Arbuscular Mycorrhizal Fungi in Agroecosystems:

Prospects for Reducing Nutrient Contamination of Waterways

Michelle A. Wenisch
M.S. Agronomy
Iowa State University
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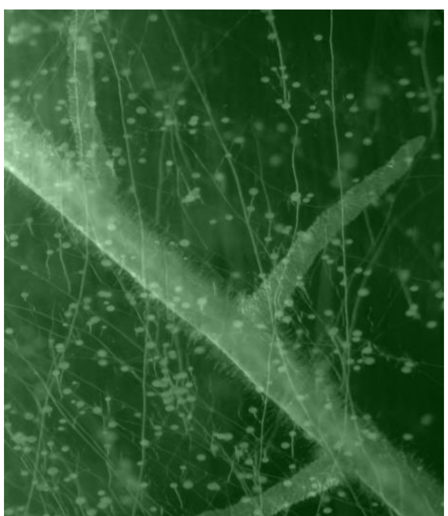
Image Source: Ian Sanders, University of Lausanne

Presentation Outline



- My Background
- Topic Selection, Introduction, Objectives
- New Approach to Soils: Soil Food Web
- Mycorrhizal Fungi & AM Fungal Biology
- Ecosystem Services Provided by AM Fungi
- AM Fungi’s Role in Reducing Nutrient Losses
- The P & N Studies: Methodologies & Outcomes
- Three Primary Lessons from the Studies
- Conclusion & Questions

My Background

- Born and raised in Michigan
- Earliest agronomy-related experiences included:

- Growing back-yard vegetables
- Riding horse through oats, field bean, maize, and alfalfa fields
- Visiting grandparents' hobby garden in Michigan's "Thumb," where enormous cabbages, cauliflowers, and other vegetables grew
- Mucking 30 horse stalls and spreading manure on fields

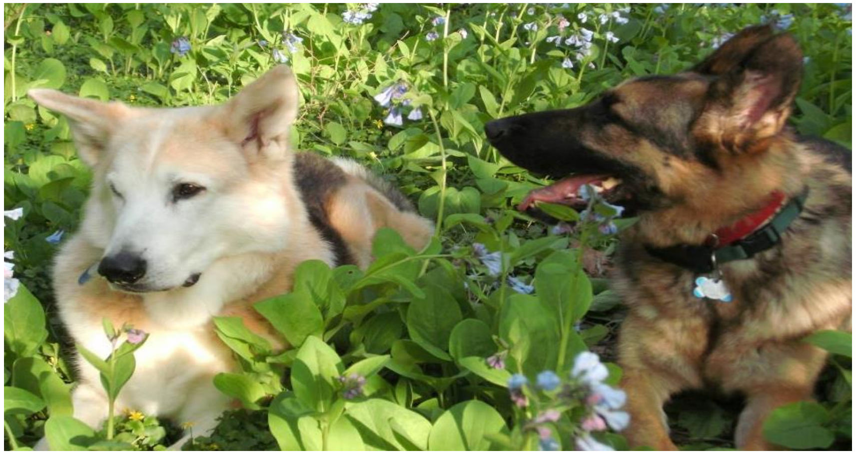
My Background

- Education & Work Experience:

  - B.S. Computer Science, University of Michigan
  - Worked as software engineer
    - Litton Guidance & Control Systems
    - Martin Marietta Astronautics
  - J.D., American U., Washington College of Law
    - Editor-in-Chief, American University Law Review
  - Worked as environmental lawyer
    - Beveridge & Diamond, PC
    - U.S. Environmental Protection Agency
      - Environmental Appeals Board
      - Office of Water

My Background

- Hobbies & Interests
  - Weed Warrior Supervisor
  - Dog Obedience Instructor
  - Butterfly Raiser
  - Master Naturalist (in training)
  - Hiker, skier, canoer, camper, equestrian, gardener
  - Dendrologist
  - Soprano, St. Patrick's Choir



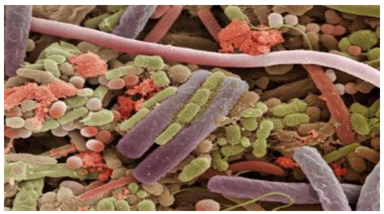
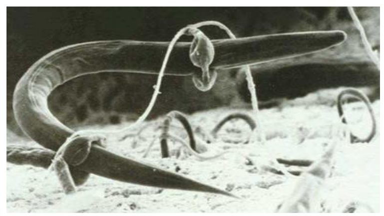
My Background

- Environmental law is inherently scientific
  - Interest always caught more by science than law
  - Questions: “**Why?**” “**How?**”

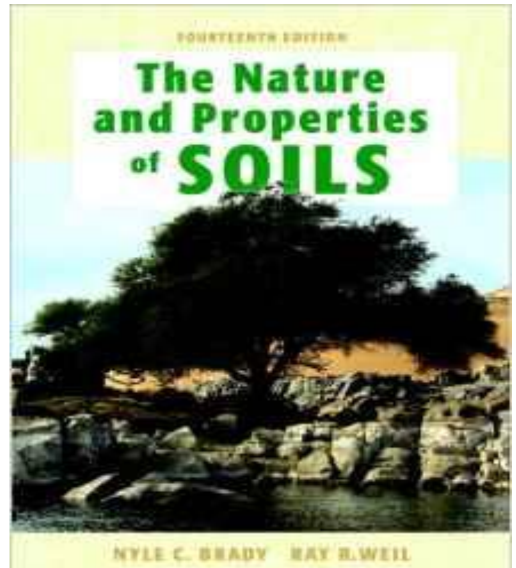
- Distance classes offered opportunity to reinvent myself yet again
- Hoping to transition to another career, this time in the natural sciences

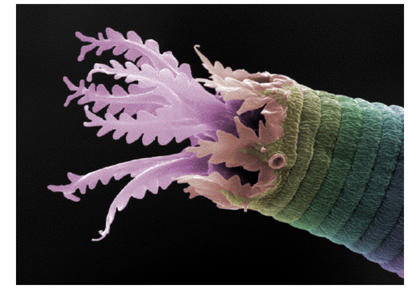
Topic Selection

- Fascinated by images of microscopic soil life

- Brady & Weil: How do they know to “hide”?



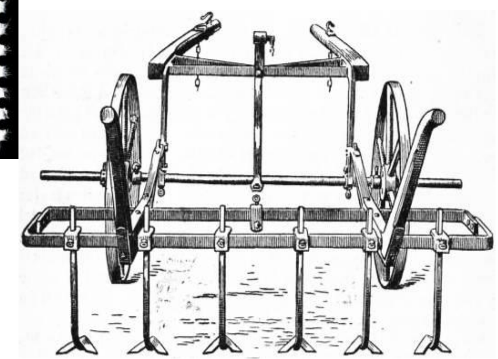


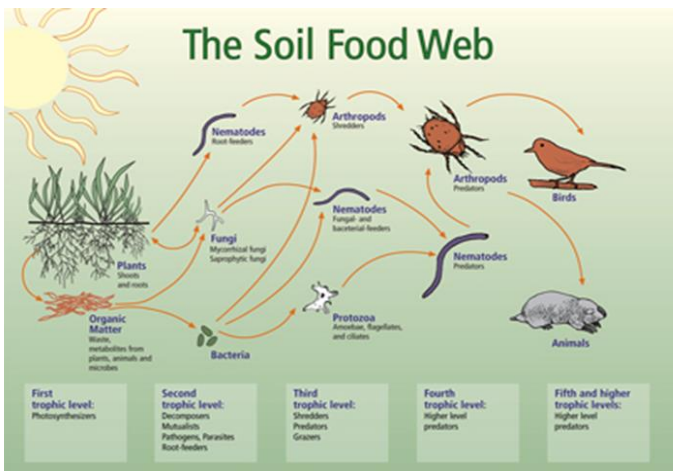

- Ray Archuleta and the aggregate stability test
- AM fungi/glomalin – superstars?!

- Worldwide water quality impairment caused by too much P and N: <u>**Can AM fungi help?**</u>

Introduction

- Soil science: a young discipline (1700s)
- Early theories of plant nutrition were based on soil chemistry/physics





- Today, new theories based on soil biology are being developed
  - Soil food web
  - Arbuscular mycorrhizal fungi: a key player
  - Soil health

Introduction

- At the same time, a **major global ecological crisis** is unfolding:
  - P and N concentrations in surface and ground water bodies **far exceed naturally occurring levels**

- Row crop agriculture is a primary source of this nutrient contamination
- <u>**Can soil biology help?**</u>

Objectives

- To understand **soil as a complex ecosystem** where biological organisms live and provide services that plants need to survive and thrive
- To learn about **arbuscular mycorrhizal fungi** and the panoply of ecosystem services they provide
- To review studies that have examined AM fungi's **capability to reduce P and N losses** to waterways
- To learn **three lessons** from the P/N studies

New Approach to Soils

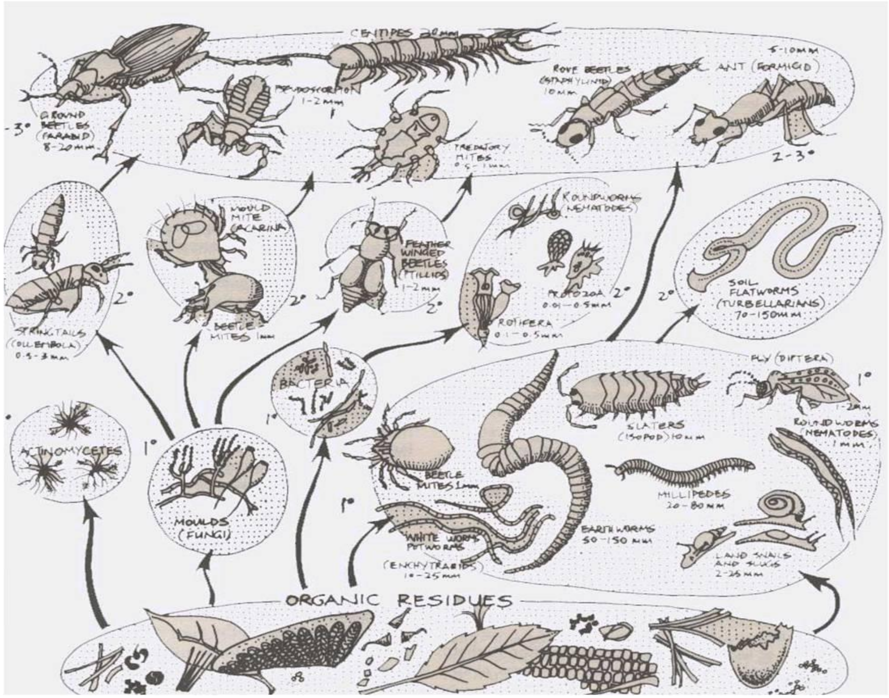
- New paradigm: A soil biology- and ecosystem-focused approach to soils leads to the concept of "**soil health**"

- **Soil health** is a soil's "continued capacity . . . to function as a vital living ecosystem that sustains plants, animals, and humans (USDA-NRCS (WV), 2016)

- Instead of viewing soil merely as "dirt" (a sterile substrate manipulated with NPK, lime, and other inputs), soil is acknowledged as a complex ecosystem where biological organisms live and provide services that plants need to survive and thrive

Soil Food Web

- Undisturbed soil teems with life forms of astonishing diversity and complexity
- All plants, including agricultural crops, depend on these life forms for their nutrition



Trophic Levels

*First: Photosynthesizers
(*Plants, Algae, Bacteria*)

*Second: Decomposers, Mutualists, Root Feeders, Pathogens & Parasites
(*Bacteria, Fungi, Nematodes, Actinomycetes, Arthropods*)

*Third: Shredders, Predators & Grazers
(*Arthropods, Earthworms, Nematodes*)

*Fourth & Above: Higher-Level Predators
(*Larger Arthropods, Nematodes*)

(Alexander, 1991; Ingham et al., 2000)

Mycorrhizal Fungi

- Of these many life forms, **mycorrhizal fungi** are particularly important to plants

- Mycorrhizal fungi are **"mutualists"** that colonize plant roots in a symbiotic association between fungus and plant
- Fungi swap essential soil nutrients for plant-produced photosynthetic carbon compounds
- The structural association between plant and fungus is called "mycorrhiza," meaning "**fungus root**"

Mycorrhizal Fungi

- Mycorrhizal fungi are **ubiquitous** in natural soils:
  - ~ **90%** of all known vascular plants
  - ~ **80%** of all known terrestrial plants

    (Nichols and Millar, 2013; Willis et al., 2013)

- Virtually all natural terrestrial ecosystems are dominated by mycorrhizal plants
  - nonmycorrhizal plants = uncommon
- Four major types:
  (1) ecto
  (2) endo
  (3) ericoid
  (4) orchid
- AM fungi, in phylum Glomeromycota, are **endomycorrhizal**
  - By far the most common mycorrhizal type, with 1-3 classes, 4-5 orders, 11-14 families, and 18-26 genera

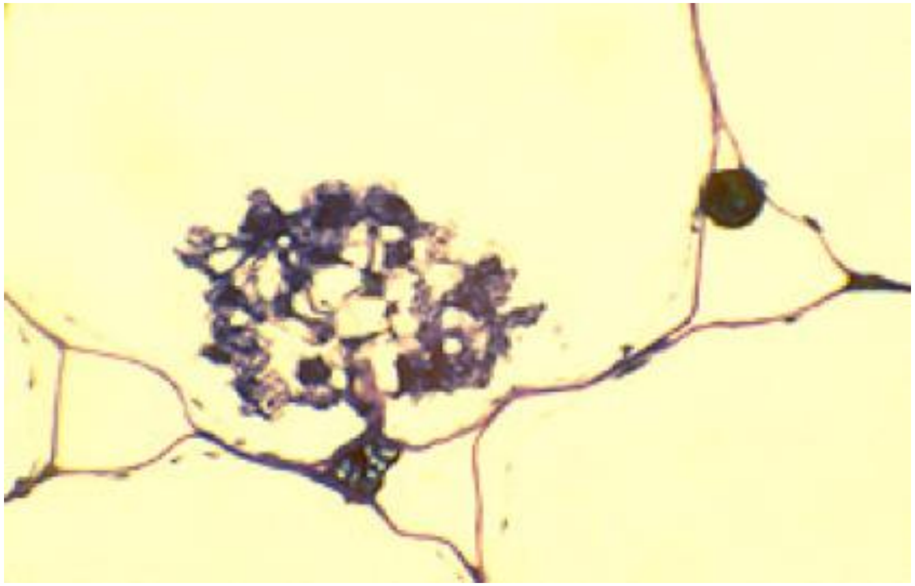
    (Smith and Read, 2008; Stürmer, 2012; Willis et al, 2013; Bagyaraj et al., 2015)

Arbuscular Mycorrhizae

- In agronomic and horticultural crops, **arbuscular mycorrhizal fungi** are the predominant symbiont
  - Virtually all crops associate with AM fungi
  - Exceptions: Plants in the *Brassicaceae* (broccoli, cabbage, canola, kale) and *Chenopodiaceae* (beets, spinach, chard) families

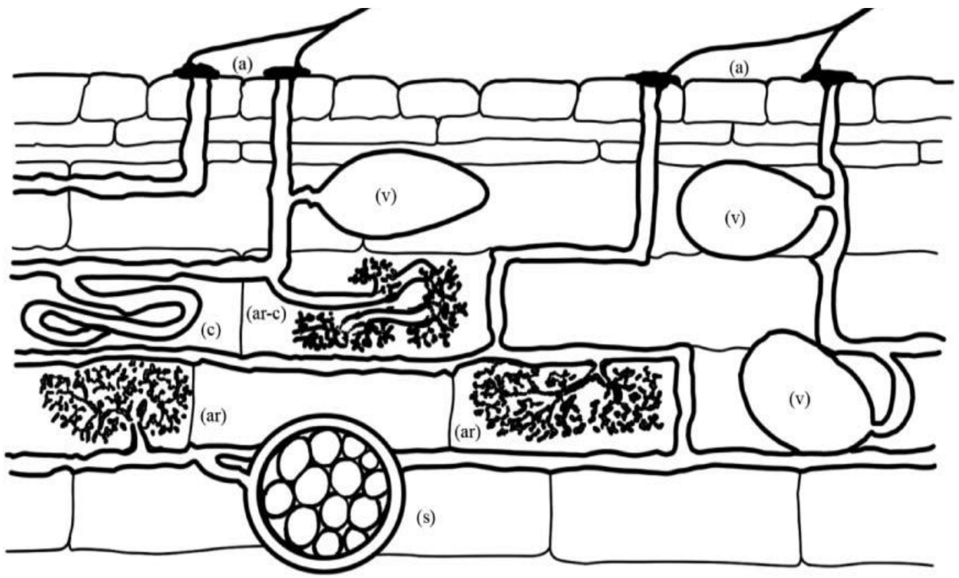
- AM fungi build *arbuscules*, tiny tree-like structures inside root cells, where fungi exchange minerals collected from soil for plant-produced carbons

- Plants offer **<u>10-20% of their photosynthates</u>** for AM fungal services



Intraradical Morphology

- AM fungal structures inside root cells include hyphae, vesicles, and/or spores
- Intraradical fungal morphology varies:
  - Two distinct types: ***“Arum*-type”** and ***“Paris*-type”**
  - Many gradations or **“intermediate” types** in between



Paris-type *Arum*-type

•*Arum*-type hyphae confined primarily to intercellular spaces; form arbuscules
•*Paris*-type hyphae grow intracellularly; form *arbusculate coils*

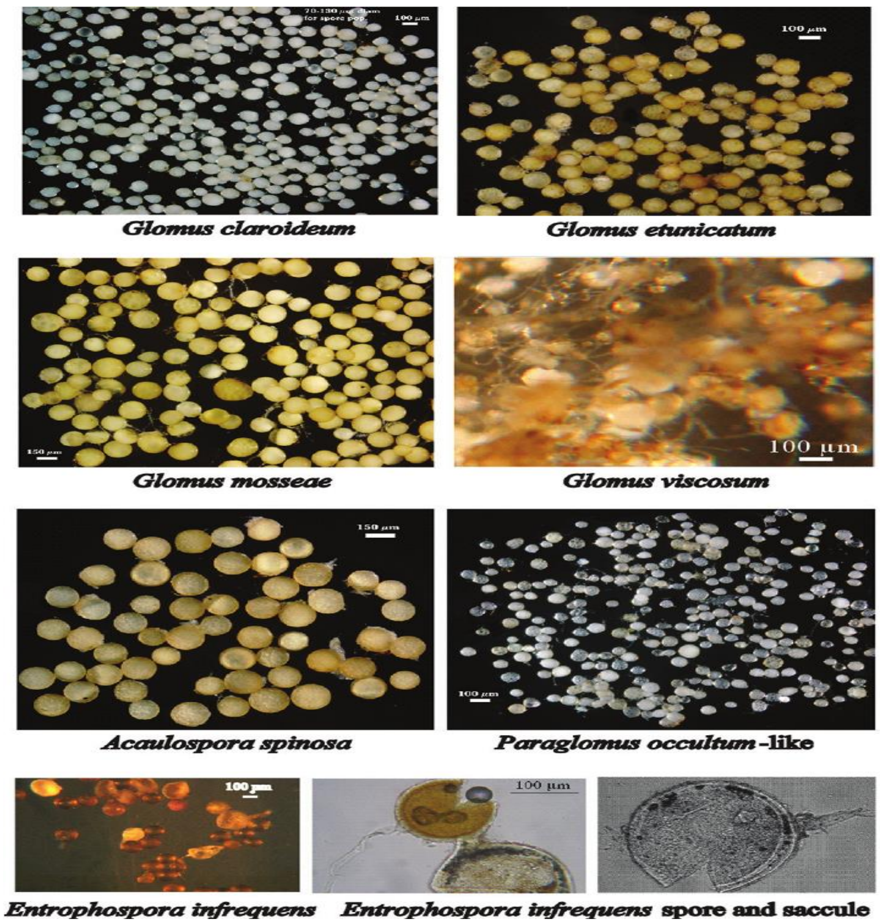
(Smith and Read, 2008; Marschner, 2012; Willis et al., 2013)

Extraradical Morphology

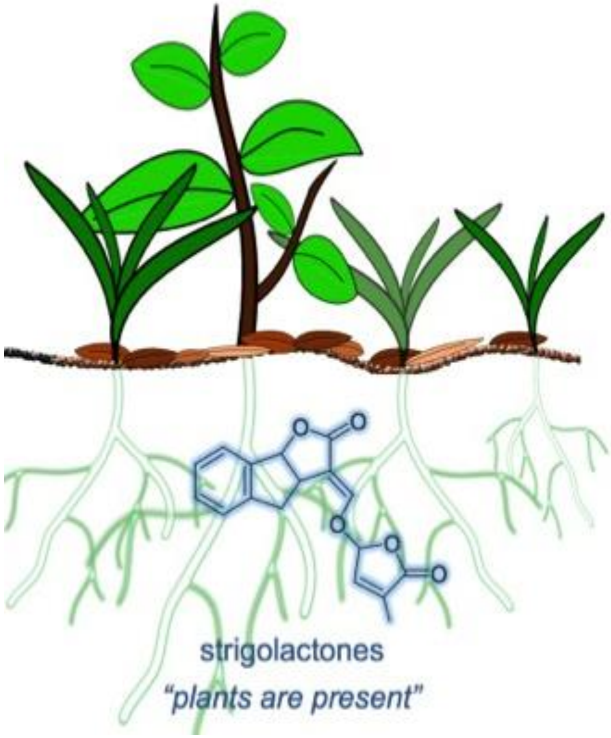
- AM fungal structures outside root cells include **extraradical hyphae** and **spores**
- Extraradical hyphae have short lifespans (5-6 days) (Neumann and Römheld, 2012)
- Hyphae extend **15 cm or more** into soil matrix (Douds and Millner, 1999; Jansa et al., 2003; Smith and Read, 2008; Martin et al., 2012)
- Spores are large, with distinctive colors and morphological features

AM Fungal Propagules

- Propagules (or inocula) exist in soil in three forms:
  - Spores
  - Extraradical hyphae
  - Colonized roots
- Most spores (70-85%) are found in top 16-18 inches of soil profile, though some are as deep as 7.2 feet (Douds and Millner, 1999; Troeh and Loynachan, 2009)



Chemical Signaling

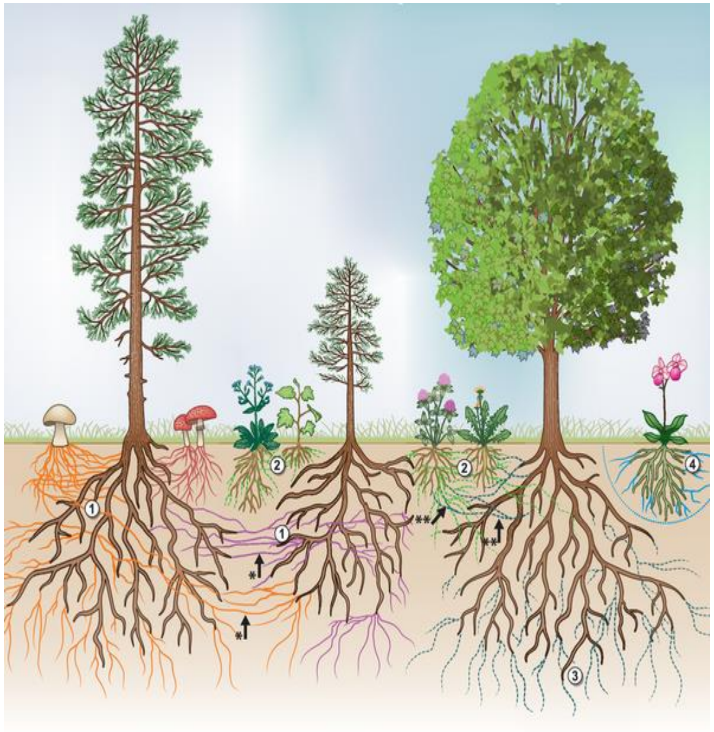


- Plants invite colonization by exuding **strigolactones** into the rhizosphere (Xie et al., 2010; Andreo-Jiminez et al., 2015)
  - Each plant species/cultivar exudes a different blend of phytohormones
- AM fungi respond by releasing **lipochitooligosaccharides**, and plants then allow them to take up residence in their roots

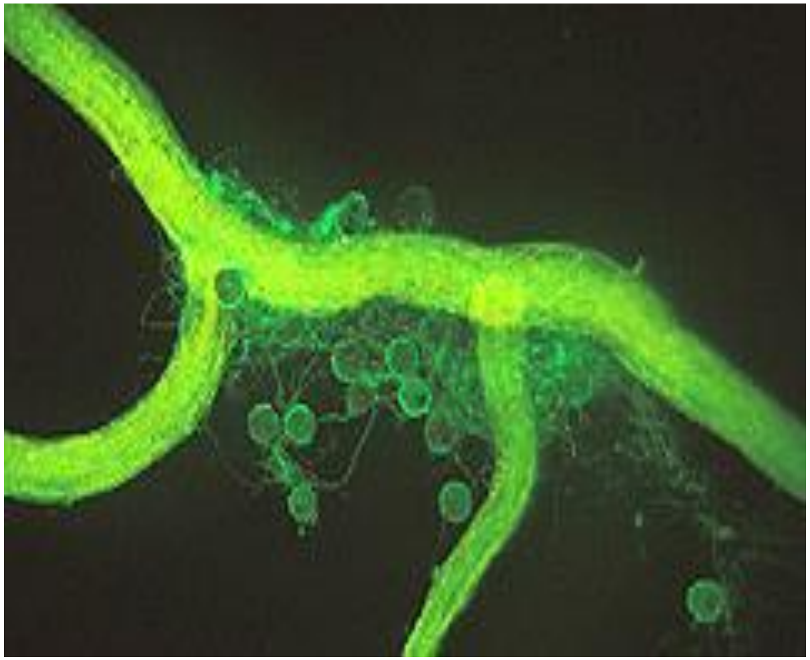
- Plants with sufficient P may choose ***not*** to invite colonization
- Some common agronomic crop cultivars are losing their inherent ability to associate with AM fungi

Underground Networks

- Different AM fungal species occupy different ecological niches, so plants are colonized by multiple species
- Fungal hyphae weave large, interconnected networks underground
  - Link neighboring plants to each other in **common mycelia network** of hyphal connections
  - May allow for chemical signaling
  - May allow for inter-plant transfers of carbon and nutrients
- Mycorrhizal networks encourage rapid seedling establishment



Glomalin



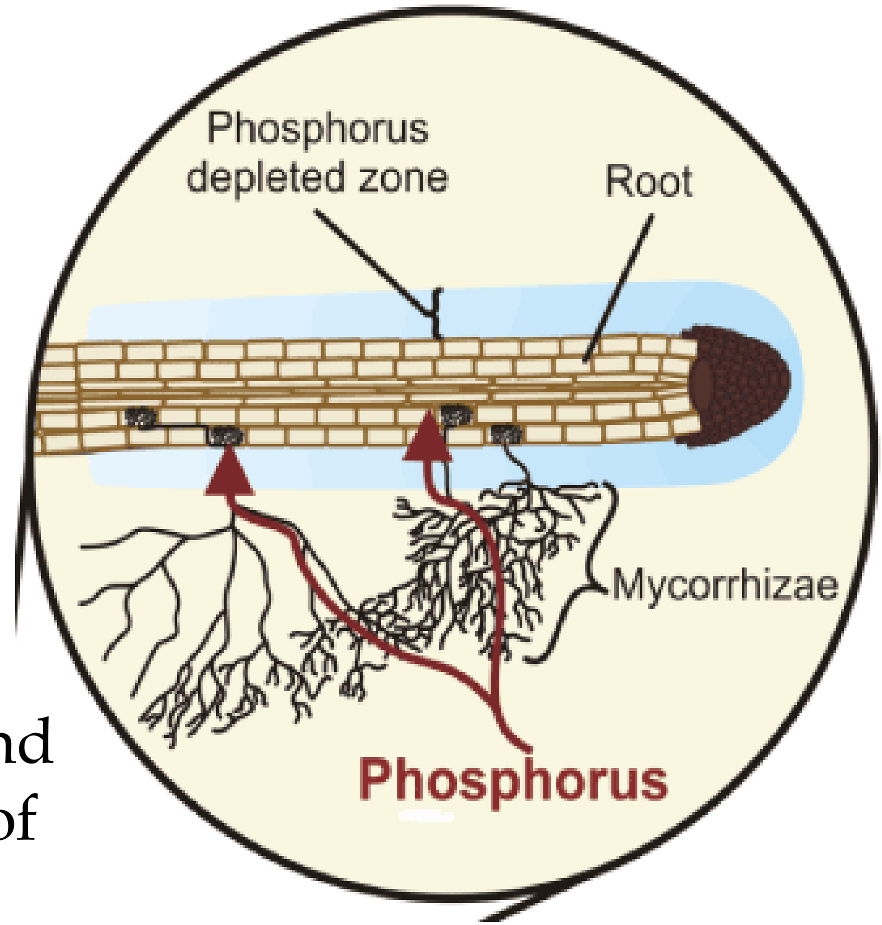
- AM fungi use the carbon they receive from host plants to produce "glomalin," a sticky, water-resistant, durable glycoprotein that protects fragile fungal hyphae and reproductive structures (Wright et al., 2000; Rillig et al., 2002; Rillig, 2004; Driver et al., 2005)
- Glomalin accumulates to high levels in soils (2-35 g/kg) (Weil and Magdoff, 2004)
- Not yet biochemically defined: quantified as glomalin-related soil proteins ("GRSP") (Rillig, 2004; Bedini et al., 2009; Wilson et al., 2009; Burrows, 2014)

Aggregate Stabilization

- Numerous studies have established that AM fungi play a central role in forming and stabilizing soil aggregates
- Extraradical hyphae enmesh soil and organic matter particles into larger aggregates
- Glomalin is "**recalcitrant**" (resistant to degradation) and **sticky**; it **keeps aggregates intact**, even when they are subjected to severe destructive forces
- AM fungi may produce more glomalin in poorly aggregated soils

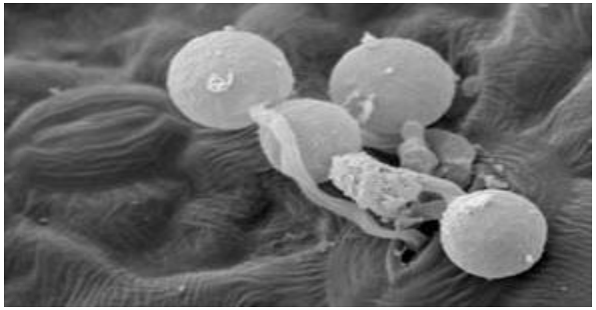
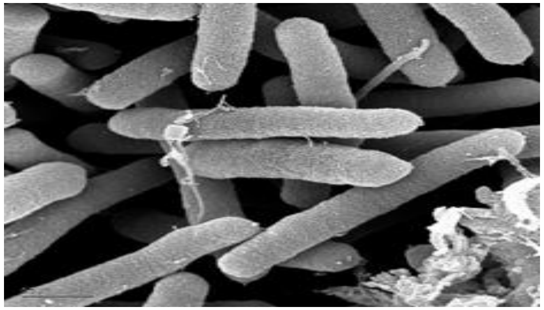
Nutrient Acquisition

- Plant's below-ground surface area is greatly enlarged by AM hyphae, **increasing its nutrient acquisition capability**
- Distant soil-immobile nutrients (**P, Zn, Cu**) become accessible
- Plants **absorb more N**, receive better nutrition, and produce higher amounts of biomass and yields



Disease Suppression

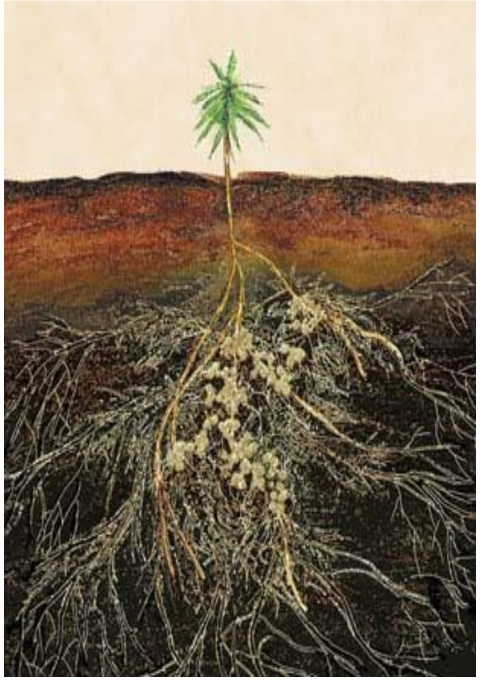
- Many studies have established that AM fungi have **suppressive effects** on **pathogenic soil organisms**
  - Outcompete disease organisms for colonization sites on plant roots ("occupied territory")
  - Mobilize plant defenses to antagonize pathogens
  - Improve plant nutritional status, allowing for compensatory growth
- New research also showing helps control insect pests


Drought Protection

- AM fungi improve plant tolerance of significant abiotic stressors
  - Drought
  - Salinity
- Some scientists credit enhanced tolerance as indirect effect of superior nutrition afforded by mycorrhizal symbiosis
- Others credit direct effects wrought by AM fungi on plant physiology (e.g., root hydraulic properties, stomatal conductance, stress hormone levels)
- Others point to improved soil structure and water storage created by AM fungi and glomalin

Carbon Sequestration

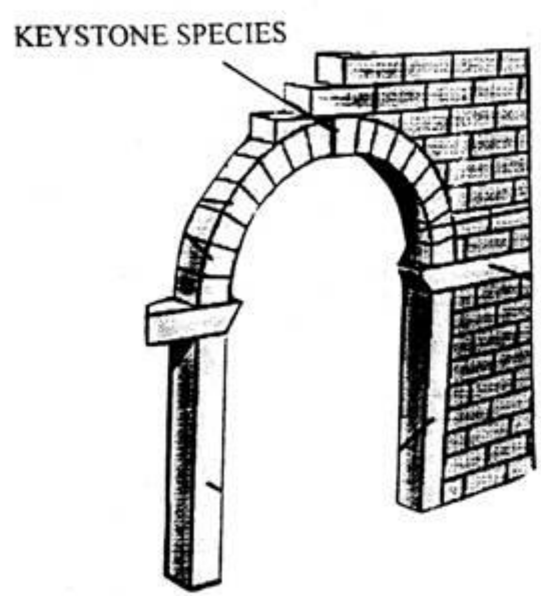


- Agricultural soils have lost one-third to three-quarters of original soil carbon
- AM fungi reverse this trajectory in two ways:
  - -- Stable soil aggregates produced by glomalin **protect organic matter, and carbon it contains**, from rapid microbial decomposition and loss to atmosphere as CO_2
  - -- Glomalin itself contains **10-35%** carbon and accounts for roughly **one- to two-thirds of all carbon in soils**
    (Treseder and Allan, 2000; Weil and Magdoff, 2004)
- If undisturbed, glomalin can persist in soil for **decades**
  (Wright et al., 2000; Willis et al., 2013)
- Sequestered carbon improves soil health and can help offset anthropogenic carbon dioxide emissions

Keystone Species

- Accumulated research has established AM fungi as likely "keystone species"

- Though microscopic in size, they have the ability to shape their environments and affect large-scale ecosystem functionality
  - Benefits themselves, their host plants, and associated above- and below-ground biomes and microbiomes



- Loss of keystone species has enormous adverse consequences for their native ecosystems

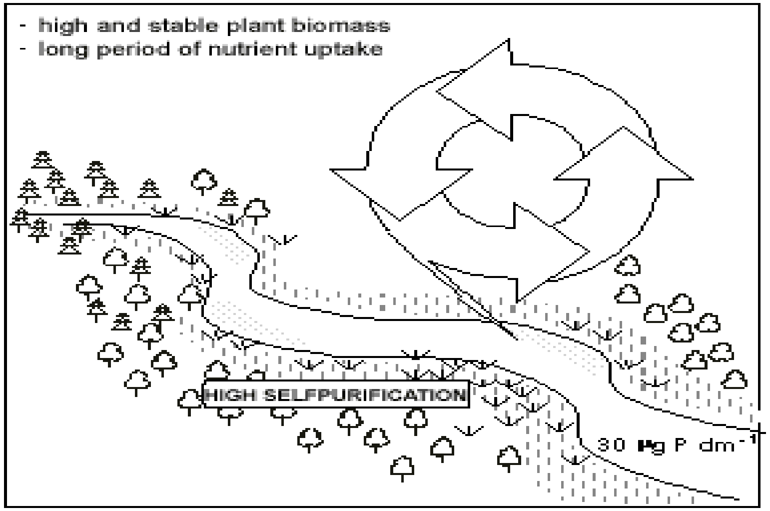
Role in Nutrient Retention

- As noted above, many ag soils have lost substantial percentages of their C content in past decades (Lal and Kimble, 1997; Douds and Millner, 1999; Weil and Magdoff, 2004; Lal et al., 2007; Kibblewhite et al., 2008; Sperow, 2016)

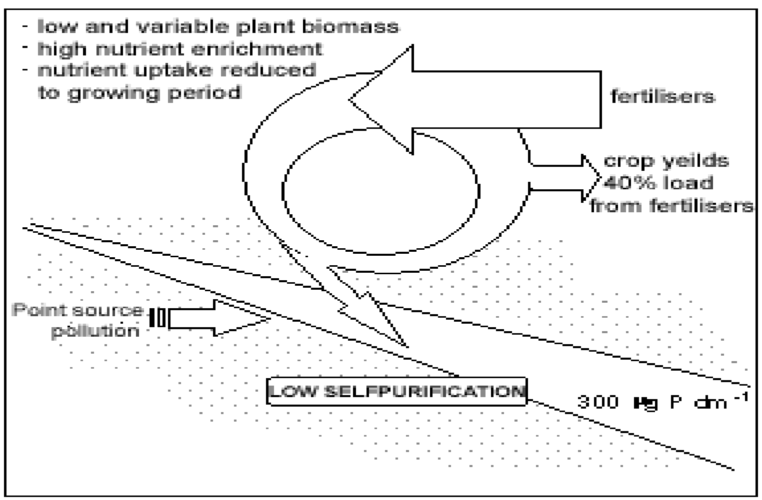
- They also have **lost AM fungi diversity and abundance** (Verbruggen et al., 2010; Lehman et al., 2012; Köhl et al., 2014; Schneider et al., 2015)

- One of many possible significant consequences: AM communities may be less able to prevent nutrients from leaching into waterways

Closed vs. Open Systems



- In undisturbed ecosystems, P and N are retained on-site rather than leached, eroded, or otherwise lost
  – "Closed" nutrient cycle



- Today's conventionally farmed agroecosystems routinely (if inadvertently) transfer nutrients from soil to aquatic ecosystems
  – "Open" nutrient cycle

Nutrient Overenrichment

- Excess P drives eutrophication of fresh water bodies
  - Algal blooms
  - Reduced dissolved oxygen levels
  - Biodiversity loss
  - Impaired water quality
- Excess N drives eutrophication of brackish/saline water bodies

The Studies

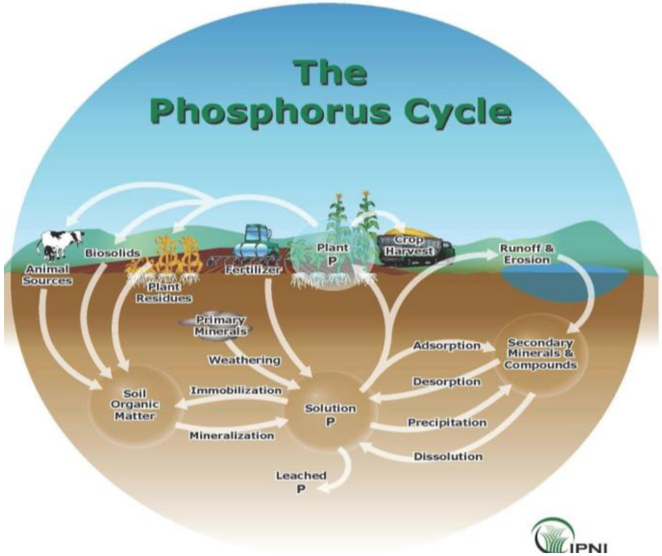
- AM fungi's role in forestalling adverse environmental and biodiversity impacts caused by P/N leaching is poorly understood
- Small groups of scientists in Switzerland, Netherlands, Australia, Iran, and other countries have begun investigating AM fungal effects on nutrient leaching




- Their research hypothesizes that AM fungi can reduce leaching of P and N to waterways
- 13 studies reviewed: 10 P studies & 11 N studies

Phosphorus

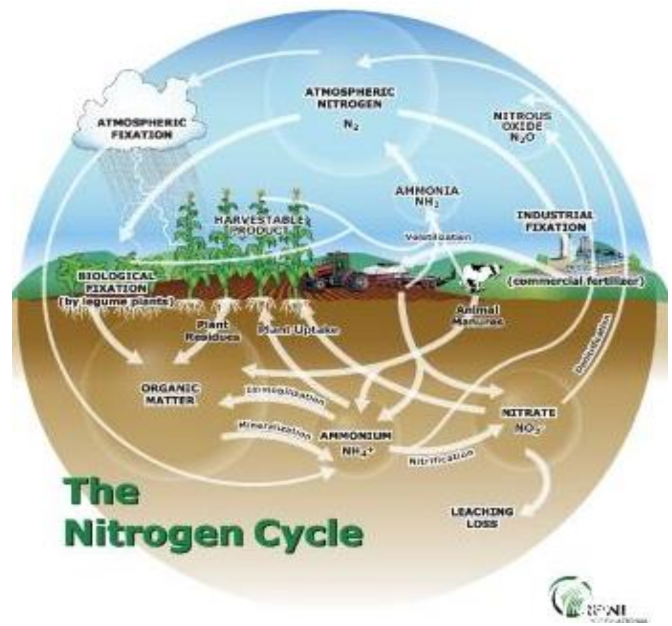
- Essential plant nutrient, but highly chemically reactive in soils
- Global stocks depleted by 2050?
- Forms and availability in soils:
  - Organic PO_4^{3-} (phytates, fatty acids)
  - Inorganic PO_4^{3-} (3 pools)
  - Soil solution pool available to plants



- Forms leached from soils:
  - Dissolved reactive P (soil solution & organic PO_4^{3-} forms)
  - Dissolved unreactive P (nucleotides, polyphosphates) (64-90%)
  - Total particulate P (P sorbed onto clays, metal oxides)
- Even small quantities of P can trigger eutrophication

Nitrogen

- Essential plant nutrient; N_2 is 78% of atmospheric gases
- N assumes diverse forms as is added, transformed, and lost in continual cycle through soil-plant-atmosphere continuum
- Forms and availability in soils:
  - Organic N (in plant, animal, microbial tissues): 95-99%
  - Inorganic NH_4^+, NO_2^-, and NO_3^- : 1-5%
  - NH_4^+ and NO_3^- are available to plants
- Forms leached from soils:
  - Dissolved inorganic NH_4^+ and NO_3^-
  - Dissolved organic N (64-97%)



Study Methodologies

- Soils sterilized with steam heat, autoclaving, or gamma radiation to kill indigenous microbial life
- Filtered washes of indigenous microbial life (without AM fungal propagules) readded
- Desired AM fungal species and plant species added
  - Many permutations in experimental design
- Fertilizers added and leachate collected
  - P and N forms and quantity in leachate measured
- AM fungi versus non-AM controls compared

Study Materials & Methods

(1) Haines and Best, 1976 (N only)

-- North Carolina forest soil (Ultisol?) with A, B, C horizons; steam sterilized

-- Small microcosms (15 cm W, >25 cm H) with 5 treatments

-- Sweetgum seedlings, soil biota filtrate, and *Funneliformis mosseae*

(2) Asghari et al., 2005 (P only)

-- Australian Calcarosol (Aridisol) mixed with sand; sterilized

-- Small microcosms (7.5 kg, 15 cm W, 40 cm H) with low/high P treatments

-- Clover, *Rhizobium*, and *Rhizoglomus irregulare*

(3) van der Heijden, 2010 (both P and N)

-- Dutch alluvial Fluvisol (Entisol) (mostly coarse sand); sterilized

-- Small microcosms (1.95 kg, 17 cm W) with high/low fertilizer treatments

-- 3 grass species, soil biota filtrate, and *Rhizoglomus irregulare*

(4) Asghari and Cavagnaro, 2011 (both P and N)

-- Australian Chromosol (Alfisol) mixed with sand; half sterilized, half not

-- Small microcosms (1.8 kg, 9 cm W, 30 cm H) with pulsed fertilizer treatments

-- Riparian grass species, soil biota filtrate (half), and indigenous AM fungi (species unspecified)

Study Materials & Methods

(5) Corkidi et al., 2011 (both P and N)

-- Soilless mix of bark, sawdust, clay, sand; sterilized
-- 1-gallon nursery pots and 3 fertilizer treatments
-- Woody perennials (CA sunflower, lemonade sumac) and *Glomus* species

(6) Asghari and Cavagnaro, 2012 (N only)

-- Australian Chromosol (Alfisol) mixed with sand; *not* sterilized
-- Small microcosms (1.8 kg, 9 cm W, 30 cm H) and pulsed fertilizer treatments
-- Mutant (mycorrhiza-defective, as non-AM control) and normal tomato cultivars, indigenous AM fungi

(7) Verbruggen et al., 2012 (P only)

-- Dutch field soil (15%) plus sand (85%) mixture; sterilized
-- Small microcosms (2.7 kg, 12 cm W, 11.5 cm H) inoculated with organically or conventionally managed Dutch maize field soil and fertilizer added
-- Maize and indigenous soil biota, including AM fungi

(8) de Vries et al., 2013 (N only)

-- Field experiments in four countries: Czech Republic, Greece, Sweden, UK
-- 60 test sites and 3 treatments: high-, medium-, low-intensity land use
-- Intact soil food webs

Study Materials & Methods

(9) Köhl et al., 2014 (both P and N)

- Soil community inoculum prepared by collecting tilled and no-tilled Swiss Cambisols (Inceptisols) from above/below plow layer; incubated 20 months
- Isolated AM fungal spores by wet sieving and centrifugation; identified 29 species and counted number of each
- Small microcosms (8.6 kg, 15 cm W, 40 cm H) with sterilized soil
- Clover/plantain/grass mix, soil biota filtrate, and AM fungi from differing depths and tilled or no-tilled soils

(10) Wagg et al., 2014 (both P and N)

- Increasingly simplified soil community inoculum prepared by collecting native soils and filtering them through sieves of successively smaller size
- Small microcosms (6 kg, 23.5 cm W, 12 cm H) with sterilized soil
- European grassland mix (10 legume, grass, forb species)

(11) Bender et al., 2015 (both P and N)

- Swiss and German Cambisols (Inceptisols) mixed with sand; gamma irradiated
- Small microcosms (7 L, 15 cm W, 40 cm H) and fertilizer
- Italian ryegrass, soil biota, and 3 AM fungal species common in Swiss soils (*F. mosseae, R. irregulare, Claroideoglomus claroideum*)

Study Materials & Methods

(12) Bender and van der Heijden, 2015 (both P and N)-

-- Swiss Cambisol (Inceptisol) mixed with sand; gamma irradiated

-- Large outdoor lysimeters (230 L, 59 cm W, 84 cm H) inoculated with either "enriched" (AM fungi + other species) or "reduced" (only organisms smaller than AM fungi) soil life inoculum

-- Two-year maize/wheat rotation

(13) Köhl and van der Heijden, 2016 (both P and N)

-- Swiss Cambisol (Inceptisol) mixed with sand; sterilized

-- Small microcosms (9.25/3.5 kg, 15/16 cm W, 40/19 cm H)

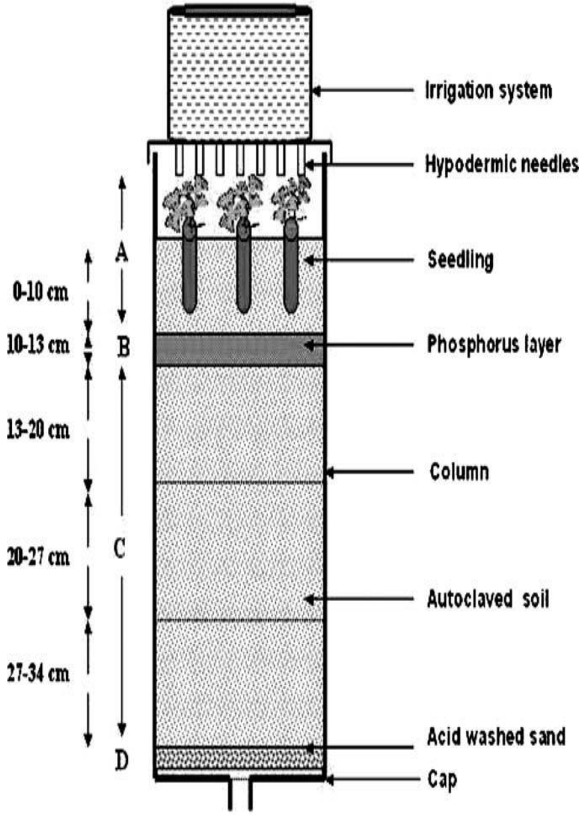
-- Italian ryegrass/red clover, soil biota wash, 4 AM treatments (*F. mosseae, R. irregulare, C. claroideum*, or none)

Study Methodologies

- Reviewed 10 greenhouse microcosm, 1 outdoor lysimeter, 1 field, and 1 nursery pot experiments

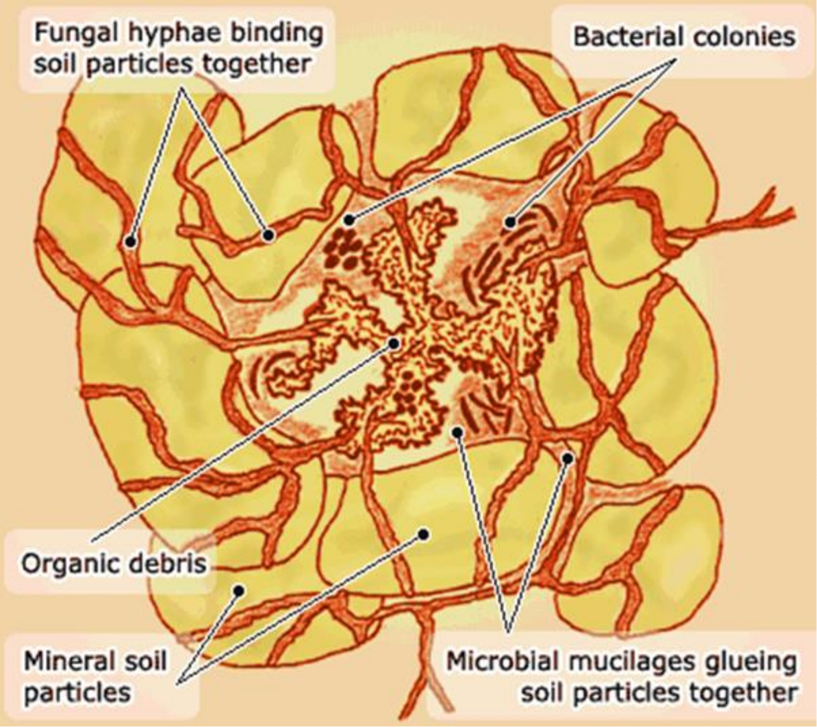
- Microcosm pot sizes ranged from:
  - 1.8 to 9.25 kg weight
  - 9 to 23.5 cm diameter
  - 11.5 to 40 cm depth

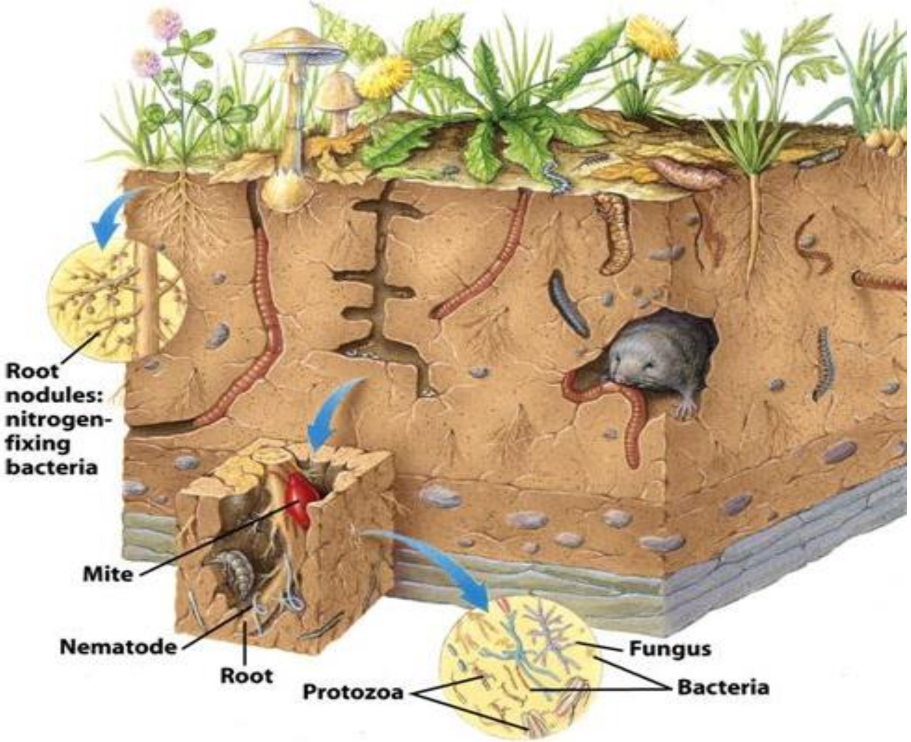
- Lysimeter sizes
  - 230 liter volume
  - 59 cm diameter
  - 84 cm depth



Study Methodologies

- Native soils collected and mixed with sand
- Soil structure (homes!) largely destroyed in most studies
  - Exceptions: field study, mutant tomato study





Phosphorus Study Outcomes

- Varied results, but **8 of the 10 P studies** demonstrated that **AM fungi reduced P leaching losses**

- Total dissolved P leaching reduced by 31 to 73%

- Dissolved reactive P (PO_4^{3-}) leaching reduced by 60%

- Dissolved unreactive P reduced 24% (this form comprised 64 to 90% of leaching losses, so important)

- Relative P leaching losses (grams leached per kg of plant uptake) reduced by 25%

Phosphorus Study Outcomes

- **Significant negative correlation** between **extraradical hyphae density** and **total dissolved P leached**
- AM fungal species differ in their effects on P leaching
- As sieve sizes decrease, AM fungi are eliminated from soil communities; plant species diversity and production decline and P leaching losses increase
- AM fungi contribute to **ecosystem sustainability** by **promoting a closed P cycle** and **reducing P leaching losses**
- **High nutrient losses** from intensively managed fields may partly result from **disruptions of soil food web**

Nitrogen Study Outcomes

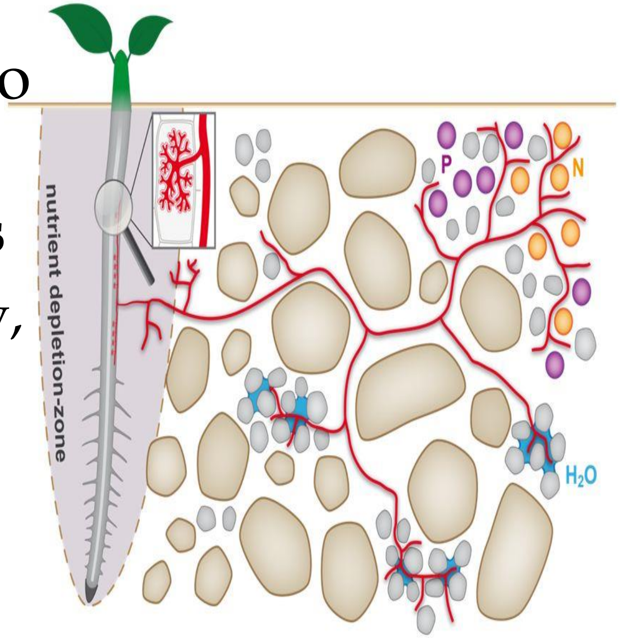
- Varied results, but **10 of the 11 N studies** demonstrated that **AM fungi reduced N leaching losses**

- Nitrate leaching reduced by 37 to 98%
- Ammonium leaching reduced by 8 to 88%
- Dissolved organic N reduced 24%
- Relative N leaching losses (grams leached per kg of plant uptake) reduced by 36%

Nitrogen Study Outcomes

- **Largest nitrate leaching reduction (98%)** reported in mutant tomato study, with **intact soil communities**
- Field study reported that **nitrogen leaching decreased with increasing biomass of AM fungi**
- AM fungi are **"ecologically significant"** as **"nitrogen conservers"**
- As soil community diversity is reduced, normal decomposition processes (e.g., N immobilization and mineralization) decline, resulting in increased N_2O emissions
- **High nutrient losses** from intensively managed fields may partly result from **disruptions of soil food web**

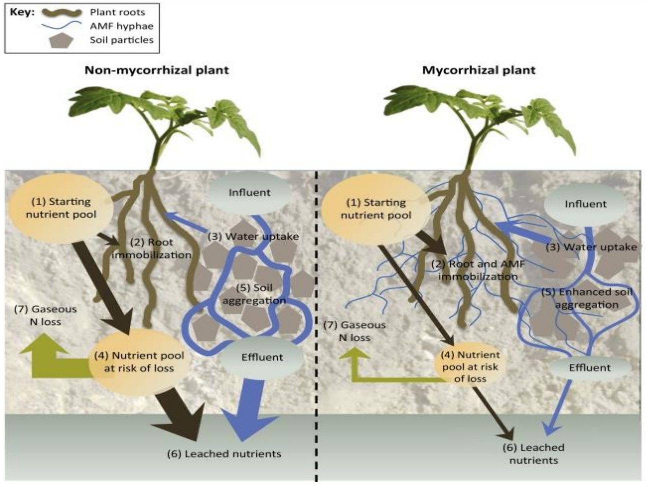
Lessons from the Studies -- #1



- AM fungi are exceptionally difficult to study; thus **large variations in experimental methods and materials**
  - Pot size, soil structure/texture/chemistry, soil sterilization, soil/plant community composition, size, diversity, etc.
  - "Strong perturbations" of soil ecosystem can cast doubt on experimental results
- Heroic attempts to simulate "home turf" (i.e., soil food web populations, soil structure) and the continual quest for a better way
- Soil texture (mostly sand): What about preferential flow losses of P?

Lessons from the Studies -- #2

- Together, the studies establish that AM fungi play a **significant role in reducing P and N leaching losses**
- Different AM fungal species may have **different abilities** to prevent leaching losses
- Possible mechanisms:
  - Exploration of larger soil volume
  - Larger plant sizes/root spread
  - P/N storage in fungal biomass
  - P/N bound in soil aggregates
  - AM fungi-engineered soil water retention improvements
  - Soil community shifts lead to more nutrient mobilization
  - P/N storage in soil food web biomass



Lessons from the Studies -- #3

- Practical steps to encourage AM fungal colonization of crop fields include:
  - Choose crop cultivars that strongly associate with AM fungi
  - Slowly reduce fertilizer inputs, especially P
  - Eliminate/reduce tillage
  - Eliminate fallowing
  - Plant cover crops
  - Increase crop diversity
  - Reduce fungicide use
  - Plant permanent strips of diverse, highly mycorrhizal native perennial plants (trees, shrubs, forbs, grasses) in/around crop fields and waterways

- Takes time to rebuild functional soil food web

Conclusions

- For most of soil science history, researchers focused on soil chemistry/physics; very few interested in soil biology (Darwin)
- New research is changing this paradigm

- The 13 studies suggest that, to the long list of ecosystem services AM fungi provide, **nutrient leaching reduction** can be added

- **<u>Policy goal</u>: Encourage use of crop production methods that foster AM fungal diversity and soil health**

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Questions?

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