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Soil Macroinvertebrates Responses to Wildfires in the Blue Ridge Mountains, USA

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SOIL MACROINVERTEBRATE RESPONSES TO WILDFIRES IN THE BLUE RIDGE MOUNTAINS, USA

by

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We hereby approve the thesis of

Soil Macroinvertebrate Responses to Wildfires in the Blue Ridge Mountains, USA

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Abstract

Fire has been a prevalent disturbance on Earth for millions of years. Around the globe there are several regions that have become fire adapted, including the Southeastern United States. There have been few studies examining the effects of wildland fires on soil macroinvertebrates in the Blue Ridge Mountains, in spite of the importance of these animals to soil processes and their contributions to the biodiversity of these ecosystems. During the fall of 2016, the Southeastern USA experienced numerous, large wildfires. These fires offered an opportunity to study the effects of wildland fire on soil macroinvertebrates. We sampled sites from three different wildfires in North Georgia and Tennessee, each site with five burned plots and five unburned plots. These sites were sampled seasonally from fall 2017 through fall 2019. At each plot, on each date, we collected macroinvertebrates by hand sorting both litter (4 m diameter plots) and mineral soil monoliths (30 x 30 x 30 cm) for 30 person-minutes each. All macroinvertebrates were identified to a coarse taxonomic level. One focal taxon, millipedes, were identified to species. We ran three-factor ANOVAs using burn status (burned vs. unburned), site, sampling date, and all the interaction terms as factors and soil fauna richness, soil fauna abundance, litter fauna richness, and litter fauna abundance as dependent variables. We analyzed millipede and macroinvertebrate datasets separately. Because sampling date was a significant main effect, we wanted to determine if it was truly the sampling date or just seasonal differences the fauna experiences. The factors for this set of ANOVAs were site, burn status, and season. Of the sixteen ANOVAs conducted, there was only one where there was a significant difference between the burned and unburned plots as a main effect. Specifically, the mean abundance of leaf litter-dwelling macroinvertebrates was higher in unburned plots than burned plots. However, in almost every ANOVA, time and site had a significant effect on abundance

and richness. Pre-fire drought conditions may have masked the effects of the fire by driving the fauna deeper into the soil, thus protecting them from the effects of the fire. Given that the taxonomic resolution for these data was coarse, there could have been responses to the fire by individual taxa. Because soil fauna was not affected by the fire, forest managers may not need to account for adverse effects of fire on soil fauna when planning for prescribed fire.

Soil Macroinvertebrate Responses to Wildfires in the Blue Ridge Mountains, USA

Introduction

Fire has been around for about 420 million years and is categorized as one of the most widespread disturbances globally that influences biological evolution and biogeochemical cycles (Abrantes 2019, Pyne 2019). Fire-adapted ecosystems - such as longleaf pine forests, grassland savannas, tallgrass prairies, and chaparrals - rely on regular fire regimes, the pattern of fire required for these ecosystems, to function normally (Brown 2000). The aspects of fire that have the biggest effects on ecosystems are intensity and severity (He et al. 2019). Intensity is measured by the heat released per unit of time (Pyne et al. 1996a) and severity focuses on the damage fire causes to the vegetation and the soil (Keeley 2009) including development of hydrophobic conditions, increased risk of erosion, and changes in soil aggregate structure (Callaham et al. 2012). Severity is typically categorized as low, moderate, or high depending on the damage to the ecosystem. The two factors are not correlated; a fire can be classified as low intensity but high severity.

Fires can have direct and indirect effects on ecosystems. Direct effects largely encompass direct mortality and injury to organisms, usually a result of their exposure to heat because most animals are unable to tolerate temperatures over 50°C (Abrantes 2019). Organisms with limited mobility are the most affected by this due to their inability to escape the heat. These direct effects typically affect the leaf litter-dwelling fauna in the O-horizon (Kauf et al. 2018). Because soil is an excellent insulator, soil-dwelling taxa such as earthworms that are able to burrow deeper into the ground are able to escape the direct impact of the fire (Iverson et al. 2002).

Indirect effects can include biological, chemical, or physical changes in soil characteristics that influence soil fauna and plant response (Callaham et al. 2012). For soil fauna specifically, one of the main indirect effects of fire is the change in habitat and food resources (Abrantes 2019) because fires remove a large portion of the upper organic horizon of the soil which is essential to many macroinvertebrate species (Coyle et al. 2017, Buckingham et al. 2019, Gongalsky et al. 2013). About 90% of terrestrial arthropods spend at least a portion of their life cycle in the surface litter layer or in the soil (Klein et al. 1988) but the overall effects of fire on macroinvertebrates varies wildly. For example, some studies show that fire can reduce abundance and richness (Buckingham et al. 2015, Buckingham et al. 2019, Gongalsky and Persson 2013, Vasconcelos et al. 2009, Lisa et al. 2015) and diversity and evenness (Gorbunova et al. 2017, Hanula and Wade 2003) of soil fauna in various ecosystems. However, other studies have shown that fire may increase abundance and diversity for some taxa (Elia et al. 2011, Moretti et al. 2006) or have little to no effect on the fauna (Andersen and Muller 2000, Trucchi et al. 2009).

There has been little research on soil fauna responses to wildfires in deciduous forests of the eastern United States though there have been some studies done after prescribed fires (e.g., Kalisz and Powell 2000, Coleman and Rieske 2006). Coleman and Rieske (2006) found that prescribed burns did not affect ground-dwelling arthropod richness and abundance, or ground-dwelling and leaf-litter arthropod diversity. Kalisz and Powell (2000) found that fire significantly reduced juvenile and adult stages of Coleoptera.

Fire History in the Southeast US

The mixed-oak forests of the southern Appalachians have experienced various different fire regimes over the past 4,000 years. Historically the fire regime consisted of frequent low-intensity fire (Van Lear 1989), which allowed oaks, pines, and chestnut to become dominant in these forest stands (Brose et al. 2001). It was not until the mid-1800s and the boom of the Industrial Revolution and the logging industry that the fire regime changed to frequent high-intensity fires. This caused changes to the forest structure: mainly conifers were unable to grow in these conditions. There were several massive wildfires that occurred in the early 1900s which caused a nationwide attitude towards fire suppression (Brose et al. 2001).

This attitude of fire suppression has had serious consequences for fire-adapted ecosystems. For example, forests have become denser which has led to shade-tolerant shrubs filling the understories. Such changes alter fire behavior due to increased diversity and abundance of fuel sources in the form of accumulation of leaf litter and organic matter (Ryan 2013). As a consequence, fires have increased in intensity (Brose et al. 2001). This accumulation of organic matter also damages the plant community structure; fine roots that are extremely vulnerable to fire will colonize and grow in the deep organic layer and are not able to penetrate deeper into the mineral soil. When these fine roots are damaged by fire this leads to delayed tree mortality, whereas under a normal fire regime these trees would be able to survive the effects of the fire (Carpenter et al. 2020).

The 2016 Wildland Fires

During the fall of 2016, numerous large wildfires burned across the Appalachian landscapes, mainly affecting northern Georgia, eastern Tennessee, and western North Carolina. In Tennessee, 2,121 residences and 53 commercial structures were burned (NICC 2016). One of the major fires in Tennessee was the Chimney Tops 2 fire which burned 17,140 acres in the Great Smoky Mountains National Park and into Gatlinburg, Tennessee (James et al. 2020). The weather conditions that preceded contributed to the severity of these fires. The southern

Appalachian Mountains were in an extreme to exceptional drought October through December (United States Drought Monitor 2021) and the total precipitation for September and October 2016 at the Coweeta Hydrologic Laboratory, in western North Carolina, was 2.4 cm, the driest fall recorded for the laboratory's 84-year history (Miniat et al. 2018).

Goals

Our goal was to determine whether these three wildfires affected soil fauna communities. Specifically, we examined whether abundance and richness of leaf litter-dwelling and soil-dwelling macroinvertebrates differed between burned and unburned plots at three locations affected by the 2016 wildfires in north Georgia and Tennessee.

Methods

Study Sites

We studied three sites affected by wildfires in Georgia and Tennessee (Table 1, Figure 1). The Chimney Tops 2 fire occurred near Gatlinburg, Tennessee. The dominant soil type in this area is Spivey-Santeetlah-Nowhere complex (Web Soil Survey 2021). The Spivey Series is classified as loamy-skeletal, isotic, mesic Humic Dystrudepts (USDA 2009). The second site was located near Dillard, Georgia at the Rock Mountain fire. The dominant soil type at this site is Ashe-Porters association and Porters association (Web Soil Survey 2021). These soils are coarse-loamy, mixed, active, and mesic Typic Dystrudepts (National Cooperative Soil Survey 2001). The Rough Ridge fire occurred in northwestern Georgia, located east of Crandall, Georgia. The dominant soil types at this field location are Cheoah-Edneytown complex and Edneytown loam (Web Soil Survey 2021). These soils are fine-loamy, mixed, active, and mesic Typic Hapludults (National Cooperative Soil Survey 2002).

Experimental Design and Collection Methods

At each site, we collected samples from ten plots, five in areas that were burned and five in areas that were unburned. We marked plot centers with a nail and spaced them thirty meters apart. We sampled once at each site during each of six time periods: Fall 2017, Spring 2018, Summer 2018, Fall 2018, Spring 2019, and Fall 2019. We were unable to sample soil during the spring and fall of 2019 in the Great Smoky Mountains National Park due to permit restrictions.

We sampled leaf litter-dwelling macroinvertebrates using hand collection over thirty person-minutes to ensure equal effort at each plot (Snyder et al. 2006) within a 2m radius of each plot center. Soil-dwelling invertebrates were hand-sorted for thirty-person minutes (Schmidt 2001) from a 30x30x30cm (2700 cm^3) soil monolith dug from a haphazardly chosen location just outside the hand collection plot. All specimens collected were preserved in 70% ethanol for long-term storage.

The invertebrates were hand-sorted and identified with a LEICA M80 dissecting microscope outfitted with a LINITRON DC12V 400mA ring light. All invertebrates were identified to a coarse taxonomic level (Table 2). Millipedes were selected as a focal taxon for this project since they are one of the most diverse groups of terrestrial invertebrates (Sierwald and Bond 2007). Additionally, the Appalachian Mountains is a biodiversity hotspot for millipedes (Means 2019). All millipedes were identified to the lowest taxonomic level possible using dichotomous keys (Hoffman 1990, Shear 1966, Shear 1999). See Appendix A for a full list of primary literature used for millipede identification.

Statistics

We ran three-factor ANOVAs using burn status (burned vs. unburned), site (Chimney Tops 2 fire, Rock Mountain fire, Rough Ridge fire), sampling date (Fall 2017, Spring 2018, Summer 2018, Fall 2018, Spring 2019, Fall 2019), and all the interaction terms as factors. Soil fauna richness, soil fauna abundance, litter fauna richness, and litter fauna abundance were the dependent variables. We analyzed millipede and macroinvertebrate datasets separately. We defined abundance as the number of individuals. Richness was defined as the number of coarse taxonomic groups for macroinvertebrates; for millipedes, richness was the number of species. Post-hoc Tukey's HSD tests were conducted when appropriate. All analyses were performed in JMP (SAS, Version 16.0.0).

Because there were significant differences in abundance and richness metrics for sampling date across leaf litter-dwelling and soil-dwelling macroinvertebrates as well as millipedes collected from the litter and soil, we opted to run an additional set of three-factor ANOVAs. The factors for this set of ANOVAs were burn status (burned vs. unburned), site (Chimney Tops 2 fire, Rock Mountain fire, Rough Ridge fire), and season (all years combined into Fall, Spring, Summer). We chose season as a factor to assess if the significant differences were due to the changes since the time the fire had occurred or were just due to seasonal differences the fauna experiences.

Results

Macroinvertebrate Data

A total of 5,518 invertebrate specimens were collected representing eight invertebrate classes. Of that total, 3,281 leaf litter-dwelling invertebrates and 2,237 soil-dwelling invertebrates were collected. When broken down by site, a total of 1,689 invertebrates were collected at our site from the Chimney Tops 2 fire, 2,193 at the Rock Mountain fire site, and 1,636 at the Rough Ridge fire site.

Leaf Litter-dwelling Invertebrates

The three-factor ANOVA conducted to examine the effect of site, burn status, and sampling date found significant differences in the abundance of leaf litter-dwelling macroinvertebrates (F=6.43, P<0.0001, Table 3, Figure 2). There was no significant three-way interaction (F=0.97, P=0.4737, Table 3). There was a significant two-way interaction between site and sampling date (F=4.08, P<0.0001, Table 3). There was a main effect that had a significant p-value, sampling date, (F=25.74, P<0.0001, Table 3, Figure 3). There was no significant difference in abundance of leaf litter-dwelling macroinvertebrates between the burned and unburned plots (F=0.19, P=0.6647, Table 3).

The three-factor ANOVA conducted to examine the effect of site, burn status, and collection season found significant differences in the abundance of leaf litter-dwelling macroinvertebrates (F=3.15, P<0.0001, Table 4). There was no significant three-way interaction (F=0.53, P=0.7169, Table 4). There were no significant two-way interactions. Each main effect had a significant p-value: site (F=7.12, P=0.001, Table 4, Figure 4), burn status (F=5.43, P=0.021, Table 4, Figure 5), and season (F=9.57, P=0.0001, Table 4, Figure 6).

The three-factor ANOVA conducted to examine the effect of site, burn status, and sampling date found significant differences in the richness of leaf litter-dwelling macroinvertebrates (F=6.95, P<0.0001, Table 3, Figure 2). There was no significant three-way interaction (F=1.05, P=0.4025, Table 3). There was a significant two-way interaction of site and sampling date (F=7.14, P<0.0001, Table 3). There were two main effects that had significant p-values: site (F=21.34, P<0.0001, Table 3, Figure 7) and sampling date (F=23.80, P<0.0001, Table 3, Figure 3). There was no significant difference in richness of leaf litter-dwelling macroinvertebrates between burned and unburned plots (F=0.24, P=0.6286, Table 3).

The three-factor ANOVA conducted to examine the effect of site, burn status, and collection season found significant differences in the richness of leaf litter-dwelling macroinvertebrates (F=2.92, P= 0.0002, Table 4). There was no significant three-way interaction (F=0.43, P=0.7872, Table 4). There were no significant two-way interactions. There were two main effects that had significant p-values: site (F=8.88, P=0.0002, Table 4, Figure 4) and season (F=10.66, P<0.0001, Table 4, Figure 6). There was no significant difference in richness between burned and unburned plots (F=0.09, P=0.7663, Table 4).

Soil-dwelling Invertebrates

The three-factor ANOVA conducted to examine the effect of site, burn status, and sampling date found significant differences in the abundance of soil-dwelling macroinvertebrates (F=3.55, P<0.0001, Table 3, Figure 8). There was no significant three-way interaction (F=1.65, P=0.1183, Table 3). There was a significant two-way interaction of site and sampling date (F=4.66, P<0.0001, Table 3). The main effect of sampling date had a significant p-value (F=17.82, P<0.0001, Table 3, Figure 9). There was no significant difference in abundance between the burned and unburned plots (F=0.003, P=0.9583, Table 3).

The three-factor ANOVA conducted to examine the effect of site, burn status, and collection season did not find significant differences in the abundance of soil-dwelling macroinvertebrates (F=0.69, P=0.8, Table 4).

The three-factor ANOVA conducted to examine the effect of site, burn status, and sampling date fire found significant differences in the richness of soil-dwelling macroinvertebrates (F=4.88, P<0.0001, Table 3, Figure 8). There was no significant three-way interaction (F=0.43, P=0.7872, Table 3). There was a significant two-way interaction of site and sampling date (F=5.38, P<0.0001, Table 3). Two of the main effects had significant p-values:

site (F=8.88, P=0.0002, Table 3, Figure 10) and sampling date (F=19.56, P<0.0001, Table 3, Figure 9). There was no significant difference in richness between the burned and unburned plots (F=0.10, P=0.7503, Table 3).

The three-factor ANOVA conducted to examine the effect of site, burn status, and collection season did not find significant differences in the richness of soil-dwelling macroinvertebrates (F=1.36, P=0.1628, Table 4).

Millipede Data

A total of 1,511 millipedes were collected, representing 7 orders and 12 families. From the two different collection methods, 246 millipedes were collected from the soil and 1,265 millipedes were collected from the litter.

When compiling species lists (Appendix B) there were some noticeable trends and differences at each site. At the Chimney Tops 2 fire, there were several species only collected from burned plots. These species were *Uroblaniulus* sp., *Pseudopolydesmus canadensis*, and *Scytonotus* sp. There was one family that was only found at this site, Zosteractinidae. In the family Zosteractinidae, we found two specimens in the genus *Ameractis*. One specimen we were able to identify to species, *Ameractis chirogona*. *Pseudopolydesmus canadensis* was also only found at this site; both specimens were found in burned plots in fall 2018. There were also two species for which we only found one representative, each, from this site (*Narceus americanus-annularis* complex and *Cambala* sp.). There were several species only found at Rock Mountain as well. We found one millipede in the genus Trichopetalidae and we found several millipedes from the species *Erdelyia saucra*.

At the Rough Ridge site, there were four species found only at this site: *Cleidogona major*, *C. inexpectata*, *Apheloria montana*., and a singular specimen of *Brachoria initialis*. All of the sites had representatives from the genus *Cambala*, however Rough Ridge was the only site to have adult males that we were able to identify to species, *Cambala annulata.* The Rough Ridge site had the most *N. americana-annularis* complex specimens, over 60 collected over the course of the study.

Litter-Dwelling Millipedes

The three-factor ANOVA conducted to examine the effect of site, burn status, and sampling date found significant differences in the abundance of millipedes collected from the leaf litter (F=10.56, P <0.0001, Table 5, Figure 11). There was a significant three-way interaction (F=1.95, P=0.0437, Table 5). There was a significant two-way interaction of site and sampling date (F=7.49, P<0.0001, Table 5). One of the main effects, sampling date, had a significant p-value (F=36.07, P<0.0001, Table 5, Figure 12). There was no significant difference in abundance of millipedes collected from the leaf litter between burned and unburned plots (F=0/65, P=0.4198, Table 5).

The three-factor ANOVA conducted to examine the effect of site, burn status, and season found significant differences in the abundance of millipedes collected from the leaf litter (F=5.71, P<0.0001, Table 6). There was no significant three-way interaction (F=1.84, P=0.1237, Table 6). There was a significant two-way interaction between site and season (F=3.51, P=0.0089, Table 6). There was a significant two-way interaction between site and burn status (F=4.06, P=0.0191, Table 6). Two of the main effects had significant p-values: site (F=20.36, P<0.0001, Table 6, Figure 13) and season (F=14.27, P<0.0001, Table 6, Figure 14). There was no significant difference in abundance of millipedes collected from the leaf litter between burned and unburned plots (F=0.44, P=0.5101, Table 6).

The three-factor ANOVA conducted to examine the effect of site, burn status, and sampling date found significant differences in the richness of millipedes collected from the leaf litter (F=4.67, P<0.0001, Table 5, Figure 11). There was no significant three-way interaction (F=1.46, P=0.1596, Table 5). Each two-way interaction was significant: site and burn status (F=7.31, P=0.0009, Table 5); site and sampling date (F=3.29, P=0.0007, Table 5); and burn status and sampling date (F=2.32, P=0.0463, Table 5). One of the main effects, sampling date, had a significant p-value (F=12.97, P<0.0001, Table 5, Figure 12); There was no significant difference between richness of millipedes collected from the leaf litter between burned and unburned plots (F=3.42, P=0.0666, Table 5).

The three-factor ANOVA conducted to examine the effect of site, burn status, and season found significant differences in the richness of millipedes collected from the leaf litter (F=4.31, P<0.0001, Table 6). There was no significant three-way interaction (F=0.69, P=0.5977, Table 6). There was a significant two-way interaction between site and season (F=3.76, P=0.0059, Table 6). Two of the main effects had significant p-values: site (F=9.45, P=0.0001, Table 6, Figure 13) and season (F=9.93, P<0.0001, Table 6, Figure 14). There was no significant difference in richness of millipedes collected from the leaf litter between burned and unburned plots (F=1.51, P=0.2204, Table 6).

Soil-Dwelling Millipedes

The three-factor ANOVA conducted to examine the effect of site, burn status, and sampling date did not find significant differences in the abundance of millipedes collected from the soil (F=1.46, P=0.0758, Table 5, Figure 15). The three-factor ANOVA conducted to examine the effect of site, burn status, and season did not find significant differences in the abundance of millipedes collected from the soil (F=1.04, P=0.4178, Table 6). The three-factor ANOVA conducted to examine the effect of site, burn status, and sampling date found significant differences in the richness of millipedes collected from the soil (F=1.87, P =0.0082, Table 5, Figure 15). There were no significant three-way or two-way interactions. The main effects did not have significant p-values. The three-factor ANOVA conducted to examine the effect of site, burn status, and season did find significant differences in the richness of millipedes collected from the soil (F=1.8648, P=0.0257, Table 6). There were no significant three-way or two-way interactions. The main effect, site, had a significant p-value (F=5.3, P=0.006, Table 6). There was no significant difference in richness of millipedes collected from the soil between the burned and unburned plots (F=2.24, P=0.1371, Table 6).

Discussion

Across the sixteen ANOVAs in our analysis, there was only one instance of a significant difference between burned and unburned plots. Specifically, the abundance of leaf litter-dwelling macroinvertebrates was higher in the unburned plots than the burned plots. This one instance follows the findings of previous studies, but overall our results contradict previous findings where fire reduced abundance and richness of the fauna (Buckingham et al. 2015, Buckingham et al. 2019, Gongalsky and Persson 2013, Vasconcelos et al. 2009, Lisa et al. 2015).

Although most of the areas where we sampled were classified as low severity burns, the organic layer was completely combusted. For example, our burned plots from the Rock Mountain fire had exposed mineral soil and there was no leaf litter layer present (Mac Callaham Jr., pers. comm.). The pre-fire drought likely contributed to the incineration of the leaf litter layer. Under normal conditions, rain and moisture will compact the leaf litter layer; the drought at the time of leaf fall led to air spaces between these fine fuels, which often leads to intense fires and more complete combustion (Pyne et al. 1996b). We anticipated that since the fire caused such complete removal of the leaf litter layer, this would cascade to an effect on the fauna.

Pre-fire conditions likely played an important role in contributing to the lack of difference in abundance and richness of leaf litter-dwelling and soil-dwelling invertebrates between burned and unburned plots. The severe drought pre-fire, which also consequently led to the fire conditions, potentially could have driven soil-dwelling fauna, such as earthworms, deeper into the soil profile to seek out optimal moisture conditions (Florian et al. 2019, Fraser et al. 2012). This would have also protected the fauna against the heat effects of the fire that could have caused direct mortality. This severe drought would have affected the leaf litter-dwelling fauna as well: species that primarily reside in the leaf litter are often capable of burrowing into the soil when conditions are not ideal (Blair et al. 1997). Although there have been no studies to date examining the combined effects of wildfire and drought on terrestrial invertebrates, Verkaik et al. (2013) conducted research on how seasonal droughts and wildfire shape macroinvertebrate communities in streams: there were no significant differences in abundance of the taxa between their control/unburned catchments and the burned catchments. They also found that drought was an overriding disturbance factor when determining the response of fauna to large-scale disturbances like wildfires because the fauna responded more to the previous spring's precipitation than the fire.

It is also possible that that fire did have an effect on our fauna, but we were unable to detect a difference in richness or abundance. First, weather factors on the day of collection such as heat, humidity, and precipitation could have an effect on the fauna's activity level (Johnson 2007). Precipitation in the days leading up to the collection date would have had an effect on soil moisture levels. Martay and Pearce-Higgins (2018) found that there was a positive correlation between earthworm abundance and soil moisture. Second, another drought during the sampling period could have confounded the results. In the Fall of 2019, North Georgia and Tennessee did experience a drought, although not as severe as the drought in 2016 (United States Drought Monitor 2021). This could have contributed to the lower abundance and richness when compared to the other collection dates. Last, because the Blue Ridge Mountains contain fire adapted ecosystems, there could have been a rapid response of the soil fauna that we missed since we started sampling a year after the fire occurred (Brose et al. 2001). For future research on wildfires, we recommend that collection start relatively quickly after the fire has passed over an area since the fauna may respond within the first few months after the fire.

Additionally, we did not look at how fire affected individual taxa. Fire may have had an effect on the macroinvertebrate fauna at a finer scale as has been shown for Coleoptera, Lepidoptera, Hymenoptera, and Aranea (Kalisz and Powell 2000, Elia et al. 2011, Moretti et al. 2006). For many taxonomic groups collected in our study, life history and phenology are not known. This may include information such as breeding season, dormant season, and what time of day the fauna is most active. It is important to keep this in mind when sampling for soil macroinvertebrates to ensure that you are sampling at different times throughout the year to get a more accurate measurement of true abundance and richness metrics of the fauna. This adds a challenge to interpreting the results of wildfire studies. There is still much information regarding soil fauna that needs to be uncovered.

An important caveat to our study is that we do not have pre-fire data, so it is possible that we did not detect difference in abundance and richness between the burned and unburned plots because the range of values for abundance and richness are typical for these sites. Wildland fires are challenging to study, in part because you cannot predict when they will occur. Because of this unpredictability, it is much more logistically feasible obtain pre-fire data for a prescribed fire than a wildfire.

Given the differences in soil, dominant trees, topography, and geologic history, the differences in abundance and richness across sites was expected. The differences in abundance and richness during the various collection seasons was expected as well (Johnson 2007). We anticipated these differences due to differences in weather patterns and behavior of the fauna during these seasons which is consistent with other studies (Silveriera et al. 2010, Auclerc et al. 2019).

In the past few decades there has been increased interest in reintroducing fire as a means of forest management. Prescribed fire helps the landscape, especially in the Appalachians, as hazard-reduction by reducing flammable fuel, controlling understory hardwood growth, regenerating pine growth, and regenerating hardwood trees (Van Lear 1989). The southern US is taking the lead in utilizing prescribed fire for land management (Ryan et al. 2013). Georgians prescribe burn about 1.4 million acres (566,560 hectares) per year (Georgia Forestry Commission 2021). Prescribed fire has also been reintroduced in the grasslands of the American Midwest for grazing and prairie restoration (Ricketts and Sandercock 2016, Hill et al. 2017). Because soil fauna in the Blue Ridge Mountains were not affected by wildland fires in our study, forest managers may not need to account for these communities when creating burn plans for prescribed fire in this region. Soil macroinvertebrates are already infrequently considered by forest managers; however, management of the diverse soil fauna communities is important for conservation of biodiversity and because of the ecosystem services soils provide (Wall and Nielsen 2021, Decaëns et al. 2006). Additionally, there is a growing interest in using prescribed fire the combat invasive earthworm species (Ikeda et al. 2015, Blackmon et al. 2019) which would help to preserve the biodiversity of native soil fauna communities (Snyder et al. 2011).

There can be differences in fire effects between prescribed and wildland fire. Depending on the goal of the prescribed fire (e.g., fuel reduction, thinning), they are often low intensity and low severity. However, wildland fires can have a lot of small spatial scale, within-fire variability in intensity and severity. The three fires in our study were likely mostly low severity with areas of moderate and high severity throughout. Given the pre-fire drought conditions and the removal of abundant fuel sources that we observed, we believe the three fires would have been high intensity as well. If the soil fauna is not affected by the variable burn severity and high intensity within a wildland fire, we would expect that they would not be affected by low severity and low intensity planned burns (Coleman and Rieske 2006, Malmstrom et al. 2008). For future research, we would recommend measuring severity of the study area and taking measures of intensity during the fire. Fire intensity is rarely measured and is an important component to be measured in the field during the fire (O'Brien et al. 2016).

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Figure 1. Satellite image of the three collection sites. Site "#1" corresponds to the location of the Chimney Tops 2 fire in Gatlinburg, TN. Site "#2" corresponds to the location of the Rock Mountain fire in Dillard, GA. Site "#3" corresponds to the Rough Ridge fire located near Crandall, GA.

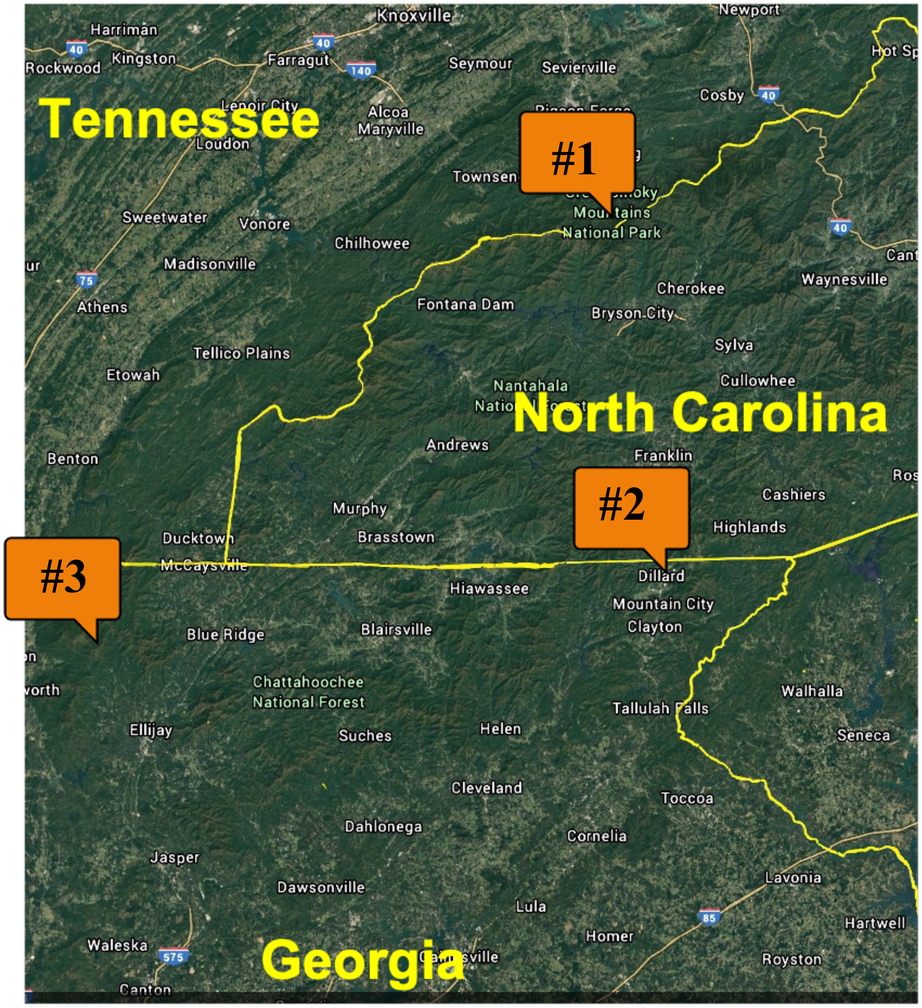


Table 1. Site description table depicting characteristics of each site

Name of the Fire	GPS Coordinates	Forest/Wilderness Area	Fire Start Date	Total Acreage Burned	Dominant Trees	Soil Order	Soil Series
Chimney Tops 2 fire	35°39’49.2” N 83° 31’17.4” W	Great Smoky Mountains National Park	13-Nov-16	17,140 acres	Hickory, Tulip Poplar, and Maple	Inceptisol	Spivey-Santeetlah-Nowhere complex
Rock Mountain fire	34°57'0" N, 83°34'12" W	Chattahoochee National Forest- Southern Nantahala Wilderness area	9-Nov-16	24,725 acres	Loblolly pine, Shortleaf pine, dry/dry-mesic Oak, and Hickory	Ultisol	Ashe-Porters association, moderately steep, and Porters association, stony, steep
Rough Ridge fire	34°51’59.0” N 84°38’37.7” W	Chattahoochee National Forest: Cohutta Wilderness area	16-Oct-16	27,870 acres	Oak, Tulip Poplar, Hemlock, Maple	Ultisol	Cheoah-Edneytown complex and Edneytown loam

Table 2. List of coarse taxonomic labeling used for identifying macroinvertebrates

Coarse Taxonomic Categories	**Class**	**Order**	**Family**
Ant	Insecta	Hymenoptera	Formicidae
Bee	Insecta	Hymenoptera	
Beetle	Insecta	Coleoptera	
Beetle Larva	Insecta	Coleoptera	
Caterpillar	Insecta	Lepitoptera	
Centipede	Chilopoda		
Cicada nymph	Insecta	Hemiptera	
Cockroach	Insecta	Blattodea	
Diplura	Entognatha	Diplura	
Earthworm	Clitellata	Opisthopora	
Fly	Insecta	Diptera	
Fly Larva	Insecta	Diptera	
Grasshopper	Insecta	Orthoptera	
Hornet	Insecta	Hymenoptera	
Insect	Insecta		
Millipede	Diplopoda		
Moth	Insecta	Lepidoptera	
Other Flying Insects	Insecta		
Rolli Polli	Malacostraca	Isopoda	
Slug	Gastropoda		
Snail	Gastropoda		
Spider	Arachnida	Araneae	
Termite	Insecta	Isoptera	
Tick	Arachnida	Ixodida	
Wasp	Insecta	Hymenoptera	
Yellow Jacket	Insecta	Hymenoptera	

Table 3. Three-factor analysis of variance combined table testing the difference between abundance and richness of leaf litter-dwelling and soil-dwelling macroinvertebrates. Factors were site, burn status, and sampling date. Significant P-values are bolded.

	Invertebrate Litter				Invertebrate Soil			
	Abundance		Richness		Abundance		Richness	
	F Ratio	P-Value	F Ratio	P-Value	F Ratio	P-Value	F Ratio	P-Value
Whole Model	6.4343	**<0.0001**	6.9463	**<0.0001**	3.5466	**<0.0001**	4.8813	**<0.0001**
Site	2.0691	0.13	21.3415	**<0.0001**	1.0305	0.3598	9.8644	**0.0001**
Burn Status	0.1886	0.6647	0.235	0.6286	0.0027	0.9583	0.1017	0.7503
Site*Burn Status	0.8175	0.4436	0.7197	0.4886	0.0338	0.9668	0.1017	0.9034
Sampling Date	25.7387	**<0.0001**	23.8047	**<0.0001**	17.821	**<0.0001**	19.5593	**<0.0001**
Site*Sampling Date	4.0801	**<0.0001**	7.1427	**<0.0001**	4.6644	**<0.0001**	5.3831	**<0.0001**
Burn Status*Sampling Date	1.4212	0.2201	1.7728	0.1221	0.3652	0.7783	0.1544	0.9267
Site*Burn Status* Sampling Date	0.9682	0.4737	1.0531	0.4025	0.3112	0.9606	1.6451	0.1183

Table 4. Three-factor analysis of variance combined table testing the difference in abundance and richness between leaf litter-dwelling and soil-dwelling macroinvertebrates. Factors were site, burn status, and season. Significant P-values are bolded.

	Invertebrate Litter				Invertebrate Soil			
	Abundance		Richness		Abundance		Richness	
	F Ratio	P-Value	F Ratio	P-Value	F Ratio	P-Value	F Ratio	P-Value
Whole Model	3.1507	**<0.0001**	2.9173	**0.0002**	0.6989	0.8	1.3648	0.1628
Site	7.1787	**0.001**	8.8763	**0.0002**	0.4899	0.6137	3.0536	0.0503
Burn Status	5.431	**0.021**	0.0887	0.7663	0.97	0.3264	0.0231	0.8794
Site*Burn Status	1.0712	0.345	0.4186	0.6587	1.0931	0.338	3.3299	**0.0386**
Season	9.5714	**0.0001**	10.6627	**<0.0001**	1.0225	0.3623	1.7444	0.1785
Site*Season	2.1083	0.0821	1.3621	0.2494	1.3661	0.2487	1.7609	0.14
Burn Status*Season	0.5793	0.5615	1.4571	0.2359	0.306	0.7369	0.0619	0.94
Site*Burn Status* Season	0.526	0.7168	0.4295	0.7872	0.2971	0.8794	1.2115	0.3086

Table 5. Three-factor analysis of variance combined table testing the difference in abundance and richness of millipedes collected from the leaf litter and the soil. Factors were site, burn status, and sampling date. Significant P-values are bolded.

	Millipede Litter				Millipede Soil			
	Abundance		Richness		Abundance		Richness	
	F Ratio	P-Value	F Ratio	P-Value	F Ratio	P-Value	F Ratio	P-Value
Whole Model	10.5617	**<0.0001**	4.6697	**<0.0001**	1.4578	0.0758	1.8721	**0.0082**
Site	0.5981	0.5512	0.8753	0.4189	1.2832	0.2807	1.5548	0.2151
Burn Status	0.6545	0.4198	3.4165	0.0666	0.0247	0.8754	0.1446	0.7043
Site*Burn Status	2.2575	0.1083	7.3129	**0.0009**	0.0247	0.9756	0.4701	0.626
Sampling Date	36.0719	**<0.0001**	12.9741	**<0.0001**	1.5973	0.1933	1.2655	0.2891
Site*Sampling Date	7.49	**<0.0001**	3.2894	**0.0007**	1.4257	0.1918	1.6136	0.127
Burn Status*Sampling Date	0.3728	0.8666	2.32	**0.0463**	2.169	0.0949	1.7959	0.1512
Site*Burn Status* Sampling Date	1.9451	**0.0437**	1.4616	0.1596	0.5022	0.8528	1.1458	0.3374

Table 6. Three-factor analysis of variance combined table testing the difference between abundance and richness of millipedes collected from the leaf litter and soil. Factors were site, burn status, and season. Significant P-values are bolded.

	Millipede Litter				Millipede Soil			
	Abundance		Richness		Abundance		Richness	
	F Ratio	P-Value	F Ratio	P-Value	F Ratio	P-Value	F Ratio	P-Value
Whole Model	5.7147	**<0.0001**	4.3091	**<0.0001**	1.0415	0.4178	1.8648	**0.0257**
Site	20.3651	**<0.0001**	9.4908	**0.0001**	2.434	0.0913	5.3016	**0.006**
Burn Status	0.4359	0.5101	1.5137	0.2204	3.2941	0.0716	2.2355	0.1371
Site*Burn Status	4.0577	**0.0191**	2.1305	0.1221	1.4484	0.2384	1.1949	0.3058
Season	14.2668	**<0.0001**	9.9332	**<0.0001**	0.158	0.854	0.0409	0.9599
Site*Season	3.5111	**0.0089**	3.7648	**0.0059**	0.4797	0.7506	1.2146	0.3073
Burn Status*Season	0.1248	0.8828	2.6809	0.0715	0.1755	0.8392	0.6529	0.5221
Site*Burn Status* Season	1.84	0.1237	0.6932	0.5977	0.9291	0.449	2.261	0.0655

Figure 2. Box plot of abundance and richness of leaf litter-dwelling macroinvertebrates depicting overall trends across site and sampling date. The three-factor ANOVA produced a significant model: abundance (F=6.4343, P<0.0001, Table 3) and richness (F=6.95, P<0.0001, Table 3).

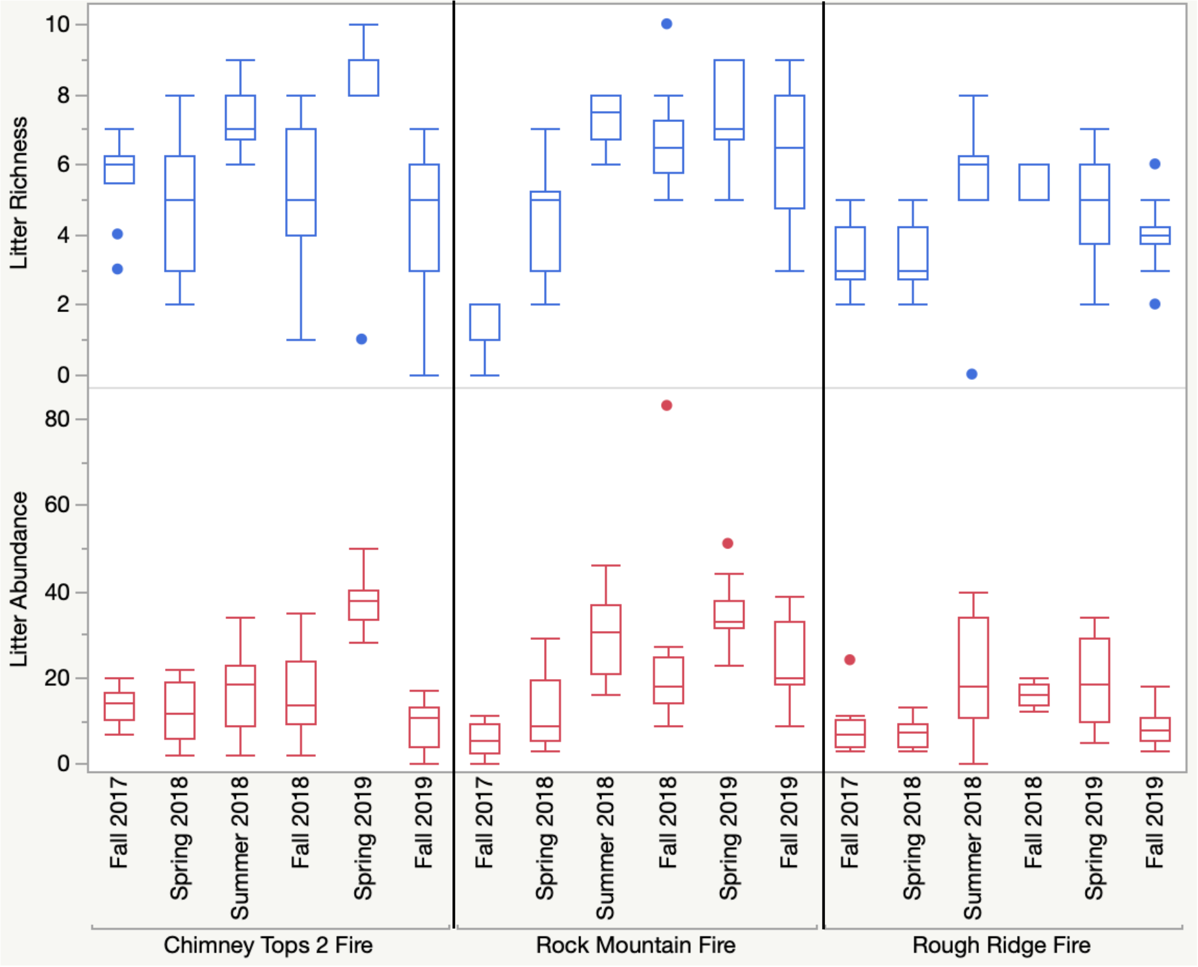


Figure 3. Box plot of abundance and richness of leaf litter-dwelling macroinvertebrates at the different sampling dates, the three field sites combined. Letters indicate the results of the post-hoc Tukey's HSD test. Different letters within a panel indicate significant differences in mean abundance or richness between different collection times since the fire had occurred.

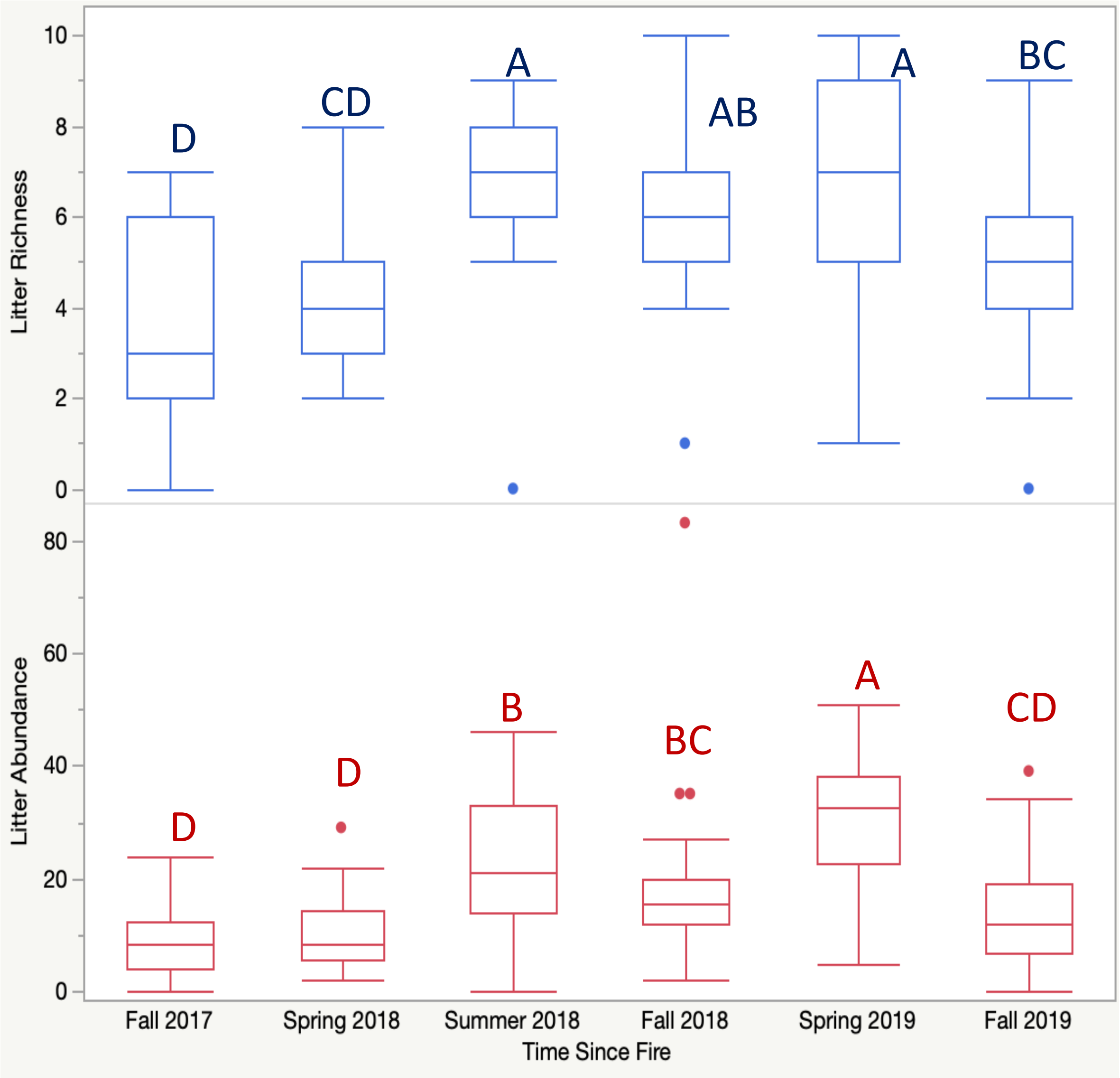


Figure 4. Box plot of abundance and richness of leaf litter-dwelling macroinvertebrates at the three different collection sites. Letters indicate the results of the post-hoc Tukey's HSD test. Different letters within a panel indicate significant differences in mean abundance or richness between different collection sites. The factors were site, burn status, and season.

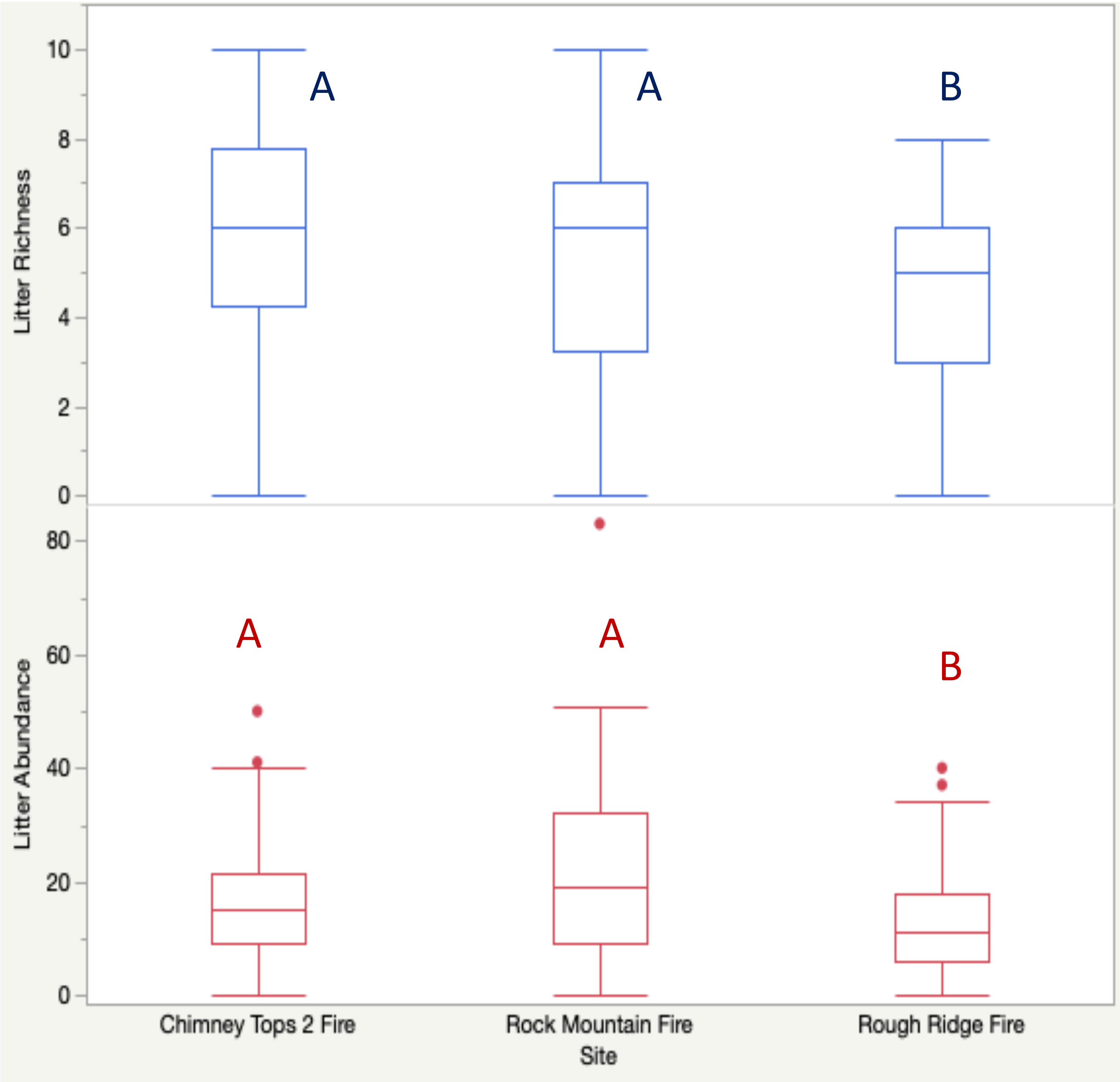


Figure 5. Box plot of abundance and richness of leaf litter-dwelling macroinvertebrates comparing burned and unburned plots. Different letters within a panel indicate significant differences in mean abundance or richness between burned and unburned plots.

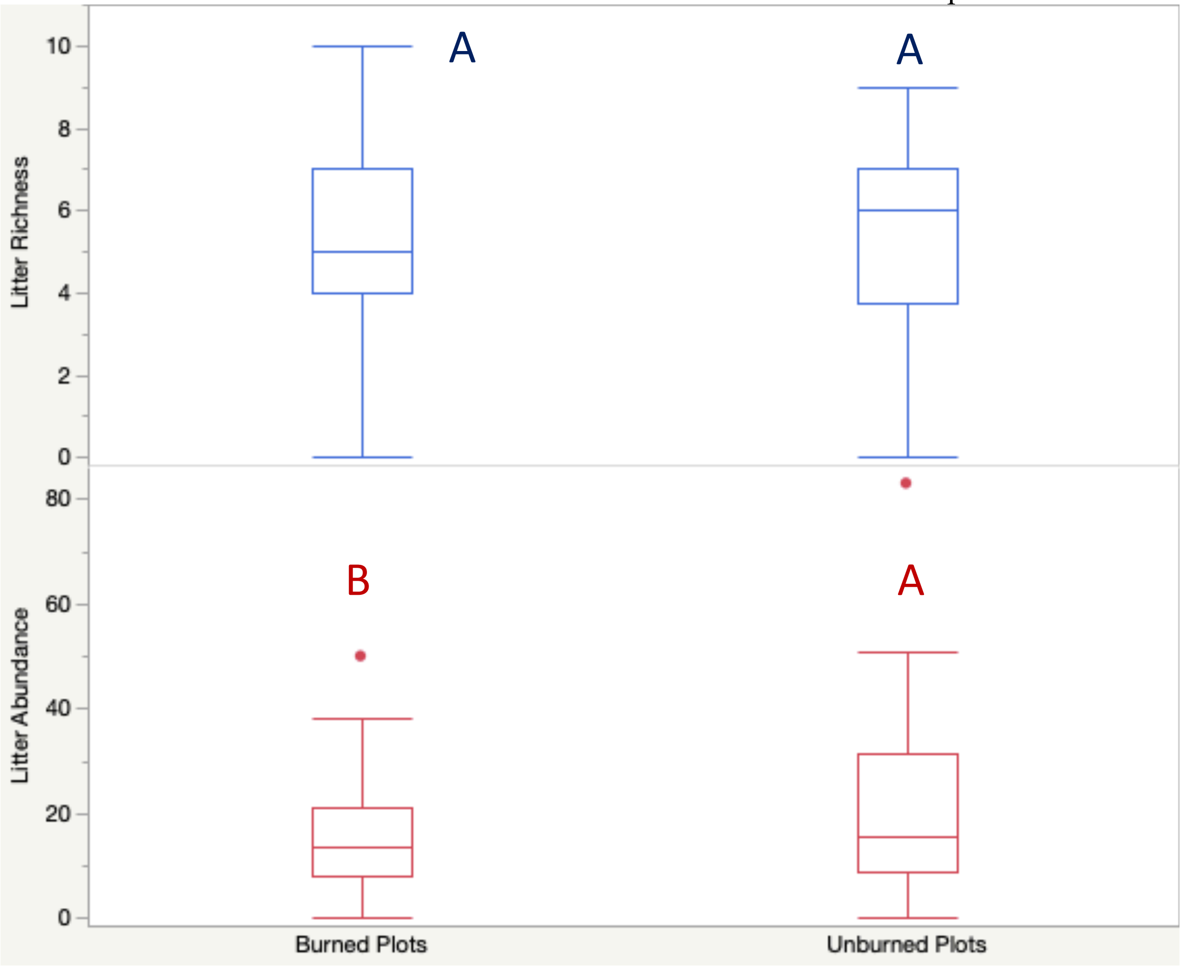


Figure 6. Box plot of abundance and richness of leaf litter-dwelling macroinvertebrates at the different collection seasons, the three sites are combined. Letters indicate the results of the post-hoc Tukey's HSD test. Different letters within a panel indicate significant differences in mean abundance or richness between different collection seasons.

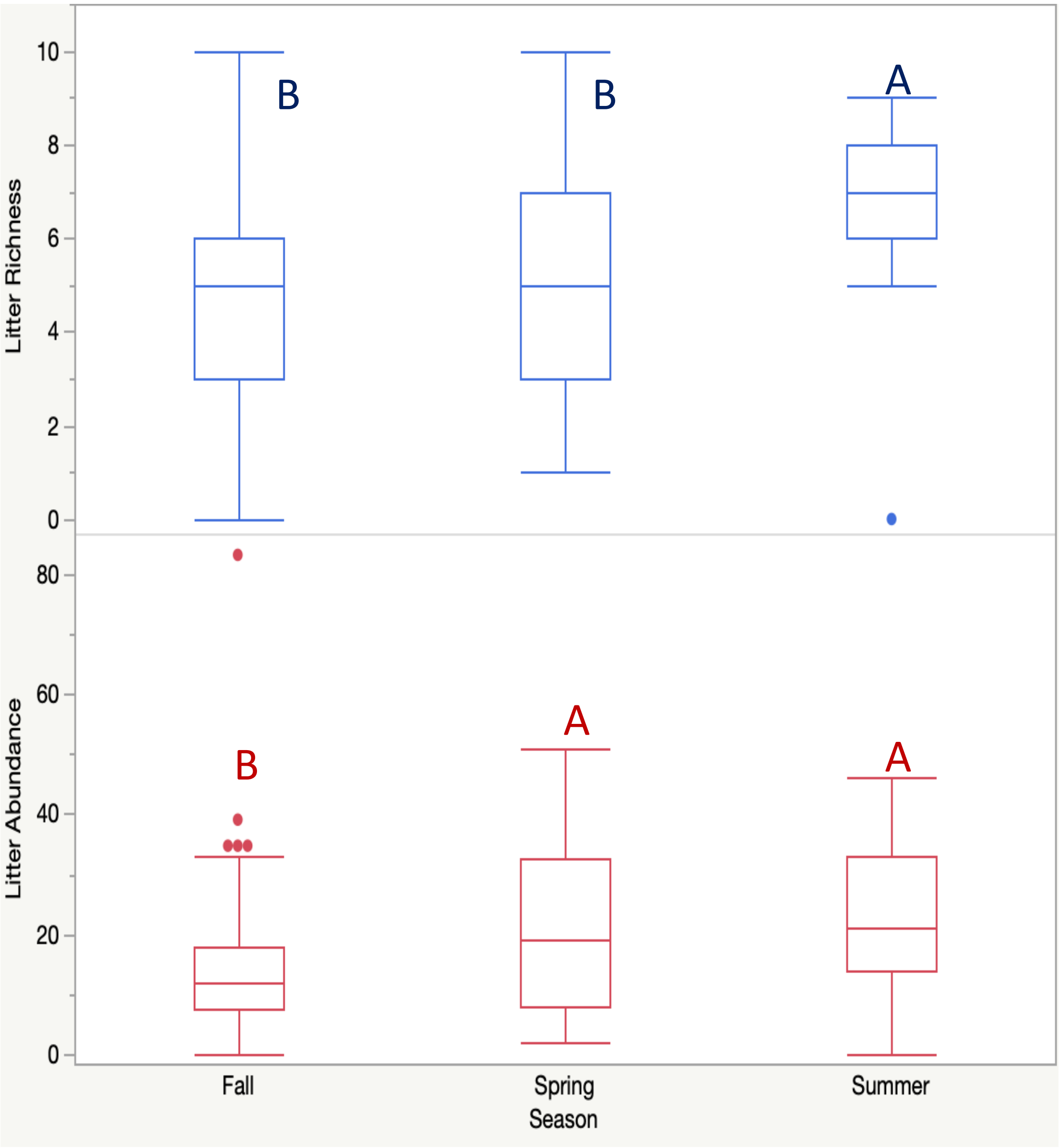


Figure 7. Box plot of abundance and richness of leaf litter-dwelling macroinvertebrates at the three different collection sites. Letters indicate the results of the post-hoc Tukey's HSD test. Different letters within a panel indicate significant differences in mean abundance or richness between different collection sites. Factors were site, burn status, and sampling date.

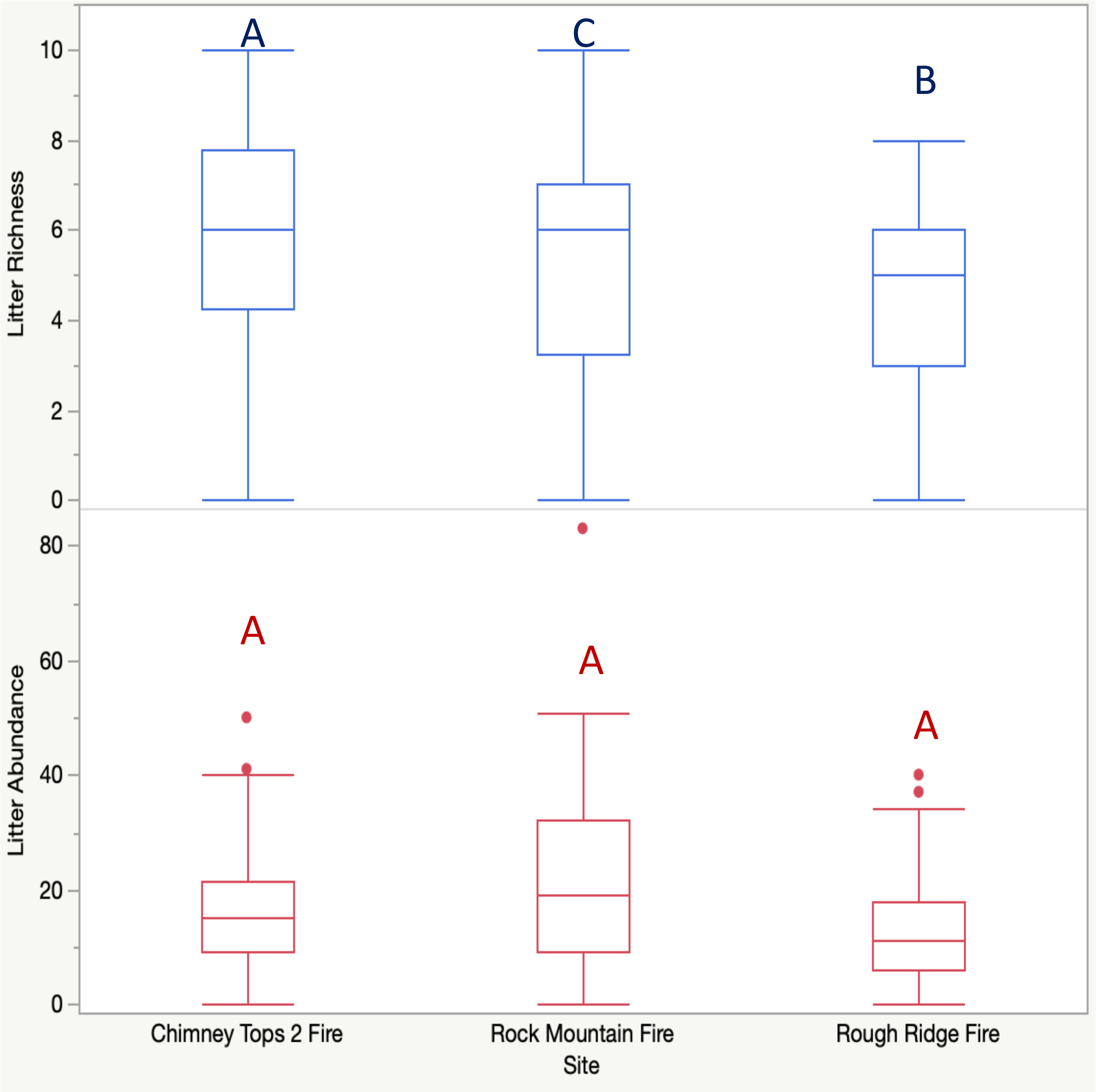


Figure 8. Box plot of abundance and richness of soil-dwelling macroinvertebrates depicting overall trends across site and sampling date. The three-factor ANOVA produced a significant model: abundance (F=3.55, P<0.0001, Table 3) and richness (F=4.88, P<0.0001, Table 3).

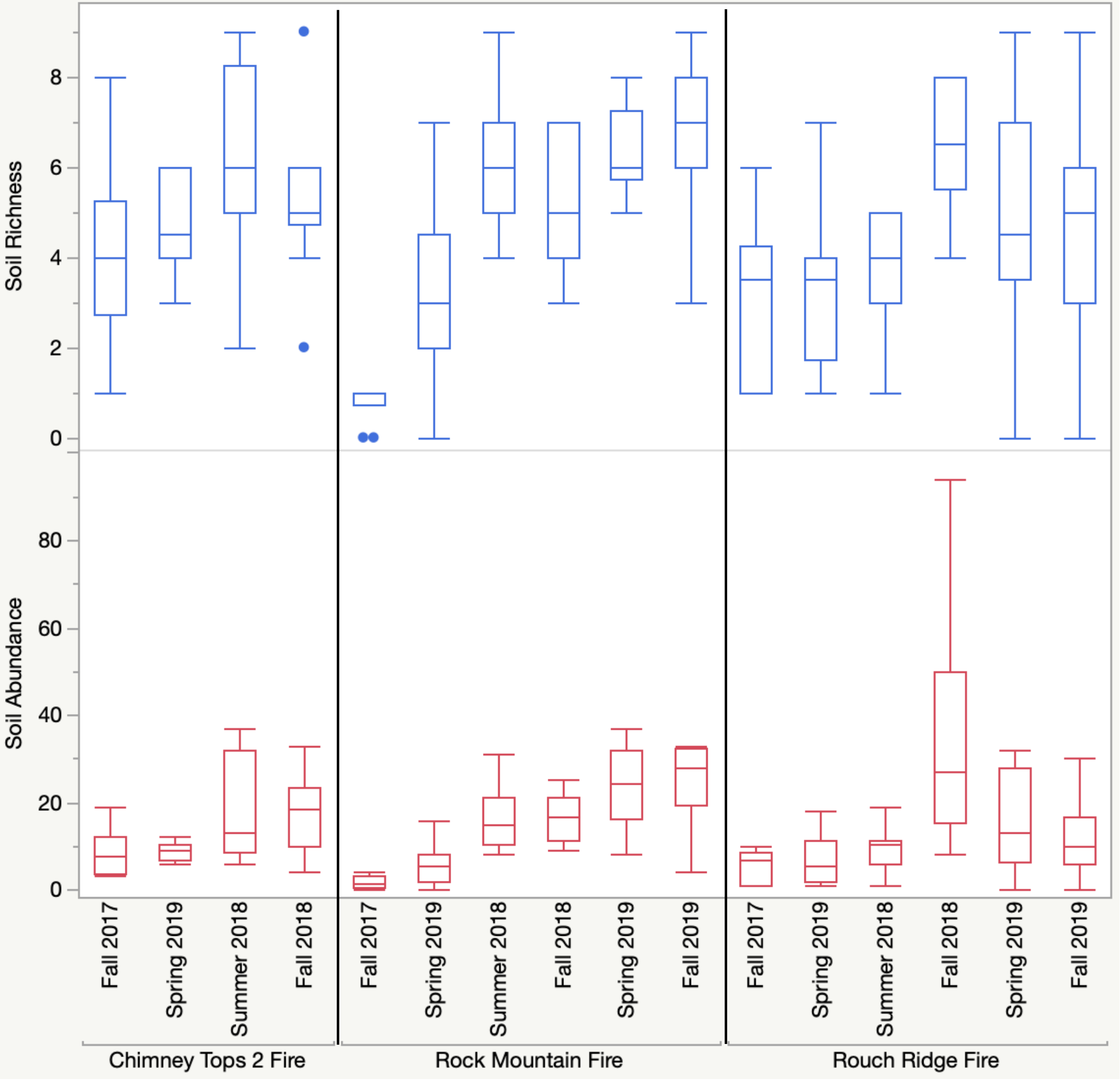


Figure 9. Box plot of abundance and richness of soil-dwelling macroinvertebrates at the different sampling dates, the three sites are combined. Letters indicate the results of the post-hoc Tukey's HSD test. Different letters within a panel indicate significant differences in mean abundance or richness between different collection times since the fire had occurred.

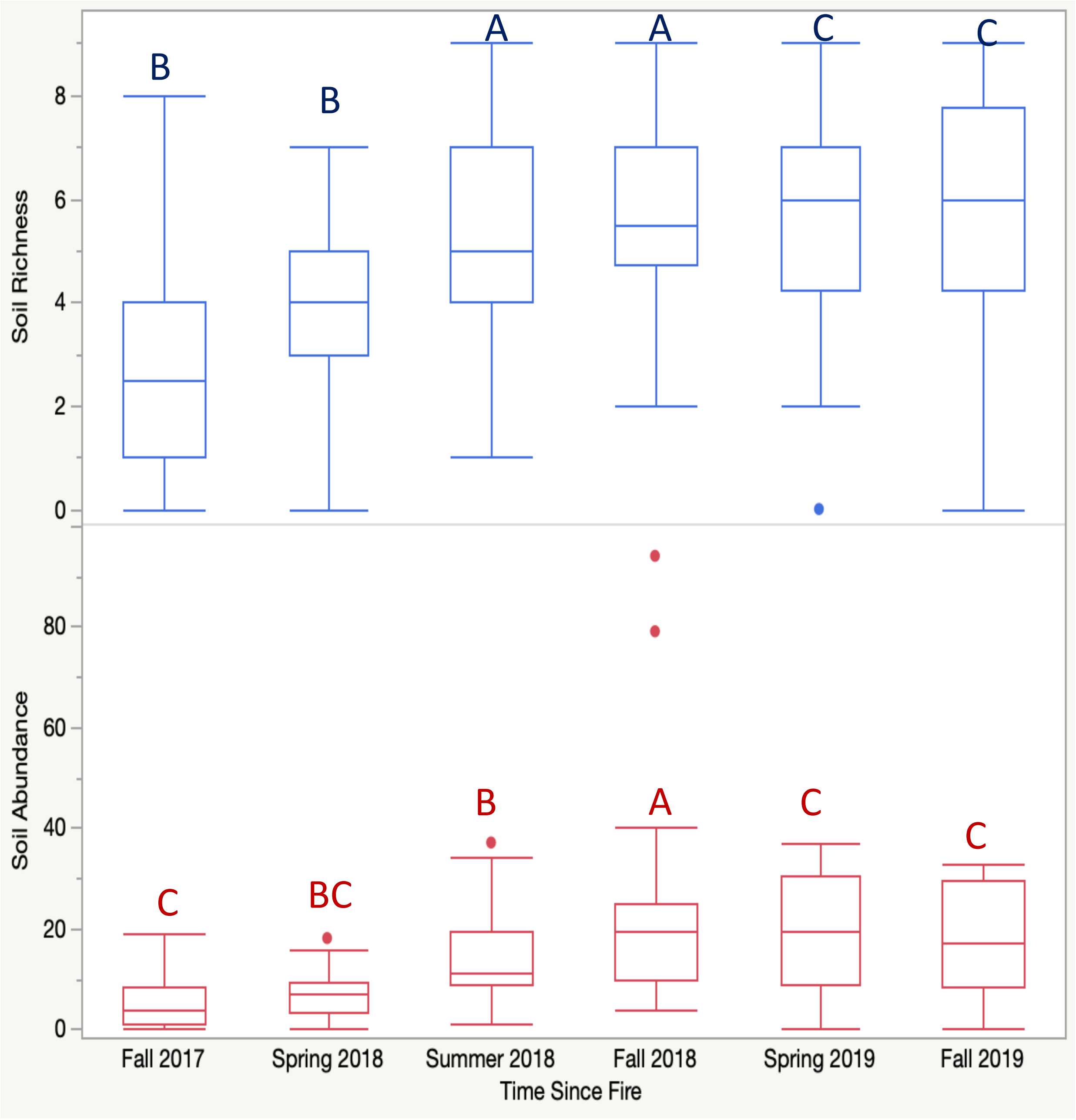


Figure 10. Box plot of abundance and richness of soil-dwelling macroinvertebrates at the three different collection sites. Letters indicate the results of the post-hoc Tukey's HSD test. Different letters within a panel indicate significant differences in mean abundance or richness between different collection sites.

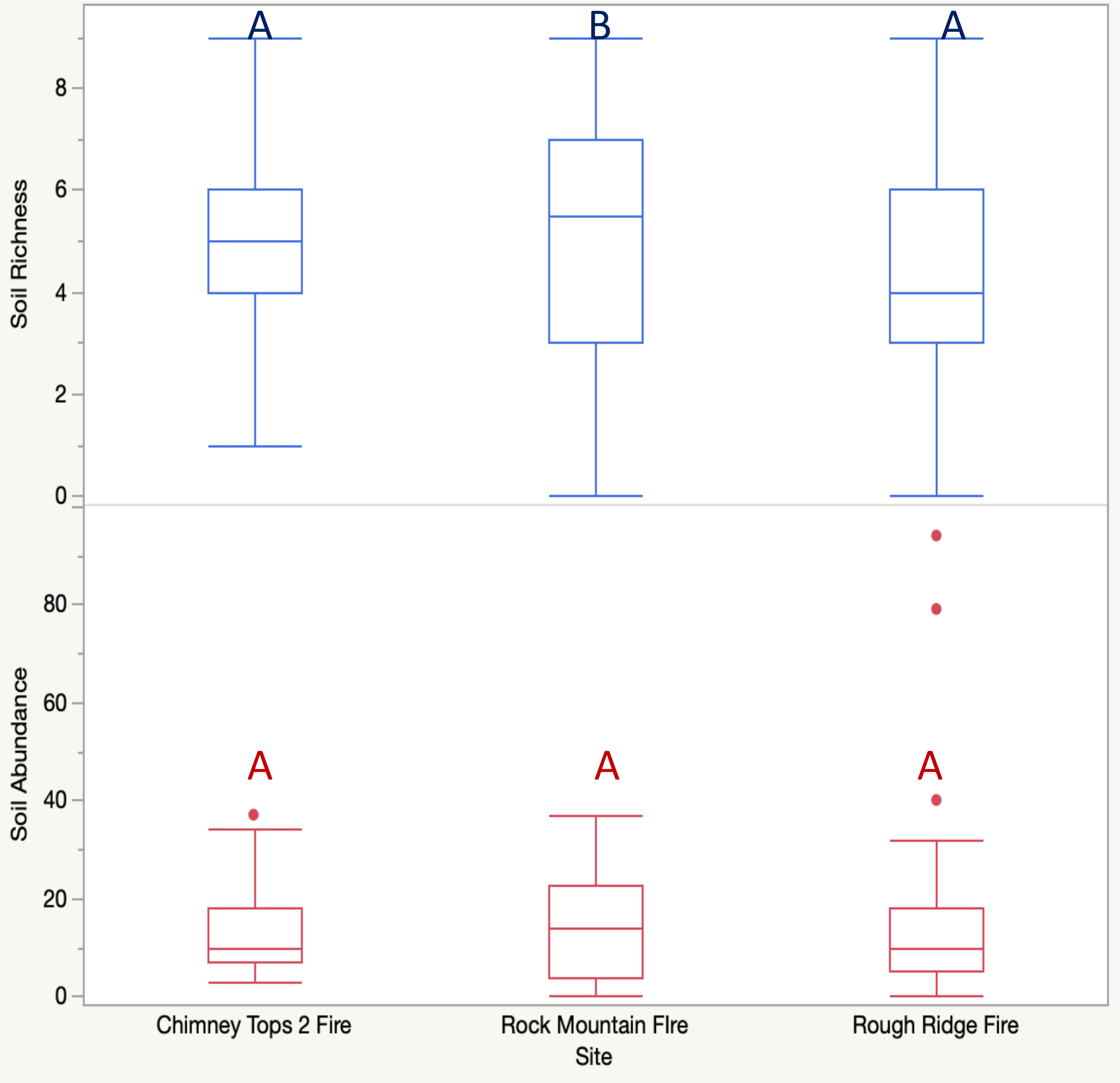


Figure 11. Box plot of abundance and richness of millipedes collected from the leaf litter depicting overall trends across site and sampling date. The three-factor ANOVA produced a significant model: abundance (F=10.56, P<0.0001, Table 5) and richness (F=4.67, P<0.0001, Table 5).

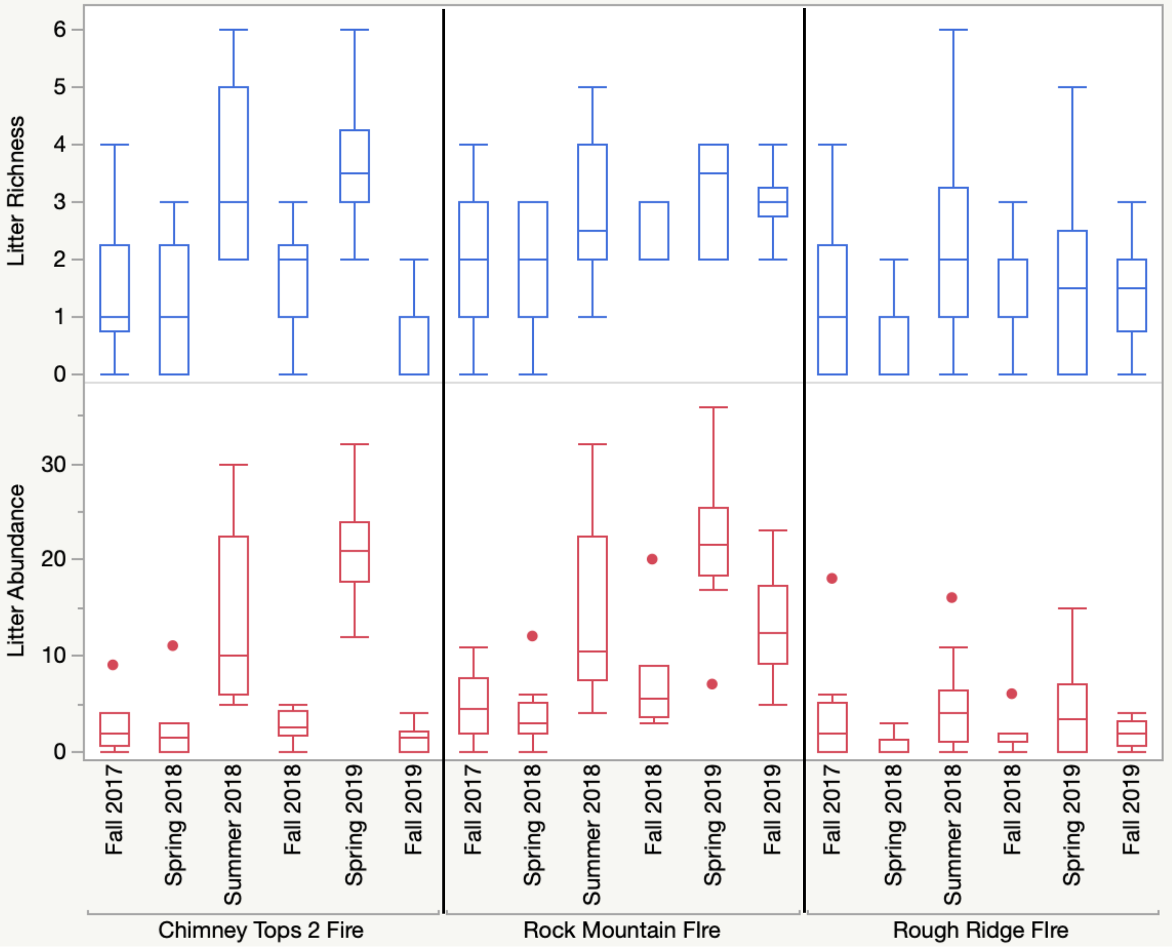


Figure 12. Box plot abundance and richness of millipedes collected from the litter at the different sampling dates, the three sites are combined. Letters indicate the results of the post-hoc Tukey's HSD test. Different letters within a panel indicate significant differences in mean abundance or richness between different collection times since the fire had occurred.

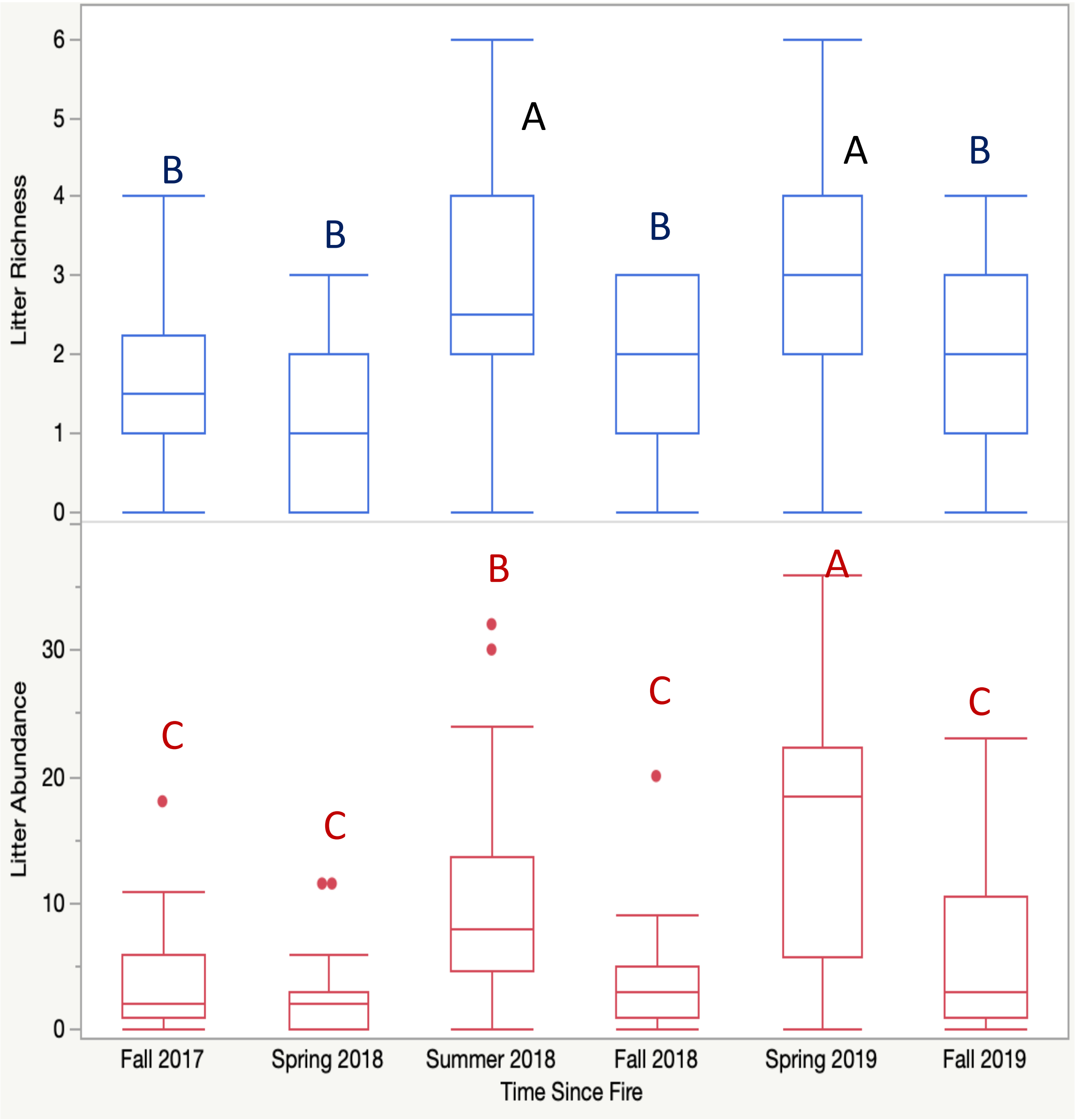


Figure 13. Box plot of abundance and richness of millipedes collected from leaf litter at the different collection sites. Letters indicate the results of the post-hoc Tukey's HSD test. Different letters within a panel indicate significant differences in mean abundance or richness between different collection sites.

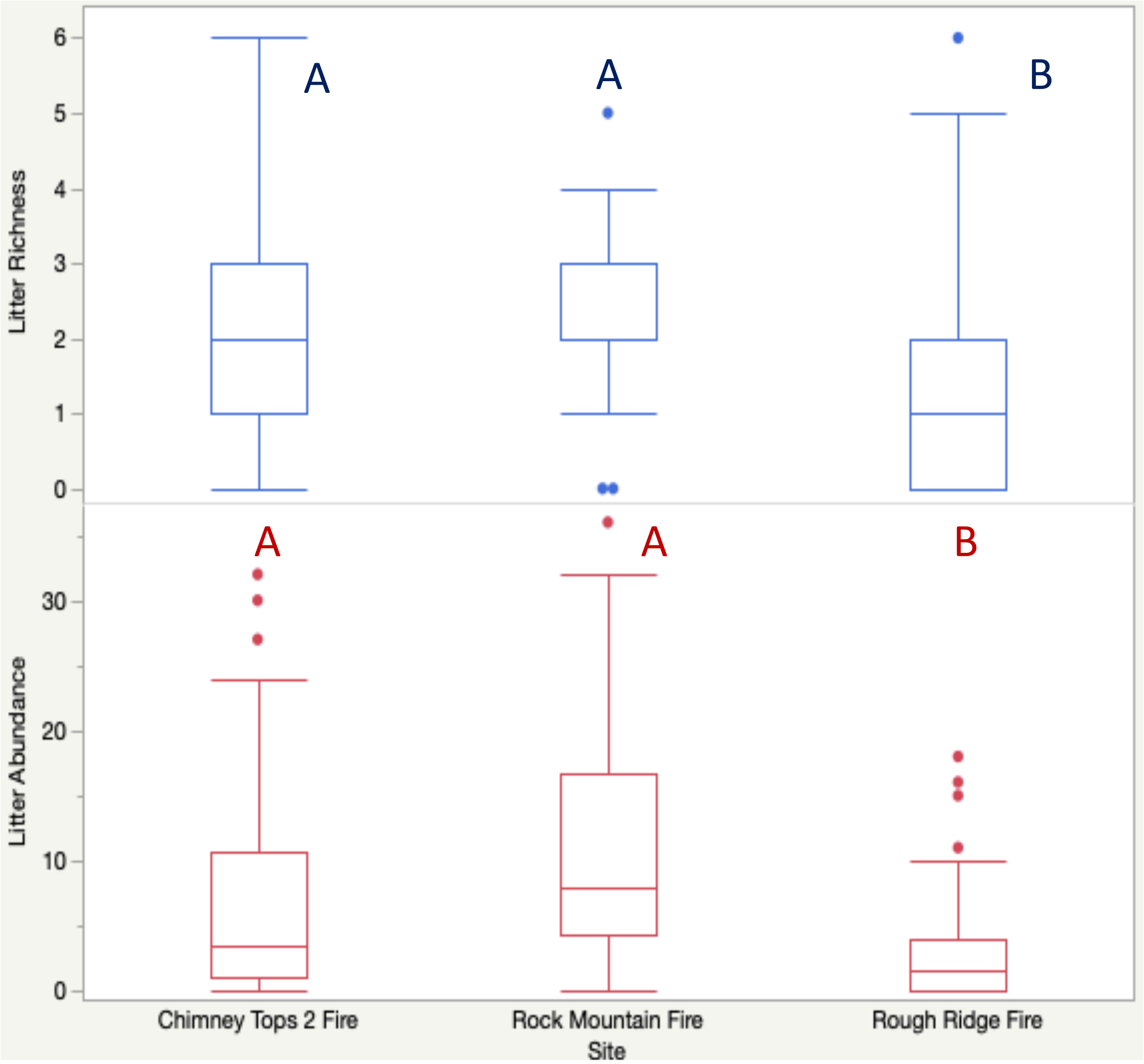


Figure 14. Box plot of abundance and richness of millipedes collected from leaf litter at the different collection seasons, the three sites are combined. Letters indicate the results of the post-hoc Tukey's HSD test. Different letters within a panel indicate significant differences in mean abundance or richness between different collection seasons.

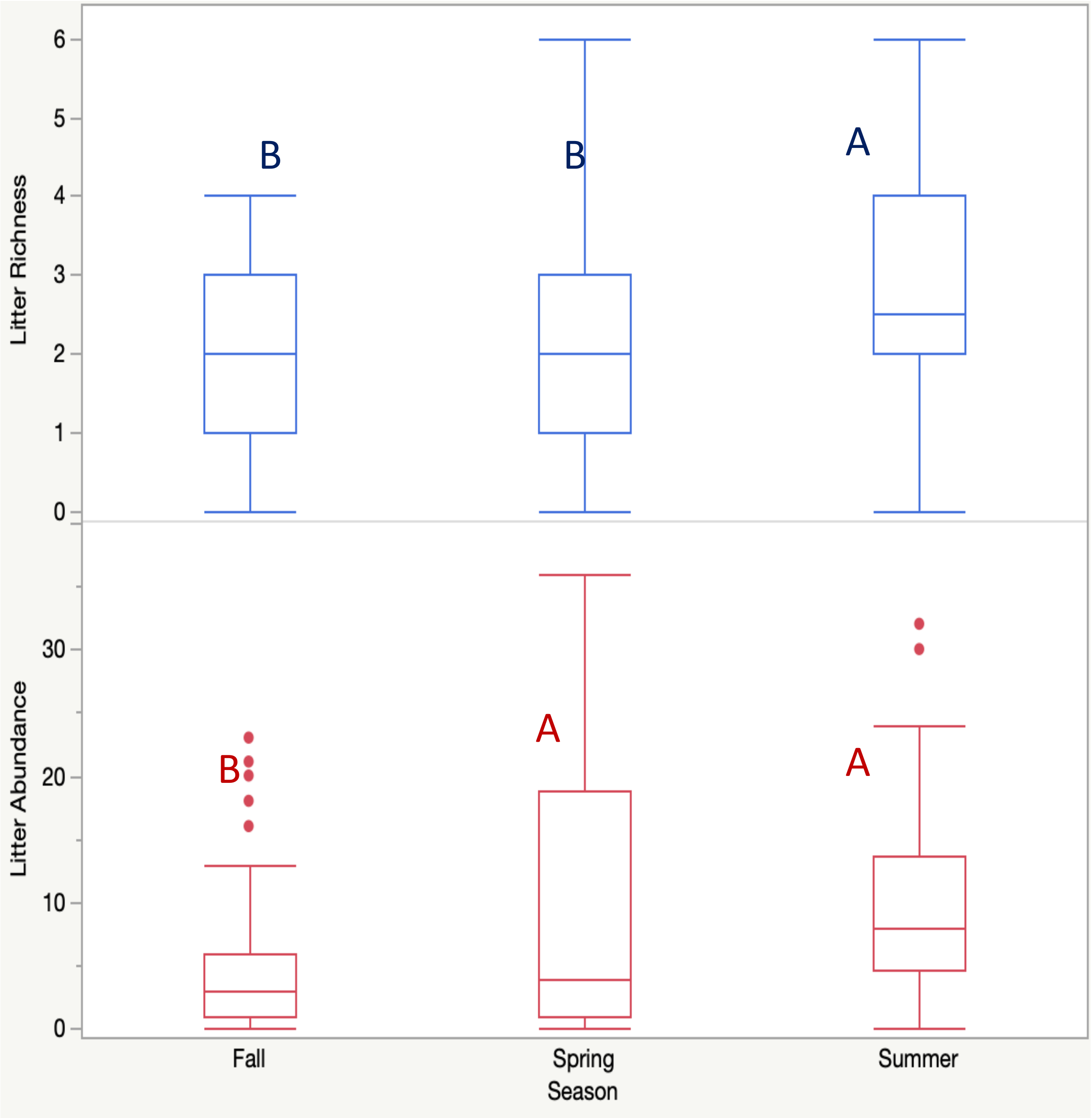
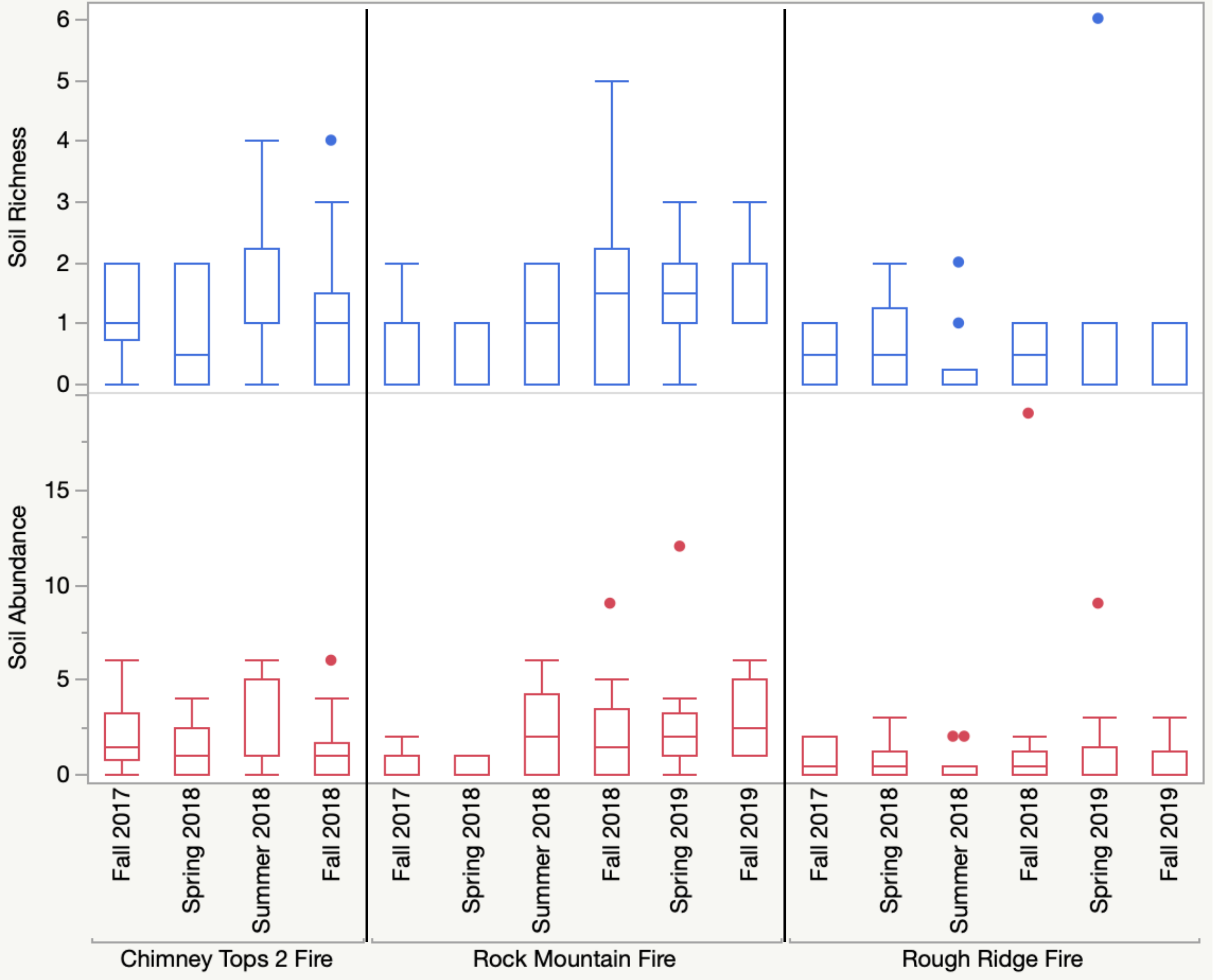


Figure 15. Box plot of abundance and richness of millipedes collected from the soil depicting overall trends across site and sampling date. The three-factor ANOVA produced a significant model: richness (F=1.87, P=0.0082, Table 5).



Appendix A

Primary Literature used for Millipede Identification

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Shear, W.A. 1999. Key to millipede families of the eastern United States.

Shelley, R.M. 1979. A revision of the millipede genus *Delophon* with the proposal of two new tribes in the subfamily Abacioninae (Callipodida: Caspiopetalidae). Proceedings of the Biological Society of Washington 92: 533-550.

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Appendix B

Species List of Millipedes for each Study Site

Great Smoky Mountains National Park

Order	Family	Genus	Species
Callipodida	Abacionidae	*Abacion*	sp.
		Abacion	*magnum*
		Delophon	sp.
		Delophon	*georgianum*
Chordeumatida	Cleidogonidae	*Cleidogona*	sp.
		Cleidogona	*margarita*
		Cleidogona	undescribed species A.
	Striariidae		
Julida	Parajulidae	*Ptyoiulius*	sp.
		Ptyoiulius	*impressus*
		Uroblaniulus	sp.
	Zosteractinidae	*Ameractis*	sp.
		Ameractis	*chirogona*
Platydesmida	Andrognathidae	*Brachycybe*	*lecontii*
		Brachycybe	*petasata*
Polydesmida	Polydesmidae	*Pseudopolydesmus*	sp.
		Pseudopolydesmus	*canadensis*
		Scytonotus	sp.
	Xystodesmidae	*Cherokia*	*georgiana georgiana*
		Nannaria	sp.
		Nannaria	*scutellaria*
Spirobolida	Spirobolidae	*Narceus*	*americanus-annularis* complex
Spirostreptida	Cambalidae	*Cambala*	sp.

Rock Mountain

Order	Family	Genus	Species
Callipodida	Abacionidae	*Abacion*	sp.
		Abacion	*magnum*
		Delophon	sp.
		Delophon	*georgianum*
Chordeumatida	Cleidogonidae	*Cleidogona*	sp.
		Cleidogona	undescribed species B.
	Striariidae		
	Trichopetalidae		
Julida	Parajulidae	*Ptyoiulus*	sp.

		Ptyoiulus	*impressus*
		Uroblaniulus	sp.
Platydesmida	Andrognathidae	*Brachycybe*	*lecontii*
Polydesmida	Euryuridae		
	Polydesmidae	*Pseudopolydesmus*	sp.
		Pseudopolydesmus	*erasus*
		Scytonotus	sp.
	Xystodesmidae	*Cherokia*	*georgiana georgiana*
		Erdelyia	*saucra*
		Nannaria	sp.
		Sigmoria	sp.
Spirobolida	Spirobolidae	*Narceus*	*americanus-annularis* complex
Spirostreptida	Cambalidae	*Cambala*	

Rough Ridge

Order	Family	Genus	Species
Callipodida	Abacionidae	*Abacion*	sp.
Chordeumatida	Cleidogonidae	*Cleidogona*	sp.
		Cleidogona	*inexpectata*
		Cleidogona	*major*
		Cleidogona	undescribed species C.
Julida	Parajulidae	*Ptyoiulus*	sp.
		Ptyoiulus	*impressus*
Polydesmida	Euryuridae		
	Polydesmidae	*Pseudopolydesmus*	sp.
		Pseudopolydesmus	*erasus*
		Scytonotus	*australis*
	Xystodesmidae	*Apheloria*	sp.
		Apheloria	*montana*
		Brachoria	*initialis*
		Cherokia	*georgiana georgiana*
		Nannaria	sp.
		Nannaria	undescribed species A.
		Sigmoria	sp.
Spirobolida	Spirobolidae	*Narceus*	*americanus-annularis* complex
Spirostreptida	Cambalidae	*Cambala*	sp.
		Cambala	*annulata*

Millipede Site Species List Notes

There were several undescribed species found through the course of the study. Each research site had an undescribed *Cleidogona* species, designated A, B, and C in our species lists. The other undescribed species was in the genus *Nannaria*, found at the Rough Ridge site. This is one of the first records of *Nannaria* in Georgia.

The Rock Mountain site had over 100 juvenile millipedes we were only able to identify to family, Xystodesmidae. Millipedes can only definitively be identified to species level using the morphology of the adult male reproductive structures, the gonopods. Without adult male specimens, many times the millipedes can only be identified to family or genus level using other external morphological characteristics. There has been a big push within the last few years to utilize DNA barcoding as a means of identification to species level for juveniles and females. However, a good foundational database of established barcodes is needed before this use can be widespread. Barcodes are a good option as a tool for identification, however we need better identification resources in general. Most of the primary literature available for millipede identification is found in individual proceedings based on genera making it extremely tedious to go through all of the literature to find the exact paper required for a specimen. Additionally, many of these are older papers lacking keys or lacking detailed drawings of relevant characters. With the advancements in photography there is a need for high-quality images of the millipedes' gonopods as well as other external morphological features.