MycoEvolve
Research Education Earthworks

Multi-functional Riparian Buffers for Myco-Phytoremediation of Phosphorus and Pollinator Habitat

A case study at Shelburne Farms

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Workshop Overview

Riparian buffers are Best Management Practices that can: slow overland flow, provide shade, reduce erosion, facilitate infiltration, reduce sediment loads to receiving waters, protect aquatic & terrestrial habitat, maintain lakeshore & floodplain stability, preserve wetland functions, & reduce pollutant loads (phosphorus, nitrogen..) to waterways (VT Agency of Natural Resources, 2005). There are many unanswered questions about long-term riparian function.

- What do we know about the variation of their efficacy over time?
- How can we support their resilience?
- Can they provide additional services?

This installation investigation, in which buckthorn (*Rhamnus cathartica*) is replaced by native vegetation, at Shelburne Farms attempts to answer these questions. In particular: how can buffer efficacy be increased when plantings are inoculated with mycorrhizae and then cyclically coppiced to remove uptaken phosphorus? And, can other ecosystem functions be restored as well such as increased pollinator habitat?

CURRENT ISSUES IN VERMONT BUFFER ECOLOGY:

Native plant survival rate in restored areas is not high. This is due to:

- Competition with non-native species and weeds
- Herbivory by voles, beavers, deer
- Minimal maintenance (watering, weeding, fences) due to lack of funding

Uncertainty on long-term water quality function

- Can saturate with phosphorus (P); transitioning from P sinks into sources of P
- Minimal monitoring (data gathering) & maintenance (*potentially coppicing*) due to lack of funding & training

Pollinator habitat establishment is not currently a priority

- Long-term data gathering is needed to track trends
- Lack of available plant palettes suitable for various riparian buffer ecosystem and soil types

SHELBURNE FARMS

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MYCORRHIZAE FUNGI

More than 400 million years ago mycorrhizae assisted plants to colonize land. Over 90% of plants share a mutualistic symbiosis with these fungi which can facilitate ecological restoration of degraded ecosystems. Some of these benefits include: plant productivity, water retention, soil aggregation, pathogen resistance, resilience in adverse conditions such as drought, toxins, and salinity. Of the seven categories of mycorrhizae, ectomycorrhizae (ECM) & endomycorrhizae (AMF) are most often found in association with agricultural & forest crops (Kendrick, 2017). While both of these are involved in this pilot, we focus on AMF in this study.

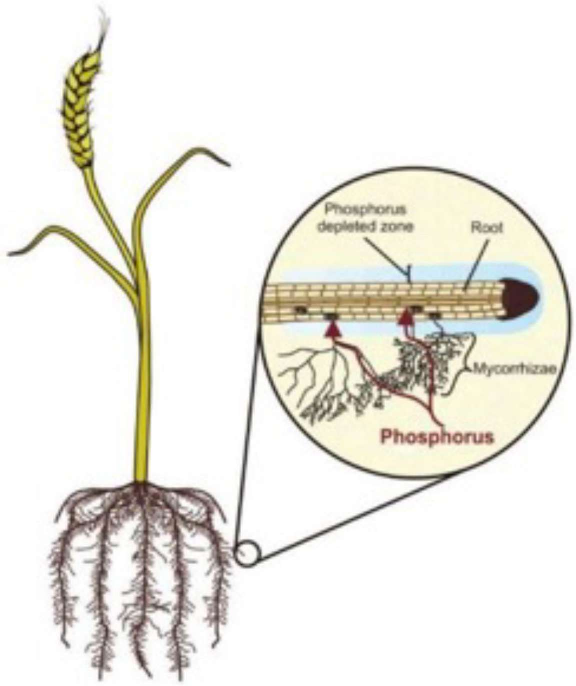


Figure 1. AMF acting as root system extensions to absorb phosphorus. Image courtesy of OMICS (Siemering, 2016).

It is established that (ECM) and (AMF) enhance the uptake of immobile soil nutrients such as P by plant hosts (Becquer et al., 2014; Bücking et al., 2012; Jones, 1998) and improve soil properties. Their facilitation of below and above-ground biodiversity with corresponding pathogen resistance improves tree and shrub survival on moisture, nutrient, and salt stressed soils (Begum et al., 2019; Diagne et al., 2020; Djighaly et al., 2020a, 2020b). In addition, they facilitate plant succession (Asmelash et al., 2016; Ortaş and Rafique, 2017). Mycorrhizae growing around or in roots utilize carbohydrates from the host, while in return supplying the host with P, (Sanders and Tinker, 1973), water and other nutrients (Policelli et al., 2020b; Smith and Read, 2010). Additionally, when planting into AMF grasslands, tree and shrub species' growth and survival is improved by inoculation with ECM specific to the species planned (Nelson and Allen, 1993). ECM support native trees to endure competition of aggressive non-native species (Policelli et al., 2020a) and play a critical role in the restoration of degraded sites (Policelli et al., 2020b).

Mycorrhizal fungi are keystone mutualists in terrestrial ecosystems (O'Neill et al., 1991) whose ecological role in assisting recovery of severely disturbed ecosystems (Dogan and Ozyigit, 2015) is evident because they enhance P plant uptake in both crops and woody plants. They may play an important role in myco-phytoremediation of phosphorus. This involves ecosystem engineering in nutrient exchange networks crucial to ecosystem succession and resilience (Zalewski, 2000). This strategy, though still relatively novel in modern landscapes, has tremendous application potential in the rising field of reconciliation ecology (Dudgeon et al., 2006), which acknowledges that, while ecosystems cannot be completely restored to their original state, they can be reestablished to reverse their degradation to a new balance (Michener, 2004).

MYCOREMEDIATION

Among many services, mycorrhizae can increase nutrient uptake of P (Rubin and Görres, 2021).

- In soils low in available P this has been proven (Khan et al., 2010; Liu et al., 2003).
- In soil high in available P the dynamic is more complicated (Asghari et al., 2005; Lambert & Weidensaul, 1991; Sandoz et al., 2020). Data currently is inconclusive.
- While P concentrations can be high, P availability to plants is generally low. It is unclear whether the benefits of the plant-fungi mutualistic symbiosis apply in these conditions.

We gather data in order to understand how the above dynamics can inform best conditions for mycorrhizal applications.

PHYTOREMEDIATION

Phytoremediation involves plants that remove pollutants such as hydrocarbons, pesticides, trace elements, toxic heavy metals, metalloids, landfill leachates (Dogan & Ozyigit, 2015; Zhang et al., 2010).

- Phytoremediation is a cost-effective, environmentally sound way to conserve soil & water resources while providing livestock with viable hay (Gotcher et al., 2014) and other resources.
- Phytoremediation can be enhanced with appropriate arbuscular mycorrhizae fungi (AMF) (Khan, 2006) and ectomycorrhizae (ECM).

Plant uptake can reduce P concentrations in soil solution and thus reduce movement of dissolved P into surface waters, especially when perennials are removed through coppicing.

MYCO-PHYTOREMEDIATION

When mycoremediation and phytoremediation are combined, a synergistic symbiosis is facilitated which also includes microbes (Li et al., 2019; Mäder et al., 2011). This form of remediation is ideal for mycorrhizae. The reported utility, in the literature, is to remediate metals & PCBs (Blagodatsky et al., 2020; Govarthanan et al., 2018; Neagoe et al., 2017; Shoaib, 2012).

To our knowledge, it has not been applied to P mitigation beyond pilot projects; case studies are rare even though it is a logical application for mycorrhizae.

WE PROPOSE:

- The time period in which a buffer functions to protect water quality can be extended by mycorrhizae and appropriate management
- Mycorrhizae can increase the uptake of P in buffer vegetation and thus remove it from the soil
- Management is needed to remove the uptaken P from the buffer by cyclically coppicing plant biomass
- While water quality is the main function of riparian buffers, they can also be managed for pollinator habitat

Research Objectives

To investigate the effectiveness of Myco-phytoremediation improving the function of a multi-purpose riparian buffer both in the field at Shelburne Farms and in lab mesocosm experiments determining:

- Soil water P extracted from the soil using lysimeters
- Plant P uptake collected by leaf harvests and coppicing
- Pollinator habitat community structure by measuring vegetation richness and diversity
- Mycorrhizal (AMF) hyphae density to check our assumptions by counts from bulk soil

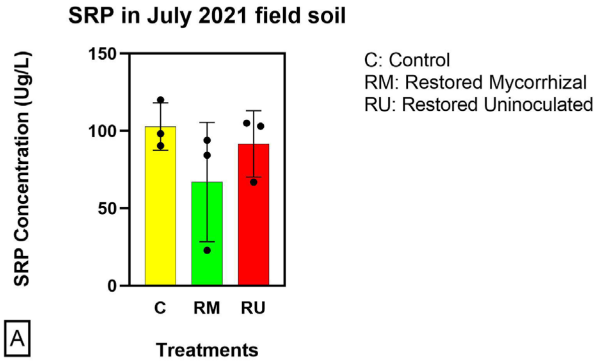


Figure 2A. Soluble Reactive Phosphorus (SRP) in July 2021 soil water from field soil. There is a trend that the restored mycorrhizal plot has the lower concentration, and the control has the highest. There is no statistically significant difference between treatments.

WHY TO PRACTICE ON MYCO-PHYTOREMEDIATION AT THIS SITE

- There is high P both in soil, soil water, (Figures 2) & runoff water (Orchard Cove is a hotspot of P).
- To enhance riparian function in a *critical source area* on a farm with degraded plant diversity (Figure 4)

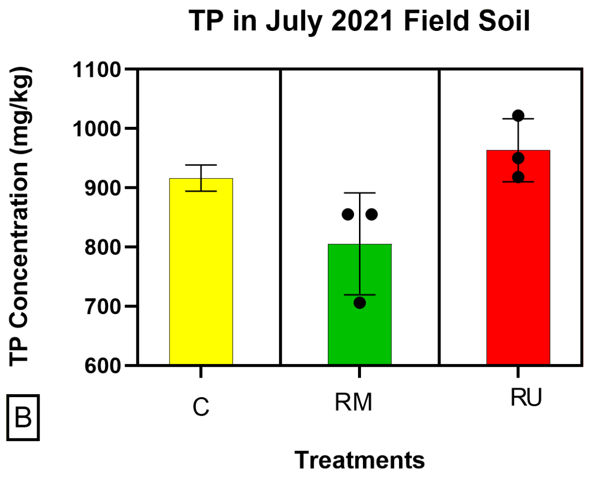


Figure 2B. TP in July 2021 pilot treatment soil. P value of .014 indicates that there is a significant difference between two treatments. A post test indicates the significant difference is between the restored mycorrhizal and restored uninoculated (p =.0407) plots. The inoculated plot has a lower concentration of TP.

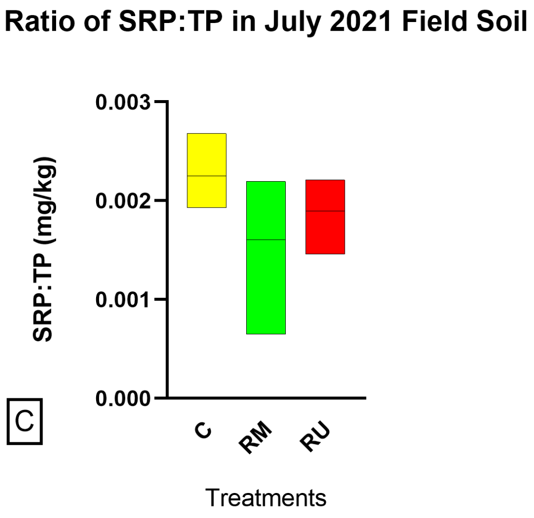


Figure 2C. SRP:TP ratio in July 2021 pilot plots. There is no statistically significant difference but the trend indicates that the restored plots have a lower SRP:TP ratio than the control plot.

HOW TO PRACTICE MYCO-PHYTOREMEDIATION AT THIS SITE

- **Remove non-native species:** which was buckthorn through chainsaw in winter to belt height, removing all stumps >4 ft from waterways with hand tools & community muscle in late winter. Cut back regrowth on stumps left, 4-5 x a year.
- Install a diverse **plant palette of cohabitating native riparian species** that provide year round pollinator habitat (Table 1).
- **Maintain** through scything grasses, weeding buckthorn and opportunistic species.
- **Monitor** the plant community, mycorrhizae, and soil water.
- **Cyclically coppice** for P removal; rotate to provide habitat: invertebrates, winter resident & early migrant birds.

The message at Got Weeds? Is simple:

- Stewardship = Presence. Our actions define our presence.
- Build on biodiversity and resilience, this begins in the soil (soil microbial community, including mycorrhizae)
- Landscapes are depleted and in need of Rehabilitation, not Restoration.
- The goal is long-term, enduring transition of lands to healthier conditions.

Pointers for Land Managers from Got WEEDS:

- Three stress events in two growing seasons is enough to kill most shrubs.
- We stress the non-native plants, observe their response, and then respond to the response.
- A five year seed life means a six year project, at minimum.
- Biosecurity is essential; tools, boots, equipment must be cleaned or used exclusively on singular project sites.

Early Findings

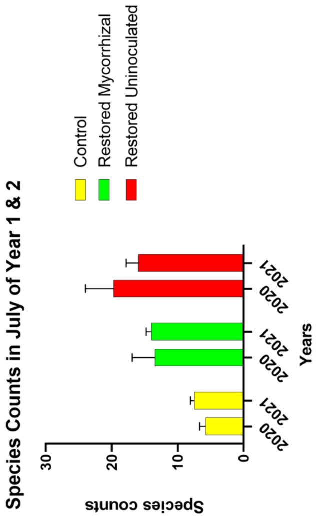
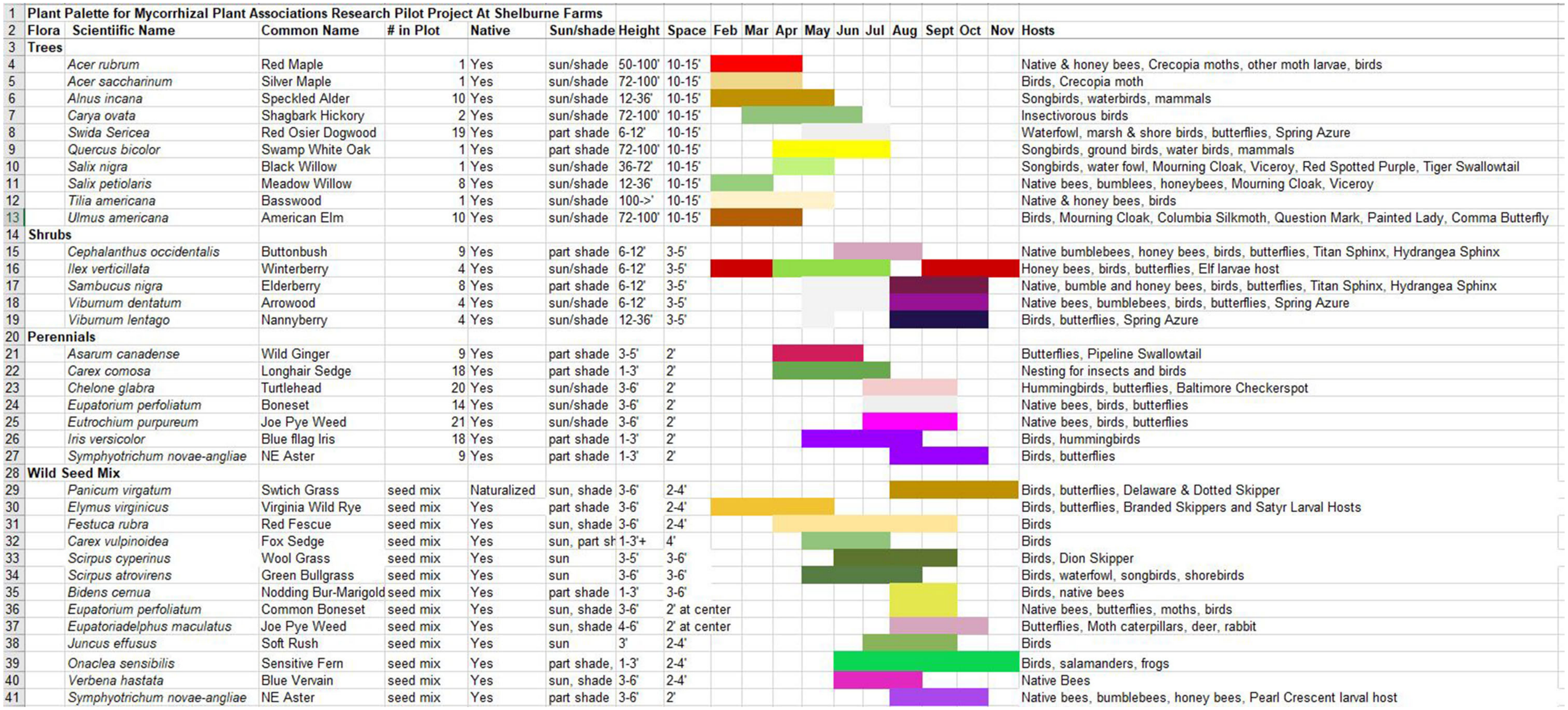


Figure 3. Plant species counts in July of year 1 & 2. Plant species richness in July of both years is statistically significant between treatments (p <0.0001) (between controlled & both restored plots) but not statistical significantly different between years. There is a statistically significant difference between the inoculated & uninoculated restored plots (p = 0.0139); there is statistically significant difference between the inoculated & control plots (p <0.0001) & between the uninoculated & control plots (p <0.0001).

- Natural nonnative species removal works.
- Restoration of plant community polycultures increases pollinator habitat, though requires maintenance.
- The number of plant species has not decreased significantly over the two years in all treatments.
- More years of data are needed to clearly see the successional trend.

Table 1. Plant palette of grasses, herbaceous species, shrubs & trees with flowering times & pollinators they host.



1	Plant Palette for Mycorrhizal Plant Associations Research Pilot Project At Shelburne Farms																	
2	Flora	Scientiific Name	Common Name	# in Plot	Native	Sun/shade	Height	Space	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Hosts
3	Trees																		
4		*Acer rubrum*	Red Maple	1	Yes	sun/shade	50-100'	10-15'	■	■	■								Native & honey bees, Crecopia moths, other moth larvae, birds
5		*Acer saccharinum*	Silver Maple	1	Yes	sun/shade	72-100'	10-15'	■	■	■								Birds, Crecopia moth
6		*Alnus incana*	Speckled Alder	10	Yes	sun/shade	12-36'	10-15'	■	■	■	■							Songbirds, waterbirds, mammals
7		*Carya ovata*	Shagbark Hickory	2	Yes	sun/shade	72-100'	10-15'		■	■	■	■						Insectivorous birds
8		*Swida Sericea*	Red Osier Dogwood	19	Yes	part shade	6-12'	10-15'				■	■	■					Waterfowl, marsh & shore birds, butterflies, Spring Azure
9		*Quercus bicolor*	Swamp White Oak	1	Yes	part shade	72-100'	10-15'			■	■	■	■					Songbirds, ground birds, water birds, mammals
10		*Salix nigra*	Black Willow	1	Yes	sun/shade	36-72'	10-15'			■	■							Songbirds, water fowl, Mourning Cloak, Viceroy, Red Spotted Purple, Tiger Swallowtail
11		*Salix petiolaris*	Meadow Willow	8	Yes	sun/shade	12-36'	10-15'	■	■									Native bees, bumblees, honeybees, Mourning Cloak, Viceroy
12		*Tilia americana*	Basswood	1	Yes	sun/shade	100->'	10-15'	■	■	■	■							Native & honey bees, birds
13		*Ulmus americana*	American Elm	10	Yes	sun/shade	72-100'	10-15'	■	■	■								Birds, Mourning Cloak, Columbia Silkmoth, Question Mark, Painted Lady, Comma Butterfly
14	Shrubs																		
15		*Cephalanthus occidentalis*	Buttonbush	9	Yes	part shade	6-12'	3-5'					■	■	■				Native bumblebees, honey bees, birds, butterflies, Titan Sphinx, Hydrangea Sphinx
16		*Ilex verticillata*	Winterberry	4	Yes	sun/shade	6-12'	3-5'	■	■	■	■	■	■		■	■	■	Honey bees, birds, butterflies, Elf larvae host
17		*Sambucus nigra*	Elderberry	8	Yes	part shade	6-12'	3-5'				■	■	■	■	■	■		Native, bumble and honey bees, birds, butterflies, Titan Sphinx, Hydrangea Sphinx
18		*Viburnum dentatum*	Arrowood	4	Yes	sun/shade	6-12'	3-5'				■	■	■	■	■	■		Native bees, bumblebees, birds, butterflies, Spring Azure
19		*Viburnum lentago*	Nannyberry	4	Yes	sun/shade	12-36'	3-5'				■			■	■	■		Birds, butterflies, Spring Azure
20	Perennials																		
21		*Asarum canadense*	Wild Ginger	9	Yes	part shade	3-5'	2'			■	■	■						Butterflies, Pipeline Swallowtail
22		*Carex comosa*	Longhair Sedge	18	Yes	part shade	1-3'	2'			■	■	■	■					Nesting for insects and birds
23		*Chelone glabra*	Turtlehead	20	Yes	sun/shade	3-6'	2'						■	■	■			Hummingbirds, butterflies, Baltimore Checkerspot
24		*Eupatorium perfoliatum*	Boneset	14	Yes	sun/shade	3-6'	2'						■	■	■			Native bees, birds, butterflies
25		*Eutrochium purpureum*	Joe Pye Weed	21	Yes	sun/shade	3-6'	2'						■	■	■			Native bees, birds, butterflies
26		*Iris versicolor*	Blue flag Iris	18	Yes	part shade	1-3'	2'				■	■	■	■				Birds, hummingbirds
27		*Symphyotrichum novae-angliae*	NE Aster	9	Yes	part shade	1-3'	2'							■	■	■		Birds, butterflies
28	Wild Seed Mix																		
29		*Panicum virgatum*	Swtich Grass	seed mix	Naturalized	sun, shade	3-6'	2-4'							■	■	■	■	Birds, butterflies, Delaware & Dotted Skipper
30		*Elymus virginicus*	Virginia Wild Rye	seed mix	Yes	part shade	3-6'	2-4'	■	■	■	■							Birds, butterflies, Branded Skippers and Satyr Larval Hosts
31		*Festuca rubra*	Red Fescue	seed mix	Yes	sun, shade	3-6'	2-4'			■	■	■	■	■	■			Birds
32		*Carex vulpinoidea*	Fox Sedge	seed mix	Yes	sun, part sh	1-3'+	4'				■	■	■					Birds
33		*Scirpus cyperinus*	Wool Grass	seed mix	Yes	sun	3-5'	3-6'					■	■	■	■			Birds, Dion Skipper
34		*Scirpus atrovirens*	Green Bullgrass	seed mix	Yes	sun	3-6'	3-6'				■	■	■	■				Birds, waterfowl, songbirds, shorebirds
35		*Bidens cernua*	Nodding Bur-Marigold	seed mix	Yes	part shade	1-3'	3-6'							■	■			Birds, native bees
36		*Eupatorium perfoliatum*	Common Boneset	seed mix	Yes	sun, shade	3-6'	2' at center							■	■			Native bees, butterflies, moths, birds
37		*Eupatoriadelphus maculatus*	Joe Pye Weed	seed mix	Yes	sun, shade	4-6'	2' at center							■	■	■		Butterflies, Moth caterpillars, deer, rabbit
38		*Juncus effusus*	Soft Rush	seed mix	Yes	sun	3'	2-4'						■	■	■			Birds
39		*Onaclea sensibilis*	Sensitive Fern	seed mix	Yes	part shade,	1-3'	2-4'					■	■	■	■	■	■	Birds, salamanders, frogs
40		*Verbena hastata*	Blue Vervain	seed mix	Yes	sun, shade	3-6'	2-4'					■	■	■				Native Bees
41		*Symphyotrichum novae-angliae*	NE Aster	seed mix	Yes	part shade	3-6'	2'							■	■	■		Native bees, bumblebees, honey bees, Pearl Crescent larval host

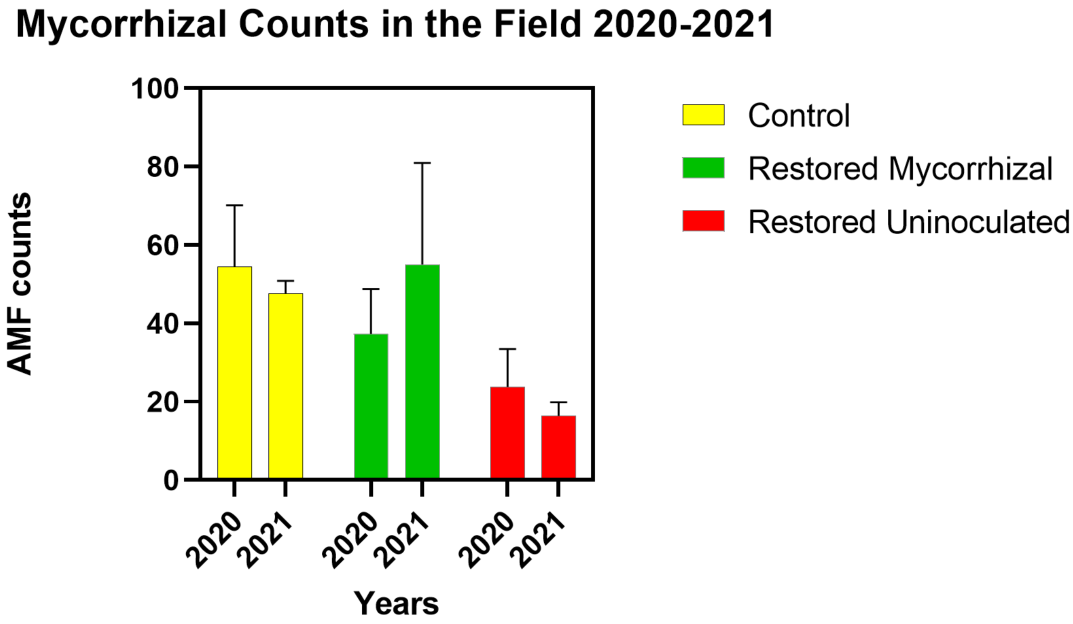


Figure 4. Endomycorrhizal (AMF) Mycorrhizal counts in July 2020 & 2021 from the field. There is a statistically significant difference between treatments (p = .0021); between the control & restored uninoculated (p = 0.0023) & between restored mycorrhizal vs. restored uninoculated (p = 0.0149). There is no statistical difference between years.

- Mycorrhizae in the inoculated restored plot increased compared to in the uninoculated restored plot.
- Mycorrhizae are already present in perennial stands, even amidst these particular nonnative species.

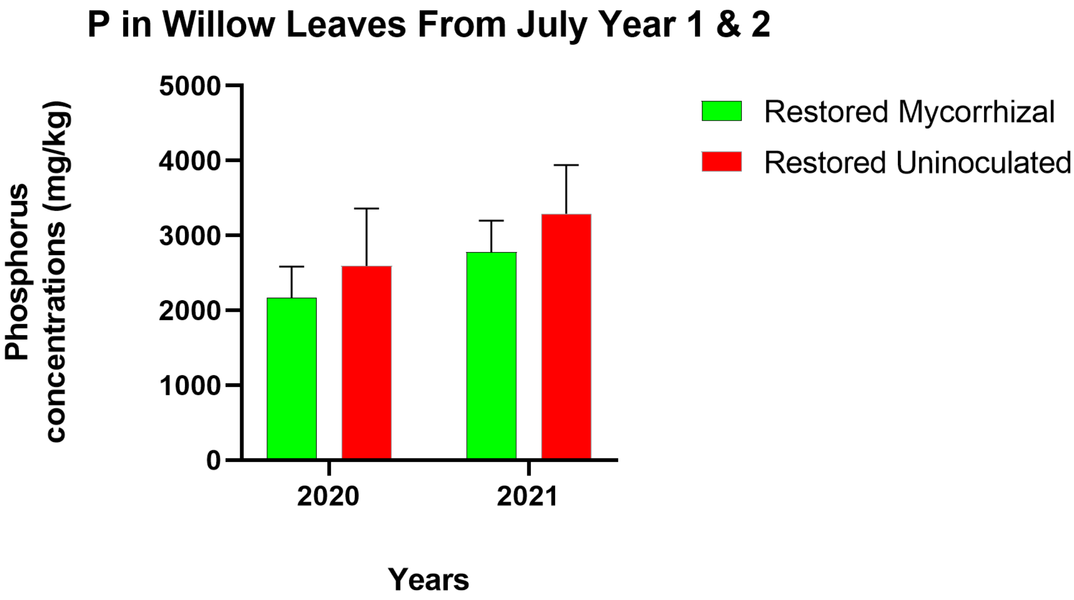


Figure 5. P concentrations in willow leaves. There is no statistically significant difference between treatments or years. It appears that P concentrations rose in year 2 across both treatments.

- In year 2, willow leaf P concentrations are greater than year 1 likely due to plants are larger & mycorrhizal networks are more developed
- This comparison between RM & RU mirrors the P concentrations in the field soil (Fig. 2).

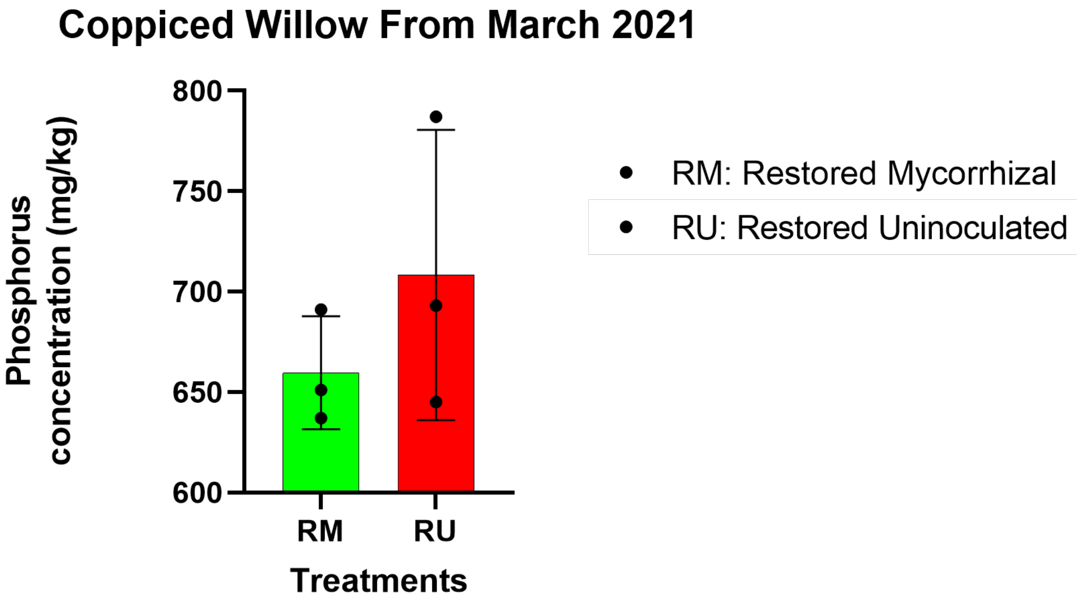


Figure 6. P concentrations in coppiced saplings from March 2021 of year 1. There is no statistically significant difference between treatments. However the RU willows seem to have uptaken more P than the RM willows.

- Coppicing for P removal may be more effective in the fall when P is still in above ground plant biomass than in winter when P is in roots.
- This trend mirrors the P concentrations in soil & water in year one (Figs. 2a & 2b) & P willow leaf uptake (Fig. 5)
- Results pending but consider alternating coppicing different trees between fall and spring.
- This is early in the study & we continue to monitor phosphate concentrations in the plant biomass.

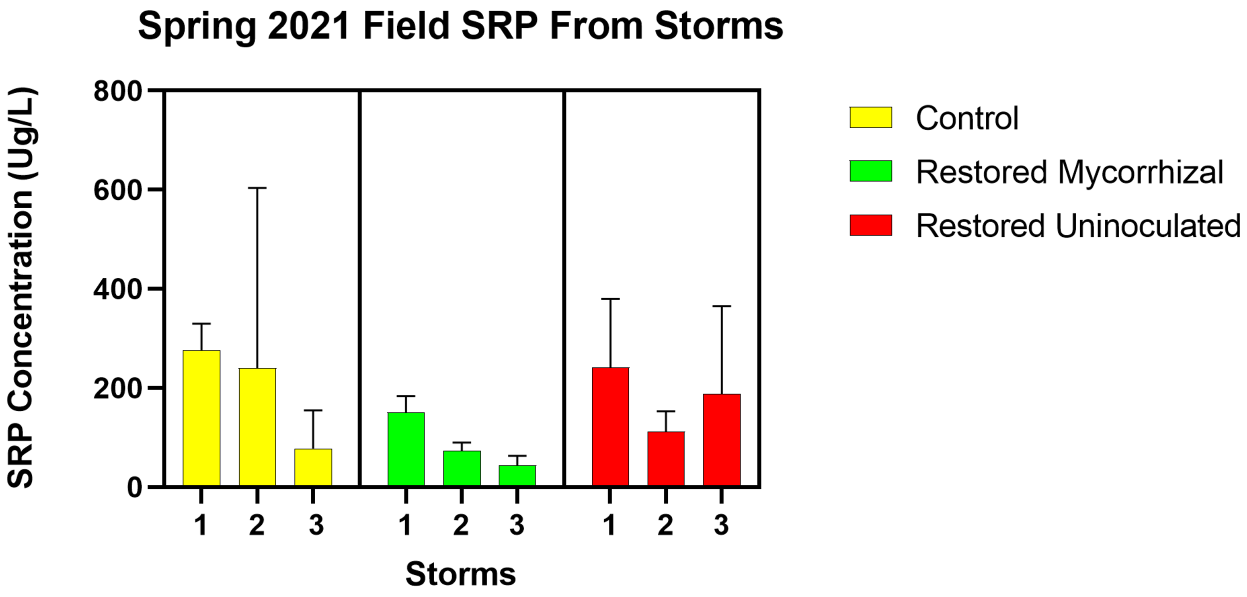


Figure 7. SRP concentrations from field pilot plots in spring 2021 rainstorms. There is no statistically significant difference between treatments.

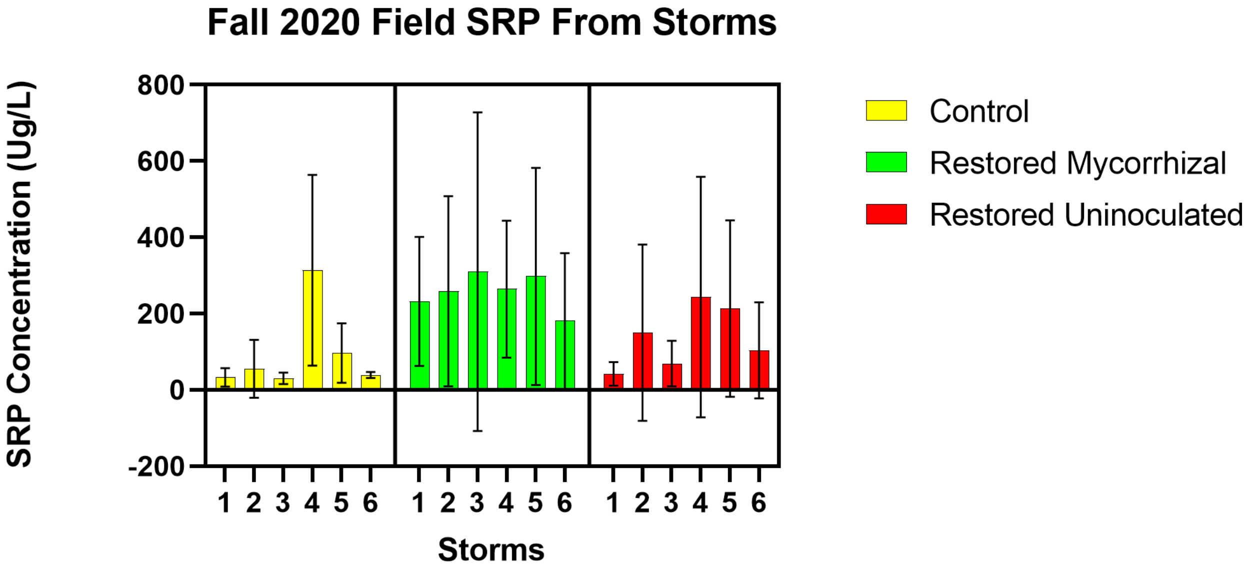


Figure 8. SRP concentrations from field pilot plots in fall 2020 rainstorms. There is no statistically significant difference between treatments. The trend indicates that RM have higher concentration of SRP while the control has the lowest.

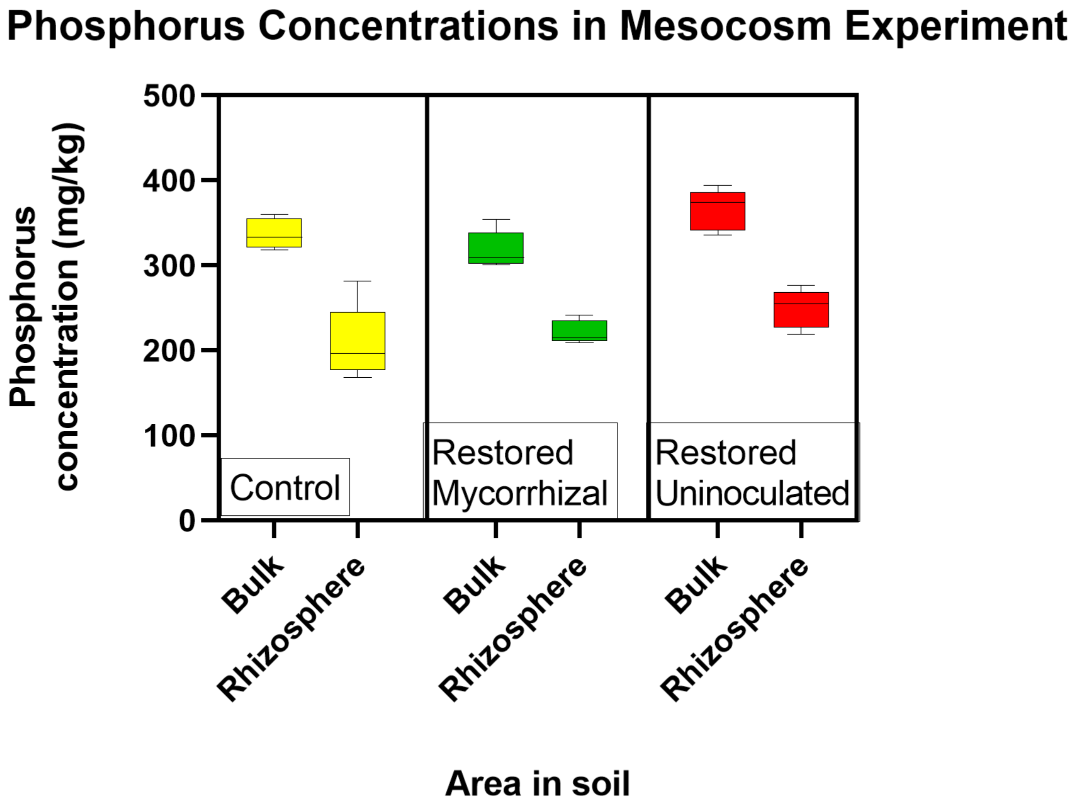


Figure 9. P concentrations in rhizosphere soil from Mesocosm experiments. There is a statistically significant difference between soil areas (rhizosphere vs. bulk) across all treatments (p<.0001). There is also a statistically significant difference in Mehlich extracted P between the overall mycorrhizal and uninocu-

lated treatments (p = .033). Specifically the Mehlich extracted P was lower in the mycorrhizal than in the uninoculated treatments.

- Higher P is detectable in bulk soil compared to in rhizosphere soil regardless of treatment.
- In the soil, the inoculated treatment has lower phosphorus concentrations than in the uninoculated treatment.
- The data indicates that the treatment (i.e. mycorrhizae) cause an effect across both bulk & rhizosphere.

Maintenance & Monitoring Conundrums

P retention in the buffer is not forever; if plant biomass is not removed then P will eventually be remobilized into water.

HOW TO COPPICE FOR BOTH MAXIMUM P REMOVAL AND PLANT LONGEVITY

Options:

- Coppice late winter (Fig. 6); most P is in roots so coppicing does not remove much
- Coppice early fall when P & nutrients have not sunk to roots; more P in plant but shocks plant

HOW TO DISCERN WHICH PLANTS TO REMOVE TO FACILITATE ABOVE & BELOW GROUND SUCCESSION

Options:

- Remove opportunistic species by scything stems but not removing roots to maintain rhizosphere web
- Track their presence & numbers over the years
- Leave untouched and observe what occurs as species battle it out

WHY LONG - TERM RESEARCH IS NEEDED:

- Year 1 data includes only 6 months of data after restoration/installation disturbance
- Mycorrhizae may not have been fully colonized
- Plants are not likely fully established
- Soil is still recovering from disturbance of restoration
- Plant, fungal, and microbial community succession takes time
- Models indicate a long lag time (of several years) between implementation & measured water quality improvements (Hamilton, 2012; Meals et al., 2010).
- Aiming for at least a decade of data.

Questions guiding next research steps

- Comparing mycorrhizal efficacy in high vs. low P soil.
- How much P mitigation (in this situation) can occur with continued upland contributions?
- How do mycorrhizae influence succession trajectory after initial restoration plantings?
- How much P can the plant community (after using what is needed for growth) extract annually?
- Is plant removal feasible while facilitating ecosystem recovery?
- Does soil P concentration affect plant diversity?
- What type of relationship exists between floral diversity recovery and P mitigation?

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FOLLOW UP QUESTIONS ABOUT:

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Notes