The missing piece of the puzzle in grassland restoration: Arbuscular Mycorrhizal Fungi

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Hundreds of plant species and more than 60 species at risk depend on Canada's grasslands.

Photo credits: World Wildlife Fund

Grassland services

- ✓ High biodiversity
- ✓ Social/cultural values
- ✓ Livestock grazing
- ✓ Water supply and flow control
- ✓ Carbon storage
- ✓ Erosion control
- ✓ Climate mitigation
- ✓ Pollination

Grasslands are in danger!

- North America – 80% of the central grasslands has been converted to cropland.

  Foley et al. 2005, Suttie et al. 2005

- Northern Europe – Over 90% of the semi-natural grasslands have been lost since the 1930s.

  Eriksson et al. 2002, Bullock et al. 2011, Pe'er et al. 2014

- Eurasia – More than 43 million hectares of the steppe have been converted into cropland.

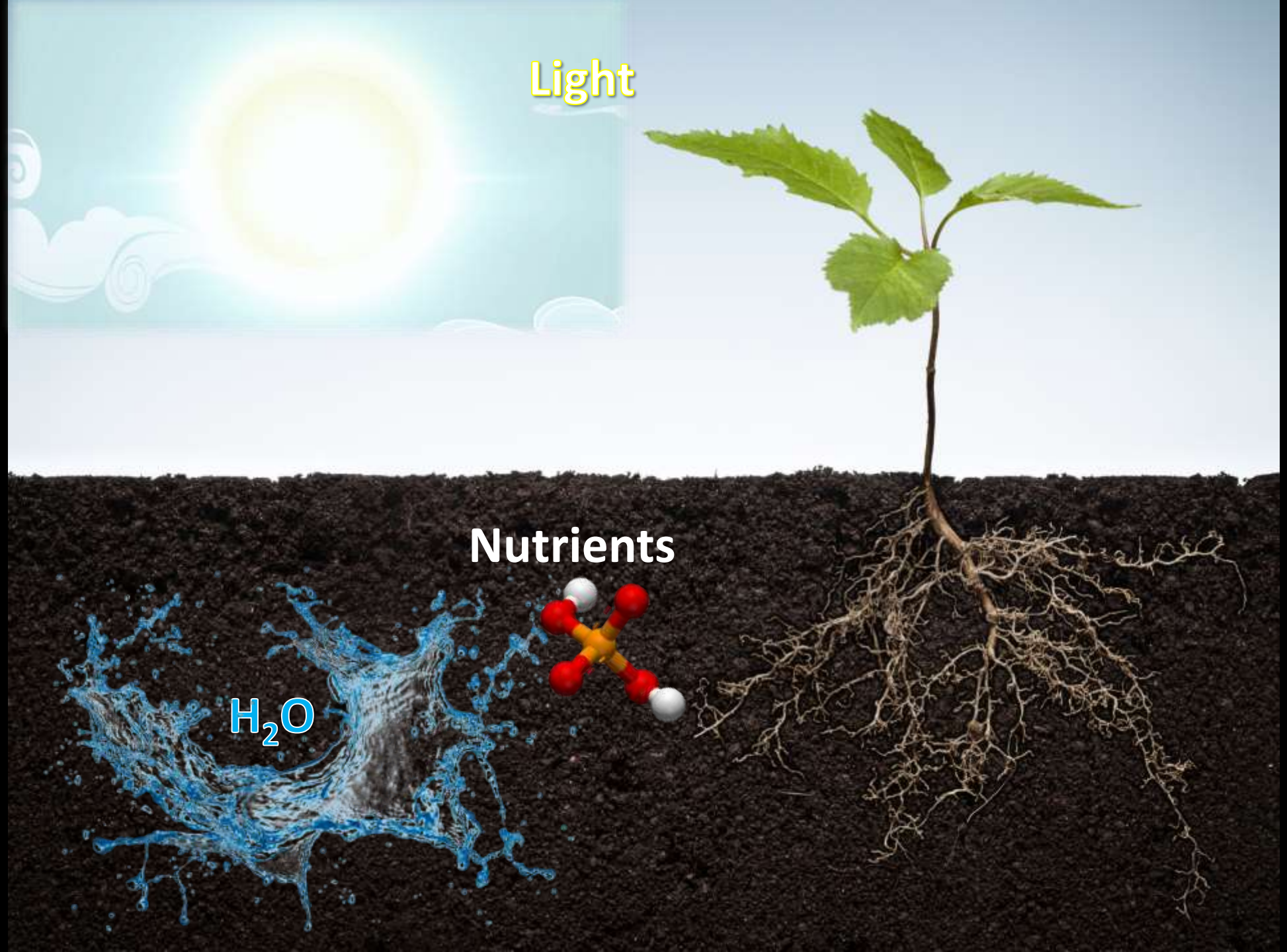
- South America – 60 to 80% of the grassland area is degraded.

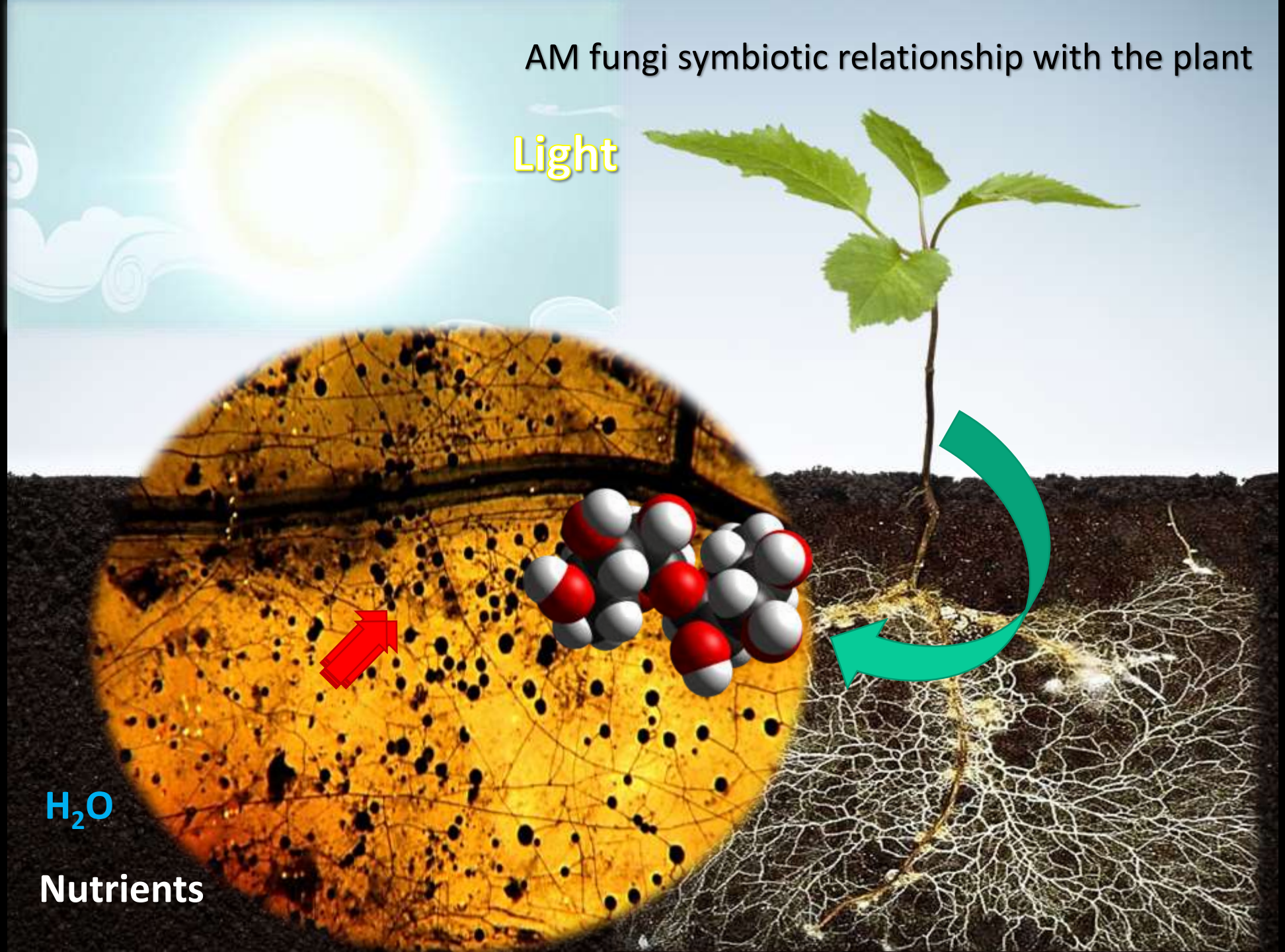
  Suttie et al. 2005

Photo credits: Grasslands - Nature Canada

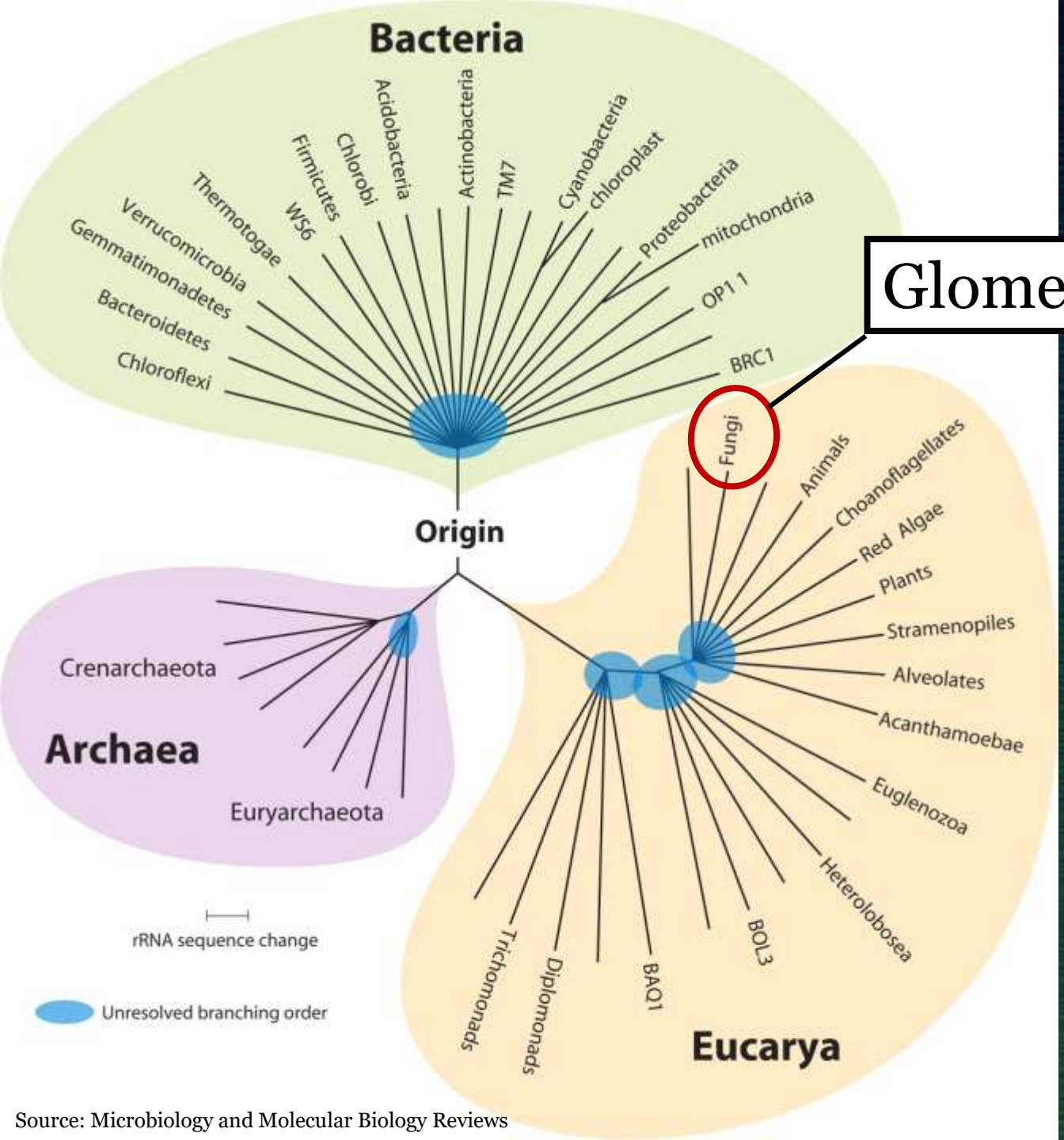
Over the past two and a half decades, Canada has lost 25 million acres of grasslands.

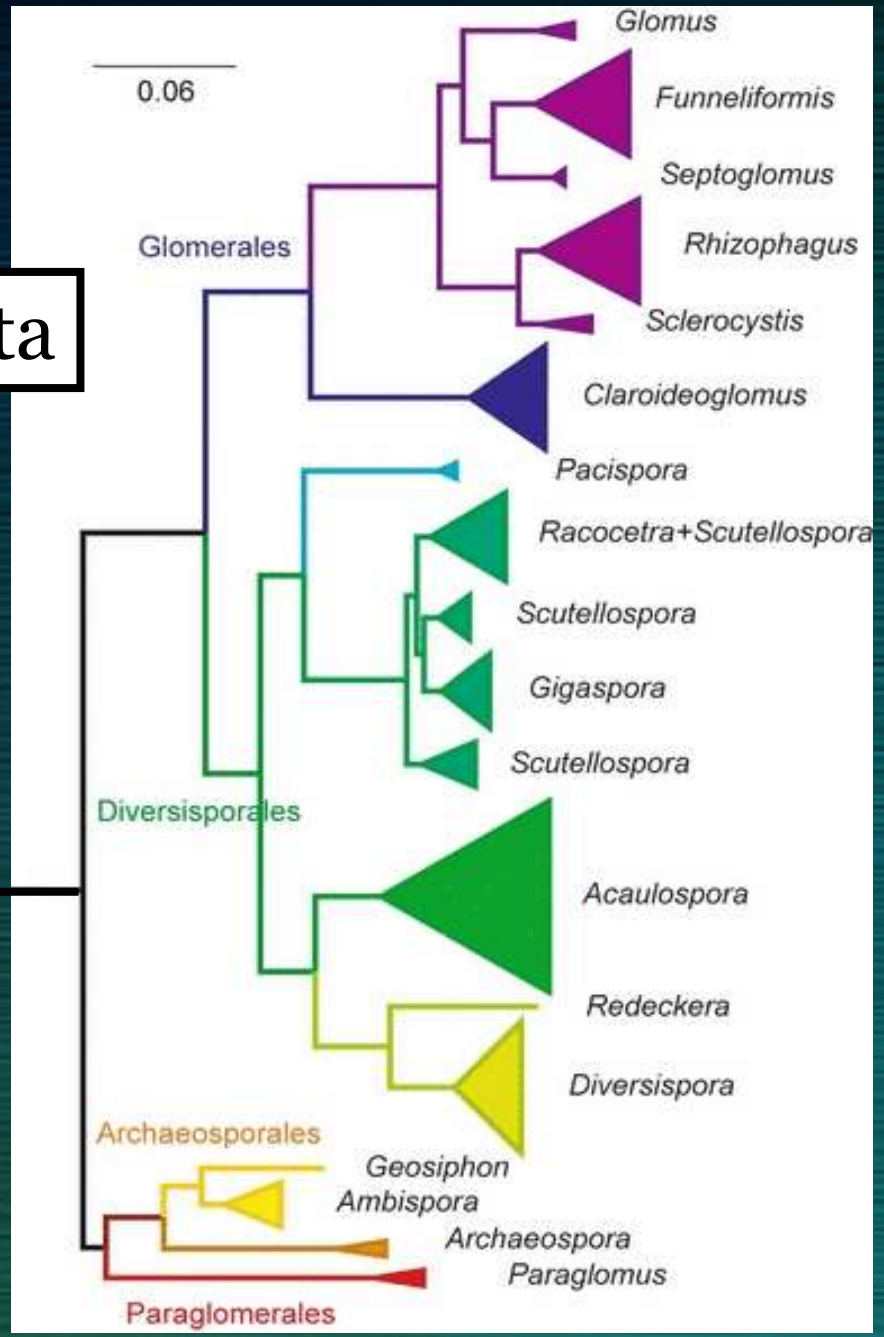
Nature Canada, 2022


Light
Nutrients
H_2O


AM fungi symbiotic relationship with the plant
Light
H_2O
Nutrients

Glomeromycota





Source: Microbiology and Molecular Biology Reviews

New Phytologist, Volume: 193, Issue: 4, Pages: 823-826, First published: 02 February 2012, DOI: (10.1111/j.1469-8137.2011.04029.x)

Figure 1 Open in figure viewer | PowerPoint

Spores of arbuscular mycorrhizal fungi (AMF) representing the different genera of the Glomeromycota. Row 1, *Glomus*, *Archaeospora*, *Redeckera*, *Pacispora*; row 2, *Acaulospora*, *Rhizophagus*, *Claroideoglomus*, *Racocetra*; row 3, *Paraglomus*, *Diversispora*, *Sclerocystis*, *Scutellospora*; row 4, *Ambispora*, *Funneliformis*, *Gigaspora*, *Entrophospora*. Bars, 200 μm. Figure courtesy of C. Walker.

AMF

Diversity
Richness
Community

New Phytologist

Commentary | Free Access

A molecular guide to the taxonomy of arbuscular mycorrhizal fungi

J. Peter W. Young

First published: 02 February 2012 | https://doi.org/10.1111/j.1469-8137.2011.04029.x | Citations: 18

Arbuscules

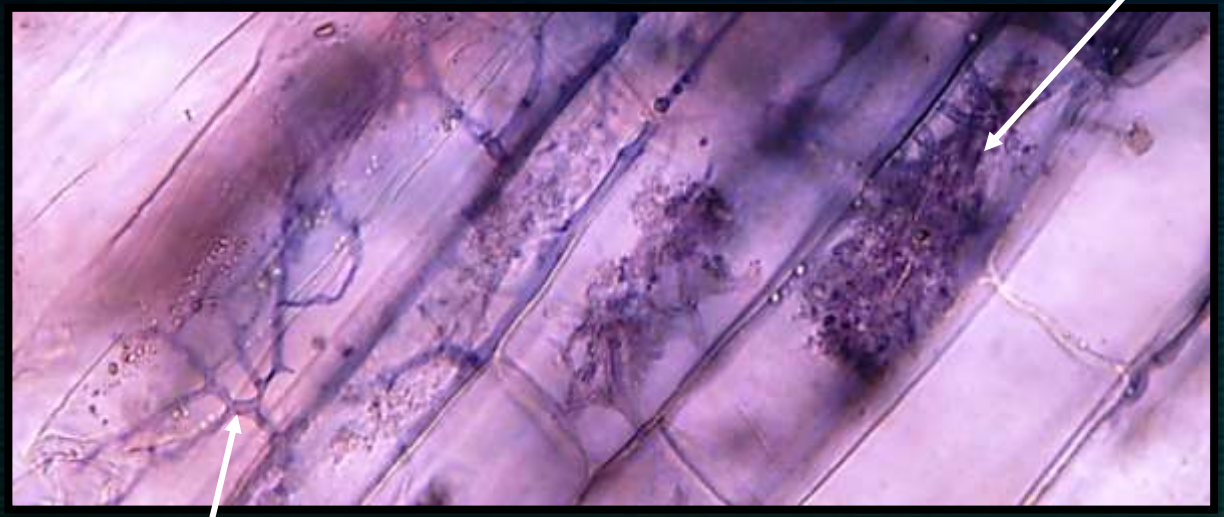


Photo credits: Navarro Borrell, A.

Hyphae*

* Groups of hyphae form the vegetative body of the fungi called **mycelia**

Hyphae*

Photo credits: Navarro Borrell, A.

AMF spore

AMF can help!

AMF ecosystem services

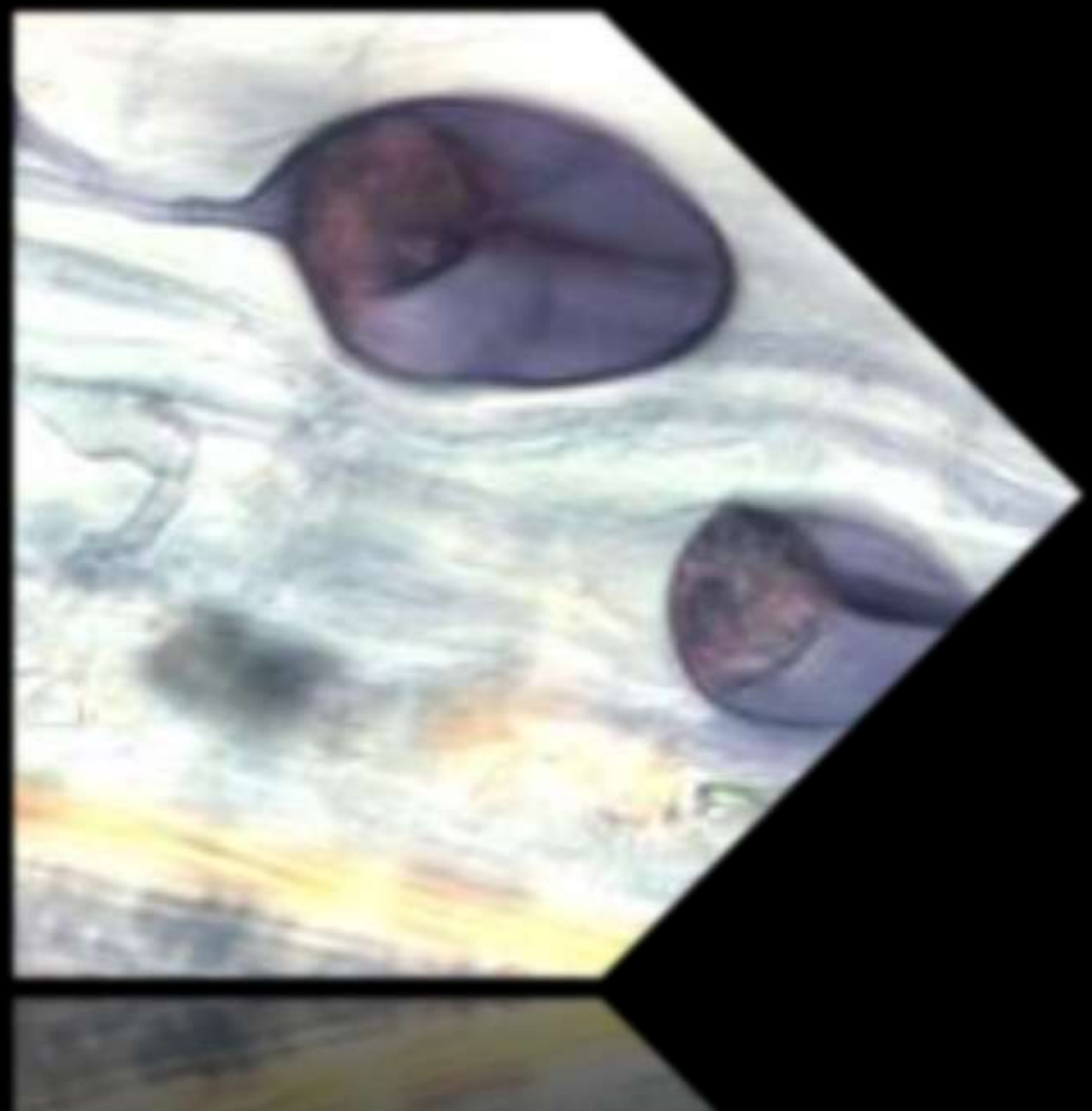
Symbiosis	Establish symbiosis with 80% of plant families
Improvement	Improve the growth of plants through increased uptake of available soil P and other non-labile mineral nutrients essential for plant growth
Stabilization	Stabilize soil aggregates
Protection	Prevent soil erosion
Tolerance	Alleviate plant stress caused by biotic and abiotic factors

AMF ecosystem services

Table 1 Overview of the main roles that the AM symbiosis can play as an ecosystem service provider

AM function	Ecosystem service(s) provided
Root morphology modification and development of a complex, ramifying mycelial network in soil	Increase plant/soil adherence and soil stability (binding action and improvement of soil structure)
Increasing mineral nutrient and water uptake by plants	Promote plant growth while reducing fertiliser requirement
Buffering effect against abiotic stresses	Increased plant resistance to drought, salinity, heavy metals pollution and mineral nutrient depletion
Secretion of 'glomalin' into the soil	Increased soil stability and water retention
Protecting against root pathogens	Increased plant resistance against biotic stresses while reducing phytochemical input (see Tables 2 and 3)
Modification of plant metabolism and physiology	Bioregulation of plant development and increase in plant quality for human health

Gianinazzi *et al.*, 2010

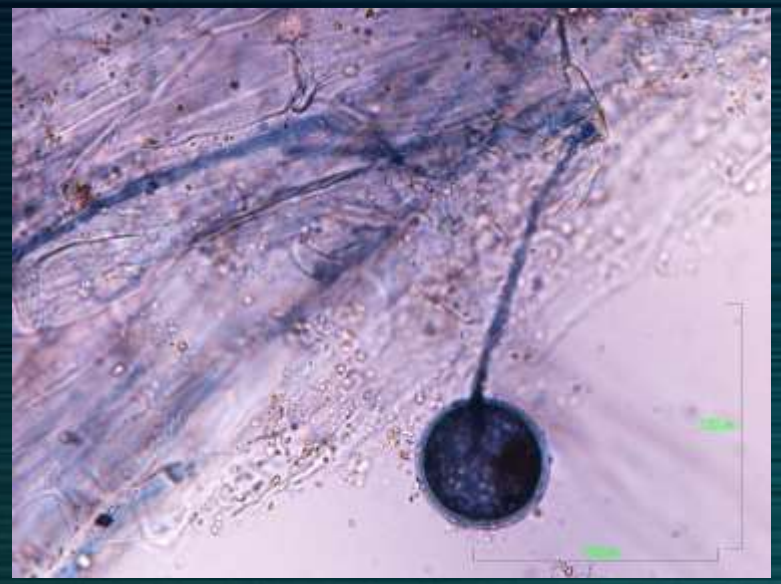


AMF identification & quantification

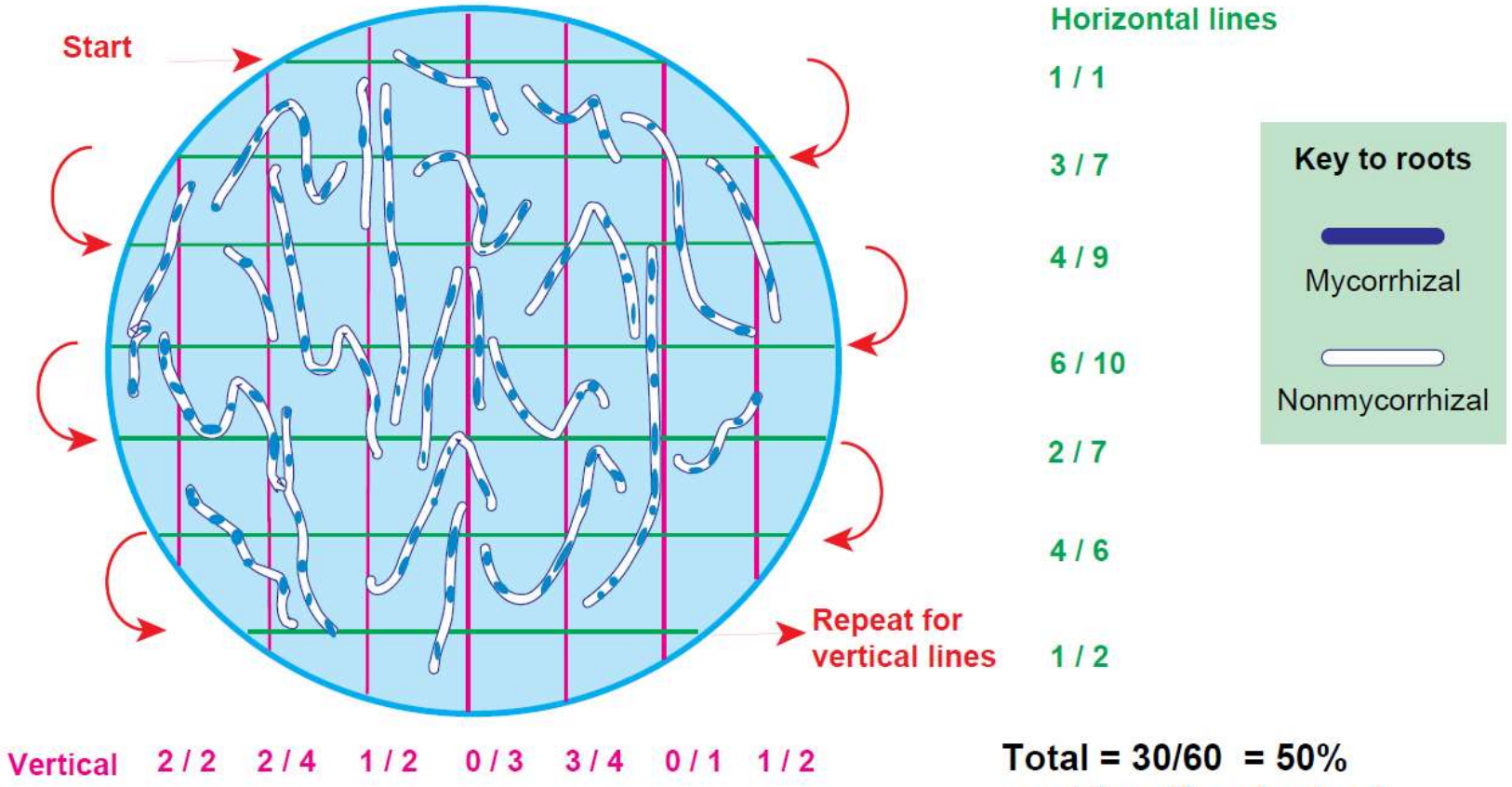
“Measure what you can measure and make measurable what cannot be measured.”

Galileo Galilei

Root colonization by AM and non-AM fungi



Gridline Intersect Method = % AMF colonization



Total = 30/60 = 50% root length colonised

Gridline intersect method using compound microscope

(McGonigle et al., 1990; also in Brundrett et al., 1996)

WinRHIZO method

Allows the examination of more replications and a larger number of samples, which would provide an insight into the significance of AMF functioning in grasslands.

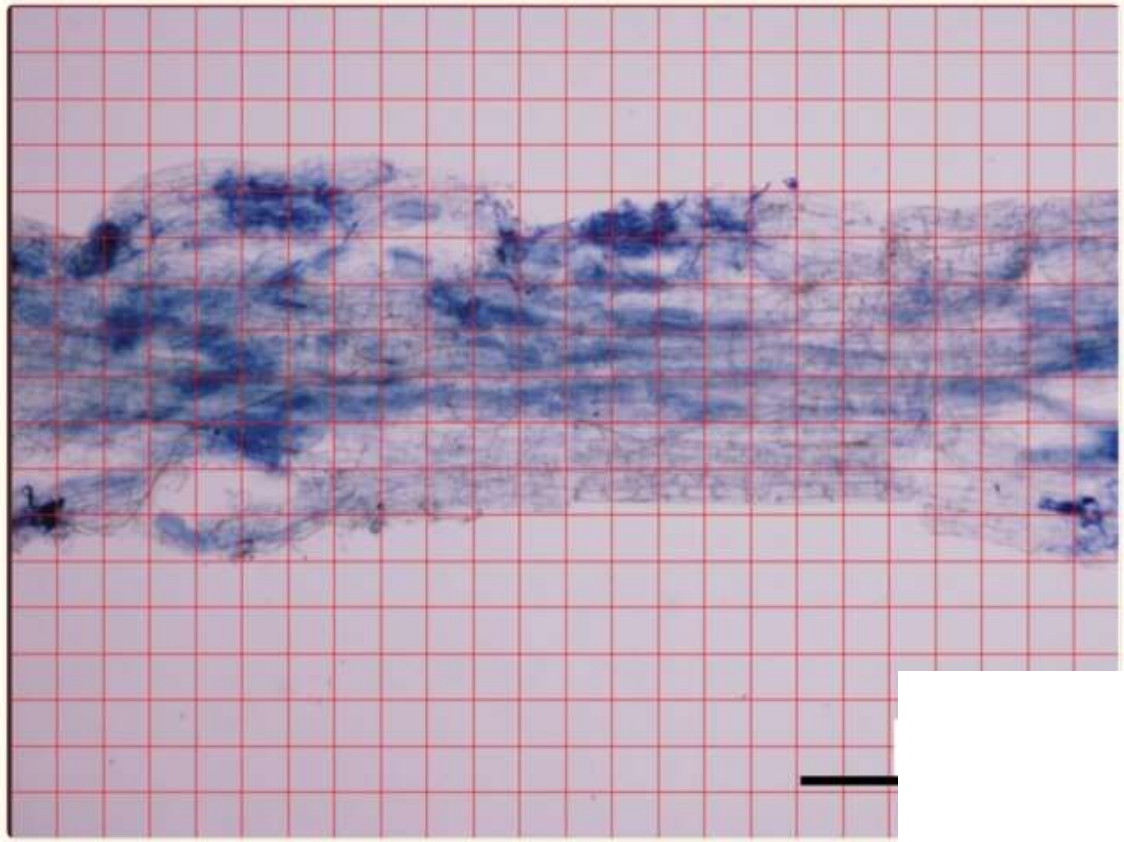


Fig. 1

Micrograph of a root fragment of *Chengiopanax sciadophylloides* seedling used in intersect method.

Mycobiology www.mycobiology.or.kr
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Published online 2017 Mar 31. doi: 10.5941/MYCO.2017.45.1.15 PMID: 28435349

Proposal of a New Estimation Method of Colonization Rate of Arbuscular Mycorrhizal Fungi in the Roots of *Chengiopanax sciadophylloides*

Seitaro Deguchi,[1] Yosuke Matsuda,[2] Chisato Takenaka,[3] Yuki Sugiura,[3] Hajime Ozawa,[4] and Yoshimune Ogata[5]

Sequencing approach

DNA extraction from roots and rhizosphere soil

PCR amplification

Gel electrophoresis

DNA purification

Library assembling

Bioinformatics and phylogenetic analysis

Primer set	Target community	Target region
ITS1F / ITS2	Non-AMF	ITS
AMV4.5NF / AMDGR	AMF	18S rDNA

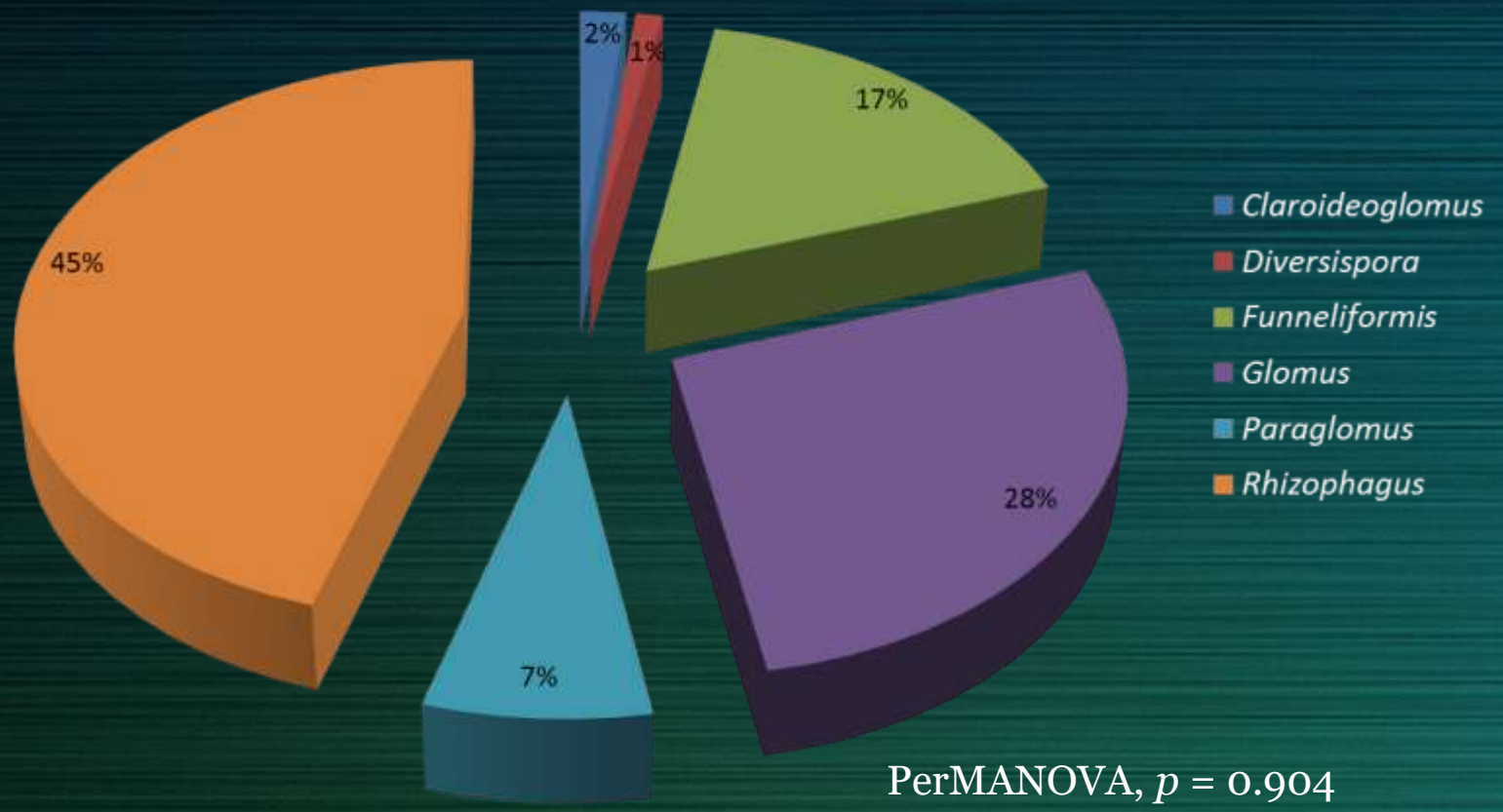
Sequencing is expensive...

Is it worth the cost?

AMF community composition in wheat roots

Flag leaf stage

Rhizophagus was the most abundant AMF colonizing wheat roots

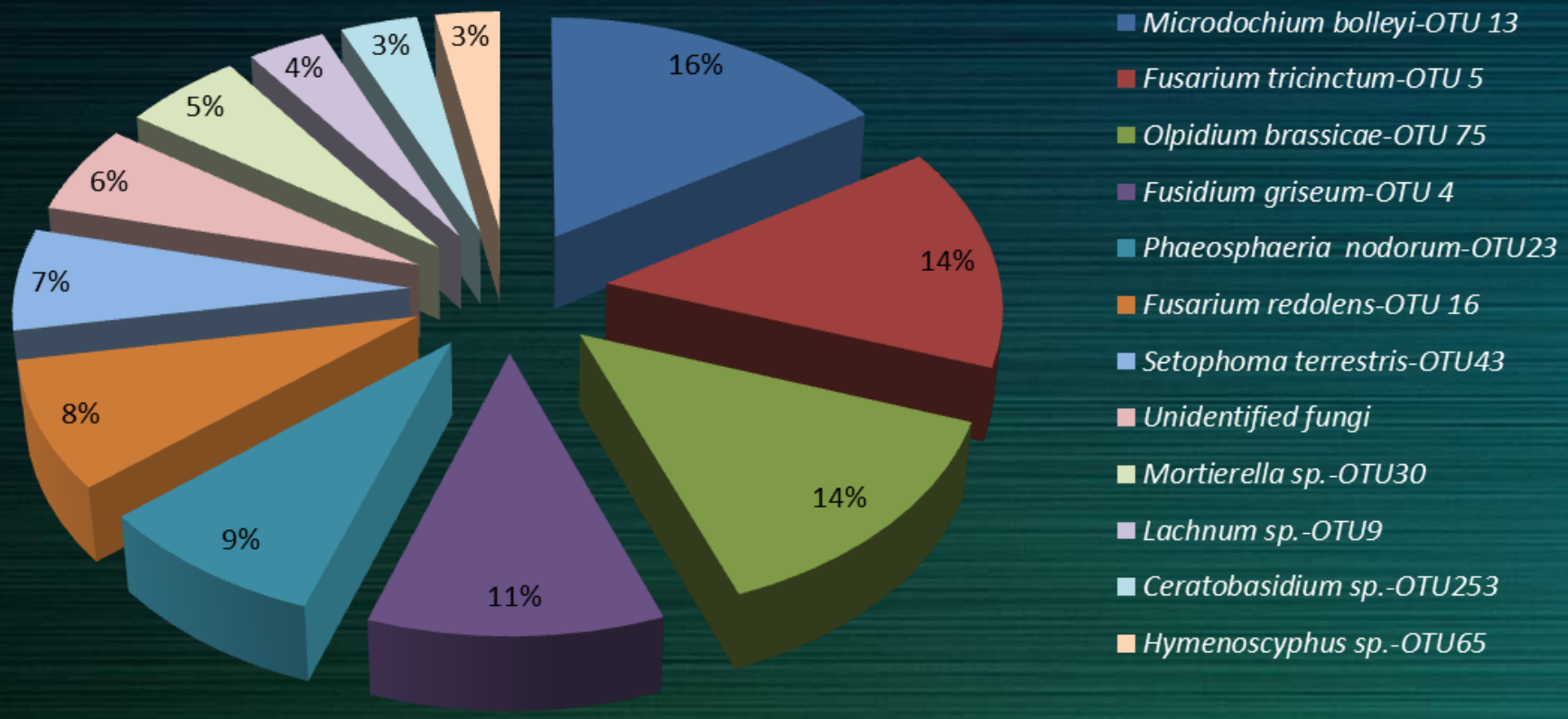


PerMANOVA, $p = 0.904$

Navarro-Borrell, 2015

Non-AMF community composition in wheat roots

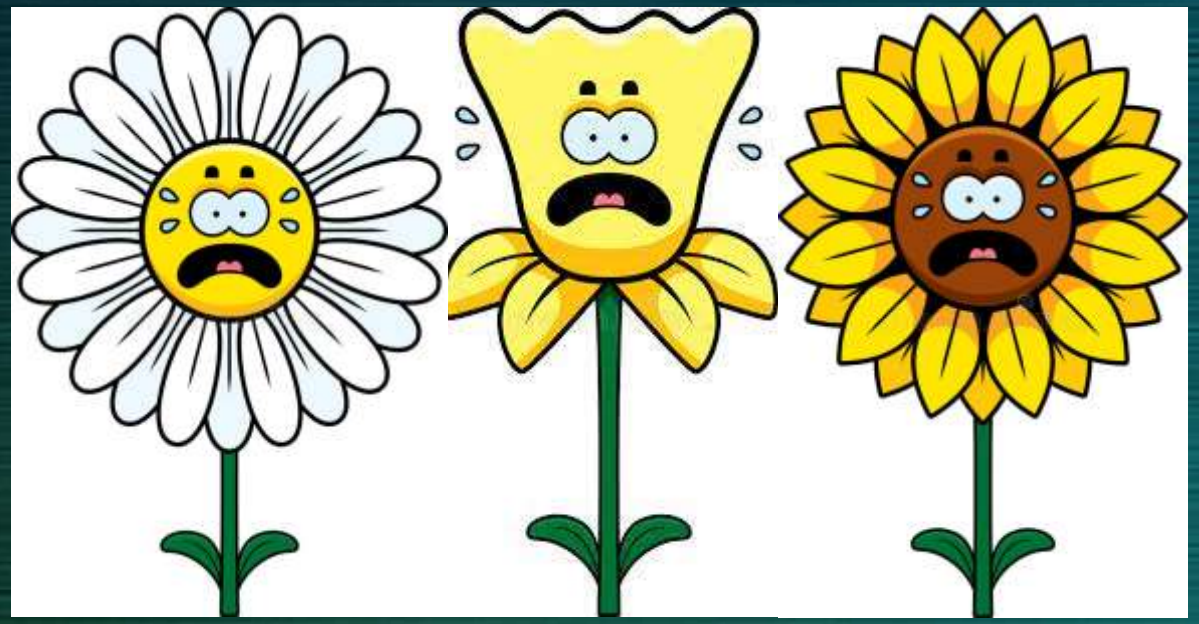
Flag leaf stage



PerMANOVA, $p = 0.521$

Navarro-Borrell, 2015

Are there "bad" mycorrhizae that should not be introduced into native perennial systems?



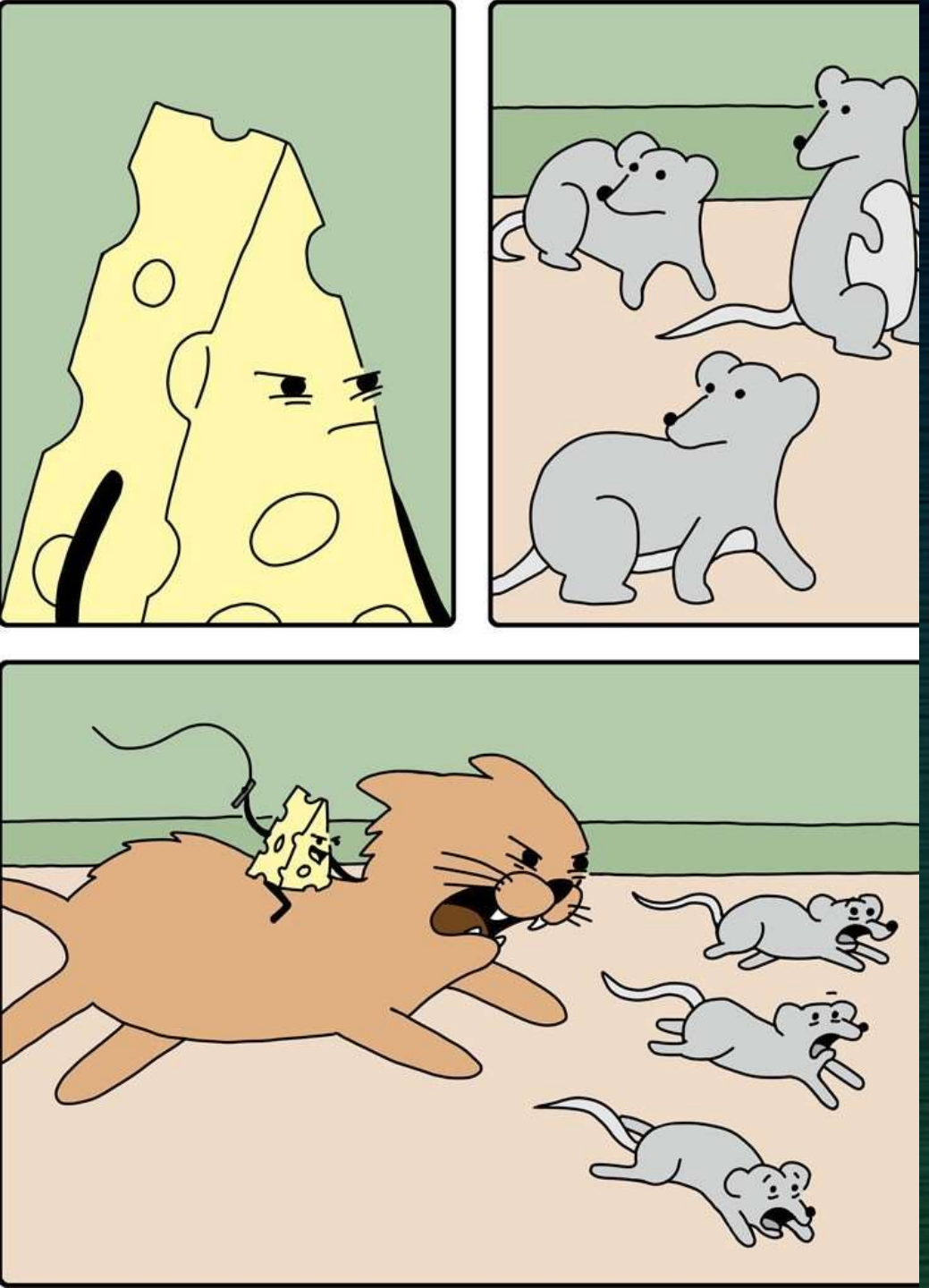
Are there "bad" mycorrhizae that should not be introduced into native perennial systems?

Positive and negative contributions of introduced AMF inoculant taxa with different origin to nutrient uptake, biomass, yield and plant productivity responses have been documented (Wilson & Hartnett, 1998; Dai *et al*., 2014; Koziol *et al*., 2015).

Examples:

- ✓ A symbiosis with *Rhizophagus irregularis* was more costly to maize than a symbiosis with *Claroideoglomus claroideum*, which improved net P uptake (Thonar *et al*., 2011)
- ✓ Glomus might be aggressive colonizers. *Glomus iranicum* and *Glomus indicum* are predominant species in the Canadian Prairies which may reduce the growth of plants under certain conditions (i.e., drought) by limiting C assimilation (Dai *et al*., 2014).

...And what about AMF benefiting invasive plant species?



The enemy of your enemy is your friend… What about the friend of your enemy?

AMF can increase competitiveness of exotic plants, potentially increasing invasion success

- Glomus and Rhizophagus dominance increased with invasion density, while overall OTU* richness decreased with invasion density.

Ecology and Evolution
Open Access

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PMCID: PMC3967904
PMID: 24683461

Grassland invaders and their mycorrhizal symbionts: a study across climate and invasion gradients

Rebecca A Bunn,[1] Ylva Lekberg,[2,3] Christopher Gallagher,[1] Søren Rosendahl,[4] and Philip W Ramsey[2]

*OTU: Operational Taxonomic Unit

Previous research shows that AMF have the potential to influence invasive plant success.

- The successful invaders *Euphorbia esula* (spurge) and *Centaurea jacea* (knapweed) have a high AMF dependency (Klironomos 2002)
- *Centaurea stoebe* becomes more competitive toward native plants when grown with AMF (Marler *et al*., 1999).
- *Centaurea jacea* (knapweed) and *Centaurea stoebe* increase the overall abundance of AMF and harbor different AMF communities than adjacent native grasslands (Lekberg *et al*., 2013).
- *Solidago canadensis* alters AMF communities in ways that promote its own growth more than a competing native plant (Zhang *et al*., 2010).

What are the effects of topsoil disturbance in native grasslands on AMF?

What are the effects of topsoil disturbance in native grasslands on AMF?

Strong differentiation of AMF communities in undisturbed grasslands, whereas AMF communities in disturbed grasslands tend to be more homogeneous.

House & Bever, 2018

AMF help plant adaptation to climate

Ecological Applications, 28(3), 2018, pp. 736–748

Disturbance reduces the differentiation of mycorrhizal fungal communities in grasslands along a precipitation gradient

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Background photo credits: Wallpaper Flare

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Soil disturbance changes arbuscular mycorrhizal fungi richness and composition in a fescue grassland in Alberta Canada

Holly J. Stover [a], M. Anne Naeth [a], Katja Boldt-Burisch [b, c]

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Highlights

- There was a shift of the AMF virtual taxa community in response to disturbance type.
- Site disturbance decreased AMF richness.
- AMF richness was positively correlated with plant richness.
- Eight AMF genera were found on undisturbed sites and three on disturbed sites.
- *Glomus* comprised the majority of virtual taxa.

- Soil disturbance and plant invasion can alter the AMF community.
- Site disturbance shifted AMF communities.
- Restoration success with native plants might be highly dependent on reintroducing...

Native AMF

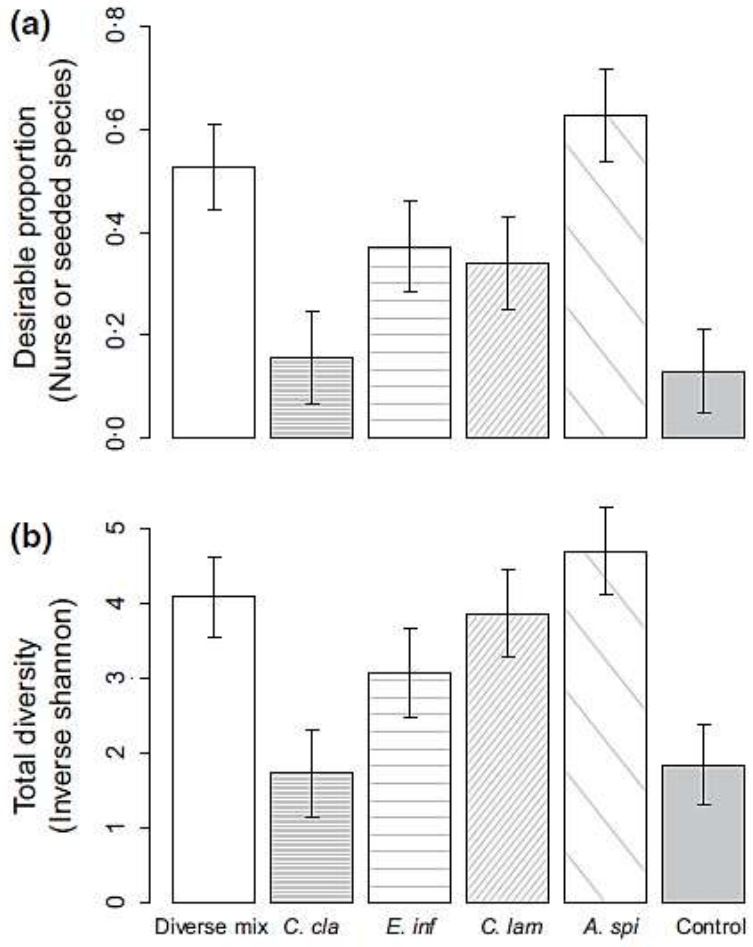

Fig. 4. (a, b) Fungal composition greatly affected both the abundance of desirable species (a) and total plot diversity (b). Total diversity and desirable abundance were greatly improved with diverse AM fungal mixture and *A. spinosa*. Bars represent the average desirable proportion of plant biomass (a) and the average total diversity (b) among plots inoculated with the six different fungal communities and error bars are standard error. Plots inoculated with *Entrophospora infrequens*, *Claroideoglomus lamellosum*, *Claroideoglomus claroideum* and *Aralia spinosa* are represented by *E. inf*, *C. lam*, *C. cla* and *A. spi*, respectively.

AMF inoculation drives plant community composition

- Plots inoculated with native AMF species were dominated by desirable prairie plants.
- Plots inoculated with other AMF species and the non-inoculated control were dominated by non-desirable plants including weeds and exotic species.
- 83% of the late successional plants only established within plots that had been inoculated with native prairie AMF inoculant.

Journal of Applied Ecology
BRITISH ECOLOGICAL SOCIETY
Journal of Applied Ecology 2017, **54**, 1301–1309 doi: 10.1111/1365-2664.12843

The missing link in grassland restoration: arbuscular mycorrhizal fungi inoculation increases plant diversity and accelerates succession

Liz Koziol*[,1,2] and James D. Bever[2]

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One size doesn't fit all!

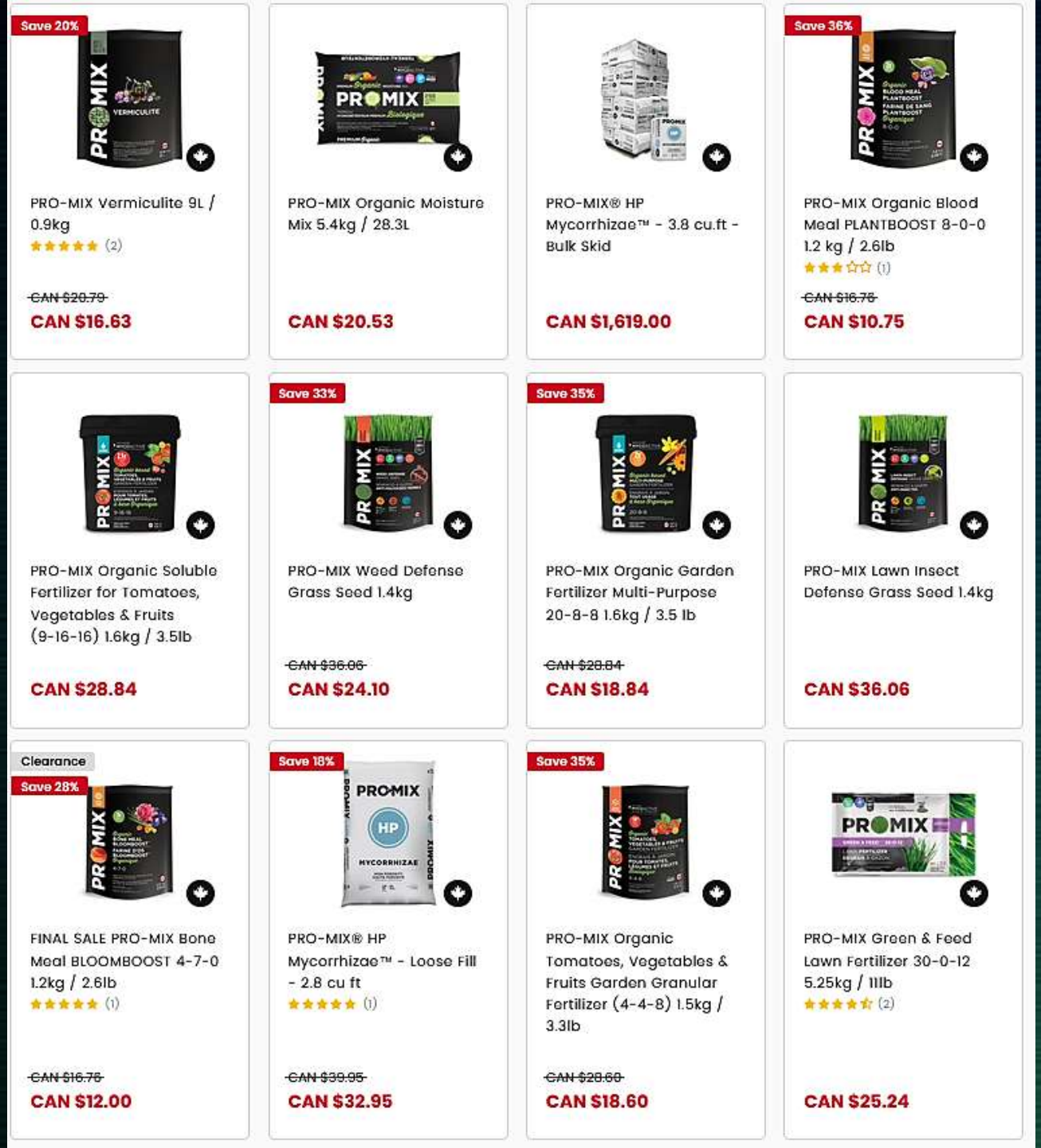
Would commercial mycorrhiza products be useful for native grassland restoration?

Glomus intraradices: 2,500 propagules/g
$119/500 g

Glomus intraradices: 6000 viable spores/g
$190 /250 g

Glomus intraradices again!

Prices from Indoor Growing Canada



AMF products

- Difficult to choose the right product
  - ✓ Similar AMF species
  - ✓ Different propagule concentration
- AMF products are expensive
- Tailored for horticulture, greenhouse and indoor cultivation.

Image source: Indoor Growing Canada

Are there practical things that can be done at industrial scales to minimize damage and promote recovery beyond current principles for minimizing disturbance?

- Ensure native grassland species are chosen.
- Adapting farm management practices to reduce agricultural spread into grasslands.
- Incentives to discourage conversion to cropland.
- Research and collaboration

Take home message

- Identification and quantification of AMF species associated with native grassland plants is essential to the grassland restoration approach.
- Site disturbance shifts AMF communities and decreases their richness.
- AMF have the potential to influence invasive plant success by enhancing their competitiveness against native plants.
- Introducing a diverse AMF community with known beneficial AMF isolates can facilitate the establishment of native prairie plants and limit the spread of undesirable/invasive species.
- AMF commercial products lack diversity of AMF species and aren't custom-made for native prairie plants, although they are excellent choices in horticulture, greenhouse crop production and indoor cultivation systems. Applying the available products to grasslands can be expensive and yield unforeseen/undesirable results.

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A longspur nest. (Photo: Melanie Toppi)

Thank you!

Questions?

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