EFFECTS OF PREY NUTRIENT CONTENT ON SPIDER EXCRETA CONTENT & SOIL-CARBON MINERALIZATION RATES

By

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Abstract:

Recent work suggests that predators can play a fundamental role in cycling nutrients throughout ecosystems. Through consumer-driven effects, including depositing excreta and uneaten parts of prey carcasses, predators can influence ecosystem function by altering the amount and type of nutrients available to soil communities and primary producers. In this study, I examined how different prey fed to a spider affected the forms and concentrations of nutrients deposited, and their subsequent effects on ecosystem function (soil-carbon mineralization rates). More specifically I examined: 1) The differences in elemental concentrations of prey remains and spider excreta when spiders fed on different species of prey (caterpillars, cockroaches, crickets, and flies). 2) If the concentrations of elements deposited by spiders differ between spring and fall. 3) The effects of spider excreta from different predator-prey interactions on soil-carbon mineralization rates. Overall, spider excreta generally had higher concentrations of many elements compared to prey remains, and whole prey. Additionally, elemental concentrations in whole prey and remains exhibited significant variation among prey species, while spider excreta had the lowest variation. Seasonally, there were significant differences in the concentrations of elements deposited between fall and spring excreta. Finally, soil-carbon mineralization rates were higher in controls than in soil with excreta from spiders fed caterpillars, cockroaches, and flies, with crickets being intermediate. The results from this study highlight the complex interactions between predator and prey physiology that determine the concentrations of elements deposited following predation. A better understanding of how other predatory-prey interactions impact nutrient feedbacks will be critical to disentangle specific consumer-driven effects on ecosystem function.

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CHAPTER I

Introduction

Ecosystems are complex and a variety of factors can affect the flow of nutrients throughout. For instance, top-down, bottom-up, or a combination of both factors can influence the amount and type of nutrients cycled through an ecosystem (Hodkinson et. al. 2001, Barrett et. al. 2005, Barnes 2010, Hawlena et. al. 2012,). In bottom-up effects, abiotic and biotic factors (e.g., wind, ocean currents, decomposing carcasses) affect nutrient availability to primary producers (Hilderbrand et.al. 1999, Romero et. al. 2006). Subsequentially, this impacts primary producer biomass and influences consumer populations at higher trophic levels (Barrett et. al. 2005). In top-down effects, consumers can reduce the abundance or biomass of lower trophic levels, which can have cascading effects on food webs (Carpenter et. al. 1985). For instance, consumption of herbivores can lead to an increase in primary producer populations (Silliman & Bertness 2002). Additionally, consumers can have both top-down and bottom-up effects; for instance, herbivory can deplete plant resources, while depositing excreta or uneaten parts of food items contributes resources to the base of food webs (Hilderbrand et. al. 1999). Recent work has suggested that animals can play important roles in biogeochemistry through these consumer-driven effects (Schmitz et al. 2018).

Numerous studies have highlighted the importance of predators in nutrient cycling across different ecosystems (Hilderbrand et. al. 1999, Hodkinson et. al. 2001, Barrett et. al. 2005, Schmitz et. al. 2010). For instance, through the consumption and release of nutrients, predator-induced herbivore foraging shifts, translocation of consumed nutrients, alteration of community composition (i.e., plant communities), and distribution of prey carcasses, predators can strongly influence the distribution of nutrients in an ecosystem (Schmitz et. al. 2010 & Schmitz et. al. 2018). For example, Hilderbrand et. al. (1999) demonstrated that brown bears distributed 83-84% of salmon-derived nitrogen to spruce foliage within 500 m of streams. Barret et. al. (2005) showed that the presence of sea birds increased coastal lizard abundance by depositing marine-derived nutrients (e.g., prey carcasses, excreta, etc.) which increased the abundance of arthropods that were prey of the lizards. While numerous studies have examined how high-density aggregations of predators mediate the translocation and alteration of nutrients in different systems (e.g., Hilderbrand et. al. 1999, Hodkinson et. al. 2001, Barrett et. al. 2005, Romero et. al. 2006, Schmitz et. al. 2010), comparatively less is known about how solitary predator-prey interactions (e.g., spider-insect) directly alter nutrient dynamics, biogeochemical cycling, and ecosystem function.

Globally, spiders are estimated to consume 400-800 million tons of insects per year, and the impacts of spider predation on nutrient flow is not well understood (Nyffeler & Birkhofer 2017). Several studies have shown that deposition of nutrients from spider-prey interactions could have strong effects on ecosystem function (Hodkinson et. al. 2001, Romero et. al. 2006, Hawlena et. al. 2012, Barnes et. al. 2019). For example, Linyphiid spiders act as early colonizers in artic environments, depositing

nutrients to the system by capturing midges and other wind-dispersed organic matter in their webs (Hodkinson et. al. 2001). Additionally, the Neotropical jumping spider, *Psecas chapado,* deposits nutrients through excreta and discarded prey remains to bromeliad plants. This leads to an 18% increase in bromeliad plant nitrogen and 15% increase in leaf production of plants with spiders compared to plants without (Romero et. al. 2006). Therefore, nutrients deposited by spiders (whether directly on the plant or the soil) could have direct effects on primary producers (Bump et. al. 2009).

A better understanding of how spider digestive processes interact with nutrient availability in prey may provide a more mechanistic understanding of how spiders influence nutrient cycling. Spiders are obligate predators that consume prey through extra-oral digestion (Foelix 1996). This process involves injecting enzymes into prey, ingesting digestible nutrients, and leaving behind indigestible prey remains. The digestibility of different prey parts affects how much of the prey nutrients will be metabolized and result in excreta production, as opposed to being deposited as indigestible prey remains, which may take much longer to decompose (Seastedt and Tate 1981, Barnes et. al. 2019). For example, the exoskeleton content of prey can affect nutrient deposition by predators, as spiders deposit more nitrogen in indigestible prey remains when feeding on prey with higher exoskeleton content (adult beetles) compared to those with less exoskeleton (larvae) (Barnes et. al. 2019).

In addition to exoskeleton, insect taxa differ considerably in nutrient content and these nutritional differences can affect the quality of insects as prey for spiders and other predators (Fagan et al. 2002, Wilder et. al. 2013, González et. al. 2020, & Reeves et. al. 2021). Hence, traits of the prey, such as exoskeleton and nutrient content, may affect the

quantity and chemical form of nutrients that spiders deposit into the soil, and how these nutrient deposits affect the flow of nutrients through an ecosystem. If predators are influencing nutrient dynamics in ecosystems through consumer-driven nutrient cycling, and if these effects change depending on the type of prey on which they feed, then it will be crucial to understand how these processes impact biogeochemical cycles and ecosystem function (Sterner 1986).

One way that even small changes in nutrient deposits to the soil can have large impacts on an ecosystem is through carbon mineralization. For example, grasshoppers stressed by the presence of spiders had a higher body carbon-to-nitrogen (C:N) ratio compared to those in the absence of spiders (Hawlena et al. 2012). Consequently, carcasses of stressed grasshoppers reduced the decomposition of added plant litter by 62% compared to unstressed grasshoppers (Hawlena et al. 2012). Further experiments with varying proportions of carbohydrates (0 to 80%) and proteins (0 to 80%) revealed that it was the nutrient content of the grasshopper bodies, especially protein, that primed soil microbial communities to decompose plant litter differently (Hawlena et. al. 2012). Higher C:N ratios impeded plant litter decomposition rates, which mirrored the results found with the stressed grasshopper carcasses (Hawlena et al. 2012). This work revealed how small but high-quality nutrient additions to the soil could have large effects on litter decomposition and carbon mineralization by priming soil microbial communities (Hawlena et. al. 2012). Spider excreta represents another type of potentially high-quality nutrient addition to soils that could similarly prime soil communities in ways that have large impacts on decomposition. Although, whether spider excreta has similar soil

priming effects, or if spider excreta from feeding on different prey groups has different effects for soil function, remains unclear.

The goal of this project was to test how feeding on different prey by a spider affected the forms and concentrations of nutrients deposited and their subsequent ecosystem function. More specifically, this study investigated three main questions: 1) Are there differences in the elemental content (e.g., N, P, Ca, Cu, Fe, K, Mg, etc.) of prey remains and spider excreta when a spider feeds on different species of prey? 2) Do the concentrations of elements deposited differ between spiders feeding in the fall (i.e., just prior to overwintering) and spring (i.e., the reproductive season)? and 3) How does spider excretion derived from these different interactions affect soil-carbon mineralization rates? To do this, I explored different predator-prey interactions between Carolina Wolf Spiders, *Hogna carolinensis*, and four species of prey: German Cockroaches (*Blattela germanica*), House Crickets (*Acheta domesticus), Flesh Flies (Sarcophaga bullata*), and Painted Lady Caterpillars (*Vanessa cardui*), and how these interactions affected spider excreta content and soil-carbon mineralization.

I hypothesized that there would be significant differences in the elemental content of spider excreta when feeding on different prey. This is due to each prey group differing in proportions of indigestible exoskeleton to body mass, which can impact nutrient availability to the spider (Lease & Wolf 2010, Wilder 2018, Barnes et. al 2019). Additionally, each prey group differs in feeding strategies, which may result in differences in the overall amounts and types of nutrients in their body. This hypothesis assumes that spiders need a fixed amount of each element and that any excess will be put in the excreta (Anderson et. al. 2005). I also hypothesized that spider excreta from

feeding on different prey would influence soil-carbon mineralization rates due to differences in the elemental content of the excreta.

CHAPTER II

Methods

Study Species

Adult female Carolina Wolf Spiders (*Hogna carolinensis*) were collected from fields in Stillwater, Oklahoma, during September-October of 2020 and April-June of 2021 (hereafter fall and spring, respectively) and housed in clear plastic containers (height:7.4 cm, diameter:16.2 cm) in the laboratory. All spiders were maintained at a constant 25 ± 1 °C and 14L:10D light regime. All spiders were fed during trials and had water provided *ad libitum*.

Prey Species

German Cockroaches, House Crickets, Flesh Flies, and Painted Lady Caterpillars were all purchased from commercial distributers (Josh's Frogs & Carolina Biological). Insect were housed at 14L:10D light regime and given water *ad libitum*. Flesh Flies and Painted Lady Caterpillars were maintained on a diet recommended by the distributer (e.g., sugar for flies and artificial diet for caterpillars). German Cockroaches and house crickets were fed dog food (Racheal Ray Nutrish Dog Food).

Feeding Trials

To standardize hunger levels prior to feeding trials, spiders were given 0.45-0.5 g of crickets, then fasted for seven days. Following the seven-day starvation period, each spider was randomly assigned a prey group (cockroaches, flies, caterpillars, crickets). Then each spider received a pre-weighed prey item(s), ranging in size from 0.3-0.35 g. Spiders were allowed to feed for a full twenty-four-hour period. Any live prey left uneaten after the allowed feeding time were removed. If the entirety of the given prey were eaten, then uneaten prey remains were collected and placed in the oven for forty-eight hours at 60°C. Prey remains were then stored dry for further analysis.

Spider Excretion Sample Collection

Spider excreta was collected 48 hours after the prey were provided to spiders. It has been demonstrated in previous work that this is allows for most spider excreta to be deposited (Barnes 2010). Distilled water (1.5 ml) was used to dissolve the excreta, which was then pipetted into 2 ml centrifuge vials. Vials were then centrifuged for 10 minutes at 13,000 RPM and placed in an oven for 48 hours at 60°C to evaporate the water. No water was removed from samples prior to drying in the oven. Samples were stored dry for further analysis.

Objective 1: Elemental Content Analysis of Spider Excretion

The elemental content of spider excreta, prey remains, and whole prey controls were measured. Carbon and nitrogen were measured by the Boston University Stable Isotope lab while other elements (B, Ca, Cu, Fe, K, Li, Mg, Mn, Na, P, S, Si, and Zn)

were measured with an ICP-OES (inductively coupled plasma – optical emission spectrometer; Thermo Scientific iCAP 7400) (n = 9 – 18 replicates per treatment).

Objective 2: Effects of Spider Excretion on Soil-Carbon Mineralization Rates

The effects of spider excreta on soil-carbon mineralization rates were measured in a process similar to Hawlena et al. (2012). The process involves adding a small sample of material, in this case spider excreta, to a small amount of soil, measuring carbon mineralization of the soil until rates of CO_2 production plateau, then adding plant litter and continuing to monitor carbon mineralization (Hawlena et al. 2012). The process required using approximately 4 mg of spider excreta, but many individual excreta were smaller than this. Hence, I typically combined 2-3 excreta samples of the same treatment type to achieve a sample that weighed 3.9-4.25 mg. I then added excreta samples from each prey group to microcosms of soil to observe carbon mineralization (n = 11 – 16 per treatment). Additionally, I included a control treatment with no excreta additions to the soil.

Soil to use in the microcosms was collected from the same fields that the Carolina Wolf Spiders inhabit in Stillwater, Oklahoma. Prior to use, soil samples were 2-mm sieved, homogenized, then air-dried at 60°C for 48 hours. Then, 3.9-4.25 mg of spider excretion from each of the different prey treatments was added to 4 g dry mass equivalent soil in 50-ml centrifuge tubes. Blank controls were also created, which contained just soil. Following an incubation procedure described by Bradford et. al. (2008) and used by Hawlena et. al. (2012), the mixtures were maintained at 65% water-holding capacity

(WHC), incubated at 25 °C, and had water addition as needed to maintain WHC (Bradford et. al. 2008, Hawlena et. al. 2012)

Then, I determined CO_2 mineralization rates, using an infrared gas analyzer (LICOR) and standard procedures for carbon mineralization measurements as demonstrated in previous studies, until the initial period of C-mineralization had plateaued (Bradford et. al. 2008, Hawlena et. al. 2012)

Data Analysis

I used 3-way ANOVAs to test the effect of treatment (excreta, remains, whole prey), prey (caterpillar, cockroach, cricket, fly), and season (spring and fall) on percent C, percent N, and C:N.

For the ICP elemental data, As, Co, and Pb were removed from analysis due to extremely low values or high numbers of zero values. The distribution of all elements was right-skewed. All elements were log-transformed as this appeared to remove the right-skew. A Principal Component Analysis (PCA) was performed on log-transformed ICP elemental content of spider excreta, prey remains, and whole prey to account for collinearity among the elements and to visual the effects of treatments on elemental content. The PCA was also used to generate principal components axis values that could be used in ANOVA analysis. A 3-way ANOVA was conducted on the first and second principal components to test the effects of treatment, prey, and season on overall elemental composition.

I used repeated measures ANOVA to analyze hourly rates of carbon mineralization over the first 17 days (prior to plant litter addition). I also calculated the

sum of all hourly carbon mineralization values for each sample and analyzed it with ANOVA. Then I used Tukey HSD to examine post hoc differences between the excreta treatments.

CHAPTER III

Results

Elemental Content

Carbon concentrations were significantly affected by prey, treatment, season, and the interaction of prey and season (Figure 1; Table 1A). Across all four prey, whole prey had the highest percent C and excreta had the lowest, with remains in between (Figure 1). For the prey by season interaction, there were some prey groups, especially caterpillar and cockroach, where C concentrations were higher in spring than in fall (Figure 1).

Similarly, nitrogen concentrations were significantly affected by prey, treatment, season, and the interactions of prey by treatment and treatment by season (Figure 2; Table 1B). For both seasons and all prey groups, spider excreta had the highest concentrations and whole prey generally had the lowest concentrations of nitrogen (Figure 2). The prey by treatment interaction appeared to result from variation among prey in the remains treatment. In some species, the remains treatment was intermediate to the whole prey and excreta, while in other species the remains treatment was closer to the whole prey (Figure 2). For the treatment by season interaction, concentrations of nitrogen were higher in excreta and remains but not the whole prey in the fall relative to the spring (Figure 2).

C:N ratios were significantly affected by all factors and interactions except the main effect of season (Table 1C). The clearest effect in the data was that the C:N ratios

were lowest in excreta, highest in whole prey and intermediate in remains (Figure 3). The interactions appeared to be due to differences among species and seasons in the effects of treatment (Figure 3).

All other elements analyzed with ICP-OES were combined into a principal components analysis. The proportion of variance explained by the first three axes were 28.12 % for PC1, 20.95 % for PC2, and 8.231% for PC3. Based on this information, I decided to only analyze PC1 and PC2 axis as they were the axes that explained the highest amounts of variation. Many elements were negatively loaded on the PC1 axis (<-0.20) including Al, B, Ba, Ca, Fe, K, Li, Mg, Ni, P, S, Si, and Sr (Table 3). For PC2, several elements were positively loaded on the axis (>0.20) including B, K, P, and S; while, several other elements were negatively loaded on the axis (<-0.20) including Ca, Cu, Mo, Mn, Sr, and Zn (Table 3).

When I plotted all prey and treatments in PCA, there were very clear effects of treatments on elemental concentrations (Figure 4). The treatments were separated in space. The excreta from all prey types were similar to each other. The different species of whole prey were also similar to each other, though less than that of excreta. The prey remains were more spread in the principal component space among the different species than were the excreta or whole prey (Figure 4).

I used ANOVA to statistically analyze PC1 and PC2 scores. There were significant effects of prey, treatment, and season on PC1 scores of the elements (Figure 4, 5, 6; Table 2A). There were also significant interactions of prey by treatment, and prey by season (Figure 5, 6; Table 1A). For the prey by treatment interaction, excreta were always lower than whole prey but the position of the remains (i.e., whether it was not

different from whole prey or excreta) varied among species (Figure 5; Table 2A). For the interaction of prey by season, all treatments were different from each other in spring, with whole prey higher than remains which was higher than excreta, while remains and excreta were not different from each other in the fall with both significantly lower than the whole prey (Figure 6; Table 2A).

For PC2, there were significant main effects of prey and treatment, with additional significant interactive effects of prey by treatment, and prey by season (Figure 4, 7, 8; Table 2B). For the prey by treatment interaction, excreta had the highest PC2 values followed by whole prey and remains but the relative differences between the treatments differed among prey species (Figure 7). For the prey by season interaction, there were no differences between season in remains and whole prey but PC2 for excreta was significantly higher in fall than spring (Figure 8; Table 2B).

Carbon Mineralization

Analysis of the results of carbon mineralization in response to excreta additions showed a significant effect of prey on carbon mineralization. (Figure 9). Overall, hourly rates of carbon mineralization were highest during the first six days, then declined for the rest of the trial (Figure 9). Based on post hoc analyses of cumulative hourly carbon mineralization, controls had the highest carbon mineralization, while flies, cockroaches, and caterpillars had the lowest. Crickets were intermediate and not significantly different from controls and the other prey.

CHAPTER IV

Discussion

These results demonstrate that there are significant differences in concentrations of a variety of elements between spider excreta, prey remains, and whole prey. Carolina Wolf Spiders who preyed upon caterpillars, cockroaches, flies, or crickets had higher concentrations of certain elements (e.g., N, P, B, K, etc.) in their excreta compared to the concentrations of these elements in prey remains and whole prey (Figure 4). Additionally, elemental concentrations in whole prey and remains exhibited significant variation among prey species (Figure 4). For instance, caterpillar prey remains had high amounts of cadmium, while the remains of crickets consisted of larger concentrations of zinc. Furthermore, there was a seasonal component to the variation of elemental composition of spider excreta, with spring excreta exhibiting higher elemental concentration than fall (Figure 5, 6, and 8). These results highlight the complex interactions between predator and prey physiology that determine the elemental content of nutrients deposited following predation.

It has been previously demonstrated that there are differences in nutrient content among insect taxa (Fagan et al. 2002, Wilder et. al. 2013, González et. al. 2020, Reeves et. al. 2021). However, less is known about other elements. For whole prey, there were differences among species in the concentrations of a variety of elements (e.g., N, C, Na,

Zn, Ca, etc.) (Figure 1, 2, 3, and 4). This is likely due to life history strategies, dietary requirements, and physiology differing among prey groups (caterpillars, cockroaches, crickets, and flies). For instance, caterpillars are holometabolous insects, feeding on carbohydrate-based plant tissue during their larval stage. Alternatively, crickets are hemimetabolous, and feed on food sources that consist of higher quantities of protein (in this case dog food that was provided *ad libitum*) (Fagan et al. 2002, Wilder et. al. 2013, González et. al. 2020, Reeves et. al. 2021). Additionally, cockroaches can store excess nitrogen as uric acid in their bodies, which results in higher N% compared to other prey (Sabree et. al. 2009). Phylogeny, ecology, and life history traits could be important factors explaining the differences in elemental concentrations that were observed between whole prey (Lease & Wolf 2010, Wilder 2018, Barnes et. al 2019). Although further work is needed to determine how and why prey species vary in a variety of nutrients.

Similarly, with prey remains there was differences among species in the concentration of elements (e.g., N, C, Na, Zn, Ca, etc.), regardless of season (Figure 4, 6, and 8). Prey remains are nearly all exoskeleton and the exoskeletons of insects are largely indigestible to predators, including spiders (Foelix 1996). Between insect taxa there are differing degrees and types of exoskeletons (i.e., soft and flexible vs. hard and protective) which may require different combinations of elements or chemicals to achieve these properties. This may directly affect the amount and types of nutrients available to predators (i.e., beetles have greater proportions of indigestible exoskeleton to body mass than caterpillars) (Reeves et. al. 2021). For instance, cricket and caterpillar remains had higher concentrations of sodium, a biologically vital element, in their indigested prey remains (Figure 4). Therefore, not only is there variation in elemental concentration of

different insect taxa, but there are also differences among species in nutrients locked in the chitinous matrix of the exoskeleton and inaccessible to predators (Reeves et. al. 2021). This poses a challenge to studying insectivore nutrition as measures of whole arthropods will not identify how much of the nutrients are digestible by the consumer. However, discarded prey remains still contain valuable nutrients (e.g., N, C, proteins, etc.) and may affect ecosystem function (i.e., plant growth, soil-carbon mineralization rates, etc.) (Bump et. al. 2009). Although, it may take years for these nutrients in the exoskeleton to be released (Seastedt and Tate 1981) and, hence, the short-term effects of prey remains on ecosystem processes remain unclear.

Unlike whole prey and prey remains, spider excreta had relatively little overall elemental variation between prey groups (Figure 1, 2, 3, and 4). Some elements present in the excreta are important for microbial communities (e.g., P, K, etc.), while others have been demonstrated to be toxic, change microbial communities, or detrimental to plant fauna at high concentrations (e.g., B, V, etc.) (Vera et. al. 2019 & Zhang et. al. 2020). Furthermore, there could be other elements present in spider excreta when they feed on other diverse insect taxa not used in this study (i.e., Coleoptera, Hymenoptera, etc.), with additional unknown effects. Overall, the consequences of trace elements on microbial communities, plant communities, and overall ecosystem function are not clear. Further studies are needed to understand how specific elements (i.e., Al, B, Ni, B, etc.) influence these communities and ecosystem function (whether beneficial or detrimental).

There were differences in the elemental content of fall and spring excreta. These differences may be explained by the life history strategies of adult female *Hogna carolinesis*. As winter approaches, the assimilation of nutrients could be key to the

spider's survival as it prepares for diapause. For example, it has been shown that female spiders that consumed supplemental dietary amino acids, produced offspring that survived overwintering conditions longer (Wilder & Schneider 2017). Alternatively, fall excreta could reflect a limited prey selection during cooler months, with spiders assimilating and retaining more nutrients than that of spring when prey abundance and diversity is higher. Spiders are opportunistic feeders, and long periods of starvation are not uncommon, hence the fall could be a vital period of nutrient retention prior to diapause (Foelix 1996). In addition, some potential explanations for higher concentrations of nutrients in excreta in the spring could be: 1) Nutrients assimilated in the fall may not be important in spring, when the focus of the organism shifts back towards reproduction. 2) Retention of high concentrations of some nutrients may hinder reproduction, and excretion of them is vital to the organism's fitness. and 3) Prey may vary seasonally in nutrient content (e.g., data on whole cockroaches (Figure 1)) (Ng et. al. 2018). However, there is little information available in the literature for seasonal nutrient requirements for arthropod predators, especially for a wide range of elements as included in the present study. Further studies will be needed to disentangle the seasonal effects that were observed and subsequent implications that could occur for ecosystem function.

In addition to measuring the elemental content of excreta, this work examined how excreta from different prey affected soil carbon mineralization rates. Carbon mineralization rates were higher in controls than in soil with excreta from caterpillars, cockroaches, and flies. Carbon mineralization from soil with cricket excreta was intermediate and not different from controls or other prey (Figure 9). I initially hypothesized that excreta from any prey group would raise carbon mineralization rates,

however the results were the opposite. It is unclear why spider excreta negatively impacted carbon mineralization rates, especially since it contains vital nutrients (e.g., P, N, C, etc.). It is possible that there are some types of nutrients in excreta that have adverse effects on microbial communities. Alternatively, the nutrients in excreta could have had positive effects on microbial communities but in ways that resulted in a decrease in carbon mineralization, which is only one limited measure of what is happening in the soil (Vera et. al. 2019 & Zhang et. al. 2020). While excreta had a direct negative effect on carbon mineralization, it is unknown how it will affect the decomposition of plant matter in the second phase of the experiment. Overall, future studies are needed to disentangle the effects of a wide range elements on soil-microbial communities, plant communities, and consequent ecological functions.

Spiders are an abundant group of terrestrial carnivores, so it is crucial to further our understanding of how spider predator-prey interactions influence ecosystem structure and function (Nyffeler & Birkhofer 2017). Spatial and temporal variations in predator or prey communities could have large implications for ecosystem function. Additionally, different habits (e.g., distance from a river, fields, deserts) can have different communities of prey. Furthermore, since I only examined the elemental concentrations of four prey types and subsequent spider excreta and remains, an increase in prey, or potentially predator, diversity could influence the amount of variation in excreta or prey remain nutrient content. Therefore, since nutrient deposition depends on prey type, then different predator-prey interactions could have various consequences for ecosystems. Furthermore, new conservation methods may need to be implemented to reflect the generalist diet of many predators, as a loss of prey biodiversity could affect the quantity

and type of nutrients being deposited (e.g., N, P, K, etc.). It will be essential for further studies to investigate other predator-prey interactions and the nutrient feedbacks associated with those interactions.

REFERENCES

Anderson, R. T., Hessen, O. D., Elser, J. J., & Urabe, J. (2005). Metabolic stoichiometry and the fate of excess carbon and nutrients in consumers. The American Naturalist 165:1 1-15

Barnes, L. C. (2010). Contribution of spider resource use to ecosystem nutrient flow and Function (unpublished doctoral thesis). Oklahoma State University.

Barnes, L. C., McCue, D. M., Hawlena, D., & Wilder, M. S. (2019). Consequences of prey exoskeleton content for predator feeding and digestion: black widow predation on larval versus adult mealworm beetles. Oecologia 190:1-9.

Barret, K., Anderson, B. W., Wait, A. D., Grismer, L. L., Polis, A. G., & Rose, D. M. (2005). Marine subsidies alter the diet and abundance of insular and coastal lizard populations. Oikos, 109:145–153.

Bradbord, A. M., Davies, A. C., Frey, D. S., Maddox, R. T., Melillo, M. J., Mohan, E. J., Reynolds, F. J., Treseder, K. K., & Wallenstein, D. M. (2008). Thermal

adaptation of soil microbial respiration to elevated temperature. Ecology Letters, 11:316-1327.

Bump, K. J., Peterson, O. R., & Vucetich, A. J. (2009). Wolves modulate soil nutrient heterogeneity and foliar nitrogen by configuring the distribution of ungulate carcasses. Ecology, 90:11, 3159-3167

Carpenter, R. S., Kitchell, F. J., & Hodgson, R. J. (1985). Cascading trophic interactions and lake productivity. BioScience, 35:10, 634-639.

Fagan, W. F., Siemann, E., Mitter, C., Denno R. F., Huberty, A. F., Woods, H. A., & Elser, J. J. (2002). Nitrogen in insects: implications for trophic complexity and species diversification. The American Naturalist, 160:6, 784-802.

Foelix, R.F. (1996) Biology of spiders, 2nd Ed. Oxford University, Press, Oxford, 330

González, A., Barnes, L. C., Wilder, M. S., & Long, M. J. (2020). Differences in macronutrient content of common aquatic macroinvertebrates available as prey for young-of-the-year *Scaphirhynchus* sturgeons in the lower Missouri river. Journal of Freshwater Ecology, 35:1, 191-202.

Hawlena, D., Strickland, S. M., Bradford, A. M., & Schmitz, J. O. (2012). Fear of predation slows plant-litter decomposition. Science, 336:6087, 1434-1438.

Hilderbrand, V.G., Hanley, A.T., Robbins, T.C., & Schwartz, C.C. (1999). Role of brown bears (*Ursus arctos*) in the flow of marine nitrogen into a terrestrial ecosystem. Oecologia, 121:546–550.

Hodkinson, D. I., Coulson, J. S., Harrison, J., & Webb, R. N. (2001). What a wonderful web they weave: spiders, nutrient capture and early ecosystem development in the high Arctic– some counter-intuitive ideas on community assembly. Oikos, 95:2, 349-352.

Lease, M. H., & Wolf, O. B., (2010). Exoskeletal Chitin Scales Isometrically With Body Size in Terrestrial Insects. Journal of Morphology, 271:759-768.

Nyffeler, M. & Birkhofer, K. (2017). An estimated 400–800 million tons of prey are annually killed by the global spider community. Sci Nat, 104:30.

Procedure for Soil Respiration, Ohio State University Soil Fertility Lab, 2019.

Reeves, T. J., Fuhlendorf, D. S., Davis, A. C., & Wilder, M. S. (2021). Arthropod prey vary among orders in their nutrient and exoskeleton content. Ecology and Evolution, 11:24, 17774-17785.

Romero, G. Q., Mazzafera, P., Vasconcellos-Neto, J., & Trivelin, P. C. (2006). Bromeliad- living spiders improve host plant nutrition and growth. Ecology, 87:4, 803-808.

Sabree, L. Z., Kambhampati, S., & Moran, A. N. (2009). Nitrogen recycling and nutritional provisioning by *Blattabacterium*, the cockroach endosymbiont. PNAS, 106: 19521-19526.

Seastedt, R. T. & Tate, M. C. (1981). Decomposition rates and nutrient contents of arthropod remains in forest litter. Ecology, 62:1, 13-19.

Schmitz, J. O., Hawlena, D., & Trussell, C. G. (2010). Predator control of ecosystem nutrient dynamics. Ecology Letters, 13:10, 1199-1209.

Schmitz, J. O., Wilmers, C. C., Leroux, J. S., Doughty, E. C., Atwood, B. T., Galetti, M., Davies B. A., & Goetz, J. S. (2018). Animals and the zoogeochemistry of the carbon cycle. Science, 362:6419.

Silliman, R. B. & Bertness, D. M. (2002). A trophic cascade regulates salt marsh primary production. PNAS, 99:16, 10500-10505.

Sterner W. R. (1986). Herbivores' direct and indirect effects on algal populations. Science, 231:4738, 605-607.

Stricland, S. M., Lauber, C., Fierer, N., & Bradford, A. M. (2009). Testing the functional significance of microbial community composition. 90:2, 441-451.

Vera, A., Moreno, L. J., Garcia, C., Morais, D., & Bastida, F. (2019). Boron in soil: The impacts on biomass, composition and activity of the soil microbial community. Science of The Total Environment, 685:564-573.

Wilder, M. S., Norris, M., Lee, W. R., Raubenheimer, D., & Simpson, J. S. (2013). Arthropod food webs become increasingly lipid-limited at higher trophic levels. Ecology Letters, 16:895-902.

Wilder, M. S. & Schneider, M. J. (2017). Micronutrient consumption by female *Argiope bruennichi* affects offspring survival. Journal of Insect Physiology, 100:128-132.

Wilder, M., Shawn (2018). Unpublished Data.

Zhang, H., Zhang, B., Wang, S., Chen, J., Jiang, B., & Xing, Y. (2020). Spatiotemporal vanadium distribution in soils with microbial community dyanics at vanadium smelting site. Environmental Pollution, 265A, 114782.

APPENDICES

Table 1. Results of ANOVA analysis a) percent carbon, b) percent nitrogen, c) C/N ratios of whole prey, prey remains, and predator excreta collected from spiders in different seasons.

A

	Df	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
Prey	3	421	140	5.223	0.0019
Treatment	2	8736	4368	162.404	< 0.001
Season	1	344	344	12.783	< 0.001
Prey:Treatment	6	319	53	1.978	0.072673
Prey:Season	3	256	85	3.167	0.026459
Treatment:Season	2	21	10	0.382	0.683398
Prey:Treatment:Season	6	56	9	0.349	0.909596
Residuals	140	3766	27		

B

	Df	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
Prey	3	725	242	8.111	< 0.001
Treatment	2	9031	4515	151.468	< 0.001
Season	1	528	528	17.726	< 0.001
Prey:Treatment	6	561	94	3.139	0.006443
Prey:Season	3	32	11	0.359	0.78294
Treatment:Season	2	433	217	7.267	< 0.001
Prey:Treatment:Season	6	153	25	0.854	0.53027
Residuals	140	4174	30		

C

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Prey	3	23	7.66	21.816	< 0.001
Treatment	2	342.1	171.06	487.04	< 0.001
Season	1	0.2	0.16	0.466	0.496046
Prey:Treatment	6	21.2	3.53	10.05	< 0.001
Prey:Season	3	4.7	1.56	4.453	0.005078
Treatment:Season	2	13.6	6.81	19.379	< 0.001
Prey:Treatment:Season	6	9.8	1.64	4.657	< 0.001
Residuals	140	49.2	0.35		

Table 2. The results of ANOVA analysis on a) PC1, and b) PC2 axis values generated by principal components analysis.

A. PC1

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Prey	3	133	44.32	18.322	< 0.001
Treatment	2	388.6	194.29	80.323	< 0.001
Season	1	55.1	55.08	22.77	< 0.001
Prey:Treatment	6	108.9	18.15	7.505	< 0.001
Prey:Season	3	12.5	4.15	1.717	0.1653
Treatment:Season	2	15.1	7.57	3.13	0.0463
Prey:Treatment:Season	6	15.5	2.58	1.068	0.3835
Residuals	170	411.2	2.42		

B. PC2

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Prey	3	17.7	5.91	5.704	< 0.001
Treatment	2	621	310.49	299.912	< 0.001
Season	1	2.7	2.73	2.639	1.06E-01
Prey:Treatment	6	13.9	2.31	2.235	4.21E-02
Prey:Season	3	1.7	0.56	0.54	0.65566
Treatment:Season	2	10.6	5.31	5.132	0.00685
Prey:Treatment:Season	6	5.5	0.91	0.878	0.51269
Residuals	170	176	1.04		

Table 3. Loading scores of elements for PC1 and PC2 other than C and N.

	PC1	PC2
Al	-0.316	0.171
B	-0.208	0.235
Ba	-0.294	-0.186
Ca	-0.227	-0.329
Cd	-0.137	-0.161
Cr	-0.148	0.030
Cu	-0.018	-0.273
Fe	-0.257	-0.123
K	-0.203	0.225
Li	-0.321	-0.036
Mg	-0.220	0.032
Mo	0.005	-0.267
Mn	-0.095	-0.375
Na	-0.079	-0.171
Ni	-0.266	0.120
P	-0.230	0.295
S	-0.236	0.270
Si	-0.318	0.009
Sr	-0.293	-0.233
Zn	-0.059	-0.360
V	-0.183	0.059

Table 4. The mean elemental values and standard errors per prey group, a) excreta, b) remains, c) whole prey.

A. 1

	Caterpillar			Cockroach			Cricket			Fly		
Al	0.69216	±	0.12662	1.10994	±	0.22219	0.45448	±	0.14557	1.08883	±	0.33879
B	0.03454	±	0.00708	0.03954	±	0.00832	0.02162	±	0.00394	0.04366	±	0.00820
Ba	0.00455	±	0.00103	0.00653	±	0.00166	0.00591	±	0.00186	0.00467	±	0.00150
Ca	3.91480	±	2.12988	3.15189	±	1.18551	1.17107	±	0.32113	1.14669	±	0.23216
Cd	0.00011	±	0.00003	0.00019	±	0.00005	0.00008	±	0.00003	0.00026	±	0.00010
Cr	0.00024	±	0.00005	0.00047	±	0.00016	0.00023	±	0.00011	0.00044	±	0.00022
Cu	0.01289	±	0.00467	0.01695	±	0.00424	0.00539	±	0.00141	0.01635	±	0.00392
Fe	0.38934	±	0.20697	0.36596	±	0.17110	0.14037	±	0.02910	1.32704	±	1.21669
K	72.77345	±	6.14020	31.25392	±	3.25320	29.14842	±	2.02339	32.22199	±	3.18455
Li	0.00029	±	0.00007	0.00034	±	0.00007	0.00014	±	0.00003	0.00028	±	0.00008
Mg	4.49431	±	3.15778	1.67573	±	0.26442	1.23864	±	0.16182	1.31962	±	0.23702
Mo	0.00051	±	0.00018	0.00051	±	0.00019	0.00120	±	0.00025	0.00054	±	0.00032
Mn	0.06982	±	0.06193	0.00695	±	0.00242	0.00588	±	0.00193	0.00654	±	0.00209
Na	5.13650	±	0.84052	8.02312	±	1.45191	7.59749	±	2.08846	8.19148	±	1.98179
Ni	0.01134	±	0.00618	0.00804	±	0.00387	0.00706	±	0.00472	0.02335	±	0.01658
P	40.56737	±	3.44024	32.23093	±	2.97038	29.68611	±	1.91446	36.61797	±	3.77006
S	12.02590	±	1.18575	11.12343	±	1.40655	10.66823	±	0.87231	14.54730	±	1.33136
Si	0.18733	±	0.03403	0.09581	±	0.01848	0.07114	±	0.03271	0.10212	±	0.02842
Sr	0.00625	±	0.00146	0.00751	±	0.00144	0.00653	±	0.00283	0.00651	±	0.00157
Zn	0.09136	±	0.02297	0.44169	±	0.38236	0.07910	±	0.02495	0.25756	±	0.09234
V	0.00189	±	0.00041	0.00624	±	0.00157	0.00214	±	0.00082	0.00532	±	0.00223

B. 2

	Caterpillar			Cockroach			Cricket			Fly		
Al	0.34561	±	0.03782	0.09279	±	0.01117	0.17295	±	0.01667	0.09478	±	0.00685
B	0.00282	±	0.00047	0.00402	±	0.00031	0.00235	±	0.00051	0.01215	±	0.00185
Ba	0.00811	±	0.00095	0.00614	±	0.00076	0.00663	±	0.00118	0.00384	±	0.00045
Ca	13.05651	±	1.70224	5.05390	±	0.62723	4.55194	±	0.92993	1.17978	±	0.07887
Cd	0.00014	±	0.00001	0.00004	±	0.00001	0.00016	±	0.00002	0.00010	±	0.00003
Cr	0.00011	±	0.00002	0.00004	±	0.00001	0.00007	±	0.00001	0.00002	±	0.00000
Cu	0.01121	±	0.00090	0.04126	±	0.00257	0.02981	±	0.00242	0.00936	±	0.00117
Fe	0.53295	±	0.06339	0.06792	±	0.00884	0.16004	±	0.02104	0.06219	±	0.00595
K	22.63524	±	1.86719	20.70837	±	1.49753	10.77108	±	0.98865	3.56049	±	0.65033
Li	0.00027	±	0.00003	0.00011	±	0.00001	0.00014	±	0.00002	0.00004	±	0.00000
Mg	1.40283	±	0.08408	0.75115	±	0.06376	0.67316	±	0.06219	0.90529	±	0.07916
Mo	0.00200	±	0.00037	0.00171	±	0.00010	0.00267	±	0.00029	0.00019	±	0.00004
Mn	0.10354	±	0.00811	0.01240	±	0.00162	0.07490	±	0.00882	0.01848	±	0.00076
Na	7.11834	±	1.46734	16.90855	±	2.26455	9.95017	±	0.87586	5.31308	±	0.56694
Ni	0.00124	±	0.00026	0.00064	±	0.00014	0.00098	±	0.00011	0.00173	±	0.00063
P	12.16628	±	1.17356	4.12020	±	0.46876	7.65472	±	0.66755	1.78347	±	0.32799
S	10.14770	±	0.63444	1.64631	±	0.16993	4.05412	±	0.33742	2.39031	±	0.26986
Si	0.16943	±	0.02219	0.02591	±	0.00296	0.05638	±	0.00972	0.02753	±	0.00298
Sr	0.01207	±	0.00180	0.00758	±	0.00117	0.00870	±	0.00149	0.00590	±	0.00078
Zn	0.26846	±	0.03896	0.22126	±	0.02000	0.53381	±	0.05159	0.13758	±	0.00964
V	0.00129	±	0.00021	0.00048	±	0.00010	0.00092	±	0.00013	0.00035	±	0.00004

C. 3

	Caterpillar		Cockroach		Cricket		Fly	
Al	0.06283 ±	0.01161	0.05230 ±	0.01713	0.03885 ±	0.00328	0.06428 ±	0.02314
B	0.00168 ±	0.00017	0.00342 ±	0.00081	0.00160 ±	0.00046	0.00323 ±	0.00055
Ba	0.00052 ±	0.00006	0.00174 ±	0.00025	0.00081 ±	0.00029	0.00057 ±	0.00019
Ca	1.83535 ±	0.16739	2.00742 ±	0.21996	1.47983 ±	0.54866	0.56303 ±	0.03787
Cd	0.00005 ±	0.00001	0.00002 ±	0.00000	0.00004 ±	0.00001	0.00028 ±	0.00002
Cr	0.00004 ±	0.00001	0.00005 ±	0.00001	0.00004 ±	0.00001	0.00003 ±	0.00001
Cu	0.00926 ±	0.00064	0.02276 ±	0.00272	0.01554 ±	0.00176	0.02653 ±	0.00170
Fe	0.07553 ±	0.00752	0.07449 ±	0.00899	0.06471 ±	0.01225	0.21940 ±	0.10942
K	32.02198 ±	9.77910	11.08601 ±	1.45552	11.25240 ±	0.38417	6.81950 ±	0.41308
Li	0.00004 ±	0.00001	0.00005 ±	0.00001	0.00004 ±	0.00001	0.00002 ±	0.00000
Mg	1.41715 ±	0.05008	1.06882 ±	0.12037	0.72289 ±	0.02037	0.84155 ±	0.03856
Mo	0.00026 ±	0.00003	0.00056 ±	0.00007	0.00078 ±	0.00010	0.00016 ±	0.00004
Mn	0.01340 ±	0.00117	0.00400 ±	0.00044	0.01999 ±	0.00136	0.00641 ±	0.00028
Na	0.89541 ±	0.04761	4.74653 ±	0.49732	10.10974 ±	3.32498	3.62503 ±	0.23307
Ni	0.00019 ±	0.00003	0.00042 ±	0.00006	0.00041 ±	0.00013	0.00019 ±	0.00008
P	12.77105 ±	0.52584	8.80500 ±	0.86897	11.36789 ±	0.31650	8.59487 ±	0.39454
S	4.70328 ±	0.16496	3.85076 ±	0.37592	4.96862 ±	0.12798	6.27936 ±	0.21500
Si	0.06170 ±	0.00714	0.00823 ±	0.00173	0.00900 ±	0.00156	0.00925 ±	0.00116
Sr	0.00156 ±	0.00015	0.00273 ±	0.00034	0.00279 ±	0.00124	0.00182 ±	0.00047
Zn	0.06943 ±	0.00381	0.23469 ±	0.02711	0.24834 ±	0.01227	0.11478 ±	0.00729
V	0.00026 ±	0.00006	0.00022 ±	0.00003	0.00024 ±	0.00005	0.00041 ±	0.00012

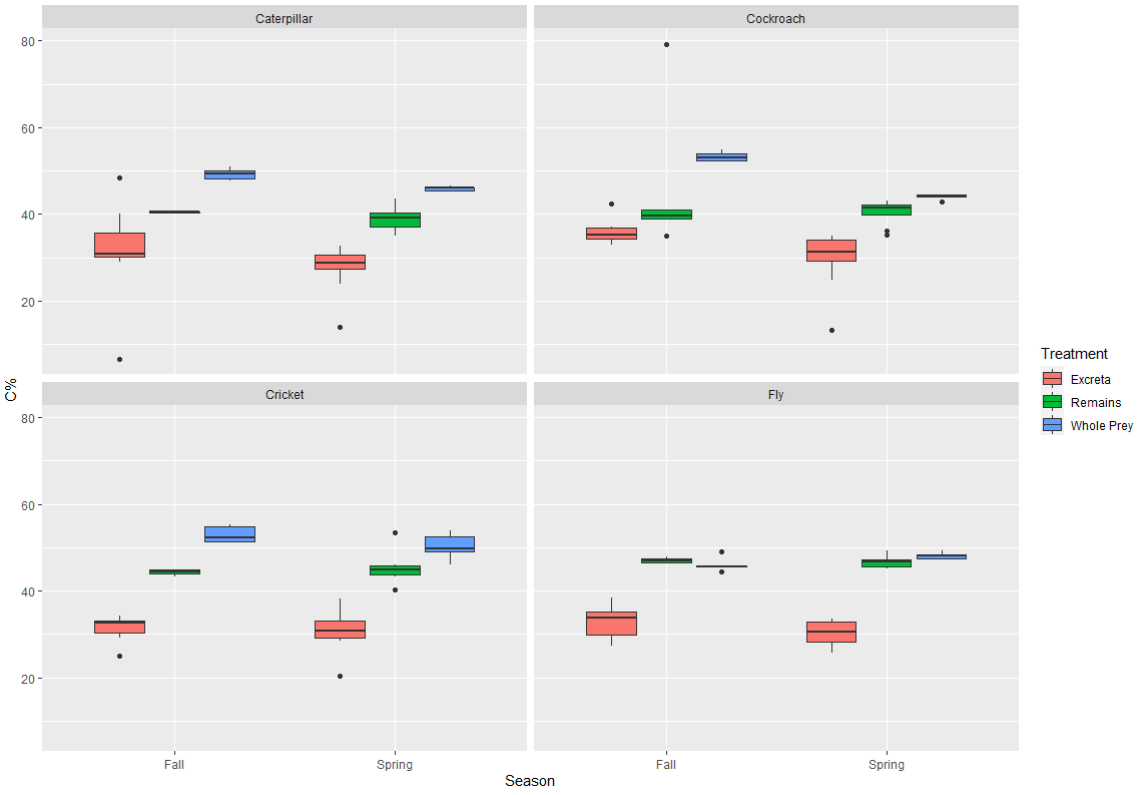

Figure 1. The effects of treatment and season on the percent carbon content of samples.

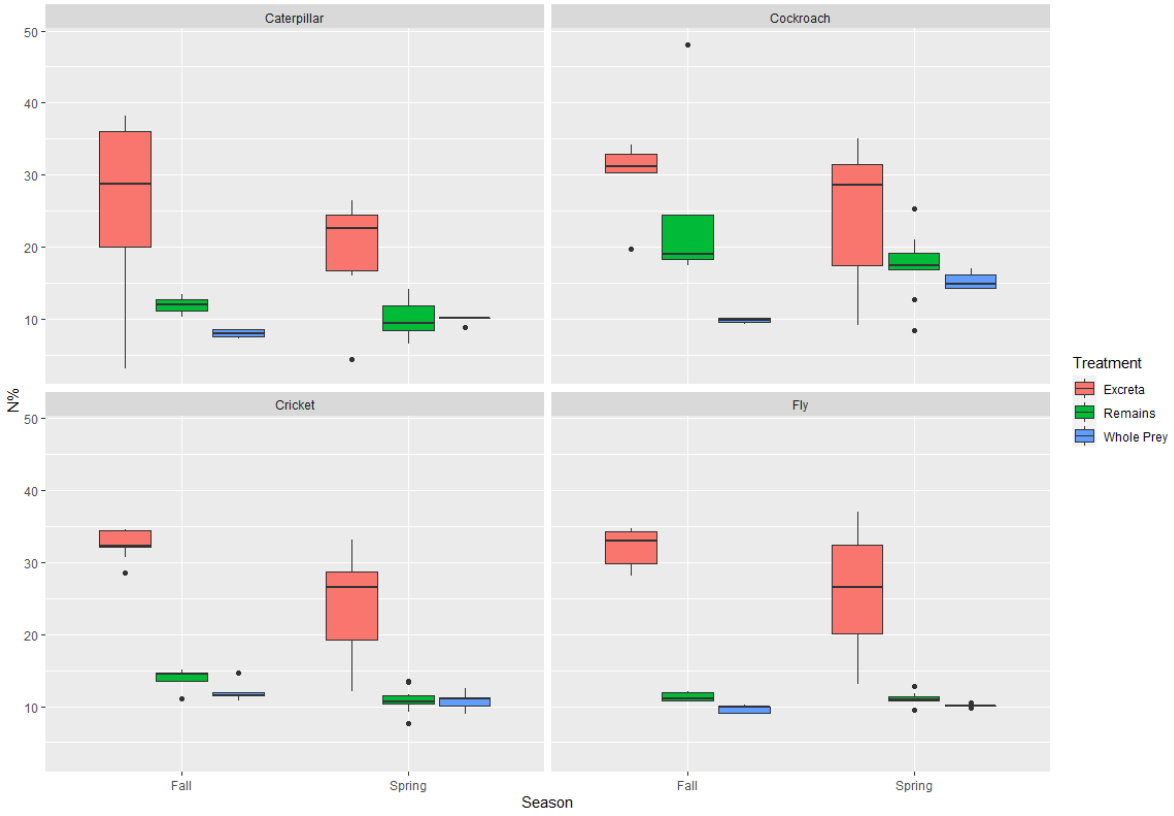

Figure 2. The effects of treatment and season on the percent nitrogen content of samples.

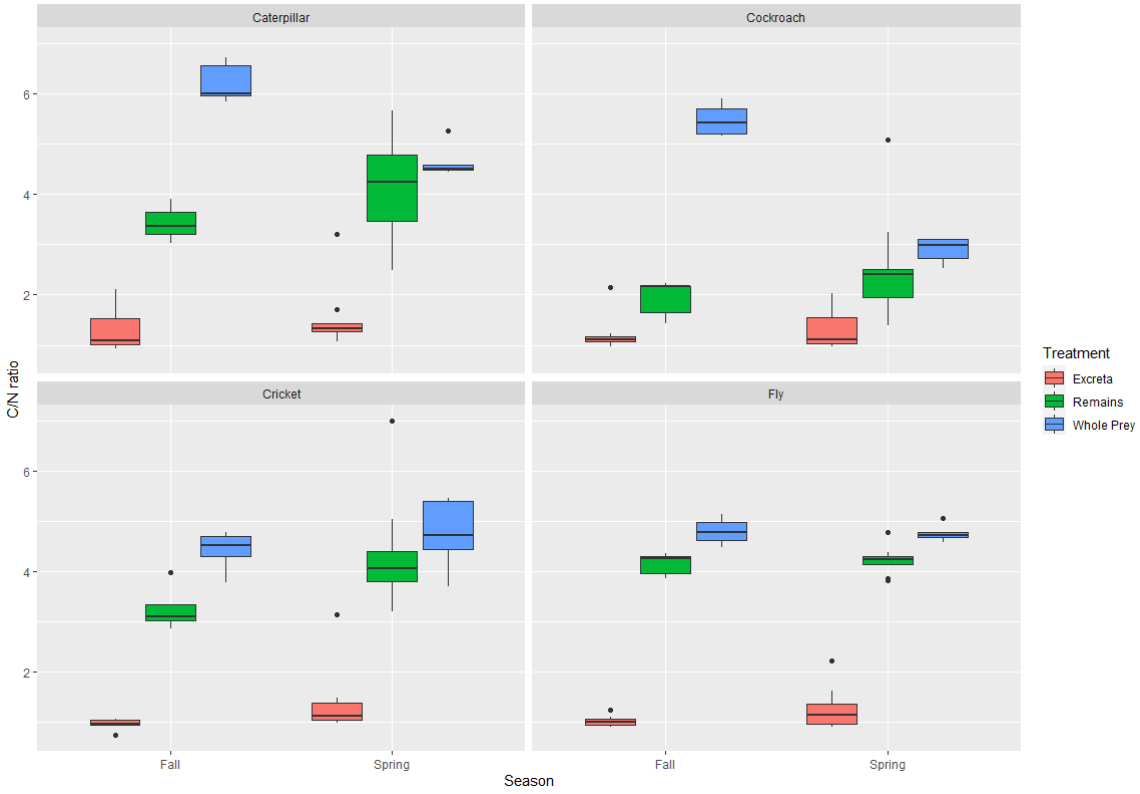

Figure 3. The effects of treatment and season on C/N ratios of samples.

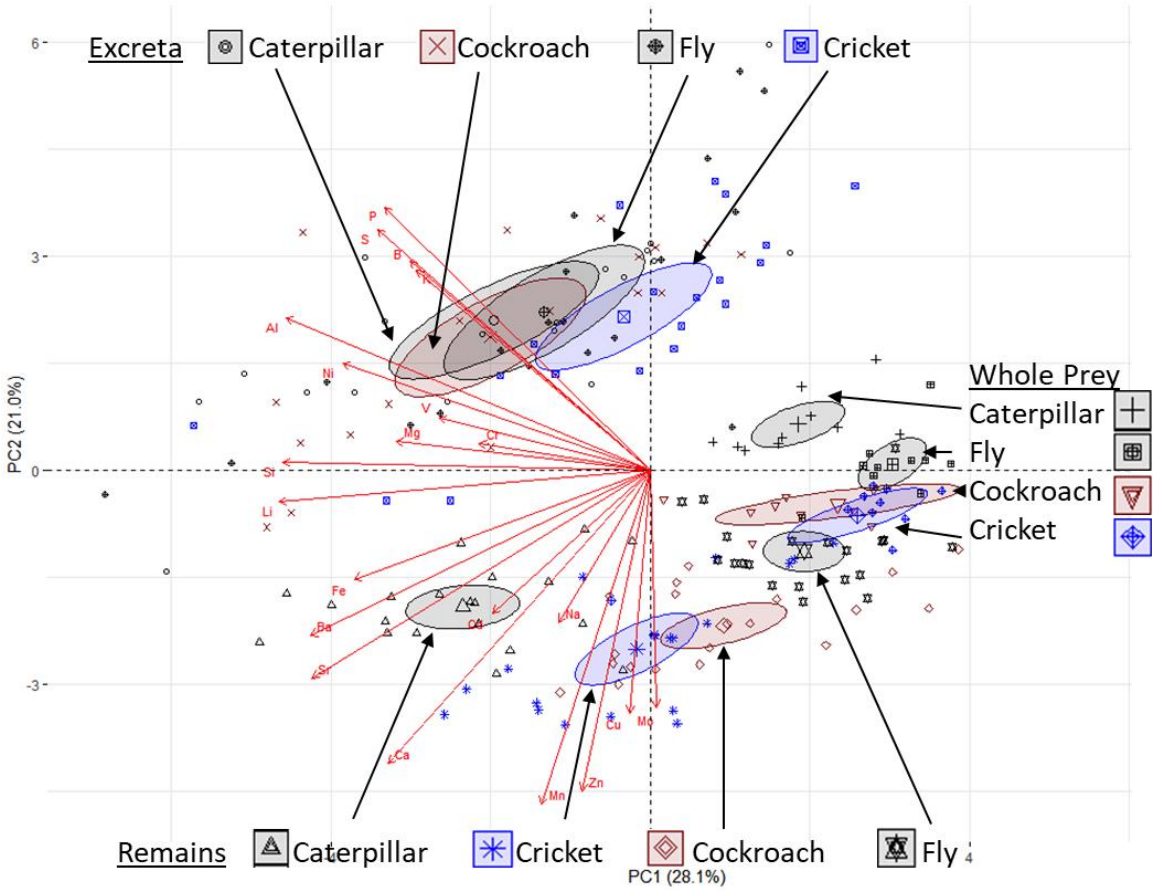

Figure 4. The effects of treatment and prey type on PC1 and PC2 values for elemental concentrations (i.e., all measured elements except for C and N).

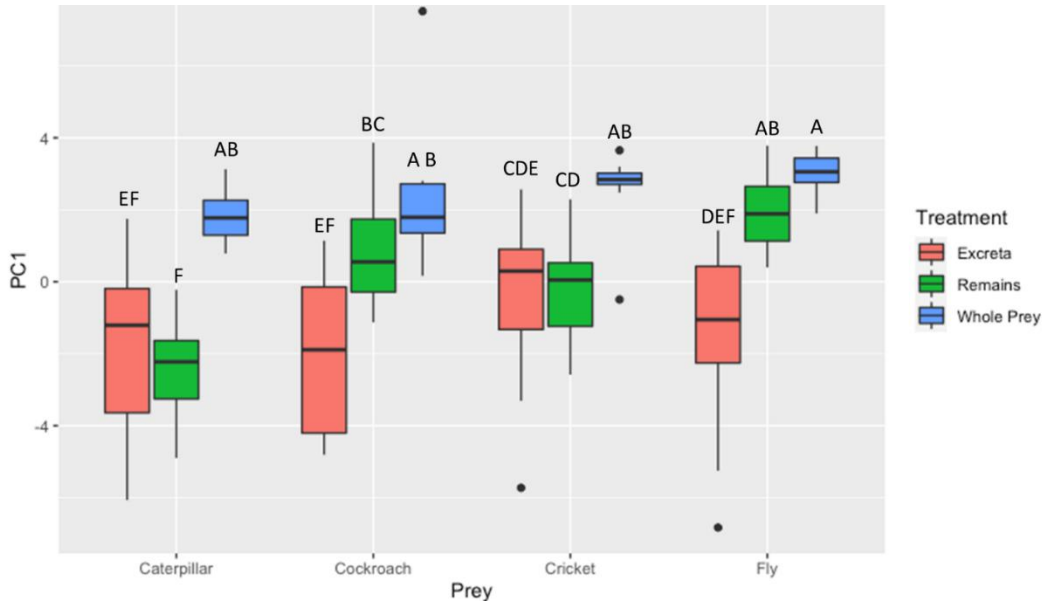

Figure 5. The effects of treatment and prey type on PC1 values for elements other than C and N. Bars with different letters were significantly different from each other in post hoc analyses.

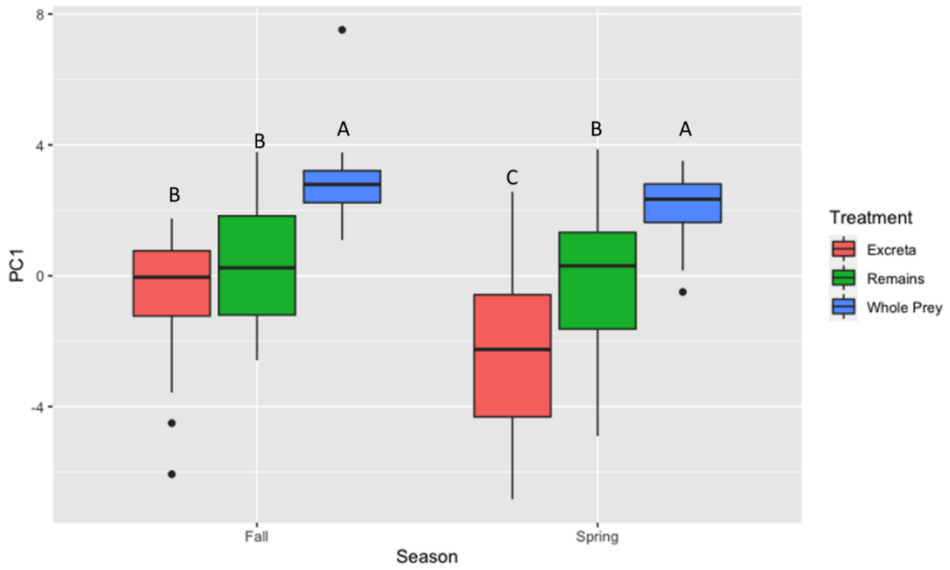

Figure 6. The effects of treatment and season on PC1 values for elements other than C and N. Bars with different letters were significantly different from each other in post hoc analyses.

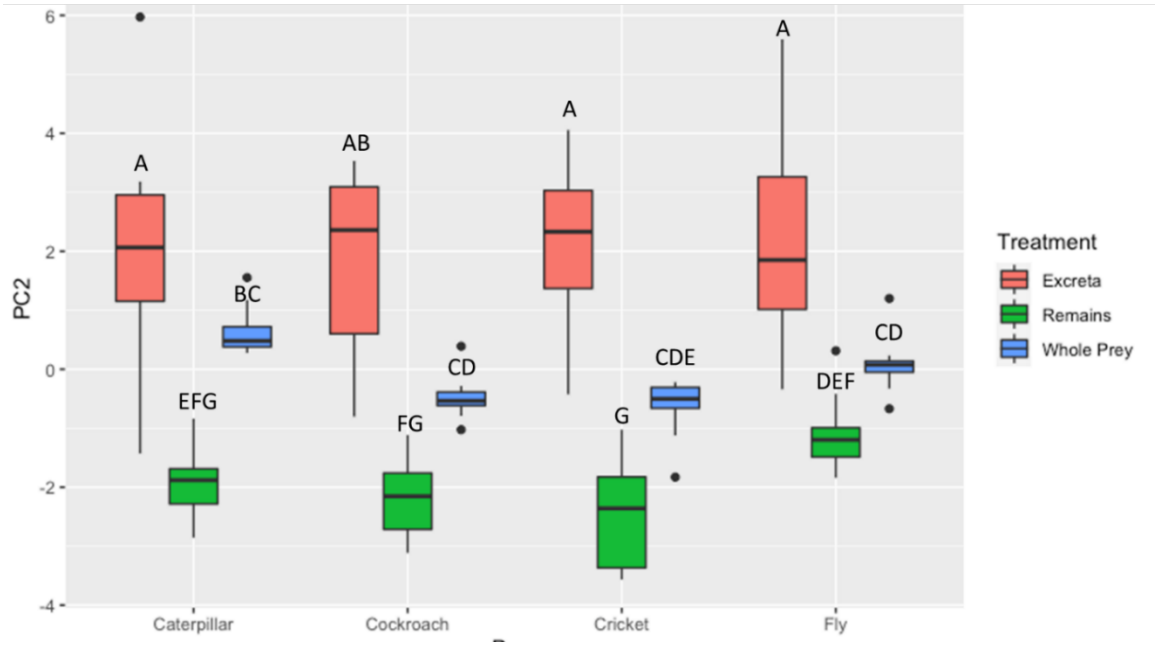

Figure 7. The effects of treatment and prey type on PC2 values for elements other than C and N. Bars with different letters were significantly different from each other in post hoc analyses.

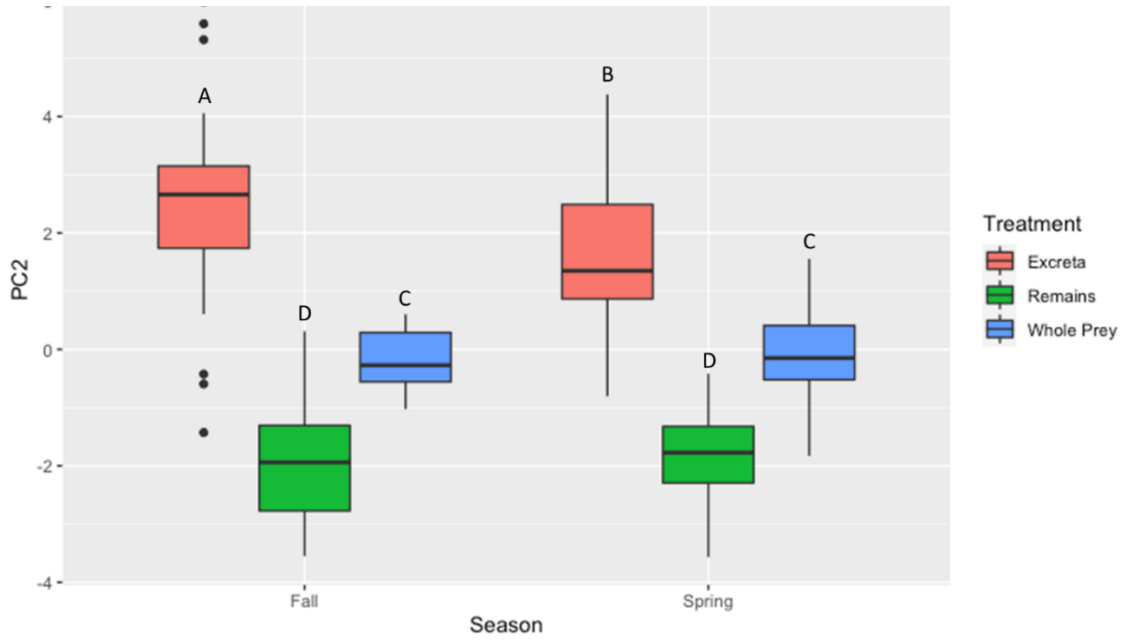

Figure 8. The effects of treatment and season on PC2 values for elements other than C and N. Bars with different letters were significantly different from each other in post hoc analyses.

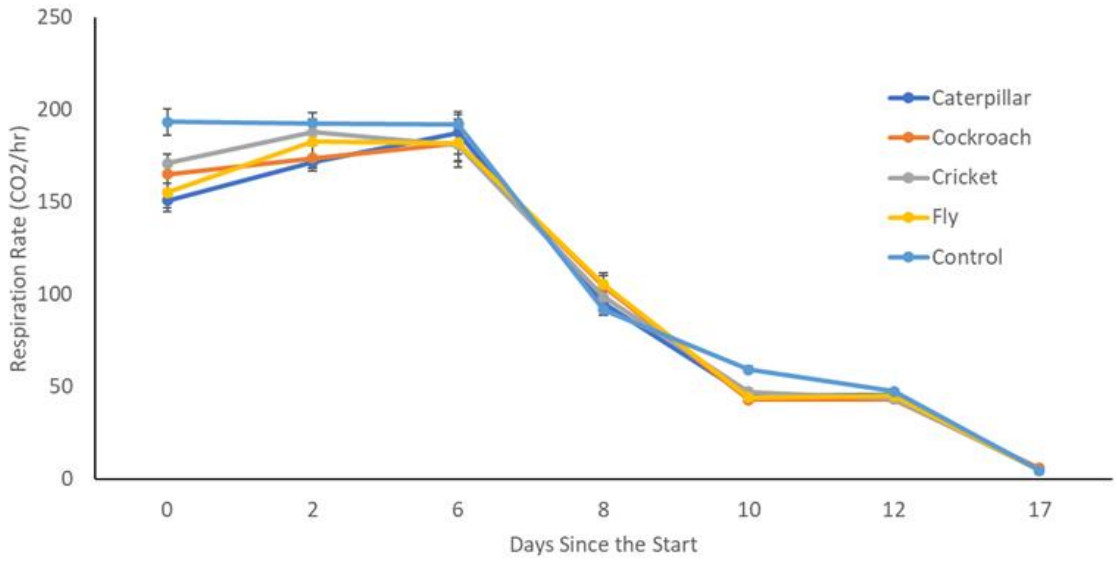

Figure 9. The effect of excreta from different prey on hourly rates of soil respiration (prior to plant litter addition).

VITA

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