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UNIVERSITÀ DI BOLOGNA

# Soil Ecology, Microbial Interactions, and Their Implications for Truffle Cultivation

Alessandra Zambonelli

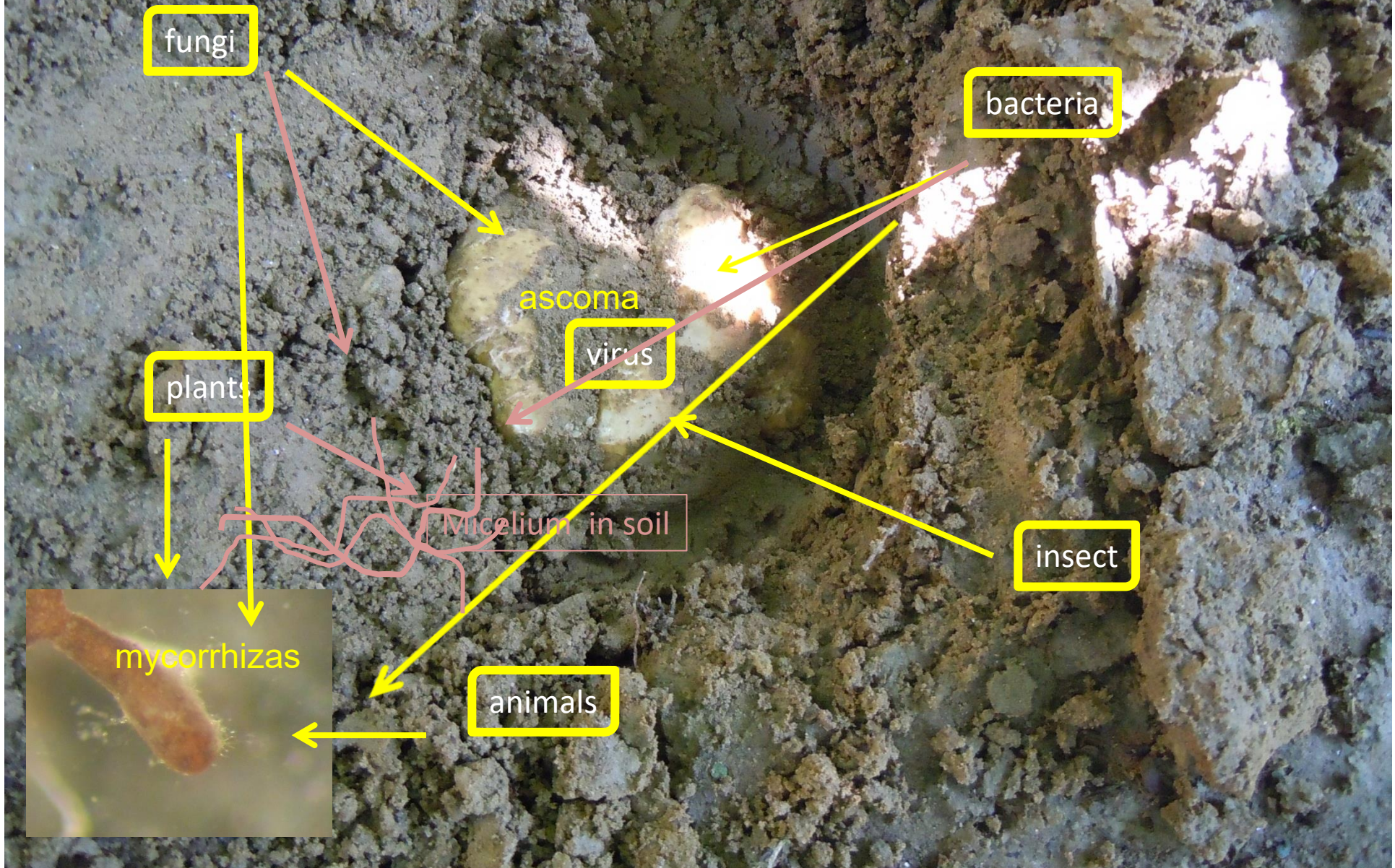
Department of Agricultural and Food Sciences (DISTAL)

## Soil is the environment where truffles live throughout their entire life cycle

- **Total limestone:** > 8%
  - **Soil pH:** 7.5–8.5
  - **C/N ratio:** ~10
  - **Texture:** preferably loamy or sandy-loam soils
  - **Available phosphorus:** < 15–20 ppm
  - **Low fertility soils** are generally preferred
- 
-  However, soil properties can be modified through appropriate management practices (e.g., liming) to improve suitability for truffle cultivation.



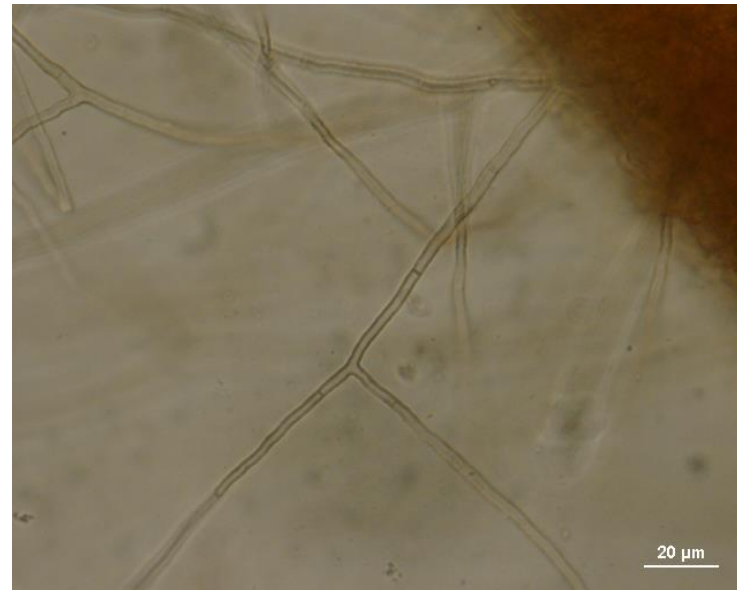
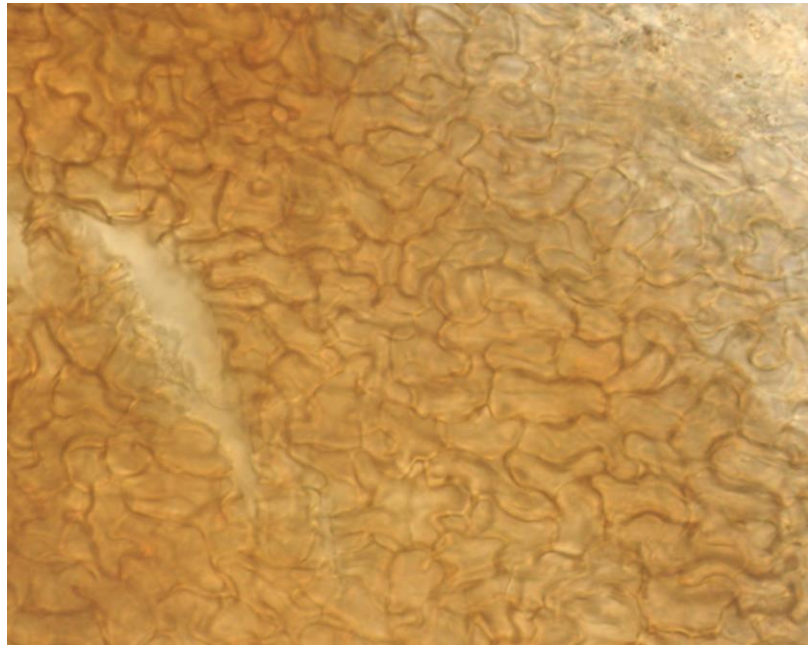
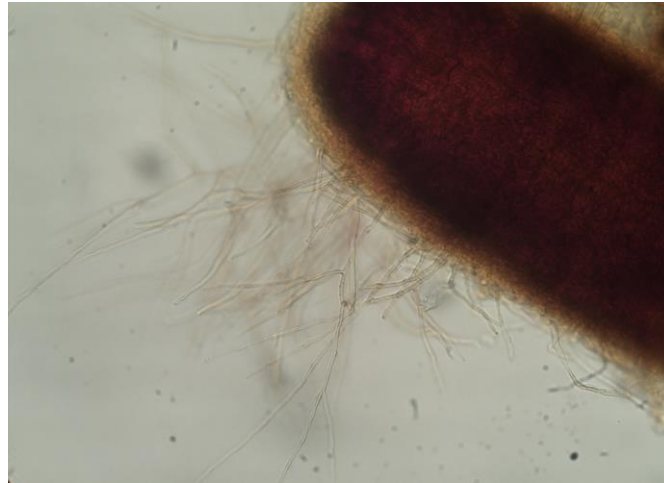
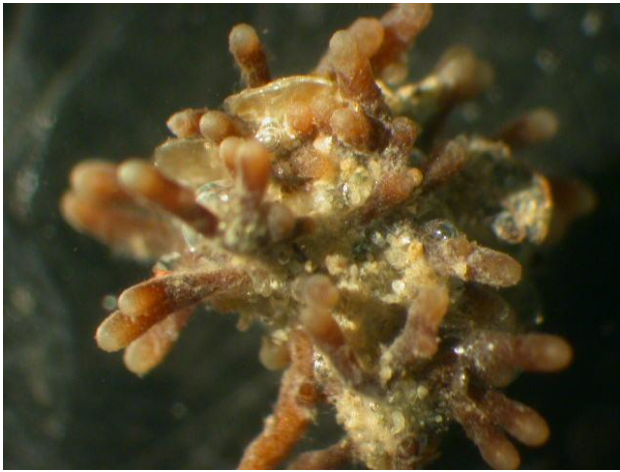
# Truffle in soil



# Plant interactions



# Ectomycorrhizas



Truffles in particular *Tuber melanosporum* (the Périgord black truffle,) interacts with herbaceous plants through a unique endophytic relationship, colonizing their roots inside the "brûlé" (burnt area) without forming true ectomycorrhizae.



Fungal Ecology 31 (2018) 59–68



Contents lists available at ScienceDirect

Fungal Ecology

journal homepage: [www.elsevier.com/locate/funeco](http://www.elsevier.com/locate/funeco)



## Is *Tuber melanosporum* colonizing the roots of herbaceous, non-ectomycorrhizal plants?



Laure Schneider-Maunoury <sup>a</sup>, Sébastien Leclercq <sup>a</sup>, Cannelle Clément <sup>a</sup>, Hervé Covès <sup>a</sup>,  
Josie Lambourdière <sup>a, b</sup>, Mathieu Sauve <sup>c</sup>, Franck Richard <sup>c</sup>, Marc-André Selosse <sup>a, d, \*</sup>,  
Elisa Taschen <sup>e</sup>

<sup>a</sup> Institut de Systématique, Évolution, Biodiversité (ISYEB – UMR 7205 – CNRS, MNHN, UPMC, EPHE), Muséum national d'Histoire naturelle, Sorbonne Universités, 57 rue Cuvier, 75005, Paris, France

<sup>b</sup> Service de Systématique Moléculaire (SSM – UMS 2700 OMSI), Muséum national d'Histoire naturelle, 43 rue Cuvier, 75005 Paris, France

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<sup>d</sup> Department of Plant Taxonomy and Nature Conservation, University of Gdansk, ul. Wita Stwosza 59, 80-308 Gdańsk, Poland

<sup>e</sup> INRA, UMR Eco&Sols, Place Viala, 34060, Montpellier, France



## Sites of study

Three *T. magnatum* natural truffle ground



Città delle Pieve,  
Umbria, Italy

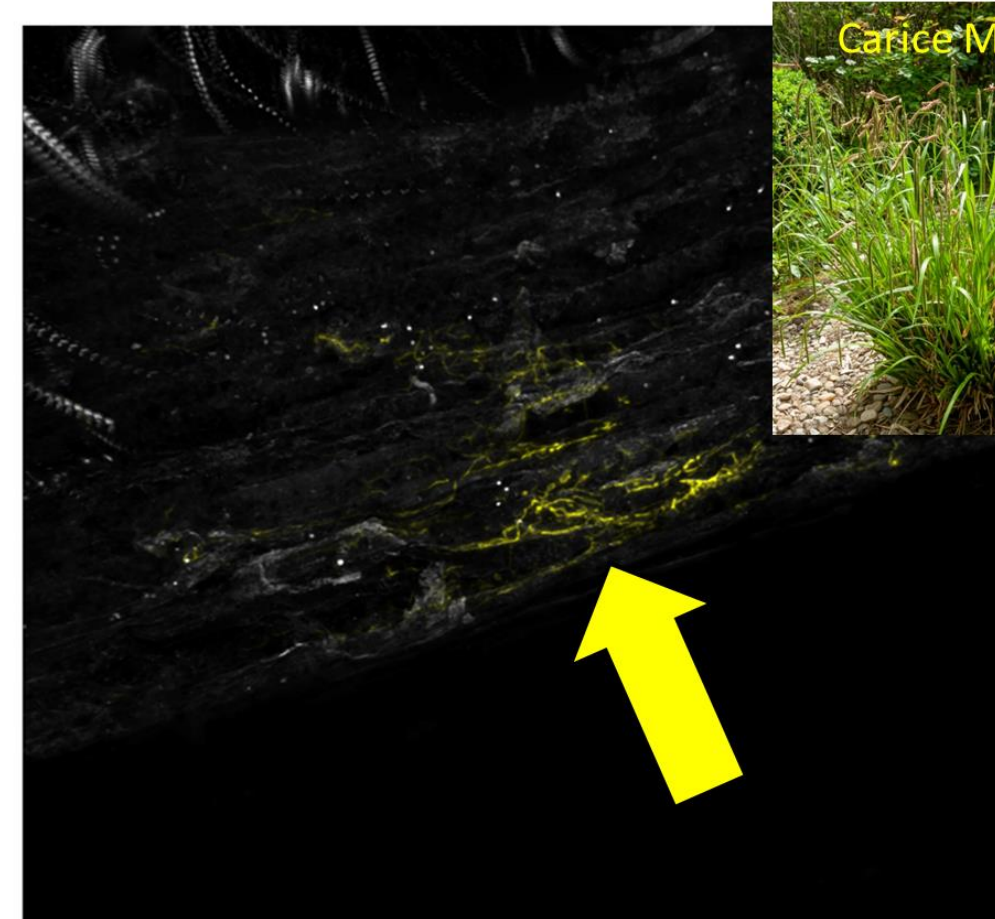
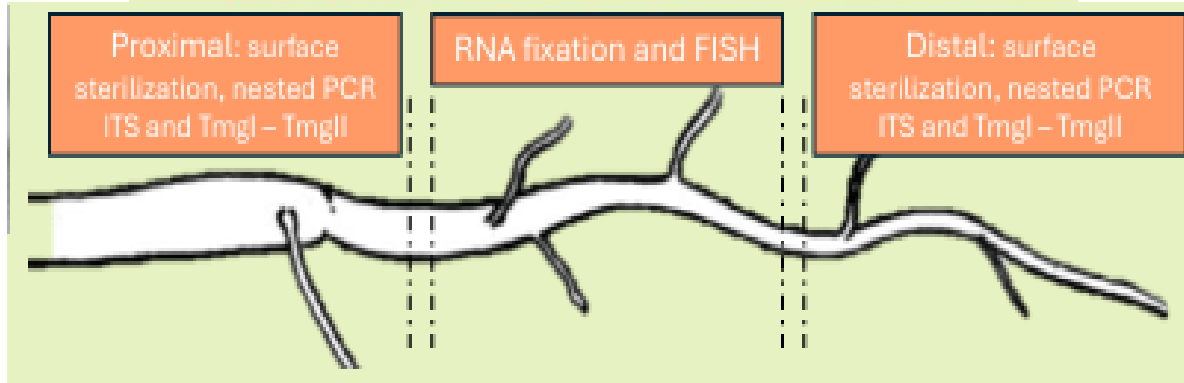


Montefalcone nel  
Sannio, Molise, Italy



Panfilia Forest, Emilia-  
Romagna, Italy

*Tuber magnatum*, a truffle species that does not form a brùlé, has also been found as an endophyte in herbaceous plants.



Microbial Ecology (2025) 88:42  
<https://doi.org/10.1007/s00248-025-02542-z>

RESEARCH



## Newly Designed Fluorescence In Situ Hybridization Probes Reveal Previously Unknown Endophytic Abilities of *Tuber magnatum* in Herbaceous Plants

Simone Graziosi<sup>1</sup> · Lara Deloche<sup>2</sup> · Mélanie Januario<sup>2</sup> · Marc-André Selosse<sup>2,3,4</sup> · Aurélie Deveau<sup>5</sup> · Cyrille Bach<sup>5</sup> · Zhixiao Chen<sup>5</sup> · Claude Murat<sup>5</sup> · Mirco Iotti<sup>6</sup> · Philippe Rech<sup>2</sup> · Alessandra Zambonelli<sup>1</sup>

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On the other hand, although there is currently no published scientific evidence to support it, some herbaceous plants, particularly aromatic species, may negatively affect the growth of the mycelium of certain *Tuber* species.

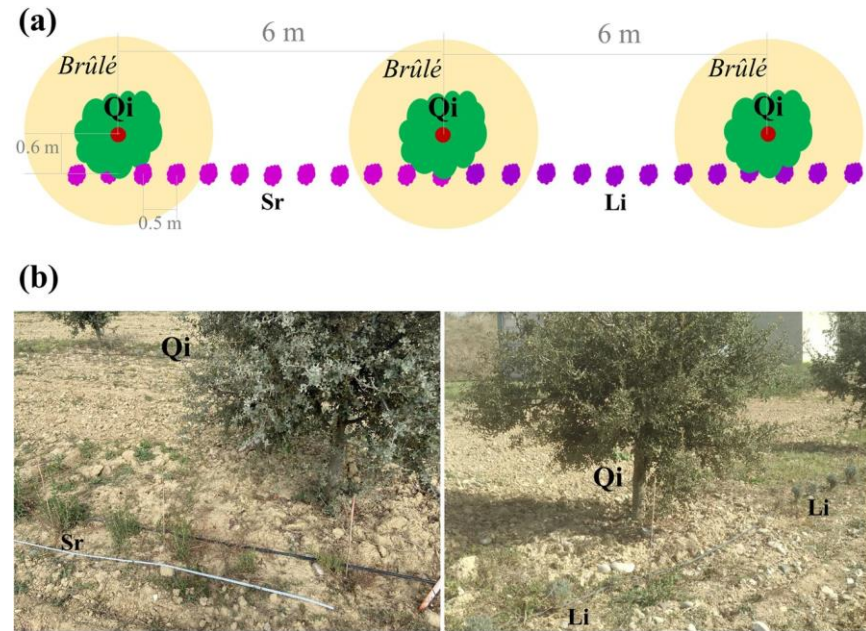
Plant Soil (2025) 506:79–89  
<https://doi.org/10.1007/s11104-023-06106-3>

RESEARCH ARTICLE



## Intercropping of aromatic plants in truffle orchards: short-term effect on extraradical truffle mycelium and aromatic plant growth

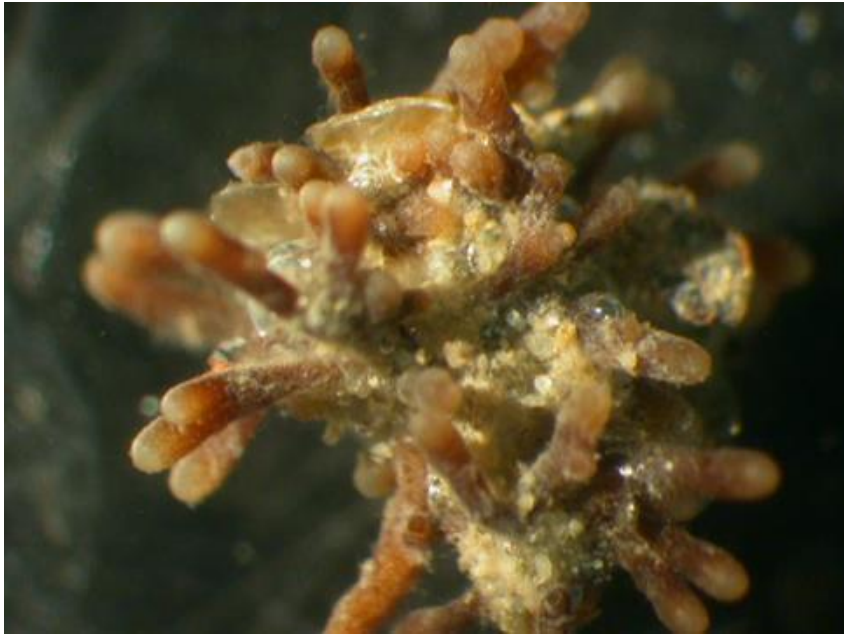
Sergi Garcia-Barreda · Juliana Navarro-Rocha · Eva Gómez-Molina · Vasiliki Barou · María Ángeles Sanz · Sergio Sánchez · Javier Parladé



From a practical perspective, it may be prudent to avoid intercropping aromatic species within truffle orchards until more conclusive evidence becomes available.



# Fungal interactions



# The underground warfare



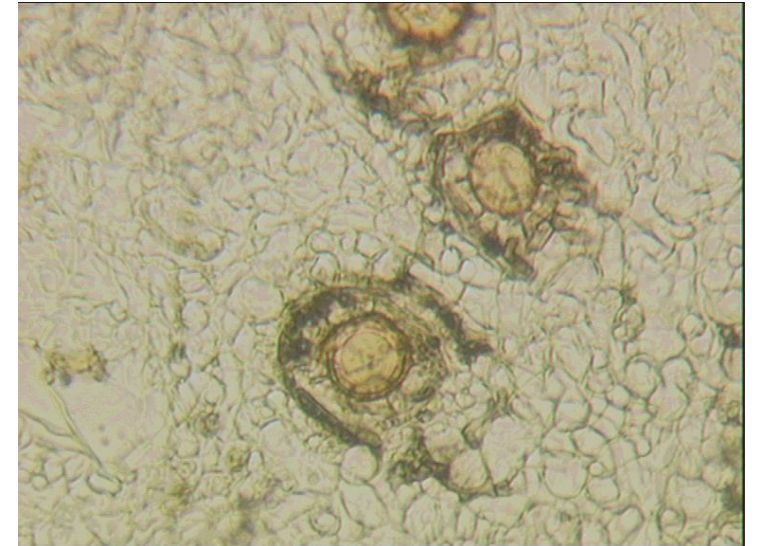
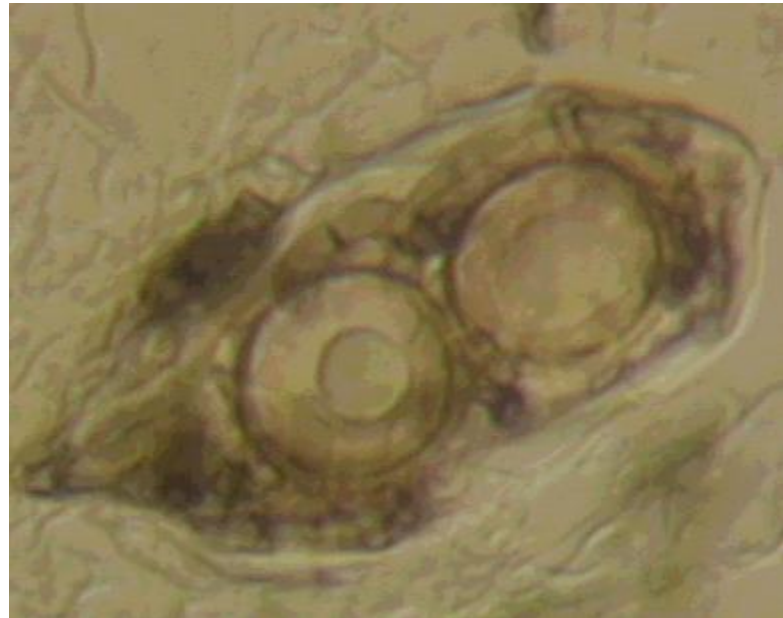
# Fungi inside the fruiting body

Arch Microbiol (2005) 184: 187–193  
DOI 10.1007/s00203-005-0043-y

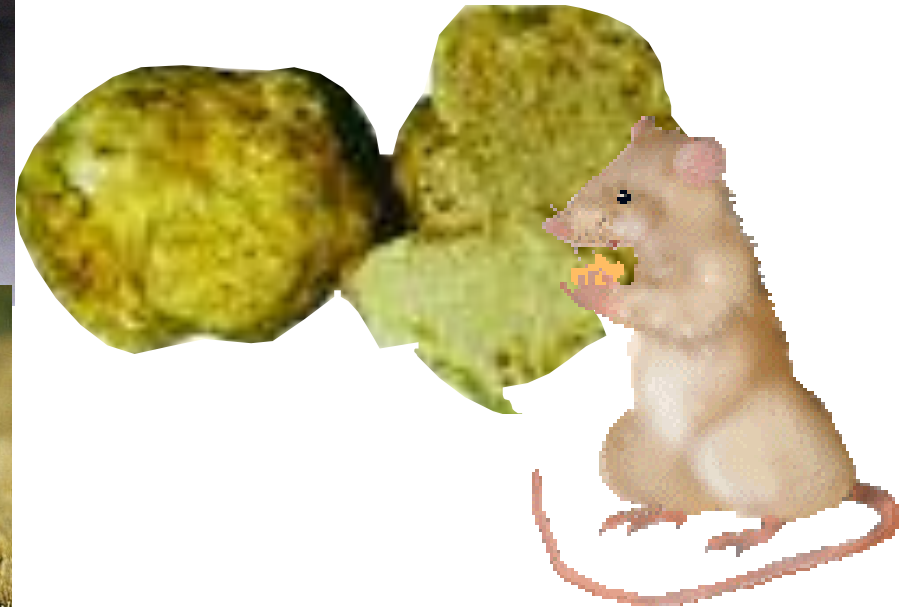
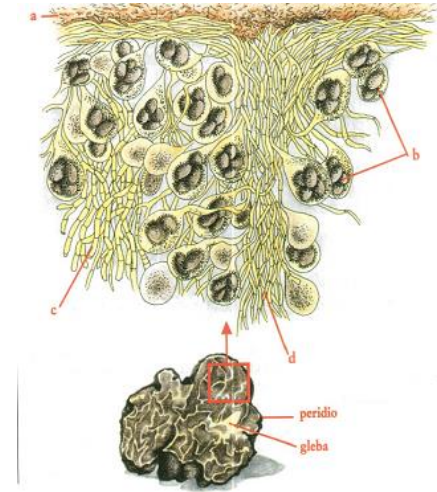
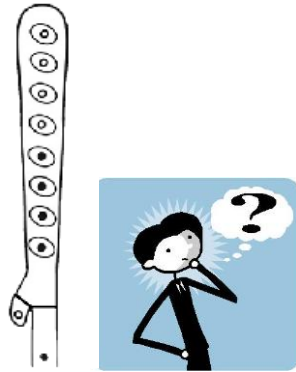
ORIGINAL PAPER

Pietro Buzzini · Chiara Gasparetti  
Benedetta Turchetti · Maria Rita Cramarossa  
Ann Vaughan-Martini · Alessandro Martini  
Ugo Maria Pagnoni · Luca Forti

**Production of volatile organic compounds (VOCs) by yeasts isolated from the ascocarps of black (*Tuber melanosporum* Vitt.) and white (*Tuber magnatum* Pico) truffles**



# Animal interactions










# Insect interactions

Environ Monit Assess (2024) 196:1239  
<https://doi.org/10.1007/s10661-024-13401-8>

RESEARCH



## Identification of Dipteran species inhabiting *Tuber aestivum* (the summer truffle) ascomata

Alessia Marino  · Marco Leonardi  · Emanuele Berrilli  ·  
Matteo Garzia  · Alessandra Zambonelli  · Pierfilippo Cerretti  ·  
Mirco Iotti 



## Searching the truffles with flies



# Mycovirus interactions



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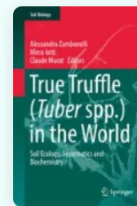
Home > [True Truffle \(Tuber spp.\) in the World](#) > Chapter

### Mycoviruses Infecting True Truffles

Chapter | First Online: 27 July 2016

pp 333–349 | [Cite this chapter](#)

[Save chapter](#)

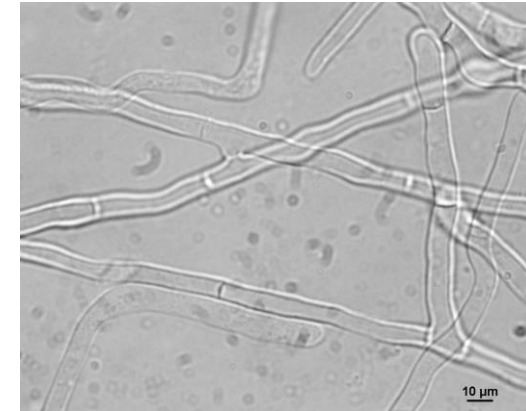


[True Truffle \(T](#)

[Claudio Ratti](#) [✉](#), [Mirco Iotti](#), [Alessandra Zambonelli](#) & [Federica Terlizzi](#)

Acc

# Bacterial interactions



# Mycorrhiza Helper Bacteria

*New Phytol.* (1994), **128**, 197–210

## Tansley Review No. 76 Helper bacteria: a new dimension to the mycorrhizal symbiosis

By J. GARBAYE

*INRA, Centre de Recherches Forestières de Nancy, 54280 Champenoux (France)*

*(Accepted 25 May 1994)*



Review

New  
Phytologist 



*Tansley review*

The mycorrhiza helper bacteria revisited

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Received: 23 April 2007  
Accepted: 17 June 2007

P. Frey-Klett<sup>1</sup>, J. Garbaye<sup>1</sup> and M. Tarkka<sup>2</sup>

<sup>1</sup>INRA, UMR1136 INRA-UHP 'Interactions Arbres/Micro-organismes', IFR 110, Centre de Nancy, 54280 Champenoux, France; <sup>2</sup>UFZ-Department of Soil Ecology, Helmholtz Centre for Environmental Research, Theodor-Lieser-Strasse 4, 06120 Halle, Germany

## Plants, Mycorrhizal Fungi, and Bacteria: A Network of Interactions

Paola Bonfante and Iulia-Andra Anca

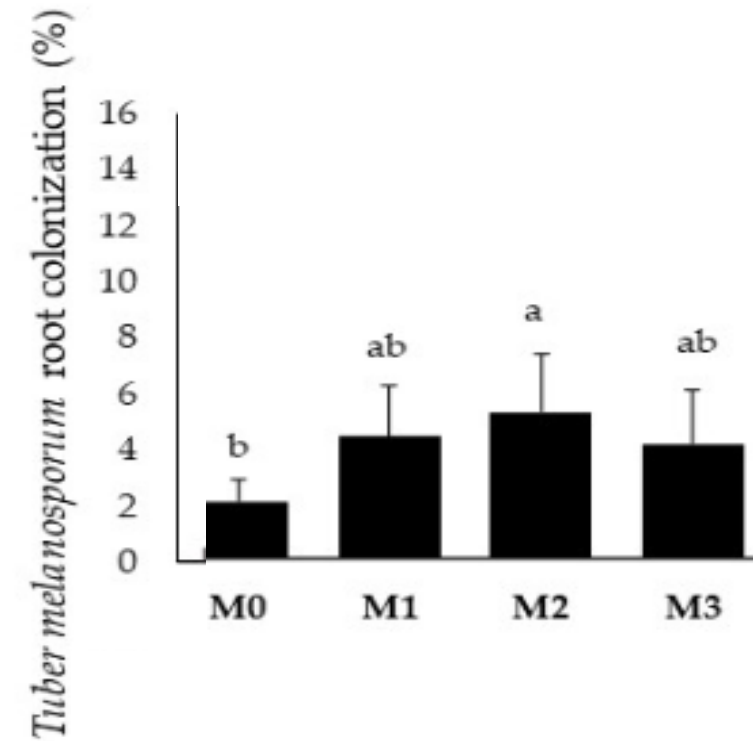
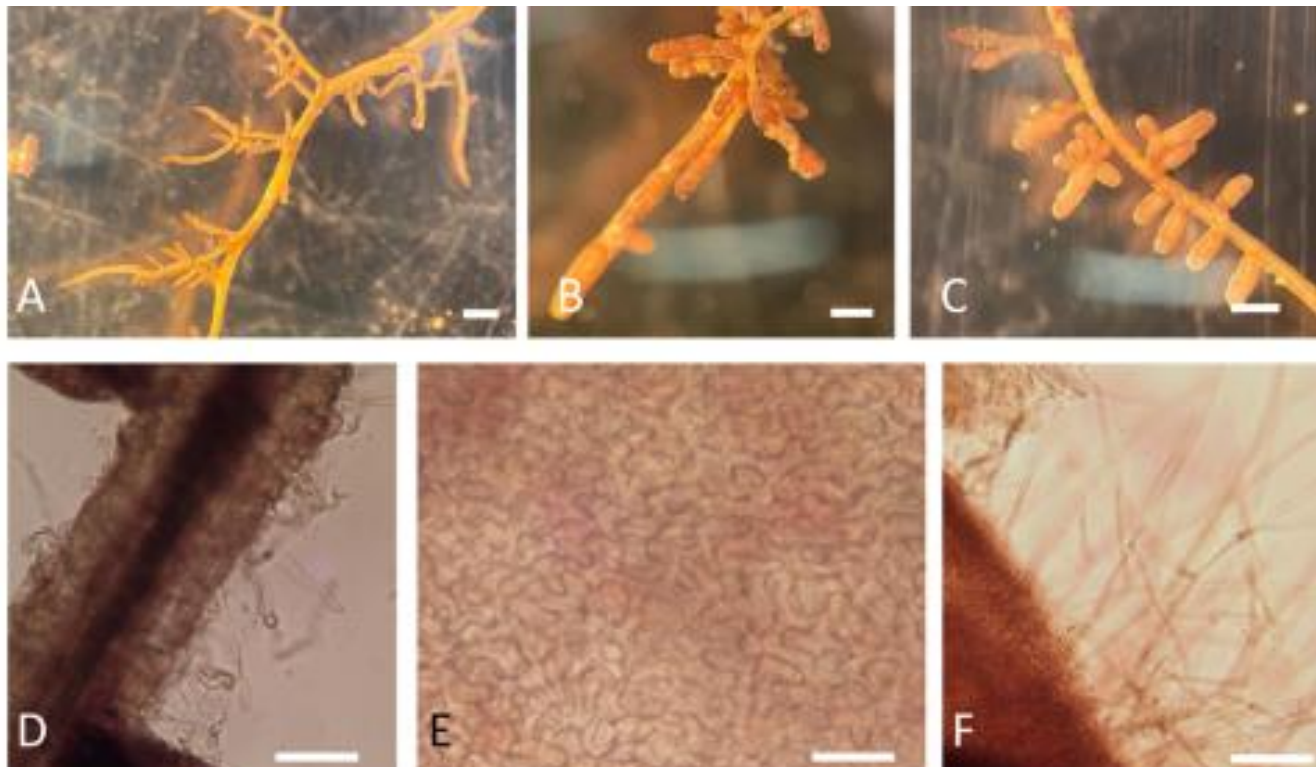
Dipartimento di Biologia Vegetale dell' Università di Torino and Istituto di Protezione delle Piantе del CNR, Sezione di Torino, 10125 Torino, Italy; email: [p.bonfante@ipp.cnr.it](mailto:p.bonfante@ipp.cnr.it)

*Annu. Rev. Microbiol.* 2009. 63:363–83



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# Bacteria promoting the establishment of mycorrhizal symbiosis with *T. melanosporum*



Article

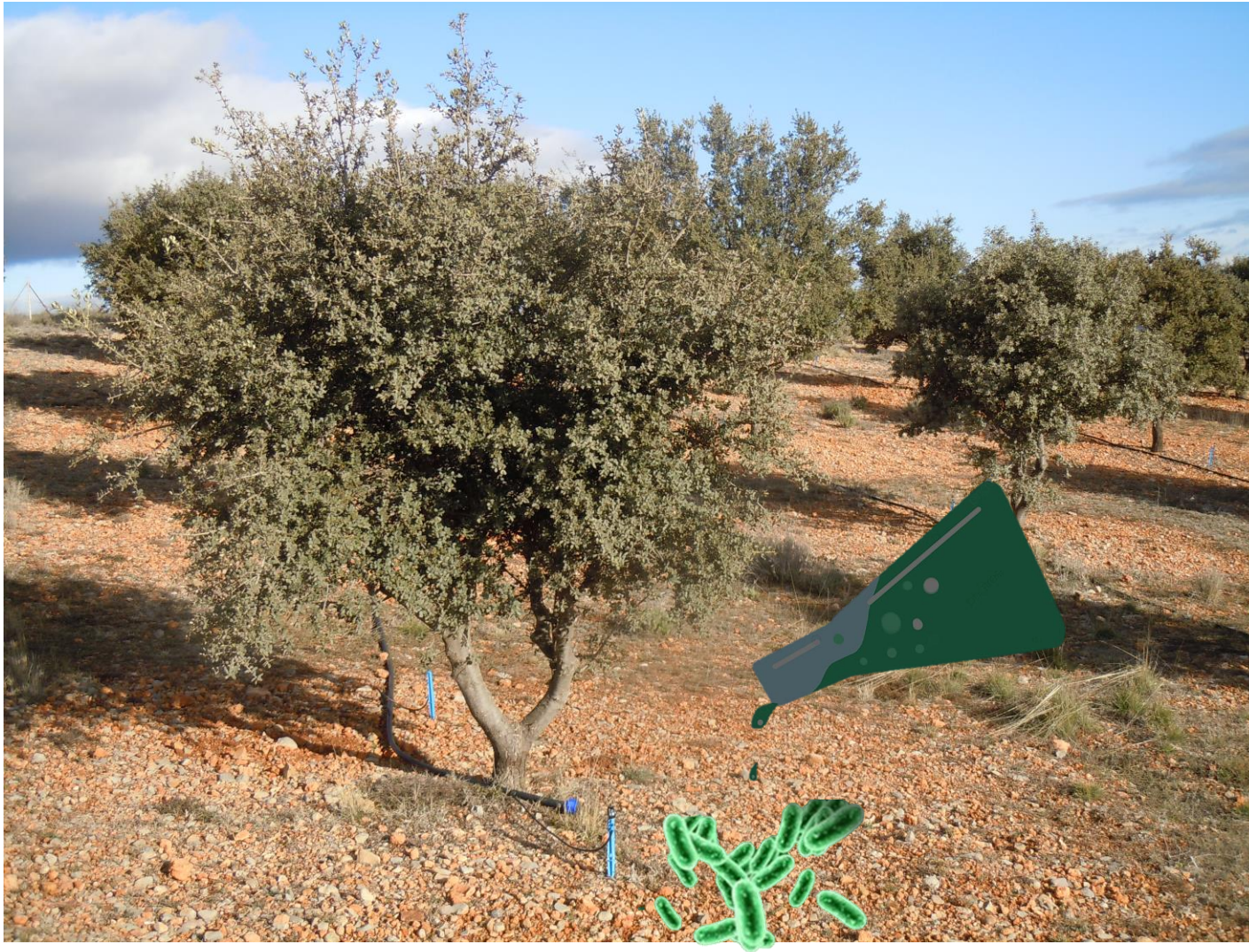
## Effect of Bacteria Inoculation on Colonization of Roots by *Tuber melanosporum* and Growth of *Quercus ilex* Seedlings

Veronica Giorgi <sup>1</sup>, Antonella Amicucci <sup>2,\*</sup>, Lucia Landi <sup>1</sup>, Ivan Castelli <sup>1</sup>, Gianfranco Romanazzi <sup>1</sup>, Cristiano Peroni <sup>3</sup>, Bianca Ranocchi <sup>2</sup>, Alessandra Zambonelli <sup>4</sup> and Davide Neri <sup>1</sup>



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# Inoculation of bacteria in the field













# Bacterial Communities within the Fruiting Body: Implications for Truffle Quality



Fungal Biology  
Volume 122, Issue 12, December 2018, Pages 1134-1141



## Microbial and pigment profile of the reddish patch occurring within *Tuber magnatum* ascomata

Antonella Amicucci <sup>a</sup> <sup>b</sup> <sup>1</sup>  , Elena Barbieri <sup>a</sup> <sup>1</sup>  , Valentina Sparvoli <sup>a</sup> ,  
Anna Maria Gioacchini <sup>a</sup> , Cinzia Calcabrini <sup>a</sup> , Francesco Palma <sup>a</sup> ,  
Vilberto Stocchi <sup>a</sup> <sup>2</sup> , Alessandra Zambonelli <sup>b</sup> <sup>2</sup> 



# Bacterial Communities within the Fruiting Body: Implications for Truffle Aroma

environmental  
microbiology



Environmental Microbiology (2015) 17(8), 2647–2660

doi:10.1111/1462-2920.12521

## Bacteria associated with truffle-fruiting bodies contribute to truffle aroma

Richard Splivallo,<sup>1,2\*</sup> Aurélie Deveau,<sup>3</sup> Nayuf Valdez,<sup>1</sup> Nina Kirchhoff,<sup>1</sup> Pascale Frey-Klett<sup>3</sup> and Petr Karlovsky<sup>1</sup>

<sup>1</sup>Molecular Phytopathology and Mycotoxin Research, Georg-August University of Goettingen, Goettingen, Germany.

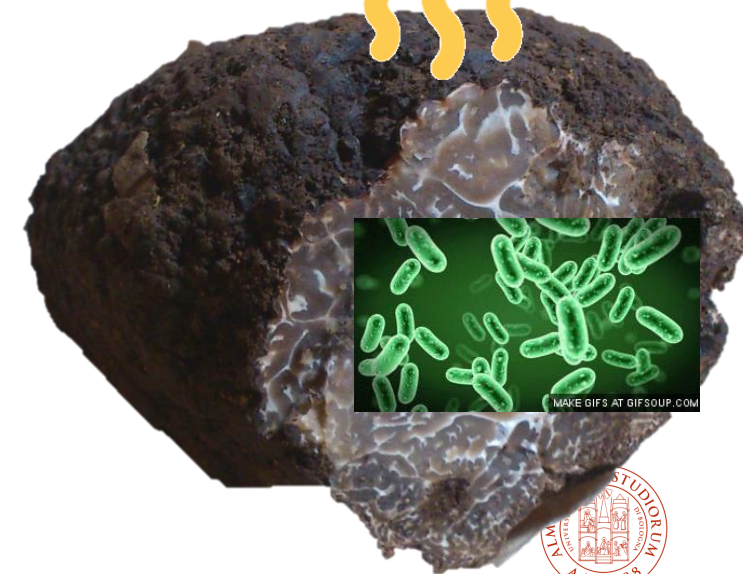
<sup>2</sup>Institute for Molecular Biosciences, Biozentrum/Campus Riedberg, Frankfurt am Main, Germany.

<sup>3</sup>INRA, UMR 1136 INRA Université de Lorraine 'Interactions Arbres/Micro-organismes', Champenoux, France.

sexual stage of truffles, thiophene volatiles are exclusively synthesized by bacteria and not by the truffle. At this stage, the origin of thiophenes precursor in *T. borchii* remains elusive and the involvement of yeasts or other bacteria cannot be excluded.

### Introduction

Truffles are symbiotic fungi that develop underground in association with plant roots, forming ectomycorrhizas (Mello *et al.*, 2006). Ectomycorrhizal associations, domi-



ELSEVIER

Contents lists available at ScienceDirect

Food Microbiology

journal homepage: [www.elsevier.com/locate/fm](http://www.elsevier.com/locate/fm)



## Are bacteria responsible for aroma deterioration upon storage of the black truffle *Tuber aestivum*: A microbiome and volatilome study

Maryam Vahdatzadeh<sup>a,b</sup>, Aurélie Deveau<sup>c</sup>, Richard Splivallo<sup>a,b,\*</sup>

<sup>a</sup>Institute of Molecular Biosciences, Goethe University Frankfurt, 60438, Frankfurt am Main, Germany

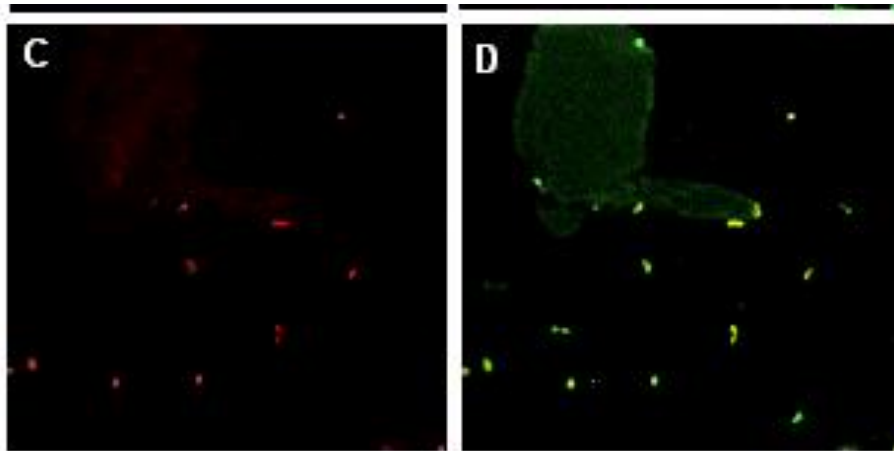
<sup>b</sup>Integrative Fungal Research Cluster, 60325, Frankfurt, Germany

<sup>c</sup>Institut national de la recherche agronomique (INRA), Unité Mixte de Recherche 1136 INRA-Université de Lorraine, Interactions Arbres/Microorganismes, Centre INRA-Grand Est-Nancy, 54280, Champenoux, France



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# Bacterial Communities within the Fruiting Body: Implications for Truffle nutrition



Barbieri E., Bertini L., Rossi I., Ceccaroli P., Saltarelli R., Guidi C., Zambonelli A., Stocchi V. (2005). New evidence for bacterial diversity in the ascocarp of the ectomycorrhizal fungus *Tuber borchii* Vittad. FEMS microbiology letters 247, 23-25.

Barbieri E., Guidi C., Bertaux, J., Frey-Klett P., Garbaye J., Ceccaroli P., Saltarelli R., Zambonelli A., Stocchi V. (2007) Occurrence and diversity of bacterial communities in *Tuber magnatum* during truffle maturation. Environmental Microbiology, 9 (9): 2234-2246.



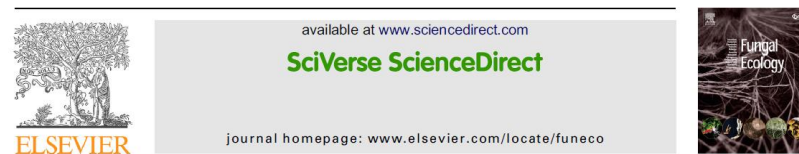
## New evidence for nitrogen fixation within the Italian white truffle *Tuber magnatum*

Elena BARBIERI<sup>a,\*</sup>, Paola CECCAROLI<sup>a</sup>, Roberta SALTARELLI<sup>a</sup>, Chiara GUIDI<sup>a</sup>, Lucia POTENZA<sup>a</sup>, Marina BASAGLIA<sup>b</sup>, Federico FONTANA<sup>b</sup>, Enrico BALDAN<sup>b</sup>, Sergio CASELLA<sup>b</sup>, Ouafae RYAHIC<sup>c</sup>, Alessandra ZAMBONELLI<sup>c</sup>, Vilberto STOCCHI<sup>a</sup>

<sup>a</sup>Dipartimento di Scienze Biomolecolari, University of Urbino "Carlo Bo", Urbino, Italy

<sup>b</sup>Dipartimento di Biotecnologie Agrarie, University of Padova, Agripolis, Legnaro, Italy

<sup>c</sup>Dipartimento di Protezione e Valorizzazione Agroalimentare, University of Bologna, Bologna, Italy



## Actinobacteria may influence white truffle (*Tuber magnatum* Pico) nutrition, ascocarp degradation and interactions with other soil fungi

Aleksandar PAVIĆ<sup>a,1</sup>, Slaviša STANKOVIĆ<sup>b</sup>, Elmira SALJNIKOV<sup>c</sup>, Đirk KRÜGER<sup>c</sup>, François BUSCOT<sup>c,d</sup>, Mika TARKKA<sup>c,d</sup>, Zakiina MARJANOVIĆ<sup>a,\*</sup>

<sup>a</sup>Institute for Multidisciplinary Research, University of Belgrade, Kneza Višeslava 1a, 11030 Belgrade, Serbia

<sup>b</sup>Chair of Microbiology, Faculty of Biology, University of Belgrade, Studentski Trg 16, 11000 Belgrade, Serbia

<sup>c</sup>UFZ – Helmholtz Centre for Environmental Research, Department of Soil Ecology, Theodor-Lieser-Str. 4, D-06120 Halle, Germany

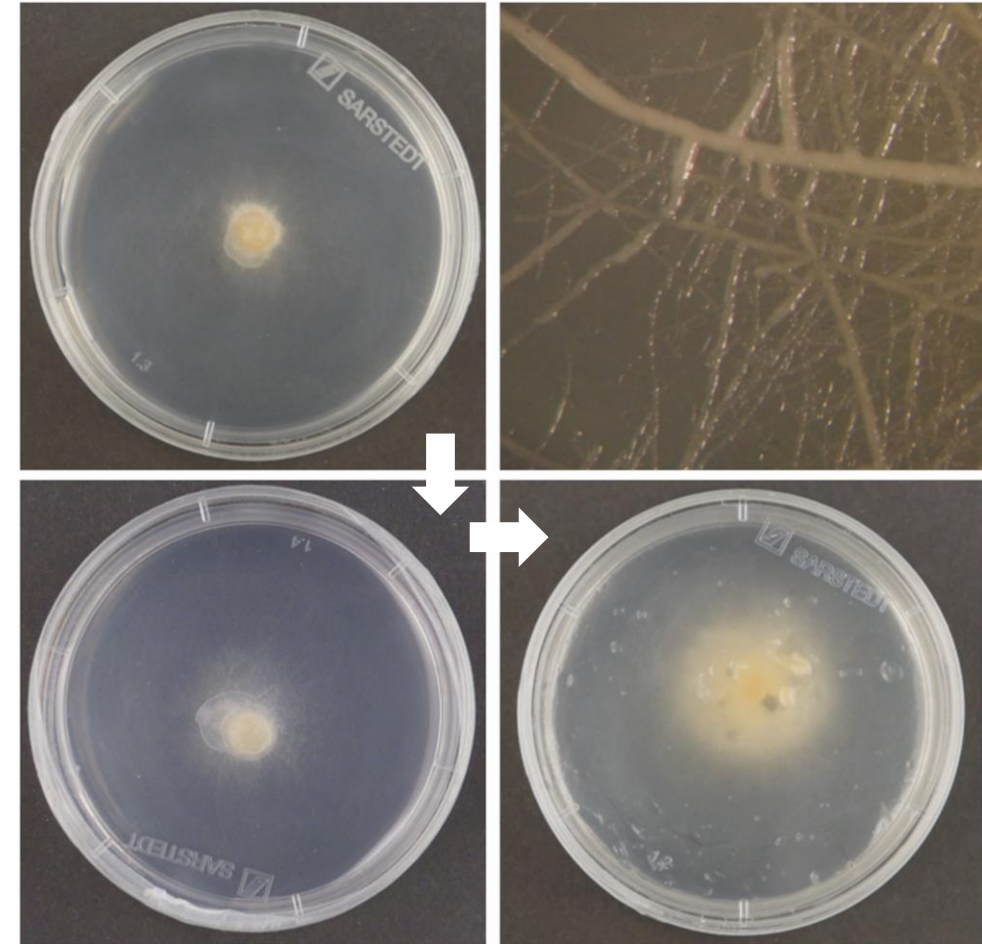
<sup>d</sup>University of Leipzig, Institute of Biology, Chair of Soil Ecology, Johannisallee 21-23, D-04103 Leipzig, Germany

<sup>e</sup>Institute for Soil Science, Teodora Dražžera 7, 11 000 Belgrade, Serbia



# Positive effects of bacteria on mycelium growth in vitro

- The mycelium of *T. magnatum* was isolated on WPMm
- Confirmed by PCR with *T. magnatum* specific primers
- Biofilm formation
  
- Three *T. magnatum* strains 5072, 5300, 5319



Received: 30 November 2023 | Accepted: 6 April 2024

DOI: 10.1111/1758-2229.13271

RESEARCH ARTICLE

ENVIRONMENTAL MICROBIOLOGY REPORTS

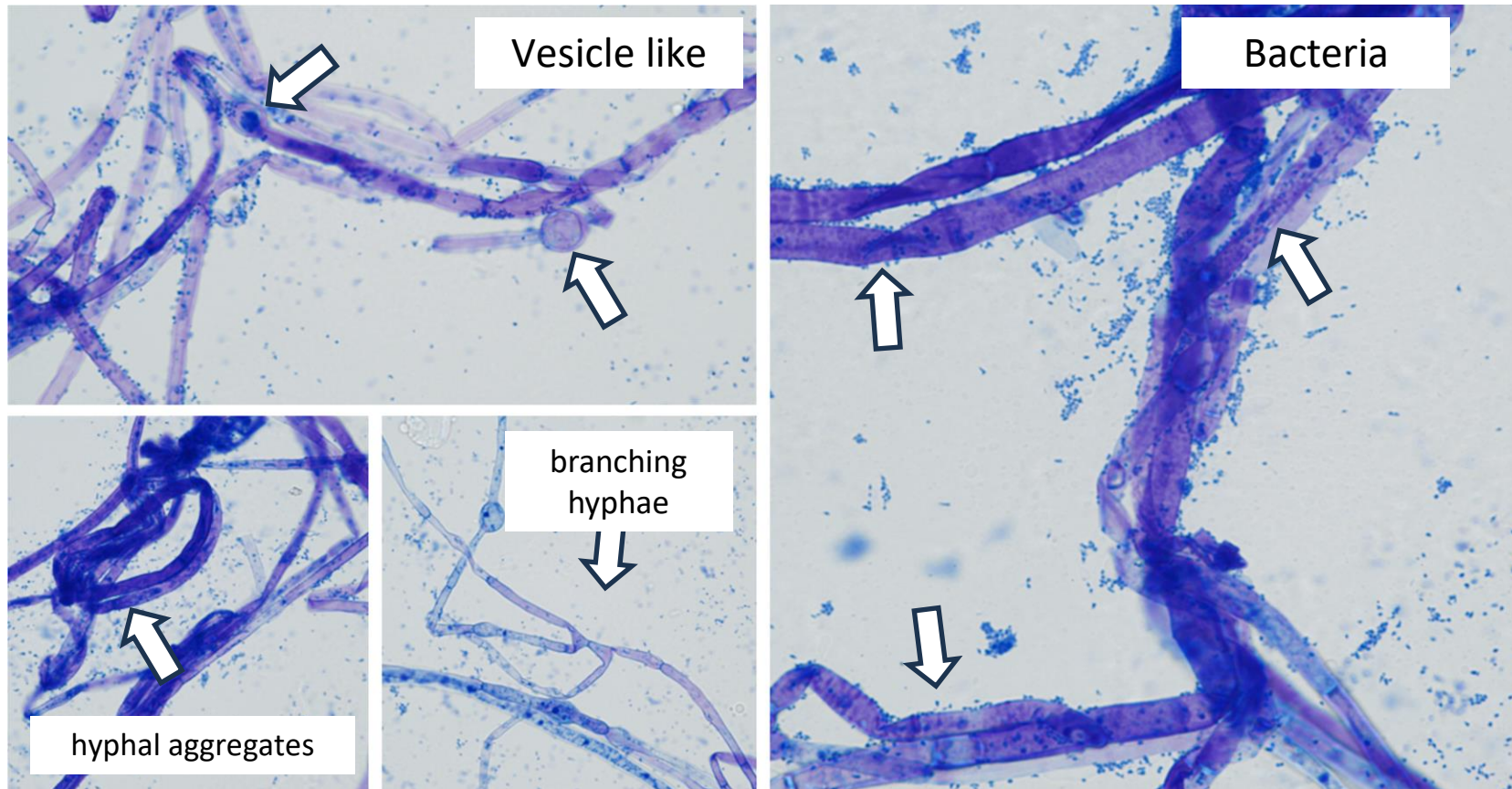
## In vitro interactions between *Bradyrhizobium* spp. and *Tuber magnatum* mycelium

Simone Graziosi<sup>1</sup> | Federico Puliga<sup>1</sup> | Mirco Iotti<sup>2</sup> | Antonella Amicucci<sup>3</sup> |  
Alessandra Zambonelli<sup>1</sup>



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# Microscopic features of the hyphae



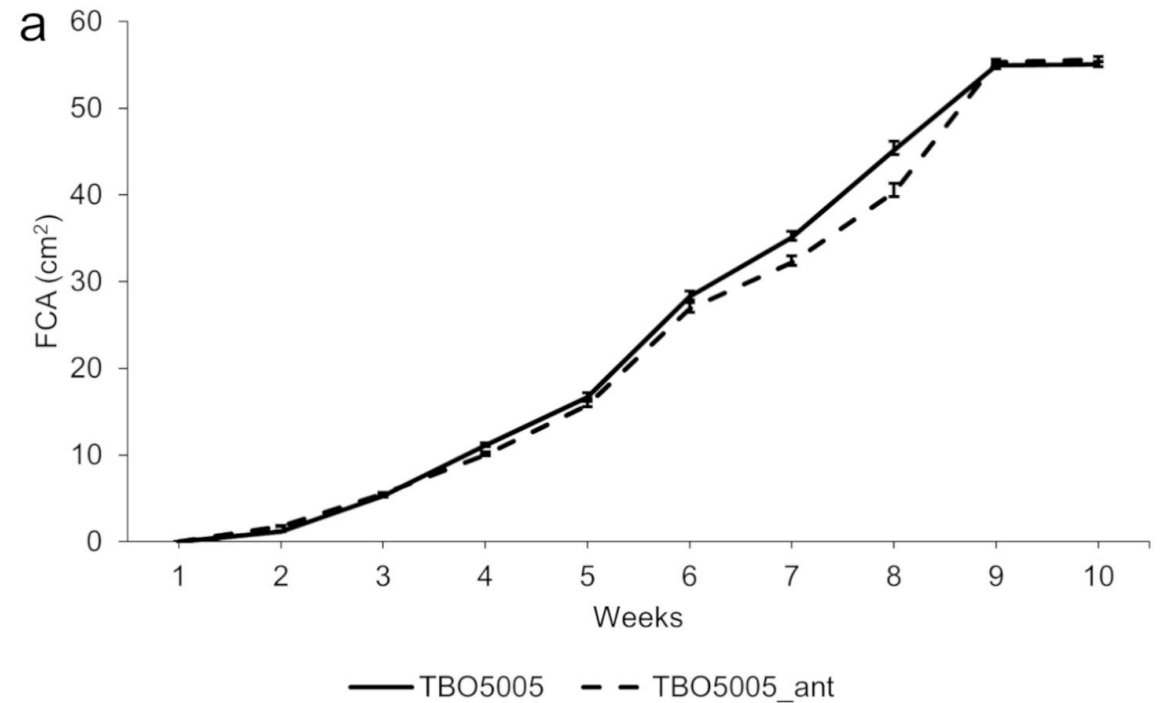
# Antibiotic treatment

- Addizion of Streptomycin, Ampicillin and Chloramphenicol to WPMm.



- Pre – test on *T. borchii*: no effects of antibiotic treatment

- Antibiotic treatment suppress *T. magnatum* mycelium and bacterial grwoth



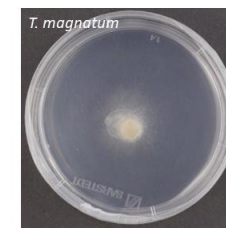
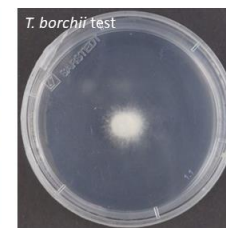
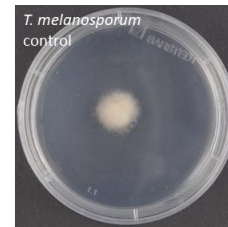
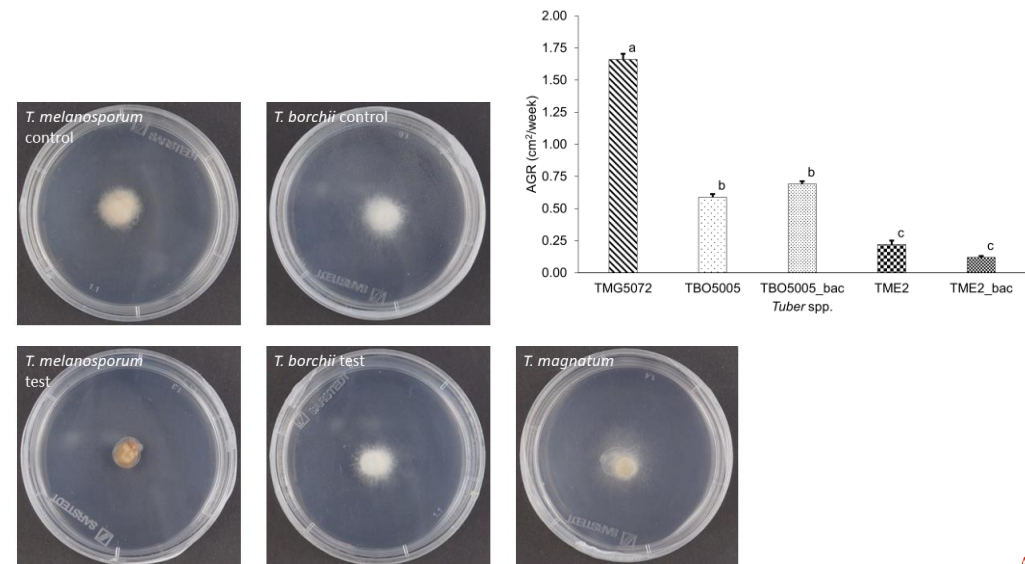
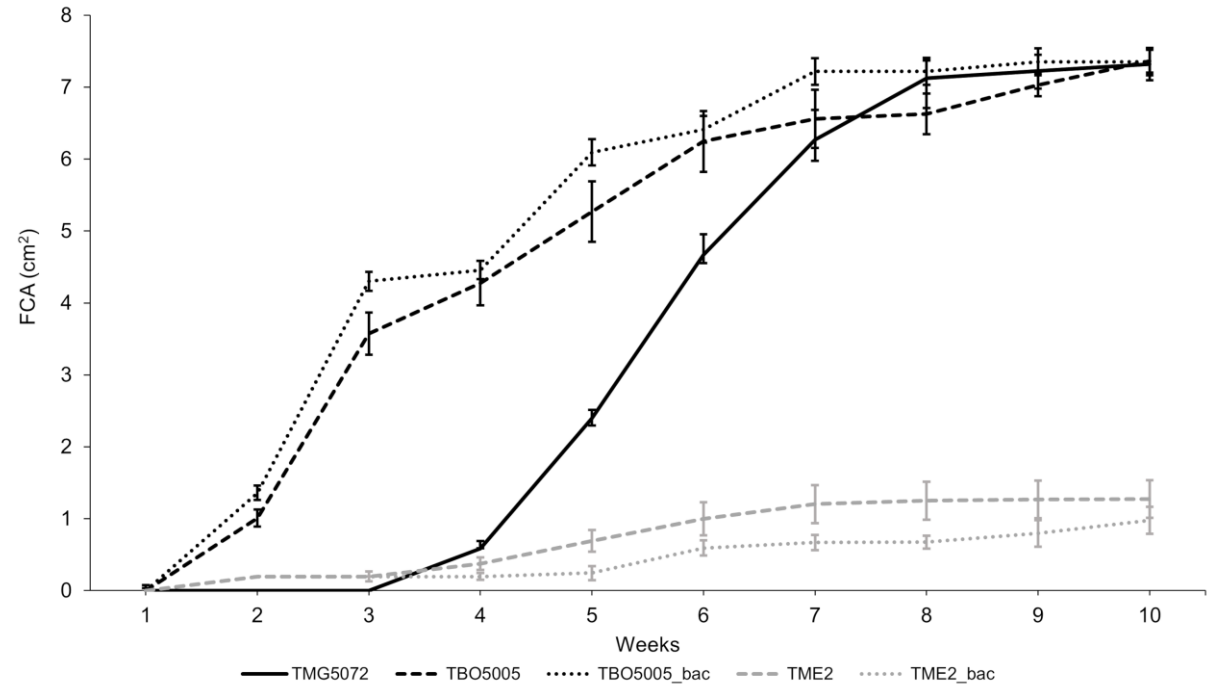
## Co-culture and specificity test

- **Co-culture assays** of *T. magnatum* and other *Tuber* species (*T. borchii* and *T. melanosporum*) on WPMM, with bacterial supplementation.

- *T. magnatum* showed significantly higher AGR values than the other species.

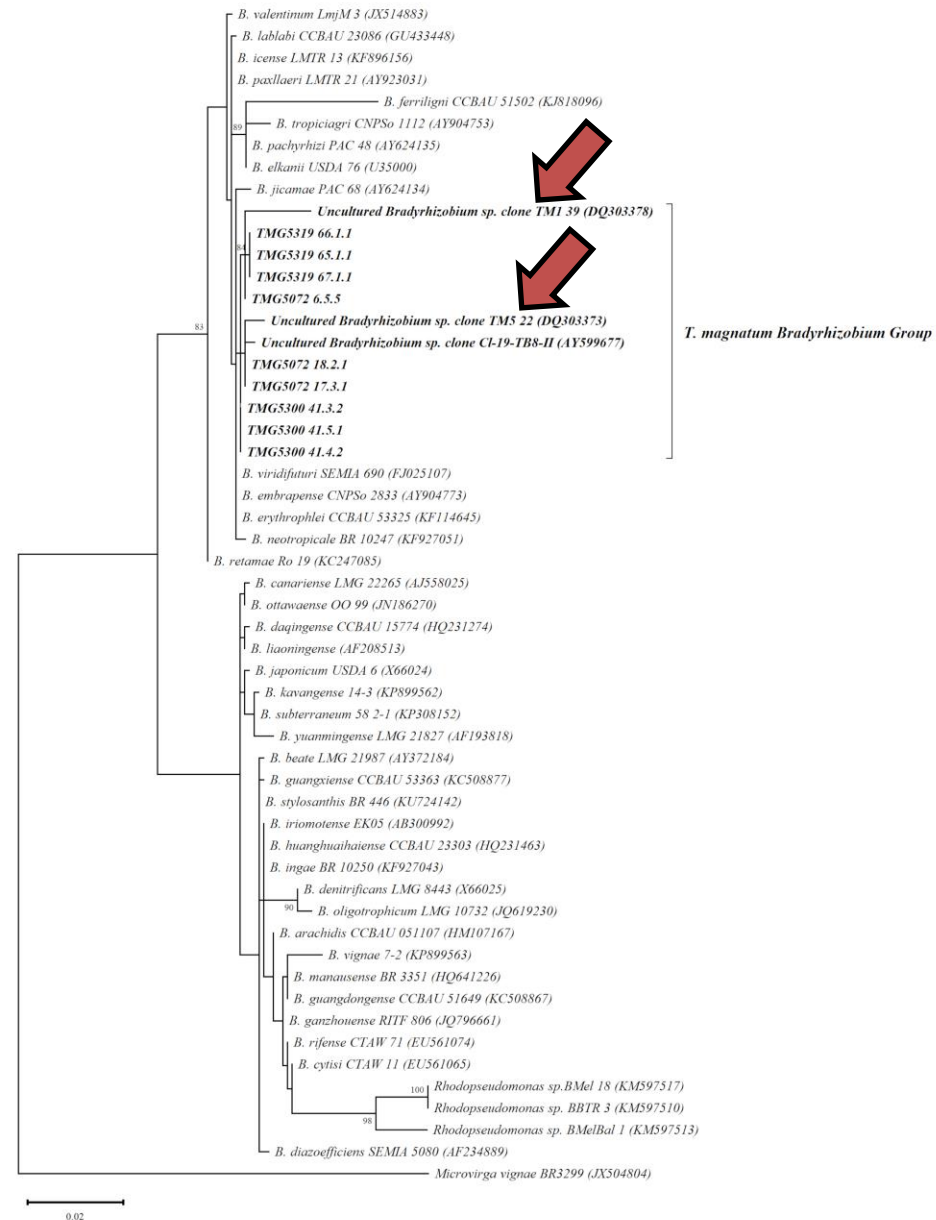
- Growth of *T. borchii* and *T. melanosporum* was not significantly affected by the presence of bacteria.

- **Conclusion:** Bacterial supplementation specifically promotes the growth of *T. magnatum*, while having little or no effect on the growth of the other *Tuber* species.

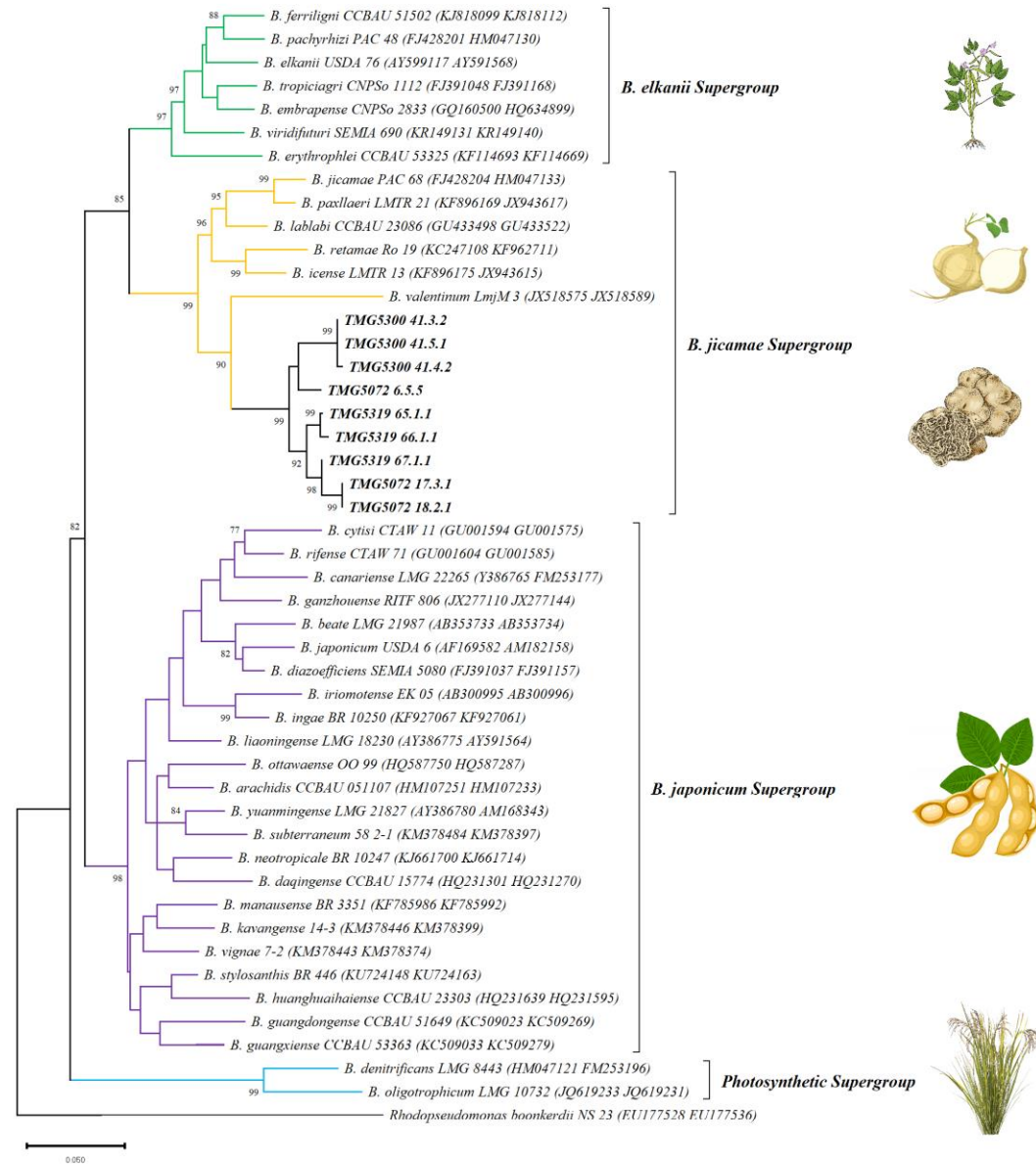


# 16S rRNA phylogenetic tree

The 16S phylogeny showed that all our isolates belong to the genus *Bradyrhizobium*. The strains clustered together with bacterial sequences previously detected in the ascomata of *T. magnatum* and *T. borchii*, as reported in earlier studies.

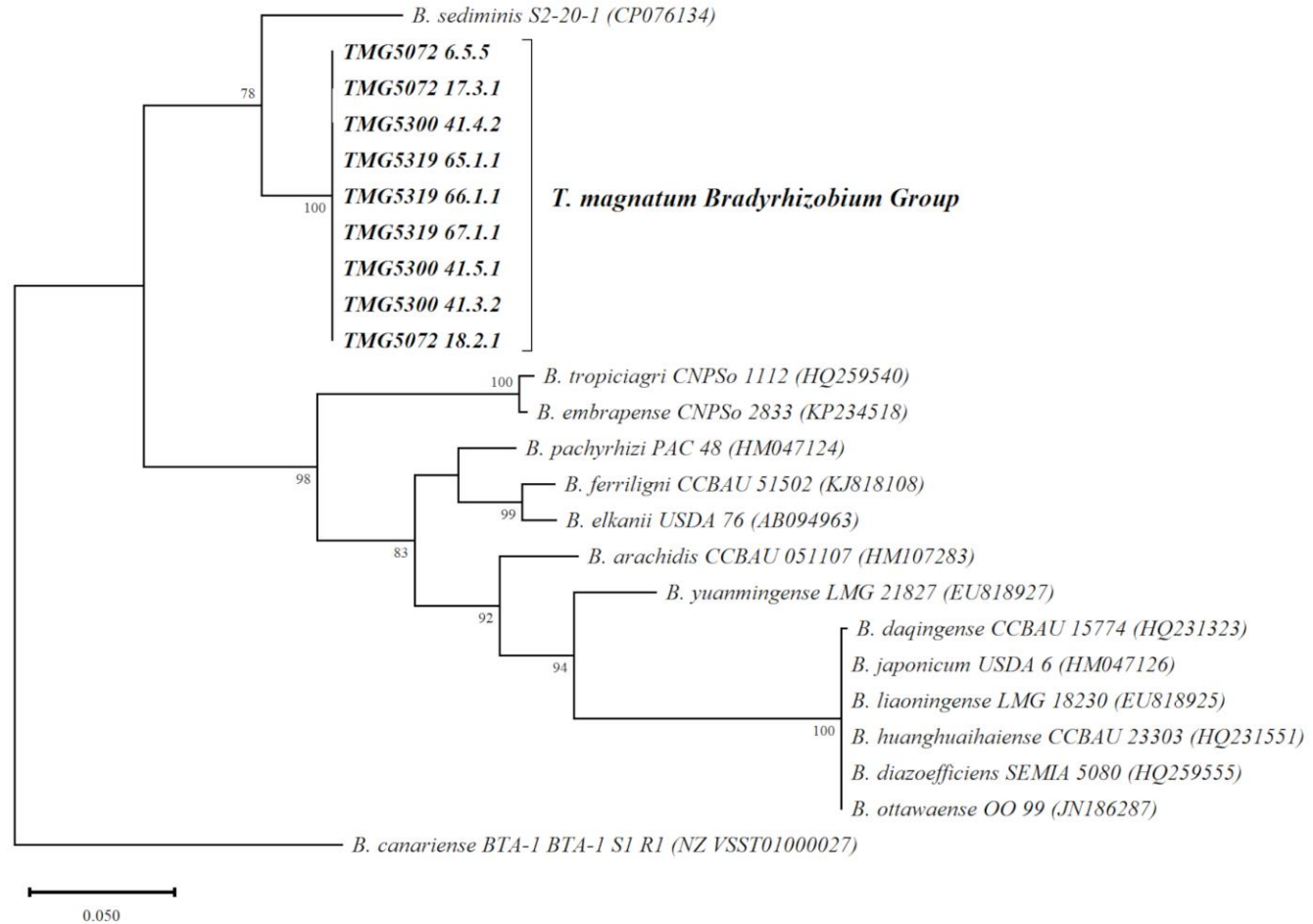


# glnII + recA MLSA

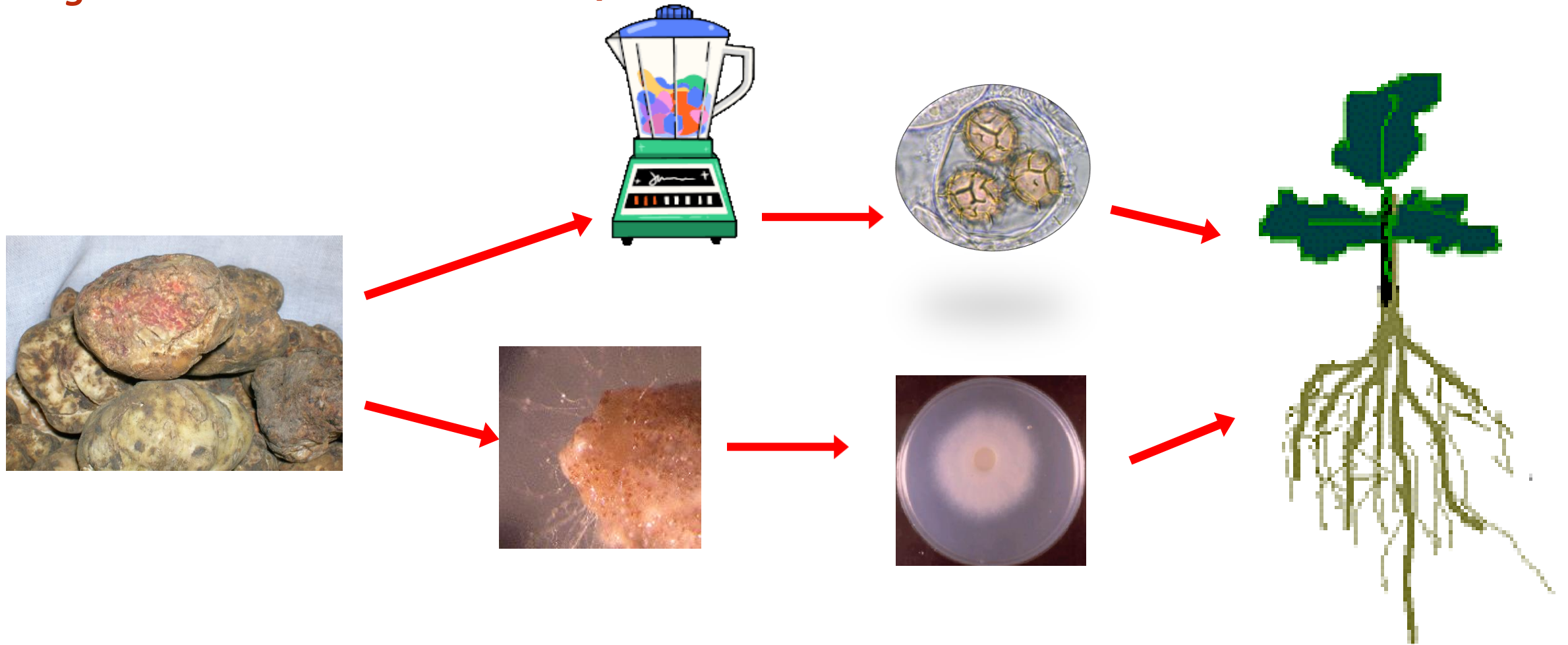


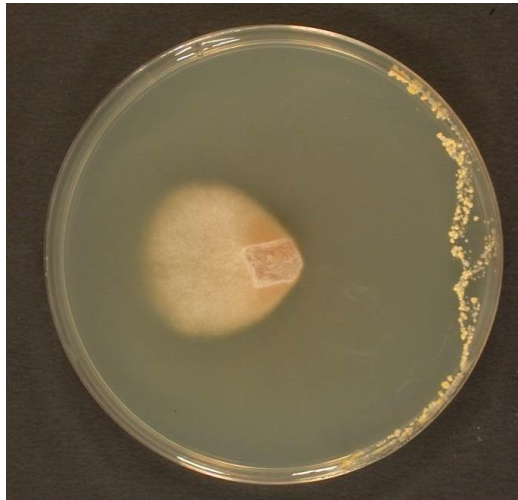
# nifH Phylogenetic tree

\*(Jin et al., 2022)



# Future Biotechnological Applications - Development of optimized culture systems for *Tuber magnatum* based on its bacterial partners





RAPID COMMUNICATIONS IN MASS SPECTROMETRY  
*Rapid Commun. Mass Spectrom.* 2005; 19: 3411–3415  
 Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/rcm.2209

**RCM**

### Determination of microbial volatile organic compounds from *Staphylococcus pasteurii* against *Tuber borchii* using solid-phase microextraction and gas chromatography/ion trap mass spectrometry

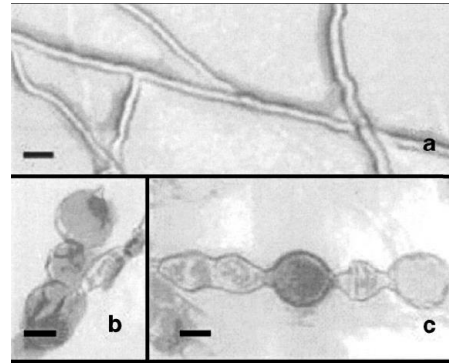
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Some bacterial species can negatively affect the development of truffle mycelium.



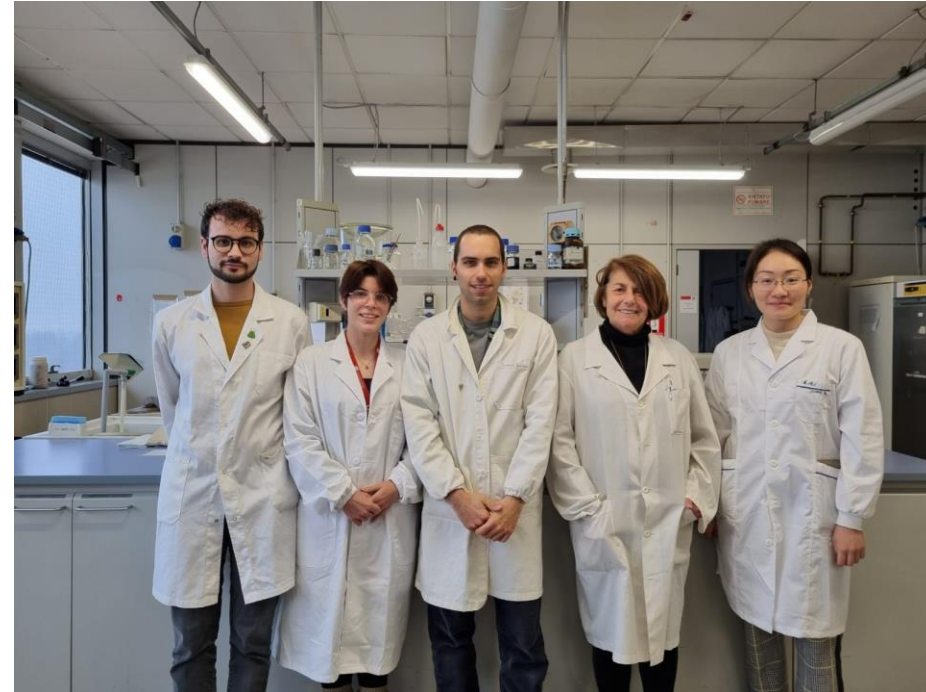
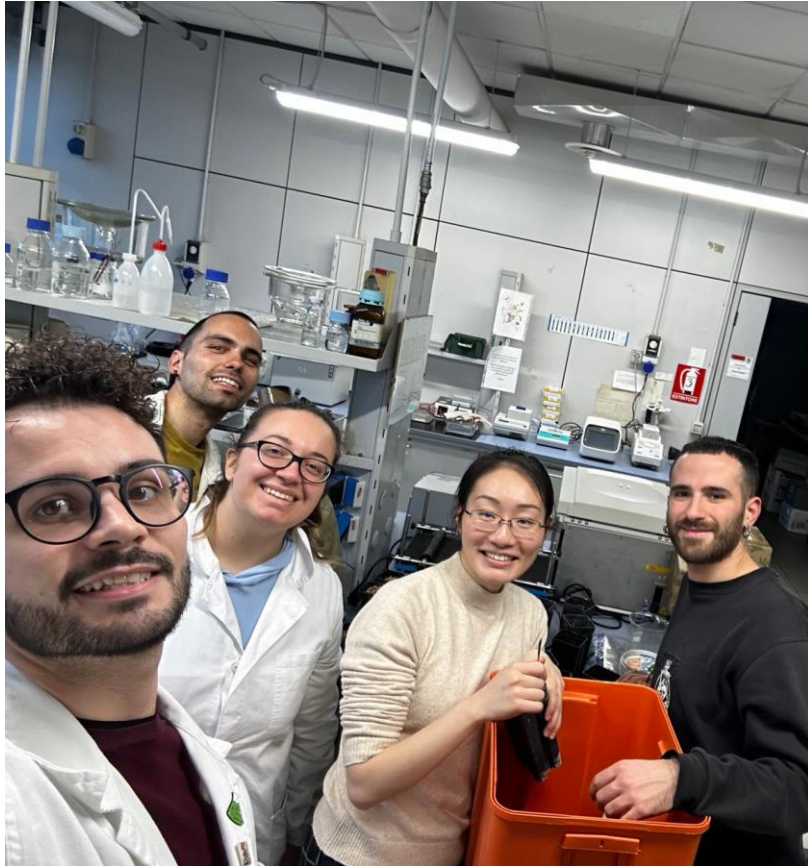
## Conclusions

- Truffles interact with a wide range of organisms within the soil ecosystem.
- Soil and environmental conditions shape microbial community composition and influence these interactions.
- Interactions can be beneficial, neutral, or detrimental to truffle development. These relationships affect all stages of the truffle life cycle, including mycelial growth, symbiosis, and fruiting body production.
- Understanding these complex interactions is essential for improving truffle cultivation.
- This knowledge may enable the development of innovative biotechnological applications for truffle cultivation based on beneficial microbial partners.

***To fully understand and cultivate truffles, we must move beyond studying the fungus alone and consider the entire soil ecosystem in which it lives.***



# My collaborators



THANK YOU  
FOR YOUR  
ATTENTION

