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Andrea Gamache

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# **Fire and periodical cicadas: impacts on soil nutrients and understory plant germination**

Gamache, A., N. Green, J. McNeal, M. Weand, and M. Bretfeld

*Kennesaw State University, Department of Ecology, Evolution, and Organismal Biology, Kennesaw, (GA), USA*

## **Abstract**

### **Purpose**

A compound disturbance, composed of a prescribed fire and a periodical cicada emergence, occurred in northern Georgia in the summer of 2021. Independently these disturbances have substantial effects on the surrounding ecosystems. This study investigated the collective impact on soil composition and seed germination.

### **Methods**

Through the soil analysis of cicada turrets, burned soil, and unburned soil, we hope to understand the composition of each soil type. Additionally, a  $2 \times 2$  factorial study was performed with those same samples, providing insight into the impact on seed germination these disturbances have. The two factors were between burned and unburned soil as a base and whether turrets were present or not.

### **Results**

Turret samples had significantly higher Ca, P, Zn, and C% concentrations, and a higher pH than burned samples. Turrets also had significantly higher concentrations of all nutrients accounted for in the soil analysis than the unburned samples. Similarly, burned samples also contained higher concentrations of nutrients than unburned samples, except both samples, had the same pH. During the  $2 \times 2$  factorial germination study, the treatment with the positive outcome on germination was the unburned soil, regardless of the turret's presence.

### **Conclusion**

Our study shows that the compound disturbance of prescribed burning and soil bioturbation due to cicada emergence significantly impacted soil chemistry, increasing nutrient availability. We also found that these two events had either no effect or a negative effect on seeds germinating during our study.

## Introduction

Environmental disturbances come in a wide range of forms and can have cascading effects on ecosystems (Johnstone et al. 2016). Disturbances are classified along many axes, including biotic or abiotic, periodic or stochastic, and individual or compound. Compound disturbances occur when individual disturbances combine to create a new phenomenon that can have novel effects on ecosystems (Buma 2015). There are numerous examples of compound disturbances that have yet to be studied thoroughly. One compound disturbance event that we were able to observe was prescribed fire followed by a periodical cicada emergence. This unique opportunity allowed us to study the interactive impacts of these disturbances on the surrounding soil and flora.

Periodical cicadas are unique insects that spend most of their life cycle underground as nymphs. There are seven known species of periodical cicadas in the genus *Magicicada*, four on a 13-year cycle (*Magicicada tredecim*, *M. tredecassini*, *M. tredecula*, and *M. neotredecim*) and three on a 17-year cycle (*M. septendecim*, *M. cassini*, and *M. septendecula*; Grant 2005). While underground, cicada nymphs burrow and forage until they finally emerge to become adults for roughly two weeks. Regional populations of cicadas with a similar emergence period are classified as brood numbers and are often parapatric (Cooley et al. 2013). Members of the same brood will emerge within seven to ten days of each other to begin a few weeks of mating and egg-laying. This aboveground period is then followed by another 13-to-17-year underground phase (William and Simon 1995). Periodic emergence creates a sudden increase in cicada population density, thought to be an anti-predator adaption known as predator satiation that reduces the per capita death rate (Toivonen and Fromhage 2019). This synchronized emergence of defenseless insects results in a fluctuation of energy and a resource pulse for ecosystems (Yang 2013). When nymphs emerge, they are often preyed upon, as they are an excellent source of protein (Wheeler et al. 1992). The cicada emergence can have substantial impacts at the ecosystem level (Whiles et al. 1991). Koenig and Liebhold (2005) found that 15 out of 24 avian predator species exhibited, an increase or decrease, in abundance closely associated with the emergence of periodical cicadas. The change was mostly during or immediately after the cicada emergence. Another study found that after emergence, the cicada carcasses act as a resource pulse for the soil. The addition of dead adult cicadas to soil increased plant biomass by 61% and increased foliar nitrogen by 20% (Yang 2013).

Cicadas have a direct impact on ecosystems through their bioturbation or movement of soil. When underground nymphs inhabit soil depths around 7 and 69 cm but will usually congregate around 7.6 to 22.5 cm (Maier 1980). While cicadas are preparing to emerge, they will sometimes produce turrets or towers made of soil at the top of their

emergence tunnel. There is currently no evidence as to why the turrets are constructed or not. Turrets are constructed with excavated sediment weeks prior to their emergence (William and Simon 1995) and are primarily constructed in the late evening and at night (Cory and Knight 1937). It is hypothesized that cicadas construct turrets in order to maintain emergence burrow humidity by reducing interior exposure to sunlight (Heath 1968 ). The turrets are short-lived and quickly desiccate due to weather conditions on the surface soon after emergence (Humphreys 1989). Cicadas may construct turrets out of the soil located in the A and B horizons (Luken and Kalisz 1989). In addition to moving soil from deeper horizons to the surface, cicadas may directly add nutrients and pH to the soil through excretion. Cicadas consume xylem fluid from the roots of plants, and it is predominately composed of potassium, sodium, calcium, magnesium, chloride, and phosphate ions (Cochard et al. 2010). The urine of cicadas contains the same ions found in xylem fluid, in the same proportions but at lower concentrations (Cheung and Marshall 1973). Cicadas use their bodily fluids, like saliva and urine, to moisten the soil and make it easier to manipulate (Luken and Kalisz 1989). This increases concentrations of nutrients in the soil that is available for absorption by the surrounding plants. After emergence, the cicada carcasses act as a resource pulse for the soil, increasing plant biomass by 61% and increasing foliar nitrogen by 20% (Yang 2013).

Fire is a frequent disturbance for terrestrial ecosystems in many parts of the Southeastern United States. Fire shapes plant community composition, structure, productivity, and nutrient circulation (Elliott et al. 1999; Fowler and Konopik 2007). Regular burning reduces fuel buildup and contributes to the biodiversity of these areas (Miller et al. 2020). Due to historical practices of fire prevention and suppression by the US government, there are drastic changes to ecosystems (Fowler and Konopik 2007; Hodgkins 2011). Fire suppression practices in Northern Georgia, caused a decline of pyrophytic species like the Shortleaf Pine (*Pinus echinate*) or the White Oak (*Quercus alba*) (Warwick 2021) due to competition between more aggressive species like loblolly and slash pine that can thrive without the presence of fire (Outcalt 2000; Miller et al. 2019). Since the 1990s, policies have shifted from suppression to using prescribed burning to maintain and restore forest communities. As the understanding of how fire impacts the ecosystem increases, so too does the proper management and care of communities, previously harmed by fire suppression practices (Welch and Waldrop 2001; Certini 2005; Pereira et al. 2018; Ling et al. 2020). Initially, fire results in a decrease of the overall nutrient pool of an ecosystem; however, the soil in the same ecosystem, may see an increase in the availability of nutrients for plants (Certini 2005). Through nutrient cycling, oxidatively break down organic matter, like leaf litter and fallen trees, and produce a water-soluble ash that contains

nutrients that are essential for plant growth and development (Gonzalez–Perez et al. 2004). This ash is then leached into the soil, where it is readily available for the surrounding flora. Fire can also transform some nutrients. For example, combustion of organic nitrogen results in ammonium, which can be nitrified to form nitrate (Wang et al. 2014), the form of nitrogen most easily used by plants, but also the form most easily lost through soil leaching (Alkharabsheh et al. 2021). Many other nutrients, like phosphorus or calcium, if not immobilized by biota following the fire, may be lost from the ecosystem through erosion and leaching (Lehmann and Schroth 2009; Verma et al. 2019). While fire makes some nutrients more available at the cost of doing so makes those nutrients more likely to be lost from the ecosystem.

Seeds are greatly affected by environmental conditions, such as water availability, temperature, and soil conditions (Parolin 2001; Roem et al. 2002; Taiz et al. 2018). If optimal, these conditions will send signals within the seeds, triggering germination (Taiz et al. 2018). However, not all species require the same conditions to germinate, though the conditions will usually contribute to growing in ideal surroundings for the individual. Temperatures act as triggers because temperature patterns are guides to season changes (Singh et al. 2018), or water as a trigger it symbolizes the end of a drought (Yi et al. 2018). There are also conditions in the soil that can contribute to germination, like acidity and possibly specific nutrients available. Some species have been shown to germinate favorably in Ca treated soils, or the addition of Al has been seen to decrease the germination of some seeds (Parolin 2001). We are not sure of the exact species that will be present, but in theory, the increased nutrients should aid in the germination of seeds.

As disturbances both fire and periodical cicadas have been independently studied. The cicadas can bring buried nutrients to the surface and add to the existing nutrient pool through their excretions. Fire further alters soil chemistry by breaking down organic matter and transforming nutrients. To our knowledge, our study is the first to investigate the potential interactive effects of these disturbances on soils and forest understory plant germination. During the summer of 2021, periodical cicadas (*Magicicada* spp.) emerged as part of Brood X in the mountains of North Georgia. Brood X is the largest brood of seventeen-year cicadas. Their emergence was immediately followed by prescribed fires performed by the United States Forest Service (USFS) to limit the buildup of fuels on the forest floor. This combination of events provided a unique opportunity to study the interactive impacts of these disturbances on a forest ecosystem. We performed a  $2 \times 2$  factorial experiment at the KSU greenhouse, using soils

sampled from burned and unburned plots in the Cooper Creek Watershed in northern Georgia, as well as cicada turrets collected in the same areas where the soil was collected.

We hypothesized that the soil that comprises cicada turrets would have greater concentrations of nutrients because of the cicadas utilizing soil where nutrients had leached from under the soil's surface (Anderson 1988) as well as the addition of cicada fluids to the soil (Luken and Kalisz 1989). We also hypothesized that, compared to both turret and unburned soils, the burned soils would have a greater carbon percentage and a higher pH due to the production of K and Na oxides, hydroxides, and carbonates relative to unburned soils, which all can increase the pH of the topsoil (Certini 2005). The carbonates produced from organic matter, for example, wood ash, should increase the overall carbon percentage (Bodi et al. 2014; Demeyer et al. 2001). Thus, we predicted a greater total carbon percentage and pH in burned samples than unburned soils. We further hypothesized both cicada emergence and fire would positively affect plant community diversity by opening space and mobilizing nutrients. Thus, we predicted that both burned soil and cicada turret presence would positively impact understory plant germination due to the increased plant-available nutrients in the soil from fire and the bioturbation of cicadas. Further, we predicted that pots with both burned soil and turrets would have both a high species richness and total individuals to germinate when compared to the other treatments.

## Methods and Materials

Soil samples were collected from mid-June to early July of 2021 within the Cooper Creek Watershed of the Blue Ridge Ranger District, part of the Chattahoochee-Oconee National Forest. The area consists of oak forests with a humid temperate climate. The soil is well-drained, and surface soil texture is fine sandy loam over clay loam or clay subsoil. Slope ranges from 6 to 25% (Baker 2018). The collection sites ran along the Mulky Gap Rd. in the Cooper Creek Watershed Management Area (34.783°N, 84.033°W). This road was used as a barrier by USFS while performing prescribed burns in 2021, with one side of the road subject to burning and the other side left unburned (Burned and Unburned treatments, Table 1).

**Table 1.** Location and elevation of soil sampling in burned or unburned treatment areas.

Plot #	Treatment	Latitude (N)	Longitude (W)	Elevation (m)
Plot 1	Burned	34.79528°	84.25750°	689.46
Plot 2	Burned	34.79806°	84.06472°	745.54
Plot 3	Burned	34.79639°	84.25250°	733.35

Plot 4	Burned	34.91722°	84.30444°	795.83
Plot 5	Burned	34.92056°	84.29694°	804.06
Plot 6	Burned	34.92556°	84.29278°	806.81
Plot 7	Burned	34.79639°	84.25611°	689.46
Plot 8	Burned	35.01278°	84.17750°	852.22
Plot 9	Burned	34.82500°	84.17306°	794.92
Plot 10	Burned	35.05111°	84.15167°	877.82
Plot 11	Unburned	35.06083°	84.08972°	833.32
Plot 12	Unburned	34.80278°	84.08861°	827.84
Plot 13	Unburned	34.81028°	84.10139°	822.66
Plot 14	Unburned	34.81167°	84.09778°	817.17
Plot 15	Unburned	34.89000°	84.04889°	761.69
Plot 16	Unburned	34.88944°	84.04528°	758.65
Plot 17	Unburned	34.89167°	84.03861°	755.29
Plot 18	Unburned	34.99944°	84.03528°	740.97
Plot 19	Unburned	34.99944°	84.03611°	717.19
Plot 20	Unburned	35.00528°	84.29028°	713.23

135

136 In each treatment area, soil samples were taken from ten plots. The plot locations were at least 50 m from the road.

137 For each plot we collected 4 or 5 soil samples randomly within a 10 m radius of the plot center. For each sample we

138 collected the top 5 cm off soil after brushing away any leaf litter and duff. All soil samples within a plot were pooled

139 to represent the entire plot.

140 We collected turrets strictly from burned plots as no turrets could be found at unburned plots. Turrets were collected

141 by hand. Turrets in the burned plots appear to have been hardened from the fire, allowing them to remain intact for

142 collection. We only found emergence holes in the unburned plots and assume that either no turrets were constructed

143 or that turrets in those plots were broken down by precipitation or other environmental factors before they could be

144 collected.



**Figure 1.** Example of four turrets collected in Chattahoochee-Oconee National Forest in northern Georgia, following a periodical cicada emergence and prescribed burning

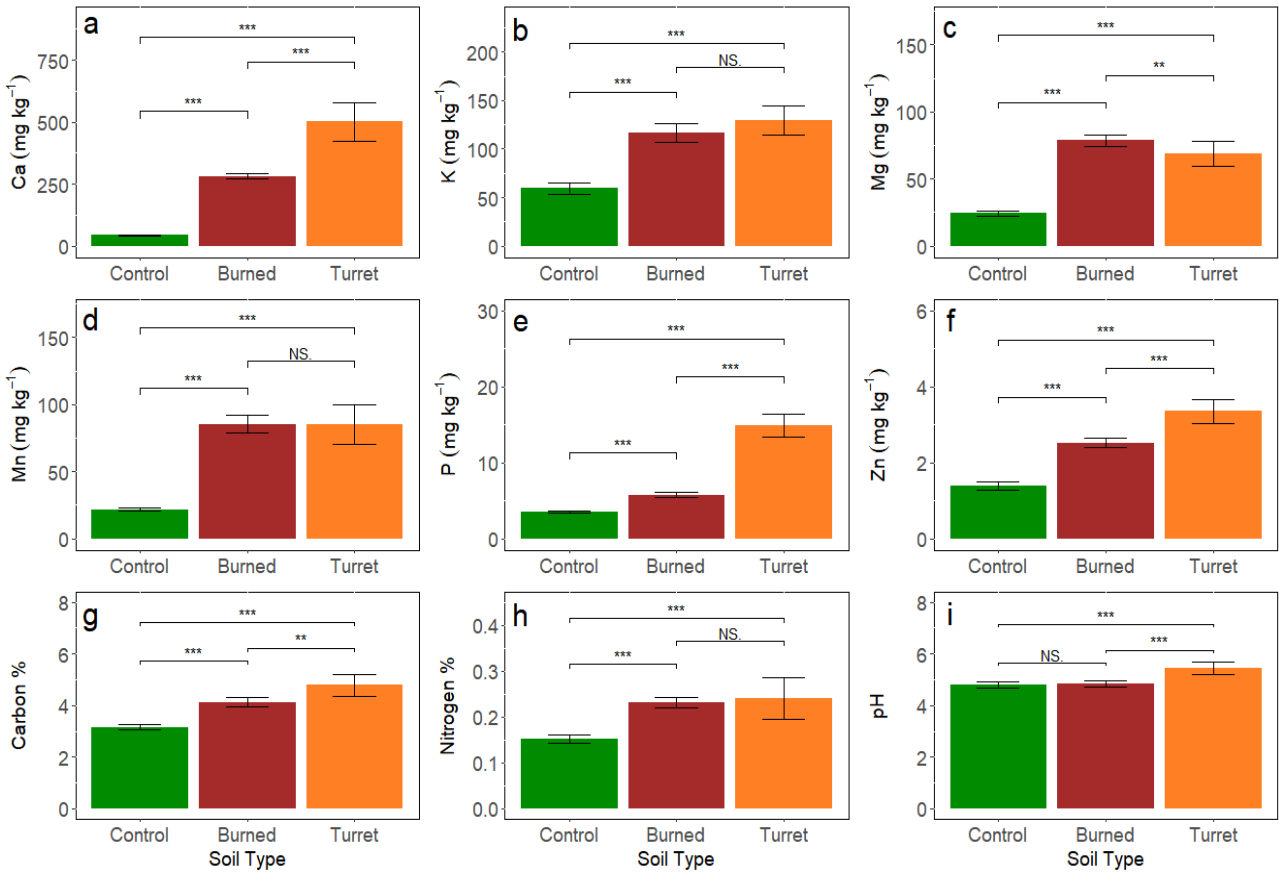
In order to test for effects of burning, turret presence, or both on post-fire emergent plant communities, we performed a germination study in a greenhouse at the Kennesaw State University Field Station (Acworth, GA), from July 2021 to November 2021. The germination study was set up as a  $2 \times 2$  factorial experiment. The two factors tested were fire (unburned vs. burned) and turret presence (absent vs. present). Soil samples from plots in the same burn treatment were combined and homogenized to remove effects of individual plots. The homogenized soils were put into 1-gallon (15.88 cm depth  $\times$  16.51 cm) pots ( $n = 48$ ; 24 unburned and 24 burned). Based on previous studies of typical cicada emergence densities (Dybas and Davis 1962; Luken and Kalisz 1989) and the soil surface area within each pot, we added 40 g of dried, crushed turret soil to the surfaces of half of the 24 pots of each treatment. At the start of the experiment, the pots were randomly positioned in the greenhouse. Each pot received the same amount of water, via an automatic watering system with a micro bubbler dispenser in each pot, which dispensed water for 1 minute twice each day at 08:00 and 16:00. The soil was not altered following collection, so the



germinated individuals were assumed to be originating from the seed bank of the original collection sites. Once an individual plant germinated, it was recorded and removed from the pot to prevent competition within each pot. Individual germinates were identified to the lowest taxonomic level possible; if an individual could not be identified, it was transplanted into a new tray to grow until it could be identified. We measured nutrient and chemical characteristics of the soils to test for effects of burning and cicada turrent presence on soil chemistry. Soil analyses were performed by the University of Georgia Extension, Agricultural & Environmental Services Labs: Soil, Plant, and Water Laboratory (Athens, GA). We tested 30 samples (10 burned, 10 unburned, and 10 turret soil samples) for lime buffer capacity (LBC), lime buffer capacity at equilibrium ( $LBC_{eq}$ ), pH, calcium (Ca), potassium (K), magnesium (Mg), manganese (Mn), Phosphorus (P), zinc (Zn), total carbon percentage (C%), and total nitrogen percentage (N%). LBC is the measure of soil acidity that must be neutralized to raise the pH, and  $LBC_{eq}$  is LBC at equilibrium after five days (Kizzel and Vendrell 2015). The 30 samples submitted to chemical analysis were drawn from the combined and homogenized soil samples used to fill pots in the germination experiment. UGS determined P, K, Ca, Mg, Mn, Zn through the Mehlich-1 extraction method. Afterward, an inductively coupled plasma spectrograph was used to determine the amount of element expressed. The pH and LBCs were determined using an automated LabFit AS-3000 pH analyzer equipped with direct titration capabilities. Lastly, to determine both the total C% and N% the samples were combusted and then the gases analyzed, for C content the gases passed through an infrared (IR) cell. When determining the N content, the gases were passed through a thermal conductivity (TC) cell (University of Georgina 2011). We used one-way analysis of variance (ANOVA) to test for univariate differences in soil chemistry variables among soil treatments. The independent variable was soil type: unburned soil, burned soil, and turret soil. Because we ran nine related tests, a Bonferroni correction was applied to reduce  $\alpha$  to account for multiple comparisons. In comparisons where soil type was statistically significant in the omnibus test, we used a Tukey's honest significant difference (HSD) post hoc test to explore which soil types had different means. We also used a principal components analysis (PCA) to characterize variation in soil characteristics. The data were standardized prior to the PCA due to differences in the magnitudes of the metrics. We then used analysis of similarity (ANOSIM) on the PCA scores to test for a multivariate difference between the soil. We used generalized linear models (GLM) with a Poisson family and log link function to analyze the richness and the total individuals germinated in each treatment. The Poisson distribution was used because these endpoints represent counts and because we saw no evidence of overdispersion (Bolker 2007). All analyses were performed in R version 4.0.2 (R

Development Core Team 2020). Within R, we used the package vegan version 2.5-7 for PCA and ANOSIM (Oksanen et al. 2020).

# Results



**Figure 2.** Comparison of soil chemistry among unburned (“Control”), burned, and cicada turret soil. Values are shown as mean ± SE: Symbols above brackets connecting each pair of soil types denote significant differences: NS for  $P > 0.05$ , \*\* for  $P < 0.01$ , and \*\*\* for  $P < 0.001$

**Table 2.** Results of Tukey test with the differences and adjusted p-value for each comparison on soil types for each nutrient and pH. Estimated difference for each comparison is the mean in the first treatment minus the mean in the second.

Endpoint	Comparison	Difference	Confidence Interval 95% [LL, UL]	<i>P</i>
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Calcium	Unburned vs. Burned	-0.027	[185, 291]	0.939
	Turret vs. Burned	0.619	[167, 273]	< 0.001
	Turret vs. Unburned	0.646	[405, 510]	< 0.001
Potassium	Unburned vs. Burned	-0.079	[45.0, 69.0]	< 0.001
	Turret vs. Burned	0.009	[0.04, 25.0]	0.772
	Turret vs. Unburned	0.088	[57.5, 82.5]	< 0.001
Magnesium	Unburned vs. Burned	-54.41	[47.4, 61.5]	< 0.001
	Turret vs. Burned	-9.76	[-16.8, -2.71]	0.005
	Turret vs. Unburned	44.65	[37.6, 51, 7]	< 0.001
Manganese	Unburned vs. Burned	-63.54	[52.6, 74.5]	< 0.001
	Turret vs. Burned	-0.49	[-11.5, 10.5]	0.993
	Turret vs. Unburned	63.05	[54.1, 74.1]	< 0.001
Phosphorus	Unburned vs. Burned	-2.193	[1.13, 3.26]	< 0.001
	Turret vs. Burned	9.113	[8.05, 10.2]	< 0.001
	Turret vs. Unburned	11.306	[10.2, 12.3]	< 0.001
Zinc	Unburned vs. Burned	-1.132	[0.896, 1.368]	< 0.001
	Turret vs. Burned	0.831	[0.595, 2.07]	< 0.001
	Turret vs. Unburned	1.963	[1.73, 2.2]	< 0.001
Carbon %	Unburned vs. Burned	-0.96	[0.652, 1.27]	< 0.001
	Turret vs. Burned	0.657	[0.349, 0.965]	0.004
	Turret vs. Unburned	1.617	[1.31, 1.92]	< 0.001
Nitrogen %	Unburned vs. Burned	-0.079	[0.047, 0.111]	< 0.001
	Turret vs. Burned	0.009	[-0.023, 0.041]	0.772
	Turret vs. Unburned	0.088	[.056, 0.12]	< 0.001
pH	Unburned vs. Burned	-0.027	[-0.17, 0.224]	0.939
	Turret vs. Burned	0.619	[0.422, 0.816]	< 0.001
	Turret vs. Unburned	0.646	[0.449, 0.843]	< 0.001

198

199 Turret soils were more alkaline and had significantly higher concentrations of most nutrients than burned soils

200 (Figure 2). This difference was greatest for Ca and P (Fig. 2a, Fig. 2e). Zinc, C%, and pH were also significantly

201 greater in turret than in burned soils, but less than Ca and P. (Fig. 2f, Fig. 2g, Fig. 2i). Nitrogen percentage, K, and

202 Mg concentrations did not differ between turret and burned soils (Fig. 2b, Fig. 2d, Fig. 2h). Magnesium

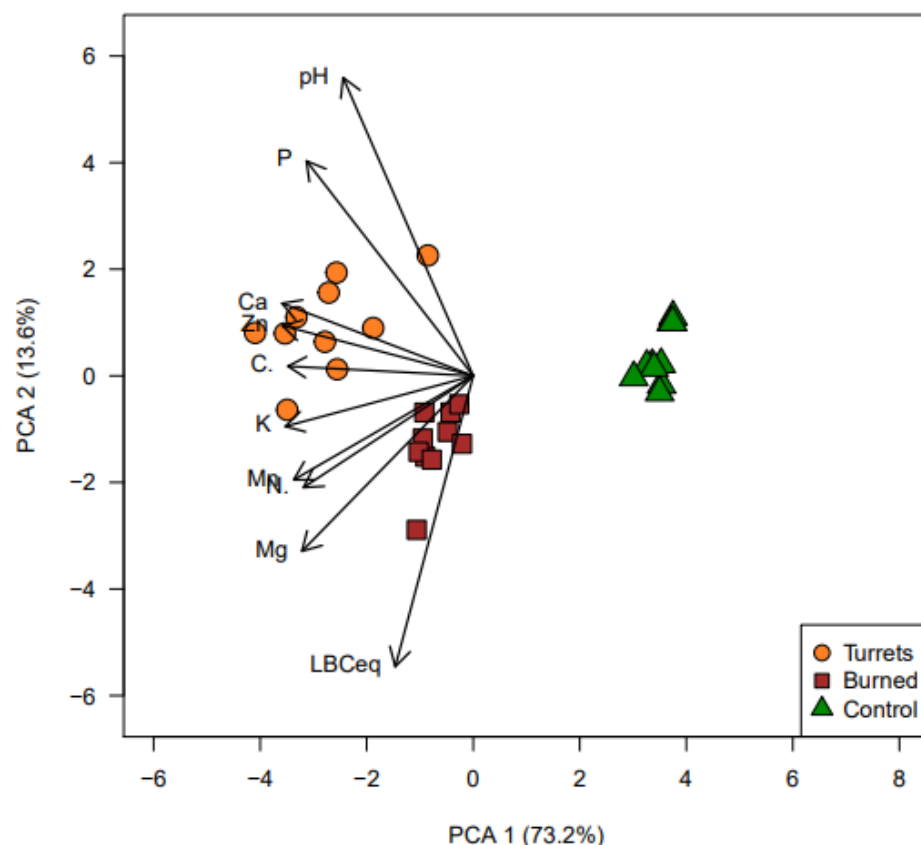
203 concentration was lower in turret soils than burned soils (Fig. 2c).

204 Soil concentrations of all nutrients tested were greater in burned soil than in unburned soil (Fig. 2;  $P < 0.001$  in all

205 comparisons). However, pH did not differ significantly between burned and unburned soil (Fig. 2i) Additionally, the

206 turret soils had significantly larger concentrations of nutrients and greater pH than the unburned soil (Fig. 2;  $P <$

207 0.001 in all comparisons).



**Figure 3.** Principal components analysis (PCA) biplot showing the variation in soil chemistry between the turret, burned and unburned (“Control”) samples. The first two principal components accounted for >86% of variation in soil chemistry. C.: Carbon Percentage, N.: Nitrogen Percentage, Ca: Calcium, K: Potassium, Mg: Magnesium, Mn: Manganese, P: Phosphorus, Zn: Zinc, LBC<sub>eq</sub>: Equilibrium Lime Buffer Capacity, pH: Potential of Hydrogen.

The first two principal components (PC) explained >86% of the variation in soil nutrients (Figure 3), with PC1 alone accounting for ≈73% of the variation. PC1 described a gradient of decreasing nutrient concentrations, particularly C%, K, Zn, and Ca. PC2 described a gradient from acidic to alkaline samples. Samples of the three soil types tended to group together within the PCA space (ANOSIM  $R = 0.222$ ,  $P < 0.001$ ).

**Table 2.** List of species that germinated in burned and unburned soils over the course of the experiment. Values show number of individuals that germinated in each treatment. The final column shows whether they are known to be germinated by the presence of smoke. Taxa with an asterisk (\*) developed sporophytes. Determination of whether the individual species is an annual and its seed length is provided by SERNEC Data Portal, 2022.

<b>Taxon</b>	<b>Burned + Turrets</b>	<b>Burned + No Turrets</b>	<b>Unburned + Turrets</b>	<b>Unburned + No Turrets</b>	<b>Annual</b>	<b>Seed Length (mm)</b>
<i>Apium graveolens</i>	0	1	0	0	No	1-2
<i>Bellis sylvestris</i>	1	1	0	0	No	<1
<i>Betula</i> spp.	0	0	3	0	No	TBD
<i>Cardamine hirsuta</i>	3	2	3	7	Yes	1-2
<i>Cerastium</i> spp.	0	0	0	1	Yes	<1
<i>Dennstaedtia</i> spp.*	1	2	5	7	No	N/a
<i>Fragaria</i> spp.	0	1	0	0	No	1-3
<i>Liriodendron tulipifera</i>	0	1	1	2	No	1-2
<i>Lobelia inflata</i>	0	0	19	24	Yes	<1
<i>Lobelia siphilitica</i>	0	1	1	1	No	<1
<i>Packera obovata</i>	0	0	27	21	No	2
<i>Rubus</i> spp.	1	1	7	0	No	1-5
<i>Scrophularia</i> spp.	0	0	0	1	Unknown	1-2
<i>Solidago</i> spp.	2	1	0	1	No	TBD
<i>Veronica peregrina</i>	2	1	1	1	Yes	<1
<i>Viola</i> spp.	0	0	2	0	No	TBD
Grass 1	0	2	2	1	Unknown	Unknown
Grass 2	0	0	2	4	Unknown	Unknown
Grass 3	0	0	1	0	Unknown	Unknown
Unknown 1	0	0	0	3	Unknown	Unknown
Unknown 2	0	1	0	0	Unknown	Unknown
Unknown 3	1	0	1	2	Unknown	Unknown
Unknown 4	0	0	1	0	Unknown	Unknown
<b>Total Individuals</b>	11	15	76	76		
<b>Taxon Richness</b>	7	12	14	14		

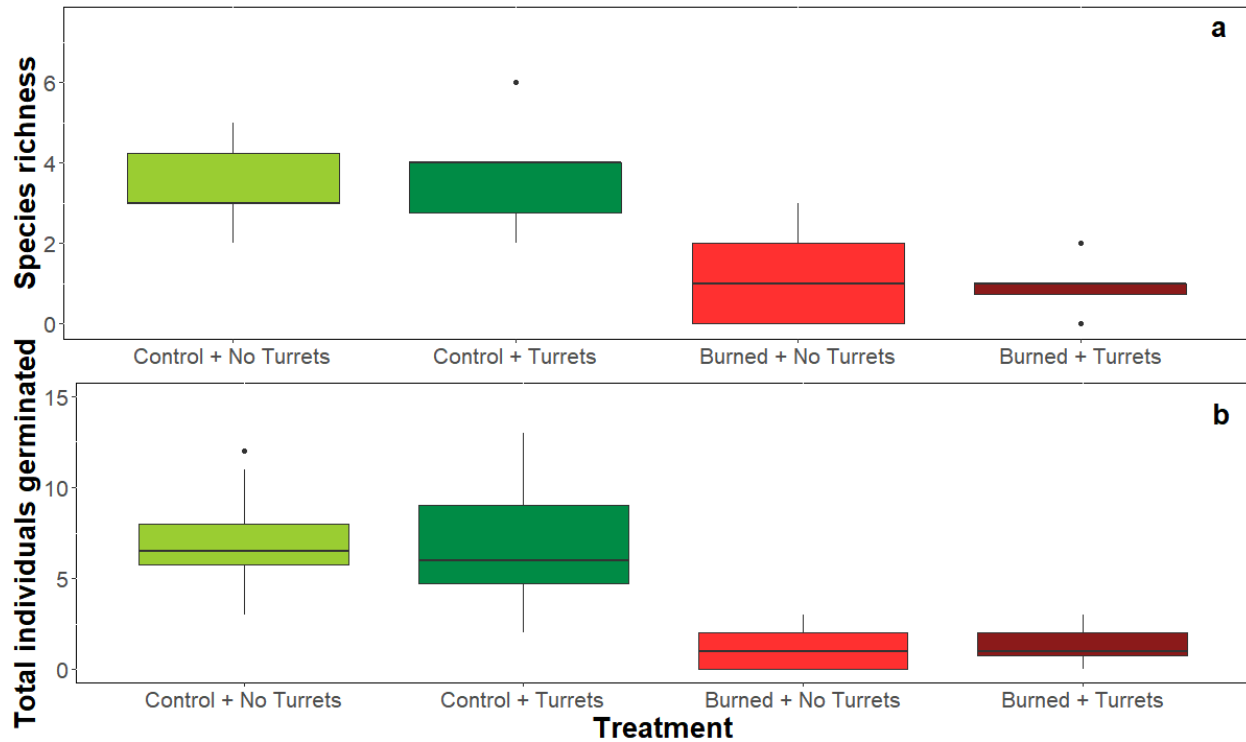
221

222 In total, 23 different species successfully germinated across all treatments. Most taxa appeared in the unburned soil

223 treatments, with 76 individuals in the unburned base + turrets treatment and 76 individuals in the unburned base +

224 no turret treatments.

225



**Figure 3. a.** Comparison of germination richness, between four possible soil treatments. Unburned (“Control”) **b.** Comparison of overall number of germinations, between four possible soil treatments. Unburned (“Control”).

**Table 3.** Parameter of the generalized linear model (GLM) with a Poisson family and log link function of the species richness and total individuals to germinate. Parameters shown are estimated mean ( $\lambda$ ) with standard error (SE) in the unburned treatment without turrets (“Intercept”) and effects of burning, turrets, and burning and turrets. Z is the test statistic and P is the P-value.

Parameter	Species Richness			Total Individuals Germinated		
	Estimate $\pm$ SE	Z	P	Estimate $\pm$ SE	Z	P
Intercept	0.080 $\pm$ 0.278	0.289	0.772	0.223 $\pm$ 0.258	0.864	0.387
Burned	1.170 $\pm$ 0.317	3.700	< 0.001	1.720 $\pm$ 0.280	6.150	< 0.001
Turrets	-0.167 $\pm$ 0.410	-0.408	0.683	0.057 $\pm$ 0.402	0.853	0.853
Burned:Turrets	0.214 $\pm$ 0.463	0.461	0.645	-0.069 $\pm$ 0.372	0.142	0.887

Species richness and number of individuals germinated were both significantly smaller in burned pots than in unburned pots regardless of turret presence (Fig. 3a, Fig. 3b;  $P < 0.001$  in both comparisons). There was no significant effect of turret presence or an interaction between turret presence and burning on species richness or number of individuals germinated (**Table 3**).

## Discussion

Our soil analysis results partially supported our first hypothesis that the soil comprising turrets will have the highest nutrient content and pH compared to the other soil samples. Turret samples had significantly higher concentrations of Ca, P, Zn, C%, and a higher pH (**Fig. 2a, Fig. 2c, Fig. 2f, Fig. 2g, Fig. 2i**) compared to both the burned and unburned soils. This tends to support the hypothesis that cicada burrowing is an important source of bioturbation. When cicadas are constructing their turrets, they use their bodily fluids, potentially from the midgut, to soften the soil making it easier to manipulate (Luken and Kalisz 1989). Thus, the increase in Ca and P may be due to the food source of cicadas, xylem fluid, which results in the midgut of cicadas containing a high concentration of these two nutrients (Cheung and Marshall 1973). Although also present in xylem fluid, the concentration of Mg was significantly lower in turret soils than in the burned soils (**Fig. 2c**). A possible reason that we saw high levels of Mg in burned soil is because the burning of organic matter released a pulse of exchangeable Mg in surface soils. Over time the nutrient pulse on the surface are either taken-up by biota or are leached further downward in the soil. However, this does not occur with the turrets since Mg leaching cannot flow against gravity and up to the turrets. The turrets will then contain the same amount of Mg they had following the fire, while the burned soil will see a spike in Mg in the weeks following the fire (Yildiz et al. 2010). Finally, the total C% was also significantly higher in the turrets than in the burned or unburned soil. A possible explanation is suggested by the appearance of the turrets (Figure 1). Many of the turrets had organic matter fused within them; it is unknown whether this occurred prior to or during the fire. Regardless, this will impact the total carbon percentage. Since we did not measure any specific form of C, the organic matter would have greatly increased the C within the turret samples. One caveat to our analysis is that turrets were only collected from burn plots, because no turrets could be located in the unburned plots. We assume that these turrets in the unburned plots were essentially the same as the turrets collected. Despite this shortcoming, our analysis does show significant differences between the soil within cicada turrets and the surrounding soil.

Our hypothesis that burned soil will contain the highest pH and largest total C% was not supported. We predicted that turrets would contain the highest nutrient content due to both the effect of fire on the soil and the additions that cicadas bring, with the exception of the total C% and pH (**Fig. 2h, Fig. 2i**). This was not supported by our data as the turrets still had the highest total C% and pH. Burned soil only had a higher total C% than the unburned soil, but

between the two soil types, there was no significant difference in pH. While many chemical byproducts of fire can raise pH, a significant increase only occurs at temperatures exceeding 450 °C (Certini 2005). The temperatures of the prescribed burning must have been lower since we did not see an increase in the pH of the burned soil. In a previous study, cicada turrets were found to have similar pH levels and had nearly halved C% compared to the soil within the top 5 cm (Luken and Kalisz 1989). However, their study did not include the turrets being heated, which may have contributed to the increase in pH. Since we only analyzed turrets that were collected from burned areas, we unfortunately cannot test this. The organic matter fused into the turrets (Figure 1) may have increased total C percentage in turret soils. Additionally, when collecting the burned soil samples, we brushed away any organic matter, like burned leaves and twigs, that could have increased the total C%.

Finally, our hypothesis that the pots with the treatment of burned soil and turrets would have a positive impact on understory plant germination was not supported. We found that the pots with the burned soil had lower species richness and numbers of individuals germinated (**Fig. 3a, Fig. 3b and Table 3**). Additionally, the turrets had no impact on germination in either soil type. The increase in nutrients brought about by both the fire and cicada emergence leads us to the conclusion that increasing nutrient availability does not directly increase germination. The burned soil contained significantly higher nutrients than the control in all circumstances (**Figure 2**), yet as seen in the germination study (**Fig. 3a, Fig. 3b and Table 3**) the increase in nutrients provided by the burned soil did not increase the germination occurring within the pots.

A possible explanation may be differences in seed size and dispersal. Many plants in our study produce small seeds, around 1-2 mm in length; these seeds get trapped in the leaf litter until they eventually sink to the surface of the soil. When collecting the soil samples, the leaf litter and other debris were moved aside. This action may have shaken the seeds into the soil, leading to increased germination. The burned sites, however, had most of the leaf litter burned away, including any seeds that may have been present at the time, and seeds that were present during the collection period may have been limited to those blown in from the unburned sites following the fire.

Another possible factor affecting germination may be moss cover and exposed mineral soil. A few weeks into the germination study, the pots began to grow patches of moss in places where the turret soil was not present. This phenomenon is likely due to a high concentration of Zn in the turrets preventing the growth of moss (Mohanasundaram and Pandey 2022). This may aid in preventing moss growth on cicada turrets, which would prevent obstruction by moss and allow for easier movement by the cicada during emergence. The turrets may result



in an extended period in which the mineral soil is exposed. Since exposed mineral soils dry faster, limiting the soil's ability to retain moisture, and chances of seeds germinating might be decreased. On the other hand, some seeds require exposed mineral soil for germination and further research is required to understand species-specific responses to fire and simultaneous cicada emergence.

We found higher concentrations of nutrients in both fire and turret soil samples, even though more seeds germinated in the unburned treatments. These results imply that increased soil nutrient concentrations had little effect on seed germination. Although we only used 40 g of the turrets in each designated pot, which was a much smaller amount of soil compared to the base soils (burned and unburned), additional nutrients may impact growth and survival rates after the seeds have broken dormancy and germinated. The ash created during the fire is deposited on the surface of the burned sites, where the nutrients can seep into the soil and be absorbed by plants (Taiz et al. 2018). The Ca and P deposited by the cicadas may increase the health of plants in the short to medium term. Calcium aids in the formation of cell walls and membranes (Thor 2019). Without it, plants lose their turgor and are unable to retain water, eventually drying out (Simon 1977). Phosphorus is crucial in the photosynthesis of plants, as it is the vital component of ATP and other crucial compounds in the production of energy (Carstensen et al. 2018). Phosphorus is also one of the most limiting nutrients for plants due to phosphorus being added only very slowly to ecosystems, by the weathering of rocks (Menge et al. 2012). So, in areas with P deficiencies, this pulse brought about by the periodical cicada emergence might temporarily increase floral productivity.

## **Conclusion**

Our study shows that the compound disturbance of prescribed burning and soil bioturbation due to cicada emergence can significantly impact soil chemistry and increase nutrient availability. We also found that these two events had either no effect or a negative effect on seeds germinating during the timeframe of our study. However, short-term increases in nutrients may impact growth and health of understory plants, and therefore plant dynamics (e.g., competition outcomes) in Georgia forests where this compound disturbance occurred. The initial plant succession that follows this compound disturbance may be a burst of vegetation from the increased nutrients and open areas for growth. For a time, these areas may support a higher number of individuals that also have larger biomasses in comparison to areas unaffected by the events. Further research should focus on the impacts of turret soil nutrient pulses on plant survival and growth, potentially by performing a physiological comparison between similar species grown in different soil treatments. Overall, our study represents one of very few studies on compound disturbances

and periodical cicadas, adding to the sparse literature and increasing our general knowledge of these subjects that can aid in forest understory plant species conservation and management.

### **Integration of Thesis Research**

Integrative research provides a broader view and allows for deeper understanding beyond a single field's limited understanding. This research integrates several disciplines and uses a variety of techniques to understand how the compound disturbance of a periodical cicada emergence and a prescribed fire impact soil composition and seed germination. The fields of study involved in this research are fire ecology, soil science, chemistry, botany, entomology, statistics, and forest management. The overall focus of our study is examining the impact both fire and insects, specifically periodical cicadas, have on soil components and seed germination. When starting with the relationship cicadas have with the surrounding soil, it was necessary to understand their life cycle and ethology through their bioturbation. The periodical cicadas deeply rely on trees as a food source, a mating ground, and a nursery for their eggs to hatch, though their lifestyles have little to no impact on the trees. The other half of this research is the prescribed burns, which act as a substantial nutrient cyclers. The fire breaks down organic matter and volatilizes compounds, creating an open forest floor for new individuals to inhabit.

After the fieldwork to collect the soil samples, we utilized UGA labs to understand the soil components in the samples. We also performed a germination study to examine any impact the altered soil may have on seed germination. Through data analysis performed with the program RStudio, we interpreted our results and gained a new understanding of these disturbances' impact.

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