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Endophytic fungal communities of ancient wheat varieties

Giulia CASINI[1,2], Thaer YASEEN[3], Ahmed ABDELFATTAH[4,5*], Franco SANTORO[6], Leonardo VARVARO[1], Sandro DRAGO[2], Leonardo SCHENA[4]

[1] *Department of Agriculture and Forestry Science, University of Tuscia, Via S. Camillo de Lellis s.n.c., 01100 Viterbo (VT), Italy*
[2] *Enbiotech S.r.l., via Quarto dei mille, 6, 90144 Palermo (PA), Italy*
[3] *Regional Office for the Near East and North Africa Region (RNE), Food and Agriculture Organization of the United Nations (FAO), Cairo, Egypt*
[4] *Dipartimento di Agraria, Università Mediterranea di Reggio Calabria, Località Feo di Vito, 89122 Reggio Calabria (RC), Italy*
[5] *Department of Ecology, Environment and Plant Sciences, Stockholm University, SE-106 91, Stockholm, Sweden*
[6] *CIHEAM – Istituto Agronomico Mediterraneo, Via Ceglie 9, 70010 Valenzano (BA), Italy*
* Corresponding author: ahmed.abdelfattah@su.se

Abstract. The fungal community composition and structure of two ancient tetraploid wheat varieties, native to the Sicilian territory of Italy, Perciasacchi (winter wheat) and Tumminia (spring wheat) were investigated using High Throughput Sequencing (HTS). This showed a predominance of Ascomycetes and Basidiomycetes including *Alternaria*, *Fusarium*, *Mycosphaerella, Filobasidium, Cystofilobasidium, Cryptococcus*, *Leucosporidium*, *Dioszegia, Puccinia*, *Sporobolomyces, Aureobasidium, Cladosporium, Holtermanniella* and *Gibberella*. Principal Coordinates Analysis (PCoA) and Linear discriminant analysis Effect Size (LEfSe) showed that *Aureobasidium, Leucosporidium* and *Puccinia* differentiated between the two wheat varieties. In addition, the microbial association analysis suggested that some endophytic taxa play important roles within the wheat fungal community. Genera such as *Cryptococcus* and *Cystofilobasidium* were shown to have consistent antagonistic activity against *Gibberella* spp., while, *Acremonium* and a group of unidentified ascomycetes had mutual exclusion relationships with *Puccinia*. Since both *Gibberella* and *Puccinia* contain several economically important pathogens of wheat, the detected fungal interactions may indicate microbial-mediated resistance in these wheat varieties.

Keywords. TS, fungal diversity, microbial ecology, microbiome, domestication.

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INTRODUCTION

Plant-associated microorganisms, collectively referred to as the plant microbiota, are known to influence plant physiological development, and response to environmental changes by conferring stress tolerance, disease resistance, and affecting interactions with competitors and predators

(Lugtenberg and Kamilova 2009; Berendsen *et al.,* 2012). Endophytes are microorganisms that can be isolated from surface-disinfested plant tissues or extracted from within plants, and that do not harm plant growth (Hallmann *et al.,* 1997). Endophytes have gained importance in basic and translational science due to their potential roles as biocontrol agents (Dorworth and Callan 1996; Massart *et al.,* 2015; Abdelfattah *et al.,* 2018).

Wheat (*Triticum* sp.) is a global staple food crop adapted to a wide variety of environmental conditions, including marginal areas (Salmon and Clark, 1913). In Italy, production of durum wheat (*Triticum durum*) accounts for more than 50% of the total European production (European Commission, Eurostat and DG Agriculture and Rural Development). Production of this crop is mainly concentrated in the hot and dry southern regions of Italy, including Sicily (https://gain.fas.usda.gov, http://dati.istat.it). Recently, local ancient wheat varieties have acquired increased importance due to their peculiar organoleptic properties, which has fueled expanding craft milling and bakery enterprises (Shewry and Hey, 2015; Jankielsohn and Miles, 2017). For example, in Sicily, the importance of local ancient tetraploid wheat landraces is increasing. This is due to the quality of their flour, their low nutritional requirements, and their ability to grow in dry environmental conditions typical of the Sicilian region. This has sparked scientific curiosity towards the molecular determinants of such adaptation capabilities.

The possibility of finding microbial strains capable of improving wheat productivity and tolerance to biotic and abiotic factors has motivated scientific studies in the last decade (Coombs and Franco, 2003; Larran *et al.,* 2007; Velazquez-Sepulveda *et al.,* 2012; Hubbard *et al.,* 2014). Advances in sequencing and computational applications, such as metabarcoding (Taberlet *et al.,* 2012), have enabled detailed characterization of microbial diversity (Turner *et al.,* 2013; Ofek-Lalzar *et al.,* 2014). Most studies have focused on the bacteria while little is known about fungi. It is becoming increasingly clear that fungal microbiota play critical roles in plant growth, development and stress tolerance (Shendure and Ji, 2008; Nicolaisen *et al.,* 2014; Abdelfattah *et al.,* 2018).

It remains unclear whether locally-adapted wheat varieties are capable of recruiting distinct microbiota, and, if so, to what extent this differential recruitment is influenced by environment and the host genotype. As a first step towards deciphering the contribution of wheat microbiota to crop adaptation and yield, we here report molecular characterization of the composition of the fungal endophytic communities of two locally adapted Sicilian tetraploid wheat varieties, the winter type Perciasacchi and the spring type Tumminia, grown in two agricultural fields.

MATERIALS AND METHODS

Experimental design

This study was carried out in two fields (Field 1 and Field 2), of almost 1 ha each, located in the Madonie area of the Sicilian inland. Field 1 (37.7813410 N, 14.2852990 E), was located at 850 m above sea level and was previously used for legume culture. Field 2 (37.740245 N, 14.239368 E) was located at 800 m above sea level and was uncultivated for almost 5 years. One half of both fields was sown with Perciasacchi in the second week of November 2015, while the other half was sown with Tumminia in the first week of February 2016. The fields and seed were not subjected to any treatments before or after sowing. Based on the field topography, 23 sampling plots were selected in Field 1 and 27 were selected in Field 2.

Plant sampling, surface sterilization and sample preparation

During the heading phase of both varieties, a total of 50 samples were collected from the selected sampling plots. Each sample consisted of ten plants including their roots. Samples were transported to the laboratory and stored at 4°C before processing. The aerial parts of each plant were cut using sterile scissors and a stem portion, of approx. 15 cm above the crown, was kept for sap extraction. Stem and root samples were surface-sterilized using 5% sodium hypochlorite (NaClO), and then rinsed in sterile water. Sap extraction was carried out with the use of a new method, the CIHEAM-IAMB patented 'Method for the extraction of sap from plant material and apparatus for carrying out the method' (https://patents.google.com/patent/WO2017017555A1/en). This method of extracting plant sap from vessels and xylem using the pressure of a syringe has the advantage of reducing plant components in the final extract that can inhibit enzyme activity, adversely affecting the results of diagnosis. Sap extract was obtained by inserting 1 mL of phosphate-buffered saline (PBS) solution into one terminal of the plant stem using a syringe. PBS with the sap extracts was collected from the other terminal of the plant stem in a sterile 1.5 mL capacity tube, which was later used for plating and DNA extraction. Twenty-eight samples were randomly chosen, seven from each half of each field (14 from each wheat variety), for high throughput sequencing (HTS).

Culture conditions for endophytic fungi and DNA extraction

Endophytic fungi were cultured by aseptically transferring 100 µL of each wheat sap extract to 9 cm diam. Petri dishes containing semi-selective Nutrient Yeast Dextrose Agar (NYDA; containing 10 g L^{-1} glucose, 5 g L^{-1} yeast extract, 8 g L^{-1} nutrient broth, 18 g L^{-1} agar, 250 mg L^{-1} streptomycin sulphate and 250 mg L^{-1} ampicillin) (Janisiewicz and Roitman, 1988). The inoculated dishes were incubated at 26°C for 48-72 h. After incubation, a small portion of the growing mycelium was transferred to new Potato Dextrose Agar (PDA). A small portion of the subsequently grown mycelium was transferred from each plate into a unique sterile 1.5 mL capacity tube, and stored at -20°C. DNA extraction was carried out from 400 µL of wheat sap extract and the mixed fungal mycelium, using the NucleoSpin® Plant II extraction kit (Macherey-Nagel) following the manufacturer's instructions and doubling the pre-lysis and lysis incubation periods.

Metabarcoding analyses

Samples for metabarcoding were grouped into the following four categories including seven biological replicates: P1 (Perciasacchi grown in Field 1), T1 (Tumminia grown in Field 1), P2 (Perciasacchi grown in Field 2) and T2 (Tumminia grown in Field 2).

Amplifications of the fungal ribosomal Internal Transcribed Spacer 2 (ITS2) region were performed using the forward primer ITS86F (Turenne *et al.,* 1999) and mix reverse primers ITS4-Mix 1, ITS4-Mix 2, ITS4-Mix 3, and ITS4-Mix 4 (Tedersoo *et al.,* 2014; 2015). Amplifications of the ITS2 region were performed using KAPA HiFi Hot Start ReadyMix kit (KAPA Biosystems), under a temperature profile of 95°C for 3 min, 35 cycles at 98°C for 20 sec, 56°C for 15 sec, 72°C for 30 sec, followed by an elongation step of 72°C for 1 min. PCR products were visualized on 2% agarose gel in order to verify the successful amplification and the absence of contamination. PCR purification was performed using Agencourt AMPure XP beads kit and following the user manual instructions (Beckman Coulter). Amplicon indexing was carried out using Nextera XT v2 Index Kit (Illumina) and followed by a second PCR purification as previously described. Amplification products were quantified by fluorimetry using Qubit (Invitrogen) and pooled in equimolar concentrations before sequencing reactions in MiSeq (Illumina) according to the manufacturer guidelines (support.illumina.com). Datasets generated during this study were deposited and are available at the National Center for Biotechnology Information (NCBI), Sequence Read Archive (SRA), under the accession number PRJNA449228 (www.ncbi.nlm.nih.gov/bioproject/ PRJNA449228).

Sequencing data analyses

Raw reads were trimmed using Trimmomatic v. 0.32 (Bolger *et al.,* 2014), using a sliding window of six bases, Q-score average of ≥ 20, and a minimum sequence length of 150 bp. The paired-end reads were then assembled using PANDAseq Assembler (Masella *et al.,* 2012), setting a minimum sequence length value of 150 bp and a minimum overlapping value of 20 bp. Reads were then checked and filtered for chimeric sequences using VSEARCH v.1.11.1 (Rognes *et al.,* 2016) and the UNITE dynamic database released on November 20, 2016 (https://unite.ut.ee). The same database was also used for creating Operational Taxonomic Units (OTUs), with a similarity threshold of 99% and for taxonomy assignments with BLAST method (Altschul *et al.,* 1990) as implemented in QIIME v. 1.9.1 (Caporaso *et al.,* 2010). These taxonomic units were collapsed to describe the fungal community at the genus, family, order, class, and phylum levels.

Downstream analyses

The downstream analyses were conducted using QIIME 1.9.1 pipeline (Caporaso *et al.,* 2010) as described by (Abdelfattah *et al.,* 2017). The OTU table was normalized by rarefaction to an even depth of 57,623 for cultured mycelium and 42,448 for sap extract samples. This was to reduce sample heterogeneity as well as to keep samples with acceptable numbers of sequences to be used in statistical and taxonomic analyses. Fungal richness and abundance were calculated through alpha-diversity analyses determined by Shannon's Diversity, Simpson, Chao1, and Observed OTUs indices. The diversity results were then compared using a nonparametric two-sample t-test, and the *P*-values were calculated through 999 Monte Carlo permutations. Beta diversity analysis was performed using Bray Curtis dissimilarity metrics and the results were used to conduct Principal Coordinates Analysis (PCoA). PCoA graphs were also implemented with the taxonomic information and plotted on a 3D graph using EMPeror (Vázquez-Baeza *et al.,* 2013). Beta diversity results were used to compare groups of samples (fields, wheat varieties and sample categories) using Permanova analyses. Differentially abundant taxa between the two fields, the wheat varieties

and the four sample categories were detected using the Linear discriminant analysis Effect Size (LEfSe) (Segata *et al.,* 2011), setting *P*-value thresholds for the factorial Kruskal-Wallis test and the pairwise Wilcoxon test of 0.05, and the logarithmic Effect Size (LDA) cut-off >2.

Fungal association network

Inferred fungal associations (co-occurrence and mutual exclusion) within each wheat variety were computed using the CoNet (v1.1.1. beta) plugin within Cytoscape (v3.6.1). The associations of OTUs present in at least 20 samples were identified using an ensemble of correlation metrics (Spearman and Pearson coefficients) and distance metrics (Bray–Curtis and Kullback–Leibler dissimilarity measures). For each association metric and each edge, 100 renormalized permutation and bootstrap scores were generated following the ReBoot procedure (Faust *et al.,* 2012). The measure-specific *P*-values from multiple association metrics were merged using the Simes method (Sarkar and Chang, 1997), and false-discovery rate corrections were performed using Benjamini–Hochberg multiple testing correction (Benjamini and Hochberg, 1995). Only 1,000 top- and 1,000 bottom-ranking edges from each association measure were kept in the network analysis, and only edges supported by at least two of the four association metrics were retained in the final network inference of associations among taxa.

Table 1. Sequencing results obtained from analyses of DNA extracted from cultured mycelium isolated from four wheat variety and field categories: P1 = Perciasacchi from Field 1; T1 = Tumminia from Field 1; P2 = Perciasacchi from Field 2; and T2 = Tumminia from Field 2.

Category	No. of reads	Total OTUs	Rarefied OTUs[a]
P1	83,331	927	927
T1	127,221	960	960
P2	57,623	615	615
T2	84,962	753	753

[a]Rarefaction depth 57,623

Table 2. Sequencing and alpha diversity results from analyses of DNA extracted from wheat sap extracted from four wheat variety and field categories: **P1** = Perciasacchi from Field 1; **T1** = Tumminia from Field 1; **P2** = Perciasacchi from Field 2; and **T2** = Tumminia from Field 2.

Category	Sequencing results			Alpha diversity metrics			
	No. of reads	Total OTUs	Rarefied OTUs[a]	Simpson	Shannon	Chao1	Observed Species
P1	459,150	5,454	3,181	0.95	5.62	1249.40	651.52
T1	299,769	2,646	1,805	0.96	5.67	1252.82	555.92
P2	566,026	4,047	2,815	0.92	4.61	1072.07	507.20
T2	949,383	6,612	3,799	0.93	5.15	908.93	498.35

[a] Rarefaction depth 42,448

RESULTS

Sequencing data processing

HTS generated a total of 1,509,410 unpaired reads from fungal mycelium and 4,492,327 unpaired reads from wheat sap extract. After trimming, pairing, quality and chimera filtering, 687,093 high-quality fungal sequences were retained from mycelium and 2,330,956 from sap extract. The resulting sequences were then assigned to 2,943 OTUs for mycelium samples and 6,885 OTUs for sap samples. A summary of the sequencing results is shown in Table 1 and Table 2.

Cultured endophytic fungi from wheat

The sequencing of the ITS2 region of the endophytic fungi isolated in culture plates provided the taxonomic composition of the cultivable endophytic fungi (Figure 1). A total of 12 species from ten genera were identified. The number of genera per sample varied from four to nine. Ascomycota was the representative phylum of all the cultured genera, where *Mycosphaerella*, *Acremonium*, *Cladosporium*, *Aureobasidium,* and *Fusarium* were shared between all the assessed samples. Other genera were detected only in some sample categories: *Fusarium* and *Alternaria* from P1, T1 and T2, *Microdochium* from P1 and T1, and *Monographella* from T1 and T2).

Endophytic fungal community composition

The taxonomic assignment of the OTUs obtained from the HTS of wheat sap samples elucidated the composition of the endophytic fungal community among the four sample categories (P1, P2, T1 and T2). Overall, 28 OTUs were identified, belonging to 26 fungal genera (Figure 2). However, the relative abundance (RA) of the detected fungal taxa showed some differences between the sample categories, at the phylum and class levels (Figure 3). For example, members of Ascomycota were predominant in P1 (60% RA) and T2 (68% RA). In contrast, Basidiomycota were the most abundant in P2 (39%

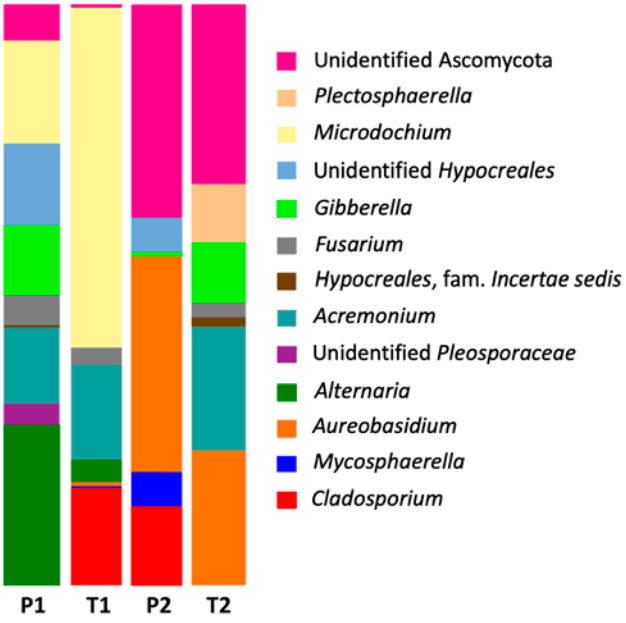

Figure 1. Taxonomic composition of cultured endophytic fungi. Genera distributions of endophytic fungi grown in cultures and analyzed using HTS, among four wheat variety and field categories: P1 = Perciasacchi from Field 1; T1 = Tumminia from Field 1; P2 = Perciasacchi from Field 2; and T2 = Tumminia from /Field 2.

RA) and T1 (59% RA). Field 2 gave the greatest number of unidentified fungal sequences, in particular in the T2 category. Dothideomycetes and Tremellomycetes were the main representative fungal classes in all the samples, with an average RA of 30%, followed by Sordariomycetes (7.5%), Agaricomycetes (2.5%), Microbotryomycetes (4.4%), Pucciniomycetes (3.4%), Leotiomycetes (1.0%), Ustilaginomycotina *incertae-sedis* (0.4%), and Taphrinomycetes (0.2%). At the genus level, *Alternaria*, *Mycosphaerella*, *Cladosporium*, *Filobasidium*, *Holtermanniella*, *Cystofilobasidium* and *Cryptococcus* were detected in all the sample categories.

Richness and diversity of endophytic fungi

The number of detected fungal OTUs per sample varied from 2,646 to 6,612. Alpha diversity indices showed that samples collected from Field 1 had greater fungal diversity compared to those from Field 2 (Table 2). The two-sample t-test based on Shannon index revealed a significant difference between the two fields ($P = 0.02$). However, there was no significant differences, either between the same variety grown in different fields or between the two varieties grown within the same field.

The PCoA plots, showing beta-diversity results, showed that samples collected from the two fields tended to segregate separately. In addition, the two wheat varieties clustered into different groups within each field (Figure 4). In agreement with PCoA and alpha diversity results, statistical comparisons using non-parametric Permanova tests showed significant variation of the fungal communities between the two fields ($P = 0.001$). In addition, the fungal communities were significantly different between the two wheat varieties ($P = 0.044$). A significant difference was also detected where the same varieties were grown in different fields ($P = 0.002$ for Field 1 and 0.005 for Field 2).

Some of the detected taxa had significantly different RAs in the investigated fields and varieties. In particular, *Mycosphaerella*, *Dioszegia*, *Filobasidium*, *Protomyces* and *Alternaria* varied significantly between the two fields (Figure 5), while *Aureobasidium*, *Leucosporidium* and *Puccinia* varied between the two varieties (Figure 6).

Fungal interactions

The co-occurrence and mutual exclusion of specific OTUs were analyzed for each wheat variety. The resulting networks, after statistical calculations and removal of unstable edges/links, were characterized, for Perciasacchi by 132 nodes (OTUs) linked with 420 edges with a clustering coefficient of 0.606, and for Tumminia, by 134 nodes (OTUs) linked with 583 edges and a clustering coefficient of 0.731 (Figure 7). Overall, the interactions between fungal phylotypes were characterized by a greater number of co-occurrences (401 in Perciasacchi and 510 in Tumminia) compared to mutual exclusions (19 in Perciasacchi and 73 in Tumminia). In both varieties, *Cryptococcus, Cystofilobasidium* and *Holtermanniella* were the dominant genera, with consistent co-occurrence interactions between each other as well as within each genus. *Cryptococcus* and *Cystofilobasidium* had mutual exclusion relationships with *Gibberella* species. On the other hand, *Gibberella*, *Acremonium* and a group of unidentified ascomycetes had a mutual exclusion interaction with *Puccinia*. *Cryptococcus* excluded *Sporobolomyces* only in Perciasacchi.

DISCUSSION

This study has demonstrated the limits of culture-dependent methods for characterization of the endophytic fungal communities. Metabarcoding analyses from crude sap extract allowed the detection of 26 genera belonging to Ascomycota and Basidiomycota, while

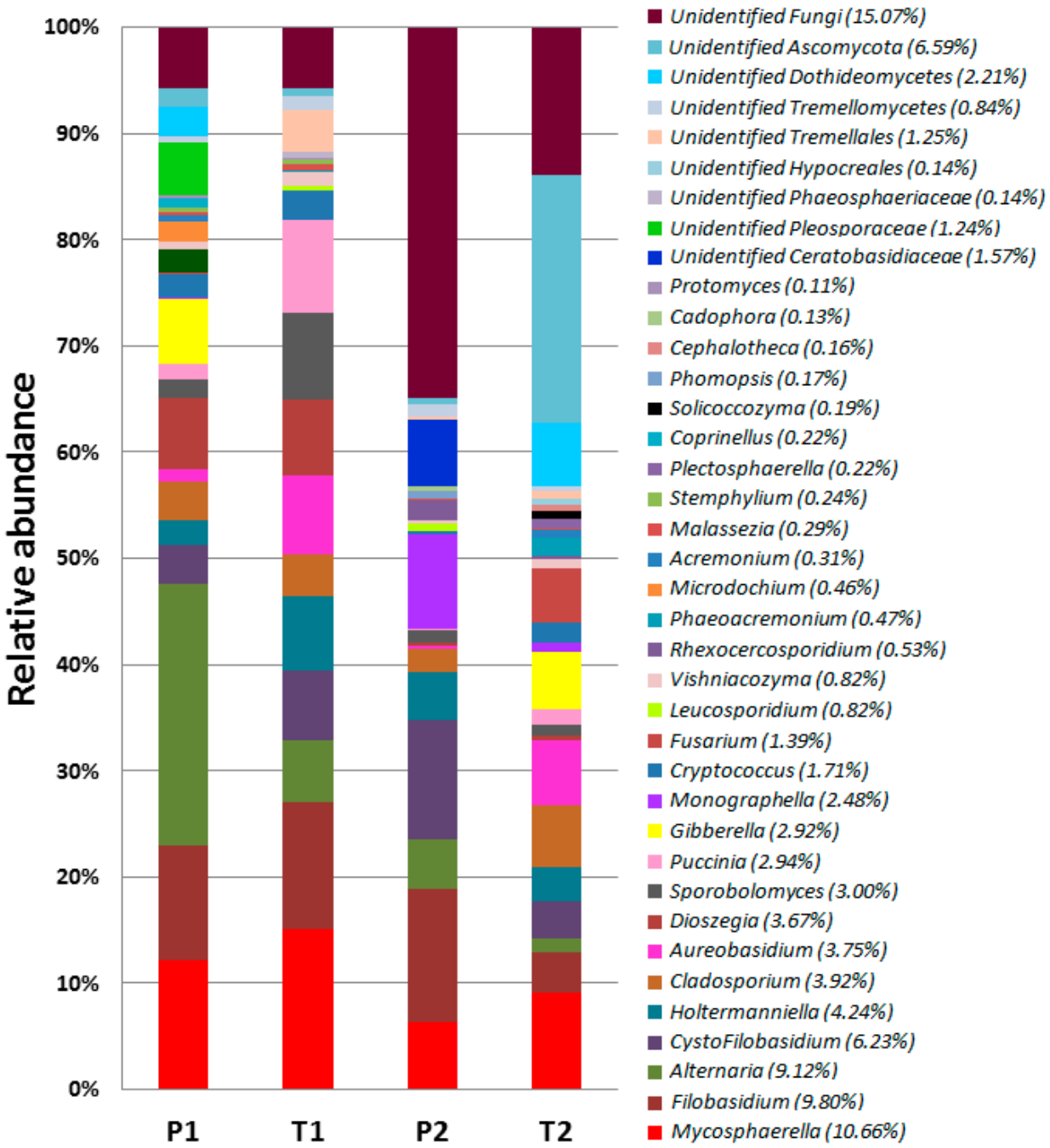

Figure 2. Endophytic fungal richness in two ancient wheat varieties. Distributions and relative abundance of the most abundant genera detected among four sample categories, from analyses of wheat sap samples. Average relative abundance for each genus is reported in parentheses. P1 = Perciasacchi from Field 1; T1 = Tumminia from Field 1; P2 = Perciasacchi fromField 2; and T2 = Tumminia from Field 2. Mean values of relative abundance are indicated in the parenthesis.

fungal isolations allowed the detection of only ten genera belonging to Ascomycota. Since isolations were carried out using one culture medium under one condition, a broader range of culturing conditions may be required to capture greater proportion of wheat endophytes. Nevertheless, nine out of 12 fungal species grown in plate cultures were also detected in the wheat sap extracts, therefore a good correspondence was obtained between the fungal taxa identified by the two methods. The variability in detection of the two methods could be due to

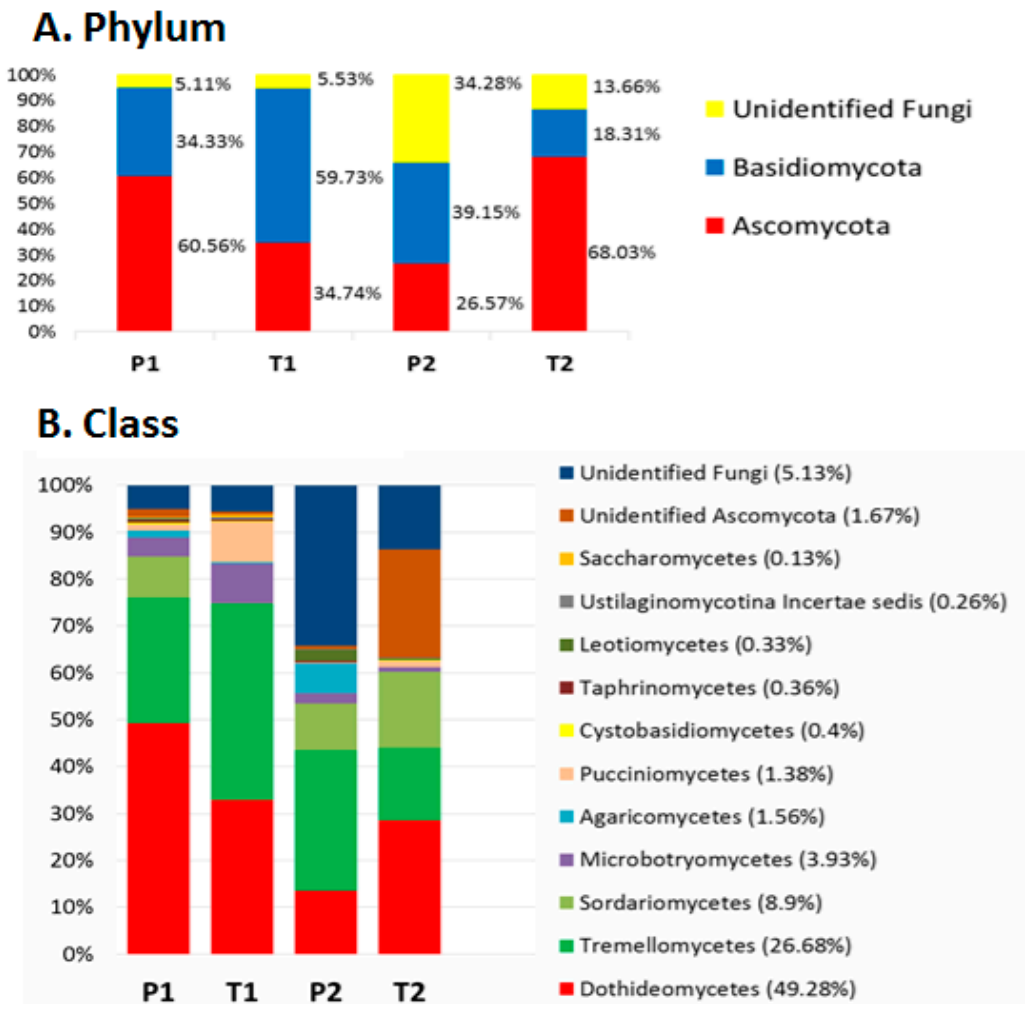

Figure 3. Structure and richness of the wheat endophytic fungal communities. Richness of fungal phyla (A) and classes (B) resulting from downstream analyses of the HTS performed on DNA extracted from wheat sap. Average relative abundance for each class is reported in parentheses. Sample categories are: P1 = Perciasacchi from Field 1; T1 = Tumminia from Field 1; P2 = Perciasacchi from Field 2; and T2 = Tumminia from Field 2.

bias of the culturing technique, which may have favored some taxa over others. Alternatively, the choice of primers for HTS analyses may have impact on the numbers of detected taxa (Tedersoo *et al.,* 2015). Moreover, it is important to highlight that the sap extract obtained with the patented method was successful in fungal isolation and molecular analyses.

The variations observed in the endophytic fungal communities between each wheat variety grown in the two fields could be explained by the different locations and agronomic conditions at the two field sites. Field 1 had been to crop rotation with legumes, while Field 2 was not cultivated for five previous years. Previous studies have reported that geographical location as well as crop management practices can affect the composition of soil microbial communities and, as consequence, the composition of endophytic microbial communities (Göre and Bucak, 2007; Sapkota *et al.,* 2017; Soman *et al.,* 2017).

The fungal communities were different between the two wheat varieties. This is not surprising since host genotype is considered to be a major factor determining the composition of endophyte communities (Sapkota *et al.,* 2015). These results highlight the importance of investigating microbial diversity of ancient crop varieties as possible sources of beneficial microorganisms.

Alternaria, *Cladosporium, Sporobolomyces, Dioszegia* and *Cryptococcus* are reported to be ubiquitous, and they have been detected in the present study as well as in the phyllospheres and grain of several commercial wheat

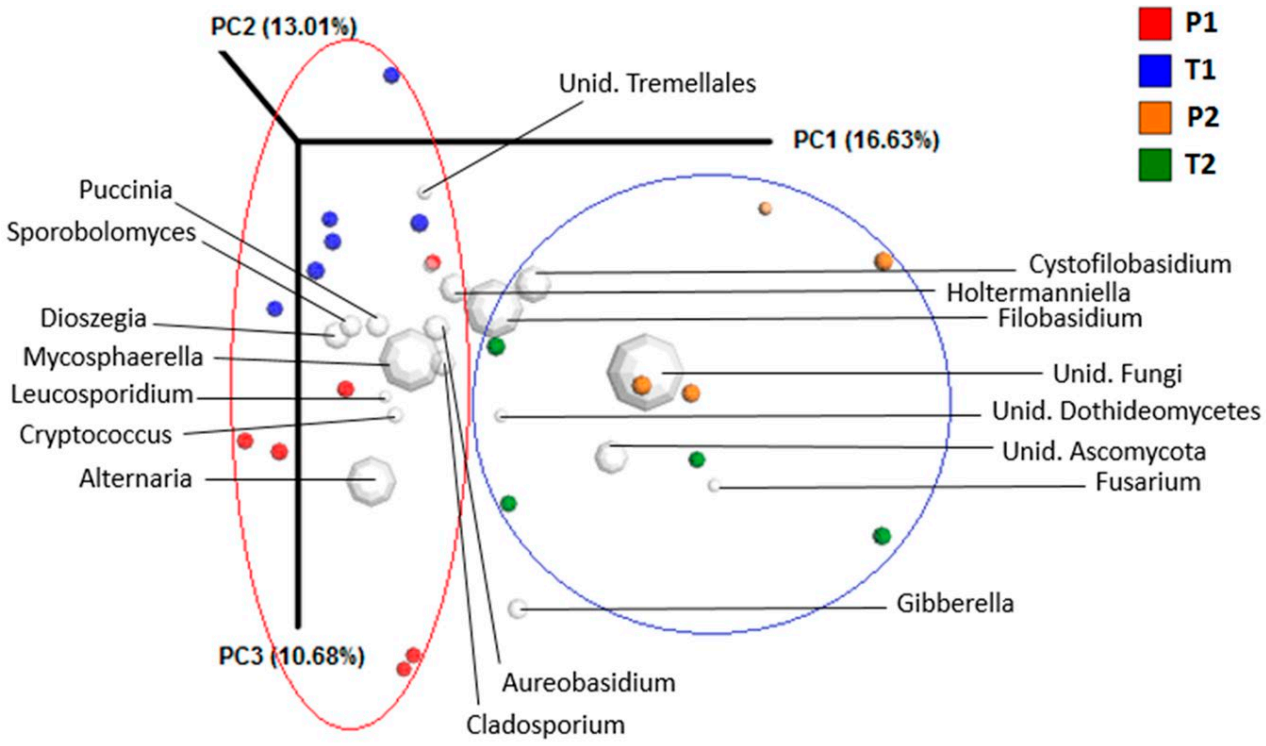

Figure 4. Principal Coordinates Analysis (PCoA). Integration of taxonomy in the PCoA graph showing the distribution of the 18 most abundant endophytic fungal genera among the four sample categories (white symbols). The taxonomic plot weight and the distance from the sample plots are proportional to the relative abundance of each genus. Red circle: cluster of Field 1 samples; blue circle: cluster of Field 2 samples. The different coloured symbols indicate the sample categories: P1 = Perciasacchi from Field 1; T1 = Tumminia from Field 1; P2 = Perciasacchi from Field 2; and T2 = Tumminia from Field 2.

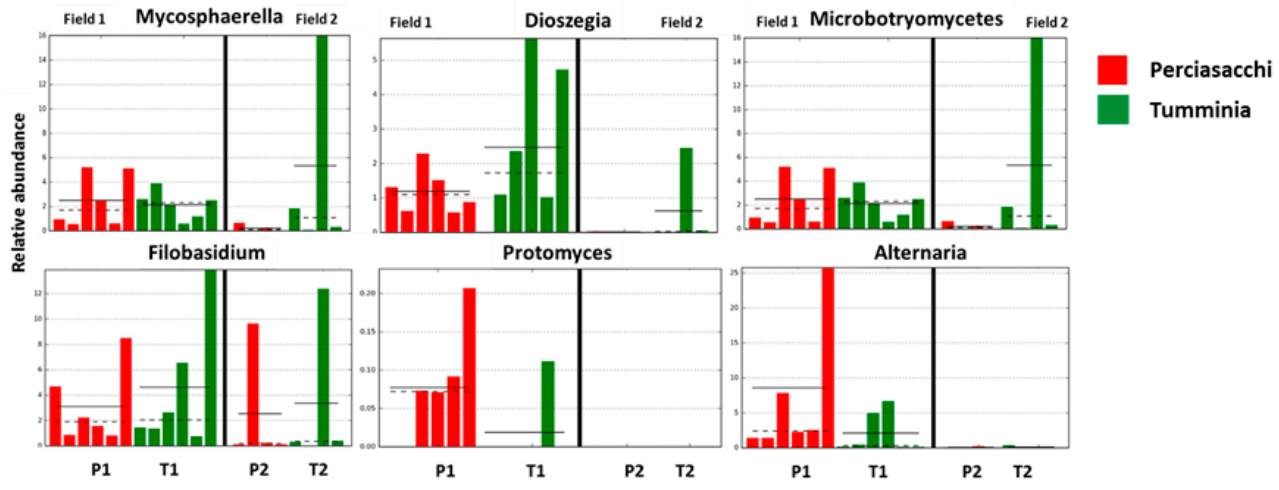

Figure 5. Differentially abundant genera between two ancient wheat varieties and two fields. Histograms indicate significant differences calculated by LEfSe analyses (Pa< 0.05 and LDA cut-off >2). P1 = Perciasacchi from Field 1; T1 = Tumminia from Field 1; P2 = Perciasacchi from Field 2; and T2 = Tumminia from Field 2. Category means (straight line) and medians (dotted line) are indicated.

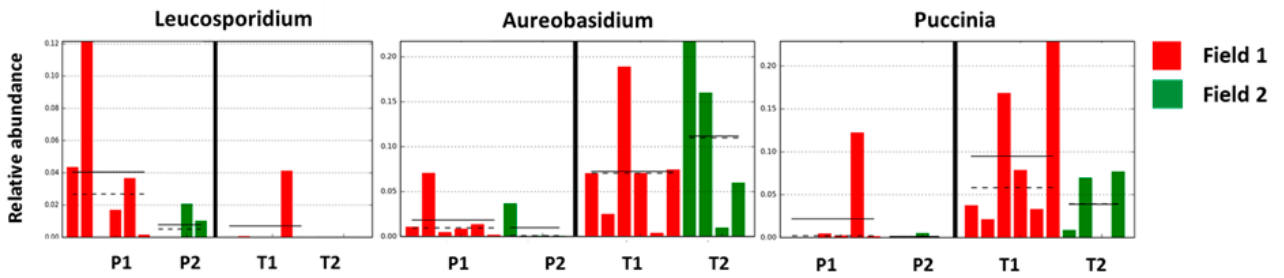

Figure 6. Discriminant fungal genera between two ancient wheat varieties. Differential genera between variety Perciasacchi (red) and Tumminia (green), resulting from LEfSe analysis ($P < 0.05$, LDA cut-off > 2). Histograms represent differential taxa distributions between the two wheat varieties and among the four sample categories: P1 = Perciasacchi from Field 1; T1 = Tumminia from Field 1; P2 = Perciasacchi from Field 2; and T2 = Tumminia from Field 2). Category means (straight line) and medians (dotted line) are indicated.

varieties (Nicolaisen *et al.,* 2014; Sapkota *et al.,* 2015). On the other hand, genera such as *Filobasidium*, *Holtermanniella* and *Cystofilobasidium* were detected in Perciasacchi and Tumminia, but have not been detected in the phyllospheres of modern cultivars. Conversely, *Pyrenophora*, *Epicoccum*, *Phoma* and *Sphaeosphaeria* belonging to core OTUs in the phyllospheres of modern wheat cultivars (Nicolaisen *et al.,* 2014) were not found in the present study. Differences between ancient and modern cultivars may be the consequence of different breeding histories as well as cultivar selection and/or growing conditions. Further research comparing varieties with contrasting pedigrees is necessary to firmly define taxa shared, or differentiating between, wheat genotypes, as previously reported for other crop species (Liu *et al.,* 2018).

Despite the presence of important fungal pathogens, no disease symptoms were observed in the collected samples from both fields. The presence of non-pathogenic fungi and plant resistant genes may account for this observation. However, an important contribution from beneficial fungi for maintaining plant health is likely. The analysis of the association networks suggested that key endophytes played roles within the wheat fungal communities detected. For instance, Basidiomycete endophytes such as *Cryptococcus* and *Cystofilobasidium* were the predominant genera and may have had consistent antagonistic activity against *Gibberella* species. Similarly, *Acremonium* and a group of unidentified ascomycetes mutually excluded *Puccinia*. Since both *Gibberella* and *Puccinia* contain several pathogens that cause economically important diseases in wheat, the detected interactions may indicate microbial-mediated resistance in wheat varieties. Furthermore, *Holtermanniella* and *Cystofilobasidium* were detected in the two ancient wheat varieties, and have not been reported to colonize modern wheat cultivars. This indicates possible negative impacts of breeding and domestication on native beneficial microbiomes.

In conclusion, the results from this study have expanded knowledge of the endophytic fungal communities associated with ancient wheat varieties, and have allowed formulation of hypotheses on the roles of the fungi in host plants. Further studies, including comparison between ancient and modern wheat microbiota, are required to clarify the roles of specific fungi, and to facilitate their exploitation as alternative means for improving plant health and resilience.

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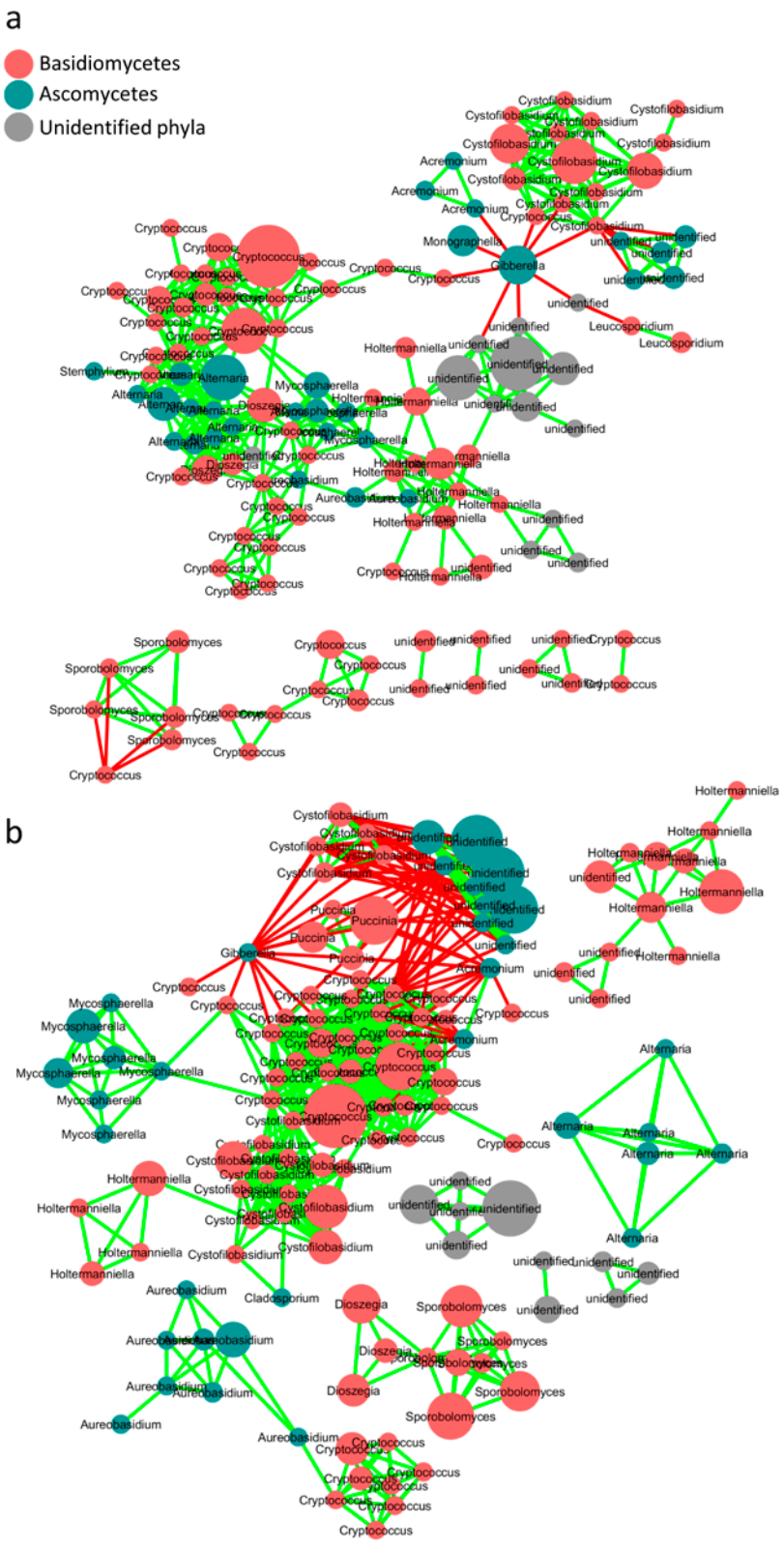

Figure 7. Microbial association network showing the interactions (co-occurrence and mutual exclusion) represented by green links for wheat variety Perciasacchi (a) and red links for Tumminia (b) The size of the nodes indicates OTU abundance. Node colours differentiate fungal phyla. Interactions were calculated by CoNet and visualised in Cytoscape 3.6.

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