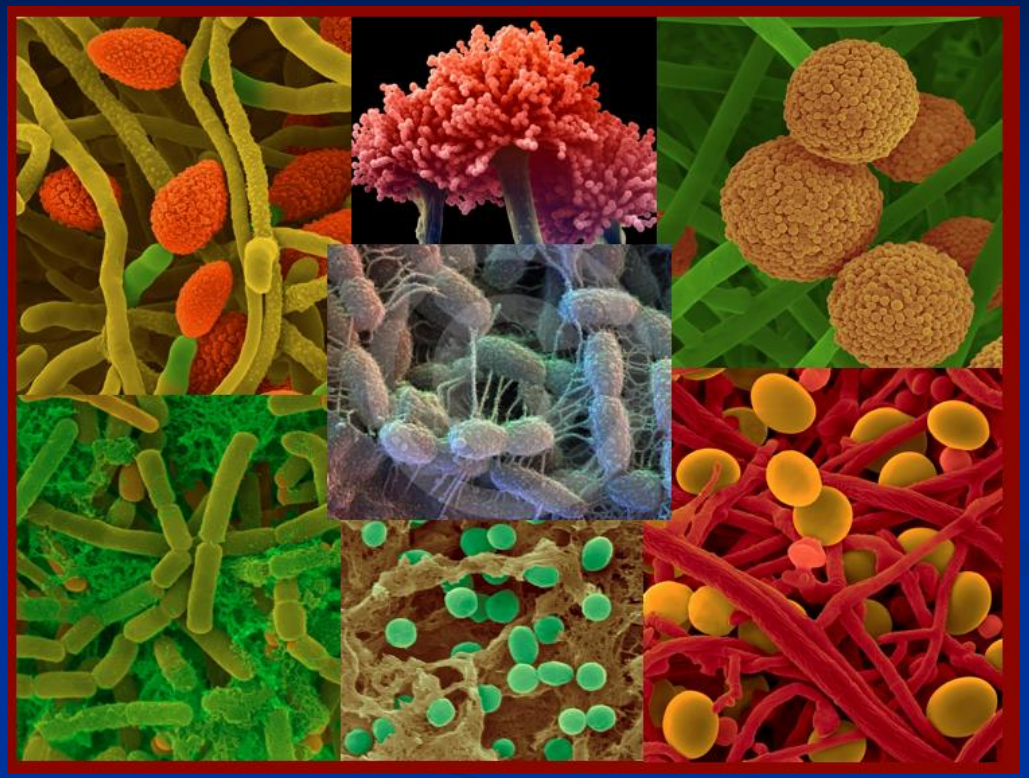
Biology Lab 2
Assessing fundamental symbiotic relationships between plants and soil microorganisms

Soil biodiversity

- Soil probably harbours most of our planet's undiscovered biodiversity (Tiedje et al., 1999)
- 1 tbsp. soil contains more microbes than there are people on earth
- "Microbial life can easily live without us; we, however, cannot survive without the global catalysis and environmental transformations it provides (Falkowski et al, 2008"



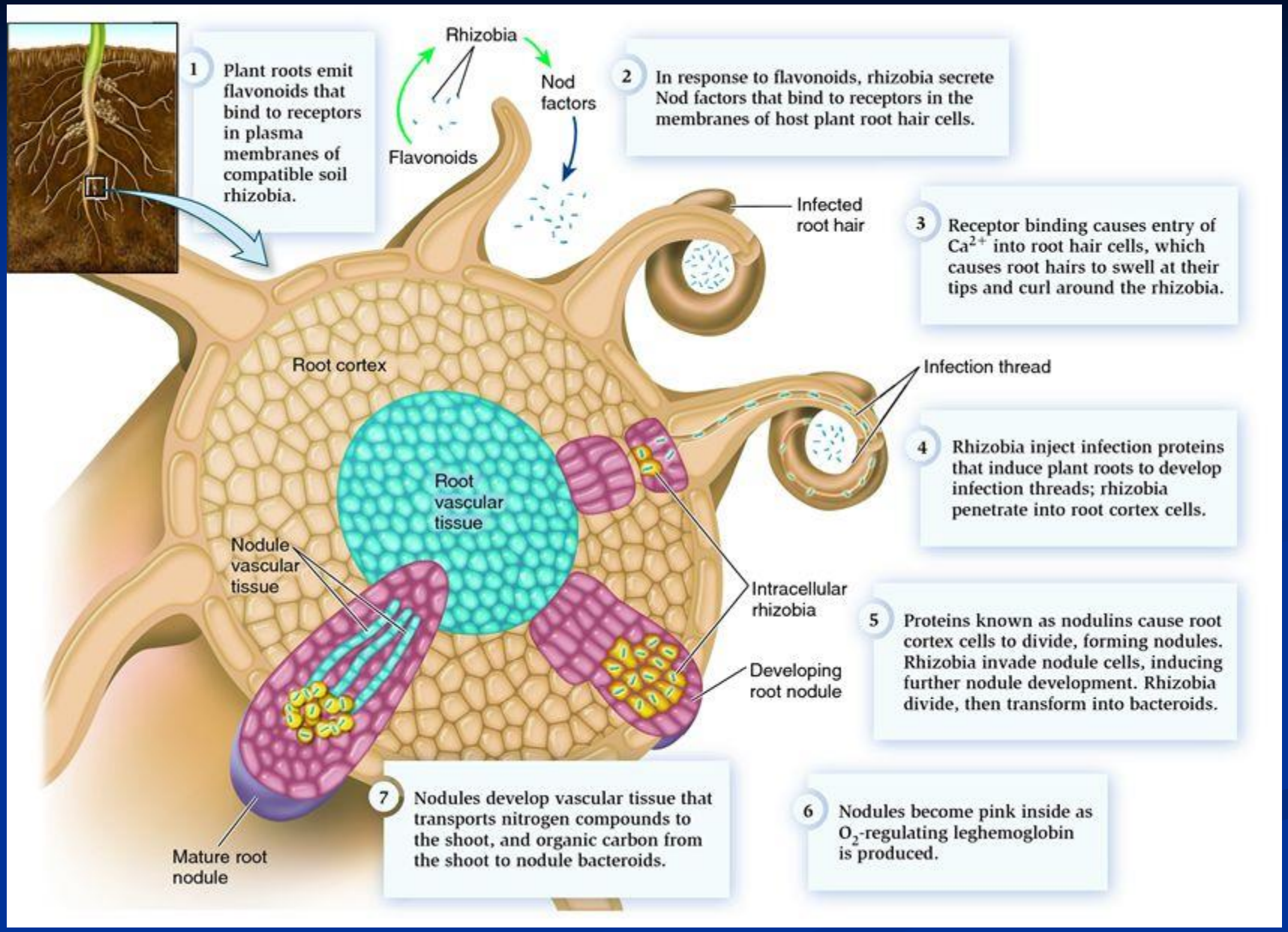
- **Microorganisms can be free-living or in symbiosis**
- **Key symbiotic associations between plants & microbes**
  - **N_2-fixing nodules (plants and bacteria)**
  - **Mycorrhizae (plants and fungi)**

Symbiotic bacteria
Non-leguminous nodules

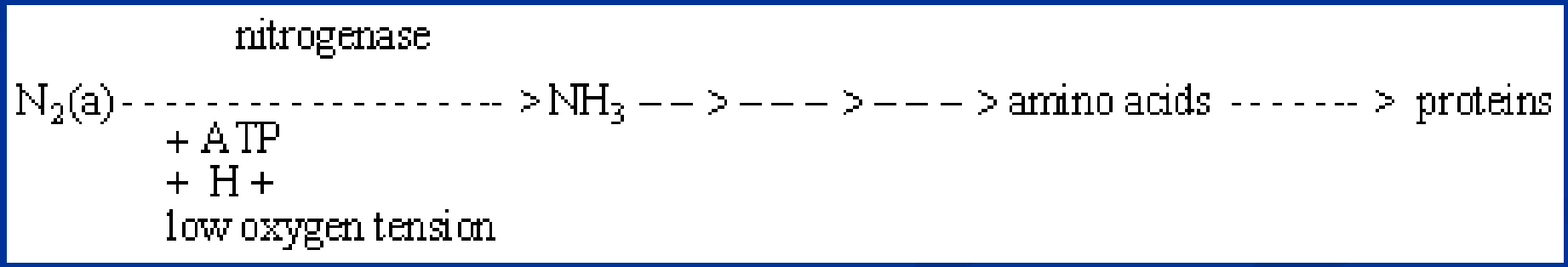
- 200 dicotyledonous plants form non-leguminous N_2 fixing nodules after infection with actinomycete - *Frankia sp.*, all but 2 are woody and found in temperate regions and tropics
- Most prominent is *Frankia sp.* with Alder

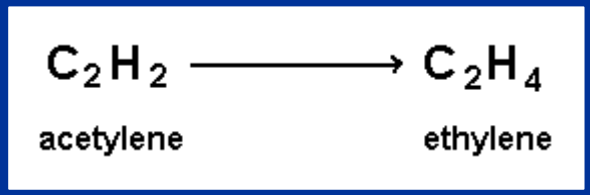
Symbiotic bacteria - Leguminous nodules

- Herbaceous legumes infection is through root hairs - host specific recognition signals recognised by Rhizobium
  - Peas
  - Beans
  - Clover
  - Vetch
  - Alfalfa
  - Soybean
  - Lentils
  - Chickpeas


1 Plant roots emit flavonoids that bind to receptors in plasma membranes of compatible soil rhizobia.
Rhizobia
Nod factors
Flavonoids
2 In response to flavonoids, rhizobia secrete Nod factors that bind to receptors in the membranes of host plant root hair cells.
Infected root hair
3 Receptor binding causes entry of Ca^{2+} into root hair cells, which causes root hairs to swell at their tips and curl around the rhizobia.
Root cortex
Infection thread
4 Rhizobia inject infection proteins that induce plant roots to develop infection threads; rhizobia penetrate into root cortex cells.
Root vascular tissue
Nodule vascular tissue
Intracellular rhizobia
Developing root nodule
5 Proteins known as nodulins cause root cortex cells to divide, forming nodules. Rhizobia invade nodule cells, inducing further nodule development. Rhizobia divide, then transform into bacteroids.
7 Nodules develop vascular tissue that transports nitrogen compounds to the shoot, and organic carbon from the shoot to nodule bacteroids.
Mature root nodule
6 Nodules become pink inside as O_2-regulating leghemoglobin is produced.

Biochemical mechanism of N_2 fixation





Nitrogenase also catalyses reduction of acetylene

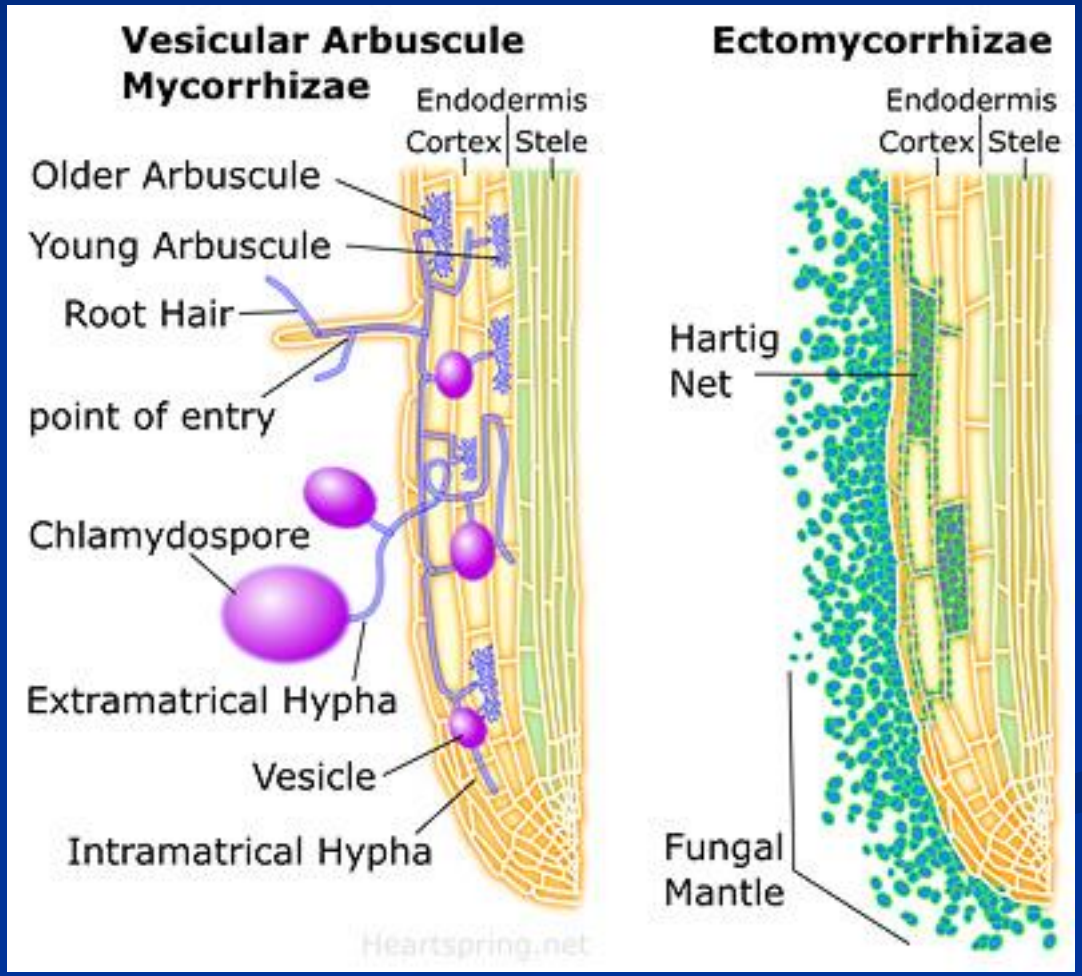
Symbiotic fungi Mycorrhizae

- Most plants are mycorrhizal: fungus receives C for growth, plant greater acquisition of nutrients
- Mycorrhizal fungi exist as spores/vegetative propagules in root fragments
- Germination & growth stimulated by root exudates chemotropism to root via exudates
- 3 types

  Arbuscular

  Ecto

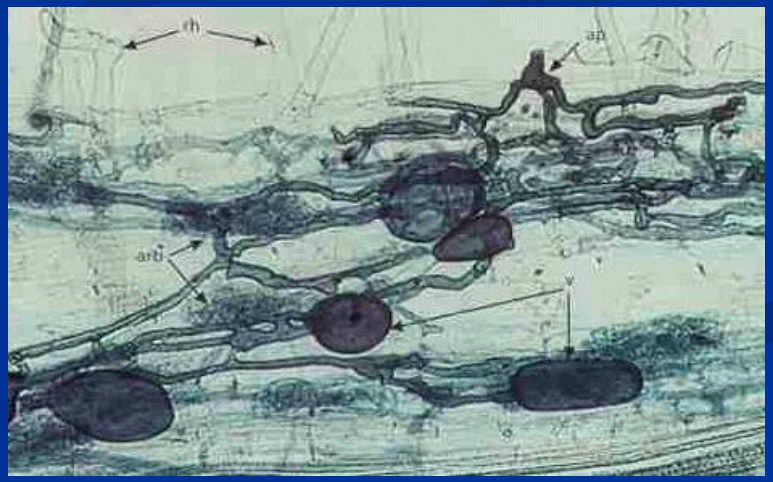
  Ericoid



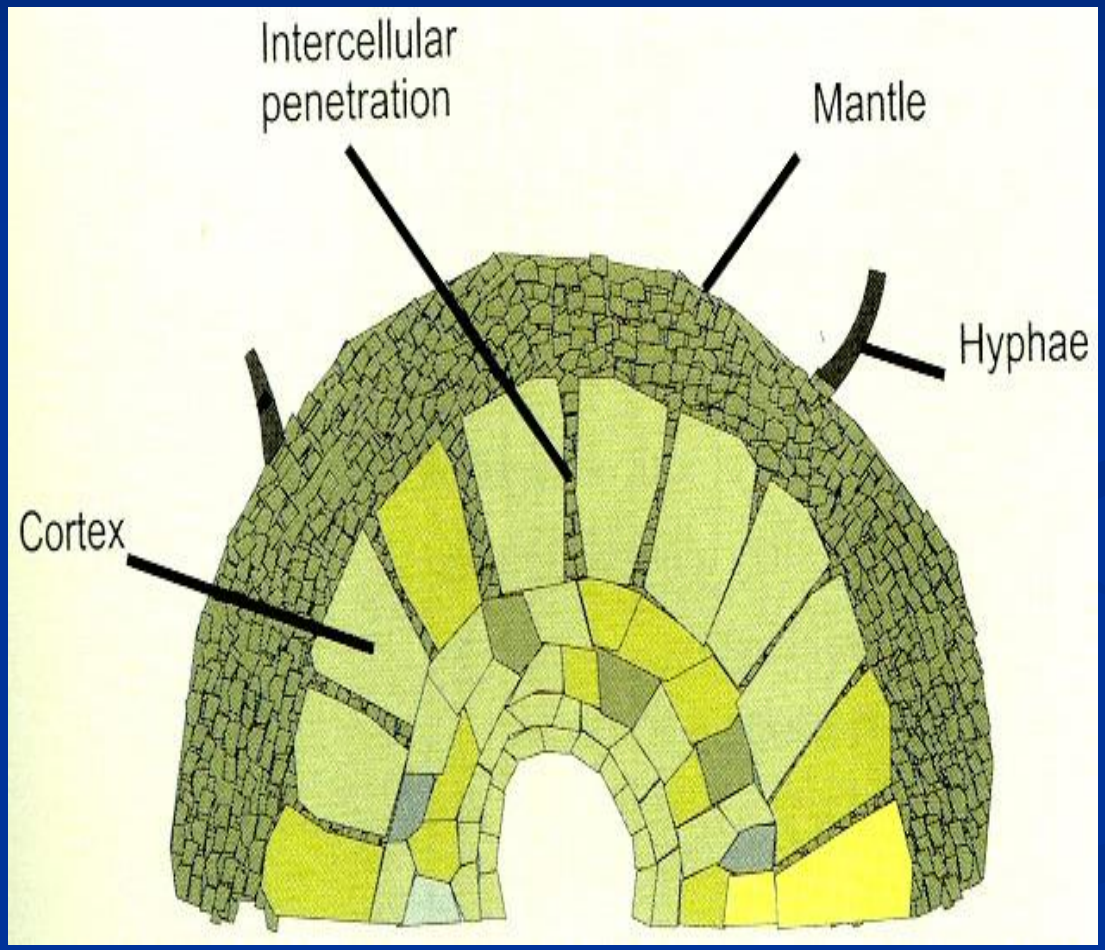
Symbiotic fungi - Arbuscular Mycorrhizae

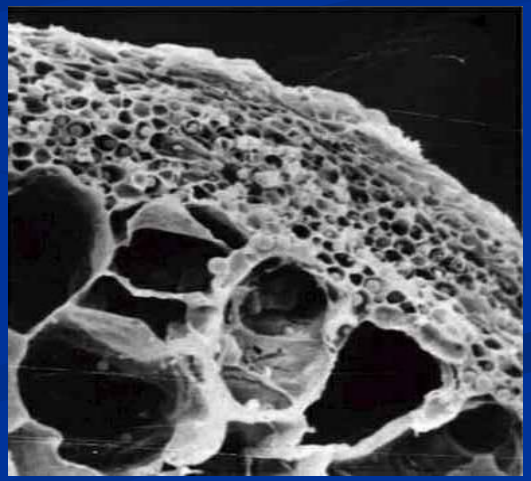
- Arbuscular Mycorrhizae
- Found on vast majority of wild and crop plants, exceptions are Brassica. Some tree species eg cedar, maple, alder, majority tropical tree species
- Fungi belong to Zygomycete group

- Majority fungal mycelium is internal penetrates cortical cells
- Site of penetration is top right, - fungus produces pre-infection swelling (**appressorium**, ap), grows between root cells and forms **arbuscules** (arb) and swollen **vesicles** (v).
- Arbuscules - sites of nutrient exchange
- Vesicles - used for storage.



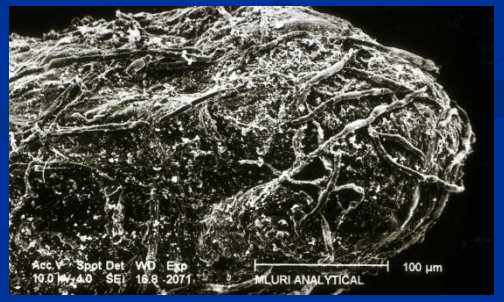
Symbiotic organisms
Ecto Mycorrhizae





Symbiotic fungi – Ecto Mycorrhizae

- Predominate in trees particularly in Pine, Beech, Spruce, Fir and Birch
- Pine & oak are obligately mycorrhizal
- Fungi belong to Ascomycete and Basidiomycete groups – many mushroom producers
- Majority of fungus is external - forming hyphal mantle around root, external mycelium can extend many metres through soil and inter-connect trees



Outline

- **Skills**
  - Isolation and estimation of % infection of plant roots with mycorrhizae, staining and microscopy techniques; morphotyping of ecto-mycorrhizae; visualization of root nodules and their viability; calculation of N_2 fixation rates

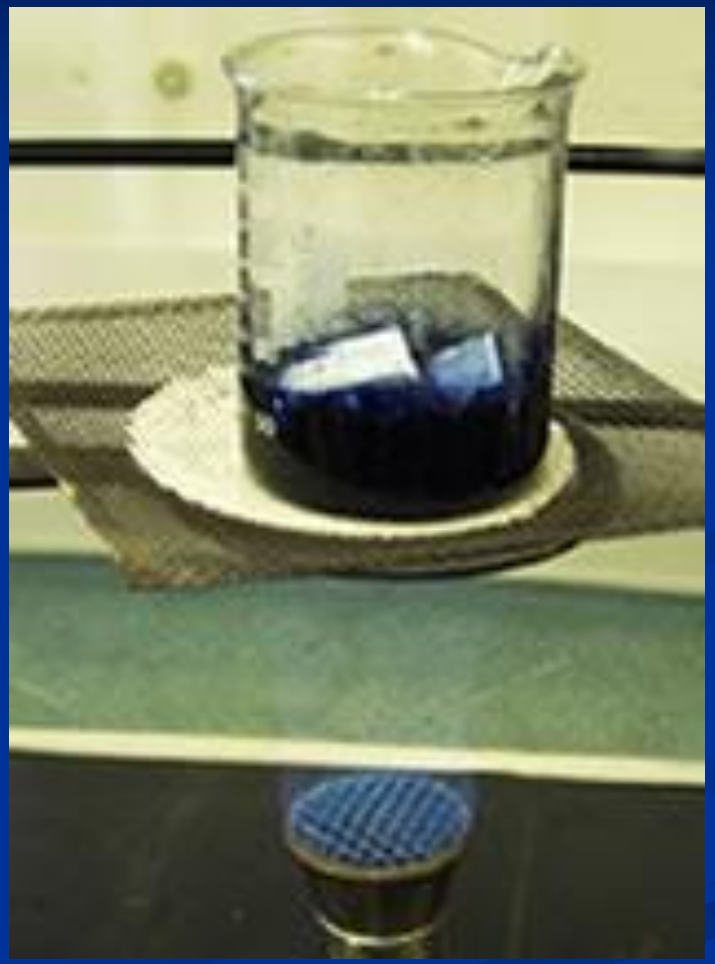
- **Objectives:**
  - **To observe, characterize and enumerate % colonization of plant roots by mycorrhizae using staining, microscopy techniques and morphotyping. To identify root nodules, enumerate and estimate N_2 fixation rates.**

Methods

- Clear (boil in KOH) and stain herbaceous roots with ink and vinegar and estimate % AM infection using microscopy and the grid-line intersection method
- Wash fine roots of trees and observe ECM root tips using a dissecting microscope. Use colour atlas of mycorrhizae to morphotype ECM and estimate % infection of roots by different mycorrhizal fungi. Estimate ECM abundance and richness.
- Wash root nodules from legumes and trees. Calculate nodule mass. Determine % active (brown/red) vs inactive (white/green) nodules by dissection.
- Use known BNF rate per nodule to calculate BNF per plant per day

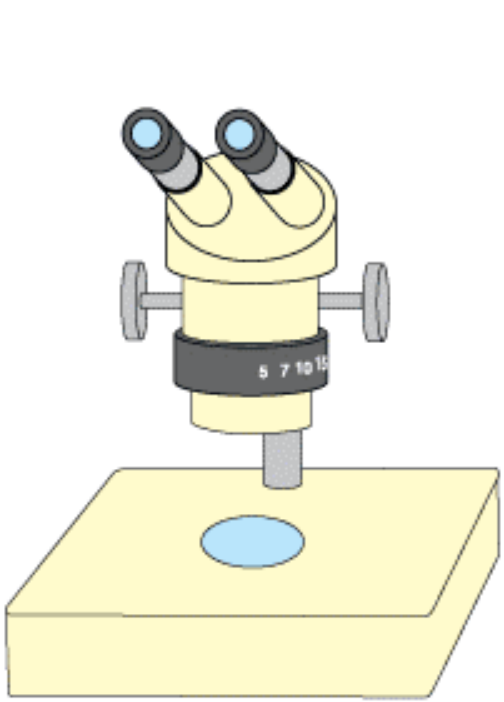
Clearing and staining of roots

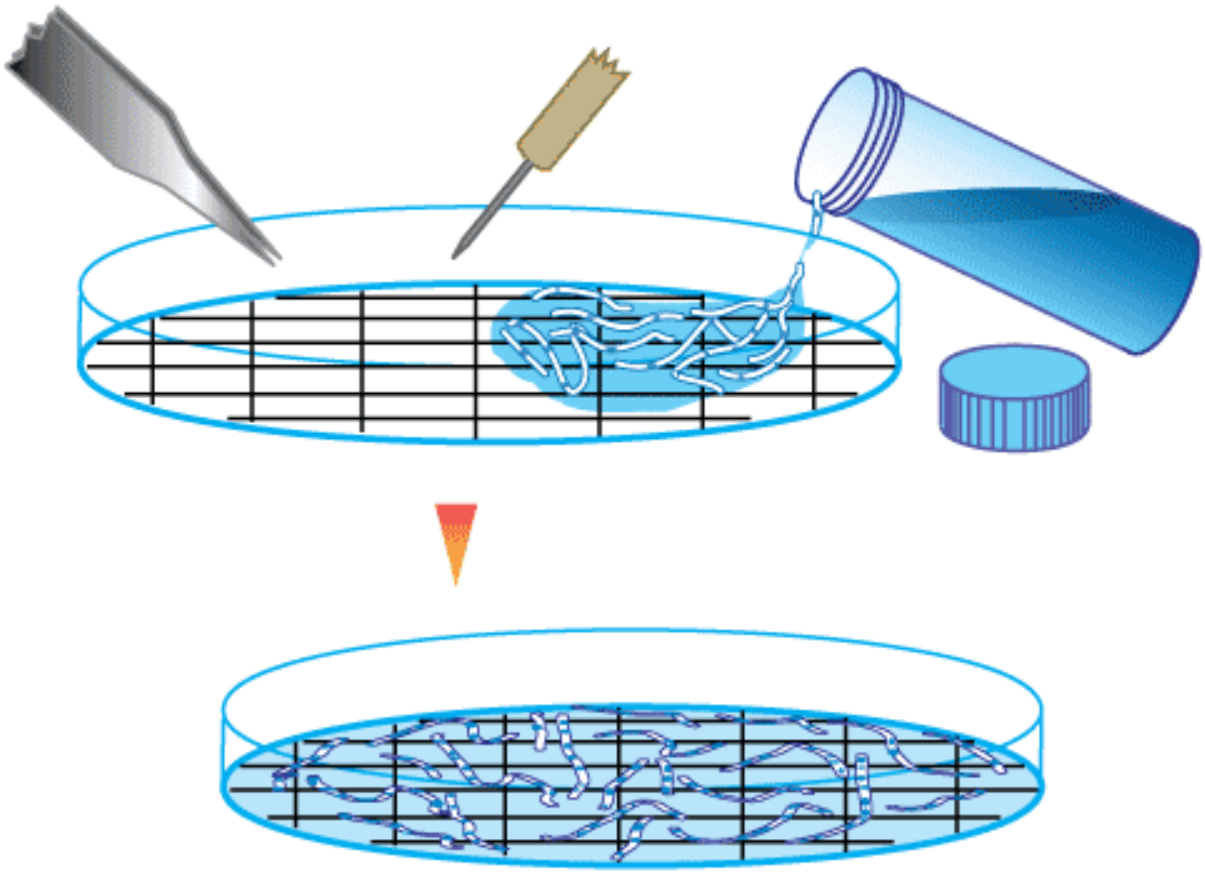
Clear with boiling KOH !



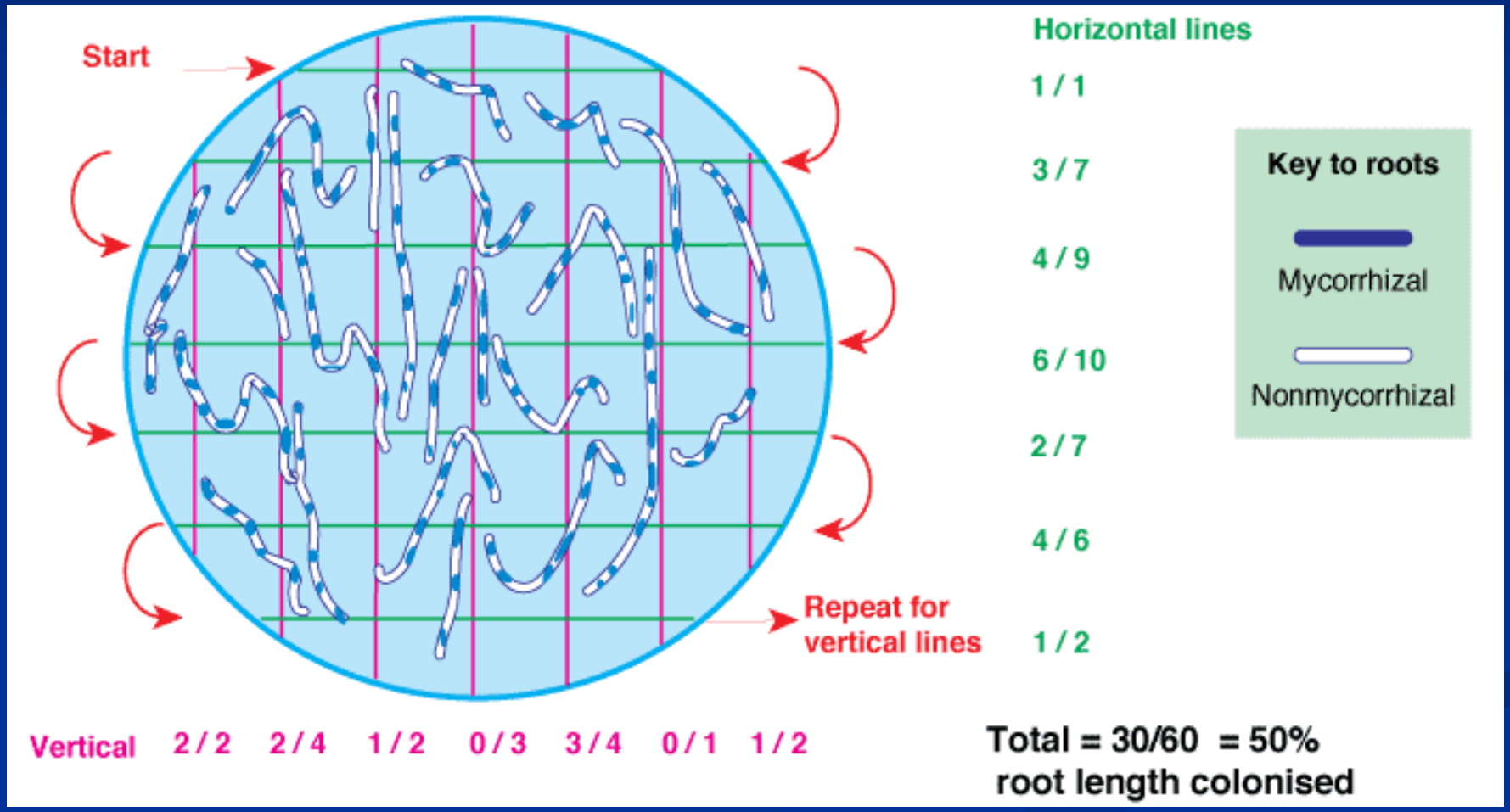
Stain with black ink and white vinegar

Put stained roots in petri dish with grid lines, randomly disperse & observe under dissecting microscope





Assess % mycorrhizal colonization using the gridline intersection method



Examples of ectomycorrhizal morphotypes

Pine & Suillus brevipes

Pine & Amanita muscaria

Pine & Boletus edulis

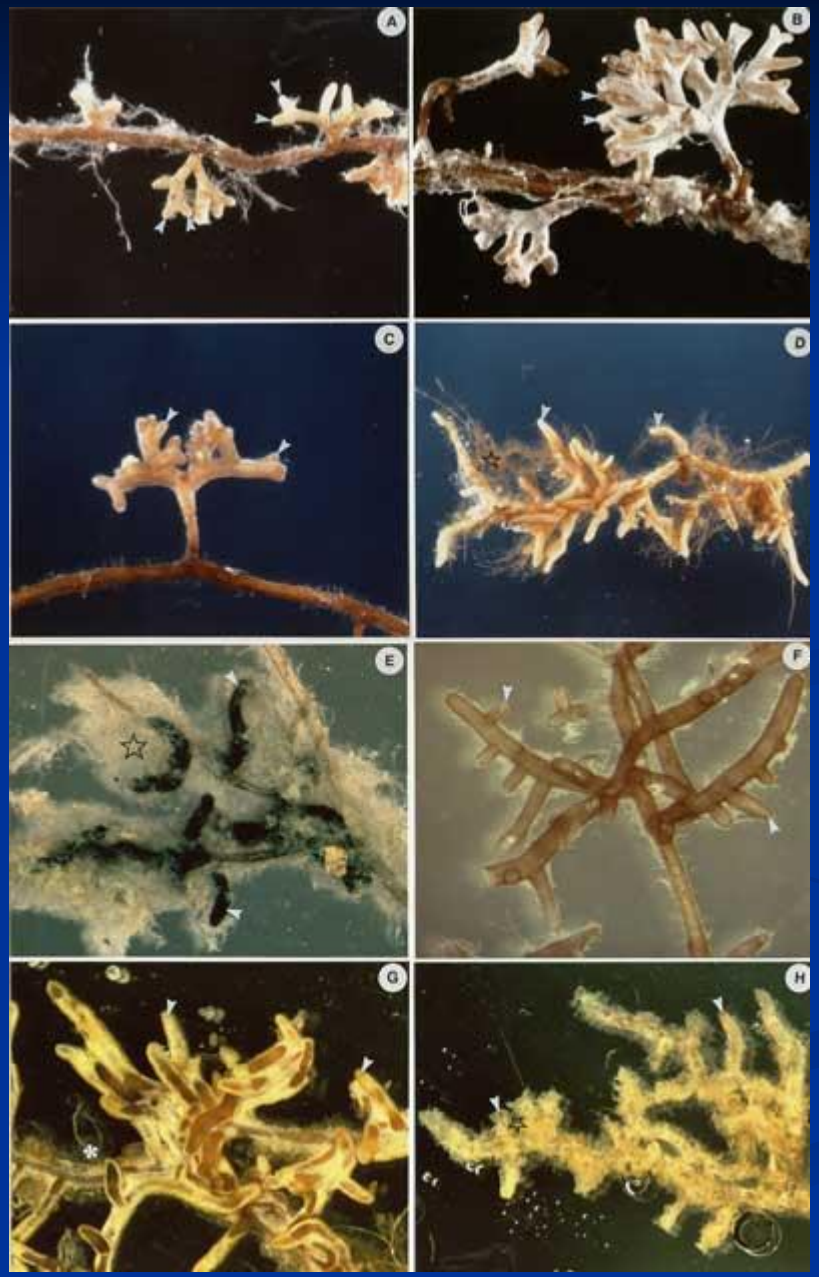
Eucalyptus & Astraeus pteridis

Eucalyptus & Tylopilus sp.

Eucalyptus & Redellomyces sp.

Eucalypt & Pisolithus sp.

Eucalypt & Amanita sp.



Nodules of trees and legumes

Alder

Clover

Nitrogenase is an O_2 sensitive enzyme. Low O_2 is realized through compartmentation in cyanobacteria, active respiration (in *Azotobacter*), synthesis of leghemoglobin (in *Rhizobium* legume).

Leghemoglobin is a macromolecule synthesized by both symbiotic partners - *Rhizobium* synthesizes the heme portion, and the plant the globine.

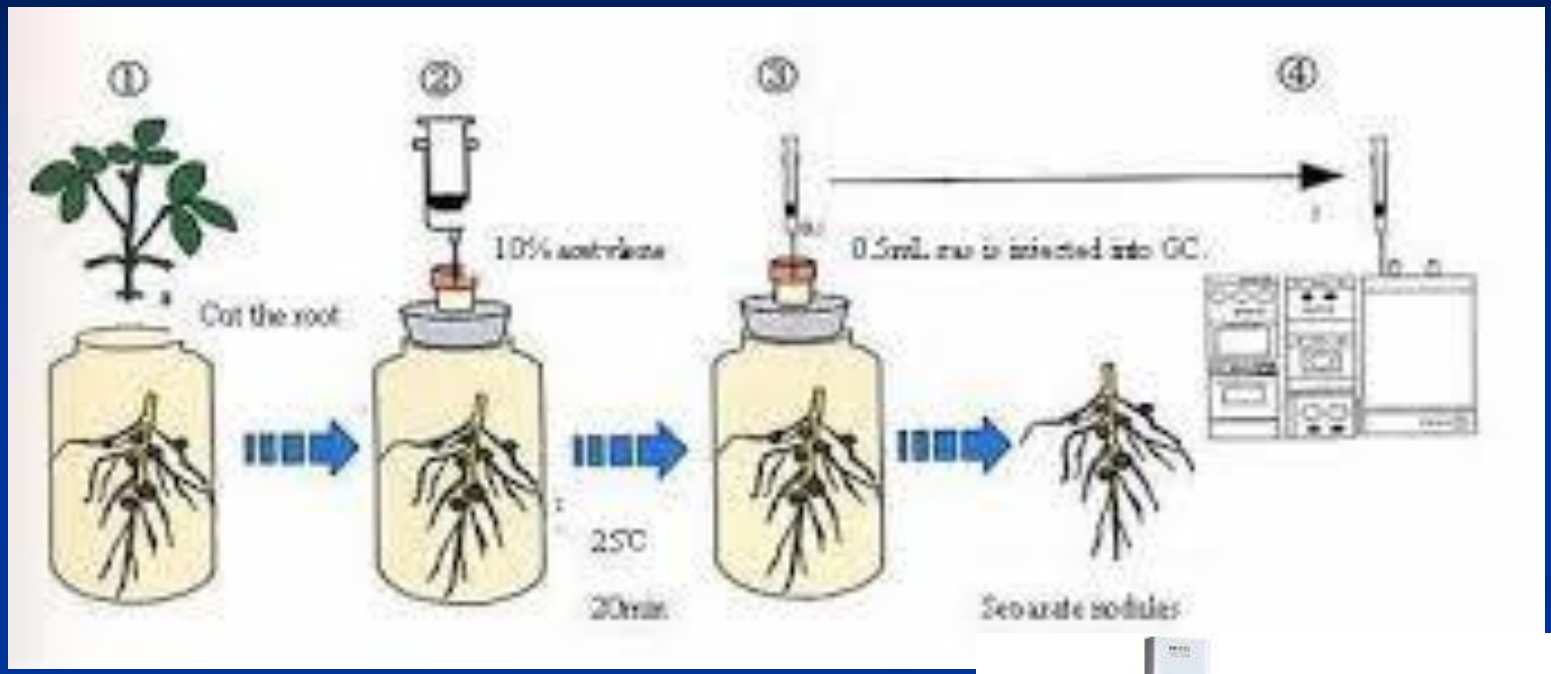
Like human hemoglobin, leghemoglobin fixes O_2.

Red color of open nodule shows healthy rhizobia

Interior of active (i.e., N_2-fixing) nodules are red or brown

Interior of non-N_2-fixing nodules are white or green as the globine has degenerated

Acetylene reduction assay



$$C_2H_2 \longrightarrow C_2H_4$$

acetylene ethylene

You have been given data on N_2 fixation rate per g nodule (will see GC in week 3 in BEG labs)

Lab week 2

- **From week 1:**
  - Identify meso-fauna using microscopy and keys
  - Determine % feeding activity in fauna in your soil from bait strips
  - Enter faunal species diversity into table on connect (so everyone can calculate E (evenness) of faunal communities) **BY TUESDAY NOV 19**
- **Week 2**
  - Stain and assess % AM infection of herbaceous roots
  - Assess diversity of ECM morphotypes on woody roots and the % ECM infection of each type
  - Count nodules on roots, dissect nodules to determine if nodules are active, weigh total nodule mass per plant
  - Enter %AM infection of roots, %ECM infection of roots, ECM diversity into table on connect (so everyone can compare different plant/soils) **BY FRIDAY NOV 22**
  - Enter nodule numbers, mass and % active into into table on connect (so everyone can compare different plant/soils) **BY FRIDAY NOV 22**
- WEEK 1 lab submission due FRIDAY NOV 22 11.59PM and WEEK 2 lab submission due MONDAY NOV 25 1PM

Further reading

- **Fundamentals of Soil Ecology, Second Edition (1994)** by David C. Coleman, Jr., D. A. Crossley, Paul F. Hendrix
- **Soil Microbiology, Ecology and Biochemistry, Third Edition (2006)** by Eldor A. Paul
- **Biological Diversity and Function in Soils (2006)** by Richard Bardgett, Michael Usher, David Hopkins
- **The Biology of Soil: A Community and Ecosystem Approach (2005)** by Richard D. Bardgett
- Falkowski PG et al., 2008. The microbial engines that drive earth's biogeochemical cycles. Science 320, 1034-1039.

- Digital learning centre for microbial ecology http://commtechlab.msu.edu/sites/dlc-me/index.html
- Fun facts about fungi http://www.herbarium.usu.edu/fungi/funfacts/factindx.htm
- The microbial world http://helios.bto.ed.ac.uk/bto/microbes/index.htm#Top