SCAP
SOCIEDADE DE CIÊNCIAS AGRÁRIAS DE PORTUGAL

Fungal community in chestnut orchards with different *Hypholoma fasciculare* aboveground abundance: potential implications for sustainable production

Comunidade fúngica de soutos com diferentes abundâncias de *Hypholoma fasciculare*: potenciais implicações para a produção sustentável de castanheiro

Francisca Reis[1], Eric Pereira, Rui M. Tavares[1], Paula Baptista e Teresa Lino-Neto[1*]

[1] *Biosystems & Integrative Sciences Institute (BioISI), Plant Functional Biology Center (CBFP), Department of Biology, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal;*
[2] *CIMO / School of Agriculture, Polytechnic Institute of Bragança, Campus Sta. Apolónia, 5300-253 Bragança, Portugal.*
*(*e-mail: tlneto@bio.uminho.pt)*
http://dx.doi.org/10.19084/RCA15153

Received/recebido: 2015.11.05
Received in revised form/recebido em versão revista: 2016.04.13
Accepted/aceite: 2016.04.20

ABSTRACT

European chestnut (*Castanea sativa* Mill.) trees have a significant impact in the Portuguese economy, due to the production of chestnuts and wood, and other related activities, such as mushroom collection and hunt. *Hypholoma fasciculare* (Huds.) is a saprophytic fungus widely distributed in Trás-os-Montes (northeast of Portugal) chestnut groves that displays an *in vitro* strong antagonistic activity against ectomycorrhizal (EMC) fungi. In this study, the above- and belowground fungal diversity was evaluated in three chestnut orchards, containing distinct *H. fasciculare* sporocarps abundances in an attempt to better understand the potential of this fungus to attain an improved chestnut tree sustainable productivity. Aboveground analysis was performed based on macrofungi collection during fruiting seasons (spring and autumn) of two consecutive years. Belowground evaluation was based on the metabarcoding of chestnut orchards soil DNA, using the fungal barcode ITS1 and a high-throughput sequencing (454-sequencing) approach. Although all collected fruitbodies were identified as being produced by Basidiomycota fungi, a more diversified fungal community was revealed by the belowground approach. Both approaches have revealed a rich and abundant ECM community in all chestnut orchards. The correlation between the abundance of *H. fasciculare* fruitbodies and specific fungal guilds fruitbodies/reads suggests that this fungus may affect soil fungal community, mainly ECM and phytoparasites, as well as species composition of fungal communities. Although not conclusive, the results suggest that *H. fasciculare* presence could be critical for sustainable chestnut ecosystems.

Keywords: *Castanea sativa*, chestnut orchard soils, ectomycorrhizal community, *Hypholoma fasciculare*, ITS metabarcoding.

RESUMO

O castanheiro europeu (*Castanea sativa* Mill.) tem um enorme impacto na economia Portuguesa, sobretudo devido à produção de castanha e de madeira, mas também de actividades relacionadas com a colheira de cogumelos e a caça. *Hypholoma fasciculare* (Huds.) é um fungo saprófita amplamente distribuído nos soutos da região de Trás-os-Montes (nordeste de Portugal) e que apresenta, em condições *in vitro*, uma forte actividade antagonista contra fungos ectomicorrízicos. Neste estudo pretendeu-se avaliar a comunidade fúngica presente na superfície e no sub-solo de soutos contendo diferentes abundâncias de carpóforos de *H. fasciculare*, numa tentativa de compreender melhor a possibilidade deste fungo melhorar a produção sustentável do castanheiro. A diversidade fúngica à superfície do solo foi avaliada recorrendo à colheita de cogumelos durante o outono e a primavera, ao longo de dois anos consecutivos. A diversidade no sub-solo foi avaliada molecularmente pela extração do DNA do solo de soutos, amplificação da região ITS1 e sequenciação de nova geração (sequenciação 454). Apesar dos cogumelos colhidos serem todos produzidos por fungos Basidiomicetes, uma comunidade fúngica mais diversificada foi revelada pela abordagem molecular. No entanto, ambas as abordagens revelaram uma comunidade rica e abundante de fungos ECM em todos os soutos. A correlação efetuada entre a abundância de cogumelos de *H. fasciculare* e os grupos tróficos dos fungos presentes, avaliada por cogumelos/sequências, sugere que este fungo pode afetar a comunidade fúngica do solo, principalmente

os fungos ectomicorrízicos e fitoparasitas, assim como a composição em espécies da comunidade. Apesar de não serem conclusivos, os resultados sugerem que a presença de *H. fasciculare* poderá ser crítica para a sustentabilidade de ecossistemas de castanheiro.

Palavras-chave: *Castanea sativa*, comunidade ectomicorrízica, *Hypholoma fasciculare*, identificação molecular por ITS, solos de soutos.

INTRODUCTION

Chestnut ecosystems are among the conservation priorities in Europe (Habitat Directive 92/43/EEC, 1993). Chestnut tree (*Castanea sativa* Mill.) has a great economic importance, mainly due to the fruit value and high quality wood. Portugal is the third largest producer in Europe of chestnut fruit (FAO, 2013), where four different Protected Designations of Origin (PDO; *Castanha da Terra Fria*, *Castanha dos Soutos da Lapa*, *Castanha da Padrela* and *Castanha de Marvão*) were attributed. Taking into account the economic and social value of this market, efforts have been made in order to preserve chestnut orchards ecosystems. The soil fungal diversity has been, not only important for mushroom collection activities, but also a major concern for chestnut tree sustainability. When evaluating the fungal diversity and fruiting pattern associated with Portuguese chestnut orchards, the high abundance of a saprotrophic fungus – *Hypholoma fasciculare* – was reported in healthy and centenary chestnut orchards (Baptista *et al*., 2010). Recently, a deeper assessment of soil fungal diversity in Trás-os-Montes chestnut orchards, which combines both traditional (fruit body collection) and molecular (next generation sequencing) approaches revealed a high fungal diversity (Baptista *et al*., 2015). Although the ectomycorrhizal (ECM) community represented more than 50% of total fungal community, *H. fasciculare* was also detected by molecular methods (Baptista *et al*., 2015). There are evidences that this lignin-saprophytic cord-forming fungus presents a significant antagonistic action against different fungi that naturally occur in the soil of chestnut groves (Pereira *et al*., 2012). Taking advantage from this high antagonistic activity, *H. fasciculare* was used in field trials for the biological control of certain pathogens, such as *Armillaria ostoyae* (Romagn.) Herink. (Chapman *et al*., 2004). Although some *H. fasciculare* antagonistic interactions could be indeed beneficial for chestnut tree sustainability, others could be detrimental for ectomycorrhizal association, as revealed by *in vitro* assays (Pereira *et al*., 2011). However, despite the recognized role of *H. fasciculare* as an antagonist, there is still a limited understanding of its effect on soil microbial diversity and consequently on sustainable crop production.

During the life cycle of many fungi, sexually reproducing structures are visible to naked eye, comprising the so-called aboveground components of a fungal community. Different molecular approaches based on the detection of fungal DNA in the soil have recently emerged and are included in the so-called belowground approaches (Yoccoz, 2012). Differences in the accuracy of traditional and molecular approaches for describing fungal community became evident in the past years and are now known to bring complementary information for ecological fungal research (Horton and Bruns, 2001; Baptista *et al*., 2015). In the present work, fruitbody surveys and soil ITS1 metabarcoding approaches are combined to study the diversity of fungi in soils of three centenary chestnut orchards displaying different abundances of *H. fasciculare.* The presence of low/high amount of this fungus was further correlated with the natural abundance of different fungal guilds in an attempt to better understand its effect on soil microbial diversity and consequently on chestnut grove sustainability.

MATERIAL AND METHODS

The sampling region was located in the Natural Park of Montesinho (Bragança, northeast of Portugal), where 100-year-old non-tilled chestnut orchards were selected. Area of sampling is characterized by having a sub-continental climate with long cold winters and short but hot and dry summers. Annual air temperature diverges among 10-14 ^{0}C (temperature ranging from 0 to 28 ^{0}C in January and July, respectively). Annual rainfall average ranges from 1000 to 1200 mm, 90% of which occurring between October and April (Baptista,

2007). Three chestnut orchards were selected (about 1.5 ha each), based on different amounts of *H. fasciculare* fruitbodies observed in each orchard from September 2002 to December 2005 (Baptista *et al*., 2010). The most abundant orchards (Ab and Md) were located in the same region (Oleiros), 500 m apart from each other (N 41º 51 W 6º 49; 899 m altitude). Ab orchard presented a higher abundance of *H. fasciculare* fruitbodies during 2002 to 2005 period than Md orchard, while the orchard Lw located in Terroso region (N 41º 52 W 6º 50; 886 m altitude) did not display any during the same period. Five non-contiguous plots (100 m^2 each) selected in each orchards were sampled for both above- and belowground analysis.

Macrofungal fruitbodies were collected within each plot, during 2010 and 2011, either weekly (from September to November and from May to June) or biweekly (December and July). Only epigeous mushrooms greater than 1 mm were collected. Corticiaceae, including widespread ECM lineages (e.g. *Tomentella*, *Sebacina*, *Tulasnella*) were not considered. Fruitbodies were identified using macro- and microscopic characters (Baptista *et al*., 2010). Collected specimens were dried in air-vented ovens (30 °C for 72 h) and deposited at the herbarium of School of Agriculture, Polytechnic Institute of Bragança (Portugal).

Soil samples for belowground analysis were collected on April 13th, 2011. Two independent soil cores were picked two meters away from the chestnut tree trunk present in each plot. Thirty samples (3 orchards x 5 plots x 2 cores) were kept at 4°C until processing. Soil samples were thoroughly mixed, sieved (2 mm mesh) and stored at -80 °C until use. DNA extraction of stored soil samples was carried out using *PowerSoil DNA Isolation Kit* (MO BIO Laboratories), according to the instructions provided by the supplier with minor modifications. DNA samples were quantified in a NanoDrop ND-1000 (NanoDrop Technologies) spectrophotometer. ITS1, the universal fungal DNA barcode, was individually amplified in triplicate, in order to ensure heterogeneous amplification of samples (Schmidt *et al*., 2013). Forward primer was common to all samples and contained the ITS1F sequence (Gardes and Bruns, 1993), whereas unique reverse primers were used for each sample and contained the multiplex identifier (MID) and ITS2 primer sequence (White *et al*., 1990). PCR using *FastStart Taq DNA Polymerase* (Roche®) included the following thermal cycle conditions: an initial denaturation at 94°C for 4 min, followed by 30 cycles of 94 °C for 30 sec, 50 °C for 45 sec and 72 °C for 45 sec and a final extension of 72 °C for 10 min. Amplicons preparation for sequencing, pyrosequencing on a *Genome Sequencer GS FLX Titanium* (Roche-454 Life Sciences, Brandford, CT, USA; service provided by BioCant, Portugal), as well as Operational Taxonomic Units (OTUs) assignment and classification, are detailed elsewhere (Baptista *et al*., 2015). Only those OTUs that presented an identity higher than 97% at genus level were used for diversity and ecological analysis, being the singletons discarded.

Taxonomic classification of genera and OTUs was performed according to Kirk *et al.* (2008). Ecological guilds were determined using genera classification of total number of fruitbodies collected (traditional approach) and considering only those OTUs that produced more than 5 reads over the whole dataset (molecular approach). Identified genera were grouped into three distinct ecological guilds, i.e. mycorrhizal, saprotrophic, and phytoparasitic. Other categories such as undetermined, non-plant pathogens, yeasts, endophytes or lichen-associated fungi were not considered to the present study. In this analysis, correlation between *H. fasciculare* and studied ecological guilds was conducted for both above- and belowground analysis. Also, richness and diversity data were used for correlation analysis (*Pearson*).

RESULTS AND DISCUSSION

Fungal community in 100-year-old non-tilled chestnut orchards was assessed by traditional and molecular approaches (Baptista *et al*., 2015). Three independent orchards were classified according to *H. fasciculare* fruitbodies abundance (Baptista *et al*., 2010). Traditional study comprised the collection of epigeous fruitbodies from two consecutive years (2010 and 2011), whereas molecular studies only used a single time point for soil collection (13th April 2011). Collected fruitbodies, 713 in total, belong all to Basidiomycota, being classified in 16 families, 21 genera and 47 species. Most representative genera were *Inocybe* (31% of total number of fruitbodies), *Hypholoma* (28%) and *Amanita* (11%).

The number of *H. fasciculare* fruitbodies identified in the three orchards validates the results previously obtained by Baptista *et al.* (2010). Ab orchard presents 403 fruitbodies, 228 of which identified as *H. fasciculare*, followed by Md with 3 *H. fasciculare* fruitbodies in a total of 196, and Lw with none *H. fasciculare* in a total of 114 collected fruitbodies.

Metabarcoding using 454 platform generated 210,291 raw reads from ITS1 sequences, 199,919 of which were fungal reads with enough quality to proceed with further analysis. From the 501 assigned OTUs, Ascomycota (49.9%) and Basidiomycota (40.5%) fungal taxa were the most represented, followed by Zygomycota (5.0%), Chytridiomycota (1.4%), and Glomeromycota (0.6%). Although Ascomycota presented higher number of identified OTUs, Basidiomycota registered a higher number of reads (77% *vs.* 16%). Basidiomycota fungi were dominated by Agaricomycetes (90%) and among them ECM species dominated in diversity [Thelephoraceae (14%), Russulaceae (13%) and Inocybaceae (7%)] and abundance [*Russula* (9%), *Inocybe* (6%), *Tomentella* (5%), *Cortinarius* (4%) and *Amanita* (3%)]. As the orchards selection was based on *H. fasciculare* fruitbodies abundance, a correlation between the reads identified in metabarcoding approach (reflecting the belowground diversity) for this fungus and the presence of fruitbodies was expected. However, a reduced number of *H. fasciculare* reads was detected, representing only 0.19% of all obtained fungal reads, despite a similar trend (34 for Ab, 2 for Md and 0 for Lw) has been found. The underrepresentation of this saprotrophic fungus by metabarcoding could be explained by two possible scenarios: (i) the amount of underground mycelium may not follow the ability of fruitbody formation, as described by Peintner *et al.* (2007), or (ii) soil sampling could have discarded the top surface organic soil layer where saprotrophic fungi are mainly located. Depth of soil cores sampling for this kind of studies has not been consensual, and could cover a very large range of soil layers (*e.g.* Buée *et al.*, 2009, Fujita *et al.*, 2010, Klaubauf *et al.*, 2010). Our sampling strategy could has influenced the saprotrophic prevalence in belowground analysis, namely for those fungi able to produce fruitbodies fungi, such as *H. fasciculare* (Voříšková *et al.*, 2014).

Above- and belowground analyses showed different results both for diversity and abundance of fungal community present in the orchards (Table 1). There are species/OTUs that were only identified by a single method, which reveals a weak correlation between both approaches as previously reported (Taschen *et al.*, 2015). The overlap between approaches is very limited once only 6.7% of fungal soil OTUs were represented by aboveground and 11% of identified OTUs generate collected fruitbodies. Accordingly, for both fungal analyses, orchards presented common and exclusive genera (Table 1 and Figure 1). From 103 molecularly identified genera, only 30 were common to all orchards. In a certain way, this was an expected result once the molecular approach has a higher detection rate than fruitbodies survey, which in turn covers a higher soil area for a longer period (Baptista *et al.*, 2015). Furthermore, ECM root tips analysis in *C. sativa* has been poorly consistent with rDNA clones obtained from soil cores (Peintner *et al.*, 2007). From our study, both approaches turned clear that ECM fungi, namely *Inocybe*, *Amanita*, and *Russula*, dominate in chestnut orchards (Table 1). Accordingly, the ECM community has been reported to be a strong sub-group of the fungal community in Fagaceae forests, such as in *Quercus suber* and *Q. ilex* forests (Richard *et al.*, 2005; Azul *et al.*, 2010).

Fungal interactions in environment are essential to the forest sustainability. Different ecological guilds play different roles, mainly symbiotic, phytoparasitic and saprotrophic. In this work, species represented by 5 and more reads were grouped in their correspondent genus, resulting in 58 genera for belowground that contrasts with the 21 genera found for aboveground. All these genera were classified as mycorrhizal, saprotroph and phytoparasitic fungi. Fruitbodies were dominated by mycorrhizal genera (58.2% of the total number of fruitbodies/11 genera), followed by saprotrophic (40.4%/8) and phytoparasitic (1.4%/2). Also, the reads identified by using the molecular approach were mainly mycorrhizal 83.8% (28 genera), but a higher amount of saprotrophs was found 9.9% (19 genera), followed by 6.3% of phytoparasitic (11 genera) (Figure 2A). In any case, the detected phytoparasites are unable to cause severe diseases in chestnut. Still, the important infection agents, such as oomycetes like *Phytophthora*, require specific primers for ITS amplification (Cooke *et al.*,

Table 1 - Fruitbodies (aboveground) or reads (belowground) abundance detected in *H. fasciculare* abundant (Ab), intermediate (Md) or low abundant (Lw) orchards

	Aboveground			Belowground		
	Ab	Md	Lw	Ab	Md	Lw
Acicuseptoria	0	0	0	30	2	441
Acremonium	0	0	0	27	25	0
Acrostalagmus	0	0	0	8	0	1
Agaricus	0	0	0	0	0	5
Amanita	7	50	38	5	105	14
Arthrinium	0	0	0	243	0	33
Aspergillus	0	0	0	2	0	2
Basidiobolus	0	0	0	2	0	2
Bionectria	0	0	0	1	17	75
Biscogniauxia	0	0	0	1	0	1
Boletus	0	0	13	1	29	0
Bovista	0	24	1	7	1	2367
Byssocorticium	0	0	0	0	14	23
Calocybe	0	2	5	0	0	0
Candida	0	0	0	5	0	0
Cantharellus	0	30	0	0	0	0
Cenococcum	0	0	0	0	31	124
Ceratobasidium	0	0	0	32	0	0
Chaetomium	0	0	0	2	0	0
Chaetosphaeria	0	0	0	38	1	61
Chloridium	0	0	0	7	4	120
Cladophialophora	0	0	0	10	42	16
Clavaria	0	0	0	0	5	1409
Clavulina	0	0	0	1	19	3
Clitocybe	0	0	5	0	0	0
Clitopilus	0	0	0	0	1	5
Coniella	0	0	0	2	2	23
Coprinellus	0	0	0	18	1	15
Coprinopsis	0	0	0	0	0	4
Cortinarius	0	0	6	18	16	329
Crinipellis	0	0	0	28	0	0
Cryptococcus	0	0	0	421	84	29
Delicatula	0	0	0	32	0	0
Devriesia	0	0	0	171	4	88
Dictyosporium	0	0	0	3	0	0
Drechslera	0	0	0	158	18	4
Entoloma	0	0	0	3	0	1
Exophiala	0	0	0	5	0	1
Fistulina	0	3	6	0	3	579
Fusarium	0	0	0	3	0	0
Ganoderma	0	0	0	3	0	0
Geminibasidium	0	0	0	0	5	26
Gibberella	0	0	0	2	0	0
Guehomyces	0	0	0	35	17	24
Hebeloma	30	3	1	0	11	2279
Hirsutella	0	0	0	0	6	2
Hormonema	0	0	0	217	7	21
Humaria	0	0	0	0	6	3
Hydnum	0	0	0	0	275	0
Hypholoma	228	3	0	37	2	14
Ilyonectria	0	0	0	9	0	2
Inocybe	126	4	5	129	2129	1
Laccaria	0	10	2	0	0	0
Lachnum	0	0	0	184	0	0
Lactarius	0	5	0	23	0	229
Lasiosphaeria	0	0	0	0	3	2

	Aboveground			Belowground		
	Ab	Md	Lw	Ab	Md	Lw
Leotia	0	0	0	0	663	0
Lepista	0	0	0	3	1	0
Lycoperdon	0	7	0	8	0	2
Lyophyllum	0	0	0	2	0	1
Macrolepiota	2	6	2	0	0	0
Meliniomyces	0	0	0	21	0	0
Metarhizium	0	0	0	430	1	0
Microdochium	0	0	0	3	0	0
Mollisia	0	0	0	2	0	2
Mortierella	0	0	0	123	82	22
Mrakia	0	0	0	2	0	0
Mucor	0	0	0	2	0	0
Mycena	0	0	2	0	0	0
Myxocephala	0	0	0	5	0	0
Neostagonospora	0	0	0	0	0	2
Ochroconis	0	0	0	13	1	0
Oidiodendron	0	0	0	57	77	0
Olpidium	0	0	0	0	3	4
Operculomyces	0	0	0	0	2	0
Pachyphloeus	0	0	0	0	3	13
Paxillus	0	0	0	2	0	3
Penicillium	0	0	0	43	6	14
Peziza	0	0	0	0	0	64
Phellodon	0	0	0	0	14	76
Phialocephala	0	0	0	1	3	12
Pholiota	0	0	0	4	0	0
Pilidium	0	0	0	0	0	2
Pisolithus	0	0	0	8	2	0
Pochonia	0	0	0	15	3	0
Preussia	0	0	0	5	2	2
Pseudeurotium	0	0	0	8	0	0
Pseudotomentella	0	0	0	0	1	1
Pulvinula	0	0	0	0	6	1
Pustularia	0	0	0	0	7	0
Ramophialophora	0	0	0	7	0	1
Rhodotorula	0	0	0	9	0	3
Russula	10	43	25	1380	650	1
Schwanniomyces	0	0	0	39	0	0
Scleroderma	0	0	1	63	3	0
Scutellinia	0	0	0	7	0	0
Sebacina	0	0	0	582	0	0
Sistotrema	0	0	0	102	13	0
Sporothrix	0	0	0	6	2	0
Stachybotrys	0	0	0	3	5	4
Tetracladium	0	0	0	79	6	0
Thanatephorus	0	0	0	17	0	0
Tomentella	0	0	0	20	305	0
Tomentellopsis	0	0	0	0	4	0
Trametes	0	0	1	0	0	0
Tremella	0	0	1	0	0	0
Tricholoma	0	6	0	1	39	0
Trichosporon	0	0	0	30	11	0
Tubaria	0	0	0	4	0	0
Tuber	0	0	0	112	91	2
Xerula	0	0	0	0	26	1
TOTAL	403	196	114	5141	4917	8611

2000) and therefore could not have been detected using this molecular approach. The disagreement in the ecological guilds pattern detected in both views (above- and belowground) was predictable, since the fruiting capacity is mainly possible by the ECM and saprotrophic fungi present in the

orchards, while all ecological guilds can be equally evaluated by molecular studies. Also, hypogeous and crustose fungal diversity were not assessed by the traditional method.

role on the determination of the fungal community structure (Castro-Sowinski *et al.*, 2007), in this work *H. fasciculare* seems to be critical to soil fungal community in chestnut groves.

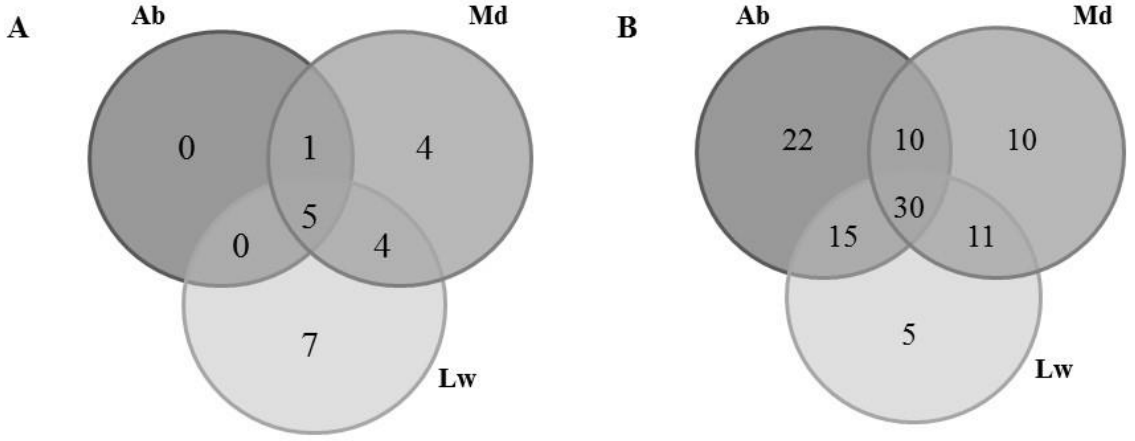

Figure 1 - Venn diagrams of shared genera between orchards evaluated by traditional (A) and molecular (B) approaches. Orchards were selected based on abundance on *H. fasciculare* from the most to the less abundant: Ab, Md and Lw.

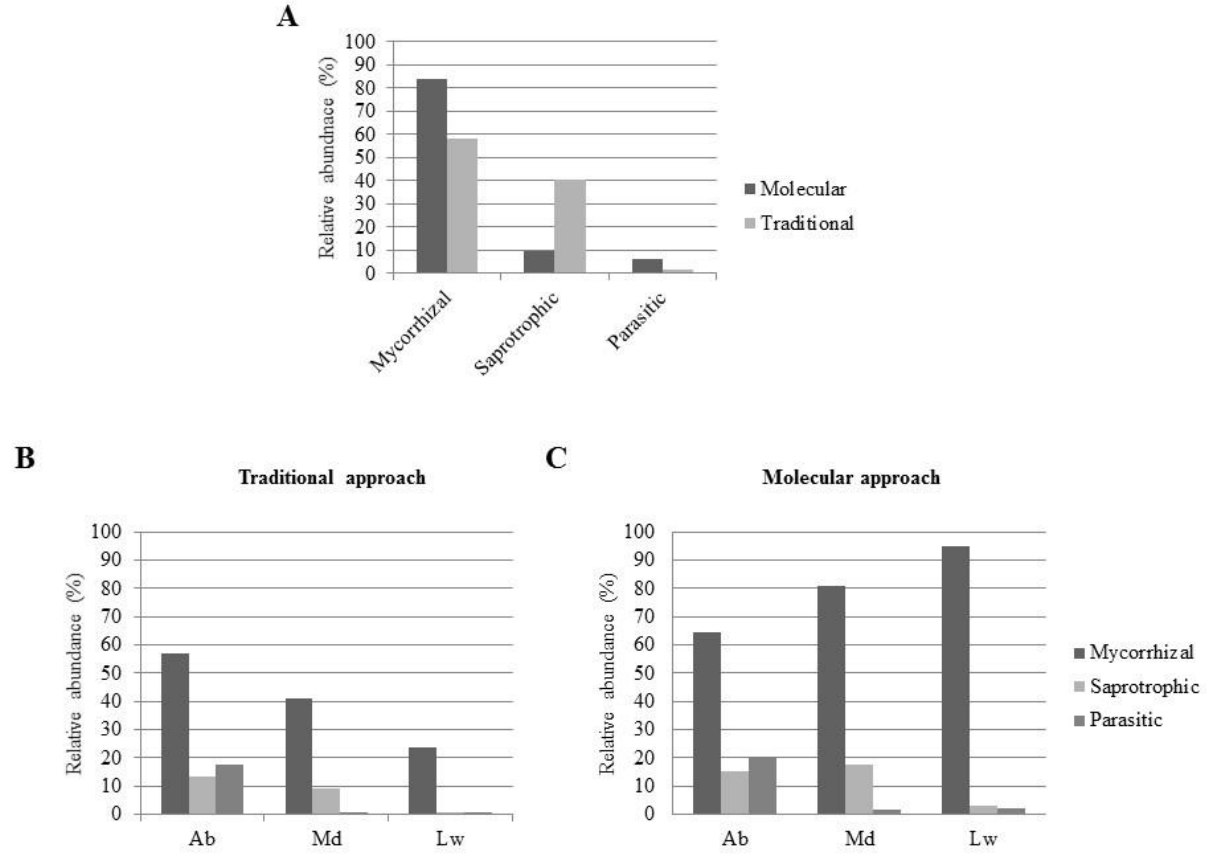

Figure 2 - Relative abundance of ecological fungal guilds in chestnut orchards. Abundance of fungal guilds detected by traditional or molecular approaches, comprising all identified mushrooms and OTUs represented by more than 5 reads (A). Ecological guilds abundance in different chestnut orchards detected by traditional (B) or molecular approaches (C). Orchards presented a gradient in the distribution of *H. fasciculare* from the most to the less abundant: Ab, Md and Lw.

ACKNOWLEDGEMENTS

This work was funded by FEDER through the Operational Competitiveness Program – COMPETE – and by national funds through the Foundation for Science and Technology – FCT – within the scope of project PTDC/AGR-AAM/099556/2008.

REFERENCES

Azul, A.M.; Sousa, J.P.; Agerer, R.; Martín, M.P. and Freitas, H. (2010) – Land use practices and ectomycorrhizal fungal communities from oak woodlands dominated by *Quercus suber* L. considering drought scenarios. *Mycorrhiza*, vol. 20, n. 2, p.73-88. http://dx.doi.org/10.1007/s00572-009-0261-2

Baptista, P. (2007) – *Macrofungos associados à cultura de castanheiro: aspectos da sua biodiversidade e da interacção de* Pisolithus tinctorius *e* Hypholoma fasciculare *com raízes de* Castanea sativa *Mill.* Doctoral thesis, University of Minho, Braga.

Baptista, P.; Martins, A.; Tavares, R.M. and Lino-Neto, T. (2010) – Diversity and fruiting pattern of macrofungi associated with chestnut (*Castanea sativa*) in the Trás-os-Montes region (Northeast Portugal). *Fungal Ecology*, vol. 3, n. 1, p. 9-19. http://dx.doi.org/10.1016/j.funeco.2009.06.002

Baptista, P.; Reis, F.; Pereira, E.; Tavares, R.M.; Santos, P.; Richard, F.; Selosse, M.A. and Lino-Neto, T. (2015) – Soil DNA pyrosequencing and fruitbody surveys reveal contrasting diversity for various fungal ecological guilds in chestnut orchards. *Environmental Microbiology Reports,* vol. 7, n. 6, p. 946-954. http://dx.doi.org/10.1111/1758-2229.12336

Buée, M.; Reich, M.; Murat, C.; Morin, E.; Nilsson, R.H.; Uroz, S. and Martin F. (2009) – 454 Pyrosequencing analyses of forest soils reveal an unexpectedly high fungal diversity. *New Phytologist*, vol. 184, n. 2, p. 449-445. http://dx.doi.org/10.1111/j.1469-8137.2009.03003.x

Castro-Sowinski, S.; Herschkovitz, Y.; Okon, Y. and Jurkevitch, E. (2007) – Effects of inoculation with plant growth-promoting rhizobacteria on resident rhizosphere microorganisms. *FEMS Microbiology Letters*, vol. 276, n. 1, p. 1-11. http://dx.doi.org/10.1111/j.1574-6968.2007.00878.x

Chapman, B.; Xiao, G. and Myers, S. (2004) – Early results from field trials using *Hypholoma fasciculare* to reduce *Armillaria ostoyae* root disease. *Canadian Journal of Botany*, vol. 82, n. 7, p. 962-969. http://dx.doi.org/10.1139/b04-078

Cooke, D.E.; Drenth, A.; Duncan, J.M.; Wagels, G. and Brasier, C.M. (2000) – A molecular phylogeny of *Phytophthora* and related oomycetes. *Fungal Genetics and Biology*, vol. 30, n. 1, p.17-32. http://dx.doi.org/10.1006/fgbi.2000.1202

Fujita, K.; Kohno, M. and Takayanagi, T. (2010) – Analysis of microbial community in Japanese vineyard soils by culture-independent molecular approach. *Journal of Wine Research*, vol. 2, p. 75-104. http://dx.doi.org/10.2147/IJWR.S13008

Gao, G.; Yin, D.; Chen, S.; Xia, F.; Yang, J.; Li, Q. and Wang Y. (2012) – Effect of biocontrol agent *Pseudomonas fluorescens* 2P24 on soil fungal community in cucumber rhizosphere using T-RFLP and DGGE. *PLoS One*, vol. 7, n. 2, p. 1-9. http://dx.doi.org/10.1371/journal.pone.0031806

Gardes, M. and Bruns, T.D. (1993) – ITS primers with enhanced specificity for basidiomycetes – application to the identification of mycorrhizae and roots. *Molecular Ecology*, vol. 2, n. 2, p. 113-118. http://dx.doi.org/10.1111/j.1365-294X.1993.tb00005.x

Horton, T.R. and Bruns, T.D. (2001) – The molecular revolution in ectomycorrhizal ecology: peeking into the black-box. *Molecular Ecology*, vol. 10, n. 8, p. 1855-1871. http://dx.doi.org/10.1046/j.0962-1083.2001.01333.x

Kirk, P.F.; Cannon, P.F.; Minter, D.W. and Stalpers, J.A. (2008) – *Dictionary of the fungi, 10th ed*. CAB International, Wallingford.

Klaubauf, S.; Inselsbacher, E.; Zechmeister-boltenstern, S.; Wanek, W.; Gottsberger, R.; Strauss, J. and Gorfer,

M. (2010) – Molecular diversity of fungal communities in agricultural soils from Lower Austria. *Fungal Diversity*, vol. 44, n. 1, p. 65-75. http://dx.doi.org/10.1007/s13225-010-0053-1

Peintner, U.; Iotti, M.; Klotz, P.; Bonuso, E. and Zambonelli, A. (2007) – Soil fungal communities in a *Castanea sativa* (chestnut) forest producing large quantities of *Boletus edulis* sensu lato (porcini): where is the mycelium of porcini? *Environmental Microbiology*, vol. 9, n. 4, p. 880-889. http://dx.doi.org/10.1111/j.1462-2920.2006.01208.x

Pereira, E.; Coelho, V.; Tavares, R.M.; Lino-Neto, T. and Baptista, P. (2011) – Effect of competitive interactions between ectomycorrhizal and saprotrophic fungi on *Castanea sativa* performance. *Mycorrhiza*, vol. 22, n. 1, p. 41-49. http://dx.doi.org/10.1007/s00572-011-0379-x

Pereira, E.; Santos, A.; Reis, F.; Tavares, R.M.; Baptista, P.; Lino-Neto, T. and Almeida-Aguiar, C. (2012) – A new effective assay to detect antimicrobial activity of filamentous fungi. *Microbiological Research*, vol. 168, n. 1, p. 1-5. http://dx.doi.org/10.1016/j.micres.2012.06.008

Richard, F.; Millot, S.; Gardes, M. and Selosse, M.-A. (2005) – Diversity and specificity of ectomycorrhizal fungi retrieved from an old-growth Mediterranean forest dominated by *Quercus ilex*. *New Phytologist*, vol. 166, n. 3, p. 1011-1023. http://dx.doi.org/10.1111/j.1469-8137.2005.01382.x

Schmidt, P.A.; Bálint, M.; Greshake, B.; Bandow, C.; Römbk, J. and Schmitt, I. (2013) – Illumina metabarcoding of a soil fungal community. *Soil Biology & Biochemistry*, vol. 65, p.128-132. http://dx.doi.org/10.1016/j.soilbio.2013.05.014

Taschen, E.; Sauve, M.; Taudiere, A.; Parlade, J.; Selosse, M.-A. and Richard, F. (2015) – Whose truffle is this? Distribution patterns of ectomycorrhizal fungal diversity in *Tuber melanosporum* brûlés developed in multi-host Mediterranean plant communities. *Environmental Microbiology*, vol. 17, n. 8, p. 2747-2761. http://dx.doi.org/10.1111/1462-2920.12741

Voříšková, J.; Brabcová, V.; Cajthaml, T. and Baldrian, P. (2014) – Seasonal dynamics of fungal communities in a temperate oak forest soil. *New Phytologist*, vol. 201, n. 1, p. 269-278. http://dx.doi.org/10.1111/nph.12481

Whipps, J.M. (2001) – Microbial interactions and biocontrol in the rhizosphere. *Journal of Experimental Botany*, vol. 52, n. S1, p. 487-511. http://dx.doi.org/10.1093/jexbot/52.suppl_1.487

White, T.J.; Bruns, T.; Lee, S. and Taylor, J. (1990) – Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In*: Innis, M.A.; Gelfand, D.H.; Sninsky, J.J. and White, T.J. (eds.) – *PRC Protocols: A guide to Methods and Applications*. Academic Press, San Diego, p. 315-322.

Yoccoz, N.G. (2012) – The future of environmental DNA in ecology. *Molecular Ecology*, vol. 21, n. 8, p. 2031-2038. http://dx.doi.org/10.1111/j.1365-294X.2012.05505