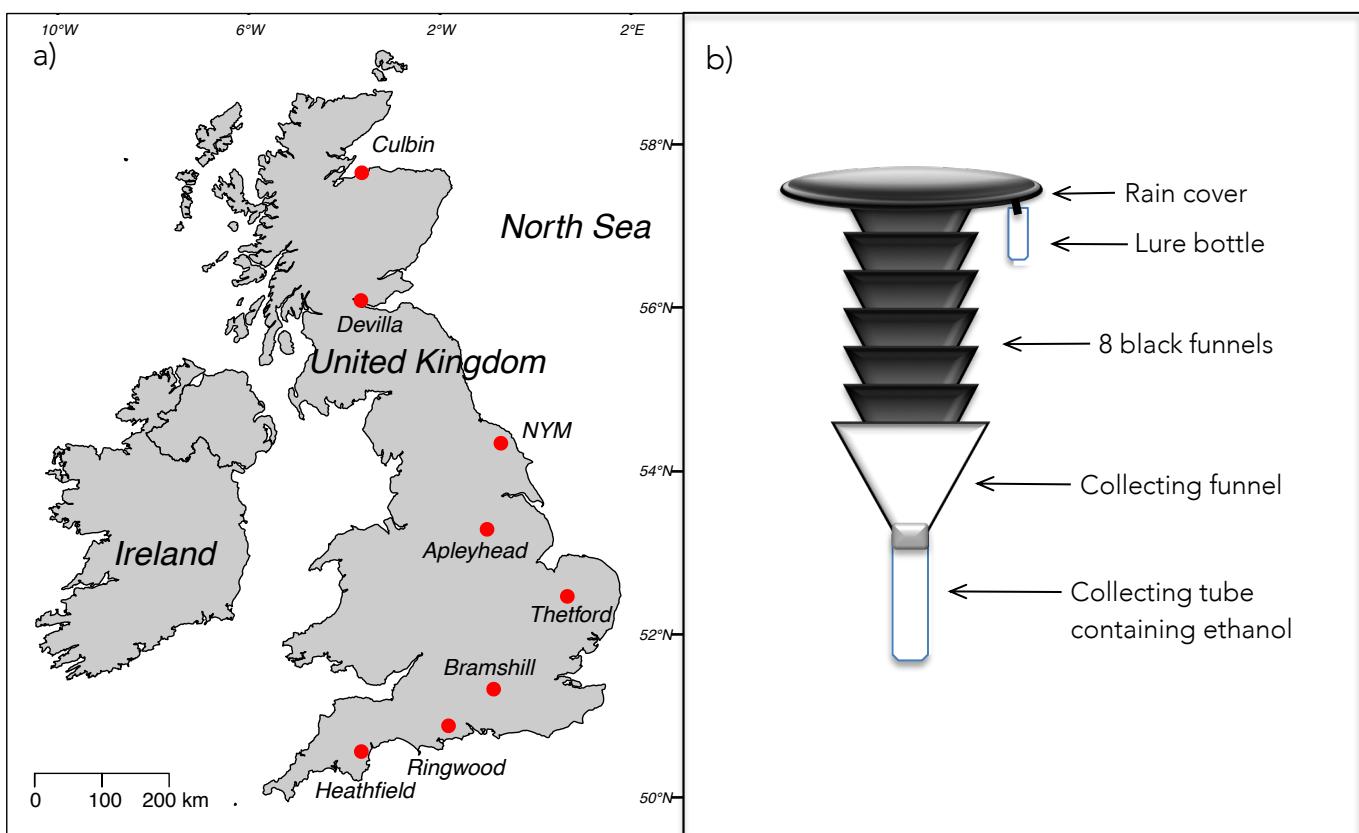


## SUPPLEMENTARY MATERIALS

S1. Geographic coordinates and stand information for each sampling site. Temperature and precipitation data from Worldclim v. 1.4 (Hijmans et al., 2005).

Locality	Latitude	Longitude	Pine Mix	Year Planted	Age	Average Temperature Annual Range (°C)	Average Annual Precipitation (mm)
'Heathfield' Great Plantation, Heathfield, Devon	50.56724	-3.66137	Corsican Pine	1962	51	18.2	898
'Ringwood' Ringwood Forest, Hampshire	50.88228	-1.83588	Corsican pine	1970	43	20.7	795
'Bramshill' Warren Heath, Bramshill Plantation, Hampshire	51.33112	-0.89135	Corsican and Scots Pine mix	1975	38	22.3	772
'Thetford' Cold Harbour, Thetford Forest, Norfolk	52.46647	0.65171	Corsican and Scots Pine mix	1969	44	21.2	595
'Apleyhead' Apleyhead Wood, Sherwood, Nottinghamshire	53.28774	-1.02952	Corsican pine	1977	40	20.4	641
'North York Moors' Pickering Moor, North Yorks (NYM)	54.34114	-0.73999	Scots Pine	1953	60	19.0	832
'Devilla' Devilla Forest, Kincardine, Falkirk	56.08857	-3.66886	Scots Pine	1958	55	19.4	890
'Culbin' Culbin Forest (Forres), Moray	57.65219	-3.65094	Corsican pine	1952	61	19.2	647



S2 (a) Map of sampling sites, (b) Schematic diagram of Lindgren multiple funnel trap for collecting bark beetles.

**Text S3: Details of sampling design**

Traps were suspended from cord strung between two trees and surrounding low-level vegetation was cut back to increase trap visibility. Plastic tarpaulins were erected above traps to reduce dilution of preserving liquid by rainwater and to prevent leaves and detritus from falling into the preservative. Alpha-Pinene is a monoterpenes compound produced by pine trees when damaged and is attractive to bark beetles (Kalinova et al. 2014).

**Text S4: Test for an effect of storage vial**

Cross-contamination between specimens stored within the same vial before DNA extraction has the potential to obscure patterns of OTU richness and composition between specimens. As such, we tested for an effect of the collection vial on the similarity of beetle mycobiota. Since each vial contained all specimens from a given site from a given week, it is expected that there would be a significant effect due to meaningful spatiotemporal variation. However, we employed PERMANOVAs and variance partitioning to divide this variation between the vial itself, the week and the site. The results of this analysis suggest that the storage vial that each specimen was stored in explained no variance independently from the week of collection. All explained variance was either shared between week and site (49.7% of explained variance) or week and vial (50.3% of explained variance) so it appears that the week of collection had the strongest effect of these three predictors and was included in the main analysis as a random effect. So, while cross-contamination cannot be ruled out entirely, we are confident that the effect on our results and conclusions was negligible.

**Text S5: Details of PCR cycling conditions and sequencing**

Each of the three PCR amplifications for ITS2 contained 2 µl template DNA, 1.6 µl dNTPs, 0.08 µl Bioline BioTaq polymerase , 4 ul MgCl<sub>2</sub>, and 0.5 µl of forward and reverse primers in a 40 µL reaction volume. The following cycling conditions were used: initial denaturation at 94°C for 300 seconds, then 30 cycles of denaturation at 94 °C for 60 seconds, annealing at 50/53/56°C for 60 seconds, extension at 72 °C for 30 seconds, and a final extension at 72 °C for 120 seconds. To confirm amplification was successful

all PCR products were visualized using GelRed<sup>TM</sup> (Biotium) on a 1% agarose gel. Purification of PCR products was done using DNA Clean & Concentrator<sup>TM</sup> (Zymo Research). To determine the concentration of double-stranded DNA present, cleaned PCR products were quantified using a Qubit 2.0 Fluorometer with the Qubit dsDNA HS Assay Kit (Invitrogen) and pooled at eqimolar concentrations.

#### ***Text S6: Detail of fungal OTUs where taxonomic ID was altered***

The main method of identifying fungal OTUs in this study was to conduct BLASTn searches with a cut-off of  $e=10^{-9}$  against the UNITE database. However, in their 2015 Fungal Biology paper, Mayers et al. describe three new species of *Ambrosiella*. Since these taxa are particularly pertinent to the current study, we manually aligned representative OTU sequences for two OTUs against representative sequences for these newly described species and found strong similarity. As such, an OTU originally identified as *Ceratocystis adiposa* was reclassified as *Ambrosiella grosmanniae* and an OTU originally identified as *Ambrosiella ferruginea* was reclassified as *Phialophoropsis ferruginea*.

#### ***S7: Results of analyses using rarefied dataset***

To assess the impact of normalisation approach on results, we re-ran analyses pertaining to the drivers of fungal species diversity and community composition using a rarefied matrix. Rarefaction is a common approach for normalising high throughput sequencing read counts between samples. However, its use has been questioned as it necessitates the removal of viable data and can increase the probability of type I errors, i.e. false positives (McMurdie & Holmes 2014). Cumulative sum scaling (CSS) normalisation approach (used for the results reported in the manuscript) is able to account for both variation in read counts between samples and the inherent uncertainty surrounding biological replicates but does not require the removal of data (Paulson et al. 2013).

As in the main analysis, reads were mapped against OTUs using UPARSE to create an OTU by sample matrix. Fungal OTUs with >1 read in negative control samples were removed from further analyses. Samples containing fewer than 100 reads in total were also removed, leaving 379 samples in total. This dataset was then rarified to 100 reads per sample using the Vegan package in R (Oksanen et al. 2008).

#### *Drivers of bark and ambrosia beetle mycobiota*

When analysing Shannon diversity, the total amount of explained variance over the full model comprising beetle, environmental and spatial predictors was 42.2% ( $F_{16, 274} = 13.69$ ,  $p < 0.0001$ ). Beetle species identity uniquely explained 38.9% ( $F_{10, 280} = 25.39$ ,  $p < 0.0001$ ) of variation, while environmental and spatial predictors explained 0.51% ( $F_{2, 288} = 6.50$ ,  $p = < 0.001$ ) and 0.33% ( $F_{2, 288} = 6.66$ ,  $p < 0.001$ ) respectively. The proportion of explained variance shared between multiple predictors was low and varied between -0.26% and 4.15%.

For the fungal community analysis, beetle host identity also explained the greatest proportion of variation for both the incidence (Sørensen) and abundance (Bray-Curtis) based analyses: 17.4%, and 38.7% respectively (Table 2). Environmental and spatial variables accounted for little variation independently (between -0.2% and 2.3%), shared variance varied between 0.08% and 1.46%, and the full models explained 20.92% and 16.36% of the total variation for incidence and abundance respectively.

As such, these results are very similar to those in the main analysis but some have with a stronger effect size (portion of explained variance).

Table 2. Results from the full PERMANOVAs modeling rarified fungal community composition against all predictor groups. Separate analyses were used for abundance-based analysis (using the Bray-Curtis dissimilarity index) and incidence-based analysis (using the Sørensen dissimilarity index). Environmental components are based on the PCA of temperature and rainfall data (S6 & S7), dbMEMs represent the spatial variability of collection sites (see methods section of main text). Bold type indicates significant p-values.

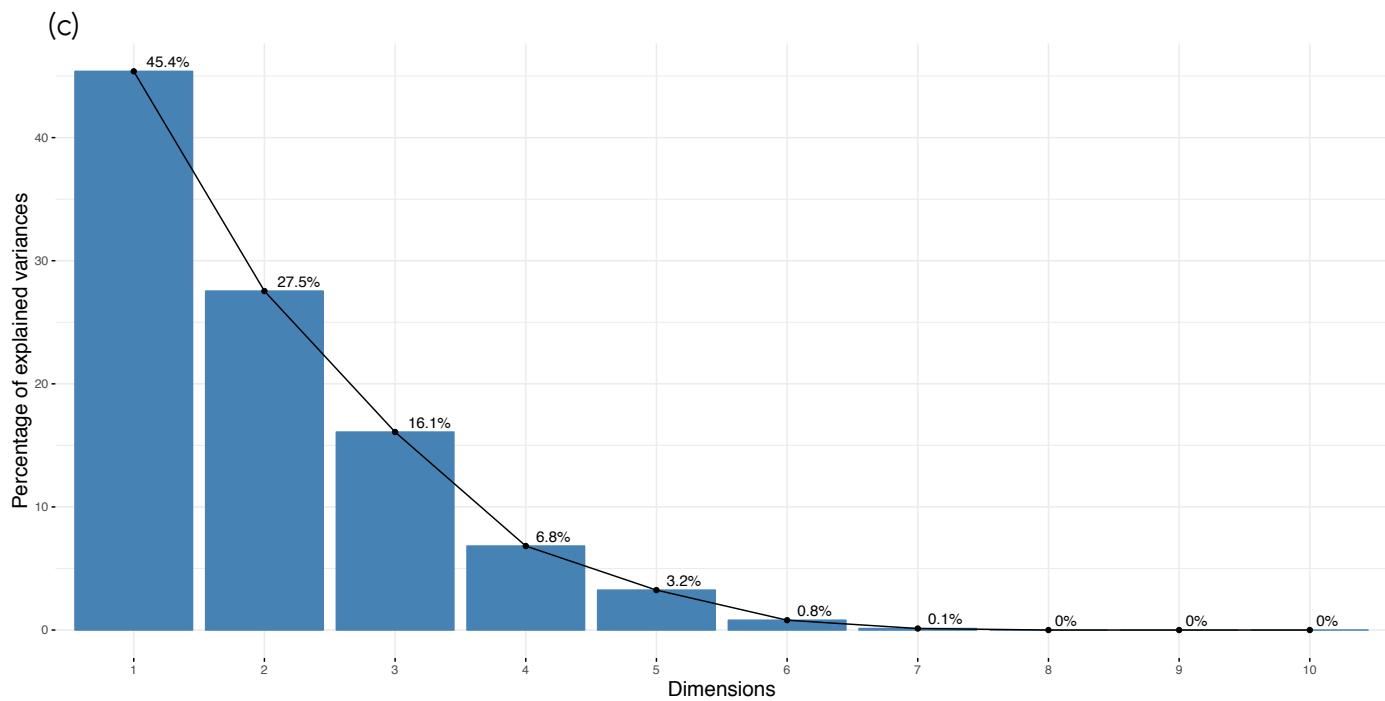
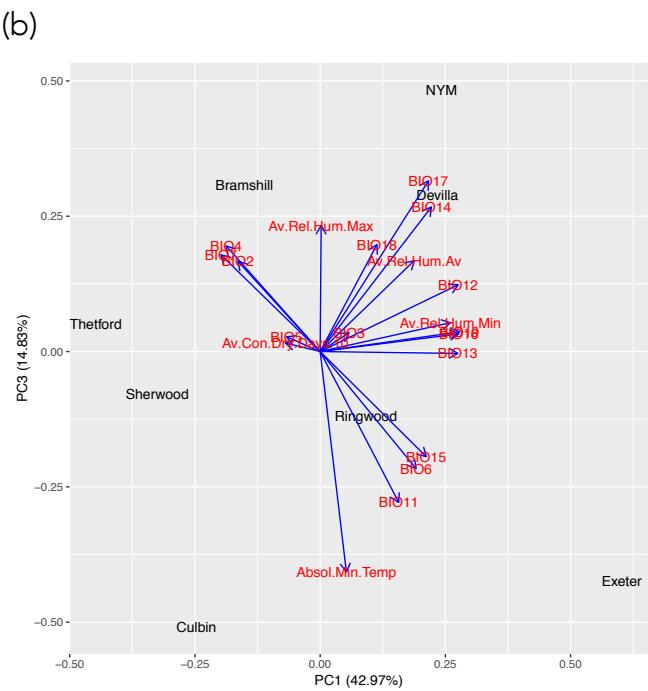
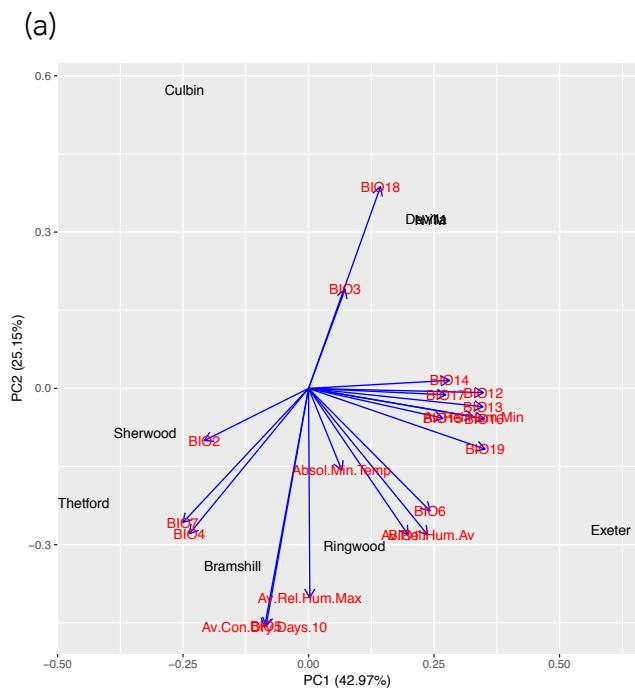
Predictor	Abundance			Incidence		
	Pseudo F	R <sup>2</sup>	p	Pseudo F	R <sup>2</sup>	p
Beetle species	18.280	0.414	<b>0.001</b>	6.1070	0.19324	<b>0.001</b>
Environment component 1	3.158	0.005	<b>0.01</b>	3.8352	0.00809	<b>0.001</b>
Environment component 2	5.760	0.009	<b>0.001</b>	2.6988	0.00569	<b>0.002</b>
Environment component 3	3.112	0.005	<b>0.021</b>	1.7824	0.00376	<b>0.011</b>
dbMEM 2	7.023	0.011	<b>0.001</b>	4.8453	0.01022	<b>0.001</b>
dbMEM 4	0.827	0.001	0.532	0.9715	0.00205	0.562
dbMEM 7	1.378	0.002	0.166	2.3193	0.00489	<b>0.001</b>

### Supplementary References

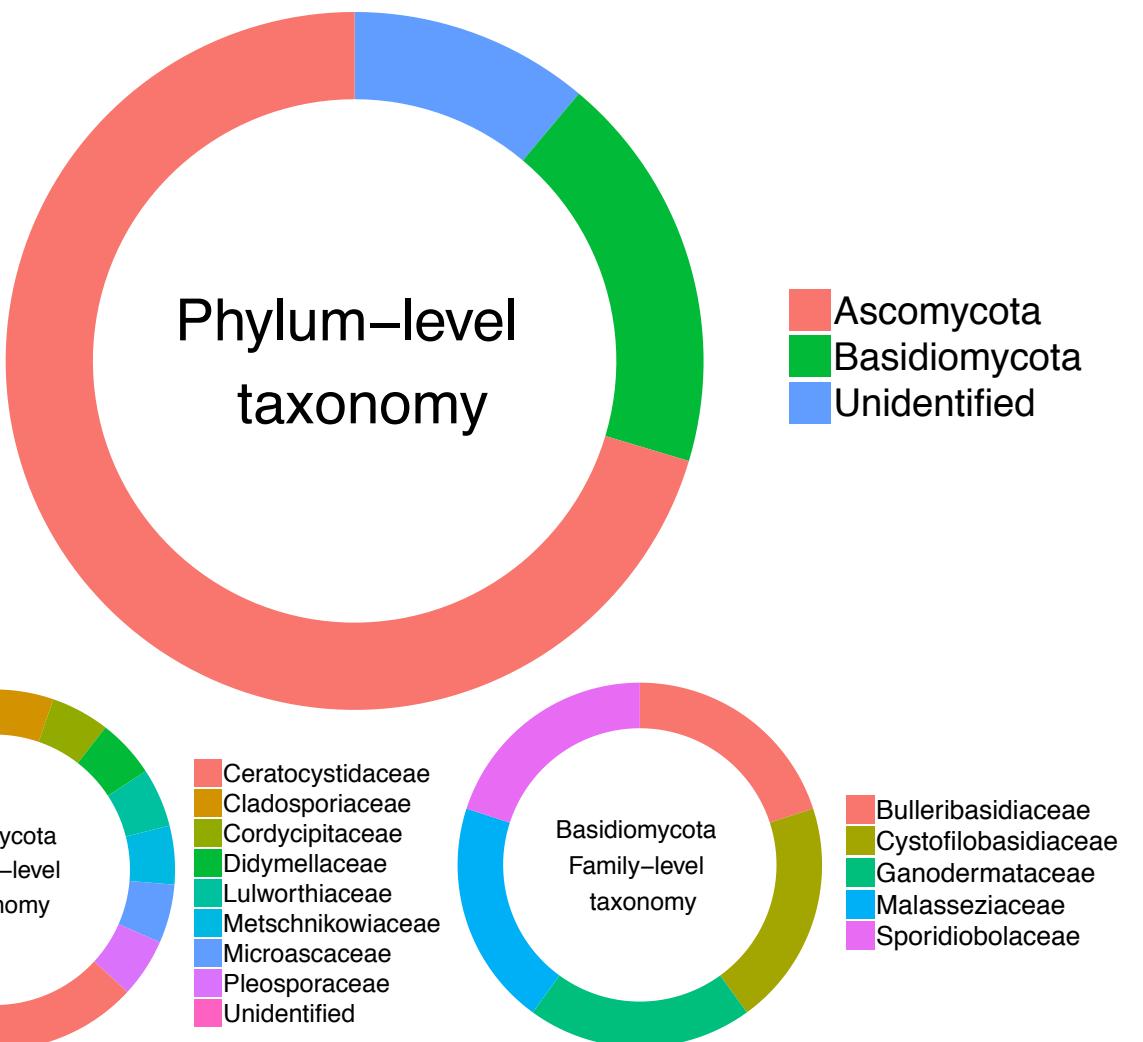
- Hijmans RJ, Cameron SE, Parra JL, Jones PG, Jarvis A (2005) Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology*, **25**, 1965–1978.
- Kalinova B, Brizova R, Knizek M, Turcani M, Hoskovec M (2014) Volatiles from spruce trap-trees detected by *Ips typographus* bark beetles: chemical and electrophysiological analyses. *Arthropod-Plant Interactions*, **8**, 305–316.
- McMurdie PJ, Holmes S (2014) Waste Not, Want Not: Why Rarefying Microbiome Data Is Inadmissible. *PLoS Computational Biology*, **10**, e1003531.
- Oksanen AJ, Kindt R, Legendre P et al. (2008) The Vegan Package.
- Paulson JN, Colin Stine O, Bravo HC, Pop M (2013) Differential abundance analysis for microbial marker-gene surveys. *Nature Methods*, **10**, 1200–1202.

Table S8. Initial environmental variables included in PCA

<b>WorldClim Dataset</b>
BIO2 = Mean Diurnal Range (Mean of monthly (max temp - min temp))
BIO3 = Isothermality (BIO2/BIO7) (* 100)
BIO4 = Temperature Seasonality (standard deviation *100)
BIO5 = Max Temperature of Warmest Month
BIO6 = Min Temperature of Coldest Month
BIO7 = Temperature Annual Range (BIO5-BIO6)
BIO11 = Mean Temperature of Coldest Quarter
BIO12 = Annual Precipitation
BIO13 = Precipitation of Wettest Month
BIO14 = Precipitation of Driest Month
BIO15 = Precipitation Seasonality (Coefficient of Variation)
BIO16 = Precipitation of Wettest Quarter
BIO17 = Precipitation of Driest Quarter
BIO18 = Precipitation of Warmest Quarter
BIO19 = Precipitation of Coldest Quarter
<b>UK Met Office Land Surface Observation Stations Dataset</b>
Av.Con.Dry.Days.10 = The number of consecutive days with precipitation $\leq$ 0.2 mm per year, averaged between 2000-2011
Av.Rel.Hum.Min = Minimum hourly relative humidity (%) averaged over each month 1961-2011
Av.Rel.Hum.Max = Maximum hourly relative humidity (%) averaged over each month 1961-2011
Absol.Min.Temp = Daily lowest air temperature between 0900-2100 is averaged across month. Lowest value of any month ( $^{\circ}$ C)
Absol.Temp.Max = Daily highest air temperature between 0900-2100 is averaged across month. Highest value of any month ( $^{\circ}$ C)



S9. Results from PCAs for environmental data showing (a) biplot for PC1 and PC2 (b) biplot for PC1 and PC3 (c) scree slope with proportion of variance captured by each component. Note: label names were changed to collection site names in these biplots, this was done to demonstrate the environmental similarity of collection sites.



S10. A total of 27 OTUs were removed from the analysis due to them being found present in the negative control samples. The majority were Ascomycetes ( $n=19$ ), but Basidiomycetes were also found ( $n=5$ ) along with unidentified fungi ( $n=3$ ). Figure shows taxonomic composition of these fungal OTUs obtained from negative control samples based upon BLAST searches against the UNITE database.