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Foliar fungal communities strongly differ between habitat patches in a landscape mosaic

Thomas Fort [Corresp., 1, 2] , **Cécile Robin** [1, 2] , **Xavier Capdevielle** [1, 2] , **Laurent Delière** [3, 4] , **Corinne Vacher** [1, 2]

[1] BIOGECO, UMR 1202, INRA, Cestas, France

[2] BIOGECO, UMR 1202, Université de Bordeaux, Cestas, France

[3] Santé et Agroécologie du Vignoble, UMR 1065, INRA, Villenave d'Ornon, France

[4] ISVV, UMR 1065 SAVE, Université de Bordeaux, Villenave d'Ornon, France

Corresponding Author: Thomas Fort
Email address: thomaslc.fort@gmail.com

Dispersal events between habitat patches in a landscape mosaic can structure ecological communities and influence the functioning of agrosystems. Here we investigated whether short-distance dispersal events between vineyard and forest patches shape foliar fungal communities. We hypothesized that these communities homogenize between habitats over the course of the growing season, particularly along habitat edges, because of aerial dispersal of spores.

We monitored the richness and composition of foliar and airborne fungal communities over the season, along transects perpendicular to edges between vineyard and forest patches, using Illumina sequencing of the ITS2 region.

In contrast to our expectation, foliar fungal communities in vineyards and forest patches increasingly differentiate over the growing season, even along habitat edges. Moreover, the richness of foliar fungal communities in grapevine drastically decreased over the growing season, in contrast to that of forest trees. The composition of airborne communities did not differ between habitats. The composition of oak foliar fungal communities change between forest edge and centre.

These results suggest that dispersal events between habitat patches are not major drivers of foliar fungal communities at the landscape scale. Selective pressures exerted in each habitat by the host plant, the microclimate and the agricultural practices play a greater role, and might account for the differentiation of foliar fugal communities between habitats.

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Thomas Fort[1,2], Cécile Robin[1,2], Xavier Capdevielle[1,2], Laurent Delière[3,4], Corinne Vacher[1,2]

[1] INRA, UMR1202 BIOGECO, F-33610 Cestas, France

[2] University of Bordeaux, BIOGECO, UMR 1202, F-33615 Pessac, France

[3] INRA, UMR1065 Santé et Agroécologie du Vignoble, F-33883 Villenave d'Ornon, France

[4] Université de Bordeaux, ISVV, UMR1065 SAVE, F-33883 Villenave d'Ornon, France

Corresponding Author :

Thomas Fort[1,2]

69 route d'Arcachon, 33612 Cestas, France

Email address: thomaslc.fort@gmail.com

Summary

Background. Dispersal events between habitat patches in a landscape mosaic can structure ecological communities and influence the functioning of agrosystems. Here we investigated whether short-distance dispersal events between vineyard and forest patches shape foliar fungal communities. We hypothesized that these communities homogenize between habitats over the course of the growing season, particularly along habitat edges, because of aerial dispersal of spores.

Methods. We monitored the richness and composition of foliar and airborne fungal communities over the season, along transects perpendicular to edges between vineyard and forest patches, using Illumina sequencing of the ITS2 region.

Results. In contrast to our expectation, foliar fungal communities in vineyards and forest patches increasingly differentiate over the growing season, even along habitat edges. Moreover, the richness of foliar fungal communities in grapevine drastically decreased over the growing season, in contrast to that of forest trees. The composition of airborne communities did not differ between habitats. The composition of oak foliar fungal communities change between forest edge and centre.

Discussion. These results suggest that dispersal events between habitat patches are not major drivers of foliar fungal communities at the landscape scale. Selective pressures exerted in each habitat by the host plant, the microclimate and the agricultural practices play a greater role, and might account for the differentiation of foliar ~~fugal~~ communities between habitats.

36 **Keywords:** Bioaerosols, Dispersal, Edges, Forest, Grapevine, Landscape, Phyllosphere,

37 Selection

Introduction

Plant leaves provide one of the largest microbial habitat on Earth (Ruinen, 1956; Morris, 2001; Vorholt, 2012). They harbour highly diverse microbial communities, including many genera of bacteria and fungi (Lindow & Leveau, 2002; Vorholt, 2012; Turner et al., 2013). The eco-evolutionary processes which shape these communities – dispersal, evolutionary diversification, selection and drift – are increasingly well understood (Hanson et al., 2012; Nemergut et al., 2013; Vacher et al., 2016). This new eco-evolutionary framework will undoubtedly have important applications in agriculture. Indeed, crop performance depends on the balance and interactions between pathogenic and beneficial microbial species (Newton et al., 2010a, 2010b). Manipulating whole foliar microbial communities, by acting on the processes shaping them, could thus greatly ~~imporve~~ crop health (Newton et al., 2010a; Xu et al., 2011). However, to reach this aim, a better understanding of the structure and dynamics of foliar microbial communities at the landscape scale is required.

The landscape plays a key role in the dynamics of macro-organism populations interacting with crops, such as arthropod pests or their natural enemies (Norris & Kogan, 2000; Chaplin-Kramer et al., 2011). In ecology, the landscape is defined as an heterogeneous geographic area, characterized by a dynamic mosaic of interacting habitat patches (Bastian, 2001). Species movements between habitat patches - referred as dispersal (Vellend, 2010) - modulates the richness, composition and function of macro-organism communities (Hurst et al., 2013; Ma et al., 2013; Lacasella et al., 2014). In agricultural landscape, species dispersal between natural and managed habitats can trigger detrimental or beneficial effects in crops (Chaplin-Kramer

et al., 2011; Blitzer et al., 2012), particularly along the edges (Thomson & Hoffmann, 2009; Lacasella et al., 2014).

The influence of dispersal events on the structure of foliar microbial ~~community~~ at the landscape scale has hardly been studied ~~so far~~. Many microbial species colonising plant leaves are horizontally transferred (i.e. from one adult plant to another) by airborne dispersal (Whipps et al., 2008; Bulgarelli et al., 2013). The foliar microbial communities of a given plant can therefore be influenced by those of its neighbours. Plant pathogens, for instance, can be transmitted from a reservoir plant to neighbouring plants (Power & Mitchell, 2004; Beckstead et al., 2010; Wilson et al., 2014). These short-distance dispersal events could have a greater effect on the foliar microbial communities of deciduous plants, because the leaves of those plants are colonised by micro-organisms every spring, after budbreak.

In this study, we analysed the structure and dynamic of foliar and airborne fungal communities in a heterogeneous landscape consisting of vineyard and forest patches. We expected the fungal communities of forest patches to be richer than those of vineyards, because the higher plant species richness and biomass in forests increase the diversity of micro-habitats available to foliar fungi. We also expected repeated dispersal events to homogenize foliar fungal communities between the two habitats over the course of the growing season, particularly along habitat edges. We thus tested the following hypotheses for both foliar and airborne fungal communities: (1) community richness is higher in forests than in adjacent vineyards, (2) community similarity between the two habitats increase over the course of the growing season and (3) is higher along habitat edges.

Materials and methods

Sampling design

Three study sites, each consisting of a forest patch and an adjacent vineyard, were selected in the Bordeaux area (France). They were located in the domains of Châteaux Reignac (N44°54′03″, O0°25′01″), Grand-Verdus (N44°47′21″, O0°24′06″) and Couhins (N44°45′04″, O0°33′53″). At each site, the edge between the forest patch and the vineyard was at least 100 m long. The width of each habitat, perpendicular to the edge, was at least 200 m. The forest patches at all three sites contained mostly deciduous species, dominated by pedunculate oak (*Quercus robur* L.). The second most frequent tree species was European hornbeam (*Carpinus betulus* L.) in Reignac and Grand-Verdus, and sweet chestnut (*Castanea sativa* Mill.) in Couhins. In the vineyards, the grapevine (*Vitis vinifera* L.) cultivar was Cabernet Sauvignon in Reignac and Grand-Verdus, and Merlot in Couhins.

At each site, leaves were collected along three parallel transects perpendicular to the forest-vineyard edge and separated by a distance of about five meters. Leaves were sampled at four locations along each transect: in the centre of the forest (100 m away from the edge), at the edge of the forest, at the edge of the vineyard and in the centre of the vineyard (100 m away from the edge). In forest patches, leaves were sampled from the two most abundant tree species. For each sampling location and each transect, a single tree of each species was selected. Three leaves oriented in different directions were collected from each tree, at a height of 7 m. In vineyards, three leaves were collected from three adjacent cloned grapevine stocks. Each of the sampled leaves was selected from the base of the cane (one-year-old shoot), to ensure the collection of

leaves of the same age on each date. The leaves were removed with scissors that had been sterilised with 96 % ethanol, and all contact of the leaves with the hands was carefully avoided. The leaves were stored in clear plastic bags containing silica gel to ensure rapid drying. In addition, grapevine leaves were placed between two sheets of sterile paper filter to ensure good dessication despite their thickness. Leaves were sampled on three dates in 2013: in May (between the 15th and 23rd), July (between the 16th and 18th) and October (3rd). The sampling dates chosen were as far removed as possible from the last chemical treatment performed in the vineyard (Supporting Information Table S1).

Airborne particles were collected along the middle transect of each site, with two Coriolis air sampler devices positioned one meter above the ground. At each sampling location, three successive 10 minute sampling sessions were carried out, with a flow rate of 200 l/min.

DNA extraction and sequencing

Sample contamination was prevented by exposing all tools and materials required for sample processing and DNA extraction to UV light for 30 minutes in a laminar flow hood. Four discs (each 8.0 mm in diameter) were cut randomly from each leaf, in the flow hood, with a hole-punch sterilised by flaming with 95 % ethanol. The four discs were placed in a single well of an autoclaved DNA extraction plate. Three wells were left empty as negative controls. Two autoclaved metallic beads were added to each well, and the plant material was ground into a homogeneous powder with a Geno/Grinder 2010 (SPEX Sample Prep, Metuchen, NJ).

123 The liquid used to collect airborne particles was transferred into sterile 15 ml centrifuge tubes.
124 Each tube was then centrifuged for 30 minutes at 13000 RCF and the supernatant was removed
125 with a sterile transfer pipette. The pellet was then transferred by resuspension to an autoclaved
126 tube and freeze-dried. A tube of unused sampling liquid was treated in the same way and used as
127 a negative control. Total DNA was extracted from each leaf and airborne sample with the
128 DNeasy 96 Plant Kit (QIAGEN). Foliar DNA samples from the same tree were pooled, as were
129 foliar DNA samples from the three adjacent grapevine stocks.

130 Fungal ITS2 (Internal Transcribed Spacer 2) was amplified with the fITS7 (forward) and ITS4
131 (reverse) primers (Ihrmark et al., 2012). Paired-end sequencing (300 bp) was then performed in a
132 single run of an Illumina MiSeq sequencer, on the basis of V3 chemistry. PCR amplification,
133 barcodes and MiSeq adapters addition, library sequencing and data preprocessing were carried
134 out by the LGC Genomics sequencing service (Berlin, Germany).

135

136 **Bioinformatic analysis**

137 Sequences were first demultiplexed and filtered. All sequences with tag mismatches, missing
138 tags, one-sided tags or conflicting tag pairs were discarded. Tags and Illumina TruSeq adapters
139 were then clipped from all sequences, and sequences with a final length fewer than 100 bases
140 were discarded. All sequences with more than three mismatches with the ITS2 primers were
141 discarded. Primers were then clipped and the sequence fragments were placed in a forward-
142 reverse primer orientation. Forward and reverse reads were then combined, and read pair
143 sequences that could not be combined were discarded.

144 The pipeline developed by Bálint et al. (2014) was used to process the sequences. The ITS2
145 sequence was first extracted from each sequence with the FungalITSextractor (Nilsson et al.,
146 2010). All the sequences were then concatenated into a single fasta file, after adding the sample
147 code in the label of each sequence. The sequences were dereplicated, sorted and singletons were
148 discarded with VSEARCH (https://github.com/torognes/vsearch). The sequences were then
149 clustered into molecular operational taxonomic units (OTUs) with the UPARSE algorithm
150 implemented in USEARCH v8 (Edgar, 2013), with a minimum identity threshold of 97 %.
151 Additional chimera detection was performed against the UNITE database (Kõljalg et al., 2013),
152 with the UCHIME algorithm implemented in USEARCH v8 (Edgar et al., 2011). The OTU
153 table, giving the number of sequences of each OTU for each sample, was created with
154 USEARCH v8.

155 OTUs were taxonomically assigned using the online BLAST web interface (Madden, 2013)
156 against the GenBank database, by excluding environmental and metagenome sequences. The
157 assignment with the lowest e-value was retained. The full taxonomic lineage of each assignment
158 was retrieved from the GI number information provided by NCBI. All the OTUs assigned to
159 plants or other organisms, and all unassigned OTUs were removed, to ensure that only fungal
160 OTUs were retained.

161

162 **Statistical analyses**

163 All statistical analyses were performed in the R environment. We computed 100 random rarefied
164 OTU matrices, using the smallest number of sequences per sample as a threshold. The number of
165 OTUs per sample (OTU richness) and the dissimilarity between samples (Bray-Curtis index

based on abundances and Jaccard index based on occurrences) were calculated for each rarefied matrix and averaged.

Type III ANOVA was used to assess the effect of host plant species (grapevine, oak, hornbeam and chestnut), sampling date (May, July, October), edge (habitat centre or edge) and their interactions on foliar OTU richness. Sampling site was included in the model as a random factor. Marginal and conditional coefficients of determination were calculated to estimate the variance explained by fixed factors (R_m^2) and fixed *plus* random factors (R_c^2). Post-hoc pairwise comparisons were then performed for each level of each factor, with Tukey's adjustment method. A similar ANOVA was performed on airborne OTU richness, including habitat (forest and vineyard), sampling date, sampling site, and their interactions.

Dissimilarities in composition between samples were represented by non-metric multidimensional scaling analysis (NMDS) and were analysed by permutational multivariate analyses of variance (PERMANOVA), including the same fixed factors as the ANOVAs, with sampling sites treated as strata. We dealt with complex interactions in PERMANOVA results, by calculating post-hoc PERMANOVAs, including sampling date, sampling site and their interaction, separately for each host plant species (or habitat for airborne samples). We then corrected the P-values for multiple testing, as described by Benjamini & Yekutieli (2001).

183

184 # Results

185 ## Taxonomic description of foliar and airborne fungal communities

186 In total, we obtained 7 946 646 high-quality sequences, which clustered into 4 360 OTUs.
187 Overall, 867 OTUs, corresponding to 4 600 179 sequences (57.9% of the raw OTU table) were
188 not taxonomically assigned to fungi by BLAST. Among them, 4 451 913 sequences were
189 assigned to plant sequences (Tracheophyta division), principally *Vitis* (59%), and *Carpinus*
190 (35%) genus, showing that fITS7-ITS4 primers are not specific of fungi. These OTUs were
191 removed. The negative controls contained 29 857 fungal sequences clustering into 337 OTUs.
192 Some of these OTUs were found in all samples and were assigned to ubiquitous fungal species
193 that had already been found on plant leaves (e.g. *Aureobasidium pullulans* or *Eppicocum*
194 *nigrum*). Because it is difficult to distinguish real contaminations from cross-contaminations
195 during the DNA extraction or sequencing process (Kircher et al., 2011; Esling et al., 2015), we
196 decided to retain all these OTUs. Two samples containing very few sequences (<300 sequences)
197 were removed. These samples corresponded to grapevine leaves collected at the Couhins site, in
198 May. The first was collected in the centre of the vineyard, and the other was collected at its edge.
199 Finally, the OTU table used for the analyses contained 196 samples and 3 487 fungal OTUs,
200 corresponding to 3 316 156 sequences. The number of sequences per sample ranged from 424 to
201 96 276, with a mean of 16 919.

202 The fungal communities of bioaerosols and leaves from forest trees and grapevines were
203 dominated by ascomycetes (Fig. 1). The sequences assigned to Ascomycota division accounted

for 82.1% of all the sequences in the rarefied dataset, followed by Basidiomycota division (14.8%). Overall, 3.1% of the total sequences remained unassigned at the division level. Airborne and foliar samples shared 905 OTUs (Fig. 2), but there was no significant difference in the composition of foliar and airborne fungal communities (PERMANOVA F=20.15, p=0.001). The ten most abundant fungal OTUs were shared by airborne, forest foliar and grapevine foliar communities, but their relative abundance differed between each compartment (Table 1).

Variations in the richness of foliar and airborne fungal communities at the landscape scale

ANOVA revealed a significant effect of the interaction between host plant species and sampling date on the richness of foliar fungal communities (Table 2). Differences in fungal community richness between plant species were not significant in May and July (Fig. 3 and Fig. S1). In October, grapevine stocks had significantly less rich foliar fungal communities than oak (post-hoc tests: $P<0.0001$; Fig. 3) and hornbeam trees ($P<0.0001$), but the richness of their fungal communities did not differ significantly from that of chestnut trees ($P=0.147$; Fig. S1). Hornbeam leaves harboured the richest communities of all the plant species considered (post-hoc tests: $P<0.0001$ between hornbeam and chestnut, $P=0.0003$ between hornbeam and oak, $P<0.0001$ between hornbeam and grapevine; Fig. S1).

ANOVA post-hoc tests also revealed a significant decrease in fungal species richness in grapevine over the course of the growing season ($P<0.0001$ for each pairwise comparison; Fig. 3). Seasonal variations in fungal richness were less marked in oak ($P=0.081$, $P=0.999$ and $P=0.004$, respectively between May and July, July and October, May and October), chestnut

(P=0.011, P=0.997 and P=0.0002, respectively) and hornbeam (P=1.00, P=0.144 and P=0.185, respectively).

ANOVA also revealed a significant effect of the interaction between host plant species and edge on the richness of foliar fungal communities (Table 2). The richness of foliar fungal communities was significantly higher at the edge in oak (P=0.002), but not in hornbeam (P=0.100), chestnut (P=0.139), or grapevine (P=0.790) (Fig. S2).

Habitat had a significant effect on the richness of airborne fungal communities (Table 2), which was significantly higher in forests than in vineyards.

Variations in the composition of foliar and airborne fungal communities at the landscape scale

PERMANOVA revealed a significant effect of the interaction between host plant species and sampling date on the composition of foliar fungal communities (Table 3). Bray-Curtis dissimilarities between oak and grapevine foliar fungal communities increased over the course of the growing season (mean ± SD; 0.47 ± 0.07 in May, 0.67 ± 0.09 in July and 0.91 ± 0.06 in October). These results are illustrated by non-metric multidimensional scaling (NMDS; Fig. 4a). Bray-Curtis dissimilarities also increased between each pair of host species (Table S2 and Fig. S3a). Similar results were obtained with the Jaccard dissimilarity index (Table S3 and Fig. S3b).

PERMANOVA also revealed significant edge effects on the composition of foliar fungal communities, in interaction with host plant species and sampling date. Post-hoc PERMANOVAs

computed separately for each host species indicated differences in community composition between the edge and centre of the forest for oak and hornbeam, in interaction with sampling date (F=1.68, P=0.031 and F=1.85, P=0.044, respectively). The composition of the fungal community did not differ between the edge and the centre of the habitat for chestnut (F=2.27, P=0.25) or grapevine (F=0.92, P=1). Finally, PERMANOVA analysis of Bray-Curtis dissimilarities revealed a significant effect of sampling date on bioaerosol composition (Table 3 and Fig. 4b). Similar results were obtained for Jaccard dissimilarity (Table S3).

Discussion

To our knowledge, this is the first time that the spatial structure and the temporal dynamic of foliar and airborne fungal communities are assessed simultaneously at the landscape scale. We studied a landscape mosaic consisting of two main habitats, vineyard and forest patches. We expected that repeated dispersal events between habitat patches would homogenize the foliar communities over the course of the growing season. We expected the homogenization to be greater along habitat edges, where grapevine stocks and forest trees are closer to each other.

Accordingly, we found that 26% of the OTUs are shared between airborne and foliar fungal communities. The most abundant ones are principally generalist species, such as *Aureobasidium pullulans*, *Cladosporium sp.* or *Eppicoccum nigrum*, which were already found as abundant in the microbiome of many species (Jumpponen & Jones, 2009; Zambell & White, 2014; Pinto & Gomes, 2016). This result confirms that many fungal species disperse through the atmosphere (Lindemann et al., 1982; Brown & Hovmøller, 2002; Bulgarelli et al., 2013). Moreover, the composition of airborne fungal communities did not differ significantly between forest patches

and adjacent vineyards, whatever the season. This result suggests that dispersal is not limiting at the landscape scale (Barberán et al., 2014), in contradiction with the results of Bowers et al. (2013). This lack of spatial variation in airborne fungal communities could account for the high similarity between foliar fungal communities of grapevine and forest tree species at the beginning of the growing season. Our results suggest that flushing leaves in May receive similar pools of fungal species through airborne dispersal, whatever the habitat and the host plant species.

Against expectation, we found that the composition of the foliar fungal communities of forest tree species and grapevine increasingly diverged from May to October. Besides, a severe decline in the richness of foliar fungal communities was observed in grapevine over the course of the growing season, but not in forest tree species. Despite an identical pool of airborne fungi in vineyards and forests, the selective pressures exerted on foliar fungal communities differ between both habitats.

Selection by the habitat can be exerted by the microclimate (Vacher et al., 2016). Harsher microclimatic conditions in vineyards than in forests, especially in the summer, could account for the decline in fungal species richness in vineyards but not in forests. Particularly, greater exposure to UV and higher air temperatures in vineyards could decrease the survival of foliar fungi on grapevine leaves. By contrast, tree cover provides a milder microclimate which could be more suitable to foliar micro-organisms.

Selection by the habitat can also be exerted by the plant host (Vacher et al., 2016). Several studies indeed revealed some host-specificity in foliar fungal communities (Kembel & Mueller, 2014; Lambais et al., 2014; Meiser et al., 2014). Our results paralleled these findings: in forest

patches, foliar fungal communities significantly differ among tree species at the end of the growing season. Seasonal variations in leaf physiology could also account for the observed temporal variations in foliar communities, especially the richness decline in grapevine fungal foliar communities. Older grapevine leaves indeed produce larger amounts of phytoalexins and tend to be more resistant to pathogens (Steimetz et al., 2012).

Finally, selection by the habitat can be exerted by agricultural practices. A few studies showed that fungicide applications can reduce the diversity and alter the composition of the foliar microbial community (Gu et al., 2010; Moulas et al., 2013; Cordero-Bueso et al., 2014; Karlsson et al., 2014). However, several other studies showed that the foliar fungal communities of grapevine are highly resilient to some chemical or biological pesticides (Walter et al., 2007; Perazzolli et al., 2014; Ottesen et al., 2015). Further research is required to assess the influence of fungicide applications on the observed decline in the richness of foliar fungal communities.

Our study also showed, for the first time, significant edge effects on foliar fungal community assemblages. A higher level of foliar fungal community richness was found in oak trees growing at the edge of the forest than in oak trees growing 100 m away. Significant differences in community composition between the edge and the centre of the forest were also found for oak and hornbeam. Variations in microclimate and leaf physiology along the forest edge (Chen et al., 1993; Zheng et al., 2005; Kunert et al., 2015) are more likely to account for this result than species dispersal from vineyards to forest patches, since the foliar fungal communities of the two habitats diverged over the course of the growing season. The absence of edge effect in grapevine foliar fungal communities suggests that dispersal of fungal species from forests to vineyards has little influence on community composition and richness. This result contrasts with the findings of many studies on macro-organisms, reporting that dispersal events between managed and non-

managed habitats shape communities and influence ecosystem functioning and services (Thomson & Hoffmann, 2009; Rusch et al., 2010; Thomson et al., 2010; Chaplin-Kramer et al., 2011; Blitzer et al., 2012).

Conclusions

Our results suggest that dispersal events between habitat patches are not major drivers of foliar fungal communities at the landscape scale. Selective pressures exerted in each habitat by the plant host, the microclimate and the agricultural practices play a greater role, and might account for the differentiation of foliar fungal communities between habitats. Our results suggest that the leaves of broad-leaf species are colonised by similar pools of airborne micro-organisms at the beginning of the growing season. The composition of foliar fungal communities then diverges between habitat patches and between plant species within the same habitat. In contrast, airborne communities remain the same between habitats. Overall, our results thus confirm the Baas-Becking statement that "Everything is everywhere, but the environment selects" (Baas Becking, 1934; De Wit & Bouvier, 2006). For fungal communities at the landscape scale, everything is everywhere in bioaerosols, but the habitat selects.

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337

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494

Tables

Table 1 Taxonomic assignment of the 10 most abundant OTUs by the online BLAST analysis against the GenBank database. The environmental and metagenome sequences were excluded. Identity is the percentage identity between the OTU representative sequence and the closest matching sequence in GenBank. Taxa followed by a * indicate representative sequences assigned to at least another species with an identical e-value. Relative abundance is the average abundance of each ~~OUT~~ over 100 rarefactions. ~~Brakets~~ contain the rank of the ~~OUT~~ in each data subset. The Project Accession Number is PRJEB13880.

Closest match			Relative abundance (rank)			
GI number	Identity	Putative taxon	Total	Airborne	Forest leaves	Grapevine leaves
1034220623	100	*Aureobasidium pullulans*	20.74	5.6 **(4)**	16.7 **(1)**	39.6 **(1)**
1031917897	100	*Cladosporium herbarum**	6.37	18.7 **(1)**	3.4 **(8)**	4.0 **(2)**
391883765	86.2	*Pseudeurotium hygrophilum**	5.03	2.4 **(8)**	7.3 **(2)**	2.1 **(9)**
1035371449	100	*Cladosporium perangustum**	3.70	9.6 **(2)**	2.5 **(10)**	2.11 **(8)**
61619908	100	*Ramularia endophylla*	3.52	1.5 **(11)**	4.7 **(4)**	2.48 **(6)**
626419142	99.5	*Taphrina carpini*	3.16	1.1 **(14)**	4.7 **(3)**	1.31 **(15)**
1024249962	100	*Erysiphe alphitoides**	2.60	1.0 **(15)**	3.7 **(5)**	1.5 **(12)**
61619940	100	*Naevala minutissima*	2.53	1.5 **(10)**	3.5 **(7)**	1.2 **(18)**
799381116	100	*Ramularia vizellae*	2.49	0.9 **(16)**	3.5 **(6)**	1.5 **(13)**
1031917850	100	*Epicoccum nigrum*	2.05	3.3 **(7)**	1.0 **(19)**	3.4 **(4)**

505 **Table 2** Effect of sampling date (May, July or October), host species (oak, hornbeam, chestnut
506 or grapevine) or habitat (vineyard or forest), edge (habitat centre or center) and their interaction
507 on OTU richness in foliar and airborne fungal communities, assessed using a type III ANOVA.
508 In both models, sampling site was included as a random variable. R_m^2 is the marginal coefficient
509 of determination (for fixed effects) and R_c^2 the conditional coefficient of determination (for fixed
510 and random effects). Bold values ares the significant ones.

	F	*P*-value	R_m^2 (R_c^2)
	Foliar OTU richness		
Date	44.49	**<0.001**	0.64 (0.71)
Species	14.97	**<0.001**	
Edge	17.21	**<0.001**	
D x S	23.42	**<0.001**	
D x E	0.11	0.894	
S x E	6.72	**<0.001**	
D x S x E	1.13	0.347	
	Airborne OTU richness		
Date	1.07	0.362	0.34 (0.52)
Habitat	10.19	**0.004**	
Edge	4.20	0.052	
D x H	0.86	0.436	
D x E	1.40	0.267	
H x E	0.01	0.912	
D x H x E	1.678	0.209	

511

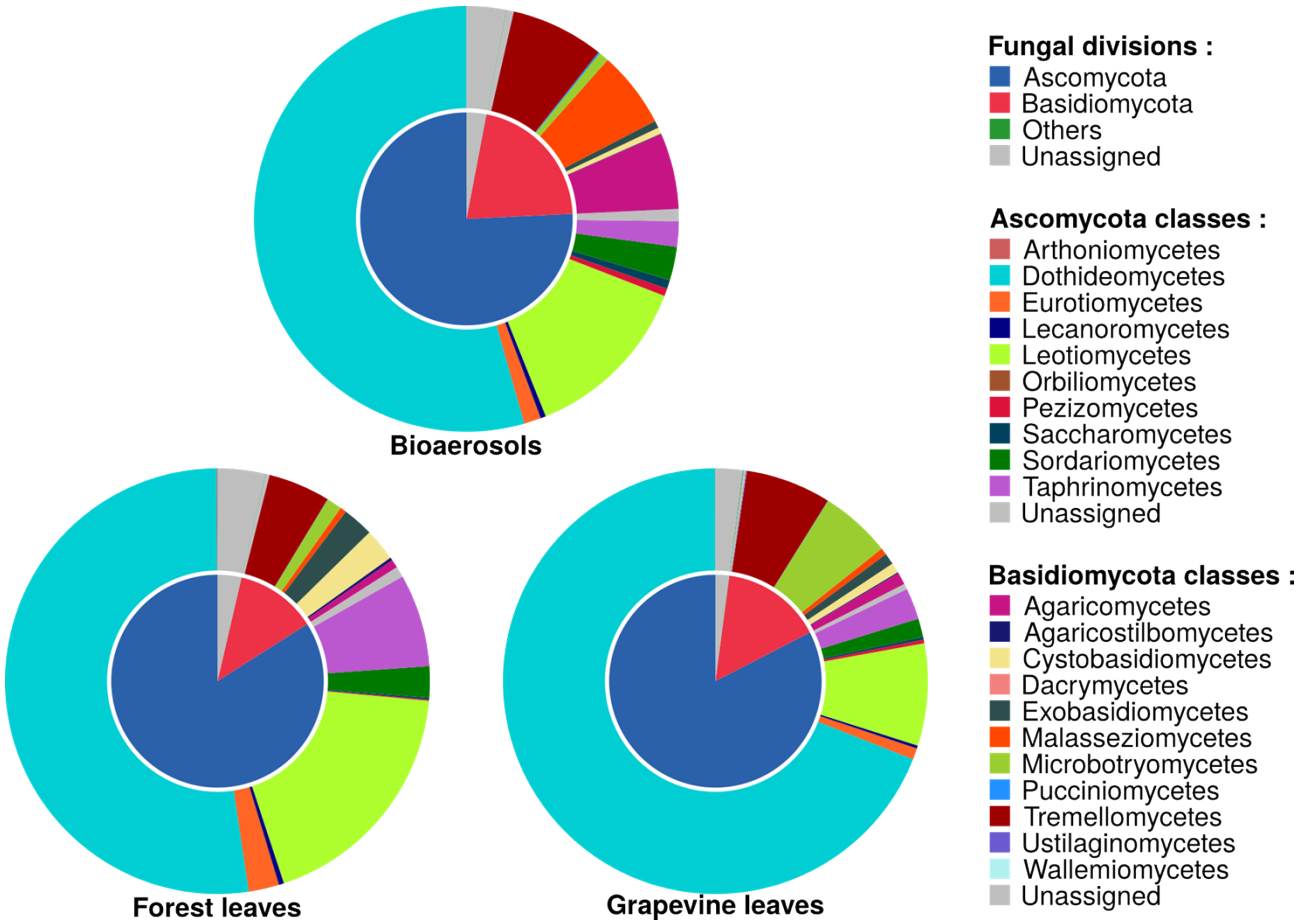
512 **Table 3** Effect of sampling date (May, July or October), host species (oak, hornbeam, chestnut
513 or grapevine) or habitat (vineyard or forest), edge (habitat centre or center) and their interaction
514 on the composition of foliar and airborne fungal communities, assessed using a PERMANOVA.
515 In both models, sampling site was included as a stratification variable. Bold values ares the
516 significant ones.

	F	R^2	*P*-value
	Foliar fungal community composition		
Date	10.13	0.078	**0.001**
Species	13.70	0.158	**0.001**
Edge	3.94	0.015	**0.001**
D x Sp	6.92	0.160	**0.001**
D x E	2.05	0.016	**0.007**
Sp x E	2.22	0.026	**0.001**
D x Sp x E	1.08	0.025	0.239
	Airborne fungal community composition		
Date	2.94	0.157	**0.001**
Habitat	1.54	0.041	0.062
Edge	0.68	0.018	0.827
D x H	0.95	0.051	0.418
D x E	0.66	0.035	0.938
H x E	0.77	0.020	0.684
D x H x E	0.71	0.038	0.878

517

Figures

Figure 1 Taxonomic composition of the airborne and foliar fungal communities in forest and vineyard habitats. The inner disc shows the proportion of sequences assigned to each taxonomic division, and the outer disc the proportion of sequences assigned to each class of the Ascomycota and Basidiomycota divisions.



527 **Figure 2** Venn diagramm giving the number of OTUs shared between the airborne, forest foliar
528 and vineyard foliar communities.

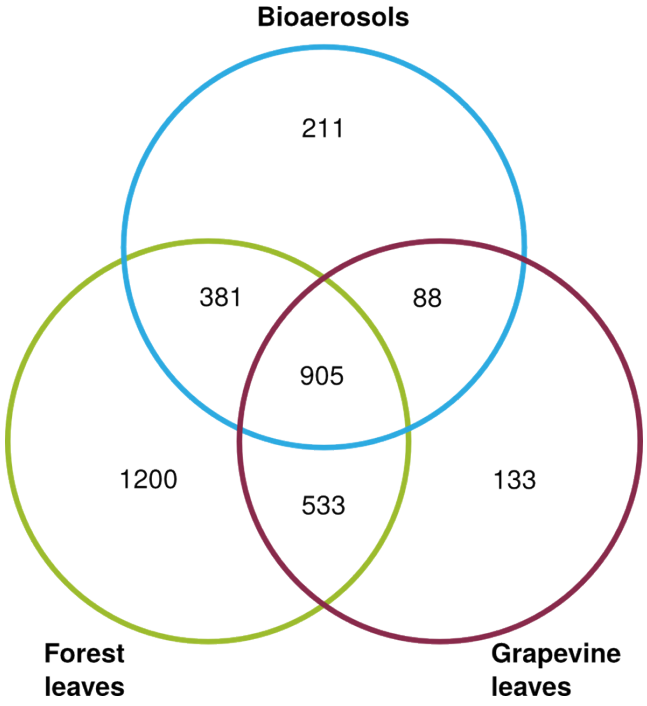


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Figure 3 Richness of foliar fungal community in oak (green) and grapevine (red), depending on the sampling date. Error bars represent the standard error.

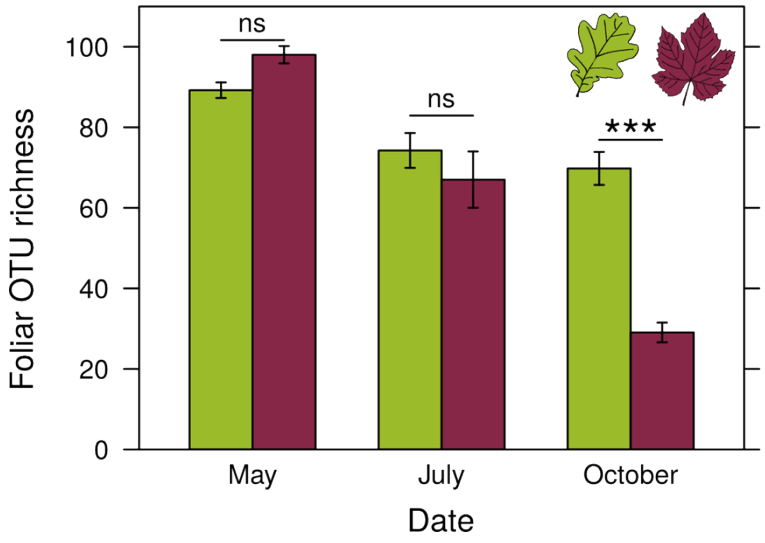

Figure 4 NMDS representing dissimilarities in the composition of fungal communities. (a) Dissimilarities in the composition of foliar fungal communities between the host species (oak in green and grapevine in red), depending on the sampling date. The other two forest species are not shown here, to make the figure easier to read, and are presented in Fig. S2. The stress value associated with this representation was 0.170. (b) Airborne fungal communities between the habitat (forest in light-blue and vineyard in dark-blue), depending on the sampling date. The stress value associated with this representation was 0.188. Dissimilarities between samples were computed with the Bray-Curtis index, averaged over 100 random rarefactions of the OTU table. The confidence ellipsoid at the 0.68 level is shown, for all combinations of these two factors.