

ORIGINAL ARTICLE

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Forest floor and Na azide effect on elements in leachate from contrasting New Hampshire and Virginia forest soils

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Abstract

As northeastern forests experience increased temperatures and fluctuations in precipitation patterns, montane soils will lose forest floor (Oi, Oe, Oa horizons) formation and their associated ecosystem services. Here, we conducted comparative laboratory soil column experiments to examine the effects of forest floor on sourcing and weathering of macroelements (Al, Si), macronutrients (Ca, Mg, P), and micronutrients (Cu and Zn) from two contrasting soils: a supraglacial-till Inceptisol (Mt. Moosilauke, NH) and colluvium Ultisol (Lesesne State Forest, VA). Forest floor addition caused a significant increase in the leaching of Ca, Mg, and Mn in the two soils. The forest floor only control leachate indicates the two mineral soils were net accumulators of Al, Si, P, Cu, and Zn from the forest floor. Using partition coefficient, K_d , values, leachate Ca and Mg could be sourced directly from the forest floor traversing the soil column. We aimed to assess the biotic influence on element release from soil using a Na azide treatment to suppress microbial activities. Under Na azide treatments, Al, Ca, Mg, Mn, and Cu leachate decreased significantly for both soils, but Na azide also did not affect or increased leachate Si, P, Cu, and Zn for the Lesesne soil. We attribute the effects from Na azide to changes in pH, dissolved organic carbon, and oxidation-reduction potential as opposed to suppression of microbes. Thus, our results suggest that the loss of the forest floor will reduce the storage of nutrients in the mineral soil, even across varying parent materials.

1 | INTRODUCTION

As shifting climate patterns, changes in tree species composition, and human management practices continue to take hold across the Northeastern United States, it is important to understand and quantify the potential impacts on forest soils from

these changes. In particular, the forest floor (the combined Oi, Oe, and Oa horizons) serves as an essential link between the aboveground forest and belowground mineral soil processes and is a key physical, chemical, and biological component of forest soils. The forest floor serves as an important reservoir for nutrients (Gosz et al., 1976; Landuyt et al., 2019; Richardson & Friedland, 2016), affects moisture and carbon storage in soils (Rawls et al., 2003), and serves as a germination location for seedlings and understory plants (Han et al., 2018; Myster, 1994). Furthermore, alterations to the forest floor may impact the release of macroelements (e.g., aluminum [Al], silicon [Si]) and nutrients (calcium [Ca], magnesium [Mg], phosphorus [P]) present in silicate minerals

Abbreviations: DOC, dissolved organic carbon; EC, electrical conductivity; FFADD, forest floor added; FFONLY, forest floor only control; K_d , partition coefficient; LOI, loss on ignition; NA-FFADD, sodium azide and forest floor added; NA-FFONLY, sodium azide added to forest floor only control; NA-NFF, sodium azide added with no forest floor added; NFF, no forest floor; OM, organic matter; ORP, oxidation-reduction potential.

of the underlying mineral soil. In addition to releasing macroelements and nutrients from decomposing plant matter (e.g., Gérard et al., 2007), organic acids leaching from the forest floor produce acidity (Schwesig et al., 2003) and chelators that enhance chemical weathering and dissolution of the mineral soil grains (Uhlig & von Blanckenburg, 2019). Conversely, organic acids released from the forest floor can generate insoluble organo-mineral complexation, which helps to bind an element to the mineral surfaces (Schmitt et al., 2017). These processes of mineral dissolution and retention by organo-mineral complexes can expand soluble and/or exchangeable nutrient pools in mineral soil from within the profile.

The effect of forest floor enhancing macroelement and nutrient release is governed by several soil properties and processes. First, the extent of weathering of the soil material can be important as less intensely weathered soils can have higher proportions of weatherable and nutrient-bearing minerals (e.g., Bailey et al., 2019), while highly weathered soils are typically dominated by nutrient-poor, more weather-resistant Al- and Si-rich minerals such as quartz, clays (e.g., kaolinite), and oxyhydroxides (e.g., boehmite, goethite, hematite; Schaller et al., 2010). Second, physicochemical properties of the soil such as pH, organic matter (OM) content, and clay content can affect element solubility, sorption capacity by the soil, and the surface area for reactions, respectively (Finlay et al., 2020; Uhlig & von Blanckenburg, 2019). The same properties can drive retention of the macroelements and nutrients and promote adsorption to solid phases, preventing their leaching from the mineral soil profile. Further, these physicochemical properties can also control water flow and gas diffusion into the soil, controlling redox states and flushing of solutions interacting with mineral surfaces (McNiccol & Silver, 2014; Schaller et al., 2010). Third, the forest floor may also indirectly cause accelerated mineral weathering by stimulating microbial community activities, which can enhance weathering of minerals (Mapelli et al., 2012; Park et al., 2002; Sokol et al., 2022). Microbial communities release enzymes, organic acids, and other forms of dissolved organic carbon (DOC), which dissolve mineral surfaces (Adeleke et al., 2017).

The main objective of this project is to determine if the addition of forest floor material to the mineral soil enhanced the dissolution of macroelements and nutrients from soils. The secondary objective was to determine if the extent of weathering of the soil materials or an anti-microbial, organic ligand would significantly alter the effect of forest floor addition. These results are important for understanding the complex plant–soil interactions that will occur with the predicted changes in climate, tree species composition of the forests, introduction of forest floor-consuming invertebrates, and management practices.

Core Ideas

- Forest floor increased Ca, Mg, and Mn in leachate but soils retained Al, Si, and P added by the forest floor.
- Leachate Ca and Mg were likely sourced from desorption or the forest floor, as partition coefficient, K_d , values indicate.
- Na azide decreased elements release from the soil but through abiotic processes, not microbial suppression.

2 | METHODS

2.1 | Soil characteristics

We examined two strongly contrasting soils underlying secondary growth deciduous forest sites: less intensively weathered, supraglacial-till derived from Mt. Moosilauke in the White Mountains of New Hampshire and highly weathered, colluvium granite from Lesesne State Forest in Blue Ridge Mountains of Virginia. Both soils were collected beneath a 225-cm² block template to a depth of 30 cm to allow for volumetric and density measurements. Soils were oven dried at 60°C, sieved to <2 mm and homogenized. Soils were analyzed for their physicochemical properties (pH, % OM, particle size distribution, mineralogy). Soil pH was determined using a 2:5 soil/water slurry method. The soil/water slurry was shaken for 1 h, settled over 8 h, and the pH of the supernatant extract was measured with a VWR 8051 pH meter. OM content of the soils was determined by loss on ignition (LOI) where 5 g of soil were weighed into crucibles and combusted in a muffle furnace at 550°C for around 8 h. Samples were analyzed for soil particle size distribution using sedimentation columns and Bouyoucos hydrometer method (Mwendwa, 2022). Exchangeable element fraction was measured by ammonium chloride extraction, and total elemental concentrations were determined by hydrofluoric-nitric acid (HF-HNO₃) strong acid digestion in Teflon vials following methodology from Richardson et al. (2022). Mineralogical characterization of the shale samples was performed by X-ray diffraction on random powder mounts and orientated mounts for in-depth clay mineral identification. For quantification, approximately 0.5 g of each sample was mounted on a glass slide and analyzed between 5 and 55° 2θ (0.02° resolution, 1°/min) using Rigaku Miniflex-2 equipped with a copper (Cu) K_α X-ray source. Quantification was performed using the Rietveld whole pattern profile fit module in PDXL2 (Rigaku Corporation 2007–2017).

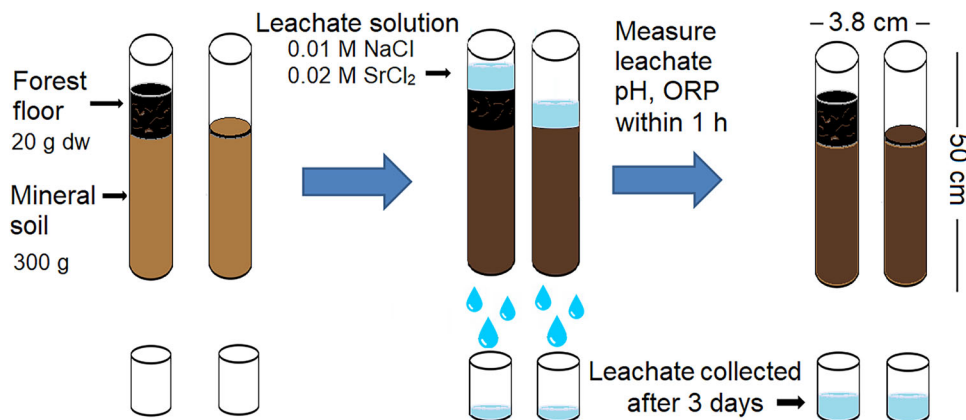


FIGURE 1 Schematic of the column experiments demonstrating how each leaching event was conducted as well as the column dimensions (50-cm long by 3.81-cm diameter). ORP, oxidation-reduction potential.

2.2 | Soil column experiments

A soil column leaching design was used to control soil properties and measure responses (Figure 1). Twelve soil columns (50-cm height, 3.81-cm diameter) were packed with 0.3 kg of soil from Mt. Moosilauke, NH (six columns) or Lesesne State Forest, VA (six columns). One column containing only 20 g of forest floor was created to serve as a control of leachate from the forest floor. Soil was secured from the bottom of each column with 20- μ m mesh. Columns were wetted from the bottom by matric forces over 24 h and once the surface was moist, a 12-cm constant head was maintained using gravimetric hydraulic pull from tubing to a large reservoir. Soil in columns that exhibited macroporosity and above-average flow rates was re-dried and re-packed. Among the six soil columns from each soil, three received either no forest floor (NFF) or 20 g of dried, shredded, homogenized Oe+Oa horizon material from Mt. Moosilauke, NH. The 20 g of forest floor per 11.4-cm² columns corresponded with the average forest floor masses currently in New England mountainous areas (Richardson & Friedland, 2016; Richardson et al., 2014). This study resulted in the following treatments: forest floor only control (FFONLY), forest floor added (FFADD), NFF, sodium (Na) azide added to FFONLY (NA-FFONLY), Na azide and FFADD (NA-FFADD), and Na azide and NFF (NA-NFF). With the one control, there were 13 columns total with three replicates of FFADD per soil and three replicates of NFF per soil.

Since the saturated flow is unrealistic for upland, extremely well-drained soils, we utilized a 3-day flushing event to simulate precipitation-drying cycles over 60-day periods. For each event, 75 mL of a solution containing 0.01 M NaCl and 0.04 M SrCl₂ was added to each column. The 1:4 ratio of NaCl and SrCl₂ was used to maintain ionic strength and allow for flocculation of clays, which prevents them from washing through

the column or clogging micropores. The solution pH was 7.04 and solution pH with Na azide was 7.28. The leachate was collected and analyzed the same day for pH, oxidation-reduction potential (ORP), and electrical conductivity (EC) using a VWR 8051 pH meter and Atlas Scientific “Industrial” ORP and EC meters.

The second part of the experiment involved the same set of columns following the first 60-day event. Here, a new solution of 75 mL of 0.01 M Na azide and 0.04 M SrCl₂ was added to the columns every 3-day event with a solution pH of 7.28. The same process was followed as described previously. All leachate samples were acidified with 0.2 mL of 15.6 M HNO₃ and analyzed for macroelements and nutrients with an Agilent 7700 ICP-MS and an Agilent 5110 ICP-OES. DOC samples were analyzed from sample leachates taken from every other leaching event for the first half of the experiment and from every third leaching event for the second part of the experiment with Na azide.

2.3 | Adsorption experiments

Adsorption experiments were also conducted to determine the distribution coefficient, K_d , of the macronutrients Ca, Mg, and P and micronutrients Cu and zinc (Zn). The solid and aqueous phase concentrations were used to obtain the K_d partitioning coefficients, by using the slope of the linear regression line. Using Darcy’s Law (Equation 1), the velocities of the elements traveling through the column were calculated (Equation 2), which was then used to calculate the duration of transport for an element’s theoretical movement through the soil column (Equation 3).

$$\text{Water velocity} = -K_{sat} \left(\frac{\Delta H}{Z} \right), \quad (1)$$

$$\text{Element velocity} = -\frac{K_{\text{sat}}}{K_d} \left(\frac{\Delta H}{Z} \right), \quad (2)$$

$$\text{Element transport time} = -\frac{\text{Column length } (Z)}{\text{Element velocity } (Ev)}. \quad (3)$$

A stock solution of 500 mg L⁻¹ was made from salts (CaCl₂, MgCl₂, ZnCl₂) or Na₃PO₄. Serial dilutions of 1, 5, 10, 25, 50, 75, and 100 mg L⁻¹ were made by using aliquots of the stock solution and diluting with deionized water to the desired concentration. For the adsorption reactions, 10 mL of diluted solution was added to 2 g of soil in 50 mL centrifuge tubes, shaken for 3 h and centrifuged at 2500 rpm for 30 min, then filtered to <0.45 μm. This was replicated three times per concentration and repeated for each soil. Placed into 15-mL centrifuge tubes, 6 mL of the supernatant was diluted with 6 mL of 0.55 M HNO₃. The soil portion was pseudototal digested with aqua regia (9:1 15.6 M HNO₃ and 12.1 M HCl). The solutions were diluted and analyzed with an Agilent 7700 ICP-MS and an Agilent 5110 ICP-OES to obtain the concentrations. The *K_d* value of each element and nutrient was the slope of a linear regression model between the solid phase and aqueous phase concentrations in the soil.

2.4 | Solution chemistry and phase modeling

The speciation of aqueous phase ions and calculation of mineral saturation indices was performed using the Geochemist's Workbench Community Edition (Aqueous Solutions LLC). Leachate stability and solid phase solutions were modeled using individual replicate solution concentrations of Al, Ca, iron (Fe), potassium (K), Si, strontium (Sr), Na, and DOC, as well as pH and ORP. Calculations did not take into account mineral surface sorption reactions, and existing OM was not included for soils. Ionic strength of the solution was calculated from inputs, and the temperature was set at 25°C and set at 1 bar pressure. For our results in the first panel, we focused on the potential precipitation of three Si-Al minerals (gibbsite, kaolinite, and opal A) and in the second panel on Mn minerals (pyrolusite, bixbyite, and hausmannite).

2.5 | Data and statistical analysis

Statistical analyses were conducted using the Kruskal–Wallis test in R Studio (R Core Team, 2021) with the base packages. The response variable was the concentration for each element with forest floor additions and Na azide treatments as categorical fixed effects. The Kruskal–Wallis test compared the forest floor addition types for each soil, which included comparisons between FFADD and NFF additions for the two soils and between NA-FFADD and NA-NFF for the two soils,

as well as FFADD and NA-FFADD and NFF and NA-NFF. Error bars generated for the mass concentrations per day comparison were determined by finding the standard deviation among the three replicate columns of the same soils with the same additions.

The cumulative total leachate mass for elements was determined by summing each leachate mass for the individual columns and determining the average among the three columns for Moosilauke and Lesesne soils under FFADD, NFF, NA-FFADD, and NA-NFF. We attempted to determine the average-event leachate mass for the elements and tested two methods. The first method was using the cumulative total leachate mass and dividing it by the total number of the events (20 leaching events over the 60 days) to establish the average-event leachate mass. Second, we determined the quasi-steady state as the arithmetic average of the largest range of log-transformed elemental masses per event leachate masses yielding a linear slope not significantly different than zero for each soil and treatment per column (see Supplemental Tables S1 and S2). Fortunately, the average-event leachate mass and quasi-steady state were not significantly different, so only the more direct average-event leached is used going forward.

3 | RESULTS

3.1 | Soil properties, leachate pH, ORP, and DOC

Soil physical properties, pH, and total elemental concentrations are shown in Table 1, and soil exchangeable nutrients are in Supplemental Table S3. Soil pH and LOI were higher for Lesesne than Moosilauke. Sand content was much lower for Lesesne, and the clay content was higher for Lesesne. Despite different locations and extent of chemical weathering, soil mineralogy was similar between the two sites due to their similar granitic bedrock lithology. The mineralogy was dominated by quartz, feldspar, mica (muscovite and biotite), and hornblende (Table 1). Total soil Al, Ca, manganese (Mn), P, and Cu were higher for Lesesne soil, while total Mg, Si, and Zn were higher for Moosilauke soil. The forest floor had higher LOI but lower Al, Mg, and Si than the Moosilauke and Lesesne soils but comparable Ca, P, Zn, and Cu concentrations as Lesesne soil. Exchangeable Al and Zn were higher for Moosilauke, while Lesesne had higher exchangeable Ca, Mg, and Mn. Exchangeable P, Si, and Cu were similar for the two soils. The forest floor had higher exchangeable Ca than the Moosilauke and Lesesne soils.

Throughout the experiment, leachate pH, ORP, and EC were all measured within 1 h of the start of each leaching event, once the solution had flowed through the column (Figure 2). This same-day method provided the closest

TABLE 1 Soil physical and chemical properties of the two soils from epipedons studied.

		Loss on ignition (LOI)	Sand	Clay	Quartz	Mica	Feldspar	Hornblende
	pH	%	%	%	%	%	%	%
Forest Floor	3.4	67	N/A	N/A	N/A	N/A	N/A	N/A
	±0.1	±2	N/A	N/A	N/A	N/A	N/A	N/A
Mt. Moosilauke	3.8	10	76	10	55	15	22	6
	±0.2	±0.1	±2	±2	±2	±3	±2	±1
Lesesne State Forest	4.9	15	58	32	51	17	24	4
	±0.2	±0.3	±3	±2	±2	±2	±2	±1
	Al	Ca	Mg	Mn	P	Si	Zn	Cu
	g/kg	mg/kg	mg/kg	mg/kg	mg/kg	g/kg	mg/kg	mg/kg
Forest Floor	0.39	1800	700	170	630	4.7	41	12
	±0.02	±175	±64	±14	±27	±0.8	±3	±1
Mt. Moosilauke	112	264	2500	130	180	290	77	8
	±11	±30	±108	±6	±7	±70	±6	±0.2
Lesesne State Forest	150	2130	1400	790	804	143	46	13
	±43	±920	±210	±110	±143	±20	±9	±3

Note: Properties of the sites including the pH, amount of organic matter determined by LOI, mineralogy by X-ray diffraction, and percent of sand, silt, and clay present. Total concentration of elements found in each soil. Total digestion was measured using HF-NO₃ digestion, except Si measured by X-ray fluorescence. Each measurement was conducted in triplicate and error values are standard error.

TABLE 2 Average-event conditions under both forest floor added and no forest floor (FFADD and NFF, respectively) and Na azide treatments for pH, dissolved organic carbon (DOC), and oxidation-reduction potential (ORP) parameters $N = 3$ per treatment and their standard errors.

	Treatment	pH	ORP (mV)	DOC (mg/L)			
Control (FF)	FFONLY	3.4	±0.1	280	±4.8	120	±40
	NA-FFONLY	4.9	±0.2	210	±13	11	±1
Mt. Moosilauke	FFADD	4.7	±0.1	200	±6.2	200	±13
	NFF	4.5	±0.1	220	±6.6	100	±1.4
	NA-FFADD	5.8	±0.04	100	±8.6	14	±0.4
	NA-NFF	5.5	±0.02	140	±3.3	10	±0.2
Lesesne State Forest	FFADD	6.2	±0.03	47	±6.2	120	±9
	NFF	6.3	±0.03	49	±16	100	±19
	NA-FFADD	6.6	±0.1	23	±7.1	13	±0.3
	NA-NFF	6.7	±0.2	59	±14	11	±0.3

Abbreviations: FFONLY, forest floor only control; NA-FFADD, Na azide and forest floor added; NA-FFONLY, Na azide added to forest floor only control; NA-NFF, Na azide and no forest floor.

estimate to conditions within the soil column. Leachate pH was nearly constant for the duration of both experiments in the Lesesne soil remaining around 6.3 for both FFADD and NFF at their average-event. The Moosilauke soil leachate gradually increased over time, but the average-event pH reached 4.7 for FFADD and 4.5 for NFF columns (Table 2). The FFONLY control had the lowest pH of 3.4. The addition of Na azide increased the leachate pH for FFONLY to 4.7 and significantly increased pH in the Moosilauke NA-FFADD and NA-NFF to 5.8 and 5.5, respectively, and the leachate

pH also significantly increased in the Lesesne NA-FFADD and NA-NFF to 6.6 and 6.7, respectively.

FFONLY ORP was highest at 280 mV, while the FFADD and NFF Moosilauke soil were significantly below that at 200 and 220 mV, respectively (Table 2). Lesesne FFADD and NFF had significantly lower ORP values than Moosilauke at 47 mV for FFADD and 49 mV for NFF. The FFADD and NA-FFADD ORP values were significantly different than the NFF and NA-FFADD counterparts for either soil.

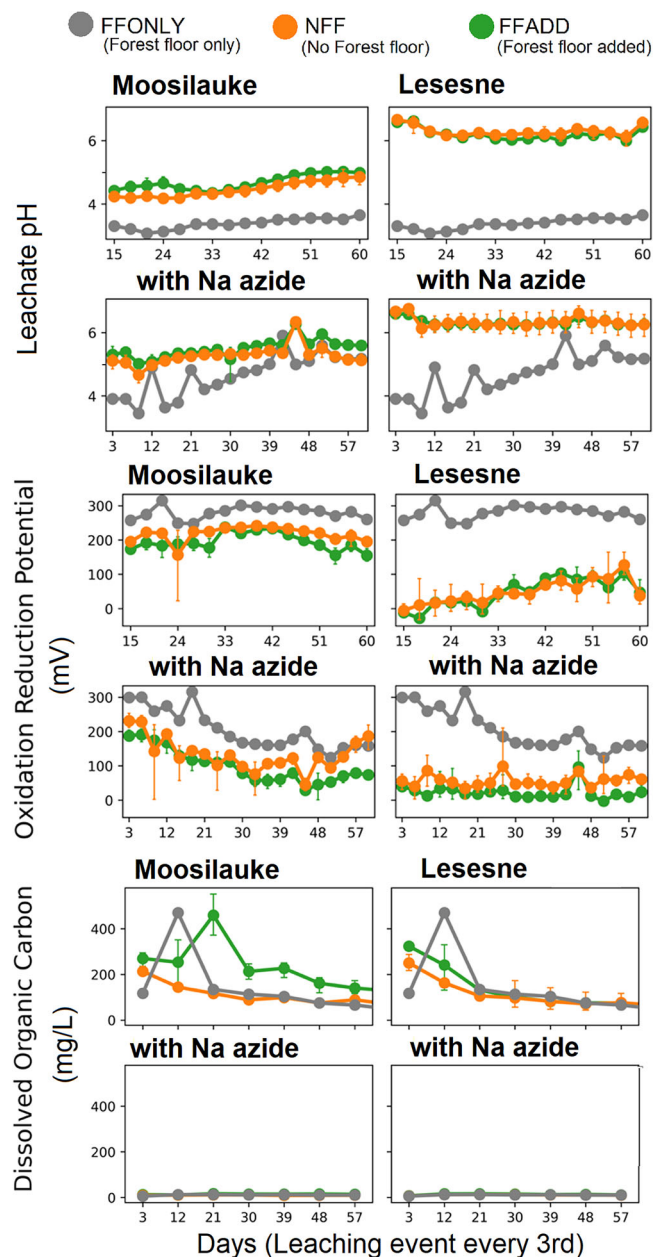


FIGURE 2 Changes in leachate pH, oxidation-reduction potential (ORP), and dissolved organic carbon (DOC) during the two 60-day column experiments measured the day of each leaching event. Average values for the three columns are shown and error bars are standard deviation.

Leachate DOC measurements show that FFONLY added 120 mg L^{-1} of DOC per average-event (Table 2). Moosilauke and Lesesne leachate were significantly different between FFADD and NFF with FFADD leaching higher DOC in both soils. However, Na azide also significantly affected the leached DOC in Moosilauke and Lesesne soils. NA-FFONLY DOC leachate was lower than FFONLY, and NA-FFADD and NA-NFF had significantly lower DOC than FFADD and NFF,

respectively, in both soils (Table 2). Na azide resulted in a 90% reduction in average DOC in the leachates.

3.2 | Macroelements (Al and Si)

The macroelements, Al and Si, served as proxies for aluminosilicate dissolution for this experiment. Changes in leachate Al and Si mass during the experiments are shown in Figure 3. Our average-event and cumulative total results show the forest floor had no effect on enhancing Al and Si dissolution (Tables 3 and 4). Average-event and cumulative total leachate Al mass for FFADD and NFF were not significantly different nor were NA-FFADD and NA-NFF, for both Moosilauke and Lesesne. However, Na azide significantly decreased Al leachate for both soils. Lesesne FFADD was much lower than FFONLY soils (Table 3). Following Na azide treatment, Al leachate mass decreased significantly for the Moosilauke and Lesesne soils. Silicon exhibited a slightly different pattern than Al. Na azide did not generate a significant effect for Moosilauke nor Lesesne average-event and cumulative Si leachate mass but Si average-event mass was significantly different between NFF and NA-NFF for both soils. NA-FFONLY had much higher Si leachate than FFONLY, which was the opposite of most other elements, which decreased with Na azide treatment.

As shown in Figure 4, the FFONLY treatment and Moosilauke soils with and without forest floor treatments generated soluble Al and Si. However, NA-FFONLY and Moosilauke with Na azide treatment had leachate solutions saturated with respect to kaolinite. Lesesne soils with and without Na azide treatment had leachate solutions saturated between kaolinite and gibbsite.

3.3 | Macronutrients (Ca, Mg, P)

Average-event and cumulative total leaching nutrients were compared between the forest floor and Na azide treatments over the 60-day experiment. Changes in leachate Ca, Mg, and P during the experiment are shown in Figure 3. Leachate Ca and Mg were significantly different between FFADD and NFF for Moosilauke and Lesesne (Tables 3 and 4). The FFONLY leachate contained 8 mg/event Ca and $570 \text{ } \mu\text{g/event Mg}$, which was comparable to Moosilauke FFADD but much lower than Lesesne FFADD. Under the Na azide treatment, the Ca and Mg leachate decreased significantly for Moosilauke, Lesesne, and the control FFONLY, except for Moosilauke NA-NFF. There was no significant effect of forest floor under Na azide treatment for Moosilauke, but there was a significant difference in Ca and Mg average-event mass in the Lesesne soil (Table 3).

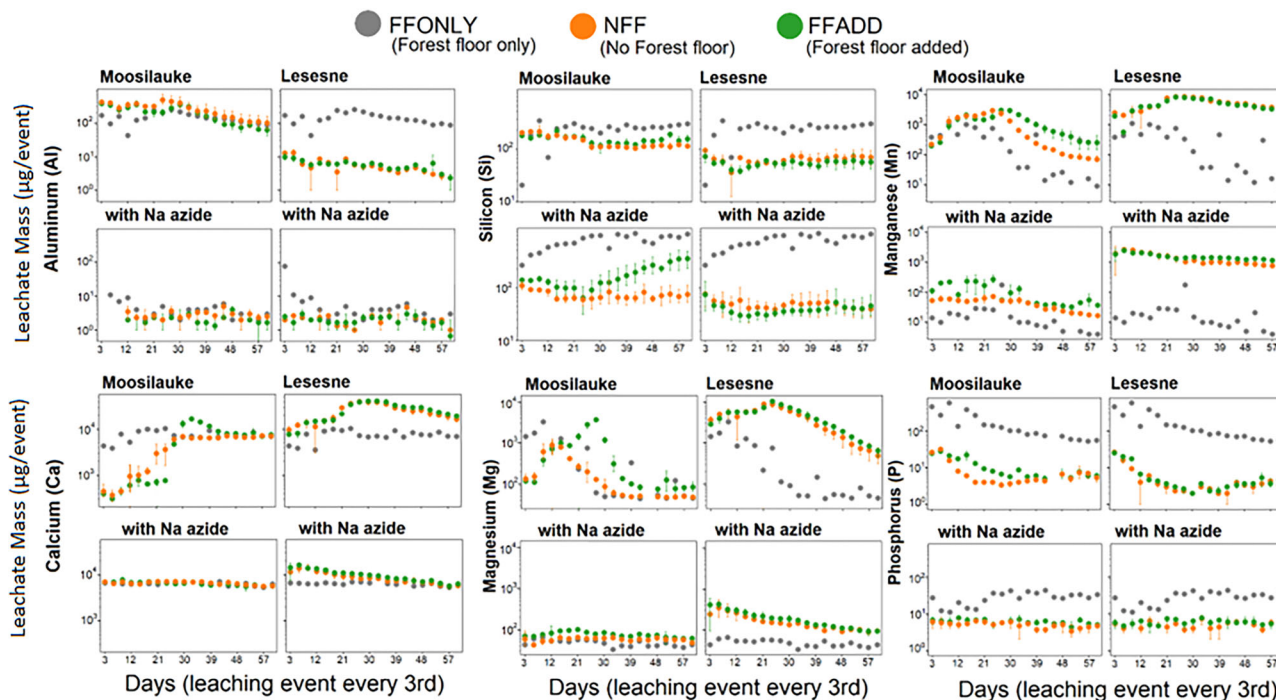


FIGURE 3 Changes in leachate Al, Si, Mn, Ca, Mg, and P during the two 60 days column experiments. Average values for the three columns are shown and error bars are standard deviation. Note the logarithmic y axis.

TABLE 3 Average-event leachate macroelements, macronutrients, and micronutrients. These represent a quasi-steady state reached by the columns. Error provided is the standard error (SE) of the three columns ($N = 3$).

		Al	Ca	Mg	Mn	P	Si	Zn	Cu
		µg/event	mg/event	µg/event	µg/event	µg/event	µg/event	µg/event	ng/event
Control	FFONLY	160	8.3	570	340	160	180	6.7	73
	NA-FFONLY	8	6.2	48	22	28	560	3.4	38
Mt. Moosilauke	FFADD	210	7.2	700	1300	12	130	5.1	110
		±14	±0.3	±51	±96	±0.5	±3	±0.02	±7
	NFF	290	5.5	240	950	12	120	7.4	130
		±48	±0.04	±34	±52	±3.3	±2	±0.7	±11
	NA-FFADD	8	6.4	77	130	6.1	160	1.9	39
		±1	±0.1	±4	±37	±0.3	±26	±0.4	±2
Lesesne State Forest	NA-NFF	21	7.6	64	63	6.2	90	3.9	44
		±8	±1	±6	±10	±1.1	±15	±0.8	±5
	FFADD	6	30	4800	5500	6	52	0.5	53
		±0.3	±0.3	±29	±130	±0.2	±5	±0.05	±2
	NFF	6	27	4200	5700	5.1	59	0.5	57
		±0.5	±0.6	±290	±420	±0.4	±3	±0.1	±5
	NA-FFADD	2	11	210	1800	6.7	51	1.6	50
		±0.4	±1	±7	±200	±0.8	±8	±0.1	±12
	NA-NFF	2	8.8	160	1400	4.8	53	1.6	41
		±0.1	±0.5	±11	±140	±0.1	±9	±0.2	±0.2

Abbreviations: FFADD, forest floor added; FFONLY, forest floor only control; NA-FFADD, Na azide and forest floor added; NA-FFONLY, Na azide added to forest floor only control; NA-NFF, Na azide and no forest floor; NFF, no forest floor.

TABLE 4 Cumulative totals and standard errors of the columns for both mineral soils and control.

		Al μg	Ca mg	Mg mg	Mn mg	P μg	Si μg	Zn μg	Cu ng
Control	FFONLY	3200	170	11	7	3200	3600	130	1500
	NA-FFONLY	160	120	1.0	0.4	560	11,000	67	760
Mt. Moosilauke	FFADD	4300	140	14	25	240	2600	150	2200
		±270	±6	±1	±2	±11	±62	±14	±150
	NFF	5800	110	5	19	230	2500	142	2600
		±950	±1	±0.7	±1.0	±65	±46	±7	±220
	NA-FFADD	160	130	2	3	120	3200	37	770
		±26	±2	±0.1	±0.7	±5	±510	±8	±39
	NA-NFF	420	150	1	1.3	120	1800	78	880
		±160	±21	±0.1	±0.2	±23	±300	±17	±90
Lesesne State Forest	FFADD	120	600	97	110	120	1000	10	1100
		±7	±6	±0.6	±3	±4	±97	±0.9	±34
	NFF	120	540	83	110	100	1200	10	1100
		±10	±12	±6	±8	±7	±58	±1.2	±94
	NA-FFADD	47	220	4	36	130	1000	33	1000
		±7	±19	±0.1	±4	±17	±150	±3	±250
	NA-NFF	44	180	3.2	28	96	1100	32	810
		±2	±9	±0.2	±3	±1	±180	±4	±4

Note: Elements are described as total mass leached out from all 60 days of the experiment. Al, P, Si, and Zn are in microgram, Cu is in nanogram, and Ca, Mg, and Mn are in milligram.

Abbreviations: FFADD, forest floor added; FFONLY, forest floor only control; NA-FFADD, Na azide and forest floor added; NA-FFONLY, Na azide added to forest floor only control; NA-NFF, Na azide and no forest floor; NFF, no forest floor.

The pattern for average-event and cumulative total leachate P was different than Ca and Mg because the FFONLY leached greater amounts of P, compared to the two soils (Tables 3 and 4). Leachate P was not significantly different between the FFADD and NFF treatments in either soil and under Na azide treatment as well. The exceptions with significant differences were Moosilauke FFADD versus NA-FFADD and Lesesne NA-FFADD versus NA-NFF. Furthermore, the FFONLY and NA-FFONLY caused an addition of 160 and 28 ug/event of P, respectively, which were much higher than leachate from Moosilauke and Lesesne soils.

3.4 | Micronutrients (Cu, Mn, Zn)

Average-event and cumulative total micronutrients Cu, Mn, and Zn had varying responses to the forest floor and Na azide treatments (Tables 3 and 4). Changes in leachate Mn are shown in Figure 3, and changes in Cu and Zn are in Supplemental Figure S1. Average-event leachate of Cu for FFADD was not significantly different than NFF for Moosilauke and Lesesne. Zn and Mn had significantly different FFADD than NFF in the Moosilauke soil, but this difference was not seen in Lesesne. FFADD had higher masses of Mn leach out, but the opposite was found for Zn where FFADD was lower than

NFF leachate mass. Under Na azide, Cu and Mn were significantly higher between NA-FFADD than NA-NFF, while Zn was significantly lower for Moosilauke. The FFONLY Cu and Zn leachate was comparable to leachate masses for NFF and FFADD. The forest floor was not an important source of Mn to the Lesesne soil, as it was 340 μg/event, while leachates ranged from 1300 to 5700 μg/event. Total cumulative leachate shows significantly higher Mn leaching from both soils than the control of FFONLY (Table 3). Total cumulative leachate Cu and Zn for FFONLY were comparable with the total FFADD and NFF of the Moosilauke soil but were much higher than in the Lesesne soil (Table 4). All treatments yielded solutions that promote soluble Mn as the pH was too acidic and ORP was too reducing to allow for the precipitation of Mn oxyhydroxide minerals (Figure 4).

3.5 | Adsorption and transport rate estimation

We conducted an adsorption experiment to estimate transport rates for elements to move through the soil to determine if forest floor elements can traverse the soil column within the duration of the experiment. Unfortunately for Cu, Zn, and P sorption was too strong to determine a *K_d* as the fraction in

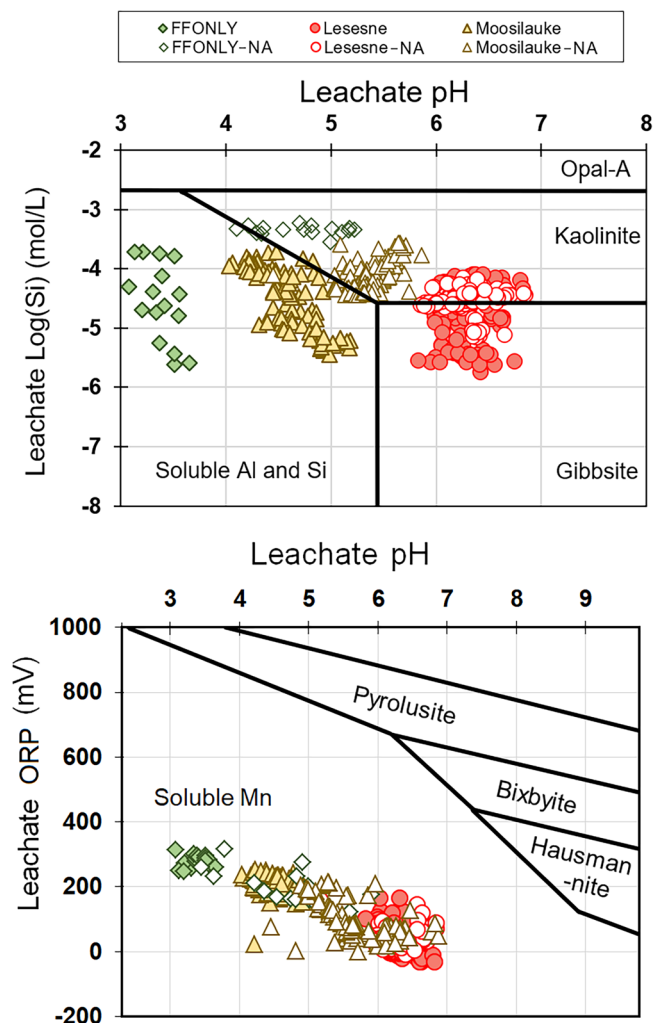


FIGURE 4 Phase diagram of leachate Si concentrations plotted with modeled opal-A, kaolinite, and gibbsite and phase diagram of leachate Mn modeled with pyrolusite, bixbyite, and hausmannite. Other species present in the models include Al, Ca, K, Na, S (assumed to be SO_4), and dissolved organic carbon were set at their median measured values for the respective soil and treatment and the model was set at 25°C, 1 bar pressure, and 800 mV. FFONLY, forest floor only control; FFONLY-NA, sodium azide added with no forest floor added.

solution was too low and indiscernible from the background for the range typically observed in soils in point-source contaminated and uncontaminated soils (Bergkvist et al., 1989). Thus, K_d was determined for Ca (Moosilauke $K_d = 319$, Lesesne $K_d = 230$) and Mg (Moosilauke $K_d = 530$, Lesesne $K_d = 580$). From measured water velocities using Cl^{-1} tracer, the Ca and Mg K_d values, and Equations 2 and 3, we determined Ca would take approximately 20 days to leach out of the Moosilauke soil and 42 days in the Lesesne soil; Mg would take about 33 days in the Moosilauke soil but 55 days in the Lesesne soil.

4 | DISCUSSION

4.1 | Forest floor additions on element leaching

For our first hypothesis, we expected forest floor addition to increase weathering and dissolution of minerals, resulting in increased masses of macroelements, macronutrients, and potentially micronutrients in the Moosilauke and Lesesne leachate. Our results show that forest floor addition (FFADD) did generate significantly higher Ca, Mg, and Mn leachate but not Al, Si, P, Cu, and Zn leachate (Tables 2, 3, and 5). However, contrary to our expected process of enhanced silicate mineral weathering, we can attribute the increases in Ca, Mg, and Mn to releases directly from the forest floor. The forest floor represented a substantial pool of Ca and Mg; the forest floor alone had a comparable average-event to Moosilauke leachate and approximately 25%–30% of average-event Lesesne leachate. Thus, the forest floor released Ca and Mg at rates that would mask any potential weathering from silicate minerals. The importance of the forest floor as a nutrient source agrees with field observations made by Dijkstra (2003) and would be most important for Lesesne soils, which had a larger pool of exchangeable Ca and Mg than Moosilauke soil and allowed for more exchange of cations with the leaching solution. Last, based upon the K_d values and transport rates, it is possible for Ca and Mg sourced from the forest floor to traverse through the soil column during the timeframe of the experiment.

The other dominant process we observed is that the forest floor was an important source for macroelements (Al, Si), macronutrient (P), and micronutrients (Cu, Zn), which appeared to have been sequestered within the mineral soil. Despite the solution chemistry suggesting soluble Si, FFONLY P and Si cumulative leachate results strongly imply that the Moosilauke and Lesesne soil had substantial adsorption of both elements onto the soil surfaces. This resulted in negligible amounts of forest floor P (3% to 8%) being leached without Na azide across Moosilauke and Lesesne soils. This storage of P can be important for understory and canopy trees, as adsorbed P onto the soil surface can be available for plant uptake (Penn & Camberato, 2019). Comparing FFONLY to NA-FFADD and NA-NFF for both soils, cumulative total Si leachate dropped between 9% and 29% from 28% and 72% without Na azide. Leachate Zn and Cu were largely unaffected by the forest floor treatment, and the size of the FFONLY leachate implies that Cu and Zn added from the forest floor could have significantly affected leachate masses. We hypothesize that Cu and Zn concentrations adsorbed to surfaces are too low in concentration or too slow in transport times to have an observed effect within the timeframe of the experiments (120 days total).

TABLE 5 Results of the Kruskal–Wallis statistical analysis.

	Al	Ca	Mg	Mn	P	Si	Zn	Cu	pH	ORP	DOC
Control											
FFONLY x NA-FFONLY	0.317	0.317	0.317	0.317	0.317	0.317	0.317	0.317	0.317	0.317	0.317
Mt. Moosilauke											
FFADD x NFF	0.127	0.049	0.049	0.049	0.513	0.127	0.049	0.275	0.049	0.049	0.049
NA-FFADD x NA-NFF	0.127	0.127	0.127	0.049	0.513	0.049	0.049	0.275	0.049	0.049	0.049
FFADD x NA-FFADD	0.049	0.049	0.049	0.049	0.049	0.513	0.049	0.049	0.049	0.049	0.049
NFF x NA-NFF	0.049	0.049	0.049	0.049	0.127	0.049	0.049	0.049	0.049	0.049	0.049
Lesesne State Forest											
FFADD x NFF	0.827	0.049	0.049	0.827	0.127	0.275	0.827	0.513	0.127	0.824	0.513
NA-FFADD x NA-NFF	0.827	0.049	0.049	0.127	0.049	0.827	0.827	0.513	0.827	0.127	0.049
FFADD x NA-FFADD	0.049	0.049	0.049	0.049	0.513	0.827	0.049	0.513	0.049	0.049	0.049
NFF x NA-NFF	0.049	0.049	0.049	0.827	0.513	0.049	0.049	0.049	0.513	0.049	0.049

Note: FFONLY compares the control between Na azide addition, FFADD and NFF were compared normally and under both treatments to assess the impact of forest floor addition, as well as compared between FFADD and NA-FFADD to assess the impacts of Na azide. *P*-values < 0.05 were considered significant and are in bold. Abbreviations: FFADD, forest floor added; FFONLY, forest floor only control; NA-FFADD, Na azide and forest floor added; NA-FFONLY, Na azide added to forest floor only control; NA-NFF, Na azide and no forest floor; NFF, no forest floor.

The forest floor was a substantial input of acidity and organic ligands to both mineral soils. As shown in Figure 2, the FFONLY had average-event leachate pH of 3.4 and added 120 mg/L DOC. Both mineral soils were able to buffer the acidity added by the forest floor as leachate pH for Moosilauke ranged from 4.7 to 5.8 and Lesesne 6.2 to 6.6 over the 60 days of leaching. Moreover, DOC inputs from the forest floor were less than DOC per average-event with FFADD greater than NFF resulting in a significant difference between FFADD and NFF. This suggests a net sequestration or mineralization of the DOC entering the mineral soil from the forest floor and net release of DOC from existing soil organic carbon (e.g., Schwesig et al., 2003), which has been observed at field scale (Borken et al., 2011). The decrease in ORP under FFADD for Moosilauke may be attributed to microbial O₂ consumption during the mineralization of forest floor and mineral soil DOC (Y. Li et al., 2022). The lower ORP likely resulted in more Mn reduction in the soil, particularly for the Lesesne soil, and would explain the Mn leachate exceeding forest floor inputs for the Lesesne soil under FFADD (e.g., H. Li et al., 2021). The solution chemistry shows that Mn was too acidic and too reducing to allow for the precipitation of Mn oxyhydroxides (Figure 4). Riedel et al. (2015) found that Mn leaching increased due to the anoxic conditions of the column incubations, which reduced the Mn into a more soluble form. Furthermore, OM-mediated oxidation-reduction reactions may also play an important role. Ma et al. (2020) show that the presence of DOC, specifically humic acids in their experiment, can alter Mn oxidation and inhibit secondary Mn mineral formation.

Last, we expected there to be a significant effect of soil material, in which less weathered supraglacial till soil from Mt. Moosilauke would likely respond more than the intensively weathered Lesesne soil. The total concentration of soil Al and Mn of Lesesne is not much higher than Moosilauke, but the lower Mg was expected in the more intensively weathered soil. Additionally, Lesesne soil had higher total concentrations of Ca and P than Moosilauke, which can be attributed to the Moosilauke local till being dominated by Conway granite with low nutrient availability (Schaller et al., 2010). Thus, our experimental design was not able to address the intensity of weathering but highlights soil parent material itself as a very important factor as leachate and soil total Ca were higher for Lesesne. The lower Al leachate for Lesesne was likely due to the formation of stable insoluble forms, such as kaolinitic clays or oxyhydroxide Al such as gibbsite (Figure 4). Moosilauke Al leachate was higher than Lesesne, implying either a higher aluminosilicate weathering rate or less formation of stable Al forms. As shown in Figure 4, Moosilauke leachate pH was more favorable for soluble Al than Lesesne leachate, supporting the later explanation. The cumulative Al leachate suggests the forest floor addition could be the primary source of Al, but the release of Al by weathering for Moosilauke is possible.

4.2 | Na azide effect on element leaching

With the addition of Na azide, we sought to reduce microbial activity to evaluate abiotic versus biotic effects on forest floor releases of DOC and mineral soil weathering. Unfortunately, Na azide strongly affected physicochemical properties and altered element solubility. First, the pH slightly increased for Moosilauke soils. Skipper and Westermann (1973) found that Na azide was able to neutralize soil acidity as it is a weak base, which may explain the increase in Moosilauke, Lesesne, and FFONLY pH. The addition of Na azide decreased DOC in leachate, which was converse to our hypothesis that it would inhibit microbes from producing more DOC. The decrease in DOC under Na azide is most likely a DOC removal via sorption to mineral surfaces and formation of organo-mineral complexes (Xue et al., 2009). The greater DOC sorption under Na azide treatment may likely be driven by the increase in soil pH and greater DOC charge sites. Kumi et al. (2013) found that the addition of Na azide to their soils resulted in a pH increase as well as an organic carbon increase. While the main objective of their study was not specifically looking at Na azide affects solution pH, it does agree with the pH increase seen in our experiment. The increase in organic carbon was attributed to the mulch present in the soil allowing for a higher microbial biomass (Kumi et al., 2013); however, it may also be possible that the Na azide played a role in sequestering the organic carbon by sorption. Last, we observed a decrease in ORP from Na azide, which is due to the weak reducing power of Na azide itself. These issues with Na azide limited our ability to discern between shutting down microbial processes and abiotic-reducing conditions.

There were significant changes with Na azide, predominantly decreases in leaching of macroelements (Al), nutrients (Ca, Mg, P for Moosilauke), and micronutrients (Mn and Cu and Zn for Moosilauke). We generally hypothesize that Na azide increased soil sorption capacity for most elements by increases in pH and creating greater organo-mineral complexes. Moreover, the Na azide promoted the formation of kaolinite for FFONLY treatment, Moosilauke soils, and Lesesne soils (Figure 4). In addition, decreases in Ca and Mg in Lesesne are likely due to direct sorption to organo-mineral complexes or its facilitation such as Ca bridging (Rowley et al., 2018). The leachate of some elements was not affected (particularly P, Si, and Cu) and even increased in the leachate (Zn). The higher forest floor Si leaching under Na azide treatments in the Moosilauke soil suggests that azide may have enhanced release of Si from exchange sites in the OM due to competitive sorption (e.g., Gimsing & Borggaard, 2002). As discussed in the previous paragraph, DOC leaching was decreased by the treatment of Na azide. Thus, we may conclude that the increased sorption of the DOC may have also promoted greater sorption and organo-metallic complexes of the macroelements and nutrients within the soils. Further investigation is warranted into the ligand-enhanced

transport of Si and other elements that saw a decrease in leachate concentrations under Na azide treatment.

5 | CONCLUSION

We conclude that the forest floor addition did impact the leaching of elements from two contrasting soils. However, instead of enhanced release of elements through aluminosilicate weathering, we were unable to separate weathering effects from the forest floor additions or release from organo-mineral complexes. We can generalize our findings as two groups of elements: (1) elements where they were sourced from the forest floor but had lower masses within leachate indicating adsorption within the mineral soil (e.g., Al, Si, P, Cu, Zn), and (2) leachate mass increase due to forest floor sourcing, increased weathering, or desorption from soil (Ca, Mg, and Mn). If we consider our results at the field scale, they highlight that the forest floor can be an important storage center for nutrients and other elements over short intervals as shown by the two 60-day experiments we conducted. In addition, the intensity of weathering of the soil materials did not matter in our study as the nutrient-poor Conway granite supraglacial-till comprising the Moosilauke soil had lower Ca than the intensively weathered Lesesne soil. Thus, the material itself as opposed to its previous exposure to chemical weathering was most important for considering the impact on nutrients in leachate. A parent material with soluble nutrient phases such as carbonates or apatite may be more susceptible to chemical weathering on the growing season timeframe (e.g., Bailey et al., 2019) and should be considered in future experiments.

In our subsequent experiment using Na azide as a microbial suppressant, the efficacy as a microbial suppressant was masked by its properties as a weak base, organic ligand, and mild reductant. We observed that nearly all elements, except Si and Lesesne Zn, had a decrease in their leachate mass. Our ORP and DOC measurements suggest that Na azide was causing greater DOC retention, creating reducing conditions, and increasing the pH, which would promote greater cation and anion retention in soil. Thus, we cannot conclusively address if microbial suppression or the weak base and reduction by Na azide abiotically enhanced element retention. We recommend future studies use alternative methods such as fumigation or irradiation to suppress microbial life. However, the decrease in leachate DOC due to Na azide suggests that DOC acting as a ligand may be quite important for the solubility and transport of elements. Future studies will need a longer duration, larger sample size, or an effective solid phase examination technique to be able to quantify increases in Al, Si, and P from mineral weathering while excluding their abundance within the forest floor materials.

AUTHOR CONTRIBUTIONS

Bailee B. Street: Conceptualization; methodology; investigation; data curation; formal analysis; project administration; writing—original draft; writing—review and editing; visualization. **Alexandrea M. Rice:** Methodology; formal analysis; software; writing—original draft; writing—review and editing. **Justin B. Richardson:** Conceptualization; formal analysis; funding acquisition; methodology; supervision; visualization; project administration; investigation; resources; writing—review and editing; writing—original draft.

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CONFLICT OF INTEREST STATEMENT

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

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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