Biologicals for a sustainable agriculture:

Introduction of a quality management framework for inoculants containing arbuscular mycorrhizal fungi

THE UNIVERSITY of ADELAIDE

Dr Matthias Salomon

matthias.salomon@adelaide.edu.au

@Wurzelkosmos

Matt Salomon

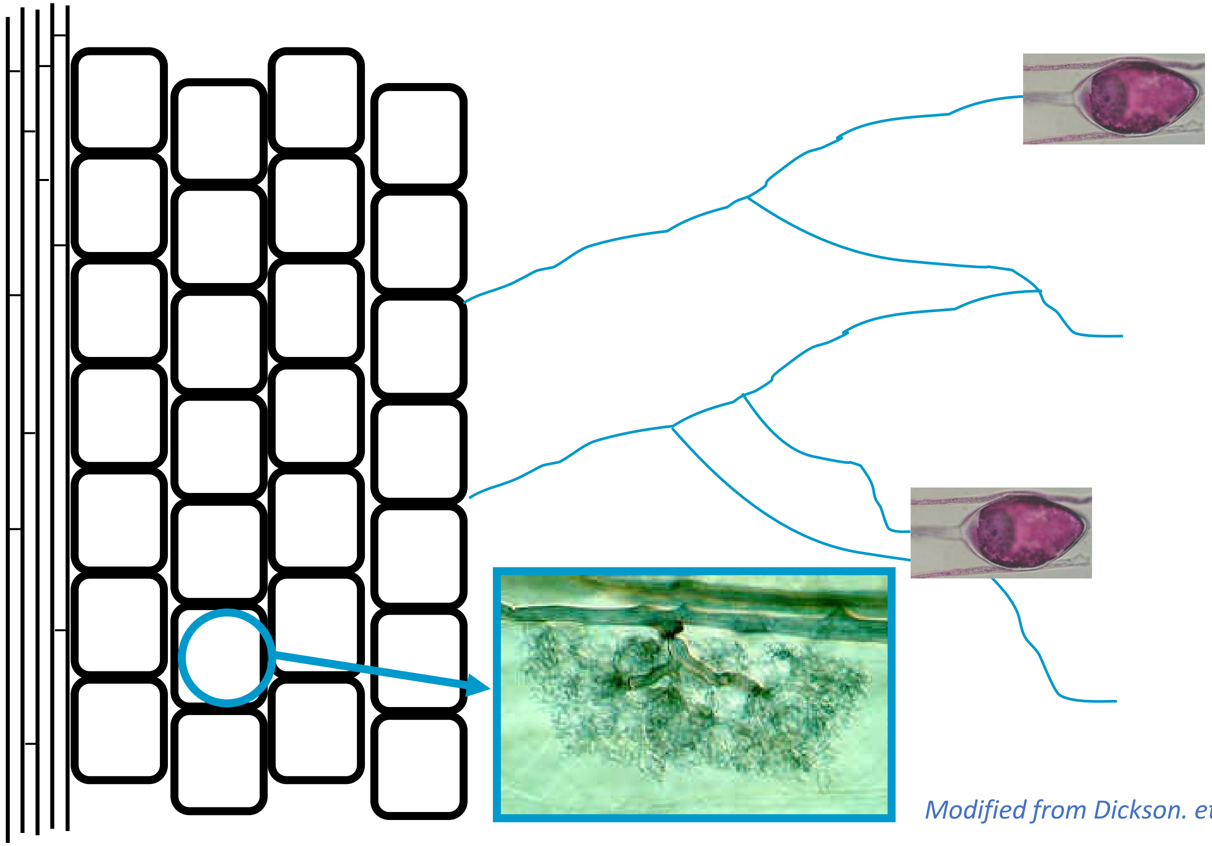

Food
Finite resources
Soil microbial community
Arbuscular mycorrhizal fungi

Arbuscular mycorrhizas

- 80% of all terrestrial plants
- Most crop plants
- Cosmopolitan distribution

- Phylum: Glomeromycota with about 230 described species
- Commercial most important: *Rhizophagus sp.*, *Glomus sp*.

- Obligate symbionts *

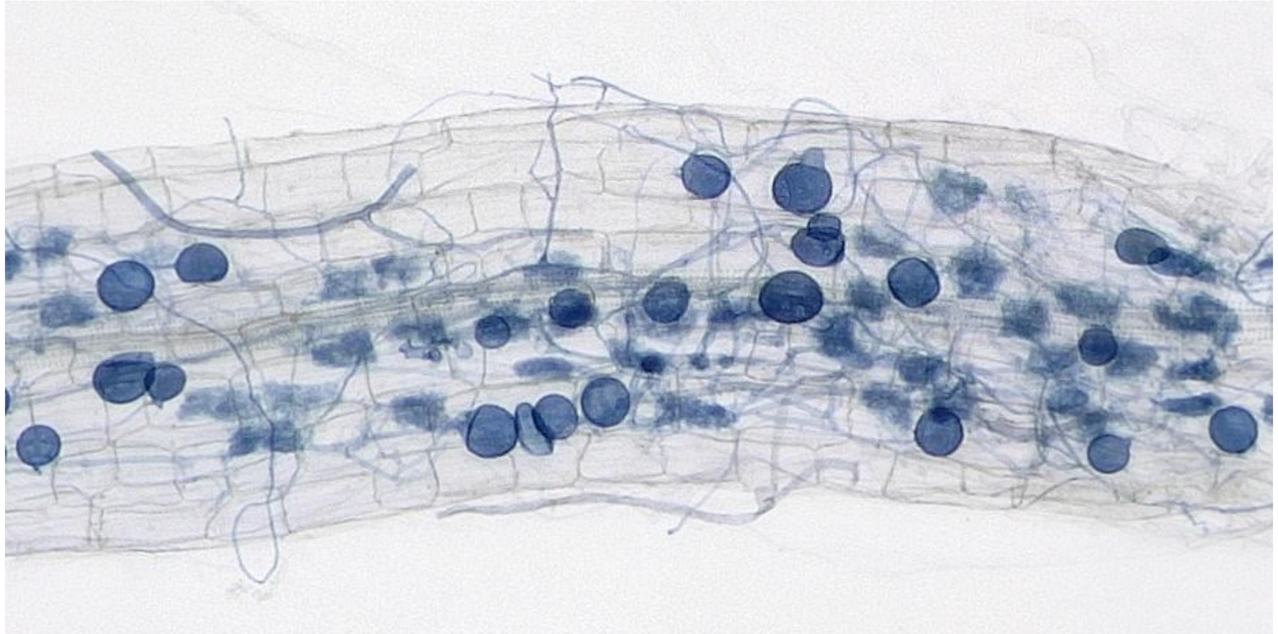


Modified from Dickson. et al.

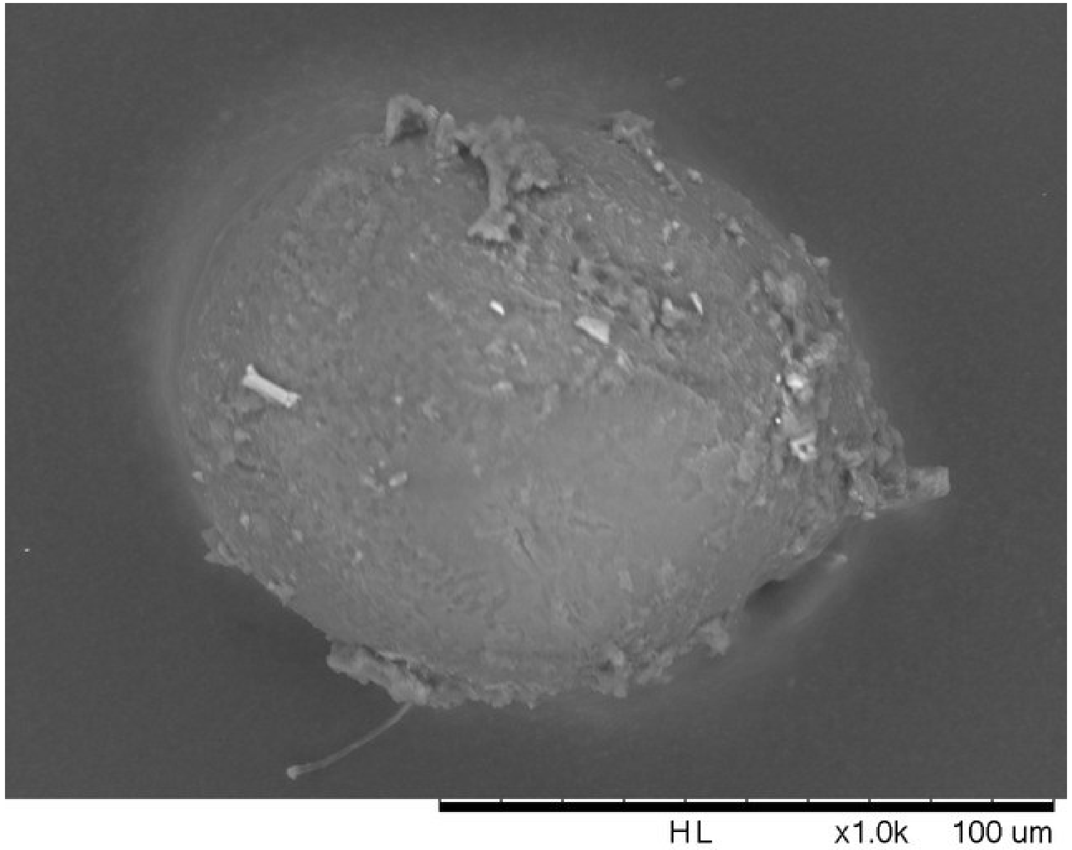
↑ Plant growth ↑ Soil health ↑ Ecosystem functions

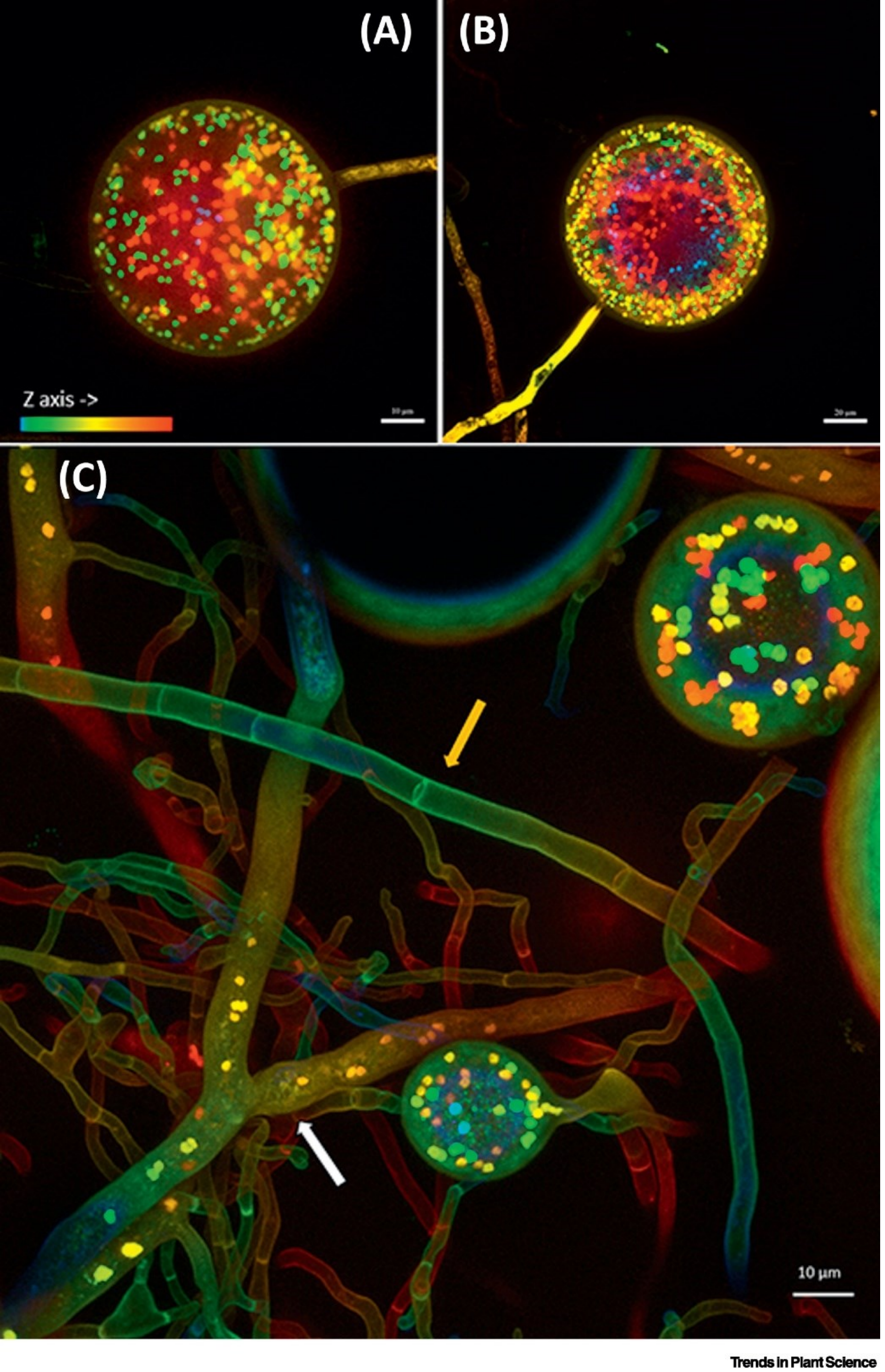
* Yuta Sugiura et al. (2020): Myristate can be used as a carbon and energy source for the asymbiotic growth of arbuscular mycorrhizal fungi. *Proceedings of the National Academy of Sciences*

Arbuscular mycorrhizas



https://www.slcu.cam.ac.uk/news/new-method-quantify-arbuscular-mycorrhizal-fungi-amf-colonisation-plant-roots

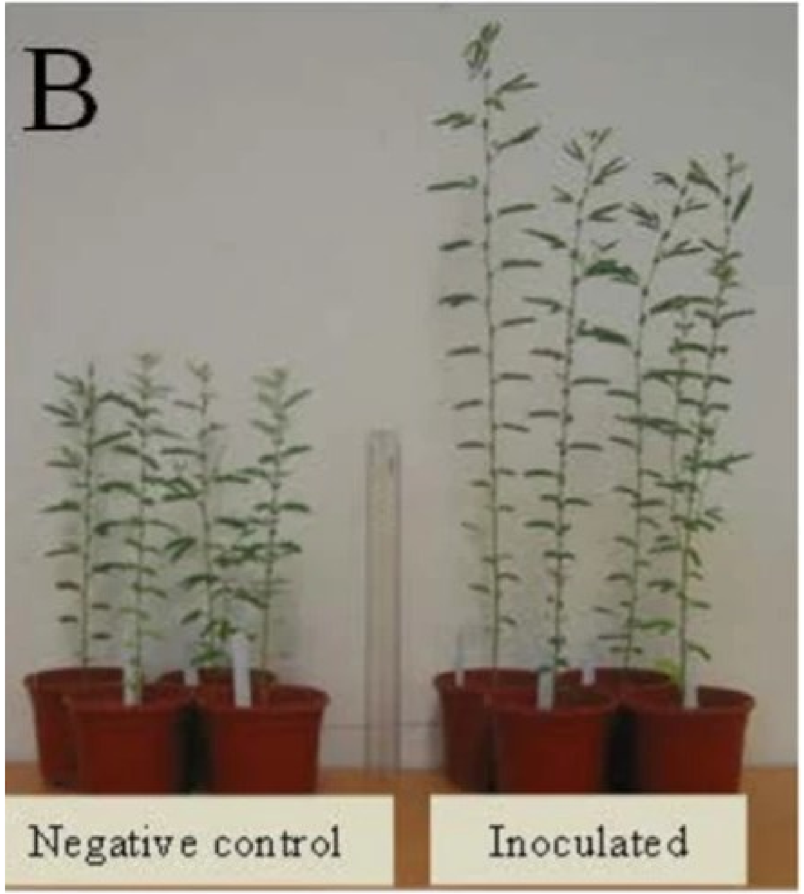




Multi-nuclei spores

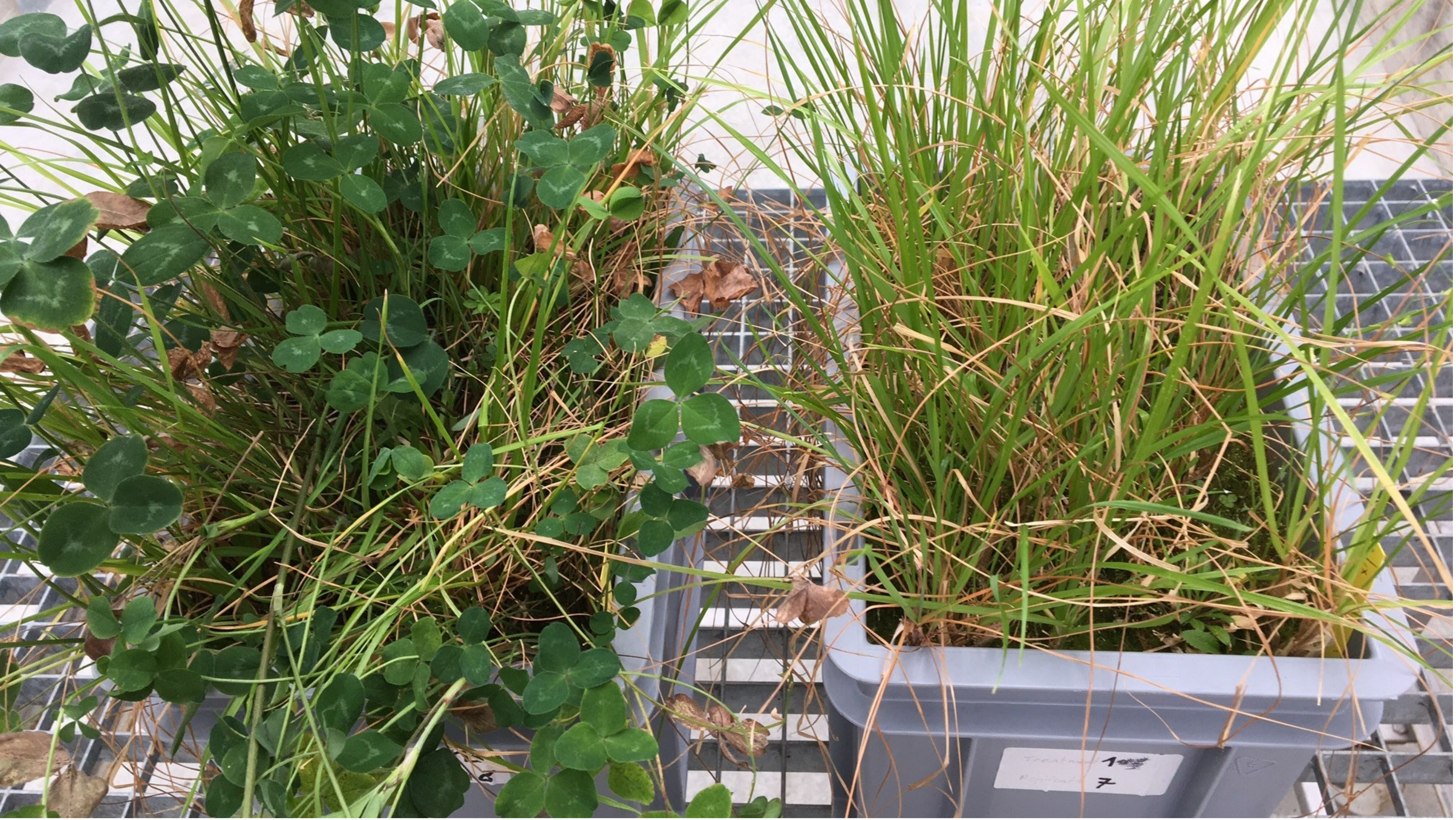
Kokkoris, Vasilis, et al. "Nuclear dynamics in the arbuscular mycorrhizal fungi." *Trends in Plant Science* 25.8 (2020): 765-778.

Arbuscular mycorrhizas - benefits



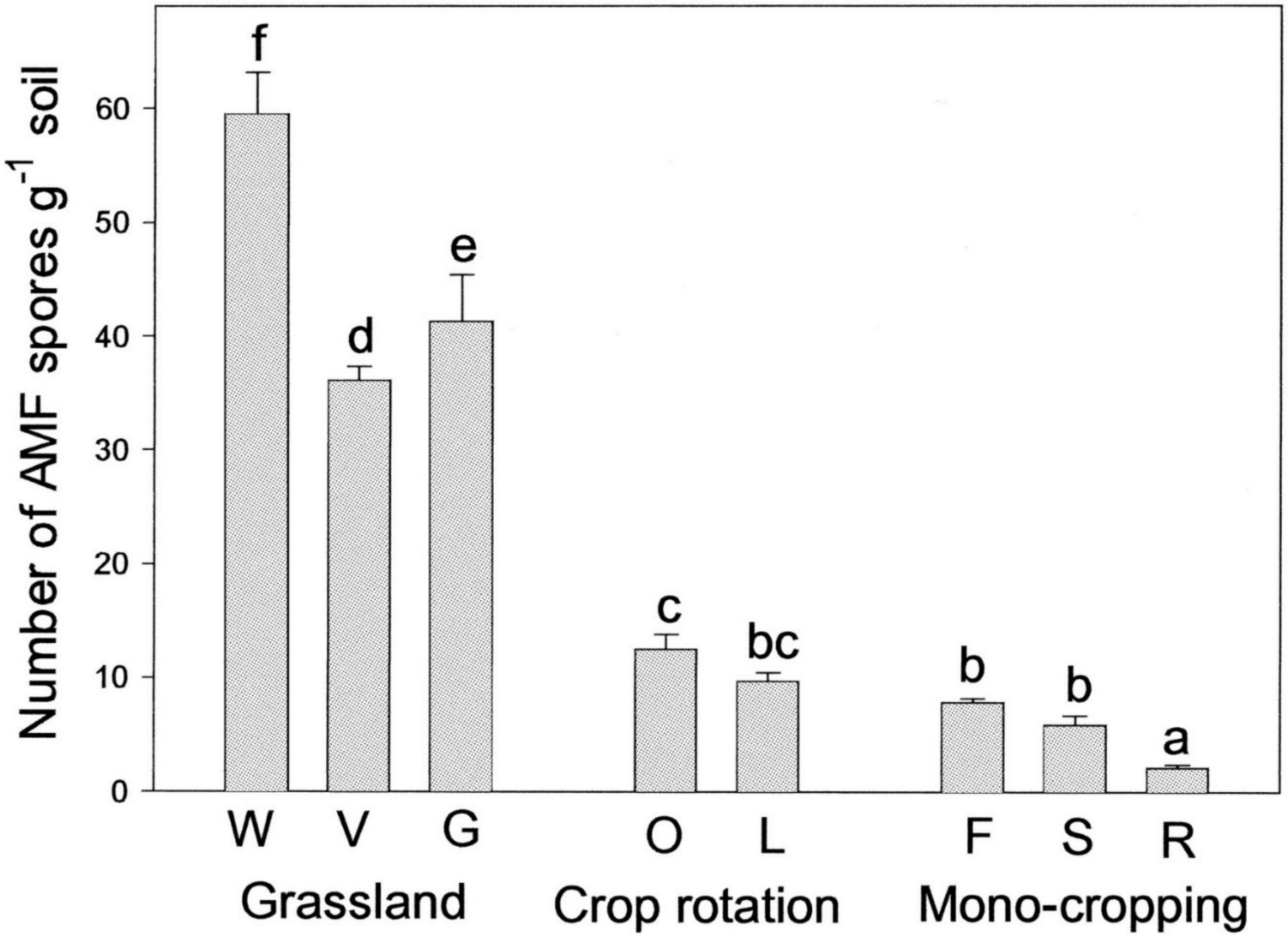
Al-Yahya'ei, M.N., Błaszkowski, J., Al-Hashmi, H. *et al.* From isolation to application: a case study of arbuscular mycorrhizal fungi of the Arabian Peninsula. *Symbiosis* **86**, 123–132 (2022)

- Improved plant growth
  - Drought resistance
  - Nutrient uptake
  - Alleviation of soil contamination
  - Disease resistance
- Increased carbon sequestration
- Reduced nutrient leaching
- Reduced greenhouse gas emissions
- ….



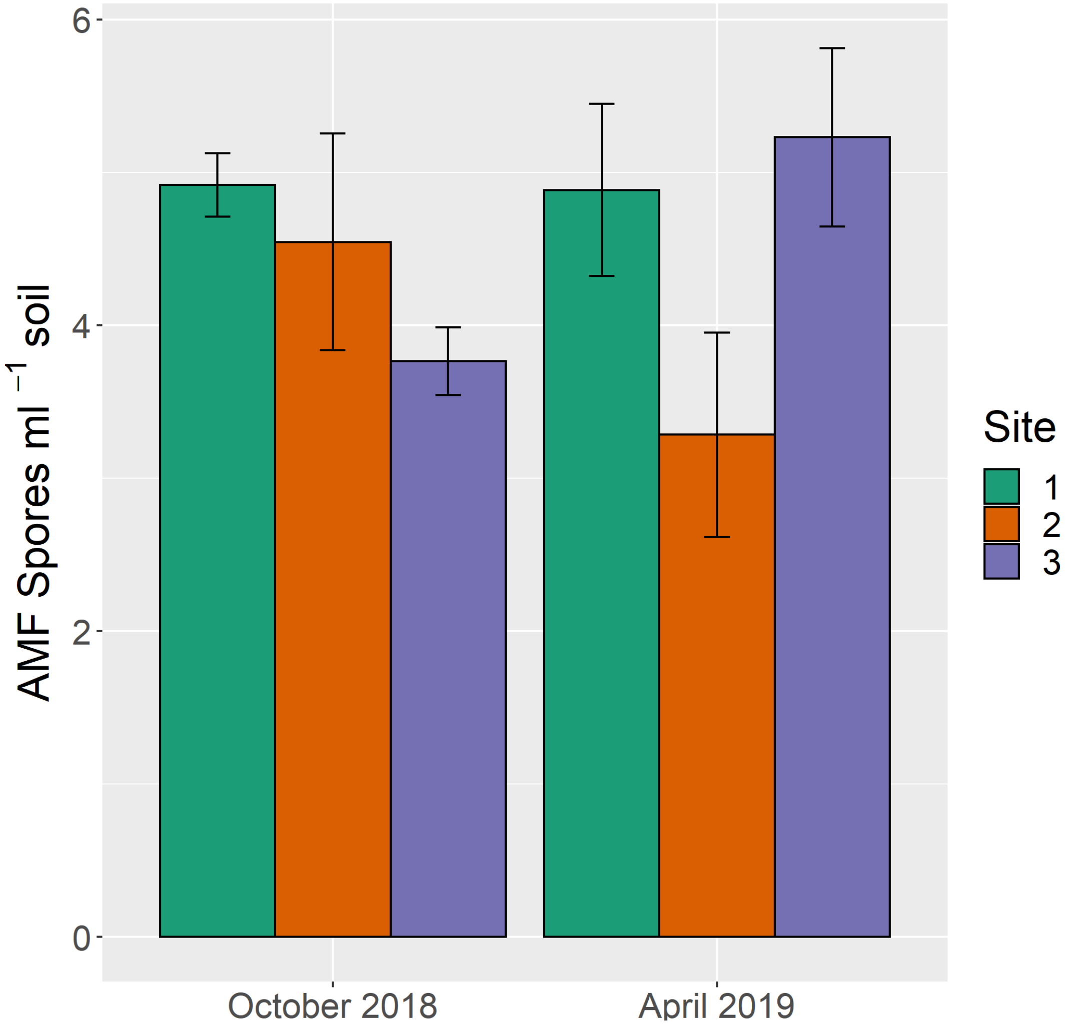
Marcel van der Heijden (Agroscope, Zurich) via Twitter

Natural occurrence of AMF



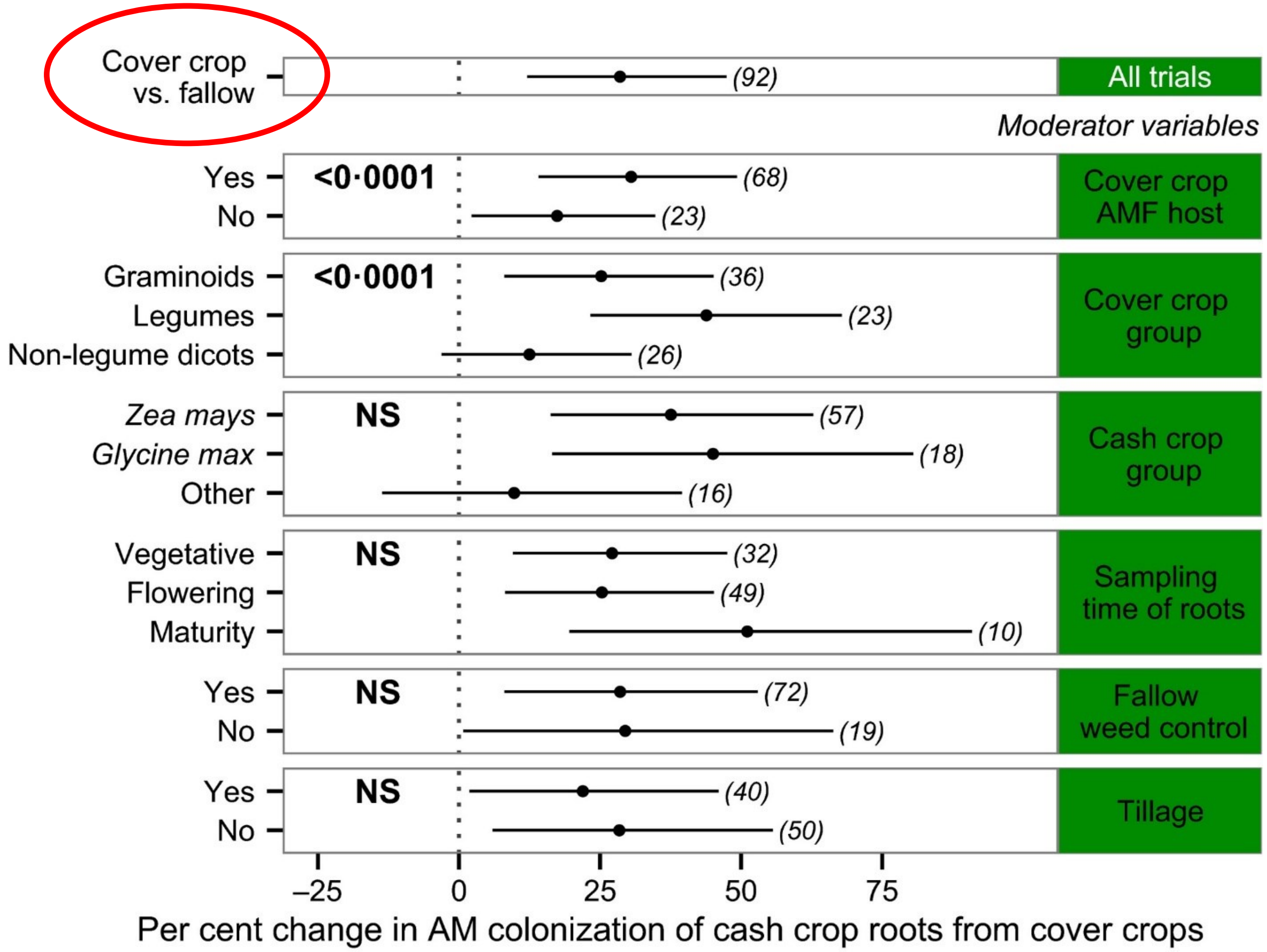
Oehl et al. (2003): Impact of Land Use Intensity on the Species Diversity of Arbuscular Mycorrhizal Fungi in Agroecosystems of Central Europe. *Applied and Environmental Microbiology*

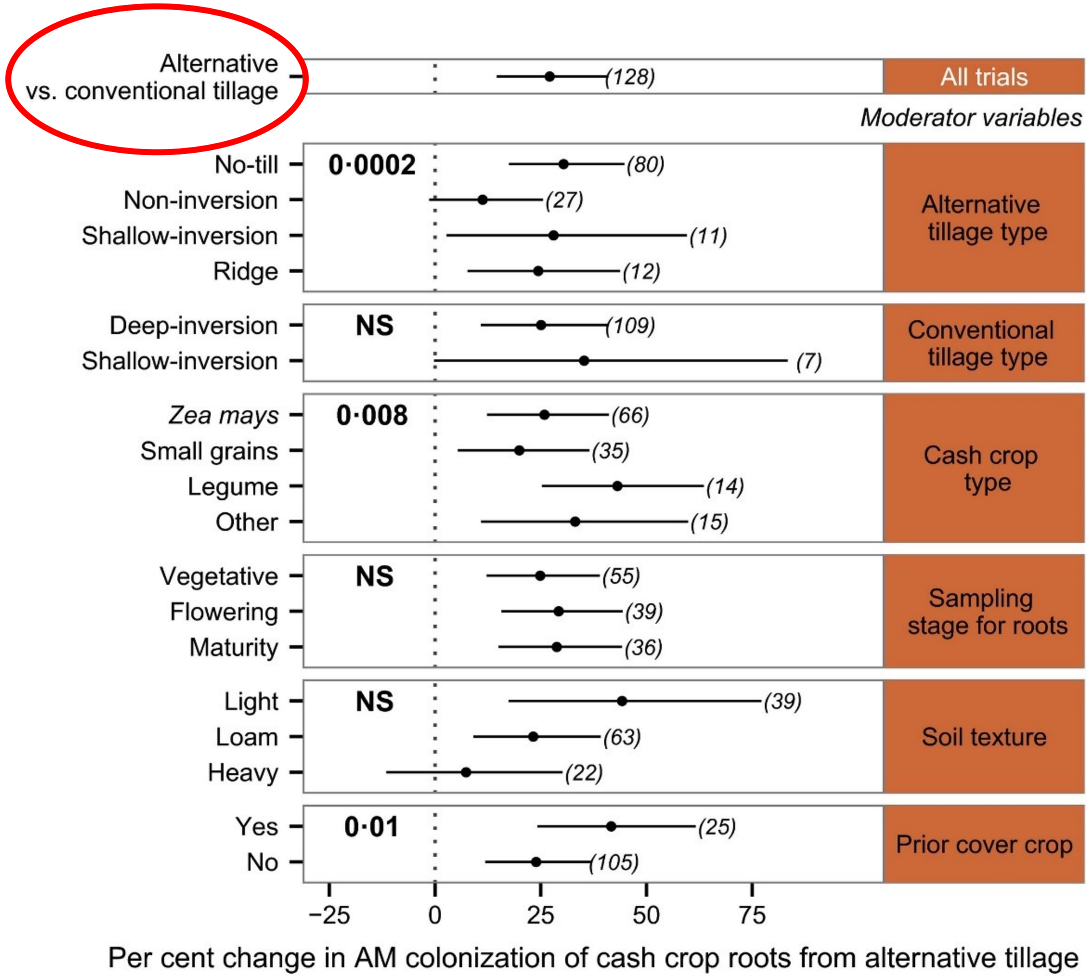
Natural occurrence of AMF



Salomon, M.J., Watts-Williams, S.J., McLaughlin, M.J. and Cavagnaro, T.R. (2022). Spatiotemporal dynamics of soil health in urban agriculture. *Science of The Total Environment*

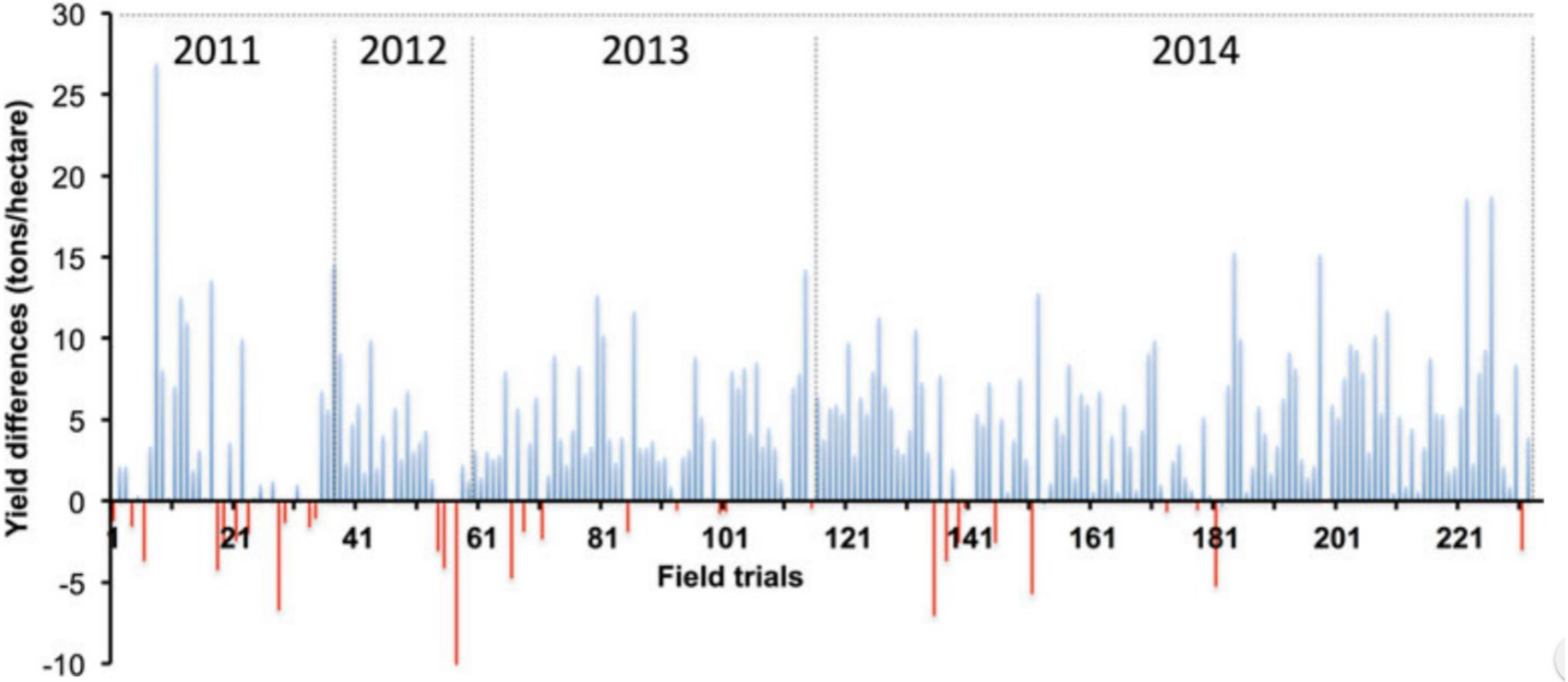
AMF-enhancing practices





Bowles et al. (2016): Ecological intensification and arbuscular mycorrhizas: a meta-analysis of tillage and cover crop effects. *Journal of Applied Ecology*

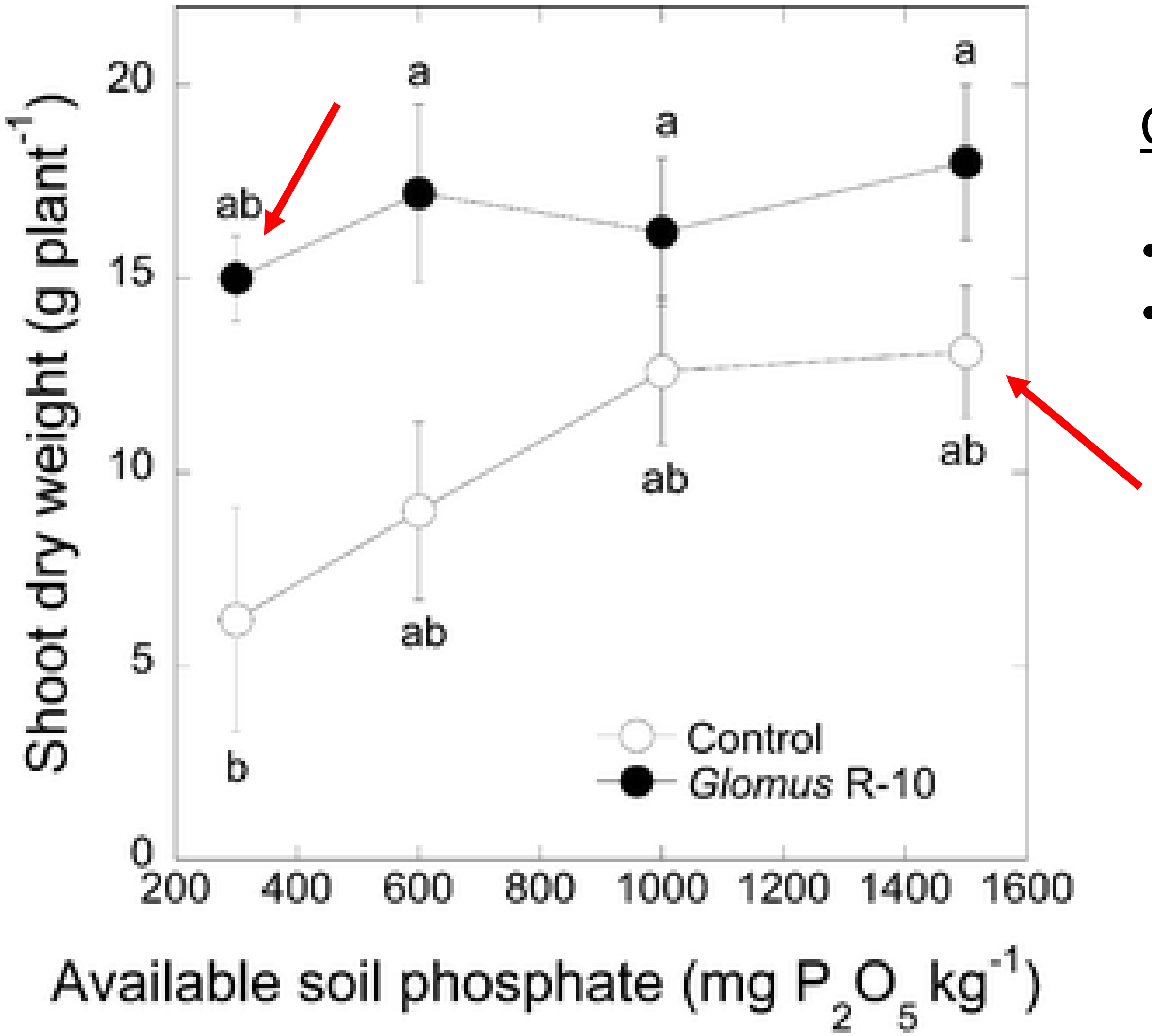
Economics of AMF field inoculation



"Inoculation was profitable with a 0.67-tons/ha increase in yield, a threshold reached in almost 79 % of all trials."

Hijri (2016): Analysis of a large dataset of mycorrhiza inoculation field trials on potato shows highly significant increases in yield. *Mycorrhiza*

Economics of AMF field inoculation



<u>Costs:</u>

- AM inoculum: 2,285 USD ha^{-1}
- Superphosphate: 5,659 USD ha^{-1}
  (1600 mg kg^{-1})

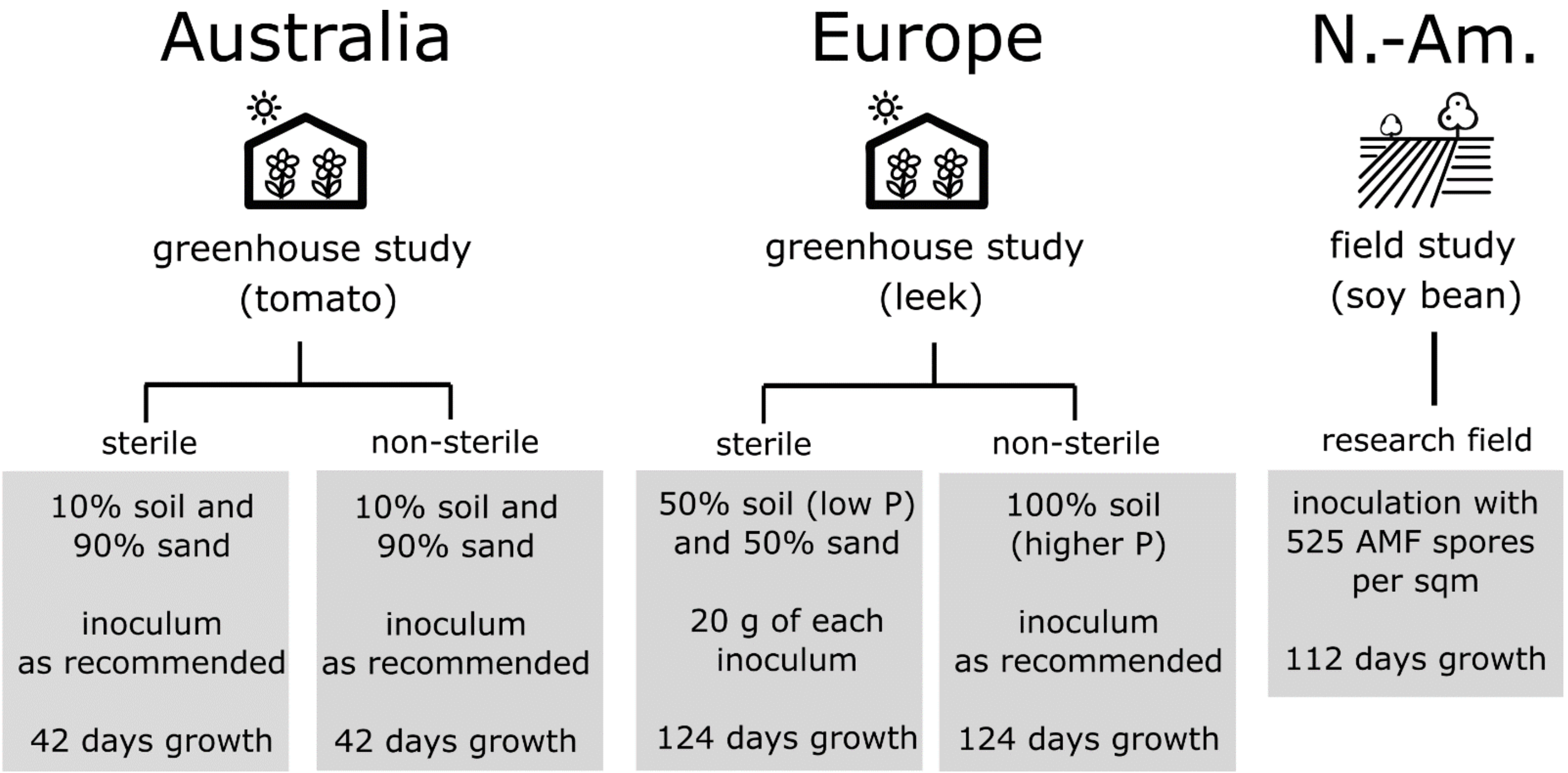
Tawaraya et al. (2012): Inoculation of arbuscular mycorrhizal fungi can substantially reduce phosphate fertilizer application to *Allium fistulosum* L. and achieve marketable yield under field condition. *Biology of Fertile Soils*

Commercial AMF inoculum

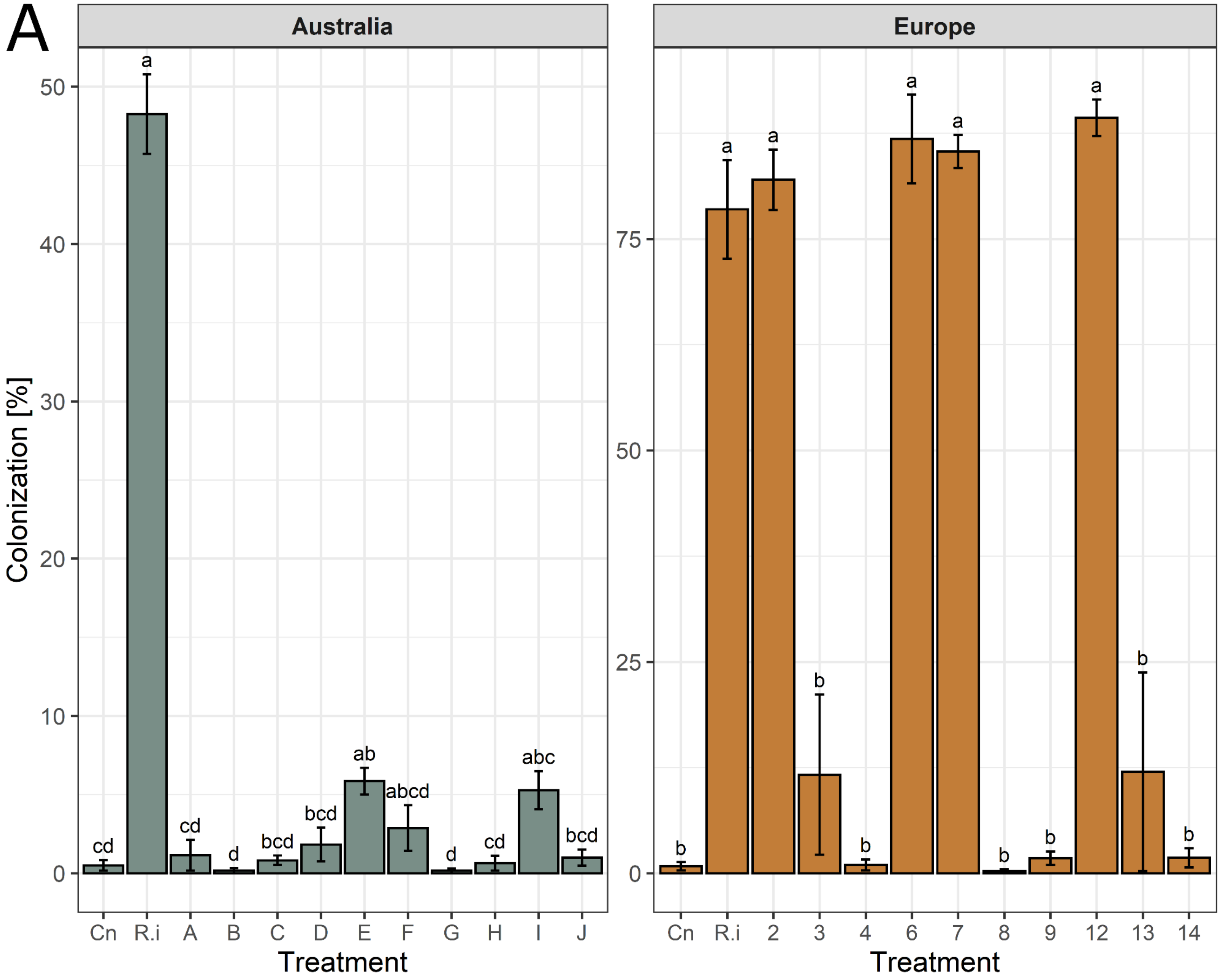
- Economic and environmental inoculation success most likely in specific host plants and environments
- Nonetheless, large economic potential → increasing number of products being released
- Research topics
  - Free of pathogens?
  - Establishment in the field?
  - Persistence in the field?
  - Effect on natural soil community?
  - Effect on plants and soil?

- Most basic question:
  - <u>Do they result in mycorrhizal root colonization?</u>

Global analysis of AMF inoculants



Salomon, M.J., Demarmels, R., Watts-Williams, S.J., McLaughlin, M.J., Kafle, A., Ketelsen, C., Soupir, A., Bücking, H., Cavagnaro, T.R. and van der Heijden, M.G., 2022. Global evaluation of commercial arbuscular mycorrhizal inoculants under greenhouse and field conditions. *Applied Soil Ecology*

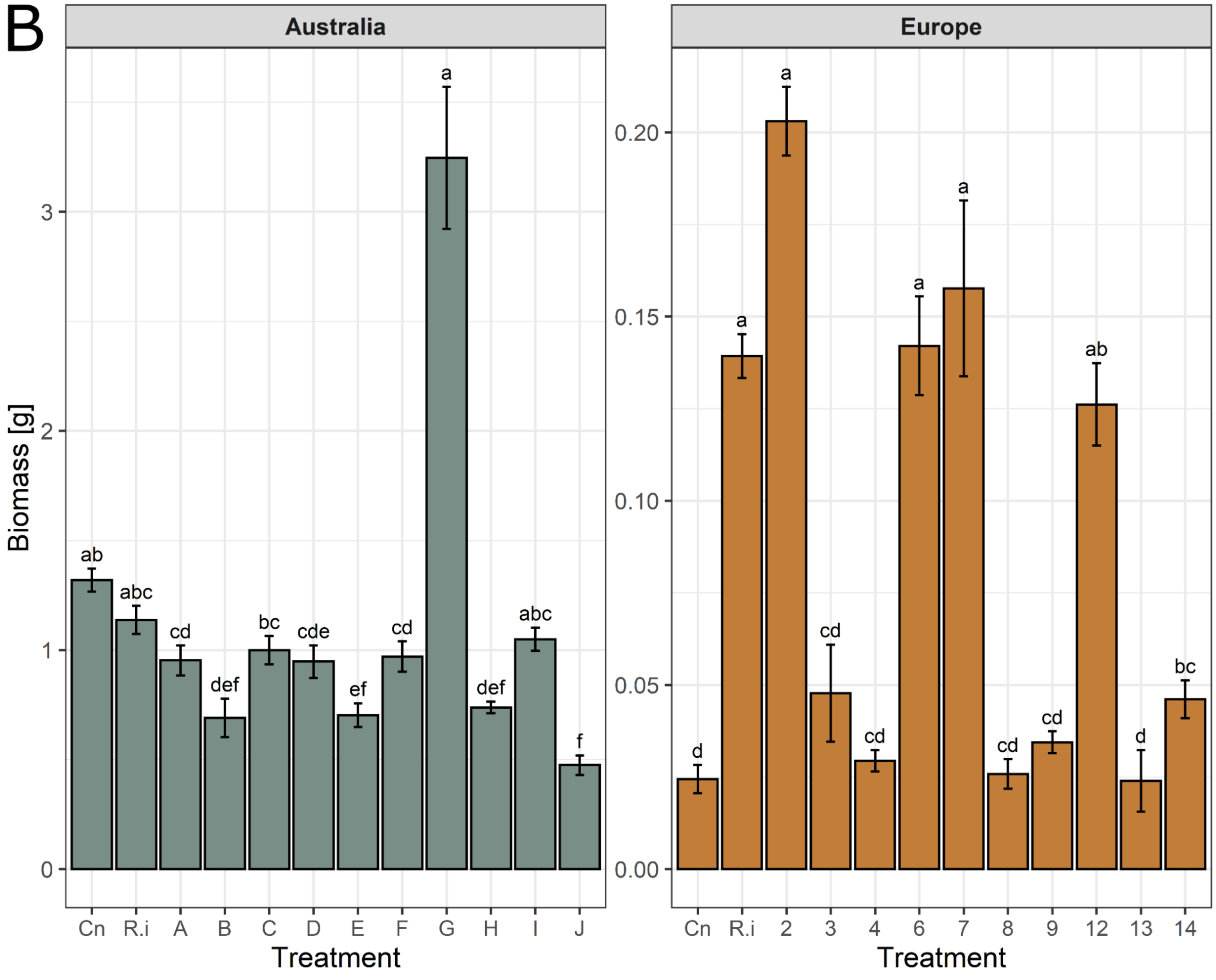


application rate as recommended

20g inoculum for all treatments

Greenhouse study using sterilized soil

Salomon et al. (2022). Global evaluation of commercial arbuscular mycorrhizal inoculants under greenhouse and field conditions. *Applied Soil Ecology*



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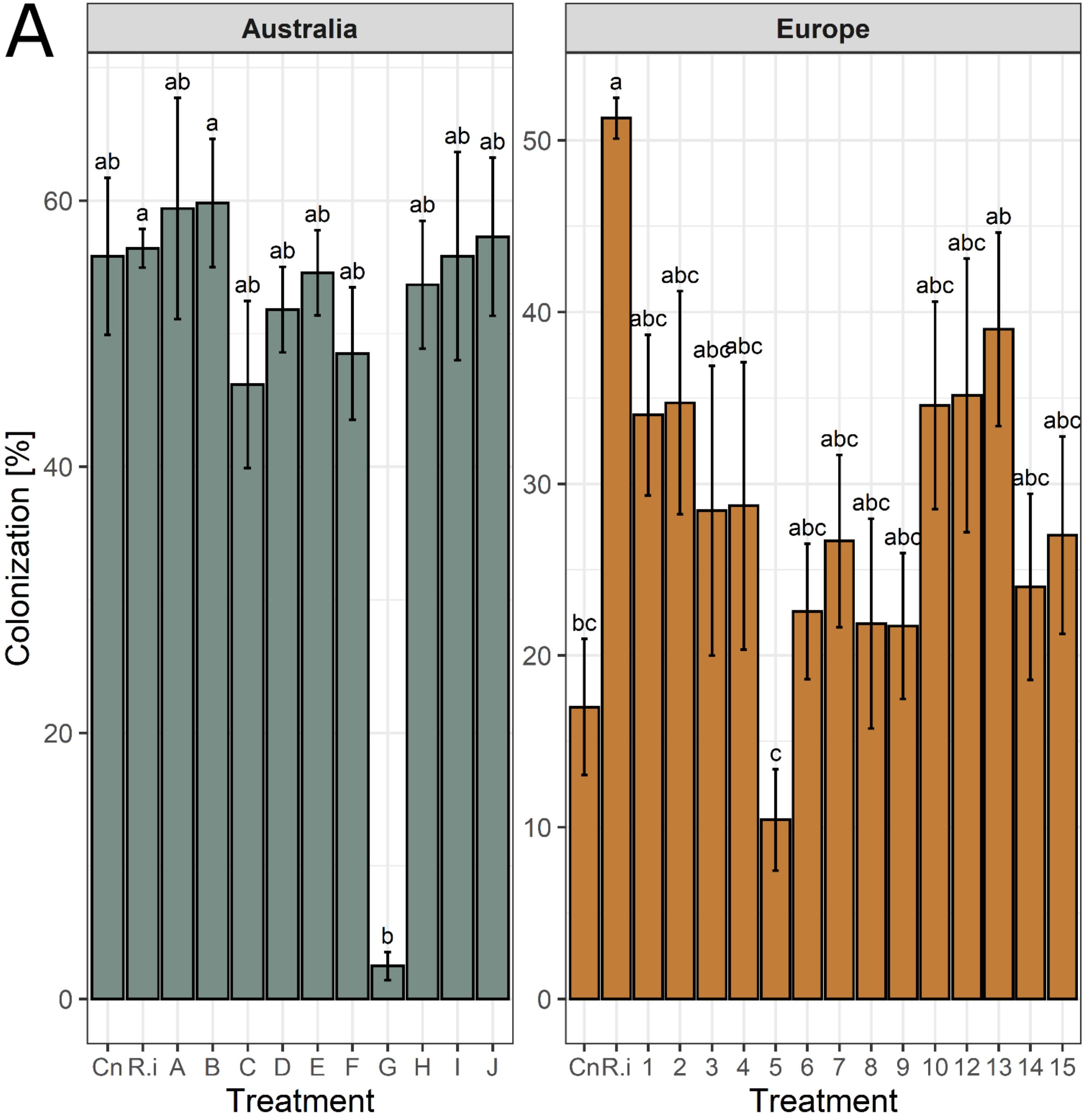
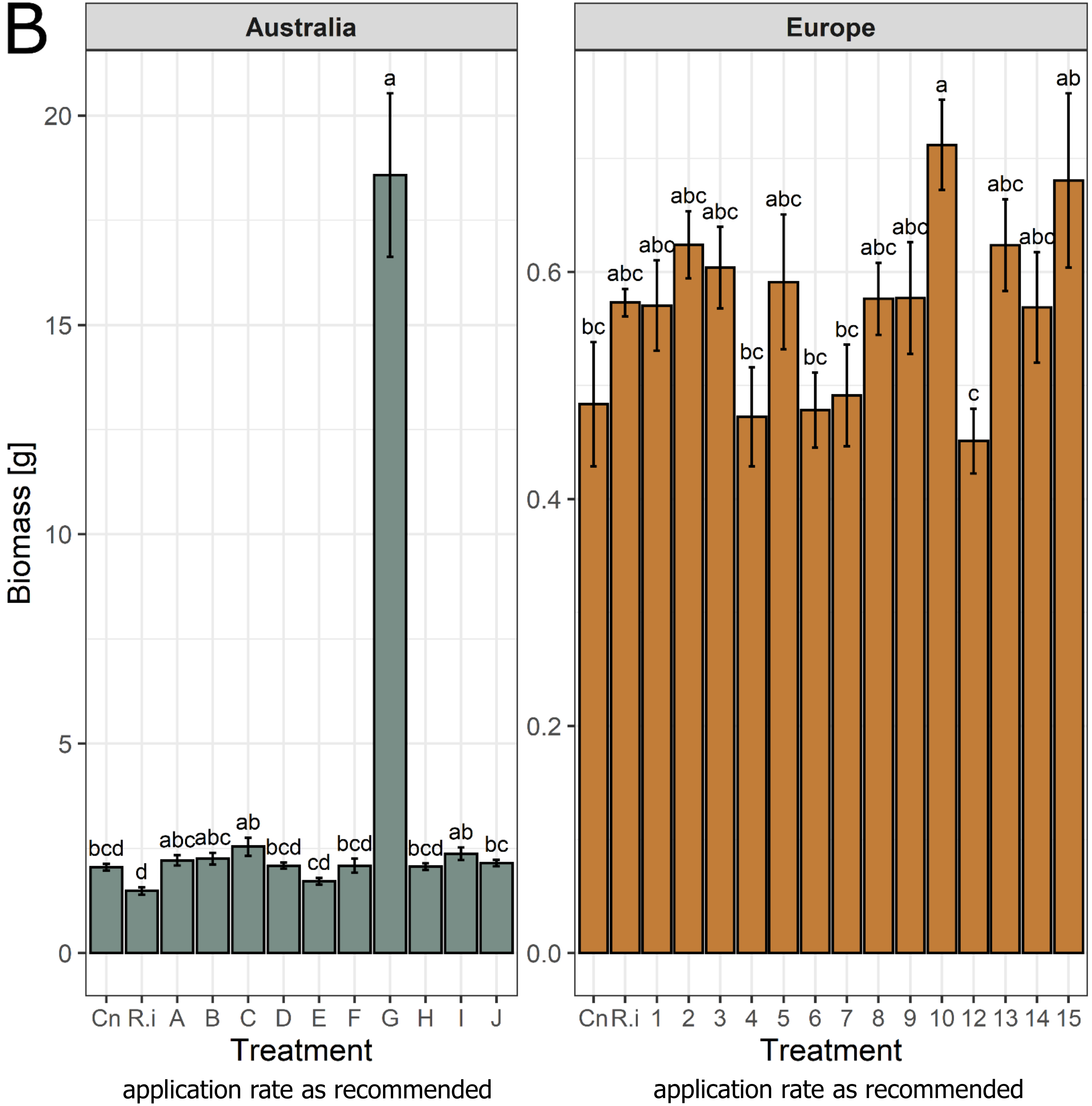


application rate as recommended

application rate as recommended

Greenhouse study using non-sterilized soil

Salomon et al. (2022). Global evaluation of commercial arbuscular mycorrhizal inoculants under greenhouse and field conditions. *Applied Soil Ecology*



Greenhouse study using non-sterilized soil

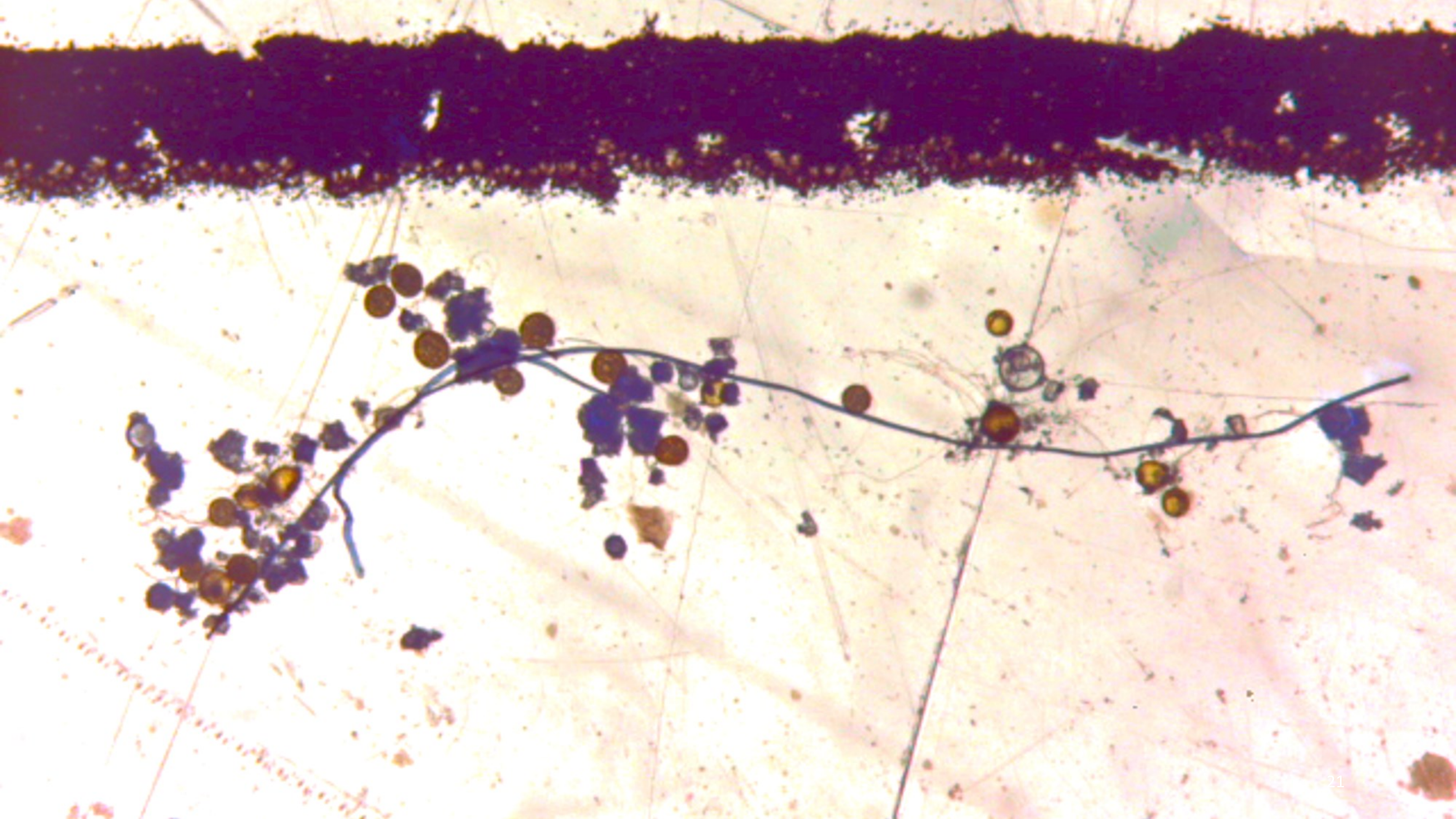
Salomon et al. (2022). Global evaluation of commercial arbuscular mycorrhizal inoculants under greenhouse and field conditions. *Applied Soil Ecology*

Spore count

Spore count

	Australia	Europe	North America
Sample size (n)	10	15	3
Spores g^{-1} (median)	18	24	280
Spores g^{-1} (range)	1 - 715	0 - 267	93 - 2744
Spores per pot (median)	34	131	525 spores m^{-2}

Salomon et al. (2022). Global evaluation of commercial arbuscular mycorrhizal inoculants under greenhouse and field conditions. *Applied Soil Ecology*

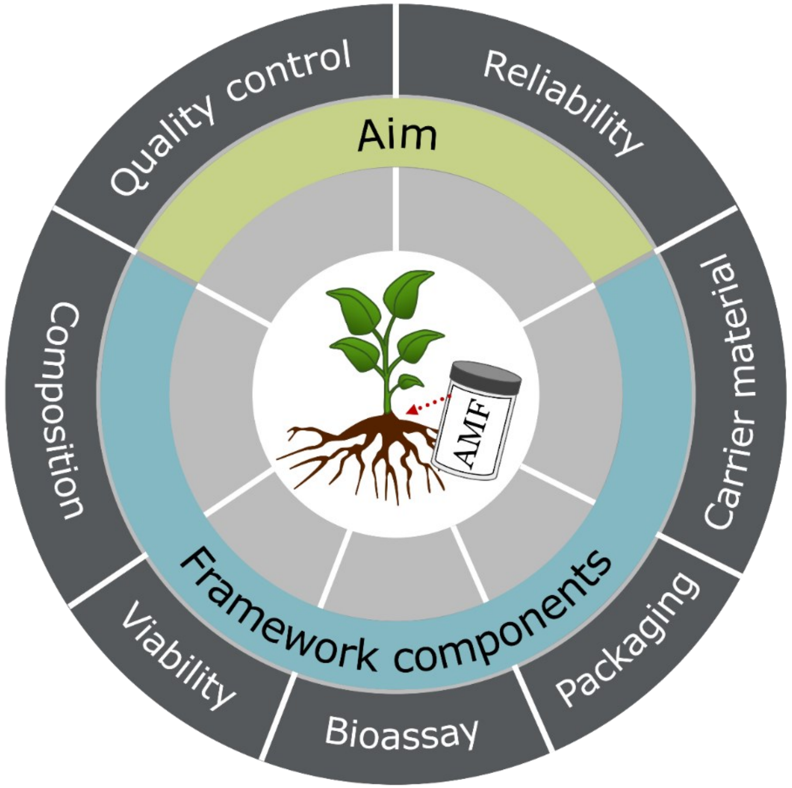


Conclusion?

- Undeclared and relatively high concentrations of plant nutrients.
- Some products without any spores or very low spore concentrations.
- Products with enough spores but reduced viability.

- But: Successful inoculants in the European study
- Clear biomass increase compared to soils without AMF propagules

Establishing a quality management framework for commercial inoculants containing arbuscular mycorrhizal fungi



Salomon, M.J., Watts-Williams, S.J., McLaughlin, M.J., Bücking, H., Singh, B.K., Hutter, I., Schneider, C., Martin, F., Vosatka, M., Guo, L.D. and Ezawa, T., Saito M., Declerck S., Zhu YG., Bowles T., Abbott L.K., Smith F.A., Cavagnaro T.R., van der Heijden M.G.A. 2022. Establishing a quality management framework for commercial inoculants containing arbuscular mycorrhizal fungi. *iScience*

Methods for AMF inoculum assessment

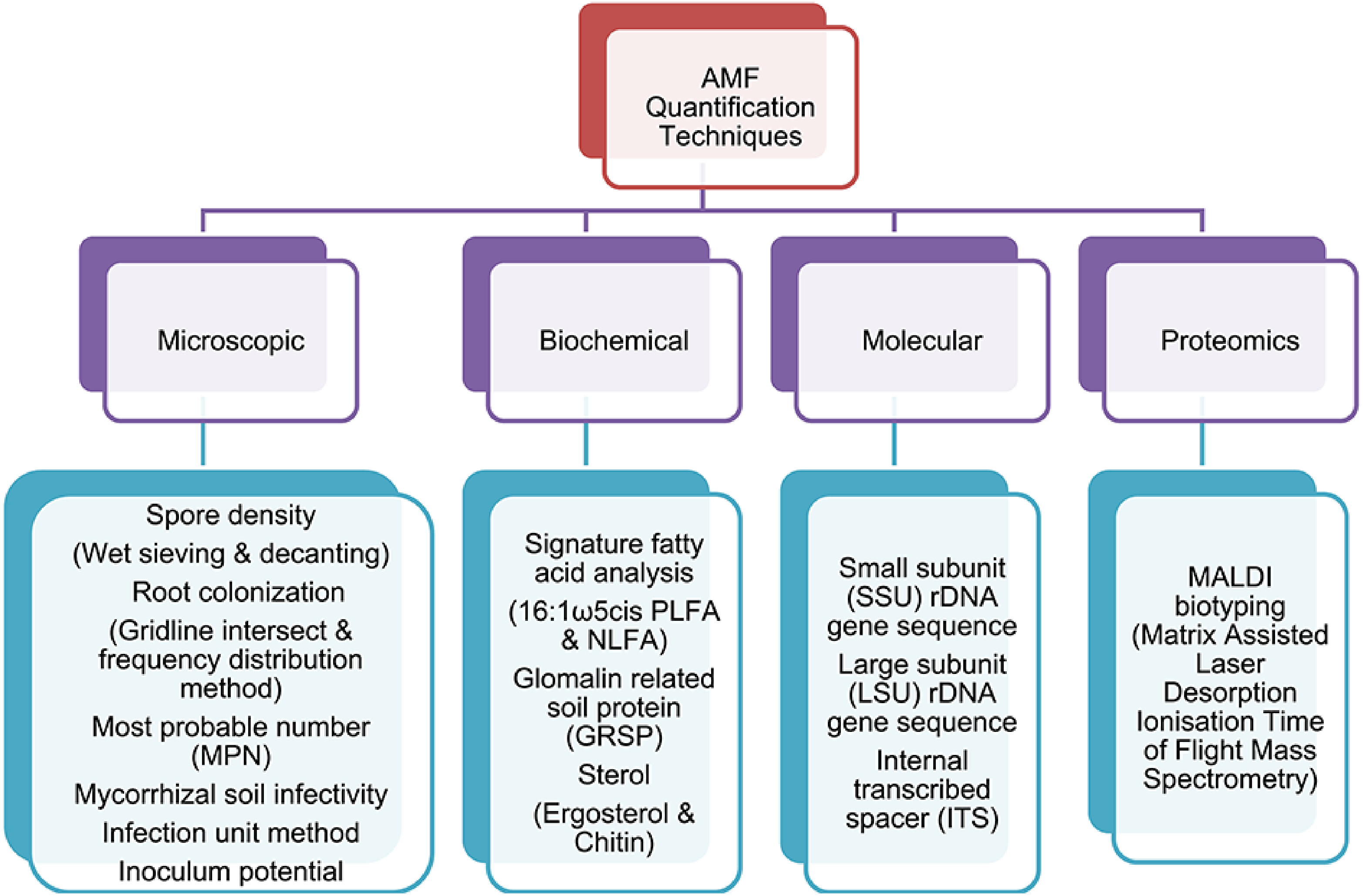


Figure by: Agnihotri, R., Sharma, M., Bucking, H. *et al.* Methods for assessing the quality of AM fungal bio-fertilizer: Retrospect and future directions. *World J Microbiol Biotechnol* **38**, 97 (2022).

Soil Productivity Improvement Act – Japan (1979)

1) Bioassay testing inoculum in vermiculite substrate
2) Four weeks greenhouse bioassay
3) Root staining and mycorrhizal quantification using intersect grid method
4) Minimum required root colonization ≥ 5%
5) Mandatory information on the product label
    - Colonization [%] in bioassay and used host plant
    - Used carrier material
    - Applicable and non-applicable plants
    - Expiration date

EU fertilizer regulation (2019/1009)

- CEN/TC 455 - Plant Biostimulants and Agricultural Micro-Organisms
  - CEN/TS 17722:2022 - Plant biostimulants - Determination of mycorrhizal fungi
- Standard is only for mycorrhizal propagule enumeration and fungal characterization
  - Protocols for different inoculum composition scenario (and feasibility to extract spores)
  - Ongoing work to improve protocols

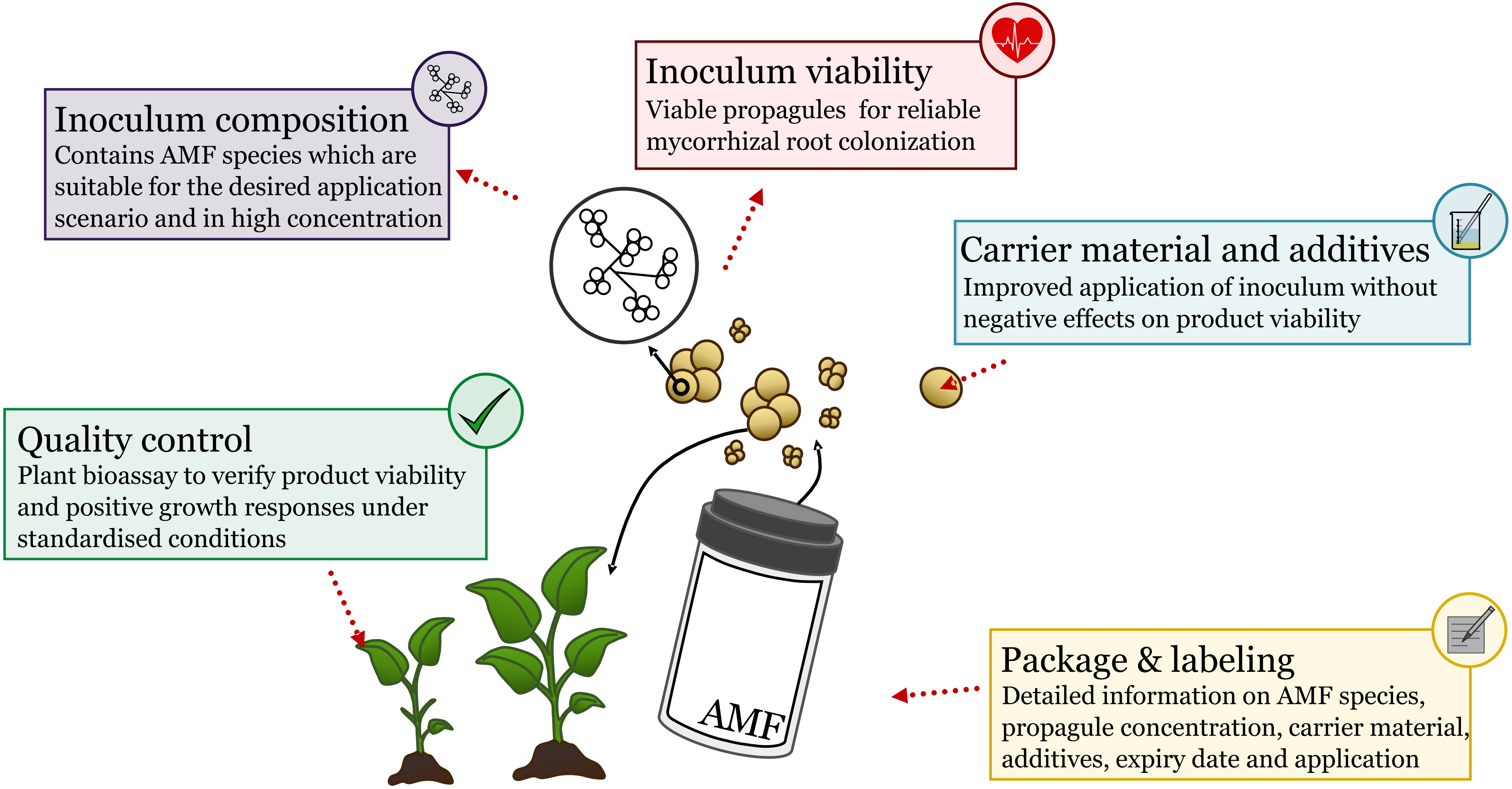
1. Whenever possible, direct counting of propagules

   (Requirement: Claimed spore count within 15% range of actual spore count)

2. Vitality staining in certain scenarios (spores only)

3. Most probable number only when spores cannot be extracted

Salomon, M.J. et al.: Establishing a quality management framework for commercial inoculants containing arbuscular mycorrhizal fungi. *iScience*



Inoculum composition
Contains AMF species which are suitable for the desired application scenario and in high concentration

Inoculum viability
Viable propagules for reliable mycorrhizal root colonization

- Inclusion of a generalist AMF species
    - Exemption applies for specialized inoculum for specific host plants
- Free of plant pathogens, weeds, and other contaminants
    - *in vitro* production
    - DNA-based pathogen detection

- High number of viable spores
- Fast distribution channels to end-consumer, e.g., via selected retailers or direct selling.
    - Suitable storage condition

Carrier material and additives

Improved application of inoculum without negative effects on product viability

- Facilitates application of inoculum
  - Liquid formulation, sticky powder, coarse granules…..
- Only suitable additives that do not interfere with the mycorrhizal development
  - Chitin, humic acid, alginates…
  - Fertilizer?

Package & labelling

Detailed information on AMF species, propagule concentration, carrier material, additives, expiry date and application

- Propagule composition (AMF isolates)
- Carrier material and other additives
- Plant-available nutrients (NPK)
- Batch number
- Production and expiration date
- Instructions on storage and application
- Documented evidence of root colonization (including picture) and plant growth stimulation on the producer's website

Quality control

Plant bioassay to verify product viability and positive growth responses under standardised conditions

- Confirmed root colonization in standardized bioassay
- Confirmed plant growth stimulation in standardized bioassay
- Visual confirmation of the absence of unwanted contaminants, such as weeds or plant pathogens

Host plants	Maize (*Zea mays*) or Sorghum (*Sorghum bicolor*)	Leek (*Allium porrum*)
Growth period (Starting from seedling emergence or transplanting of seedlings)	6 weeks	10 weeks
Minimum pot size	2 litres	1 litre
Plants per pot	1	1
Minimum replicates per treatment	6	
Soil : sand dilution (using fine sand and agricultural soil that is typical for the region where the inoculant is tested)	1:9	
Soil sterilization	Autoclavation for 60 minutes at 121 °C or steaming for 60 minutes at 80 °C or gamma sterilization	
Phosphorus addition	20 mg P kg^{-1} substrate, in form of 88.4 mg CaH_2PO_4 kg^{-1} substrate	
Nutrient solution (Long Ashton -P) (lacking phosphorus)	Weekly, 10 mL per L^{-1} substrate	Every second week, 10 mL per L^{-1} substrate
Watering (Reverse Osmosis or distilled H_2O)	Every second day to field capacity	
Temperature	18 °C (night) to 30 °C (day)	
Daylight average light intensity	> 600 µmol m^{-2} s^{-1}	

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Limitations and future research

- Expensive and time intensive compared to other methods.
- Cannot be repeated for many batches (variability within product?)
- Issues with specialised AMF species when using maize or leek.
- Using AMF-favourable conditions → realistic?

- Future research for AMF <u>inoculum</u>
  - Developing AMF application models to predict inoculation success and yield responses
  - Understanding the establishment of introduced AMF under field conditions and its effects on indigenous AMF communities (potential hazards?)
  - Development of advanced production methods to achieve highly concentrated and contaminant free inoculants
    - And it’s effect on AMF functioning and genetic stability

Conclusion

- Introduction of mandatory regulatory standards urgently required
- AMF inoculum comes in many shapes and forms
  - Best assessment method and protocol?
- Economical viable and scientifically meaningful results

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Matt Salomon

Prof Timothy Cavagnaro

Timothy.cavagnaro@adelaide.edu.au

@IDigSoil

Prof Marcel van der Heijden

marcel.vanderheijden@
agroscope.admin.ch

@vandeheijdenlab