylum by their relative abundance. Shown are SHs identified to genus or species level. For the full list of species, including unidentified SHs, see Supporting Information Tables S3 and S5.

experimental studies involving several tree species and spanning a range of genetic identities, thereby exploring which specific plant traits may explain genetic variation in foliar fungal community composition.

Conclusions

We observed a strong seasonal change in foliar fungal community composition, with basidiomycetous yeasts gradually replacing

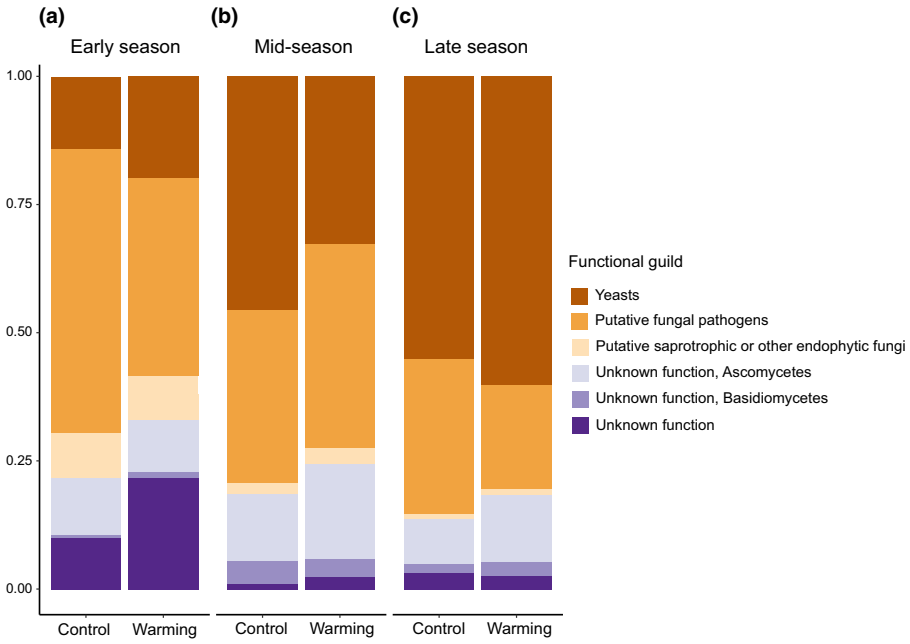

Fig. 4 The distribution of fungal functional guilds in the warming and control treatments in the early (a), mid- (b) and late seasons (c). The relative abundance of each fungal guild in each sample is presented as the ratio between the added number of reads of all species hypothesis in the guild and the total number of reads.

ascomycetous endophytes and pathogens towards the end of the growing season. We also found that experimental climate warming had a major impact on the seasonal dynamics of foliar fungal communities, whereas tree genotype and warming × genotype interactions explained only a minor part of the variation. These findings have important implications for predicting the eco-evolutionary dynamics of leaf fungal communities and their hosts under changing climate conditions. From an ecological perspective, we may expect a decrease in the foliar fungal species richness and a change in community composition, with implications for plant health, interactions between plants and higher trophic levels, and ecosystem functions and services (e.g. litter decomposition). Given the range of responses detected, warming may impose some rather complex changes in community composition. There may then be no single, general direction that pathogens will take in a warmer future – and this complexity in itself seems a message as important as any. From an evolutionary perspective, the weak effect of plant genotype and warming × genotype interactions makes it unlikely that the evolutionary response of oak trees to climate-mediated changes in the foliar fungal communities and warming will keep pace with climate warming.

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Author contributions

MF, AJMT and TR conceived and designed the experiment. MF conducted the empirical work. MF carried out the molecular work. MF, AA and BDL conducted the bioinformatic analyses. MF analysed the data with support from AA, CV, PH, BDL, FGB and AJMT. MF wrote the first draft and all authors contributed to the final manuscript.

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Data availability

Data are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.2jm63xsp9.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Rarefaction curves for fungi in leaves.

Fig. S2 Principal coordinate analysis (PCoA) showing the impact of warming on the foliar fungal community of *Quercus robur* during the growing season.

Fig. S3 Partitioning of the total β diversity (βJAC) between the parts of the season into the components of species turnover (βJTU) and change in species numbers (βJNE).

Fig. S4 Partitioning of the total β diversity (βJAC) between the treatments in each part of the season into the components of species turnover (βJTU) and change in species numbers (βJNE).

Fig. S5 The change in the relative abundance of fungal species hypotheses (SHs) between the parts of the season.

Fig. S6 The change in the relative abundance of fungal species hypotheses (SHs) between the treatments in each part of the season.

Methods S1 Detailed description of molecular methods and bioinformatics.

Table S1 Definitions of functional guilds among fungal taxa identified in the leaves of *Quercus robur*.

Table S2 Species hypotheses (SHs) identifications, functional assignment and representative sequences.

Table S3 A summary of the models and model terms fitted for analyses.

Table S4 Test statistics and degrees of freedom from ANOVA and PERMANOVA analyses of the impact of warming, tree genotype, season and their interactions on the foliar fungal community of *Quercus robur*.

Table S5 Test statistics and degrees of freedom from ANOVA and PERMANOVA analyses of the impact of warming, tree genotype and their interaction on the foliar fungal community of *Quercus robur*.

Table S6 List of differentially abundant taxa between different parts of the season as identified by differential abundance analysis (DESeq2).

Table S7 Test statistics, degrees of freedom and *P*-values from the impact of warming, tree genotype, season and their interactions on the relative abundance of fungal functional guilds.

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