USING TERRESTRIAL ARTHROPODS AS RECEPTOR SPECIES TO DETERMINE

TROPHIC TRANSFER OF HEAVY METALS IN A RIPARIAN ECOSYSTEM

by

GREGORY NEWTON O'QUINN

(Under the Direction of Paul M. Bertsch)

ABSTRACT

Four taxa of terrestrial arthropods (Carabidae, Gryllidae, Gryllacrididae, Lycosidae) were collected to quantify metal body concentration (Uranium, Nickel, Copper, Cadmium, Mercury, Titanium) in a riparian ecosystem on the Department of Energy's Savannah River Site, Aiken, SC. Metal concentrations for taxa collected at the test site (Tims Branch) were compared to a control site (Boggy Gut) to determine if significant differences in metal concentrations existed among taxa. A General Linear Model (GLM) was used to determine if metal concentration was dependent on species and if the number of individuals was affected by metal concentrations. The interaction of taxa and number of individuals caught was used to determine if metal concentration affected the number of individuals caught for each taxa and if species interactions were significant. Stable isotopes were used to determine trophic structure of taxa as well as evaluate the integrity of grouping members of Carabidae into morphospecies. Metal concentrations varied among taxa between sites for each metal. Uranium and Nickel were the only metals that were significantly different for all taxa between both sites. Additionally, Uranium and Nickel were the only metals that were significantly different among all taxa collected at Tims Branch. $\delta^{13}C$ values and $\delta^{15}N$ values were used to establish trophic positions for taxa and helped to identify groups of morphospecies. $\delta^{13}C$ values and $\delta^{15}N$ values from invertebrates in this study were compared to similar isotope values for cotton mice (*Peromyscus gossypinus*) collected from the same experimental site to provide insight into potential food sources for these small mammals. These feeding habits could possibly affect the flow of contaminants within this system.

INDEX WORDS: Carabidae, Gryllidae, Gryllacrididae, Lycosidae, morphospecies, stable isotopes, trophic level, metal burden.

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DEDICATION

I would like to dedicate this thesis to my fiancée Sadika Baladi, who knew long before I did what I was about to undertake, whose support, patience, and belief in my abilities enabled me to endure, and without whom I never, ever could have done this. To my mother, who showed me the true meaning of perseverance during difficult times.

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CHAPTER 1

INTRODUCTION

Many studies have been performed regarding the bioaccumulation of sediment bound pollutants in ecosystems using species from various vertebrate taxa. Generally, these studies have used taxa that represent higher trophic levels, such as mammals and birds. However, in terrestrial environments, invertebrates are the gateway to contaminant transport to higher trophic compartments. Only by understanding how contaminants are transported through invertebrate communities will we gain a better understanding of how contaminants move through an entire system. That is, understanding why some contaminants are highly mobile, while others are not, requires knowledge of the interaction between the soil biogeochemistry and the primary producers and consumers. Although there have been numerous studies of contaminants in invertebrates such as earthworms [1, 2], few studies have focused on terrestrial arthropods. Although earthworms can be ecologically significant as a food source to many vertebrate species, certain taxa of terrestrial arthropods constitute an even larger portion of vertebrate diets [3]. Moreover, studies have shown the importance of arthropods in the bioavailability of metals. Specifically, Hunter et al. [4] used various invertebrate taxa (Carabidae, Orthoptera, and Lycosidae, among others) to show how cadmium and copper bioaccumulate within a grassland ecosystem. This study showed that invertebrates had body: diet concentration factors of 2-4 times for copper and 3-5 times for cadmium. Similarly, Rabitsch [5] studied how Formicidae, Arachnida, and Coleoptera species inhabiting a smelter site could potentially accumulate lead, cadmium, copper and zinc. The results from this study indicated that metal concentrations of

species increased with site contamination levels. The concentrations of certain metals in taxa were within the range of pooled specimens reported in the Hunter study. Additionally, Heikens et al. [6] used Coleoptera, Arachnida, Formicidae, as well as other arthropods, to determine how lead, cadmium, copper, and zinc accumulate within invertebrate communities. They found significant differences in accumulation levels, by a factor of 2-12, between taxonomic groups. All of these studies focused on endpoint species that use different foraging strategies. The relationships between detritivores, herbivores, omnivores, and carnivores can influence contaminant mobility. Feeding behavior may ultimately influence pathways for introducing toxicants into the food web. For example, detritivores may feed on contaminated decaying plant material. Herbivores could accumulate toxicants by consuming plant material that has contaminants incorporated into the tissue or adsorbed to the outside. Omnivores feed on detritus, plants, and, in some instances, animals as well. Ultimately, contaminants could be passed on to carnivores feeding on any of these trophic groups.

Newman [7] defines bioaccumulation as "the accumulation of a toxicant in or on an individual". Therefore, regardless of whether the contaminant is present internally or externally, it is still potentially mobile. The previously mentioned studies have shown that terrestrial arthropods do accumulate contaminants, particularly heavy metals, due to their interaction with the soil. This suggests that terrestrial arthropods have a higher potential for bioaccumulation of sediment bound contaminants than non-ground dwelling taxa, such as arboreal arthropods. This is due to the fact that soil particles tend to adhere to the bodies of ground dwelling organisms as a result of normal movement along the soil. Further, some arthropods, both larval forms and adults, may accumulate metals by feeding on detritus. Both of these are potentially direct pathways for the movement of contaminants into insectivorous species.

It is well known that metal contamination in both aquatic and terrestrial environments is heterogeneously distributed, which makes field experiments addressing how these toxicants move through the food web extremely difficult. However, it is extremely important to know if a contaminant is bioavailable, and how it is accumulated. To model risk, the appropriate hierarchical scale must be identified. The contamination in the soil must be quantified, and the receptor species that may promote the mobility of the toxicant must be identified. Invertebrates, especially terrestrial arthropods, are ideal receptor species to determine the potential mobility of metals in the soil. .

In quantifying the accumulation of metals in contaminated systems, it is necessary to understand the mechanisms of trophic transfer within a food web. For example, if invertebrates are not accumulating contaminants above ambient levels, the contaminants may not be bioavailable, possibly due to geochemical properties within the soil system or to the particular speciation of the metals. Alternatively, physiological processes, such as sequestering metals, unique to a species may preclude any accumulation of a metal within tissues. It is also possible that the lack of accumulation could be due to avoidance of contaminated areas, or merely lack of food availability. Understanding these relationships is extremely important to long-term management of contaminated sites. At government facilities such as those run by the Department of Defense (DOD) and the Department of Energy (DOE), there is a legacy of environmental perturbations including the contamination of ecosystems from industrial by-products. If bioaccumulation of heavy metals is occurring in terrestrial arthropods, there is potential for transfer to higher trophic levels.

Due to the enormous diversity of terrestrial arthropods, selecting representative taxa to study can be difficult without understanding their functional niches and life histories. Not only

are terrestrial arthropods an important food source for a variety of upper level vertebrate consumers, they also function as pollinators, vectors, parasitoids, decomposers, herbivores, and predators [3]. These characteristics should be considered when selecting taxa to investigate trophic transfer of toxicants. Feeding behaviors vary among taxa, so determining how multiple feeding behaviors influence trophic transfer is extremely important. For example, one possible route of trophic transfer is through predation on detritivores and herbivores by certain generalist predators, vertebrate or invertebrate. The movements of these individuals based on these foraging strategies could influence the spatial distribution of metals in the environment.

It has been estimated that invertebrates (specifically, arthropods) represent about 5/6 of known organism [8]. Due to this extremely large number, it is likely that many species have not been described. Additionally, it has been stated that, due to the enormous diversity of invertebrate taxa, some species may become extinct even before they are characterized [9-11]. The concept of placing organisms into specific groups based on similar morphological characteristics is quite common among invertebrate studies due to the vast diversity of terrestrial arthropods. Several authors have explored the concept of using morphotypes to functionally group species. Oliver and Beattie [12, 13] discuss the use of morphospecies (taxa that are readily separated by morphological differences that are obvious to individuals without extensive taxonomic training) as a possible method of rapid assessment of biodiversity and ecosystem function. Identifying morphospecies also has the potential to clarify trophic interactions among invertebrates. Due to the vast diversity of terrestrial arthropods, many different types of food web interactions exist. Predator-prey interactions at the arthropod level probably exceed the interactions exhibited by higher vertebrate groups, such as mammals. It has been shown that nearly half of all the insect taxa have members that are top predators in certain habitats even after

vertebrates are factored in [3]. This indicates that insects do not just occupy middle links in ecosystems, but also occupy local top predator positions. Morphospecies may establish certain food web compartments, which in turn may reveal potential contaminant pathways. However, merely classifying individual taxa into morphospecies is not adequate to determine the structure of an ecosystem. This method only hypothesizes trophic pathways. Other methods must be used to actually identify what foods are assimilated. That is, terrestrial arthropods occupy a larger number of niches and exhibit wider trophic breadth than vertebrate taxa. As a result, it is much more difficult to identify predator-prey interactions.

One of the most widely used methods for gaining insight into trophic positions and interactions among taxa is the use of stable isotope analysis, particularly carbon and nitrogen isotope analysis. Isotope compositions are measured as ratios (e.g. $^{15}N/^{14}N$ for nitrogen and $^{13}C/^{12}C$) and are reported in standard delta notation as $\delta^{a}x=[(r_{sa}/r_{std})-1]\ 1000$, where r_{sa} and r_{std} are isotope ratios in a sample (sa) and standard reference material (std) and $^{a}x=^{13}C$ or ^{15}N refers to carbon or nitrogen isotopes, respectively.

The $\delta^{13}C$ of an organism typically falls within the range of foods it eats. For plants, $\delta^{13}C$ values are related to the photosynthetic pathways by which carbon is fixed in plant biomass [14]. Plants using the C_3 photosynthetic pathway have $\delta^{13}C$ values between –18‰ and –36‰, whereas plants that fix carbon via the C_4 pathway have a $\delta^{13}C$ range of -7‰ to -18‰ [15, 16]. The whole plant $\delta^{13}C$ is heavily dependent on environmental conditions, such as water availability, light, and temperature [17]. Animals have $\delta^{13}C$ values that are similar to the foods they eat [14]. Therefore, differences in $\delta^{13}C$ composition of an organism can be attributed to different diets.

Nitrogen stable isotope compositions for organisms are commonly utilized to elucidate the relative trophic position an individual occupies in an ecosystem. Animal nitrogen is enriched

in ^{15}N relative to the dietary nitrogen incorporated by about 3-4‰ [18]. This is due to protein formation, degradation, and excretion of nitrogen. Animals with relatively high $\delta^{15}N$ values are considered to feed at higher trophic levels for ecosystems that have nitrogen bases that are isotopically similar.

Carbon and nitrogen isotopes can help gain insight as to how an organism assimilates energy within a functional food web. For example, stable isotope enrichment of ^{15}N and ^{13}C has been used to study a generalist invertebrate predator (*Pardosa lugubris*, Lycosidae) that was fed prey of different quality [19]. For this study, experimental food chains were established to incorporate three trophic positions: prey media, prey organism, and predator. Different life stages of *P. lugubris* were fed different types of prey, resulting in varying patterns of stable isotope enrichment. Quinn et al [20] used nitrogen isotopes to analyze the trophic transfer of metals in stream food webs. The authors used nitrogen stable isotope ratios to measure food chain length, mean trophic level, taxa richness and trophic position of stream macroinvertebrates in relation to metal bioavailability. $\delta^{15}N$ values were measured for taxa in order to determine their trophic position. This study showed that the relationship between trophic position and metal accumulations differed considerably among certain metals (Fe, Cu, Zn).

Stable isotope analysis can also aid in determining the accuracy of placing individuals into morphospecies groups. Large differences in the isotope composition within a particular morphotype may indicate that individuals were improperly categorized. Alternatively, differences may be attributed to resource availability. To date, some studies have combined the techniques of using morphospecies and stable isotope analyses to better understand the relationship between contaminant movement within aquatic ecosystems disturbed from industrial toxicants [20]. However, no studies presently address the issue using terrestrial ecosystems.

Stable isotope analysis of soil-inhabiting invertebrates could provide insight into the pathway through which contaminants reach upper level vertebrate species. This information is critical for ecological risk assessments and the development of remediation strategies in areas of known contamination. This is particularly important at DOE and DOD federal facility sites where there is greater potential for release of toxicants based on past anthropogenic activities.

The purpose of this study is to determine how metals, specifically, uranium (U), nickel (Ni), copper (Cu), cadmium (Cd) and mercury (Hg) assimilate into the soil arthropod foodweb in the Tims Branch ecosystem, an area of known soil contamination on the DOE's Savannah River Site (SRS). These metals, and others, were present in processed wastewater discharged into the Tims Branch corridor [21]. This effluent discharge could also potentially have an impact on the Upper Three Runs Integrator Operable Unit (IOU) via surface water pathways. Titanium (Ti) was included in this study because it is a good marker for soil, and can indicate possible accumulation of soil particles in or on arthropods [22]. These metals have been the subject of studies conducted at Tims Branch [23, 24] to determine how contaminants move within the ecosystem. Previous studies on small mammals and amphibians from the Tims Branch ecosystem have shown elevated concentrations of metals can be quantified in vertebrate taxa [24, 25]. However, the mechanisms responsible for these elevated concentrations are not fully understood. These particular taxa feed on invertebrates. Therefore, invertebrates may be the link between metals in the soil and higher trophic levels. The four taxa chosen were families Gryllidae (crickets), Gryllacrididae (camel crickets), Carabidae (ground beetles), and Lycosidae (wolf spiders). These taxa represent different trophic positions within terrestrial arthropod foodwebs (omnivores and generalist predators). These taxa can serve as indicator species to determine the bioavailability of metals. Based on this focus, the specific objectives were to: (1)

collect three different taxa in order to determine if metal accumulation is occurring in an area of known soil contamination and if these levels differ among taxa; (2) use stable isotopes to determine if morphospecies can be distinguished based on trophic position; (3) determine if invertebrate contaminant levels are related to trophic structure based on morphospecies and as identified through stable isotopic analyses; and (4) determine if invertebrate metal concentration is correlated with soil gamma activity from U daughter products.

Description of taxa:

The order Orthoptera, in particular, the families Gryllidae (crickets) and Gryllacrididae (cave and camel crickets) contain many common and well-known species [26]. Sizes for representatives of the family Gryllidae range from 15-17 mm (*Acheta domesticus*) to 14-30 mm (*Gryllus pennsylvanicus*). Gryllacrididae range from 14-19 mm (*Ceuthopolis maculates*) to 30-50 mm (*Stenopelmatus fuscus*) [27]. Both families are primarily short winged or wingless, and have chewing mouthparts for feeding on plant material, although some species are detritivores. Typically, cricket life cycles begin with an egg; after hatching the young develop through a series of instars which progressively look more like the adult [28]. In habitats such as caves and the tropics, where typically there are no seasons, individuals can be in any particular life stage at any time of year. In temperate regions, species may over winter as eggs, mid to late juveniles, or even as adults. The majority of the life cycle is spent crawling on the ground, with brief periods of flight in search of other habitats when necessary. For some species, the only flight is an initial migratory flight upon reaching the adult phase. Ground movement patterns also are species dependent. One study showed that bush crickets (*Metrioptera bicolor*) had mean daily movements of 2.25 m for males and 2.16 for females, with a maximum daily movement distance of 40.2 m [29]. However, for the most part, little is known of daily movements for most species.

Most crickets are omnivorous, but depending on genus, they may be entirely herbivorous or largely predacious. Ground inhabiting crickets that are herbivores primarily cut and eat small or young plants with their chewing mouthparts. These characteristics, along with abundance and relatively large body mass, make these two particular families good representatives of low-level consumers.

The order Coleoptera is another taxa well represented among the terrestrial arthropods. This order contains more species than any other extant taxa, vertebrate or invertebrate, with approximately a quarter of a million species described to date [26]. Beetles are primarily plant feeders and scavengers; however some are predators of insects and other invertebrates [27]. The family Carabidae (ground beetles) is one of the most abundant families within this order with over 40,000 described species [30], and almost all species are predacious. The subfamily Harpalinae accounts for nearly 85% of the entire Carabid family [31]. Representatives of this subfamily range in size from 8-8.5mm (*Pterostichus leconteianus* Lut.) to 20-25mm (*Dicaelus dilatatus*). This diversity sometimes makes it difficult to fully understand their life histories, feeding behaviors, and movement patterns. Generally, ground beetles develop from a single egg deposited in the soil, from which a larva emerges. Typically, the larva is free moving, undergoing three stages to pupation and then becomes an adult, usually within a year. As an adult, ground beetles can move either by crawling or flying. Due to the energy costs of flight, they typically move by crawling. Movement patterns are species and habitat dependent, with most members being nocturnal. One study used radio telemetry to examine the dispersal of *Carabus coriaceus* L.[32] and found a maximum linear dispersal range of 51.25 m in a beech-pine forest within a 12-hour period, with a maximum total distance of 387.8 m in 17.5 days and 191.1 m in 12 days. However, it should be noted that these particular individuals were quite

large (30-40 mm), which could indicate a larger dispersal range compared to species with smaller body sizes. Another study showed the effect of temperature on dispersal [33]. As temperature increased, the maximum radius sampled by a trap in one day increased, with the maximum distance traveled 29 m at 20^{o} C. Many studies have shown that carabid beetles are quite voracious and feed on a wide variety of food items, including other beetles and spiders [30]. Other prey items include springtails, aphids, and fly larvae. This wide range of feeding habits indicates that this particular taxa is a good representative for a mid level consumer.

In order to fully establish relationships among trophic levels, a top-level predator is required. Perhaps the best example of this in the arthropod community is spiders (order Aranae,). Wolf spiders (Lycosidae) are generalist predators that are exclusively carnivorous, generally seizing only live animals [34]. The life cycle of spiders can be divided into four main periods. The first period consists of an embryonic state from egg to emergence that occurs in the cocoon. The second phase is a gregarious phase that lasts about 4 to 5 days. These juveniles are incomplete and not able to hunt, relying on either egg yolk or undeveloped eggs. Dispersal occurs shortly thereafter as the spiderlings become capable of hunting, beginning the third solitary phase. The final period is the adult molt to death phase. This phase is primarily dedicated to reproduction. These life cycles are dependent on environmental conditions such as temperature and other geographically determined conditions. The family Lycosidae is a very common group of spiders that have the capacity to produce silk, although they do not build webs. Rather, they are predators that actively forage for prey, either as sit and wait ambush predators or as wandering predators. They may either hide in burrows or under objects such as rocks or leaves, or they may be seen running along the ground. They can either be diurnal or nocturnal. Although spiders do not travel very far, distances of more than 100-200m per night

can be covered by some wandering nocturnal individuals [35]. However, two species of lycosidae, *Hogna helluo* and *Pardosa milvina*, have been shown to occupy ranges of 1 per m^2 and 2 per m^2, respectively [36]. Representatives from the family Lycosidae range in size from 3.3-3.7mm (*Pirata minutus*) to 22-35mm (*Lycosa carolinensis*). (It should be noted that some taxonomists use the genus *Lycosa* in place of *Hogna*). As generalist predators, spiders typically feed on any type of invertebrate that is available. Since they are top macroinvertebrate predator, they can have a substantial trophic impact on a wide variety of primary and secondary consumers [37]. Orthopterans (specifically Gryllidae, crickets) have been used as live prey items in feeding experiments using wolf spiders [38, 39]. Wolf spiders have strong jaws that crush insects, as digestive fluid is regurgitated, leaving behind only a small mass of undigestible material such as chitinous elements [34]. This type of feeding behavior is quite unlike the general perception of weaker jawed spiders using fangs to inject venom into their prey, extracting the liquefied tissues, and leaving behind the empty shell. This is an important concept to consider when looking at contaminant uptake. The method utilized by the wolf spider provides more complete consumption of whole prey, leaving behind only a small amount of material, and therefore possibly picking up more contaminants.

CHAPTER 2

MATERIALS AND METHODS

Study Site:

This study focuses on understanding the mobility of soil-bound metals in a contaminated riparian area on the Department of Energy's (DOE) Savannah River Site (SRS). The SRS is a 777 km^2 former nuclear weapons production and facility located in the Upper Atlantic Coastal plain in west-central SC, established in 1951 by the Atomic Energy Commission. Although the production of weapons material has ceased, the facility is still used to house nuclear materials and waste processing sites and for research. Since the establishment of SRS, numerous ecosystems and watersheds have become contaminated by site operations.

Beginning in 1954, aluminum-clad Uranium targets were produced in A and M areas of the SRS. For three decades metals were released via effluent discharge into a nearby holding reservoir called Steed Pond (SP). Steed Pond is a former farm pond located on the Tims Branch (TB) corridor and is part of the SP-TB system, a small second-order black water stream that drains portions of the Upper Three Runs watershed, which drains into the Savannah River. This effluent discharge continued for three decades until 1982, when the discharges ceased due to implemented waste management practices. The M-area settling basin was removed from service, stabilized, and capped in 1989 [40]. In 1984, the man-made dam at SP breached, and the reservoir released its contents downstream. This caused downstream flooding, and resulted in another area, Pond 25, essentially becoming a settling basin for the contaminants present in SP. This allowed the deposition of contaminants to occur as well. As the flooded basin drained,

these contaminants were left behind. Not too long after the breach of the SP dam, the dam at Pond 25 also breached, resulting in contaminant transport even further downstream.

The specific portion of the SP-TB corridor used in this study is Pond 25 and will be subsequently be referred to as Tims Branch. Whipple et al [41] described the major components of the SP-TB system as: 1) bottomland hardwood forest including numerous beaver ponds; 2) the floodplain of Steed Pond, which exhibits early successional growth; and 3) Tims Branch, which displays mature growth at an advanced successional stage. There have been previous studies on plant flora and soil sediments that show metals are present in varying concentrations throughout the Tims Branch basin [23]. These studies have shown that the contamination is heterogeneously distributed in the SP-TB system and is constrained by stream morphology [42]. Studies of cotton mice [24] have indicated elevated levels of metals, and willow trees have shown accumulation of metals as well [23, 43].

A control site, Boggy Gut (BG), was chosen due to its similar geomorphological characteristics and comparable habitats to the SP-TB system . This site is upstream from the SP-TB system within the Upper Three Runs watershed on the SRS, but is not impacted by site activities.

Invertebrate collections:

At both Tims Branch and Boggy Gut, 40 trap sites were established along 4 transect lines approximately 23 m apart, paralleling the stream that runs through the basin at each site. Transect lines contained 10 trap sites that were approximately 10 meters apart (in some instances, traps were greater than or less than 10 m apart due to tree roots hindering digging in the ground). Invertebrates were collected using pitfall traps. This sampling method was used

primarily because it provided a passive means of trapping, and is a highly efficient method for collecting terrestrial organisms [44]. Pitfall traps were constructed with aluminum flashing and plastic drinking cups. Drift fences for the traps were made from the aluminum flashing and were approximately 30 cm long by 13 cm high. A hole was dug in the ground using a long handled bulb planter and a plastic cup was placed in the hole. Four pieces of flashing were placed at 90^{o} intervals from one another around the hole. Each drift fence was placed 3 cm into the ground to ensure stability. 50 mL of a 10% formalin solution were added to each trap to act as a killing agent as well as a preservative. A square (15 mm x 15 mm) ceramic bathroom tile was placed over the cup to deter any direct rainfall from collecting in the trap. However, in periods of heavy rainfall, water accumulated in traps set up in areas prone to flooding or where the ground was consistently saturated. Since traps were checked weekly, this dilution of formalin did not cause any deterioration of arthropods collected in the trap. The contents of each trap were poured into pint size Mason jars for transport back to the laboratory. The plastic cups were then inverted over the hole to render the trap inactive until the following trapping session. Trapping seasons were conducted in the fall and spring. The first fall trapping season began during the final week of August 2002 and concluded the third week of November. The spring trapping season began during the last week of March 2003 and concluded the third week of June 2003. A second fall trapping season was initiated during the first week of September 2003 and concluded the last week of November 2003. Trapping seasons originally were scheduled to last 6-7 weeks, with traps activated for 1 week then closed for 1 week.

Samples were transported back to the laboratory in jars and the contents were sorted. The contents from each individual trap site were poured into a plastic weighing boat and specimens were sorted out according to family as follows:

1. Coleoptera: Carabidae (ground beetles)
2. Orthoptera: Family Gryllidae (crickets) and Family Gryllacrididae (camel crickets)
3. Aranae: Family Lycosidae (wolf spiders)

Due to their enormous diversity and consequent difficulty in identification, specimens of Coleoptera were placed in morphologically similar groups (morphospecies) based on body part structure, color, and/or size. This method yielded eight groups of morphospecies (A, B, C, D, E, F, G, K). Single specimens from these morphospecies were placed in individual vials to provide a reference for future collections. Identification keys were used to separate Orthoptera and Lycosidae representatives to family with relative ease. These specimens were placed in clean vials and labeled with the taxa (order, family) and trap site location (Ex., Coleoptera, Carabidae A, TB 1-1). Numbers of individuals from each taxa collected were recorded according to trap site. All other types of invertebrates that were collected were placed in vials and labeled as "miscellaneous". Representatives for all taxa were verified by Dr. Cecil Smith, curator of the entomology department for the Natural History Museum at the University of Georgia. The verified specimens were then used as references for subsequent collections. Due to a pilot study to determine the best taxonomic identification system, no morphospecies were identified for the first 16 trap sites (transect 1, traps 1-1 through 1-10 and transect 2, traps 2-1 through 2-6) for trap season one, Fall 2002. Collections for the remaining sessions for the first trap season and subsequent seasons were sorted and identified as described above.

Metals analysis:

Metals analysis was conducted on invertebrates collected from season one (fall 2002) and season two (spring 2003). The contents for each vial containing identified individuals for each

taxa collected at each trap site were pooled and analyzed as individual samples. Samples were air dried on paper towels in a fume hood for 15 – 20 minutes to allow any residual formalin to evaporate. Samples were weighed to determine a wet weight and placed in an oven set at 60^{o} C. To determine the appropriate drying time, samples were oven-dried for 24 hours, reweighed, oven dried for another 24 hours, and weighed again. Since there was no significant weight change between the two drying times, a 48 hr drying period was deemed sufficient. After drying, samples were crushed using a mortar and pestle to ensure a homogeneous mixture. Approximately 100mg of sample was weighed and placed in Teflon^{TM} PFA microwave vessels with 2.5 mL of trace metal grade (Fisher Scientific) HNO_3 and 2.5 mL of Milli-Q water. Vessels were then capped and digested using increasing power applied over a 20-minute program in a microwave digestion apparatus (MDS-2000, CEM Corporation, Matthews, NC). Samples were digested in batches of 12. Sample material was placed in 10 vessels; a reference material was placed in the 11th vessel, and a digest blank consisting of 2.5 mL of trace metal HNO_3 and 2.5 mL of Milli-Q water. Digested solution was added to 15 mL Falcon^{TM} tubes and diluted to 10 mLs with Milli-Q water was placed in the twelfth. This solution was further diluted 1:5 for analysis. Vessels were cleaned with concentrated HNO_3 between digestions. Samples were analyzed for, Cd, Cu, Ni, U, Hg, and Ti by an Inductively Coupled Plasma Mass Spectrometer (ICP-MS) using a Perkin Elmer SCIEX (ELAN DRC plus) in standard operating mode. The procedure followed methodology outlined in EPA method 6020 and quality control procedures were based on EPA procedure SW-846. Standard reference material for analysis was lobster hepatopancreas (Tort-2), a lyophilized invertebrate tissue purchased from the National Research Council of Canada.

Stable Isotope analysis:

Stable isotope analysis was conducted on samples collected from season three (fall 2003). Elemental analysis-isotope mass spectrometry was used to quantify $^{15}N/^{14}N$ and $^{13}C/^{12}C$ ratios for individuals from a single trap site and individuals pooled from trap sites. Samples were dried and weighed as described above in the metals section. Approximately 2mg of crushed sample were placed in individual pre-cleaned tin capsules and weighed using an ultramicrobalance. Capsules were then sealed and placed in the auto sampler of a Carlo Erba Elemental Analyzer (NA2500) interfaced to a continuous flow isotope ratio mass spectrometer [Finnigan Delta +XL (Finnigan-MAT, San Jose, CA)]. Samples were combusted to N_2 and CO_2 in oxidative-reduction furnaces, separated by gas chromatography, and then measured for $^{15}N/^{14}N$ and $^{13}C/^{12}C$ ratios on a mass spectrometer. A N_2 (g) working standard was admitted prior to each sample combustion for calibration to an international AIR standard [45]. Stable isotope ratios were reported in per mil units (‰) using standard delta (δ) notation [46]. External working standards of dorm and acetanilide (National Research Council of Canada) were analyzed to determine external precision to $\pm$ 0.2‰ (1 standard deviation) for both carbon and nitrogen isotopes.

Differences within a single trap site and among trap sites were examined. In order to determine variation within a trap site, specimens from one trap site were analyzed as individual samples. For other trap sites, all specimens were pooled by site. For Carabidae Morphotype A, ten individuals from trap site 4-9 were analyzed as individual samples. At nineteen other trap sites, specimens of morphotype A were pooled together and analyzed as a single sample (three replicates). For Gryllidae, ten individual specimens from trap site 1-8 were analyzed. Gryllidae specimens from 15 other trap sites were pooled together by site for analysis. Nine specimens of Lycosidae were analyzed from trap site 4-4 as individuals. Pooled samples from sixteen other

trap sites were analyzed for Lycosidae. For the other Carabidae morphotypes, specimens from each selected trap site were analyzed as individuals to determine the variance within a trap site. Means for each trap site were used to compare differences between trap sites.

Soil analysis:

Grab samples were taken for soil from each trap site and placed in plastic zip lock bags. Soil samples were dried in an oven at 60^{o} Celsius for 48 hours to determine dry weights. Approximately 250mg of sample was weighed out and digested with 9 mLs of HNO_3 and 3 mLs of Hydrofluoric acid (HF), using the same protocol as for invertebrate samples for the metal analysis. Digested solutions were added to 50 mL Falcon tubes and diluted to 50mLs with Milli-Q water. Samples were analyzed for the same metals as invertebrates using the same procedures.

Gamma activity in the soil was measured using a hand held Geiger counter. Gamma emitting daughter products of uranium were measured as counts per minute (cpm). Measurements were taken approximately 2-3 inches above the soil at each trap site at Tims Branch for approximately one minute or until a constant reading was recorded.

Formalin analysis:

There were concerns that formalin preservation might cause certain undesirable effects on stored samples [47, 48]. Among these concerns was the possibility of contaminants leaching into or out of the samples. In order to determine possible leaching into the storage medium, formalin solution from the sample vials was analyzed to determine the presence of metals. Additionally, three samples of 10% formalin solution and three samples of 4% formalin solution were analyzed to establish background levels of metals in solutions used in the field and in the storage vials in the laboratory, respectively. These concentrations were compared to

concentrations from the metals analysis of the arthropod samples to determine if any leaching of metals occurred.

Below Detection Limits (BDLs):

Samples having concentrations below detection limits (BDL) were encountered during metal analyses. These BDLs were based on method detection limits calculated as 3 times the standard deviation of the blanks plus the average value for the blank samples. The BDLs (in mg/kg dry weight) were: Ni=0.430, Cu=1.143, Cd=0.008, Hg=0.004, U=0.285, and Ti 0.036. When a BDL was encountered, the detection limit was entered as the value for statistical purposes [7].

Statistical Analyses:

We first examined metal and isotope distributions using Shapiro-Wilk statistics (PROC UNIVARIATE, version 8.1; SAS Institute). Tests that the data were random samples from normal distributions were rejected ($P<0.05$) and stem-and-leaf plots suggested a log-transformation of data prior to analysis. All log-transformed variables were normally distributed.

A Generalized Linear Model (GLiM; Dobson 2002) assuming a Poisson distribution, was used to determine whether raw gamma counts associated with Uranium daughter products were spatially correlated with metal concentrations in terrestrial arthropods from the Tim's Branch Site

To determine if arthropod metal concentrations were dependent upon taxa, the number of individuals of each taxa, and their interaction, a General Linear Model (GLM; PROC GLM; SAS Institute) was used. Further, a GLM was also used to examine the relationships between metal concentration in arthropods and site, ^{15}N, ^{13}C and their interactions. If the interactions were not significant, they were dropped from the model. For all tested models, Type III (partial) sums of

squares and associated *F*-statistics were interpreted and least-squares means procedures were used to provide estimates of dependent variables that were adjusted for all effects in the models and to provide mean separation tests. All statistical tests were considered significant at $P<0.05$. Means and standard errors are presented as back-transformed values of log least-squares means estimates (i.e., geometric means). Statistical comparisons of related data (the $\delta^{13}C$ and $\delta^{15}N$ values of morphotypes of Carabidae) were made using the *K* neighbor-statistic [49-51]. A value of 4 was used for *K* for all pairwise comparisons. All comparisons were considered statistically significant at $P \leq 0.05$.

In order to quantify relationships between contaminant concentrations in soil and invertebrates (i.e., the source of contamination and the receptors) in this study, transfer factors (TFs) were calculated. Transfer factors are often utilized as a simple estimator in risk assessment, and can be used as a crude indicator of bioavailability [23]. Transfer factors were established between soils and invertebrates to identify the potential mobility of contaminants between compartments within the Tims Branch basin. Metal concentrations of all invertebrates were divided by mean soil concentrations for all trap sites to calculate TFs. Transfer factors were also calculated using data from other studies [23, 24] to examine how contaminants move from the soil through plants and invertebrates into upper level vertebrates, such as small mammals.

CHAPTER 3

RESULTS

Taxa Diversity:

A total of 8413 specimens were trapped over all three seasons at both Tims Branch and Boggy Gut. Season one (Fall 2002) yielded 3062 specimens. Season two (Spring 2003) yielded 2915 specimens. Season three (Fall 2003) yielded 2436 specimens (Table 1).

For season one, Carabidae was the most abundant taxa (n=1467), followed by Gryllidae (n=786), Lycosidae (n=573), and Gryllacrididae (n=236). As mentioned, due to a preliminary pilot study, specimens were not grouped into morphotypes for all traps along transect one and the first six traps of transect 2. The total number of Carabidae recorded was 1467, of which, 1271 were characterized by morphotype. The difference of 196 specimens can be accounted for by individuals collected during the pilot study from transect 1 and half of transect 2. The total number of individuals in each Carabidae morphotype were not equal, with morphotype A being the most abundant (n=746), followed by C (n=184), B (n=119), D (n=80), F (69), G (55), and E (18). No specimens were recorded for morphotype K during this season.

For season two, Lycosidae was the most abundant taxa (n=1726), followed by Carabidae (n=800), Gryllacrididae (n=224), and Gryllidae (n=165). The most abundant morphotype was morphotype A (n=324), followed by G (n=107), D (n=91), B (n=67), K (n=63), E (n=54), F (n=50) and C (n=44).

For season three, Carabidae was again the most abundant taxa (n=990), followed by Gryllidae (n=916), Lycosidae (n=398), and Gryllacrididae (n=132). The most abundant

morphotype was again morphotype A (n=519), followed by B (n=183), D (n=107), C (n=72), G (n=56), F (n=45), K (n=5), and E (n=3).

Below Detection Limits (BDLs):

Samples having metal concentrations below detection limits were encountered during the analysis. Percentages of BDLs encountered for metal concentrations were calculated for all taxa (Table 2). Metals that had high percentages of BDLs were Ni (24.28% for Boggy Gut), Hg (22.11% for Tims Branch and 19.51% for Boggy Gut), and U (49.13% Boggy Gut).

Formalin analysis:

Because of concerns that the formalin solution might cause metals to leach into or out of collected specimens, leftover storage formalin was analyzed for relevant metals. Metal concentrations (mg/L) were multiplied by the storage vial volume to obtain the mass of each metal in solution in each vial. Similarly, the total mass of metal in each pooled invertebrate sample was calculated for each vial. Comparison of total metal in the formalin to total metals in the pooled invertebrate samples showed that less than 1% of any metal had leached into formalin. Analysis of the formalin used for preservation (4% and 10%) showed that metal concentrations were below detection. These data show that little or no leaching or contamination occurred as a result of formalin storage.

Metal Concentrations:

The GLiM showed that there were significant relationships between gamma emitting daughter products in the soil along each transect and invertebrate metal concentrations (Table 3). Only two metals, Hg and U, showed a positive relationship while all other metals showed a negative relationship. As gamma counts increased, Hg and U invertebrate concentrations

increased, while the concentrations for the other metals decreased. However, in each case, the slope was nearly zero. The overall p-value for the model was <0.0001, indicating that each relationship was significant at the $p \leq 0.05$ level.

There were no significant differences in metal concentrations among taxa for each season; therefore, all taxa from both seasons were combined. For all taxa, concentrations of Ni, U, and Ti, were significantly different between Tims Branch and Boggy Gut, but Cu, Cd, and Hg were not different (Table 4). For the metals that were significantly different, Ni and U were higher in Tims Branch taxa, whereas Ti was higher in Boggy Gut taxa. However, metal concentrations varied among taxa for both sites (Table 5, Figure 2). Ni and U were significantly higher in all taxa collected at Tims Branch, while the other metals differed for each taxa relative to site. Carabidae had significantly higher Cu concentrations, Gryllidae had significantly higher Ti concentrations, and Lycosidae had significantly higher Cd concentrations at Boggy Gut (Figure 3).

Metal concentration varied with the type of taxa collected for all metals for both seasons (Table 6). Additionally, the number of individuals caught was dependent on concentrations of Ni, Cd and U at Tims Branch, but not for any metals at Boggy Gut. The interaction of taxa and number of individuals varied between sites. Bonferroni multiple comparisons were used to test species relationships based on metal concentrations for each taxa (Table 7).

Soil metal concentrations (Table 8) are presented as means for all trap sites at Tims Branch. Based on calculated transfer factors (TFs) between trap site soil means and means of all invertebrates, only two metals had values greater than one (Cu and Cd, Table 8). Additionally, soil means from this study were used to calculate TFs to other compartments (plant and small

mammal tissue), based on the data from other studies conducted at Tims Branch (Table 8). Only transfer factors for Cd and Hg exceeded one for soils to plants and small mammals.

Isotope ratios:

Stable isotope ratios for carbon and nitrogen were determined for taxa Carabidae, Gryllidae, and Lycosidae trapped at Tims Branch during the fall of 2003. Due to limitations in sample size, representatives from taxa Gryllacrididae were not analyzed. Additionally, due to the small sample size, only 5 of the 8 morphotypes (A, B, C, D, G) from Carabidae were analyzed. $\delta^{15}N$ values varied for all taxa collected (Table 9). While there were large ranges for $\delta^{15}N$ values, there was no significant difference among taxa. The range of $\delta^{13}C$ values was relatively small, and there was a significant difference among taxa (F-Value = 94.75, P-Value <0.0001, DF = 2). Multiple comparisons showed significant differences among all taxa except Lycosidae-Gryllidae. There were no significant relationships between $\delta^{15}N$ and $\delta^{13}C$ values and any metals, meaning that transfer of metals among taxa could not be determined using nitrogen and carbon isotopes as variables. $\delta^{15}N$ and $\delta^{13}C$ values were variable within taxa (Figure 3). In Carabidae, morphotypes B and D had the highest mean values for $\delta^{15}N$. Morphotype A had the highest mean value for $\delta^{13}C$ with all other taxa having mean $\delta^{13}C$ values between –27.00 and –25.00. Morphotypes C and G had the lowest mean values for $\delta^{15}N$.

Morphotype A appeared to have two dissimilar feeding behaviors (Figure 4). One behavior was associated with a small $\delta^{13}C$ range while the other increased in $\delta^{13}C$ values. Morphotypes B and D had individuals with the most overlap, which may indicate that individuals from these two groups utilize similar resources.

The K nearest neighbor test was used to establish significant relationships between morphospecies based on their position in space relative to their $\delta^{15}N$ and $\delta^{13}C$ values. All

pairwise comparisons showed that morphotypes were significantly different, with $P<0.0001$; the one exception was between morphotype B and morphotype D, where $P=0.5618$.

CHAPTER 4

DISCUSSION

To understand the potential risk of trophic transfer of contaminants within an ecosystem, there are many relationships that need to be considered. To fully characterize a system, not only can no compartment be ignored, but their relationships must also be considered. This study explored the relationships between invertebrate diversity, contaminant bioavailability, and trophodynamics within a disturbed system. Small mammals trapped in this study area, as well as other areas of known soil contamination, do accumulate metals [24, 43, 52]. This suggests that terrestrial arthropods, which are a staple food item of these and other vertebrates, may play a major role in toxicant mobility in this system.

This study showed a high variation in contaminant concentrations among and within invertebrate taxa, which may partially be explained by their life cycle. Ontogeny and longevity can be highly variable among invertebrates. Further, within taxa, life cycles can also be variable. Most exhibit a heterodynamic life cycle, that is, adults appear for a limited time during a particular season, and some life stages pass the winter in a state of dormancy [26]. This over wintering stage could be the egg (most Orthoptera), the nymph (many Orthoptera), or adult (many Coleoptera). Most taxa have one generation a year, some require two years or more to complete their life cycle, and some have more than one generation a year, which may influence both the numbers of individuals caught each season and their contaminant concentrations. These parameters may help explain why there were no consistent patterns of diversity among collection periods. For example, Lycosidae, which have a yearly life cycle, had the highest numbers for

any one season (n=1726, spring 2003). This was three times higher than the previous fall (n=573, fall 2002), possibly due to reproduction or prey availability. In contrast, crickets typically live for 1-2 years [37], so higher numbers in both fall seasons (Gryllidae n=786, 2002; n=916, 2003), compared to the spring season (n=165), suggests one generation per year, with adults present in the fall, laying eggs, and dying as winter approaches. Additionally, cyclic fluctuations between Lycosidae and Gryllidae could be related to predator-prey interactions, since Lycosidae feed on Gryllidae [38, 39]. Furthermore, carnivorous species tend to be over-represented in pitfall traps due to their active hunting behavior at the ground surface [44].

Similar numbers of Gryllacrididae were collected in fall 2002 (n=236) and spring 2003 (n=224), possibly indicating that they over wintered as adults. More Carabidae were collected in the fall of 2002 (n=1467), than spring 2003 (n=800) and fall 2003 (n=990), which may be due to the fact that carabids generally develop from an egg to adult in less than one year, reproduce once, and then die [30]. Depending on the species, this cycle can last anywhere from one to two years. These temporal dynamics could explain the relatively high numbers for each season, but do not offer insight as to why the first season yielded so many more specimens than the other two seasons. It is possible that there may have been more resources available for these individuals during the 2002 fall trapping season.

These fluctuations in life cycles among taxa potentially could influence contaminant mobility. Since certain taxa exist in the egg or larval stage at different times of the year, resource availability may be limited for other types of predacious taxa. The life cycles of these taxa may alternate such that prey species are in the pre-adult stage while the predators are adults. Lycosidae, which have members that are sit-and-wait ambush predators [37] feed on actively moving prey. If Gryllidae are present as eggs while of Lycosidae are in the adult stage, then the

Lycosidae are probably feeding on other resources. Depending on the potential among prey taxa to accumulate contaminants, metal uptake by the Lycosidae could be influenced. Alternatively, Carabidae, as generalist predators or scavengers, may feed on taxa in pre-adult stages. As a result, Carabidae may be feeding on individuals that have not had time to accumulate contaminants, resulting in low contaminant exposure. Moreover, habitat and feeding behaviors may shift based within life cycles [5]. Adult forms of these taxa, particularly Carabidae, may have completely different habits compared to larval forms, which may cause a shift in nutritional requirements during development.

Metal concentrations (particularly Ni and U) in taxa were expected to be higher at Tims Branch than at Boggy Gut because of the known effluent discharge that occurred within the Tims Branch-Steed Pond corridor. However, Cu, Cd, and Ti were higher at Boggy Gut for all taxa. The higher concentrations of these metals in arthropods from Boggy Gut compared to Tims Branch may be related to environmental conditions such as pH, moisture, and soil composition, which can affect metal speciation and bioavailability. For all taxa, Lycosidae had the highest concentrations of Cu and Cd, while Carabidae had the lowest concentrations for these two metals (Figure 2). Hunter et al [4] demonstrated that spiders (specifically, Lycosidae) had higher levels of both copper and cadmium compared to beetles (specifically, Carabidae). They concluded that these elevated levels might be due to certain homeostatic controls, or even differences in developmental histories. This study showed similar patterns for these two taxa (Table 5), and may be explained similarly.

Contaminant concentrations in animals are very difficult to predict, especially in natural environments. Physiological processes specific to a particular organism facilitate or inhibit contaminant accumulation. Some metals are important constituents for physiological processes

in certain invertebrate taxa. For example, Cu is a main component of hemocyanin, a respiratory pigment in spider hemolymph [53]. This may account for the high concentration of Cu in this taxa. Certain detoxification processes, such as sequestering of metals, may allow organisms to redistribute contaminants among tissues and / or eliminate contaminants via feces, which may be related to the speciation of the metal. Cu regulation has been suggested in this manner for certain carabids [54]. This may account for Carabidae having the lowest concentrations of Cu among taxa in this study. The biological half-life of a particular element also contributes to its potential for accumulation. Hook and Yates [55] found that Cd had a long biological half-life in lycosid spiders, compared to crickets. This could explain why Lycosidae had higher cadmium levels compared to the other taxa.

Another possible method for the elimination of toxicants is by molting of the exoskeleton. It is possible that metals could be sequestered in the developing exoskeleton and ultimately discarded when the exoskeleton is shed. In this study we were interested in trophic mobility of contaminants, and did not separate exoskeletons from other body components. However, this means of excretion has been reported in woodlice [56], particularly for Ni and Cr. Insect growth and development is strictly dependent on the capability to remodel chitinous structures [57]. In insects, chitin-degrading enzymes play a crucial role in postembryonic development, especially during larval molt and pupation. The reincorporated constituents seem to be recycled and used to produce the new procuticle. Due to this re-absorption, it is quite possible that metals incorporated into the exoskeleton could be reabsorbed into the animal, thus preventing elimination and increasing biological effects. Additionally, some larvae may ingest the shed exuvia, thereby recycling contaminants into the gut.

Although some $\delta^{13}C$ values were consistent with a carbon source ultimately derived from a C_4 pathway, all taxa means were consistent for C_3 sources (Table 9), indicating that most individuals foraged on prey that consumed C_3 plant material. However, it is important not to ignore other potential foraging strategies. Specifically, both omnivores (Orthoptera) and predacious species (Carabidae and Lycosidae) were analyzed in this study. Due to specimen availability of the orthopterans, only Gryllidae could be used for isotope analysis. Carabidae exhibited variable $\delta^{15}N$ values and $\delta^{13}C$ values. Morphotypes B and D showed higher $\delta^{15}N$ values than other taxa, which may indicate that these individuals were higher in the food chain compared to the other taxa. These morphotypes (B and D) may be feeding at the highest trophic level compared to the other taxa, even Lycosidae, which are considered to be the top predators in the arthropod community [3].

Morphotype C had the smallest ranges for both $\delta^{15}N$ and $\delta^{13}C$. This morphotype may feed on a particular type of prey and it may be a specialist that feeds on one specific taxa or plant type, resulting in small ranges for both isotopes. The range of $\delta^{15}N$ values for Gryllidae indicated that these individuals possibly fed over a wide trophic range, and may be related to foraging strategies. However, the relatively small range of $\delta^{13}C$ values (-26.6‰ to –27.9‰) may be attributed to consumption of food items that originated through the C_3 photosynthetic pathway. Additionally, studies have shown that there are differences between the $\delta^{13}C$ value of chitin and an animal's diet, ranging up to 3.5‰ on individuals in a species raised on the same diet [14]. This value may increase for individuals that feed on a variety of prey, whether it is animal or plant.

Animals that are considered generalist omnivores most likely do not discriminate when foraging for food items. Any potential food source, whether it is plant or animal, may be consumed. With this type of foraging, prey items may vary on a daily basis. Ostrum et al [24] showed how dietary changes on a short time scale (daily) influenced isotope values in ladybird beetles. Additionally, their results suggested that ladybird beetles are able to exploit different habitats on a seasonal basis. This may explain why certain taxa exhibit broad ranges in isotope values. Lycosidae had high $\delta^{15}N$ values. This is expected because they are considered to be generalist predators. Some species act as "sit and wait" predators, while others are active hunters. Regardless of the manner in which they acquire their prey, they may be limited in the types of prey they can capture. This could potentially limit them to only a few types of organisms. This may account for Lycosidae having a smaller range of $\delta^{15}N$ values compared to morphotype D, which may actively pursue prey, but also may act as a scavenger, therefore resulting in either higher $\delta^{15}N$ values or a greater range in $\delta^{15}N$ values.

The $\delta^{15}N$ values showed that there was large variation within, as well as between, morphotypes. Morphotype A appeared to utilize two possible feeding pathways (Figure 4). One pathway yielded a small ^{13}C range while the other appeared to increase ^{13}C values. This suggests this particular morphotype is a more opportunistic feeder that feeds on consumers that obtain their carbon from a wider range of plants, or feeds directly on the plants themselves. Morphotype B and D had individuals with the most overlap. This suggests that these two groups occupy the same trophic level and utilize identical food sources. Further, while morphotype C did had lowest $\delta^{15}N$ values, individuals from this group somewhat overlapped morphotypes B and D. This shows that, while taxa can have variable ranges of δ^{15} N values, individuals within these groups are feeding at trophic levels that overlap. That is, while different food sources are

available and are utilized, certain groups may be feeding on the same type of prey. This is expected since carabids are considered to be generalist predators and would potentially consume any prey items they might encounter. Therefore, the foraging strategies of these taxa are too complex to detect with relationships with metal concentrations.

Since trophic structure of the invertebrate community in Tims Branch is difficult to quantify, using isotopes as a covariate in metal uptake models for this system may not help explain variation in contaminant uptake by these invertebrate taxa. Specifically, community structure can be hard to establish since these arthropods exhibit many different feeding behaviors (detritivores, herbivores, omnivores, carnivores). Further, the abundance of food sources available to individuals that are opportunistic feeders complicates predator-prey interactions. This is also confounded by the fact that organisms may either be feeding on deconcentrators or accumulators. However, isotope analysis did prove useful in better understanding invertebrate foraging strategy, which is useful in an integrated risk assessment. That is, metal transfer through trophic positions becomes clearer when more detail on food items are known. Taxa that are species specific in their feeding behavior could possibly provide the best means for tracking contaminants through trophic levels. The use of stable isotope analysis in this study did show that individuals placed into groups of morphotypes could be distinguished as separate groups based on their isotopic ratios.

With the exception of a few studies, knowledge of foraging activities of arthropods is very limited. Laboratory feeding experiments that include these morphotypes and their potential food sources might reveal the pathways by which contaminants move from the soil into the invertebrate community, and ultimately into higher trophic levels. However, more field studies need to be conducted in order to determine the spatial dynamics of these arthropods. One

possible method is to try and follow taxa by some means of tracking, either by radio transmitters or mark and recapture methods. This would give an idea of the range of these individuals, and possibly the prey they are feeding on and habitats they are occupying. This study looked at a variety of taxa that were only identified to family to determine if any metal accumulation occurred. Focusing on one group of species within each of these families might limit the amount of variation that was seen, and may also yield a clearer understanding of these predator-prey interactions. By focusing on particular species, life cycles, foraging activities, and behaviors might be better understood, which in turn may give a better understanding of these interactions. Additionally, individuals could be separated into juveniles and adults, or males and females. These factors may influence feeding behaviors, and if so, could almost certainly affect contaminant uptake. These ideas could be investigated either in the field as trapping experiments, or in the laboratory as feeding experiments. Once these predator-prey interactions are established, the use of stable isotope analysis may provide a clearer idea of the potential pathway for contaminants to move through trophic levels.

When assessing the potential risk associated with contaminated sites (in this case the Tims Branch corridor on the DOE's Savannah River Site), links between contaminated soil and higher trophic levels need to be established. Studies have shown that small mammals accumulate metals [24, 43, 52] and that small mammals are important as indicators for the bioavailability of contaminants, particularly omnivorous taxa. Therefore, the quantification of metals in invertebrates at Tims Branch was necessary to understand how small mammals accumulated contaminants. However, the Tims Branch system was more complex than this simple model assumed. Since invertebrates exhibit patterns of omnivory, there is a wide range of variability and interaction among taxa. Further, variability is increased because the

contamination is hetereogeneously distributed throughout this ecosystem. Individuals may be feeding on a variety of food items in areas with different levels of soil contamination, making it very difficult to track contaminant movement. One possible method for tracking contaminant movement is by comparing the ratios of metals in the soil to the metal concentrations in the invertebrates. This can determine if metal concentrations in the invertebrates were a result of soil contamination or ingestion of other organisms. The data from this study showed that the U:Ni ratio for Tims Branch soil was 3.70 and the U:Ni ratio for the invertebrates was 0.70. The higher U:Ni ratio in soil suggests that soil contamination may not be an important factor in accumulation. Additionally, Ni appears to be more bioavailable than U.This study also showed that generally, as metal concentrations went up, numbers of individuals increased. This may be explained by individuals exhibiting different foraging strategies, patterns of life history cycles, or individuals occupying an area of contamination longer than other individuals. Furthermore, individuals may inhabit areas such as depressions in the terrain that may hold more organic material, but also may have higher levels of contamination due to metals settling in these depressions.

Small mammals (specifically, *Peromyscus gossypinus,*) are considered to be generalist omnivores that feed on a variety of invertebrates [24, 52] Therefore, quantifying metal levels in these terrestrial arthropods may explain how small mammals accumulate contaminants. Previous work on *P. gossypinus* at Tims Branch showed that season exerted the most significant effect on metal concentration in rodent tissue, with metal concentrations during the fall greater than those in spring. This seasonal difference may reflect increased ingestion of invertebrates. $\delta^{15}N$ values of 4‰ were reported for *P. gossypinus* collected from Tims Branch [24]. Three invertebrate taxa collected from Tims Branch (Morphotypes B, D, and Lycosidae) had $\delta^{15}N$ values greater than

6‰. While *P. gossypinus* may be feeding on invertebrates in the Tims Branch ecosystem, these three taxa may not be among the prey items consumed. All other taxa from this study potentially could be food items for *P. gossypinus* based on their $\delta^{15}N$ values. Lycosidae had the highest concentrations for Cd, Cu, and Hg among all taxa (Figure 2). If Lycosidae is not considered to be a prey item of *P. gossypinus*, then any of these metals into the tissues of *P. gossypinus* must be coming from other food sources. Carabidae generally had the lowest concentrations of metals among all taxa, but *P. gossypinus* most likely did not accumulate metals as a result of feeding on individuals of this taxa. While the $\delta^{15}N$ values of Morphotypes B and D were well above the $\delta^{15}N$ values of *P. gossypinus*, the other morphotypes (A, C, G) had $\delta^{15}N$ values that were close to those of this mammal. Gryllacrididae and Gryllidae had the highest and second highest concentrations, respectively, for U and Ni among all taxa. Additionally, these two taxa had $\delta^{15}N$ values that were close to *P. gossypinus*. *P.gossypinus* may have been utilizing these two taxa as a food source, which could result in the accumulation of U and Ni. This study is consistent with the literature showing that *P. gossypinus* primarily feeds on animal material during the spring/summer season.

Transfer factors (TFs) for metal concentrations in soils were calculated for other compartments at Tims Branch using soil means from this study (Table 8). TFs for soil to invertebrates indicate that transfer of metals occurred for some contaminants (Cu and Cd) but did not occur for other metals (Ni, U, Hg, and Ti). Based on this data, it would appear that invertebrates accumulated only Cu and Cd while showing no accumulation of the other metals. However, data from the metals analysis clearly showed a significant difference in Ni and U concentrations in invertebrates for Tims Branch and Boggy Gut (Table 4). Moreover, individual taxa did not show a significant accumulation of Cu, and only Lycosidae showed a significant

accumulation of Cd (Table 5). Additionally, individual taxa show that there was accumulation for other metals. Areas of high contaminant concentration in one type of media may cause an increase in the calculated transfer factors for other types of media that have low concentrations. This can result in overestimating, or even underestimating, the mobility of a particular contaminant. Therefore, transfer factors may be misleading and should be used with caution in estimating ecological risks.

Due to the omnivorous lifestyle of terrestrial invertebrates coupled with heterogeneously distributed contaminants, it is difficult to distinguish any pathway for contaminants in the soil to move through invertebrate taxa into upper level vertebrates. Quantifying the metals in invertebrates is useful because it indicates the presence of contaminants that potentially could be transferred to higher trophic levels, regardless of whether the contamination is internal or external. The use of stable isotopes did separate taxa into identifiable morphotypes and showed that these groups occupied different trophic levels. However since they are fed upon indiscriminately by upper level vertebrate taxa, establishing these groups may not be necessary, from a trophic transfer aspect. A few taxa have been isolated that most likely are not consumed and therefore should not be used to model trophic transfer. Excluding these taxa, all other invertebrates could be grouped into a single compartment. In terms of ecological risk assessment, the use of morphotypes as groupings may be too narrow a focus when the omnivorous habits of organisms are considered. Perhaps the best way to model the system would be to treat invertebrates as an entire compartment (minus the previously mentioned taxa) based on the nondiscriminatory feeding habits of certain vertebrate taxa. Further, due to the non-mobility of certain metals, there may not be a risk associated with the presence of contaminants. Perhaps the best remediation for Tims Branch is to not disturb the soil. If the contaminants are

not mobile, then there potentially is no ecological risk of any concern. However, due to the heterogeneous distribution of contamination, both spatially and chemically, this may not be the case. Further studies that show how certain contaminants interact with one another should be conducted. Moreover, additional studies need to be conducted using organisms that occupy multiple trophic positions within an ecosystem to fully understand contaminant mobility from the soil through other compartments.

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Figure 1. Map of the Department of Energy's Savannah River Site (SRS) located near Aiken, SC.

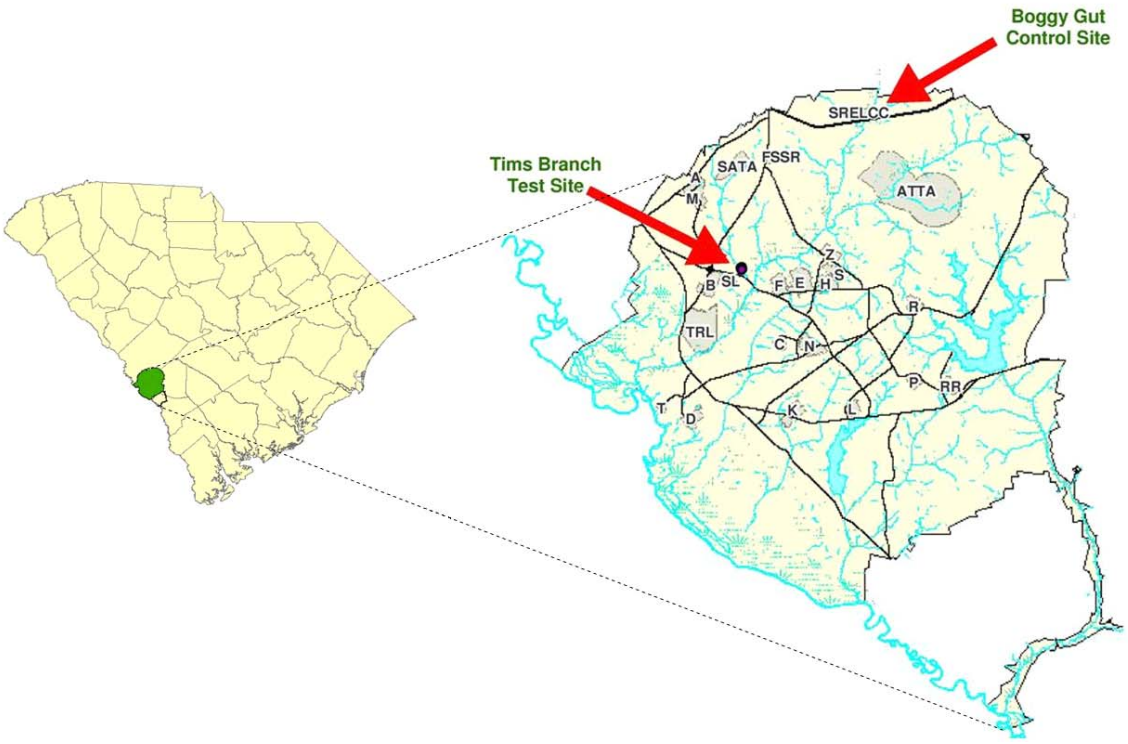


Table 1. The total number of individuals collected for each taxa at Tims Branch and Boggy Gut on the Department of Energy's Savannah River Site, Aiken, SC for Fall 2002 (Season 1), Spring 2003 (Season 2), and Fall 2003 (Season 3). Specimens for family Carabidae (beetles) were separated into eight "morphotypes" (individuals grouped together based on similar morphological characteristics). Families Gryllidae (crickets), Gryllacrididae (cave and camel crickets) and Lycosidae (wolf spiders) had individuals grouped together for their respective family. Individuals for each taxa were pooled together for each trap site along the four transect lines at both sites. Asterisk (*) indicates individuals from family Carabidae collected from transect 1 and 2 that were not identified to morphotype.

Fall 2002: Tims Branch and Boggy Gut

	Tims Branch	Boggy Gut	Grand Total
Morphotype A	210	536	746
Morphotype B	27	92	119
Morphotype C	65	119	184
Morphotype D	60	20	80
Morphotype E	15	3	18
Morphotype F	0	69	69
Morphotype G	0	55	55
Morphotype K	0	0	0
Total Number of Morphotypes	377	894	1271
Carabidae not identified to Morphotype	196	0	196
Total Carabidae	573	894	1467
Gryllidae	522	264	786
Gryllacrididae	180	56	236
Lycosidae	166	407	573
Grand Total	1441	1621	3062

Spring 2003: Tims Branch and Boggy Gut

	Tims Branch	Boggy Gut	Grand Total
Morphotype A	106	218	324
Morphotype B	49	18	67
Morphotype C	5	39	44
Morphotype D	47	44	91
Morphotype E	19	35	54
Morphotype F	4	46	50
Morphotype G	17	90	107
Morphotype K	12	51	63
Total Carabidae	259	541	800
Gryllidae	123	42	165
Gryllacrididae	120	104	224
Lycosidae	861	865	1726
Grand Total	1363	1552	2915

Table 1 continued

Fall 2003: Tims Branch and Boggy Gut

	Tims Branch	Boggy Gut	Grand Total
Morphotype A	244	275	519
Morphotype B	61	122	183
Morphotype C	31	41	72
Morphotype D	89	18	107
Morphotype E	1	2	3
Morphotype F	2	43	45
Morphotype G	3	53	56
Morphotype K	2	3	5
Total Carabidae	433	557	990
Gryllidae	420	496	916
Gryllacrididae	34	98	132
Lycosidae	175	223	398
Grand Total	1062	1374	2436

Table 2. Percentages of invertebrates that were Below Detection Limits (BDLs) for metal concentrations for four families (n=692; Carabidae, Gryllidae, Gryllacrididae, Lycosidae) collected during two trapping seasons (Fall 2002 and Spring 2003) from Tims Branch and Boggy Gut on the Savannah River Site, Aiken, SC. BDLs are in mg/kg dry weight.

Metals	Tims Branch	Boggy Gut	BDL (mg/kg)
Ni	3.90%	24.28%	0.430
Cu	0.00%	0.00%	1.143
Cd	0.00%	0.00%	0.008
Hg	22.11%	19.51%	0.004
U	4.91%	49.13%	0.285
Ti	0.00%	0.00%	0.036

Table 3. Poisson regression results showing relationship between gamma emitting daughter products in soil and metal concentrations in arthropod taxa collected during two trapping seasons (Fall 2002 and Spring 2003) from Tims Branch on the Savannah River Site, Aiken, SC (n=257).

Parameter	Estimate	Standard Error	95 % Confidence Limits		Chi square	P-value
Intercept	5.4920	0.0099	5.4726	5.5114	306604	<0.0001
Ni	-0.0053	0.0007	-0.0066	-0.0040	59.75	<0.0001
Cu	-0.0008	0.0002	-0.0011	-0.0005	27.11	<0.0001
Cd	-0.0580	0.0088	-0.0752	-0.0408	43.64	<0.0001
Hg	0.4092	0.0588	0.2939	0.5245	48.40	<0.0001
U	0.0859	0.0016	0.0828	0.0889	3018.51	<0.0001
Ti	-0.0256	0.0018	-0.0291	-0.0222	213.04	<0.0001

Table 4. Comparison of metal concentrations for four families (n=692; Carabidae, Gryllidae, Gryllacrididae, Lycosidae) collected during two trapping seasons (Fall 2002 and Spring 2003) from Tims Branch and Boggy Gut on the Savannah River Site, Aiken, SC. The geometric mean (mean) and standard errors (SE) are presented with associated F and P-values from the ANOVA model. Units are in mg/kg dry weight.

	Tims Branch		Boggy Gut			
Metals	Mean	SE	Mean	SE	F-Value	P-Value
Ni	2.69	1.10	0.14	1.22	180.26	<0.0001
Cu	34.79	1.07	38.64	1.06	1.35	0.2453
Cd	0.24	1.09	0.29	1.13	1.38	0.2398
Hg	0.06	1.10	0.05	1.12	1.49	0.2223
U	1.07	1.14	0.00	1.21	701.76	<0.0001
Ti	1.14	1.10	2.05	1.08	5.75	0.0168

Table 5. Differences in metal concentrations of four families (Carabidae, Gryllidae, Gryllacrididae, Lycosidae) collected during two trapping seasons (Fall 2002 and Spring 2003) from Tims Branch and Boggy Gut on the Savannah River Site, Aiken, SC. The geometric mean (mean) and standard errors (SE) are presented with associated F and P-values from the ANOVA model. Units are in mg/kg dry weight.

Carabidae

	Tims Branch (n-127)		Boggy Gut (n=150)			
Metals	Mean	SE	Mean	SE	F-Value	P-Value
Ni	1.07	1.20	0.08	1.40	43.11	<0.0001
Cu	16.38	1.03	17.98	1.02	6.49	0.0114
Cd	0.06	1.09	0.07	1.16	0.63	0.4298
Hg	0.03	1.21	0.02	1.23	2.27	0.1327
U	1.24	1.16	0.00	1.33	303.01	<0.0001
Ti	1.02	1.18	1.39	1.12	2.55	0.1119

Gryllidae

	Tims Branch (n=76)		Boggy Gut n=54)			
Metals	Mean	SE	Mean	SE	F-Value	P-Value
Ni	4.90	1.25	0.74	1.21	37.64	<0.0001
Cu	22.42	1.27	29.98	1.04	1.06	0.3051
Cd	0.29	1.12	0.33	1.24	0.22	0.6380
Hg	0.05	1.20	0.07	1.15	2.17	0.1435
U	1.38	1.34	0.00	1.59	171.36	<0.0001
Ti	1.66	1.26	3.84	1.19	7.15	0.0089

Table 5 continued

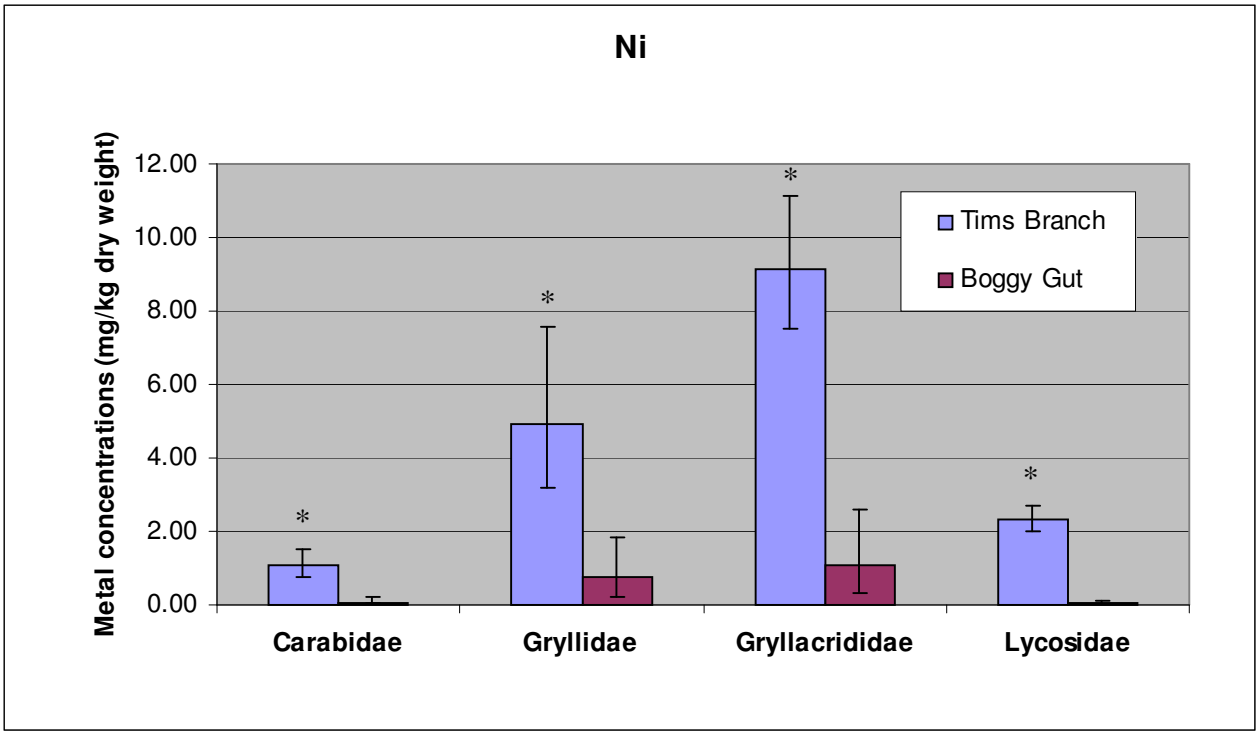
Gryllacrididae

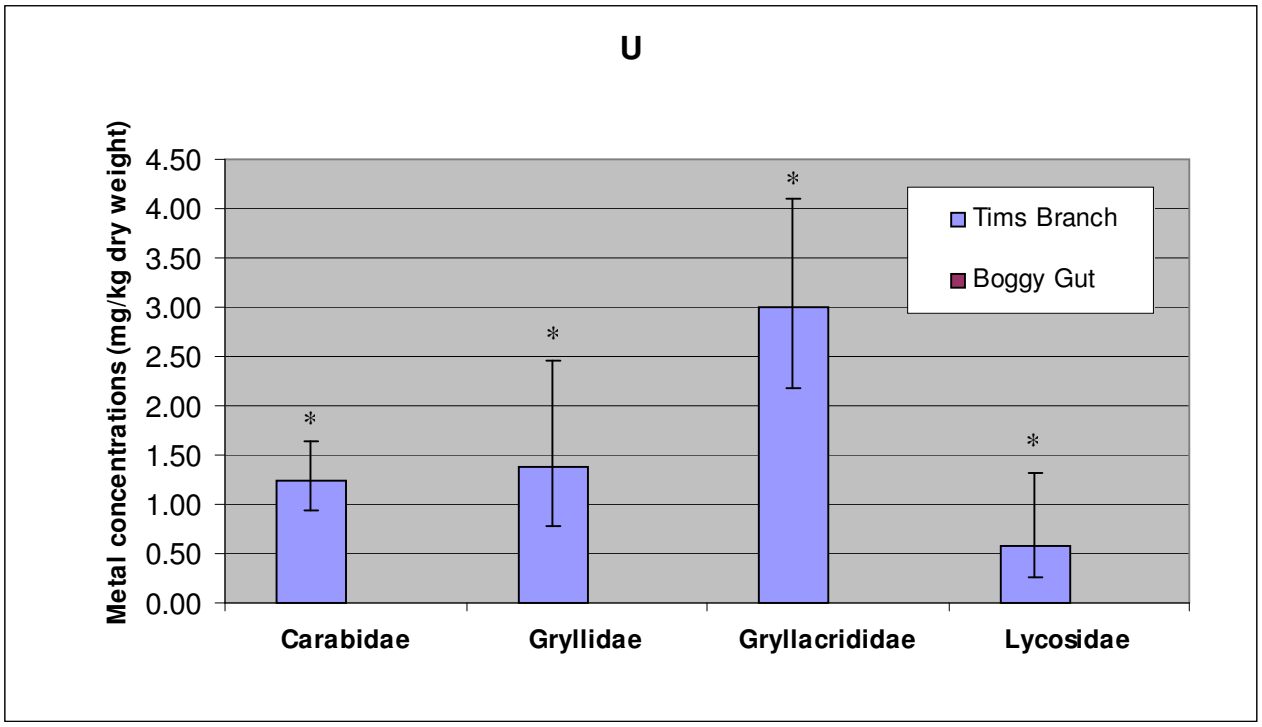
	Tims Branch (n=68)		Boggy Gut (n=59)			
Metals	Mean	SE	Mean	SE	F-Value	P-Value
Ni	9.14	1.11	1.07	1.20	117.57	<0.0001
Cu	37.56	1.03	39.00	1.04	0.52	0.4735
Cd	0.26	1.11	0.23	1.19	0.47	0.4926
Hg	0.07	1.08	0.05	1.26	1.97	0.1631
U	3.00	1.17	0.00	1.57	229.79	<0.0001
Ti	1.91	1.29	2.56	1.21	0.83	0.3645

Lycosidae

	Tims Branch (n=78)		Boggy Gut (n=80)			
Metals	Mean	SE	Mean	SE	F-Value	P-Value
Ni	2.32	1.08	0.03	1.58	85.23	<0.0001
Cu	170.07	1.05	191.08	1.06	2.24	0.1365
Cd	1.81	1.17	4.67	1.11	26.63	<0.0001
Hg	0.22	1.19	0.25	1.13	0.43	0.5120
U	0.59	1.52	0.00	1.48	78.87	<0.0001
Ti	2.25	1.13	2.43	1.13	0.21	0.6502

Figure 2. Differences in metal concentrations between four families (Carabidae, Gryllidae, Gryllacrididae, Lycosidae) collected during two trapping seasons (Fall 2002 and Spring 2003) from Tims Branch and Boggy Gut on the Savannah River Site, Aiken, SC. The natural logs of the data have been back transformed with the geometric means of concentration represented by the columns and the bars representing 95 % confidence limits. Asterisks indicate significant differences based on ANOVA models.





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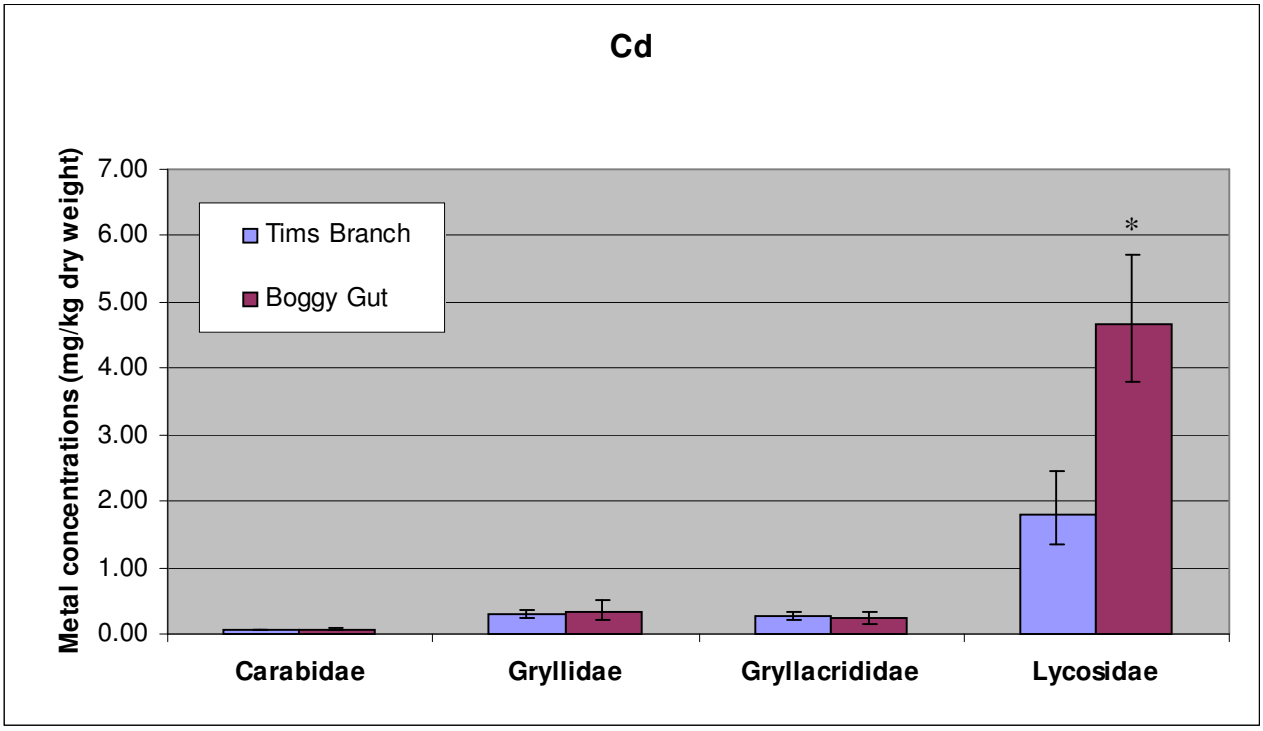

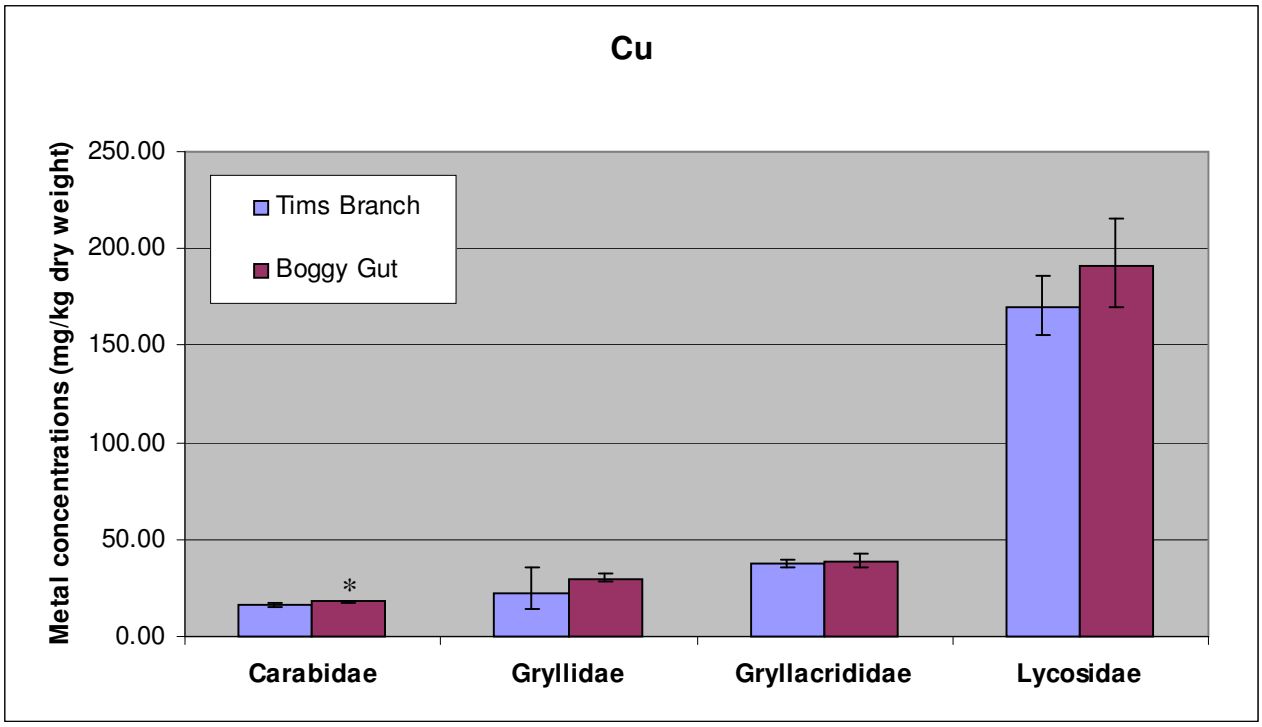

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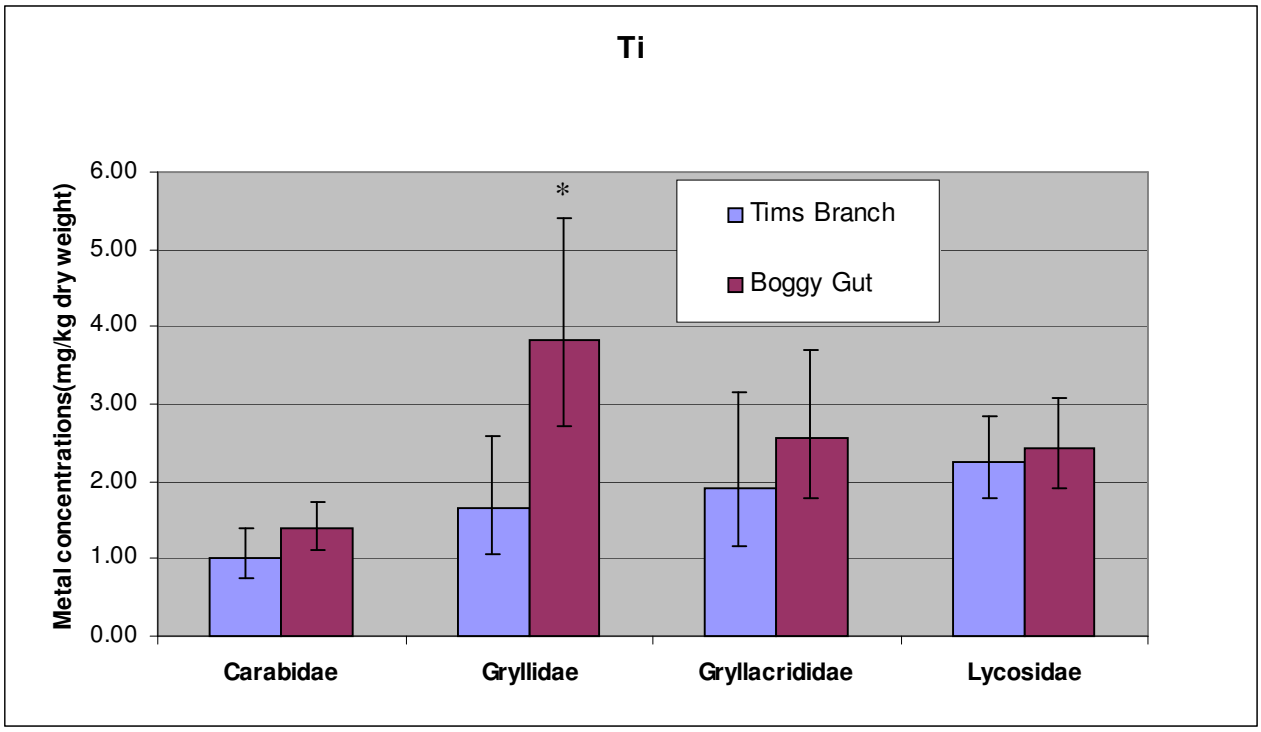


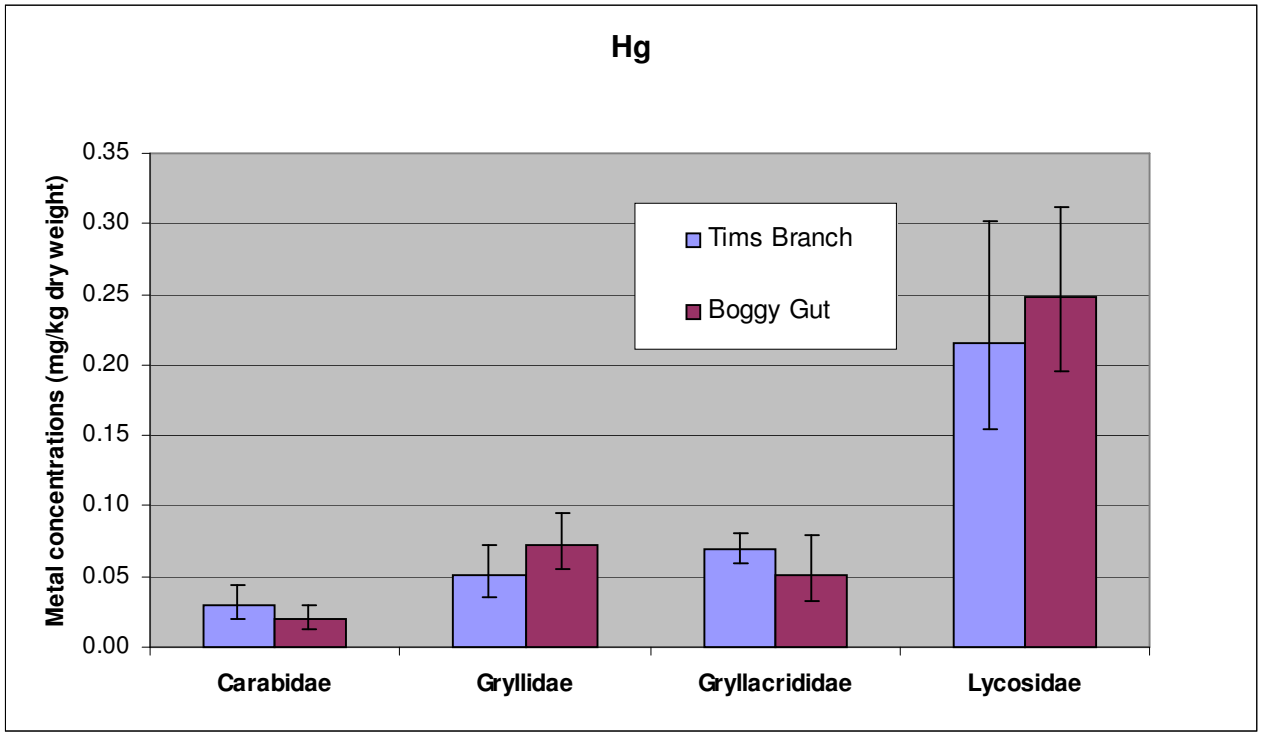


Table 6. General Linear Model results showing the effects of metal concentration on the type of taxa collected (SPP), number of individuals within taxa (NUMIND) and their interactions (NUMIND*SPP) for four families (Carabidae, Gryllidae, Gryllacrididae, Lycosidae) collected during two trapping seasons (Fall 2002 and Spring 2003) from Tims Branch and Boggy Gut on the Savannah River Site, Aiken, SC. "SPP" data were used to determine if metal concentrations were dependent on the type of taxa caught. "NUMIND" data were used to determine if metal concentrations affected the number of individuals captured. "NUMIND*SPP" data were used to determine if metal concentration was dependent on the number of individuals collected and if that changed based on taxa. *F*-values are given with *P*-values in parentheses (n=349 for Tims Branch and n=343 for Boggy Gut).

Tims Branch

Metals	SPP		NUMIND		NUMIND*SPP	
Ni	21.14	(<0.0001)	3.89	(0.0495)	3.02	(0.0300)
Cu	44.30	(<0.0001)	1.03	(0.3104)	0.78	(0.5050)
Cd	58.55	(<0.0001)	9.26	(0.0025)	1.14	(0.3329)
Hg	4.93	(0.0023)	0.71	(0.4009)	4.28	(0.0056)
U	11.14	(<0.0001)	5.54	(0.0192)	4.12	(0.0068)
Ti	9.80	(<0.0001)	3.50	(0.0626)	10.68	(<0.0001)

Boggy Gut

Metals	SPP		NUMIND		NUMIND*SPP	
Ni	8.98	(<0.0001)	0.00	(0.9781)	0.43	(0.7333)
Cu	347.57	(<0.0001)	0.09	(0.7702)	3.87	(0.0096)
Cd	57.38	(<0.0001)	0.21	(0.6507)	1.97	(0.1178)
Hg	9.15	(<0.0001)	0.61	(0.4343)	0.76	(0.5156)
U	6.95	(0.0002)	0.55	(0.4584)	14.57	(<0.0001)
Ti	14.98	(<0.0001)	0.07	(0.7937)	11.09	(<0.0001)

Table 7. Comparison of species relationships for metal concentrations of four families (Carabidae, Gryllidae, Gryllacrididae, Lycosidae) collected during two trapping seasons (Fall 2002 and Spring 2003) from Tims Branch on the Savannah River Site, Aiken, SC. Bonferroni multiple comparison tests were used to determine if metal concentrations differed between taxa (n=349). Asterisks (*) indicate comparison of taxa is significant; "ns" indicates not significant.

Metal	*F*-Value	(*P*-value)	Ga-Gr	Ga-Ca	Ga-Lyc	Gr-Ca	Gr-Lyc	Ca-Lyc
Ni	21.14	(<0.0001)	ns	*	*	*	*	*
Cu	44.30	(<0.0001)	*	*	*	ns	*	*
Cd	58.55	(<0.0001)	ns	*	*	*	*	*
Hg	4.93	(0.0023)	ns	*	*	ns	*	*
U	11.14	(<0.0001)	ns	ns	*	ns	ns	ns
Ti	9.80	(<0.0001)	ns	ns	ns	ns	ns	*

Table 8. Transfer factors (TFs) for metals in soil, plants, invertebrates, and, cotton mice (*Peromyscus gossypinus*) tissues (kidney, liver, and muscle) collected from Tims Branch on the Savannah River Site, Aiken, SC. No data is represented by "nd".

				P. gossypinus		
Metals	Soil	Plant	Invertebrates	Kidney	Liver	Muscle
	Mean	TF	TF	TF	TF	TF
Ni	155.70	0.61	0.04	0.00	0.00	0.00
Cu	20.15	0.46	3.11	0.67	0.64	0.22
Cd	0.31	nd	2.87	3.91	0.81	0.08
Hg	0.27	nd	0.48	1.03	3.19	0.18
U	575.84	0.26	0.01	0.00	0.00	0.00
Ti	3410.23	nd	0.00	nd	nd	nd

Table 9. Summary statistics for $\delta^{15}N$ and $\delta^{13}C$ values of three families (Carabidae, Gryllidae, Lycosidae) collected during one trapping season (Fall 2003) at Tims Branch on the Savannah River Site, Aiken, SC. Family Carabidae is represented by five morphotypes (A, B, C, D, G) along with families Gryllidae and Lycosidae. Morphotype A had ten specimens from trap site 4-9 analyzed as individual samples. Additionally, for morphotype A, there were nineteen other trap sites that had all individuals pooled and analyzed in triplicate for each trap site. All other morphotypes (B, C, D, G) had specimens from each trap site analyzed as individual samples.

		N-15	C-13
Morphotype A	MEAN	3.49	-23.65
Site	ST DEV	3.09	3.89
4-9	MIN	-0.57	-27.16
(n=10)	MAX	7.10	-16.89

		N-15	C-13
Morphotype A	MEAN	5.62	-21.60
(n=202)	ST DEV	1.15	1.88
	MIN	2.71	-25.12
	MAX	6.96	-18.58

		N-15	C-13
Morphotype B	MEAN	8.48	-25.82
(n=35)	ST DEV	1.45	0.76
	MIN	5.03	-27.93
	MAX	10.63	-24.51

		N-15	C-13
Morphotype C	MEAN	3.39	-26.95
(n=13)	ST DEV	1.65	0.34
	MIN	0.99	-27.63
	MAX	5.67	-26.55

		N-15	C-13
Morphotype D	MEAN	7.39	-25.55
(n=34)	ST DEV	2.16	1.87
	MIN	3.07	-27.42
	MAX	10.07	-17.44

		N-15	C-13
Morphotype G	AVG	3.21	-25.74
(n=3)	ST DEV	1.95	0.83
	MIN	1.13	-26.63
	MAX	4.98	-24.97

		N-15	C-13
Gryllidae	MEAN	4.63	-27.47
Site	ST DEV	1.52	0.47
1-8	MIN	0.99	-28.20
(n=10)	MAX	6.08	-26.74

		N-15	C-13
Gryllidae	MEAN	5.64	-27.34
All sites	ST DEV	1.24	0.33
(n=130)	MIN	2.36	-27.90
	MAX	7.29	-26.58

		N-15	C-13
Lycosidae	MEAN	5.77	-26.17
Site	ST DEV	2.16	0.36
4-4	MIN	3.17	-26.63
(n=9)	MAX	9.50	-25.63

		N-15	C-13
Lycosidae	MEAN	6.29	-26.41
All sites	ST DEV	1.15	0.51
(n=104)	MIN	5.14	-27.84
	MAX	9.79	-25.79

Figure 3. $\delta^{13}C$ and $\delta^{15}N$ values for all taxa (Carabidae morphotypes, Gryllidae, Lycosidae) collected during one season (Fall 2003) at Tims Branch located on the Savannah River Site, Aiken, SC. Data points represent means with error bars representing standard deviation. Family Carabidae is represented by five morphotypes (Morph A, B, C, D, G) along with families Gryllidae and Lycosidae. Means are all trap sites combined for each taxa.

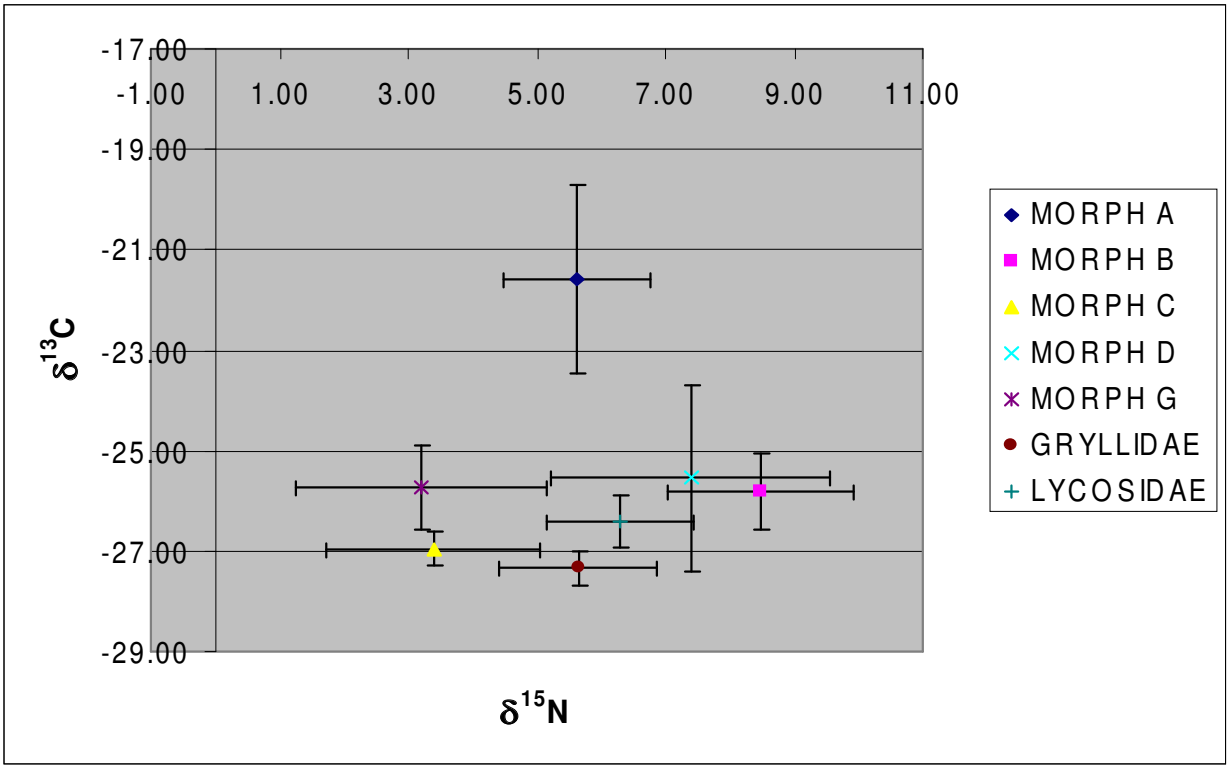


Figure 4. $\delta^{13}C$ and $\delta^{15}N$ values for individuals from Carabidae morphotypes collected during one season (Fall 2003) from Tims Branch located on the Savannah River Site (SRS) Aiken, SC. Data for Morphotype A are individuals from one trap site (4-9). All other morphotypes are individuals from multiple trap sites.

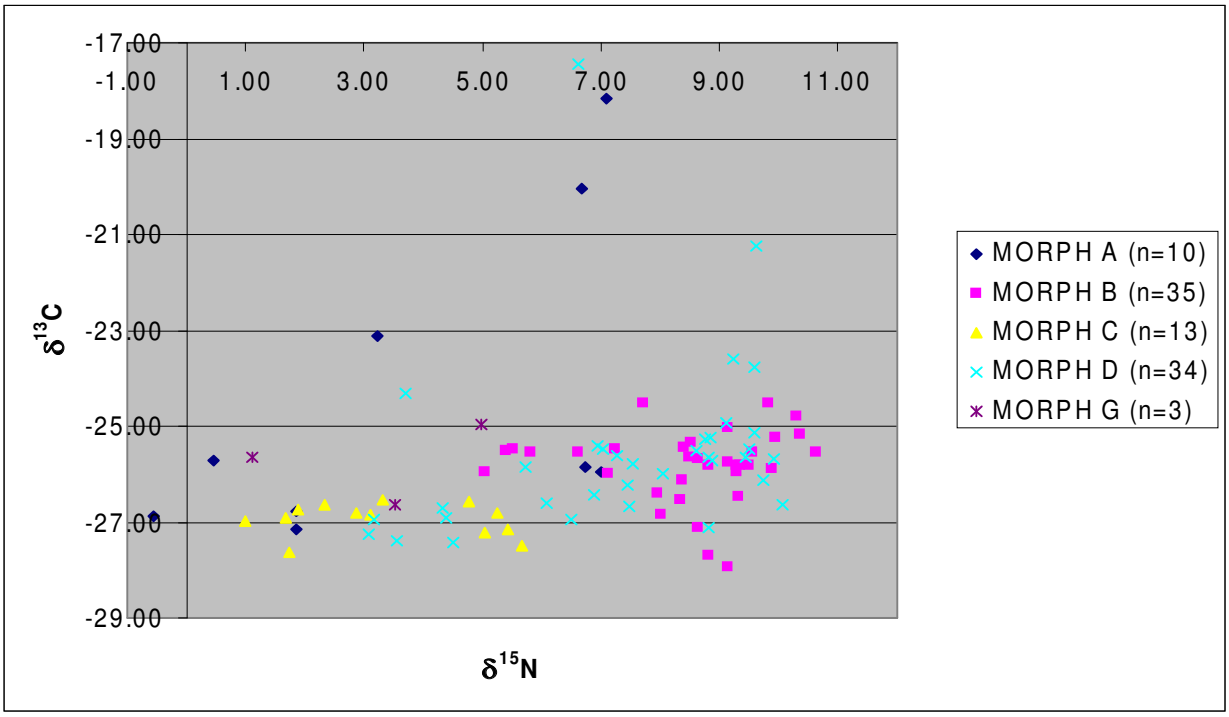


Figure 5. Metal concentrations for soil, plant tissue (*Salix nigra* leaves), invertebrate taxa (Carabidae, Gryllidae, Gryllacrididae, Lycosidae) and *Peromyscus gossypinus* tissue (kidney, muscle, liver) collected from Tims Branch on the Department of Energy's (DOE) Savannah River Site (SRS), Aiken, SC. Mean metal concentrations are listed above bars (mg/kg dry weight).

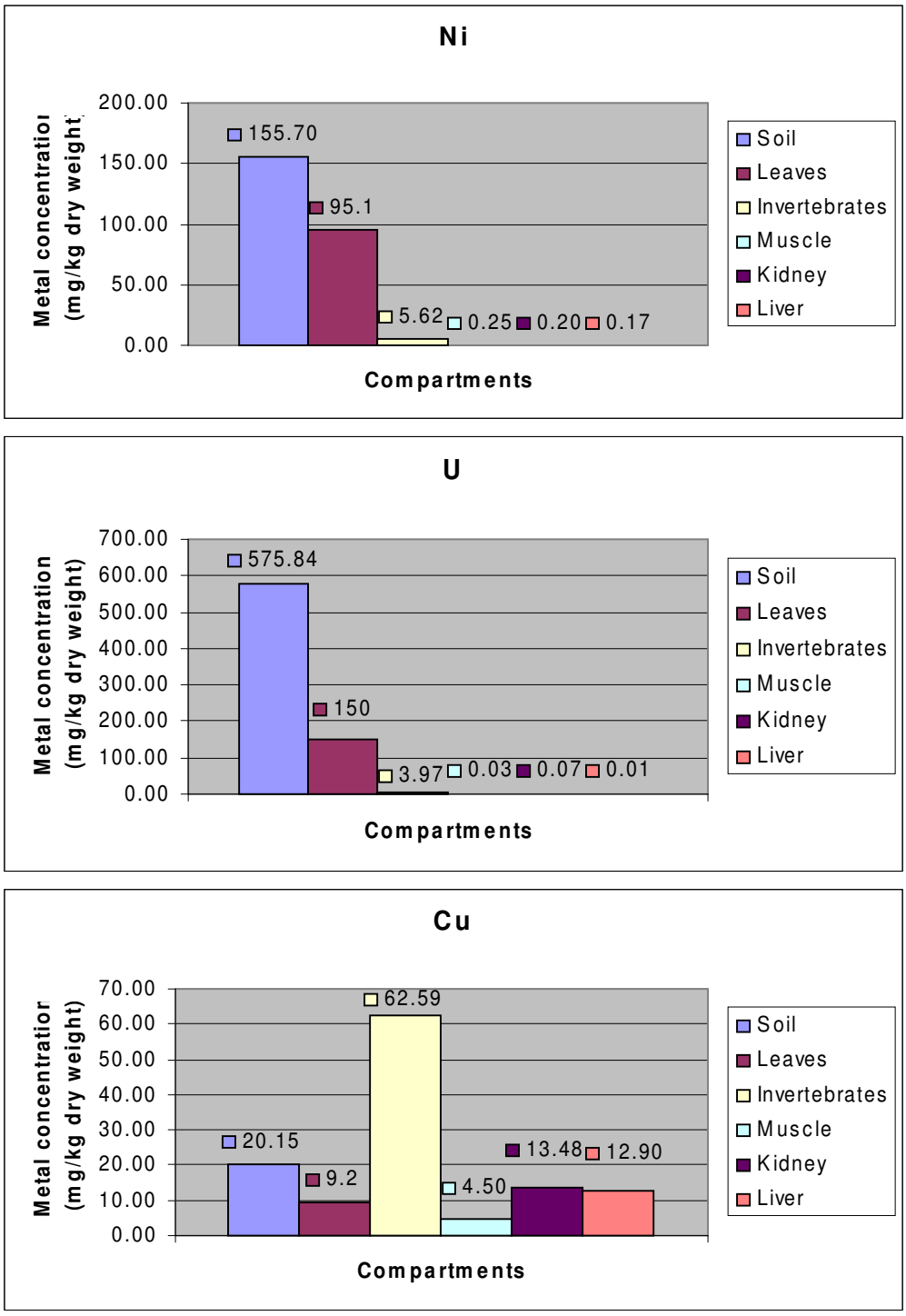
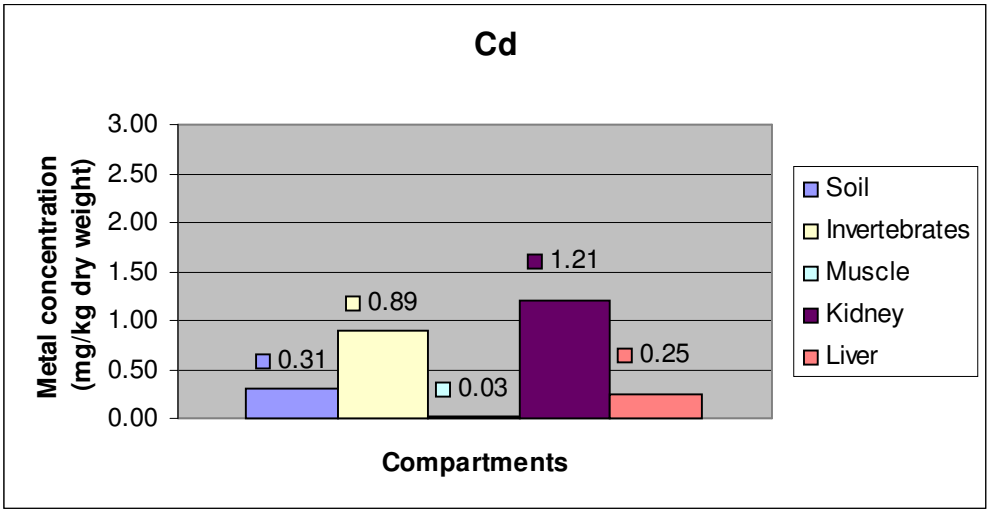
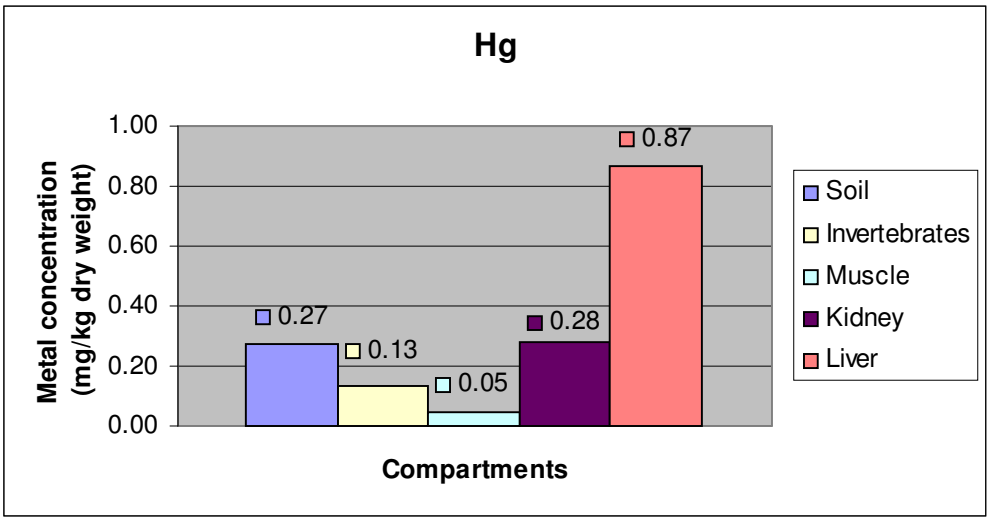


Figure 5 continued





Appendix 1. Summary statistics for metal concentrations for four families (Carabidae, Gryllidae, Gryllacrididae, Lycosidae) collected during Season 1 (Fall 2002). Concentrations are in mg/kg dry weight.

SEASON 1 FALL 2002
Carabidae
(n=1467)

	Site	Mean	Standard Deviation	Median	Minimum	Maximum
Ni	Tims Branch	2.57	1.90	2.05	0.43	9.33
	Boggy Gut	1.18	2.41	0.43	0.43	17.16
Cu	Tims Branch	16.07	4.90	15.10	8.84	34.67
	Boggy Gut	17.86	5.10	16.61	9.56	37.33
Cd	Tims Branch	0.07	0.07	0.05	0.01	0.45
	Boggy Gut	1.63	6.50	0.07	0.01	32.07
Hg	Tims Branch	0.03	0.03	0.03	0.00	0.13
	Boggy Gut	0.03	0.04	0.02	0.00	0.18
U	Tims Branch	2.36	1.81	2.15	0.28	9.85
	Boggy Gut	0.29	0.01	0.28	0.28	0.40
Ti	Tims Branch	4.11	1.15	4.01	1.96	6.27
	Boggy Gut	7.43	2.57	6.77	3.67	14.32

SEASON 1 FALL 2002
Gryllidae
(n=786)

	Site	Mean	Standard Deviation	Median	Minimum	Maximum
Ni	Tims Branch	8.58	5.42	8.45	0.43	25.88
	Boggy Gut	1.33	1.44	1.20	0.43	8.84
Cu	Tims Branch	26.54	8.59	27.76	1.14	38.38
	Boggy Gut	32.09	9.35	30.56	17.18	61.25
Cd	Tims Branch	0.58	0.39	0.51	0.01	1.89
	Boggy Gut	2.64	6.96	0.70	0.01	31.00
Hg	Tims Branch	0.06	0.07	0.04	0.00	0.38
	Boggy Gut	0.09	0.05	0.08	0.00	0.23
U	Tims Branch	6.18	12.61	1.99	0.28	75.76
	Boggy Gut	0.28	0.00	0.28	0.28	0.28
Ti	Tims Branch	7.83	3.13	7.83	0.16	13.02
	Boggy Gut	11.60	2.91	11.48	6.59	17.46

Appendix 1 continued

SEASON 1 FALL 2002
Gryllacrididae
(n=236)

	Site	Mean	Standard Deviation	Median	Minimum	Maximum
Ni	Tims Branch	12.67	8.45	10.45	2.95	38.24
	Boggy Gut	1.71	1.16	1.46	0.43	5.01
Cu	Tims Branch	41.82	12.13	42.75	16.49	61.86
	Boggy Gut	42.07	17.40	37.57	23.33	114.18
Cd	Tims Branch	0.57	0.53	0.41	0.14	2.32
	Boggy Gut	1.68	5.11	0.25	0.01	27.79
Hg	Tims Branch	0.07	0.07	0.05	0.01	0.35
	Boggy Gut	0.09	0.12	0.05	0.00	0.63
U	Tims Branch	7.38	10.97	3.49	0.50	51.53
	Boggy Gut	0.28	0.00	0.28	0.28	0.28
Ti	Tims Branch	8.22	2.01	8.00	5.09	11.65
	Boggy Gut	13.86	5.03	12.36	6.83	26.86

SEASON 1 FALL 2002
Lycosidae
(n=573)

	Site	Mean	Standard Deviation	Median	Minimum	Maximum
Ni	Tims Branch	1.95	1.39	1.49	0.45	6.72
	Boggy Gut	0.55	0.19	0.47	0.43	1.34
Cu	Tims Branch	224.36	118.17	205.64	73.15	606.80
	Boggy Gut	299.28	145.99	276.59	100.02	748.13
Cd	Tims Branch	3.12	6.46	1.76	0.01	30.39
	Boggy Gut	7.97	8.74	4.49	0.01	30.70
Hg	Tims Branch	0.27	0.25	0.23	0.00	1.21
	Boggy Gut	0.37	0.27	0.22	0.00	1.00
U	Tims Branch	1.85	2.19	0.84	0.28	7.74
	Boggy Gut	0.34	0.32	0.28	0.28	2.33
Ti	Tims Branch	8.08	2.03	7.80	5.46	14.66
	Boggy Gut	8.74	1.38	8.30	6.69	12.15

Appendix 2. Summary statistics for metal concentrations for four families (Carabidae, Gryllidae, Gryllacrididae, Lycosidae) collected during Season 2 (Spring 2002). Concentrations are in mg/kg dry weight.

SEASON 2 SPRING 2003
Carabidae
(n=800)

	Site	Mean	Standard Deviation	Median	Minimum	Maximum
Ni	Tims Branch	1.84	1.90	1.11	0.48	9.95
	Boggy Gut	2.24	8.64	0.48	0.48	71.97
Cu	Tims Branch	18.60	9.21	16.21	9.53	53.29
	Boggy Gut	19.51	6.40	17.60	12.64	51.54
Cd	Tims Branch	0.09	0.06	0.06	0.06	0.30
	Boggy Gut	0.10	0.09	0.06	0.06	0.60
Hg	Tims Branch	0.10	0.02	0.09	0.09	0.18
	Boggy Gut	0.10	0.01	0.09	0.09	0.18
U	Tims Branch	2.38	3.11	1.47	0.10	20.35
	Boggy Gut	0.10	0.00	0.10	0.10	0.10
Ti	Tims Branch	1.06	0.73	0.87	0.41	4.18
	Boggy Gut	0.66	0.23	0.60	0.39	1.90

SEASON 2 SPRING 2003
Gryllidae
(n=165)

	Site	Mean	Standard deviation	Median	Minimum	Maximum
Ni	Tims Branch	8.09	9.82	5.52	1.27	58.35
	Boggy Gut	0.89	1.10	0.48	0.48	5.31
Cu	Tims Branch	37.23	9.36	37.22	18.66	51.91
	Boggy Gut	29.36	7.64	27.90	16.77	45.00
Cd	Tims Branch	0.25	0.16	0.22	0.07	0.88
	Boggy Gut	0.15	0.13	0.11	0.06	0.58
Hg	Tims Branch	0.10	0.08	0.09	0.09	0.55
	Boggy Gut	0.09	0.00	0.09	0.09	0.09
U	Tims Branch	3.52	3.77	1.95	0.14	15.39
	Boggy Gut	0.10	0.00	0.10	0.10	0.10
Ti	Tims Branch	1.18	0.71	0.91	0.50	3.62
	Boggy Gut	1.86	1.12	1.50	0.54	5.14

Appendix 2 continued

SEASON 2 SPRING 2003
Gryllacrididae
(n=224)

	Site	Mean	Standard deviation	Median	Minimum	Maximum
Ni	Tims Branch	11.82	10.52	9.29	0.48	41.21
	Boggy Gut	1.28	0.91	1.10	0.48	4.16
Cu	Tims Branch	35.90	7.76	35.15	21.69	64.37
	Boggy Gut	40.13	12.93	38.66	14.32	80.75
Cd	Tims Branch	0.21	0.16	0.16	0.06	0.64
	Boggy Gut	0.19	0.10	0.19	0.06	0.56
Hg	Tims Branch	0.09	0.00	0.09	0.09	0.12
	Boggy Gut	0.09	0.01	0.09	0.09	0.13
U	Tims Branch	5.49	7.94	2.87	0.10	41.54
	Boggy Gut	0.10	0.00	0.10	0.10	0.10
Ti	Tims Branch	1.78	2.00	1.03	0.39	11.05
	Boggy Gut	1.31	0.70	1.07	0.36	3.88

SEASON 2 SPRING 2003
Lycosidae
(n=1726)

	Site	Mean	Standard deviation	Median	Minimum	Maximum
Ni	Tims Branch	3.71	1.66	3.12	1.27	7.94
	Boggy Gut	0.50	0.11	0.48	0.48	0.96
Cu	Tims Branch	150.79	32.30	147.30	102.90	245.15
	Boggy Gut	148.30	74.04	142.17	74.53	516.81
Cd	Tims Branch	3.07	0.65	3.01	1.44	4.61
	Boggy Gut	4.90	2.56	4.26	2.09	14.89
Hg	Tims Branch	0.37	0.10	0.35	0.21	0.69
	Boggy Gut	0.27	0.12	0.25	0.09	0.54
U	Tims Branch	5.11	4.34	4.33	0.41	15.95
	Boggy Gut	0.10	0.00	0.10	0.10	0.10
Ti	Tims Branch	1.36	0.53	1.22	0.69	3.42
	Boggy Gut	1.33	0.39	1.26	0.88	3.13

Appendix 3. Transfer factors (TF) for soil means and invertebrates collected from Tims Branch on the Savannah River Site, Aiken, SC. Soil means are for all trap sites from Tims Branch.

TB	Soil	TB	Carabidae			TB	Gryllacrididae	
	Mean			Mean	TF		Mean	TF
Ni	155.70		Ni	2.13	0.01	Ni	12.25	0.08
Cu	20.15		Cu	17.52	0.87	Cu	38.95	1.93
Cd	0.31		Cd	0.08	0.26	Cd	0.40	1.28
Hg	0.27		Hg	0.07	0.25	Hg	0.08	0.30
U	575.84		U	2.37	0.00	U	6.47	0.01
Ti	3410.23		Ti	1.70	0.00	Ti	3.85	0.00

TB	Soil	TB	Gryllidae			TB	Lycosidae	
	Mean			Mean	TF		Mean	TF
Ni	155.70		Ni	8.34	0.05	Ni	2.85	0.02
Cu	20.15		Cu	31.74	1.58	Cu	186.63	9.26
Cd	0.31		Cd	0.42	1.36	Cd	3.10	9.99
Hg	0.27		Hg	0.08	0.31	Hg	0.32	1.19
U	575.84		U	4.89	0.01	U	3.52	0.01
Ti	3410.23		Ti	3.50	0.00	Ti	3.45	0.00

Appendix 4. Transfer factors (TF) for separate invertebrate taxa and *Perromyscus gossypinus* tissues.

TB	Carabidae		*P. gossypinus*		TB	Gryllacrididae		*P. gossypinus*	
		Kidney	Liver	Muscle			Kidney	Liver	Muscle
	Mean	TF	TF	TF		Mean	TF	TF	TF
Ni	2.13	0.09	0.08	0.12	Ni	12.25	0.02	0.01	0.02
Cu	17.52	0.77	0.74	0.26	Cu	38.95	0.35	0.33	0.12
Cd	0.08	15.29	3.17	0.33	Cd	0.40	3.06	0.64	0.07
Hg	0.07	4.10	12.70	0.70	Hg	0.08	3.41	10.54	0.58
U	2.37	0.03	0.00	0.01	U	6.47	0.01	0.00	0.00

TB	Gryllidae		*P. gossypinus*		TB	Lycosidae		*P. gossypinus*	
		Kidney	Liver	Muscle			Kidney	Liver	Muscle
	Mean	TF	TF	TF		Mean	TF	TF	TF
Ni	8.34	0.02	0.02	0.03	Ni	2.85	0.07	0.06	0.09
Cu	31.74	0.42	0.41	0.14	Cu	186.63	0.07	0.07	0.02
Cd	0.42	2.87	0.60	0.06	Cd	3.10	0.39	0.08	0.01
Hg	0.08	3.33	10.31	0.57	Hg	0.32	0.87	2.69	0.15
U	4.89	0.01	0.00	0.01	U	3.52	0.02	0.00	0.01

Appendix 5. Transfer factors for all taxa means combined and *Perromyscus gossypinus* tissues.

						P. gossypinus		
	Inverts		Kidney	Liver	Muscle	Kidney	Liver	Muscle
	Mean		Mean	Mean	Mean	TF	TF	TF
Ni	5.62	Ni	0.20	0.17	0.25	0.04	0.03	0.05
Cu	62.59	Cu	13.48	12.90	4.50	0.22	0.21	0.07
Cd	0.89	Cd	1.21	0.25	0.03	1.36	0.28	0.03
Hg	0.13	Hg	0.28	0.87	0.05	2.13	6.60	0.36
U	3.97	U	0.07	0.01	0.03	0.02	0.00	0.01

Appendix 6. Transfer factors for *Salix nigra* leaves and invertebrates.

S. nigra		Inverts		
	Mean	All taxa	Mean	TF
Ni	95.1	Ni	5.62	0.06
Cu	9.2	Cu	62.59	6.80
U	150	U	3.97	0.03
S. nigra		Carabidae		
	Mean		Mean	
Ni	95.1	Ni	2.13	0.02
Cu	9.2	Cu	17.52	1.90
U	150	U	2.37	0.02
S. nigra		Gryllidae		
	Mean		Mean	
Ni	95.1	Ni	8.34	0.09
Cu	9.2	Cu	31.74	3.45
U	150	U	4.89	0.03
S. nigra		Gryllacrididae		
	Mean		Mean	
Ni	95.1	Ni	12.25	0.13
Cu	9.2	Cu	38.95	4.23
U	150	U	6.47	0.04
S. nigra		Lycosidae		
	Mean		Mean	
Ni	95.1	Ni	2.85	0.03
Cu	9.2	Cu	186.63	20.29
U	150	U	3.52	0.02