# Mycoremediation for Watershed Restoration

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Stropharia rugoso - annulata

# Background

Pathogens and excess nutrients flowing from industry, agriculture, and wastewater threaten Lake Champlain's watershed health. Beaches frequently close after heavy rains due to *E. coli* levels > 235 colony forming units (CFUs)/100ml water. Fungi's primary role of decomposition happens via mycelium. In mycoremediation, fungi degrade or absorb environmental pollutants (Cotter 2014). Fungi destroy pathogens through mycelial 'sweat.' Respiration releases chemicals which denature microbes that die and contribute carbonaceous mass to mycelial nets (Stamets 2005).



# Objectives

Rhizomorphic hyphae of King Stropharia

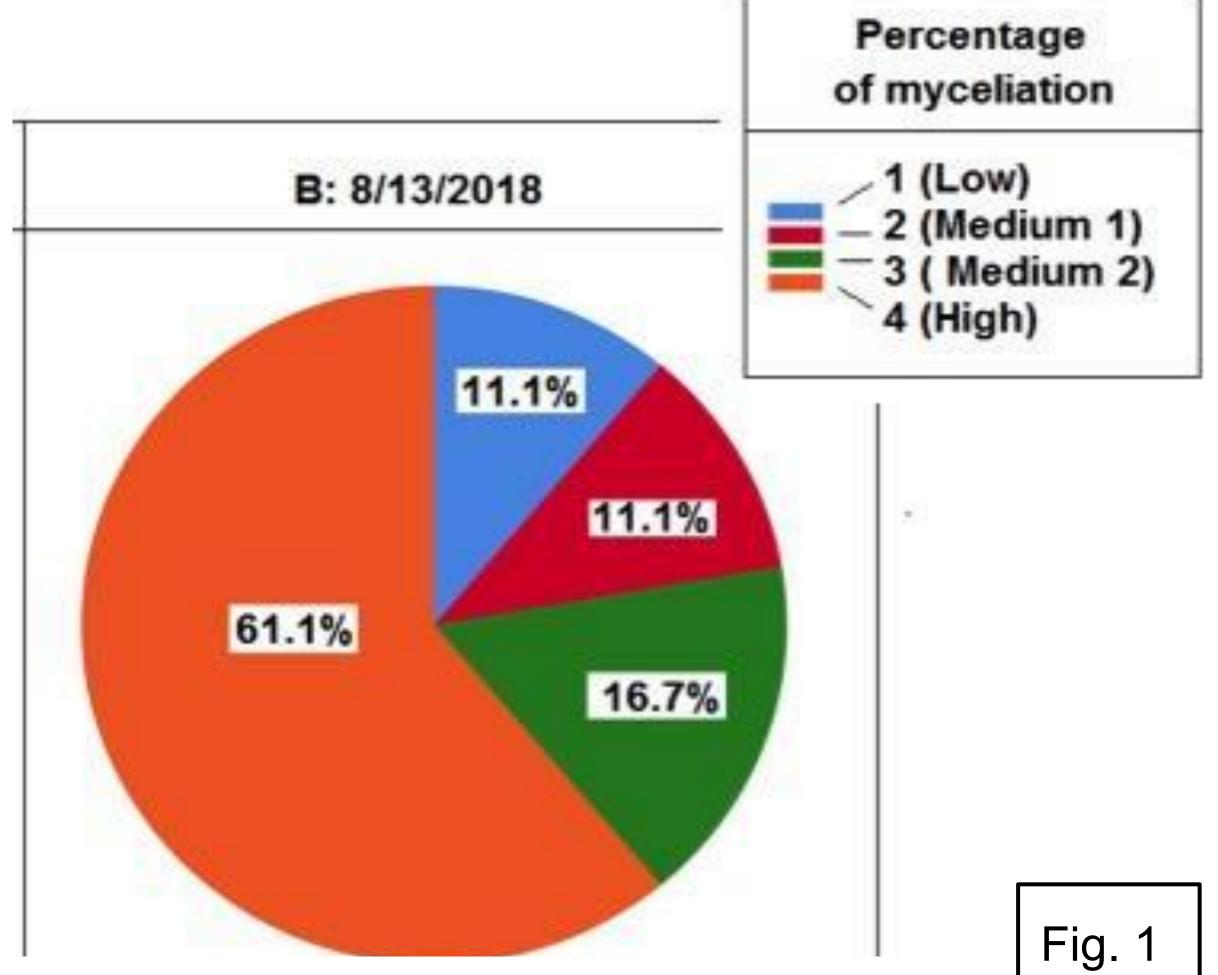
- assess how King Stropharia (Stropharia rugosoannulata) fungi form mycelial mats
- determine if mycelial mats affect nutrient cycling
- assess if mycelial mats effectively reduce CFU's of *E. coli* from manure slurry
- discern if E. coli CFU's drop when exposed to mycelial eluent

### Methods

- Greenhouse-based methods grew mats in 36 66-quart bins of hardwood chips, half and full, myceliated and nonmyceliated. 10 lbs of mushroom spawn inoculated each yard of wood chips. The manure slurry was filtered at 3 concentrations: low *E. coli* = 125 bacteria/100 mL water, middle = 250 bacteria/100 mL, high = 500 bacteria/100 mL; influent was compared to effluent after 22 hr dwell time.
- Laboratory-based mesocosm experiments compared *E.coli* concentrations after passing through 100 g mycelial mats to those after mixing with mat eluent over five minutes.

#### Results

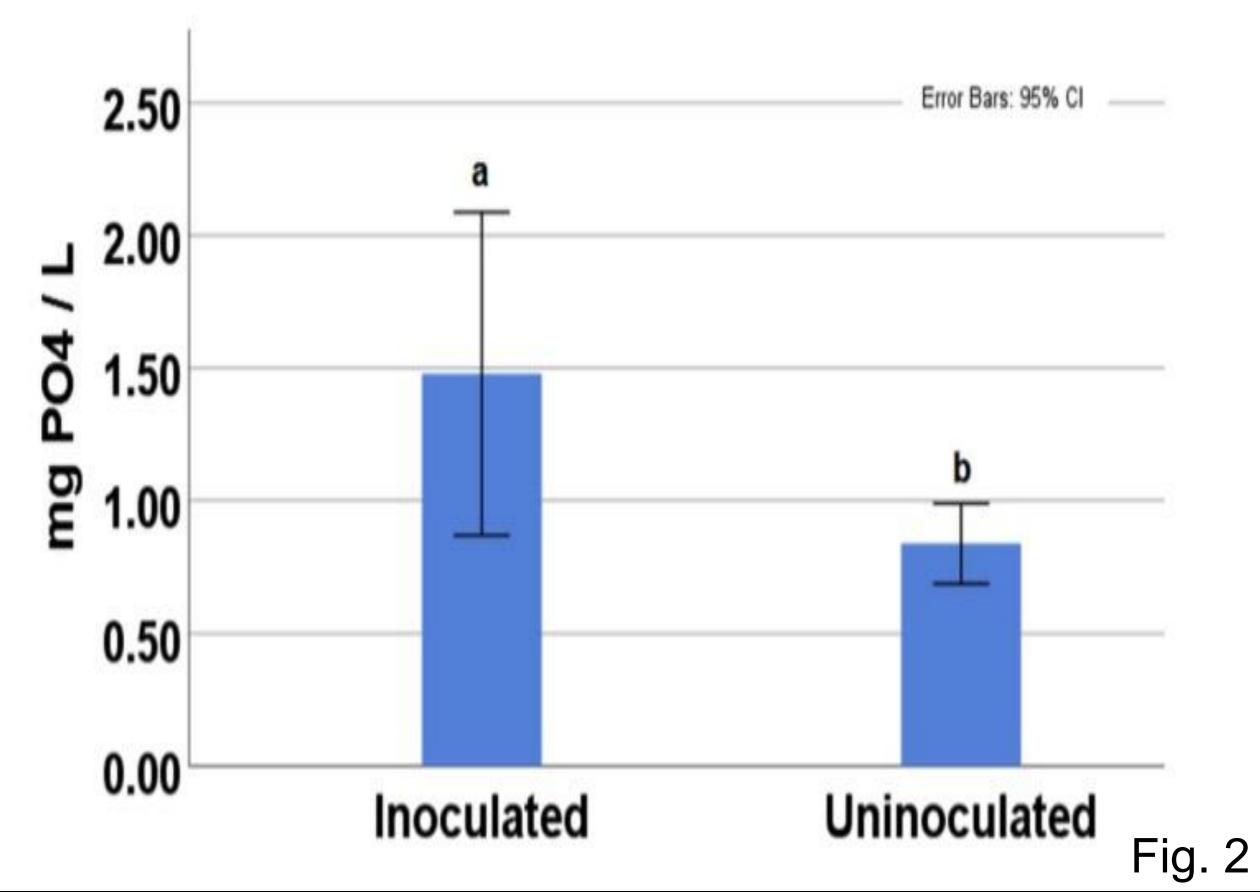
- → Greenhouse experiments revealed patchy colonization. (Fig.1)
- → Laboratory-based experiments:
- Outflow plated, incubated, and counted using Coliscan Membrane Filtration kits indicated lower E. coli counts than inflow (Table 1).
- ❖ There was no difference between *E. coli* concentrations after passing slurry through mycelial mats or adding it to eluent. (Table 1).
- ❖ In a separate experiment, filter from mycelial mats was enriched in phosphorus and other bacteria. (Fig 2).



#### Table 1

Number of *E. coli* CFU's from medium level manure slurry before and after filtration by mesocosm mycelial mat and after exposure to buffered eluent

# of <i>E.coli</i> CFUs	250 <i>E. coli</i> /100ml Slurry		Eluent
	Mat inflow	Mat outflow	Solution outflow
Average	11.5	1	0.66
Minimum	3	0	0
Maximum	20	2	1
The average of controls (DI water solution)	0.5	0.5	



#### Conclusions

- Stropharia rugoso annulata may reduce E. coli in runoff.
- Stropharia rugoso annulata mats may not effectively filter water due to patchy monomitic hyphal structure.
- Mycelial mats may provide substrate for bacterial growth and increased phosphorus cycling
- Mycelial mats may be reservoirs for microbes and sources of nutrients.
- Questions arise concerning tradeoffs of Stropharia
   rugoso annulata killing E.coli vs. mineralizing nutrients
   promoting E.coli growth.

## Further research questions:

- Could fungi with trimitic hyphal structure improve mat density?
- → How frequently do mycelial installations need to be replenished to remain active?
- → Can technology be developed that only uses eluent to successfully mitigate *E.coli?*
- → Can nutrient release caused by fungal decomposition of woody substrate be mitigated by plant uptake?

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