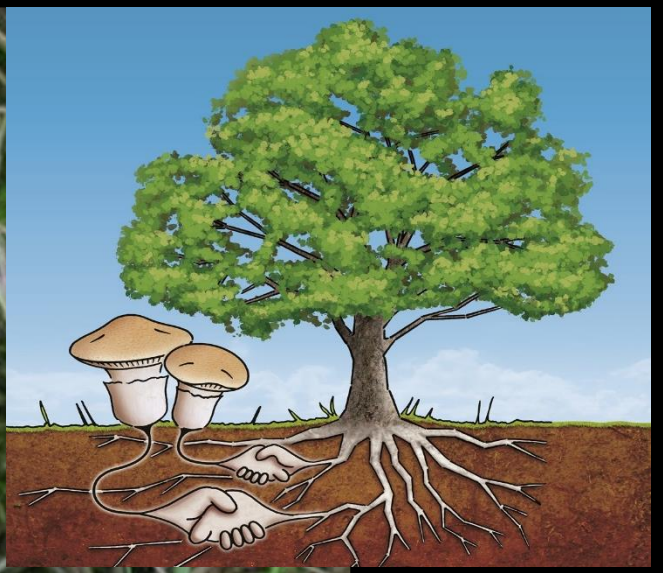
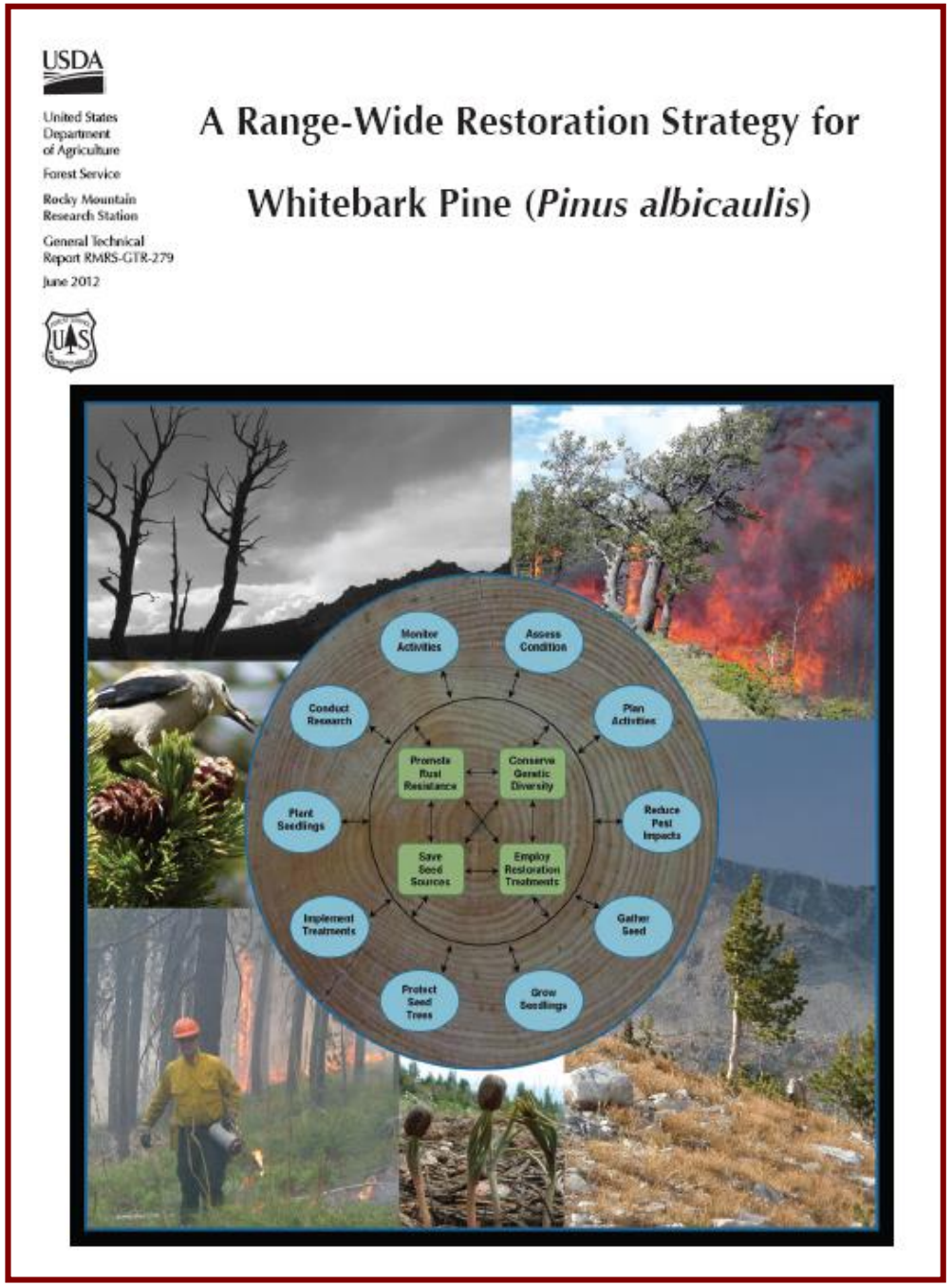
Mycorrhizal Fungi and Whitebark Pine: an update



Cathy Cripps

MONTANA STATE UNIVERSITY



Section on: Ectomycorrhizal Fungi (EMF)

- **Awareness**

  All pines need mycorrhizal fungi to survive in nature!

- **Conservation** – minimize negative impacts

  tree cutting, soil removal, mechanical disturbance, liming, N-deposition, fertilization, removal of woody debris & nurse trees, high intensity fire

- **Strategies for maintaining EMF**

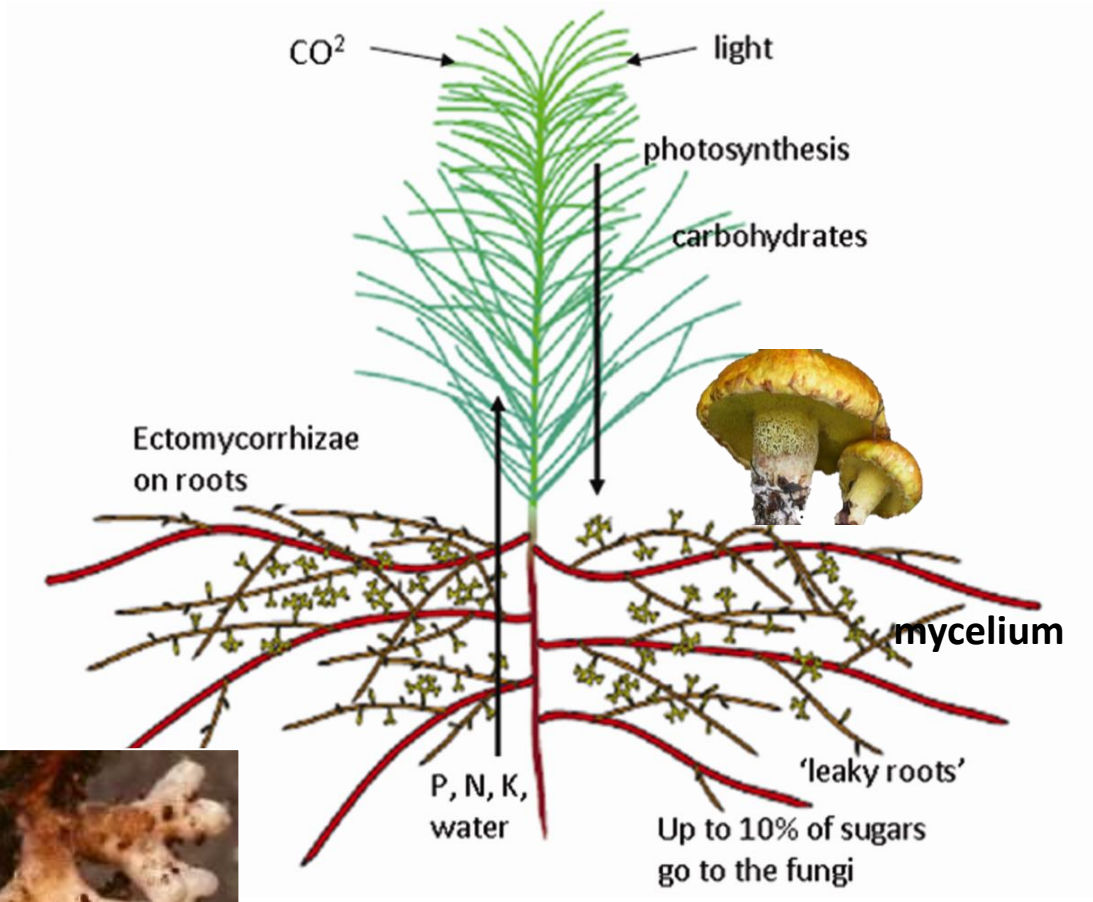
  plant seedling near living whitebark pines

  plant seedlings within a year of disturbance

  assess soils for EMF, monitor for EMF

  inoculate if necessary with **native fungi**
  (do not use commercial inoculum)

How do Ectomycorrhizal Fungi benefit trees?



ectomycorrhizae

- **enhance nutrient uptake**
  especially nitrogen
- **provide protection**
  drought
  pathogens
  grazers
  heavy metals
- **aggregate the soil**

In nature:

Many different fungi on roots of each tree

Cripps, CL 2002. Mycorrhiza. In: Pscheidt & Ocamb, Pacfic NW Plant Disease Management Handbook

Over 50 species of Ectomycorrhizal Fungi associate with whitebark pine

Generalists: are they enough? weedy species, that associate with many tree species

Specialists: are they necessary?
Host specific: only associate with pines, 5-needle pines, whitebark pine

Mostly no fruiting bodies

Amanita alpinicola Cripps **Cortinarius bridgei** Cripps

Only in whitebark pine forests

Suilloids

Host specific

On seedlings

On mature trees

Can be used in restoration

Not all mycorrhizal fungi are the same:
Each fungus functions differently and provides different benefits to the trees

Suilloids with whitebark pine (Pinus albicaulis)

Primarily nitrogen uptake

Suillus sibiricus Singer: with 5-needle pines in section strobus N. hemisphere

Suillus subalpinus Moser: endemic, with whitebark pine, on fungal conservation list

Suillus discolor (A.H. Smith, Thiers & O.K. Miller) N.H. Nguyen with 5 needle pines (S. tomentosus var. discolor)

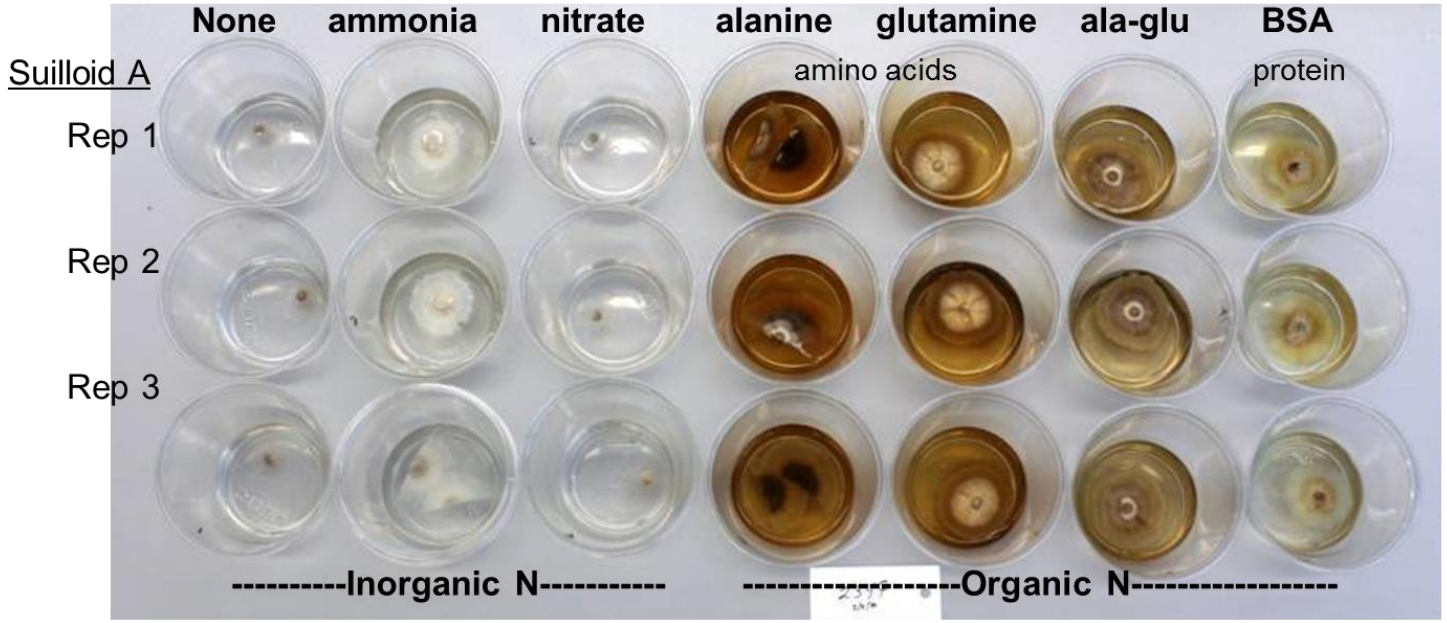
Rhizopogon evadens: 5-needle and 2-3 needle pines

Suilloids are important in accessing nitrogen for whitebark pine. What nitrogen sources can they access? Are all fungal species the same?

Culture study: Examined utilization of organic and inorganic nitrogen sources by 28 Suilloids from whitebark pine forests

Results: Growth rates, densities, pigment production varied among species

Species	CLC	Amm	Nit	Ala	Gln	Ala-Gln	BSA
Rhizopogon evadens	2035	0.62	0.09	0.15	0.27	0.26	0.10
Rhizopogon evadens	2451	0.24	0.33	0.23	0.47	0.33	0.37
Rhizopogon milleri	2544	0.21	-0.04	0.38	0.37	0.34	0.07
Rhizopogon roseolus	2469	0.88	0.08	0.76	1.10	1.23	0.28
Rhizopogon roseolus	2475	0.30	0.23	0.22	0.26	0.44	0.32
Rhizopogon roseolus	2489	0.63	0.09	0.35	0.58	0.48	0.18
Rhizopogon sp.	Hyp1	1.70	0.26	0.52	1.64	1.83	0.29
Suillus cf. placidus	2473	0.92	-0.07	0.83	1.60	1.34	0.56
Suillus discolor	2422	1.11	0.12	0.33	0.64	0.42	0.16
Suillus discolor	2433	0.22	0.28	0.70	1.32	0.87	0.11
Suillus discolor	2441	1.39	0.90	1.89	2.11	1.81	1.37
Suillus discolor	2500	0.98	0.10	1.58	1.53	1.45	0.51
Suillus discolor	2510	0.92	1.55	1.60	1.91	1.31	0.65
Suillus discolor	2539	0.92	0.19	1.05	1.41	0.81	0.88
Suillus sibiricus	2421	1.17	0.82	1.17	1.18	1.14	0.63
Suillus sibiricus	2449	1.22	0.50	1.21	1.35	1.06	0.66
Suillus sibiricus	2450	0.73	0.00	0.33	0.96	1.01	0.55
Suillus sibiricus	2484	1.27	-0.03	0.85	1.52	1.48	0.19
Suillus sibiricus	2540	0.65	-0.13	0.15	0.30	0.16	0.15
Suillus sp.	2199	1.08	0.11	0.08	0.89	1.23	0.08
Suillus sp.	2467	1.06	0.10	0.93	0.96	0.85	0.34
Suillus sp.	2480	2.07	0.90	0.69	2.06	2.10	0.42
Suillus subalpinus	2347	0.80	-0.04	0.67	0.58	0.56	0.29
Suillus subalpinus	2487	0.40	-0.12	0.22	0.39	0.25	0.12
Suillus subalpinus	2505	0.36	-0.05	0.12	0.24	0.16	0.03
Suillus subalpinus	2508	0.35	-0.10	0.20	0.44	0.19	0.05
Suillus subalpinus	2533	1.11	0.13	0.20	0.97	0.68	0.32
Suillus subalpinus	2536	0.35	-0.09	0.18	0.33	0.14	0.07



- **Most did best on ammonia and amino acids, and not as well on inorganic nitrogen sources (nitrate)**
- **There is functional diversity among ectomycorrhizal fungi!**
- If EMF communities change, overall mycorrhizal functioning changes

Antibus, Hobbie and Cripps 2018

When appropriate ectomycorrhizal fungi are reduced or lacking in soil, do we need to inoculate seedlings for restoration?

High intensity fire
(Wiensczyk et al. 2002)

Beetle infestation (Treu et al. 2014)

Rust infection?
(how long can EMF survive in ghost forests)

Non-forested areas
(absent)

Soil disturbance

Optimal conditions for recovery after fire

After fire
lower EMF diversity
lower EMF species richness
shift in EMF communities
(Trusty and Cripps 2010)

- Adjacent mature forest
- Seed source
- Nutcrackers present
- Source of fungal inoculum
- Mammal vectors present

Teeter-totter Model

Time to full recovery?

If appropriate Ectomycorrhizal Fungi are lacking, there may be a need to inoculate.

We developed a method for inoculation with native ectomycorrhizal fungi from whitebark pine forests

Here's the method!

Collect <u>appropriate</u> native mycorrhizal fungi from WBP forests

Suilloids!

Keep cool!

Remove pore surface (contains spores) for Suillus

From this

Whole mushroom

To this

Save the Pores—spongy layer (contain spores)


For Rhizopogon,
slice them up!
Or dry them on a
food dehydrator

Making a spore slurry from fresh pores (spongy layer)

Movie clip

Dilute spore slurry to 1 million spores/ml

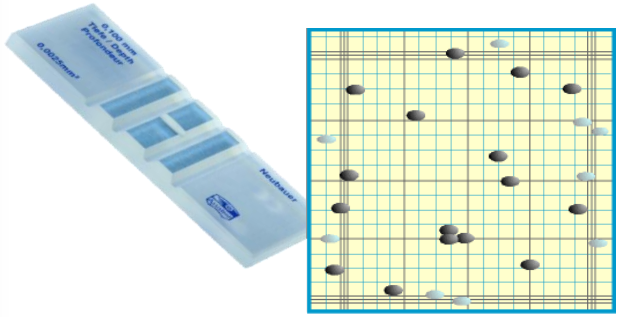
Slurry color chart

Dark Chocolate **Milk Chocolate** **Tea color**

or

Use a counting chamber

(Haemocytometer & microscope)



Then calculate the number of spores/ml …..

Typically this means diluting the spores slurry 10 or 20 times.

Store in refrigerator
How long does it last?

How to inoculate Seedlings with Spore Slurries

We use 3 ml of slurry (@ 1 million spores/ml) = 3 million spores/container

Drip method using a pippette

or

Inoculation gun

On soil surface near base of seedling

Cattle vaccination gun

Use of Native Ectomycorrhizal Fungi to inoculate whitebark pine seedlings for restoration purposes

Screened 28 strains of native EMF in the greenhouse and examined conditions to optimize colonization in the greenhouse

- species/isolates of fungi
  Suillus sibericus (certain strains)
- types of inoculum/ how much
  spore slurries
- when to inoculate
  older seedlings
- substrate type
  composted bark mix
- inoculation method
  injection
- fertilizer regime
  low nitrogen fertilizer

Cripps & Grimme 2010; Lonergan & Cripps 2013

Webinar link: Cripps Whitebark pine and native mycorrhizal fungi
UTube https://www.youtube.com/watch?v=JlHKCoaSBIw

Inoculation versus Colonization!

Not all seedlings become colonized after inoculation

Some seedlings that are not inoculated can become colonized!

Idaho Nursery: 5-needle pine orchard in back of greenhouse with whitebark pine seedlings

Full of Suillus making spores!

DNA

How does *Suillus sibiricus* affect nitrogen uptake in whitebark pine seedlings <u>in burn soil</u>?

A greenhouse study of **<u>colonized</u>** and **<u>uncolonized</u>** whitebark pine seedlings

Methods

Soil from the Eureka Basin Burn, SW MT

Planted Uncolonized and colonized in burn soil

Unsterilized Soil Sterilized Soi	Uncolonized	Colonized
	A1 n = 17	**B1** n = 26
	A2 n = 19	**B2** n = 20

Seedling planting treatments.

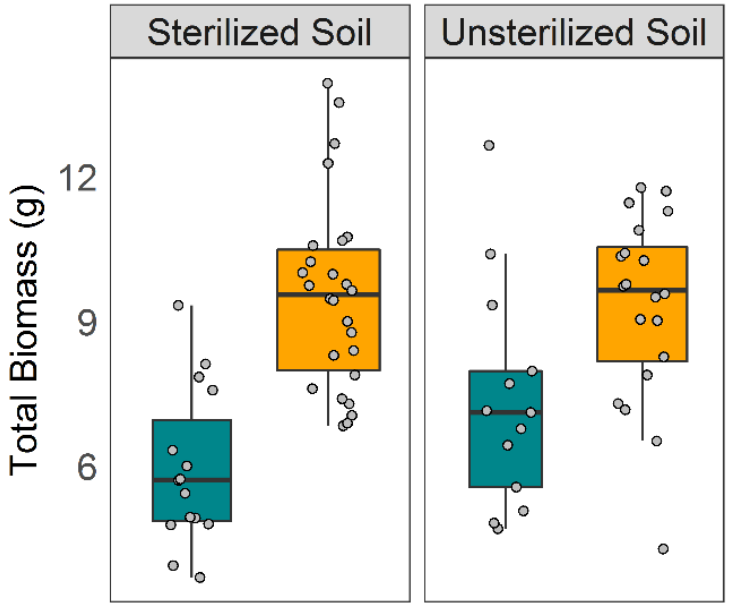
Study 3: How does *Suillus sibiricus* affect nitrogen uptake in whitebark pine seedlings in burn soil?

Results

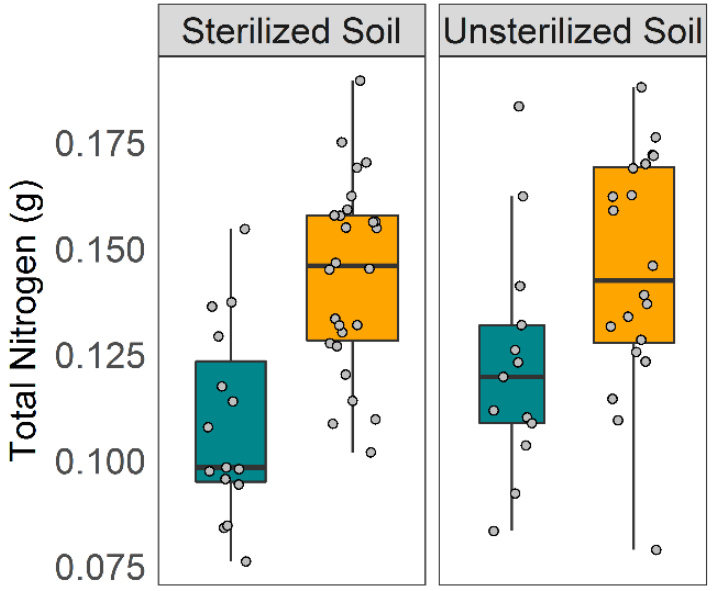
Uncolonized
Colonized

For colonized seedlings after 6 months:

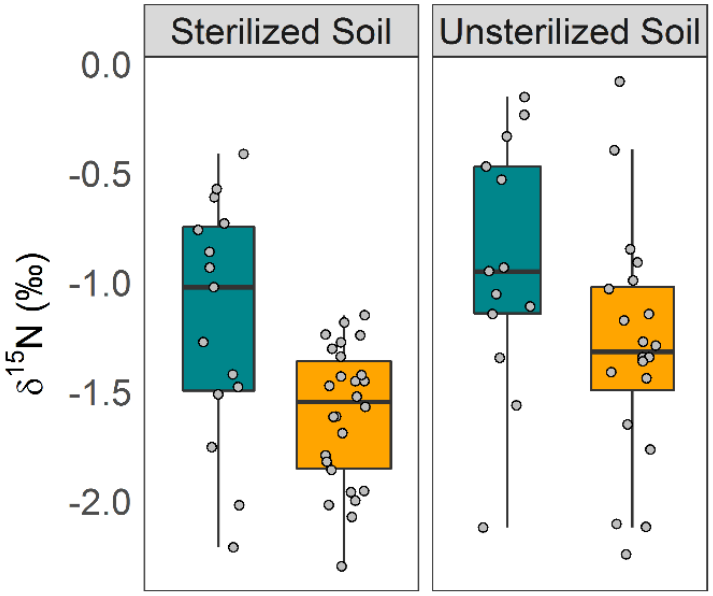
Biomass 61% greater



Total foliar nitrogen 25% higher



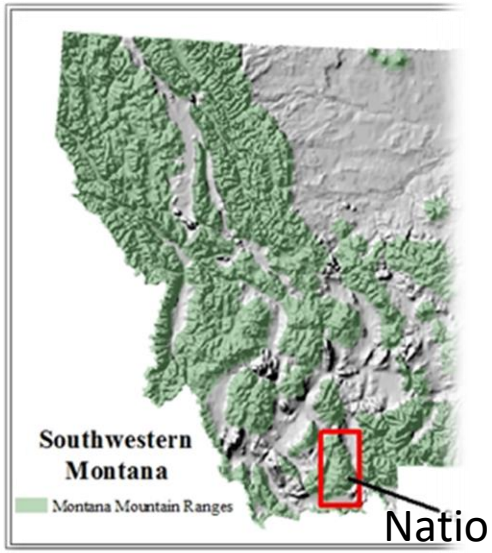
$\delta^{15}N$ lower



Jenkins, Cripps, Germain-Gains 2018.

Direct evidence that ECF are responsible for increased foliar N

Does colonization with *Suillus sibericus* improve seedling survival on a <u>severe</u> burn?

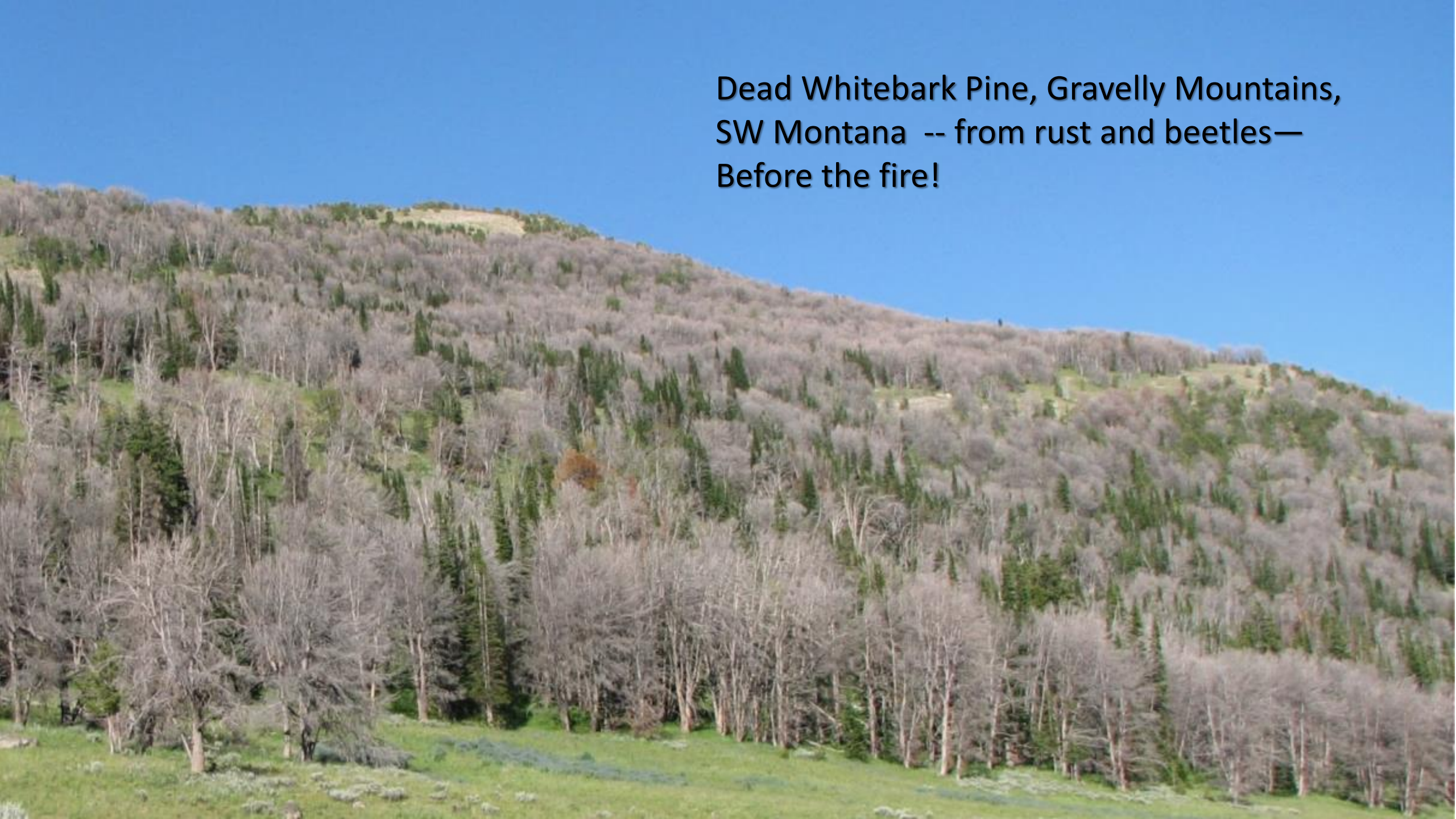


Eureka Basin
Burn Site
Gravelly Mountains, MT

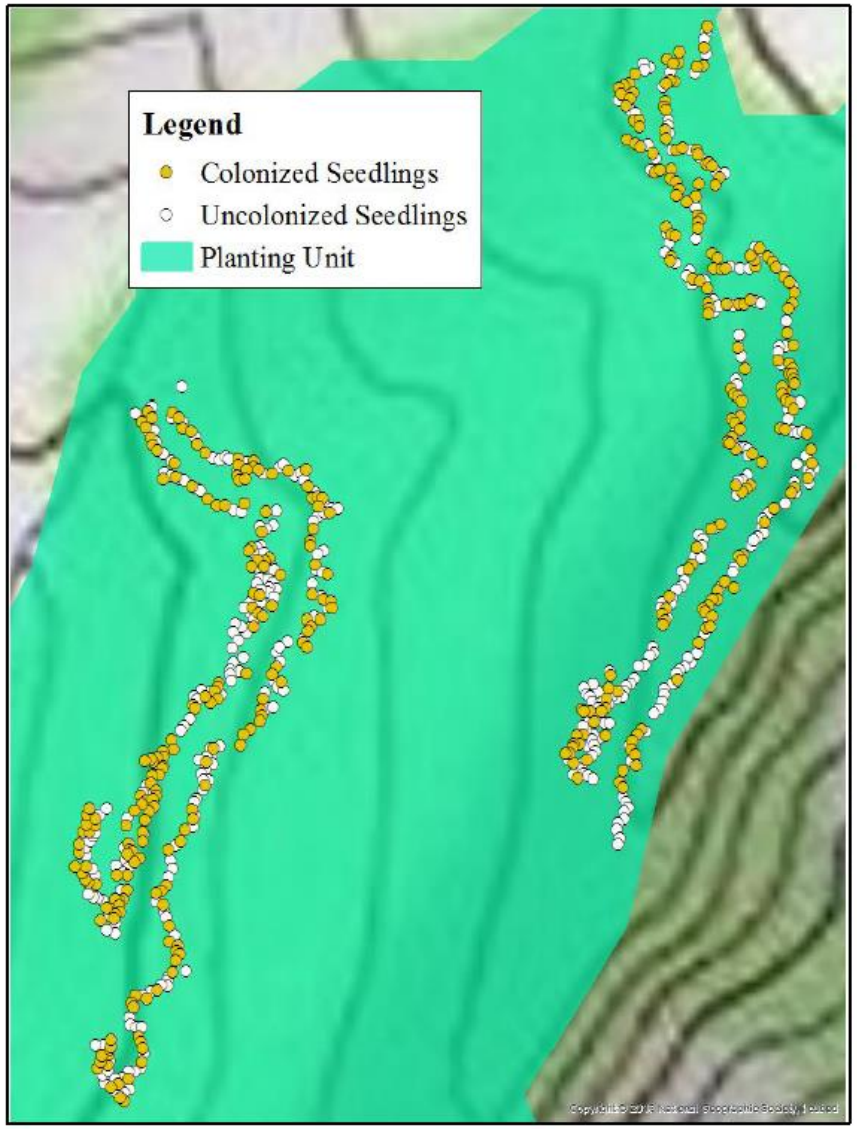
4,000 acres burned in 2013

U.S. Forest Service planting 36,000 whitebark pine seedlings/yr, starting in 2015

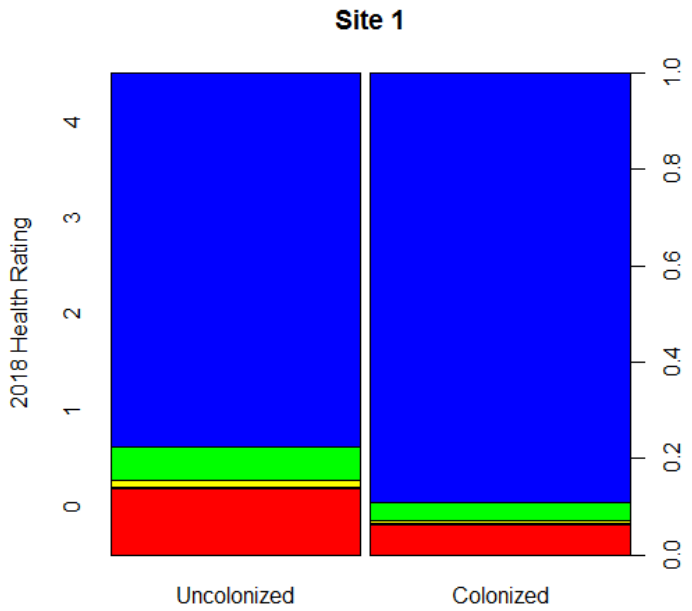
800 + 800 seedlings monitored, half **<u>colonized</u>** with *Suillus*

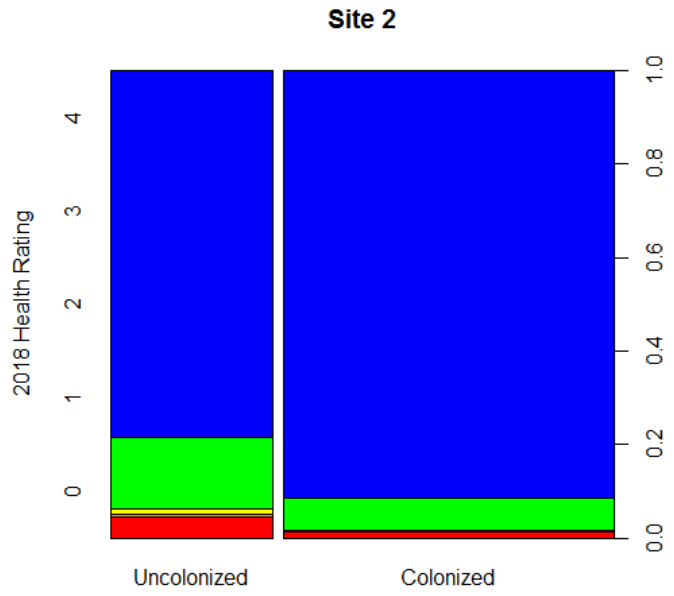

Dead Whitebark Pine, Gravelly Mountains,
SW Montana -- from rust and beetles—
Before the fire!

Early results of health effects 2018



Seedling mycorrhizal status across 2 transects on site 1 in ArcMap for 800 seedlings





<u>Health rating</u>

Blue = healthy seedlings
Green = yellowing needles
Yellow = partially dead
Red = dead

- Colonized seedlings have statistically significant higher health rating
- Seedling health data attached to GPS points using ArcMap Software
- Relate to site factors including colonization, location, wetness, aspect, etc.

High and dry looks better so far!

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High Country News for featuring our work.

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Full paper

Sporocarp $\delta^{15}N$ and use of inorganic and organic nitrogen *in vitro* differ among host-specific suilloid fungi associated with high elevation five-needle pines

Robert K. Antibus [a,*], Erik A. Hobbie [b], Cathy L. Cripps [c]

Plant Soil (2018) 425:113–131
https://doi.org/10.1007/s11104-018-3577-x

REGULAR ARTICLE

Scorched Earth: *Suillus* colonization of *Pinus albicaulis* seedlings planted in wildfire-impacted soil affects seedling biomass, foliar nutrient content, and isotope signatures

Martha L. Jenkins · Cathy L. Cripps · Leslie Gains-Germain

forests

MDPI

Article

Designer Niches Promote Seedling Survival in Forest Restoration: A 7-Year Study of Whitebark Pine (*Pinus albicaulis*) Seedlings in Waterton Lakes National Park

Cathy L. Cripps [1,*], Genoa Alger [2] and Robert Sissons [3]

High Country News

Montana mycologist fights fungus with fungus To save whitebark pines, apply slippery jack.
Ben Goldfarb Dec. 22, 2014

Webinar link: **Cripps Whitebark pine and native mycorrhizal fungi** UTube
https://www.youtube.com/watch?v=JlHKCoaSBlw

3 papers & extended abstracts