Technical Seminar

LIFE MycoRestore

Vallombrosa (Reggello)

16/6/2022

30 years of bringing green ideas to LIFE

“Use of native mycological resources as Biocontrol Agent of Forest Pathogens and Resilience of Forests to Climate Change”

Funghi ectomicorrizici: aspetti ecologici e biologici

Dr. Antonietta Mello

ADVANCES IN THE IDENTIFICATION OF ECTOMYCORRHIZAL FUNGI
INSIGHTS IN THEIR BIOLOGICAL AND ECOLOGICAL ASPECTS

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June 16, 2022

Bonfante 2018

New Phytologist

Tansley review

The future has roots in the past: the ideas and scientists that shaped mycorrhizal research

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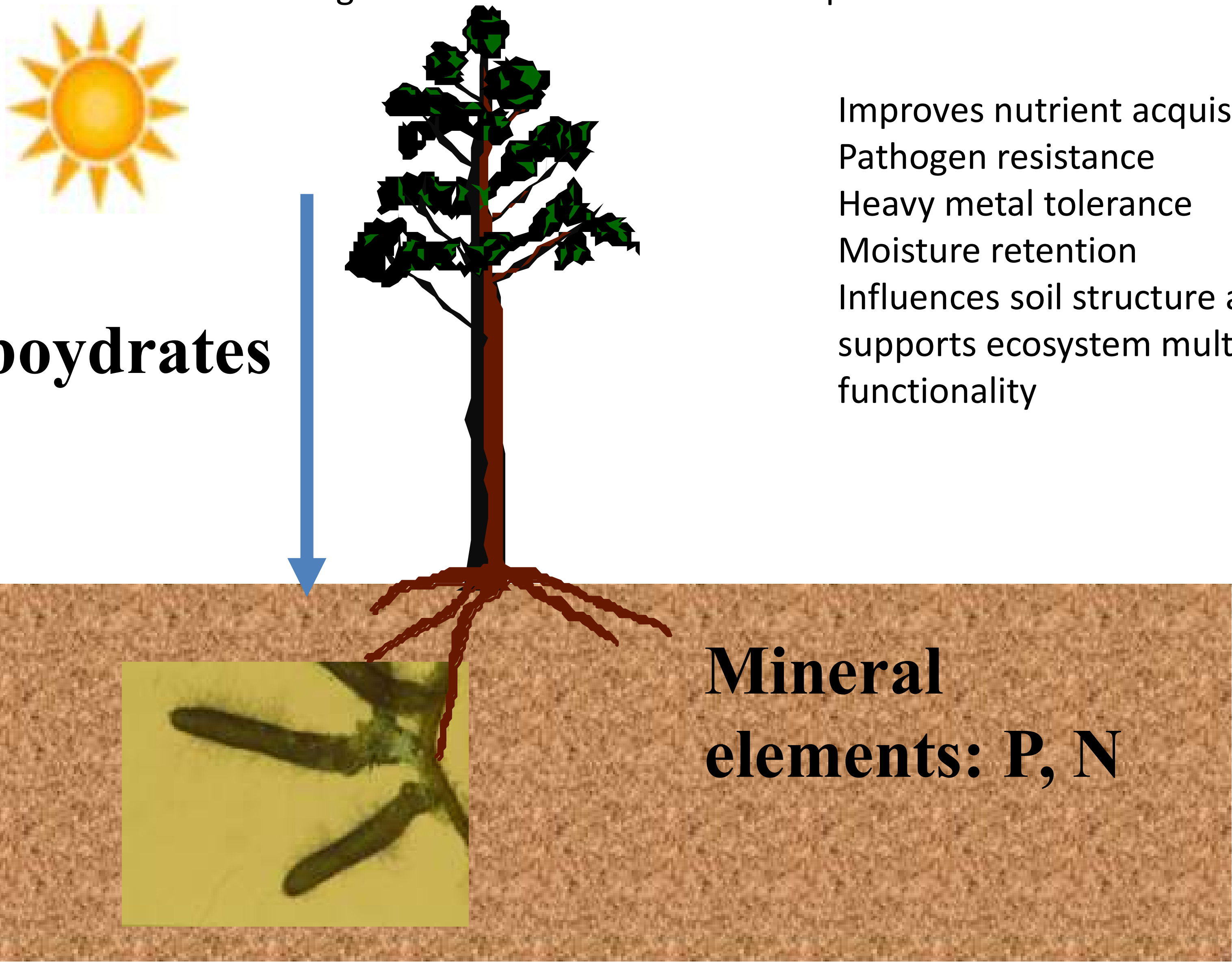
What are mycorrhizae?

The term mycorrhiza comes from the Greek words *mykós* for “fungus” and *riza* for “root” and describes diverse root-fungus associations in which both partners benefit

Improves nutrient acquisition
Pathogen resistance
Heavy metal tolerance
Moisture retention
Influences soil structure and supports ecosystem multi-functionality

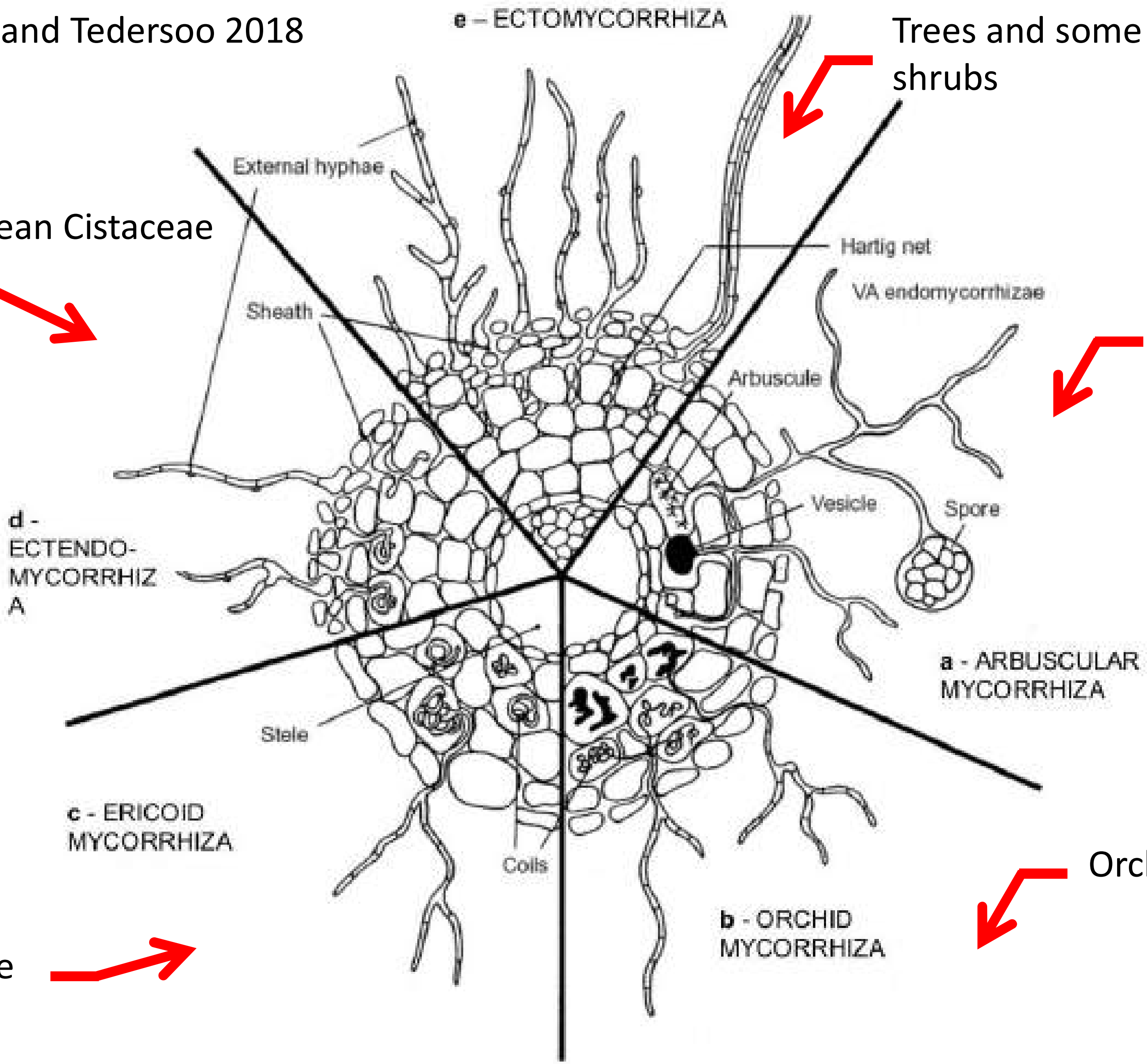


Brundrett and Tedersoo 2018

mediterranean Cistaceae

Trees and some shrubs

crops



Orchidaceae

Ericaceae

Transversal section of a root apex

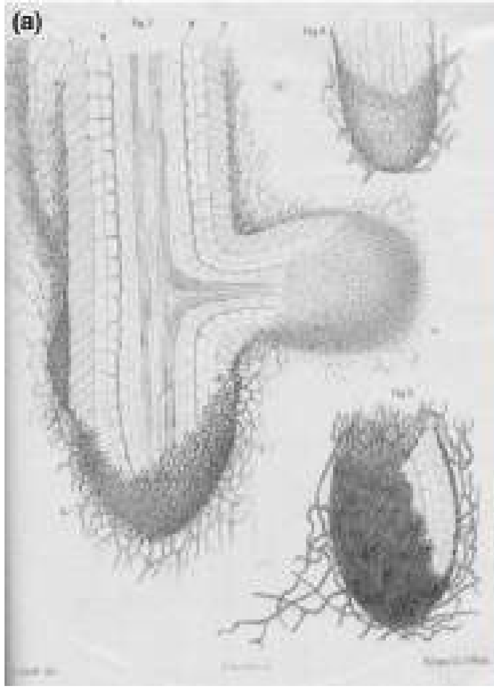
Our knowledge of mycorrhizas dates back to at least 150 years ago

A.B. Frank (1839-1900)

coined the term “mycorrhiza”

E. Melin (1889-1979)

started the study of the physiology of ECM fungi in Uppsala



G. Gibelli (1831-1898)

O. Mattirolo (1856-1947)

exhibition held in Turin in 1911

J.L. Harley (1911-1990)

Wrote the first book devoted to mycorrhiza and allowed to publish mycorrhiza research in New Phytologist

His students D. Read and S. Smith (reference book Read and Smith, 1997)

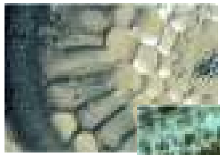
At the end of 19th century also herbs and flowering plants were investigated and pioneering observations on endotrophic mycorrhizas started: P.A. Dangeard (1862–1947), J.M. Janse (1860–1938), L. Petri (1875–1946) and J.Gallaud (1905)

Evolutionary history of mycorrhizal symbioses and global host plant diversity

Brundrett and Tedersoo 2018

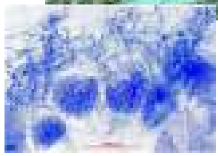
72% of vascular plants are arbuscular mycorrhizal

2% are ectomycorrhizal



1.5% are ericoid mycorrhizal

10% are orchid mycorrhizal



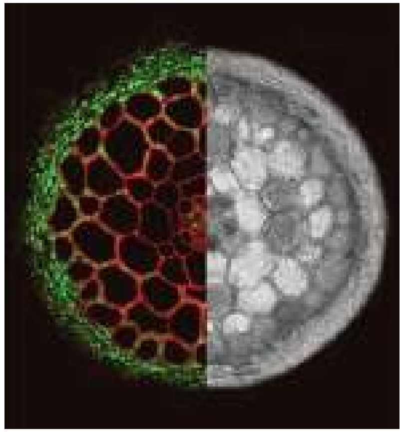
Just 8% of plants are completely nonmycorrhizal

and 7% have inconsistent nonmycorrhizal-arbuscular mycorrhizal associations (habitat/nutritional specialists)

How can we study this impressive biodiversity?

ECTOMYCORRHIZAL LIFE STYLE

>20,000 species of EM Basidiomycetes and Ascomycetes
~6,000 tree species



Although the majority of EM fungi share a typical anatomical pattern, the phenotypic diversity is broad, as host species and ecological specialization

Ramaria

Suillus

Tomentella

While many fungi have been studied from the ecological point of view, a few have been used as model in *in vitro* associations: *Laccaria bicolor* with poplars, *Hebeloma cylindrosporum* with pine, *Tuber melanosporum* with hazel, and *Pisolithus* spp. with eucalypts an important contribution to the knowledge of the mechanisms involved in the symbiosis has been given by the microscopy that has documented the development of the ectomycorrhiza

The development of ectomycorrhizae involves a series of events described in *in vitro* mycorrhizal systems since the end of 1990

-the vicinity of a mycorrhizal fungus to the root stimulates the development of lateral short root ending in a round apex (a)

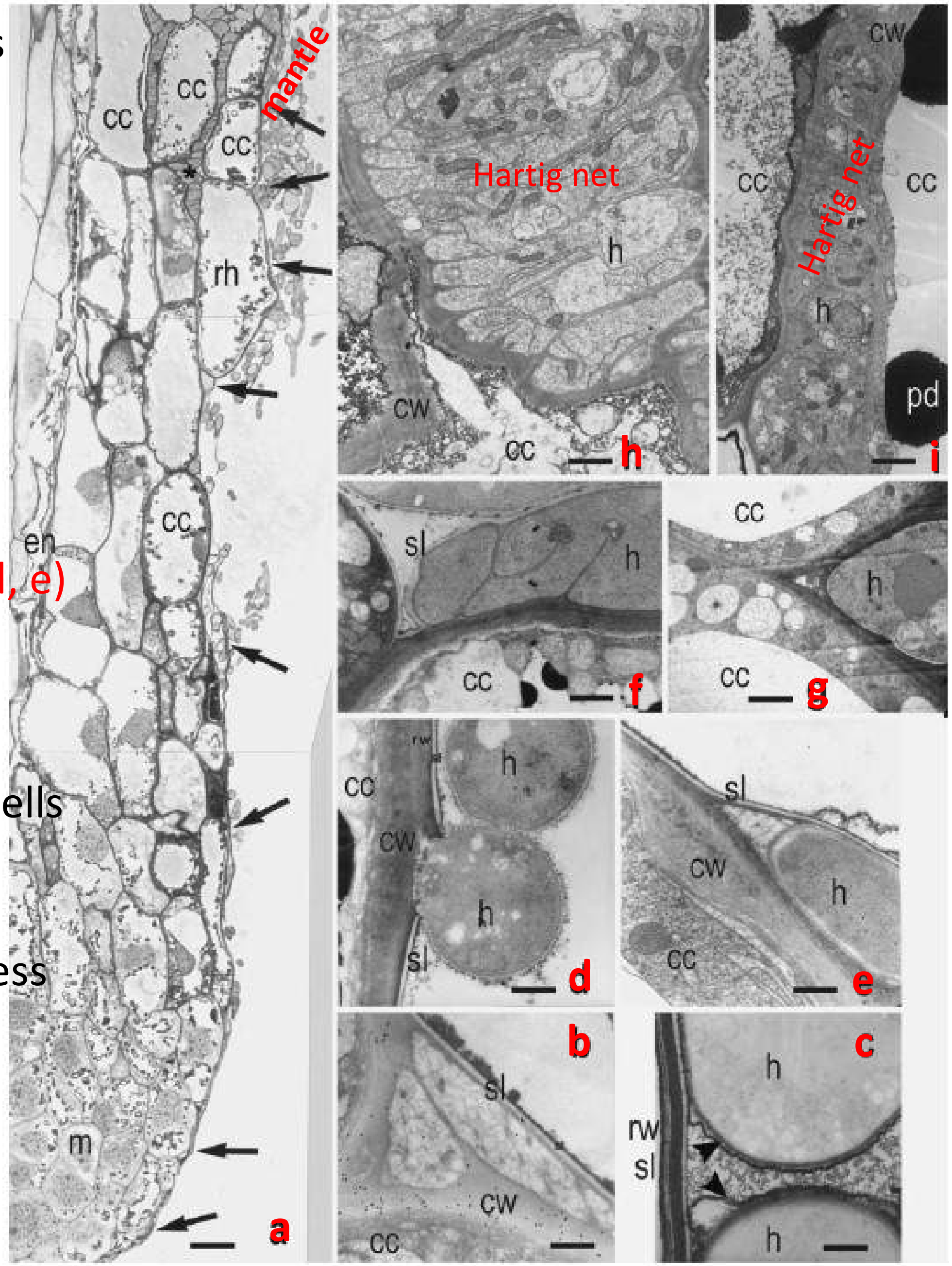
-hyphal attachment to the root surface (b, c, d, e)

-changes in the hyphal growth and branching

-hyphal ingression between the root cortical cells that establish the Hartig net (g)

-remodelling of the cell walls during this process

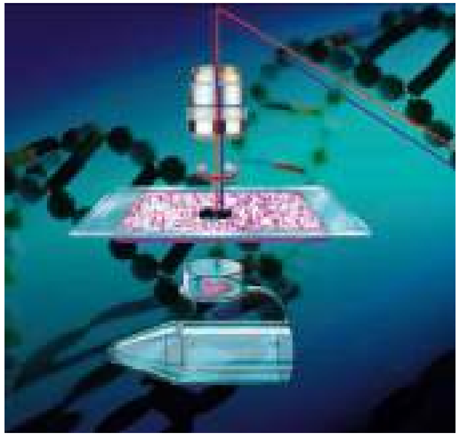
-formation of the hyphal mantle (a)



Early stage of mycorrhizal development at TEM (b-i) between *Picea abies* and *Laccaria amethystine* (Balestrini and Kottke, 2017)

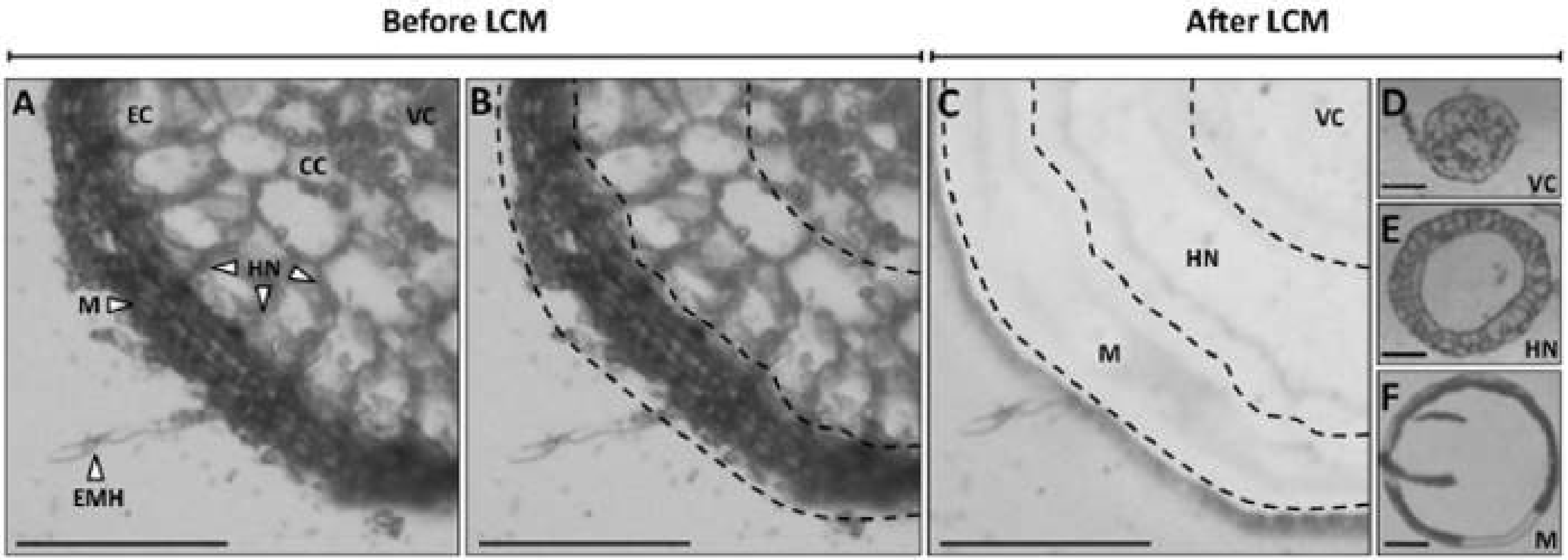
Nehls et al. (2001) separated by forceps the fungal mantle of ectomycorrhizas of *Amanita muscaria* from the remaining poplar roots still containing Hartig net

Hacquard et al. (2013) collected the two ECM fungal compartments by a laser microdissection and from microarray gene expression analysis revealed a functional specificity for the two ECM compartments:
-the mantle is the responsible for nitrogen and water acquisition from soil
-the Hartig net shows enhancement of transport activities



Laser Microdissection (LMD)

Laser capture microdissection (LCM) of *Tuber melanosporum*/*Corylus avellana* ectomycorrhizas



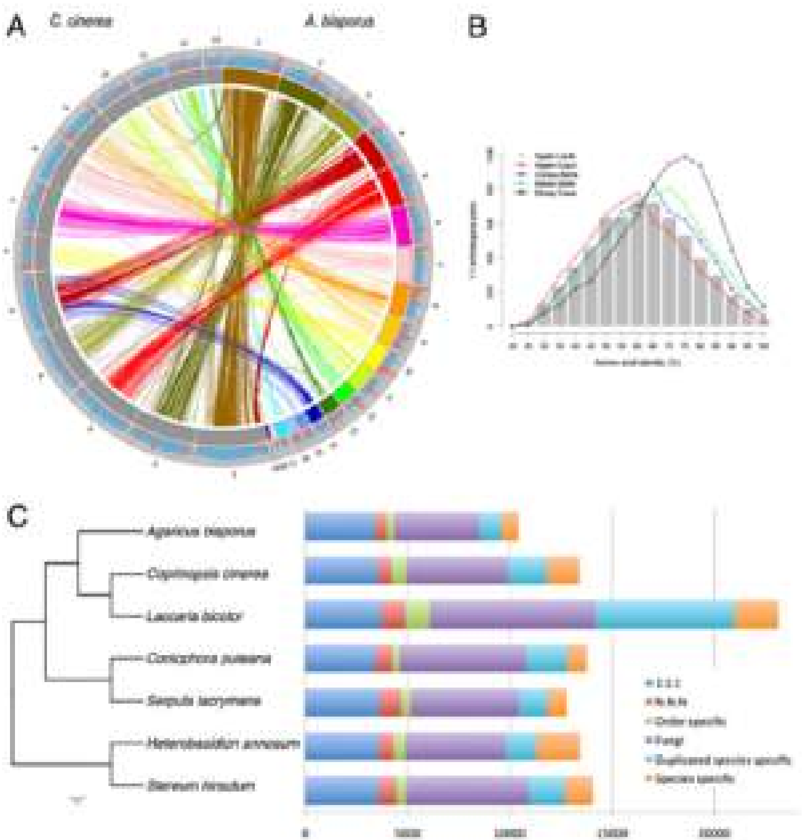
Transversal section of an ectomycorrhiza

Selection of areas for LCM

Section after LCM of the 3 compartments

D. Microdissected root cells
E. Microdissected Hartig net
F. Microdissected mantle

An improved knowledge of the mechanisms involved in the symbiosis emerged from **genomics** applied to EM fungi, **transcriptomics** and **comparative genomics** with fungi having different life styles



sequenced genomes produced within the JGI-INRA Mycorrhizal Genomics Initiative

	Species	Genome size	Gene no.
Arbuscular mycorrhizal fungi			
1	*Rhizophagus irregularis*	91 083 792	28 232
Ectomycorrhizal fungi			
2	*Amanita muscaria*	40 699 759	18 153
3	*Boletus edulis*	46 637 611	16 933
4	*Cenococcum geophilum*	177 557 160	14 748
5	*Choiromyces venosus*	126 035 033	17 986
6	*Clavulina* sp.	41 635 769	15 452
7	*Cortinarius glaucopus*	63 450 306	20 377
8	*Gyrodon lividus*	43 048 674	11 779
9	*Hebeloma cylindrosporum*	38 226 047	15 382
10	*Laccaria amethystina*	52 197 432	21 066
11	*Laccaria bicolor*	60 707 050	23 132
12	*Morchella conica*	48 213 273	11 600
13	*Meliniomyces bicolor*	82 384 847	18 619
14	*Paxillus involutus*	58 301 126	17 968
15	*Paxillus rubicundulus*	53 011 005	22 065
16	*Piloderma croceum*	59 326 866	21 583
17	*Pisolithus microcarpus*	53 027 657	21 064
18	*Pisolithus tinctorius*	71 007 534	22 701
19	*Rhizopogon vinicolor*	36 102 320	14 469
20	*Scleroderma citrinum*	56 144 862	21 012
21	*Suillus brevipes*	51 712 595	22 453
22	*Suillus luteus*	37 014 302	18 316
23	*Terfezia boudieri*	63 234 573	10 200
24	*Tricholoma matsutake*	175 759 688	22 885
25	*Tuber aestivum*	131 544 163	9344
26	*Tuber magnatum*	192 781 443	9433
27	*Tuber melanosporum*	124 945 702	7496
28	*Wilcoxina mikolae*	117 288 895	13 093
Ericoid mycorrhizal fungi			
13	*Meliniomyces bicolor*	82 384 847	18 619
29	*Meliniomyces*	55 857 776	20 389
30	*Rhizoscyphus ericae*	57 408 471	16 784
31	*Tulasnella calospora*	62 392 858	19 659
Orchid mycorrhizal fungi			
32	*Tulasnella calospora*	62 392 858	19 659
33	*Sebacina vermifera*	38 094 242	15 312

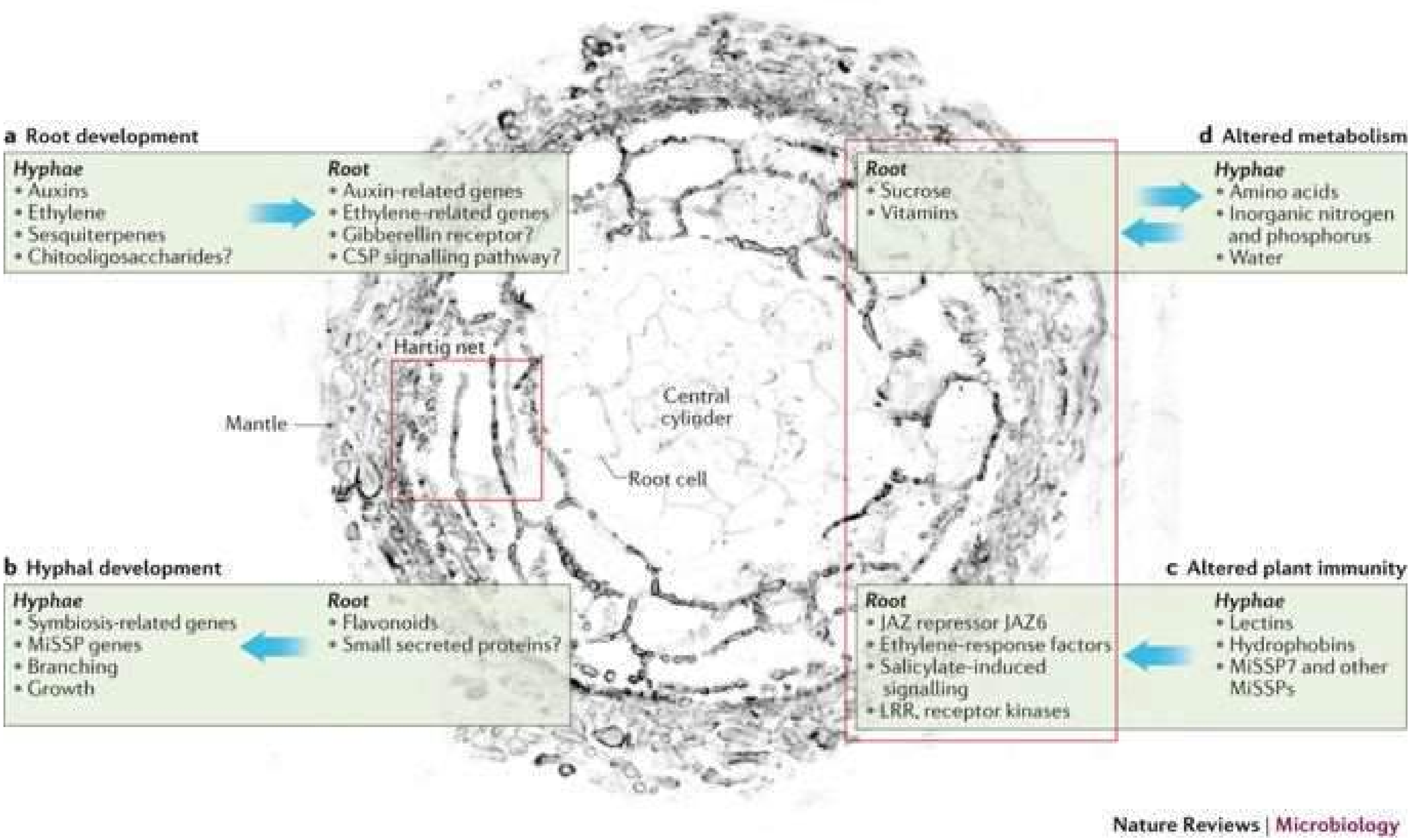
from Vander Heijden et al. 2015

phylogenomic relationships

EM fungi have a reduced repertoire of plant cell wall (PCW)-degrading enzymes, and consequently degradative abilities, compared to saprotrophic and pathogenic fungi.

The loss of lignocellulose-degrading enzymes in the EM fungi made them dependent on their host plant photoassimilates as a C source, rather than decay the organic matter. Thus, the evolution of EM fungi is first characterized by a loss-of-function. However species-specific traits have been identified.
The EM fungal lifestyle has evolved multiple times from saprotrophic lineages through convergent evolution

Adaptation to the EM lifestyle has also required the acquisition of mycorrhiza-induced small secreted proteins (MiSSPs) that facilitate interactions with plant hosts



A model for the establishment of ectomycorrhizal symbioses

Martin et al. 2016

four steps that must be achieved by the fungus: modulation of host root development; hyphal development; suppression of plant defences; and modulation of the metabolism of host root cells

But beyond these studies, most performed on *in vitro* cultures, how researchers have detected all the phases of the complex life cycle of the EM fungi in field?

The identification of ECM fungi has generally been focused on the examination of fruiting bodies

Amanita rubescens

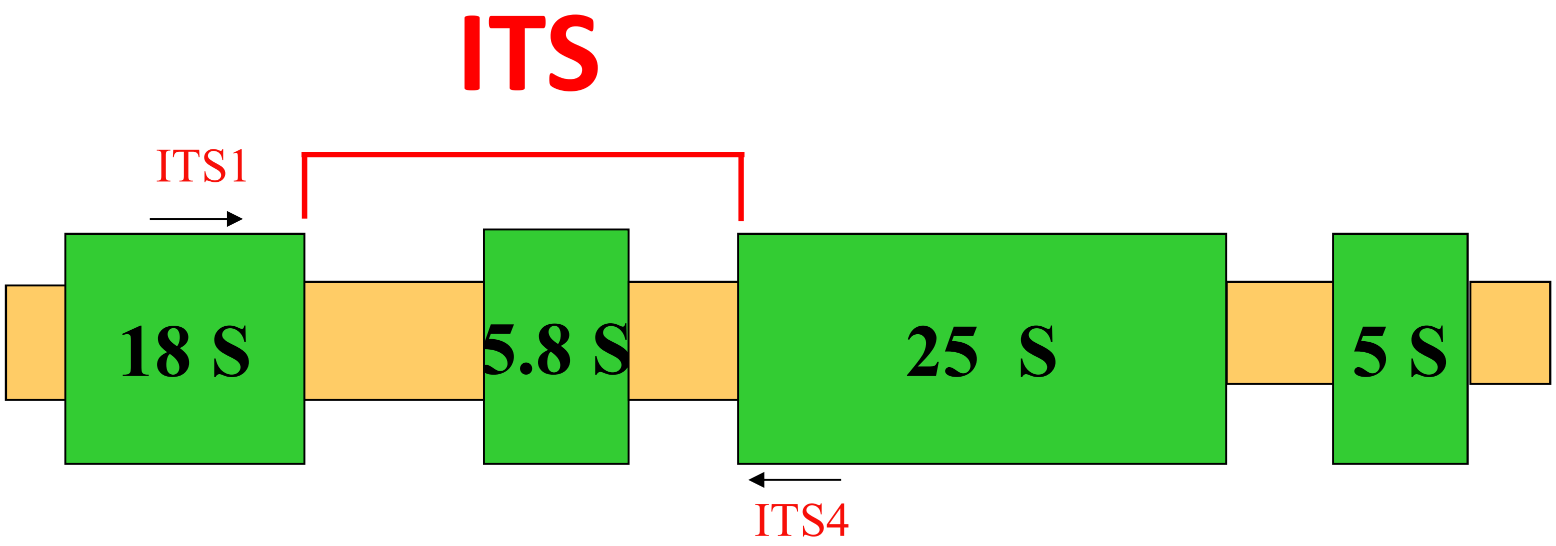
Boletus edulis

only since the early 1990s fruiting bodies have also been characterized by DNA-based methods

ITS-RFLP ⟶ Database from fruitbodies

ITS sequences ⟶ deposit in GenBank and UNITE

ITS primers



typing the ECM tips
after sorting these in morphotypes

Lactarius chrysorrheus/Quercus robur

Thelephora terrestris/Picea abies

Laccaria amethystina/Fagus sylvatica

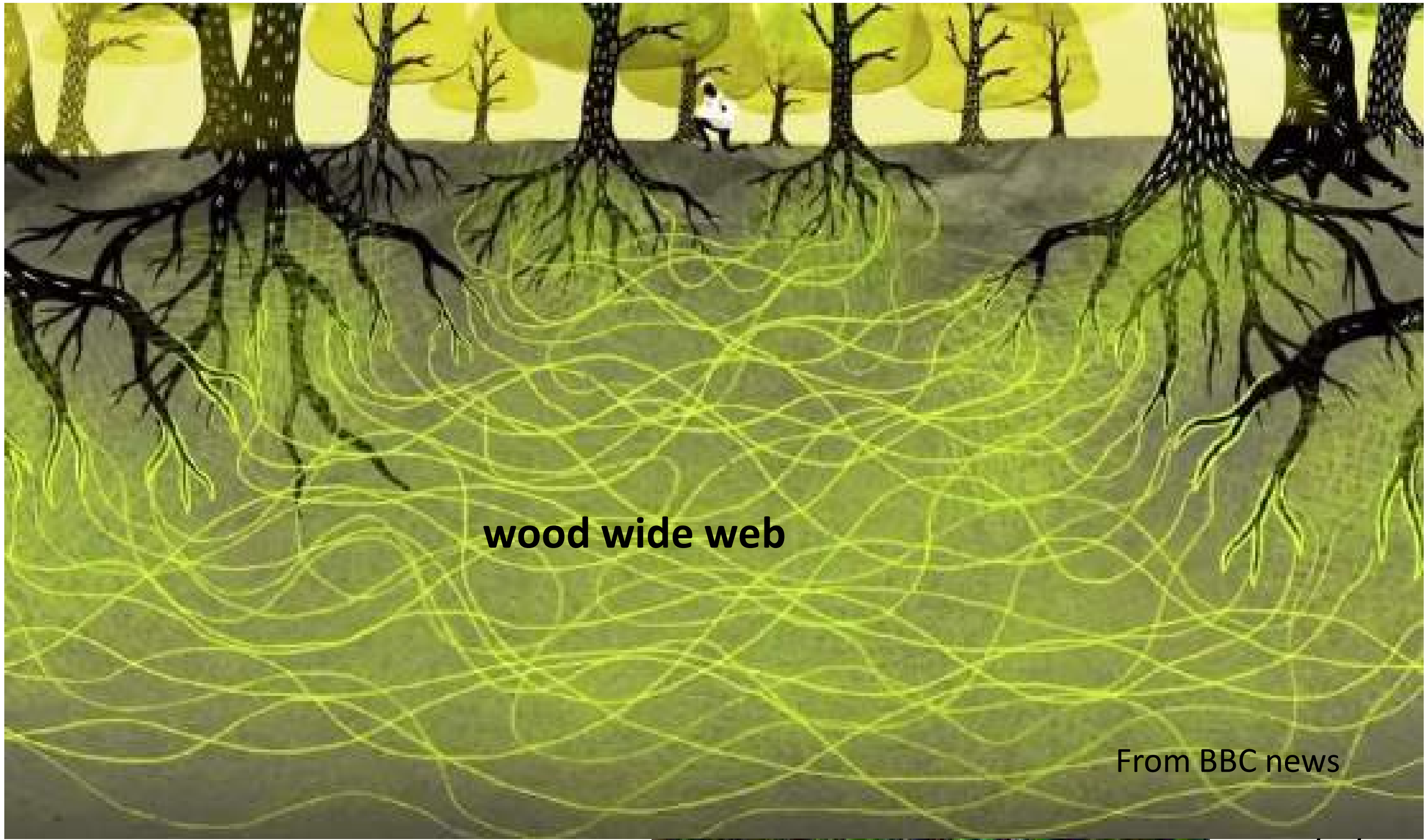
Method used in many studies on ECM community structure and spatial distribution since the pioneering work of Gardes et al. (1991)

↓

there is not a direct linkage between mycorrhizae and fruiting bodies

Detection of the mycelium

Thanks to the direct extraction and amplification of DNA from soil



Hebeloma cylindrosporum was the first ECM fungus to be detected in soil (Guidot et al., 2002)

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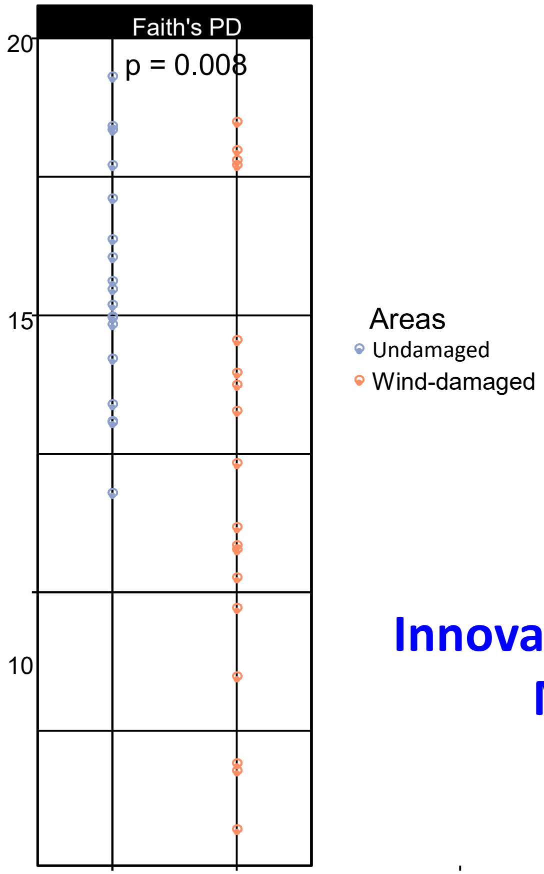
Vallombrosa

Ectomycorrhizal fungi



Higher diversity in undamaged

Undamaged

Wind-damaged

Innovative use of mycological resources for resilient & productive Mediterranean forests threatened by climate change

30 years of bringing green ideas to LIFE

LIFE MycoRestore

Thank You!

Gianni Della Rocca

Alvaro Peix

Carabinieri Forestali