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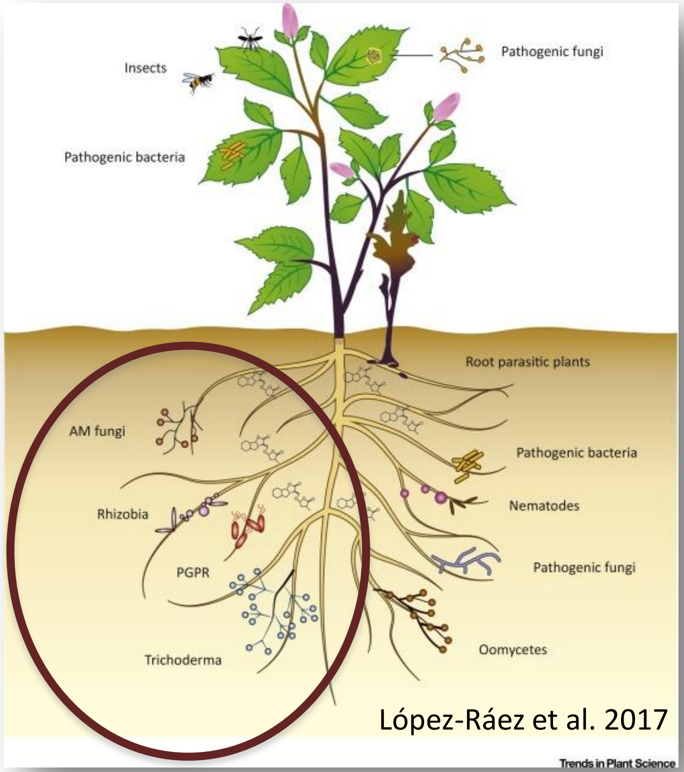
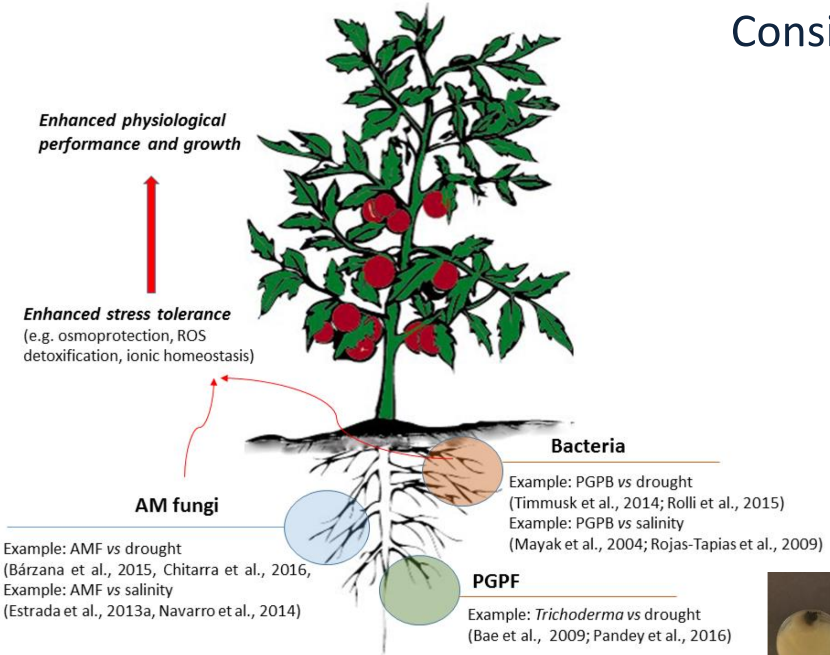
Gli effetti del climate change: il microbioma del suolo

Raffaella Balestrini
CNR-IPSP, Torino

All plants, in all environments, depend on microbes, and therefore, potentially all crops, no matter where they are grown, could benefit from optimization of their microbial partners.

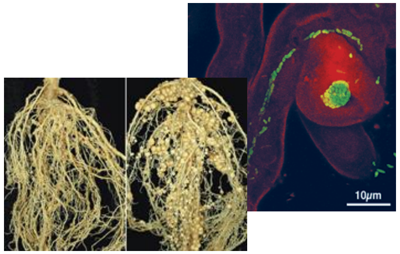


Beneficial root-associated microorganisms



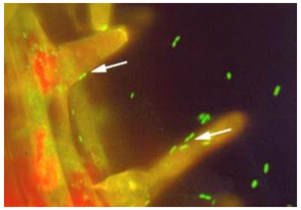
Considered as a key factor for managing crop production

Maintain soil fertility and support crops

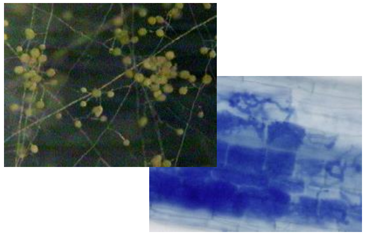


Root nodules (rhizobia-legumes)

PGPB or PGPR



Trichoderma spp.

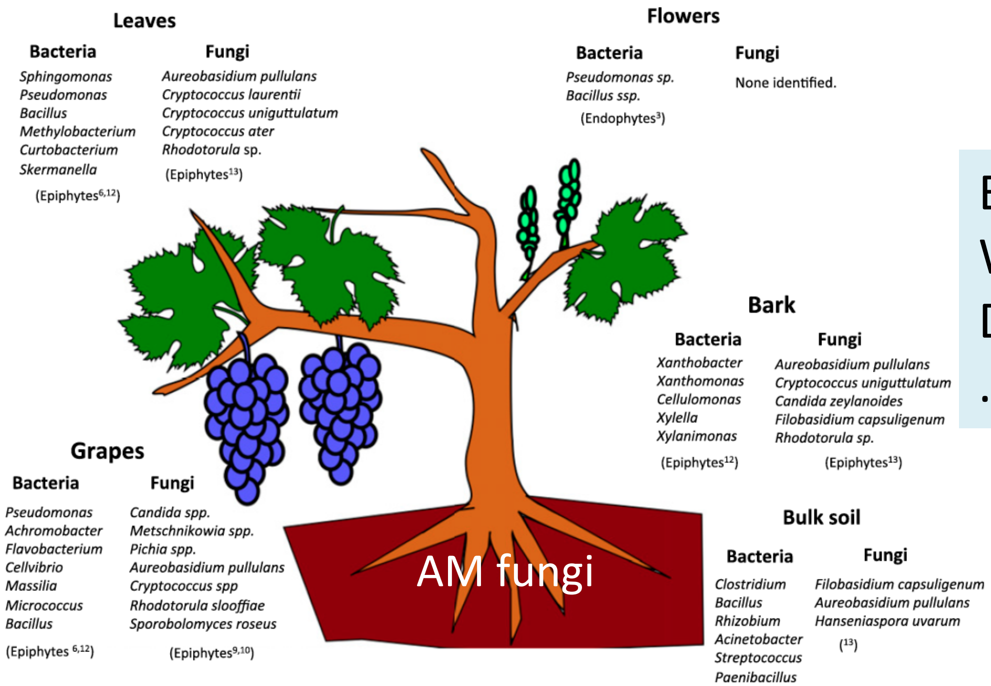


Arbuscular mycorrhizal (AM) fungi

The outcome of the several interactions can be environmental- and species-dependent, and the effects are often not sufficiently stable for practical application

Soil microbes, climate change, *terroir*…

…definisce l'interazione tra più fattori, come terreno, disposizione, clima, viti, viticoltori e come questa interazione porti alla realizzazione di un vino specifico e unico per la sua **territorialità**



Role in intial fermentation steps

Flavor, color, quality

Fig. 1. Diagrammatic representation of some of characteristic bacteria and fungi known to show associations with the different tissues of *Vitis vinifera*.

COMMENTARY

PNAS 2018

Microbial *terroir* for wine grapes

Elevated CO_2
Warming
Drought
…

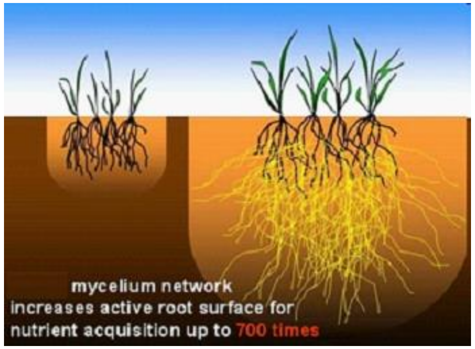
The soil microbiome is undoubtedly of importance but still remains less studied

Involved in the mitigation of the impact of environmental stress

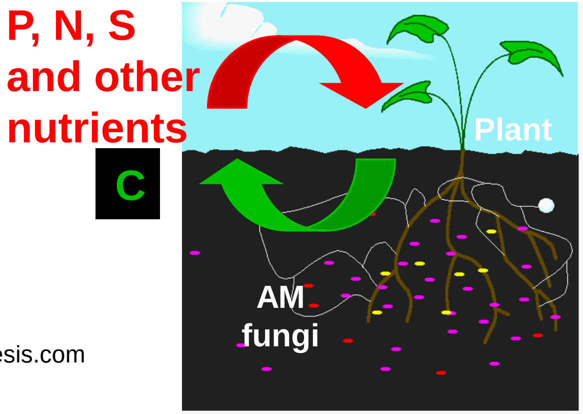
AMs: the symbioses 'that help feed the world'

- most widespread terrestrial symbiosis formed by ~80% of land plant species
- formed with obligate biotrophic fungi
- the genome of an AM fungus has been recently sequenced

Agricultural ecosystems



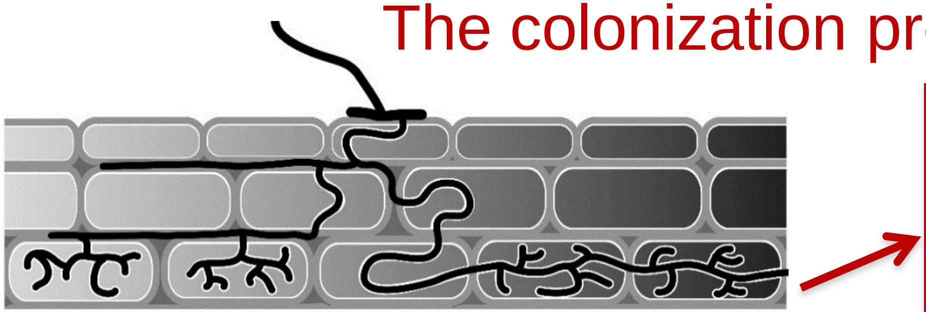
http://www.agro-genesis.com

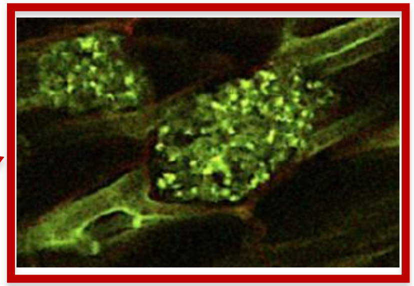


Benefits:
- Improved mineral nutrition
- Increased tolerance to abiotic and biotic stresses

Biofertilizers: the exploitation of these plant-beneficial symbionts in agro-environments is of high relevance

The colonization process





arbuscule

Phosphate transporter genes

MPMI Vol. 20, No. 9, 2007, pp. 1055–1062. doi:10.1094/MPMI-20-9-1055. © 2007 The American Phytopathological Society e-Xtra*

Laser Microdissection Reveals That Transcripts for Five Plant and One Fungal Phosphate Transporter Genes Are Contemporaneously Present in Arbusculated Cells

Raffaella Balestrini, Jorge Gómez-Ariza, Luisa Lanfranco, and Paola Bonfante

Istituto Protezione Piante, CNR and Dipartimento di Biologia Vegetale, Università di Torino, Viale Mattioli, 25-10125 Torino, Italy

Submitted 16 February 2007. Accepted 23 April 2007.

Plants:

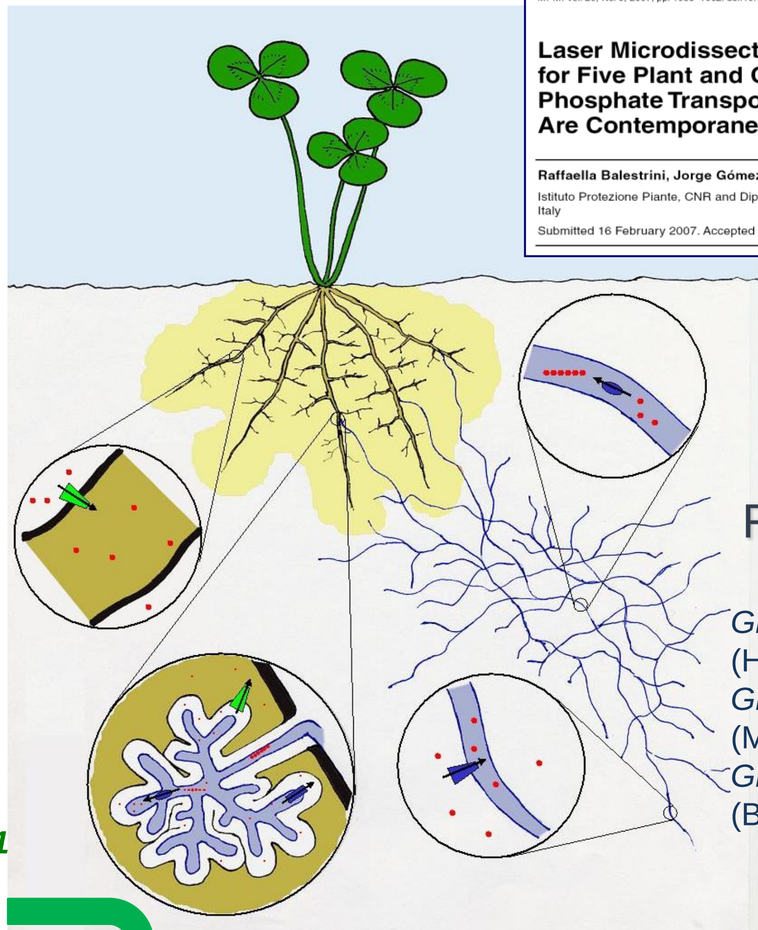
- Medicago
- Tomato
- Potato
- Rice
- Barley
- Wheat
- Poplar

MtPT1
StPT2
LePT1
LePT2

Bucher, 2007
Nagy et al., 2005
Javot et al., 2007

Plant

MtPT4
StPT3
OsPT11
LePT3
LePT4



Fungus

Glomus versiforme **GvPT**
(Harrison & van Buuren, 1995)
Glomus intraradices **GiPT**
(Maldonado-Mendoza et al. 2001)
Glomus mosseae **GmosPT**
(Benedetto et al., 2005)

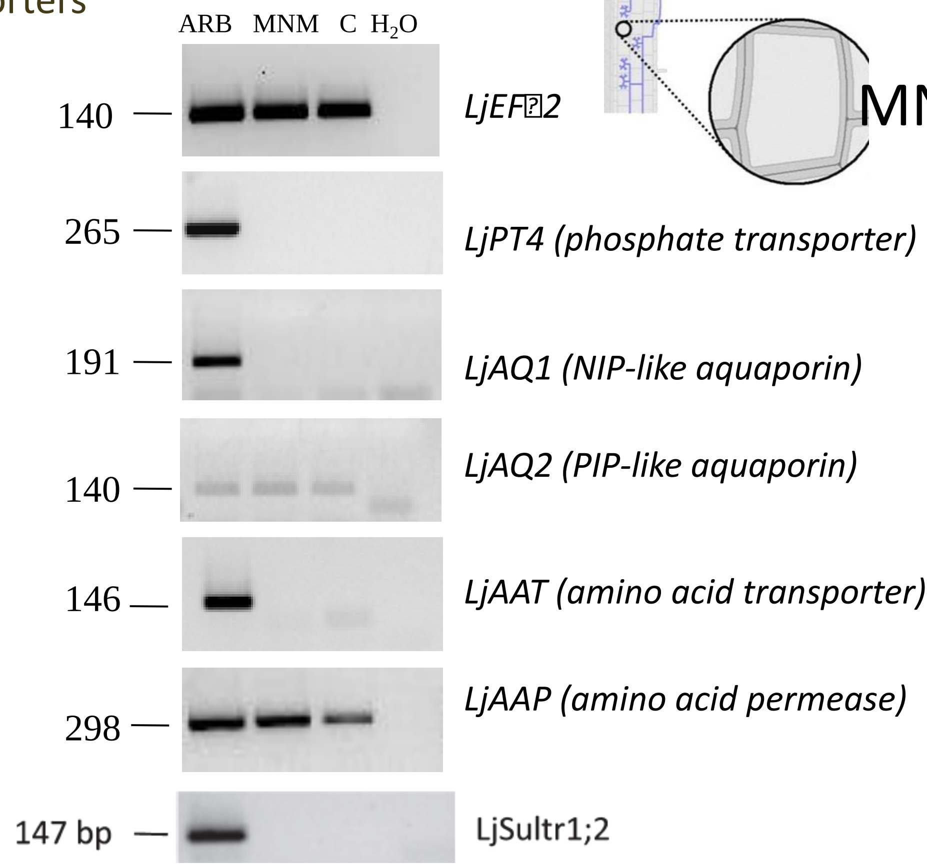
Plants possess PTs which are mycorrhiza specific

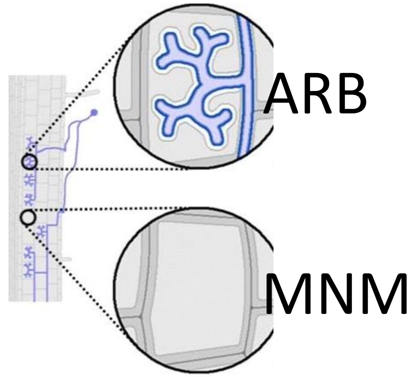
Global transcriptomic analyses showed that AMFs impact mineral plant nutrition, changing the expression of a huge number of nutrient transporters

Putative annotation	Number of genes
Phosphate transporters	1
Peptide transporters	7
Ammonium transporters	1
Nitrate transporters	4
Amino acid transporters	3
Potassium transporters	1
Sulfate transporters	3
Aquaporins/water channels	5
Sugar transporters	2
Zinc transporter	1
Other metal ion transporters	1

Lotus japonicus + *Gigaspora margarita*

LMD and RT-PCR

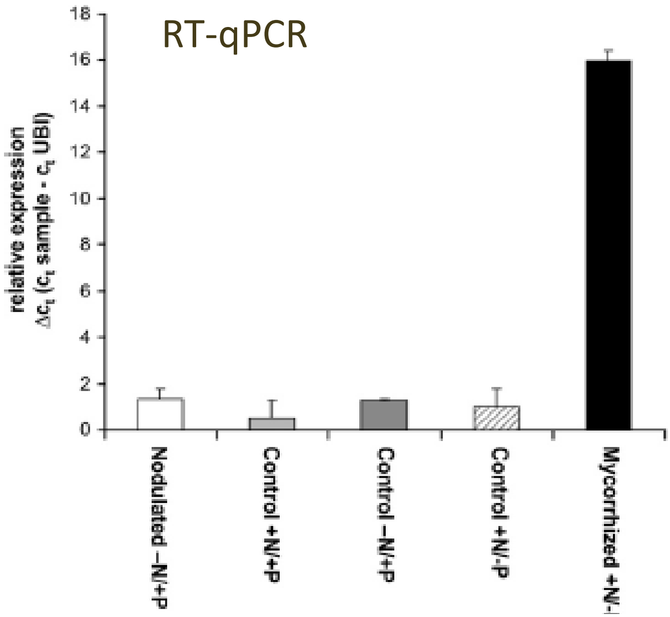




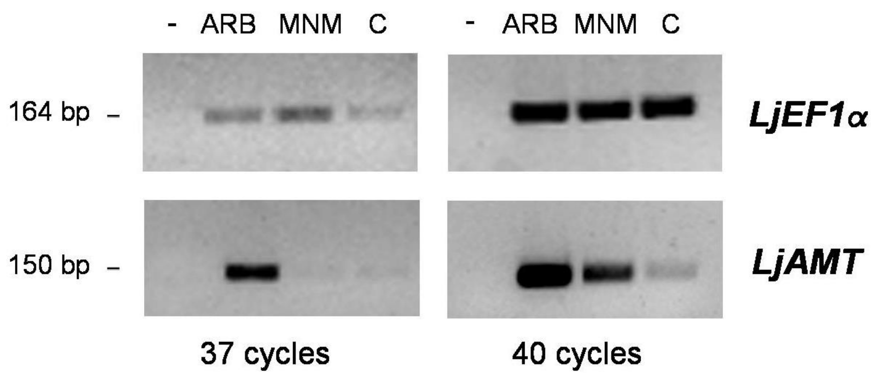
Guether *et al.* 2009, 2011
Giovannetti *et al.* 2012, 2014

LjAMT 2;2: an Ammonium-Transporter Type 2

LjAMT2;2 is specifically expressed in mycorrhizal roots



LjAMT2;2 is predominatly expressed in arbusculated cells



The finding of a plant mycorrhiza-dependent AMT transporter opens new speculation: mycorrhizal fungi could optimize the uptake of N from fertilizers dispersed on agricultural soils and release it as ammonium to the plant

Guether *et al.* 2009 – Plant Physiology

AM symbiosis in vineyards

Agron. Sustain. Dev. (2015) 35:1449–1467
DOI 10.1007/s13593-015-0329-7

CrossMark

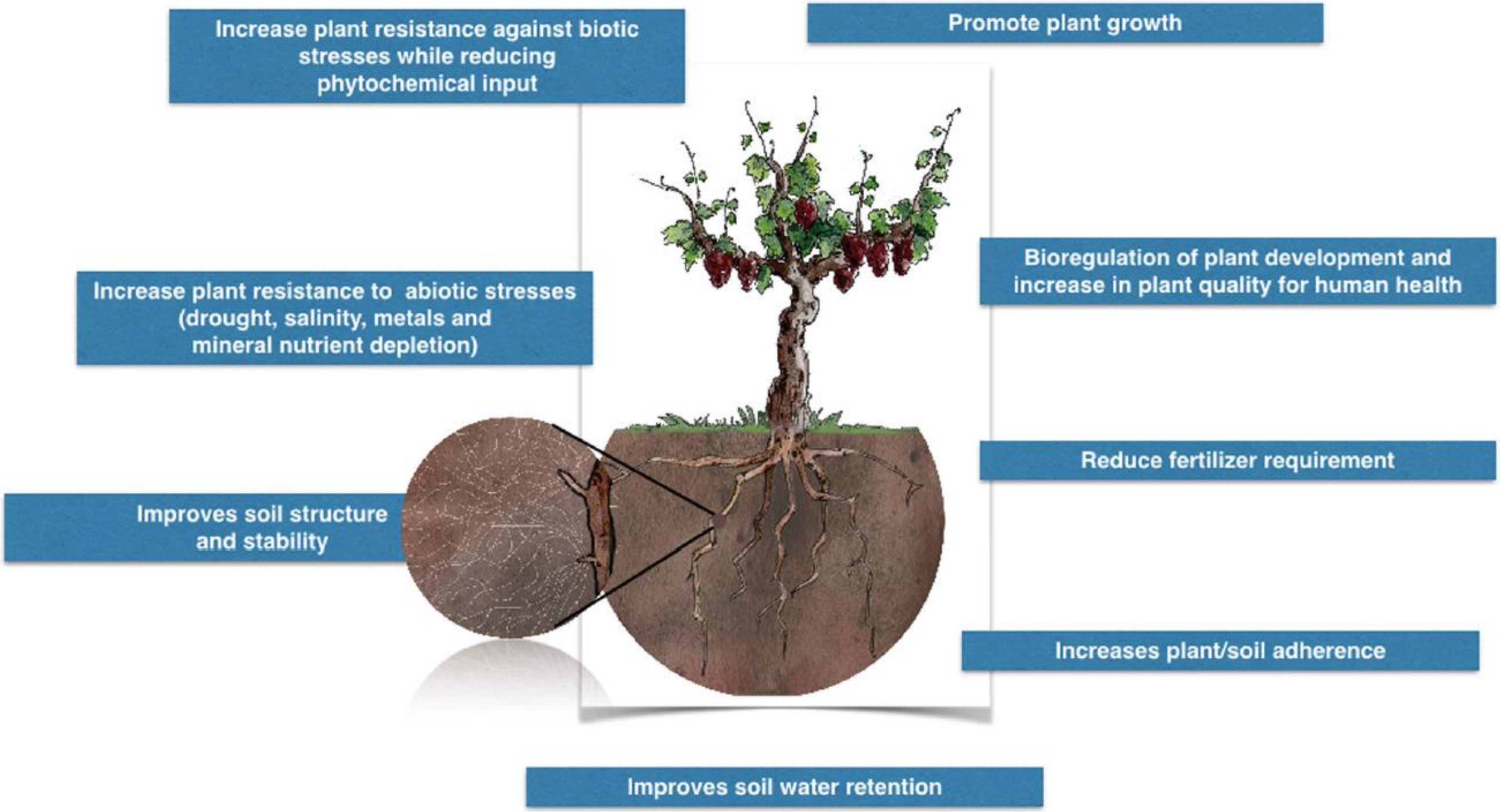
REVIEW ARTICLE

Arbuscular mycorrhiza symbiosis in viticulture: a review

Sophie Trouvelot[1] · Laurent Bonneau[1] · Dirk Redecker[1] · Diederik van Tuinen[2] · Marielle Adrian[1] · Daniel Wipf[1]

There is increasing interest from winegrowers, technical institutes, and the scientific community for a better knowledge of the possible ecosystemic services AM symbiosis could provide with respect to adaptation to climate evolution and development towards sustainable viticulture.

Ecosystem services provided by AM symbiosis



Exploitation and application of mycorrhizal fungi in agricultural programs

↓

✓ Identification of the events that lead to the establishment of a functional symbiosis, including the mechanisms involved in nutrient transfer and in the improved tolerance to several environmental stresses (abiotic, pathogens, pests)

✓ Identification of the best efficient microbial species. Cooperation between host plants and AM fungi is often related to the symbiotic partners, and it depends on several factors, such as environmental conditions, resources, plant/fungus functional diversity

CNR AQUA Project: Impact of the AM symbiosis on the **tomato** tolerance to **water stress** alone or in combination with a biotic stress such as aphid attacks or nematode infection.

Rhizophagus intraradices
Funneliformis mosseae

Lycopersicon esculentum
(San marzano nano)

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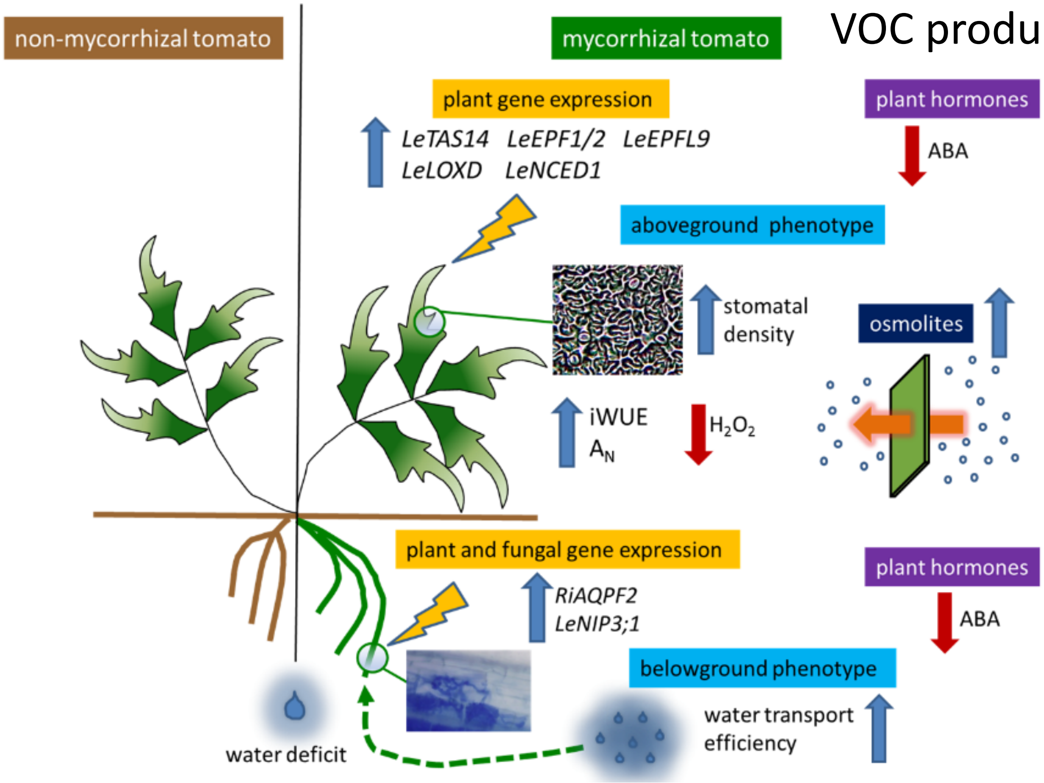
The association with two different AM fungi differently affects water stress tolerance in tomato



VOC products

PT genes

Chitarra et al. 2016
Volpe et al. submitted

AM symbiosis positively affects the tolerance to water deficit in tomato

Two different AM fungal species were used, confirming a species-specific impact on belowground/aboveground interactions in tomato

Different approaches:
Eco-physiological approaches
Transcriptomics (RT-qPCR, RNAseq)
Metabolomics
VOC emissions
Biochemical analyses

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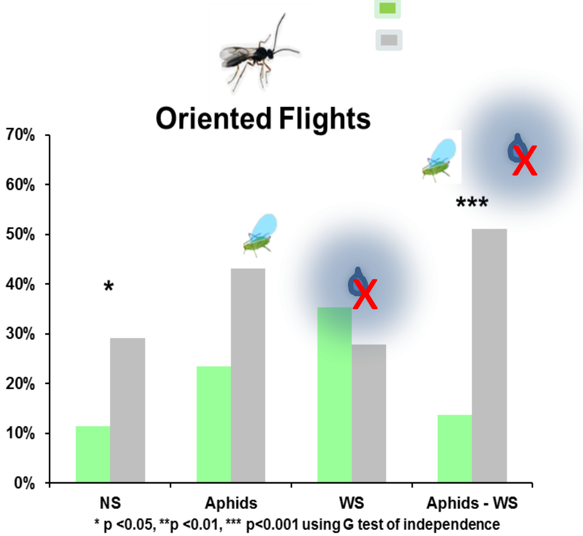
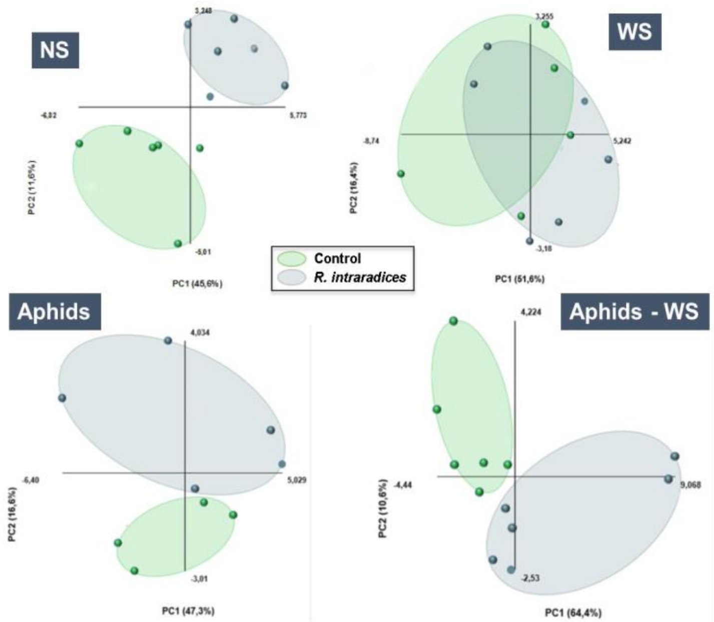
Plants are subject to multiple stresses: the role of AM symbiosis on tomato subjected to a combination of abiotic and biotic stresses

Parassitoid attractiveness



Macrosiphum euphorbiae–Aphidius ervi

PCA on VOC data



The variation in VOC emission mirrors the attractivity in AM-colonized plants respect to non-colonized plants, suggesting an enhanced plant tolerance to a combined stress condition (moderate WS and aphids)

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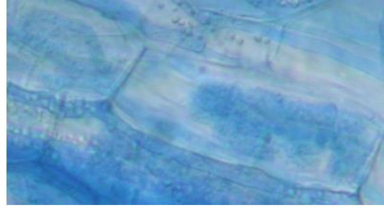
What about grapevine?



✓ Impact on growth and nutrient uptake (different rootstock, AM fungi, controlled and field conditions)

SYMBIOSIS (2006) 41, 127–133 ©2006 Balaban, Philadelphia/Rehovot ISSN 0334-5114

Drought responses of arbuscular mycorrhizal grapevines

A.J. Valentine[1*], P.E. Mortimer[1], M. Lintnaar[1], and R. Borgo[2]

Journal of Horticultural Science & Biotechnology (2003) **78** (1) 113–118

Cytokinin content and water relations of 'Cabernet Sauvignon' grapevine exposed to drought stress

By N. A. NIKOLAOU[1]*, M. KOUKOURIKOU[1], K. ANGELOPOULOS[2] and N. KARAGIANNIDIS[3]
[1]Aristotle University of Thessaloniki, Department of Agriculture, 54124 Thessaloniki, Greece
[2]University of Partas, Department of Biology, 26500 Patras, Greece
[3]National Agricultural Research Foundation, Soil Science Institute of Thessaloniki, 57001 Thessaloniki, Greece
(e-mail: nicolaou@agro.auth.gr) *(Accepted 11 November 2002)*

Mycorrhiza (2007) 17:551–562
DOI 10.1007/s00572-007-0128-3

ORIGINAL PAPER

Deficit irrigation promotes arbuscular colonization of fine roots by mycorrhizal fungi in grapevines (*Vitis vinifera* L.) in an arid climate

R. Paul Schreiner · Julie M. Tarara · Russell P. Smithyman

…but also in presence of biotic stress such as nematode infection (Hao et al. 2012, J Exp Bot)

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What about grapevine?

- Biodiversity in vineyards and in vine roots, considering:

✓ Different soil management (e.g. covered *vs* tilled)
Lumini et al. 2010; Orgiazzi et al. 2012
✓ Different soil characteristics/environments
Schreiner & Mihara 2009; Balestrini et al. 2010; Magurno et al. 2011; Holland et al. 2014
✓ Extreme environments
Berruti et al. 2018

AIM: to identify AM species/isolate specific for grapevine

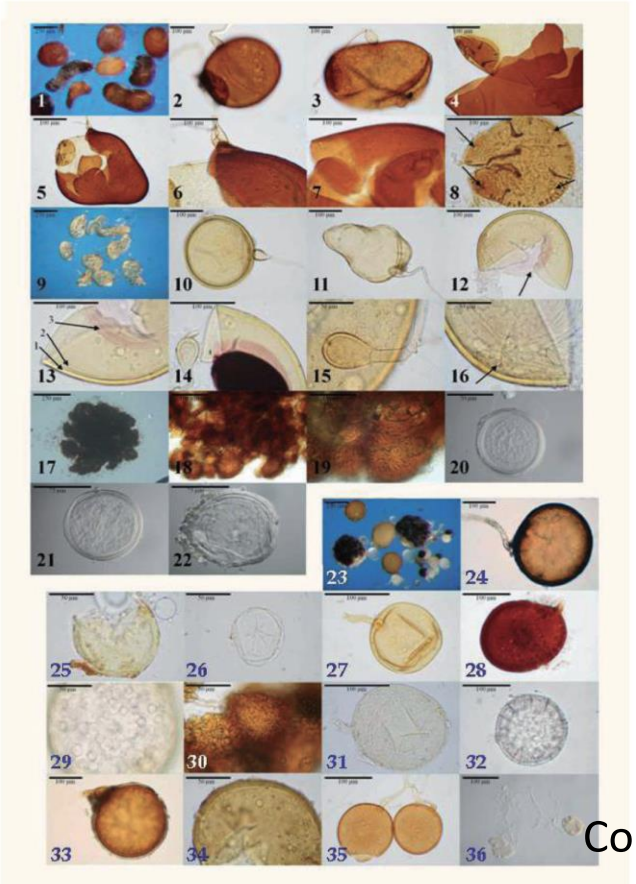
- Functional aspects of the interaction

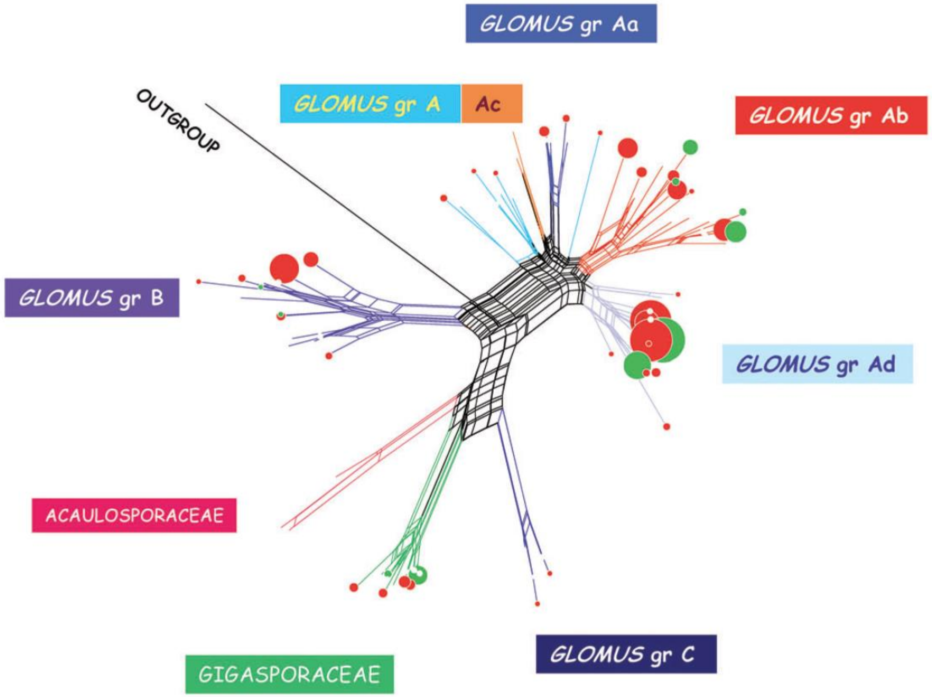
✓ Transcriptome profiles in roots (rootstock)
Balestrini et al. 2018
✓ Proteomics in roots (rootstock)
Cangahuala-Inocente et al. 2011

AMF biodiversity in vineyards

To analyse the composition of AMF communities living in symbiosis with grapevine **(soils and roots)**

Morphological and molecular analyses





Collaboration with Chris Walker

tech 4 wine

environmental microbiology reports

Environmental Microbiology Reports (2010) **2**(4), 594–604 doi:10.1111/j.1758-2229.2010.00160.x

Cohorts of arbuscular mycorrhizal fungi (AMF) in *Vitis vinifera*, a typical Mediterranean fruit crop

Raffaella Balestrini,[1†] Franco Magurno,[1†] Christopher Walker,[2] Erica Lumini[1] and Valeria Bianciotto[1*]

LESSONA and NEIVE: high biodiversity, comparable with the one found in natural/seminatural ecosystems

Comparing the communities from the two sites, remarkable differences in phylotypes composition were found, suggesting an impact of the soil characteristics on AMF communities

LESSONA/NEIVE: agreement between SOIL and ROOT communities

Metabarcoding analysis of AM fungal assemblages

HUMAN INPUT : low input management maintains an higher biodiversity

ECOLOGICAL TRAITS : AMF in the cork-oak are replaced by other symbiotic fungal species more likely associated to trees and shrubs

Impact of **soil management** on AMF fungal diversity

Impact of **ecological traits** (plant coverage) on AMF fungal diversity

Lumini *et al.* 2010 Environmental Microbiology 12 (8): 2165-2179

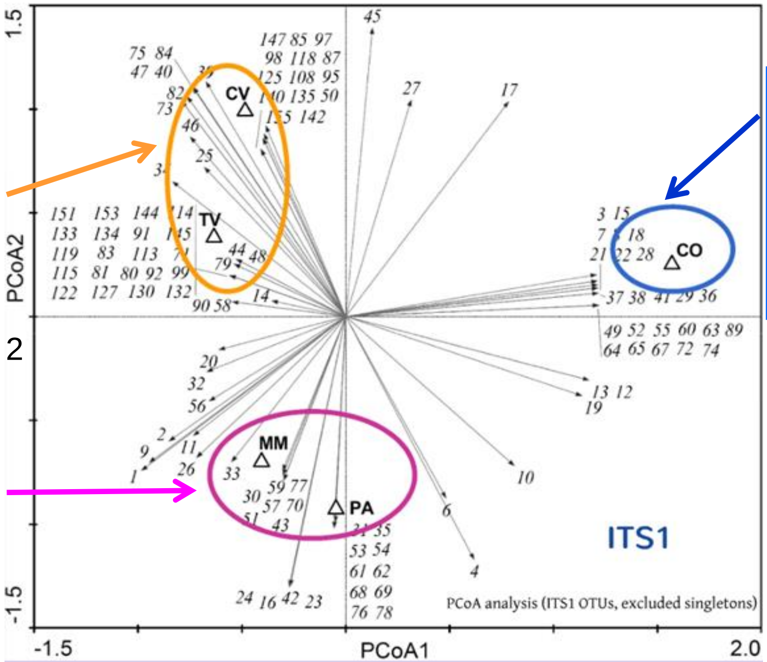
Metabarcoding analysis of overall fungal assemblages

May fungi be used as bioindicators in ecologically different soil?

fungal assemblages from **vineyard** soils (CV & TV) are **similar**

axis 2

grass-covered soils (PA and MM) showed **typical** fungal OTUs

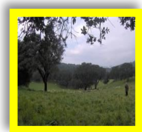


woodland soil (CO) is separated from all the others because of **ectomycorrhizal** fungal OTUs

axis 1

similar results considering ITS2 region

CORK-OAK FORMATION



MANAGED MEADOW

PASTURE

TILLED VINEYARD

COVERED VINEYARD

Low-input grazing area:
Geoglossum spp. & *Hygrocybe* spp.

Coprophilous fungi:
Podospora spp. & *Thelebolus* spp.

Fungi growing on substrates rich in sugars:
Mortierella spp.

Orgiazzi A et al. (2012) PLoS ONE 7: e34847

Progetto Vitinnova: vitigni della Valle d'Aosta

Le caratteristiche del suolo e del microbiota sono parametri che influiscono sulla catena viti-vinicola

Esistono associazioni specifiche tra la vite ed alcuni funghi AM?

Quali fattori ambientali potrebbero influenzare la simbiosi?

Siti di campionamento

Ottin (Saint Christophe): South exposure, 623 m.a.s.l.; Slope 10-15%

IAR (Institut Agricole Régional - Vallée d'Aoste): South-East exposure, 780 m.a.s.l.; Slope 40%

Anselmet (Saint-Pierre) South exposure, 812 m.a.s.l.; Slope 50%

27 campioni di radici e 27 di suolo
Estrazione di DNA, Illumina Miseq

Il sequenziamento massivo attraverso l'analisi di:

MiSeq Output

63.818 sequenze ITS (Funghi)

62.152 sequenze 18S (Funghi Micorrizico Arbuscolari)

Ha permesso :

1) Identificare le sequenze di DNA "marker" tipiche per i diversi taxa/phyla di organismi

Diversità tassonomica e filogenetica

2) Monitorare e valutare e la diversità dei campioni

Ricchezza e abbondanza di specie

Berruti et al. 2018

Chi c'è nell'Ecosistema Vigneto? numeri e nomi…

Rhizophagus/Sclerocystis group (64.08%)
(OTU001, OTU002, and OTU004)

Funneliformis/Septoglomus group (11.60%)
(OTU005, OTU009, and OTU010)

Diversispora genus (6.07%)
(OTU006, less frequent)

Rhizophagus/Sclerocystis group (57.27%)
(OTU001, OTU002, OTU003, OTU004, and OTU005)
with a preponderance of *Rhizophagus irregularis* DAOM181602
(5.17%)

Funneliformis/Septoglomus group (15.41%)

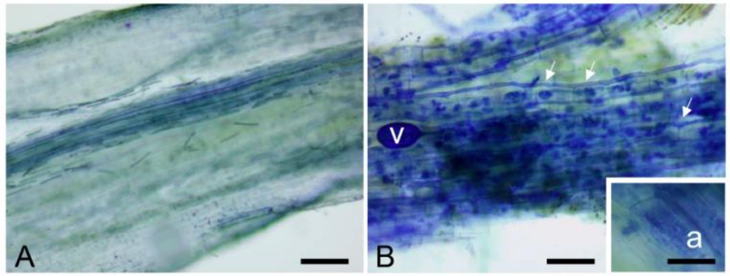
Berruti et al. 2018

Cosa fanno i funghi AM:
analisi del trascrittoma radicale

Effects of a single microbe *versus* a complex microbial inoculum on grapevine roots

Ref.	Product
InoLab	*Glomus mosseae* granular inoculum

Microrganismi che compongono il microbiota per 100g di prodotto:	
40%	Funghi simbionti (*Glomus* spp. GB 67, *G. viscosum* GC 41, *G. mosseae* GP 11)
21,6% C.F.U./g: 4,85 x 10^7	Batteri della rizosfera (*Bacillus subtilis* BA 41, *Pseudomonas fluorescens* PN 53, *Pseudomonas* spp. PT 65, *Streptomyces* spp. SA 51, *Streptomyces* spp. SB 14 e *Streptomyces* spp. ST 60) Funghi saprofiti (*Pochonia chlamydosporia* PC 50, *Trichoderma harzianum* TH 01 e *Trichoderma viride* TV 03).
Supporti inerti qb a 100	



Mixed inoculum — *F. mosseae* inoculum

Coltura delle piante effettuata dai ROERO VITI VIVAI

Balestrini *et al.* 2018 - Mycorrhiza

Experimental Planning

Vitis vinifera plants grafted on 110 Richter rootstock:

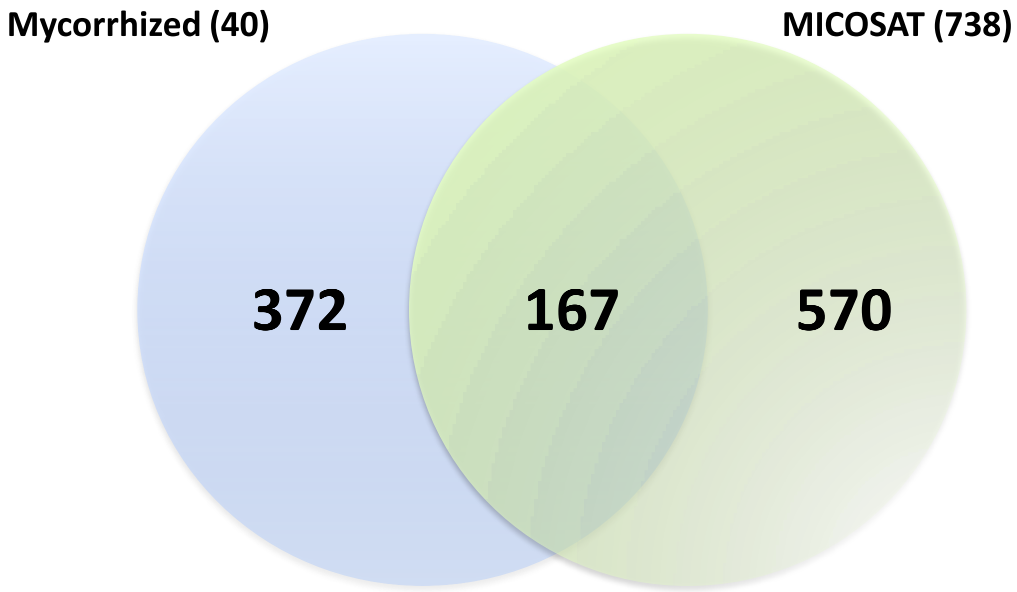
- ✓ non-inoculated
- ✓ inoculated with *Funneliformis mosseae*
- ✓ inoculated with a commercial inoculum (Micosat)

grown in sterilized natural soil in pots, with or without the inoculum, for about 3 months.

→ AMF root colonization (cotton blue staining)

→ RNA extraction
- → RNAseq → Bioinformatics
- → RT-qPCR

How the vine roots respond to AM colonization?



Mycorrhizal roots	76	297
Micosat	40	517
Common	54	127

The mixed inoculum lead to the regulation of a higher number of genes compared to the AMF inoculum. Most of regulated genes resulted to be down-regulated in both treatments *versus* the control condition.

Concluding remarks

After 3 months, roots resulted to be mycorrhized exclusively after the *F. mosseae* treatment, and consequently RNAseq analysis revealed several AM marker genes to be up-regulated (e.g., a phosphate transporter gene).

The commercial inoculum did not lead to any colonization by AMF, but elicited a more important transcriptional regulation, which was probably due to the dominant presence of plant-growth-promoting bacteria.

LMD as tool to study AMF biodiversity inside the mycorrhizal roots

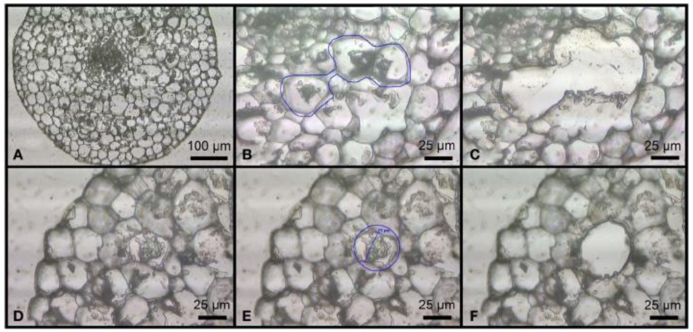
frontiers in
PLANT SCIENCE

METHODS ARTICLE
published: 09 May 2013
doi: 10.3389/fpls.2013.00135

Application of laser microdissection to identify the mycorrhizal fungi that establish arbuscules inside ro

*Andrea Berruti[1], Roberto Borriello[1], Erica Lumini[1], Valentina Scariot[2], Valeria Bianciotto[1] and Raffaella Balestrini[1]**

[1] National Research Council, Plant Protection Institute - Turin UOS, Torino, Italy
[2] Department of Agricultural, Forest and Food Sciences, University of Torino, Torino, Italy



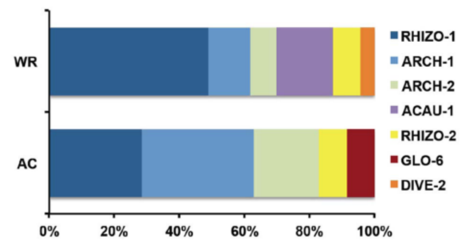
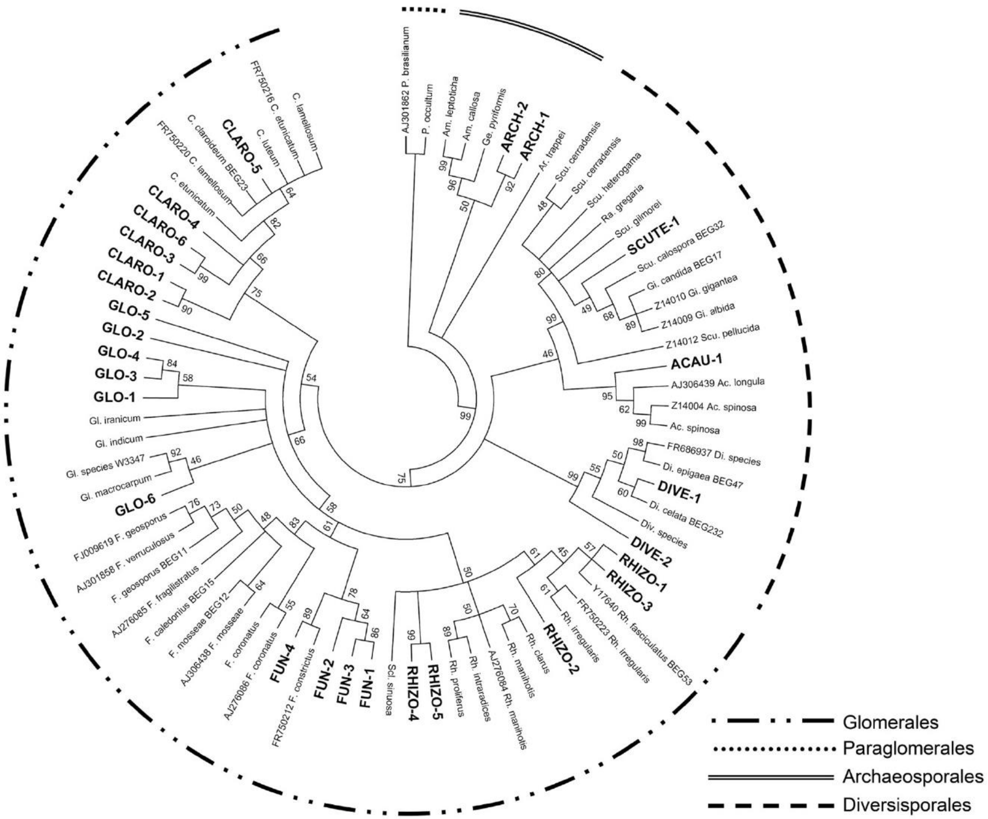


FIGURE 7 | RFLP profile percentage distribution for the whole root cumulative community (R1 + R2 = WR) and the arbusculated cell cumulative community (A300 + A500 = AR).



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Marasco *et al. Microbiome* (2018) 6:3
DOI 10.1186/s40168-017-0391-2

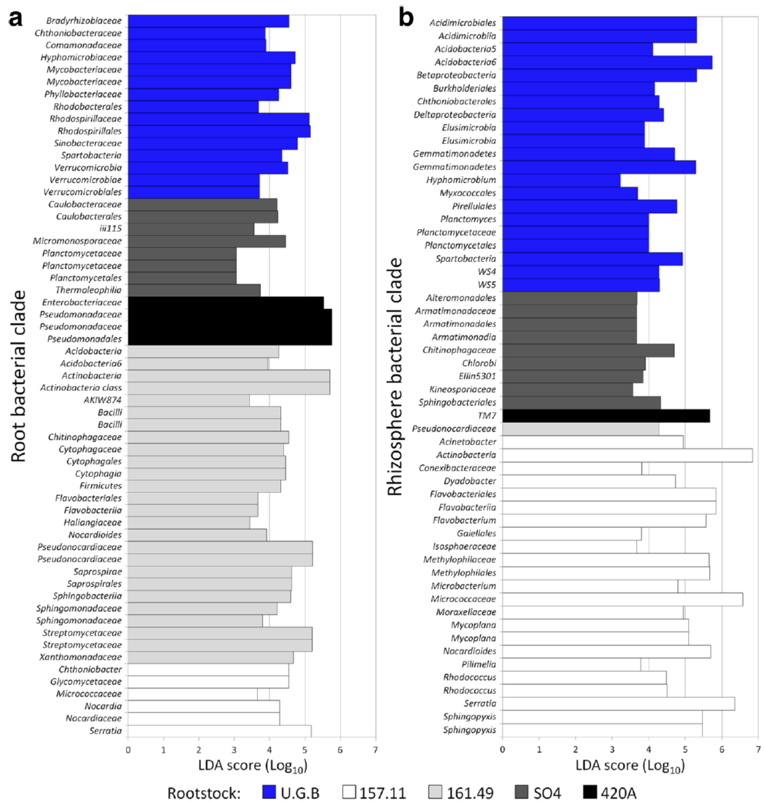
Microbiome

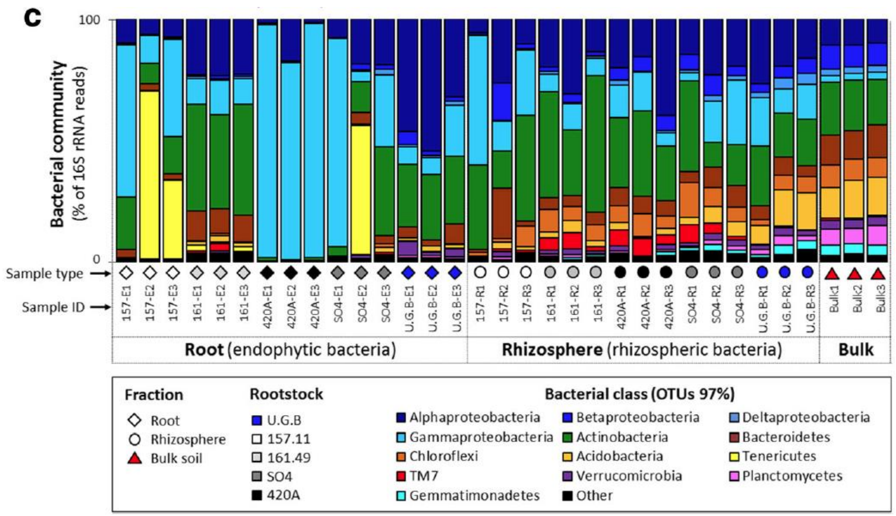
RESEARCH Open Access

CrossMark

Grapevine rootstocks shape underground bacterial microbiome and networking but not potential functionality

Ramona Marasco[1*†], Eleonora Rolli[2†], Marco Fusi[1], Grégoire Michoud[1] and Daniele Daffonchio[1,2*]





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Geophysical Research Abstracts
Vol. 19, EGU2017-9875, 2017
EGU General Assembly 2017

Comparative response of six grapevine rootstocks to inoculation with arbuscular mycorrhizal fungi based on root traits

Antreas Pogiatzis (1), Pat Bowen (2), Miranda Hart (1), Taylor Holland (1), and John Klironomos (1)
(1) University of British Columbia, Okanagan, Kelowna, BC, Canada, (2) Pacific Agri-food Research Center, Summerland, BC, Canada

Aboveground growth was enhanced by AM fungi, with differences among the rootstocks

Cosa fanno i funghi AM: impatto su diversi portainnesto

Varietà **Glera** (Prosecco) su due portinnesti con comportamento opposto:

1103 Paulsen (vigoroso e mediamente resistente alla siccità e stress abiotici),
SO4 (poco vigoroso ma resistente a stress biotici)

Prove in vaso + pieno campo

Trattamenti

1. No inoculo AM e no zucchero (controllo)
2. Inoculo AM (*R. irregularis* + *F. mosseae* prodotto da INOQ)
3. Inoculo AM + zucchero (biostimolante)
4. Zucchero

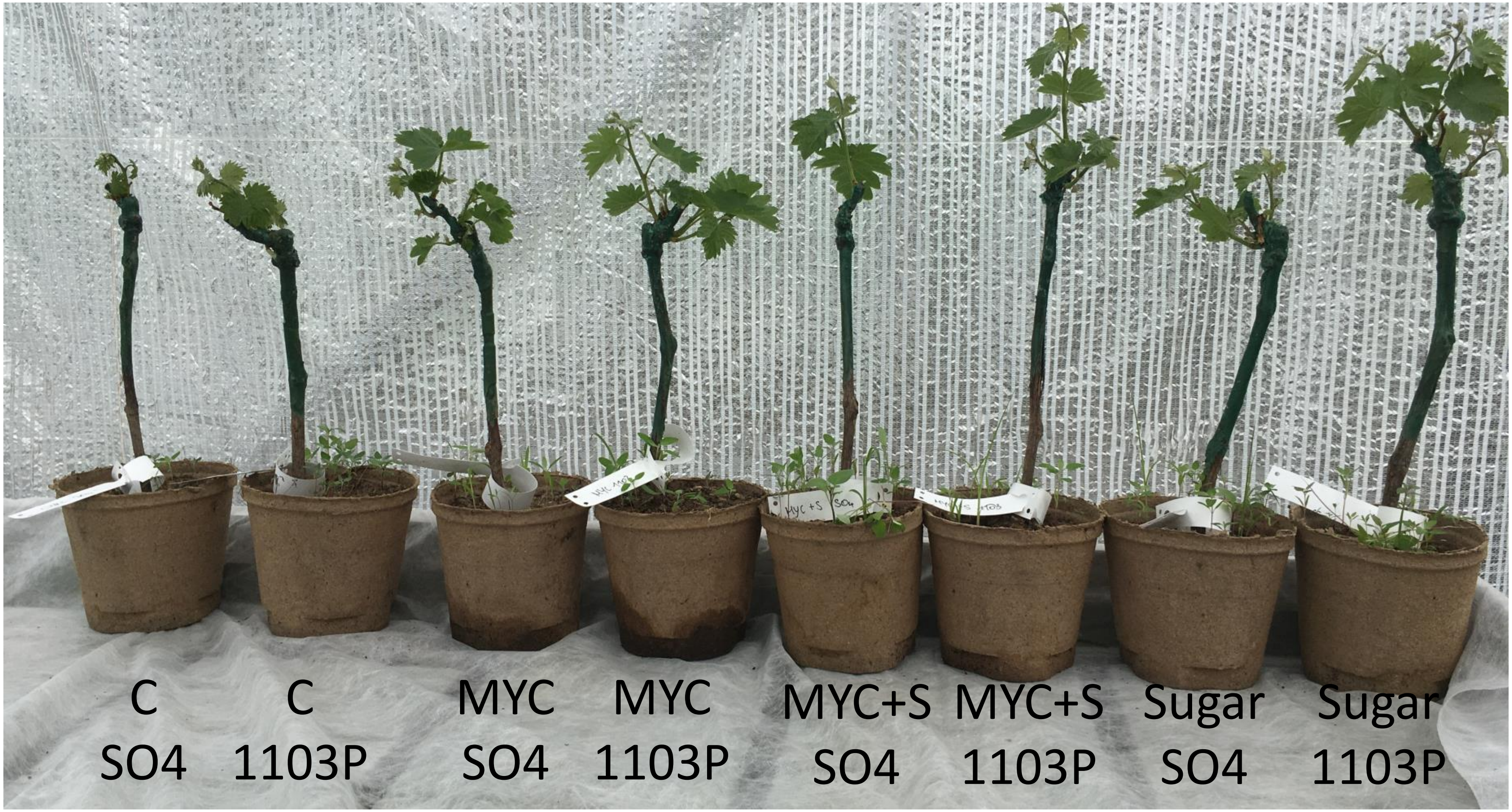
Collaboration with CREA-Conegliano
Walter Chitarra
Luca Nerva
Diego Tomasi

unpublished

Principali attività previste

- ✓ Valutare la colonizzazione in vaso e/o campo;
- ✓ Valutare le performance fisiologiche ed agronomiche delle piante sottoposte ai diversi trattamenti (incluso assorbimento azoto);
- ✓ Estrazione di RNA ed analisi molecolari;
- ✓ Valutare eventuale induzione di resistenza ai principali patogeni fungini della vite (es. peronospora), e.g. analisi e quantificazione dei metaboliti di difesa quali stilbeni (resveratrolo, viniferina) e prove in laboratorio per mezzo di dischetti fogliari.

Primi risultati…



> crescita e % di attecchimento rispetto ai controlli

Systemic effects

Fruit from tomato and strawberry respond to AM symbiosis

Zouari et al. 2014 (tomato)

Robinson Boyer et al. 2016 (strawberry)

Have the AM symbiosis an impact on fruit organoleptic characteristics and on *terroir*?

frontiers in Plant Science

REVIEW
published: 29 June 2018
doi: 10.3389/fpls.2018.00897

Arbuscular Mycorrhizal Symbiosis as a Promising Resource for Improving Berry Quality in Grapevines Under Changing Environments

*Nazareth Torres, M. Carmen Antolín and Nieves Goicoechea**

Perspectives

- ✓ Identify microbial species/isolates adaptated to a specific environment, with the aim to improve and drive agricultural practices and to protect ecosystems/crops in the climate change scenario.
- ✓ Move from lab to field to verify the effects in a more complex natural environment and the impact on the natural communities in soil.

Obtaining an overview of the occurrence, functioning and benefits of AFM in the vineyard, and the use of these microorganisms in a context of sustainable viticulture, still remain a true issue

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Walter Chitarra

fondazione banfi

SANGUIS JOVIS
ALTA SCUOLA DEL SANGIOVESE