FINAL REPORT

Title: Assessing the contribution of soil faunal complexity to ecosystem services after restoration with thinning and fire

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List of Abbreviations/Acronyms

MRPP: Multi-response permutation procedure
PCA: Principal components analysis
VALL: Valles Caldera National Preserve

Keywords

Soil, Mites, Collembolans, Ponderosa Pine, Thinning, Prescribed Fire, Faunal Complexity, Decomposition, Soil Organic Matter, Nitrogen, Fungal Communities, Field Mesocosms

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Abstract

While managed fire often produces clear changes in aboveground functional diversity, we know little about how fire affects belowground fauna and their mediation of biogeochemical processes. Because soil micro- and mesofauna, particularly nematodes, collembolans and mites, are significant contributors to nutrient and carbon cycling in forest systems, understanding how managed fire changes soil faunal assemblages and their contributions to ecosystem function is important. We successfully manipulated soil faunal complexity over two growing seasons in field mesocosms installed in thinned/burned and untreated control management units within a second-growth ponderosa pine forest at Valles Caldera National Preserve, New Mexico. The method we developed was designed to minimize treatment side effects while allowing repeated internal measurements of mesocosms, and it should aid future field investigations of faunal communities. These studies are sorely needed, both to illuminate the roles of soil fauna in forests and to adequately assess forest management impacts on soil ecological functions. Five years post-fire, densities and species richness of microarthropods >300 μm were markedly reduced in mesocosms within the thinned/burned unit relative to the untreated control unit. However, we did not find evidence that faunal complexity influenced fungal community composition, nitrogen mineralization, or soil organic matter formation in either forest management unit. Decomposition appeared to be affected by faunal complexity only within the thinned/burned unit, despite reduced faunal complexity in that unit. We speculate that the biotic and abiotic context of faunal complexity may be more important for modulating decomposition than faunal complexity per se, or that abundance of fauna must be severely reduced before decomposition effects are evident in this system.

1. Objectives

While managed fire often produces clear changes in aboveground functional diversity, we know little about how fire affects belowground fauna and their mediation of biogeochemical processes. Because soil micro- and mesofauna, particularly nematodes, collembolans and mites, are significant contributors to nutrient and carbon cycling in forest systems, understanding how managed fire changes soil faunal communities and their contributions to ecosystem function is important. **Our objective was to assess what concomitant changes in ecological function may occur with shifts in soil food web structure after restoration of a ponderosa pine forest.** This objective was met: **we successfully manipulated soil faunal complexity** in field mesocosms installed in adjacent thinned/burned and untreated control ponderosa pine management units at Valles Caldera National Preserve (VALL), New Mexico, **and we elucidated how faunal complexity modulates fungal community structure, decomposition, soil organic matter, and nitrogen availability** in the treated and untreated units. Since thinning and burning alter both the abiotic context of faunal interactions with decomposer organisms and the composition of faunal assemblages, **we hypothesized that the functional importance of faunal complexity would differ between untreated and thinned/burned units.** We further predicted that lignin decomposition would be primarily affected by modulation of competitive interactions between saprotrophic and ectomycorrhizal fungi (the Gadgil effect (Fernandez and Kennedy, 2016)), while cellulose decomposition would be primarily affected by comminution (Neher and Barbercheck, 2019). Our predictions regarding the direction and relative strength of faunal complexity effects on focal ecosystem functions are summarized in **Table 1**.

This project was undertaken to address the JFSP topic area of *managed fire effects and post-fire recovery*. Factors governing decomposition rates, nitrogen cycling, and fungal community dynamics are of interest to land managers, as these processes help determine tree growth rates, forage nutritional quality, ground fuel accumulation, water holding capacity, and carbon storage. Our study aids in evaluating restoration of soil ecological function with thinning and managed fire, focusing on key players in soil food webs that are often neglected in forest restoration research. In addition, comparing ecosystem services provided by simple soil faunal communities to those provisioned by more complex communities can allow us to make inferences about impacts to ecosystem services when soil fauna are decimated by high-severity wildfire.

Table 1. Predictions regarding the effects of increasing soil faunal complexity (more microarthropod species and individuals) on ecological functions in untreated and thinned/burned ponderosa pine management units.

More complex faunal assemblages will:	
Untreated Control	**Thinned/Burned**
Inhibit decomposition of recalcitrant substrate (lignin) if microarthropods preferentially graze on saprotrophic over ectomycorrhizal hyphae, influencing competitive interactions among fungi (the Gadgil effect). Effect expected to be **stronger** because more microarthropods are likely present in this unit, and because competition between ectomycorrhizal and saprotrophic fungi is predicted to be greater.	**Inhibit decomposition of recalcitrant substrate (lignin)** if microarthropods preferentially graze on saprotrophic over ectomycorrhizal hyphae. Effect expected to be **weaker** because there are likely fewer microarthropods and less competition between ectomycorrhizal and saprotrophic fungi. Alternatively, if low fungal abundance is limiting decomposition and fauna are dispersing fungi to the substrate, increased faunal complexity could promote decomposition.
Increase decomposition of labile substrate (cellulose) because comminution (fragmentation) increases substrate surface area, and because a wide range of saprotrophic microorganisms (including many bacteria) can break down cellulose. This effect is expected to be **stronger** because there are more microarthropods.	**Increase decomposition of labile substrate (cellulose)**. Effect expected to be **weaker** because there are likely fewer microarthropods.
Decrease nitrogen availability, if there are more bacterivore nematodes in mesocosms with less faunal complexity (in other words, if there are top-down effects of faunal complexity on nematode abundance). Nematodes are the most important soil fauna in mineralizing nitrogen, and more nitrogen is immobilized in microbial biomass in the control unit.	**Not affect nitrogen availability**, because top-down effects on nematodes should not be strong (few predators, few nematodes) and because mineral nitrogen is abundant, with relatively little nitrogen immobilized in microbial biomass.
Affect fungal communities, mainly because of grazing impacts on competitive outcomes (due to preferential grazing) and on hyphal morphology (stimulation of hyphal growth/branching).	**Affect fungal communities**, mainly because of dispersal.

2. Background

The potentially millions of nematodes (Yeates, 2007) and tens to hundreds of thousands of collembolans and mites (e.g., Marra and Edmonds, 2005) which can, along with other soil fauna, inhabit a square meter of forest soil contribute both directly and indirectly to the fates of carbon and nutrient inputs to the pedosphere. Directly, comminution of litter by mesofauna increases the surface area available to microbial decomposers and enhances leaching of low-molecular weight compounds into the soil, which may increase formation of stable SOM fractions (Soong, 2014). Bacterivores (especially nematodes) and fungivorous micro- and mesofauna liberate nutrients from immobilization in bacterial and fungal biomass (Chen and Ferris, 1999; Freckman, 1988). Indirectly, soil fauna can regulate carbon and nutrient cycling by altering microbial abundance, community composition, and function (e.g., Kaneda and Kaneko, 2008; Trap et al., 2016). Their feeding may favor unpalatable, poisonous, or structurally-protected microbial taxa, altering the enzymatic capabilities of soil communities. For instance, many fungivores appear to prefer saprotrophic to mycorrhizal hyphae, and melanized over hyaline hyphae (Klironomos and Kendrick, 1996). Fungivore preferences thus have the potential to intensify the Gadgil effect - the oft-cited but highly variable depression of decomposition rates by ectomycorrhizal fungi (reviewed in Fernandez and Kennedy, 2016). Grazing at low intensities, however, may stimulate growth of fungal taxa (Janoušková et al., 2018) and/or modify mycelial morphology and resource allocation (Ngosong et al., 2014). Bacterivores, while usually observed to decrease bacterial biomass, may also increase it by liberating resources bound in senescent cells (Trap et al., 2016). Finally, the feces, exuviae, eggs, and corpses of mesofauna can serve as nuclei for aggregate formation, sequestering occluded particulate organic matter and increasing soil carbon storage (Maaß et al., 2015).

The magnitude and even the direction (positive or negative) of net faunal effects on soil processes is dependent upon biotic and abiotic context, but environmental drivers of functional outcomes still remain poorly understood (Briones, 2014). In fire-adapted dry forests of the Southwest, restoration with thinning and low-severity burning is likely to alter many biotic and abiotic characteristics relevant to determination of food web function. Soil nutrient availability and other physicochemical characteristics changed by fire may govern the magnitude and functional consequences of top-down controls (Lenoir et al., 2007). As faunal size classes can have opposing effects on fungal community composition and decomposition (Crowther et al., 2011), net outcomes may also depend on the relative resilience of broad faunal groups. Soil fauna could perhaps play an oversized role in structuring microbial communities in recently burned soils by facilitating propagule dispersal as they return to scorched soil patches from unburned or little-affected patches in the typical post-fire habitat mosaic, providing a recolonization advantage to favored taxa or specialized hitchhikers.

3. Materials and Methods

3.1 Study sites

The study was conducted at VALL in the Banco Bonito area (**Figure 1**). Soils in this area are sandy loams to loamy sands formed by a rhyolite lava flow ~68,000 years ago (Goff, 2009) and belonging to the Totavi-Jemez-Rock Outcrop association (Hacker and Banet, 1987). Annual

precipitation averaged 590 mm from 1981–2010, and the mean annual temperature for this period was 6.6 °C, with monthly mean temperatures ranging from -2.3 °C in January to 17 °C in July (PRISM, 2021). Mesocosms were installed within two ponderosa pine forest management units, hereafter referred to as the thinned/burned unit and the untreated control unit. In 2012, the thinned/burned unit was thinned to an average tree density of ~60 trees ha^{-1}. Marketable timber was removed, and remaining woody residues were masticated. A low-intensity broadcast burn occurred in October of 2015. The untreated control unit was located across an old logging road and retained ~300 trees ha^{-1}. Adjacent study areas with similar topography and soil texture, measuring approximately 0.75 ha and 1.25 ha, respectively, were established within these units.

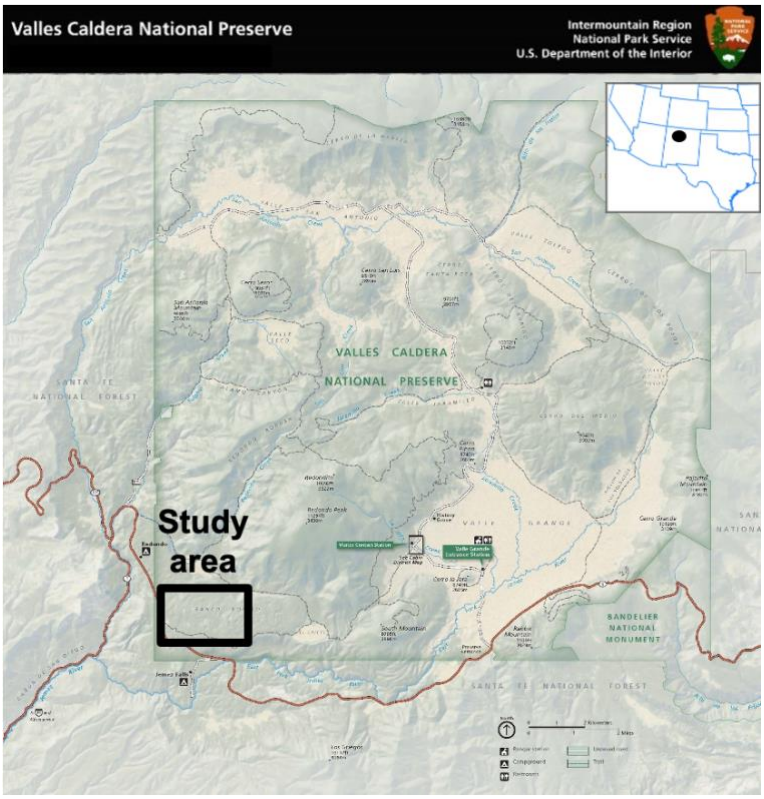

Figure 1. Location of study area within Valles Caldera National Preserve and New Mexico.

3.2 Experimental design

Twelve trees per study area with diameters at breast height between 34.4-55.4 cm were selected to serve as blocks. At each of these trees, we installed defaunated mesocosms designed to permit recolonization by either small microfauna only, small and medium sized-microfauna (including most nematodes, but not mites), all micro- and mesofauna but not roots, or all micro- and mesofauna and roots. One replicate of each mesocosm type was installed at each tree for a total of 96 experimental mesocosms. Six of these trees per study area were randomly selected to receive one additional replicate of each mesocosm type for periodic destructive monitoring of recolonization by soil biota (hereafter “sacrificial mesocosms”, 48 in total). Four of the remaining trees in each study area were selected for installation of three types of controls (24 control units in total) to quantify any side effects of mesocosm physical structure and root

severing on soil properties and processes. Severed disturbed controls and unsevered disturbed controls consisted of mesocosm-sized holes refilled with defaunated mineral and organic horizons and covered with defaunated litter, with or without root severing, and undisturbed controls consisted of intact forest floor equivalent in area to a mesocosm.

3.3 Mesocosm construction and installation

Five large windows were cut in the sides of 25 cm long sections of PVC sewer pipe with an internal diameter of 20.32 cm, then polyester mesh with hole sizes of 21 µm, 41 µm, or 1 mm was wrapped around the exterior of the mesocosms (**Figure 2 A**). These mesh sizes were selected because diameters of common small nematode taxa at this site range from 12.5-15 µm and most nematodes are at least 25 µm in diameter, while the smallest mites are approximately 46 µm in diameter, and no mite or collembolan taxa measuring > 1 mm in diameter were encountered in the course of our earlier work at this site. The bottoms and caps of all mesocosms were constructed identically to equalize drainage, infiltration, and albedo. Mesocosm bottoms were fitted with 21 µm mesh and covered with PVC-coated fiberglass window screen to provide additional structural support (**Figure 2 B**). Tight-fitting removable caps (**Figure 2 C** and **Figure 2 D**) were assembled from vinyl flashing and 41 µm mesh, chosen for its superior permeability to precipitation versus 21 µm mesh and because few soil microfauna were expected to colonize mesocosms from aboveground. Mesh was glued to surfaces with all-weather 100% silicone caulk. A ring of Fluon PTFE insect barrier (byFormica, Warner Robins, Georgia, USA) was applied above the windows to reduce the probability of arthropods entering the tops of the mesocosms.

Mesocosms were installed in June 2019 at tree driplines in semi-circular arrays centered on their southern aspects, at intervals of 50 cm, or greater if necessary to avoid understory vegetation (**Figure 3**). If woody debris longer than mesocosm width but not covering more than half of the mesocosm footprint was present, the debris was moved. At each mesocosm location, litter and soil O horizon depths were measured, then these layers were collected separately using a segment of the sewer pipe with a sharpened end as a coring guide. Holes for the mesocosms were dug to 15 cm below the mineral soil surface with a 20.32 cm soil auger, and excavated soil was sieved to 5.6 mm and homogenized. Mineral and O horizon soil and litter were defaunated following a modified version of Franco et al.'s (2017) method for nematode exclusion. Soil and litter were placed in aluminum steam table pans, were pre-wetted and allowed to incubate for 24 hours (12 hours if already well wetted by rain) at ambient temperature, then were heated at 65 °C for 3 days. Following this treatment, litter and O horizon soil were additionally frozen to -20 °C for at least 48 hours, since we expected fauna in these layers would be more resistant to heating than fauna in mineral soil. Mineral soil was sieved once more to 1.25 cm to break up hardened blocks that resulted from heating.

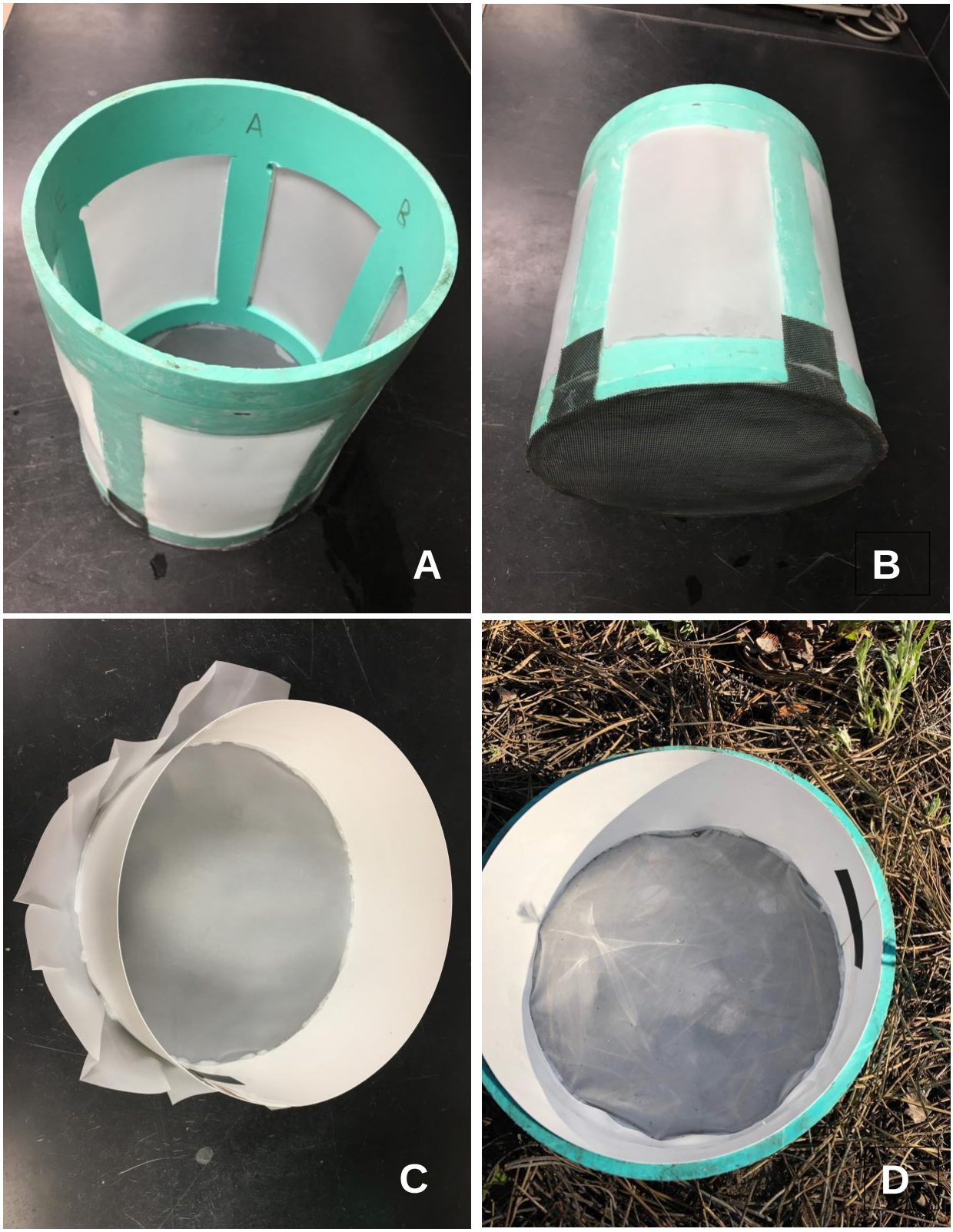

Figure 2. Top (**A**) and bottom (**B**) views of mesocosms assembled from PVC sewer pipe, polyester mesh, and PVC-coated fiberglass window screen. Caps constructed from 40 µm mesh and vinyl flashing before (**C**) and after (**D**) installation of mesocosms.

Figure 3. Sacrificial and experimental mesocosms at the dripline of a tree in the thinned/burned study area. Other trees in the study, flagged with orange tape and marked with stars, are visible in the background.

Mesocosms were buried to a depth of 15 cm below the mineral soil surface and capped until defaunation of soil and litter was completed. Immediately prior to refilling, the empty interiors of the mesocosms were sprayed with 70% ethanol to ensure no live fauna were present inside. Defaunated mineral soil, O horizon soil, and litter layers were returned to their trees of origin and used to fill mesocosms and holes designated for disturbed controls. Soil was leveled and litter was distributed so as to maximize contact between interior and exterior soil and litter layers. Decomposition bags containing standard substrates composed predominantly of lignin or cellulose (described below) were placed between the litter and O horizon layers. Finally, the mesocosm caps were replaced, and petroleum jelly was applied between the flashing and the interior walls of each mesocosm to prevent fauna from entering through this crevice. During the growing season, we severed roots monthly to a depth of 20 cm around the root-exclusion treatment 1 mm mesh mesocosms (smaller mesh sizes did not allow root entry) and around disturbed severed controls.

We verified defaunation efficacy by extracting nematodes (which were presumed would be more resistant to the defaunation procedure than microarthropods) from subsamples of mineral soil. Subsamples from each tree were retained during mesocosm installation, transported on ice to Flagstaff, Arizona, and stored at 4 °C until extraction 10-19 days after mesocosms were filled. Nematodes were extracted from 100 cc soil using a combination of decanting and sieving and modified Baermann trays ("nematode rafts", see Gibson et al. (2019)) and were collected after 48 and 72 hours. Resulting samples were refrigerated unpreserved and examined for nematodes within one week of extraction. No nematodes were detected in 22 of the 24 samples, but the two remaining samples each contained one nematode. The effects of sieving and defaunation on nitrogen availability were determined from analysis of three replicate samples of unsieved, sieved, and defaunated soil collected from each study site. Ammonium and nitrate were extracted according to Keeney and Nelson (1982) and analyzed via colorimetry (Wendt, 1999).

We used balsa wood (predominantly lignin) and museum board (predominantly cellulose) as standard labile and recalcitrant substrates (Neher et al., 2003) to illuminate the influence of faunal complexity on decomposition processes. Balsa wood (~1 mm thick) and museum board (~1.75 mm thick) disks with a diameter of 2.22 cm were dried at 60 °C, weighed, and sealed in polyester mesh bags with openings of 1 mm. Each bag contained one disk of each type. We buried five decomposition bags in each mesocosm (except sacrificial units) beneath the litter layer. Decomposition bags were also placed at control locations, over which we secured chicken wire to reduce disturbance of the bags.

3.4 Mesocosm monitoring and measurement of response variables

One replicate decomposition bag was collected from within each mesocosm and control in September 2019 (T1; the end of the monsoon season), April 2020 (T2; the end of winter), July 2020 (T3; the end of the dry season), August 2020 (T4; the height of the monsoon season), and September 2020 (T5; one year after the first sampling). All measurements and sampling activities were performed with tools and gloved hands that were sanitized with 70% ethanol between mesocosms. To quantify potential microclimate differences across mesocosm treatments, we measured soil moisture at each of these timepoints using a portable probe (ML3 ThetaProbe with HH2 soil moisture meter; Delta-T Devices, Cambridge, UK), and soil

temperature at the first timepoint. (No temperature differences were detected, so we did not measure temperature at subsequent timepoints.) We also destructively sampled sacrificial mesocosms for nematodes and microarthropods at T1 and T3. Samples for nematode extraction were collected from the uppermost 10 cm of soil (including mineral and O horizons, but not litter) using a 2.54 cm diameter probe, and samples for microarthropod extraction including the top 10 cm of soil and the overlying litter layer were obtained using a 5.08 cm diameter corer. Microarthropod and nematode soil samples were transported on ice to Northern Arizona University and stored at 4°C until processing. Nematodes were extracted as described above and preserved in DESS solution (Yoder et al., 2006), then enumerated under a dissecting microscope at 40X magnification. Microarthropods were extracted using Tullgren funnels with 15 watt bulbs over 7 days. Light intensity was gradually increased over 5 days, then held at maximum brightness for two days. Extracted animals were preserved in 70% ethanol. Microarthropods were counted at 40-50X and categorized as mites, collembolans, or others (all other arthropods); additionally, we noted whether mites in the suborder Brachypylina were present (this is a diverse and often numerically dominant group of oribatids with adult body sizes >200 µm).

We destructively sampled all mesocosms and controls (with the exception of two mesocosms in the thinned/burned management unit which were compromised by mammal activity) at T5 to determine final faunal complexity, fungal community composition, and ammonium, nitrate, and soil organic matter (SOM) content. We used diversity of microarthropods >300 µm as a proxy for soil faunal complexity. This size cutoff is used by the Alberta Biodiversity Monitoring Institute (2009) in their microarthropod inventories, and separates juveniles from identifiable adults for most taxa in the most speciose order of mites, the Oribatida. Microarthropods were extracted and preserved as detailed previously, then were filtered using a 300 µm sieve. Microarthropods captured on this sieve were counted, and mites and collembolans were sorted to morphospecies. Soil samples for SOM, ammonium, and nitrate content determination were collected in envelopes and stored in closed containers with silica beads to air dry in the field, then were sieved to 2 mm prior to analysis. Soil organic matter was measured by loss on ignition of 5 g soil at 450 °C for 24 hours (Bisutti et al., 2004). Soil for fungal community analysis was transported from the field on dry ice and stored at -20 °C upon return to Northern Arizona University. We extracted DNA from these samples using Qiagen DNeasy PowerSoil kits.

Fungal community analysis was performed at the Arizona State University Genomics Core via next generation sequencing of the ITS region using the MiSeq Illumina platform. We used the barcoded primer set ITS1f-ITS2, designed by Smith and Peay (2014), and followed the Earth Microbiome Project protocol (http://www.earthmicrobiome.org/emp-standard-protocols/) for library preparation. PCR amplifications for each sample were done in duplicate, then pooled and quantified using Accublue® High sensitivity dsDNA Quantitation Kit (Biotium). A no-template sample was included during the library preparation as a control for extraneous nucleic acid contamination. 200 ng of DNA per sample was pooled and then cleaned using QIA quick PCR purification kit (QIAGEN). The pool was quantified by Illumina library Quantification Kit ABI Prism® (Kapa Biosystems); diluted to a final concentration of 4 nM; then denatured and diluted to a final concentration of 4 pM with a 25% of PhiX. Finally, the DNA library was loaded in the MiSeq Illumina and run using the version 2 module, 2x250 paired-end, following the directions of the manufacturer.

3.4 Statistical analyses

All analyses were performed in R (R Core Team, 2020) unless otherwise noted. Effects of mesh treatment, ponderosa restoration treatment, and mesh x ponderosa restoration treatment combinations were assessed with ANOVA, if assumptions could be met, or Kruskal-Wallis H tests, if they could not be met. Where necessary, response variables were log transformed (after adding 1 to all observations if there were 0 values) to meet normality requirements for parametric analysis. P-values for multiple pairwise comparisons were adjusted using Tukey's HSD for ANOVA or with the Benjamini-Hochberg method for Wilcoxon rank sum tests. Data were visualized with ggplot2 (Wickham, 2011).

Multivariate analyses of faunal communities, and of interactions between fauna and fungi, were executed in PC-ORD version 5.1 (McCune and Mefford., 2006). We used multi-response permutation procedure (MRPP) to test whether mesocosm mesh treatments had produced different faunal communities. This non-parametric test for differences among *a priori* groups yields the statistic *A*, the chance-corrected proportion of between-sample distances explained by group identity. *A* will equal 1 when all samples within groups are internally identical, but groups are distinct, and 0 if heterogeneity within groups is equal to the chance expectation. $A < 0$ indicates more within-group heterogeneity than should be expected to occur by chance, while $A > 0$ if there is more agreement within groups than should occur by chance (i.e., if differences between groups are likely to exist). Mantel tests of correlation between fungal and faunal communities were conducted using the Monte Carlo method with 999 randomized runs. For microarthropod communities, oth MRPP and Mantel tests were performed using Bray-Curtis distance after adding a dummy variable column containing 1 for all rows, since some samples contained no microarthropods > 300 µm. Weighted UniFrac was used as the distance measure for fungal communities.

Analysis of ITS sequence data was accomplished using Qiime2 at the Arizona State University Biodesign Institute's Bioinformatics Core. Briefly, the DADA2 tool was used to merge paired-end fastq files, denoise them, purge chimeras, and infer sample sequences. Group differences were visualized with principal components analysis and tested with PERMANOVA using weighted UniFrac distance, and Faith phylogenetic diversity values were calculated.

We assessed the effects of forest restoration treatment, mesocosm treatment, and soil characteristics (moisture, ammonium, and organic matter) on decomposition using analysis of covariance models. To address non-normality, we applied the logit transformation to the proportion of original mass remaining for both substrates (Warton and Hui, 2011). This transformation requires positive values between 1 and 0 noninclusive. For balsa disks, approximately 14.4% of which gained an average of 2.42% of their original mass despite our cleaning procedure, we removed observations with mass gain >6.7% (outliers which were at least 1.5 times the interquartile range below the 25^{th} percentile of the distribution). These observations represented 3.3% of the 590 balsa disks collected. We then subtracted the next highest value of mass gain (6.5%) from the remaining balsa mass loss observations prior to transformation to constrain proportions between 0 and 1. One outlying museum board disk observation which had gained a significant amount of weight was also discarded, and values for 5 museum board disks which had gained a small amount of mass were changed to 0% mass loss

(these constituted <1% of the data set). Finally, we subtracted 0.025 from each final mass proportion so that all values were below 1.

We fit the linear models *balsa wood mass remaining ~ timepoint + restoration treatment* and *museum board mass remaining ~ timepoint + restoration treatment*. We used the timepoint number (1, 2, 3, 4, 5) instead of days in the field for these analyses, as we did not expect daily decomposition rates to be consistent across seasons. If restoration treatment was a significant predictor of mass loss for a substrate, we assessed the advisability of including an interaction between *restoration treatment* and *timepoint* using an F-test. We then compared the preferred model to models additionally including all possible combinations of *average soil moisture Z score* (the average Z score of soil moisture for each mesocosm across five measurement timepoints), *soil organic matter*, *ammonium concentration*, and/or *mesh treatment* using the Akaike information criterion (AIC). Mesocosm controls were excluded because they were not replicated at all trees and were subject to the influence of UV and additional disturbance, which appear to have speeded mass loss; however, selected models run with and without controls yielded broadly similar parameter estimates (**Table S3**). Prior to beginning the selection procedure for each model, we also tested for an interaction between mesh size and timepoint, but the additional explanatory power did not justify inclusion in the model. Predictor variables were checked for collinearity prior to inclusion in candidate models, and soil organic matter and ammonium concentration were log transformed to address non-normality. In the event that models tied for minimum AIC (i.e., AIC values differed by <2), we chose the most parsimonious model that also included soil moisture (which we knew to be confounded with mesh size).

We investigated faunal complexity effects on soil organic matter and ammonium content with linear mixed effects modeling using the packages "lme4" (Bates et al., 2015) and "lmerTest" (Christensen et al., 2017). We did not model nitrate content, because this nutrient was very low in most mesocosms and could not be transformed to meet model assumptions. In addition, we expected ammonium to better reflect mineralization by soil fauna (Chen and Ferris, 1999). We modeled *mesh treatment* (excluding mesocosm controls) and *average soil moisture Z score* as fixed effects, and *tree* as a random effect (since mesocosms in each tree block had identical soil characteristics at the outset of the study). We fit a version of this model for all trees, untreated control trees only, and thinned/burned trees only. We then tested each model for significance against a simpler model omitting mesh treatment with the "anova()" function.

4. Results and Discussion

4.1 Manipulation of soil faunal complexity

4.1.1 Monitoring of sacrificial mesocosms

Monitoring of sacrificial mesocosms at T1 and T3 showed some colonization of fine mesh mesocosms by very small microarthropods but indicated continued efficacy of mesh treatments at maintaining microarthropod community complexity differences (**Table 2**). Importantly, mites in the speciose and abundant oribatid suborder Brachypylina were absent from all but one of the fine mesh mesocosms. Examination of nematodes collected at T1 revealed that 21 µm mesh mesocosms were already contaminated by large nematode taxa, and nematode community

composition did not differ among mesocosm mesh treatments (MRPP based on morphospecies: $A<0.01$, $P>0.3$ for pairwise comparisons of 21 µm vs. larger mesh sacrificial mesocosms in untreated control and thinned/burned units). Nematode communities were therefore not compared at subsequent sampling timepoints, and subsequently we focused on microarthropod community differences.

Table 2. Abundances of microarthropods in all size classes and presence of adult higher oribatids (number of units in which Brachypylina occurred/total units sampled) in sacrificial mesocosms sampled in September 2019 (T1) and July 2020 (T3). N=3 per restoration treatment unit.

	T1 Microarthropods			*T3 Microarthropods*		
	Mean mites	Mean collembolans	Presence of Brachypylina	Mean mites	Mean collembolans	Presence of Brachypylina
21 µm	84.3	11.2	0/6	51.2	6.8	0/6
41 µm	225.8	0.5	0/6	220.3	16	1/6
1 mm Sev	239.2	56.2	6/6	139.8	9.2	6/6
1 mm	310.8	79.2	6/6	208.3	8.8	5/6

4.1.2 Final community complexity

Examination of microarthropods > 300 µm at the conclusion of our study confirmed that the mesocosm treatments had successfully manipulated soil faunal complexity, producing more complex microarthropod communities in 1 mm mesh mesocosms and in mesocosm controls than in mesocosms with fine mesh sizes (21 µm and 41 µm). Abundance and species richness of mites > 300 µm was higher in 1 mm mesh mesocosms and controls than in fine mesh mesocosms (**Figure 4 A** and **B**), and fine meshes were especially successful at excluding mites in the order Oribatida (**Figure 4 C**). Densities of other microarthropods (including Protura, minute spiders, termites, and macroarthropod larvae) also tended to be lower in fine mesh mesocosms, although these animals were encountered infrequently in general (**Figure 4 D**). Mesh treatments were less effective at excluding collembolans, however, and species richness and abundance of these animals did not differ across mesocosm treatments (**Figure 4 E** and **F**).

Multi-response permutation procedure (MRPP) analysis of microarthropod species abundances revealed that communities in fine mesh mesocosms were distinct from those of coarse mesh mesocosms and controls (**Table 3**). Of the 43 mite and collembolan taxa observed in the >300 µm fraction (**Table S1**), only one was a strong indicator (IV > 25) for any of the mesh treatments: a collembolan in the family Entomobryidae, which had maximum IV values in the 21 µm mesh treatment. We suspect that this species, which has a very large furcula (spring), was especially well equipped to colonize mesocosms through small tears in lids that appeared between maintenance trips, or while lids were temporarily removed for soil moisture monitoring and collection of decomposition disks.

Table 3. Multiresponse permutation procedure (MRPP) results for soil fauna species >300 µm, grouped by mesocosm mesh treatment and based on Bray-Curtis distance. Note that mesocosm controls were not replicated at all trees, and *p*-values are not corrected for multiple comparisons.

All trees

	41 µm		1 mm Sev		1 mm		Control	
	A	*p*	*A*	*p*	*A*	*p*	*A*	*p*
21 µm	0.015	0.098	**0.052**	**0<.01***	**0.063**	**<0.001***	**0.064**	**<0.001***
41 µm			**0.059**	**<0.001***	**0.077**	**<0.001***	**0.061**	**<0.001***
1 mm Sev					-0.006	0.903	-0.001	0.449
1 mm							0.002	0.273

4.1.3 Non-target treatment effects

Defaunation, but not sieving, increased soil ammonium concentrations immediately after treatment, especially in soil from the untreated control units (**Figure S1 A**). Nitrate was below detectable levels in all but two defaunated samples from the thinned/burned unit. This pulse in available nitrogen had dissipated by the end of the study, at which time ammonium concentrations were very similar in mesocosm controls with and without defaunated soil (**Figure S1 B**). No differences in soil temperature between mesocosm mesh treatments were detected at T1, and temperature was not monitored at later sampling points. Mesocosm mesh size and root severing did influenced moisture retention, however, with 21 µm mesh mesocosms retaining the most, and 1 mm unsevered mesocosms the least, soil moisture on average (**Figure S2**). Median soil moisture differences between 21 µm and 1 mm unsevered mesocosms ranged from 1.2% at T3 to 6.2% at T5. We accounted for these moisture differences to the best of our ability in our models of decomposition as described below.

4.2 Forest management treatment effects

4.2.1 Effects of thinning/burning on soil fauna

Five years after burning, microarthropod communities differed in the two management units (MRPP: A=0.081, $p<0.001$). Both abundance and species richness of mites and collembolans >300 µm, as well as abundance of other microarthropods in this size class, were reduced in thinned/burned mesocosms and controls relative to those in the untreated control management unit (**Figure 5**). Oribatids, which comprised approximately two thirds of examined microarthropods in 1 mm mesocosms and in mesocosm controls, appeared more sensitive to thinning and burning than mites as a whole (**Figure 5 C)**. This finding supports those of Camann et al. (2012), who found evidence of continuing oribatid decline two years after prescribed fire in ponderosa pine-dominated stands in the California Cascade Range. Four microarthropod species were strong indicators for the untreated management unit (IV>25), but none appeared to prefer mesocosms in the thinned/burned unit (**Table S1**).

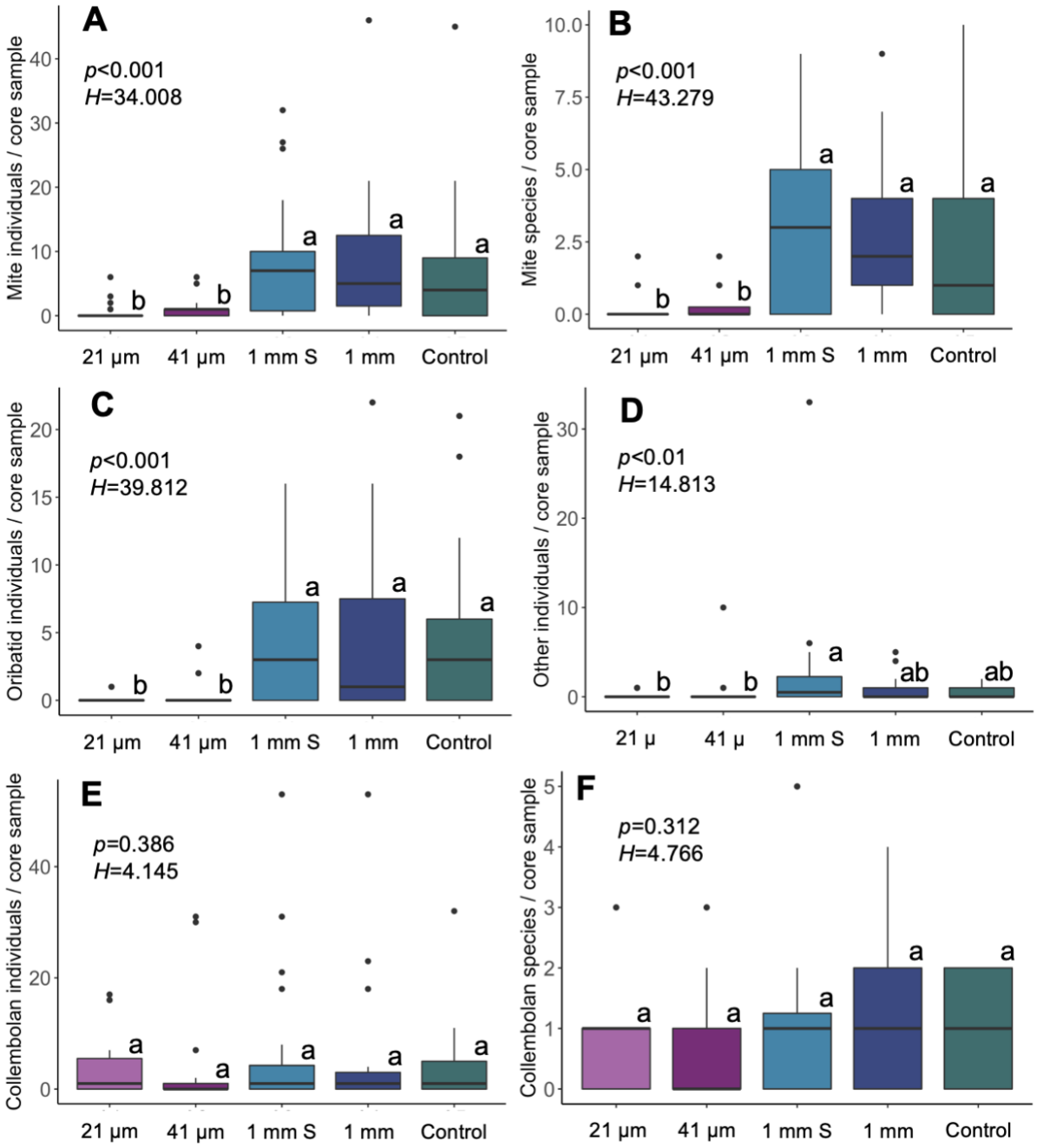

Figure 4. Abundance and species richness of soil fauna >300 µm extracted from combined soil/litter core samples, grouped by mesocosm mesh treatment: 21 µm, 41 µm, 1 mm with root severing (1 mm S), 1 mm without root severing (1 mm), and mesocosm controls without mesh or pipe. (**A**) Abundance and (**B**) species richness of mites; **(C)** abundance of oribatids; **(D)** abundance of other microarthropods not belonging to Acari or Collembola; **(E)** abundance and **(F)** species richness of collembolans. Boxes with different letters are significantly different at $\alpha<0.05$ according to Wilcoxon rank sum tests, after application of the Benjamini-Hochberg correction for multiple comparisons. Model *P*-values were calculated from Kruskal-Wallis H-tests.

4.2.2 Effects of thinning/burning on soil fungi

Fungal communities diverged in mesocosms in the thinned/burned and untreated control management units (**Figure 6;** PERMANOVA based on weighted UniFrac distance: pseudo-*F*: 7.579, *p*=0.001). Sequences belonging to the fungi in the phylum Basidiomycota were observed more frequently in mesocosms within the untreated control unit, while subphylum Glomeromycotina sequences were more abundant in the thinned/burned unit and phylum Ascomycota sequences were similarly common in both management units (**Figure S3**). Glomeromycotina comprises arbuscular mycorrhizal fungi (AMF) which are obligate symbionts of graminoids and many forbs. Host species for AMF typically increase following restoration treatments in ponderosa pine forests (indeed, promoting herbaceous understory vegetation is often an explicit goal of these restoration treatments), and positive responses of AMF to thinning and prescribed burning of ponderosa forests have been documented by others (Korb et al., 2003). The Basidiomycota and Ascomycota include both saprotrophic and ectomycorrhizal taxa. Ascomycota in ponderosa pine forests may be more resilient to fire effects than Basidiomycota, as Reazin et al. (2016) also reported rapid replacement of dominant Basidiomycota by Ascomycota after burning small-scale experimental plots in this forest type.

In contrast to our findings regarding restoration treatment effects on diversity of microarthropod communities, the thinned/burned management unit hosted more diverse fungal communities as quantified by Faith's phylogenetic diversity index (**Figure S4**), a measure of the total branch length of the phylogenetic tree formed by operational taxonomic units in a sample (Faith, 1992). (Note, however, that this is a fundamentally different measure of diversity than that used to compare microarthropod communities.) Increased phylogenetic diversity in the thinned/burned mesocosms may be the product of an interaction between mesocosm artifacts and restoration treatments, because mesocosm controls in the thinned/burned and untreated units did not differ. On the other hand, mesocosm controls were replicated at only three trees per management unit, so our power to detect differences in diversity between the management treatments was reduced for these experimental units.

4.2.3 Effects of thinning/burning on soil properties and ecological functions

Soil organic matter was reduced (**Figure S5 A**), and soil ammonium tended to be lower (**Figure S6 A**), in mesocosms within the thinned/burned unit than in those within the untreated control unit. Median nitrate concentration was an order of magnitude lower than ammonium concentration and did not differ between restoration treatment units (W=1970, *p*=0.156). Soil moisture was reduced by thinning and burning (*F*=4.678, *p*=0.032). Parameter estimates for selected models explaining decomposition of balsa wood and museum board substrates are shown in **Table 4** and **Table 5**. Decomposition of both balsa wood and museum board was lower on average in the thinned/burned management unit than in the untreated control management unit (significant intercept offset), but interactions between restoration treatment and timepoint were not significant and were not included in the models.

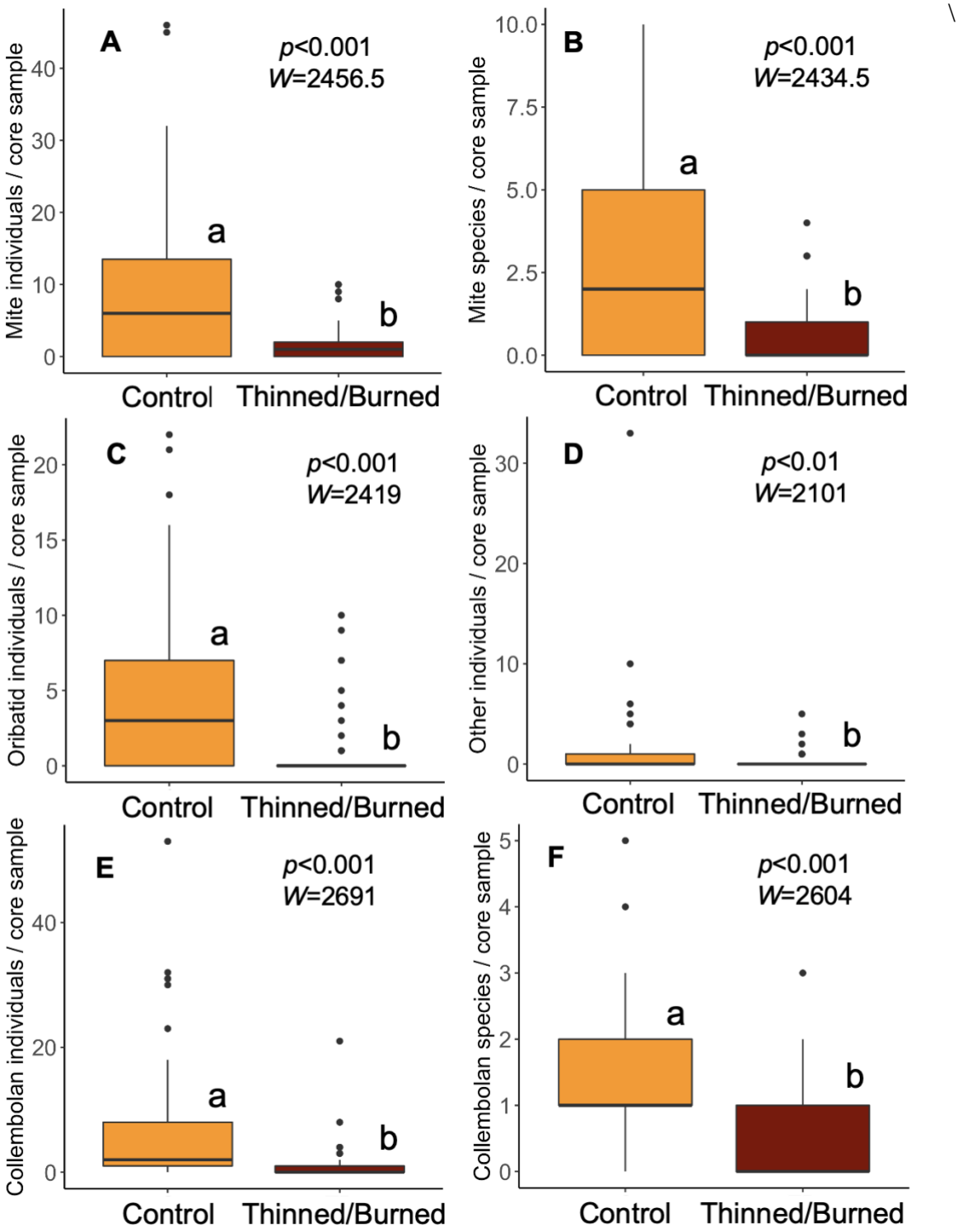

Figure 5. Abundance and species richness of microarthropods in combined soil and litter core samples from mesocosms and mesocosm controls in the thinned/burned management unit and the untreated control management unit. (**A**) Species richness and (**B**) abundance of collembolans. (**C**) Species richness and (**D**) abundance of mites. (**E**) Abundance of mites in the order Oribatida. (**F**) Abundance of other microarthropods apart from mites and collembolans. *P*-values and test statistics are from Wilcoxon rank-sum tests.

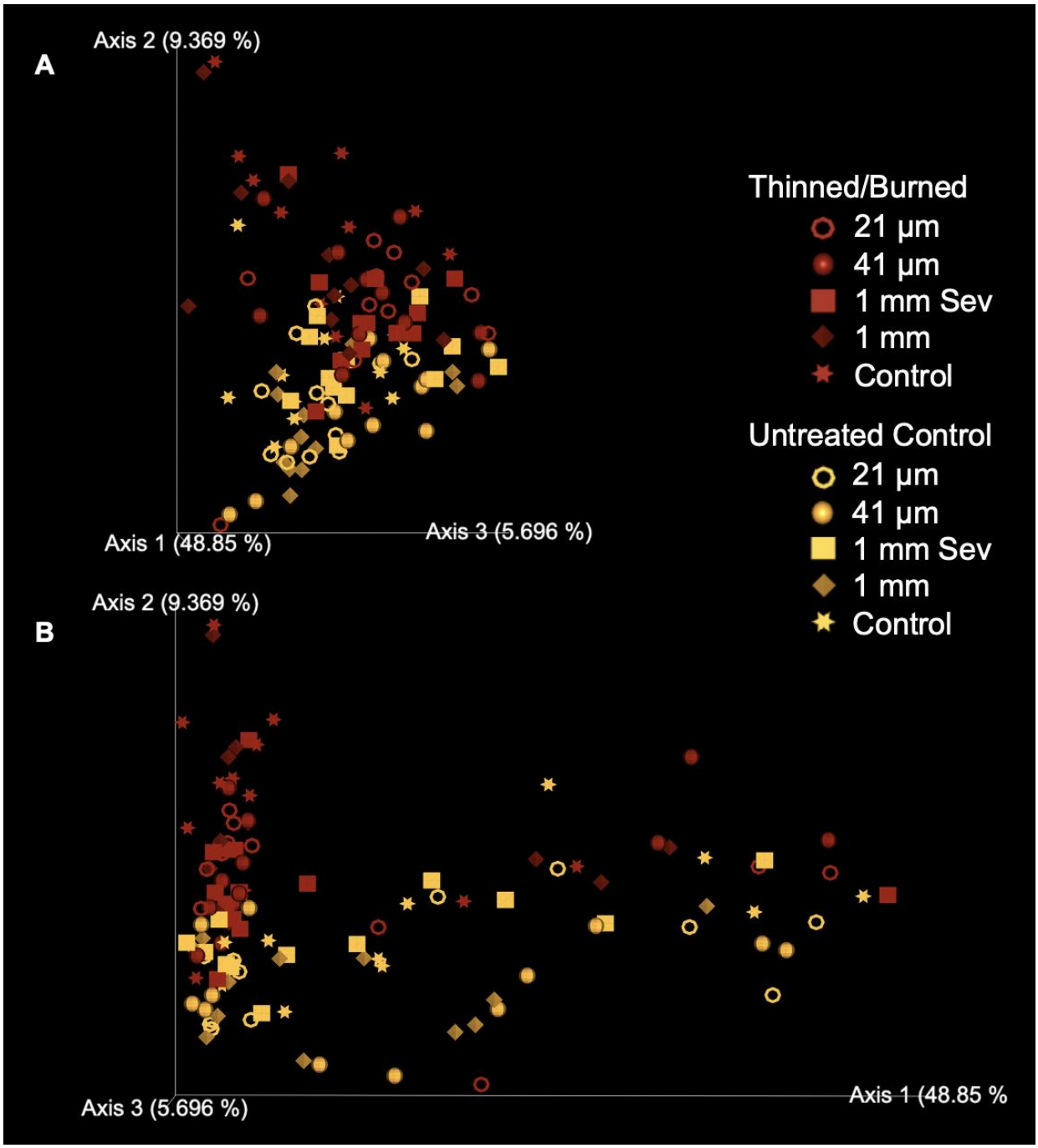

Figure 6. Three dimensional principal component analysis (PCA) ordination of fungal communities based on weighted UniFrac distance, visualized with Emperor Qiime2View. Colors correspond to ponderosa restoration treatment, and shapes designate mesh treatment: 21 µm, 41 µm, 1 mm with root severing (1mm Sev), 1 mm without root severing (1 mm), and mesocosm controls without mesh or pipe. (**A)** Variation along PCA axes 2 and 3. (**B)** Variation along PCA axes 1 and 2.

In addition to warmer and drier conditions, altered microbial community composition and decreased fungal biomass (we observed more hyphae within the untreated control unit when harvesting balsa and museum board disks in the field) may have contributed to slower mass loss in the thinned/burned unit. Others comparing decomposition in thinned and/or burned and untreated second growth ponderosa pine forests have observed conflicting patterns, from increased mass loss of a lignin-rich standard substrate in burned and thinned/burned plots (Gundale et al., 2005; up to two years post-fire) to slightly faster decomposition of ponderosa pine needles in untreated units (Monleon and Cromack Jnr, 1996; along a chronosequence ranging from months to 12 years post-fire). Higher N availability immediately after fire may ephemerally enhance decomposition (Gundale et al., 2005), especially of more recalcitrant substrates, but mineral nitrogen was no longer elevated in the soils of our thinned/burned mesocosms 5 years after fire. The artificiality of our mesocosms does limit our inferences regarding effects of thinning and burning on decomposition at the stand level, however.

Restoration treatment unit was the strongest predictor of decomposition across all trees for balsa wood, but for museum board, the model indicated a strong positive effect of soil moisture and a strong negative effect of soil organic matter in addition to the effect of restoration treatment. Increased soil moisture enhances activity of saprotrophs where moisture is limiting, as it is in xeric forest types. We are uncertain what mechanism may underlie the apparent retardation of cellulose decomposition with increasing soil organic matter. This pattern seemed to be driven by trees in the thinned/burned management unit, and soil organic matter was not selected as an important covariate in the cellulose decomposition model for the untreated control unit alone. One possibility is that locations with more soil organic matter were less impacted by fire and hosted more ectomycorrhizal fungi relative to strictly saprotrophic fungi. If so, the Gadgil effect could be at play, where decomposition rates are suppressed by competition of ectomycorrhizal with saprotrophic fungi.

4.3 Faunal complexity effects on fungi and ecosystem functions

Mantel tests based on weighted UniFrac distance revealed that fungal and faunal communities were correlated across all trees (r=-0.087, p=0.019) and within the thinned/burn unit alone (r=-0.188, p=0.009) but not within the untreated control management unit alone (r=-0.033, p=0.208). However, fungal community composition did not differ according to mesh treatments at either the stand level (PERMANOVA based on weighted UniFrac distance: pseudo-F: 0.9578, p=0.448) or management treatment level (p>0.10 for all pairwise comparisons among mesh treatments within thinned/burned and control stands, respectively) (**Figure 6**). Although phylogenetic diversity did vary by mesh treatment (**Figure S4 B** and **Figure S4 C**), these differences did not accord with measured variation in the complexity of faunal communities among the mesh treatments. Together, these findings suggest that faunal community complexity is not a strong force regulating fungal communities in this system, and that instead fauna simply respond to fungal community characteristics, or that common factors drive fungal and faunal community differences, or both.

Table 4. Results from selected analysis of covariance models for decomposition of balsa wood disks in mesocosms at all trees, untreated control unit trees only, and thinned/burned (TB) unit trees only. Note that unsevered 1 mm mesh mesocosms are the reference group for models including mesh size.

Balsa wood (predominantly lignin)
logit (proportion mass remaining)

All trees

	Estimate	**Std. error**	***t* value**	**Probability <|t|**
Intercept	2.09179	0.06050	34.575	< 2e-16***
Timepoint	-0.08587	0.01357	-6.330	5.97e-10***
Restoration treatment: TB	0.14734	0.03978	3.704	0.000239***
1 mm mesh severed	0.08061	0.05679	1.419	0.156514
41 µm mesh	-0.02786	0.06234	-0.447	0.125520
21 µm mesh	-0.10027	0.06533	-1.535	0.655147
Soil moisture (*average Z score*)	-0.02518	0.03148	-0.800	0.424267
		Residual standard error		0.4105
			Adjusted R^2	0.1254
			***F* (d.f.=)**	11.83 (6, 447)
			Model *P*	2.519e-12***

Untreated control trees only

	Estimate	**Std. error**	***t* value**	**Probability <|t|**
Intercept	2.09912	0.06791	30.908	< 2e-16 ***
Timepoint	-0.08853	0.02050	-4.319	2.34e-05 ***
Soil moisture (*average Z score*)	-0.07205	0.03230	-2.231	0.0266 *
		Residual standard error		0.442
			Adjusted R^2	0.08487
			***F* (d.f.=)**	11.76 (2, 230)
			Model *P*	1.375e-05***

Thinned/burned trees only

	Estimate	**Std. error**	***t* value**	**Probability <|t|**
Intercept	2.31056	0.07785	29.680	< 2e-16***
Timepoint	-0.08329	0.01717	-4.850	2.36e-06***
1 mm mesh severed	0.08373	0.07212	1.161	0.24693
41 µm mesh	-0.15799	0.08413	-1.878	0.06175 .
21 µm mesh	-0.25929	0.07911	-3.278	0.00122**
Soil moisture (*average Z score*)	0.08791	0.05441	1.616	0.10761
		Residual standard error		0.3646
			Adjusted R^2	0.1648
			***F* (d.f.=)**	9.681 (5, 215)
			Model *P*	2.367e-08***

Table 5. Results from selected analysis of covariance models for decomposition of museum board disks in mesocosms at all trees, untreated control unit trees only, and thinned/burned (TB) unit trees only.

Museum board (predominantly cellulose)
logit (proportion mass remaining)

All trees

	Estimate	**Std. error**	***t* value**	**Probability <|t|**
Intercept	1.14731	0.29954	3.830	0.000146 ***
Timepoint	-0.44262	0.03482	-12.712	< 2e-16 ***
Restoration treatment: TB	0.22057	0.10416	2.118	0.034747 *
Soil moisture (*average Z score*)	-0.37166	0.06604	-5.628	3.17e-08 ***
log % **Soil organic matter**	0.49691	0.15530	3.200	0.001470 **
		Residual standard error		1.062
			Adjusted R^2	0.3148
			***F* (d.f.=)**	54.53 (4, 462)
			Model *P*	< 2.2e-16

Untreated control trees only

	Estimate	**Std. error**	***t* value**	**Probability <|t|**
Intercept	2.10192	0.16810	12.504	< 2e-16 ***
Timepoint	-0.46827	0.05048	9.276	< 2e-16 ***
Soil moisture (*average Z score*)	-0.43692	0.07841	-5.572	6.84e-08 ***
		Residual standard error		1.101
			Adjusted R^2	0.3246
			***F* (d.f.=)**	58.2 (2, 236)
			Model *P*	< 2.2e-16***

Thinned/burned trees only

	Estimate	**Std. error**	***t* value**	**Probability <|t|**
Intercept	0.21269	0.42094	0.505	0.613866
Timepoint	-0.41536	0.04656	-8.920	< 2e-16 ***
1mm mesh severed	0.21460	0.19390	1.107	0.269621
41 µm mesh	0.34738	0.22667	1.533	0.126820
21 µm mesh	0.68006	0.21434	3.173	0.001725 **
Soil moisture (*average Z score*)	-0.49196	0.14702	-3.346	0.000963 ***
log % **Soil organic matter**	0.97593	0.23223	4.202	3.83e-05 ***
		Residual standard error		0.9931
			Adjusted R^2	0.3266
			***F* (d.f.=)**	19.35 (6, 221)
			Model *P*	< 2.2e-16***

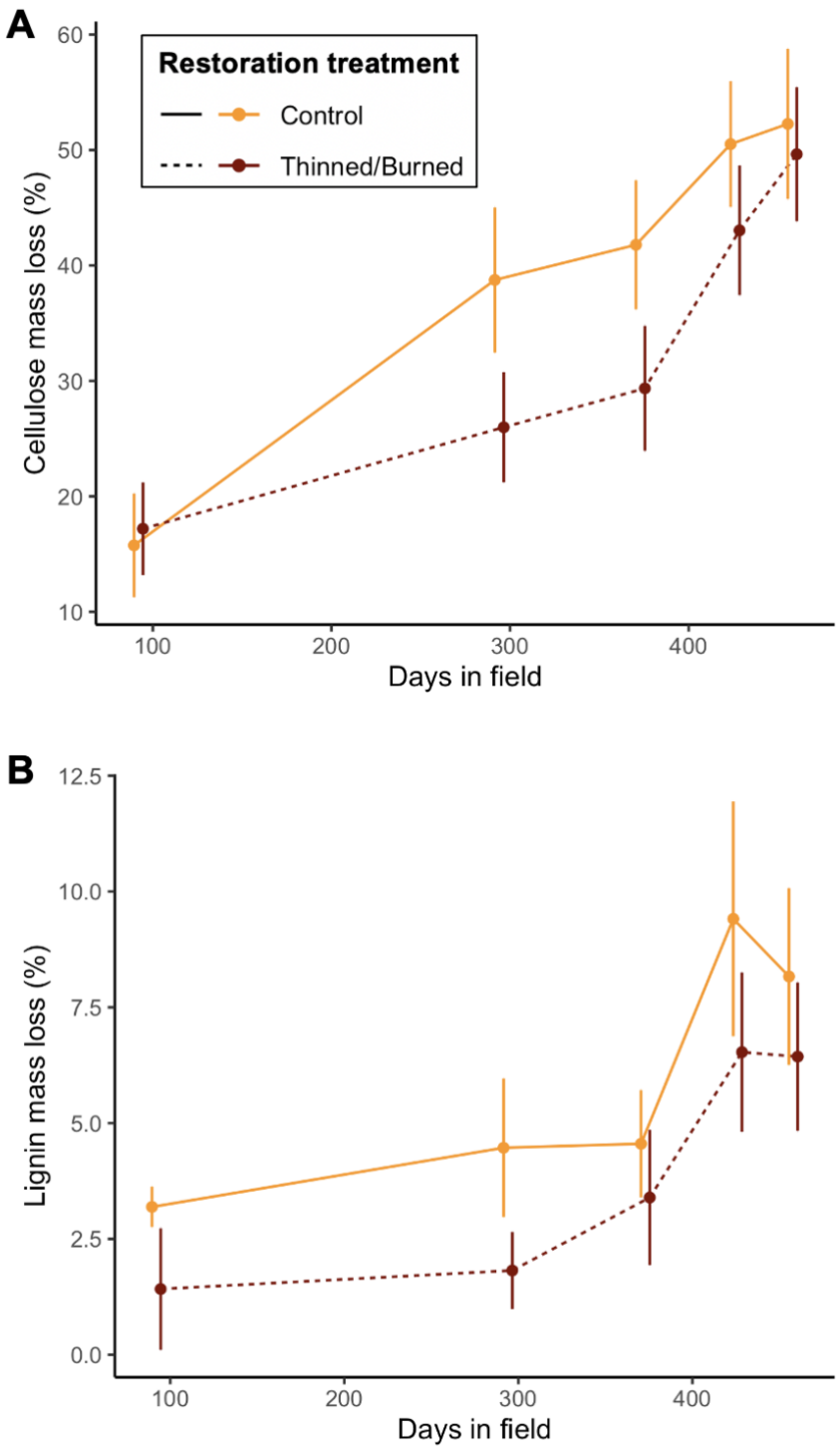

Figure 7. Decomposition of recalcitrant and labile standard substrates in thinned/burned and untreated control ponderosa pine forest management units. (**A)** Mass loss of museum board (predominantly cellulose) over time. (**B)** Mass loss of balsa wood (predominantly lignin) over time. Error bars represent 95% confidence intervals.

The importance of faunal complexity for decomposition varied between untreated control and thinned/burned forest management units (**Table 4, Table 5, Figure 8, Figure 9**). Faunal complexity does not appear to have affected decomposition of either the lignin-rich or the cellulose-rich substrates in the untreated control unit: our model selection procedure indicated that mesh treatment differences in decomposition were better explained by soil moisture within this forest management unit. Mesh size did emerge as an important predictor of decomposition of both substrates in the thinned/burned unit, however. For both balsa wood and museum board decomposition in this forest management unit, only the 21 µm mesh treatment had a significant effect on mass loss. Compared to 1 mm mesh mesocosms, the 21 µm mesh treatments increased remaining museum board by an estimated 15.6% of the initial mass, and decreased remaining balsa wood by an estimated 2.36% of the initial mass. The 41 µm mesh treatment produced weaker effects of the same directionality as the 21 µm mesh. Faunal complexity in the 21 µm and 41 µm mesh treatments did not differ according to our assessment of microarthropods >300 µm, but data from the sacrificial mesocosms suggests that the 41 µm mesh mesocosms may have hosted considerably more small microarthropods than did the 21 µm mesh mesocosms.

We also saw no evidence that faunal complexity influenced mineral ammonium content (**Figure S6 C**) or soil organic matter (**Figure S5 C)**: mesh treatment was not a significant predictor of either of these soil characteristics in mixed models accounting for between-tree variation ($p>0.3$ for all null model comparisons). We expected that nematodes (and especially bacterivorous nematodes) would influence nitrogen mineralization more strongly than microarthropods (Neher et al., 2012), and our mesocosms do not appear to have directly manipulated nematode community complexity. We would not expect to see an influence of faunal complexity on soil organic matter in the absence of effects on fungal communities, nitrogen mineralization, or decomposition—at least not over the timespan of this study. Given that we observed faunal complexity effects on decomposition only in the thinned/burned unit, where relatively little organic material was available for saprotrophs to break down, more sensitive analyses may have been required to detect changes in soil organic matter there. On the other hand, Grandy et al. (2016) argue that faunal effects on decomposition may not necessarily translate to effects on soil organic matter dynamics.

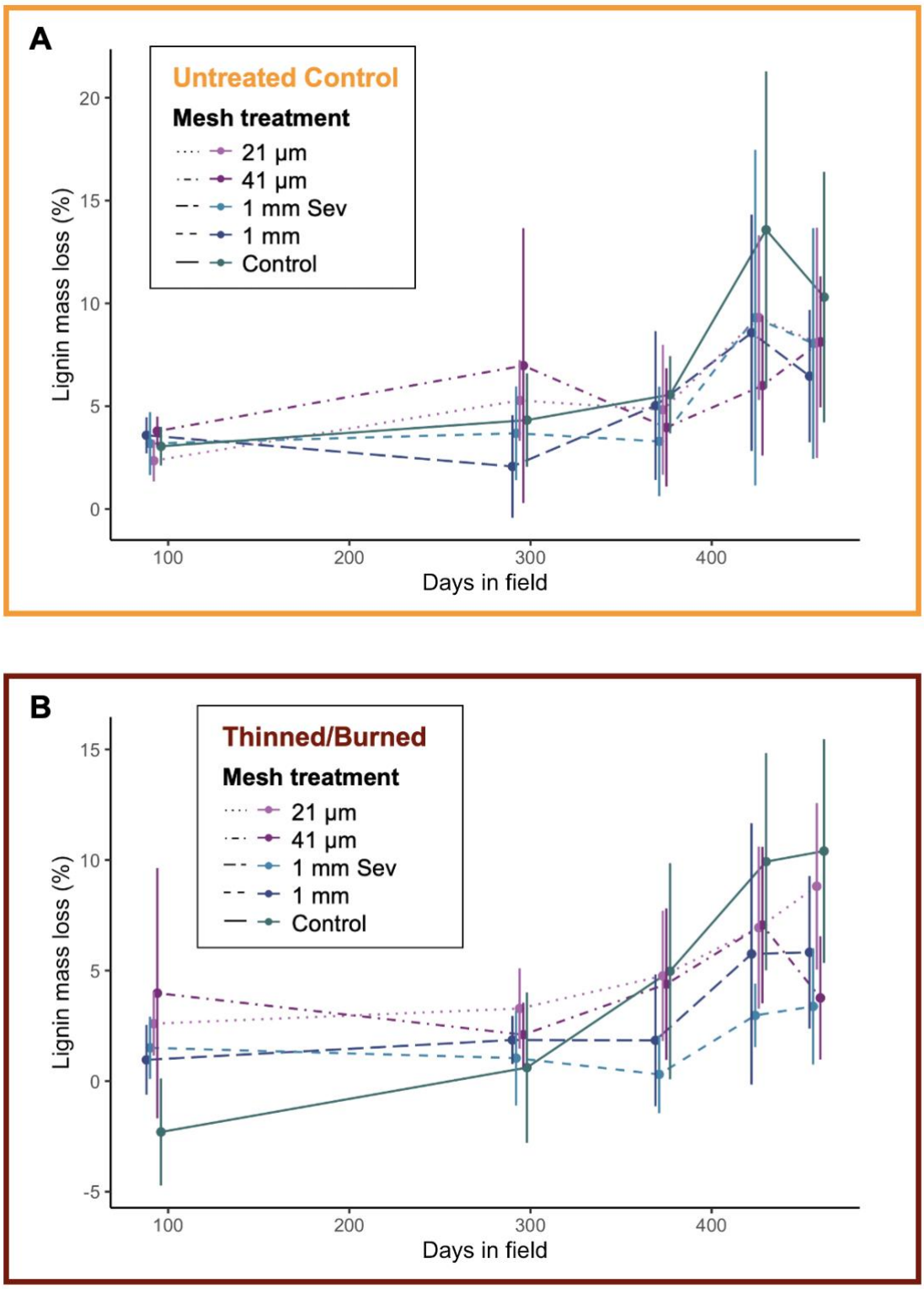

Figure 8. Decomposition of balsa wood (predominantly lignin) in (**A)** untreated control and (**B)** thinned/burned ponderosa pine forest management units. Error bars represent 95% confidence intervals.

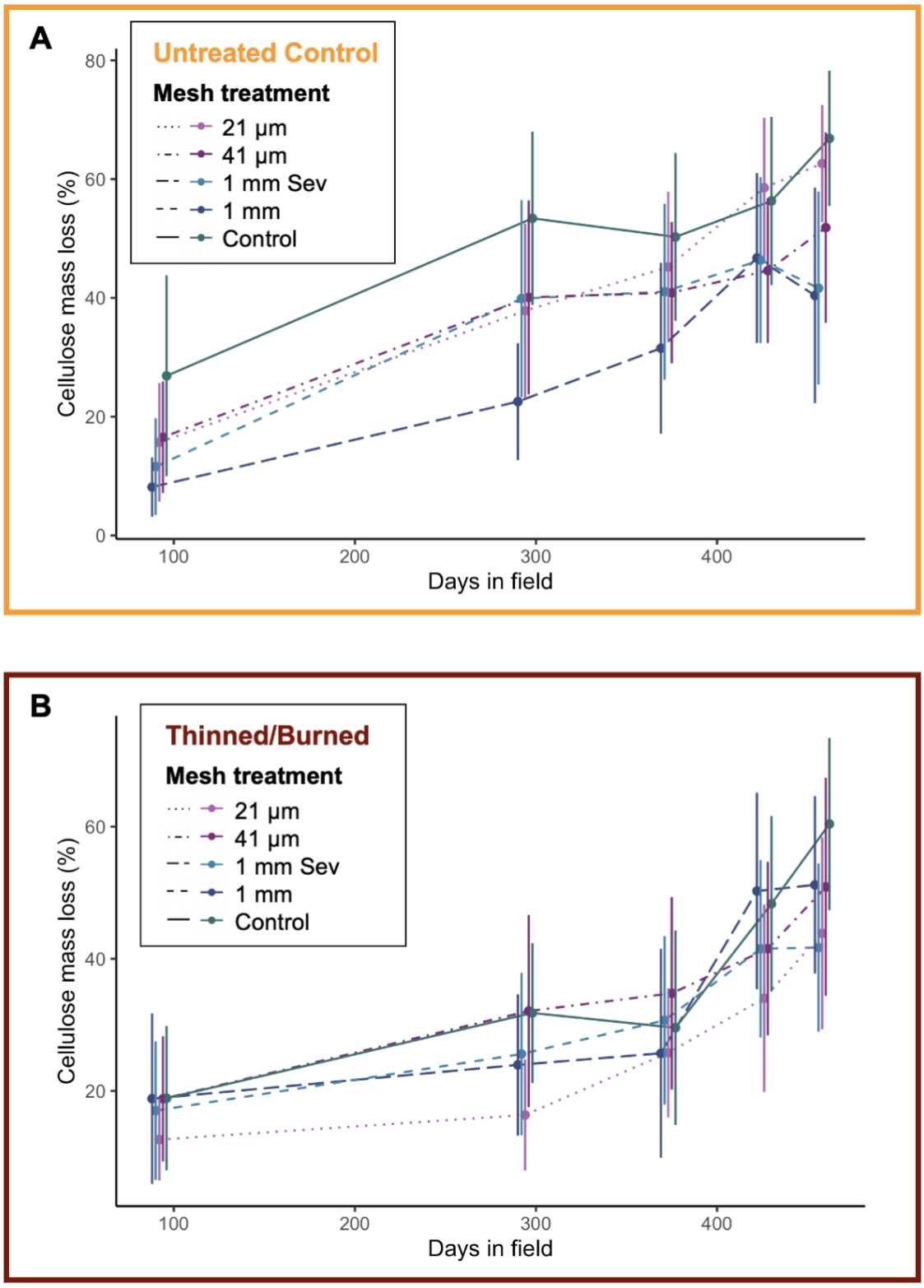

Figure 9. Decomposition of balsa wood (predominantly lignin) in (**A)** untreated control and (**B)** thinned/burned ponderosa pine forest management units. Error bars represent 95% confidence intervals.

Table 6. Evidence for hypotheses regarding the effects of increasing soil faunal complexity (more microarthropod species and individuals) on ecological functions in untreated and thinned/burned ponderosa pine management units.

More complex faunal assemblages will:	
Untreated Control	**Thinned/Burned**
Inhibit decomposition of recalcitrant substrate (lignin) Effect expected to be **stronger**	**Inhibit decomposition of recalcitrant substrate (lignin)** Effect expected to be **weaker**
Not supported*: no effect of mesh treatment on mass loss of balsa wood in untreated control unit after accounting for variation in soil moisture*	***Partially supported:*** *mass loss was slower in coarse than in fine mesh mesocosms in thinned/burned unit*
Increase decomposition of labile substrate (cellulose) Effect expected to be **stronger**	**Increase decomposition of labile substrate (cellulose)** Effect expected to be **weaker**
Not supported: *no effect of mesh treatment on mass loss of museum board after accounting for variation in soil moisture*	***Partially supported****: mass loss was faster in mesocosms with more faunal complexity*
Decrease nitrogen availability	**Not affect nitrogen availability**
Not supported*: no effect of mesh size on soil ammonium content*	***Supported:*** *no effect of mesh size on soil ammonium content*
Affect fungal communities	**Affect fungal communities**
Not supported*: no effect of mesh size on fungal communities, and faunal and fungal communities were not correlated*	***Not supported:*** *although fungal and faunal communities were correlated, mesh size was not a significant predictor of fungal communities*

4.4 Methods development

In a meta-analysis of 40 years of litterbag experiments testing microarthropod effects on decomposition, Kampichler and Bruckner (2009) made the disconcerting observation that none of the 101 experiments surveyed had taken potential side effects of faunal exclusion treatments into account. Noting that the mean effect size estimate from studies employing insecticide treatments was approximately twice that of those using mesh size differences, and that applying a correction factor for mesh size side effects could change the sign of microarthropod effects, they concluded that the real contribution of microarthropods to decomposition could not be assessed. A later meta-analysis (García-Palacios et al., 2013) of faunal exclusion litterbag studies

downplayed the importance of potential treatment side effects (which researchers had evidently continued to ignore), noting that exclusion method (mesh or insecticide) and, for mesh size-based exclusion experiments, mesh size choices did not alter the finding that soil fauna increased decomposition. However, this meta-analysis did not distinguish between studies testing the effects of mesofauna from those examining macrofauna. (In fact, many of the faunal exclusion treatments included in this broader meta-analysis used fine mesh sizes that would have included all microarthropods.) This discrimination is important, because these faunal size classes differ in their predominant functional roles. To our knowledge, no relevant meta-analyses have been published since, but it appears that these issues remain largely unaccounted for in studies concerning mesofauna effects on decomposition. Minimizing and controlling for side effects in manipulative studies of faunal functions is especially important when working in high elevation xeric forests, where water is a limiting resource and UV radiation is strong.

Our study endeavored to address the most likely side effects resulting from manipulation of faunal complexity by mesh size: 1) altered infiltration, leaching, and UV penetration (by using the same mesh size for lids and bottoms); 2) relatedly, differences in microclimate between substrates with and without focal fauna (by repeatedly measuring soil moisture); and 3) the increased loss of litter fragments through coarse mesh (by controlling the presence or absence of fauna in an entire soil/litter system, not just on the substrate). Our novel mesocosm design enabled us to perform repeated measurements within mesocosms while maintaining differences in faunal complexity over two growing seasons. Although side effects were not eliminated, they were quantified, and we are confident that they were lower than would have occurred in this system with use of other faunal exclusion mesocosm designs (e.g. Vedder et al., 1996). Side-effects could be further reduced if these mesocosms were installed in locations that allowed frequent servicing, in which case any measured soil moisture differences could be equalized through water addition or rain-out shelters. Moisture differences may be negligible, however, if these mesocosms are used in a more mesic forest type. We also note that our mesocosms are compatible with LI-COR chambers, facilitating measurement of gas fluxes. We hope that development of this mesocosm method will invite future field manipulations of soil faunal complexity.

4.4 Planned and completed science delivery activities

Some of our science delivery activities were delayed due to the COVID-19 pandemic.

1. Presentation at CFLRP All Hands Meeting (cancelled in 2020 and 2021, will present at next meeting in 2022)
2. Presentation at relevant forest management conference (delayed)
3. JFSP fact sheet
4. Dissertation chapter (forthcoming)
5. Peer-reviewed publication (forthcoming)

5. Conclusions, Management Implications, and Future Research

We successfully manipulated faunal complexity within thinned/burned and untreated control ponderosa pine management units over two growing seasons using mesocosms of novel design. These mesocosms were constructed to minimize side effects of manipulation while permitting

repeated sampling and measurement of internal conditions. We are hopeful that the method we developed can aid in resolving relationships between soil faunal communities and ecological functions across forest types and management treatments. Field data on these relationships that can be causally dissected are scarce, but critical for informing soil biogeochemical models (Grandy et al., 2016).

Our study revealed persistent effects of thinning and burning on microarthropod assemblages five years after treatment, but did not indicate significant ramifications for several soil ecological functions known to be influenced by faunal communities: regulation of fungal communities, nitrogen mineralization, soil organic matter formation, and decomposition (Kanters et al., 2015; Soong and Nielsen, 2016; Verhoef and Brussaard, 1990). The sole exception was decomposition, which appears to have been increased by faunal complexity for a labile substrate (cellulose) and decreased by faunal complexity for a recalcitrant substrate (lignin)—but only in the thinned/burned unit. These differences in decomposition were only marked when comparing the simplest communities (21 µm mesh mesocosms) to the most complex communities (1 mm mesh mesocosms). We can think of two possible explanations for this:

1. Paradoxically, faunal complexity may be more important for decomposition after restoration treatments (despite simpler microarthropod communities) than in untreated stands (where microarthropod diversity and abundance are higher) because the biotic and abiotic context of faunal activities matters, and that context changes with restoration treatments. For example, in thinned/burned forests the ratio of faunal to microbial biomass could be higher, strengthening grazing effects; the ratio of saprotrophic to ectomycorrhizal fungi could be higher, enabling fauna to intensify the Gadgil effect (Fernandez and Kennedy, 2016); and spatial and temporal connectivity of resources could be lower, increasing the importance of micro-scale propagule dispersal and nutrient translocation by microarthropods which "wake up" soil microbes (Briones, 2018).

2. An extreme reduction in microarthropod densities, not merely a simplification of faunal communities, must occur in this system before decomposition is significantly impacted. If this is the case, the effects of thinning and burning on faunal mediation of decomposition may be negligible. Although we did not enumerate microarthropods < 300 µm in all mesocosms at the end of the study, data collected earlier from a subset of sacrificial mesocosms indicate that 21 µm mesh mesocosms in the untreated control unit had total mite densities similar to those of the 1 mm mesh mesocosms in the thinned/burned unit (**Figure S8 A**). In other words, the most effective "microarthropod exclusion" mesh treatment in the untreated control unit achieved total microarthropod densities similar to the "microarthropod inclusion" mesh treatment in the thinned/burned unit.

Our results must be interpreted in the context of historically anomalous climate behavior in the region. While our mesocosms were deployed, the area received only an estimated ~60% of its average precipitation over that timespan, and only ~50% of its average precipitation during the growing season (PRISM Climate Group, 2021). In an already xeric ecosystem type, these precipitation deficits likely had profound implications for microarthropod communities and their performance of ecological functions. However, increasing aridity is expected in the region with

climate change, so our findings may be more reflective of future than of past conditions. We also note that our fungal community data represent a single snapshot in time, and are derived from minute subsamples of soil, while our mineral nitrogen and soil organic matter analyses were probably only capable of detecting relatively large effects of faunal complexity. It is also critical to remember that the reduced complexity of faunal communities in the thinned/burned unit may or may not be representative of pre-fire-exclusion conditions, because impacts of thinning and prescribed fire are likely more extreme than those of historic low-intensity burns (e.g. due to disturbance from logging machinery and increased heat transfer to soil from higher fuel loads). On the other hand, the untreated control unit in our study was undeniably a very unnatural ecological stage for microarthropod communities. It is thus difficult to gauge the "desirability" of these changes from a management perspective.

References

Alberta Biodiversity Monitoring Institute, 2009. Processing Mites (Oribatids) and Springtails (Collembola).

Bates, D., Maechler, M., Bolker, B.M., Walker, S., 2015. Fitting Linear Mixed-Effects Models using {lme4}. J. Stat. Softw. https://doi.org/10.18637/jss.v067.i01

Briones, M.J.I., 2018. The serendipitous value of soil fauna in ecosystem functioning: The unexplained explained. Front. Environ. Sci. https://doi.org/10.3389/fenvs.2018.00149

Camann, M., Lamoncha, K., Gillette, N., 2012. Oribatid Mite Community Decline Two Years after Low-Intensity Burning in the Southern Cascade Range of California, USA. Forests 3, 959–985. https://doi.org/10.3390/f3040959

Chen, J., Ferris, H., 1999. The effects of nematode grazing on nitrogen mineralization during fungal decomposition of organic matter. Soil Biol. Biochem. 31, 1265–1279. https://doi.org/10.1016/S0038-0717(99)00042-5

Christensen, A.K., Brockhoff, P.B., Rune, H.B., 2017. lmerTest package: Tests in linear mixed effects models. J. Stat. Softw. 82, 1–26.

Faith, D.P., 1992. Conservation evaluation and phylogenetic diversity. Biol. Conserv. 61, 1–10. https://doi.org/10.1016/0006-3207(92)91201-3

Fernandez, C.W., Kennedy, P.G., 2016. Revisiting the "Gadgil effect": Do interguild fungal interactions control carbon cycling in forest soils? New Phytol. 209, 1382–1394. https://doi.org/10.1111/nph.13648

García-Palacios, P., Maestre, F.T., Kattge, J., Wall, D.H., 2013. Climate and litter quality differently modulate the effects of soil fauna on litter decomposition across biomes. Ecol. Lett. 16, 1045–1053. https://doi.org/10.1111/ele.12137

Goff, F., 2009. Valles Caldera: a geologic history. UNM Press.

Grandy, A.S., Wieder, W.R., Wickings, K., Kyker-Snowman, E., 2016. Beyond microbes: Are fauna the next frontier in soil biogeochemical models? Soil Biol. Biochem. 102, 40–44. https://doi.org/10.1016/j.soilbio.2016.08.008

Gundale, M.J., DeLuca, T.H., Fiedler, C.E., Ramsey, P.W., Harrington, M.G., Gannon, J.E., 2005. Restoration treatments in a Montana ponderosa pine forest: Effects on soil physical, chemical and biological properties. For. Ecol. Manage. 213, 25–38. https://doi.org/10.1016/j.foreco.2005.03.015

Hacker, L., Banet, C., 1987. Soil Survey of Sandoval County Area, New Mexico.

Kanters, C., Anderson, I.C., Johnson, D., 2015. Chewing up the wood-wide web: Selective

grazing on ectomycorrhizal fungi by collembola. Forests 6, 2560–2570. https://doi.org/10.3390/f6082560
Korb, J.E., Johnson, N.C., Covington, W.W., 2003. Arbuscular mycorrhizal propagule densities respond rapidly to ponderosa pine restoration treatments. J. Appl. Ecol. 40, 101–110. https://doi.org/10.1046/j.1365-2664.2003.00781.x
McCune, B., Mefford., M.J., 2006. PC-ORD. Multivariate Analysis of Ecological Data. Version 5.10, MjM Software, Gleneden Beach, Oregon, U.S.A. https://doi.org/10.1890/0012-9623(2005)86[6a:MAOEDU]2.0.CO;2
Monleon, V.J., Cromack, K.J., 1996. Long-term effects of prescribed underburning on litter decomposition and nutrient release in ponderosa pine stands in central Oregon. For. Ecol. Manage. 81, 143–152. https://doi.org/10.1016/0378-1127(95)03658-x
Neher, D.A., Barbercheck, M.E., 2019. Soil microarthropods and soil health: Intersection of decomposition and pest suppression in agroecosystems. Insects. https://doi.org/10.3390/insects10120414
Neher, D.A., Weicht, T.R., Barbercheck, M.E., 2012. Linking invertebrate communities to decomposition rate and nitrogen availability in pine forest soils. Appl. Soil Ecol. 54, 14–23. https://doi.org/10.1016/j.apsoil.2011.12.001
PRISM Climate Group, 2021. PRISM Climate Data [WWW Document]. Prism Clim. Gr.
R Core Team, 2020. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
Reazin, C., Morris, S., Smith, J.E., Cowan, A.D., Jumpponen, A., 2016. Fires of differing intensities rapidly select distinct soil fungal communities in a Northwest US ponderosa pine forest ecosystem. For. Ecol. Manage. 377, 118–127. https://doi.org/10.1016/j.foreco.2016.07.002
Smith, D.P., Peay, K.G., 2014. Sequence Depth, Not PCR Replication, Improves Ecological Inference from Next Generation DNA Sequencing. PLoS One 9, 90234. https://doi.org/10.1371/journal.pone.0090234
Soong, J.L., Nielsen, U.N., 2016. The role of microarthropods in emerging models of soil organic matter. Soil Biol. Biochem. 102, 37–39. https://doi.org/10.1016/j.soilbio.2016.06.020
Vedder, B., Kampichler, C., Bachmann, G., Bruckner, A., Kandeler, E., 1996. Impact of faunal complexity on microbial biomass and N turnover in field mesocosms from a spruce forest soil. Biol. Fertil. Soils 22, 22–30. https://doi.org/10.1007/BF00384428
Verhoef, H.A., Brussaard, L., 1990. Decomposition and nitrogen mineralization in natural and agro-ecosystems: The contribution of soil animals. Biogeochemistry 11, 175–211. https://doi.org/10.1007/BF00004496
Warton, D.I., Hui, F.K.C., 2011. The arcsine is asinine: the analysis of proportions in ecology. Ecology 92, 3–10.
Wickham, H., 2011. ggplot2. Wiley Interdiscip. Rev. Comput. Stat. https://doi.org/10.1002/wics.147
Yoder, M., Tandingan, I., Ley, D., King, I.W., Mundo-Ocampo, M., Mann, J., Blaxter, M., Poiras, L., De Ley, P., 2006. DESS : a versatile solution for preserving morphology and extractable DNA of nematodes. Nematology 8, 367–376.

Appendix A: Contact Information for Key Project Personnel

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Appendix B: List of Completed/Planned Scientific/Technical Publications/Science Delivery Products

Some of our science delivery products were delayed due to the COVID-19 pandemic.

1. Presentation at CFLRP All Hands Meeting (cancelled in 2020 and 2021, will present at next meeting in 2022)
2. Presentation at relevant forest management conference (delayed)
3. JFSP fact sheet
4. Dissertation chapter (forthcoming)
5. Peer-reviewed publication (forthcoming)

Appendix C: Metadata

Data and metadata will be made publicly available at the Forest Service Research Data Archive (www.fs.usda.gov/rds/archive). These data will include microarthropod abundances from sacrificial mesocosms; abundances of microarthropods > 300 µm from remaining mesocosms destructively sampled at the end of the experiment; fungal community Illumina sequence data; soil moisture measurements; mass loss of balsa and museum board disks; and soil ammonium, nitrate, and organic matter content. We were not able to collect soil respiration data due to the time consuming nature of these measurements when performed through mesocosm lids, the remote location of our study site relative to our university, and the difficulty of correcting for litter and duff in the chamber headspace. We also did not quantify mass loss from root, grass, and pine needle litter because we deemed that available space in the mesocosms was insufficient to include them without significantly altering habitat for soil fauna. Finally, soil phosphate was not quantified because the cost of other data collection activities exceeded our initial projections, and we concluded that based on previous analyses of soils from this site phosphate is likely not limiting in this system.

Appendix D: Supplemental Tables and Figures

Table S1. Mite and collembolan species encountered in the > 300 µm size fraction and results of indicator species analyses for management treatment groups (untreated control (UC) vs. thinned/burned (TB)) and mesh treatment groups. Bolded statistics represent strong indicators for the specified group with IV>25 (Dufrene and Legendre, 1997).

	Management Treatment			**Mesh Treatment**		
	IV	*p*	Max Group	IV	*p*	Max Group
Collembola						
Hypogastruridae sp. 1						
Hypogastruridae sp. 2						
Onychiuridae sp.						
Tullbergiidae sp.						
Isotomidae sp.	33.6	<0.001	UC			
Entomobryidae sp.	43.8	<0.001	UC	25.6	0.0134	21 µm
Entomobryomorpha sp. 1						
Entomobryomorpha sp. 2						
Entomobryomorpha sp. 3						
Entomobryomorpha sp. 4						
Acari						
Euphthiracaroidea sp.						
Trhypochthoniidae sp.						
Nothrus sp.						
Eremaeus cf. *boreomontanus*	38.4	<0.001	UC			
Odontodamaeus sp.	28.8	<0.001	UC			
Propelops cf. *canadensis*						
Damaeidae sp.						
Oribatida sp. 1						
Oribatida sp. 2						
Oribatida sp. 3						
Oribatida sp. 4						
Oribatida sp. 5						
Gymnodamaeidae sp. 1						
Eupodidae sp.						
Cunaxidae sp. 1						
Cunaxidae sp. 2						

Cunaxidae sp. 3
Cunaxidae sp. 4
Spinibdella sp.
Biscirus sp. (?)
Bdella cf. *muscorum*
Bdelloidea sp. 1
Prostigmata sp. 1
Prostigmata sp. 2
Prostigmata sp. 3
Prostigmata sp. 4
Prostigmata sp. 5
Prostigmata sp. 6
Prostigmata sp. 7
Prostigmata sp. 8
Prostigmata sp. 9
Mesostigmata sp. 1
Mesostigmata sp. 2

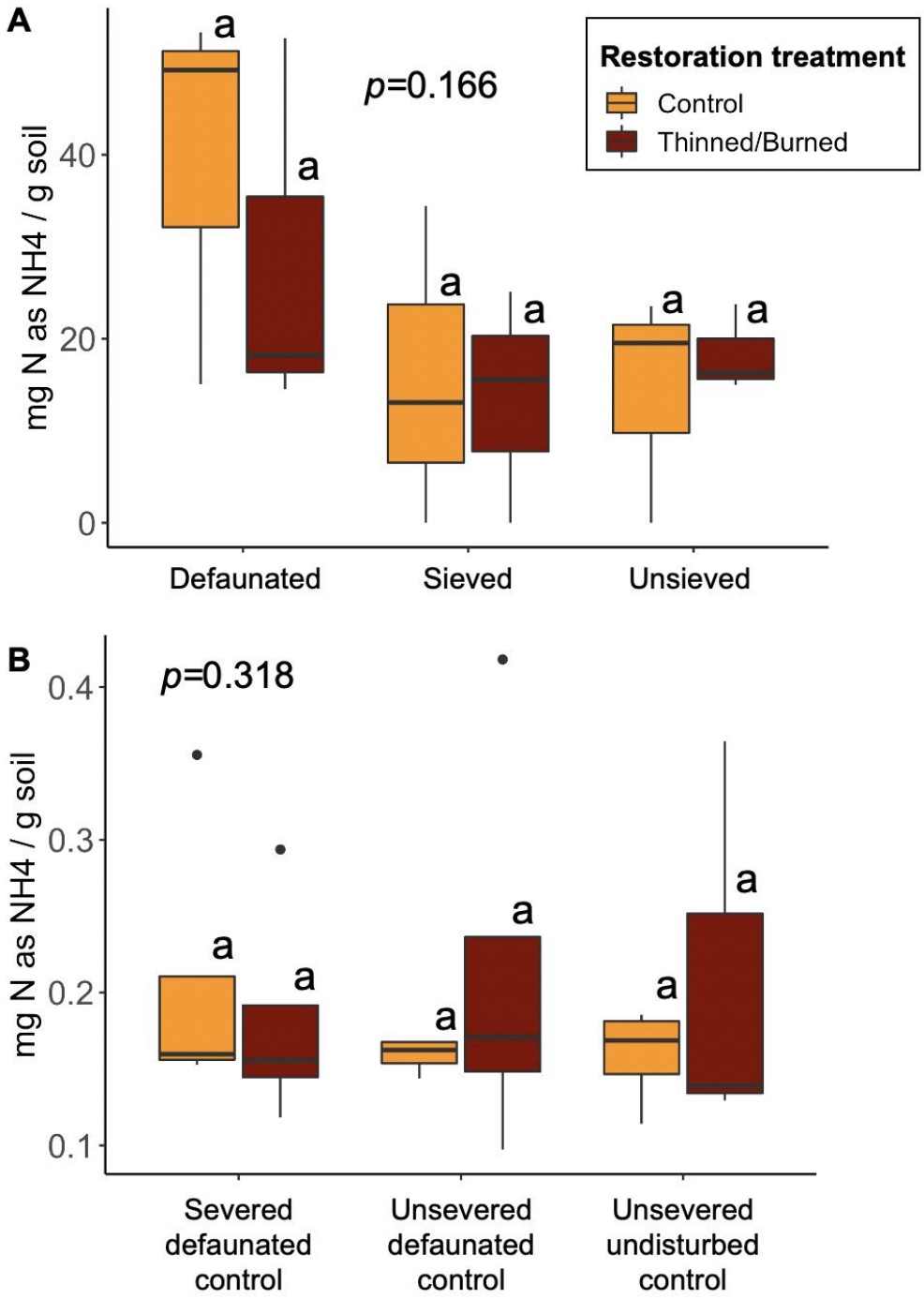

Figure S1. Effects of soil defaunation and sieving treatments on ammonium content. **A.** Ammonium content of defaunated (sieved and heated wet), sieved only, and unsieved (and unheated) soil collected from three trees per forest management unit prior to installation of mesocosms. Model *p*-value was calculated by Kruskal-Wallis H test. **B.** Ammonium content of soil in the three types of mesocosm controls at the end of the study. Model P-value was calculated by ANOVA on log-transformed values.

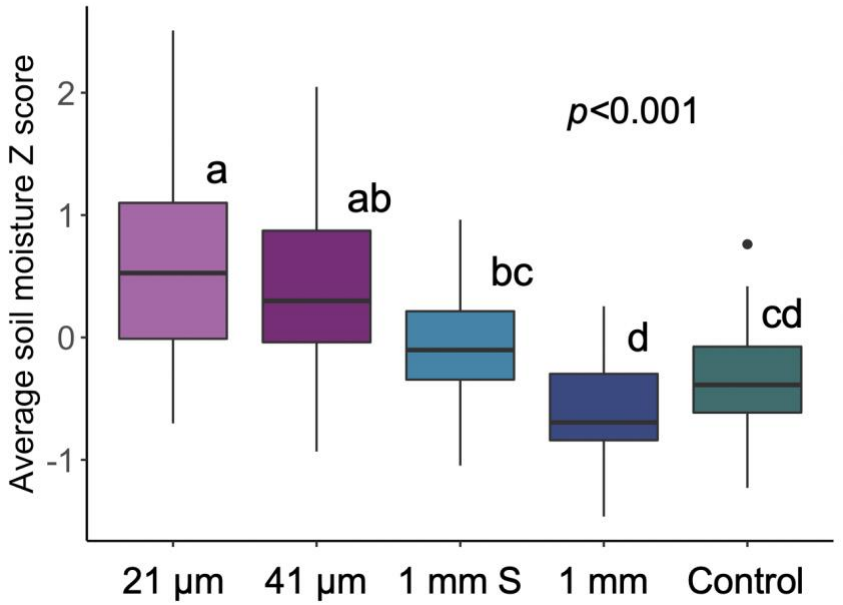

Figure S2. Average soil moisture Z-scores for mesocosm mesh treatments (21 µm, 41 µm, 1 mm with root severing (1 mm S), 1 mm without root severing (1 mm), and mesocosm controls without mesh or pipe) across five monitoring timepoints. ANOVA: F=14.52.

Table S2. Multi-response permutation procedure (MRPP) results for soil fauna species >300 µm within untreated control and thinned/burned management units, grouped by mesocosm mesh treatment and based on Bray-Curtis distance. Note that mesocosm controls were not replicated at all trees, and *p*-values are not corrected for multiple comparisons.

Untreated control only

	41 µm		1 mm Sev		1 mm		Control	
	A	*p*	*A*	*p*	*A*	*p*	*A*	*p*
21 µm	0.034	0.065	0.117	<0.001*	0.085	<0.001*	0.165	<0.001*
41 µm			0.086	<0.01*	0.070	<0.01*	0.155	<0.001*
1 mm Sev					-0.003	0.566	0.030	0.011*
1 mm							0.015	0.091

Thinned/burned only

	41 µm		1 mm Sev		1 mm		Control	
	A	*p*	*A*	*p*	*A*	*p*	*A*	*p*
21 µm	-0.009	0.535	0.052	0.048*	0.101	<0.01*	0.032	0.071
41 µm			0.054	0.034*	0.104	<0.01*	0.016	0.147
1 mm Sev					-0.014	0.787	0.010	0.222
1 mm							0.037	0.036*

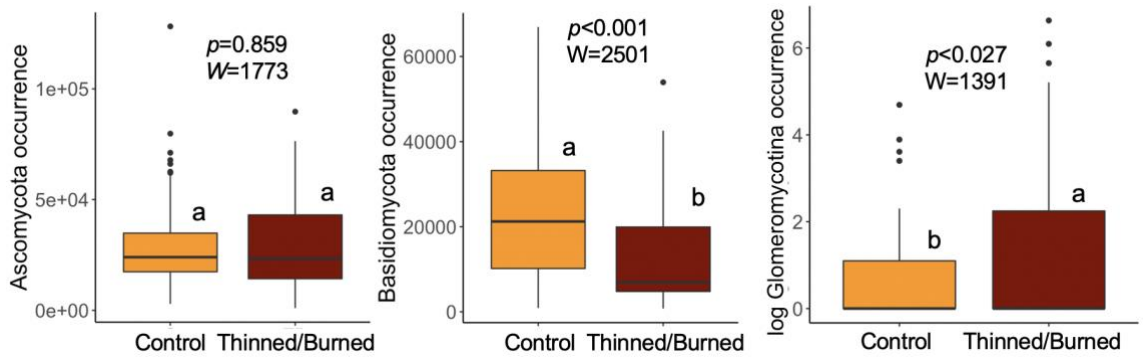

Figure S3. Observations (per sample) of sequences belonging to the fungal phyla Ascomycota and Basidiomycota and the subphylum Glomeromycotina in mesocosm soil core samples from the untreated control management unit and the thinned/burned management unit. *P*-values and test statistics are from Wilcoxon rank-sum tests.

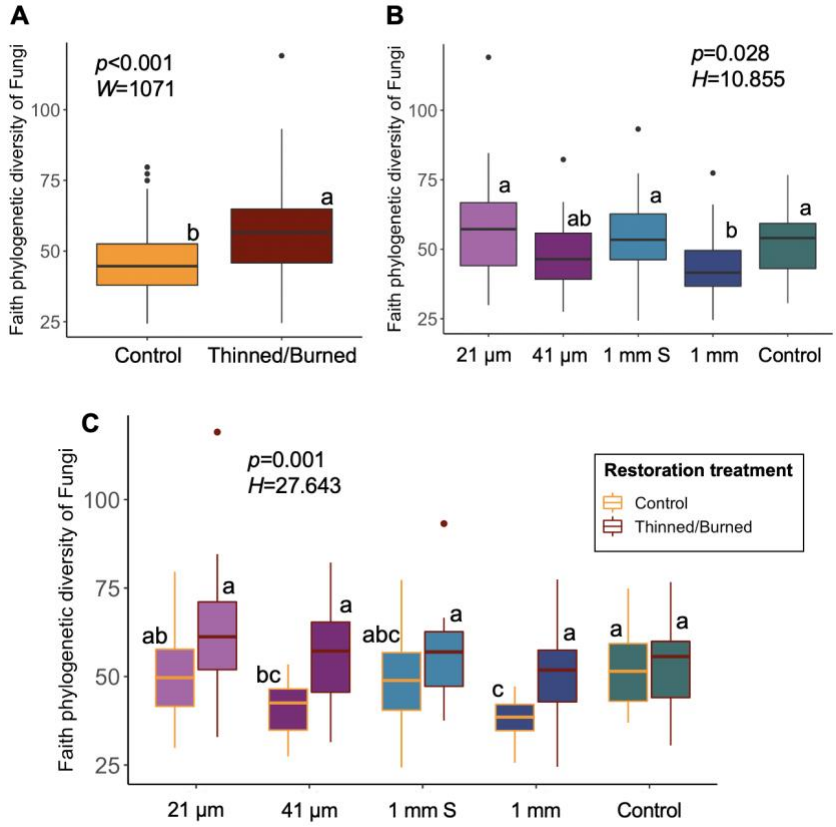

Figure S4. Faith phylogenetic diversity of fungal communities by **A.** forest management treatment; **B.** mesocosm mesh treatment (21 µm, 41 µm, 1 mm with root severing (1 mm S), 1 mm without root severing (1 mm), and mesocosm controls without mesh or pipe); and **C.** mesh treatment by forest management treatment. Boxes with different letters are significantly different at $\alpha<0.05$ according to Wilcoxon rank sum tests, after application of the Benjamini-Hochberg correction for multiple comparisons. Model *P*-values were calculated by Kruskal-Wallis H-test for mesh and mesh by forest management unit comparisons, and by Wilcoxon rank sum tests for forest management comparisons alone.

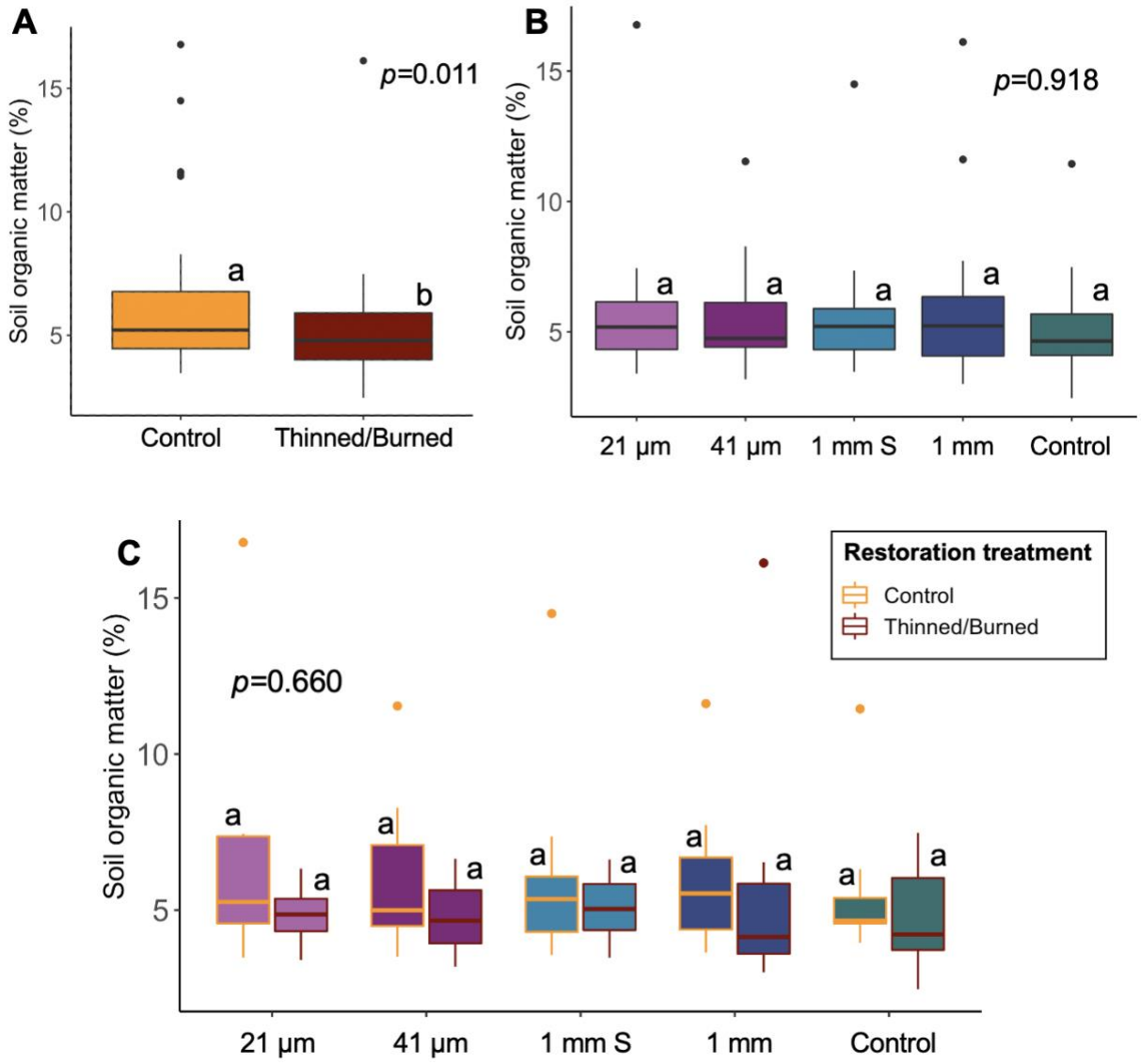

Figure S5. Soil organic matter by (**A)** forest restoration treatment unit (*F*=6.575); (**B)** mesocosm mesh treatment (*H*=0.940); (**C)** forest restoration treatment x mesocosm mesh treatment (*H*=6.780). P-values are from ANOVA on log-transformed data for ponderosa restoration treatment, and Kruskal-Wallis H tests for mesh and mesh by restoration treatment comparisons.

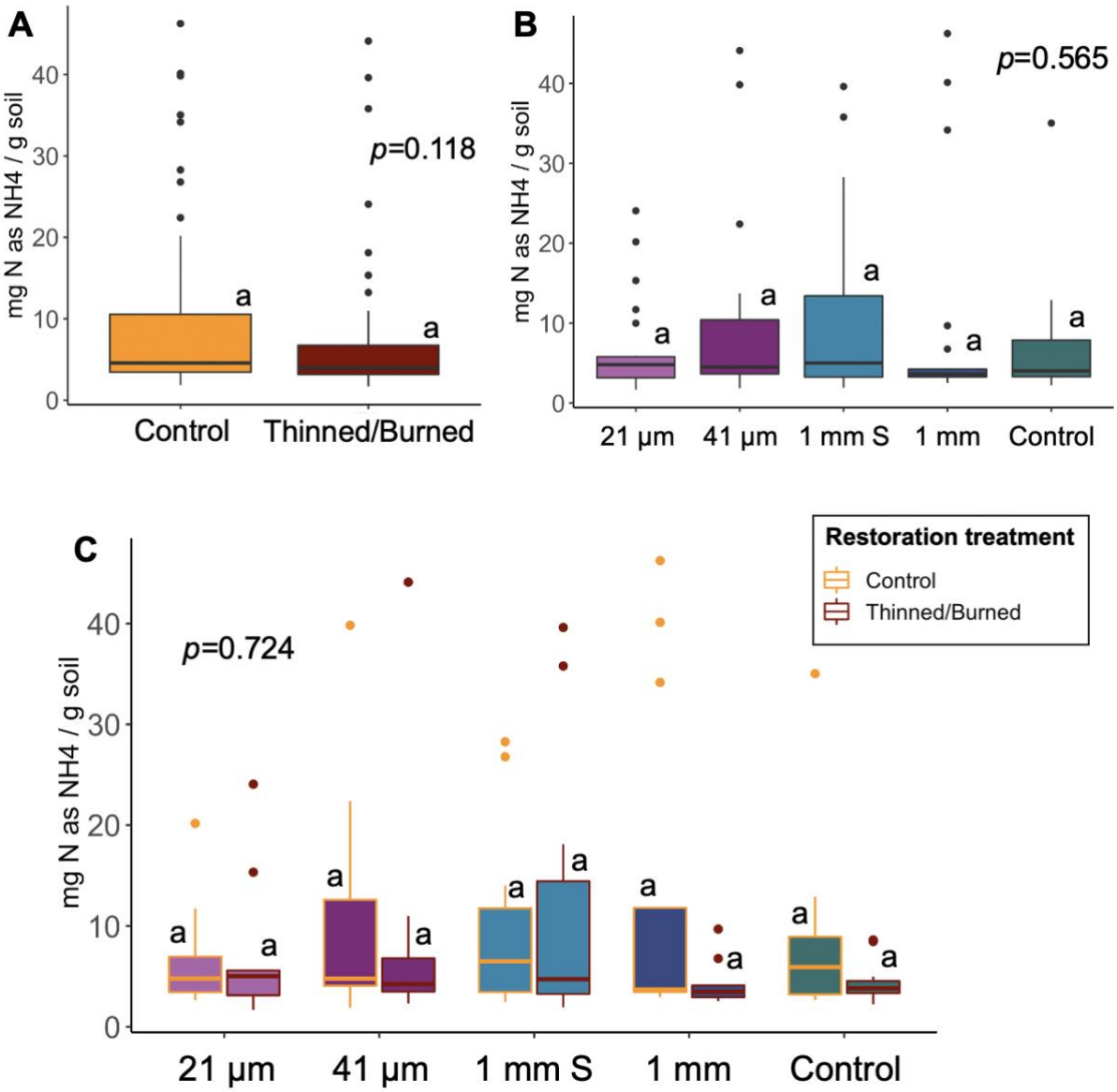

Figure S6. Soil ammonium content by (**A)** forest restoration treatment unit (*H*=2.432); (**B)** mesocosm mesh treatment (*H*=2.954); (**C)** forest restoration treatment x mesocosm mesh treatment (*H*=6.153). Model *p*-values are from Kruskal-Wallis H tests.

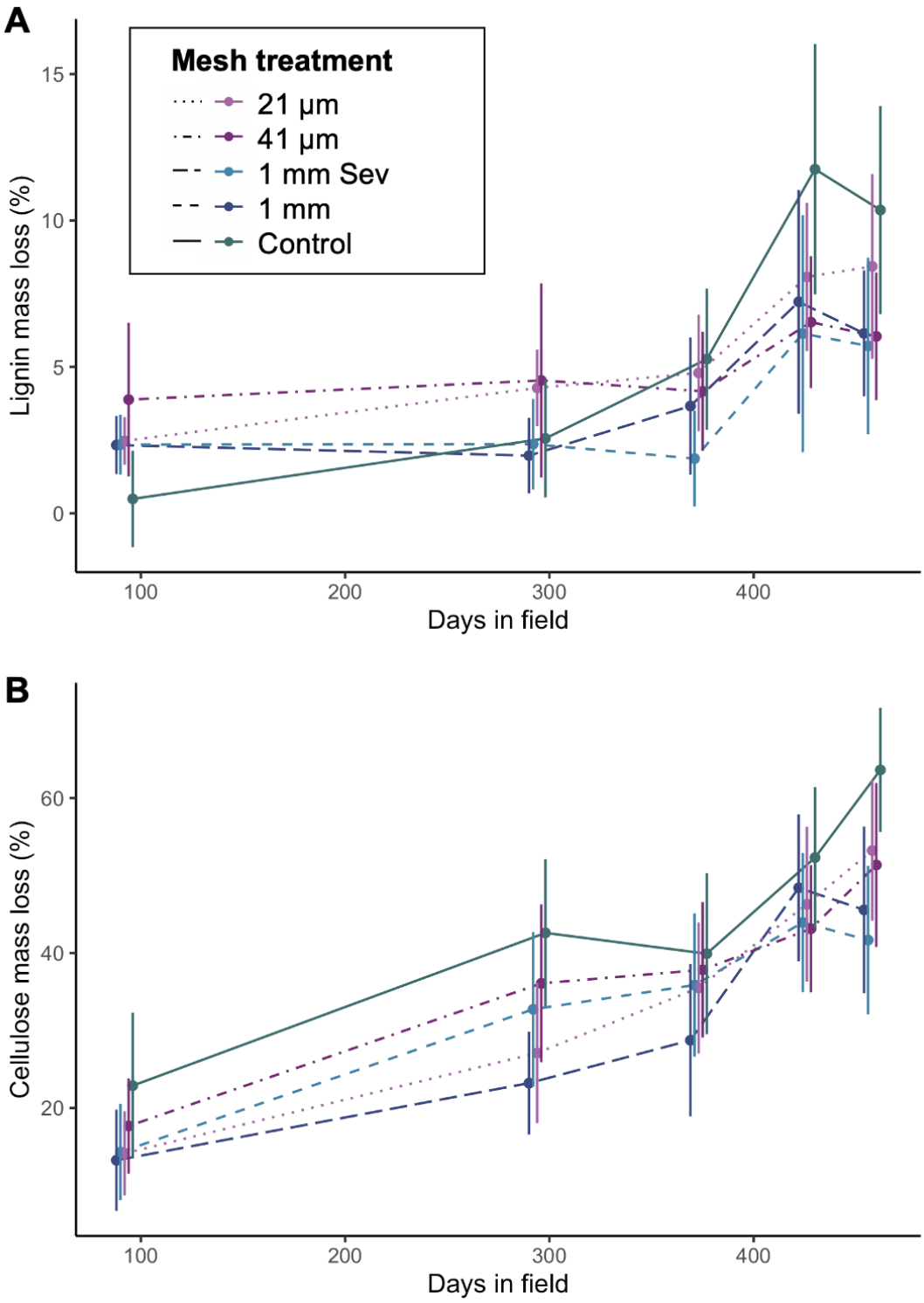

Figure S7. Decomposition of (**A)** balsa wood (predominantly lignin) and (B) museum board (predominantly cellulose) in untreated control by mesocosm mesh treatment. Error bars represent 95% confidence intervals.

Table S3. Results from selected analysis of covariance models for decomposition of balsa wood and museum board disks in mesocosms at all trees, untreated control unit trees only, and thinned/burned (TB) unit trees only, run with mesocosm controls included. Note that unsevered 1 mm mesh mesocosms are the reference group for models including mesh size.

Balsa wood (predominantly lignin)

logit (proportion remaining)

All trees

	Estimate	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	2.1548	0.06076	35.463	< 2e-16 ***
Timepoint	-0.11373	0.01299	-8.752	< 2e-16 ***
Restoration treatment: TB	0.16719	0.03793	4.408	1.25e-05 ***
1 mm mesh severed	0.0891	0.0602	1.48	0.139
21 µm mesh	-0.07891	0.06827	-1.156	0.248
41 µm mesh	-0.01009	0.06539	-0.154	0.877
Control	-0.08631	0.05889	-1.466	0.143
Soil moisture *(average Z score)*	-0.04479	0.0313	-1.431	0.153
			Residual standard error	0.4373
			Adjusted R^2	0.1625
			***F* (d.f.=)**	16.66 (7, 558)
			Model *P*	< 2.2e-16***

Untreated control trees only

	Estimate	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	2.1073	0.05999	35.129	< 2e-16 ***
Timepoint	-0.10202	0.01822	-5.6	5.01e-08 ***
Soil moisture *(average Z score)*	-0.07343	0.0296	-2.481	0.0137 *
			Residual standard error	0.4371
			Adjusted R^2	0.109
			***F* (d.f.=)**	18.69 (2, 287)
			Model *P*	2.35E-08***

Thinned/burned trees only

	Estimate	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	2.44304	0.08767	27.866	< 2e-16 ***
Timepoint	-0.12647	0.01833	-6.9	3.72e-11 ***

1 mm mesh severed	0.08031	0.08522	0.942	0.34682
21 µm mesh	-0.26289	0.0928	-2.833	0.00496 **
41 µm mesh	-0.16501	0.09812	-1.682	0.09379 .
Control	-0.04931	0.08333	-0.592	0.55448
Soil moisture *(average Z score)*	0.09337	0.06108	1.529	0.12754
			Residual standard error	0.4324
			Adjusted R^2	0.1784
			***F* (d.f.=)**	10.95 (6, 269)
			Model *P*	6.52E-11***

Museum board (predominantly lignin)

logit (proportion remaining)

All trees

	Estimate	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	1.03566	0.27481	3.769	0.000181 ***
Timepoint	-0.44846	0.03224	-13.909	< 2e-16 ***
Restoration treatment: TB	0.32894	0.09595	3.428	0.000651 ***
Soil moisture *(average Z score)*	-0.30319	0.06289	-4.821	1.82e-06 ***
log % **Soil organic matter**	0.46488	0.14448	3.218	0.001365 **
			Residual standard error	
			Adjusted R^2	0.2929
			***F* (d.f.=)**	61.46 (4 and 580)
			Model *P*	< 2.2e-16***

Untreated control trees only

	Estimate	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	1.87849	0.15694	11.969	< 2e-16 ***
Timepoint	-0.46013	0.04739	-9.709	< 2e-16 ***
Soil moisture *(average Z score)*	-0.36824	0.07632	-4.825	2.25e-06 ***
			Residual standard error	
			Adjusted R^2	0.2788
			***F* (d.f.=)**	58.42 (2, 295)
			Model *P*	< 2.2e-16***

Thinned/burned trees only

	Estimate	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	1.13764	0.39395	2.888	0.00418 **
Timepoint	-0.43646	0.04331	-10.079	< 2e-16 ***
1 mm mesh severed	0.2242	0.20104	1.115	0.26571
21 µm mesh	0.65247	0.22076	2.956	0.00339 **
41 µm mesh	0.30865	0.23216	1.329	0.18478
Control	-0.14396	0.19522	-0.737	0.46147
Soil moisture (average Z score)	-0.47777	0.14449	-3.307	0.00107 **
log % **Soil organic matter**	0.43954	0.21052	2.088	0.03772 *
			Residual standard error	
			Adjusted R^2	0.2934
			***F* (d.f.=)**	17.96 (7, 279)
			Model *P*	< 2.2e-16***

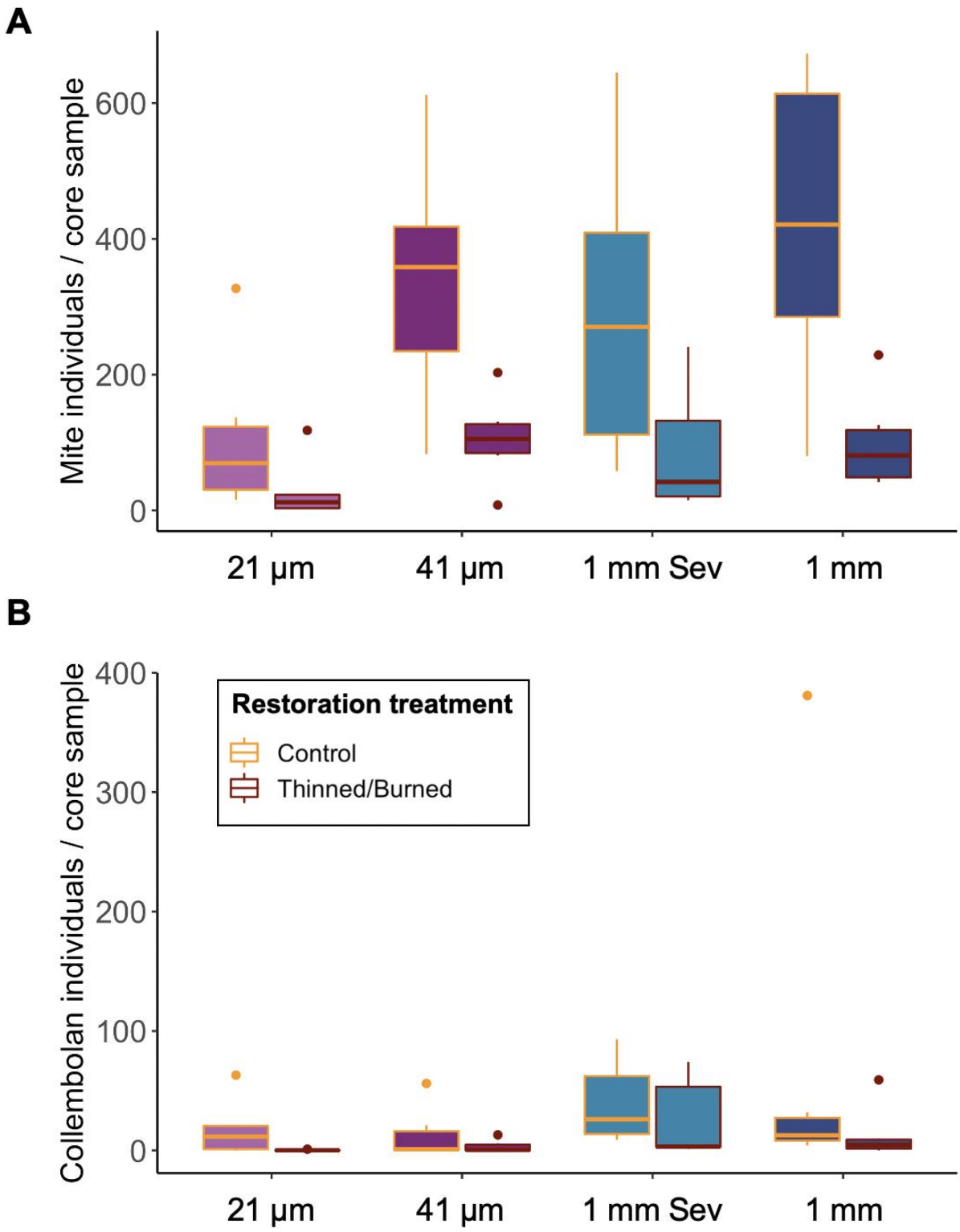

Figure S8. Abundances of mites (**A**) and collembolans (**B**) in sacrificial mesocosms sampled at T1 and T3. Data from both timepoints are pooled in these figures. N=3 mesocosms per restoration treatment per timepoint.