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Evidence that pyrophilous fungi aggregate soil after forest fire

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ABSTRACT

Forest fire is an important occurrence in western landscapes where it helps drive ecosystem processes, and prescribed fire is a common forest management strategy. An initial consequence of fire is burned ground with reduced biodiversity. Numerous studies have documented how forests recover after fire in terms of plant regeneration and animal colonization, but little is known of the ecological roles fungi play in this process. Pyrophilous (burn-loving) fungi are documented to reliably produce large fruitings and copious mycelium on burns after fire in North America, Europe, Asia, and Australia. We hypothesize that pyrophilous fungi help bind and stabilize soil after forest fires via their extensive mycelial network. Three pyrophilous fungi, *Geopyxis carbonaria*, *Pyronema omphalodes*, and *Morchella septimelata* were tested for their ability to aggregate burned soil. The fungi were isolated from burn sites, grown in vitro, and inoculated onto sterilized soil from a natural burn. The ability of each species to aggregate soil in comparison to non-inoculated controls was assessed after 10, 20, 30, and 40 days, using a wet sieve aggregate stability test. All three fungi increased soil aggregation after 10 days, and this increase was maintained for the 40-day period. The burned soil was up to 30% more aggregated when a fungus was present; results provide the first direct evidence that pyrophilous fungi aggregate burn soil. This further implies that these fungi play a role in reducing soil erosion and enhancing soil moisture soon after fire in burned forests. Pyrophilous fungi also decompose charred material, sequester carbon, and capture transient nitrogen pulses after fire. This overlooked group of fungi may be critical in enhancing conditions for plant regeneration after forest fire at an early stage in recovery. Consideration should be given to avoiding or delaying restoration activities that disturb this natural process, especially those that contribute to soil compaction, during early post-fire recovery when these fungi are proliferating.

1. Introduction

Forest fires are an important phenomenon in western landscapes where they help drive ecosystem process (Pausas and Keeley, 2009; He et al., 2019), and prescribed fires are a common strategy for forest management (Hunter and Robles, 2020). Forest fires open up the landscape to new growth and regeneration by resetting the successional clock (Heinselman, 1981). The initial consequence for both wild (Walker and del Moral, 2003) and prescribed fire (Esquilin et al., 2007) is burned ground almost devoid of life or with reduced biodiversity depending on fire intensity and other factors (Neary et al., 1999). Subsequent recolonization by plants and animals has been well documented (Keeley et al., 2011; Pausas, 2018); however, less is known about how fungi respond to fire and the ecological roles they play in the recovery process. It has been hypothesized that a certain group of fungi serve to stabilize soil, reduce erosion, and enhance moisture retention on burns after fire through natural process (Claridge et al. 2009); our goal is to

provide evidence for this hypothesis. The timing of post-fire restoration strategies that contribute to soil compaction during an early sensitive recovery phase might need to be reconsidered in light of new information.

Fire typically reduces the microbial biomass in soil, and the fungal portion is particularly sensitive to burning (Guerrero et al., 2005; O'Dea, 2007; Dooley and Treseder, 2011; Holden and Treseder, 2013; Pressler et al., 2019). Fire has the potential to negatively impact pre-existing ectomycorrhizal communities and subsequent colonization of regenerating seedlings (Cairney and Bastias, 2007; Mataix-Solera et al., 2009; Dove and Hart, 2017; Taudière et al., 2017). Soil saprophytes also can be decimated (Widden and Parkinson, 1975), but these fungi appear less susceptible and may recover faster after fire (Treseder et al., 2004; Sun et al., 2015; Holden et al., 2016). Overall, less is known about their response (Kauki and Salo, 2020). However, one particular group of fungal saprophytes consistently responds positively to fire and shows up early in the forest recovery phase, before mycorrhizal fungi and their

hosts have established.

The pyrophilous fungi (also called burn, postfire, fireplace, carbonicolous, or phoenicoid fungi) produce a striking phenomenon when they fruit prolifically and extensively across burned forest landscapes one and two years after a fire (Carpenter and Trappe, 1985). They typically fruit following snowmelt in the spring or after the first significant rain event, but can be visible as soon as one or two weeks after fire (Vrålstad, et al. 1998; Watts et al., 2018, Bruns et al., 2020). It has been estimated that they have the potential to produce up to 100,000 fruiting bodies per hectare (Claridge et al., 2009) and fruiting bodies have been counted at 1000 per square meter (Vrålstad, 2004). This amazing display has been well documented in the western North America, but occurs on a global scale after forest fires and is reported in Australia and Eurasia (McMullan-Fisher et al., 2011; Watts et al., 2018). The most well-known fungi in this group are the burn morels (*Morchella* species) that fruit in abundance after fire (Greene et al., 2010; Larson et al., 2016). Burn morels are a non-timber product with high economic value, particularly in western North America (Alexander et al., 2002; Wurtz et al., 2005; McFarlane et al., 2005). However, a whole cadre of pyrophilous fungi has been documented on burned soil and charred wood after forest fires (Petersen, 1970; Claridge et al., 2009; Hughes et al., 2020). Many, such as *Pyronema, Anthracobia* and *Geopyxis* species, are cup fungi (Ascomycota) that fruit prolifically and consistently after fire (Greene et al., 2010; Bruns et al., 2020; Raudabaugh et al., 2020). A recent molecular study showed that pyrophilous fungi were the one fungal guild that responded favorably to fire (Kauki and Salo, 2020). The species of pyrophilous fungi are well-known, but their ecology and the functional roles they play in post-fire systems are not.

As saprophytes, these fungi restore nutrients to the soil and promote the conditions for plant succession to begin, but they may provide additional ecological services as well. Claridge et al. (2009) hypothesized that the pyrophilous fungi play a role in soil stabilization on burns, but provided no evidence other than direct observation. In addition to fruiting bodies, these fungi produce extensive mats of mycelium across burned areas that appear to bind the soil (Claridge et al., 2009; Bruns et al., 2020). Mycelium comprises the main body of fungi and consists of long, stringy strands of chitinous material; on burns it is interwoven with soil particles. If these fungi are found to aggregate soil, the broader implications are that they play a role in reducing erosion and increasing moisture-retention in soil on burns during a sensitive early forest recovery phase.

Structure is an important feature of soil and is derived from the interplay of aggregates and pore space (Lehmann et al., 2020). Aggregates are the stable association of individual particles (mineral and organic), as a result of grain-size composition, biological ineractions, and physical-chemical properties of soil (Mataix-Solera et al., 2011). There is ample evidence that fungi are important in the aggregation and stabilization of soil in non-burned landscapes (Lynch and Bragg, 1985; Degens et al., 1996, Tisdall et al., 1997; Lehmann et al., 2017, 2020). In agricultural soils, aggregation by microfungi has been confirmed to contribute to soil health crop productivity (Tisdall and Oades, 1982; Tisdall, 1991; Eash, 1993). In forests, ectomycorrhizal fungi on roots are known to promote soil aggregation (Mataix-Solera et al., 2009) and soil stabilization particularly on slopes (Graf et al., 2019); arbuscular mycorrhizal fungi produce glue-like glomulin which aggregates soil (Wright and Upadhyaya, 1998; Rillig and Mummey, 2006). In open areas, microbial crusts, which can include fungi, protect against wind erosion as a layer on top of soil (Belnap and Gillette, 1998). However, few studies have examined the role saprophytic fungi play in the aggregation of soil on forest burns, and none have addressed the pyrophilous fungi in particular.

Fire can disaggregate soil as a consequence of organic matter destruction due to combustion or conversely increase aggregation by mineralization of certain soils; both processes are dependent on the intensity of the fire (Mataix-Solera et al., 2011). The reduction of aggregate stability in most soils is an important factor that influences post-fire

erosion and soil restoration (Mataix-Solera et al., 2009). Intense fire especially can reduce the aggregate size above the 2 mm fraction (Andreu et al., 2001; Mataix-Solera et al., 2009). In one of the few studies that examined soil aggregation after fire, the amount of live fungal biomass was found to be a strong predictor of soil loss from wind erosion after prescribed burning in a semi-arid non-forested area (O'Dea, 2007). The live fungal fraction of the soil organic carbon strongly and positively influenced soil structural stability and reduced erosion in grassland savannas (O'Dea, 2007). We could find no studies that examined the aggregation of burned soil by fungi in previously forested systems.

While fungi appear to be one of the microorganism groups most sensitive to fire, pyrophilous fungi consistently proliferate within weeks to a year or two after fire (Claridge et al., 2009; Bruns et al., 2020). While it seems likely that this set of fungi have the ability to aggregate soil on burns, previously there was no experimental evidence to confirm this. The goal of this research is to evaluate the potential impact of pyrophilous fungi on the aggregation of burned soil. Three pyrophilous fungi (*Morchella septimelata, Geopyxis carbonaria, Pyronema omphalodes*) were isolated from burns, and evaluated individually for their ability to aggregate sterilized soil from the same burn using a wet sieve soil aggregation test. This was done in vitro with time series assessments at 10, 20, 20 and 40 days post-inoculation as a proxy for mycelial accruement. Mycelial mass could not be measured, but was inferred to increase over time. If these fungi function to aggregate burned soil, implications are that they help stabilize soil, prevent erosion, and enhance moisture retention; this is in addition to their known ability to release nutrients through decomposition. All of these ecological roles serve to promote plant establishment in early forest succession. Results have implications for consideration of various post-fire restoration strategies, particularly those that contribute to soil compaction at an early successional stage in the recovery of burned forests (Jennings et al., 2012).

2. Methods

2.1. Collection and processing of fungi and soil

The TenMile Fire near Trego, MT occurred in August 2018 and burned 694 acres; it was not assessed for severity. Burn fungi were collected on the burn in 2019, one year after the fire. Fruiting bodies of ascomycota *Geopyxis carbonaria* and a *Morchella septimelata* were transported to the lab and cultured onto Malt Extract Agar in petri dishes. *Pyronema* was also found on the TenMile burn, but was not successfully isolated, so a culture of *Pyronema omphalodes* isolated from a burn in Tennessee, was obtained from Dr. Karen Hughes. It was important to include this fungus as it is known to produce copious amounts of mycelium on burns. The morel was identified by sequencing the ITS region followed by a Blast Search (Gardes and Bruns 1993), and others were identified morphologically. Cultures were allowed to grow out until they filled Petri dishes which was only a few days, as they are fast growers. Soil was collected from the TenMile Burn in buckets in 2019 and transferred to the lab where larger particles were removed using a one-inch mesh screen (Fig. 1). The burn soil was used to fill jars for the experiment.

2.2. Study design

In all, 72 wide-mouth pint canning jars of soil were used in the experiment. Each jar was filled with 280 mL of burn soil, 20 mL of deionized water, and 20 mL of liquid malt extract to initiate fungal growth. A 3/4'' hole was punched in the center of each lid which was underlain with filter paper to allow for fungal respiration and to prevent contamination. Jars with soil, media and water were autoclaved for 30 min to sterilize soil and kill any resident microbes. After cooling, 36 jars were left uninoculated as controls. One plug of mycelium from a cork borer was used to inoculate each of 36 jars with fungi as follows: twelve

Fig. 1. Pyrophilous fungi on burnt ground. Top left: cup fungi *Geopyxis carbonaria* and top right *Pyronema omphalodes*. Bottom left fungal mycelium binding soil and bottom right the burn morel *Morchella septimelata*. Photos by C. Cripps, except P.B. Matheny for *Pyronema*.

jars with *Pyronema,* twelve with *Morchella,* and twelve with *Geopyxis.* Jars were placed in a dark box to simulate the original environment and fungi were allowed to grow through the soil. Every 10 days following inoculation, 3 jars from each treatment (and 3 control jars) were selected, and the soil in each was assessed for aggregation. Soil in selected jars was processed after 10, 20, 30 and 40 days. The time series was used on the assumption that mycelium would accrue over time and that jars processed first would have less mycelium and less aggregation.

2.3. Assessment of soil aggregation

A Wet Sieve Aggregate Stability Test was used to determine the amount of aggregated soil in each sample (Kemper and Rosenau, 1986; Vanek, 2018). The burned soil in each jar was washed through a series of sieves (2 mm, 0.5 mm, and 0.25 mm) as follows: soil was placed on the 2 mm sieve in a container filled with water 4 cm deep and soaked for 5 min; the sieve was then dunked in and out of the water for 2 min at half second intervals; soil remaining on the sieve (aggregated soil) was removed and dried. The soil slurry that had traversed the first sieve was then carefully poured though the 0.5 mm sieve, and the dunking process repeated for 2 min.; the soil left on the second sieve was removed and dried. This was repeated for the 0.25 mm sieve. Soil segregates were dried in a plant dryer room for 7 days and weighed. The method was repeated for controls without fungi (Fig. 2). All was repeated every 10 days for the same number of treatment and control jars.

2.4. Statistical analysis

The weight of soil portions on each sieve was recorded for each treatment and control jar. The portion of soil on the first (2 mm) sieve was considered the aggregated soil; thus a larger amount of soil on the first (2 mm) sieve was equated with higher aggregation.

Fig. 2. Wet sieve soil aggregation test. Soil added to the sieve set is soaked and dunked twice per min for 2 min. Soil on top sieve is then dried and weighed. The process is repeated for the next sieves.

Table 1
Aggregation of burned soil by pyrophilous fungi. Percentage of aggregated soil (in the 2 mm sieve) for 3 fungal treatments after 10, 20, 30, and 40 days, and standard error. Means with different superscript letters are different at p < 0.05.

	10 days (%)	20 days (%)	30 days (%)	40 days (%)
Geopyxis carbonaria	63.67 (0.74) [A]	82.01 (1.32) [A]	84.67 (1.10) [A]	83.14 (1.10) [A]
Pyronema omphalodes	67.92 (0.56) [A]	68.54 (0.39) [B]	71.96 (2.83) [B]	77.71 (4.46) [AB]
Morchella septimelata	54.84 (2.67) [AB]	69.18 (1.63) [B]	73.82 (0.34) [B]	65.57 (5.27) [BC]
Control	47.16 (3.82) [B]	47.11 (2.37) [C]	54.08 (1.77) [C]	55.39 (2.16) [C]

% aggregation = dry weight of soil on sieve/total weight of soil in jar × 100

Results were plotted on a bar graph as % aggregation for each sieve size for each fungal species and controls were pooled for each assessment time (10, 20, 30, 40 days). Results were plotted for each 10-day assessment as % aggregation for each sieve size for each treatment over time. An analysis for the best model was conducted to assess the potential difference in soil aggregation over time for each treatment (R Core Team, 2020). The variables of interest considered were fungal species, treatment type, sieve size, days since inoculation, and percent aggregation. Initial alterations started with the removal of sieve size from the analysis, as observations were not independent from one another, and the 2 mm sieve was driving results. Model diagnostics and AIC values were used to assess a series of subsequent models until the final simple linear model below was selected. This mixed effects model represents percent aggregation as a function of the fixed effects: day since inoculation, fungal species (compared to the uninoculated control), and their interaction, with jar as a random effect.

$\mu\{Percent: Days, Treatment\} = \beta_0 + \beta_2 Days + \beta_2 I_{trt1} + \beta_3 I_{trt2} + \beta_4 I_{trt3} + \beta_5 Days * I_{trt1} + \beta_6 Days * I_{trt2} + \beta_7 Days * I_{trt3} + b_k + \epsilon_{jk} + Jar_{jk}$
With $Jar_{jk} \sim N(0, \sigma^2_{jar})$, independent of $\epsilon_{jk} \sim N(0, \sigma)$; Trt1 = treatment with Geopyxis; Trt2 = treatment with Morchella; and Trt3 = treatment with Pyronema.

Statistical analyses were done using R 3.6.1 (R Core Team, 2020), the ggplot2 (v3.2.1; Wickham, 2016), the car (v3.0–5; Fox & Weisberg, 2019), the effects (v4.1–4; Fox et al., 2018, 2019), the grid (v3.6.1; R Core Team, 2020), the Gmisc (v1.9.1; Max 2019), the readxl (v 1.3.1; Wickham & Bryan, 2019), the knitr (v 1.26; Xie, 2014; 2015, 2021), and the MuMIn (v 1.43.10; Barton, 2020) packages. Transformation of data was explored using log transformations and changes in the model fits were then visually observed. Based on results, transformation was determined to add unnecessary complexity without a difference in fit.

Analysis of variance was then used to assess differences in aggregation among treatments for each assessment time which was considered to be independent (different jars were used) at 10, 20, 30 and 40 days (R Core Team, 2020). The variables of interest were percentage of aggregation and treatment. For each analysis, a subset of the original data was used; only measurements taken on the relevant day and with the 2 mm sieve were considered, and data for other sieves were not included. ANOVA results confirmed there were differences among treatments for each assessment. Therefore, an ad-hoc tukey's HSD analysis was performed to determine significant differences among treatments and superscripts A-D were used to delineate statistically different groups at p ≤ 0.05.

3. Results

For the overall model, there was no evidence of interaction between days and treatment (chi-square: 2.02, p-value: 0.57), and very strong evidence against the null hypothesis that there is no difference in aggregation over time, regardless of treatment (chi-square: 25.13, p-value: 5.36e-07). Overall and over time, soil in jars with *Geopyxis* was

aggregated 20.1% more than controls (95% CI: 9.20, 30.96). *Morchella* aggregated soil 13.6%, over controls (95% CI: 2.74, 24.50) and *Pyronema* aggregated soil 20.3% over controls (95% CI: 9.20, 30.96). Average daily increases over controls were 0.29% for *Geopyxis* (95% CI: −0.10, 0.69), 0.5% for *Morchella* (95% CI: −0.35, 0.45), and 0.01% for *Pyronema* (95% CI: −0.39, 0.41).

For individual assessments, the amount of soil remaining in the 2 mm sieve is presented as a percentage of the total amount of soil in the jar for the assessments at 10, 20, 30, and 40 days in Table 1. In most cases, the amount of soil aggregated in the fungal treatments was significantly larger or near significantly larger than in the controls (p ≤ 0.05) for the 2 mm sieve, and correspondingly there was more soil in the smaller sieves for the control. Aggregation in the 2 mm sieve ranged from 8% to 30% higher for fungal treatments with the largest differences at 20–40 days for *Geopyxis*. The pyrophilous fungi tested grew rapidly, and by 10 days aggregation increased for *Pyronema* by 21% (p = 0.05), for *Geopyxis* by 17% (p = 0.10), and for *Morchella* by 8% (p = 0.30), near significant for two of the species over controls. By day 20, the soil was significantly more aggregated by all three fungi, which was also true at the 30-day assessment, and for two of the fungi at the 40 day assessment. While the soil was 22% more aggregated by *Morchella,* this was not significant (p = 0.17) at 40 days. Differences among fungal treatments are indicated by superscripts for statistical groupings, with *Geopyxis* aggregating the most soil for this time period.

Graphical representations of the data were generated to visually compare aggregation over time for treatments and controls to elucidate any observable differences by treatment (Fig. 3 and Fig. 4). Fig. 3 shows the percentage of soil in all three sieve sizes with error bars and shows that more soil was captured in the 2 mm sieve for fungal treatments, and that more soil was captured in the 0.5 mm and 0.25 mm sieves for the control. The visualization over time, shows that the amount of aggregated soil (in 2 mm sieve) increased rapidly from 10 to 20 days for *Geopyxis* and *Morchella*, and by inference from 0 to 10 days for all three fungi (Fig. 4). Data on rate increases per day are given in the overall model results.

4. Discussion

Forest recovery after fire is a complex process that involves the interplay of numerous organisms, including fungi. There is growing evidence that a pulse of fire-responsive fungi appears quickly and proliferates across burned soil soon after fire in conifer forests. This phenomenon has been recently documented by new molecular probing techniques that detect mycelial and spore presence, so that evidence no longer depends solely on fruiting body occurrence, giving a broader picture of fungal distribution (Watts et al., 2018; Kauki and Salo, 2020; Smith et al., 2021). According to molecular results, pre-fire fungal communities dominated by basidiomycota are replaced by fire-responsive ascomycete fungi (including *Pyronema* and *Morchella*), as soon as one week after fire (Watts et al., 2018). At least some of these fungi (*Pyronema, Geopyxis*, and *Warcupia*) may be restricted to burned conifer forests after fire (Smith et al., 2021).

Fungal assemblages after a large-scale disturbance have functionally important roles in forest dynamics (Kauki and Salo, 2020), and there is increasing evidence that pyrophilous fungi have important functions on
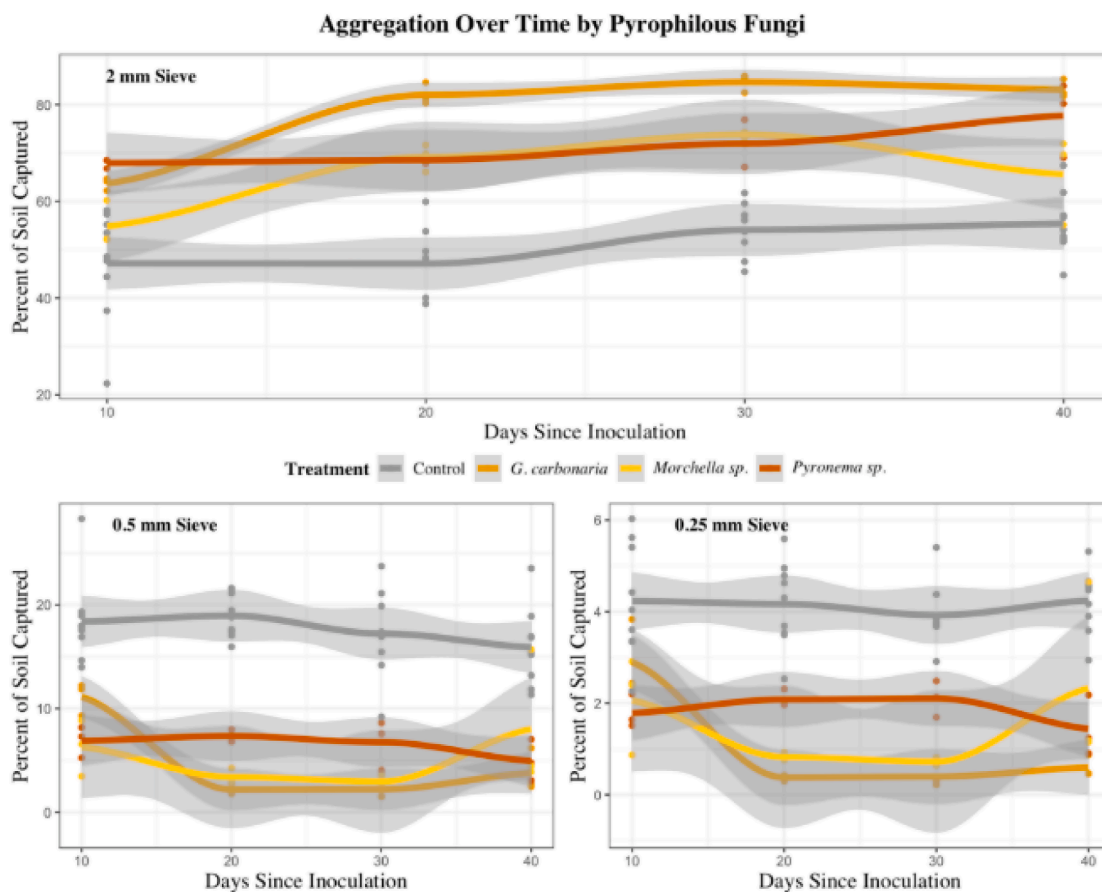
Aggregation Over Time by Pyrophilous Fungi
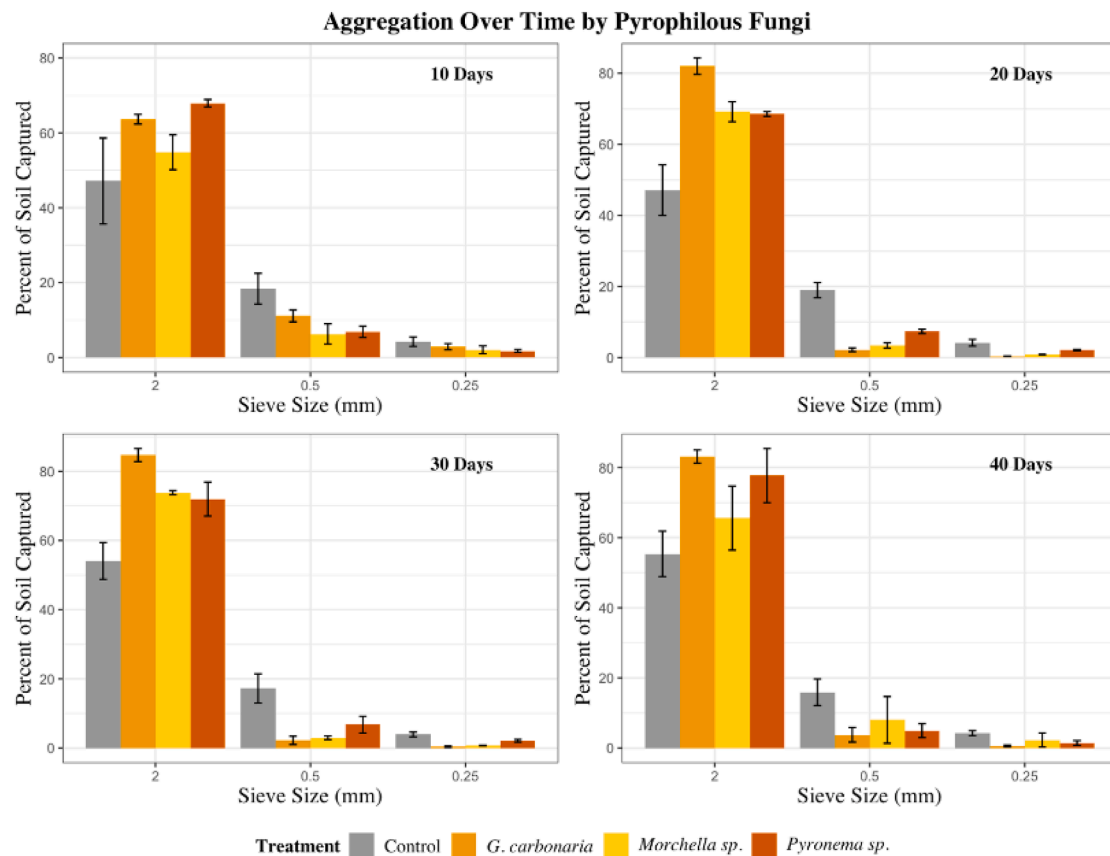
Fig. 3. Aggregation of soil by 3 species of pyrophilous fungi: *Geopyxis carbonaria, Morchella septimelata,* and *Pyronema omphalodes.* Results show a statistically higher % of soil aggregation in the larger (2 mm) sieve by the three fungi over controls at 20, 30, and 40 days.

forest burns. Some associate with charred woody material (Kauki and Salo, 2020) and could be critical in releasing nutrients from recalcitrant substrates during initial forest recovery. Pyrophilous ascomycetes have been shown to capture nitrogen from the transient pulse that occurs after fire (Egger, 1986). The fruiting bodies of these fungi sequester carbon, and their decay releases available nutrients to the soil (Claridge et al., 2009). *Geopyxis carbonaria* appears to have an association with red conifer needles on the forest floor, and other ascomycete fungi are biotrophic with mosses (Raudabaugh et al., 2020). Fire-dependent insects breed inside the fungus *Daldinia concentrica* which fruits on burned wood (Wikars, 2001). The fungivorous insect *Aradus funestus* is attracted to burns and may feed on morels (Werner, 2002). Fire-adapted insects in turn attract birds to burned areas. Here we provide evidence that pyrophilous fungi also have the ability to aggregate soil on burns. From direct and experimental observation, soil aggregation can occur a week or two after a fire, but usually is observed within one year. This aggregation stabilizes the soil which, by inference, can help hold moisture for seed germination. The importance of this guild of fire-adapted fungi functioning at a critical stage in forest recovery has been previously overlooked.

Here we provide direct evidence that at least three pyrophilous species (*Geopyxis carbonaria, Pyrnonema* sp, and *Morchella septimelata*) have the ability to grow in burn soil and bind it. This ability may not have been previously assessed because it is difficult to isolate these fungi from burns into pure culture and measures of soil aggregation are not standardized (Mataix-Solera et al., 2011). Aggregation of soil happened within 10 days of inoculation—very quickly for our group of fungi. The mycelial growth of each living fungus increased soil aggregation over controls within 10 days by 8–20%, in 20 days by 20–35%, after which increases leveled off. These fungi are fast-growers as observed in Petri dishes and in burned soil in jars; and they are known to proliferate

quickly after fire (Bruns et al. 2020). The initial soil was highly aggregated, and this may be due to prior binding by fungal hyphae on the burn site before soil was collected. Autoclaving would not necessarily break up this aggregation as fungal mycelium remains intact on Petri dishes or in jars after being heat-killed. Alternatively, although less likely, mineralization from the forest fire could have pre-aggregated the soil (Mataix-Solera et al., 2011). There were slight differences among species. *Geopyxis* showed a steeper initial curve and the highest aggregation levels over time. *Morchella* also showed a steep initial growth phase but aggregation levels were not as high as for *Geopyxis*. *Pyronema* produced the highest aggregation level at 10 days among the fungi followed by a slow rise for the next 30 days. Differences among fungi were significant in a few cases. Explaining differences at this point is speculation, but may reflect differing growth rates and mycelial branching patterns. We do know that *Pyronema* fruits first on burns, followed by *Geopyxis* and then *Morchella*. Larger trials with more strains and in different soil types are warranted to confirm species differences.

Soil aggregation tests are usually done on a large scale in the field and involve measuring only total soil biomass. Few studies have tested individual fungi for their ability to aggregate any kind of soil; However, in one laboratory experiment, jars of agricultural soil were inoculated with the soil microfungus *Chaetomium* sp. and its ability to aggregate soil was assessed with both wet and dry sieve methods (Eash, 1993). Results showed that the *Chaetomium* species increased soil aggregation for both the wet sieve method (measures soil erodibility by water) and for the dry soil sieve method (measures erodibility by wind). Further, the amount of aggregation was not affected by repeated freeze–thaw or wet-dry cycles (Eash 1993). In another study, six saprophytic fungi (*Chaetomium, Mucor, Stemphylium, Curvularia,* 2 unknown fungi) inoculated onto soil all enlarged aggregates by cross-linkage and entanglement of particles (Tisdall et al., 2012). A greenhouse experiment using burned soil

Aggregation Over Time by Pyrophilous Fungi

Fig. 4. Percent aggregation of soil by 3 pyrophilous fungi (*Geopyxis carbonaria, Morchella septimelata,* and *Pyronema omphalodes*) over time. Higher weights in the 2 mm sieve indicate more soil aggregation by all three species of fungi over controls; there is a slightly different pattern for each species.

investigated the effect of fertilizer on microbial biomass and subsequent aggregation of soil in plastic pots (Villar et al., 2004). This was done to simulate restoration strategies on burns that use fertilizers to stimulate plant growth. A high positive and significant relationship between the amount of soil microbial biomass and aggregate stability was observed in the burn soil, and there were differences among fertilizers used. Further, it was suggested that the labile fraction of the organic matter (fungal hyphae, microbial mucilages) rather than the total organic matter might be the responsible binding agent for the formation of macroaggregates (Villar et al., 2004). The method of aggregation assessment was similar to ours, but is not comparable due to differences in sieve sizes used. Recently, molecular studies have revealed that different fungal communities exist within different size aggregates, showing micro-niches further define how fungi inhabit the soil (Bach et al., 2018; Fox et al., 2018).

The mechanisms by which pyrophilous fungi aggregate soil were not examined in our study. However, aggregation of soil by fungi can be through (i) biophysical, (ii) biochemical, and (iii) biological mechanisms (Rillig and Mummey, 2006; Lehmann et al., 2020). Biophysical aggregation is basically physical attachment of mycelium to soil particles in which the particles are enmeshed in the tangle of hyphae (Tisdall & Oades, 1982). Aggregate formation would then depend on the properties of the individual fungal networks (e.g., hyphae diameter, density, and interconnectivity) and the tensile strength of the different strains of fungal hyphae (Rillig and Mummey, 2006). Lehmann et al. (2020) found that fungi with denser mycelial growth produced more soil aggregation under Petri dish conditions. Our pyrophilous fungi have comparatively less dense mycelium than most fungi (Pers. Observation), however their fast growth may be a compensation in the field as they can cover large areas quickly. For biochemical processes, fungi are known to produce

"glue-like" glomulin (Rillig and Mummey, 2006), polysaccharide compounds or hydrophobin proteins which alter wettability (Mataix-Solera et al., 2009). Other biological mechanisms might include the production of fungal exudates that foster bacterial growth, which in turn increases aggregate formation (Rillig and Mummey, 2006). Further, fungal mycelium has been shown to withstand sonification without a reduction in aggregation ability, despite the hyphal network being disrupted, showing that at least in some cases binding may be more biochemical than physical (Aspiras et al., 1971; El Mountassir et al., 2018). The mechanisms of aggregation of soil by fungi are reviewed in Lehmann et al. (2020). While we now have evidence that that pyrophilous fungi have the ability to bind burn soil, the mechanisms by which they do so, remain unknown.

In conclusion, the overlooked pyrophilous fungi could play a key role in stabilizing soil on burns after fire, and as such their establishment should be considered in forest management and remediation strategies after fire. These fungi could be critical in determining successional pathways in young post-fire forests (Kauki and Salo, 2020). Erosion control programs such as grass seeding are often ineffective and are expensive (Robichaud et al., 2000). Consideration might be given to avoiding or delaying activities in particular burn areas that contribute to soil compaction such as mechanical harvesting or use of mechanized vehicles for other restoration measures (Jennings et al. 2012. More *in situ* research on a larger scale could examine the conditions necessary to promote fungal activity after fire, and document the effects of this natural process on a wider scale.

CRediT authorship contribution statement

Olivia Filialuna: Investigation, Writing – original draft, Writing –

review & editing, Funding acquisition. **Cathy Cripps:** Conceptualization, Methodology, Writing – original draft, Writing – review & editing, Supervision, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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