Post-Fire Microbial Inoculation

Methods and Protocols for PFMI Kits

This guide was assembled in November, 2024
and should be updated as appropriate

Background

In the past three decades, wildfires have increased in size and severity throughout Western North America, posing far-reaching ecological consequences (1). Fire impacts both above- and belowground communities, shifting microbial richness or diversity and altering soil carbon dynamics (2, 3). Fungi and the ecosystems they support may benefit from post-fire microbial inoculation (PFMI) after severe wildfire. As PFMI can support plant growth (4, 5) it may also reduce sedimentation (6, 7) and prevent contaminants from reaching sources of drinking and irrigation water. Incorporating microbes that control erosion could reduce the likelihood of landslide events, thereby reducing excessive damage to roads and commerce. Because of the ability of some soil microbes to absorb heavy metals (7, 9), this active ecosystem restoration approach may also mitigate the impact of burned structures and associated contamination for nearby homeowners.

When using PFMI as a restoration tool, several factors are to be considered for successful and ecologically beneficial inoculation (10, 11). In some situations, there are risks to altering the soil biology of an area and caution is necessary (12, 13). In this guide we outline the major factors one should consider when deciding to use PFMI, primary methods used, and detailed protocols for each.

Photo: These specimens of *Crassisporium funariophilum* were found growing in soil on the side of a trail within San Vicente Redwoods (mixed hardwood/conifer forest). This area burned at high severity in the 2020 CZU fire and at time of collection was dominated by early successional *Eriodictyon californicum* and *Ceanothus spp*. See [iNaturalist] for more details.

Things to consider when deciding to use PFMI

Land ownership

Whether the fire occurred on public or private land may restrict the type of PFMI that can be employed. For example, public lands may require special permits to conduct any type of active restoration, including the use of a microbial inoculum to stimulate soil productivity. However, on private lands, there are less (or no) restrictions.

Structures burned

When a man made structure is burned in a fire, there is often a problem with toxic runoff and risk of contaminants entering watersheds. Some species of fungi are capable of absorbing these contaminants (e.g. heavy metals and polycyclic aromatic hydrocarbons). If using these fungi, toxins can accumulate in fungal tissue, be harvested, and then properly disposed of to remove toxins from the environment.

Fire type

If the burn was low-intensity (e.g. a prescribed fire or a fire in a grassland or a previously managed site with low fuel load), the soil microbial community likely did not face high mortality and will recover quickly. In this case, no PFMI treatment would be necessary, but you could add carbon resources (e.g. an uninoculated wattle) to provide an easily accessible food source for those local soil microbes. If the burn was high-intensity (e.g. a wildfire that burned a wooded/forest area), then soil microbial communities may take decades to recover to their pre-burn levels of complexity and may greatly benefit from PFMI to kick-start the recovery process.

Photos: PFMI wattles deployed around a structure fire (left), and in a burned woodland (right)

Primary methods of PFMI

(1) Inoculation of wattle with a single strain of native fungi

With this method, native fungi are collected (or a culture of native fungi is acquired from a local mycologist) and the spawn (e.g. cultivated mycelium) is used for inoculation. There are several ways which these native fungi can be used to inoculate soil at the restoration site, and the choice of which fungal species to use is very important. One must be very careful to choose a local species or strain of fungi to use because some species, such as the Eurasian golden oyster mushroom (14), can be highly invasive outside of their native range (12, 13).

(2) Inoculation of wattle with soil from reference site

With this method, soil from relatively intact nearby habitat (an area that was not burned during the high-severity fire) is collected, tested for presence of pathogens, and used for inoculation. Reference soil can be mixed with soil at the restoration site in several different ways. One must be careful to not transplant harmful soil pathogens, such as *Phytophtyora* species, which is an invasive pathogenic microbe from East Asia known to cause sudden oak death in the Western US (15).

(3) Inoculation of wattle with a compost tea

With this method, compost or a compost extract (tea) is used for inoculation. Like the reference soil method, there are several ways one could use compost to inoculate the wattle at the restoration site. Also, the choice of where to source compost for your project depends on site-specific characteristics and availability. When sourcing compost, think local (as with the above two methods), and consider using a compost that has a complete soil food web (16, 17). Compost input and output quality can vary dramatically, either yielding a final product that is biodiverse with bioavailable nutrients for plants or a product that is void of microbial life with less ecological value.

(4) Use an uninoculated wattle to stimulate soil microbes

With this method, wattles filled with some form of cellulose (e.g. jute or straw) are implemented at the reference site without any form of additional inoculum (e.g. soil or fungal spawn). This protocol is the most straight-forward, as it does not involve manipulation of biological organisms. However, just because it is a simple method, does not mean that it is ineffective. In many degraded habitats, soils are lacking in organic matter and thus soil microbes do not have sufficient resources to form complex communities. The simple addition of cellulose, which is very easy for soil microbes to metabolize, will stimulate microbial growth and activity (17).

For each of the above methods, see our detailed protocols and/or contact us for more information about how to successfully apply the method at your site.

Photo: wattles filled and ready to deploy!

Choosing a method

With the above considerations and methods at your disposal, it is time to make a choice for which PFMI method(s) you are going to use. With our FENiXS study, we compared plant and microbial response to each of the above 4 methods. Although each inoculation method altered microbial communities in some way, there are a multitude of variables to consider and our data analysis to date has yet to reveal any clear cut best practices for using PFMI. We are trying to untangle the ecological nuances and mysteries with our research, but it is an endless process of discovery and we need to try many different approaches to discern all the variables. Much more experimentation and monitoring needs to be done to deepen our understanding of which method would be best to use in a given habitat, with a given disturbance history, and available resources or constraints. However, based on our study and field experiences, we can recommend these protocols to follow when setting up your own PFMI experiment.

For our FENiXS study, we constrained ourselves to rigid protocols in order to reduce bias in our experimental design. Ultimately there are many ways these methods can be employed, and the protocols outlined below are merely guides for you to follow and adjust based on your land and resource scenarios. We encourage you to experiment (after you have done your homework) and report back to us your experiences and observations.

Photo: Fungi and other microbes are first on the scene after fire combusts vegetation; they kick-start successional processes that govern ecological resilience to disturbances. However, not all microbes are able to metabolize products of fire (e.g. ash, charcoal & "biochar"). Certain "pyrogenic" fungi have evolved to consume these post-fire resources and this ability is considered to be a fire-adapted trait. Here you can see these bright beauties hard at work in the field! They are likely a species of genus *Anthracobia* from the family *Pyronemataceae*.

Materials & Protocols:

Kit contents

- Wood fiber wattle socks
    - 3 - 1 meter long
- Wooden stakes
    - 2 stakes per wattle

Photo: sample kit

Extra materials

- Substrate to fill the sock
    - If you choose method 1 (single strain of fungi), your substrate should match the fruiting preferences of that fungi (see protocol below); for other methods, you can be flexible
    - Ex: straw, wood chips, sawdust, compost, leaf litter, or local understory duff
- Bucket(s) or tub(s) for mixing materials and/or storing soil
    - Optional & depending on chosen method
- Shovel or trowel for digging the furrow
- Hammer or mallet to secure stakes in the ground
- Microbial inoculant (no need for method 4)
    - Choose an inoculant based on the method you chose

Photos: wattles are placed in dug-out furrows (left); we use a special machine to fill wattles for large-scale projects (right), but you can also fill them by hand. Tie the ends of your wattles closed with a simple bow knot.

Site selection & installation

Wattles are commonly used to mitigate erosion at construction sites and the goal with PFMI is to build on that concept by increasing the capacity of this biological barrier to capture rainwater as it runs downslope in recently burned areas. If a man made structure was burned in the fire, you may want to install the wattles downhill of that structure to mitigate toxic runoff.

Most pre-made erosion control wattles are 25 feet long and cover the entire contour of a slope to capture as much water runoff as possible. However, our PFMI wattles are much smaller as they are designed for experimentation and we space them out to test different methods (photo below, left). If you have extra resources available, you can expand on the kit to cover more ground and capture more runoff (photo below, right). Some counties will even assist landowners by applying wattles post-fire.

After you have chosen the PFMI method you would like to use, and have filled your wattles with the corresponding substrate and inoculum, you will install your wattles at your site. To install your wattle, dig a shallow furrow or trench in the ground (approx. 4 inches deep) that is as long as your wattle. Place the wattle in the furrow, stake in place, and (optionally) cover with displaced soil and/or wood chips as mulch. Note, if heavy water runoff is expected, mulch will likely not remain in place. You may even choose to water your wattle initially (and/or regularly) just as you would do for a recently transplanted plant. This may be especially useful if it is not yet the rainy season. Also, the filled wattle(s) may be heavy to transport from assembly to installation areas - consider using a wheelbarrow.

Photos: wattles can be placed on a slope (left), or on flat ground (right); they can be covered with displaced soil (left) or not (right); they can be spread out (left) or aligned end-to-end (right)

Method 1 Protocol:

Inoculation of wattle with single strain of native fungi

Notes and special considerations:

(1) As mentioned above, it is very important that you choose a fungal species or strain that is local to the region where you plan to employ your PFMI wattle. Like plants and animals, fungi can be invasive and negatively impact ecological communities that they did not evolve with (12-14).
    (a) You can use the Fungal Diversity Database on iNaturalist to help discover local fungi that may suit your needs and/or contact a local mycologist for advice

(2) If you choose the single strain method, we recommend that you first learn some basic information about fungal life cycles and common approaches to DIY mushroom cultivation. After all, with this method, you're growing mushrooms in your wattle to create a biofilter. There are many resources you can use, including (but not limited to):
    (a) Organic Mushroom Farming and Mycoremediation, a book by Tradd Cotter
    (b) The Mushroom Cultivator, a book by Paul Stamets
    (c) Youtube channels such as: Boomer Shroomer, North Spore, and FreshCap Mushrooms *just to name a few, but here is a more complete list of resources*

Step-by-step instructions:

(1) Acquire spawn of native fungi
    (a) You can do this by
        (i) Collecting your own specimens in the wild and culturing them
        (ii) Finding a local mushroom cultivator who already has spawn
    (b) You can use different forms of spawn
        (i) Grain spawn - this is a common intermediate growing media for fungi and is packed with nutrients
            1) However, if using grain spawn to inoculate wattles in the field, you may run the risk of having animals (e.g. rodents) chew through your wattles to eat the delicious grain
            2) If you use grain spawn, inoculate your wattles indoors and let them colonize before installing outdoors
            3) This is the protocol we used in our FENiXS study
        (ii) Sawdust spawn - this is also a common intermediate growing media, but is only suitable for some species of fungi
        (iii) Pre-colonized straw and/or wood chips can also be used as spawn to inoculate wattles - depending on the metabolic preference of the fungi you're using
    (c) Common relatively easy fungi to grow
        (i) *Pleurotus spp* (oyster mushrooms) are known to be easy to grow because they are not picky eaters and many species are tolerant to adverse environmental conditions (e.g. drought, heat, frost). However, you must find a local strain if you

are releasing spawn in the environment. Golden oyster (*Pleurotus citrinopileatus*) is a commonly cultivated mushroom, but has become invasive in natural areas across North America (14) and it is very important we do not spread it further.

(ii) Depending on your area, there may also be local varieties of other commonly cultivated mushrooms that you could choose from, such as shiitake or lion's mane

(d) Our PFMI staff will help you choose the right spawn during your consultation

(2) Pasteurization of substrate - prepare your wattle for inoculation

(a) This carbon resource will be quickly colonized by microbes when exposed to the open air. With this method, we want to promote the colonization and growth of a single fungal strain and thus need to give it a head start/clean slate

(b) To do this, you want to pasteurize your substrate before inoculation so there are little to no other microbes competing for this resource

(c) There are many ways to pasteurize the substrate - choose a method based on the type of mushroom you are working with & resources available to you

(d) Here are two common approaches for outdoor mushroom cultivation

(i) Heat pasteurization - e.g. soak straw/etc in a large pot/bucket/etc, completely submerged in hot water (140-160° F) for 2-4 hours, covered

(ii) Cold water fermentation - e.g. soak straw/etc in a large pot/bucket/etc, completely submerged in cold water for 7-10 days

1) Soaking kills aerobic microbes (require oxygen), draining and exposing to air kills anaerobic microbes (require absence of oxygen)

2) This is the protocol we used for our FENiXS study

(3) Inoculate your substrate & fill your wattle

(a) Use clean (e.g. washed with soap and/or sterilized with ethanol) hands, surfaces and tools to reduce contamination when inoculating

(b) Timing - you can either

(i) Inoculate/fill & install same day

1) Pros: quick; less need for space

2) Cons: colonization of the substrate by the fungal mycelium takes time - if outdoors, exposure to elements may encourage colonization by other microbes that are more competitive than your intended strain, and your wattle is more likely to dry out (which can delay colonization)

(ii) Inoculate/fill & let incubate indoors/covered before installation

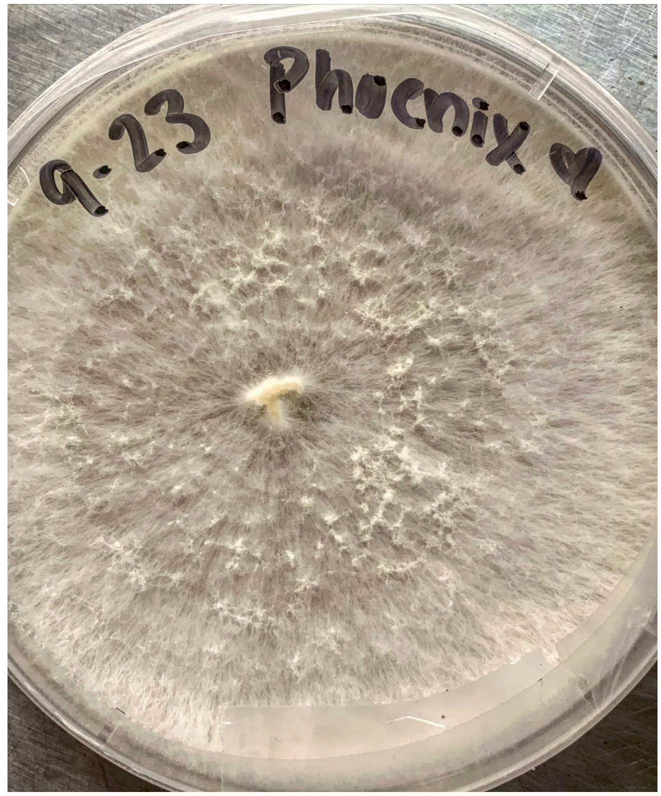
1) Pros: the colonization process can be more controlled - you can keep it moist more easily, many fungi also benefit from being covered/in the dark (as they would be underground or in woody tissue in the wild)

2) Cons: more hands-on/time/space needed

(c) Inoculate by either

(i) Mixing substrate with spawn (e.g. in a sterilized bin/ on a tarp) and fill wattle

(ii) Adding substrate/spawn to your wattle in layers



Photos: Oyster mushrooms (*Pleurotus sp.*) found in California habitats (top left) are cultured in petri dishes (top right) by mycologists (bottom)

Method 2 Protocol:

Inoculation of wattle with soil from reference site

Notes and special considerations:

(1) As mentioned above, your choice of reference location to collect soil is very important
   (a) Here is a thorough video of this process created by Point Blue Conservation Science
   (b) You want to choose a natural area that is as similar as possible to the area you are planning to install your PFMI wattles (12-15)
      (i) Consider: habitat type (plant community), microclimate (quantity and type of precipitation), land-use history (avoid areas that have been used for intensive agriculture), and even topography/aspect
   (c) Ideally your reference area will not have been burned in the same recent fire that impacted the land you're using PFMI on
      (i) However, wildfires can burn in a patchy way, with some areas burning at low-intensity (e.g. areas that had previously been cleared of heavy understory fuels). These areas may be excellent options, especially if they have native older trees, because soil microbes likely recovered quickly and are thriving with their fire-adapted plant hosts
   (d) Consider permissions! Do not collect soil on private land without permission from landowners and equally, consult land managers of public lands

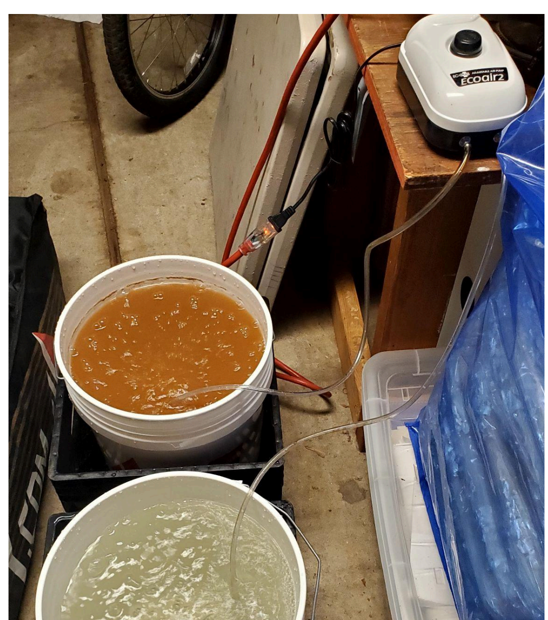
(2) Also as mentioned above, this method comes with the risk of transferring soil pathogens
   (a) The primary pathogen to consider in the Western US is *Phytophtyora*, which is an invasive pathogenic microbe from East Asia known to cause sudden oak death in the Western US (15). However, it is important to know that this blight can parasitize many other plants, including both native plants (in wild and in nurseries), as well as agricultural crops - beware and take precautions!
   (b) You can mitigate risk of transferring *Phytophtyora* by following some best-management practices outlined by OSU Extension Service, the California Oak Mortality Task Force, and the University of California ANR
      (i) Look for symptoms on plants in areas you are considering as reference sites
      (ii) Avoid collecting soils in areas that accumulate water after rains
      (iii) Avoid collecting soils in areas with dense understory vegetation (living and dead)
      (iv) Sterilize collection tools (e.g. shovel, bucket, hands/gloves) before & between use
   (c) You can send soil to a lab to test for *Phytophtyora*
      (i) The National Plant Diagnostic Network last a list of accredited labs
      (ii) Also see the Cal. Dept. of Food and Agriculture (CDFA) diagnostic center
   (d) You can also perform some simple diagnostic tests on your own, but they are not as accurate as a full lab diagnostic

        (i) Immunoassay test kits (e.g. Agdia's ELISA kit) can be purchased. However, these are designed for plant tissue (including roots) and not specifically soil. If there are symptomatics plants nearby, this could be a helpful tool
        (ii) The pear-bait test can be used. This involves soaking soil in a bag/bucket for several days with a nearly-perfect pear. After a few days, see what happens to the pear and certain physical indicators are diagnostics
            1) Here are detailed protocols from Phytosphere, and the California Native Plant Society (CNPS). Also see the video linked in 1-a above
            2) We used this protocol for our FENiXS study
    (e) Besides known pathogens, soil translocation can also spread other non-native microbes - for example, those that have become naturalized in agricultural settings (12, 13)

<u>Step-by-step instructions:</u>

(1) Locate a reference site
    (a) See above notes about site selection & precautions
(2) Collect soil from reference site
    (a) Volume - this could be highly variable
        (i) For each wattle, you will want 1-3 gallons of soil
(3) Test for *Phytophthora*
    (a) See above notes for testing resources
(4) Optional - make a compost tea with your reference soil
    (a) See method 3 below for more details
(5) Add soil to wattle and/or directly to ground at installation
    (a) There are many different ways to use soil as a source of inoculum - all need to be tested and we encourage you to experiment and report back to us what you observe
    (b) For the FENiXS study, we used a combination of the following
        (i) Mix/layer reference soil with substrate as you fill the wattle
        (ii) Sprinkle reference soil on top of soil at installation location, after furrow is dug
        (iii) Sprinkle reference soil on top of wattle (and/or mixed with displaced soil) after wattle is placed in the furrow
        (iv) Pour a compost tea made with reference soil over the installed wattle

Photo: soil and/or compost aerating in buckets using an air bubbler



Method 3 Protocol:

Inoculation of wattle with a compost tea

Step-by-step instructions:

(1) Find compost
  (a) You can either
    (i) Make your own
    (ii) Source a local compost
      1) For the FENiXS project, we used a commercial product that had only 2 species of microbes found in the region
  (b) Considerations
    (i) As mentioned above, compost quality is variable and depends on the quality of source material, compost method used, level of attention/monitoring, etc (16, 17)
    (ii) Our best-practice recommendation is to source as local & as high quality (e.g. as biodiverse) as possible
    (iii) There is still a need to consider introducing invasive soil microbes - see method 2 for details about *Phytophtyora*
    (iv) Also, soil microbes that are composting agricultural products, livestock residues, and/or kitchen waste will not necessarily be the same as microbes that would be best for landscape restoration

(2) Optional - make a compost tea with your reference soil
  (a) Compost teas can be aerated or non-aerated
  (b) If using a commercial product, follow the provided instructions
    (i) For the FENiXS study, we aerated the compost in a bucket with a bubbler
  (c) If using your own or a locally-sourced compost, follow a guide and do your homework
    (i) Rodale Institute guide
    (ii) University of Vermont article & guide
    (iii) USDA article cautioning use of additives in compost tea
    (iv) Washington State University article

(3) Apply compost
  (a) There are many different ways to use compost as a source of inoculum - all need to be tested and we encourage you to experiment and report back to us what you observe
  (b) For the FENiXS study, we used a combination of the following
    (i) Mix/layer compost with substrate as you fill the wattle
    (ii) Sprinkle compost on top of soil at installation location, after furrow is dug
    (iii) Fully submerge the filled wattle into the bucket with compost tea
    (iv) Pour a compost tea over the installed wattle

Method 4 Protocol:

Use an uninoculated wattle to stimulate growth

Step-by-step instructions:

(1) Stuff wattle with substrate and install - it's as simple as that!
   (a) See notes above about choice of substrate and installation location
   (b) For this method, we recommend that the wattle is soaked with water ahead of time, as a moist environment will be more attractive to soil microbes, and the wet substrate will be easier for microbial metabolism

Photo: uninoculated wattles still deliver easy-to-metabolize carbon nutrients to soil microbes in areas that may otherwise have little resources available after fire or other disturbances. Microbes in the environment will quickly invade the wattle and begin to decompose the added substrate. Just add water!

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