BIOACCUMULATION AND COMMUNITY COMPOSITION OF SMALL TERRESTRIAL BIOTA EXPOSED TO COAL COMBUSTION RESIDUAL CONTAMINANTS

by

KELLY E. HOLLAND

(Under the Direction of Tracey D. Tuberville and James C. Beasley)

ABSTRACT

Anthropogenic pollutants from energy production pose a major threat to the health of ecosystems worldwide. Potentially toxic trace elements in coal combustion residues (CCRs) can persist in the environment for decades and readily bioaccumulate in animal tissues leading to deleterious effects. We reported contamination levels and small vertebrate community metrics in a terrestrial area that has not received CCR inputs in over five decades within the Department of Energy's Savannah River Site (Jackson, South Carolina). We 1) quantified current trace element concentrations in soils and target biota, 2) compared concentrations in soils and biota to reference site samples and historical data, 3) quantified stable isotope ratios in biota and compared to elemental concentrations, and 4) measured species richness, diversity, and dissimilarity along an estimated CCR exposure gradient. Our results broadly suggest the continued bioavailability and accumulation of trace elements in exposed biota may not be strongly impacting community metrics of small terrestrial vertebrates.

INDEX WORDS: Ecotoxicology; coal combustion residues, fly ash; trace elements; bioavailability; biomagnification; community-level effects; terrestrial biota; herpetofauna; small mammals

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Energy production from burning fossil fuels continues to produce large quantities of pollutants worldwide despite known extensive negative environmental impacts (Page et al. 1979, Rowe et al. 2002, Luther 2010, Briffa et al. 2020). Fly ash, the solid byproduct that makes up about 85% of coal combustion residues (CCRs), contains high concentrations of potentially toxic elements including arsenic (As), copper (Cu), mercury (Hg) and selenium (Se). In 1997, over 60 million tons of fly ash was generated in the U.S., 97% of which was either disposed of in landfills or sluiced with water into ash settling basins, also known as surface impoundments or ash ponds. At that time, coal accounted for approximately 50% of U.S. energy production (Rowe et al. 2002). Today, coal-fired power plants produce only about 20% of the energy in the U.S. (EIA 2021*a*) but still result in nearly 100 million tons of CCR yearly. While the U.S. may be on a downward trend of coal usage, developing countries, such as China and India, are increasing consumption as they struggle to keep up with energy demands (Miricioiu and Niculescu 2020).

Traditional disposal methods of CCR can facilitate environmental exposure. In recent years, the beneficial use of CCR in building materials such as cement or sheetrock has become increasingly common (Tenenbaum 2009). Utilization rates for CCR and strategies of CCR disposal vary among countries, but in the U.S. only about 42% of CCR is reused (Heidrich et al. 2013). Therefore, the majority of CCR produced is being disposed of either in landfills or ash settling basins, creating a potential pathway of contaminant exposure to biota (Rowe et al. 2002). These basins are often accessible by wildlife, and thus can then serve as ecological sinks to

species using them due to high levels of contaminants (Rowe and Hopkins 2003, Snodgrass et al. 2004). Additionally, the basins often discharge into the surrounding watershed, facilitating transport of pollutants throughout ecosystems (Rowe et al. 2002).

There is also risk of unintentional releases of contaminants due to structural failure of impoundments. For example, following an impoundment breech in 2008, Tennessee Valley Authority's (TVA's) Kingston Fossil Plant unintentionally released over 4 million m^3 of fly ash slurry into the Clinch and Emory Rivers, tributaries of the Tennessee River (Ruhl et al. 2009, Meyer et al. 2015). This accident created an ecological disaster costing an estimated $1.2 billion in clean-up efforts. Following national attention, this event also stimulated the Environmental Protection Agency (EPA) to update federal guidelines for the disposal of CCR (Luther 2010).

In 2015 the "Disposal of Coal Combustion Residuals (CCR) from Electric Utilities" final rule was created by the EPA and included regulations concerning risk reduction, groundwater protection, general operations, and record keeping within coal-fired plants. The rule also established in-place or by-removal remediation options for noncompliant ash ponds and their surrounding environment (EPA 2015). Capping surface impoundments in place by draining water, grading them, and covering the CCR with a liner has been the preferred remediation method, which is not without flaws. Leaching of contaminants into the groundwater is possible if the contained ash is near an aquifer, and caps can fail and release ash during large storms or floods (Magruder and Haun 2021). As regulations increase and cleaner renewable energy sources become more efficient and cost-effective, there are plans to retire 28% of the current coal-fired plants operating in the U.S. by 2035 (EIA 2021*b*). In other developed countries, such as those in the European Union, coal consumption is declining overall as of 2020 (IEA 2021). However, plants that utilized on-site disposal of CCR for decades are now faced with the question of what

to do with their residual contaminants. Understanding the transport, fate, and effects of these contaminants within the environment will help inform remediation decisions as closures continue.

As an alternative to fly ash removal, natural attenuation may reduce the bioavailability of contaminants, particularly legacy contaminants, within polluted sites. Natural attenuation is the process by which contaminants are contained and reduced in the environment without human intervention (EPA 1999). There are various natural processes that enable attenuation — including degradation, volatilization, dispersion, and dilution — that depend on the pollutant and its environmental fate and flow characteristics (Mulligan and Yong 2004). For example, inorganic metals within CCR such as As, Cu, and Nickle (Ni) do not biodegrade but can become immobilized over time under certain conditions through sorption onto soil organic matter (Mulligan and Yong 2004, Briffa et al. 2020). In the Clinch and Emory rivers that were contaminated during the TVA fly ash spill, bioavailable trace elements associated with more mobile sediment fractions were reduced to near reference levels in 780 days due to weathering, despite total trace element concentrations remaining similar after dredging efforts (Markwiese et al. 2014). If sites have favorable conditions, the use of natural attenuation as an in situ remediation effort is often less costly than removal operations but requires a long-term investment of time and environmental monitoring (Mulligan and Yong 2004).

Soil conditions influence the movement and availability of pollutants and should be considered when weighing remediation options for sites contaminated with CCR. A mixture of high organic matter and low pH at the surface level of the soil supports the occurrence of surface leeching, which would facilitate trace elements moving down through soil horizons and potentially becoming immobilized and less toxic (Sandhu and Mills 1991, Briffa et al. 2020). By

comparing previous soil trace element concentrations to current concentrations, longitudinal studies may give insight into the current state of natural attenuation of contaminants and the potential for reduced effects among wildlife. For example, Metts et al. 2012 compared trace element bioaccumulation in larval southern toads (*Anaxyrus terrestris*) reared with three different soil substrates: one collected from a site that underwent natural attenuation for 50 years after CCR contamination, one collected from an active ash basin, and one reference soil as a control. Larval toads reared in substrate from the ash basins negatively impacted survival, larval period, mass, and growth rate compared to toads reared with the naturally attenuated substrate (Metts et al. 2012). Therefore, natural attenuation may reduce concentrations or bioavailability of trace elements and associated sublethal effects in southern toads, but further research is required to determine if a similar response occurs in other taxa. If a similar response is seen in other taxa, natural attenuation may be an appropriate strategy for remediating sites with legacy contaminants when attenuation is facilitated by local site conditions.

Some ash-associated trace elements including Cu, Se, and Zn are essential for biological function, but they can become toxic at elevated levels (EPA 2007). Wildlife exposed to CCR have been shown to accumulate associated trace elements, which have been correlated with adverse effects on survival, development, and recruitment in a variety of taxa (Rowe 2014). Biological effects of elevated concentrations and consequential biological effects have been studied in a variety of taxa, but particularly in aquatic and semi-aquatic species, as the highest concentrations of contaminants associated with CCR often occur within aquatic ash settling basins (Rowe et al. 2002). Experimental mesocosm and laboratory studies have been important for distinguishing between effects of CCR-associated trace elements and other environmental factors (Rowe and Dunson 1994). For example, grass shrimp (*Palaemonetes pugio*) and lake

chubsucker (*Erimyzon sucetta*) showed reduced growth and survival in CCR exposure periods of two months or less (Hopkins et al. 2004, Kuzmick et al. 2007). Exposure to CCR in ash basins resulted in elevated contaminant levels and lowered hatching success, viability, and overall maternal reproductive success for southern toads in comparison to those reared from reference sites (Hopkins et al. 1998, Metts et al. 2013). Similarly, eastern narrow-mouthed toads (*Gastrophryne carolinensis*) exposed to CCR experienced reduced hatch success and viability and increased developmental abnormalities (Hopkins et al. 2006). In contrast, longer lived reptile species exposed to CCR, including mud turtles (*Kinosternon subrubrum*), yellow-bellied sliders (Trachemys scripta scripta), and American alligators (*Alligator mississippiensis*), accumulated elevated levels of trace elements but with no apparent negative health effects among the parameters tested (Finger et al. 2016, Haskins et al. 2017, Cochran et al. 2018).

Field studies have also documented effects of CCR exposure in terrestrial species (Peles and Barrett 1997, Sample and Suter 2002, Hernández et al. 2017). In a revegetated ash pond that received fly ash for over three decades in Tennessee, trace element concentrations were elevated in water, plant, and small mammal samples when compared to reference samples (Sample and Suter 2002). The authors concluded that the reported trace element levels may be detrimental to the survival and reproduction of small mammal species such as mice and shrews that maintain smaller home ranges because of increased exposure, but that larger terrestrial species appeared to be at lesser risk because they can move freely in and out of the contaminated area (Sample and Suter 2002). Within CCR contaminated sites in South Carolina, elevated levels of ash-associated contaminants, particularly arsenic, were found in cotton rats (*Sigmodon hispidis*), marsh rice rats (*Oryzomys palustris*), and raccoons (*Procyon lotor*) when compared to reference sites (Peles and Barrett 1997, Hernández et al. 2017). In Southern France, elevated As concentrations were

correlated with poor body condition in wood mice (*Apodemus sylvaticus*) inhabiting contaminated and partially remediated mining sites (Drouhot et al. 2014). Additionally, the high variability in trace element concentrations in soils and organs of small mammals collected from contaminated, remediated, and reference sites suggests other factors, such as diet and vagility, may be impacting contaminant accumulation in small mammals and require further investigation.

Several studies have also described population level-effects resulting from CCR exposure in wildlife. Recruitment declined in multiple anurans and an ambystomid (mole) salamander species that were exposed to CCR (Rowe et al. 2001, Snodgrass et al. 2004, Hopkins et al. 2006, Roe et al. 2006). In amphibians, larvae have been shown to accumulate the highest concentrations of contaminants (Hopkins et al. 2000, Metts et al. 2013) of any life stage, potentially reducing the number of individuals surviving to breeding age. In North Carolina, in a reservoir that received CCR effluents for 12 years, researchers observed complete reproductive failure in multiple fish species within the first year of operation and ultimately the extirpation of all but one species (Lemly 2002).

Observing community-level effects in a field study introduces environmental variables that are difficult to control, but it also increases the study's ecological relevance. Individual and population-level effects of exposure to CCR have been shown in a variety of taxa but can vary among species, suggesting the potential for community-level impacts. Studies of community-level effects due to contaminants typically rely on comparing estimates of abundance, the presence or absence of sensitive species, and measures of species diversity including evenness and richness, at contaminated sites in comparison to reference sites (Clements and Newman 2002). In terms of CCR exposure, invertebrate, bacterial, and aquatic communities have been commonly explored. For example, high alkalinity and salinity associated with fly ash reduced

bacterial diversity in both composting piles amended with fly ash and an ash-contaminated forested wetland (Fang and Wong 2000, Brofft et al. 2002). In contrast, benthic invertebrate communities consisting primarily of insect larva, bivalves, and aquatic worms were seemingly unaffected by the TVA fly ash spill when sampled less than six months after the remediation despite the large-scale exposure and predictions that ash-impacted benthic communities could take up to a decade to recover after such an event (Buys et al. 2015). The inconsistency in community-level effects and paucity of data by which to evaluate the effects of CCR contaminants highlights the need for further research. Additionally, CCR-specific effects among terrestrial vertebrate communities have received little attention, leaving a significant knowledge gap.

In the following chapters I present two concurrent studies aimed to inform future remediation efforts of a CCR contaminated site by assessing the effects of associated legacy contaminants on small terrestrial vertebrates. In Chapter 2, I quantify and compare wildlife contaminant loads, patterns of bioaccumulation and biomagnification, as well as trace element concentrations in soils to a reference site and to historical data. In Chapter 3, I compare community diversity and composition of small terrestrial vertebrates over a gradient of CCR exposure. These studies build on current knowledge of the impacts CCR exposure has on terrestrial vertebrates and attempt to better understand how these impacts matriculate into a higher level of biological organization.

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CHAPTER 2

ACCUMULATION OF TRACE ELEMENTS IN SOIL AND BIOTA WITHIN A SITE HISTORICALLY CONTAMINATED WITH COAL COMBUSTION RESIDUES

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ABSTRACT

Legacy contaminants tied to energy production are a worldwide concern. Coal combustion residues (CCRs) contain high concentrations of potentially toxic trace elements such as arsenic (As), mercury (Hg), and selenium (Se), which can persist for decades after initial contamination. CCR disposal methods, including aquatic settling basins and landfills, can facilitate environmental exposure through intentional and accidental releases. Coal combustion residue exposure in wildlife has shown negative impacts on development, reproduction, and survival. In the current study, concentrations of As, Hg, Se, and strontium (Sr) were quantified and compared within soils and target biota from a historically CCR-contaminated site and a reference site within the United States Department of Energy's Savannah River Site (Jackson, SC, USA). Target biota included three vertebrate species; green anole (*Anolis carolinenesis*), southern toad (*Anaxyrus terrestris*), and cotton mouse (*Peromyscus gossypinus*), and three invertebrate taxa; worms (Oligochaetes), camel crickets (*Ceuthophilus uhleri*), and wolf spiders (*Tigrosa georgicola*). Study objectives were to 1) compare current concentrations of trace elements in soil and resident biota to reference levels, 2) assess natural attenuation of elements in soils by comparing current concentrations to historic levels, and 3) evaluate biomagnification potential via body burden and trophic position correlations among biota. Trace element concentrations were higher in contaminated soils than reference soils (all $p \leq 0.004$). Similar concentrations from 2003 to 2022 suggested no natural attenuation of trace elements. In fact, Se increased significantly ($p < 0.001$) within that period. Additionally, target biota had elevated As, Se, and Sr levels in comparison to reference samples (all $p \leq 0.001$). A positive correlation between Sr concentrations and trophic position, as assessed by nitrogen stable isotope ratios, within southern toads sampled ($p = 0.005$) demonstrated potential for elemental biomagnification

within our study system. Elevated trace element accumulations in biota suggest continued bioavailability within soils and the potential for biomagnification within higher trophic level taxa in a historically CCR-contaminated site.

INTRODUCTION

The increasing demand for energy across the world has resulted in the concomitant increase in production of associated waste products. Despite known negative environmental impacts, coal usage continues to increase in developing countries where it remains an important source of energy production, making its pollutants a global concern (Miricioiu and Niculescu 2020). In 2016, the leading countries in coal consumption—China, India, and the United States—were estimated to have produced over 860 million metric tons of coal combustion waste products (Harris et al. 2019). Coal combustion residues, or CCRs, are the collective solid waste products of coal combustion and are primarily made up of fly ash. Fly ash is a fine-grained particulate containing potentially toxic elements including metals and metalloids such as arsenic (As), selenium (Se), lead (Pb), and mercury (Hg). Among countries producing the highest levels of fly ash, utilization rates of generated ash in the production of materials like cement ranges from 60 – 70% (Miricioiu and Niculescu 2020). Remaining fly ash not utilized for materials production, and its associated contaminants, are typically disposed of into the environment in landfills or aquatic settling basins (Rowe et al. 2002). Thus, understanding the transport, fate, and impacts of these contaminants within an ecosystem is important for both disposal regulations and remediation decisions.

Clean energy competition and waste regulations have contributed to downward trends in coal consumption in developed countries such as the U.S. and those in the European Union. Currently, the U.S. plans to retire almost 25% of its coal-fired capacity by 2030 (EIA 2022),

which will trigger remediation efforts of on-site fly ash disposal areas. Areas of legacy contamination from coal-fired power plants require assessment to identify the best outcomes from remediation efforts. Remediation of fly ash contaminated areas involves its removal to landfills, incorporation into construction materials, or by filling and capping aquatic surface impoundments (Magruder and Haun 2021). In less contaminated areas, the natural attenuation of elements within CCR may be a sufficient means for remediation by allowing contaminants to be reduced or immobilized by natural processes. Soil acts as a sink for trace elements but contamination levels and bioavailability depend on physio-chemical properties of both the contaminant and the sediments (EPA 1999). For example, heavy metals are typically insoluble in water, but acidic soil pH has been shown to increase the solubility of Pb, cadmium (Cd), and zinc (Zn), facilitating their movement within water (Chuan et al. 1996). Inorganic metals found in CCR are non-biodegradable and persistent but can become immobilized or volatilized over time by sorption or precipitation (Mulligan and Yong 2004). Soil organic matter content can also impact the adsorption of heavy metals to soil particles. Multiple studies have shown that the removal of soil organic matter greatly reduces soil adsorption of Zn and chromium (Cr) (Yu et al. 2023). However, the time frame for natural attenuation processes varies greatly by site, which can result in adverse impacts on environmental resources if left unmonitored (EPA 1999).

While numerous trace elements found in CCR are essential to biological function, all can become toxic at elevated levels (EPA 2007). Exposure to CCR has been shown to have a range of sublethal effects on wildlife depending on species and exposure routes and rates (Smith et al. 2007, Chen et al. 2024). Elevated trace element accumulation in producers and invertebrates from contaminated water and sediments also highlights the possibility of trophic transfer and bioaccumulation of trace elements in predatory species (Cherry and Guthrie 1977). In both

terrestrial and aquatic fly ash contaminated sites, wildlife have exhibited elevated levels of many ash-associated trace elements. Among elements found in CCR, As, Se, and strontium (Sr) often accumulate at significantly higher levels in comparison to reference sites (Rowe et al. 2002). Arsenic and Se toxicosis can be expressed through a range of effects, including organ cancer in mammals and reduced hatch success in birds (Mann et al. 2011). Aquatic or semi-aquatic species are especially vulnerable to trace element accumulation, as they often inhabit contaminated aquatic settling basins (Rowe et al. 2002). In Ambystomatid salamanders, high accumulations of As, Se, Sr, and Vanadium (V) extended the larval period and reduced survival to metamorphosis, thereby impacting recruitment (Roe et al. 2006). Ranid frogs and Bufonid toads displayed similar effects, as well as decreased larval growth and development rates (Snodgrass et al. 2004, Metts et al. 2012).

Amphibians and other small terrestrial vertebrates such as reptiles and small mammals are vital components of ecosystems, can be relatively abundant, and live long enough to bioaccumulate contaminants, making them valuable biosentinels of environmental contaminants (Talmage and Walton 1991, Grillitsch and Schiesari 2010, Hopkins and Rowe 2010). Further, small terrestrial vertebrates serve as secondary and tertiary consumers within food webs and may biomagnify trace elements among trophic levels (Talmage and Walton 1991, Mann et al. 2011). Small terrestrial vertebrates are likely directly and chronically exposed to localized soil contaminants, such as CCR, dermally and dietarily due to their fossorial behaviors and limited vagility (Talmage and Walton 1991, Smith et al. 2007). However, the presence of trace elements in the soil does not necessarily mean they are bioavailable. Site-specific research exploring contamination levels of multiple exposed species, their prey items, and the environment they inhabit can help elucidate the relationship among the three.

Our study aims to understand current contamination levels of soils and biota as well as patterns of contaminant uptake in a CCR contaminated site that has not received CCR effluents in over 5 decades. Our objectives were to: 1) quantify and compare concentrations of ash-associated trace elements in soil and target biota collected from a CCR contaminated area and a reference site, 2) determine the extent of natural attenuation by comparing contemporary soil concentrations to historical data, and 3) test for correlations between body burden of trace elements and trophic position within species sampled.

METHODS

Study Area

We conducted this study on the U.S. Department of Energy's Savannah River Site (SRS), a 780 km^2 property located along the Savannah River in west-central South Carolina, USA (Figure 2.1). Originally established to produce nuclear weapon materials during the Cold War era, the SRS houses numerous industrial facilities, including five nuclear reactors and several coal-fired steam power plants that have been retired from operations but are associated with legacy waste and contaminated sites (White and Gaines 2000). Among these, the D-Area coal-fired power plant provided steam power from the early 1950s until its closure in 2012. During the plant's operation, sluiced fly ash was deposited into aquatic settling basins, which have since been remediated by removing and relocating residual CCR to an offsite landfill and capping the empty basins (Office of Environmental Management 2018). During operation, effluents moved from primary to secondary basins, and due to both intentional release and an accidental breech, were released into the adjacent bottomland forest, creating an expansive plume within the terrestrial environment adjacent to the basins (Hopkins et al. 1999). This 40-ha plume of ash deposition (henceforth D-Area Ash Plume or DAP) is a well-defined area of CCR contamination,

with ash reaching depths of up to 2.7 m. Since the cessation of effluent discharge over five decades ago, DAP has undergone natural succession and about three cm of soil have accumulated on the surface throughout most of the plume. We used DAP as our contaminated site as it has not received any remediation effort. Since contamination, DAP has revegetated into a mid-successional bottomland forest with a canopy dominated by American sweetgum (*Liquidambar styraciflua*), American sycamore (*Platanus occidentalis*) and bald cypress (*Taxodium distichum*). Although it is largely unknown how natural attenuation and ecological succession have affected the local distribution and bioavailability of contaminants, studies in the area during the early 2000s and 2010s reported elevated CCR concentrations in soils and biota, including amphibians, reptiles, small mammals, and invertebrates (see below).

Lethal and sublethal effects of fly ash exposure have been extensively studied in the D-area aquatic settling basins in a variety of taxa, including aquatic invertebrates (Rowe et al. 2001*b*, Kuzmick et al. 2007, Fletcher et al. 2014*b*, 2017), fish (Hopkins et al. 2000, 2004, Staub et al. 2004, Fletcher et al. 2014a), amphibians (Hopkins et al. 1998, Hopkins et al. 2000, Roe et al. 2006, Metts et al. 2012, 2013, Flynn et al. 2019), snakes (Hopkins et al. 1999) and long-lived reptiles such as turtles and alligators (Finger et al. 2016, Tuberville et al. 2016, Haskins et al. 2017, Cochran et al. 2018), birds (Bryan et al. 2012, Oldenkamp et al. 2017, Leaphart et al. 2020, Tisdale et al. 2021), and mammals (Burger et al. 2002, Hernández et al. 2017, Oldenkamp et al. 2017, Borchert et al. 2019). However, the surrounding terrestrial environment, including the DAP, has been comparatively understudied. Limited evidence (Metts et al. 2012) suggests reported that most trace element concentrations in sediments from the DAP were intermediate in concentration compared to sediments from the settling basins and those from an uncontaminated site.

For our study, we selected a nearby (~4 km) bottomland forest adjacent to Upper Three Runs Creek, a tributary of the Savannah River as a reference site (Figure 2.1). We chose this site based on similarities in vegetation, elevation, and hydrology to DAP. The reference area has no known history of CCR contamination.

Study species

We targeted small vertebrate species that were common, widespread, and known to occur within the DAP and surrounding landscape: cotton mouse (*Peromyscus gossypinus;* omnivorous mammal), green anole (*Anolis carolinensis*; insectivorous reptile), and southern toad (*Anaxyrus terrestris*; insectivorous amphibian as adults). Additionally, we sampled invertebrate taxa likely to be included in the diet of our target vertebrate species: wolf spiders (*Tigrosa georgicola*; insectivorous arthropod), camel crickets (*Ceuthophilus uhleri*; omnivorous arthropod) and worms (Oligochaetes; detritivorous annelid). We attempted to collect 10 individuals of each species from both the DAP and the reference site.

Sample collection

In October 2022 we collected soil cores along transects that were spaced 100 m apart and were associated with biota collection arrays (2 at the reference site, 3 at DAP). Along each transect, we collected 5 cores approximately every 100 m using a soil core sampler (JMC Soil Samplers, Newton, IA) with 46 cm plastic tubes. We collected cores on the same day to limit variation due to precipitation. We brought cores back to the lab where we measured total depth then noted observable soil and ash layers and their respective depths, color (i.e. brown, gray, yellow) and textural differences (i.e. sandy, clay, powdery). We photo-documented all cores for visual comparisons and archival use.

From August 2022 to November 2023, we collected target biota from 3 collection arrays within DAP and 2 arrays in the reference site, as well as through opportunistic captures. The DAP arrays were constructed in the center of the plume, ~100 m interior from the plume edge with ~100 m between arrays. We constructed reference site arrays >100 m from each other in similar habitat as our contaminated site and where we believed seasonal flooding to be minimal (Figure 2.1). Collection arrays consisted of three 7.5 m sections of aluminum flashing positioned as a Y-shape drift fence converging on a central pitfall trap (sunken 5-gal/18.9 L plastic bucket). We placed double-ended funnel traps along both sides of each flashing section for a total of 6 funnel traps per array (Figure 2.2). For small mammal captures, we used Sherman live-traps (H.B. Sherman Traps Inc., Tallahassee, FL) baited with sunflower seeds covered in peanut butter oil (Wildlife Control Supplies, Suffield, CT). We placed 2 Sherman traps 5 m apart along a transect between each pair of drift fence arms, for a total of 6 traps per array. We collected worms by hand under nearby woody debris as they were rarely captured in pitfall traps. Our sampling frequency varied seasonally based on when we believed biota to be most active; we opened traps for 5 days each month during August – December 2022 and for 10 days each month during March – July 2023. Between August and November 2023, we added 4 supplemental capture days targeting mice and anoles to reach our target sample sizes. We brought captured specimens back to the lab, where they were humanely euthanized. We measured wet weight (g) and snout-vent length [amphibians and reptiles (mm)] or hind foot length [small mammals (mm)], rinsed them with DI water, and stored specimens individually at -70°C until analysis. All animal handling and euthanasia procedures were carried out under South Carolina DNR collecting permit #SC-08-2022 and in accordance with University of Georgia Institutional

Animal Care and Use Committee guidelines under protocols A2022 05-016-Y1-A0, A2022 06-012-A1, and A2021 12-001.

Sample preparation and elemental analysis

We cut soil core tubes open and transferred samples into 24 oz sterile plastic bags (Fisher Scientific, Waltham, MA) and mechanically homogenized them by hand. To evaluate baseline soil conditions, we first assessed percent moisture and pH. To calculate percent moisture, we placed 3 subsamples of approximately 5 g from each core in separate aluminum tins and recorded wet weight to the closest 0.1 mg. We placed aluminum tins in a drying oven overnight at 105°C. The following day, we recorded dry weight, which was used to calculate average percent moisture for each core. For soil pH, we placed 2 subsamples of approximately 10 g from each core in separate 50 mL centrifuge tubes and mixed them each with 20 mL of deionized water. We stirred tubes intermittently for 30 minutes before using a pH probe (SevenExcellence S400; Mettler Toledo, Columbus, OH) to obtain soil pH values used to calculate average pH for each core.

We freeze dried and then further homogenized the remaining soil from each core using 2 mm plastic sieves. We then conducted trace element analysis for a suite of ash-associated contaminants of concern, including As, Se, Sr, and total mercury (THg, hereafter Hg). For analysis, we used microwave-assisted acid digestions (MARS 6; CEM Corporation, Matthews, NC) with weighed subsamples of up to 0.25 g from each soil core using 10 mL of trace metal grade nitric acid (70% HNO_3) in pre-cleaned Teflon PFA vessels. After acid digestion, we made initial dilutions by adding the digested sample to 30 mL of Milli-Q water (18MΩ; Millipore) in 50 mL metal-free centrifuge tubes and brought the samples up to a volume of 50 mL (20% HNO_3). We made final dilutions by adding 1 mL of digested sample to 9 mL of Milli-Q water

(2% HNO_3) in 15 mL metal-free centrifuge tubes for analysis with inductively coupled plasma mass spectroscopy (ICP-MS) (NexlON 300X ICP-MS; Perkins Elmer U.S. LLC, Shelton, CT). For quality control, we included 2 replicates of 2 different certified reference materials (PACS-3 marine sediment, National Research Council, Ottawa, ON, Canada; Montana II Soil, National Institute of Standards and Technology, Gaithersburg, MD, USA), 2 blanks, and 2 sample replicates in our analysis.

We analyzed Hg concentrations in soil samples using approximately 150 mg subsamples of dried, homogenized soil. We weighed samples in nickel boats to the nearest 0.1 mg and analyzed them by thermal decomposition, catalytic conversion, amalgamation, and atomic absorption spectrophotometry (DMA-80 EVO; Milestone Inc, Shelton, CT). The run included 23 samples and in each half of the samples we included a replicate, blanks, and 2 replicates of 2 different certified reference materials (PACS-3 and TORT-3 lobster hepatopancreas; National Research Council, Ottawa, ON, Canada).

To compare historical and contemporary contamination levels, we obtained archived data of trace element concentrations from soil cores collected and analyzed in 2003 as part of another study (McLeod, unpublished data). Of the 73 short core (30 cm) soil samples taken in 2003, we included in our comparison the 36 that fell within the DAP boundary, based on GPS coordinates associated with those historical samples. Elemental concentrations were obtained from core composites similarly to methods described above.

For whole-body contaminant concentrations in biota, we conducted trace element analysis for the same suite of ash-associated contaminants as soil samples. Prior to analysis, we freeze dried samples to a constant mass, recorded dry weight to the nearest 0.1 mg and

homogenized them individually with a mechanical grinder. For smaller worms, we analyzed a composite sample of 2-5 individuals to achieve a sample weight of at least 85.0 mg for analysis.

For microwave-assisted acid digestions of biota, we weighed out a subsample of approximately 50 mg from each homogenized sample, weighed it to the nearest 0.1 mg, transferred it into pre-cleaned Teflon PFA vessels and added 1 mL of trace metal grade nitric acid (70% HNO_3) before microwave digestion. Each run of digestions included up to 34 biota samples and 2 each per run of sample replicates, blanks, and certified reference material (TORT-3) for quality control. After microwave digestion, we made initial dilutions by transferring samples into 15 mL metal-free centrifuge tubes and adding 4 mL of Milli-Q water to bring samples to a volume of 5 mL (20% HNO_3). For the final dilution, we added 1.5 mL of the diluted sample to 3.5 mL of Milli-Q water (6% HNO_3) which was used for analysis with ICP-MS.

We quantified Hg in biota using approximately 50 mg subsamples of dried homogenized samples, weighed to the nearest 0.1 mg. We weighed samples in nickel boats and analyzed them in the DMA-80 EVO. Each run included 2 sample replicates, 2 nickel boat blanks, and 2 replicates of both certified reference materials (TORT-3 and PACS-3) for quality control. For soils and biota, we report all elemental concentrations as means ± 1 SD in μg/g dry weight.

For stable isotope analysis, we sent subsamples of 8-10 DAP collected individuals from all target vertebrate and invertebrate taxa to University of Florida's Department of Geological Sciences (Gainesville, FL). We analyzed samples with isotope ratio mass spectrometry using a Thermo Electron Delta V Advantage spectrometer (ThermoFisher Scientific, Waltham, MA) with a ConFlo IV interface attached to an ECS 8020 elemental analyzer (N.C. Technologies, Milan, IT). We report all nitrogen isotopic ratios in standard delta notation, $\delta^{15}N$. We used an increase of 3 $\delta^{15}N$ to indicate a change in trophic position (Ehleringer et al. 1986).

Statistical Analyses

We excluded any elements with >50% of samples below detection limit (BDL) from further analysis. For the remaining elements, we replaced all samples below detection limit (BDL) values with 50% of the calculated minimum detection limit of the element (Fletcher et al. 2014*b*). We performed all statistical analyses in R Studio statistical software (Version 4.3.1, The R Foundation for Statistical Computing) and considered a p-value of <0.05 to be significant. Elemental concentrations, soil pH and percent moisture, and isotopic delta values were tested for normality using a Shapiro-Wilk test before analysis. For soil pH and moisture, we analyzed differences between sites using Welch's two sample t-tests. For soil and biota elemental concentration comparisons we used non-parametric Kruskal-Wallis one way analysis of variance tests due to non-normal distributions of elements. For soils, we analyzed differences in trace element concentrations between our DAP and reference site samples in addition to current and historical DAP soil samples. For biota, we tested for differences in mean elemental concentrations of each species between reference-collected and DAP-collected individuals. To assess biomagnification, we used analysis of variance (ANOVA) followed by Tukey HSD pairwise comparisons to compare $\delta^{15}N$ values among species. Adjusted p-values are reported to account for multiple comparisons. We then used linear regression models to compare concentrations of As, Se, Sr, and Hg as a function of $\delta^{15}N$ values separately for each species and element combination.

RESULTS

Soil

We collected and analyzed 15 soil samples from our fly ash contaminated site (DAP) and 8 from our reference site (Table 2.1). Ash was present in all samples collected from DAP and

none of the reference samples. In DAP samples where soil layers were prominent, ash was present, on average, 4.17 cm (SD = 1.25) below the surface. Most DAP samples had ash at the deepest point of the core, but, in 3 samples a brown clay layer was found below the layer of ash deposition at 14, 33 and 35 cm, respectively. Soil percent moisture in DAP averaged 34.0% (SD = 6.4%) and was significantly higher than the reference site, which averaged 14.9% (SD = 5.9%) (Table 2.1; $t = 7.21$, $df = 15.47$, $p = <0.001$). Soil pH was also significantly higher in DAP, averaging 5.8 (SD = 0.2) in comparison to the reference site's average pH of 4.5 (SD = 0.6) (Table 2.1; $t = 5.53$, $df = 8.02$, $p = <0.001$). All trace elements were significantly higher in DAP in comparison to the reference site (Figure 2.3). Trace element concentration averages were relatively similar to 2003 reported levels with the exception of Se, which increased significantly (Figure 2.4).

Biota

We collected and quantified trace elements in 81 individuals from DAP and 81 individuals from our reference site (Table 2.2). Sample sizes varied from 10 to 16 individuals per taxa per site. DAP collected individuals had significantly higher concentrations of As, Se and Sr when compared to reference individuals across all species. Invertebrate species accumulated higher concentrations of As (1.42 – 18.22 μg/g) than the vertebrate species tested (0.25 – 1.12 μg/g). DAP worms accumulated 10 to 73 times more As than all other species ($n = 15$, $\bar{x} = 18.22$ μg/g). Invertebrate species also accumulated higher average Se levels (1.75 – 26.59 μg/g) than vertebrate species (1.30 – 14.48 μg/g), with DAP worms also having the highest average ($n = 15$, $\bar{x} = 26.59$ μg/g). For Sr, vertebrates accumulated higher average levels (18.65 – 404.95 μg/g) than invertebrates (5.54 – 242.50 μg/g), with DAP toads accumulating the highest levels ($n = 16$,

$\bar{x}$ = 404.95 µg/g). In contrast, concentrations of Hg in reference collected worms, crickets, spiders, and mice were significantly higher than their DAP counterparts (Figure 2.5; Table 2.2).

Isotopes

We quantified $\delta^{15}N$ values from 57 DAP collected individuals. An ANOVA showed $\delta^{15}N$ values differed significantly among species ($F_{5,51}$ = 31.53, p = <0.001). Post-hoc pairwise comparisons showed average $\delta^{15}N$ values significantly differed by at least 3 $\delta^{15}N$ values among our three invertebrate species, suggesting increasing trophic levels from worms (n = 10, $\bar{x}$ = 1.76 $\delta^{15}N$) to crickets (n = 9, $\bar{x}$ = 5.42 $\delta^{15}N$, *adj* p = <0.001) to spiders (n = 10, $\bar{x}$ = 8.53 $\delta^{15}N$, *adj* p = <0.001). Among vertebrate species, average $\delta^{15}N$ values increased from anoles (n = 8, $\bar{x}$ = 5.52 $\delta^{15}N$) to mice (n = 10, $\bar{x}$ = 7.67 $\delta^{15}N$, *adj* p = 0.026) to toads (n = 10, $\bar{x}$ = 7.93 $\delta^{15}N$, *adj* p = 0.998). As vertebrates differed by less than 3 $\delta^{15}N$ values, there was no evidence of trophic position variation among the three species. Arsenic, Se, and Sr increased as $\delta^{15}N$ increased for both toads and spiders; however, only Sr in toads had a significant positive correlation (Figure 2.6; *adj* R^2 = 0.60, p = 0.005).

DISCUSSION

Our study showed that legacy contaminants are still present at elevated levels at a site that has not received fly ash effluents in over 50 years. Elevated concentrations of trace elements were evident in both soil as well as in a diversity of small terrestrial biota inhabiting DAP, demonstrating that ash-associated trace elements continue to be bioavailable to terrestrial species inhabiting the area. Further, elemental concentrations in soils have remained similar to levels documented in 2003 (20 years prior to the present study), except for selenium, which appears to have increased in concentration in recent decades. Collectively, these data suggest there has been

limited natural attenuation of trace elements in soils and thus there is continued potential for bioaccumulation in biota and biomagnification among trophic levels within the system.

In comparison to the reference site, soils from the ash-contaminated site had elevated levels of contaminants of concern, including As, Hg, Se, and Sr. While DAP's elevated ash-associated contaminants in comparison to a reference site was an expected finding due to historic CCR inputs and fly ash presence within soil cores, the lack of natural attenuation of trace elements over the last 20 years and increased Se concentrations was unexpected. Our finding of increased Se contradicts a previous study assessing trace element concentrations in sediments within the Belews Lake Reservoir in North Carolina that saw a decrease in Se concentrations 11 years after the cessation of fly ash effluent inputs (Lemly 1997*a*). However, aquatic systems have increased sediment mobility and may not be accurate comparisons to floodplain sites. Further, our sampling effort was focused near the original ash deposition area and along the downstream flow towards the Savannah River in DAP, whereas the previous 2003 study in DAP spanned its entirety. Therefore, differences in sample size and distribution of trace elements throughout the plume could explain the difference in Se soil concentrations in our samples compared to those collected in 2003.

Although we did not find evidence of natural attenuation based solely on trace element concentrations, soil properties in DAP are favorable for immobilization through soil adsorption, which would decrease their bioavailability to living organisms in the system (Mulligan and Yong 2004, Usman et al. 2023). The trace elements tested are likely not being reduced within DAP because they are inorganic, do not biodegrade, and tend to be sequestered in sediments. Studies have shown increased pH can facilitate precipitation and adsorption of some trace elements, thereby immobilizing them and decreasing bioavailability (Usman et al. 2023). DAP's higher pH

in comparison to the reference site supports the potential for trace element immobilization. Additionally, increased organic matter, which was visually observed to be forming above the ash layer within DAP soil cores, can improve adsorption and decrease bioavailability to wildlife.

In our study, we found elevated concentrations of As, Sr, and Se in all DAP collected biota when compared to the reference site. Of the species we examined, southern toads bioaccumulated the highest concentrations of Hg ($\bar{x}$ = 0.21 µg/g) and Sr ($\bar{x}$ = 404.95 µg/g). Compared to our study, adult female southern toads collected in 2009 from DAP had similar levels of Hg but Sr levels were 50% lower (Metts et al. 2012), supporting our finding of trace elements remaining within the system rather than attenuating, thereby continuing to bioaccumulate in wildlife. Oligochaete worms had the highest concentrations of As ($\bar{x}$ = 18.22 µg/g) and Se ($\bar{x}$ = 26.59 µg/g). Earthworms in the genus *Eisenoides* have been commonly used in toxicity tests and have experienced maximal accumulation capacities of 50 to 56x our reported As levels (Romero-Freire et al. 2015). As a food source for small terrestrial vertebrates, invertebrates such as worms can introduce high contaminant loads into food webs which may transfer and biomagnify among trophic levels (Edwards and Bohlen 1996). In our study, invertebrates on average accumulated higher concentrations of As, Hg, and Sr. As mostly primary consumers, they can then introduce these elevated concentrations to higher trophic levels, further contributing to bioaccumulation.

High accumulations of our tested elements (As, Hg, Se, and Sr) in wildlife exposed to CCR has been documented in a variety of species (reviewed in Lemly 1997*b*, Rowe et al. 2002, Mann et al. 2011, Chen et al. 2024). While biota have biological defenses against bioaccumulation of non-essential trace elements through sequestration and excretion, when exposed to high concentrations of these elements, detoxification abilities are hindered and can

result in toxicity (Mann et al. 2011). Studies of species inhabiting ash basins have reported deleterious effects such as failed larval recruitment in southern toads (Rowe et al. 2001*a*), reduced hatch success and increased developmental abnormalities in eastern narrow-mouth toads (*Gastrophryne carolinensis*) (Hopkins et al. 2006), and increased metabolic rate in banded water snakes (*Nerodia fasciata*) (Hopkins et al. 1999). Bioaccumulation patterns and subsequent lethal and sublethal effects vary between species, and even among members of the same species making direct toxicity comparisons difficult. Among vertebrates, we found As concentrations almost 5x lower than the maximum concentration associated with no survival, growth, or developmental effects in exposed larval leopard frogs (*Rana pipiens*) (Chen et al. 2009). However, for all species besides cotton mice, Se levels were found to ≥14.5 μg/g, which exceeds the biological effects threshold (4 μg/g) for health and reproduction of freshwater fish (Lemly 1997*b*). The discrepancy of our sampled biota reaching concentrations considered of biological concern for some elements and not others, highlights the uncertainty of biological effects.

Though associated sublethal and lethal effects have been reported in CCR exposed wildlife, particularly in those inhabiting primary disposal sites such as ash basins, other studies have failed to document negative biological effects resulting from elevated elemental concentrations. For example, juvenile American alligators (*Alligator mississippiensis*) fed CCR contaminated diets accumulated As and Se in kidney and liver tissues, but growth rate and body condition were not affected (Tuberville et al. 2016). In a maternal transfer study, fence lizards (*Sceloporus occidentalis*) even accumulated Se levels (14.1 μg/g female gonad) reaching near threshold values for reproductive toxicity when fed Se enriched crickets but had no evidence of lethal or sublethal effects (Hopkins et al. 2005). Though we found wildlife inhabiting a legacy CCR contaminated site accumulating trace elements, accumulation does not always correlate

with deleterious effects of biological concern and thus additional studies are needed to quantify sub-lethal effects of contaminant exposure among biota inhabiting this system.

Due to high variation in calculated $\delta^{15}N$ values among individuals of each species, trophic position did not consistently correlate with elemental concentrations. Of the six species sampled, southern toads were the highest trophic position of vertebrates and we found evidence that ash-associated trace elements biomagnify among southern toads sampled. We did however find a significant positive correlation between southern toads $\delta^{15}N$ values and Sr concentrations suggesting the potential for biomagnification of this element. Although Sr has been found to bioaccumulate (Burger et al. 2002, Snodgrass et al. 2004, Roe et al. 2006), it has not previously been reported to biomagnify in terrestrial systems. Additionally, we found the highest concentrations of Hg and Sr in southern toads. Accumulations at the highest level of our simplified terrestrial food chain suggests the potential for trophic transfer of trace elements in higher level predatory vertebrates. While we found few correlations, our study contributes to the limited existing literature of trace element biomagnification in terrestrial systems.

Our study found 1) elevated concentrations of tested elements of concern within the CCR contaminated site soils and biota when compared to the reference site, 2) limited evidence for natural attenuation of trace elements, and 3) potential biomagnification of trace elements within terrestrial biota. Quantification of contemporary contaminant levels in soil and biota in a historically well-studied system allows better understanding of how contaminants are interacting with the environment over time. Temporal changes in exposure and concentrations can aid informed remediation decisions. Our finding of elevated trace elements in soils and biota in an area that hasn't received ash effluents in over 5 decades highlights the need for continued monitoring of historically CCR contaminated areas. Beyond documenting the potential for

chronic exposure to elevated levels of trace elements, monitoring efforts should evaluate the effects of exposure at multiple levels of biological organization. We found concentration levels in soil and biota to vary greatly within the ash plume. Inter- and intraspecific variation in body burden and isotopic ratios among biota, in addition to small scale spatial heterogeneity in contaminant levels, emphasizes the importance of including multiple species and sampling locations in contaminant assessments. Historically, studies have focused on individual level effects, making population and community-level effects much less understood. Further research is required to assess if elevated body burdens of trace elements are negatively impacting communities of biota inhabiting contaminated sites. Additional efforts focused on the conditions affecting the bioavailability of ash-associated elements could also elucidate the mechanisms driving variation in bioaccumulation that we observed. The complexity of interactions between trace elements, biota, and their environment make predicting movement and fate of contaminants complicated but important in the decision-making process for remediation.

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Table 2.1: Percent moisture, pH, and trace element concentrations (µg/g dry weight) in soils collected from the D-Area Ash Plume (DAP) in 2003 and 2022 and a reference site (Ref) in 2022 within the Savannah River Site, South Carolina. P-values are for 2003 DAP versus 2022 DAP comparisons and 2022 Ref versus 2022 DAP comparisons. Asterisks signify significant differences. NR = Not Recorded.

	2003 DAP (*n* =36)			2022 DAP (*n* =15)		2022 Ref (*n* =8)		
Variable	**Mean**	**SD**	**p-value**	**Mean**	**SD**	**Mean**	**SD**	**p-value**
Moisture (%)	NR	NR	NR	34.0	6.4	14.9	5.9	< 0.001 *
pH	NR	NR	NR	5.8	0.2	4.5	0.6	< 0.001 *
Element	**Mean**	**SD**	**p-value**	**Mean**	**SD**	**Mean**	**SD**	**p-value**
Arsenic (As)	29.08	14.27	0.231	33.59	15.35	1.94	1.65	< 0.001 *
Mercury (Hg)	0.21	0.13	0.871	0.18	0.06	0.04	0.01	< 0.001 *
Selenium (Se)	5.03	2.19	< 0.001 *	9.87	3.34	4.93	2.27	0.004 *
Strontium (Sr)	154.11	80.48	0.591	143.00	70.54	10.24	6.90	< 0.001 *

Table 2.2: Trace element (As, Hg, Se, Sr) concentrations (µg/g dry weight) in species collected from the D-Area Ash Plume (DAP) and a reference site (Reference) in 2022. Invertebrate species included Oligochaetes (Worm), *Ceuthophilus uhleri* (Cricket), and *Tigrosa georgicola* (Spider). Vertebrate species included *Anolis carolinensis* (Anole), *Anaxyrus terrestris* (Toad), and *Peromyscus gossypinus* (Mouse). Asterisks signify significant difference between sites.

		DAP			Reference			
Element	**Species**	**N**	**Mean**	**SD**	**N**	**Mean**	**SD**	**p-value**
Arsenic	Worm	15	18.22	7.76	15	0.73	0.33	< 0.001 *
(As)	Cricket	15	1.42	1.10	13	0.13	0.08	< 0.001 *
	Spider	15	1.72	3.14	15	0.26	0.18	< 0.001 *
	Anole	10	0.55	0.55	15	0.07	0.04	< 0.001 *
	Toad	16	1.12	1.18	13	0.08	0.03	< 0.001 *
	Mouse	10	0.25	0.14	10	0.05	0.03	< 0.001 *
Mercury	Worm	15	0.14	0.03	15	0.35	0.24	< 0.001 *
(Hg)	Cricket	15	0.06	0.02	13	0.10	0.06	0.020 *
	Spider	15	0.13	0.04	15	0.39	0.19	< 0.001 *
	Anole	10	0.06	0.02	15	0.08	0.03	0.096
	Toad	16	0.21	0.15	13	0.16	0.06	0.645
	Mouse	10	0.03	0.01	10	0.10	0.03	< 0.001 *
Selenium	Worm	15	26.59	8.67	15	5.62	3.56	< 0.001 *
(Se)	Cricket	15	18.35	5.60	13	1.90	1.90	< 0.001 *
	Spider	15	23.77	4.81	15	1.75	1.00	< 0.001 *
	Anole	10	15.26	6.74	15	1.30	0.53	< 0.001 *
	Toad	16	14.48	5.62	13	1.84	0.36	< 0.001 *
	Mouse	10	5.44	1.01	10	2.13	0.95	< 0.001 *
Strontium	Worm	15	242.50	61.33	15	41.09	24.82	< 0.001 *
(Sr)	Cricket	15	54.80	30.14	13	5.54	3.32	< 0.001 *
	Spider	15	51.02	14.91	15	7.20	2.38	< 0.001 *
	Anole	10	211.42	59.19	15	34.39	7.73	< 0.001 *
	Toad	16	404.95	175.45	13	99.16	55.78	< 0.001 *
	Mouse	10	76.61	37.50	10	18.65	6.49	< 0.001 *

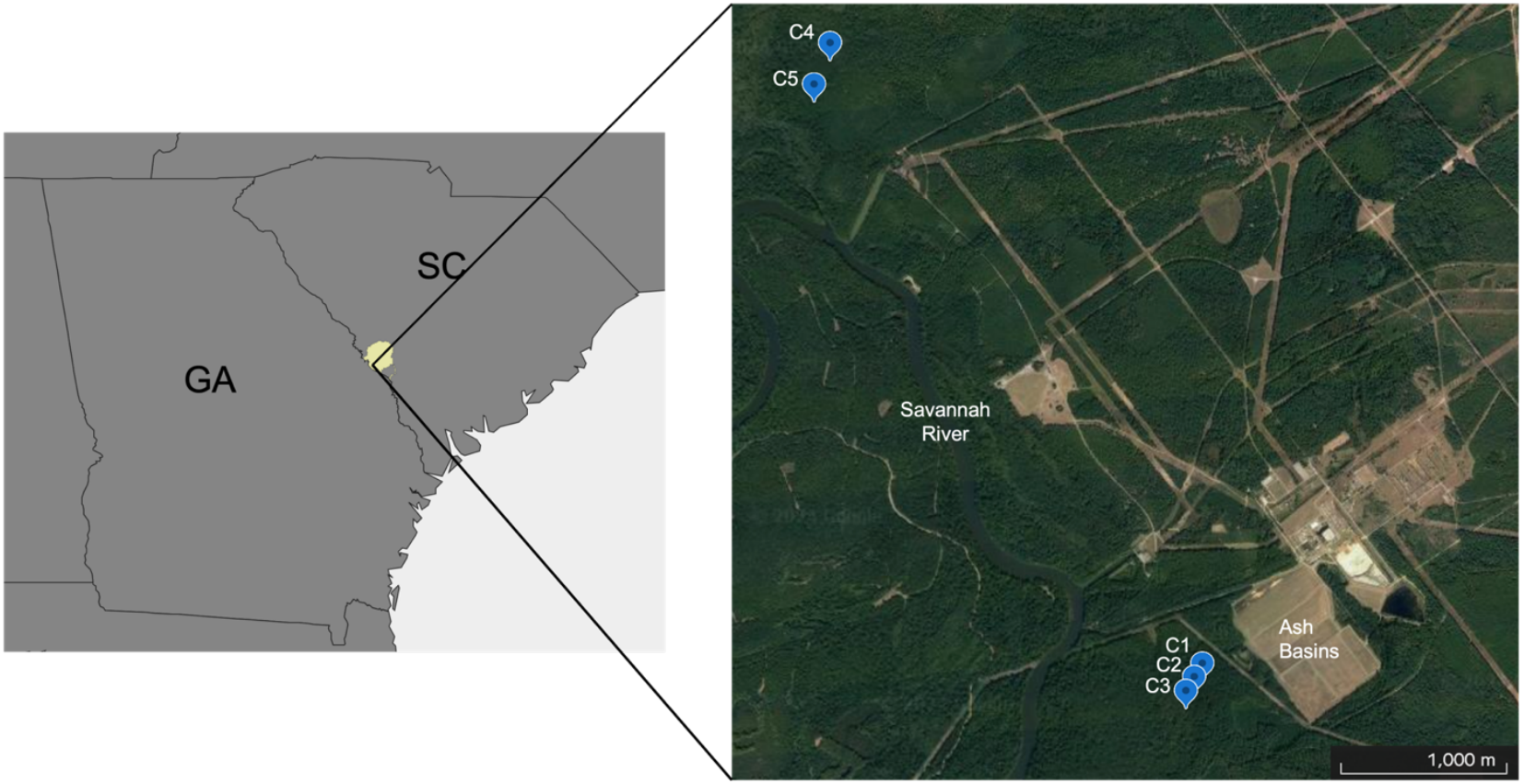


Figure 2.1: Map of study site within U.S. Department of Energy's Savannah River Site in Jackson, SC. Contaminated biota samples were collected from collection arrays C1, C2,and C3 within the D-Area Ash Plume (DAP) southwest of the remediated ash basin area ("Ash Basins"). Reference biota samples were collected from collection arrays C4 and C5 in the reference site about 4 km northeast of DAP.

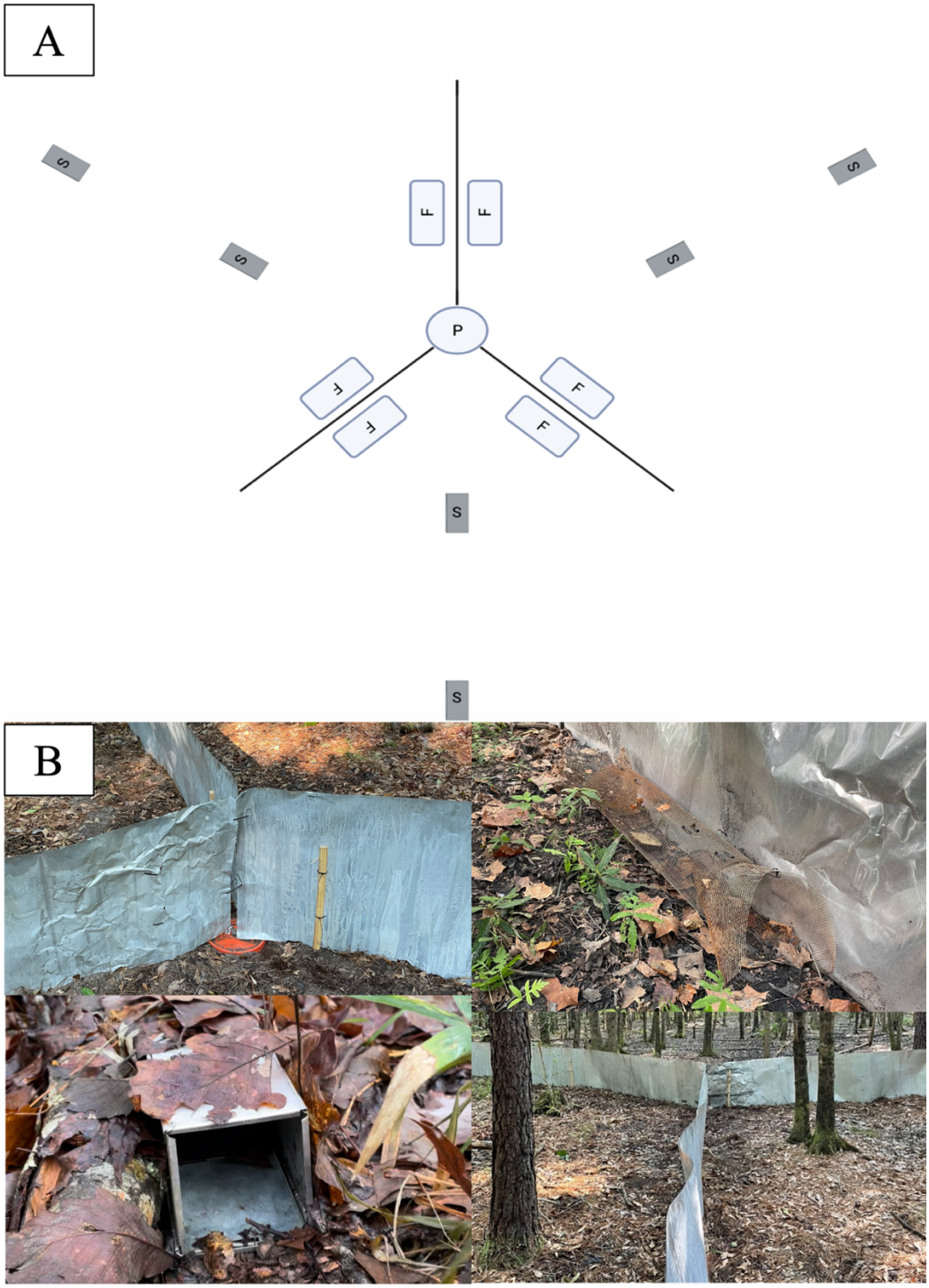


Figure 2.2: A) Diagram of array layout used to collect biota samples in Savannah River Site, Jackson, SC between August 2022 and November 2023. Arrays were made of three 7.5 m drift fence arms of aluminum flashing with a central pitfall trap (P), 6 funnel traps (F) on each side, and 2 Sherman traps (S) 5 and 10 m away from the pitfall between each drift fence arm for a total of 6. B) Photos of a collection array with associated traps (left to right: closed pitfall, funnel, Sherman trap, drift fence) in the field.

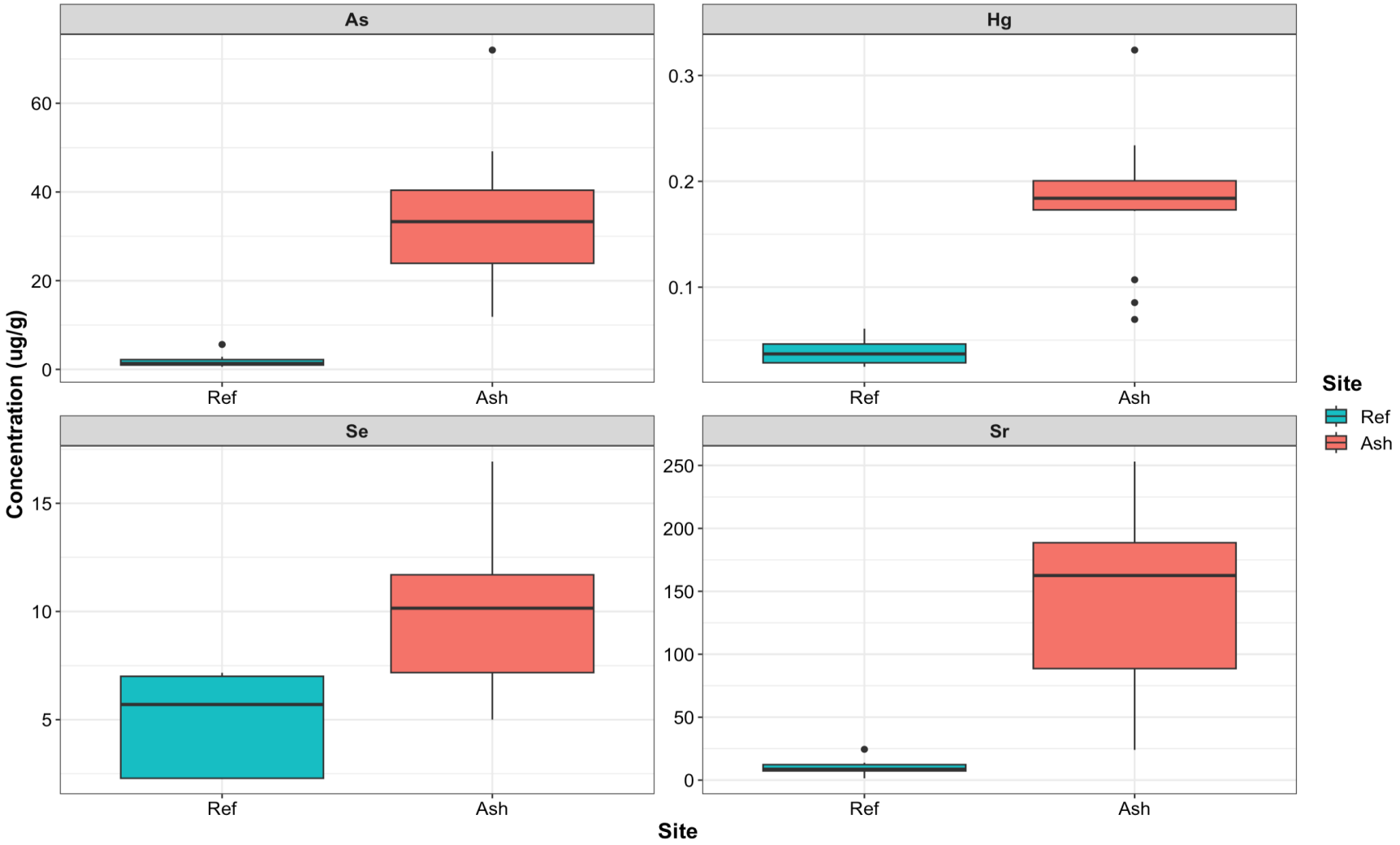


Figure 2.3: Trace element (As, Hg, Se, and Sr) concentration (µg/g dry weight) comparisons in soil samples collected from the D-Area Ash Plume (Ash, *n*=15) and a reference site (Ref, *n*=8) within the Savannah River Site, SC. All samples were collected in October 2022.

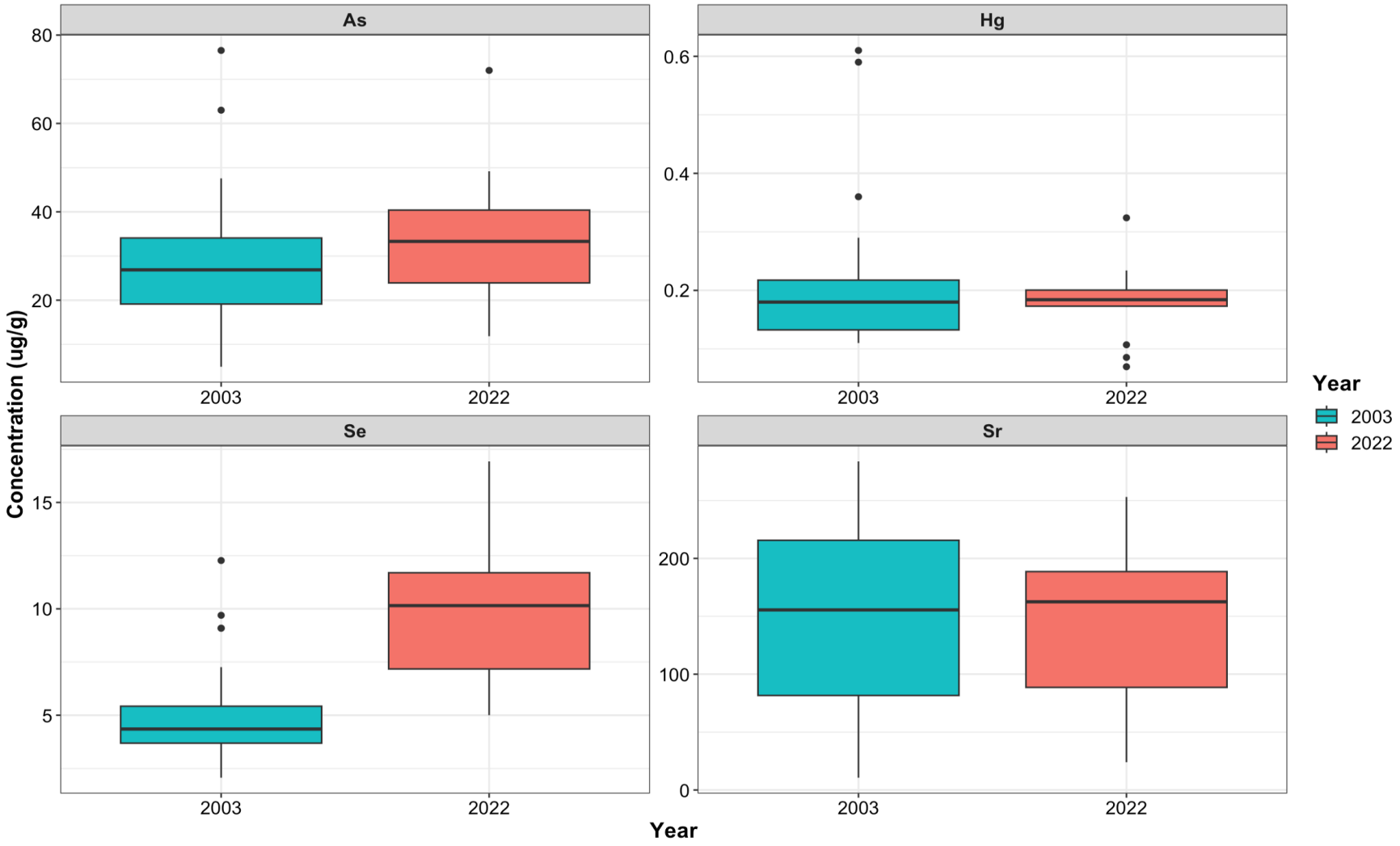


Figure 2.4: Comparison of trace element (As, Hg, Se, and Sr) concentrations (µg/g dry weight) quantified from soil samples taken in 2003 (*n*=36) and 2022 (*n*=15) in the D-Area Ash Plume (DAP) within the Savannah River Site, SC.

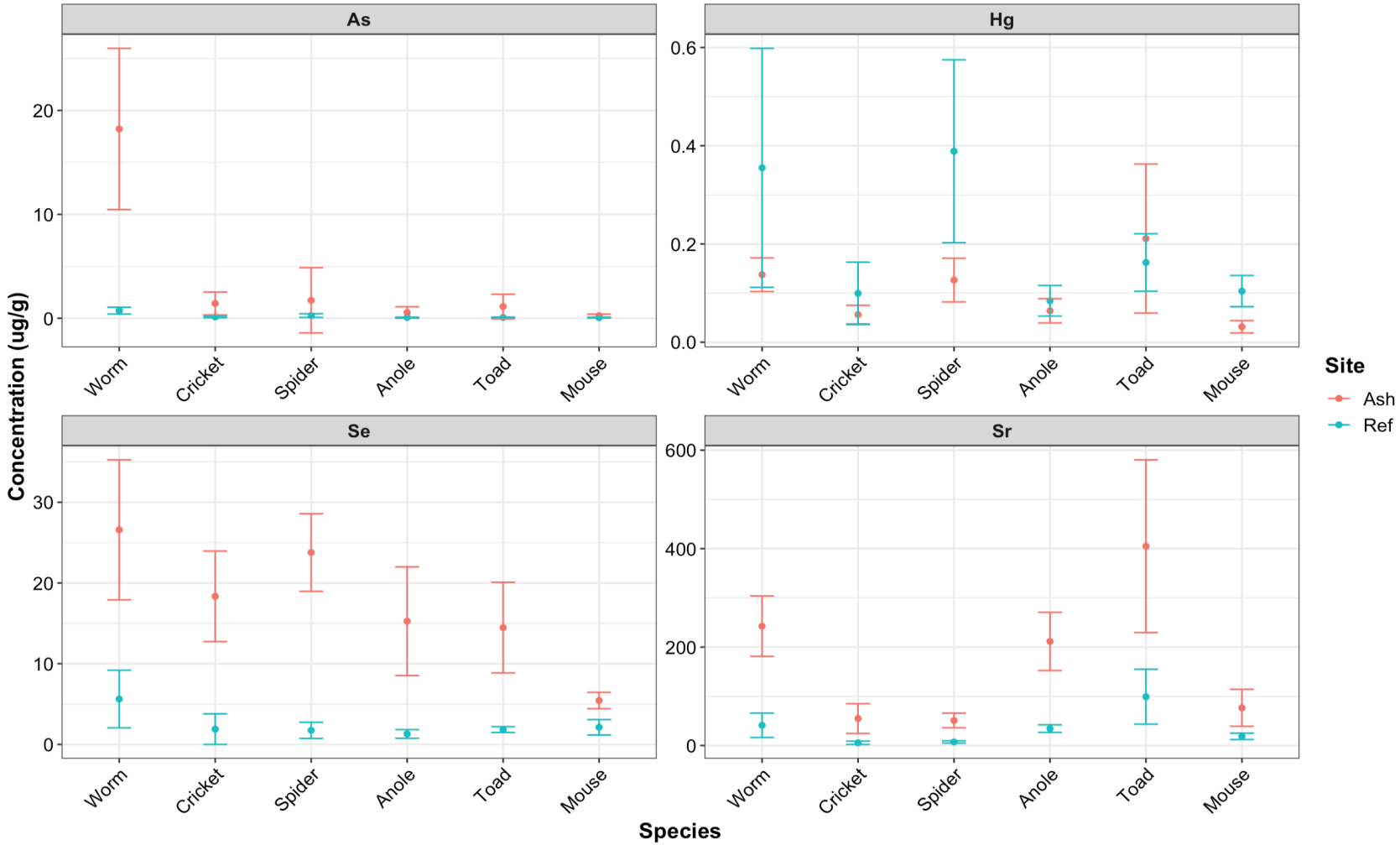


Figure 2.5: Average concentrations (µg/g dry weight) of trace elements (As, Hg, Se, and Sr) with standard deviations (± 1 SD) in six species collected from the D-Area Ash Plume (Ash) and a reference site (Ref) within the Savannah River Site, South Carolina. Invertebrate species included Oligochaetes (Worm), *Ceuthophilus uhleri* (Cricket), and *Tigrosa georgicola* (Spider). Vertebrate species included *Anolis carolinensis* (Anole), *Anaxyrus terrestris* (Toad), and *Peromyscus gossypinus* (Mouse).

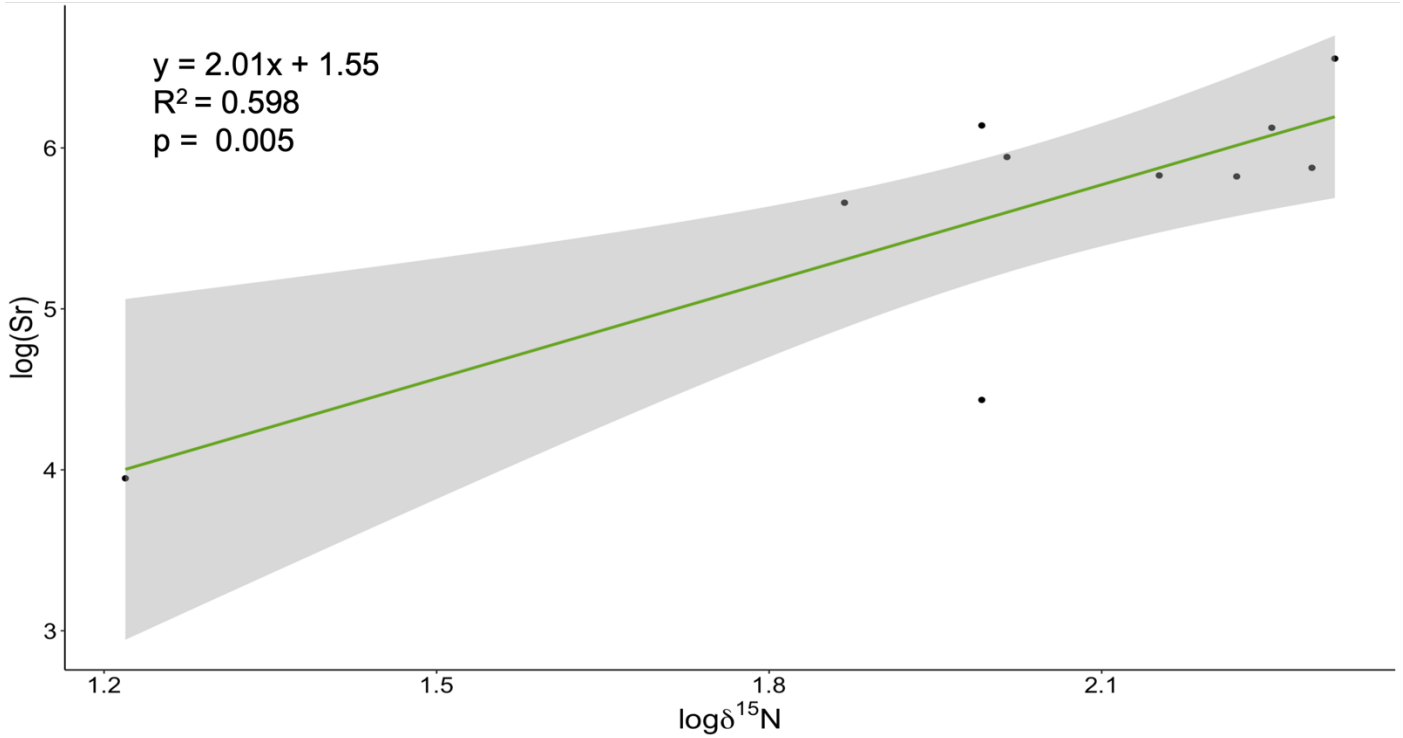


Figure 2.6: Linear regression model showing a significant positive relationship (p= 0.0053) between log Sr concentrations and log $\delta^{15}N$ values of southern toads sampled from the D-Area Ash Plume in Savannah River Site, South Carolina.

CHAPTER 3

COMMUNITY COMPOSITION OF SMALL VERTEBRATES IN A TERRESTRIAL SITE HISTORICALLY CONTAMINATED WITH COAL COMBUSTION RESIDUES

Holland, K.E., J.C. Beasley, and T.D. Tuberville. To be submitted to *Journal of Environmental Management*

ABSTRACT

Coal combustion residues (CCRs) are anthropogenic pollutants associated with coal-fired energy production throughout the world and contain potentially toxic trace elements such as arsenic (As), selenium (Se), and mercury (Hg). Historically underregulated disposal of CCR has facilitated environmental exposure. Negative effects of CCR exposure on survival, growth, reproduction, and recruitment have been documented across a variety of taxa, but community-level effects require additional attention. We evaluated the current community structure of small vertebrates within a terrestrial site that received CCR effluents in the early 1900s on the U.S. Department of Energy's Savannah River Site (SRS). Our objectives were to 1) quantify herpetofauna and small mammal communities through mark-recapture and camera trap surveys and 2) compare measures of alpha and beta diversity of small vertebrate communities among sites representing a gradient of presumed CCR exposure. We measured species richness, diversity, and similarity of herpetofauna and small mammals occurring within the core of the CCR contaminated area, an intermediate site (the interface of uncontaminated and contaminated area), and reference sites. Between July 2022 and July 2023, we had 837 live captures of 34 species from mark-recapture and 3026 individual detections of 31 species from camera traps. We found strong evidence of seasonal effects on all community metrics measured and limited but existing evidence of CCR exposure and environmental variables potentially influencing differences in species composition between sites. Our results suggest that community level metrics in biota can be influenced by numerous environmental factors. Comprehensive and long-term studies will be important in understanding the extent of CCR exposure within historically contaminated sites. This research contributes to the growing knowledge of community-level CCR impacts in terrestrial systems.

INTRODUCTION

Globally, loss of biodiversity is one of the greatest threats to functioning ecosystems (Hooper et al. 2005). Among the many threats to biota, pollution of air, water, and soil is often cited as one of the main factors contributing to localized and widespread species loss (Jaureguiberry et al. 2022). In particular, energy production and use causes widespread pollution across both terrestrial and aquatic ecosystems, with known negative impacts on the environment (Dincer 1998). Among sources of energy production, burning of coal accounts for about one third of the world's energy production (IEA 2021) and contributed over 600 million metric tons of waste in 2010 (Heidrich et al. 2013). Coal combustion emits harmful nitrogen oxides, carbon dioxide and particulates that exacerbate air pollution (Dincer 1998) and creates solid byproducts known as coal combustion residues (CCRs).

CCR includes fly ash, bottom ash, and slag that can contaminate waterways and sediments if not used for materials manufacturing or disposed of properly (Rowe et al. 2002). Fly ash, the primary component of CCR, contains high concentrations of non-essential trace elements such as arsenic (As), lead (Pb), and mercury (Hg) that can be extremely toxic at high concentrations to both plant and animal life (EPA 1988). CCR is typically disposed of in surface impoundments, known as aquatic settling basins, or in landfills which can facilitate environmental exposure through leachate, runoff, and unintentional releases (Carlson and Adriano 1993, Rowe et al. 2002). Most settling basins also are accessible to biota, and thus represent a potential pathway for contaminant exposure to a broad diversity of taxa (Rowe et al. 2002, Rowe 2014) and can even serve as ecological sinks (Rowe and Hopkins 2003). Increased regulatory legislature regarding CCR disposal (e.g., EPA 2015) and advancing clean energy technology have pushed the U.S. and other developed countries towards decreasing their reliance

on coal and retiring aging coal power plants (Gielen et al. 2019, Li et al. 2020, EIA 2022). However, these countries then face the issue of what to do with legacy contaminants generated by decades of coal combustion. Gaining an understanding of how legacy CCR waste affects resident biota can help inform disposal methods and remediation decisions to protect ecosystems from negative impacts of exposure.

Ash settling basins are the historically preferred fly ash disposal method due to low cost and labor demand (Carlson and Adriano 1993). However, resident wildlife can be chronically exposed to CCR contaminants and experience lethal or sublethal effects (Rowe et al. 2002) due to physical and chemical changes of water and sediments in comparison to non-contaminated wetlands (Rowe and Hopkins 2003). Studies of invertebrates (Buys et al. 2015, Fletcher et al. 2017), fish (Hopkins et al. 2000, Lemly 2002), amphibians (Snodgrass et al. 2004, Roe et al. 2006), reptiles (Nagle et al. 2001, Finger et al. 2016, Cochran et al. 2018), waterfowl (Leaphart et al. 2020), and semi-aquatic mammals (Borchert et al. 2019) have demonstrated that exposure to CCR facilitates bioaccumulation of trace elements (Cherry and Guthrie 1977), resulting in impacts to survival (Hopkins et al. 2004, Kuzmick et al. 2007), reproduction (Hopkins et al. 1998, Metts et al. 2013), and abundance (Lemly 2002) of numerous species. However, research on the effects of CCR exposure on wildlife within terrestrial systems requires more attention as most research has focused on aquatic systems. CCR pollutants have also been found to persist in sediments decades after disposal, potentially impacting ecosystems at multiple levels of biological order over time (See Chapter 2, Meduni et al. 2018).

Further, ecotoxicological studies on wildlife have largely focused on individual or population-level effects of contaminants, whereas comparatively little research has integrated community- or ecosystem-level approaches (Rhodes et al. 2020). By incorporating ecological

complexities such as environmental and inter-species interactions, the increased biological relevance of community-level studies can provide better understanding of ecosystem functioning in contaminated and remediated environments (Clements and Newman 2002). Negative impacts of pollutants at the community-level can be indicated by decreased biodiversity, shifts in species composition, or absence of rare or sensitive species. If toxicity thresholds are exceeded, direct loss of species can occur from lethality. For example, the resident fish community in Belews Lake in North Carolina, USA was reduced from 20 species to a single species after receiving wastewater from a coal-fired power plant (Lemly 2002). Pollution tolerant species can also replace sensitive species. The small mammal community at a Superfund site in Oklahoma, USA, experienced a compositional shift, with one tolerant species dominating in comparison to reference sites (Phelps and Mcbee 2009). Fly ash as a soil contaminant in terrestrial systems makes small terrestrial vertebrates such as small mammals valuable biosentinels of local contamination levels and impacts (Adamus and Brandt 1990, Talmage and Walton 1991). Studying exposure effects of CCR on a community of small terrestrial vertebrates can give insight into contamination levels and the potential for larger scale environmental effects.

In the present study, we compared community composition of terrestrial small vertebrates along a CCR exposure gradient on the U.S. Department of Energy's Savannah River Site (SRS) in South Carolina, USA. Ash deposition from fly ash effluents occurred at the CCR-contaminated site for over two decades, although the site has also not received fly ash effluents in over 50 years and has been allowed to naturally revegetate. Therefore, our study aims to inform future remediation decisions by investigating community level effects of legacy CCR. Our objective was to quantify and compare measures of richness, diversity, and similarity of terrestrial small vertebrate communities among sites along a contamination gradient of presumed

CCR exposure, accounting for natural variation among sites through measuring biologically relevant environmental variables. We hypothesized that richness and diversity would be lowest in the contaminated site and that contaminated and reference site species composition would be dissimilar if the presence of CCR was impacting community assemblages.

METHODS

Study Site

We conducted our study within the SRS, a 780 km^2 property located along the Savannah River in South Carolina, USA. (Figure 3.1). Originally established to produce nuclear weapon materials during the Cold War era, the SRS supports a variety of terrestrial and aquatic habitats, including areas of known contamination (White and Gaines 2000). The SRS's "D-Area" housed a coal-fired steam power plant that functioned from the early 1950s until its closure in 2012. While operational, effluents of fly ash slurry were released into primary and secondary aquatic settling basins. Until the 1970s effluents were also released into a nearby bottomland hardwood forest, including one accidental breech event, creating a plume of ash sediments on the surface. The 40-hectare D-Area Ash Plume (DAP) is a well-defined waste unit within SRS, with the boundaries of ash deposition identified through historical and contemporary soil sampling and trace element quantification (see Chapter 2). The DAP has not received new effluents in over five decades but fly ash persists under several centimeters of leaf litter and organic soil that have accumulated since releases have ceased. The plume has since revegetated into a mid-successional floodplain forest dominated by American sweetgum (*Liquidambar styraciflua*), bald cypress (*Taxodium distichum*), and American sycamore (*Platanus occidentalis*). With the power plant closed and ash basins remediated, research has shifted to how residual legacy ash within DAP is currently impacting the terrestrial ecosystem. We surveyed small terrestrial vertebrates

within DAP and from three nearby reference sites. Reference sites had no known history of CCR contamination and were chosen based on habitat similarities to DAP including elevation, dominant vegetation types (mixed hardwood floodplain forest), and hydrology as identified by LiDAR imagery. Reference sites were approximately 1.5, 3, and 4 km from DAP.

Sampling

We constructed sampling arrays along a gradient of presumed contaminant exposure, targeting small (< 300g) terrestrial vertebrates in Core (n = 5), Intermediate (n = 5) and Reference (n = 7) areas (Figure 3.1). Core arrays (or "Ash" arrays) were at least 100 m inside the outer edge of the plume to limit the extent to which home ranges of surveyed species could have incorporated areas outside of the contaminated area. We considered individuals caught at core arrays those most frequently exposed to CCR. We constructed Intermediate arrays outside the eastern edge of the plume at its interface with the surrounding forest but within 50 m of the plume boundary (Figure 3.1), increasing the likelihood individuals were moving between both contaminated and non-contaminated areas. We assumed individuals caught in Intermediate arrays were less chronically exposed to CCR compared to individuals from Ash arrays. We established 5 additional arrays in Reference site 1, which had no known CCR contamination (see Chapter 2). We added Reference sites 2 and 3, each with 1 additional sampling array, 4 months into the study to increase reference replicates and spatial scale of sampling. We assumed individuals caught at reference arrays to have spent little to no time exposed to CCR based on the spatial ecology of our target species. All arrays were systematically placed 100 m or more away from each other to limit overlap but allow multiple arrays within the DAP.

At each of the 17 sampling arrays we used both passive and active sampling techniques to quantify community composition of terrestrial small vertebrates, including amphibians,

reptiles, and mammals. Arrays consisted of three 7.5 m wings of aluminum flashing installed to create a Y-shaped drift fence. We placed funnel traps on each side of the flashing and the three wings converged on a central pitfall trap (sunken 5-gal bucket). Modified camera traps were placed at the terminal end of each drift fence arm to passively sample small vertebrates (Martin et al. 2017, Amber et al. 2021). Additionally, for small mammals we set a 5 X 4 (n = 20 traps) grid of Sherman live-traps (H.B Sherman Traps Inc., Tallahassee, FL) spaced 10 m apart and centered on the array (Figures 3.2a and 3.2b). We baited Sherman traps with sunflower seeds covered in peanut butter oil (Wildlife Control Supplies, Suffield, CT).

Active sampling periods consisted of a mark-recapture period that occurred during the first full week of each month from July 2022 to June 2023. On the first day we opened all traps and monitored them daily for five consecutive days. Each day we identified individuals to species, recorded age class, and if they were new or recaptured. We used taxa-specific techniques for marking new individuals. We assigned larger bodied snakes, turtles, and small mammals with individual identification codes via PIT tags, notching, and ear tags, respectively. All other species were given an array-specific code rather than unique identification codes due to higher quantities of captures. For array-specific codes we toe-clipped amphibians and injected visual implant elastomer in reptiles. These marks facilitated identification of recaptured individuals and detection of movement of marked individuals between arrays.

For passive sampling, we placed three modified trail cameras at each sampling array, which remained active continuously from July 2022 - July 2023. We mounted infrared trail cameras (Stealth Cam QS20; GSM Outdoors, Irving, TX) in inverted 5-gallon buckets with openings cut at opposite sides allowing movement through the bucket and photos taken to document species (Martin et al. 2017, Amber et al. 2021). We checked cameras every week for

the first month then every 2-3 weeks for malfunction and to change batteries and SD cards. We set cameras on the highest detection sensitivity with 3 photo bursts on a 30 second delay to increase likelihood of detection and identification. We uploaded all photos containing target vertebrates into Camelot software (Camelot v1.6.13; Hendry and Mann 2018) and, when possible, identified detections to species. To be recorded as an individual detection event, photos from the same camera of the same identified species had to be at least 1 minute apart. One minute was chosen to balance limiting recounting individuals while not missing detections.

Environmental variables

At each array we recorded environmental variables, including elevation, canopy density, ground cover density, abundance of coarse woody debris, and basal area of canopy trees. Elevation was determined at sampling arrays (± 4 m accuracy) using a hand-held GPS device (GPSMAP® 64 series, Garmin Ltd., Schauffhausen, Switzerland). Canopy density was calculated using a spherical densiometer at the center of the array and expressed in percent. Ground cover density was measured ordinally from 0-5 based on percent cover (0-20%, 21-40%, 41-60%, 61-80%, 81-100%) in a 1 m^2 plot 5 m away from the array in a randomly generated direction. For basal area we recorded species and DBH (>10 cm) of trees within a 10 m radius and converted to m^2/hectare. We also recorded the number and species of understory trees (<10 cm) within a 7.5 m radius of the center of the array. We recorded the number of coarse woody debris >10 cm DBH in a 7.5 m radius from the center of the array.

Statistical Analysis

All statistical analyses were performed in R Studio (R Core Team 2023) using packages nlme (Pinheiro et al. 2023) and vegan (Oksanen et al. 2022). We tested variables measured for normality using Shapiro-Wilks test. For all tests a p-value of <0.05 was considered significant.

We first assessed habitat differences among sites, using analysis of variance (ANOVA) to compare elevation, canopy density, woody debris, and basal area among Ash, Intermediate, and Reference sites 1, 2, and 3. We used a non-parametric Kruskal-Wallis statistical test to compare ground cover among sites due to its non-normal distribution. We used any environmental variables that significantly differed among sites in subsequent models to account for site variability. We also assigned each array an ash exposure score (hereafter "ash exposure") based on the site type: 0 for Reference arrays, 1 for Intermediate arrays, and 2 for Ash arrays.

We analyzed mark-recapture and camera data separately for all community metrics due to varied surveying frequency and to compare their effectiveness in species detections. For consistency, we organized capture data from mark-recapture and detection data from camera traps in the same manner. We first organized number of captures and detections by species per day for each array. Due to limited captures from mark-recapture, we then pooled daily captures by month per array. We also assigned each month a season to assess perceived seasonal trends. July - August 2022 and June of 2023 were considered summer, September – November 2022 as fall, December 2022 – February 2023 as winter, and March 2023 – May 2023 as spring.

We calculated alpha and beta diversity measures based on monthly captures per array. For species richness, we calculated rarified richness rather than raw richness to account for uneven sampling period among sites. Rarefaction calculates species richness based on a random subsample of individuals (a "sample"). Typically, the sample number used would less than the total community size, or number of species, detected within the sampling period (Shimadzu 2018). Because we had a few months with one or more arrays having no captures or detections, we instead based our rarefaction on 5 samples, or species within the community, a low but robust enough number for calculations for most arrays. Rarified richness values for Reference sites 2

and 3 were aggregated with Reference site 1. We log transformed resulting richness values to improve non-normality, then calculated average richness over time, standard deviation, and 95% confidence intervals by site (Ash, Intermediate and Reference) and within each season to visualize trends.

To calculate diversity, we used inverse Simpson's index which considers species richness and evenness but is less sensitive to presence of rare species than other indices. Similarly to above, diversity values were calculated per month by array, Reference sites 2 and 3 were aggregated with Reference site 1, and all values were log transformed prior to analysis. We calculated average diversity, standard deviation, and 95% confidence intervals seasonally by site type including Ash, Intermediate, and Reference, to visualize trends.

We created linear mixed effects models to assess effects of ash exposure, as well as season and environmental variables, on richness and inverse Simpson's diversity calculated from both capture methods. For mark-recapture and camera trap methods, we used richness and inverse Simpson's diversity as our response variables and ash exposure, season, and environmental variables as explanatory variables, totaling four models. We included array nested within site type as a random effect in all models.

For similarity, we used Bray-Curtis dissimilarity index within vegan to quantify species compositional differences between sites and used non-metric multidimensional scaling (NMDS) to visualize effects of ash exposure and season. We used NMDS to ordinate communities and added 95% confidence ellipses grouped by ash exposure. Additionally, we calculated multiple regressions of environmental variables and species using the envfit function and incorporated them into our dissimilarity ordinations. We followed our ordination with permutational multivariate analysis of variance (PERMANOVA) to statistically ask how much variation in

community composition could be attributed to differences in season, ash exposure, and environmental variables. We used 999 permutations and maintained the representation within site type (Ash, Intermediate, or Reference) to preserve the original structure of the data.

RESULTS

Environmental variables

Of the five environmental variables recorded, we found significant differences in elevation and ground cover among Ash, Intermediate, Reference 1, 2, and 3 sites (Table 3.1). Canopy density, coarse woody debris and basal area did not show significant differences between sites. Pairwise comparisons showed Reference site 1 arrays were located at significantly lower elevations than Ash (Tukey HSD; $df = 4$, $adj\ p = 0.004$) and Intermediate arrays ($df = 4$, $adj\ p = 0.001$) although differences were small (~4 m). Additionally, pairwise comparisons showed Ash arrays had significantly more ground cover than Reference site 1 arrays (Dunn Test; $Z = 2.93$, $adj\ p = 0.034$).

Mark-Recapture

We had 837 captures of 34 species including 15 reptile, 15 amphibian, and 4 small mammal species (Table 3.2). We recorded 22 species at the Ash site, 16 from the Intermediate site, and 11-21 species from the three Reference sites. Eleven of 34 detected species were found at the Ash, Intermediate, and at least one Reference site. We did not have enough recaptures to make any strong inferences on species abundances or movement between arrays. Of our 55 total recaptures, seven were from a different array than their original capture array. Of the seven individuals recaptured in different arrays, only two (southern toads, *Anaxyrus terrestris*) moved between site types. None of the recaptured individuals moved out of the Ash site, but the two southern toads moved from an Intermediate array to an Ash array. Recorded movement patterns

supported both our hypothesis of chronic exposure in the Ash site and that individuals caught within the Intermediate site could have some level of exposure to CCR. However, with such a small sample size we cannot confidently say that movement wasn't reciprocal from the Ash site to the Intermediate site as well. There was no effect of site on the number of species observed per array ($F_{4,12} = 1.13$, $p = 0.387$). The most abundant and specious taxa caught were frogs, with 13 species recorded. Eastern spadefoot toads (*Scaphiopus holbrookii*), southern toads, and southern leopard frogs (*Lithobates sphenocephalus*) together made up over 50% of captures.

Rarified richness averaged within season and grouped by site ranged from 0.29 – 1.02 in the Ash site, 0.53 – 0.72 in the Intermediate site, and 0.38 – 1.09 in the Reference sites (Figure 3.3a). Overall, fall had the highest average rarified richness for all sites. Spring had the lowest average rarified richness for Ash and Intermediate sites and winter was lowest for Reference sites. Seasonal averages of inverse Simpson's diversity ranged from 0.28 – 0.97 in the Ash site, 0.49 – 0.64 in the Intermediate site, and 0.34 – 1.05 in Reference sites (Figure 3.3b). All sites had highest diversity in the fall. Both Ash and Intermediate sites had lowest diversity in the spring while Reference sites had the lowest in the winter. Linear mixed effect models showed season had a significant effect on richness ($F_{3,135} = 7.88$, $p = <0.001$) and diversity ($F_{3,135} = 7.46$, $p = <0.001$) but neither ash exposure nor environmental variables did (Figures 3.4a and 3.4b). Fall had a significant positive effect on species richness and diversity while winter had a significant negative effect on both measures.

When assessing beta diversity, characterized by Bray-Curtis dissimilarity, our PERMANOVA results revealed season ($F_{3,145} = 4.04$, $p = 0.001$), ash exposure ($F_{2,157} = 5.54$, $p = 0.001$), and elevation ($F_{1,145} = 2.58$, $p = 0.004$) had a significant effect on species composition, as visualized in Figure 3.5a. In NMDS visualization (stress < 0.08), Ash and Intermediate sites

seemed to be more similar to each other than either were to reference sites; however, 95% confidence ellipses overlapped for all 3 site types (Figure 3.5a). Dissimilarity was driven by outlier species and environmental variables. Species drivers included those caught ≤3 times, including the five-lined skink (*Plestiodon fasciatus*) and copperhead (*Agkistrodon contortrix*). Elevation ($R^2 = 0.058$, $p = 0.007$) and ground cover ($R^2 = 0.049$, $p = 0.011$) were found as environmental drivers of dissimilarity among sites. Neither species nor environmental variables could be shown on ordination plots because point coordinates were outside of axis range required to view fine scale patterns.

Camera Trapping

Detection events were derived from 12,400 photos of biota, including 3570 photos of non-target species (i.e, birds, mesomammals, and invertebrates), and 610 photos for which species could not be identified. After removal of non-target species, unknown or unidentifiable species, and repeat detections, we had 3026 individual captures of 31 species including 19 reptile, 7 amphibian, and 5 small mammal species (Table 3.3). Snakes were the most specious taxa with 12 species captured. Rodents made up the most abundant taxa with cotton mice (*Peromyscus gossypinus*) alone representing over 50% of detections. Fourteen of 31 detected species were found in the Ash, Intermediate, and at least one Reference site. Cameras detected a significantly higher number of species than mark-recapture ($F_{1,32} = 18.31$, $p = <0.001$). Site had a significant effect on the average number of species observed per array ($F_{4,12} = 3.47$, $p = 0.042$), with 15.4, 14.2, 11.8, 15, and 13 average species recorded from the Ash, Intermediate, and Reference site 1, 2, and 3 arrays, respectively. A pairwise comparison showed that significantly more species were recorded from arrays in the Ash site compared to at arrays in Reference site 1 (Tukey HSD; $df = 4$, $adj\ p = 0.027$). No other sites were found to be significantly different.

Seasonal rarified richness averages ranged from 0.43 – 1.02 in the Ash site, 0.49 – 0.99 in the Intermediate site, and 0.39 – 0.86 in Reference sites (Figure 3.3c). Ash and Intermediate sites had highest richness in the summer and Reference sites had highest richness in the spring. Winter had the lowest rarified richness averages for all sites. Inverse Simpson's diversity averages ranged from 0.00 – 1.47 in Ash arrays, 0.27 – 1.22 in Intermediate arrays, and 0.15 – 1.01 in Reference sites (Figure 3.3d). Ash and Intermediate sites had highest diversity in the summer, and Reference sites had highest diversity in the spring. Winter had the lowest diversity averages for all sites. Linear mixed effect models showed season had a significant effect on richness ($F_{3,162} = 14.35$, $p = <0.001$) and diversity ($F_{3,162} = 10.75$, $p = <0.001$) but that ash exposure and environmental variables did not (Figures 3.4c and 3.4d). Winter had a significant negative effect on species richness and diversity.

When assessing beta diversity, characterized by Bray-Curtis dissimilarity, our PERMANOVA results revealed season ($F_{3,173} = 6.54$, $p = 0.001$), ash exposure ($F_{2,173} = 2.45$, $p = 0.001$), and ground cover ($F_{3,173} = 1.91$, $p = 0.007$) had a significant effect on species composition, which can be visualized in Figures 3.5b and 3.5c. In NMDS visualization (stress < 0.22), 95% confidence ellipses overlapped for all 3 site types (Figures 3.5b and 3.5c). Dissimilarity based on camera trap data was driven by both species and environmental variables. Species dissimilarity was driven by abundant species like cotton mice in the Ash site, eastern woodrats (*Neotoma floridana*) in the Reference sites, and green anoles (*Anolis carolinensis*) in Ash and Reference sites (Figure 3.5b). The main environmental driver of dissimilarity among sites was ground cover (Figure 3.5c; $R^2 = 0.053$, $p = 0.008$).

DISCUSSION

Our study assessed the impacts of ash exposure on community metrics of terrestrial small vertebrates within a site historically contaminated with CCR effluents. We found strong evidence for seasonal differences in all community metrics based on both mark-recapture and camera trapping methods. However, we found little evidence that the presence of ash-associated contaminants in DAP are impacting alpha diversity measures such as species richness and diversity. Our findings suggest beta diversity as measured by dissimilarity in species composition could be impacted by ash but there are also environmental differences between sites. Together, our data found that the small vertebrate community may be altered within the contaminated site, but we have no evidence to confirm this alteration is negative.

Although we found evidence of ash exposure impacting species composition, there was broad overlap in species composition among reference and ash-contaminated sites and broadly speaking, the species of small terrestrial vertebrates we documented were reflective of a typical southeastern floodplain forest. About one third of species detected in mark-recapture and almost half of species detected from camera traps were found at the Ash, Intermediate, and at least one Reference site. The most abundant species captured or detected included eastern spadefoot toads, southern toads, southern leopard frogs, green anoles, cotton mice, and eastern woodrats, all of which are habitat generalists, native, and common to the southeast (Dorcas and Gibbons 2008, Gibbons et al. 2009, Best and Hunt 2020). Habitat generalists tend to be adaptable to disturbed habitats where they often outcompete species with more specialized requirements. For example, reptile richness peaked in human-altered Indonesian agroforests (Wanger et al. 2010), and abundances peaked in human developed areas (Paudel et al. 2023). In our study, anurans were among the most abundant species in the ash-contaminated site despite previous studies showing

amphibian vulnerability to habitat alterations (Wanger et al. 2010, Banville and Bateman 2012), particularly pollutants (Egea-Serrano et al. 2012). Previous research has also suggested certain small mammal species are relatively resilient to environmental stressors like heavy metals. A Superfund site study reported compositional differences between contaminated and reference site communities, with white-footed mice (*Peromyscus leucopus*) dominating the contaminated area (Phelps and Mcbee 2009). We found similar results with abundance of cotton mice, a close relative to the white-footed mouse, playing a key role in compositional differences between our contaminated and reference sites. The extent to which tolerant species could have replaced species sensitive to anthropogenic pollutants in DAP is unknown. Local adaptions to contaminants within species are also possible. Evidence of adaption to CCR exposure has previously been reported in southern toads when an exposed population had increased survival and larval growth within a contaminated environment in comparison to a naïve population (Flynn et al. 2021). Still, we did not detect any rare or sensitive species at any sites including reference sites, suggesting limited species replacement due to legacy CCR contaminants. We would need a more replicated design of both contaminated and reference sites to assess if significant differences in species composition are indicative of ash presence or a result of natural differences between sites.

We found strong evidence that season affected richness, diversity, and dissimilarity of small vertebrates based on both mark-recapture and camera trap data. Spring and summer had the highest richness and diversity for camera traps while winter had a strong negative effect on richness and diversity for both capture methods in our study. Similar seasonal trends have been reflected in community-level studies concerning related taxa. As ectotherms, herpetofaunal activity is largely influenced by seasonally variable environmental factors such as temperature

and photoperiod. A herpetofaunal study in Southern Texas, reported species richness, diversity, and evenness significantly varying among seasons with spring and summer having the highest richness and diversity (Ruthven et al. 2002). Small mammal abundance has also been reported to change seasonally, with lower abundances recorded during cold dry periods (Ramahlo et al. 2022, Richard et al. 2022), coinciding with the southeastern U.S. winter season. Additionally, food availability can drive activity trends in wildlife. As many of our target species are primary or secondary consumers, this coincides with plant and invertebrate biomass availability peaks in warmer months of the year (Hicke et al. 2002, Shaftel et al. 2021).

Targeting a wide array of taxa within our community of interest prompted us to implement both active and passive capture techniques. We found that using live traps, including pitfalls and funnels, was more effective for amphibian captures and our adapted camera traps were more effective for reptiles, specifically squamate, and small mammal detections. Previous studies implementing adapted camera traps with drift fences also reported higher reptile and mammal species detections versus amphibian species (Martin et al. 2017, Dundas et al. 2019, Amber et al. 2021). While mark-recapture and camera traps each possess their own challenges and biases, using both methods allowed us to create more comprehensive species lists. Live traps facilitated confident species identification of individuals by having them in hand. Mark-recapture can also contribute to understanding species abundance and movement patterns when enough recaptures are obtained (Lettink and Armstrong 2003). Our recaptures were limited but supported our prediction of little movement among arrays and sites by individuals. Camera trap use allowed us to continually obtain data with less field hours but was more difficult to identify individuals to species. We recommend similar community-level studies implement multiple trap types when possible to comprehensively sample target multiple taxa.

Community ecotoxicology presents a unique challenge in separating impacts due to anthropogenic disturbance, such as CCR contamination, from natural environmental variation. To account for natural variation within and among sites, we measured multiple environmental variables that can impact microhabitat used by small terrestrial vertebrates. Environmental variables varied minimally among sites, and although elevation and ground cover differed between sites, we found no evidence that any environmental variables we measured affected species richness or diversity. Overall, elevation averages differed by ≤4 m among sites and while statistically different, such modest differences are likely not biologically meaningful in our study system. Ground cover density was greater at our Ash sampling sites compared to Reference site 1, which may be attributed to the enriched availability of essential trace elements to plants growing within the ash plume (Usman et al. 2023). In contrast, we did find evidence of environmental variables effecting dissimilarity between sites. Elevation and ground cover effect on species compositional differences could be at least in part due to habitat variation among site types. One habitat variable we noticed but were unable to quantify was average array distance to water within sites. Periodic flooding of reference sites prevented accurate measurement of distance to water measurements for each array; thus, we could not determine the influence of proximity to water on community metrics. As water is required for portions of the lifecycle of most amphibians, it may be an important factor in shaping species composition in our target community. Paudel et al. (2023) reported an inverse relationship between amphibian abundances and distance to the nearest water source. Additionally, our detections of aquatic species unique to reference sites such as water snakes (*Nerodia spp*.) and eastern mud turtles (*Kinosternon subrubrum)*, reflect proximity to water could be driving species trends. Future community-level

studies aiming to distinguish contaminant effects from environmental effects should carefully consider variables included.

Despite previous work finding elevated concentrations of trace elements within soil and biota in our target community (Chapter 1), our results demonstrate that CCR exposure within a historically contaminated site has limited impact on community-level metrics. Instead, our data suggests season consistently impacts richness, diversity, and composition of small terrestrial vertebrates. Additionally, environmental factors may influence species compositional differences between contaminated and reference sites. Our contaminated site has not received contaminant inputs in over five decades, and therefore these results may not be reflective of sites experiencing more recent CCR contamination. However, this research contributes to the growing knowledge of community-level effects concerning ecotoxicology and specifically contributes to limited knowledge of CCR impacts on terrestrial systems. Long-term monitoring of terrestrial communities and the incorporation of biological effects, such as age class and abundance assessments, could provide more complete understanding of community-level trends within CCR contaminated sites.

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Table 3.1: Comparisons of environmental variables recorded at 5 sites within Savannah River Site (SRS), South Carolina, August 2023. An asterisk (*) indicates significant p-value. Letters indicate significant differences between sites using post-hoc tests. [1] indicates variable was recorded on ordinal scale showing median with upper and lower quartiles.

Variable	ASH (n=5) Mean ± SD	INT (n=5) Mean ± SD	REF 1 (n=5) Mean ± SD	REF 2	REF 3	Test	p-value
Elevation (m)	11.45 ± 0.97^{A}	12.07 ± 1.14^{A}	7.75 ± 1.62^{B}	9.45	9.75	ANOVA	0.002*
Canopy Density (%)	86.90 ± 2.16	90.22 ± 2.28	88.14 ± 5.13	80.24	85.44	ANOVA	0.157
Ground Cover[1]	3 (3-4)^{A}	1 (.5-2)	1 (0-1)^{B}	0	1	Kruskal-Wallis	0.023*
Woody Debris	9.0 ± 3.81	13.8 ± 6.76	17.8 ± 12.36	20	14	ANOVA	0.541
Basal Area (m^2/hectare)	218.41 ± 115.71	301.37 ± 178.78	390.85 ± 98.98	372.75	187.37	ANOVA	0.339

Table 3.2: Number of individuals captured of each species detected at each site (ash, intermediate, reference 1-3) during mark-recapture in July 2022 - June 2023 within the Savannah River Site, South Carolina. Species in order of highest to lowest total captures within larger taxonomic groups.

			Site					
Taxa	**Species**	**Common Name**	**ASH**	**INT**	**REF1**	**REF2**	**REF3**	**Total**
Salamanders	*Ambystoma opacum*	Marbled salamander	1	6	5	37	5	54
	Plethodon glutinosus	Slimy salamander			1			1
							Subtotal	55
Frogs	*Scaphiopus holbrookii*	Eastern spadefoot toad	9	10	110	4	35	168
	Anaxyrus terrestris	Southern toad	67	56	35	3	5	166
	Lithobates sphenocephalus	Southern leopard frog	12	41	46	5	3	107
	Gastrophryne carolinensis	Eastern narrowmouth toad	25	41	7	1	2	76
	Lithobates clamitans	Green frog	4	39	2			45
	Acris gryllus	Cricket frog	8	14	8		1	31
	Pseudacris crucifer	Spring peeper	5	7		1		13
	Dryophytes chrysoscelis	Grey treefrog	2		3			5
	Hyla squirella	Squirrel treefrog	2	3				5
	Hyla cinerea	Green treefrog	2	1				3
	Hyla gratiosa	Barking treefrog	2		1			3
	Lithobates catesbeianus	American bullfrog	2				1	3
	Pseudacris ornata	Ornate chorus frog					1	1
							Subtotal	626
Lizards	*Anolis carolinensis*	Green anole	13	7	29			49
	Scincella lateralis	Little brown skink	4		1			5
	Plestiodon laticeps	Broadhead skink	2	1				3
	Plestiodon fasciatus	Common five-lined skink	1		1			2
							Subtotal	59
Snakes	*Storeria dekayi*	Dekay's brown snake	4					4
	Agkistrodon contortrix	Copperhead	2		1			3
	Agkistrodon piscivorous	Cottonmouth				2	1	3
	Virginia striatula	Rough earth snake		1	2			3
	Coluber constrictor	Black racer		1			1	2
	Nerodia erythrogaster	Plain-bellied water snake				2		2
	Pantherophis alleghaniensis	Eastern rat snake			2			2
	Diadophis punctatus	Ring-necked snake			1			1
	Storeria occipitomaculata	Redbelly snake				1		1
							Subtotal	21
Turtles	*Kinosternon subrubrum*	Eastern mud turtle				1	1	2
	Terrepene carolina	Eastern box turtle					1	1
							Subtotal	3
Rodents	*Peromyscus gossypinus*	Cotton mouse	18	9	11	2		40
	Neotoma floridiana	Eastern woodrat			10			10
							Subtotal	50
Shrews	*Blarina carolinensis*	Short-tailed shrew	4	6	9			19
	Sorex longirostris	Southeastern shrew	2		2			4
							Subtotal	23
		Total	191	243	287	59	57	837

Table 3.3: Number of individual detections of species seen at each site (Ash, Intermediate, Reference 1-3) through camera traps from July 2022 – July 2023 within the Savannah River Site, South Carolina. Species in order of highest to lowest total captures within larger taxonomic groups. *Genus spp* indicates individuals that could not be confidently identified to species and were instead identified to genera.

			Site					
Taxa	**Species**	**Common Name**	**ASH**	**INT**	**REF1**	**REF2**	**REF3**	**Total**
Salamanders	*Ambystoma opacum*	Marbled salamander		2			Subtotal	2
Frogs	*Anaxyrus terrestris*	Southern toad	27	20	14	1	3	65
	Scaphiopus holbrookii	Eastern spadefoot toad	7		28		17	52
	Lithobates spp	True frog spp	6	11	9	9	3	38
	Gastrophryne carolinensis	Eastern narrowmouth toad	5	2		2	1	10
	Hyla spp	Tree frog spp	2	1				3
	Acris gryllus	Cricket frog	1					1
							Subtotal	169
Lizards	*Anolis carolinensis*	Green anole	180	121	175	1	4	481
	Plestiodon spp	Skink spp	42	39	45	3	3	132
	Scincella lateralis	Little brown skink	18	9	5			32
							Subtotal	645
Snakes	*Coluber constrictor*	Black racer	23	13	27	1	13	77
	Agkistrodon contortrix	Copperhead	10	11	18	2	2	43
	Pantherophis alleghaniensis	Eastern rat snake	14	8	18			40
	Agkistrodon piscivorous	Cottonmouth	7	3	10	5		25
	Thamnophis sirtalis	Common garter snake	1	17				18
	Nerodia spp	Water snake species		4	7	1		12
	Lampropeltis getula	Eastern kingsnake	6	3				9
	Storeria dekayi	Dekay's brown snake	5				2	7
	Crotalus horridus	Timber rattlesnake		1	1			2
	Diadophis punctatus	Ring-necked snake	1			1		2
	Heterodon platirhinos	Eastern hognose snake				1		1
	Lampropeltis triangulum	Eastern milksnake		1				1
							Subtotal	237
Turtles	*Terrapene carolina*	Eastern box turtle	20	11	1		2	34
	Kinosternon subrubrum	Eastern mud turtle				1	2	3
	Sternotherus odoratus	Eastern musk turtle					2	2
	Trachemys scripta	Pond slider	1	1				2
							Subtotal	41
Rodents	*Peromyscus gossypinus*	Cotton mouse	685	507	289	102	100	1683
	Neotoma floridana	Eastern woodrat	18	56	88			162
	Sigmodon hispidus	Hispid cotton rat	3					3
							Subtotal	1848
Shrews	*Blarina carolinensis*	Short-tailed shrew	23	29	22	2	1	77
	Sorex longirostris	Southeastern shrew	5		2			7
							Subtotal	84
		Total	1110	870	759	132	155	3026

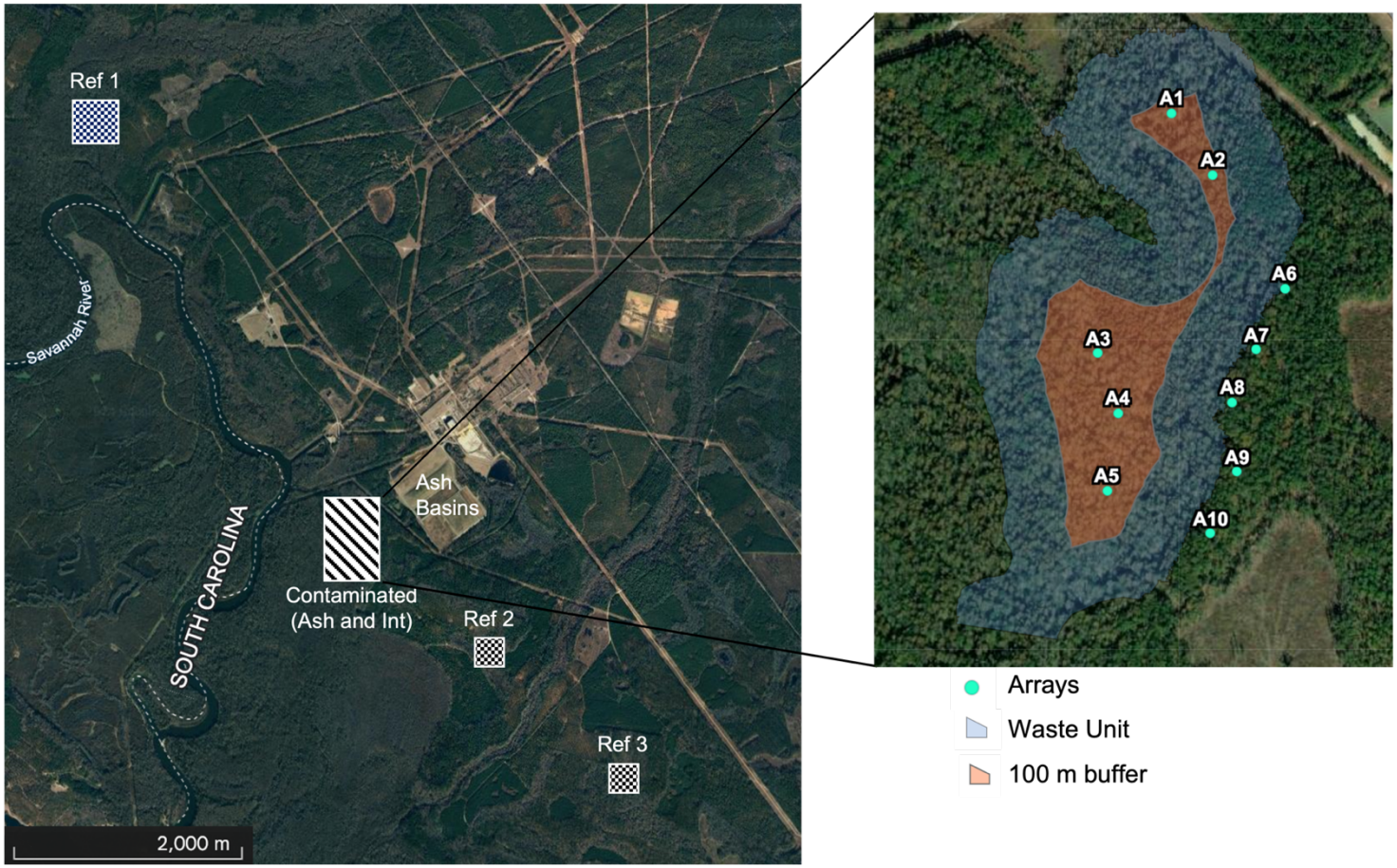


Figure 3.1: Map of all sites (Ash, Int, Ref 1, Ref 2, and Ref 3) were community sampling occurred within Savannah River Site (SRS) in South Carolina, USA. Zoomed in map of the contaminated area shows the outline of the D-Area Ash Plume ("Waste Unit"), a 100 m buffer from the edge designating the ash plume core containing ash arrays (A1-A5), and intermediate arrays (A6-A10) within 50 m of the edge of the plume.

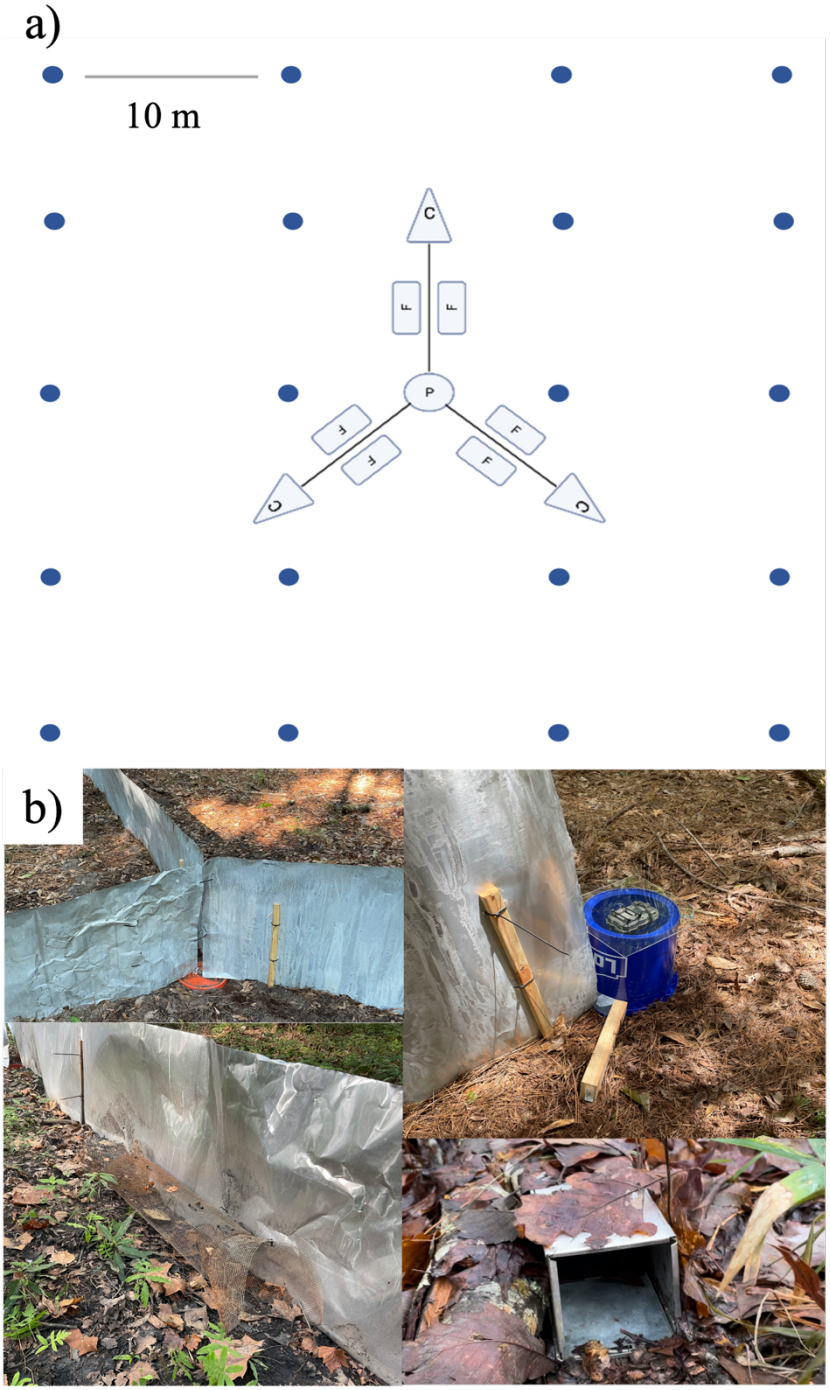


Figure 3.2: Sampling arrays used at ash, intermediate, and reference sites within Savannah River Site, South Carolina. a) Diagram of sampling array layout with trap placement; C = camera traps, F = funnel traps, P = pitfall trap, dot = 5 x 4 Sherman trap grid spaced 10 m apart. b) Photos of traps included in sampling arrays; left to right: inactive pitfall trap with lid, modified bucket camera trap, wire mesh funnel trap, and Sherman live trap in situ.

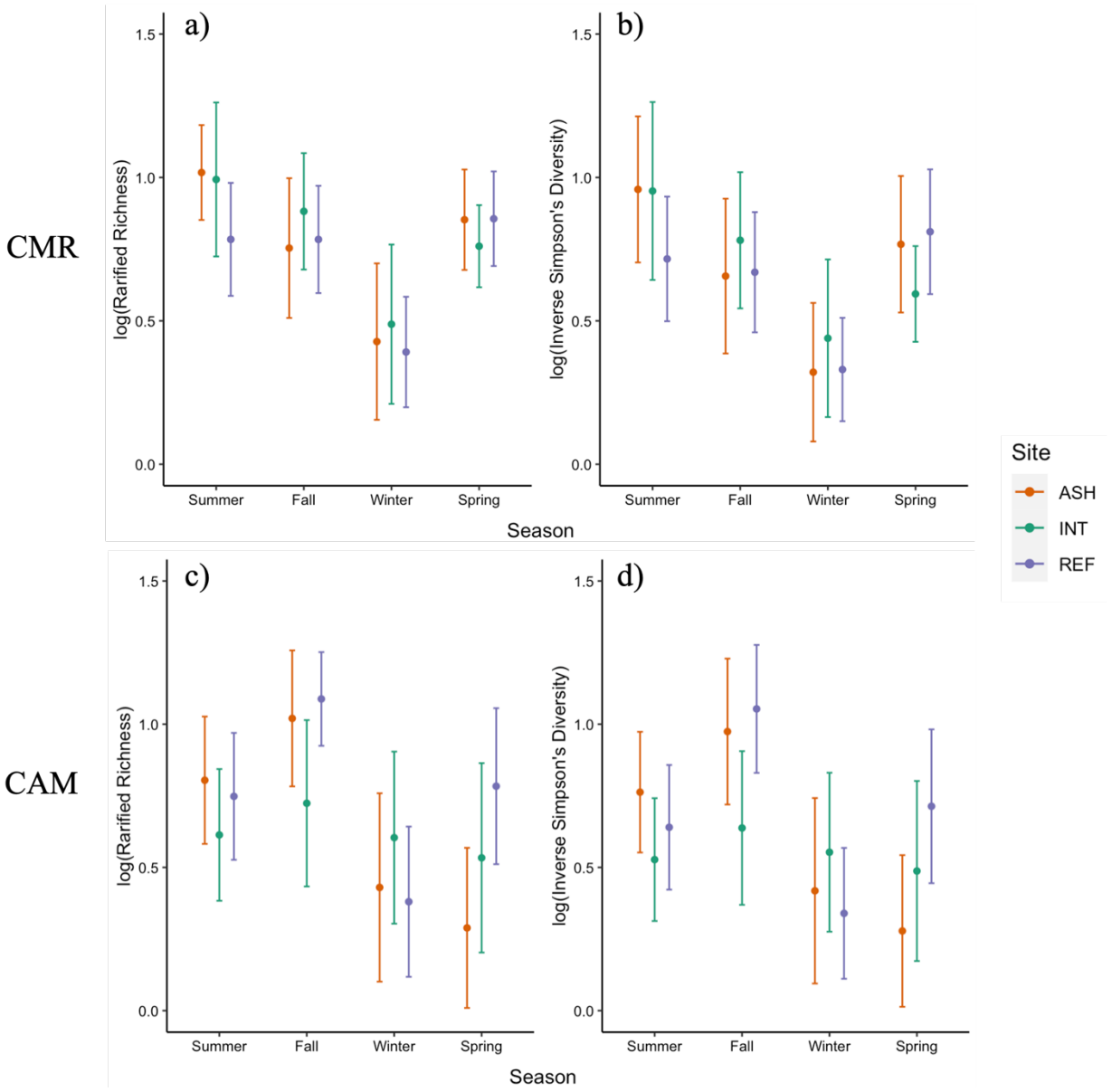


Figure 3.3: Small terrestrial vertebrate log transformed richness and diversity values from mark-recapture (CMR) and camera traps (CAM) within Savannah River Site, South Carolina. Rarified richness (left) and inverse Simpson's diversity averages (right) plotted with 95% confidence intervals for each site by season. ASH = Ash site, INT = Intermediate site, REF = Reference sites 2 and 3 aggregated into Reference 1 site averages.

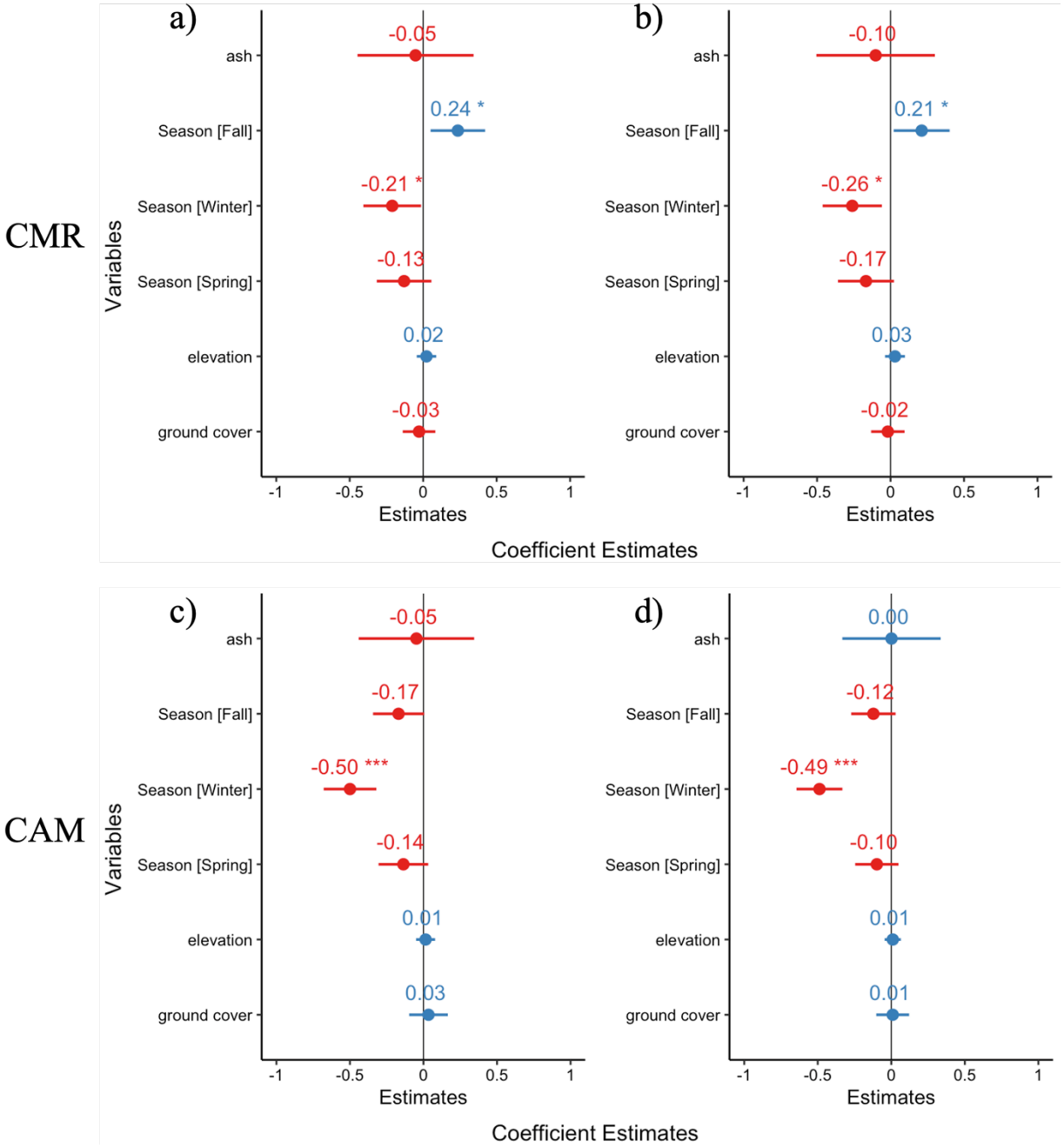


Figure 3.4: Coefficient estimates of fixed variables including ash exposure, season, and environmental variables (elevation and ground cover) on richness (right) and inverse Simpson's diversity values (left) derived from mark- recapture captures (CMR) and camera trap detections (CAM). Asterisks (*) depict significance. Winter had a significant negative effect on richness and diversity for both capture methods. Fall had a significant positive effect on richness and diversity While no significant effect was detected, ash exposure coefficient estimates had wide CI ranges.

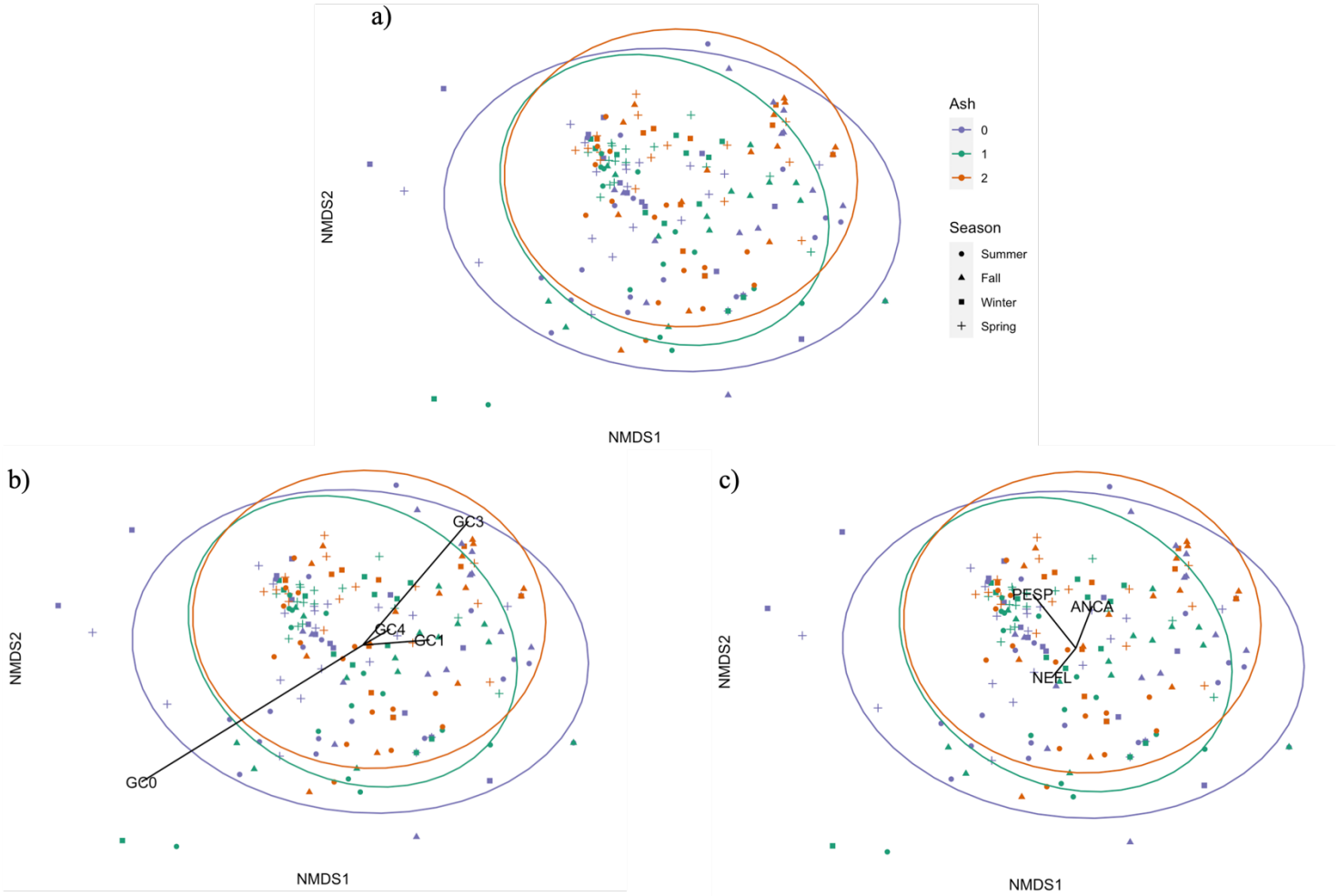


Figure 3.5: Non-metric multi-dimensional scaling plot of monthly a) mark-recapture captures and b-c) camera trap detections per array by ash exposure level (Ash = 2, Intermediate = 1, Reference = 0) and season using averaged Bray-Curtis dissimilarity distances. 95% confidence ellipses around ash exposure levels. a) 3 outlier points omitted to view finer scale patterns, b) *Peromyscus gossypinus* (PESP), *Anolis carolinensis* (ANCA), and *Neotoma floridana* (NEFL) were significant species driving compositional patterns, c) Ground cover also drove dissimilarity patterns (GC0 – GC4 = ground cover percentages on ordinal scale 1 thru 4).

CHAPTER 4

SUMMARY AND CONCLUSIONS

This thesis explores the impacts of legacy contaminants from coal combustion residues (CCR) on terrestrial biota. The preceding chapters explore patterns of persistence, uptake, and accumulation of potentially toxic elements and their possible community-level effects in a historically contaminated site. These studies contribute to limited literature focusing on terrestrial CCR contamination and its possible effects at the community-level. I present this research to inform future remediation decisions of our study site as well as similar sites containing legacy contaminants.

In Chapter 2, I reported current levels of ash-associated trace elements of concern—arsenic (As), mercury (Hg), selenium (Se), and strontium (Sr)—within soil and biota from a historically contaminated floodplain forest. Soil testing showed elevated levels of all measured trace elements in comparison to the reference site. I found no evidence of trace elements naturally attenuating within the site, despite not receiving CCR effluents in over five decades. In fact, in comparison to concentrations reported in 2003, contamination levels have remained the same except for Se, which increased. Body burdens from four vertebrate and three invertebrate species showed elevated levels of most elements, suggesting continued bioavailability and bioaccumulation of potentially toxic elements. Additionally, I found a positive relationship between trophic position, represented by stable isotope ratios, and concentrations of trace elements in some species. The potential for elemental biomagnification among higher trophic levels highlights the need for continued monitoring and inclusion of higher-level taxa in future

studies. The continued bioavailability of trace elements as demonstrated by elevated levels in sampled biota 50 years after CCR input cessation, emphasizes the persistence of these elements in the environment and importance of long-term studies within historically contaminated sites. This chapter also contributed to the limited knowledge of CCR-specific uptake and accumulation patterns among terrestrial vertebrates.

In Chapter 3, I investigated community metrics of biota within three site types—contaminated, intermediate, and reference—representing a presumed CCR exposure gradient. I used mark-recapture methods and camera traps to survey the small terrestrial vertebrate community including amphibians, reptiles, and small mammals. I then quantified community metrics, richness, diversity, and similarity, for each site type. I found no evidence that ash exposure negatively impacted species richness or diversity, and limited evidence that it impacts species composition. Instead, season appeared to be the most influential driver of all community metrics. I found species typical to southeastern U.S. floodplain habitats at all sites with over one third of species detected common to all three site types, suggesting the ash and intermediate sites capacity for supporting small terrestrial vertebrate communities. Community-level research on CCR exposure is limited and often focuses on species inhabiting aquatic disposal sites, therefore our research contributes to a significant knowledge gap in terrestrial community impacts of CCR exposure.

RECOMMENDATIONS

Based on the present study, I make the following recommendations to stakeholders involved in the management and remediation of terrestrial CCR contaminated sites. I recommend to 1) implement or continue contamination level monitoring of sediments and biota, 2) incorporate long-term studies, when possible, to assure impacts are not increasing over time, 3)

conduct similar sampling methods among higher trophic level taxa to monitor biomagnification potential, and 4) reduce and/or contain further contamination from entering areas surrounding disposal sites.

APPENDIX A

CHAPTER 2 SUPPLEMENTAL INFORMATION

Supplemental tables are provided reporting additional trace elements tested within soil and biota collected in Chapter 2 from the Savannah River Site, South Carolina in 2022-2023. Table A.1 shows the averages and standard deviations of the remaining 12 elements tested within soils including barium, beryllium, cobalt, chromium, copper, iron, molybdenum, nickel, lead, thallium, vanadium, and zinc. All elements were significantly higher in the D-Area Ash Plume (DAP) aside from iron and lead. Table A.2 reports average concentrations and standard deviations from the same suite of trace elements within our seven target species collected. Any species with >50% of samples below detection limits for an element were excluded. Table A.3 reports concentrations from 36 soil cores collected in DAP as part of a 2003 study (McLeod et al., unpublished data). These values were used in comparison to our contemporary soil samples to assess extent of natural attenuation of elements within the site in a near 20-year period.

Table A.1: Additional trace element concentrations (µg/g dry weight) in soils collected from the D-Area Ash Plume (DAP) and a reference site (Ref) in 2022 within the Savannah River Site, South Carolina. Asterisks signify significant differences.

	DAP (*n* =15)		Reference (*n* =8)			
Element	**Mean**	**SD**	**Mean**	**SD**	**p-value**	
Barium (Ba)	359.30	164.23	86.43	49.30	< 0.001	*
Beryllium (Be)	2.56	0.81	0.80	0.40	< 0.001	*
Cobalt (Co)	8.65	3.05	1.20	0.87	< 0.001	*
Chromium (Cr)	21.24	4.57	7.93	5.93	0.001	*
Copper (Cu)	29.01	7.22	2.88	1.69	< 0.001	*
Iron (Fe)	10119.19	3605.75	6269.96	5194.50	0.081	
Molybdenum (Mo)	3.51	2.56	0.29	0.00	< 0.001	*
Nickel (Ni)	21.12	6.18	3.86	1.44	< 0.001	*
Lead (Pb)	12.42	2.54	11.08	4.75	0.519	
Thallium (Tl)	0.90	0.27	0.10	0.06	< 0.001	*
Vanadium (V)	42.59	8.80	17.93	15.65	0.002	*
Zinc (Zn)	19.35	5.94	6.43	3.21	< 0.001	*

Table A.2: Additional trace element concentrations (μg/g dry weight) in species collected from the D-Area Ash Plume (DAP) and a reference site (Reference) in 2022-2023 from the Savannah River Site, South Carolina. Invertebrate species included Oligochaetes (Worm), *Ceuthophilus uhleri* (Cricket), and *Tigrosa georgicola* (Spider). Vertebrate species included *Anolis carolinensis* (Anole), *Anaxyrus terrestris* (Toad), *Peromyscus gossypinus* (Mouse), and short-tailed shrews (*Blarina carolinensis*). Asterisks signify significant difference in species between sites.

		DAP			Reference				
Element	**Species**	**N**	**Mean**	**SD**	**N**	**Mean**	**SD**	**p-value**	
Barium (Ba)	Worm	15	507.98	288.60	15	182.08	169.80	< 0.001	*
	Cricket	15	18.99	10.50	13	10.00	6.49	0.012	*
	Spider	15	5.80	2.46	15	3.93	2.25	0.033	*
	Anole	10	26.84	8.01	15	30.34	14.66	0.542	
	Toad	16	58.89	21.59	13	50.45	18.28	0.357	
	Mouse	10	27.15	12.82	10	26.82	13.56	0.940	
	Shrew	2	44.65	9.99	10	24.87	11.83	0.014	*
Beryllium (Be)	Worm	15	0.21	0.12	15	0.18	0.06	0.468	
	Cricket	15	0.03	0.03	13	0.03	0.04	0.035	*
Cadmium (Cd)	Worm	15	1.48	0.80	15	1.28	0.56	0.756	
	Cricket	15	0.25	0.23	13	0.21	0.31	0.333	
	Spider	15	1.86	0.72	15	3.17	1.21	0.005	*
	Anole	10	0.22	0.10	15	0.12	0.04	0.008	*
	Toad	16	0.20	0.18	13	0.20	0.09	0.254	
	Mouse	10	0.11	0.03	10	0.11	0.04	0.910	
	Shrew	2	0.25	0.09	10	0.20	0.17	0.416	
Chromium (Cr)	Worm	15	4.81	2.71	15	13.17	10.55	0.005	*
	Cricket	15	22.78	15.90	13	4.51	4.62	< 0.001	*
	Spider	15	0.65	0.45	15	0.72	0.47	0.868	
	Anole	10	2.38	1.73	15	10.07	7.41	< 0.001	*
	Toad	16	1.44	0.83	13	9.36	17.08	0.930	
	Mouse	10	0.51	0.26	10	1.15	0.69	0.028	*
	Shrew	2	0.82	0.57	10	1.77	0.82	0.099	
Cobalt (Co)	Worm	15	1.95	0.85	15	3.67	1.75	0.004	*
	Cricket	15	0.28	0.12	13	0.22	0.22	0.045	*
	Spider	15	0.14	0.09	15	0.19	0.21	1.000	
	Anole	10	0.10	0.06	15	0.23	0.12	0.004	*
	Toad	16	0.25	0.19	13	0.20	0.12	0.313	
	Mouse	10	0.06	0.02	10	0.07	0.03	0.307	
	Shrew	2	0.18	0.04	10	0.15	0.08	0.372	
Copper (Cu)	Worm	15	18.69	4.13	15	8.85	1.78	< 0.001	*

	Cricket	15	42.69	17.56	13	37.65	10.81	0.504	
	Spider	15	116.30	40.10	15	158.85	66.52	0.071	
	Anole	10	8.47	4.93	15	4.13	0.84	< 0.001	*
	Toad	16	19.17	8.74	13	14.69	6.89	0.096	
	Mouse	10	10.45	2.05	10	10.14	2.30	0.940	
	Shrew	2	12.23	2.85	10	12.54	5.15	0.884	
Iron (Fe)	Worm	15	2532.79	1475.49	15	2195.03	1170.46	0.694	
	Cricket	15	431.96	201.91	13	226.89	146.59	0.002	*
	Spider	15	165.06	74.37	15	211.94	115.72	0.165	
	Anole	10	111.85	26.06	15	167.14	61.21	0.007	*
	Toad	16	236.90	186.28	13	268.94	174.47	0.630	
	Mouse	10	209.58	35.59	10	234.27	56.40	0.290	
	Shrew	2	963.67	70.76	10	609.86	223.14	0.184	
Lead (Pb)	Worm	15	4.20	2.64	15	31.52	17.05	< 0.001	*
	Cricket	15	0.30	0.17	13	2.58	3.50	0.011	*
	Spider	15	0.80	1.08	15	0.56	0.47	0.885	
	Anole	10	0.27	0.44	15	0.34	0.32	0.166	
	Toad	16	0.90	0.96	13	1.32	1.44	0.119	
	Mouse	10	0.17	0.16	10	0.44	0.31	0.076	
	Shrew	2	0.48	0.14	10	3.48	2.94	0.056	
Molybdenum (Mo)	Worm	15	2.76	1.46	15	0.51	0.18	< 0.001	*
	Cricket	15	0.63	0.26	13	0.15	0.04	< 0.001	
	Spider	15	0.57	0.53	15	0.13	0.05	< 0.001	*
	Anole	10	0.40	0.24	15	0.13	0.05	< 0.001	*
	Toad	16	0.31	0.17	13	0.16	0.09	0.004	*
	Mouse	10	0.46	0.06	10	0.28	0.08	< 0.001	*
	Shrew	2	0.87	0.03	10	0.34	0.13	0.061	
Nickel (Ni)	Worm	15	5.51	1.46	15	2.84	1.24	< 0.001	*
	Cricket	15	1.56	0.48	13	0.67	0.30	< 0.001	*
	Spider	15	0.86	0.34	15	0.60	0.37	0.007	*
	Anole	10	0.93	0.81	15	3.39	2.85	0.007	*
	Toad	16	0.87	0.52	13	0.83	0.74	0.293	
	Mouse	10	1.10	0.37	10	1.12	0.46	1.000	
	Shrew	2	0.52	0.28	10	0.94	0.48	0.273	
Thallium (Tl)	Worm	15	0.72	0.20	15	0.09	0.02	< 0.001	*
	Cricket	15	0.05	0.03	13	0.01	0.01	< 0.001	*
	Spider	15	0.07	0.03	15	0.02	0.02	< 0.001	*
	Anole	10	0.05	0.01	15	0.02	0.04	< 0.001	*
	Toad	16	0.10	0.06	13	0.02	0.01	< 0.001	*
	Mouse	10	0.07	0.04	10	0.01	0.01	< 0.001	*

Vanadium (V)	Shrew	2	0.34	0.17	10	0.03	0.01	0.080	
	Worm	15	7.34	4.40	15	5.01	2.75	0.152	
	Cricket	15	0.80	0.56	13	0.48	0.53	0.002	*
	Spider	15	0.35	0.23	15	0.23	0.35	0.014	*
	Anole	10	0.09	0.07	15	0.09	0.05	0.542	
	Toad	16	0.53	0.60	13	0.34	0.28	0.335	
	Mouse	10	0.07	0.04	10	0.11	0.11	0.705	
Zinc (Zn)	Shrew	2	0.86	0.10	10	0.33	0.18	0.120	
	Worm	15	148.76	17.16	15	113.77	29.28	< 0.001	*
	Cricket	15	182.03	28.74	13	169.86	42.87	0.084	
	Spider	15	373.94	52.99	15	590.50	290.11	0.003	*
	Anole	10	178.00	43.37	15	137.69	24.17	0.011	*
	Toad	16	153.50	43.29	13	138.97	54.33	0.380	
	Mouse	10	165.64	137.08	10	281.09	205.06	0.131	
	Shrew	2	129.83	41.20	10	113.70	16.16	0.954	

Table A.3: Elemental concentrations from short cores taken within D-Area Ash Plume in the Savannah River Site, SC in 2003.

Sample ID	As	Cu	Fe	Hg	Mo	Ni	Pb	Se	Sr	V	Zn
D10	15.64	40.02	20747.00	0.36	1.65	21.20	16.10	5.48	68.71	47.29	56.02
E9	18.67	45.28	39001.56	0.29	4.23	22.00	29.79	12.27	43.70	75.34	73.45
E10	10.88	52.90	15660.47	0.61	1.74	18.30	13.61	4.72	55.50	32.72	51.22
F5	9.14	20.61	43565.00	0.11	1.32	23.12	27.30	3.34	42.32	94.52	54.30
F6	33.29	33.69	14309.00	0.29	4.40	30.72	17.52	7.00	181.29	66.07	30.40
F7	43.08	42.27	8213.42	0.11	2.91	17.57	16.26	9.09	224.82	33.51	44.35
F8	19.91	24.30	9380.00	0.19	1.08	21.02	11.18	3.43	215.95	43.45	21.26
F9	18.43	22.44	8563.00	0.13	1.10	18.76	8.68	4.28	214.81	37.48	17.34
F10	14.76	53.04	23388.00	0.59	2.01	26.27	21.99	4.99	57.61	49.98	92.43
G5	26.92	29.87	14425.00	0.21	4.67	25.52	14.63	5.19	151.00	46.62	33.17
G6	34.55	51.68	8253.47	0.11	4.48	17.35	16.21	6.68	277.07	28.64	41.28
G7	33.15	47.62	6533.03	0.11	1.87	15.05	15.09	5.02	275.96	26.06	42.13
G8	16.24	25.79	9358.00	0.17	1.36	21.94	10.97	3.69	215.56	42.51	30.59
G9	18.17	24.53	9700.00	0.19	2.96	21.80	9.47	4.01	186.40	40.53	21.66
G10	25.79	30.70	10421.00	0.25	5.30	23.56	14.09	9.09	160.09	53.77	37.69
H5	63.00	28.85	13168.00	0.20	3.39	25.77	10.70	3.18	164.05	51.55	29.98
H6	42.97	47.17	11597.54	0.11	4.48	18.44	13.50	6.95	270.44	29.81	41.49
H7	29.02	30.15	9419.82	0.20	8.59	25.80	9.95	4.26	214.26	37.10	21.84
H8	25.41	36.95	5219.23	0.11	7.94	10.33	12.55	2.90	281.14	24.52	27.33
H9	28.44	27.90	9516.00	0.22	2.58	23.41	11.93	4.53	230.06	50.06	26.50
I2	19.28	13.44	9477.00	0.11	1.00	11.03	9.99	3.28	53.52	30.53	19.67
I3	23.57	23.93	11215.00	0.13	1.36	22.22	9.66	3.83	143.99	39.87	21.39
I4	47.57	27.04	11756.00	0.16	1.88	22.46	9.65	3.85	224.04	43.94	32.61
I5	30.80	21.14	12237.00	0.11	2.38	18.76	6.60	2.96	107.09	32.19	29.62
I6	24.38	23.12	9397.71	0.11	6.06	8.96	15.65	5.40	50.69	28.15	54.58
I7	26.95	38.82	5595.24	0.11	4.54	12.67	12.46	4.42	283.77	24.29	33.00
I8	25.60	34.50	6342.45	0.11	3.03	12.52	9.85	3.68	192.11	22.70	28.24
J2	34.87	24.62	10763.00	0.14	3.28	19.83	9.23	4.24	120.96	39.63	27.36
J3	26.82	23.16	11098.00	0.14	1.25	20.56	8.72	2.96	146.20	38.78	23.51
J4	32.99	24.09	10325.00	0.14	4.70	19.16	8.67	4.48	105.75	39.38	25.67
J5	26.27	21.02	11658.00	0.11	3.23	17.39	6.67	4.09	85.69	33.04	19.67
J6	33.92	37.74	7340.24	0.11	1.78	13.47	10.04	9.70	202.58	23.20	33.15
J7	4.94	6.11	4445.28	0.11	0.22	2.96	10.49	2.06	10.63	14.27	13.45
K3	41.77	25.72	13575.00	0.17	3.09	21.95	9.05	4.75	115.78	42.93	24.13
K4	43.27	25.16	11930.00	0.13	3.52	16.95	7.57	4.17	69.57	37.69	17.98
K5	76.52	34.07	16868.40	0.19	6.07	22.17	9.48	7.26	104.98	45.83	17.40