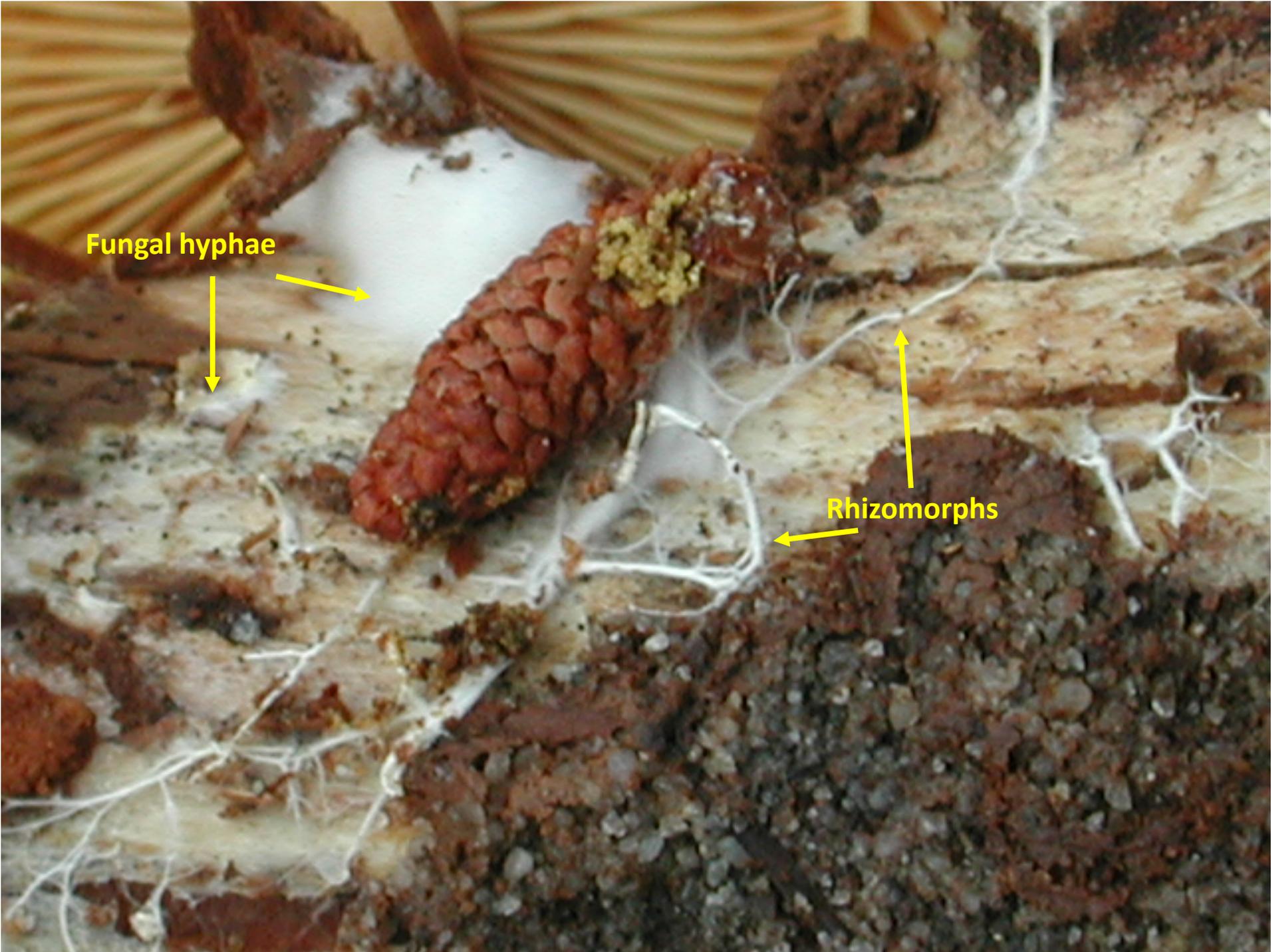


# **Beneficial fungi in the forest and impacts of pollution**

**John Dighton**

Rutgers Pinelands Field Station, New Lisbon, NJ



Fungal hyphae

Rhizomorphs

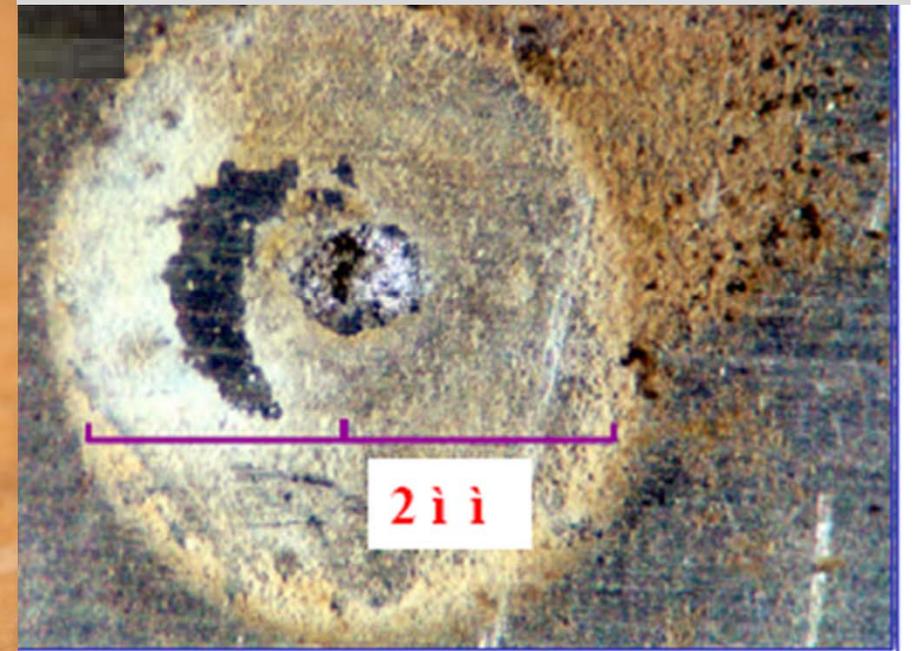
*Serpula lachrymans*  
(Wikipedia)



Fungal damage to an  
ancient manuscript



Fungal damage to aluminum  
structure Mir space station



*Penicilium*

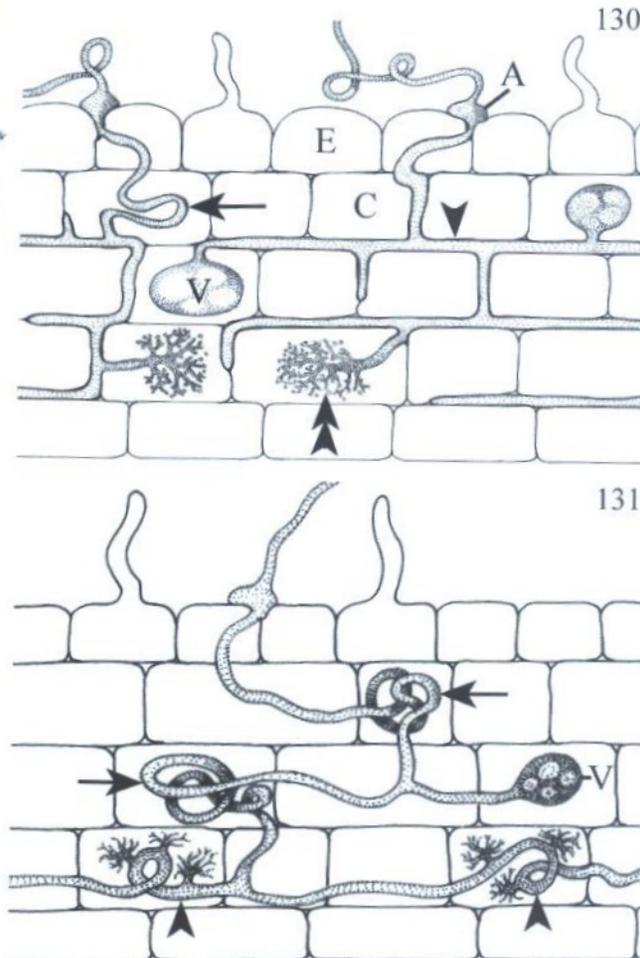


# Arbuscular mycorrhizae

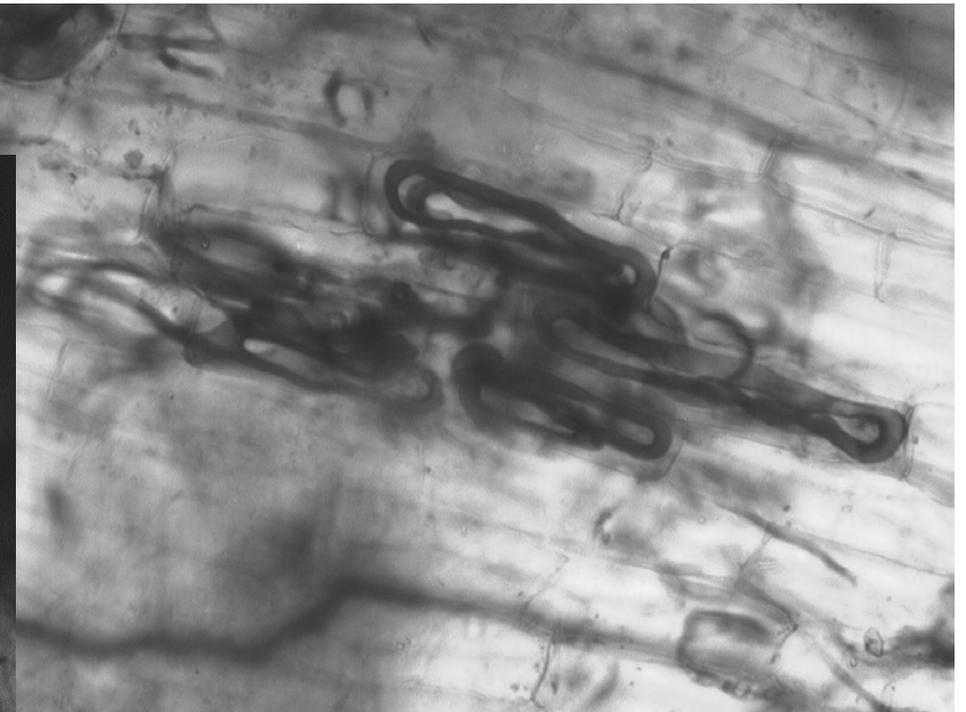
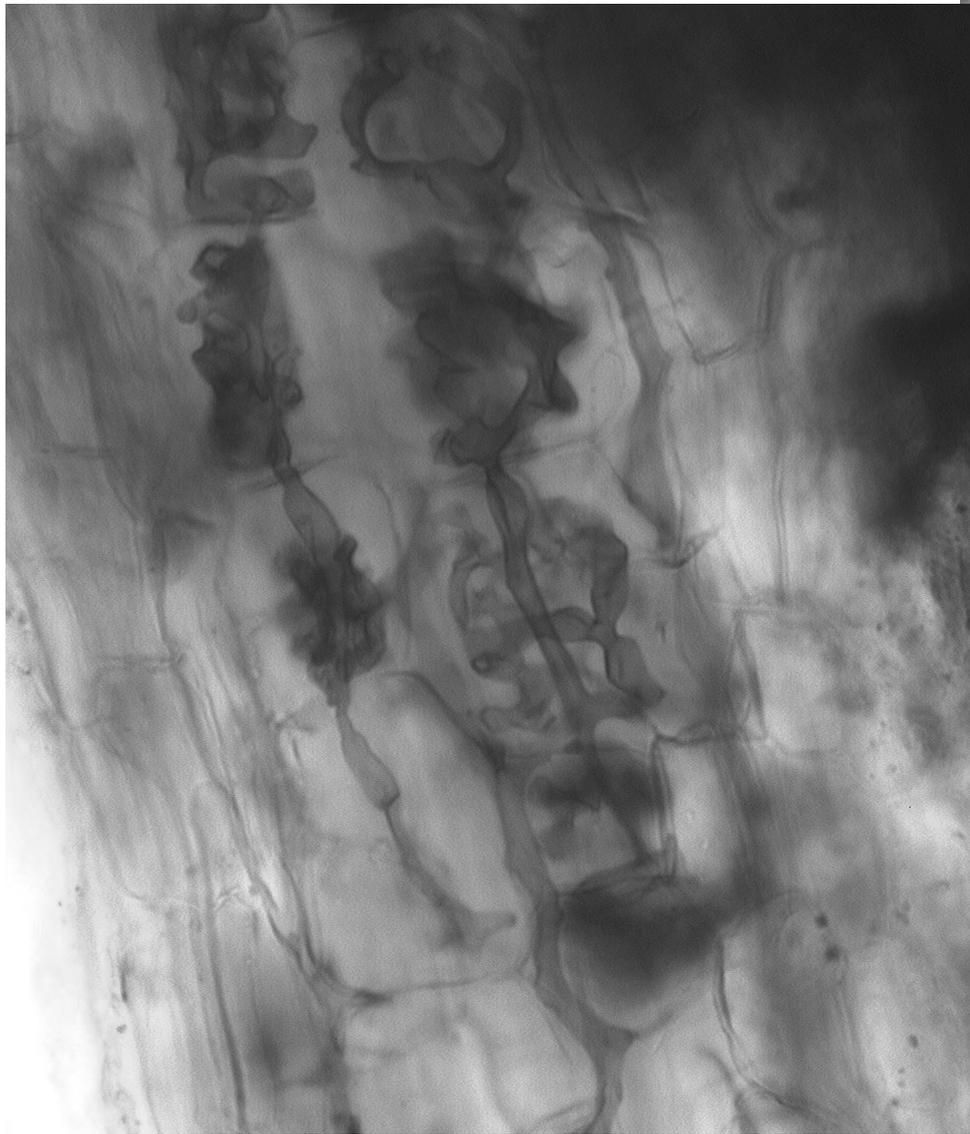
- Associated mainly with **herbaceous** plants and some trees (particularly tropical)
- A limited range of **zygomycete** fungal species associated with a large range of host plant species
- Fungal component penetrates epidermis and cortex cell walls
- Develops and **arbuscule** by invagination of the cortex cell plasmolemma
- Limited hyphal network within the root.
- Produces spores within or outside the root

Arbuscular mycorrhizas

Colonization of a new root from a previously colonized root



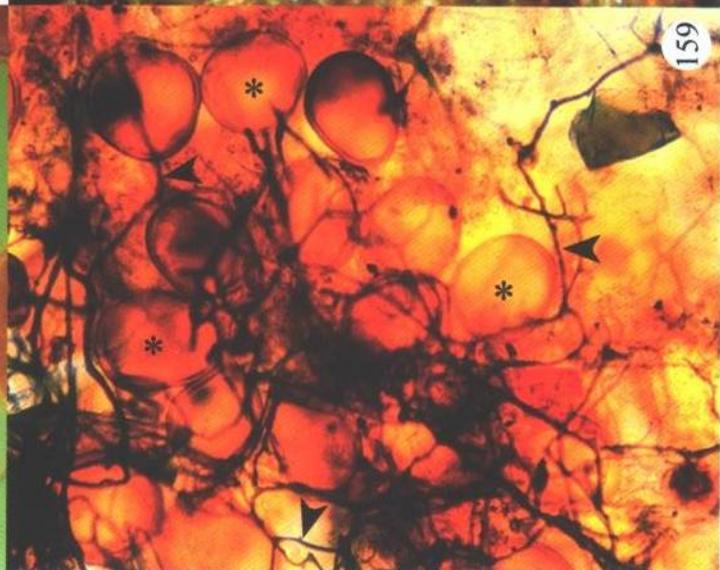
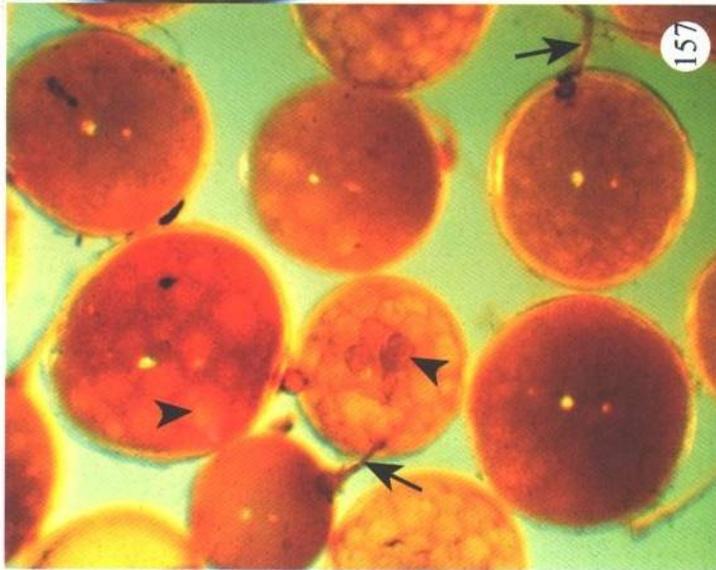
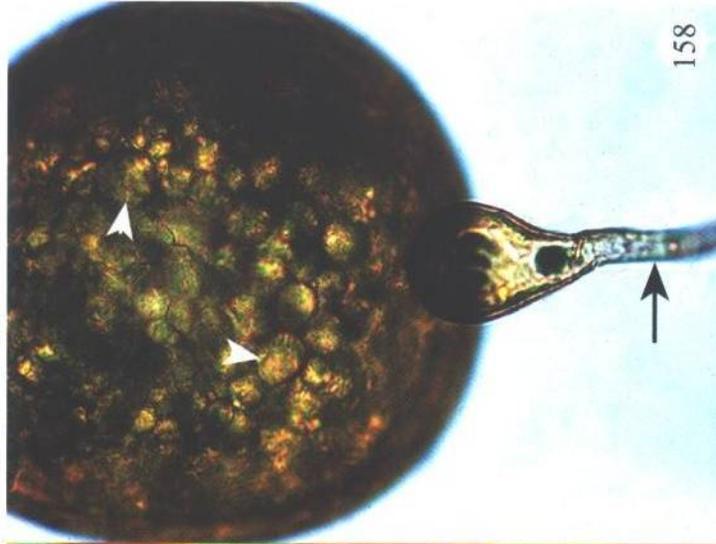
AM structures in *Rhynchospora knieskernii*



Dighton *et al.* (2013) *Bartonia*

# Arbuscular mycorrhizal spores

Arbuscular mycorrhizas



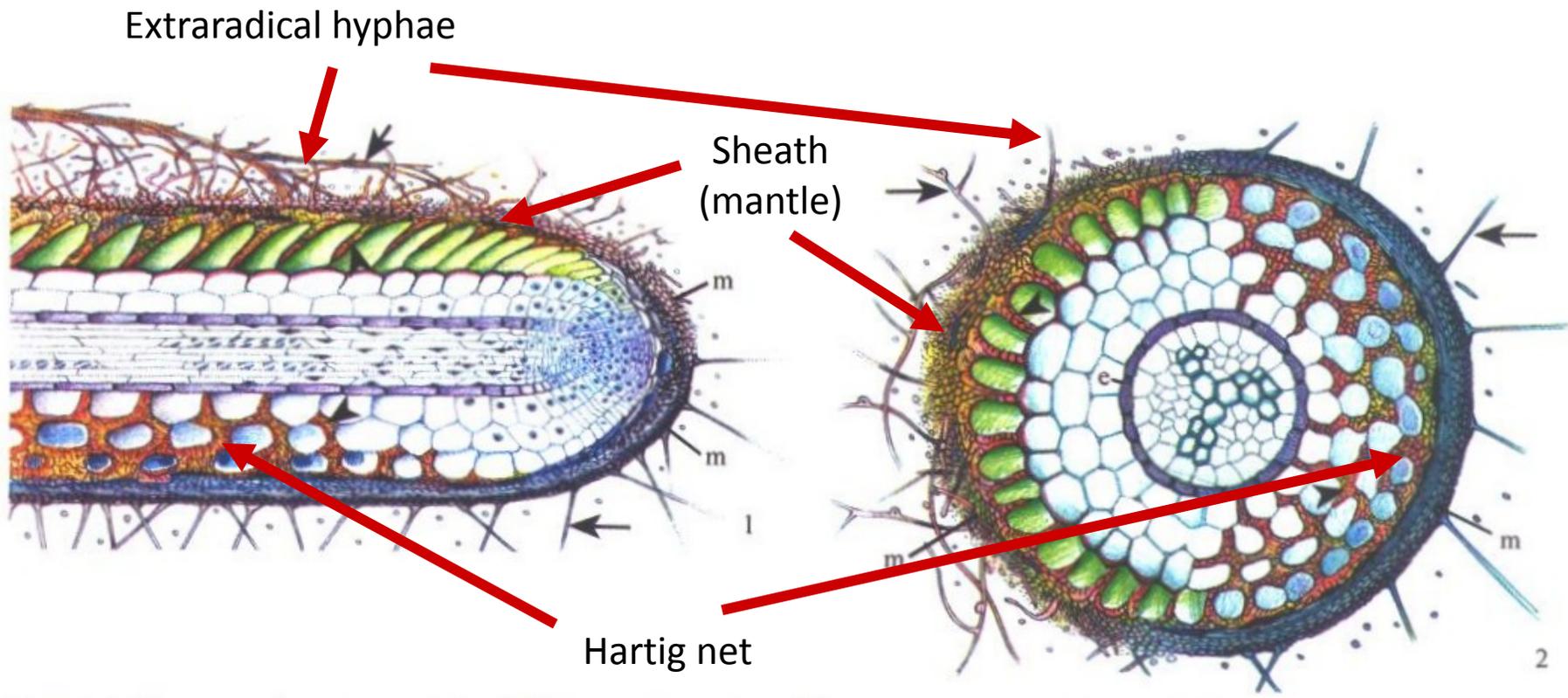
# Ectomycorrhizae

- Associated with **tree** species and some shrubs
- Large range of **basidiomycete** and **ascomycete** fungi in association with a small diversity of plant species
- Fungal component does not penetrate host plant cells. Surface covering of fungal biomass = **sheath** and hyphal penetration between cortical cells to the endodermis = **Hartig Net**
- Extensive hyphal network within and on surface of root
- Spores produced in specialized structures away from the root

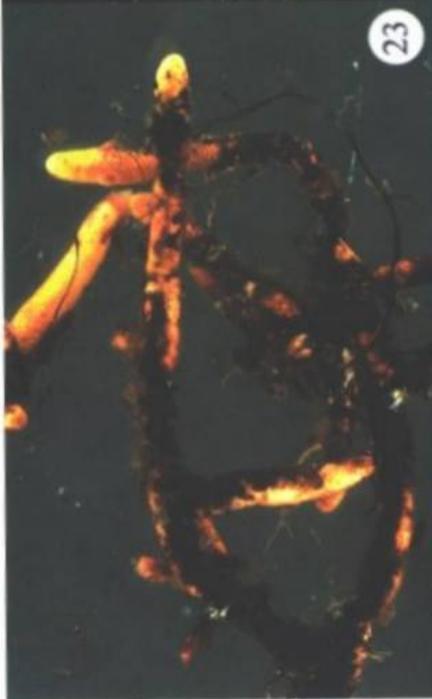
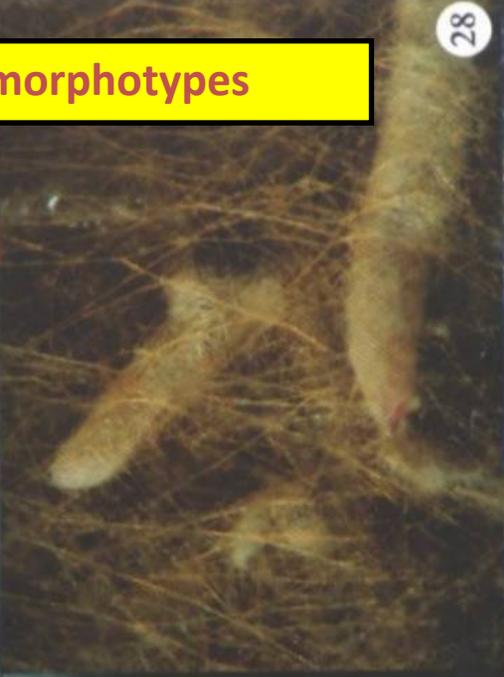




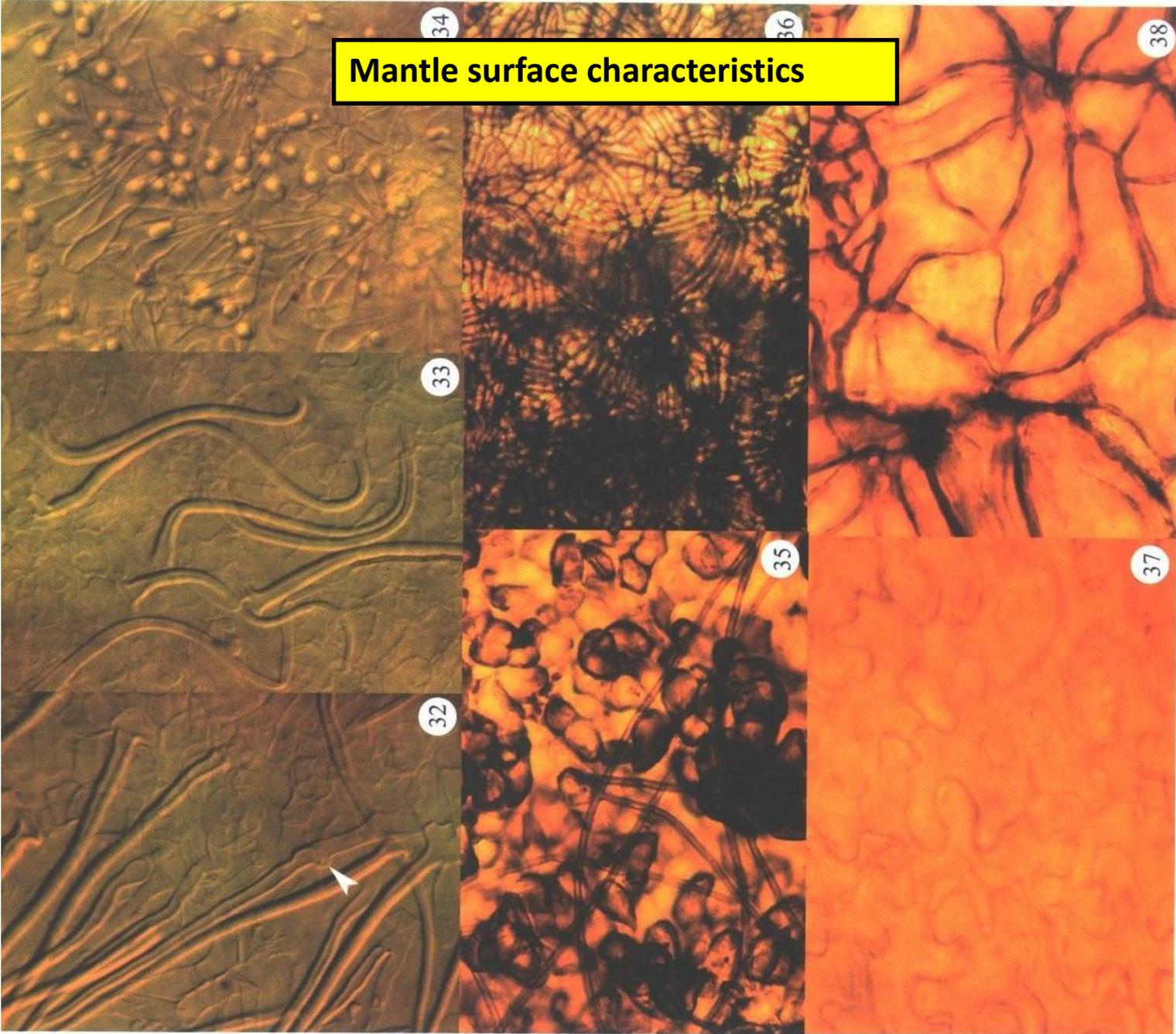
# Ectomycorrhizae



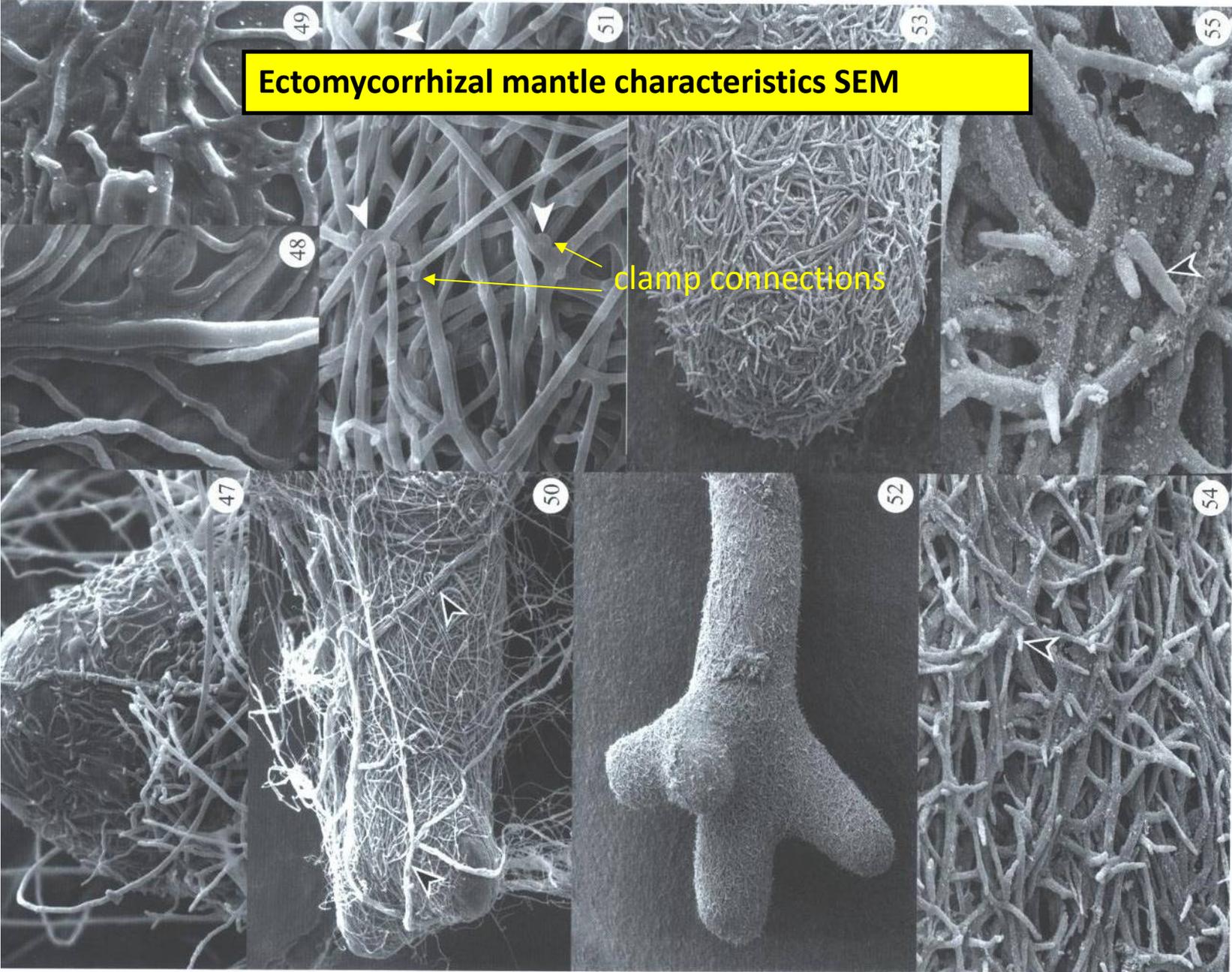
Ectomycorrhizal morphotypes



Mantle surface characteristics



**Ectomycorrhizal mantle characteristics SEM**

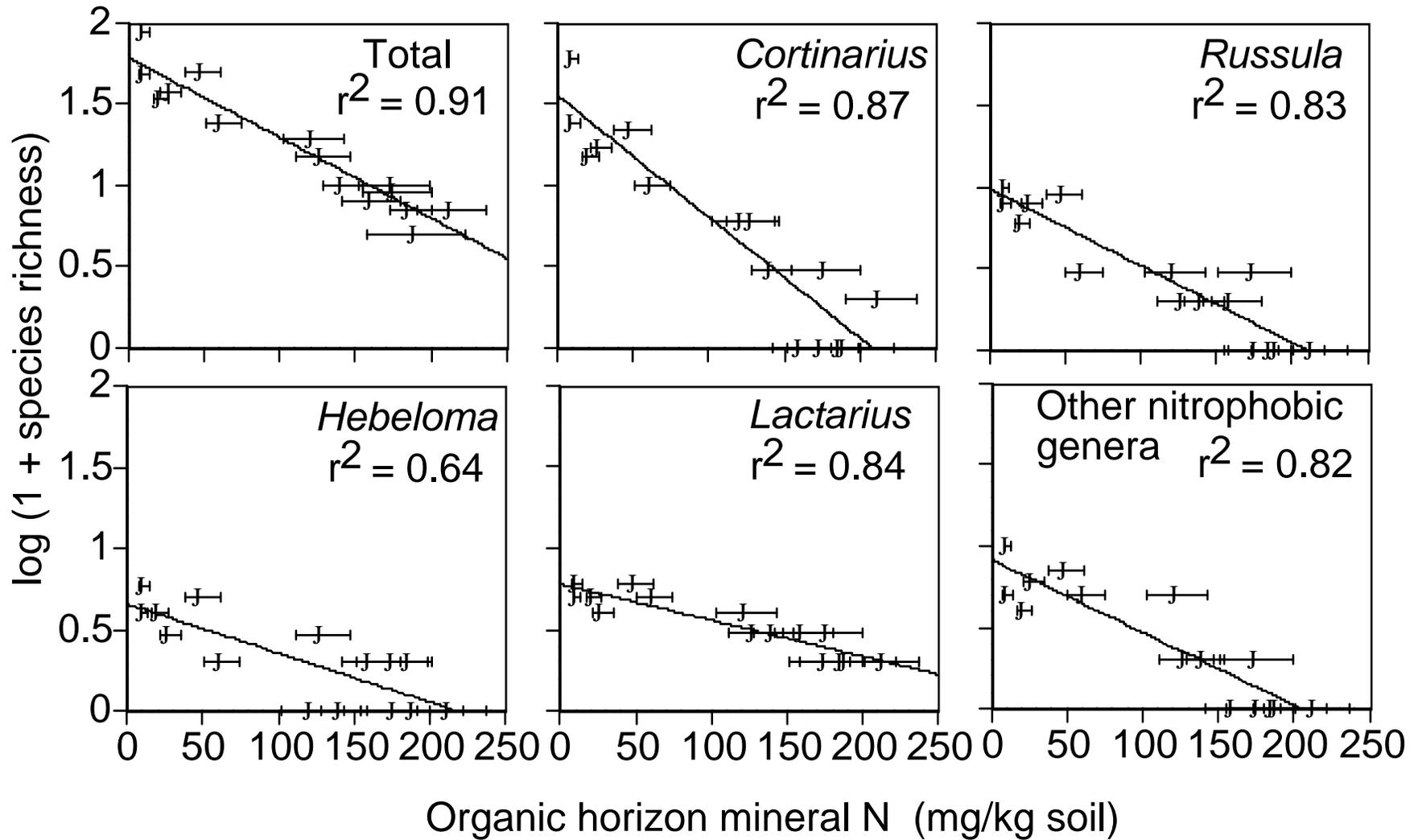


# Function of Ectomycorrhizae

- Help host plant take up nutrients
  - Mineral nutrients
  - Organic nutrients via enzymes
- Help host plant access water
- Protect host plant from pathogens and root grazers
- Protect host plant from heavy metal and radionuclide pollutants
- Species composition on roots is influenced by changes in nutrient and water status and pollutants
  - Useful as bioindicators

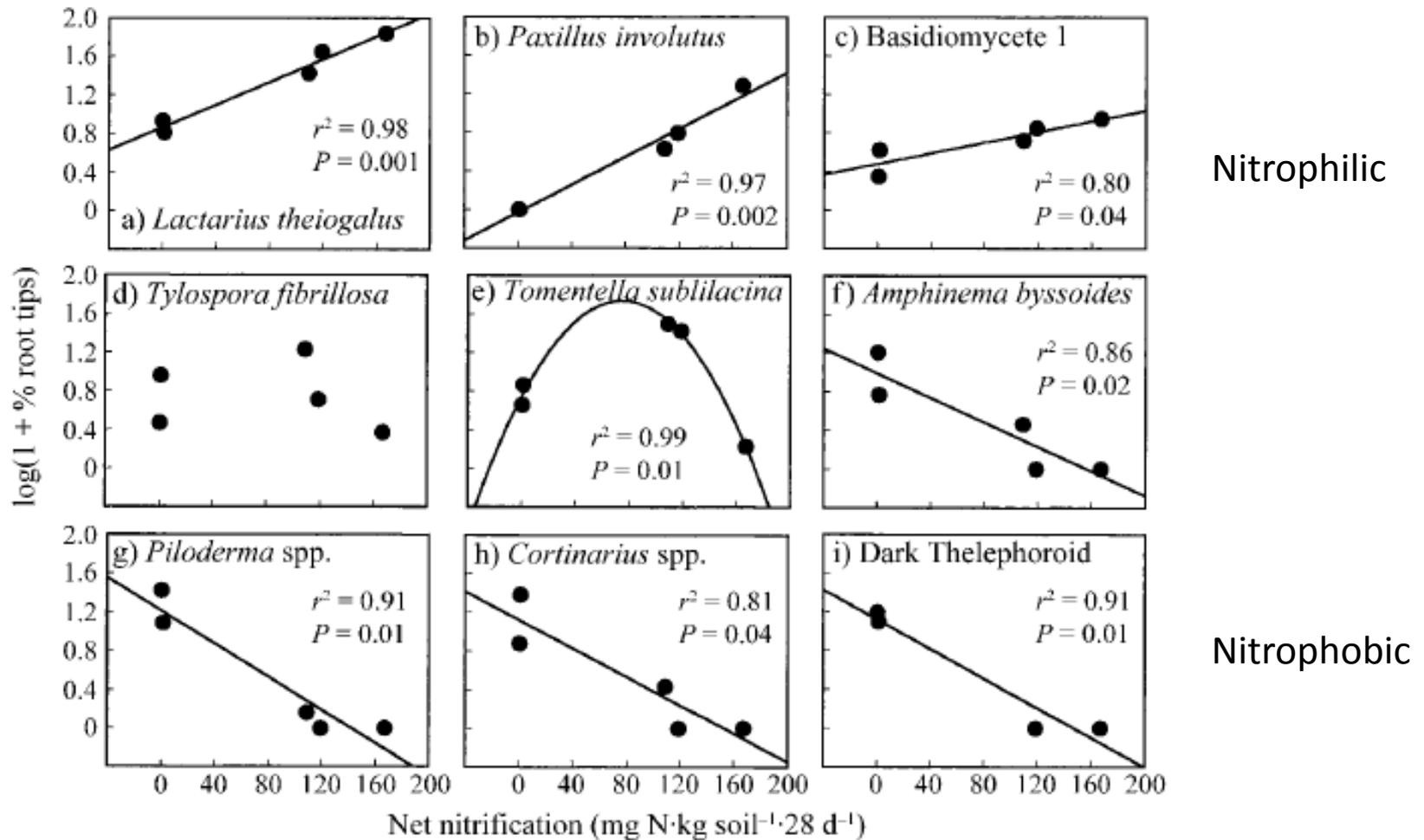
# Effect of N Addition on ECM Fungal Fruiting under Scots Pine

- Termorshuizen (1990) showed that application of ammonium or nitrate N:
  - Reduced number of mycorrhizal fruitbodies
  - Reduced fruitbody biomass
  - Had different effects on different species
    - Decreased *Lactarius rufus*
    - Increased *Laccaria proxima*



**Soil extractable N effects on mycorrhizal sporocarp abundance**

From Lilleskov et al. (2001) Ecol. App. 11: 397-410



**Change in abundance of ectomycorrhizae of white spruce in Alaska in relation to nitrification (N deposition rates from 1 to 14 kg N ha<sup>-1</sup> y<sup>-1</sup>)**

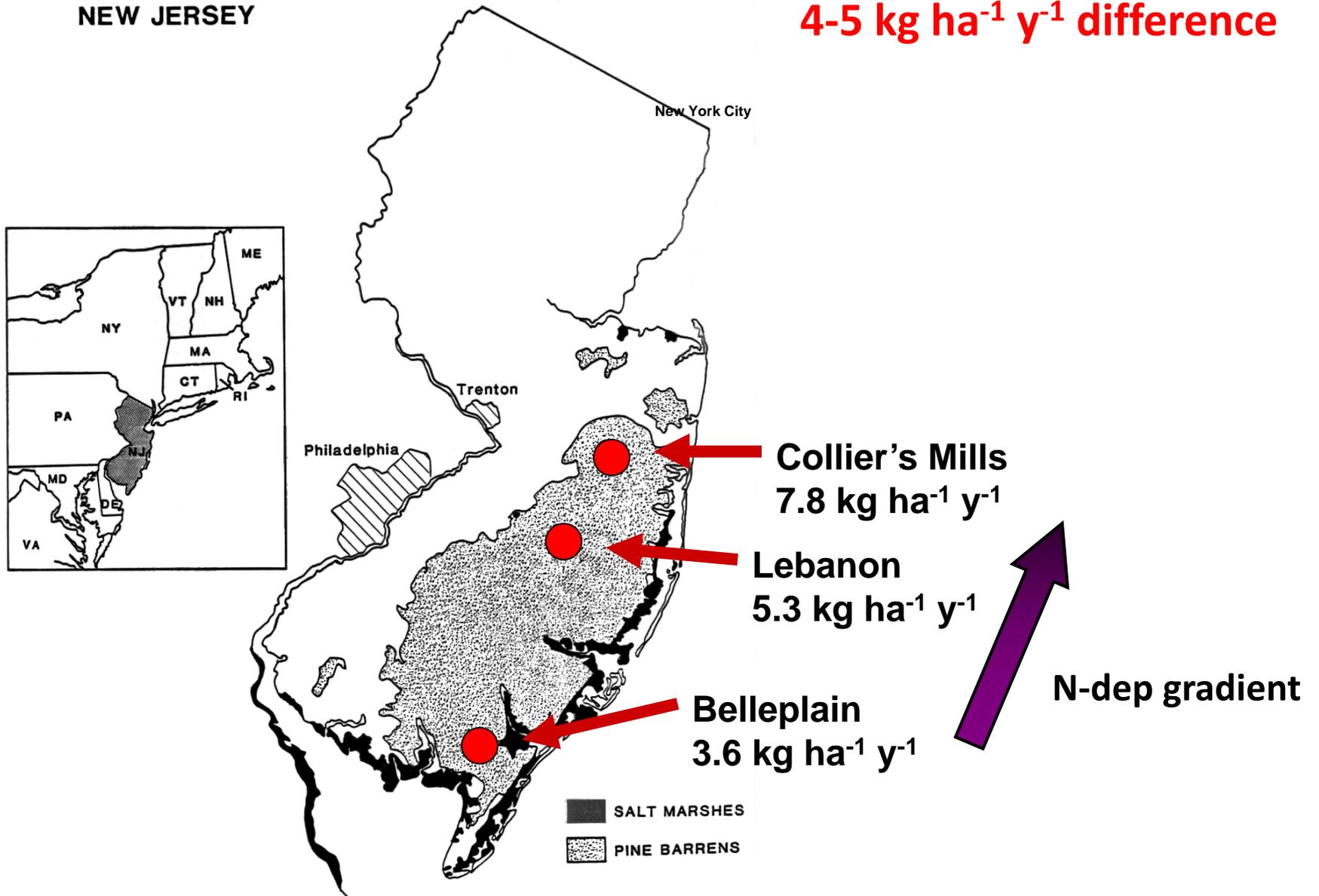
From Lilleskov *et al.* (2002) Ecology 83: 104-115

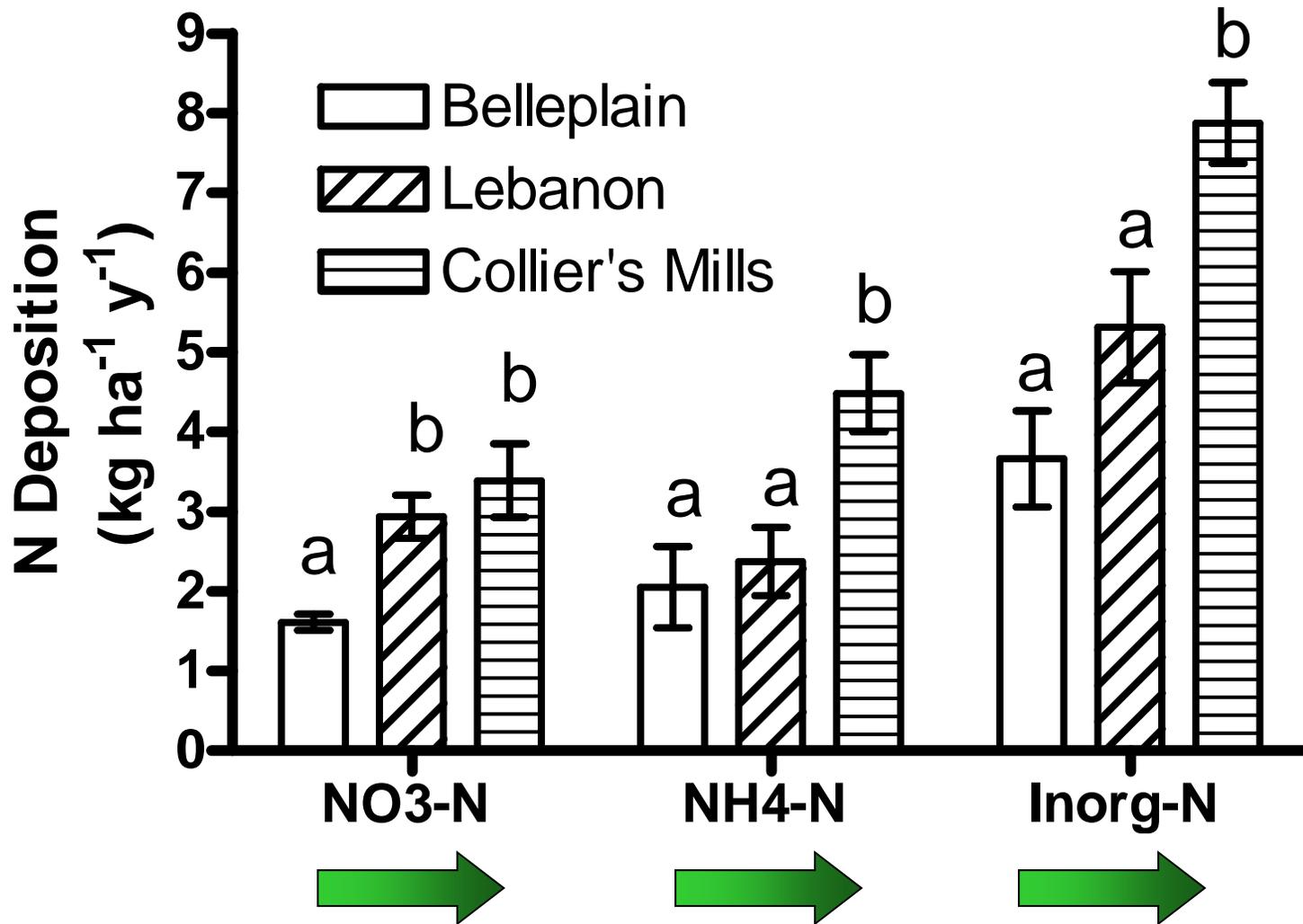
# **Atmospheric N-Deposition on NJ Pine Barrens Ectomycorrhizal Communities**

**Effects in an oligotrophic sandy soil  
system**

# Chronic N deposition

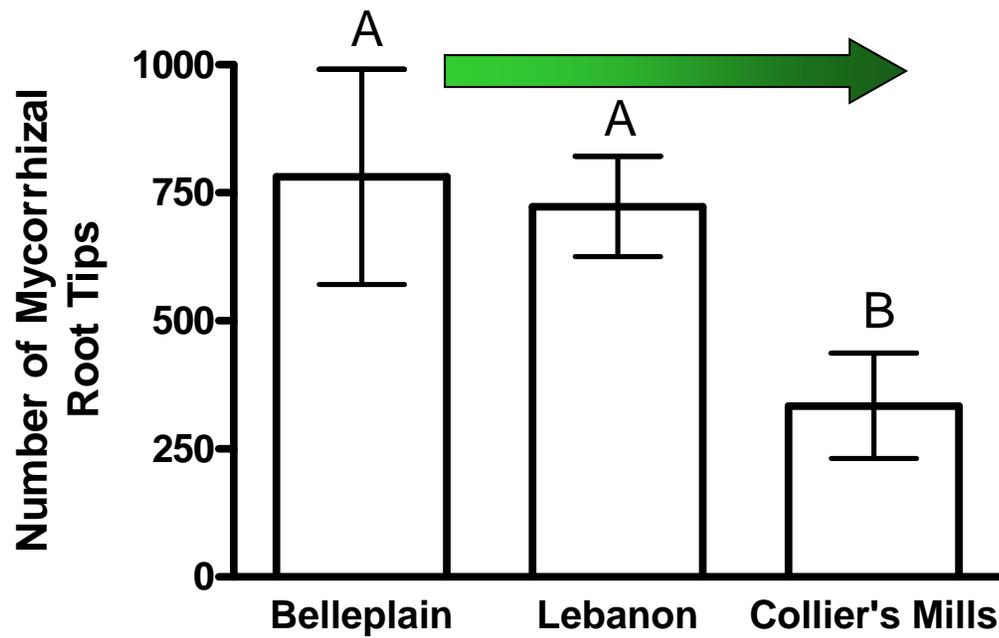
NEW JERSEY





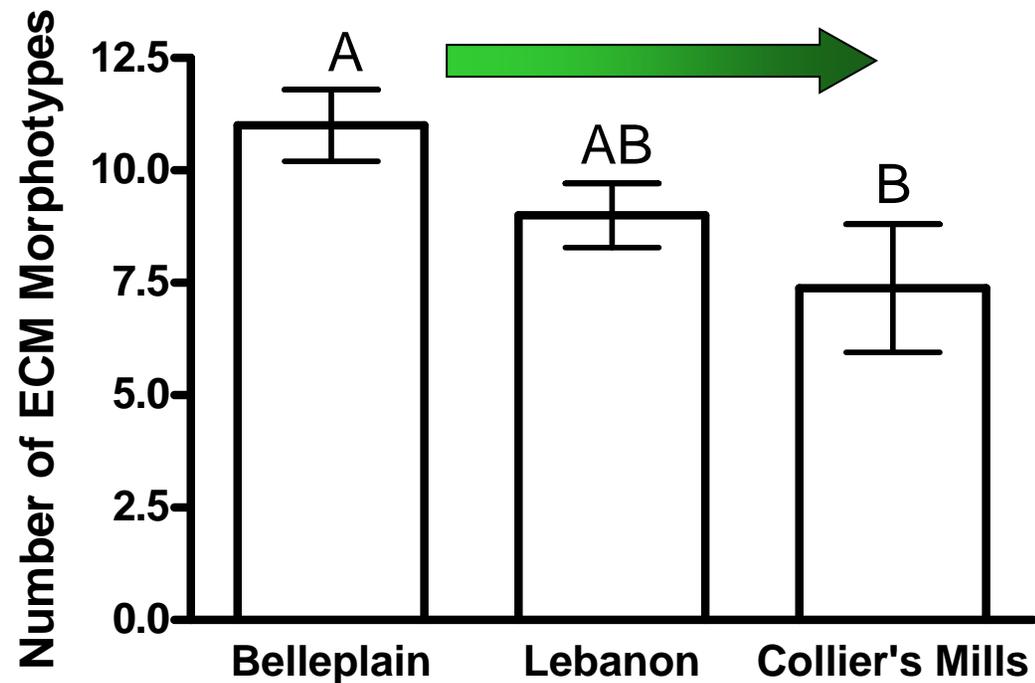
**Annual N Deposition at Each Site Measured in Bulk Precipitation**

From Dighton *et al.* (2004) *For. Ecol. Manage.* 201: 131-144



## Abundance and Richness of Ectomycorrhizal Root Tips at Each Site

From Dighton *et al.* (2004) For. Ecol. Manage. 201: 131-144



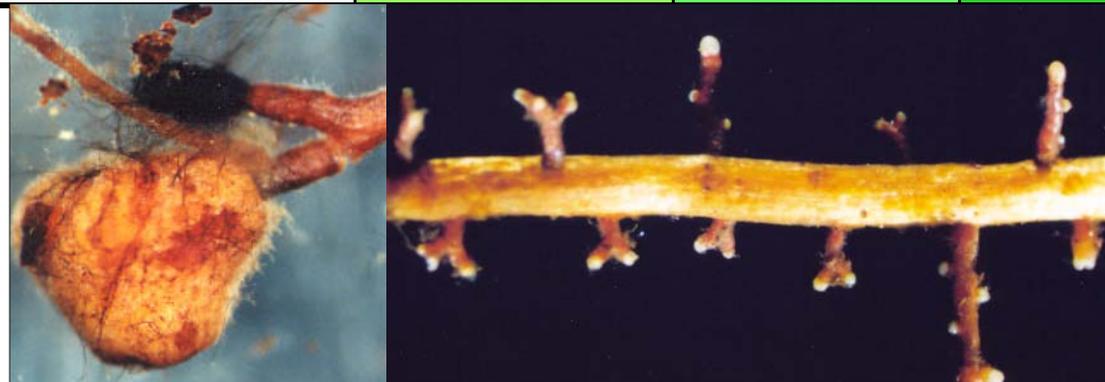
Abundance (tips per core) of potentially nitrophobic ectomycorrhizal morphotypes from field sites in relation to N-deposition ( $\text{kg ha}^{-1} \text{y}^{-1}$ )

	Belleplain	Lebanon	Colliers Mills
	3.7	5.3	7.8
Brown/white – DB ( <i>Russula</i> )	672	232	179
Green/ yellow	178	68	0
Yellow ( <i>Lactarius</i> )	509	10	0
Brown, bristly pinnate	71	0	0
Chartreuse	97	0	0
Yellow- bulbous	123	0	0



Abundance (tips per core) of potentially nitrophilic ectomycorrhizal morphotypes from field sites in relation to N-deposition ( $\text{kg ha}^{-1} \text{y}^{-1}$ )

	Belleplain 3.7	Lebanon 5.3	Colliers Mills 7.8
<b>Coral</b>	0	0	418
<b>Pumpkin (Suilloid)</b>	11	24	49
<b>White/brown (<i>Rhizopogon</i>)</b>	0	0	90
<b>Y/Br (Ascomycete)</b>	693	1066	1391
<b>Yellow/white</b>	0	0	49
<b>Yellow - tuber</b>	4	8	79

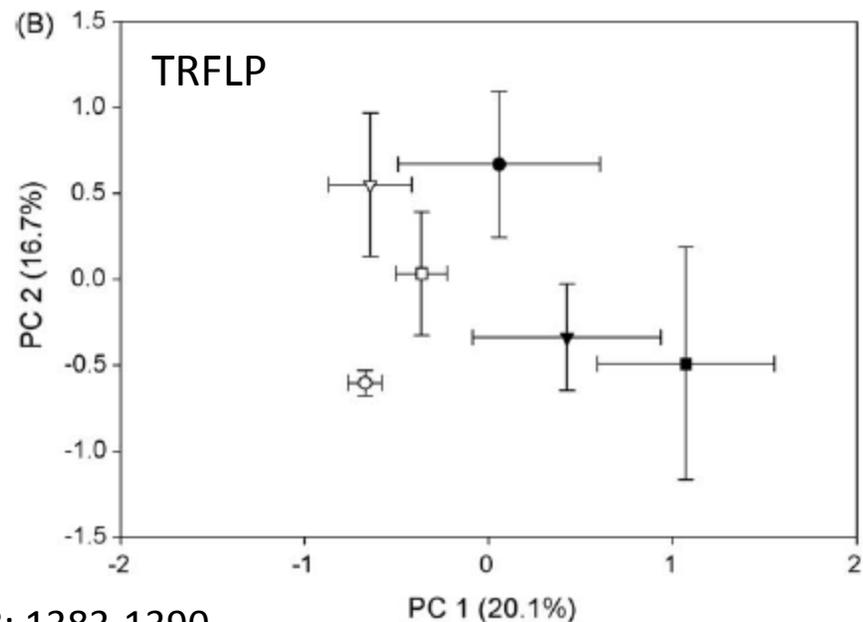
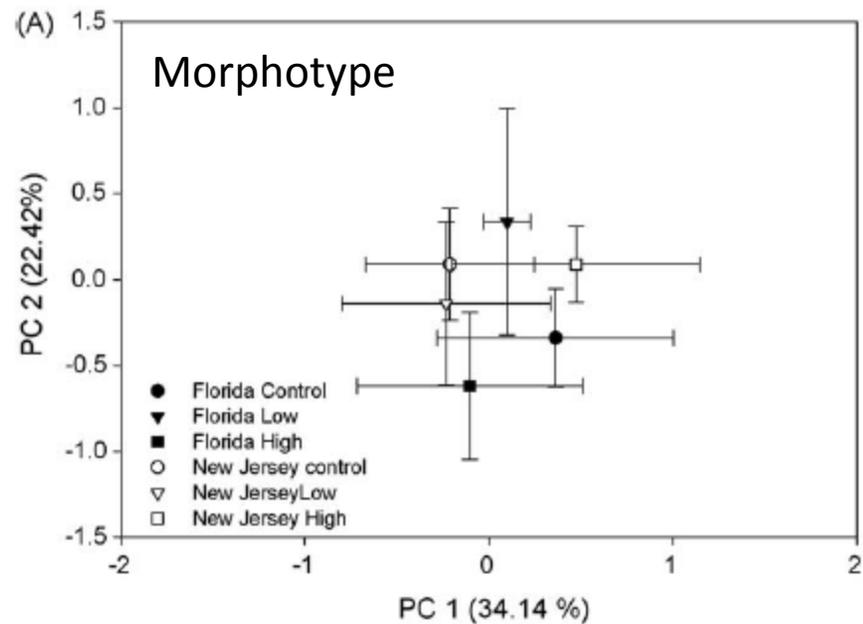


## Acute N deposition

Ectomycorrhizal community composition from ECM morphology (A) and molecular TRFLP (B) from NJ and Florida under 3 N additions (0, 35 and 70 kg ha<sup>-1</sup> above ambient) for 1 year.

No significant difference between sites or N level

No difference in richness between sites or between N levels at either site

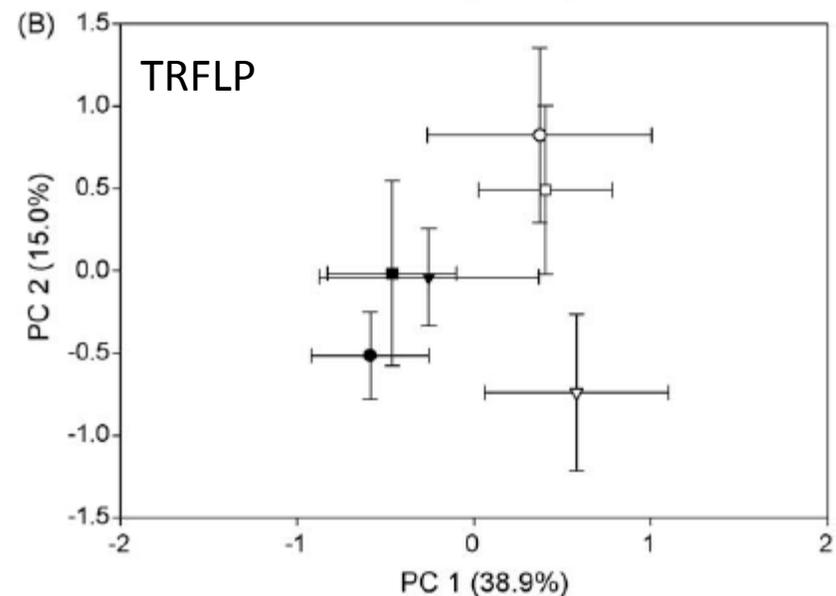
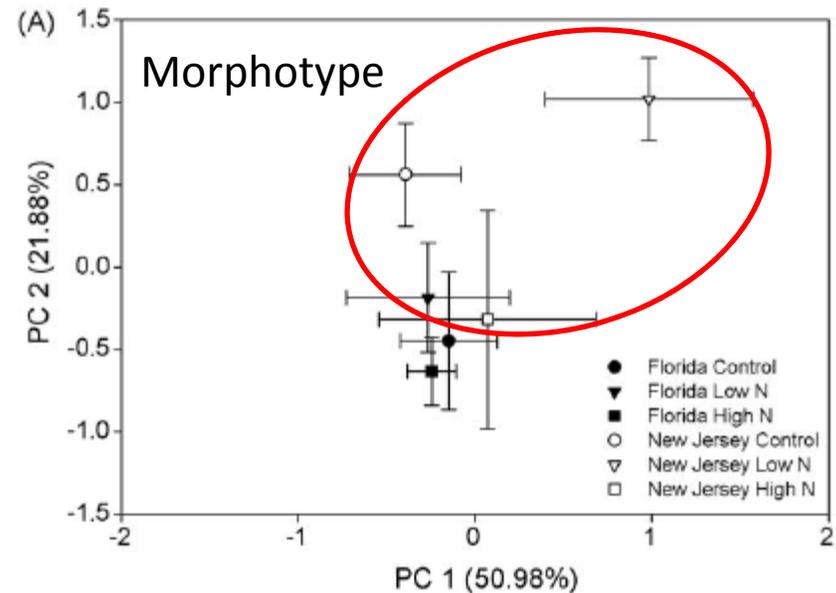


From Krumins *et al.* (2009) *For. Ecol. Manage.* 258: 1383-1390.

**Bacterial community composition from colony morphology (A) and molecular TRFLP (B) from NJ and Florida under 3 N additions (0, 35 and 70 kg ha<sup>-1</sup> above ambient) for 1 year.**

**Significant difference between sites and between N levels within NJ by morphotyping only**

**Bacterial richness significantly higher in FL than NJ and increasing with N addition**



From Krumins *et al.* (2009) *For. Ecol. Manage.* 258: 1383-1390.

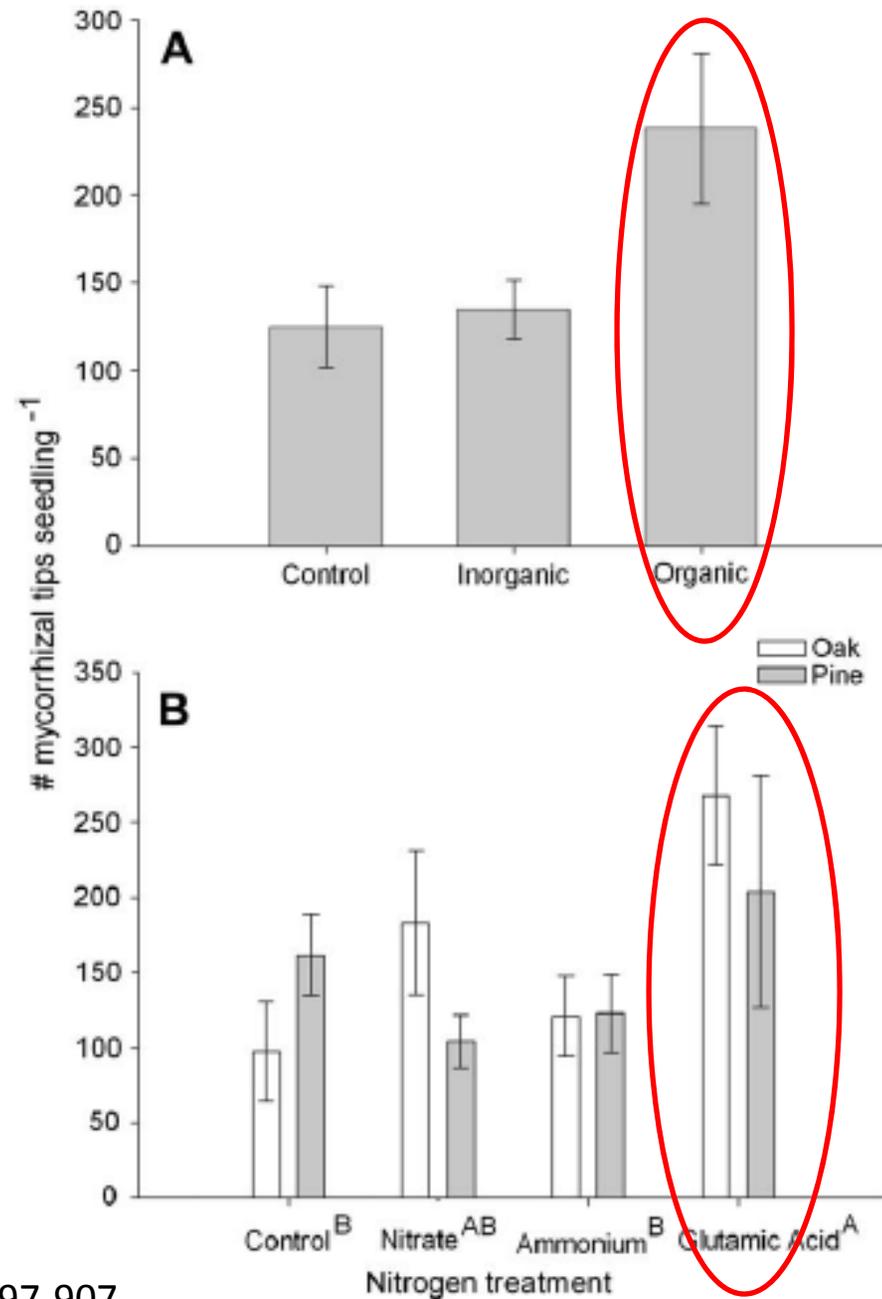
But .....

About 1/3rd of N deposition is from organic N

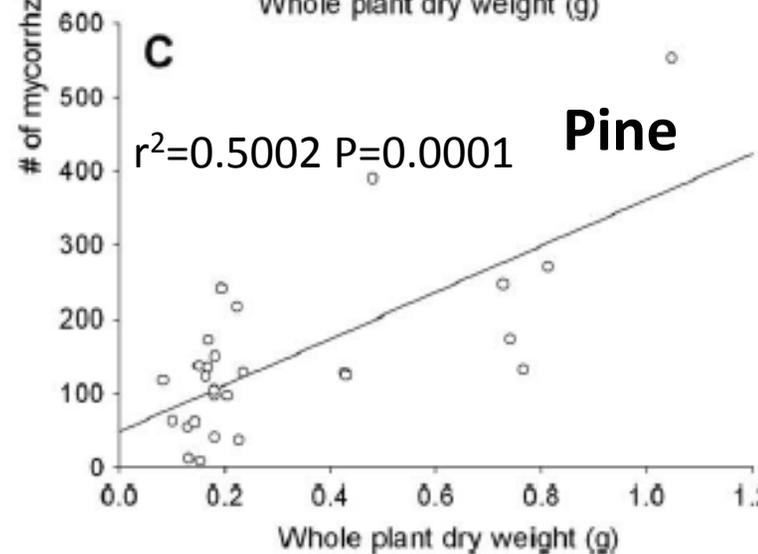
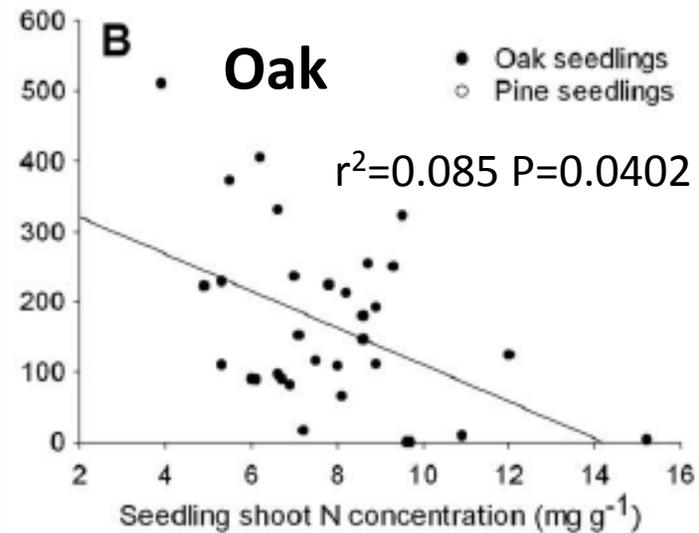
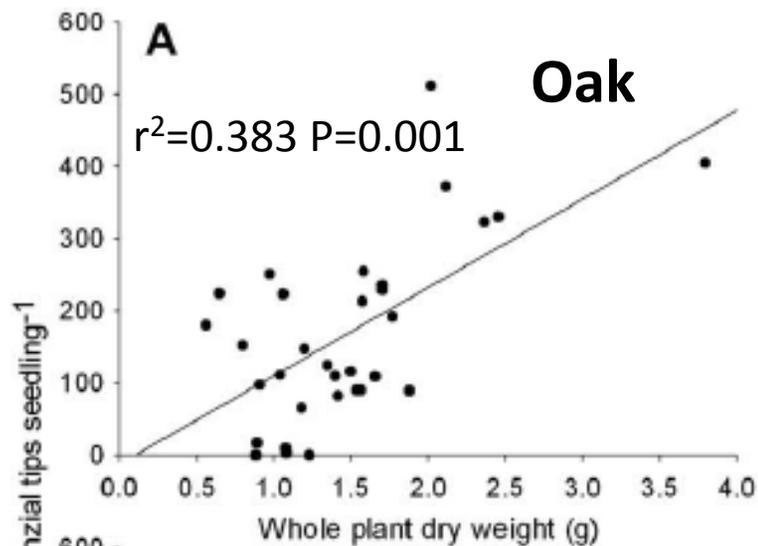
(Tamm *et al.* 2002 Biogeochem 57/58: 99-136)

Most studies have been on inorganic N  
deposition

**Ectomycorrhizal root colonization of pitch pine and black oak seedlings in NJ pine barrens soil amended with inorganic or organic N (227.5 kg ha<sup>-1</sup> y<sup>-1</sup> equivalent over 10 weeks)**



From Avolio *et al.* (2009) Mycol. Res. 113: 897-907.



**Mycorrhizal colonization by pine is correlated only to plant mass.**

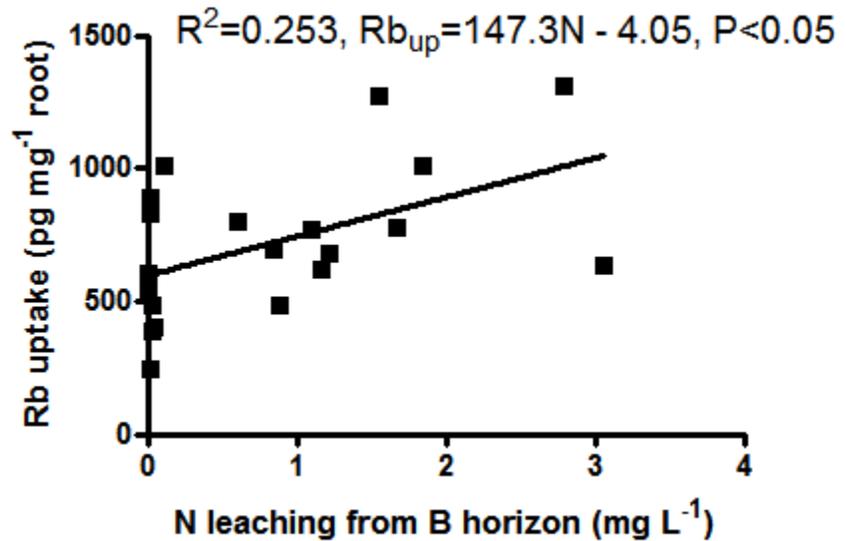
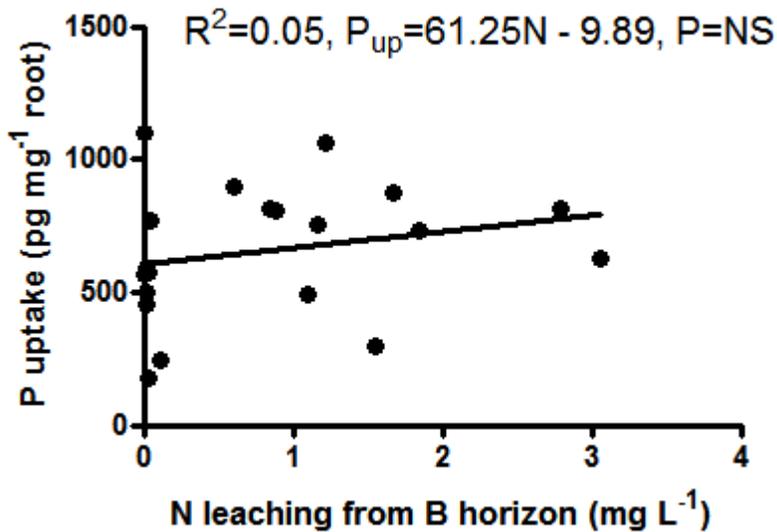
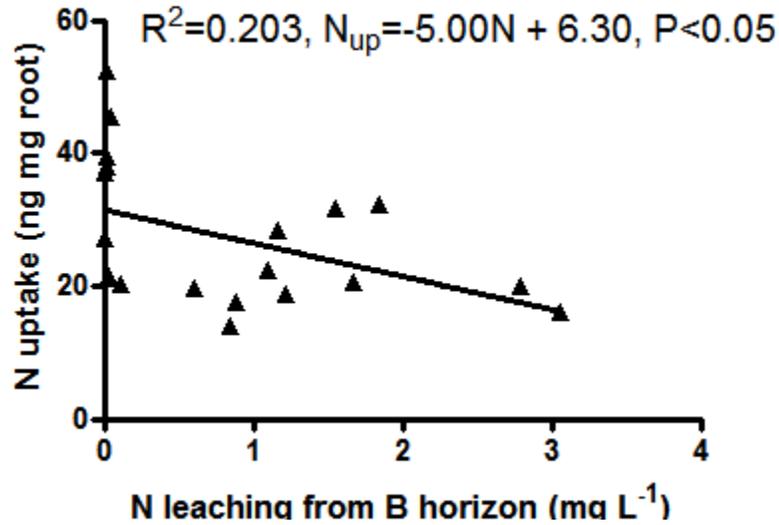
**Colonization of oak is related to both plant mass and shoot N concentration**

So .... are trees selecting mycorrhizal associates and root colonization rate in relation to their growth and nutrient status differentially in order to optimize their nutrient ratios?

Plant type	Suggested optimum nutrient ratios				
	N	P	K	Ca	Mg
Deciduous plants *	100	14.6	64.6	7.0	9.4
Coniferous plants *	100	15.0	47.5	8.0	7.5
Herbaceous plants *	100	14.3	68.3	8.3	8.7
Birch **	100	8	30	2	4

From Knecht & Goransson (2004) Tree Physiol. 24: 447-460

N dep = 20-30 kg ha<sup>-1</sup> y<sup>-1</sup>  
N demand = 56 kg ha<sup>-1</sup> y<sup>-1</sup>



## Effect of N deposition on N, P and K demand by Sitka spruce forests in Wales

Data reworked from Harrison *et al.* (1995) For. Ecol. Manage. 76: 139-148

# Critical Loads

- Critical loads for ecosystem are based on the N saturation point of soil – N leaching
- For ectomycorrhizae, Wallander & Kottke (1998) cite examples where  $35 \text{ kg N ha}^{-1} \text{ y}^{-1}$  cause changes in sporophore communities
- Suggested setting critical load at  $15\text{-}20 \text{ kg N ha}^{-1} \text{ y}^{-1}$  for sporophores
- They suggest that mycorrhizal roots are less sensitive to N changes, so critical load at  $40\text{-}60 \text{ kg N ha}^{-1} \text{ y}^{-1}$

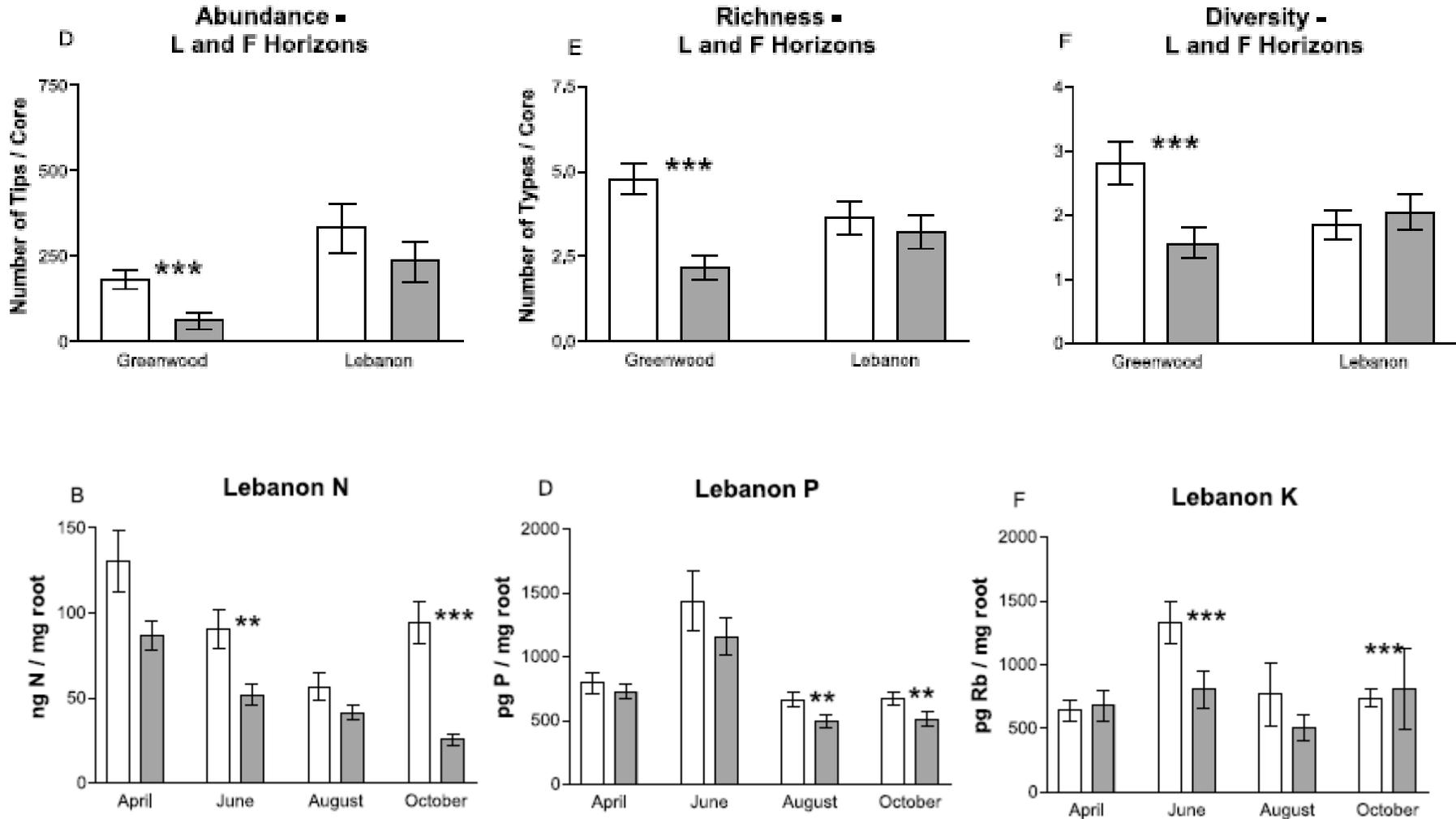
# Pine Barrens Oligotrophic Ecosystem

- We have demonstrated changes in ectomycorrhizal root communities over a range of  $\sim 5 \text{ kg N ha}^{-1} \text{ y}^{-1}$  with max of  $8 \text{ kg N ha}^{-1} \text{ y}^{-1}$  chronic N deposition
- So should critical loads for mycorrhizae be adjusted according to soil type
- We have not been able to cause a change in ECM community structure on pine with acute N deposition, but have with oak.
- So, are we forest species dependent in response

# The Balance of Nutrients

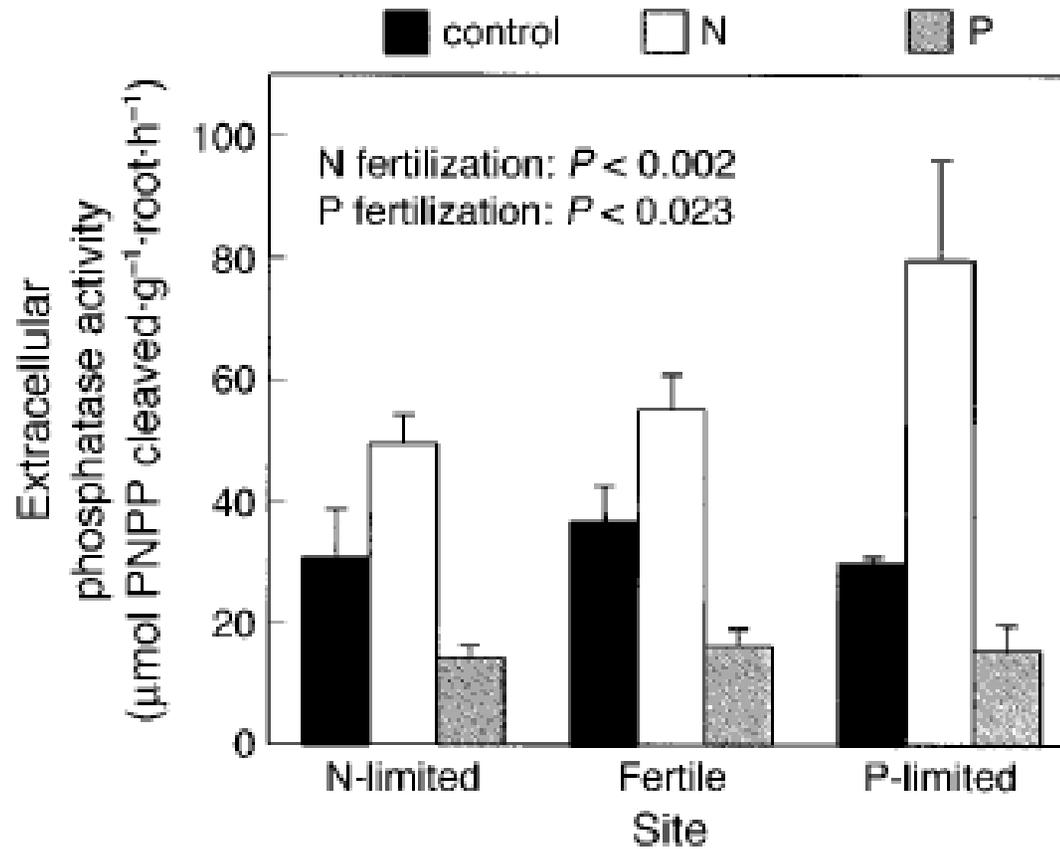
- Lessons from Control Burning
  - Effects on soil nutrients
  - Effects on ectomycorrhizae
  - Root bioassays – what the tree thinks

# Effect of prescribed burning on ectomycorrhizae and changes in nutrient demand by trees



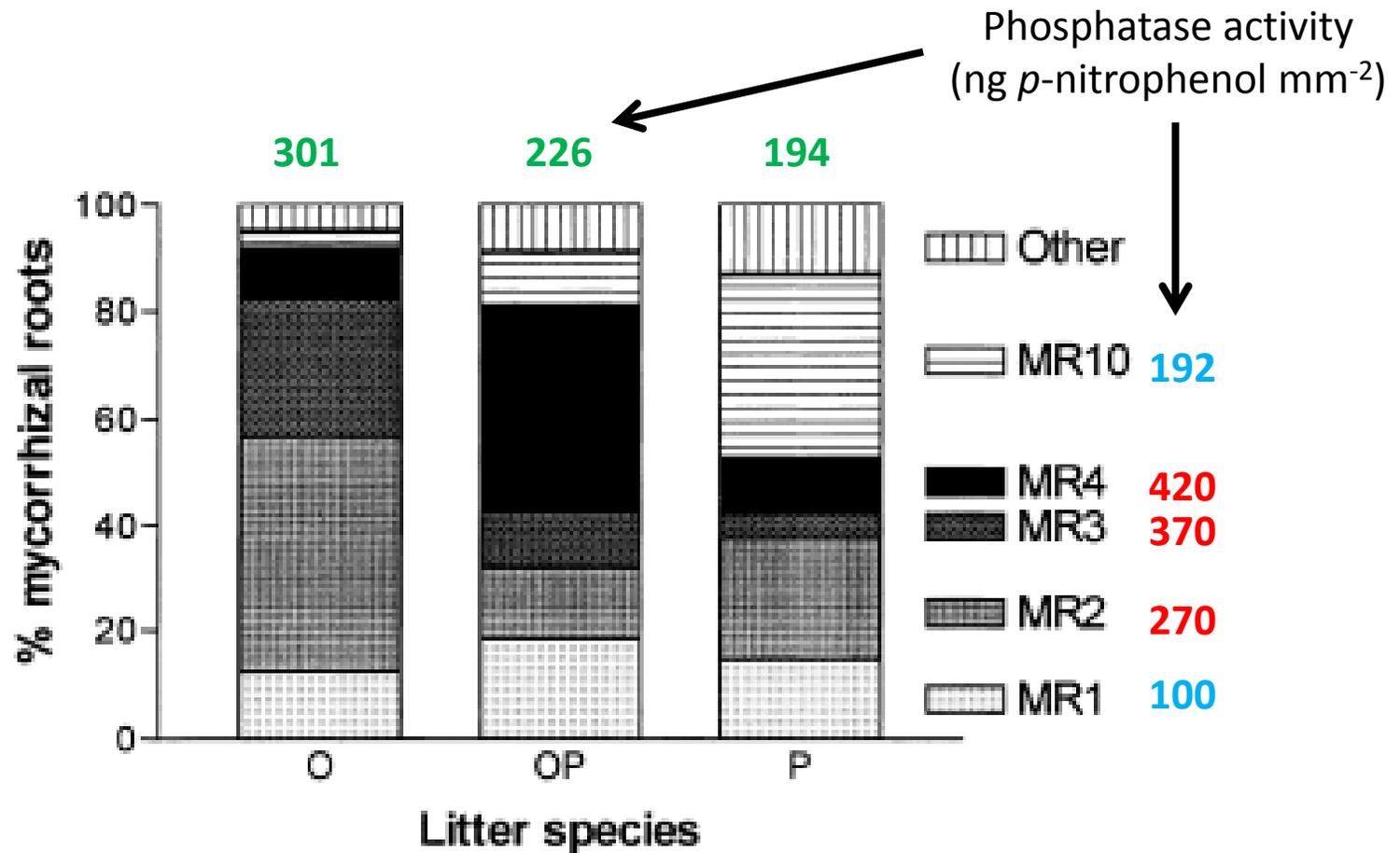
From Tuininga & Dighton (2004) Can. J. For. Res. 34: 1755-1765

N & P added at 100 kg ha<sup>-1</sup> y<sup>-1</sup>



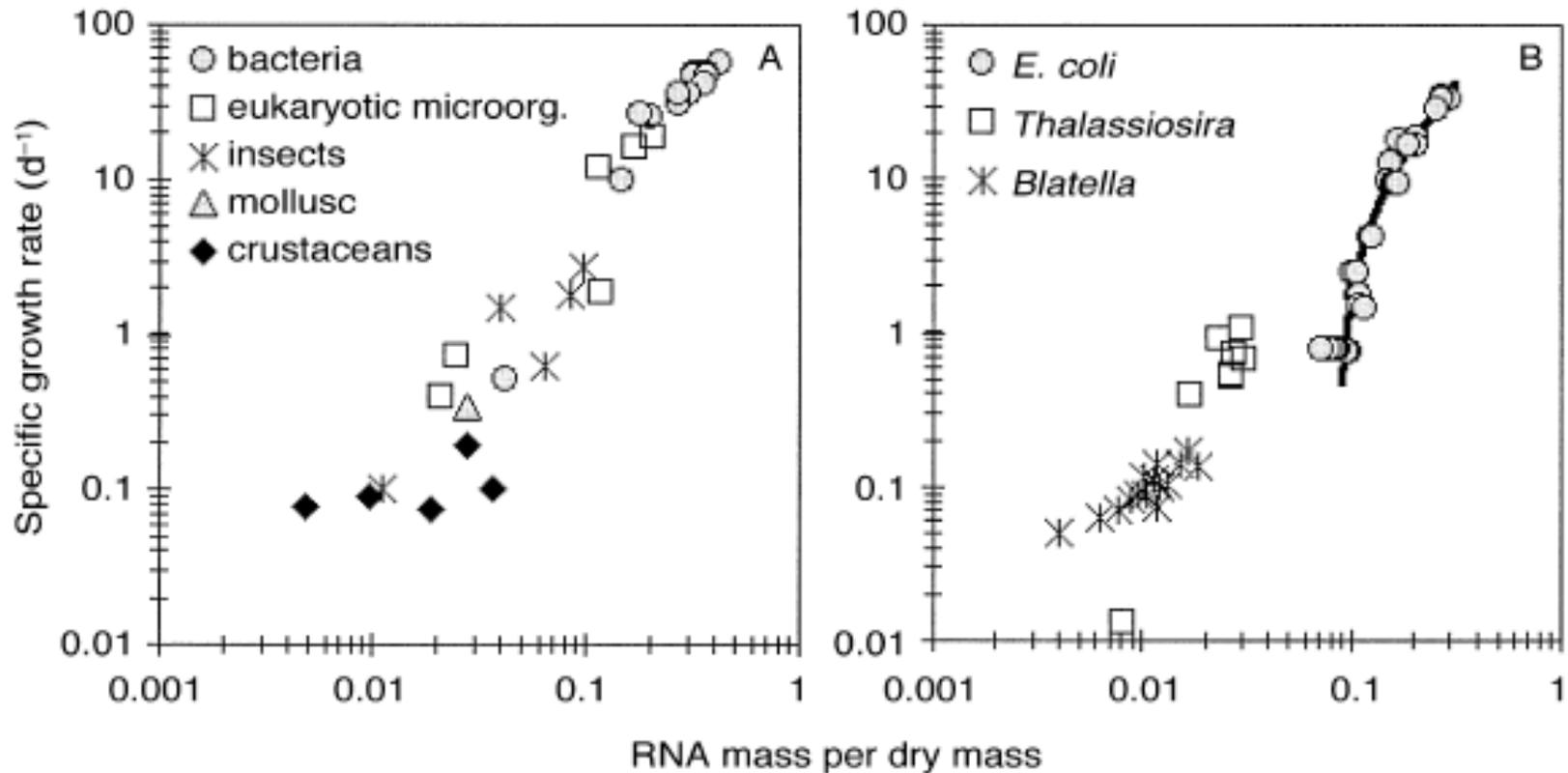
**Root/arbuscular-mycorrhizal phosphatase production with N and P addition to soils of contrasting fertility in evergreen forest in Hawaii**

From Treseder & Vitousek (2001) Ecology 82: 946-954



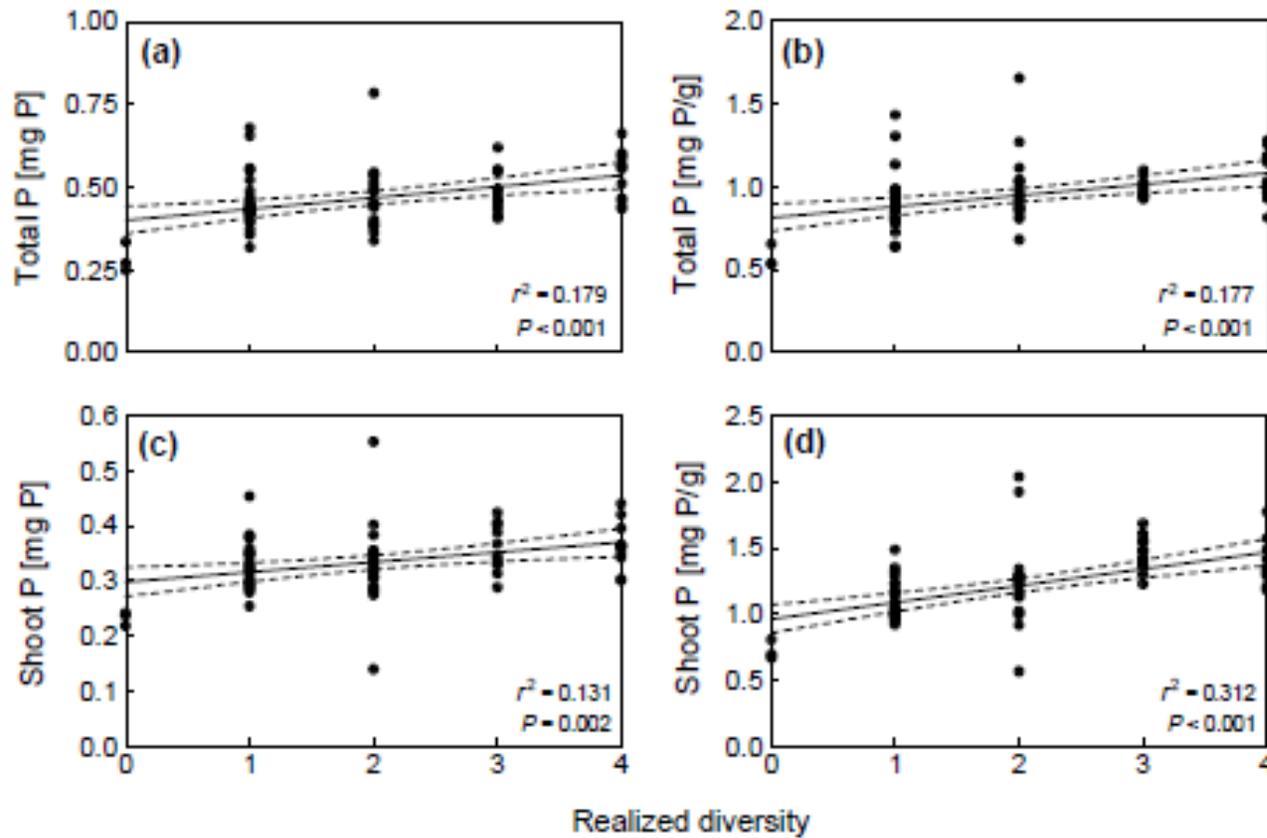
## Effect of leaf litters on ectomycorrhizal community composition and acid phosphatase activity

From Conn & Dighton (2000) Soil Biol. Biochem. 32: 489-496



**Relationship between organism growth rate and RNA content, which is highly dependent on C:N:P stoichiometry – particularly important for rubisco production in autotrophs (Vrede *et al.* (2004) Ecology 85: 1217-1229)**

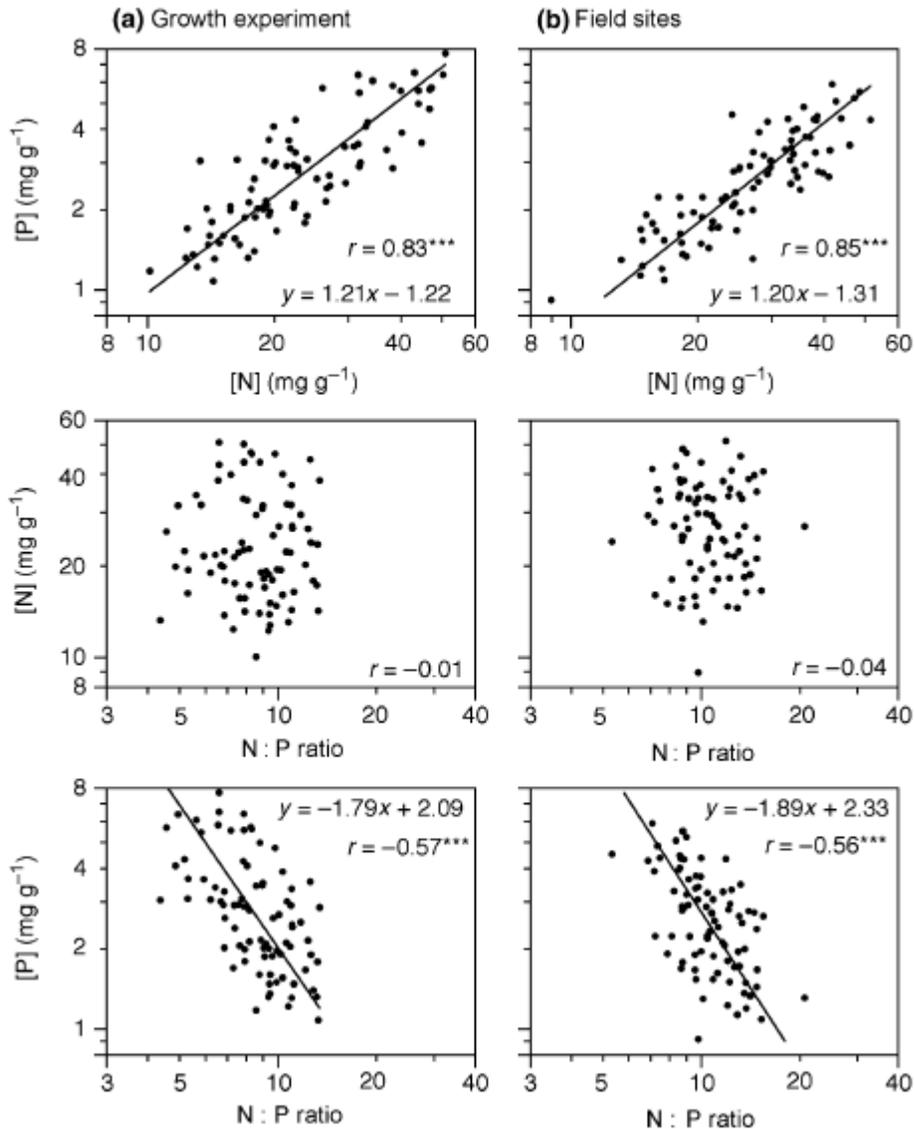
**But in pine, rubisco production is better correlated to P availability than N availability (Warren & Adams (2004) Tree Physiol. 22: 11-19)**



**Effect of mycorrhizal diversity on host plant P content**

**No effect on N content**

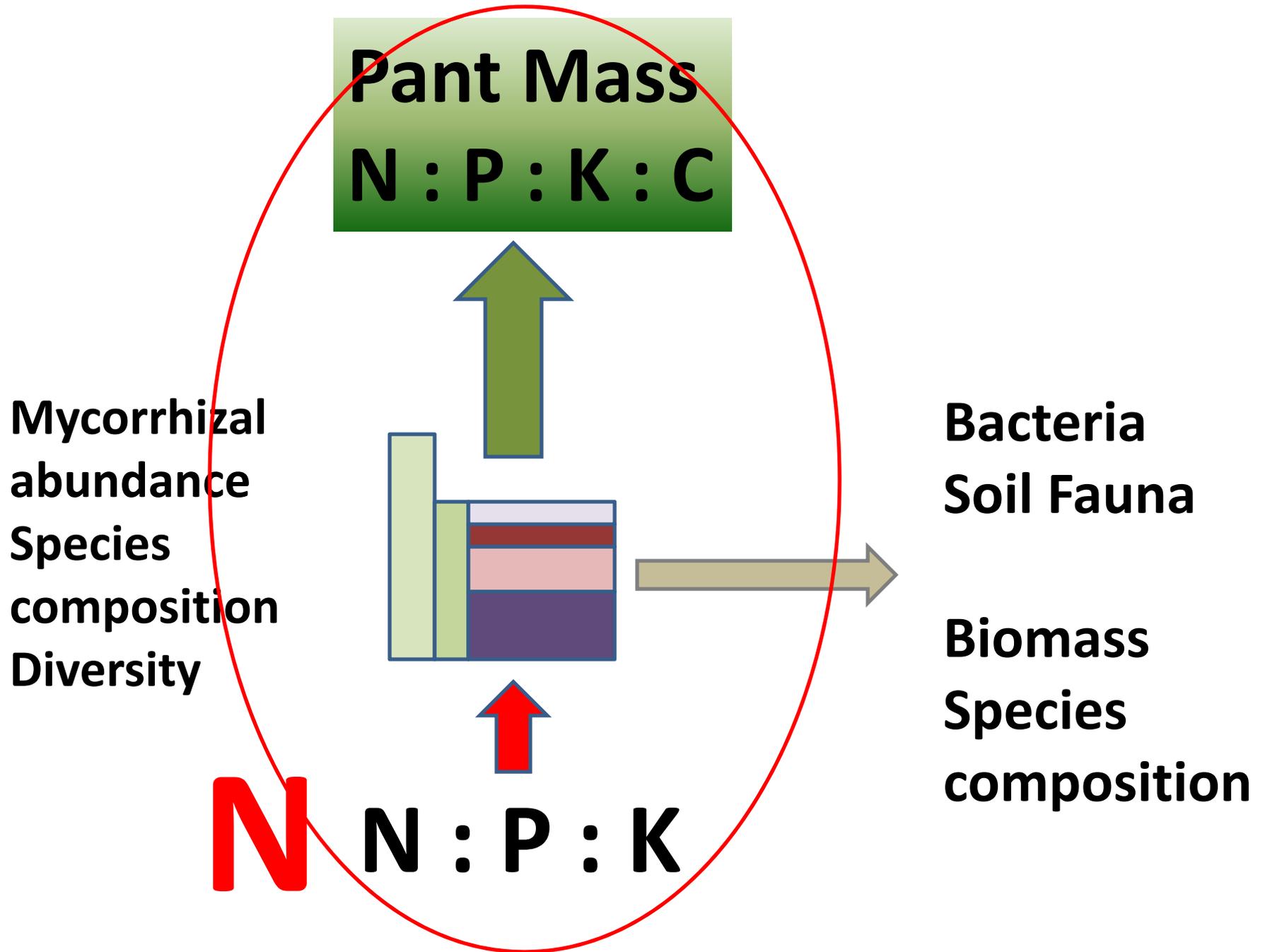
From Baxter & Dighton (2001) *New Phytol.* 152: 139-149



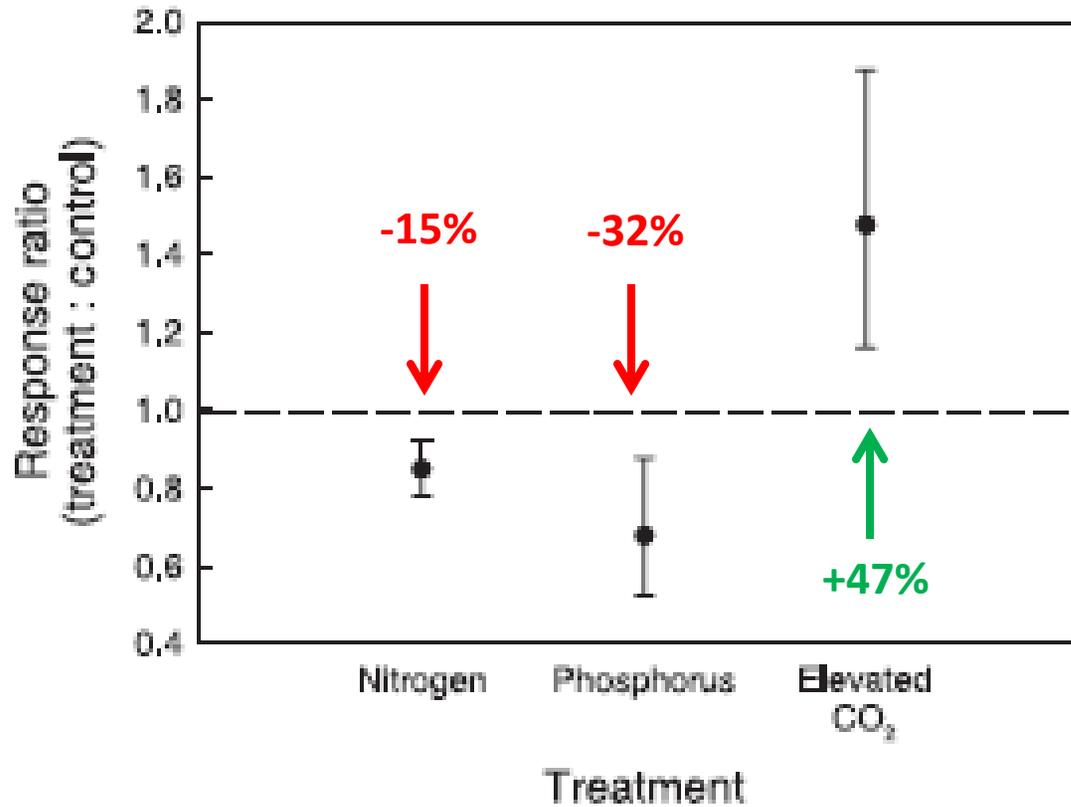
**Effect of N and N:P ratio supply on P and N content of woody plants in experimental and field conditions**

**N:P ratio influences P acquisition, not N**

From Guswell (2004) *New Phytol.* 164: 243-266



# Relative importance of N as regulator of mycorrhizae



**Mycorrhizal responses to N or P addition and elevated CO<sub>2</sub> from a meta-analysis of data from the literature**

From Treseder (2004) *New Phytol.* 164: 347-355

# Conclusions

- N deposition affects both mycorrhizal colonization of roots (abundance and richness) and fruiting structures (sporocarps) – differential levels
- The level of N dep to have an effect differs between soil types – oligotrophic soils are more sensitive
- The impact of N dep may be more important to other groups of soil organisms by direct and indirect effects
- Stoichiometric considerations are probably more important than concentrating on a single nutrient element
- There may be stoichiometric interactions that influence attainment of optimum plant nutrient stoichiometry and plant fitness – may be important in competition and invasiveness

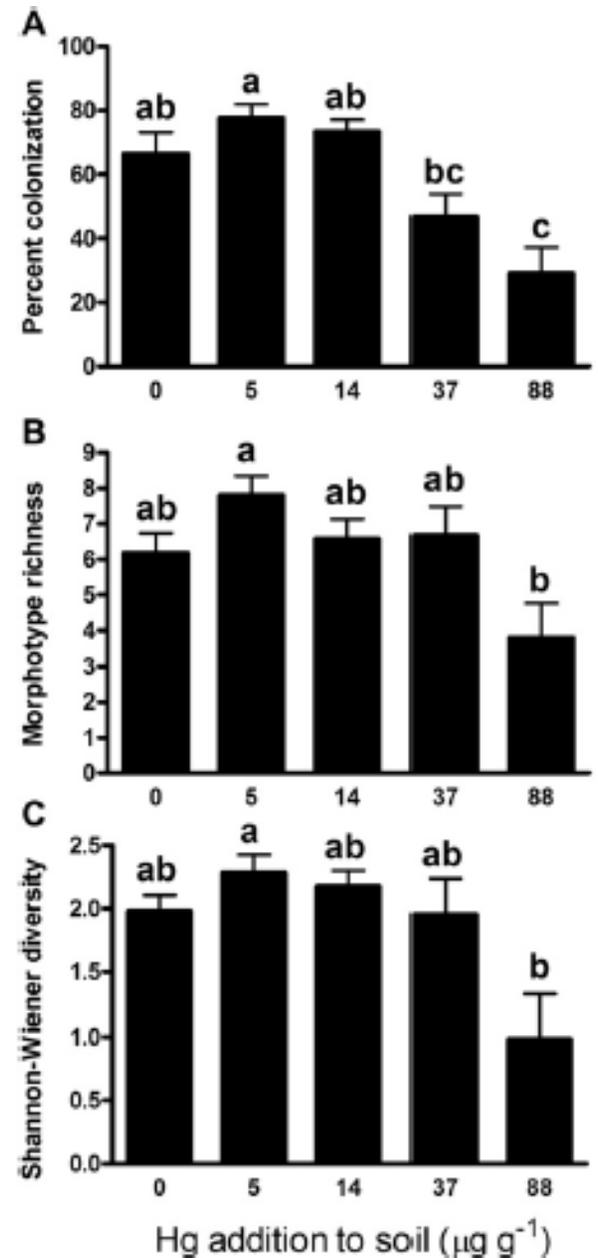
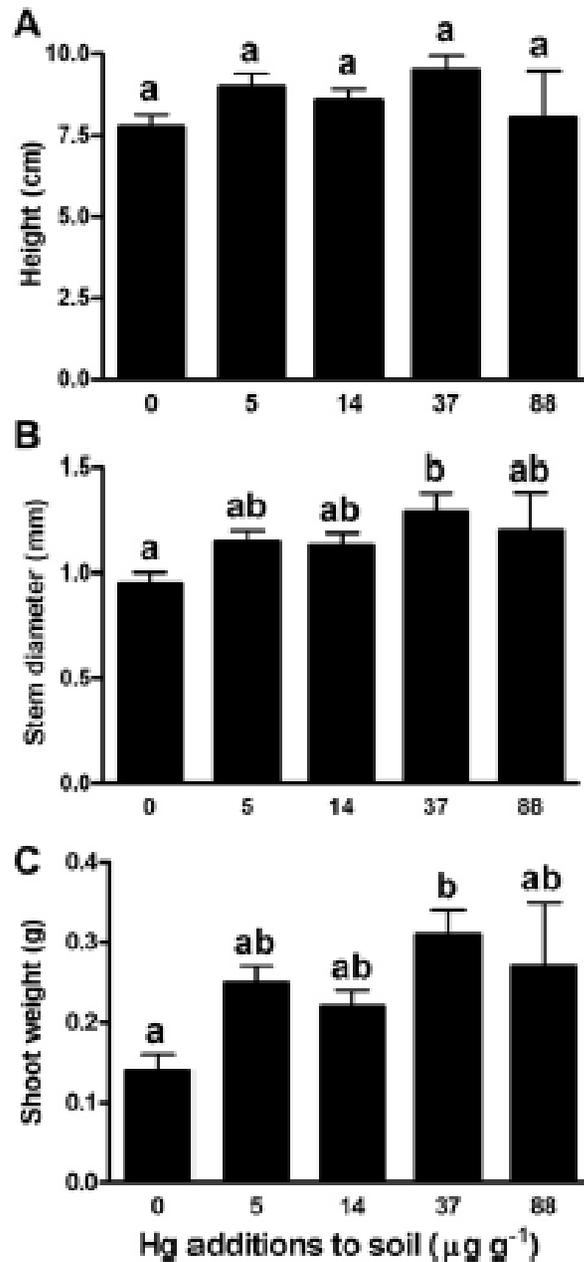
# Hg pollution

- Interactions with mycorrhizae
- Interactions in phylloplane

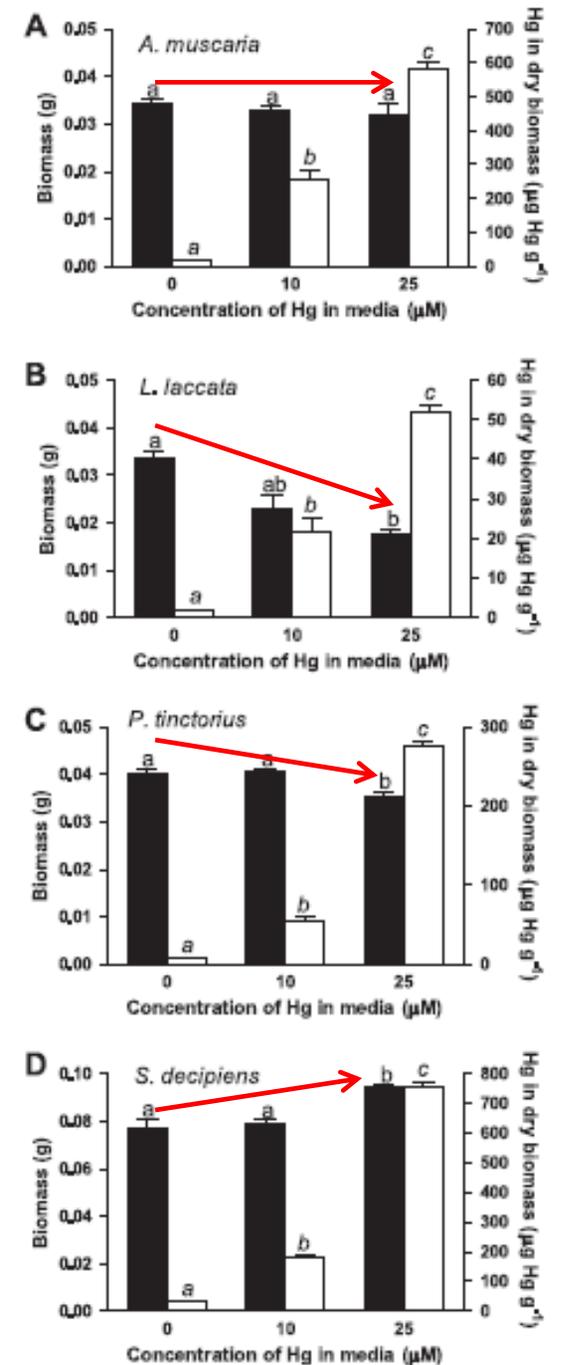
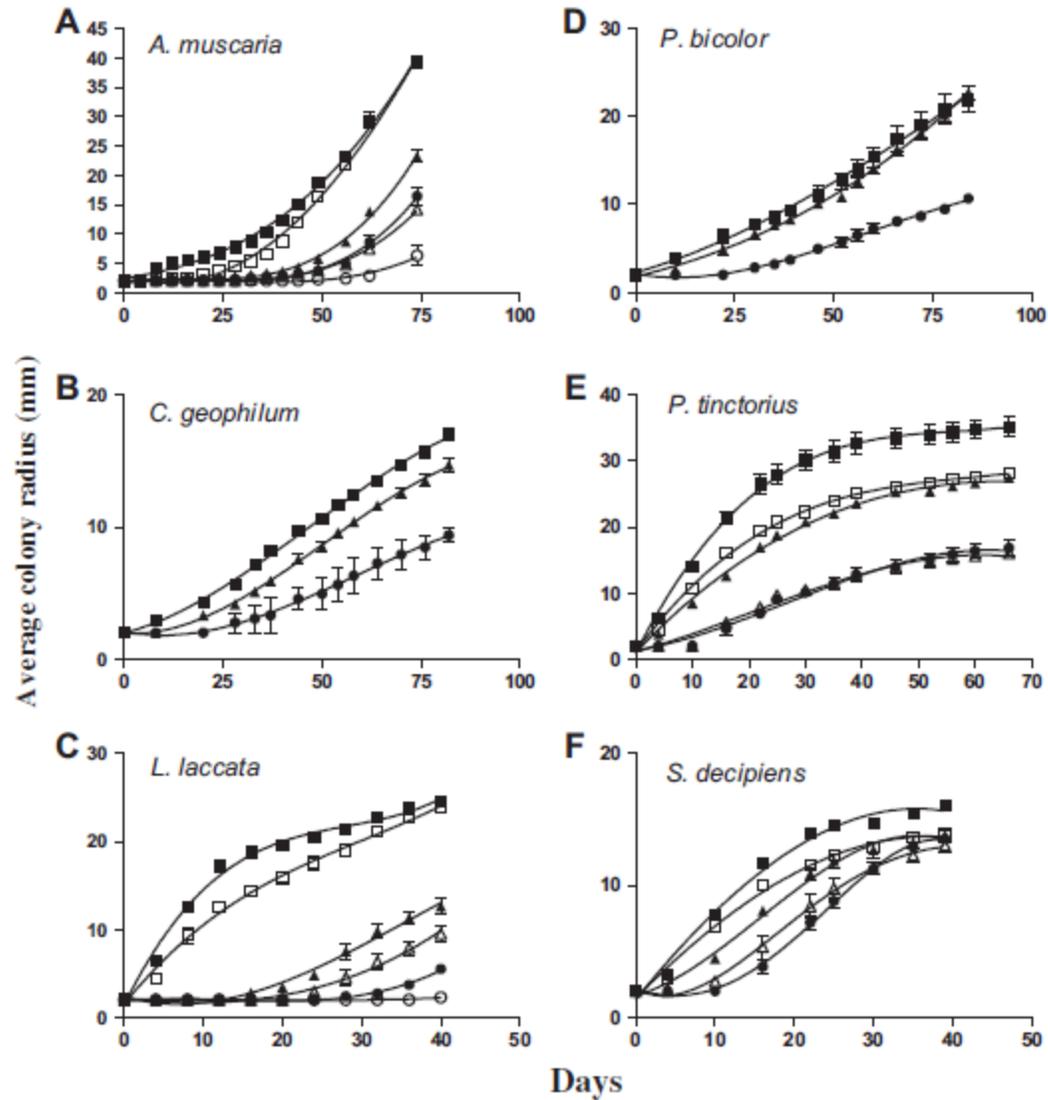
# Effects of Hg concentration in soil on pitch pine seedling growth and mycorrhizal colonization of roots

## Possible threshold response of mycorrhizae

From Crane et al. (2012)  
Fungal Ecol. 5: 245-251



# Growth trajectories of ECMF at different Hg levels and effects on plant growth



From Crane et al. (2010) Fungal Biol. 114: 873-880

# Hg and phylloplane fungi

See next seminar

Katie Malcolm

# The Chernobyl Accident

- Was a world-wide experiment
- The fate of Cs in the environment could be tracked as:
  - $^{137}\text{Cs}$ : $^{134}\text{Cs}$  ratio was 2:1
  - $^{137}\text{Cs}$  half life = 28 yrs
  - $^{134}\text{Cs}$  half life = 2.2 yrs
- Thus able to identify source of  $^{137}\text{Cs}$  in the environment

# Radiocaesium in Ecosystem Components in Swedish Boreal Forest at Deposition of 220 kBq m<sup>-2</sup>

(Guillette *et al.*, 1994)

Ecological component	<sup>137</sup> Cs content (kBq kg <sup>-1</sup> )
Leaf litter and A horizon	40
Total organic soil horizon	19
Facultative mycorrhizae	230
Obligate mycorrhizae	120
Saprotrophic fungi	140
Lichens	36
Mosses	19
Ferns	16
Angiosperms	8

# Percentage of $^{137}\text{Cs}$ in Mushrooms, not Derived from Chernobyl

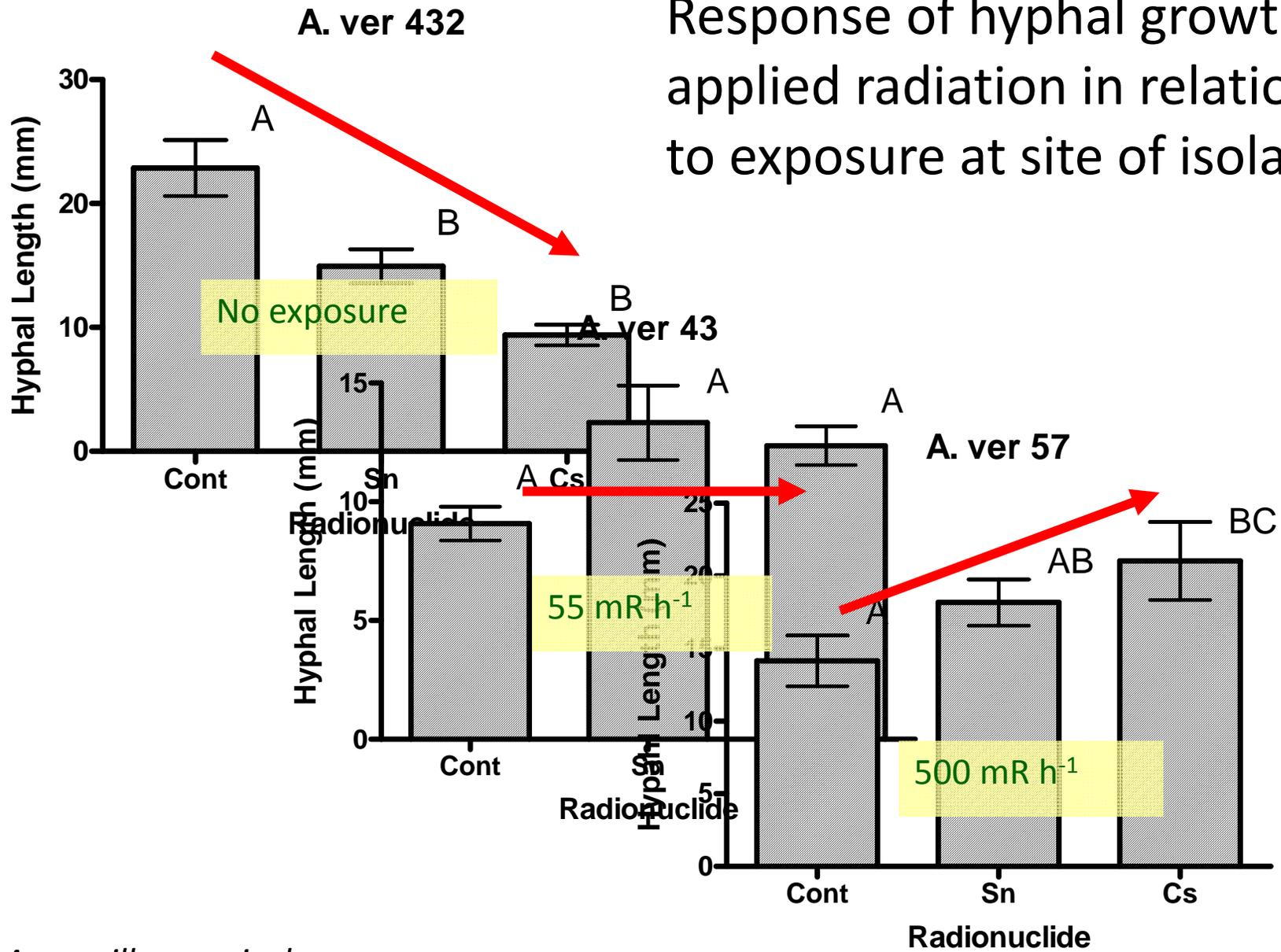
(Dighton & Horrill, 1988)

Fungal Species	Location	% of $^{137}\text{Cs}$
<i>Lactarius rufus</i>	MH86	92
	MH87	81
	St	74
	S2	67
	SB	73
	B	25
<i>Inocybe longicystis</i>	SB	75
	S4	83

# Adaptive Properties of Fungi

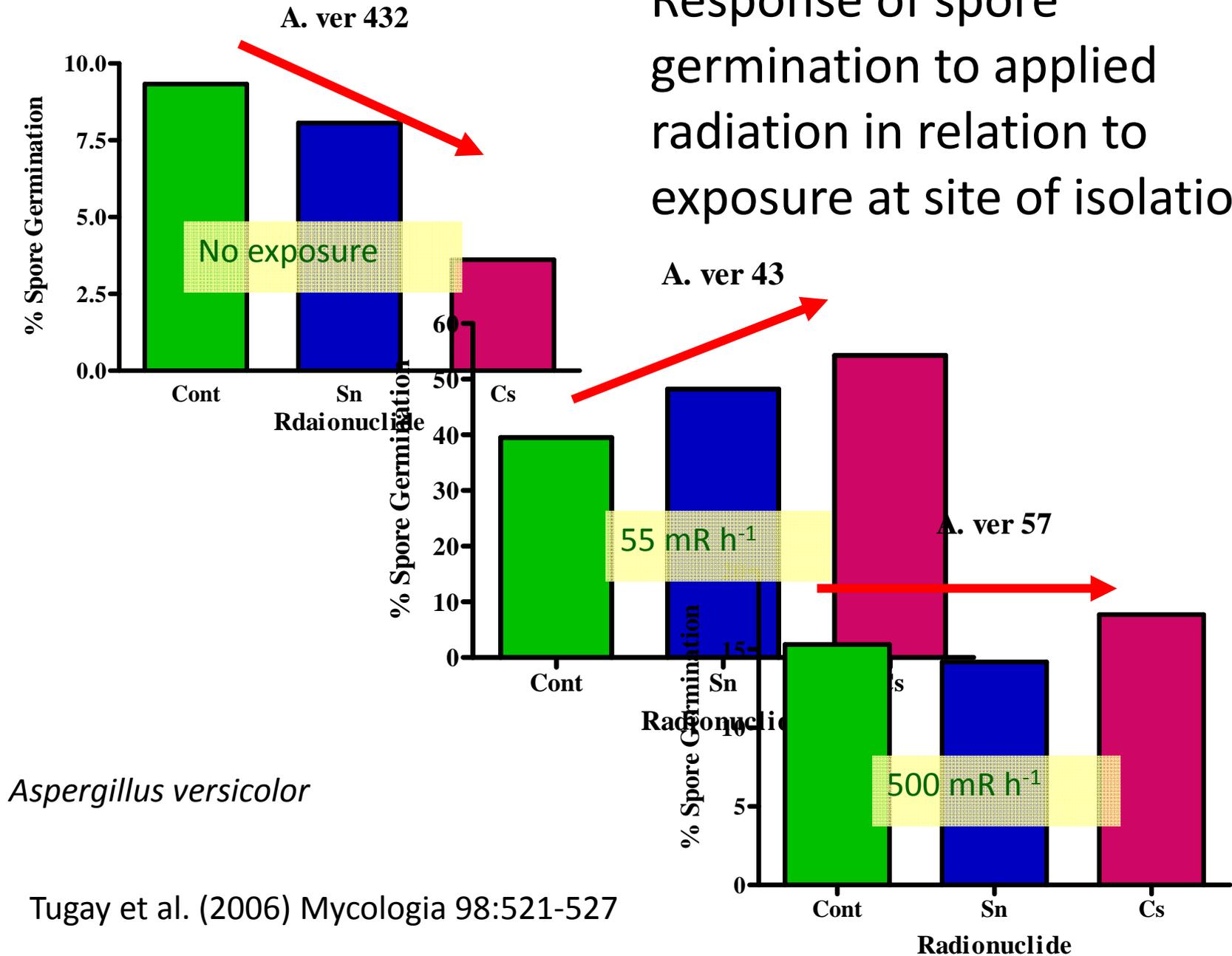
- When previously exposed to radiation, we have found that fungi appear to adopt properties that fungi from non-radioactive area do not possess. These adaptive properties include:
  - **Enhanced spore germination**
  - **Enhanced hyphal growth**
  - **Directional growth towards sources of radiation (radiotropism)**

Response of hyphal growth to applied radiation in relation to exposure at site of isolation



*Aspergillus versicolor*

# Response of spore germination to applied radiation in relation to exposure at site of isolation

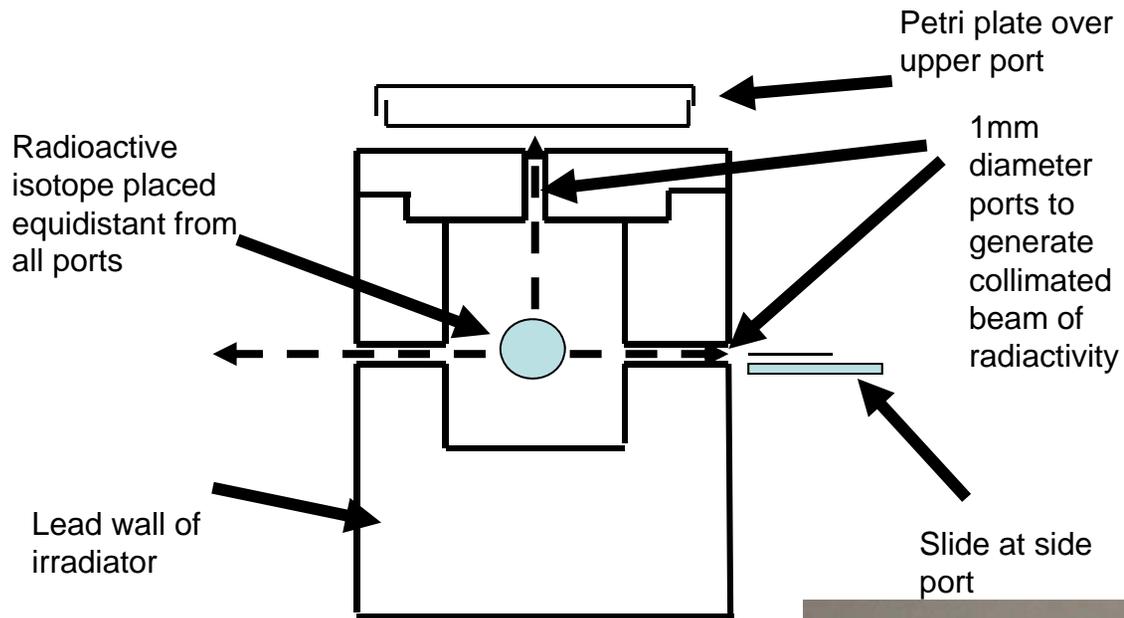


# Summary

- Exposure to radiation has contrasting effects on spore germination and hyphal extension
  - Prior exposure to radiation can stimulate responses
  - Responses are dose related
  - We can see hormetic responses
  - Responses are genus and species specific

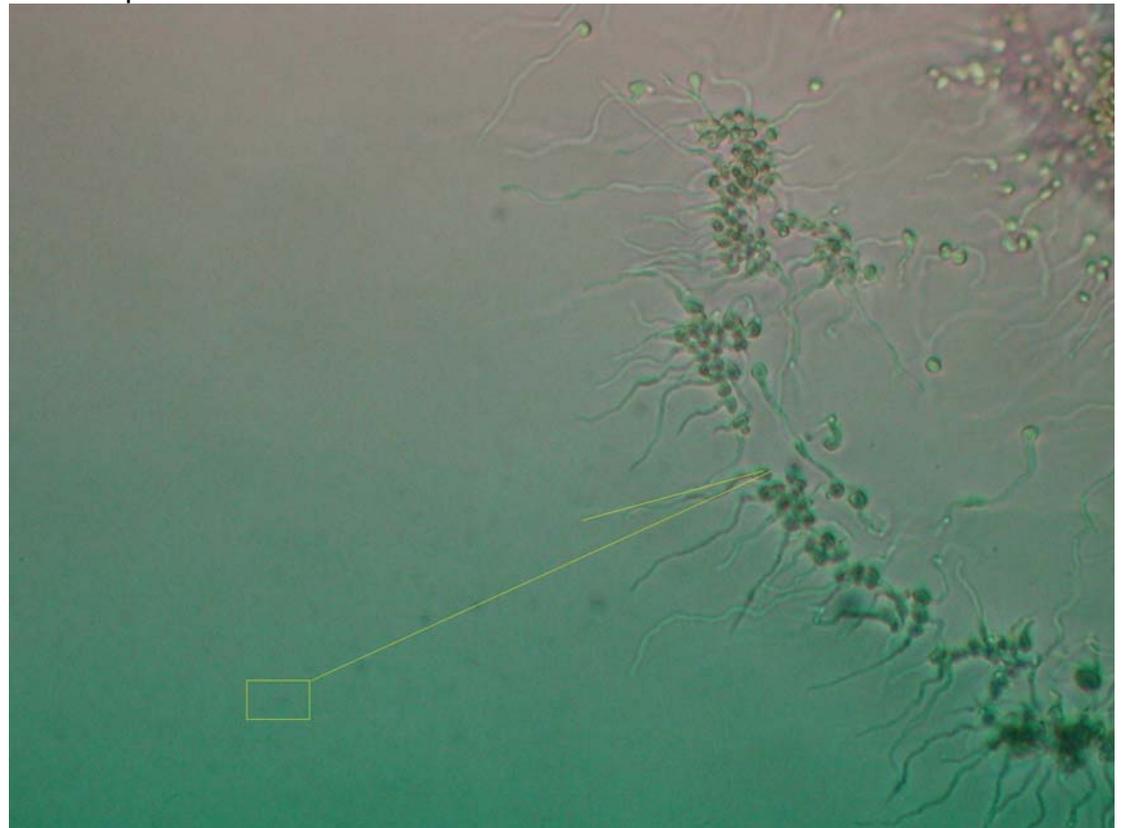
# Radiotropism

- Or directed growth of hyphae towards radiation



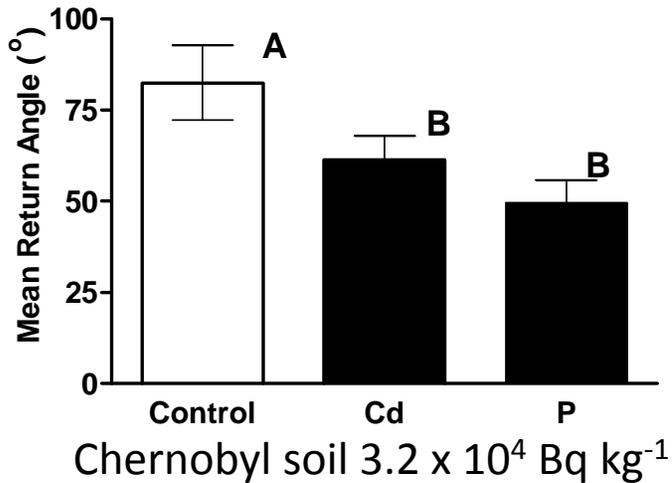
Assembly for delivering a collimated beam of ionizing radiation to fungi

Calculation of 'return angle'

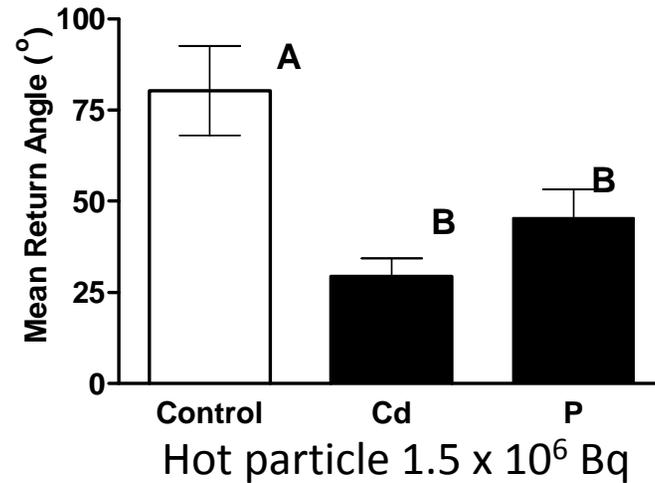


# Hyphal Return Angle When Exposed to $^{109}\text{Cd}$ ( $\gamma$ ) or $^{32}\text{P}$ ( $\beta$ ) Collimated Beam of Radiation

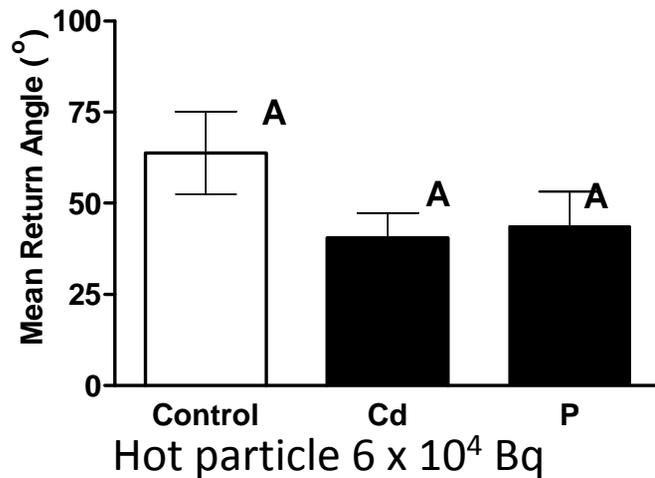
*P. lil* 1941



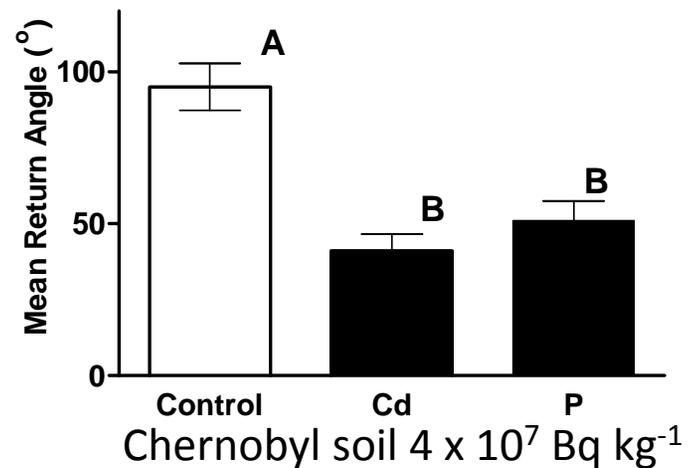
*P. hirz* 3



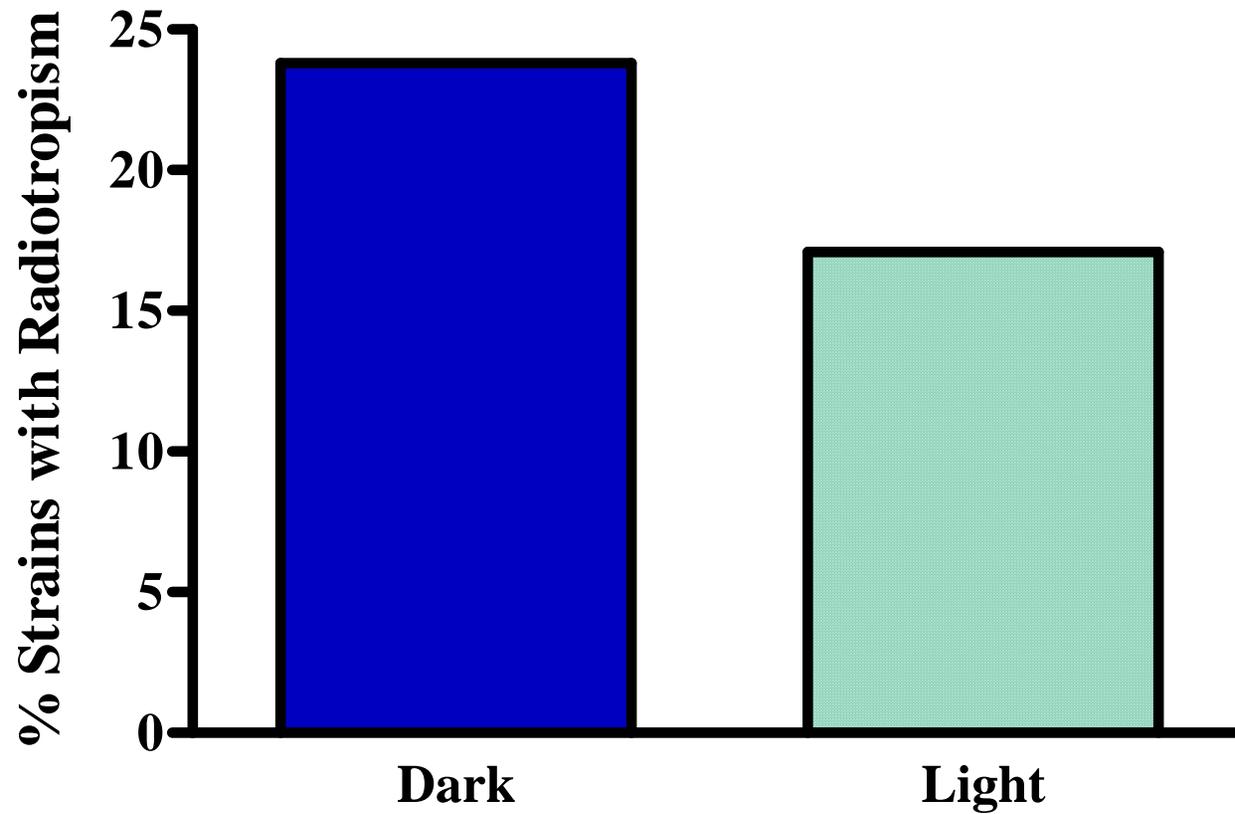
*P. hirz* 1



*Cl. cl* 4



## Frequency of Occurrence of Radiotropism in Fungal Isolates Containing Melanin (dark) or Not



# Summary

- Prior exposure to radiation elicited a directional growth of hyphae
- No prior exposure generally resulted in no response
- Magnitude of responses were species dependent
- Directional growth is more prevalent in melanized fungal isolates