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Fungal endophyte communities differ between chestnut galls and surrounding foliar tissues

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ABSTRACT

Foliar endophytic fungi are present in almost all vascular plants. The composition of endophyte communities varies among plant individuals. Likely, but understudied, sources of this variation are the species composition of plant community and initial attacks by insect herbivores. We addressed these issues by characterizing fungal endophyte communities on leaves of chestnut (*Castanea sativa)* grown in pure *vs.* mixed stands. We used ITS metabarcoding methods to identify endophytic fungi associated with galls caused by the invasive wasp, *Dryocosmus kuriphilus,* and with surrounding chestnut leaf tissues. We found 1,378 different OTUs. The richness, diversity and composition of endophyte communities differed between galls and surrounding leaf tissues but were independent of forest stand composition. Fungal endophyte richness was lower in galls than in surrounding leaf tissues. Most differences in the composition of fungal endophyte communities between galls and foliar tissues were due to OTU turnover. These results suggest that insect-induced galls provide a particular habitat condition for endophytic microorganisms, regardless of forest species composition. A better understanding of endophyte biology is important to improve their use as biocontrol agents of galling insects.

Key words: endophytes, galls, diversity, plan-microbe-insect interactions, next generation sequencing

INTRODUCTION

All major lineages of vascular plants worldwide are colonized by endophytic fungi (Arnold, 2007; Hardoim et al., 2015). Endophytic fungi live inside living plant tissue without causing visible disease symptoms, at least during a part of their life cycle (Partida-Martínez and Heil, 2011). In some cases, their presence may increase plant fitness through improved plant resistance to abiotic (e.g. drought or salinity tolerance, Redman et al., 2002; Sherameti et al., 2008) and biotic stressors (e.g., resistance to insects and fungal pathogens, Clay, 1996; Ownley et al., 2010; Combès et al., 2012) . In these cases they are considered 'beneficial' microbes (e.g. Pineda et al., 2013). However, endophytic fungi can also turn into pathogenic forms, depending on biotic and abiotic factors, such as plant and microbe genotypes or environmental conditions (Hardoim et al., 2015). Because of the complexity of the endophyte-pathogen-saprotroph continuum in fungi (Arnold, 2007; Delaye et al., 2013), the outcomes of plant-endophyte interactions remain elusive. Experimental studies on plant-endophyte interactions are commonly carried out in controlled conditions manipulating one endophyte species under limited biotic and abiotic conditions. In the wild, plants host a large diversity of endophytic organisms (bacteria, fungi, archaea…), but also interact with many other organisms including other plants, non-endophytic microbes and herbivores. Endophytes and other plant-associated organisms may also interact with each other. These interactions are likely key drivers of the structure of endophytic communities. In this study, we focus on how insect and tree species mixtures may modify foliar endophytic fungi communities in one host tree species.

Endophytic fungi are one of the key drivers of plant-herbivore interactions, with several studies reporting negative impacts of endophytic fungi on herbivorous insects (Clay, 1996; Clay and Schardl, 2002; but see Kuldau and Bacon, 2008; Rudgers and Clay, 2008; Saikkonen et al., 2010; Faeth and Saari, 2012; Fernandez-Conradi et al., 2018). However, reciprocal studies on the impact of insect herbivores on fungal endophytes are comparatively scarce. Insects induce changes in host plant quality and production of defensive compounds, which may impact endophytic fungi. Particularly, gall-forming insects can manipulate and reprogram host plant development, inducing spectacular morphological and physiological changes in host plant tissues (Giron et al., 2016). Galls may act as plant metabolic sinks, thus affecting host plant quality for fungi. Furthermore, insect larvae inside galls can represent supplementary source of nutriments for endophytic fungi, since several endophytes are entomopathogens (Wilson, 1995). However, studies on the impact of gall-makers on endophytic fungi are still scarce (but see Lawson et al., 2014; Washburn and Van Bael, 2017). For instance, Lawson et al. (2014) showed that endophytic fungal communities in poplars (*Populus spp*.) differed between aphid-induced galls and intact leaves. This result is consistent with those found by Washburn and Van Bael (2017) on galls formed by midges on bald cypress. Furthermore, these authors also found differences between fungal diversity and composition of galls with and without insect emergence, suggesting that insects might bring some fungi into their galls.

Independently of herbivore attack, plant diversity may also be an important factor driving plant-endophyte interactions (Saikkonnen et al. 2007; Nguyen et al. 2016; Jactel et al. 2017). Endophytic fungi of trees are usually horizontally transmitted, i.e. from one tree to its neighbour, by contrast to grass endophytic fungi which are vertically transmitted, i.e. from one plant to its offspring (Partida-Martínez and Heil, 2011). Increasing tree diversity in forest stands may thus result in a 'dilution effect', as non-host tree species may act as barriers to spore transmission of specialist fungi thus reducing the number of fungi on a given target tree. In contrast, for generalist fungi, increase in tree diversity may result in a 'contagion' process from other neighboring species present in mixed plots, thus resulting in higher diversity of fungal communities in mixed-species forests. These two different mechanisms may explain why studies testing the effect of tree species diversity on endophytic fungal communities provide inconsistent results. For instance, endophytic fungal diversity in Norway spruce (*Picea abies*) needles was higher in pure spruce stands than in spruce mixtures with Pubescent birch (*Betula pubescens*) or Scots pine (*Pinus Sylvestris*; Müller and Hallaksela 1998). By contrast, Nguyen et al. (2016) showed that the significant effect of tree diversity on the structure of communities of foliar fungi infecting Norway spruce trees in European forests depended on the forest type. They suggested that tree species identity and tree species composition could blur the sole effect of tree diversity, but this question remains to be explored.

In this study, we analyzed endophytic fungal communities in leaf galls induced in chestnut (*Castanea sativa*) leaves by the Asian chestnut gall wasp *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera: Cynipidae), and compared them to communities inhabiting the surrounding foliar tissues. We hypothesized that endophytic fungal communities are (i) different between galls and surrounding foliar tissues because of differences in nutrients and secondary compounds concentration between gall and foliar tissues; and (ii) affected by tree neighbors' diversity. To test this hypothesis we sampled galled chestnut leaves in natural mature forest plots where chestnuts were growing alone or mixed with pine (*Pinus pinea*), oak (*Quercus cerris*) or ash (*Fraxinus ornus*). We characterized endophytic fungi communities by ITS meta-barcoding. By addressing the above, this study builds towards a better knowledge of foliar endophytic fungal communities associated with *D. kuriphilus* galls and more generally a better understanding of the forces driving plant-endophyte interactions (specifically insect and plant diversity effects).

MATERIALS ANDS METHODS

Model system

The Asian chestnut gall wasp, *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera: Cynipidae) is a micro-Hymenoptera native to China and considered the most important invasive pest of *Castanea* species worldwide (Moriya et al., 2003). This univoltine species, which reproduces by parthenogenesis, is the only known insect forming galls in chestnuts. In Europe, between mid-June

and mid-August, short-living females emerge from galls. They immediately lay eggs in chestnut buds, in which larvae hatch in summer. First instar larvae overwinter within dormant buds until the following spring (Bernardo et al. 2013). At the time of chestnut bud-burst (around mid-April in Europe), larval development prevents normal plant organogenesis and induces the formation of galls, which developed on buds, leaves, stipulas or shoots (Maltoni et al. 2012). Gall formation can reduce leaf photosynthetic area, which can result in a reduced vigor of infected shoots (Ugolini et al. 2014). Severe and repeated infestations on young trees can even lead to tree death (Moriya et al. 2003).

Dryokosmus kuriphilus populations are mainly regulated by hymenopteran parasitoids (Cooper and Rieske 2007). Morever, research on the potential use of entomopathogenic fungi like *Fusarium proliferatum* as biocontrol agents for *D. kuriphilus* is increasing (Tosi et al., 2015). Recently, necrosis found in *D. kuriphilus*-induced galls in Italy was attributed to the endophytic fungus *Gnomoniopsis castanea,* which is seen as another putative biocontrol agent (Vannini et al., 2017).

Sampling design

The study was performed in old coppice stands of Southern Tuscany (Italy) already described in Fernandez-Conradi et al. (2017a). These forests are mainly composed of European chestnut (*Castanea sativa*), Hop hornbeam (*Ostrya carpinifolia*), oaks (*Quercus ilex*, *Q. petraea*, *Q. suber, Q. pubescens* and *Q. cerris*), ash (*Fraxinus ornus*) and maritime pine (*Pinus pinaster*). We sampled 28 chestnut plots consisting of eight chestnut monocultures and 20 two-species mixtures, *C. sativa* with *Q. cerris* (n = 8), *F. ornus* (n = 8) or *P. pinaster* (n = 4). In each plot, in June 2015, we selected three chestnut trees and sampled three leaves bearing fresh galls (one per branch, three branches located in different orientations). Leaves were introduced into hermetic plastic bags and dried with silica gel and kept frozen at -80°C until DNA extraction. We also haphazardly sampled three leaves per plot from chestnut associated tree species (oak, ash or pine, Supplementary material, Appendix A).

DNA extraction and sequencing

In a microbiological safety workbench, four small pieces (of 1 mm^2 each) were cut from the center of each gall and two leaf discs (each 8 mm diameter) were sampled from the foliar tissues surrounding the gall. The punches and razors used were sterilized between each gall and leaf sample using 70 % ethanol and a flame. With the aim of studying endophytic fungi and not epiphytic or commensal fungi, gall and leaf discs were sterilized according to Bàlint et al. (2015): they were first immersed in 4% sodium hydrochloride solution for 1 min and then washed twice in a sterile solution containing 0.1% of Tween ® 20 (Sigma-aldrich company) to break surface tension. Total DNA for each foliar and gall tissue sample was extracted with the DNeasy 96 Plant Kit (QIAGEN). A DNA extraction was also performed from two tubes left opened in the bench during the whole extraction operation, to serve as negative controls. Before DNA amplification, the three “gall tissue” DNA extracts from one tree and the three “associated foliar tissue” DNA extracts were pooled to have two single DNA samples per

tree, one from gall and one from associated foliar tissues. Fungal Internal Transcribed Spacer 1 (ITS1) barcode was amplified with the ITS1 (forward) and ITS2 (reverse) primer pair (Gardes and Bruns, 1993). Paired-end sequencing (300 bp) was performed in a single run of an Illumina MiSeq sequencer, on the basis of V3 chemistry. Polymerase Chain Reaction (PCR) amplification, barcodes and MiSeqadapters addition, library sequencing and data preprocessing were carried out by the LGC Genomics sequencing service (Berlin, Germany). Sequences were deposited in the European Nucleotide Archive (ENA) database, under the PRJEB30131 project accession number (http://www.ebi.ac.uk/ena/data/view/PRJEB30131).

Bioinformatic analysis

Sequences were first demultiplexed and filtered. All sequences with tag mismatches, missing tags, one-sided tags or conflicting tag pairs were discarded. Tags and Illumina TruSeq adapters were then clipped from all sequences, and sequences with final length shorter than 100 bases were discarded. All sequences with more than three mismatches with the ITS1 and ITS2 primers were discarded. Primers were then clipped and sequence fragments were placed in forward-reverse primer orientation. Forward and reverse reads were then combined with the BBmerge software. Read pair sequences that could not be combined were discarded. See also appendix B (Supplementary material) for detailed information about reads number per sample, along bioinformatic process.

The remaining high quality sequences were processed following the pipeline developed by Bálint et al. (2014). The ITS1 sequence was first extracted from each read with the FungalITSextractor (Nilsson et al., 2010). All the sequences were then concatenated in a single fasta file, after adding the sample code in the label of each sequence. The sequences were dereplicated, sorted and singletons were discarded with VSEARCH (https://github.com/torognes/vsearch). The sequences were then clustered into operational taxonomic units (OTUs) with the UPARSE algorithm implemented in USEARCH v8 (Edgar, 2013), with a minimum identity threshold of 97%. Additional chimera detection was performed against the UNITE database (Kõljalg et al., 2013), with the UCHIME algorithm implemented in USEARCH v8 (Edgar et al., 2011). The curated OTU table, giving the number of sequences in each OTU for each sample, was created with USEARCH v8. OTUs were taxonomically assigned with the online BLAST web interface (Madden, 2013) against the GenBank database, by excluding environmental and metagenome sequences. Only the assignment with the lowest *e*-value was retained. The full taxonomic lineage of each assignment was retrieved from the GI number information provided by NCBI. All the OTUs assigned to plants or other organisms, and all unassigned OTUs were removed, to ensure that only fungal OTUs were retained.

Statistical analyses

All statistical analyses were performed in *R 3.4.1*. To account for unequal number of reads per sample, one hundred random rarefied OTU matrices were computed, using the smallest number of sequences

per sample (717) as rarefaction threshold. OTU richness (number of OTUs), Shannon diversity index (taking into account both richness and abundance) and Bray-Curtis dissimilarity index were calculated for each rarefied matrix, and averaged across the 100 matrices (Fort et al., 2016; Jakuschkin et al., 2016). Linear mixed effect models were used to test the effect of tissue (leaf vs. gall) and tree species composition on OTU richness and diversity, including stand and chestnut tree nested within stand as random factors to account for the non-independence of the three leaf samples per tree and the three tree replicates per stand (Schielzeth and Nakagawa, 2013). We applied model simplification by removing non-significant interactions prior estimating significance of principal effects. We used the *lmer* function from the *lmer4* package (Bates et al., 2015) after linearization of the index (richness and Shannon diversity) to obtain the effective OTUs number (Jost, 2006). Package *car* (Fox and Weisberg 2011) was used to estimate the significance of fixed effects.

Permutational Analysis of Variance (PERMANOVA) was carried out to compare endophytic fungal communities present in chestnut galls vs. in surrounding foliar tissues, and to study the effect of tree species mixture on community composition. Sampling plot was included as strata factor in the PERMANOVA.

Beta diversity between samples was assessed using Bray-Curtis dissimilarities (average from rarefied tables) and visualized with Principal Component Analysis (PCoA). Beta-diversity partitioning was used to separate turnover from nestedness-resultant components of endophytic community composition between chestnut galls and surrounding foliar tissues (Baselga, 2010).

RESULTS

Taxonomic description of sequences and OTUs

We found 6,401,652 high-quality sequences, clustered into 1,378 OTUs. We discarded 97 OTUs (accounting for 55,306 sequences, i.e. 0.86% of the raw OTU table) that were not taxonomically assigned to fungi with the BLAST interface against NCBI database. Among them, a single OTU was assigned to the Eumetazoa kingdom, another one to the Chlorophyta division and five to the Tracheophyta division, from *Quercus*, *Castonopsis* and *Fraxinus* genera. The total OTU table used for analyses contained 6,336,807 sequences representing 1,274 fungal OTUs and distributed within 181 samples, with 97 foliar (84 from chestnut leaves, and 13 from chestnut heterospecific neighbors) and 84 gall communities. The mean number of sequences per sample was 35,010 ranging from 717 to 188,765, except in five samples that contained less than 500 sequences and were discarded. The 1,274 OTUs were assigned to 670 different fungal species in NCBI database. The studied communities were largely dominated by Ascomycota (Figure 1, Table 1). Other divisions and unassigned sequences at the division level represented together 1.5% of the sequences.

The number of reads and OTUS detected in the negative control samples were used to assess the quality of DNA extraction and amplification. Only 8,745 sequences (0.14% of the total fungal sequences) and 22 OTUs were found in these negative controls. One sample contained 491 reads and 6

fungal OTUs. The second one contained 8,491 sequences, among which 2,397 and 1,979 reads represented two dominant OTUs that were assigned to *Diplodia seriata* and *Cryptococcus spp*. respectively. There is no current consensus on how to deal with sequences in negative controls (Nguyen, 2015). We thus decided to keep those OTUs in the dataset.

We found 763 different fungal OTUs in chestnut leaves and *D. kuriphylus* galls. Among them, a single OTU (OTU 1, Table 1) represented 55.89% of the reads. This OTU was assigned to *Gnomoniopsis castanea* (Sordariomycetes, Ascomycota). OTUs 955, 1 051, 1 058, 1 112, 1 165 and 1 168 were also assigned to this species. Twelve other OTUs detected in galls represented more than 1% of the reads (Table 1).

Fungal diversity in *D. kuriphilus* galls and surrounding foliar tissues

Both richness and Shannon diversity of fungal OTUs were significantly lower in galls than in surrounding foliar tissues ($\chi^2 = 12.778$; $df = 1$; $P < 0.001$ and $\chi^2 = 78.038$; $df = 1$; $P < 0.001$ respectively, Figure 2).

The first two PCoA axes explained 28.2 and 6.7% of variance in OTU composition of fungal communities inhabiting galls and surrounding foliar tissues (Figure 3). The two communities were significantly different (PERMANOVA: $F = 15.31$; $R^2 = 0.09$; $P = 0.001$). The β-diversity partitioning revealed that dissimilarity between these two fungal communities was mostly explained by turnover (87.1% of total dissimilarity) with low nestedness (12.9%), indicating that the lower OTU richness and diversity in galls as compared to foliar tissues were not primarily due to exclusion of some OTUs.

Effect of stand composition on leaf and galls endophyte communities

Endophytes OTU richness and Shannon diversity in chestnut galls were unaffected by tree species composition of sampled forests ($F = 0.42$; $df = 3$; $P = 0.738$ and $F = 0.24$; $df = 3$; $P = 0.867$ respectively). Likewise, OTU richness and Shannon diversity in chestnut foliar tissues were independent of tree species composition ($F = 1.90$; $df = 3$; $P = 0.135$ and $F = 0.99$; $df = 3$; $P = 0.403$ respectively). Tree species composition had no effect on the structure of endophytic fungal communities neither in galls (Figure 4a; $F = 1.21$; $R^2 = 0.04$; $P = 1.000$) nor in foliar tissues (Figure 4b; $F = 1.38$; $R^2 = 0.04$; $P = 1.000$).

DISCUSSION

Our study provides the first environmental data describing communities of endophytic fungi inhabiting insect-induced galls in chestnut. They show that foliar endophytic fungal communities can be shaped by attacks of galling insects, irrespective of forest diversity or composition.

We identified a large amount of fungal sequences in galls caused by *D. kuriphilus* in chestnut foliar tissues, clustered in 763 different fungal OTUs. This number of fungal OTUs is much higher than the

number of morphotypes found by Lawson *et al.* (2014) in aphid-induced galls and leaves in poplars (2014, 23 morphotypes) and the number of OTUs found by Washburn and Van Bael (2017) in midge-induced galls in bald cypress (34 OTUs defined on isolated strains). In addition to species specific effects, these differences may be due to the technique used in these studies (culture – based) as compared to our use of meta-barcoding with new generation sequencing technique, which enables identification of fungal strains that are reluctant to grow on agar medium.

Interestingly, the most common OTU present in our chestnut gall samples (representing 56% of the reads) was assigned to *Gnomoniopsis castanea* (Gnomoniaceae, Diaporthales). This species is now considered a latent pathogen, which can infect flowers, leaves and chestnut branches and can cause necrosis in branches, leaves and fruits (reviewed in Lione et al. 2018). It has also been associated with chestnut gall necrosis (Magro et al., 2010; Seddaiu et al., 2017; Vannini et al., 2017), but its effectiveness in regulating *D. kuriphilus* populations remains controversial (Lione et al. 2016; Vannini et al. 2017). We found *G. castanea* in all living galls even if they were not necrotic. This suggests that *G. castanea* is not an efficient at regulating *D. kuriphylus*. The genus *Fusarium* represented about 1.5% of OTU sequences amplified in our samples. *F. proliferatum* was shown to be associated with high *D. kuriphilus* mortality rates in the laboratory and to be nonpathogenic to chestnut trees (Tosi et al., 2015). However, in our samples, the amplified sequences matched with *F. lateritium* (also found in galls by Seddaiu et al., 2017), *F. ciliatum*, *F. oxysporum* and other unidentified *Fusarium spp*. but not with *F. proliferatum*. This might be due to the low frequency of *Fusarium* in galls or to methodological problems in identifying and differentiating species of this genus.

Foliar endophytic fungi communities differed between galls and surrounding foliar tissues

Fungal communities in galls differed, by their diversity and composition, from those inhabiting surrounding foliar tissues. Most differences between the two tissues were due to species turnover, indicating a low overlap between gall and leaf inhabiting species, which was also found by Washburn and Van Bael (2017). However, contrary to our expectation, OTU richness and diversity were lower in galls than in surrounding leaf tissues. This result contrasts with those reported by Lawson et al. (2014) and by Washburn and Van Bael (2017), who found similar richness and diversity in galls and surrounding tissues (13 *vs* 15 and 18 *vs* 20 species, respectively).

Differences in the composition of endophytic communities between chestnut galls and surrounding foliar tissues may have two causes. First, different external sources of endophytic fungi may explain different fungal composition in galls and foliar tissues. Such external inoculum, not currently associated with the plant in the absence of galls, may be aerial and deposited on galls in development or brought by gall-making insects during oviposition (Wilson, 1995; Washburn and Van Bael, 2017). This hypothesis is consistent with the observation that in several cases, insects can act as vectors of

plant pathogenic fungi (Kluth et al., 2002) or are themselves infected by entomopathogenic fungi. However, this hypothesis has received only indirect support (see Washburn and Van Bael, 2017). Second, endophyte composition may be dependent on tissue traits (particularly primary and secondary metabolites), which differs between *D. kuriphylus* gall and foliar tissues (Fernandez-Conradi et al. 2017b). Galling insects are able to divert plant nutrients to the galls (Allison and Schultz, 2005; Giron et al., 2016) or to modify levels of secondary metabolites acting as defence compounds, which may affect endophytic fungi. Altogether, these considerations suggest that galls and foliar tissues provided different habitat conditions to the local pool of endophytes. How these changes can modify the physical and chemical requirements of endophyte infection requires more investigations.

The lack of effect of tree species mixture on foliar endophytic fungi communities in gall and surrounding foliar tissues

We found no overall effect of forest specific composition on the diversity and composition of endophytic communities in *D. kuriphylus* galls or chestnut foliar tissues. Tree diversity effects may be dependent on the host specificity of fungi. For specialist fungi, non-host tree species may acts as barriers to spore transmission, resulting in a 'dilution effect' for fungal inoculum and thus reduced fungal species richness. For generalist fungi, several tree species may act as alternative hosts and thus increase the probability of successful establishment, leading to higher fungal species richness in forest mixtures. In this study, we did not focus only on one fungal species but on the whole endophytic community of chestnut. So, the lack of overall effect of forest stand composition, could result from endophytic communities being a mix of generalist and specialist endophytes, which are differently affected by tree diversity (Saikkonen, 2007; Nguyen 2017). In fact, when analyzing endophyte communities of foliar tissues from tree neighbors in the stand (pines, oaks and ashes), we found that they differed from chestnut galls and foliar tissues communities (see Appendix A), with several specific and shared OTUs (Figure 5, Appendix A). This may suggest that tree diversity effect, if there is any, may be blurred at the fungal community level.

CONCLUSION

Our study contributes to a better understanding on how galling insects and plant diversity may affect endophytic fungal communities of chestnut. Endophytic communities in insect galls differ from those in surrounding leaf tissues both in terms of richness and composition. This suggests that physiological changes in plant tissues triggered by gall induction are important determinants of endophyte assemblages. How these changes can modify the physical and chemical requirements of endophyte infection remains elusive. The fact that fungal endophyte communities in both galls and associated tissues were independent of tree species composition of forest stands is probably due to the large variability in endophytes species traits, particularly their host specificity. More research on functional

diversity of leaf-inhabiting fungi is clearly needed to better disentangle the mechanisms underlying plant- endophytic fungi interactions in forests.

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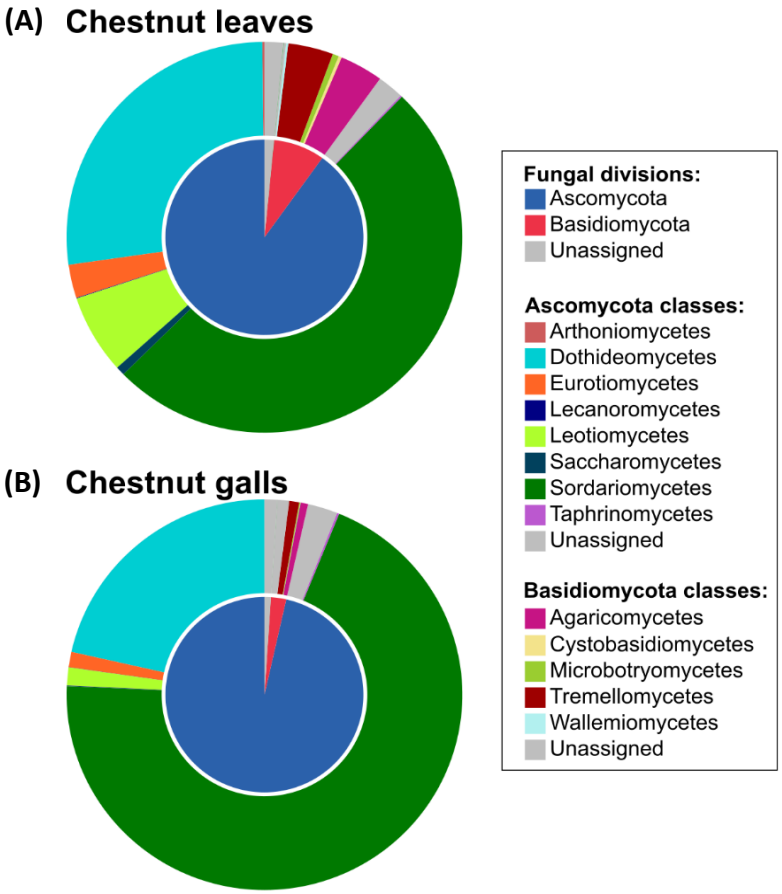
Figure 1. Taxonomic composition of chestnut leaf (A) and *Dryocosmus kuriphylus* gall-inhabiting fungi (B). The inner disc shows the proportion of sequences assigned to each taxonomic division and the outer disc the proportion of sequences assigned to the most abundant classes in each division.

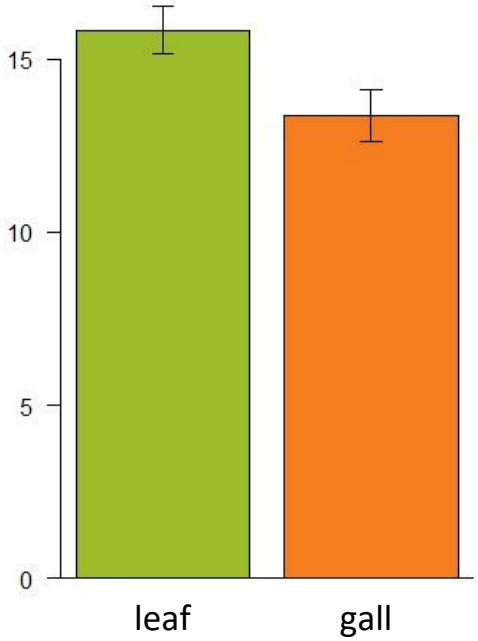
Figure 2. OTU richness (A) and Shannon diversity (B) of fungal endophytic communities in *Dryocosmus kuriphylus* galls and surrounding leaves tissues. Error bars represent the standard error of the mean (n= 84 leaves and 84 galls).

Figure 3. Principal Coordinate Analysis showing OTU distribution in chestnut galls (in orange) and surrounding leaf tissues (in green). Dissimilarities between samples were computed with the Bray-Curtis index, averaged over 1000 random permutations.

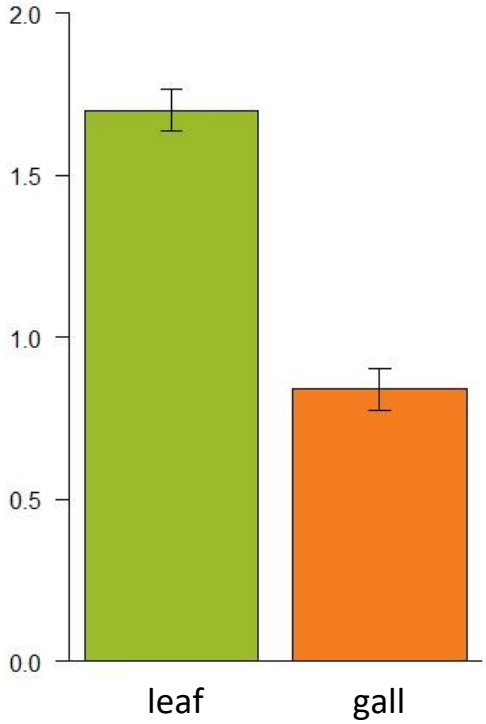
Figure 4. Principal Coordinates Analyses representing dissimilarities in the composition of fungal endophytic communities in (A) chestnut galls and (B) surrounding leaf tissues. Different colors and ellipses represent the composition of tree species in the plot. Dissimilarities between samples were computed with the Bray-Curtis index, averaged over 1000 random permutations.

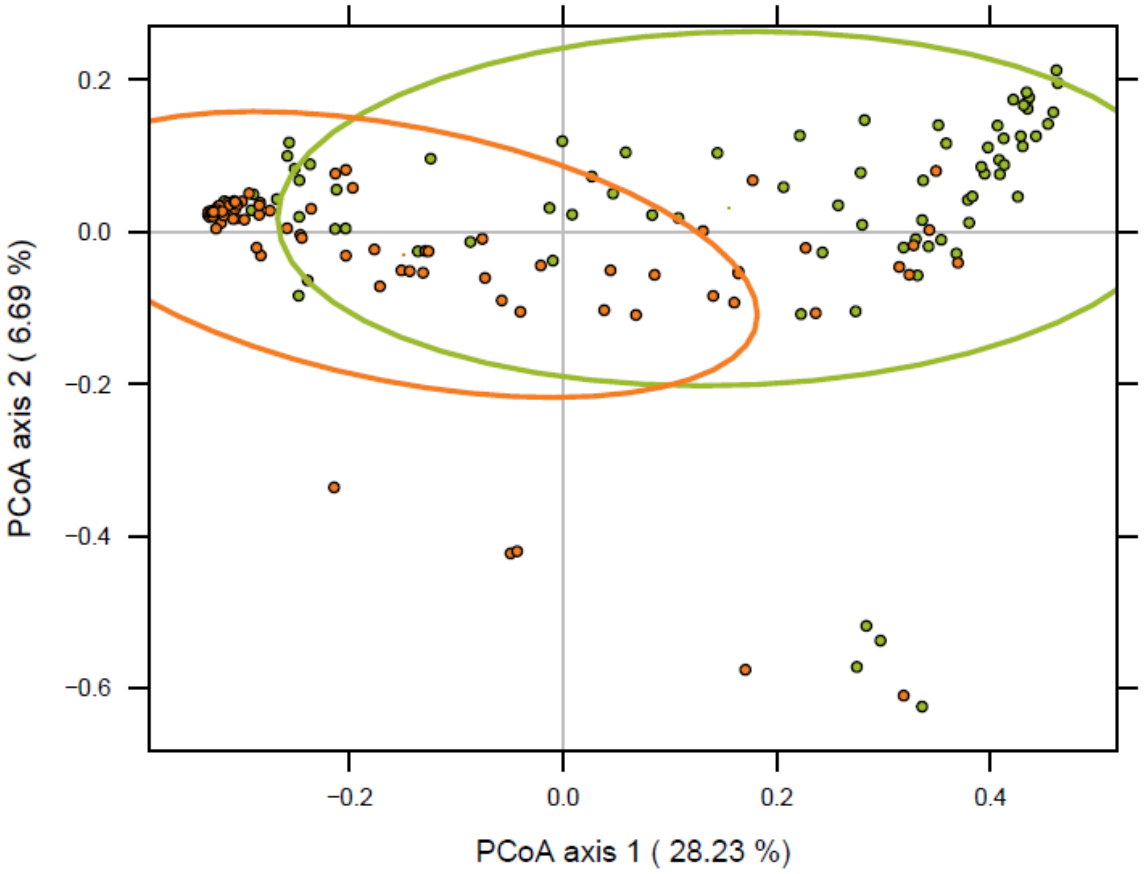

(A) Chestnut leaves
(B) Chestnut galls
Fungal divisions:
Ascomycota
Basidiomycota
Unassigned
Ascomycota classes:
Arthoniomycetes
Dothideomycetes
Eurotiomycetes
Lecanoromycetes
Leotiomycetes
Saccharomycetes
Sordariomycetes
Taphrinomycetes
Unassigned
Basidiomycota classes:
Agaricomycetes
Cystobasidiomycetes
Microbotryomycetes
Tremellomycetes
Wallemiomycetes
Unassigned

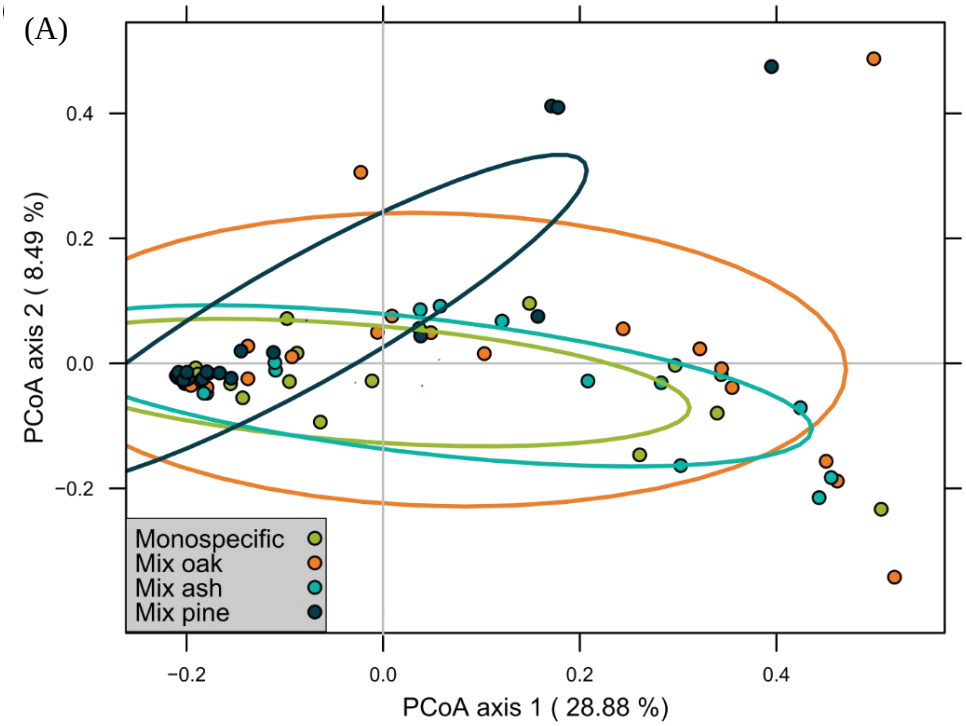
(A) Richness



(B) Shannon diversity




0.2
0.0
−0.2
−0.4
−0.6
PCoA axis 2 (6.69 %)
−0.2
0.0
0.2
0.4
PCoA axis 1 (28.23 %)


(A)
PCoA axis 2 (8.49 %)
PCoA axis 1 (28.88 %)
Monospecific
Mix oak
Mix ash
Mix pine

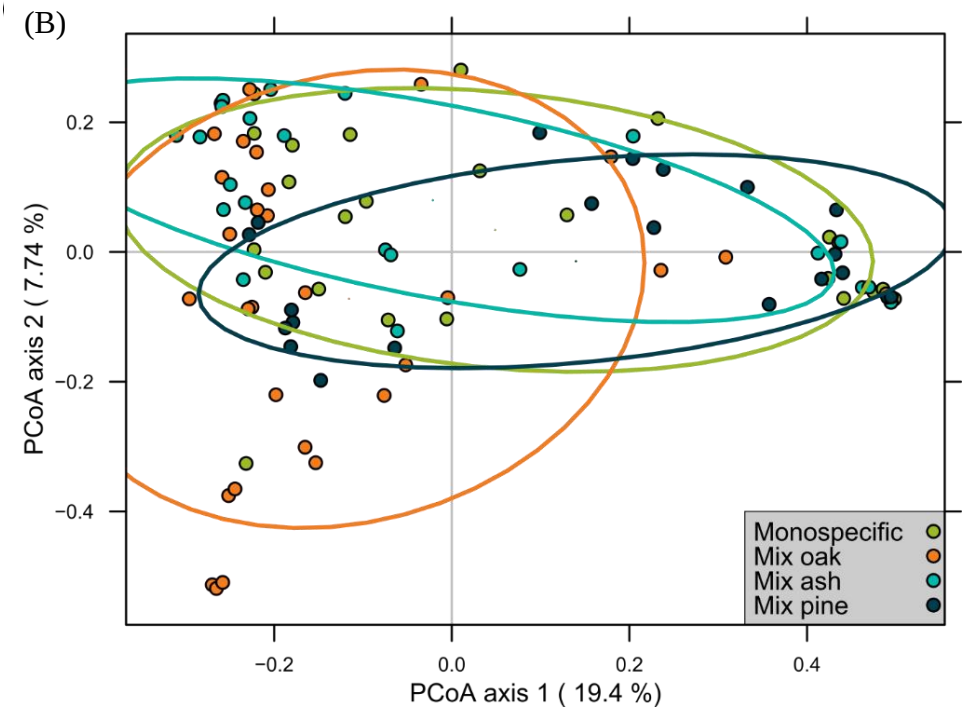

(B)
PCoA axis 2 (7.74 %)
PCoA axis 1 (19.4 %)
Monospecific
Mix oak
Mix ash
Mix pine

Table 1. Taxonomic assignment of the 12 OTUs, representing more than 1% of total sequences, found in chestnut galls and leaves (online BLAST analysis against the NCBI database).

OTU number	Putative class	Putative species/taxon	Total abondance (reads number)	Relative abondance (%)
1	Sordariomycetes	*Gnomoniopsis castanea*	1, 845,964	55.89
2	Sordariomycetes	*Trichothecium roseum*	187,145	5.67
3	Dothideomycetes	*Alternaria sp.*	170,375	5.16
4	Dothideomycetes	*Diplodia seriata*	111,741	3.38
7	Dothideomycetes	*Stemphylium vesicarium*	86,460	2.62
14	Unknown	*Ascomycota sp.* D7	73,807	2.23
10	Dothideomycetes	*Botryosphaeria dothidea*	69,926	2.12
6	Sordariomycetes	*Sordario sp.*	66,545	2.01
13	Dothideomycetes	*Pyrenochaeta cava*	53,327	1.61
15	Sordariomycetes	*Trichothecium roseum*	50,226	1.52
22	Sordariomycetes	*Diaporthe sp.* G360	44,255	1.34
5	Letiomycetes	*Botryotinia pelargonii*	43,446	1.32