

We would like to thank the reviewers for taking the time to review our manuscript, and for their thoughtful and constructive comments. Their feedback has been invaluable in enhancing the clarity, rigour and overall quality of our work.

Below, we provide detailed, point-by-point responses to each of the reviewer's comments.

We have also uploaded the final version of the manuscript, figures, and supplementary files as attached documents.

Specific comments Reviewer 1

Specific comments

Materials and methods. The criteria and distance between the tree gaps are not mentioned (line 127). This may be relevant to support or discuss the lack of differences.

We thank the reviewer for highlighting this detail. The distance between the sampling stations (locations), which is now included in the article (Materials and methods section and as new supplementary figure 3.3), was only about 40 to 60 metres. Since these distances are not very great—unlike, for example, distances measured in kilometers—this could be one of the reasons why no differences were observed. We address this point in the Discussion section.

Three different sets of primers were employed for NGS libraries. Is a novel approach? If not, please cite previous works. It is not specified whether the sequences were or not pooled. I assume they were, but please clarify. Also, if this is a novel approach, the differences found in richness or diversity between them should be mentioned and a recommendation for the specific set of primers yielding the best resolution.

We thank the reviewer for this comment. The use of multiple primer pairs in metabarcoding studies is not novel and has been previously proposed to improve taxonomic coverage (e.g. Tedersoo et al., 2015). In our study, three primer pairs were selected based on this work to maximise the detection of fungal diversity while maintaining cost efficiency. All PCR reactions were performed on the same DNA extract, and the resulting amplicons were pooled prior to sequencing. Because amplicons from different primer pairs were not individually barcoded, it was not possible to assess or compare the performance of each primer combination in terms of richness or diversity. Therefore, no primer-specific comparisons or recommendations can be made for our samples. We have clarified these points in the

Methods section, including the papers that have previously demonstrated the efficiency of the primers used here.

For the altitudes employed, I doubt about the relevance of the mass air trajectories and the accuracy, especially within a forest. Please, discuss and justify.

We thank the reviewer for this comment. We agree that, at the sampling heights considered and within a forested environment, the accuracy and relevance of backward air mass trajectories are limited, and local processes are likely to dominate. In our study, trajectory analysis was used only as a qualitative assessment to verify whether sampling heights were exposed to comparable air masses, and thus to support the decision to merge samples across heights. It was not used to infer precise source regions or transport processes. We have clarified this point in the Discussion.

Results. A figure or table indicating the taxa of the core mycobiome would be appreciated and relevant for future comparable studies.

We thank the reviewer for this comment. We have now added Table S3.4.

Figure 3 can be improved (legend names are cut and units are not necessary).

Done, legend names are written in full and units have been removed.

A statistical test must be added as support for Figure 4 and statement in lines 273-275.

We thank the reviewer for this comment. We have now added to the text: "Differences in relative abundance between warm and cold months were assessed using a Wilcoxon rank-sum test. Ascomycota showed significantly higher relative abundance during the warm season, whereas Basidiomycota was significantly more abundant during colder months (Wilcoxon test, $p = 6.68 \times 10^{-5}$ and $p = 2.0 \times 10^{-6}$, respectively)."

Line 283. ..." in several other studies". Discuss the relevance (similar or different environment?). In addition, this and many lines in page 11 are more suitable for the Discussion section.

We thank the reviewer for their comment, we have now moved these lines to the Discussion and clarified this point in this way: "In line with this, the dominance of genera such as *Cladosporium*, *Alternaria*, and *Epicoccum* observed in this study is consistent with previous works conducted in temperate environments, where these taxa are commonly reported as major components of airborne fungal communities (Almaguer et al. 2014; Sadyś et al. 2015; Akgül et al. 2016; Grinn-Gofroń et al. 2018; Antón et al. 2019; Ščevková and Kováč 2019; Grinn-Gofroń et al. 2020). These three genera are also some of the best-known sources of allergic reactions and plant diseases (Bavbek et al. 2006; Abuley and Nielsen 2017; Nowakowska et al. 2019; Grinn-Gofroń et al. 2020). Their increased abundance during the

summer months supports the hypothesis that Ascomycota are favoured under warmer conditions, likely reflecting enhanced sporulation and dispersal during these periods.

In contrast, members of Basidiomycota, particularly within the class Tremellomycetes, were more prevalent during colder periods, indicating a seasonal pattern opposite to that observed for Ascomycota. The detection of genera such as *Vishniacozyma* and *Itersonilia*, both associated with plants as endophytes or pathogens (Liu et al., 2025; Gandy, 1966), further supports the role of surrounding vegetation as a source of airborne fungal propagules.

At a finer taxonomic resolution, the species *Vishniacozyma tephrensensis*, characterised as psychrotolerant and psychrophilic, was predominantly detected during colder periods, with higher abundance in late autumn. Its occurrence under these conditions reflects its tolerance to low temperatures and environmental stress (Vishniac 2002; Wei et al., 2022). In this context, it is worth noting that, while environmental factors such as temperature, humidity, precipitation, and wind speed...”

Discussion. Paragraph 408-429 can be shorter.

Done, the paragraph has been revised and shortened to improve clarity and readability, while adding key ecological interpretations to address reviewer’s 2 comments on seasonality. The paragraph now reads as follow: “In this context, the detection in our dataset of the plant pathogen *Erysiphe alphitoides*, a widespread pathogen causing oak powdery mildew in Europe (Marçais et al. 2014; Tăut et al. 2024), is consistent with the presence of suitable host plants (*Quercus* spp.) and likely reflects seasonal patterns in host phenology and canopy development (Andrew et al. 2017). More broadly, the occurrence of multiple phytopathogenic taxa, including species such as *Zymoseptoria tritici*, which affects wheat plantations (Torriani et al., 2015), suggests that surrounding vegetation and nearby agricultural areas represent potential sources of airborne fungal propagules. These observations indicate that seasonal changes in plant communities and phenology play a key role in shaping airborne fungal assemblages, alongside the influence of meteorological variables. In addition, it is important to note that climate change may further modulate these patterns by altering host plant phenology (Piao et al., 2019). In this context, we argue that disentangling the effects of meteorological conditions from broader seasonal dynamics remains challenging, as previously reported (Cáliz et al. 2018; Bowers et al. 2012), and that more robust assessments of these drivers require multi-year sampling.

Interestingly, the detection of taxa that have been previously reported primarily outside Europe e.g. *Alanphillipsia aloetica* from South Africa (Crous et al., 2013), further highlights the

complexity of airborne fungal communities and the potential for long-distance dispersal. Taken together, these findings provide a baseline for assessing temporal dynamics and the impacts of environmental change in airborne fungal assemblages, thereby corroborating the role of this study as an important benchmark for future works.”

Lines 134-139 are confusing. Please simplify.

We thank the reviewer for this comment, this has now been simplified to: “For this study, we analysed one week of samples for each month between March 2019 and February 2020 (Table S2.2). Specifically, the 4th week of each month was selected due to logistical and financial constraints and to ensure comparability with meteorological records across all months”.

Line 96. ... “we would expect?”

This has now been changed with: “only limited divergence in the composition of fungal aerosols collected within the canopy layer is expected.”

Lines 334, 335: italics for the cited genera.

Done.

Figure S2. A mix of languages in the figures

We thank the reviewer for this comment, this was changed to English.

Technical details – reviewer 2

General comment

We thank the reviewer for this thoughtful and constructive comment. We agree that seasonal dynamics, including vegetation phenology and fungal life cycles, are likely to play a major role in shaping airborne fungal communities, alongside meteorological variables. In response, we have revised the Discussion to more explicitly acknowledge the contribution of seasonal processes and to clarify that the observed patterns likely reflect an interplay between meteorological conditions and broader seasonal dynamics. We have also expanded the ecological interpretation of key taxa. In particular, we now discuss the occurrence of phytopathogenic species such as *Erysiphe alphitoides* in relation to host plant phenology, and interpret the detection of *Zymoseptoria tritici* as likely reflecting nearby agricultural sources. Additionally, we have moderated statements regarding taxa with uncertain biogeographic distributions (e.g. *Alanphillipsia aloetica*) by emphasising the potential role of long-distance dispersal and limitations in species-level taxonomic resolution.

Finally, we now explicitly acknowledge that disentangling the direct effects of meteorological variables from broader seasonal processes remains challenging within the scope of a single-year study, and that multi-year sampling would be required for a more robust assessment. These revisions align the manuscript more closely with the seasonal patterns evident in the data, while maintaining the relevance of meteorological drivers.

We provide detailed, point-by-point responses to each of the reviewer's comments.

We have also uploaded the final version of the manuscript, figures, and supplementary files as attached documents.

Line 145-152: Please list the primer pairs in a table and indicate which ITS region they are amplifying

We thank the reviewer for raising this point. We have clarified in the Material and Methods section that multiple primer pairs targeting different portions of the fungal ITS region were used. As a result, the dataset includes sequences from both ITS1 and ITS2 regions, and we have revised the text accordingly to refer more generally to the fungal ITS region. The text reads now as: "The fungal internal transcribed spacer (ITS) region was amplified using three different primer combinations (Table 1). These were selected based on previous work aiming to maximise fungal taxonomic coverage (Tedersoo et al., 2015; White et al., 1990). PCR reactions were performed on the same DNA extract, and the resulting amplicons were pooled prior to sequencing (see below for specific details). Because amplicons

from different primer pairs were not individually barcoded, it was not possible to assess primer-specific differences in richness or diversity.”

We have also added a table listing all primer pairs used and indicating the corresponding fungal ITS region amplified by each pair (Table 1).

While the use of multiple primer pairs may introduce some amplification bias, this does not affect the main ecological patterns reported, which are based on relative differences in community composition across samples.

Line 188: How was the data normalized?

We thank the reviewer for this comment. We have clarified that normalisation was performed using total-sum scaling (relative abundance transformation), whereby ASV counts were divided by the total number of reads per sample and expressed as percentages.

Line 233: To stay consistent “areas” should probably be changed to “sources”

Thank you, this has now been changed to “sources”.

Line 218: it says that data is grouped into 760 ASVs, according to Table S3 1,877 ASVs were identified (Table S3). Please explain this discrepancy.

We thank the reviewer for pointing out this apparent discrepancy. Table S3 reported the total number of ASVs identified after removal of contaminants (1,877 ASVs). In contrast, the 760 ASVs reported in line 218 correspond to the subset retained after additional filtering steps applied prior to downstream analyses. Specifically, ASVs with low abundance (≤ 2 reads) and low prevalence (present in less than 1% of samples) were removed to reduce noise and improve comparability among samples. This filtering step substantially reduced the number of ASVs while retaining the vast majority of sequence reads. We have clarified this distinction in the revised manuscript (new text: “Our quality-filtered dataset consisted of 1,421,177 fungal sequencing reads, with an average of 13,198 reads per sample. After removal of contaminants, 1,877 ASVs were identified. Following additional filtering of low-abundance and low-prevalence ASVs, 760 ASVs were retained for downstream analyses (Table S3.1).”) and modified the Table S3.1 accordingly.

Table S3: Column (sample?) labeling should be clarified, month and/or dates should be added. Heights and locations should be explained. It is unclear why some entries are highlighted or colored.

We thank the reviewer for this comment. We have clarified the sample naming convention in the caption of Table S3. Sample identifiers now follow the format *tXhY_YYMM*, where *tX* indicates the sampling station location, *hY* the sampling height (3 m, 15 m, and 28 m), and *YYMM* the last two digits of the year and the month of sampling. An example (*t1h1_1903*) has been included in the caption of the table for clarity. Conditional formatting has been disabled from the entire sheet.

In Figure 2, it is unclear why the correlation between wind speed and Shannon diversity is not significant, while the correlation with Gini-Simpson diversity is significant. Shannon and Gini-Simpson indices are highly correlated. This may need some discussion.

We thank the reviewer for this comment. We agree that Shannon and Gini-Simpson indices are strongly related and, in our dataset, both showed a positive correlation with wind speed. However, the two tests yielded slightly different significance levels: Shannon (Spearman's $\rho = 0.56$, $p = 0.058$) and Gini-Simpson (Spearman's $\rho = 0.61$, $p = 0.034$). We therefore interpret this as a borderline difference around the significance threshold rather than a biological contradiction. Because the analyses were conducted on monthly pooled data, the sample size was limited, and small differences in index formulation and rank structure were sufficient to place one result just above and the other just below $p = 0.05$. This has been clarified in the main text.

Figure S1: what does the different colours of the trajectories indicate?

Explanation **is** **needed**

We thank the reviewer for this comment. We have clarified in the caption of Figure S1 that colours represent trajectories corresponding to different days of the sampling period.