



## Localized reshaping of the fungal community in response to a forest fungal pathogen reveals resilience of Mediterranean mycobiota

Francesco Venice<sup>a</sup>, Alfredo Vizzini<sup>a,b</sup>, Arcangela Frascella<sup>c</sup>, Giovanni Emiliani<sup>c</sup>, Roberto Danti<sup>c</sup>, Gianni Della Rocca<sup>c</sup>, Antonietta Mello<sup>a,\*</sup>

<sup>a</sup> Institute for Sustainable Plant Protection (IPSP)—SS Turin—National Research Council (CNR), Viale Mattioli 25, 10125 Turin, Italy

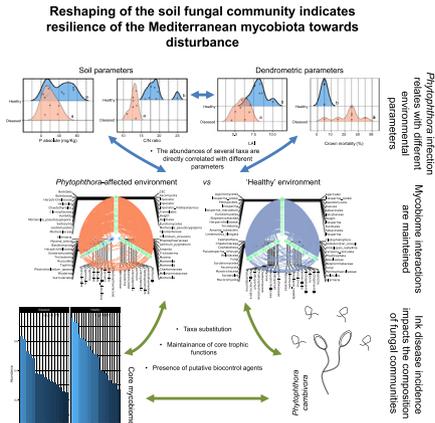
<sup>b</sup> Department of Life Sciences and System Biology, University of Turin, Viale Mattioli 25, 10125 Turin, Italy

<sup>c</sup> Institute for Sustainable Plant Protection (IPSP)—National Research Council (CNR), Via Madonna del Piano 10, 50019 Sesto F.no (FI), Italy

### HIGHLIGHTS

- Mediterranean forests are increasingly threatened by *Phytophthora* pathogens.
- Monitoring mycobiota is fundamental to preserve ecosystems.
- Metabarcoding reveals the resilience of Mediterranean Chestnut mycobiota upon stress.
- Environmental parameters and key-stone species shape the soil fungal network.
- Taxa substitutions sustain fungal diversity associated with the diseased environment.

### GRAPHICAL ABSTRACT



### ARTICLE INFO

#### Article history:

Received 25 May 2021

Received in revised form 16 July 2021

Accepted 7 August 2021

Available online 10 August 2021

Editor: Manuel Esteban Lucas-Borja

#### Keywords:

*Castanea sativa*

Ink disease

Metabarcoding

*Phytophthora cambivora*

### ABSTRACT

Mediterranean forests are facing the impact of pests such as the soilborne *Phytophthora cambivora*, the causal agent of Ink disease, and this impact is made more severe by global changes. The status and resilience of the soil microbial ecosystem in areas with such a disturbance are little known; however, the assessment of the microbial community is fundamental to preserve the ecosystem functioning under emerging challenges. We profile soil fungal communities in a chestnut stand affected by ink disease in Italy using metabarcoding, and couple high-throughput sequencing with physico-chemical parameters and dendrometric measurements. Since the site also includes an area where the disease symptoms seem to be suppressed, we performed several analyses to search for determinants that may contribute to such difference. We demonstrate that neither pathogen presence nor trees decline associate with the reduction of the residing community diversity and functions, but rather with microbial network reshaping through substitutions and new interactions, despite a conservation of core taxa. We predict interactions between taxa and parameters such as soil pH and C/N ratio, and suggest that disease incidence may also relate with disappearance of pathogen antagonists, including ericoid- and ectomycorrhizal (ECM) fungi. By combining metabarcoding and field studies, we infer the resilient status of the fungal community towards a biotic stressor, and provide a benchmark for the study of other threatened ecosystems.

© 2021 Elsevier B.V. All rights reserved.

\* Corresponding author.

E-mail address: [antonietta.mello@ipsp.cnr.it](mailto:antonietta.mello@ipsp.cnr.it) (A. Mello).

had their specific keystone taxa. The keystone taxa in the diseased network, however, had a lower importance (ranking), compared with those of the healthy area, probably indicating less established relationships, and therefore more plasticity. Our study suggests that the decline of the health status of the trees in the investigated site did not reduce diversity and functioning of the fungal community: indeed, the presence of biotic stress in the investigated site can be defined as a long-term disturbance (Mandakovic et al., 2018), as ink disease symptoms have been observed for 15–20 years. The studied fungal community was likely not resistant (insensitive) to the disturbance, but rather apparently resilient in that, although reshaped, it maintained core taxa, and ecosystem services exerted by saprotrophic and mycorrhizal guilds. This was accompanied by the apparent persistence of the native mycobiota, since the dominating fungi in the diseased stand are those that are known to inhabit Mediterranean chestnut stands (Ambrosio and Zotti, 2015; Blom et al., 2009; Laganà et al., 2002; Peintner et al., 2007), contrary to other cases where dominance of invasive fungal species was observed after different disturbances, including climate change (Anthony et al., 2017; Dickie et al., 2016; Garbelotto and Pautasso, 2012; Vizzini et al., 2008). Our study also showed that specific soil and plant characteristics (such as soil humidity, C/N ratio and plant height) and fungal community composition are correlated, although our results do not allow the establishment of causal relationships between these parameters, a “chicken or the egg” quandary which is common in the understanding of tree decline (Sapsford et al., 2017). One of the general outcomes of these complex dynamics may also be that a pedological condition adverse to the pathogen is established. Outliers were present, in both areas, in terms of soil and tree parameters, and of taxa composition (for example, the heterogeneity in Mortierellomycetes abundance). This phenomenon requires more insights, and may depend on further partitioning into microhabitats that, at least for the diseased condition, could be due to the pathogen acting in patches. Due to the proximity of the two sub-areas, we tend to exclude the possibility that microclimatic factors are the prominent drivers of fungal community assemblage. The present results will hopefully provide support for management and preservation of chestnut orchards, and be useful for benchmarking resilience of fundamental mycobiota that supports Mediterranean forests.

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2021.149582>.

### CRedit authorship contribution statement

**Francesco Venice:** Formal analysis, Writing – original draft, Investigation. **Alfredo Vizzini:** Validation, Data curation. **Arcangela Frascella:** Investigation, Writing – original draft. **Giovanni Emiliani:** Resources. **Roberto Danti:** Resources. **Gianni Della Rocca:** Conceptualization, Funding acquisition. **Antonietta Mello:** Conceptualization, Writing – original draft, Supervision.

### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

### Acknowledgements

The research was funded thanks to the EU LIFE program in the framework of the LIFE MycoRestore project (LIFE18/CCA/ES/001110). The authors want to thank the coordinator of the LIFE project, Alvaro Peix, the farm ‘Le Casine di Salieri Giuseppe’ for hosting the demonstrative project areas, the technicians Vincenzo di Lonardo and Stefano Secci for helping in the field work. We also appreciate, for the various support to the project and to the research, the Tuscany Region, the Foreste Casentinesi, Monte Falterona e Campigna National Park, the Unione

dei Comuni Valdarno e Valdisieve, the Consorzio Promozione Tutela Marrone del Mugello IGP, the municipality of San Godenzo and the Montagne Fiorentine Model Forest.

### References

- Ambrosio, E., Zotti, M., 2015. Mycobiota of three *Boletus edulis* (and allied species) productive sites. *Sydowia* 67, 197–216. <https://doi.org/10.12905/0380.SYDOWIA67-2015-0197>.
- Andrews, S., 2012. FastQC: a quality control tool for high throughput sequence data. <http://www.bioinformatics.babraham.ac.uk/projects/fastqc/>.
- Anthony, M.A., Frey, S.D., Stinson, K.A., 2017. Fungal community homogenization, shift in dominant trophic guild, and appearance of novel taxa with biotic invasion. *Ecosphere* 8 (9), e01951. <https://doi.org/10.1002/ecs2.1951>.
- Badali, H., Gueidan, C., Najafzadeh, M.J., Bonifaz, A., van den Ende, A.H.G.G., de Hoog, G.S., 2008. Biodiversity of the genus *Cladophialophora*. *Stud. Mycol.* 61, 175–191. <https://doi.org/10.3114/sim.2008.61.18>.
- Baptista, P., Reis, F., Pereira, E., Tavares, R.M., Santos, P.M., Richard, F., Selosse, M.-A., Lino-Neto, T., 2015. Soil DNA pyrosequencing and fruitbody surveys reveal contrasting diversity for various fungal ecological guilds in chestnut orchards. *Environ. Microbiol. Rep.* 7 (6), 946–954. <https://doi.org/10.1111/1758-2229.12336>.
- Bengtsson-Palme, J., Ryberg, M., Hartmann, M., Branco, S., Wang, Z., Godhe, A., Wit, P.D., Sánchez-García, M., Ebersberger, I., de Sousa, F., et al., 2013. Improved software detection and extraction of ITS1 and ITS2 from ribosomal ITS sequences of fungi and other eukaryotes for analysis of environmental sequencing data. *Methods Ecol. Evol.* 4 (10), 914–919. <https://doi.org/10.1111/2041-210X.12073>.
- Birkebak, J., Mayor, J., Ryberg, M., Matheny, P., 2013. A systematic, morphological and ecological overview of the clavariaceae (Agaricales). *Mycologia* 105. <https://doi.org/10.3852/12-070>.
- Bisanz, J.E., 2018. Qiime 2R: importing QIIME2 artifacts and associated data into R sessions. <https://github.com/jbisanz/qiime2R>.
- Blom, J.M., Vannini, A., Vettraino, A.M., Hale, M.D., Godbold, D.L., 2009. Ectomycorrhizal community structure in a healthy and a *Phytophthora*-infected chestnut (*Castanea sativa* mill.) stand in Central Italy. *Mycorrhiza* 20 (1), 25–38. <https://doi.org/10.1007/s00572-009-0256-z>.
- Bolyen, E., Rideout, J.R., et al., 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nat. Biotechnol.* 37, 852–857. <https://doi.org/10.1038/s41587-019-0209-9>.
- Bosshard, W., 1986. *Couronnes d'arbres: Le chiome degli alberi. Eidgenössische Anstalt für das Forstliche Versuchswesen, Birmensdorf*, p. 98.
- Branzanti, M.B., Rocca, E., Pisi, A., 1999. Effect of ectomycorrhizal fungi on chestnut ink disease. *Mycorrhiza* 9 (2), 103–109. <https://doi.org/10.1007/s005720050007>.
- Bray, R.H., Kurtz, L.T., 1945. Determination of total, organic, and available forms of phosphorus in soils. *Soil Sci.* 59 (1), 39–46. <https://doi.org/10.1097/00010694-194501000-00006>.
- Breiman, L., 2001. Random forests. *Mach. Learn.* 45 (1), 5–32. <https://doi.org/10.1023/A:1010933404324>.
- Bruzzese, S., Blanc, S., Brun, F., 2020. Strategies for the valorization of chestnut resources in Italian mountainous areas from a sustainable development perspective. *Resources* 9 (5), 60. <https://doi.org/10.3390/resources9050060>.
- Burgess, T.I., Scott, J.K., McDougall, K.L., Stukely, M.J.C., Crane, C., Dunstan, W.A., Brigg, F., Andjic, V., White, D., Rudman, T., Arentz, F., Ota, N., Hardy, G.E.S.J., 2017. Current and projected global distribution of *Pytophthora cinnamomi*, one of the world's worst plant pathogens. *Glob. Chang. Biol.* 23 (4), 1661–1674. <https://doi.org/10.1111/gcb.13492>.
- Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., Holmes, S.P., 2016. DADA2: high-resolution sample inference from illumina amplicon data. *Nat. Methods* 13 (7), 581–583. <https://doi.org/10.1038/nmeth.3869>.
- Camacho, C., Coulouris, G., Avagyan, V., Ma, N., Papadopoulos, J., Bealer, K., Madden, T.L., 2009. BLAST: architecture and applications. *BMC Bioinformatics* 10, 421. <https://doi.org/10.1186/1471-2105-10-421>.
- Campanella, V., Ippolito, A., Nigro, F., 2002. Activity of calcium salts in controlling *Phytophthora* root rot of citrus. *Crop Prot.* 21, 751–756. [https://doi.org/10.1016/S0261-2194\(02\)00032-7](https://doi.org/10.1016/S0261-2194(02)00032-7).
- Chen, W., Simpson, J., Levesque, C.A., 2018. RAM: R for amplicon-sequencing-based microbial ecology. <https://CRAN.R-project.org/package=RAM>.
- Conedera, M., Tinner, W., Krebs, P., de Rigo, D., Caudullo, G., 2016. *Castanea sativa* in Europe: Distribution, Habitat, Usage and Threats.
- Corcobado, T., Cech, T.L., Brandstetter, M., Daxer, A., Hüttler, C., Kudláček, T., Horta Jung, M., Jung, T., 2020. Decline of European beech in Austria: involvement of *Phytophthora* spp. and contributing biotic and abiotic factors. *Forests* 11 (8), 895. <https://doi.org/10.3390/f11080895>.
- Crous, P.W., Schumacher, R.K., Wingfield, M.J., Akulov, A., Denman, S., Roux, J., Braun, U., Burgess, T.I., Carnegie, A.J., Váczy, K.Z., Guatimosim, E., Schwartsburd, P.B., Barreto, R.W., Hernández-Restrepo, M., Lombard, L., Groenewald, J.Z., 2018. New and interesting fungi. 1. *Fungal Systematics and Evolution*. 1, pp. 169–216. <https://doi.org/10.3114/fuse.2018.01.08>.
- de Mattos-Shiple, K.M.J., Ford, K.L., Alberti, F., Banks, A.M., Bailey, A.M., Foster, G.D., 2016. The good, the bad and the tasty: the many roles of mushrooms. *Stud. Mycol.* 85, 125–157. <https://doi.org/10.1016/j.simyco.2016.11.002>.
- Defrenne, C.E., Philpott, T.J., Guichon, S.H.A., Roach, W.J., Pickles, B.J., Simard, S.W., 2019. Shifts in ectomycorrhizal fungal communities and exploration types relate to the environment and fine-root traits across interior Douglas-fir forests of Western Canada. *Front. Plant Sci.* 10. <https://doi.org/10.3389/fpls.2019.00643>.